UNIVERSITAT DE BARCELONA

Mayotlide: synthetic approaches and structural elucidation

Jesús Herraiz Cobo

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Memoria presentada por

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Mayotlide: Synthetic Approaches and Structural Elucidation

Dirigida por:

Dr. Fernando Albericio Palomera
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UNIVERSITAT DE BARCELONA

FACULTAT DE QUÍMICA

DEPARTAMENT DE QUÍMICA ORGÀNICA

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Jesús Herraiz Cobo

2017

“La diferencia entre un investigador bueno y uno malo es muy clara: el investigador bueno se da cuenta de cosas que el investigador malo no se da cuenta.”

-Fernando Albericio Palomera

“Cada uno vive como puede, o como le dejan”

-Francisco Estevan Estevan

“Everything is going to be fine in the end.
If it's not fine it's not the end.”

-Oscar Wilde

“An educated man is not, necessarily, one who has an abundance of general or specialized knowledge. An educated man is one who has so developed the faculties of his mind that he may acquire anything he wants, or its equivalent, without violating the rights of others.”

-Napoleon Hill

“Sekai heiwa”

-Takemi Takayasu

“Get busy living or get busy dying”

-Stephen King

AGRADECIMIENTOS DE CORAZÓN

Me gusta llamarlos agradecimientos de corazón, porque hay unas cuantas personas, que me han ayudado en esta vida a conseguir mis objetivos sin pedirme absolutamente nada a cambio, sólo buscando mi propio bien, como podrían haber hecho con cualquier otra persona.

Obviamente, los primeros en esta lista son mi familia: mi abuela, mi padre, mi madre y mi hermana. Os quiero muchísimo. Gracias por apoyarme y siempre aconsejarme sobre lo que vosotros pensabais que era correcto. Gracias por alimentarme bien, tanto mi cuerpo como mi mente, por enseñarme valores, a ser bueno nieto, hijo, hermano, y apoyarme en mi carrera profesional y siempre estar a mi lado.

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I would like to thank Julia Hahn (née Julia Neumann) for your time and dedication during my internship in Bayer CropScience. I learnt a lot with you, and I wish you my best to your husband and your kid.

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Patricia Argibay, la brujita. ¡Cómo voy a echar de menos que me llames ¡EmPrEdAtOr! Mil gracias por dedicarme tu tiempo cuando he estado mal. Tu punto de vista de la vida y tus conocimientos han evitado que me volviera un adoctrinado del sistema. Una de las mejores personas que pululan con por el PCB, de las más sensibles y de las más inteligentes. Espero que tu vida empresarial sea fructífera. Quién sabe si nuestras vidas se cruzarán en algún momento. Cuídate mucho.

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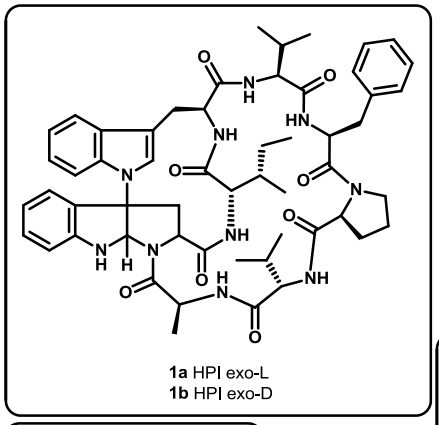
GENERAL INDEX

ACRONYMS

$[\alpha]_D$	optical rotation
a-549	human lung adenocarcinoma epithelial cell line
aa/AA	amino acid
aq.	aqueous
ala	alanine
alloc	allyloxycarbonyl
amu	atomic mass unit
Bn	benzyl
Boc	*tert*-butoxycarbonyl
bs	broad singlet
cat.	catalytic
δ	chemical shift
d	doublet
DCM	dichloromethane
dd	doublet of doublet
ddd	doble doble doblete
DIC/ DIPCDI	*N,N*'-diisopropylcarbodiimide
DIPEA/ DIEA	*N*,*N*-diisopropylethylamine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
dt	doublet of triplets
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
eq.	equivalent
ESI	electrospray ionization
Et	ethyl
Fmoc	9-fluorenylmethoxycarbonyl
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HMBC	heteronuclear multiple-bond correlation
HPI	hexahydropyrroloindole
HPLC	high performance liquid chromatography
HTS	high throughput screening
HOAt	1-hydroxy-7-azabenzotriazole
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single-quantum correlation spectroscopy
HT-29	human colorectal adenocarcinoma cell line
Ile	Isoleucine
*i*Pr	isopropyl
IR	infrared
J	coupling constant
m	multiplet
M	molar
MDA-MB 231	human breast cancer cell line

M.p.	melting point
Me	methyl
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscpy
nOe	nuclear Overhauser effect
Ph	phenyl
Phe	Phenylalanine
ppm	parts per million
Pro	Proline
PyAOP	(7-Azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
q	quadruplet
RT/rt	room temperature
s	singlet
sat.	saturated
spps	solid phase peptide synthesis
t	triplet
*t*Bu	*tert*-butyl
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TLC	thin layer chromatography
TMS	trimethylsilyl
TOCSY	total correlation spectroscopy
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
Z	benzyloxycarbonyl

INDEX OF COMPOUNDS



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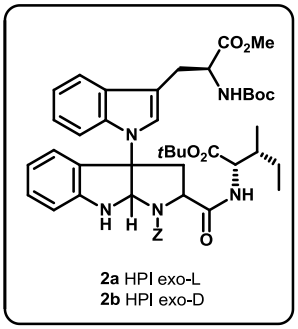
1b HPI exo-D



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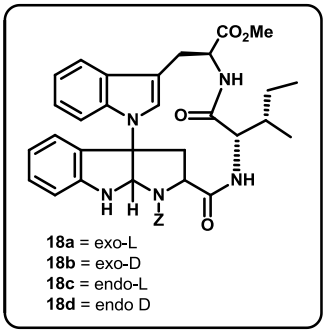

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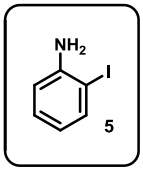
18b = exo-D
18c = endo-L
18d = endo D



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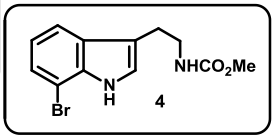

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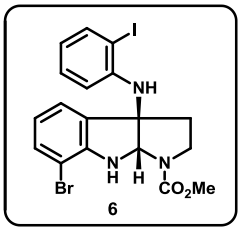

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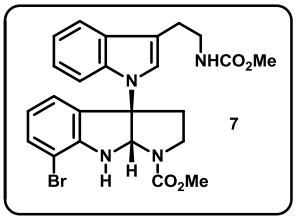

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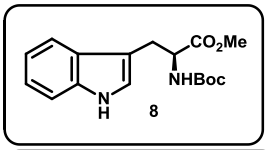

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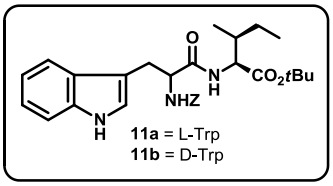

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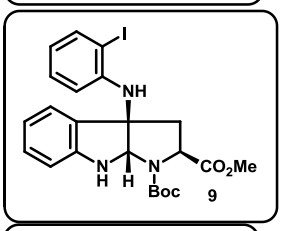
11b = D-Trp



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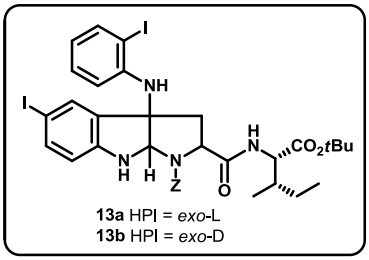
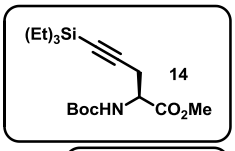


13a HPI = *exo*-L
13b HPI = *exo*-D



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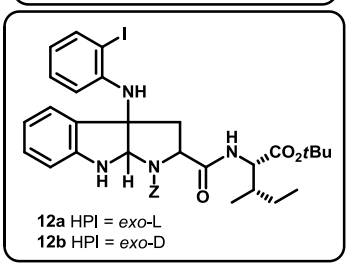
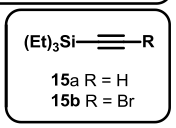
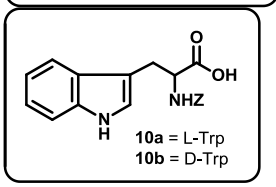


12a HPI = *exo*-L
12b HPI = *exo*-D



15a R = H
15b R = Br



10a = L-Trp
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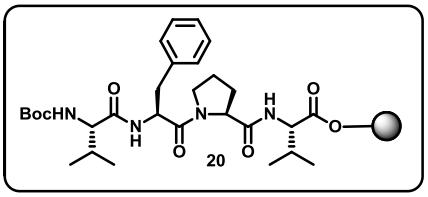

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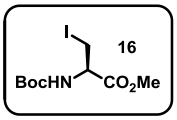

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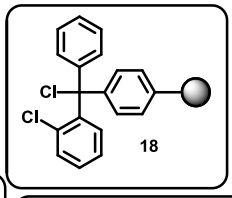

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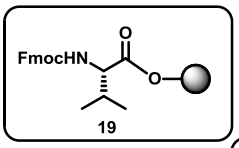

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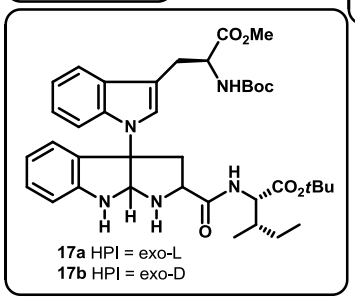
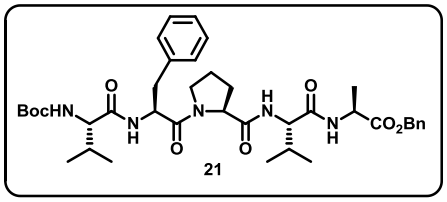


17a HPI = exo-L
17b HPI = exo-D



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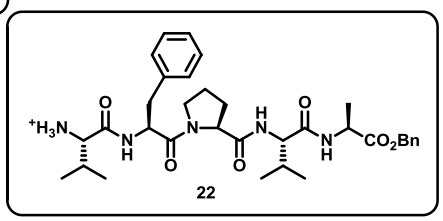

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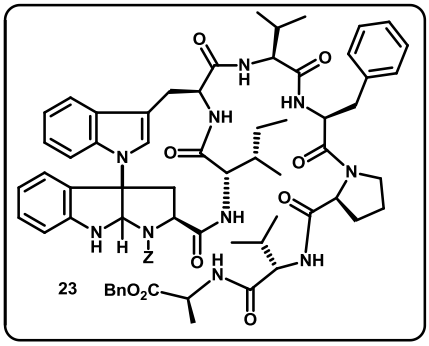

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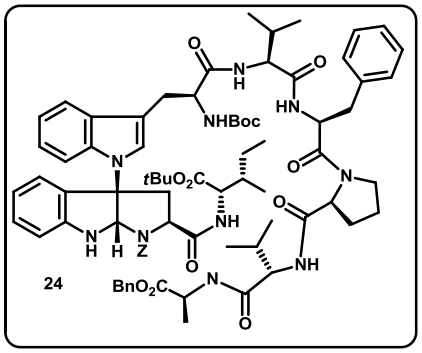

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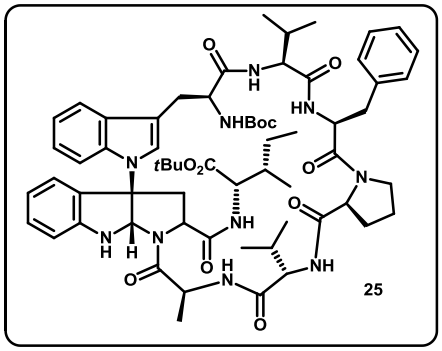

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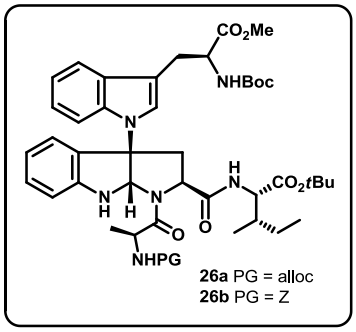
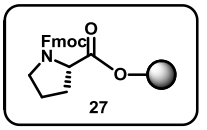


26a PG = alloc
26b PG = Z



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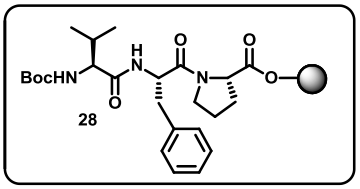

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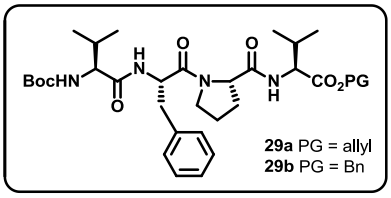
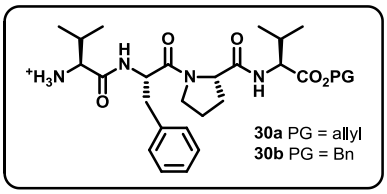


29a PG = allyl
29b PG = Bn



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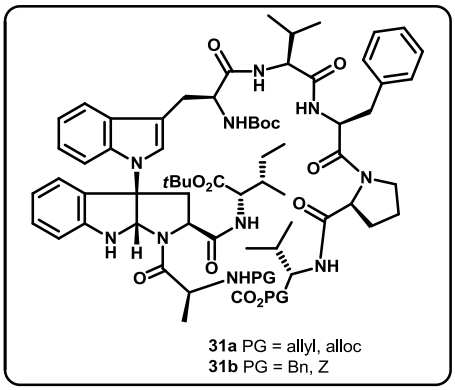
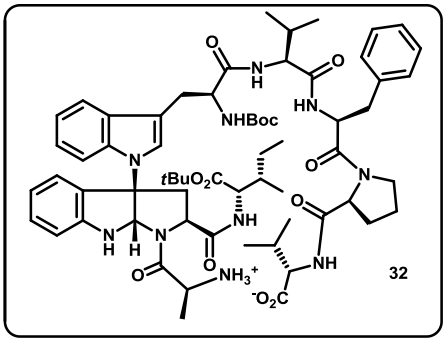
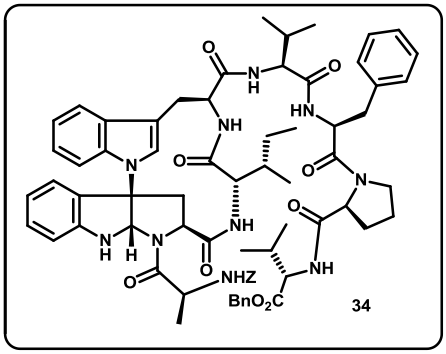
30b PG = Bn



31a PG = allyl, alloc
31b PG = Bn, Z



32



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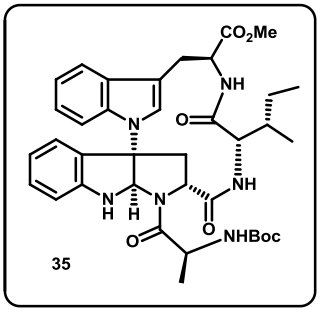

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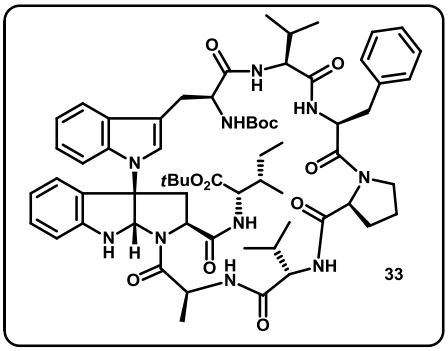

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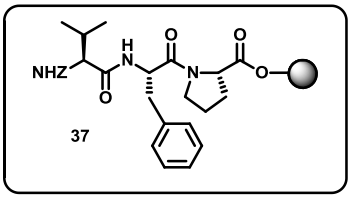
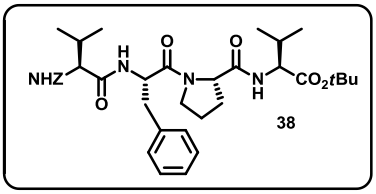


37



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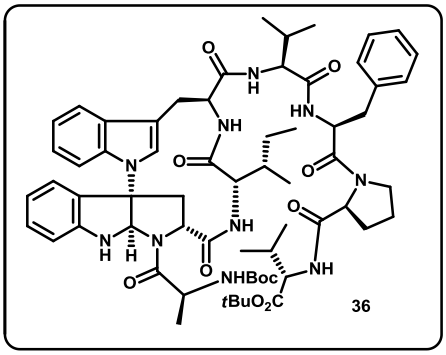

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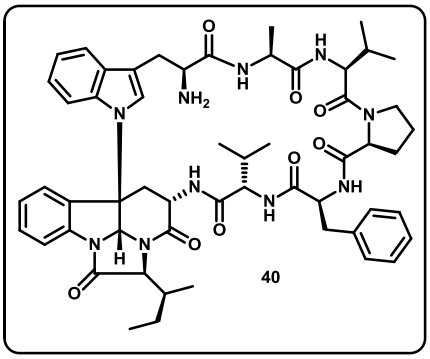

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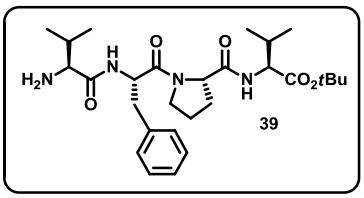

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CHAPTER 1:

Introduction and Objectives

1 Introduction

The term natural product refers to a molecule produced by a living organism, which exhibit interesting biological properties for the treatment of a certain disease. In general terms, they are secondary metabolites with small molecular weight (<1500 amu).[1]

The history of natural products is as ancient as humankind. There are some evidences that suggest that, more than 60,000 years ago, Neanderthals were aware of the medicinal properties of several plants.[2]

The Sumerians were the first civilization that wrote an evidence plants' usage with medicinal interest 5.000 years ago. It was performed on a clay slab and compiled 12 recipes for drug preparation regarding more than 250 plants.[3]

The Chinese Emperor Shen Nung, circa 2.500 BC, wrote the book on roots and grasses "Pen T'Sao", which describes 365 drugs and plants. Most of them are still used nowadays.

Another interesting document, the Ebers Papyrus, was written by the ancient Egyptians circa 1550 BC. It contains information on over 700 plant medicines.

Successively, civilizations as the Indians, Greeks, Romans and Slavics enhanced the libraries of medicinal plants and the Arabs gathered and combined all their knowledge during the Middle Age.

Nevertheless, the great revolution in natural products took place when Friedrich Sertürner isolated morphine from an opium extract, *Papaver somniferum*.[4] That crucial moment established that the natural products could be isolated, purified, identified and commercialized. Merck released morphine into the market in 1827, becoming the first commercially available drug from a natural product.

Another relevant instance concerns the aspirin. The Greek physician Hippocrates of Kos, "the father of modern medicine", related the el use of the white willow bark, *Salix alba*, as medicine against fever on the V century b.C.. Finally, in 1899, Bayer marketed aspirin, the first pure semisynthetic drug based on a natural product.

Another historical contribution to the word of natural products was situated in 1928, concretely the discovery of penicillin by Alexander Fleming from several species of *penicillium*

fungi.[5] Penicillin became the first antibiotic resulting from a microorganism, and Fleming, Florey and Chain were awarded with the Nobel Prize in 1945 for this achievement.

The discovery of penicillin led to the "Golden Age" of antibiotic discovery, between 1940 and 1962. During this period of time, massive studies of microorganisms that could provide new biological molecules were performed.[6]

Regarding the last decades, around 40% of the 520 new approved drugs between 1983 and 1994 were natural products or derivatives, and 60–80% of antibacterial and anticancer drugs were from natural sources. In 2000, about 60% of all drugs in clinical trials for cancer treatment were natural products. In 2001, 8 of the 30 top selling drugs (simvastatin, pravastatin, amoxycillin, clavulanic acid, azithromycin, ceftriaxone, cyclosporin, and paclitaxel) were natural products or derivarives.[7] In less than a century, natural products had revolutionized medicine and enhanced the life expectancy of human being in more than 30 years.

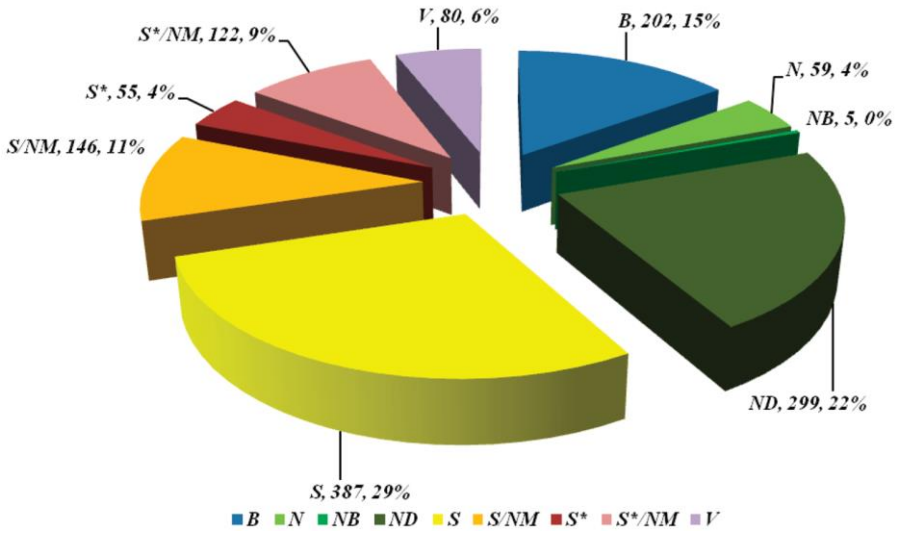
However, on the 90's decade, the interest for the chemistry of natural products decreased significantly. The factors that led to the estrangement by the pharmaceutical industry from the study of natural products were:[8]

- The process of acquisition of a natural product is long and expensive: expeditions, extraction, isolation, purification, structural determination of the active molecule and to conclude the performance of the biological assays. Thus, on the 90's decade, the interest on natural products declined on behalf of High Throughput Screening (HTS) methodologies. HTS allows to test on a fast and efficacy way combinatorial libraries of chemical compounds with a wide range of structures in different therapeutic targets.
- Progresses in molecular biology, cell biology and genomics that augmented the number of molecular targets and demanded shorter times for drug discovery.
- The lack of interest of pharmaceutical companies on the treatment of infectious diseases (classic therapeutic target of natural products).[9]

However, HTS shows inefficacy for successfully discover new drug leads in significant numbers, and hence the most efficient way to find new drug candidates consist on the synergy of natural products with modern technology.

This occurrence has a simple explanation: the creation of synthetic combinatorial libraries has been determined by the availability of reagents and adequate reactions, in contrast with natural products' diversity, which is based on the context of biological utility. Most natural products have a biological function, and therefore the biosynthetic routes which generate these chemical entities have coevolved along with the requirements of ligand functionality. Several studies state that combinatorial chemistry should mimic certain properties of natural products[10] and avoid unnecessary modifications as chirality, conformational constraint elimination and structure simplifications, and recommend the use of natural products as templates for library construction.[11]

Thus, nowadays there is still a great interest on the development of natural products as drugs.[12] Actually, just the 10% of the terrestrial biodiversity has been explored. The relevance of natural products is reflected on the analysis of worldwide approved drugs with all source categories between 1/1/1981 and 12/31/2010[12d] (fig. 1.1), which shows that natural sources are not just important for the discovery of new bioactive molecules, but also are an inspiration for reaching new structures:



B: Biological; usually a large peptide or protein (>45 aa), either isolated from an organism/cell line or produced by biotechnological means in surrogate host.

N: Natural Product.

NB: Natural Product (Botanical).

ND: Derivative from a natural product (usually a semisynthetic modification).

S: Totally synthetic drug (often found by random screening/modification of an exist agent).

S*: Made by total synthesis, but the pharmacophore is/was from a natural product.

V: Vaccine.

/NM Subcategory: Natural product mimic.

Fig. 1.1. New approved drugs from 1/1/1981 to 12/31/2010 (N= 1335).

The main conclusion extracted from this analysis is that natural products play a dominant role in drug development, even though several scientist argue that the introduction of categories such as “S/NM” and “S*/NM” is an overstatement of the role played by natural products in the drug discovery process. On the contrary, these divisions serve to illustrate the inspiration provided by natural products in devising innovative syntheses of structural mimics. Even whether these categories are eliminated, the contribution of natural products to the expansion of chemical libraries is continuous and overwhelmed.

Some researchers have compared the molecular characteristics of natural products opposite to purely synthetic products. On the structure of natural products it is remarkable:[10,13]

- High chemical diversity (functional groups).
- More complex chemical structures.
- More stereocenters.
- More oxygen atoms and, in general terms, heteroatoms.
- Lower ratio of atoms in aromatic rings versus heavy atoms.
- More electron-donating and withdrawing atoms for establishing hydrogen bonds.
- More molecular rigidity.

In addition, natural products display some other biological activities that frequently are selective, specific and have permitted to discover the mechanisms of action.[14]

To sum up, natural products can be described as a group of privileged molecules selected by evolution in order to interact with a large diversity of proteins and other biological targets with a specific objective. That is, they can be considered as a potential group for drug candidates.

1.1 Natural products of marine origin

Unlike terrestrial sources, the study of marine depths didn't start until the middle of the twentieth century, decade in which the necessary technological development for accessing into the ocean depths and initiate the isolation, development and synthesis of natural products of marine origin was achieved.[15]

In 1951, W. Bergman presented two nucleosides isolated from a marine sponge, *Cryptotethia cripta*, which displayed an unknown structure at that time.[16] The following investigations performed by J. Faulkner, P. J. Scheuer, M. H. G. Munro or W. Fenical among others have increased the study, and therefore, the knowledge on this field.

The chemistry of natural products is more complex than the terrestrial ones because they exhibit some additional difficulties. Thus, the main disadvantages encountered when working with marine products are:

- Difficult reproducibility, because changes on the environment of the living being can modify his organism and modify the production of the natural product that we are looking for.
- Possible extinction of the source of extraction and the restrictions about the recollection of biomaterials, as it was stated on the Convention of Biological Diversity in Rio in 1.992.[17]
- Difficulties concerning the access into the places were the marine organisms live. On the surface, it is difficult to reach to several plants due to the topography, but on the deep seas this adversity is increased. The immersions into large profundities

require sophisticated scuba diving equipment, or even underwater equipment with robotic arms.

- Difficulties associated with the reproduction of the growing medium of the natural product. Hence, due to the impossibility of growing a lot of organisms, like sponges and their microbial fauna, they must be recollected in order to achieve valuable compounds for synthesize.
- High quantity of the source of the natural product required for obtaining an analyzable quantity of extract that contains the pure bioactive substance. As well as the inherent difficulties of the isolation and purification of the product
- Difficulties on the synthesis of the natural product, due to the complexity of the structures and the presence of a large number of stereocenters.

Indeed, extracting compounds from oceans is much more troublesome than land ones, but on the other hand is the incentive for working with marine natural products, because of their exotic environment where they live and the wide range of flora and fauna, including microorganisms.

It is predicted that there may be around 300.000 marine species on Earth, [18] although it can oscillate considerably depending on the source. The oceanic diversity is lower than terrestrial one because there are limiting factors like sunlight, which homogenizes the species that inhabit on it.

Nonetheless, there are some other factors that potentiate the interest of marine products in front of terrestrial ones:

- Just one third of the ocean profundity has been explored. Therefore, there are a lot of marine species to be discovered.
- Life was born on the seas, and thus it is more evolved than on the terrestrial surface.
- The high competition level that can exist in some marine ecosystems like coral reefs has as a consequence that the species which evolve their surviving mechanism have higher probabilities of persisting.

Referring to these last points, one of the most important mechanisms that living organisms have developed is the secondary metabolism. The secondary metabolism is a biological machinery which has been developed throughout evolution, giving place to the creation of bioactive molecules which help the organism into surviving. Because of the unfavorable environmental conditions of the marine species, they have improved significantly that metabolism. Hence, they exhibit more sophisticated metabolic pathways and provide molecules with great structural complexity.

For all these reasons, the interest in marine natural products has grown exponentially.

Currently, several marine-origin drugs are distributed into the market, which demonstrates the progress and development to overcome the previously enumerated limitations.[20] Likewise, in the near future it is expected that the amount of marine-origin drugs increases, since the compounds in preclinical and clinical phases have augmented considerably.[21]

1.2 Natural products which contain a hexahydropyrrolo[2,3-*b*]indole moiety

The hexahydropyrrolo[2,3-*b*]indole (HPI) moiety has been found in several naturally-occurring marine natural products. Basically, it is derived from a tryptophan whose amine cyclizes towards the C-2 of the indole ring (fig. 1.2).[22]

The first described natural products that contained the HPI were alkaloids. However, the improvement in the isolation and characterization of natural products allowed to discover another peptidic structures with interesting biological activities.

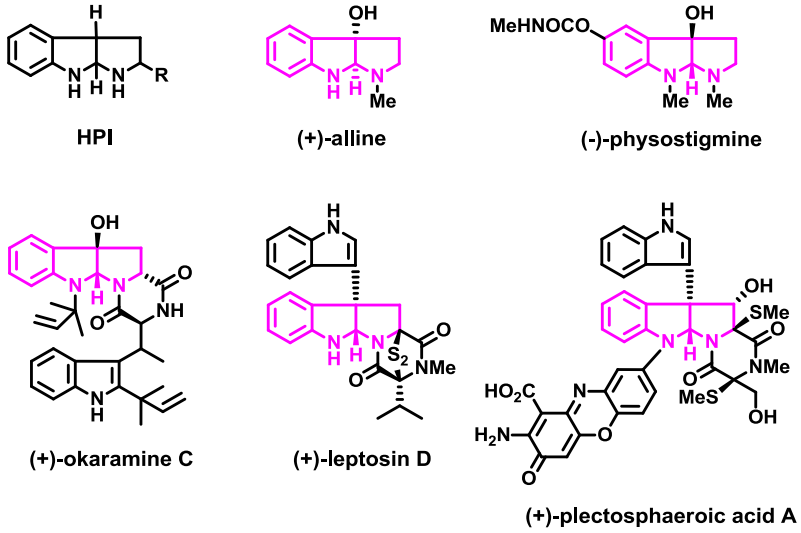


Fig. 1.2. Natural products which contain a HPI unit (marked in purple).

(+)-alline is the smallest natural product with a HPI system.[23] (-)-Physostigmine (also known as eserine, from éséré, the West African name for the Calabar bean) is an important alkaloid extracted from the Calabar bean, *Physostigma venenosum.* This alkaloid, a reversible chlolinesterase inhibitor, has been used on the treatment of cholinergic disorders, myasthenia gravis, Alzheimer, mydriasis and glaucoma (fig. 1.2.).[24]

Some other natural products contain the HPI condensed with an aminoacid, forming a diketopiperazine (DKP). That's the case of the family of the okaramines,[25] isolated from the fungus *Penicillium simplicissum*, leptosin D[26], or the family of the plectosphaeroic acid,[27] among others (fig. 1.2).

Another category of natural products comprises those who have more than a HPI unit. The amauromine[28] exhibits two HPI units condensed by means of a DKP, the family of the chimonantines,[29] which have the two HPI units connected by the respective C^{3a}. The (+)-WIN 64821 and (+)-WIN 64745, which are neurokinin antagonist, contains, furthermore, a DKP into each HPI cycle.[30] Idiosmermuline contains three HPI units,[31] quadrigemine C four,[32] until the caledonine, which contains seven HPI units (fig. 1.3).[33]

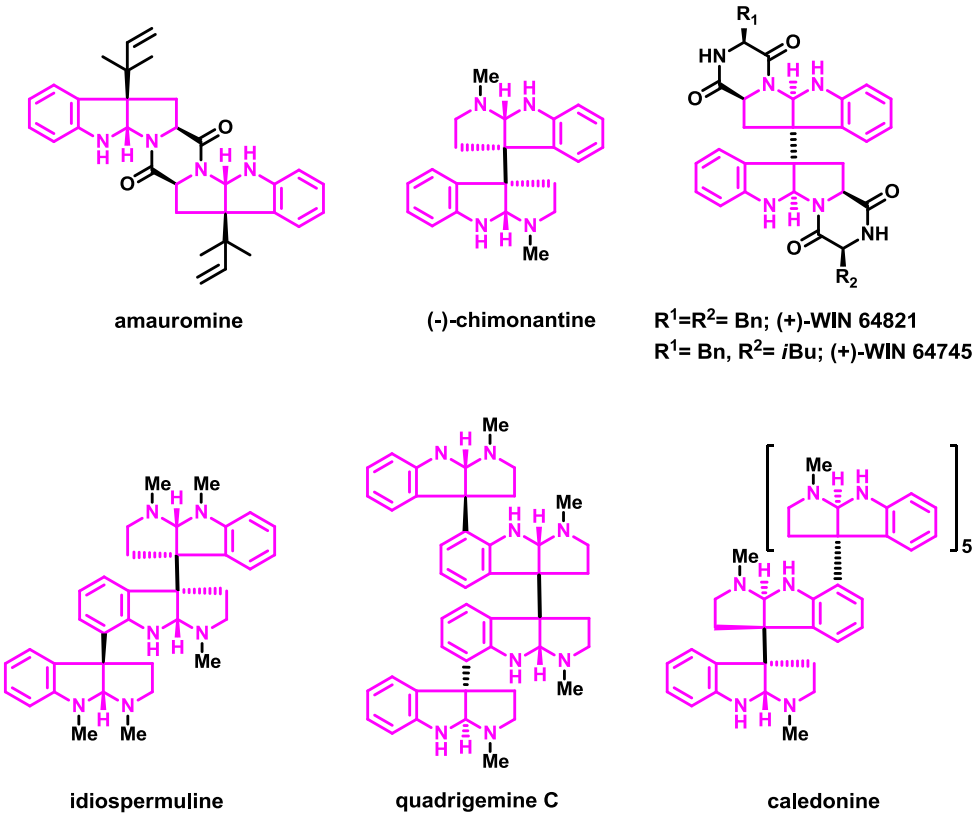


Fig. 1.3 Natural products with more than a HPI unit (marked in purple).

In another natural products, the HPI is integrated into a peptidic chain, as the omphalotine D,[34] the phakellistatin 3[35] or the himastatine (fig. 1.4).[36]

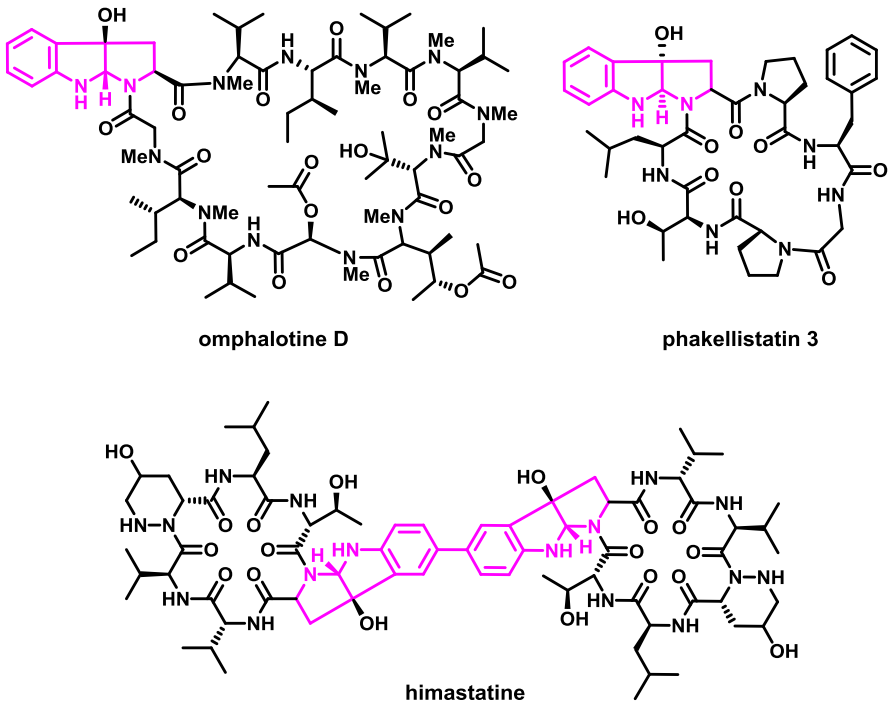


Fig. 1.4 Natural products with the HPI (marked in purple) involved in a peptidic chain.

1.3 Natural products with two Trp united via N^1-C^{3a} bond

In the recent years, a new group of molecules have been isolated, which contain a Trp tied with a modified tryptophan or tryptamine by means of a N^1-C^{3a} bond. Some instances comprises the alkaloids psychotrimine[37] and psychotetramine,[38] the family of the epipolithiodioxopiperazines, with the chetomin[39] and the chaetocochines A and B,[40] among others, which exhibit a Trp-HPI motif. They present interesting bioactivities as: antimicrobial, antitumoral, cytotoxicity and antinematodal (fig. 1.5).

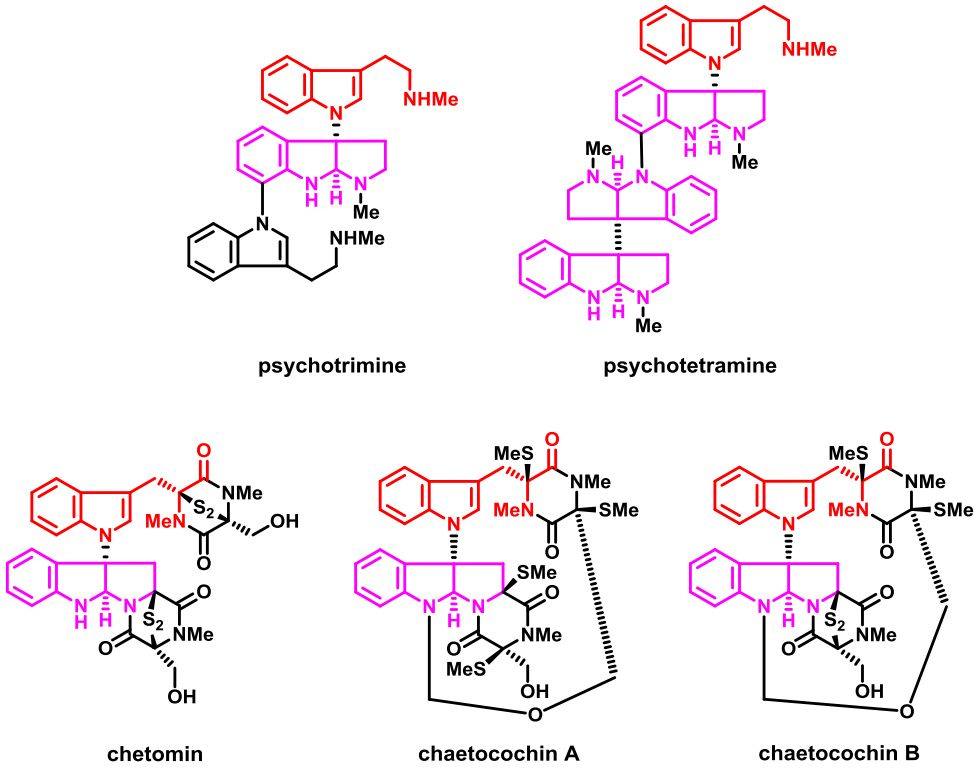


Fig. 1.5 Natural products with a N^1-C^{3a} bond between a Trp (marked in red) and HPI (marked in purple).

Another category of natural products with two Trp connected with a N^1-C^{3a} bond comprises the α-carbolines. The first reported natural products which contained the α-carbonile framework were the grossularines in 1989, isolated from the tunicate *Dendrodoa grossularia* (fig 1.6).[41]

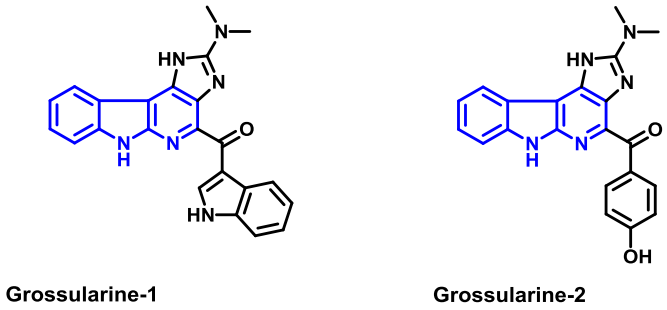


Fig. 1. 6 Grossularines, first natural product with α-carboline motif (marked in blue).

Later on, Nakao and coworkers isolated from the spongia *Cribrochalina olemda* a family of cyclic peptides which contained a non peptidic bond beween a Trp and a Trp-derivative α-carboline, which was part of a fused tetracyclic system. Because of this feature, they were called "kapakahines" (kapakahi means on Hawaiian language lopsided, crooked) (fig. 1.7).[42]

Kapakahines B, C, E, F and G showed moderate cytotoxicity against P388 murine leukemia cells.

