UNIVERSITÀ
DEGLI STUDI
FIRENZE

DOTTORATO DI RICERCA IN SCIENZE CHIMICHE

SETTORE SCIENTIFICO DISCIPLINARE CHIM/06

XXVI CICLO

STEREOSELECTIVE SYNTHESIS OF MODIFIED LENTIGINOSINES AS PROAPOPTOTIC AGENTS

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2011-2013

DEDICATED
TO
MY Late Father

INDEX

Chapter 1: Introduction

1.1 Iminosugars 1

1.2 Structure of iminosugars 1

1.2.1 Monocyclic structures 2

1.2.2 Bicyclic structures 4

1.3 Biological activity of iminosugars 6

1.3.1 Inhibition of digestive glycosidases 8

1.3.2 Inhibition of lysosomal storage diseases 10

1.3.3 Processing glycosidase 11

1.3.4 Anti-cancer activity and anti-viral activity 13

1.3.5 Anti-insecticide activity 14

1.4 Mechanism of action of glycosidases 14

1.5 Lentiginosine and its unnatural enantiomer 19

1.6 Aim of the thesis 26

1.6.1 The mechanism of interaction between (+)-lentiginosine and glucoamylase 27

1.6.2 Synthetic Targets 31

1.6.3 Importance of aromatic ring 33

1.6.4 Medicinal chemistry aspects of aromatic ring 34

Chapter 2: Revisited synthesis of 3, 4-bis-*tert*-butoxypyrroline *N*-oxide and (-)-7-OH-lentiginosine: synthesis and proapoptotic activity

2.1 Introduction 35

2.2 Nitrone 36

2.2.1 1,3 dipolar cycloaddition mechanism 37

2.2.2 1,3 dipolar cycloaddition: regiochemistry and stereochemistry 38

2.2.3 Cyclic nitrone (3,4-bis-*tert*-butoxypyrroline *N*-oxide) 42

2.3 Result and discussion 42

2.3.1 Synthesis of 3, 4-bis-*tert*-butoxypyrroline *N*-oxide 42
2.3.2 Synthesis of (-)-(1*R*-2*R*-7*S*-*8aR*)-1, 2, 7-trihydroxyindolizidine 48
2.3.2.1 Old approach: (-)-(1*R*-2*R*-7*S*-*8aR*)-1, 2, 7-trihydroxyindolizidine 48
2.3.2.2 New approach: (-)-(1*R*-2*R*-7*S*-*8aR*)-1, 2, 7-trihydroxyindolizidine 51
2.4 Biological activity 53
2.5 Conclusion 57

Chapter 3: Stereoselective synthesis of a highly hydroxy functionalize benzo[*e*]indolizidine via Cu (I) Catalyzed Ullmann reaction

3.1 Introduction 59
3.2 Result and discussion 60
3.2.1 Synthesis of benzo[*e*]indolizidine from nitrone, derived from tartaric acid 60
3.2.1.1 Synthesis of isoxazolidine 60
3.2.1.2 Opening of isoxazolidine ring 64
3.2.1.3 Closure of benzoindolizidine 64
3.2.1.3.1 Buchwald-Hartwig Amination reaction 65
3.2.1.3.2 Copper mediated cyclization by Ullmann reaction 68
3.2.1.3.3 Intramolecular amination reactions 69
3.2.1.4 Deprotection of Hydroxy benzo[*e*]indolizidine 75
3.2.1.5 Synthesis of benzo[*e*]indolizidine 76
3.2.2 Synthesis of benzo[*e*]indolizidine from nitrone, derived from malic acid 77
3.2.2.1 Synthesis of (S)-4-*tert*-butoxy-3,4-dihydro- 2H-pyrrole 1 oxide 78
3.2.2.2 Synthesis of (3*S*)-1,2,3,3a,4,5-hexahydropyrrol [1,2-*a*]quinolin-3-ol 79
3.3 Biological activity 83
3.4 Conclusion 84

Chapter 4: Development of new synthetic methodology towards substituted tetrahydroquinolines

4.1 Introduction 85

4.2 Result and discussion 86

4.2.1 Synthesis of (2*S**,4*R**)-1-benzyl-2-phenyl-1,2,3,4-tetrahydroquinolin-4-ol 86

4.2.2 (2*S**,4*R**)-and(2*S**,4*S**)-2-(4-methoxyphenyl)-1-methyl-1,2,3,4 tetrahydroquinolin-4-ol 90

4.2.3 Synthesis of (2*S**,4*R**)-2-(4-aminophenyl)-1-methyl-1,2,3,4 tetrahydroquinolin-4-ol 95

4.2.4 Synthesis of (2*S**,3*R**,4*R**)-ethyl 4-hydroxy-2-(4-methoxyphenyl)-1-methyl-1,2,3,4-tetrahydroquinoline-3-carboxylate 98

4.2.5 Synthesis of (2*R**,3*S**,4*S**)-ethyl 4-hydroxy-1-methyl-2-phenyl-1,2,3,4-tetrahydro-1,8-naphthyridine-3-carboxylate 103

4.3 Conclusion 107

Chapter 5: Synthesis of benzo[*f*]indolizidine and benzo[*g*]indolizidine

5.1 Stereoselective synthesis of benzo[*f*]lentiginosine via Pd catalyzed Intramolecular carbonylation of secondary amine. 108

5.1.1 Introduction 108

5.1.2 Result and discussion 110

5.1.2.1 Synthesis of benzo[*f*]lentiginosine from nitrone derived from tartaric acid 110

5.1.2.2 Synthesis of benzo[*f*]indolizidine from nitrone derived from Malic acid 118

5.2 Stereoselective synthesis of benzo[*g*]lentiginosine via coupling of Arynes with Pyrroline-*N*-oxides 121

5.2.1 Introduction 121

5.2.2 Result and discussion 125
5.2.2.1 Synthesis of isoxazolidine ring via cycloaddition of nitrone with aryne 125
5.2.2.2 Reductive cleavage of isoxazolidine ring 128
5.2.2.3 Rest of synthesis is in progress……. 128
5.3 Conclusion 129

Experimental section 130
References 208
Acknowledgement 217

1. Introduction[1]

1.1 Iminosugars

Molecules, that mimic natural or synthetic carbohydrates, in which, the endocyclic oxygen atom has been replaced by a nitrogen atom, are called as iminosugars or azasugars.

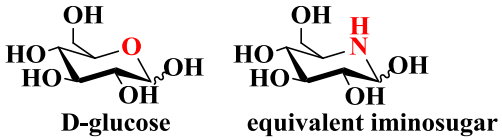


Figure 1.1

The iminosugar motif is present in several classes of monocyclic and bicyclic compounds, leading to a large and structurally diverse class of molecules. Iminosugars have the potential to block or modify glycosidase enzymes, which are involved in a wide range of biological processes and they have therefore attracted a great deal of interest since they were first discovered. This interest has focused on the isolation of new iminosugars, the study of their biological activity and, indeed, their synthesis.

1.2 Structure of iminosugars

The first iminosugar, nojirimycin (**1**, NJ) [Figure 1.3], was discovered in 1966[2a] and numerous iminosugars have since been isolated from plants and microorganisms.[2b] Naturally occurring sugar mimics with a nitrogen in the ring are classified into six structural classes: These molecules may have a pyrroline (**a**), pyrrolidine (**b**), piperidine (**c**), pyrrolizidine (**d**), indolizidine (**e**) or *nor*-tropane (**f**) skeleton and these can therefore be grouped as monocyclic or bicyclic structures as shown in [Figure 1.2].

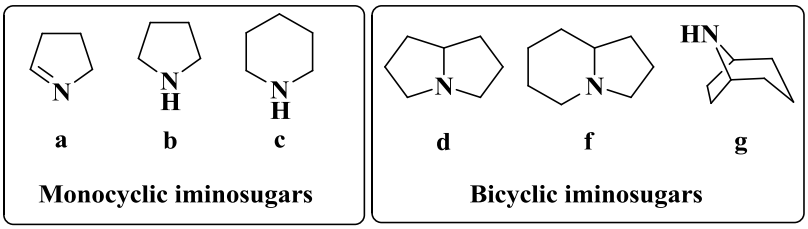


Figure 1.2 General Iminosugars core structures

1.2.1 Monocyclic structures

There are three classes of monocyclic iminosugars, pyrroline, piperidines and pyrrolidines with general structures shown in [Figure 1.3], Nojirimycin (or 5-amino-5- deoxy-D-glucopyranose) (NJ, **1**) [Figure 1.3] is the main representative of the piperidine class of monocyclic iminosugars. It is a polyhydroxylated piperidine corresponding to glucose in the pyranose form and was first described as an antibiotic produced in bacterial cultures of *Streptomyces roseochromogenes* R-468 and *Streptomyces nojiriensis* SF-426.[3] As a hemiacetal, NJ is an inherently unstable structure and therefore its corresponding 1- deoxy analogue, 1-deoxynojirimycin (DNJ; **2**) [Figure1.3], was synthesised by reduction with $NaBH_4$ by Paulsen and coworkers.[3b,4] DNJ was later isolated from the roots of mulberry leaves and was called moranoline.[5] DNJ is also produced by many strains of the *Bacillus* and *Streptomyces* genera. DNJ is related to another natural product, 1- deoxymannojirimycin (DMJ, **3**) [figure 1.3], where the hydroxyl group at C-2 has the opposite stereochemistry, mimicking the pyranose form of D-mannose. DMJ was found to be produced by *Streptomyces lavendulae* SF-425 and has recently been extracted from *Adenophora triphylla*.[5] A number of other piperidine iminosugars have since been isolated from a range of plants and bacterial cultures and these are shown in [Figure 1.3].[2] Iminosugars with a hydroxyl group at C-1, nojirimycin B (*manno*-NJ, **4**) and galactostatin (*galacto*-NJ, **5**), were also isolated from

species of *Streptomyces*. Fagomine, 1,2- dideoxynojirimycin (**6**) has been isolated from the seeds of Japanese buckwheat (*Fagopyrum esculentum*)[6] as well as the Moreton Bay Chestnut and *Castanospermum australe*.[7]

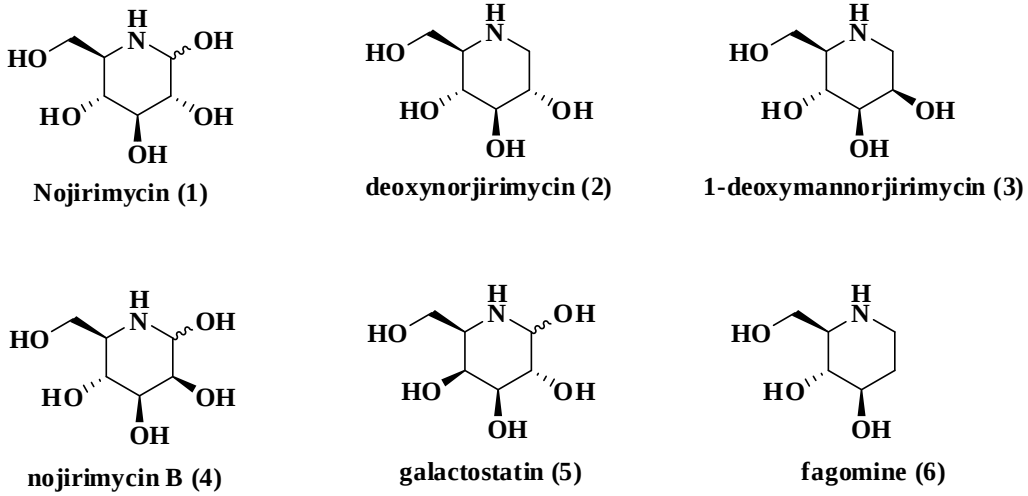


Figure 1.3 Naturally occurring piperidines

Polyhydroxylated pyrrolines and pyrrolidines resemble sugars in the furanose form and can be potent inhibitors of the corresponding glycosidases specific for carbohydrates with a matching pattern of hydroxyl substitution and stereochemistry. Some of the naturally occurring pyrrolines and pyrrolidines are shown in [Figure 1.4]

In 1976, 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP, **7**), a naturally occurring analogue of β-D-fructofuranose was found in the leaves of the legume, *Derris elliptica*.[8, 9] This compound is a very common metabolite as it has since been isolated from various other plants and microorganisms.[10] A second example of a pyrrolidine analogue is 1,4-dideoxy-1,4-imino-D-arabinitol (D-AB1, **8**), which resembles **7** but has only one hydroxymethyl group. D-AB1 was initially isolated from the fruits of *Angylocalyx boutiqueanus* but, like DMDP, has since been isolated from different species of plants.[11, 12] The 6-deoxy derivative of DMDP (6-deoxy-

DMDP, 2,5,-imino-1,2,3-trideoxy-Dmannitol, **9**) was isolated from the seeds of *Anglocalyx pynaertii.* [13] and polyhydroxypyrroline nectrisine (**10**) was isolated from a broth culture of the fungus *Nectria ludica*.[2]

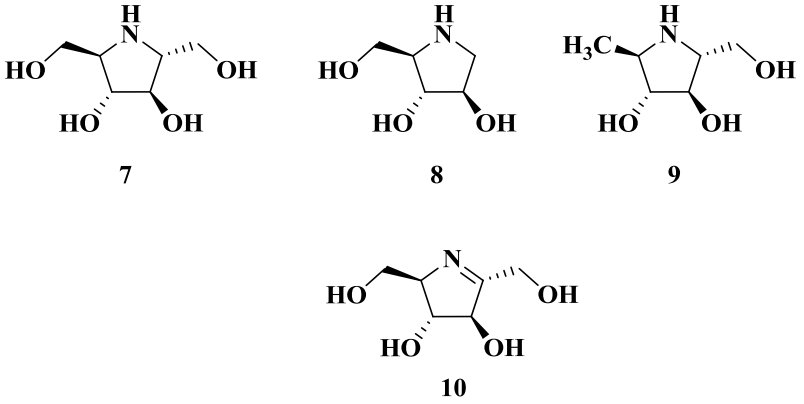


Figure 1.4 Naturally occurring pyrrolines and pyrrolidines iminosugars

1.2.2 Bicyclic structures

There are three classes of natural bicyclic iminosugars: indolizidines, pyrrolizidines and nortropanes, which have the general structures as shown in [Figure 1.2]. The first bicyclic iminosugar, an indolizidine, was isolated from a legume called *Swainsona canescens* in 1979 and was named swainsonine (**11**) [Figure 1.5].[14] It has since been isolated from locoweeds (*Astragalus* and *Oxytropis* species) which cause the disorder 'locoism' in animals. Another similar alkaloid, castanospermine (**12**) [Figure 1.5], was later isolated from the seeds of the tree *Castanospermum australe*.[15]

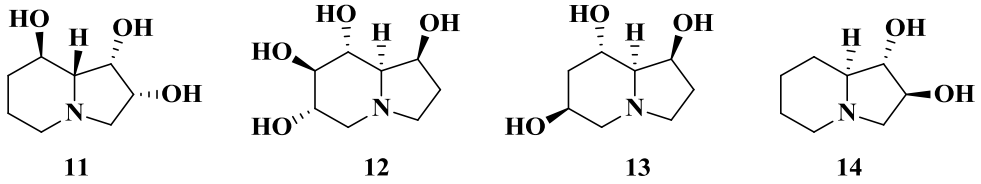


Figure 1.5 Naturally occurring indolizidines iminosugars

It was found to share the same core indolizidine structure as **11** but differs in the substitution pattern of the hydroxyl groups. The seeds of *Castanospermum australe* were also found to produce 7-deoxy-6-*epi*-castanospermine (**13**).[15]

Lentiginosine **14**, isolated from the leaves of *Astragalus Lentiginous, a* plant found in western part of North America, was more recently discovered and characterized for the first time in 1990 by Pastuszak et al.[43]

Figure 1.6 The plant of *Astragalus Lentiginous*

(+)-Lentiginosine (**14**) is the least hydroxylated naturally occurring indolizidine derivative possessing a glycosidase inhibitor activity: it shows highly selective inhibitory activity against amyloglucosidase (see section 1.6 below). Owing to the growing interest in hydroxylated indolizidines as different glycosidase inhibitors, there has been a plethora of publications concerning the synthesis of hydroxylated indolizidines including lentiginosine.

The first polyhydroxylated pyrrolizidines, alexine (**15**) and australine (or 7a*epi*-alexine, **16**) [Figure 1.7] were isolated in 1988 from the legumes, *Alexa leipetala* and *Castanospermum australe*.[16] These structures differ from the long known necine bases, pyrrolizidine alkaloids, with a hydroxymethyl side chain at the C-1 position.

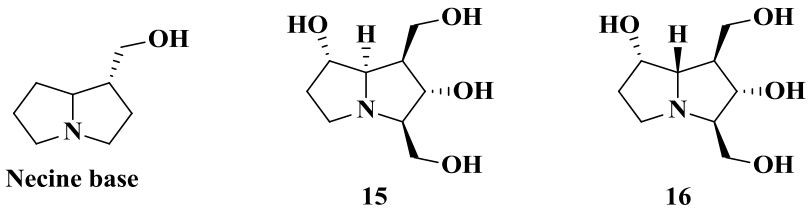


Figure 1.7 Naturally occurring pyrrolizidines iminosugars

Polyhydroxylated nortropanes were the final class of naturally occurring bicyclic iminosugars to be discovered when calystegines were found to be secondary metabolites in plants and were implicated in the maintenance of plant-bacterium relationships.[17] A shown in [Figure 1.8],

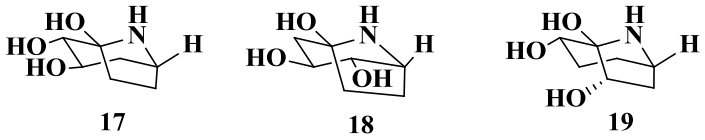


Figure 1.8 Naturally occurring nortropanes iminosugars

Calystegines have a nortropane ring system, two to four hydroxyl functions of varying stereochemistry and position and a tertiary alcohol group at the bridgehead carbon C-1 of the bicyclic ring. The first calystegines, A3 (**17**), B1 (**18**) and B2 (**19**), were extracted from root cultures of *C. sepium*.[17]

1.3 Biological activity of iminosugars [1,18]

Iminosugars present a wide structural diversity in the basic skeleton (five-membered ring, six-membered ring, simple or fused ring), in the number of hydroxy functions and in the relative and absolute 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 belonging 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 biological processes such as degradation of polysaccharides and glycoconjugates (glycoproteins, glycolipids, proteoglycans) in nearly all life forms[18] 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), 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 1.9].

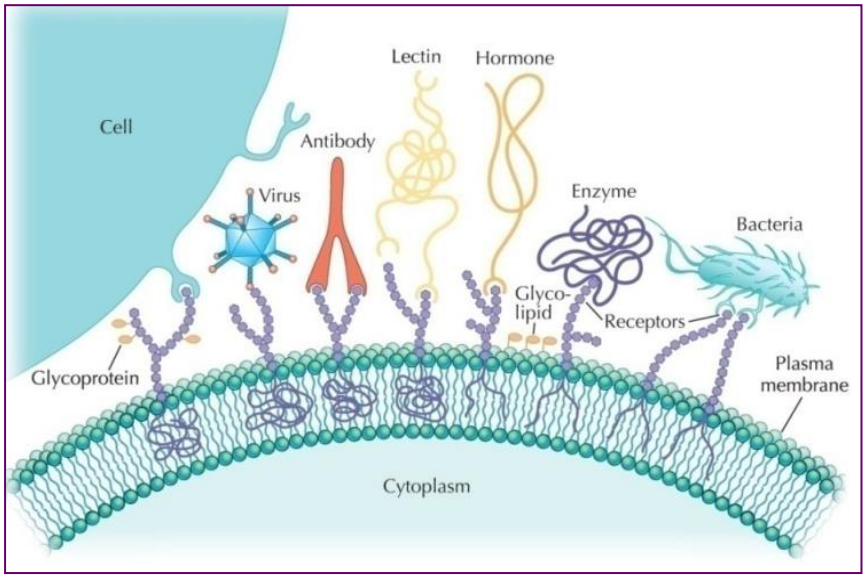


Figure 1.9 Glycoconjugates and molecular recognition mechanisms

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.

The first example involved the first isolated iminosugar NJ (**1**) which was reported to inhibit both α-glucosidases and β-glucosidases.[3] The reduced form of NJ, DNJ , exhibited activity against glycosidases I and II, whereas DMJ and its bicyclic counterpart Swainsonine (**11**) were reported to inhibit lysosomal α- mannosidases.[2, 9] Castanospermine (**12**) has been shown to be a powerful inhibitor of the α- and β-glucosidases in the mammalian gut.[15] Australine was found to be a good inhibitor of the α-glucosidases, amylglucosidase and subsequently the glycoprotein enzyme glucosidase I.[19]

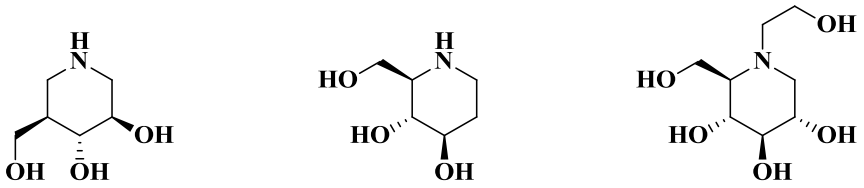
Unsurprisingly, the inhibition of glycosidase and glycosyltransferase enzymes can affect the digestion and metabolism of polysaccharides as well as the maturation, transportation and secretion of glycoconjugates. Glycocongugates expressed at the cell surface – often possessing complex, branched extracellular carbohydrate structures are involved in several fundamental biological functions such as cell-cell recognition, cell adhesion and signalling. The role of these biomacromolecules in cell differentiation, immune response, oncogenesis, tumor metastasis and viral infections has prompted considerable interest in iminosugars as a class of compounds for therapeutic intervention, with the emphasis on their potential applications in the treatment of diabetes, cancer and viral disease.

1.3.1 Inhibition of digestive glycosidases

Digestive glycosidases, located in the small intestine, are enzymes that hydrolyze dietary carbohydrates to monosaccharides, which are then absorbed through the intestine wall. It was

thought that treatment of non insulin dependent diabetes (type II diabetes) could be achieved by means of blocking these enzymes, thus regulating the absorption of carbohydrates through the intestine wall. The original discovery of DNJ (**2**) resulted from investigations prompted by the knowledge that extracts of mulberry were able to suppress the rise in blood glucose that follows eating, suggesting that this activity could be beneficial for diabetes treatment.[9] DNJ (**2**) was subsequently found to have a good inhibitory effect on mammalian α- glucosidase *in vitro* and was thus considered to be a promising agent for treatment of diabetes.[18, 20] Unfortunately, the compound lacked efficacy *in vivo* for this clinical indication but these studies stimulated the synthesis and evaluation of many derivatives of DNJ and *N*-alkylated analogues, such Miglitol (**22**), were subsequently found to be potent inhibitors of the glycogenolysis induced by glucagen in studies with hepatocytes.[21] Miglitol (GLYSETTM) is now used in the treatment of type II diabetes and is available in the USA and Canada.

In type II diabetes, an increase in hepatic glucose production and blood glucose levels is observed. Inhibiting hepatic glycogen phosphorylase could prevent this from occurring. Isofagomine (**20**) [Figure 1.10] was recently found to be a good inhibitor of liver glycogenphosphorylase, blocking glycogen degradation in hepatocytes.[22] The specificity of iminosugars is well illustrated by the fact that some *N*-alkylated derivatives of isofagomine (**20**) are active inhibitors at micromolar concentrations, whereas Fagomine (**21**) and DNJ (**2**) lack activity [Figure 1.10].[23]



20 Isofagomine

21 fagomine

22 Miglitol

Figure 1.10

1.3.2 Inhibition of lysosomal storage diseases

Disorders in the biosynthesis or catabolism of glycolipids in the cell have an impact on so called lysosomal storage diseases like type I Gaucher disease or Fabry disease.[24] In normal cells there is a balance between the degradation of glycosphingolipids (GSLs) in the lysosome and their biosynthesis in the endoplasmic reticulum (ER)/Golgi system. The rates of influx of GSLs and efflux of metabolites are in equilibrium. In a lysosomal storage cell, enzyme activity in the lysosome is so low that GSLs accumulate. However, although the catalytic activity of enzymes is reduced, it is not totally eliminated. Thus, drugs that could regulate the biosynthesis of GSLs to a concentration that fits well in the residual enzymatic activity could prevent storage. Studies have thus been carried out with *N*-alkylated DNJs, which are inhibitors of ceramide specific glycosyltransferases, [25] and *N*-butyl DNJ (**23**) [Figure 1.11], marketed as Zavesca®, is now used in the treatment of type1 Gaucher's disease.

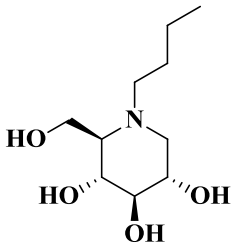


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Figure 1.11 N-butyl DNJ

The discovery that swainsonine (**11**) can induce a reversible phenocopy of the genetic lysosomal storage disease, mannosidosis, in animals has led to the use of chemically induced deficiencies of lysosomal hydrolyses as models for studying the pathogenesis of lysosomal storage disease.[26, 27] Mannosidosis is characterised by the accumulation in cells, and excretion in urine, of mannose rich oligosaccharides resulting from a deficiency of lysosomal α-mannosidase. Treatment with swainsonine (**11**) is particularly effective in mimicking lysosomal storage disease because of the compound's potent inhibitory activity against lysosomal α-mannosidase and its lysosomotropic behaviour. It is a weak base with a pKa of 7.4, which ensures that it is taken up rapidly into cells by permeation and, once inside the lysosomes, becomes protonated and becomes concentrated. Swainsonine (**11**) has since been granted orfan drug status for the treatment of gaucher disease.

1.3.3 Processing glycosidase

The α-D-glucosidases and α-D-mannosidases involved in the post-translational processing of asparagine-linked glycans of glycoproteins can be selectively inhibited by various iminosugars.[28] Using these inhibitors, the consequences of altering the glycosylation of a particular glycoprotein or the glycotype of cells can be studied in a defined way. Indeed,

castanospermine (**12**), DMJ (**3**) and swainsonine (**11**), become the standard commercially available reagents used in biochemical studies for inhibition of α-glucosidases I, α-mannosidases I and α- mannosidases II. These compounds are not completely specific, however, and they do not inhibit processing unless they are present in relatively high concentrations, which in itself increases lack of specificity. Consequently, there is current interest in the discovery of more specific glycosidase inhibitors and this has prompted structure activity relationship studies. For example, *N*-methyl-DNJ (**24**) [Figure 1.12] has been found to be a poorer inhibitor of α-glucosidases I than DNJ (**2**) and conversely for α-glucosidases II. Castanospermine also exhibited a similar inhibition of α-glucosidases I to *N*-methyl- DNJ (**24**) suggesting that substitution of the piperidine nitrogen favours inhibition of α-glucosidases I.

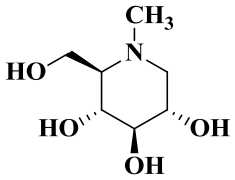

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Figure 1.12 N-Methyl DNJ

DMJ (**3**) was found to inhibit α-mannosidase I, which is particularly susceptible to iminosugar pyranose analogues. Although swainsonine (**11**) has been shown to be a potent inhibitor of α-mannosidase II, it does not inhibit other processing α-mannosidases in human cell cultures.[9] The specificity of synthetic iminosugars for the various processing glycosidases has not yet been exhaustively evaluated, but the available information suggests that many are more specific *in vitro* than their parent compounds. The effect of the common glycoprotein processing inhibitors on the biosynthesis, intracelluar transport and function of most well

characterised glycoproteins has been thoroughly evaluated however. Indeed, their effects on many cellular processes have been evaluated, but perhaps the two areas of most excitement and interest relate to anti-cancer and antiviral applications.

1.3.4 Anti-cancer activity and anti-viral activity

In the case of the development of the tumor metastasis, the role of the glycoproteins is absolutely crucial.[29] Cell migration is an essential process of the mechanism of 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.[30] 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.

1.3.5 Anti-insecticide activity

Nojirimycin and pyrrolidine-based iminosugar derivatives are evaluated as potential inhibitors of porcine and insect trehalases with selectivity towards the insect glycosidase. Trealose, hydrolyzed into glucose (vital for insect flight) by the enzyme α-trehalase (EC 3.2.1.28), an inverting glycosidase. Trehalose is not found in mammalian cells, but humans possess the enzyme trehalase, probably to handle ingested trehalase. Trehalase inhibitors have great potential as novel insecticides and fungicides. Selectivity toward insects' trehalases are a main issue to be addressed.[34]

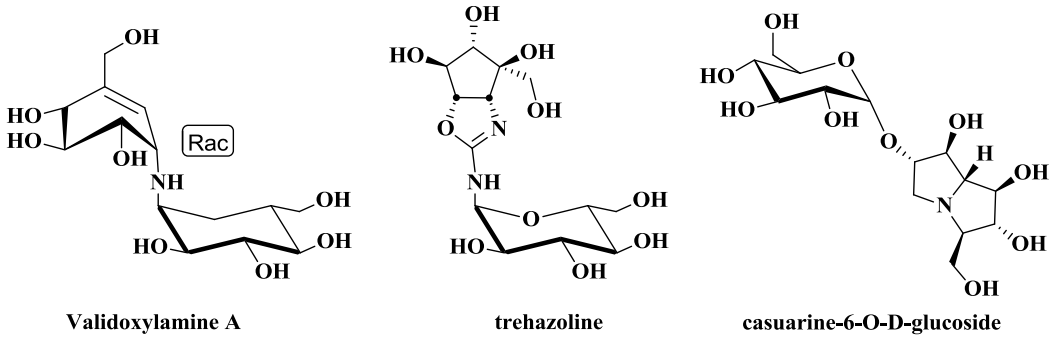


Figure 1.13 Some example of Trehalase inhibitors

1.4 Mechanism of action of glycosidases

As we already said, glycosidases 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 catalyze the cleavage of glycosidic bonds involving terminal glucose connected at the site of cleavage through an □-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.[31] Since competitive inhibition is observed with a lot of inhibitors, probably both conformational and electrostatic influences are important in the active site binding. During glycosidic bond cleavage reaction can occurs with one or two stereo chemical outcomes, inversion or retention of configuration through double or single nucleophilic substitution. Both of this enzymatic mechanisms involve key oxonium ion intermediate generated in the transition state preferably in its half-chair conformation **b** than in the chair conformation **a** [Figure 1.14].

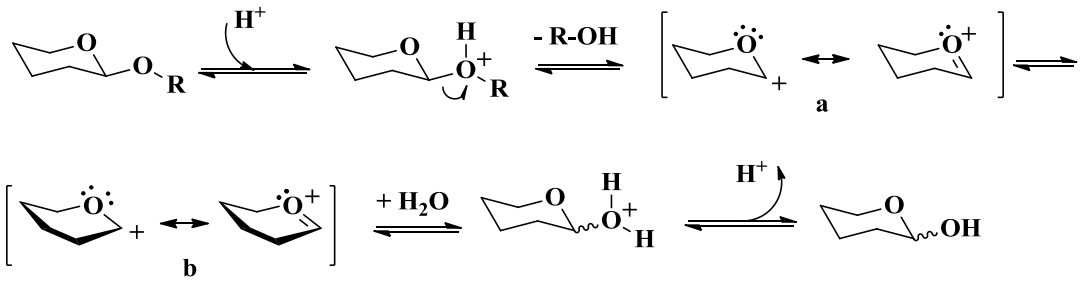
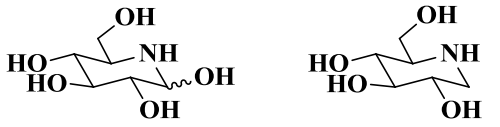


Figure 1.14

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.[32]



Nojirimycin **1-Deoxinojirimycin**

Figure 1.15

Nojirimycin and 1-Deoxinojirimycin 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 1.16].

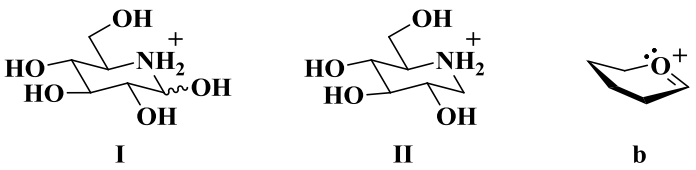


Figure 1.16

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** cannot 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 catalytic sites.

The shape, pattern of hydroxyl substitution and stereochemistry for iminosugars selectivity, albeit important, is not always easily predictable.[35] It was initially thought that inhibitors of glucosidases and mannosidases should have structures closely resembling glucose and mannose respectively. For example DNJ (**2**) has a structure similar to that of glucose and DMJ (**3**) has a structure similar to mannose, and indeed these compounds are known to inhibit α-glucosidase and α-mannosidase respectively, demonstrating that the differing stereochemical arrangement of the hydroxyl groups plays a key role in controlling inhibitory selectivity. This

argument often holds for glucosidase inhibitors, which typically do have structures with hydroxyl substitution patterns similar to glucose.[36] However, close mimicry between the structure of an inhibitory iminosugar and the enzyme's substrate is not always seen. As an example, (+)-Lentiginosine (**14**), has only two hydroxy groups positioned on the five-membered ring. Beside the presence of the basic nitrogen atom, other two features are essential for the bio-activity: the *trans* configuration of the two hydroxy groups and the *S,S* absolute configuration of the carbons bearing these groups (see section 1.7.1). In another example, 6-*epi*castanospermine (**25**) has a structure closely resembling the D-mannosyl cation (**26**), but was found to inhibit α- glucosidases and not the expected α-mannosidase.[37] With the exception of DMJ (**3**); α-D-mannosidase inhibitors in reality generally do not have structures that closely resemble mannose. For example, swainsonine (**11**) exhibits potent inhibition of α-mannosidases although its structural resemblance to α-D mannose is not obvious. Winkler and Holan were able to offer an explanation for these inconsistencies based on the results of molecular orbital calculations and structure-activity relationship studies with α-D-mannose analogues and mannosidase inhibitors.[38] These calculations and studies showed that there are two optimized half chair geometries of the mannosyl cation, a "flap up" form (**C1**) and the "flap down" form (**C2**), the former being the lower in energy [Figure 1.17]. This study showed that the best inhibitors resembled the "flap up" half chair geometry of the mannosyl cation. This model was also able to rationalize why 6-*epi*-castanospermine (**25**), which superficially resembles mannose, is actually a poor mannosidase inhibitor; it has the incorrect ring conformation for good superimposition onto the mannosyl cation in the "flap up" half chair conformation.[39]

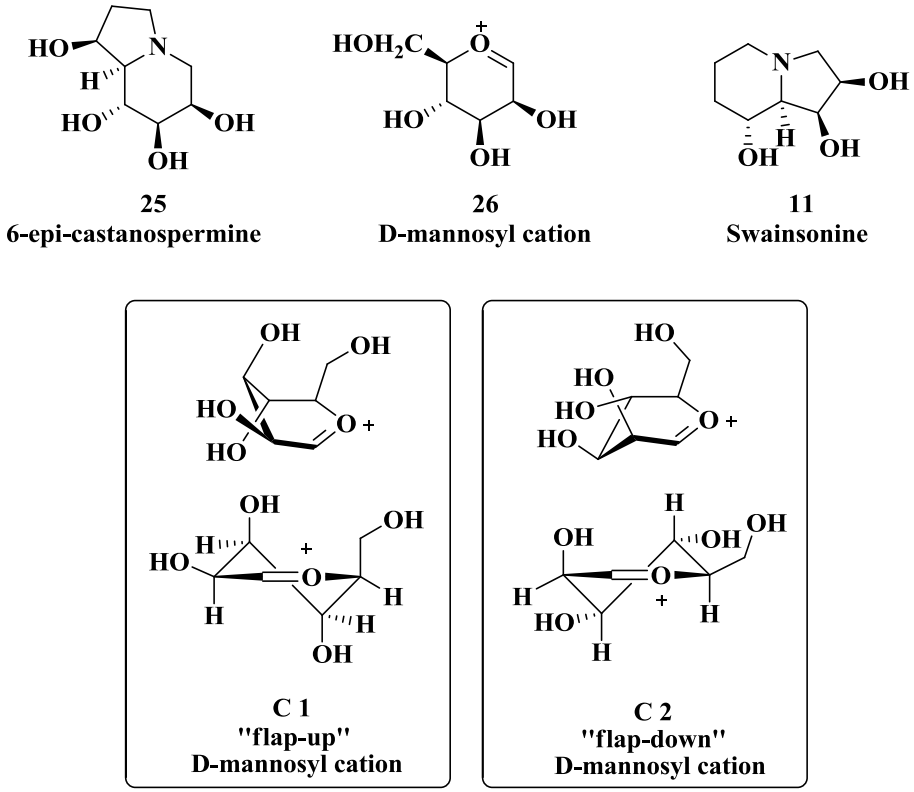


Figure 1.17 Structural configurations of D-mannosyl cations

Nevertheless, in many cases, further exploration is required to establish a proper understanding of structure activity relationships for carbohydrate processing enzymes. Thus, for example, the basis for selective mannosidase-inhibitory activity of protonated DNJ (**II**) remains unclear since this compound does not appear to closely mimic the structure of the "flap up" mannosyl cation (**26**). A possible explanation might be that binding in the mannosidase catalytic pocket is in this case dominated by ionic interactions between the protonated DNJ structure and the catalytic acidic residues lining the pocket.[40]

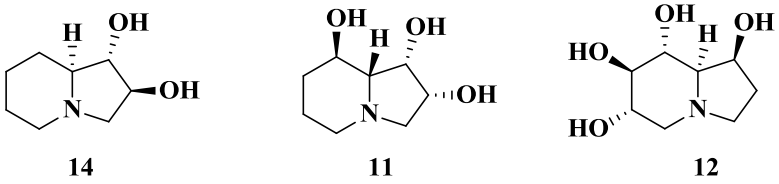
Polyhydroxylated pyrrolidine, piperidine and indolizidine alkaloids are able to inhibit the hydrolytic reaction.[41] The spatial distribution of the hydroxy groups of these compounds allows the formation of a series of hydrogen bonds inside the catalytic enzymatic cavity that

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.

Given the promising enzyme inhibitory activity of iminosugars in general, considerable effort has been made over the last three decades to develop efficient synthetic routes to these compounds. This work has been prompted both by the need to provide material for biological assessment and clarification of structure activity relationships and also in the hope that synthesis of new structures might provide compounds wth improved activity and selectivity.

1.5 Lentiginosine and its unnatural enantiomer

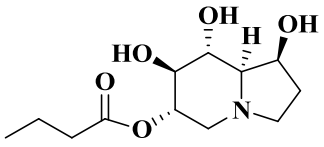
Iminosugars such as (+)-lentiginosine (**14**), (–)-swainsonine (**11**) and (+)-castanospermine (**12**) are polyhydroxylated alkaloids with an indolizidine structure [Figure 1.18].



14 11 12

Figure 1.18

Iminosugar has potential for the treatment of viral infection, [45] cancers[46] and diabetes.[47] Celgosivir,[48] a 6-butanoil derivative of castanospermine is already on the market as a drug against Gaucher's disease.

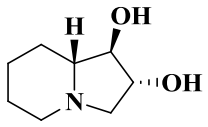


Celgosivir

(+)-Lentiginosine [(1*S*,2*S*,8a*S*)-octahydro-1,2-indolizinediol] (**14**) shown selective inhibitory activity against amyloglucosidase, higher than that of castanospermine.[49] Indeed, enzymatic tests carried out on natural (+)-lentiginosine **14** showed that it has inhibitory activity against *Aspergillus niger* amyloglucosidases with an half maximal inhibitory concentration (IC_{50}) equal to 5 μg/mL (amyloglucosidase from *Aspergillus niger*), synthetic (+)-Lentiginosine **14** 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 (+)-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).[49c, 65] 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.[50] In deseases such as chronic pancreatic tumors[51] or breast cancer[52] is observed an enhanced Hsp 90 expression. The unnatural enantiomer of (+)-lentiginosine (**14**) is (–)-lentiginosine (**27**) [Figure 1.19].[53]



27

Figure 1.19

This compound has an activity profile very different with 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.[54] Experiments performed on healthy donors and tumoral cell cultures, showed that (–)-lentiginosine (**27**), when present in concentration in the order of 200 μM, possesses a proapoptotic activity but, at the same time, has a cytotoxicity clearly lower in respect to that of a chemotherapic agent such as SN38 (7-ethyl-10-hydroxycamptothecin) [Table1, Figure 1.20].

Table 1

Cell lines	Compounds	$MAIC_{50}$± SD (μM) [a]
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 (humancolorectal adenocarcinoma cells)	(–)-lentiginosine	577 ± 101.3
	(+)-lentiginosine	>1000
	SN38	<1
PBMCs (healthy peripheral blood mononuclear cells)	(–)-lentiginosine	384.52 ± 49.02
	(–)-lentiginosine	>1000
	SN38	<1

a) $MAIC_{50}$ Value = Metabolic activity cytotoxic inhibitory concentration 50%

Figure 1.20 Effect of compound **14, 27** and **SN38** on cell viability in tumour cell line.

For the experimental tests four cell lines were used. In the table, the levels of cytotoxicity of (–)-lentiginosine, (+)-lentiginosine and SN38 are compared. The cytotoxicity 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 1.21] is showed a comparison of the proapoptotic activity of SN38, (–)-lentiginosine (**27**) and (+)-lentiginosine (**14**) on the four cell lines. The activity is expressed as % of hypodiploid nuclei.

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 caspases (*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.

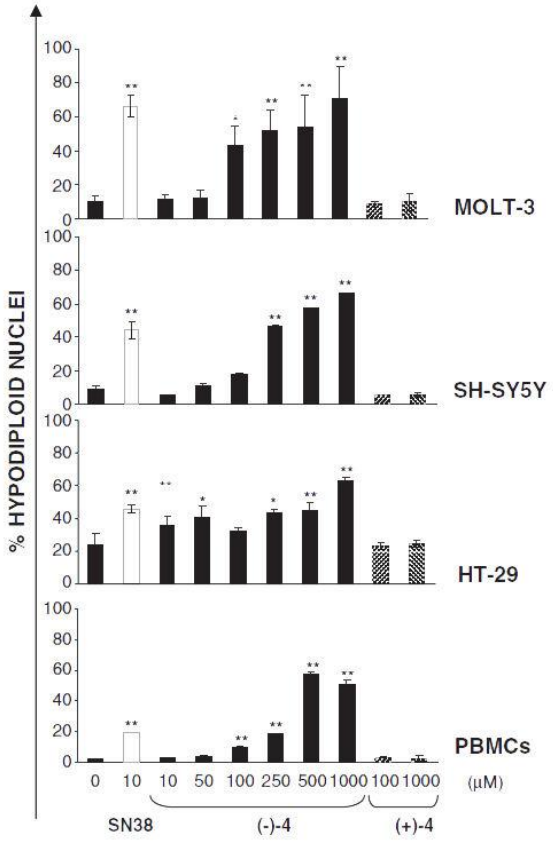
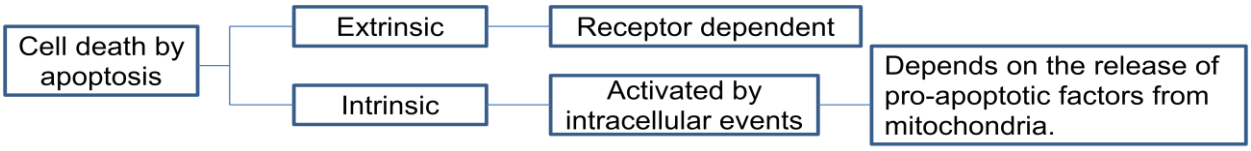


Figure 1.21

Apoptosis induced by D-(–)-lentiginosine in MOLT-3, HT-29 and SH-SY5Y tumour cell line. The results showed that D-(–)-lentiginosine increased caspase 9 expression at 18 h in all the cell lines from 1.5–3.1 folds. Cytochrome-C in the cytoplasm was found to be increased from 2.3–2.6 folds in treated cells with respect to control cells. These effects were accompanied by a remarkable collapse of the mitochondrial membrane potential and by the down regulation of anti-apoptotic genes, as well as the up regulation of pro-apoptotic genes of the Bcl-2 family. Thus, the study establishes that the enantiomer of a natural iminosugar is endowed with a possible anti-tumorigenic effect that might be ascribed not only to its capacity to inhibit glycosidases, but also to other unknown mechanisms. These data encourage further investigation on similar compounds to make them an interesting platform for the generation of new anticancer drugs.

Cancer cell death by apoptosis occurs through two types of mechanism a) Extrinsic or b) Intrinsic.



D-(–)-lentiginosine induced apoptosis involves the intrinsic pathway. [Figure 1.22] The expression of **caspase 9** (a) was assessed by western blot analysis after 3 and 18 h of treatment [Figure 1.22].

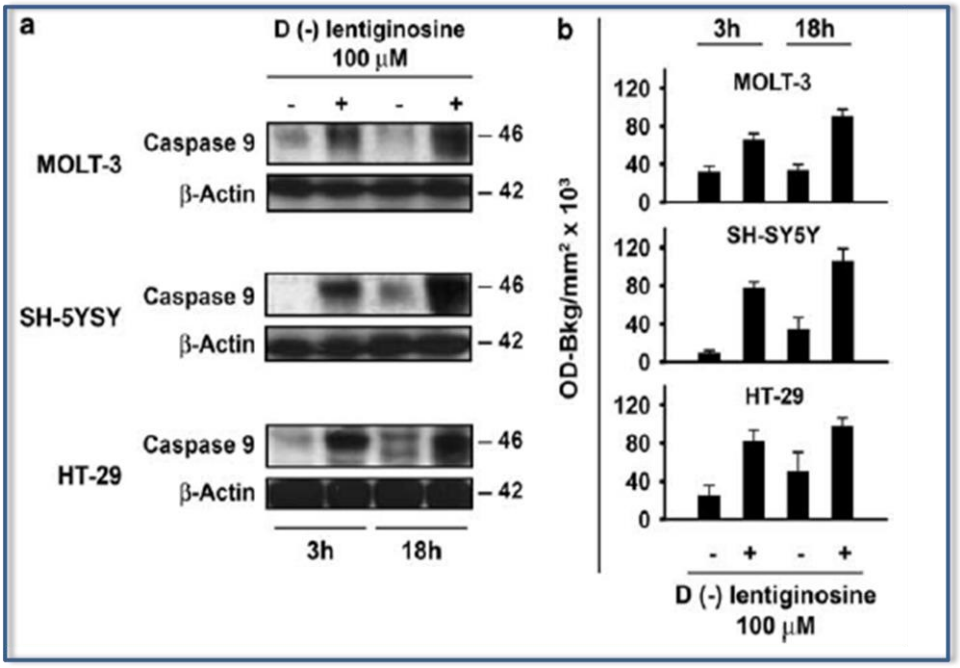


Figure 1.22 Effect of Cytochrome-C release, measured on the treatment of MOLT-3, SH-SY5Y and HT-29 cells with D(–) lentiginosine at 100 μM for 3 and 18 h.

Cytochrome-C production was assayed by enzyme-linked immunosorbent assay (ELISA) and the results are shown in [Figure 1.23]. The results are the cumulative mean values of three independent experiments. After 3 h of treatment, a noticeable, but not significant, increase in Cytochrome-C occurred in all the three cell lines. After 18 h of treatment the release of Cytochrome-C was highly significantly increased by 2.3 folds in MOLT-3 and HT-29 cells and by 2.6 folds in SH-SY5Y cells, respectively, in comparison with respective control cells.

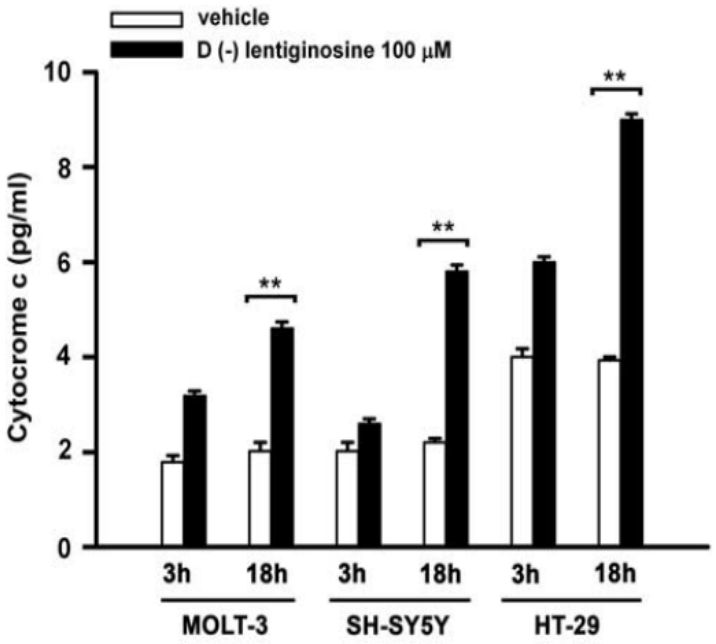


Figure 1.23 The data are represented as mean values±S.D. of three independent experiments. Asterisks (**P <0.001) indicate highly significant differences between treated and control cells.

1.6 Aim of the thesis

Owing to the very interesting and broad biological profile 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 aromatic and heteroaromatic structures, hydrophilic and hydrophobic functionalities.

A series of derivatives of the lentiginosine have been synthesized with the aim to define and optimize very simple, efficient and versatile synthetic methods. 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, besides the finding of new more potent proapoptotic agents, to the study of its mechanism of action, in particular to find its exact target.

1.6.1 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).[55] This enzyme cleaves the α-1,4-glucosidic bond preferentially and, at a slower rate, the α-1,6-glucosidic bond.[53] Glucoamylase is inhibited by sugar analogues having a structure which resembles that of the enzyme's natural substrate, amylose in this case.

In [Figure 1.24][56] are compared the natural glucoamylase substrate amylose, (+)-lentiginosine (**14**) and 1-deoxynojirimycin (**DNJ**), maybe the most investigated iminosugar.[57]

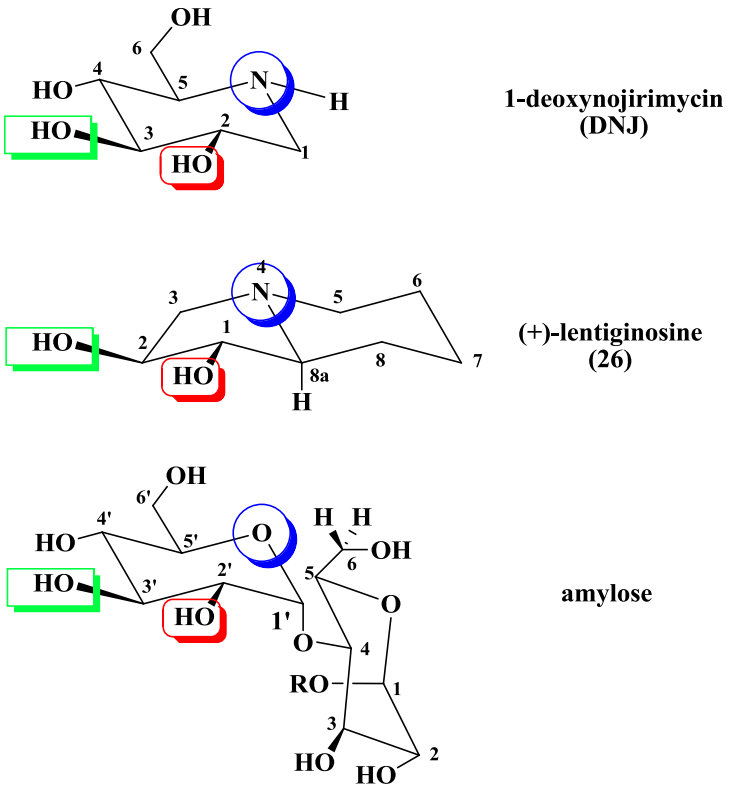


Figure 1.24

As we already said, one of the most important features of iminosugars is that they have a nitrogen atom instead of the oxygen atom present in the sugar structure. In contrast of **DNJ** and of natural substrate amylose, (+)-lentiginosine (**14**) 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 analogy of (+)-lentiginosine with the natural substrate is not immediate. But, if we observe [Figure 1.24], 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 possess a hydroxy function at C-2 or that have a *cis*-dihydroxypyrrolidine unit are inactive.[55,

[58] Furthermore, (–)-lentiginosine, which has an *R,R* absolute configuration on the C-1 and C-2 carbons, is 35 times less active than (+)-lentiginosine.[53]

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).[59] Before performing the computational studies, an evaluation of the protonation state was carried out (pH 7± 0.2).

In [Figure 1.25] is shown the catalytic site of the enzyme.

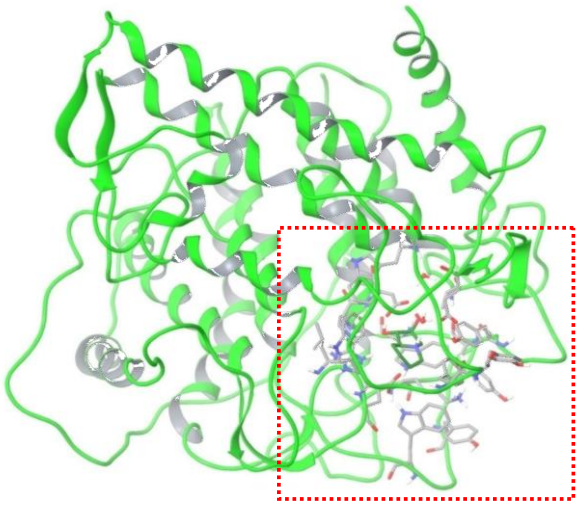


Figure 1.25

In [Figure 1.26] is illustrated the interaction model between the enzyme and (+)-lentiginosine.

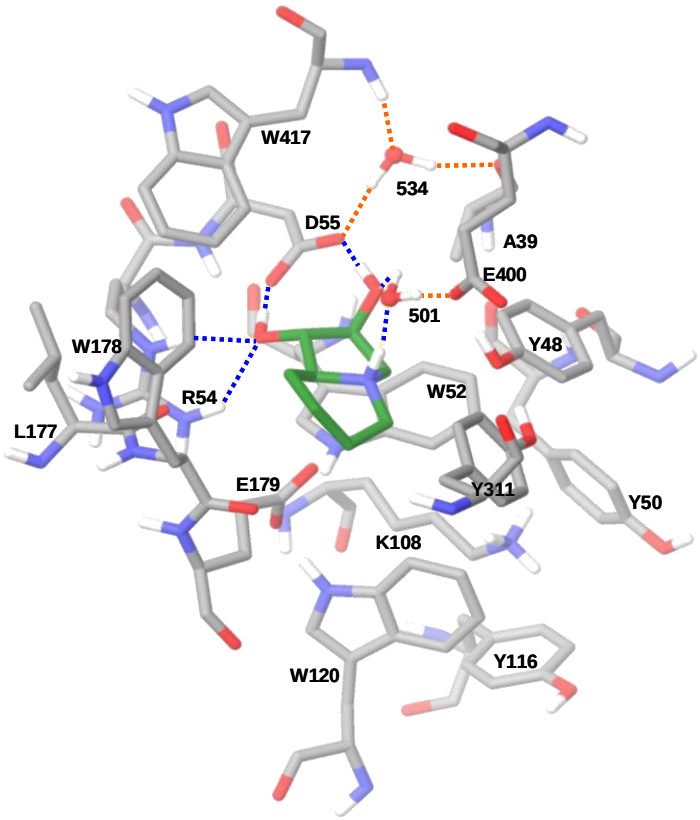
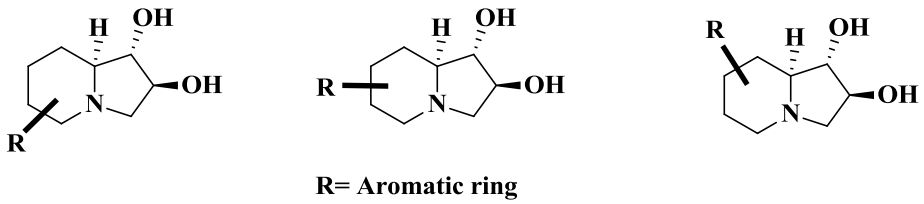


Figure 1.26

As we can see [Figure1.26], the two hydroxy groups establish two fundamental hydrogen bonds with two amino acid 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 moiety 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, 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 introduction of new functionalities on the lentiginosine structure.

Computational docking studies with glucoamylase suggested that an **aromatic ring** fused to the six-member ring (+)-**lentiginosine** could be favorably accommodated in the enzyme cavity and increase the affinity of the ligand towards the enzyme. [Figure 1.27]



R= Aromatic ring

Figure 1.27

1.6.2 Synthetic Targets

Computational docking studies have been performed by Prof. Paola Gratteri, university of Florence, on the basis of these studies following synthetic targets were designed and synthesized from L- and D- tartaric acids [Figure 1.28].

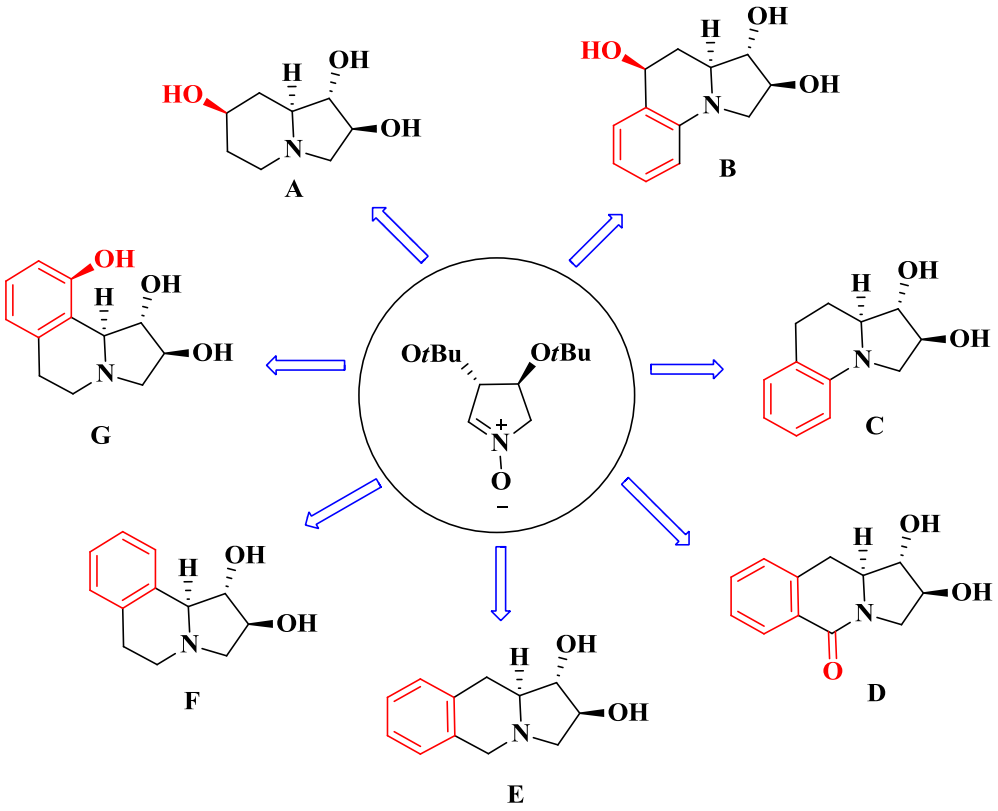


Figure 1.28

The computational *docking* studies of our synthetic targets **B** are illustrated in [Figure 1.29].

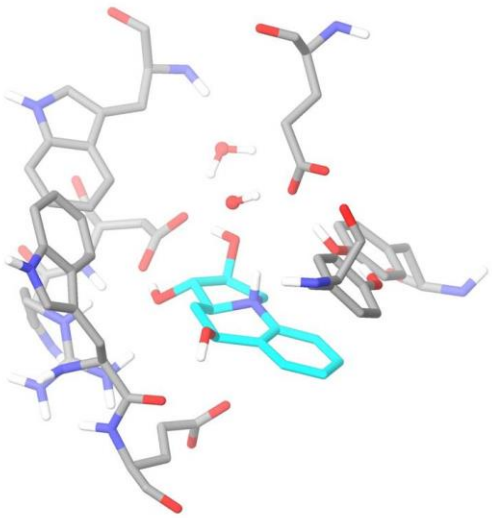


Figure 1.29

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.

1.6.3 Importance of aromatic ring[60]

- Benzene ring (or a phenyl group) is a neutral moiety its presence in molecule does not affect its *pKa.*
- In spite of its low H:C ratio, these rings confer exceptional chemical stability compared with simple unsaturated congeners *(e.g*. the cycloalkene or the open chain hexatriene).
- The high thermodynamic and chemical stability of the system is attributed to the delocalization of p-orbitals containing 6π electrons (resonance stabilization of a conjugated cyclic triene).
- Effect on the Polar surface area (PSA).
- Effect on the clogP
    a) +ve value of clogP indicate the substituent makes phenyl ring more lipophilic and increases the logP of the molecule.
    b) –ve value of clogP indicates the substituent makes the molecule more polar by decreasing its lipophilicity.
- Functional group modification has effect on the biological activity and Metabolism.

