1. INTRODUCTION

1.1 The Iminosugars

Polyhydroxylated alkaloids are a wide category of natural compounds present in plants or bacteria that presents very interesting and different biological activities. These molecules may have a pyrroline (**a**), pyrrolidine (**b**), piperidine (**c**), pyrrolizidine (**d**), indolizidine (**e**) or *nor*-tropane (**f**) skeleton (Figure 1.1).

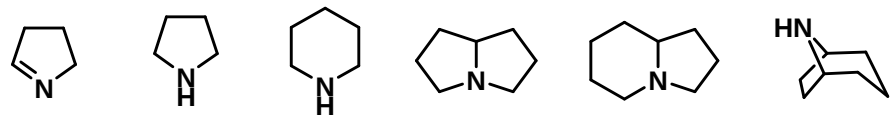


Figure 1.1

In Figure 1.2 is shown one example of compound for each core structure of Figure 1.1.

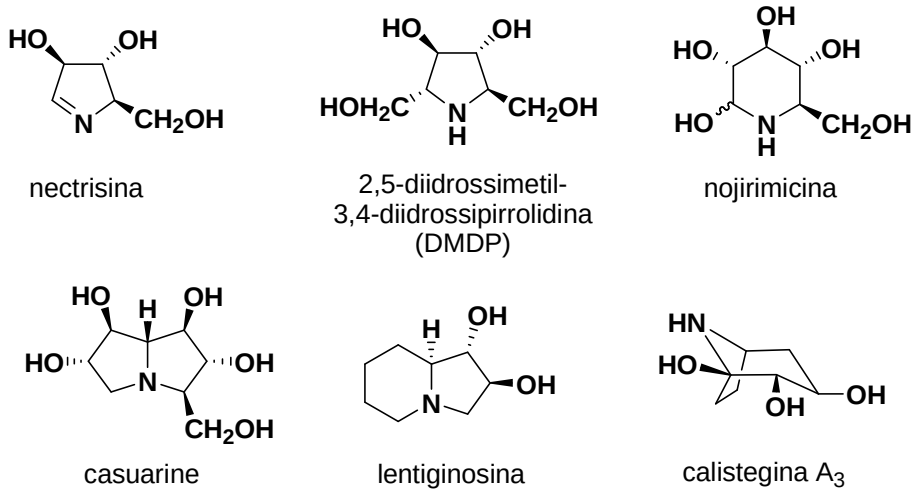


Figure 1.2

As we can see, these compounds present a strong resemblance with sugars. Indeed, in these structures the oxygen atom of the furanose or of the pyranose is replaced by a

nitrogen atom. For this reason, these structures are also called *iminosugars*. This feature produces in many case a very potent and selective activity as competitive and reversible inhibitors of glycosidases,[1] enzymes that break in a specific way the *O*-glycosidic or *N*-glycosidic bonds between two or more carbohydrates, or between a carbohydrate and a non-carbohydrate mojety, and glycosyltransferases,[2] enzymes that transfer a monosaccharide unit from an activated nucleotide sugar to a glycosyl acceptor molecule, usually an alcohol. The substrates of these enzymes are molecules such as pentose and hexose sugars, disaccharides, oligosaccharides and glycoconjugates. Therefore we can say that iminosugars are also *sugar mimetic.* Since the proved involvement of these enzymes in diseases such as viral infection[3] and tumor metastasis,[4] in the last years these molecules have been attracting vast attention owing to their potential role as therapeutic agents. Unfortunately, these compounds are present in a very low concentration in the organisms that produce them, and, because of their hydroxylation, their extraction is very difficult and inefficient, therefore is very important to find a short and economic synthesis to obtain them.

1.2 The biological activities of Iminosugars

Iminosugars present a wide structural diversity in the basic skeleton (five-membered ring, six-membered ring, simple or fused ring), in the number of hydroxylic functions and in the absolute and relative configuration of the stereocenters. Of course, this structural diversity modulates the kind and the potency of the activity of each one of them. Anyway, the most important feature that many molecules that belong to this class of compounds present is the ability to inhibit the glycosidases in a reversible and competitive manner. These families of ubiquitous enzymes are involved in a lot of key

[1] Watson, A.A.; Fleet, G.W.J.; Asano, N.; Molyneux, R.J.; Nash, R.J. *Phytochemistry* **2001**, *56*, 265-295.
[2] Compain, P.; Martin, O.R. *Bioorg. Med. Chem.***2001**, *9*, 3077.
[3] Mehta, A.; Ziztmann, N.; Rudd, P.M.; Block, T.M.; Dwek, R.A. *FEBS Lett.***1998**, *430*, 17-22.
[4] Dennis, J.W.; White, S.L.; Freer, A.M.; Dime, D. *Biochem. Pharmacol.* **1993**, *46*, 1459-1466.

biological processes such as degradation of polysaccharides and glycoconjugates and overall in the biosynthesis, transformation and degradation of one of the most important class of biological molecules, the membrane glycoproteins.

Glycosylation is a form of co-translational and post-translational modification in which glycosidases build a specific sugar antenna on the protein which is forming. The number and the typology of the involved monosaccharides and the kind of the linkage establish an accurate biological message.

Glycoproteins are complexes in which a sugar portion is covalently linked to an asparagine residue (*N*-glycans) or a serine or threonine residue (*O*-glycans) of a protein. These molecules are involved in a lot of crucial biological processes such as cell-cell adhesion and exogen molecules recognition, but also in a lot of pathologic methabolic pathways like viral and bacterial infection and tumor metastasis (Figure 1.3).

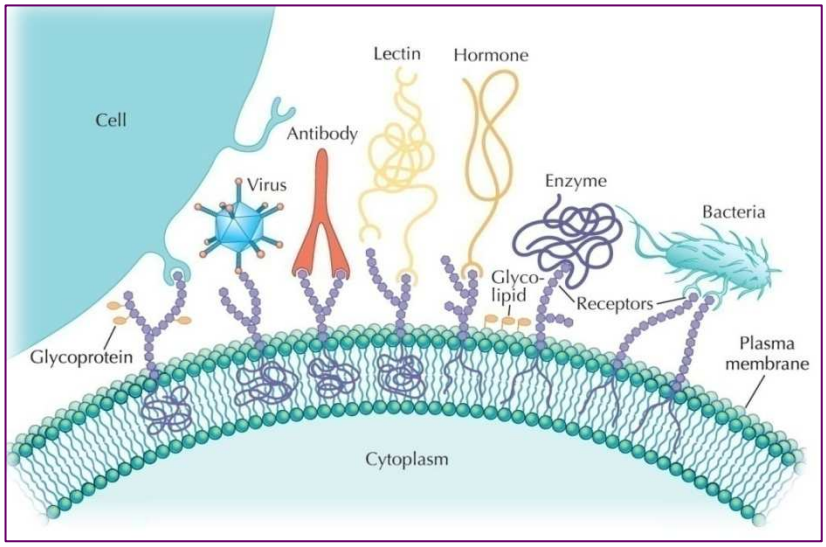


Figure 1.3

The oligosaccharide chains of these molecules play an important role in the correct functioning of these proteins by stabilizing them and ensuring that they have the correct

conformation. They may also be involved in the targeting mechanism of certain proteins.

Among the most important biological functions of the membrane glycoproteins, we find the process of cell recognition. One very interesting example is represented by the neuronal adherence mediated by *N-CAM* membrane proteins.[5] *N-CAM* is a proteic component of cellular surface that has a key role in the neuronal aggregation. This structure is constituted of three regions: an extra-cellular mojety, a portion that crosses the cellular membrane and a cytoplasmatic domain. The *N-CAM* molecules on adjacent cells interact one with each other building links between cells. A very crucial feature of this interaction is that *N-CAM* protein can be made adhesive or repulsive through modification with a sialic acid unit, an elaborate saccharide (10 carbon atoms) which presents a negative charge. The molecules of sialic acid are covalently linked in the extra-cellular domain of the protein and can drastically reduce the adhesiveness of a cell respect to another one. Cells with relatively low amounts of sialic acid linked to the *N-CAM* proteins aggregate four times more quickly than those that contains high levels of sialic acid. During the progressive growth of the embryo, most of the *N-CAM* proteins mature from an high sialic acid content form to a low sialic acid content form that stabilizes mature tissues. In this way, *N-CAM* proteins play a role both in stimulating two near cells to make contact regions and in suppressing this contact. If the two cells express the low sialic acid content form of *N-CAM* protein, a strong adherence is promoted. *Vice versa*, if the cells express the high sialic acid content form, the adherence will be weak or inhibited (Figure 1.4).

[5] Hoffman, S.; Edelman, G.M. *Adv. Exp. Med. Biol.* **1984**, *181*, 147-160.

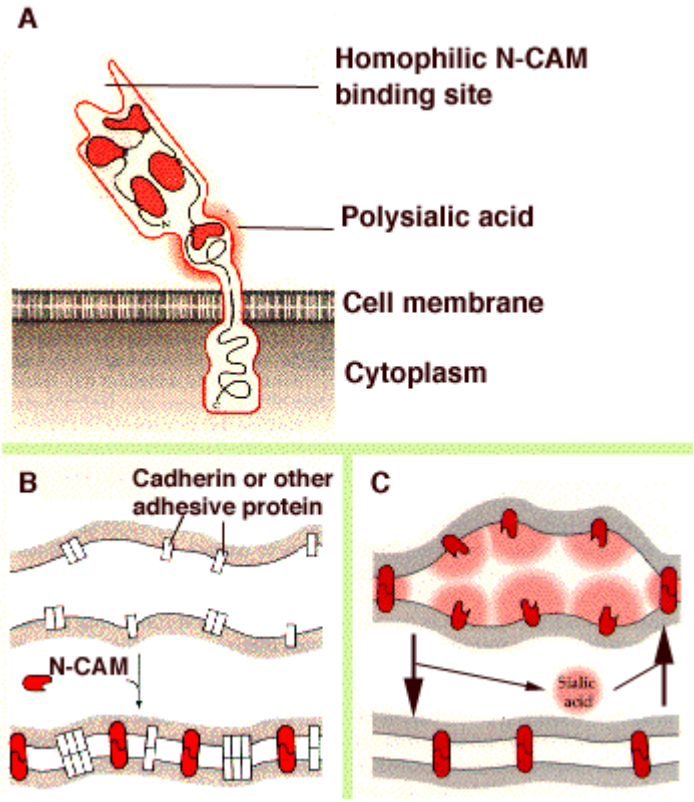


Figure 1.4

Morover, carbohydrate mojety of blood glycoproteins and of glycoproteic hypophysis hormones are involved in the kidney clearance and in the addressing of the hormones to their relative target organs.

Therefore, glycoproteins are an aggregation tool for the cells that constitute a multicellular organism, but, at the same time, they also are involved in pathologic mechanisms such as metastasis processes and, because they are expressed on the cellular surfaces of the cells that coat organs (like windpipe, stomach and intestine), in the development of viral infections.

In the case of the development of the tumor metastasis, the role of the glycoproteins is absolutely crucial.[6] Cell migration is an essential process of the mechanism of

[6] Janik, M.E.; Lityńska, A.; Vereecken, P. *Bioch. Bioph. Acta Gen. Subj.* **2010**, *1800*, 545-555.

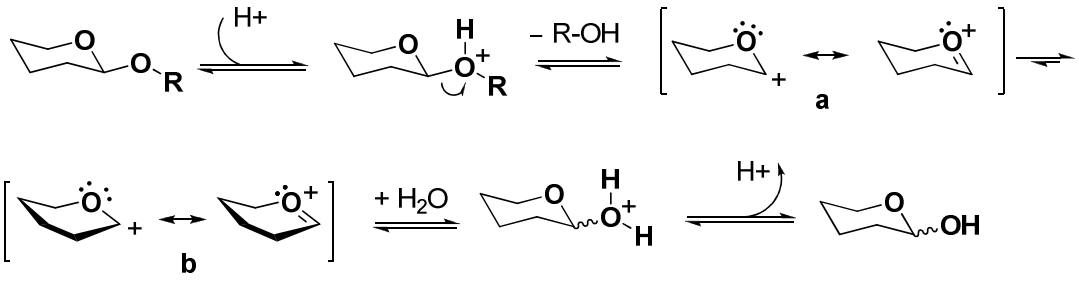
metastasis. The extra cellular matrix (ECM) represents the molecular scaffold for cell migration. Therefore, adhesion of the cells to the ECM is critical. Engagement of integrin receptors with ECM ligands gives rise to the formation of complex multiproteins which link the ECM to the cytoplasmic actin skeleton. Both ECM proteins and the adhesion receptors are glycoproteins, and it is well accepted that *N*-glycans modulate their conformation and activity, thereby affecting cell-ECM interactions. Likely targets for glycosylation are the integrins, whose ability to form functional dimers depends upon the presence of *N*-linked oligosaccharides. Cell migratory behaviour may depend on the level of expression of adhesion proteins, and their *N*-glycosylation that affect receptor-ligand binding.

In regard to the involvement of glycoproteins in viral infections, in human immunodeficiency virus (HIV) and in hepatitis B virus (HBV), *N*-linked oligosaccharides play a crucial role in the folding of viral glycoproteins by mediating interactions with the lectin-like chaperone proteins calnexin and calreticulin with nascent glycoproteins.[3] These interactions can be prevented by inhibitors of the α-glucosidases and this causes some proteins to be misfolded and retained within the endoplasmic reticulum, interfering with the viral life cycle.

1.3 Mechanism of action of glycosidases

As we already said, glycosidases are enzymes that catalyze the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates. It is possible to make a classification on the basis of the number, the position or the configuration of the hydroxy groups in the substrate. Glycosidases that are able to catalyze the cleavage of glycosidic bonds involving terminal glucose connected at the site of cleavage through an α-linkage at the anomeric center are classified as α-glucosidases. In the same way, if the configuration

of the anomeric carbon is β, we can talk about β-glucosidases. The great potency and specificity of the iminosugars as glycosidase inhibitors is due to their capacity to mimic the transition state of the pyranosidic or furanosidic units of the natural glycosidase substrates.[7] Since competitive inhibition is observed with a lot of inhibitors, probably both conformational and electrostatic influences are important in the active site binding. In Scheme 1.1 is shown the cleavage of a glycosidic bond catalyzed by β-glycosidases. Iminosugars or polyhydroxylated alkaloids can mimic the conformation and charge of the oxycarbenium ion intermediate, generated in the transition state during the glycosidic bond cleavage (see Scheme 1.1, **a** and **b**), preferably in its half-chair conformation **b** than in the chair conformation **a.** Through double or single nucleophilic substitution it is possible to obtain a retention or an inversion of the anomeric carbon configuration.

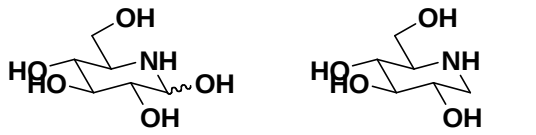


Scheme 1.1

The partial cleavage of the glycosidic bond enhances the positive charge generated on the oxygen or anomeric carbon of the natural substrate. The substitution of one of the two atoms by protonated nitrogen will mimic, in the transition state, the charge in these centers.[8]

[7] Borges de Melo, E.; da Silveira Gomes, A.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277-10320.
[8] Szczepina, M.G.; Johnston, B.D.; Yuan, Y.; Svensson, B.; Pinto, M.B. *J. Am. Chem. Soc.* **2004**, *126*, 12458-12469.

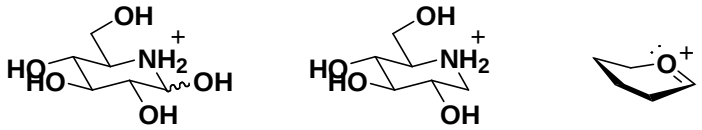
Nojirimycin was isolated from microorganisms (*Streptomyces)* from Ishida et al.[9] Afterwards, 1-deoxinojirimycin was synthesized by Inouye et al (Figure 1.5).[10]



Nojirimycin 1-Deoxinojirimycin

Figure 1.5

Both these molecules are potent glucosidase inhibitors, in fact they are able to mimic when protonated (**I** and **II**) the charge development of the transition state resembling glucosyl oxycarbenium ion **b,** but they have a chair conformation instead of the expected half-chair conformation of **b** (Figure 1.6).



I **II** **b**

Figure 1.6

It is not possible that the transition state has a chair conformation when there is a charge on the ring oxygen. Therefore, **I** and **II** can not be expected to be perfect transition state analogues.

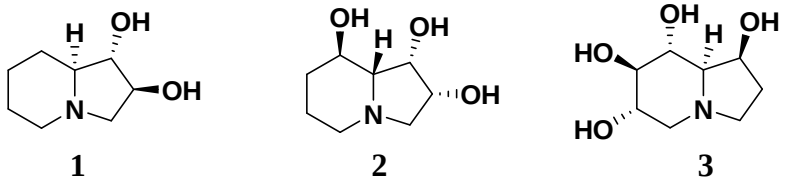
In substance, the significant factors to obtain the inhibition of glycosidases are the charge and the shape of the transition state, defined by the hybridization and the conformation of the pyranose ring in the natural substrate and piperidine ring in the inhibitors. It is fundamental that inhibitors are protonated to interact, through hydrogen bonds, with the catalytic site.

[9] Ishida, N.; Kumagai, K.; Niida, T.; Tsuruaka, T.; Yumato, H. *J. Antibiot.* **1967**, *20*, 66-71.
[10] Inouye, S.; Tsuruaka, T.; Ito, T.; Niida, T. *Tetrahedron* **1968**, *24*, 2125-2144.

Polyhydroxylated pyrrolidine, piperidine and indolizidine alkaloids are able to inhibit the hydrolytic reaction.[11] The spatial distribution of the hydroxy groups of these compounds allows the formation of a series of hydrogen bonds inside the catalytic enzymatic cavity that ease the recognition of the alkaloid as natural substrate. However, the presence of the nitrogen atom in the ring instead of oxygen determines the fundamental difference on which is based the inhibition mechanism of this class of compounds. At a physiological pH the nitrogen atom is protonated, therefore the alkaloid works like the hydrolysis intermediate of the natural sugar, stabilizing itself in the enzymatic cavity. This kind of structure cannot be hydrolyzed because there is no more electronic availability on the heteroatom. In consequence, enzymatic activity is stopped.

1.4 Lentiginosine and its unnatural enantiomer

Iminosugars such as (+)-lentiginosine (**1**), (–)-swainsonine (**2**) and (+)-castanospermine (**3**) are polyhydroxylated alkaloids with an indolizidine structure (Figure 1.7).



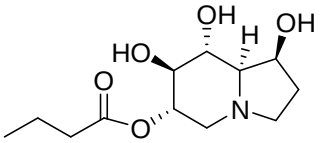
1 **2** **3**

Figure 1.7

(–)-Swainsonine (**2**) is a natural compound isolated in 1973 from the fungus *Rhizoctonia leguminicolain*[12] and is a potent inhibitor of α-D–mannosidase that can interferes with virus proliferation and the metastatic process.[13]

[11] (a)Legler, G. *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 319; (b) Asano, N.; Kizu, H.; Oseki, K.; Tomioka, E.; Matsui, K.; Okamoto, M.; Baba, M. *J. Med. Chem.* **1995**, *38*, 2349.
[12]a) Guengerich, F.P.; DiMari, S.J.; Broquist, H.P. *J. Am. Chem. Soc.* **1973**, *95*, 2055; b) Schneider, M.J.; Ungemach, F.S.; Broquist, H.P.; Harris, T.M. *Tetrahedron* **1983**, *39*, 29-32.

(+)-Castanospermine (**3**) is another natural indolizidine alkaloid and was isolated from the seeds of the Australian legume *Castanospermum australe.*[14] This iminosugar has potential for the treatment of viral infection[15], cancers[16] and diabetes.[17] Celgosivir,[18] a 6-butanoil derivative of castanospermine is already on the market as a drug against Gaucher's disease.



Celgosivir

(+)-Lentiginosine [(1*S*,2*S*,8a*S*)-octahydro-1,2-indolizinediol] (**1**) was discovered in the extract of the leafs of *Astragalus lentiginosus* and characterized for the first time in 1990 from Pastuszak et al..[19] It is the least hydroxylated compound of the series and the most recent to be studied. This compound has shown a very promising and selective inhibitory activity against amyloglucosidase, higher than that of castanospermine.[20] Indeed, enzymatic tests carried out on the molecule showed that it has inhibitory activity against *Aspergillus niger* and *Rhizopus* mold amyloglucosidases (EC 3.2.1.3) with an half maximal inhibitory concentration (IC_{50}) equal to 0.43 μg/mL (amyloglucosidase from *Aspergillus niger*) and to 0.48 μg/mL (amyloglucosidase from *Rhizopus* mold). Furthermore, with amyloglucosidase from *Aspergillus niger* synthetic

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[13] a) Olden, K.; Breton, P.; Grzegorzewski, K.; Yasuda, Y.; Gause, B.L.; Oredipe, O.A.; Newton, S.A.; White, S.L. *Pharmacol. Ther.* **1991**, *50*, 285-290; b) Galustian, C.; Foulds, S.; Dye, J.F., Guillou, P.G. *Immunopharmacology* **1994**, *27*, 165-172.

[14] Hohenschutz, L. D.; Bell, E. A.; Jewess, P. J.; Leworthy, D. P.;Pryce, R. J.; Arnold, E.; Clardy, J. *Phytochemistry* **1981**, *20*, 811-814**.**

[15] Durantel, D. *Curr. Opi.Investig. Drugs* **2009**, *8*, 860-870.

[16] Yee, C. S.; Schwab, E. D.; Lehr,J. E.; Quigley, M.; Pienta, K. J. *Anticancer Res.* **1997**, *17*, 3659-3663.

[17] Rhinehart, B. L.; Robinson, K. M.; Payne, A. J.; Wheatley, M. E.; Fisher,J. L.; Liu, P. S.; Cheng, W. *Life Sci*. **1987**, *41*, 2325-2331**.**

[18] Sorbera, L.A.; Castaner, J.; Garcia-Capdevila, L. *Drugs of the Future* **2005**, *30*, 545.

[19]Pastuszak, I.; Molyneux, R. J.; James, L. F.; Elbein, A. D. *Biochemistry* **1990**, *29*, 1886.

[20] a) Brandi, A.; Cicchi, S.; Cordero, F.M.; Frignoli, R.; Goti, A.; Picasso, S.; Vogel, P. *J. Org. Chem.* **1995**, *60*, 6806-6812; b) Cardona, F.; Goti, A.; Brandi, A. *Eur. J. Org. Chem.*, **2007**, 1551-1565.

(+)-lentiginosine showed inhibition (K_i = 2 μM) five times stronger than that reported for natural (+)-lentiginosine. Therefore, (+)-lentiginosine is the most potent and selective inhibitor of amyloglucosidases among azasugars with indolizidine structure.

Amyloglucosidase is not the only target of this very interesting molecule. A recent research performed by the group of Prof. Nunziatina de Tommasi of the Pharmaceutical Sciences Department of the University of Salerno showed that (+)-lentiginosine has an inhibitory activity against the human *Heat Shock Protein* 90 (Hsp 90). This protein has an important role in the cell proliferation, differentiation and apoptosis. For this reason, there is a growing interest towards this molecule as a target for potential anticancer drugs.[21] In deseases such as chronic pancreatic tumors[22] or breast cancer[23] is observed an enhanced Hsp 90 expression. Several inhibitors of this protein are already in advanced clinical phase (Figure 1.8).

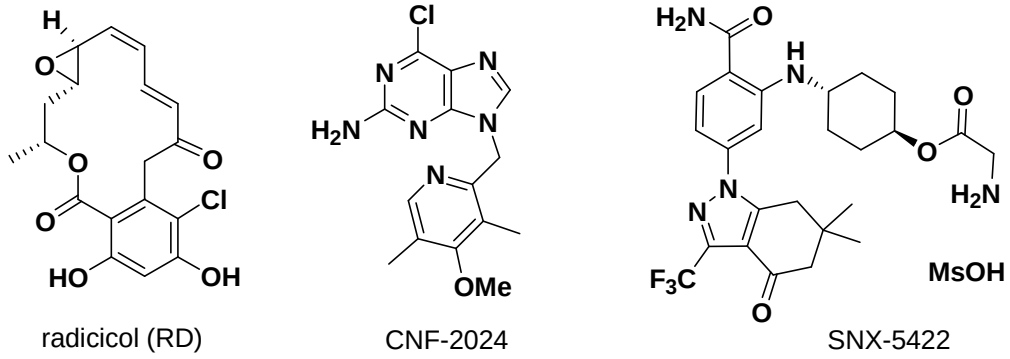
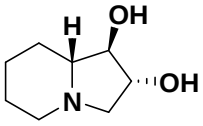


Figure 1.8

Radicicol (RD) has been extracted from *Monosporium bonorden* and represented the point of departure for the development of synthetic inhibitors such as CNF-2024 and SNX-5422.

The unnatural enantiomer of (+)-lentiginosine is (-)-lentiginosine (**4**) (Figure 1.9).[20a]

[21] Sreedhara, A. M.; Kálmara, É.; Csermely, P.; Shen, Y.-F. *FEBS Lett.* **2004**, *562*, 11.
[22] Ogata, M.; Naito, Z.; Tanaka, S.; Moriyama, Y.; Asano, G. *J. Nippon Med. Sch*. **2000**, *67*, 177-185.
[23] Jameel, A.; Skilton, R.A.; Campbell, T.A.; Chander, S.K.; Coombes, R.C.; Luqmani, Y.A. *Int. J. Cancer* **1992**, *50*, 409-415.



4

Figure 1.9

This compound has an activity profile very different in respect to (+)-lentiginosine. Its inhibition activity against amyloglucosidase from *Aspergillus niger* is much lower than that of (+)-lentiginosine (IC_{50} =17 μg/ml) and is not able to inhibit Hsp 90 protein. Despite that, this molecule showed capacity to induce apoptosis.[24] Experiments performed on healthy donors and tumoral cell culture, showed that (-)-lentiginosine, when present in concentration in the order of 200 μM, possesses a proapoptotic activity but, at the same time, has a citotoxicity clearly lower in respect to that of a chemoterapic agent such as SN38 (7-ethyl-10-hydroxycamptothecin) (Table 1.1).

Cell lines	**Compounds**	**$MAIC_{50}$± SD (μM)**
MOLT3 (human acute lymphoblastic T cells)	(-)-lentiginosine	213.33 ± 96.62
	(+)-lentiginosine	>1000
	SN38	14 ± 2
SHSY5Y (neuroblastoma cells)	(-)-lentiginosine	95.5 ± 19.09
	(+)-lentiginosine	>1000
	SN38	<0.1
HT29 (human colorectal adenocarcinoma cells)	(-)-lentiginosine	577 ± 101.3
	(+)-lentiginosine	>1000
	SN38	<1

[24] Macchi, B.; Minutolo, A.; Grelli, S.; Cardona, F.; Cordero, F.M.; Mastino, A.; Brandi, A. *Glycobiology* **2010**, *20*, 500-506.

PBMCs (healthy peripheral blood mononuclear cells)	(-)-lentiginosine	384.52 ± 49.02
	(+)-lentiginosine	>1000
	SN38	<1

Table 1.1

For the experimental tests four cell lines were used. In the table, the levels of citotoxicity of (-)-lentiginosine, (+)-lentiginosine and SN38 are compared. The citotoxicity is expressed as the necessary concentration to inhibit 50% of mitochondrial enzymatic activity ($MAIC_{50}$). As high as is $MAIC_{50}$ value, minor will be the toxicity of the compound.

In Figure 1.10 is showed a comparison of the proapoptotic activity of SN38, (-)-lentiginosine (**4**) and (+)-lentiginosine (**1**) on the four cell lines. The activity is expressed as % of hypodiploid nuclei.

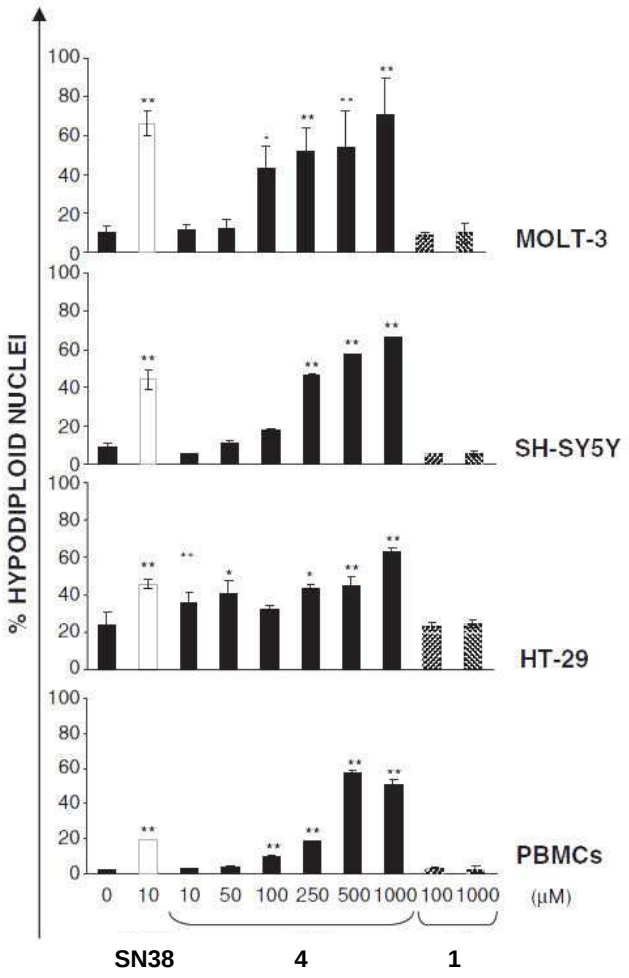


Figure 1.10

The study of the activity of (-)-lentiginosine has shown that cell death induced by the molecule has the typical characteristic of apoptosis, because of the incremented expression and activity of caspase (*c*ysteine-*asp*artic prote*ases*), enzyme that plays an essential role in apoptosis (but also in necrosis and inflammation). Unfortunately, the sequence of events in the caspase cascade induced by (-)-lentiginosine is not yet defined. Further investigation is needed to clarify the relationship between apoptotic, toxic effects of (-)-lentiginosine and events.

1.5 Aim of the thesis

Because of the very interesting biological activities of (+)-lentiginosine and of its enantiomer (-)-lentiginosine, the principal goal of this thesis was to design a

straightforward synthetic method to obtain derivatives of lentiginosine starting from the convenient intermediates of its synthesis carried out in Prof. A. Brandi's group. The derivatives planned were intended to conjugate the lentiginosine structure with amino acids, aromatic and heteroaromatic structures, hydrophilic and hydrophobic functionalities.

A series of derivatives of the two molecules have been synthesized with the aim to define and optimize a very simple, efficient and versatile synthetic method to obtain derivatives of the two compounds. In the case of (+)-lentiginosine, of which is exactly known the target, the synthesis of new derivatives is directed to find molecules which can have more potency in the inhibition of amyloglucosidase. In the case of (-)-lentiginosine, the obtaining of new derivatives was finalized to the study of its mechanism of action, in particular to find its exact target.

1.5.1 The mechanism of interaction between (+)-lentiginosine and glucoamylase

The choice of the derivatives to synthesize has been made on the basis of computational studies performed by the group of Prof. Paola Gratteri of the Pharmaceutical Sciences Department of the University of Florence.

At this point it is appropriate to illustrate the interaction mechanism of our lead compound, (+)-lentiginosine.

Glucoamylase is a glucosidase which catalyzes the hydrolysis of β-D-glucose from the non-reducing ends of starch (and other related oligo- and polysaccharides).[25] This enzyme cleaves the α-1,4-glucosidic bond preferentially and, at a slower rate, the α-1,6-

[25] (a) Weill, C. E.; Burch, R. J.; Van Dyk, J. W. *Cereal Chem.* **1954**, *31*, 150-158. (b) Manjunath, P.; Shenoy, B. C.; Raghavendra Rao, M. R. *J. Appl. Biochem.* **1983**, *5*, 235-260.

glucosidic bond.[26] Glucoamylase is inhibited by sugar analogues having a structure which resembles that of the enzyme's natural substrate, amylose in this case.

In Figure 1.11[27] are compared the natural glucoamylase substrate amylose, (+)-lentiginosine (**1**) and 1-deoxynojirimycin (**DNJ**), maybe the most investigated iminosugar.[28]

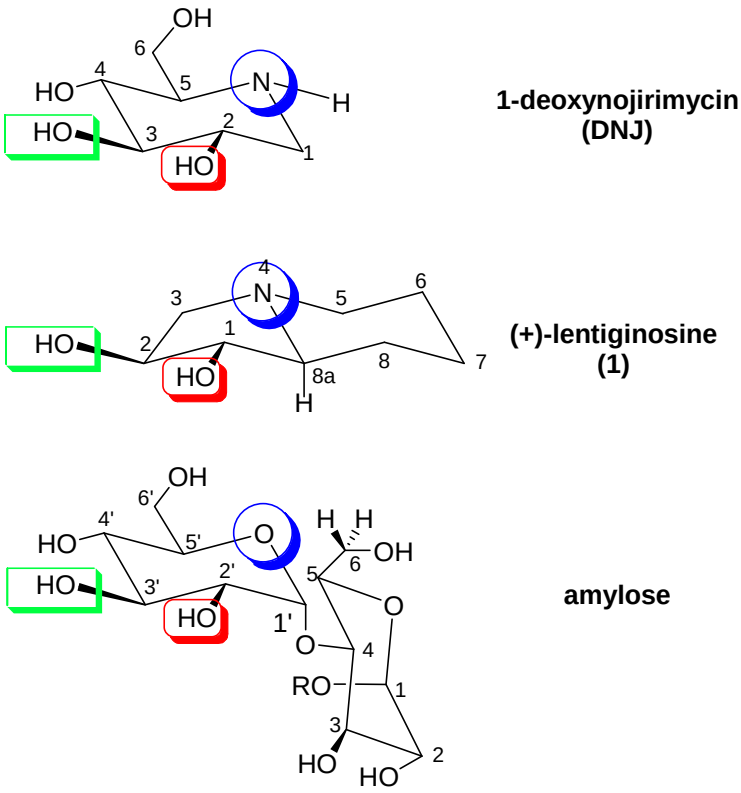


Figure 1.11[27]

As we already said, one of the most important feature of iminosugars is that they have a nitrogen atom instead of the oxygen atom presents in the sugar structure. In contrast of **DNJ** and of natural substrate amylose, (+)-lentiginosine (**1**) has only two hydroxy groups and these functional groups are not positioned on the six-membered ring, like in the case of the other two compounds, but on the five-membered ring. Accordingly, the

[26] Abdullah, M.; Fleming, I. D.; Taylor, P. M.; Whelan, W. J. *Biochem. J.* **1963**, *89*, 35-36.
[27] Cardona, F.; Goti, A.; Brandi, A.; Scarselli, M.; Niccolai, N.; Mangani, S. *J. Molec. Model.* **1997**, *3*, 249-260.
[28] *Iminosugars—From Synthesis to Therapeutic Applications*; Compain, P., Martin, O.R., Eds.; Wiley: Chichester**, 2007**.

analogy of (+)-lentiginosine with the natural substrate is not immediate. But, if we observe Figure 1.11, it is immediately clear that, beside the presence of the basic nitrogen atom, other two features are essential for the activity: the *trans* configuration of the two hydroxy groups and the *S,S* absolute configuration of the carbons bearing these groups. Indeed, compounds that do not posses a hydroxy function on C-2 or that have a *cis*-dihydroxypyrrolidine unit are inactive.[19,29] Furthermore, (-)-lentiginosine, which has an *R,R* absolute configuration on the C-1 and C-2 carbons, is 35 times less active than (+)-lentiginosine.[20a]

The computational docking studies were performed using the Glucoamylase II (α-1,4-D-glucan glucohydrolase, EC 3.2.1.3, *inverting glycosidase*) from *Aspergillus awamori* (PDB: DOG1; structure determined by X ray as complex with 1-deoxynojirimycin).[30] Before performing the computational studies, an evaluation of the protonation state was carried out (pH 7± 0.2).

In Figure 1.12 is shown the catalytic site of the enzyme.

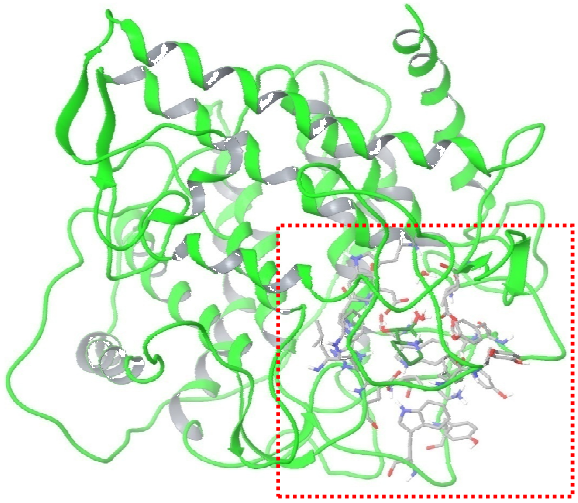


Figure 1.12

[29] Goti, A.; Cardona, F.; Brandi, A.; Picasso, S.; Vogel, P. *Tetrahedron: Asymmetry* **1996**, *7*, 1659-1674.
[30] Harris, E. M. S.; Aleshin, A. E.; Firsov, L. M.; Hontzatko, R. B.*Biochemistry* **1993**, *32*, 1618-1626.

In Figure 1.13 is illustrated the interaction model between the enzyme and (+)-lentiginosine.

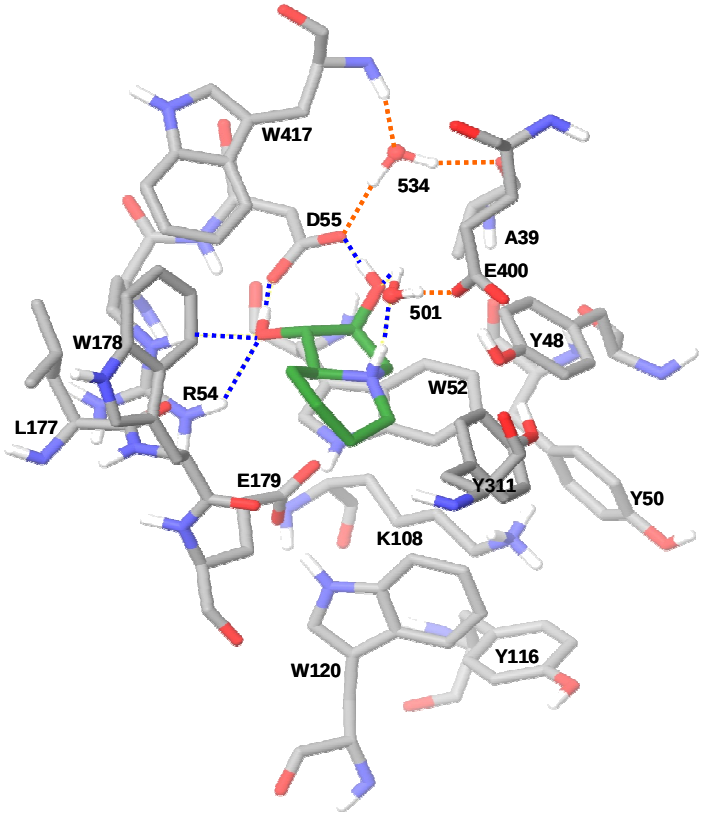
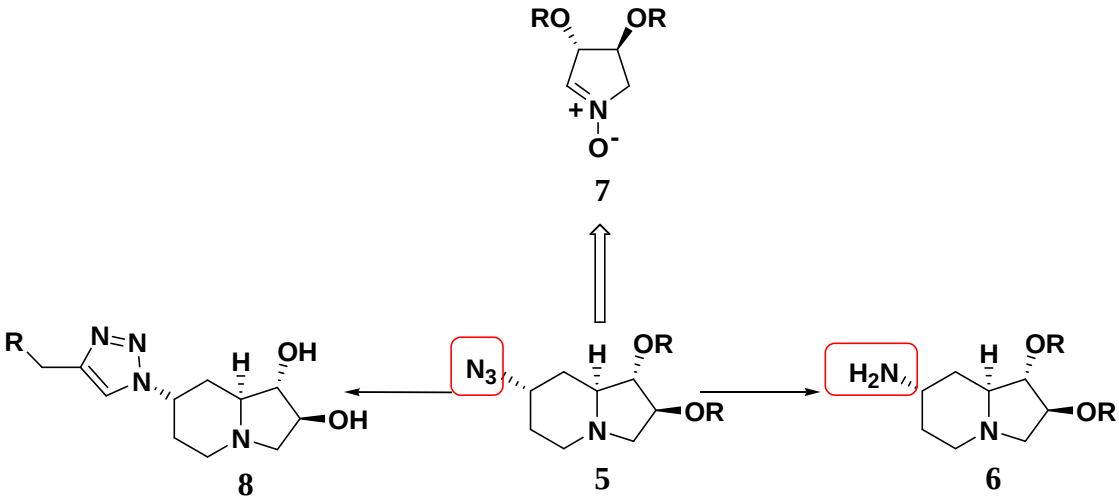


Figure 1.13

As we can see in the figure, the two hydroxy groups establish two fundamental hydrogen bonds with two aminoacid residues of the cavity, the **D55** aspartate and the **R54** arginine. The nitrogen atom forms a hydrogen bond with the water molecule **W501**, which has a catalytic role. This type of interaction suggested that a substitution on the mojety of the molecule not crucially involved in the interaction with the enzyme, that is the six-membered ring, should not interfere with the favourable interaction, but on the contrary could represent a tool to obtain derivatives, possibly more active than the lead compound, through the connection of groups that could improve the interaction with the target. Therefore, we considered the possibility of synthesising a new indolizidine functionalized on the six-membered ring that, apart being at the same time a new derivative by itself, could be a tool for the easy and stable indroduction of new

functionalities on the lentiginosine structure. The new 7*S*-azidolentiginosine (**5**) and 7*S*-aminolentiginosine (**6**) were, then, synthesized (Scheme 1.2, Section 2).