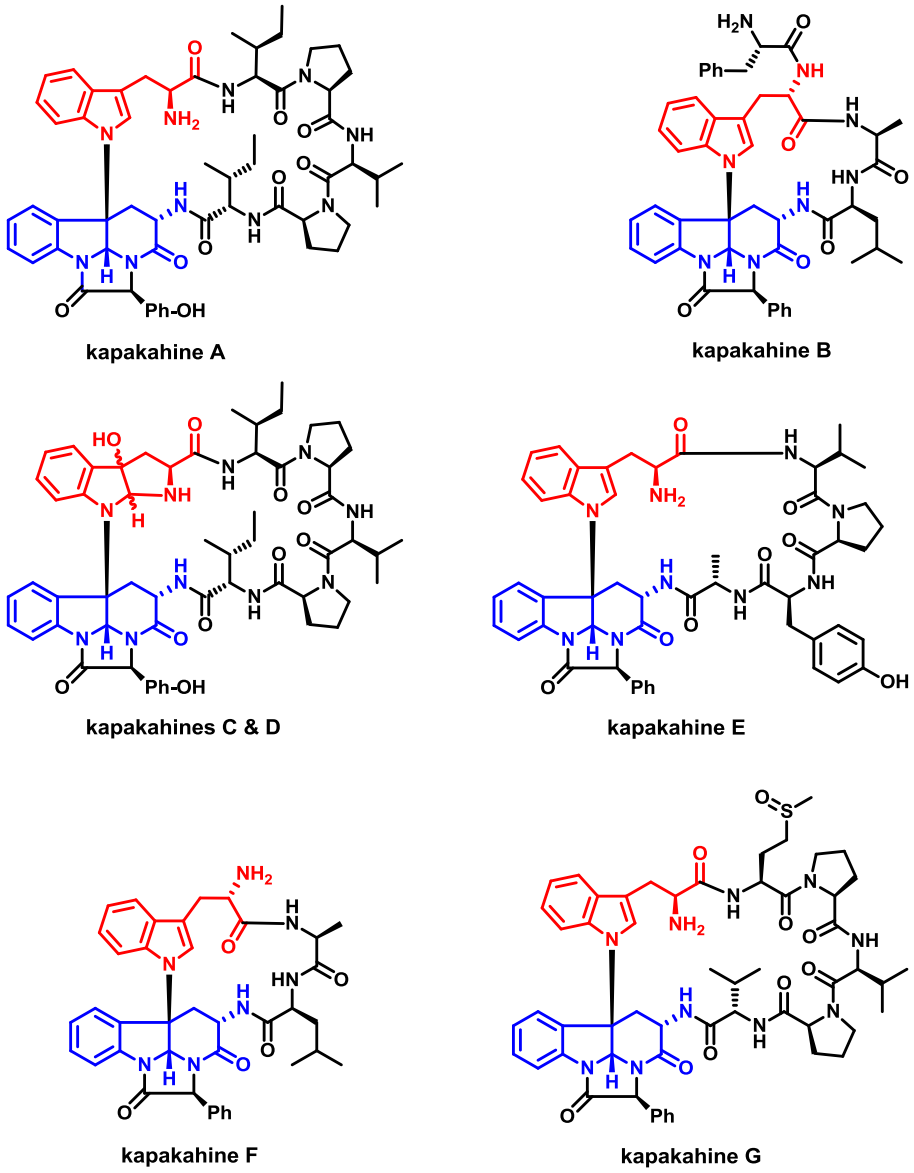


Fig. 1.7 The family of the kapakahines, with a N^1-C^{3a} bond between a Trp (marked in red) and an α-carboline (marked in blue).

1.4 Mayotlide

Mayotlide (**1**) is a marine naturally-ocurring peptide isolated by PharmaMar from an extract of *Spongia sp* (fig. 1.8). It presents cytotoxic activity against the following human cancer cells: MDA-MB-231, A-549 and HT-29, in micromolar concentrations.

In order to elucidate the structure of mayotlide, exhausting studies were performed: mass spectrometry, NMR studies and Marfey degradation and aminoacid analysis.[43] They provided the following data:

- There are two linked tryptophanes, one of them cyclized, establishing a N^1-C^{3a} bond.
- All the aminoacids have L configuration but the cyclized tryptophan, which remains unknown.
- The linear sequence was H-Ile-Trp-Val-Phe-Pro-Val-Ala-Trp-OH

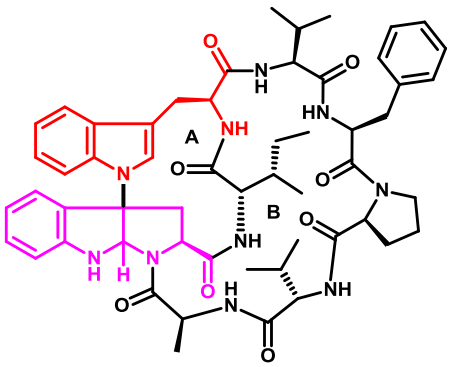


Fig 1.8 First structure proposal for mayotlide (**1**).

Hence, the first proposal of mayotlide contained two macrocyclic rings: the ring B (aka big ring) is constituted by the 8 aminoacids of the molecule, tied by amide bond, with two tryptophan residues on positions *I* and *i+2*. The other cycle (cycle A or small cycle) is established by the two Trp and one Ile, with the two tryptophans forming a Trp-HPI unit via N^1-C^{3a} bond.

1.5 Synthetic approaches for mayotlide

There are three aminoacids that belong to both cycles: the two Trp and the Ile, which are the three aminoacids of the ring A. therefore, it would be interesting to achieve a robust synthon which contains those aminoacids and that allow us to freely select the points of

aminoacid coupling and/or cyclization without any compromise. Such synthon would be tripeptide **2** (fig. 1.9).

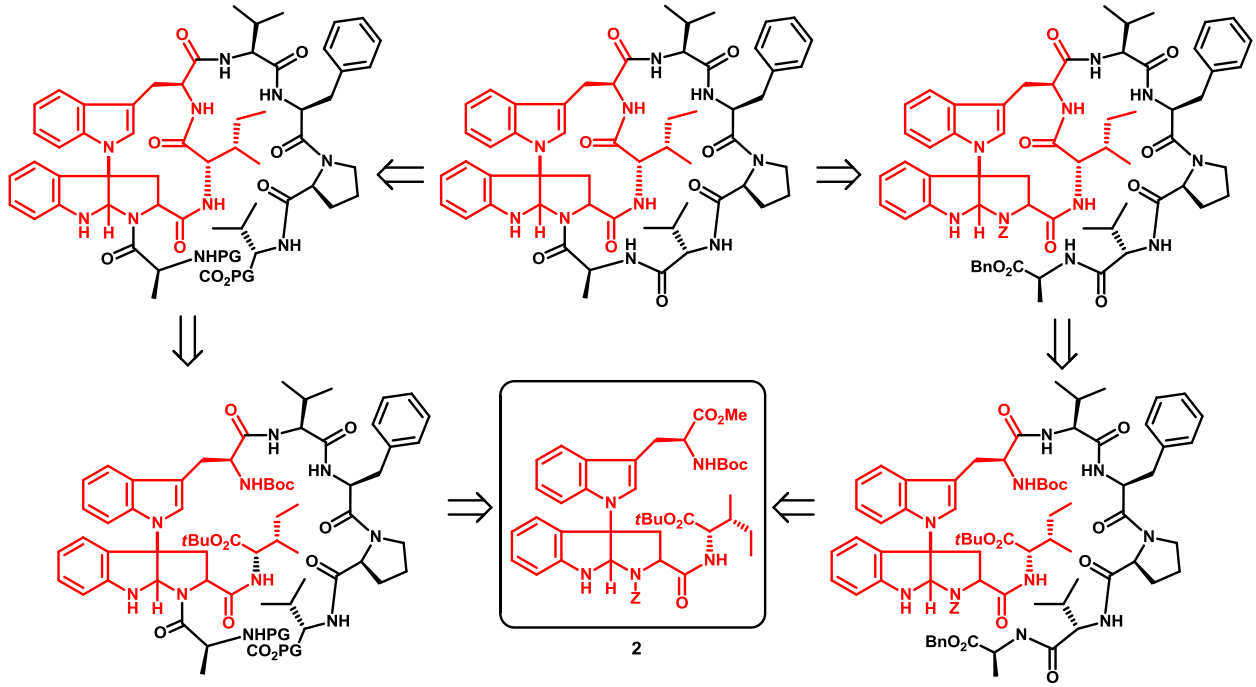


Fig 1.9 Retrosynthetic scheme of mayotlide proposal starting with tripeptide **2.**

Thus, the key points for the synthesis of mayotlide are:

- Find a proper strategy that allows to reach, on a facile manner, the intermediate **2**, which is the simplest scaffold that contains the three aminoacids that are common in both cycles.
- Make and adequate selection of the cyclization points. In order of being successful on this point, it is necessary to make and adequate and proper use of the protecting groups for the N and C terminus of the peptide sequences.

2 Objectives

The main goal of this work is the achievement of the total synthesis of mayotlide, in order to confirm the structure of the molecule and confirm the absolute stereochemistry of the HPI unit. In order to reach the target adequately, these are the steps that should be completed:

- **Synthesis of the Trp-HPI system.** The formation of the tripeptide **2** takes place in two steps: 1) a convenient Trp undergoes a tandem N-C2 cyclization - 2-iodoaniline oxidative addition to form a HPI-2-iodoaniline intermiediate; 2) the 2-iodoaniline, along with a disubstituted alkyne, condense together to reach the Trp-HPI framework through the Larock reaction.

- **Synthesis of the ring A or mayotlide**. In order to study the stereochemistry of the HPI, it is easier to do it on the ring A than on the whole molecule, attempting to find any similarity with the spectra of the natural product.

- **Synthesis of mayotlide.** The tripeptide **2** offers different possibilities. Hence, a correct use of the protecting groups and an appropriate selection of the cyclization points is essential for the total synthesis.

- **Discussion of results.** The experimental results of mayotlide will be compared with the natural one and the conclusions will be extracted from them.

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CHAPTER 2:

Synthetic and Stereochemical Studies of the Ring A

2.1 Towards the synthesis of the ring A

On the introduction it has been discussed the structural particularities of mayotlide. This molecule is constituted by two rings: on the ring B, all the aminoacids are linked via amide bond, and on the ring A it appears this exotic Trp-HPI scaffold.

It is known by the aminoacids' analysis the L configuration of all of them except the HPI. Therefore, it is necessary to make an extensive study of this aminoacid before starting the syntesis.

2.2 What is the HPI?

The HPI (hexahydropyrroloindole) is a tricyclic structure constituted by a Trp whose amino terminus has cyclized through the C2 of the indole system.

In 1981, Taguichi and Hino reported the N-C2 cyclization of a Trp in acidic conditions.[1] During the HPI cyclization, two new stereocenters are generated in positions C^{3a} and C^{8a}. Both substituents adopted always a *cis* conformation because of the conformation of the ring, and hence can be considered as one block of stereocenters (fig. 2.1).

Taguichi and Hino also studied the relationship among the three stereocenters:

- Whether the substituents in C^{3a} and C^{8a} are in *trans* conformation concerning the R^1 substituent, the HPI has ***endo*** configuration, which follows a **thermodynamic control**.
- Whether the substituents in C^{3a} and C^{8a} are in *cis* conformation concerning the R^1 substituent, the HPI has ***exo*** configuration, which follows a **kinetic control**. The kinetic adduct undergoes isomerization towards the thermodynamic *endo* adduct as they are in equilibrium (fig. 2.1). The explanation to this phenomena is the steric hindrance between the R^1, C^{8a} and C^{3a} surrogates when all of them are positioned in *cis* on the HPI ring.

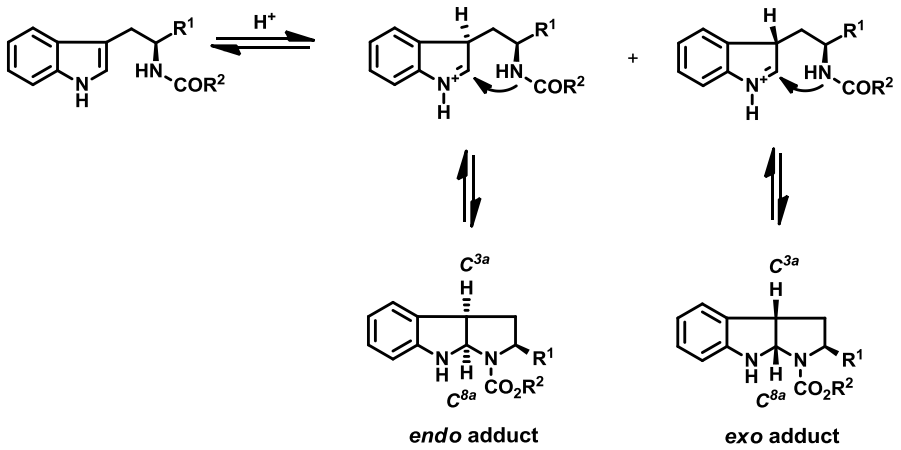

Fig. 2.1. *Endo* and *exo* adducts of the HPI from Trp.

In order to justify the thermodynamic differences, the pre *endo-exo* equilibrium is depicted on fig. 2.2. On the transition state of the pre-*endo* ensemble, the R^1 substituent has a steric conflict with the aromatic ring of the indole, which doesn't occur on the pre-*exo* ensemble, which is the kinetically favored.

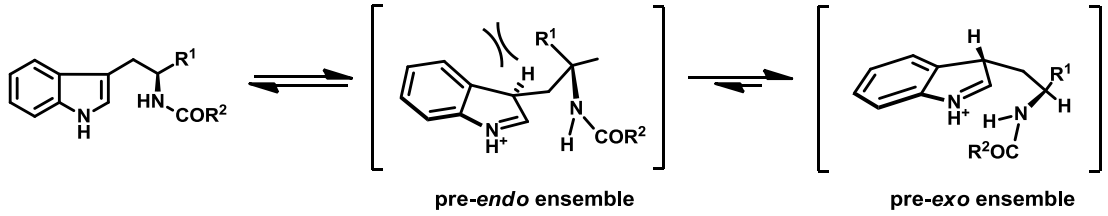

Fig. 2.2. Pre *endo-exo* ensembles**.**

2.3 Preliminary studies of the ring A

As it was described on the previous point, depending on the relationship between the R^1 substituent and the surrogates in C^{3a} and C^{8a}, there can be two adduct possibilities: *endo* and *exo*. Furthermore, the configuration of the Cα of the Trp that forms the HPI is also unknown, which can be L or D. Hence, we have "like" two stereocenters, which forms $2^2 = 4$ diasteromers of the HPI unit (fig 2.3):

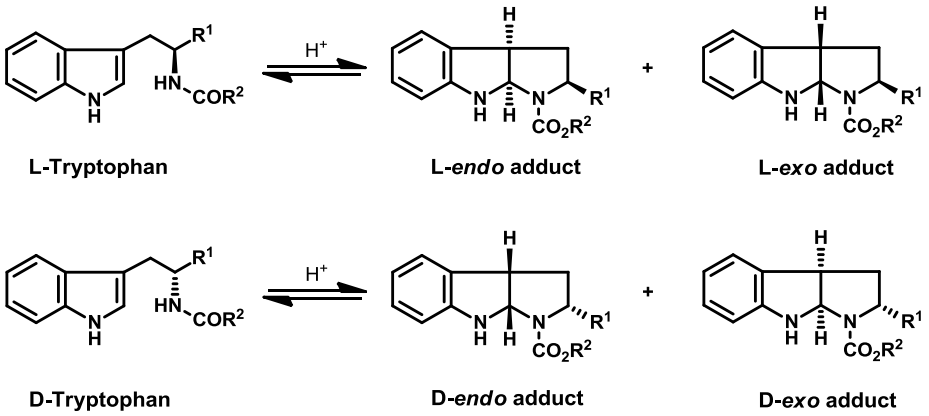

Fig. 2.3. Four possible diasteromers of the HPI unit.

To the best of our knowledge, there were not any experimental evidences about which of the HPI diasteromers could be more stable for macrocyclic rings. Hence, before starting the experimental procedures, it was recommendable to discard which diasteromers are less favorable. A computational study with the MOE 2015.8 software (from the Chemical Computing Group)[2] analyzed the energy of the ring A with the four HPI possibilities. Both *exo* L and D adducts (**3a** and **3b**) have the lower energies and the *endo* ones (**3c** and **3d**) seem to be more unstable. Therefore there were discarded (fig. 2.4).

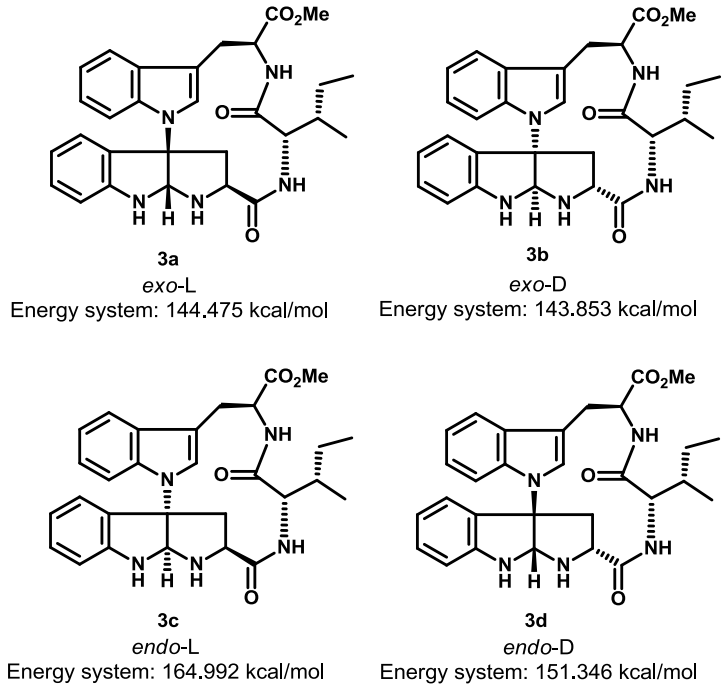

Fig. 2.4. Computational analysis of the Ring A (**3**) possibilities.

At that point, with two candidates instead of four, another computational study was performed but this time with the whole molecule (**1a** and **1b**) (fig. 2.5):

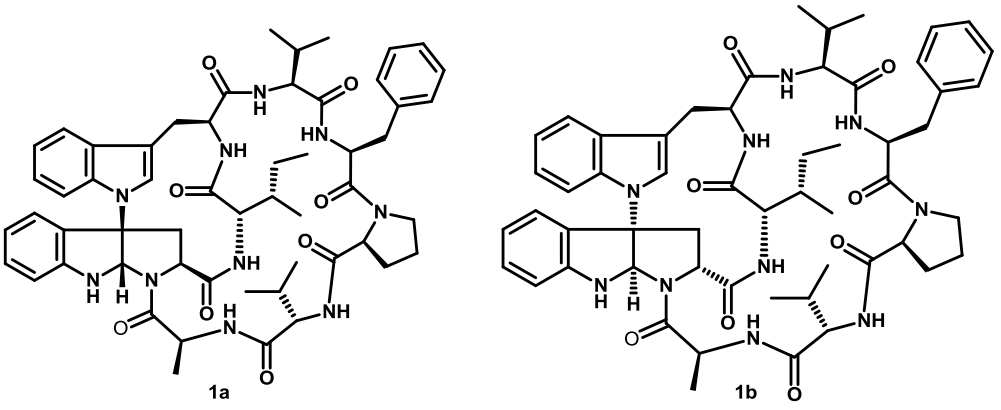

Fig 2.5. Conformational studies of *exo* L and D *endo* mayotlides (**1**).

Albeit the *exo*-D ring A has lower energy, it seems that the mayotlide with the *exo*-L HPI is more favored. Hence, the first mayotlide approach would be in that direction.

Now that there was a hint about the possible structure of mayotlide, the synthetic methodology should be selected. But before, it is necessary to analyze the biosynthetic pathways for searching a good methodology that could mimic the natural synthetic process.

2.4 Biosynthetic studies of the Trp-HPI system

Although the biogenetic mechanism for dimeric indole alkaloids linked at carbon are presumed to proceed via a radical dimerization process,[3] the genesis of nitrogen-carbon linked relatives in this family is still unknown.

There are some possibilities about how nature creates this exceptional connectivity. The most straightforward possibility would be a radical- or cationic-based, oxidative dimerization to directly form the N^1-C^{3a} bond (fig. 2.6 A).

A second possibility, lies on the propensity of a nitrogen substituent to migrate from *C2* to *C3* of indole via a [1,5] sigmatropic rearrangement (fig. 2.6 B).[4]. Such situation would require a N^1-C^2 dimer of tryptophan units to afford a N^1-C^{3a} linked compound. Such Naturally occurring alkaloids are unknown to the best of our knowledge, although imidazole heteroatom linkage at C^2 of tryptophan has precedents, as celogentin C.[5]

A third option would involve direct nucleophilic displacement of a halide by an indole nitrogen (fig. 2.6 C). Rainier and coworkers have demonstrated that this process can happen,

but it requires a strong base and proceeds to give the *endo* diasteromer, the opposite output that is required for the hypothesis of mayotlide, via epimerization of the HPI α-carbon.[6]

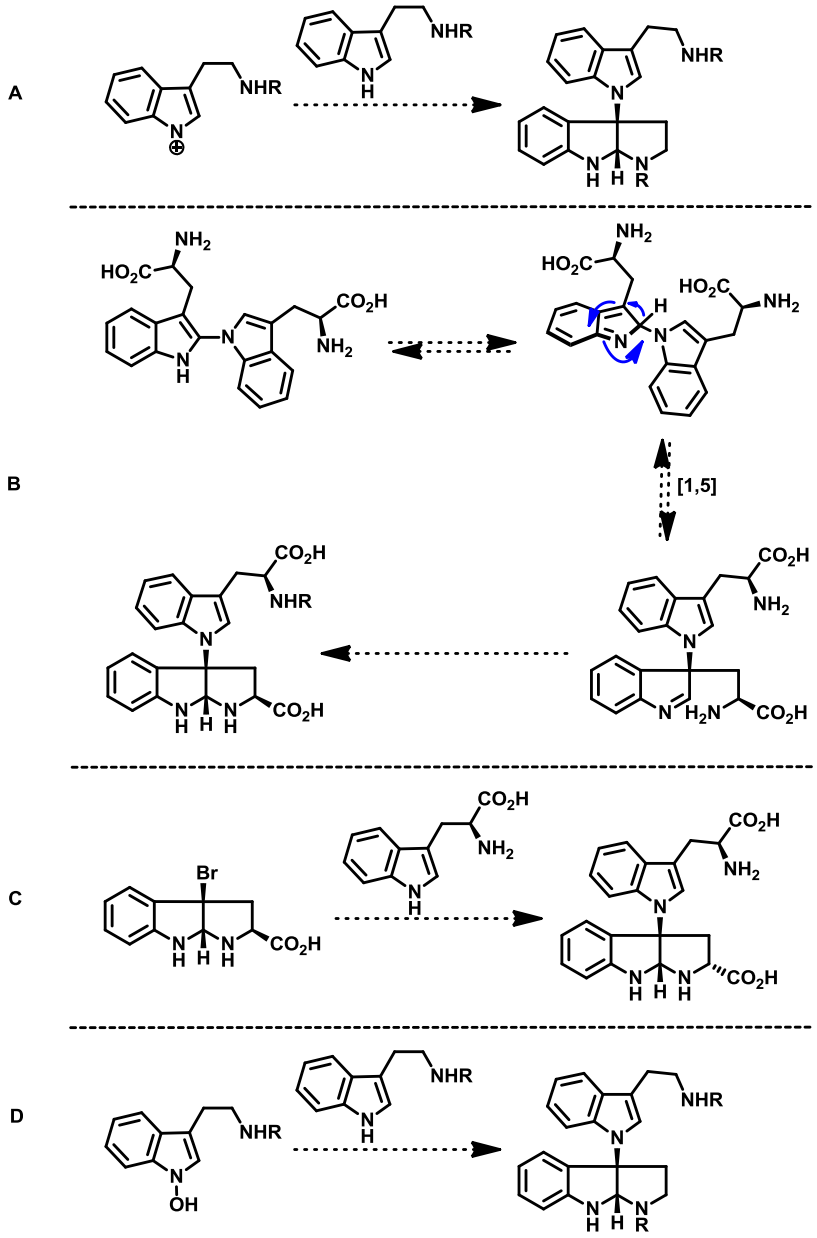

Fig 2.6. Biosynthetic possibilites for Trp-HPI molecules containing the *N*1-C3 bond.

The final hypothesis was established by Somei, via N-hydroxyindoles (fig. 2.6 D) with concomitant loss of water.[7a] Somei has demonstrated that in certain cases this kind of linkage formation is reliable,[7b] but a quaternary center cannot be obtained in more than traces via this process.[7c]

2.5 Synthetic methodology for the Trp-HPI fragment

Baran and coworkers have developed a methodology based on the biosynthetic pathway A for the synthesis of molecules such as the psychotrimine[8] (fig. 2.7) and the kapakahines B and F.[9]

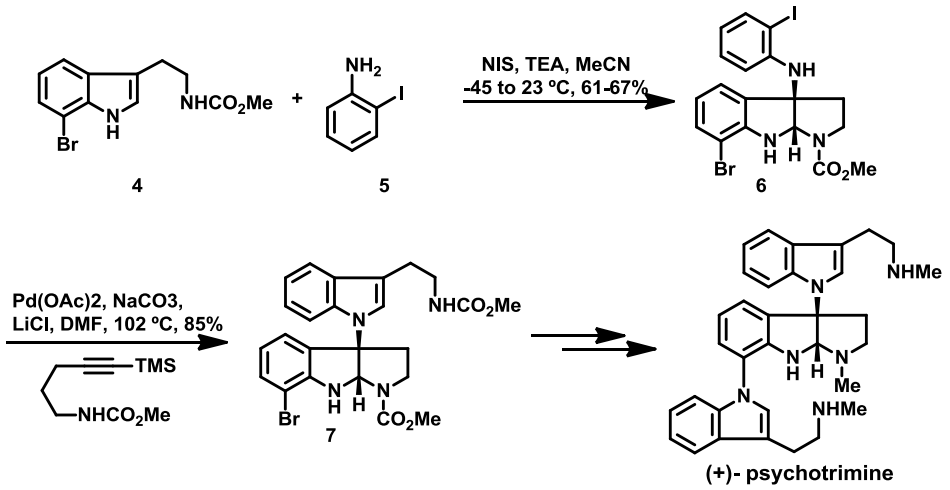

Fig. 2.7. Total synthesis of psychotrimine by Baran and coworkers.

With two simple reactions the Trp-HPI framework could be accomplished.

1. **Tandem HPI cyclization – 2-iodoaniline oxidative addition:** with this reaction, a Trp or triptamine (**4**) cyclizes intro the *exo* adduct exclusively, at the same time that the N^1-C^{3a} bond with the 2-iodoaniline (**5**) is established.
2. **Larock indole heteroannulation:** the 2-iodoaniline (**6**), along with a disubstituted alkyne, condense together and form the upper Trp (**7**).

This methodology was fascinating: it was simple (two steps), exhibited high yields and the tandem reaction conducted exclusively to the *exo* adduct, the one that we were looking for. Hence, it was adapted it for the synthetic approaches of mayotlide.

2.6 Mechanisms of the combo tandem - Larock strategy

The tandem HPI cyclization – 2-iodoaniline oxidative addition is inspired on the first biosynthetic possibility for Trp-HPI framework molecules via N^1-C^{3a} linkage, the cationic-based oxidative dimerization, described on section 2.4 (fig. 2.8). [10]

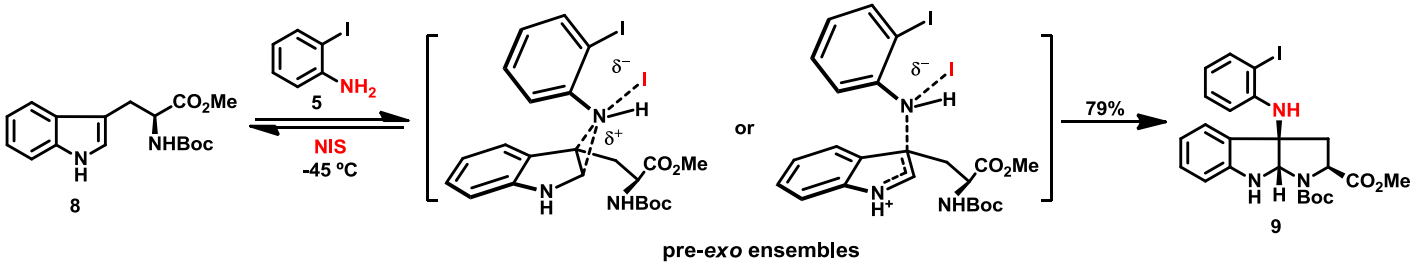

Fig. 2.8. Tandem HPI cyclization – 2-iodoaniline oxidative addition.

Trp o triptamine (**8**) reacts with a NIS-oxidized 2-iodoaniline species, forming a pre-*exo*-ensemble. That complex receive a nucleophilic attack from the carbamate, obtaining exclusively the *exo* diasteromer, at the same time that the bond N^1-C^{3a} with the 2-iodoaniline is formed.

To explain the high degree of diasteroselectivity for the C3-quaternization, a reversible formation of diasteromeric intermediates followed by preferential formation of the *exo* adduct (**9**) must be invoked.

PPTS can be optionally supplied to enhance the keto-enolic equilibrium of the carbamate and increase the nucleophilia of the nitrogen.

It is important to remark that the tandem reaction doesn't work with amides because of the lower nucleophilia of such functionality in comparison with carbamates.

The second step concerns the transformation of the 2-iodoaniline intro a Trp by means of the Larock reaction.[11] The Larock indole synthesis, also known as the Larock heteroannulation (fig. 2.9), is a one-pot palladium-catalyzed heteroannulation of a *o*-iodoaniline and an internal alkyne for the synthesis of 2,3-disubstituted índoles. The reaction was shown to be a high regioselective process giving the bulky substituent of the alkyne in position two of the resulting indole ring. Larock modified the annulation process to access 3-substituted indoles by employing silyl-substituted alkynes. In this case, the bulky silyl group dominates the regioselectivity of the annulation and thus serves as a phantomdirecting group in the heteroannulation step. Silylated alkynes provide 2-silyl-3-substituted indoles with excellent regioselectivity. Subsequent desilylation affords 3-substituted indoles in good yield.

The scope and mechanism of palladium-catalyzed annulation of internal alkynes to give 2,3-disubstituted indoles, the effect of substituents on the aniline nitrogen or on the alkynes, as well as the effect of the salts such as LiCl or n-Bu_4NCl were studied by Larock and coworkers.[12] The mechanism they propose for indole synthesis proceeds as follows: (a) reduction of the $Pd(OAc)_2$ to Pd(0); (b) coordination of the chloride to form a chloride-ligated zerovalent palladium species; (c) oxidative addition of the aryl iodide to Pd(0); (d) coordination of the alkyne to the palladium atom of the resulting arylpalladium intermediate and subsequent regioselective syn-insertion into the arylpalladium bond; (e) nitrogen displacement of the halide in the resulting vinyl palladium intermediate to form a six-membered, heteroatom-

containing palladacycle; and (f) reductive elimination to form the indole and to regenerate Pd(0).[13]

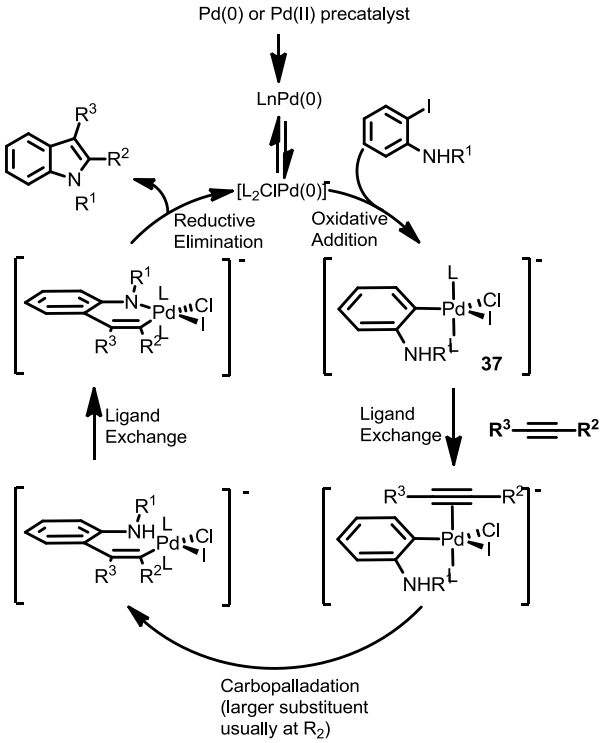

Fig. 2.9. Catalytic cycle of the Larock Reaction.

2.7 Synthesis and stereochemical studies of the *exo*-L ring A

Once that the synthetic strategy and the HPI adduct were selected, the final preliminary step consisted on the retrosynthetic analysis of the ring A (**3a**) (fig. 2.10).

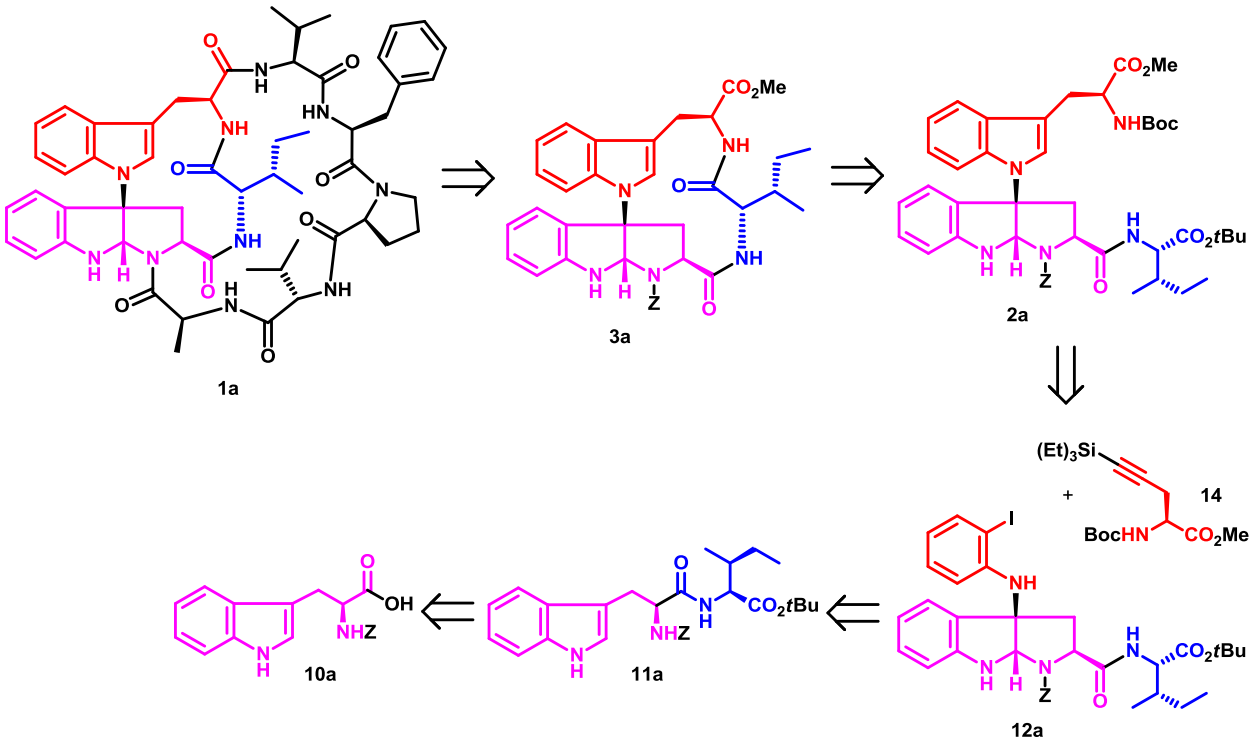

Fig. 2.10. Retrosynthetic scheme of **3a**.

One important issue on peptide chemistry in the correct selection of the protecting groups,[14] as the Trp-HPI scaffold has several functionalities (two carboxylic acids and three amines). As compound has already Boc and methyl ester protecting groups, the remaining protecting groups are:

- Z or benzyloxycabonyl for the amine of the HPI precursor Trp: due to its robustness and high compatibility with acid and basic media. It cleaves with Pd under hydrogen atmosphere.
- *Tert*-butyl ester for the carboxylic acid of the Ile: the *C*-terminus of the Ile binds the *N*-terminus of the Trp, which will be protected with Boc. Thus, both protecting groups can be eliminated with the same conditions and save reagents and time.

The synthesis started with the coupling of Z-L-Trp-OH (**10a**) with H-L-Ile-*t*Bu, using EDC·HCl and oxyma pure as coupling reagent (fig. 2.11).

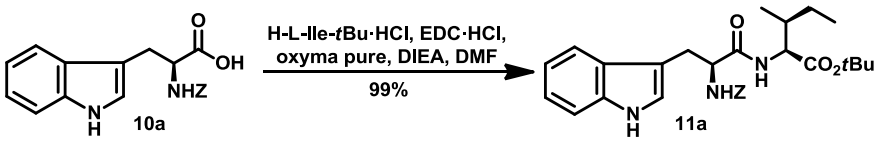

Fig. 2.11. Synthesis of dipeptide **11a**.

Secondly, the tandem cyclization-oxidative addition was performed to achieve intermediate **12a**. Such reaction was already depicted on previous sections: oligopeptide **11a**, PPTS and 2-iodoaniline are dissolved in MeCN and cooled down to -45 °C. Then, NIS is added dropwise and the reaction was let to warm up to -35 °C. Whether NIS is not sufficiently slowly added, it may appear the iodinated compound **13a** (fig. 2.12).

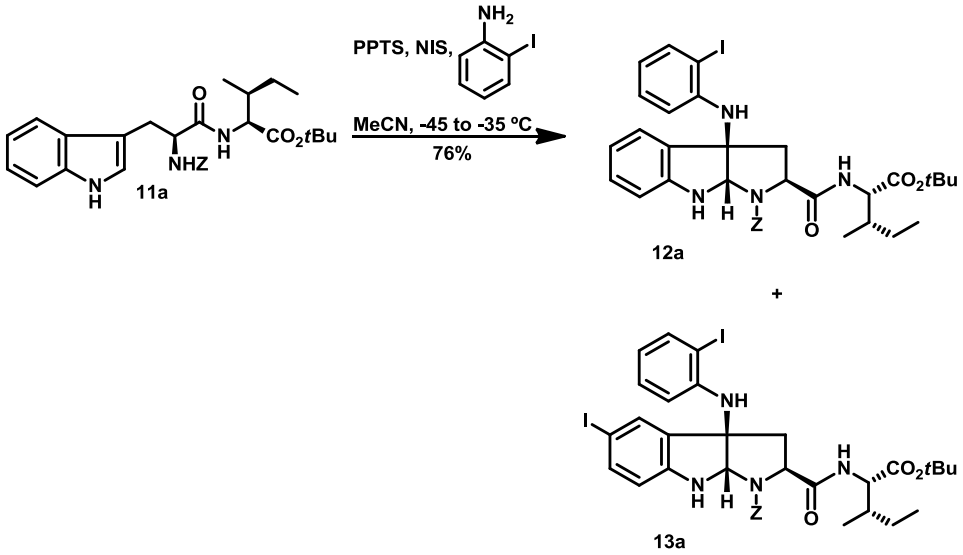

Fig. 2.12. Tandem reaction of oligopeptide **12a**.

Compounds **12a** and **13a** and have the same r_f in normal phase TLC (SiO_2 silica gel), but they have different retention time on HPLC. Hence, they had to be separated using Combi Flash Isco reverse Phase (C18 silica gel). The position of the second iodine in compound **13a** was studied by NMR spectroscopy (HSQC and HMBC experiments).

The yield for this tandem reaction is quite good and leads a comfortable intermediate for the next step.

For the Larock reaction it was necessary to prepare the complementary disubstituted alkyne that, along with the *o*-iodoaniline, would lead to the indole ring of the upper tryptophan. This aminoacid was prepared by the methodology developed by Rutges and coworkers (fig. 2.13).[15]

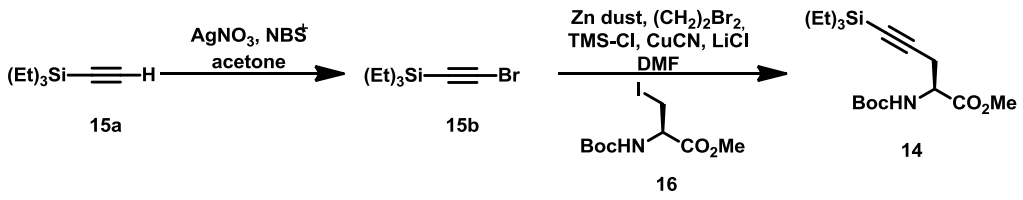

Fig. 2.13. Preparation of aminoacid **14**.

Tripeptide **2a** was achieved by condensation of previous dipeptide **12a** and disubstituted alkyne **14**. The triethyl silyl protecting group used as a phantom director is removed during acidic aqueous workup, converting the 2-iodoaniline into a 3-substituted indole with outstanding yields (fig. 2.14).

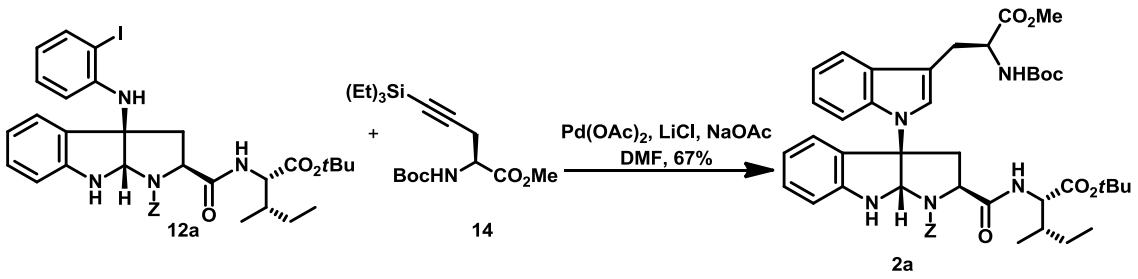

Fig. 2.14. Larock reaction for the consecution of tripeptide **2a**.

The overall yield is 50% in three steps.

At this point, there were three points that were mandatory to be tested:

- The stereochemistry of the Trp-HPI: due to the Larock reaction takes place at 100 °C in basic media, it may happen that the kinetic *exo* adduct isomerizes into the thermodynamic *endo* diasteromer.[16] Unfortunately, the presence of rotamers in a 1:1 ratio blocked a NOESY study (fig. 2.15).