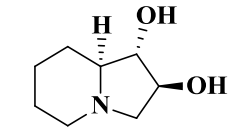
1.6.4 Medicinal chemistry aspects of aromatic ring[61, 62]

- Aromatic ring preferred for replacements of linear and branched alkyl/ cyclo alkyl groups.
- Aromatic ring gives rigidity to the molecule and potentially increases the non-covalent interaction.
- Involvement in π-π stacking, O-H/π and cation- π interactions.
- Drug design perspective: aromatic rings and their functionalized counterparts can be considered as versatile pharmacophores that increase these individual non-bonding and electrostatic interactions between the ligand and macro-molecules. Hence, incorporation of these rings into a molecule can improve the binding affinity and therefore the potency of the drug increased.
- The presence of an aromatic ring in a molecule provides a platform for functionalizations of a compound. It serves as a core that helps to orient other groups in a structure in the right direction and therefore enhance the interaction with functionalities in the receptor.
- An introduction of phenyl group into a molecule addresses important physicochemical property such as lipophilicity and therefore influences the absorption, distribution and clearance (excretion and metabolism) properties of a compound.

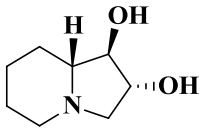
Revisited synthesis of 3,4-bis-*tert*-butoxypyrroline *N*-oxide and [(–)-7*S*-OH-Lentiginosine]: synthesis and proapoptotic activity

2.1 Introduction

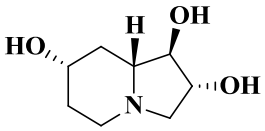
Among the natural Indolizidine iminosugars, (+)-lentiginosine[63] is one of the more recently discovered, but has already attracted large interest because of the potent glycosidase inhibition activity despite of its simple dihydroxylated structure, and also because of the debate that arose about its absolute configuration[64] [Figure 2.1].



(+)-lentiginosine (14)



(-)-lentiginosine (27)



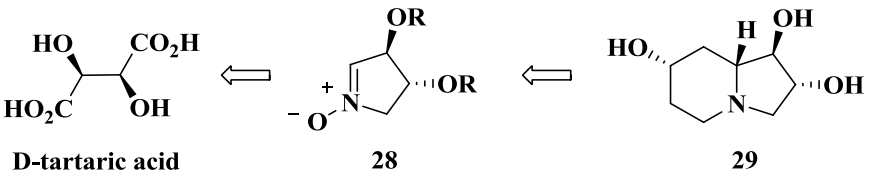
(-)-(1R,2R,7S,8aR)-1,2,7-trihydroxyindolizidine
[(-)-7S-OH-lentiginosine, 29]

Figure 2.1

Our long-lasting interest on (+)-lentiginosine **14** has reinforced recently with the discovery of a potent alternative bioactivity of this compound as potent inhibitor of the HSP90 protein.[65] Moreover, very recently it was found that the enantiomeric (–)-lentiginosine **27** is a potent proapoptotic agent against different strains of cancer cells, but with very low cytotoxicity.[66] In search of new candidates which display the same bioactivity, we decided to examine another indolizidine, the (–)-(1*R*,2*R*,7*S*,8a*R*)-1,2,7-trihydroxyindolizidine [(–)-7*S*-OH-lentiginosine, **29**] as a proapoptotic agent. The enantiomer of **29** is a non-natural compound, in its protected form, is an advanced intermediate in two syntheses of lentiginosine,[67] and has been employed

to produce lentiginosine conjugates.[68] The important biological profile of these compounds in both enantiomeric forms suggested, at first, the improvement of the synthesis of **29** to render it more available for biological tests and other applications. We report here a revisited synthesis of (–)-7*S*-OH-lentiginosine **29**, which benefits of a novel efficient and practical approach to the key intermediate 3,4-bis-*tert*-butoxypyrroline *N*-oxide **28**, and of a convergent and cascade version of the previous synthesis of the indolizidine skeleton. The effects of **29** on cell viability and apoptosis of cell lines of different origin are, then, described.

In this chapter, is described the synthesis of 7*S*-OH-lentiginosine (**29**) starting from D-tartaric acid through a seven step strategy based on diastereoselective 1,3 dipolar cycloaddition of 3,4-bis-*tert*-butoxypyrroline *N*-oxide **28** (Scheme 2.1). Analogously, the enantiomer (7*R*-OH-lentiginosine) has been obtained from L-tartaric acid following same sequence.[67,69]



Scheme 2.1

2.2 Nitrone

Nitrones are *N*-oxides of an imine and a functional group in organic chemistry. The general structure is $R_1R_2C=NR_3^+O^-$, where R_3 is different from H. Nitrones are very reactive species: they exhibit a broad reactivity profile and are recognized as versatile synthetic intermediates due to their ability to undergo numerous useful reactions [Figure 2.2].[70-75]

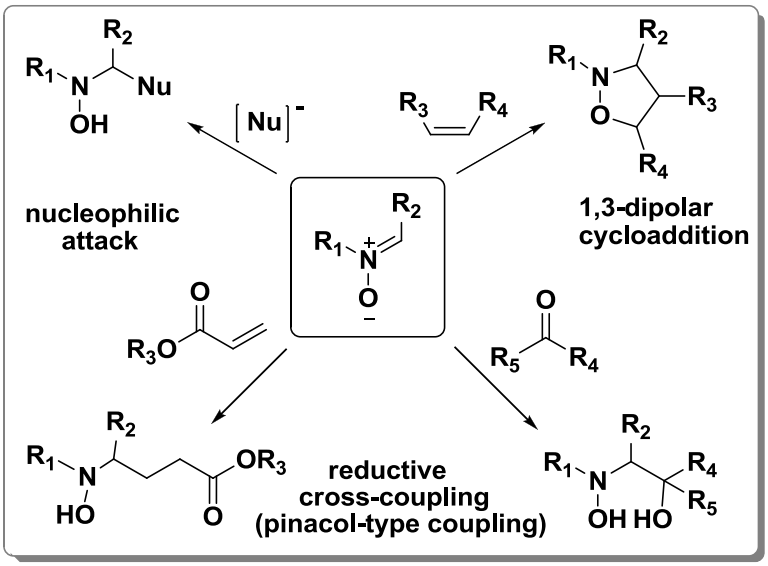


Figure 2.2

2.2.1 1, 3 dipolar cycloaddition mechanism

The nitrone-alkene 1,3-dipolar cycloaddition is useful method for obtaining isoxazolidine ring systems, with highly selective regio- and stereocontrol in a single [Figure 2.3].[76]

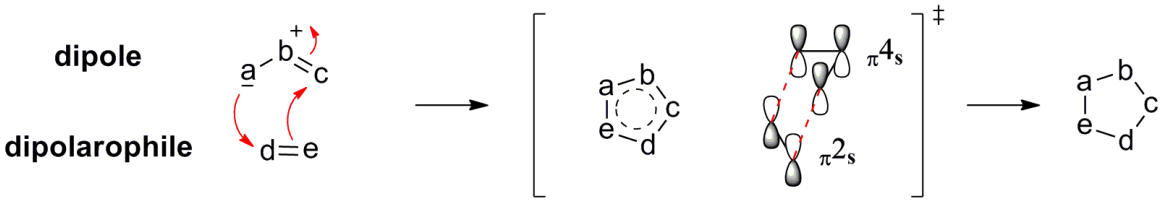


Figure 2.3

It is possible to generate up to 3 new stereocenters using this 1,3-dipolar cycloaddition, and the isoxazolidine N-O bond can be reductively cleaved to afford synthetically versatile amino alcohols. The nitrone 1,3-dipolar cycloaddition is best described as a concerted reaction between 2π and 4π electron addends.[77] As with the Diels-Alder reaction, the thermally allowed

1,3-dipolar cycloaddition is a suprafacial [π2s+π4s] process. Regio- and stereocontrol in nitrone 1,3-dipolar cycloadditions have been reviewed in detail in the literature[76, 78] and the scope of the following discussion will therefore be limited to 1,3- dipolar cycloadditions of cyclic nitrones with alkenes.

In 1,3-dipolar cycloadditions different substituents on the dipole do not vary the cycloaddition rate. Moreover, there is no solvent effect, i.e. the solvent polarity has a little effect on cycloaddition rate. Stereochemistry of 1,3-dipolar cycloaddition reactions is always stereospecific with respect to dipolarophile, supporting the concerted pericyclic reaction mechanism. Cycloadditions experience a large negative entropy of activation, therefore transition states are highly ordered.

2.2.2 1,3 dipolar cycloaddition: regiochemistry and stereochemistry

There can be two regiochemical outcomes for the cycloaddition of a cyclic nitrone with a substituted alkene. In principle, a C-4 or C-5 substituted isoxazolidine can be obtained [Figure 2.4]. However, it has been shown that steric and electronic factors conspire to control the regioselectivity of the reaction which substantially favour a 5-substituted isoxazolidine product from alkenes bearing both electron donating and electron withdrawing substituent's.[79]

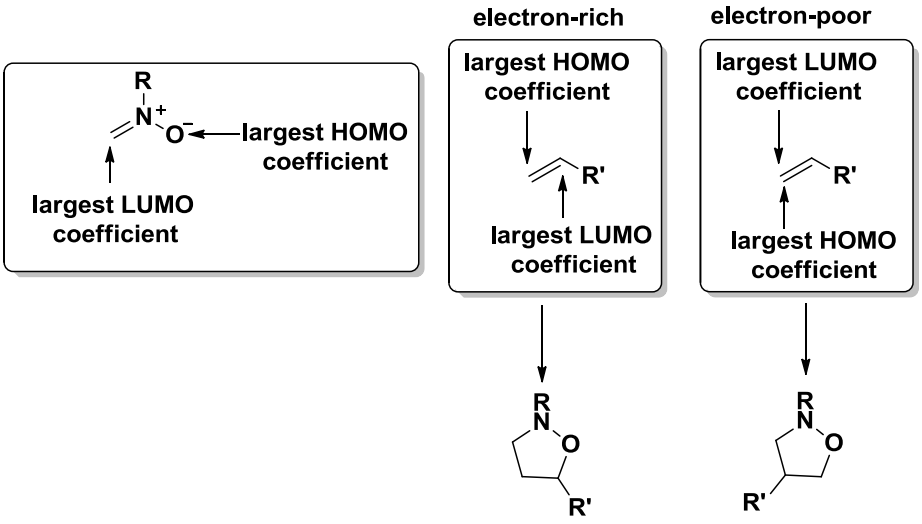
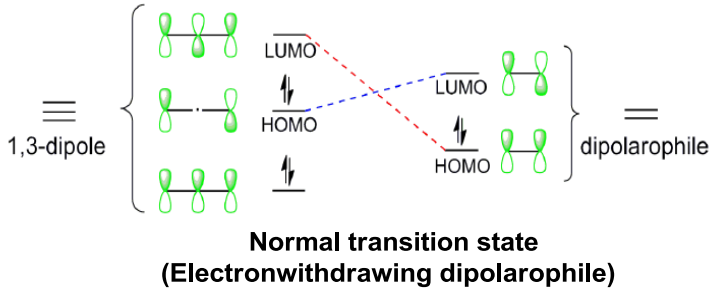


Figure 2.4 Regiochemical outcomes of 1,3-dipolar cycloaddition

As regards the electronic control of nitrone cycloaddition, regiochemistry is typically rationalized by analysis of frontier molecular orbital interactions, where maximum orbital overlap contributing to the transition state involves interaction of the most energetically proximal HOMO-LUMO pair combination [Figure 2.5], and determines the reaction outcome. For nitrone-alkene cycloadditions, both sets of interactions favour a 5-substituted isoxazolidine product except in the case of alkenes carrying the most electron demanding of substituents (such as nitroethylene).[80] Reactions of monosubstituted alkenes with 5- membered ring nitrones, such as 1-pyrroline-*N*-oxide, behave accordingly and 5-substituted isoxazolidine are predominantly formed. [81]



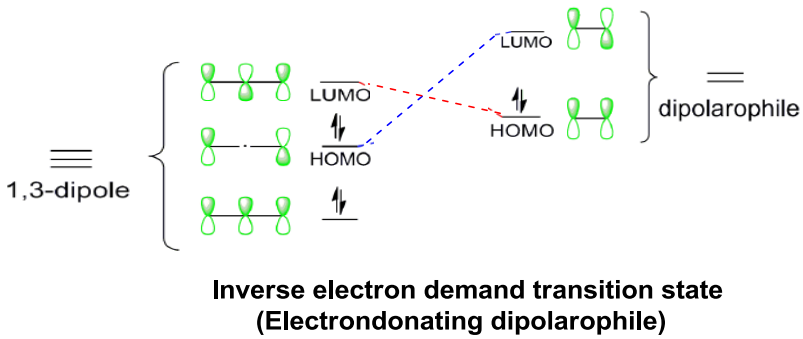


Figure 2.5 Frontier molecular orbital interactions in 1,3-dipolar cycloadditions

The 1,3-dipolar cycloaddition of a cyclic nitrone with a mono-substituted alkene can proceed with formation of two new stereogenic centers at C-3 and C-5 (Isoxazolidine numberings), which can potentially yield four different diastereoisomers, when 5-substituted isoxazolidines are formed.[82] As cyclic nitrones do not have the capacity to exhibit *E/Z* isomerism, the stereocontrol in cycloadditions of these nitrones is typically higher than for their acyclic counterparts. It is the dipolarophile orientation (*endo* or *exo*) and direction of approach (*anti* or *syn*) towards the 1,3-dipole that determines the product stereochemistry. The *endo*/*exo* selectivity of cycloaddition reactions can be influenced by the interactions of secondary π orbitals [Figure 2.6].[83] In Diels-Alder reaction, the *endo* isomer is usually dominant with dienophiles carrying functionality that allows formation of favourable secondary orbital interactions in the transition state. In 1,3-dipolar cycloadditions of nitrones, the interaction of the *N*-nitrone p_z orbital with a vicinal p_z orbital is small.[84] Therefore, unlike Diels-Alder reactions, the *endo/exo* selectivity in nitrone cycloadditions is largely controlled by steric factors, and hindrance between the nitrone and alkene substituents disfavours the *endo* transition state. The 1,3-dipolar cycloaddition of cyclic nitrones generally occurs *via* an *exo* transition state therefore.

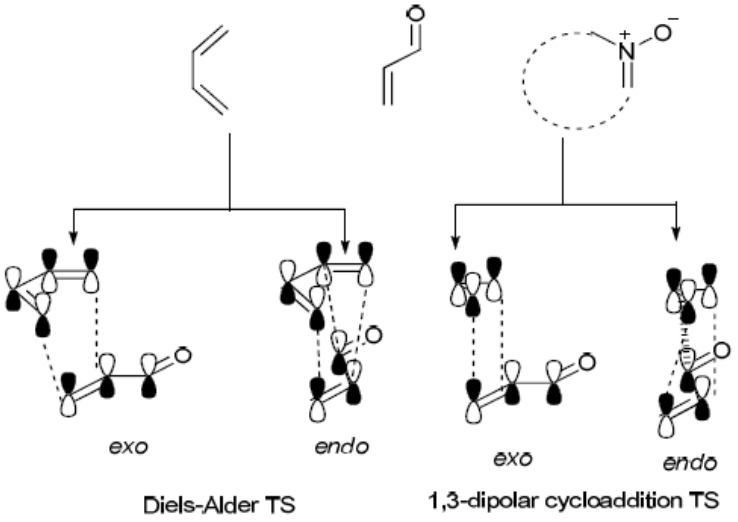


Figure 2.6 Orbital interactions of *exo* and *endo* approach

The stereochemical outcome of the cycloaddition is further complicated for cyclic nitrones bearing an existing stereogenic feature in the form of a substituent at one of the ring sp^3 carbons. In this case, cycloaddition with a mono-substituted alkene may proceed to give a 5-substituted isoxazolidine product through four possible transition state combinations [Figure 2.7] and *anti/syn* selectivity, determined by the face from which the alkene preferentially attacks the cyclic nitrone, also has to be considered. This selectivity is usually determined by steric factors, with the dipolarophile attacking from the least sterically demanding *anti* face of the cyclic nitrone. The most favourable transition state for a 1,3-dipolar cycloaddition of this type is therefore generally *exo-anti*, and this has been observed by many groups.[85,86,87] Minor products resulting from the *endo-anti* and *exo-syn* are often seen, whereas the highly sterically demanding *endo-syn* product is rarely observed.

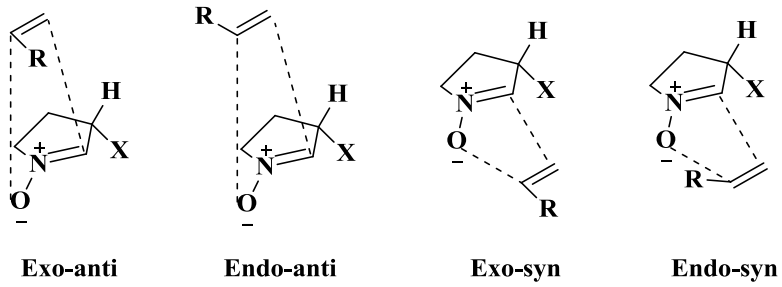


Figure 2.7 Possible approaches of dipolarophile in 1,3-dipolar cycloaddition.

2.2.3 Cyclic Nitrone (3,4-bis-*tert*-butoxypyrroline *N*-oxide)

Cyclic nitrone (3, 4-bis-*tert*-butoxypyrroline *N*-oxide) **28** has been synthesized in the group of Prof. A. Brandi in both the enantiomeric forms starting from the easily available chiral pool compounds L- and D-tartaric acids. [Figure 2.8][68a, 89,100]

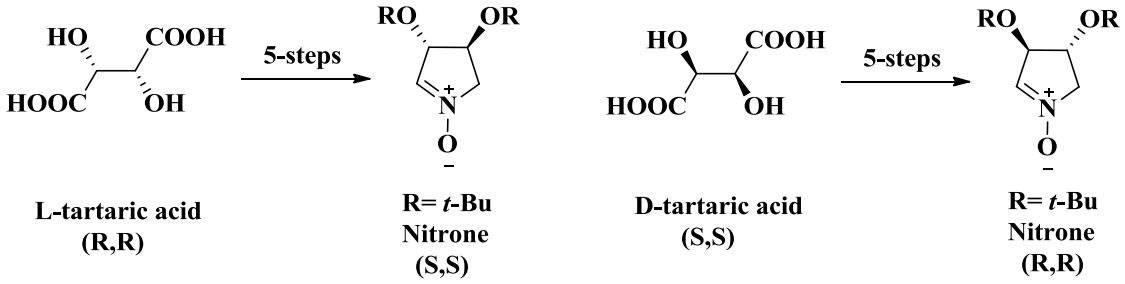


Figure 2.8

2.3 Result and discussion

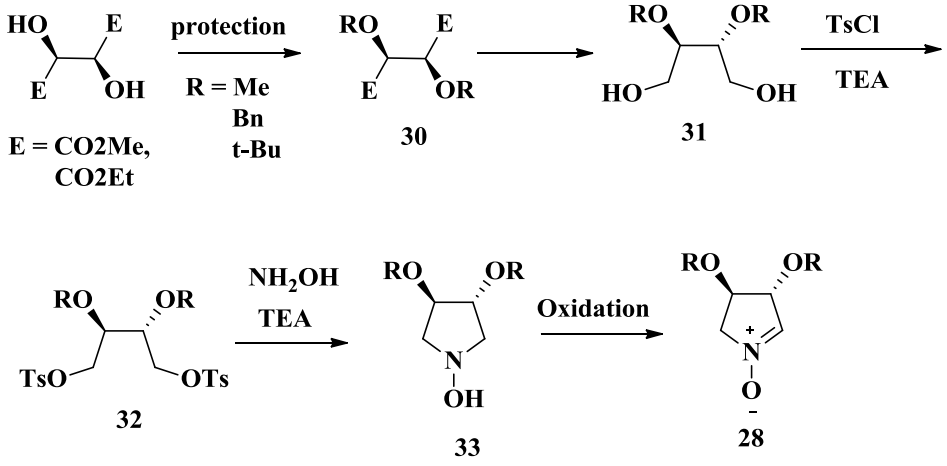
2.3.1 Synthesis of 3, 4-bis-*tert*-butoxypyrroline *N*-oxide (28)

In literature, there are some reports to synthesis of different cyclic or acyclic nitrones with different methods. Synthesis of 3-4-bis-methoxymethylpyrroline *N*-oxide was earlier reported by Prof. Petrini, University of Camerino, Italy starting from L-tartaric acid.[90b] Synthesis of 3-4-bis-O -protected pyrroline *N*-oxide (**28**) with several protecting groups have been used later on to mask the two *trans*-related hydroxyl moieties (Me, Bn, *t*-But, MOM, TBDPS, TBDMS,

and Bz) of easily available chiral pool compounds L- and D-tartaric acids. The appropriate protecting group was chosen on the basis of the desired final product and the nature of the reaction necessary to obtain it. Among them the most used is the *tert*-butyl group[91] because of its high stability under many different reaction conditions, it's easy and traceless hydrolysis, and its bulkiness that can induce superior diastereofacial control compared to other smaller protecting groups. Two general and complementary approaches use to synthesize nitrone **28** are commonly employed (Scheme 2.2).[90, 92]

In one approach, a tartaric ester is firstly protected then reduced to the diprotected tetrol **31**. The activation of the primary hydroxyl group via mesylation or tosylation **32** followed by cyclization with hydroxylamine afford the *N*-hydroxypyrrolidine **33** that can be oxidized to **28** by reagents such as HgO,[91] NaOCl[93] and MnO_2[94] (Scheme 2.2).

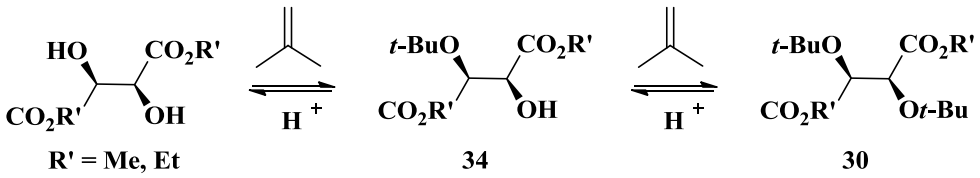
In this approach, the overall yield 25-35% over 5 steps.



Scheme 2.2[91-93]

Tert-Butylation of tartaric ester is achieved with isobutene catalyzed by acid. The reaction can be carried out by bubbling isobutene through an acidic tartaric ester solution or by condensing

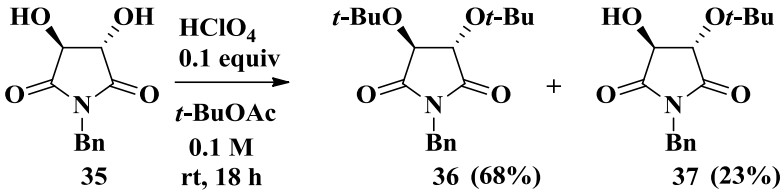
the gas at low temperature in a high pressure vessel, then running the reaction at room temperature for 2 days. Under these conditions, the di-*tert*-butyl ether of dimethyl tartrate **30** (R'=Me) was obtained in 39% yield in addition to the monoester **34** (R'=Me) in 23% yield.[95] This reaction, however, is not easily reproducible, the tricky step being neutralization of the reaction mixture, because in absence of a large amount of isobutene even a trace of acid can quickly shift the equilibrium from **30** towards the monoprotected diol **34** (Scheme 2.3).



Scheme 2.3

Recently, Occhiato et al,[96] reported *tert*-butylation of primary and secondary alcohols by using *tert*-butyl acetate in the presence of catalytic $HClO_4$ at room temperature. Under these very practical conditions, dimethyl tartaric acid afforded the di- and mono-*tert*-butyl ether in 43% and 41% yields respectively, after 44 h. The major drawback was the need of a very high diluted solution (0.0025M) to form equimolar amount of the di- and monoether, otherwise, the monoether derivative was the principal product. Under the reported conditions, cyclic alcohols react with *tert*-butyl acetate better than the acyclic ones; tested this procedure on a cyclic derivative of tartaric acid such as imide **35. (**Scheme 2.4)

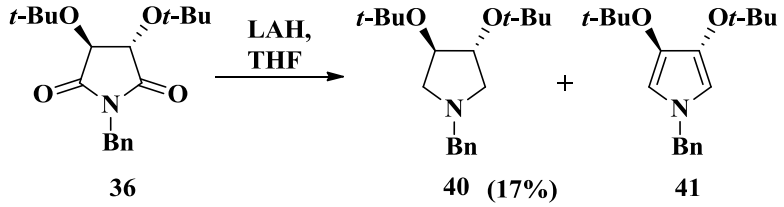
Benzylimide **35** was prepared by treating tartaric acid with benzylamine to get benzylammonium salt of tartaric acid as recently reported by Rosenberg.[97] Benzylammonium salt of tartaric acid was refluxed in xylene, with removal of water to obtain benzylimide **35**.



Scheme 2.4

A reaction performed on imide **35** in a 0.1 M solution of *t*-BuOAc in the presence of a catalytic amount of $HClO_4$ (0.1 eq) after 18 h afforded the ethers **36** and **37** in 3:1 ratio with 91% overall yield (Scheme 2.3). This excellent result of protection step convenience us to the feasibility of another approach towards synthesize 3-4-bis-*tert*-butoxypyrroline *N*-oxide (**28**).

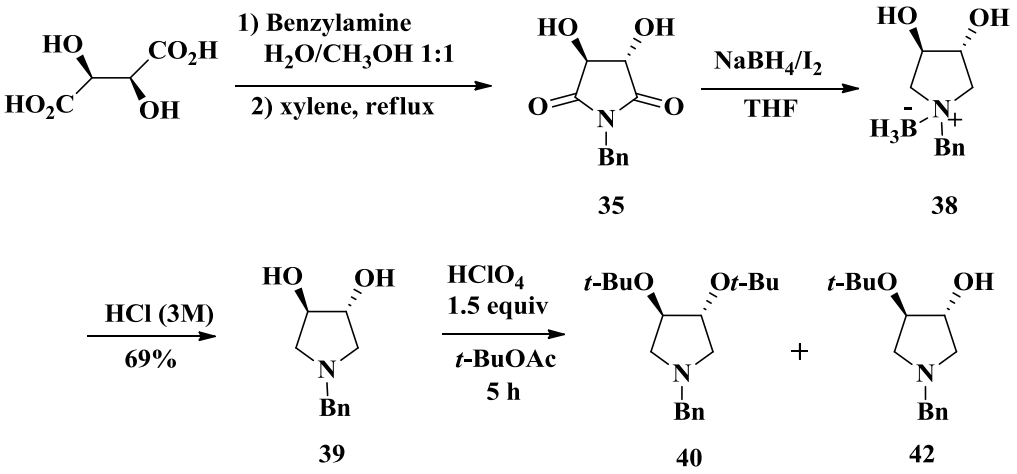
Unfortunately, reduction of imide **36** with $LiAlH_4$ in refluxing THF afforded the corresponding benzyl pyrrolidine **40** (R = *t*-Bu) in very low yield (17%), along with 1-benzyl-3,4-di-*tert*-butoxy-1*H*-pyrrole **41**(Scheme 2.5).



Scheme 2.5

The aromatization of other succinimide derivatives with $LiAlH_4$ was already reported, and could be avoided using milder reducing reagents such as borane.[99] However it was decided to first produce the N-benzylpyrrolidine **39** (scheme 2.6) then to carry out the protection. Benzylimide **35** was treated with $NaBH_4$ and I_2 at 0 °C under anhydrous condition, heated at the reflux temperature for 6 h afforded **38**. The borazine **38** was isolated and characterized. This compound does not have symmetry elements and the diastereotopic carbinolic protons 3-

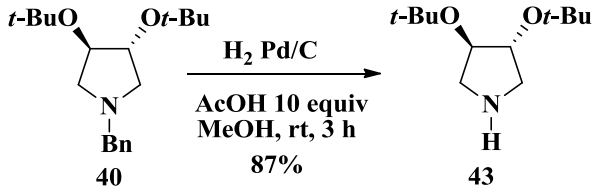
H and 4-H resonate at different frequencies. To remove borane borazine **38** was treated with HCl to get **39** as a white solid**.** The protection of dihydroxy etherification of the dihydroxy pyrrolidine **39**[68, 96] with *t*-BuOAc/$HClO_4$ resulted to be even more efficient affording a 5.6:1 mixture of di- and mono protected pyrrolidine **40** and **42**, respectively. Analogously to the previous case, the reaction could be run on gram scale (2.8 g of **39**) using a 0.1 M concentration with the exception of the use of an excess of $HClO_4$ (1.5 equiv), because of the presence of the basic nitrogen atom. Both the reagent/solvent *t*-BuOAc and the side product **42** could be recovered and reused. It should be stressed here that obtainment of **40** by the isobutene method was never possible in our hands. (Scheme 2.6)



Scheme 2.6

Unprecedented hydrogenolysis of the benzyl group in **40** was quite tricky. In the presence of a catalytic amount of Pd/C or $Pd(OH)_2/C$ and H_2 (1 Atm) in MeOH, debenzylation was slow and surprisingly a significant amount of 4-*tert*-butoxypyrrolidin-3-ol that is monodeprotection, was formed. Luckily, in the presence of AcOH (10 eq), debenzylation was complete after 2.5

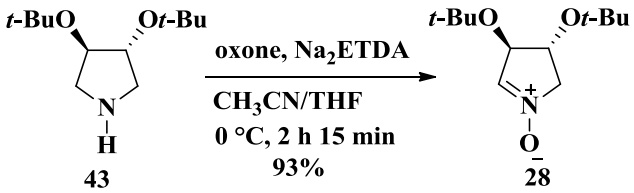
h and ether hydrolysis was suppressed (Scheme 2.7). After neutralization, **43** was obtained in 87% yield.



Scheme 2.7

The oxidation of the secondary amine **43**, has been accomplished with different reagents such as H_2O_2 or urea hydroperoxide catalyzed by SeO_2, Na_2WO_4, Na_2MoO_4 or MTO, alkyl hydroperoxide in the presence of a (trialkanolaminato)titanium(I) complex, or Oxone®.[68,98] The key oxidation of C_2 symmetrical pyrrolidine **43** was performed using oxone.[68, 98] The reaction was performed in a solvent system constituted by Na_2EDTA aqueous solution, CH_3CN and THF in 1:1:0.25 ration in presence of $NaHCO_3$ at 0 °C afforded 3,4-bis-*tert*-butoxypyrroline *N*-oxide (**28**) in 93% yield. Crude nitrone **28** was obtained sufficiently pure to be used in the next step without further purification. Starting from L-tartaric acid the enantiomeric nitrone *ent*-(+)-**28** was prepared following the same procedure **(**Scheme 2.8).

In this approach, the overall yield is 46% over 5 steps.[100]



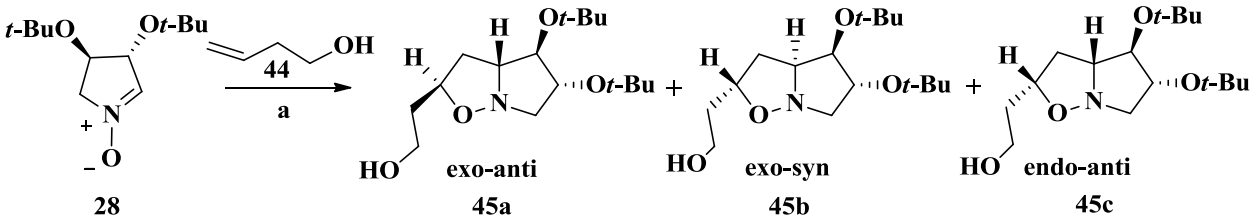
Scheme 2.8

2.3.2 Synthesis of (-)-(1*R*-2*R*-7*S*-*8aR*)-1, 2, 7-trihydroxyindolizidine [(-)-7*S*-OH-lentiginosine]

In this chapter, we are going to discuss the synthesis (–)-7*S*-OH-lentiginosine **(29)** carried out by the old approach as well as new approach. In literature, Prof. A. Brandi reported synthesis of 7*S*-OH-lentiginosine in 2000.[67c] We compared both approach towards synthesis of (–)-7*S*-OH-lentiginosine **(29)** transformed.

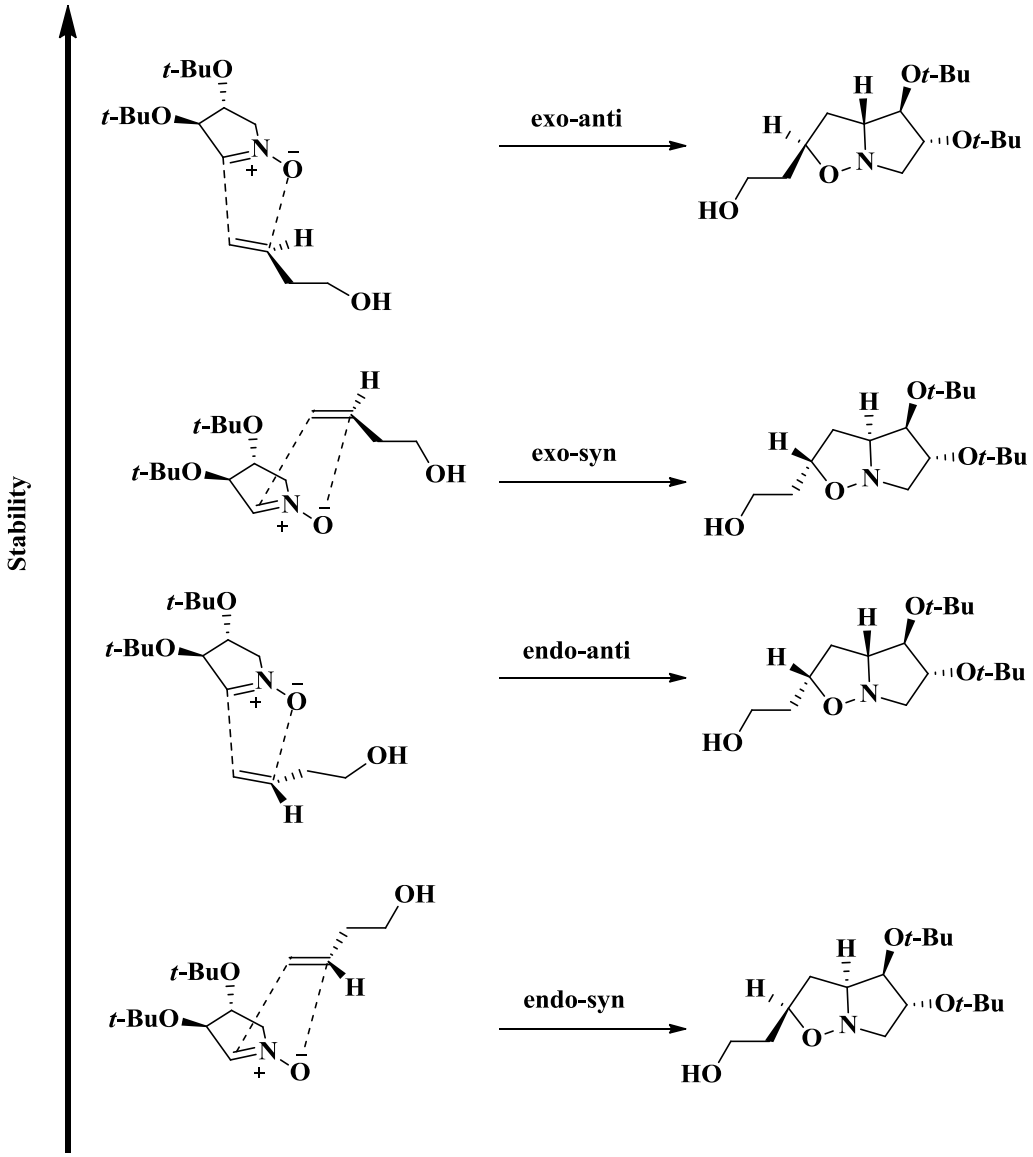
2.3.2.1 Old approach towards Synthesis of (-)-(1*R*-2*R*-7*S*-*8aR*)-1,2,7 trihydroxyindolizidine

In the old approach, synthesis of (–)-7*S*-OH-lentiginosine via 1,3-DC reaction of 3,4-bis-*tert*-butoxypyrroline *N*-oxide (**28**) with butenol **44** (5 eq) in benzene at 60 °C for two days afforded a mixture of diastereomeric cycloadducts **45a, 45b** and **45c** in 10:2:1 ratio with complete regioselectivity (Scheme 2.9).[67c] The major exo-[4-O*t*Bu] anti adduct **45a** was obtained in 74% yield after chromatography.



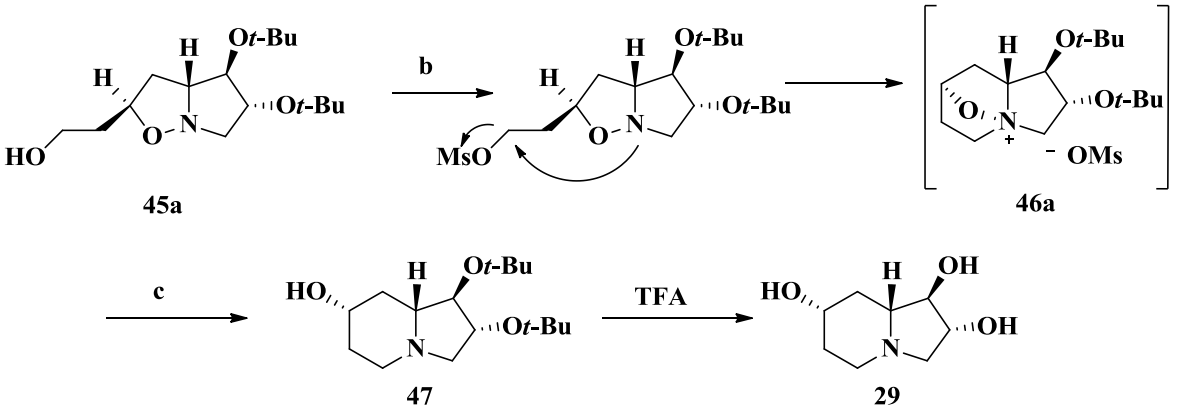
Scheme 2.9

The similar results could be obtained in shorter time at higher temperature. In particular at 100 °C in the presence of 5 equiv of butenol, nitrone was consumed in 1.5 hours. Adduct **45a** was isolated in 75% yield after chromatography on silica gel, and the ratio between the two main cycloadducts was ca. 5:1 as in the previous case.



Scheme 2.9 Possible approaches of dipolarophile in 1,3-dipolar cycloaddition

Possible approaches of 1,3-DC of dipolarophile with nitrone are described above (Scheme 2.10). The *exo-anti* approach is highly favored, because of the hindering effect of the ring and its substituents in an *endo* approach. *endo-syn* product is rarely observed.



Scheme 2.10

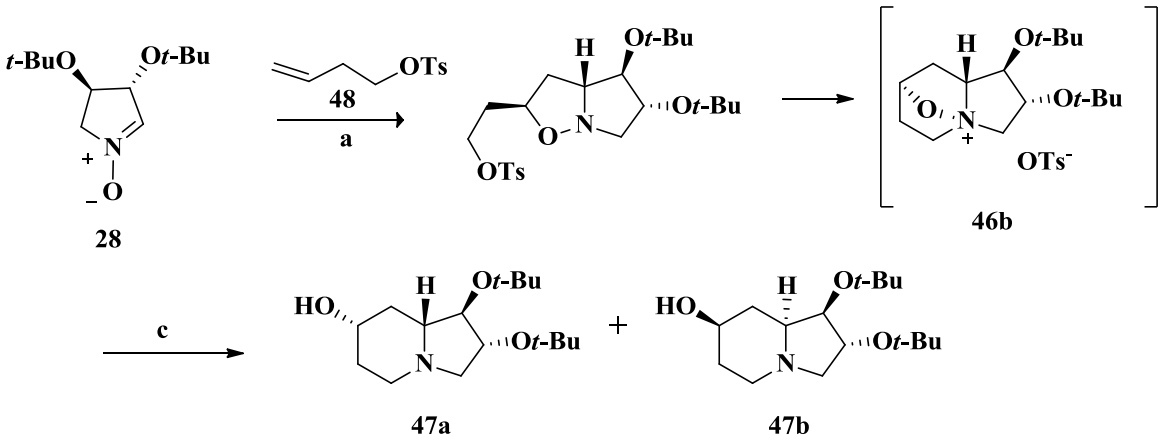
The cycloadduct **45a** was converted into indolizidine through activation of primary hydroxy group by mesylation and subsequent spontaneous cyclization to salt **46a**, that was reduced, without isolation, with H_2 in the presence of catalytic amount of Pd/C to obtain protected 7-OH-lentiginosine **47** [Table 2.1] . Final deprotection of **47** affords (–)-7*S*-OH-lentiginosine (**29**).

Table 2.1

Reagent and condition of 1,3-DC/Cyclization/N-O bond reduction sequence				
	Step a	**Step b**	**Step c**	**Yield of 47**
1	**44** (5 eq), C_6H_6, 60 °C, 2 days	TsCl, TEA, dry DCM	H_2, Pd/C, MeOH, 1 day	64%
2	**44** (5 eq), toluene, 100 °C, 1.5 h	MsCl, TEA, dry DCM	H_2, Pd/C, MeOH, 15 h	64%

2.3.2.2 New approach towards the Synthesis of (–)-(1*R*-2*R*-7*S*-*8aR*)- 1,2,7 trihydroxyindolizidine.[100]

In our new revised approach, another possible change to make the process more convergent was to use a dipolarophile with the hydroxyl functionality activated in advance towards the nucleophilic substitution, such as 3-butenyl tosylate **48**, and running a one pot three step reaction in a sealed Sovirel flask. The two-step domino process consisting of 1,3-DC/nucleophilic substitution was carried out, in the presence of an excess amount of dipolarophile **48** (4 equiv) at room temperature for three days, because of the thermal instability of salt **46b**. After 3 days, activated Zn, AcOH and Et_2O was added to the white slurry of the salt **46b** and the mixture kept at room temperature under stirring for one more day. Neutralization and purification by flash chromatography on silica gel afforded three diastereomeric indolizidines **47a** and **47b** in ca. 5:1:0.2 ratios (60%, 12% and 2% yield respectively) and the excess of dipolarophile **48** that was recovered in 95% yield in the very early chromatographic fractions (Scheme 2.11).[100]



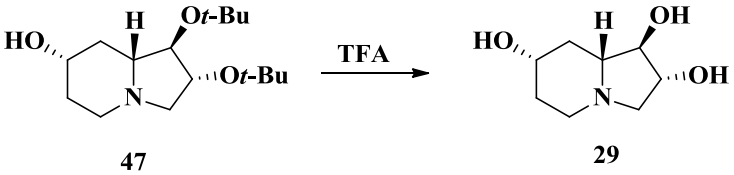
Scheme 2.11

Table 2.2

Reagent and condition of 1,3-DC/Cyclization/N-O bond reduction sequence				
	Step a	**Step b**	**Step c**	**Yield of 47a-c**
1	**48** (4 eq), RT, 3 days		Zn, AcOH, Et_2O	60%, 12% 2%

Reductive cleavage of the isoxazolidine N-O bond with H_2 over Pd/C afforded analogous results, except for the hydrogenation of the double bond of the excess dipolarophile that could not be recovered.

This one pot procedure has some advantages over the previous one. In particular, it is more convergent, is run at rt, the excess of dipolarophile can be easily recovered and reused, does not require anhydrous conditions and special handling, and the overall yield of **47** is similar to the previous ones (60% vs. 64%). The only drawback of this variation is the overall long reaction time (4 days). The same process was carried out starting from ***ent*-28** to obtain the enantiomeric ***ent*-29**.



47 29

Scheme 2.12

Eventually, deprotection of indolizidine **47** by treatment with TFA afforded pure triol **29** in 87% yields. (Scheme 2.12) In conclusion, the overall new process allowed a more straightforward access to (–)-7*S*-OH-lentiginosine **29** which is very promising in the light of its interesting biological activity.

2.4 Biological activity[100]

The effect of **29** on both cell viability and apoptosis of tumour cell lines of different origins was investigated by the group of Dr. Beatrice Macchi at the University of Tor Vergata, Rome. The cytotoxic effect of **29** was assayed towards MOLT-3, SH-SY5Y, HT-29, MCF- 7, and M-14 cell lines [Table 2.3]. HT-29 cells were slightly more sensitive. Conversely, the well-known cytotoxic chemotherapeutic agent SN38, used as a positive control, was highly toxic towards all the tested cell lines [Table 2.3].

Cell lines	Compounds	$MAIC_{50}$±SD(μM)
MOLT3 (human acute lymphoblastic T cells)	**(-)-lentiginosine**	**213.33 ± 96.62**
	(+)-lentiginosine	**>1000**
	(-)-7S-OH lentiginosine	**485±16.62**
	SN38	**14±2**
SHSY5Y (neuroblastoma cells)	**(-)-lentiginosine**	**95.5 ± 19.09**
	(+)-lentiginosine	**>1000**
	(-)-7S-OH lentiginosine	**396±9.09**
	SN38	**<0.1**
HT29 (human colorectal adenocarcinoma cells)	**(-)-lentiginosine**	**577 ± 101.3**
	(+)-lentiginosine	**>1000**
	(-)-7S-OH lentiginosine	**285±35.3**
	SN38	**<1**
PBMCs (peripheral blood mononuclear cells)	**(-)-lentiginosine**	**384.52 ± 49**
	(+)-lentiginosine	**>1000**
	SN38	**<1**

[a] **$MAIC_{50}$** value=metabolic activity cytotoxic inhibitory concentration 50%, **SD**=standard deviation.

Table 2.3. Effects of compounds **29** and **SN38** on viability, as assessed by the MTS assay in tumour cell lines.

It was important to verify, whether the effect of the tested compound on cell viability could be ascribed to the induction of cell death by apoptosis. To this aim, cell lines were treated for 24 hours with **29** at concentrations ranging from 1 to 10^3 µM. The results of apoptosis expressed as a percentage of hypodiploid nuclei (hypodiploid nuclei are nuclei that contain less number of chromosomes than normal cell nuclei) are shown in [Figure 2.9], the values being the mean±SD of three independent experiments. The results underline a dose effect response in the presence of a concentration of **29** from 1 to 10^3 µM in all the tested cell lines. However, the assayed cell lines exhibited different susceptibility to compound-induced apoptosis. The MOLT-3, MCF-7, and M-14 cells were highly susceptible to apoptosis induced by **29**, with differences in comparison with control cells that were significant at a concentration as low as 10 µM. Conversely, SH-SY5Y and HT-29 cells treated with **29** showed apoptosis levels that were significantly different from control cells at a concentration of 10^2 µM or higher. The reference positive control SN38 was effective in inducing apoptosis in all the cell lines assayed at a concentration of 10 µM, as expected.

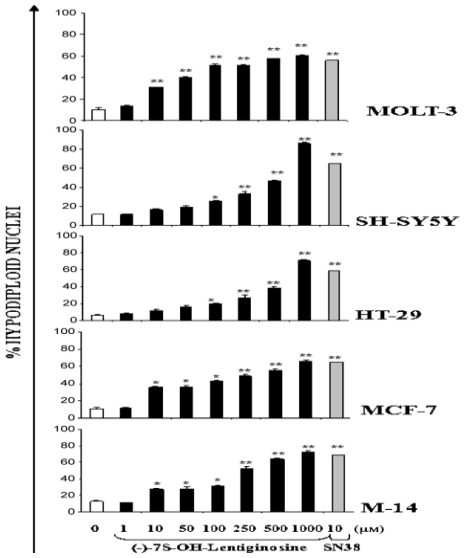


Figure 2.9 A) Effects of the compound (–)-7S-OH-lentiginosine **29** on apoptosis in cell lines

To further characterize apoptosis induced by **29**, effects of compound **29** on both upstream and downstream caspases was investigated. To this purpose, MOLT-3, SH-SY5Y, HT-29, MCF-7, and M-14 cells were treated with **29** at 10^2 μM that is, at the minimum compound concentration equally able to induce apoptosis in all the cell lines. The expression of both pro and cleaved caspase 3 and caspase 8 after 6 and 24 hours of culture was investigated by immunoblotting. The results, reported in [Figure 2.10], show that Pro-caspase 3 was constitutively expressed both in untreated and in treated cells except in MCF-7 and in M-14, where it was expressed only after treatment with compound **29**. Densitometry analysis values indicate that pro-caspase 3 expression was increased, following 6 hours of treatment, by 2- and 4-fold only in MCF-7 and in M-14 cells, respectively. Conversely, the cleaved form of 17/24 KDa was highly expressed with respect to the untreated control as early as after 6 hours and this expression was increased after 24 hours by 1.2- to 2-fold in SHSY5Y, HT-29, MOLT-3, and M-14 cells. Moreover, pro-caspase 8 expression was constitutive in MOLT-3, SH-SY5Y, MCF-7, and M- 14 cells. At 6 hours following treatment with **29**, the expression of pro-caspase 8 increased from 1.5- to 3-fold in the entire cell lines except for HT-29 cells, in which a 7-fold increase was observed.

These results highlight that **29**, which is an enantiomer of a synthetic glycosidase inhibitor, is endowed with a well detectable, caspase-related pro-apoptotic activity on a broad range of tumour cell lines of lymphoid as well as epithelial origin. The present study further extends previous data indicating that (–)-lentiginosine (**27**), which is the synthetic enantiomer of a natural product, was able to induce apoptosis in different tumour cells in vitro.[66] Although triol **29** [(–)-7S-OH-lentiginosine] differs only for the presence of the OH group at C7 with respect to **27**, its biological activity presented two distinct aspects. It was able to induce appreciable

apoptosis in MOLT-3 cells starting from half the concentration with respect to (–)-lentiginosine (**27**).

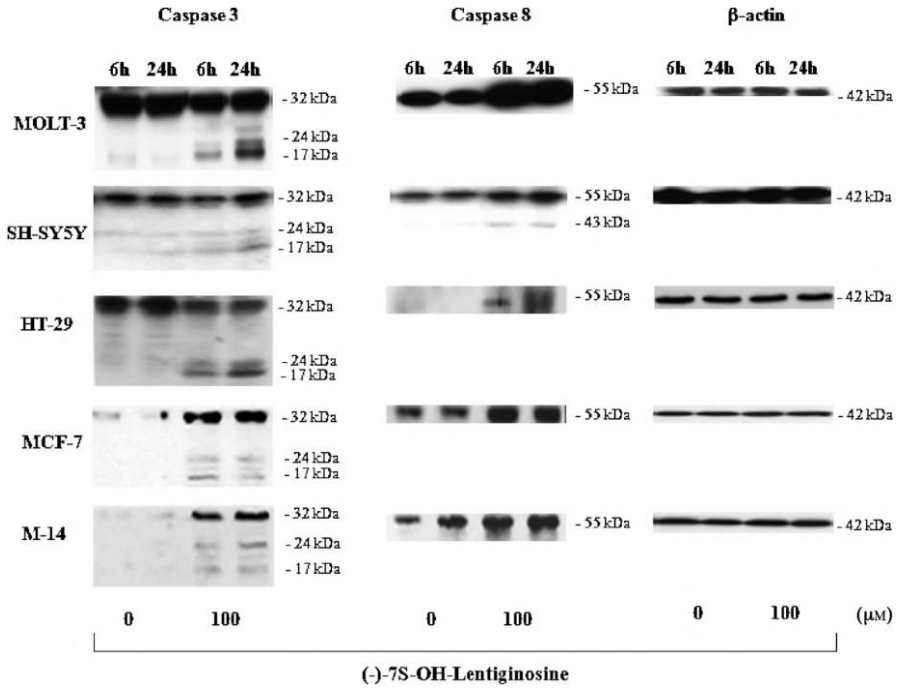


Figure 2.10 Caspase 3 and caspase 8 expression in cells line after incubation of 100 μM of **29**

Apoptosis induced by **29** was well detectable starting from 24 hours after treatment, meanwhile (–)-lentiginosine induced apoptosis as early as 18 hours after treatment. The reason underlying this slightly different biological behaviour is not clear. However, we can notice that the delayed time of **29** in inducing apoptosis with respect to (–)-lentiginosine, fits with the fact that caspase 3 and caspase 8 expression peaked at 24 hours after treatment. In addition, maximal cytotoxic activity, as detected by the MTS assay, also occurred 24 hours after treatment. The comparison of results of the MTS assay with those of apoptosis suggests that most of the cytotoxic activity exerted by **29** towards the different tumour cell lines was attributable to apoptosis, except for HT- 29 cells that were quite resistant to apoptosis while

more sensitive than the other cell lines to metabolic activity inhibition. Observe that compound **29** is 50–100 times less potent as a pro-apoptotic agent than etoposide. However, the concentration of **29** necessary to cause a cytotoxic effect ($MAIC_{50}$ value) is 40 to 400 times higher than that of etoposide, accordingly, indolizidine **29** is less cytotoxic than etoposide analogously to the parent (–)-lentiginosine (**27**).[66]

2.4 Conclusion

The synthesis of the enantiopure 1,2,7-trihydroxyindolizidine **29** was revisited leading to a sensible overall improvement particularly in the preparation of nitrone **28**. In the previous approach, the overall yield of nitrone synthesis reached 25-30%, but the reaction, particularly the protection step, was not easily reproducible. In the new approach 46% overall yield of the nitrone was achieved over 5 steps and all reaction are reproducible. The revisited synthesis of 1,2,7-trihydroxyindolizidine **29** was also more convenient because all the reactions were carried out at room temperature, do not require anhydrous conditions, and is more atom economical, because the excess of dipolarophile is recovered, even if overall yield of **29** is similar (60 v 64%). It has been proved that (–)-7S-OH-lentiginosine **29** is a proapoptotic agent versus different tumor cell lines, and it has similar potency to that of the parent compound **27**. The results lead us to a more insightful investigation to assess the potential antitumor effect of the enantiomer of lentiginosine and its derivatives. Taken together these data demonstrate how these compounds, with a structure resembling that of sugar mimetic glycosidase inhibitors, endowed with previously unknown pro-apoptotic activity, could constitute a new distinct class of pro-apoptotic agents. Actually, recent data have shown how iminosugars, in general, can be endowed both with cytotoxic and glycosidase inhibition activity on human cancer cell lines of

different origin.[101b] These studies suggest that a more deep investigation should be aimed to assess whether inhibition of glycosidase might directly be involved in induction of apoptosis by these compounds.

Stereoselective synthesis of a highly hydroxy functionalized benzo[*e*]indolizidine via Cu (I) catalyzed Ullmann reaction

3.1 Introduction

Lentiginosine [(1*S*,2*S*,8a*S*)-octahydroindolizine-1,2-diol] (**14**), one of the least substituted indolizidine iminosugars, has shown an interesting activity in addition to the glycosidase inhibition. In particular, it was shown to be a potent inhibitor of HSP90.[101] Moreover, its enantiomer [(1*R*, 2R, 8aR)-octahydroindolizine-1,2-diol] (**27**)[4,102] and the corresponding 7-OH derivative [(1*R*,2*R*,7*S*,8a*R*)-octahydroindolizine-1,2,7-triol](**29**)[100] have a low cytotoxicity effect, but can induce apoptosis in cancer cell strains.

The search for new, more selective and potent candidates of this important class of compounds suggested the modification of the indolizidine skeleton through its conjugation with relevant substructures, like functionalized chains, or amino acid structures. The six membered ring of lentiginosine was the target of these functionalizations.[68] Proceeding on this idea, the synthesis of benzosubstituted lentiginosines was taken in account, supported by computational studies that suggested a better interaction of a benzolentiginosine with the enzymatic cavity. It was, then decided to fuse a benzene ring at six-membered ring of lentiginosine, at the [*e*]-bond position. The importance of benzene rings in medicinal chemistry is mentioned in the literature.[61, 62] All the more, natural aryl substituted polyhydroxylated pyrrolidines, like codonopsine or radicamine,[103] show interesting biological activities.

In this chapter, we discussed the synthesis of benzosubstituted lentiginosines [Figure 3.1] starting from L- tartaric acid. The strategy is based on the diastereoselective 1,3-dipolar cycloaddition of 3,4-bis-*tert*-butoxypyrroline *N*-oxide (**28**) with halogen substituted styrenes described in (Scheme 3.1).

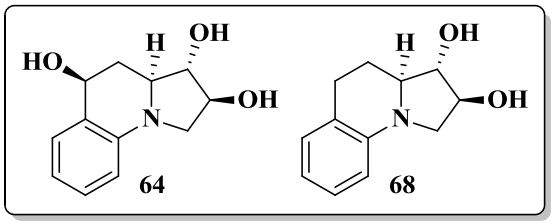
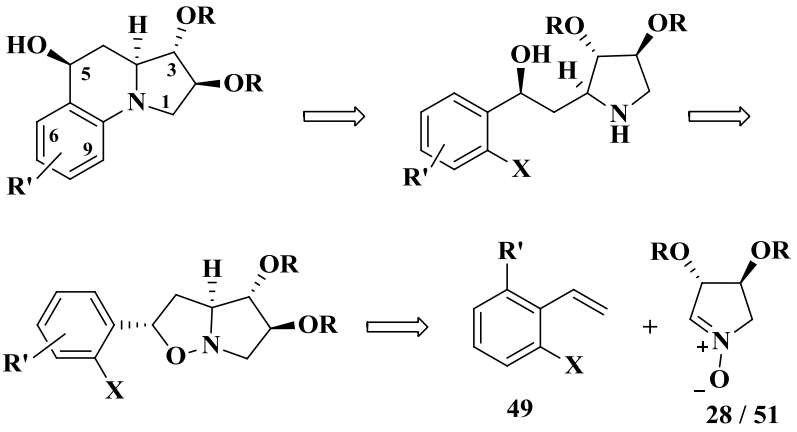


Figure 3.1

One particular advantage of this approach was anticipated to be the control of the regio- and stereochemistry of the 1,3-dipolar cycloaddition (1,3-DC), and possibility of carrying out a metal catalyzed ring closing amination in the final step (Scheme 3.1).



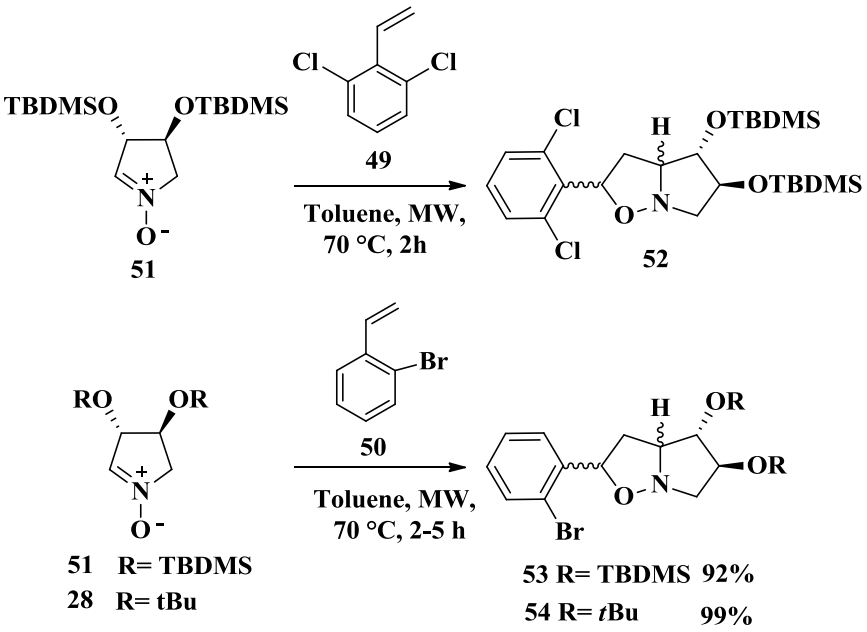
Scheme 3.1

3.2 Result and discussion

3.2.1 Synthesis of benzo[*e*]indolizidine from nitrone (28), which derived from tartaric acid

3.2.1.1 Synthesis of isoxazolidine

The cycloaddition of nitrones **28** and **51** with two functionalized styrenes, 2,6-dichlorostyrene (**49**) and 2-bromostyrene (**50**) was carried out (Scheme 3.2).[123]



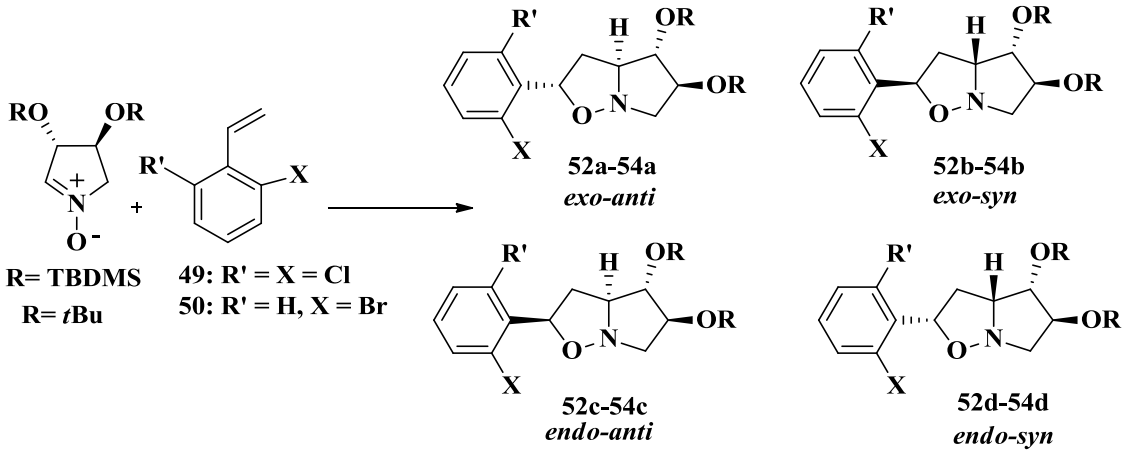
Scheme 3.2

Reagents	Reaction time	adduct	Yield	*exo-anti*	*exo-syn*	*endo-anti*
51 + 49	1h 45’	52	94%	6.8	6.4	1
51 + 50	2 h	53	92%	18.4	11.6	1
28 + 50	5 h	54	99%	12	3.5	1

Table 1 Cycloaddition between halogenated styrene and nitrones.

