UNIVERSITÀ
DEGLI STUDI
FIRENZE

DOTTORATO DI RICERCA IN SCIENZE CHIMICHE

CICLO XXVI

COORDINATORE Prof. Andrea Goti

Novel syntheses of iminosugars and their conjugation to gold nanoparticles for biological studies

Settore Scientifico Disciplinare CHIM/06

Dottorando
Dott. Camilla Matassini

Tutore
Prof. Andrea Goti

Cotutore
Dr. Marco Marradi

Anni 2011/2013

Summary

Preface

This PhD Thesis deals with the synthesis of iminosugar alkaloids with pyrrolidine, piperidine and pyrrolizidine structures, their biological evaluation towards a range of commercially available glycosidases and their conjugation to gold glyconanoparticles in order to build novel multivalent iminosugars.

Iminosugars are very attractive carbohydrate mimetics in which the oxygen endocyclic atom is replaced by a nitrogen atom. Many of these structures have been isolated from plants and microorganisms, and showed a potent inhibitory activity towards glycosidases, enzymes involved in a variety of physiological events. Due to the important role of glycosidases, their inhibition can be the key to develop new drugs for the treatment of several pathologies such as cancer, virus and bacterial infections, diabetes and genetic metabolic disorders (e.g. lysosomal storage disorders), as well as in the crop protection field as novel insecticides and fungicides.

The possibility to create multivalent iminosugar structures has recently expanded the scope of iminosugars, showing that the concept of multivalency is important not only to study the interaction between multivalent ligands and lectins, but it can be applied to increase the biological response towards enzymes with a deep enzyme cavity. Therefore, in recent years the multivalency concept has been also applied to glycosidase inhibition.

Chapter 1 describes the principal applications of iminosugars as potential drugs, their natural occurrence and a selection of the main synthetic strategies aimed at their synthesis and that of their structural analogs. Indeed, total synthesis is an important tool both for the structural confirmation of the natural compounds and also to provide new unnatural analogs with increased biological activity.

Chapter 2 deals with the synthetic strategies developed in our group at the University of Florence to build pyrrolidine and pyrrolizidine iminosugars starting from enantiopure cyclic nitrones derived from carbohydrate precursors. A novel oxidation procedure to oxidize carbohydrate derived hydroxylamines to nitrones has been also developed. Using these strategies, two novel compounds suitable for further multimerization were synthesized. The synthesis of new pseudo disaccharides pyrrolidine iminosugars inhibitors of insect and porcine trehalase was reported using the CM (cross metathesis) strategy. This work has

been partially published (D. Bini, M. Forcella, L. Cipolla, P. Fusi, C. Matassini, F. Cardona, *Eur. J. Org. Chem.* **2011**, 3995-4000).

Chapter 3 reports a novel straightforward synthetic methodology to obtain diversely funtionalized piperidine iminosugars through the double reductive amination (DRA) or the Strecker reaction followed by reductive amination of a key aldehyde intermediate derived from mannose. This latter strategy allowed the synthesis of trihydroxypipecolic acids and of aminomethylpiperidine iminosugars. This work has been partially published (C. Matassini, S. Mirabella, A. Goti, F. Cardona, *Eur. J. Org. Chem*.**2012**, 3920-3924; C. Matassini, S. Mirabella, X. Ferhati, C. Faggi, I. Robina, A. Goti, E. Moreno-Clavijo, A. J. Moreno-Vargas, F. Cardona, *Submitted*).

Chapter 4 deals with the construction of novel multivalent iminosugars (whose synthesis was described in *Chapters 2* and *3*) by their conjugation to gold glyconanoparticles. This work has been achieved thanks to the nine months of the PhD Course spent in the Laboratory of GlycoNanotechnology of CIC biomaGUNE (San Sebastián, Spain) as a result of the collaboration with Prof. Soledad Penadés and Dr. Marco Marradi. In this Chapter, the preparation and characterization of novel gold glyconanoparticles decorated with piperidine and pyrrolizidine iminosugars is described. These new hybrid gold glyconanoparticles are under biological evaluation thanks to the collaboration with Prof. Inmaculada Robina (University of Sevilla, Spain).

Chapter 1

Iminosugar alkaloids: potential therapeutic and industrial applications

Iminosugars are structural analogues of carbohydrates where the endocyclic oxygen is replaced by a nitrogen atom (Figure 1.1).

Figure 1.1: General carbohydrate structure compared with general iminosugar structure.

At physiological pH the nitrogen atom of the iminosugar is protonated and this charged species perfectly mimics the oxocarbenium ion involved in the transition state of the hydrolysis catalyzed by glycosidase enzymes (Figure 1.2).

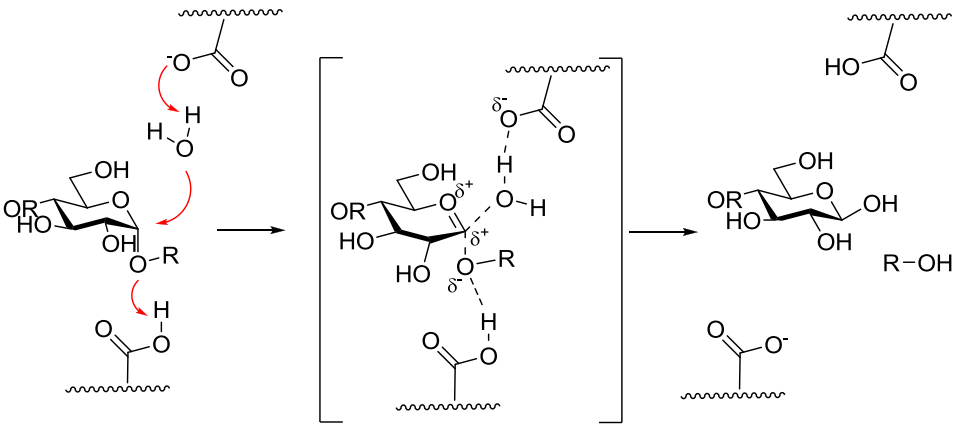


Figure 1.2: Example of an inverting glycosidase mechanism. An oxocarbenium ion like transition state is always involved in the hydrolytic process.

For this reason iminosugars can inhibit or moderate the activity of a wide range of this kind of enzymes,[1] that can act on carbohydrates and can affect the function of other carbohydrate-recognising proteins. These effects can be exploited to modify the glycosylation of eukaryotic cells, the metabolism of carbohydrate and glycoconjugates, the carbohydrate-dependent properties of glycoproteins such as folding and transport and the carbohydrate-mediated interactions of the host cells with the infective agents.[2] This is a

[1] For recent reviews, see: a) T. M. Gloster, G. J. Davies, *Org. Biomol. Chem.* **2010**, ***8***, 305–320; b) A. E. Stütz, T. M. Wrodnigg, *Adv. Carbohydr. Chem. Biochem.* **2011**, *66*, 187-298.
[2] *Iminosugars: from synthesis to therapeutic applications* (Eds.: P. Compain, O. R. Martin), Wiley-VCH, Weinheim, **2007.**

very active area of research both in academia and industry,[3] and several international patents have recently appeared on the use of iminosugars in the treatment of relevant pathologies such as cystic fibrosis, hyperglycemia, diabetes and several viral infections.[4]

1.1 Intestinal glycosidases, obesity and diabetes

Many natural α-glucosidase inhibitors, such as deoxynojirimycin (DNJ, **1**) and castanospermine (**2**) can inhibit intestinal glycosidases and delay postprandial hyperglycemia, making them potential antidiabetic and obesity drugs (Figure 1.3). The synthetic derivative of DNJ, *N*-hydroxyethyl DNJ or Miglitol® (**3**), is approved for use in type II diabetes. It decreases moderately blood glucose, insulin and glycated hemoglobin levels but causes considerable gastrointestinal discomfort due to undigested polysaccharides. The pseudotetrasaccharide acarbose (**4**), which is not appreciably absorbed, has a similar action and was introduced by Bayer onto the German market in 1990, under the name of Glucobay®.

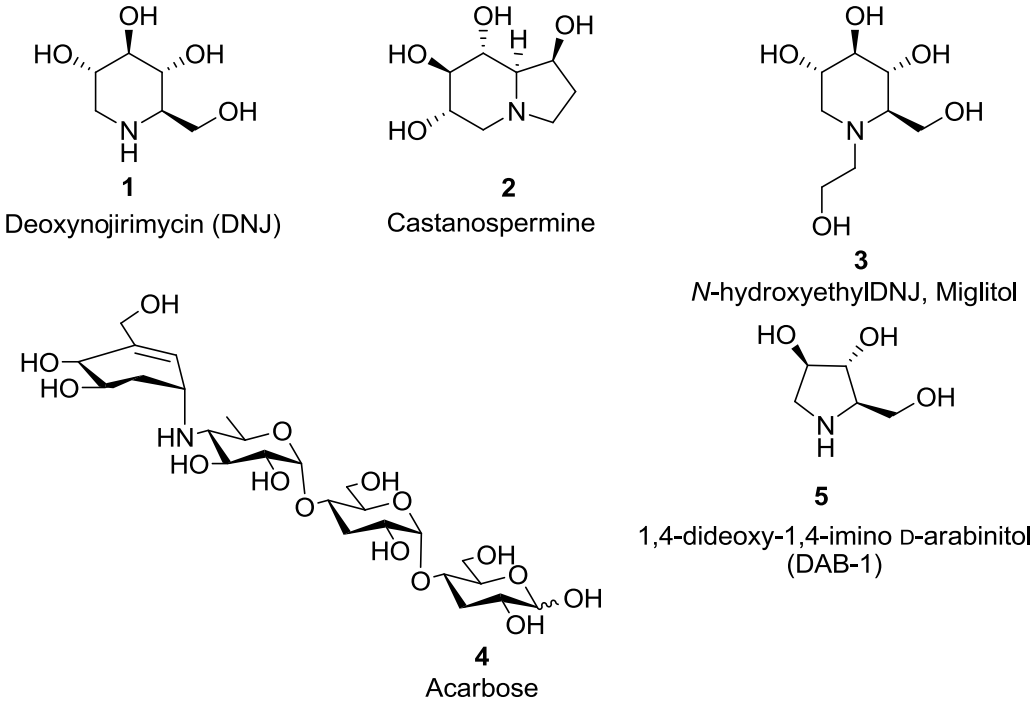


Figure 1.3: Antibiabetic (type II) agents.

[3] G. Winchester, *Tetrahedron: Asymmetry* **2009**, *20*, 645-651.

[4] a) G. W. J. Fleet. Preparation of pyrrolidine imino-sugars used in the treatment of cystic fibrosis, PCT Int. Appl. (2011), WO 2011086347. b) A. Kato, I. Adachi, H. Takahata, T. Imahori. Agent for ameliorating postprandial hyperglycemia, and pyrrolidine iminosugar or salt thereof, PCT Int. Appl. (2011), WO 2011058975 A1 20110519. c) F. X. Wilson, R. J. Nash, G. Horne, R. Storer, J. M. Tinsley, A. G. Roach. Treatment of energy utilization diseases. d) U. Ramstedt, B. Klose, N. Zitzmann, R. Dwek, T. D. Butters. Iminosugars and methods of treating viral diseases, PCT 2010, WO 2010096764 A1 20100826.

The blood glucose level can also be regulated by controlling glycogenolysis. The pyrrolidine 1,4-dideoxy-1,4-imino D-arabinitol (DAB-1, **5,** Figure 1.3) is another potential drug for type II diabetes because it can decrease glucagon-induced and spontaneous hyperglycemia by inhibition of hepatic glycogen phosphorylase.[5] Miglitol® has also shown to inhibit glycogenolysis and the effects of several other iminosugars on glycogenolysis have been studied in vitro.[6]

1.2 Glycoprotein processing

Iminosugars can also inhibit specific processing glycosidases in the endoplasmic reticulum (ER) and in the Golgi apparatus, altering the cellular ensemble of N-linked (or asparagine-linked) glycans on glycoproteins. For instance, the inhibition of molecules **6-10** is indicated in Figure 1.4.

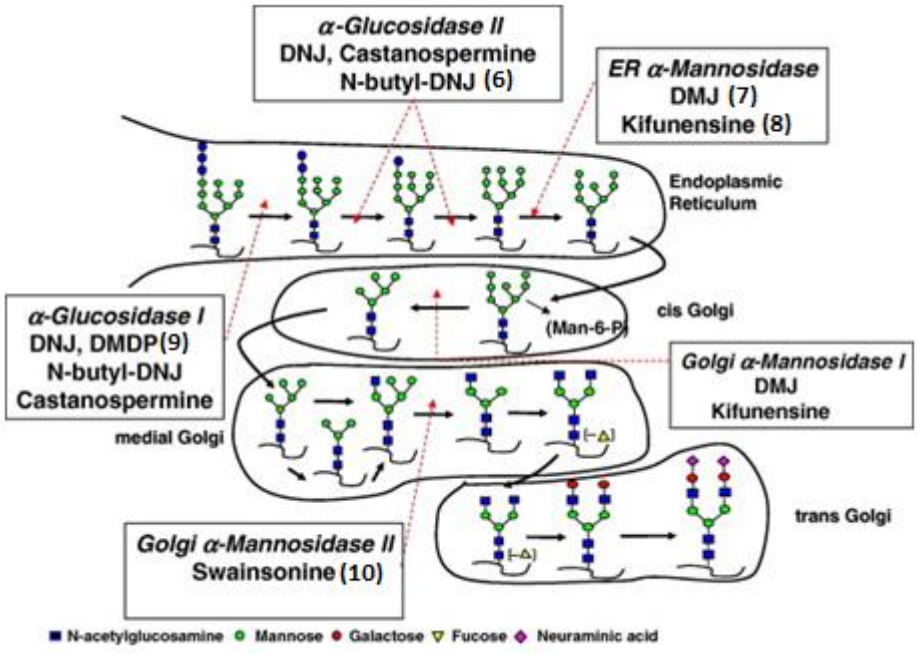


Figure 1.4: Inhibition of processing glycosidases. The structures of compounds **6-10** are reported in Figure 1.5. Taken from Ref. [3]

Inhibition of an early enzymatic step in processing can block the formation of a precursor for a certain glycan, leading the processing to an alternative pathway. This has been extensively exploited to investigate the function of the glycosylation of specific

[5] T. Latsis, B. Andersen, L. Agius, *Biochem. J.* **2002**, *368*, 309–316.
[6] N. Wang, S. Minatoguchi, X. Chen, Y. Uno, M. Arai, C. J. Lu, G. Takemura, T. Fujiwara, H. Fujiwara, *Br. J. Pharmacol.* **2004**, *142*, 983–990.

glycoproteins and to alter cellular glycosylation for therapeutic purposes such as a modulation of the immune response,[7] cancer therapy[8] and anti-viral activity.[9]

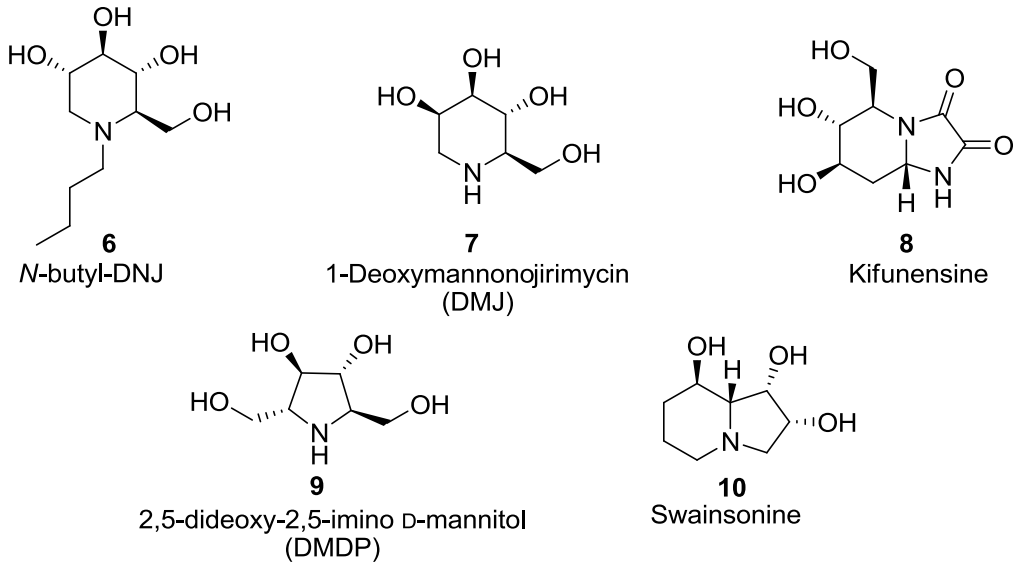


Figure 1.5: Structures of iminosugar inhibitors of processing glycosidases.

1.3 Anti-infective drugs

Specific inhibition of an enzyme, which is expressed in an infecting organism but not in the host, is an ideal drug target. Such an enzyme is the elongating α-D-mannosylphosphate transferase of the *Leishmania* parasite. This enzyme is involved in the synthesis of cell surface phosphoglycans, essential for the survival and the infectivity of the parasite but not expressed in the mammalian hosts. Novel iminosugars (1-oxabicyclic β-lactams, Figure 1.6) **11** have been synthesized based on the transition state of the enzyme and have shown to be potent inhibitors of the enzyme.[10] Pyrrolidine analogue of D-galacto-furanose and L-galactofuranose **12**-**15** can inhibit *Mycobacterium* galactan biosynthesis (Figure 1.6).[11]
Attempts to find iminosugars with antifungal activity have been less successful. [12]
On the contrary, the piperidine derivative, Siastatin B (**16**, Figure 1.6), which can be isolated from *Streptomyces*, inhibits mammalian, viral and bacterial neuraminidase (a glycosidase

[7] B.Winchester, The Development of Iminosugars as Drugs. In *Glycobiology*; C. Sansom, O. Markman, Eds.; Scion Publishing Limited, Bloxham: Oxfordshire, UK, **2006**; 308–324.
[8] T. M. Wrodnigg, A. J. Steiner, B. Ueberbacher*, J. Anticancer Agents Med. Chem.* **2008**, *8*, 77–85.
[9] a) P.Greimel; J. Spreitz, A. Stutz, E.; T. M. Wrodnigg, *Curr. Top. Med. Chem*. **2003**,*3*, 513–523. b) C. N. Scanlan, J. Offer, N. Zitzmann, R. A. Dwek, *Nature* **2007**, *446*, 1038–1045.
[10] D. Ruhela, P.Chatterjee, R. A. Vishwakarma, *Org. Biomol. Chem.* **2005**, *3*, 1043–1048.
[11] a) R. E. Lee, M. D. Smith, R. J. Nash, R. C. Griffiths, M. McNeil, R. K Grewal, W. Yan, G. S. Besra, P. J. Brennan, G. W. J. Fleet, *Tetrahedron Lett.* **1997**, *38*, 6733–6736. B) S. Cren, C. Wilson, N. R. Thomas, *Org. Lett*. **2005**, *7*, 3521–3523.
[12] I. Gautier-Lefebvre, J.-B. Behr, G. Guillerm, M. Muzard*, Eur. J. Med. Chem.* **2005**, *40*, 1255–1261.

that cleaves the glycosidic bond of neuraminic acid) and its 3-epimer is a particulary good inhibitor of influenza virus neuraminidase.[13]

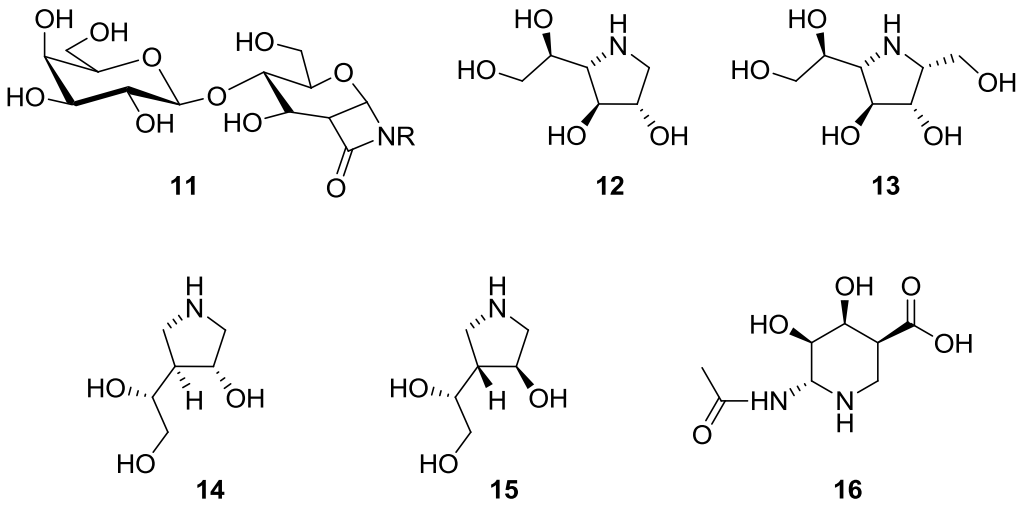


Figure 1.6: Anti-infective drugs structures.

1.4 Lysosomal storage disorders

Lysosomal storage disorders (LSDs) are a group of genetic diseases caused by defects of activity, transcription, post-translational modifications and trafficking of soluble lysosomal hydrolases. These defects cause impaired intracellular turnover and lead to accumulation of a variety of substrates such as glycosphyngolipids, glycogen, mucopolysaccharides and glycoproteins.[14] Deficiencies in metabolic enzymes required for the degradation of such substances provoke inflammations, cell damages, and tissue dysfunctions.

More than 50 disorders of this type have been identified[15] and Gaucher disease is the most common LSD, with an occurrence of approximately 10000 patients in developed countries. Most of LSDs are neuronopathic, with Central Nervous System (CNS) involvement, progressive neurodegeneration and cognitive impairment. In all LSDs clinical heterogeneity is present. The most severe forms occur in infants with onset in utero (i.e. Sialidosis, Galactosialidosis, GM1 gangliosidosis, Gaucher type II, MPSIVA) or within 6 months and outcome in the second year of life. In the less severe forms, clinical symptoms eventually appear during adulthood and are the cause of reduced life expectation.

[13] Y. Nishimura, *Curr. Top. Med. Chem*. **2003**, *3*, 575–591.
[14] B. Winchester, A. Vellodi, E. Young, *Biochem. Soc. Trans.* **2000**, *28*, 150.
[15] a) P. J. Meikle, J. J. Hopwood, A. E. Clague, W. F. Carey, *JAMA* **1999**, *281*, 249. b) J. A. Rider, D. L. Rider, *Mol. Gen. Metab.* **1999**, *66*, 231. c) P. Santavuori, *Brain Dev.* **1988**, *10*, 80.

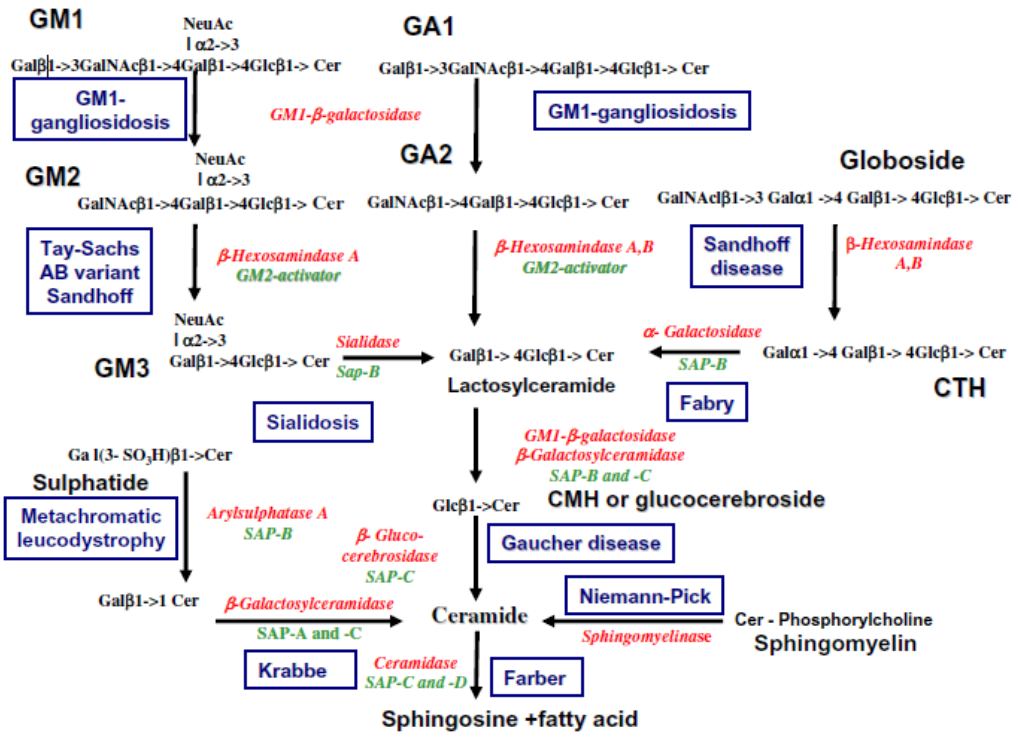


Figure 1.7: The degradation of glycosphingolipids occurs in lysosomes and involves various enzymes which are responsible for the removal of each terminal sugar unit. The deficiency in one enzyme results in the accumulation of the undegraded substrate, leading to a specific lysosomal storage disease. Taken from Ref.[3]

In particular the sphyngolipidosis are characterized by the deficiency of the glycosidases involved in the catabolism of glycosphingolipids in the lysosome (Figure 1.7).

Over the last 20 years, several therapeutic approaches have been developed for the treatment of LSDs such as increasing of enzyme activity by Enzyme Replacement Therapy (ERT) and/or by reduction of accumulated substrates known as Substrate Reduction Therapy (SRT).[16] More recently, an emerging therapeutic strategy using small molecules as potential oral drugs has received great attention: the active-site-specific chaperone (ASSC) therapy, also referred to as pharmacological chaperone therapy.[17]

Surprisingly, all the three different therapeutic approaches involve the use of iminosugars alkaloids.

[16] G. Parenti *EMBO Mol. Med.* **2009**, *1*, 268.

[17] a) J.-Q.Fan, *Trends Pharmacol. Sci.* **2003**, *24*, 355–360. b) R. J. Desnick, E. H. Schuchman, *Nat. Rev. Genet* **2002**, *3*, 954–966.

1.4.1 Preparation of human recombinant therapeutic enzymes with modified glycosylation

Ingestion of iminosugars was originally considered to cause a lysosomal storage disease by inhibition of lysosomal hydrolases in situ, for example, in swainsona toxicosis or locoweed poisoning. This phenomenon was used to provide evidence for the feasibility of enzyme replacement therapy by showing that the lysosomal storage induced in cells by swainsonine was reversed when the inhibitor was removed and endogenous activity restored.

Recombinant human lysosomal enzymes produced in mammalian cells for enzyme replacement therapy contain a mixture of N-linked glycans, including complex, hybrid and at least one high mannose with the mannose-6-phosphate motif for delivery of the enzyme to the lysosome by the mannose-6-phosphate receptor system. However, enzyme replacement therapy for Gaucher disease does not utilize the mannose-6-phosphate receptor system but targets the enzyme to the mannose-receptor on macrophages. For this reason, recombinant β-glucocerebrosidase is processed chemically and with high costs, after production to expose mannose-residues on its N-linked glycans. Therefore the development of a technology which allowed to produce recombinant proteins with defined patterns of glycosylation has been a challenge for a long time. Today this goal is achieved producing recombinant β-glucocerebrosidase with high mannose chains by including an iminosugar in the fermentor culture medium.[3]

1.4.2 Substrate reduction therapy

The first evidence that iminosugars could affect the metabolism of glycosphingolipids came from treating normal fibroblasts in culture with castanospermine (**2**), a powerful inhibitor of α-and β-glucosidases. The appearance of two additional major glycosphingolipids in the treated cells, which were not present in untreated normal cells or in cells from a patient with Gaucher's disease, suggested that glycosphingolipid biosynthesis had been disturbed or that the castanospermine was inhibiting an unknown enzyme in the catabolic pathway. Subsequently, Butter co-workers showed that α-glucosidase inhibitor *N*-butyl-DNJ (**6**) inhibited ceramide glucosyl-transferase (the first step in the biosynthesis of most glycosphingolipids) acting as a mimic of ceramide.[18] This suggested that N-butyl-DNJ could be a generic drug for decreasing the rate of synthesis of all glycosphingolipids for which glucosylceramide is the precursor and, in consequence, decreasing the rate of accumulation of these glycosphingolipids in the glycosphingolipidoses in which their catabolism is

[18] F. M. Platt, G. R. Nieses, G. Reinkensmeir, M. J. Townsend, V. H. Perry, R. L. Proia, B. Winchester, R. A. Dwek, T. D. Butter, *Science* **1997**, *276*, 428–431.

impaired (Figure 1.7). This is the basis of substrate reduction therapy (SRT). On the basis of an encouraging clinical trial, *N*-butyl-DNJ (also known as OGT 918, Zavesca or Miglustat) has been licensed in Europe (2002) and USA (2003) for treatment of non-neuronopathic Gaucher disease type 1, in which there is some residual ceramide glucosyltransferase activity. Unfortunatly *N*-butyl-DNJ did not have any appreciable effect on the neurological manifestations of neuronopathic Gaucher disease type 3.[19] Clinical trials of SRT with *N*-butyl-DNJ for other glycosphingolipidoses, such as Fabry, Tay-Sachs and Sandhoff diseases did not show clinical benefit and have been abandoned. Several other compounds including some iminosugars are being evaluated in preclinical trials.[20]

1.4.3 Pharmacological chaperone therapy

It had been common knowledge among enzymologists for many years that the addition of simple sugars protected glycosidases during their extraction from tissues and that imino- and amino-sugars were excellent ligands for affinity chromatographic media for the purification of glycosidases. It had also been observed that sometimes iminosugars could activate rather than inhibit glycosidases in assays in vitro, suggesting in all cases that the tight binding of the iminosugars to the active sites of glycosidases stabilized them.

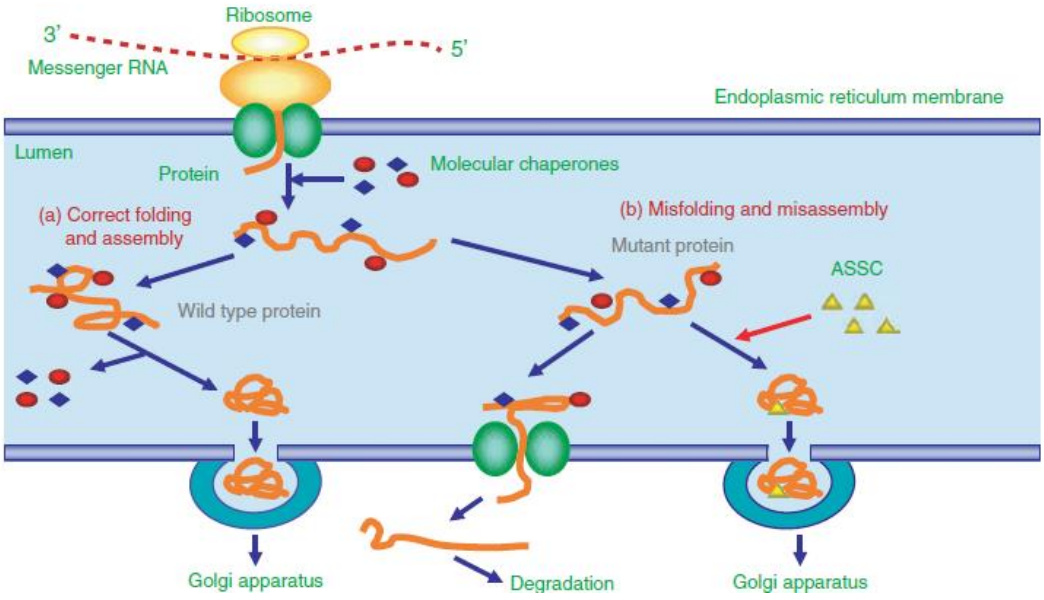
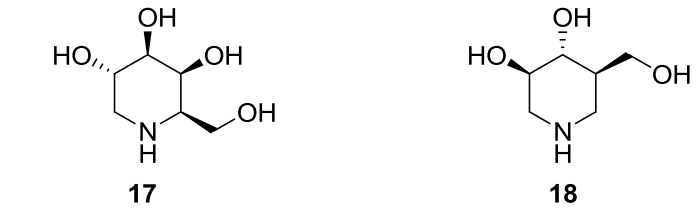


Figure 1.8: Endoplasmic reticulum (ER) quality-control system and active-site-specific chaperone (ASSC) therapy. Taken from Ref.[2]

[19] R. Schiffmann, E. J.Fitzgibbon, C.Harris, C.DeVile, E. H.Davies, L. Abel, I. N. van Schaik, W. Benko, M.Timmons, M. Ries, A.Vellodi, *Ann. Neurol*. **2008**, *64*, 514–522.
[20] T. M. Cox, *Acta Paedtr*. **2005**, *94*, 69–75.

However, it was not until 1999 that Fan and co-workers introduced the concept of active site-specific chaperone therapy (ACCS therapy or pharmacological chaperone therapy) for lysosomal storage diseases using iminosugars to prevent misfolding and premature degradation of mutant enzymes in the endoplasmic reticulum (Figure 1.8).[21]

They showed that sub-inhibitory concentrations of the potent α-galactosidase inhibitor, deoxygalactonojirimycin (DGJ, **17**, Figure 1.9) increased the residual activity in lymphoblasts from patients with Fabry disease by seven- to eightfold over 5 days, whereas higher concentrations decreased the activity.



17 Deoxygalactonojirimycin (DGJ)

18 Isofagomine (IFG)

Figure 1.9: Iminosugars for active-site-specific chaperone (ASSC) therapy.

Comparison of a series of derivatives of DGJ indicated a correlation between potency as an inhibitor and effectiveness as a chaperone.[22]

Even infusions of galactose, the product of the enzymic reaction and a weak inhibitor of the enzyme, led to clinical improvement in a Fabry patient with residual α-galactosidase activity.[23] A Phase I clinical trial of DGJ in healthy volunteers was started by a company in 2004 and a Phase II/III clinical trial in Fabry patients was carried out in 2005-2007. The preliminary results are encouraging. As iminosugars and their derivatives are usually water soluble and inert, can be taken orally and can cross the blood–brain barrier, they are attractive drugs for attempting to treat lysosomal storage diseases, especially those with CNS involvement. Several iminosugars and derivatives are being tested as chaperones for Gaucher's disease, in particular Phase I and II clinical trials with isofagomine (IFG, **18**, Figure 1.9) are in progress.[24]

[21] J.-Q. Fan, S. Ishii, N. Asano, Y.Suzuki, *Nat. Med*. **1999**, *5*, 112–115.

[22] N. Asano, S. Ishii, H. Kizu, K. Ikeda, K. Yasuda, A. Kato, O. R. Martin, J. Q. Fan, *Eur. J. Biochem*. **2000**, *267*, 4179–4186.

[23] A. Frustaci, C. Chimenti, R. Ricci, L. Natale, M. A. Russo, M. Pieroni, C. M. Eng, R. J. Desnick, *N. Engl. J. Med.* **2001**, *345*, 25–32.

[24] R. E. Boyd, G. Lee, P. Rybczynski, E. R. Benjamin, R. Khanna, B. A. Wustman, K. J. Valenzano, *J. Med. Chem.* **2013**, *56*, 2705−2725.

1.5 Agrochemical: trehalase inhibitors

Glycosidase inhibitors also find applications in the field of crop protection and specifically many iminosugar alkaloids have been shown a potent inhibition activity towards trehalase. Trehalose is a blood sugar in insects and a major storage sugar in fungi and yeast. Trehalase hydrolizes trehalose into two units of glucose and therefore plays the essential role in the transport of glucose in insects and fungi, while vertebrates do not depend on the hydrolysis of trehalose.[25]

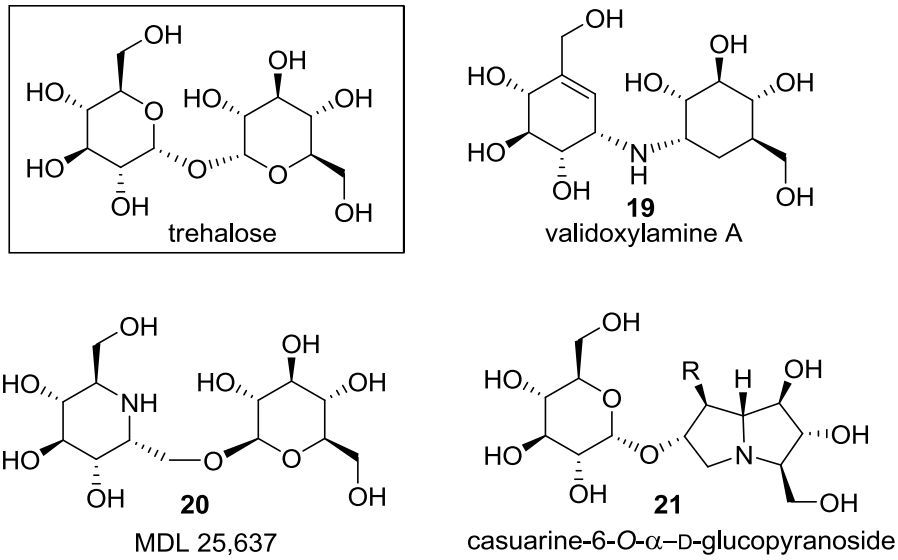


Figure 1.10: Structures of trehalose and its inhibitors: **20** and **21** contain an iminosugar alkaloid unit.

For this reason trehalase inhibitors are expected to have potential as nontoxic insecticides or fungicides. All powerful trehalase inhibitors reported to date are pseudodisaccharide type of inhibitors, such as validoxylamine A (**19**),[26] MDL 25637 (α-homonojirimycin-7-*O*-β-D-glucopyranoside, **20**)[27] and casuarine-6-*O*-α-D-glucopyranoside (**21**,[28] Figure 1.10). These compounds are powerful competitive inhibitors of porcine kidney trehalase. The extremely high affinity of pseudodisaccharide inhibitors derives from the synergistic interactions of an alkaloid unit and a sugar unit with two enzyme's subsites.[29] However, these trehalase inhibitors are not practical as insecticides because they have poor percutaneous absorption

[25] a) Elbein, A. D. *Adv.Carbohydr. Chem. Biochem.* **1974**, *30*, 227-256. b) Elbein, A. D.; Pan, Y. T.; Pastuszak, I.; Carroll, D. *Glycobiology* **2003***, 13,* 17R-27R.
[26] a) N. Asano, T. Yamaguchi, Y. Kameda, K. Matsui, *J. Antibiot* **1987**, *40*, 526-532. b) Y. Kameda, N. Asano, T. Yamaguchi, K. Matsui, *J. Antibiot* **1987**, *40*, 563-565.
[27] P. B. Anvenzano, L. J. Creemer, J. K. Daniel, C. –H. R. King, P. S. Liu, *J. Org. Chem* **1989**, *54*, 2539-2542.
[28] Kato, A.; Kano, E.; Adachi, I.; Molyneux, R. J.; Watson, A. A.; Nash, R. J.; Fleet, G. W. J.; Wormald, M. R.; Kizu, H.; Ikeda, K.; Asano, N. *Tetrahedron: Asymmetry* **2003**, *14*, 325-331.
[29] Asano, N.; Kato, A.; Matsui, K. *Eur. J. Biochem.* **1996**, *240*, 692-698.

through the insect skin. The design of trehalase inhibitors or prodrugs with good percutaneous absorption will lead to practical use as insecticides.

1.6 Synthesis of Iminosugar Alkaloids

In the past fifty years, more than 100 polyhydroxylated alkaloids have been isolated from plants and microorganisms, with structures belonging to the classes of polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines and nortropanes (Figure 1.11). Iminosugar alkaloids have been widely investigated and their potential therapeutic applications in many different fields have interested many research groups.[30]

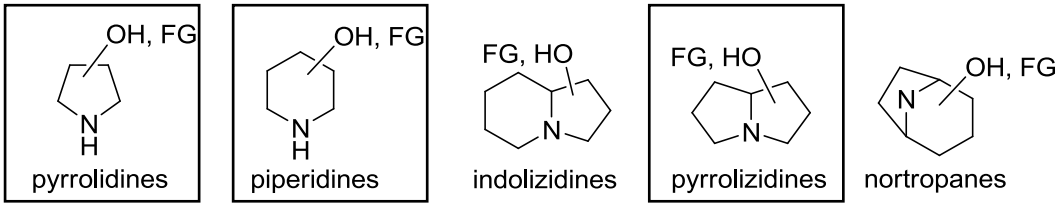


Figure 1.11: Classes of natural occurring polyhydroxylated alkaloids.

In particular, since the synthesis and the biological evaluation of pyrrolidine, piperidine and pyrrolizidine alkaloids represents the subject of this research project, the natural occurrence and the main synthetic methodologies for the obtainment of iminosugars (natural and not natural analogues) belonging to these three families will be presented more in detail in this introduction. Indeed, total synthesis is important not only for the stereochemical confirmation of the natural compounds, but also to access unnatural analogues for SAR studies.

1.6.1 Pyrrolidine Alkaloids

Natural occurrence: 2,5-Dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP, **9** Figure 1.5) was the first pyrrolidine iminosugar isolated.[31] It was extracted from the leaves of *Derris elliptica* in 1976, however its biological activity was not discovered until several later years.[32] Afterwards it was shown to be present in many different species of plants and microorganism,[33] indicating that this is a common metabolite. In 1991, 2,3-dideoxy-2,5-

[30] a) Iminosugars: from synthesis to therapeutic applications. Ed. P. Compain, O.R. Martin, **2007** Wiley-VCH. b) R. J. Nash, A. Kato, C.-Y. Yu, G. W. J. Fleet, Iminosugars as therapeutic agents: recent advances and promising trends. *Fut. Med. Chem.* **2011**, *3*, 1513-1521; c) G. Horne, F. X. Wilson, *Progr. Med. Chem.* **2011**, *50*, 135-176.
[31] A. Welter, J. Jadot, G. Dardenne, M. Marlier, J. Casimir, *Phytochemistry* **1976**, *15*, 747-749.
[32] A. D. Elbein, M. Mitchell, B. A. Sanford, L. E. Fellows, S. V. Evans, *J. Biol, Chem.* **1984**, *259*, 2409-2413.
[33] Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochemistry.* **2001**, *56*, 265-295.

imino-D-glucitol (DGDP, **22**) was synthesized and was shown to have potent α- and β-glucosidase inhibition. Since then DGDP has been isolated from the Thai traditional medicine "Non tai yak" (*Stemona tuberosa*).

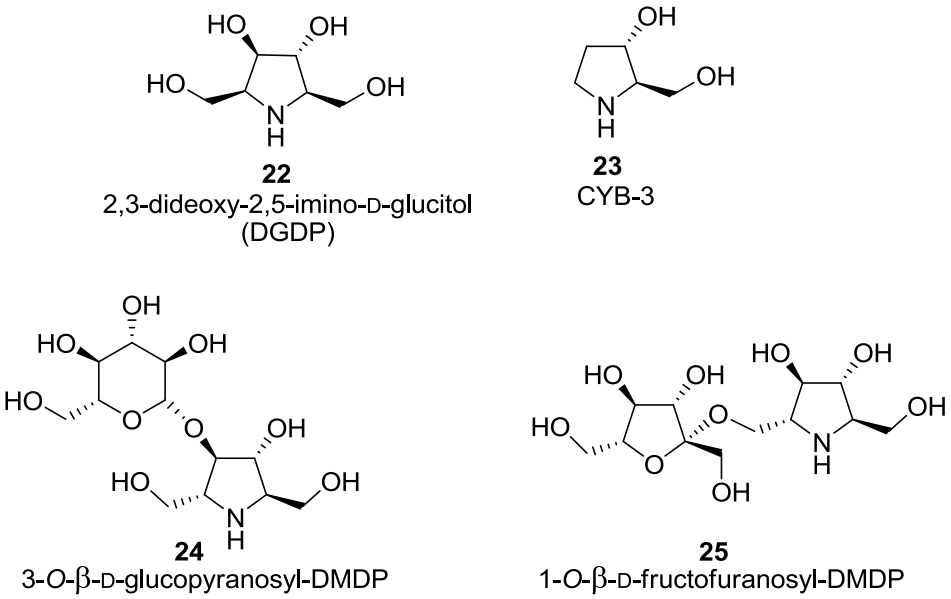


Figure 1.12: Naturally occurring pyrrolidine iminosugars.

The 1,4-dideoxy-1,4-imino-D-arabinol DAB-1 (**5**, Figure 1.3) was originally isolated from the fruit of *Anglylocalyx boutiqueanus*[34] and subsequently in many other species of plants as well as DMDP.[33] The first isolated 1,2-dideoxy iminosugar, 2-hydroxymethyl-3-hydroxypyrrolidine (CYB-3, **23**), was extracted from *Castanospermum australe* in 1985. [35] More recently, a number of new glycosyl pyrrolidine iminosugars have also been isolated. In 2005 Asano and coworkers extracted Thai medicinal plants and, in combination with several previously identified iminosugars, isolated 3-*O*-β-D-glucopyranosyl-DMDP (**24**).[36] In 2008, Kato and coworkers extracted ten iminosugars from the leaves of the African medicinal tree *Baphia nitida* and discovered the novel 1-*O*-β-D-fructofuranoside of DMDP (**25**).[37]

Synthetic strategies: in 1984 Fleet reported the synthesis of 1,4-dideoxy-1,4-imino-D-mannitol (**26,** DIM),[38] the first pyrrolidine iminosugar able to inhibit a sugar processing enzyme. Until that point the mimicry of the pyranose substrates of these enzymes was

[34] R. J. Nash, E. A. Bell, J. M. Williams, *Phytocheistry.* **1985**, 24 1620-1622.
[35] R. J. Nash, E. A. Bell, G. W. J. Fleet, R. H. Jones, J. M. Williams, *J. Chem. Soc. Chem. Commun*. **1985**, 738-740.
[36] N. Asano, T. Yamauchi, K. Kgamifuchi, N. Shimizu, S. Takahashi, H. Takatsuka, K. Ikeda, H. Kizu, W. Chuakul, A. Ketawan, T. Okamoto*, J. Nat. Prod.* **2005**, *68*, 1238-1242.
[37] A. Kato, N. Kato, S. Miyauchi, Y. Minoshima, I. Adachi, K. Ikeda, N. Asano, A. A. Watson, R. J. Nash, *Phytochemistry* **2008**, *69*, 1261-1265.
[38] G. W. J. Fleet, P. W. Smith, S. V. Evans, L. E. Fellows, *Chem. Commun*. **1984**,1240-1241.

ascribed only to six-membered piperidines such as DNJ (**1**). It is now clear, through the synthesis of many potent inhibitors, that pyrrolidines such as DIM (**26**) are at least as good, if not better than their piperidine counterparts, probably due to their close conformational resemblance to the postulated half-chair conformation of the transition state of glycosidases (Figure 1.13) and acid–base interactions with catalytic residues in active sites.[39]

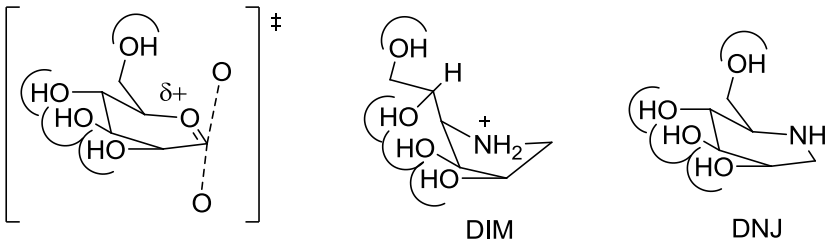


Figure 1.13: Conformational resemblance between the postulated transition state of glycosidases and pyrrolidine DIM (**26**) or piperidine DNJ (**1**), elaborated from Ref.39.

The synthesis of DIM was obtained in 32% from benzyl mannoside, suitable protected to allow the introduction of an azido moiety at C-4. Reduction of this key intermediate formed an amine at C-4, deprotected C-1 and then allowed intramolecular reductive amination to form DIM (**26**) in very good yield (Figure 1.14).

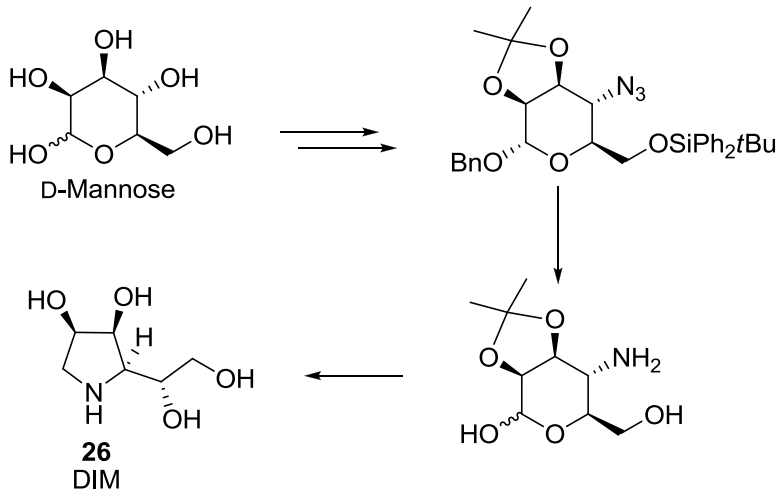


Figure 1.14: Synthesis of DIM (**26**), reported by Fleet in 1984.

Ever since, reductive amination continues to be a popular method for the synthesis of a variety of polyhydroxylated pyrrolidines. For example improved syntheses of 2,5 dideoxy-2,5-imino-D-mannitol (DMDP, **9**) and 2,5-dideoxy-2,5-imino-D-glucitol (DGDP, **22**) were recently developed by Fernández and co-workers.[40] In this case, an effective intramolecular benzyl protection/delivery system was employed (Figure 1.15) using the D-fructose-derived

[39] B. G. Davis, *Tetrahedron: Asymmetry* **2009**, *20*, 652-671.
[40] M. I. Garca-Moreno, M. Aguilar, C. O. Mellet, J. M. G. Fernández, *Org. Lett.* **2006**, *8*, 297–299.

3-*O*-(2-bromomethyl)benzyl ether **27** to form the alcohol **28** that possessed a fused eight-membered ring *o*-xylylene tether between the C-3 and C-4 hydroxy groups.

Conversion of alcohol **28** into the iodide and subsequent treatment with sodium azide gave azide **29** with net retention of stereochemistry. Acidic conditions removed the acid labile protecting groups in **29** and catalytic hydrogenation effected a one-pot reductive amination-cyclisation to yield DMDP **9** in excellent yield. Conversely, elaboration of the 5-isomer of **29** (formed via azide substitution of the triflate of **28**) led to the isolation of DGDP (**22**, Figure 1.12).

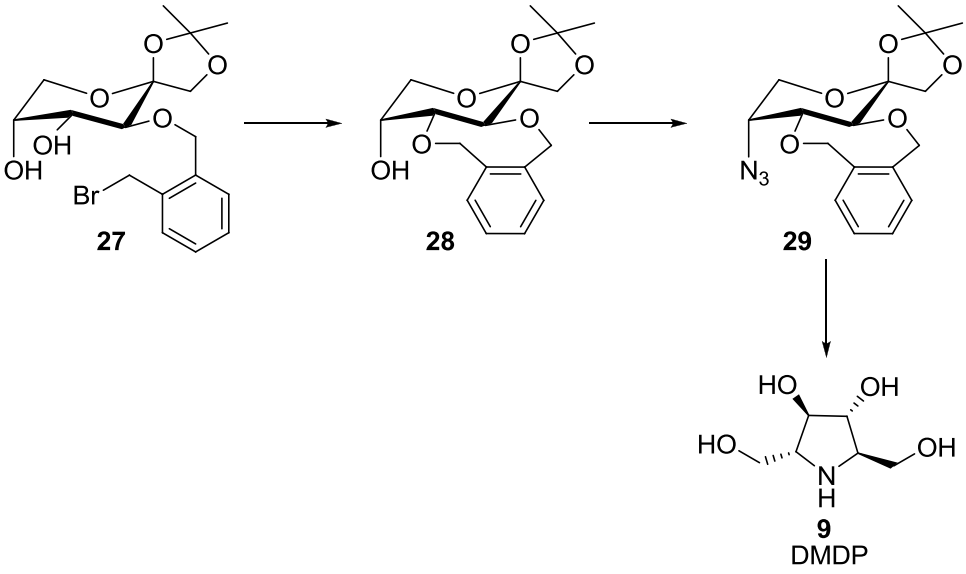


Figure 1.15: Recent synthesis of DMDP (**9**), by Fernández and co-workers.

Among the amination methodologies, nucleophilic substitution reactions involving the displacement of good leaving groups by primary and secondary amines represent a method of choice for the formation of iminosugars. In particular, the introduction and subsequent displacement of a mesyl group by an amine has been largely exploited by many different groups.[41]

In an interesting application of mesylate displacement, Moriarty et al. recently reported a facile synthesis of five membered ring iminosugars from pentonolactones (Figure 1.16).[42] Here, alkylation of lactone **30** with Grignard reagents (e.g. MeMgBr, *n*BuMgBr, *n*-

[41] Selected examples: a) J. S. Reddy, A. R. Kumar, B. V. Rao, *Tetrahedron: Asymmetry* **2005**, *16*, 3154–3159; b) A. J. Moreno-Vargas, A. T. Carmona, F. Mora, P. Vogel, I. Robina, *Chem. Commun.* **2005**, 4949–4951. c) J. S. Reddy, B. V. Rao, *J. Org. Chem.* **2007**, *72*, 2224–2227; d) M. Bosco, P. Bisseret, P. Constant, J. Eustache, *Tetrahedron Lett.* **2007**, *48*, 153–157; e) J.-B. Behr, G. Guillerm, *Tetrahedron Lett.* **2007**, *48*, 2369–2372; f) M. A. Alam, A. Kumar, Y. D. Vankar, *Eur. J. Org. Chem.* **2008**, *29*, 4972–4980; g) A. E. Håkansson, J. van Ameijde, L. Guglielmini, G. Horne,R. J. Nash, E. L. Evinson, A. Kato, G. W. J. Fleet, *Tetrahedron: Asymmetry* **2007**, *18*, 282–289.

[42] R. M. Moriarty, C. I. Mitan, N. Branză-Nichita, K. R. Phares, D. Parrish, *Org. Lett.* **2006**, *8*, 3465-3467.

$C_9H_{19}MgBr$) produced keto sugars **31**, which were subsequently reacted with ammonia to yield the *endo*-imine **33** following rearrangement of the initially formed *exo*-amine **32**. Imine products **33** could also be readily hydrogenated to provide the saturated pyrrolidine product, some of which showed potent antiviral activity.