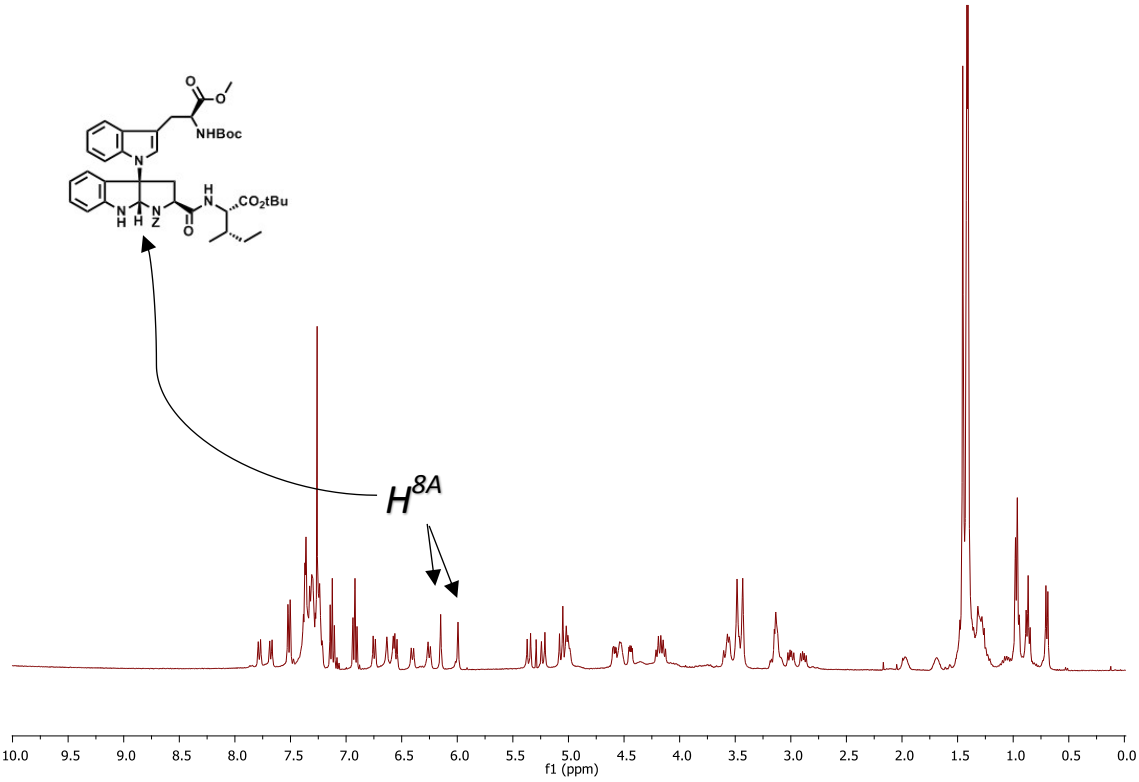

Fig. 2. 15. ^{1}H-RMN of tripeptide **2a** in $CDCl_3$.

- Ring A cyclization: treatment of **2a** with 60% TFA in DCM and subsequent cyclization in high dilution with PyAOP as coupling reagent[17] afforded the ring A *exo*-L **3a** (fig. 2.16).

Phosphonium derivatives of HOAt such as PyAOP are frequently useful for the preparation of a range of peptides that include hindered amino acids, difficult short sequences, and cyclic peptides. An advantage relative to uronium salts is that excess PyAOP does not undergo the undesired guanidinium formation at the amino terminus which blocks further chain assembly.

Because of its coupling strength and the *N* terminus of the Ile is forming an amide, PyAOP may prompt epimerization. The reaction takes place at 0 °C to prevent it.[17]

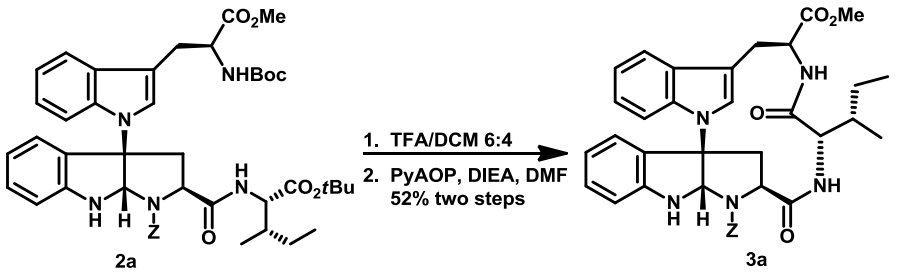

Fig. 2.16. Intramolecular cyclization of ring A (**3a**).

This is a very important key intermediate for the synthesis of mayotlide. Again, it was analyzed by NMR spectroscopy and, surprisingly, the presence of regioisomers were detected although in minor range (8:2) than tripeptide **2a** (fig. 2.17).

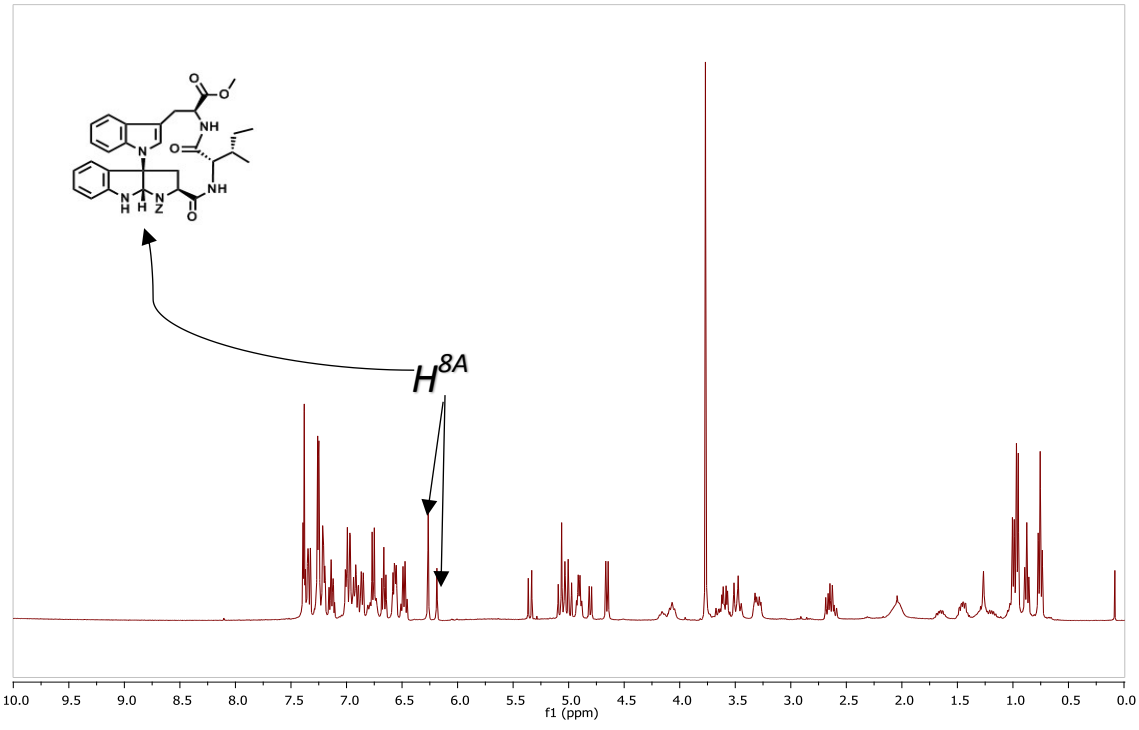

Fig. 2.17. ^{1}H-NMR of ring A in $CDCl_3$.

- Deprotection of Z (**17a**): the cleavage of the benzyloxycarbonyl runs with cat. Pd black over H_2 atmosphere. It is a grateful reaction, considering the absence of purification and no loss of matter (fig. 2.18).

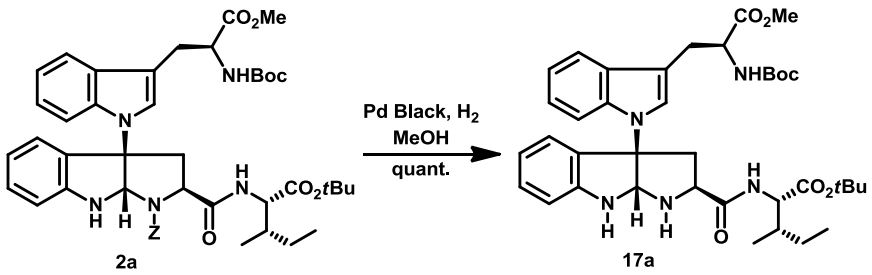

Fig. 2.18. Z hydrolysis of tripeptide **2a**.

Fortunately, the last single mayotlide intermediate has a main regioisomer in high proportion (fig. 2.19) and hence TOCSY and NOESY studies may reveal the configuration of the stereocenters of the HPI.

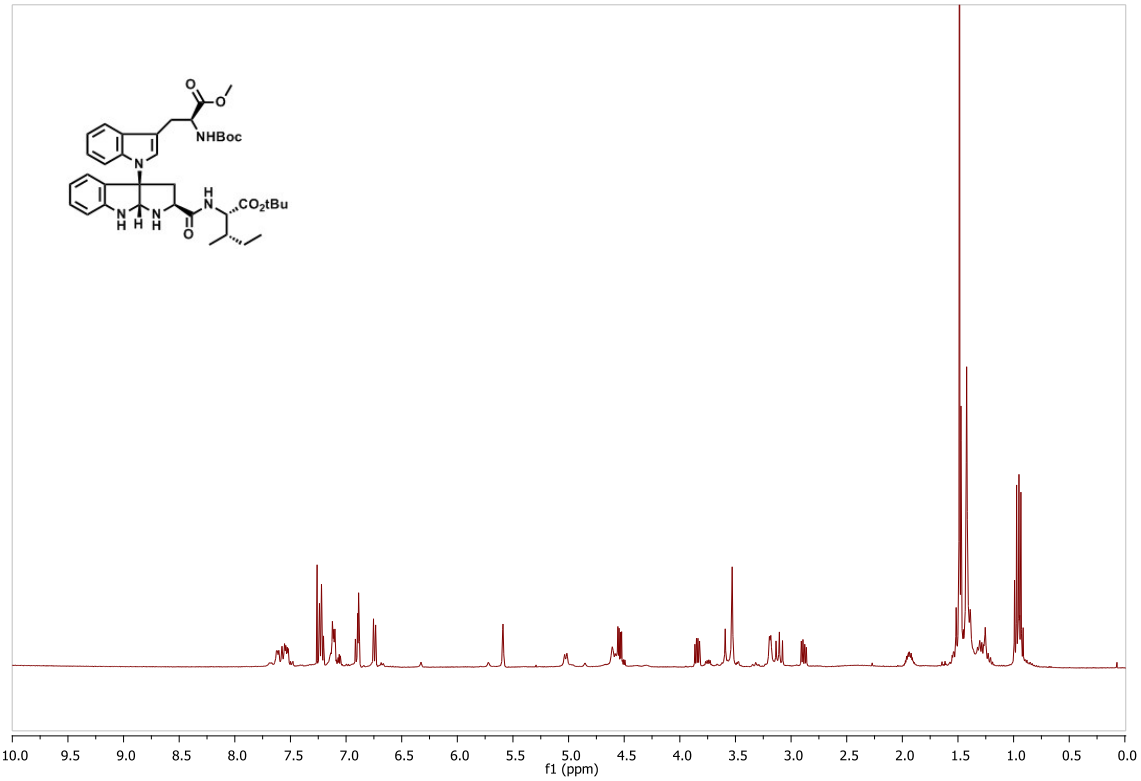

Fig. 2.19 ^{1}H-NMR of **17a** in $CDCl_3$.

With NOESY experiment it was studied whether existed spatial correlation between the H^{8a} and the α-H of the HPI. TOCSY experiment would reveal which is the the α-H of the HPI as the alpha hydrogens are undistinguishable by ^{1}H-NMR (fig. 2.20).

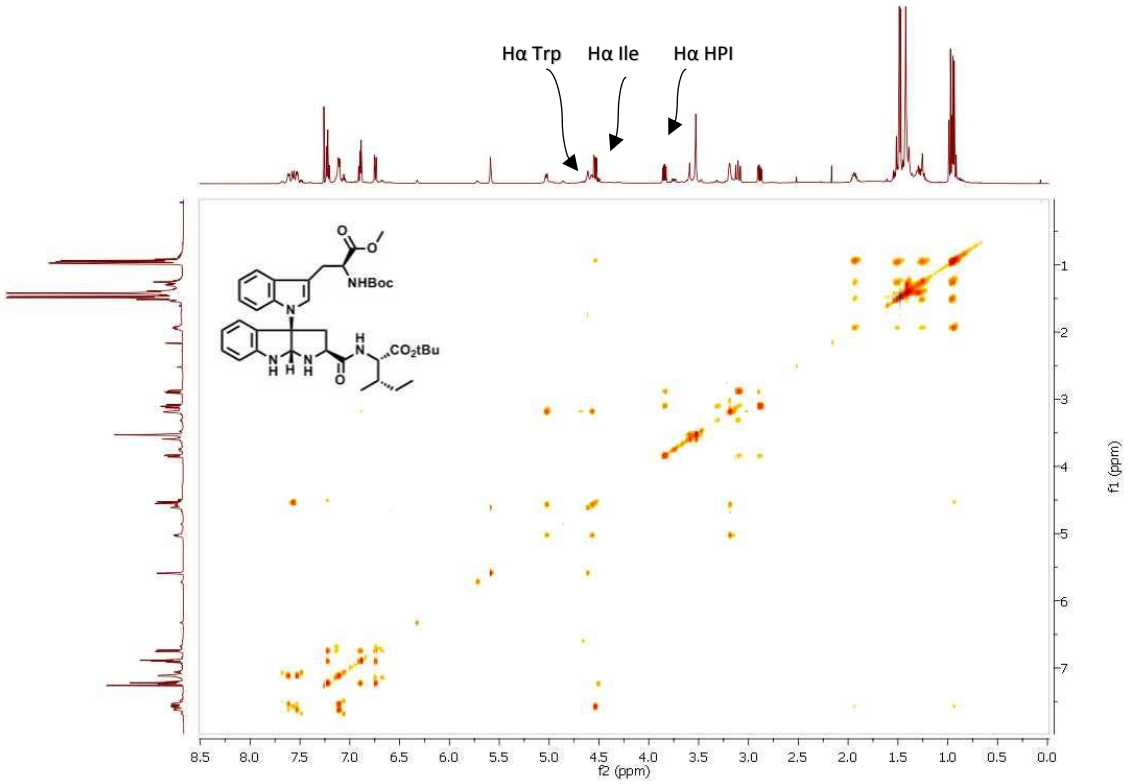

Fig. 2.20. TOCSY experiment of **17a**.

Once that the alpha protons were situated, two correlations that were observed revealed the stereochemistry of the molecule:

- NOE between the H8a and the Hβ2 of the HPI.
- NOE between the Hα of the HPI and the Hβ1 of the HPI.

That has completely sense. Whether the H8a and the Hα of the HPI are situated in opposite spatial directions, it is obvious that Hα correlates with Hβ1 and H8a correlates with Hβ2 (fig. 2.21)

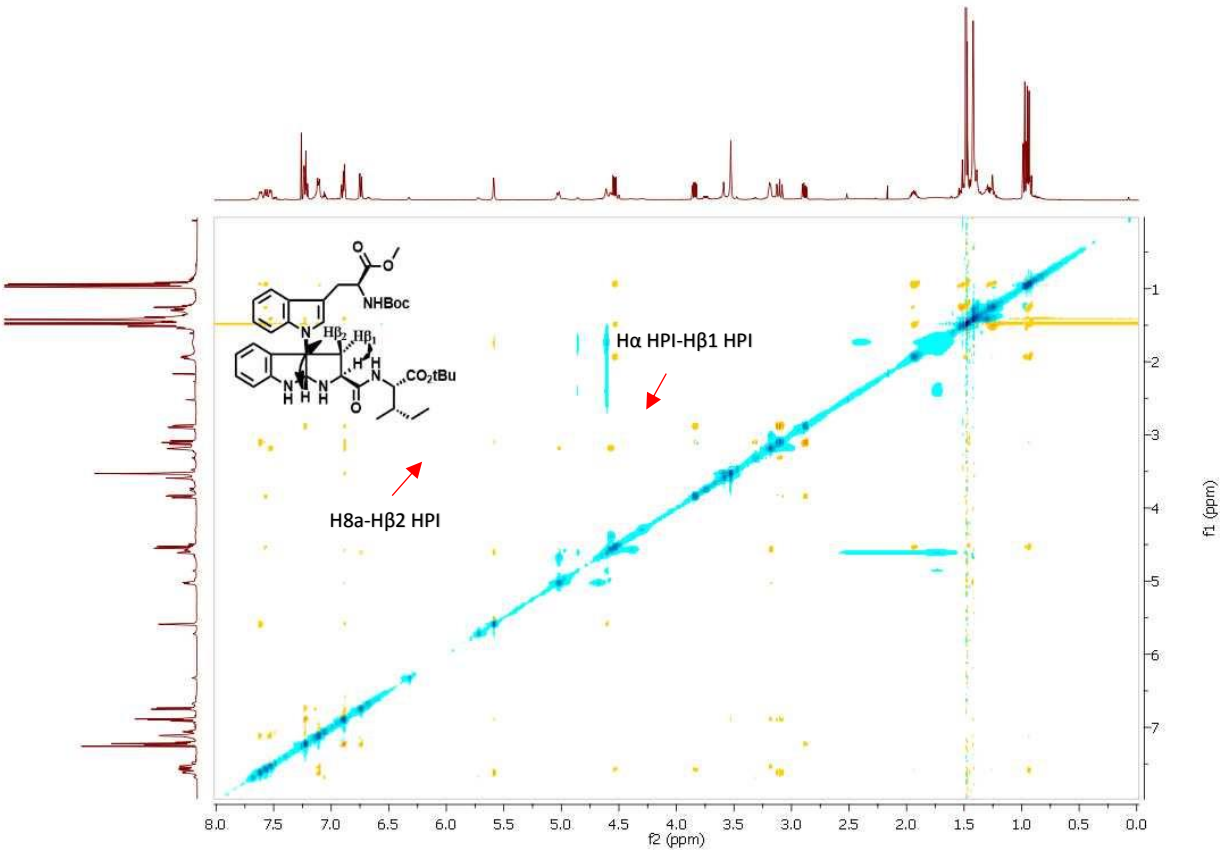

Fig. 2.21. Correlation of H8a and Hα with the Hβ hydrogens of the HPI *exo*-L.

No correlation between the H^{8a} and the α-H of the HPI was observed. Thus, it is possible to state that the *exo* configuration of the HPI maintains unmodified during the Larock reaction.

The next chapter describes the final synthetic approaches towards the total synthesis of mayotlide.

2.8 Conclusions chapter 2

- There are four HPI diasteromers of the HPI. Computational studies demonstrated that the mayotlide with the *exo*-L HPI has the lower energy according to MOE program.
- To the best of our knowledge, the methodology developed by Baran for the synthesis of Trp-HPI containing molecules via N^1-C^{3a} bond seemed the most appropriate. It was possible to assemble the three aminoacids wich are common among both rings A and B in three steps with 50% yield with retention of the configuration of the HPI, studied by NMR spectroscopy.

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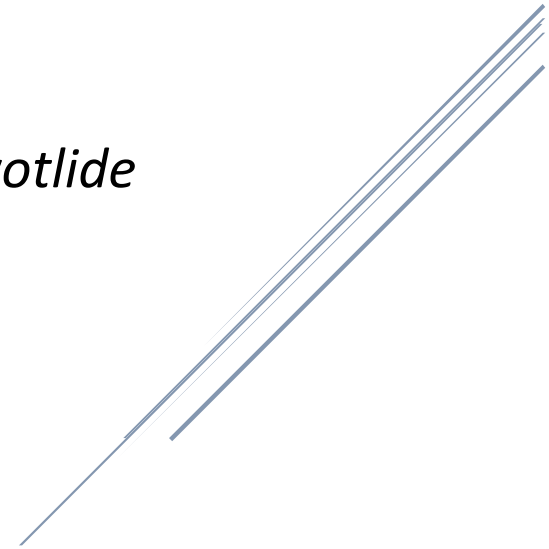
CHAPTER 3:

Synthetis of exo-L Mayotlide



3.1 Ring A…got it!

On the lasf part of the previous chapter, the synthetic procedure for the consecution of the ring A has been discussed, as well as the the streochemistry of the Trp-HPI framework, confirming that it doesn't suffer any alteration after the Larock reaction.

On the following chapter, the differentes synthetic approaches will be described, along with the obtained experimental results.

3.2 Total synthesis approach to mayotlide from the ring A

Having the ring A in our hands, it just remained to prepare the linear pentapeptide that contain the remaining amioacids of the ring B.

Taking advantage of the spps,[1] tetrapeptide **20** was prepared over 2-chlorotrityl chloride resin **18**,[2] utilizing the Fmoc/*t*Bu strategy. DIC-oxyma pure was the coupling reagent cocktail and 20% piperidine in DMF was the Fmoc deprotection mixture. Couplings were evaluated by ninhydrin and chloranyl tests, as appropriate, and the peptide was cleaved from the resin with a treatment with 2% TFA in DCM solution. The cleaved tetrapeptide **20** and H-L-Ala-OBn were coupled in solution with EDC·HCl/oxyma pure/DIEA in DMF, with an overall yield of 53%. The Boc protecting group was hydrolyzed with a treatment of 50% TFA in DCM, affording the *N*-deprotected peptide **22** (fig. 3.1).

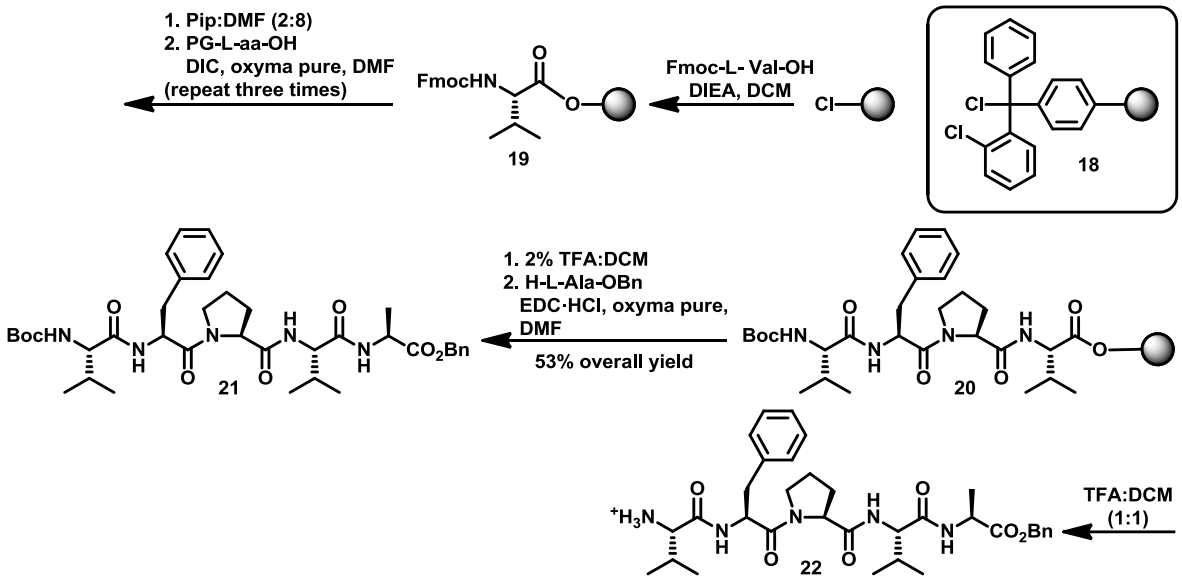


Fig. 3.1. Synthesis of pentapeptide **22**.

The ring A was saponified with LiOH and peptide **22** were coupled with EDC·HCl and oxyma pure to prevent epimerization, achieving peptide **23** with 57% yield (fig. 3.2).

The finall deprotection and cyclization steps were lamentably unsuccessful. The following reasons may justify the fact that the cyclization did not work (fig. 3.2):

- The free amine of the HPI is very hindered.
- The carboxylic acid of a peptide is less reactive than a N-carbamate protected aminoacid.[3]

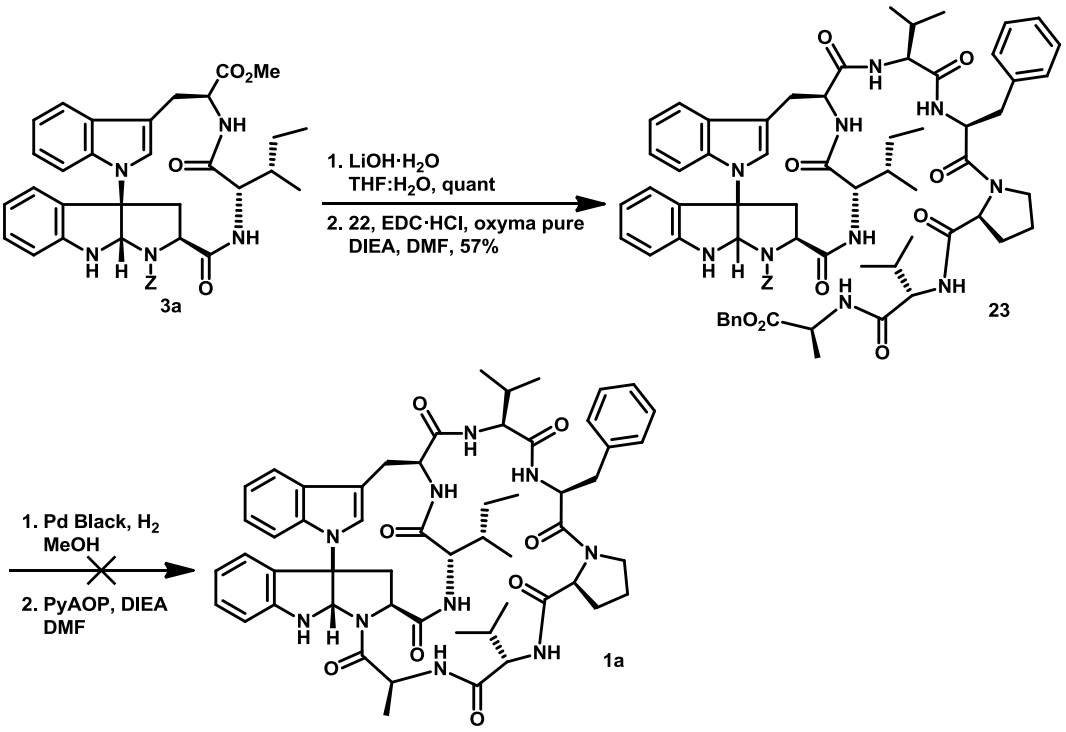


Fig. 3.2. Unsuccessful strategy to achieve mayotlide from ring A.

The possibility to reach the mayotlide was blocked on this direction. It was better to give a step back to tripeptide **2a** and redesign the synthetic approaches. The main peculiarity of compound **2a** is that it is the simplest compound that contains the three aminoacids that are common in both rings.

The retrosynthetic approaches that were settled up were depicted on fig. 3.3:

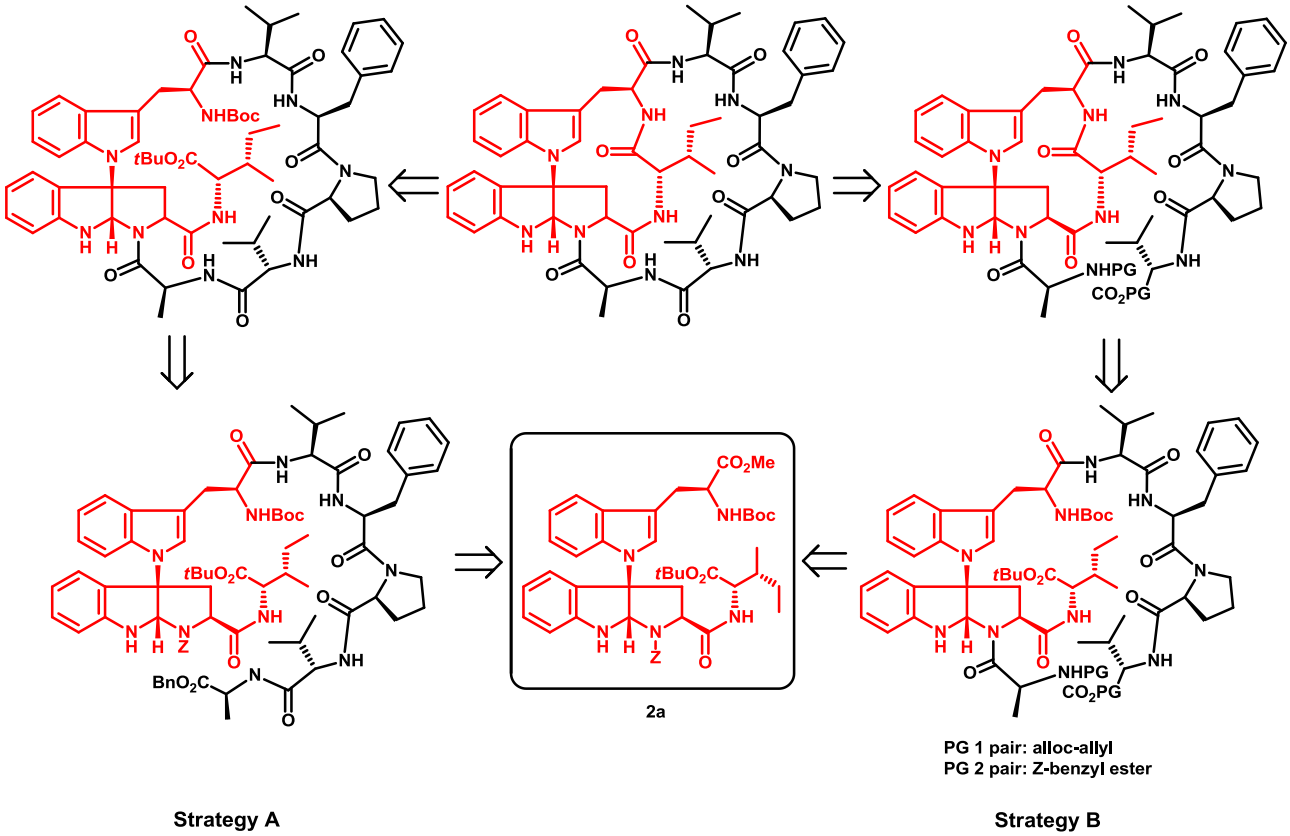


Fig. 3.3. New strategy from **2a**.

Take in account that, on strategy B, the protecting groups in both N and C terminus are related, and therefore can be erased under the same conditions for avoid time and reagents' consuming.

- For strategy A, the order of the sequence would be: 1) deprotection of Z-benzyl ester and cyclization for ring B formation; b) deprotection of Boc-*tert* butyl ester and cyclization for obtaining the ring A.
- Strategy B, would be slightly different: a) the first step would be the coupling of the first aminoacid of the peptidic chain of ring B; b) depending on the cyclization tests results, it would be possible either to make first the cycle A or the cycle B.

Strategy A: tripeptide **2a** was saponified, and using the same pentapeptide (**22**) than in the previous case, intermediate was achieved. Again, saponification hydrogenation of the protecting groups and cyclization failed (fig. 3.4).

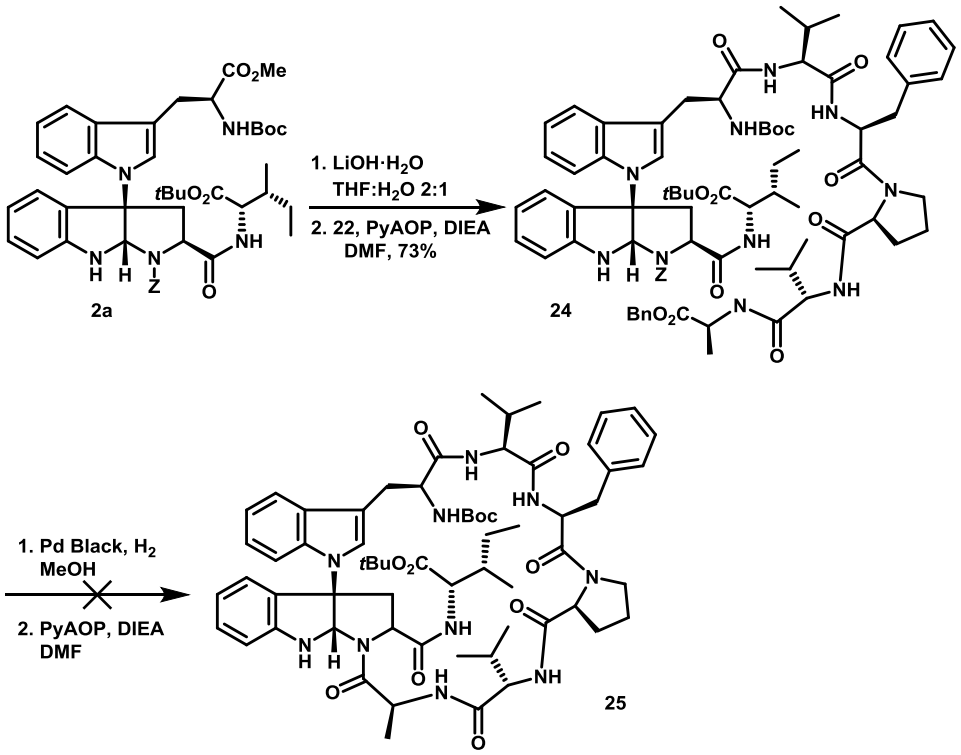


Fig. 3.4 Failure for achieving ring B from **2a**.

At this point, the route B seemed to be more reliable, due to it seems easier for the ring B consecution to cyclize with a primary amine rather than the HPI secondary amine. From tripeptide **2a**, incorportation of N-protected alanine would be the the following step (fig. 3.5).

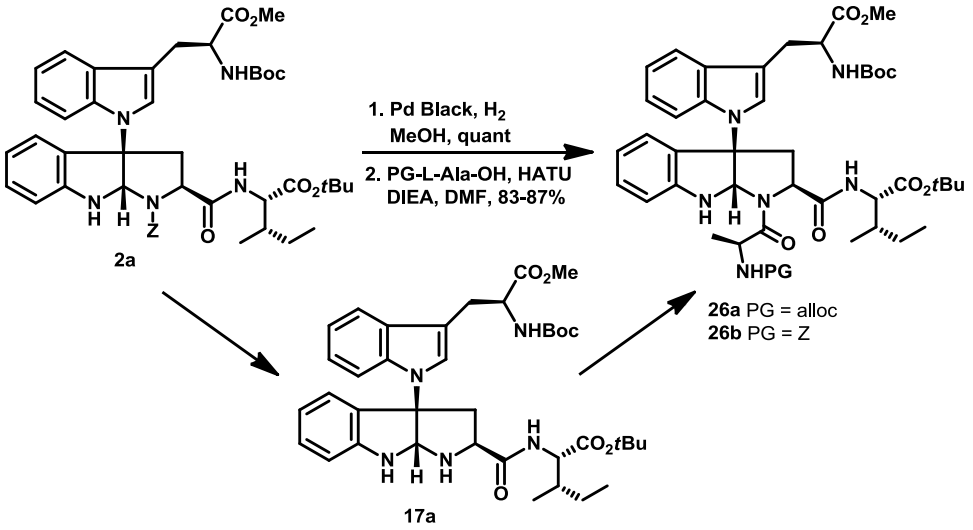


Fig. 3.5. Coupling of differents PG-L-Ala-OH to **2a**

Deprotected tripeptide **17a** contains three protecting groups: Boc-*t*Bu and methyl ester. The *N*-terminus protecting group of the Ala should be compatible with these ones. The chosen ones were Z and alloc.

This coupling was especially troublesome due to the low reactivity of the free amine of the HPI. It was necessary to add five equivalents twice with HATU as coupling reagent to reach full conversion of **17a**.

The complementary peptides were prepared as peptide **22**: part in solid phase and part in solution (fig. 3.6):

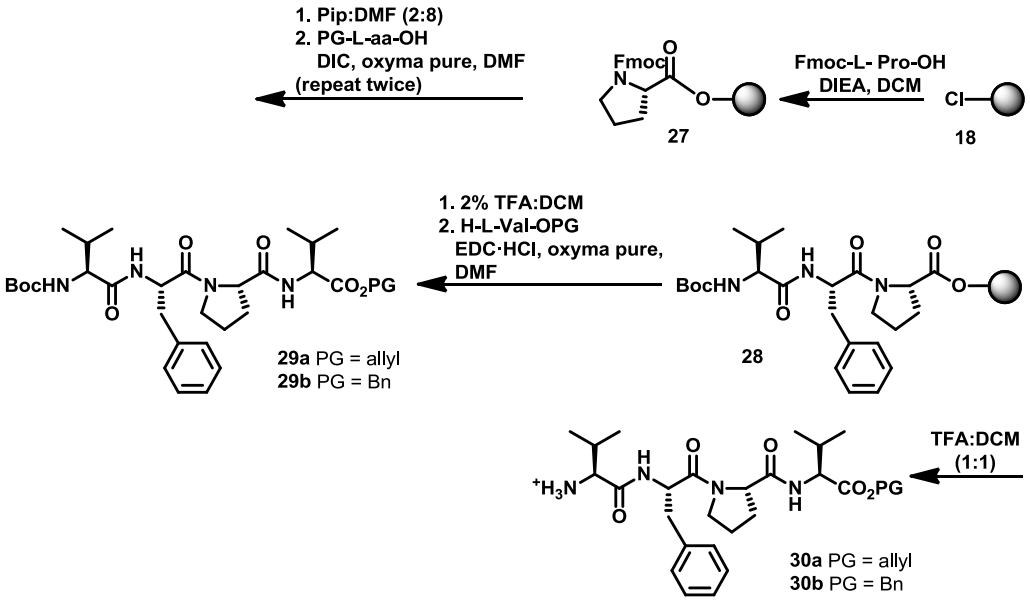


Fig. 3.6 Preparation of complementary peptides for compounds **26a** and **b**.

Compounds **26** were saponified and the peptides **30** were coupled as prior instances (fig. 3.7).

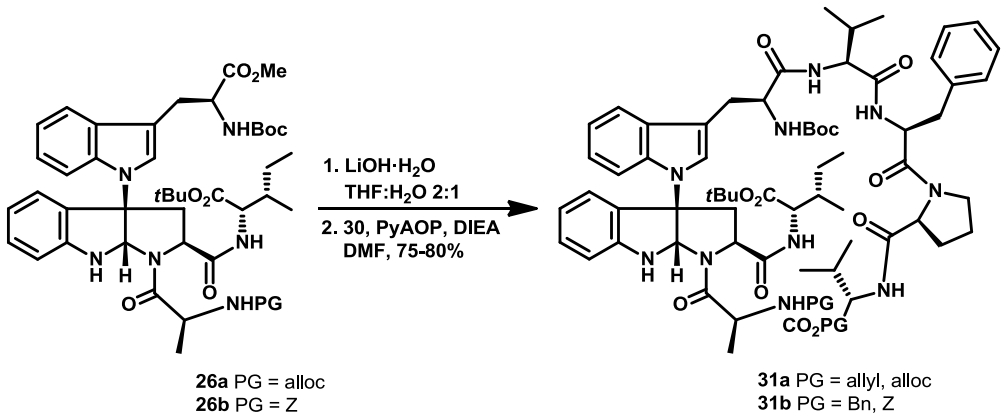


Fig. 3.7. Octapeptides **31** with all *N* and *C* terminus protected.

It is described on the literature that deprotection of alloc-allyl esters on the presence of tryptophan provide side-allylation reactions.[4] Then, cyclization conditions were tested. Deprotection treatment with $Pd(PPh_3)_4$ afforded fully deprotected compound **32**. Nevertheless, on cyclization conditions, the afforded product contained the cyclized mass +42 **33** (fig. 3.8).

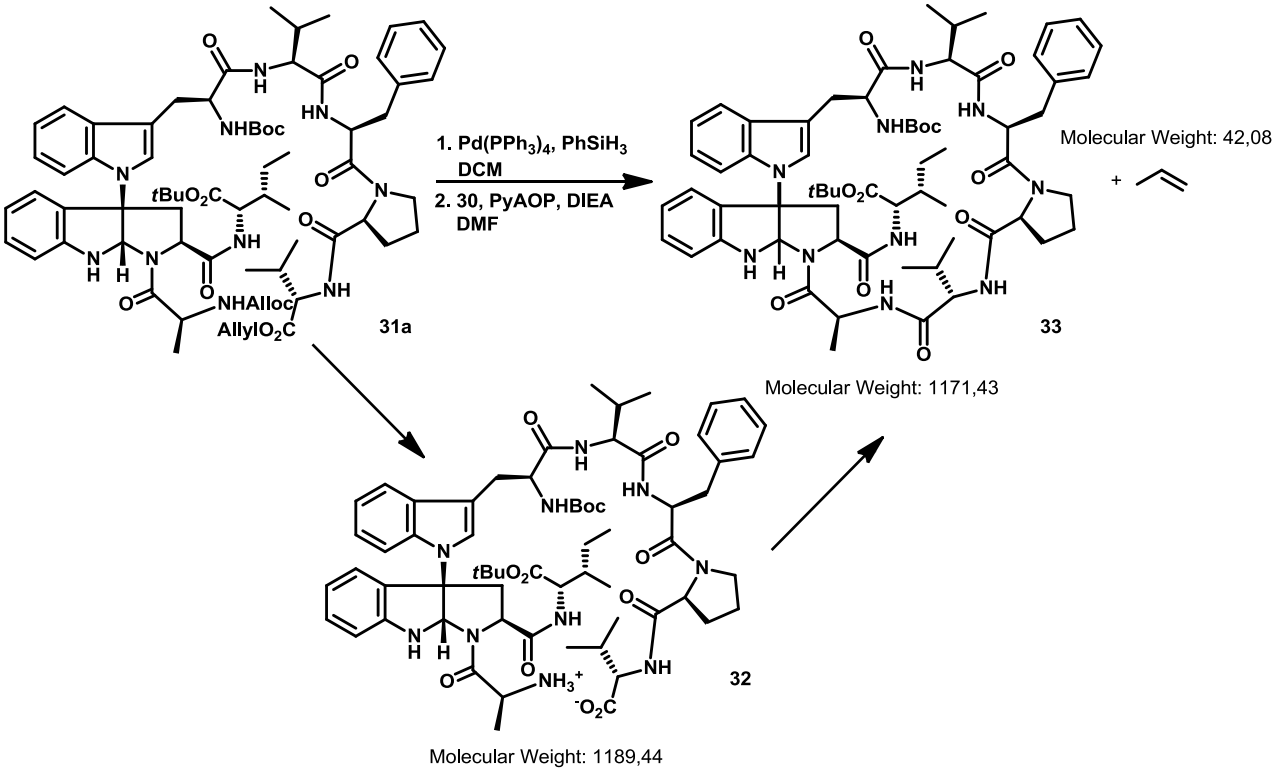


Fig. 3.8. Experimental procedure for the ring B cyclization with alloc-allyl protecting groups.