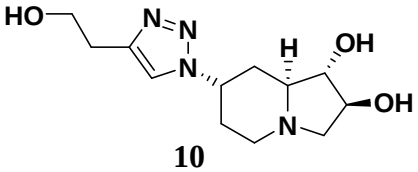
Scheme 1.2

The amino group on the C-7 of **6** is usable for coupling reactions to obtain, for example, amino acid conjugates. Furthermore, this compound is itself a derivative of lentiginosine and was texted too. However, the most capitalized intermediate in the search of new derivatives was the azide **5.** First of all, the azide is a precursor of the 7-amino derivative. Moreover, it was possible to use the compound to carry out a series of very efficient and simple 1,3-dipolar cycloadditions (see Section 2) between the azide and some different alkynes that led to a series of different derivatives all containing a triazole ring that has, by itself, some biomimetic character.[31] The performed computational studies suggested that various substituents could be favourably accommodated in the enzyme cavity (hydrophilic, lipophilic, aromatic).

One of the first hypothesized structures was that in which the indolizidine core of the lentiginosine was joined with a medium-sized chain bringing at the end a hydrophilic

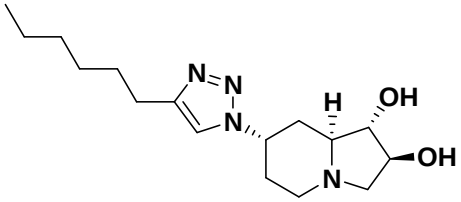
[31] Kolb, H.C.; Sharpless, K.B. *Drug Discovery Today* **2003**, *8*, 1128-1137.

group. Starting from the azide **5** was carried out a Huisgen cycloaddition between the azide and the available 3-butyn-1-olo (**9**), with the obtainment of the structure **10**.

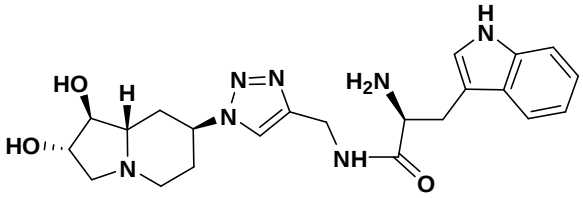


10

With the same synthetic strategy were obtained the molecules **11** and **12**.

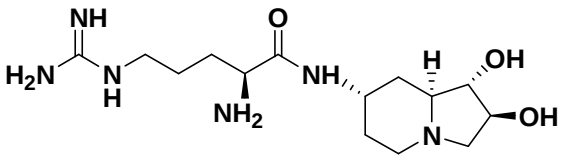


11



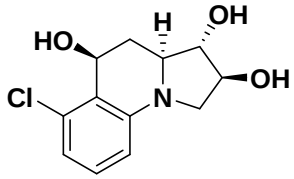
12

Furthermore, we considered the possibility of performing a coupling reaction between the *7S*-aminolentiginosine and the natural amino acid arginine to produce the compound **13**.

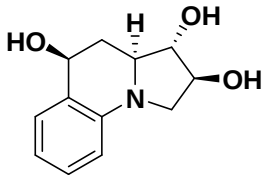


13

Moreover, it was hypothesized that the presence of a fused aromatic system in the *e* position of the indolizidine structure could stabilize the inhibitor in the enzymatic cavity. For this reason we synthesized the two compounds **14** and **15** on the basis of the computational *docking* studies illustrated in Figure 1.14 and Figure 1.15.



14



15

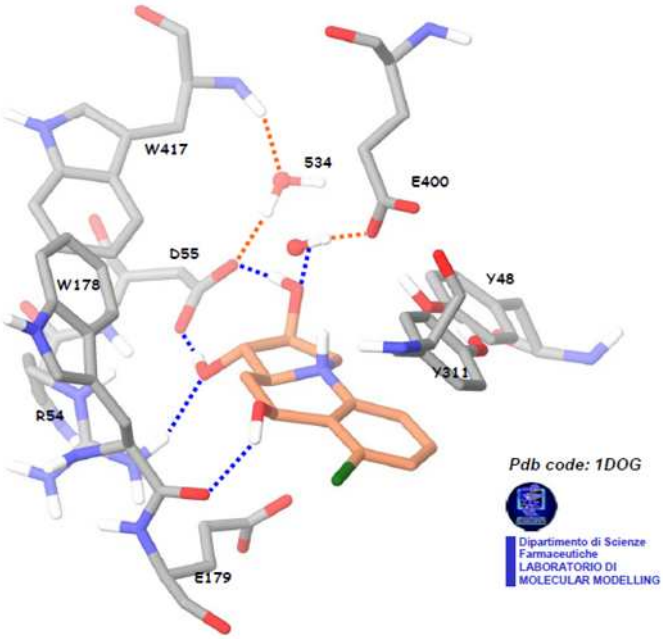


Figure 1.14

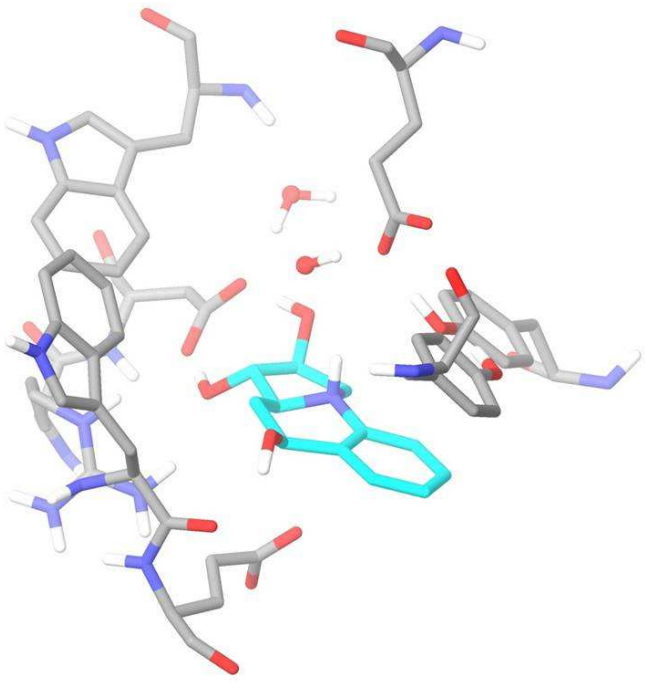
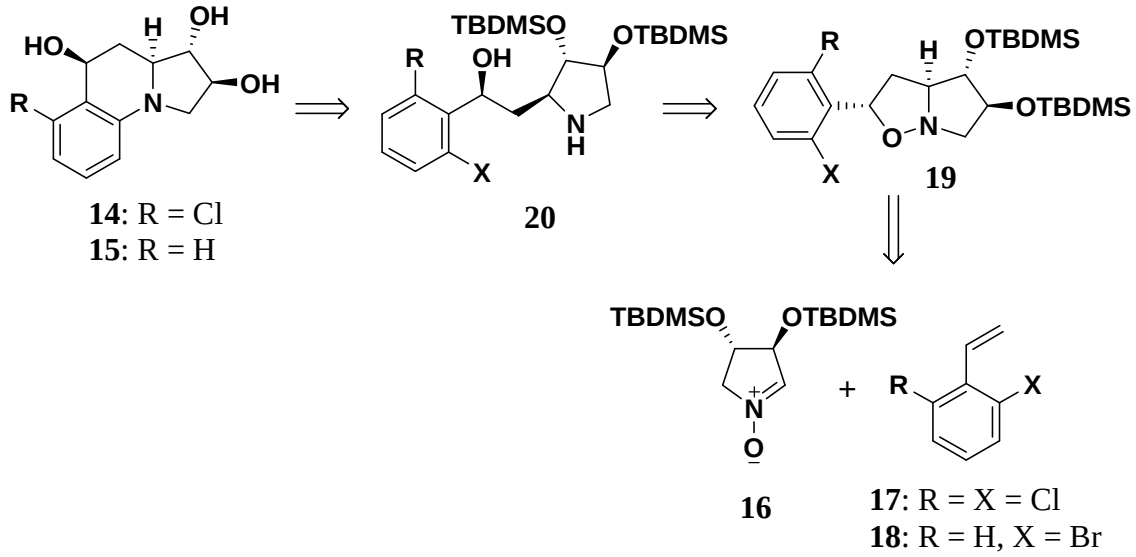


Figure 1.15

The π-π *stacking* interaction between the benzofused system and a phenylalanine residue positioned at the access of the enzymatic pocket promises to stabilize the interaction of the compound with the enzyme. To obtain compounds **14** and **15** we decided to carry out a 1,3-dipolar cycloaddition between the nitrone **16** and a conveniently functionalized styrene, in our case 2,6-dichlorostyrene (**17**) and 2-bromostyrene (**18**) (Scheme 1.3).



14: R = Cl
15: R = H

20

16

17: R = X = Cl
18: R = H, X = Br

Scheme 1.3

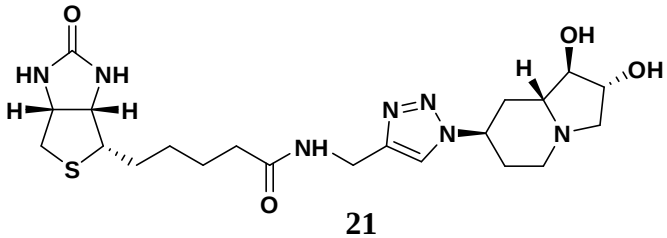
It was supposed that **14** and **15** could be obtained by the cyclization between the fragment containing the aryl halide and the one containing the pyrrolidinic unit of the compound **20** through a metal catalysed aromatic intramolecular amination (reaction of Buchwald - Hartwig), followed by the deprotection of the hydoxy functions. Compound **20** can be obtained by reductive opening of isoxazolidine **19,** attainable by 1,3-dipolar cycloaddition of nitrone **16** with a functionalized styrene.

1.5.2 A new derivative of (-)-lentiginosine to investigate its activity

Functional studies of biologically active molecules can be greatly facilitated by small molecules probes that covalently label the molecule. This application is widely diffused

in drug discovery, target identification and discovery of previously uncharacterized enzyme activity.[32]

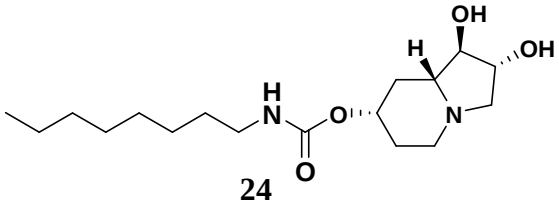
For this reason the derivative **21** containing the marker biotin was synthesized**.**



21

Compound **21** was synthesized starting from the 7*R*-azidolentiginosine (**22**) through a Huisgen cycloaddition between the azide and a propargyl amide of biotin (**23**) (see Section 2).

To test the length of the carbon chain, to which has to be linked an opportune marker, and the compatibility of a carbamate junction, also compound **24** was synthesized.

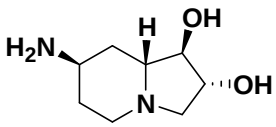


24

The new compound **24** was obtained through a nucleophilic addition of the 7*S*-OH intermediate **25** to octyl isocyanate.

Finally, after reduction of **22** followed by deprotection of hydroxy groups the new derivative 7*R*-aminolentiginosine (**26**) was synthesized and tested.

[32] Evans, M.J.; Cravatt, B.F. *Chem. Rev.* **2006**, *106*, 3279-3301.

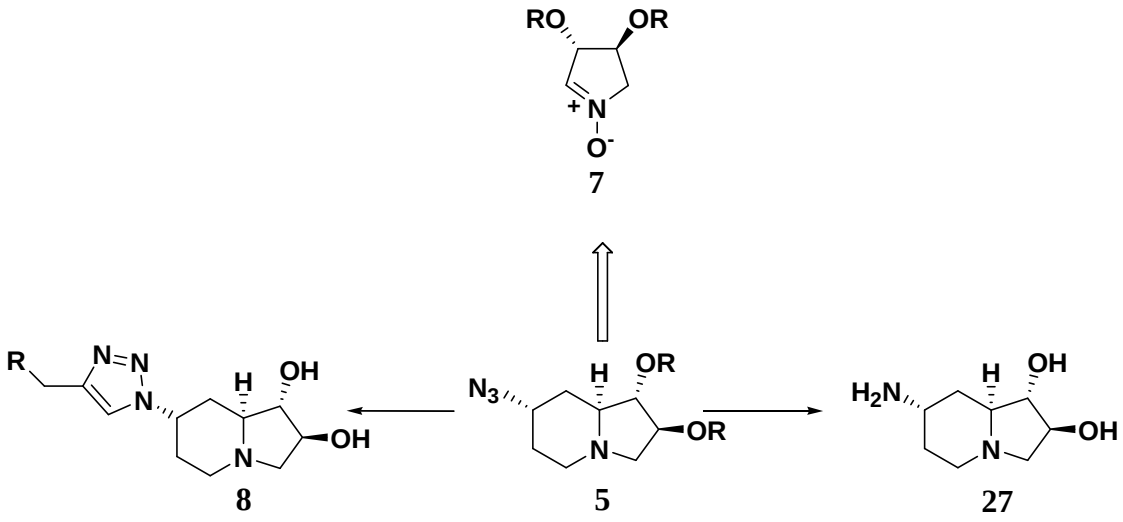


26

2. EFFICIENT AND VERSATILE METHODS TO OBTAIN NEW DERIVATIVES OF (+)-LENTIGINOSINE AND OF ITS UNNATURAL ENANTIOMER (-)-LENTIGINOSINE

2.1 Introduction

In this research project, the new 7*S*-aminolentiginosine (**27**) has been synthesized starting from L-tartaric acid through a ten-step strategy based on the diastereoselective 1,3-dipolar cycloaddition of 3,4-dihydroxylated pyrroline *N*-oxide **7** (Scheme 2.1).[33,34] Analogously, the enantiomer 7*R*-aminolentiginosine **26** has been obtained from D-tartaric acid following the same sequence.

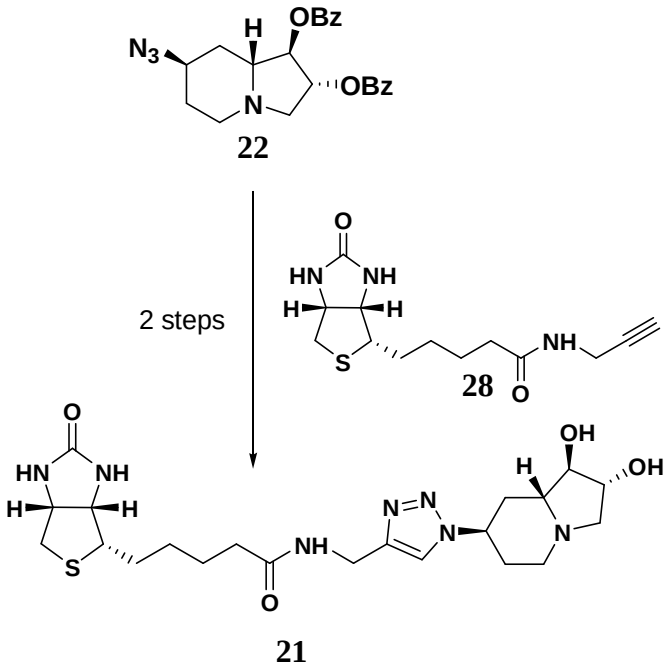


Scheme 2.1

The intermediate azido compound **5** has been chosen as a possible versatile tool for the synthesis of a variety of 7-functionalyzed lentiginosines by running a Huisgen's 1-3 dipolar cycloaddition[35] of the azide with a variety of alkynes (Scheme 2.1).

[33] Brandi, A.; Cardona, F.; Cicchi, S.; Cordero, F.M.; Goti, A. *Enantiopure Pyrroline-N-oxides for the Synthesis of Pyrrolizine and Indolizine Alkaloids*, in: *Current Trends in Organic Synthesis*, (Eds.: C. Scolastico, F. Nicotra), Kluwer Academic/Plenum Publishers, New York, **1999**, pp231-220.
[34] Cordero, F.M.; Bonanno, P.; Neudeck, S.; Vurchio, C.; Brandi, A. *Adv. Synth. Catal.* **2009**, *351*,1155
[35] Huisgen, R. in *1,3-Dipolar Cycloaddition Chemistry;* Padwa, A., Ed.; Wiley: New York, **1984**.

Analogously, the enantiomeric 7*R*-azidolentiginosine (**22**) was employed in the synthesis of the compound **21,** obtained by cycloaddition of the azide with the propargyl amide of biotine (**28**) (Scheme 2.2).



Scheme 2.2

The successful coupling of the amino group of 7*S*-aminolentiginosine **27** with the natural amino acid L-arginine **29** proved the possibility of synthesizing a peptide conjugate containing the indolizidine moiety of lentiginosine **13** (Figure 2.1).

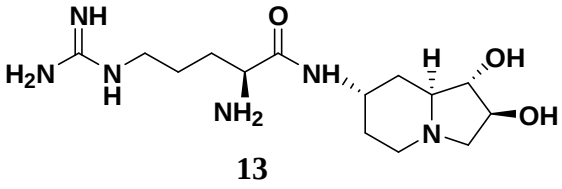
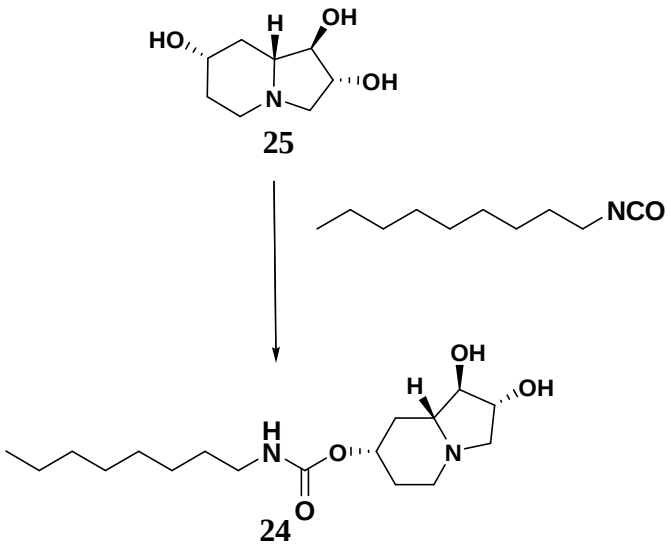


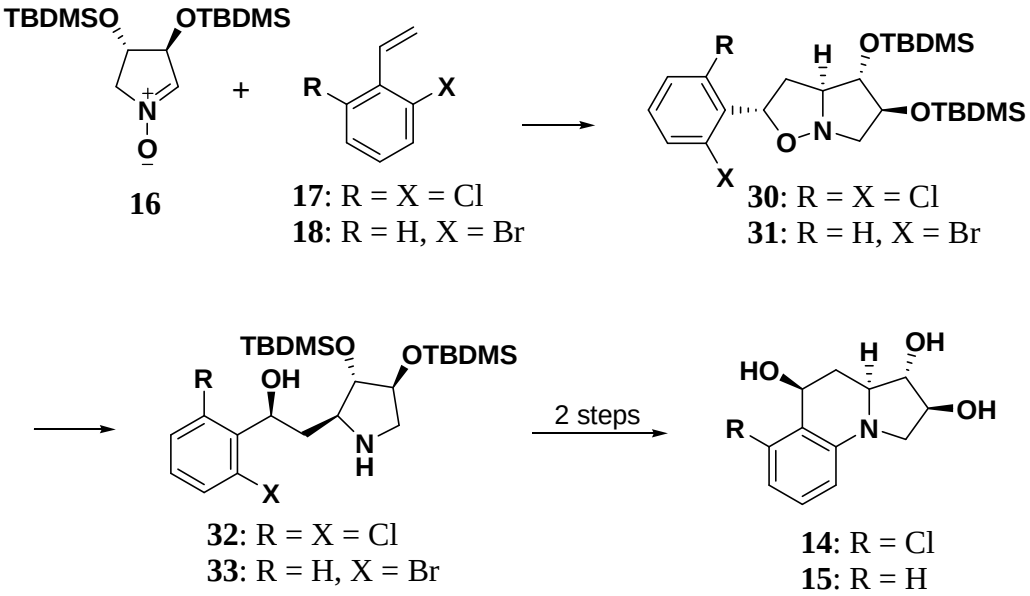
Figure 2.1

Furthermore, the nucleophilic addition of the alcoholic intermediate **25** to octyl isocyanate gave compound **24** (Scheme 2.3).



Scheme 2.3

Finally, starting from the nitrone derived from L-tartaric acid and protected with *tert*-butyldimethylsilyl group (**16**),[36,37] a synthesis of the benzocondensated derivatives **14** and **15** has been studied. In particular, these compounds were prepared by 1,3-dipolar cycloaddition between **16** and a suitably functionalized styrene, such as 2,6-dichlorostyrene **17** and 2-bromostyrene **18**, followed by reductive opening of the isoxazolidine intermediates **30** and **31** and a transition metal catalysed intramolecular amination of **32** and **33** (Scheme 2.4).



Scheme 2.4

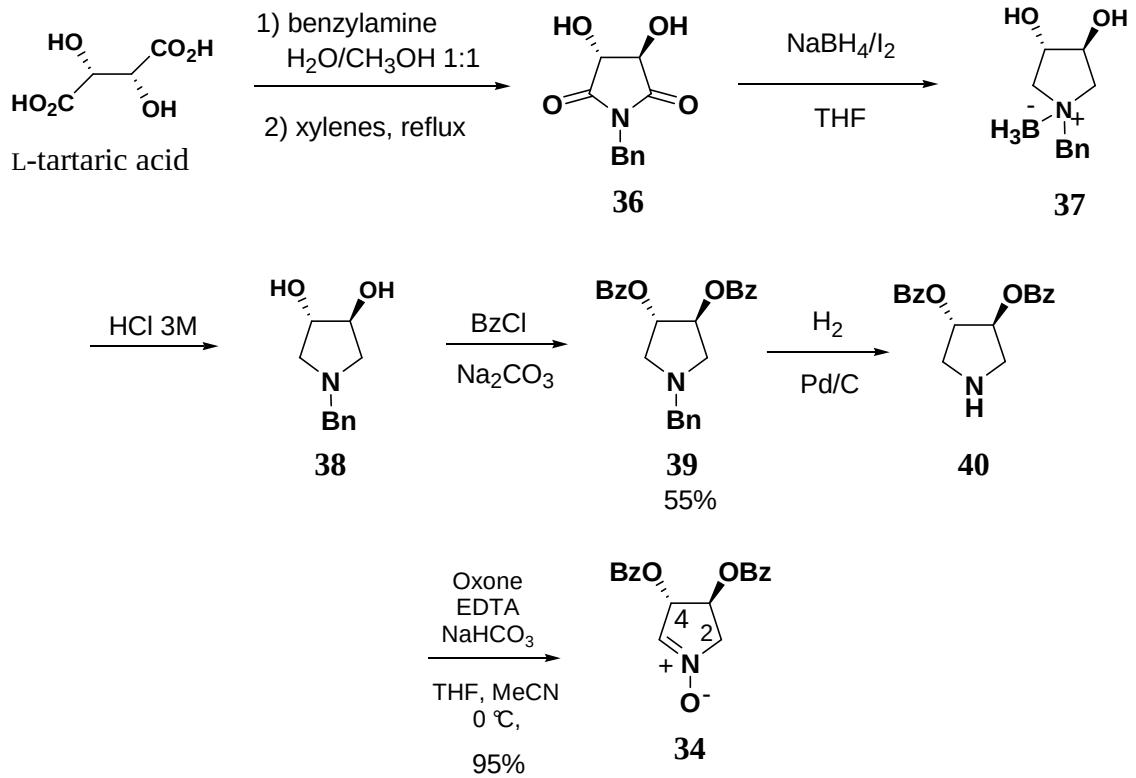
[36] Cicchi, S.; Höld, I.; Brandi, A. *J. Org. Chem.* **1993**, *58*, 5274
[37] Ballini, R.; Marcantoni, E.; Petrini, M. *J. Org. Chem.***1992,** *57*, 1316.

2.2 Synthesis of variously protected dihydroxypyrroline *N*-oxides

Three different protecting groups were used to mask the two hydroxy functions in the nitrone and in the synthetic intermediates, i.e. the benzoate,[34] the *tert*-butyl [36] and the *tert*-butyldimethylsilyl group.[29,38] The appropriate protecting group was chosen on the basis of the desired final product and the nature of the reactions necessary to obtain it.

2.2.1 Synthesis of the dihydroxypyrroline *N*-oxide protected by benzoyl group

The new enantiopure (bis)benzoylated nitrone **34** was obtained in a very efficient way starting from L-tartaric acid (Scheme 2.5). Analogously, the enantiomeric nitrone **35** was prepared starting from D-tartaric acid.



Scheme 2.5

[38] (a) Forcato, M.; Mba, M.; Nugent, W. A.; Licini, G. *Eur. J. Org. Chem.* **2010**, 740; (b) Soldaini, G.; Cardona, F.; Goti, A. *Org. Lett.* **2007**, *9*, 473.

Benzylimide **36** was prepared by heating the benzylammonium salt of tartaric acid in refluxing xylenes, with azeotropic removal of water, as recently reported by Rosenberg et al.[39] The *N*-Benzyldihydroxypyrrolidine **38** was obtained by reduction of the imide **36**. In particular, **36** was treated with $NaBH_4$ and I_2 at 0 °C under anhydrous conditions and then heated at the reflux temperature for 6 h to reach a full conversion. After destroying the excess of borane with MeOH at rt, the pyrrolidine-borane adduct **37** was washed with water to remove NaI salt, and then treated with an acidic aqueous solution to achieve **38** in 77% yield. In this step the final extraction of the molecule from the aqueous phase is very critical, because of its high hydrosolubility.

The borazine **37** can be isolated and characterized. This compound does not have symmetry elements and the diastereotopic carbinolic protons 3-H and 4-H resonate at different frequencies (δ = 4.67 ppm and δ = 4.04 ppm). The four methylene protons at the 2 and 5 positions give four different signals [**2-H$_\beta$**: 2.85 (dd, *J* = 11.5, 6.3 Hz), **5-H$_\beta$**: 3.14 (br d, *J* = 12.0 Hz), **5-H$_\alpha$**: 3.33 (dd, *J* = 12.0, 7.1 Hz), **2-H$_\alpha$**: 3.54 (ddd, *J* = 11.5; 6.6; 1.1 Hz)] in the ^{1}H NMR spectrum. Unlike **37**, the C2-symmetric pyrrolidine **38** contains three pairs of enantiotopic hydrogens on the ring which generate only three signals in the ^{1}H NMR spectrum.

Before to adopt acidic work-up, borazine's destruction was carried out by heating with methanol at 40 °C for 20 h. At the end of the treatment, the pyrrolidine **38** was usually obtained as a colorless oil. On the contrary, by the treatment with hydrochloric acid instead of methanol, the pyrrolidine was obtained as a white crystalline solid.

[39] Rejman, D.; Kočalka, P.; Buděšínskỳ, M.; Pohl, R.; Rosenberg, I. *Tetrahedron* **2007**, *63*, 1243-1253.

The next synthetic step was the protection of the hydroxy functional groups of the pyrrolidine. In particular, **38** was benzoylated[40] by treatment with 2.48 molar equivalents of benzoylchloride in dichloromethane in the presence of a 1.5 M aqueous solution of sodium carbonate. With the introduction of the acidic work-up in the synthesis of **38,** instead of the neutral conditions, the benzoylated intermediate **39** was obtained in better yields (from 70 to 87% after recrystallyzation against 43-57%) and optimal purity. The ^{1}H-NMR spectrum shows the presence of the new aromatic signals corresponding to the protons of the introduced benzoyl groups [δ = 8.09-8.04 (m, 4H; Bz); 7.60-7.54 (m, 2H; Bz); 7.48-7.41 (m, 4H; Bz)].

The removal of the *N*-benzyl protecting group was obtained through hydrogenolysis. The reaction was carried out under hydrogen atmosphere, in methanol at rt overnight, with the catalysis of palladium on carbon at 10% (0.062 molar equivalents), to give **40** in 91% yield. The ^{1}H-NMR spectrum shows the disappearance of the signals corresponding to the benzylic protons (δ = 7.40–7.23).

The key oxidation of the C_2 symmetrical pyrrolidine **40** was performed using oxone®, an oxidizing reagent recently employed by Font and co-workers.[41] Oxone® is a stabilized mixture of potassium monopersulfate ($KOSO_2OOH$), potassium hydrogen sulfate ($KHSO_4$) and potassium sulfate (K_2SO_4) salts in 2:1:1 ratio. The reaction was performed in a solvent system constituted by 0.01 M Na_2EDTA aqueous solution, CH_3CN and THF in 1:1:0.25 ratio in the presence of $NaHCO_3$ at 0 °C. The nitrone **34** was obtained with this reagent in excellent yield (95%). Previously, this oxidation was carried out by

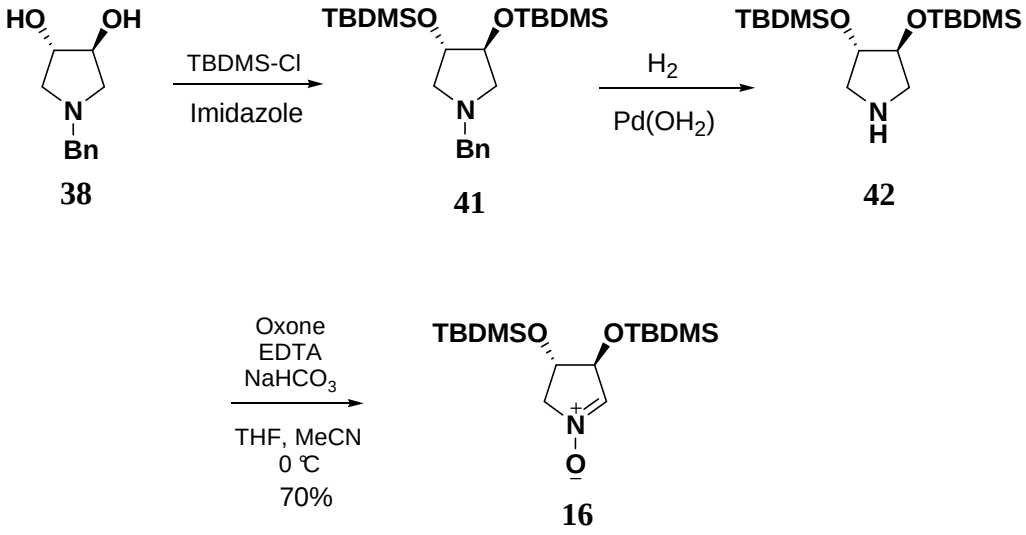
[40] Folkersen, B.M.; Lundt, I.; Foged, C.; Valsborg, J.S. *J. Labelled Cpd.Radiopharm.* **1999**, *42*, 1145-1159.
[41] Sánchez-Izquierdo, F.; Blanco, P.; Busqué, F.; Alibés, R.; de March, P.; Figueredo, M.; Font, J.; Parella, T. *Org. Lett.* **2007**, *9*, 1769-1772.

several methods, such as SeO_2/ H_2O_2[42] and MTO/H_2O_2[43], which were not satisfying with regard to the reaction yields, or *N*-sulfonyloxaziridine,[44] not practical in large-scale syntheses for the high cost and low atom economy.

In conclusion, the nitrone **34** was obtained in a 30% overall yield starting from imide **36**.

2.2.2 Synthesis of the dihydroxypyrroline *N*-oxide protected by the *tert*-butyldimethylsilyl group

The procedure used to obtain nitrone **16** was the same to that applied to the synthesis of nitrone **34** discussed in section 2.2.1, except for the protection of pyrrolidine **38** by the *tert*-butyldimethylsilyl group (Scheme 2.6).



Scheme 2.6

[42].Murahashi, S.-I.; Shiota, T. *Tetrahedron Lett.* **1987**, *28*, 2383-2386.
[43] Goti, A.; Nannelli, L. *Tetrahedron Lett.* **1996**, *37*, 6025-6028.

[44] a) Zajac Jr., W.W.; Walters, T.R.; Darcy, M.G. *J. Org. Chem.* **1988**, *53*, 5856–5860. b) McCaig, A.E.; Wightman, R.H. *Tetrahedron Lett.* **1993**, *34*, 3939–3942. c) Cicchi, S.; Nunes Jr., J.; Goti, A.; Brandi, A. *Eur. J. Org. Chem.* **1998**, 419–421. d) Cicchi, S.; Ponzuoli, P.; Goti, A.; Brandi, A. *Tetrahedron Lett.* **2000**, *41*, 1583–1587.

In particular, **38** was treated with 2.5 molar equivalents of *tert*-butyldimethylsilyl chloride in the presence of a large excess of imidazole (3.0 molar equivalents) for 2 h in *N*,*N*-dimethylformamide (DMF) at 60 °C under anhydrous conditions, obtaining **41** in quantitative yield. The ^{1}H-NMR spectrum of the product shows the presence of the new aliphatic signals of the methyl groups (δ = 0.25-0.10 ppm) and of the *tert*-butyl group (δ = 0.82-1.00 ppm).

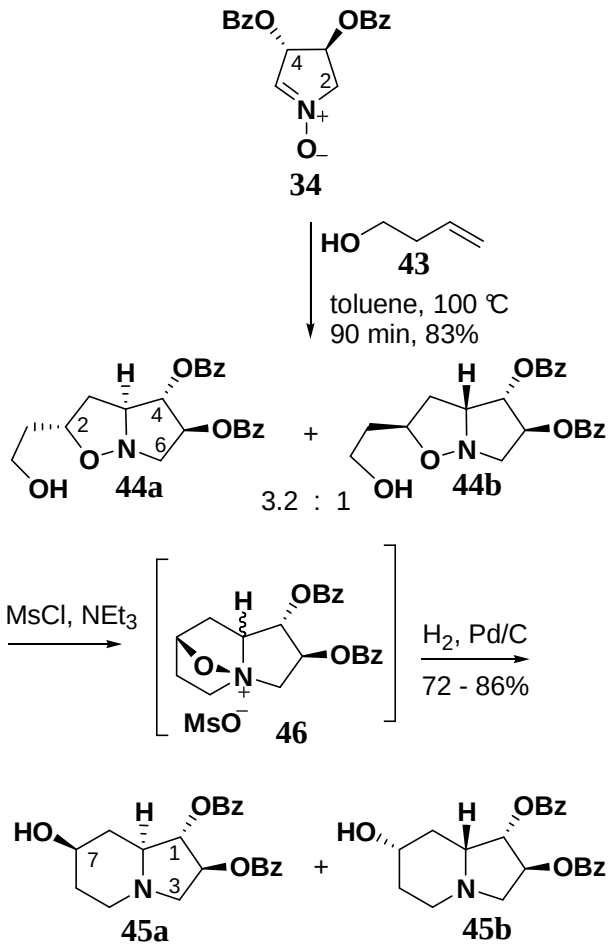
The removal of the *N*-benzyl protecting group was carried out using the same conditions of **39,** but using a different catalyst, palladium hydroxide on carbon at 20% (Pearlman's catalyst), that afforded 85% yield of pyrrolidine **42**. The ^{1}H-NMR spectrum shows the disappearance of the signals corresponding to the benzyl protons (δ = 7.45–7.15 ppm).

Oxidation of **42** was performed using oxone® under the same conditions of the reaction that produced nitrone **34**. In conclusion, **16** was obtained in 34% overall yield starting from immide **36**.

2.3 Synthesis of variously protected azidolentiginosine

2.3.1 Synthesis of the benzoyl protected azidolentiginosine

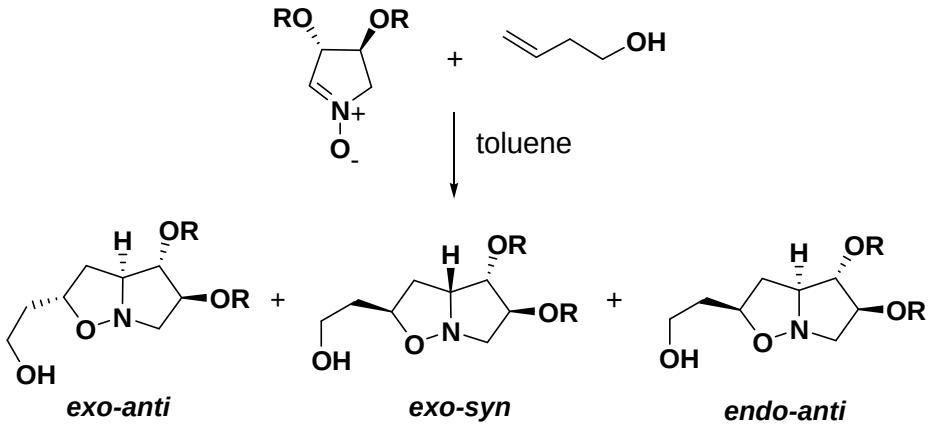
The cycloaddition of **34** with butenol **43** (Scheme 2.7), carried out in toluene at 100 °C for 90 min in a microwave oven, gave two main cycloadducts **44a** and **44b**, besides a third one in traces, in 83% overall yield.



Scheme 2.7

The ratio of the two main cycloadducts **44a** and **44b,** 3.2:1 (^{1}H 400 MHz NMR monitoring), is somewhat lower than that obtained by the cycloaddition of *t*-Bu protected nitrone to **43** (5:1), but similar to that obtained with TBDMS-protected nitrone (Scheme 2.8; Table 2.1).[2945]

[45] Goti, A.; Cardona, F.; Brandi, A. *Synlett* **1996**, *7*, 761-763.