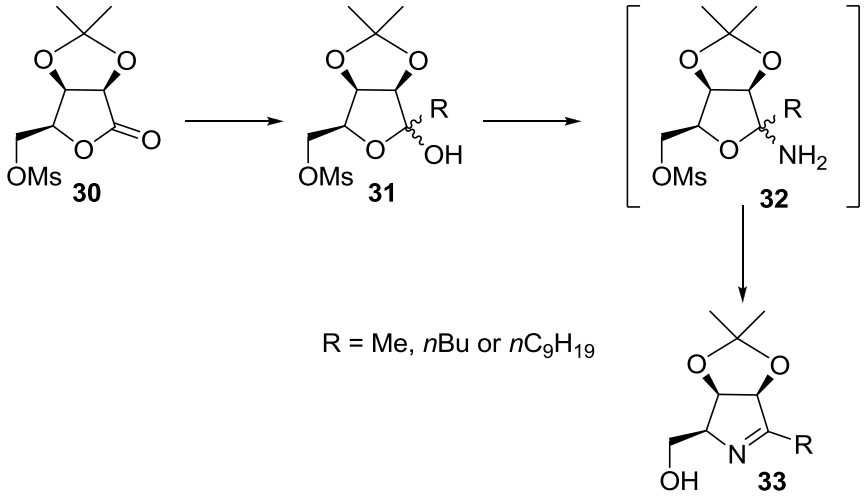


Figure 1.16: An example of mesylate displacement strategy, reported by Moriarty and co-workers.

The aldol reaction represents another example of well-precedented procedure, which has been recently extended to its enzymatically catalysed version, allowing the preparation of a variety of pyrrolidine iminosugars in remarkable few steps.

This methodology, employing different aldolases and different donors, has been investigated essentially by the two groups of Wong and Clapés, allowing the synthesis of different iminocyclitols, such as L-iminoglucitol (**34**, Figure 1.17)[43] or DAB-1 and 5-deoxy DAB-1,[44] respectively.

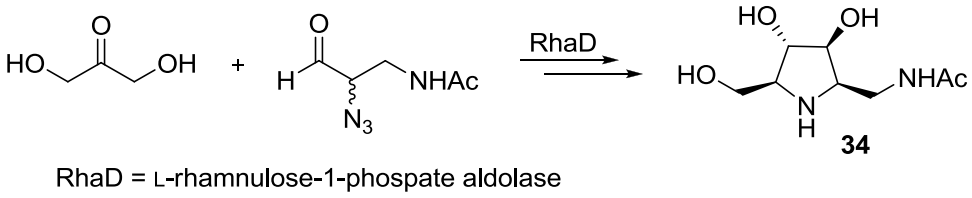


Figure 1.17: Synthesis of L-iminoglucitol (**34**) through RhaD catalysed aldol reaction, by Wong and co-workers.

With the advent of more potent, functional group tolerant, and commercially available catalysts (Figure 1.18), the Ring-Closing Metathesis (RCM) reaction has become a popular

[43] a) M. Sugiyama, Z. Hong, L. J. Whalen, W. A. Greenberg, C.- H. Wong, *Adv. Synth. Catal.* **2006**, *348*, 2555–2559; b) M. Sugiyama, Z. Hong, P.-H. Liang, S. M. Dean, L. J. Whalen, W. A. Greenberg, C.-H. Wong, *J. Am. Chem. Soc.* **2007**, *129*, 14811–14817.

[44] a) L. Espelt, J. Bujons, T. Parella, J. Calveras, J. Joglar, A. Delgado, P. Clapés, *Chem. Eur. J.* **2005**, *11*, 1392–1401; b) A. L. Concia, C. Lozano, J. A.Castillo, T. Parella, J. Joglar, P. Clapés, *Chem. Eur. J.* **2009**, *15*, 3808–3816.

strategy to synthesise cyclic molecules. In the construction of iminosugars, the combination of RCM and *cis*-hydroxylation has become an especially valuable methodology.

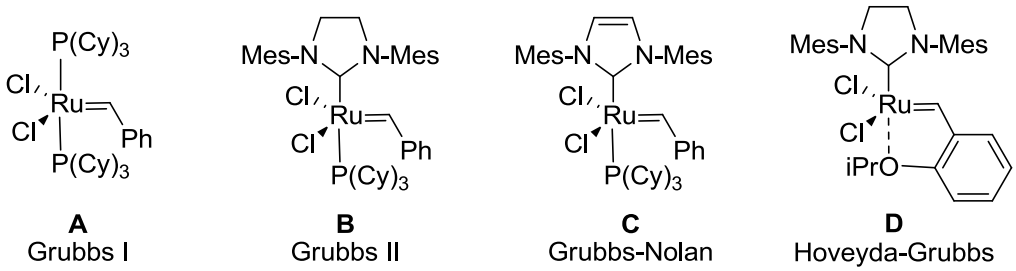


Figure 1.18: Metathesis catalysts.

A large number of 1,4-dideoxy-1,4-imino-hexitols have been prepared using this dual strategy with a typical procedure for the preparation of an iminohexitol involving the RCM of a suitably functionalized nitrogen containing diene to yield a dehydropyrrolidine that is subsequently dihydroxylated, often using OsO_4. To synthesise the required diene, a variety of reaction conditions have been used. For example, Rao and co-workers[45] used a Grignard addition to prepare the diene of desired stereochemistry for the synthesis of 1,4-dideoxy-1,4-imino-D-allitol **39** (Figure 1.19). Here, (*R*)-2,3-*O*-isopropylidine glyceraldehyde (**35**) was condensed with benzylamine and subjected to a Grignard reaction with vinylmagnesium bromide to provide alkene **36**. The nitrogen in **36** was then Boc-protected, debenzylated, and allylated to give diene **37**. RCM of the nitrogen tethered diene provided pyrrole **38**, which was subsequently dihydroxylated and finally deprotected to give imino-D-allitol **39**. The syntheses of 1,4-dideoxy-1,4- imino derivatives of L-allitol and D-talitol were also accomplished using similar methodology.

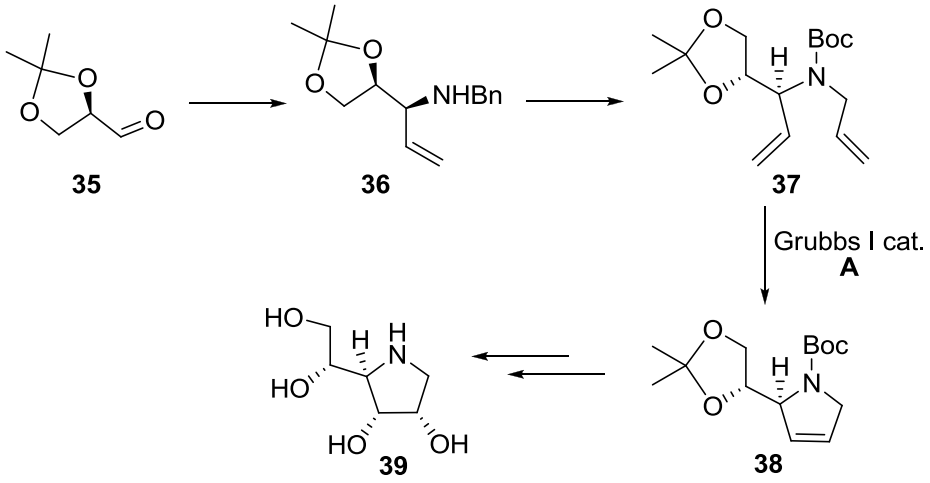


Figure 1.19: Example of Ring-Closing Metathesis methodology by Rao and co-workers.

[45] B. Chandrasekhar, A. Madhan, B. V. Rao, *Tetrahedron* **2007**, *63*, 8746–8751.

Nucleophilic addition to nitrones, imides and imines has been well documented and recently exploited as a key methodology for the synthesis of a variety of polyhydroxylated pyrrolidines.[46] In particular, the *anti*-addition of an organometallic reagent to a nitrone carbon atom represents one of the strategy investigated by our group and it has been addressed in depth in *Chapter 2.1.1.*

Conversely, a novel and efficient approach to the stereoselective construction of densely functionalised pyrrolidines, based on the addition to aldehydes with in situ cyclisation, was recently reported by Somfai and co-workers.[47]

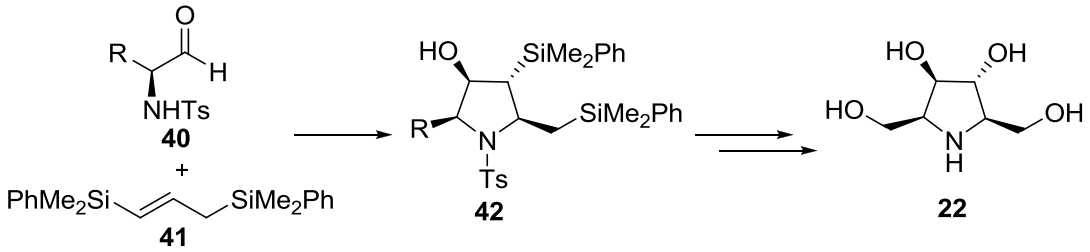


Figure 1.20: Addition to aldehyde with in situ cyclisation methodology, by Somfai and co-workers.

Here they employed a Lewis acid promoted [3+2]-annulation of *N*-tosyl-α-amino aldehydes **40** and 1,3-bis-(silyl)propenes. In this strategy, silane **41** functioned as a 1,2-dipole equivalent, and stereoselectively reacted with amino aldehyde **40** to yield a β-silyl cation that underwent a subsequent annulation reaction to form pyrrolidine **42** (Figure 1.20). A number of *N*-*p*Ts-α-amino aldehydes were amenable to these reaction conditions and gave a variety of substrates suitable for further synthetic transformations. For example, the addition of silane **41** to **40** (where R = CH_2OTBS) yielded pyrrolidine **42** (R = CH_2OTBS) which was subsequently transformed into DGDP (**22**) following desilylation, a stereospecific Tamao–Fleming oxidation, and detosylation.

Finally, the recent growth in environmental concern and awareness has seen a development of more efficient and less hazardous chemical processes. This also holds true for the synthesis of iminosugars with several groups having developed methodologies that allow for the synthesis of iminosugars without the need for protecting groups. In Stocker and co-workers protecting group free synthesis of pyrrolidines, two novel reaction

[46] Selected examples: T. Machan, A. S. Davis, B. Liawruangrath, S. G. Pyne, *Tetrahedron* **2008**, *64*, 2725–2732; b) S. Lauzon, F. Tremblay, D. Gagnon, C. Godbout, C. Chabot, C. Mercier-Shanks, S. Perreault, H. DeSève, C. Spino, *J. Org.Chem.* **2008**, *73*, 6239–6250;c) Y. Ichikawa, T. Ito, M. Isobe, *Chem. Eur. J.* **2005**, *11*, 1949– 1957; d) T. Sengoku, Y. Satoh, M. Oshima, M. Takahashi, H. Yoda, *Tetrahedron* **2008**, *64*, 8052–8058; e) D. Declerck, S. Josse, A. Nguyen Van Nhien, D. Postel, *Tetrahedron Lett.* **2009**, *50*, 2171–2173; f) M. Moura, S. Delacroix, D. Postel, A. Nguyen Van Nhien, *Tetrahedron* **2009**, *65*, 2766–2772.

[47] a) P. Restorp, A. Fischer, P. Somfai, *J. Am. Chem. Soc.* **2006**, *128*, 12646–12647; b) P. Restorp, M. Dressel, P. Somfai, *Synthesis* **2007**, 1576–1583.

methodologies were developed: the formation of primary amines via a modified Vasella/reductive amination reaction and the stereoselective formation of cyclic carbamates from alkenylamines.[48]

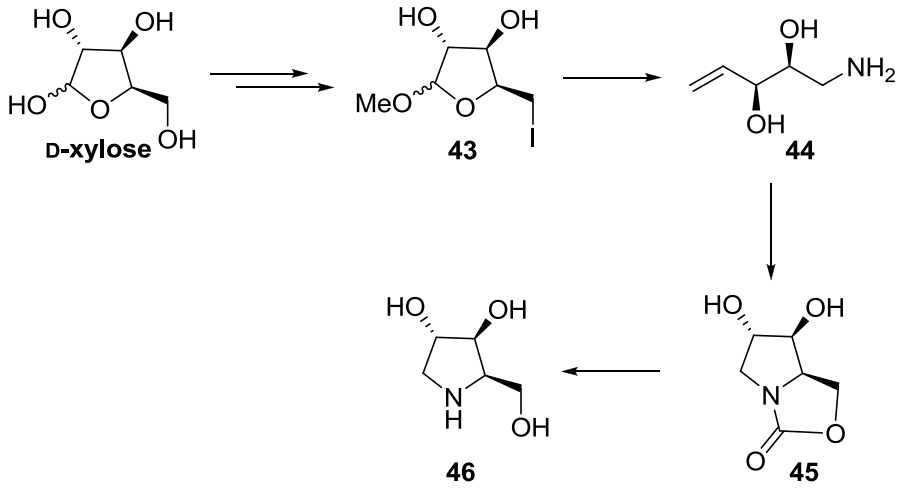


Figure 1.21: An example of protecting group-free synthesis by Stocker and co-workers.

The synthesis of the D-*xylo*-pyrrolidine **46** started with the conversion of D-xylose into the corresponding methyl iodoglycoside **43** (Figure 1.21). The modified Vasella/reductive amination protocol, which involves the addition of satd. NH_4OAc and NH_3 to a solution of methyl glycoside **43** in ethanol, gave linear alkenylamine **44** as the only product. Alkenylamine **44** was then subjected to aqueous $NaHCO_3$ and iodine to give carbamate **45** via an unprecedented, highly diastereoselective, iodine-mediated carbamate annulation reaction. Hydrolysis of carbamate **45** then gave D-*xylo* pyrrolidine **46** in excellent yield. In summary, 1,4-dideoxy-1,4-imino-D-xylitol **46** was prepared in five steps and in a 57% overall yield. 1,4-Dideoxy- 1,4-imino-L-lyxitol and 1,2,4-trideoxy-1,4-imino-L-xylitol were also prepared via similar methodology and in good overall yields.

1.6.2 Pyrrolizidine Alkaloids

Natural occurrence: pyrrolizidine structure is composed by two fused pyrrolidines with a bridgehead nitrogen atom. The first compound of this group to be isolated from natural sources was alexine (**47**, Figure 1.22), extracted in 1988 from the seeds of *Alexa leiopetala*.[49] At about the same time, australine (**48**, Figure 1.22), the 7a-epimer of alexine, was isolated from the seeds of *Castanospermum australe*.[50] Pyrrolizidines **47** and **48** present a pattern of substitution, on the pyrrolidine ring A, that is repeated in a large

[48] E. M. Dangerfield, M. S. M. Timmer, B. L. Stocker, *Org. Lett.* **2009**, *11*, 535–538.
[49] R. J.Nash, L. E. Fellows, J. V. Dring, G. W. J Fleet, A. E. Derome, T. A.Hamor, A. M. Scofield, D. J. Watkin, *Tetrahedron Lett.* **1988**, *29*, 2487-2490.
[50] R. J. Molyneux, M. Benson, R. Y. Wong, J. H. Tropea, A. D. Elbein, *J. Nat. Prod*. **1988**, *51*, 1198-1206.

number of other pyrrolizidines and which reminds the structure of DMDP (**9**, Figure 1.5) and castanospermine (**2**, Figure 1.22).

Another very interesting pyrrolizidine is casuarine (**49,** Figure 1.22). This molecule was found, with its 6-O-α-glucoside (**21**, Figure 1.10), in the barks of *Casuarina equisetifolia*, a plant used in traditional medicine in Samoa for the treatment of breast cancer. Both compounds were also isolated from the leaves *of Eugenia jambolana*, which is a well-known tree in India for the therapeutic value of its seeds, leaves and fruits against diabetes and bacterial infections.[51] More recently a series of pyrrolizidines with highly diversified structures were isolated from *Hyacinthoides non-scripta* and *Scilla campanulata* (both from *Hyacinthaceae* family) and named hyacinthacines (for instance, hyacinthacine C_1 **50** or hyacinthacine A_2 **51**, Figure 1.22).[52]

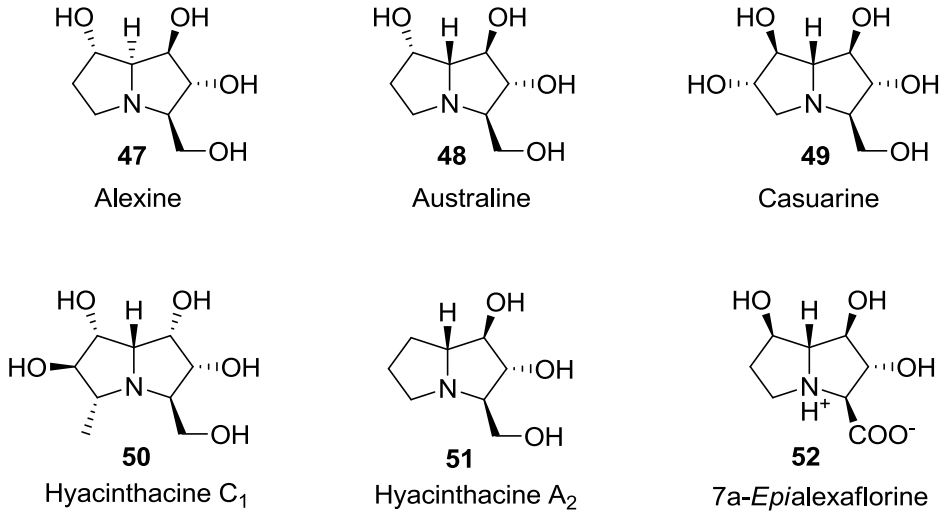


Figure 1.22: Naturally occurring pyrrolizidine iminosugars.

Finally an important example is 7a-epialexaflorine (**52**, Figure 1.22), extracted from *Alexa grandiflora*,[53] in which the castanospermine hydroxymethyl group has been replaced with a carboxyl group. This is the first example of a natural amino acid in which the carboxyl group is in position 3 of the pyrrolizidine nucleus.

Synthetic strategies: since pyrrolizidines are pyrrolidine-containing compounds, all the methodologies reported in *Chapter 1.6.1* for the synthesis of pyrrolidine iminosugars (amination, aldol condensation, organometallic catalysis, nucleophilic addition, protecting

[51] a) R. J. Nash, P. I. Thomas, R. D. Waigh, G. W. J. Fleet, M. R. Wormald, P. M.de Q. Lilley, D. J. Watkin, *Tetrahedron Lett.* **1994**, *35*, 7849–7852; b) M. R. Wormald, R. J. Nash, A. A. Watson, B. K. Bhadoria, R. Langford, A. Sims, G. W. J. Fleet, *Carbohydr. Lett*. **1996**, *2*, 169–174.

[52] A. Kato, I. Adachi, M. Miyauchi, K. Ikeda, T. Komae, H. Kizu, Y. Kameda, A. A. Watson, R. J. Nash, M. R. Wormald, G. W. J. Fleet, N. Asano*, Carbohydr. Res.* **1999**, *316*, 95-103.

[53] A. C. de S. Pereira, M. A. C. Kaplan, J. G. S. Maia, O. R Gottlieb, R. J. .Nash, G. W. J. Fleet, L. Pearce, D. J. Watkin, A. M. Scofield, *Tetrahedron* **1991**, *47*, 5637-5640.

group-free strategies) could be successfully employed also in the synthesis of pyrrolizidine iminosugars. Therefore only few strategies exclusively related to these bicyclic compounds synthesis will be reported herein.

For example, the Wittig reaction is a versatile means by which to synthesise a variety of pyrrolizidine and indolizidine iminosugars where, in general, the olefination reaction is used to extend a suitably protected pyrrolidine with an alkyl chain that is subsequently used for the generation of a second ring. Notably, Izquierdo and coworkers have used this methodology to create a number of pyrrolizidines from the hyacinthacine A and casuarine families whereby D-fructose was used as the starting material.[54]

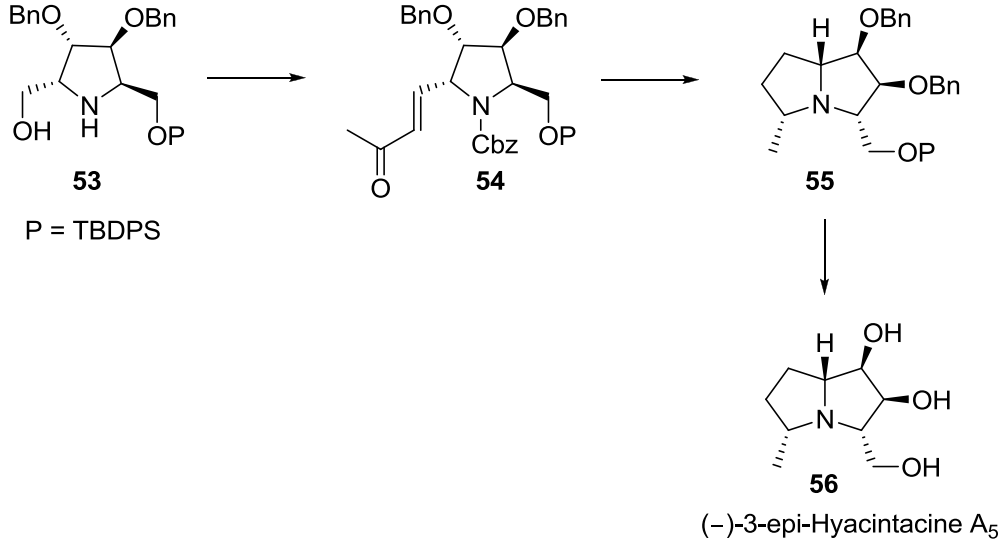


Figure 1.23: Synthesis of (–)-3-*epi*-hyacinthacine A_5 (**56**), by Izquierdo and co-workers.

As reported in Figure 1.23 for the synthesis of (**–**)-3-*epi*-hyacinthacine A_5 **Iba**, pyrrolidine **53** was Cbz-protected, oxidized, and subjected to a Wittig olefination with 1-(triphenylphosphoranylidene)-2-propanone to give the α,β-unsaturated ketone **54.** Ketone **54** was then hydrogenated, which resulted in the one-pot reduction of the double bond, *N*-deprotection and in situ cyclisation to give the protected pyrrolizidine **55** Global deprotection then gave (–)-3-*epi*-hyacinthacine A_5 (**56**).

Though less common than other amination strategies, radical reactions have nonetheless been elegantly used during the synthesis of several bicyclic iminosugars, as recently reported by Chabaud et al.[55] and Chen and Tsai.[56] In Chabaud's synthesis of (+)-

[54] a) I. Izquierdo, M. T. Plaza, J. A. Tamayo, F. Sánchez-Cantalejo, *Eur. J. Org. Chem.* **2007**, 6078–6083; b) I. Izquierdo, M. T. Plaza, J. A. Tamayo, V. Yáñez, D. L. Re, F. Sánchez-Cantalejo, *Tetrahedron* **2008**, *64*, 4613–4618; c) I. Izquierdo, M. T. Plaza, J. A. Tamayo, M. Rodríguez, A. Martos, *Tetrahedron* **2006**, *62*, 6006–6011.

[55] L. Chabaud, Y. Landais, P. Renaud, *Org. Lett.* **2005**, *7*, 2587–2590.

[56] M.-J. Chen, Y.-M. Tsai, *Tetrahedron Lett.* **2007**, *48*, 6271–6274.

hyacinthacine A_1 **60** and its 3-epimer, the stereocontrolled carboazidation of chiral allylsilane **57** with xanthate **58** provided the required carbon framework **59** (Figure 1.24). C–Si bond oxidation and reduction of the azide, with ring-closure, completed the total synthesis and established the absolute configuration of (+)-hyacinthacine A_1 **60**.[57]

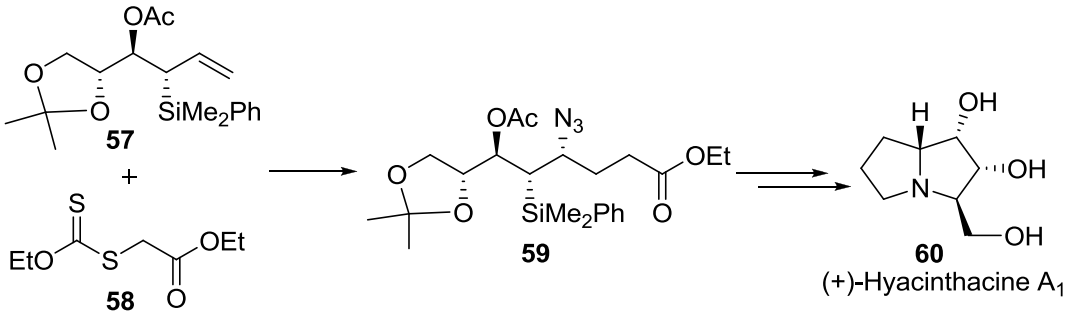


Figure 1.24: Synthesis of (+)-hyacinthacine A_1 (**60**), by Chabaud and co-workers.

In the field of organometallic catalysis, of particular note in the past few years has been the use of RCM as an essential tool in determining the correct structure of uniflorine A. Following seminal work by Pyne and co-workers who eluded to differences in the spectroscopic data for the isolated and synthesised uniflorine A,[58] a number of groups have sought to resolve this disparity.

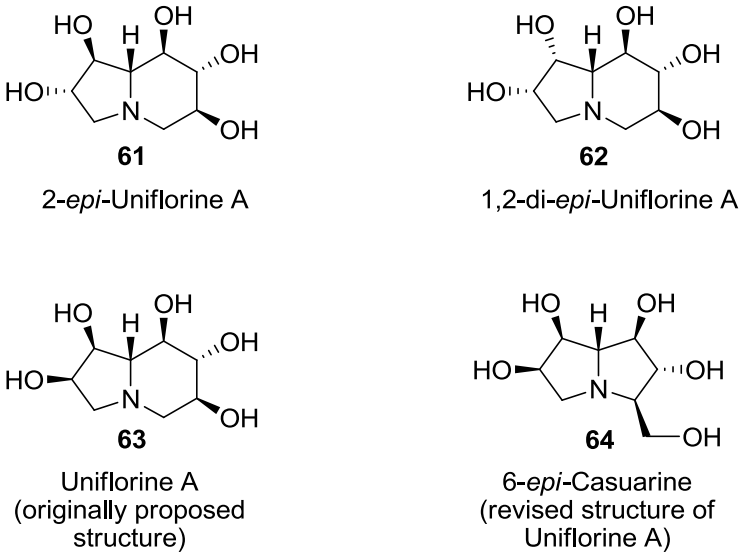


Figure 1.25: Proposed and revised structures of Uniflorine A.

Dhavale and co-workers employed RCM of a D-glucose derived diene-substrate containing a nitrogen functionality, followed by asymmetric dihydroxylation to afford a variety of "uniflorine A" analogues, however all had different spectroscopic data to that of the

[57] D. Díez, M. T. Benéitez, M. J. Gil, R. F. Moro, I. S. Marcos, N. M. Garrido, P. Basabe, J. G. Urones, *Synthesis* **2005**, 565–568.
[58] A. S. Davis, S. G. Pyne, B. W. Skelton, A. H. White, *J. Org. Chem.* **2004**, *69*, 3139–3143.

isolated material.[59] In 2008, Pyne and co-workers used RCM and a stereoselective osmium-catalysed dihydroxylation reaction to achieve the synthesis of the 2-epimer **61** and the 1,2-di-epimer **62** of the putative structure of uniflorine A (**63**) (Figure 1.25).[60] From a comparison of the NMR spectroscopic data of uniflorine A with that of casuarine and known synthetic pyrrolizidine isomers, the structure of uniflorine A was thus suggested to be 6-*epi*-casuarine (**64**). In addition, Pyne and co-workers showed that uniflorine B is in fact the known alkaloid casuarine (**49**, Figure 1.22).

Moreover the synthesis of another important pyrrolizidine alkaloid, the hyacinthacine A_2 (**51**, Figure 1.22), was accomplished by using a Cross-Methathesis (CM) reaction as key step.[61] Dewi-Wulfing and Blechert employed CM between allylamine **65** (prepared via the enzymatic resolution of (±)-*N*-Cbz-vinylglycine) and enone **66** using the Hoveyda–Grubbs ruthenium catalyst (**D**, Figure 1.18) to obtain the (*E*)-alkene which was subsequently dihydroxylated with AD-mix-β to yield diol **67** (88% *de*, Figure 1.26).

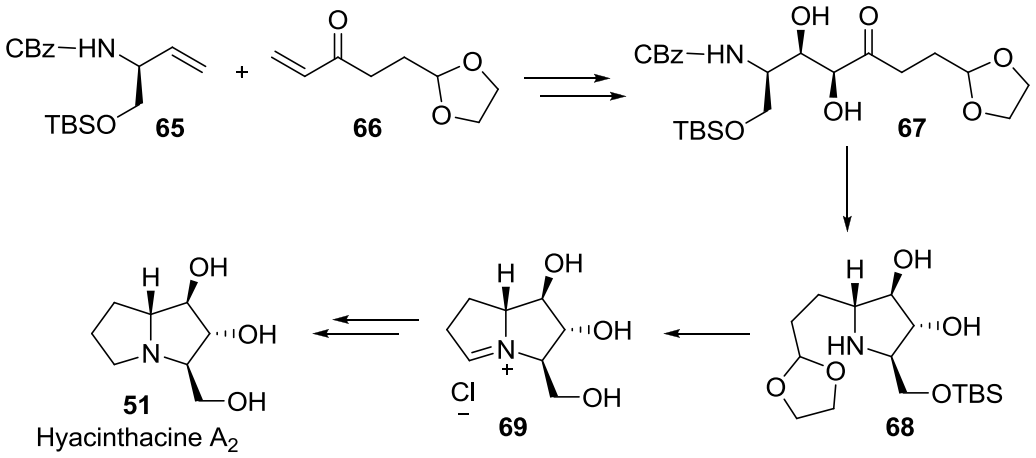


Figure 1. 26: Synthesis of hyacinthacine A_2 (**51**), by Dewi-Wulfing and Blechert.

Attempts to cyclise diol **67** were first made using a one-pot four-step procedure, however this resulted in low yields and the formation of side products. A sequential double reductive cyclisation protocol was then employed involving hydrogenation to afford **68**, followed by treatment with HCl to cleave the dioxolane and the TBS protecting groups, which gave iminium salt **69**. Imine **69** was further hydrogenated, and then neutralised via the addition of amberlite (OH^- form), to give hyacinthacine A_2 (**51**) in an overall yield of 39% for the four steps.

Polyhydroxylated enantiopure cyclic nitrones derived from sugar are attractive key building blocks for the synthesis of pyrrolizidine alkaloids. For instance the addition of lithiated

[59] N. S. Karanjule, S. D. Markad, D. D. Dhavale, *J. Org. Chem.* **2006**, *71*, 6273–6276.
[60] A. S. Davis, T. Ritthiwigrom, S. G. Pyne, *Tetrahedron* **2008**, *64*, 4868–4879.
[61] P. Dewi-Wulfing, S. Blechert, *Eur. J. Org. Chem.* **2006**, 1852–1856.

allene **71** to nitrone **70** furnished the oxazine **72** which was subjected to hydroboration/oxidation to yield **73** (Figure 1.27). Subsequently ring cleavage and cyclization afforded the most straightforward and high yielding total synthesis of casuarine reported to date (84% overall yield and six steps from nitrone **70**).[62]

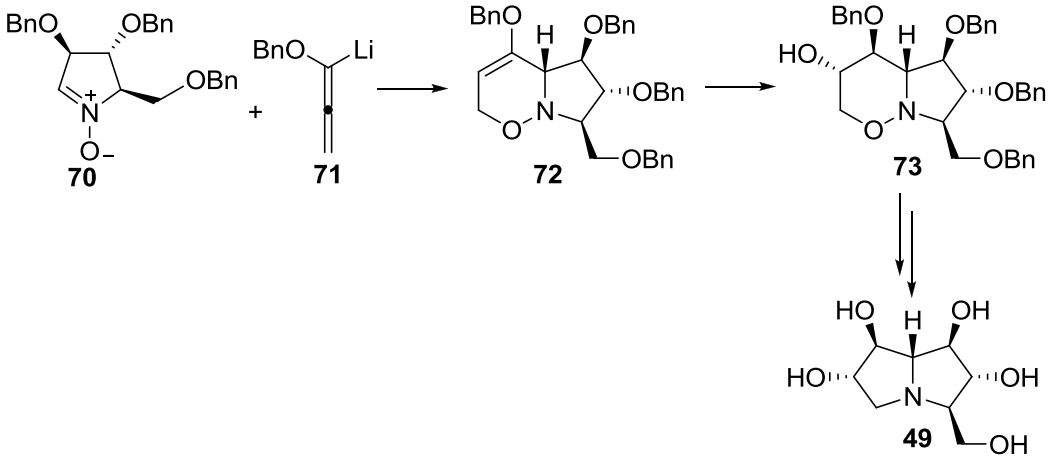


Figure 1.27: Total synthesis of casuarine (**49**), by employing addition of lithiated allene **71** to nitrone **70** as the key step.

Lastly, pericyclic reactions have to be considered one of the most potent tools for the synthesis of polyhydroxylated bicyclic alkaloids, allowing access to a large variety of structures with optimal control of relative and absolute configuration of the stereogenic centers, with few simple and selective steps. In particular, our group achieved the synthesis of many different pyrrolizidine and indolizidine iminosugars by a general strategy consisting of a highly stereoselective 1,3-dipolar cycloaddition of polyhydroxylated nitrones, followed by simple transformations of the isoxazolidine adducts.[63] Some examples of this methodology will be presented in detail in *Chapter 2.1.2.*

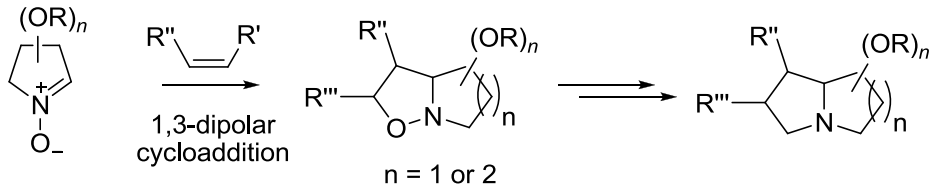


Figure 1. 28: Stereocontrolled cyclic nitrone cycloaddition strategy by Goti and co-workers.

1.6.3 Piperidine Alkaloids

Natural occurrence: in 1966, Inouye et al. discovered the first natural polyhydroxylated alkaloid, nojirimycin (**74**, NJ, Figure 1.29).[64] Isolated from a Streptomyces filtrate, it was shown to actively inhibit α- and β-glucosidases and was therefore the first natural glucose

[62] C. Parmeggiani, F. Cardona, L. Giusti, H.-U. Reissig, A.Goti, *Chem. Eur. J*. **2013**, *19*, 10595–10604.
[63] A. Brandi; F. Cardona; S. Cicchi; F. M. Cordero, A. Goti, *Chem. Eur. J.* **2009**, *15*, 7808-7821.
[64] S.Inouye, T. Tsuruoka, T. Niida, *J. Antibiot.* **1966**, *19*, 288-292.

mimic. Nojirimycin B (**75**, commonly called mannonojirimycin, Figure 1.29) and galactostatin (**76**, galactonojirimicyn, Figure 1.29) were isolated soon after.[65,66] However these iminosugars with a hydroxyl group at C-1 were found to be relatively difficult to isolate and handle due to their low stability.

1-Deoxynojirimycin (**1**, DNJ, Figure 1.3) was first prepared by chemical synthesis from L-sorbofuranose in 1966[67] and by reduction of nojirimycin in 1968[68] but later isolated from the roots of Mulberry trees[69] as well as *Streptomyces* cultures.[70] Its mannose analogue, 1-deoxy-mannojirimycin (**7**, DMJ, Figure 1.5) was isolated from the seeds of the legume *Lonchocarpus sericeus* and later from the neotropical liana *Omphalea diandra* as well as from *Streptomyces* cultures.[71]

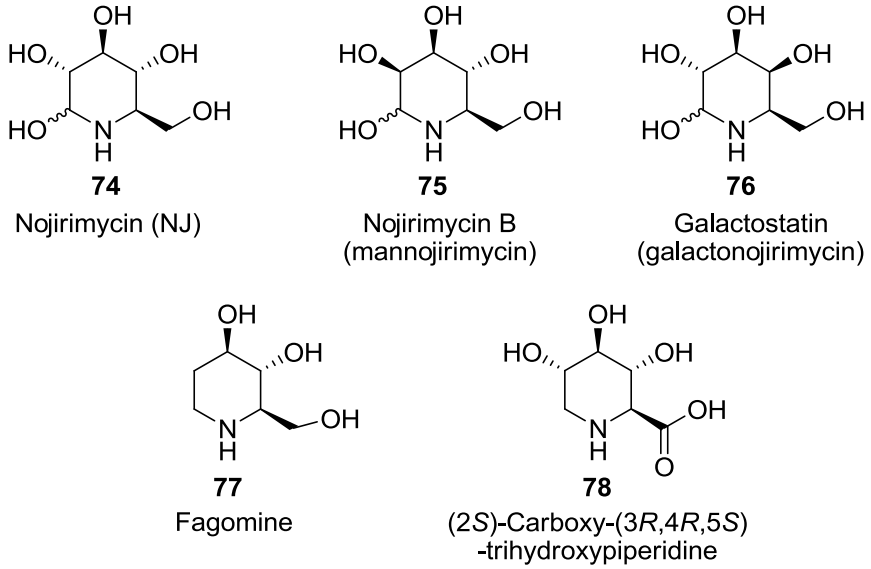


Figure 1.29: Naturally occurring piperidine iminosugars.

A further DNJ analogue is the 1,2-dideoxynojirimycin, fagomine (**77**, Figure 1.29). Initially found in the seeds of *Fagopyrum escuelentum*,[72] it was later discovered in the leaves and roots of *Xanthocercis zambesiaca* together with other fagomine analogues and derivatives.[73]

[65] T. Niwa, T. Tsouruoka, H. Goi, Y. Kodama, J. Itoh, S. Inouye, Y. Yamada, T. Niida, M. Nobe, Y. Ogawa, *J. Antibiot.* **1984**, *37*, 1579-1586.
[66] Y. Miyake, M. Ebata, *Agric. Biol. Chem.* **1988**, *52*, 661-666.
[67] H. Paulsen, *Angew. Chem. Int. Ed. Engl.* **1966**, *78*, 495-511.
[68] S. Inouye, T. Tsuruoka, T. Ito, T. Niida, *Tetrahedron* **1968**, *24*, 2125-2144.
[69] M.Yagi, T.Kouno, Y. Aoyagi, H.Murai, *Nippon Nogei Kagaku Kaishi* **1976**, *50*, 571-572.
[70] S. Murao, S. Miyata, *Agric. Biol. Chem.* **1980**, *44*, 219-221.
[71] a) L. E. Fellows, E. A. Bell, D. G. Lynn, F. Pikiewicz, I. Miura, K. Nakanishi, *J. Chem Soc. Chem. Commun*. **1979**, 977-979. b) G. C. Kite, L. E. Fellows, G. W. J. Fleet, P. S. Liu, A. M. Scofield, N. G. Smith, *Tetrahedron Lett*. **1988**, *29*, 6483-6486. c) D. J. Hardick, D. W. Hutchinson, S. J. Trew, E. M. S. Wellington, *Tetrahedron* **1992**, *48*, 6285-6295.
[72] M. Koyama, S. Sakamura, *Biol. Chem.* **1974**, *38*, 1111-1112.
[73] A. Kato, N. Asano, H. Kizu, K. Matsui, A. A. Watson, R. J. Nash, *J. Nat. Prod.* **1997**, *60*, 312-314.

In 1984, Cenci di Bello et al. extracted from the seeds of *Baphia racemosa* the first naturally occurring mimic of glucuronic acid, (2S)-carboxy-(3R,4R,5S)-trihydroxypiperidine (**78**, Figure 1.29), which was found to inhibit the human liver β-D-glucuronidase and α-L-iduronidase.[74]

Synthetic strategies: Since nojirimycin (**74**) was isolated from natural source, many different polyhydroxylated piperidines have been synthesized and evaluated towards glycosidases, in order to find compounds with higher potency and selectivity. As a consequence, a great number of synthetic approaches has been proposed, most of which employ carbohydrates as starting materials.[75] Generally the conversion of a sugar into an iminosugars occurs through the introduction of an amino moiety and subsequent amino cyclization. Actually, there are quite a few combinations of reactions that can be exploited to reach the target molecule. Aldoses, alditols, or glyconolactones can be used as substrates, the nitrogen atom can be introduced variously as an amine, as a hydroxylamine, or as an azide, and the amino-cyclization can be performed by exploiting an intramolecular nucleophilic displacement, a reductive amination, an epoxide ring-opening or a nucleophilic attack on an activated double bond. The strategies from carbohydrates, even though very popular, usually lack of versatility, since the utilization of chiral pool compounds enforce to change starting material for each synthesis. This drawback has been overcome by developing methodologies which allow access to libraries of compounds, employing a common synthetic pathway or functionalizing a common scaffold compound in many different ways. The generation of libraries of molecules is particularly useful in the search for powerful and specific inhibitors of a selected target enzyme, since the rational design of inhibitors is often extremely difficult due to the limited information regarding the structure of enzyme active sites. The first application of this concept was reported by Lin and coworkers, with the preparation of a library of fuconojirimycin derivatives,[76] through condensation of the amino group of fuconojirimycin derivative **79** (Figure 1.30) with 60 aliphatic and aromatic carboxylic acids in a microtiter plate.

[74] I. Cenci di Bello, P. Dorling, L. E. Fellows, B. Winchester, *FEBS Lett.* **1984**, *176*, 61-64.

[75] B. La Ferla, F. Nicotra, *Iminosugars as Glycosidase Inhibitors* (Ed.: A. Stütz), Wiley–VCH Verlagsgesellschaft GmbH, 68-92, **1998**; b) L. Cipolla , B. La Ferla , F. Nicotra , *Curr. Topics Med. Chem.*, **2003**, *3*, 485 –511; c) L. Cipolla , B. La Ferla , M. Gregori, *Combinatorial Chemistry & High-throughput screening*, **2006**, *9*, 571–582; d) Y. Nishimura, *Heterocycles* **2006**, *67*, 461–488; e) T. Ayad, Y. Genisson, M. Baltas, *Curr. Org. Chem*. **2004**, *8*, 1211–1233.

[76] C.-Y. Wu, C.-F. Chang, J. S.-Y. Chen, C.-H. Wong, C.-H. Lin, *Angew. Chem. Int. Ed.*, 2003, **42**, 4661–4664.

Figure 1.30: Generation of a library of fuconojirimycin inhibitors**.**

As an alternative to classical synthesis of iminosugars from carbohydrates, many total synthesis from non carbohydrate, chiral and nonchiral, starting materials have been also reported in recent years. Malacria and coworkers[77] reported the synthesis of 1-deoxymannojirimycin (**7**, Figure 1.5) starting from the silylated butendiol dicarbonates **80**, which was converted in intermediate **81**, through a chemo- and stereoselective palladium-catalysed amination (Figure 1.31). Two more steps, the conversion into the epoxyaldehyde **83** and the subsequent intramolecular aldolization, were necessary to obtain the piperidine skeleton (**83**, major compound). Regioselective epoxide ring opening and Tamao–Flemming oxidation of the C-Si bond led to the protected 1-deoxymannojirimycin **84** which was then completely deprotected.

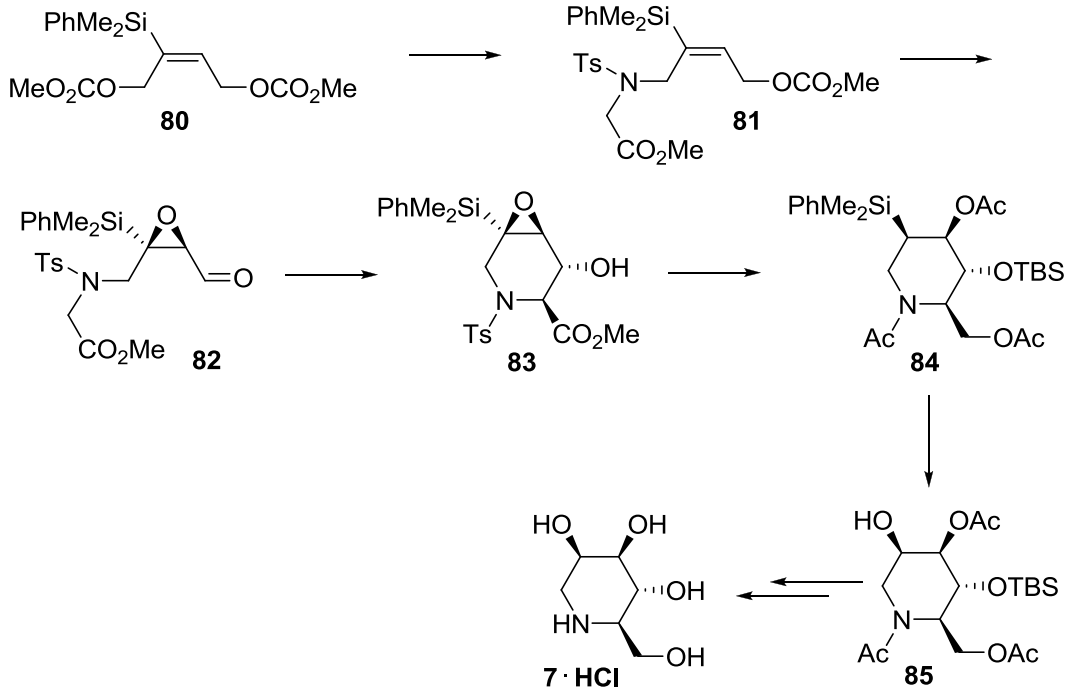


Figure 1.31: Synthesis of 1-deoxymannojirimycin (**7**) hydrochloride, by Malacria and co-workers.

Moreover, chiral synthons such as enantiomerically pure 2-(1'-amino-2'-hydroxyalkyl)furans, are very useful tools in total synthesis. In particular, Yao and coworkers[78] accessed piperidine derivative **90** starting from compound **86**, in turn obtained with an efficient route from L-serine (Figure 1.32). The oxidation of furan **86** followed by

[77] C. Boglio, S. Stahlke, S. Thorimbert, M. Malacria, *Org. Lett.* **2005**, 7, 4851–4854.
[78] X. Cong, K.-G. Liu, Q.-J. Liao, Z. J. Yao, *Tetrahedron Lett* **2005**, *46*, 8567-8571.

hemiaminal trapping afforded ketone **87** which was stereoselectively reduced to compound **88**. Then, the ethoxy group was removed, leading to the allylic alcohol **89** which was stereoselectively hydroxylated and deprotected to the final piperidine derivative **90**.

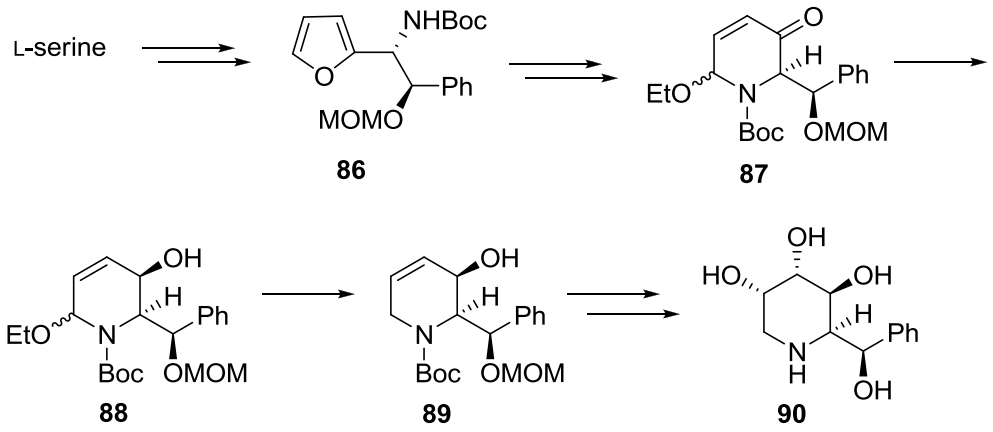


Figure 1.32: Synthesis of piperidine derivative **90**, by Yao and co-workers.

Han and coworkers reported the use of a readily available olefin (**91**) as valuable starting material for the synthesis of hydroxyl piperidines **95**.[79] As generally shown in Figure 1.33, a regioselective asymmetric aminohydroxylation (AA) reaction led to the formation of a syn-aminoalcohol with excellent stereoselectivity (>20:1). Then the aminoalcohol **92** was converted to the intermediate **93** which, through an RCM reaction, afforded the key cyclic olefin **94** which was dihydroxylated and converted to a series of 1-deoxyiminosugars.

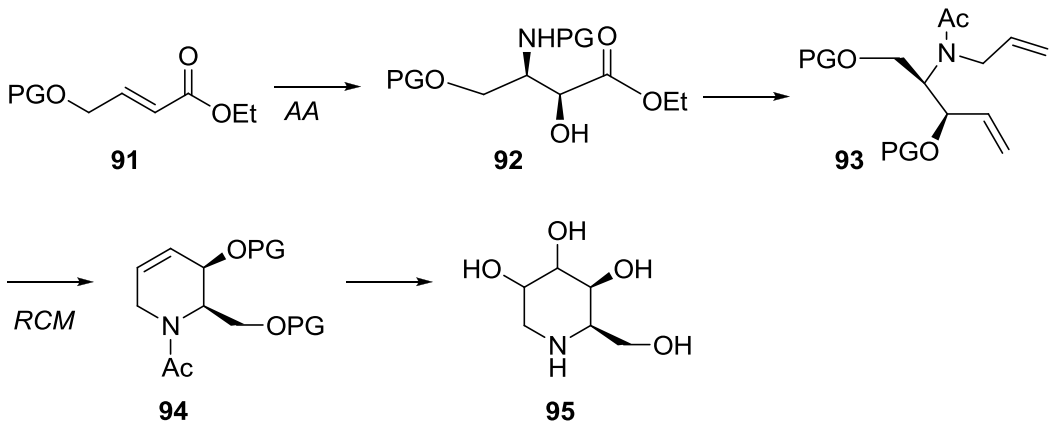


Figure 1.33: General approach to 1-deoxyiminosugars from olefin **91**, by Han and co-workers..

Another interesting example is the *de novo* synthesis of six membered-ring iminosugars reported by Còrdova and coworkers. The one-pot, tandem organocatalytic asymmetric Mannich–Wittig olefination performed on aldehyde **96**, is the key step of the whole methodology (Figure 1.34). The subsequent diastereoselective dihydroxylation allowed access to compound **99,** precursor of the final δ-lactam **100**. Only three steps were

[79] O. V. Singh, H. Han, *Tetrahedron Lett* **2003**, *44*, 2387-2391.

necessary in this case to access the piperidine skeleton, with a high control of the newly formed stereocenters.

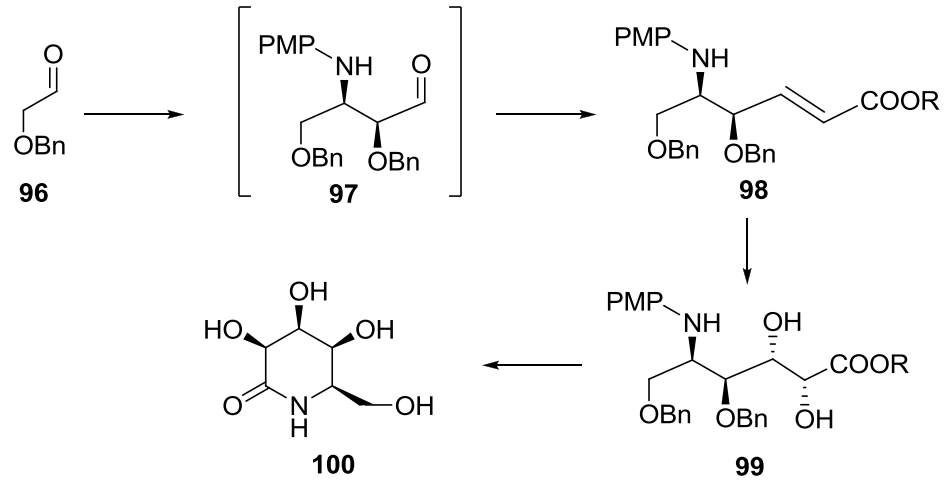


Figure 1.34: De novo synthesis by Còrdova and co-workers.

Lastly, difluoromethylenated analogues of polyhydroxylated piperidines can be prepared to evaluate the influence of each hydroxyl group in the interaction with the enzyme active site. These structures are able to modify the binding characteristics by changing the pKa of the nitrogen: when the CF_2 group was introduced in place of a CHOH group important for binding, a decrease in binding affinities was observed.[80] An example for the synthesis of such useful compounds was reported by Qing and coworkers: starting from (R)-glyceraldehyde acetonide **101** and 3-bromo-3,3-difluoro propene **102**, the 1,4-dideoxy-4,4-difluoro-D-mannonojirimycin **105** was obtained (Figure 1.35).

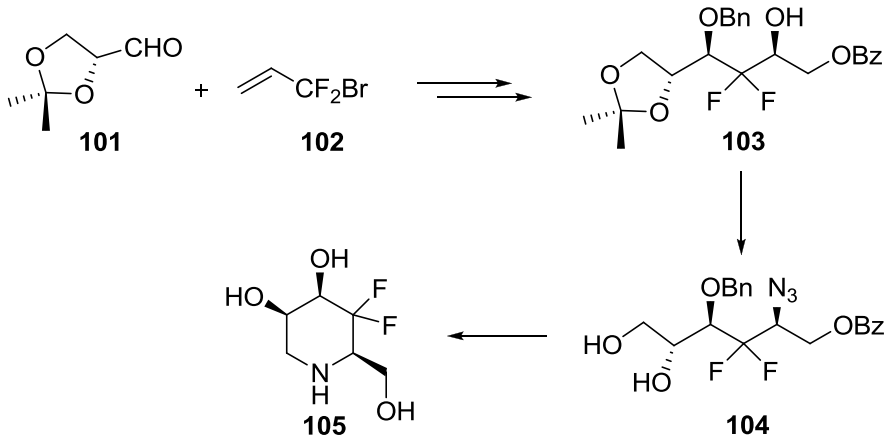


Figure 1.35: Synthesis of 1,4-dideoxy-4,4-difluoro-D-mannonojirimycin **105**, by Qing and co-workers.

[80] R.-W. Wang, X.-L. Qing, M. Bols, F. Ortega-Caballero, F.-L. Qing, *J Med. Chem* **2006**, *68*, 9026-9033.

1.7 Aim of the work

Iminosugars continue to be of great synthetic interest, due to the intrinsic pharmacological potential of many of them and the need for more active and selective compounds. Because of the difficulties encountered in their synthesis, the development of novel synthetic approaches remains a main challenge.

The first part of this work is aimed to investigate novel synthetic strategies for the synthesis of diversely functionalized analogues of iminosugars belonging to the class of pyrrolidine, pyrrolizidine and piperidine alkaloids. Indeed, versatile methodologies coupled with accessible biological screening of inhibitory activities, can provide important informations on the interactions with glycosidase active sites. All the presented methodologies are highly stereoselective and employ readily available starting materials.

In *Chapter 2*, methodologies well established in our group, which employ polyahydroxylated cyclic nitrones as key building blocks, were exploited for the synthesis of novel iminosugar-based trehalose mimetics and for further nitrogen functionalization of natural pyrrolidine-containing alkaloids.

In *Chapter 3*, a completely new synthetic route was developed for the preparation of diversely funtionalized trihydroxypiperidines, by employing a D-mannose derived dialdehyde as starting material. All the novel iminosugars compounds reported in these chapters were also tested on commercial glycosidases to evaluate a specific inhibitions (i.e. trehalase inhibitors) or to investigate the role played by different functionalization in the inhibitory activity.