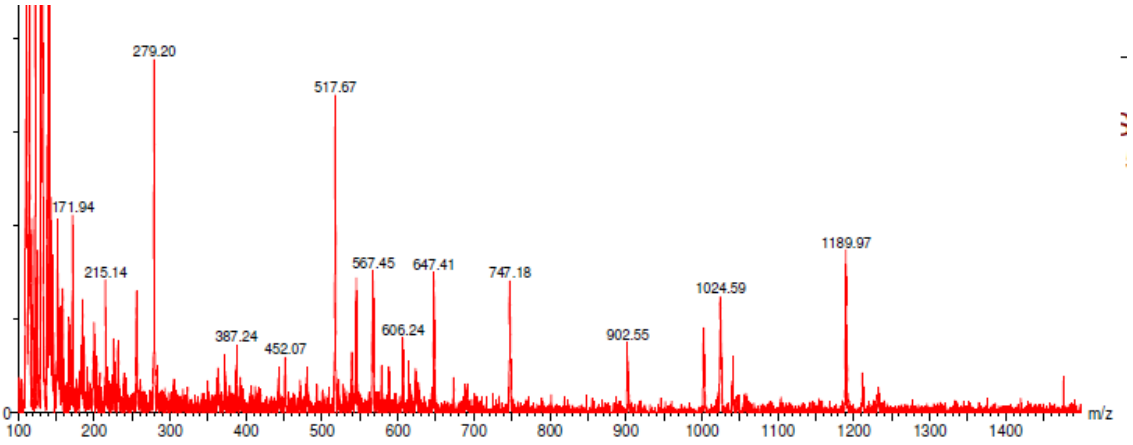


Fig. 3.9. Mass spectrometry of **32**.

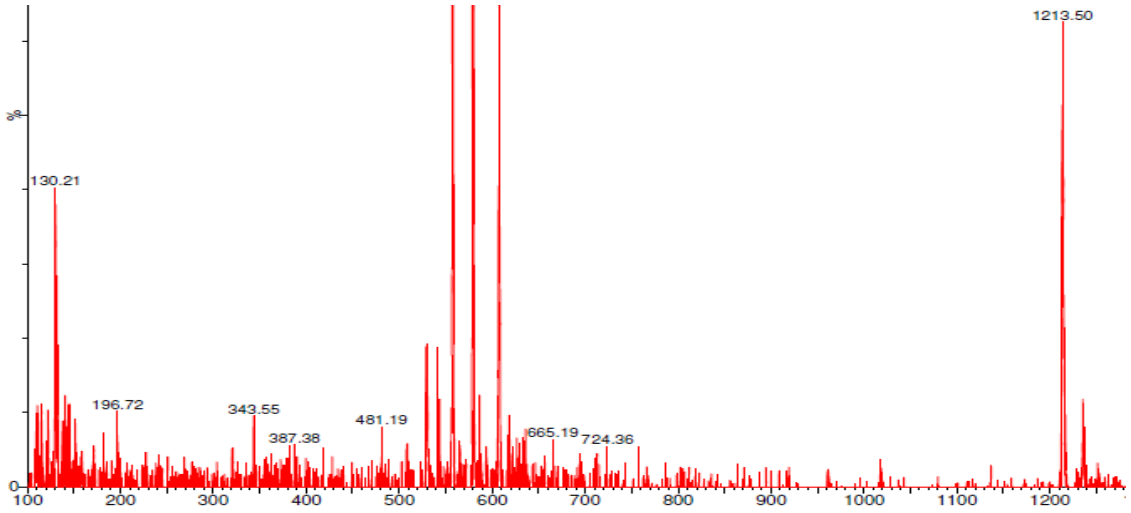


Fig. 3.10. Mass spectrometry of **33**.

During the deprotection conditions, the mass of the unprotected peptide +42 was not found. Therefore, the coupling reactions should activate the allylation somehow.

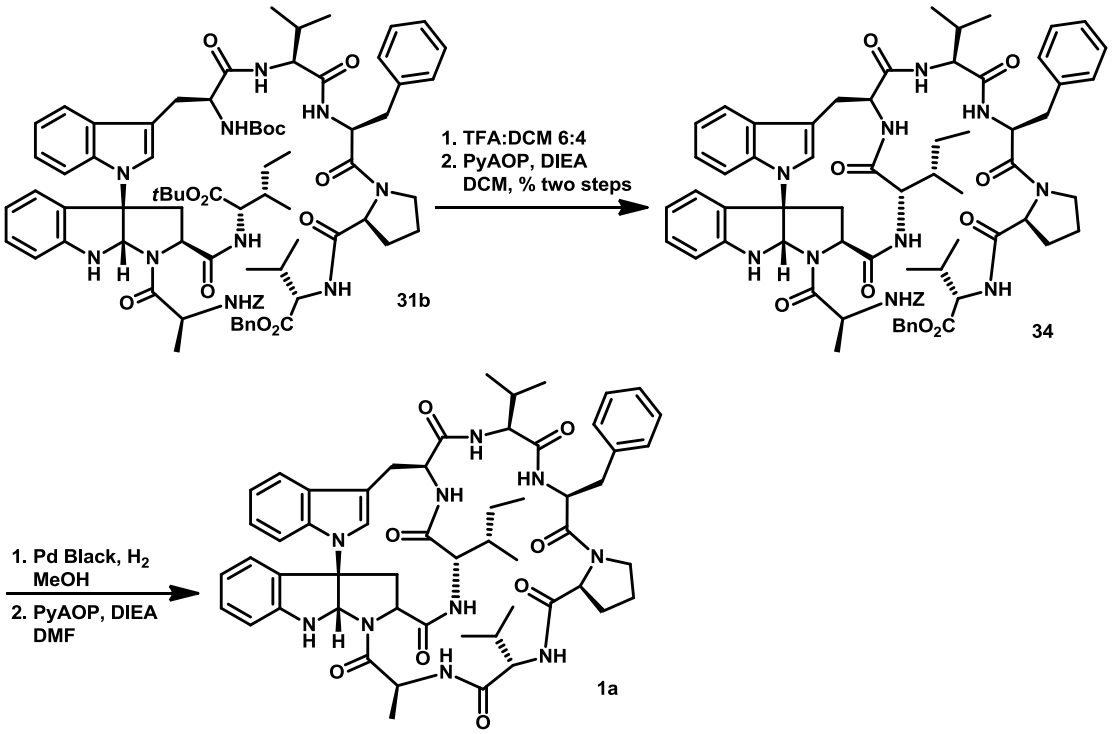
Even adding 10 equivalents of the scavenger phenyl silane, the allylated sideproduct appeared. Then the solution would beeither purify after deprotection or adding an indole as scavenger. None of these solutions were satisfactory, because the function of the protecting groups must be provide a free functionality with a clean reaction, or in other words, without purification. If purification was necessary, it was not fulfilling the requirements of the protecting group. In the case of adding an indole scavenger, as *N*-methyl indole or indole: a) these scavengers would compete with our substrate and would not guarantee the allylated ring B; b) these indole scavengers must be eliminated from the crude mixture and would difficult the purification and hence, the final yield would be severely damaged.

At this point the information that was compiled from the diverse experiments were:

- The ring A can be achieved on a comfortable manner.
- The ring B via HPI-Ala cyclization doesn't give good results.
- The alloc-allyl ester protecting groups are not well compatible with the Trp-HPI substrate.

Hence the safest option consisted on, from compound **31b**, reach the ring A, and from this point, check different cyclization conditions for ring B.

Treatment with 60% TFA in DCM and cyclizatio afforded macrocycle **34**. Hydrogenation and cyclization afforded the final objective, the mayotlide with the *exo*-L configuration (fig.3.9).



Firg. 3.11. Accomplishing of *exo*-L mayotlide

3.3 Analysis of *exo*-L mayotlide

The *exo*-L were analyzed by exhaustive NMR spectra (1H, 13C, TOCSY, HMBC, NOESY) and MS-MS.

Apparently, the NMR spectra looks correct although they are different to the natural product (fig. 3.12). On the experimental section all the spectral data of the natural and synthetic mayotlide were compiled into a table.

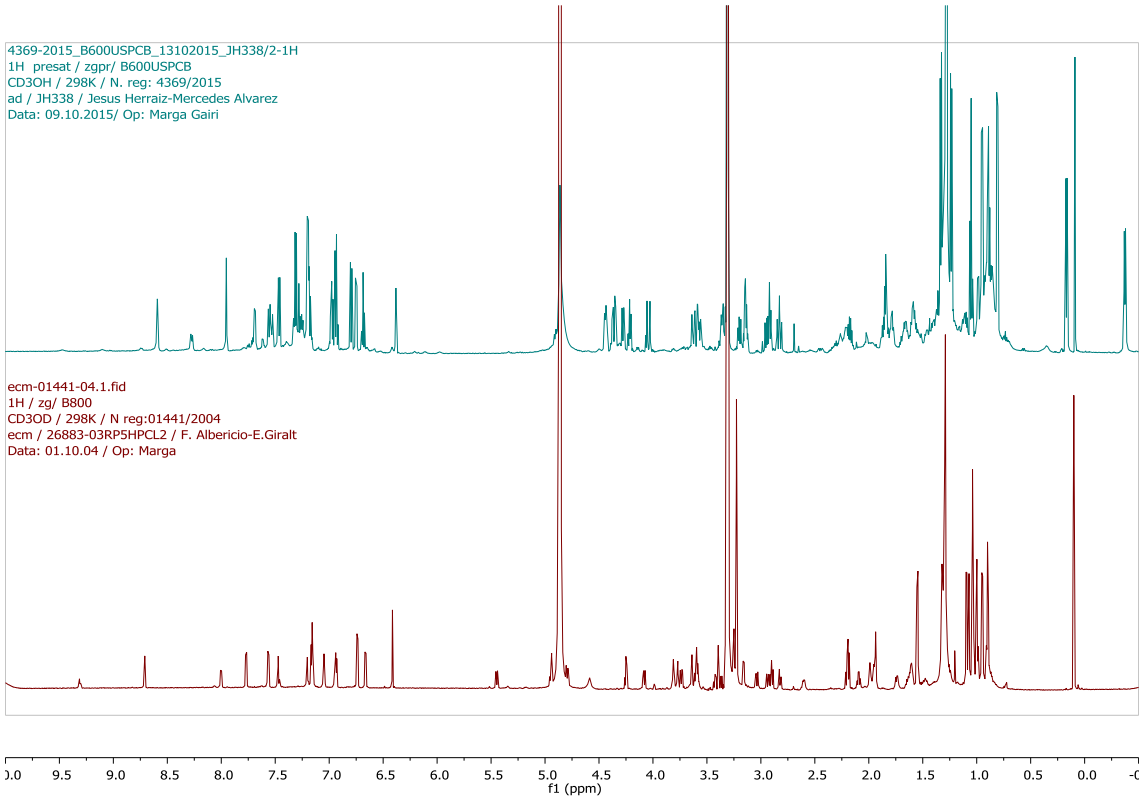


Fig. 3.12. ^{1}H-NMR of the synthetic product (above) and natural mayotlide (below).

The main conclusions that can be extracted from the spectrum are the following ones:

- The Trp-HPI framework keeps its structure, as it can be observed from the H^{8a} signal. In addition, HMBC spectra detects correlation between the C^{3a} of the HPI and the H2 of the Trp.
- The aromatic signals are not as defined as the natural product. That range was compared with the natural mayotlide, the synthetic *exo*-L and the ring A *exo*-L(fig. 3.13):

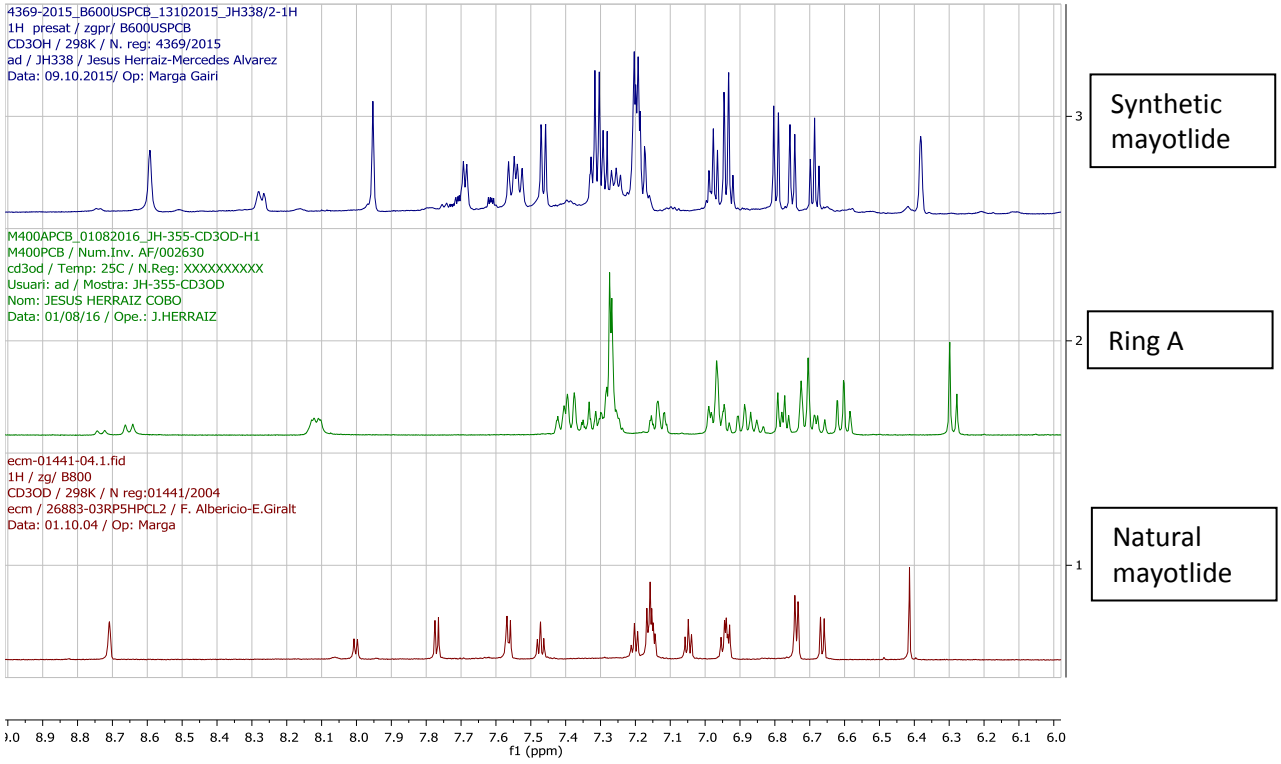


Fig. 3.13. Aromatic area of the ring A, synthetic and natural mayotlide

- On the aliphatic area, two methyl signals coming from a Val have anomalous low chemical shifts. This phenomena could be because they have fallen into the anisotropy cone of an aromatic ring, in principal the Phe.

Concerning the MS-MS, the fragmentations were studied. On fig. 3.12, the upper spectra corresponds to the natural mayotlide and the lower one to the synthetic *exo*-L mayotlide.

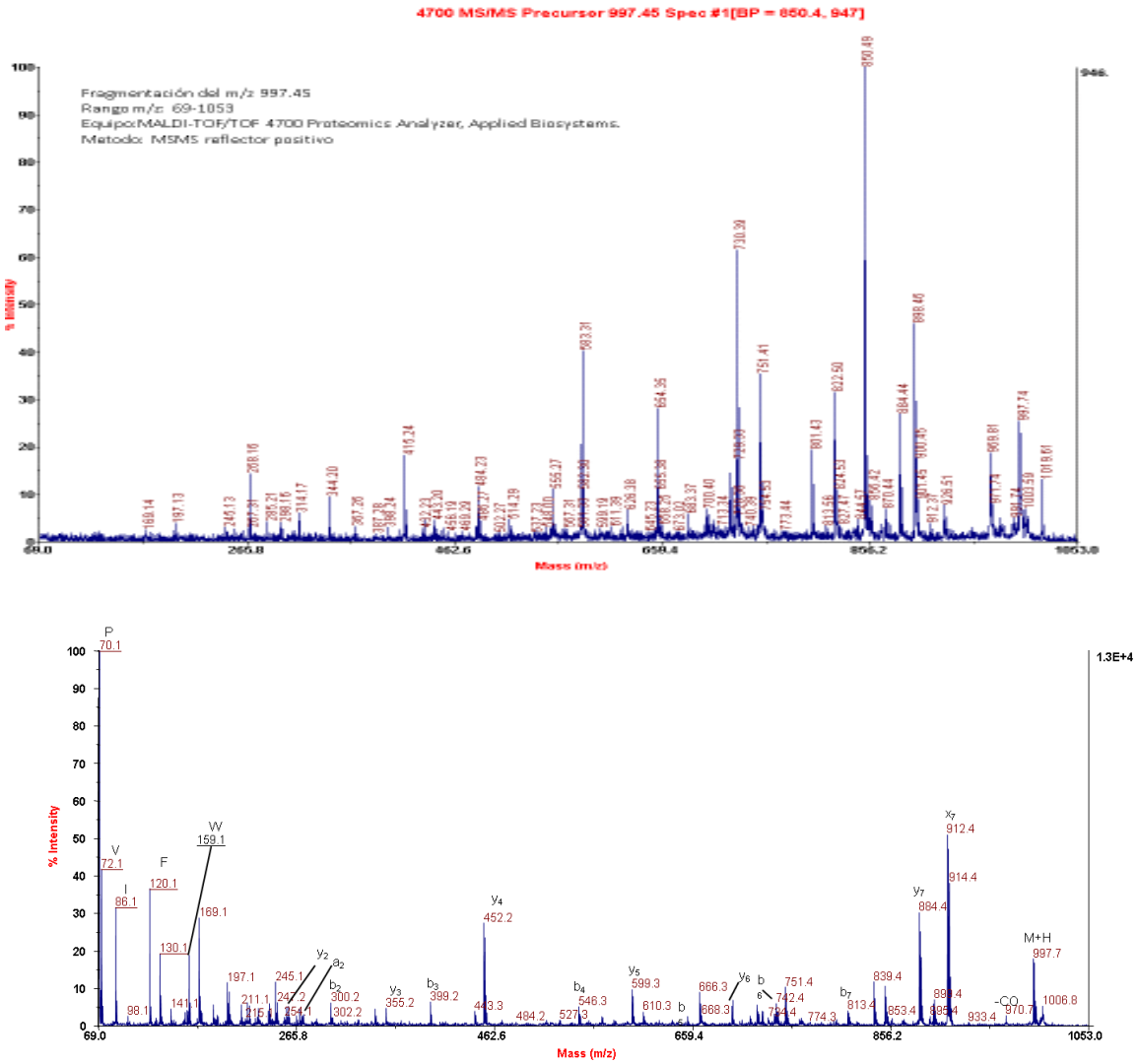


Fig. 3.12. MS-MS MALDI-TOF spectra of synthetic (up) and natural (down) mayotlide.

At first sight, the fragmentation pattern is different. Whereas on the natural one the main fragmentation corresponds to the Ile (912 and 884), on the synthetic one corresponds to the Phe (850).

All the fragmentations were placed into a table and were studied (fig. 3.13):

Synthetic *exo*-L mayotlide

PM (1er AA)	113,08		186,08		99,07		147,07		97,05		99,07		71,04		184,1	
Secuencia	1		2		3		4		5		6		7		8	
iones																
b1	Ile	114,08	Trp	187,08	Val	100,07	Phe	148,07	Pro	**98,05**	Val	100,07	Ala	72,04	trp*	185,07
b2	W	**300,16**	V	**286,15**	F	**247,14**	P	**245,12**	V	**197,12**	A	171,11	w	256,11	I	298,15
b3	V	**399,23**	F	433,22	P	**344,19**	V	344,19	A	268,16	w	355,18	I	369,19	W	484,23
b4	F	**546,3**	P	530,27	V	443,26	A	415,23	w	452,23	I	468,26	W	555,27	V	583,3
b5	P	643,35	V	629,34	A	514,3	w	599,3	I	565,31	W	654,34	V	654,34	F	730,37
b6	V	**742,42**	A	700,38	w	698,37	I	712,38	W	751,39	V	753,41	F	801,41	P	827,42
b7	A	**813,46**	w	884,45	I	811,45	W	898,46	V	850,46	F	900,48	P	898,46	V	926,49
b8	w	997,53	I	997,53	W	997,53	V	997,53	F	997,53	P	997,53	V	997,53	A	997,53
a2		272,16		258,15		219,14		217,12		169,12				228,11		
y1		185,07		114,08		187,08		100,07		148,07		98,05		100,07		72,04
y2		256,11		298,15		300,16		286,15		247,14		245,12		197,12		171,11
y3		**355,18**		369,19		484,23		399,23		433,22		344,19		344,19		268,16
y4		**452,23**		468,26		555,27		583,3		546,3		530,27		443,26		415,23
y5		**599,3**		565,31		**654,34**		654,34		730,37		643,35		629,34		514,3
y6		698,37		712,38		751,39		753,41		801,41		827,42		742,42		700,38
y7		884,45		811,45		**898,46**		**850,46**		900,48		898,46		926,49		813,46
y8		997,53		997,53		997,53		997,53		997,53		997,53		997,53		997,53

Natural mayotlide

PM (1er AA)	113,08		186,08		99,07		147,07		97,05		99,07		71,04		184,1	
Sequence	1		2		3		4		5		6		7		8	
ions																
b1	Ile	114,08	Trp	187,08	Val	100,07	Phe	148,07	Pro	**98,05**	Val	100,07	Ala	72,04	trp*	185,07
b2	W	**300,16**	V	**286,15**	F	**247,14**	P	**245,12**	V	**197,12**	A	171,11	w	256,11	I	298,15
b3	V	**399,23**	F	433,22	P	**344,19**	V	344,19	A	268,16	w	355,18	I	369,19	W	484,23
b4	F	**546,3**	P	530,27	V	443,26	A	415,23	w	452,23	I	468,26	W	555,27	V	583,3
b5	P	643,35	V	629,34	A	514,3	w	599,3	I	565,31	W	654,34	V	654,34	F	730,37
b6	V	**742,42**	A	700,38	w	698,37	I	712,38	W	751,39	V	753,41	F	801,41	P	827,42
b7	A	**813,46**	w	884,45	I	811,45	W	898,46	V	850,46	F	900,48	P	898,46	V	926,49
b8	w	997,53	I	997,53	W	997,53	V	997,53	F	997,53	P	997,53	V	997,53	A	997,53
a2		272,16		258,15		219,14		217,12		169,12				228,11		
y1		185,07		114,08		187,08		100,07		148,07		98,05		100,07		72,04
y2		256,11		298,15		300,16		286,15		247,14		245,12		197,12		171,11
y3		**355,18**		369,19		484,23		399,23		433,22		344,19		344,19		268,16
y4		**452,23**		468,26		555,27		583,3		546,3		530,27		443,26		415,23
y5		**599,3**		565,31		**654,34**		654,34		730,37		643,35		629,34		514,3
y6		698,37		712,38		751,39		753,41		801,41		827,42		742,42		700,38
y7		884,45		811,45		**898,46**		**850,46**		900,48		898,46		926,49		813,46
y8		997,53		997,53		997,53		997,53		997,53		997,53		997,53		997,53

Fig. 3.13. Fragmentation of synthetic *exo*-L (upper table) and natural (lower table) mayotlides.

It is quite interesting that, although many of the fragmentations are in common, the most abundant sequence don't coincide. On the natural mayotlide corresponds to IWVFPVAW (sequence 1), whereas the synthetic one corresponds to WIWVFPVA (sequence 8).

3.4 Conclusions chapter 3

- The synthetic *exo*-L mayotlide has achieved. During the process, a nice and efficient approach allowed to test different cyclization points and protecting groups.
- The NMR spectra corroborate the Trp-HPI framework, but there are big differences between the synthetic and the natural mayotlide.
- The MS-MS spectra demonstrates that the sequence is correct, but the fragmentation pattern is different.
- The next alternative would be the synthesis of mayotlide but with the *exo*-D adduct.

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(1) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.

(2) Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiu, G.; Sotiriu, P.; Wenqing, Y.; Schäfer, W. *Tetrahedron Lett.* **1989**, *30*, 3943–3946.

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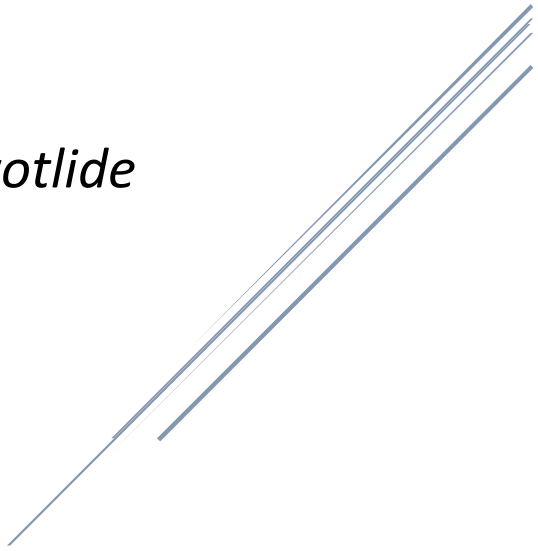
CHAPTER 4:

Synthetis of exo-D Mayotlide



4.1 What do we do now?

On chapter 3, the objective of the *exo*-L synthetic product was accomplished, albeit the ^{1}H-NMR does not match with the natural one. The stereochemistry was not modified after the several manipulations, the NMR revealed the Trp-HPI structure was intact and the sequence of the aminoacids were correct according to the MS-MS.

On this chapter is related which alternative was followed afterwards.

4.2 Maybe the *exo*-D adduct of the HPI?

it has been reported on the literature that several natural products contain D-aminoacids.[1] It could be the possibility that the configuration of the tryptophan that forms the HPI may be D instead of L. after the NMR and MS-MS studies of the *exo*-L mayotlide, we were not able to figure out which was the real structure of the mayotlide. The most immediate step to attempt was the synthesis of the mayotlide with the HPI in *exo*-D configuration.

The strategy for the mayotlide with the *exo*-D HPI was similar to the prior methodology, targeting this time the tripeptide **2b** as the first objective (fig. 4.1):

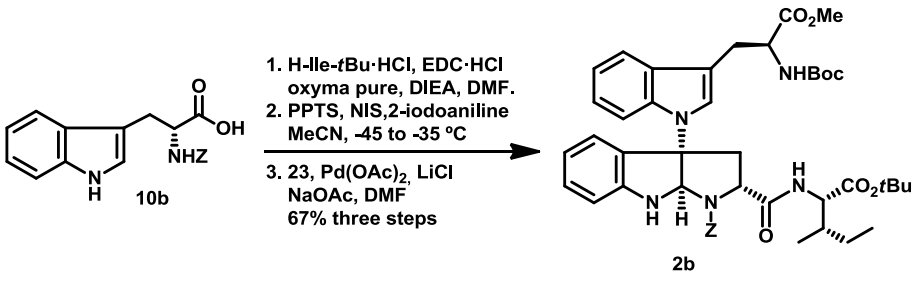


Fig. 4.1. Synthesis of peptide **2b**.

The overall yield for the synthesis of **2b** were improved respectively to **2a**. The HPI – *N*-C^{3a} bond this time afforded no iodinated compound, and the equivalents of alkyne for the Larock were 5 instead of three, improving the yield from 67 to 85%.

To reach **1b** the followed strategy was slightly different (fig. 4.2).

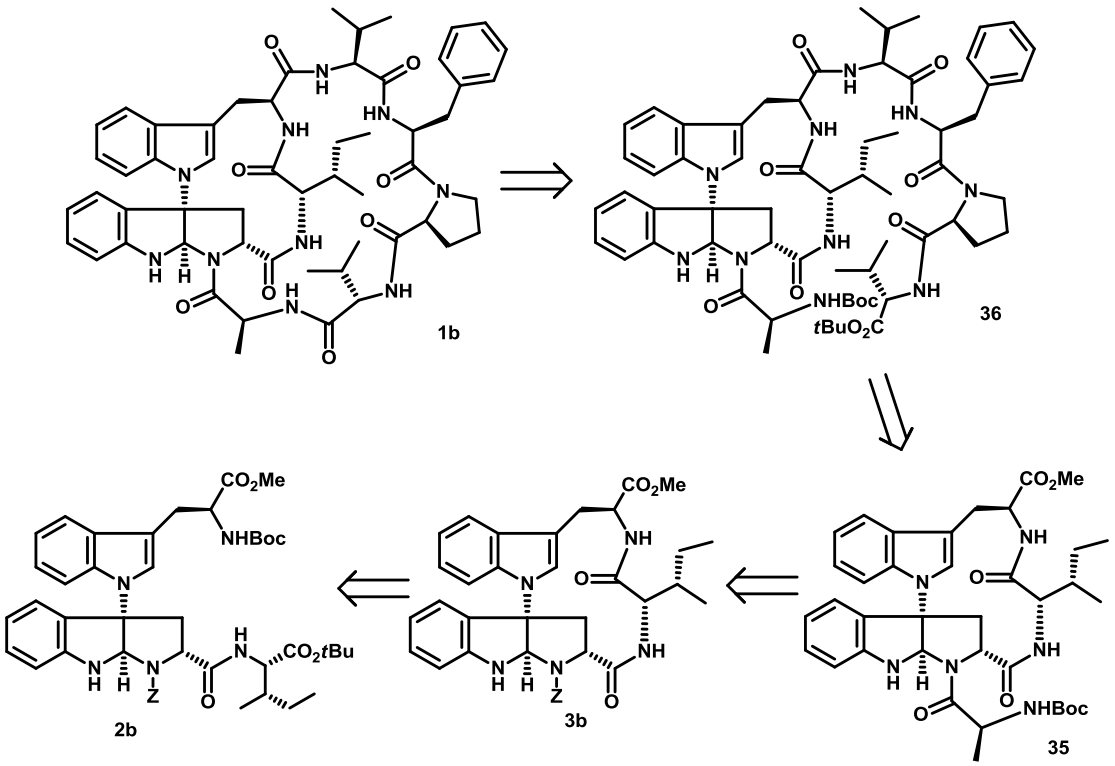


Fig. 4.2. Retrosynthetic scheme for the synthesis of **1b**.

This time the strategy will be different: the protecting groups of the *N* and *C* terminus of tripeptide **2b** are Boc-*t*Bu. With the formation of the ring A, they are eliminated and hence they can be reintroduced with the coupling of Boc-L-Ala-OH and the remaining tetrapeptide **39**, which was build up as the former linear peptides (fig. 4.3):

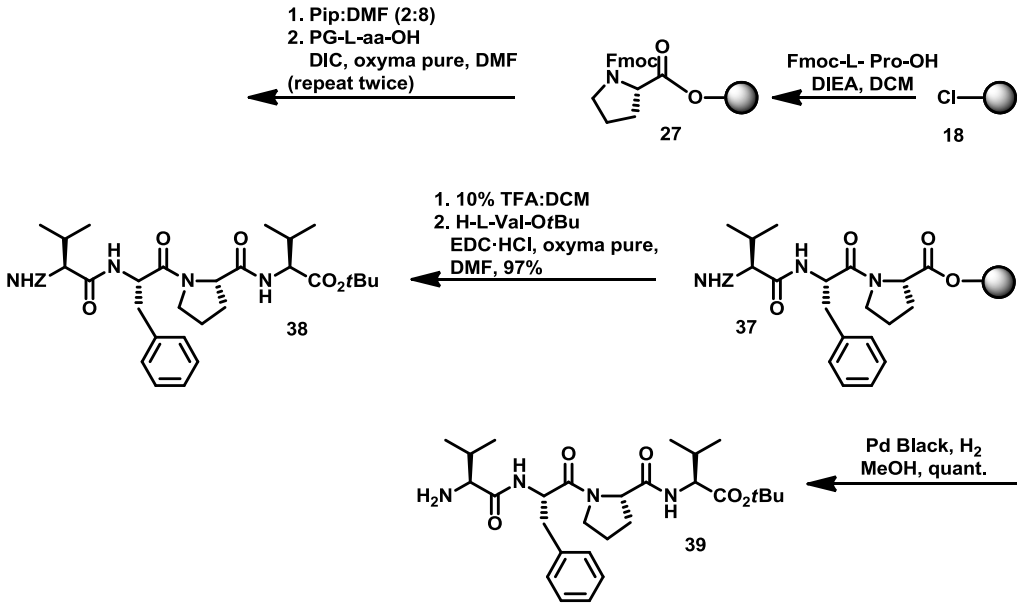


Fig. 4.3. Synthesis of tetrapeptide **39**.

On the other hand, tripeptide **2b** was converted into compound **35** in four steps (fig. 4.4).

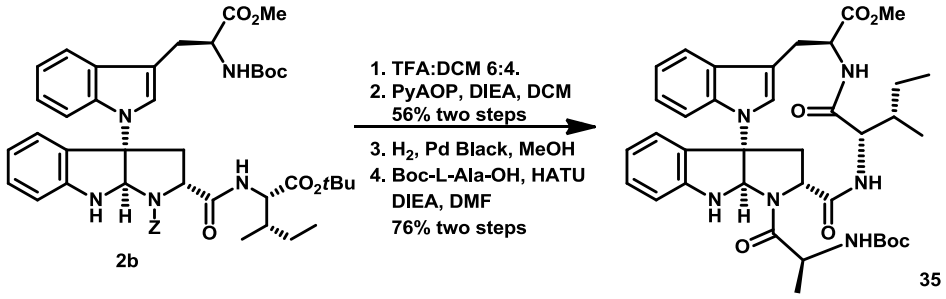


Fig. 4.4. Synthesis of **35**.

35 and **39** were coupled to achieve **36**. From here, **1b** were attempted (fig. 4.5).

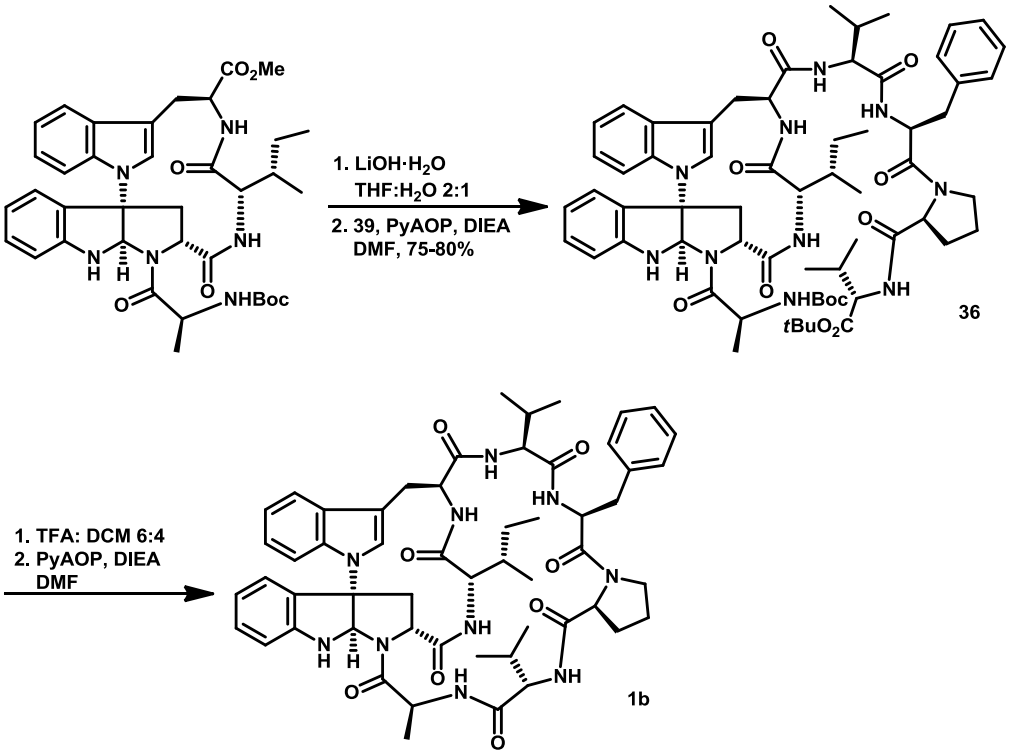


Fig. 4.5. Final step for **1b**.

The final product was not purified, due to the complexity of the crude and that another more probable structure was elucidated during the synthetic process. The new structure will be discussed on the next chapter.

References

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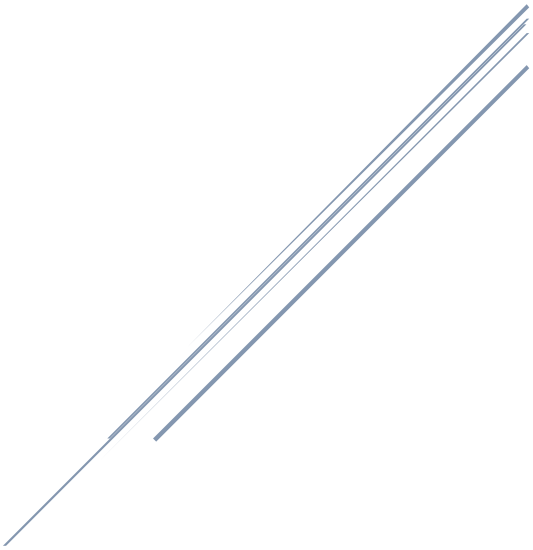
CHAPTER 5:

Revision of Mayotlide



5.1 Mayotlide is mayotlide?

During the synthesis of the *exo*-D mayotlide, the bibliography and the analytical data of the natural product were carefully examined. Albeit there were some point in favor to the first proposal, there were some others that don't, and hence and extensive revision was done. This is going to be the objective of the last chapter of the thesis.

5.2 The points of the discord

The points in favor to the first structure proposal are the following ones:

- The linear sequence established by the MS-MS patterns fixes with the natural product.
- The NMR shows two tryptophans linked by N^1-C^{3a} bond. The H8a singlet signal is indicative of this feature.

As the title indicates, the following points question that the original structure of mayotlide is the right one:

- There were no precedents of macrocyclic peptides with a Trp-HPI framework via N^1-C^{3a} bond. The more related natural products are the kapakahine family.[1]
- The natural mayotlide decomposed on the NMR tube after two days of measurements. The synthetic *exo*-L was stable, at least, to that period of time. Previously, Baran reported decomposition problems during the synthesis of the kapakahines B and F.[2]
- The ring A *exo*-L and the synthetic *exo*-L mayotlide NMR spectra have significant differences, especially on the aromatic area and a couple methyl valine signals on the aliphatic area.
- The fragmentation patern on the MS-MS were different.
- The main fragmentation on the natural product belongs to the Ile: 884 concerns to the loss of the complete aminoacid and 912 is the heptapeptide with the carbonyl

of the Ile. The most relevant fragmentation on the *exo*-L mayotlide corresponds to the loss of Phe.

- If you take a look to the fragmentation table on fig. 5.1, you can realize that, on the natural product, among the eight sequences settled, just in sequence 1 the Ile appears as *C*-terminus of a b-ion. On the first proposal, the ile is one of the three aminoacids that are common in both rings. Therefore, according to that structure proposal, it should appear more frequently on the diverse fragmentations.

Sequence	1		2		3		4		5		6		7		8	
ions																
b1	Ile	114,08	Trp	187,08	Val	100,07	Phe	148,07	Pro	**98,05**	Val	100,07	Ala	72,04	trp*	185,07
b2	W	**300,16**	V	**286,15**	F	**247,14**	P	**245,12**	V	**197,12**	A	171,11	w	256,11	I	298,15
b3	V	**399,23**	F	433,22	P	**344,19**	V	344,19	A	268,16	w	355,18	I	369,19	W	484,23
b4	F	**546,3**	P	530,27	V	443,26	A	415,23	w	452,23	I	468,26	W	555,27	V	583,3
b5	P	643,35	V	629,34	A	514,3	w	599,3	I	565,31	W	654,34	V	654,34	F	730,37
b6	V	**742,42**	A	700,38	w	698,37	I	712,38	W	751,39	V	753,41	F	801,41	P	827,42
b7	A	**813,46**	w	884,45	I	811,45	W	898,46	V	850,46	F	900,48	P	898,46	V	926,49
b8	w	997,53	I	997,53	W	997,53	V	997,53	F	997,53	P	997,53	V	997,53	A	997,53

Fig. 5.1. Absence of isoleucine on the Ms-MS fragmentation pattern.

To sum up, the three questions that were not solved with the first proposal were the following ones:

- Why the mayotlide was asigned as peptide wich two macrocyclic rings when there were no precedents of them?
- Why the main fragmentation on the Ms-Ms is placed through the Ile? for instance, Pro is a quite offen fragmentation, but Ile didn't have to be so.
- Why the Ile just appears on one fragmentation sequence on the Ms-Ms when it is forming part of two cycles?

5.3 Towards the real? mayotlide

Concerning the precedents of naturally-ocurring peptides , the most related family of natural products to mayotlide are the kapakahines. Kapakahines contain a tetracyclic system, very tensioned. In fact, kapakahine E has a very similar aminoacid sequence (fig. 5.2).[3]

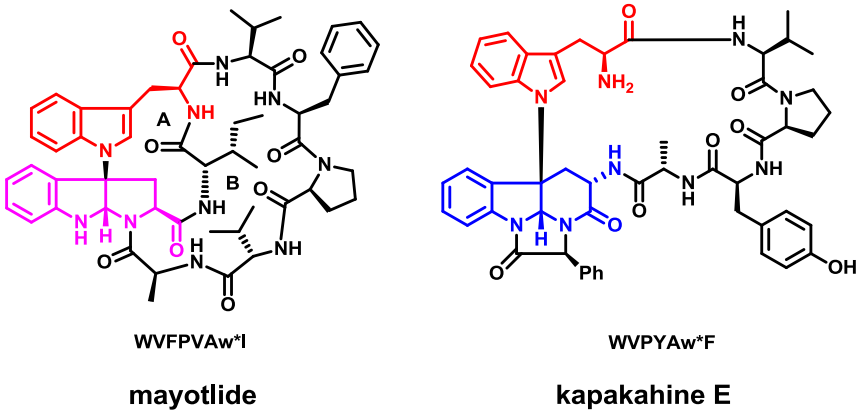


Fig. 5.2. Comparison of linear sequences among mayotlide and kapakahine E.

The first proposal of mayotlide with a α-carbonile structure was the following one, relabelled as "kapakahine H". The main changes are: a) the HPI system is substituted by an α-carboline tetracyclic system formed by one Trp and the Ile; b) there is one macrocyclic ring instead of two. Nevethelss, the α-carbolina also exhibits the H^{8a} (fig. 5.3).