The 1,3-DC of nitrone **51** with dichlorostyrene **49** in toluene as solvent was performed in the MW oven at the temperature 75 °C for 2 h. A higher temperature for the reaction should be avoided to prevent the polymerization of styrene and the degradation of the starting materials. The 1,3-DC of **51** with **49** was less diastereoselective than the corresponding reaction with bromostyrene **50** (ca 1:1 vs 1.6:1 *exo-anti/exo-syn* ratio). 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. Likely, the mono-substituted bromostyrene **50** can better accommodate the substituted phenyl ring in an *anti* approach. The highest stereoselectivity was obtained with the dipolarophile **50** and di-*tert*-butoxy nitrone **28** that afforded the adduct *exo*-(3-*OR*)-*anti* **54a** in 73% yield (calculated). Diastereomeric adducts *exo-syn* and *endo-anti* were not easily separable by chromatography on silica gel, and were separated at later steps. In all the cases, the *exo*-(3-*OR*)-*anti* diastereoisomer (**52a**-**54a**) (Scheme 3.3), deriving from the *exo* attack of the alkene on the opposite face of the C-3 substituent of the nitrone, was the major adduct. The second and third isomers were, respectively, the *exo*-(3-*OR*)-*syn* and the *endo*-(3-*OR*)-*anti* isomers (**52b**-**54b** and **52c**-**54c**).



Scheme 3.3 Possible diastereisomers can derive from cycloaddition of **49, 50** with nitrone.

The cycloaddition reaction between **28 / 51** and styrenes **49** / **50** could give four diastereoisomer; the *exo-anti* diastereoisomer should be the most favoured, whereas the *endo-syn* diastereoisomer should be the less favoured [Figure 3.2].

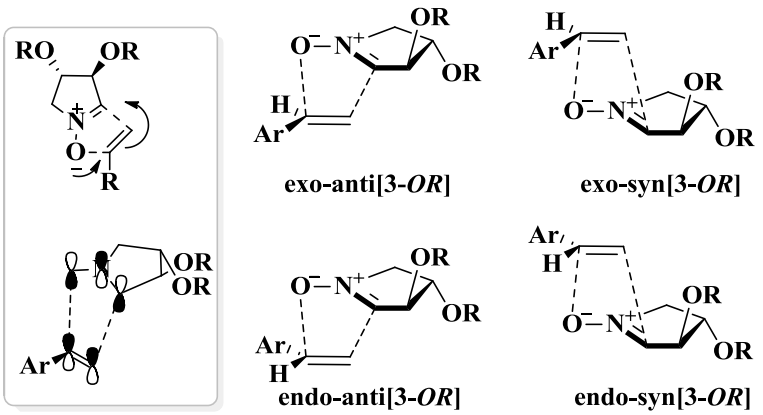


Figure 3.2

The stereochemistry of cycloadducts **52-54** was assigned by NMR analyses and then confirmed by the analysis of the corresponding derivatives. In particular, a NOE interaction was present between 2-H and 4-H in **54a** and between 2-H and 5-H in **54b** in agreement with an *exo*-preferred approach [Figure 3.3]. The *trans* relationship between the 3a-H and 4-H protons in the 3-(O*t*-Bu)-*anti* adducts **54a** and **54c** was indicated by the small $^{2}J_{3a\text{-}H/4\text{-}H}$ coupling value (4.1 and 3.5 Hz, respectively).

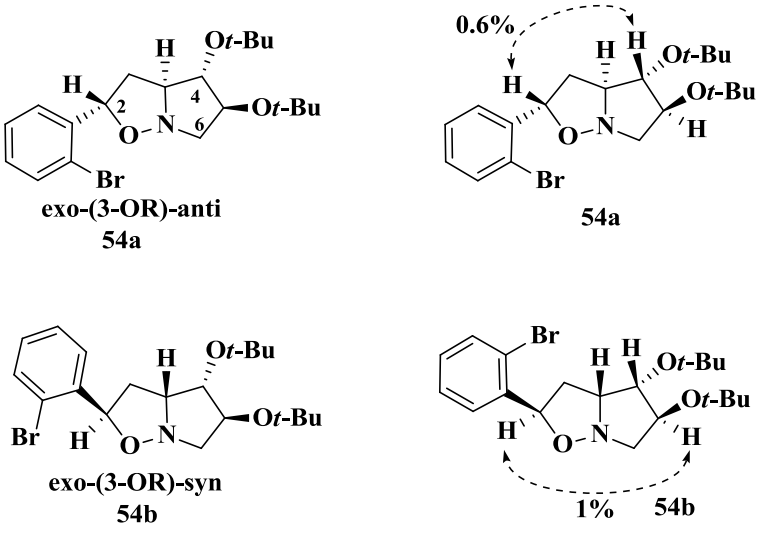
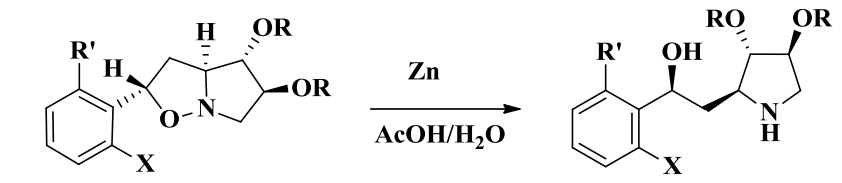


Figure 3.3 Diagnostic NOE interactions in adducts **54a** and **54b**

3.2.1.2 Opening of isoxazolidine ring

The selective reduction of the isoxazolidine N-O bond was carried out with Zn and acetic acid and afforded the corresponding pyrrolidines in good yields (Scheme 3.4).



52a	**R'=X=Cl; R=TBDMS**	**60 °C, 4 h**	**72%**	**55a**	**R'=X=Cl; R=TBDMS**
53a	**R'=H, X=Br, R=TBDMS**	**60 °C, 4 h**	**88%**	**56a**	**R'=H, X=Br, R=TBDMS**
54a	**R'=H, X=Br, R=t-Bu**	**80 °C, 5 h**	**96%**	**57a**	**R'=H, X=Br, R=t-Bu**

Scheme 3.4 Only major distereoisomers are reported

NOE experiments on pyrrolidines derived from adduct **57a** and **57b** confirmed the assigned configuration. In particular, a NOE interaction between 2-H and 4-H was observed only in **57a** and the interaction between the vicinal 2-H and 3-H proton was smaller in pyrrolidines **57a** than in the corresponding isomers **57b** in accord with the assigned relative configuration [Figure 3.3].

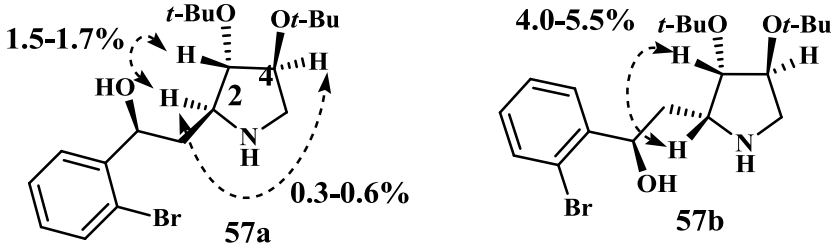
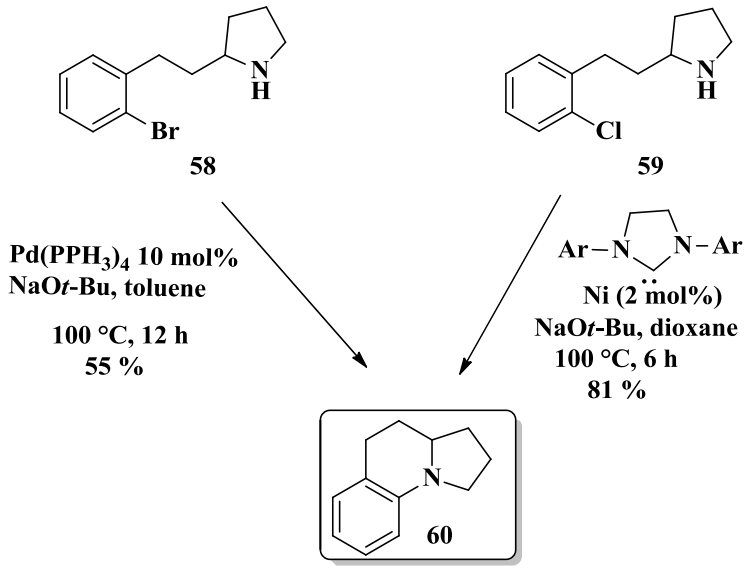


Figure 3.4 Diagnostic NOE interactions in adducts **57a** and **57b**

3.2.1.3 Closure of benzoindolizidine ring

The halogen atom present 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 literature

several examples of cyclizations of model compounds are reported (Scheme 3.6). Buchwald et al [104c] synthesized 1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoline (**60**) in 55% yield by intramolecular Pd catalyzed nucleophilic aromatic substitution of the 2-Br-phenethylpyrrolidine (**58**) (Scheme 3.6). Fort et al,[105c] have carried out the same cyclization of the chloro derivative **59** in the presence of a Ni catalyst in 81% yield (Scheme 3.6).



Scheme 3.6

Although our substrates **55-57** are highly functionalized, but the skeleton is quite similar with these model reactions, we decided, then, to apply the same reaction conditions used by Buchwald and Fort with our substrate in order to achieve our next goal.

3.2.1.3.1 Buchwald-Hartwig Amination reaction

The formation of aniline starting from aromatic halides and primary or secondary amines is a reaction strategically very important for the synthesis of amino-aromatic nucleus, which is basically important for compounds of pharmaceutical interest. The first reaction of amination

of aryl starting from aromatic halides has been addressed by the group of Migita in 1983, which used a stoichiometric amount of tetrakis-triphenylphosphine palladium [Pd (PPh_3) $_4$] for the reaction between various bromobenzene and (*N*, *N* - diethylamino) tributylstannane. [104]

Buchwald[104] and Hartwig[105] 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 a 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 [Figure 3.5] is illustrated the amination mechanism, [106] useful to understand the importance of the role of the ligand.

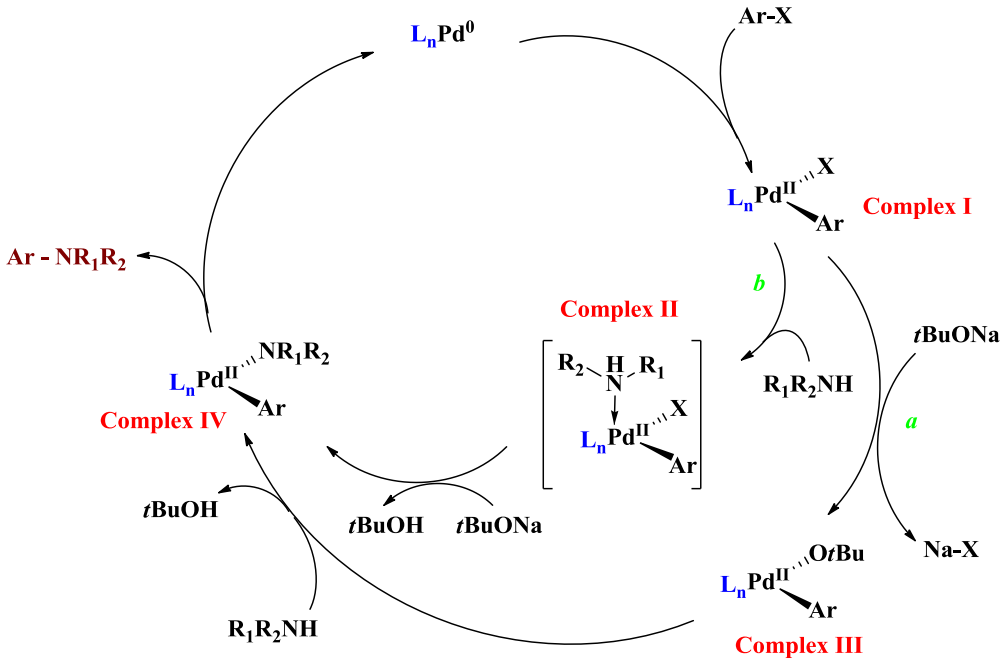


Figure 3.5

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.[107] 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.[108]

Usually, the arylbromides and -iodides undergo the Buchwal–Hartwig reaction at lower temperatures, in shorter reaction time and affording better yields than the corresponding arylchlorides, which often do not react at all. To obtain benzocondensated derivatives of the lentiginosine **61-63,** initially a Buchwald–Hartwig reaction was attempted [Figure 3.6].

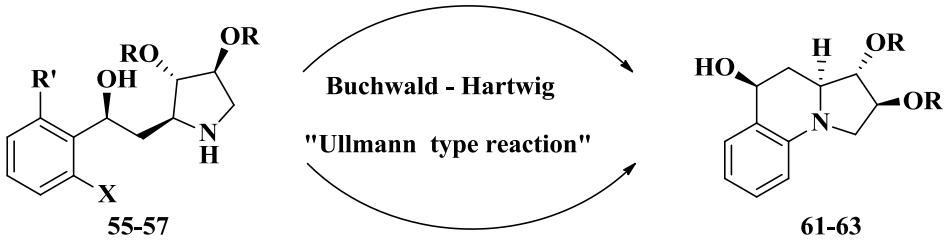
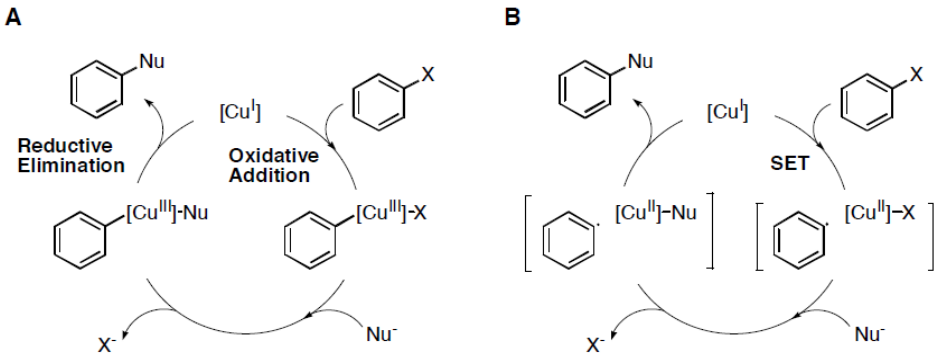


Figure 3.6

In the cyclization reaction of **55a** and **56a** 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.[109,110] 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 **50** was also used. Furthermore, the bromoderivative **56a** was a suitable substrate for an Ullmann-type reaction catalysed by Cu(I), accordingly, also this option was explored.

3.2.1.3.2 Copper mediated cyclization by Ullmann reaction

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).[111] 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 (**A**, **B**) or without (**C**, **D**) variation of the copper oxidation state [Figure 3.7].



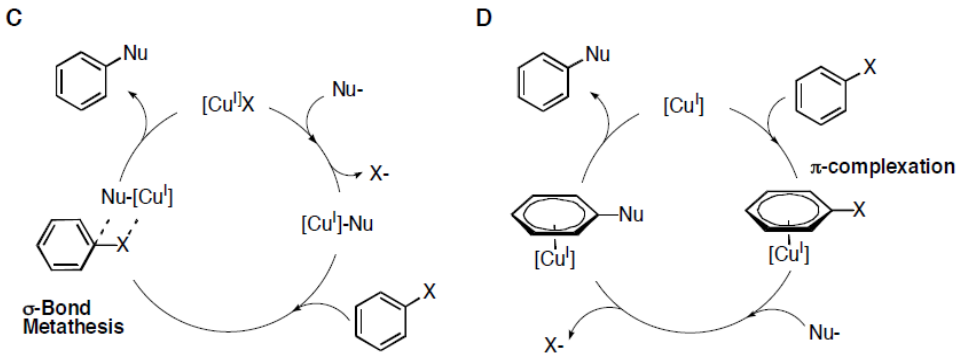
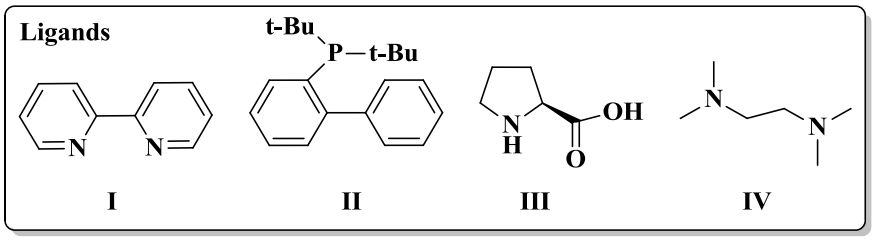


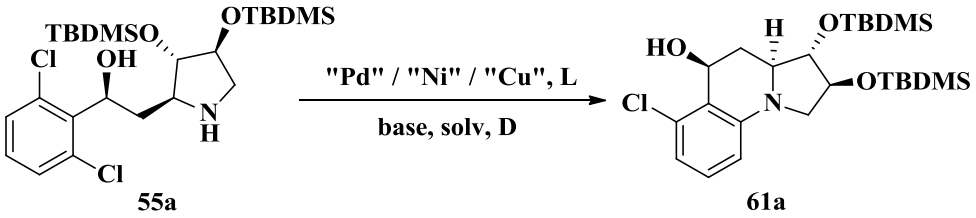
Figure 3.7

The oxidative addition [Figure 3.7, **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 *ortho* 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.

3.2.1.3.3 Intramolecular amination reactions

The cyclization reactions carried out on substrate **55a** were numerous: only a summary is reported in Table 2. The ligands used were: 2,2'-bipyridyl (**I**), (2-biphenyl)di-*tert*-butylphosphine (**II**), L-proline (**III**) and tetramethylethylenediamine (**IV**) (Scheme 3.7, Table 2).





Scheme 3.7

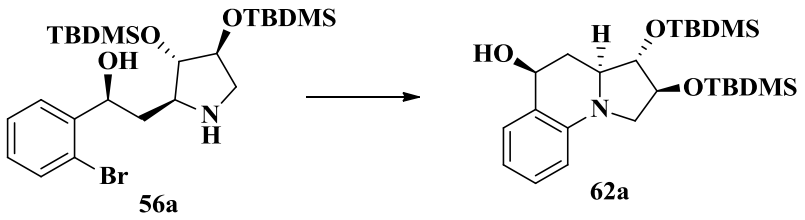
	Ar-X	Scale (mg)	M source (mol. equiv. %)	L[a]	M:L	Base (mol. equiv.)	Solv.	T (°C)	T (h)	Yield (%)
1	**55a**	30	$Ni(OAc)_2$ (5)	bpy	1 : 3	*t*BuOH (0.1)/NaH (1.2)	THF	90	5	0
2	**55a**	66	$Pd(OAc)_2$ (10)	bptbp	1 : 2	*t*BuOK (1.2)	toluene	110	21	0
3	**55a**	30	-	-	-	*t*BuOK (2.5)	toluene	130	5	0
4	**55a**	27	$PdCl_2$TEMED (10)	bptbp	1 : 2	*t*BuOK (1.2)	THF	90	5	0
5	**55a**	218	CuI (10)	Pro	1 : 2	K_2CO_3 (2.0)	DMF	100	48	5

Table 2 Intramolecular N-arylation by Buchwald-Hartwig reaction conditions

All the reactions under Buchwald-Hartwig conditions failed to give any cyclization product. Bisphenylphosphine **II** [112-114] used in the experiments 2 and 4 with Pd (0) did 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, protection of hydroxy group 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.[115-117] The use of manganese oxide (IV) also failed,[118] in accord with a reduced reactivity of benzylic OH group. Because of the role of *t*BuOH in combination with sodium hydride, we supposed that alcoholic functions can be deprotonated and the formed alkoxide interferes with the vicinal metal coordinated with the benzene ring quenching its catalytic effect. The presence of an intramolecular hydrogen bond in apolar solvents was proved by the persistence of the broad band in the 3100-3550 cm^{-1} region of the IR spectra of diluted solutions of **55a** (30–5 mM in $CDCl_3$). Attempts to convert the free OH group in silyl ether with TBDMSCl and TMSCl in DMF, or TMSOTf in DCM failed or gave very small yields of the desired compound. To verify this hypothesis, the cyclization under Ullmann reaction condition was examined, because the highly polar solvents used in Cu(I) catalyzed C-N coupling should effectively reduce the strength of the internal hydrogen bond in substrates **55-57**, and shield the reactivity of the hydroxy group.

Finally, only under Ullmann conditions, using Cu(I) 10 mol% as catalyst, proline as ligand and potassium carbonate as base, the formation of the cyclization product **61** was observed even if in very small amount (5% yield) [Table 2. Entry 5]. Somewhat better results were obtained by switching to the bromoderivative **56a**. In [Table 3] are described the cyclization reactions performed on **56a**.



Scheme 3.8

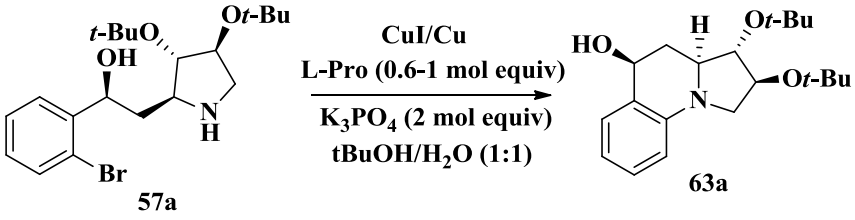
CuI/Cu/L-Pro (mol %)	Base	Solvent	*T*	*t*	yield
10/100/20	K_3PO_4	Dry DMF	100 °C	48 h	37%
30/30/60	DBU	*t*-BuOH	100 °C (MW)	10 h	30%
30/30/60	K_3PO_4	*t*-BuOH/H_2O/THF	100 °C (MW)	5 h	46%

Table 3 Intramolecular *N*-arylation by Ullmann reaction conditions

Cyclization of pyrrolidine **56a** in the presence of copper salts was tested using various combinations of catalyst sources, with or without proline, an organic or inorganic base, under anhydrous or aqueous conditions. It was found that CuI in the presence of metallic Cu was more efficient as catalyst than CuI alone or the $CuSO_4$/Cu couple and that the use of proline as copper ligand increased the yield. The catalytic effect of copper was proved by analyzing the thermal cyclization of **56a** (DMF, DBU or K_3PO_4, 120 °C, 2 h) that afforded only traces of **62a** along with a mixture of decomposition products. DBU and K_3PO_4 were more efficient than K_2CO_3 in DMF, but in protic solvents a higher yield was obtained when K_3PO_4 was used as base. Unfortunately, the desired compound **62a** could be isolated in only 30-46% yields at

best (Scheme 3.8, table 3). A significant amount of decomposition products was always detected in the crude reaction mixtures even when conversion was not complete. Generally, with longer reaction times or a higher temperature, decomposition increased with detrimental effect on the overall yield in accord with a relative instability of **62a** under the reaction conditions. ^{1}H NMR spectra of the crude mixtures showed a large excess of high field signals suggesting that the TBDMS protecting group could be part of the problem. In fact, it has been reported that the TBDMS group can be removed in the presence of copper salts.[120]

Then, the copper-mediated cyclization of the di-*tert*-butyl derivative **57a** was studied using the best reaction conditions found, i.e. CuI/Cu/L-Pro as catalyst mixture, K_3PO_4 as base and a 1:1 mixture of *t*BuOH/H_2O as solvent (Scheme 3.9, Table 4).



Scheme 3.9

No	CuI/Cu (%)	T (°C)	t (h)	RSM (%)[b]	Yield (%)[c]
1	30/30	100 (MW) [a]	3.5	61	36 (90)
2	30/30	120	1.5	32	48 (71)
3	50/50	90 (MW) [a]	10	n.d.	75
4	50/50	90	10	n.d.	65
5	80/80	90 (MW) [a]	13	15	62 (73)
6	80/80	110	2	n.d.	21
7	80/80	100 (MW) [a]	3.5	30	31 (44)
8	50/50	95	10	28	58 (81)
9	50/50	90	12	n.d.	74
10	50/50	90	12	7[(d)]	77 (82)[d]

[**a**] MW: Microwave heating. [**b**] RSM: recovered starting material (**57a**) after chromatography; n.d: not determined [**c**] Isolated yield after chromatography; values in parentheses refer to yields based on conversion. [d] The reaction was made on ***ent*-57a**

Table 4 Intramolecular N-arylation by Ullmann reaction conditions

The replacement of the protecting group led to a significant reduction of decomposition products. Preliminary tests on **57a** revealed that a higher yield based on converted starting material could be achieved when the reaction is run at lower temperature and for longer time (Table 4 entries 1-2 and 5-7). Half mol equiv of the CuI/Cu couple was found to be the best compromise between conversion percentage, overall yield and a reasonable reaction time (10-12 h) when the reaction was carried out at 90 °C. Due to the long reaction time, conventional

heating was preferred over microwave irradiation that was only slightly more efficient (Table 4, entries 3, 4 and 9). Finally, the best reaction conditions (Table 4, entries 9 and 10) were applied to both the enantiomeric pyrrolidines **57a** and *ent*-**57a.** The corresponding tricyclic products **63a** and *ent*-**63a** were obtained in 74 and 77% yield, respectively, demonstrating no major effect of the use of the chiral L-proline as catalyst ligand with different enantiomers.

Under the same conditions, the dichloro derivative **55a** proved to be less reactive affording only traces of the corresponding benzoindolizidine after 48 h at 100 °C. The known reduced reactivity of chloro aromatic compounds in comparison with the corresponding bromo derivatives and the steric hindrance of the densely functionalized phenethyl pyrrolidines **55** compared to **56** and **57**, seem to justify the lack of reactivity.

NOE experiments on tricyclic compound **63a** confirmed the assigned configuration. In particular, hydrogen 3a-H showed NOE interactions with both 2-H and 5-H [Figure 3.8].

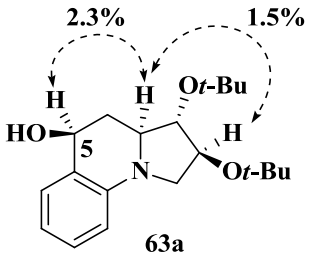
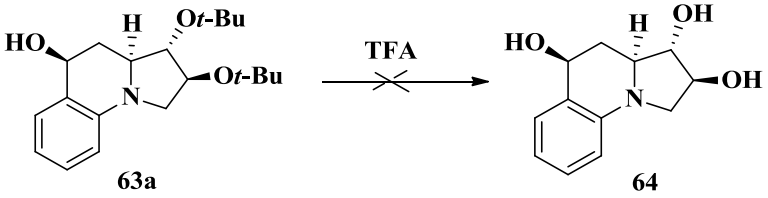


Figure 3.8 Diagnostic NOE interactions in adducts **63a**.

3.2.1.4 Deprotection of benzo[*e*]indolizidine

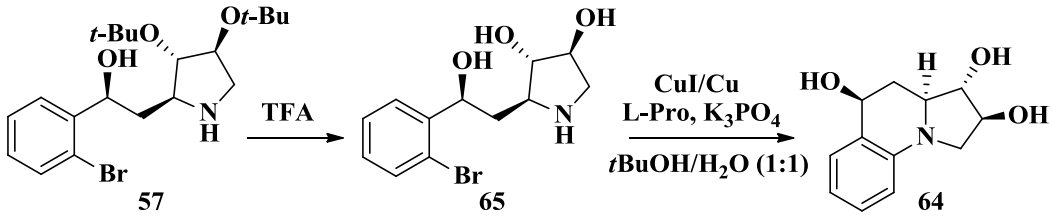
The last step of the synthesis, the deprotection of the protecting groups on the hydroxy functions in position 2 and 3 of tricyclic product **63a**, was carried out with TFA at 0 °C- rt. However, the reaction did not afford any product **64** but only decomposition products.

Probably the presence of the OH group on benzylic position renders the compound unstable **(**Scheme 3.10).



Scheme 3.10

In order to avoid this drawback, deprotection of **57a** was carried out in the same conditions before cyclization in order to get **65**. The tri-hydroxy derivative **65**, without purification was, then, subjected for intramolecular aryl amination under the best reaction conditions, which gave compound **64** in 40% overall yield over the two steps (Scheme 3.11).

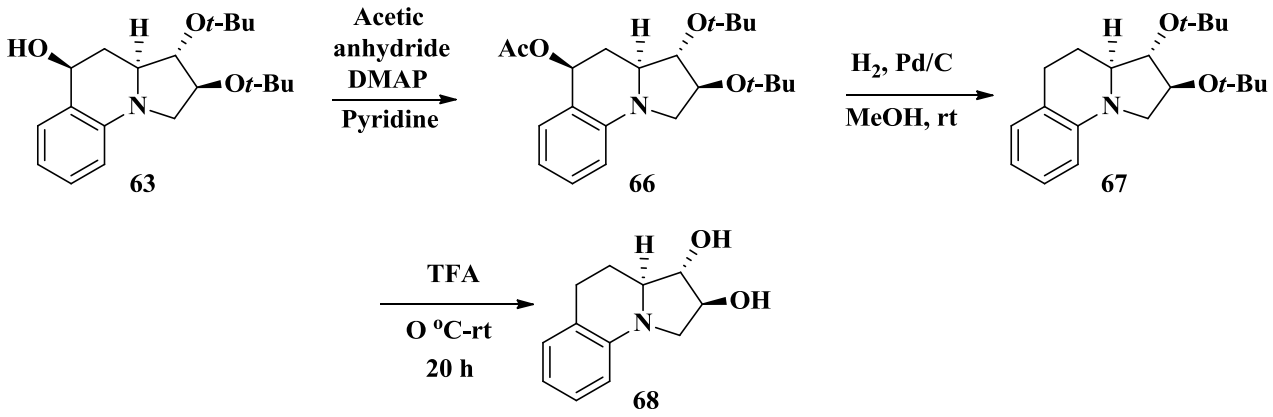


Scheme 3.11

3.2.1.5 Synthesis of benzo[*e*]indolizidine (64)

Having in hand the indolizidine **63** it was needed to remove the hydroxyl functionality to obtained benzolentiginosine. The removal of OH group was achieved by first transformation in the corresponding acetate followed by reduction with H_2 on Pd. The acetylation of **63** was carried with acetic anhydride in pyridine at 0 °C to obtain **66**.[121] The protected tricyclic

product **66** was then treated with Pd/C under H_2 to get diprotected benzo[*e*]lentiginosine **67** in 83% yield (Scheme 3.12).



Scheme 3.9

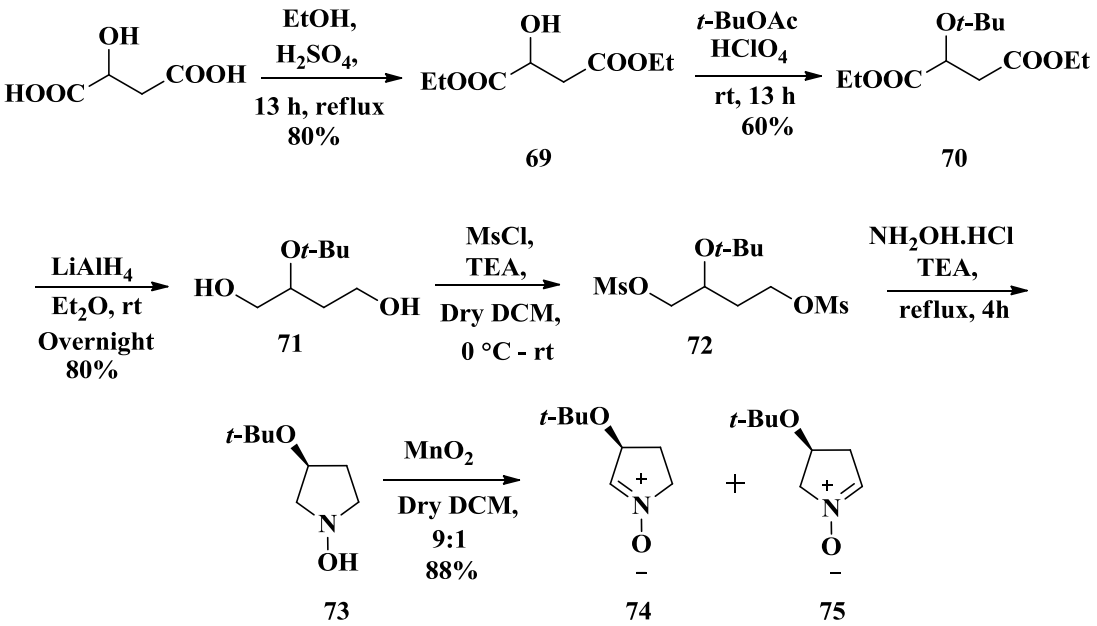
Deprotection of diol **67** with TFA at 0 °C gave benzo[*e*]lentiginosine **68** in 91% yield. The synthesis of benzo[*e*]indolizidines **64** and **68** was, then, complete in satisfactory with good yield in both enantiomeric forms, to explore their biological activity.

3.2.2 Synthesis of benzo[*e*]indolizidine from nitrone (74), which derived from malic acid

We decided to test the optimized Ullmann reaction condition on an analogous system bearing only an alkoxy group on the pyrrolidine ring. Therefore, we decided to synthesize another benzo[*e*]indolizidine starting from nitrone **74**, which can be easily derived from malic acid.

3.2.2.1 Synthesis of (*S*)-4-*tert*-butoxy-3,4-dihydro-2H-pyrrole 1-oxide (74).[127]

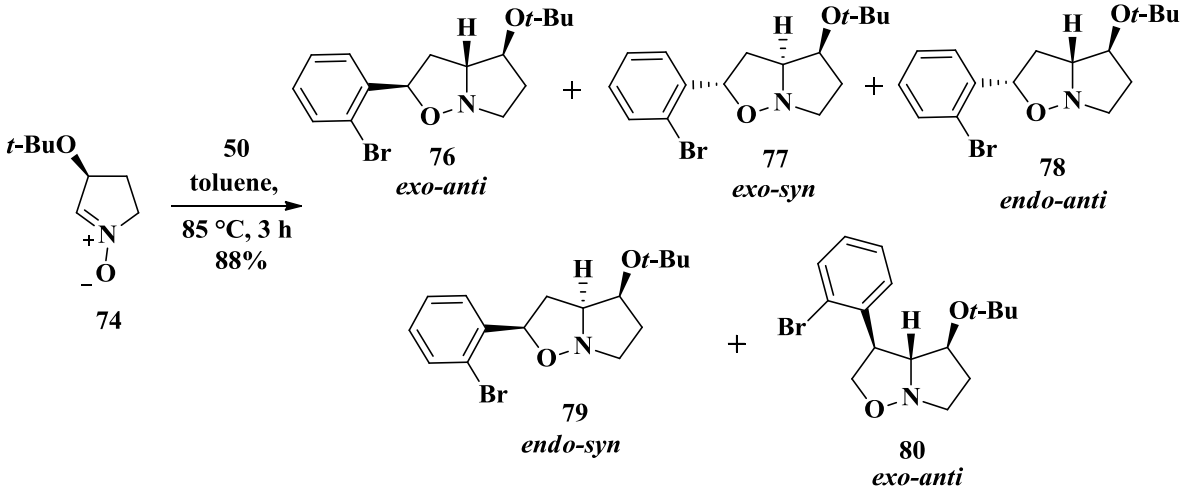
The optically pure chiral five membered 4-*tert*-butoxy pyrrolidine *N*-oxide (**74**) was prepared through a convenient six step procedure starting from L-malic acid (Scheme 3.10). In particular, the diethyl ester of malic acid **69** was treated with *tert*-butyl acetate in the presence of a catalytic amount of $HClO_4$ to protect the free hydroxyl group,[96] then the diester **70** was reduced to the corresponding diol **71**, which was in turn mesylated **72**. Cyclization with hydroxylamine afforded the *N*-hydroxypyrrolidine **73** through a double-nucleophilic displacement of the (bis)mesylate. The final step consists of a regioselective MnO_2 oxidation of hydroxylamine **73** that can afford two regioisomeric nitrones. The two nitrones **74** and **75** were obtained in 9:l ratio by treatment of **73** with MnO_2 in dichloromethane at room temperature. The assignment of the structure to the two nitrones was easily made on the basis of the coupling pattern of the HC=N proton in the ^{1}H NMR spectra (δ 6.79 ppm, quartet, J = 1.8 Hz for **74,** and δ 6.83, multiplet for **75**).



Scheme 3.10

3.2.2.2 Synthesis of (3*S*)-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinolin-3-ol (83 and 84).

The 1,3-DC of (*S*)-4-*tert*-butoxy-3,4-dihydro-2*H*-pyrrole 1-oxide (**74**) with 2-bromostyrene **50** in toluene at 85 ^{o}C for 3 h afforded two major diastereomeric adducts **76**, **77** along with two other diastereoisomers (**78**, and **79)** and a regioisomer **80** in traces amounts (50:14:4:2:1 ratio) as colourless liquids in 88% overall yield (Scheme 3.11). The observation of the regioisomer **80**, albeit in very minute amount, is remarkable for this kind of 1,3-DC that is commonly highly regioselective. It must be due to some influence of the Br electron withdrawing substituent on the styrene dipolarophile. It is well known that electron withdrawing substituents on alkene dipolarophiles favour the 4-regioisomers. Adducts **76** and **78** could not be separated by chromatography on silica gel and were obtained 67% yield (ca: 12:1 mixture), whereas the adduct *exo-syn* (**77**) , *endo-syn* (**79**) and a regioisomer **80** could be separated by chromatography on silica gel.



76/**77**/**78**/**79**/**80** = 50:14:4:2:1

Scheme 3.11 Diastereomeric adducts derived from 1,3-DC of nitrone **74** with **50**

The structure and stereochemistry of the adducts were assigned by NMR analysis.

In particular,

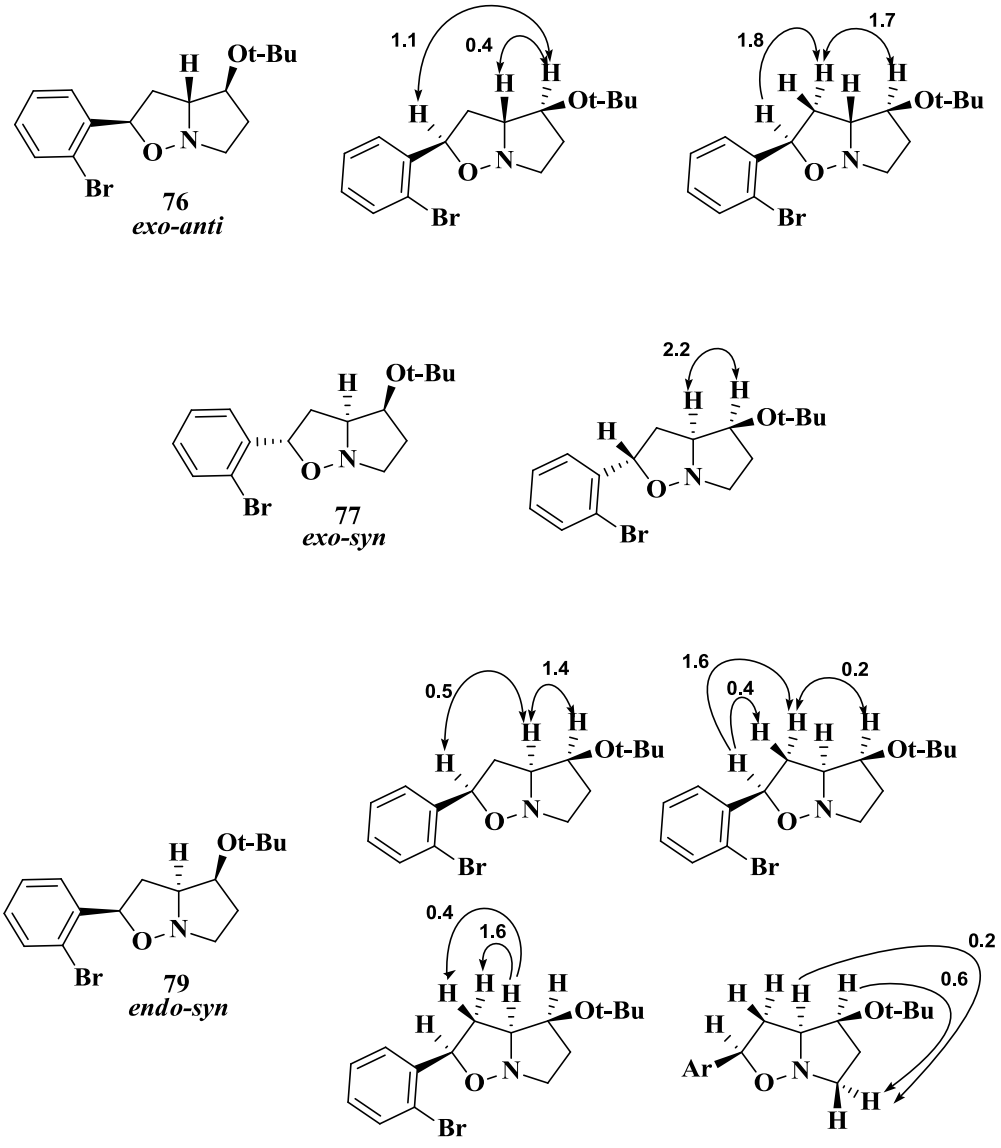
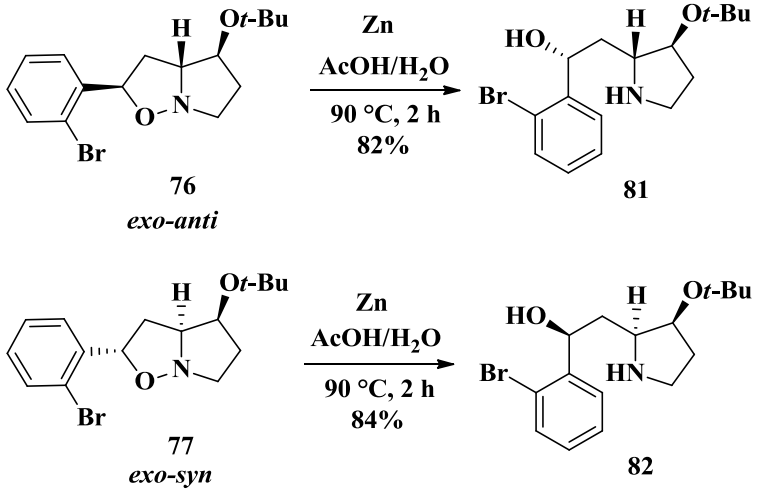


Figure 3.9 Selected NOEs for the determination of relative configuration in pyrrolo[1,2-*b*]isoxazolidines **76**, **77**, and **79**.

The cycloaddition reaction between **74** and **50** could give four diastereoisomeric 2-substituted pyrrolo[1,2-*b*]isoxazolidines. This selectivity is usually determined by steric factors, and analogously to the previous cases, the *exo*-(3-*OR*)-*anti* diastereoisomer (**76**) (Scheme 3.11),

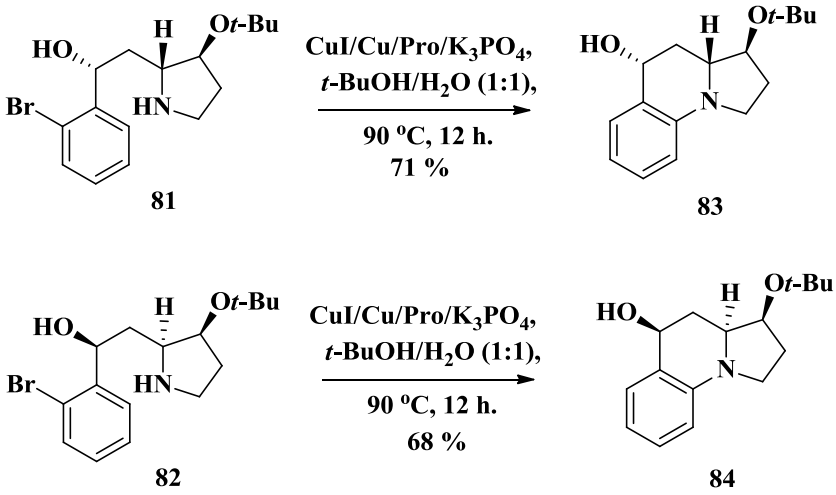
deriving from the *exo* attack of the alkene on the opposite face of the C-3 substituent of the nitrone, was the major adduct. The second is the *exo*-(3-*OR*)-*syn* isomer (**77**) and third *endo*-(3-*OR*)-*anti* isomer (**78**), whereas the *endo*-*syn* diastereoisomer (**79**) should be the less favoured.

Reduction of the major diastereoisomers **76** (12:1 mixture with **78**) and **77** with Zn in acetic acid/ H_2O gave the 2-(bromophenethyl) pyrrolidines **81** and **82** as white solids in 82% and 84 % yield, respectively (Scheme 3.12).



Scheme 3.12

2-Bromophenyl-*tert*-butoxypyrrolidines **81** and **82** were subjected to intramolecular amination reaction by using the Ullmann reaction condition as previously, and the protected benzo[*e*]indolizidines **83** and **84** were obtained as colourless oil in 71% and 68% yield respectively, after chromatography on silica gel (Scheme 3.13).



Scheme 3.13

The structure and stereochemistry tricyclic protected benzo[*e*]indolizidines **83** and **84** was assigned by NMR analysis. In particular NOE experiments on benzo[*e*]indolizidine **83** shown interaction with both 4-H and 5-H protons, whereas NOE interactions was present between 3-Ha with 3-H and 5-H protons in benzo[*e*]indolizidine **84** [Figure 3.10].

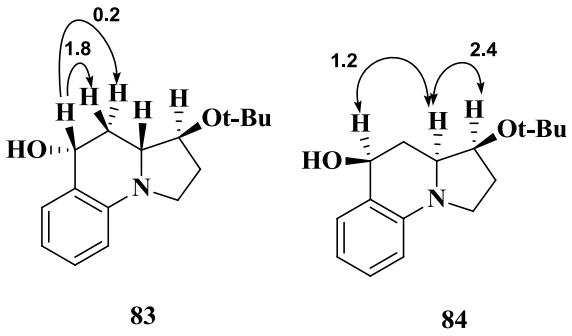
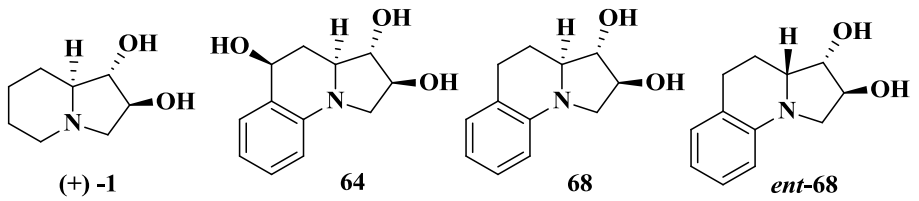


Figure 3.10 Diagnostic NOE interactions in **83** and **84**.

The synthesis of protected benzo[*e*]indolizidines **83**and **84** was, then, complete in satisfactory good yield.

3.3 Biological activity

The synthesized compound were tested on glycosidase inhibition activity and compared with (+)lentiginosine **1**. The result are given in below [Table 5].



	(+) 1	**64**	**68**	***ent*-68**
α–glucosidase EC 3.2.1.20 yeast	0	0	0	29
β–glucosidase EC 3.2.1.21 Almonds	0	0	35	15
β–mannosidase EC 3.2.1.25 Snail	0	17	20	0

Table 5 Inhibition (in **%**) toward glycosidases at **1 mM** concentration of benzo-lentiginosines.

Benzo[*e*]lentiginosine **64** and **68** are completely inactive towards amyloglucosidase. Benzo[*e*]lentiginosine **64** shows some degree of activity only towards β-mannosidase, whereas **68** show a limited activity towards β glycosidase and β-mannosidase. Interestingly the enantiomer of **68** shows the similar low activity, however towards α–glucosidase and β-glycosidase. The parent (+)-Lentiginosine is inactive towards all these glycosidases.

The glycosidase inhibition activity was not good as expected. One possible explanation is the fact that the nitrogen atom is not as basic as in lentiginosine, because of the conjugation of the

nitrogen lone pair with the aromatic ring, and is less prone to protonation to form the intermediate **85** that mimics the transition state for glycoside bond hydrolysis [Figure 3.11].

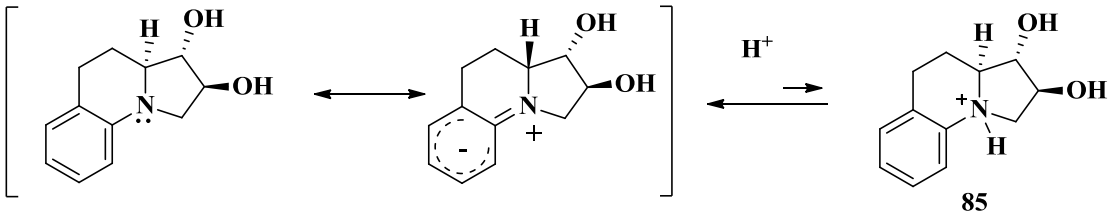


Figure 3.11

3.4 Conclusion

1,3-DC of enantiopure 3,4-dialkoxy-pyrroline *N*-oxide with 2-bromostyrene, followed by reduction of the isoxazolidine N-O bond and copper-catalyzed intramolecular N- arylation provides a convenient method for the synthesis of highly functionalized benzo[*e*]indolizidines. The bromo-phenyl pyrrolidines undergo cyclization under Ullmann reaction conditions (copper catalyst, protic solvents) whereas Pd and Ni catalysts, which operate in apolar solvents, failed to give the same reaction. The required reaction conditions involving a high temperature (90 °C), stoichiometric copper, and a base are well tolerated by *t*-butyl ether groups whereas TBDMS ethers suffer a partial decomposition. Our optimized Ullmann reaction condition could be nicely applied to the nitrone derived from malic acid affording the corresponding benzo[*e*]indolizidines or hexahydropyrrolo[1,2-*a*]quinolines **83** and **84** with the same efficiency of tartaric acid derivatives. The method provides access to benzocondensed derivatives of iminosugars which demonstrated only low glycosidase inhibitor activity towards α-glycosidase, β- glycosidase and β-mannosidase.

Development of new synthetic methodology towards synthesis of substituted tetrahydroquinolines

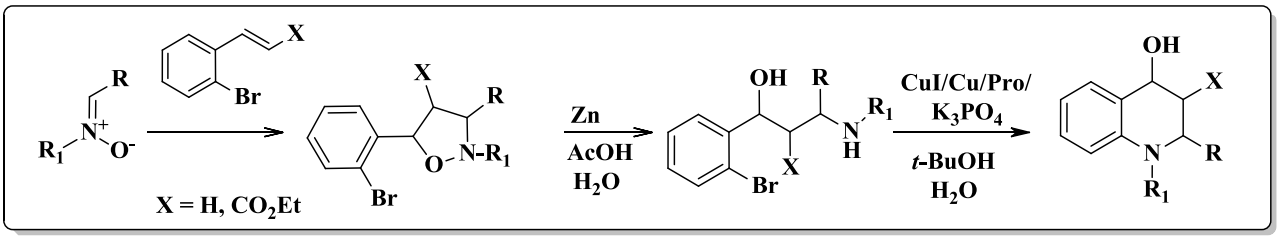
4.1 Introduction

Heterocyclic ring systems are among the most important organic compounds and they have always had a significant role in organic synthesis, because of their being abundant in nature. Heterocyclic rings are present in compounds involved in many biological processes like vitamins, coenzymes, hormones, porphyrins, DNA, RNA.

Heterocyclic compounds, especially aza-heterocycles, constitute the most important class of compounds in the pharmaceutical and agrochemical industries, with heterocycles comprising around 60% of all drug substances. Many commercial pharmaceuticals are also derived from aza-heterocyclic scaffolds as pyridines, indoles, quinolines, azepines, and pyrimidines. Therefore, as key building blocks in pharmaceutical research, heterocycles have continued to be important subjects in medicinal chemistry from the beginning. It is not surprising if this class of compounds has received special attention by chemists that always have shown interest in finding new synthetic methods to introduce enormous variety of structural features in order to increase the potential of these molecules. The transformation of heterocycles to other more complex molecules is nowadays an established process in organic synthesis.

The quinoline heterocyclic scaffold in general and the tetrahydroquinoline ring system in particular, are very common structural motives and are found in numerous biologically active natural products and pharmacologically relevant therapeutic agents. Because of the significance of these scaffolds in drug discovery and medicinal chemistry, the development of new methodologies for the synthesis of tetrahydroquinolines derivatives continues to be a very active field of research.[122]

In the previous chapter, we described an optimized intramolecular amination reaction under Ullmann's reaction conditions that give access to the tetrahydroquinoline core structure.[123] Accordingly, we considered to extend our work and develop a new general methodology towards the synthesis of substituted tetrahydroquinolines. The proposed approach is a three-step sequence consisting of a nitrone 1,3-dipolar cycloaddition (1,3-DC), reduction of isoxazolidine ring, and ring closure by intramolecular copper mediated Ullmann reaction, which is the key step of our synthetic methodology (Scheme 4.1).



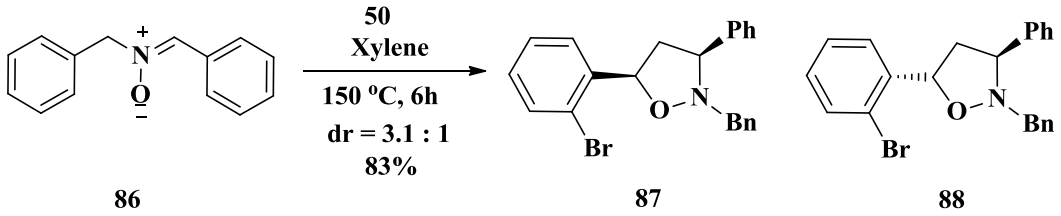
Scheme 4.1 General approach towards tetrahydroquinolines.

To determine the scope of this approach, we studied some examples starting from different acyclic nitrones. Either electron rich and electron poor acyclic nitrones were used in order to study the electronic effect on the 1,3-DC. Moreover, the effect of the presence of an ethoxycarbonyl group on the styrene double bond was considered.

4.2 Result and discussion

4.2.1 Synthesis of (2*S**,4*R**)-1-benzyl-2-phenyl-1,2,3,4-tetrahydroquinolin-4-ol (91)

1,3-DC of phenyl benzyl nitrone **86**[124] and bromostyrene **50** in xylene at 150 °C for 6 h afforded a mixture of diastereomeric cycloadducts **87** and **88** in 3.1:1 ratios (Scheme 4.2). The two adducts were inseparable by column chromatography.



Scheme 4.2

The relative configuration of cycloadducts **87** and **88** was assigned by NMR analyses. In particular, a NOE interaction was present between 5-H and 3-H, only in the *cis*-isomer **87** [Figure 4.1].

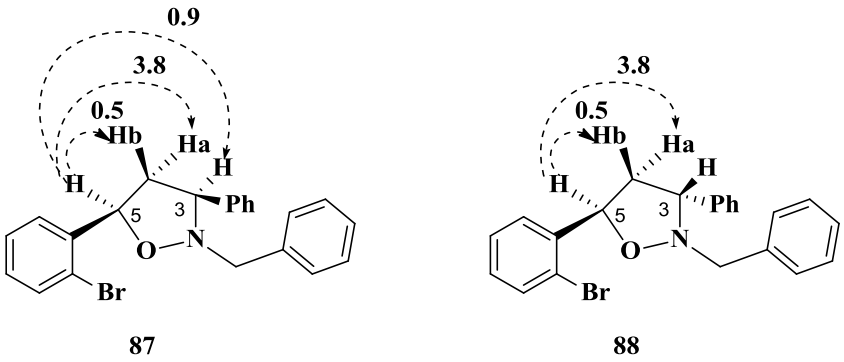


Figure 4.1 Diagnostic NOE interactions in adducts **87** and **88**

Therefore, nitrone **86** and dipolarophile **50** interact preferentially through an *exo* transition state (TS) considering the reacting nitrone in its *Z*-configuration [Figure 4.2].

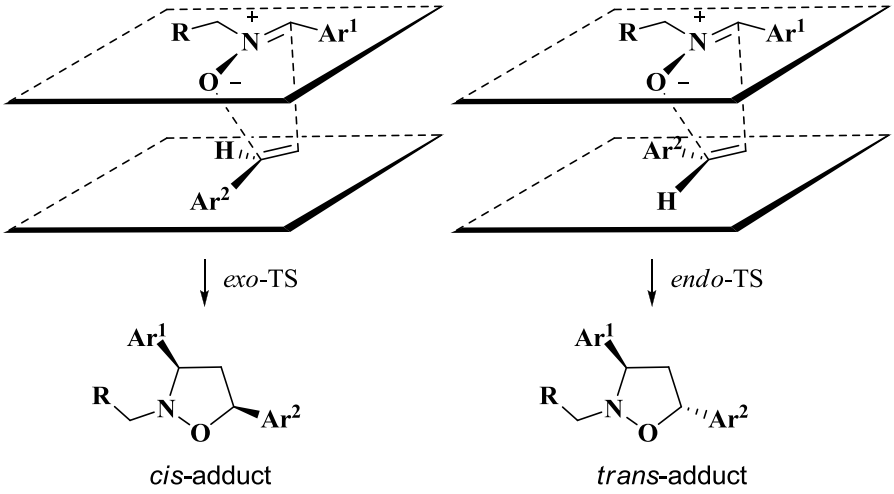
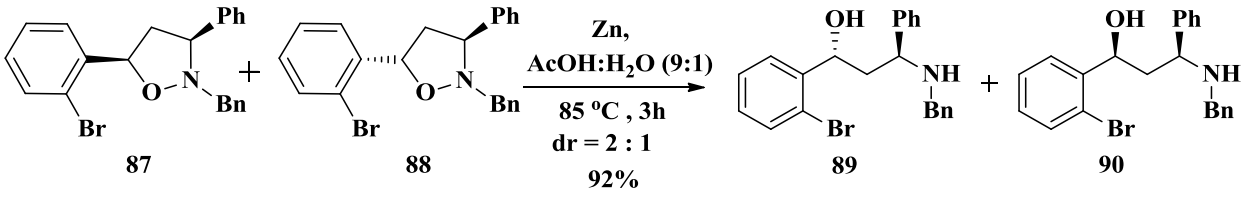


Figure 4.2

The mixture of isoxazolidine **87** and **88** (3.1:1 ratio) was reduced with Zn in acetic acid/H_2O (1:1) to get amino alcohols **89** and **90** (ca. 2:1 ratio) in 92% overall yield (Scheme 4.3). Amino alcohols **89** and **90** could be only partially separated by column chromatography.



Scheme 4.3

NOE experiments on **89**, which derived from adduct **87**, showed the presence of a strong interaction between the following couple of hydrogens: 1-H/3-H (3.3%), 1-H/2-Ha (2.5%), 3-H/2-Ha (2.7%) and 3-H/CH_2Ph (3.2%) [Figure 4.3]. These data suggest that in $CDCl_3$, amino

alcohol **89** is mainly in a cyclic conformation that is stabilized by an intramolecular hydrogen bond [Figure 4.3, structure II].