Finally, at the light of outstanding results recently reported in the context of multivalent glycosidases inhibitors, we embarked on the multimerization of some of our iminosugars analogues, by employing different scaffolds. In particular, the immobilization of multiple copies of pyrrolizidine and piperidine iminosugars on gold glyconanoparticles was reported in *Chapter 4*.

Chapter 2

Synthesis of pyrrolidine-containing iminosugars: exploiting past experience for new functionalization

2.1 Introduction

Given the enormous therapeutic potential of iminosugars, the development of improved synthetic methodologies for their synthesis has become the objective of many synthetic chemists. In particular, among the four structural classes of iminosugars containing a five-membered ring, pyrrolidine and pyrrolizidine alkaloids hold a prominent role.The post 2005 developments in the synthesis of pyrrolidine containing iminosugars have been exhaustively reported by Timmer and co-workers in a recent Microreview, classifying the synthetic methodologies on the basis of their key steps.[81]

Our research group contributed to the synthesis of a variety of natural iminosugars and related structural analogues by preparing enantiopure five-membered ring cyclic nitrones starting from chiral pool building blocks, such as L-malic, D- and L-tartaric acids or D- and L-protected pentoses.

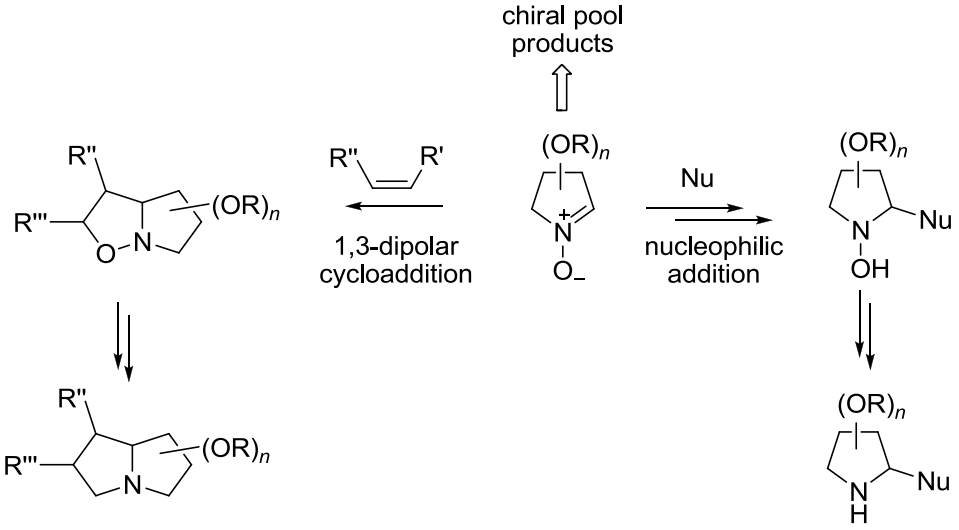


Figure 2.1: Dual synthetic strategy starting from polyhydroxylated enantiomerically pure cyclic nitrones.

[81] B. L. Stocker, E. M. Dangerfield, A. L. Win-Mason, G. W. Haslett, M.S.M. Timmer, *Eur. J. Org. Chem.* **2010**, 1615–1637.

The cyclic nitrones themselves proved to be versatile precursors for the synthesis of both pyrrolidine and pyrrolizidine iminosugars (Figure 2.1). In the former case these nitrones have been used as susbstrate for highly diastereoselective nucleophilic additions to afford enantiomerically pure polyhydroxylated pyrrolidines, after reduction of the hydroxylamine adducts. On the other hand, nitrones have been conveniently used in 1,3-dipolar cycloaddition reactions with different dipolarophiles for the synthesis of bicyclic pyrrolizidine alkaloids of the casuarine and hyacinthacine families.

2.1.1 Pyrrolidine Alkaloids

The addition of different reagents to five membered enantiopure cyclic nitrones **106, 107** and **70**[82,83] derived from L-malic acid and D-arabinose (Figure 2.2) has been investigate over the last decade, providing a straightforward approach to the synthesis of diversely substituted chiral pyrrolidines.

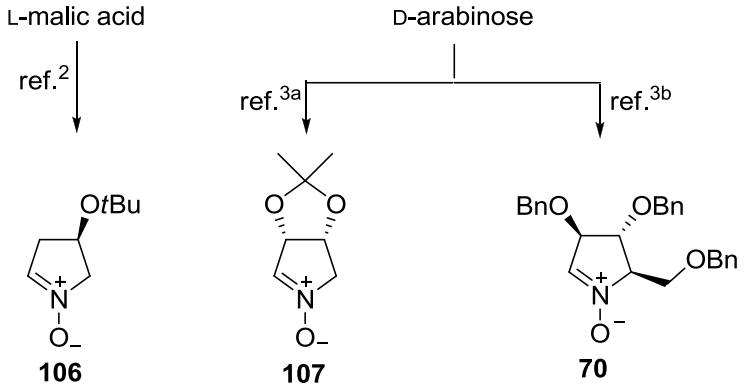


Figure 2.2: Five membered cyclic nitrones **106**, **107** and **70** derived from L-malic acid and D-arabinose.

As the starting point, C-2 epimers of 2-aminomethyl and 2-hydroxymethyl pyrrolidines were synthesised by the stereocontrolled addition of TMSCN or $LiCH_2OMOM$ to the nitrone **107**, easily accessible from D-arabinose, and subjection of the resulting hydroxylamine to an oxidation-reduction inversion sequence.[84] On the basis of these preliminary results a more general synthetic strategy was developed to access 2-substituted polyhydroxylated pyrrolidines with abundant configurational diversity.[85]

[82] a) S. Cicchi, A. Goti, A. Brandi, *J. Org. Chem.* **1995**, *60*, 4743–4748; b) R. Saladino, V. Neri, F.Cardona, A. Goti, *Adv. Synth.Catal.* **2004**, *346*, 639–647.
[83] a) S. Cicchi, M. Marradi, P. Vogel, A. Goti, *J. Org. Chem.* **2006**, *71*, 1614–1619; b) F. Cardona, E. Faggi, F. Liguori, M. Cacciarini, A. Goti, *Tetrahedron Lett.* **2003**, *44*, 2315–2318.
[84] M. Marradi, S. Cicchi, J. Ignacio Delso, L. Rosi, T. Tejero, P. Merino, A. Goti, *Tetrahedron Lett.* **2005**, *46*, 1287–1290.
[85] P. Merino, I. Delso, T. Tejero, F. Cardona, M. Marradi, E. Faggi, C. Parmeggiani, A. Goti, *Eur. J.Org. Chem.* **2008**, *17*, 2929–2947.

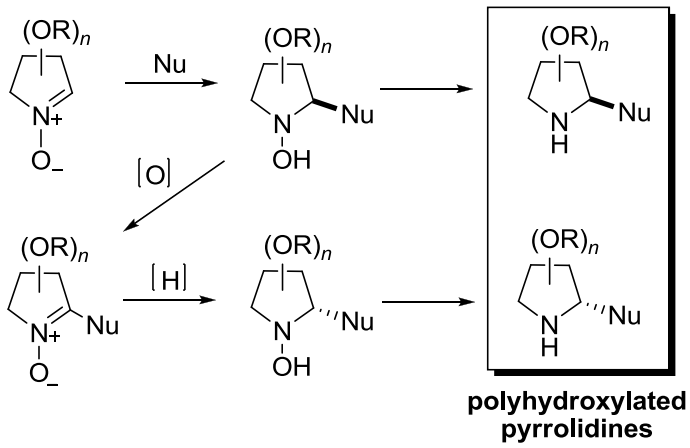


Figure 2.3: Synthetic strategy for the synthesis of polyhydroxylated pyrolidines

The adopted methodology, based on an oxidation/reduction protocol involving hydroxylamine/nitrone pairs (Figure 2.3) allowed access to DMDP, 6-deoxy-DMDP, DAB-1, CYB-3, nectrisine and radicamine B (Figure 2.4).

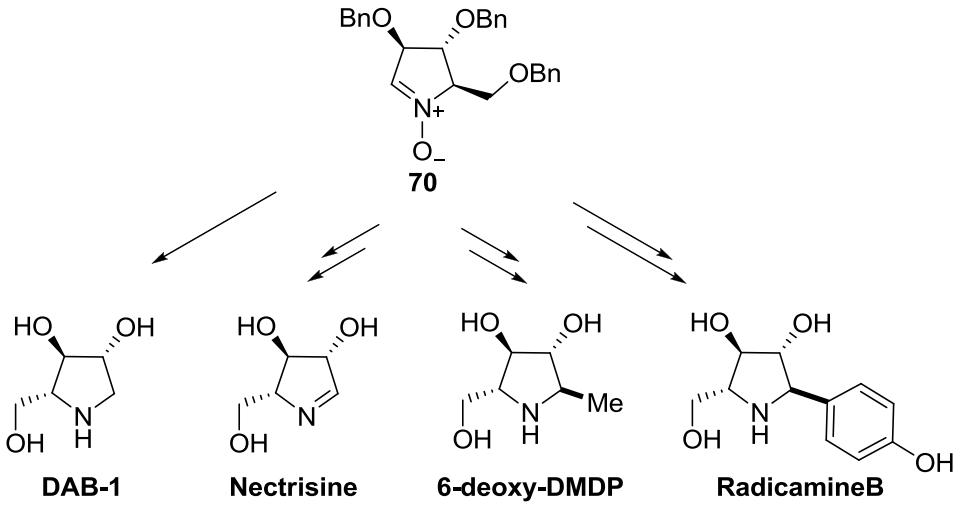


Figure 2.4: Synthesis of natural polyhyroxylateted pyrrolidine alkaloids from nitrone **70**.

Several analogues of these compounds with inverted configuration at one or more stereocenters were also prepared, demonstrating the use of reagent- and substrate-derived stereocontrol.

2.1.2 Pyrrolizidine Alkaloids

As depicted in Figure 2.5, nitrone **70** has also the correct stereochemistry of ring A of many natural pyrrolizidine alkaloids and could be easily prepared in four steps starting from D-arabinose. For this reason our group has employed this compound as starting material for the total synthesis of casuarine (**49**), hyacinthacine A_2 (**51**), (−)-uniflorine A (**64**) and their structural analogues, through the 1,3-dipolar cycloaddition strategy.

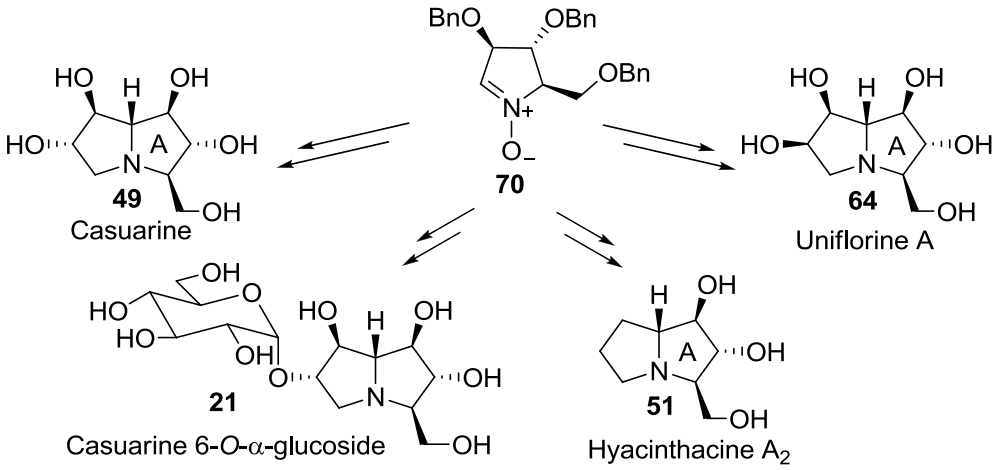


Figure 2.5: Synthesis of natural polyhyroxylateted pyrrolizidine alkaloids from nitrone **70**.

The general synthetic strategy followed for the synthesis of the these pyrrolizidine alkaloids is outlined in Figure 2.6. We took advantage of a stereocontrolled cyclic nitrone cycloaddition strategy[63] employing polyfunctionalized nitrone **70** and suitable (*Z*)−configurated dipolarophiles **108**, that afforded isoxazolidine adducts readily converted into pyrrolizidinone derivatives **110** by reductive ring opening/cyclization. Intermediates **110** bear a free hydroxyl group on C-6 of the pyrrolizidine ring, thus allowing selective glucosylation in this position. A good choice of the dipolarophile is crucial for the success of the strategy.

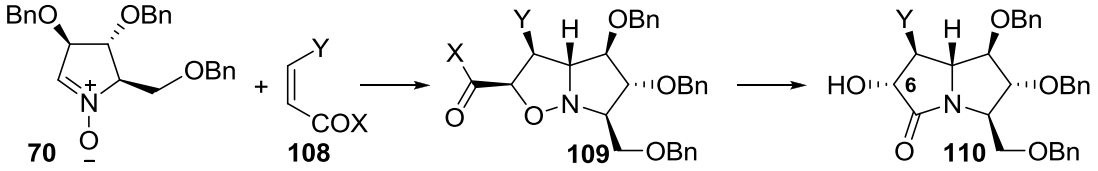


Figure 2.6: Synthetic strategy for the synthesis of pyrrolizidines

The most preferred approach of alkenes to nitrone **70** occurs through *exo-anti* transition states (Figure 2.7).

Indeed, the stereoselectivity of the cycloaddition is in general controlled by the C-3 stereocenter of the five-membered nitrone. An approach of the dipolarophile *anti* to the alkoxy group at C-3 is by far the most preferred at the transition state over the other possible *syn* approach. The extent of diastereoselectivity is accordingly influenced by the bulkiness of the alkoxy group. The *exo* approach of the dipolarophile is also the preferred pathway; the ratio again depends on the bulkiness and nature of the protecting group.

The characteristic feature of the process is the transfer of stereochemical information from the nitrone stereocenter to the newly formed stereocenters in the cycloadduct, with the bridgehead H assuming a cis-relationship with respect to the vicinal alkoxy group.

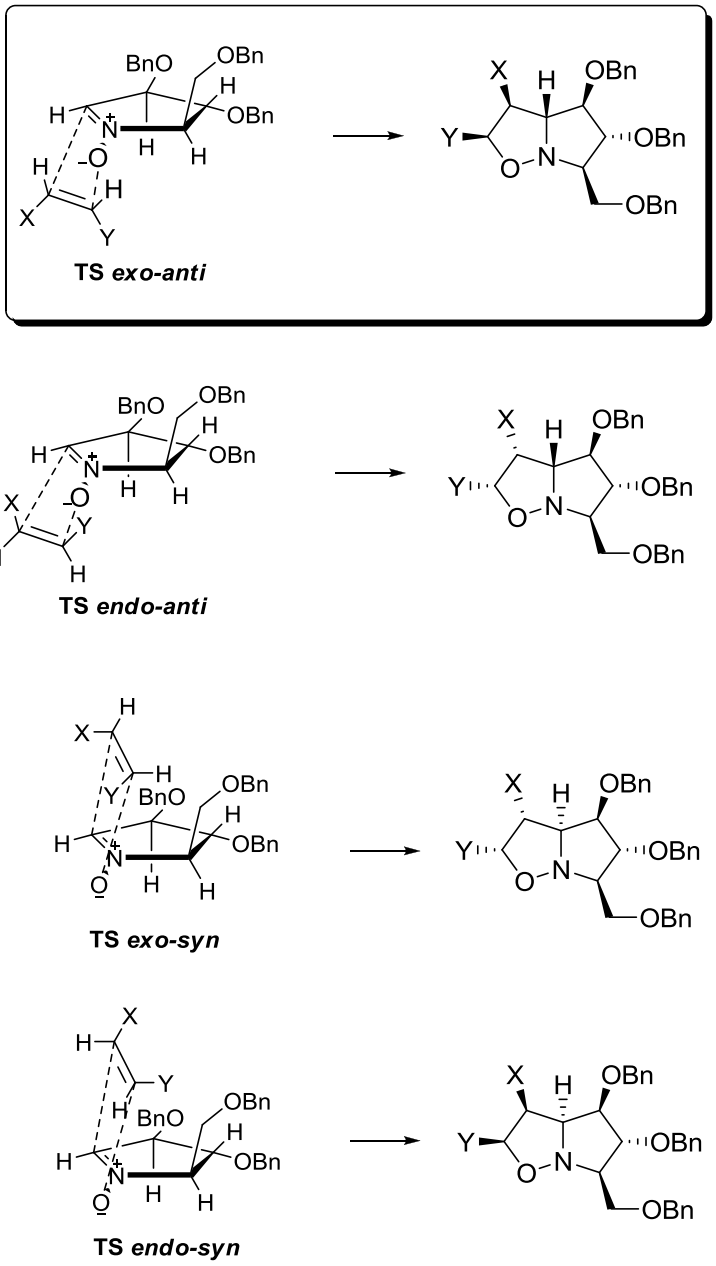


Figure 2.7: Favorite transition state of cycloaddition

In the case of nitrone **70**, the substituents at C-3 and C-5 allow to obtain exclusively *anti* cycloadducts and the steric bulk of substituent at C-4 ensures an excellent *exo* selectivity. The complete stereoselectivity of the 1,3-dipolar cycloaddition, which allowed the selective installation of the three new stereocenters (corresponding to C-6, C-7 and C-7a in the target molecules) in one step with the required configuration, may be ascribed to the peculiar all-*trans* disposition of the benzyloxy groups in nitrone **70**, which hampered any *endo* or *syn* approaches.[83b,86]

[86] a) J. Revuelta; S. Cicch.; A. Goti; A. Brandi, *Synthesis* **2007**, *4*, 485-504; b) A. T. Cramona,; R. H. Wightman, I. Robina; P. Vogel, *Helv. Chim. Acta* **2003**, *86*, 3066-3073; c) S. Desvergnes; S. Py; Y. Vallée, *J. Org. Chem.* **2005**, *70*, 1459-1462.

The 1,3-dipolar cycloaddition of nitrone **70** to dimethylacryl amide **111** was the first key-step for the synthesis of hyacintacine A_2 (**51**) and 7-deoxycasuarine **114** (Figure 2.8).[83b,87] The cycloaddition reaction gave regio- and stereo-selectively isoxazolidine **112** in very good yield and the subsequent N-O bond cleavage afforded lactam **113**, which was then converted into 7-deoxycasuarine **114**, through reduction and catalytic hydrogenation.

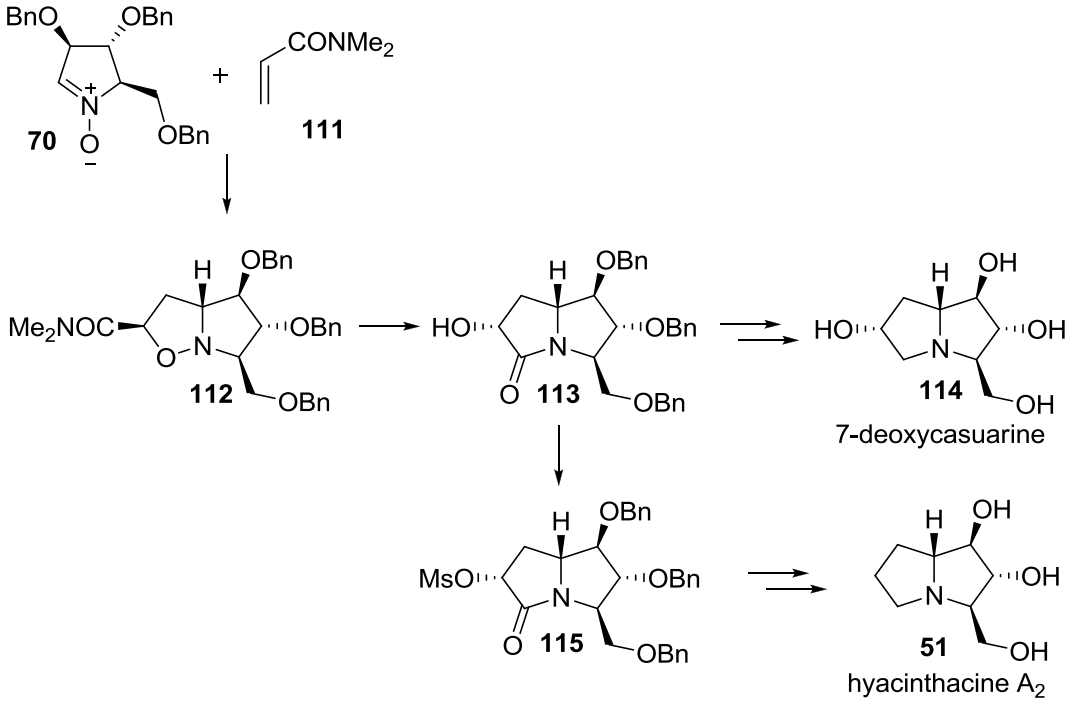


Figure 2.8: Synthesis of 7-deoxycasuarine (**114**) and hyacintacine A_2 (**51**).

Deoxygenation at C-6, necessary to afford hyacinthacine A_2 (**51**), was obtained by mesylation of intermediate **113** followed by treatment of mesylate **115** with $LiAlH_4$ in refluxing THF. Finally catalytic hydrogenation allowed access to natural hyacinthacine A_2.

The same methodology, with the introduction of a further hydroxy group at C-7, allowed an efficient synthesis of casuarine and its 6-*O*-α-D-glucoside **21**. In this case, in order to obtain the proper regioselectivity, dipolarophile **116** was chosen for the cycloaddition reaction (Figure 2.9).[87,88] The 1,3 dipolar cycloaddition reaction of **70** with acrylate **116** provided the isoxazolidine **117** with complete stereoselectivity and excellent regioselectivity. Reduction of the alkoxyamine in **117** led to spontaneous cyclisation into lactam **118**. Finally, oxidative cleavage of the silane and reduction of the carbonyl gave diol **120**, which, following deprotection, yielded casuarine (**49**). The binding of casuarine and casuarine 6-*O*-α-D-

[87] C. Bonaccini, M. Chiocchioli, C. Parmeggiani, F. Cardona, D. Lo Re, G. Soldaini, P. Vogel, C. Bello, A. Goti, P. Gratteri, *Eur. J. Org. Chem.* **2010**, 5574-5585.
[88] F. Cardona, C. Parmeggiani, E. Faggi, C. Bonaccini, P. Gratteri, L. Sim, T. M. Gloster, S. Roberts, G. J. Davies, D. R. Rose, A. Goti, *Chem. Eur. J.* **2009**, *15*, 1627–1636.

glucoside **21** to glucoamylase NtMGAM and trehalase Tre37A, respectively, was then investigated and revealed interesting similarities in the catalytic sites of these two enzymes.[88]

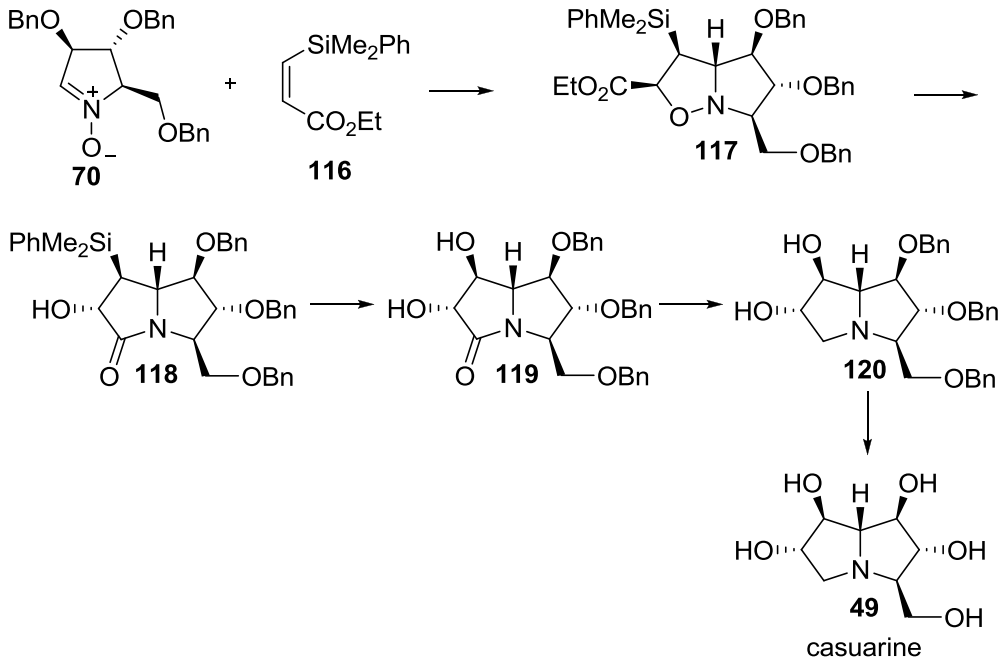


Figure 2.9: Synthesis of casuarine (**49**).

By manipulating isoxazolidine **117** in a different way, the total synthesis of (−)-uniflorine A (**64**) was also attained,[89] providing evidence that (−)-uniflorine A is 6-*epi*casuarine, as previously proposed by Pyne and co-workers.[58]

In this case, the synthesis proceeded via inversion of configuration of the OH group at C-6 achieved through a Mitsunobu reaction with benzoic acid on the key intermediate **121** (Figure 2.10), previously synthesized by *in situ* protection and Tamao-Fleming reaction on isoxazolidine **117.**

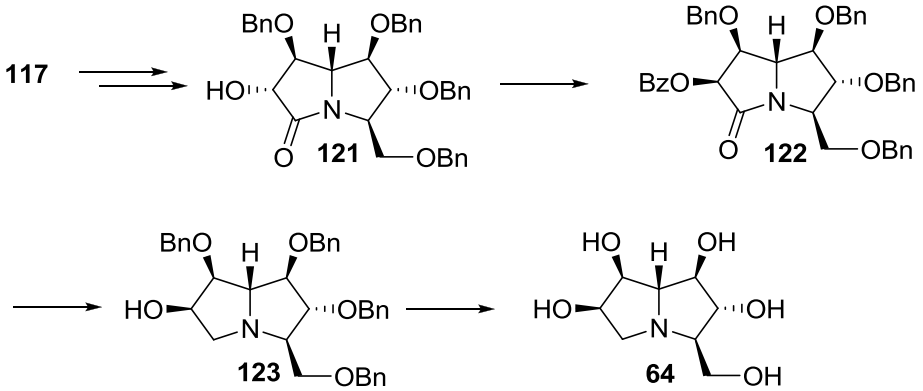


Figure 2.10: Synthesis of (−)-uniflorine A (**64**).

[89] C. Parmeggiani, D. Martella, F. Cardona, A. Goti, *J. Nat. Prod.* **2009**, *72*, 2058–2060.

More recently, the total synthesis of natural (+)-hyacinthacine A_1 **124**, (+)-7a-*epi* hyacinthacine A_1 **125** and their 6-hydroxy analogues was achieved[90] using the same nitrone cycloaddition strategy, but employing the parent D-ribose-derived cyclic nitrone **126**[91] as the dipole and *tert*-butyl acrylate **127** as the dipolarophile.

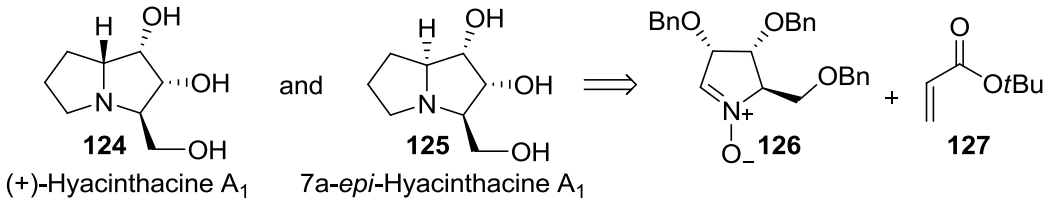


Figure 2. 11: Nitrone **126** as the key building block for the synthesis of (+)-hyacinthacine A_1 (**124**) and 7a-*epi*-hyacinthacine A1(**125**)**.**

All the reported examples highlight how the success of our strategies mainly depends on a practical access to enantiomerically pure nitrones, whose synthesis remains one of the primary goals of our group. The most widely used nitrone **70** has been obtained through *N*-alkylation of the tribenzyl D-arabinose derived oxime, with a double inversion of configuration (Figure 2.12).

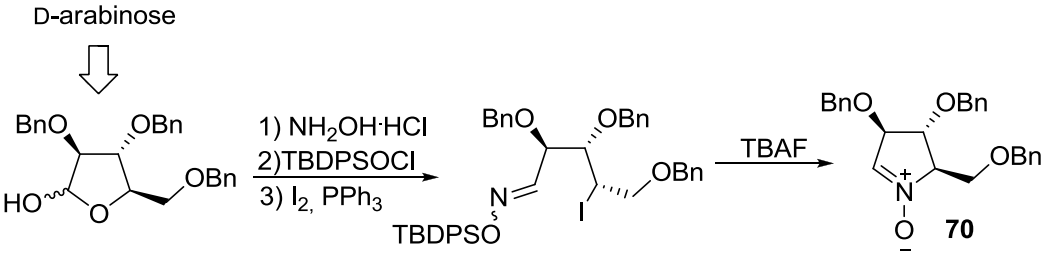


Figure 2. 12: Synthesis of nitrone **70**, starting from tribenzyl D-arabinose

Although this strategy resulted very efficient and versatile (parent nitrones, such as **126** could be obtained by employing different starting sugar derivatives), many other methods for the synthesis of polyfunctionalized nitrones are described in the literature.[86a] Among them, oxidative methods have recently received a renovated interest in order to develop environmentally friendly synthetic strategies, which avoid the use of toxic oxidants and prefer metal-catalyzed processes in which O_2 or air are used as oxidants and the catalyst could be recovered and reused.

[90] G. D'Adamio, A. Goti, C. Parmeggiani, E. Moreno-Clavijo, I. Robina, F. Cardona *Eur. J. Org. Chem.* **2011**, 7155–7162.
[91] E.-L. Tsou, Y.-T. Yeh, P.-H. Liang, W.-C. Cheng, *Tetrahedron* **2009**, *65*, 93–100.

In this context we envisaged in hypervalent iodine(V) reagents, extensively used for the oxidation of alcohols, potentially good candidates to develop a metal-free oxidation strategy to obtain nitrones from hydroxylamines, in a regio- and stereoselective manner.

2.2 Synthesis of nitrones through metal-free oxidation of hydroxylamines with hypervalent iodine reagents.

Cyclic hypervalent iodine reagents (Figure 2.13), such as Dess-Martin periodinane (DMP), 2-iodoxybenzoic acid (IBX) and diacetoxyiodobenzene (DIB) have broad applications in a number of oxidation processes.[92] However, to the best of our knowledge, the use of cyclic hypervalent iodine reagents for the synthesis of nitrones has no precedent in the literature.

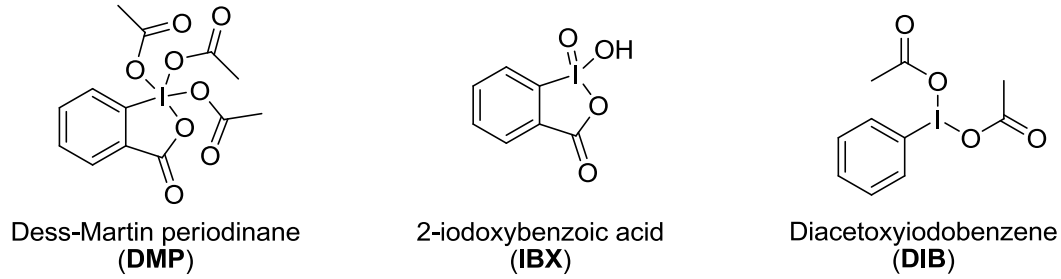


Figure 2.13: Structures of hypervalent iodine reagents employed in our study. Dess-Martin periodinane (DMP) and 2-iodobenzoic acid (IBX) belong to iodine (V) compounds while diacetoxyiodobenzene (DIB) is a iodine (III) compound.

As a first attempt we studied the feasibility of the oxidation of commercial or readily available symmetric hydroxylamines, by screening the three different reagents reported in Figure 2.13. Since 1991,[93] DMP has emerged as the reagent of choice for the oxidation of primary and secondary alcohols to aldehydes and ketones, respectively. The mild reaction conditions (room temperature and neutral pH), high chemoselectivity, and convenience of use have made this reagent especially suitable for the oxidation of substrates containing sensitive functional groups.[94] Moreover an example of oxidation of hydroxamic acids, *N*-hydroxyureas and *N*-hydroxycarbamates with the Dess-Martin periodinane to generate the corresponding acyl nitroso compounds has been reported.[95]

---

[92] a) T. Wirth, *Angew. Chem. Int. Ed*. **2001**, 40, 2812-2814; b) V. V. Zhdankin, P. J. Stang., *Chem. Rev*. **2002**, 102, 2523-2584; c) U. Ladziata, V. V. Zhdankin, *Synlett* **2007**, 527-537; d) *V. Satam*, A. Harad, R. Rajule, H. Pati, *Tetrahedron **2010**, 66, 7659-7706*; e) V. V. Zhdankin, J. Org. Chem 2011, 76, 1185-1197; f) *R. Bernini*, G. Fabrizi, L. Pouységu, D. Deffieux, S. Quideau, *Curr. Org. Synth. **2012**, 9, 650-669.*

[93] D. B. Dess, J. C. Martin, *J. Am. Chem. Soc*. **1991**, 113, 7277-7287.

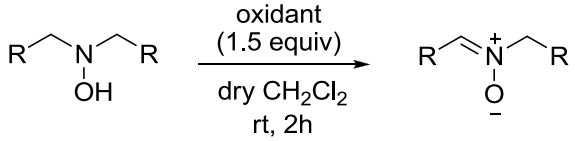
[94] A. Speicher, V. Bomm, T. Eicher, *J. Prakt. Chem.* **1996**, *338*, 588-590; b) S. S. Chaudhari, *Synlett* **2000**, 278-278.

[95] N. E. Jenkins, R. W. Ware Jr., R. N. Atkinson, S.B. King, *Synthetic Commun*., **2000**, 30, 947-953.

Although synthesized by Hartmann and Meyer[96] more than 100 years ago, IBX has attracted increasing interest as mild and selective oxidizing reagent only in recent years. The synthetic applications of IBX have been summarized in an excellent review by Duschek and Kirsch,[97] where the great versatility of this reagent was underlined. In particular, the oxidation of two hydroxylamines to the corresponding oximes, reported by Nicolaou and co-workers[98] resulted of great interest to our purpose.

Despite its low reactivity towards alcohols,[92b] we decided to include also DIB in our study since it resulted much more accessible (ten times cheaper respect to IBX) and stable than DMP and IBX.

The following general procedure was followed for the oxidation reactions in order to compare the effectiveness of the three reagents: the hydroxylamine was treated with 1.5 equivalents of oxidation reagent in dry dichloromethane (0.09 M) at room temperature for two hours. The results with hydroxylamines **128-130** are reported in Table 2.1.



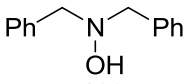
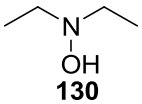
Entry	Substrates	Nitrone yield %[a]		
		DMP	IBX	DIB
1	 **128**	67%	96%[b]	77%
2	 **129**	75%	88%	79%
3	 **130**	71%	96%	72%

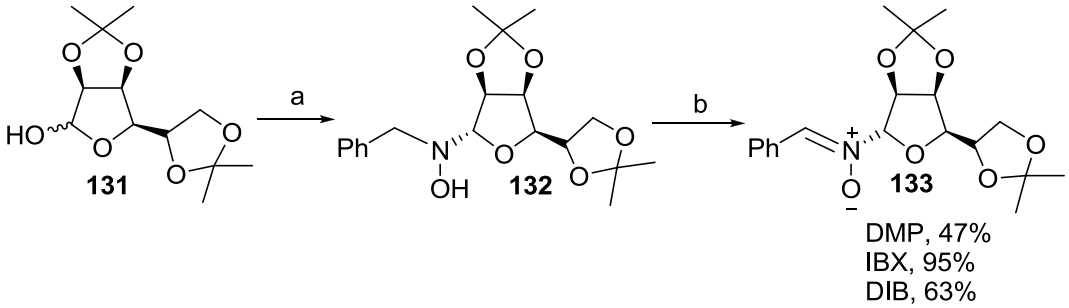
Table 2.1: [a] Isolated yield after flash column chromatography; [b] Yield of the crude, >95% purity as evaluated by ^{1}H NMR spectroscopy.

The reaction mixture was subsequently washed several times with a saturated solution of Na_2CO_3 and the crude nitrones purified by flash chromatography. These preliminary data,

[96] C. Hartmann, V. Meyer, *Ber. Dtsch. Chem. Ges.* **1893**, 26, 1354-1370.
[97] F. *Kirsch,* A. *Duschek, Angew*. *Chem*. *Int*. *Ed.* ***2011****, 50, 1524* –1552.
[98] K. C. Nicolaou, C. J. N. Mathison, T. Montagnon, *J. Am. Chem. Soc.* **2004**, 126, 5192-5201.

summarized in Table 2.1, proved that cyclic hypervalent iodine reagents are able to oxidize hydroxylamines to nitrones with very good to excellent yields. The best results have been obtained with IBX, which in a particular case (Table 2.1 entry 1) allowed to access the corresponding nitrone in 96% yield after basic quench, without any further purification. Interestingly, DIB provided similar or even better results with respect to DMP, suggesting that the more accessible DIB could be easily employed for routinary oxidations.

These encouraging results prompted us to test the protocol on more complex and challenging non symmetric substrates, in order to evaluate the regioselectivity of the reaction for the three different oxidation agents. The chiral hydroxylamine **132** was thus synthesized starting from D-mannose derivative **131** (Scheme 2.1), modifying the protocol usually employed in our group,[99] to avoid the use of pyridine as solvent. Under these cleaner conditions, the desired hydroxylamine was obtained with a higher yield (96% vs 73%) and purification by flash column chromatography resulted unnecessary. The following oxidation to nitrone, occurred cleanly and with excellent yield only with IBX. The oxidation reaction with DIB and DMP, although the formation of the less favored nitrones was not observed, afforded nitrone **133** with lower yields after column chromatography purification (Scheme 2.1).

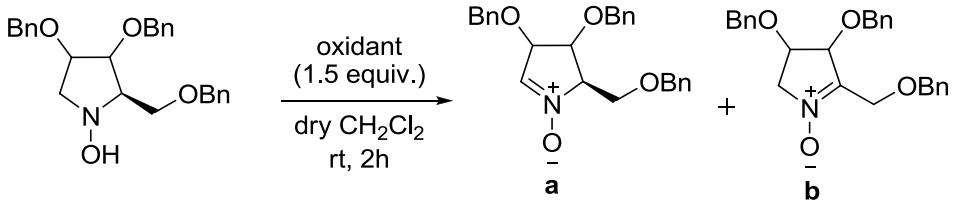


Scheme 2.1: Synthesis of chiral hydroxylamine **zb5** and subsequent oxidation with hypervalent iodine reagents DMP, IBX and DIB. Reagents and conditions: a) $BnNH_2OH{\cdot}HCl$, Na_2SO_4, NEt_3, CH_2Cl_2, rt, 3 days, 96%; b) oxidant (1.5 equiv.), CH_2Cl_2, rt, 2h.

We then extended our study to a panel of non symmetric hydroxylamines, available in our laboratory for different applications, ie, cyclic hydroxylamines **134-136** derived from tribenzyl pentoses, which possess the configuration of D-xylose, D-ribose and D-arabinose, respectively. With these hydroxylamines, the formation of aldo-nitrones **a** should be favored by statistic and steric effects; on the other hand, formation of keto-nitrones **b** should be favored on a thermodynamic ground. The results obtained with IBX (Table 2.2) agree with the theoretical prediction based on steric considerations in all of three cases, since the reactions afforded selectively aldo-nitrones **a** in very good yields. In particular

[99] S. Cicchi, M. Marradi, M. Corsi, C. Faggi, A. Goti, *Eur. J. Org. Chem*. **2003**, 4152-4160.

with hydroxylamine **135** (Table 2.2, entry 2), the oxidation occurred in a completely regioselective manner and only the nitrone **a** was observed.



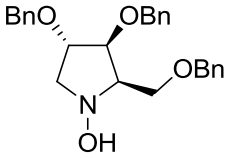
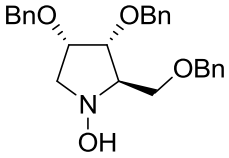
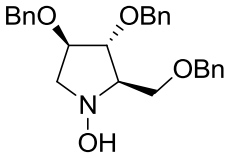
Entry	Substrates	a : b ratio (total yield %)		
		DMP	IBX[a)]	DIB
1	 **134**	< 5 : 95 [b)] (8%)	6 : 1[c)] (100%)	3.5: 1[b)] (36%)
2	 **135**	2 : 1 (42%)	> 95 : 5 [c)] (94%)	1.1 : 1 [b)] (38%)
3	 **136**	1 : 1 [c)] (35%)	5 : 1 [c)] (89%)	1 : 1.2 [c)] (44%)

Table 2.2: a) Yield of the crude, > 95% purity as evaluated by ^{1}H NMR spectroscopy; b) a:b ratio determined on the basis of isolated yields after flash column chromatography; c) a:b ratio determined by ^{1}H NMR spectroscopic analysis of the crude mixture.

Conversely, both DMP and DIB have proved to be poorly regio- and chemo-selective reagents for oxidation of this hydroxylamine class: almost equal mixture of aldo- and keto-nitrones and very low yields were generally observed. Moreover, in case of compound **134** (Table 2.2, entry 1) a completely inverted selectivity was observed for DMP with respect to

IBX and DIB, since only keto-nitrone **b** was isolated, however only in 8% yield. An attempt to increase the selectivity in the oxidation of **136**, performing the reaction at -20 °C and slowly raising the temperature to 10 °C during 2 hours, was unsuccessful, since these conditions affected negatively both the conversion and the regioisomeric ratio, leading to only 33% conversion and 4.2:1 **a:b** ratio.

These preliminary results show that IBX is a superior reagent among the screened hypervalent iodine reagents, and can be efficiently employed for the regioselective oxidation of non symmetric hydroxylamines.

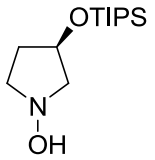
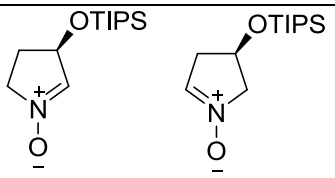
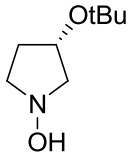
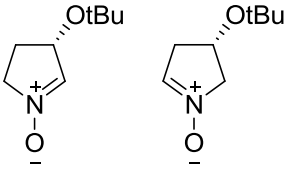
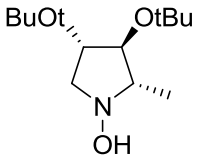
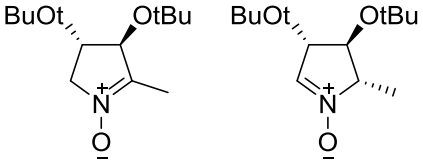
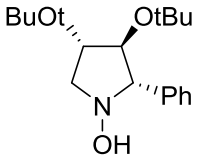
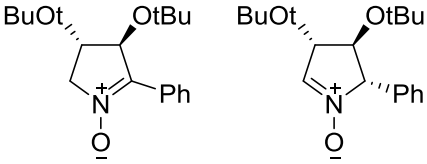
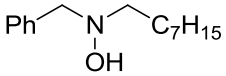
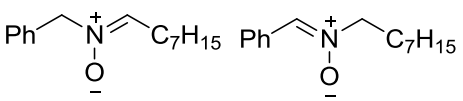
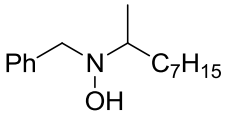
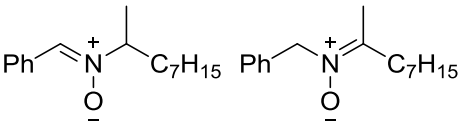
Entry	Substrate	Products	ratio	yield
1			3.7 : 1	92%
2			6 : 1	38%
4			1 : 4.4	100%
5			1 : 4.2	91%
6			1 : 1	100%
7			69 : 1	73%

Table 2.3: Preliminary results for the oxidation of different hydroxylamines with IBX. For reaction conditions see Table 2.2.

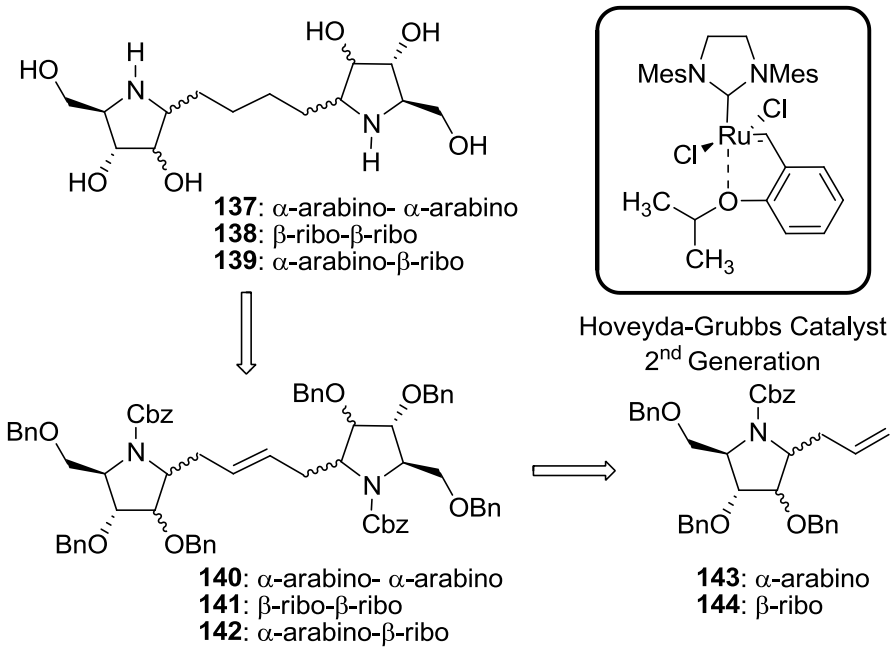
For this reason, other hydroxylamines, differently substituted, cyclic and acyclic, have been prepared in our laboratories and evaluated as substrates for the oxidation with IBX. We carried out the oxidation of different hydroxylamines, as reported in Table 2.3, with IBX under the optimal conditions (room temperature, 2 h). The regioisomeric ratios and yields were determined by ^{1}H NMR spectroscopic analysis of the crude mixtures. The selectivity of oxidation strongly depends on the substituents position. Selectivity among differently substituted methylene groups in acyclic hydroxylamines is null (entry 6). In case of 3-alkoxy substituted *N*-hydroxypyrrolidines (entries 1 and 2) the abstraction of the vicinal proton is favored, but selectivity is significantly lower compared to other oxidants, such as MnO_2. However, when a further substituent is present in the α-position the aldo-nitrone is the major product, independently on the steric hindrance and the electronic effects of the substituent (entry 4 vs entry 5). Remarkably, with substrate in entry 5 we found a good enhancement of the selectivity with respect to the use of MnO_2, previously reported for the oxidation of the same substrate.[100] Magnification of this effect was obtained with the acyclic hydroxylamine in entry 7, which gave an impressive 69:1 selectivity in favor of the less hindered aldo-nitrone. These preliminary results demonstrate the potential of polyvalent iodine reagents to replace toxic transition metal reagents for this transformation, an hot topic in synthesis which is continuing to generate enormous interest.

2.3 Synthesis of novel iminosugar-based trehalase inhibitors by cross metathesis reactions

Following our ongoing interest in the synthesis of trehalase inhibitors for agrochemical applications and due to the reported activity against trehalases of some pyrrolidine iminosugars such as **DAB-1** (**5**), we envisaged the possibility to synthesize novel trehalase inhibitors containing pyrrolidine iminosugars derived from cyclic nitrones **70** and **126**. As briefly described in *Chapter 1*, being trehalose a disaccharide, the most potent trehalase inhibitors reported to date are pseudodisaccharides. Thus, in order to mimic the disaccharidic structure, two iminosugars units could be linked by a short and flexible alkyl bridge. In particular, the Grubbs Ru-carbene-catalyzed cross-metathesis (CM) reaction[101]

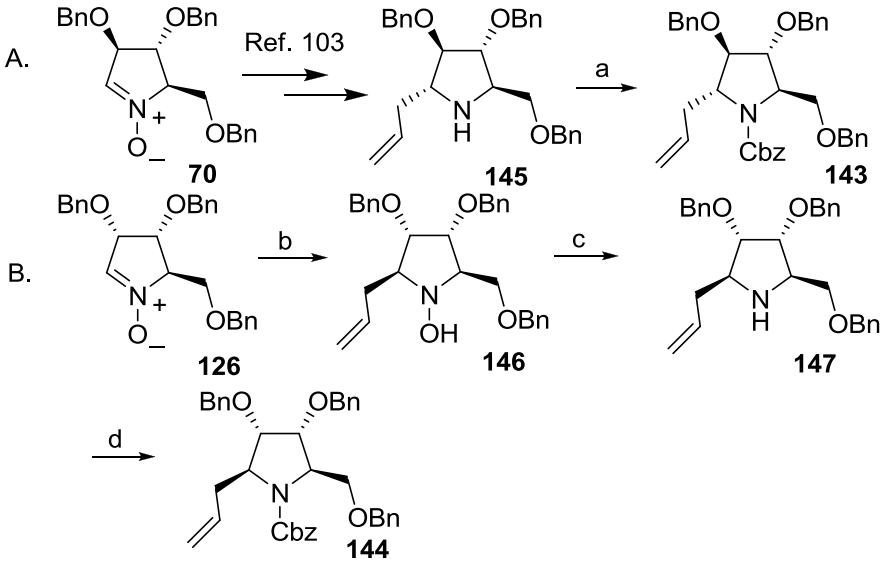
[100] A. Goti, S. Cicchi, V. Mannucci, F. Cardona, F. Guarna, P. Merino, T Tejero, *Org. Lett.* **2003,** 5, 4235-4238.
[101] See, for examples: a) D. V. Jarikote, P. V. Murphy, *Eur. J. Org. Chem*. **2010**, 4959–4970; b) R. H. Grubbs, *Tetrahedron* **2004**, *60*, 7117–7140.

was used to connect the two moieties suitably functionalised with a double bond as outlined in the retrosynthetic scheme (Scheme 2.2).[102]



Scheme 2.2: Retrosynthetic scheme for the synthesis of trehalase inhibitors.

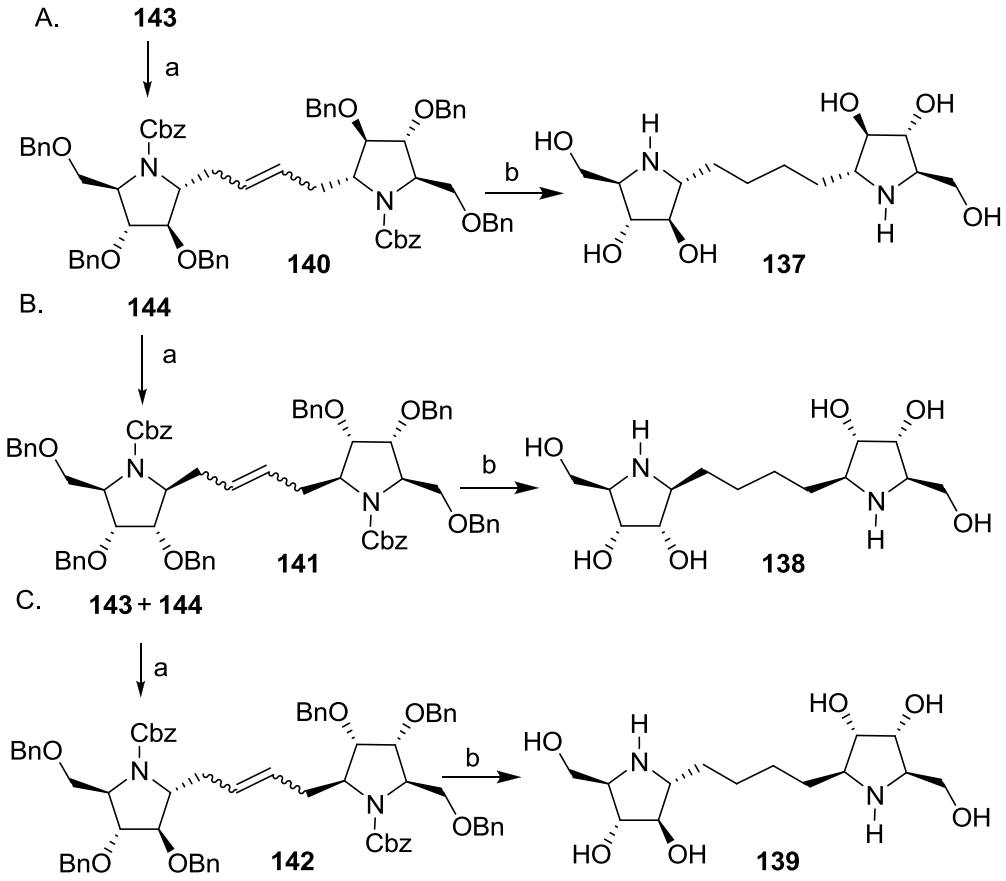
Two different allyl-iminofuranoses were synthesised and used for the CM reaction (Scheme 2.3), possessing a 1,4-dideoxy-1,4-imino-D-arabinitol or a 1,4-dideoxy-1,4-imino-D-ribitol *core,* respectively, and with the endocyclic nitrogen atom protected as a carbamate.



Scheme 2.3: Synthesis of the pyrrolidine scaffolds. Reagents and conditions*:* a) CbzCl, H_2O/dioxane, $NaHCO_3$, rt, 16 h, 80%; b) Allylmagnesium bromide, THF, 0 °C, 3h, quant. yield; c) Zn, AcOH/H_2O, rt, 40 min., 80%; d) CbzCl, H_2O/dioxane, $NaHCO_3$, rt, 20 h, 79%.

[102] D. Bini, M. Forcella, L. Cipolla, P. Fusi, C. Matassini and F. Cardona *Eur. J. Org. Chem*., **2011**, 3995-4000**.**

The synthesis of the α-C-allyl iminoarabinofuranosyl derivative **143** (Scheme 2.3A) proceeded via Grignard addition to nitrone **70**, as recently described.[103] Final carbamoylation of intermediate **145** by Cbz-chloride in water/dioxane[104] afforded **143** (80%).[103] On the contrary, the synthesis of the β-C-allyl iminoribofuranosyl derivative, which had not precedents in literature, was obtained starting from nitrone **126**,[91] as illustrated in Scheme 2.3B. The Grignard reaction on nitrone **126** occurred with total stereoselectively and only the *anti* isomer was formed, according to the expected stereochemical outcome; reduction of hydroxylamine **146** to the corresponding amine **147**, followed by carbamoylation afforded the β-C-allyl iminoribofuranosyl derivative **144** suitable to be employed in the CM reaction. The two scaffolds were then used for homodimerisation and heterodimerisation reaction by CM, as outlined in Scheme 2.4.