Scheme 2.8

R	**overall yield** (%)	**diastereomeric ratio**		
		exo-anti	*exo-syn*	*endo-anti*
t-Bu	83	10	2	1
Bz	91	24	7	1
TBDMS	70	6	2	1

Table 2.1

This reaction is completely regioselective, affording exclusively 5-substituted isoxazolidines. Regarding the diastereoselectivity, the minor diastereoisomer **44b** derives from an *exo-syn* approach of the dipolarophile to the C-4 BzO substituent of the nitrone, less favoured than the common *exo-anti* approach (Figure 2.2).[20]

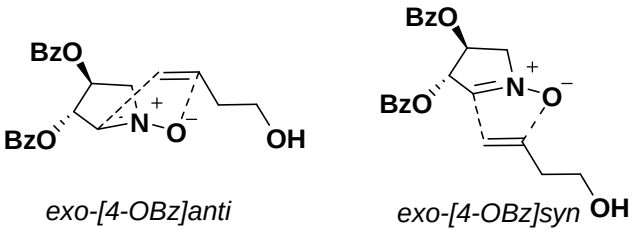


Figure 2.2

The *exo* approach is highly favoured, because of the hindering effect of the ring and its substituents in an *endo* approach. Evidence for the assignment derives from proton NMR coupling constants of the bridgehead proton 3a-H with the vicinal 4-H, 3.1 Hz in

44a compared to 6.7 Hz in **44b**, diagnostic for a *cis* and *trans* relationship, respectively. The low diastereoselectivity can be ascribed to the relative flatness of the benzoyl group exerting a lower steric hindrance compared to a *t*-Bu group. Also different reaction conditions were tested, such as heating in a standard oven or in a microwave reactor (MW), different rection temperatures and different solvents, but no improvement of selectivity and reaction times was observed (Table 2.2).

Method	Temperature	Time	Solvent	Diast. Ratio
MW	60 °C 80 °C	20 min 20 min	dichloromethane	3.5:1
Oven	60 °C	3 days	toluene	3.1:1
MW	80 °C	1 h 10 min	dichloromethane	3.6:1
MW	80 °C	2 h 10 min	acetonitrile	3.47:1
MW	90 °C	34 min	dichloromethane	3.5:1
MW	90 °C	1 h	1,2-dichloroethane	3.18:1
MW	90 °C	1 h	acetonitrile	3.15:1
MW	100 °C	20 min	chloroform	3.71:1
MW	100 °C	20 min	1,2-dichloroethane	2.08:1
MW	100 °C	20 min	acetonitrile	3.48:1
MW	100 °C	30 min	toluene	2.80:1
MW	100 °C	30 min	chloroform	3.34:1
Oven	100 °C	1 h	toluene	3.14:1
Oven	100 °C	1h 30 min	toluene	3.2:1

Table 2.2

Cycloadducts **44a** and **44b** could be only partially separated by chromatography on silica gel, unlike the *tert*-butyl- and *tert*-butyldimethylsilyl-protected adducts. Therefore, it has been more convenient to carry out the next step on the isomeric mixture as the corresponding diastereomeric indolizidines **45a** and **45b** were easily separable (Scheme 2.7). The indolizidine formation was carried out under anhydrous conditions by adding methanesulfonyl chloride (1.1 molar equivalents) to a dichloromethane solution of the two main diastereomeric cycloadducts and triethylamine. The reaction mixture was stirred at 0 °C for 1 h, then the produced inner

salts **46**, which are not isolable, were immediately reduced by H_2 on Pd/C to indolizidines **45a** and **45b**. The yields of this reaction carried out on different amounts of adducts (1.0-3.5 g) vary in the 72-86% range, and the yields of the single diastereomeric protected 7-hydroxy-lentiginosines are calculated on the basis of the diastereomeric ratio of the corresponding starting cycloadduct mixture monitored by ^{1}H 200 MHz spectra (Table 2.3).

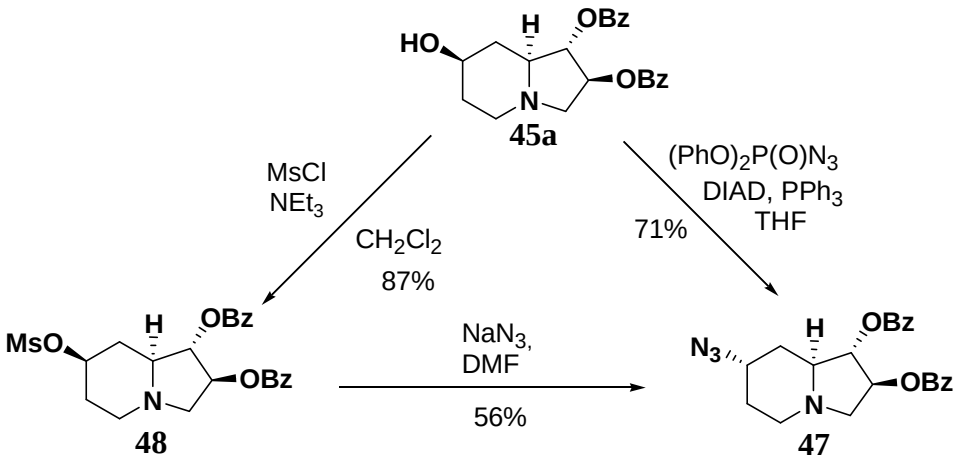
Scale	**Diastereomeric Cycloadducts Ratio** (*exo-anti*:*exo-syn*)	**45a**	**45b**	**Reaction Yield**
		Yields		
1g	3.6:1	66%	92%	72%
1.4 g	2.3:1	95%	56%	85%
1.4 g	2.26:1	82%	73%	80%
2.2 g	2.8:1	82%	95%	86%
3.5 g	2.6:1	77%	62%	73%

Table 2.3

The synthesis of 7*S*-azidolentiginosine **47** (and of its enantiomer **22**) was carried out through two different ways (Scheme 2.9):

- mesylation of the free 7-OH of the indolizidine **45a** followed by a nucleophilic substitution of the mesylate with NaN_3;[46]
- direct nucleophilic substitution of the free 7-OH of **45a** under the Mitsunobu's reaction conditions.[47]

[46] Cordero, F.M.; Pisaneschi, F.; Meschini Batista K.; Valenza, S.; Machetti, F.; Brandi, A. *J. Org. Chem.* **2005**, *70*, 856-867.
[47] Lawton, G.R.; Ji. H.; Silverman, R.B. *Tetrahedron Lett.* **2006**, *47*, 6113-6115.

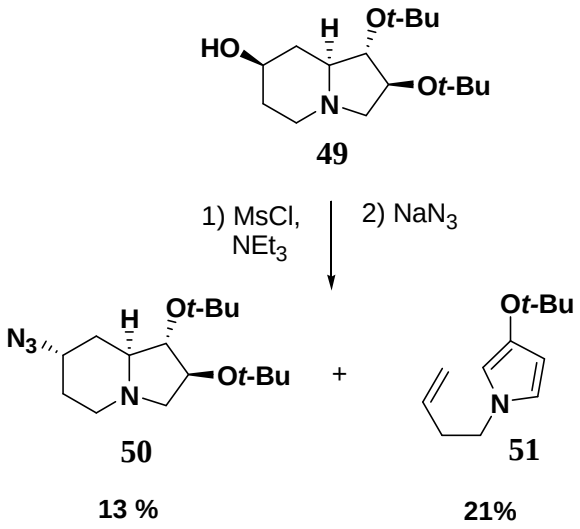


Scheme 2.9

The mesylation of **45a** was carried out under anhydrous conditions using 2 molar equivalents of methanesulfonyl chloride. The mixture was stirred under nitrogen at rt for 2 h and after work-up the intermediate **48** was obtained in 87% yield. After treatment with sodium azide (2.5 molar equivalents) in *N*,*N*-dimethylformamide at 80 °C for 22 h, the corresponding azide **47** was obtained in 56 % yield (49% overall yield). The synthesis of **47** under the Mitsunobu's reaction conditions was done using 1.4 molar equivalents of diphenyl phosphoryl azide in the presence of 1.2 molar equivalents of triphenylphosphine and 1.4 molar equivalents of diisopropyl azodicarboxylate (DIAD) in dry THF. The reaction mixture was stirred at rt overnight and gave **47** in 71% yield after purification. The synthesis of **47** under the Mitsunobu's conditions allowed to avoid the step of mesylation of **45a** and to obtain a better yield of the product respect to the two steps procedure.

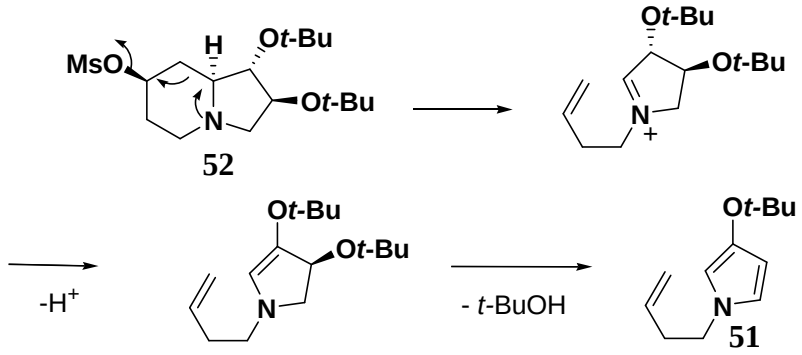
2.3.2 Synthesis of the *tert*-butyl protected azidolentiginosine

The reaction of mesylation followed by nucleophilic substitution was performed also on the 1,2-(bis)-*t*-Bu protected indolizidinetriol **49**.[42,29] Mesylation of **49** with NaN_3 in DMF at 80 °C for 2.5 h gave a mixture of two products (Scheme 2.10).



Scheme 2.10

Besides the expected azide **50** obtained in poor 13% yield, a new product **51** was obtained in 21% yield to which the fragmented and pyrrole aromatized structure **51** was assigned on the basis of NMR and mass spectrometry data. The product **51** must derive from a Grob's fragmentation-type process,[48] followed by monodeprotection and elimination/aromatization of the pyrrole ring (Scheme 2.11).



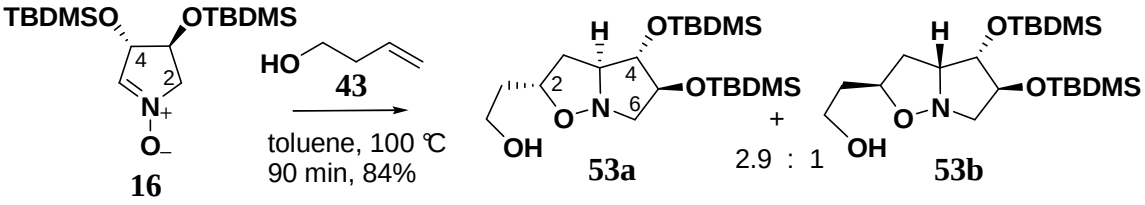
Scheme 2.11

Changing the conditions of temperature and reaction times did not significantly affect much the results. The same fragmentation problem is not observed with the benzoylated indolizidine **45a**.

[48] a) Grob, C.A.; Ostermayer, F.; Raudenbusch, W. *Helv. Chim. Acta* **1962**, *45*, 1672-1682; b) Grob, C.A.; Schwarz, W. *Helv. Chim. Acta*, **1964**, *47*, 1870-1878.

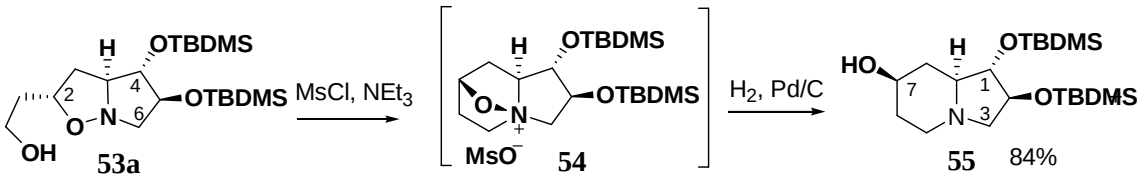
2.3.3 Synthesis of the *tert*-butyldimethylsilyl protected azidolentiginosine

The cycloaddition of **16** with butenol **43** (Scheme 2.12) was carried out in toluene at 100 °C for 90 min in a microwave oven analogously to the cycloaddition of nitrone **34** (see Section 2.3.1) and gave two main cycloadducts **53a** and **53b** besides traces of a third one, in 84% overall yield.



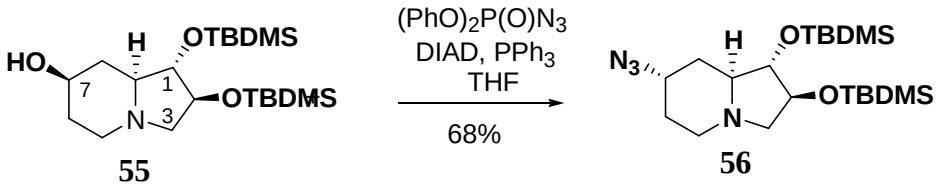
Scheme 2.12

Unlike the benzoyl protected cycloadducts **44a** and **44b**, **53a** and **53b** were easily separated by flash chromatography and were obtained in a 2.9:1 ratio (see Table 2.1, section 2.3.1). Therefore, the next reaction was carried out on only one diastereomeric cycloadduct, i.e. **53a** (Scheme 2.13), under the same reaction conditions used for the synthesis of the diastereomeric indolizidines **45a** and **45b**.



Scheme 2.13

Starting from indolizidine **55**, the azide **56** was synthesized using exclusively the more convenient Mitsunobu's reaction conditions (Scheme 2.14).

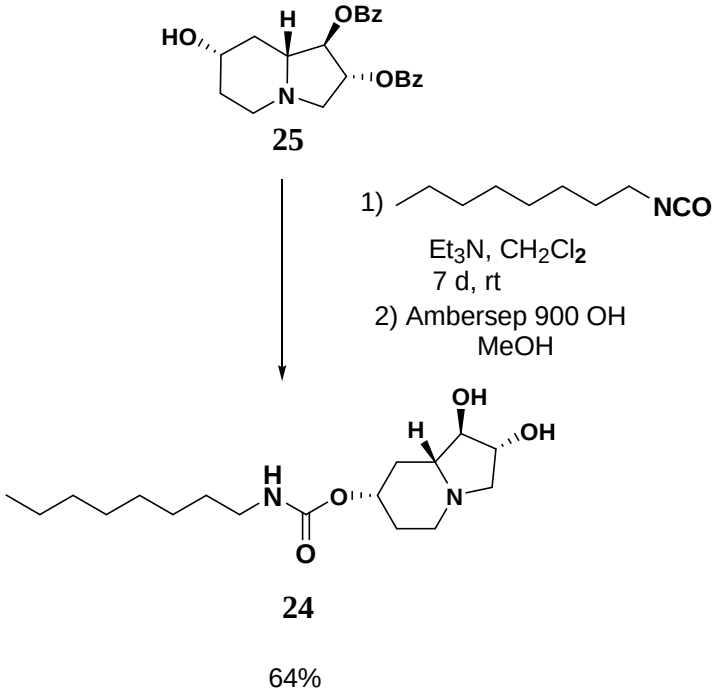


Scheme 2.14

The adopted procedure, identical to that used to obtain azide **47,** gave **56** in slightly lower yield (68%).

2.4 Synthesis of a new derivative of indolizidinetriol 25

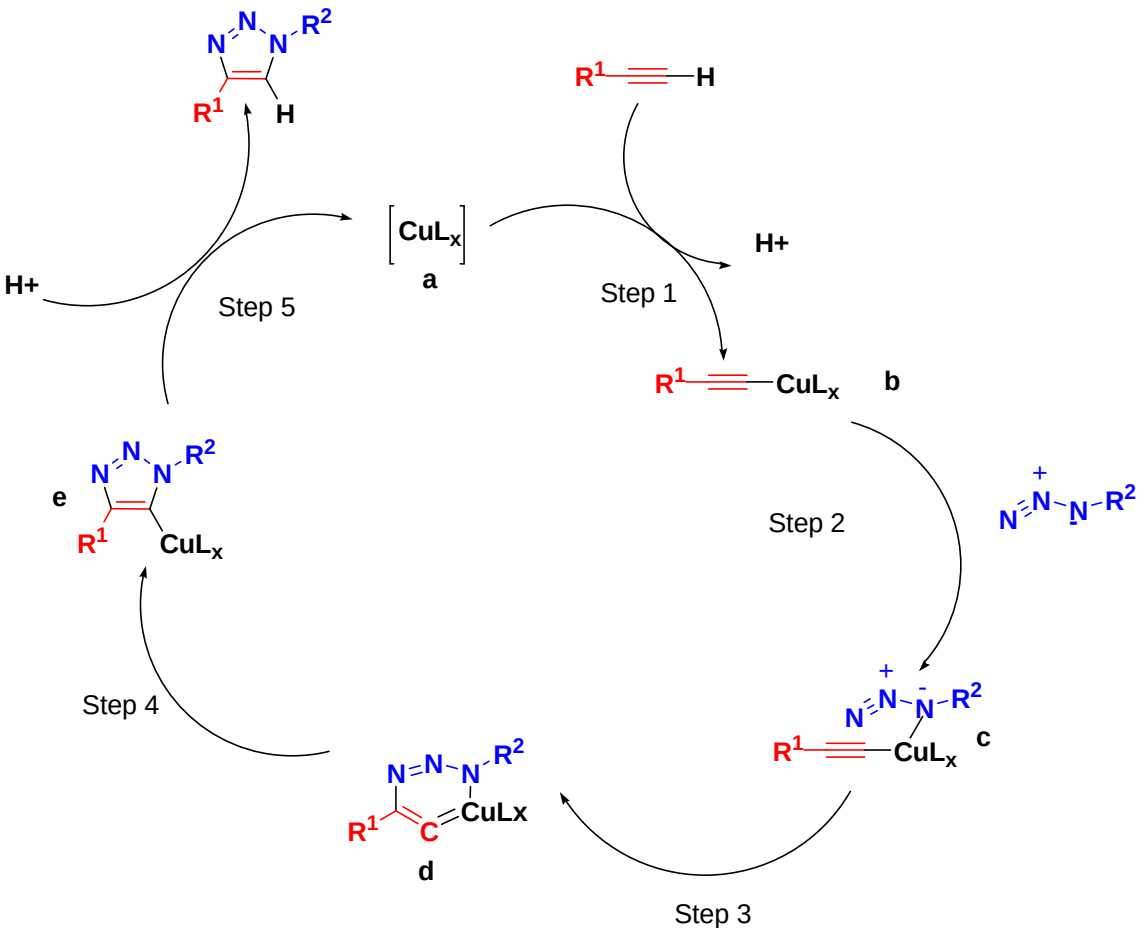
Starting from the major diastereomeric 7-hydroxy-lentiginosine **25,** the derivative **24** was obtained in two steps by reaction with octyne isocyanate in the presence of TEA, followed by deprotection with alkaline Ambersep resin (Scheme 2.15).



Scheme 2.15

2.5 Huisgen's cycloaddition of protected azidolentiginosine with alkynes

In the last years "click chemistry" has emerged as a fast and efficient approach to synthesis of novel compounds with desired function.[49] The formation of the azide **47** and of its enantiomer allowed a very good approach for conjugating the alkaloid to other substrates via the formation of 1,2,3-triazoles by running Huisgen's cycloaddition.[50] The copper(I) catalysed version[49a,51,52] of the cycloaddition confers a high chemo- and regioselectivity to the process. In the Scheme 2.16 is showed a proposed catalytic cycle for the cycloaddition.[52e,53]



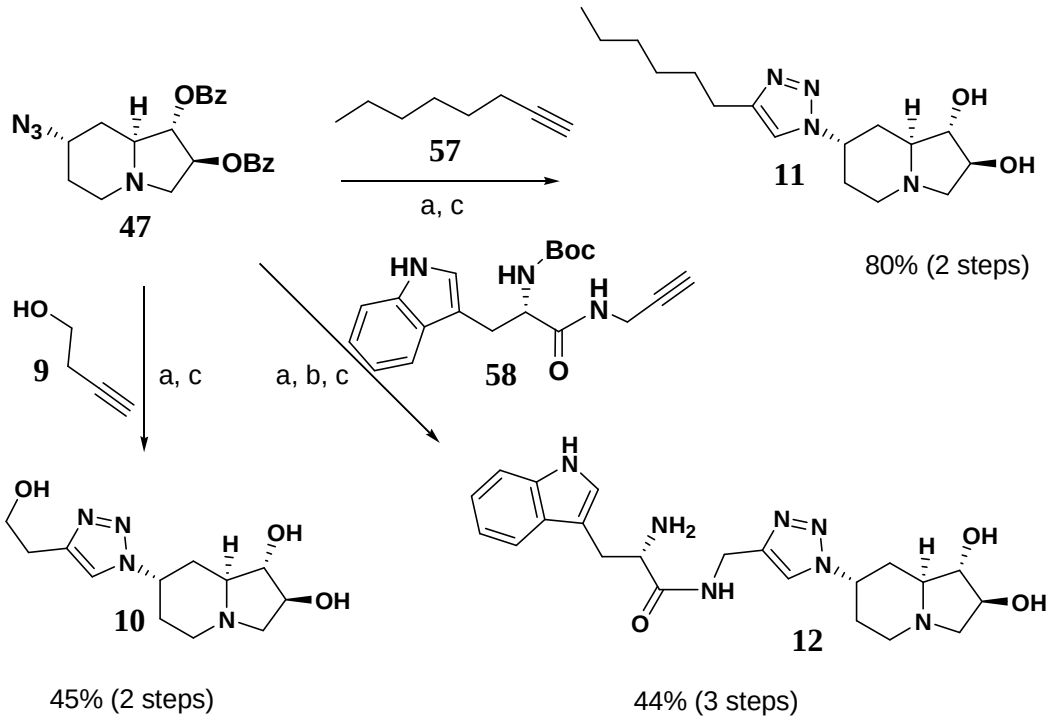
Scheme 2.16

[49] a) Kolb, H.C.; Finn, M.G., Sharpless, K.B. *Angew. Chem.* **2001**, *113*, 2056-2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021; b) Kolb, H.C.; Sharpless, K.B. *Drug Discovery Today* **2003**, *8*, 1128-1137.
[50] Huisgen, R. in *1,3-Dipolar Cycloaddition Chemistry;* Padwa, A., Ed.; Wiley: New York, **1984**.
[51] a)Rostovtsev, V.V.; Green, L.G.; Fokin, V.V.; Sharpless, K.B. *Angew. Chem* **2002**, *114*, 2708-2711; *Angew. Chem. Int. Ed.* **2002,** *41*, 2596-2599; b) Tornøe, C.W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.

[52] a) Bock, V.D.; Hiemstra, H.; van Maarsveen, J.H. *Eur. J. Org. Chem.* **2006**, 51-68; b) Lutz, J.F.*Angew. Chem.* **2007**, *119*, 1036-1043; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018-1025; c) Gil, M.V.; Arevalo, M.J.; Lopez, O. *Synthesis* **2007**, 1589-1620; d) Moses, J.E. ; Moorhouse, A.D. *Chem. Soc. Rev.* **2007**, *36*, 1249-1262; e) Wu, P.; Fokin, V.V. *Aldrichimica Acta* **2007**, *40*, 7-17.
[53] Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman,L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*,210.

The lack of reactivity of internal alkynes suggested the early formation of the Copper(I) acetilydes as the first step of the cycle. A concerted 1,3-dipolar cycloaddition of the azide to copper acetylide has a high calculated potential energy barrier (23.7 kcal/mol), thus the metal must play an additional role. In the step 2 the azide is coordinated to copper, with the formation of intermediate **c**. When L is a water molecule this step is nearly thermoneutral (2.0 kcal/mol). In the key step 3 the unusual six-membered copper metallacycle **d** is formed. This step is endothermic by 12.6 kcal/mol with a calculated barrier of 18.7 kcal/mol, which is considerably lower than the barrier for the uncatalyzed reaction (26 kcal/mol), thus accounting for the enormous rate acceleration accomplished by Cu(I). Therefore, the regiospecificity of this reaction is determined by the binding of both azide and alkyne to copper prior to the formation of C-C bond. Protonolysis of **e** releases the triazole product, thereby completing the catalytic cycle.

Starting from 7*S*-azidolentiginosine **47** a set of reactions was performed in which three different kind of alkynes were used: 3-butyn-1-ol (**9**), 1-octyne (**57**) and a derivative of the natural amino acid L-tryptophane (**58**), obtained by a coupling reaction between the Boc-protected amino acid and propargylamine (Scheme 2.17).



Scheme 2.17 Reaction conditions: a) alkyne **9** or **57** or **58,** $CuSO_4$, Cu powder, H_2O/*t*-BuOH 1:1, THF, 80 °C, 45-120 min, MW; b) TFA, thiophenol; c) Ambersep 900 OH, MeOH.

As we can see, all the products obtained are 1,4-disubstituted-1,2,3-triazoles and are easily isolated by simple filtration. Microwave irradiation significantly reduces the time of reaction.[54] In all the cycloadditions, the azide and the alkyne were allowed to react in a solvent system made up of THF and a 1:1 mixture of *t*-BuOH and water.[54] The Cu(I) catalyst was prepared *in situ* by comproportionation of Cu(0) (copper powder) and Cu(II) (copper sulfate).[54] The best conditions were found to be 100 W irradiation power of the MW oven and 80 °C.

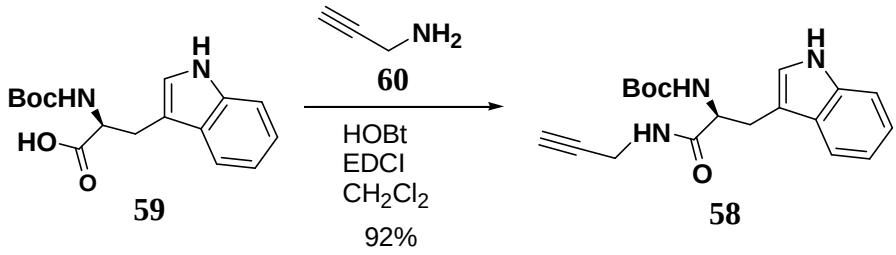
Regarding the cycloaddition of **9** with azide **47,** at the beginning of the reaction, less than equivalent of 3-butyn-1-ol (**9**) was added (0.155 mmol against 0.172 mmol of **47**) to avoid that it could react with itself.[55] After a run in the MW reactor at 80 °C for 30 minutes, a second quantity of alkyne (0.075 mmol) was added, followed by a second run in the MW reactor under the same conditions of time and temperature. The reaction was then monitored through *TLC* and was stopped when all the azide was consumed.

[54] Appukkuttan, P.; Dehaen, W.; Fokin, V.V.; Van der Eycken, E. *Org. Lett.* **2004**, *6*, 4223-4225.
[55] Siemens, P.; Livingston, R.C.; Diederich, F. *Angew. Chem. Int. Ed.* **2000**, *39*, 2632-2657.

Overall, 1.34 molar equivalents of alkyne were added in two times, and, after deprotection of the benzoyl groups with Ambersep 900 OH in methanol, the triazole **10** was achieved in 45% over the two steps.

In the synthesis of triazole **11**, an excess of octyne **57** (3.6 molar equivalents) was added portionwise at regular intervals of 30 minutes and each addition was followed by heating in the MW oven. Also in this case the deprotection was performed by treatment with Ambersep 900 OH in methanol and **11** was obtained in 80% yield over the two steps.

Finally, the cycloaddition of the azide with **58,** the propargylamide of the Boc-protected natural amino acid tryptophane prepared by coupling of N_{α}-Boc-L-tryptophan **59** and propargylamine **60,** was carried out (Scheme 2.18).[56]



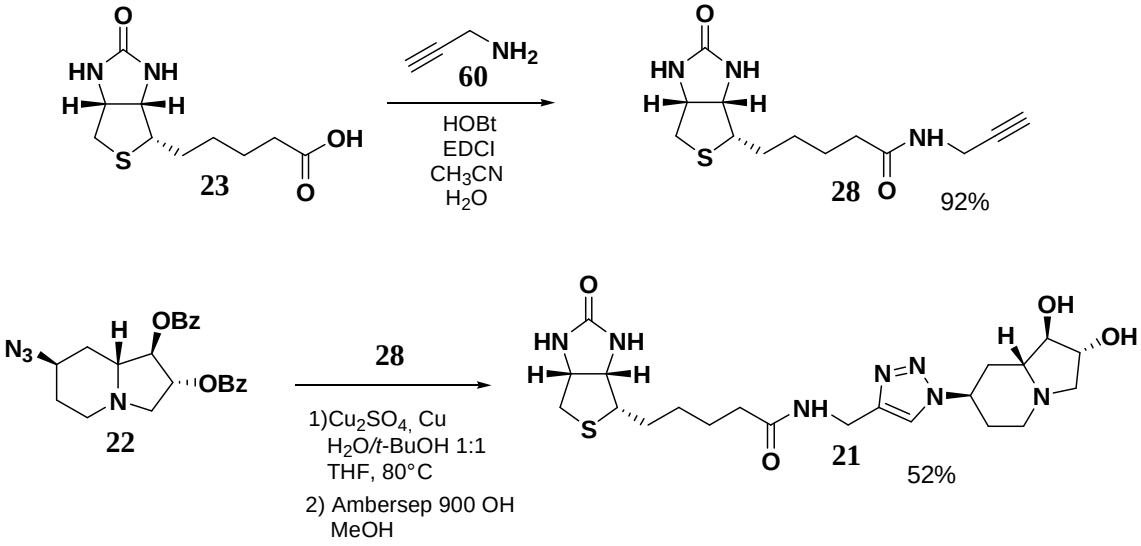
Scheme 2.18

The coupling reaction of **59** with propargylamine **60** (1.58 mol equiv), anhydrous hydroxybenzotriazole (HOBt, 1.51 mol equiv) and *N*′-ethylcarbodiimide hydrochloride (EDCI, 1.56 mol equiv) in dichloromethane, is very fast (only 30 minutes) and does not need anhydrous conditions, resulting very simple and efficient (92% yield). Compound **58** was then allowed to react with azide **47** under the same conditions of the previously discussed cycloadditions. Only a small excess of **58** (1.37 mol equiv) was necessary, and it was added portionwise at regular intervals of 30 minutes followed by heating in the MW reactor. The two protecting groups (Boc and benzoyl groups) were sequentially

[56] Lau, K.-N.; Chow, H.-F.; Chan, M.-C.; Wong, K.-W. *Angew. Chem. Int. Ed.* **2008**, *47*, 6912-6916.

removed by treatment with trifluoroacetic acid with 1% of thiophenol, followed by a basic treatment carried out with Ambersep 900 OH that gave **12** in 44% yield over the three steps.

The enantiomeric compound 7*R*-azidolentiginosine **22** was also coupled with the propargyl amide of biotin via the Huisgen cycloaddition (Scheme 2.19).



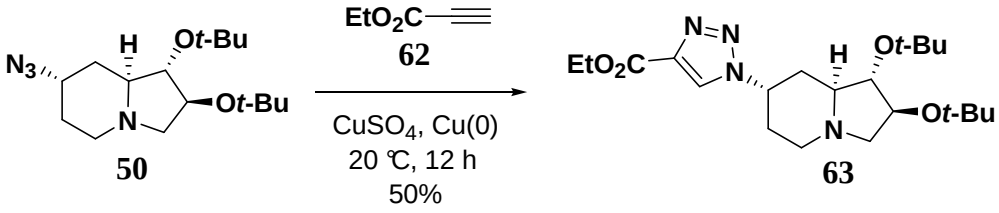
Scheme 2.19

Compound **28,** already known in the literature,[57] was synthesized employing the same coupling reagents used in the preparation of **58** (HOBt, EDCI) whereas a mixture of acetonitrile and water 1:1 was used as solvent instead of dichloromethane, because of the polarity of biotin **23**. Under these conditions, amide **28** was obtained with a very good yield (92%), comparable to that reported in the literature [i) *N*-hydroxysuccinimmide, EDCI, DMF; ii) propargylamine, triethylamine, DMF, 98%].

The cycloaddition reaction was carried out as described above by adding the alkyne **28** (1.4 mol equiv) portionwise at regular intervals of 30 minutes followed by heating in the MW oven in the presence of Cu (I). After treatment with Ambersep 900 OH in methanol **21** was obtained with a yield of 52% over the two steps.

[57] Lin, P.-J.; Ueng, S.-H.; Yu, S.-C.; Jan, M.-D.; Adak, A.K.; Yu, C.-C.; Lin, C.-C. *Org. Lett.* **2007**, *9*, 2131-2134.

Finally, starting from the *tert*-butyl protected azide **50,** another example of click chemistry was carried out (Scheme 2.20).

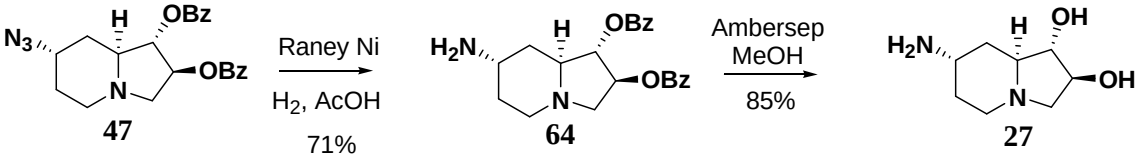


Scheme 2.20

The cycloaddition of azide **50** and ethyl propiolate **62** occurs at rt, because of the higher reactivity of **62,** affording **63** in 50% yield. Measurable traces of the regioisomeric cycloaddition product were also observed in this case, probably because the reactivity of the dipolarophile allows also the uncatalysed reaction.

2.6 Synthesis of 7-aminolentiginosine

Reduction of **47** (and of its enantiomer **22**) with hydrogen in the presence of Raney Ni, followed by hydrolysis of benzoates by treatment of **64** with Ambersep in methanol, afforded 7*S*-aminolentiginosine **27** (Scheme 2.21) in 60% yield over the two steps.

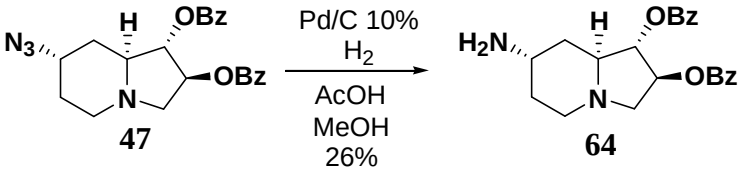


Scheme 2.21

The hydrogenation was carried out in the presence of acetic acid (10 mol equiv) to avoid that the basicity of the catalyst Raney Ni could induce a partial hydrolysis of the benzoyloxy groups. The reaction was carried out in methanol by stirring the reagents for

1 h at 0 °C and then for 7 h at rt. At the end, the protected amine **64** was obtained in good yield (71%).

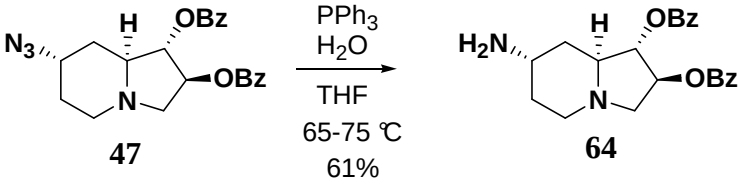
Other catalysts and reductive reagents were also investigated, but they were shown to be less efficient than hydrogenation with Raney Ni. The reduction catalized by 10% palladium on carbon in the presence of 10 molar equivalents of acetic acid was also tested (Scheme 2.22).



Scheme 2.22

After stirring overnight at rt the corresponding amine **64** was obtained in only 26% yield.

The reduction of azide **47** was also tried through a Staudinger reaction (Scheme 2.23).[58]

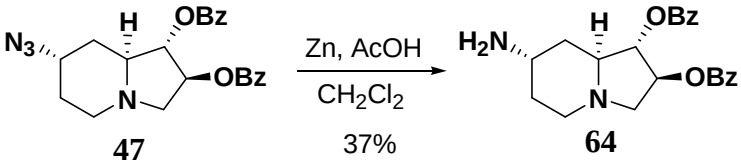


Scheme 2.23

In this reaction a mixture of **47** and triphenylphosphine (1.2 molar equivalents) in THF in a closed vial was heated at 65 °C in an oven until all the azide was consumed (*TLC* monitoring). Then water was added (10 molar equivalents) and the reaction was heated at 65-75 °C for 2 h. After purification, **64** was obtained in 61% yield, lower than the hydrogenation with Raney Ni.

[58] Clare, J.P.; Ayling, A.J.; Joos, J.-B.; Sisson, A.L.; Magro, G.; Pérez-Payàn, M.N.; Lambert, T.N.; Rameshwer, S.; Smith, B.D.; Davis, A.P. *J. Am. Chem. Soc.* **2005**, *127*, 10739-10746.

A further attempt of reduction was carried out using zinc and acetic acid as reductive system (Scheme 2.24).[59]



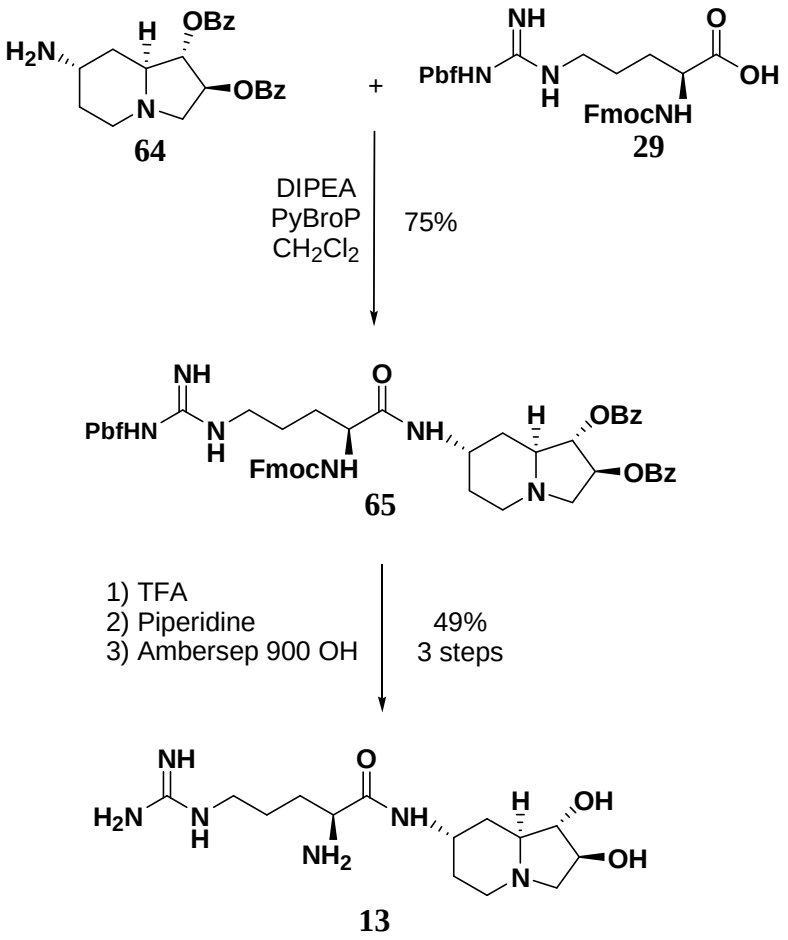
Scheme 2.24

To a solution of **47** in dichloromethane was added a very large excess of zinc (320 molar equivalents) and acetic acid (2.8 molar equivalents). The reaction was stirred overnight at rt and gave **64** in only 37% yield after purification.

2.7 Synthesis of an amino acid conjugate of 7-aminolentiginosine

The amino group of **64** is amenable for further transformations such as, for example, the coupling with amino acids for the synthesis of peptide conjugates. In particular, we obtained compound **13** via coupling of **64** with the protected natural amino acid Fmoc-Arg(Pbf)-OH **29** (Scheme 2.25).[46]

[59] Takano, Y.; Kojima, N.; Nakahara, Y.; Hojo, H.; Nakahara, Y. *Tetrahedron* **2003**, *59*, 8415-8427.