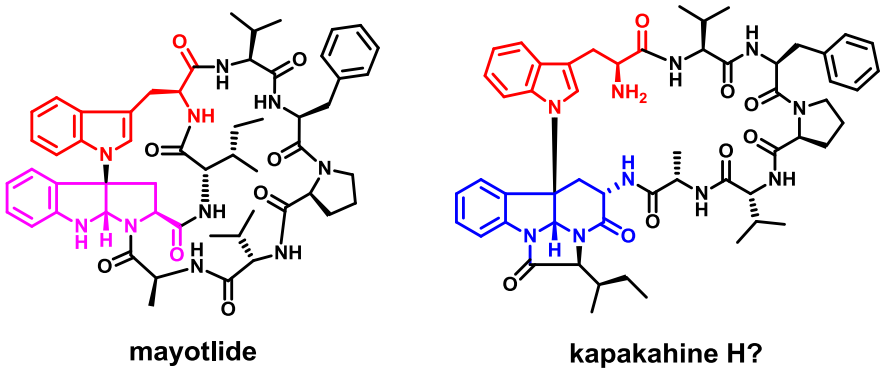


Fig. 5.3. First proposal of kapakahine H.

There was necessary to establish the fragmentation pattern for the new proposed structure. It was taken in consideration the two following points:

- The biosynthesis of the kapakahines: on section 2.4 there were described four alternatives for the Trp-HPI synthesis. The option one, the oxidative dimerization of two tryptophans, suits better with hour experimental experience. Hence, the fragmentation that leads the linear sequence of the peptide could be the one depicted on fig. 5.4.

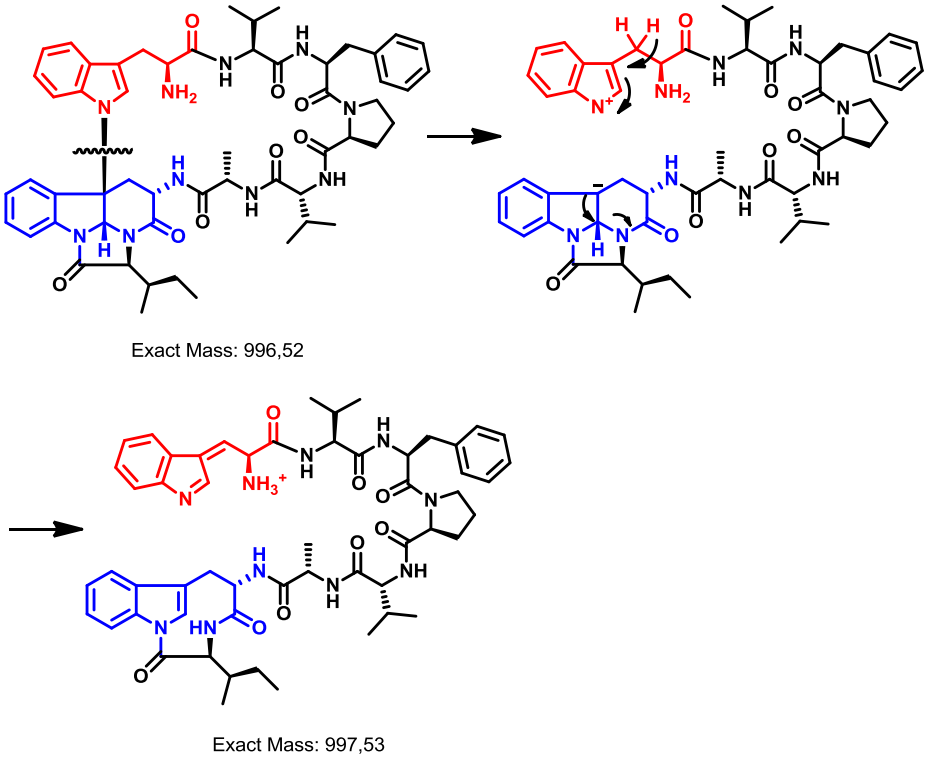


Fig. 5.4. Main fragmentation for kapakahines.

Such fragmentation would convert the upper indole into an electron-deficient system, the aromaticity of the lower indole is restituted and the Ile gets release from the α-carboline ring.

- The fragmentation pattern of macrocyclic peptides: in 2012, Niedermeyer and coworkers published a software that reproduces the fragmentation of cyclic peptides.[4] It is based on the formation of oxazolones on the *C*-terminus of the b ions. That oxazolone fragmentation pattern can be exported in our case, for instance, for the release of the Ile from the cycle that forms with the lower Trp (fig. 5.5).

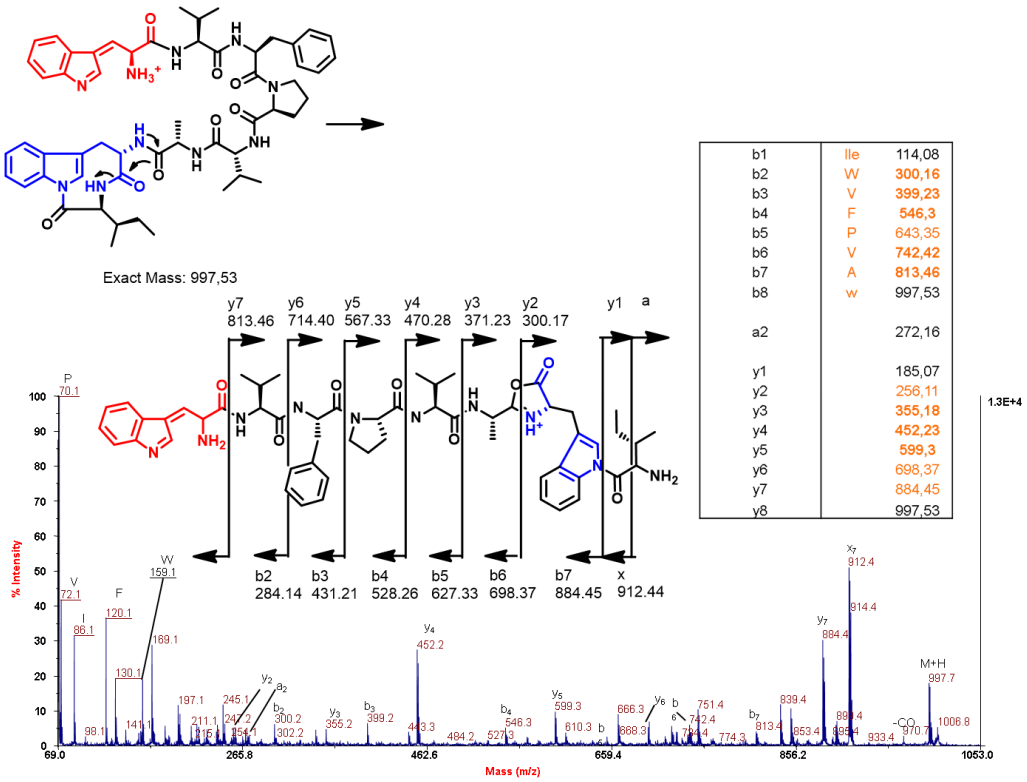


Fig. 5.5. Fragmentations for the first proposal of kapakahine H.

This structure justifies the intense fragmentation of the Ile. The carboxylic acid of that aminoacid is coupled with the *N*-indole of the Trp which forms together the α-carboline. That amide bond is not naturally occurring, and hence explains the fragmentation of the Ile into x and a-ions.

The problem of this proposal is that the linear sequence doesn't correspond to the natural product.

Probably, the two Trp could be exchanged by confusion, and then, the central aminoacids have the inverse sequence. The second proposal was the following one (fig. 5.6):

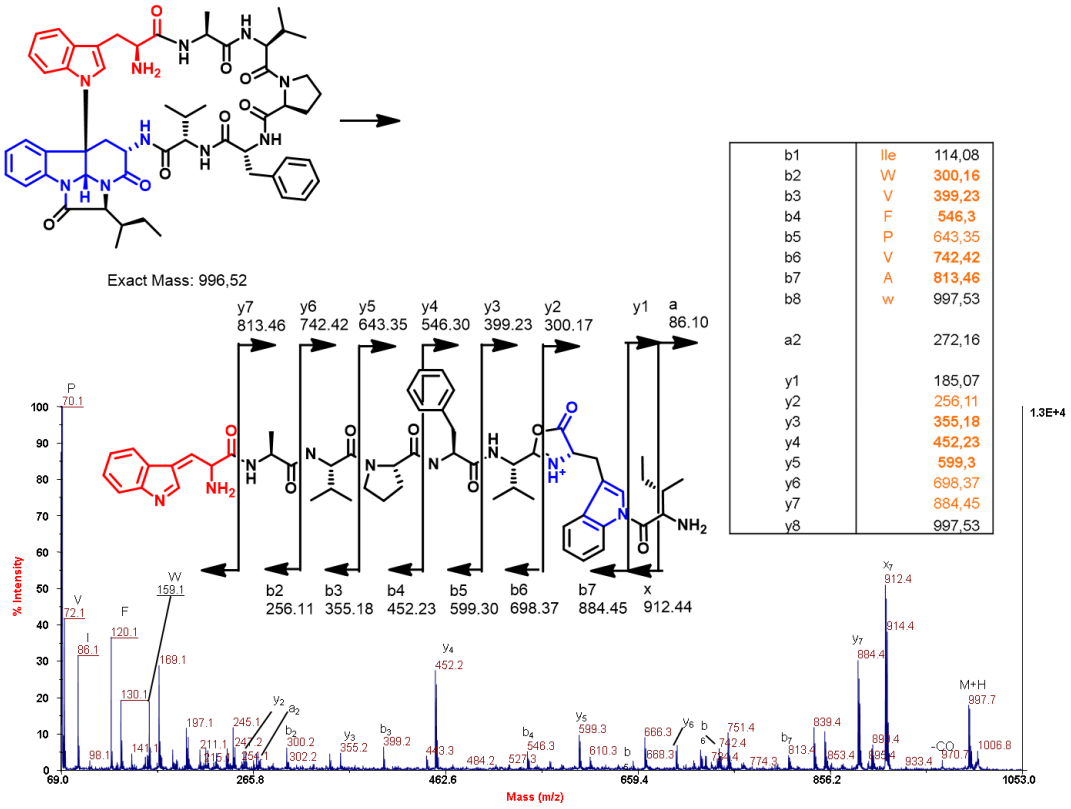


Fig. 5.6. Fragmentations for the second proposal of kapakahine H.

Bingo! This structure is correct from the point of view of the exact mass, Ile fragmentation and the main fragmentation sequences. Due to the peculiarity of the fragmentation, with the formation of oxazolones, the ions were also exchanged (b-ions are y ions and on the other way around). On the experimental data are compiled all the fragmentations by the formation of oxazolones.

All the fragmentation data were reported to PharmaMar and they have corroborated hour hypothesis

In addition, the fragmentation pattern can give an idea about a biosynthetic pathway, depicted on fig. 5.7. The main steps would be: a) linear sequence of the octapeptide; b) intramolecular cyclization of the C-terminus of the Ile with the *N*-indole of the lower Trp; and c) intramolecular attack of the N-terminus of the Ile to the C2 of the lower Trp with concomitant loss of aromaticity. The resulting free electron pair con C^{3a} attacks the N-oxidized Trp.

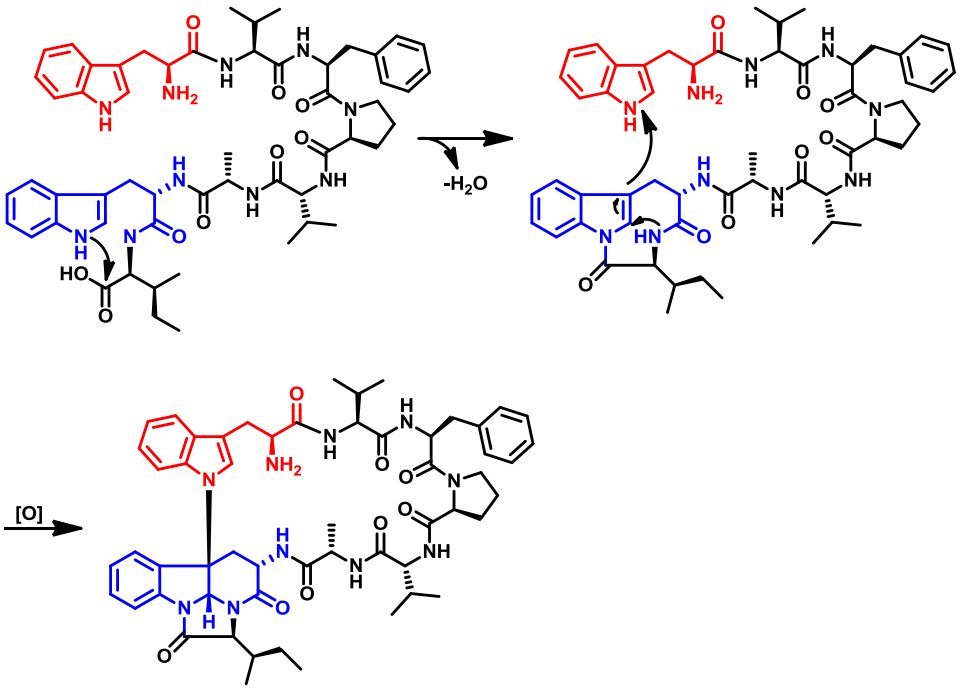


Fig. 5.7. Possible biosynthesis of kapakahine H.

There is just one detail missing. After checking the ^{1}H-NMR spectra of the kapakahines, the singlet of the H^{8a} appears around δ 5.7 - 5.8. On figure fig. 5.7 is attached the ^{1}H-NMR of the kapakahine E as an instance (fig. 5.8).[5]

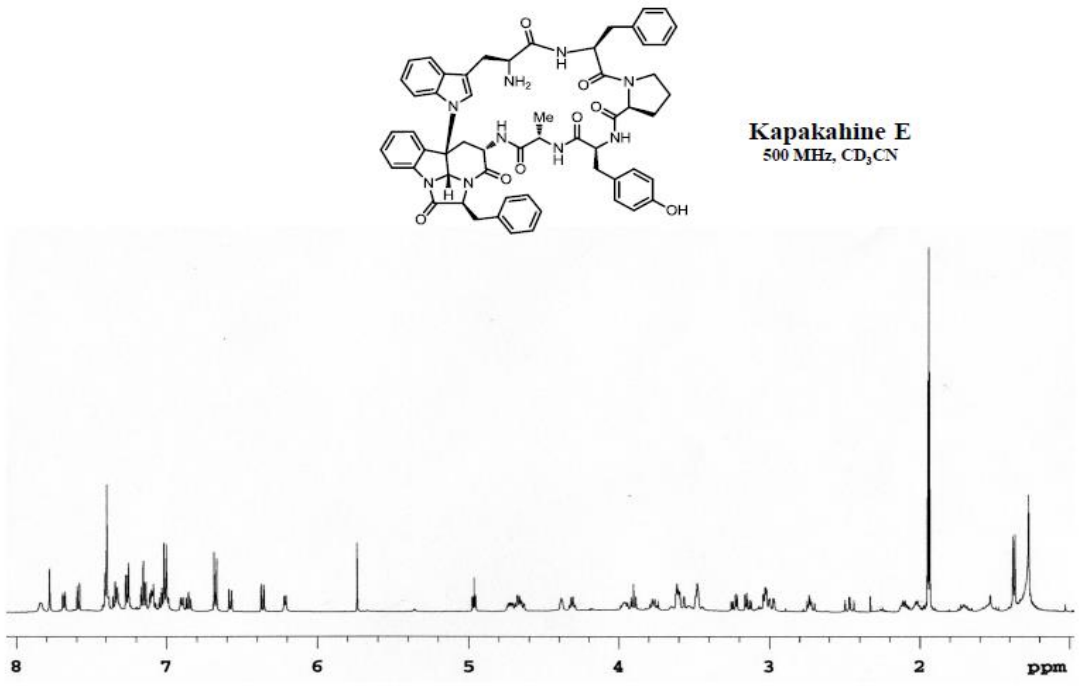


Fig. 5.8. ^{1}H-NMR spectra of kapakahine E, with the δ H^{8a} at 5.7 approx.

The δ H^{8a} on kapakahine H lies around 6.4. Obviating the difference of the solvent, 0,7 ppm is a significant difference for molecules with a very close structure. On the kapakahines A-G, always an aromatic aminoacid (Tyr or Phe) forms the bridge for the tetracyclic system. On the case of the kapakahine H, this aminoacid is Ile, whose residue is aliphatic. It could be that, on kapakahines A-G, the H^{8a} falls into the anisotropy cone of the aromatic ring of Phe/Tyr, displacing the signal into higher fields.

To conclude, fig. 5.9 contains the first structure of mayotlide, the first proposal for kapakahine H and the definitive one.

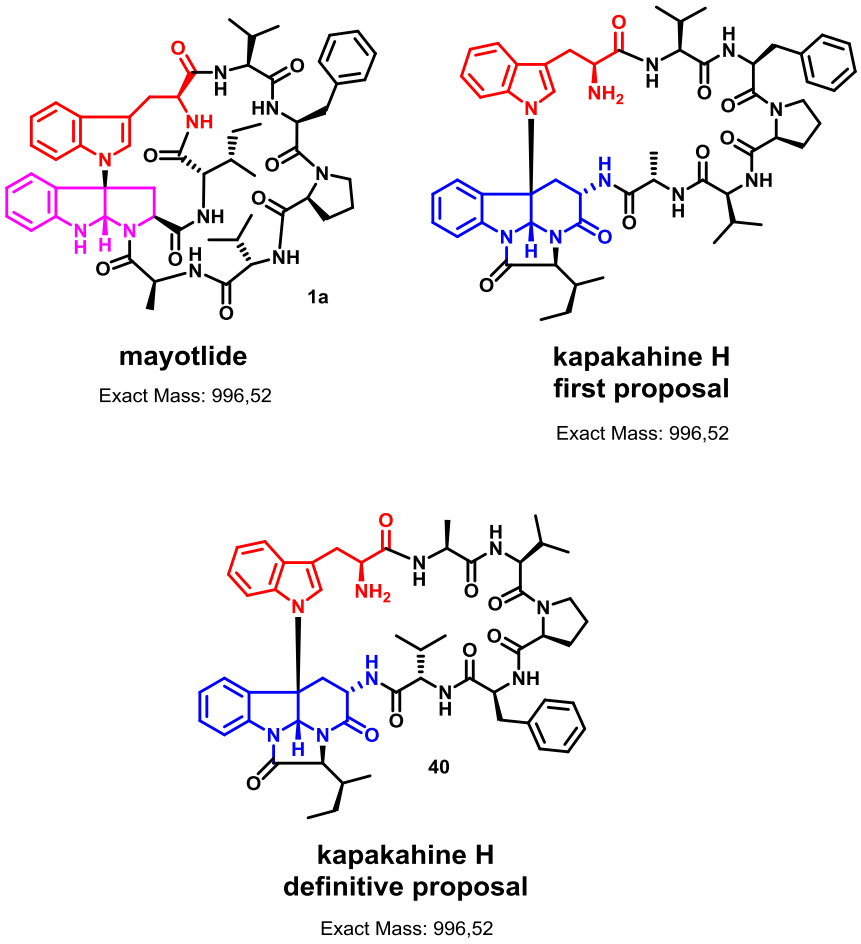


Fig. 5.9. The three analyzed molecules on this chapter.

5.4 Conclusions chapter 5

- During the synthesis of the *exo*-D, the bibliography and the analytical data of the natural product were extensively revised.
- There were no precedents of peptidic natural products with the Trp-HPI structure.
- Although the NMR spectra may fix with the first proposal, there were considerable divergences on the MS-MS.
- The structure of the molecule was completely redesigned: instead of two macrocycles there is just one, instead of a HPI there is an α-carboline on its place, and the central aminoacid sequence was inverted.
- The new proposal seems more likely to be the correct one, according to the analytical data, that the one proposed in 2004-2005. It has been relabeled as “kapakahine H”.

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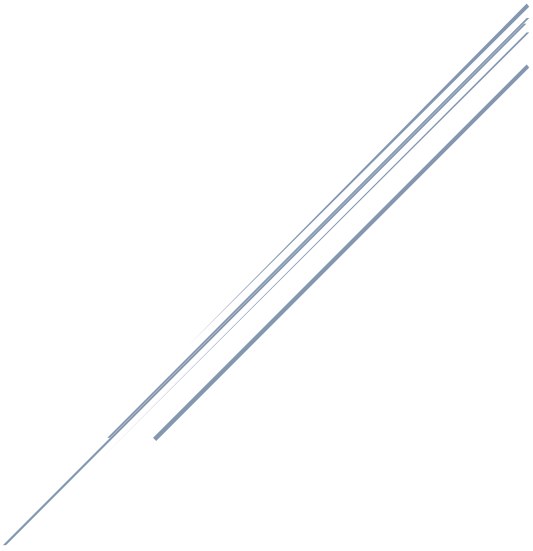
CONCLUSIONS



Conclusions

- The marine-origin peptide mayotlide was reported as an octapeptide peptide with two macrocycles, one of them containing a Trp-HPI unit. The aminoacid analysis reported the L-configuration of all the aminoacids except the HPI, which remains unknown.

- The HPI aminoacid has been deeply studied. It contains three stereocenters, two of them are always in *cis* conformation due to the configuration of the tricyclic system. Therefore, there are four possible diasteromers of HPI.

- In order to select, among the four diasteromers, which of them may be more stable within mayotlide, a computational study with the MOE program was performed, with the four ring A-containing HPI possibilities, revealing that the *exo* adducts have lower energy, according to the mentioned program.

- The methodology developed by Baran and coworkers for the synthesis of Trp-HPI containing molecules was adapted for the synthetic purposes. Just two steps are necessary for achieving the Trp-HPI framework:

    - Under reaction conditions, the starting Trp cyclizes, forming a HPI unit with the *exo* configuration exclusively, at the same time that a new bond between the C^{3a} of the HPI and the *N* of 2-iodoaniline is formed.
    - The Larock reaction provides the upper Trp by means of the iodoaniline and a disubstituted alkyne via Pd catalytic cycle. The indole formation takes places with retention of the stereochemistry of the HPI.

- The tripeptide **2a**, the smallest scaffold that contains the three aminoacids that are common in both cycles, was achieved in acceptable conditions (three steps, 50%-65% yield). This intermediate will serve as a platform for all the synthetic approaches for the *exo*-L and D mayotlides.

- During the synthetic approaches, three pairs of protecting groups were used: alloc-allyl, Z-Bn and Boc-*t*Bu. The alloc-allyl were not well compatible with the molecule due to the reactivity of the Trp with the allyl deprotection process, which needs of purification or scavenger addition. The two remaining protecting groups don't offer particular and can be used, as appropriate, depending of the selectec synthetic pathway.

- The *exo*-L mayotlide was achieved, but the ^{1}H-NMR doesn't coincide, as well as the MS-MS fragmentation pattern and the main fragmentations.

- As an alternative, the mayotlide with the *exo*-D adduct was explored. However, during the synthesis of this molecule, the analytical data and the bibliography were intensively revised, reaching the conclusion that the first structure proposal was incorrect.

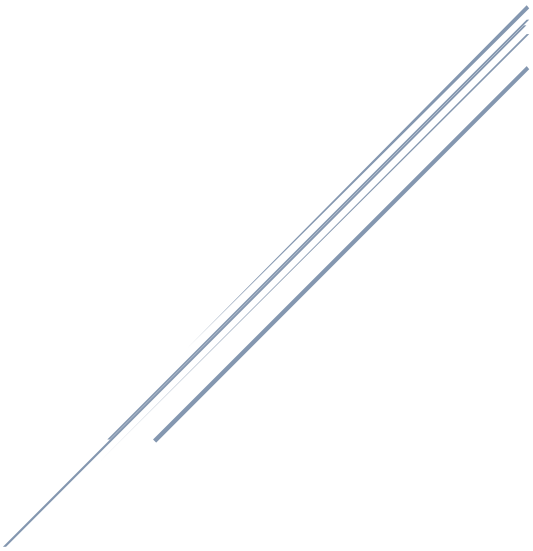
- The evidences that were stated for the revision of the structure of mayotlide are the following ones:

    - There are no precedent of naturally-occurring peptides with the Trp-HPI moiety. The closest peptide family to mayotlide are the kapakahines, which have a Trp-α carboline as central motif, instead of Trp-HPI, and the aminoacids establish one macrocycle instead of two.

    - The MS-MS presents critical divergences between the natural product one and the synthetic *exo*-L. The fragmentation pattern was different and the main fragmentations of the natural one, regarding the Ile, were not justified with the Trp-HPI molecule proposal.

- The structure of mayotlide was redesigned, changing the Trp-HPI for a Trp-α carboline, and the order of the central sequence of the aminoacids were inverted. The new structure proposal justifies completely the NMR and MS-MS experimental evidences.

EXPERIMENTAL SECTION



General Information

N,*N*-dimethylformamide (DMF) was dried using a PureSolv solvent purification system. All other solvents and reagents were used as purchased without further purification, unless otherwise indicated.

Flash column chromatography in normal phase was performed on SDS silica gel (60A 35-70 µm) as stationary phase. Reverse phase column chromatography was performed on a Teledyne Isco Combiflash R_f

Analytical thin layer chromatography was performed using aluminium-backed plates (0.2 mm, 20x20 cm) coated with Merck KIesegel 60 F_{254}; compounds were visualized under a UV lamp (254 and 360 nm) or with $KMnO_4$ solution.

Polarimetry studies were performed on a Perkin-Elmer 241 of Jasco P-2000 polarimeter.

IR spectra were recorded on a Thermo Nicolet FT-IR Nexus spectrometer.

Melting points were performed in a Büchi B-540 melting point apparatus.

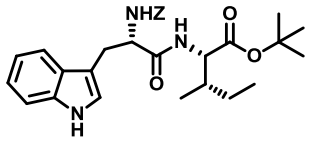
^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Mercury 400MHz or Bruker 600MHz spectrometer. Chemical shifts are reported in ppm referenced to the residual solvent peaks ($CDCl_3$, DMSO-d_6 or CD_3OD) and coupling constants are reported in Hz. Multiplicity of the carbons was assigned with gHSQC experiments. Standard abbreviations for off-resonance decoupling were employed: (s) singlet, (d) doublet, (t) triplet, (q) quadruplet. The same abbreviations were also used for the multiplicity of signals in ^{1}H NMR, as well as, (bs) broad singlet, (bd) broad doublet,(dd) doublet of doublets, (dt) doublet of triplets, (m) multiplet.

Reverse-phase analytical HPLC was carried out in a Waters Alliance separation module 2695 equipped with a Waters XBridge C18 columns (4.6x100 mm, 3.5µm) and a Waters 996 PDA with a photodiode array detector, using MeCN (0.036% TFA) and H_2O (0.045% TFA) as mobile phases.

High Resolution Mass Spectroscopy (HRMS) was performed an Agilent LC/MSD-TOF 2006 using ESI-MS technique.

MS-MS MALDI-TOF was performed on a ABSciex 4700 Maldi Tof Tof Mass Spectrometer 4700 Proteomics Analyzer.

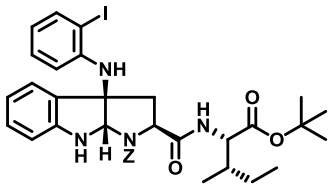
Tert-butyl ((benzyloxy)carbonyl)-L-tryptophyl-L-isoleucinate (**11a**)



Z-L-Trp-OH (**10a**) (5g, 14.78 mmol), H-L-Ile-*t*-Bu·HCl (3.64 g, 16.26 mmol), EDC·HCl (3.4 g, 17.74 mmol) and oxyma pure (2.95 g, 20.69 mmol) were dissolved in DMF (30 ml) and stirred at 0 °C. DIEA (7.7 ml, 44.33 mmol) was added dropwise and the mixture was let to warm up to RT over 3h. The crude mixture was diluted with AcOEt and washed successively with 1M HCl, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The afforded product (**11a**) was a white foam with mass 7.4 g (99% yield).

M.p. 61-63 °C; **$[\alpha]_D^{20}$** -28.1° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d,* 1:4 mixture of rotamers) δ 0.73 and 0.81 (d, *J* = 6.9 Hz, 3H), 0.86 and 0.90 (t, *J* = 7.3 Hz, 3H), 0.98-1.09 (m, 1H), 1.29-1.38 (m, 1H), 1.42 (s, 9H), 1.67-1.79 and 1.81-1.92 (bs, 1H), 3.20 (dd, *J* = 14.5, 7.2 Hz, 1H), 3.39–3.27 (m, 1H); 4.35 (dd, *J* = 8.2, 4.6 Hz, 1H), 4.52-4.58 (m, 1H), 5.11 5.19 and 5.27 (s and 2d, *J* = 12.0 Hz,2H), 5.50 (d, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 2.3 Hz, 1H), 7.11 (t, *J* = 7.4 Hz, 1H), 7.19 (t, *J* = 7.0 Hz, 1H), 7.28-7.40 (m, 6H), 7.63-7.70 (m, 1H), 8.12 (bs); **^{13}C NMR** (100 MHz, Chloroform-*d*) δ 11.8 (q), 15.2 (q), 25.5 (t), 28.2 (t), 38.1 (d), 55.6 (d), 57.1 (d), 67.1 and 68.4 (t), 82.1 (s), 110.5 (s), 111.3 (d), 119.0 (d), 119.9 (d), 122.4 (d), 123.5 (d), 128.2 (d), 128.3 (d), 128.6 (d), 136.4 (s), 156.1 (s), 170.4 (s), 171.1 (s); **IR** (NaCl film, cm^{-1}) 3322.83, 2966.88, 2929.26, 1713.83, 1685.84; **HRMS** (ESI+) m/z Calcd for $C_{29}H_{38}N_3O_5$ [M+H] 508.2806, found 508.2809.

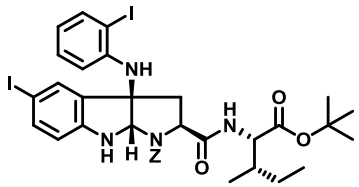
Benzyl (2S,3aR,8aS)-2-(((2S,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)-3a-((2-iodophenyl)amino)-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**12a**)



11a (1.5 g, 3.0 mmol mmol), PPTS (754 mg, 3.0 mmol) and 2-iodoaniline (755 mg, 3.5 mmol) were dissolved in MeCN (15 ml) and cooled down to -45 °C with an acetone - dry ice bath. NIS (1.06 g, 4.7 mmol) dissolved in MeCN (15 ml) was added dropwise and the mixture was allowed to warm to -35 °C over one hour. The crude mixture was poured on a separatory funnel and quenched with sat. $Na_2S_2O_4$ solution. The organic phase was separated and the water phase was extracted with AcOEt. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude was purified by Combiflash ISCO reverse phase C18 (water:MeCN, 2:8 – 0:100), affording a white foam with mass 1.6 g (76% yield) and 153 mg of **13a** as a side product (6% yield).

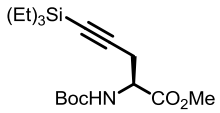
M.p. 100-102 °C; **$[\alpha]_D^{20}$** -124.0° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d*, 85:15 mixture of rotamers) δ 0.70 and 0.83 (d, *J* = 6.8 Hz, 3H); 0.90 (t, *J* = 7.4 Hz, 3H); 1.14 (ddd, *J* = 13.1, 8.8, 6.8 Hz, 1H); 1.37 and 1.42 (m, 1H), 1.45 and 1.48 (s, 9H), 1.73-1.82 and 1.83-1.92 (m, 1H); 2.34 and 2.58 (dd, *J* = 14.0, 8.9 Hz, 1H); 2.99 and 3.22 (dd, *J* = 13.9, 1.6 Hz, 1H); 4.41 and 4.64 (dd, *J* = 8.9, 1.5 Hz, 1H); 4.49 and 4.55 (dd, *J* = 8.6, 4.2 Hz, 1H); 4.80 (bs), 5.11 and 5.22 (d, *J* = 12.2 Hz, 1H), 5.14 and 5.28 (d, *J* = 12.1 Hz, 1H), 5.91 and 6.04 (s, 1H), 6.00 and 6.25 (d, *J* = 8.2 Hz, 1H), 6.33 and 6.38 (t, *J* = 7.5 Hz, 1H), 6.54 (d, *J* = 8.2 Hz, 1H), 6.61 and 6.66 (d, *J* = 7.9 Hz, 1H), 6.73 (t, *J* = 7.4 Hz, 1H), 6.85 and 6.95 (t, *J* = 7.8 Hz, 1H), 7.08 (d, *J* = 7.9 Hz, 1H), 7.13 (t, *J* = 7.7 Hz, 1H), 7.27 and 7.42 (m, 6H), 7.67 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.73 (d, *J* = 8.6 Hz, 1H); **^{13}C NMR** (100 MHz, Chloroform-*d*) δ 12.0 and 12.1 (q), 15.3 and 15.7 (q), 25.5 and 25.7 (t), 28.2 (q), 38.2 and 38.3 (d), 41.2 and 43.5 (t), 57.0 and 57.3 (d), 60.3 and 60.6 (d), 68.1 and 68.3 (t), 71.8 and 73.2 (s), 79.9 and 80.2 (d), 81.9 and 82.3 (s), 86.1 and 87.0 (s), 109.9 and 110.0 (d), 113.2 and 113.5 (d), 118.7 and 119.5 (d), 119.8 (d), 122.7 and 122.9 (d), 128.4 , 128.5 (d), 128.7 , 128.9 (d), 129.4 , 129.9 , 135.8 and 135.9 (s), 139.7 and 140.1 (d), 144.6 and 145.2 (s), 146.4 and 147.4 (s), 155.2 and 156.6 (s), 170.6 and 170.8 (s); **IR** (NaCl film, cm^{-1}) 3383.60, 3319.94, 2946.62, 1748.55, 1710.93, 1647.27; **HRMS** (ESI+) m/z Calcd for $C_{35}H_{42}IN_4O_5$ [M+H] 725.2194, found 725.2182.

Benzyl (2S,3aR,8aS)-2-(((2S,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)-5-iodo-3a-((2-iodophenyl)amino)-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**13a**)



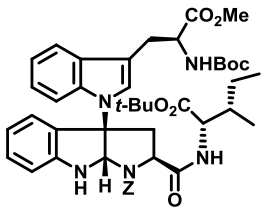
M.p. 100-102 °C; **$[\alpha]_D^{20}$** -92.9° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d*, 7:3 mixture of rotamers) δ 0.69 and 0.81 (d, *J* = 6.9 Hz, 3H), 0.84 and 0.89 (t, *J* = 7.4 Hz, 3H), 1.07-1.19 (m, 1H), 1.38-1.44 (m, 1H), 1.45 and 1.48 (s, 9H), 1.74-1.80 and 1.82-1.90 (m, 1H), 2.28 (dd, *J* = 14.0, 9.0 Hz, 1H), 3.20 (d, *J* = 14.0 Hz, 1H), 4.42 and 4.64 (d, *J* = 8.7 Hz, 1H), 4.48 and 4.54 (dd, *J* = 8.5, 4.1 Hz, 1H), 4.85 and 5.45 (bs, 1H), 4.90 (bs), 5.19 (d, *J* = 12.1 Hz, 1H), 5.27 (d, *J* = 12.1 Hz, 1H), 5.46 (bs), 5.89 and 6.01 (s, 1H), 5.95 and 6.15 (d, *J* = 8.1 Hz, 1H), 6.33-6.46 (m, 2H), 6.88 and 6.96 (t, *J* = 7.8 Hz, 1H), 7.31-7.43 (m, 6H), 7.69 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.73 (d, *J* = 8.6 Hz, 1H); **^{13}C NMR** (100 MHz, Chloroform-*d*) δ 12.1 (q), 15.3 and 15.7 (q), 25.5 (t), 28.2 (q), 38.3 (d), 41.3 (t), 57.1 and 57.3 (d), 60.0 and 60.4 (d), 68.2 and 68.4 (t), 72.9 (s), 79.6 and 80.0 (d), 80.7 (s), 82.0 (s), 86.1 (s), 111.9 and 112.1 (d), 113.1 and 113.3 (d), 119.0 and 119.7 (d), 131.2 and 131.4 (d), 128.5 (d), 128.6 (d), 128.7 (d), 128.9 (d), 132.8 (s), 135.8 (s), 138.1 (d), 140.2 (d), 144.9 (s), 145.9 (s), 156.5 (s), 170.4 (s), 170.5 (s); **IR** (NaCl film, cm^{-1}) 3348.87, 2963.99, 2929.26, 1722.51, 1696.46, 1600.96; **HRMS** (ESI+) m/z Calcd for $C_{35}H_{41}I_2N_4O_5$ [M+H] 851.1161, found 851.1149.

(S)-methyl 2-((tert-butoxycarbonyl)amino)-5-(triethylsilyl)pent-4-ynoate (**14**)



Aminoacid **14** was prepared following the experimental part described on: Newhouse, T.; Lewis, C. A.; Eastman, K. J.; Baran, P. S. *J. Am. Chem. Soc.* **2010**, *132*, 7119–7137.

Benzyl (2S,3aR,8aS)-2-(((2S,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)-3a-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-1H-indol-1-yl)-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**2a**)

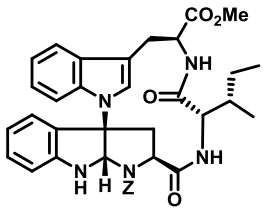


A Schlenk flask containing compound **12a** (2g, 2.76 mmol), aminoacid **14** (2.83 g, 8.29 mmol), $Pd(OAc)_2$ (0.12g, 0.55 mmol), anhydrous NaOAc (1.58 g, 19.33 mmol) and anhydrous LiCl (0.12g, 2.76 mmol) was evacuated and backfilled with nitrogen. Under nitrogen atmosphere, DMF (27 mL) was added. In the next step, the reaction mixture was degassed via iterative freeze-pump-thaw cycles. After heating the mixture to 100 °C during 24 h, the reaction mixture was cooled to room temperature, diluted with toluene, filtered through Celite with EtOAc flushing and concentrated. The residue was taken up with AcOEt and washed vigorously with aqueous 1M HCl. The layers were separated, and the aqueous layer was extracted additionally with AcOEt. The combined organic layers were washed successively with Sat. $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated. The crude material was purified by flash column chromatography (Hexane:AcOEt 9:1 to 3:7). The excess of alkyne **14** (1.84 g) and unreacted **12a** (480 mg) could be recovered during chromatography. The afforded product (**2a**) was a brown foam with mass 1.5 g (67 % yield).

M.p. 100-102 °C; $[\alpha]_D^{20}$ -54.3° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d*, 1:1 mixture of rotamers) δ 0.70 and 0.97 (d, *J* = 6.9 Hz, 3H); 0.86 and 0.96 (t, *J* = 7.3 Hz, 3H); 1.01-1.13 and 1.18 and 1.25 (2m, 1H) 1.39-1.47 (m, 18H); 1.69 and 1.98 (bs, 1H); 2.89 and 3.00 (dd, *J* = 13.3, 7.2 Hz, 1H); 3.43 and 3.48 (2s, 3H); 3.44-3.50 and 3.53-3.61 (2m, 1H); 4.09 – 4.25 (m, 1H); 4.44 and 4.59 (dd, *J* = 8.6, 4.0 Hz, 1H); 4.49-4.56 (m, 1H); 5.06 (d, *J* = 12.0 Hz, 1H); 5.23 and 5.36 (d, *J* = 12.2 Hz, 1H); 5.99 and 6.15 (s, 1H); 6.25 and 6.40 (d, *J* = 8.5 Hz, 1H); 6.55 and 6.75 (d, *J* = 7.9 Hz, 1H); 6.57 and 6.63 (bs, 1H); 6.92 (t, *J* = 7.6 Hz, 1H); and 7.12 (t, *J* = 7.5 Hz, 1H); 7.20 and 7.41 (m, 9H); 7.51 (d, *J* = 8.0 Hz, 1H); 7.67 and 7.78 (d, *J* = 8.4 Hz, 1H); **^{13}C NMR** (100 MHz, Chloroform-*d*) δ 12.0 (q), 15.2 and 15.4 (q), 25.5 and 25.6 (t), 27.8 and 27.9 (t), 28.2 (q), 28.5 (q), 38.3 and 39.2 (t), 38.5 (d), 52.0 (q), 54.5 (d), 56.8 and 57.1 (d), 61.1 and 61.7 (d), 67.7 and

67.9 (t), 72.9 and 73.8 (s), 79.1 (s), 79.8 and 80.1 (d), 82.4 and 82.5 (s), 108.9 and 109.0 (s), 110.8 and 111.1 (d), 112.3 and 112.5(d), 119.6 (d), 119.8 and 120.0 (d), 122.4 and 122.5 (d), 125.5 (d), 125.7 (d), 126.3 and 126.5 (s), 127.7 (d), 128.2 (d), 128.6 (d), 129.0 (d), 130.6 (s), 131.2 (d), 134.7 (s), 135.9 (s), 148.8 and 149.2 (s), 154.2 and 154.6 (s), 155.2 (s), 170.3 and 170.7 (s), 170.8 and 171.0 (s), 172.4 and 172.5 (s); **IR** (NaCl film, cm^{-1}) 3305.47, 3053.70, 2958.20, 2932.15, 1739.87, 1696.46, 1681.99, 1655.95, 1609.65; **HRMS** (ESI+) m/z Calcd for $C_{46}H_{58}N_5O_9$ [M+H] 824.4229, found 824.4226.

11-benzyl 4-methyl (12S,13aR,18aS,21S,4S,7S,Z)-7-((S)-sec-butyl)-6,9-dioxo-11,12,13,13a,18,18a-hexahydro-21H-5,8-diaza-1(3a,2)-pyrrolo[2,3-b]indola-2(1,3)-indolacyclononaphane-11,4-dicarboxylate (**3a**)

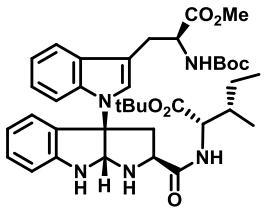


Compound **2a** (1.17g, 1.42 mmol) was dissolved in a mixture (4 mL) containing 50% TFA/2% isopropyl sulfide in DCM and stirred during 6h at RT. The solvents were concentrated under reduced pressure, the mixture was redissolved in the minimum amount of DMF and DCM (1.4 l, 1 mM) was added. The mixture was cooled down to 0 °C and PyAOP (1.48 g, 2.85 mmol) and DIEA (1.48 ml, 8.54 mmol) was added dropwise. The mixture was stirred overnight. The solvents were concentrated under reduced pressure and the crude mixture was washed successively with 1M HCl, $NaHCO_3$ and brine, dried over MgSO4, filtered and concentrated. The product was purified by flash column chromatography (Hexane:AcOEt 1:1). The obtained product (**3a**) was a pink foam with mass 472 mg (52% yield).