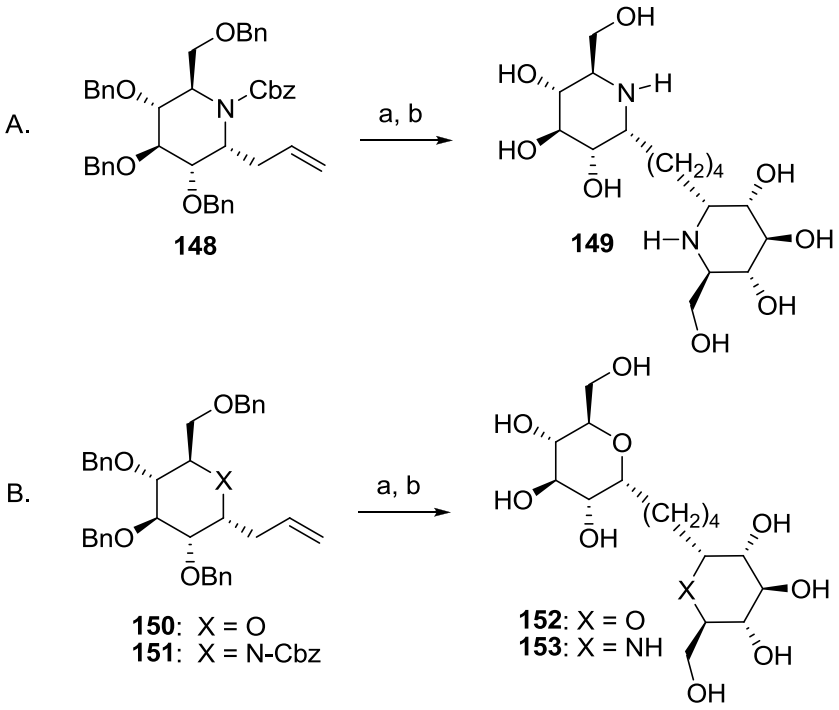
Scheme 2.4: Dimerisation of pyrrolidine structures by cross-metathesis. Reagents and conditions: a) Hoveyda-Grubbs Catalyst 2nd Generation, DCM, rt, overnight; b) $Pd(OH)_2/C$, H_2, EtOAc/EtOH 1:1.

The cross metathesis reactions were performed in anhydrous CH_2Cl_2 at room temperature, employing Grubbs 1,3-dimesityl-4,5-dihydroimidazol-2-ylideneruthenium carbene (Hoveyda-Grubbs Catalyst 2nd Generation, 5% in weight) and afforded dimers **140**, **141** and

[103] I. Delso, T. Tejero, A. Goti, P. Merino, *Tetrahedron* **2010**, *66*, 1220-1227.
[104] F. Sladojevich, A. Trabocchi, A. Guarna, *Org. Biomol. Chem*. **2008**, *6*, 3328-3333.

142 in 54%, 42% and 12% yields, respectively. Actually, the lower yield of heterodimerisation was expected due to the typical formation of homodimerization and starting material by-products. Finally, hydrogenolysis afforded deprotected compounds **137**, **138** and **139**.

Thanks to a collaboration with Prof. Laura Cipolla group (University of Milan-Bicocca), we had the opportunity to synthesize also the corresponding iminopiranose homodimer with a nojirimycin *core* (**149**, Scheme 2.5A), by following the same protocol. Moreover, in order to assess the relevance of the nitrogen atom in the inhibition assays with iminosugar-based trehalase inhibitors, the homodimer of the α-*C*-allyl glucoside **152** and the heterodimer containing one nojirimycin unit and one glucose unit **153** were synthesised (Scheme 2.5B).



Scheme 2.5: A) Homodimerization of nojirimycin derivative. B) Homo and heterodimerization of glucose derivative. Reagents and conditions: a) Hoveyda-Grubbs Catalyst 2nd Generation, DCM, rt, overnight; b) $Pd(OH)_2/C$, H_2, EtOAc/EtOH 1:1.

All the deprotected compounds (i.e. **137**-**139, 149**, **152, 153**,) were tested for their inhibitory activity against porcine trehalase. To examine the potential of each member of the library as trehalase inhibitor, preliminary screening assays at a fixed concentration (1 mM) of potential inhibitors was carried out, and dose-response curves were established for most active compounds in order to determine the Ki values. Experiments were performed at fixed substrate concentration, close to the K_m value (2.5 mM), in the presence of increasing inhibitor concentrations. The inhibitory activity is shown as a percentage at the fixed concentration in Figure 2.14.

Compound **149**, that is a nojirimycin dimer, resulted to be the most active derivative of the series, showing a K_i = 44 μM. At 1 mM concentration compounds **137** ,**138**, **139** and **153** showed, 14%, 28%, 43% and 25%of inhibition, respectively, with respect to the control in the absence of inhibitor. On the other hand, reference glucose dimer **152** did not show any inhibition at 1 mM concentration.

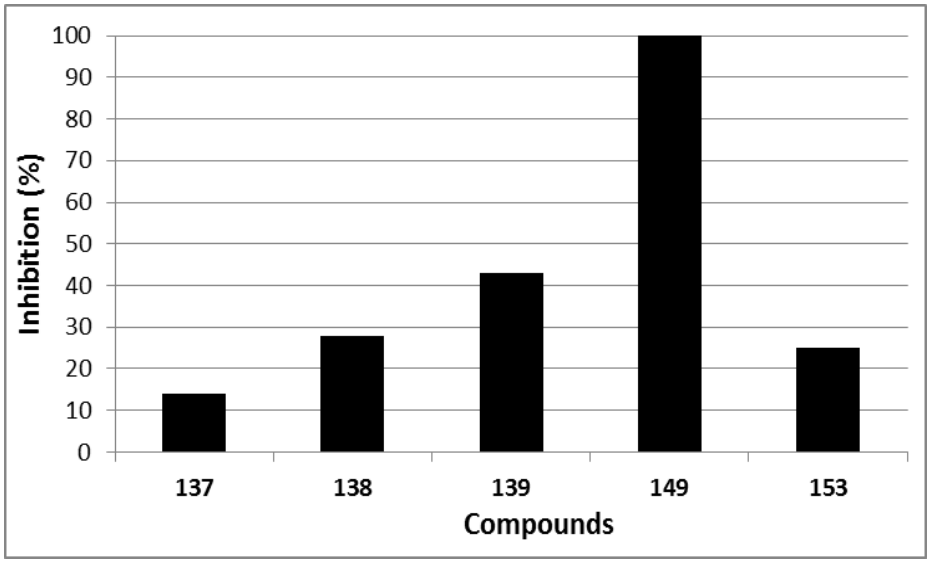


Figure 2.14: Inhibition data of the synthesised compounds **137**, **138**, **139**, **149** and **153** against porcine trehalase observed at 1 mM inhibitor concentration.

2.4 Functionalization of pyrrolidine-containing iminosugars for the construction of multivalent systems

The concept of multivalency, essentially unexplored concerning specific glycosidase inhibition using iminosugar as glycomimetics up to 2010, has recently received a newly born interest from the scientific community (see *Chapter 4.1* for a more detailed discussion). The ensemble of reported results highlights the importance of building new and diversified multivalent iminosugars in the search for more selective glycosidase inhibitors. Following our interest in this field, we have recently started exploring the multimerization of our biologically active iminosugars by employing as scaffolds both dendrmeric alkynes and gold nanoparticles.In the former case the functionalization of imosugars with an azido moiety was required to perform Cu^{I}-catalyzed azide-alkyne cycloadditions (CuAAC), while in the second case the presence of an amino group was necessary for further functionalization with suitable linkers in order to attach the molecules to gold nanoparticles. We reasoned that the best strategy to pursue was the synthesis of azido derivatives, which in turn could

be converted into the corresponding amino derivatives, through a simple reduction reaction (Figure 2.15).

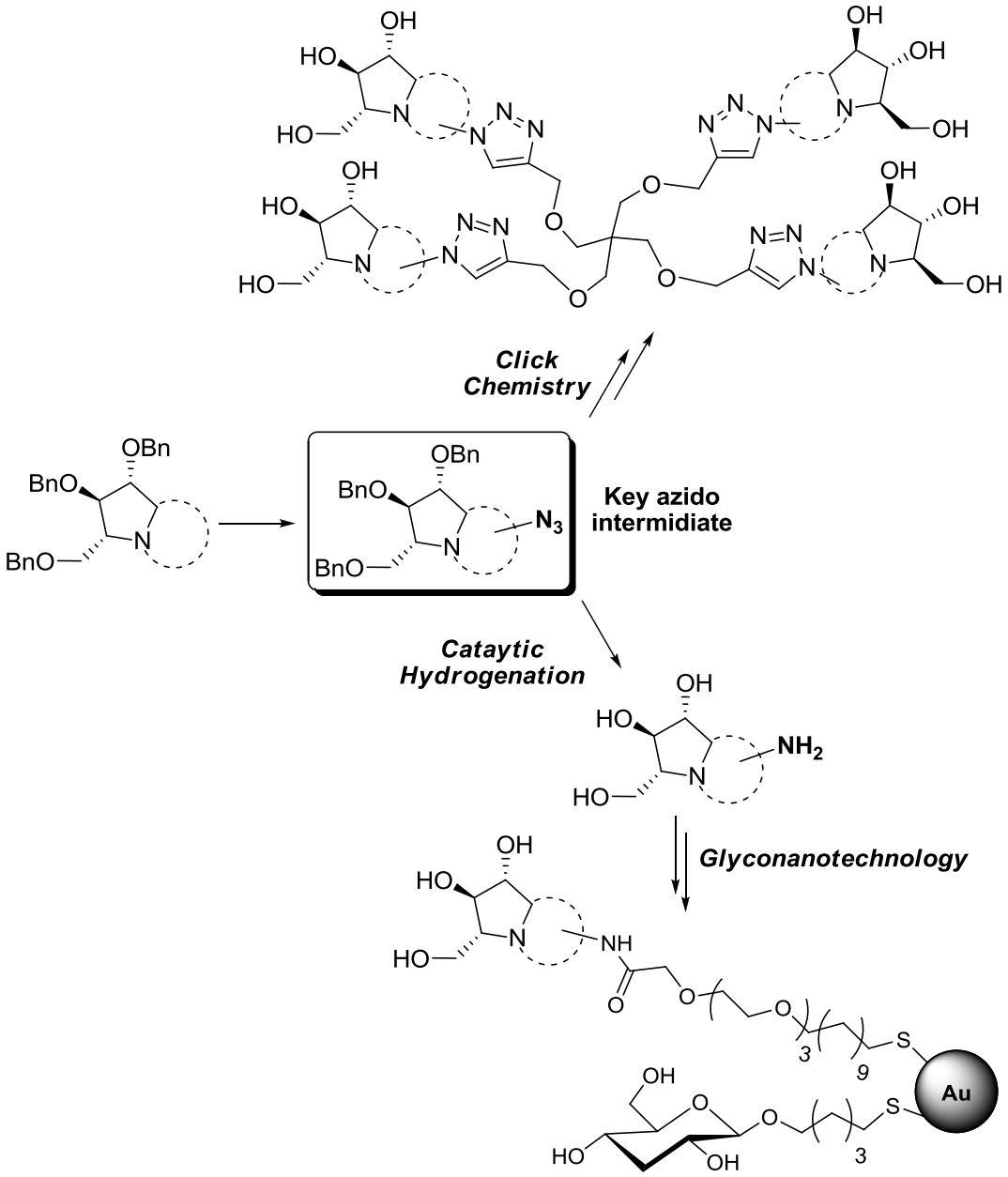


Figure 2.15: Synthetic strategy for preparation of multivalent iminosugar inhibitors, by employing alkyne or gold nanoparticles scaffolds.

A pyrrolidine *N*-alkyl analogue of DAB-1 and a pyrrolizidine analogue of casuarine have been selected as peripheral ligands for the construction of multivalent structures. Their functionalization with azido and amino moieties are reported hereafter.

2.4.1 Pyrrolidine derivatives

Compared to the parent iminosugars, the *N*-alkylated structures tend to exhibit better biological activities in vivo, partially due to the improvement of lipophilicity of the

compounds, and sometimes because the alkylated chains contribute additional binding to the pocket of the receptor.[105] Indeed the *N*-octyl analogue of DAB-1, whose synthesis has been recently reported by our group,[106] resulted to inhibit (Figure 2.16) not only commercial α-glucosidases but also human α-mannosidase, the most promising target to evaluate the multivalent effect in glycosidase inhibition.

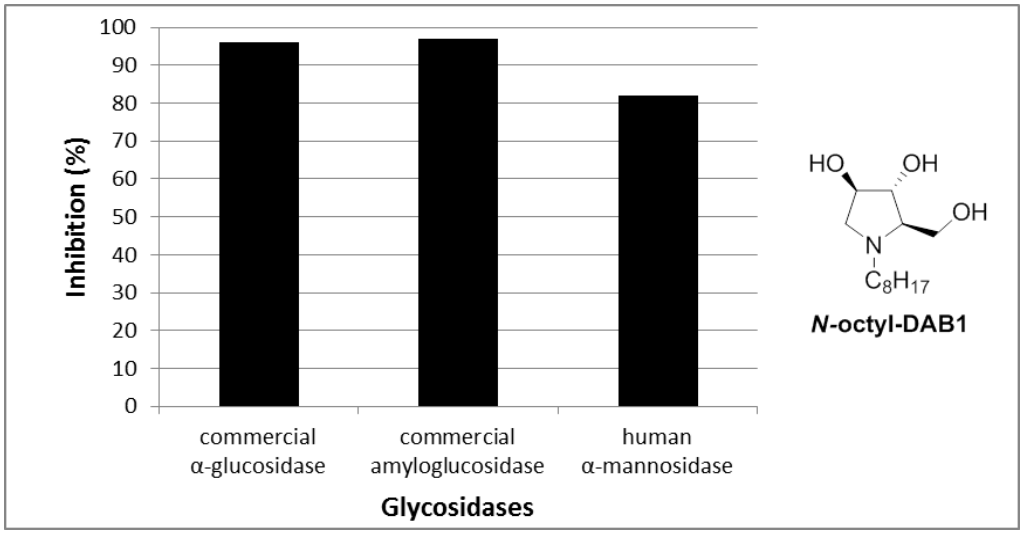
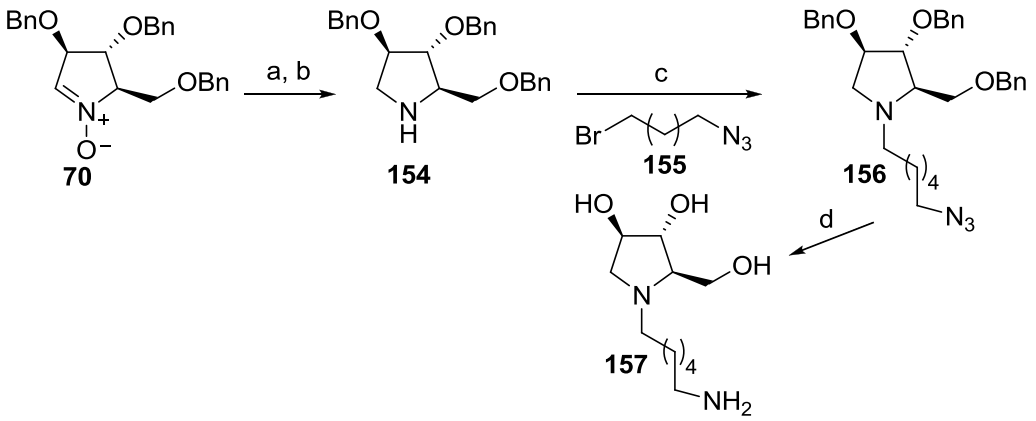


Figure 2.16: Inhibition data of N-octyl-DAB1 against commercial and human glycosidases observed at 1 mM inhibitor concentration.

According to these preliminary results, we decided to functionalize DAB-1, by introducing the azido moiety at the end of the *N*-alkyl chain. In particular, the *N*-6-azidohexyl derivative of DAB-1 **156** has been prepared starting from nitrone **70** (Scheme 2.6). The conversion of nitrone **70** into corresponding protected amine was achieved through a stepwise reduction with $LiAlH_4$, followed by treatment with Zn in acetic acid. The crude compound **154** resulted pure enough to be employed in the following step without any further purification. The alkylation reaction with 1-azido-6-bromohexane[107] **155** was performed in a MW reactor at 150° for 4h, since complete conversion of the starting material was not observed at room temperature. Under these conditions compound **156** was obtained in 86% yield after flash column chromatography and employed in the synthesis of tri- and tetravalent compounds through click reactions with dendrimeric alkyne scaffolds.[108] The simple catalytic hydrogenation of compound **156** allowed access to amino derivative **157**, which has

[105] Wang G.-N., L. Yang, L.-H. Zhang, X.-S.-Ye, *J. Org. Chem*. **2011**, *76*, 2001-2009.
[106] D. Bini, F. Cardona, M. Forcella, C. Parmeggiani, P. Parenti, F. Nicotra, L. Cipolla, *Beilstein J. Org. Chem.* **2012**, *8*, 514-521.
[107] F, Coutrot, E. Busseron, *Chem. Eur. J.* **2009**, *15*, 5186-5190.
[108] F. Cardona, G. D'Adamio, C. Matassini, A. Goti, "Synthesis of multivalent iminosugars", *26th International Carbohydrate Symposium, ICS*, Madrid, July 22nd-27th **2012**.

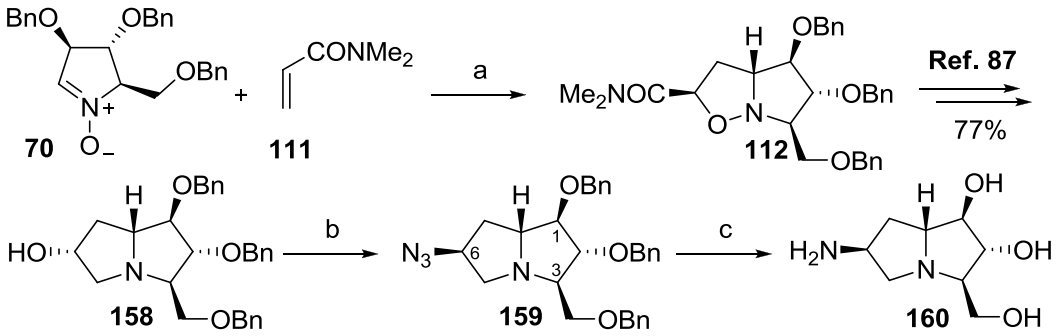
subsequently been coupled with a suitable thiol-ending linker for further multimerization on gold nanoparticle scaffolds (see *Chapter 4.2*).



Scheme 2.6: Synthesis of the pyrrolidine derivatives **156** and **157**. Reagents and conditions*:* a) $LiAlH_4$, dry THF, 0° C to reflux, 1.5 h, b) Zn, $AcOH/H_2O$, rt, 2 h, 79% over two steps; c) K_2CO_3, CH_3CN, MW, 150° C, 2h, 86% d) Pd/C, H_2, MeOH, HCl, rt, 3 days then DOWEX 50WX8, 65%.

2.4.2 Pyrrolizidine derivatives

The azide "casuarine-like" key intermediates **159** was obtained exploiting the cyclic polyhydroxylated nitrone cycloaddition chemistry of nitrone **70**,[63] previously presented in *Chapter 2.1.2* (Figure 2.8).



Scheme 2.7: Synthesis of the pyrrolizidine derivatives **159** and **160**. Reagents and conditions:a) CH_2Cl_2, rt, 3 d, 78%; b) DPPA (1.3 equiv.), PPh_3 (1.1 equiv.), DIAD (1.1 equiv), dry THF, rt, 2 h, 87%; c) H_2, Pd/C, MeOH, HCl, rt 1 day, then Dowex 50WX8-200, quant. yield.

Briefly, cycloaddition of D-arabinose derived nitrone **70** to *N,N*-dimethylacrylamide **111** afforded isoxazolidine **112**[83b,87] with 78% yield. This compound, after N-O bond cleavage with Zn in acetic acid and reduction of the C=O bond with $LiAlH_4$ in THF afforded compound **158**[87] with a 77% overall yield. Straightforward introduction of the azido group was achieved by direct Mitsunobu reaction of **158** with 1.3 equivalents of diphenylphosphoryl azide (DPPA) in the presence of 1.1 equiv. of PPh_3 and diisopropyl azodicarboxylate (DIAD)

at room temperature in dry THF,[109] which furnished the 6-azido intermediate **159** in 87% yield (Scheme 2.7). Confirmation of the occurred inversion of configuration at C-6 was given by 1D NOESY correlations between H-6 and H-1 and between H-6 and H-3 observed for compound **159**. The "casuarine like" azide **159** has been used in the CuAAC cycloaddition with different alkyne scaffolds for the synthesis of the first examples of multivalent pyrrolizidine iminosugars.[110] Catalytic hydrogenation in acidic MeOH followed by basic treatment furnished (6*S*)-6-aminohyacinthacine A2 **160** in quantitative yield (Scheme 2.7), subsequently incorporated onto gold nanoparticles (see *Chapter 4.2*).

Thanks to a collaboration with the group of Prof. I. Robina (University of Seville), the amino derivatives **157** and **160** were assayed toward a panel of eleven commercially available glycosidases (Table 2.4).

ENZYME \ COMPOUND	**157**	**160**
α-L-fucosidase EC 3.2.1.51 1-bovine kidney	n.i.	n.i.
α-galactosidase EC 3.2.1.22 2-coffee beans	n.i.	n.i.
β-galactosidase EC 3.2.1.23 5-*Escherichia coli* 8-*Aspergillus orizae*	 n.i. n.i.	 n.i. n.i.
α-glucosidase EC 3.2.1.20 10-yeast 11-rice	 78 18	 n.i. n.i.
amyloglucosidase EC 3.2.1.3 13-*Aspergillus niger*	98 (12.9 μM) Ki =**6.5 μM**	98 (15.3 μM) Ki=**18.2 μM**
β-glucosidase EC 3.2.1.21 15-almonds	n.i.	24
α-mannosidase EC 3.2.1.24 16-jack beans	74	n.i.
β-mannosidase EC 3.2.1.25 18-snail	n.i.	n.i.
β-N-acetylglucosaminidase EC 3.2.1.30 21-jack beans	n.i.	44

Table 2.4: Inhibitory activities of compounds **157** and **160** toward glycosidases. Percentage of inhibition at 1 mM, IC_{50} (in parenthesis, μM). Optimal pH, 35°. [a)] For conditions of measurements see Ref.[111]; [b)] n.i.: no inhibition was detected at 1 mM concentration of the corresponding compound.

[109] K. Kamikawa, T. Watanabe, A. Daimon, M. Uemura, *Tetrahedron* **2000**, *556*, 2325-2337.
[110] A. Goti, F. Cardona et al., Manuscript in preparation.
[111] a) R. Saul, J. P. Chambers, R. J. Molyneux and A. D. Elbein, *Arch. Biochem. Biophys.* **1983**, *221*, 593-597; b) A. Brandi, S. Cicchi, F. M. Cordero, R. Frignoli, A. Goti, S. Picasso and P. Vogel, *J. Org. Chem.* **1995**, *60*, 6806-6812.

Both compounds showed to be potent and competitive inhibitors of amyloglucosidase from Aspergillus niger, with IC_{50} values in the μM range. Compound **157** also modestly inhibits α-glucosidases from yeast (78% at 1mM) and α-mannosidase from jack beans (74% at 1mM). While compound **160** inhibits, although to a lesser extent, β-glucosidase from almonds and β-N-acetylglucosaminidase from jack beans (24% and 44% at 1mM, respectively).

2.5 Conclusions

In this chapter different applications related to the use of polyhydroxylated cyclic nitrones in the synthesis of pyrrolidine-containing iminosugars have been reported. First of all, a reactivity study aimed to obtained these key intermediates *via* hydroxylamine oxidation with iodine hypervalent reagents was presented. Then, we described the synthesis of iminosugar-based trehalase inhibitors possessing a pseudodisaccharidic structure, obtained by cross-metathesis reactions between suitably functionalized pyrrolidine iminosugars. Final dimeric structures were preliminarily assayed for their activity against porcine trehalase. Lastly, the functionalization with azido and amino moieties was carried out on two different polyhyroxilated alkaloids: a *N*-alkyl derivative of DAB1 and a casuarine analogue. These modified compounds have been subsequently employed as *active components* in the preparation of gold glyconanparticles (see *Chapter 4*).

2.6 Experimental Section

General methods: Commercial reagents were used as received. All reactions were carried out under magnetic stirring and monitored by TLC on 0.25 mm silica gel plates (Merck F254). Column chromatographies were carried out on Silica Gel 60 (32-63 □m) or on silica gel (230–400 mesh, Merck). Yields refer to spectroscopically and analytically pure compounds unless otherwise stated. ^{1}H NMR spectra were recorded on a Varian Mercury-400 or on a Varian INOVA 400 instruments at 25 °C. ^{13}C NMR spectra were recorded on a Varian Gemini-200 or on a Varian Gemini-300. Chemical shifts are reported relative to TMS (1H: δ=0.00 ppm) and $CDCl_3$ (13C: δ=77.0 ppm). Integrals are in accordance with assignments, coupling constants are given in Hz. For detailed peak assignments 2D spectra were measured (COSY, HSQC, NOESY, and NOE as necessary). Small scale microwave-assisted syntheses were carried out in a Microwave apparatus for synthesis CEM Discover with an open reaction vessel and external surface sensor. IR spectra were recorded with a BX FT-IR Perkin–Elmer System spectrophotometer. Mass spectra were recorded with a System Applied Biosystems MDS SCIEX instrument (Q TRAP, LC–MS–MS, turbon ion spray)

or with a System Applied Biosystem MDS SCIEX instrument (Q STAR elite nanospray). ESI-MS spectra were recorded with a Thermo Scientific™ LCQ Fleet Ion Trap Mass Spectrometer. Elemental analyses were performed with a Perkin-Elmer 2400 analyzer. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter.

General Procedure for the Cross-Metathesis Reaction: To a 0.1 m solution of the appropriate allyl monomers in anhydrous CH_2Cl_2 was added the Grubbs catalyst (5% in weight). The mixture was stirred overnight at room temperature and concentrated. The residue was purified directly on a silica gel column using a suitable eluent. All the products obtained by CM were the (*E*) isomers (>95% as determined by NMR).

General Procedure for the Hydrogenolysis Reaction: A 0.02 m solution of the appropriate dimer dissolved in AcOEt/EtOH (1:1) was treated with Pd(OH)2/C (100% in weight). The reaction was stirred for 5 d under a H_2 atmosphere. Palladium was then removed by filtration through a Celite pad followed by washing with EtOH and water. Evaporation of the solvents afforded the corresponding deprotected compounds in quantitative yields.

Homodimer 137: ^{1}H NMR (D_2O): δ = 4.19–3.05 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.88–1.58 (m, 4 H, CH-C*H*$_2$-CH$_2$), 1.48–1.27 ppm (m, 4 H, CH$_2$-C*H*$_2$-C*H*$_2$-CH$_2$); ^{13}C NMR (D_2O): δ = 79.1, 73.7 (C-2, C-3), 64.9, 64.0 (C-1, C-4), 60.6 (C-5), 27.4 (CH-*C*H$_2$-CH$_2$), 26.5 ppm (CH$_2$-*C*H$_2$-*C*H$_2$-CH$_2$); $C_{14}H_{28}N_2O_6$ (320.39): calcd. C 52.48, H 8.81, N 8.74; found C 52.41, H 8.80, N 8.75.

Homodimer 138: ^{1}H NMR (D_2O): δ = 4.30–3.17 (m, 12 H, 1-H, 2- H, 3-H, 4-H, 5-H), 1.80–1.49 (m, 4 H, CH-C*H*$_2$-CH$_2$), 1.47–1.26 ppm (m, 4 H, CH$_2$-C*H*$_2$-C*H*$_2$-CH$_2$); ^{13}C NMR (D_2O): δ = 75.8, 73.0 (C-2, C-3), 65.2, 62.9 (C-1, C-4), 59.9 (C-5), 32.2 (CH-*C*H$_2$-CH$_2$), 27.8 ppm (CH$_2$-*C*H$_2$-*C*H$_2$-CH$_2$); $C_{14}H_{28}N_2O_6$ (320.39): calcd. C 52.48, H 8.81, N 8.74; found C 52.43, H 8.80, N 8.73.

Heterodimer 139: ^{1}H NMR (D_2O): δ = 4.17–3.19 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.82–1.50 (m, 4 H, CH-C*H*$_2$-CH$_2$), 1.48–1.27 ppm (m, 4 H, CH$_2$-C*H*$_2$-C*H*$_2$-CH$_2$); ^{13}C NMR (D_2O): δ = 80.7, 76.9, 75.8, 73.0 (C-2, C-2', C-3, C-3'), 64.9, 64.9 63.9, 63.9 (C-1, C-1', C-4, C-4'), 60.6, 59.9 (C-5, C-5'), 32.2, 32.2 (CH-*C*H$_2$-CH$_2$), 27.7, 27.7 ppm (CH$_2$-*C*H$_2$-*C*H$_2$-CH$_2$); $C_{14}H_{28}N_2O_6$ (320.39): calcd. C 52.48, H 8.81, N 8.74; found C 52.52, H 8.82, N 8.74.

Protected Homodimer 140: Flash column chromatography (PE/AcOEt, 87.5:12.5). ^{1}H NMR ($CDCl_3$): δ = 7.48–6.97 (m, 40 H, ArH), 5.39–5.25 (m, 2 H, CH=CH), 5.23–4.98 (m, 4 H, CH$_2$ Cbz), 4.69–4.21 (m, 12 H, OCH$_2$Ph), 4.21–3.40 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.26 ppm (t, *J* = 7.1 Hz, 4 H, *CH*2-CH=CH). ^{13}C NMR ($CDCl_3$): δ = 154.4 (C=O, Cbz), 138.7–136.7 (C Ar),

129.9 (CH=CH), 128.7–127.6 (CH Ar), 83.6, 82.7, 64.5, 63.0 (C-1, C-2, C-3, C-4), 73.2, 71.2 (OCH_2Ph), 68.9, 68.0 (C-5,5'), 67.1 (CH_2 Cbz), 29.9 ppm (*C*H2-CH=CH). MS (TOF): *m*/*z* = 1127.54 $[M + H]^+$; found 1127.20. $C_{72}H_{74}N_2O_{10}$ (1127.38): calcd. C 76.71, H 6.62, N 2.48; found C 76.75, H 6.61, N 2.48.

Protected Homodimer 141: Flash column chromatography (PE/AcOEt, 80:20). ^{1}H NMR ($CDCl_3$): δ = 7.36–7.11 (m,40 H, ArH), 5.51–5.25 (m, 2 H, CH=CH), 5.17–5.03 (m, 4 H, CH2 Cbz), 4.58–4.22 (m, 12 H, OCH_2Ph), 4.21–3.41 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.25 ppm (t, *J* = 7.1 Hz, 4 H, C*H*2-CH=CH); ^{13}C NMR ($CDCl_3$): δ = 155.9 (C=O, Cbz), 138.5–136.9 (C Ar), 128.7–127.7 (CH=CH, CH Ar), 77.6, 76.6, 61.9, 61.3 (C-1, C-2, C- 3, C-4), 73.3, 71.7 (OCH_2Ph), 68.3 (C-5), 67.1 (CH_2 Cbz), 30.0 ppm (*C*H2-CH=CH); MS (TOF): *m*/*z* = 1127.54 $[M + H]^+$; found 1127.30. $C_{72}H_{74}N_2O_{10}$ (1127.38): calcd. C 76.71, H 6.62, N 2.48; found C 76.77, H 6.63, N 2.48.

Protected Heterodimer 142: Flash column chromatography (PE/AcOEt, 85:15). ^{1}H NMR ($CDCl_3$): δ = 7.56–7.00 (m, 40 H, ArH), 5.54–5.24 (m, 2 H, CH=CH), 5.23–4.94 (m, 4 H, CH_2 Cbz), 4.69–4.21 (m, 12 H, OCH_2Ph), 4.20–3.40 (m, 12 H, 1-H, 2-H, 3-H, 4-H, 5-H), 1.29–1.24 ppm (m, 4 H, CH_2-CH=CH); ^{13}C NMR (CDCl3): δ = 156.3 (C=O, Cbz), 138.5–136.9 (C Ar), 128.7– 127.8 (CH=CH, CH Ar), 82.9, 76.7, 62.6, 61.3 (C-1, C-2, C-3, C-4), 73.3, 71.4 (OCH_2Ph), 68.9 (C-5), 67.1 (CH_2 Cbz), 29.9 ppm (*C*H_2-CH=CH); $C_{72}H_{74}N_2O_{10}$ (1127.38): calcd. C 76.71, H 6.62, N 2.48; found C 76.67, H 6.63, N 2.48.

(2S,3S,4R,5R)-2-Allyl-3,4-bis(benzyloxy)-5-(benzyloxy-methyl)pyrrolidine-1-carboxylate (144): Allyl ammine **147** (575 mg, 1.3 mmol) was dissolved in water (5 mL) and $NaHCO_3$ (218 mg, 2.6 mmol) was added. The suspension was stirred until complete dissolution of the salt, then dioxane (6 mL) was added. The resulting mixture was cooled at 0 °C with an ice bath and Cbz-Cl (186 µL, 1.3 mmol) was added dropwise. After 10 minutes the ice bath was removed and the reaction mixture was stirred for 20 h at room temperature. After have added EtOAc (27 mL) and water (8 mL) the aqueous layer was separated and washed with EtOAc (3 x 10 mL); the combined organic extracts were washed with 1 M HCl (2 x 10 mL), brine, and dried over Na_2SO_4. The solvent was evaporated and the residue purified by flash column cromatography to afford pure **144** (590 mg, 1.02 mmol, 79 %) as a colorless oil. R_f=0.74 (PE/AcOEt 2:1); $[\alpha]_D^{23}$ = −10.3 (c =0.93, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.51-7.10 (m, 20 H, ArH), 5.73-5.29 (m, 1 H, CH_2CH=CH_2), 5.18-5.11 (m, 2 H, CH_2 Cbz), 5.05-4.82 (m, 2 H, CH_2CH=CH_2), 4.53-4.40 (m, 6 H, OCH_2Ph), 4.20-4.01 (m, 3 H, H-1, H-2, H-4) 3.79 (t, J = 4.2 Hz, 1 H, H-3), 3.72-3.46 (m, 2 H, H-5), 2.50-2.32 ppm (m, 2 H, CH_2CH=CH_2); ^{13}C NMR ($CDCl_3$, 50

MHz): δ = 151.1 (C=O Cbz), 137.9-136.3 (C Ar), 133.8 ($CH_2CH=CH_2$), 128.1-127.0 (C Ar), 117.2 ($CH_2CH=CH_2$), 78.4 (C-3), 77.4 (C-2), 72.9, 71.3, 71.0 (OCH_2Ph), 67.8 (C-5), 66.6 (CH_2 Cbz), 61.5-60.43 (C-1, C-4), 36.4-35.9 ppm ($CH_2CH=CH_2$). MS (ESI) m/z (%): 600.54 (79) $[M + Na]^+$. IR ($CDCl_3$): ν =3672, 3089, 3067, 3033, 2973, 2929, 2868, 2248, 1952, 1875, 1809, 1695, 1641, 1497, 1103 cm^{-1}; elemental analysis calcd (%) for $C_{37}H_{39}NO_5$ (577.71): C, 76.92; H, 6.80; N, 2.42; found: C, 76.85; H, 6.81; N, 2.42.

(2S,3S,4R,5R)-2-Allyl-3,4-bis(benzyloxy)-5-(benzyloxy-methyl)pyrrolidin-1-ol (146): To a cooled (0 °C) solution of nitrone **70** (960 mg, 2.3 mmol) in anhydrous THF (30 mL), allylmagnesium bromide (6.9 mL of a 1.0 M solution in diethyl ether, 6.9 mmol) was added dropwise. After stirring for 3 h at 0 °C the reaction was quenched with saturated aqueous NH_4Cl (20 mL). The reaction mixture was diluted with diethyl ether (25 mL), the organic layer was separated and the aqueous layer was extracted with diethyl ether (2 x 20 mL). The combined organic extracts were washed with brine (20 mL), dried over Na_2SO_4, filtered, and the solvent was eliminated under reduced pressure to afford the crude product **146** (1.05 g, quantitative) which was employed in the following step without further purification. A sample of crude product was purified by FCC to give pure **146** as a white solid, necessary to the characterisation. R_f=0.54 (PE/AcOEt 3:1); Mp 63-65 °C; $[\alpha]_D^{24}$ = -22.1 (c =1.035, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.32-7.29 (m, 15 H, ArH), 5.94-5.83 (m, 1 H, $CH_2CH=CH_2$), 5.08-5.00 (m, 2 H, $CH_2CH=CH_2$), 4.58-4.51 (m, 5 H, OCH_2Ph), 4.43 (d, J = 11.7 Hz, 1 H, OCH_2Ph), 3.72 (t, J = 5.4 Hz, 1 H, H-3), 3.56 (dd, J = 5.3, 2.3 Hz, 2 H, H-5), 3.47 (dd, J = 7.3, 5.8 Hz, 1 H, H-2), 3.39-3.30 (m, 2 H, H-1, H-4), 2.46-2.32 ppm (m, 2 H, $CH_2CH=CH_2$); ^{13}C NMR ($CDCl_3$): δ = 138.2, 138.1, 138.0 (C Ar), 135.5 ($CH=CH_2$), 128.4-127.7 (C Ar), 116.7 ($CH=CH_2$), 77.3 (C-2), 75.4 (C-3), 73.3 (C-4), 71.9, 71.7, 71.4 (OCH_2Ph), 70.1 (C-1), 69.7 (C-5), 36.0 ppm ($CH_2CH=CH_2$); MS (ESI) m/z (%): 482.42 (100) $[M + Na]^+$. IR ($CDCl_3$): ν =3576, 3087, 3067, 3031, 2866, 2246, 1951, 1810, 1496, 1097 cm^{-1}; elemental analysis calcd (%) for $C_{29}H_{33}NO_4$ (459.58): C, 75.79; H, 7.24; N, 3.05; found: C, 75.47; H, 6.91; N, 3.41.

(2S,3S,4R,5R)-2-Allyl-3,4-bis(benzyloxy)-5-(benzyloxy-methyl)pyrrolidine (147): A solution of hydroxylamine **146** (923 mg, 2.0 mmol) in acetic acid (10 mL) and water (10 mL) was treated with Zn powder (2.6 g, 40.0 mmol). The resulting mixture was stirred at room temperature for 40 minutes, diluted with water (50 mL) and treated with Na_2CO_3 (9.3 g) until bubbling of CO_2 stopped. The reaction mixture was extracted with DCM (3 x 50 mL) and the combined organic extracts were washed with NaOH 3 M (50 mL) and brine, dried (Na_2SO_4) and evaporated under reduced pressure to give pure ammine **32** (704 mg, 1.6

mmol, 80%), as a colorless oil, which did not need further purification. An analytically pure sample was obtained after purification by FCC . R_f=0.41 ($CHCl_3$/Et_2O 2:1); $[\alpha]_D^{23}$ = -14.5 (c =0.99, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.36-7.24 (m, 15 H, ArH), 5.78 (ddt, J = 17.1, 10.1, 7.0 Hz, 1 H, CH_2CH=CH_2), 5.08-5.01 (m, 2 H, CH_2CH=CH_2), 4.60-4.45 (m, 6 H, OCH_2Ph), 3.74 (t, J=5.3 Hz, 1 H, H-3), 3.51- 3.40 (m, 4 H, H-2, H-4, H-5), 3.35-3.30 (m, 1 H, H-1), 2.41-2.35 (m, 1 H, CH_2CH=CH_2), 2.08 ppm (td, J = 14.2, 6.9 Hz, 1H, CH_2CH=CH_2); ^{13}C NMR ($CDCl_3$): δ = 138.3-138.2 (C Ar), 135.2 (CH=CH_2), 128.3-127.6 (C Ar), 117.1 (CH_2CH=CH_2), 81.3 (C-2), 78.2 (C-3), 73.2, 71.8, 71.6 (OCH_2Ph), 71.1 (C-5), 61.6 (C-4), 60.6 (C-1), 38.4 (CH_2CH=CH_2); MS (ESI) m/z (%): 444.42 (100) $[M + H]^+$; IR ($CDCl_3$): ν =3067, 3031, 2865, 2246,1710, 1640, 1496, 1434, 1364, 1027 cm^{-1}; elemental analysis calcd (%) for $C_{29}H_{33}NO_3$ (443,58): C, 78.52; H, 7.50; N, 3.16; found: C, 78.59; H, 7.50; N, 3.15.

Synthesys of (3*R*,4*R*,5*R*,)-1-(6-azidohexyl)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl] pyrrolidine (156): To a suspension of $LiAlH_4$ (183 mg, 4.89 mmol) in dry THF (40 mL) a solution of **70** (680 mg, 1.63 mmol) in dry THF (40 mL) was added dropwise at 0 °C, under N_2 atmosphere. The reaction mixture was heated to reflux and left stirred for one hour and half. The reaction mixture was cooled to room temperature and then a saturated solution of Na_2SO_4 was added dropwise, under vigorous stirring. The mixture was extracted with AcOEt, dried over anydrous Na_2SO_4 and the solvent was evaporated to obtain 607 mg (1.45 mmol, 89%) of corresponding hydroxylamine as the only product. To a solution of crude hydroxylamine in AcOH (7.4 mL) Zn powder (1.89 g, 29.00 mmol) and H_2O (7.4 mL) were added. The mixture was left stirring for 2 hours at room temperature and then diluted with 40 mL of H_2O. A saturated solution of $NaHCO_3$ was added until complete neutralization of AcOH (pH = 7) and the mixture was extracted with CH_2Cl_2 (3 x 40 mL) The combined organic layers were washed with 3M NaOH and brine, dried over anydrous Na_2SO_4 and the solvent was evaporated under vacuum, affording 520 mg (1.29 mmol, 89%) of compound **2**,[112] which was employed in the following step without further purification.

To a solution of **154** (173 mg, 0.43 mmol) and 1-azido-6-bromohexane **155** (163 mg, 0.77 mmol) in CH_3CN (4.2 mL) K_2CO_3 (77 mg, 0.56 mmol) was added. The reaction mixture was left stirring in the microwave at 150 °C, for two hours and then filtered over Celite®, washing with CH_2Cl_2. The filtrate was concentrated under vacuum and the residue was purified by FCC affording **156** (195 mg, 0.37 mmol) as a yellowish oil in 86% yield. R_f=0.48 (EP/AcOEt 5:1); $[\alpha]_D^{30}$ = −25.1 (c =1.30, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.35-7.25 (m,

[112] Spectroscopic data in agreement with: X. Zhou, W.-J. Liu, J.-L. Ye, P.-Q. Huang, *Tetrahedron* **2007**, *63*, 6346–6357.

15H, Ar), 4.57-4.42 (m, 6H, Bn), 3.92 (d, *J* = 4.8 Hz, 1H, 3-H), 3.88 (d, *J* = 3.9 Hz, 1H, 4-H), 3.62-3.50 (m, 1H, 6-Ha), 3.52 (dd, *J* = 9.8, 6.3 Hz, 1H, 6-Hb), 3.24 (t, *J* = 7.1 Hz, 2H, 6'-H), 3.24-3.19 (m, 1H, 2-Ha), 2.88-2.81 (m, 1H, 1'-Ha), 2.71 (*b* s, 1H, 5-H), 2.55 (dd, *J* = 10.3, 4.9 Hz, 1H, 2-Hb), 2.38-2.31 (m, 1H, 1'-Hb), 1.59 (quin, *J* = 7.1 Hz, 2H, 5'-H), 1.54-1.48 (m, 2H, 2'-H), 1.41-1.25 ppm (m, 4H, 3'-H + 4'-H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 138.5, 138.4, 138.3 (s, 3C, Ar), 128.3-127.6 (d, 15C, Ar), 85.5 (d, C-4), 81.7 (d, C-3), 73.2 (t, CH_2Bn) 71.3 (t, CH_2Bn), 71.2 (t, CH_2Bn), 71.0 (t, C-6), 69.4 (d, C-5), 57.3 (t, C-2), 55.5 (t, C-1'), 51.4 (t, C-6'), 28.8 (t, C-5'), 28.0 (t, C-2'), 27.0, 26.6 ppm (t, 2C, C-3' + C-4'); MS (ESI): *m/z* (%) = 529.42 (100) $[M+H]^+$, 551.42 (38) $[M+Na]^+$; IR ($CDCl_3$): ν = 3088, 3066, 3032, 3009, 2937, 2862, 1496, 1453, 1365, 1261 cm^{-1}; elemental analysis calcd (%) for $C_{32}H_4N_4O_3$ (504.49): calcd. C, 72.70; H, 7.63; N, 10.60; found C 72.32, H 7.23, N 10.59.

Synthesys of (3*R*,4*R*,5*R*)-1-(6-aminohexyl)-5-(hydroxymethyl)pyrrolidine-3,4-diol (157): To a solution of **156** (89 mg, 0.17 mmol) in MeOH (5 mL) and HCl 12M (6 drops) Pd /C (45 mg) was added and the reaction mixture was left stirring at room temperature under H_2 atmosphere for three days. The catalyst was filtered through Celite® and the filtrate was concentrated under vacuum, to yield quantitative the hydrochloride of **3** which was eluted through an ion-exchange resin (DOWEX® 50XW8-100) with MeOH, H_2O and 6% NH_4OH to give the free amine **157** (25 mg, 0.11 mmol, 65%). $[\alpha]_D^{20}$ **=** − 49.4(c =0.69, H_2O); ^{1}H-NMR (400 MHz, D_2O) δ = 3.98 (dt, *J* = 5.4, 2.4 Hz, 1H, 3-H), 3.80 (dd, *J* = 4.9, 2.9 Hz, 1H, 4-H), 3.63-3.54 (m, 2H, 6-H), 2.87 (dd, *J* = 11.2, 2.4 Hz, 1H, 2-Ha), 2.72-2.65 (m, 3H, 1'-Ha, 6'-H), 2.60 (dd, *J* = 11.2, 5.8 Hz, 1H, 2-Hb), 2.39 (dd, *J* = 10.5, 5.1 Hz, 1H, 5-H), 2.24 (td, *J* = 10.7, 5.4 Hz, 1H, 1'-Hb), 1.48-1.19 ppm (m, 8H, 2'-5'-H); ^{13}C-NMR (50 MHz, D_2O) δ = 79.3 (d, C-4), 75.5 (d, C-3), 71.9 (d, C-5), 61.4 (t, C-6), 58.3 (t, C-2), 55.3 (t, C-1'), 39.8 (t, C-6'), 28.2-25.5 ppm (4C, t, C2'-C5'); MS (ESI): *m/z* (%) = 233.23 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{11}H_{24}N_2O_3$ (232.32): C 56.87, H 10.41, N 12.06; found C 56.36, H 10.31, N.11.84.

(1*R*,2*R*,3*R*,6*S*,7a*R*)-1,2-Bis(benzyloxy)-3-[(benzyloxy)methyl]-6-azido-hexahydro-1*H*-pyrrolizidine (159): To a solution of **158** (914 mg, 1.99 mmol) in dry THF (19 ml) under nitrogen atmosphere triphenylphosphine (626 mg, 2.39 mmol) was added. The reaction mixture was cooled to 0 °C and DIAD (470 µL, 2.39 mmol) was added dropwise, forming a yellow precipitate. After addition of diphenylphosphorilazide (823 mg, 2.99 mmol), the suspension was raised to room temp. and stirred under nitrogen atmosphere for 2 h, until TLC analysis (CH_2Cl_2:MeOH 10:1) showed disappearance of the starting material (R_f=0.22) and formation of a new product (R_f=0.95). The solvent was removed under reduced

pressure and the crude was purified by FCC affording pure **159** (838 mg, 1.73 mmol, 87% yield) as a yellow oil. R_f=0.21 (PE/AcOEt 4:1); $[\alpha]_D^{27}$ = +3.96 (c =1.06, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=7.30-7.18 (m, 15H, H-Ar), 4.60-4.48 (AB system, *J*=11.7 Hz, 2H, H-Bn), 4.50-4.41 (AB system, *J*=11.7 Hz, 2H, H-Bn), 4.48-4.42 (AB system, *J*=12.2 Hz, 2H, H-Bn), 4.10-4.06 (m, 1H, H-6), 3.96 (dd, *J*=6.3, 5.4 Hz, 1H, H-2), 3.76 (t, *J*=4.9 Hz, 1H, H-1), 3.59-3.54 (m, 1H, H-7a), 3.50 (dd, *J*=9.2, 5.3 Hz, 1H, Ha-8), 3.43 (dd, *J*=9.2, 6.3 Hz, 1H, Hb-8), 3.07 (dd, *J*=11.7, 3.4 Hz, 1H, Ha-5), 2.98 (dd, *J*=11.7, 5.4 Hz, 1H, Hb-5), 2.93 (q, *J*=6.3 Hz, 1H, H-3), 1.96 (ddd, *J*=13.2, 6.8, 3.4 Hz, 1H, Ha-7), 1.81 ppm (ddd, *J*=13.6, 8.3, 6.3 Hz, 1H, Hb-7); ^{13}C-NMR (50 MHz, $CDCl_3$): δ=138.4, 138.2, 137.9 (s, C-Ar), 128.4-127.5 (d, 15C, C-Ar), 88.3 (d, C-1), 85.9 (d, C-2), 73.3 (t, C-Bn) 72.4 (t, 2C, C-Bn, C-8), 71.9 (t, C-Bn), 69.2 (d, C-3), 66.7 (d, C-7a), 62.1 (d, C-6), 59.9 (t, C-5), 37.0 ppm (t, C-7); IR ($CDCl_3$): ν =3081, 3064, 3032, 2926, 2864, 2102, 1496, 1453, 1365, 1264, 1099 cm^{-1}; MS (ESI): *m/z* (%) = 485.33 (100) [$M+H^+$]; elemental analysis calcd (%) for $C_{29}H_{32}N_4O_3$ (484.59): C 71.88, H 6.66, N 11.56; found: C 71.45, H 6.54, N 11.68.

(1*R*,2*R*,3*R*,6*S*,7a*R*)-6-Amino-3-hydroxymethylhexahydro-1*H*-pyrrolizidine-1,2-diol (160): To a solution of **159** (325 mg, 0.67 mmol) in 25 ml of methanol 163 mg of 10% Pd/C and five drops of 37% HCl were added under nitrogen atmosphere, then the mixture was stirred under hydrogen atmosphere at room temp. for 1 day. TLC analysis (PE:AcOEt 3:1) showed the disappearance of the starting material (R_f=0.35) and formation of a new product (R_f=0.00). The mixture was filtered through Celite® and the solvent was removed under reduced pressure affording a crude yellow oil (150 mg). Free amine was obtained by passing the hydrochloride through a Dowex 50WX8 ion-exchange resin. Elution with 6% NH_4OH afforded the free base **160** (126 mg, 0.67 mmol, 100% yield over two steps) as a waxy brown solid. $[\alpha]_D^{23}$=+16.7 (c =1.01, MeOH); ^{1}H-NMR (400 MHz, D_2O): δ=3.66-3.59 (m, 3H, H-1, H-2, Ha-8), 3.49 (dd, *J*=11.7, 6.3 Hz, 1H, Hb-8), 3.46-3.40 (m, 1H, H-6), 3.15 (td, *J*=7.8, 3.9 Hz, 1H, H-7a), 2.85 (dd, *J*=11.7, 6.3 Hz, 1H, Ha-5), 2.59 (ddd, *J*=9.8, 6.3, 3.9 Hz, 1H, H-3), 2.47 (dd, *J*=11.7, 7.8 Hz, 1H, Hb-5), 1.96 (ddd, *J*=12.7, 6.4, 3.9 Hz, 1H, Ha-7), 1.62 ppm (dt, *J*=12.7, 8.3 Hz, 1H, Hb-7); ^{13}C-NMR (50 MHz, D_2O): δ=80.3 (d, C-1) 76.6 (d, C-2), 69.4 (d, C-3), 65.1 (d, C-7a), 62.9 (t, C-8), 60.9 (t, C-5), 49.4 (d, C-6), 37.7 ppm (t, C-7); MS (ESI): *m/z* 189.09 (100) [$M+H^+$]; elemental analysis calcd (%) for $C_8H_{16}N_2O_3$ (188.22): C 51.05, H 8.57, N 14.88; found: C 51.66, H 8.98, N 15.08.

Chapter 3

Polyhydroxylated piperidine iminosugars and pipecolic acid analogs: synthesis from a D-mannose derived aldehyde

3.1 Introduction

Polyhydroxylated piperidines such as 1-deoxynojirimycin (**1**) and 1-deoxymannojirimycin (**7**) (Figure 3.1) are natural products widely found essentially in plants and microorganisms and are among the most attractive carbohydrate mimetics reported to date. Their inhibitory activity against glycosidic enzymes has prompted an intense research for the synthesis of the natural products and unnatural analogs in order to develop new therapies against cancer, virus, diabetes and hereditary metabolic disorders (See *Chapter 1*).

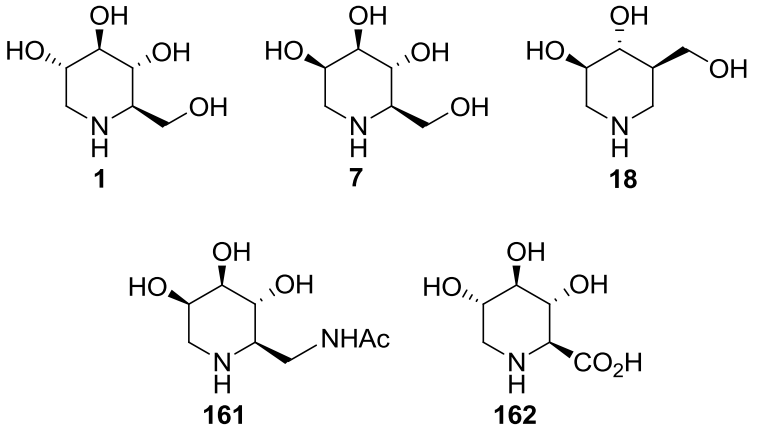


Figure 3.1: Natural and synthetic piperidine iminosugars **1**, **7**, **18**, amino derivative **161** and polyhydroxypipecolic acid **162**.

Synthetic amino derivatives such as 6-acetylamino-1,6-dideoxymannojirimycin (**161**, Figure 3.1) also showed a very high activity as glycosidase inhibitors.[113] More recently, the antibacterial activity of synthetic hydroxyamino piperidines and other iminosugar derivatives was discovered.[114] Polyhydroxypipecolic acid **162,** the only natural

[113] I. McCort, S. Fort, A. Duréault, J.-C. Depezay, *Bioorg. Med. Chem.* **2000**, *8*, 135-143.

[114] a) N. L. Segraves, P. Crews, *J. Nat. Prod.* **2005**, *68*, 118-121; b) Y. Zhou, V. E. Gregor, B. K. Ayida, G. C. Winters, Z. Sun, D. Murphy, G. Haley, D. Bailey, J. M. Froelich, S. Fish, S. E. Webber, T. Hermann, D. Wall, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1206-1210; c) L. Zhang, F. Sun; Q. Wang, J. Zhou, L.-H. Zhang, X.-L. Zhang, X.-S. Ye, *ChemMedChem* **2009**, *4*, 756-760; d) D. Rejman, A. Rabatinová, A. R. Pombinho, S. Kovačková, R. Pohl, E. Zborníková, M. Kolář, K. Bogdanová, O. Nyč, H. Šanderová, T. Látal, P. Bartůněk, L. Krásný, *J. Med. Chem.* **2011**, *54*, 7884-7898.

trihydroxypipecolic acid known, was also isolated from the seeds of *Bathia racemosa*[115] and proved to be a specific inhibitor of human liver β-D-glucuronidase,[116] and displayed anti HIV[117] as well as antimetastatic properties.[118]

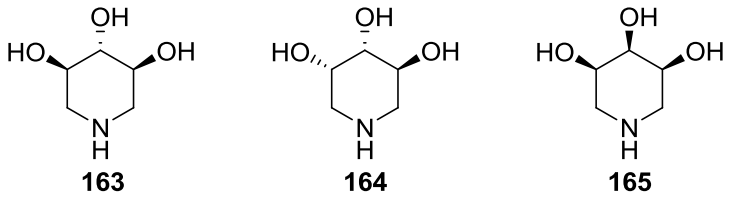


Figure 3.2: Natural glycosidase inhibitor trihydroxypiperidine alkaloids.