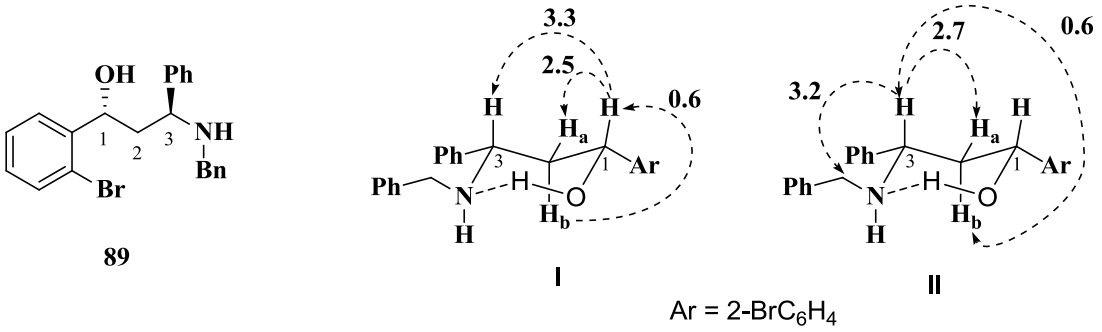
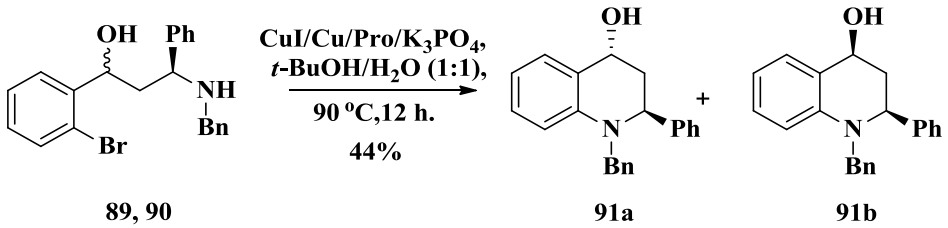


Figure 4.3 Diagnostic NOE interactions in major isomer **89**

The mixture of secondary amines **89** and **90** (ca 1:1 ratio) was subjected to intramolecular cyclization under Ullmann's reaction condition and afforded a mixture of phenyl substituted quinolines **91a** and **91b** in ca 4:1 ratio. After chromatographic separation an enriched fraction of quinoline **91a** (isomeric ratio 10:1) was obtained in 44% overall yield along with recovery of starting material **89** (30%) (Scheme 4.4). The relative configuration of the major quinoline could not be established by NMR analysis, but in analogy with the other studied examples (see below) it could be assigned as *trans*. Moreover, the partial conversion of amino alcohol **88** (70%) was in accord with a lower reactivity of this isomer. Accordingly, the yield of the Ullmann reaction was 80% for **90** that afforded the *trans*-quinoline **91a**, whereas amino alcohol **89** was converted into the less stable *cis*-quinoline **91b** in 11% yield (70% conversion).

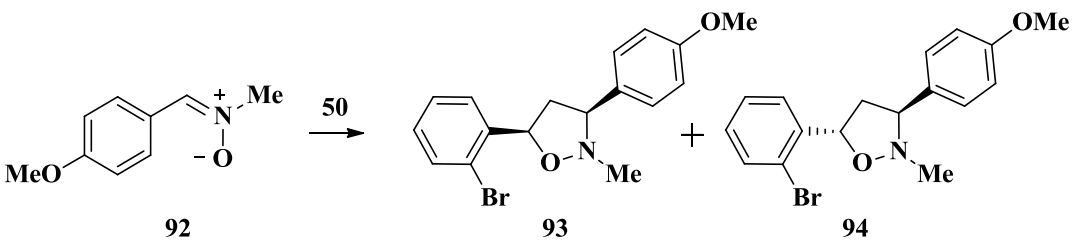


89, 90 91a 91b

Scheme 4.4

4.2.2 (2*S**,4*R**)- and (2*S**,4*S**)-2-(4-methoxyphenyl)-1-methyl-1,2,3,4-tetrahydroquinolin-4-ol (97and 98)

The nitrone *N*-(4-methoxybenzylidene) methanamine oxide (**92**) containing an electron donating group on the C-Ar group was synthesized following the literature procedure and then reacted with bromostyrene **50** overnight in toluene at 110 °C by standard heating (oil bath). Under these reaction conditions, the two diastereoisomeric adducts **93** and **94** were formed in 1:1 ratio and quantitative overall yield (Scheme 4.5). The diastereoselectivity of the cycloaddition was lower compared to nitrone **86** (ds 50% vs 71%). The same reaction carried out at lower and higher temperature (90 °C and 150 °C) afforded the products in lower yield and different ratio. In particular, the formation of the *cis*-adduct **93** was slightly favored at 90 °C whereas the *trans*-isomer **94** was favored at 150 °C (Scheme 4.5).



92: **50**	solvent, temp., time	**93:94**	overall yield
2:1	toluene, 90 °C, overnight	1.2:1	74%
2:1	toluene, 110 °C, overnight	1:1	100%
2:1	*o*-DCB, 150 °C, 3 h	1:1.3	83%
1:1	*o*-DCB, 150 °C, 3 h	1:1.1	69%

Scheme 4.5

The differences in stereoselectivity are very low and suggest that the *cis*-adduct **92** could be the kinetically favored isomer whereas *trans*-adduct **93** was thermodynamically more stable isomer. Anyway, the lower recovery of the *cis*-adduct **92** at 150 °C could be also explained by a lower thermal stability of *cis* isomer, that could undergo decomposition more easily than its *trans* isomer **93**.

The stereochemistry of cycloadducts **93** and **94** was assigned by NMR analysis. In particular, a NOE interaction between protons 5-H and 3-H (0.8%) was present only in the *cis*-adduct **93** [Figure 4.4].

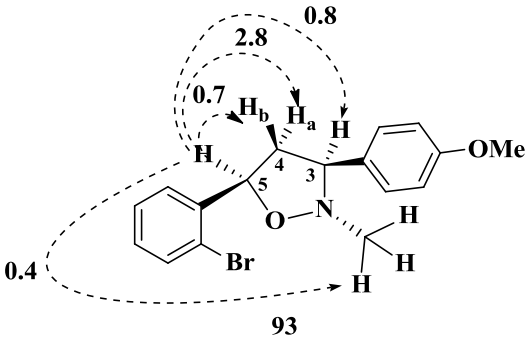
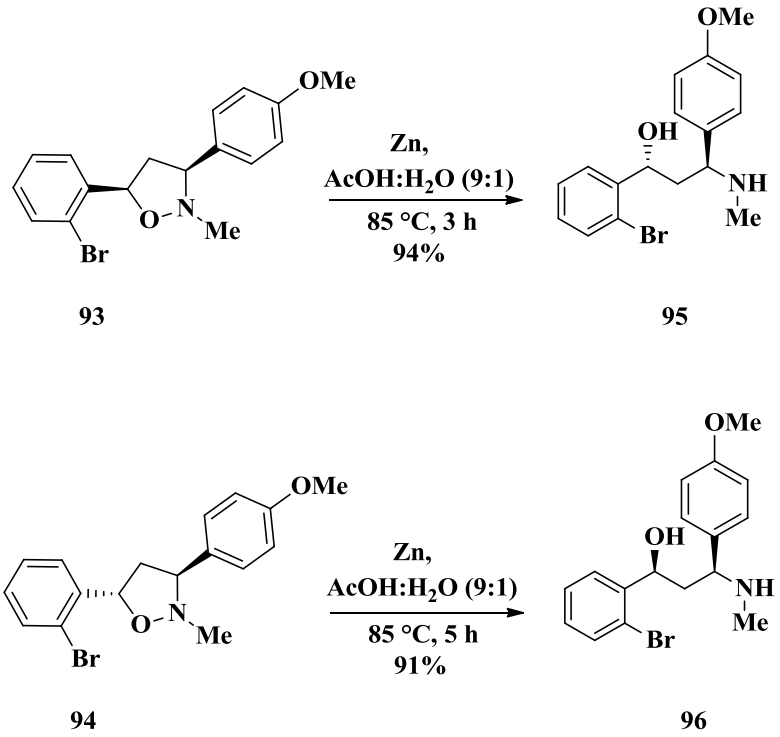


Figure 4.4 Diagnostic NOE interactions in isomer **93**

Cycloadducts **93** and **94** were separated by chromatography on silica gel and then reduced with Zn in acetic acid/H_2O (9:1) at 85 °C for 3 h to afford **95** and **96** with 94% and 91% yields respectively (Scheme 4.6).



Scheme 4.6

Analogously to aminoalcohol **89**, the NOESY-1D spectra of **95** recorded in $CDCl_3$ show a significant NOE interaction between protons 1-H and 3-H (3.0%) in accord with the presence of a cyclic structure stabilized by an intramolecular H-bond [Figure 4.5].

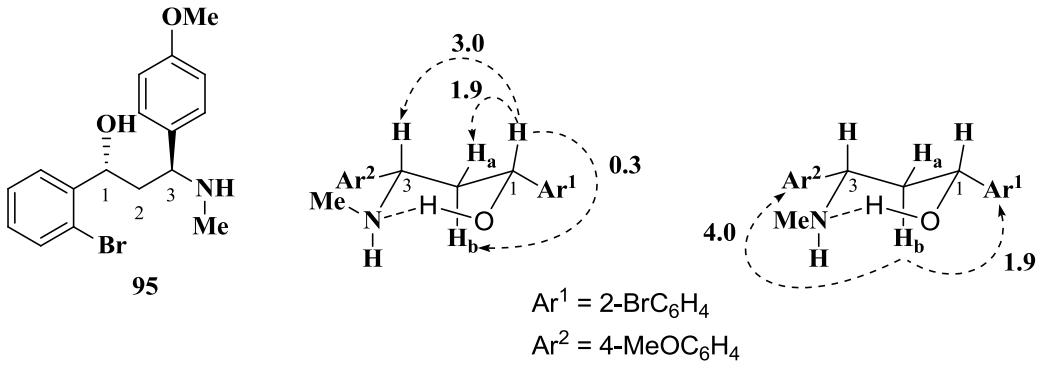
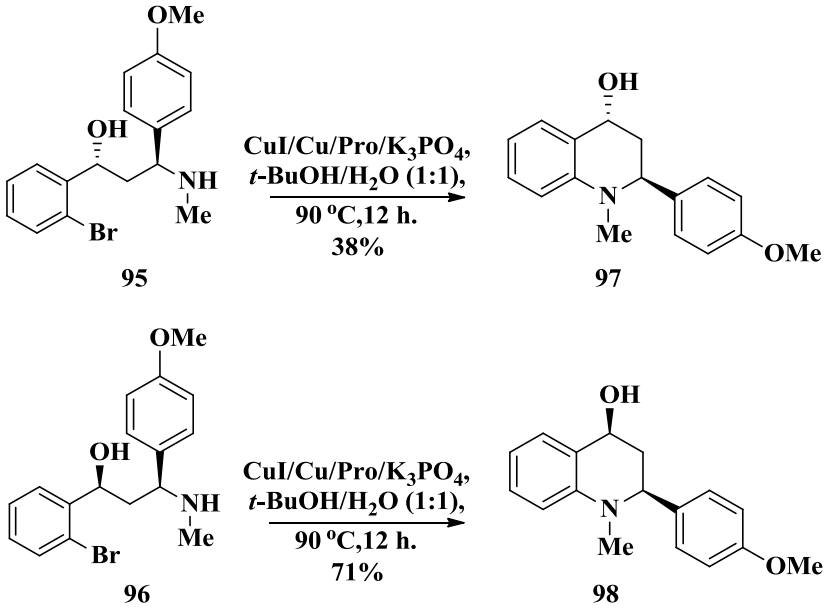


Figure 4.5

Under the usual Ullmann reaction condition, amines **95** and **96** undergo intramolecular cyclization to give **97** and **98** in 38% and 71% yield, respectively (Scheme 4.7).



Scheme 4.7

The relative configuration of stereogenic centers in **97** and **98** was confirmed by NMR analysis. In particular, a NOE interaction between protons 4-H and 2-H (0.8%) was present only in the *cis*-adduct **97**. Moreover, the *trans*-relationship between 4-H and 2-H in **97** was indirectly validated by their NOE interactions with 3-H protons [Figure 4.6].

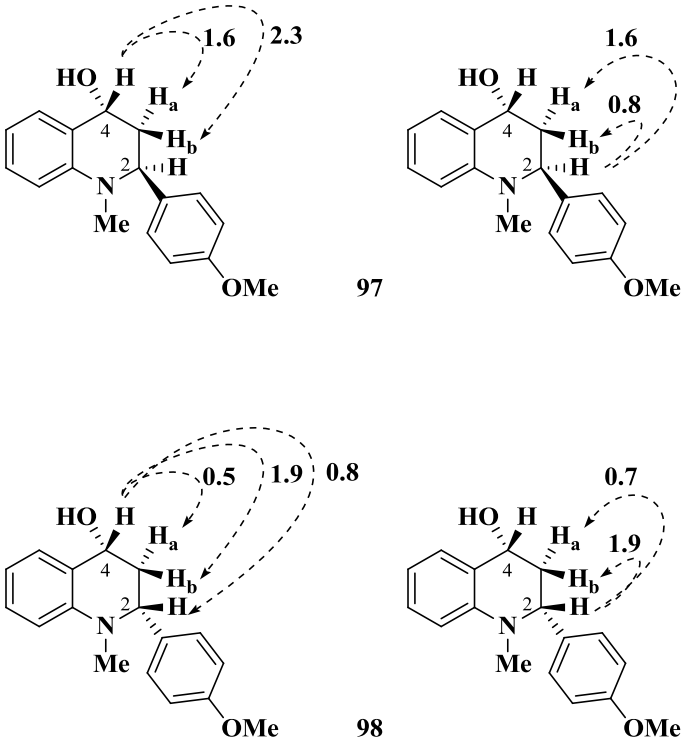
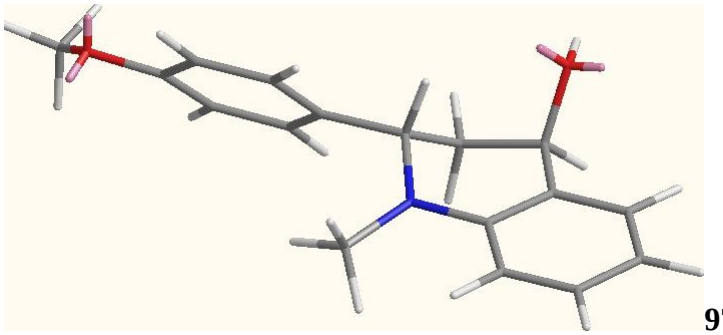


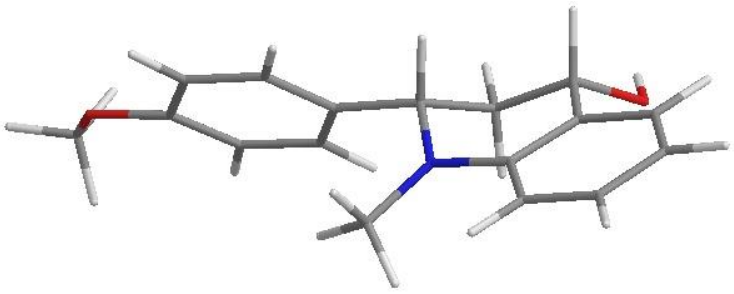
Figure 4.6

The Ullmann reaction on **95** and **96** show that the relative configuration of substituents on C-1 and C-3 largely affect the capability of cyclization of this type of substrate. In fact, **95** is stabilized in a chair conformation by hydrogen bonding. Compound **96** lacks this stabilization and likely requires lower activation energy to reach TS of the reaction. Moreover, the lower efficiency in the formation of the *trans*-disubstituted tetrahydroquinolines **97** in comparison with the *cis*-isomer **98** can be ascribed to its lower stability caused by the presence of one of the two substituents in a pseudo-axial orientation on the piperidine ring. As shown in [Figure

4.7], isomer **97** experiences a 1,3-diaxial interaction between 2-H and 4-OH that hampers its formation, whereas in **98** there is not such steric interaction because both the substituents can occupy pseudo-equatorial positions. The stereochemistry effect on the cyclization reaction is even more critical when a third substituent is present (see below).



97



98

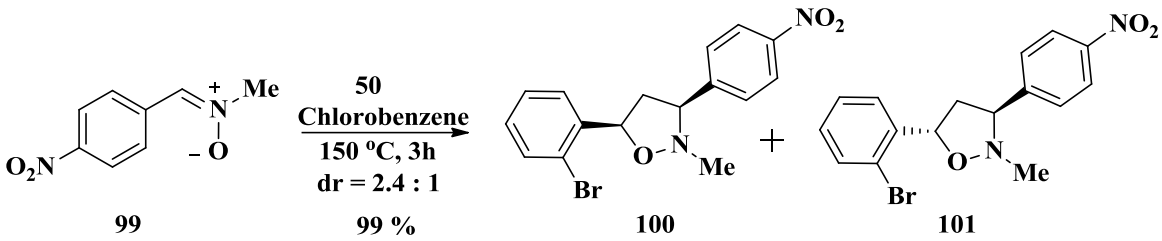
Figure 4.7 The three Dimensional structures of diastereomers **97** and **98**

4.2.3 Synthesis of (2*S**,4*R**)-2-(4-aminophenyl)-1-methyl-1,2,3,4-tetrahydroquinolin-4-ol (104)

In this example, we studied the effect on the cycloaddition of an electron withdrawing group on the C-aryl group of the nitrone. The *N*-(4-nitrobenzylidene)methanamine oxide (**99**) was synthesized following the procedure reported in the literature.

The reaction of nitrone **99** with bromostyrene **50** in chlorobenzene at 150 °C for 3 h gave the adducts **100** and **101** in 2.4:1 ratio, respectively. The two adducts were inseparable by column

chromatography. A pure sample of the major compound **100** was obtained after repeated purification and used for the characterization (Scheme 4.8).



Scheme 4.8

The stereochemistry of cycloadducts **100** and **101** was assigned by NMR analysis. In particular, a NOE interaction was present between hydrogens 5-H and 3-H (0.8%) in accord with a *cis*-relationship in cycloadduct **100** [Figure 4.8].

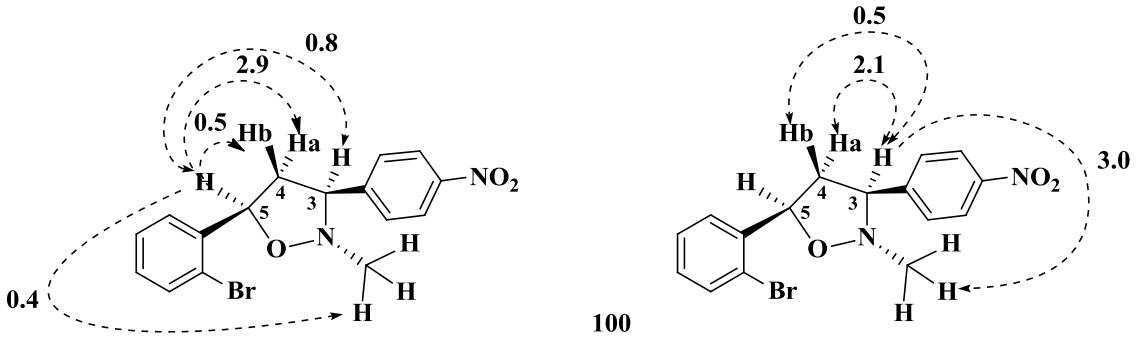
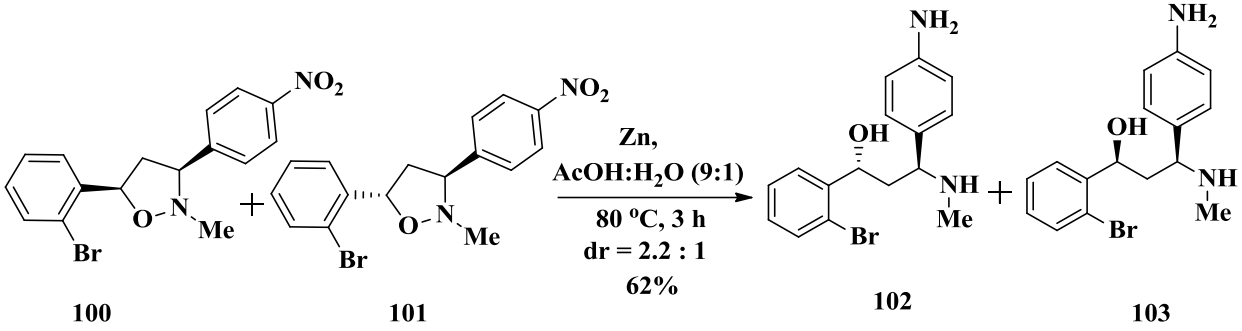


Figure 4.8

Accordingly, the stereoselectivity of the 1,3-DC of nitrone **99** with **50** was higher than nitrone **92** and similar to nitrone **86**.

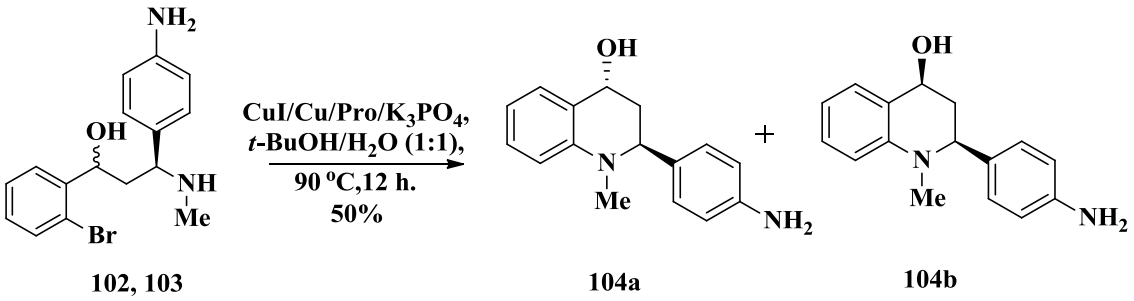
Treatment of the mixture of isoxazolidines **100** and **101** (2.4:1 ratio**)** with Zn in acetic acid/H_2O (1:1) caused the reductive opening of the isoxazolidine ring and the concomitant

reduction of the nitro group affording amino alcohol **102** and **103** (2.2:1 ratio) in 62% yield. The Mixture of amino alcohol **102**and **103** could be only partially separated by column chromatography (Scheme 4.9).



Scheme 4.9

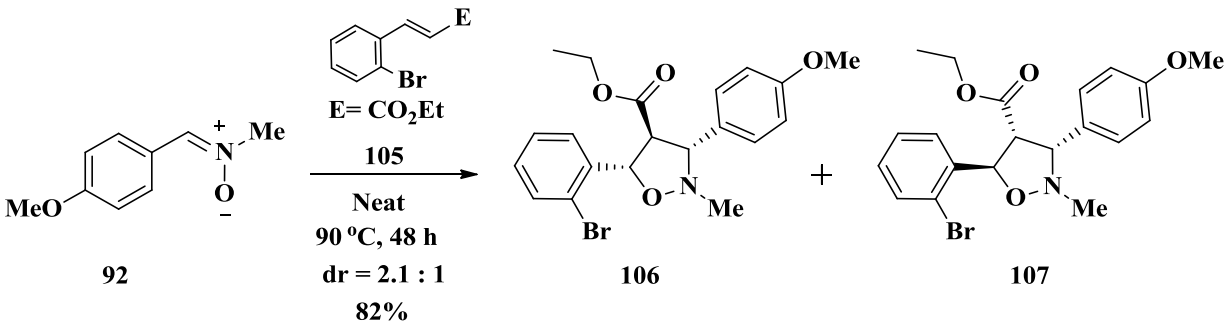
Cyclization of mixture of amino alcohol **102** and **103** (1:1 ratio) under the usual Ullmann reaction conditions afforded the mixture of *trans-cis* disubstituted tetrahydroquinoline **104a** and **104b** (7:1 ratio) in 50% yield (Scheme 4.10).



Scheme 4.10

4.2.4 Synthesis of (2*S**,3*R**,4*R**)-ethyl 4-hydroxy-2-(4-methoxyphenyl)-1-methyl-1,2,3,4-tetrahydroquinoline-3-carboxylate (110)

The application of the method to the synthesis of more substituted tetrahydroquinoline derivatives was then studied. The *trans*-disubstituted alkene (*E*)-ethyl 3-(2-bromophenyl) acrylate (**105**) was synthesized following a literature procedure[125] and then used as dipolarophile in the cycloaddition with nitrone **92**. The 1,3-DC was carried out without solvent at 90 ^{o}C for 48 h and afforded the two diastereomeric adducts **106** and **107** with complete regioselectivity. Cycloadducts **106** and **107** were obtained in 2.1:1 ratio and 82% overall yield and could be separated by column chromatography on silica gel (Scheme 4.11).



Scheme 4.11

The relative configuration of cycloadducts **106** and **107** was assigned by NMR analysis. In particular, a NOE interaction between hydrogen 5-H and 3-H (0.7%) was present only in the major isomer **106** attesting the *cis*-relationship between these two atoms [Figure 4.9]. A significant NOE interaction between 4-H and the 4-MeOC_6H_4 group (2.5%) of the same isomer **106** was also in accord with the reported structure.

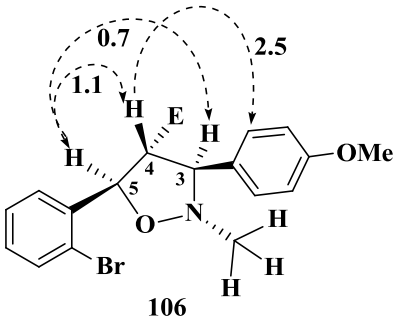


Figure 4.9

Therefore the 1,3-DC of **92** with **105** occurred preferentially through the *exo*(4-$MeOC_6H_4$),*endo*(CO_2Et)-TS [Figure 4.10].

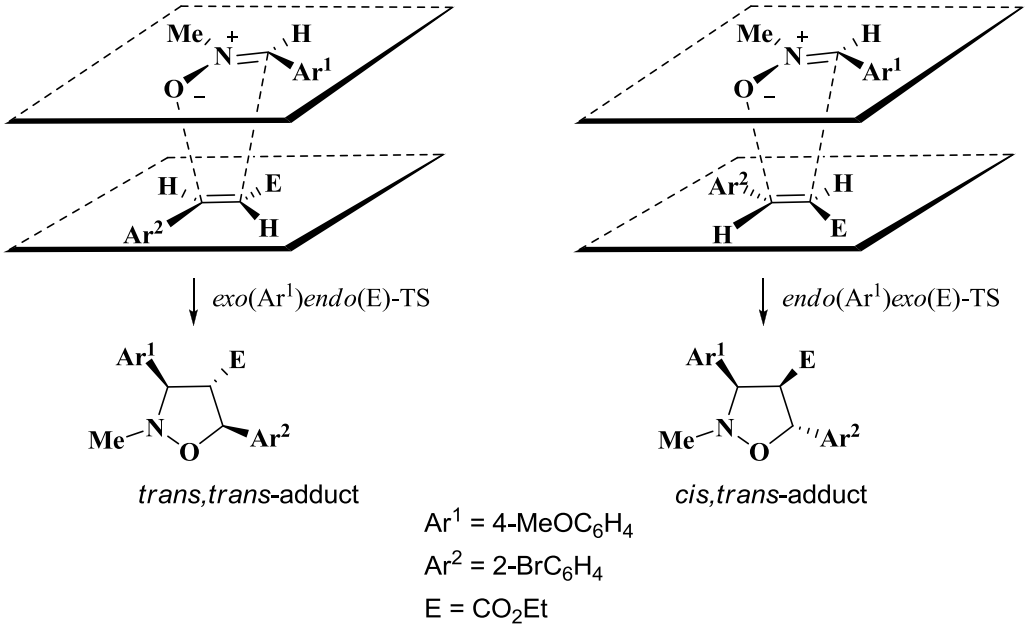
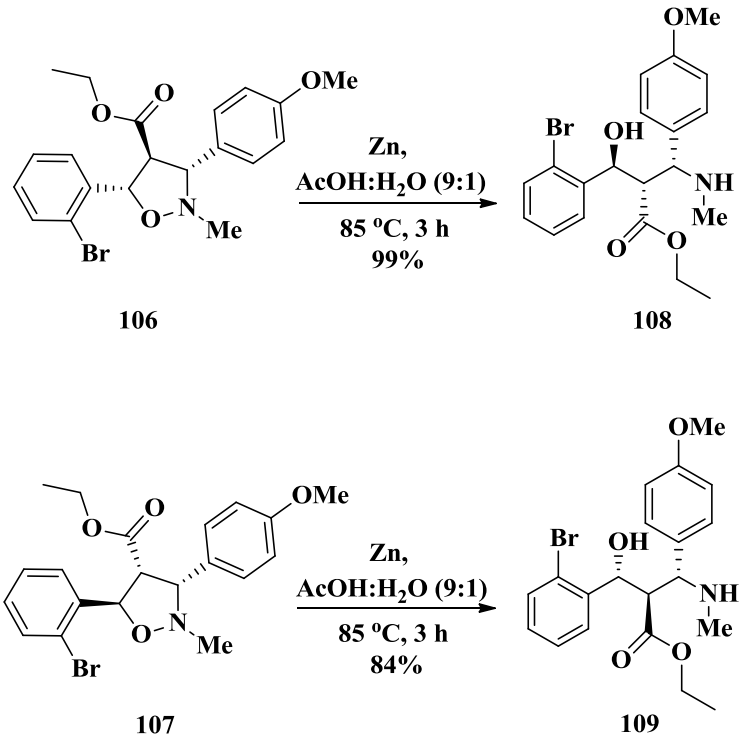


Figure 4.10

Reduction of separated cycloadducts **106** and **107** with Zn in acetic acid: H_2O (9:1) afforded the corresponding amino alcohols **108** and **109** in 99% and 84% yield respectively (Scheme 4.12).



Scheme 4.12

Analogously to the previous cases, NOESY 1D spectrum of a $CDCl_3$ solution of **108** is in accord with a cyclic conformation stabilized by an intramolecular H-bond [Figure 4.11].

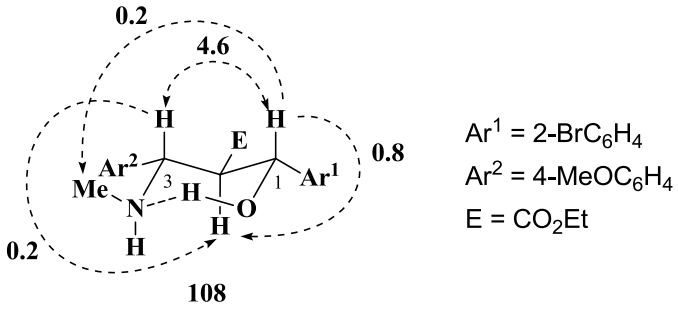
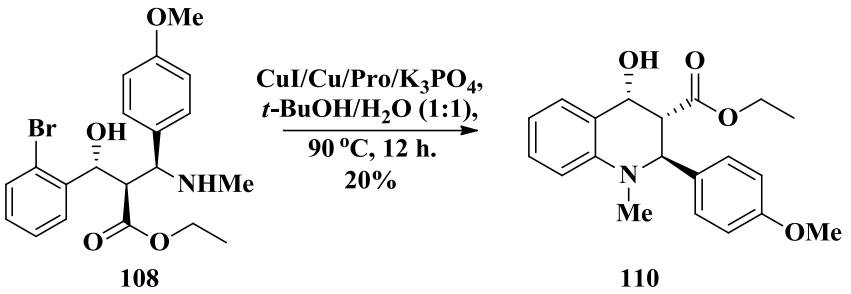


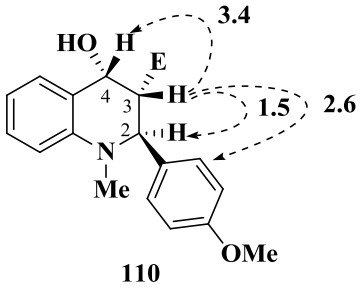
Figure 4.11

Under the usual reaction conditions, amino alcohol **108** underwent intramolecular cyclization affording the trisubstituted tetrahydroquinoline **110** in low yield (20%) (Scheme 4.13).



Scheme 4.13

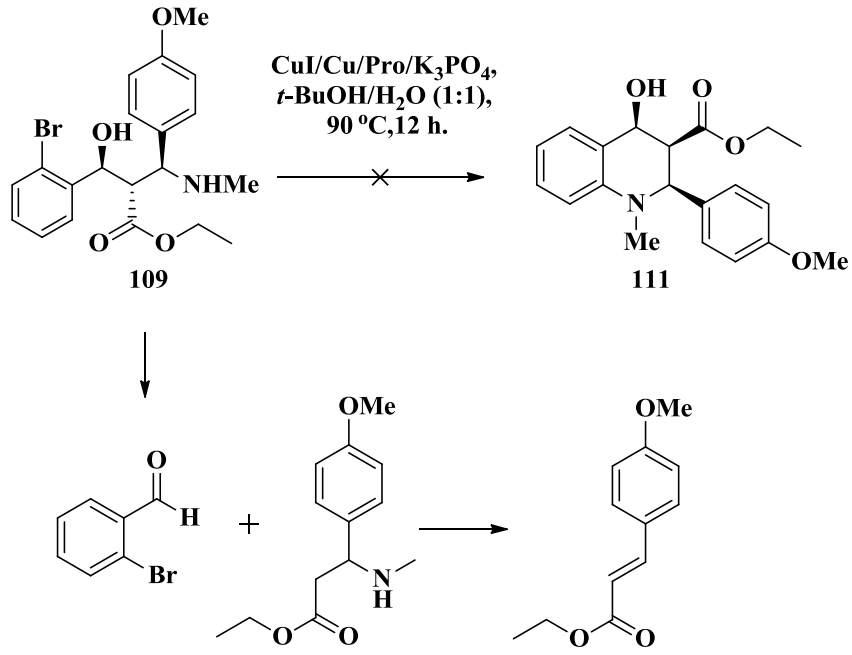
The stereochemistry of the final product **110** was confirmed by NMR analysis. In particular, the value of NOE interactions between 3-H and 2-H and 4-H were in accord, respectively, with a *trans* and a *cis* relationship [Figure 4.12].



E= CO_2Et

Figure 4.12

Under the usual condition, diastereoisomer **109** failed to give the corresponding tetrahydroquinoline derivative **111** but was converted into a complex mixture of decomposition products including 2-bromobenzaldehyde and (*E*)-ethyl 3-(4-methoxyphenyl)acrylate. These side products likely formed through retro-aldol and retro-Michael type reactions (Scheme 4.14).



Scheme 4.14

As in the case of tetrahydroquinoline **97**, the low yield of **110** can be rationalized by steric factors that hamper the ring closure. In the case of compound **111**, the presence of three substituents on the same face of the ring system prevents completely the intramolecular cyclization favoring the fragmentation processes [Figure 4.13]

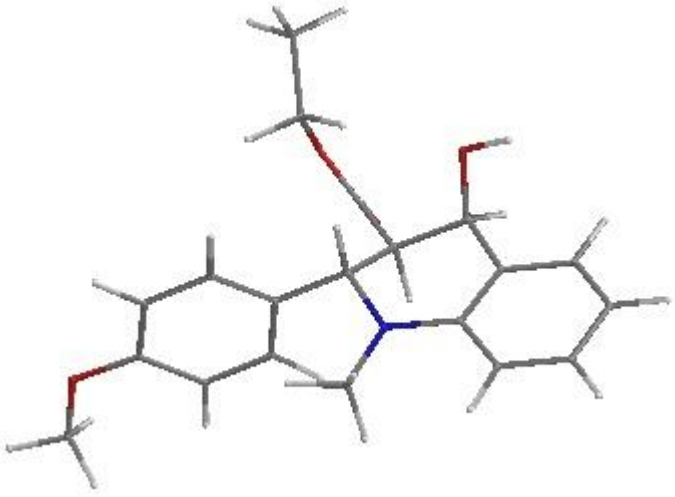
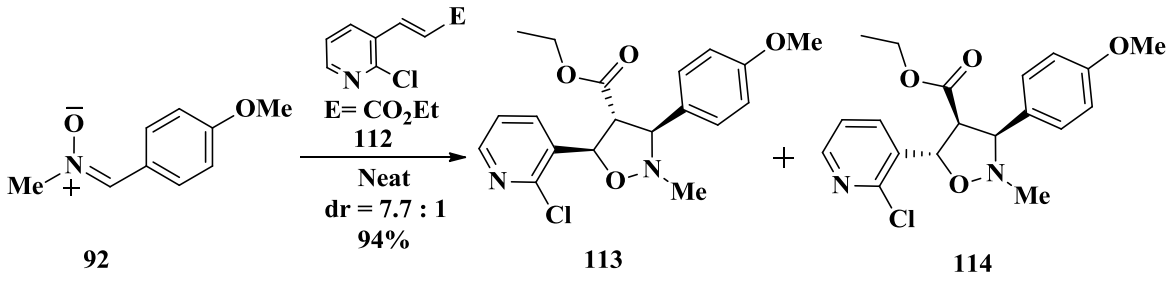


Figure 4.13 The three Dimensional structures of diastereoisomer **111**

4.2.5 Synthesis of (2*R, 3*S**,4*S**)-ethyl 4-hydroxy-1-methyl-2-phenyl-1,2,3,4-tetrahydro-1,8-naphthyridine-3-carboxylate (117)**.

Finally we studied the application of the reported 3-step sequence to the synthesis of a naphthyridine derivative. Naphthyridines are interesting molecules that can be used as ligands for biological and pharmacological applications.[125b] Treatment of a mixture of nitrone **91** and (*E*)-ethyl 3-(2-chloropyridin-3-yl) acrylate (**112**)[125] at 90 °C for 48 h under solvent free conditions afforded diastereomeric cycloadduct **113** and **114** in 7.7:1 ratio and 94% overall yield (Scheme 4.15). Analogously to the 1,3-DC of **92** with **105** [see Figure 4.10], the favored approach between **92** and **112** occurs through an *exo*(Ar)*endo*(CO_2Et)-TS, but in this case the diastereoselectivity was higher (ds 89% vs 68%) and the major adduct, the *trans-trans*-trisubstituted isoxazolidine **113**, was obtained after chromatographic separation in 94% yield.



Scheme 4.15

The relative configuration of **113** and **114** was assigned by analysis of their NOESY-1D spectra. In particular, in both the isomers the hydrogen 5-H and 4-H are *trans*-oriented (NOE 1.1%). Moreover, hydrogen 5-H has a NOE interaction with 3-H (0.7%) in **113** and with the 4-OMe-C_6H_4 (0.6%) in **114** [Figure 4.14].

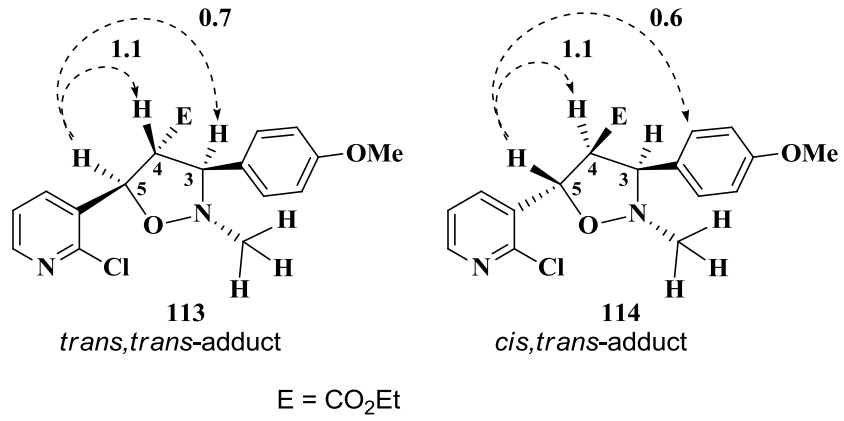
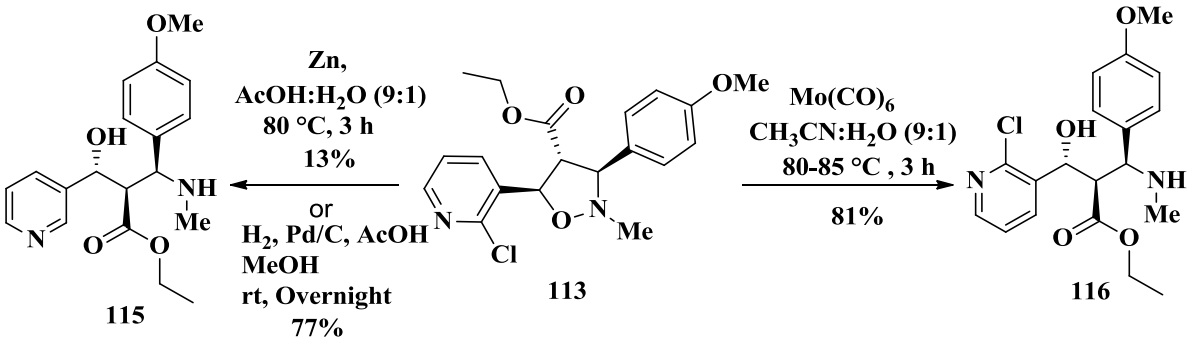


Figure 4.14

The usual reduction conditions (Zn in acetic acid/H_2O) could not be used to open the isoxazolidine ring in **113** because under these conditions the pyridine-chlorine bond was also cleaved (Scheme 4.16). An analogous result was obtained by hydrogenation in the presence of Pd/C and acetic acid in MeOH.

We tried, then, to reduce the cycloadduct in milder reaction conditions. A chemoselective reduction of the N-O bond could be finally achieved following a methodology developed in the Chemistry Department by Prof. A. Brandi's research group i.e. using $Mo(CO)_6$[126] as reducing agent in acetonitrile/water at the reflux temperature. Under this milder reaction conditions amino alcohol **116** was obtained in high yield (81%) (Scheme 4.16).



Scheme 4.16

Analogously to the previous cases, NOESY 1D spectrum of a $CDCl_3$ solution of **93** is in accord with a cyclic conformation stabilized by an intramolecular H-bond [Figure 4.15].

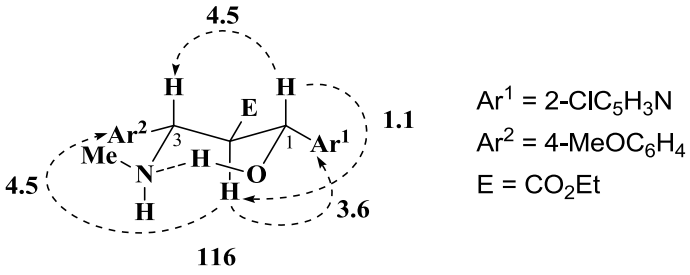
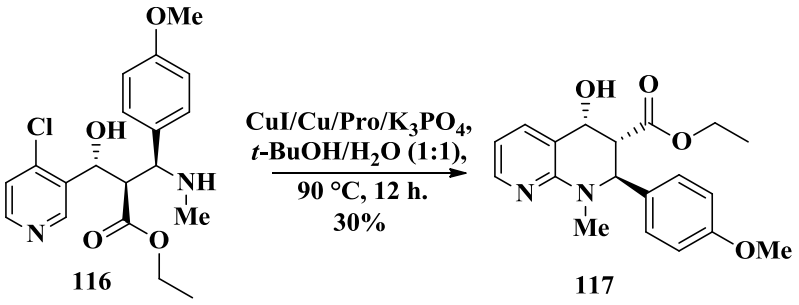


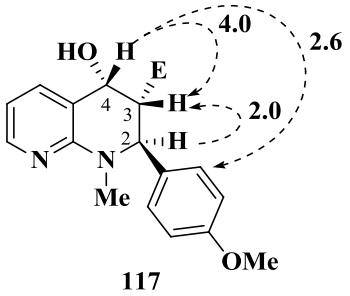
Figure 4.15

Finally, **116** was cyclized under the usual Ullmann reaction conditions to get highly functionalized naphthyridine **117** in 33% yield (Scheme 4.17). The cyclization yield was only modest, but higher than the corresponding trisubstituted tetraidroquinoline **110** (see Scheme 4.17). This last example prove that the three step approach can be applied also to prepare naphthyridine derivatives and that in this case the presence of the electron poor aromatic ring allow also the use of chlorine as leaving group in the copper catalyzed cyclization.



Scheme 4.17

The stereochemistry of the final product **117** was confirmed by NMR analysis. In particular, the value of NOE interactions between 3-H and 2-H and 4-H were in accord, respectively, with a *trans* and a *cis* relationship [Figure 4.16].

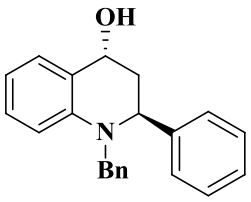


117

E= CO_2Et

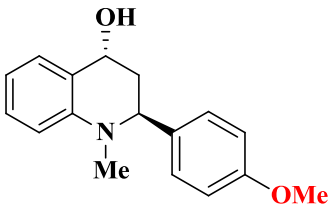
Figure 4.16

By using our three step reaction approach towards synthesis of tetrahydroquinoline, were synthezed the following molecules by using nitrones [Figure 4.17].



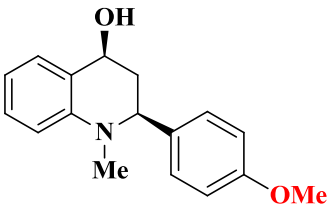
91

44%



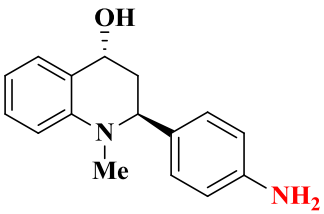
97

38%



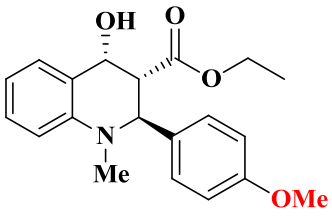
98

71%



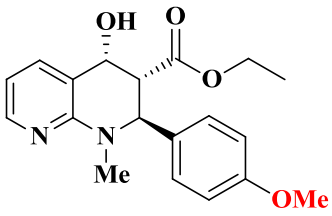
104

50%



110

20%



117

33%

Figure 4.17

4.3 Conclusion

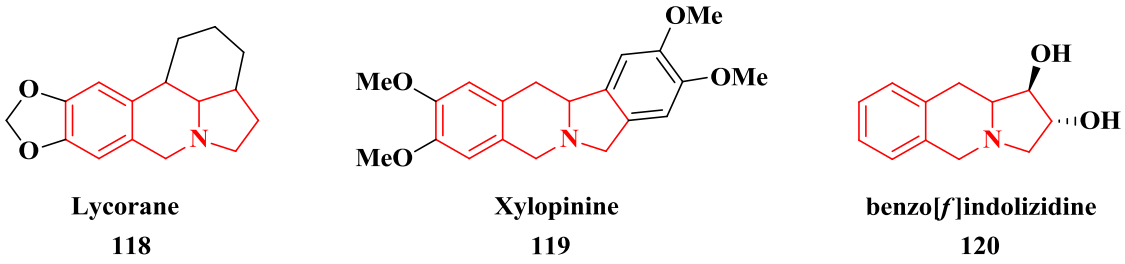
The new synthetic methodology described demonstrated useful for the synthesis of complex heterocyclic compound for natural product synthesis or for biological application. Our three step reaction sequence is useful to contruct substituted tetrahydroquinolines which are used in material chemistry, natural chemistry and medicinal chemistry.We applied our developed methodology to acyclic *C*-Aryl nitrones that led to synthesis of highly functionalized tetrahydroquinolines and a naphtiridine.

Stereoselective synthesis of benzo[*f*]indolizidine and benzo[*g*]indolizidine

5.1 Stereoselective synthesis of benzo[*f*]lentiginosine via Pd catalyzed intramolecular carbonylation of secondary amine.

5.1.1Introduction

Heterocyclic compounds, especially aza-heterocycles, are ubiquitous in natural products, pharmaceuticals and represent the most important class of key structural units in large number of bioactive molecules. The amaryllidaceae alkaloids, such as Lycorane and crinane, The Protoberberines alkaloids are the large group of naturally occurring alkaloids contains same basic core structure like benzo[*f*]indolizidine **120** [Figure 5.1].[128]



Lycorane **118**

Xylopinine **119**

benzo[*f*]indolizidine **120**

Figure 5.1

Indolizidine alkaloids, lentiginosine and its stereoisomers are widespread in plants and microorganisms. Their intriguing structure combined with their potent ability to inhibit glycosidase as well as anticancer activity, anti-HIV and immunoregulatory activity. Aim of our thesis is to synthesis the modified derivative of lentiginosine to improved biological activity of lentiginosine. In chapter 3, it was discussed synthesis of benzo[*e*]indolizidines **64** and **68**, and their biological activity. Benzo[*e*]indolizidine **64, 68** are not more biologically active, likely because nitrogen atom is in conjugation with aromatic ring and lone pair of

nitrogen is not available for protonation to form the tetrahedral mimic of transition state enzyme hydrolysis. We decided, then to synthesize benzo[*f*]indolizidine **120** to check biological activity as well as hypothesis based on computational docking studies with amyloglucosidase [Figure 5.2].

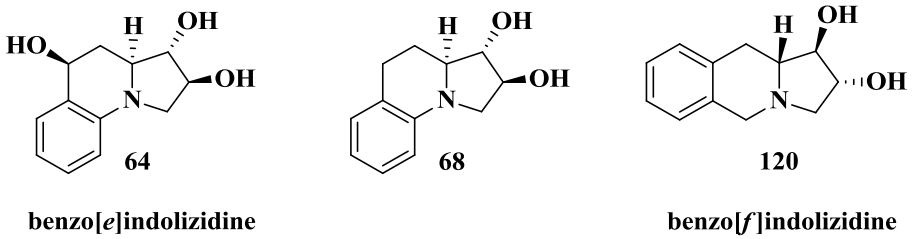
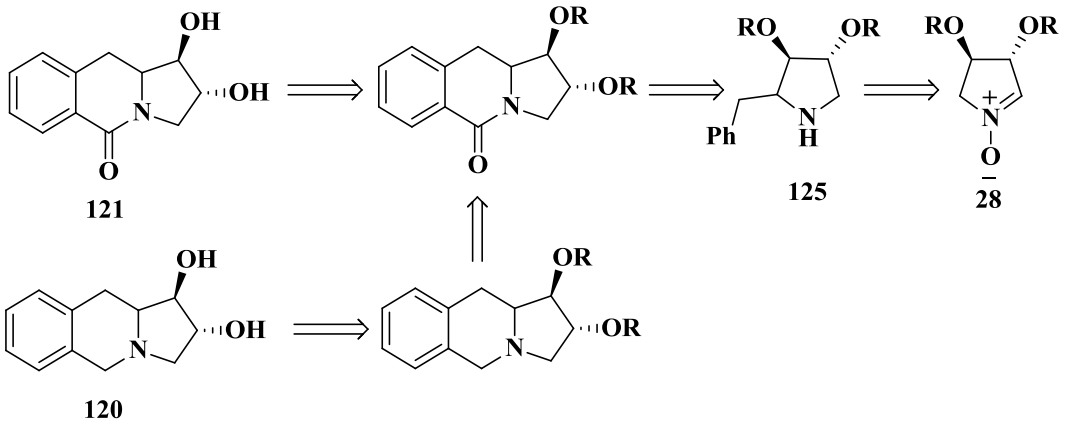


Figure 5.2

Our approach towards the enantiomer of benzo[*f*]lentiginosines **120** starts from the enantiopure pyrrolidine-*N*-oxides **28** derived from L- and D-tartaric acid. Benzo[*f*]lentiginosine **120** was obtained by alkylation of pyrrolidine *N*-oxides **28** with a suitable Grignard reagent followed by hydroxylamine reduction, ring-forming carbonylation, amide reduction and OH-deprotection (Scheme 5.1).

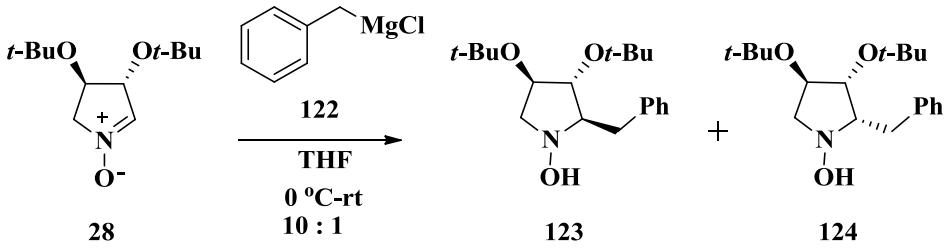


Scheme 5.1

5.1.2 Result and discussion

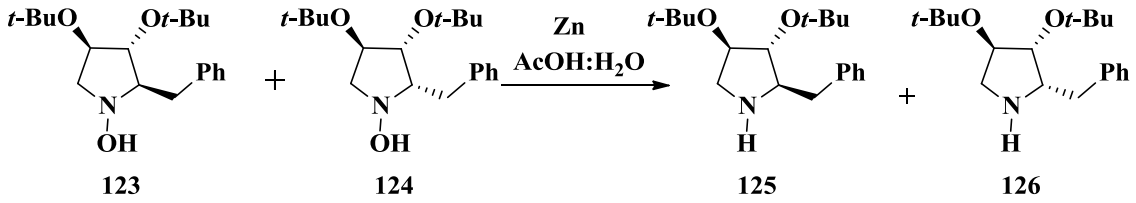
5.1.2.1 Synthesis of benzo[*f*]lentiginosine from 3,4-bis-*tert*-butoxypyrroline *N*-oxide (28) via carbonylation of secondary amine.

Alkylation of pyrrolidine-*N*-oxides **28** which is derived from D-tartaric acid was carried out with benzylmagnesium chloride **122** at 0 °C in THF to afford mixture of hydroxylamines **123** and **124** in 10:1 ratio, respectively, not separable by column chromatography (Scheme 5.2).



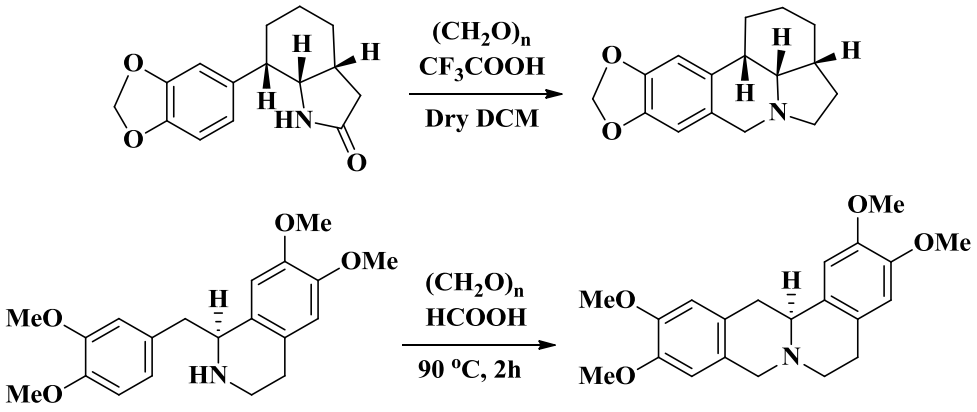
Scheme 5.2

Subsequent reduction of the mixture of hydroxylamines **123** and **124** with Zn in acetic acid/water (1:1) at room temperature for 2 h gave pyrrolidines **125** and **126** with 93% yield. Pyrrolidine **125** and **126** were partially separated by column chromatography (Scheme 5.3).



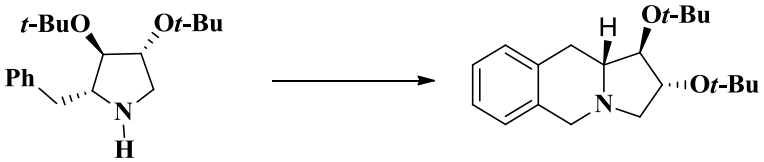
Scheme 5.3

Several reports can be found in the literature for the next goal: the cyclization of a secondary amine with aromatic ring with one carbon insertion (Scheme 5.4). In literature reports, paraformaldehyde was used as the source of carbon homologation, in a Pictet-Spengler type cyclization. All reactions were carried out in different reaction conditions in order to obtain cyclized product. Some model reaction are given below (Scheme 5.4).[129, 130]



Scheme 5.4

We applied same the reaction condition with our substrate, but unfortunately we were not able to obtain our desired product (Scheme 5.5, Table 1).



125 **127**

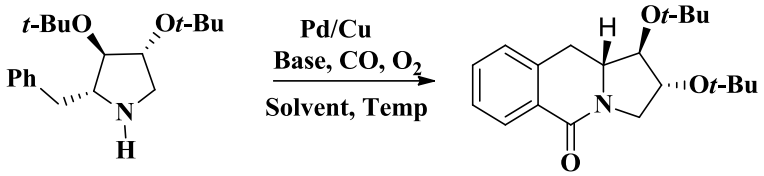
Scheme 5.5

Table 1

No	Catalyst	Temp (T ^{o}C)	Time (Hours)	Product
1	HCHO, HCOOH	90	7	-
2	HCHO, $Sc(OTf)_3$, CH_3CN:H_2O	rt	24	ND
3	HCHO, CF_3COOH, Dry DCM	rt	24	ND

One likely reason for the failure in the obtainment of **127** is the nature of the aromatic ring. In fact the aromatic ring in **125** is not activated towards an electrophilic intramolecular cyclization of the intermediate methyleniminium cation. The activation of aromatic ring needs some activating group, for ex. OR groups, like the examples of Scheme 5.4. As we wanted the unsubstituted aromatic ring, we had to find another possible way to close the ring.

In the literature were found some reports on carbonylative cyclization of amines.[131] Carbonylation of primary amines has been done by many groups, but the carbonylation of secondary amines is reported in very few reports, especially for cyclic amines.[132] The carbonylation of amines by $Pd(OAc)_2$ catalysis under CO atmosphere was studied by Orito et al.[132] We then applied the condition reported for carbonylation of **125**. The catalyzed carbonylation was carried out under CO/O_2 atmosphere in the presence of Pd(II) catalyst in combination with a Cu(II) salt and gave as product the amine carbonylation product followed by cyclization on the aromatic ring (Scheme 5.6, Table 2).



125 128

Scheme 5.6

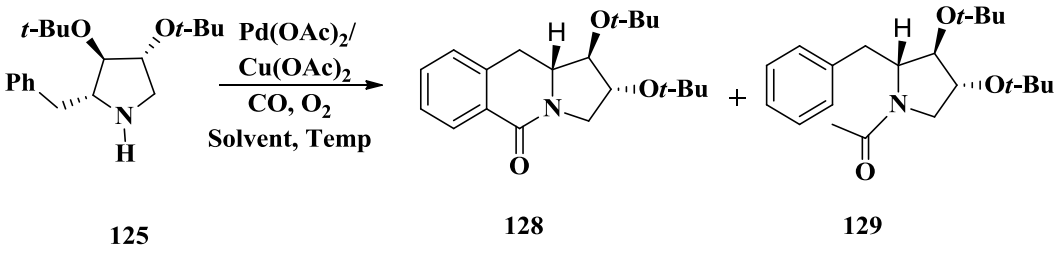
Table 2

	M Source (Mol eq)	Base (eq)	Solvent	T (^oC)	t (h)	Yield (%)
1	$PdCl_2$ (0.05) /$CuSO_4$(1.5)		Dioxane:H_2O	100	4	0
2	$PdCl_2$ (0.1) /$Cu(OAc)_2$ (3)		Toluene	110	6	0
3	$PdCl_2$ (0.1) / $CuCl_2$ (3)		Toluene	110	5	0
4	$Pd(OAc)_2$(0.1)/$Cu(TFA)_2$(1),		Dioxane	100		0
5	$Pd(OAc)_2$(0.2) /$Cu(OAc)_2$(3),	*t*-BuOK (2.5)	DMF	140	6	0
6	$Pd(MeCN)_2Cl2$(0.3)/$Cu(OAc)_2$(3),	*t*-BuOK (2.5)	Toluene	110	6	21
7	$Pd(MeCN)_2Cl2$(0.2)/$Cu(OAc)_2$(3),	*t*-BuOK (2)	Dioxane	110	15	52
8	$Pd(OAc)_2$(0.05) /$Cu(OAc)_2$(0.5),		Toluene	110	4	43
9	$Pd(OAc)_2$(0.1) /$Cu(OAc)_2$(3),	DBU (2)	Toluene	110	4	31
10	$Pd(OAc)_2$(0.2) /$Cu(OAc)_2$(3),	*t*-BuOK (2.5)	Dioxane	110	7	56
11	$Pd(OAc)_2$(0.5) /$Cu(OAc)_2$(4),	*t*-BuOK (3)	Dioxane	110	24	72

Table 2 Intramolecular Pd catalyzed carbonylation reaction of secondary amine.