M.p. 171-173 °C; $[\alpha]_D^{20}$ -170.4° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d,* 2:1 mixture of rotamers) δ 0.75 and 0.88 (t, *J* = 7.4 Hz, 3H); 0.96 and 1.00 (d, *J* = 6.5 Hz, 3H); 1.00-1.06, 1.13-1.23, 1.42-1.50 and 1.60-1.70 (m, 1H), 1.97-2.14 (m, 1H); 2.58 and 2.70 (m, 1H); 3.26-3.35 (m, 1H); 3.42-3.53 (m, 1H), 3.56-3.67 (m, 1H); 3.77 (s, 3H); 4.07 and 4.16 (bt, *J* = 9.6 Hz, 1H); 4.66 and 4.80 (d, *J* = 8.6 Hz, 1H); 4.91 (q, *J* = 6.3 Hz, 1H); 4.96-5.10 and 5.35 (m and d, *J* = 12.3 Hz, 2H); 6.19 and 6.26 (s, 1H); 6.48 (dd, *J* = 7.3 Hz, 1H); 6.53-6.80 (m, 3H), 6.83-7.02 (m, 3H); 7.10-7.16 (m, 1H), 7.18-7.28 (m, 3H); 7.32-7.40 (m, 3H); **^{13}C NMR** (100 MHz, Chloroform-*d*) δ 10.4 and 10.6 (q); 15.9 and 16.0 (q); 24.9 and 25.0 (t); 25.9 and 26.0 (t); 33.9 and 34.0 (d); 41.0 and 41.8 (t); 52.1 and 52.2 (d); 52.9 and 52.9 (q); 58.7 (d); 59.7 and 60.0 (d); 67.7 and 67.9 (t); 74.5 and 75.8 (s); 82.6 and 83.6 (d); 107.8 (s); 109.8 and 110.0 (d); 112.0 and 112.1 (d); 118.7 and 118.8 (d); 119.6 and 119.8 (d); 119.8 and 119.9 (d); 121.9 and 122.2 (d); 122.8 and 122.9 (d); 124.0 and 124.4 (d); 127.9 and 128.2 (d); 128.3 and 128.4 (d); 128.5 and 128.8 (d); 129.5 and 129.6 (s); 129.7 and 129.9 (s); 130.1 and 130.2 (s); 135.4 and 135.5 (s); 135.9 and 136.2 (s);

146.8 and 147.3 (s); 155.0 and 155.7 (s); 170.1 and 170.2 (s); 170.9 (s); 171.7 (s); **IR** (NaCl film, cm^{-1}) 3305.47, 3053.70, 2958.20, 2932.15, 1739.87, 1696.46, 1681.99, 1655.95, 1609.65; **HRMS** (ESI+) m/z Calcd for $C_{37}H_{40}N_5O_6$ [M+H] 650.2973, found 650.2960.

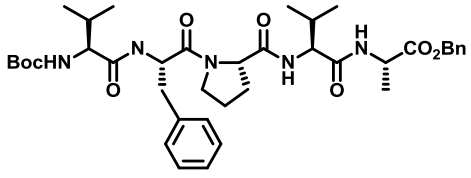
Tert-butyl ((2S,3aR,8aR)-3a-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-1H-indol-1-yl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl)-L-isoleucinate (**17a**)



Compound **2a** (100 mg, 0.145 mmol) and Pd Black (20 mg, 20% weight) were stirred in MeOH (10 mL). The mixture was purged with N_2, backfilled with H_2 and stirred during 2 h. The mixture was filtered over Celite and concentrated. The product (**17a**) was light green foam with mass 84 mg (quantitative yield).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** -58.8° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d,* 8:2 mixture of rotamers) δ 0.91-1.00 (m, 6H); 1.23-1.32 (m, 1H); 1.38-1.50 (m, 18H); 1.52-1.56 (m, 1H); 1.87-2.00 (m, 1H); 2.89 (dd, *J* = 12.3, 5.7 Hz, 1H); 3.09 (t, *J* = 11.2 Hz, 1H); 3.19 (bd, *J* = 4.9 Hz, 2H); 3.53 and 3.59 (s, 3H); 3.75 and 3.84 (dd, *J* = 11.0, 5.7 Hz, 1H); 4.49-4.60 (m, 2H); 5.03 (d, *J* = 7.9 Hz, 1H); 5.59 and 5.72 (s, 1H); 6.74 (d, *J* = 7.7 Hz, 1H); 6.87-6.92 (m, 2H); 7.04-7.15 (m, 2H); 7.20-7.25 (m, 2H); 7.47-7.70 (m, 2H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 12.0 (q), 15.7 (q), 25.4 (t), 28.0 (t), 28.2(q), 28.5 (q), 38.5 (d), 43.2 (t), 52.1 (d), 54.5 (d), 56.3 (d), 61.0 (d), 76.6 (s), 79.9 (s), 81.4 (d), 82.3 (s), 108.9 (s), 111.4 (d), 111.8 (d), 119.5 (d), 119.8 (d), 120.4 (d), 121.9 (d), 125.4 (d), 126.0 (d), 127.7 (s), 130.4 (s), 130.8 (d), 134.9 (s), 150.1 (s), 155.2 (s), 171.2 (s), 172.2 (s); **IR** (NaCl film, cm^{-1}) 3351.77, 3053.70, 2969.77, 2932.15, 2871.38, 1713.83, 1670.42, 1603.86; **HRMS** (ESI+) m/z Calcd for $C_{38}H_{52}N_5O_7$ [M+H] 690.3861, found 690.3867.

Benzyl (tert-butoxycarbonyl)-L-valyl-L-phenylalanyl-L-prolyl-L-valyl-L-alaninate (**21**)



On a 20 mL polypropylene syringe, 2-chlorotrityl chloride resin (0.7 g) was washed sequentially with DMF and DCM five times. The resin was swelled with DCM during 10 min. Thereafter, the solvent was filtrated and the resin was loaded with Fmoc-L-Val-OH (238 mg,

0.7 mmol) dissolved in DCM (3 ml aprox) and DIEA (0.6 mL, 3.5 mmol) and stirred during 1h. The mixture was filtrated and the mixture was stirred with MeOH (5 ml) during 10 minutes. The aminoacid was deprotected with 20% piperidine in DMF (three treatments, 3ml each; 1, 2 and 3 min respectively). The resin was washed successively with DMF and DCM, swelled with DCM during 1 minute and filtered.

Fmoc-L-Pro-OH and Fmoc-L-Phe-OH were successively coupled following this strategy: Fmoc-aa-OH (1 mmol), oxyma pure (142 mg, 1 mmol) and DIC (0.158 µl, 1 mmol) were dissolved in DMF (3 mL) and stirred during 5 min. The crude mixture was added to the resin and the syringe was stirred during 15 min. The solvent was purged and the coupling process was repeated for another 15 min. The results of the coupling were verified with ninhydrin (primary amines) and chloranyl (secondary amines) test, as appropriate. Fmoc deprotection was carried out with 20% piperidine solution in DMF (three treatments, 3ml each; 1, 2 and 3 minutes respectively). After deprotection process, the resin was washed with DMF and DCM, swelled with DCM during 1 minute and filtered. Boc-L-Val-OH was coupled utilizing the previous methodology, avoiding the final deprotection step.

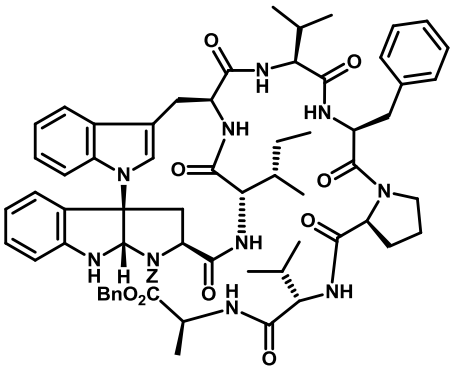
The resin was washed successively with DMF, 2-propanol and DCM (one syringe volume) five times.

The tetrapeptide was cleaved from the resin by treatment with 2% TFA in DCM (five syringe volumes) over 5% $NaHCO_3$ solution in water. The resin was washed with DCM (5 syringe volumes) the solvents were concentrated under reduced pressure and the remaining solvents were removed by lyophilization.

Boc-V-F-P-V-OH, H-L-Ala-OBn·HCl (216 mg, 1 mmol), EDC·HCl (192 mg, 1 mmol) and oxyma pure (142 mg, 1 mmol) were dissolved in DMF (5 ml) and cooled down to 0 °C. DIEA (3 mmol) was added and the mixture was stirred overnight. The peptide was precipitated with cold water and centrifuged. The precipitate was dissolved in AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated under reduced pressure. The product was a yellow foam with mass 191 mg (53% yield).

M.p. 96-98 °C; **$[\alpha]_D^{20}$** -57.8° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d*) 0.75 (t, *J* = 13.8 Hz, 3H); 0.84-0.96 (M, 9H); 1.35 and 1.41 (d, *J* = 7.2 Hz, 3H); 1.44 (s, 9H); 1.85-2.22 (m, 6H); 2.94 (dd, *J* = 13.8, 6.9 Hz, 1H); 3.05 (dd, *J* = 13.7, 6.7 Hz, 1H); 3.17-3.25 (m, 1H); 3.60-3.69 (m, 1H); 4.04 (t, *J* = 7.5 Hz, 1H); 4.26 (dd, *J* = 8.3, 6.4 Hz, 1H); 4.53-4.58 (m, 1H); 4.63 (t, *J* = 7.3 Hz, 1H); 4.95-5.03 (m, 1H); 5.13 (d, *J* = 12.3 Hz, 1H); 5.20 (d, *J* = 12.3 Hz, 1H); 6.61 (d, *J* = 7.5 Hz, 1H); 6.99 (d, *J* = 7.7 Hz, 1H); 7.17-7.25 (m, 5H); 7.30-7.38 (m, 5H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 17.4 (q), 18.2 (q), 18.4 (q), 19.4 (q), 19.4 (q), 25.2(t), 28.3 (t), 28.4 (q), 31.0 (d), 31.1 (d), 39.0 (t), 46.6 (t), 47.7 (d), 48.3 (d), 52.1(d), 58.9 (d), 60.3 (d), 67.3 (t), 127.1 (d), 128.3 (d), 128.6 (d), 128.7 (d), 128.7 (d), 128.8 (d), 129.1 (d), 129.6 (d), 135.4 (s), 136.1 (s), 155.9 (s), 170.7 (s), 171.4 (s), 172.6 (s); **IR** (NaCl film, cm^{-1}) 3302.57, 2963.99, 2932.15, 2871.38, 1739.87, 1719.61, 1655.95, 1629.90, 1537.30; **HRMS** (ESI+) m/z Calcd for $C_{39}H_{56}N_5O_8$ [M+H] 722.4123, found 722.4113.

Benzyl (12S,13aR,18aS,21S,4S,7S,Z)-4-(((S)-1-(((S)-1-((S)-2-(((S)-1-(((S)-1-(benzyloxy)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamoyl)pyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamoyl)-7-((S)-sec-butyl)-6,9-dioxo-11,12,13,13a,18,18a-hexahydro-21H-5,8-diaza-1(3a,2)-pyrrolo[2,3-b]indola-2(1,3)-indolacyclononaphane-11-carboxylate (**23**)



Compound **3a** (100 mg, 0.15 mmol) was dissolved in a mixture of THF:H_2O 2:1 (3 mL). LiOH·H_2O (63mg, 1.5 mmol) was added and the reaction mixture was stirred overnight. Aqueous 2M HCl was added dropwise until no precipitation was observed. The crude was extracted with EtOAc, the combined organic layers were washed with brine, dried over MgSO4, filtered and concentrated under reduced pressure. The afforded product was a brown foam with mass 98 mg (quant. yield).

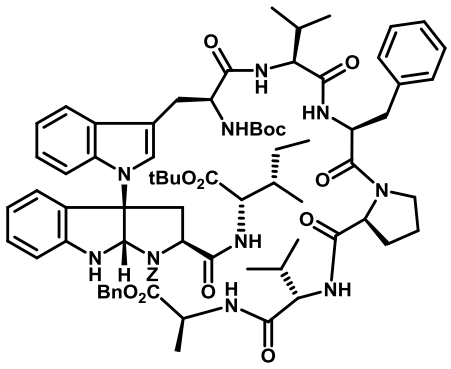
On the other hand, peptide **21** (0.22 mmol) was dissolved on an mixture of DCM:TFA 1:1 (5 mL) during 30 minutes. The mixture was concentrated under reduced pressure and the residue was coevaporated with toluene. The afforded product was a white foam.

The previously unprotected compounds, EDC·HCl (58 mg, 0.3 mmol) and oxyma pure (43 mg, 0,3 mmol) were dissolved in DMF (4 ml) and cooled down to 0 °C. DIEA (0,2 mL, 1.15 mmol) was added and the mixture was stirred overnight. The peptide was precipitated with cold water (80 mL) and centrifuged. The residue was dissolved with AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified by Combiflash ISCO reverse phase C18 (water:MeCN, 1:1 – 0:100), affording a brown foam (**23**) with mass 106 mg (57% yield) .

M.p. 208-210 °C; $[\alpha]_D^{20}$ -34.9° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (500 MHz, Methanol-d_4) δ 0.77 (t, *J* = 7.4 Hz, 3H), 0.85-0.91 (m, 9H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.98-1.02 (m, 1H), 1.39 (d, *J* = 7.3 Hz, 3H), 1.44-1.49 (m, 1H), 1.76-2.09 (m, 7H), 2.84–2.93 (m, 1H), 3.03–3.19 (m, 2H), 3.50–3.59 (m, 1H), 3.27-3.34 (m, 1H), 3.50–3.59 (m, 1H), 3.71–3.84 (m, 1H), 4.00 (d, *J* = 10.9 Hz, 1H), 4.05 (d, *J* = 11.0 Hz, 1H), 4.20 (d, *J* = 7.0 Hz, 1H), 4.30 – 4.36 (m, 1H), 4.45 (q, *J* = 7.3 Hz, 1H), 4.47-4.51 (m, 1H), 4.63 (t, *J* = 8.5 Hz, 1H), 4.97-5.23 (m, 2H), 6.30 and 6.33 (s, 1H), 6.65 and 6.69 (d, *J* = 8.4 Hz, 1H), 6.76 (d, *J* = 8.3 Hz, 1H), 6.84 and 6.94 (m, 2H), 6.98 (d, *J* = 7.7 Hz, 1H), 7.03-7.15 (m, 2H), 7.22-7.37 (m, 10H), 7.39 – 7.43 (m, 1H), 7.46 (dd, *J* = 7.9, 3.5 Hz, 1H); **^{13}C NMR** (126 MHz, Methanol-d_4) δ 11.1 (q), 15.9 (q), 17.3 (q), 18.8 (q), 18.8 (q), 19.7 (q), 19.8 (q), 26.0 and 26.2 (t), 30.4 and 30.8 (t), 32.4 (d), 32.7 (d), 35.9 (d), 38.3 (t), 48.9 (t), 49.7 (d), 54.1 (d), 59.8 (d), 59.8 (d), 60.0 (d), 61.1 (d), 61.4 (d), 67.9 and 68.4 (t), 75.8 (s), 77.1 (s), 84.2 and 85.1 (d), 110.1 (s), 111.0 (d), 112.9 (d), 122.6 (d), 123.0 (d), 123.4 (d), 127.9 (s), 128.5 (d), 128.9 (d), 129.0 (d), 129.3 (d), 129.5 (d), 129.6 (d), 130.4 (d), 131.5 (s), 136.7 (s), 137.2 (s),

137.5 (s), 138.6 (s), 149.3 (s), 157.2 (s), 172.1 (s), 172.7 (s), 173.2 (s), 173.3 (s), 173.8 (s), 174.0 (s); **IR** (NaCl film, cm^{-1}) 3319.94, 2972.67, 2932.15, 2871.38, 1716.72, 1696.46, 1606.75, 1511.25; **HRMS** (ESI+) m/z Calcd for $C_{70}H_{83}N_{10}O_{11}$ [M+H] 1239.6237, found 1239.6215.

Benzyl (2S,3aR,8aS)-3a-(3-((S)-3-(((S)-1-(((S)-1-((S)-2-(((S)-1-(((S)-1-(benzyloxy)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamoyl)pyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)-1H-indol-1-yl)-2-(((2S,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**24**)



Compound **2a** (174 mg, 0.21 mmol) was dissolved in a mixture of THF:H_2O 2:1 (3 mL). LiOH·H_2O (88 mg ,2.1 mmol) was added and the reaction mixture was stirred overnight. Aqueous 2M HCl was added dropwise until no precipitation was observed. The crude was extracted with EtOAc, the combined organic layers were washed with brine, dried over MgSO4, filtered and concentrated under reduced pressure. The afforded product was a brown foam with mass 170 mg (quant. yield).

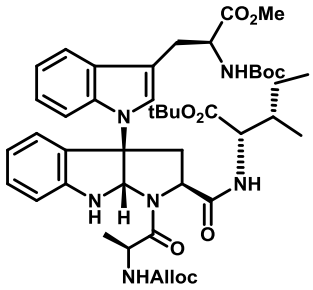
On the other hand, peptide **21** (0.21 mmol) was dissolved on an mixture of DCM:TFA 1:1 (5 mL) during 30 minutes. The mixture was concentrated under reduced pressure and the residue was coevaporated with toluene. The afforded product was a white foam.

The previously unprotected compounds and PyAOP (110 mg, 0.21 mmol) were dissolved in DMF (1.5 mL) and cooled down to 0 °C. DIEA (0.22 mL, 1.26 mmol) was added and the mixture was allowed to warm up to RT and stirred during 2h. The peptide was precipitated with cold water (40 mL) and centrifuged. The residue was dissolved with AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified by flash column chromatography (SiO_2, Hexane:AcOEt 3:7 to 1:9), affording a yellow foam with mass 211 mg (73% yield).

M.p. 142-144 °C; $[\alpha]_D^{20}$ -82.2° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d,* 6:4 mixture of rotamers) 0.60-0.98 (m, 18H); 1.0-1.10 (m, 1H), 1.19-1.47 (m, 23H); 1.62-2.16 (m, 7H); 1.80-2.18 (m, 6H); 2.82-3.67 (m, 6H), 4.17 (d, *J* = 8.8 Hz, 1H), 4.22 (dd, *J* = 8.3, 6.4 Hz, 1H), 4.29 (dd, *J* = 14.2, 6.2 Hz, 1H), 4.43 (dd, *J* = 8.2, 4.1 Hz, 1H), 4.85 (bs, 1H), 4.93 (q, *J* = 7.3 Hz, 1H), 5.01-5.38 (m, 4H), 5.63 (bs, 1H), 6.02 and 6.17 (s, 1H), 6.31 and 6.48 (d, *J* = 8.4 Hz, 1H), 6.50-6.77 (m, 3H), 6.88 (t, *J* = 7.5 Hz, 1H), 7.01-7.42 (m, 15H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.69 and 7.79 (d, *J* = 8.6 Hz, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 12.0 (q), 15.2 and 15.4 (q), 17.4 (q), 18.2 (q), 18.4 (q), 19.2 (q), 19.4 (q), 25.5 (t), 28.1 (t), 28.4 (q), 30.7 (d), 38.2 (d), 38.4 (t), 47.6 (t), 48.2 (d), 52.3

(d), 55.2 (d), 56.8 (d), 57.1 (d), 58.1 (d), 58.9 (d), 60.4 (d), 61.6 (d), 67.3 (t), 73.8 (s), 79.0 and 80.0 (d), 82.3 (s), 109.4 (s), 110.8 (d), 112.4 (d), 119.7 (d), 119.9 (d), 120.1 (d), 122.6 (d), 125.7 (d), 125.8 (d), 127.0 (d), 127.7 (s), 128.1 (d), 128.3 (d), 128.5 (d), 128.6 (d), 129.0 (d), 129.5 (d), 130.2 (s), 131.2 (d), 135.0 (s), 135.4 (s), 136.0 (s), 136.2 (s), 149.6 (s), 155.8 (s), 155.9 (s), 170.5 (s), 170.7 (s), 170.9 (s), 171.5 (s), 171.9 (s), 172.6 (s); **IR** (NaCl film, cm^{-1}) 3311.25, 3068.17, 2963.99, 2929.26, 2874.28, 1687.78, 1650.16, 1627.01, 1525.72, 1453.38; **HRMS** (ESI+) m/z Calcd for $C_{79}H_{101}N_{10}O_{14}$ [M+H] 1413.7493, found 1413.7481.

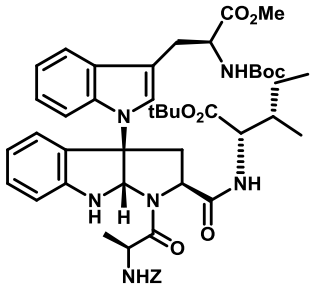
Tert-butyl ((2S,3aR,8aS)-1-(((allyloxy)carbonyl)-L-alanyl)-3a-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-1H-indol-1-yl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl)-L-isoleucinate (**26a**)



Compound **17a** (84 mg, 0.145 mmol), Alloc-L-Ala-OH·DCHA (250 mg, 0.72 mmol) and HATU (330 mg, 0.87 mmol) were dissolved in DMF (2 ml) and cooled down to 0 °C. DIEA (0.25 ml, 1.45 mmol) was added and the mixture was stirred during 1h at RT. Thereafter, the reaction was cooled down to 0 °C and Alloc-L-Ala-OH·DCHA (250 mg, 0.72 mmol), HATU (330 mg, 0.87 mmol) and DIEA (0.25 ml, 1.45 mmol) were added. The mixture was stirred during another hour. The crude was diluted with EtOAc and washed successively with 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified flash column chromatography (SiO2, Hexane:AcOEt 7:3 to 1:1), affording a white foam with mass 102 mg (83% yield).

M.p. 113-115 °C; **$[\alpha]_D^{20}$** -46.4° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*, 8:2 mixture of isomers) δ 0.90-0.96 (m, 6H), 1.16-1.29 (m, 1H), 1.30-1.49 (m, 22H), 1.86-1.97 (m, 1H), 2.88 (dd, *J* = 12.9, 6.9 Hz, 1H), 3.09-3.19 (m, 2H), 3.47 and 3.59 (s, 3H), 3.54 (dd, *J* = 13.0, 10.3 Hz, 1H), 4.09 and 4.18 (dd, *J* = 10.3, 6.8 Hz, 1H), 4.46 – 4.62 (m, 5H), 5.02 (d, *J* = 8.2 Hz, 1H), 5.20 and 5.23 (q, *J* = 1.3 Hz, 1H), 5.28 and 5.32 (q, *J* = 1.5 Hz, 1H), 5.43 and 5.47 (d, *J* = 8.6 Hz, 1H), 5.88 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H), 6.11 and 6.15 (s, 1H), 6.18 and 6.23 (d, *J* = 8.7 Hz, 1H), 6.69 (s, 1H), 6.74 (d, *J* = 7.9 Hz, 1H), 6.91 (t, *J* = 7.5 Hz, 1H), 7.09 and 7.14 (t, *J* = 7.5 Hz, 1H), 7.19-7.29 (m, 2H), 7.30 and 7.39 (d, *J* = 7.6 Hz, 1H), 7.51 and 7.54 (d, *J* = 7.7 Hz, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 12.0 (q), 15.3 (q), 18.6 (q), 25.5 (t), 27.9 (t), 28.3 and 28.5 (q), 38.4 (d), 39.0 (t), 48.7 (d), 52.0 (q), 54.6 (d), 57.1 (d), 61.6 (d), 66.3 (t), 75.0 (s), 79.4 and 79.8 (d), 82.3 (s), 109.1 (s), 111.1 (d), 112.2 (d), 118.2 (t), 119.6 (d), 119.8 (d), 120.0 (d) 122.6 (d), 125.4 (d), 125.5 (d), 125.7 (s), 127.1 (s), 131.4 (d), 132.4 (d), 134.8 (s), 149.7 (s), 156.8 (s), 169.4 (s), 171.0 (s), 173.8 (s); **IR** (NaCl film, cm^{-1}) 3351.39, 3303.51, 3046.67, 2977.03, 2929.14, 2876.90, 1697.22, 1605.80, 1510.04; **HRMS** (ESI+) m/z Calcd for $C_{45}H_{59}N_6O_{10}$ [M-H] 843.4298, found 843.4295.

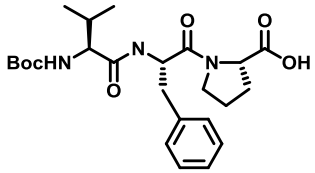
Tert-butyl ((2S,3aR,8aS)-1-(((benzyloxy)carbonyl)-L-alanyl)-3a-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-1H-indol-1-yl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl)-L-isoleucinate (**26b**)



Compound **17a** (84 mg, 0.145 mmol), Z-L-Ala-OH (162 mg, 0.72 mmol) and HATU (330 mg, 0.87 mmol) were dissolved in DMF (2 ml) and cooled down to 0 °C. DIEA (0.25 ml, 1.45 mmol) was added and the mixture was stirred during 1h at RT. Thereafter, the reaction was cooled down to 0 °C and Z-L-Ala-OH (162 mg, 0.72 mmol), HATU (330 mg, 0.87 mmol) and DIEA (0.25 ml, 1.45 mmol) were added. The mixture was stirred during another hour. The crude was diluted with EtOAc and washed successively with 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified flash column chromatography (SiO2, Hexane:AcOEt 7:3 to 1:1), affording a white foam with mass 113 mg (87% yield).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** -34.9° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*, 9:1 mixture of isomers) δ 0.89-0.94 (m, 6H), 1.15-1.27 (m, 1H), 1.31-1.45 (m, 22H), 1.86-1.97 (m, 1H), 2.87 (dd, *J* = 13.1, 6.8 Hz, 1H), 3.10-3.21 (m, 2H), 3.47 and 3.60 (s, 3H), 3.54 (dd, *J* = 13.0, 10.3 Hz, 1H), 4.20 (dd, *J* = 10.3, 6.8 Hz, 1H), 4.46 – 4.61 (m, 3H), 5.04 (d, *J* = 12.2 Hz, 1H), 5.13 (d, *J* = 12.2 Hz, 1H), 5.54 and 5.60 (d, *J* = 8.5 Hz, 1H), 6.11 and 6.16 (s, 1H), 6.28 and 6.33 (d, *J* = 8.5 Hz, 1H), 6.66-6.73 (m, 2H), 6.91 (t, *J* = 7.4 Hz, 1H), 7.09 and 7.14 (t, *J* = 7.5 Hz, 1H), 7.22 – 7.26 (m, 1H), 7.26 – 7.41 (m, 8H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 12.0 (q), 15.3 (q), 18.6 (q), 25.5 (t), 27.8 (t), 28.2 and 28.5 (q), 38.3 (d), 38.8 (t) 48.7 (d), 52.0 (q), 54.6 (d), 57.1 (d), 61.6 (d), 67.4 (t), 75.0 (s), 79.4 and 79.8 (d), 82.3 (s), 109.0 (s), 111.1 (d), 112.1 (d), 119.6 (d), 119.8 (d), 120.0 (d), 122.6 (d), 125.4 (d), 125.5 (d), 125.7 (s), 128.2 (d), 128.4 (d), 128.7 (d), 130.7 (s) 131.3 (d), 134.7(s), 136.0 (s), 149.7 (s), 155.2 (s), 156.8 (s), 169.4 (s), 171.0 (s), 172.4 (s), 173.8 (s); **IR** (NaCl film, cm^{-1}) 3319.94, 2972.67, 2932.15, 2871.38, 1716.72, 1696.46, 1606.75, 1511.25; **HRMS** (ESI+) m/z Calcd for $C_{49}H_{63}N_6O_{10}$ [M+H] 895.4600, found 895.4601.

(Tert-butoxycarbonyl)-L-valyl-L-phenylalanyl-L-proline (**28**)



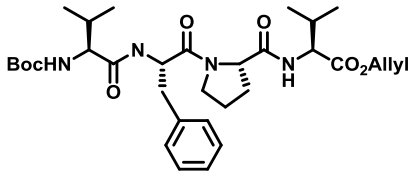
On a 60 mL polypropylene syringe, 2-chlorotrityl chloride resin (4.3 g) was washed sequentially with DMF and DCM five times. The resin was swelled with DCM during 10 min. Thereafter, the solvent was filtrated and the resin was loaded with Fmoc-L-Pro-OH·H_2O (507 mg, 4.3 mmol) dissolved in DCM (10 ml aprox) and DIEA (3.7 ml, 21 mmol) and stirred during 1h. The mixture was filtrated and the mixture was stirred with MeOH (20 ml) during 10 minutes. The aminoacid was deprotected with 20% piperidine in DMF (three treatments, 10ml each; 1, 2 and 3 min respectively). The resin was washed successively with DMF and DCM, swelled with DCM during 1 minute and filtered.

Fmoc-L-Phe-OH (6 mmol), oxyma pure (852 mg, 6 mmol) and DIC (0.95 ml, 6 mmol) were dissolved in DMF (10 mL) and stirred during 5 min. The crude mixture was added to the resin and the syringe was stirred during 15 min. The solvent was purged and the coupling process was repeated for another 15 min. The results of the coupling were verified with chloranyl test. Fmoc deprotection was carried out with 20% piperidine solution in DMF (three treatments, 10ml each; 1, 2 and 3 minutes respectively). After deprotection process, the resin was washed with DMF and DCM, swelled with DCM during 1 minute and filtered. Boc-L-Val-OH was coupled utilizing the previous methodology, avoiding the final deprotection step. The results of the coupling were verified with ninhydrin test.

The resin was washed successively with DMF, 2-propanol and DCM (one syringe volume) five times.

The tripeptide was cleaved from the resin by treatment with 2% TFA in DCM (five syringe volumes) over 5% $NaHCO_3$ solution in water. The resin was washed with DCM (5 syringe volumes) the solvents were concentrated under reduced pressure and the remaining solvents were removed by lyophilization.

Allyl (tert-butoxycarbonyl)-L-valyl-L-phenylalanyl-L-prolyl-L-valinate (**29a**)

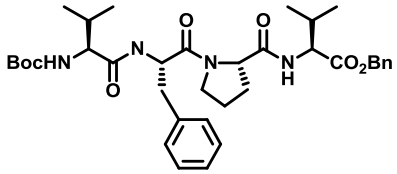


Boc-V-F-P-OH (460 mg, 1mmol), H-L-Val-OAllyl·*p*-TS (659 mg, 2 mmol), EDC·HCl (384 mg, 2 mmol) and oxyma pure (284 mg, 2 mmol) were dissolved in DMF (5 ml) and cooled down to 0 °C. DIEA (0.52 ml, 3 mmol) was added and the mixture was stirred overnight. The peptide was precipitated with cold water and centrifuged. The precipitate was dissolved in AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated under reduced pressure. The product was a yellow foam with mass 336 mg (57% overall yield).

M.p. 100-102 °C; $[\alpha]_D^{20}$ -54.6° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.77 (d, *J* = 6.7 Hz, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.92 (dd, *J* = 6.9, 2.3 Hz, 3H), 0.96 (d, *J* = 6.9 Hz, 3H), 1.44 (s,

9H), 1.86-1.97 (m, 2H), 1.99-2.09 (m, 2H), 2.13-2.24 (m, 2H), 2.94 (dd, *J* = 13.7, 7.0 Hz, 1H), 3.05 (dd, *J* = 13.8, 6.7 Hz, 1H), 3.21 (bs, 1H), 3.63 (d, *J* = 8.2 Hz, 1H), 4.05 (bs, 1H), 4.50 (dd, *J* = 8.4, 4.9 Hz, 1H), 4.53 – 4.58 (m, 1H), 4.60-4.70 (m, 1H), 4.96-5.07 (m, 1H), 5.26 (dq, *J* = 10.4, 1.3 Hz, 1H), 5.35 (dq, *J* = 17.1, 1.5 Hz, 1H), 5.91 (ddt, *J* = 17.2, 10.4, 5.9 Hz, 1H), 7.14 – 7.25 (m, 5H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 17.4 (q), 18.0 (q), 19.2 (q), 19.4 (q), 25.1 (t), 28.0 (t), 28.4 (q), 31.1 (d), 31.3 (d), 39.0 (t), 47.7 (t), 52.1 (d), 57.7 (d), 59.7 (d), 60.2 (d), 66.0 (t), 80.2 (s), 119.1 (t), 127.1 (d), 128.7 (d), 129.6 (d), 131.8 (d), 136.0 (s), 156.0 (s), 171.1 (s), 171.6 (s); **IR** (NaCl film, cm^{-1}) 3311.25, 2961.09, 2935.05, 2877.17, 1777.49, 1742.77, 1684.69, 1629.90, 1522.93, 1447.59; **HRMS** (ESI+) m/z Calcd for $C_{32}H_{49}N_4O_7$ [M+H] 601.3596, found 601.3608.

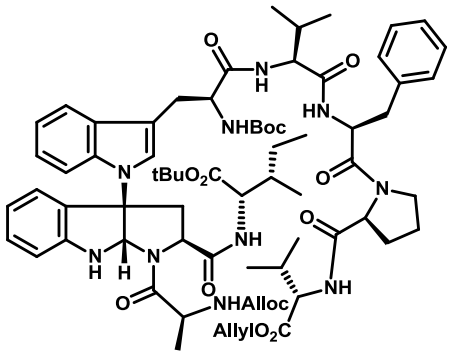
Benzyl (tert-butoxycarbonyl)-L-valyl-L-phenylalanyl-L-prolyl-L-valinate (**29b**)



Boc-V-F-P-OH (460 mg, 1mmol), H-L-Val-OBn·HCl (487.86 mg, 2 mmol), EDC·HCl (384 mg, 2 mmol) and oxyma pure (284 mg, 2 mmol) were dissolved in DMF (5 ml) and cooled down to 0 °C. DIEA (0.52 ml, 3 mmol) was added and the mixture was stirred overnight. The peptide was precipitated with cold water and centrifuged. The precipitate was dissolved in AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated under reduced pressure. The product was a yellow foam with mass 345 mg (54% overall yield).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** -57.8° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.77 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 7.1 Hz, 6H), 0.93 (d, *J* = 6.9 Hz, 3H), 1.45 (s, 9H), 1.78 – 1.93 (m, 2H), 1.94 – 2.10 (m, 2H), 2.11 – 2.24 (m, 2H), 2.93 (dd, *J* = 13.7, 6.8 Hz, 1H), 3.03 (dd, *J* = 13.7, 6.8 Hz, 1H), 3.12-3.21 (m, 1H), 3.59 (q, *J* = 7.9 Hz, 1H), 4.03-4.10 (m, 1H), 4.50-4.57 (m, 2H), 4.95-5.04 (m, 1H), 5.12 (d, *J* = 12.2 Hz, 1H), 5.23 (d, *J* = 12.2 Hz, 1H), 6.95 (d, *J* = 8.3 Hz, 1H), 7.38 – 7.15 (m, 10H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 17.4 (q), 18.0 (q), 19.3 (q), 19.4 (q), 25.1 (t), 27.9 (t), 28.5 (q), 31.2 (d), 31.3 (d), 39.2 (t), 47.6 (t), 52.0 (d), 57.6 (d), 59.6 (d), 60.0 (d), 67.1 (t), 80.0 (s), 127.1 (d), 127.7 (d), 128.5 (d), 128.6 (d), 128.7 (d), 129.0 (d), 129.5 (d), 129.6 (d), 135.6 (s), 136.1 (s), 155.9 (s), 171.0 (s), 171.1 (s), 171.2 (s), 171.8 (s); **IR** (NaCl film, cm^{-1}) 3302.57, 2963.99, 2932.15, 2871.38, 1739.87, 1719.61, 1655.95, 1629.90, 1537.30; **HRMS** (ESI+) m/z Calcd for $C_{36}H_{51}N_4O_7$ [M+H] 651.3752, found 651.3747.

Tert-butyl ((2S,3aR,8aS)-3a-(3-((S)-3-(((S)-1-(((S)-1-((S)-2-(((S)-1-(allyloxy)-3-methyl-1-oxobutan-2-yl)carbamoyl)pyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)-1H-indol-1-yl)-1-(((allyloxy)carbonyl)-L-alanyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl)-L-isoleucinate (**31a**)



Compound **26a** (150 mg, 0.18 mmol) was dissolved in a mixture of THF:H_2O 2:1 (3 mL). LiOH·H_2O (76mg, 1.8 mmol) was added and the reaction mixture was stirred overnight. Aqueous 2M HCl was added dropwise until no precipitation was observed. The crude was extracted with EtOAc, the combined organic layers were washed with brine, dried over MgSO4, filtered and concentrated under reduced pressure. The afforded product was a brown foam with mass 147 mg (quant. yield).

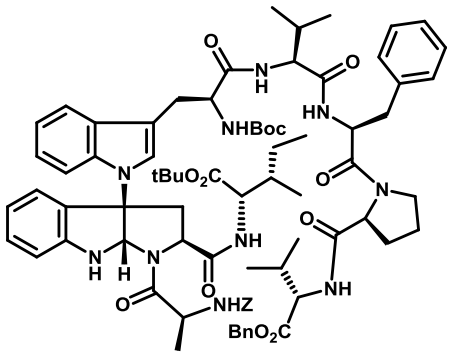
On the other hand, peptide **30a** (108 mg, 0.18 mmol) was dissolved on an mixture of DCM:TFA 1:1 (5 mL) during 30 minutes. The mixture was concentrated under reduced pressure and the residue was coevaporated with toluene. The afforded product was a white foam.

The previously unprotected compounds and PyAOP (94 mg, 0.18 mmol) were dissolved in DMF (2 ml) and cooled down to 0 °C. DIEA (0,2 mL, 1.15 mmol) was added and the mixture was stirred overnight. The peptide was precipitated with cold water (40 mL) and centrifuged. The residue was dissolved with AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified by flash column chromatography (SiO^2, Hexane:AcOEt 3:7 to 1:9), affording a light yellow foam with mass 177 mg (75% yield).

M.p. 158-160 °C; **$[\alpha]_D^{20}$** -57.8° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.59 (d, *J* = 6.8 Hz, 3H), 0.67 (d, *J* = 6.7 Hz, 3H), 0.88-0.93 (m, 9H), 0.95 (d, *J* = 6.9 Hz, 3H), 1.22-1.29 (m, 2H), 1.37 (s, 9H), 1.42 (s, 9H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.81-1.93 (m, 4H), 1.94-2.05 (m, 1H), 2.07-2.25 (m, 2H), 2.86 (ddd, *J* = 19.5, 13.3, 6.6 Hz, 2H), 3.00 (dd, *J* = 13.8, 7.5 Hz, 1H), 3.03-3.11 (m, 1H), 3.11-3.21 (m, 2H), 3.51 (dd, *J* = 12.9, 10.3 Hz, 1H), 3.60 (q, *J* = 8.3 Hz, 1H), 4.26-4.37 (m, 3H), 4.45 (dd, *J* = 8.3, 5.0 Hz, 1H), 4.48-4.60 (m, 6H), 4.63 (ddt, *J* = 10.0, 5.8, 1.4 Hz, 2H), 4.93 (q, *J* = 7.3 Hz, 1H), 4.99 (d, *J* = 7.5 Hz, 1H), 5.18-5.38 (m, 4H), 5.89 (dddt, *J* = 17.5, 13.6, 10.4, 5.8 Hz, 2H), 6.15 (s, 1H), 6.55-6.63 (m, 1H), 6.70 (d, *J* = 7.9 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 6.88 (d, *J* = 7.5 Hz, 1H), 7.08-7.30 (m, 13H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.65 (d, *J* = 7.9 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 12.1 (q), 15.3 (q), 17.5 (q), 18.1 (q), 18.7 (q), 19.3 (q), 25.1 and 25.6 (t), 27.9 (t), 28.2 (q), 28.4 (q), 29.8 (t), 31.2 (d), 38.4 (d), 39.0

(t), 39.2 (t), 47.6 (t), 48.7 (d), 52.2 (d), 55.1 (d), 57.2 (d), 57.8 (d), 58.0 (d), 60.1 (d), 61.6 (d), 65.8 (t), 66.3 (t), 75.1 (s), 79.6 (d), 82.3 (s), 109.5 (s), 111.2 (d), 112.3 (d), 118.3 (t), 118.9 (t), 119.7 (d), 119.9 (d), 120.2 (d), 122.7 (d), 125.6 (d), 125.7 (d), 127.1 (d), 128.6 (d), 129.5 (d), 130.3 (s), 131.3 (d), 131.9 (d), 132.5 (d), 135.0 (s), 136.2 (s), 149.7 (s), 156.9 (s), 169.6 (s), 170.6 (s), 171.0 (s), 171.6 (s), 172.0 (s), 174.0 (s); **IR** (NaCl film, cm^{-1}) 3302.57, 2963.99, 2932.15, 2871.38, 1739.87, 1719.61, 1655.95, 1629.90, 1537.30; **HRMS** (ESI+) m/z Calcd for $C_{71}H_{97}N_{10}O_{14}$ [M+H] 1313.718, found 1313.717.

Tert-butyl ((2S,3aR,8aS)-3a-(3-((S)-3-(((S)-1-(((S)-1-((S)-2-(((S)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)carbamoyl)pyrrolidin-1-yl)-1-oxo-3-phenylpropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)amino)-2-((tert-butoxycarbonyl)amino)-3-oxopropyl)-1H-indol-1-yl)-1-(((benzyloxy)carbonyl)-L-alanyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl)-L-isoleucinate (**31b**)



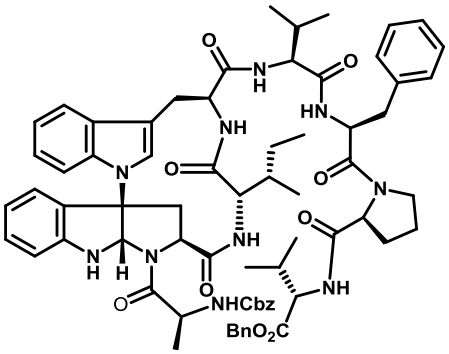
Compound **26b** (150 mg, 0.11 mmol) was dissolved in a mixture of THF:H_2O 2:1 (3 mL). LiOH·H_2O (76mg, 1.8 mmol) was added and the reaction mixture was stirred overnight. Aqueous 2M HCl was added dropwise until no precipitation was observed. The crude was extracted with EtOAc, the combined organic layers were washed with brine, dried over MgSO4, filtered and concentrated under reduced pressure. The afforded product was a brown foam with mass 147 mg (quant. yield).