In particular, 3,4,5-trihydroxypiperidines **163-165** (Figure 3.2), also referred to as 1-*N*-azasugars or isofagomine (**18**) analogues, were isolated in 1995 from *Eupatorium fortunei TURZ*[119] and have shown to be the active components of the extracts of this plant, traditionally used in Chinese and Japanese folk medicine as a diuretic, antipyretic and antidiabetic agent. As a consequence, in recent years there has been a great deal of interest not only in the synthesis of the natural products themselves, but also in the synthesis of chemically modified analogues.

All the methodologies described in the literature can be divided into two big general families: "chiral pool" and "enantioselective" strategies.

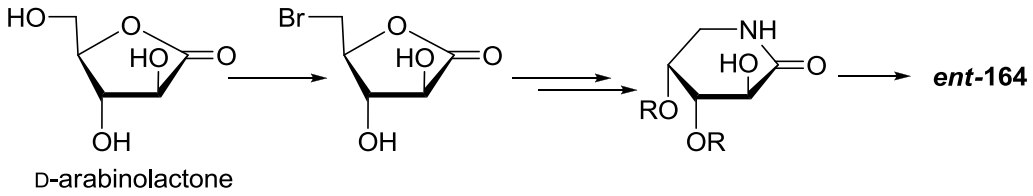
3.1.1 Chiral pool strategies

Before their natural occurrence was discovered, compounds **163**, **164** and the unnatural ***ent*-164**, were synthesized by Ganem[120] starting from D-glucose, D-galactose and D-mannose, respectively. After this first example, many other "chiral pool" strategies, employing a carbohydrate as starting material have been reported.

In 1996 Winchester and co-workers[121] presented a simple method for the preparation of all trihydroxypiperidines **163**, **164**, ***ent*-164** and **165** from 5-substituted aldonolactones/aldonic acid derivatives. When the latter compounds were treated with aqueous ammonia, 5-amino-5-deoxy-1,5-lactams were formed in a single step and subsequently reduced to target molecules (Scheme 3.1). It is worth noting that in this synthesis only cheap and ready available reagent have been used, and all compounds have been crystallized directly.

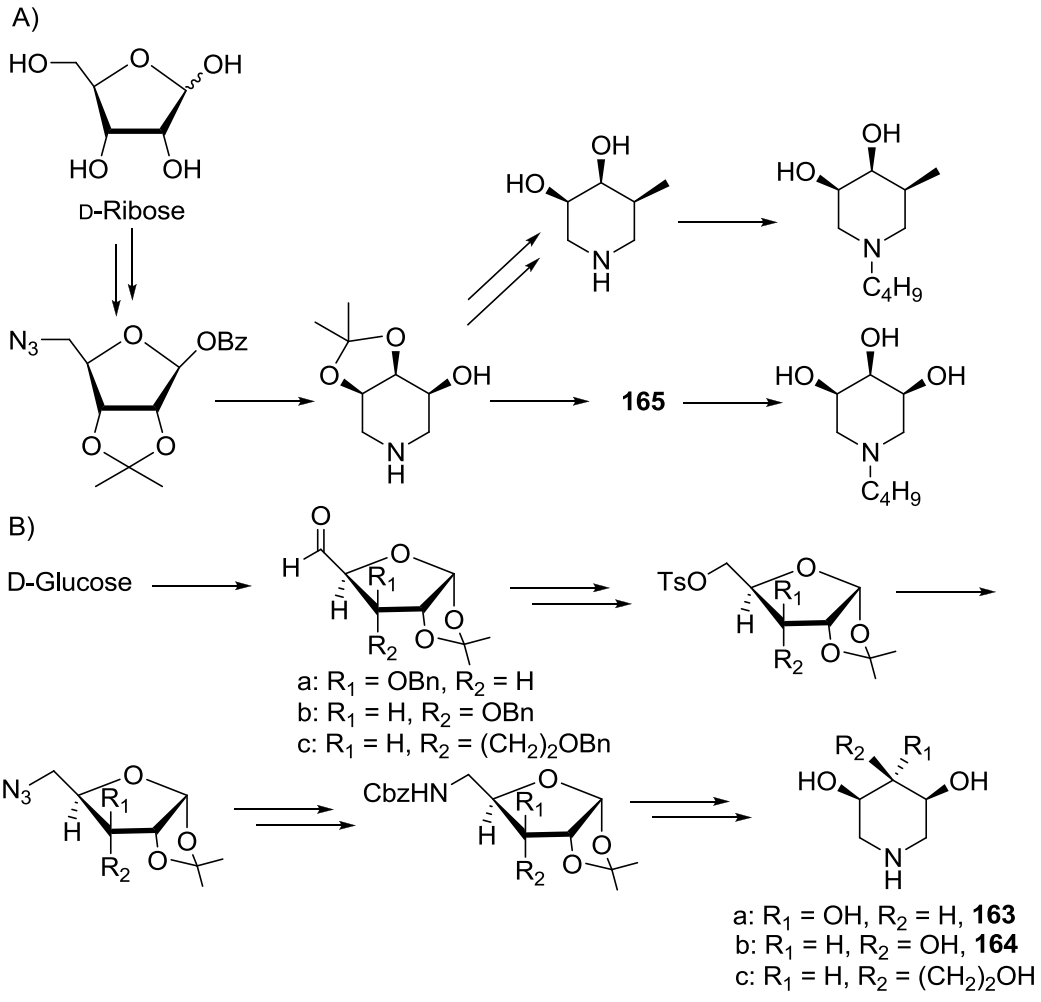
---

[115] K. S. Manning, D. G. Lynn, J. Shabanovitz, L. E. Fellows, M. Singh, B. D. Schrire, *J. Chem. Soc. Chem. Commun.* **1985**, 127-129.
[116] I. C. de Bello, P. Dorling, L. E. Fellows, B. Winchester, *FEBS Lett.* **1984**, *176*, 61-64.
[117] G. M. Makeev, M. I. Kumskov, N. D. Zelinsky, *Mendelev Commun.* **1996**, *1*, 27-29.
[118] T. Tsuruoka, H. Fukuyasu, M. Ishii, T. Usui, S. Shibahara, S. Inouye, *J. Antibiot.* **1996**, *49*, 155-161.
[119] T. Sekioka, M. Shibano, G. Kusano, *Nat. Med.* **1995**, *49*, 332–335.
[120] R. C. Bernotas, G. Papandreou, J. Urbach, B. Ganem, *Tetrahedron Lett.* **1990**, *31*, 3393–3396.
[121] M. Godskesen, I. Lundt, R. Madsen, B. Winchester, *Bioorg. Med. Chem.* **1996**, *4*, 1857–1865.



Scheme 3.1: Synthetic strategy reported by Winchester. In a similar manner compounds **164** and **165** were obtained from D-ribonolactone while D-xylonic acid was converted in **163** in five steps.

At the same time, Ichikawa and co-workers[122] evaluated the glycosidase inhibitor activity of many natural trihydroxypiperidines and their synthetic analogues obtained through intramolecular reductive amination of azido derivatives, resulting from different pentoses such as D-ribose (Scheme 3.2A) or D-lyxose.

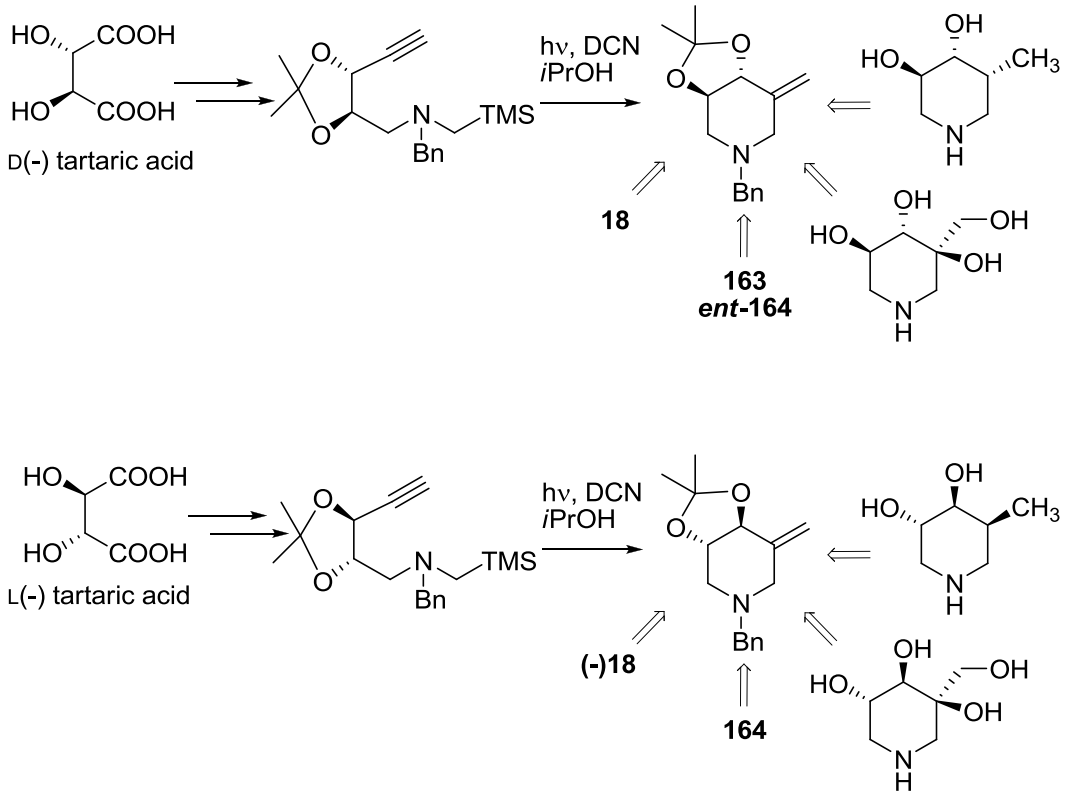


Scheme 3.2: Example of "chiral pool" synthetic strategy by Ichikawa (A) and Dhavale (B).

[122] a) Y. Igarashi, M. Ichikawa, Y. Ichikawa, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 553–558; b) Y. Ichikawa, Y. Igarashi, M. Ichikawa, Y. Sahara, *J. Am. Chem. Soc.* **1998**, *120*, 3007–3008.

Furthermore, Dhavale and co-workers[123] exploited a very similar azido intermediate to synthesize trihydroxypiperidines **163**, **165** and its analogue with a hydroxyethyl substituent on C-4, starting from D-glucose (Scheme 3.2B).

From 2002, Pandey group[124] also provided examples of "chiral pool" synthesis not employing carbohydrates as starting materials, in order to overcome the typical lack of versatility of these strategies. In particular, two general precursors were obtained using enantiopure tartaric acids. The piperidine skeleton was synthesized *via* PET (photoinduced electron transfer)-mediated cyclization of α-trimethylsilylmethylamine radical cation to a tethered acetylene functionality (Scheme 3.3). Starting from D- tartaric acid complete synthesis of isofagomine, natural compounds **163**, ***ent*-164** and other analogues functionalized at C-5 were reported. In a similar manner, employing L-tartaric acid as starting material the corresponding enantiomeric azasugars were prepared.



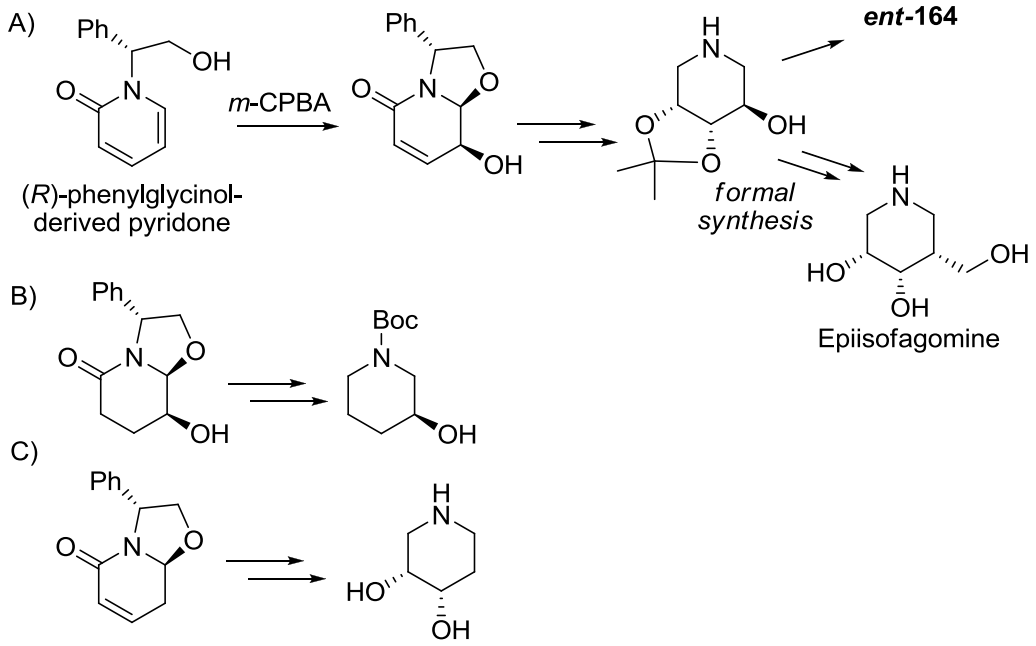
Scheme 3.3: Example of "chiral pool" synthetic strategy starting from D- and L-tartaric acid, from the Pandey group.

[123] N. T. Patil, S. John, S. G. Sabharwal, D. D. Dhavale, *Bioorg. Med. Chem.* **2002**, *10*, 2155–2160.
[124] a) G. Pandey, M. Kapur, *Org. Lett* **2002**., *4*, 3883-3886; b) G. Pandey, M. Kapur, M. I. Khan, S. M. Gaikwad, *Org. Biomol. Chem.* **2003**, *1*, 3321–3326; c) G. Pandey, K. C. Bharadwaj, M. I. Khan, K. S. Shashidhara, V. G. Puranik, *Org. Biomol. Chem.* **2008**, *6*, 2587–2595.

3.1.2 Enantioselective strategies

The syntheses of polyhydroxylated piperidines starting from carbohydrates require, in general, a large number of steps to reach the specific target and lack generality for diversity oriented syntheses. Thus, the development of new methods for the enantioselective synthesis of these compounds have involved many groups and still constitutes an area of great interest.

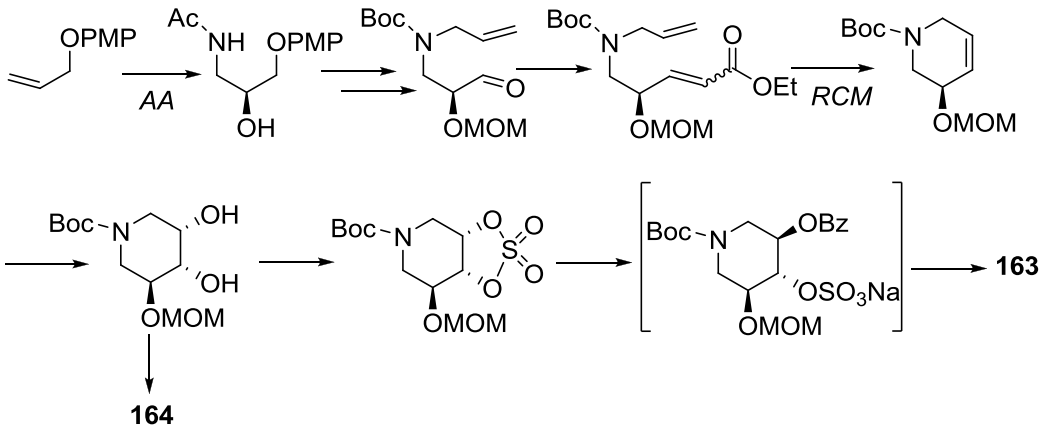
In the early 2000, Bosh and co-workers[125] demonstrated the potential of chiral non racemic bicyclic lactams derived from (*R*)-or (*S*)-phenylglycinol as versatile building block for the enantioselective synthesis of ***ent*-164**, as well as of the monohydroxy (C-3) and dihydroxy analogues (Scheme 3.4A-C).



Scheme 3.4: Example of enantioselective synthesis employing nonracemic bicyclic lactams by Bosh group.

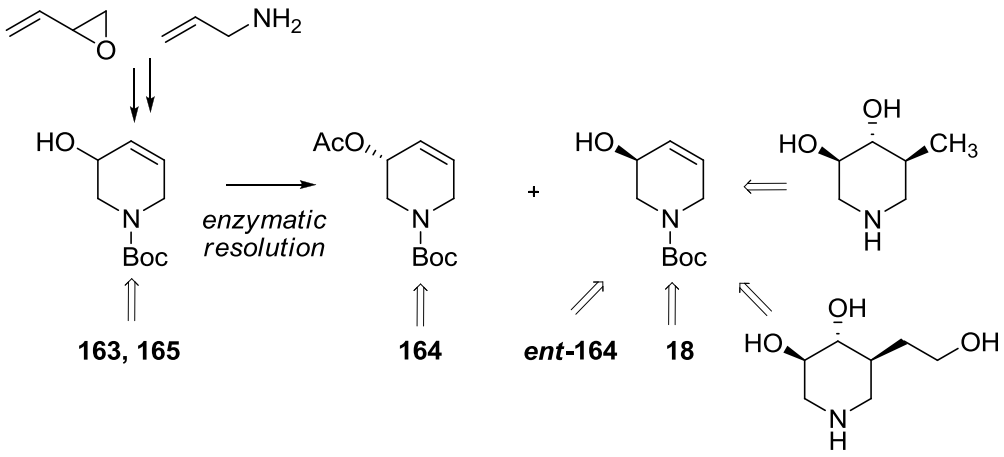
A few years later a complete asymmetric methodology for the synthesis of compounds **163** and **164** was developed by Han:[126] a readily available achiral olefin was transformed into the target molecules via regioselective asymmetric aminohydroxylation (AA), ring-closing metathesis (RCM), and diastereoselective dihydroxylation reactions by installing all stereocenters in a highly stereocontrolled fashion (Scheme 3.5).

[125] M. Amat, N. Lior, M. Huguet, E. Molins, E. Espinosa, J. Bosch, *Org. Lett* **2001**, *3*, 3257-3260.
[126] H. Han, *Tetrahedron Lett.* **2003**, *44*, 1567–1596.



Scheme 3.5: A complete asymmetric synthesis of polyhydroxypiperidines **163** and **164** by Han.

In 2005 Takahata and co-workers[127] provided a very general method for the synthesis of all the natural 3,4,5-trihydroxypiperidines **163-165** and some unnatural analogues starting from a chiral *N*-Boc-5-hydroxy-3-piperidene. This precursor was readily obtained in its racemic form from butadiene monoxide and allylamine in two steps and good yield. Its enzymatic resolution (achieved through the *Pseudomonas cepacia* lipase) allowed the synthesis of isofagomine, homoisofagomine and 5'-deoxyisofagomine via stereoselective epoxidation and regioselective ring-cleavage, together with the stereoselective synthesis of 3,4,5-trihydroxypiperidines **163**, **164**, ***ent*-164** and **165** (Scheme 3.6).

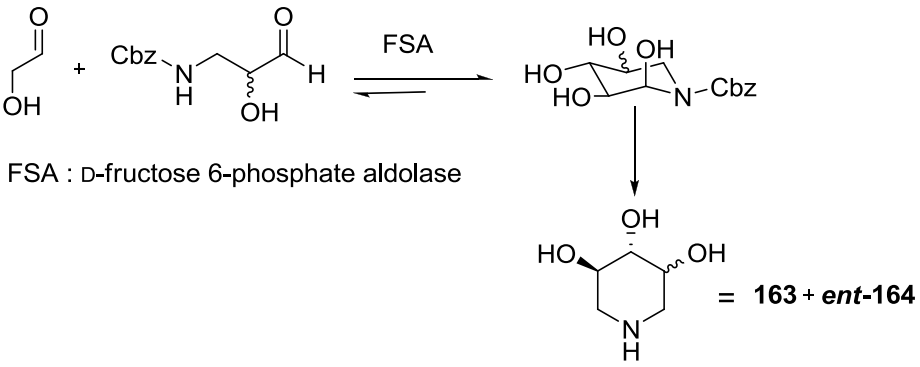


Scheme 3.6: Example of enantioselective synthesis by Takahata and co-workers.

A completely different approach for the synthesis of **163** and **164** was recently reported by Clapés and co-workers[128] in a more extensive study on the synthesis of polyhydroxylated compounds catalyzed by D-fructose 6-phosphate aldolase (FSA). In particular, a suitably *N*-protected aminohydroxy propanale resulted a good acceptor for a FSA catalyzed

[127] H. Ouchi, Y. Mihara, H. Takahata*, J. Org. Chem.* **2005***, 70*, 5207-5214.
[128] X. Garrabou, J. A. Castillo, C. Guérard-Hélaine, T. Parella, J. Joglar, M. Lemaire, P. Clapés, *Angew. Chem.* **2009**, *121*, 5629; *Angew. Chem. Int. Ed.* **2009**, *48*, 5521–5225.

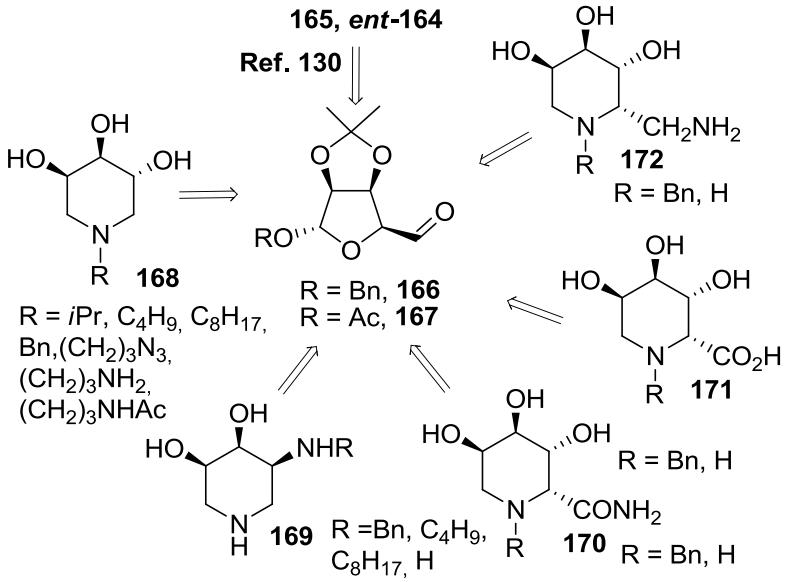
asymmetric cross-aldol reaction of glycolaldehyde, affording desired iminosugar cyclic derivatives in quantitative conversion. Subsequent removal of Cbz protecting group followed by reductive amination led to final compounds **163** and **164** (Scheme 3.7).



Scheme 3.7: Example of asymmetric cross-aldol reaction catalyzed by D-fructose-6-phosphate aldolase (FSA) reported by Clapés and co-workers.

3.1.3 Our Synthetic Strategy: Double Reductive Amination and Selective Strecker Reaction of a D-Lyxaric Aldehyde

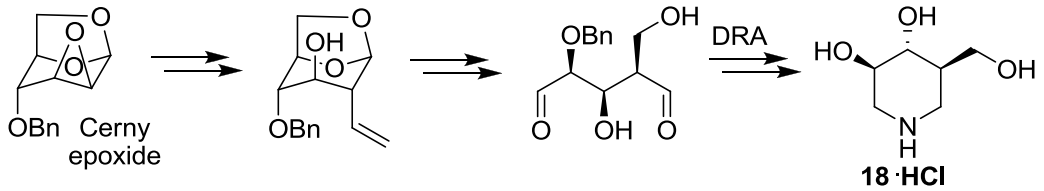
Following our ongoing interest in the synthesis of polyhydroxylated alkaloids and unnatural analogs, we recently envisaged in the D-mannose derived aldehyde **166**[129] a possible common building block for the synthesis of natural **165, *ent*-164** and some new unnatural *N*-alkylated analogs (Scheme 3.8) using a double reductive amination procedure (DRA).[130]



Scheme 3.8: Retrosynthetic analysis for the synthesis of polyhydroxyamino piperidines and pipecolic acid analogs from D-mannose derived aldehydes **166** and **167**.

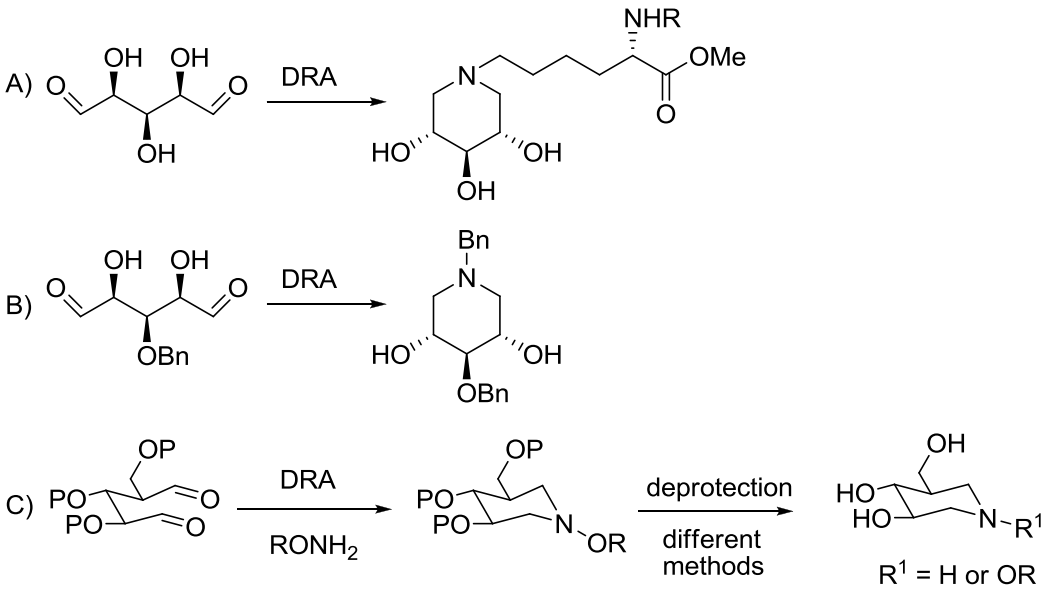
[129] F.-E. Chen, J.-F. Zhao, F.-J. Xiong, B. Xie, P. Zhang, *Carbohydr. Res.* **2007**, *342*, 2461–2464.
[130] Preliminary communication: C. Matassini, S. Mirabella, A. Goti, F. Cardona, *Eur. J. Org. Chem.* **2012**, 3920-3924.

While many examples of this reaction have been reported on diketones or ketoaldehyde derivatives,[131] the same approach with dialdehydes has been less exploited. In 1994 Bols reported the first synthesis of isofagomine, using ammonia as nitrogen source in the cyclization of a pentadialdose derived from Cerny epoxide, with a total 6% yield over 10 steps (Scheme 3.9).[132]



Scheme 3.9: Synthesis of isofagomine by Bols group: the double reductive amination reaction was performed employing ammonia as amine source and hydrogen as the reducing agent

In more recent years other few examples have been reported.[133] Wroding and coworkers exploited the cyclization via double reductive amination on *xylo*-pentadialdose with the respective amino group of lysine and serine derivatives, in order to synthesize some iminosugar-amino acid hybrids, with excellent inhibitory activity against β-xylosidase (Scheme 3.10A).[133a]



Scheme 3.10: Double reductive amination (DRA) strategies with dialdehydes reported until today by Wroding (A), Martin (B) and Crich (C).

[131] For a recent review, see: P. Compain, V. Chagnault, O. R. Martin, *Tetrahedron: Asymmetry* **2009**, *20*, 672-711.

[132] T. M. Jespersen, M. Bols, M. R. Sierks, T. Skrydstrup, *Tetrahedron* **1994**, *50*, 13449-13460.

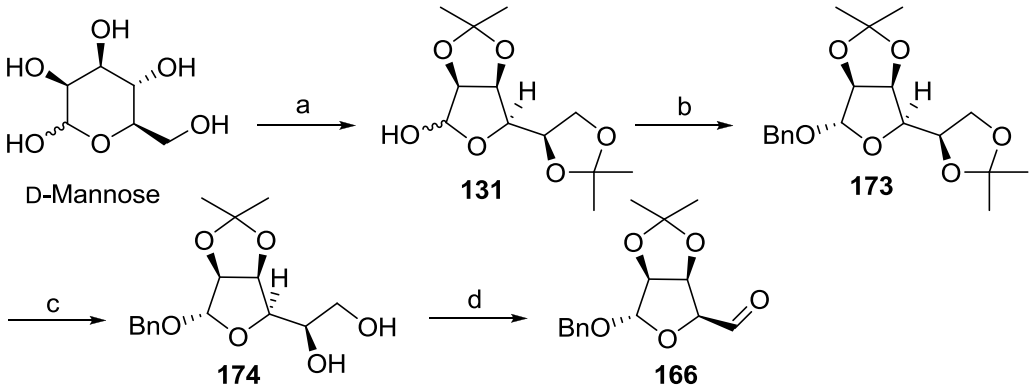
[133] a) A. J. Steiner, A. E. Stütz, C. A. Tarling, S. G. Withers, T. M. Wrodnigg, *Aust. J. Chem.* **2009**, *62*, 553-557; b) F. Oulaïdi, S. Front-Deschamps, E. Gallienne, E. Lesellier, K. Ikeda, N. Asano, P. Compain, O. R. Martin, *ChemMedChem* **2011**, *6*, 353-361; c) G. Malik, X. Guinchard, D. Crich, *Org. Lett.* **2012**, *14*, 596-599; d) G. Malik, A. Ferry, X. Guinchard, T. Cresteil, D. Crich, *Chem. Eur. J.* **2013**, *19*, 2168-2179.

A properly protected analogue of the same dihaldehyde has been subsequently employed by Martin and coworkers for the synthesis of a series of 2-*O*-alkyl-iminoxylitol derivatives, with promising chaperoning activity towards xylosidase (Scheme 3.10B).[133b] Finally, Crich and co-workers exploited the double reductive amination strategy on functionalized 1,5-dialdehydes with various hydroxylamines en route to the synthesis of polyhydroxylated *N*-alkoxypiperidines xylosidase (Scheme 3.10C).[133c,d]Our early encouraging results[130] led us to continue with the exploitation of the scope of this strategy, which allowed a versatile access to novel 1-*N*-alkyl polyhydroxypiperidines **168** and amino piperidines **169** (Scheme 3.8). A change in the anomeric protecting group on aldehyde **166** gave us the opportunity to exploit the Strecker reaction on aldehyde **167** en route to the synthesis of new polyhydroxypipecolic acid analogs **170-171** and 6-aminopiperidine iminosugars **172** (Scheme 3.8). The results of the Strecker reaction on **167** also furnished a firm proof of the mechanism proposed for the stereoselective formation of 2-cyano piperidines as side products of the double reductive amination reaction.[130]

3.2 Results and Discussion

3.2.1 Synthesis of N-alkyl piperidines and 5-amino piperidines vi the DRA strategy

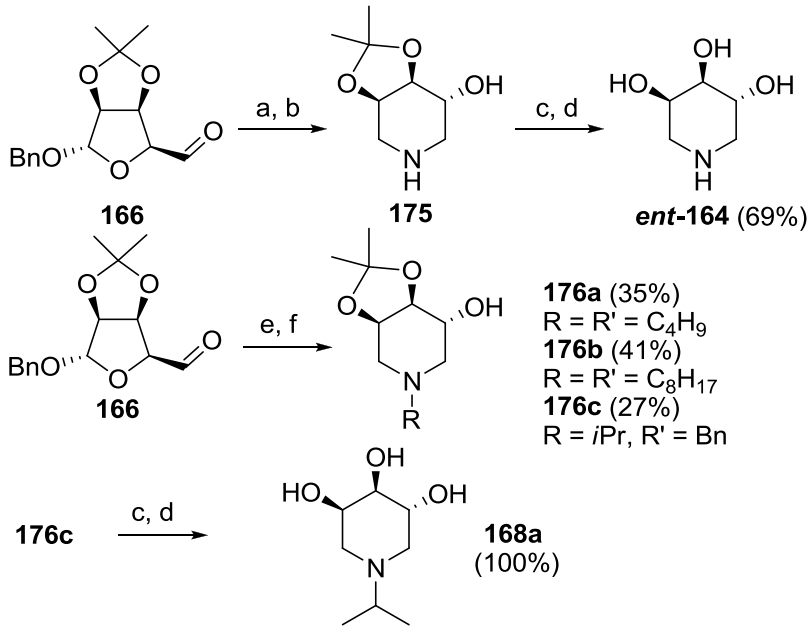
The aldeyde **166** was easily synthesized in four steps and 80% overall yield from D-mannose without the need of any chromatographic purification, by following a slight modification of the published procedure[129] (Scheme 3.11).



Scheme 3.11: Synthesis of aldehyde **166** from D-mannose. Reagents and conditions: a) Acetone, I_2, rt, 2h; b) KOH, BnBr, THF, 18-crown-6, rt, 16h; c) CH_3COOH, H_2O, rt, 18 h; d) Silica Gel Supported, $NaIO_4$, CH_2Cl_2, rt, 50 min, 80% over four steps.

Our first aim was the introduction of lipophilic chains on the nitrogen atom of the target piperidine iminosugars, which is expected to improve the pharmacokinetics properties in vivo and the suitability of such molecules as pharmacological chaperones.[134]

We initially investigated the reductive amination reaction on **166** using different amine sources and hydrogen as the reducing agent, since this could allow removal of the benzyl protecting group at the anomeric position of **166** in the same step and therefore the liberation of the second aldehyde moiety necessary to perform the required cyclization. However, this reaction suffered from low reproducibility in our hands, it proved to be scarcely versatile and also showed unexpected drawbacks. Most of the problems encountered probably derived from the inclination of **166** to undergo quick hydration by air humidity, that hampers the reaction. Indeed, the reaction of **166** with benzylamine (1 equiv.) in MeOH in the presence of 3 Å molecular sieves, followed by treatment with $Pd(OH)_2/C$ and H_2 (balloon) for 25 h was successful, affording piperidine ***ent*-164** after deprotection of the acetonide protecting groups (**175**) in HCl/MeOH in 69% overall yield (Scheme 3.12).[130]

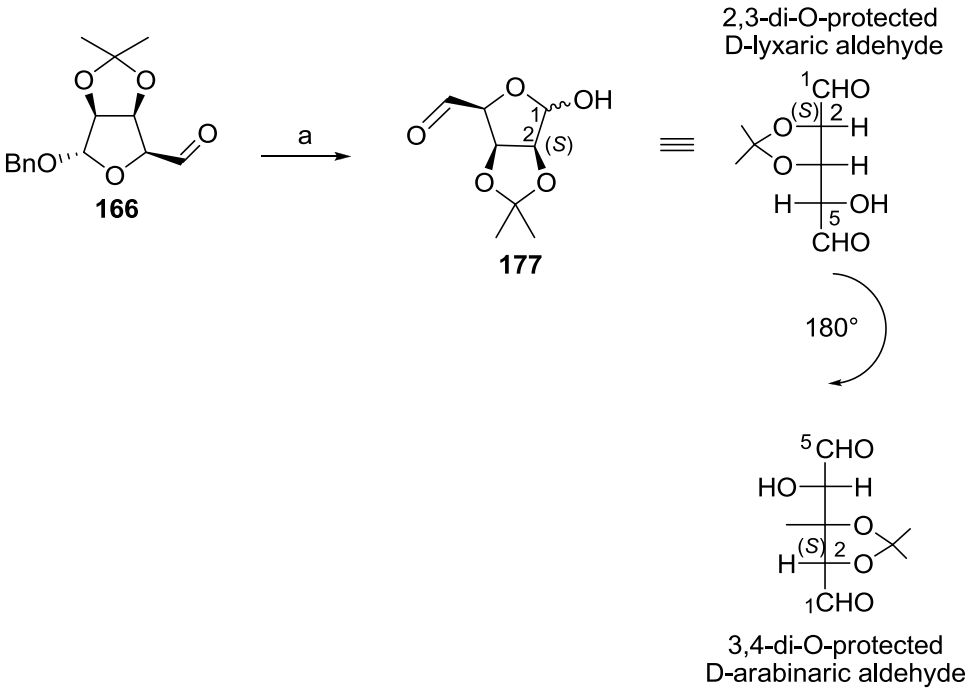


Scheme 3.12: The double reductive amination strategy using aldehyde **166** and hydrogen as reducing agent. Reagents and conditions: a) $BnNH_2$, MeOH, 3 Å MS, rt, 40 min; b) $Pd(OH)_2/C$, MeOH, H_2, rt, 25 h; c) MeOH, 1M HCl; d) DOWEX 50WX8, 100%; e) R'NH2, MeOH, 3 Å MS, CH_3CO_2H, f) $Pd(OH)_2/C$, MeOH, H_2, rt, 25 h; rt, 7 days.

[134] D. S. Alonzi, R. A. Dwek, T. D. Butters, *Tetrahedron: Asymmetry* **2009**, *20*, 897-901; b) G. Godin, P. Compain, O. R. Martin, K. Ikeda, L. Yu, N. Asano, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5991-5995; c) T. S. Rasmussen, A. Allman, G. Twigg, T. D. Butters, H. H. Jensen, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1519-1522; d) L. Yu, K. Ikeda, A. Kato, I. Adachi, G. Godin, P. Compain, O. Martin, N. Asano, *Bioorg. Med. Chem.* **2006**, *14*, 7736-7744; e) Z. Yu, A. R. Sawkar, L. J. Whalen, C.-H. Wong, J. W. Kelly, *J. Med. Chem.* **2007**, *50*, 94-100.

However, the same protocol employing butylamine or octylamine required more prolonged reaction times (7 days) and the presence of 2 equiv. of AcOH to achieve complete conversion of the starting aldehyde, and piperidines **176a** and **176b** were obtained in moderate overall yields (35% and 41%, respectively). Moreover, upon reaction of **166** with benzylamine and AcOH for 4 days, *N*-isopropyl piperidine **176c** was isolated in 27% yield (Scheme 3.12), probably deriving from partial acetonide deprotection and subsequent reductive amination of the acetone liberated in situ with the formed piperidine. Therefore, in this case a one-pot triple reductive amination occurred, together with debenzylation and acetonide deprotection. The low yield, however, in a process composed of six consecutive reactions which may afford a 50% maximum yield, may be ascribed to formation of oligomers by cross reductive amination reactions. Deprotection of **176c** performed as usual afforded compound **168a** in quantitative yield.

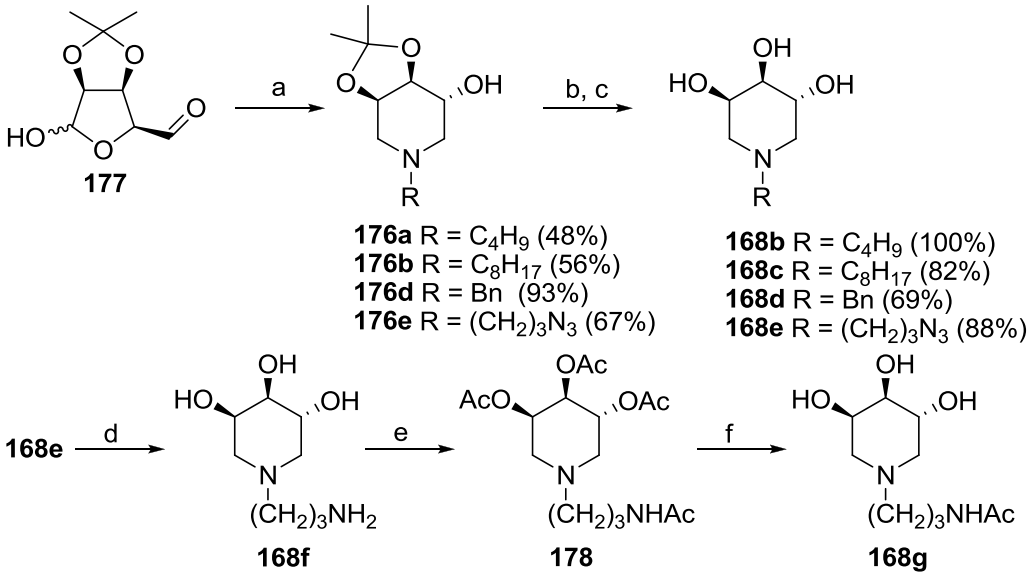
Due to problems encountered with the reductive amination strategy on aldehyde **166**, we sought to perform the same task by working on dialdehyde **177**, obtained quantitatively by catalytic hydrogenation of **166**. It is worth noting that this compound can be viewed (in its open-chair form) either as a 2,3-di-*O*-protected D-lyxaric aldehyde or a 3,4-di-*O*-protected D-arabinaric aldehyde (Scheme 3.13).



Scheme 3.13: Deprotection of aldehyde **166** led to dialdehyde **177**, here represented in its open–chair forms. Reagents and conditions: a) $Pd(OH)_2/C$, H_2, MeOH, rt, 4 h, 100%.

In solution, the ^{1}H NMR spectrum of **177** was rather complicated, showing the presence of a complex mixture of different forms.

In our hands, the reaction of dialdehyde **177** with different amine sources (0.9 equiv.), $NaBH_3CN$ (3.0 equiv.) and AcOH (2 equiv.)[133b] was much more reliable and reproducible than the above mentioned hydrogenation procedure on masked dialdehyde **166**, affording protected piperidines **176a-b** in slightly higher yields (48% and 56%, respectively) and the *N*-benzyl substituted piperidine **176d,** obviously not achievable through a catalytic hydrogenation procedure, in excellent yield (93%). Deprotection of **176a-b** and **176d** with methanolic HCl followed by ion-exchange resin afforded the *N*-alkylated piperidines **168b-d** (Scheme 3.14). The use of $NaBH_3CN$ instead of H_2 as the reducing agent for the double reductive amination on **177** also allowed the synthesis of piperidines functionalized on the alkyl chain. The reaction of **177** with 1 equivalent of 3-azidopropyl-1-amine,[135] $NaBH_3CN$ (3.0 equiv.) and AcOH (2 equiv.) afforded *N*-alkylated piperidine **176e** in 67% yield (Scheme 3.14), which upon usual deprotection performed gave compound **168e** in 90% yield.



Scheme 3.14: The double reductive amination strategy using dialdehyde **177** and $NaBH_3CN$ as the reducing agent. Reagents and conditions: a) RNH_2, MeOH, 3 Å MS, $NaBH_3CN$, CH_3CO_2H, rt, 3-7 days; b) MeOH, HCl, rt; 18; c) DOWEX 50WX8; d) $Pd(OH)_2/C$, MeOH, HCl, rt, 3 days, 100%; e) Ac_2O, Py, rt, 18 h, 86%; f) Ambersep® 900-OH, MeOH, rt 2h, 71%.

Catalytic hydrogenation followed by ionic exchange resin eluting with 15% NH_4OH afforded the primary amine **168f**, which curiously presented split signals (both in ^{1}H and ^{13}C NMR spectra) for the propyl chain atoms. This phenomenon can be ascribed to an high tendency of this compound to protonation, possibly through reaction with atmospheric CO_2 as

[135] O. E. Vercillo, C. K. Z. Andrade, L. A. Wessjohann, *Org. Lett.* **2008**, *10*, 205-208.

evidenced by the ^{13}C NMR spectra that showed signals around δ = 160 ppm. Moreover an intramolecolar cyclization, via hydrogen bond, of the endocyclic nitrogen with the primary amine of the propyl chain (Figure 3.3) to form a six-membered ring, cannot be excluded.

Complete acetylation by reaction of **168f** with an excess of acetic anhydride in pyridine afforded **178** in 86% yield. Final treatment with the strongly basic resin Ambersep 900 OH in MeOH gave the trihydroxypiperidine **168g** in quantitative yield.

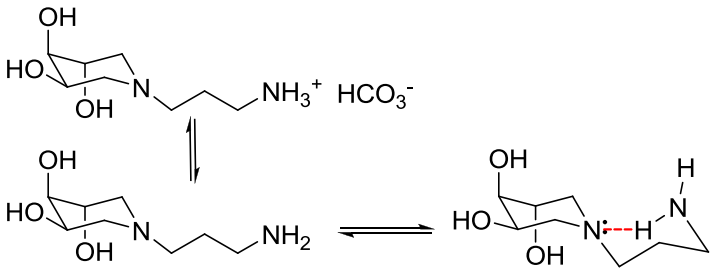
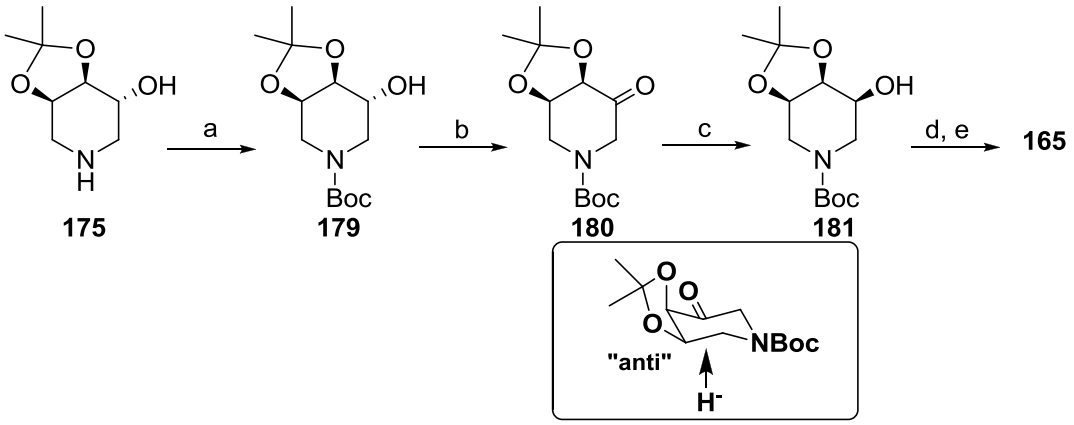


Figure 3.3: Hypothetical salification and cyclization of amino-propyl chain of compound **168f.**

Compound **176e** has been successively employed in the synthesis of a multivalent compound with an alkyne tetravalent scaffold (see below *Chapter 3.2.3*), while the amino derivative **168f** has been conjugate with a proper thiol linker for further multimerization on gold nanoparticles (see *Chapter 4*).

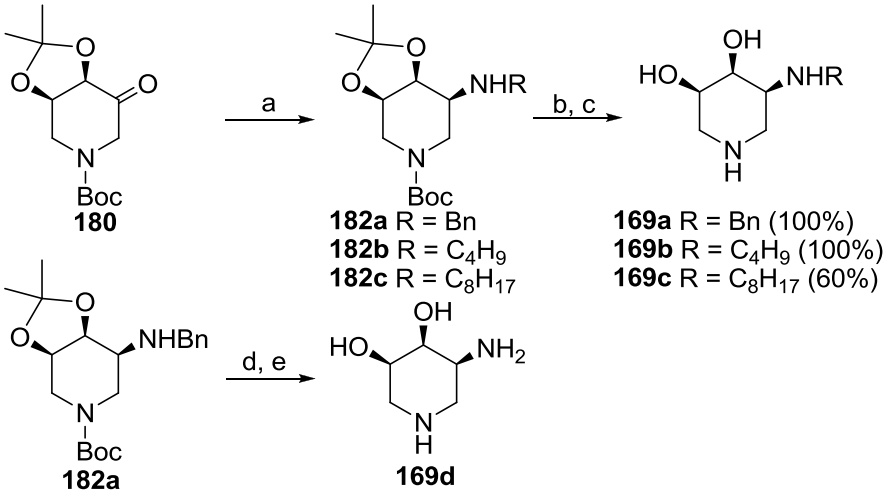
In order to access natural trihydroxypiperidine **165**, an inversion of configuration at C5-OH in compound **179** was required to access This was achieved through an oxidation-reduction procedure, since Mitsunobu conditions failed on the Boc protected piperidine **179**. Therefore, oxidation of **179** with the Dess-Martin periodinane followed by $NaBH_4$ reduction of ketone **180** afforded stereoselectively alcohol **181**, that gave **165** upon deprotection in acidic medium (Scheme 3.15).



Scheme 3.15: The oxidation-reduction procedure: synthesis of natural **165**. Reagents and conditions: a) $NaHCO_3$, Boc_2O, $MeOH/H_2O$, rt, 48 h, 93%; b) Dess-Martin, CH_2Cl_2, rt, 2h, 98%; c) $NaBH_4$, EtOH, rt, 18 h, 76%; d) MeOH, HCl, rt, 18 h; e) DOWEX 50WX8, 100%

We envisaged in ketone **180** a suitable intermediate to introduce an exocyclic amino moiety in the piperidine skeleton affording piperidines **182**. Indeed, the high stereoselectivity observed in the reduction of **180** to **181** by the hydride anion, deriving from a favoured axial attack to C=O at C-5 anti to the vicinal C-O bond according to a Felkin-Anh model, could be exploited in the reduction of a C=N bond formed by reaction of **180** with different amines.

Therefore, we investigated the reductive amination reaction on ketone **180** (Scheme 3.16). We initially employed the best reaction conditions previously found for the synthesis of piperidine **176d** from dialdehyde **177**: dry MeOH, 3Å molecular sieves, $BnNH_2$ (0.9 equiv.), $NaBH_3CN$ (3.0 equiv.) and AcOH (2.0 equiv.). After 6 days the conversion was complete, but unexpectedly a 1:2 mixture of the desired aminopiperidine **182a** together with alcohol **181** was recovered (Table 3.1, Entry 1). Employing a slight excess of $BnNH_2$ (1.1 equiv.) and waiting 40 minutes before addition of $NaBH_3CN$ in lower excess (1.5 equiv.) gave a similar result (Table 3.1, Entry 2). The critical point in this reaction was the formation of the imine intermediate, since the chemoselectivity of $NaBH_3CN$ in this reaction appeared quite low. The formation of alcohol **181** could be completely suppressed by stirring a mixture of **180**, $BnNH_2$ (1.5 equiv.) and AcOH (2 equiv.) for 3 hours before the addition of $NaBH_3CN$, thus assuring complete formation of the imine intermediate (Table 3.1, Entry 3) prior to hydride attack.



Scheme 3.16: The oxidation-reduction procedure, synthesis of amino piperidines **169**. Reagents and conditions: a) RNH_2, Reducing agent (see Table 3.1); b) MeOH, HCl, rt, 18 h; c) DOWEX 50WX8; d) Pd/C, H_2, HCl, MeOH, rt, 6 days; e) DOWEX 50WX8, 100%.

Under these conditions, amine **182a** could be recovered in a satisfying 48% yield after purification on silica gel. Due to the low chemoselectivity of $NaBH_3CN$, alkylpiperidines **182b** and **182c** were more easily accessed by employing hydrogen as the reducing agent. Ketone

180 was left stirring in MeOH, with 3Å molecular sieves and the suitable amine for 40 min, then $Pd(OH)_2/C$ was added and the mixture was stirred under H_2 (baloon) for 20-24 h. Both reactions (Table 3.1, Entries 4-5) gave the desired amines in good yields. As a matter of fact, hydrogenation also showed complete diastereofacial selectivity, as a consequence of steric hindrance to attack at the opposite face.

Entry	RNH_2 (equiv.)	Reducing agent (equiv.)	Time (h)	182/181[a)] ratio	Yield of 182 (%)
1[b)]	$BnNH_2$ (0.9)	$NaBH_3CN$ (3.0)	144	1:2	17
2[c)]	$BnNH_2$ (1.1)	$NaBH_3CN$ (1.5)	20	1:2	19
3[d)]	$BnNH_2$ (1.1)	$NaBH_3CN$ (3.0)	25	>98:2	48
4	$C_4H_9NH_2$ (1.5)	$Pd(OH)_2/C$, H_2	24	>98:2	68
5	$C_8H_{17}NH_2$ (1.5)	$Pd(OH)_2/C$, H_2	20	>98:2	53

Table 3.1: The reductive amination of ketone **180** in MeOH. [a)]Determined by isolation of compounds after flash column chromatography. [b)]All reagents, including 3Å MS and AcOH (2.0 equiv.) were mixed together at the same time. [c)]Ketone, amine and 3Å MS were left stirring for 40 min before addition of $NaBH_3CN$ and AcOH (2.0 equiv.). [d)]Ketone, amine, 3Å MS and AcOH (2.0 equiv.) were left stirring for 3 h before addition of $NaBH_3CN$.

Final treatment with methanolic HCl followed by ion-exchange resin afforded the new *N*-alkylamino piperidines **169a-c** (Scheme 3.16) in good to quantitative yields. Moreover, 5-amino piperidine **169d** was also accessed in quantitative yield by catalytic hydrogenation under acid conditions of compound **182a**: the acetonide protective group, the Boc protection and the benzyl group were all removed in the same step.

The structure of the aminopiperidines **169** was established on the basis of a careful analysis of their ^{1}H NMR and 1D NOESY spectra. In particular, for **169d** a strong NOE correlation peak between 3-H and 5-H was observed (Figure 3.4), which also established the structure of **169a** since both compounds derive from **182a**. Moreover, the ^{1}H NMR spectrum of **169d** showed a pseudo triplet with J = 2.5 Hz for 4-H, in agreement with two *eq-ax* relationships with both 3-H and 5-H. For piperidines **169b** and **169c**, due to the superimposition of corresponding NMR signals, 1D NOESY spectra were not useful for structural assignment. However, the analysis of the ^{1}H NMR spectrum showed a broad singlet for 4-H in both **169b** and **169c**, that is in agreement with two *eq-ax* relationships with both 3-H and 5-H. In

contrast, the ^{1}H NMR spectrum of **168** (with the opposite configuration at C-5) showed a dd for 4-H with *J* = 8.3 Hz and *J* = 2.9 Hz, in agreement with an *ax-ax* relationship and an *ax-eq* relationship with the vicinal protons (Figure 3.4).

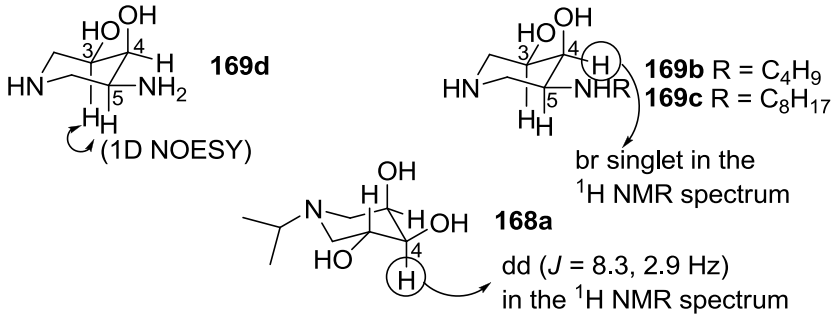


Figure 3.4: Structural assignments by 1D NOESY and ^{1}H NMR spectra of piperidines **169** and **168**. Only the most likely chair conformations with two equatorial and one axial substituents are reported. The alternative chair conformation does not affect the reported conclusion.