Scheme 2.25 Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl group.

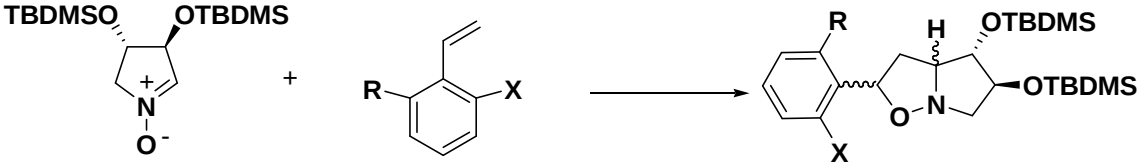
To a solution of **64** in dichloromethane were added subsequently the protected amino acid **29** (1.1 molar equivalents), diisopropylethylamine (DIPEA, 1 molar equivalent) and PyBroP® (bromotripyrrolidinophosphonium hexafluorophosphate, 1 molar equivalent). The reaction was then stirred overnight at rt and after purification **65** was obtained in 75% yield. Removal of the three ortogonal protecting groups was carried out without purification of the intermediates. The first group to be removed was the Pbf[60] by using TFA and water in 95:5 ratio.[61] The solution was stirred at rt for 30 minutes and, after washing with diethyl ether and concentration, the crude product was directly treated with piperidine (20% in dichloromethane) to remove the Fmoc

[60] Carpino, L.A.; Shroff, H.; Triolo, S.A.; Mansour, E.-S. M.E.; Wenschuh, H.; Albericio, F. *Tetrahedron Lett.* **1993**, *39*, 7829-7832.
[61] Far, S.; Kossanyi, A.; Verchère-Béaur, C.; Gresh, N.; Taillandier, E.; Perrée-Fauvet, M. *Eur. J. Org. Chem.* **2004**, *8*, 1781-179

protective group.[62] The reaction was stirred at 0 °C for 1 h and 30 min, concentrated under reduced pressure and sequentially washed with petroleum ether, diethyl ether and ethyl acetate. After concentration in vacuo the obtained crude product was dissolved in methanol and treated with Ambersep 900 OH. Arginine conjugate **13** was obtained in 49% yield over three steps starting from **65**.

2.8 Synthesis of isoxazolidines 30 and 31

The cycloaddition of nitrone **16** with two functionalized styrenes, 2,6-dichlorostyrene (**17**) and 2-bromostyrene (**18**) was carried out (Scheme 2.26).



16

17: R = X = Cl
18: R = H, X = Br

30: R = X = Cl
31: R = H, X = Br

Scheme 2.26

Because of the electron releasing nature of the aromatic group of styrenes, only one regioisomer is obtained, the one with the aromatic ring attached to the 5 position of isoxazolidine, in accord to the transition state showed in Figure 2.3.[63]

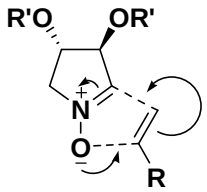


Figure 2.3

[62] a) Haag; T.; Hughes, R.A.; Ritter, G.; Schmidt, R.R. *Eur. J. Org. Chem.* **2007**, *36*, 6016-6033; b) Wen, S.; Packham, G.; Ganesan, A. *J. Org. Chem.* **2008**, *23*, 9353-9361.
[63] Houk, K. N. *Acc. Chem. Res.* **1975**, *8*, 361-369.

With regard to the diastereoselectivity of the reaction, also in this case the C-4 substituent of the nitrone determines which face of the molecule will be more easily attacked (see Section 2.3.1). The cycloaddition reaction between **16** and styrenes **17** and **18** could give four diastereoisomers; the *exo-anti* diastereoisomer should be the most favoured, whereas the *endo-syn* diastereoisomer should be the less favoured (Figura 2.4).

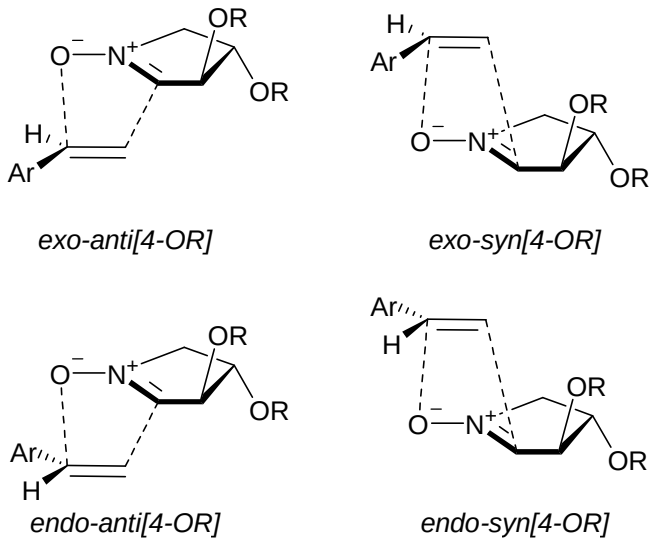
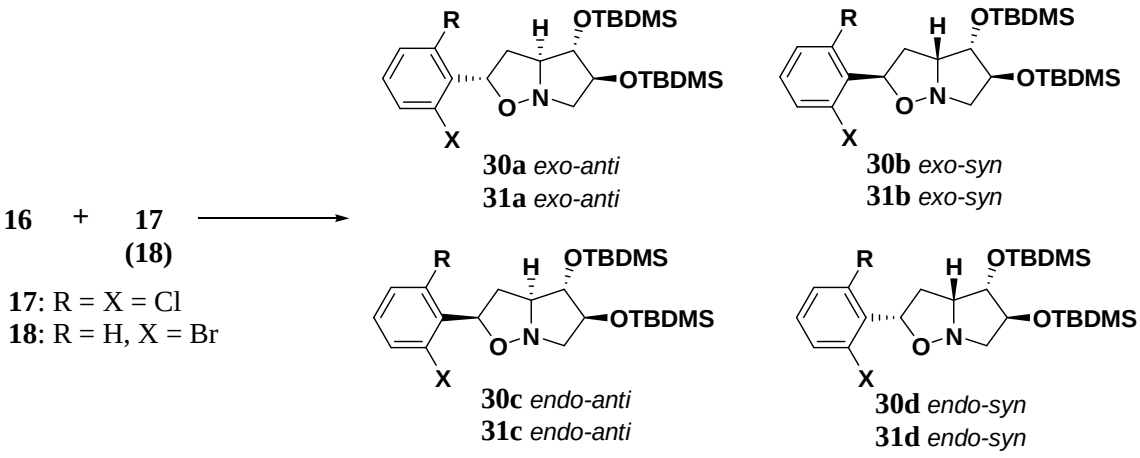


Figure 2.4

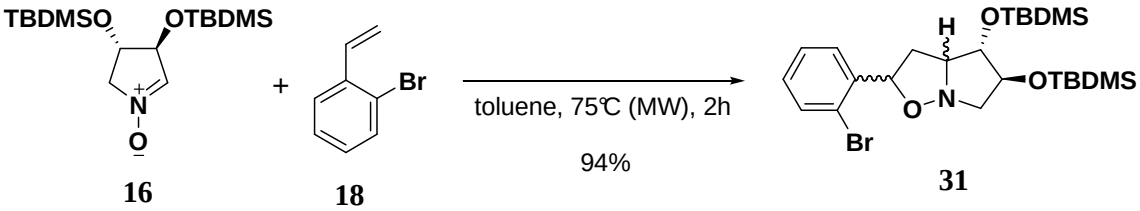
In Scheme 2.27 are shown the four possible diastereoisomers that can derive from the cycloaddition between **16** and the styrenes **17** and **18**.



Scheme 2.27

The cycloaddition of nitrone **16** and 2,6-dichlorostyrene **17** was performed under different conditions. Toluene as solvent and 1 molar equivalent of **17** were used at first. The reaction was carried out in oven at 75 °C and was monitored via *TLC* until disappearing of the nitrone (5.5 h). Another attempt was done in the MW oven at the same temperature (75 °C) for 2 h. The shorter reaction time required once again underlines the advantage of the MW irradiation as heating system. Finally, a trial was carried out in the MW reactor at 130 °C. The reaction ended after only 15 minutes, but the advantages were limited, and this reaction condition was discarded to avoid the polymerization of styrene and the degradation of the starting materials. The reaction afforded three diastereoisomers in 7.5:6.9:1 ratio (^{1}H 400 NMR monitoring) with 92% overall yield. The major diastereoisomer (*exo-anti*), obtained in 48% yield, was partially isolated by chromatography and completely characterized; the others two diastereisomers were only detected in mixture.

The cycloaddition with 2-bromostyrene (**18**) gave after heating in the MW oven at 75 °C for 2 h and in toluene as the solvent, a very good yield (94%) of three diastereoisomers in 18.4:11.6:1 ratio (^{1}H NMR 400 monitoring) (Scheme 2.28).



16 **18** **31**

Scheme 2.28

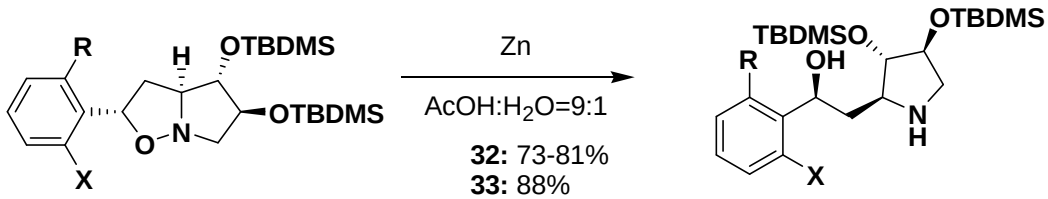
Also in this case the major diastereoisomer was only partially separated by chromatography. The cycloaddition of **16** with **18** is more diastereoselective than with **17**. The different steric hindrance of the two dipolarophiles could be a possible

explanation of the different diastereoselectivity. The presence of two *o*-Cl atoms could hamper the coplanarity between the vinylic group and the benzene ring, reducing the stereofacial preference induced by the C-4 TBDMSO substituent.

The *endo-syn* adduct was never observed in the crude reaction mixtures. However, only in the case of the dichloro derivative, traces of the fourth diastereoisomer were observed after reductive opening of the isoxazolidine ring. Likely, it was not possible to detect it in the crude cycloaddition mixture besides the other diastereoisomers.

2.9 Opening of the isoxazolidine ring

The reductive cleavage of the N-O bond of the major isoxazolidines was carried out by treatment of the cycloadducts **30** and **31** with zinc in a 1:9 mixture of water and acetic acid for 4 h at 60 °C. Pyrrolidines **32** and **33** were obtained in 73-88% yield after purification by column chromatography on silica gel (Scheme 2.29).



30a: R = X = Cl
31a: R = H, X = Br

32a: R = X = Cl
33a: R = H, X = Br

Scheme 2.29 Only the major diastereoisomers are reported

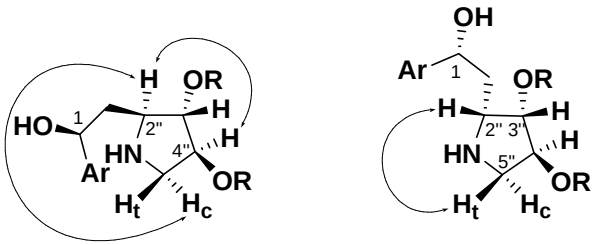
Reduction of a mixture of isomers of **30** afforded four diastereoisomers of **32** in 24.5:22.6:4.8:1 ratio (^{1}H-NMR monitoring) and 81% overall yield. As said above, the minor cycloadduct **30d** was probably formed in very small amount and could not be detected in the mixtures with the other adducts.

The reduction of the single major adduct *exo-anti* **30a** under the same conditions gave the corresponding pyrrolidine **32a** with a slight lower yield (73%).

The two major diastereoisomers of **32** were separated and characterized. The two minor diastereoisomers were obtained only in mixture with other isomers.

The reduction of bromo-substituted cycloadducts **31** under the same reaction conditions gave **33** in 88% yield. It was possible in this case to separate and characterize the two major diastereisomes.

1D-NOESY experiments allowed to determine the configuration of the new C-2 stereocenter of the products (see Figure 2.5).



diast. I (1*S*,2"*S*,4"*S*,5"*S*)

diast. II (1*R*,2"*R*,4"*S*,5"*S*)

32: Ar = 2,6-$Cl_2C_6H_3$
33: Ar = 2-BrC_6H_4

Figure 2.5

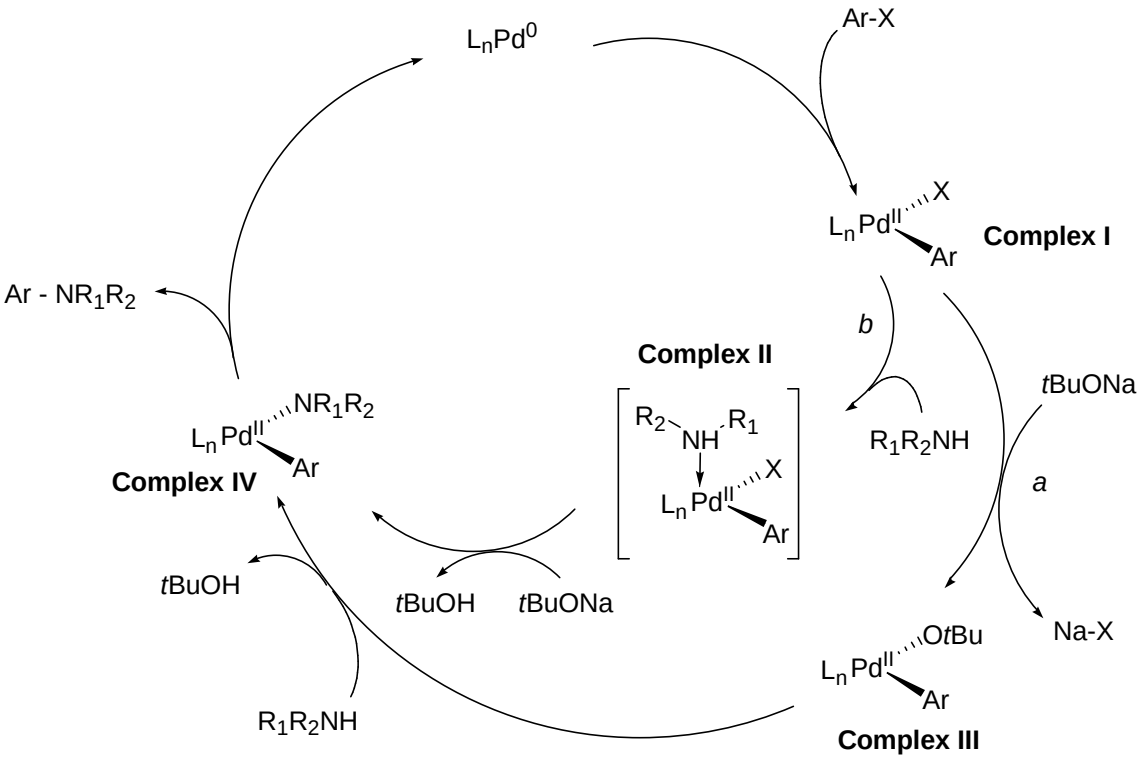
In particular, the irradiation of the 2''-H hydrogen of the major diastereoisomers **I** of **32** and **33** produces a NOE positive effect on the 4''-H and 5''-H_{cis} hydrogen atoms, in agreement with an *S* configuration of the C-2''stereocenter. In the diastereoisomers **II** a NOE effect on 2''-H is observed when 5''-H_{trans} is irradiated, in agreement with the assigned structures.

2.10 Closure of the benzoindolizidine ring

The halogen atom presents on the aromatic ring is crucial for the last step of the synthesis, that is a nucleophilic aromatic substitution promoted by an appropriate catalyst in a Buchwald-Hartwig reaction or in an Ullmann reaction.

2.10.1 Buchwald-Hartwig Amination Reaction

Buchwald[64] and Hartwig[65] developed the first general procedure of aryl amination catalyzed by palladium tetrakis-triphenylphosphine [$Pd(PPh_3)_4$]. This reaction allows the insertion of amines, alcohols, acetyl esters and methyl ketones in aromatic structures on which an halogen atom (except fluorine) or a trifluoroacetate group (OTf) is present. The catalytic reaction conditions require the use of a metal in an oxidation state (0), a ligand of the metal, a base and a suitably high boiling solvent. The ligand choice is crucial as it can determine a very significant change in the yield of the reaction (from 0 to 90%). In Scheme 2.30 is illustrated the amination mechanism,[66] useful to understand the importance of the role of the ligand.



Scheme 2.30

[64] Guram, A. S.; Rennels, R. A.; Buchwald, S. L. *Angew. Chem. Int. Ed., Eng.* **1995**, *34*, 1348.
[65] (a)Paul, F.; Patt, J.; Hartwig, J. F. *J. Am. Chem. Soc.* **1994**, *116*, 5969; (b) Mann, G.; Hartwig, J. F. *Tetrahedron Lett.* **1995**, *36*, 3609.
[66] Wolfe, J. P.; Wagaw, S.; Buchwald, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 7215.

The active catalyst is a palladium complex in an oxidation state (0). This complex is very oxygen labile, therefore it is necessary a deoxygenation of the reaction medium.

The first step of the mechanism is the formation of the complex I, derived from an oxidative addition of the aromatic halide to the palladium activated complex. The ligand hindrance plays a key role in the formation of the activated catalyst complex.[67] The oxidative addition is the *rate determining step* (rds) of the reaction. Then, two possible reaction steps can occur depending on which among the base or the amine reacts at first (*a* and *b* pathways, respectively). In any case, the deprotonation of the amine is very important for the formation of the complex IV.

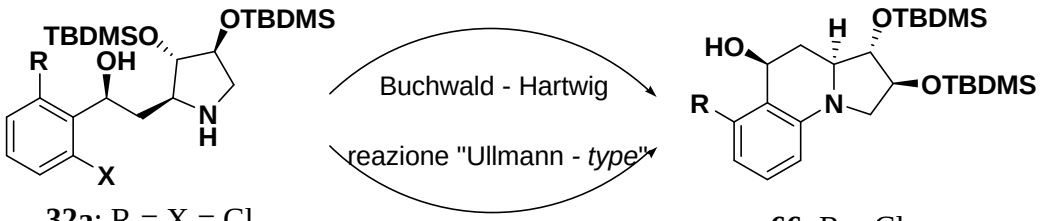
Through a reductive elimination, complex IV is split in the aniline and in the regenerated catalyst. During this step, an electron donating effect of the ligand can promote the elimination of the product and decrease the rate of the competitive β-elimination reaction on the amines which have removable protons in this position.[68]

Usually, the expensive arylbromides and -iodides undergo the Buchwal–Hartwig reaction at lower temperatures, in shorter reaction times and affording better yields than the corresponding arylchlorides, which often do not react at all.

To obtain benzocondensated derivatives of the lentiginosine **66** and **67,** initially a Buchwald–Hartwig reaction was attempted (Scheme 2.31).

[67] a) Tsou, T. T.; Kochi, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 6319; b) Amatore, C.; Broeker, G.; Jutand, A.; Khalil, F. *J. Am. Chem. Soc.* **1997**, *119*, 5176.

[68] (a) Reddy, N. P.; Tanaka, M. *Tetrahedron Lett.* **1997**, *38*, 4807; (b) Old, D. W.; Wolfe, J. P.; Buchwald, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 9722; (c) Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 1158.



32a: R = X = Cl
33a: R = H, X = Br

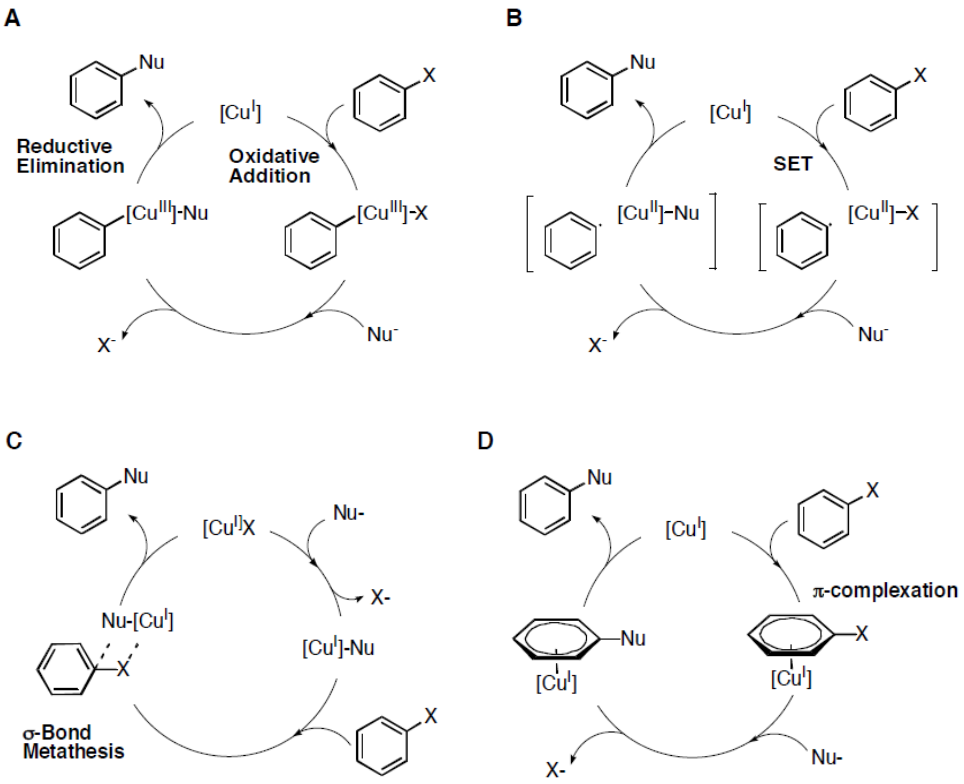
66: R = Cl
67: R = H

Scheme 2.31

In the cyclization reaction of **32a** and **33a** were employed complexes of both palladium and nickel as catalysts, in fact nickel complexes have been shown to be more efficient in the amination of arylchlorides.[69,70] A series of tests, to find the best reaction conditions, were also performed using pyrrolidine with the model compound 1-(2-chlorophenyl)ethanol (racemic). In order to facilitate the oxidative addition of the substrate to the metal, 2-bromostyrene **18** was also used. Furthermore, the bromoderivative **33a** was a suitable substrate for an Ullmann-type reaction catalysed by Cu(I), accordingly, also this option was explored.

The Ullmann reaction leads to formation of biphenyls starting from aromatic iodides (or bromides) with the employment of stoichiometric amounts of metallic copper at high temperatures (>200 °C).[71] The active catalytic species is Cu(I). The nature of the organometallic intermediates that are formed in the catalytic cycle and the copper oxidation state in these intermediates are not well defined yet. Two different kinds of mechanisms are hypothesized, with or without variation of the copper oxidation state (Scheme 2.32).

[69] Gradel, B.; Brenner, E.; Schneider, R.; Fort, Y. *Tetrahedron Lett.* **2001**, *42*, 5689.
[70] Desmarets, C.; Schneider, R.; Fort, Y. *J.Org. Chem.* **2002**, *67*, 3029.
[71] Ullmann, F.; Bielecki, J. *Chem. Ber.* **1901**, *34*, 2174.



Scheme 2.32

The oxidative addition (Scheme 2.32, **A**), a key step in the Pd-catalysed reaction, maybe can not occur because: i) the contemporary presence of Cu(III) and X^- in the same reaction ambient is unknown in the literature (because of the reduction potential of the two ions); ii) Ullmann-type reactions smoothly occur with *orto* substituted aromatic systems, unlike Pd-catalysed reactions; iii) aromatic triflates do not react under the Ullmann type conditions, but react very easily in Pd-catalysed reactions. However, it is not possible to exclude anyone of the proposed mechanisms.

2.10.2 Intramolecular amination reactions

The cyclization reactions carried out on substrate **32a** are reported in Table 2.4. The ligands used were: 2,2'-bipyridyl (**I**), (2-biphenyl)di-*tert*-butylphosphine (**II**), L-proline (**III**) and tetramethylethylenediamine (**IV**) (Figure 2.6).

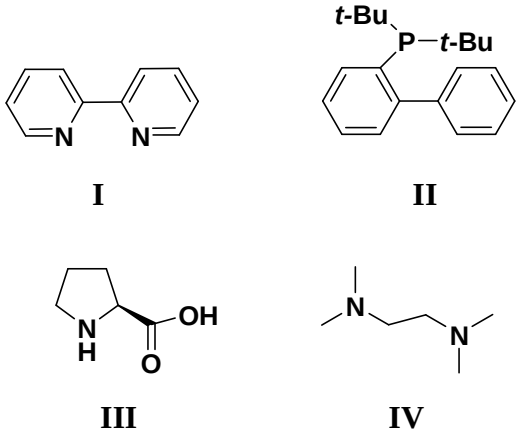
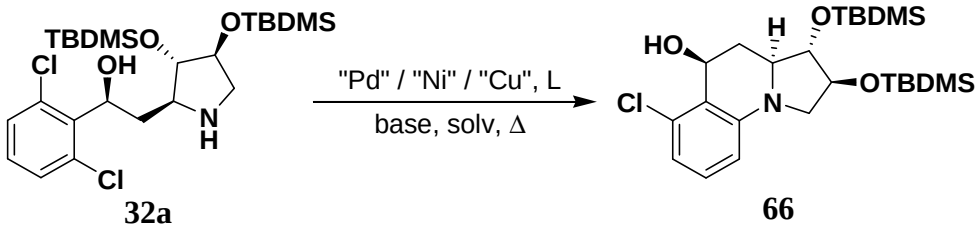


Figure 2.6



Scheme 2.33

#	Ar-X	Scale (mg)	M source (mol. equiv. %)	L[a]	M:L	Base (mol. equiv.)	Solv.	T (°C)	T (h)	Yield (%)
1	**32a**	30	$Ni(OAc)_2$ (5)	bpy	1 : 3	*t*BuOH (0.1)/NaH (1.2)	THF	90	5	0
2	**32a**	66	$Pd(OAc)_2$ (10)	bptbp	1 : 2	*t*BuOK (1.2)	toluene	110	21	0
3	**32a**	30	-	-	-	*t*BuOK (2.5)	toluene	130	5	0
4	**32a-O-TBDMS**	27	$PdCl_2$TEMED (10)	bptbp	1 : 2	*t*BuOK (1.2)	THF	90	5	0
5	**32a**	218	CuI (10)	Pro	1 : 2	K_2CO_3 (2.0)	DMF	100	48	5

Table 2.4 The general procedure was the following: substrate, catalyst, base and ligand were dissolved in a freshly distilled solvent in a Schlenk vial under nitrogen atmosphere. All the reagents were dried, distilled or recrystallizated before their use. [a] bpy = 2,2'-bipyridyl; bptbp = (2-biphenyl)di-*tert*-butylphosphine; Pro = L–proline; TEMED = tetramethylethylenediamine.

All the reactions under Buchwald-Hartwig conditions failed to give any cyclization product. Bisphenylphosphine **II**[72,73,74] used in the experiments 2 and 4 with Pd(0) did

[72] Wolfe, J. P.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **1999**, *38*, 2413.
[73] Tomori, H.; Fox, J. M.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 5334.

not activate the metallic center. The Ni-catalyzed reaction did not give any transformation of the substrate. A possible reason for the absence of reactivity of the substrate could be the presence of the free hydroxy group that could give rise to a strong hydrogen bond with the nitrogen atom lowering its nucleophilicity and, on the other hand, moving it away from the aromatic ring, hampering an intramolecular attack. Anyway, the protection of the free hydroxy group of **32a** with TBDMS (reaction 4) did not bring any benefit to the product formation.

It was also considered to oxidize the hydroxy group. Unfortunately the presence of the amino group limited the reagent choice for a direct oxidation.[75,76,77] The use of manganese oxide (IV) in dichloromethane and 1,2-dichloroethane was tested,[78] but after one night at rt the starting material was recovered unchanged.

Because of the role of *t*BuOH in combination with sodium hydride, we supposed that alcoholic functions can be deprotonated during the reaction and can interfere in the coordination of the amine to the metal. Furthermore, the hydroxy group in benzylic position is in a very favoured spatial position to bring to the chelation of the metal after the oxidative addition of the aromatic fragment. This chelation could bring to the spatial isolation of the pyrrolydine substituent, preventing the cyclization of the substrate.

Finally, only under Ullman conditions, using Cu(I) 10 mol% as catalyst, proline as ligand and potassium carbonate as base, the formation of the cyclization product **66** was observed even if in very small amount (5% yield).

Somewhat better results were obtained by switching to the bromoderivative **33**. In Table 2.5 are described the cyclization reactions performed on **33**.

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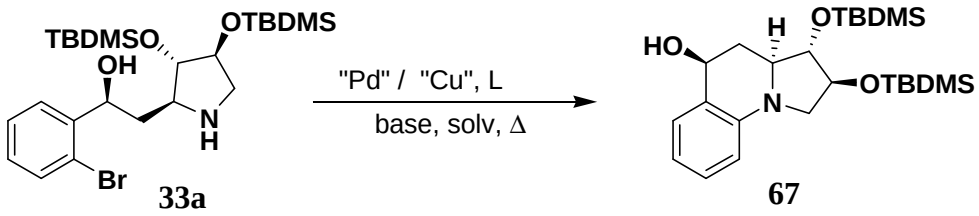
[74] Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 1158.
[75] Lou, J.-D.; Xu, Z.-N. *Tetrahedron Lett.* **2002**, *43*, 6149.
[76] Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. Per un applicazione: Ma, S.; Sun, H. *Org. Lett.* **2000**, *2*, 2503.
[77] Griffith, W. P.; Ley, S. V. *Aldrichimica Acta* **1990**, *23*, 13.
[78] Shimbashi, A.; Ishikawa, Y.; Nishiyama, S. *Tetrahedron Lett.* **2004**, *45*, 939.

Unfortunately, the replacement of the chlorine atom with a bromine (experiments 1 and 2) did not result in any improvement in the cyclization reaction under the Buchwald-Hartwig reaction conditions. Instead, the Ullmann-type reaction gave somewhat better results in the aromatic amination of **33**.



33a **67**

Scheme2.33

#	33 Diast (mg)	M source (% mol equiv)	L[a]	L:M	Base (mol equiv)	Solv.[b]	[33]	T (°C)	t (h)	conv (%)	yield (%)
1	a/b (30)	$Pd(OAc)_2$ (16)	bptbp	2:1	*t*BuOK (1.6)	THF‡	0.14 M	90	24	0	0
2	a (40)	$Pd(PPh_3)_4$(10)	-	-	*t*BuOK/ K_2CO_3(2/1.6)	THF‡	0.2 M	100	17	0	0
3	a/b (50)	$Cu^{(I)/(0)}$ (10)	Pro	2:1	K_3PO_4 (2)	DMF‡	1.7 M	100	48	nd	15
4	a (100)	$Cu^{(I)/(0)}$ (10)	Pro	2:1	K_3PO_4 (2)	DMF‡	0.63 M	100	48	nd	37
5	b (20)	$Cu^{(I)/(0)}$ (30)	Pro	2:1	K_3PO_4 (2)	DMF	0.63 M	120 MW	2	nd	38
6	a (20)	$Cu^{(I)/(0)}$ (30)	Pro	2:1	K_3PO_4 (2)	*t*BuOH:H_2O (1:1) / THF	0.05 M	80-100 MW	6.5	60	46[c]
7	a (20)	$Cu^{(II)/(0)}$ (30)	Pro	2:1	K_3PO_4 (2)	DMF	0.05 M	80 MW	4	nd	10
8	a (20)	$Cu^{(II)/(0)}$ (30)	Pro	2:1	K_3PO_4 (2)	DMF	0.05 M	100	4	nd	10
9	a (40)	$Cu^{(II)/(0)}$ (30)	Pro	2:1	K_3PO_4 (2)	DMF	0.05 M	120 MW	2	34	15
10	a (20)	$Cu^{(II)/(0)}$ (30)	Pro	2:1	K_3PO_4 (2)	DMF	0.05 M	80	14 d	nd	10
11	a (20)	$Cu^{(II)/(0)}$ (30)	Pro	2:1	K_2CO_3 (2)	DMF	0.05 M	120 MW	2	27	87
12	a (20)	$Cu^{(II)/(0)}$ (30)	Pro	2:1	DBU (2)	DMF‡	0.05 M	120 MW	2	43	61
13	a (20)	-	-	-	K_3PO_4 (2)	DMF‡	0.05 M	120 MW	2	34	40
14	a (20)	$Cu^{(II)/(0)}$ (30)	-	-	DBU (2)	DMF‡	0.05 M	120 MW	2	60	52
15	a (20)	-	-	-	DBU (2)	DMF‡	0.05 M	120 MW	2	34	22
16	a (50)	$Cu^{(II)/(0)}$ (10)	-	-	DBU (2)	DMF‡	0.05 M	120 MW	4	69	34
17	a (70)	$Cu^{(II)/(0)}$ (10)	Pro	2:1	DBU (2)	*t*BuOH	0.05 M	100 MW	10	nd	30
18	a (70)	$Cu^{(I)/(0)}$ (10)	Pro	2:1	K_3PO_4 (2)	*t*BuOH:H_2O (1:1)/ THF	0.05 M	100 MW	7	0	0

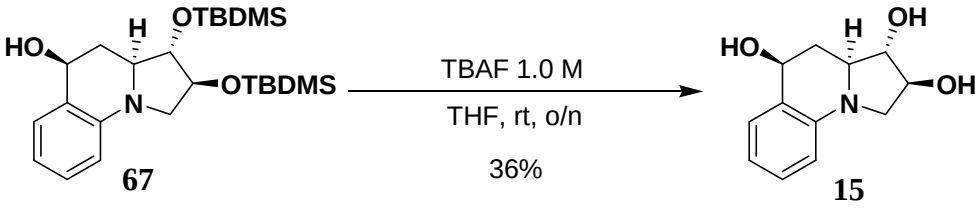
Table 2.5 General procedure: substrate, catalyst, base and ligand were dissolved in the solvent (freshly distilled or not) in a Schlenk vial or microwave vial under nitrogen atmosphere. When the reaction was carried out under anhydrous conditions, all the reagents and solvents were previously dried, distilled or recrystallizated to minimize the water and oxygen contents; [a] bptbp = (2-biphenyl)di-*tert*-butylphosphine; Pro = L–proline; [b] solvents that present the ‡ simbol were freshly distilled; [c] %yield after chromatographic column.
Note: all the reported yields are referred to the converted starting material (except [c]).

The search of the best reaction conditions led to some interesting results: a) the use of the catalytic systems Cu(I)/Cu(0) or Cu(II)/Cu(0) allows the continuous regeneration of the activated form in slightly oxidating conditions too [see Cu(I)-catalyzed Huisgen cycloaddition, Section 2.5]; b) organic bases like DBU can replace the inorganic ones described in the literature, making the base more available in apolar solvents and allowing to use a solvent different from the usually employed DMF; c) proline, used to promote the solubilization of the copper salts, does not seem to have a significative effect; d) copper is necessary for the catalysis of the reaction: the nucleophilic aromatic substitution promoted only by the temperature (experiments 13 and 15) affords the product in very lower yields and under these conditions, it seems that tribasic phosphate is better as a base than DBU in DMF; e) anhydrous conditions gave better yields; f) at 80 °C the reaction is very slow, either by microwave and oil bath heating (experiments 7 and 10); in the MW reactor it is necessary to heat at 120 °C to get acceptable yields; g) experiment 6 gave the best yield (46%), but in a bigger scale (experiment 18) the product was not obtained. In conclusion, the target benzolentiginosine **67** has been obtained in 30-45% yield. The analysis of the spectra of the crude reaction mixtures shows signals of the TBDMS moiety derived from desilylation processes. Desilylated products were also obtained as fractions in the flash-chromatography purifications. Actually, the used conditions for cyclizations with copper salts and bases could induce the partial deprotection of the TBDMS group. Accordingly, the choice of a different, more stable, protecting group of the hydroxy groups on the pyrrolidine ring should result in a more efficient synthesis of benzolentiginosine **67**.

2.11 Final deprotection

The last step of the synthesis, the removal of the protecting groups on the hydroxy functions in position 2 and 3, was carried out with tetra-*n*-butylammonium fluoride

(TBAF)[79] in THF at rt and afforded the deprotected benzolentiginosine **15** in a not optimized 36% yield (Scheme 2.34).



Scheme 2.34

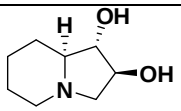
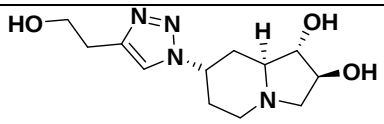
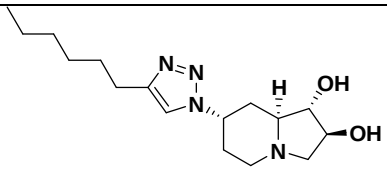
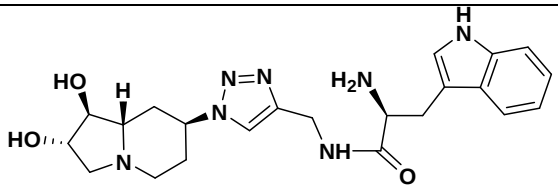
[79] Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

3. ENZYMATIC TESTS AND COMPUTATIONAL DOCKING STUDIES ON THE NEW DERIVATIVES. CONCLUSIONS AND PERSPECTIVES

In this section the results of the enzymatic tests performed on the new synthesized derivatives and the computational docking studies made on them will be discussed.

3.1 (+)-Lentiginosine Derivatives

The new synthesized derivatives of (+)-lentiginosine were tested on a commercial glucosidase (amyloglucosidase EC 3.2.1.3 from *Aspergillus niger*) by the research group of Prof. Inmaculada Robina of the Organic Chemistry Department of the University of Seville. In the Table 3.1 are shown the results of the trials.

Compound	**Structure**	**% Inhibition at 1[mM]**	**IC_{50} (μg/mL)**	**K_i (μM)**
1		100	0.43[20a]	2[20a]
10		26	-	-
11		16	-	-
12		40	-	-

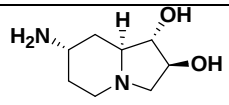
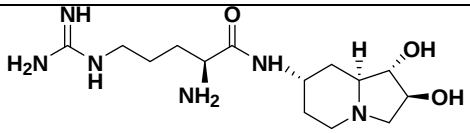
27		40	-	-
13		-	-	-

Table 3.1

In the table three values are showed: the percentage of inhibition of enzymatic activity, the concentration of inhibitor required for obtain a 50% inhibition of enzyme activity (IC_{50}) and the equilibrium constant of the link of the inhibitor to the enzyme (K_i). This last value is calculated when the 100% of inhibition of the enzymatic activity is reached with a concentration of the inhibitor of 1 mM. As we can see in the table, *no one of the derivatives can reach this value*. As we already said in the section 1.5, the principal aim of this thesis was to elaborate an efficient and versatile synthetic method to obtain derivatives of our lead compound, on the basis of general and vaguely indicative computational studies. The not excellent biological results that the tested molecules provided represent a very important starting point in the research of new more active derivatives of (+)-lentiginosine. Through our synthetic method and a more accurate study of the computational interaction between the planned derivative and the enzymatic catalytic site hopefully the goal of the discovery of new potent inhibitors can be reached. For these reasons, we thought that a deeper interaction study of our inactive derivatives was very important to carry out. In Figure 3.1 is showed the interaction between molecule **10** and the enzymatic cavity.