On the other hand, peptide **30b** (117 mg, 0.18 mmol) was dissolved on an mixture of DCM:TFA 1:1 (5 mL) during 30 minutes. The mixture was concentrated under reduced pressure and the residue was coevaporated with toluene. The afforded product was a white foam.

The previously unprotected compounds and PyAOP (94 mg, 0.18 mmol) were dissolved in DMF (2 ml) and cooled down to 0 °C. DIEA (0,2 mL, 1.15 mmol) was added and the mixture was stirred overnight. The peptide was precipitated with cold water (40 mL) and centrifuged. The residue was dissolved with AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified by flash column chromatography (SiO_2, Hexane:AcOEt 3:7 to 1:9), affording a light yellow foam with mass 198 mg (78% yield).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** -70.9° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.60 (d, *J* = 6.6 Hz, 3H), 0.67 (d, *J* = 6.6 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 7.1 Hz, 9H), 1.14-1.24 (m, 1H), 1.37 (s, 9H), 1.43 (s, 9H), 1.44-1.46 (m, 1H), 1.47 (d, *J* = 7.0 Hz, 4H), 1.71-2.01 (m, 6H), 2.08–2.23 (m, 2H), 2.80–2.93 (m, 2H), 2.95-3.07 (m, 1H), 3.09-3.19 (m, 1H), 3.45 – 3.63 (m, 2H), 4.25-4.35 (m, 3H), 4.44-4.58 (m, 3H), 4.92 (q, *J* = 7.1 Hz, 1H), 5.03-5.25 (2d, *J* = 12.3 Hz, 2H), 5.87 (bs), 6.16 (s, 1H), 6.67 (d, *J* = 7.8 Hz, 1H), 6.81 (s, 1H), 6.88 (t, *J* = 7.4 Hz, 1H), 7.08-7.38 (m, 19H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 12.0 (q), 15.3 (q), 18.0 (q), 18.7 (q), 19.3 (q), 25.1 (t), 25.5 (t), 27.8 (t), 28.2 (q), 28.4 (q), 31.2 (d), 38.4 (d), 39.0 (t), 47.6 (t), 48.8 (d), 52.2 (d), 55.3 (d), 57.2 (d), 57.8 (d), 58.1 (d), 60.2 (d), 61.6 (d), 67.0 (d), 67.5 (d), 75.1 (s), 79.5 (d), 82.3 (s), 109.5 (s), 112.3 (d),119.7 (d), 120.0 (d), 120.2 (d), 122.7 (d), 125.6 (d), 125.7 (d), 127.1 (d), 128.3 (s), 128.4 (d), 128.5 (d), 128.6 (d), 128.7 (d), 128.7 (d), 129.5 (d), 131.4 (d), 135.0 (s), 135.6 (s), 136.0 (s), 136.1 (s), 149.7 (s), 157.0 (s), 171.0 (s), 171.7 (s), 173.9 (s); **IR** (NaCl film, cm^{-1}) 3288.10, 3062.38, 2929.26, 2871.38, 1728.30, 1690.68, 1641.48, 1531.51, 1453.38; **HRMS** (ESI+) m/z Calcd for $C_{79}H_{101}N_{10}O_{14}$ [M+H] 1413.7493, found 1413.7481.

Benzyl ((12S,13aR,18aS,21S,4S,7S,Z)-11-(((benzyloxy)carbonyl)-L-alanyl)-7-((S)-sec-butyl)-6,9-dioxo-11,12,13,13a,18,18a-hexahydro-21H-5,8-diaza-1(3a,2)-pyrrolo[2,3-b]indola-2(1,3)-indolacyclononaphane-4-carbonyl)-L-valyl-L-phenylalanyl-L-prolyl-L-valinate (**34**)

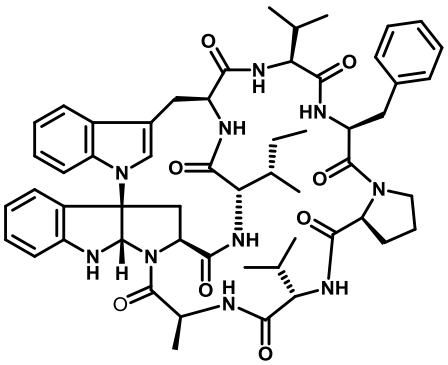


Compound **31b** (1.47 g, 1.04 mmol mmol) was dissolved in a mixture (10 mL) containing a TFA:DCM mixture during 3h at RT. The solvents were concentrated under reduced pressure, the mixture was coevaporated with toluene, the mixture was redissolved in the minimum amount of DMF and DCM (1l, 1 mM) was added. The mixture was cooled down to 0 °C and PyAOP (1.1 g, 2.08 mmol) and DIEA (0.9 ml, 5 mmol) was added dropwise. The mixture was stirred overnight. The solvents were concentrated under reduced pressure and the crude mixture was washed successively with 1M HCl, $NaHCO_3$ and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified by Combiflash ISCO reverse phase C18 (water:MeCN, 1:1 – 0:100), affording a brown solid with mass 490 mg (38% yield).

M.p. 208-210 °C; **$[\alpha]_D^{20}$** -34.9° (*c* 1.0, DMSO); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.77 (t, *J* = 7.4 Hz, 3H), 0.85-0.91 (m, 9H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.98-1.02 (m, 1H), 1.39

(d, *J* = 7.3 Hz, 3H), 1.44-1.49 (m, 1H), 1.76-2.09 (m, 7H), 2.84–2.93 (m, 1H), 3.03–3.19 (m, 2H), 3.50–3.59 (m, 1H), 3.27-3.34 (m, 1H), 3.50–3.59 (m, 1H), 3.71–3.84 (m, 1H), 4.00 (d, *J* = 10.9 Hz, 1H), 4.05 (d, *J* = 11.0 Hz, 1H), 4.20 (d, *J* = 7.0 Hz, 1H), 4.30 – 4.36 (m, 1H), 4.45 (q, *J* = 7.3 Hz, 1H), 4.47-4.51 (m, 1H), 4.63 (t, *J* = 8.5 Hz, 1H), 4.97-5.23 (m, 2H), 6.30 and 6.33 (s, 1H), 6.65 and 6.69 (d, *J* = 8.4 Hz, 1H), 6.76 (d, *J* = 8.3 Hz, 1H), 6.84 and 6.94 (m, 2H), 6.98 (d, *J* = 7.7 Hz, 1H), 7.03-7.15 (m, 2H), 7.22-7.37 (m, 10H), 7.39 – 7.43 (m, 1H), 7.46 (dd, *J* = 7.9, 3.5 Hz, 1H); **^{13}C NMR** (126 MHz, DMSOl-d_6) δ 11.1 (q), 15.9 (q), 17.3 (q), 18.8 (q), 18.8 (q), 19.7 (q), 19.8 (q), 26.0 and 26.2 (t), 30.4 and 30.8 (t), 32.4 (d), 32.7 (d), 35.9 (d), 38.3 (t), 48.9 (t), 49.7 (d), 54.1 (d), 59.8 (d), 59.8 (d), 60.0 (d), 61.1 (d), 61.4 (d), 67.9 and 68.4 (t), 75.8 (s), 77.1 (s), 84.2 and 85.1 (d), 110.1 (s), 111.0 (d), 112.9 (d), 122.6 (d), 123.0 (d), 123.4 (d), 127.9 (s), 128.5 (d), 128.9 (d), 129.0 (d), 129.3 (d), 129.5 (d), 129.6 (d), 130.4 (d), 131.5 (s), 136.7 (s), 137.2 (s), 137.5 (s), 138.6 (s), 149.3 (s), 157.2 (s), 172.1 (s), 172.7 (s), 173.2 (s), 173.3 (s), 173.8 (s), 174.0 (s); **IR** (NaCl film, cm^{-1}) 3319.94, 2972.67, 2932.15, 2871.38, 1716.72, 1696.46, 1606.75, 1511.25; **HRMS** (ESI+) m/z Calcd for $C_{70}H_{83}N_{10}O_{11}$ [M+H] 1239.6237, found 1239.6215.

***Exo*-L mayotlide** (**1a**)

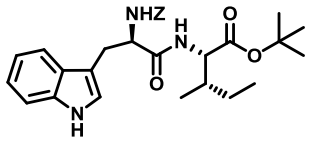


34 (0.44 g, 0.35 mmol) was dissolved on a mixture of DCM-*i*-propanol 2:1 (12 mL). Pd Black (88 mg, 20% weight) was added, the mixture was purged with N_2, backfilled with H_2 and stirred during 2 h. The mixture was filtered over Celite, washed with *i*-propanol and concentrated. The mixture was diluted with DMF (350 ml, 0.1 M) and DMSO (2%, 7ml). The mixture was cooled down to 0 °C and PyAOP (365 mg, 0.70 mmol) and DIEA (0.36 ml, 2.1 mmol) were added successively. The mixture was stirred overnight.

The solvents were concentrated and the crude product was washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified by Combiflash ISCO reverse phase C18 (water:MeCN, 7:3 – 0:100), affording a white solid with mass 12 mg (3% yield) .

					Exo -L Mayotlide	
aa	ssignmen	δ C	δ H	J	HMBC	NOE
Ala	Cα	47,6	4,36	4.37 (q, *J* = 7.4 Hz, 1H)	17,6; 175,6	1,33 (CβALA)
	Cβ	17,6	1,33	1.33 (d, *J* = 7.4 Hz, 1H)	47,6; 175,6	4,36(Cαala); 4,89(CαHPI)
	NH		7,52	47,6;	17,6; 47,6; 170,5	0,81;0,95(Cγ VAL1); 1,33 (CβALA); 2,17 (CβVAL1); 4,21(CαVAL1)
	CO	175,6				
Val 2	Cα	58,3	4,28	4.28 (d, *J* = 10.3 Hz, 1H)	17,2; 20,3; 28,1; 171,5; 174	0,17 (CγVal2); 1,83(CβVal2)
	Cβ	28,1	1,83	(m, 1H)	17,2; 20,3; 28,1; 58,3; 171,5	-0,37(CγVal2); 1,83(CβVal2); 3,18(Cβ trp) 4,28 (CαVal2)
	Cγ	20,3	0,17	0.17 (d, *J* = 7.4 Hz, 3H)	17,2; 28,1; 58,3	-0,37(CγVal2); 1,83(CβVal2); 4,28 (CαVal2); 4,89(CαHPI)
	Cγ	17,2	-0,37	(d, *J* = 7.3 Hz, 3H)	20,3; 28,1; 58,3	0,17 (CγVal2); 1,83(CβVal2); 2,17 (CβVAL1)4,28 (CαVal2)
	NH		7,53	m, 1H	58,3;	
	CO	171,5				
Val 1	Cα	59,8	4,21	4.21 (d, *J* = 7.1 Hz, 1H)	18,9; 20,3; 29,7; 170,4; 174	0,81-0,95 (Cγ VAL1); 2,17 (CβVAL1)
	Cβ	29,7	2,17	2.20 – 2.14 (m, 1H)	18,9; 20,3; 59,8; 170,4	0,81-0,95 (Cγ VAL1); 4,21 (CαVAL1);
	Cγ	20,3	0,95	0.95 (d, *J* = 7.1 Hz, 3H)	18,9; 29,7; 59,8	2,17 (CβVal1); 4,21 (CαVal1)
	Cγ	18,9	0,81	0.81 (d, *J* = 7.0 Hz, 3H)	18,9; 29,7; 59,8	-0,37(CγVal2); 2,17 (CβVal1); 4,21 (CαVal1)
	NH		8,27	8.27 (Bd, *J* = 7,1 Hz, 1H)	125,2; 134,5; 173,9	0,81-0,95 (Cγ VAL1); 2,17 (CβVAL1)2,9(αpro), 3,36 (δ pro, + PEQUEÑO); 4,21 (CαVAL1); 4,44
	CO	170,4				
Ile	Cα	64,4	4,35	4,34-4,36(m, 1H)	15,5; 26,6; 37,5; 170,4	1,05 (CδILE); 1,33 (CβALA); 1,58-1,78 (CγCH2 ILE); 2,22 (CβILE); 4,89 (Cα HPI)
	Cβ	37,5	2,22	2.21 (m, 1H)	12,4; 15,5; 64,4	1,24 (Cδ ILE); 4,28 (Cα val2)
	Cγ CH2 1	26,6	1,78	1.80 – 1.75 (m, 1H)	12,4; 15,5; 37,5; 64,4	3,18(Cβ1trp)?
	Cγ CH2 2	26,6	1,58	1.55-1,61 (m, 1H)	12,4; 15,5; 37,5; 64,4	?, señal azul
	Cγ CH3	15,5	1,24	1.24 (d, *J* = 7.2 Hz, 3H)	26,6 ;37,5 ;64,4;	2,22(Cβile); 4,35 (Cαile)
	Cδ	12,4	1,05	1.05 (t, *J* = 7.6 Hz, 3H)	26,6; 37,5	1,78 (Cγ CH2 Ile);
	NH		8,59	8.59 (d, *J* = 2.3 Hz, 0H)	37,5; 64,4; 173,7	1,24 (Cδ ILE); 1,58-1,78 (CγCH2 ILE); 2,22 (CβILE) 4,04(CH2HPI); 4,35 (Cα ILE); 4,89 (Cα HPI)
	CO	173,6			173,20	
Pro	Cα	61,5	2,92	2.92 (t, *J* = 8.5 Hz, 1H)	22,6; 31,9; 47,7; 173,6	1,09(Cβpro)?
	Cβ	31,9	1,87	1.87 (m, 1H)	22,6; 47,7; 61,5; 173,6	1,09(Cβpro); 2,83 (CβPHE)
	Cβ	31,9	1,09	1.08 – 1.15 (m, 1H)	22,6; 31,9; 47,7; 61,5; 173,6	1,87(Cβpro)
	Cγ	22,6	1,67	1.62 – 1.71 (m, 1H)	22,60	3,36(CδPRO)
	Cγ	22,6	1,4	1.41 (m, 1H)		3,36(CδPRO)
	Cδ	47,7	3,36	3.32 – 3.38 (m, 2H)	22,6; 31,9; 61,5; 173,6	1,09(CβPRO); 1,4 + 1,6(CγPRO);
	CO	173,6				
Phe	Cα	56,25	4,44	4,41-4,46(m, 1H)	38,43; 173	COINCIDEN DOS ALFAS
	Cβ1	38,43	3,57	3.59 – 3.54 (m, 1H)	56,25; 130; 137; 173	2,83(Cβ phe)
	Cβ1	38,43	2,83	2.83 (t, *J* = 12.8 Hz, 1H)	56,25; 130; 137; 173	3,57(Cβ phe)
	NH		7,68			
	CO	173,1				
TRP	Cα	54,9	4,44	4,41-4,46(m, 1H)	25,2; 109,3	COINCIDEN DOS ALFAS
	Cβ1	25,2	3,59	3.62 (dd, *J* = 14.2, 2.9 Hz, 1H)	54,9; 109,3; 124,3; 130,8	3,18(Cβ1trp)
	Cβ1	25,2	3,18	3.18 (dd, *J* = 15.8, 6 Hz, 1H)	54,9; 109,3; 124,3; 130,8;	3,59(Cβ1trp)
	C2	124,3	7,95	7.95 (s, 1H)	25,2; 74,4; 109,3; 112,8; 120,5; 130,8; 136,6	1,24 (CγVAL1)2,94 (AZUL); 3,18 (Cβ TRP); 4,04 (CH2HPI); 4,89(Cα HPI)
	C3	109,3				
	C3A	130,8				
	C4	120,9	6,98	6.98 (t, *J* = 8.1 Hz, 1H)	112,8; 130,8	-0,37; 0,16 (ch3 val2),
	C5	112,8	6,75	6.75 (d, *J* = 8.4 Hz, 1H)	120,9; 130,8	-0,37(CγVal2)
	C6	123,1	6,92	6.93 (t, *J* = 8.4 Hz, 3H)	120,5; 136,6	DOS PROTONES JUNTOS
	C7	120,5	7,46	7.46 (d, *J* = 8.3 Hz, 1H)	109,3; 122,8; 130,7; 136,6	Γvaline2; 3,59(ch2trp);
	C7A	136,5				
	NH		7,69	7.69 (d, *J* = 6.3 Hz, 1H)	25,2; 54,9; 173,0	1,22(CγILE)/3.18(CH2 HPI)/4,36(αILE); 7,95(C2TRP)
	CO	172,9				
HPI	Cα	60,7	4,89		43,3; 74,4; 85,1	1,24(CγILE); 7,69 (NHTRP) 7,95(C2TRP); 8,59(NHILE)
	Cβ1	43,3	4,04	4.04 (d, *J* = 12,0 Hz, 1H)	74,4; 85,1; 174	2,94(CβHPI)
	Cβ1	43,3	2,94	2.94 (dd, *J* = 12.0, 9.1 Hz, 1H)	60,7; 74,4; 130,4	4,04(CβHPI)
	C3A	74,4				
	C3B					
	C4	130,4	7,17	7,17-7,20(m, 1H)	110,9; 123,2; 148,8	SEVERAL SIGNALS OVERLAPPED
	C5	119,8	6,69	6.68 (t, *J* = 7.6 Hz, 1H)	74,4; 110,92; 123,2; 130,1; 148,8	NO NOE
	C6	123,2	6,93	6.94 (d, *J* = 8.5 Hz, 1H)	74,4; 110,9; 130,4; 148,9	TWO PROTONS TOGETHER
	C7	110,9	6,8	6.80 (d, *J* = 8.3 Hz, 1H)	74,4; 119,8; 130,4	6,38(C8AHPI)
	C7A	148,9				
	C8A	85,1	6,38	6.38 (s, H)	43,3; 60,7; 74,4; 148,8; 175,9	6,8(C7HPI)
	NH8		6,58	6.58 (bs, 1H)	74,4; 130,4	
	CO	175,9				(CγILE)

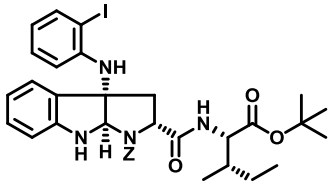
Tert-butyl ((benzyloxy)carbonyl)-D-tryptophyl-L-isoleucinate (**11b**)



Z-D-Trp-OH (**10b**) (5g, 14.78 mmol), H-L-Ile-*t*-Bu·HCl (3.64 g, 16.26 mmol), EDC·HCl (3.4 g, 17.74 mmol) and oxyma pure (2.95 g, 20.69 mmol) were dissolved in DMF (30 ml) and stirred at 0 °C. DIEA (7.7 ml, 44.33 mmol) was added dropwise and the mixture was let to warm up to RT over 3h. The crude mixture was diluted with AcOEt and washed successively with 1M HCl, $NaHCO_3$ and brine, dried over $MgSO_4$, filtered and concentrated. The afforded product (**11b**) was a white foam with mass 7.4 g (99% yield).

M.p. 100-102 °C; $[\alpha]_D^{20}$ +19.4° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.62 (d, *J* = 6.87 Hz, 3H), 0.81 (t, *J* = 7.0 Hz, 3H), 0.83 – 0.94 (m, 1H), 1.18 – 1.29 (m, 1H), 1.40 (s, 9H), 1.53 – 1.67 (m, 1H) 3.21 (dd, *J* = 14.42, 7.66 Hz, 1H), 3.29 (bs, 1H), 4.37 (dd, *J* = 8.44, 4.40 Hz, 1H), 4.57 (bs, 1H), 5.07 (d, *J* = 12.3 Hz, 1H), 5.11 (d, *J* = 12.3 Hz, 1H), 5.46 (bs, 1H), 6.24 (d, *J* = 8.38 Hz, 1H), 7.02 (d, *J* = 2.32 Hz, 1H), 7.11 (t, *J* = 7.49 Hz, 1H), 7.18 (ddd, *J* = 8.16, 7.02, 1.20 Hz, 1H), 7.27 – 7.39 (m, 6H), 7.65 (d, *J* = 5.9 Hz, 1H), 8.19 (s, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 11.8 (q), 15.0 (q), 25.2 (t), 28.1 (q), 28.7 (t), 38.1 (d), 55.8 (d), 56.8 (d), 67.1 (t), 82.1 (s), 110.5 (s), 111.4 (d), 118.9 (d), 119.9 (d), 122.4 (d), 123.1 (d), 127.4 (q), 128.2 (d), 128.2 (d), 128.6 (d), 136.4 (s), 156.1 (s), 170.5 (s), 170.9 (s); **IR** (NaCl film, cm^{-1}) 3406.75, 3322.83, 3062.38, 2963.99, 2929.26, 2874.28, 1722.51, 1661.74, 1517.04, 1453.38; **HRMS** (ESI+) m/z Calcd for $C_{29}H_{38}N_3O_5$ [M+H] 508.2806, found 508.2809.

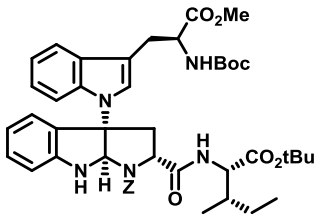
Benzyl (2R,3aS,8aR)-2-(((2S,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)-3a-((2-iodophenyl)amino)-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**12b**)



12b (1.5 g, 3.0 mmol mmol), PPTS (754 mg, 3.0 mmol) and 2-iodoaniline (755 mg, 3.5 mmol) were dissolved in MeCN (15 ml) and cooled down to -45 °C with an acetone - dry ice bath. NIS (1.06 g, 4.7 mmol) dissolved in MeCN (15 ml) was added dropwise and the mixture was allowed to warm to -35 °C over one hour. The crude mixture was poured on a separatory funnel and quenched with sat. $Na_2S_2O_4$ solution. The organic phase was separated and the water phase was extracted with AcOEt. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude was purified by flash column chromatography (Hexane:AcOEt 8:1 to 1:1), affording a brown foam with mass 1.74 g (80%).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** +141.5° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.74 and 0.95 (d, *J* = 6.9 Hz, 3H), 0.87 and 0.95 (d, *J* = 7.3 Hz, 3H), 1.17 – 1.30 (m, 1H), 1.35 and 1.37 (s, 9H), 1.39 – 1.54 (m, 1H), 1.82 and 1.96 (m, 1H), 2.36 and 2.57 (dd, *J* = 13.9, 8.9 Hz, 1H), 2.97 and 3.19 (d, *J* = 13.8 Hz, 1H), 4.50 and 4.67 (d, *J* = 7.8 Hz, 1H), 4.53 (dd, *J* = 8.5, 4.2 Hz, 1H), 4.87 (d, *J* = 4.1 Hz, 1H), 5.02 – 5.42 (m, 2H), 5.72 (s, 1H), 5.93 and 6.08 (d, *J* = 4.1 Hz, 1H), 5.96 (dd, *J* = 8.3, 1.4 Hz, 1H), 6.31 and 6.36 (td, *J* = 7.5, 1.4 Hz, 1H), 6.60 and 6.66 (d, *J* = 7.8 Hz, 1H), 6.72 (td, *J* = 7.4, 0.9 Hz, 1H), 6.83 and 6.93 (t, *J* = 7.7 Hz, 1H), 7.07 (d, *J* = 7.2 Hz, 1H), 7.12 (td, *J* = 7.7, 1.4 Hz, 1H), 7.46 – 7.28 (m, 5H), 7.60 and 3.63 7.63 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d,* mixture of rotamers 7:3) δ 12.0 (q), 15.2 and 15.6 (q), 25.5 (t), 28.1 (q), 38.1 and 38.6 (t), 41.6 and 44.0 (t), 57.6 (d), 60.6 (d), 68.1 and 68.4 (d) , 73.2 (s), 79.8 and 80.3 (d), 81.7 (s), 86.4 (s), 110.0 (d), 113.1 (d), 118.8 (d), 119.8 (d), 122.7 (d), 128.6 (d), 128.9 (d), 129.4 (d), 129.8 (s), 135.9 (s), 139.9 (d), 144.9 (s), 146.4 (s), 156.7 (s), 170.2 (s), 170.6 (s); **IR** (NaCl film, cm^{-1}) 3386.50, 3334.41, 2963.99, 2929.26, 1722.51, 1690.68, 1612.54, 1583.60, 1511.25; **HRMS** (ESI+) m/z Calcd for $C_{35}H_{42}IN_4O_5$ [M+H] 725.2194, found 725.2182.

Benzyl (2R,3aS,8aR)-2-(((2S,3S)-1-(tert-butoxy)-3-methyl-1-oxopentan-2-yl)carbamoyl)-3a-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-1H-indol-1-yl)-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (**2b**)

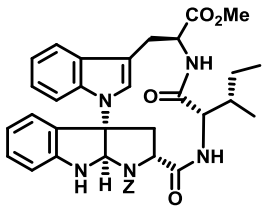


A Schlenk flask containing compound **12b** (1g, 1.38 mmol), aminoacid **14** (2.36 g, 6.90 mmol), $Pd(OAc)_2$ (0,06 g, 0.276 mmol), anhydrous NaOAc (0.79 g, 9.66 mmol) and anhydrous LiCl (0.06g, 1.38 mmol) was evacuated and backfilled with nitrogen. Under nitrogen atmosphere, DMF (14 ml) was added. In the next step, the reaction mixture was degassed via iterative freeze-pump-thaw cycles. After heating the mixture to 100 °C during 24 h, the reaction mixture was cooled to room temperature, diluted with toluene, filtered through Celite with EtOAc flushing and concentrated. The residue was taken up with AcOEt and washed vigorously with aqueous 1M HCl. The layers were separated, and the aqueous layer was extracted additionally with AcOEt. The combined organic layers were washed successively with Sat. $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered and concentrated. The crude material was purified by flash column chromatography (Hexane:AcOEt 9:1 to 3:7). The excess of alkyne **14** (1.79 g) and unreacted **12b** (112 mg) could be recovered during chromatography. The afforded product (**2b**) was a brown foam with mass 0.96 g (85 % yield).

M.p. 101-103 °C; **$[\alpha]_D^{20}$** +68.1° (*c* 1.0, CH_2Cl_2); **^{1}H RMN** (400 MHz, Chloroform-*d*, 6:4 mixture of rotamers) δ 0.74-0.99 (m, 6H), 1.08 and 1.20 (m, 1H), 1.35-1.49 (m, 19H), 1.77-1.85 and 1.86-

1.94 (m, 1H), 3.13 (qd, *J* = 14.6, 5.3 Hz, 2H), 3.53 (q, *J* = 12.3, 11.3 Hz, 1H), 3.61 (s, 3H); 4.09 and 4.19 (t, *J* = 8.4 Hz, 1H), 4.41-4.58 (m, 2H), 4.41-4.58 (m, 2H), 4.99 (d, *J* = 8.2 Hz, 1H), 5.12 and 5.36 (s and d, *J* = 12.3 Hz, 2H), 5.62 (s, 1H), 6.00 and 6.10 (s, 1H), 6.35 (d, *J* = 7.9 Hz, 1H), 6.56 and 6.75 (d, *J* = 8.1 Hz, 0H), 6.58-6.61 (m, 1H), 6.91 (t, *J* = 7.6 Hz, 0H), 7.13 (t, *J* = 7.6 Hz, 0H), 7.23-7.38 (m, 5H), 7.49 and 7.53 (d, *J* = 7.9 Hz, 1H), 7.65 – 7.75 (m, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 11.9 (q), 15.2 (q), 25.7 (t), 27.8 (t), 28.2 and 28.5 (q), 38.4 (d), 39.1 (t) 52.2 (q), 54.5 (d), 56.7 and 57.0 (d), 61.2 (d), 67.6 (t), 72.9 (s), 79.8 and 80.1 (d), 82.5 (s), 109.0 (s), 111.3(d), 112.4 (d), 119.6 (d), 119.8 (d), 120.0 (d), 122.5 (d), 125.4 (d), 125.5 (d), 126.5 (s), 127.8 (d), 128.2 (d), 128.6 (d), 129.0 (d), 130.6 (s), 131.3 (d), 134.8 (s), 135.9 (s), 149.3 (s), 154.6 (s), 155.2 (s), 170.6 (s), 172.7 (s); **IR** (NaCl film, cm^{-1}) 3363.34, 3056.59, 2966.88, 2932.15, 1733.83, 1609.65, 1502.57; **HRMS** (ESI+) m/z Calcd for $C_{46}H_{58}N_5O_9$ [M+H] 824.4229, found 824.4226.

11-benzyl 4-methyl (12R,13aS,18aR,21R,4S,7S,Z)-7-((S)-sec-butyl)-6,9-dioxo-11,12,13,13a,18,18a-hexahydro-21H-5,8-diaza-1(3a,2)-pyrrolo[2,3-b]indola-2(1,3)-indolacyclononaphane-11,4-dicarboxylate (**3b**)

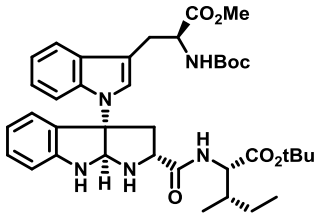


Compound **2b** (300 mg, 0.36 mmol) was dissolved in a mixture (5 mL) containing 60% TFA in DCM and stirred during 3h at RT. The solvents were concentrated under reduced pressure and coeavaporated with toluene, the mixture was redissolved in the minimum amount of DMF and DCM (0.36 l, 1 mM) was added. The mixture was cooled down to 0 °C and PyAOP (380 mg, 0.72 mmol) and DIEA (0.5 ml, 48 mmol) was added dropwise. The mixture was stirred overnight. The solvents were concentrated under reduced pressure and the crude mixture was washed successively with 1M HCl, $NaHCO_3$ and brine, dried over MgSO4, filtered and concentrated. The product was purified by flash column chromatography (Hexane:AcOEt 1:1). The obtained product was a pink foam with mass 131 mg (56% yield).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** +167.6° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.60 and 0.81 (t, *J* = 7.4 Hz, 1H), 0.74 and 0.80 (d, *J* = 6.6 Hz, 1H), 0.84-0.95 and 1.03-1.15 (m, 1H), 1.19-1.30 and 1.44-1.55 (m, 1H), 1.57 and 1.72 (m, 1H), 2.55 – 2.88 (m, 1H), 3.40 – 3.66 (m, 1H), 4.21 (dt, *J* = 21.0, 10.5 Hz, 1H), 4.78 and 4.82 (d, *J* = 10.0 Hz, 1H), 4.88-4.99 (m, 1H), 4.96 and 5.05 (d, *J* = 12.1 Hz, 1H), 5.31 and 5.37 (d, *J* = 12.1 Hz, 1H), 6.37 and 6.51 (s, 1H), 6.55-6.79 (m, 3H), 6.88-6.98 (m, 1H), 7.12-7.23 (m, 2H), 7.28-7.37 (m, 3H), 7.40-7.49 (m, 2H) 7.61 (d, *J* = 7.9 Hz, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 9.9 and 10.3 (q), 15.1 and 15.4 (q), 24.2 (t), 29.7 and 30.1 (t), 34.5 and 34.7(d), 40.1 and 41.2 (t), 51.3 and 51.4 (d) 52.8 (q), 57.9 and 58.0 (d),

61.3 and 61.8 (d), 68.4 and 68.5 (t), 74.2 (s), 75.4 (s), 83.7 and 84.7(d), 109.2 and 109.3 (s), 110.0 and 110.1 (d), 112.4 and 112.5 (d), 118.6 and 118.7(d), 119.8 and 120.1 (d), 120.2 and 120.2 (d), 122.0 and 122.2 (d), 122.4 and 122.5 (d), 124.9 (d), 125.4 (d), 128.2 (s), 128.6 and 128.7 (d), 128.8 and 129.0 (d), 129.6 (s), 130.0 and 130.1 (d), 135.3 and 135.5 (s), 135.6 and 135.7 (s), 145.8 and 146.1 (s), 155.3 and 155.4(s), 170.2 and 170.6(s), 171.0 and 171.2 (s), 172.1 and 172.2 (s); **IR** (NaCl film, cm^{-1}) 3395.18, 3308.36, 3059.49, 2961.09, 2929.26, 1742.77, 1710.93, 1647.27, 1609.65, 1525.72; **HRMS** (ESI+) m/z Calcd for $C_{37}H_{40}N_5O_6$ [M+H] 650.2973, found 650.2960.

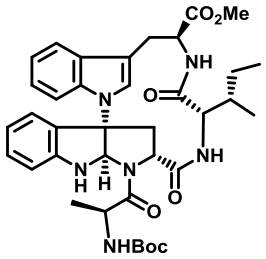
***Tert*-butyl ((2R,3aS,8aS)-3a-(3-((S)-2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)-1H-indol-1-yl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl)-L-isoleucinate** (**17b**)



Compound **2b** (150 mg, 1.42 mmol) and Pd Black (30 mg, 20% weight) were stirred in MeOH (10 mL). The mixture was purged with N_2, backfilled with H_2 and stirred during 2 h. The mixture was filtered over Celite and concentrated. The Product was light green foam with mass 126 mg (quantitative yield).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** +103.7° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.60 and 0.81 (t, *J* = 7.4 Hz, 1H), 0.74 and 0.80 (d, *J* = 6.6 Hz, 1H), 0.84-0.95 and 1.03-1.15 (m, 1H), 1.19-1.30 and 1.44-1.55 (m, 1H), 1.57 and 1.72 (m, 1H), 2.55 – 2.88 (m, 1H), 3.40 – 3.66 (m, 1H), 4.21 (dt, *J* = 21.0, 10.5 Hz, 1H), 4.78 and 4.82 (d, *J* = 10.0 Hz, 1H), 4.88-4.99 (m, 1H), 4.96 and 5.05 (d, *J* = 12.1 Hz, 1H), 5.31 and 5.37 (d, *J* = 12.1 Hz, 1H), 6.37 and 6.51 (s, 1H), 6.55-6.79 (m, 3H), 6.88-6.98 (m, 1H), 7.12-7.23 (m, 2H), 7.28-7.37 (m, 3H), 7.40-7.49 (m, 2H) 7.61 (d, *J* = 7.9 Hz, 1H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 11.9 (q), 15.6 (q), 25.5 (t), 28.0 (t), 28.2(q), 28.5 (q), 38.2 (d), 43.2 (t), 52.3 (d), 54.5 (d), 56.5 (d), 60.8 (d), 76.6 (s), 80.2 (s), 81.3 (d), 82.2 (s), 108.8 (s), 111.2 (d), 112.2 (d), 119.3 (d), 119.8 (d), 120.3 (d), 122.2 (d), 125.3 (d), 125.8. (d), 128.1 (s), 130.3 (s), 130.7 (d), 135.2 (s), 150.0 (s), 155.3 (s), 170.8 (s), 172.1 (s); **IR** (NaCl film, cm^{-1}) 3340.19, 2966.88, 2932.15, 2874.28, 171961, 1673.31, 1606.75, 1508.36; **HRMS** (ESI+) m/z Calcd for $C_{38}H_{52}N_5O_7$ [M+H] 690.3861, found 690.3867.

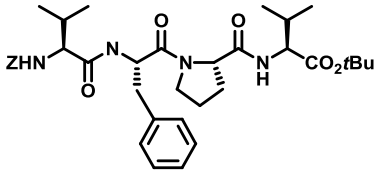
Methyl (12R,13aS,18aR,21R,4S,7S,Z)-11-((tert-butoxycarbonyl)-L-alanyl)-7-((S)-sec-butyl)-6,9-dioxo-11,12,13,13a,18,18a-hexahydro-21H-5,8-diaza-1(3a,2)-pyrrolo[2,3-b]indola-2(1,3)-indolacyclononaphane-4-carboxylate (**35**)



Compound **3b** (84 mg, 0.145 mmol), Boc-L-Ala-OH (162 mg, 0.72 mmol) and HATU (330 mg, 0.87 mmol) were dissolved in DMF (2 ml) and cooled down to 0 °C. DIEA (0.25 ml, 1.45 mmol) was added and the mixture was stirred during 1h at RT. Thereafter, the reaction was cooled down to 0 °C and Boc-L-Ala-OH (162 mg, 0.72 mmol), HATU (330 mg, 0.87 mmol) and DIEA (0.25 ml, 1.45 mmol) were added. The mixture was stirred during another hour. The crude was diluted with EtOAc and washed successively with 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified flash column chromatography (SiO2, Hexane:AcOEt 7:3 to 1:1), affording a white foam with mass 113 mg (87% yield).

M.p. 100-102 °C; $[\alpha]_D^{20}$ +53.8° (*c* 1.0, CH_2Cl_2); ^{1}**H NMR** (400 MHz, Chloroform-*d*) δ 0.60 and 0.81 (t, *J* = 7.4 Hz, 1H), 0.74 and 0.80 (d, *J* = 6.6 Hz, 1H), 0.84-0.95 and 1.03-1.15 (m, 1H), 1.19-1.30 and 1.44-1.55 (m, 1H), 1.57 and 1.72 (m, 1H), 2.55 – 2.88 (m, 1H), 3.40 – 3.66 (m, 1H), 4.21 (dt, *J* = 21.0, 10.5 Hz, 1H), 4.78 and 4.82 (d, *J* = 10.0 Hz, 1H), 4.88-4.99 (m, 1H), 4.96 and 5.05 (d, *J* = 12.1 Hz, 1H), 5.31 and 5.37 (d, *J* = 12.1 Hz, 1H), 6.37 and 6.51 (s, 1H), 6.55-6.79 (m, 3H), 6.88-6.98 (m, 1H), 7.12-7.23 (m, 2H), 7.28-7.37 (m, 3H), 7.40-7.49 (m, 2H) 7.61 (d, *J* = 7.9 Hz, 1H); ^{13}**C NMR** (101 MHz, Chloroform-*d*) δ 9.9 and 10.3 (q), 15.1 and 15.4 (q), 24.2 (t), 29.7 and 30.1 (t), 34.5 and 34.7(d), 40.1 and 41.2 (t), 51.3 and 51.4 (d) 52.8 (q), 57.9 and 58.0 (d), 61.3 and 61.8 (d), 68.4 and 68.5 (t), 74.2 (s), 75.4 (s), 83.7 and 84.7(d), 109.2 and 109.3 (s), 110.0 and 110.1 (d), 112.4 and 112.5 (d), 118.6 and 118.7(d), 119.8 and 120.1 (d), 120.2 and 120.2 (d), 122.0 and 122.2 (d), 122.4 and 122.5 (d), 124.9 (d), 125.4 (d), 128.2 (s), 128.6 and 128.7 (d), 128.8 and 129.0 (d), 129.6 (s), 130.0 and 130.1 (d), 135.3 and 135.5 (s), 135.6 and 135.7 (s), 145.8 and 146.1 (s), 155.3 and 155.4(s), 170.2 and 170.6(s), 171.0 and 171.2 (s), 172.1 and 172.2 (s); **IR** (NaCl film, cm^{-1}) 3395.18, 3308.36, 2963.99, 2932.15, 1739.87, 1693.57, 1650.16, 1517.04. **HRMS** (ESI+) m/z Calcd for $C_{37}H_{47}N_6O_7$ [M+H] 687.3501, found 687.3500.

***Tert*-butyl ((benzyloxy)carbonyl)-L-valyl-L-phenylalanyl-L-prolyl-L-valinate (38)**



On a 20 mL polypropylene syringe, 2-chlorotrityl chloride resin (0,7 g) was washed sequentially with DMF and DCM five times. The resin was swelled with DCM during 10 min. Thereafter, the solvent was filtrated and the resin was loaded with Fmoc-L-Pro-OH·H_2O (507 mg, 0,7 mmol) dissolved in DCM (10 ml aprox) and DIEA (0,6 ml, 3.5 mmol) and stirred during 1h. The mixture was filtrated and the mixture was stirred with MeOH (5 ml) during 10 minutes. The aminoacid was deprotected with 20% piperidine in DMF (three treatments, 5 ml each; 1, 2 and 3 min respectively). The resin was washed successively with DMF and DCM, swelled with DCM during 1 minute and filtered.

Fmoc-L-Phe-OH (387 g, 1 mmol), oxyma pure (142 mg, 1 mmol) and DIC (0.16 ml, 1 mmol) were dissolved in DMF (3 mL) and stirred during 5 min. The crude mixture was added to the resin and the syringe was stirred during 15 min. The solvent was purged and the coupling process was repeated for another 15 min. The results of the coupling were verified with chloranyl test. Fmoc deprotection was carried out with 20% piperidine solution in DMF (three treatments, 5 ml each; 1, 2 and 3 minutes respectively). After deprotection process, the resin was washed with DMF and DCM, swelled with DCM during 1 minute and filtered. Z-L-Val-OH was coupled utilizing the previous methodology, avoiding the final deprotection step. The results of the coupling were verified with ninhydrin test.

The resin was washed successively with DMF, 2-propanol and DCM (one syringe volume) five times.

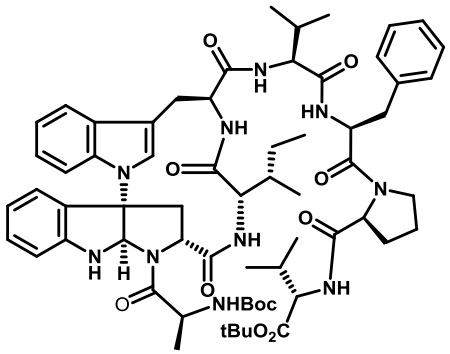
The tripeptide was cleaved from the resin by treatment with 10% TFA in DCM (five syringe volumes. The resin was washed with DCM (5 syringe volumes) and the solvents were concentrated under reduced pressure.