3.2.2 Stereoselective Strcker Reaction

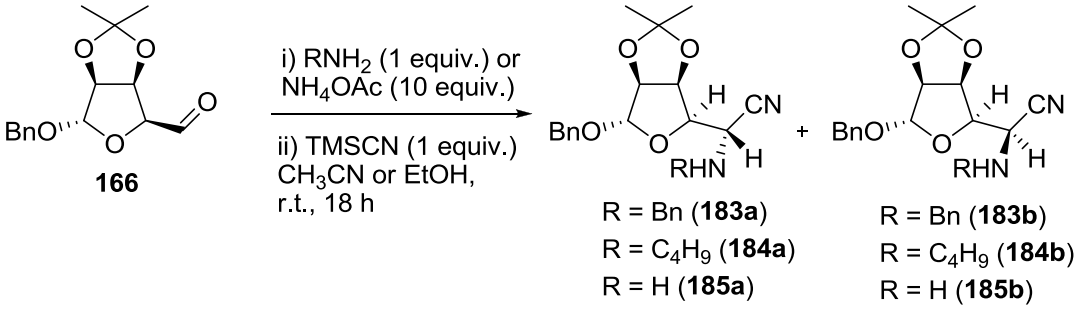
Another synthetic strategy which could provide access to functionalized piperidines from aldehyde **166**, and in particular to compounds **172-170** (Scheme 3.9), was the Strecker reaction followed by deprotection at the anomeric carbon and cyclization. Indeed, we envisaged that aldehyde **166** would afford piperidines functionalized at the α-position with a cyano group,[136] a motif also found to occur in natural products with antitumor activity such as Saframycin A, and in related synthetic hybrids.[137] We reasoned that such compounds could be obtained regioselectively by a Strecker reaction performed on the "masked" dialdehyde **166**.

The Strecker reaction, discovered in 1850, [138] is one of the most efficient and straightforward methods for the synthesis of aminonitriles,[139] which are equivalent synthones to imminium moieties and acyl anions. These compounds are versatile intermediates for the synthesis of α-amino acids, 1,2-diamines, amides, and various nitrogen-containing heterocycles such as thiazoles and imidazoles. Moreover, due to the importance of obtaining optically active α-amino acids, enantioselective version of Strecker reaction has been widely investigated, exploiting chiral auxiliary based methods (i.e.

[136] D. Enders, J. P. Shilvock, *Chem. Soc. Rev.* **2000**, *29*, 359-373.
[137] E. J. Martinez, E. J. Corey, *Org. Lett.* **1999**, *1*, 75-77.
[138] A. A. Strecker, *Ann. Chem. Pharm.* **1850**, *75*, 27-45.
[139] a) D. Enders, J. P. Shivock, *Chem. Soc. Rev*. **2000**, *29*, 359-373; b) M. North, *Angew. Chem. Int. Ed*. **2004**, *43*, 4126-4128.

carbohydrate auxiliary, chiral amine auxiliary and cyclic ketamine auxiliary) or catalytic asymmetric synthesis (by using metal-based or metal-free catalysts).[140]

In our case, due to the chirality of **166**, we expected to obtain diastereoselective Strecker reaction. α-Aminonitriles are generally prepared by the nucleophilic addition of cyanide ion to the imines using different Lewis acid or base catalysts.[140a,141] A variety of cyanating agents such as trimethylsiloxy nitriles and diethylphosphorocyanidate have been used for the preparation of α-amino nitriles under various conditions.[142] In particular, we chose trimethylsilyl cyanide (TMSCN) since is a safe, easy-to handle and very effective cyanide anion source for the one pot synthesis of α-amino nitriles.[143]



Entry	RNH_2 /$AcONH_4$	Solvent	a/b[a)]	Yield of a (%)[b)]
1	$BnNH_2$	MeCN[c)]	>95/5	100[d)] (80)[e)]
2	$BuNH_2$	MeCN[c)]	>95/5	100[d)] (75)[e)]
3[f)]	$AcONH_4$	MeCN	62:38	-
4[f)]	$AcONH_4$	EtOH	81:19	61

Table 3.2: Strecker reaction of aldehyde **166**. a) Determined by ^{1}H NMR spectroscopic analysis of the crude mixture; b) Isolated yield after flash column chromatography; c) Anhydrous solvent and 3 Å MS added; d) Yield of the crude, >95% purity as evaluated by ^{1}H NMR spectroscopy; e) Addition of 1.0 mol% of $Cu(OTf)_2$; f) 1.2 equiv. of TMSCN.

[140] a) H. Gröger, *Chem. Rev.* **2003**, *103*, 2795-2827; b) P. Merino, P. E. Marqués-López, T. Tejero, R. P. Herrera, *Tetrahedron* **2009**, *65*, 1219-1234.

[141] a) B. A. B. Prasad, A. Bisai, V. K. Singht, *Tetrahedron Lett*. **2004**, *45*, 9565-9567; b) J. S. Fossey, C. J. Richards, *Tetrahedron Lett.* **2003**, *44*, 8773-8776; c) E. Takahashi, H. Fujisawa, T. Yanai, T. Mukaiyama, *Chem. Lett*. **2005**, *34*, 318-319.

[142] a) K. Mai, G. Patil, *Tetrahedron Lett.* **1984**, *25*, 4583-4586; b) S. Harusawa, Y. Hamada, T. Shioiri, *Tetrahedron Lett.* **1979**, *20*, 4663-4666**.**

[143] a) T. K. Chakraborty, G. V. Readdy, K. A. Hussain, *Tetrahedron Lett.* **1991**, *32*, 7597-7600; J. P. Leblanc, H. W. Gibson, *Tetrahedron Lett.* **1992**, *33*, 6295-6298.

Aldehyde **166** was first treated with benzylamine (1.0 equiv.) and TMSCN (1.0 equiv.) in dry acetonitrile with the addition of 1.0 mol% of $Cu(OTf)_2$ as catalyst.[144] We were delighted to observe that mainly one diastereoisomer was formed, in remarkable diastereomeric excess (>95:5; Table 3.2, Entry 1). A similar result was observed with $BuNH_2$ as the amine source (Table 3.2, Entry 2). However, the presence of a Lewis acid catalyst was found unnecessary (Table 3.2, Entries 1 and 2), with cleaner products obtained under these conditions that did not require further purification by flash column chromatography. By employing 10 equiv. of NH_4OAc[145] as source of ammonia in acetonitrile (Table 3.2, Entry 3), adduct **185a** was obtained in 62:38 diastereoselectivity. A change to EtOH led to an improvement in the diastereoselectivity (81:19; Table 3.2, Entry 4) and a quite good isolated yield (61%) of **185a** was also achieved. The (*S*) configuration at the newly formed stereocentre was unambiguously assigned for **185a** on the basis of a single crystal X-ray analysis (Figure 3.5) and attributed accordingly to **183a**, **184a**.

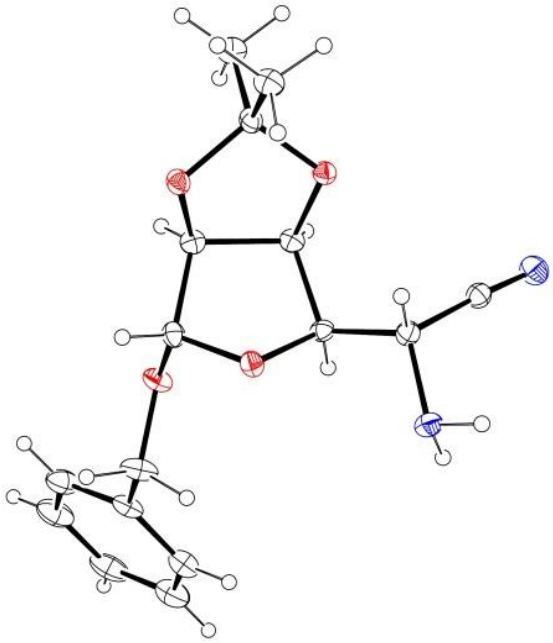


Figure 3.5: X-ray crystal structure of **185a**. These data can be obtained free of charge from The cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[144] A. S. Paraskar, A. Sudalai, *Tetrahedron Lett.* **2006**, *47*, 5759–5762.

[145] a) A. L. Win-Mason, E. M. Dangerfield, P. C. Tyler, B. L. Stocker, M. S. M. Timmer, *Eur. J. Org. Chem.* **2011**, 4008–4014; b) A. L. Win-Mason, S. A. K. Jongkees, S. G. Withers, P. C. Tyler, M. S. M. Timmer, B. L. Stocker, *J. Org. Chem.* **2011**, *76*, 9611–9621.

This stereochemical outcome was in agreement to our expectation and can be rationalized by a preferred approach of cyanide through a Cram chelated transition state (Figure 3.6), where chelation involves the iminium proton and the endocyclic oxygen atom of the sugar to give a five-membered ring. According to this model, the cyanide anion would attack from the less hindered *Si* face of the double bond, thus affording adducts **183a, 184a** and **185a** with the *S* absolute configuration at the newly formed stereocentre with high preference.

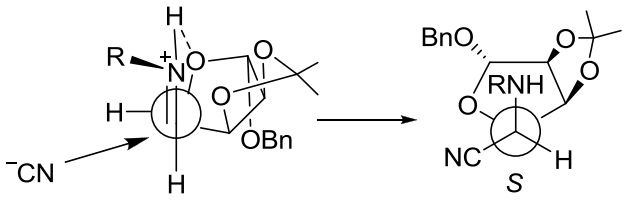
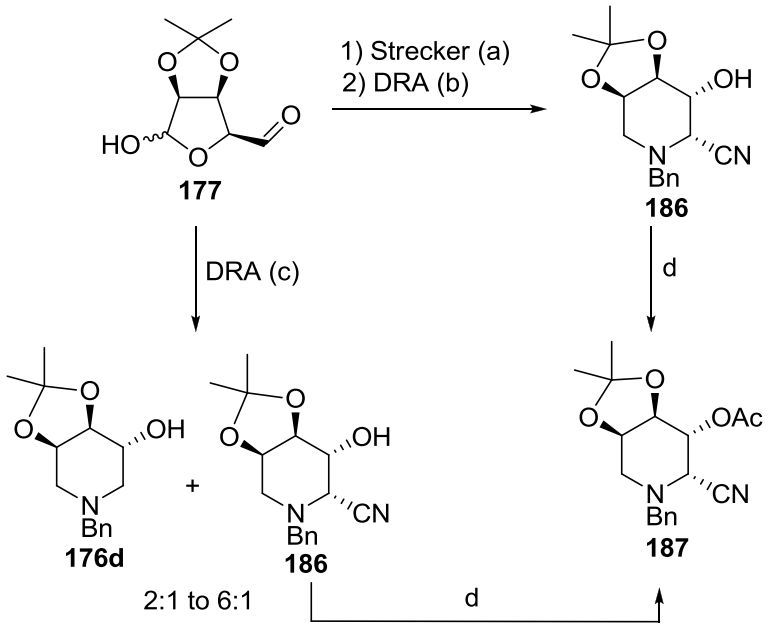


Figure 3.6: Cram chelate model for the Strecker reaction on **166** affording stereoselectively α-aminonitriles **183a**, **184a**, **185a**.

This stereochemical outcome is in agreement to a recent proposal for the nucleophilic attack to acyclic polyhydroxylated aldeyhdes.[145]

Unfortunately, attempted deprotection at the anomeric position by catalytic hydrogenation on α-aminonitriles **183a-185a**, which would induce subsequent cyclization, failed both under neutral and acidic conditions and employing different solvents (MeOH, EtOH, CH_3CN), probably due to catalyst deactivation by the cyanide group. However, the direct Strecker reaction with benzylamine on dialdehyde **177** followed by cyclization and in situ reduction of the iminium ion intermediate by $NaBH_3CN$, afforded highly regio- and stereoselectively the 2-cyano piperidine **186** which was recovered in 50% yield (Scheme 3.17) after purification over silica gel. Compound **186** had been previously observed as side product formed in the reductive amination of **177** with $NaBH_3CN$ under neutral conditions.[130] Their identity was ascertained by ^{1}H NMR comparison of the corresponding acetylated derivative **187** (Scheme 3.17). The formation of cyano-substituted compounds as by-products during reductive amination reactions with $NaBH_3CN$ under neutral or basic reaction conditions has been occasionally reported.[146] Following these results, we considered that the regio- and stereoselective formation of compound **186** from **177** during the reductive amination derived from a Strecker reaction occurring at the more reactive aldehyde at C-5 followed by intramolecular reductive amination at C-1.

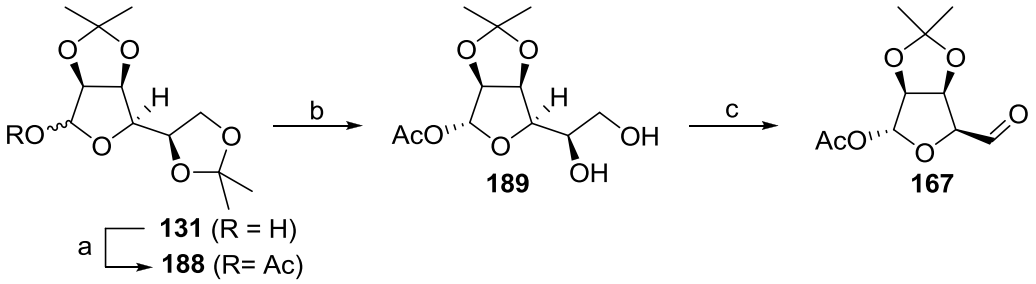
[146] a) J. Neumann, S. Weingarten, J. Thiem, *Eur. J. Org. Chem.* **2007**, 1130–1144; b) J.-P. Liou, Z.-Y. Wu, C.-C. Kuo, C.-Y. Chang, P.-Y. Lu, C.-M. Chen, H.-P. Hsieh, J.-Y. Chang, *J. Med. Chem.* **2008**, *51*, 4351–4355.



Scheme 3.17: Synthesis of 2-cyano piperidine **186** through direct Strecker reaction on **177** and as by-product in the reductive amination of **177** in neutral conditions. Reagents and conditions: a) $BnNH_2$, 3 Å MS, TMSCN, dry CH_3CN, rt, 18 h; b) $NaBH_3CN$, CH_3CO_2H, EtOH, 3 days, 50% over two steps; c) $BnNH_2$ (1.2 equiv.), $NaBH_3CN$ (2.0 to 3.0 equiv.), MeOH, rt, 2-5 days; d) Ac_2O, py, rt, 48 h.

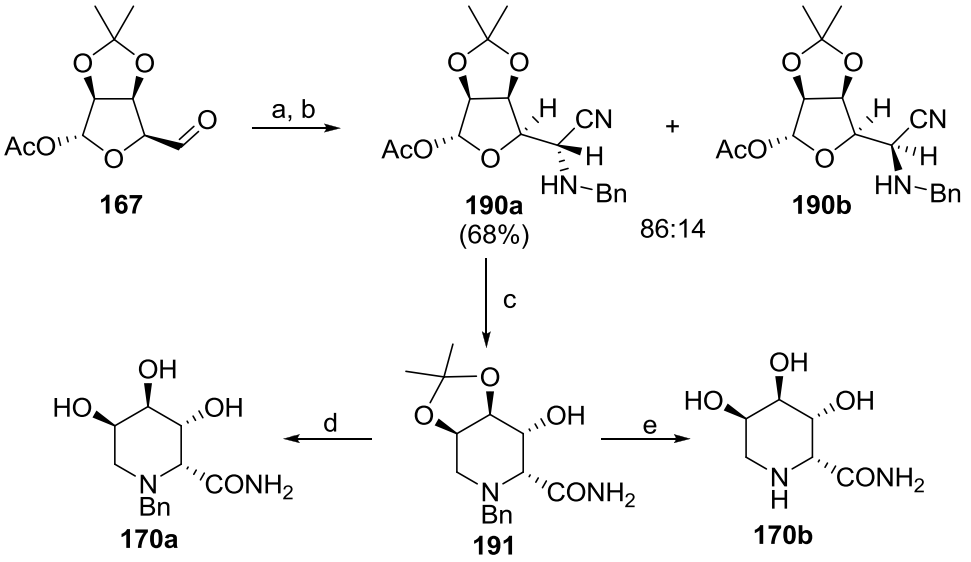
To get a solid evidence, we modified the anomeric protecting group of the starting sugar derivative, in order to demonstrate that the product **183a** of the Strecker reaction affords piperidine **186** upon a reductive amination/ring closure procedure. A different protecting group would eventually allow to perform the cyclization to piperidines functionalized at C-2 that failed starting from α-aminonitriles **183-185.** The simplest protecting group, that could be orthogonally removed with respect to the acetonide moiety, was envisaged in the acetyl group. Therefore, the differently protected "masked" dialdehyde **167** was synthesized in three steps from protected D-mannose **131** (Scheme 3.18). Compound **131** was acetylated at the anomeric position affording **188**,[147] that was deprotected at C-5 and C-6 hydroxy groups in aqueous acetic acid affording **189**.[147a] Subsequent oxidative cleavage of glycol **189** using Silica-Gel-supported sodium metaperiodate in dichloromethane[148] led to aldehyde **167**[149] in 65% overall yield calculated over three steps (Scheme 3.18). We were delighted to observe that also the Strecker reaction on **167** was highly stereoselective. Indeed, the reaction of **167** with $BnNH_2$ in the presence of TMSCN afforded the two diastereoisomers **190a** and **190b** in 86:14 ratio.

[147] a) D. Vonlanthen, C. J. Leumann, *Synthesis* **2003**, 1987-1090; b) M. I. García-Moreno, P. Díaz-Pérez, C. Ortiz-Mellet, J. M. García Fernández, *J. Org. Chem.* **2003**, *68*, 8890-8901.
[148] Y.-L. Zhong, T. K. M. Shing, *J. Org. Chem.* **1997**, *62*, 2622-2624.
[149] K. Bischofberger, A. Jordaan, M. Potgieter, P. L. Wessels, *S. Afr. J. Chem.* **1981**, *34*, 33-40.



Scheme 3.18: Synthesis of aldehyde **167**. Reagents and conditions: a) Ac_2O, py, rt 3h; b) CH_3CO_2H, H_2O, rt, 18 h; c) Silica Gel Supported $NaIO_4$, CH_2Cl_2, rt, 1h, 65% over three steps.

The major isomer **190a** could be isolated in 68% yield after chromatography on silica gel (Scheme 3.19). Structural assignment to the major product as **190a** rests on similarity of its spectroscopic data to those of **183a** but was firmly established by successive transformations (see below). A reason for the lower diastereoselectivity of the Strecker reaction on aldehyde **167** with respect to its relative **166** may be ascribed to the higher hyperconjugative effect given by the acetoxy group, which makes the anti lone pair of the endocyclic oxygen less available for intramolecular H-bonding.

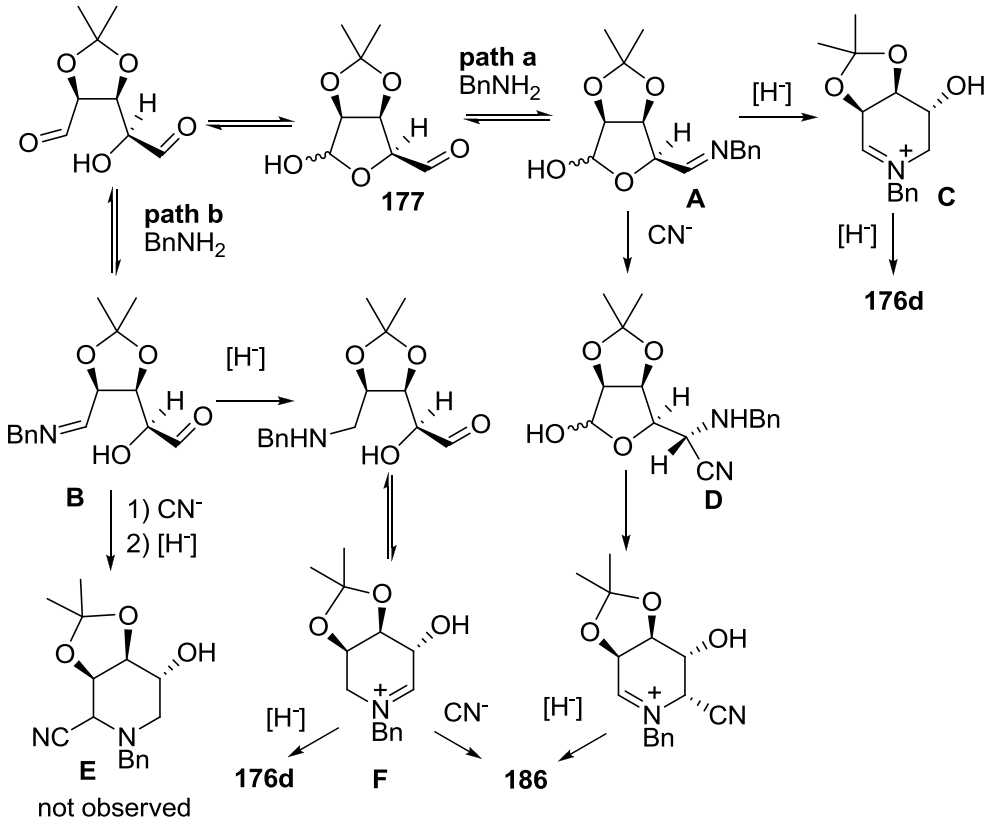


Scheme 3.19: The Strecker reaction on aldehyde **167** and synthesis of 2-carbamido piperidines **170a** and **170b** by reductive amination. . Reagents and conditions: a) $BnNH_2$ (1.0 equiv.), dry CH_3CN, rt, 40 min.; b) TMSCN, rt, 18 h; c) Ambersep®900-OH, MeOH, rt, 18 h, 79%; d) HCl, MeOH, rt, 18 h, DOWEX 50WX8, 80%; e) Pd/C, H_2, HCl, MeOH, rt, 48 h, DOWEX 50WX8, 82%.

A solution of **190a** in MeOH was left stirring with Ambersep® 900-OH ion exchange resin at room temperature to remove the acetyl group at the anomeric position. After 2 h, the ion exchange resin was simply filtered off, $NaBH_3CN$ (1.1 equiv.) and AcOH (2.0 equiv.) were added to perform the reductive amination reaction and the reaction mixture was stirred at room temperature overnight. To our pleasure, the ring closure finally worked, leading to 2-amido-piperidine **191** in 79% yield (Scheme 3.19). Formation of this compound instead of

the expected 2-cyanopiperidine **186** was probably due to concomitant hydrolysis of the cyano group under basic conditions during treatment with the resin.[150] Treatment with methanolic HCl or catalytic hydrogenation in acidic conditions of **191** afforded the hydrochloride salts of **170a** and **170b,** respectively. Final elution through ion-exchange resin (DOWEX® 50XW8-100) led to the corresponding free amines in 80% and 82% yields, from **191** (Scheme 3.19). The structure of **191** was assigned on the basis of the analysis of its 1D and 2D NMR spectra. The ^{1}H NMR spectrum showed for 2-H a doublet with J = 4.4 Hz, in agreement with an *eq-ax* relationship with 3-H, and therefore of a *cis* relationship between the amido group and the free hydroxy group at C-3. Remarkably, the relative stereochemistry of C-2 in **191** is the same found for the 2-cyanopiperidine **186**. This finding supports the hypothesis that the selective formation of **186** during the reductive amination (see Scheme 3.17) is driven by a Strecker reaction, combined with the following double reductive amination, in agreement with our expectation.

On the basis of these data, the plausible mechanism for the regio- and stereoselective formation of compound **186** in the reductive amination of dialdehyde **177** under neutral conditions is reported in Scheme 3.20.

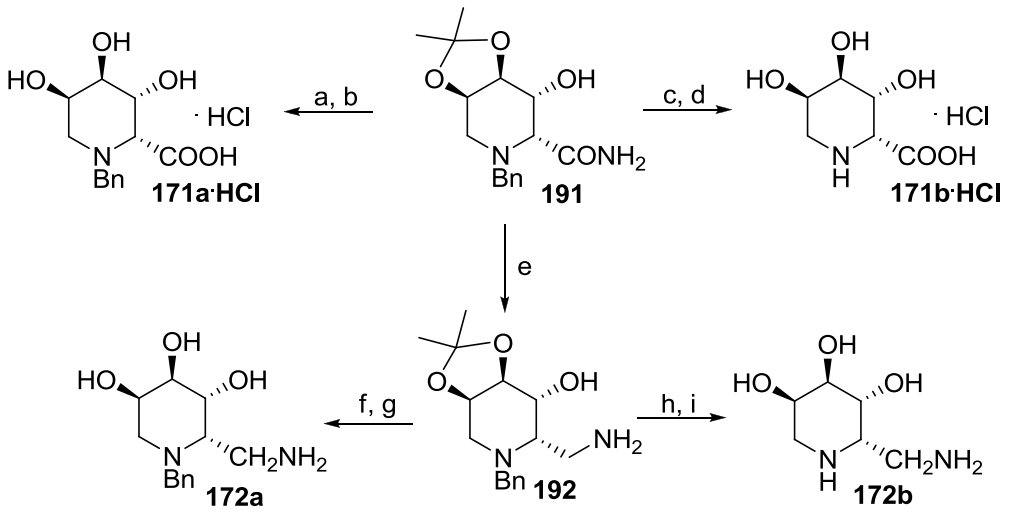


Scheme 3.20: Proposed mechanism for the formation of **186** during the reductive amination of **177** through intermediate cyanide **D**.

[150] T. Tu, Z. Wang, Z. Liu, X. Feng, Q. Wang, *Green Chem.* **2012**, *14*, 921-924.

Likely, imine **A**, formed preferentially with respect to imine **B** (path a *vs* b, Scheme 3.20), leads to adduct **C** through attack of the hydride ion and therefore to piperidine **176d** as the major product. However, imine **A** can undergo attack of the cyanide ion according to the Cram chelate model stereoselectively affording α-amino nitrile **D** and, after cyclization and reduction by the hydride ion, the 2-cyano substituted piperidine **186**. The alternative pathway (path b, Scheme 3.20) would involve reaction of the open-chain form of aldehyde **177** to afford imine **B**. Attack of the cyanide ion on **B** can be ruled out, since piperidines **E** were never observed. Alternatively, reduction of imine **B** and formation of iminium ion **F** might afford piperidine **186** through cyanide addition at this level occurring stereoselectively, which seems unlikely, albeit it cannot be completely excluded.

Amide **191** is also a suitable intermediate for the synthesis of the new trihydroxypipecolic acid **171a**, the recently synthesized **171b**[151] and of 2-aminomethylpiperidine iminosugars **165a** and **165b** (Scheme 3.21). Hydrolysis of the amido moiety and concomitant deprotection of acetonide group of **191** was achieved upon heating in a 6 M HCl solution for 16 h.[152] Purification of this hydrochloride salt was achieved by stirring in an aqueous NaOH solution for 1 h, followed by elution on an anion exchange resin (Ambersep® 900 OH) eluting sequentially with MeOH, H_2O and a 6 M solution of HCl. Concentration of the acidic fractions afforded pure **171a·HCl** in 71% yield (Scheme 3.21).



Scheme 3.21: Synthesis of trihydroxypipecolic acids **171a** and **171b** and of aminopiperidines **172a** and **172b**. Reagents and conditions: a) 6 M HCl, reflux, 16 h, b) Ambersep®900-OH, 71%; c) Pd/C, H_2, HCl, MeOH, rt; d) 6 M HCl, reflux, 16 h, 100%; e) $LiAlH_4$, reflux, 1.5 h, 79%; f) MeOH, HCl, rt, 48 h, g) DOWEX 50WX8, 89%; h) Pd/C, H_2, HCl, MeOH, rt, 3 days; i) DOWEX 50WX8, 95%.

[151] Y. Yoshimura, C. Ohara, T. Imahori, Y. Saito, A. Kato, S. Miyauchi, I. Adachi, H. Takahata, *Bioorg. Med. Chem.* **2008**, *16*, 8273-8286.
[152] A. Fadel, N. Lahrache, J. Org. Chem. **2007**, *72*, 1780-1784.

Alternatively, catalytic hydrogenation followed by heating in a 6 M HCl solution for 16 h gave **171b·HCl** in 100% yield.

This compound was characterized as the hydrochloride salt since previous obtainment as the free amine[151] showed great variability in the optical rotation measurement. On the other hand, reduction of **191** with $LiAlH_4$ in refluxing THF gave the diamine **192** in 79% yield. Treatment with methanolic HCl or catalytic hydrogenation under acidic conditions afforded the hydrochloride salts of **165a** and **165b**,[153] respectively. Final elution through ion-exchange resin (DOWEX® 50XW8-100) led to the corresponding free amines in 89% and 95% yields, respectively (Scheme 3.21).

Compounds **165, *ent*-164, 168a-g, 169a-d, 170a-b, 171a-b·HCl** and **172a-b** were assayed as glycosidase inhibitors towards a panel of commercially available glycosidases (see Table 3, *Chapter 3.3*).

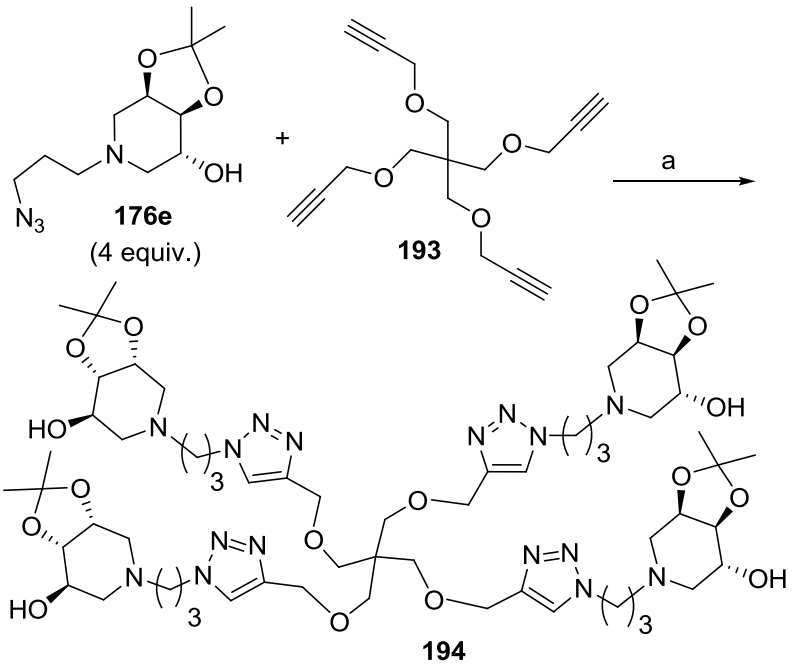
3.2.3 Synthesis of Multivalent Piperidine Iminosugars

In analogy with what reported in *Chapter 2.4* for pyrrolidine-containing iminosugars, an example of multivalent piperidine-based compound was also synthesised by Cu^{I}-catalyzed azide-alkyne cycloaddition (CuAAC).

First of all the tetravalent scaffold **193** was synthesized by propargylation of pentaerythritol with propargyl bromide and NaH as previously reported.[154] The reaction of 4 equivalents of azido derivative **176e** with scaffold **193** afforded the expected tetravalent iminosugar derivative **194** in 88% yield (Scheme 3.22). Deprotection of adduct **194** was not a trivial task, due to the high basicity and hydrophilicity of the deprotected compound.

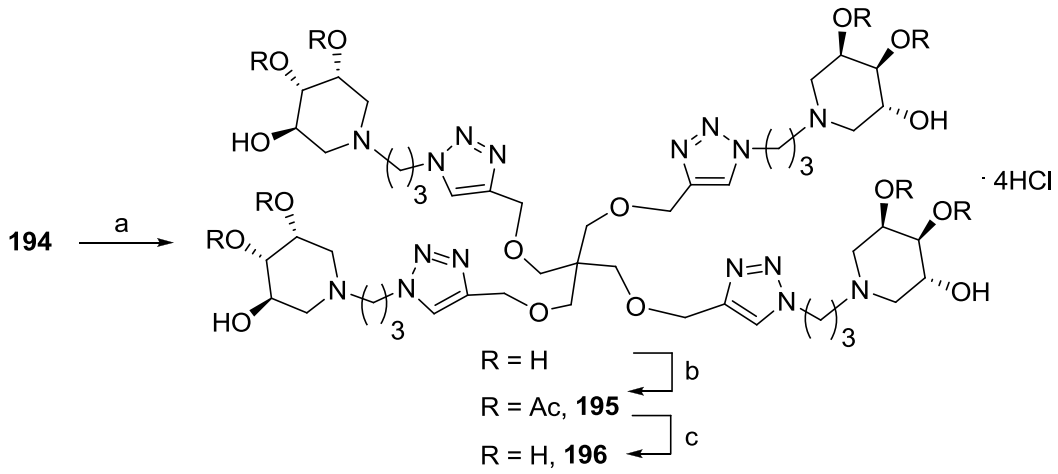
Treatment of **194** in acidic MeOH at room temperature for 18 hours gave the hydrochloride salt, that was passed onto a ion exchange resin Dowex 50WX8-200 eluting successively with MeOH, H_2O and 6% aqueous ammonia. However, most of the compound was eluted in the first fraction with MeOH as hydrochloride salt, and only a small amount with ammonia in the final fraction as free amine. The methanolic fraction was then acetylated by treatment with pyridine and acetic anhydride, affording compound **195** with 91% yield after flash column chromatography. After treatment with strongly basic resin Ambersep 900 OH, pure **196** was obtained in 86% yield.

[153] M. Ganesan, R. Vilas Salunke, N. Singh, N. G. Ramesh, *Org. Biomol. Chem.* **2013**, *11*, 599-611.
[154] I. Papp, J. Dernedde, S. Enders, R. Haag, *Chem. Commun.* **2008**, 5851-5853.



Scheme 3.22: Synthesis of the tetravalent piperidine **194.** Reagents and conditions: a) $CuSO_4$ (0.3 equiv.), sodium ascorbate (0.6 equiv.), THF/H_2O 2:1 MW 80 °C, 45 min, 88%.

This compound and the free amine previously recovered by DOWEX elution of **194** with 6% aqueous ammonia were proved to be identical by ^{1}H NMR analysis (Scheme 3.23). Preliminary biological evaluation of compound **196** and its hydrocholide salt (**196 HCl**) were carried out by measuring their inhibitory activity towards a panel of commercially available glycosidases, in the laboratories of Prof. I. Robina of University of Seville (see Table 3.3, *Chapter 3.3*).



Scheme 3.23: Deprotection and purification the tetravalent piperidine **194**. Reagents and conditions: a) MeOH, 12M HCl, rt, 18 h, 100%; b) Ac_2O, Py, rt 18 h, 91%; c) MeOH, Ambersep 900 OH rt, 18h, 86%.

The tetravalent compound, both as free amine and hydrochloride salt, showed a very similar activity respect to the monovalent iminosugar **176e**. All three compounds resulted modest inhibitors of α-fucosidase, being the monovalent compound a little bit more potent than multivalent one (62% vs 45%). This may be ascribed to the nature of the scaffold or the linker, which prevents the bioactive piperidine molecule to reach the active site. Curiously, the tetravalent *free amine **196*** resulted less selective with respect to monovalent compound showing an increased inhibitory activity towards amyloglucosidase (57% vs 27%) and some kind of activity also towards α-glucosidase and α-galactosidase.

These preliminary results show that the choice of appropriate scaffold and linker is crucial.[155]

3.3 Biological Evaluation

Since natural compounds **165** and **ent-164** are non-selective moderate inhibitors of glycosidases[122b] it was expected that piperidines derivatives bearing alkyl, aryl or additional amino moieties, may reinforce the enzymatic inhibitory properties through allosteric interactions or acting as transition state mimetics reinforcing the interaction with the glycosidase active site.[156] Compounds **165**, **ent-164**, **168a-g**, **169a-d**, **170a-b**, **171a-b·HCl and 172a-b** were assayed as glycosidase inhibitors towards a panel of commercially available glycosidases. The results are summarized in Table 3.3. The compounds tested did not exhibit significant activity against β-galactosidase from *Escherichia coli* β-glucosidases from rice, β-mannosidase from snail and β-N-acetylglucosaminidase from Jack bean. In our hands, known compound **ent-164**[122b] presents the best activity towards α-L-fucosidase showing a moderate value, IC_{50} = 90.3 μM, towards this enzyme. Previous results for this compound showed worst results with an IC_{50} = 450 μM towards the same enzyme, and no selectivity.[157] *N*-substitution of compound **ent-164** reduces the inhibitory activity with a greater extent in the isopropyl derivative **11a**. The *N*-butyl derivative **168b** showed a moderate activity, which is in accordance with the data reported[122b] for this compound. The

[155] C. Decroocq, A. Joosten, R. Sergent, T. M. Barragán, C. Ortiz Mellet, P. Compain, *ChemBiochem* **2013**, *14*, 2038-2049.

[156] a) S. Gerber-Lemaire, F. Popowycz, E. Rodríguez-Garcia, A. T. Carmona, and I. Robina, *ChemBioChem.* **2002**, *5*, 466-470; b) A.T. Carmona, F. Popowycz, S. Gerber-Lemaire, E. Rodríguez-García, C. Schütz, P. Vogel, and I. Robina, *Bioorg. & Med. Chem.*, **2003**, *11*, 4897-4911; c) F. Popowycz, S. Favre, C. Schütz, P. Vogel, S. Gerber-Lemaire, and L. Juillerat-Jeanneret, *J. Med. Chem.* **2005**, *48*, 4237-4246; d) E. Moreno-Clavijo, A. T. Carmona, Y. Vera-Ayoso, A. J. Moreno-Vargas, C. Bello, P. Vogel, and I. Robina, *Org. Biomol. Chem.* **2009**, *7*, 1192-1202.

[157] Inhibitory activity against β-galactosidase from coffee bean IC_{50} = 70 μM, β-galactosidase from *Aspergillus orizae* IC_{50} = 50 μM and β-glucosidase from almonds IC_{50} = 100 μM, for details see Ref. [122b].

presence of a free amino group or an acetamido group at the 1-*N*-alkyl side chain is detrimental for α-L-fucosidase inhibition as shown in compounds **168f** and **168g**. These compounds displayed weak-to-moderate inhibitory activity towards β-galactosidase from coffee beans.

Enzymes % of inhibition at 1 mM (IC_{50}, μM)							
Compound	**α-L-Fuc-ase**	**α-Gal-ase**	**β-Gal-ase**	**α-Gluc-ase**	**Amylogluc-ase**	**β-Gluc-ase**	**α-Man-ase**
***ent*-164**	89 (90.3)	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
168a	46	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
168b	72	22	n.i.	n.i.	n.i.	n.i.	n.i.
168c	77	n.i.	n.i.	50	36	n.i.	n.i.
168d	n.i.	n.i.	n.i.	n.i.	n.i.	48	n.i.
168e	62	n.i.	n.i.	n.i.	27	n.i.	n.i.
168f	n.i.	32	n.i.	n.i.	n.i.	n.i.	n.i.
168g	n.i.	64	n.i.	15	n.i.	n.i.	n.i.
165	38	n.i.	47[d)]	n.i.	n.i.	79	17
169a	n.i.	n.i.	n.i.	n.i.	24	83 (65.3)	n.i.
169b	n.i.	n.i.	n.i.	n.i.	21	75	35
169c	n.i.	n.i.	n.i.	n.i.	16	66	n.i.
169d	n.i.	n.i.	n.i.	n.i.	35	n.i.	n.i.
170a	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
170b	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
171a·HCl	18	n.i.	n.i.	n.i.	37	n.i.	70
171b·HCl	n.i.	n.i.	n.i.	20	n.i.	35	n.i.
172a	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
172b	37	n.i.	n.i.	n.i.	n.i.	n.i.	51
196	45	39	n.i.	29[e)]	57	n.i.	n.i.
196·HCl	56	n.i.	n.i.	n.i.[e)]	27	n.i.	n.i.

Table 3.3: Inhibitory activities of compounds ***ent*-164**, **168a-g**, **165**, **169a-d**, **170a-b**, **171a-b·HCl**, **172a-b** and tetravalent compound **196** (free amine and hydrochloride salt) toward glycosidases. Optimal pH, 35 ºC [a, b, c]. a) For conditions of measurements see Ref.[111]; b) n.i.: no inhibition was detected at 1 mM concentration of the corresponding compound; c) α-L-fucosidase from bovine kidney, α-galactosidase from coffee beans, β-galactosidase from *Aspergillus orizae*, α-glucosidase from rice, Amyloglucosidase from *Aspergillus niger*, β-glucosidase from almonds, α-mannosidase from Jack bean; d) β-galactosidase from *Aspergillus orizae* and from *Escherichia coli*; e) α-glucosidase from yeast.

The inversion of configuration at 5-OH, as in epimer **165**, greatly disminished the activity towards α-L-fucosidase, showing also weak to moderate inhibition against other enzymes.[158] The substitution of an OH group in **165** by an amino moiety reduced the inhibitory activity towards the panel of tested enzymes displaying only a poor inhibition against amyloglucosidase from *Aspergillus niger*.

Interestingly, the introduction of an aromatic moiety as in compound **169a**, increased the inhibitory activity towards β-glucosidase from almonds to an IC_{50} = 65.3 μM, which is in agreement with the data reported for other aryl iminosugars.[156,159] Changing the aryl moiety to alkyl chains (**169b**, **169c**), the inhibitory activity against β-glucosidase is slightly reduced, although is higher than in the aminopiperidine **169d**. The inhibitory activity of a series of 3,4,5-trihydroxypipecolic acids have been evaluated[151] showing weak inhibitory properties. In our hands known compound **171b·HCl** showed only weak inhibition to α- and β-glucosidases.[160] The benzyl derivative **171a·HCl** significantly presented a moderate inhibition against α-mannosidases, which is abolished in the *N*-benzyl aminomethyl derivative **172a** and a little reduced in aminomethyl derivative **172b**. Changing the acid group to an amide group in **170a** and **170b** lead to a complete reduction of the inhibitory activity.

Finally, the tetravalent compound **196**, both as free amine and as hydrocholide salt (**196 HCl**), did not present any increase of inhibitory activity respect to the monovalent compound **176e**.

3.4 Conclusions

In conclusion, a straightforward synthetic strategy for the obtainment of diversely functionalized polyhydroxy and polyhdyroxyamino piperidines, among which natural products **165** and ***ent*-164** is presented in this chapter, that employs the double reductive amination strategy or the Strecker reaction on D-mannose-derived aldeydes **166** and **167**.

---

[158] According to Ichikawa and co-workers (see Ref. [122b]): inhibitory activity against β-galactosidase from *Aspergillus orizae* IC_{50} = 40 μM, β-glucosidase from almonds IC_{50} = 8.8 μM and α-mannosidase from Jack bean IC_{50} = 360 μM.

[159] a) Wu, C.-Y.; Chang, C.-F.; Chen, S.-Y. J.; Wong, C.-H.; Lin, C.-H. *Angew. Chem. Int. Ed*. **2003**, *42*, 4661; b) Chang, C. F.; Ho, C. W.; Wu, C.-Y.; Chao, T.-A.; Wong, C.-H.; Lin, C.-H. *Chem. Biol.* **2004**, *11*, 1301.

[160] According to Takahata and coworkers (see Ref.[151]) this compound exhibited weak activity against β-*N*-acetylglucosaminidase from bovine kidney and from human placenta, α-*N*-acetylgalactosaminidase from chicken liver and against β-glucuronidase from bovine liver and from *E. Coli*.

This strategy also allowed the synthesis of trihydroxypipecolic acids **171a,b** and of aminomethylpiperidine iminosugars **172a,b**.

The exploitation of the CuAAC cycloaddition on key azido intermediate **176e** with a tetravalent alkyne scaffold also allowed the synthesis of the multivalent compound **196**.

Biological evaluation of all the synthesized compounds towards a range of commercially available glycosidases has been also reported.

3.5 Experimental Section

General methods: Commercial reagents were used as received. All reactions were magnetically stirred and monitored by TLC on 0.25 mm silica gel plates (Merck F_{254}) and column chromatography was carried out on Silica Gel 60 (32-63 μm). Yields refer to spectroscopically and analytically pure compounds unless otherwise stated. ^{1}H NMR spectra were recorded on a Varian Mercury-400, Varian INOVA-400 or Bruker AVANCE-500. ^{13}C NMR spectra were recorded on a Varian Gemini-200 or Bruker AVANCE-500. Infrared spectra were recorded with a Perkin-Elmer Spectrum BX FT-IR System spectrophotometer. ESI full MS were recorded on a Thermo LTQ instrument by direct inlet; relative percentages are shown in brackets. Elemental analyses were performed with a Perkin-Elmer 2400 analyzer. Optical rotation measurements were performed on a JASCO DIP-370 polarimete.

Synthesis of benzyl 2,3-*O*-(1-methylethylidene)-α-D-*lyxo*-pentodialdo-1,4-furanoside (166): To a suspension of D-mannose (5.0 g, 27.80 mmol) in acetone (250 mL), iodine (1.47 g, 5.80 mmol) was added and the mixture was stirred for 2 h at room temperature. The reaction mixture was quenched at 0 °C with saturated solutions of sodium thiosulfate (70 mL) and sodium bicarbonate (70 mL), then extracted with chloroform (200 mL). After treatment of the organic phase with a saturated solution of sodium bicarbonate (3x50 mL), the organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **131** as a pale yellow solid (6.76 g, 26.0 mmol, 93%), which was used without further purification in the next step. To a solution of **131** (500 mg, 1.92 mmol) in dry THF (3.84 mL), freshly powdered KOH (194 mg, 3.46 mmol), 18-crown-6 (21 .0 mg, 0.08 mmol) and BnBr (0.25 mL, 2.11 mmol) were added sequentially. The mixture was stirred at room temperature for 16 h and then diluted with CH_2Cl_2 and washed several times with water. Evaporation of the organic phase furnished pure product **173** with quantitative yield. A solution of **173** (295 mg, 0.84 mmol), glacial AcOH (6.4 mL) and water (2.7 mL) was stirred for 18 h at room temperature and concentrated under reduced pressure. The residue was dissolved in AcOEt (14 mL), washed sequentially with saturated aqueous solution of

$NaHCO_3$ (2x4 mL), water (2x2 mL) and brine (2x3 mL), dried (Na_2SO_4) and the solvent was concentrated to afford **174** (240 mg, 0.77 mmol, 92%) as a colorless oil. To a vigorously stirred suspension of silica-gel supported NaIO4[161] reagent (1.54 g) in CH_2Cl_2 (3.85 mL), a solution of **174** (240 mg, 0.77 mmol) in CH_2Cl_2 (3.85 mL) was added and stirred at room temperature for 50 minutes. The reaction mixture was then filtered, washing the residue with $CHCl_3$. The combined filtrates were concentrated under reduced pressure and allowed to stand at 5 °C overnight to give **166** (200 mg, 0.72 mmo, 93%) as a solid, m.p. 80-81 °C, which was pure enough to be employed in the subsequent steps (80% over four steps from D-mannose).

Synthesis of (3*R*,4*S*,5*R*)-5-hydroxy-3,4-*O*-(1-methylethylidene)-piperidine (175): Aldehyde **166** (126 mg, 0.45 mmol) and benzylamine (50 µL, 0.45 mmol) were dissolved in MeOH (5.0 mL) and 60 mg of 3Å pellets molecular sieves were added. The reaction mixture was left stirring at room temperature for 40 minutes (formation of imine is attested by ^{1}H NMR control), then $Pd(OH)_2/C$ (63 mg) was added and the mixture was left stirring at room temperature under H_2 atmosphere for 25 hours. The catalyst and the molecular sieves were filtered, washing several times with MeOH and then the solvent was evaporated under vacuum. The crude product was purified using silica gel gradient flash chromatography (CH_2Cl_2/MeOH/NH_4OH 6% from 40:1:0.1 to 10:5:0.5) to give compound **175** as a white solid (54 mg, 0.31 mmol, 69%), m.p. 117-119 °C. $[\alpha]_D^{24}$ = - 84.0 (c = 1.01, MeOH). ^{1}H-NMR (400 MHz, CD_3OD) δ = 4.18 (dt, *J* = 5.5, 3.2 Hz, 1H, 3-H), 3.88 (t, *J* = 5.9 Hz, 1H, 4-H), 3.67 (ddd, *J* = 9.4, 6.5, 4.5 Hz, 1H, 5-H), 3.10 (dd, *J* = 14.7, 2.2 Hz, 1H, 2-Ha), 2.94-2.89 (m, 1H, 6-Ha), 2.90 (dd, *J* = 14.6, 3.4 Hz, 1H, 2-Hb), 2.34 (dd, *J* = 13.3, 9.4 Hz, 1H, 6-Hb), 1.49 (s, 3H, Me), 1.34 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, CD_3OD) δ = 107.7 (s, acetal), 78.0 (d, C-4), 72.0 (d, C-3), 68.4 (d, C-5), 47.3 (t, C-6), 44.7 (t, C-2), 26.3 (q, Me), 24.4 (q, Me) ppm. IR (KBr): ν = 3299, 3162, 2981, 2935, 2897, 1557, 1456, 1383, 1373, 1220, 1089, 1054, cm^{-1}. MS (ESI): *m/z* (%) = 173.98 (100) $[M+H]^+$. $C_8H_{15}NO_3$ (173.21): calcd. C 55.47, H 8.73, N 8.09; found C 55.26, H 8.79, N 8.05.

Synthesis of (3*R*,5*R*)-3,4,5-trihydroxy-piperidine (*ent*-164): A solution of **175** (49 mg, 0.28 mmol) in MeOH (5.0 mL) and HCl 1M (10 drops) was left stirring at room temperature overnight. The crude mixture was concentrated to yield the hydrochloride of ***ent*-164** which was eluted through an ion-exchange resin (DOWEX® 50XW8-100) with MeOH, H_2O and 6% NH_4OH to give the free amine ***ent*-164** (37 mg, 100%). $[\alpha]_D^{26}$ **=** - 65.7 (c = 1.00, MeOH). ^{1}H-

[161] Y. L. Zhong, T. K. M. Shing, *J. Org. Chem*. **1997**, *62*, 2622-2624.

NMR (400 MHz, D_2O) δ = 3.88 (*br* s, 1H, 3-H), 3.67 (td, *J* = 8.8, 4.4 Hz, 1H, 5-H), 3.45 (dd, *J* = 8.6, 2.9 Hz, 1H, 4-H), 2.96 (dd, *J* = 13.0, 4.3 Hz, 1H, 6-Ha), 2.82 (d, *J* = 13.9 Hz, 1H, 2-Ha), 2.61 (d, *J* = 14.0 Hz, 1H, 2-Hb), 2.30 (dd, *J* = 12.8, 9.8 Hz, 1H, 6-Hb) ppm. ^{13}C-NMR (50 MHz, D_2O) δ = 72.8 (d, C-4), 67.6 (d, C-3), 67.3 (d, C-5), 47.6 (t, C-6), 47.3 (t, C-2) ppm. MS (ESI): *m/z* (%) = 156.16 (100) $[M+Na]^+$. $C_5H_{11}NO_3$ (133.15): calcd. C 45.10, H 8.33, N 10.52; found C 45.32, H 7.98, N 10.39.

General procedure for the synthesis of *N*-alkyl piperidines (176a and 176b): Aldehyde **166** and the appropriate amine (1.0 equiv) were dissolved in MeOH (0.1 M) and 60 mg of 3Å pellets molecular sieves were added. The reaction mixture was left stirring at room temperature for 40 minutes and, when the complete conversion to imine was observed (^{1}H NMR control), $Pd(OH)_2/C$ (50% wt) and CH_3COOH (2.0 equiv) were added and the mixture was left stirring at room temperature under H_2 atmosphere for 7 days. The catalyst and the molecular sieves were filtered, washing several times with MeOH and then the solvent was evaporated under vacuum.

(3*R*,4*S*,5*R*)-1-Butyl-5-hydroxy-3,4-*O*-(1-methylethylidene)-piperidine (176a). Application of this procedure to 168 mg (0.60 mmol) of **166** with butylamine (59 µL, 0.60 mmol) furnished, after purification by flash column chromatography (CH_2Cl_2/MeOH/NH_4OH 6% from 15:1:0.1 to 10:1:0.1), compound **176a** (48 mg, 0.21 mmol, 35%) as a white solid, m.p. 21-23 °C. $[\alpha]_D^{24}$ **=** + 16.4 (c = 1.07, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.27 (t, *J* = 7.2, 6.0 Hz, 1H, 3-H), 4.03 (t, *J* = 4.4 Hz, 1H, 4-H), 3.94-3.91 (m, 1H, 5-H), 2.73 (ddd, *J* = 11.7, 6.2, 1.5 Hz, 1H, 2-Ha), 2.55 (dd, *J* = 11.7, 2.8 Hz, 1H, 6-Ha), 2.46 (ddd, *J* = 11.7, 5.3, 1.4 Hz, 1H, 6-Hb), 2.41-2.30 (m, 3H, 2-Hb, 1'-H), 1.49 (s, 3H, Me), 1.47-1.39 (m, 2H, 2'-H), 1.34 (s, 3H, Me), 1.32-1.24 (m, 2H, 3'-H), 0.91-0.87 (t, *J* = 7.4 Hz, 3H, 4'-H) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 109.1 (s, acetal), 77.3 (d, C-4), 72.2 (d, C-3), 67.8 (d, C-5), 57.5 (t, C-1'), 55.6 (t, C-2), 55.3 (t, C-6), 28.8 (q, Me), 28.2 (q, Me), 26.3 (t, C-2'), 20.5 (t, C-3'), 13.9 (q, C-4') ppm. MS (ESI): *m/z* (%) = 230.35 (99) $[M+H]^+$, (10) $[M+Na]^+$. IR ($CDCl_3$): ν = 3604, 3499, 2958, 2935, 2247, 1686, 1456, 1383, 1219, 1058 cm^{-1}. $C_{12}H_{23}NO_3$ (229.32): calcd. C 62.85, H 10.11, N 6.11; found C 63.12, H 9.78, N 6.40.

(3*R*,4*S*,5*R*)-1-Octyl-5-hydroxy-3,4-*O*-(1-methylethylidene)-piperidine (176b): Application of this procedure to 140 mg (0.50 mmol) of **166** with octylamine (83 µL, 0.50 mmol) furnished, after purification by flash column chromatography (CH_2Cl_2/MeOH/NH_4OH 6% from 15:0.5:0.1 to 10:1:0.1), compound **176b** (59 mg, 0.21 mmol, 41%) as a white solid, m.p. 39-41 °C. $[\alpha]_D^{23}$ **=** + 27.0 (c = 0.76, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.27 (dt, *J* = 7.6, 5.1 Hz,

1H, 3-H), 4.08 (dd, *J* = 4.9, 3.9 Hz, 1H, 4-H), 3.95 (dd, *J* = 7.6, 3.8 Hz, 1H, 5-H), 2.82 (ddd, *J* = 7.7, 6.3, 1.5 Hz, 1H, 2-Ha), 2.56 (d, *J* = 2.4 Hz, 2H, 6-H), 2.43-2.34 (m, 3H, 2-Hb, 1'-H), 1.50 (s, 3H, Me), 1.48-1.41 (m, 4H) 1.35 (s, 3H, Me), 1.33-1.20 (m, 8H), 0.88 (t, *J* = 6.8 Hz, 3H, 8'-H) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 109.2 (s, acetal), 77.0 (d, C-4), 72.1 (d, C-3), 67.6 (d, C-5), 57.8 (t, C-1'), 55.8 (t, C-2), 55.5 (t, C-6), 31.8, 29.4, 29.2 (t, 3C), 28.2 (q, Me), 27.3 (q, Me), 26.8, 26.3, 22.6 (t, 3C), 14.0 (q, C-8') ppm. MS (ESI): *m/z* (%) = 286.25 (100) $[M+H]^+$. IR ($CDCl_3$): ν = 3610, 3472, 2987, 2953, 2927, 2249, 1467, 1219, 1059 cm^{-1}. $C_{16}H_{31}NO_3$ (285.42): calcd. C 67.33, H 10.95, N 4.91; found C 67.31, H 10.73, N 4.54.