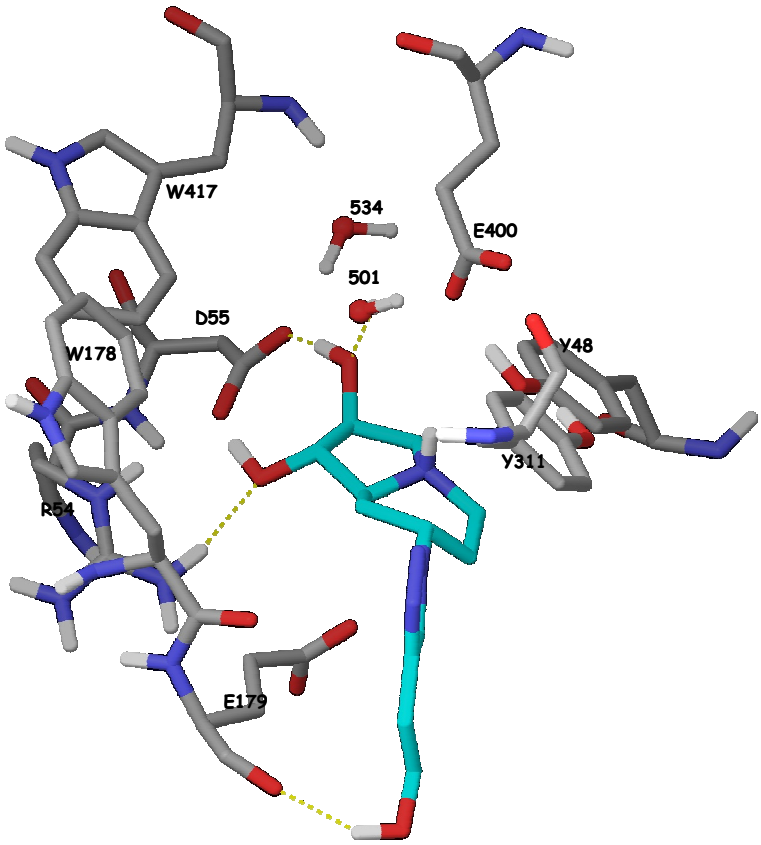


Figure 3.1

If compared to the interaction proposed for (+)-lentiginosine (see section 1.5), also in this case the two hydroxy groups seem to form two hydrogen bonds with the same amino acidic residues that interact with (+)-lentiginosine (the **D55** aspartate and the **R54** arginine). However, the nitrogen atom is not involved in any hydrogen bond with no amino acidic residue and no water molecule. Indeed, **W501** here interacts with the OH function on C-2 carbon. Furthermore, the OH function on the lateral alkyl chain forms another hydrogen bond with the glutamate **E179,** and this fact probably hampers the correct insertion of the molecule into the cavity.

The molecule **11** gave a percentage of inhibition of 16. As it is showed in Figure 3.2, besides the expected hydrogen bonds of the two hydroxyl functions with the two residues **D55** and **R54**, no others constructive interactions are developed by the molecule, maybe due to the presence of the long alkyl chain attached to the molecule.

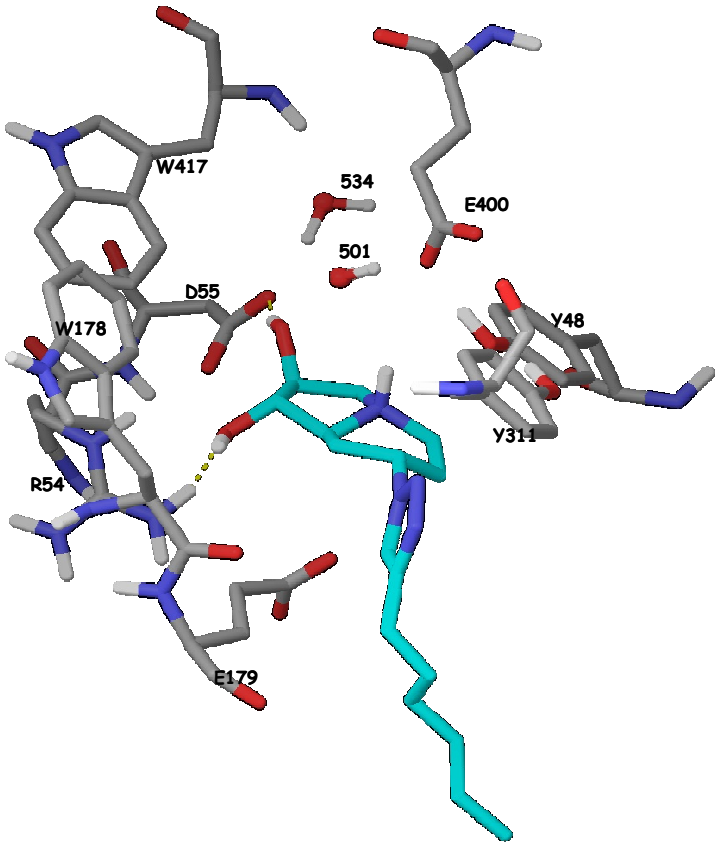


Figure 3.2

The molecule **12** gave a better inhibition (40%) respect to the two precedent compounds. It's docking with the enzyme cavity is reported in Figure 3.3.

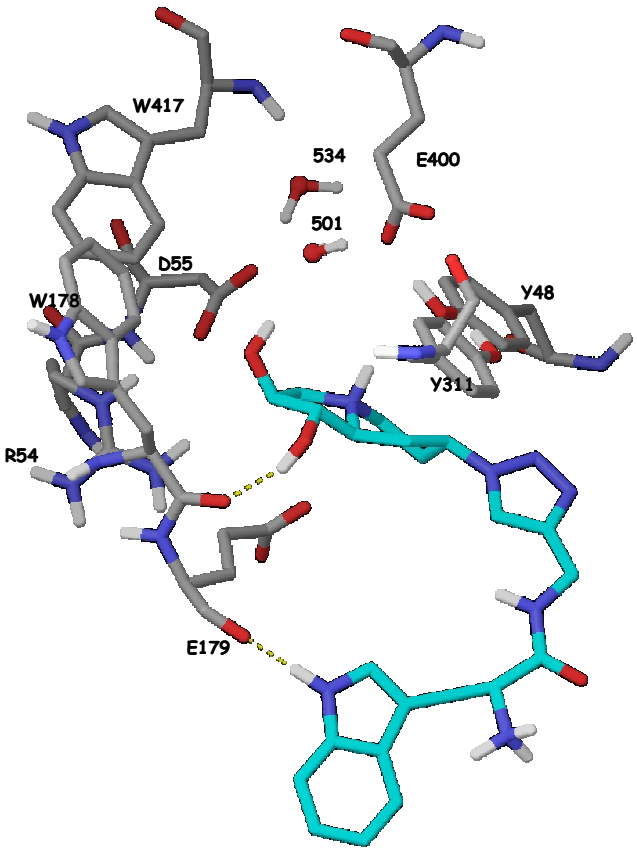


Figure 3.3

Also in this case are not measurable beneficial interactions of the aromatic fragment on the indolizidine mojety with the enzyme cavity. However, this compound gave better results in the enzymatic tests compared to **10** and **11**. This result demonstrates that is rather difficult to predict the enzymatic activity of the compounds on the basis of a simple computational study and that the collection of data of a large library of candidate inhibitors is necessary to find the important features that allow to design a good inhibitor .

27 gave the same percentage of inhibition of **12** (40%). In Figure 3.4 is illustrated its supposed interaction.

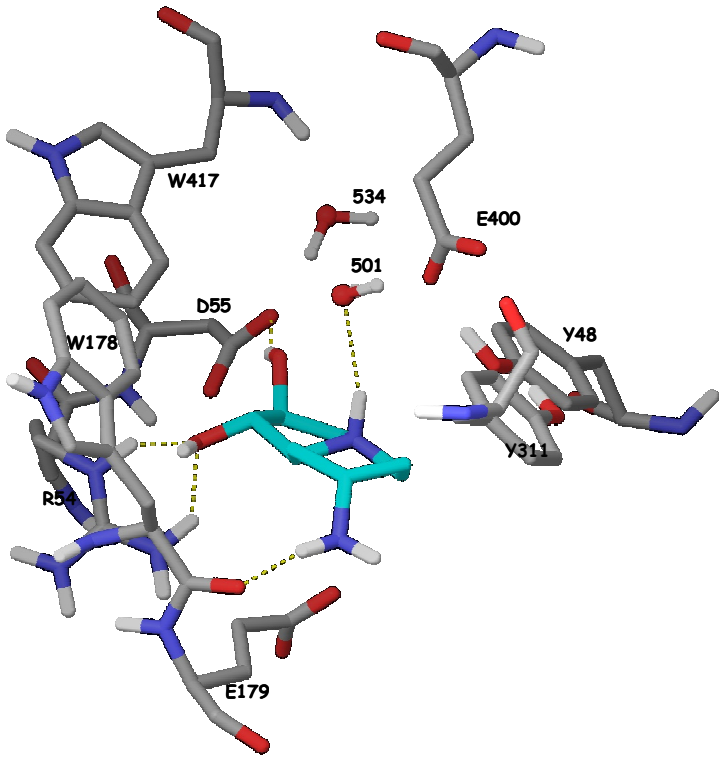


Figure 3.4

Finally, in Figure 3.5 is showed the inadequate level of interaction between compound **13** and the enzymatic cavity, that amply justifies the total lack of activity of the compound.

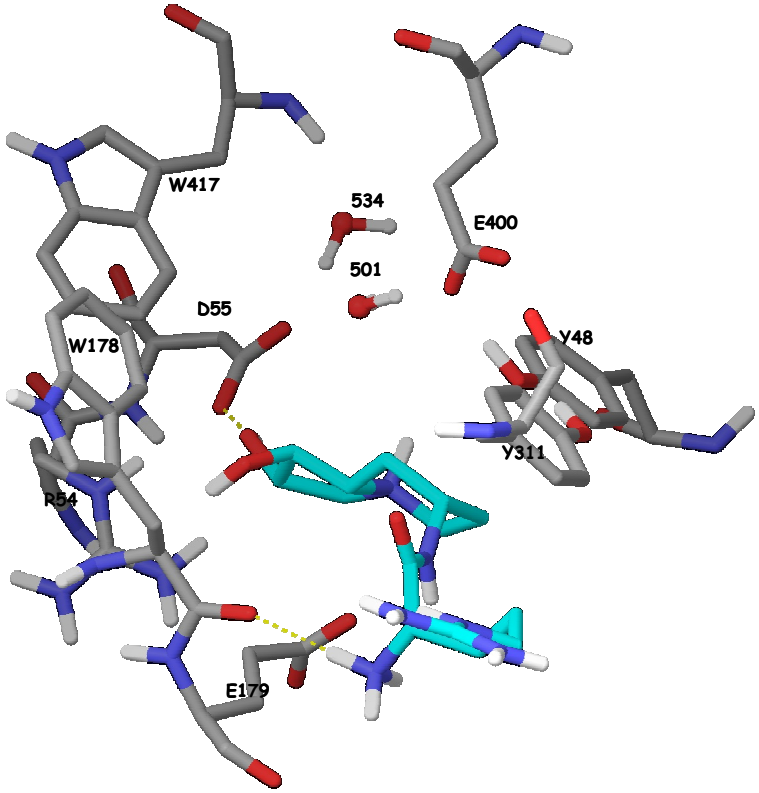


Figure 3.5

The computational studies of docking of **15** with the enzyme cavity suggest a good interaction. Unfortunately, enzymatic tests of compound **15** are not yet available (see Section 1.5).

3.2 (–)-Lentiginosine Derivatives

In section 1.4 the proapoptotic activity of (–)-lentiginosine is illustrated. In search of new active derivatives of this compound and, above all, with the aim of investigate the mechanism of action of (–)-lentiginosine, three new derivatives were synthesised: **26**, **21** and **24** (Figure 3.6).

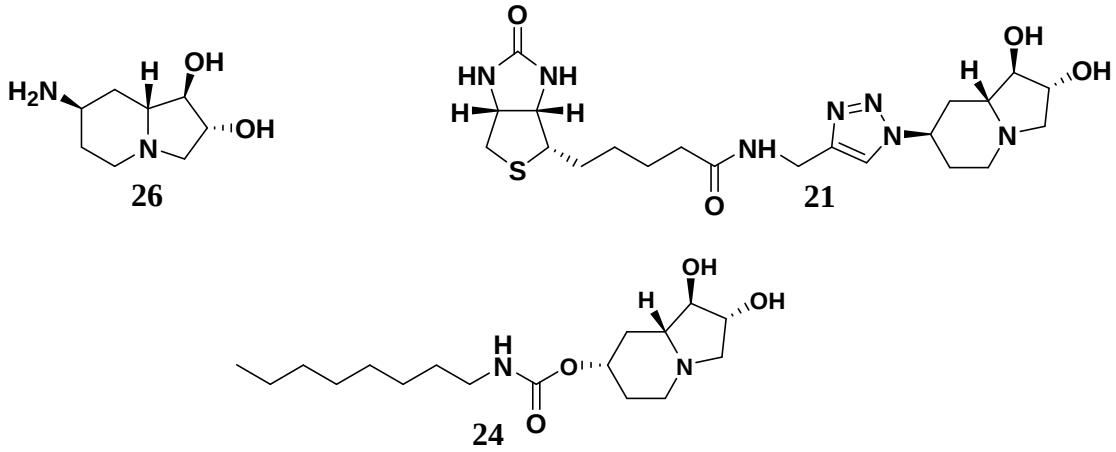


Figure 3.6

In particular, compound **21** represents an effort of obtaining a labelled derivative of (–)-lentiginosine that could be a tool for the enlightenment of its mechanism of action. Activity-based protein profiling (ABPP) has been shown to be a versatile tool for the investigation of assignment of structure and functions of a lot of life crucial importance proteins. A typical probe for ABPP represents a mechanism-based inactivator specific to the active site of the enzyme under investigation conjugated via a noncleavable or cleavable linker to biotin which allows for affinity chromatography or fluorescence spectroscopy.[80] In general, the biotin-(strept)avidin interaction has been proven to be an indispensable tool for biorecognition in the context of diagnostics, biotechnology and nanotechnology.[81]

Compounds **26** and **21** have been subjected to biological tests performed by the research group of Dr. Beatrice Macchi of the Neoroscience Department of University of Rome Tor Vergata. Unfortunately, no one of the two compounds showed any proapoptotic activity on the employed cell lines.

Therefore, we thought to synthesize a derivative in which could be present a medium-sized linker that could keep the biotin portion as long as possible from the indolizidine

[80] Uttamchandani, M.; Li, J.; Sun, H.; Yao, S. Q. *ChemBioChem* **2008**, *9*, 667-675.
[81] Wilchek, M.; Bayer, E.A.; Livnah, O. *Immunology Lett.* **2006**, *103*, 27-32.

mojety of lentiginosine. For this reason, starting from the intermediate **25** we obtained the molecule **24**. Unfortunately, enzymatic tests of compound **24** are not yet available.

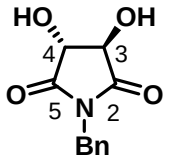
4. EXPERIMENTAL SECTION

All the reactions requiring anhydrous conditions were carried out under nitrogen, and the solvents were appropriately dried before use. R_f values refer to TLC on 0.25 mm silica gel plates (Merck F254, Macherey-Nagel precoated sheets). Melting points (m. p.) were determined on a Thiele Electrothermal apparatus. Polarimetric measurements were performed on a JASCO DIP-370. NMR spectra were measured on Varian Gemini (^{1}H, 200 MHz, ^{13}C, 50 MHz) and Varian INOVA (^{1}H, 400 MHz, ^{13}C, 100 MHz) nuclear magnetic resonance spectrometers; $CDCl_3$ was used as solvent in NMR analyses if not otherwise specified. The NMR data are reported in δ (ppm) from TMS at 25 °C and peak assignments were made on the basis of ^{1}H–^{1}H COSY and HMQC experiments. IR spectra were recorded with a Perkin-Elmer Spectrum BX FT-IR System spectrophotometer on $CDCl_3$ solutions. Mass spectra were recorded on a QP5050 Shimadzu spectrometer with a GC; relative percentages are shown in parentheses. Elemental analyses were performed with a Perkin-Elmer 2400 analyzer. Accurate mass spectra were recorded on a LTQ-Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source.

The two series of enantiomeric compounds were synthesized starting from respectively (2*R*, 3*R*)-(+)-tartaric acid (e.e. ≥99.5%) and (2*S*, 3*S*)-(-)-tartaric acid (e.e.99%) purchased from Sigma-Aldrich.

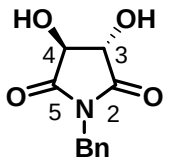
Synthesis of (3*S*,4*S*)- and (3*R*,4*R*)-1-Oxido-3,4-dihydro-2*H*-pyrrole-3,4-diyl dibenzoate (34 and 35)

(3R,4R)-1-Benzyl-3,4-dihydroxypyrrolidine-2,5-dione (**36**)



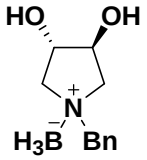
Benzylamine (18.21 mL, 0.167 mol) was slowly added to a suspension of (3*R,4R*)-(+)-tartaric acid (25.02 g, 0.167 mol) in 50% aqueous methanol (34 mL). The viscous mixture was concentrated under reduced pressure. Xylene (445 mL) was added to the obtained white solid and the reaction mixture was refluxed in a Dean-Stark apparatus set in an oil bath at 150 °C for 8 h. During that period, additional xylene (4x50 mL) was added. The resulting mixture was cooled, filtered under vacuum and the solid washed with petroleum ether. The crude imide **36** was obtained in 81% yield (29.97 g, 0. 135 mol) as a white solid and was used in the next step without further purification. The NMR properties of **36** are identical to those reported in the literature. [39]

(3S,4S)-1-Benzyl-3,4-dihydroxypyrrolidine-2,5-dione (**68**)



Compound **68** was prepared from (3*S,4S)*-(–)-tartaric acid (25.11 g, 0.167 mol) using the same procedure as for compound **36** and was obtained in 74% yield (27.34 g, 0.124 mol) as a white solid after recrystallization from ethanol. The NMR properties of **68** are identical to those reported in the literature.[39]

(3S,4S)-1-N-Benzyl-3,4-dihydroxypyrrolidine-1-borane (**37**)

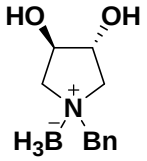


A solution of iodine (21 g, 83 mmol) in THF (100 mL) was added dropwise to a vigorously stirred ice bath cooled suspension of $NaBH_4$ (6.24 g, 165 mmol) and **36** (7.25 g, 33 mmol) in THF (153 mL) during 2 hours under nitrogen. The reaction mixture was refluxed for 6 hours and then the excess of borane was carefully destroyed with MeOH until no further effervescence was observed (35 mL). The obtained clear solution was concentrated under reduced pressure and the resulting white mixture was dissolved in MeOH and concentrated under reduced pressure three times (3x90 mL). Finally, to eliminate the last traces of MeOH, the product was sequentially treated and concentrated under reduced pressure first with EtOAc (30 mL) and then with *i*Pr_2O (30 mL). Deionized H_2O (70 mL) was added to the obtained white residue and the mixture was first washed with petroleum ether (5x20 mL) and then extracted with EtOAc (8x20 mL). The combined EtOAc phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **37** as a white solid that was used in the next step without further purification. A sample purified by chromatography on silica gel (eluent: petroleum ether / EtOAc 2:3) afforded analytically pure **37**.

37 R_f = 0.36; m. p. 98 – 99 °C; $[\alpha]_D^{25}$= + 21.2 (*c* = 0.525, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.43–7.35 (m, 5H, Ph), 4.67 (dt, *J* = 1.7; 6.5 Hz, 1H, 3-H), 4.09 (A part of an AB system, *J* = 13.2 Hz, 1H, C*H*HPh), 4.05 (B part of an AB system, *J* = 13.2 Hz, 1H, C*H*HPh), 4.02 (br d, *J* = 7.1 Hz, 1H, 4-H), 3.54 (ddd, *J* = 11.5; 6.6; 1.2 Hz, 1H, 2-H_a), 3.33 (dd, *J* = 12.0, 7.1 Hz, 1H, 5-H_a), 3.14 (br d, *J* = 12.0 Hz, 1H, 5-H_b), 2.85 (dd, *J* = 11.5, 6.3 Hz, 1H, 2-H_b) ppm; ^{13}C-NMR (50 MHz): δ = 132.6 (d; 2C, Ph), 130.7 (s; Ph),

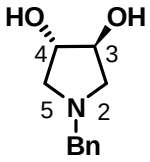
129.3 (d; Ph), 128.4 (d; 2C, Ph), 78.8 (d; C-4), 78.5 (d; C-3), 67.2 (t; CH_2Ph), 64.5 (t; C-5), 63.8 (t; C-2) ppm; ^{11}B NMR (64 MHz) δ = -8.71 ppm; IR ($CDCl_3$): ν = 3610, 3390 (br), 2955, 2381 (B-H st), 1455 (B-N st), 1169, 1107, 1052 cm^{-1}; anal. calcd. or $C_{11}H_{18}BNO_2$ (207.1): C 63.80, H 8.76, N 6.76; found: C 63.70, H 9.08, N 6.73.

(3R,4R)-1-N-Benzyl-3,4-dihydroxypyrrolidine-1-borane (**69**)



Compound **69** was prepared starting from imide **68** (8.03 g, 36 mmol) following the same procedure as for compound **37** and was used in the next step without further purification. A sample purified by chromatography on silica gel (eluent: petroleum ether / EtOAc 2:3) afforded analytically pure **69**. The NMR properties of **69** are identical to those of the enantiomer **37**.

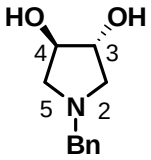
(3S,4S)-1-Benzylpyrrolidine-3,4-diol (**38**)



A 3 M HCl aqueous solution (26 mL) was added to an ice bath cooled suspension of the crude pyrrolidine-borane adduct **37** (6.80 g) in diethyl ether (30 mL). The suspension was stirred at room temperature until no further effervescence and no solid at the interphase were observed. Then the two layers were separated and the aqueous solution

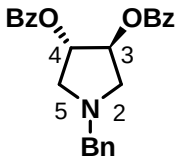
was washed with EtOAc (3x10 mL), treated with a 3 M NaOH aqueous solution (75 mL) at 0 °C, and then extracted with EtOAc (6x30 mL). The combined organic phases were dried over Na_2SO_4. After filtration and concentration under reduced pressure, crude pyrrolidine **38** was obtained as a white solid that was directly esterified.

(3R,4R)-1-Benzylpyrrolidine-3,4-diol (**70**)



Pyrrolidine **70** was prepared starting from the pyrrolidine-borane adduct **69** following the same procedure as for compound **38**. The NMR properties of **70** are identical to those of the enantiomer **38**.

(3S,4S)-1-Benzylpyrrolidine-3,4-diyl dibenzoate (**39**)

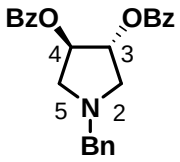


To a solution of **38** (4.9 g, 25.36 mmol) in CH_2Cl_2 (40 mL) was added a solution of Na_2CO_3 (6,46 g, 60.98 mmol in 40 mL H_2O). The mixture was stirred vigorously at 0 °C. Benzoylchloride (7.29 mL, 62.85 mmol) was added dropwise at 0 °C and stirring was continued at rt overnight. The phases were separated and the aqueous one was extracted with CH_2Cl_2 (3·30 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4 and concentrated to give a white solid residue.

Recrystallization from *i*Pr_2O yielded **39** as a white crystalline solid (7.20 g, 17.95 mmol, 54% overall yield from imide **36**).

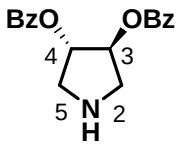
39 ^{1}H NMR (400 MHz): δ = 8.09-8.04 (m, 4H, Bz); 7.60-7.54 (m, 2H, Bz); 7.48-7.41 (m, 4H, Bz), 7.40–7.23 (m, 5H, Ph), 5.58-5.53 (m, 2H, CHO), 3.73 (A part of an AB system, *J* = 13.0 Hz, 1H, C*H*HPh), 3.68 (B part of an AB system, *J* = 13.0 Hz, 1H, C*H*HPh), 3.28 (dd, *J* = 10.3, 6.2 Hz, 2H, C*H*HN), 2.75 (dd, *J* = 10.3, 4.6 Hz, 2H, C*H*HN) ppm.

(3R,4R)-1-Benzylpyrrolidine-3,4-diyl dibenzoate (**71**)



Compound **71** was prepared starting from pyrrolidine **70** following the same procedure as for **39** and was obtained in 65% overall yield from **68** (9.404.6 g, 23 mmol). The NMR properties of **71** are identical to those of the enantiomer **39**.

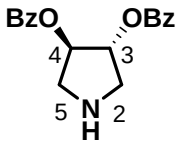
(3S,4S)-Pyrrolidine-3,4-diyl dibenzoate (**40**)



N-Benzyl-pyrrolidine **39** (4.6 g, 11.45 mmol) was suspended in MeOH (74 mL). Acetic acid (6.5 mL, 114.5 mmol) was added under stirring at 0 °C. Then the solution was treated with hydrogen gas (1 Atm) in the presence of 10% Pd/C (0.753 g, 0.70 mmol Pd) at rt overninght. Then, the catalyst was filtered off through a short pad of Celite in

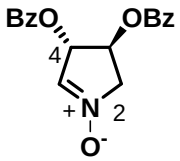
vacuum and the MeOH solution obtained was concentrated under reduced pressure. The resulting white solid was dissolved in EtOAc (40 mL) and treated with a saturated aqueous solution of Na_2CO_3 (50 mL) at 0 °C. The two layers were separated and the aqueous solution was extracted with EtOAc (3x20 mL). The combined organic phases were washed with brine (40 mL) and dried over Na_2SO_4. After filtration and concentration under reduced pressure the product was obtained as a colorless oil (3.226g, 11 mmol, 91%) which was directly oxidazed. The NMR properties of **40** are identical to those reported in the literature.[40]

(3R,4R)-Pyrrolidine-3,4-diyl dibenzoate (**72**)



Compound **72** was prepared starting from pyrrolidine **71** (4 g, 10 mmol) following the same procedure as for compound **40** and was obtained in 92% yield (2.84 g, 10 mmol). It was used in the next step without further purification. The NMR properties of **72** are identical to those of the enantiomer **40.**

(3S,4S)-1-Oxido-3,4-dihydro-2H-pyrrole-3,4-diyl dibenzoate (**34**)

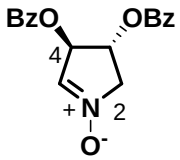


$NaHCO_3$ (4.35 g, 51.84 mmol) was added to a stirred solution of amine **40** (3.23 g, 10.37 mmol) in acetonitrile-THF (4:1, 20 mL) and Na_2EDTA (0.01 M, 15 mL). The mixture was then cooled in an ice bath and Oxone® (9.47 g, 15.39 mmol) was added

portionwise over 5 h and 35 min. The mixture was then diluted with EtOAc (50 mL) and deionized H_2O (100 mL) was added. The two phases were separated and the aqueous one was extracted with EtOAc (3x30 mL). The combined organic phases were washed with brine (30 mL), dried over Na_2SO_4, and filtered. The solution was concentrated under reduced pressure to give a white solid in 95% yield (3,22 g, 10 mmol). A sample purified by chromatography on silica gel (eluent: petroleum ether/EtOAc, 2:1) afforded analytically pure **34**.

34 R_f =0.38; m.p. 155 – 158 °C (with decomposition); $[\alpha]_D^{25}$= +220.53 (*c* = 0.525, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.10-8.00 (m, 4H, Ph), 7.65–7.57 (m, 2H, Ph), 7.50–7.43 (m, 4H, Ph), 7.17–7.15 (m, 1H, 5-H), 6.11–6.09 (m, 1H, 4-H), 5.74 (dm, J = 6.3, 1H, 3-H), 4.73 (dddd, J = 15.7, 6.3, 2.1, 1.2 Hz, 1H, 2-H_a), 4.07 (dddd, J = 15.7, 2.1, 1.2, 0.7 Hz, 2-H_b); ^{13}C-NMR (50 MHz): δ = 165.4 (s; CO), 165.3 (s; CO), 133.9 (d; Ph), 133.8 (d; Ph), 130.3 (d; C-2), 129.8 (d; 4C, Ph), 128.6 (d; 4C, Ph), 128.4 (s; Ph), 128.3 (s; Ph), 78.2 (d; C-3), 72.3 (d; C-4), 67.2 (t; C-5) ppm; IR (CDCl3) 1725, 1579, 1452, 1317, 1263, 1105 cm−1; MS (EI): *m/z (%)* = 325 (1, M^+), 203 (10), 105 (100), 82 (11), 77 (30); anal. calcd. for $C_{18}H_{15}NO_5$ (325.31): C 66.46, H 4.65 , N 4.31; found C 66.32, H 4.36, N 4.25.

(3R,4R)-1-Oxido-3,4-dihydro-2H-pyrrole-3,4-diyl dibenzoate (**35**)



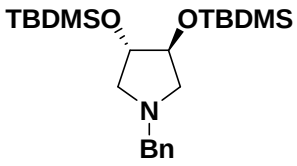
Compound **35** was prepared starting pyrrolidine **72** (2.62 g, 8.42 mmol) following the same procedure as for the compound **34** and was obtained in 95% yield as a white solid

(2.59 g, 8 mmol). A sample purified by chromatography on silica gel (eluent: petroleum ether/EtOAc, 2:1) afforded analytically pure **35**.

35:$[\alpha]_D^{24}$= – 218.58 (*c* = 0.430, $CHCl_3$); anal calcd. for $C_{18}H_{15}NO_5$ (325.31): C 66.46, H 4.65 , N 4.31; found C 66.26, H 4.50, N 4.19. Spectral properties are identical to those of **34.**

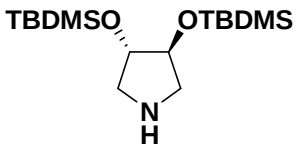
Synthesis of (3*S*,4*S*)-3,4-Bis{[tert-butyl(dimethyl)silyl]oxy}-3,4-dihydro-2H-pyrrole 1-oxide (16)

(3S,4S)-1-Benzyl-3,4-bis{[tert-butyl(dimethyl)silyl]oxy}pyrrolidine (**41**)



Anhydrous DMF (42 mL) was added to a mixture of **38** (3.481 g, 18.01 mmol) and imidazole (3.681 g, 54.03 mmol) under nitrogen atmosphere. The solution was then cooled in an ice bath and TBDMS-Cl (6.778 g, 44.97 mmol) was added portionwise. At the end of the addition the solution was heated at 60 °C for 2 h and monitored by *TLC* every 30 min. Then, distilled H_2O (40 mL) was added to the mixture at rt and the solution was extracted with petroleum ether (4 x 20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduce pressure. A colorless oil was obtained (7.408 g, quantitative yield). The NMR properties of **41** are identical to those reported in the literature.[29]

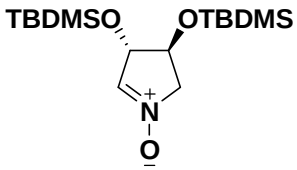
(3S,4S)-3,4-Bis{[tert-butyl(dimethyl)silyl]oxy}pyrrolidine (**42**)



A mixture of pyrrolidine **41** (5.76 g, 0.014 mmol) $Pd(OH)_2/C$ at 20% (50% water) (2.02 g, 0.14 molar equivalents of palladium hydroxyde) in MeOH (71 mL) was stirred at rt overnight under a hydrogen atmosphere (1 Atm). The solution was then filtered through a short pad of celite® and concentrated under reduced pressure to give **42** in 85% yield (3.83 g, 0.011 mmol).

42 ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.92-4.00 (m, 2H, 3-H, 4-H), 3.10 (dd, *J*= 12.1, 3.8 Hz, 2H, 2-Ha, 5-Ha), 2.67 (br d, *J*= 12.1 Hz, 2H, 2-Hb, 5-Hb), 2.50 (br s, 1H, NH), 0.87 (s, 18H, *t*-Bu), 0.06 (s, 6H, CH_3), 0.05 (s, 6H, CH_3).

(3S,4S)-3,4-Bis{[tert-butyl(dimethyl)silyl]oxy}-3,4-dihydro-2H-pyrrole 1-oxide (**16**)

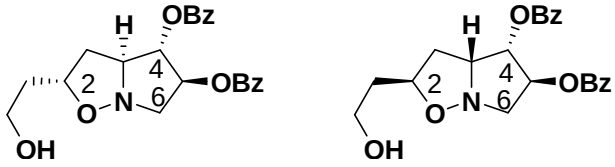


A 0.01 M aqueous solution of Na_2-EDTA (8.3 mL) was added under stirring to a solution of **42** (1.868 g, 5.633 mmol) in a 1:4 mixture of THF and CH_3CN (10.8 mL). The solution was then cooled in an ice bath and $NaHCO_3$ (2.366 g, 28.16 mmol) was added. Then, oxone® (3.636 g, 5.914 mmol) was added portionwise during 2 h. The reaction was monitored via *TLC* during the addition of oxone®. At the end, the reaction mixture was diluted with H_2O (10 mL) and EtOAc (10 mL). The aqueous phase was separated from the organic one and extracted with CH_2Cl_2 (2x10 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified through a column on silica gel (eluent: EtOAc

/petroleum ether: 1:1). Nitrone **16** was obtained in 70% yield (1.426 g, 4. 12 mmol) as a deliquescent white solid. The spectral properties of **16** are identical to those reported in the literature.[29]

Synthesis of (1*S*,2*S*,7*R*,8a*S*)- and (1*R*,2*R*,7*S*,8a*R*)-7-Hydroxyoctahydroindolizine-1,2-diyl dibenzoate (45a and 25a)

(2S,3aS,4S,5S)- and (2R,3aR,4S,5S)-2-(2-Hydroxyethyl)hexahydropyrrolo[1,2-b]isoxazole-4,5-diyl dibenzoate (**44a** and **44b**).



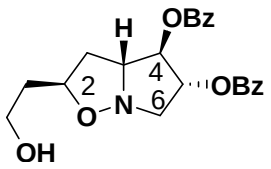
Butenol **43** (3.6 mL, 42 mmol) was added to a suspension of the crude nitrone **34** (2.72 g, 8.37 mmol) in toluene (8 mL) and the reaction mixture was heated in a sealed vial in oven at 100 °C for 1 h 30 min. The solvent and the excess of butenol were removed under reduced pressure and a mixture of the three major diastereoisomers in 23.7:7.4:1 ratio was obtained (^{1}H NMR 400 MHz monitoring). After flash chromatography on silica gel (eluent: EtOAc /petroleum ether 2:1) the adducts were obtained in 83% yield (2.76 g, 7 mmol) as a dark brown oil. The diastereomeric mixture was used in the next step without separation of the diastereoisomers. The two major diastereoisomers were obtained in a 3.2:1 ratio (^{1}H NMR 400 MHz monitoring).

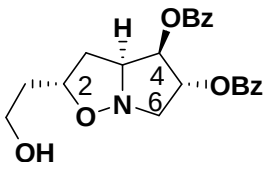
44a ^{1}H NMR (400 MHz): δ = 8.06-7.99 (m, 4H, Ph), 7.61-7.54 (m, 2H, Ph), 7.48-7.41 (m, 4H, Ph), 5.61 (pseudo quintet, *J* = 2.9 Hz, 1H, 5-H), 5.47 (pseudo t, *J* = 3.1 Hz, 1H, 4-H), 4.56 (pseudo quintet, *J* = 6.4 Hz, 1H, 2-H), 3.89-3.83 (m, 1H, 3a-H), 3.84 (dd, *J* =14.6, 5.9 Hz, 1H, 6-H$_a$), 3.79-3.72 (m, 2H, C*H*$_2$OH), 3.58 (br dd, *J* = 14.6, 3.2 Hz, 1H,

6-H_b), 2.78 (ddd, *J* = 12.7, 6.7, 3.4 Hz, 1H, 3-H_a), 2.41 (ddd, *J* = 12.7, 8.4, 7.1 Hz, 1H, 3-H_b), 1.86 (pseudo q, *J* = 5.8 Hz, 2H, C*H*$_2$CH$_2$OH) ppm; ^{13}C-NMR (100 MHz): δ = 165.8 (s; C=O), 165.6 (s; C=O), 133.5 (d; Ph), 133.4 (d; Ph), 129.9 (d, 2C; Ph), 129.7 (d, 2C; Ph), 129.4 (s; Ph), 129.1 (s; Ph), 128.5 (d, 4C; Ph), 83.6 (d; C-4), 78.6 (d; C-5), 75.6 (d; C-2), 71.1 (d; C-3a), 60.0 (t; CH_2OH), 59.9 (t; C-6), 39.9 (t; C-3), 37.1 (t; *C*H$_2$CH$_2$OH) ppm.

44b ^{1}H NMR (400 MHz, detectable signals): δ = 5.80 (dt, *J* = 3.7, 6.1 Hz, 1H, 5-H), 5.68 (dd, *J* = 6.7, 3.7 Hz, 1H, 4-H), 4.41 (pseudo quintet, *J* = 6.74 Hz, 1H, 2-H), 4.30 (ddd, *J* = 8.5, 6.7, 2.3 Hz, 1H, 3a-H), 3.38 (dd, *J* = 14.0, 5.8 Hz, 1H, 6-H_b), 2.41 (ddd, *J* = 12.9, 7.1, 2.3 Hz, 1H, 3-H_a), 2.15 (ddd, *J* = 12.9, 8.5, 7.0 Hz, 1H, 3-H_b) ppm.

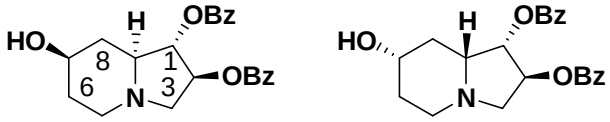
(2R,3aR,4R,5R)- and (2S,3aS,4R,5R)-2-(2-Hydroxyethyl)hexahydropyrrolo[1,2-b]isoxazole-4,5-diyl dibenzoate (**73a** and **73b**)





The mixture of the two main diastereoisomers **73a** and **73b** was prepared starting from nitrone **35** (2.59g, 7.97 mmol) following the same procedure as for the mixture of the **44a** and **44b.** The diastereomeric adducts were obtained in 70% overall yield (2.2g, 5.5 mmol).

(1S,2S,7R,8aS)- and (1S,2S,7S,8aR)-7-Hydroxyoctahydroindolizine-1,2-diyl dibenzoate (**45a** and **45b**)



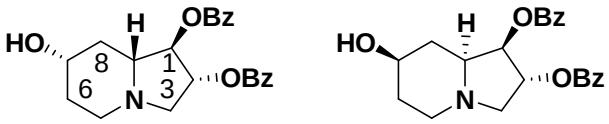
Cold freshly distilled MsCl (0.3 mL, 3.987 mmol) was added dropwise to a solution of the diastereomeric cycloadducts **44a** and **44b** (1.44 g, 2.51 mmol of **44a** and 1.11 mmol of **44b**) and NEt_3 (0.7 mL, 5 mmol) in CH_2Cl_2 (distilled over P_2O_5, 12 mL) at 0 °C under nitrogen atmosphere. The mixture was stirred for 1 h at 0 °C, and then concentrated under reduced pressure. The residue was diluted with THF (9 mL) and reconcentrated for two times. The residue was dissolved in MeOH (40 mL), treated with a catalytic amount of 10% Pd/C (230 mg) and reacted under H_2 atmosphere (1 Atm) overnight. The reaction mixture was filtered through a short pad of Celite and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (15 mL) and washed with a saturated aqueous $NaHCO_3$ solution (15 mL). The aqueous solution was extracted with CH_2Cl_2 (3x15 mL) and the combined organic phases washed with H_2O (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification and separation of the crude product by chromatography on silica gel (eluent: EtOAc / petroleum ether 3:1) gave **45a** (783.4 mg, 2.05 mmol, 82%) and **45b** (311.5 mg, 0.82 mmol, 73%) as colorless solids.