The carbonylation reaction was carried out by using $PdCl_2$ in combination with $CuSO_4$, $Cu(OAc)_2$ and $CuCl_2$, but the catalyst was not even partially soluble in reaction solvent, and decomposition of catalyst occurred[Table 2, entries 1-3]; The starting material was just recovered. Increasing the catalyst loading didn't cause any change in the reaction. The use of $Pd(OAc)_2$with $Cu(TFA)_2$ [Table 2, entry 4], gave the same solubility problems and decomposition of the catalyst. We tried to make some more variation with Pd catalyst in combination with $Cu(OAc)_2$, because $Cu(OAc)_2$ showed better solubility than other Cu salts. Different Pd catalyst were used [Table 2, entry 2, 6 and 8] with varied molar amounts, but $Pd(OAc)_2$ /$Cu(OAc)_2$ [Table 2, entry 8] gave the most satisfactory results than other combination of Pd catalyst with $Cu(OAc)_2$. Knowing the best combination of catalyst for this reaction, then variations were made of the solvent, temperature and reaction time. All this reactions needed high temperature, so an high boiling solvent with good solubility of the catalyst is necessary. We carried out one trial in DMF at 140 °C with $Pd(OAc)_2$/$Cu(OAc)_2$ [Table 2, entry 5] , but here decomposition of catalyst and starting material occurs. It was necessary to reduced the temperature of the reaction, some trials were carried out in toluene as well as in 1,4-dioxane at 110 °C: dioxane gave better result than toluene. The $Pd(OAc)_2$ (0.5 equiv) $Cu(OAc)_2$ (4 equiv) in dioxane is the best combination of catalyst found, showing a better solubility than other cases, albeit the amount of Pd catalyst is excessive [Table 2, entry 11]. In this sense the conditions in entry 10 appear the best compromise. The molar amount of $Cu(OAc)_2$ is high, almost ten times that of Pd, because for the reaction is required a Pd (II) catalyst, and $Cu(OAc)_2$ oxidizes Pd (0) back to Pd (II) [see below the mechanism, Figure 5.4]. Applying the best reaction condition for carbonylation, the product **128** was recovered along with the side product **129** (scheme 5.7). When the reaction was carried out without base, the

side product **129** increased at the expense of **128.** Since in the reaction using $Pd(OAc)_2$ [see below the mechanism, Figure 5.4] is formed acetic acid, it reacts with the pyrrolidine **125** to produce amide **129**, which, then, cannot undergo catalyzed cyclization on the aromatic ring.



125 **128** **129**

Scheme 5.7

A base is then necessary to avoid the formation of side product **129**. However, some acidic condition are necessary for the reaction to occur, then the base is added in a lower molar amount than $Cu(OAc)_2$. The best reaction conditions for carbonylation of secondary amine, then, are those in entry 10 and 11[Table 2]. The use of a lower amount of Pd (0.2 equiv) afforded 56% yield of **128** in 7 h. Higher amount of catalyst (0.5 equiv) of course afforded an higher yield (72%), but this yield was also obtained in much longer reaction time.

Assignment of structure to compound **128** was confirmed by NMR spectroscopy [Figure 5.3]

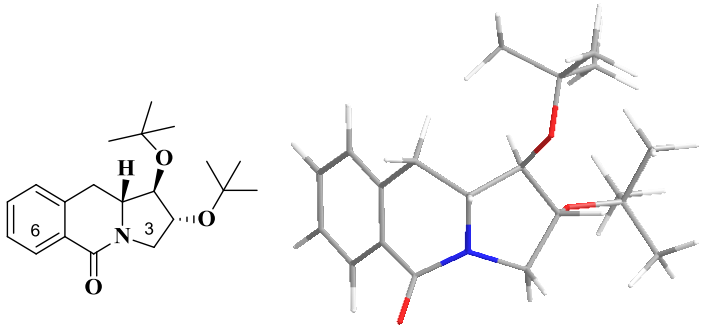


Figure 5.3

Due to presence of carbonyl group on six member ring connected with the five membered ring, the structure of **128** is rigid and there is very little conformational freedom. In ^{1}H NMR spectra, 6-H aromatic proton resonated at δ 8.01 (dd, J = 7.6, 1.4 Hz), more deshielded than other aromatic protons, because of the presence of the vicinal amide group, this is a confirmation of with the ring closure and bond formation between carbonyl group and aromatic ring. Effect of carbonyl group also deshielded 3-H protons at δ 3.60 and δ 3.44, compared to lentiginosine (δ 2.76 and δ 2.22).

Possible mechanism of Pd catalyzed carbonylation reaction is given below [Figure 5.4].

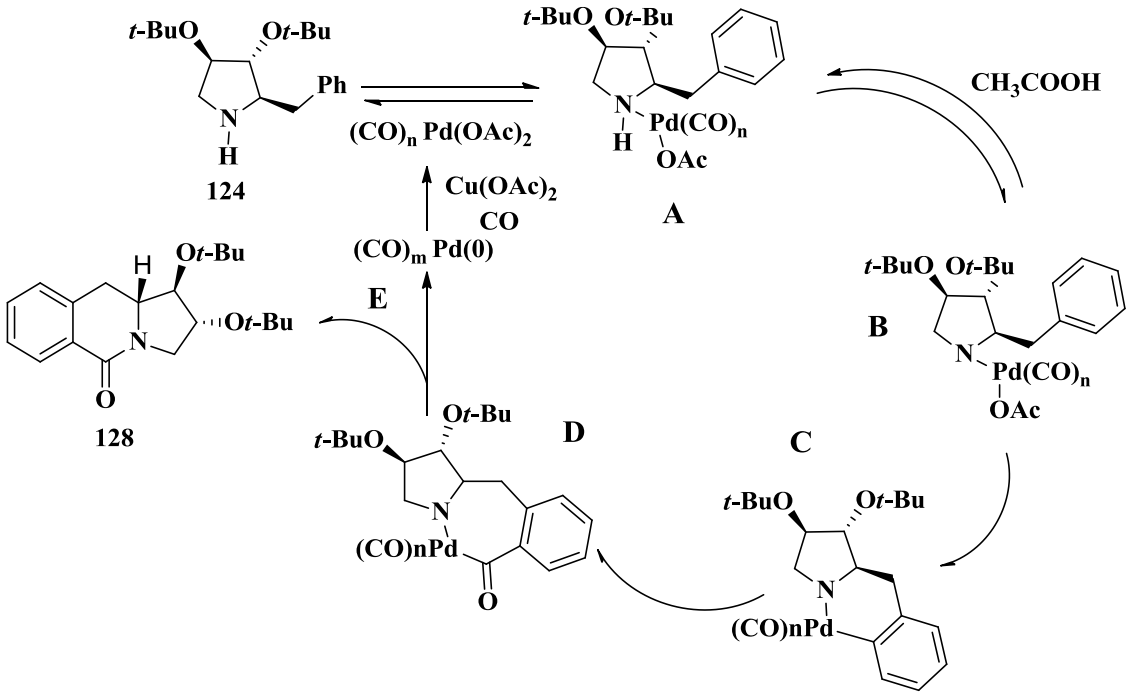
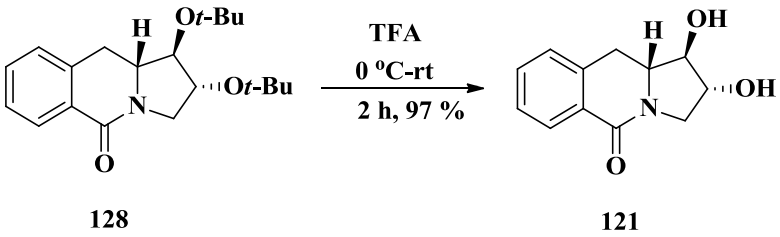


Figure 5.4

Pd (II), first coordinate with nitrogen to formed complex **A** by reduction of Pd (II) into Pd (I). Then elimination of acetic acid to get the complex **B**. Pd (I) convert into Pd (0) by coordinate with phenyl ring in order to get complex **C.** Incorporation of CO to formed to formed complex **D**. Subsequent elimination of product **128** with Pd (0), continue cycle by oxidation of Pd (0) into Pd (II) with Cu (OAc) $_2$.

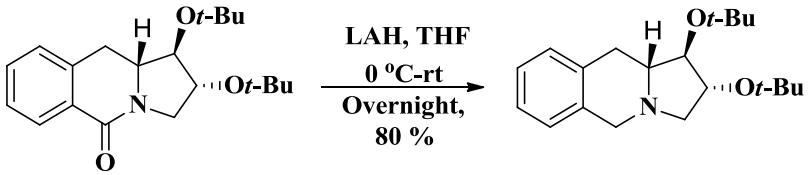
The deprotection of **128** with TFA at 0 °C– rt for 2 h, afforded benzo[*f*]derivative of lentiginosine **121** as a white solid with 97 % yield (Scheme 5.8).



128 121

Scheme 5.8

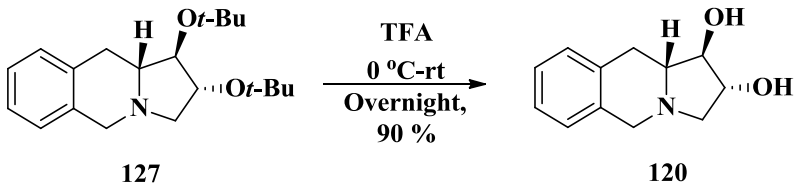
Protected amide **128** was reduced with LAH in THF at 0 °C- rt overnight and afforded **127** in 80% yield (Scheme 5.9).



128 127

Scheme 5.9

Final deprotection of **127** with TFA at 0 °C to rt overnight gave benzo[*f*]lentiginosine **120** as a white solid in 90% yield (Scheme 5.10).



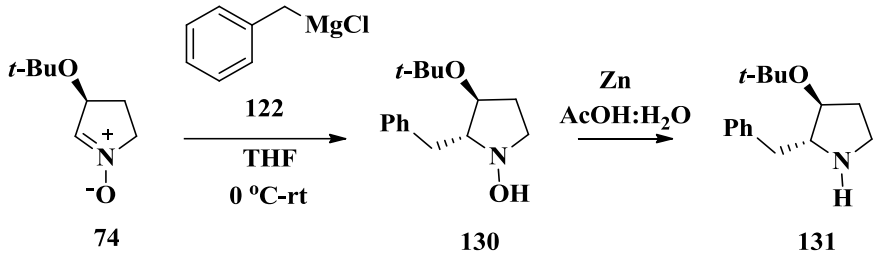
127 **120**

Scheme 5.10

The same reaction sequence was followed to synthesize ***ent*-120** and ***ent*-121**.

5.1.2.2 Synthesis of benzo[*f*]indolizidine from Nitrone (74) which derived from Malic acid

The synthesis of benzo[*f*]indolizidine was extended by the use of another enantiomerically pure pyrrolidine *N*-oxide **74**, derived from L-Malic acid. Alkylation of pyrrolidine *N*-oxide **74** was carried out with benzylmagnesium chloride **122** at 0 °C in THF to obtain the single isomer **130** with 100% yield. This reaction was more selective than reaction with pyrrolidine-*N*-oxides **28.** Reduction of hydroxylamine **130**with Zn in acetic acid/water (1:1) at room temperature for 2 h afforded pyrrolidine **131** with 85% yield (Scheme 5.11).



Scheme 5.11

The stereochemistry of **131** was assigned by NMR analyses. In particular, a strong NOE interaction was present between 3-H and 4b-H, which could be assigned without doubt because of the proximity of *tert*-BuO group. Lower the NOE interaction, as expected, were present between 3-H and 2-H, and 4a-H [Figure 5.5].

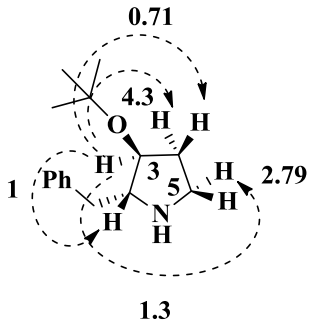
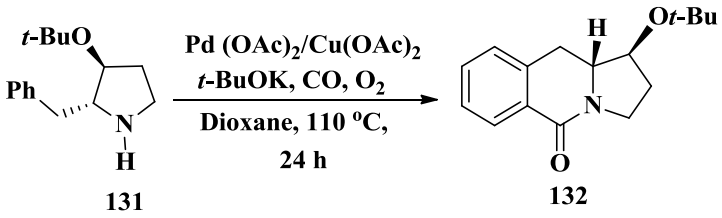


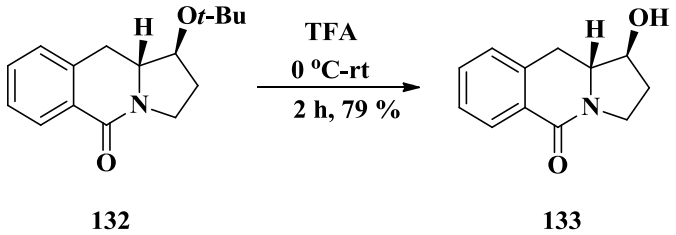
Figure 5.5

Applying the best conditions for carbonylation of secondary amine by using Pd/Cu metal catalysis, the carbonylation reaction was carried out on pyrrolidine **131** to obtain **132** in 45 % yield (Scheme 5.12).



Scheme 5.12

Final deprotection of amide **132** was carried with TFA at 0 °C to rt for 1 h to obtain the benzo[*f*]indolizidine **133** in 79 % yield derived from malic acid. (Scheme 5.13)



Scheme 5.13

5.2 Stereoselective synthesis of benzo[*g*]lentiginosine via coupling of Arynes with Pyrroline-*N*-oxides

5.2.1 Introduction

Arynes, in the past were once mainly the subject of structural curiosity. Within 14 years of the seminal experiments of J. D. Roberts leading to the first proposal of the structure of benzyne [Figure 5.4], synthetic organic chemists recognized the potential to exploit this highly reactive intermediate (and its substituted variants) in the total synthesis of natural products. More specifically, it was recognized that arynes offered the strategic advantage of rapidly functionalizing an aromatic ring by forming multiple carbon-carbon or carbon-heteroatom bonds in a single operation, often in a regioselective manner. Initially, the scope of synthetic applications was somewhat limited by the harsh conditions required to produce the aryne species. Many of these methods required strong bases, such as n-BuLi, or high temperatures. However, with the development of milder methods for the generation of arynes came increased interest in employing them in the synthesis of more complex polycyclic systems [Figure 5.6].[133,134]

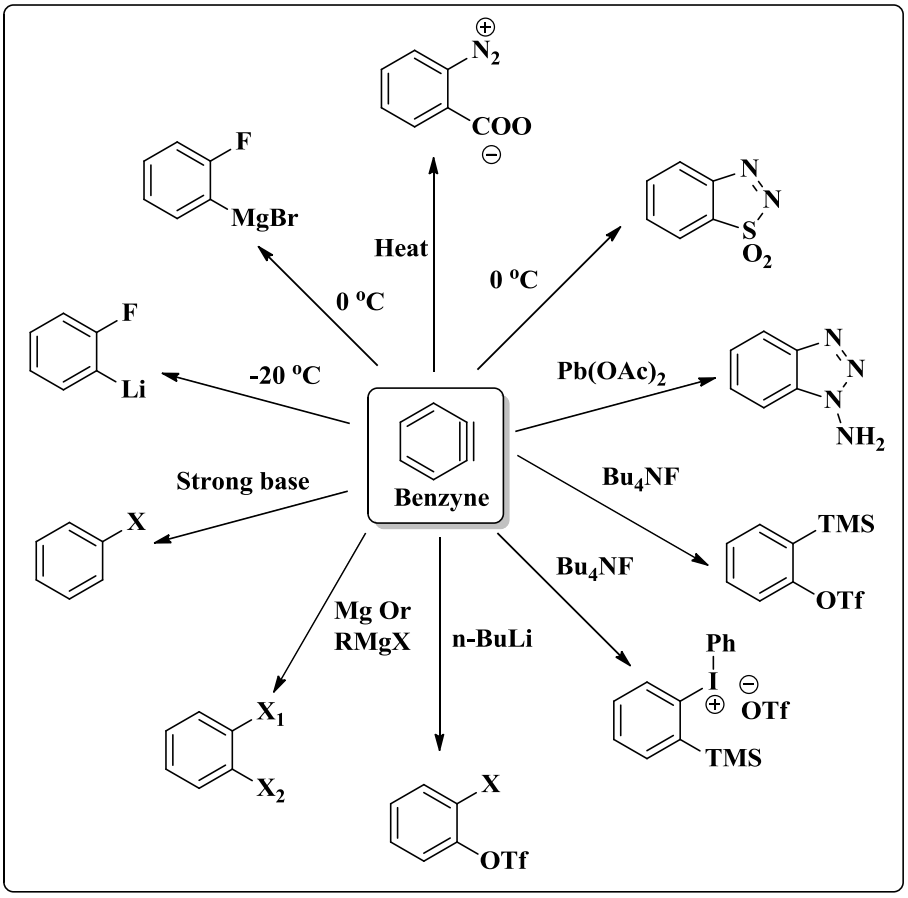


Figure 5.6 Methods for generation of benzyne

Most recently, the use of o-silyl aryl triflates as aryne precursors has allowed generation of the reactive intermediate under almost neutral conditions.[135] The great advantage of generating a benzyne by the Kobayashi route is the ability to control the rate of benzyne generation by varying the concentration of the fluoride ion in solution. Thus, one can instantly generate a benzyne in THF using tetrabutylammonium fluoride (TBAF), which is quite soluble in THF. It is possible to slow down the rate of benzyne generation by employing CsF in MeCN (at room and elevated temperatures) or CsF in THF (at elevated temperatures). To further slow down the formation of the benzyne, CsF in toluene – MeCN mixtures can be used. The nature and amount of the added fluoride source, the solvent, and the temperature of the reaction can have

a profound effect on the overall rate and success of such aryne reactions. Arynes undergo numerous organic reactions [Figure 5.7].[133, 134]

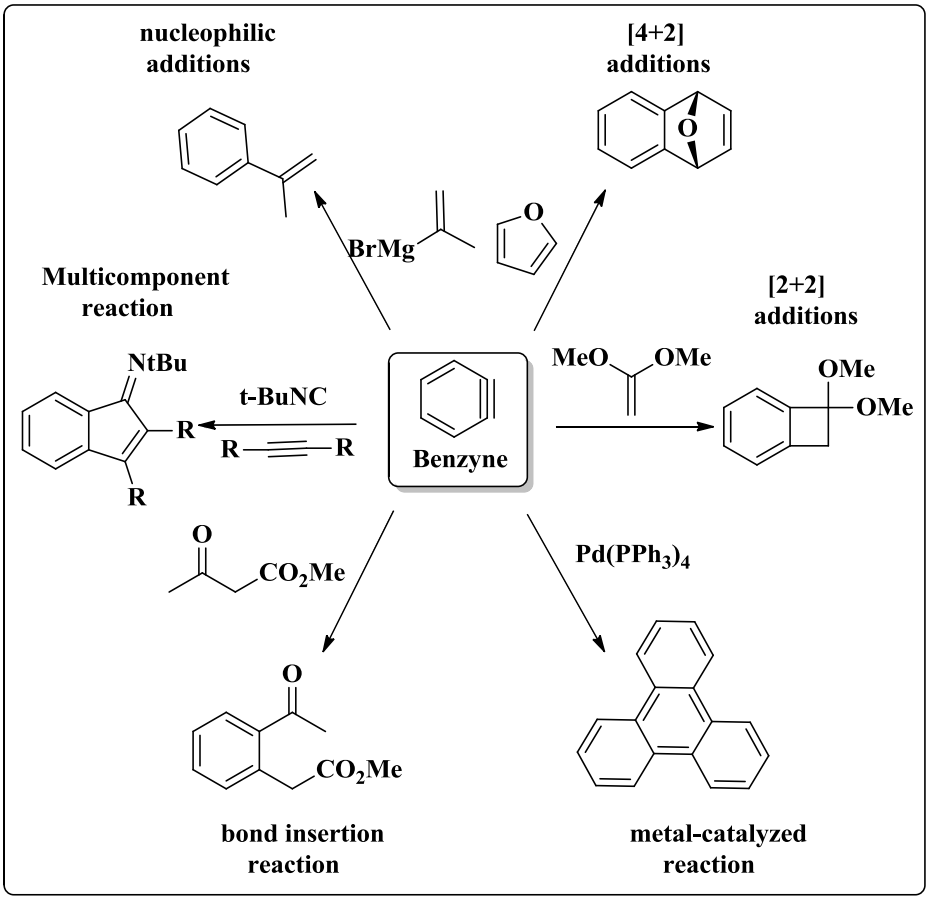
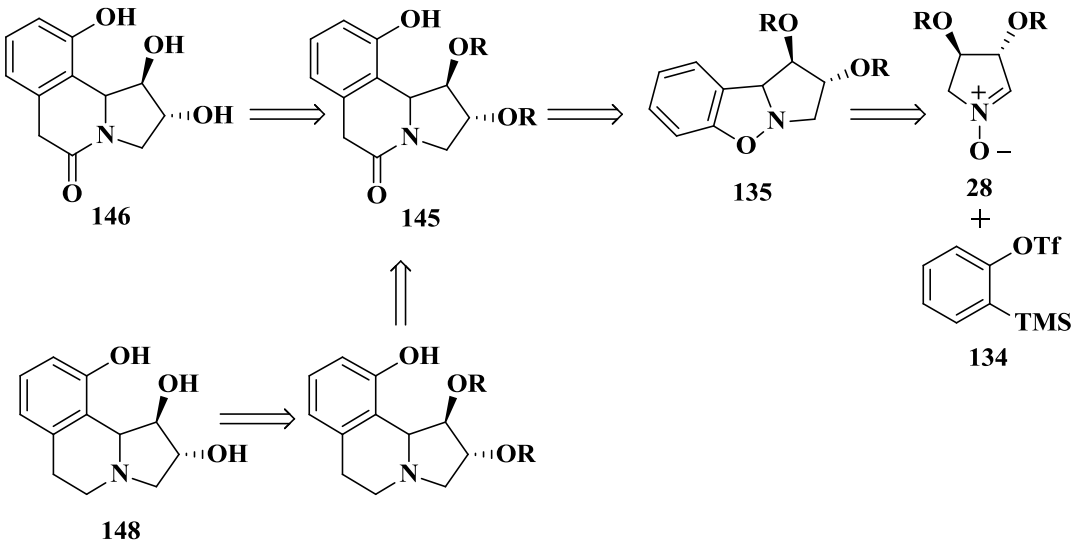


Figure 5.7 Representative reactions of benzyne.

The possibility of arynes to undergo 1,3-dipolar cycloaddition reactions deserve special mention owing to their ability to produce pharmaceutically important nitrogen containing heterocycles. Several dipoles have been systematically studied for their reactivity with arynes, leading to interesting heterocycles. However, only a few nitrones, mostly achiral ones, have been investigated as dipole partners for arynes.[136-138]

We choosed to employ aryne strategy for our synthesis of benzo[*g*]lentiginosine via coupling with nitrone. Nitrones have long been used as building blocks in organic synthesis. Having explored tartaric acid derived cyclic nitrones for highly diastereoselective 1,3-dipolar cycloaddition reactions, we envisioned that the reaction of these nitrones with benzyne would lead to benzoisoxazolines **135**, which upon cleavage of *N*–O bond would afford *o*-hydroxyaryl-substituted pyrrolidines **143**. Pyrrolidines, by treatment with chloroacetyl chloride and subsequent ring closure mediated by Lewis acid could form the tricyclic core structure **145,** which by reduction and deprotection could afford the target benzo[*g*]lentiginosine **148** (Scheme 5.14).

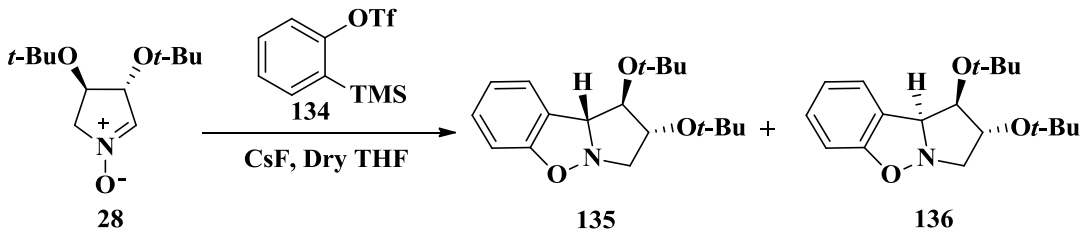


Scheme 5.14

5.2.2 Result and Discussion

5.2.2.1 Synthesis of isoxazolidine ring via cycloaddition of nitrone with aryne

The 1,3-dipolar cycloaddition of 3,4-bis-*tert*-butoxypyrroline *N*-oxide (**28)** with 2-(trimethylsilyl)phenyl trifluoromethanesulfonate (**134**) afforded two diastereisomers **135** and **136** in 1.3:1 ratio (Scheme 5.15).



Scheme 5.15 Cycloaddition of nitrone **28** with benzyne

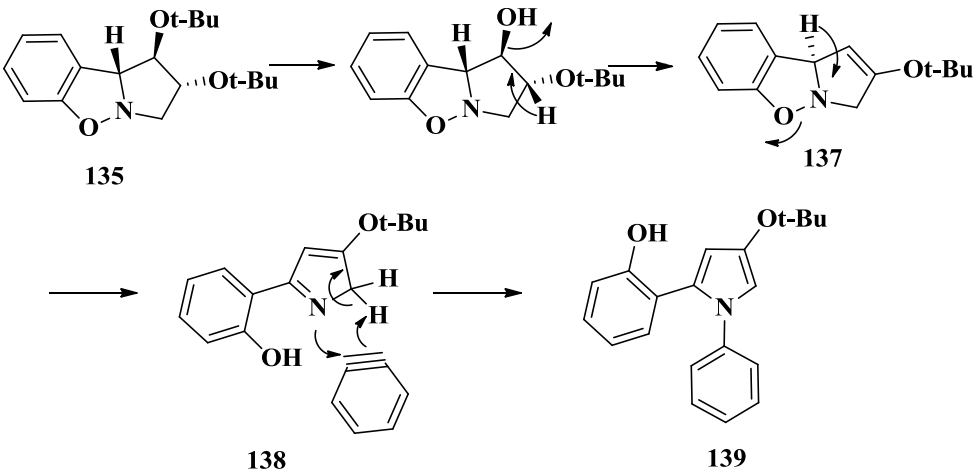
Table 1

No	Aryne equiv	Fluoride source (equiv)	Condition	Time	Yield (%)
1	1.6	CsF (6)	Dry THF, rt	5 days	58
2	2.4	CsF (6)	Dry THF, 60 °C	2 days	56
3	3	CsF (6)	Dry THF, rt	6 days	71
4	3	CsF (6)	Dry MeCN, rt	1 day	16
5	1.5	CsF (6)	MeCN:Toluene (1:1), 55 °C	3 days	36
6	1.5	Bu_4NF (4)	Dry DMF, rt	5 h	68
7	1.5	Bu_4NF (4)	Dry DMF , rt	2.5 h	59

The reactions were conducted in the same conditions used in precedent literature reports.[138] In all trials however, were obtained lower yields than literature reports on similar cyclic nitrones, [138] because of the formation of a side product in the reaction with a yield ranging from 40% to 90%. The best condition resulted in carrying out the reaction in dry THF (entry 3, Table 1), where the reaction is slower and requires 3 equiv of the aryne, or in dry DMF, with Bu_4NF as the fluoride source, only 1,5 equiv of the aryne, 5 h and 2.5 h reaction time at rt (entry 6 and 7, Table 1). Going through complete analysis of the side product of the cycloaddition reaction, it was assigned to it the structure **139**.

The compound, indeed, contains a phenol substituted pyrrole ring, a substructure that recalls the occurrence of a cycloaddition process, but the pyrrolidine ring, besides aromatization, has lost a *t*-BuO substituent, and, moreover, has undergone a N-phenyl substitution. All these hints suggested that the side product **139** could derive from a decomposition of the primary cycloadduct **135**.

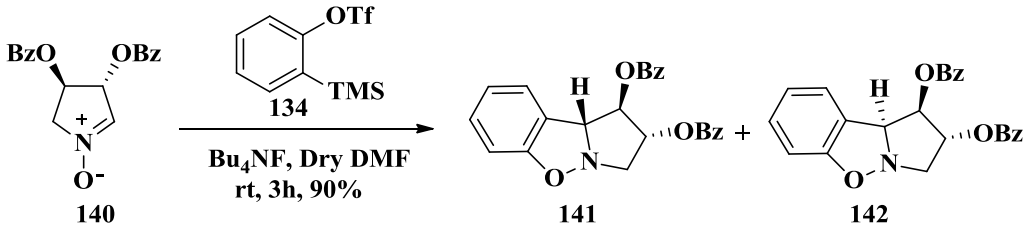
A plausible mechanism is given below (Scheme 5.16).



Scheme 5.16 Plausible mechanisms for conversion of cycloadduct **135** into side product **139**

In the proposed mechanism, one of protected hydroxy group might undergo deprotection due to acidic conditions of the reaction. Subsequent elimination of water molecule affords a pyrroline ring **137** which opens to **138**. This process must be driven by the consequent possible aromatization of the pyrroline ring. Finally the intermediate pyrroline **138** can react with aryne to form the N-phenyl pyrrole **139**. This process is probably favored by the presence of the two bulky *t*-BuO groups on the cycloadduct. Work is in progress to understand if with only one *t*-BuO group (nitrone derived from malic acid) this side reactivity could be suppressed, or to understand the role of protecting groups.

In order to check role of protecting group, one more trial was carried out by using benzoyl protected nitrone. The 1,3-DC of (bis)benzoylated nitrone **140** with 2-(trimethylsilyl) phenyl trifluoro-methanesulfonate (**134**) afforded two diastereisomers **141** and **142** in 90% yield without obtaining side product (Scheme 5.17).

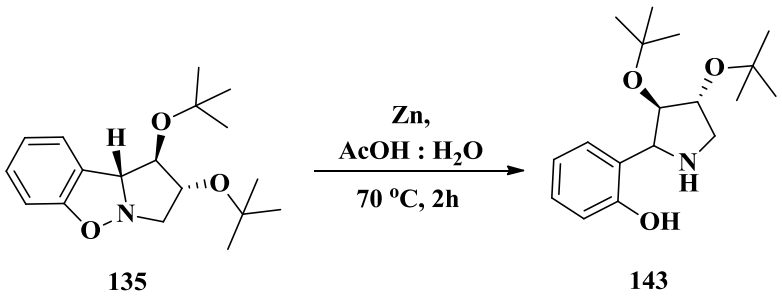


Scheme 5.17

The protecting group play more crucial role in 1,3-DC of nitrone with benzyne. We are successfully able to suppress the formation of side product which occurs in 1,3-DC reaction nitrone **28** with benzyne. The replacement of protecting group from *tert*-butyl to benzoyl afforded good yield of product in short reaction time.

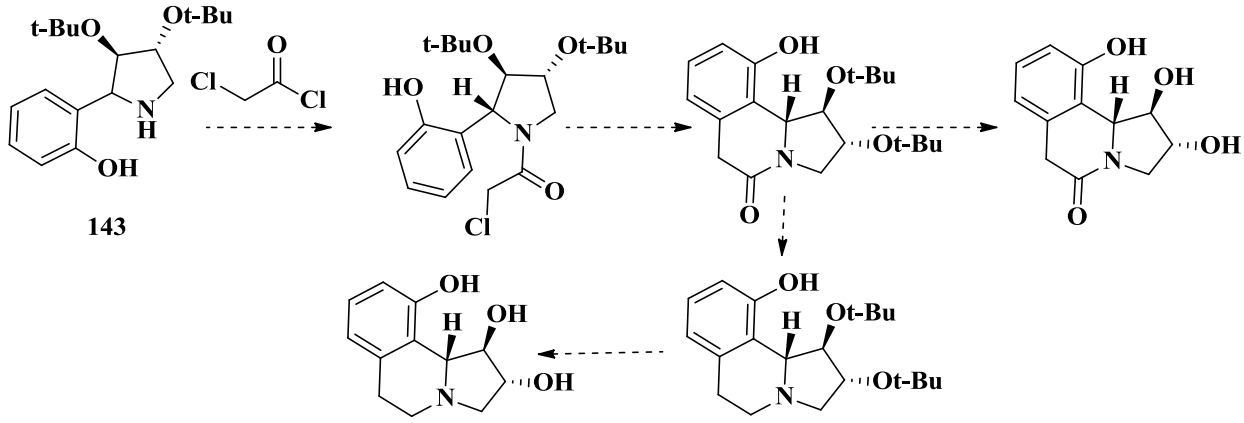
5.2.2.2 Reductive cleavage of isoxazolidine ring

Cycloadduct **135** was reduced with Zn in acetic acid/water (1:1) at 70 ^{o}C for 2 h to obtain *o*-hydroxyaryl pyrrolidines **143** with 75% yield (Scheme 5.18).



Scheme 5.18 Reduction of Isoxazolidine ring

Further synthesis of benzo[*g*]lentiginosine is in progress (Scheme 5.18).



Scheme 5.18

5.3 Conclusion

In this chapter, it was discussed the synthesis of benzo[*f*]lentiginosine, by using Pd/Cu metal catalyzed carbonylation reaction of a secondary amine. The best reaction conditions for carbonylation of a cyclic secondary amine, a not trivial goal, and its further cyclization on an unactivated aromatic ring were indentified . The new methodology could be nicely developed for carbonylation of other secondary amines. By using this approach, were synthesized tricyclic structures which are the core structure of many natural products. In preliminary biological test, benzo[*f*]lentiginosine **120** and and lactam **121**show only limited proapoptotic activity against different strain of cancer cells. Their glycosidase activity is still under scrutiny.

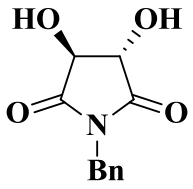
By using aryne chemistry approach, we envisioned an entry to the benzo[*g*]indolizidine ring system, on the way to benzo[*g*]lentiginosine, that is still in progress in our laboratorie

Experimental section

General Remarks: All the reactions requiring anhydrous conditions were carried out under nitrogen, and the solvents were appropriately dried before use. Chromatographic purifications were performed on silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM, Merk) using flash-column technique; R_f values refer to TLC on 0.25 mm silica gel plates (Merck F254, Macherey-Nagel precoated sheets) with the same eluant indicated for column chromatography unless otherwise stated. Melting points (m.p.) were determined on a RCH Kofler apparatus. Polarimetric measurements were performed on a JASCO DIP-370 polarimeter. NMR spectra were recorded on Varian Gemini (^{1}H, 200 MHz), Varian Mercury (^{1}H, 200 MHz), Varian Mercuryplus (^{1}H, 400 MHz) instruments using $CDCl_3$ as solvent and were referenced internally to solvent reference frequencies. Assignments were made on the basis of ^{1}H–^{1}H COSY, HMQC, HSQC and HMBC 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 or direct inlet (70-eV ionizing voltage); relative percentages are shown in parentheses. Elemental analyses were performed with a Perkin-Elmer 2400 analyzer. Microwave-assisted reactions were carried out in a CEM Discover™ single mode microwave reactor with IR temperature sensor.

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.

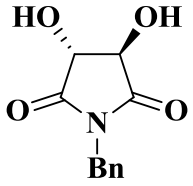
(3S,4S)-1-Benzyl-3,4-dihydroxypyrrolidine-2,5-dione (**35**)



35

Benzylamine (18.21 mL, 0.167 mol) was slowly added to a suspension of (3*S*,*4S*)-(–)-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 **35** 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 **35** are identical to those reported in the literature.

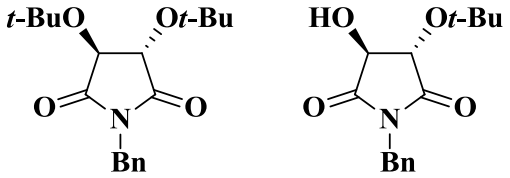
(3R,4R)-1-Benzyl-3,4-dihydroxypyrrolidine-2,5-dione (***ent*-35**)



***ent*-35**

Following the same procedure as for **35**, the enantiomeric ***ent*-35** was prepared starting from (3*R,4R)*-(+)-tartaric acid. The NMR properties of ***ent*-35** are identical to those reported in the literature. [97]

(3*S*,4*S*)-1-Benzyl-3,4-di-*tert*-butoxy-2,5-pyrrolidinedione (36) and (3*S*,4*S*)-1-benzyl-3-*tert*-butoxy-4-hydroxy-2,5-pyrrolidinedione (37).



36 37

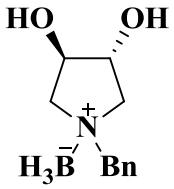
$HClO_4$ (60%, 49.2 µL, 0.4 mmol) was added to a suspension of imide **35** (1 g, 4.5 mmol) in *tert*-butyl acetate (45 mL, 0.1 M) at 0 °C and the reaction mixture was stirred at rt for 18 h. The obtained clear solution was basified to pH 9 by slow addition of a saturated aqueous solution of Na_2CO_3 (20 mL) and solid Na_2CO_3 at 0 °C. The two layers were separated and the two phases treated as follow. The *tert*-butyl acetate solution was washed with brine, dried over Na_2SO_4, filtered and partially distilled under reduced pressure at room temperature for recovering the majority of the solvent (ca 60-65%) that could be recycled or used for other protection reaction. The Na_2SO_4 used to dry the solution was thoroughly washed with CH_2Cl_2 and the obtained solution combined with the distillation residue. The aqueous solution was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by chromatography on silica gel (eluent: CH_2Cl_2/ MeOH first 99.5: 0.5, then 99:1 and 98:2) of all the combined residues afforded **36** (1 g, 68%) as a yellow solid and **37** (0.29 g, 23%) as a white solid.

36: R_f = 0.7 (CH_2Cl_2 / MeOH 99:1); mp 56.2–59 °C; $[\alpha]_D^{24}$= –155.1 (*c* = 0.665, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.40–7.35 (m, 2H, H_{Ph}), 7.33–7.24 (m, 3H, H_{Ph}), 4.64 (A part of an AB system, *J* = 14.0 Hz, 1H, C*H*HN), 4.60 (B part of an AB system, *J* = 14.0 Hz, 1H,

C*H*HN), 4.36 (s, 2H, 3-H + 4-H), 1.30 (s, 18H, CH_3 x 6) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 173.3 (s; 2C, C-2 + C-5), 135.4 (s; CPh), 129.0 (d; 2C, CHPh), 128.6 (d; 2C, CHPh), 127.9 (d; CHPh), 76.4 (s; 2C, CMe_3), 75.0 (d; 2C, C-3 + C-4), 42.3 (t; CH_2), 28.5 (q; 6C, CH_3) ppm; IR ($CDCl_3$): *v* = 2980, 1718, 1393, 1371, 1118, 1080, 1025 cm^{-1}; MS (EI): *m/z* (%) = 333 (M^+, 0.5), 277 (7), 221 (28), 192 (11), 164 (5), 91 (36), 57 (100), 41 (33); $C_{19}H_{27}NO_4$ (333.4): calcd.: C, 68.44; H, 8.16; N, 4.20; found C, 68.57; H, 8.02; N, 4.17.

37: R_f = 0.36 (CH_2Cl_2 / MeOH 98:2); mp 103.8–105.4 °C; $[\alpha]_D^{23}$= –132.4 (*c* = 0.70, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.40-7.27 (m, 5H, H_{Ph}), 4.05 (s, 2H, CH_2), 4.45 (br A part of an AB system, *J* = 5.3 Hz, 1H, 4-H), 4.41 (B part of an AB system, *J* = 5.3 Hz, 1H, 3-H), 3.17 (br s, OH), 1.32 (s, 9H, CH_3 x 3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 174.6 (s; *C*=O), 173.1 (s; *C*=O), 135.0 (s; C_{Ph}), 129.0 (d; 2C, CH_{Ph}), 128.7 (d; 2C, CH_{Ph}), 128.2 (d; CH_{Ph}), 76.5 (s; 2C, *C*Me_3), 75.1 (d; *C*HO), 75.0 (d; *C*HO), 42.7 (t; *C*H_2), 28.2 (q; 3C, *C*H_3) ppm; IR ($CDCl_3$): *v* = 3577, 2981, 1720, 1394, 1371, 1104, 1078 cm^{-1}; MS (EI): *m/z* (%) = 277 (M^+, 1), 262 (6), 221 (32), 192 (8), 164 (5), 91 (55), 71 (34), 57 (100), 41 (33); $C_{15}H_{19}NO_4$ (277.3): calcd.: C, 64.97; H, 6.91; N, 5.05; found C, 64.97; H, 7.22; N, 4.97.

(3*R*,4*R*)-1-N-Benzyl-3,4-dihydroxypyrrolidine-1-borane (38)



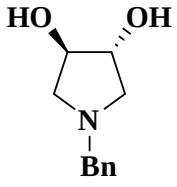
38

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 **35** (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 **38** 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 **38**.

38: 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): □ = 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 ($CDCl_3$): *v* = 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-Benzylpyrrolidine-3,4-diol (**39**)



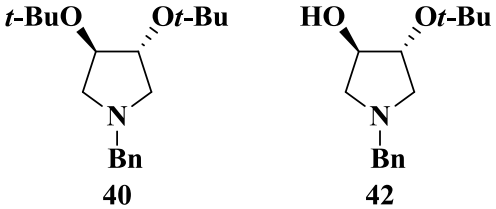
39

A 3 M HCl aqueous solution (26 mL) was added to an ice bath cooled suspension of the crude pyrrolidine-borane adduct **38** (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 **39** was obtained as a white solid that was directly esterified.

(3S, 4S)-1-Benzylpyrrolidine-3,4-diol (***ent*-39**)

Following the same procedure as for **39**, pyrrolidine ***ent*-39** was prepared starting from the pyrrolidine-borane adduct ***ent*-38**.

(3*R*,4*R*)-1-Benzyl-3,4-di-*tert*-butoxypyrrolidine (40) and (3*R*,4*R*)-1-benzyl-4-*tert*-butoxy-3-pyrrolidinol (42).



40 **42**

$HClO_4$ (60%, 2.4 mL, 21.5 mmol) was added to a mixture of pyrrolidine **39** (2.8 g, 14.3 mmol) in *tert*-butyl acetate (145 mL, 0.1 M) at 0 °C. The reaction mixture was stirred at rt for 5.5 h, cooled at 0 °C and then basified to pH 9 by slow addition of a saturated aqueous solution of Na_2CO_3 (20 mL) and solid Na_2CO_3. The two layers were separated and the two phases treated as follow. The *tert*-butyl acetate solution was washed with brine, dried over Na_2SO_4, filtered and partially distilled under reduced pressure at room temperature for recovering the majority of the solvent (ca 60-65%) that could be recycled or used for other protection reaction. The Na_2SO_4 used to dry the solution was thoroughly washed with CH_2Cl_2 and the obtained solution combined with the distillation residue. The aqueous solution was extracted with CH_2Cl_2 (3x20 mL) and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by chromatography on silica gel (eluent CH_2Cl_2/MeOH first 99: 1, followed by 97: 3 and CH_2Cl_2/MeOH 95:5) giving **40** (3.4 g, 78%) as a yellow waxy solid and **42** (0.51 g, 14%) as a white solid.

40: R_f = 0.33 (CH_2Cl_2/MeOH 97:3); $[\alpha]_D^{23}$= – 88 (*c* = 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.34-7.20 (m, 5H, H_{Ph}), 3.97-3.92 (m, 2H, 3-H + 4-H), 3.63 (A part of an AB system, *J* = 13.1 Hz, 1H, C*H*HPh), 3.49 (B part of an AB system, *J* = 13.1 Hz, 1H, C*H*HPh), 2.78 (pseudo dd, *J* = 9.4, 6.4 Hz, 2H, 2-H_a + 5-H_a), 2.39 (pseudo dd, *J* = 9.4, 5.1 Hz, 2H, 2-H_b + 5-H_b), 1.16 (s, 18H, C*H*$_3$ x 6) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 138.3 (s; C_{Ph}), 128.9 (d; 2C, CH_{Ph}), 128.1 (d; 2C, CH_{Ph}), 126.9 (d; CH_{Ph}), 78.2 (d; 2C, C-3 + C-4), 73.5 (s; 2C, *C*Me_3), 60.6 (t; *C*H_2Ph), 60.3 (t; 2C, C-2 + C-5), 28.6 (q; 6C, *C*H_3) ppm; IR ($CDCl_3$): *v* = 2977, 2797, 1391, 1366, 1186 cm^{-1}; MS (EI): *m/z* (%) = 305 (M^+, 9), 248 (16), 232 (3), 192

(28), 158 (6), 91 (100), 57 (92); $C_{19}H_{31}NO_2$ (305.2): calcd.: C, 74.71; H, 10.23; N, 4.59; found C, 74.37; H, 10.24; N, 4.95.

42: R_f = 0.23 (CH_2Cl_2/MeOH 95:5); mp = 78.6–81.8 °C; $[\alpha]_D^{23}$= – 10 (*c* = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35-7.24 (m, 5H, H_{Ph}), 4.03-3.98 (m, 2H, 3-H + 4-H), 3.70 (A part of an AB system, *J* = 12.9 Hz, 1H, C*H*HPh), 3.66 (B part of an AB system, *J* = 12.9 Hz, 1H, C*H*HPh), 3.26 (dd, *J* = 10.1, 6.7 Hz, 1H) and 2.82 (broad d, *J* = 10.4 Hz, 1H) (2-H_a and 5-H_a), 2.68 (dd, *J* = 10.4, 5.1 Hz, 1H) and 2.34 (dd, *J* = 10.1, 5.4 Hz, 1H) (2-H_b and 5-H_b), 1.19 (s, 9H, C*H*$_3$ x 3) ppm; ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 137.3 (s; CPh), 128.9 (d; 2C, CHPh), 128.3 (d; 2C, CHPh), 127.4 (d; CHPh), 78.6 (d) and 78.2 (d) (C-3 and C-4), 74.1 (s; *C*Me_3), 60.6 (t) and 60.4 (t) (C-2 and C-5), 60.1 (t; *C*H_2Ph), 28.4 (q; 3C, *C*H_3) ppm; IR ($CDCl_3$): *v* = 2978, 2801, 1392, 1366, 1192, 1090 cm^{-1}; MS (EI): *m/z* (%) = 249 (M^+,6), 192 (27), 91 (100), 57(19); $C_{15}H_{23}NO_2$ (249.3): calcd.: C, 72.25; H, 9.30; N, 5.62; found C, 71.99; H, 9.18; N, 5.54.

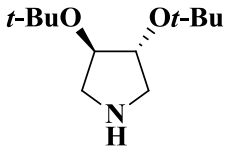
(3*S*,4*S*)-1-Benzyl-3,4-di-*tert*-butoxypyrrolidine (*ent*-40) and (3*S*,4*S*)-1-benzyl-4-*tert*-butoxy-3-pyrrolidinol (*ent*-42).

Following the same procedure as for **40** and **42**, the enantiomeric pyrrolidines *ent*-**40** and *ent*-**42** were prepared starting from the L-tartaric acid derivative *ent*-**39.**

ent-**40:** $[\alpha]_D^{25}$= + 83 (*c* = 0.7, $CHCl_3$); $C_{19}H_{31}NO_2$ (305.4): calcd.: C, 74.71; H, 10.23; N, 4.59; found C, 74.41; H, 10.41; N, 4.97. Spectral properties are identical to those of **40**.

ent-**42:** $[\alpha]_D^{27}$= + 8 (*c* = 0.4, $CHCl_3$); $C_{15}H_{23}NO_2$ (249.3): calcd.: C, 72.25; H, 9.30; N, 5.62; found C, 72.54; H, 9.57; N, 5.85. Spectral properties are identical to those of **42**.

(3*R*,4*R*)-3,4-Di-*tert*-butoxypyrrolidine (43)



43

AcOH (4.3 mL, 75 mmol) was added to a mixture of pyrrolidine **40** (2.3 g, 7.5 mmol) and 10% Pd/C (0.493 g, 0.46 mmol) in MeOH (48 mL) at 0 °C. The reaction mixture was stirred under a H_2 atmosphere (1 Atm) at rt for 3 h, then filtered through a short pad of Celite washing with MeOH. The filtrate was concentrated under reduced pressure and the resulting oil was dissolved in CH_2Cl_2 (20 mL) and basified to pH = 9 with a saturated aqueous Na_2CO_3 solution (40 mL) at 0 °C. The two layers were separated, and the aqueous solution was extracted with CH_2Cl_2 (3x40 mL). The combined organic phases were washed with brine (60 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford **43** (1.4 g, 87%) as an oil, that was used in the next step without further purification.

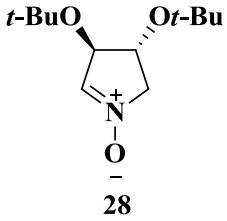
43: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.86–3.81 (m, 2H, 3-H + 4-H), 3.09 (dd, *J* = 12.0, 5.3 Hz, 2H, 2-H_a + 5-H_a), 2.66 (dd, *J* = 12.0, 3.5 Hz, 2H, 2-H_b + 5-H_b), 2.41 (br s, NH), 1.17 (s, 18H, C*H*$_3$ x 6) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 79.1 (d; 2C, C-3 + C-4), 73.5 (s; 2C, *C*Me_3), 53.9 (t; 2C, C-2 + C-5), 28.6 (q; 6C, *C*H_3) ppm.

(3*S*,4*S*)-3,4-Di-*tert*-butoxypyrrolidine (*ent*-43).

Following the same procedure as for **43**, the enantiomeric pyrrolidine *ent*-**43** was prepared starting from *ent*-**40**.

ent-**43:** Spectral properties are identical to those of **43**.

(3*R*,4*R*)-3,4-Di-*tert*-butoxy-3,4-dihydro-2*H*-pyrrole 1-oxide (28).



28

$NaHCO_3$ (4 g, 47.6 mmol) was added to a stirred solution of crude **43** (2 g, 9.3 mmol) in a 4:1 mixture of CH_3CN–THF (18 mL) and aqueous Na_2EDTA (0.01 M, 13 mL). The reaction mixture was cooled at 0 °C and Oxone (7.6 g, 12 mmol) was added portionwise over 5 h. The mixture was stirred at 0 °C for 30 min and then diluted with EtOAc (30 mL) and deionised H_2O (50 mL). The two phases were separated and the aqueous solution was sequentially extracted with EtOAc (2x20 mL) and CH_2Cl_2 (3x20 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford spectroscopically pure **28** (2.023 g, 93%) as a yellow solid that can be used in the next step without further purification. Purification by chromatography on silica gel [eluent: CH_2Cl_2/MeOH (1% NH_4OH98:2] afforded analytically pure **28** (1.7 g, 78%) as a white solid.

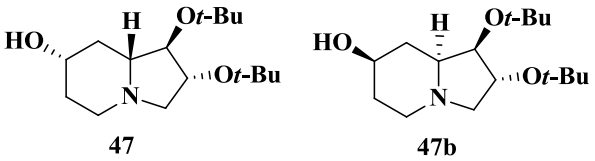
28: R_f = 0.32; mp = 75.4–77.2 °C; $[\alpha]_D^{27}$= – 166 (*c* = 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 6.78–6.76 (m, 1H, 5-H), 4.59-4.56 (m, 1H, 4-H), 4.20–4.12 (m, 2H, 2-H_a + 3-H), 3.71–3.64 (m, 1H, 2-H_b), 1.21 (s, 9H, C*H*$_3$ x 3), 1.19 (s, 9H, C*H*$_3$ x 3) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 134.9 (d; C-5), 79.0 (d; C-4), 74.8 (s; *C*Me$_3$), 74.7 (s; *C*Me$_3$), 74.2 (d; C-3), 68.3 (t; C-2), 28.2 (q; 6C, *C*H$_3$) ppm; IR ($CDCl_3$): *v* = 2978, 2935, 1588, 1392, 1367, 1260, 1187, 1086 cm^{-1}; MS (EI): *m/z* (%) = 229 (M^+, 1), 173 (16), 117 (39), 100 (18), 88 (31), 70 (43), 57

(100), 41 (42); $C_{12}H_{23}NO_3$ (229.3): calcd.: C, 62.85; H, 10.11; N,6.11; found C, 63.14; H, 10.06; N, 6.31.

(3*S*,4*S*)-3,4-Di-*tert*-butoxy-3,4-dihydro-2*H*-pyrrole 1-oxide (*ent*-28).[93] Following the same procedure as for **28**, the enantiomeric nitrone *ent*-**28** was prepared starting from *ent*-**43**.

ent-**28:** $[\alpha]_D^{23}$= + 151 (*c* = 0.8, $CHCl_3$); $C_{12}H_{23}NO_3$ (229.3): calcd.: C, 62.85; H, 10.11; N,6.11; found C, 62.59; H, 10.49; N, 6.02. Spectral properties are identical to those of **28**.

1*R*,2*R*,7*S*,8a*R*) and (1*R*,2*R*,7*R*,8a*S*)-1,2-Di-*tert*-butoxyoctahydro-7-indolizinol (47a and 47b).



A mixture of nitrone **28** (229 mg, 1 mmol) and alkene **48** (905 mg, 4 mmol) was stirred at rt for 3 days. The reaction mixture was suspended in Et_2O (10 mL), cooled at 0 °C, treated with glacial AcOH (2 mL) and activated Zn dust (981 mg, 15 mmol) and then stirred at rt for 24 h. The excess of Zn dust was filtered off and the solution basified to pH 9 with a 3 M aqueous NaOH solution at 0 °C. The two layers were separated and the aqueous one was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford a diastereomeric mixture of indolizidines. Purification by column chromatography on silica gel [eluent: CH_2Cl_2/MeOH (1% NH_4OH) first 97 : 3 then 95 : 5] afforded indolizidine **47a** (170 mg, 60%) as a white solid and a mixture of **47b** and a third diastereomer (40 mg, 14%) in 6:1 ratio. Further separation

afforded a sample of pure **47b**. The excess of dipolarophile **48** could be recovered in the early chromatography fractions (644 mg, 95%) and reused.

47a: R_f = 0.11 [CH_2Cl_2/MeOH (1% NH_4OH) 97:3]; mp = 117.5-119.5; $[\alpha]_D^{26}$= – 45 (*c* = 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 3.83 (ddd, *J* = 7.1, 4.0, 1.7 Hz, 1H; 2-H), 3.67 (dd, *J* = 8.5, 4.0 Hz, 1H; 1-H), 3.58 (pseudo tt, *J* = 10.9, 4.6 Hz, 1H; 7-H), 2.93 (ddd, *J* = 11.2, 4.5, 2.5 Hz, 1H; 5-H_a), 2.89 (dd, *J* = 10.1, 1.7 Hz, 1H; 3-H_a), 2.42 (dd, *J* = 10.1, 7.1 Hz, 1H; 3-H_b), 2.21-2.15 (m, 1H; 8-H_a), 1.96 (pseudo dt, *J* = 2.6, 11.8 Hz, 1H; 5-H_b), 1.90–1.77 (m, 2H; 6-H_a + 8a-H), 1.61 (pseudo ddt, *J* = 11.2, 4.5, 12.3 Hz, 1H; 6-H_b), 1.27 (pseudo q, *J* = 11.2 Hz, 1H; 8-H_b), 1.20 (s, 9H, C*H*$_3$ x 3), 1.16 (s, 9H, C*H*$_3$ x 3) ppm; ^{13}C-NMR ($CDCl_3$,100 MHz): δ = 83.2 (d; C-1), 77.9 (d; C-2), 73.8 (s; *C*Me_3), 73.7 (s; *C*Me_3), 69.7 (d; C-7), 65.4 (d; C-8a), 61.1 (t; C-3), 50.5 (t; C-5), 37.9 (t; C-8), 34.2 (t; C-6), 29.2 (q; 3C, *C*H_3), 28.7 (q; 3C, *C*H_3) ppm; IR ($CDCl_3$): *v* = 3689, 3608, 2978, 2257, 1602, 1391, 1367, 1216, 1190, 1067 cm^{-1}; MS (EI): *m/z* (%) = 285 (M^+, 2), 228 (51), 172 (18), 128 (9), 113 (46), 100 (16), 57 (100), 41 (54); $C_{16}H_{31}NO_3$ (285.42): calcd: C,67.33 ; H, 10.95; N,4.91; found C, 67.29 ; H, 11.21 ; N, 4.77.

47b: R_f = 0.23 [CH_2Cl_2/MeOH (1% NH_4OH) 95:5]; $[\alpha]_D^{23}$= – 19 (*c* = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$,400 MHz): δ = 3.98 (pseudo dt, *J* = 1.0, 6.3 Hz, 1H; 2-H), 3.73 (dd, *J* = 5.0, 1.0 Hz, 1H; 1-H), 3.68 (pseudo tt, *J* = 11.0, 4.7 Hz, 1H; 7-H), 3.40 (dd, *J* = 9.4, 6.8 Hz, 1H; 3-H_a), 3.04 (ddd, *J* =11.2, 4.2, 2.7 Hz, 1H; 5-H_a), 2.16–2.08 (m, 1H; 8a-H), 2.00 (pseudo dt, *J* = 1.9, 11.8 Hz, 1H; 5-H_b), 1.92 (dd, *J* = 9.4, 5.8 Hz, 1H; 3-H_b), 1.91–1.81 (m, 2H; 6-H_a + 8-H_a), 1.63–1.51 (m, 2H; 6-H_b + 8-H_b), 1.18 (s, 9H, C*H*$_3$ x 3), 1.16 (s, 9H, C*H*$_3$ x 3) ppm; ^{13}C-NMR ($CDCl_3$,100 MHz): δ = 79.9 (d; C-1), 79.7 (d; C-2), 73.7 (s; *C*Me_3), 73.4 (s; *C*Me_3), 70.0 (d; C-7), 64.5 (d; C-8a), 63.0 (t; C-3), 50.6 (t; C-5), 35.0 (t; C-8), 34.4 (t; C-6), 28.7 (q; 3C, *C*H_3),

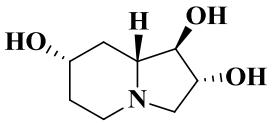
28.6 (q; 3C, *C*H_3) ppm; IR ($CDCl_3$): ν = 2978, 2934, 1468, 1391, 1366, 1191, 1070 cm^{-1}; MS (EI): *m/z* (%) = 285 (M^+, 4), 228 (85), 212 (8), 172 (25), 113 (58), 100 (22), 70 (9), 57 (100), 41(56) ; $C_{16}H_{31}NO_3$ (285.4): calcd.: C, 67.33 ; H, 10.95 ; N, 4.91 ; found C, 67.19; H, 11.13; N, 4.94.

(1*S*,2*S*,7*R*,8a*S*)- and (1*S*,2*S*,7*S*,8a*R*)-1,2-Di-*tert*-butoxyoctahydro-7-indolizinol (*ent*-47a and *ent*-47b).

Following the same procedure as for **47a** and **47b**, the enantiomeric indolizidines *ent*-**47a** and *ent*-**47b** were prepared starting from nitrone *ent*-**28**

ent-**47a:** $[\alpha]_D^{23}$= + 49 (*c* = 0.4, $CHCl_3$), {lit. $[\alpha]_D^{23}$= + 53.0 (*c* = 1.02, $CHCl_3$); $[\alpha]_D^{23}$= + 49.7 (*c* = 0.45, $CHCl_3$); $C_{16}H_{31}NO_3$ (285.4): calcd.: C, 67.33; H, 10.95; N, 4.91; found C, 67.22 ; H, 11.20; N,.5.01.

(1*R*,2*R*,7*S*,8a*R*)-Octahydroindolizine-1,2,7-triol (29).



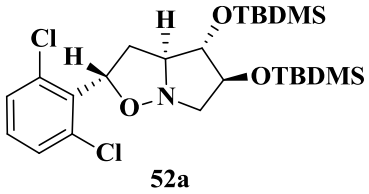
29

A solution of **47a** (55 mg, 0.193 mmol) in TFA (0.83 mL) was stirred at rt for 24 h. After this time the reaction mixture was concentrated under reduced pressure. The obtained crude material was dissolved in MeOH and Ambersep 900-OH was added at 0 °C. The mixture was slowly stirred at 0 °C for 10 min and then shaken at rt for 80 min on a flat shaker at 180 rpm. After this time the reaction mixture was filtered through cotton wool and concentrated under

reduced pressure. Purification of the crude product by chromatography on silica gel (eluent: CH_2Cl_2/MeOH/NH_4OH 35:19:1) afforded **29** (29 mg, 87%) as a white solid.

29: R_f = 0.5; mp = 180–182 °C (dec); $[\alpha]_D^{26}$= – 3 (*c* = 0.47, MeOH), {lit. *ent*-**29**: $[\alpha]_D^{22}$= + 2.1 (*c* = 0.36, MeOH); $[\alpha]_D^{21}$= + 1.8 (*c* = 0.81, MeOH); ^{1}H NMR (D_2O, 400 MHz): δ = 4.11 (ddd, *J* = 7.5, 3.9, 1.8 Hz, 1H; 2-H), 3.70 (dd, *J* = 8.5, 3.9 Hz, 1H; 1-H), 3.65 (pseudo tt, *J* = 11.1, 4.6 Hz, 1H; 7-H), 2.97 (ddd, *J* = 11.2, 4.5, 2.5 Hz, 1H; 5-H_a), 2.84 (dd, *J* = 11.2, 1.8 Hz, 1H; 3-H_a), 2.65 (dd, *J* = 11.2, 7.5 Hz, 1H; 3-H_b), 2.24–2.04 (m, 3H, 5-H_b + 8-H_a + 8a-H), 1.97–1.89 (m, 1H; 6-H_a), 1.47 (pseudo ddt, *J* = 11.3, 4.5, 12.6 Hz, 1H; 6-H_b), 1.28 (pseudo q, *J* = 11.4 Hz, 1H; 8-H_b) ppm; ^{13}C-NMR (D_2O, 50 MHz): δ = 82.8 (d; C-1), 77.0 (d; C-2), 68.7 (d; C-7), 67.3 (d; C-8a), 59.8 (t; C-3), 50.1 (t; C-5), 36.5 (t; C-8), 33.1 (t; C-6) ppm; HRMS (ESI): calcd for $C_8H_{16}NO_3$ $[M+H]^+$ 174.11247; found 174.11242.