Synthesis of (3*R*,4*S*,5*R*)-5-Hydroxy-1-isopropyl-3,4-isopropylidene- dioxypiperidine (176c): Aldehyde **166** (570 mg, 2.05 mmol) and benzylamine (220 µL, 2.05 mmol) were dissolved in MeOH (90 mL) and 300 mg of 3Å pellets molecular sieves were added, under N_2 atmosphere. The reaction mixture was left stirring at room temperature for 40 minutes, then $Pd(OH)_2$/C (285 mg) was added and the mixture was left stirring at room temperature under H_2 atmosphere for 4 days (the disappearance of benzyl signals was attested by ^{1}H NMR control). The catalyst and the molecular sieves were filtered, washing several times with MeOH, and the solvent was evaporated under vacuum. Purification of the residue by flash column chromatography on silica gel afforded pure **176c** (121 mg, 0.56 mmol, 27%). R_f=0.70 (CH_2Cl_2/MeOH/NH_4OH 6% 10:1:0.1); $[\alpha]_D^{24}$=+23.0 (c=1.04 in $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=4.30-4.25 (m, 1H; 3-H), 4.08 (dd,^3J(H,H)=5.0, 3.6 Hz, 1H; 4-H), 3.92 (dd, ^3J(H,H)=6.4, 3.6 Hz, 1H; 5-H), 2.84-2.76 (m, 2H; 2a-H, CH-*i*Pr), 2.61-2.59 (m, 2H; 6-H), 2.38 (dd, ^2J(H,H)=11.6, ^3J(H,H)=8.0 Hz, 1H; 2b-H), 1.49 (s, 3H; Me), 1.34 (s, 3H; Me), 1.00 (d, ^3J(H,H)=6.6 Hz, 3H; CH_3-*i*Pr), 0.99 ppm (d, ^3J(H,H)=6.6 Hz, 3H; CH_3-*i*Pr); ^{13}C-NMR (50 MHz, $CDCl_3$): δ=108.9 (s; acetal), 77.3 (d, C-4), 71.9 (d, C-3), 66.5 (d, C-5), 54.0 (d, N*C*H(CH_3)$_2$), 51.7 (t; C-2), 50.3 (t, C-6), 28.0 (q, Me), 26.1 (q, Me), 18.0 (q, NCH(*C*H_3)$_2$) 17.5 ppm (q, NCH(*C*H_3)$_2$); IR ($CDCl_3$): $\tilde{\nu}$=3469, 2938, 2970, 2837, 2249, 1660, 1459, 1405, 1383, 1325, 1291, 1253, 1219, 1163, 1058 cm^{-1}; MS (ESI): *m/z* (%): 216.11 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{11}H_{21}NO_3$ (215.29): C 61.37, H 9.83, N 6.51; found C 61.57, H 10.02, N 6.31.

Synthesis of (3*R*,4*S*,5*R*)-1-Isopropyl-3,4,5-trihydroxypiperidine (168a): A solution of **176c** (40 mg, 0.19 mmol) in MeOH (10.0 mL) was left stirring with 12M HCl (8 drops) at room temperature for 24 h. The crude mixture was concentrated to yield the hydrochloride salt of **168a**. The corresponding free amine was obtained by passing the hydrochloride salt through a DOWEX® 50XW8-100 ion-exchange resin. Elution with 6% ammonia afforded the

free base **168a** (33 mg, 0.19 mmol, 100%). $[\alpha]_D^{24}$**=-**41.9 (c=0.65 in H_2O); ^{1}H-NMR (400 MHz, D_2O): *δ*=3.86 (m, 1H; 3-H), 3.72 (td, ^3J(H,H)=8.8, 4.4 Hz, 1H; 5-H), 3.34 (dd, ^3J(H,H)=8.3, 2.9 Hz, 1H; 4-H), 2.77-2.64 (m, 3H; 2a-H, 6a-H, CH-*i*Pr), 2.31 (dd, ^2J(H,H)=12.4, ^3J(H,H)=1.7 Hz, 1H; 2b-H), 2.12 (t, *J*(H,H)=10.0 Hz, 1H; 6b-H), 0.91 (d, ^3J(H,H)=9.2 Hz, 3H; CH_3-*i*Pr), 0.89 ppm (d, ^3J(H,H)=9.2 Hz, 3H; CH_3-*i*Pr); ^{13}C-NMR (50 MHz, D_2O): *δ*=73.6 (d, C-4), 67.7 (d, C-5), 67.5 (d, C-3), 54.0 (d, N*C*H(CH_3)$_2$), 52.2 (t, C-6), 50.6 (t, C-2), 17.6 (q, NCH(*C*H_3)$_2$), 16.2 ppm (q, NCH(*C*H_3)$_2$); MS (ESI): *m/z* (%): 198.17 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_8H_{17}NO_3$ (175.23): C 54.84, H 9.78, N 7.99; found C 55.15, H 9.92, N 7.68.

Synthesis of 2,3-*O*-(1-methylethylidene)-α-D-*lyxo*-pentodialdo-1,4-furanose or 3,4-*O*-(1-methylethylidene)-D-*arabino*-pentodialdo-2,5-furanose (177): To a solution of **166** (514 mg, 1.85 mmol) in MeOH (36 mL), $Pd(OH)_2$/C (257 mg) was added and the reaction mixture was left stirring at room temperature under H_2 atmosphere for 4 h. The catalyst was filtered through Celite® and the filtrate was concentrated under vacuum, to yield quantitative **177** (348 mg). ^{1}H NMR of **177** showed the presence of a complex mixture or different forms.

General Procedure for the synthesis of *N*-alkyl piperidines 176a, 176b and 176d by double reductive amination on 177: A 0.06 M solution of dialdehyde **177** in dry MeOH was stirred in the presence of 3Å molecular sieves powder for 15 min, under nitrogen atmosphere. Then $NaBH_3CN$ (3.0 equiv) was added and the reaction mixture cooled at 0°C. Finally R-NH_2 (R = Bn, Bu, Octyl, 0.9 equivalents) and AcOH (2 equiv) were added and the mixture was allowed to warm to room temperature and stirred for a total of 3-7 days, under nitrogen atmosphere. The molecular sieves were removed by filtration through Celite and the filtrate was concentrated under vacuum. The residue was purified by flash chromatography (CH_2Cl_2/MeOH or CH_2Cl_2/MeOH/6% NH_4OH) affording **176a**, **176b** and **176d** in 48-93% yields.

Application of the general procedure to 100 mg of **177** (0.53 mmol) and butylamine (47 µL, 0.48 mmol) afforded compound **176a** (52 mg, 0.23 mmol) in 48% yield after purification by flash column chromatography (CH_2Cl_2/MeOH/NH_4OH 6%, 15:0.5:0.1).

Application of the general procedure to 104 mg of **177** (0.55 mmol) and octylamine (83 µL, 0.50 mmol) afforded compound **176b** (75 mg, 0.28 mmol) in 56% yield after purification by flash column chromatography (CH_2Cl_2/MeOH/NH_4OH 6%, 15:0.5:0.1).

Application of the general procedure to 122 mg of **177** (0.65 mmol) and benzylamine (64 µL, 0.59 mmol) afforded compound **176d** (144 mg, 0.55 mmol) in 93% yield after purification by flash column chromatography (CH_2Cl_2/MeOH/NH_4OH 6%, 15:0.5:0.1

General procedure for the synthesis of unnatural alkylated piperidines (168b, 168c and 168d): A 0.05 M solution of **176a**, **176b** and **176d** in MeOH was left stirring with HCl 1M (10 drops) at room temperature overnight. The crude mixture was concentrated to yield the hydrochloride salts of **168b**, **168c**, **168d**. The corresponding free amine was obtained by passing the hydrochloride salt through a DOWEX® 50XW8-100 ion-exchange resin. Elution with 6% ammonia afforded the free bases **168b**, **168c**, **168d**.

(3*R*,5*R*)-1-Butyl-3,4,5-trihydroxy-piperidine (168b): Application of this procedure to 60 mg (0.26 mmol) of **176a** furnished the free amine **168b** (49 mg, 100%) as a waxy solid. $[\alpha]_D^{27}$ **=** - 42.5 (c = 0.93, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ = 3.94-3.91 (m, 1H, 3-H), 3.79 (dt, *J* = 13.2, 4.4 Hz, 1H, 5-H), 3.45-3.43 (m, 1H, 4-H), 2.90-2.83 (m, 2H, 2-Ha, 6-Ha), 2.47-2.35 (m, 3H, 2-Hb, 1'-H), 2.14-2.18 (m, 1H, 6-Hb), 1.43-1.33 (m, 2H, 2'-H), 1.19 (td, *J* = 14.6, 7.3 Hz, 2H, 3'-H), 0.78 (t, *J* = 7.3 Hz, 3H, 4'-H) ppm. ^{13}C-NMR (50 MHz, D_2O) δ = 72.2 (d, C-4), 66.3 (d, 2C, C-3, C-5), 56.3 (t, C-1'), 55.1 (t, C-6), 54.4 (t, C-2), 26.4 (t, C-2'), 19.2 (t, C-3'), 12.4 (q, C-4') ppm. MS (ESI): *m/z* (%) = 190.17 (100) $[M+H]^+$. $C_9H_{19}NO_3$ (189.25): calcd. C 57.12, H 10.12, N 7.40; found C 56.77, H 10.39, N 7.84.

(3*R*,5*R*)-1-Octyl-3,4,5-trihydroxy-piperidine (168c): Application of this procedure to 20 mg (0.07 mmol)of **176b** furnished the free amine **168c** as a pale yellow oil (14 mg, 0.06 mmol, 82%). $[\alpha]_D^{27}$ **=** - 19.6 (c = 0.52, MeOH). ^{1}H-NMR (400 MHz, CD_3OD) δ = 3.94-3.92 (m, 1H, 3-H), 3.82 (td, *J* = 7.4, 3.9 Hz, 1H, 5-H), 3.48-3.42 (m, 1H, 4-H), 2.90-2.83 (m, 2H, 2-Ha, 6-Ha), 2.52-2.46 (m, 3H, 2-Hb, 1'-H), 2.35-2.22 (m, 1H, 6-Hb), 1.55-1.53 (m, 2H, 2'-H), 1.36-1.25 (m, 10 H), 0.89 (t, *J* = 7.1 Hz, 3H, 8'-H) ppm. ^{13}C-NMR (50 MHz, CD_3OD) δ = 71.7 (d, C-4), 66.6 (d, C-5), 65.7 (d, C-3), 56.7 (t, C-1'), 54.7 (t, C-2), 54.2 (t, C-6), 30.7, 28.2 (t, 2C), 28.1 (t, C-2'), 26.1, 24.5, 21.4 (t, 3C), 12.2 (q, C-8') ppm. MS (ESI): *m/z* (%) = 246.25 (100) $[M+H]^+$, 268.17 (24) $[M+Na]^+$. $C_{13}H_{27}NO_3$ (245.36): calcd. C 63.64, H 11.09, N 5.71; found C 63.88, H 11.06, N 5.32.

(3*R*,5*R*)-1-Benzyl-3,4,5-trihydroxy-piperidine (168d): Application of this procedure to 17 mg (0.07 mmol)of **176d** furnished the free amine **168d** (10 mg, 0.05 mmol, 69%) as an oil.

$[\alpha]_D^{23}$ **=** - 25.7 (c = 0.97, MeOH).[162] ^{1}H-NMR (400 MHz, CD_3OD) δ = 7.38-7.25 (m, 5H, Ar), 3.91 (dt, *J* = 5.9, 2.9 Hz, 1H, 3-H), 3.81 (dt, *J* = 7.6, 3.9 Hz, 1H, 5-H), 3.72-3.64 (m, AB system, 2H, Bn), 3.43 (*br* s, 1H, 4-H), 2.89-2.87 (m, 1H, 6-Ha), 2.81-2.77 (m, 1H, 2-Ha), 2.44-2.41 (m, 1H, 2-Hb), 2.28-2.14 (m, 1H, 6-Hb) ppm. ^{13}C-NMR (50 MHz, CD_3OD) δ = 135.1 (s, Ar), 128.4-126.4 (d, 5C, Ar), 72.4 (d, C-4), 67.1 (d, C-5), 66.4 (d, C-3), 60.7 (t, Bn), 55.0 (t, C-6), 54.6 (t, C-2) ppm. MS (ESI): *m/z* (%) = 246.12 (100) $[M+Na]^+$. $C_{12}H_{17}NO_3$ (223.27): calcd. C 64.55, H 7.67, N 6.27; found C 64.90, H 7.83, N 6.41.

Synthesis of (3*R*,4*S*,5*R*)-1-(3-Azidopropyl)-5-hydroxy-3,4-isopropyli- denedioxypiperidine (176e): To a solution of **177** (147 mg, 0.78 mmol) in dry MeOH (10 mL) a solution of 3-azidopropyl-1-amine (78 mg, 0.78 mmol) in dry MeOH (13 mL) and 3Å powdered molecular sieves were added. The mixture was stirred under N_2 atmosphere for 4 h (the aldehyde disappearence was attested by ^{1}H NMR control), then $NaBH_3CN$ (147 mg, 2.34 mmol) and AcOH (90 µL, 1.56 mmol) were added. The reaction mixture was left stirring at room temperature for a week and then concentrated under vacuum. The crude product was purified by gradient FCC (from DCM/MeOH 20:1 to 10:1), affording 132 mg (0.52 mmol, 67%) of **176e** as a yellow pale oil. R_f=0.26 (CH_2Cl_2/MeOH 20:1); $[\alpha]_D^{23}$**=+**16.3 (c=0.92 in $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ=4.29 (*p*q, ^3J(H,H)=5.9 Hz, 1H; 3-H), 4.04 (*p*t, ^3J(H,H)=4.7 Hz, 1H; 4-H), 3.96-3.93 (m, 1H; 5-H), 3.34 (t, ^3J(H,H)=6.8 Hz, 2H; 3'-H), 2.73 (ddd, ^2J(H,H)=12.0, ^3J(H,H)=6.0, ^4J(H,H)=1.5 Hz, 1H; 2-Ha), 2.60 (dd, ^2J(H,H)=11.7, ^3J(H,H)=2.9 Hz, 1H; 6-Ha), 2.50-2.41 (m, 4H; 2-Hb, 6-Hb, 1'-H), 1.75 (quint, ^3J(H,H)=6.8 Hz, 2H; 2'-H), 1.50 (s, 3H; Me), 1.36 ppm (s, 3H; Me); ^{13}C-NMR (50 MHz, $CDCl_3$): δ=109.0 (s, acetal), 76.7 (d, C-4), 71.8 (d, C-3), 67.5 (d, C-5), 55.3 (t, C-6), 55.2 (t, C-2), 54.3 (t, C-1'), 49.2 (t, C-3'), 28.0 (q, Me), 26.0 (t, C-2'), 25.9 ppm (q, Me); MS (ESI): *m/z* (%): 279.14 (100) $[M+Na]^+$, 257.27 (20) $[M+H]^+$; IR ($CDCl_3$): $\tilde{\nu}$=3677, 3608, 3487, 2987, 2944, 2823, 2237, 2099, 1456, 1378, 1219, 1060 cm^{-1}; elemental analysis calcd (%) for $C_{11}H_{20}N_4O_3$ (256.30): C 51.55, H 7.87, N 21.86; found C 51.91, H 7.94, N 21.61.

Synthesis of (3*R*,5*R*)-1-(3-Azidopropyl)-3,4,5-trihydroxypiperidine (168e): A solution of **176e** (44 mg, 0.17 mmol) in MeOH (2.0 mL) was left stirring with 12M HCl (142 µL, 1.7 mmol) at room temperature for 18 h. The crude mixture was concentrated to yield the hydrochloride salt of **168e**. The corresponding free amine was obtained by passing the

[162] This value is in agreement to what reported for its enantiomer ($[\alpha]_D^{23}$ **= +**26.7 (MeOH, c= 0.3), G. Pandey, K. C. Bharadwaj, M. I. Khan, K. S. Shashidhara, V. G. Puranik, *Org. Biomol. Chem.* **2008**, *6*, 2587-2595.

hydrochloride salt through a DOWEX® 50XW8-100 ion-exchange resin. Elution with 6% ammonia afforded the free base **168e** (33 mg, 0.15 mmol, 88%). $[\alpha]_D^{21}$**=-**21.2 (c=0.74 in MeOH); ^{1}H-NMR (400 MHz, CD_3OD): δ=3.89 (dt, ^3J(H,H)=5.8, 2.9 Hz, 1H; 3-H), 3.78 (td, ^3J(H,H)=7.8, 4.3 Hz 1H; 5-H), 3.40-3.38 (m, 1H; 4-H), 3.36 (t, ^3J(H,H)=6.8 Hz, 2H; 3'-H), 2.82-2.74 (m, 2H; 6-Ha, 2-Ha) 2.46 (t, ^3J(H,H)=6.8 Hz, 2H; 1'-H), 2.31 (d, ^2J(H,H)=11.2, 1H; 2-Hb), 2.12-2.09 (m, 1H; 6-Hb), 1.76 ppm (quint, ^3J(H,H)=6.8 Hz, 2H; 2'-H); ^{13}C-NMR (50 MHz, CD_3OD): δ=72.9 (d, C-4), 67.2 (d, C-5), 66.8 (d, C-3), 55.8 (t, C-6), 55.2 (t, C-2), 53.7 (t, C-1'), 48.2 (t, C-3'), 24.8 ppm (t, C-2'); MS (ESI): *m/z* (%): 217.17 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_8H_{16}N_4O_3$ (216.24): C 44.44, H 7.46, N 25.91; found C 44.29, H 7.79, N 25.71.

Synthesis of (3*R*,5*R*)-1-(3-Aminopropyl)-3,4,5-trihydroxypiperidine (168f): To a solution of **168e** (40 mg, 0.18 mmol) in MeOH (3 mL) and HCl 12M (6 drops), Pd/C (25 mg) was added and the reaction mixture was left stirring at room temperature under H_2 atmosphere for 3 days. The catalyst was filtered through Celite® and the filtrate was concentrated under vacuum to yield the hydrochloride salt of **168f** which was passed through an ion-exchange resin (DOWEX® 50XW8-100). Elution with MeOH, H_2O and 15% NH_4OH afforded the amine **168f** in quantitative yield (34 mg, 0.18 mol)**.** $[\alpha]_D^{23}$**=-**37.1 (c=0.77 in H_2O); ^{1}H-NMR (500 MHz, CD_3OD) δ=3.94 (td, ^3J(H,H)=5.5, 2.6 Hz, 1H; 3-H), 3.81 (td, ^3J(H,H)=8.0, 4.1 Hz, 1H; 5-H), 3.42 (*br* s, 1H; 4-H), 3.11 (t, ^3J(H,H)=6.6 Hz, 0.5H; 3'-H of HCO_3^- salt), 2.90 (t, ^3J(H,H)=6.6 Hz, 1.5H; 3'-H of free amine), 2.87-2.78 (m, 2H; 6-Ha, 2-Ha), 2.59-2,49 (m, 1.5H; 1'-H of free amine), 2.48-2.41 (m, 0.5H; 1'-H of HCO_3^- salt), 2.37-2.24 (m, 1H, 2-Hb), 2.21-2.10 (m, 1H, 6-Hb), 1.76 (dquint, ^2J(H,H)=13.9, ^3J(H,H)=6.6 Hz, 1.5H; 2'-H of free amine) 1.68 ppm (quint, ^3J(H,H)= 7.1 Hz, 0.5 H, 2'-H of HCO_3^- salt); ^{13}C-NMR (125 MHz, CD_3OD): δ=164.5, 160.1 (s, H$\underline{C}O_3^-$), 74.0 (d, C-4), 68.3(d, C-5), 67.9 (d, C-3), 56.9 (t, C-6), 56.0 (t, C-2), 55.8, 55.4 (C-1', HCO_3^- and free amine), 39.9, 39.6 (C-3', HCO_3^- salt and free amine), 27.2-26.0 ppm (C-2', HCO_3^- salt and free amine). ^{1}H-NMR (400 MHz, D_2O) δ=3.90-3.87 (m, 1H; 3-H), 3.77-3.71 (m, 1H; 5-H), 3.39-3.37 (m, 1H; 4-H), 2.89 (t, ^3J(H,H)=6.8 Hz, 0.8 H; 3'-H of HCO_3^- salt),2.78 (t, ^3J(H,H)=6.8 Hz, 1.2 H; 3'-H of free amine), 2.80-2.69 (m, 2H; 6-Ha, 2-Ha), 2.48-2.26 (m, 2H; 1'-H), 2.21 (*br* d, ^2J(H,H)=11.7 Hz, 1H; 2-Hb), 2.60-1.94 (m, 1H; 6-Hb), 1.65 (quint, ^3J(H,H)=7.3 Hz, 1.3H; 2'-H of free amine), 1.52 ppm (quint, ^3J(H,H)= 7.3 Hz, 0.7H, 2'-H of HCO_3^- salt); ^{13}C-NMR (50 MHz, D_2O): δ=73.6 (d, C-4), 67.6 (d, C-3), 67.5 (d, C-5), 56.4 (t, C-6), 55.5 (t, C-2), 54.7 (C-1', HCO_3^- and free amine), 39.4, 38.8 (C-3', HCO_3^- salt and free amine), 26.4-25.5 ppm (C-2', HCO_3^- salt and free amine). MS (ESI): *m/z* (%): 191.08 (100) $[M+H]^+$.

Synthesis of (3*R*,5*R*)-1-[3-(Acetylamino)propyl]piperidine-3,4,5-tri-*O*-acetyl-3,4,5-trihydroxypiperidine (178): A solution of **168f** (13 mg, 0.07 mmol) in pyridine (0.3 mL) and Ac_2O (0.2 mL) was left stirring at room temperature for 18 h and then concentrated under vacuum. The crude product was purified by flash column chromatography on silica gel (DCM/MeOH 15:1) affording 22 mg (0.06 mmol, 86%) of **178** as a colorless oil. R_f=0.32 (DCM/MeOH 15:1); $[\alpha]_D^{25}$**=-**58.5 (c=0.93 in $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 6.65 (*br* s, 1H; NH), 5.30 (dt, ^3J(H,H)= 5.2, 2.7 Hz, 1H; 3-H), 5.11 (dt, ^3J(H,H)=8.6, 4.3 Hz, 1H; 5-H), 4.95 (dd, ^3J(H,H)=8.6, 3.6 Hz, 1H; 4-H), 3.46 (td, ^2J(H,H)=12.1, ^3J(H,H)=5.8 Hz, 1H; 3'-Ha), 3.20-3.12 (m, 1H; 3'-Hb), 3.04-3.02 (m, 1H; 6-Ha), 2.86-2.83 (m, 1H; 2-Ha), 2.46 (t, ^3J(H,H)=6.3 Hz, 2H; 1'-H), 2.44 (d, ^2J(H,H)=11.7 Hz, 1H; 2-Hb), 2.24-2.18 (m, 1H; 6-Hb), 2.08 (s, 3H; Me), 2.04 (s, 3H; Me), 2.02 (s, 3H; Me), 1.98 (s, 3H; Me), 1.64 ppm (quint, ^3J(H,H)=6.3 Hz, 2H; 2'-H); ^{13}C-NMR (50 MHz, $CDCl_3$): δ=173.5-169.9 (s, 4C, $\underline{C}OCH_3$), 70.7 (d, C-4), 67.9 (d, C-5), 67.7 (d, C-3), 56.0 (t, C-1'), 54.0 (t, C-6), 53.8 (t, C-2), 38.9 (t, C-3'), 25.5 (t, C-2'), 23.2-20.7 ppm (q, 4C, $CO\underline{C}H_3$); MS (ESI): *m/z* (%): 381.41 (100) $[M+Na]^+$; IR ($CDCl_3$): $\tilde{\nu}$=3689, 3606, 3451, 3312, 3950, 2825, 2257, 2245, 1743, 1660, 1522, 1372, 1230, 1050 cm^{-1}; elemental analysis calcd (%) for $C_{16}H_{26}N_2O_7$ (358.39): C 53.62, H 7.31, N 7.82; found C 53.20, H 7.42, N 8.16.

Synthesis of (3*R*,5*R*)-1-[3-(acetylamino)propyl]-3,4,5-trihydroxy-piperidine (168g): To a solution of compound **178** (74 mg, 0.21 mmol) in MeOH (7 mL), 300 mg of Ambersep®900-OH were added and the mixture was stirred at room temperature. After 2 h, the ion-exchange resin was filtered washing with MeOH. The solvent was evaporated under vacuum affording the pure compound **168g** (48 mg, 0.15 mmol, 71%). $[\alpha]_D^{24}$**=-**33.1 (c=1.17 in H_2O); ^{1}H-NMR (400 MHz, D_2O): δ=3.88-3.86 (m, 1H; 3-H), 3.73 (td, ^3J(H,H)= 8.8, 4.4 Hz, 1H; 5-H), 3.37 (dd, ^3J(H,H)=8.3, 2.4 Hz 1H; 4-H), 3.06 (t, ^3J(H,H)=6.9 Hz, 2H; 3'-H), 2.77-2.71 (m, 2H; 6-Ha; 2-Ha), 2.36-2.25 (m, 2H; 1'-H), 2.18 (d, ^2J(H,H)=12.2 Hz, 1H; 2-Hb), 2.02-1.92 (m, 1H; 6-Hb), 1.85 (s, *3*H; Me), 1.57 ppm (quint, ^3J(H,H)=6.8 Hz, 2H; 2'-H); ^{13}C-NMR (50 MHz, D_2O): δ=173.91 (s, $\underline{C}OCH_3$), 75.6 (d, C-4), 67.6 (d, C-3), 67.5 (d, C-5), 56.4 (t, C-1'), 55.5 (t, C-2), 54.5 (t, C-6), 37.6 (t, C-3'), 25.1 (q, $CO\underline{C}H_3$), 21.8 ppm (t, C-2'); MS (ESI): *m/z* (%): 255.08 (100) $[M+Na]^+$, 233.17 (72) $[M+H]^+$; elemental analysis calcd (%) for $C_{10}H_{20}N_2O_4$ (232.28): C 51.71, H 8.68, N 12.06; found C 51.61, H 8.39, N 12.10.

Synthesis of (3*R*,4*R*)-5-oxo-3,4-*O*-(1-methylethylidene)-*N*-Boc-piperidine (180): To a stirred solution of **175** (75 mg, 0.43 mmol) and $NaHCO_3$ (56 mg, 0.67 mmol) in H_2O (1.0 mL), MeOH (1.0 mL), and Boc_2O (141 mg, 0.65 mmol) were added. The mixture was stirred at room temperature for 48 h, concentrated and extracted with AcOEt (3x20 mL). The

combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated to give **179** (109 mg, 0.40 mmol, 93%) pure enough to be employed in next step without further purification. Dess-Martin periodinane (280 mg, 0.66 mmol) was added to a solution of **179** (119 mg, 0.44 mmol) in dry CH_2Cl_2 (4.7 mL) at room temperature. The reaction mixture was stirred for 2 h then washed with a $NaHCO_3$ saturated solution, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel flash chromatography (PE/AcOEt 2:1) to give **180** (117 mg, 0.43 mmol, 98%), m.p. 63-65 °C. $[\alpha]_D^{24}$ **=** + 19.6 (c = 1.17, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.65 (*br* s, 1H, 3-H), 4.40 (d, *J* = 7.3 Hz, 1H, 4-H), 4.38-4.25 (m, 1H, 6-Ha), 3.94 (d, *J* = 18.4 Hz, 1H, 6-Hb), 3.85-3.68 (m, 1H, 2-Ha), 3.60 (dd, *J* = 14.7, 3.9 Hz, 1H, 2-Hb), 1.47 (s, 12H, Me, tBu), 1.38 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 203.1 (s, C=O), 154.4 (s, N<u>C</u>OO), 111.5 (s, acetal), 80.8 (s, OCtBu), 77.5 (d, C-4), 74.9 (d, C-3), 53.8, 53.1 (t, C-6, two rotamers), 45.8, 44.7 (t, C-2, two rotamers), 28.2 (q, 3C, *t*Bu), 26.6 (q, Me), 25.0 (q, Me) ppm. MS (ESI): *m/z* (%) = 294.03 (20) $[M+Na]^+$. IR (KBr): ν = 3461, 2978, 2935, 2254, 1740, 1692, 1417, 1370, 1245, 1210, 1155 cm^{-1}. $C_{13}H_{21}NO_5$ (271.31): calcd. C, 57.55; H, 7.80; N, 5.16; found C 57.68, H 7.54, N 4.98.

Synthesis of (3*R*,4*S*,5*S*)-5-hydroxy-3,4-*O*-(1-methylethylidene)-*N*-Boc-piperidine (181): A solution of **180** (21 mg, 0.08 mmol) in EtOH (0.5 mL) was cooled to 0°C and $NaBH_4$ (7.6 mg, 0.20 mmol) was slowly added. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. Then, water (0.1 mL) and MeOH (0.3 mL) were added and mixture was stirred for 7 h at room temperature and concentrated under reduced pressure. The crude product was purified by silica gel flash chromatography (PE/AcOEt 2:1) to give **181** (16 mg, 0.06 mmol, 76%). $[\alpha]_D^{22}$ **=** + 3.54 (c = 0.96, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 4.35 (*br* s, 2H, 3-H, 4-H), 3.92-3.84 (m, 1H, 5-H), 3.63-3.58 (m, 1H, 6-Ha), 3.55-3.44 (m, 2H, 2-H), 3.16 (dd, *J* = 12.4, 9.6 Hz, 1H, 6-Hb), 2.29 (d, *J* = 8.3 Hz, 1H, O*H*), 1.50 (s, 3H, Me), 1.46 (s, 9H, *t*Bu), 1.37 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 154.7 (s, N<u>C</u>OO), 109.2 (s, acetal), 79.7 (s, O<u>C</u>*t*Bu), 73.3, 71.9 (d, 2C, C-3, C-4), 65.5 (d, C-5), 43.8, 43.1 (t, C-6, two rotamers), 42.6, 41.5 (t, C-2, two rotamers), 28.1 (q, 3C, *t*Bu), 26.5 (q, Me), 24.6 (q, Me) ppm. MS (ESI): *m/z* (%) = 296.08 (100) $[M+K]^+$. IR (KBr): ν = 3565, 2978, 2935, 2246, 1684, 1409, 1163 cm^{-1}. $C_{13}H_{23}NO_5$ (273.33): calcd. C 57.13, H 8.48, N 5.12; found C 57.61, H 8.54, N 5.06.

Synthesis of (3*R*,4*s*,5*S*)-3,4,5-trihydroxy-piperidine (165): A solution of **181** (17 mg, 0.06 mmol) in MeOH (4.0 mL) and HCl 1M (5 drops) was left stirring at room temperature overnight. The crude mixture was concentrated to yield the hydrochloride of **165**, which

was eluted through an ion-exchange resin (DOWEX® 50XW8-100) with MeOH, H_2O and 6% NH_4OH to give the corresponding free amine **165** (8 mg, 100%), m.p. 163-165 °C. $[\alpha]_D^{22}$ = + 0.0 (c = 0.60, MeOH). ^{1}H-NMR (400 MHz, D_2O) δ = 3.87 (t, *J* = 2.4 Hz, 4-H), 3.66-3.62 (m, 2H, 3-H, 5-H), 2.68 (dd, *J* = 12.7, 4.4 Hz, 2H), 2.61 (dd, *J* = 12.7, 8.8 Hz, 2H). ppm. ^{13}C-NMR (50 MHz, D_2O) δ = 69.5 (d, C-4), 67.4 (d, 2C, C-3, C-5), 43.9 (t, 2C, C-2, C-6) ppm. MS (ESI): *m/z* (%) = 156.06 (100) $[M+Na]^+$. $C_5H_{11}NO_3$ (133.15): C 45.10, H 8.33, N 10.52; found C 44.97, H 8.39, N 10.43.

Synthesis of (*3R,4S,5S)-N*-Boc-3,4-*O*-(1-methylethylidene)-5-*N*-benzyl-aminopiperidine (182a): A solution of ketone **180** (69 mg, 0.25 mmol) in dry MeOH (4.5 mL) was stirred in the presence of 3Å molecular sieves powder,under nitrogen atmosphere, for 15 min before adding benzylamine (43 µL, 0.39mmol) and AcOH (22 µL, 0.39 mmol). The reaction mixture was left stirring for 3 h and then $NaBH_3CN$ (49 mg, 0.78 mmol) and more AcOH (8 µL, 0.13 mmol) were added. The mixture was stirred for 25 h, under nitrogen atmosphere. The molecular sieves were removed by filtration through Celite® and the filtrate was concentrated under vacuum. The residue was purified by flash chromatography (EP/AcOEt/NEt_3 2: 1:0.1) affording **182a** (43 mg, 0.12 mmol) in 47% yield. $[\alpha]_D^{25}$ = +16.25 (c= 0.8, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm= 7.39-7.23 (m, 5H, Ar), 4.41 (dd, *J* = 6.8, 2.5 Hz, 1H, 4-H), 4.27 (*br* s, 1H, 3-H), 3.90 (m, 2H, Bn), 3.66-3.55 (m, 2H, 2-Ha, 6-Ha), 3.28 (dd, *J* =14.0, 3.9 Hz, 1H, 2-Hb), 2.98 (*p*t, *J* = 11.7, 1H, 6-Hb), 2.84 (m, 1H, 5-H), 1.45 (s, 12H, *t*-Bu, Me), 1.34 (s, 3H, *Me*). ^{13}C-NMR (50 MHz, $CDCl_3$) δ ppm = 155.2 (s, C=O), 139.9 (s, Ar), 128.4-127.1 (d, 5C, Ar), 108.8 (s, aceal), 79.7 (s, O*C*tBu), 72.4 (d, 2C, C-3, C-4), 52.4 (d, C-5), 50.5 (t, Bn), 42.5 (t, 2C, C-2, C-6), 28.4 (3C, *t*-Bu), 26.9 (q, Me), 24.9 (q, Me). MS (ESI): *m/z* (%) = 385.25 (100) $[M+Na]^+$. IR ($CDCl_3$) ν = 3333, 2981, 2933, 2248, 1685, 1454, 1413, 1163 cm^{-1}.

General procedure for the synthesis of *N*-alkyl aminopiperidines (182b-182c): Ketone **180** and the appropriate amine (1.5 equiv) were dissolved in MeOH (0.05 M) and 25 mg of 3Å pellets molecular sieves were added. The reaction mixture was left stirring at room temperature for 50 minutes and, when the complete conversion to imine was observed (^{1}H NMR control), $Pd(OH)_2/C$ (50% wt) was added and the mixture was left stirring at room temperature under H_2 atmosphere for 24 h. The catalyst and the molecular sieves were filtered, washing several times with MeOH and then the solvent was evaporated under vacuum.

(*3R,4S,5S)-N*-Boc-3,4-*O*-(1-methylethylidene)-5-*N*-butyl- aminopiperidine (182b):

Application of this procedure to 68 mg (0.25 mmol) of **180** with butylamine (37 µL, 0.38 mmol) furnished, after purification by flash column chromatography (EP/AcOEt 2:1), compound **182b** (57 mg, 0.17 mmol, 68%) as a colorless oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ ppm= 4.46 (m, 1H, 4-H), 4.29 (*br* s, 1H, 3-H), 3.59-3.50 (m, 2H), 3.31, (dd, *J* = 14.3, 3.7 Hz, 1H), 2.98-2.65 (m, 4H), 1.56-1.16 (m, 19H, *t*-Bu*,* Me*,* 2'-H, 3'-H), 0.86 (t, *J* = 7.1 Hz, 3H, 4'-H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ ppm= 155.2 (s, C=O), 108.8 (s, acetal), 79.7 (s, O<u>C</u>*t*Bu), 72.4 (d, 2C, C-3, C-4), 53.5, 46.6, 42.8 (4C, C-2, C-5, C-6, C-1'), 32.4-20.3 (7C, *t*-Bu*,* Me*,* C-2', C-3'), 13.9 (q, C-4'). MS (ESI): *m/z* (%) = 329.25 (100) $[M+H]^+$.

(*3R,4S,5S)*-*N*-Boc-3,4-*O*-(1-methylethylidene)-5-*N*-octyl-aminopiperidine (182c):
Application of this procedure to 52 mg (0.19 mmol) of **180** with butylamine (47 µL, 0.28 mmol) furnished, after purification by gradient flash column chromatography (EP/AcOEt from 5:1 to 2.1), compound **182c** (39 mg, 0.10 mmol, 53%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ ppm= 4.44 (m, 1H, 4-H), 4.30 (*br* s, 1H, 3-H), 3.79-3.55 (m, 2H), 3.32 (dd, *J* = 14.1, 3.5 Hz, 1H), 2.98-2.68 (m, 4H), 1.99 (m, 2H, 2'-H), 1.53-1.13 (m, 25H, 3'-7'-H, Me*, t*-Bu), 0.87 (t, *J* =6.3 Hz, 3H, 8'-H). ^{13}C-NMR (50 MHz, $CDCl_3$): δ ppm= 155.2 (s, C=O), 108.8 (s, acetal), 79.6 (s, O<u>C</u>*t*Bu), 72.4 (d, 2C, C-3, C-4), 53.5, 46.9 (2C, C-5, C-1'), 42.4 (t, 2C, C-2, C-6), 31.8-22.6 (11 C, C-2' -7', Me*, t*-Bu), 14.0 (q, C-8'). MS (ESI): *m/z* (%) = 385.33 (100) $[M+H]^+$.

General procedure for the synthesis of completely deprotected *N*-alkyl aminopiperidines (169a, 169b and 169c): A 0.02 M solution of **182a-c** in MeOH was left stirring with HCl 12M (5 drops) at room temperature overnight. The crude mixture was concentrated to yield the hydrochloride salts of **169a-c**. The corresponding free amine was obtained by passing the hydrochloride salt through a DOWEX® 50XW8-100 ion-exchange resin. Elution with 6% ammonia afforded the free bases **169a-c**.

(3*R*,4*S*,5*S*)-3,4-dihydroxy-5-*N*-benzyl aminopiperidine (169a): Application of this procedure to 28 mg (0.08 mmol) of **182a** furnished the free amine **169a** (17 mg, 100%). $[\alpha]_D^{25}$ **=** - 3.6 (c = 0.74, MeOH). ^{1}H-NMR (400 MHz, D_2O) δ ppm= 7.32-7.22 (m, 5H, Ar), 4.04 (*br* s, 1H, 4-H), 3.76 (d, *J* = 13.1 Hz, Bn), 3.69 (d, *J* = 13.1 Hz, Bn), 3.53 (ddd, *J* = 10.5, 4.7, 1.9 Hz, 1H, 3-H), 2.77-2.64 (m, 3H, 6-Ha, 2-Ha, 5-H), 2.55 (t, *J* = 11.5 Hz, 1H, 2-Hb), 2.43 (t, *J* = 11.5 Hz, 6-Hb).^{13}C-NMR (50 MHz, D_2O) δ ppm= 137.4 (s, Ar), 127.8-126.6 (d, 5C, Ar), 67.6 (d, C-3), 67.2 (d, C-4), 54.4 (d, C-5), 48.2 (t, Bn), 43.15 (t, C-2), 41.6 (t, C-6). MS (ESI): *m/z* (%) = 223.17 (100) $[M+H]^+$.

(3*R*,4*S*,5*S*)-3,4-dihydroxy-5-*N*-butyl aminopiperidine (169b): Application of this procedure to 20 mg (0.06 mmol) of **182b** furnished the free amine **169b** (12 mg, 100%) as a colorless oil. $[\alpha]_D^{26}$ **=** - 8.1 (c = 0.44, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ ppm= 4.04 (*br* s, 1H, 4-H), 3.56 (m, 1H, 3-H), 2.77-3.89 (m, 7H, 2-H, 5-H, 6-H, 1'-H), 1.38 (quint, *J* = 7.4 Hz, 2H, 2'-H), 1.22 (sest, *J* = 7.3 Hz, 2H, 3'-H) 0.78 (t, *J*= 7.3 Hz, 3H, 4'-H).^{13}C-NMR (50 MHz, D_2O) δ ppm= 68.6 (d, C-3), 67.9 (d, C-4), 56.2 (d, C-5), 44.9 (t, C-1') 44.3, (t, C-2), 42.3 (t, C-6), 30.1 (t, C-2'), 19.7 (t, C-3') 13.1 (q, C-4'). MS (ESI): *m/z* (%) = 189.17 (100) $[M+H]^+$.

(3*R*,4*S*,5*S*)-3,4-dihydroxy-5-*N*-octyl aminopiperidine (169c): Application of this procedure to 38 mg (0.10 mmol) of **182c** furnished the free amine **169c** (14 mg, 0.06 mmol, 60%) as a colorless oil. $[\alpha]_D^{24}$ **=** - 2.5 (c = 0.36, MeOH). ^{1}H-NMR (400 MHz, D_2O) δ ppm= 4.03 (*br* s, 1H, 4-H), 3.57 (d, *J* = 7.2 Hz, 1H, 3-H), 2.78-2.47 (m, 7H, 2-H, 5-H, 6-H, 1'-H), 1.42 (*br* s, 2H, 2'-H), 1.17 (m, 10H, 3'-7'-H), 0.74 (t, *J* = 6.8 Hz, 3H, 8'-H). ^{13}C-NMR (50 MHz, D_2O) δ ppm= 67.7 (d, C-3), 66.8 (d, C-4), 55.6 (d, C-5), 44.8 (t, C-1'), 43.7 (t, C-2), 41.4 (t, C-6), 30.6-21.4 (t, 6C,), 12.8 (q, C-8'). MS (ESI): *m/z* (%) = 245.25 (100) $[M+H]^+$.

Synthesis of (3*R*,4*S*,5*S*)-3,4-dihydroxy-5- aminopiperidine (169d): To a solution of **182a** (35 mg, 0.10 mmol) in MeOH (5 mL) and HCl 12M (3 drops) Pd /C (18 mg) was added and the reaction mixture was left stirring at room temperature under H_2 atmosphere for 6 days. The catalyst was filtered through Celite® and the filtrate was concentrated under vacuum, to yield quantitative the hydrochloride of **169d** which was eluted through an ion-exchange resin (DOWEX® 50XW8-100) with MeOH, H_2O and 6% NH_4OH to give the free amine **36** (13 mg, 100%). $[\alpha]_D^{25}$ **=** +3.84 (c = 0.25, MeOH). MS (ESI): *m/z* (%) = 155.11 (100) $[M+Na]^+$, 133.09 (32) $[M+H]^+$.

General Procedure for the Strecker Reaction: A 0.2 M solution of compound **166** and the appropriate amine source ($BuNH_2$, $BnNH_2$, 1.0 equiv) in dry CH_3CN with 3Å pellets molecular sieves was stirred at room temperature for 40 minutes. Trimethylsilyl cyanide(1.0 equiv) and $Cu(OTf)_2$ (1.0 mol%) were added and the resulting reaction mixture was stirred at room temperature for 18 hours, then diluted with AcOEt and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude products as a >95:5 mixture of two diastereoisomers (as determined by ^{1}H NMR analysis), which were purified by flash chromatography (PE/AcOEt) to afford pure major diastereoisomers **183a** and **184a**.

1-*O*-Benzyl-5-benzylamino-5-cyano-5-deoxy-2,3-*O*-(1-methylethylidene)-β-L-erythro-pentofuranose (183a): Application of the general procedure to 110 mg (0.40 mmol) of

aldehyde **166** and benzylamine (44 µL, 0.40 mmol) afforded a crude mixture, that was purified by column chromatography on silica gel (PE/AcOEt 8:1) to afford the pure major diastereoisomer **183a** with 80% yield (125 mg, 0.32 mmol) as a colorless oil. $[\alpha]_D^{25}$ = +75.5 (c = 1.00, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.37-7.17 (m, 10 H, Ar), 5.12 (s, 1H, 1-H), 4.76 (dd, *J* = 5.8, 3.9 Hz, 1H, 3-H), 4.60 (d, *J* = 5.9 Hz, 1H, 2-H), 4.56 (d, *J* = 12.0 Hz, 1H, O-Bn), 4.41 (d, *J* = 12.0 Hz, 1H, O-Bn), 4.10 (dd, *J* = 7.8, 3.9 Hz, 1H, 4-H), 4.03 (d, *J* = 12.9 Hz, 1H, N-Bn), 3.91 (d, *J* = 7.8 Hz, 1H, 5-H), 3.79 (d, *J* = 12.9 Hz, 1H, N- Bn), 1.93 (*br* s, 1H, NH), 1.45 (s, 3H, Me), 1.23 (s, 3H, Me) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 138.1, 137.0 (s, 2C, Ar), 128.6-127.6 (d, 10C, Ar), 118.0, 113.6 (s, 2C, CN + acetal), 105.7 (d, C-1), 84.7 (d, C-2), 79.2 (d, C-3), 78.4 (d, C-4), 69.5 (t, O-Bn), 51.8 (t, N-Bn), 49.6 (d, C-5), 25.2 (q, Me), 24.5 (q, Me) ppm. MS (ESI): *m/z* (%) = 417.00 (71) $[M+Na]^+$. IR ($CHCl_3$): ν = 3335, 3067, 3025, 3010, 2240, 2944, 1952, 1604, 1497, 1455, 1376, 1262, 1027 cm^{-1}. $C_{23}H_{26}N_2O_4$ (394.46): calcd. C 70.03, H 6.64, N 7.10; found C 69.73, H 6.80, N 7.18.

1-*O*-Benzyl-5-butylamino-5-cyano-5-deoxy-2,3-*O*-(1-methylethylidene)-β-L-erythro-pentofuranose (184a): Application of the general procedure to 155 mg (0.56 mmol) of aldehyde **166** and butylamine (44 µL, 0.40 mmol) afforded a crude mixture, that was purified by column chromatography on silica gel (PE/AcOEt 8:1) to afford the pure major diastereoisomer **184a** with 75% yield (152 mg, 0.42 mmol) as a colorless oil. $[\alpha]_D^{26}$ = + 59.7 (c = 0.95, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.29-7.18 (m, 5 H, Ar), 5.12 (s, 1H, 1-H), 4.75 (dd, *J* = 6.3, 3.4 Hz, 1H, 3-H), 4.61 (d, *J* = 6.3 Hz, 1H, 2-H), 4.59 (d, *J* = 11.7 Hz, 1H, Bn), 4.43 (d, *J* = 11.7 Hz, 1H, Bn), 4.10 (dd, *J* = 8.3, 3.4 Hz, 1H, 4-H), 3.89 (d, *J* = 8.3 Hz, 1H, 5-H), 2.83 (ddd, *J* = 10.8, 7.8, 6.4 Hz, 1H, 1'-Ha), 2.57 (ddd, *J* = 11.2, 8.2, 6.3 Hz, 1H, 1'-Hb), 1.51-1.27 (m, 4H, 2'-H + 3'-H), 1.45 (s, 3H, Me), 1.24 (s, 3H, Me), 0.87 (t, *J* = 7.3 Hz, 3H, 4'-H) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 137.1 (s, Ar), 128.5-128.0 (d, 5C, Ar), 118.2, 113.5 (s, 2C, CN, acetal), 105.9 (d, C-1), 84.7 (d, C-2), 79.2 (d, C-3), 78.5 (d, C-4), 69.4 (t, Bn), 50.5 (d, C-5), 47.7 (t, C-1'), 31.7 (t, C-2'), 25.3 (q, Me), 24.5 (t, C-3'), 20.3 (q, Me), 13.9 (q, C-4') ppm. MS (ESI): *m/z* (%) = 382.82 (100), $[M+Na]^+$. IR ($CDCl_3$): ν = 3334, 3067, 3033, 2959, 2874, 2249, 1455, 1376, 1210, 1084 cm^{-1}. $C_{20}H_{28}N_2O_4$ (360.45): calcd. C 66.64, H 7.83, N 7.77; found C 66.94, H 7.85, N 7.93.

Synthesis of 1-*O*-benzyl-5-amino-5-cyano-5-deoxy-2,3-*O*-(1-methylethylidene)-β-L-erythro-pentofuranose (185a): To a solution of aldehyde **166** (79 mg, 0.28 mmol) in EtOH (3.0 mL), NH_4OAc (216 mg, 2.80 mmol)was added. The mixture was stirred at room temperature for 40 min, then TMSCN (43 µL, 0.34 mmol) was added dropwise. The solution

was stirred at room temperature for 18 h, then diluted with AcOEt, washed with sat. aq. $NaHCO_3$, H_2O and brine, dried (Na_2SO_4), filtered and concentrated under vacuum. ^{1}H NMR analysis of the crude mixture showed the formation of two diasteroisomers in 81:19 ratio. Purification of the crude product using silica gel gradient flash chromatography afforded 52 mg (0.17 mmol, 61%) of the major diastereoisomer **185a**. Crystallization of **185a** (EtOAc) yielded fine white needles, m.p. 115-117 °C, which were subjected to a single-crystal X-ray diffraction. $[\alpha]_D^{26}$ **=** + 74.8 (c = 0.52, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.30-7.21 (m, 5H, Ar), 5.12 (s, 1H, 1-H), 4.77 (dd, *J* = 6.3, 3.4 Hz, 1H, 3-H), 4.62 (d, *J* = 6.2 Hz, 1H, 2-H), 4.60 (d, *J* = 11.7 Hz, 1H, Bn), 4.40 (d, *J* = 11.7 Hz, 1H, Bn), 4.07 (d, *J* = 7.8 Hz, 1H, 5-H), 4.02 (dd, *J* = 7.8, 3.4 Hz, 1H, 4-H), 1.69 (br s, 2H, NH_2), 1.45, (s, 3H, Me), 1.25 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 136.7 (s, Ar), 128.2-127.7 (d, 5C, Ar), 119.4, 113.1 (s, 2C, CN, acetal), 105.4 (d, C-1), 84.5 (d, C-2), 79.7 (d, C-4), 78.8 (d, C-3), 69.2 (t, Bn), 43.2 (d, C-5), 25.1 (q, Me), 24.1 (q, Me) ppm. MS (ESI): *m/z* (%) = 327.06 (100), $[M+Na]^+$, 361.11 (12), $[M+H]^+$. IR (KBr): ν = 3368, 3261, 3161, 2932, 2360, 2235, 1595, 1376, 1081 cm^{-1}. $C_{16}H_{20}N_2O_4$ (304.14): C 63.14, H 6.82, N 9.20; found C 63.12, H 6.49, N 8.97.

Synthesis of (3*R*,4*S*,5*R*)-1-benzyl-5-hydroxy-3,4-*O*-(1-methylethylidene)-piperidine (176d) and (2*S*,3*R*,4*S*,5*R*)-1-benzyl-2-cyano-3-hydroxy-4,5-*O*-(1-methyethylidene)-piperidine (186), Scheme 3.17: To a solution of dialdehyde **177** (323 mg, 1.72 mmol) in MeOH (3.6 mL), benzylamine (225 µL, 2.06 mmol) and $NaBH_3CN$ (216 mg, 3.44 mmol) were added. The mixture was stirred at room temperature for a total of five days, until the complete starting material disappearance (TLC control) was observed. ^{1}H NMR analysis of the crude mixture showed the formation of **176d** and **186** in 6:1 ratio. The solvent was evaporated under vacuum and the residue was purified using silica gel flash chromatography (CH_2Cl_3/Et_2O 7:1) to give 147 mg of a 2.7: 1 mixture of **176d** and **186** and 51 mg of pure **176d** (0.19 mmol, 11%).

176d[163]**:** $[\alpha]_D^{24}$ **=** + 10.8 (c = 1.16, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.34-7.24 (m, 5H, Ar), 4.29 (dd, *J* = 6.9, 5.8 Hz, 1H, 3-H), 4.06 (t, *J* = 4.8 Hz, 1H, 4-H), 3.93 (dd, *J* = 7.1, 4.2 Hz, 1H, 5-H), 3.59-3.51 (m, 2H, Bn), 2.80 (ddd, *J* = 11.7, 6.1, 1.5 Hz, 1H, 2-Ha), 2.58-2.48 (m, 2H, 6-H), 2.39 (dd, *J* = 11.7, 7.4 Hz, 1H, 2-Hb), 1.50 (s, 3H, Me), 1.35 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 137.5 (s, Ar), 129.0-127.4 (d, 5C, Ar), 109.3 (s, acetal), 77.0 (d, C-4), 72.1 (d, C-3), 67.7 (d, C-5), 62.1 (t, Bn), 55.5 (t, C-2), 55.1 (t, C-6), 28.3 (q, Me), 26.4 (q, Me) ppm. MS (ESI): *m/z* (%) = 264.31 (100) $[M+H]^+$, 286.19 (35) $[M+Na]^+$. IR ($CDCl_3$): ν = 3602, 3497, 2988,

[163] Zhi-cai, S.; Chun-min, Z.; Guo-qiang, L. *Heterocycles* **1995**, *41*, 277-287.

2938, 2827, 2420, 2248, 1601, 1494, 1454, 1383, 1219, 1058 cm^{-1}. $C_{15}H_{21}NO_3$ (263.33): calcd. C 68.42, H 8.04, N 5.32; found C 68.21, H 7.85, N 5.33.

Synthesis of (2*S*,3*R*,4*R*,5*R*)-1-benzyl-2-cyano-3-*O*-acetyl-4,5-*O*-(1-methyethylidene)-piperidine (187) as by-product in the reductive amination (Scheme 3.17): A mixture of **176d** and **186** was dissolved in Py:Ac_2O (3:2 v/v) and stirred at r.t. overnight, then the solvent was evaporated under reduced pressure and the crude purified by flash chromatography (CH_2Cl_2/MeOH from 50:1 to 20:1) affording pure **187**. $[\alpha]_D^{24}$ **=** - 37.3 (c = 0.59, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.30-7.19 (m, 5H, Ar), 4.93 (dd, *J* = 8.4, 4.8 Hz, 1H, 3-H), 4.32-4.26 (m, 1H, 5-H), 4.18 (dd, *J* = 8.4, 5.2 Hz, 1H, 4-H), 3.91 (d, *J* = 4.8 Hz, 1H, 2-H), 3.73 (d, *J* = 13.4 Hz, 1H, Bn), 3.52 (d, *J* = 13.4 Hz, 1H, Bn), 3.29 (d, *J* = 14.0 Hz, 1H, 6-Ha), 2.81 (dd, *J* = 14.0, 2.8 Hz, 1H, 6-Hb), 2.04 (s, 3H, acetyl), 1.49 (s, 3H, Me), 1.30 (s, 3H, Me). ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 170.1 (s, C=O), 135.6 (s, Ar), 128.8-128.1 (d, 5C, Ar), 113.4 (s, CN), 110.1 (s, acetal), 74.3 (d, C-4), 72.9 (d, C-5), 71.1 (d, C-3), 59.2 (t, Bn), 53.3 (d, C-2), 49.3 (t, C-6), 27.0 (q, Me), 26.2 (q, Me), 20.8 (q, CH_3CO) ppm. MS (ESI): *m/z* (%) = 353.06 (100) $[M+Na]^+$. IR ($CDCl_3$): ν = 2989, 2938, 2832, 2257, 1752, 1496, 1455, 1379, 1220 cm^{-1}. $C_{18}H_{22}N_2O_4$ (330.38): calcd. C 65.44, H 6.71, N 8.48; found. C 65.61, H 6.93, N 8.37.