45a: R_f = 0.31; m. p. 148.4 – 149.5 °C; $[\alpha]_D^{24}$ = +110.052 (*c* = 0.385, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.11-8.02 (m, 4H, Ph), 7.60-7.53 (m, 2H, Ph), 7.47-7.40 (m, 4H, Ph), 5.46-5.40 (m, 2H, 1-H, 2-H), 3.71-3.62 (m, 1H, 7-H), 3.19 (br d, *J* =11.2 Hz, 1H, 3-H_a), 3.06 (ddd, *J* = 11.3, 4.4, 2.5 Hz, 1H, 5-H_a), 2.87 (dd, *J* = 11.2, 6.6 Hz, 1H, 3-H_b), 2.36-2.26 (m, 2H, 8-H_a, 8a-H), 2.16 (dt, *J* = 2.7, 11.8 Hz, 1H, 5-H_b), 1.96 (dm, *J* = 12.4 Hz, 1H, 6-H_a), 1.66 (ddt, *J* = 11.1, 4.6, 12.4 Hz, 1H, 6-H_b), 1.58 (q, *J* = 11.4 Hz, 1H; 8-H_b) ppm; ^{13}C-NMR (100 MHz): δ = 166.4 (s; C=O), 165.8 (s; C=O), 133.3 (d; Ph), 133.2 (d; Ph), 129.9 (d, 2C; Ph), 129.8 (d, 2C; Ph), 129.7 (s; Ph), 129.6 (s; Ph), 128.4 (d, 2C;

Ph), 128.3 (d, 2C; Ph), 81.7 (d; C-1), 78.3 (d; C-2), 69.2 (d; C-7), 65.9 (d; C-8a), 58.9 (t; C-3), 49.8 (t; C-5), 38.0 (t; C-8), 33.9 (t; C-6) ppm. IR (CDCl3): *v* = 3608, 2950, 2804, 1718, 1451, 1280, 1113 cm^{-1}. MS (EI): *m/z (%)* = 380 (0.07, M^+), 259 (4), 138 (100), 120 (23), 105 (70), 77 (40); anal. calcd. for $C_{22}H_{23}NO_5$ (381.4): C 69.28, H 6.08, N 3.67; found C 68.99, H 5.81, N 3.66.

45b: R_f = 0.23; ^{1}H NMR (400 MHz): δ = 8.12-8.08 (m, 2H, Ph), 8.06-8.02 (m, 2H, Ph), 7.60-7.53 (m, 2H, Ph), 7.47-7.41 (m, 4H, Ph), 5.59 (dd, *J* = 5.2, 1.3 Hz, 1H, 1-H), 5.46 (dt, *J* = 1.3, 7.0 Hz, 1H, 2-H),), 3.84 (dd, *J* =9.8. 7.3 Hz, 1H, 3-H_a), 3.75 (tt, *J* = 10.9, 4.6 Hz, 1H, 7-H), 3.17(ddd, *J* = 11.4, 4.3, 2.5 Hz, 1H, 5-H_a), 2.45 (ddd, *J* = 11.3, 5.2, 2.4 Hz, 1H, 8a-H), 2.26 (dd, *J* =9.8. 6.7 Hz, 1H, 3-H_b), 2.15 (dt, *J* = 2.6, 11.9 Hz, 1H, 5-H_b), 2.08 (ddt, *J* = 11.9, 4.5, 2.2 Hz, 1H, 8-H_a), 2.00-1.93 (m, 1H; 6-H_a), 1.63(d pseudo q, *J* = 4.3, 11.9 Hz, 1H, 6-H_b), 1.50 (pseudo q, *J* = 11.4 Hz, 1H, 8-H_b) ppm. ^{13}C-NMR (100 MHz): δ = 166.0 (s; C=O), 165.7 (s; C=O), 133.3 (d, 2C; Ph), 129.9 (d, 2C; Ph), 129.7 (d, 2C; Ph), 129.5 (s; Ph), 129.4 (s; Ph), 128.4 (d, 2C; Ph), 128.3 (d, 2C; Ph), 79.1 (d; C-1), 78.6 (d; C-2), 69.4 (d; C-7), 64.7 (d; C-8a), 58.8 (t; C-3), 50.0 (t; C-5), 34.3, 34.1 (t; C-8, C-6) ppm.

(1R,2R,7S,8aR)- and (1R,2R,7R,8aS)-7-Hydroxyoctahydroindolizine-1,2-diyl dibenzoate (**25a** and **25b**)

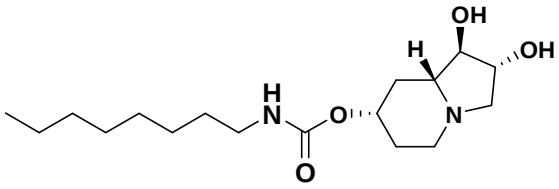


The two diastereomers **25a** and **25b** were prepared with the same procedure of **45a** and **45b** starting from 2.2 g of diastereomeric cycloadducts (3.97 mmol of **73a** and 1.52 mmol of **73b**). **25a** was obtained in 77% yield (1.16 g, 3.05 mmol). **25b** was obtained

in 62% yield (0.359 g, 0.942 mmol). Spectral properties are identical to those of **45a** and **45b.**

25a $[\alpha]_D^{23}$ = -108.175 (*c* = 0.575, $CHCl_3$); anal calcd. for 3 $C_{22}H_{23}NO_5$ 1 H_2O (1162.3): C 68.20, H 6.16, N 3.62; found: C 67.97; H 6.15; N 3.50.

(1R,2R,7S,8aR)-1,2-dihydroxyoctahydroindolizin-7-yl octylcarbamate (**24**)



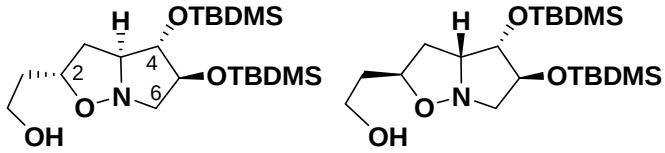
To a solution of **25a** (30 mg, 0.08 mmol) in 0.7 mL of dry CH_2Cl_2 were added triethylamine (1 μL, 0.008 mmol) and ethyl isocyanate (0.084 mL, 0.48 mmol) respectively. The reaction mixture was stirred at rt for 7 d and then concentrated. Ambersep 900-OH was then added to a solution of the crude product in MeOH (4 mL) and the mixture was agitated at rt for 3 h on a flat shaker at 150 rpm. The reaction mixture was filtered through cotton wool and concentrated under reduced pressure. Chromatography on silica gel (eluent: CH_2Cl_2/MeOH 5:1) afforded **24** in 64% yield over two steps (16.5 mg, 0.050 mmol).

24 ^{1}H NMR (CD_3OD, 400 MHz): δ = 4.63-4.46 (m, 1H, 7-H), 3.99 (ddd, *J* = 7.0, 3.3, 1.3 Hz, 1H, 2-H), 3.63 (dd, *J* = 8.3, 3.3 Hz, 1H, 1-H),), 3.07 (t, *J* = 7.0 Hz, 2H, CH_2NHCO), 2.97 (ddd, *J* = 11.3, 4.1, 2.5 Hz, 1H, 5-Ha), 2.86 (d, *J* = 10.6 Hz, 1H, 3-H_a), 2.55 (dd, *J* = 10.5, 7.1 Hz, 1H, 3-Hb), 2.34-2.27 (m, 1H, 8-Ha), 2.10 (dt, *J* = 2.2, 11.9 Hz, 1H, 5-H_b), 2.01-1.80 (m, 2H, 8a-H and 6-Ha), 1.61 (qd, *J* = 12.1, 4.4 Hz, 1H, 6-Hb), 1.53-1.42 (m, 2H, CH_2CH_2NHCO), 1.41-1.21 (m, 11H, 8-Hb, $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2$NHCO), 0.90 (t, *J* = 7.0 Hz, 3H,

CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NHCO) ppm; ^{13}C-NMR (CD_3OD, 50 MHz): δ = 158.4 (s; C=O), 84.7 (d; C-1), 78.5 (d; C-2), 72.7 (d; C-7), 69.2 (d; C-8a), 61.8 (t; C-3), 51.2 (t; C-5), 41.7 (t; CONH*CH*$_2$), 35.5 (t; C-8), 33.0 (t; chain), 31.8 (t; C-6), 30.9 (t; CONHCH$_2$*CH*$_2$), 30.4 (t; 2C, chain), 27.9 (t; chain), 23.8 (t; chain), 14.5 (t; chain) ppm; MS (ESI): *m/z* = 329.44, calcd. for $C_{17}H_{33}N_2O_4$ $[M + H]^+$: 329.24.

Synthesis of (1*S*,2*S*,7*R*,8a*S*)-1,2-Bis{[tert-butyl(dimethyl)silyl]oxy}octahydroindolizin-7-ol (55)

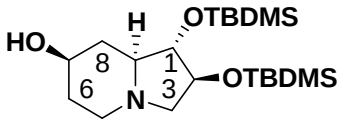
2-((2S,3aS,4S,5S)- and 2-((2R,3aR,4S,5S)-4,5-Bis{[tert-butyl(dimethyl)silyl]oxy}hexahydropyrrolo[1,2-b]isoxazol-2-yl)ethanol (**53a** and **53b**).



Butenol **43** (2 mL, 24.3 mmol) was added to a suspension of the crude nitrone **16** (1.09 g, 0.003 mmol) in toluene (3 mL) and the mixture was heated in sealed vial in oven at 100 °C for 1 h 30 min. The solvent and the excess of butenol were removed under reduced pressure and a mixture of the two major diastereoisomers in 2.86:1 ratio was obtained (^{1}H NMR 400 MHz monitoring). The two diastereoisomeric cycloadducts were separated by flash chromatography on silica gel (eluent: EtOAc /petroleum ether 1:1). The adducts **53a** (62% yield, 781 mg, 1.87 mmol) and **53b** (22% yield, 273 mg, 0.653 mmol) were obtained as dark brown oils. The total yield of the cycloaddition

reaction is 84%. The spectral properties of the adducts are identical to those reported in the literature.[82]

(1S,2S,7R,8aS)-1,2-Bis{[tert-butyl(dimethyl)silyl]oxy}octahydroindolizin-7-ol (**55**)



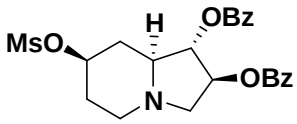
Cold freshly distilled MsCl (0.158 mL, 2.05 mmol) was added dropwise to a solution of **53a** (781 mg, 1.8 mmol) and NEt_3 (0.362 mL, 2.6 mmol) in CH_2Cl_2 (distilled over P_2O_5, 6 mL) at 0 °C under nitrogen. The mixture was stirred for 1 h at 0 °C, and concentrated under reduced pressure. The residue was diluted with THF (3 mL) and reconcentrated for two times. The residue was dissolved in MeOH (22 mL), treated with a catalytic amount of 10% Pd/C (115 mg) and reacted under H_2 atmosphere (1 Atm) overnight. The reaction mixture was filtered through a short pad of Celite and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (30 mL) and washed with a saturated aqueous $NaHCO_3$ solution (40 mL) added at 0 °C. The aqueous solution was extracted with CH_2Cl_2 (3x30 mL) and the combined organic phases were washed with brine (60 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel (eluent: EtOAc / petroleum ether 1:1) gave **55** in 84% yield (611 mg, 1.52 mmol). The spectral properties of the adducts are identical to those reported in the literature.[82]

Synthesis of (1*S*,2*S*,7*S*,8a*S*)- and (1*R*,2*R*,7*R*,8a*R*)-7-Azidooctahydroindolizine-1,2-diyl dibenzoate (47 and 22)

[82] Cardona, F.; Goti, A.; Picasso, S.; Vogel, P.; Brandi, A. *J. Carbohydrate Chemistry* **2000**, *19*, 585-601.

Method A:

(1S,2S,7R,8aS)-7-[(Methylsulfonyl)oxy]octahydroindolizine-1,2-diyl dibenzoat (**48**)

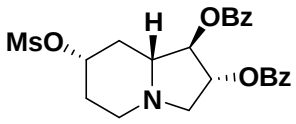


Methanesulfonyl chloride (MsCl, 0.327 mL, 4.24 mmol) was added dropwise to a solution of **45a** (809 mg, 2.12 mmol) and triethylamine (1.462 mL, 10.5 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred under nitrogen at rt for 2 h and the resulting suspension was diluted with CH_2Cl_2 (9 mL) and H_2O (9 mL). The two phases were separated and the aqueous phase extracted with CH_2Cl_2 (2x9 mL). The collected organic phases were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was filtered through a short pad of silica gel (eluent: EtOAc / petroleum ether 1:1) and evaporation of the solvent afforded the mesylate **48** (848 mg, 87%) as a white solid, which was used in the next step without further purification.

48 R_f = 0.35; m. p. 134.4 – 134.7 °C; $[\alpha]_D^{25}$ = + 97.477 (*c* = 0.325, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.11-8.02 (m, 4H; Ph), 7.61-7.54 (m, 2H; Ph), 7.48-7.41 (m, 4H; Ph), 5.67-5.40 (m, 2H; 1-H, 2-H), 4.72-4.62 (m, 1H; 7-H), 3.19 (br d, J =11.3 Hz, 1H; 3-H_a), 3.14-3.08 (m, 1H; 5-H_a), 3.03 (s, 3H; CH_3), 2.87 (dd, *J* = 11.3, 6.5 Hz, 1H; 3-H_b), 2.47 (dm, *J* = 11.6 Hz, 1H; 8-H_a), 2.41-2.33 (m, 1H; 8a-H), 2.27-2.12 (m, 2H; 5-H_b, 6-H_a), 1.95 (dq, *J* = 4.5, 12.1 Hz, 1H; 6-H_b), 1.88 (q, *J* = 11.6 Hz, 1H; 8-H_b) ppm; ^{13}C-NMR (100 MHz): δ = 166.3 (s; C=O), 165.8 (s; C=O), 133.4 (d; Ph), 133.2 (d; Ph), 129.9 (d, 2C; Ph), 129.8 (d, 2C; Ph), 129.6 (s; Ph), 129.3 (s; Ph), 128.4 (d, 2C; Ph), 128.3 (d, 2C; Ph), 81.5 (d; C-1), 78.3 (d; C-7), 78.1 (d; C-2), 65.6 (d; C-8a), 58.6 (t; C-3), 49.3 (t; C-

5), 38.9 (q, CH_3), 35.5(t; C-8), 31.5 (t; C-6) ppm. IR (CDCl3): ν = 3050, 2948, 2942, 1720, 1332, 1279, 1178, 1112 cm^{-1}; anal. calcd. for $C_{23}H_{25}NO_7S$ (459.5): C 60.12, H 5.48, N 3.05; found C 60.33, H 5.49, N 3.03.

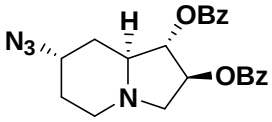
(1R,2R,7S,8aR)-7-[(Methylsulfonyl)oxy]octahydroindolizine-1,2-diyl dibenzoate (**74**)



Following the same procedure, the enantiomeric compound **74** was prepared starting from **25a** (272 mg, 0.715 mmol) and was obtained in 93% yield.

74: anal calcd. for $C_{23}H_{25}NO_7S$ (459.5): calcd. C 60.12, H 5.48, N 3.05; found C 60.39, H 5.84, N 3.25.

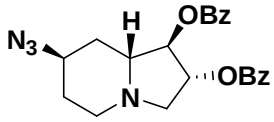
(1S,2S,7S,8aS)-7-Azidooctahydroindolizine-1,2-diyl dibenzoate (**47**)



A mixture of the mesylate **48** (591 mg, 1.29 mmol) and NaN_3 (209 mg, 3.2 mmol) in DMF (3.1 mL) was heated at 80 °C for 22 h. The reaction mixture was diluted with EtOAc (8 mL) and H_2O (9 mL), the two phases were separated and the aqueous phase was extracted with EtOAc (9 x 8 mL). The collected organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (eluent: CH_2Cl_2) to afford azide **47** (296 mg, 56%) as a colorless oil.

47 R_f = 0.34; $[\alpha]_D^{25}$= + 109.70 (*c* = 0.500, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.10-8.03 (m, 4H; Ph), 7.60-7.52 (m, 2H; Ph), 7.48-7.40 (m, 4H; Ph), 5.43-5.36 (m, 2H; 1-H, 2-H), 4.07-4.02 (m, 1H; 7-H), 3.19 (d, J =11.2 Hz, 1H; 3-H_a), 2.95-2.86 (m, 2H; 3-H_b, 5-H_a), 2.55 (ddd, *J* = 11.1, 8.4, 2.4 Hz, 1H; 8a-H), 2.42 (dt, *J* = 3.0, 11.6 Hz, 1H; 5-H_b), 2.13 (dm, *J* = 13.6 Hz, 1H; 8-H_a), 1.98-1.77 (m, 3H; 6-H, 8-H_b) ppm; ^{13}C-NMR (100 MHz): δ = 166.4 (s; C=O), 165.9 (s; C=O), 133.3 (d; Ph), 133.1 (d; Ph), 129.9 (d, 2C; Ph), 129.8 (d, 2C; Ph), 129.7 (s; Ph), 129.5 (s; Ph), 128.4 (d, 2C; Ph), 128.3 (d, 2C; Ph), 81.9 (d; C-1), 77.4 (d; C-2), 61.7 (d; C-8a), 59.5 (t; C-3), 55.5 (d; C-7), 47.3 (t; C-5), 33.2 (t; C-8), 28.6 (t; C-6) ppm; IR ($CDCl_3$): *v* = 2930, 2815, 2098, 1718, 1602, 1451, 1276, 1112 cm^{-1}; HRMS: calcd for $C_{22}H_{23}N_4O_4$ $[M+H]^+$ 407.17138, found 407.17102; anal. calcd. for $C_{22}H_{22}N_4O_4$ (406.4): C 65.01, H 5.46, N 13.78; found: C 64.70, H 5.08, N 13.55.

(1R,2R,7R,8aR)-7-Azidooctahydroindolizine-1,2-diyl dibenzoate (**22**)



Following the same procedure, the enantiomeric azide **22** was prepared starting from **74** (283 mg, 0.6 mmol) and was obtained in 54% yield (132 mg, 0.32 mmol).

22: $[\alpha]_D^{23}$= - 111.38 (*c* = 0.460, $CHCl_3$); anal. calcd. for $C_{22}H_{22}N_4O_4$ (406.4): C 65.01, H 5.46, N 13.78; found: C 64.91, H 5.07, N 13.53.

Method B:

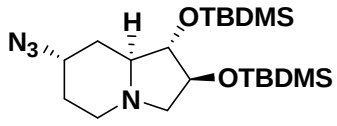
Diisopropyl azodicarboxylate (0.620 mL, 3.15 mmol) was added dropwise to a solution of triphenylphosphine (708 mg, 2.7 mmol) in dry THF (45 mL) under nitrogen atmosphere. After 5 minutes of stirring at room temperature the solution was added via cannula to a solution of **45a** (835 mg, 2,19 mmol) in dry THF (45 mL). Then diphenyl phosphoryl azide (0.681 mL, 3.15 mmol) was added dropwise to the reaction mixture and the solution was stirred overnight at rt under nitrogen atmosphere. The mixture was concentrated under reduced pressure, and the crude product was purified by flash chromatography on silica gel (eluent: petroleum ether/EtOAc 10:1, then petroleum ether/ EtOAc 4:1) to obtain **47** as a colorless oil in 71% yield (694 mg, 1.56 mmol).

47: anal. calcd. for $C_{22}H_{22}N_4O_4$ (406.4): C 65.01, H 5.46, N 13.78; found: C 65.12, H 5.69, N 13.56.

Following the same procedure, the enantiomeric azide **22** was prepared starting from **25a** (964 mg, 2.53mmol) and was obtained in 75% yield (764 mg, 1.9 mmol).

22: anal. calcd. for $C_{22}H_{22}N_4O_4$ (406.4): C 65.01, H 5.46, N 13.78; found: C 65.39, H 5.6, N 14.05.

(1S,2S,7S,8aS)-7-Azido-1,2-bis{[tert-butyl(dimethyl)silyl]oxy}octahydroindolizine (**56**)



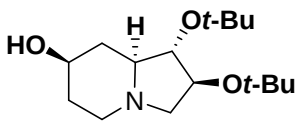
Diisopropyl azodicarboxylate (0.413 mL, 2.1 mmol) was added dropwise to a solution of triphenylphosphine (472 mg, 1.8 mmol) in dry THF (30 mL) under nitrogen atmosphere. After 5 minutes of stirring at rt, the solution was added via cannula to a solution of **55** (611 mg, 1,5 mmol) in dry THF (30 mL). Then diphenyl phosphoryl

azide (0.454 mL, 2.1 mmol) was added dropwise to the system and the solution was stirred overnight at rt under nitrogen atmosphere. After concentration under reduced pressure the crude product was purified by flash chromatography on silica gel (eluent: petroleum ether/ EtOAc 4:1) and was obtained as a colorless oil in 68% yield (435 mg, 1.02 mmol).

56 R_f : 0.60; $[\alpha]_D^{24}$= + 23.359 (*c* = 0.780, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 4.05-3.97 (m, 2H, 7-H, 2-H), 3.71 (dd, *J* = 8.4, 3.8 Hz, 1H, 1-H), 2.84 (d, *J* = 10.1 Hz, 3-Ha), 2.80-2.71 (m, 1H, 5-Ha), 2.60 (dd, *J* = 9.9, 7.8 Hz, 1H, 3-Hb), 2.25 (bt, 1H, 5-Hb), 2.15 (bt, 1H, 8a-H), 2.02 (ddd, *J* = 13.3, 5.0, 2.5 Hz, 1H, 8-Ha), 1.94-1.80 (m, 1H, 6-Hb), 1.80-1.64 (m, 1H, 6-Ha), 1.52 (bt, 1H, 8-Hb), 0.89 (s, 18 H, *t*-Bu), 0.06 (dd, *J* = 8.4, 7.0 Hz, 12 H, CH_3) ppm; ^{13}C-NMR (100 MHz): δ = 85.1 (d; C-1), 78.0 (d; C-2), 62.4 (d; C-8a), 61.8 (t; C-3), 55.5 (d; C-7), 47.7 (t; C-5), 32.5 (t; C-8), 28.7 (t; C-6), 25.9 (q; 3C, *t*-Bu), 25.8 (q; 3C, *t*-Bu), 17.9 (s; *t*-Bu), 17.8 (s; *t*-Bu), -4.07 (q; CH_3), -4.28 (q; CH_3), -4.31 (q; CH_3), -4.67 (q; CH_3), ppm; IR ($CDCl_3$): *v* = 2955, 2929,2856, 2813, 2161, 1471, 1360, 1258, 1150 cm^{-1}; MS (EI): *m/z* (%) = 426 (6, M^+), 411 (3), 398 (3), 384 (11), 369 (11), 138 (76), 73 (100); anal. calcd.: C, 65.01; H, 5.46; N, 13.78; found: C, 64.70; H, 5.08; N, 13.55.

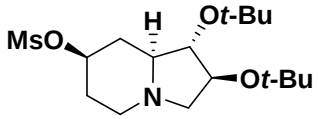
Synthesis of (1*S*,2*S*,7*S*,8a*S*)-7-Azido-1,2-di-tert-butoxyoctahydroindolizine (50)

(1S,2S,7R,8aS)-1,2-Di-tert-butoxyoctahydroindolizin-7-ol (**49**)



Cold freshly distilled MsCl (0.3 mL, 3.87mmol) was added dropwise to a solution of 2-[(2*S*,3a*S*,4*S*,5*S*)-4,5-di*tert*-butoxyhexahydropyrrolo[1,2-*b*]isoxazol-2-yl]ethanol (1.6 g, 3.52 mmol) and NEt_3 (0.69 mL, 4.93 mmol) in CH_2Cl_2 (distilled over P_2O_5, 16.4 mL) at 0 °C under N_2. The mixture was stirred for 1 h at 0 °C, and then concentrated under reduced pressure. The residue was diluted with THF (9 mL) and reconcentrated for two times. The residue was dissolved in MeOH (42 mL), treated with a catalytic amount of 10% Pd/C (187 mg) and reacted under H_2 atmosphere (1 Atm) overnight. The reaction mixture was filtered through a short pad of Celite and concentrated under reduced pressure. The crude product was dissolved in CH_2Cl_2 (7 mL) and washed with a saturated aqueous $NaHCO_3$ solution (7 mL). The aqueous solution was extracted with CH_2Cl_2 (3x10 mL) and the combined organic phases washed with H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give crude **49** (970 mg, 96%) as a yellow waxy solid, which was used in the next step without further purification.

(1S,2S,7R,8aS)-1,2-Di-tert-butoxyoctahydroindolizin-7-yl methanesulfonate (**52**)



Methanesulfonyl chloride (0.52 mL, 6.73 mmol) was added dropwise to a solution of **49** (960 mg, 3.36 mmol) and triethylamine (2.34 mL, 16.8 mmol) in CH_2Cl_2 (4.8 mL) at 0 °C. The mixture was stirred under nitrogen at rt for 2 h and the resulting suspension was diluted with CH_2Cl_2 (15 mL) and H_2O (15 mL). The two phases were separated and the aqueous phase extracted with CH_2Cl_2 (2x15 mL). The collected organic phases were washed with brine (3x10 mL), dried over Na_2SO_4 and concentrated under reduced

pressure. The crude mesylate **52** (1.2 g, 98%) was obtained as an orange oil and was used in the next step without further purification.

52 R_f = 0.47; ^{1}H NMR (400 MHz): δ = 4.59 (tt, *J* = 11.1, 4.8 Hz, 1H; 7-H), 3.84 (ddd, *J* = 7.0, 3.9, 1.7 Hz, 1H; 2-H), 3.68 (dd, *J* = 8.4, 3.9 Hz, 1H; 1-H), 3.01 (s, 3H; SCH_3), 3.00-2.94 (m, 1H; 5-H_a), 2.89 (dd, *J* = 10.1, 1.6 Hz, 1H; 3-H_a), 2.44 (dd, *J* = 10.1, 7.0 Hz, 1H; 3-H_b), 2.32 (dm, *J* = 11.4 Hz, 1H; 8-H_a), 2.10-1.98 (m, 2H; 5-H_b, 6-H_a), 1.95-1.83 (m, 2H; 6-H_b, 8a-H), 1.57 (q, *J* = 11.4 Hz, 1H; 8-H_b), 1.19 (s, 9H; *t*-Bu), 1.17 (s, 9H; *t*-Bu) ppm; HRMS: calcd for $C_{17}H_{34}NO_5S$ $[M+H]^+$ 364.21522, found 364.21493

(1S,2S,7S,8aS)-7-Azido-1,2-di-tert-butoxyoctahydroindolizine and 1-but-3-en-1-yl-3-tert-butoxy-1H-pyrrole (**50** and **51**)

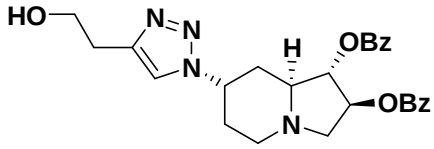
A mixture of the mesylate **52** (1.2 g, 3.3 mmol) and NaN_3 (429 mg, 6.6 mmol) in DMF (7.9 mL) was heated at 40 °C for 4 h and then at 80 °C for 2.5 h. The reaction mixture was diluted with H_2O (25 mL) and extracted with petroleum ether (3x30 mL). The collected organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (eluent: petroleum ether / EtOAc initially 19:1 then 4:1) to afford azide **50** (129 mg, 0.42 mmol, 13%) as a pale yellow waxy solid and compound **51** (138 mg, 0.7 mmol, 21%) as a brown oil.

50 R_f = 0.11; ^{1}H NMR (400 MHz): δ = 3.99 (quintet, *J* = 3.0 Hz, 1H; 7-H), 3.81 (ddd, *J* = 7.0, 4.0, 1.6 Hz, 1H; 2-H), 3.60 (dd, *J* = 8.5, 3.9 Hz, 1H; 1-H), 2.89 (ddd, *J* = 10.1, 1.4 Hz, 1H; 3-H_a), 2.75 (ddd, *J* = 11.1, 4.6, 2.2 Hz, 1H; 5-H_a), 2.49 (dd, *J* = 10.1, 7.1 Hz,

1H; 3-H_b), 2.22 (dt, *J* = 2.9, 11.8 Hz, 1H; 5-H_b), 2.11-1.97 (m, 2H; 8-H_a, 8a-H), 1.87 (dddd, *J* = 14.1, 12.4, 4.6, 3.4 Hz, 1H; 6-H_a), 1.73 (d quintet, *J* = 14.1, 2.5 Hz, 1H; 6-H_b), 1.59-1.46 (m, 1H; 8-H_b), 1.19 (s, 9H; *t*-Bu), 1.17 (s, 9H; *t*-Bu) ppm; ^{13}C-NMR (100 MHz): δ = 83.4 (d; C-1); 76.8 (d; C-2), 73.7 (s; *t*-Bu), 73.6 (s; *t*-Bu), 61.7 (t; C-3), 61.0 (d; C-8a), 55.9 (d; C-7), 47.9 (t; C-5), 32.7 (t; C-8), 29.3 (q, 3C; *t*-Bu), 28.8 (q, 3C; *t*-Bu), 28.7 (t; C-6) ppm; HRMS: calcd for $C_{16}H_{31}N_4O_2$ $[M+H]^+$ 311.24415, found 311.24373.

51 R_f = 0.68; ^{1}H NMR (400 MHz): δ = 6.39-6.37 (m, 1H; 2-H), 6.31-6.29 (m, 1H; 5-H), 5.78 (dd, *J* = 2.8, 1.9 Hz, 1H; 4-H), 5.72 (ddt, *J* = 17.1, 10.2, 6.9 Hz, 1H; C=CH), 5.04 (dm, *J* = 17.1 Hz, 1H; C=C*H*H), 5.02 (dm, *J* = 10.2 Hz, 1H; C=C*H*H), 3.81 (t, *J* = 7.1 Hz, 2H; NCH_2), 2.46 (qm, *J* = 7.0 Hz, 2H; C$H_2$$CH_2$N), 1.28 (s, 9H; *t*-Bu) ppm; ^{13}C-NMR (50 MHz): δ = 140.6 (s; C-3), 134.4 (d; H*C*=C), 117.2 (d; C-2), 116.9 (t; *C*H_2=C), 111.5 (d; C-5), 104.0 (d; C-4), 76.7 (s; *t*-Bu), 49.6 (t, NCH_2), 35.9 (t, NCH_2*C*H_2), 28.3 (q, 3C; *t*-Bu) ppm; MS (EI): m/z (%) = 193 (7, M^+), 178 (2), 137 (50), 120 (15), 96 (100); HRMS: calcd for $C_{12}H_{20}NO$ $[M+H]^+$ 194.15394, found 194.15464.

(1S,2S,7S,8aS)-7-[4-(2-Hydroxyethyl)-1H-1,2,3-triazol-1-yl]octahydroindolizine-1,2-diyl dibenzoate (**75**)

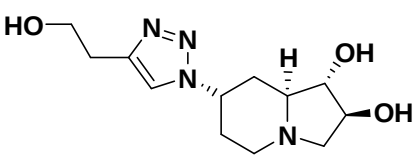


In a microwave reaction tube were added a mixture of azide **47** (70 mg, 0.172 mmol) and 97% pure 3-butin-1-ol (**9**, 0.012 mL, 0.155 mmol) in a 1:1 mixture of water and *t*-BuOH (0.8 mL) and THF (0.1 mL), copper powder (4.44 mg, 0.07 mmol) and copper sulfate (19.51 mg. 0.12 mmol). The mixture was stirred for 30 min at 80 °C, using an

irradiation power of 100 W. Then, other one portion of **9** (0.006 mL) was added and the mixture was heated at 80 °C for 15 min. To the reaction mixture was then added CH_2Cl_2 (2 mL) and Na_2SO_4. After filtration the reaction mixture was concentrated under reduced pressure and the crude product was purified by chromatography on silica gel (EtOAc/MeOH 14:1) to afford analytically pure triazole **75** (70 mg, 85%) as a colorless oil.

75 R_f = 0.26; $[\alpha]_D^{24}$ = +118.96 (*c* = 0.49, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.10-8.06 (m, 2H; Ph), 8.04-8.01 (m, 2H; Ph), 7.59-7.54 (m, 2H; Ph), 7.46-7.41 (m, 5H; Ph, triazole), 5.46-5.39 (m, 2H; 1-H, 2-H), 4.80-4.74 (m, 1H; 7-H), 3.99-3.92 (m, 2H; C*H*$_2$OH), 3.22 (d, J =11.2 Hz, 1H; 3-H_a), 3.01 (ddd, *J* = 11.3, 4.7, 2.4 Hz, 1H; 5-H_a), 2.96 (dd, *J* = 11.2, 6.7 Hz, 1H; 3-H_b), 2.94 (t, *J* = 5.7 Hz, 2H; C*H*$_2$CH$_2$OH), 2.75-2.67 (m, 2H; 8-H_a, 8a-H), 2.63 (dt, *J* = 2.9, 11.8 Hz, 1H; 5-H_b), 2.57 (br s, 1H; OH), 2.43-2.27 (m, 2H; 6-H), 2.22 (ddd, *J* = 14.5, 11.8, 4.4 Hz, 1H; 8-H_b) ppm; ^{13}C-NMR (100 MHz): δ = 166.3 (s; C=O), 166.0 (s; C=O), 145.4 (s, triazole), 133.3 (d; Ph), 133.2 (d; Ph), 129.8 (d, 4C; Ph), 129.6 (s; Ph), 129.3 (s; Ph), 128.4 (d, 2C; Ph), 128.3 (d, 2C; Ph), 121.0 (d, triazole), 82.1 (d; C-1), 77.4 (d; C-2), 61.6 (t; *C*H$_2$OH), 61.5 (d; C-8a), 59.3 (t; C-3), 53.8 (d; C-7), 47.6 (t; C-5), 33.5 (t; C-8), 29.2 (t; C-6), 28.6 (t, *C*H$_2$CH$_2$OH) ppm; IR ($CDCl_3$): *v* = 3627, 3456 br, 2957, 2836, 1718, 1603, 1451, 1280, 1113 cm^{-1}; HRMS: calcd for $C_{26}H_{29}N_5O_4$ $[M+H]^+$ 477.21325, found 477.21286; anal. calcd. for $C_{26}H_{28}N_4O_5$(476.52): C 65.53, H 5.92, N 11.76; found: C 65.11, H 5.95, N 11.40.

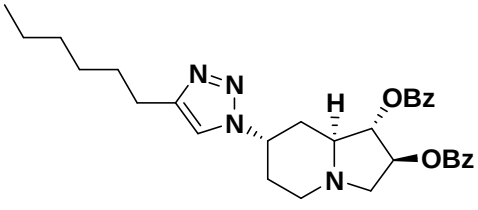
(1S,2S,7S,8aS)-7-[4-(2-hydroxyethyl)-1H-1,2,3-triazol-1-yl]octahydroindolizine-1,2-diol (**10**)



Ambersep 900 OH was added to a solution of **75** (70 mg, 0.15 mmol) in MeOH (5 mL) and the mixture was stirred at rt for 2 hours. Then the solution was filtered and concentrated under reduced pressure. The crude product was purified by column on silica gel (eluent: MeOH, 2% NH_4OH) and pure **10** was obtained in 52% yield (23.92 mg, 0.09 mmol).

10 R_f: 0.40; $[\alpha]_D^{24}$:+13.488 (*c* = 0.215, CH_3OH); ^{1}H NMR (400 MHz): δ= 7.87(s, 1H, triazole), 4.87-4.79 (m, 1H, 7-H), 3.94 (ddd, *J*= 7.0, 3.4, 1.5 Hz, 1H, 2-H), 3.81 (t, *J*=6.7 Hz, 2H, CH_2OH), 3.65 (dd, *J*= 8.2, 3.4 Hz,1H, 1-H), 2.97-2.85 (m overlapped with triplet at 2.91, 2H, 5-Ha, 3-Ha), 2.91 (t, *J*=6.7 Hz, 2H, CH_2CH_2OH), 2.65 (ddd, *J*= 13.9, 4.8, 2.4 Hz, 1H, 8-Ha), 2.58 (dd, *J*= 10.6, 7.0 Hz, 1H, 3-Hb), 2.50-2.39 (m, 2H, 6-Ha, 5-Hb), 2.31-2.16 (m, 1H, 6-Hb), 2.12 (ddd, *J*= 11.5, 8.3, 2.5 Hz, 1H, 8a-H), 1.97 (ddd, *J*= 13.9, 11.5, 4.1 Hz, 1H, 8-Hb) ppm; ^{13}C-NMR (100 MHz): δ = 145.9 (s; triazole), 123.3 (d; triazole), 85.0 (d; C-1), 77.6 (d; C-2), 64.7 (d; C-8a), 62.5 (t; C-3), 62.1 (t; CH_2OH), 55.3 (d; C-7), 49.3 (t; C-5), 34.0 (t; C-8), 29.9 (t; CH_2CH_2OH), 29.6 (t; C-6) ppm; MS (EI): *m/z* (%) = 144 (9), 57 (9), 44 (73); anal. calcd. for $C_{12}H_{20}N_4O_3.H_2O$ (286.33): C 50.34, H 7.74, N 19.57; found: C 50.69, H 7.35, N 19.21.

(1S,2S,7S,8aS)-7-(4-Hexyl-1H-1,2,3-triazol-1-yl)octahydroindolizine-1,2-diyl dibenzoate (**76**)

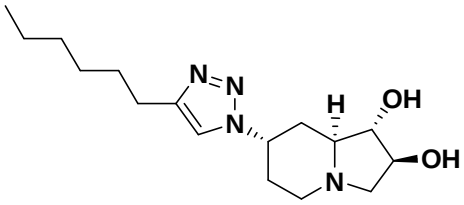


In a microwave reaction tube were added a mixture of azide **47** (61 mg, 0.150 mmol) and 98% pure 1-octyne (**57**, 0.020 mL, 0.135 mmol) in a 1:1 mixture of water and *t*-

BuOH (0.8 mL) and THF (0.1 mL), copper powder (3.81 mg, 0.06 mmol) and copper sulfate (16.75 mg, 0.10 mmol). The mixture was stirred for 30 min at 80 °C, using an irradiation power of 100 W. Then, other three portions of **57** (0.020 mL) were added and the mixture was heated at 80 °C for 30 min after each addition. Then, the reaction mixture was filtered and concentrated under reduced pressure and the crude product was purified by chromatography on silica gel (EtOAc/petroleum ether 1:1) to afford analytically pure triazole **76** (76 mg, o.147 mmol, 98%) as a white solid.