(2*S*, 3a*S*,4*S*,5*S*)-2-(2,6-Dichlorophenyl)-4,5-bis (*tert*-butyldimethylsilyloxy) hexahydropyrrolo[1,2-*b*][1,2]oxazole (52a):



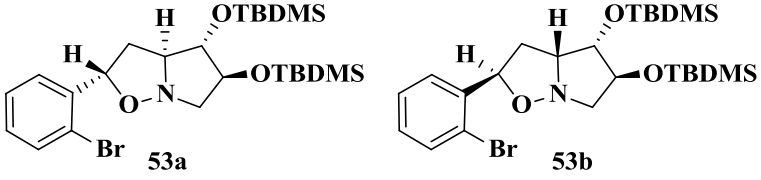
52a

2,6-Dichlorostyrene (**49**, 506 mg, 2.92 mmol) was added to a suspension of nitrone **51** (1.0 g, 2.89 mmol) in toluene (0.2 mL) and the reaction mixture was heated at 70 °C for 1 h 15 min in a microwave reactor (MW) using an irradiation power of 100 W. A second portion of nitrone (310 mg, 0,9 mmol) was added and the reaction mixture was heated in MW at 70 °C for 30 min. The solvent was evaporated under a nitrogen stream and the residue was purified by

chromatography on silica gel [eluent: EtOAc (1% Et_3N)/petroleum ether 1:10]. Enriched fractions of the diastereoisomeric adducts were obtained in 94% overall yield (1.43 g, *exo-anti*:*exo-syn*:*endo-anti* ratio = 6.8:6.4:1). The major *exo-anti* diastereoisomer **52a** was obtained in 46% combined yield (696 mg), calculated by ^{1}H NMR of the mixed fractions, as a white solid.

52a (17:1 mixture with **52b**)**:** R_f = 0.43; m.p.= 72-73 °C; $[\alpha]_D^{22}$ = + 52.2 ($CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.29 (pseudo d, *J* = 8.0 Hz, 2H, H_{Ar}), 7.13 (dd, *J* = 8.5, 7.5 Hz, 1H, H_{Ar}), 5.91 (pseudo t, *J* = 8.8 Hz, 1H, 2-H), 4.09-4.04 (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, 4.6 Hz, 1H, 6-Ha), 3.12-3.06 (m, 1H, 6-Hb), 2.83 (ddd, *J* = 12.2, 10.4, 9.4 Hz, 1H, 3-Ha), 2.52 (ddd, *J* = 12.2, 8.3, 4.3 Hz, 1H, 3-Hb), 0.93 [br s, 9H, $C(CH_3)_3$], 0.90 [br s, 9H, $C(CH_3)_3$], 0.12 (br s, 6H, $SiCH_3$), 0.10 (br s, 6H, $SiCH_3$) ppm; ^{13}C-NMR (50 MHz): δ = 135.7 (s; 2C, C_{Ar}), 132.9 (s; C_{Ar}), 129.3 (d; 3C, CH_{Ar}), 83.8 (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: *ν* = 2956, 2931, 2857, 1564, 1472, 1439, 1259, 1108 cm^{-1}; anal. calcd. for $C_{24}H_{41}Cl_2NO_3Si_2$ (518.7): C 55.58, H 7.97, N 2.70; found: C 55.52, H 8.23, N 2.79.

(2*S*,3a*S*,4*S*,5*S*)-and(2*R*,3a*R*,4*S*,5*S*)-2-(2-Bromophenyl)-4,5-bis(*tert*-butyldimethylsilyloxy)hexahydropyrrolo[1,2-*b*][1,2]oxazole (53a and 53b)



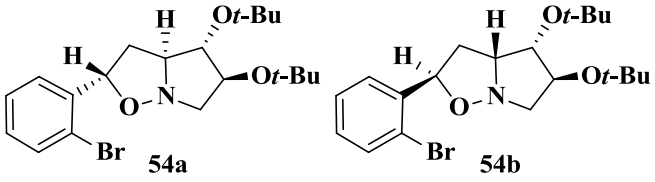
2-Bromostyrene (**50**, 609 mg, 3.32 mmol) was added to a suspension of nitrone **51** (1.147 g, 3.32 mmol) in toluene (2.5 mL) and the reaction mixture was heated at 70 °C for 3 h in MW using an irradiation power of 100 W. The solvent was co-evaporated with MeOH under reduced pressure and the residue was purified by chromatography on silica gel (eluent: EtOAc/petroleum ether 1:14). Enriched fractions of the diastereoisomeric adducts were obtained in 92% overall yield (1.618 g, *exo-anti*:*exo-syn*:*endo-anti* = ca 18.4:11.6:1). The *exo-anti* diastereoisomer **53a** was obtained in 55% combined yield (959 mg), calculated by ^{1}H NMR of the mixed fractions, as a white solid.

53a (ca 7:1 mixture with two minor isomers)**:** R_f = 0.50; m.p.= 52–53 °C; $[\alpha]_D^{22}$= + 6.1 ($CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.62 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}), 7.51 (dd, *J* = 8.0, 1.1 Hz, 1H, H_{Ar}), 7.31 (pseudo dt, *J* = 1.1, 7.6 Hz, 1H, H_{Ar}), 7.12 (pseudo dt, *J* = 1.6, 7.6 Hz, 1H, H_{Ar}), 5.44 (pseudo t, *J* = 7.4 Hz, 1H, 2-H), 4.09 (pseudo dt, *J* = 4.1, 5.5 Hz, 1H, 5-H), 4.02 (pseudo t, *J* = 3.8 Hz, 1H, 4-H), 3.64 (dd, *J* = 12.1, 5.4 Hz, 1H, 6-Ha), 3.67-3.61 (m, 1H, 3a-H), 3.14 (dd, *J* = 12.1, 5.7 Hz, 1H, 6-Hb), 2.83 (ddd, *J* = 12.4, 6.7, 4.2 Hz, 1H, 3-Ha), 2.21 (ddd, *J* = 12.4, 9.2, 8.1 Hz, 1H, 3-Hb), 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_{Ar}), 132.5 (d; CH_{Ar}), 128.8 (d; CH_{Ar}), 127.7 (d; CH_{Ar}), 127.3 (d; CH_{Ar}), 121.7 (s; C_{Ar}), 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; IR: *ν* =2955, 2930, 2858, 1471, 1440, 1361, 1258, 1110 cm^{-1}; MS (EI): *m/z* (%) = 529 [$(M+2)^+$, 6], 527 (M^+, 5), 286 (3), 241 (5), 212 (3), 187 (3), 185 (3), 171 (19), 147 (25), 133 (10), 128 (10), 115 (11),

73 (100), 55 (76); anal. calcd. for $C_{24}H_{42}BrNO_3Si_2$ (528.7): C 54.52, H 8.01, N 2.65; found: C 54.66, H 8.27, N 2.61.

53b: ^{1}H NMR (400 MHz) (18:1 mixture with **53a**): δ = 7.65 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.50 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}), 7.30 (pseudo dt, *J* = 1.2, 7.8 Hz, 1H, H_{Ar}), 7.11 (pseudo dt, *J* = 1.7,7.6 Hz, 1H, H_{Ar}), 5.27 (dd, *J* = 8.8, 6.4 Hz, 1H, 2-H), 4.25 (pseudo dt, *J* = 3.1, 4.9 Hz, 1H, 5-H), 4.08-4.00 (m, 2H, 3a-H, 4-H), 3.37 (dd, *J* = 12.5, 5.2 Hz, 1H, 6-Ha), 3.22 (dd, , *J* = 12.5, 4.7 Hz, 1H, 6-Hb), 2.93 (ddd, *J* = 12.4, 6.4, 2.0 Hz, 1H, 3-Ha), 2.03 (pseudo dt, *J* = 12.4, 8.7 Hz, 1H, 3-Hb), 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;

(2*S*,3a*S*,4*S*,5*S*)- and (2*R*,3a*R*,4*S*,5*S*)-,2-(2-Bromophenyl)-4,5-di-*tert*-butoxyhexahydropyrrolo[1,2-*b*][1,2]oxazole (54a, 54b):



2-Bromostyrene (**50**, 595 mg, 3.27 mmol) was added to a suspension of nitrone **28** (0.5 g, 2.18 mmol) in toluene (1.16 mL) and the reaction mixture was heated at 70 °C for 5 h in MW 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 1:4), affording fractions enriched of diastereoisomers, especially the minor ones in 99% overall yield (0.889 g). The *exo-anti* adduct **54a** and the *exo-syn* isomer **54b** were obtained in

73% and 22% yield, respectively (677 and 191 mg), calculated by ^{1}H NMR of the mixed fractions, as colourless oils.

54a (6.7:1 mixture of **54a** and **54c**)**:** R_f = 0.40; $[\alpha]_D^{25}$= + 12.8 (*c* = 0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.59 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.50 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}), 7.30 (pseudo dt, *J* = 1.2, 7.6 Hz, 1H, H_{Ar}), 7.11 (pseudo dt, *J* = 1.7, 7.7 Hz, 1H, H_{Ar}), 5.51 (pseudo t, *J* = 7.3 Hz, 1H, 2-H), 3.97 (pseudo dt, *J* = 8.0, 5.6 Hz, 1H, 5-H), 3.87 (dd, *J* = 5.3, 4.1 Hz, 1H, 4-H), 3.57 (dd, *J* = 10.7, 5.9 Hz, 1H, 6-Ha), 3.60-3.55 (m, 1H, 3a-H), 3.02 (dd, *J* = 10.7, 8.0 Hz, 1H, 6-Hb), 2.82 (ddd, *J* = 12.5, 7.1, 5.3 Hz, 1H, 3-Ha), 2.23 (ddd, *J* = 12.5, 9.0, 7.5 Hz, 1H, 3-Hb), 1.22 (s, 9H, CH_3x3), 1.19 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 140.1(s; C_{Ar}), 132.4 (d; CH_{Ar}), 128.8 (d; CH_{Ar}), 127.7 (d; CH_{Ar}), 127.3 (d; CH_{Ar}), 121.8 (s; C_{Ar}), 81.6 (d; C-4), 77.3 (d; C-2), 76.2 (d; C-5), 74.1 (s; Me_3CO), 74.0 (s; Me_3CO), 70.1 (d; C-3a), 60.3 (t; C-6), 41.6 (t; C-3), 28.7 (q; 3C, CH_3), 28.5 (q; 3C, CH_3) ppm; IR ($CDCl_3$): *v* = 2977, 2935, 2872, 1472, 1390, 1365, 1190, 1105 cm^{-1}; MS (EI): *m/z* (%) = 413 [$(M+2)^+$, 6], 411 (M^+, 6), 356 (12), 354 (12), 300 (6), 298 (6), 284 (2), 282 (2), 228 (7), 226 (8), 116 (8), 57 (100), 55 (36); anal. calcd. for $C_{20}H_{30}BrNO_3$ (412.4): C 58.25, H 7.33, N 3.40; found C 57.94, H 7.41, N 3.24.

54b (6.9:1 mixture of **54b** and **54d**)**:** R_f = 0.22; $[\alpha]_D^{25}$= + 58.2 (*c* = 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.65 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.50 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}), 7.30 (pseudo dt, *J* = 1.2, 7.6 Hz, 1H, H_{Ar}), 7.10 (pseudo dt, *J* = 1.7, 7.7 Hz, 1H, H_{Ar}), 5.29 (pseudo t, *J* = 7.3 Hz, 1H, 2-H), 4.11 (pseudo dt, *J* = 5.1, 6.5 Hz, 1H, 5-H), 3.96-3.86 (m, 2H, 3a-H, 4-H), 3.40 (dd, *J* = 13.1, 6.3 Hz, 1H, 6-Ha), 3.09 (dd, *J* = 13.1, 6.8 Hz, 1H, 6-Hb), 3.02 (ddd, *J* = 12.4, 6.8, 2.9 Hz, 1H, 3-Ha), 2.01 (pseudo dt, *J* = 12.4, 8.0 Hz, 1H, 3-Hb), 1.22 (s, 9H, CH_3x3), 1.20 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 140.9(s; C_{Ar}), 132.4

(d; CH_{Ar}), 128.6 (d; CH_{Ar}), 127.6 (d; CH_{Ar}), 127.2 (d; CH_{Ar}), 121.7 (s; C_{Ar}), 78.6 (d; C-2), 77.4 (d; C-4), 76.4 (d; C-5), 74.0 (s; Me_3CO), 73.6 (s; Me_3CO), 66.6 (d; C-3a), 60.7 (t; C-6), 38.7 (t; C-3), 28.5 (q; 6C, CH_3) ppm; IR ($CDCl_3$): ν = 2977, 2934, 2907, 2872, 1568, 1472, 1390, 1365, 1193, 1096, 1020 cm^{-1}; MS (EI): *m/z* (%) = 413 [$(M+2)^+$, 3], 411 (M^+, 3), 356 (8), 354 (7), 300 (5), 298 (5), 284 (2), 282 (2), 228 (5), 226 (6), 116 (7), 57 (100), 55 (36); anal. calcd. for $C_{20}H_{30}BrNO_3$ (412.4): C 58.25, H 7.33, N 3.40; found C 58.40; H 7.35; N 3.60.

54c: (6.7:1 mixture of **54a** and **54c**): ^{1}H NMR (CDCl3, 400 MHz, discernible signals): δ = 7.74 (dd, *J* = 7.8, 1.7 Hz, 1 H, HAr), 7.49 (dd, *J* = 7.9, 1.2 Hz, 1 H, HAr), 7.29 (pseudo dt, *J* = 1.2, 7.5 Hz, 1 H, HAr), 5.28 (dd, *J* = 9.9, 6.1 Hz, 1 H, 2-H), 4.05 (pseudo dt, *J* = 386 4.1, 5.7 Hz, 1 H, 5-H), 3.84 (pseudo t, *J* = 3.8 Hz, 1 H, 4-H), 3.70 (pseudo dt, *J* = 3.5, 8.3 Hz, 1 H, 3a-H), 3.53 (dd, *J* = 11.8, 5.6 Hz, 1 H, 6-Ha), 3.25 (dd, *J* = 11.8, 5.9 Hz, 1 H, 6-Hb), 3.06 (ddd, *J* = 12.2, 7.8, 6.1 Hz, 1 H, 3-Ha), 2.01 (ddd, *J* = 12.2, 9.9, 8.6 Hz, 1 H, 3-Hb), 1.21 [s, 9 H, C(CH3)3], 1.16 [s, 9 H, C(CH3)3] ppm. 13C 391 NMR (CDCl3, 100 MHz, discernible signals): δ = 82.4 (d; C-4), 80.1 (d; C-2), 79.0 (d; C-5), 73.9 (s; Me3*C*O), 72.6 (d; C-3a), 61.8 (t; C-6), 41.8 (t; C-3), 28.6 (q; 3 C, CH3), 28.5 (q; 3 C, CH3) ppm.

54d: (6.9:1 mixture of **54b** and **54d**): ^{1}H NMR (CDCl3, 400 MHz, discernible signals): δ = 7.57 (dd, *J* = 7.8, 1.7 Hz, 1 H, HAr), 7.13 396 (pseudo dt, *J* = 1.7, 7.7 Hz, 1 H, HAr), 5.20 (dd, *J* = 9.8, 6.2 Hz, 1 H, 2-H), 4.27 (pseudo dt, *J* = 9.1, 6.2 Hz, 1 H, 5-H), 3.55 (dd, *J* = 13.9, 6.4 Hz, 1 H, 6-Ha), 2.90 (dd, *J* = 13.9, 9.1 Hz, 1 H, 6-Hb), 2.65 (ddd, *J* = 12.4, 8.6, 6.2 Hz, 1 H, 3-Ha), 2.35–2.27 (m, 1 H, 3- Hb), 1.21 [s, 9 H, C(CH3)3], 1.13 [s, 9 H, C(CH3)3] ppm. 13C NMR 401 (CDCl3, 100 MHz, discernible signals): δ = 132.5 (d; CHAr), 128.9 (d; CHAr),

127.5 (d; CHAr), 127.3 (d; CHAr), 78.9 (d; C-2), 77.6 (d; C-4), 76.0 (d; C-5), 66.5 (d; C-3a), 58.9 (t; C-6), 37.9 (t; C-3) ppm.

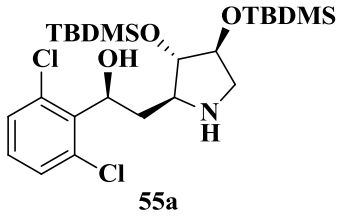
(2*R*,3a*R*,4*R*,5*R*)- and (2*S*,3a*S*,4*R*,5*R*)-,2-(2-bromophenyl)-4,5-di-*tert*-butoxyhexahydropyrrolo[1,2-*b*][1,2]oxazole (*ent*-54a and *ent*-54b):

Following the same procedure used to prepare **54a** and **54b,** synthesized ***ent*-54a** and ***ent*-54b**

***ent*-54a:** $[\alpha]_D^{25} = -12.844$ (*c* = 0.545, $CHCl_3$);

***ent*-54b:** $[\alpha]_D^{25} = -64.704$ (*c* = 0.525, $CHCl_3$);

(1*S*)-1-(2,6-Dichlorophenyl)-2-[(2*S*,3*S*,4*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)pyrrolidin-2-yl]ethanol (55a):



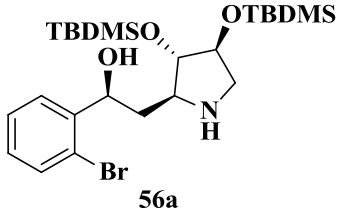
55a

A 17:1 mixture of isoxazolidines **52a** and **52b** (63 mg, 0.119 mmol of **52a** and 3 mg of **52b**) was mixed with zinc powder (36 mg, 0.56 mmol) in AcOH:H_2O = 9:1 (1.08 mL) and the suspension was heated in an oil bath at 60 °C for 4 h and then filtered through cotton wool. The solid residue was washed with EtOAc, the solution was basified to pH=9 with a saturated aq. $NaHCO_3$ solution and then 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 **55a** in 72% yield (45 mg) as a white solid.

55a: *Rf* = 0.30 (CH_2Cl_2/MeOH with 1% NH_4OH 15:1); m.p.= 113-114 °C; $[\alpha]_D^{25}$= + 14.2 ($CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.27 (pseudo d, *J* = 8.0 Hz, 2H, H_{Ar}), 7.10 (dd, *J* = 8.4, 7.6 Hz, 1H, H_{Ar}), 5.69 (dd, *J* = 9.7, 3.2 Hz, 1H, C*H*OH), 4.00-3.97 (m, 1H, 4-H), 3.87-3.84 (m, 1H, 3-H), 3.24 (pseudo dt, *J* = 8.7, 3.6 Hz, 1H, 2-H), 3.11 (dd, *J* = 11.9, 4.6 Hz, 1H, 5-Ha), 2.84 (dd, *J* = 11.9, 2.4 Hz, 1H, 5-Hb), 2.46 (ddd, *J* = 14.5, 9.7, 3.8 Hz, 1H, C*H*HCHOH), 1.80 (ddd, *J* = 14.5, 8.6, 3.1 Hz, 1H, C*H*HCHOH), 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_{Ar}), 134.2 (s; 2C, C_{Ar}), 129.3 (d; 2C, CH_{Ar}), 128.4 (d; CH_{Ar}), 83.7 (d; C-3), 79.9 (d; C-4), 70.0 (d; *C*HOH), 64.6 (d; C-2), 53.4 (t; C-5), 36.3 (t; *C*H_2CHOH), 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: *v* = 3600, 3200 (br), 2954, 2923, 2862, 1582, 1562, 1472, 1437, 1258, 1089 cm^{-1}; MS (EI): *m/z* (%) = [521 $(M+2)^+$, 2]; 519 (M^+, 2), 504 (1), 484 (1), 462 (2), 352 (2), 330 (5), 276 (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.

(1*S*)-and(1*R*)-1-(2-Bromophenyl)-2-[(2*S*,3*S*,4*S*)-3,4-bis(*tert*-butyldimethylsilyloxy)pyrrolidin-2-yl]ethanol (56a and 56b):



56a

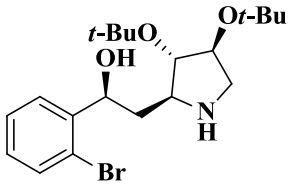
Following the same procedure used to prepare **55a**, pyrrolidines **56a** and **56b** were obtained starting from a mixture of cycloadducts **53a** and **53b**.

56a: white solid, 88% yield, *Rf* = 0.34 [eluent: CH_2Cl_2/MeOH (1% NH_4OH) 20:1]; m.p.= 102-103 °C; $[\alpha]_D^{25}$= + 12.1 ($CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.65 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.48 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{Ar}), 7.33 (pseudo dt, *J* = 1.2, 7.5 Hz, 1H, H_{Ar}), 7.09 (pseudo dt, *J* = 1.7, 7.6, Hz, 1H, H_{Ar}), 5.28 (dd, *J* = 6.4, 3.2 Hz, 1H, C*H*OH), 3.98-3.94 (m, 1H, 4-H), 3.82-3.80 (m, 1H, 3-H), 3.16 (dd, *J* = 11.9, 4.9 Hz, 1H, 5-Ha), 3.02 (pseudo dt, *J* = 9.6, 2.7 Hz, 1H, 2-H), 2.89 (dd, *J* = 11.9, 2.8 Hz, 1H, 5-Hb), 2.14 (ddd, *J* = 14.6, 9.6, 3.2 Hz, 1H, C*H*HCHOH), 1.88 (ddd, *J* = 14.6, 6.4, 3.3 Hz, 1H, C*H*HCHOH), 0.89 [s, 9H, $C(CH_3)_3$], 0.79 [s, 9H, $C(CH_3)_3$], 0.08 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.00 (s, 3H, $SiCH_3$), -0.07 (s, 3H, $SiCH_3$) ppm; ^{13}C-NMR (100 MHz): δ = 143.7 (s; C_{Ar}), 132.5 (d; CH_{Ar}), 128.2 (d; CH_{Ar}), 127.9 (d; CH_{Ar}), 127.3 (d; CH_{Ar}), 121.2 (s; C_{Ar}), 84.0 (d; C-3), 79.9 (d; C-4), 71.6 (d; CHOH), 64.4 (d; C-2), 53.2 (t; C-5), 36.2 (t; *C*H_2CHOH), 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; $SiCH_3$), -4.67 (q; $SiCH_3$), -4.71 (q; $SiCH_3$), -4.8 (q; $SiCH_3$) ppm. IR: ν = 3661, 3190, 2930, 2858, 1471, 1463, 1258, 1088, 1082 cm^{-1}; MS (EI): *m/z* (%) = 531 [$(M+2)^+$, 1], 529 (M^+, 1), 450 (2), 432 (5), 171 (54), 97(44), 83 (45), 71 (59), 57 (100); anal. calcd. for $C_{24}H_{44}BrNO_3Si_2$ (530.69): C 54.32, H 8.36, N 2.64; found: C 54.32, H 8.28, N 2.68.

56b: white solid; m.p.= 155-156 °C; $[\alpha]_D^{26}$= + 46.2 ($CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.65 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}), 7.49 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{Ar}), 7.33 (pseudo dt, *J* = 1.2, 7.5

Hz, 1H, H_{Ar}), 7.10 (pseudo dt, *J* = 1.6, 7.6, Hz, 1H, H_{Ar}), 5.31 (pseudo t, *J* = 4.6 Hz, 1H, C*H*OH), 3.94 (pseudo dt, *J* = 4.6, 1.6 Hz, 1H, 4-H), 3.74 (dd, *J* = 4.1, 1.6 Hz, 1H, 3-H), 3.24 (dd, *J* = 12.4, 4.6 Hz, 1H, 5-Ha), 3.19 (pseudo dt, *J* = 11.3, 3.6 Hz, 1H, 2-H), 2.69 (dd, *J* = 12.4, 1.6 Hz, 1H, 5-Hb), 2.11 (ddd, *J* = 14.7, 11.3, 4.2 Hz, 1H, C*H*HCHOH), 1.93 (ddd, *J* = 14.7, 5.0, 3.1 Hz, 1H, C*H*HCHOH), 0.87 [s, 9H, $C(CH_3)_3$], 0.82 [s, 9H, $C(CH_3)_3$], 0.033 (s, 3H, $SiCH_3$), 0.031 (s, 3H, $SiCH_3$), 0.02 (s, 3H, $SiCH_3$), -0.01 (s, 3H, $SiCH_3$) ppm; ^{13}C-NMR (100 MHz): δ = 143.7 (s; C_{Ar}), 132.7 (d; CH_{Ar}), 128.3 (d; CH_{Ar}), 127.8 (d; CH_{Ar}), 127.3 (d; CH_{Ar}), 121.4 (s; C_{Ar}), 80.4 (d; C-3), 78.4 (d; C-4), 71.5 (d; CHOH), 57.4 (d; C-2), 52.9 (t; C-5), 32.8 (t; *C*H_2CHOH), 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; $SiCH_3$), -4.67 (q; $SiCH_3$), -4.68 (q; $SiCH_3$), -4.9 (q, $SiCH_3$) ppm.

(1*S*)- and (1*R*)-1-(2-bromophenyl)-2-[(2*S*,3*S*,4*S*)-3,4-di-*tert*-butoxypyrrolidin-2-yl]ethanol (57a and 57c)*:*



57a

A 6.7:1 mixture of isoxazolidines **54a** and **54c** (686 mg, 1.66 mmol) and zinc powder (544 mg, 8.32 mmol) in AcOH:H_2O = 9:1 (15.9 mL) was heated in an oil bath at 80 °C for 5 h and then diluted with MeOH and filtered through cotton wool. The filtrate was concentrated under reduced pressure, the residue was dissolved in CH_2Cl_2, and the solution was basified to pH=9 with a saturated aq. $NaHCO_3$ solution and solid Na_2CO_3 at 0 °C. The obtained suspension was sequentially extracted with CH_2Cl_2 (3x30 mL) and EtOAc (2x30 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under

reduced pressure. Filtration on silica gel [eluent: CH_2Cl_2/MeOH (1% NH_4OH) 95:5] afforded a 6.2:1 mixture of pyrrolidines **57a** and **57c** in 96% yield (658 mg) as a yellow viscous oil.

57a (6.2:1 mixture with **57c**)**:** *Rf* = 0.20; $[\alpha]_D^{25}$= – 15.1 (*c* = 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.66 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.48 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{Ar}), 7.33 (pseudo dt, *J* = 1.2, 7.5 Hz, 1H, H_{Ar}), 7.09 (pseudo dt, *J* = 1.7, 7.6 Hz, 1H, H_{Ar}), 5.31 (dd, *J* = 5.9, 3.5 Hz, 1H, C*H*OH), 4.94-4.15 (br s, 2 H, NH, OH) 3.85-3.80 (m, 1H, 4-H), 3.66 (pseudo t, *J* = 2.2 Hz, 1H, 3-H), 3.16 (dd, *J* = 12.1, 5.6 Hz, 1H, 5-Ha), 3.03 (pseudo dt, *J* = 10.2, 2.7 Hz, 1H, 2-H), 2.92 (dd, *J* = 12.1, 4.3 Hz, 1H, 5-Hb), 2.21 (ddd, *J* = 14.7, 10.2, 3.5 Hz, 1H, C*H*HCHOH), 1.96 (ddd, *J* = 14.7, 5.9, 3.2 Hz, 1H, C*H*HCHOH), 1.18 (s, 9H, CH_3x3), 1.06 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 143.5 (s; C_{Ar}), 132.5 (d; CH_{Ar}), 128.2 (d; CH_{Ar}), 128.1 (d; CH_{Ar}), 127.3 (d; CH_{Ar}), 121.1 (s; C_{Ar}), 83.1 (d; C-3), 79.2 (d; C-4), 73.9 (s; Me_3CO), 73.8 (s; Me_3CO), 71.8 (d; CHOH), 63.0 (d; C-2), 52.3 (t; C-5), 34.8 (t; CH_2CHOH), 28.6 (q; 3C, CH_3), 28.5 (q; 3C, CH_3) ppm; IR ($CDCl_3$): *v* = 3500-2500 br, 3068, 2979, 2934, 2873, 1464, 1391, 1365, 1235, 1191, 1071, 1023 cm^{-1}; MS (EI): *m/z* (%) = 415 [$(M+2)^+$, 1], 413 (M^+, 1), 358 (1), 356 (1), 342 (1), 340 (1), 334 (1), 260 (5), 228 (3), 187 (5), 185 (6), 148 (9), 116 (34), 98 (12), 57 (100).

57c (6.2:1 mixture of **57a/57c**)**:** ^{1}H NMR ($CDCl_3$, 400 MHz, discernible signals): δ = 5.13 (dd, *J*= 10.2, 1.1 Hz, 1H, C*H*OH), 3.87 (pseudo dt, *J*= 2.3, 5.9 Hz, 1H, 4-H), 3.60 (pseudo t, *J*= 2.2 Hz, 1H, 3-H), 3.35 (pseudo dt, *J*= 12.1, 2.2 Hz, 1H, 2-H), 3.24 (dd, *J*= 12.6, 6.4 Hz, 1H, 5-Ha), 2.83 (dd, *J*= 12.6, 5.5 Hz, 1H, 5-Hb), 1.69-1.58 (m, 1H, C*H*HCHOH), 1.19 (s, 9H, CH_3x3), 1.15 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz) discernible signals: δ = 143.8

(s; C_{Ar}), 132.3 (d; CH_{Ar}), 128.2 (d; CH_{Ar}), 127.6 (d; CH_{Ar}), 127.5 (d; CH_{Ar}), 121.4 (s; C_{Ar}), 84.1 (d; C-3), 79.9 (d; C-4), 74.1 (s; Me_3CO), 73.9 (d; CHOH), 73.6 (s; Me_3CO), 66.5 (d; C-2), 52.2 (t; C-5), 37.0 (t; CH_2CHOH), 28.7 (q; 3C, CH_3), 28.5 (q; 3C, CH_3) ppm.

(1*R*)-1-(2-bromophenyl)-2-[(2*R*,3*S*,4*S*)-3,4-di-*tert*-butoxypyrrolidin-2-yl]ethanol(57b):

Following the same procedure used to prepare **57a**, the diastereomeric pyrrolidine **57b** was obtained starting from a mixture of cycloadducts **54b** and **54d**.

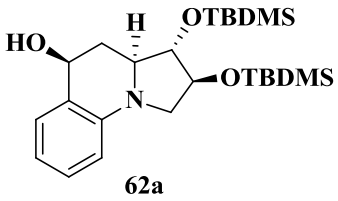
57b: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64 (dd, *J* = 7.7, 1.7 Hz, 1H, H_{Ar}), 7.48 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{Ar}), 7.33 (pseudo dt, *J* = 1.2, 7.6 Hz, 1H, H_{Ar}), 7.09 (pseudo dt, *J* = 1.7, 7.7 Hz, 1H, H_{Ar}), 5.29 (pseudo t, *J* = 4.3 Hz, 1H, C*H*OH), 4.20-2.92 (br s, 2 H, NH, OH) 3.87 (pseudo dt, *J* = 6.2, 3.5 Hz, 1H, 4-H), 3.64 (dd, *J* = 5.7, 3.6 Hz, 1H, 3-H), 3.26 (dd, *J* = 12.5, 6.2 Hz, 1H, 5-Ha), 3.06 (pseudo dt, *J* = 9.4, 5.4 Hz, 1H, 2-H), 2.66 (dd, *J* = 12.5, 3.4 Hz, 1H, 5-Hb), 2.06-2.00 (m, 2H, C*H*$_2$CHOH), 1.14 (s, 9H, CH_3x3), 1.08 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 143.8 (s; C_{Ar}), 132.6 (d; CH_{Ar}), 128.2 (d; CH_{Ar}), 128.0 (d; CH_{Ar}), 127.2 (d; CH_{Ar}), 121.2 (s; C_{Ar}), 80.0 (d; C-3), 77.9 (d; C-4), 73.7 (s; Me_3CO), 73.5 (s; Me_3CO), 72.1 (d; CHOH), 56.8 (d; C-2), 51.8 (t; C-5), 31.8 (t; CH_2CHOH), 28.6 (q; 3C, CH_3), 28.3 (q; 3C, CH_3) ppm.

(1*R*)-1-(2-bromophenyl)-2-[(2*R*,3*R*,4*R*)-3,4-di-*tert*-butoxypyrrolidin-2-yl]ethanol(*ent* 57a):

Following the same procedure used to prepare **57a**, the diastereomeric pyrrolidine ***ent*-57a** was obtained starting from a mixture of cycloadducts **ent-54a**.

$[\alpha]_D^{25}$= + 17.748 (*c* = 0.835, $CHCl_3$).

(2*S*,3*S*,3a*S*,5*S*)-2,3-Bis(*tert*-butyldimethylsilyloxy)-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinolin-5-ol (62a):



62a

Method A: DBU (40 μL, 0.267 mmol) and degassed *t*BuOH (2.6 mL) were added to a mixture of **56a** (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 MW and then concentrated. The crude product was dissolved in $CHCl_3$, filtered through a short pad of Celite® and concentrated under reduced pressure. Purification by chromatography on silica gel (eluent: petroleum ether/EtOAc 20:1) afforded **62a** in 30% yield (18 mg) as a white solid.

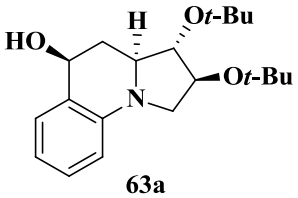
Method B: Degassed DMF (0.3 mL) was added to a mixture of **56a** (100 mg, 0.19 mmol), CuI (4.5 mg, 0.024 mmol), copper powder (14 mg, 0.22 mmol), K_3PO_4 (81 mg, 0.38 mmol), and L-proline (4.7 mg, 0.04 mmol) under nitrogen atmosphere. The reaction mixture was heated at 100 °C for 48 h in an oil bath and then concentrated. The crude product was dissolved in CH_2Cl_2, filtered through a short pad of celite®/Na_2SO_4 and concentrated under reduced pressure. Purification by chromatography on silica gel afforded **62a** in 37% yield (31 mg) as a white solid.

Method C: Degassed *t*BuOH/H_2O (1:1, 0.6 mL) and THF (0.15 mL) were added to a mixture of **56a** (22 mg, 0.04 mmol), CuI (2.4 mg, 0.013 mmol), copper powder (1.0 mg, 0.015 mmol), K_3PO_4 (17 mg, 0.08 mmol), and L-proline (2.8 mg, 0.024 mmol) under nitrogen atmosphere in a microwave vial. The reaction mixture was heated at 100 °C for 5 h in MW and then filtered through a short pad of Celite®/Na_2SO_4. The solution was concentrated under reduced pressure. Purification by chromatography on silica gel afforded **62a** in 46% yield (8 mg) as a white solid.

62a: R_f = 0.45 (petroleum ether/EtOAc 9:1); m.p.= 142-144 °C; $[\alpha]_D^{24}$= + 67.2 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (400 MHz): δ = 7.38 (pseudo dt, *J* = 7.6, 1.2 Hz, 1H, 6-H), 7.12 (dt, *J* = 8.1, 7.3, 1.5, 0.6 Hz, 1H, 8-H), 6.67 (pseudo dt, *J* = 0.8, 7.4 Hz, 1H, 7-H), 6.30 (dd, *J* = 8.1, 0.8 Hz, 1H, 9-H), 4.86 (br ddd, *J* = 11.0, 8.8, 5.5 Hz, 1H, 5-H), 4.23 (pseudo dt, *J* = 7.7, 6.7 Hz, 1H, 2-H), 3.78 (dd, *J* = 8.0, 6.7 Hz, 1H, 3-H), 3.51 (dd, *J* = 9.4, 7.7 Hz, 1H, 1-Ha), 3.38 (ddd, *J* = 11.9, 8.0, 3.0 Hz, 1H, 3a-H), 3.01 (dd, *J* = 9.4, 6.7 Hz, 1H, 1-Hb), 2.47 (ddd, *J* = 11.5, 5.5, 3.0 Hz, 1H, 4-Ha), 1.71 (d, *J* = 8.8 Hz, 1H, OH), 1.58 (pseudo q, *J* = 11.5 Hz, 1H, 4-Hb), 0.92 [s, 9H, $C(CH_3)_3$], 0.91 [s, 9H, $C(CH_3)_3$], 0.14 (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_{Ar}), 128.6 (d; C-8), 125.3 (d; C-6), 124.7 (s; C_{Ar}), 115.9 (d; C-7), 109.4 (d; 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: ν = 3586, 2955, 2929, 2857, 1605, 1500, 1472, 1462, 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 $C_{24}H_{43}NO_3Si_2$ (449.77): C 64.09, H 9.64, N 3.11; found: C 64.48, H 9.36, N 2.76.

(2*S*,3*S*,3a*S*,5*S*)-2,3-Di-*tert*-butoxy-1,2,3,3a,4,5-hexahydropyrrolo[1,2-a]quinolin-5-ol (63a):



63a

The Mixed solvent *t*BuOH/H_2O (1:1) (3 mL) were added to a mixture of **57a/57c** (103 mg, 0.248 mmol of **57a** and 14 mg of **57c**), CuI (23.6 mg, 0.124 mmol), copper powder (7.9 mg, 0.124 mmol), K_3PO_4 (105.5 mg, 0.497 mmol) and L-proline (28.6 mg, 0.248 mmol) under nitrogen atmosphere in a vial. Nitrogen was bubbled through the reaction mixture to remove oxygen. The reaction mixture was heated at 90 °C in an oil bath for 12 h and then was filtered through a small pad of celite. The solution was diluted with H_2O and sequentially extracted with CH_2Cl_2 (3x20 mL) and EtOAc (2x20 mL). The combined organic phases were washed with NH_4OH (1% H_2O solution) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel [eluent: CH_2Cl_2/MeOH (1% NH_4OH) 98:2] afforded **63a** in 74% yield (61.2 mg) as a white solid.

63a: R_f = 0.42; m.p. = 167-169 °C; $[\alpha]_D^{23}$= + 151.76 (*c* = 0.42, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38 (pseudo dt, *J* = 7.5, 1.3 Hz, 1H, 6-H); 7.12 (dddd, *J* = 8.1, 7.4, 1.5, 0.6 Hz,

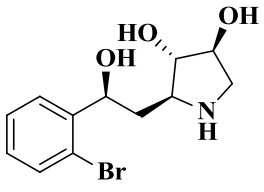
1H, 8-H), 6.67 (pseudo dt, *J* = 0.9, 7.4 Hz, 1H, 7-H), 6.31 (dd, *J* = 8.1, 0.9 Hz, 1H, 9-H), 4.92-4.82 (m, 1H, 5-H), 4.09-4.03 (m, 1H, 2-H), 3.74 (dd, *J* = 8.6, 7.2 Hz, 1H, 3-H), 3.45 (dd, *J* = 9.6, 7.9 Hz, 1H, 1-Ha), 3.33 (ddd, *J* = 11.8, 8.6, 2.8 Hz, 1H, 3a-H), 3.04 (dd, *J* = 9.6, 6.5 Hz, 1H, 1-Hb), 2.56 (ddd, *J* = 11.5, 5.7, 2.8 Hz, 1H, 4-Ha), 1.73 (br d, *J* = 7.9 Hz, 1H, OH), 1.59 (pseudo q, *J* = 11.5 Hz, 1H, 4-Hb) 1.26 (s, 9H, CH_3x3), 1.24 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 143.4 (s; C_{Ar}), 128.6 (d; C-8), 125.5 (d; C-6), 124.8 (s; C_{Ar}), 115.9 (d; C-7), 109.5 (d; C-9), 80.3 (d; C-3), 75.2 (d; C-2), 74.3 (s; Me_3CO), 73.9 (s; Me_3CO), 67.2 (d; C-5), 57.9 (d; C-3a), 52.1 (t; C-1), 35.9 (t; C-4), 29.4 (q; 3C, CH_3), 28.6 (q; 3C, CH_3) ppm; IR ($CDCl_3$): *v* = 3619, 3587, 3069, 3043, 2934, 2977, 2934, 1605, 1497, 1462, 1364, 1193, 1091 cm^{-1}; MS (EI): *m/z* (%) = 333 (M^+, 21), 314 (1), 276 (12), 258 (5), 220 (2), 202 (22), 186 (4), 161 (13), 130 (10), 71 (14), 69 (15), 57 (100); anal. calc. for $C_{20}H_{31}NO_3$ (333.4): C 72.04, H 9.37, N 4.20; found C 71.79, H 9.75, N 4.40.

(2*R*,3*R*,3a*R*,5*R*)-2,3-Di-*tert*-butoxy-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinolin-5-ol (*ent*-63a):

Following the same procedure as for **62a**, the enantiomeric trihydroxy-benzo[*e*]indolizidine ***ent*-63a** was prepared starting from **28** and nitrone *ent*-**28**.

$[\alpha]_D^{23}$= – 150.80 (*c* = 0.42, $CHCl_3$).

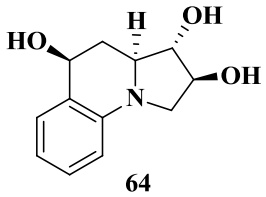
(3*S*,4*S*)-2-[(S)-2-(2-bromophenyl)-2-hydroxyethyl] pyrrolidine-3,4-diol (65)



65

Pyrrolidine **57a** (38 mg, 0.092 mmol) was dissolved in TFA (0.5 mL) at 0 °C and the solution was stirred at rt for 2 h. The excess of TFA was co-evaporated with toluene (4 × 3 mL) under reduced pressure (each addition of toluene was done at 0 °C) and then the residue was co-evaporated with MeOH (3 × 3 mL) to remove the last traces of toluene.The crude product **65** (25 mg) was used in the next step without further purification.

(2*S*, 3*S*, 3a*S*, 5*S*)-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoline-2,3,5-triol (64) –



64

The mixed solvents *t*BuOH: H_2O (1:1) (0.8 mL) were added to a mixture of **65** (25 mg, 0.083 mmol), CuI (7.9 mg, 0.041 mmol), copper powder (2.63 mg, 0.041 mmol), K_3PO_4 (35.13 mg, 0.165 mmol) and L-proline (9.53 mg, 0.083 mmol) under nitrogen atmosphere in a vial. Nitrogen was bubbled through the reaction mixture to remove oxygen. The reaction mixture was heated at 90 °C for 12 h in an oil bath and then was filtered through a small pad of Celite washing with MeOH. The solution was diluted with H_2O and sequentially extracted with CH_2Cl_2 and EtOAc. The combined organic phases were washed with NH_4OH (1% H_2O solution) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1% NH_4OH) 90:10] to afford **64** in 40% yield (7.3 mg, 0.033mmol) as a white solid.

64: *Rf* = 0.25; m.p. 161–163 °C (dec.). $[\alpha]_D^{25}$= + 85.06 (*c* = 0.45, MeOH); 1H NMR (CD3OD, 400 MHz): δ = 7.31 (pseudo dt, *J* =7.6, 1.3 Hz, 1 H, 6-H); 7.05–7.00 (m, 1 H, 8-H), 6.59

(pseudo dt, *J*= 0.9, 7.5 Hz, 1 H, 7-H), 6.30 (dd, *J* = 8.1, 0.9 Hz, 1 H, 9-H), 4.79(br. dd, *J* = 11.4, 5.5 Hz, 1 H, 5-H), 4.20 (pseudo dt, *J* = 7.9,6.9 Hz, 1 H, 2-H), 3.66 (dd, *J* = 8.3, 7.1 Hz, 1 H, 3-H), 3.53 (dd, *J* = 9.7, 7.9 Hz, 1 H, 1-Ha), 3.56–3.29 (m, 1 H, 3a-H), 3.02 (dd, *J*= 9.7, 6.7 Hz, 1 H, 1-Hb), 2.46 (ddd, *J* = 11.6, 5.5, 2.9 Hz, 1 H, 4-Ha), 1.58 (pseudo q, *J* = 11.6 Hz, 1 H, 4-Hb) ppm.

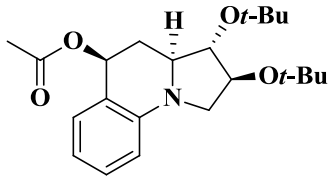
13C NMR(CD_3OD, 100 MHz): δ = 144.8 (s; CAr), 129.2 (d; C-6), 126.6 (d;C-8), 126.4 (s; CAr), 116.9 (d; C-7), 110.6 (d; C-9), 82.8 (d; C-3),76.6 (d; C-2), 67.5 (d; C-5), 61.3 (d; C-3a), 52.8 (t; C-1), 36.4 (t; C-4) ppm.

(2*R*,3*R*,3a*S*,5S)-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoline-2,3,5-triol (*ent*-64) –

Following the same procedure as for **64a**, the enantiomeric trihydroxy-benzo[*e*]indolizidine *ent*-**64a** was prepared starting from **50** and nitrone ***ent*-28**.

$[\alpha]_D^{25}$= – 88.19 (*c* = 0.455, MeOH).

(2*S*,3*S*,3a*S*,5*S*)-2,3-di-*tert*-butoxy-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinolin-5-yl acetate (66)



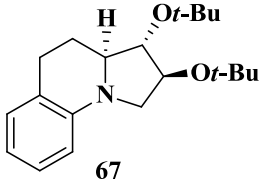
66

Quinolin-5-ol **63a** (55 mg, 0.165 mmol) and a catalytic amount of DMAP were dissolved in dry pyridine (1.6 mL) at 0 °C under nitrogen atmosphere. After 10 min, acetic anhydride (0.237 mL, 0.33 mmol) was added dropwise to the reaction mixture at 0 °C under nitrogen

atmosphere. The reaction mixture was stirred at 0 °C for 20 min, then at rt for 3 h, and then was concentrated under reduced pressure. The residue was sequentially co-evaporated with toluene (3 × 3 mL) and MeOH (2 × 3 mL) under reduced pressure to facilitate the removal of all volatiles. The residue was dissolved in CH_2Cl_2, the insoluble salts were filtered through cotton, and the filtrate was concentrated under reduced pressure. Crude **66** (60 mg, 0.16 mmol) was obtained as a colourless oil and was used in the next step without further purification. Acetate **66** undergoes a S_N2 hydrolysis of C-5 acetate group on silica gel affording the epimerized alcohol in low yield along with decomposition products.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.12 (dddd, *J* = 8.1, 7.4, 1.5, 0,7 Hz, 1H, 8-H); 7.04 (pseudo dt, *J* = 7.6, 1.2 Hz, 1H, 6-H), 6.64 (pseudo dt, *J* = 0.9, 7.5 Hz, 1H, 7-H), 6.35 (dd, *J* = 8.1, 0.9 Hz, 1H, 9-H), 6.02 (br dd, *J* = 10.9, 6.0 Hz, 1H, 5-H), 4.07 (ddd, *J* = 7.9, 7.0, 6.3 Hz, 1H, 2-H), 3.75 (dd, *J* = 8.4, 7.0 Hz, 1H, 3-H), 3.46 (dd, *J* = 9.6, 7.9 Hz, 1H, 1-Ha), 3.33 (ddd, *J* = 12.1, 8.4, 2.7 Hz, 1H, 3a-H), 3.06 (dd, *J* = 9.6, 6.3 Hz, 1H, 1-Hb), 2.60 (ddd, *J* = 11.4, 6.0, 2.7 Hz, 1H, 4-Ha), 2.18 (s, 3H, CH_3CO), 1.63 (pseudo q, *J* = 11.5 Hz, 1H, 4-Hb) 1.24 [s, 9H, $C(CH_3)_3$], 1.23 [s, 9H, $C(CH_3)_3$] ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 171.1 (s; C=O), 143.9 (s; C_{Ar}), 128.8 (d; C-8), 125.9 (d; C-6), 120.1 (s; C_{Ar}), 115.8 (d; C-7), 109.9 (d; C-9), 80.4 (d; C-3), 75.3 (d; C-2), 74.2 (s; Me_3CO), 73.9 (s; Me_3CO), 69.0 (d; C-5), 57.6 (d; C-3a), 52.2 (t; C-1), 31.3 (t; C-4), 29.4 [q; 3C, $C(CH_3)_3$], 28.6 [q; 3C, $C(CH_3)_3$], 21.4 (q; CH_3CO) ppm.

(2*S*,3*S*,3a*S*)-2,3-di-*tert*-butoxy-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoline (67)



67

Crude quinolin-5-yl acetate **66** (60 mg, 0.16 mmol) was dissolved in MeOH (1.9 mL) and hydrogenated in the presence of 10% Pd/C (0.34 mg, 0.32 mmol) at rt and atmospheric pressure overnight. Then reaction mixture was filtered through a short pad of Celite washing with MeOH. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography (eluent: EtOAc/petroleum ether 5:95) to afford quinoline **67** (41.9 mg, 0.132 mmol) as a white solid and in 83% yield based on protected triol **63**.

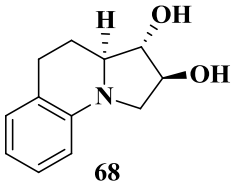
R_f = 0.5 (petroleum ether/EtOAc, 95:1); m.p. 87-89 °C; $[\alpha]_D^{25}$= + 143.33 (*c* = 0.375, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ= 7.05 (pseudo dt, *J* = 1.1, 7.7 Hz, 1H, H_{Ar}); 7.25 (br d, *J* = 7.3 Hz, 1H, H_{Ar}), 6.56 (pseudo dt, *J* = 0.9, 7.3 Hz, 1H, H_{Ar}), 6.34 (br d, *J* = 8.0 Hz, 1H, H_{Ar}), 4.09 (pseudo q, *J* = 7.2 Hz, 1H, 2-H), 3.69 (dd, *J* = 8.5, 7.3 Hz, 1H, 3-H), 3.50 (dd, *J* = 9.5, 8.0 Hz, 1H, 1-Ha), 3.19 (ddd, *J* = 11.2, 8.5, 3.0 Hz, 1H, 3a-H), 3.06 (dd, *J* = 9.5, 6.6 Hz, 1H, 1-Hb), 2.86-2.72 (m, 2H, 5-H), 2.30-2.24 (m, 1H, 4-Ha), 1.52 (pseudo dq, *J* = 6.0, 11.7 Hz, 1H, 4-Hb), 1.26 (s, 9H, CH_3x3), 1.24 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 144.2 (s; C_{Ar}), 128.5 (d; CH_{Ar}), 127.1 (d; CH_{Ar}), 121.2 (s; C_{Ar}), 115.4 (d; CH_{Ar}), 109.3 (d; CH_{Ar}), 80.5 (d; C-3), 75.2 (d; C-2), 74.1 (s; Me_3CO), 73.8 (s; Me_3CO), 59.4 (d; C-3a), 52.2 (t; C-1), 29.5 (q; 3C, CH_3), 28.6 (q; 3C, CH_3), 27.5 (t; C-5), 25.5 (t; C-4) ppm; IR ($CDCl_3$): *v* = 2977, 2933, 2845, 1602, 1501, 1475, 1460, 1391, 1363, 1190, 1088 cm^{-1}; MS (EI): *m/z* (%) = 317 (M^+, 45), 260 (30), 204 (48), 170 (12), 145 (46), 132 (27), 117 (12), 57 (100) ; anal. calcd. for $C_{20}H_{31}NO_2$ (317.4): C, 75.67; H, 9.84; N, 4.41; found C, 75.78.; H, 10.06; N, 4.43.

(2*R*,3*R*,3a*S*)-2,3-di-*tert*-butoxy-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoline (*ent*-67)

Following the same procedure as for **67**, the enantiomeric di-*tert*-hydroxy-benzo[*e*]indolizidine *ent*-**67** was prepared.

$[\alpha]_D^{22}$= – 146.07 (*c* = 0.42, $CHCl_3$).

(2*S*,3*S*,3a*S*)-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoline-2,3-diol (68)



68

Ether **67** (40.5 mg, 0.127 mmol) was dissolved in TFA (1.27 mL) at 0 °C and the solution was stirred at rt for 20 h. The excess of TFA was co-evaporated with toluene (4 × 3 mL) under reduced pressure (each addition of toluene was done at 0 °C) and then the residue was co-evaporated with MeOH (3 × 3 mL) to remove the last traces of toluene. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1% NH_4OH) 95:5] gave **68** (27.3 mg, 0.133 mmol) as a white solid in 91% yield.

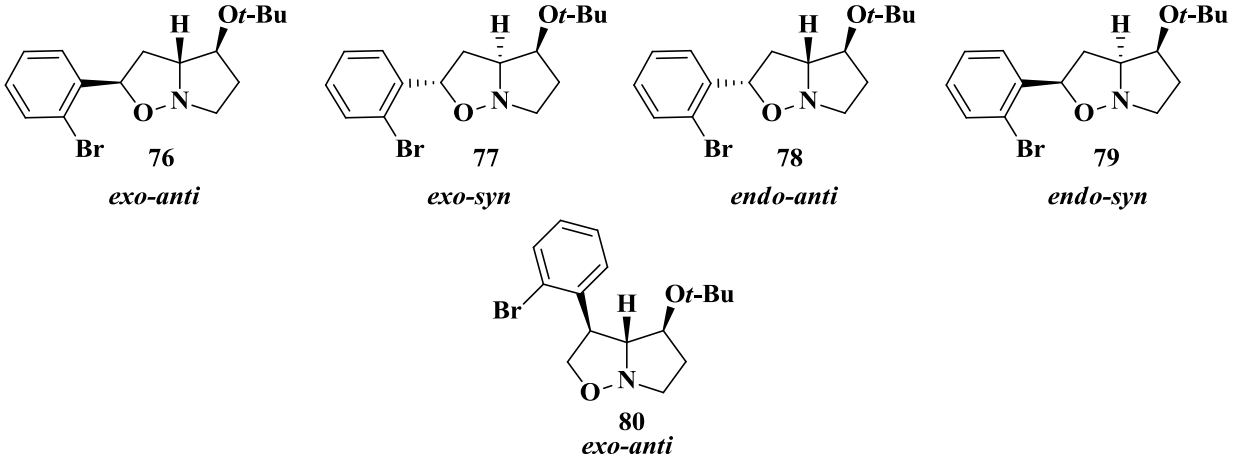
R_f = 0.25; m.p. 144 -146 °C; $[\alpha]_D^{24}$= + 113.47 (*c* = 0.69, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.00-6.94 (m, 1H, H_{Ar}); 6.89 (dm, *J* = 7.4 Hz, 1H, H_{Ar}), 6.50 (pseudo dt, *J* = 1.0, 7.4 Hz, 1H, H_{Ar}), 6.32 (br d, *J* = 7.8 Hz, 1H, H_{Ar}), 4.22 (pseudo dt, *J* = 7.9, 6.9 Hz, 1H, 2-H), 3.62 (dd, *J* = 8.2, 7.2 Hz, 1H, 3-H), 3.56 (dd, *J* = 9.7, 7.9 Hz, 1H, 1-Ha), 3.17 (ddd, *J* = 11.2, 8.2, 3.1 Hz, 1H, 3a-H), 3.04 (dd, *J* = 9.7, 6.7 Hz, 1H, 1-Hb), 2.84-2.71 (m, 2H, 5-H), 2.31-2.23 (m, 1H, 4-Ha), 1.49 (pseudo dq, *J* = 6.6, 11.4 Hz, 1H, 4-Hb) ppm; ^{13}C-NMR (CD_3OD, 100 MHz): δ = 145.6 (s; CAr), 129.5 (d; CHAr), 128.1 (d; CHAr), 122.5 (s; CAr), 116.8 (d; CHAr), 110.8 (d; CHAr), 83.0 (d; C-3), 76.6 (d; C-2), 63.0 (d; C-3a), 53.0 (t; C-1), 28.5 (t; C-5), 26.7 (t; C-4) ppm; MS (EI): *m/z* (%) = 205 (M^+, 63), 185 (1), 167 (6), 145 (100), 132 (36), 130 (45), 117 (73), 91 (19), 51 (15).

(2*R*,3*R*,3a*S*)-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinoline-2,3-diol (*ent*-68)

Following the same procedure as for **68**, the enantiomeric di-hydroxy-benzo[*e*]indolizidine ***ent*-68** was prepared.

$[\alpha]_D^{28}$= – 114.88 (*c* = 0.5, MeOH);

(2*R*,3a*R*,4*S*)-, (2*S*,3a*S*,4*S*)-, (2*S*,3a*R*,4*S*)-, and (2*R*,3a*S*,4*S*)-2-(2-bromophenyl)-4-(*tert*-butoxy)hexahydropyrrolo[1,2-*b*]isoxazole (76, 77, 78, and 79) and (3*S*,3a*R*,4*S*)-3-(2-bromophenyl)-4-(*tert*-butoxy)hexahydropyrrolo[1,2-*b*]isoxazole (80)



2-Bromostyrene (**50**, 524 mg, 2.86 mmol) was added to a solution of nitrone **74** (0.3 g, 1.91 mmol) in toluene (1.01 mL) and the reaction mixture was heated at 85 °C for 3 h in the MW 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 3:7) to give a 12:1 mixture of the major adduct *exo-anti*-**76** with *endo-anti*-**78** (437 mg, 67%),

the regioisomer **80** (8 mg, 1%), *endo-syn*-**79** (15 mg, 2%), and the second major adduct *exo-syn*-**77** (114 mg, 18%) obtained as colourless oils.

76 (***exo-anti***)**:** (12:1 mixture with **78**)**:** R_f = 0.5; $[\alpha]_D^{22}$ = + 34.64 (*c* = 0.83, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.70 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.49 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}), 7.31 (pseudo dt, *J* = 1.2, 7.6 Hz, 1H, H_{Ar}), 7.11 (pseudo dt, *J* = 1.7, 7.6 Hz, 1H, H_{Ar}), 5.30 (pseudo t, *J* = 7.1 Hz, 1H, 2-H), 4.01 (pseudo dt, *J* = 6.9, 4.6 Hz, 1H, 4-H), 3.56 (ddd, *J* = 8.0, 4.0, 3.0 Hz, 1H, 3a-H), 3.45 (pseudo dt, *J* = 12.7, 7.3 Hz, 1H, 6-Ha), 3.39 (pseudo dt, *J* = 12.7, 6.6 Hz, 1H, 6-Hb), 2.76 (ddd, *J* = 12.7, 7.0, 3.0 Hz, 1H, 3-Ha), 2.36-2.22 (m, 2H, 3-Hb, 5-Ha), 1.80-1.70 (m, 1H, 5-Hb), 1.21 (s, 9H, CH_3x3) ppm;^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 140.9 (s; C_{Ar}), 132.3 (d; CH_{Ar}), 128.7 (d; CH_{Ar}), 127.7 (d; CH_{Ar}), 127.2 (d; CH_{Ar}), 121.3 (s; C_{Ar}), 73.6 (s; *C*Me_3), 72.8 (d; C-3a), 55.7 (t; C-6), 41.8 (t; C-3), 33.7 (t; C-5), 28.5 (q; 3C, CH_3) ppm; IR ($CDCl_3$): *v* = 2977, 1568, 1466, 1440, 1390, 1364, 1253, 1188, 1044, 1019 cm^{-1}; MS (EI): *m/z* = 340.26, calcd. for $C_{16}H_{22}BrNO_2$ $[M + H]^+$: 342.13; $[M + Na]^+$: 361.97; anal. calcd. for $C_{16}H_{22}BrNO_2$ (340.26): C, 56.48; H, 6.52; N, 4.12; found C 56.15, H 6.68, N 4.37.