Z-V-F-P-OH (0.5 mmol), H-L-Val-O*t*Bu·HCl (210 mg, 1 mmol), EDC·HCl (192 mg, 1 mmol) and oxyma pure (142 mg, 1 mmol) were dissolved in DMF (5 ml) and cooled down to 0 °C. DIEA (0.5 ml, 3 mmol) was added and the mixture was stirred overnight. The peptide was precipitated with cold water and centrifuged. The precipitate was dissolved in AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated under reduced pressure. The product was a yellow foam with mass 316 mg (97% overall yield).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** +42.3° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 0.77 (d, *J* = 6.8 Hz, 3H), 0.87 (2d, *J* = 7.7 Hz, 6H), 0.94 (d, *J* = 6.9 Hz, 3H), 1.47 (s, 9H), 1.97 – 2.09 (m, 2H), 1.79 – 1.97 (m, 2H) 2.11-2.20 (m, 2H), 2.91 (dd, *J* = 13.8, 6.8 Hz, 1H), 3.05 (dd, *J* = 13.8, 6.6 Hz, 1H), 3.26 (t, *J* = 9.1 Hz, 1H), 3.62 (q, *J* = 8.3 Hz, 1H), 4.21 – 4.32 (m, 1H), 4.42 (dd, *J* = 8.5, 4.4 Hz, 1H), 4.62 (d, *J* = 7.2 Hz, 1H), 5.21 – 4.97 (m, 1H) 5.38 (bs, 1H), 7.14-7.24 (m, 5H), 7.27 – 7.39 (m, 5H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 17.3 (q), 17.9 (q), 19.2 (q), 19.5 (q), 25.0 (t) , 28.1 (t), 28.2(q), 31.5 (d), 31.6 (d), 39.3 (t), 47.6 (t), 52.0 (d), 57.8 (d), 59.8 (d), 60.0 (d), 67.2 (t), 81.9 (s),

127.0 (d) , 128.2 (d), 128.3 (d), 128.6 (d), 128.7 (d), 129.7 (d), 136.1 (s), 136.4 (s), 156.6 (s), 170.7 (s), 170.9 (s), 171.1 (s); **IR** (NaCl film, cm^{-1}) 3299.88, 2963.99, 2929.26, 2868.49, 1728.30, 1679.10, 1629.90, 1528.62, 1450.48; **HRMS** (ESI+) m/z Calcd for $C_{36}H_{51}N_4O_7$ [M+H] 651.3752, found 651.3747.

tert-butyl ((12R,13aS,18aR,21R,4S,7S,Z)-11-((tert-butoxycarbonyl)-L-alanyl)-7-((S)-sec-butyl)-6,9-dioxo-11,12,13,13a,18,18a-hexahydro-21H-5,8-diaza-1(3a,2)-pyrrolo[2,3-b]indola-2(1,3)-indolacyclononaphane-4-carbonyl)-L-valyl-L-phenylalanyl-L-prolyl-L-valinate (36)



Compound **35** (150 mg, 0.11 mmol) was dissolved in a mixture of THF:H_2O 2:1 (3 mL). LiOH·H_2O (76mg, 1.8 mmol) was added and the reaction mixture was stirred overnight. Aqueous 2M HCl was added dropwise until no precipitation was observed. The crude was extracted with EtOAc, the combined organic layers were washed with brine, dried over MgSO4, filtered and concentrated under reduced pressure. The afforded product was a brown foam with mass 147 mg (quant. yield).

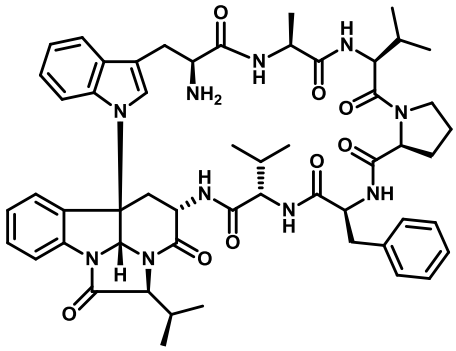
On the other hand, peptide **38** (150 mg, 1.42 mmol) and Pd Black (30 mg, 20% weight) were stirred in MeOH (10 mL). The mixture was purged with N_2, backfilled with H_2 and stirred during 2 h. The mixture was filtered over Celite and concentrated. The Product was light green foam with mass 126 mg (quantitative yield).

The previously unprotected compounds and PyAOP (94 mg, 0.18 mmol) were dissolved in DMF (2 ml) and cooled down to 0 °C. DIEA (0,2 mL, 1.15 mmol) was added and the mixture was stirred overnight. The peptide was precipitated with cold water (40 mL) and centrifuged. The residue was dissolved with AcOEt and washed successively with aqueous 2M HCl, sat. $NaHCO_3$ sol. and brine, dried over MgSO4, filtered and concentrated. The crude mixture was purified by flash column chromatography (SiO_2, Hexane:AcOEt 3:7 to 1:9), affording a light yellow foam with mass 198 mg (78% yield).

M.p. 100-102 °C; **$[\alpha]_D^{20}$** -5.20° (*c* 1.0, CH_2Cl_2); **^{1}H NMR** (400 MHz, DMSO-d_6) δ 0.77 (d, *J* = 6.8 Hz, 3H), 0.87 (2d, *J* = 7.7 Hz, 6H), 0.94 (d, *J* = 6.9 Hz, 3H), 1.47 (s, 9H), 1.97 – 2.09 (m, 2H), 1.79 –

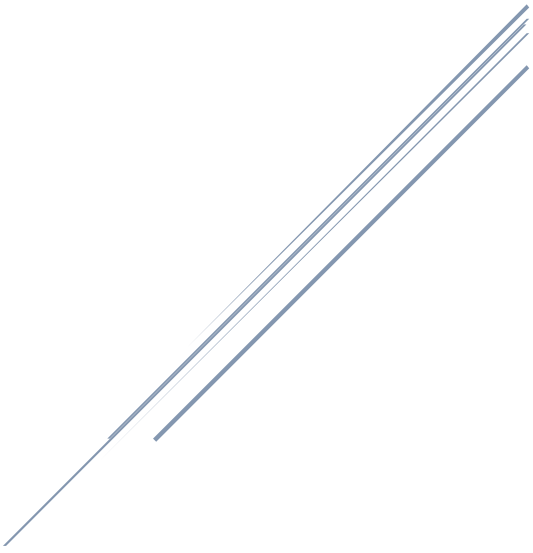
1.97 (m, 2H) 2.11-2.20 (m, 2H), 2.91 (dd, *J* = 13.8, 6.8 Hz, 1H), 3.05 (dd, *J* = 13.8, 6.6 Hz, 1H), 3.26 (t, *J* = 9.1 Hz, 1H), 3.62 (q, *J* = 8.3 Hz, 1H), 4.21 – 4.32 (m, 1H), 4.42 (dd, *J* = 8.5, 4.4 Hz, 1H), 4.62 (d, *J* = 7.2 Hz, 1H), 5.21 – 4.97 (m, 1H) 5.38 (bs, 1H), 7.14-7.24 (m, 5H), 7.27 – 7.39 (m, 5H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 17.3 (q), 17.9 (q), 19.2 (q), 19.5 (q), 25.0 (t) , 28.1 (t), 28.2(q), 31.5 (d), 31.6 (d), 39.3 (t), 47.6 (t), 52.0 (d), 57.8 (d), 59.8 (d), 60.0 (d), 67.2 (t), 81.9 (s), 127.0 (d) , 128.2 (d), 128.3 (d), 128.6 (d), 128.7 (d), 129.7 (d), 136.1 (s), 136.4 (s), 156.6 (s), 170.7 (s), 170.9 (s), 171.1 (s). **IR** (NaCl film, cm^{-1}) 3305.47, 2961.09, 2929.26, 2871.38, 1725.40, 1684.89, 1641.48, 1627.01, 1522.83, 1456.27. **HRMS** (ESI+) m/z Calcd for $C_{64}H_{87}N_{10}O_{11}$ [M+H] 1171.6550, found 1171.6547.

Kapakahine H (40)



	Kapakahine H			
aa	Assignment	δ C	δ H	J
Ala	Cα	60,7	4,94	m, 1H
	Cβ	14,9	1,55	1.55 (d, J = 7.3 Hz, 3H)
Val 2	Cα	60	4,08	4.08 (d, *J* = 10.5 Hz, 1H)
	Cβ	27,7	2,6	m, 1H
	Cγ	15	1,08	1.08 (d, *J* = 6.8 Hz, 3H)
	Cγ	18,9	0,95	0.95 (d, *J* = 6.7 Hz, 3H)
Val 1	Cα	59,1	4,25	4.25 (d, *J* = 7.1 Hz, 1H)
	Cβ	33,4	2,09	2.12 – 2.07 (m, 1H)
	Cγ	18,6	1,03	1.03 (d, *J* = 6.9 Hz, 3H)
	Cγ	18,6	1,04	1.04(d, *J* = 6.9 Hz, 3H)
Ile	Cα	59,5	3,74	3.74 (d, J = 11,5, 4.9 Hz, 0H)
	Cβ	38	1,99	m, 1H
	Cγ CH2 1	22,3	1,74	1.77 – 1.71 (m, 1H)
	Cγ CH2 2	22,3	1,47	1.48 (m, 1H)
	Cγ CH3	15	1,08	1.08 (d, *J* = 6.8 Hz, 6H)
	Cδ	11,2	0,99	0.99 (t, *J* = 7.3 Hz, 3H)
Pro	Cα	63,4	3,6	m, 1H
	Cβ	31	1,96	m, 2H
	Cγ	23	1,96	m, 2H
	Cδ	51,5	3,25	bs, 1H
Ar1	Cα	53	5,42	5.42 (d, *J* = 12.0, 3.8 Hz, 1H)
	Cβ1	48,9	2,9	
	Cβ1	48,9	3,72	
Ar2	Cα	48,6	4,8	
	Cβ1	36,6	3,25	
	Cβ1	36,6	2,29	
Ar3	Cα	56,5	3,74	3.75 (dd, J = 11.5, 5.1 Hz, 1H)
	Cβ1	38	2,94	
	Cβ1	38	2,84	
				6.42 (s, 1H)
				6.66 (d, *J* = 8.4 Hz, 1H)
				6.74 (d, *J* = 7.4 Hz, 2H)
				6.94 (t, *J* = 7.7 Hz, 2H)
				7.05 (t, *J* = 7.5 Hz, 1H)
				7.15 (m, 5H)
				7.20 (t, *J* = 7.5 Hz, 1H)
				7.47 (t, *J* = 7.8 Hz, 1H)
				7.57 (d, *J* = 7.8 Hz, 1H)
				7.77 (d, *J* = 8.1 Hz, 1H)
				8.70 (s, 1H)

RESUMEN EN CASTELLANO



INTRODUCCIÓN

1.1 Productos Naturales

El término productos natural se refiere a una molécula procedente del metabolismo secundario de un ser vivo, de no más de 1500 uma en términos generales, y que tiene un potencial interés terapéutico.[1]

La historia de los productos naturales es tan antigua como el hombre. Numerosas civilizaciones a lo largo de la historia han usado las plantas con una función medicinal y/o curativa.

En los últimos tiempos, las aportaciones de la ciencia de los productos naturales en la medicina han sido tan remarcables como la extracción de la morfina de *Papaver somniferum*,[2] la síntesis de la aspirina utilizado el ácido salicílico como producto de partida, o el descubrimiento de la penicilina en especies de hongos *penicillium*[3], entre otros.

En la actualidad, se trata de combinar la síntesis de productos naturales con los avances en síntesis orgánica, con el propósito de poder obtener análogos que exhiban mayor actividad con menor cantidad de efectos secundarios.

1.2 Productos Naturales de Origen Marino

Desde mediados del siglo XX, se están explorando los fondos oceánicos en busca de organismos que puedan albergar nuevas moléculas que ofrezcan estructuras y propiedades alternativas a las ya conocidas por el hombre. El principal factor de interés a la hora de buscar nuevas especies marina radica en la posesión de un metabolismo secundario más avanzado que sus análogos terrestres.[4,5]

1.3 Productos Naturales que contienen una unidad de hexahidropirrolo[2,3-*b*]indol

Es frecuente encontrar en los productos naturales familias de compuestos que compartan un motivo central. El HPI es uno de estos motivos,[6] encontrándose en moléculas tales como la (+)-alline,[7] que es el producto natural más pequeño hasta la fecha que contiene una unidad de HPI, hasta la caledonine que tiene siete unidades de éstos;[8] formando dicetopiperazinas como la amauromine,[9] o en péptidos como la omphalotine D (fig. 1).[10]

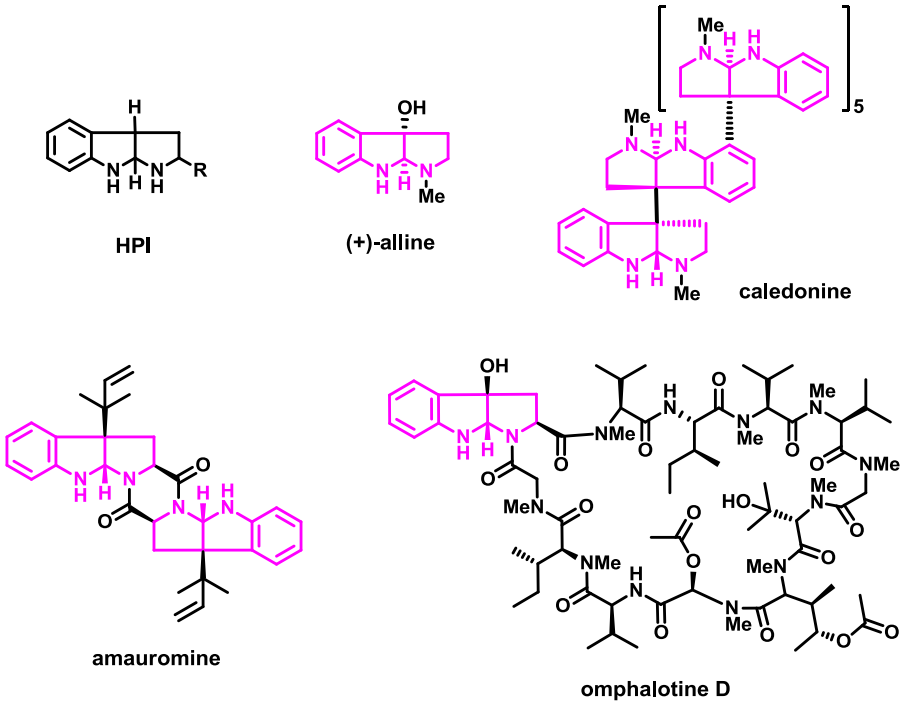


Fig. 1. Productos naturales con una unidad de HPI (marcada en morado)

Para terminar esta sección introductoria, también cabe resaltar productos naturales con dos triptófanos unidos mediante enlace N^1-C^{3a}, como la psychotrimine, con una estructura Trp-HPI o la familia de las kapahahines (fig 2).

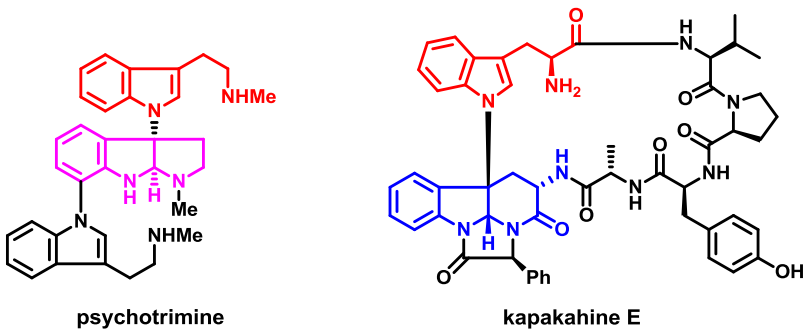


Fig. 2 Psychotrimine y kapakahine E, dos ejemplos de diversos productos naturales con dos Trp unidos por enlace N^1-C^{3a}. Trp (rojo), HPI (morado) y α-carbolina (azul).

OBJETIVOS

Esta tesis fue concebida con el objetivo principal de abordar la síntesis total del péptido natural de origen marino mayotlida, así como del estudio de su estereoquímica.

Los datos experimentales de RMN y MS-MS afloraron la secuencia lineal de los ocho aminoácidos contenidos en la molécula, dos de los cuales eran triptófanos formando la estructura Trp-HPI.

En la estructura inicial propuesta, los aminoácidos estaban dispuestos de tal manera que formaban dos anillos macrocíclicos, uno de ellos formado por una isoleucina y cerrado por el enlace N^{1}-C^{3a} del núcleo Trp-HPI (fig. 3).

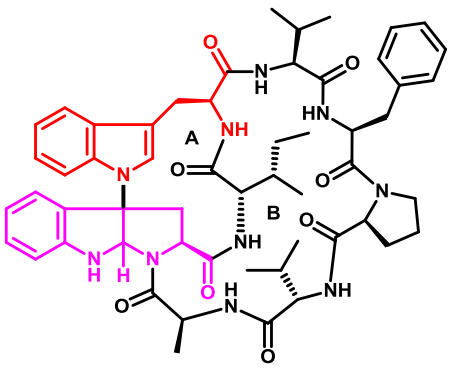


Fig.3 Primera propuesta de estructura de la mayotlida, Trp (rojo), HPI (morado).

Del análisis de aminoácidos se pudo conocer que todos los aminoácidos tienen configuración L salvo el HPI, que queda desconocida.

En el proceso de ciclación del amino terminal del Trp sobre el *C2* del indol, tiene lugar la formación de dos estereocentros nuevos en las posiciónes C^{3a} y C^{8a}, cuyos sustituyentes siempre se encuentran en cis por la propia configuración del anillo y pueden ser considerados como un grupo. Por lo tanto, este proceso de ciclación puede dar lugar a la aparición de cuatro diasterómeros, dificultando en primera instancia la elección de cuál de ellos podría ser el que forma parte de la mayotlida (fig.4).

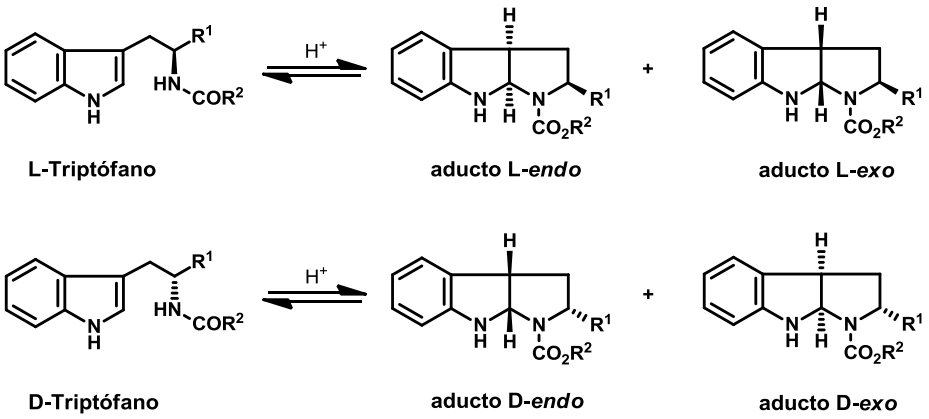


Fig. 4. Cuatro posibles diasterómeros de HPI.

Debido a que no existían evidencias empíricas que pudieran esclarecer cuál de los cuatro aductos de HPI es más probable que forme parte de la estructura de la mayotlida, se realizó un estudio computacional de la energía del anillo A con los cuatro diasterómeros de HPI, obteniendo como resultado final una tendencia más favorable a que pudiera ser cualquiera de los dos aductos *exo*. Los aductos *endo*, con una energía mayor, fueron descartados (fig. 5).

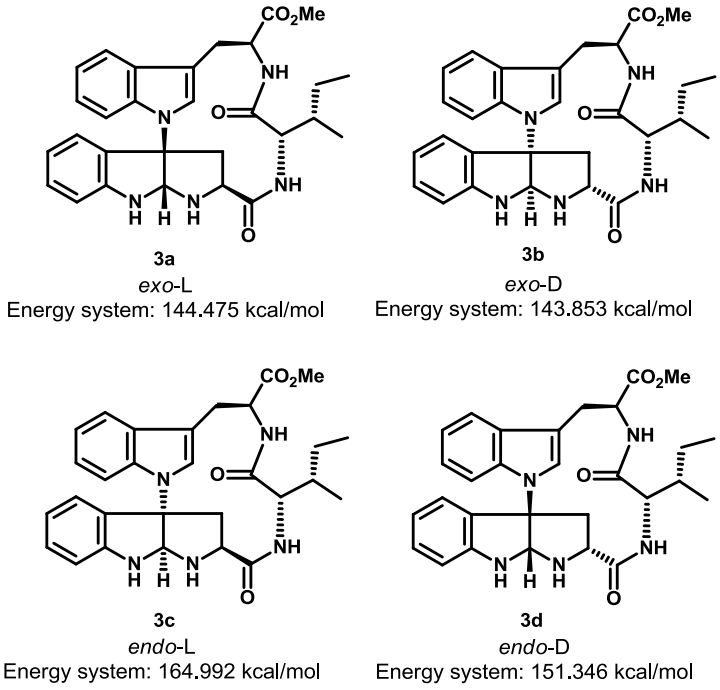


Fig.5 Estudio computacional del anillo A

Los aductos *exo* del anillo A tienen una energía muy similar, por lo que fue necesario integrar los anillos A *exo* dentro de la estructura total de la mayotlida, obteniendo como

resultado que el aducto *exo*-L proporciona una mayotlida con una energía ligeramente más baja que el *exo*-D (fig.)

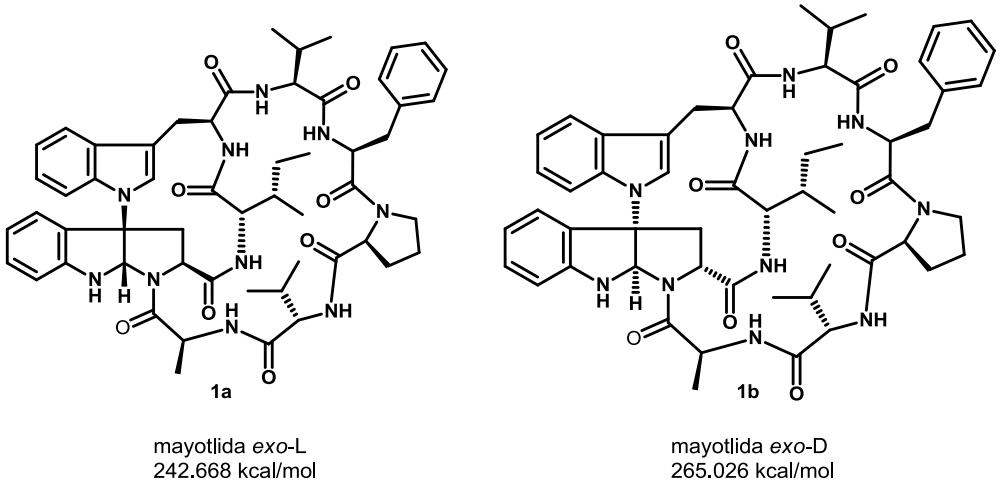


Fig.6 Análisis de mayotlida con los aductos exo de HPI.

Una vez establecido que los aductos *exo* son más estables que los *endo*, había que encontrar una metodología adecuada a las circunstancias. Baran y colaboradores han desarrollado una estrategia sintética para, empezando por un Trp adecuadamente protegido, sintetizar el fragmento Trp-HPI en dos pasos, obteniendo exclusivamente el aducto *exo* del HPI, y con buenos rendimientos. Fascinados por su eficiencia y su estereoespecificidad, se tomó la decisión de adaptarla a nuestras necesidades.

El primer objetivo sintético de la presente tesis consistía en la obtención del anillo A y estudio de su estereoquímica, puesto que resulta desconocida. Para ello, se transformaría el dipéptido **11a** en el tripéptido **2a** siguiendo la estrategia de Baran, el cual constituye el sintón más pequeño que contiene los tres aminoácidos que son comunes a ambos anillos de la mayotlida.

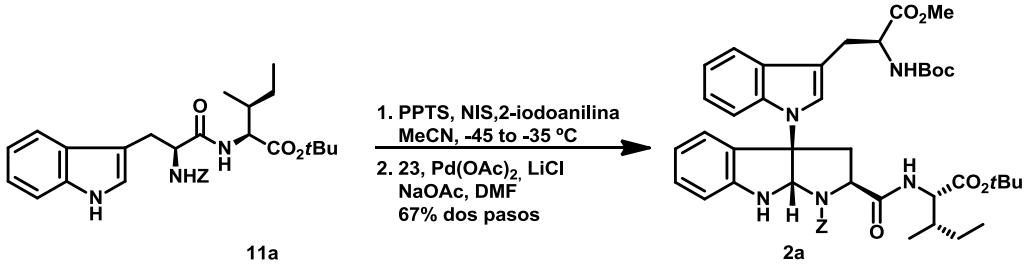


Fig. 7 Procedimiento sintético para alcanzar el tripéptido **3a**.

El ^{1}H-RMN de **2a** ofrecía un elevado radio de rotámeros (1:1), dificultando el estudio de su estereoquímica para poder comprobar si durante la reación de Larock el aducto *exo* había isomerizado en favor del aducto termodinámicamente más estable *endo*. Quizás el anillo A,

producto de la ciclación de **2a**, ofrecería un mejor resultado, pero la relación de rotámeros (8:2) seguía siendo alta. El producto de desprotección de Z de **2a**, el compuesto, **17a,** presentaba un nivel de rotámeros óptimo para el estudio de la estereoquímica del HPI, dando como resultado final la no isomerización del aducto *exo* durante la reacción de Larock (fig.)

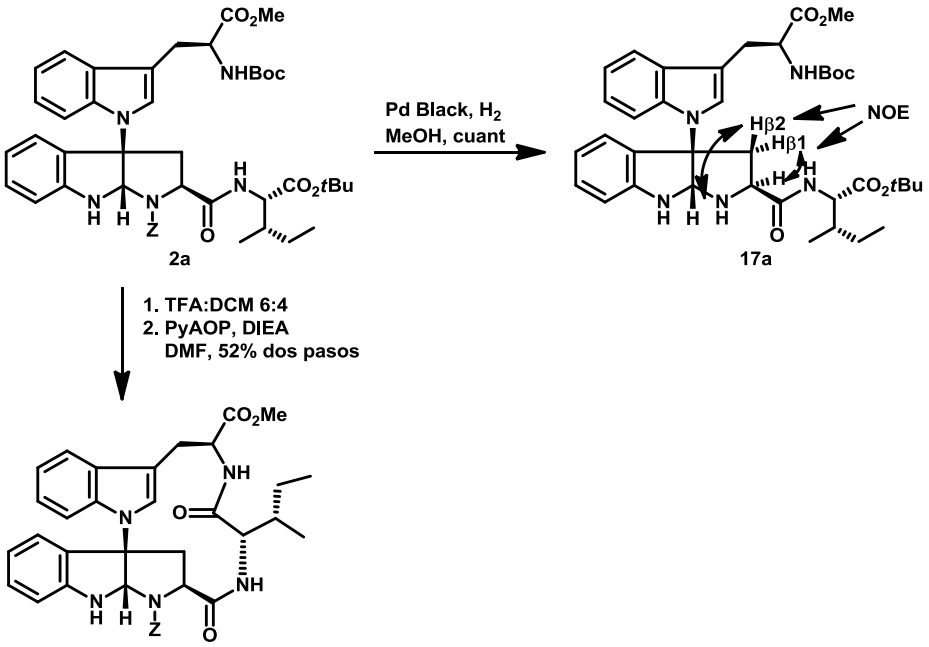


Fig. 8 Síntesis del anillo A y estudio de la estereoquímica de **17a**.

A partir del compuesto 2a se realizaron una serie de aproximaciones sintéticas, siendo la más fructífera la descrita en la fig. 9.

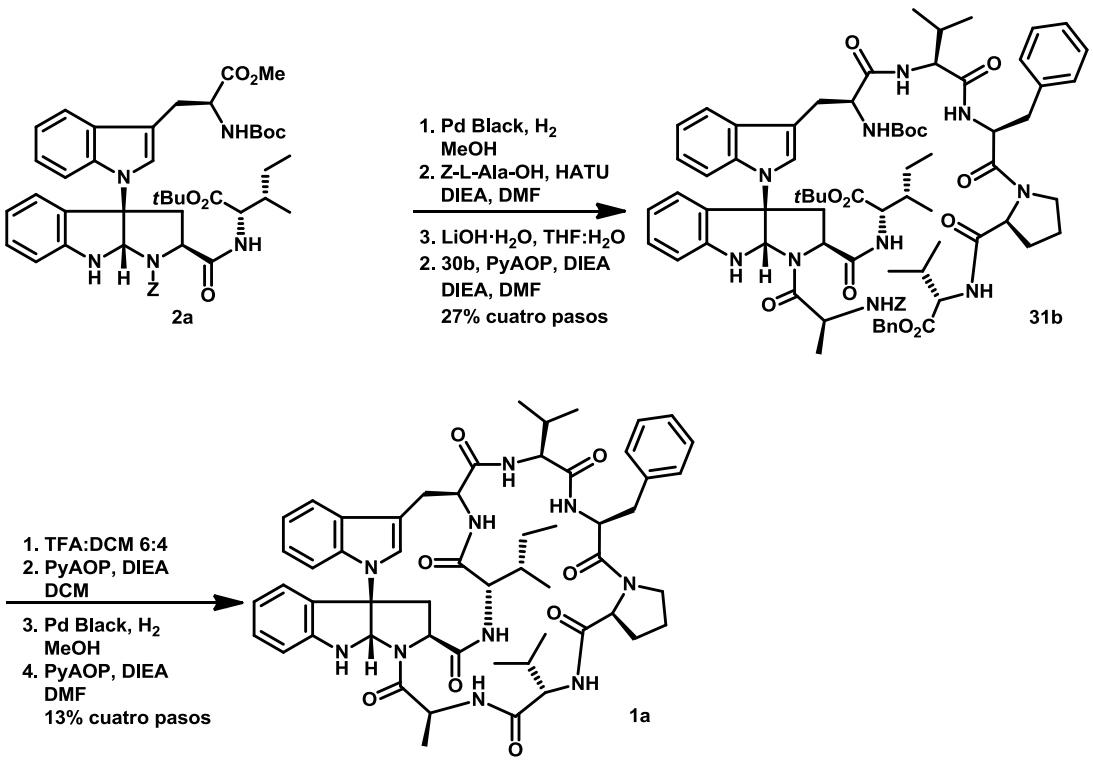


Fig.9. Síntesis de la *exo*-L mayotlida (**1a**) desde el tripéptido **2a**.

Desafortunadamente, ni los espectros de RMN ni el de MS-MS coincidían (fig. 10).

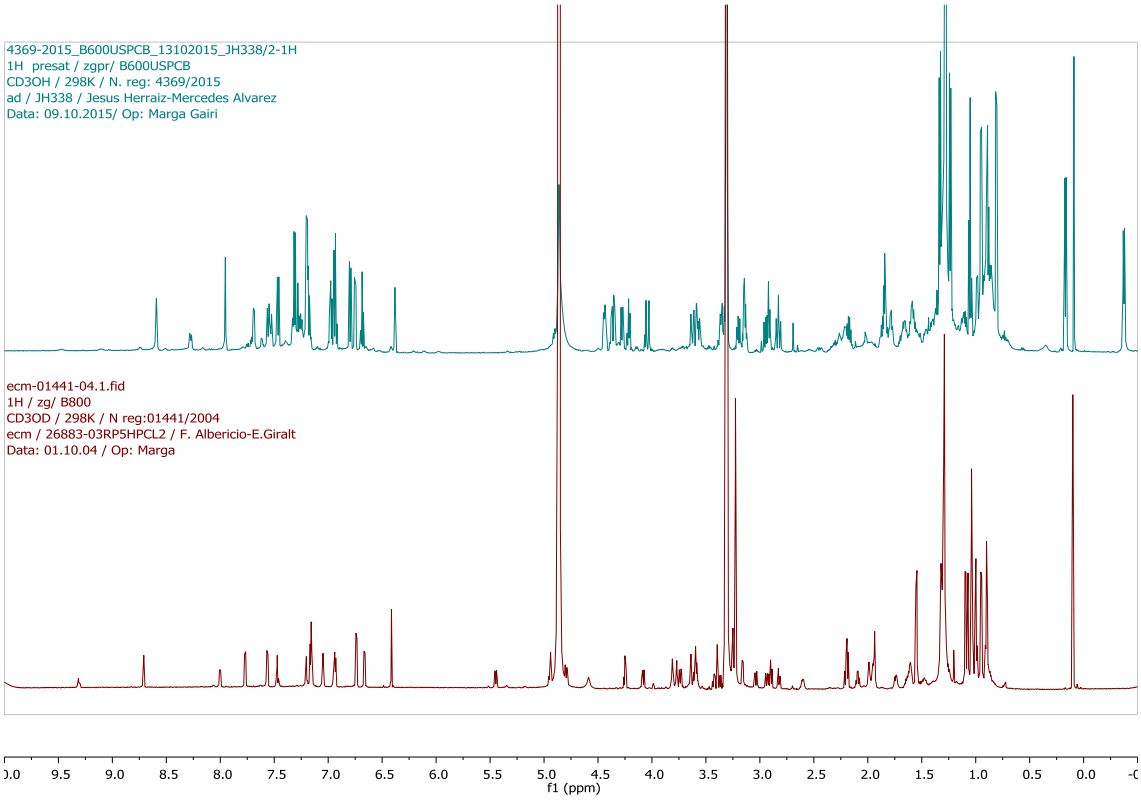


Fig. 10. ^{1}H-RMN del producto sintético (arriba) y el product natural (abajo).

No supimos interpretar cuál era el origen de esta discordancia de resultados, por lo que el nuevo objetivo sintético consistiría en abordar la síntesis de la mayotlida, esta vez con el aducto *exo*-D, que computacionalmente hablando, era el producto de menor energía después del *exo*-L (fig. 11).

Desde el compuesto **10b** se alcanzaría el tripéptido **2b**, en una metodología análoga a la síntesis de la mayotlida *exo*-L. Esta vez, sin embargo, se cambiaría ligeramente la secuencia sintética, de tal manera que se reduciría la variabilidad de los grupos protectores, usando los mismos después de haber desprotegido y ciclado para obtener el anillo A.

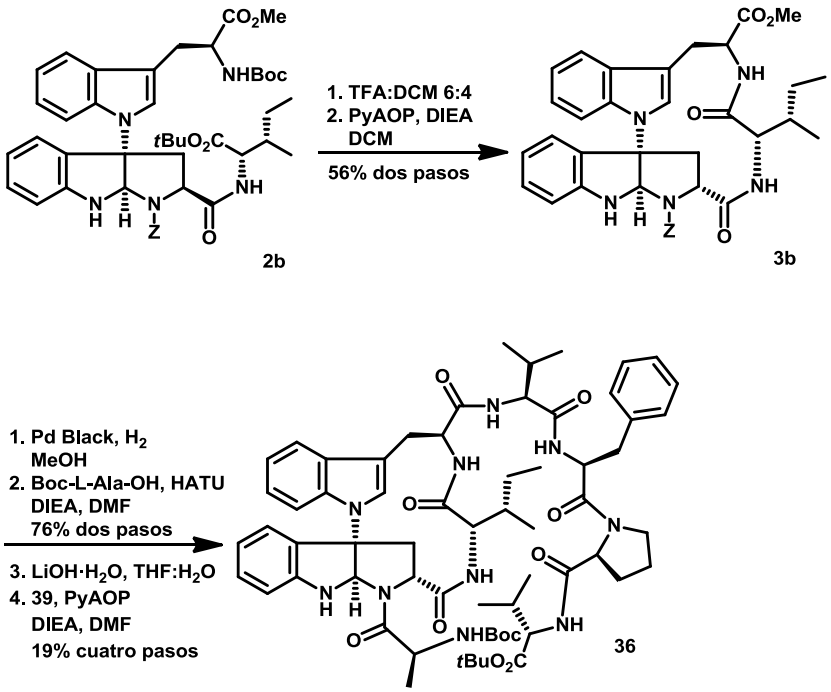


Fig. 11 Síntesis de la mayotlida con el aducto *exo*-D del HPI.

El último paso, la desprotección Boc-tBu, se llevó a cabo aunque no se purificó, debido a la complejidad de su crudo y que, paralelamente a la síntesis de la mayotlida *exo*-D, se revisaron exhaustivamente tanto la bibliografía como los datos analíticos del producto natural, llegando a la conclusión de que la estructura propuesta podía ser errónea. En términos generales, los puntos críticos en contra de la estructura inicial de la mayotlida eran los siguientes:

- No existen precedentes de productos naturales peptídicos con la estructura Trp-HPI. Las kapakahines son péptidos similares, que en lugar de tener una estructura Trp-HPI, tienen una estructura Trp-α carbolina.
- El espectro de MS-MS presenta divergencias críticas con respecto a la estructura inicial, sobre todo con respecto al aminoácido isoleucina, que es el aminoácido que forma el puente entre ambos ciclos de la mayotlida.

Así por tanto, tras modificar el motivo central, es decir, cambiar el HPI por una α carbolina, e invertir la secuencia central de los aminoácidos, se ha propuesto una alternativa que encaja perfectamente con los datos analíticos obtenidos, rebautizada como kapakahina H (fig. 12).

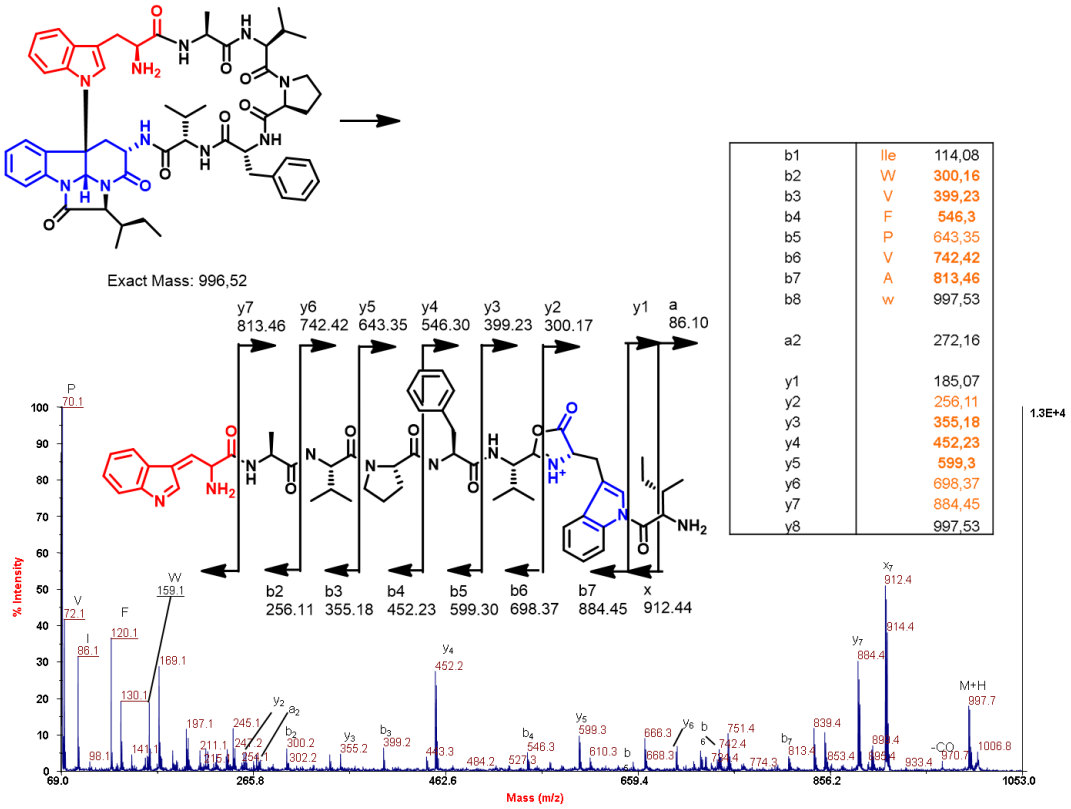


Fig. 12. kapakahina H, con su espectro de Ms-Ms y sus patrones de fragmentación.

Por último, la fig. 13 recoge la propuesta inicial de 2005 y la propuesta de esta tesis.

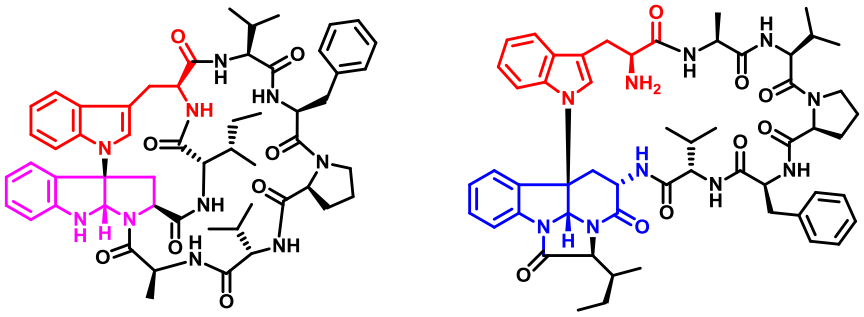


Fig. 13 Estructura de la mayotlida y de la kapakahina H.

Conclusiones

- La mayotlida, un producto natural de origen marino, se estableció como un péptido con dos anillos macrocíclicos, uno de los cuales contenía una unidad Trp-HPI, con la configuración de este último desconocida.
- Se estudió en profundidad la estereoquímica del HPI, y cómo dicha estereoquímica podía influir energéticamente a través de un cálculo teórico de energías realizado con el programa MOE, llegando a la conclusión de que los aductos *exo* tienen una energía más reducida que los *endo*.
- Para poder abordar con éxito la síntesis de la mayotlida, se adaptó una metodología desarrollada en el laboratorio de Phil Baran para moléculas similares a la mayotlida. Dicha estrategia permite, en dos pasos y con rendimientos aceptables, transformar un Trp deseado en una unidad Trp-HPI, con el HPI en configuración *exo* estereoespecíficamente.
- Se consiguió la síntesis total de la mayotlida *exo*-L, aunque desafortunadamente, el espectro de hidrógeno mostraba diferencias severas con respecto al natural.
- Como alternativa, se planteó la síntesis de la mayotlida con el aducto *exo*-D, sin llegarse a concluir, ya que, durante la síntesis de la misma, se estipuló que la estructura propuesta para mayotlida no era correcta.
- No se hallaron precedentes de productos naturales análogos a la mayotlida en la bibliografía, y la estructura inicial presentaba varias incongruencias que indicaban la posibilidad de una reorganización de los aminoácidos podía conducir a una propuesta que encajara mejor.
- Dicha propuesta, rebautizada como kapakahina H, se ajusta perfectamente, tanto a los espectros de RMN, como al espectro de MS-MS, habiendo sido confirmada por el departamento de síntesis de productos naturales de PharmaMar.

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