76 R_f: 0.26; m.p. 119.5-120.7 °C; $[\alpha]_D^{22}$ = + 83.092 (*c* = 0.76, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.11-8.05 (m, 2H, Ph), 8.04-7.99 (m, 2H, Ph), 7.65-7.50 (m, 2H, Ph), 7.48-7.36 (m, 4H, Ph), 7.30 (s, 1H, triazole), 5.49, 5.38 (m, 2H, 1-H, 2-H), 4.79-4.70 (m, 1H, 7-H), 3.24 (d, *J*= 11.4 Hz, 1H, 3-Ha), 3.04 (ddd, *J*= 11.3, 4.6, 2.5 Hz, 1H, 5-Ha), 2.96 (dd, *J*= 11.4, 6.8 Hz, 1H, 3-Hb), 2.80-2.59 (m, 5H, 5-Hb, $CH_2CH_2CH_2CH_2CH_2CH_3$, 8a-H, 8-Ha), 2.41 (d, *J*= 14.4 Hz, 1H, 6-Ha), 2.36-2.17 (m, 2H, 6-Hb, 8-Hb), 1.65 (q, *J*= 7.5 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2CH_3$), 1.40-1.21 (m, 6H, $CH_2CH_2CH_2CH_2CH_2CH_3$), 0.86 (t, *J*= 7.0 Hz, 3H, $CH_2CH_2CH_2CH_2CH_2CH_3$) ppm; ^{13}C-NMR (50 MHz): δ = 166.4 (s; C=O), 166.1 (s; C=O), 148.4 (s; triazole), 133.4 (d; Ph), 133.2 (d; Ph), 129.9 (d, 4C, Ph), 129.7 (s; Ph), 129.4 (s; Ph), 128.5 (d; 2C, Ph), 128.4 (d; 2C, Ph), 120.0 (d; triazole), 82.1 (d; C-1), 77.5 (d; C-2), 61.8 (d; C-8a), 59.5 (t; C-3), 53.7(d; C-7), 47.8 (t; C-5), 33.6 (t; C-8), 31.7 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 29.5 (t; C-6), 29.3 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 29.1 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 25.8 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 22.7 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 14.2 (q; $CH_2CH_2CH_2CH_2CH_2CH_3$) ppm; IR($CDCl_3$): *v* = 2929, 2857, 2249, 1716, 1603, 1458, 1271, 1177, 1114, 1027 cm^{-1}; MS (EI): *m/z* 394 (2), 273 (5), 241 (2), 120 (100), 105 (38), 77 (15); anal. calcd. $C_{30}H_{36}N_4O_4$ (516.63): C 69.74, H 7.02, N 10.84; found C 69.46, H 6.92, N 10.64.

(1S,2S,7S,8aS)-7-(4-hexyl-1H-1,2,3-triazol-1-yl)octahydroindolizine-1,2-diol (**11**)

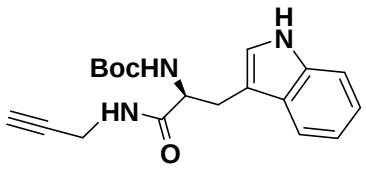


Ambersep 900 OH was added to a solution of **76** (63 mg, 0.122 mmol) in MeOH (5 mL) and the mixture was stirred at rt for 4 hours. Then, the reaction mixture was filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel (eluent: MeOH) afforded **11** in 82% yield (31 mg, 0.1 mmol) as a waxy solid.

11 R_f: 0.43; $[\alpha]_D^{24}$ = + 14.435 (*c* = 0.965, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.82 (s, 1H, triazole), 4.83-4.78 (m, 1H, 7-H), 3.93 (ddd, *J*= 7.0, 3.4, 1.4 Hz, 1H, 2-H), 3.64 (dd, *J*= 8.2, 3.4 Hz, 1H, 1-H), 2.97-2.85 (m, 2H, 3-Ha, 5-Ha), 2.69 (t overlapped with ddd at 2.66, *J*= 7.7 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2CH_3$,), 2.66 (ddd overlapped with t at 2.69, *J*= 13.9, 4.6, 2.3 Hz, 1H, 8-Ha), 2.56 (dd, *J*= 10.6, 7.0 Hz, 1H, 3-Hb), 2.49-2.36 (m, 2H, 6-Ha, 5-Hb), 2.29-2.14 (m, 1H, 6-Hb), 2.08 (ddd, *J*= 11.5, 8.3, 2.4 Hz, 1H, 8a-H), 1.95 (ddd, *J*= 13.9, 11.5, 4.1 Hz, 1H, 8-Hb), 1.67 (q, *J*= 7.7 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2CH_3$), 1.43-1.25 (m, 6H, $CH_2CH_2CH_2CH_2CH_2CH_3$), 0.90 (t, *J*= 6.93 Hz, 3H, $CH_2CH_2CH_2CH_2CH_2CH_3$) ppm; ^{13}C-NMR (50 MHz): δ = 148.9 (s; triazole), 122.5 (d; triazole), 85.0 (d; C-1), 77.6 (d; C-2), 64.8 (d; C-8a), 62.5 (t; C-3), 55.3 (d; C-7), 49.4 (t; C-5), 34.0 (t; C-8), 32.7 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 30.6 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 29.9 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 29.6 (t; C-6), 26.3 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 23.6 (t; $CH_2CH_2CH_2CH_2CH_2CH_3$), 14.4 (q; $CH_2CH_2CH_2CH_2CH_2CH_3$) ppm; MS (EI): *m/z* (%) = 290 (2), 206 (2), 191 (1), 154 (14), 152 (2), 137 (61), 120 (100); ESI: *m/z* = 309.17, calcd. for $C_{16}H_{29}N_4O_2$ $[M + H]^+$

309.23; anal. calcd. $C_{16}H_{28}N_4O_2$ (308.42): C 62.31, H 9.15, N 18.17; found C 62.58, H 9.51, N 18.45.

N-(tert-Butoxycarbonyl)-N-prop-2-yn-1-yl-L-tryptophanamide (**58**)

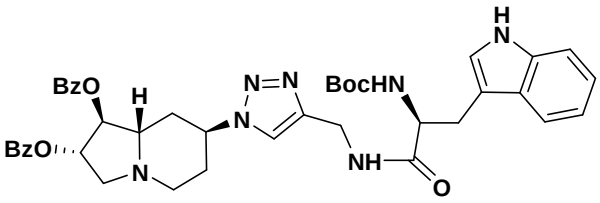


Propargylamine **60** (0.118 mL, 1.74 mmol) was added to a stirred ice bath cooled solution of *N*-Boc-L-tryptophan (**59**) (335 mg, 1.1 mmol) in CH_2Cl_2 (42 mL, distilled from CaH_2). Then hydroxybenzotriazole anhydrous (224.43 mg, 1.66 mmol) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (330 mg, 1.72 mmol) were added at rt. The reaction mixture was attired for 30 minutes and then concentrated under reduced pressure. Purification of the crude product by flash chromatography on silica gel (eluent: EtOAc /petroleum ether 3:1) gave **58** as a white solid in 92 % yield (345.3 mg, 1.01 mmol).

58 R_f: 0.56; m.p. 112 - 114 °C; $[\alpha]_D^{25}$ = - 2.164 (*c* = 0.61, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.20(s, 1H, NH-indole), 7.64 (d, *J*= 7.8 Hz, 1H, 4-H indole), 7.36 (d, *J*= 8.0 Hz, 1H, 7-H indole), 7.20 (ddd, *J*= 8.1, 7.0, 1.2 Hz, 1H, 5-H indole), 7.13 (ddd, *J*= 7.9, 7.0, 0.9 Hz, 1H, 6-H indole), 7.05 (d, *J*= 2.2 Hz, 1H, 2-H indole), 6.13-5.97 (m, 1H, *NH*CH_2C≡≡CH), 5.13 (br s, 1H, Boc*NH*), 4.43 (br s, 1H, *CH*NHBoc), 3.99 (s, 2H, *CH_2*C≡≡CH), 3.31 (d, *J*= 14.5 Hz, 1H, *CH*-indole), 3.18 (dd, *J*= 14.4, 7.1 Hz, 1H, *CH*-indole), 2.14 (t, *J*= 2.5 Hz, 1H, CH_2C≡≡*CH*), 1.42 (s, 9H, *t*-Bu) ppm; ^{13}C-NMR (100 MHz): δ = 171.1 (s; C=O), 155.1 (s; C=O), 135.9 (s; C-7a indole), 127.1 (s; C-3a indole), 122.9 (d; C-2 indole), 121.9 (d; C-5 indole), 119.4 (d; C-6 indole), 118.5 (d; C-4 indole), 110.9 (d; C-7 indole), 110.0 (s; C-3 indole), 80.0 (s; CH_2*C*≡≡CH), 78.8 (s; $C(CH_3)_3$), 71.2 (d; CH_2C≡≡*CH*), 54.9 (d; *CH*NHBoc), 28.8 (t; *CH_2*C≡≡CH), 28.2 (t;

CH$_2$-indole; q; 3C, *t*-Bu) ppm; IR($CDCl_3$): *v* = 3477, 3434, 3308, 2981, 2931, 2250, 1704, 1680, 1490, 1368, 1251, 1165 cm^{-1}; MS (EI): *m/z* 341 (3), 285 (0.35), 259 (0.19), 240 (0.23), 224 (12), 223 (10), 159 (6), 130 (100), 57 (37); anal. calcd. for $C_{19}H_{23}N_3O_3$(341.40): C 66.84, H 6.79, N 12.31; found C 66.49, H 6.58, N 12.12.

(1S,2S,7S,8aS)-7-[4-({[N-(tert-Butoxycarbonyl)-L-tryptophyl]amino}methyl)-1H-1,2,3-triazol-1-yl]octahydroindolizine-1,2-diyl dibenzoate (**77**)

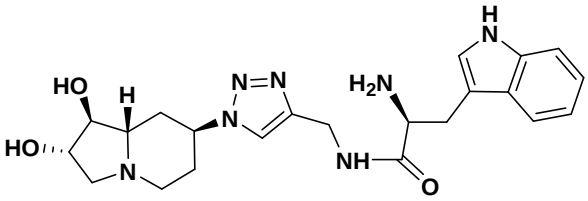


In a microwave reaction tube were added azide **47** (80.5 mg, 0.198 mmol) and **58** (24 mg, 0.07 mmol) in a 1:1 mixture of H_2O and *t*-BuOH (1 mL) and THF (0.3 mL), copper powder (5.20 mg, 0.08 mmol) and copper sulfate (22 mg, 0.14 mmol). The mixture was stirred for 30 min at 80 °C, using an irradiation power of 100 W. Then, other two portions of **58** (24 mg) were added and the mixture was heated at 80 °C for 30 min after each addition. The reaction mixture was then filtered and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to afford analytically pure triazole **77** (90 mg, 0.120 mmol, 61% yield) as a white solid.

77 R_f: 0.26; m.p. 223-224 °C; $[\alpha]_D^{25}$ = +74.711 (*c* = 0.745, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.40 (s, 1H, *NH* indole), 8.08 (dd, *J*= 8.4, 1.3 Hz, 2H Ph), 8.01 (dd, *J*= 8.4, 1.3 Hz, 2H, Ph), 7.61 (d, *J*= 7.9 Hz, 1H, 4-H indole), 7.59-7.51 (m, 2H, Ph), 7.42 (q, *J*= 7.6 Hz, 4H, Ph), 7.33 (d, *J*= 8.1 Hz, 1H, 7-H indole), 7.17 (t, *J*= 7.6 Hz, 1H, 5-H indole), 7.14-7.05 (m, 2H, 6-H indole, triazole), 6.91-6.85 (m, 1H, 2-H indole), 6.48-6.31 (m, 1H, CH_2*NH*CO), 5.46-5.36 (m, 2H, 1-H, 2-H), 5.17 (br s, 1H, CH*NH*CO), 4.62

(s, 1H, 7-H), 4.44-4.28 [m, 3H, triazoleCH_2, CO*CH*(CH2-indole)NHCO], 3.26 (dd overlapped with a d at 3.20, *J*= 14.3, 4.9 Hz, 1H, C*H*-indole), 3.20(doverlapped with a dd at 3.26 , *J*= 11.38 Hz, 1H, 3-Ha), 3.13 (dd, *J*= 14.5, 7.5 Hz, 1H, C*H*-indole), 3.02-2.87 (m, 2H, 5-Ha, 3-Hb), 2.73-2.51 (m, 3H, 8a-H, 6-Ha, 5-Hb), 2.35-2.10 (m, 3H, 6-Hb, 8-Ha, 8-Hb), 1.38 (s, 9H, *t*-Bu) ppm; ^{13}C-NMR (100 MHz): δ = 171.7 (s; C=O), 166.3 (s; C=O), 166.2 (s; C=O), 155.4 (s; C=O), 144.2 (s; triazole), 136.1 (s; C-7a indole), 133.4 (d; Ph), 133.2 (d; Ph), 129.8 (d; 4C, Ph), 129.6 (s; Ph), 129.2 (s; Ph), 128.5 (d; 2C, Ph), 128.3 (d; 2C, Ph), 127.5 (s; C-3a indole), 123.1 (d; C-2 indole), 122.2 (d; C-5 indole), 121.7 (d; triazole), 119.7 (d; C-6 indole), 118.9 (d; C-4 indole), 111.2(d; C-7 indole), 110.4 (s; C-3 indole), 82.2 (d; C-1), 77.4 (d; C-2), 61.4 (d; C-8a), 59.2 (t; C-H), 53.8 (d; C-7), 47.5 (t; C-5), 34.9 (t; triazole-CH_2NHCO), 33.6 (t; C-8), 29.0 (t; C-6), 28.3 (t; *CH2*-indole), 28.2 (q; 3C, *t*-Bu) ppm; IR($CDCl_3$): *v* =3475, 3431, 2980, 2933, 2257, 2246, 1716, 1672, 1316, 1281, 1160, 1114 cm^{-1}; MS (EI): *m/z* (%) = 663 (1), 647 (1), 441 (1), 359 (1), 316 (1), 267 (12), 116 (8), 98 (45); anal. calcd. for $C_{41}H_{47}N_7O_7$ (747.84): C 65.85, H 6.07, N 13.11; found C 65.54, H 5.60, N 13.58.

N-({1-[(1S,2S,7S,8aS)-1,2-Dihydroxyoctahydroindolizin-7-yl]-1H-1,2,3-triazol-4-yl}methyl)-L-tryptophanamide (**12**)

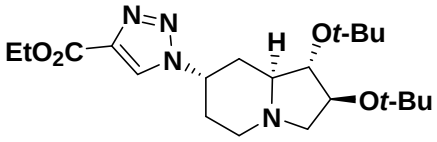


A 1% solution of thiophenol in trifluoroacetic acid (3 mL) was added to **77** (85 mg, 0.11 mmol) at 0 °C. The obtained solution was stirred at rt for 40 min. The reaction was then concentrated under reduce pressure and the residue was dissolved in MeOH (10 mL).

Ambersep 900–OH was added at 0 °C and the reaction mixture was stirred overnight at rt. Then, the solution was concentrated under reduced pressure and the obtained crude product was purified by flash chromatography on silica gel [eluent: CH_2Cl_2/MeOH (1% NH_3) 1:1]. **12** was obtained in 73% yield (35.3 mg, 0.08 mmol) as a white waxy solid.

12 R_f : 0.21 $[\alpha]_D^{24}$= + 17.045 (*c* =0.995, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.60 (d, *J*= 8 Hz, 1H, indole), 7.37-7.32 (m, 2H, indole, triazole), 7.13-7.08 (m, 1H, indole), 7.06 (s, 1H, indole), 7.05-6.98 (m, 1H, indole), 4.75-4.63 (m, 1H, 7-H), 4.35 (q, *J* = 15.3 Hz, 2H, triazole-CH_2), 3.93 (ddd, *J* = 7.0, 3.3, 1.4 Hz, 1H, 2-H), 3.69-3.58 (m, 2H, 1-H, *CH*CH_2-indole), 3.15 (dd, *J* = 14.1, 6.8 Hz, 1H, *CH*-indole), 3.04 (dd, *J* = 14.2, 6.4 Hz, 1H, *CH*-indole), 2.92-2.83 (m, 2H, 3-Ha, 5-Ha), 2.60-2.49 (m, 2H, 3-Hb, 8-Ha), 2.39 (dt, *J* = 11.9, 2.7 Hz, 1H, 5-Hb), 2.34-2.26 (m, 1H, 6-Ha), 2.24-2.11 (m, 1H, 6-Hb), 2.06 (ddd, *J* = 11.5, 8.2, 2.4 Hz, 1H, 8-Hb), 1.93 (ddd, *J* = 13.9, 11.5, 4.1 Hz, 1H, 8a-H) ppm; ^{13}C-NMR (CD_3OD, 100 MHz): δ = 177.1 (s; C=O), 145.7 (s; triazole), 137.9 (s; C-7a, indole), 128.8 (s; C-3a, indole), 124.7 (d; C-2, indole), 123.3 (d; triazole), 122.4 (d; C-5, indole), 119.8 (d; C-6, indole), 119.5 (d; C-4, indole), 112.2 (d; C-7, indole), 111.2 (s; C-3, indole), 85.0 (d; C-1), 77.6 (d; C-2), 64.6 (d; C-8a), 62.5 (t; C-3), 57.0 (d; *CH*CH_2-indole), 55.4 (d; C-7), 49.3 (t; C-5), 35.6 (t; CH*CH_2*-indole), 34.1 (t; C-8), 32.2 (t; triazole- *CH_2*), 29.6 (t; C-6) ppm; MS (EI): *m/z*(%) = 267 (2), 239 (2), 154 (3), 134 (10), 98 (19); ESI: *m/z* =440.33 , calcd. for $C_{22}H_{30}N_7O_3$ $[M + H]^+$ 440.24; anal. calcd. for $C_{22}H_{29}N_7O_3$ (439.5): C 60.12, H 6.65; N 22.31; found: C 60.33, H 6.26, N 22.42.

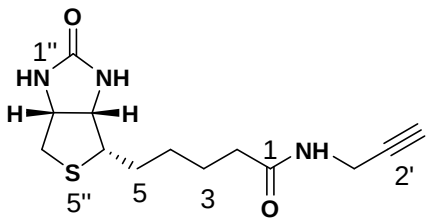
Ethyl 1-[(1S,2S,7S,8aS)-1,2-di-tert-butoxyoctahydroindolizin-7-yl]-1H-1,2,3-triazole-4-carboxylate (**63**)



Copper powder (3.6 mg, 0.06 mmol) and copper sulfate (28.3 mg. 0.177 mmol) were added to a mixture of azide **50** (70 mg, 0.22 mmol) and ethyl propiolate (**62**, 0.039 mL, 0.38 mmol) in a 1:1 mixture of H_2O and *t*-BuOH (1 mL) cooled at 0 °C. The reaction mixture was stirred at rt overnight then concentrated, diluted with CH_2Cl_2, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by chromatography on silica gel (eluent: initially petroleum ether / EtOAc 4:1, then EtOAc/ MeOH 10:1) to afford **63** (52 mg, 50%) as a pale yellow solid.

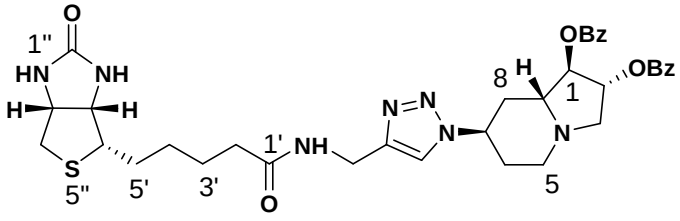
63: R_f = 0.16 (EtOAc); ^{1}H NMR (400 MHz): δ = 8.18 (s, 1H; triazole), 4.91-4.86 (m, 1H; 7-H), 4.43 (q, *J* = 7.1 Hz, 2H; C*H*$_2$CH$_3$), 3.80 (ddd, *J* = 7.0, 3.9, 1.5 Hz, 1H; 2-H), 3.66 (dd, *J* = 7.7, 3.9 Hz, 1H; 1-H), 2.96-2.88 (m, 2H; 3-H$_a$, 5-H$_a$) 2.60-2.54 (m, 1H; 8-H$_a$), 2.48 (dd, *J* = 10.2, 7.0 Hz, 1H; 3-H$_b$), 2.46-2.40 (m, 1H; 6-H$_a$), 2.35-2.22 (m, 2H; 5-H$_b$, 6-H$_b$), 2.02-1.91 (m, 2H; 8-H$_b$, 8a-H), 1.42 (t, *J* = 7.1 Hz; 3H; CH$_2$C*H*$_3$); 1.20 (s, 9H; *t*-Bu), 1.17 (s, 9H; *t*-Bu) ppm; ^{13}C-NMR (100 MHz): δ = 160.7 (s, CO), 139.7 (s; triazole), 126.6 (d; triazole), 83.6 (d; C-1); 76.7 (d; C-2), 74.0 (s; *t*-Bu), 73.9 (s; *t*-Bu), 61.7 (t; C-3), 61.3 (t, OCH$_2$), 60.8 (d; C-8a), 54.8 (d; C-7), 48.0 (t; C-5), 33.2 (t; C-8), 29.3 (q, 3C; *t*-Bu), 28.8 (q, 3C; *t*-Bu), 28.6 (t; C-6), 14.5 (dq, CH$_3$) ppm; HRMS: calcd for $C_{21}H_{37}N_4O_4$ $[M+H]^+$ 409.28093, found 409.28182.

5-[(3aS,4S,6aR)-2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]-N-prop-2-yn-1-ylpentanamide (**28**)



Propargylamine (**60**, 0.044 mL, 0.648 mmol) was added to a stirred ice bath cooled solution of biotin (**23**, 100 mg, 0.41 mmol) in CH_3CN/H_2O 1:1 (18 mL). Then anhydrous hydroxybenzotriazole (84 mg, 0.62 mmol) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (123 mg, 0.64 mmol) were added at rt and the mixture was stirred overnight. The solution was concentrated under reduced pressure and the crude product was purified by flash chromatography (eluent: CH_2Cl_2/MeOH 15:1, 12:1, 10:1). A white solid was obtained in 92 % yield (105.8 mg, 0.376 mmol). The NMR properties of **28** are identical to those reported in the literature.[57]

(1R,2R,7R,8aR)-7-{4-[({5-[(3aS,4S,6aR)-2-Oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanoyl}amino)methyl]-1H-1,2,3-triazol-1-yl}octahydroindolizine-1,2-diyl dibenzoate (**78**)

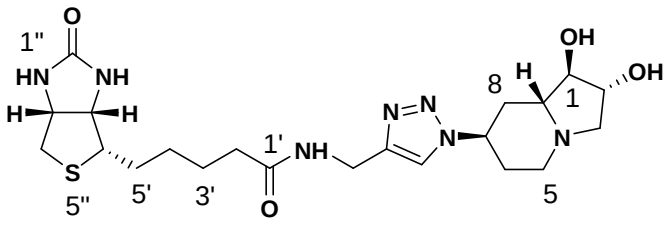


In a microwave reaction tube were added a mixture of azide **22** (141 mg, 0.347 mmol) and **28** (35 mg, 0.123 mmol) in a 1:1 mixture of H_2O and *t*-BuOH (1.5 mL) and THF (0.1 mL), copper powder (9 mg, 0.141 mmol) and copper sulfate (39 mg, 0.246 mmol). The mixture was stirred for 30 min at 80 °C, using an irradiation power of 100 W. Then, other three portions of **28** (35 mg) were added and the mixture was heated at 80 °C for

30 min after each addition. The reaction mixture was then filtered and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (CH_2Cl_2 / MeOH 10:1) to afford analytically pure triazole **78** (126.3 mg, 0.184 mmol, 53% yield) as a waxy solid.

78 R_f: 0.28; $[\alpha]_D^{25}$ = - 44.372 (*c* = 0.565, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.12-7.98 (m, 4H, Ph), 7.71 (t, *J*= 5.8 Hz, 1H, CH_2*NH*CO), 7.65 (s, 1H, triazole), 7.60-7.51 (m, 2H, Ph), 7.47-7.39 (m, 4H, Ph), 7.05 (s, 1H, *NH* biotin), 6.42 (s, 1H, *NH* biotin), 5.47-5.35 (m, 2H, 1-H, 2-H), 4.76 (s, 1H, 7-H), 4.56-4.43 (m, 2H, triazole *CH*, 6’’a-H biotin), 4.38 (dd, *J*= 15.0, 5.6 Hz, 1H, triazole *CH*), 4.26 (dd, *J*= 7.6, 4.8 Hz,1H, 3’’a-H biotin), 3.20 (d, *J*= 11.2 Hz, 1H, 3-Ha), 3.05 (td, *J*= 7.2, 4.7 Hz, 1H, 4’’-H biotin), 2.98 (ddd, *J*= 11.3, 4.0, 2.7 Hz, 1H, 5-Ha), 2.93 (dd, *J*= 11.4, 6.5 Hz, 1H, 3-Hb), 2.82(dd, *J*= 12.8, 4.8 Hz, 1H, 6’’-Ha biotin), 2.77-2.60 (m, 3H, 8-Ha, 6’’-Hb biotin, 8a-H), 2.52 (td, *J*= 11.4, 3.1 Hz, 1H, 5-Hb), 2.40-1.85 (m, 5H, 6-Ha, 6-Hb, 8-Hb, 2’-Ha, 2’-Hb), 1.77-1.50 (m, 4H, 3’-Ha, 3’-Hb, 5’-Ha, 5’-Hb), 1.48-1.29 (m, 2H, 4’-Ha, 4’-Hb) ppm; ^{13}C-NMR (50 MHz): δ = 173.3 (s; C=O), 166.2 (s; C=O), 165.9 (s; C=O), 164.5 (s; C=O), 144.8 (s; triazole), 133.3 (d; Ph), 133.1 (d; Ph), 129.8 (d; 4C, Ph), 129.5 (s; Ph), 129.2 (s; Ph), 128.4 (d; 2C, Ph), 128.3 (d; 2C, Ph), 121.9 (d; triazole), 82.0 (d; C-1), 77.3 (d; C-2), 61.6 (d; C-8a), 61.5 (d; C-3’’a biotin), 60.2 (d; C-6’’a biotin), 59.3 (t; C-3), 55.7 (d; C-4’’ biotin), 53.4 (d; C-7), 47.5 (t; C-5), 40.5 (t; C-6’’ biotin), 35.7 (t; C-2’), 34.3 (t; triazole CH_2), 33.2 (t; C-8), 29.2 (t; C-6), 28.1 (t; C-4’), 27.9 (t; C-5’), 25.4 (t; C-3’)ppm; IR($CDCl_3$): ν = 3257, 2932, 2245, 1717, 1699, 1467, 1280, 1113 cm^{-1}; MS (EI):*m/z* (%)= 244 (1), 226 (1), 197(1), 142 (3), 137 (4), 125 (6), 57 (199); anal. calcd. for $C_{35}H_{41}N_7O_6S$ (687.81): C 61.12, H 6.01, N 14.25; found C 60.82, H 5.6, N 13.99.

N-({1-[(1R,2R,7R,8aR)-1,2-Dihydroxyoctahydroindolizin-7-yl]-1H-1,2,3-triazol-4-yl}methyl)-5-[(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl]pentanamide (**21**)

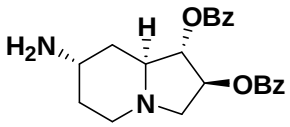


Ambersep 900 OH was added to a solution of **78** (75.2 mg, 0.109 mmol) in MeOH (10 mL). The mixture was stirred at rt for 3 h and then filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel (eluent: CH_2Cl_2/MeOH 5:1) afforded **21** in 98% yield (51.3 mg, 0.107 mmol) as a waxy solid.

21 R_f: 0.29 (methanol); $[\alpha]_D^{22}$ = + 29.933 (*c* = 0.750, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.94 (s, triazole), 4.91-4.76 (m overlapped with s at 4.84, 1H, 7-H), 4.49 [dd, *J*= 7.8, 4.3 Hz, 1H, 6’’a-H biotin], 4.43 (s, 2H, triazole-*CH*$_2$), 4.30 [dd, *J*= 7.9, 4.3 Hz, 1H, 3’’a-H], 3.94 (ddd, *J*= 7.0, 3.4, 1.4 Hz, 1H, 2-H), 3.64 (dd, *J*= 8.2, 3.3 Hz, 1H, 1-H), 3.23-3.15 [m, 1H, 4’’-H biotin], 2.97-2.83 (m, 3H, 6’’-Ha biotin, 5-Ha, 3-Ha), 2.70 (d, *J*= 12.7 Hz, 1H, 6’’-Hb biotin), 2.65 (dq, *J*= 13.9, 2.2 Hz, 1H, 8-Ha), 2.56 (dd, *J*= 10.5, 7.1 Hz, 3-Hb), 2.47-2.35 (m, 2H, 5-Ha, 6-Ha), 2.29-2.15 (m, 3H, 6-Hb, 2’-Ha, 2’-Hb), 2.09 (ddd, *J*= 11.4, 8.3, 2.3 Hz, 1H, 8a-H), 1.96 (ddd, *J*= 13.9, 11.5, 4.1 Hz, 1H, 8-Hb), 1.78-1.51 (m, 4H, 3’-Ha, 3’-Hb, 5’-Ha, 5’-Hb), 1.42 (q, *J*= 7.5 Hz, 2H, 4’-Ha, 4’-Hb); ^{13}C-NMR (CD_3OD, 50 MHz): δ = 175.8 (s; C=O), 166.0 (s; C=O), 145.9 (s; triazole), 123.7 (d; triazole), 85.0 (d; C-1), 77.6 (d; C-2), 64.7 (d; C-8a), 63.3 [d; C-3’’a biotin], 62.5 (t; C-3), 61.6 [d; C-6’’a biotin], 57.0 [d; C-4’’ biotin], 55.5 (d; C-7), 49.3 (t; C-5), 41.1 (t; C-6’’ biotin), 36.5 (t; C-2’), 35.6 (t; triazole *CH*$_2$), 34.0 (t; C-8), 29.6 (t;

C-6), 29.4 (t; 2C, C-4', C-5'), 26.7 (t; C-3') ppm; MS (EI): *m/z* (%) = 325 (1), 267 (2), 239 (2), 226 (1), 154 (2), 143 (3), 134 (6), 98 (13); anal. calcd. for $C_{21}H_{33}N_7O_4S$ (479.6): C 52.59, H 6.94, N 20.44; found C 52.84, H 7.05, N 20.05.

(1S,2S,7S,8aS)-7-Aminooctahydroindolizine-1,2-diyl dibenzoate (**64**)



Method A:

A water suspension of activated Raney-Ni was added dropwise to an ice bath cooled solution of **47** (395 mg, 0.97 mmol) in MeOH (11 mL). The mixture was stirred at 0 °C for 1 h and then at rt for 45 minutes under hydrogen atmosphere (1 Atm). After this time, the solution was filtered through a short pad of Celite and MeOH was evaporated under reduced pressure. The aqueous phase was treated with CH_2Cl_2 (4 mL) and the two phases were separated. The aqueous phase was extracted with CH_2Cl_2 (6 x 4 mL). The combined organic phases were dried on Na_2SO_4, filtered and concentrated under reduced pressure. The residue was then purified by flash chromatography on silica gel (eluent: CH_2Cl_2/MeOH 7:1) and the product **64** was obtained in 71% yield as a colorless oil (262 mg, 0.688 mmol).

Method B:

Acetic acid (0.085 mL, 1.5 mmol) was added to an ice bath cooled solution of **47** (63 mg, 0.15 mmol) in MeOH (2 mL). The solution was treated with hydrogen gas (1 Atm)

in the presence of 10% Pd/C (9.6 mg, 0.009 mmol Pd) at rt overnight. *TLC* analysis showed the presence of unreacted azide along with the desired amine, therefore the reaction mixture was added of 10% Pd/C (10 mg) and of acetic acid (0.085 mL) and stirred at rt for another 6 h under hydrogen atmosphere (1 Atm). The mixture was then filtered through a short pad of Celite, and concentrated under reduced pressure. The obtained residue was dissolved in a mixture of saturated aqueous solution of Na_2CO_3 (4 mL) and CH_2Cl_2 (4 mL). The two phases were separated and the aqueous one was extracted with CH_2Cl_2 (3x5 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel (eluent: CH_2Cl_2/MeOH 7:1) afforded the amine **64** in 26% yield (15 mg, 0.04 mmol) as a colorless oil.

Method C:

Triphenylphosphine (30 mg, 0.11 mmol) was added to a solution of **47** (34 mg, 0.08 mmol) in THF (2 mL) and the mixture was heated in an oven at 65 °C overnight. H_2O (0.016 mL, 0.9 mmol) was added, the reaction mixture was heated at 75 °C for another 2 h and then concentrated under reduced pressure. Purification by flash chromatography afforded **64** was obtained in 61% yield (18.8 mg, 0.05 mmol) as a colorless oil.

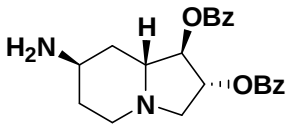
Method D:

Acetic acid (0.16 mL, 0.28 mmol) and zinc (2.25 g, 34 mmol) were added to an ice bath cooled solution of **47** (39 mg, 0.10 mmol) in CH_2Cl_2 (16 mL). The solution was stirred at rt overnight, then filtered through a short pad of Celite, and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (10 mL) and a saturated aqueous

solution of Na_2CO_3 (9 mL) was added. The two phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3x5 mL). The combined organic phases were washed with H_2O (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel afforded **64** in 37% yield (13.5 mg, 0.035 mmol) as a colorless oil.

64 R_f : 0.24; $[\alpha]_D^{24}$= + 77.528 (*c* = 0.390, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 8.13- 8.02 (m, 4H, Ph), 7.60-7.51 (m, 2H, Ph), 7.48-7.39 (m, 4H, Ph), 5.44-5.36 (m, 2H, 1-H, 2-H), 3.45-3.39 (m, 1H, 7-H), 3.19 (d, *J* = 11.6 Hz, 1H, 3-Ha), 2.94 (dd, *J* = 11.3, 6.5 Hz, 1H, 3-Hb), 2.84 (ddd, *J* = 11.1, 4.7, 2.3 Hz, 1H, 5-Ha), 2.67 (ddd, *J* = 11.0, 8.3, 3.1 Hz, 1H, 8a-H), 2.56-2.47 (m, 1H, 5-Hb), 1.99-1.77 (m, 3H, 8-Ha, 6-Ha, 8-Hb), 1.59-1.50 (m, 1H, 6-Hb) ppm; ^{13}C-NMR (100 MHz): δ = 166.6 (s, C=O), 166.1 (s, C=O), 133.3 (d, Ph), 133.2 (d, Ph), 130.0 (d, 2C, Ph), 129.9 (d, 2C, Ph), 129.8 (s, 2C, Ph), 128.5 (d, 2C, Ph), 128.4 (d, 2C, Ph), 83.4 (d, C-1), 77.8 (d, C-2), 61.2 (d, C-8a), 59.9 (t, C-3), 47.1 (t, C-5), 43.9 (d, C-7), 36.2 (t, C-8), 31.9 (t, C-6) ppm; IR($CDCl_3$): *v* = 2926, 2247, 1717, 1451, 1114 cm^{-1}; MS (EI): *m/z* (%) = 381 (1), 257 (4), 182 (4), 137 (24), 120 (13), 105 (14), 57 (100), 43 (100); HRMS: calcd for $C_{22}H_{25}N_2O_4$ $[M+H]^+$ 381.18088, found 381.18190; anal. calcd. for $2.C_{22}H_{24}N_2O_4$ H_2O (778.89): C 67.85 H 6.47, N 7.19, found: C 68.11,H 6.77, N 7.41.

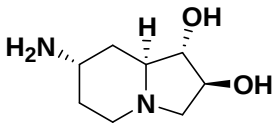
(1R,2R,7R,8aR)-7-Aminooctahydroindolizine-1,2-diyl dibenzoate (**79**)



The corresponding enantiomeric compound **79** was prepared starting from **22** (48 mg, 0.118 mmol) using method A and was obtained in 71% yield (32 mg, 0.084 mmol).

79 $[\alpha]_D^{23}$= – 79.308 (*c* = 0.265, $CHCl_3$); anal. calcd. for $3.C_{22}H_{24}N_2O_4\ H_2O$ (1159.3): C 68.38, H 6.43, N 7.25; found: C 68.35, H 6.7, N 6.92. NMR properties are identical to those of **64**.

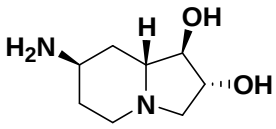
(1S,2S,7S,8aS)-7-Aminooctahydroindolizine-1,2-diol (**27**)



Ambersep 900-OH was added to a solution of **64** (40 mg, 0.10 mol) in MeOH (2 mL) and the mixture was agitated at rt for 1 h on a flat shaker at 150 rpm. The reaction mixture was filtered through cotton wool and concentrated under reduced pressure. Chromatography on silica gel (eluent: MeOH with 1% of NH_4OH) afforded **27** in 85% yield (15 mg, 0.085 mmol).

27 R_f : 0.19; $[\alpha]_D^{24}$= – 2.382 (*c* = 0.340, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ = 3.97 (ddd, *J* = 7.2, 3.5, 1.5 Hz, 1H; 2-H), 3.60 (dd, *J* = 8.4, 3.5 Hz, 1H; 1-H), 3.29-3.24 (m, 1H; 7-H), 2.85 (dd, *J* =10.6, 1.5 Hz, 1H; 3-H_a), 2.76 (ddd, *J* = 11.4, 4.7, 2.6 Hz, 1H; 5-H_a), 2.63 (dd, *J* =10.6, 7.2 Hz, 1H; 3-H_b), 2.37 (dt, *J* =2.9, 12.4 Hz, 1H; 5-H_b), 2.18 (ddd, *J* =11.6, 8.4, 2.7 Hz, 1H; 8a-H), 1.92 (dq, *J* =13.3, 2.4 Hz, 1H; 8-H_a), 1.84 (br dt, *J* = 4.4, 13.1 Hz, 1H; 6-H_a), 1.63-1.54 (m, 2H; 6-H_b, 8-H_b) ppm; ^{13}C-NMR (CD_3OD, 100 MHz): δ = 85.1 (d; C-1), 77.8 (d; C-2), 64.1 (d; C-8a), 62.6 (t; C-3), 48.2 (t; C-5), 45.2 (d; C-7), 35.9 (t; C-8), 32.2 (t; C-6) ppm; MS (EI): *m/z* (%) = 154 (19), 137 (34), 120 (23), 95 (23), 57 (48); HRMS: calcd for $C_8H_{17}N_2O_2$ $[M+H]^+$ 173.1294, found 173.1290 ; anal. calcd.for $C_8H_{16}N_2O_2\ H_2O$ (190.2): C 50.51, H 9.54, N 14.73; found: C 50.66, H 9.76, N 15.02.

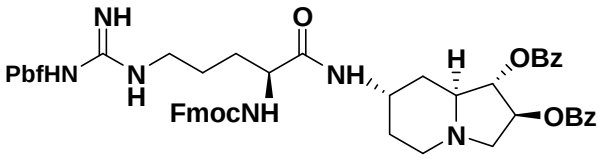
(1R,2R,7R,8aR)-7-Aminooctahydroindolizine-1,2-diol (**26**)



Following the same procedure, the enantiomeric compound **26** was prepared starting from **79** (50 mg, 0.13 mmol) and was obtained in 83% yield (19 mg, 0.11 mmol).

26 $[\alpha]_D^{22}$= + 3.720 (*c* = 0.500, CH_3OH); anal. calcd. for $3.C_8H_{16}N_2O_2\ H_2O$ (534.7): C 53.91, H 9.43, N 15.72; found: C 53.58, H 9.18, N 16.02. NMR properties are identical to those of **27**.

(1S,2S,7S,8aS)-7-{[N²-[(9H-Fluoren-9-ylmethoxy)carbonyl]-N⁵-(imino{[(2,2,4,6,7-pentamethyl-2,3-dihydro-1-benzofuran-5-yl)sulfonyl]amino}methyl)-L-ornithyl]amino}octahydroindolizine-1,2-diyl dibenzoate (**65**)

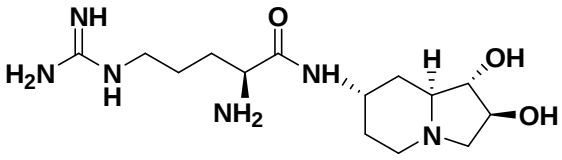


Bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP®) (289.03 mg, 0.62 mmol) was added to a solution of **64** (235.6 mg, 0.62 mmol), Fmoc-L-Arg(Pbf)-OH (**29**, 441.16 mg, 0.68 mmol), and *N*,*N*-diisopropylethylamine (0.106 mL, 0.62mmol) in CH_2Cl_2 (0.62 mL, freshly distilled from P_2O_5) with cooling in an ice/water bath. Then, the reaction mixture was stirred at rt under nitrogen atmosphere overnight and concentrated under reduced pressure. The residue was dissolved in EtOAc (5 mL), filtered and concentrated under reduced pressure. Purification by chromatography on

silica gel (eluent: EtOAc/triethylamine 98:2) afforded **65** in 75% yield (471 mg, 0.466 mmol) as a white solid.