78 (***endo-anti***)**:** (1:12 mixture with **76**) Detectable signals**:** ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 5.20 (dd, *J*= 9.0, 6.9 Hz, 1H, 2-H), 3.91 (pseudo dt, *J*= 6.6, 2.6 Hz, 1H, 4-H), 3.68 (ddd, *J* = 8.8, 5.1, 2.8 Hz, 1H, 3a-H), 2.48-2.37 (m, 1H, 5-Ha), 1.13 (s, 9H, CH_3x3) ppm;

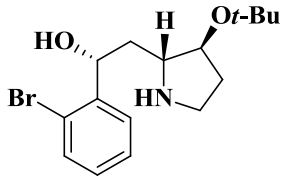
77 (***exo-syn***) **:** R_f = 0.15; $[\alpha]_D^{23}$ = – 39.22 (*c* = 0.72, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.65 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.50 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}), 7.30 (pseudo dt, *J* = 1.2, 7.6 Hz, 1H, H_{Ar}), 7.10 (pseudo dt, *J*= 1.7, 7.6 Hz, 1H, H_{Ar}), 5.30 (dd, *J* = 8.5, 6.3 Hz, 1H, 2-H), 4.11 (m, 1H, 4-H), 3.79 (ddd, *J* = 8.3, 6.5, 2.5 Hz, 1H, 3a-H), 3.34-3.18 (m, 2H, 6-H x

2), 3.00 (ddd, *J* = 12.1, 6.3, 2.5 Hz, 1H, 3-Ha), 1.98-1.88 (m, 3H, 3-Hb, 5-H x 2,) 1.23 (s, 9H, CH_3x3);^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 140.8 (s; C_{Ar}), 132.4 (d; CH_{Ar}), 128.6 (d; CH_{Ar}), 127.6 (d; CH_{Ar}), 127.1 (d; CH_{Ar}), 121.8 (s; C_{Ar}), 78.5 (d; C-2), 73.8 (s; *C*Me_3), 71.4 (d; C-4), 68.9 (d; C-3a), 54.1 (t; C-6), 38.5 (t; C-3), 33.4 (t; C-5), 28.3 (q; 3C, CH_3) ppm; IR ($CDCl_3$): *v* = 2976, 1568, 1472, 1440, 1390, 1364, 1185, 1108, 1047, 1016 cm^{-1}; MS (EI): *m/z* = 340.26, calcd. for $C_{16}H_{22}BrNO_2$ $[M + H]^+$: 342.14; $[M + Na]^+$: 361.98; anal. calcd. for $C_{16}H_{22}BrNO_2$ (340.26): C, 56.48; H, 6.52; N, 4.12; found C 55.51, H 6.47, N 4.72.

79(***endo-syn***)**:** R_f = 0.28; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}), 7.50 (dd, *J* = 8.0, 1.1 Hz, 1H, H_{Ar}), 7.28 (pseudo dt, *J* = 0.9, 7.5 Hz, 1H, H_{Ar}), 7.11 (pseudo dt, *J* = 1.7, 7.7 Hz, 1H, H_{Ar}), 5.25 (dd, *J* = 9.6, 6.4 Hz, 1H, 2-H), 4.11 (dd, *J* = 15.0, 7.5 Hz, 1H, 3a-H), 3.85-3.78 (m, 1H, 4-H), 3.49 (ddd, *J* = 11.3, 9.1, 4.7 Hz, 1H, 6-Ha), 2.96 (ddd, *J* = 13.6, 11.3, 6.3 Hz, 1H, 6-Hb), 2.65 (ddd, *J* = 12.4, 8.8, 6.4 Hz, 1H, 3-H), 2.32 (ddd, *J* = 12.4, 9.6, 6.0 Hz, 1H, 3-H), 2.11 (dddd, *J* = Hz, 1H, 5-Ha), 1.96 (dddd, *J* = Hz, 1H, 5-Hb), 1.13 (s, 9H, CH_3x3).

80 (**regioisomer**)**:** ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.55 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}), 7.53 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}), 7.32 (pseudo dt, *J* = 1.2, 7.6 Hz, 1H, H_{Ar}), 7.09 (ddd, *J*= 8.0, 7.4, 1.6 Hz, 1H, H_{Ar}), 4.23 (dd, *J*= 8.5, 7.2 Hz, 1H, 2-Ha), 4.12-4.05 (m, 2H, 3-H, 4-H), 3.69 (dd, *J*= 8.5, 6.2 Hz, 1H, 2-Hb), 3.56 (pseudo t, *J* = 3.6 Hz, 1H, 3a-H), 3.43-3.30 (m, 2H, 6-H x 2), 2.76 (dddd, *J* = 12.9, 8.9, 7.3, 6.6 Hz, 1H, 5-Ha), 1.71 (dddd, *J* = 12.9, 6.5, 4.5, 3.6 Hz, 1H, 5-Hb), 2.36-2.22 (m, 2H, 3-Hb, 5-Ha), 1.11 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 140.8 (s; C_{Ar}), 132.6 (d; CH_{Ar}), 128.7 (d; CH_{Ar}), 128.3 (d; CH_{Ar}), 128.2 (d; CH_{Ar}), 124. 1 (s; C_{Ar}), 81.1 (d; C-3a), 77.7 (d; C-4), 73.9 (s; *C*Me_3), 72.9 (t; C-2), 55.4 (t; C-6), 53.3 (d; C-3), 33.2 (t; C-5), 28.4 (q; 3C, CH_3) ppm;

(*R*)-1-(2-bromophenyl)-2-[(2*R*,3*S*)-3-(*tert*-butoxy)pyrrolidin-2-yl]ethanol (81)



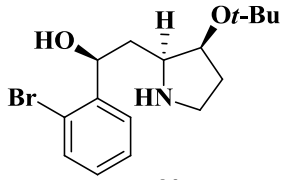
81

A mixture of isoxazolidines **76** and **78** (12:1 ratio; 78 mg, 0.23 mmol) and zinc powder (75 mg, 1.15 mmol) in AcOH/H_2O (9:1) (2.20 mL) was heated in an oil bath at 90 °C for 2 h. The reaction mixture was diluted with MeOH, filtered through cotton wool and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and the solution was basified to pH = 9 with a saturated aq. $NaHCO_3$ solution and solid Na_2CO_3 at 0 °C. The two phases were separated. The aqueous phase was sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2/MeOH (1% NH_4OH) 90:10] afforded pyrrolidines **81** (64.3 mg) in 82% yield as a white solid.

81: *Rf* = 0.31; m.p = 99-101 °C; $[\alpha]_D^{24}$= + 74.92 (*c* = 0.79, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.66 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.48 (dd, *J* = 7.9, 1.1 Hz, 1H, H_{Ar}), 7.33 (pseudo dt, *J* = 1.1, 7.5 Hz, 1H, H_{Ar}), 7.09 (dt, *J* = 1.7, 7.6 Hz, 1H, H_{Ar}), 5.25 (dd, *J* = 7.1, 3.2 Hz, 1H, C*H*OH), 4.32-3.66 (m, 2H, NH, OH), 3.96 (pseudo dt, *J* = 6.8, 3.7 Hz, 1H,3-H), 3.17-2.99 (m,3H, 2-H,5-Ha, 5-Hb), 2.14-2.03 (m, 1H, 4-Ha), 1.96 (ddd, *J* = 14.5, 7.7, 3.2Hz, 1H, C*H*HCHOH), 1.84 (ddd, *J* = 14.5, 7.1, 3.8 Hz, 1H, CH*H*CHOH), 1.74-1.65 (m, 1H, 4-Hb), 1.13 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 143.6 (s; C_{Ar}), 132.4 (d; CH_{Ar}), 128.3 (d; CH_{Ar}), 128.0 (d; CH_{Ar}), 127.4 (d; CH_{Ar}), 121.1 (s; C_{Ar}), 73.7 (d; C-3), 71.8 (d; CHOH), 63.8 (d, C-2), 44.4 (t, C-5), 35.7 (t, *C*H_2CHOH), 35.1 (t, C-4), 28.5 (q, 3C, CH_3)

ppm; IR ($CDCl_3$): ν = 2976, 1601,1567, 1464, 1440, 1391, 1236, 1190, 1122, 1087, 1023 cm^{-1}; MS (EI): *m/z* (%) = 341.10, calcd. for $C_{16}H_{22}BrNO_2$ $[M + H]^+$: 342.10; $[M + Na]^+$: 364.16; anal. calcd. for $C_{16}H_{24}BrNO_2$ (341.10): C, 56.15; H, 7.07; N, 4.09; found C 55.90, H 7.30, N 3.87.

(*S*)-1-(2-bromophenyl)-2-[(2*S*,3*S*)-3-(*tert*-butoxy)pyrrolidin-2-yl]ethanol (82)

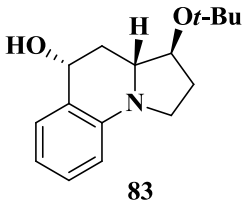


82

Following the same procedure used to prepare **81**, pyrrolidine **82** (62mg, 0.18 mmol) was obtained in 84% yield as a white solid starting from **77** (73 mg, 0.22 mmol).

82: *Rf* = 0.21; m.p = 58-60 °C; $[\alpha]_D^{25}$= – 45.78 (*c* = 1.025, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.64 (dd, *J* = 7.7, 1.5 Hz, 1H, H_{Ar}), 7.49 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{Ar}), 7.32 (dt, *J* = 1.1, 7.5 Hz, 1H, H_{Ar}), 7.09 (dt, *J* = 1.7, 7.6 Hz, 1H, H_{Ar}), 5.29 (pseudo t, *J* = 4.7Hz, 1H, C*H*OH) 4.97-3.81 (m, 2H, NH, OH), 3.93-3.87 (m, 1H, 3-H), 3.17 (ddd, *J* = 11.7, 9.0, 5.5 Hz, 1H, 5-Ha), 2.94 (pseudo dt, *J* = 9.9, 4.6 Hz, 1H, 2-H), 2.83 (ddd, *J* = 11.7, 9.1, 6.4 Hz, 1H, 5-Hb), 2.10-1.85 (m, 3H, C*HH*CHOH, 4-Ha), 1.71(dddd, *J* = 13.2, 9.0, 6.4, 4.2 Hz, 1H, 4-Hb), 1.1 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 144.2 (s; C_{Ar}), 132.6 (d; CH_{Ar}), 128.2 (d; CH_{Ar}), 127.9 (d; CH_{Ar}), 127.2 (d; CH_{Ar}), 121.4 (s; C_{Ar}), 73.6 (d; C-3), 71.6 (d; CHOH), 59.1 (d, C-2), 43.7 (t, C-5), 34.3 (t, *C*H_2CHOH), 33.7 (t, C-4), 28.3 (q, 3C, CH_3) ppm; IR ($CDCl_3$): ν = 2976, 1601,1567, 1464, 1438, 1390, 1364, 1231, 1191, 1112, 1078, 1023 cm^{-1}; anal. calcd. for $C_{16}H_{24}BrNO_2$ (341.10): C, 56.15; H, 7.07; N, 4.09; found C 56.10, H 6.97, N 4.05.

(3*S*,3a*R*,5*R*)-3-(*tert*-butoxy)-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinolin-5-ol (83)



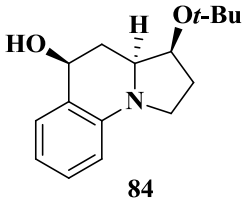
83

The mixed solvent *t*BuOH/H_2O (1:1) (1.65 mL) was added to a mixture of **81** (28 mg, 0.082 mmol), CuI (7.8 mg, 0.041 mmol), copper powder (2.62 mg, 0.041 mmol), K_3PO_4 (34.9 mg, 0.165 mmol) and L-proline (9.47 mg, 0.082 mmol) under nitrogen atmosphere in a vial. Nitrogen was bubbled through the reaction mixture to remove oxygen. The reaction mixture was heated at 90 °C in an oil bath for 12 h and then was filtered through a small pad of celite®. The solution was diluted with H_2O and sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x10 mL). The combined organic phases were washed with NH_4OH (1% H_2O solution) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel [eluent: CH_2Cl_2/MeOH (1% NH_4OH) 98:2] afforded **83** (15.2 mg) in 71% yield as a colourless oil.

83: R_f = 0.32; $[\alpha]_D^{23}$= – 58.179 (*c* = 0.56 , $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38 (pseudo dt, *J* = 7.5, 1.3 Hz, 1H, H_{Ar}), 7.13 (dddd, *J* = 8.1, 7.4, 1.5, 0.7 Hz, 1H, H_{Ar}), 6.67 (pseudo dt, *J* = 1.0, 7.4 Hz, 1H, H_{Ar}), 6.35 (dd, *J* = 8.1, 1.0 Hz, 1H, H_{Ar}), 4.96-4.85 (m, 1H, 5-H), 3.82-3.72 (m, 1H, 3-H), 3.36-3.28 (m, 2H, 3-Ha, 1-Ha), 3.22 (pseudo dt, *J* = 7.9, 9.2 Hz, 1H, 1-Hb), 2.56 (ddd, *J* = 11.5, 5.6, 2.9 Hz, 1H, 4-Ha), 2.28 (ddt, *J* = 12.4, 2.5, 7.5 Hz, 1H, 2-Ha), 1.89 (pseudo dq, *J* = 12.4, 9.3 Hz, 1H, 2-Hb), 1.70 (br d, *J* = 7.4 Hz, 1H, OH)1.50 (pseudo q, *J* = 11.5 Hz, 1H, 4-Hb), 1.24 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 143.7 (s; C_{Ar}), 128.6 (d; CH_{Ar}), 125.4 (d; CH_{Ar}), 124.8 (s; C_{Ar}), 115.7 (d; CH_{Ar}), 109.4 (d; CH_{Ar}), 73.7 (d; C-3), 67.3 (d; C-5), 59.8 (d, C-3a), 44.4 (t, C-1), 35.8 (t, C-4), 32.7 (t, C-2),

28.6 (q, 3C, CH_3) ppm; IR ($CDCl_3$): ν = 2977, 2854, 1605,1501, 1481, 1461, 1364, 1189, 1096, 1045 cm^{-1}; anal. calc. for $C_{16}H_{23}NO_2$ (261.17): C, 73.53; H, 8.87; N, 5.36; found C, 73.15; H, 8.55; N, 5.04.

(3*S*,3a*S*,5*S*)-3-(*tert*-butoxy)-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinolin-5-ol (84)

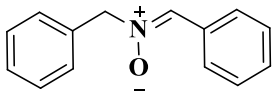


84

Following the same procedure used to prepare **83**, quinoline **84** (14.7 mg, 0.06 mmol) was obtained in 68% yield as a colourless oil starting from **82** (28.5 mg, 0.08 mmol).

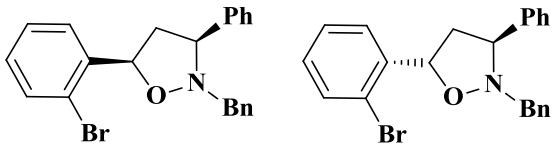
84: R_f = 0.42; $[\alpha]_D^{23}$= + 104.29 (c = 0.585, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.38 (pseudo dt, J = 7.5, 1.3 Hz, 1H, H_{Ar}), 7.11 (dddd, J = 8.1, 7.3, 1.6, 0.7 Hz, 1H, H_{Ar}), 6.64 (pseudo dt, J = 0.9, 7.4 Hz, 1H, H_{Ar}), 6.39 (dd, J = 8.1, 0.9 Hz, 1H, H_{Ar}), 4.94 (dd, J = 11.2, 5.5 Hz, 1H, 5-H), 4.11 (pseudo dt, J = 1.1, 4.0 Hz, 1H, 3-H), 3.62 (pseudo dt, J = 11.6, 3.6 Hz, 1H, 3a-H), 3.34 (pseudo dt, J = 7.9, 9.0, 1H, 1-Ha), 3.24 (pseudo dt, J = 2.5, 8.8, 1H, 1-Hb), 2.12 (ddd, J = 12.0, 5.5, 3.4 Hz, 1H, 4-Ha), 2.08-1.93 (m, 2H, 2-Ha, 2-Hb), 1.94 (pseudo q, J = 11.6 Hz, 1H, 4-Hb), 1.87-1.69 (m, 1H, OH), 1.19 (s, 9H, CH_3x3) ppm; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 144.2 (s; C_{Ar}), 128.5 (d; CH_{Ar}), 125.2 (d; CH_{Ar}), 124.8 (s; C_{Ar}), 115.2 (d; CH_{Ar}), 110.2 (d; CH_{Ar}), 71.8 (d; C-3), 67.8 (d; C-5), 59.9 (d, C-3a), 45.2 (t, C-1), 33.5 (t, C-4), 32.6 (t, C-2), 28.3 (q, 3C, CH_3) ppm; IR ($CDCl_3$): ν = 2976, 2858, 1605,1502, 1476, 1461, 1364, 1319, 1187, 1105, 1042 cm^{-1}.

(*Z*)-*N*-benzylidene-1-phenylmethanamine oxide (86) [124]



$NaHCO_3$ (1.06 g, 12.67 mmol) was added to a stirred solution of dibenzylamine (0.5 g, 2.53 mmol) in a 4:1 mixture of CH_3CN–THF (4.68 mL) and aqueous Na_2EDTA (0.01 M, 3.54 mL). The reaction mixture was cooled at 0 °C and then oxone (2.18 g, 3.55 mmol) was added portionwise over 1.5 h. The mixture was stirred at 0 °C for another 1hour. The reaction mixture was diluted with EtOAc (20 mL) and deionised H_2O (30 mL). The two phases were separated and the aqueous solution was sequentially extracted with EtOAc (2x20 mL) and CH_2Cl_2 (3x20 mL) respectively. The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel (eluent: EtOAc/petroleum ether 5:5) afforded pyrrolidine **86** (0.512 g, 2.43 mmol) in 96% yield as a white solid.

(3*S,5*R**) and (3*S**,5*S**)-2-benzyl-5-(2-bromophenyl)-3-phenylisoxazolidine (87 and 88)**



87 **88**

2-Bromostyrene (0.254 mL, 1.962 mmol) was added to a solution of the nitrone **86** (0.2 g, 0.95 mmol) in xylene (1.9 mL) and the reaction mixture was heated at 150 °C for 6 h in oil bath. The solvent was evaporated under a nitrogen stream to obtain a mixture of adducts **87**

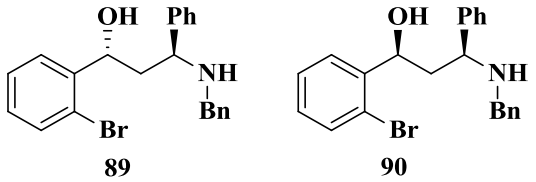
and **88**. The residue was purified by flash chromatography on silica gel (eluent: EtOAc/petroleum ether 3:97) to afford an inseparable mixture of **87** and **88** (0.309 g, 0.78 mmol, ca. 3.1:1 ratio) in 83% yield as a colourless oil.

87 (ca 3.1:1 mixture with isomers **88):** R_f = 0.35; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.65 (d, *J* = 7.8 Hz, 1H, H_{Ar}), 7.54-7.22 (m, 12H, H_{Ar}), 7.07 (pseudo dt, *J* = 1.8 Hz, 7.5 Hz, 1H, H_{Ar}), 5.44 (dd, *J* = 8.4, 6.5 Hz, 1H, 5-H), 4.08 (A part of an AB system, *J* = 14.3 Hz, CH_2Ph), 4.00 (dd, *J* = 9.4, 7.3 Hz, 1H, 3-H), 3.82 (B part of an AB system, *J* = 14.0 Hz, CH_2Ph), 3.41 (ddd, *J* = 12.6, 8.4, 7.3 Hz, 1H, 4-Ha), 2.22 (ddd, *J* = 12.6, 9.4, 6.5 Hz, 1H, 4-Hb). ^{13}C-NMR (CDCl3,100 MHz): δ = 143.4 (s; C_{Ar}), 139.0 (s; C_{Ar}), 137.8 (s; C_{Ar}), 132.2 (d; CH_{Ar}), 129.0 (d; 2C, CH_{Ar}), 128.6 (d; 2C, CH_{Ar}), 128.2 (d; CH_{Ar}), 128.1 (d; 2C, CH_{Ar}), 127.9 (d; CH_{Ar}), 127.8 (d; 2C, CH_{Ar}), 127.5 (d; CH_{Ar}), 127. 2 (d; CH_{Ar} x 2), 120.9 (s; C_{Ar}), 77.3 (d; C-5), 71.2 (d; C-3), 60.2 (t; CH_2Ph), 47.7 (t; C-4) ppm; IR: *v* = 3030, 2984, 2926, 2870, 1603, 1568, 1495, 1354, 1202, 1120 cm^{-1}; MS (EI): *m/z* (%) = 396 (M^{+2},16), 394 (M,15), 273 (39), 271 (41), 194 (17), 192 (100), 92 (73), 77 (69), 51 (45); anal. calcd. for $C_{22}H_{20}BrNO$ (394.30): C, 67.01; H, 5.11; N, 3.55; found C, 66.66.; H, 5.14; N, 3.57.

88: ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.65 (d, *J* = 7.8 Hz, 1H, H_{Ar}), 7.54-7.22 (m, 12H, H_{Ar}), 7.13 (pseudo dt, *J* = 1.8, 7.7 Hz, 1H, H_{Ar}), 5.52 (dd, *J* = 8.8, 5.6 Hz, 1H, 5'-H), 4.10 (A part of an AB system, *J* = 14.3 Hz, CH_2Ph), 3.95-3.89 (m, 1H, 3-H), 3.92 (B part of an AB system, *J* = 14.3 Hz, CH_2Ph'), 2.98 (pseudo dt, *J* = 9.0, 12.5 Hz, 1H, 4-Ha), 2.43 (ddd, *J* = 12.5, 7.8, 5.6 Hz, 1H, 4-Hb). ^{13}C-NMR (CDCl3, 100 MHz): δ =141.5 (s; $C_{Ar'}$), 132.5 (d; $CH_{Ar'}$), 129.0 (d; 2C, CH_{Ar}), 128.7 (d; 2C, CH_{Ar}), 128.6 (d; CH_{Ar}), 128.2 (d; 2C, CH_{Ar}), 127.9 (d; CH_{Ar}), 127.8

(d; 2C, CH_{Ar}), 127.3 (d; CH_{Ar}), 127. 2 (d; CH_{Ar} x 2), 121.5 (s; C_{Ar}), 77.4 (d; C-5), 69.8 (d; C-3), 59.8 (t; CH_2Ph), 46.7 (t; C-4) ppm;

(1*R,3*S**) and (1*S**,3*S**)-3-(benzylamino)-1-(2-bromophenyl)-3-phenylpropan-1-ol (89 and 90)**



89 **90**

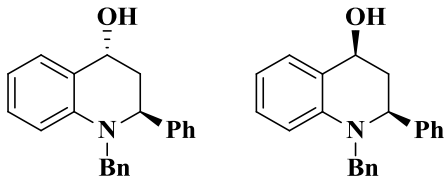
A mixture of isoxazolidines **87** and **88** (3.1:1 ratio; 0.22 g, 0.56 mmol) and zinc powder (0.18 g, 2.8 mmol) in AcOH:H_2O [9:1 (v/v), 5.4 mL] was heated in an oil bath at 85 °C for 3 h. The reaction mixture was diluted with MeOH, filtered through cotton wool and then filtrate was concentrated under reduced pressure. The white residue was dissolved in CH_2Cl_2, and the solution was basified up to pH = 9 with a saturated aq. $NaHCO_3$ solution and solid Na_2CO_3 at 0 °C. The two phases were separated. The aqueous phase was sequentially extracted with CH_2Cl_2 (3x30 mL) and EtOAc (2x20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a crude mixture of the two diastereomeric amino alcohols **89** and **90** in ca 2:1 ratio (calculated by ^1H NMR). Purification of the crude product by chromatography on silica gel [eluent: EtOAc (1% TEA) /petroleum ether 20:80] afforded **89** (93 mg, 42%) and a ca 1:1 mixture of **89** and **90** (111 mg, 50%) in 92% overall yield as yellow viscous oils.

89: R_f = 0.29; ^1H NMR ($CDCl_3$, 400 MHz): δ = 7.71 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.46 (dd, *J* = 7.9, 1.1 Hz, 1H, H_{Ar}), 7.42-7.24 (m, 11H, H_{Ar}), 7.08 (pseudo dt, *J* = 1.8, 7.6, 1H, H_{Ar}), 5.30 (dd, *J* = 10.3, 1.8 Hz, 1H, 1-H), 4.04 (dd, *J* = 11.3, 2.5 Hz, 1H, 3-H), 3.72 (A part of an AB

system, *J* = 12.6 Hz, CH_2Ph), 3.65 (B part of an AB system, *J* = 12.6 Hz, CH_2Ph), 2.14 (pseudo dt, *J* = 14.5, 2.2 Hz, 1H, 2-Ha), 1.73 (ddd, *J* = 14.5, 11.3, 10.3 Hz, 1H, 2-Hb). ^{13}C-NMR ($CDCl_3$,100 MHz): δ =143.6 (s; C_{Ar}), 142.7 (s; C_{Ar}), 138.9 (s; C_{Ar}), 132.3 (d; CH_{Ar}), 128.9 (d; 2C, CH_{Ar}), 128.6 (d, 2C, CH_{Ar}), 128.4 (d, 3C, CH_{Ar}), 127.6 (d; CH_{Ar}), 127.5 (d; CH_{Ar}), 127.4 (d; CH_{Ar}), 127.3 (d; CH_{Ar}), 126.3 (d; 2C, CH_{Ar}), 121.4 (s, C_{Ar}), 74.1 (d; C-4), 63.2 (d; C-2), 51.1 (t; CH_2Ph), 44.2 (t; C-3) ppm; IR: *v* = 3209, 3107, 3066, 3029, 2911, 2856, 1601, 1568, 1494, 1453, 1201, 1124, 1095, 1025 cm^{-1}; MS (EI): *m/z* (%) = 196 (93), 194 (3), 157 (1), 155 (1), 108 (6), 106 (12), 91 (100), 79 (4), 77 (16); anal. calcd. for $C_{22}H_{22}BrNO$ (396.32): C, 66.67; H, 5.60; N, 3.53; found C, 66.29.; H, 5.64; N, 3.43

90: ^{1}H NMR ($CDCl_3$, 300 MHz) Detectable signal: δ = 5.26-5.21 (m, 1H, 1-H), 3.82 (dd, *J* = 9.1, 2.9 Hz, 1H, 3-H), 2.26 (ddd, *J* = 14.7, 9.2, 3.8 Hz, 1H, 2-H).

(2*S,4*R**) and (2*S**,4*S**)-1-benzyl-2-phenyl-1,2,3,4-tetrahydroquinolin-4-ol (91a and 91b)**



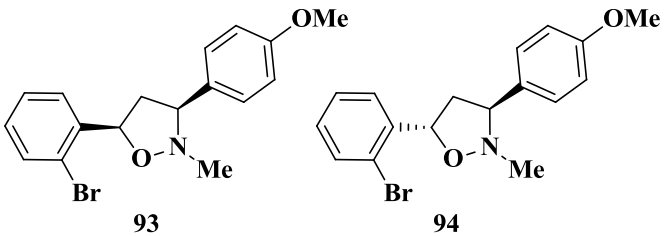
91a **91b**

The Mixed solvent *t*BuOH/H_2O (1:1) (1.34 mL) were added to a mixture of **89** and **90** (ca 1:1 ratio; 26.5 mg, 0.067 mmol), CuI (6.4 mg, 0.033 mmol), copper powder (2.12 mg, 0.033 mmol), K_3PO_4 (28.4 mg, 0.134 mmol) and L-proline (7.7 mg, 0.067 mmol) under nitrogen atmosphere in a vial. The nitrogen was bubbled through the reaction mixture to remove oxygen. The reaction mixture was heated at 90 °C for 12 h in oil bath and then was filter through small pad of Celite®. The solution was diluted with H_2O and sequentially extracted

with CH_2Cl_2 (3x10 mL) and EtOAc (2x10 mL). The combined organic phases were sequentially washed with NH_4OH (1% H_2O solution) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography (eluent: CH_2Cl_2) afforded **91a** and **91b** (10:1 *trans-cis* diastereomeric ratio; 9.2 mg, 0.029 mmol) in 44% overall yield as a yellow oil along with recovered **89** (4 mg, 30%).

91 (ca. 10:1 mixture)**:** R_f = 0.32; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.36-7.12 (m, 12H, H_{Ar}), 6.73 (pseudo dt, *J* = 1.0, 7.3 Hz, 2H, H_{Ar}), 4.80 (dd, *J* = 9.0, 4.8 Hz, 1H, 4-H), 4.79 (A part of an AB system, *J* = 17 Hz, CH_2Ph), 4.71 (dd, *J* = 11, 5.5 Hz, 1H, 2-H), 4.18 (B part of an AB system, *J* = 17 Hz, CH_2Ph), 2.51 (pseudo ddd, *J* = 11.5, 9.0, 4.8 Hz, 1H, 3-Ha), 1.35 (d, *J* = 9.0 Hz, 1H, 3-Hb). ^{13}C-NMR ($CDCl_3$,100 MHz): δ =144.4 (s; C_{Ar}), 142.5 (s; C_{Ar}), 138.3 (s; C_{Ar}), 129.3 (d; CH_{Ar}), 128.9 (d; 2C, CH_{Ar}), 128.8 (d, CH_{Ar}), 128.5 (d, 2C, CH_{Ar}), 127.2 (d; CH_{Ar}), 126.8 (d; CH_{Ar}), 126.6 (d; 2C, CH_{Ar}), 126.5 (d; 2C, CH_{Ar}), 125.1 (s; C_{Ar}), 116.6 (d, CH_{Ar}), 111.8 (d; CH_{Ar}), 66.5 (d; C-4), 59.6 (d; C-2), 53.1 (t; CH_2Ph), 39.4 (t; C-3) ppm; IR: *v* = 3107, 3064, 3029, 2924, 1604, 1574, 1495, 1452, 1346, 1226, 1124, 1053 cm^{-1}; MS (EI): *m/z* (%) = 315 (M^+), 297 (15), 296 (12), 220 (33), 206 (23), 205 (15), 128 (14), 91 (100); anal. calcd. for $C_{22}H_{21}NO$ (315.41): C, 83.78; H, 6.71; N, 4.44; found C, 83.03.; H, 6.39; N, 4.20

(3*S,5*R**) and (3*S**,5*S**)-5-(2-bromophenyl)-3-(4-methoxyphenyl)-2-methylisoxazolidine (93 and 94)**



93 **94**

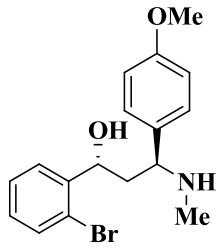
2-Bromostyrene **50** (0.078 mL, 0.605 mmol) was added to a solution of nitrone **92** (200 mg, 1.21 mmol) in toluene (2.4 mL) and the reaction mixture was heated at 110 °C overnight in oil bath. The solvent was evaporated under a nitrogen stream and the residue was purified by flash chromatography on silica gel (eluent: EtOAc/petroleum ether 5:95) to give the *cis* diastereoisomer **93** (106.5 mg, 0.306 mmol) in 51% yield and the *trans* diastereoisomer **94** (103.5 g, 0.297 mmol) in 49% yield as colourless oils

93: R_f = 0.24; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.89 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}), 7.51 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{Ar}),7.37 (pseudo dt, *J* = 1.2, 7.6 Hz, 1H, H_{Ar}), 7.20 (m, 2H, H_{Ar}), 7.12 (pseudo dt, *J* = 1.7, 7.6 Hz, 1H, H_{Ar}), 6.85-6.80 (m, 2H, H_{Ar}), 5.44 (dd, *J* = 8.3, 6.6 Hz, 1H, 5-H), 3.77 (s, 3H, OCH_3), 3.71-3.60 (m, 1H, 3-H), 3.33 (ddd, *J* = 12.6, 8.3, 7.1 Hz, 1H, 4-Ha), 2.67 (s, 3H, NCH_3), 2.19 (ddd, *J* = 12.6, 9.8, 6.6 Hz, 1H, 4-Hb); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 159.3 (s; C_{Ar}), 143.5 (s; C_{Ar}), 132.3 (d; CH_{Ar}), 130.3 (s; C_{Ar}), 128.9 (d; 2C, CH_{Ar}), 128.3 (d, CH_{Ar}), 127.6 (d, CH_{Ar}), 127.1 (d; CH_{Ar}), 120.9 (s; C_{Ar}), 114.0 (d; 2C, CH_{Ar}), 77.2 (d; C-5), 73.5 (d; C-3), 55.2 (q; OCH_3), 48.0 (t; C-4), 43.2 (q; NCH_3) ppm; IR: *v* = 3069, 2960, 2838, 1611, 1513, 1465, 1439, 1248, 1173, 1036, 1020 cm^{-1}; MS (EI): *m/z* (%) = 350 (M+2, 1), 348 (M^+, 2), 303 (7), 301 (7), 165 (100), 166 (11), 164 (97), 135 (22), 133 (10), 91 (17), 77 (28); anal. calcd. for $C_{17}H_{18}BrNO_2$ (348.23): C, 58.63; H, 5.21; N, 4.02; found C, 58.75; H, 5.20; N, 3.94

94: R_f = 0.15; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.69 (dd, *J* = 7.8, 1.5 Hz, 1H, H_{Ar}), 7.54 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{Ar}), 7.38 - 7.30 (m,3H, H_{Ar}), 7.15 (pseudo dt, *J* = 1.7, 7.7 Hz, 2H, H_{Ar}), 6.92-6.87 (m, 2H, H_{Ar}), 5.51 (dd, *J* = 8.9, 5.6 Hz, 1H, 5-H), 3.81 (s, 3H, OCH_3), 3.67 - 3.48 (m,1H, 3-H), 3.00 - 2.88 (m, 1H, 4-Ha), 2.71 (s, 3H, NCH_3), 2.40 (ddd, *J* = 12.6, 7.7, 5.6 Hz, 1H, 4-Hb); ^{13}C-NMR ($CDCl_3$,100 MHz): δ = 159.4 (s; C_{Ar}), 141.3 (s; C_{Ar}), 132.6 (d; CH_{Ar}),

130.5 (s; C_{Ar}), 128.9 (d; 2C, CH_{Ar}), 128.7 (d, CH_{Ar}), 127.4 (d, CH_{Ar}), 127.1 (d; CH_{Ar}), 121.7 (s; C_{Ar}), 114.1 (d; 2C, CH_{Ar}), 77.2 (d; C-5), 72.3 (d; C-3), 55.2 (q; OCH_3), 46.7 (t; C-4), 43.1 (q; NCH_3) ppm; IR: ν = 3069, 2960, 2838, 1611, 1513, 1470, 1442, 1304, 1278, 1175, 1035 cm^{-1}; MS (EI): *m/z* (%) = 349 (M+1, 4), 347 (M^+, 4), 303 (4), 301 (5), 185 (18), 183 (17), 165 (100), 164 (88), 91 (22), 89 (13), 77 (40), 75 (16); anal. calcd. for $C_{17}H_{18}BrNO_2$ (348.23): C, 58.63; H, 5.21; N, 4.02; found C, 58.71; H, 5.20; N, 3.99.

(1*R,3*S**)-1-(2-bromophenyl)-3-(4-methoxyphenyl)-3-(methylamino)propan-1-ol (95)**

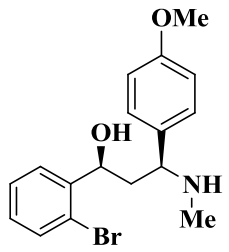


95

A mixture of isoxazolidine **93** (0.222 g, 0.637 mmol) and zinc powder (0.208 g, 3.19 mmol) in AcOH:H_2O [9:1(v/v), 5.5 mL] was heated in an oil bath at 85 °C for 3 h. The reaction mixture was diluted with MeOH and filtered through cotton wool. The filtrate was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2. The solution was basified to pH = 9 with a saturated aq. solution of $NaHCO_3$ and solid Na_2CO_3 at 0 °C. The two phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3x30 mL) and EtOAc (2x20 mL). The combined organic phases were sequentially washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1%NH_4OH) 93:7] afforded **95** (210 mg, 0.6 mmol) in 94% overall yield as a colourless viscous oil.

95: R_f = 0.31; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.70 (dd, *J* = 7.8, 1.7 Hz, 1H, H_{Ar}), 7.46 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}), 7.36-7.31 (m, 1H, H_{Ar}), 7.17-7.12 (m, 1H, H_{Ar}), 7.08 (dt, *J* = 1.7, 7.6 Hz, 1H, H_{Ar}), 6.90-6.85 (m, 2H, H_{Ar}), 5.29 (dd, *J* = 10.3, 1.9 Hz, 1H, 1-H), 3.80 (dd, *J* = 11.2, 2.6, Hz, 1H, 3-H), 3.80 (s, 3H, OCH_3), 2.31 (s, 3H, NCH_3), 2.08 (pseudo dt, *J* = 14.4, 2.3 Hz, 1H, 2-Ha), 1.68 (ddd, *J* = 14.4, 11.2, 10.3, 1H, 2-Hb), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ =158.9 (s; C_{Ar}), 143.8 (s; C_{Ar}), 134.7 (s; C_{Ar}), 132.3 (d; CH_{Ar}), 128.3 (d; 2C, CH_{Ar}), 127.6 (d, CH_{Ar}), 127.4 (d, CH_{Ar}), 127.3 (d; CH_{Ar}), 121.4 (s; C_{Ar}), 114 (d; 2C, CH_{Ar}), 74.1 (d; C-1), 64.8 (d; C-3), 55.3 (q; OCH_3), 43.7 (t; C-2), 33.2 (q; NCH_3) ppm; IR: *v* = , 2952, 2909, 2803, 1609, 1513, 1464, 1442, 1306, 1248, 1177, 1034 cm^{-1}; MS (EI): *m/z* (%) = 350 (M), 187 (4), 185 (7), 165 (1), 163 (1), 151 (50), 91 (22), 77 (51), 42 (100); anal. calcd. for $C_{17}H_{20}BrNO_2$ (350.25): C, 58.30; H, 5.76; N, 4.00; found C, 58.08; H, 5.59; N, 3.79;

(1*S*,3R)-1-(2-bromophenyl)-3-(4-methoxyphenyl)-3-(methylamino)propan-1-ol (96)**



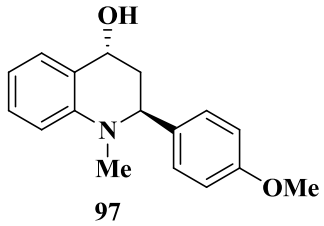
96

Following the same procedure to prepare **95**, the diastereomeric amino alcohol **96** (0.23 g, 0.66 mmol, 91% yield, white solid) was obtained starting from **94** (0.25 g, 0.72 mmol).

96: R_f = 0.48; m.p = 100–102 °C ;^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.71 (dd, *J* = 7.7, 1.7 Hz, 1H, H_{Ar}), 7.46 (dd, *J* = 7.9, 1.2 Hz, 1H, H_{Ar}),7.37 (pseudo dt, *J* = 1.2, 7.7 Hz, 1H, H_{Ar}), 7.16-7.11 (m, 2H, H_{Ar}), 7.11 (pseudo dt, *J* = 1.7, 7.5 Hz, 1H, H_{Ar}), 6.89-6.84 (m, 2H, H_{Ar}), 5.22 (dd, *J* = 6.0, 3.6 Hz, 1H, 1-H), 3.80 (s, 3H, OCH_3), 3.64 (dd, *J* = 8.8, 3.0, Hz, 1H, 3-H), 2.35 (s,

3H, NCH_3), 2.22 (ddd, *J* = 14.7, 8.8, 3.6 Hz, 1H, 2-Ha), 2.04 (ddd, *J* = 14.7, 6.0, 3.0 Hz, 1H, 2-Hb), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ =158.8 (s; C_{Ar}), 143.7 (s; C_{Ar}), 133.8 (s; C_{Ar}), 132.6 (d; CH_{Ar}), 128.3 (d; CH_{Ar}), 127.7 (d; CH_{Ar}), 127.6 (d; 2C, CH_{Ar}), 127.3 (d; CH_{Ar}), 121.3 (s; C_{Ar}), 113.9 (d; 2C, CH_{Ar}), 71.5 (d; C-1), 61.5 (d; C-3), 55.3 (q; OCH_3), 40.1 (t; C-2), 33.3 (q; NCH_3) ppm; IR: *ν* = , 3065, 2954, 2913, 2838, 1610, 1513, 1464, 1442, 1305, 1240, 1177, 1036 cm^{-1}; MS (EI): *m/z* (%) = 350 (M), 318 (3), 320 (3), 187 (3), 185 (6), 152 (4), 150 (100), 91 (19), 77 (42); anal. calcd. for $C_{17}H_{20}BrNO_2$ (350.25): C, 58.30; H, 5.76; N, 4.00; found C, 58.23; H, 5.89; N, 3.95

(2*S,4*R**)-2-(4-methoxyphenyl)-1-methyl-1,2,3,4-tetrahydroquinolin-4-ol (97)**



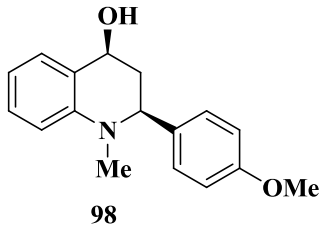
97

The mixed solvent *t*BuOH: H_2O [1:1 (v/v), 1.45 mL] was added to a mixture of **95** (25.5 mg, 0.073 mmol), CuI (6.93 mg, 0.036 mmol), copper powder (2.31 mg, 0.036 mmol), K_3PO_4 (30.91 mg, 0.147 mmol) and L-proline (8.38 mg, 0.073 mmol) under nitrogen atmosphere in a vial. Nitrogen was bubbled through the reaction mixture to remove oxygen. The reaction mixture was heated at 90 °C for 12 h in an oil bath and then was filtered through a small pad of Celite®. The solution was diluted with H_2O and sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x5 mL). The combined organic phases were washed with NH_4OH (1% H_2O solution) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography on silica gel [eluent: EtOAc/petroleum ether (1% TEA)

20:80] afforded **97** (7.4 mg, 0.027 mmol) in 38% yield as a yellow liquid along with unreacted starting material **95** (8.6 mg, 0.024 mmol).

97: R_f = 0.21; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.26 -7.19 (m, 4H, H_{Ar}), 6.88 (Pseudo dt, *J* = 2.5, 9.3 Hz, 2H, H_{Ar}), 6.76 - 6.70 (m, 2H, H_{Ar}), 4.66 (dd, *J* = 5.3, 3.5 Hz, 1H, 4-H), 4.42 (dd, *J* = 9.5, 4.3, Hz, 1H, 2-H), 2.81 (s, 3H, OCH_3), 2.76 (s, 3H, NCH_3), 2.18 (ddd, *J* = 11.6, 7.2, 6.1 Hz, 1H, 3-Ha), 2.08 (dt, *J* = 9.6, 3.5 Hz, 1H, 3-Hb), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ =158.8 (s; C_{Ar}), 146.4 (s; C_{Ar}), 135.6 (s; C_{Ar}), 129.5 (d; CH_{Ar}), 128.3 (d; CH_{Ar}), 127.9 (d; 2C, CH_{Ar}), 124.3 (s; CH_{Ar}), 116.2 (d; CH_{Ar}), 114.1 (d; 2C, CH_{Ar}), 112.1 (d; CH_{Ar}), 65.3 (d; C-4), 58.8 (d; C-2), 55.2 (q; OCH_3), 39.6 (t; C-3), 37.2 (q; NCH_3) ppm; IR: *v* = 2956, 2927, 2838, 1605, 1511, 1501, 1373, 1247, 1173, 1040 cm^{-1}; MS (EI): *m/z* (%) = 269 (M^+), 251 (32), 250 (47), 220 (2), 144 (100), 91 (2), 77 (9), 51 (5); anal. calcd. for $C_{17}H_{19}NO_2$ (269.34): C, 75.81.30; H, 7.11; N, 5.20; found C, 75.38; H, 7.04; N, 4.58

(2*S,4*S**)-2-(4-methoxyphenyl)-1-methyl-1,2,3,4-tetrahydroquinolin-4-ol (98)**



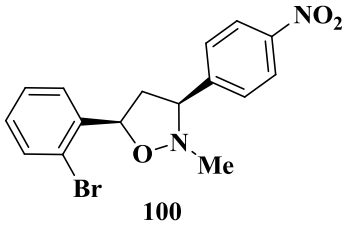
98

Following the same procedure to prepare **97**, the diastereomeric quinoline **98** (17 mg, 0.063 mmol, 71% yield, yellow waxy solid) was obtained starting from **96** (31 mg, 0.09 mmol).

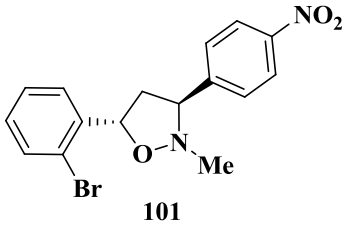
98: R_f = 0.42; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35 (Pseudo dt, *J* = 1.4, 7.3 Hz, 1H, H_{Ar}), 7.23 (dt, *J* = 7.3, 8.6 Hz, 1H, H_{Ar}), 7.14 (pseudo dt, *J* = 2.5, 9.3 Hz, 2H, H_{Ar}), 6.86 (pseudo dt, *J* = 2.5, 9.3 Hz, 2H, H_{Ar}), 6.77 - 6.70 (m, 2H, H_{Ar}), 4.83 (dd, *J* = 12.3, 7.5 Hz, 1H, 4-H), 4.45

(dd, *J* = 7.7, 4.8, Hz, 1H, 2-H), 3.79 (s, 3H, OCH_3), 2.80 (s, 3H, NCH_3), 2.43 (dt, *J* = 4.7, 6.0 Hz, 1H, 3-Ha), 2.24 (dt, *J* = 7.6, 13.2 Hz, 1H, 3-Hb), ^{13}C-NMR ($CDCl_3$, 100 MHz): δ =158.7 (s; C_{Ar}), 145.7 (s; C_{Ar}), 134.8 (s; C_{Ar}), 129.1 (d; CH_{Ar}), 127.6 (d; 2C, CH_{Ar}), 127.3 (d; CH_{Ar}), 125.7 (s; CH_{Ar}), 116.5 (d; CH_{Ar}), 114.2 (d; 2C, CH_{Ar}), 111.5 (d; CH_{Ar}), 66.6 (d; C-4), 61.2 (d; C-2), 55.2 (q; OCH_3), 41.1 (t; C-3), 37.7 (q; NCH_3) ppm; IR: *v* = 2957, 2838, 1606, 1510, 1455, 1339, 1247, 1171, 1097, 1037 cm^{-1}; MS (EI): *m/z* (%) = 269 (M^+), 251 (27), 250 (39), 206 (6), 144 (100), 77 (8); anal. calcd. for $C_{17}H_{19}NO_2$ (269.34): C, 75.81.30; H, 7.11; N, 5.20; found C, 75.52; H, 7.23; N, 5.41

(3*S,5*R**) and (3*S**,5*S**)-5-(2-bromophenyl)-2-methyl-3-(4-nitrophenyl) isoxazolidine (100 and 101)**



100



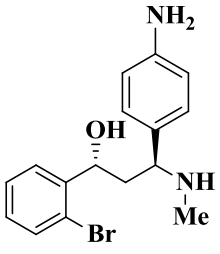
101

2-Bromostyrene **50** (0.079 mL, 0.61 mmol) was added to a solution of nitrone **99** (220 mg, 1.22 mmol) in chlorobenzene (2.4 mL) and the reaction mixture was heated at 150 °C for 3 h in an oil bath. The solvent was evaporated under a nitrogen stream and the residue was purified by flash chromatography on silica gel (eluent: EtOAc/petroleum ether 10:90), to afford an inseparable mixture of **100** and **101** (2.4:1 ratio; 219.5 mg, 0.604 mmol) in 99% overall yield as a yellow oil. A pure sample of the major isomer **100** was obtained after repeated purification and used for the characterization.

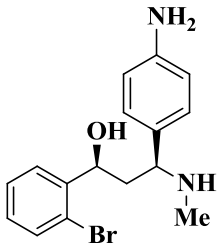
100: R_f = 0.4; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.17 – 8.13 (m, 2H, H_{Ar}), 7.80 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}),7.50–7.46 (m, 3H, H_{Ar}), 7.37 (pseudo dt, *J* = 1.0, 7.8 Hz, 1H, H_{Ar}), 7.13 (pseudo dt, *J* = 1.7, 7.7 Hz, 1H, H_{Ar}), 5.49 (dd, *J* = 8.2, 6.4 Hz, 1H, 5-H), 3.88 (pseudo t, *J* = 8.3 Hz, 1H, 3-H), 3.46 (ddd, *J* = 12.7, 8.2, 7.7 Hz, 4-Ha), 2.74 (s, 3H, NCH_3), 2.15 (ddd, *J* = 12.7, 9.0, 6.4 Hz, 1H, 4-Hb); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ =147.0 (s; C_{Ar}), 146.9 (s; C_{Ar}), 142.5 (s, C_{Ar}), 132.4 (d; CH_{Ar}), 128.6 (d; CH_{Ar}), 128.4 (d; 2C, CH_{Ar}), 127.6 (d, CH_{Ar}), 126.8 (d, CH_{Ar}), 123.9 (d; 2C, CH_{Ar}), 121 (s; C_{Ar}), 77.3 (d; C-5), 72.7 (d; C-3), 48.0 (t; C-4), 43.5 (q; NCH_3) ppm; IR: *ν* = 3065, 2960, 2852, 1606, 1524, 1466, 1440, 1351, 1109, 1042, 1021 cm^{-1}; MS (EI): *m/z* (%) = 365 (M^{+2}, 2), 263 (M^+, 3), 318 (30), 316 (29), 272 (17), 270 (18), 193 (3), 191 (21), 181 (8), 179 (100), 77 (42);

101: ^{1}H NMR ($CDCl_3$, 300 MHz) Detectable signal: δ = 8.26 – 8.20 (m, 2H, H_{Ar}), 7.67 (dd, *J* = 7.1, 1.6 Hz, 1H, H_{Ar}), 7.17 (dt, *J* = 1.7, 7.5 Hz, 1H, H_{Ar}), 5.00 (dd, *J* = 8.8, 6.2 Hz, 1H, 5-H), 3.79 (pseudo t, *J* = 7.9 Hz, 1H, 3-H), 2.90 (pseudo dt, *J* = 12.6, 8.7 Hz, 4-H), 2.50 (ddd, *J* = 12.6, 8.1, 6.2 Hz, 4-H).

(1*S,3*R**) and (1*S**,3*S**)-3-(4-aminophenyl)-1-(2-bromophenyl)-3-(methylamino)propan-1-ol (102 and 103)**



102



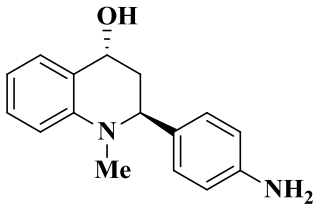
103

A mixture of isoxazolidines **100** and **101** (2.4:1 ratio; 0.04 g, 0.11 mmol) and zinc powder (0.037 g, 0.56 mmol) in AcOH:H_2O [9:1 (v/v), 0.98 mL] was heated in an oil bath at 80 °C for 3 h. The reaction mixture was diluted with MeOH and filtered through cotton wool. The filtrate was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2, The solution was basified to pH = 9 with a saturated aq. $NaHCO_3$ solution and solid Na_2CO_3 at 0 °C. The two phases were separated. The aqueous phase was sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (2% NH_4OH) 9:1] afforded a mixture of **102** and **103** (2.2:1 ratio; 0.025 g, 0.07 mmol) in 62% yield as a yellow viscous oil. A pure sample of the major compound **102** was obtained after repeated purification and used for the characterization.

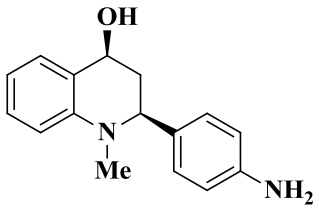
102: R_f = 0.4; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.69 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}), 7.45 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}),7.32 (pseudo dt, *J* = 1.0 7.5 Hz, 1H, H_{Ar}), 7.07 (pseudo dt, *J* = 1.7, 7.6 Hz, 1H, H_{Ar}), 7.04-7.00 (m, 2H, H_{Ar}), 6.69-6.62 (m, 2H, H_{Ar}), 5.27 (dd, *J* = 10.4, 1.8 Hz, 1H, 1-H), 3.76 (dd, *J* = 11.2, 2.6 Hz, 1H, 3-H), 2.65 (br s, 1H, NH), 2.31 (s, 3H, NCH_3), 2.08 (pseudo dt, *J* = 14.4, 2.3 Hz, 1H, 2-Ha); 1.71 (pseudo dt, *J* = 14.4, 10.8 Hz, 1H, 2-Hb); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 145.3 (s; C_{Ar}), 143.5 (s; C_{Ar}), 132.0 (d; CH_{Ar}), 131.7 (s; C_{Ar}), 127.9 (d; CH_{Ar}), 127.2 (d; 2C, CH_{Ar}), 127.0 (d, CH_{Ar}), 121.1 (d, CH_{Ar}), 119.8 (s; C_{Ar}),114.8 (d; 2C, CH_{Ar}), 73.7 (d; C-1), 64.5 (d; C-3), 43.1 (t; C-2), 32.8 (q; NCH_3) ppm; IR: *v* = 2950, 2914, 1624, 1586, 1466, 1442, 1275, 1180, 1074, 1017 cm^{-1}; MS (EI): *m/z* (%) = 359, 357, 337, 335, 306, 304, 136 (10), 134 (100), 91 (5), 77 (10); anal. calcd. for C16H17BrN_2O_3 (364.04): C, 57.32 ; H, 5.71; N, 8.36; found C, 56.50 ; H, 5.36 ; N, 7.57.

103: ^{1}H NMR ($CDCl_3$, 300 MHz) Detectable signal: δ = 5.23 (dd, *J* = 5.9, 3.5 Hz, 1-H), 3.82 (dd, *J* = 11.1, 2.5 Hz, 3-H), 2.34 (s, 3H, NCH_3).

(2*S,4*R**)- and (2*S**,4*S**)-2-(4-aminophenyl)-1-methyl-1,2,3,4-tetrahydroquinolin-4-ol (104a and 104b)**



104a



104b

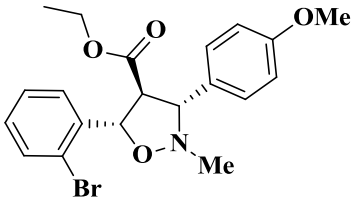
The mixed solvent *t*BuOH: H_2O (1:1) (1.49 mL) was added to a mixture of **102** and **103** (1:1 ratio; 25 mg, 0.07 mmol), CuI (7.1 mg, 0.037 mmol), copper powder (2.37 mg, 0.037 mmol), K_3PO_4 (31.66 mg, 0.15 mmol) and L-proline (8.6 mg, 0.07 mmol) under nitrogen atmosphere in a vial. Nitrogen was bubbled through the reaction mixture to remove oxygen. The reaction mixture was heated at 90 °C for 12 h in an oil bath and then was filtered through a small pad of Celite®. The solution was diluted with H_2O and sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x5 mL). The combined organic phases were washed with NH_4OH (1% H_2O solution) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography [eluent: EtOAc/petroleum ether (1% TEA) 6:4] afforded **104a** and **104b** (7:1 *trans-cis* diastereomeric ratio; 9.3 mg, 0.036 mmol) in 50 % overall yield as yellow oils.

104a (ca. 7:1 mixture with **104b**)**:** R_f = 0.34; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.17-7.13 (m, 2H, H_{Ar}), 6.99 (pseudo dt, *J* = 2.3, 8.8 Hz, 2H, H_{Ar}), 6.61-6.54 (m, 4H, H_{Ar}), 4.58 (dd, *J* = 5.4, 3.5 Hz, 1H, 4-H), 4.28 (dd, *J* = 9.5, 4.4 Hz, 1H, 2-H), 2.68 (s, 3H, NCH_3), 2.08 (ddd, *J* = 11.5,

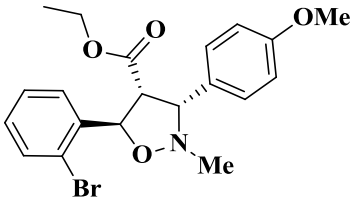
9.4, 6.3 Hz, 1H, 3-Ha), 2.01 (dd, *J* = 9.4, 3.6 Hz, 1H, 3-Hb); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ =146.2 (s; C_{Ar}), 145.4 (s; C_{Ar}), 129.4 (d; CH_{Ar}), 129.0 (s; C_{Ar}), 128.2 (d; CH_{Ar}), 127.9 (d, 2C, CH_{Ar}), 127.5 (d; CH_{Ar}), 116.3 (s; C_{Ar}), 116.0 (d; CH_{Ar}), 115.2 (d; 2C, CH_{Ar}), 65.40 (d; C-4), 58.91 (d; C-2), 39.66 (t; C-3), 29.73 (q; NCH_3) ppm; IR: *v* = 3380, 2925, 1622, 1515, 1495, 1372, 1264, 1099, 1042 cm^{-1}; MS (EI): *m/z* (%) = 255 (M^{+1}, 3), 254 (M^+, 22), 235 (10), 220 (11), 144 (12), 119 (82), 91 (10), 77 (16), 43 (100); anal. calcd. for $C_{16}H_{18}N_2O$ (254.14): C, 75.56 ; H, 7.13; N, 11.01; found C, 74.40; H, 6.80; N, 10.71.

1**04b** (ca. 1:7 mixture with **104a**), detectable signal of *cis*-isomer **:** ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.35 (dm, *J* = 7.4, 1H, H_{Ar}), 7.02-6.98 (m, 2H, H_{Ar}), 4.81 (dd, *J* = 7.3, 4.8 Hz, 1H, 4-H), 2.80 (s, 1H, N-Me), 2.41 (pseudo dt, *J* = 13.3, 4.8 Hz, 1H, 3-H), 2.24 (pseudo dt, *J* = 13.3, 7.5 Hz, 1H, 3-Hb).

(3*R,4*S**,5*S**)- and (3*R**,4*R**,5*R**)-ethyl-5-(2-bromophenyl)-3-(4-methoxyphenyl)-2-methylisoxazolidine-4-carboxylate (106 and 107)**



106



107

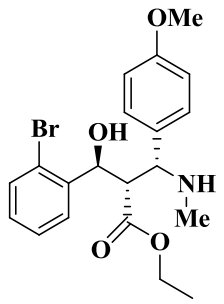
A mixture of 2-bromophenylacrylate **105** (100 mg, 0.392 mmol) and nitrone **92** (323.7 mg, 1.96 mmol) was heated without solvent at 90 °C for 48 h in an oil bath. The reaction mixture was purified by flash chromatography on silica gel (eluent: EtOAc/petroleum ether 8:92) to afford the *trans-trans* adduct **106** (92.4 mg, 0.22 mmol) in 56% yield and the *cis-trans* adduct **107** (43.2 mg, 0.10 mmol) in 26% yield as colourless oils.

106: R_f = 0.27; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.94 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}), 7.50 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}),7.39 (pseudo dt, *J* = 1.2, 7.8 Hz, 1H, H_{Ar}), 7.21-7.16 (m, 2H, H_{Ar}), 7.14 (pseudo dt, *J* = 1.7, 7.7 Hz, 1H, H_{Ar}), 6.86-6.81 (m, 2H, H_{Ar}), 5.69 (d, *J* = 5.2 Hz, 1H, 5-H), 4.22-4.14 (m, 2H, CH_2), 3.79 (d, *J* = 8.9 Hz, 1H, 3-H), 3.78 (s, 3H, OCH_3), 3.31 (dd, *J* = 8.9, 5.2 Hz, 1H, 4-H), 2.69 (s, 3H, NCH_3), 1.20 (t, *J* = 7.1 Hz, 3H, $CH_2C\mathit{H}_3$); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ =171.6 (s; CO), 159.7 (s; C_{Ar}), 142 (s; C_{Ar}), 132.3 (d; CH_{Ar}), 128.9 (d; 2C, CH_{Ar}), 128.7 (d; CH_{Ar}), 128.5 (s; C_{Ar}), 128.0 (d; CH_{Ar}), 127.2 (d; CH_{Ar}), 121.1 (s; C_{Ar}), 114.1 (d; 2C, CH_{Ar}), 80.1 (d; C-5), 78 (d; C-3), 65.8 (d; C-4), 61.2 (t; C-CH_2), 55.2 (q; OCH_3), 42.7 (q; NCH_3), 14.2 (q; $CH_2\mathit{C}H_3$Me) ppm; IR: *v* = 2963, 2846, 1729, 1612, 1514, 1248, 1175, 1034, 1021 cm^{-1}; MS (EI): *m/z* (%) = 421 (M+1, 8), 420 (M^+, 1), 223 (1), 221 (2), 167 (1), 165 (100), 91 (3), 77 (10); anal. calcd. for $C_{20}H_{22}BrNO_4$ (420.30): C, 57.15; H, 5.28; N, 3.33; found C, 57.18; H, 5.45; N, 3.32

107: R_f = 0.14; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.69 (dd, *J* = 7.7, 1.0 Hz, 1H, H_{Ar}), 7.55 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}), 7.36 (pseudo dt, *J* = 1.1, 7.6 Hz, 1H, H_{Ar}), 7.29-7.24 (m, 2H, H_{Ar}), 7.17 (pseudo dt, *J* = 1.7, 7.6 Hz, 1H, H_{Ar}), 6.89-6.84 (m, 2H, H_{Ar}), 5.89 (d, *J* = 5.6 Hz, 1H, 5-H), 3.94 (br d, *J* = 8.7 Hz, 1H, 3-H), 3.85- 3.76 (m, 1H, C*H*H), 3.79 (s, 3H, OCH_3), 3.74-3.64 (m, 1H, C*H*H), 3.48-3.39 (m, 1H, 4-H), 2.74 (s, 3H, NCH_3), 0.85 (t, *J* = 7.1 Hz, 3H, $CH_2C\mathit{H}_3$); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ =170 (s; CO), 159.2 (s; C_{Ar}), 139.2 (s; C_{Ar}), 132.9 (d; CH_{Ar}), 129.2 (d; 2C, CH_{Ar}),129 (d; CH_{Ar}), 127.8 (d; CH_{Ar}), 127.1 (d; CH_{Ar}), 126.5 (s; C_{Ar}), 121.9 (s; C_{Ar}), 113.4 (d; 2C, CH_{Ar}), 79.4 (d; C-5), 74.9 (d; C-3), 61.6 (d; C-4), 60.4 (t; CH_2), 54.9 (q; OCH_3), 42.8 (q; NCH_3), 13.4 (q; $CH_2\mathit{C}H_3$) ppm; IR: *v* = 2936, 2839, 1731, 1612, 1513, 1466, 1438, 1380, 1250, 1176, 1035 cm^{-1}; MS (EI): *m/z* (%) = 421 (M+1, 5), 420 (M^+, 1), 223 (1),

221 (1), 167 (1), 165 (100), 91 (4), 77 (8); anal. calcd. for $C_{20}H_{22}BrNO_4$ (420.30): C, 57.15; H, 5.28; N, 3.33; found C, 57.18; H, 5.45; N, 3.32

(2*S,3*S**)-ethyl-3-(2-bromophenyl)-3-hydroxy-2-[(*R**)-(4-methoxyphenyl) (methylamino)methyl]propanoate (108)**.



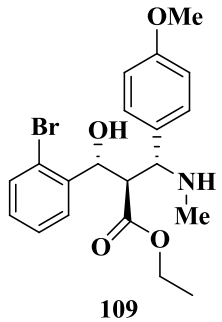
108

The mixture of isoxazolidine **106** (221.5 mg, 0.53 mmol) and zinc powder (172.2 mg, 26.35 mmol) in AcOH:H_2O [9:1 (v/v), 5.1 mL] was heated in an oil bath at 85 °C for 3 h. The reaction mixture was diluted with MeOH, filtered through cotton wool and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and the solution was basified to pH = 9 with a saturated aq. $NaHCO_3$ solution and solid Na_2CO_3 at 0 °C. The two phases were separated. The aqueous phase was sequentially extracted with CH_2Cl_2 (3x30 mL) and EtOAc (2x20 mL). The combined organic phases were washed with brine, 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% TEA) 6:4] afforded **108** (222 mg, 0.52 mmol) in 99% yield as a white solid.

108: R_f = 0.29; m.p. 106-108 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.58 (dd, *J* = 7.8, 1.6 Hz, 1H, H_{Ar}), 7.48 (dd, *J* = 8.0, 1.2 Hz, 1H, H_{Ar}),7.32 (pseudo dt, *J* = 1.2, 7.6 Hz, 1H, H_{Ar}), 7.21-7.16 (m, 2H, H_{Ar}), 7.09 (ddd, *J* = 8.0, 7.4, 1.6 Hz, 1H, H_{Ar}), 6.88-6.82 (m, 2H, H_{Ar}), 5.62 (d, *J*

= 9.3 Hz, 1H, 1-H), 4.00 (d, *J* = 10.8, Hz, 1H, 3-H), 3.78 (s, 3H, OCH_3), 3.61-3.44 (m, 2H, CH_2), 3.15 (dd, *J* = 10.8, 9.2 Hz, 1H, 2-H), 2.32 (s, 3H, NCH_3), 0.67 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ =169.6 (s; CO), 159.2 (s; C_{Ar}), 140.4 (s; C_{Ar}), 132.7 (d; CH_{Ar}), 131.3 (s; C_{Ar}), 129.6 (d; CH_{Ar}), 129.2 (d; CH_{Ar}), 128.3 (d; 2C, CH_{Ar}), 127.5 (d; CH_{Ar}), 123.1 (s; C_{Ar}), 113.9 (d; 2C, CH_{Ar}), 75.9 (d; C-5), 67.2 (d; C-3), 60.1 (d; C-4), 58.1 (t; CH_2), 55.2 (q; OCH_3), 33.3 (q; NCH_3), 13.5 (q; CH_2CH_3) ppm; IR: *v* = 2983, 2926, 1727, 1610, 1513, 1466, 1442, 1301, 1250, 1178, 1033 cm^{-1}; MS (EI): *m/z* (%) = 422 (M), 185 (1), 183 (1), 150 (100), 91 (2), 77 (7); anal. calcd. for $C_{20}H_{24}BrNO_4$ (422.31): C, 56.88; H, 5.73; N, 3.32; found C, 56.89; H, 5.74; N, 3.27

(2*R,3*R**)-ethyl-3-(2-bromophenyl)-3-hydroxy-2-[(*R**)-(4-methoxyphenyl) (methylamino)methyl]propanoate (109)**.



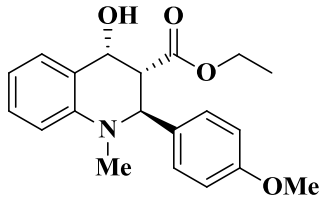
109

Following the same procedure to prepare **108**, the amino alcohol **109** (68 mg, 0.16 mmol) was obtained in 84% yield as a white solid starting from **107** (80 mg, 0.19 mmol).

109: R_f = 0.21; m.p. 124-126 ^{0}C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.76 (dd, *J* = 7.7, 1.4 Hz, 1H, H_{Ar}), 7.60 (dd, *J* = 7.9, 1.1 Hz, 1H, H_{Ar}), 7.43 (pseudo dt, *J* = 0.9, 7.5 Hz, 1H, H_{Ar}), 7.20 (pseudo dt, *J* = 1.6, 7.6 Hz, 1H, H_{Ar}), 6.97-6.92 (m, 2H, H_{Ar}), 6.82-6.77 (m, 2H, H_{Ar}), 5.54 (d, *J* = 2.6 Hz, 1H, 1-H), 4.25-4.04 (m, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.52 (d, *J* = 2.6 Hz, 1H, 3-H), 3.24 (pseudo t, *J* = 2.6 Hz, 1H, 2-H), 2.27 (s, 3H, NCH_3), 1.22 (t, *J* = 7.1 Hz, 3H,

CH_2CH_3); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 172.0 (s; CO), 158.5 (s; C_{Ar}), 142.1 (s; C_{Ar}), 133.1 (d; CH_{Ar}), 131.2 (s; C_{Ar}), 128.8 (d; CH_{Ar}), 128.3 (d; CH_{Ar}), 128 (d; 2C, CH_{Ar}), 127.3 (d; CH_{Ar}), 121.2 (s; C_{Ar}), 113.7 (d; 2C, CH_{Ar}), 75.5 (d; C-5), 61.1 (d; C-3), 60.7 (d; C-4), 55.2 (t; CH_2), 53.0 (q; OCH_3), 33.5 (q; NCH_3), 14.1 (q; CH_2CH_3) ppm; IR: *v* = 2983, 2938, 1733, 1716, 1608, 1558, 1456, 1373, 1251, 1181, 1033 cm^{-1}; MS (EI): *m/z* (%) = 422 (M), 185 (1), 183 (1), 150 (100), 91 (2), 77 (7); anal. calcd. for $C_{20}H_{24}BrNO_4$ (422.31): C, 56.88; H, 5.73; N, 3.32; found C, 57.07; H, 5.88; N, 3.33

(2*S,3*R**,4*R**)-ethyl-4-hydroxy-2-(4-methoxyphenyl)-1-methyl-1,2,3,4-tetrahydroquinoline-3-carboxylate (110)**.



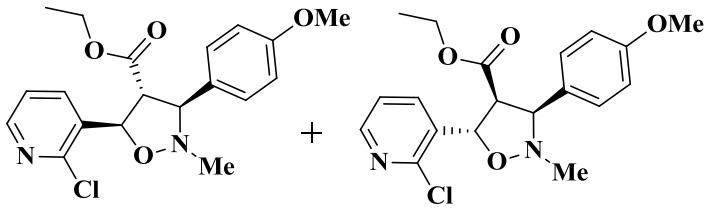
110

The mixed solvent *t*BuOH: H_2O (1:1) (1.89 mL) was added to a mixture of **108** (40 mg, 0.095 mmol), CuI (9.02 mg, 0.047 mmol), copper powder (3.01 mg, 0.047 mmol), K_3PO_4 (40.21 mg, 0.19 mmol) and L-proline (10.9 mg, 0.095 mmol) under nitrogen atmosphere in a vial. Nitrogen was bubbled through the reaction mixture to remove oxygen. The reaction mixture was heated at 90 °C for 12 h in an oil bath and then was filtered through a small pad of Celite®. The solution was diluted with H_2O and sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x5 mL). The combined organic phases were washed with NH_4OH (1% H_2O solution) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography [eluent: EtOAc/petroleum ether (1% TEA) 20:80],

afforded **110** (6.6 mg, 0.02 mmol) in 20% yield as a colourless oil, along with recovery of the starting material **108** (3.4 mg, 0.008 mmol).

110: R_f = 0.31; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.34 (dd, *J* = 7.4, 1.0 Hz, 1H, H_{Ar}), 7.23 (dt, *J* = 1.6, 8.5 Hz, 1H, H_{Ar}),7.16 (pseudo dt, *J* = 2.5, 9.3 Hz, 2H, H_{Ar}), 6.85 (Pseudo dt, *J* = 2.5, 9.3 Hz, 2H, H_{Ar}), 6.74 (dt, *J* = 1.0, 7.4 Hz, 1H, H_{Ar}), 6.68 (d, *J* = 8.2 Hz, 1H, H_{Ar}), 4.80 (d, *J* = 7.2 Hz, 1H, 2-H), 4.76 (dd, *J* = 7.2 , 3.5 Hz, 1H, 4-H), 4.04 (dd, *J* = 14.2, 7.1 Hz, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.06 (dd, *J* = 7.2, 3.5 Hz, 1H, 3-H), 2.79 (s, 3H, NCH_3), 1.06 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ =172.4 (s; CO),159 (s; C_{Ar}), 144.9 (s; C_{Ar}), 132.9 (s; C_{Ar}), 129.3 (d; CH_{Ar}), 128.2 (d; 2C, CH_{Ar}), 127.3 (d; CH_{Ar}), 123.3 (s; C_{Ar}), 116.4 (d; CH_{Ar}), 113.9 (d; 2C, CH_{Ar}), 111.1 (d; CH_{Ar}), 65.9 (d; C-2), 61.4 (d; C-4), 60.9 (t; CH_2), 55.3 (q; OCH_3), 51.4 (d; C-3), 37.3 (q; NCH_3), 14 (q; CH_2CH_3) ppm; IR: *v* = 2935, 2854, 1718, 1607, 1511, 1499, 1374, 1247, 1175, 1035 cm^{-1}; MS (EI): *m/z* (%) = 342 (M+1, 10), 341 (M+, 51), 250 (100), 161 (25), 91 (9), 77 (23); anal. calcd. for $C_{20}H_{23}NO_4$ (341.4): C, 70.36; H, 6.79; N, 4.10; found C, 70.02; H, 6.81; N, 3.95

(3*S,4*R**,5*R**) and (3*S**,4*S**,5*S**)-ethyl 5-(2-chloropyridin-3-yl)-3-(4-methoxyphenyl)-2-methylisoxazolidine-4-carboxylate (113, 114)**.



113 **114**

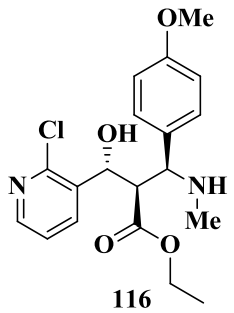
The mixture of (*E*)-ethyl 3-(2-chloropyridin-3-yl) acrylate **112** (100 mg, 0.47 mmol), nitrone **92** (390.26 mg, 2.36 mmol) was heated under solvent free condition at 90 °C for 48 h in an oil

bath. Purification by flash chromatography on silica gel [eluent: EtOAc/petroleum ether (2% TEA) 2:8] afforded the *trans-trans* adduct **113** (132.2 mg, 0.35 mmol) in 74% yield, the *cis-trans* adduct **114** (35.1 mg, 0.09 mmol) in 20% yield as a colourless oil along with unreacted nitrone **92** (283.2 mg, 1.71 mmol).

113: R_f = 0.14; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.32 (dd, *J* = 4.7, 1.9 Hz, 1H, H_{Ar}), 8.25 (dd, *J* = 7.7, 1.9 Hz, 1H, H_{Ar}),7.33 (dd, *J* = 7.7, 4.7 Hz, 1H, H_{Ar}), 7.16-7.11 (m, 2H, H_{Ar}), 6.85-6.80 (m, 2H, H_{Ar}), 5.66 (d, *J* = 4.9 Hz, 1H, 5-H), 4.19 (q, *J* = 7.1 Hz, 1H, CH_2), 3.78 (s, 3H, OCH_3), 3.76 (br d, *J* = 8.8 Hz, 4-H), 3.30 (dd, *J* = 4.9, 8.0 Hz, 1H, 3-H), 2.67 (s, 3H, NCH_3), 1.20 (t, *J* = 7.1 Hz, 3H, $CH_2C\mathit{H}_3$); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 171.4 (s; CO), 159.8 (s; C_{Ar}), 148.5 (s; C_{Ar}), 148.4 (d; CH_{Ar}), 137.3 (s; C_{Ar}), 136.8 (d; CH_{Ar}), 128.9 (d; 2C, CH_{Ar}), 128.1 (s; C_{Ar}), 122.7 (d; CH_{Ar}), 114.2 (d; CH_{Ar}), 78 (d; C-5), 77.4 (d; C-3), 65.3 (d; C-4), 61.3 (t; C-CH2), 55.5 (q; OCH_3), 42.4 (q; NCH_3), 14.1 (q; $CH_2\mathit{C}H_3$) ppm; IR: *v* = 2996, 2839, 1731, 1615, 1514, 1407, 1373, 1305, 1250, 1182, 1082, 1033 cm^{-1}; MS (EI): *m/z* (%) = 378 (M^{+2}, 3), 376 (M, 7), 222 (3), 167 (1), 165 (100), 91 (4), 77 (8); anal. calcd. for $C_{19}H_{21}ClN_2O_4$ (376.12): C, 60.56; H, 5.62; N, 7.43; found C, 59.06; H, 5.55; N, 7.59.

114 R_f = 0.16 ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.32 (dd, *J* = 4.8, 1.9 Hz, 1H, H_{Ar}), 8.03 (dd, *J* = 7.7, 1.9 Hz, 1H, H_{Ar}), 7.30 (dd, *J* = 7.7, 4.8 Hz, 1H, H_{Ar}), 7.27-7.22 (m, 2H, H_{Ar}), 6.90-6.83 (m, 2H, H_{Ar}), 5.83 (d, *J* = 5.7 Hz, 1H, 5-H), 3.89 (br s, 1H, 4-H), 3.86-3.76 (m, 1H, C*H*H), 3.79 (s, 3H, OCH_3), 3.72-3.63 (m, 1H, CH*H*), 3.42 (br s, 1H, 3-H), 2.71 (s, 3H, NCH_3), 0.84 (t, *J* = 7.2 Hz, 3H, $CH_2C\mathit{H}_3$); IR: *v* = 2964, 2838, 1733, 1612, 1567, 1531, 1409, 1380, 1250, 1181, 1082, 1034.

(2*R,3*R**)-ethyl-3-(2-chloropyridin-3-yl)-3-hydroxy-2-[(*S*)-(4-methoxyphenyl) (methylamino)methyl]propanoate (116)**.



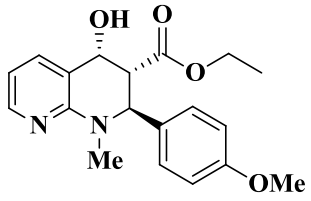
116

A mixture of isoxazolidine **113** (96 mg, 0.255 mmol) and $Mo(CO)_6$ (67.25 mg, 0.255 mmol) in CH_3CN:H_2O [9:1 (v/v), 5.09 mL] was refluxed in an oil bath for 3 h and then was concentrated under a nitrogen stream. The obtained residue was diluted with CH_2Cl_2, and the solution was added with silica gel. The slurry was leaved in the presence of air for 1 h and then directly loaded on a chromatography column. Chromatography on silica gel [eluent: DCM/MeOH (1% NH_4OH) 97:3] afforded **116** (77.8 mg, 0.205 mmol) in 81% yield as a colourless viscous solid.

116: R_f = 0.29; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.29 (dd, *J* = 4.7, 2.0 Hz, 1H, H_{Ar}), 7.94 (dd, *J* = 7.7, 1.9 Hz, 1H, H_{Ar}),7.26 (dd, *J* = 7.6, 4.7 Hz, 1H, H_{Ar}), 7.19-7.15 (m, 2H, H_{Ar}), 6.88-6.83 (m, 2H, H_{Ar}), 5.59 (d, *J* = 9.4 Hz, 1H, 1-H), 4.00 (d, *J* = 10.8 Hz, 1H, 3-H), 3.78 (s, 3H, OCH_3), 3.63-3.50 (m, 2H, CH_2), 3.07 (dd, *J* = 10.8, 9.4 Hz, 1H, 2-H), 2.32 (s, 3H, NCH_3), 0.70 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ =168.0 (s; CO), 159.3 (s; C_{Ar}), 149.5 (s; C_{Ar}), 149.2 (d; CH_{Ar}), 138 (d; CH_{Ar}), 135.8 (s; C_{Ar}), 131.1 (s; C_{Ar}), 128 (d; 2C, CH_{Ar}), 123.2 (d; CH_{Ar}), 112.4 (d; 2C,CH_{Ar}), 73.5 (d; C-1), 67 (d; C-3), 60.1 (t; CH_2), 57.8 (q; OCH_3), 55.2 (d; C-2), 33.5 (q; NCH_3), 13.2 (q; CH_2CH_3) ppm; IR: *v* = 2983, 2936, 2839, 1723, 1610, 1580, 1513, 1464, 1372, 1305, 1251, 1178, 1095 cm^{-1}; MS (EI): *m/z* (%) = 342,

251 (3), 190 (2), 161 (6), 150 (100), 133 (3), 78 (4), 42 (20) ; anal. calcd. for $C_{19}H_{23}ClN_2O_4$ (378.85): C, 60.24; H, 6.12; N, 7.39; found C, 60.99; H, 6.08; N, 7.60

(2*S,3*R**,4*R**)-ethyl-4-hydroxy-2-(4-methoxyphenyl)-1-methyl-1,2,3,4-tetrahydro-1,8-naphthyridine-3-carboxylate (117)**.



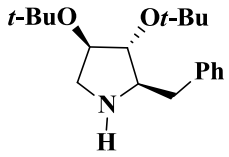
117

The mixed solvent *t*BuOH: H_2O (1:1) (1.95 mL) was added to a mixture of **116** (37 mg, 0.098 mmol), CuI (27.90 mg, 0.146 mmol), copper powder (9.31 mg, 0.146 mmol), K_3PO_4 (41.46 mg, 0.195 mmol) and L-proline (11.24 mg, 0.098 mmol) under nitrogen atmosphere in a vial. Nitrogen was bubbled through the reaction mixture to remove oxygen. The reaction mixture was heated at 90 °C for 12 h in oil bath and then was filtered through small pad of Celite®. The solution was diluted with H_2O and sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x5 mL). The combined organic phases were washed with NH_4OH (1% H_2O solution) and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography [eluent: EtOAc/petroleum ether (1% TEA) 5:5] afforded **117** (11 mg, 0.032 mmol) with 33% yield as a white solid along with unreacted starting material **116** (3.6 mg, 0.009 mmol).

117: R_f = 0.32; m.p. 123-125 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.13 (dd, *J* = 5, 1.8 Hz, 1H, H_{Ar}), 7.59 (pseudo dt, *J* = 1.8, 7.2 Hz, 1H, H_{Ar}),7.12 (pseudo dt, *J* = 2.5, 9.3 Hz, 2H, H_{Ar}), 6.62 (Pseudo dt, *J* = 2.5, 9.3 Hz, 2H, H_{Ar}), 4.96 (d, *J* = 5.2 Hz, 1H, 2-H), 4.64 (dd, *J* = 8.1 , 4.2 Hz,

1H, 4-H), 4.09 (t, *J* = 7.1 Hz, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.10 (pseudo t, *J* = 5.2 Hz, 1H, 3-H), 3.04 (s, 3H, NCH_3), 1.12 (t, *J* = 7.1 Hz, 3H, CH_2CH_3); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 172.1 (s; CO), 159.1 (s; C_{Ar}), 147.5 (s; C_{Ar}), 133.9 (d; CH_{Ar}), 131.7 (s; C_{Ar}), 131.0 (s; C_{Ar}), 127.6 (d; 2C, CH_{Ar}), 118.7 (s; C_{Ar}), 114.1 (d; 2C,CH_{Ar}), 112.2 (d; C_{Ar}), 64.6 (d; C-2), 61.4 (d; C-4), 61.2 (t; CH_2), 55.3 (q; OCH_3), 49.6 (d; C-3), 35.4 (q; NCH_3), 14.1 (q; CH_2CH_3) ppm ; IR: *v* = 2935, 2839, 1713, 1602, 1512, 1494, 1406, 1338, 1250, 1176, 1034 cm^{-1}; MS (EI): *m/z* (%) = 343 (M, 5), 342 (M, 26), 251 (100), 17 (7), 161 (46), 108 (45), 91 (6); anal. calcd. for $C_{19}H_{22}N_2O_4$ (342.39): C, 66.65; H, 6.48; N, 8.18; found C, 66.57; H, 6.13; N, 7.87.

(2*R*, 3*R*, 4*R*)-2-benzyl-3,4-di-*tert*-butoxypyrrolidine (125)



125

Benzylmagnesium chloride **122** (2.0 M solution in THF, 2.10 mL) was added dropwise to a solution of nitrone **28** (0.5 g, 2.18 mmol) in dry THF (8.5 mL) at 0 °C under nitrogen atmosphere. The resulting mixture was stirred for 3 h at rt and then the reaction was quenched with saturated aqueous NH_4Cl at 0 °C. The two phases were separated and the aqueous phase was sequentially extracted with CH_2Cl_2 (3x30 mL) and EtOAc (3x20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The obtained mixture of crude compounds **123** and **124** (0.7 g, 2.18 mmol) was dissolved in AcOH: H_2O [1:1(v/v), 21.2 mL] at 0 °C and then activated zinc powder (2.8 g, 43.6 mmol) was added. The suspension was stirred at rt for 2 h, diluted with MeOH and

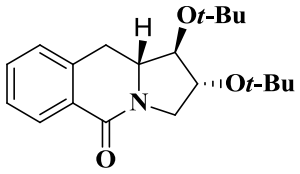
filtered through cotton wool. The filtrate was concentrated under reduced pressure and the residue was dissolved in EtOAc. The solution was basified to pH = 9 with a saturated aq. $NaHCO_3$ solution and solid Na_2CO_3 at 0 °C. The two phases were separated and the aqueous phase was sequentially extracted with EtOAc (3x30 mL) and CH_2Cl_2 (2x30 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1% NH_4OH) 92:8] afforded the major isomer **125** (0.44 g, 1.43mmol) in 66% yield as a colourless oil and a mixture of both isomers **125** and **126** (0.18 g, 0.6 mmol, 3:1 ratio) in 27% yield.

125: R_f = 0.39; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.32-7.22 (m, 4H, H_{Ar}), 7.21-7.16 (m, 1H, H_{Ar}), 3.82 (pseudo dt, *J* = 5.4, 2.5 Hz, 1H, 4-H), 3.64 (pseudo t, *J* = 2.7 Hz, 1H, 3-H), 3.14-3.06 (m, 1H, 2-H), 3.10 (dd, *J* = 11.6, 5.4 Hz, 1H, 5-Ha), 2.99 (dd, *J* = 13.3, 6.6 Hz, 1H, CH_2Ph), 2.85 (dd, *J* = 11.6, 2.9 Hz, 1H, 5-Hb), 2.70 (dd, *J* = 13.3, 8.5 Hz, 1H, CH_2Ph), 2.15 (br s, 1H, NH), 1.20 (s, 9H, CH_3x3), 1.09 (s, 9H, CH_3x3),; ^{13}C-NMR (50 MHz): δ =139.8 (s; C_{Ar}), 129.1 (d; 2C, CH_{Ar}), 128.2 (d; 2C, CH_{Ar}), 125.9 (d; CH_{Ar}), 82.1 (d; C-3), 79.1 (d; C-4), 66.4 (d; C-2), 53.2 (t; C-5), 39.2 (t; C-CH_2Ph), 28.6 (q; 3C, C-CH3), 28.5 (q; 3C, C-CH3) ppm; IR ($CDCl_3$): *v* = 2977, 1455, 1390, 1366, 1191, 1076, 1023 cm^{-1}; MS (EI): *m/z* (%) = 306 (M+1), 248 (1), 214 (37), 192 (16), 102 (56), 91 (15), 57 (100); anal. calcd. for $C_{19}H_{31}NO_2$ (305.24): C, 74.71; H, 10.23; N, 4.59; found C, 74.53; H, 10.03; N, 4.09

(2*S*,3*S*,4*S*)-2-benzyl-3,4-di-*tert*-butoxypyrrolidine (*ent*-125)

Following the same procedure used to prepare pyrrolidine **125**, the enantiomeric ***ent*-125** was obtained starting from nitrone ***ent*-28**.

(1*R*, 2*R*, 10a*R*)-1,2-di-*tert*-butoxy-2,3,10,10a-tetrahydropyrrolo[1,2-*b*]isoquinolin-5(1H)-one (128)



128

1,4-Dioxane (2.8 mL) was added to a mixture of **125** (87.5 mg, 0.286 mmol), $Pd(OAc)_2$ (32.1 mg, 0.14 mmol), $Cu(OAc)_2$ (208.1 mg, 1.14 mmol), and *t*-BuOK (96.4 mg, 0.86 mmol). The reaction mixture was heated in an oil bath at 110 °C for 24 h under CO atmosphere and then was filtered through a small pad of Celite ®. The filtrate was diluted with H_2O and sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x10 mL). The combined organic phases were washed with NH_4OH solution (2% H_2O solution) as well as brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: EtOAc (1% TEA) /petroleum ether 30:70] afforded **128** (68.7 mg, 0.21 mmol) in 72% yield as a yellow waxy solid.

128: R_f = 0.47; .). $[\alpha]_D^{24}$= +76.80 (*c* = 0.62, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.02(dd, *J* = 7.6, 1.4 Hz, 1H, H_{Ar}), 7.40 (pseudo dt, *J* = 7.4, 1.4 Hz, 1H, H_{Ar}), 7.32 (pseudo tt, *J* = 7.5, 1.0 Hz, 1H, H_{Ar}), 7.19 (br d, *J* = 7.5 Hz, 1H, H_{Ar}), 4.00-3.85 (m, 3H, 1-H, 2-H, 3-Ha), 3.61 (ddd, *J*= 13.4, 7.4, 4.1 Hz, 1H,10a-H), 3.45 (dd, *J* = 11.7, 5.8 Hz, 1H, 3-Hb), 3.06 (dd, *J* = 15.0, 4.1 Hz, 10-Ha), 2.91 (br pseudo t, *J* = 14.2 Hz, 1H, 10-Hb), 1.27 (s, 9H, CH_3x3), 1.22 (s, 9H, CH_3x3); ^{13}C-NMR (50 MHz): δ =163.1 (s; CO), 137.2 (s; C_{Ar}), 131.5 (d; CH_{Ar})129.6 (s; C_{Ar}), , 127.5 (d; CH_{Ar}), 127.2 (d; CH_{Ar}), 127 (d; CH_{Ar}), 80.9 (d; C-1), 74(d; C-2), 59.1 (d; C-10a), 49.4 (t; C-3), 33.1 (t; C-10), 29.3 (q; 3C, C-CH3), 28.5 (q; 3C, C-CH3) ppm; IR

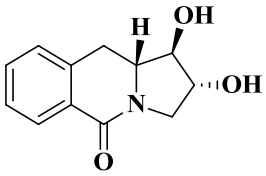
($CDCl_3$): ν = 2978, 1644, 1464, 1366, 1192, 1115, 1090 cm^{-1}; MS (EI): *m/z* (%) = 331 (M^+, 7), 274 (18), 218 (87), 158 (6), 91 (4), 57 (100) ; anal. calcd. for $C_{20}H_{29}NO_3$ (331.21): C, 72.47; H, 8.82; N, 4.23; found C, 72.54; H, 8.46; N, 4.40.

(1*S*,2*S*,10a*S*)-1,2-di-*tert*-butoxy-2,3,10,10a-tetrahydropyrrolo[1,2-*b*]isoquinolin-5(1H)-one (*ent*-128)

Following the same procedure used to prepare pyrrolidine **128**, the enantiomeric ***ent*-128** was obtained starting from ***ent*-125**.

$[\alpha]_D^{26}$= -74.98 (*c* = 1.0, $CHCl_3$)

(1*R*,2*R*,10a*R*)-1,2-dihydroxy-2,3,10,10a-tetrahydropyrrolo[1,2-*b*]isoquinolin-5(1H)-one (121)



121

The compound **128** (28 mg, 0.08 mmol) was dissolved in TFA (0.8 mL) at 0 °C and the solution was stirred at rt for 2 h. The excess of TFA was co-evaporated with toluene (4 × 3 mL) under reduced pressure (each addition of toluene was done at 0 °C) and then the residue was co-evaporated with MeOH (3 × 3 mL) to remove the last traces of toluene. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1% NH_4OH) 90:10] gave **121** (18 mg, 0.08 mmol) in 97% yield as a white solid.

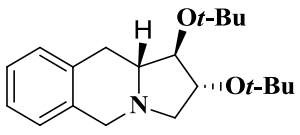
121: R_f = 0.41;m.p. 220-222 °C (Decomposed); $[\alpha]_D^{26}$= +374.30 (*c* = 0.5, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.90 (dd, *J* = 7.7, 1.1 Hz, 1H, H_{Ar}), 7.48 (pseudo dt, *J* = 1.4, 7.5 Hz, 1H, H_{Ar}), 7.35 (pseudo tm, *J* = 7.6 Hz, 1H, H_{Ar}), 7.31 (br d, *J* = 7.5, 1H, H_{Ar}), 4.16 (pseudo q, *J* = 6.6 Hz, 1H, 2-H), 3.92 (pseudo t, *J* = 6.7 Hz, 1H, 1-H), 3.88 (dd, *J* = 12.5, 7.3 Hz, 1H, 3-Ha), 3.68 (ddd, *J* = 13.6, 7.1, 4.2 Hz, 1H, 10a-H), 3.50 (dd, *J* = 12.5, 6.4 Hz, 1H, 3-Hb), 3.16 (dd, *J* = 15.4, 4.2 Hz, 1H, 10-Ha), 2.95 (br pseudo t, *J* = 13.9 Hz, 1H, 10-Hb); ^{13}C-NMR (50 MHz): δ =165.3 (s; CO), 139.2 (s; C_{Ar}), 134.9 (s; C_{Ar}), 133.3 (d; CH_{Ar}), 129.7 (d; CH_{Ar}), 128.7 (d; CH_{Ar}), 128.1 (d; CH_{Ar}), 82.6 (d; C-1), 75.1(d; C-2), 62.4 (d; C -10a), 50.7 (t; C-3), 33.6 (t; C-10) ppm; MS (EI): *m/z* (%) = 219 (M^+, 27), 176 (22), 132 (100), 103 (10), 91 (27), 78 (5); anal. calcd. for $C_{20}H_{29}NO_3$ (219.09): C, 65.74; H, 5.98; N, 6.39; found C, 65.71; H, 5.58; N, 6.04

(1*S*,2*S*,10a*S*)-1,2-dihydroxy-2,3,10,10a-tetrahydropyrrolo[1,2-*b*]isoquinolin-5(1H)-one (*ent*-121)

Enantiomeric ***ent*-121** was prepared following the same procedure used for the synthesis of pyrrolidine **121**.

$[\alpha]_D^{25}$= -375.49 (*c* =0.55, MeOH).

(1*R*,2*R*,10a*R*)-1,2-di-*tert*-butoxy-1,2,3,5,10,10a-hexahydropyrrolo[1,2-*b*]isoquinoline (127)



127

A solution of lithium aluminium hydride (1.0 M solution in THF, 1.8 mL) was added dropwise to a solution of **128** (100 mg, 0.30 mmol) in dry THF (5.5 mL) at 0 °C under nitrogen atmosphere. The reaction mixture was stirred overnight at rt and then the reaction was quenched with saturated aqueous NH_4Cl at 0 °C. The two phases were separated and the aqueous phase was sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: EtOAc (1% TEA) /petroleum ether 15:85] afforded **127** (76 mg, 0.24 mmol) in 80% yield as a white solid.

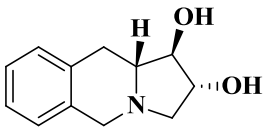
127: R_f = 0.24; m.p. = 105–107 °C; $[\alpha]_D^{25}$= +43.98 (*c* = 0.58, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.15-7.07 (m, 3H, H_{Ar}), 7.04-6.99 (m, 1H, H_{Ar}), 4.01 (d, *J* = 14.6 Hz, 1H, 5-Ha), 3.94 (ddd, *J* = 6.8, 3.6, 1.8 Hz, 1H, 2-H), 3.80 (dd, *J* = 7.7, 3.6 Hz, 1H, 1-H), 3.38 (br d, *J* = 14.6 Hz, 1H, 5-Hb), 3.13 (dd, *J* = 10.2, 1.8, Hz, 3-Ha), 3.01 (dd, *J* = 15.6, 3.9 Hz, 1H, 10-Ha), 2.81 (br dd, *J* = 15.6, 10.9 Hz, 1H, 10-Hb), 2.62 (br pseudo t, *J* = 8.0 Hz, 1H, 3-Hb), 2.36-2.24 (m, 1H, 10a-H), 1.27 (s, 9H, CH_3x3), 1.20 (s, 9H, CH_3x3); ^{13}C-NMR (50 MHz): δ =134.3 (s; C_{Ar}), 134.2 (s; C_{Ar}), 128.9 (d; CH_{Ar}), 126.3 (d; CH_{Ar}), 126 (d; CH_{Ar}), 125.5 (d; CH_{Ar}), 84.8 (d; C-1), 77.3 (d; C-2), 64.1 (d; C-10a), 62 (t; C-3), 56.1 (t; C-5), 33.5 (t; C-10), 29.3 (q; 3C, CH_3), 28.8 (q; 3C, CH_3) ppm; IR ($CDCl_3$): *v* = 2978, 2935, 1395, 1366,1258, 1190, 1161, 1072, 1021 cm^{-1}; MS (EI): *m/z* (%) = 317 (M^+, 8), 274 (1), 218 (7), 204 (34), 158 (3), 144 (20), 91 (5), 57 (100); anal. calcd. for $C_{20}H_{31}NO_2$ (317.24): C, 75.67; H, 9.84; N, 4.41; found C, 75.59; H, 9.89; N, 4.45.

(1*S*,2*S*,10a*S*)-1,2-di-*tert*-butoxy-1,2,3,5,10,10a-hexahydropyrrolo[1,2-*b*]isoquinoline (*ent*-127)

Enantiomeric ***ent*-127** was prepared following the same procedure used for the synthesis of pyrrolidine **127**.

$[\alpha]_D^{21}$= +45.26 (*c* = 1.04, $CHCl_3$)

(1*R*,2*R*,10a*R*)-1,2,3,5,10,10a-hexahydropyrrolo[1,2-*b*]isoquinoline-1,2-diol (120)



120

Ether **127** (60 mg, 0.19 mmol) was dissolved in TFA (1.9 mL) at 0 °C and the solution was stirred at rt for overnight. The excess of TFA was co-evaporated with toluene (4 × 3 mL) under reduced pressure (each addition of toluene was done at 0 °C) and then the residue was co-evaporated with MeOH (3 × 3 mL) to remove the last traces of toluene. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1% NH_4OH) 90:10] gave **120** (34.8 mg, 0.17 mmol) in 90% yield as a white solid.

120: R_f = 0.29;m.p. 154-156 °C; (Decomposed) ; $[\alpha]_D^{25}$= + 276.2 (*c* = 0.67, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ =7.16-7.04 (m, 4H, H_{Ar}), 4.09 (ddd, *J* = 7.0, 3.6, 2.1 Hz, 1H, 2-H), 4.01 (d, *J* = 14.6 Hz, 1H, 5-Ha), 3.77 (dd, *J* = 7.7, 3.5 Hz, 1H, 1-H), 3.42 (br d, *J* = 14.6 Hz, 1H, 5-Hb), 3.10 (dd, *J* = 15.8, 4.1, Hz, 10-Ha), 3.08 (dd, *J* = 10.6, 2.1, 1H, 3-Ha), 2.81 (br dd, *J* = 15.8, 10.9, 1H, 10-Hb), 2.73 (dd, *J* = 10.6, 7.0 Hz, 1H, 3-Hb), 2.34 (ddd, *J* = 10.9, 7.7, 4.1 Hz, 1H, 10a-H); ^{13}C-NMR (50 MHz): δ =135.1 (s; C_{Ar}), 135.15 (s; C_{Ar}), 130.1 (d; CH_{Ar}), 127.6 (d; 2C, CH_{Ar}), 127.1 (d; CH_{Ar}), 85.9 (d; C-1), 77.7 (d; C-2), 67.7 (d; C-10a), 62.5 (t; C-3), 56.8 (t; C-5), 34.1 (t; C-10) ppm; MS (EI): *m/z* (%) = 205 (M^+,55), 132 (24), 117 (56),

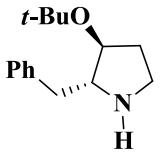
91(8), 78 (12). anal. calcd. for $C_{20}H_{31}NO_2$ (205.11): C, 70.22; H, 7.37; N, 6.82; found C, 70.30; H, 7.03; N, 6.67

(1*S*,2*S*,10a*S*)-1,2,3,5,10,10a-hexahydropyrrolo[1,2-*b*]isoquinoline-1,2-diol (*ent*-120)

Enantiomeric ***ent*-120** was prepared following the same procedure used for the synthesis of pyrrolidine **120**.

$[\alpha]_D^{25}$= - 275.6 (*c* = 0.67, MeOH)

(2*S*,3*R*)-2-benzyl-3-*tert*-butoxypyrrolidine (131)



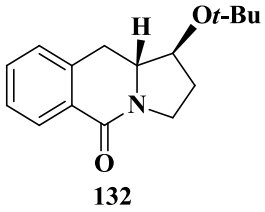
131

A solution of benzylmagnesium chloride **122** (2.0 M solution in THF, 1.54 mL) was added dropwise to a solution of the pyrrolidine *N*-oxide **74** (0.3 g, 1.9 mmol) in dry THF (6.34 mL) at 0 °C under nitrogen atmosphere. The resulting mixture was stirred for 2 h at rt and then the reaction was quenched with saturated aqueous NH_4Cl at 0 °C. The two phases were separated and the aqueous phase was sequentially extracted with CH_2Cl_2 (3x30 mL) and EtOAc (3x20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The obtained crude product **130** (0.47 g, 1.91 mmol) was dissolved in AcOH:H_2O [1:1(v/v),18.6 mL] at 0 ^oC and activated zinc powder (2.5 g, 38.1 mmol) was added. The reaction mixture was stirred at rt for 2 h, diluted with MeOH, filtered through cotton wool and then concentrated under reduced pressure. The residue was dissolved in EtOAc, and the solution was basified to pH = 9 with a saturated aq. $NaHCO_3$ solution and

solid Na_2CO_3 at 0 °C. The two phases were separated and the aqueous phase was sequentially extracted with EtOAc (3x20 mL) and CH_2Cl_2 (2x20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1% NH_4OH) 93:7] afforded **131** (0.38 g, 1.63 mmol) in 85% yield as a colourless oil.

131: R_f = 0.26; $[\alpha]_D^{24}$= + 53.70 (*c* = 0.64, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.33-7.18 (m, 5H, H_{Ar}), 3.77 (ddd, *J* = 7.7, 5.5, 4.6 Hz, 1H, 3-H), 3.10 (pseudo dt, *J* = 9.2, 5.2 Hz, 1H, 2-H), 3.02-2.94 (m, 3H, 5-H, CH_2Ph), 2.60 (dd, *J*= 13.5, 9.1 Hz, 1H, CH_2Ph-H), 2.52 (br s, 1H, NH), 2.09 (pseudo dq, *J* = 13.1, 8 Hz, 1H, 4-Ha), 1.72-1.63 (m, 1H, 4-Hb), 1.16 (s, 9H, CH_3x3), ^{13}C-NMR (50 MHz): δ =139.7 (s; C_{Ar}), 128.9 (d; 2C, CH_{Ar}), 128.3 (d; 2C, CH_{Ar}), 126.1 (d; CH_{Ar}), 75.8 (d; C-3), 66.3 (d; C-2), 44.2 (t; C-5), 39.5 (t; C-CH_2Ph), 34.1 (t; C-4), 28.5 (q; 3C, CH_3) ppm; MS (EI): *m/z* = 233.18, calcd. for $C_{15}H_{23}NO$ $[M + H]^+$: 234.10; $[M + Na]^+$: 256.11; IR ($CDCl_3$): *v* = 2976, 2935, 1602, 1495, 1453, 1390, 1364, 1189, 1091 cm^{-1}; anal. calcd. for $C_{15}H2_3NO$ (233.18): C, 77.21; H, 9.93; N, 6.00; found C, 76.50; H, 9.25; N, 6.72

(1*R*,10a*S*)-1-(*tert*-butoxy)-2,3,10,10a-tetrahydropyrrolo[1,2-*b*]isoquinolin-5(1H)-one (132)



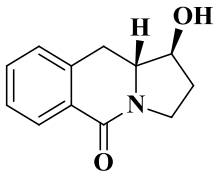
132

1,4-Dioxane (2.1 mL) was added to a mixture of **131** (50 mg, 0.214 mmol), $Pd(OAc)_2$ (24 mg, 0.11 mmol), $Cu(OAc)_2$ (155.7 mg, 0.86 mmol), and *t*-BuOK (72.13 mg, 0.64 mmol). The

reaction mixture was heated in an oil bath at 110 °C for 24 h under CO atmosphere and then was filtered through a small pad of Celite ®. The filtrate was diluted with H_2O and sequentially extracted with CH_2Cl_2 (3x10 mL) and EtOAc (2x10 mL). The combined organic phases were washed with NH_4OH solution (2% H_2O solution) as well as brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: EtOAc (1% TEA) /petroleum ether 1:1] afforded **132** (25 mg, 0.1 mmol) in 45% yield as a brown solid.

132: R_f = 0.36; m.p. 95-97 ^{0}C; $[\alpha]_D^{25}$= +118.05 (*c* = 0.66, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.03 (dd, *J* = 7.6, 1.3 Hz, 1H, H_{Ar}), 7.40 (pseudo dt, *J* = 1.5, 7.4 Hz, 1H, H_{Ar}), 7.32 (pseudo tt, *J* = 7.5, 1.0 Hz, 1H, H_{Ar}), 7.19 (dm, *J* = 7.4 Hz, 1H, H_{Ar}), 3.95 (ddd, *J* = 9.0, 8.1, 6.8 Hz, 1H, 1-H), 3.80 (ddd, *J* = 12.5, 9.9, 2.2 Hz, 1H, 3-Ha), 3.66 (ddd, *J* = 12.5, 9.9, 7.5 Hz, 1H, 3-Hb), 3.60 (ddd, *J* = 14.3, 8.1, 4.1 Hz, 1H,10a-H), 3.10 (dd, *J* = 15.1, 4.1 Hz, 1H, 10-Ha), 2.79 (pseudo t, *J* = 14.3 Hz, 1H, 10-Hb), 2.25 (dddd, *J* = 12.5, 7.7, 6.8, 2.3 Hz, 1H, 2-Ha), 1.82 (dq, *J* = 12.5, 9.9 Hz, 1H, 2-Hb), 1.26 (s, 9H, CH_3x3); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ =164.4 (s; CO), 137 (s; C_{Ar}), 131.6 (d; CH_{Ar}), 130 (s; C_{Ar}), 127.5 (d; CH_{Ar}), 127.2 (d; CH_{Ar}), 127 (d; CH_{Ar}), 76.9 (t; C-1), 60.4 (d; C-10a), 42.2 (d; C-3), 32.9 (t; C-10), 31.6 (t; C-2), 28.5 (q; 3C,CH_3) ppm; IR ($CDCl_3$): *v* = 2978, 1641, 1467, 1436, 1365, 1191, 1093 cm^{-1}; MS (EI): *m/z* = 259.16, calcd. for $C_{16}H_{21}NO_2$ $[M + H]^+$: 260.18; $[M + Na]^+$: 282.11; anal. calcd. for $C_{16}H_{21}NO_2$ (259.16): C, 74.10; H, 8.16; N, 5.40; found C, 74.23; H, 8.55; N, 5. 05.

(1*R*,10a*S*)-1-hydroxy-2,3,10,10a-tetrahydropyrrolo[1,2-*b*]isoquinolin-5(1H)-one (133)

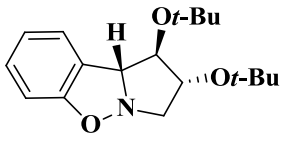


133

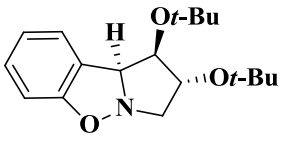
Amide **132** (32 mg, 0.12 mmol) was dissolved in TFA (1.23 mL) at 0 °C and the solution was stirred at rt for 1 h. The excess of TFA was co-evaporated with toluene (4 × 3 mL) under reduced pressure (each addition of toluene was done at 0 °C) and then the residue was co-evaporated with MeOH (3 × 3 mL) to remove the last traces of toluene. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1% NH_4OH) 94:6] gave **133** (19.9 mg, 0.1 mmol) in 79 % yield as a white solid.

133: R_f = 0.33; m.p. 198-200 ^{0}C; $[\alpha]_D^{25}$= +88.95 (*c* = 0.65, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ = 7.90 (br d, *J* = 7.3, 1H, H_{Ar}), 7.47 (pseudo dt, *J* = 1.0, 7.5 Hz, 1H, H_{Ar}), 7.35 (pseudo tm, *J* = 7.6 Hz, 1H, H_{Ar}),7.30 (dm, J = 7.5 Hz, 1H, H_{Ar}), 4.14 (pseudo dt, *J* = 9.0, 7.2 Hz, 1H, 1-H), 3.74 (ddd, *J* = 12.2, 9.5, 2.5 Hz, 1H, 3-Ha), 3.66-3.53 (m, 2H, 3-Hb, 10a-H), 3.20 (dd, *J* = 15.4, 4.1 Hz, 1H, 10-Ha), 2.85 (pseudo t, J = 14.4 Hz, 1H, 10-Hb), 2.31 (pseudo ddt, *J* = 12.3, 2.5, 6.9 Hz, 1H, 2-Ha), 1.86 (pseudo dq, *J* = 12.3, 9.6 Hz, 1H, 2-Hb); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ =165.5 (s; CO), 139.2 (s; C_{Ar}), 133.4 (d; CH_{Ar}), 130.7 (s; C_{Ar}), 128.8 (d; CH_{Ar}), 128.2 (d; CH_{Ar}), 128.1 (d; CH_{Ar}), 77.5 (d; C-1), 63.6 (t; C-10a), 43.4 (d; C-3), 33.5 (t; C-10), 32.3 (t; C-2) ppm; anal. calcd. for $C_{12}H_{13}NO_2$ (203.09): C, 70.92; H, 6.45; N, 6.89; found C, 69.84; H, 6.33; N, 6.40.

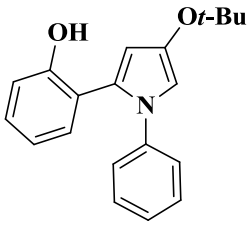
(1*R*,2*R*,9b*R*)- and (1*R*,2*R*,9b*S*)-1,2-di-*tert*-butoxy-1,2,3,9b-tetrahydrobenzo[d]pyrrolo[1,2-*b*]isoxazole (135 and 136) and 2-(4-(*tert*-butoxy)-1-phenyl-1H-pyrrol-2-yl)phenol (139)



135



136



139

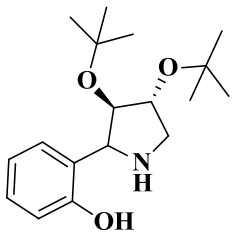
Bu_4NF (220 µL, 0.87 mmol) was added to a solution of nitrone **28** (50 mg, 0.22 mmol) in dry DMF (2 mL) and then a solution of the aryne precursor **134** (79 µL, 0.33 mmol) was added. The reaction mixture was stirred at room temperature for 2.5 h. After completion of the reaction, the DMF was evaporated in a nitrogen stream. The obtained residue was absorbed on silica gel and loaded on a column chromatography. Purification by chromatography on silica gel (eluent: EtOAc /petroleum ether 2:98) afforded the major adduct **135** (21.9 mg, 0.07 mmol) in 33 % yield, and the minor adduct **136** (17 mg, 0.06 mmol) in 26% as yellow oils along with side product **139** (28.7 mg, 0.09 mmol) as a white solid.

135: R_f = 0.4; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.25 (dm, *J* = 7.4 Hz, 1H, H_{Ar}), 7.17-7.12 (m, 1H, H_{Ar}), 6.90 (pseudo dt, *J* = 0.9, 7.4 Hz, 1H, H_{Ar}), 6.73 (br d, *J* = 8.0 Hz, 1H, H_{Ar}), 4.77 (br s, 1H, 9b-H), 4.13-4.10 (m, 1H, 1-H), 3.96 (ddd, *J* = 6.1, 4.9, 3.6 Hz, 1H, 2-H), 3.58 (dd, *J* = 11.6, 4.9 Hz, 1H, 3-Ha), 3.16 (ddm, *J* = 11.6, 6.1 Hz, 1H, 3-Hb), 1.29 (s, 9H, CH_3x3), 1.06 (s, 9H, CH_3x3); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 156.1 (s; C_{Ar}), 128.3 (d; CH_{Ar}), 126.8 (s; C_{Ar}), 123.1 (d; CH_{Ar}), 120.6 (d; CH_{Ar}), 107.1 (d; CH_{Ar}), 81.8 (d; C-1), 74.2 (d; C-2), 73.4 (d; C-9b), 61.8 (t; C-3), 28.4 (q; 3C, CH_3), 28.0 (q; 3C, CH_3)ppm; IR ($CDCl_3$): *ν* = 2977, 2831, 1597, 1480, 1456, 1390, 1365, 1253, 1190, 1099, 1079 cm^{-1}; anal. calcd. for $C_{18}H_{27}NO_3$ (305.20): C, 70.79; H, 8.91; N, 4.59; found C, 69.53; H, 8.76; N, 5.29.

136: R_f = 0.28; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.32 (br d, *J* = 7.5 Hz, 1H, H_{Ar}), 7.17(pseudo tm, *J* = 7.7 Hz, 1H, H_{Ar}), 6.91 (pseudo dt, *J* = 0.9, 7.4 Hz, 1H, H_{Ar}), 6.76 (br d, *J* = 8.0 Hz, 1H, H_{Ar}), 4.87 (br d, *J* = 6.9 Hz, 1H, 9b-H), 4.24 (pseudo t, *J* = 7.2 Hz, 1H, 1-H), 3.86-3.78 (m, 1H, 2-H), 3.49 (dd, *J* = 14.0, 7.6 Hz, 1H, 3-Ha), 3.23 (dd, *J* = 14.0, 8.6 Hz, 1H, 3-Hb), 1.28 (s, 9H, CH_3x3), 1.19 (s, 9H, CH_3x3); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 157.0 (s; C_{Ar}), 128.2 (d; CH_{Ar}), 125.7 (d; CH_{Ar}), 125.0 (s; C_{Ar}), 120.5 (d; CH_{Ar}), 107.0 (d; CH_{Ar}), 77.4 (d; C-1), 72.7 (d; C-2), 68.1 (d; C-9b), 62.3 (t; C-3), 28.2(q; 6C, CH_3) ppm; IR: 2977, 2935, 1593, 1474, 1458, 1390, 1365, 1236, 1192, 1119, 1016 cm^{-1}; anal. calcd. for $C_{18}H_{27}NO_3$ (305.20): C, 70.79; H, 8.91; N, 4.59; found C, 70.51; H, 9.12; N, 4.56.

139: R_f = 0.37; NMR ($CDCl_3$, 400 MHz): δ = 7.32-7.24 (m, 2H, H_{Ar}), 7.23-7.17 (m, 1H, H_{Ar}), 7.13-7.00 (m, 4H, H_{Ar}), 6.84 (d, *J* = 3.2 Hz, 1H, 5-H), 6.55 (dddd, *J* = 14.6, 9.2, 7.8, 1.4 Hz, 2H, H_{Ar}), 6.14 (d, *J* = 3.2 Hz, 1H, 3-H), 1.23 (s, 9H, CH_3x3); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 140.8 (s; C_{Ar}), 139.6 (s; C_{Ar}), 130.8 (d, CH_{Ar}), 128.9 (d; 2C, CH_{Ar}), 128.0 (d; 2C, CH_{Ar}), 126.3 (d; CH_{Ar}), 125.3 (d; 2C, CH_{Ar}), 122.4 (s; C-5), 119.6 (d; CH_{Ar}), 118.3 (d; CH_{Ar}), 105.1 (s, C-3), 28.0 (q; 3C, CH_3) ppm.

2-[(3*R*,4*R*)-3,4-di-*tert*-butoxypyrrolidin-2-yl]phenol (143).



143

A mixture of isoxazolidine **135** (167 mg, 0.55 mmol) and zinc powder (1.3 g, 21.9 mmol) in AcOH:H_2O [1:1 (v/v), 16.4 mL) was heated in an oil bath at 70 °C for 2 h. The reaction mixture was diluted with MeOH, filtered through cotton wool and then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and the solution was basified to pH = 9 with a saturated aq. $NaHCO_3$ solution and solid Na_2CO_3 at 0 °C. The two phases were separated. The aqueous phase was sequentially extracted with CH_2Cl_2 (3x30 mL) and EtOAc (2x30 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel [eluent: CH_2Cl_2 / MeOH (1% NH_4OH) 98:2] afforded **143** (127 mg, 0.41 mmol) in 75% yield as a white solid.

143: R_f = 0.31; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.15-7.09 (m, 1H, H_{Ar}), 7.00(dd, *J* = 7.5, 1.6 Hz, 1H, H_{Ar}), 6.80 (dd, *J* = 8.1, 1.0 Hz, 1H, H_{Ar}), 6.74 (pseudo dt, *J* = 1.2, 7.4 Hz, 1H, H_{Ar}), 4.08 (dd, *J* = 7.8 , 5.4 Hz, 1H, 3-H), 3.98 (ddd, *J* = 7.4, 5.5, 4.4 Hz, 1H, 4-H), 3.93 (d, *J* = 7.8 Hz, 1H, 2-H), 3.29 (dd, *J* = 10.6, 7.4 Hz, 1H, 5-Ha), 3.00 (dd, *J* = 10.6, 4.4 Hz, 1H, 5-Hb), 1.19 (s, 9H, CH_3x3), 0.93 (s, 9H, CH_3x3); ^{13}C-NMR ($CDCl_3$, 50 MHz): δ = 158.0 (s; C_{Ar}), 129.6 (d; CH_{Ar}), 128.7 (d; CH_{Ar}), 123.3 (s; C_{Ar}), 118.4 (d; CH_{Ar}), 116.7 (d; CH_{Ar}), 80.7.4 (d; C-3), 76.3 (d; C-4), 66.6 (d; C-2), 51.0 (t; C-3), 28.6 (q; 3C, CH_3), 28.5 (q; 3C, CH_3) ppm; IR: 2977, 2935, 1589, 1489, 1392, 1367, 1257, 1190, 1106, 1068 cm^{-1}.

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Acknowledgement

Though only my name appears on the cover of this dissertation, many others have contributed to its production. I therefore take this opportunity to thanks all those people from the bottom of my soul, who have made this thesis possible and because of whom my PhD experience has been one that I will cherish for the rest of my life.

First and foremost, my hearty thanks and deep sense of gratitude to my supervisors Prof. Alberto Brandi and Prof. Franca M. Cordero, who without their inspiration, knowledge, guidance and motivation, none of this would be possible. Prof.Alberto Brandi, I have greatly appreciated your confidence in me and in this project. I am grateful for being given the opportunity to work in your group. I would like to thanks to my supervisors for the excellent guidance during my Ph.D period at University of Florence (Italy). I have been extremely fortunate to have supervisors who not only educated me in Chemistry but also taught me discipline and shown unique ways to achieve my goals. This has helped me to overcome many obstacles along the path of my PhD. The humanity and scientific curiosity were a constant source of inspiration. I am grateful for being a scientific discussion with Prof. Franca Cordero during my Ph.D period and constant support, and encouragement especially during the last few weeks of writing my thesis. I am grateful to have been part of Prof. Brandi and Prof.Cordero research group; I feel so lucky to do work with those great scientists as well as great human being.

I thank to Dr. Beatrice Macchi, University of Rome Tor Vergata for the enzymatic and biological tests carried out on the molecules which I synthesized in laboratory. I would like to thanks our technical staff Brunella Innocenti, Maurizio Passaponti for their support. I express

my profound gratitude to Prof. Stefano Cicchi, Prof. Fabrizio Machetti, Prof. Francesca Cardona and Prof. Donatella Giomi for their support and encouragement. I thanks to for all my colleagues in the laboratory for their valuable discussions, suggestions, support and their friendly nature in laboratory. I specially thanks Dr. Paola Bonanno, Dr. Lucca Guideri, Dr. Carolina Vurchio, Dr. Luisa Lascialfari, Dr. Marino Malavolti, Andrea Martinelli, Claudia Vinattieri, Giampiero D'Adamio and Stefano Fedeli. I also owe a deep-felt gratitude to the Giorgia Casoni, Giacomo Biagiotti, Aydan Özden and Nesrin Yumitkan for friendship and good natured teasing have made this stage of my life so much more enjoyable than I ever could have imagined. Only we share in the knowledge of the feelings of sheer jubilation and crushing defeat that chemistry offers on a daily basis.

I thankful to Prof. Andrea Goti (Ph.D Coordinator) for providing this opportunity to work at University of Florence and constant support as well as valuable guidance.

I am also sincerely thankful to my friends Milind and his family, Rajesh (my roommate), Vasant and Avinash from Florence. Suresh, Santosh, Rakesh, Tushar and remaining friends in Italy which made my stay in Italy, very enjoyable and memorable.

It gives me great pleasure to express my deep sense of esteem gratitude to my supervisors in India. Dr. Ganesh Pandey, Director, Centre Of Biomedical Research, Lucknow (India) and Dr. D.D. Dhavale, Head, chemistry Department, University of Pune, for their inspiring guidance, never diminishing encouragement, support and their complete dedication during the progress of my research project and Master degree. They provide me excellent environment in research which creates an enthusiasm in me to do further research. I also would like to thanks to

Dr.Santosh Mhaske (Scientist, NCL), for gave me nice opportunity to do work under his valuable guidance.

I also consider myself blessed in that I got to spend a major chunk of my time at University of Pune and NCL with Dr. Prasanna Kumara Chikkade and Shivkumar Burugu with whom I started my research experience. I would like to thanks to my all NCL and Pune university friends who directly and indirectly play important role in my life, particularly I would like to thanks to Dr. Nilkant Aher and his family. They have always been and will continue to be an inspiration to me. We were of one mind in many things and therefore shared special memories.

I would like to acknowledge my Teachers, Classmates and Senior Colleagues from my Satana college during my graduation for their helping hands and brotherly affection Dr. Pawar, Dr.Shah, Dr. Pagar, Mr. Gunjal, Mr.Hire and Mr. Zoman throughout my tenure in college. I would also like to thank my friends Dinesh, Narendra, Amol, Pramod, Rahul, Sandip, Nilesh, and Balu.

My family is always source of inspiration and great moral support for me in perceiving my education, it is impossible to express my sense of gratitude for my family, Aai, My late father, Grandfather and Grandmother in mere words. Whatever I am and whatever I will be in future is because of their enormous blessings, hard work, commitments to my ambitions, and their selfless sacrifices. It was their single minded pursuit of the cause of my education that gave me the strength and will continue to guide my future. Although this eulogy is insufficient, I preserve an everlasting gratitude for them. Words fall short to thank my brothers Sachin (Pappu), Yogesh (Babu) they educate me and made me strong, and for their constant support.

My sister-in-laws Sunita and Nilima Vahini and my cousins Bharti, Yogesh and Sagar for their never ending encouragement and support. I really grateful to have nephews like Indrajeet (shiv) and Indrasen (shambhu) who are full of happiness, Joy and Curiosity, they brought everlasting cheerfulness in my life.

At last but not the least, I thank whole heartedly, the omnipotent God, the illimitable superior spirit, for the strength and determination to put my chin up when faced with hardships in life.

Bhushan Balasaheb Khairnar