65 R_f : 0.17; m.p. 127 – 130 °C; $[\alpha]_D^{22}$= + 44.247 (*c* = 0.445, $CHCl_3$); ^{1}H NMR (400 MHz, detectable signals): δ = 8.11-8.03 (m, 2H, Ph), 7.98-7.90 (m, 2H, Ph), 7.78-7.67 (m, 2H, Fmoc), 7.59-7.48 (m, 2H, Fmoc), 7.47-7.37 (m, 4H, Ph), 7.37-7.30 (m, 2H, Fmoc), 7.29-7.24 (m, 2H, Fmoc), 7.23-7.19 (m, 2H, Ph), 6.26 (br s, 3H, *NH*C(=*NH*)*NH*), 5.32 (dd, *J* = 8.1, 3.1 Hz, 1H, 1-H), 5.28 (dd, *J* = 6.4, 2.9 Hz, 1H, 2-H), 4.30 (d overlapped with br s at 4.22, *J* = 7.2 Hz, 2H, Fmoc), 4.22 (br s, 1H, 7-H), 4.11 (t, *J* = 7.1 Hz, 1H, Fmoc), 3.37-3.19 [br s, 1H], 3.05 (d, *J* = 11.2 Hz, 1H, 3-Ha), 2.89 (s, 2H, CH_2 Pbf), 2.80-2.64 (m, 2H, 3-Hb, 5-Ha), 2.58 (s, 3H, CH_3 Pbf), 2.51 (s, 3H, CH_3 Pbf), 2.45 (br t, 1H, 8a-H), 2.30 (br t, 1H, 5-Hb), 2.14 – 2.01 (m, 4H, 8-Ha or 6-Ha, CH_3Pbf), 1.97-1.46 (m, 9H, 8-Ha or 6-Ha, 8-Hb, 6-Hb, CH*CH*$_2$*CH*$_2$*CH*$_2$NH), 1.41 (s, 6H, CH_3 Pbf) ppm; ^{13}C-NMR (100 MHz): δ = 171.6 (s; C=O), 166.4 (s; C=O), 165.9 (s; C=O), 158.9 (s; Pbf), 156.8 (s; C=NH), 156.4 (s; C=O), 143.7 (s; 2C, Fmoc), 141.3 (s; 2C, Fmoc), 138.4 (s; Pbf), 133.2 (d; 2C, Ph), 132.7 (s; Pbf), 132.3 (s; Pbf), 129.9 (d; 2C, Ph), 129.8 (d; 2C, Ph), 129.6 (s; Ph), 128.8 (s; Ph), 128.5 (d; 4C, Ph), 127.8 (d; 2C, Fmoc), 127.2 (d; 2C, Fmoc), 125.2 (d; 2C, Fmoc), 124.8 (s; Pbf), 120.1 (d; 2C, Fmoc), 117.7 (s; Pbf), 107.9 (s; Pbf), 82.3 (d; C-1), 77.6 (d; C-2), 67.4 (d; C-7), 62.0 (d; C-8a), 59.4 (t; C-3), 54.7 [d; CO*CH*(CH_2)NHFmoc], 47.6 (t; C-5), 47.1 (d; Fmoc), 43.3 (t; 2C, Pbf and Fmoc), 40.7 (t; CH_2CH_2*CH*$_2$NH), 33.2 (t; C-3), 28.9 (t; C-6), 28.7 (q; 2C, Pbf), 25.8 (t; 2C, *CH*$_2$*CH*$_2$$CH_2$NH), 19.6 (q; Pbf), 18.2 (q; Pbf), 12.7 (q; Pbf) ppm; IR ($CDCl_3$): *ν* = 2930, 2248, 1718, 1666, 1554, 1451, 1281 cm^{-1}; MS (EI): *m/z* (%) = 647 (1), 441 (1), 267 (11), 257 (2), 242 (4), 178 (100), 57 (55); anal. calcd. for $C_{56}H_{62}N_6O_{10}S$ (1011.2): C 66.52, H 6.18, N 8.31; found: C 66.76, H 6.54, N 8.56.

N*^{1}*-[(1S,2S,7S,8aS)-1,2-dihydroxyoctahydroindolizin-7-yl]-L-argininamide (**13**)

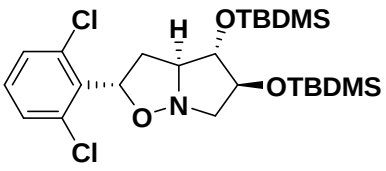


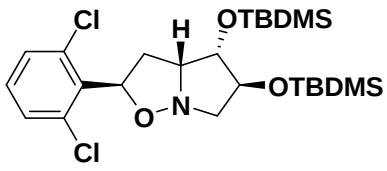
A solution of **65** (187.5 mg, 0.185 mmol) in a 95:5 mixture of trifluoroacetic acid and water (3 mL) was stirred at rt for 30 minutes. Then, diethyl ether (8 mL) was added and the precipitated white solid was filtered and washed with diethyl ether. The white solid residue was dissolved in CH_2Cl_2 (2.2 mL), cooled in an ice bath and treated with freshly distilled piperidine (0.43 mL, 0.004 mmol). The reaction mixture was stirred at 0 °C for 1 h and 30 min, concentrated under reduced pressure, and the residue was sequentially washed with petroleum ether (5 mL), diethyl ether (5 mL) and EtOAc (5 mL). The crude intermediate was dissolved in MeOH (15 mL) and treated with Ambersep 900 OH at rt for 2 h. Then, the mixture was filtered through a short pad of Celite, concentrated under reduced pressure, diluted with deionized water (0.5 mL) and acidified with 3 N aqueous HCl until pH = 1 under magnetic stirring at 0 °C. The aqueous phase was sequentially washed with petroleum ether (3x0.5 mL), EtOAc (3x0.5 mL) and CH_2Cl_2(3x0.5 mL) and then concentrated under reduced pressure. The residue was dissolved in MeOH and the solution was basified with Ambersep 900 OH until pH = 10, filtered through a short pad of Celite and concentrated under reduced pressure. The product **13** was obtained in 49% overall yield (3 steps starting from **65**) (29.6 mg, 0.09 mmol) as a white waxy solid and was characterized without further purification.

13 $[\alpha]_D^{24}$= (–) 15.996 (*c* = 0.760, CH_3OH/H_2O 1:1); ^{1}H NMR (D_2O, 400 MHz): δ 4.21-4.09 (m, 2H, 7-H, 2-H), 4.06-4.00 [m, 1H, CO*CH*(CH_2)NH], 3.70 (t, *J* = 8.4, 3.8 Hz, 1H, 1-H), 3.22 [q, *J* = 6.9 Hz, 2H, *CH_2*NHC(NH)NH_2], 2.96-2.84 (m, 2H, 3-Ha, 5-Ha), 2.71 (dt, *J* = 11.2, 7.1 Hz, 1H, 3-Hb), 2.33 (tt, *J* = 12.2, 3.3 Hz, 1H, 5-Hb), 2.19-2.04 (m, 2H, 8a-H, 8-Ha), 1.94-1.73 (m, 3H, 6-Ha, 6-Hb, 8-Hb), 1.72-1.49 [m, 4H,

$NHCH_2CH_2CH_2CH(NH_2)CO$] ppm; ^{13}C-NMR (D_2O, 50 MHz): δ = 174.5 (s, C=O), 155.9 (s, C=NH), 81.7 (d, C-1), 74.7 (d, C-2), 62.4 (d, C-8a), 59.3 (t, C-3), 54.6 [d, $COCH(NH_2)CH_2$], 46.6 (t, C-5), 42.3 (d, C-7), 40.0 [t, $CH_2NHC(NH)NH_2$], 30.7 (t, C-6), 28.2 [t, $COCH(NH_2)CH_2$], 27.0 (t, C-8), 23.6 [t, $NHCH_2CH_2CH_2CH(NH_2)CO$] ppm; MS (EI): *m/z* (%) = 261 (4), 187 (100), 159 (55), 130 (8), 116 (11), 98 (41), 73 (31), 57 (31); ESI: *m/z* = 329.33, calcd. for $C_{14}H_{29}N_6O_3$ $[M + H]^+$ 329.23; anal calcd. for $C_{14}H_{28}N_6O_3 H_2O$: C 48.54, H 8.73, N 24.26; found: C 48.93, H 9.02, N 24.45.

(2S,3aS,4S,5S)- and (2R,3aR,4S,5S)-4,5-bis{[tert-butyl(dimethyl)silyl]oxy}-2-(2,6-dichlorophenyl)hexahydropyrrolo[1,2-b]isoxazole (**30a** and **30b**)



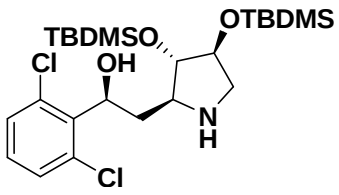


2,6-Dichlorostyrene (**17**, 0.400 mL, 2.89 mmol) was added to a suspension of the crude nitrone **16** (1.0 g, 2.893 mmol) in toluene (0.2 mL) and the reaction mixture was heated at 70 °C for 2 h in a microwave reactor using an irradiation power of 100 W. The solvent was evaporated under a nitrogen stream and the residue was purified by flash chromatography [eluent: EtOAc(+1% Et_3N)/petroleum ether 1:10]. The diastereoisomeric adducts were obtained in 92% overall yield (1.373 g, 2.6 mmol) (ratio among the three major adducts: *exo-anti*:*exo-syn*:*endo-anti* = 7.5:6.9:1) and were only partially separated. The major *exo-anti* diastereoisomer was obtained in 48% combined yield (724 mg, 1.39 mmol, calculated in the mixed fractions by ^{1}H NMR) as a white solid.

30a R_f : 0.43; m.p.= 72 °C; $[\alpha]_D^{22}$= + 52.2; ^{1}H NMR (400 MHz): δ = 7.29 (d, *J* = 8.0 Hz, 2H, 3'-H, 5'-H), 7.13 (dd, *J* = 8.5, 7.5 Hz, 1H, 4'-H), 5.91 (dd, *J* = 9.4, 8.4 Hz, 1H,

2-H), 4.04-4.09 (m, 2H, 4-H, 5-H), 3.91 (ddd, *J* = 10.4, 4.3, 1.8 Hz, 1H, 3a-H), 3.69 (dd, *J* = 12.5, 4.7 Hz, 1H, 6-Hb), 3.06-3.12 (m, 1H, 6-Ha), 2.83 (ddd, *J* = 12.2, 10.4, 9.4 Hz, 1H, 3-Hb), 2.52 (ddd, *J* = 12.2, 8.4, 43. Hz, 1H, 3-Ha), 0.93 [s, 9H, $C(CH_3)_3$], 0.90 [s, 9H, $C(CH_3)_3$], 0.13 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.10 (s, 6H, $SiCH_3$) ppm; ^{13}C-NMR (50 MHz): δ = 135.7 (s; C-6'), 132.9 (s; C-2'), 129.5 (s; C-1'), 129.3 (d; 3C, C-3', C-4', C-5'), 83.5 (d; C-4), 77.8 (d; C-5), 74.7 (d; C-2), 73.2 (d; C-3a), 62.6 (t; C-6), 39.0 (t;C-3), 25.9 [q; 3C, $C(CH_3)_3$], 25.8 [q; 3C, $C(CH_3)_3$], 18.2 [s; $C(CH_3)_3$], 18.0 [s; $C(CH_3)_3$], -4.5 (q; 2C, $SiCH_3$), -4.7 (q, 2C, $SiCH_3$) ppm; IR ($CDCl_3$): *v* = 3869, 3746, 3584, 2956, 2931, 1857, 1564, 1472, 1463, 1439, 1362, 1259, 1108 cm^{-1}; anal. calcd. for $C_{24}H_{41}Cl_2NO_3Si_2$: C 55.58, H 7.97, N 2.70; found: C 55.52, H 8.23, N 2.79.

(1S)-2-((2S,3S,4S)-3,4-bis{[tert-butyl(dimethyl)silyl]oxy}pyrrolidin-2-yl)-1-(2,6-dichlorophenyl)ethanol (**32a**)



Isoxazolidine **30a** (62 mg, 0.119 mmol) was dissolved in a mixture of AcOH:H_2O = 9:1 (1.08 mL). Then zinc (39 mg, 0.60 mmol) was added. The reaction mixture was heated in an oil bath at 60 °C for 4 h and then filtered through cotton wool. The filtrate was basified to pH=9 with a saturated aqueous solution of $NaHCO_3$ and the obtained suspension was extracted with EtOAc (5x10 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Chromatography on silica gel (eluent: initially CH_2Cl_2, then CH_2Cl_2/MeOH with 1% NH_4OH 15:1) afforded pyrrolidine **32a** in 73% yield (45 mg, 0.09 mmol).

32a R_f : 0.30; m.p.= 113 °C; $[\alpha]_D^{25}$= + 14.2; ^{1}H NMR (400 MHz): δ = 7.27 (d, *J* = 8.0 Hz, 2H, 3'-H, 5'-H), 7.10 (dd, *J* = 8.4, 7.61 Hz, 1H, 4'-H), 5.69 (dd, *J* = 9.7, 3.1 Hz, 1H, 1-H), 3.99 (ddd, *J* = 4.3, 2.3, 2.0 Hz, 1H, 4"-H), 3.84-3.87 (m, 1H, 3"-H), 3.24 (dt, *J* = 8.6, 3.7 Hz, 1H, 2"-H), 3.11 (dd, , *J* = 11.9, 4.6 Hz, 1H, 5"-H), 2.84 (dd, *J* = 11.9, 2.4 Hz, 1H, 5"-H), 2.46 (ddd, *J* = 14.5, 9.7, 3.7 Hz, 1H, 2-H), 1.80 (ddd, *J* = 14.5, 8.6, 3.1 Hz, 1H, 2-H), 0.87 [s, 9H, $C(CH_3)_3$], 0.86 [s, 9H, $C(CH_3)_3$], 0.07 (s, 3H, $SiCH_3$), 0.066 (s, 3H, $SiCH_3$), 0.058 (s, 3H, $SiCH_3$), 0.04 (s, 3H, $SiCH_3$) ppm;^{13}C-NMR (50 MHz): δ = 138.1 (s; C-1'), 134.2 (s; 2C, C-2', C-6'), 129.4 (d; 2C, C-3', C-5'), 128.4 (d; C-4'), 83.7 (d; C-3''), 79.9 (d; C-4''), 70.0 (d; C-1), 64.6 (d; C-2''), 53.4 (t; C-5''), 36.3 (t; C-2), 25.8 [q; 6C, $C(CH_3)_3$], 18.0 [s; 2C, $C(CH_3)_3$], -4.4 (q; $SiCH_3$), -4.5 (q; 2C, $SiCH_3$), -4.6 (q; $SiCH_3$) ppm; IR ($CDCl_3$): *v* = 3692, 3600, 3200, 2954, 2923, 2862, 1582, 1562, 1472, 1463, 1436, 1361, 1258, 1089 cm^{-1}; MS (EI): *m/z* (%) = 521 ($M+2^+$, 2); 519 (M^+, 2), 506 (1), 504 (1), 486 (1), 484 (1), 464 (1) 462 (2), 353 (1), 352 (2), 330 (5), 171 (63), 73 (88), 56 (100); anal. calcd. for $C_{24}H_{43}Cl_2NO_3Si_2$ (520.68): C 55.36, H 8.32, N 2.69; found: C 55.04, H 8.05, N 2.62.

(1R)-2-((2R,3S,4S)-3,4-bis{[tert-butyl(dimethyl)silyl]oxy}pyrrolidin-2-yl)-1-(2,6-dichlorophenyl)ethanol (**32b**)

Following the same procedure used to prepare **32a**, the diastereomeric pyrrolidine **32b** was obtained starting from the cycloadduct *exo-syn* **30b.**

32b: m.p.= 119 °C; $[\alpha]_D^{26}$= - 0.84; ^{1}H NMR (400 MHz): δ = 7.27 (d, 2H, *J* = 8.0 Hz, 3'-H, 5'-H), 7.11 (dd, *J* = 8.5, 7.5 Hz, 1H, 4'-H), 5.64 (dd, *J* = 8.3, 5.0 Hz, 1H, 1-H), 4.02 (dt, *J* = 4.9, 1.3 Hz, 1H, 4"-H), 3.91 (dd, *J* = 3.3,1.3 Hz, 1H, 3"-H), 3.50-3.45 (m, 1H, 2"-H), 3.36 (dd, *J* = 12.2, 4.9 Hz, 1H, 5"-H), 2.75 (dd, *J* = 12.2, 1.3 Hz, 1H, 5"-H), 2.32 (ddd, *J* = 14.3, 8.3, 4.7 Hz, 1H, 2-H), 2.01 (ddd, *J* = 14.3, 9.3, 5.0 Hz, 1H, 2-H), 0.87 [s,

9H, $C(CH_3)_3$], 0.86 [s, 9H, $C(CH_3)_3$], 0.09 (s, 3H, $SiCH_3$), 0.06 (s, 6H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$) ppm; ^{13}C-NMR (50 MHz): δ = 138.0 (s; C-1'), 134.4 (s; 2C, C-2', C-6'), 129.4 (d; 2C, C-3', C-5'), 128.6 (d; C-4'), 79.8 (d; C-3''), 78.2 (d; C-4''), 70.0 (d; C-1), 58.4 (d; C-2''), 53.2 (t; C-5''), 33.7 (t; C-2), 25.8 [q; 3C, $C(CH_3)_3$], 25.7 [q; 3C, $C(CH_3)_3$], 18.0 [s; $C(CH_3)_3$], 17.9 [s; $C(CH_3)_3$], -4.4 (q; $SiCH_3$), -4.6 (q; $SiCH_3$), -4.7 (q; $SiCH_3$), 4.8 (q; $SiCH_3$) ppm.

(1R)-2-((2S,3S,4S)-3,4-bis{[tert-butyl(dimethyl)silyl]oxy}pyrrolidin-2-yl)-1-(2,6-dichlorophenyl)ethanol (**32c**)

Following the same procedure used to prepare **32a**, the pyrrolidine **32c** was obtained in mixture with **32a** and **32b** starting from a mixture of adducts **30a, 30b** and **30c**.

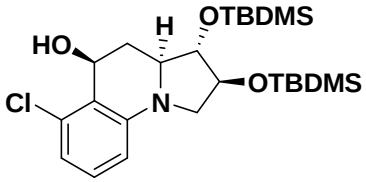
32c ^{1}H NMR (400 MHz) (in mixture with **32a** and **32b**; detectable signals): δ = 3.79 (m, 1H, 3"-H), 3.33 (m, 2H, 2"-H, 5-H), 2.97 (dd, *J* = 12.5, 3.1 Hz, 1H, 5"-H), 2.64 (dt, *J* = 14.4, 11.3 Hz, 1H, 2-H), 1.65 (dt, *J* = 14.4, 3.1 Hz, 1H, 2-H).

(1S)-2-((2R,3S,4S)-3,4-bis{[tert-butyl(dimethyl)silyl]oxy}pyrrolidin-2-yl)-1-(2,6-dichlorophenyl)ethanol (**32d**)

Following the same procedure used to prepare **32a**, the pyrrolidine **32d** was obtained in mixture with **32b** starting from a mixture of adducts **30b** and**30d**.

32d ^{1}H NMR (400 MHz) (in mixture with **32b**; detectable signals): δ = 3.79 (m, 1H, 3"-H), 3.33 (m, 2H, 2"-H, 5-H), 2.97 (dd, *J* = 12.5, 3.1 Hz, 1H, 5"-H), 2.64 (dt, *J* = 14.4, 11.3 Hz, 1H, 2-H), 1.65 (dt, *J* = 14.4, 3.1 Hz, 1H, 2-H).

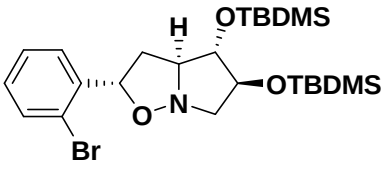
(2S,3S,3aS,5S)-2-{[tert-butyl(dimethyl)silyl]oxy}-6-chloro-3-[(trimethylsilyl)oxy]-1,2,3,3a,4,5-hexahydropyrrolo[1,2-a]quinolin-5-ol (**66**)

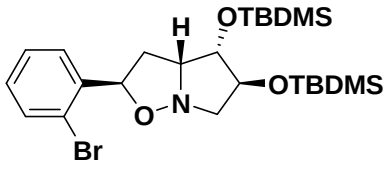


Degassed DMF (0.82 mL) was rapidly added to a mixture of **32a** (218 mg, 0.419 mmol), K_2CO_3 (116 mg, 0.837 mmol), CuI (8 mg, $4.2 \cdot 10^{-5}$ mol), L-proline (10 mg, $8.37 \cdot 10^{-5}$ mol) and a copper wire under nitrogen atmosphere in a Schlenk tube. The mixture was heated at 70 °C for 48 h under nitrogen atmosphere, diluted with deionized water (5 mL) and filtered through a short pad of silica gel. The filtrate was extracted with EtOAc (5x6 mL) and the combined organic phases were dried over Na_2SO_4, filtered through cotton wool and concentrated under reduced pressure. Purification by flash chromatography on silica gel (eluent: petroleum ether/EtOAc 20:1) afforded **66** in 5% yield (10.15 mg, 0.021 mmol) as a white solid.

66 ^{1}H-NMR (300 MHz) (selection of signals) : δ = 7.00 (tm, *J* = 8.0 Hz, 1H, 8-H), 6.60 (dm, *J* = 7.9 Hz, 1H, 7-H or 9-H), 6.27 (dm, *J* = 8.2 Hz, 1H, 7-H or 9H), 5.21-5.29 (m, 1H, 5-H) ppm; MS (EI): *m/z* (%) = 485 ($M+2^+$,9) 483 (M^+,19), 426 (9), 408 (5), 147 (27), 73 (100).

(2S,3aS,4S,5S)- and (2R,3aR,4S,5S)-4,5-bis{[tert-butyl(dimethyl)silyl]oxy}-2-(2-bromophenyl)hexahydropyrrolo[1,2-b]isoxazole (**31a** and **31b**)





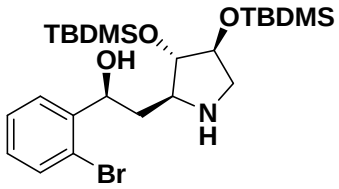
2-Bromostyrene (**18**, 0.430 mL, 3.319 mmol) was added to a suspension of the crude nitrone **16** (1.147 g, 3.319 mmol) in toluene (2.5 mL) and the reaction mixture was heated at 70 °C for 3 h in a microwave reactor using an irradiation power of 100 W. The solvent was evaporated under a nitrogen stream and the residue was purified by flash chromatography on silica gel (eluent: EtOAc/petroleum ether 1:14) with a 94% yield (1.647 g, 2.6 mmol). The three major diastereoisomers were only partially separated (*exo-anti*:*exo-syn*:*endo-anti* = 18.4:11.6:1). The *exo-anti* diastereoisomer was obtained in 55% combined yield (959 mg, 1.81 mmol, calculated in the mixed fractions by ^{1}H NMR) as a white solid.

31a R_f : 0.50; m.p.= 52 °C; $[\alpha]_D^{22}$= + 6.1; ^{1}H NMR (400 MHz): δ = 7.63 (dd, *J* = 7.8, 1.6 Hz, 1H, 3'-H or 6'-H), 7.51 (dd, *J* = 8.0, 1.1 Hz, 1H, 3'-H or 6'-H), 7.31 (dt, *J* = 1.1, 7.6 Hz, 1H, 4'-H or 5'-H), 7.12 (dt, *J* = 1.6, 7.8 Hz, 1H, 4'-H or 5'-H), 5.44 (pseudo t, *J* = 7.4 Hz, 1H, 2-H), 4.09 (dt, , *J* = 4.1, 5.5 Hz, 1H, 5-H), 4.02 (pseudo t, *J* = 3.9Hz, 1H, 4-H), 3.64 (dd, *J* = 12.1, 5.4 Hz, 1H, 6-Hb), 3.61 - 3.67 (m, 1H, 3a-H), 3.14 (dd, *J* = 12.1, 5.7 Hz, 1H, 6-Ha), 2.83 (ddd, *J* = 12.4, 6.7, 4.2 Hz, 1H, 3-Hb), 2.21 (ddd, *J* = 12.4, 9.2, 8.1 Hz, 1H, 3-Ha), 0.92 [s, 9H, $C(CH_3)_3$], 0.88 [s, 9H, $C(CH_3)_3$], 0.12 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$), 0.08 (s, 3H, $SiCH_3$) ppm; ^{13}C-NMR (100 MHz): δ = 139.9 (s; C-1'), 132.5 (d; C-3' or C-6'), 128.8 (d; C-4' or C-5'), 127.7 (d; C-4' or C-5'), 127.3 (d; C-3' or C-6'), 121.7 (s; C-2'), 83.6 (d; C-4), 78.0 (d; C-5), 77.3 (d; C-2), 71.8 (d; C-3a), 62.2 (t; C-6), 41.5 (t; C-3), 25.9 [q; 3C, $C(CH_3)_3$], 25.8 [q; 3C, $C(CH_3)_3$], 18.1 [s, $C(CH_3)_3$], 17.9 [s, $C(CH_3)_3$], -4.4 (q; $SiCH_3$), -4.5 (q; $SiCH_3$), -4.6 (q; $SiCH_3$), -4.7 (q, $SiCH_3$) ppm.

31b ^{1}H NMR (400 MHz): δ = 7.65 (dd, *J* = 7.8, 1.6 Hz, 1H, 3'-H or 6'-H), 7.50 (dd, *J* = 8.0, 1.2 Hz, 1H, 3'-H or 6'-H), 7.30 (dt, *J* = 7.8, 1.2 Hz, 1H, 4'-H or 5'-H), 7.11 (dt, *J* = 7.6, 1.7 Hz, 1H, 4'-H or 5'-H), 5.27 (dd, *J* = 8.8, 6.4 Hz, 1H, 2-H), 4.25 (dt, *J* = 4.9, 3.1

Hz, 1H, 5-H), 4.00 - 4.08 (m, 2H, 3a-H, 4-H), 3.37 (dd, *J* = 12.5, 5.2 Hz, 1H, 6-H), 3.22 (dd, , *J* = 12.5, 4.7 Hz, 1H, 6-H), 2.93 (ddd, *J* = 12.4, 6.4, 2.0 Hz, 1H, 3-H), 2.03 (dt, *J* = 12.4, 8.7 Hz, 1H, 3-H), 0.95 [s, 9H, $C(CH_3)_3$], 0.90 [s, 9H, $C(CH_3)_3$], 0.14 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.09 (s, 6H, $SiCH_3$) ppm; IR($CDCl_3$): *ν* =2955, 2930, 2886, 2858, 1471, 1440, 1390, 1361, 1258, 1110 cm^{-1}; MS (EI): *m/z* (%) = ($M+2^+$, 6), 527 (M^+, 5), 241 (5), 171 (19), 147 (25), 133(10), 73 (100), 55 (76).

(1S)-2-((2S,3S,4S)-3,4-bis{[tert-butyl(dimethyl)silyl]oxy}pyrrolidin-2-yl)-1-(2-bromophenyl)ethanol (**33a**)



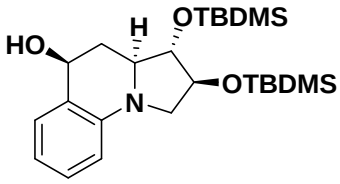
Isoxazolidine **31a** (0.982 mg, 1.86 mmol) was dissolved in a mixture of AcOH:H_2O = 9:1 (19 mL). Then zinc (607 mg, 9.29 mmol) was added. The reaction mixture was heated in an oil bath at 60 °C for 4 h and then filtered through cotton wool. The filtrate was basified to pH=9 with a saturated aqueous solution of $NaHCO_3$ and the obtained suspension was extracted with EtOAc (3x20 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Chromatography on silica gel (eluent: CH_2Cl_2/MeOH with 1% NH_4OH 20:1) afforded pyrrolidine **33a** in 88% yield (866 mg, 1.63 mmol).

33a R_f : 0.34; m.p.= 102 °C; $[\alpha]_D^{25}$= + 12.1; ^{1}H NMR (400 MHz): δ = 7.70 (dd, *J* = 8.2, 1.6 Hz, 1H, 6'-H), 7.48 (dd, *J* = 8.2, 1.2 Hz, 1H, 3'-H), 7.33 (dt, *J* = 7.6, 1.2 Hz, 1H, 5'-H), 7.09 (dt, *J* = 7.8, 1.6 Hz, 1H, 4'-H), 5.28 (dd, *J* = 3.1 Hz, 1H, 1-H), 3.96 (m, 1H, 4"-H), 3.81 (m, 1H, 3"-H), 3.16 (dd, *J* = 11.9, 4.9 Hz, 1H, 5"-H), 3.02 (dt, *J* = 9.4, 2.7 Hz,

1H, 2"-H), 2.89 (dd, *J* = 11.9, 2.9 Hz, 1H, 5"-H), 2.14 (ddd, *J* = 14.6, 9.5, 3.3 Hz, 1H, 2-H), 1.89 (ddd, *J* = 14.4, 6.4, 3.3 Hz, 1H, 2-H), 0.89 [s, 9H, $C(CH_3)_3$], 0.79 [s, 9H, $C(CH_3)_3$], 0.07 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.00 (s, 3H, $SiCH_3$), -0.08 (s, 3H, $SiCH_3$) ppm; ^{13}C-NMR (50 MHz): δ = 143.7 (s; C-1), 132.5 (d; C-5 or C-4), 128.2 (d; C-6 or C-3), 127.9 (d; C-4 or C-5), 127.3 (d; C-3 or C-6), 121.1 (s; C-2), 84.0 (d; C-3''), 79.9 (d; C-4''), 71.6 (d; C-1), 64.4 (d; C-2''), 53.2 (t; C-5''), 36.2 (t; C-2), 25.8 [q; 3C, $C(CH_3)_3$], 25.7 [q; 3C, $C(CH_3)_3$], 17.9 [s; $C(CH_3)_3$], 17.8 [s; $C(CH_3)_3$], - 4.6 (q; $SiCH_3$), -4.7 (q; 2C, $SiCH_3$), -4.8 (q; $SiCH_3$) ppm.

33b m.p.= 155 °C; $[\alpha]_D^{26}$= + 46.2; ^{1}H NMR (400 MHz): δ = 7.65 (dd, *J* = 7.8, 1.6 Hz, 1H, 5-H' or 4'-H), 7.50 (dd, *J* = 8.1, 1.2 Hz, 1H, 4'-H or 5'-H), 7.33 (dt, *J* = 7.6, 1.2 Hz, 1H, 6-H' or 3'-H), 7.10 (dt, *J* = 7.6, 1.8 Hz, 1H, 3'-H o 6'-H), 5.31 (t, *J* = 4.7 Hz, 1H, 1-H), 3.94 (dt, *J* = 4.7, 1.6 Hz, 1H, 4"-H), 3.74 (dd, *J* = 4.3, 1.6 Hz, 1H, 3"-H), 2.24 (dd, *J* = 12.1, 4.3 Hz, 1H, 5"-H), 3.19 (dt, *J* = 11.3, 3.5 Hz, 1H, 2"-H), 2.69 (dd, *J* = 12.3, 1.4 Hz, 1H, 5"-H), 2.11 (ddd, *J* = 14.8, 11.3, 4.3 Hz, 1H, 2-H), 1.93 (ddd, *J* = 14.8, 5.1, 3.1 Hz, 1H, 2-H), 0.87 [s, 9H, $C(CH_3)_3$], 0.83 [s, 9H, $C(CH_3)_3$], 0.03 (s, 6H, $SiCH_3$ x2), -0.01 (s, 3H, $SiCH_3$), -0.02 (s, 3H, $SiCH_3$) ppm; ^{13}C-NMR (100 MHz): δ = 143.6 (s; C-1), 132.6 (d; C-5 or C-4), 128.3 (d; C-6 or C-3), 127.8 (d; C-4 or C-5), 127.3 (d; C-3 or C-6), 121.4 (s; C-2), 80.4 (d; C-3"), 78.4 (d; C-4"), 71.5 (d; C-1), 57.4 (d; C-2''), 52.9 (t; C-5''), 32.8 (t; C-2), 25.8 [q; 3C, $C(CH_3)_3$], 25.7 [q; 3C, $C(CH_3)_3$], 18.0 [s; $C(CH_3)_3$], 17.8 [s; $C(CH_3)_3$], -4.6 (q; $SiCH_3$), -4.7 (q; 2C, $SiCH_3$), -4.9 (q, $SiCH_3$) ppm; IR ($CDCl_3$): ν = 3661, 3600, 3190, 2955, 2930, 2858, 1471, 1463, 1441, 1362, 1258, 1088, 1082 cm^{-1}; MS (EI): *m/z* (%) = 531 ($M+2^+$, 2), 529 (M^+, 1), 450 (2), 432 (5), 171 (54), 97(44), 83 (45), 71 (59), 57 (100).

(2S,3S,3aS,5S)-2,3-Bis{[tert-butyl(dimethyl)silyl]oxy}-1,2,3,3a,4,5-hexahydropyrrolo[1,2-a]quinolin-5-ol (**67**)

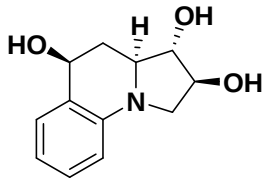


DBU (40 μL, 0.267 mmol) and degassed *t*BuOH (2.6 mL) were added to a mixture of **33a** (69.8 mg, 0.132 mmol), CuI (2.5 mg, 0.013 mmol), copper powder (1.0 mg, 0.015 mmol) and L-proline (3.0 mg, 0.026 mmol) under nitrogen atmosphere in a microwave vial. The reaction mixture was heated at 100 °C for 10 h in a microwave reactor with TLC monitoring every hour. The solvent was evaporated under an air stream and the crude product was dissolved in $CHCl_3$, filtered through a short pad of celite® and concentrated under reduced pressure. Purification by flash chromatography (eluent: initially petroleum ether/EtOAc 9:1, then 5:1) afforded **67** in 30% yield (18 mg, 0.040 mmol) as a white solid.

67 R_f = 0.45; m.p.= 142 °C; $[\alpha]_D^{24}$= + 67; ^{1}H NMR (400 MHz): δ = 7.38 (dt, *J* = 7.6, 1.2 Hz, 1H, 6-H or 9-H), 7.12 (tm, *J* = 8.1 Hz, 1H, 7-H or 8-H), 6.67 (dt, *J* = 7.4, 0.9 Hz, 1H, 7-H or 8-H), 6.30 (br d, *J* = 8.1 Hz, 1H, 6-H or 9-H), 4.82-4.90 (m, 1H, 5-H), 4.22 (dt, *J* = 7.6, 6.7 Hz, 1H, 2-H), 3.78 (dd, *J* = 8.0, 6.7 Hz, 1H, 3-H), 3.51 (dd, *J* = 9.4, 7.6 Hz, 1H, 1-H), 3.38 (ddd, *J* = 11.8, 8.0, 3.0 Hz, 1H, 3a-H), 3.01 (dd, *J* = 9.4, 6.7 Hz, 1H, 1-H), 2.47 (ddd, *J* = 11.5, 5.5, 3.0 Hz, 1H, 4-H), 1.71 (d, *J* = 8.8 Hz, 1H, OH), 1.58 (q, *J* = 11.5 Hz, 1H, 4-H), 0.92 [s, 9H, $C(CH_3)_3$], 0.91 [s, 9H, $C(CH_3)_3$], 0.13 (s, 3H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.10 (s, 3H, $SiCH_3$) ppm; ^{13}C-NMR (100 MHz): δ = 143.2 (s; C-5a), 128.6 (d; C-7 or C-8), 125.3 (d; C-6 or C-9), 124.7 (s; C-9a), 115.9 (d; C-7 or C-8), 109.4 (d; C-6 or C-9), 82.2 (d; C-3), 76.6 (d; C-2), 67.1 (d; C-5), 59.5 (d; C-3a), 52.2 (t; C-1), 35.8 (t; C-4), 25.9 [q; 3C, $C(CH_3)_3$], 25.8 [q; 3C, $C(CH_3)_3$], 18.0 [s; $C(CH_3)_3$], 17.9 [s; $C(CH_3)_3$], -3.8 (q; $SiCH_3$), -4.2 (q; $SiCH_3$), -4.4 (q; $SiCH_3$), -

4.5 (q, $SiCH_3$) ppm; IR ($CDCl_3$): ν = 3586, 2955, 2929, 2857, 1605, 1500, 1472, 1462, 1389, 1361, 1350, 1259, 1156, 1113 cm^{-1}; MS (EI): *m/z* (%) = 449 (M^+, 65), 434 (2), 430 (7), 392 (12), 374(10), 168 (56), 161 (69), 143 (48), 132 (19), 73 (100); anal. calc. for $2.C_{24}H_{43}NO_3Si_2\ H_2O$ (449.28): C 62.83, H 9.67, N 3.05; found: C 63.22, H 9.39, N 2.70.

(2S,3S,3aS,5S)-1,2,3,3a,4,5-Hexahydropyrrolo[1,2-a]quinoline-2,3,5-triol (**15**)



A 1 M solution of TBAF in THF (0.26 mL, 0.26 mmol) was added to a solution of **67** (25.8 mg, 0.0574 mmol) in dry THF (3 mL) under nitrogen atmosphere. The reaction mixture was stirred overnight at rt under nitrogen atmosphere. Purification of the crude product by flash chromatography on silica gel [eluent: CH_2Cl_2/MeOH (+ 1% NH_4OH) 9:1] afforded **15** in 36% yield (5 mg, 0.021 mmol).

15 ^{1}H-NMR (CD_3OD, 400 MHz): δ = 7.32 (dt, *J* = 7.4, 1.2 Hz, 1H), 7.19 (t, *J* = 8.0 Hz, 1H), 6.60 (dt, *J* = 7.4, 0.8 Hz, 1H), 6.31 (dd, *J* = 8.0, 0.8 Hz, 1H), 4.79 (dd, *J* = 10.9, 5.5 Hz, 1H, 5-H), 4.20 (dt, *J* = 7.8, 6.8 Hz, 1H, 2-H), 3.67 (dd, *J* = 8.4, 7.0 Hz, 1H, 3-H), 3.53 (dd, *J* = 9.7, 7.8 Hz, 1H, 1-H), 3.34 (dt, *J* = 8.4, 3.0 Hz, 1H, 3a-H), 3.03 (dd, *J* = 9.7, 6.8 Hz, 1H, 1-H), 2.47 (ddd, *J* = 11.7, 5.5, 3.0 Hz, 1H, 4-H), 1.58 (pseudo q, *J* = 11.7 Hz, 1H, 4-H) ppm; ^{13}C-NMR (CD_3OD, 100 MHz): δ = 143.4 (s; C-5a), 127.8 (d; C-7 or C-8), 125.2 (d; C-6 or C-9), 125.0 (s; C-9a), 115.5 (d; C-7 or C-8), 109.2 (d; C-6 or C-9), 81.3 (d; C-3), 75.1 (d; C-2), 66.0 (d; C-5), 59.9 (d; C-3a), 51.4 (t; C-1), 34.7 (t; C-4).