Synthesis of (2*S*,3*R*,4*R*,5*R*)-1-benzyl-2-cyano-3-*O*-acetyl-4,5-*O*-(1-methyethylidene)-piperidine (187), through Strecker reaction (Scheme 3.17): To a solution of dialdehyde **177** (132 mg, 0.70 mmol) in dry CH_3CN (1.4 mL), benzylamine (77 µL, 0.70 mmol) and 3Å molecular sieves were added under N_2 atmosphere. The reaction mixture was stirred at room temperature for one hour, then TMSCN (71 µL, 0.70 mmol) was added dropwise. The solution was stirred at room temperature for 18 h, then, once the starting material disappeared (TLC control), $NaBH_3CN$ (132 mg, 2.1 mmol), EtOH (1.0 mL) and AcOH (80 µL, 1.40 mmol) were added. The reaction mixture was left stirring at room temperature for 3 days under nitrogen atmosphere and then concentrated under vacuum. The crude compound was fast purified by silica gel flash chormatography (eluent $CHCl_3$/Et_2O 7:1) to obtain **186** (101 mg, 0.35 mmol, 50% yield). A small portion of **186** (14 mg, 0.05 mmol) was dissolved Py:Ac_2O (3:2 v/v) and stirred at r.t. overnight, then the solvent was evaporated under reduced pressure and the crude purified by flash chromatography (Et_2O/AcOEt 5:1) affording pure **187** (10 mg, 0.03 mmol, 62%). **186:** $[\alpha]_D^{24}$ **=** + 4.7 (c = 0.71, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.35-7.27 (m, 5H, Ar), 4.33-4.31 (m, 1H, 5-H), 4.08 (dd, *J* = 7.8, 5.4 Hz, 1H, 4-H), 3.86-3.83 (m, 1H, 3-H), 3.82 (d, *J* = 13.0 Hz, 1H, Bn) 3.78(d, *J* = 5.3 Hz, 1H, 2-H), 3.53 (d, *J* = 13.0 Hz, 1H, Bn), 3.35 (d, *J* = 14.2 Hz, 1H, 6-Ha), 2.91 (dd, *J* = 14.2, 3.2 Hz, 1H, 6-Hb), 1.53 (s, 3H, Me), 1.38 (s, 3H, Me). ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 135.9 (s, Ar), 129.0-

128.0 (d, 5C, Ar), 113.9 (s, CN), 109.9 (s, acetal), 77.7 (d, C-4), 73.0 (d, C-5), 70.5 (d, C-3), 59.2 (t, Bn), 55.3 (d, C-2), 49.9 (t, C-6), 28.2 (q, Me), 26.2 (q, Me) ppm. MS (ESI): *m/z* (%) = 311.0 (100) $[M+Na]^+$. IR ($CDCl_3$): ν = 3602.3, 3031.2, 2990.2, 2957.5, 2832.7, 2255.2, 1730.6, 1603.1, 1455.2, 1383.7, 1218.3, 1090.7, 1045.6 cm^{-1}. $C_{16}H_{20}N_2O_3$ (288.34): calcd. C 66.65, H 6.99, N 9.72; found. C 66.32, H 7.24, N 9.58.

Synthesis of acetyl 2,3-*O*-(1-methylethylidene)-α-D-*lyxo*-pentodialdo-1,4-furanoside (167): Compound **131** (7.22 g, 27.8 mmol) was dissolved in pyridine (12 mL) and Ac_2O (8 mL) and stirred at r.t. overnight. The reaction mixture was diluted in AcOEt (100 mL) and washed sequentially with a 1M CH_3COOH solution (2x150 mL), saturated aqueous solution of $NaHCO_3$ (2x60 mL) and brine (1x60 mL). The combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **188** (8.21 g, 27.2 mmol, 98%). A solution of **188** (8.21 g, 27.15 mmol), glacial AcOH (102 mL) and water (44 mL) was stirred for 18 h at room temperature and concentrated under reduced pressure. The residue was dissolved in AcOEt (200 mL), washed sequentially with saturated aqueous solution of $NaHCO_3$ (2x70 mL), water (2x40 mL) and brine (2x50 mL), dried (Na_2SO_4) and the solvent was concentrated to afford **189** (5.81 g, 22.2 mmol, 82%) as a colorless oil. To a vigorously stirred suspension of silica-gel supported $NaIO_4$ reagent (12.1 g) in CH_2Cl_2 (25 mL), a solution of **189** (1.57, 5.99 mmol) in CH_2Cl_2 (25 mL) was added and stirred at room temperature for 1 h. The reaction mixture was then filtered, washing the residue with $CHCl_3$. The combined filtrates were concentrated under reduced pressure and the residue was purified by gradient flash chromatography (AcOEt: PEt from 1:1 to 7:1), to give aldehyde **167** as a colorless oil (1.12 g, 4.87 mmol, 81%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 9.59 (d, *J* = 1.6 Hz, 1H, aldehyde), 6.29 (s, 1H, 1-H), 5.11 (dd, *J* = 5.8, 4.3 Hz, 1H, 3-H), 4.73 (d, *J* = 5.8 Hz, 1H, 2-H), 4.42 (dd, *J* = 4.3, 1.6 Hz, 1H, 4-H), 2.05 (s, 3H, OAc), 1.43 (s, 3H, Me), 1.28 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 197.7 (d, H<u>C</u>=O), 169.2 (s, O<u>C</u>=OCH_3), 114.0 (s, acetal), 100.1 (d, C-1), 85.3 (d, C-4), 84.5 (d, C-2), 80.9 (d, C-3), 25.8 (q, Me), 24.5 (q, Me), 20.9 (q, OC=O<u>C</u>H_3). ppm. MS (ESI): *m/z* (%) = 285.17 (100) $[(M+MeOH)+Na]^+$.

Synthesis of 1-*O*-Acetyl-5-benzylamino-5-cyano-5-deoxy-2,3-*O*-(1-methylethylidene)-β-L-erythro-pentofuranose(190a): To a solution of aldehyde **167** (505 mg, 2.19 mmol) in dry CH_3CN (11.0 mL), $BnNH_2$ (240 µL, 2.19 mmol) and 3Å pellets molecular sieves were added, under N_2 atmosphere. The mixture was stirred at room temperature for 40 min, then TMSCN (274 µL, 2.19 mmol) was added dropwise. The solution was stirred at room temperature for 18 h, then diluted with AcOEt, washed with sat. aq. $NaHCO_3$, H_2O and

brine, dried (Na_2SO_4), filtered and concentrated under vacuum. ^{1}H NMR analysis of the crude mixture showed the formation of two diasteroisomers in 86:14 ratio. Purification of the crude product using silica gel gradient flash chromatography (AcOEt/EP from 1:2 to 1:1) afforded 516 mg (1.49 mmol, 68%) of the major diastereoisomer **190a**, as a white solid. m.p. 80-82 °C. $[\alpha]_D^{19}$ **=** + 70.2 (c = 0.98, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.16-7.04 (m, 5H, Ar), 6.00 (s, 1H, 1-H), 4.68 (dd, *J* = 5.8, 3.9 Hz, 1H, 3-H), 4.50 (d, *J* = 5.8 Hz, 1H, 2-H), 4.03 (dd, *J* = 7.8, 3.9 Hz, 1H, 4-H), 3.89 (d, *J* = 12.9 Hz, NBn), 3.75 (d, *J* = 7.8 Hz, 1H, 5-H), 3.64 (d, *J* = 12.9 Hz, 1H, NBn), 1.83 (s, 3H, OAc), 1.32 (s, 3H, Me), 1.11 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 169.1 (s, O<u>C</u>=OCH_3), 138.0 (s, Ar), 128.6-127.7 8d, 5C, Ar), 117.7, 114.2 (2C, CN, acetal), 100.3 (d, C-19, 84.5 (d, C-2), 80.3 (d, C-49, 78.8 (d, C-3), 51.7 (t, NBn), 49.2 8d, C-5), 25.1 (q, Me), 24.5 (q, Me), 21.0 (q, OC=O<u>C</u>H_3). ppm. MS (ESI): *m/z* (%) =369.08 (100) $[M+Na]^+$. IR (KBr): ν = 3483, 3350, 2988, 2943, 2920, 2359, 2231, 1747, 1605, 1460 cm^{-1}. $C_{18}H_{22}N_2O_5$ (346.38): C 62.42, H 6.40, N 8.09; found C 62.79, H 6.27, N 8.05.

Synthesis of (2*S*,3*R*,4*S*,5*R*)-1-benzyl-2-carboxamide-3-hydroxy-4,5-*O*-(1-methyethylidene)-piperidine (191): To a solution of compound **190a** (376 mg, 1.09 mmol) in MeOH (43 mL), 8.5 g of Ambersep®900-OH were added and the mixture was stirred at room temperature. After 2 h, the ion-exchange resin was filtered washing with MeOH and $NaBH_3CN$ (75 mg, 1,20 mmol) and CH_3COOH (125 µL, 2.18 mmol) were added. The solution was stirred at room temperature for 18 h, until a ^{1}H NMR analysis of the crude mixture attested the complete formation of an only new product. The solvent was evaporated under vacuum and the residue was purified using silica gel flash chromatography (CH_2Cl_2/MeOH/NH_4OH 6% 10:1:0.1) to give compound **191** as a waxy solid (262 mg, 0.86 mmol, 79%).

191: $[\alpha]_D^{29}$ **=** - 5.6 (c = 0.76, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.35-7.25 (m, 5H, Ar), 5.99 (brs, 1H, NH), 4.29 (dt, *J* = 6.9, 3.4 Hz, 1H, 5-H), 4.24 (t, *J* = 6.4 Hz, 1H, 4-H), 4.17 (m, 1H, 3-H), 4.02 (d, *J* = 13.2 Hz , 1H, Bn), 3.86 (d, *J* = 13.2, 1H, Bn), 3.51 (d, *J* = 4.4 Hz, 1H, 2-H), 3.15 (dd, *J* = 14.2, 3.0 Hz, 1H, 6-Ha), 2.94 (dd, *J* = 14.2, 3.7 Hz, 1H, 6-Hb), 1.56 (s, 3H, Me), 1.34 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 176.4 (s, C=O), 138.2 (s, Ar), 128.9-127.5 (d, 5C, Ar), 109.2 (s, acetal), 76.0 (d, C-4), 73.6 (d, C-5), 68.8 (d, C-3), 62.3 (d, C-2), 61.0 (t, Bn), 45.9 (t, C-6), 27.4 (q, Me), 24.6 (q, Me) ppm. MS (ESI): *m/z* (%) = 329.33 (100) $[M+Na]^+$. IR ($CDCl_3$): ν = 3687, 3608, 3498, 3364, 3031, 2991, 2935, 1676, 1384, 1210, 1049 cm^{-1}. $C_{16}H_{22}N_2O_4$ (306.36): calcd. C 62.73, H 7.24, N 9.14; found C 62. 76, H 7. 24, N 9.19.

Synthesis of 1-benzyl-3,4,5-trihydroxypiperidine-2-carboxamide (170a): A solution of **191** (17 mg, 0.05 mmol) in MeOH (4.0 mL) and HCl 1M (4 drops) was left stirring at room

temperature overnight. The crude mixture was concentrated to yield the hydrochloride of **170a** which was eluted through an ion-exchange resin (DOWEX® 50XW8-100) with MeOH, H_2O and 6% NH_4OH to give the free amine **170a** (11 mg, 80%). $[\alpha]_D^{26}$ **= +**17.0 (c = 0.56, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ = 7.30-7.21 (5H, Ar) 3.90-3.85 (m, 3H, 3-H, 4-H, 5-H), 3.71 (d, *J* = 13.5 Hz, 1H, Bn), 3.45 (d, *J* = 13.5 Hz, 1H, Bn), 3.26 (*br* s, 1H,2-H), 2.86 (dd, *J* = 12.2, 3.4 Hz, 1H, 6-Ha), 2.36 (*p*t, *J* = 9.0 Hz, 1H, 6-Hb) ppm. ^{13}C-NMR (50 MHz, D_2O) δ = 175.7 (s, C=O), 136.4 (s, Ar), 129.8-127.9 (d, 5C, Ar), 69.7, 69.4, 66.1 (3C, d, C-3, C-4, C-5), 64.6 (d, C-2), 59.2 (t, Bn), 50.5 (t, C-6) ppm. MS (ESI): *m/z* (%) = 289.13 (100) $[M+Na]^+$, 267.33 (48) $[M+H]^+$. $C_{13}H_{18}N_2O_4$ (266.29): calcd. C 58.63, H 6.81, N 10.52; found C 58.15, H 7.26, N 10.32.

Synthesis of 3,4,5-trihydroxypiperidine-2-carboxamide (170b): To a solution of **191** (50 mg, 0.16 mmol) in MeOH (4 mL) and HCl 1M (30 drops) Pd /C (25 mg) was added and the reaction mixture was left stirring at room temperature under H_2 atmosphere for 48 h. The catalyst was filtered through Celite® and the filtrate was concentrated under vacuum, to yield quantitative the hydrochloride salt of **170b** which was eluted through an ion-exchange resin (DOWEX® 50XW8-100) with MeOH, H_2O and 6% NH_4OH to give the free amine **170b** (23mg, 0.13mmol, 82%). $[\alpha]_D^{21}$ **=** + 27.2(c =0.38, H_2O). ^{1}H-NMR (400 MHz, D_2O) δ = 4.02 (dd, *J* = 4.3, 2.4 Hz, 1H, 3-H), 3.90 (*p*t, *J* = 3.7Hz, 1H, 4-H), 3.83 (ddd, *J* = 11.0, 5.1, 3.1 Hz, 1H, 5-H), 3.55 (d, *J* = 2.4 Hz, 1H, 2-H), 2.84 (dd, *J* = 12.7, 5.1 Hz, 1H, 6-Ha), 2.62 (dd, *J* = 12.7, 11.0 Hz, 1H, 6-Hb) ppm. ^{13}C-NMR (50 MHz, D_2O) δ = 174.6 (s, C=O), 69.7 (d, C-3), 69.1 (d, C-4), 63.9 (d, C-5), 55.7 (d, C-2), 42.7 (t, C-6) ppm. MS (ESI): *m/z* (%) = 199.08 (100) $[M+Na]^+$,. $C_6H_{12}N_2O_4$ (176.16): calcd. C 40.91, H 6.87, N 15.90; found C 40.35, H 6.85, N.16.05.

Synthesis of (2*R*,3*R*,4*R*,5*R*)-1-benzyl-2-carboxy-3,4,5-trihydroxypiperidinium chloride (171a): A solution of **191** (50 mg, 0.17 mmol) in 1 mL of HCl 6M was heated to gentle reflux. After 16 h the reaction was complete, as attested by a TLC control. The solution was cooled to room temperature and then extracted with Et_2O (3x4 mL) to remove ether soluble material. The hydrochloric acid was evaporated to dryness under reduced pressure, then it was dissolved in MeOH (5 mL) and NaOH (13 mg, 0.32 mmol) was added. The reaction mixture was left stirring for 1h in order to obtain the corresponding sodium salt, which was passed over Ambersep® 900-OH, eluting with MeOH, water and a 6M HCl solution. This procedure allowed us to obtained pure **171a** (35 mg, 0.12 mmol) as a waxy solid in 71% yield. $[\alpha]_D^{23}$ **= +**26.6 (c = 0.32, MeOH). ^{1}H-NMR (400 MHz, D_2O) δ = 7.44-7.35 (5H, Ar) 4.54 (d, *J* = 12.7 Hz, 1H, CH_2Ph), 4.27 (d, *J* = 11.7 Hz, 2H, CH_2Ph, H-2), 4.10-4.07 (m, 1H, 5-H), 3.91 (pt, *J* = 3.9 Hz, 1H, 4-H), 3.90 (d, *J* = 11.7 Hz, 1H, 3-H), 3.16 (dd, *J* = 11.7 Hz, 1H, 6-Ha), 3.02

(t, *J* = 11.7 Hz, 1H, 6-Hb) ppm. ^{13}C-NMR (50 MHz, D_2O) δ = 169.0 (s, COOH), 130.9 (s, Ar), 129.6-126.7 (d, 5C, Ar), 68.7 (d, C-2), 67.0 (d, C-4), 62.2 (d, C-3), 61.0 8d, C-5), 59.7 (t, Bn), 47.1 (t, C-6). ppm. MS (ESI): *m/z* (%) = 266.33 (100) $[M-H]^+$. $C_{13}H_{18}ClNO_5$ (303.74): calcd. C 51.41, H 5.97, N 4.61; found C 51.57, H5.83, N 4.50.

(2*R*,3*R*,4*R*,5*R*)-2-carboxy-3,4,5-trihydroxypiperidinium chloride [(2*R*,3*R*,4*R*,5*R*)-3,4,5-trihydroxypipecolid acid hydrochloride salt] (171b·HCl): To a solution of **191** (49 mg, 0.16 mmol) in MeOH (4 mL) and 1M HCl (15 drops) Pd/C (25 mg) was added and the reaction mixture was left stirring at room temperature under H_2 atmosphere for 16 h. The catalyst was filtered through Celite and the filtrate was concentrated under vacuum, then 1 mL of a 6M HCl solution was added and the mixture heated to gentle reflux. After 16 h reflux the reaction was complete, as attested by a TLC control. The solution was cooled at room temperature and then extracted one time with Et_2O (4 mL) to remove ether soluble material. The aqueous layer was evaporated and dried under vacuum to obtain **171b·HCl** (34 mg, 0.16 mmol) in 100% yield. A little portion of the product was dissolved in MeOH and treated with excess NaOH. The reaction mixture was left stirring for 1 h in order to obtain the corresponding sodium salt, which was passed over Ambersep 900-OH, eluting with MeOH, water and a 6M HCl solution. This procedure allowed us to obtain pure **171b·HCl**. $[\alpha]_D^{25}$=-79.1 (c=0.32, H_2O); ^{1}H-NMR (400 MHz, CD_3OD): δ=4.33 (br m, 1H; 3-H), 4.15 (br m, 2H; 5-H, 2-H), 3.93 (br m, 1H; 4-H), 3.04 ppm (br m, 2H; 6-Ha, 6-Hb); ^{13}C-NMR (100 MHz, CD_3OD): δ=168.6 (s, COOH), 69.0 (d, C-3), 68.7 (d, C-4), 61.9 (d, C-5), 55.9 (d, C-2), 42.0 (t, C-6); MS (ESI): *m/z* (%): 212.00 (100) $[M-H]^+$.

Synthesis of (2*S*,3*R*,4*S*,5*R*)-2-Aminomethyl-1-benzyl-3-hydroxy-4,5-isopropylidenedioxy-piperidine- (192): A solution of **191** (103 mg, 0.33 mmol) in dry THF (15 mL), was cooled to 0 °C and $LiALH_4$ (0.99 mL of a 1M solution in THF) was added. The reaction mixture was heated to reflux and stirred for one hour and half, under N_2 atmosphere, until the complete disappearance of the starting material was attested by a TLC control. The reaction solution was cooled at room temperature, then an aqueous saturated solution of Na_2SO_4 was added and left stirred for 15 minutes. The mixture was extracted with AcOEt (3x10 mL), the combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography (CH_2Cl_2/MeOH/NH_4OH 6% 10: 2:0.2) affording **192** (76 mg, 0.26 mmol) in 79% yield. $[\alpha]_D^{25}$ **= +**24.3 (c = 1.01, $CHCl_3$). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.35-7.21 (5H, Ar), 4.31 (dt, *J* = 6.8, 3.4 Hz, 1H, 5-H), 4.16-4.12 (m, 2H, 4-H, 3-H), 3.89 (s, 2H, CH_2Ph), 3.26-3.20 (m, 2H, 6-Ha, CH_2NH_2), 2.88 (dt, *J* = 3.9, 2.0 Hz, 1H, 2-H), 2.84-2.80 (m, 2H,

6-Hb, C<u>H</u>$_2$NH$_2$), 1.54 (s, 3H, Me), 1.34 (s, 3H, Me) ppm. ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 140.4 (s, Ar), 128.4-126.9 (d, 5C, Ar), 108.4 (s, acetal), 74.9 (d, C-4), 73.3 (d, 2C, C-5, C-3), 60.4 (t, Bn), 56.5 (d, C-2), 47.9 (t, C-6), 43.3 (t, CH_2NH_2), 27.2 (q, Me), 24.2 (q, Me). ppm. MS (ESI): *m/z* (%) = 293.25 (100) $[M+H]^+$. IR ($CDCl_3$) ν = 3689, 3604, 3399, 3086, 3065, 3029, 2991, 2922, 2868, 2247, 1601, 1561, 1383, 1209, 1027 cm^{-1}. $C_{16}H_{24}N_2O_3$ (292.37): calcd. C 65.73, H 8.27, N 9.58; found C 66.12, H 8.12, N 9.22.

Synthesis of (2*S*,3*R*,4*R*,5*R*)-2-Aminomethyl-1-benzyl-2-3,4,5-tri-hydroxypiperidine (172a): A solution of **192** (25 mg, 0.09 mmol) in MeOH (2.3 mL) was left stirring with 12M HCl (75 µL, 0.9 mmol) at room temperature for 48 h. The crude mixture was concentrated to yield the hydrochloride salt of **172a**. The corresponding free amine was obtained by passing the hydrochloride salt through a DOWEX® 50XW8-100 ion-exchange resin. Elution with 33% ammonia afforded the free base **172a** (20 mg, 0.08 mmol) in 89% yield. $[\alpha]_D^{24}$**=+**21.2 (c=1.29 in MeOH); ^{1}H-NMR (400 MHz, D_2O): δ=7.33-7.24 (m, 5H; Ar), 4.00 (dd, ^3J(H,H)=6.9, 3.9 Hz, 1H; 3-H), 3.89 (dt, ^3J(H,H)=7.2, 3.6 Hz 1H; 5-H), 3.85 (d, ^2J(H,H)= 13.1 Hz, 1H; Bn), 3.70 (dd, ^3J(H,H)=6.9, 3.6 Hz, 1H; 4-H), 3.62 (d, ^2J(H,H)=13.1 Hz, 1H; Bn), 2.99 (d, ^3J(H,H)=6.4 Hz, 2H; C<u>H</u>$_2$NH$_2$), 2.76-2.73 (m, 1H; 2-H), 2.64 (dd, ^2J(H,H)=13.0, ^3J(H,H)= 3.4 Hz, 1H; 6-Ha), 2.46 ppm (dd, ^2J(H,H)=13.0, ^3J(H,H)=7.2 Hz, 1H; 6-Hb); ^{13}C-NMR (50 MHz, D_2O): δ=137.6 (s, Ar), 129.7-127.6 (d, 5C, Ar), 70.1 (d, C-4), 69.0 (d, C-3), 67.1 (d, C-5), 59.1 (d, C-2), 57.7 (t, Bn), 49.9 (t, C-6), 36.7 ppm (t, CH_2NH_2); MS (ESI): *m/z* (%): 253.28 (100) $[M+H]^+$, 275.29 (27) $[M+Na]^+$; elemental analysis calcd (%) for $C_{13}H_{20}N_2O_3$ (252.31): C 61.88, H 7.99, N 11.10; found C 61.38, H 8.04, N 10.72.

Synthesis of (2*S*,3*R*,4*R*,5*R*)-2-Aminomethyl-3,4,5-trihydroxy-piperidine (172b): To a solution of **192** (19 mg, 0.07 mmol) in MeOH (4 mL) and 12M HCl (7 drops) Pd/C (15 mg) was added and the reaction mixture was left stirring at room temperature under H_2 atmosphere for 3 days. The catalyst was filtered through Celite® and the filtrate was concentrated under vacuum to yield the hydrochloride salt of **172b** which was eluted through an ion-exchange resin (DOWEX® 50XW8-100) with MeOH, H_2O and 15% NH_4OH to give the free amine **172b** (10 mg, 0.06 mmol, 95%). The NMR spectra established that a mixture of free amine and the corresponding protonated form was recovered $[\alpha]_D^{23}$**=**+4.30 (c=0.58 in H_2O); ^{1}H-NMR (400 MHz, D_2O): δ=3.89-3.72 (m, 3H; 3-H, 4-H, 5-H), 3.01-2.57 ppm (m, 5H; 6-H, 2-H, C<u>H</u>$_2$NH$_2$); ^{13}C-NMR (50 MHz, D_2O): δ=69.4, 69.0, 68.8, 68.2 (d, 2C, C-3, C-4), 64.9, 64.6 (d, 1C, C-5), 52.4, 52.0 (d, 1C, C-2), 43.3, 43.2 (t, 1C, C-6), 40.2, 39.6 ppm (t, 1C, CH_2NH_2); MS (ESI): *m/z* (%): 185.10 (100) $[M+Na]^+$.

Protected tetravalent iminosugars (194): To a solution of **176e** (89 mg, 0.35 mmol) in 1.8 mL of a 2:1 THF/H_2O mixture, $CuSO_4$ (30 mol %, 3.8 mg, 0.024 mmol), sodium ascorbate (60 mol %, 9.5 mg, 0.048 mmol) and **193** (23 mg, 0.08 mmol) were added. The reaction mixture was heated in MW reactor at 80°C for 45 min, until TLC analysis showed disappearance of the starting material. After filtration through Celite®, the solvent was removed under reduced pressure and the crude was purified by gradient FCC (from CH_2Cl_2/MeOH 10:1 to CH_2Cl_2/MeOH/NH_4OH 6% 10:1:0.1) affording pure **194** (92 mg, 0.07 mmol, 88%) as a pale yellow oil. R_f=0.32 (CH_2Cl_2/MeOH/NH_4OH 6% 10:1:0.1); $[\alpha]_D^{24}$ = +4.88 (c =1.92, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.67 (s, 4H, H-Triazole), 4.54 (s, 8H, O*CH*$_2$Triazole), 4.44 (t, *J* = 6.6 Hz, 8H, 3'-H), 4.32 (dd, *J* = 11.7, 5.3 Hz, 4H, 3-H), 4.05 (t, *J* = 4.7 Hz, 4H, 4-H), 3.98-3.95 (m, 4H, 5-H), 3,42 (s, 8H, C*CH*$_2$O), 2.71 (dd, *J* = 12.0, 5.3 Hz, 4H, 2a-H), 2.59 (dd, *J* = 11.7, 2.9 Hz, 4H, 6a-H), 2.47-2.41 (m, 8H, 2b-H, 6b-H), 2.36 (t, *J* = 6.6 Hz, 8H, 1'-H), 2.11-2.03 (m, 8H, 2'-H), 1.49 (s, 12H, Me), 1.35 ppm (s, 12H, Me); ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 145.1 (s, 4C, C-Triazole), 123.3 (d, 4C, C-Triazole), 109,0 (s, 4C, acetal), 77.3 (d, 4C, C-4), 72.2 (d, 4C, C-3), 68,9 (t, 4C, C*CH*$_2$O), 68.0 (d, 4C, C-5), 64.8 (t, 4C, O*CH*$_2$Triazole), 56.1 (t, 4C, C-6), 54.8 (t, 4C, C-2), 53.4 (t, 4C, C-1'), 47.7 (t, 4C, C-3'), 45.2 (s, *C*CH_2O), 28.3 (q, 4C, Me), 27.3 (t, 4C, C-2'), 26.3 ppm (q, 4C, Me); MS (ESI): *m/z* (%) = 1335.83 (100) $[M+Na]^+$; IR ($CDCl_3$): ν = 3629, 3416, 3143, 2988, 2939, 2826, 2247, 1724, 1665, 1550, 1468, 1383, 1220, 1058 cm^{-1}; elemental analysis calcd (%) for $C_{61}H_{100}N_{16}O_{16}$ (1313.54): calcd. C, 55.78; H, 7.67; N, 17.06; found C 55.74, H 7.66, N 16.83.

Peracetylated tetravalent iminosugars (195): To a solution of **194** (85 mg, 0.065 mmol) in 35 mL of methanol, 0.22 mL (10.0 equiv,) of 37% HCl were added and the mixture was stirred at room temp. for 18 hours. After that a TLC analysis showed disappearance of the starting material, the solvent was removed under reduced pressure. Successively elution with MeOH, H_2O and 6% aqueous ammonia onto a ion exchange resin Dowex 50WX8-200 afforded 9 mg (0.008 mmol) of corresponding free amine and 42 mg (0.032 mmol) of hydrochloride salt (MeOH fraction). This fraction was dissolved in pyridine (1.2 mL) and acetic anhydride (0.8 mL) was added. The solution was stirred at room temperature for 18 hours. Then, after concentration under reduced pressure, the crude mixture was purified by gradient FCC (from CH_2Cl_2/MeOH 20:1 to 5:1) affording pure **195** (48 mg, 0.029 mmol, 91%) as an oil. R_f=0.22 (CH_2Cl_2/MeOH 10:1); $[\alpha]_D^{22}$ = -42.1 (c =1.45, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.55 (s, 4H, H-Triazole), 5.22 (dt, *J* = 5.4, 2.7 Hz, 4H, 3-H), 5.07 (dt, *J* = 8.2, 4.4 Hz, 4H, 5-H), 4.90 (dd, *J* = 8.8, 3.4 Hz, 4H, 4-H), 4.48 (s, 8H, O*CH*$_2$Triazole), 4.42-4.29 (m, 8H, 3'-H), 3.39 (s, 8H, C*CH*$_2$O), 2.92 (d, *J* = 8.3 Hz, 4H, 6a-H), 2.77 (dd, *J* = 12.1, 3.9 Hz, 4H, 2a-H), 2.40-2.33 (m, 12H, 2b-H, 1'-H), 2.21-2.15 (m, 4H, 6b-H), 2.03 (s, 12H, Ac), 2.01-1.93

(m, 8H, 2'-H), 1.99 (s, 12H, Ac), 1.97 ppm (s, 12H, Ac); ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 169.7 8 (s, 12C, OAc), 144.8 (s, 4C, C-Triazole), 122.6 (d, 4C, C-Triazole), 70.6 (d, 4C, C-4), 68.8 (t, 4C, C*CH$_2$*O), 67.8 (d, 4C, C-5), 67.5 (d, 4C, C-3), 64.7 (t, 4C, O*CH$_2$* Triazole), 53.6 (t, 4C, C-2), 53.2 (t, 4C, C-6), 53.0 (t, 4C, C-1'), 47.4 (t, 4C, C-3'), 45.3 (s, *C*CH$_2$O), 27.2 (t, 4C, C-2'), 20.8-20.5 ppm (q, 12C, OAc); MS (ESI): *m/z* (%) = 1679.92 (100) $[M+Na]^+$; IR ($CDCl_3$): ν = 3451, 3145, 2960, 2874, 2825, 2258, 2246, 1743, 1663, 1470, 1437, 1372, 1231, 1049 cm^{-1}; elemental analysis calcd (%) for $C_{73}H_{108}N_{16}O_{28}$ (1657.73): calcd. C, 52.89; H, 6.57; N, 13.52; found C 52.52, H 6.25, N 13.90.

Polyhydroxylated tetravalent iminosugar (196): A suspension of **195** (31 mg, 0.019 mmol) and ion exchange resin Ambersep 900 OH (500 mg) in 10 mL of methanol was slowly stirred at room temp. for 16 h. After filtration of resin on Celite®, the solvent was removed under reduced pressure affording pure **196** (19 mg, 0.016 mmol, 86% yield) as a waxy solid. $[\alpha]_D^{23}$ = -13.0 (c =1.1, H_2O); ^{1}H-NMR (400 MHz, D_2O): δ=7.83 (s, 4H, H-Triazole), 4.38 (s, 8H, O*CH$_2$*Triazole), 4.31 (t, *J*=6.8 Hz, 8H, 3'-H), 3.86-3.82 (m, 4H, 3-H), 3.72 (td, *J*=8.8, 4.4 Hz, 4H, 5-H), 3.58-3.34 (m, 4H, 4-H), 3.25 (s, 8H, C*CH$_2$*O), 2.72, 2.65 (m, 8H, 2a-H, 6a-H), 2.28-2.11 (m, 12H, 2b-H, 7-H), 2.00-1.91 ppm (m, 12H, 6b-H, 2'-H). ^{13}C-NMR (50 MHz, D_2O): δ=143.2 (s, 4C, C-Triazole), 124.0 (d, 4C, C-Triazole), 72.8 (d, 4C, C-4), 67.1(t, 4C, C*CH$_2$*O), 66.8(d, 4C, C-3), 66.6 (d, 4C, C-5), 62.6 (t, 4C, O*CH$_2$*Triazole), 55.7 (t, 4C,C-6), 54.8 (t, 4C, C-2), 52.9 (t, 4C, C-1'), 47.7 (t, 4C, C-3'), 43.1(s, 4C, *C*CH$_2$O), 25.4 ppm (t, 4C, C-2').; MS (ESI): *m/z* (%) = 1175.79 (100); elemental analysis calcd (%) for $C_{49}H_{84}N_{16}O_{16}$ (1153.29): C 51.03, H 7.34, N 19.43; found: C 50.71, H 7.46, N 19.62.

Chapter 4

Hybrid gold glyconanoparticles: iminosugar-based multivalent systems as potential glycosidases inhibitors

4.1 Introduction

The multivalent display of carbohydrate ligands on a scaffold is currently an area of great interest, since carbohydrates bind only weakly to their complementary proteins, while modulation and enhancement of the biological response can be achieved by means of multiple interactions established by multivalent carbohydrates. The multivalent or cluster glycoside effect is defined as the affinity enhancement obtained with multivalent ligands compared to their monovalent counterparts.[164] This concept has been widely investigated for studying carbohydrate-lectin interactions,[165,166] but has remained essentially unexplored concerning specific glycosidase inhibition [167] using iminosugars as glycomimetics up to 2010. This can be ascribed to the difficulty of preparing functionalized iminosugars grafted to a scaffold and to the enzyme receptors themselves, which usually have a single and deep active site and therefore do not seem prone to accept multivalent substrates. However, although a strong multivalent effect is generally associated with the interaction of receptors bearing multiple recognition sites with multivalent sugar ligands, significant affinity enhancements have also been observed for systems where the receptors possess a single binding centre.[165c]

Some examples of relatively low valent (2-4 iminosugar units) monocyclic iminosugars were already reported in the literature, but early glycosidase inhibition tests were not

[164] M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem. Int. Ed. Engl*. **1998**, *37*, 2755–2794.

[165] For reviews, see: a) R. T. Lee, Y. Ichikawa, T. Kawasaki, K. Drickamer, Y. C. Lee, *Arch. Biochem. Biophys.* **1992**, *299*, 129–136; b) Y. C. Lee, R. T. Lee, *Acc. Chem. Res*. **1995**, *28*, 321–327; c) J. J. Lundquist, E. J. Toone, *Chem. Rev.* **2002,** *102*, 555–578; d) A. Imberty, Y. M. Chabre, R. Roy, *Chem. Eur. J.* **2008**, *14*, 7490–7499; e) R. Roy, Trends *Glycosci. Glycotechnol*. **2003**, *15*, 291–310; f) R. Roy, M.-G. Baek, *Rev. Mol. Biotechnol*. **2002**, *90*, 291–309; g) M. Lahmann, *Top. Curr. Chem.* **2009**, *288,* 17–65; h) T. K. Dam, C. F. Brewer, *Adv. Carbohydr. Chem. Biochem*. **2010**, *63*, 139–164; i) D. Deniaud, K. Julienne, S. G. Gouin, *Org. Biomol. Chem*. **2011**, *9*, 966–979; j) R. J. Pieters, *Org. Biomol. Chem.* **2009**, *7*, 2013–2025; k) V. Roldós, F. J. Cañada, J. Jiménez-Barbero, *ChemBioChem.* **2011**, 12, 990-1005.

[166] a) P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read, D. R. Bundle, *Nature* **2000**, *403*, 669–672; b) H. C. Hang, C. R. Bertozzi, *Acc. Chem. Res.* **2001**, *34*, 727–736; c) B. T. Houseman, M. Mrksich, *Top Curr. Chem* **2002**, *218*, 1–44; d) L. L. Kiessling, J. E. Gestwicki, L. E. Strong, *Angew. Chem. Int. Ed. Engl*. **2006**, *45*, 2348–2368.

[167] S.-K. Choi, Synthetic Multivalent Molecules: Concepts and Biomedical Applications, John Wiley & Sons, Hoboken, **2004**.

encouraging.[168] In our group we also made some preliminary efforts aimed to synthesizing multivalent pyrrolidine-based ligands on calyxarene cores.[169] However, the first promising example of multivalent effect on a glycosidase was recently reported for a trivalent 1-nojirimycin derivative, which showed a 6-fold affinity enhancement towards jack bean α-mannosidase.[170]

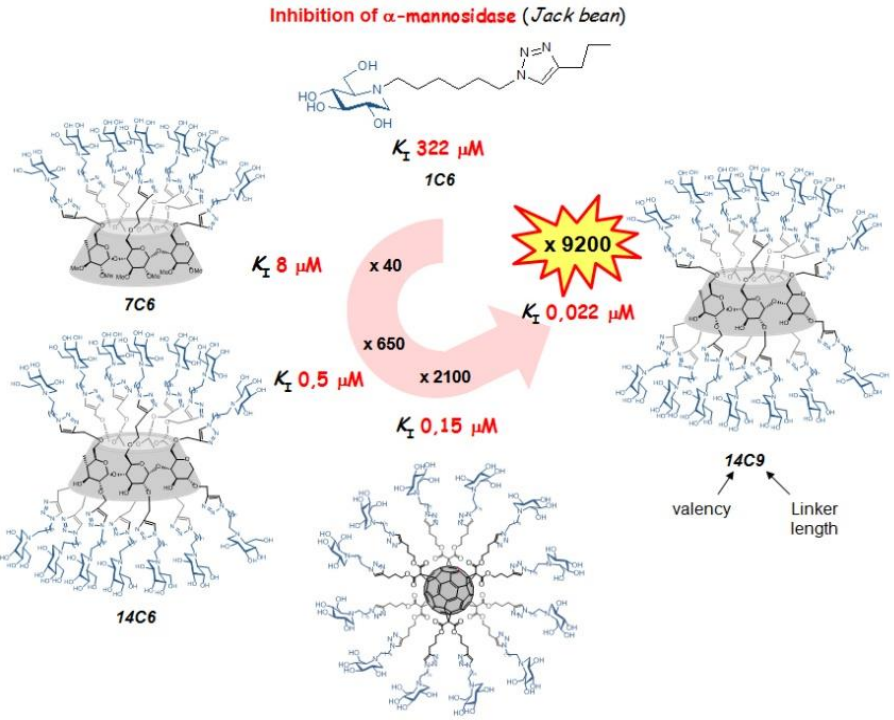


Figure 4.1: Recent examples of iminosugar based multivalent systems by Nierenengarten and Compain groups, taken from an authors adaptation (Ref. [171] and [172]).

Much greater effects were demonstrated for fullerene decorated with 12 iminosugar residues[171] and for a series of cyclodextrins conjugated with 7 or 14 iminosugars.[172] In both examples the selected iminosugar is an *N*-alkyl analogue of 1-deoxynojirimycin and the best effects were reported with jack bean α-mannosidase. In the case of the 14-valent system, an inhibition enhancement up to four orders of magnitude over the corresponding monovalent ligand was found (Figure 4.1). A strong multivalent effect was also observed

[168] a) T. Wennekes, R. J. B. H. N. van den Berg, K. M. Bonger, W. E. Donker-Koopman, A. Ghisaidoobe, G. A. van der Marel, A. Strijland, J. M. F. G. Aerts, H. S. Overkleeft, *Tetrahedron: Asymmetry* **2009**, *20*, 836–846; b) A. Lohse, K. B. Jensen, K. Lundgren, M. Bols, *Bioorg. Med. Chem*. **1999**, *7*, 1965 – 1971; c) B. A. Johns, C. R. Johnson, *Tetrahedron Lett*. **1998**, 39, 749–752.

[169] a) M. Marradi, S. Cicchi, F. Sansone, A. Casnati, A. Goti, *Beilstein J. Org. Chem.* **2012**, *8*, 951-957; b) F. Cardona, G. Isoldi, F. Sansone, A. Casnati, A. Goti, *J. Org. Chem.* **2012**, *77*, 6980-6988.

[170] J. Diot, M. I. García-Moreno, S. G. Gouin, C. O. Mellet, K. Haupy, J. Kovensky, *Org. Biomol. Chem.* **2009**, *7*, 357-363.

[171] P. Compain, C. Decroocq, J. Iehl, M. Holler, D. Hazelard, T. M. Barragán, C. O. Mellet, J.-F. Nierengarten, *Angew. Chem. Int. Ed.* **2010**, *49*, 5753-5756.

[172] C. Decroocq, D. Rodríguez-Lucena, V. Russo, T. M. Barragán, C. O. Mellet, P. Compain, *Chem. Eur. J.* **2011**, *17*, 13825-13831.

with deoxynojirimycin-decorated cyclodextrins towards a β-glucosidase of therapeutic interest, namely human GCase, thus showing the applicability of the multivalence concept in the development of chaperones for the treatment of Gaucher's disease[173]. Relatively small trivalent and tetravalent *N*-butyl deoxynojirimycin analogues were also recently found to act as more potent CFTR (cystic fibrosis transmembrane conductance regulator) correctors than the monovalent relative iminosugar, thus representing promising new candidates for the treatment of cystic fibrosis (CF).[174]

All these results suggested the existence of a multivalent effect beyond the expected statistical rebinding and local concentration effect that should favor the ligand recapture by the glycosidases. This inhibitory multivalent effect may found explication in mechanisms involving either secondary binding interactions in regions around the active site (sub-site binding), or favorable non-specific interactions in proximity to the enzyme surface .Related to the latter issue, a recent work of Nierengarten and co-workers tried to give some insights on the importance of non-glycone binding sites in glycosidase inhibition by multivalent systems (Figure 4.2) [175]

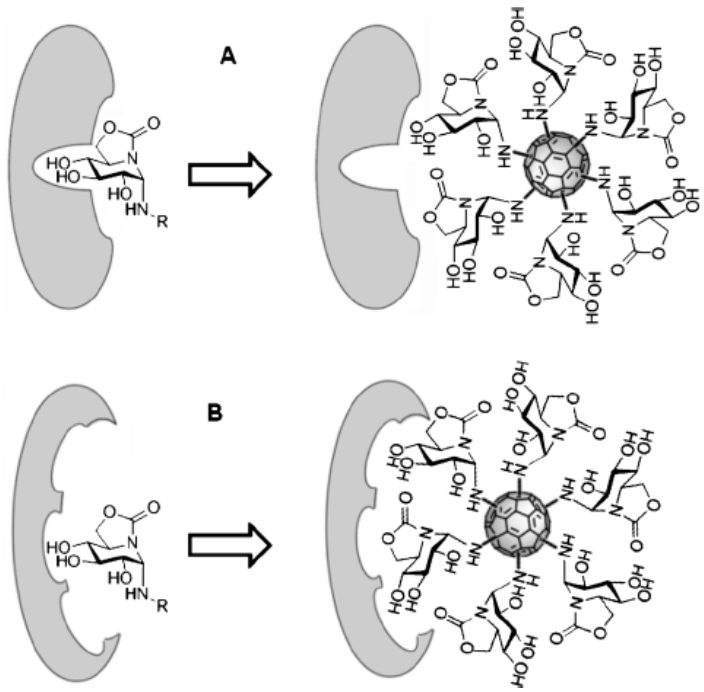


Figure 4.2: Schematic representations of the shift in binding modes after multivalent presentation of inhibitors for enzymes having A) a deep catalytic site but accessible aglycone sites or B) accessible catalytic as well as aglyconic sites. Taken from Ref. [175]

[173] C. Decroocq, D. Rodríguez-Lucena, K. Ikeda, N. Asano, P. Compain, *ChemBioChem* **2012**, *13*, 661-664.

[174] P. Compain, C. Decroocq, A. Joosten, J. de Sousa, D. Rodríguez-Lucena, T. D. Butters, J. Bertrand, R. Clément, C. Boinot, F. Becq; C. Norez, *ChemBioChem* **2013**, *14*, 2050-2058.

[175] R. Rísquez-Cuadro, J. M. García Fernandez, J.-F. Nierengarten, C. O. Mellet, *Chem. Eur. J.* **2013**, DOI: 10.1002/chem.201303158.

In another very recent paper [176]the dimeric nature of the Jack Bean α-mannosidase (JBαMan) has been recognized as a key characteristic for promoting intermolecular cross-linking interactions. For this reason, similarly to lectin mechanisms, the spatial presentation of iminosugar epitopes can modulate the strength of the multivalent effects. These examples show that the rationalization of the mechanisms involved in multivalent iminosugar-based systems and glycosidases is far from being achieved because the binding modes operating depend on the topology of the multivalent iminosugar system as well as on the type of the enzyme under study (e.g. whether the glycosidic site is deep or shallow, hidden or exposed, proximal to other binding sites or not). The high inhibitory activity enhancements reported for these relatively few examples, together with the need for better understanding the interactions of these systems with their enzymatic counterparts, highlight the interest in synthesizing multivalent iminosugar-conjugates with well-defined structures. In this context, we decided to study the feasibility of using glyconanoparticles (GNPs) as versatile scaffolds to multimerize iminosugar analogues, an approach which has no precedent in the literature.

GNPs are water-soluble and biofunctional gold nanoclusters with a three-dimensional polyvalent carbohydrate display and globular shape, chemically well-defined composition and an exceptionally small core size (Figure 4.3).[177]

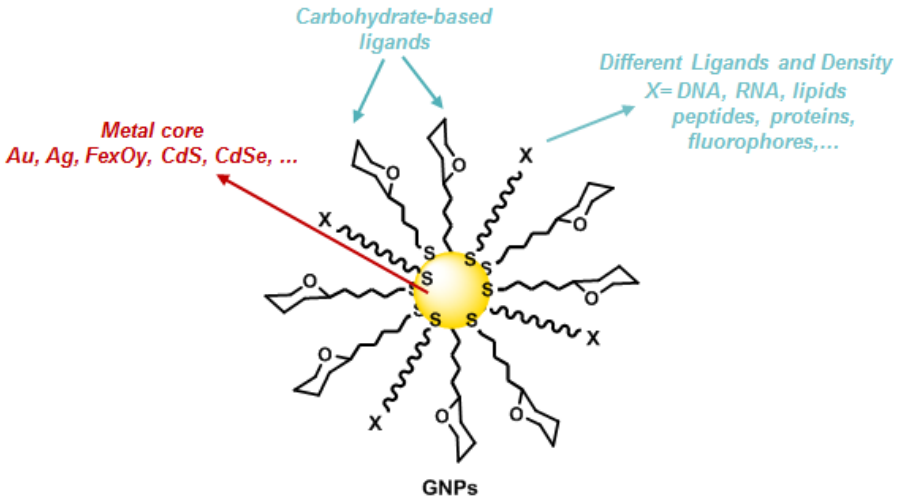


Figure 4.3: General scheme of gold glyconanoparticles (GNPs). GNPs consist of an inorganic core of nanometric size which is functionalized with suitably-derivatized carbohydrates (for example, in the case of gold nanoparticles, thiol ending neoglycoconjugates). The inorganic core can harbor other different types of ligands in addition to carbohydrates.

[176] Y. Brissonnet, C. Ortiz Mellet, S. Morandat, M. I. Garcia Moreno, D. Deniaud, S. E. Matthews, S. Vidal, S. Šestá, K. El Kirat, S. G. Gouin, J. Am. Chem. Soc. **2013**, DOI: 10.1021/ja406931w.

[177]a) J. M. de La Fuente, A. G. Barrientos, T. C. Rojas, J. Rojo, J. Cañada, A. Fernández, S. Penadés, *Angew. Chem.* **2001**, *113*, 2317–2321; *Angew. Chem. Int. Ed.* **2001**, *40*, 2257–2261; b) A. G. Barrientos, J. M. de La Fuente, T. C. Rojas, A. Fernández, S. Penadés, *Chem. Eur. J*. **2003**, 9, 1909–1921; c) J. M. de La Fuente, S. Penadés, *BBA-Gen. Subjects* **2006**, *1760*, 636–651.

They can display a large number of carbohydrates on a reduced surface with a high local concentration of sugars or lower densities. GNPs were originally designed and prepared to demonstrate the existence of carbohydrate–carbohydrate interactions by a chemical systems.[177a] In particular, gold nanoparticles coated with the trisaccharide Lewis X (Le^X) were used to unveil the specific, multivalent, calcium-dependent and reversible Le^X self-interaction in water by different techniques including transmission electron microscopy (TEM), isothermal titration calorimetry (ITC), surface plasmon resonance (SPR), and atomic force microscopy (AFM). [178] This new integrated approach based on the use of carbohydrate self-assembled monolayers (SAMs) on two- and three-dimensional gold surfaces was termed *glyconanotechnology*. GNPs also constitute a good biomimetic model to intervene in carbohydrate-mediated biological processes. The first demonstration that these nanotools can behave as anti-adhesion agents was reported against progression of lung metastasis in mice: GNPs functionalized with lactose neoglycoconjugates were able to inhibit the metastasis of melanoma in mice lungs by up to 70%.[179]

Another important characteristic of GNPs is the multifunctionality, namely the possibility of simultaneously inserting on the gold nanoplatform more the one ligand and to modulate the presentation of the ligands. In this sense, the glyconanoparticle platform offers advantageous alternatives to protein, polymer, or dendrimer scaffolds.

Glyconanotechnology allows the preparation of a great variety of water-soluble glycoclusters with high (approximately 100 molecules on a 2 nm core gold) and low ligands loadings and variable linkers to modulate rigidity and flexibility and to confer accessibility to the ligands.[180] Some examples of the application of this technology to solve biological problems include gold nanoparticles coated with (oligo)mannosides (*manno*-GNPs) which inhibit DC-SIGN-mediated HIV-1 *trans*-infection of human T cells[181] and the anti-HIV antibody 2G12-mediated neutralization of HIV-1 infection towards T cells.[182] In the last decade, many examples of multivalent and multifunctional GNPs have been reported to study carbohydrate-based interactions.[180,183]

---

[178] J. M. de la Fuente, S. Penadés, *Glycoconj. J*. **2004**, *21*, 149–163.

[179] J. Rojo, V. DÍaz, J. M. de La Fuente, I. Segura, A. G. Barrientos, H. H. Riese, A. Bernad, S. Penadés, *ChemBioChem* **2004**, *5*, 291 –297.

[180] M. Marradi, F. Chiodo, I. García, S. Penadés, *Chem. Soc. Rev*. **2013**, *42*, 4728-4745.

[181] O. Martínez-Ávila, L. M. Bedoya, M. Marradi, C. Clavel, J. Alcamí, S. Penadés, *ChemBioChem* **2009**, *10*, 1806-1809.

[182] M. Marradi, P. Di Gianvincenzo, P. M. Enríquez-Navas, O. M. Martínez-Ávila, F. Chiodo, E. Yuste, J. Angulo, S. Penadés, *J. Mol. Biol.* **2011**, *410*, 798-810.

[183] M. Marradi, M. Martín-Lomas, S. Penadés, Adv. *Carbohydr. Chem Biochem.* **2010**, *64*, 212-290.

Conversely, only two works where GNPs have been faced to enzymes are present in the literature. It has been demonstrated that carbohydrates tailored on GNPs display higher enzymatic stability respect to the free species. Gold nanoparticles of 2 nm coated with lactose conjugates were resistant to *E. coli* β-galactosidase-mediated hydrolysis even under drastic conditions (enzyme concentration, time, temperature).[184] Steric crowding of the lactose moieties on the nanoparticle surface probably inhibited enzymatic recognition and degradation. By decreasing the lactose density (from 100% to 30% and 5%) on the gold nanoparticles, different rates of hydrolysis were achieved. However, the specific activity was still very low in comparison with the free neolactoconjugate. Another recent work by Jensen's group demonstrated that maltotriose oxy-amine-GNPs were stable towards the glucoamylase-mediated enzyme hydrolysis, while the corresponding maltotriose oxime-GNPs showed partial hydrolysis of the maltotriose.[185] The explanation of this phenomenon may reside in the density of sugars on GNPs recognition by enzymes, in fact maltotriose oxy-amine derivatives self-assembled on gold nanoparticles in a three time higher amount than maltotriose oxime derivatives.

These results point out that GNPs are generally resistant to enzyme degradation and thus simple monosaccharides can be employed as *inner component* in the preparation of multivalent glycosidase inhibitor systems, bearing iminosugars as *active component* (Figure 4.4).

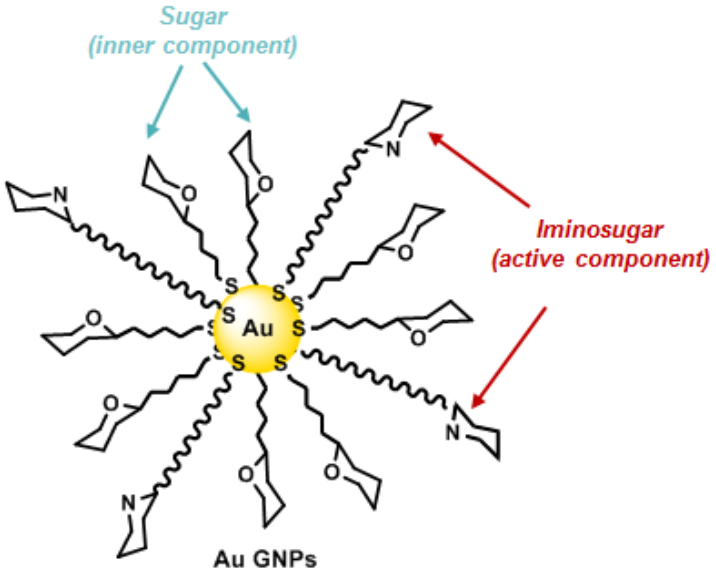


Figure 4.4: Schematic representation of an hybrid Au-GNP coated with sugar (inner component) and iminosugar (active component) derivatives.

[184] A. G. Barrientos, J. M. de la Fuente, M. Jiménez, D. Solís, F. J. Cañada, M. Martín-Lomas, S. Penadés, *Carbohydr. Res*. **2009**, *344*, 1474-1478.
[185] B. Thygesen, J. Sauer, K. J. Jensen, *Chem._Eur. J*. **2009**, *15*, 1649-1660.

In this Chapter, we describe how iminosugars can be multimerized on 2 nm gold nanoparticles with the aim of increasing their inhibitory potency towards glycosidases. The synthesis and characterization of these multivalent tools will be presented together with the preliminary biological tests with commercially available glycosidases.

4.2 Results and Discussion

In this PhD Thesis, the first example of gold GNPs coated with iminosugars is reported. Hybrid Au-GNPs consisting of a nanometric gold core (around 2 nm) coated with different percentage of selected iminosugar derivatives as *active components* and simple monosaccharides as *inner components* have been designed and prepared (Figure 4.5), following a reported protocol.[186]

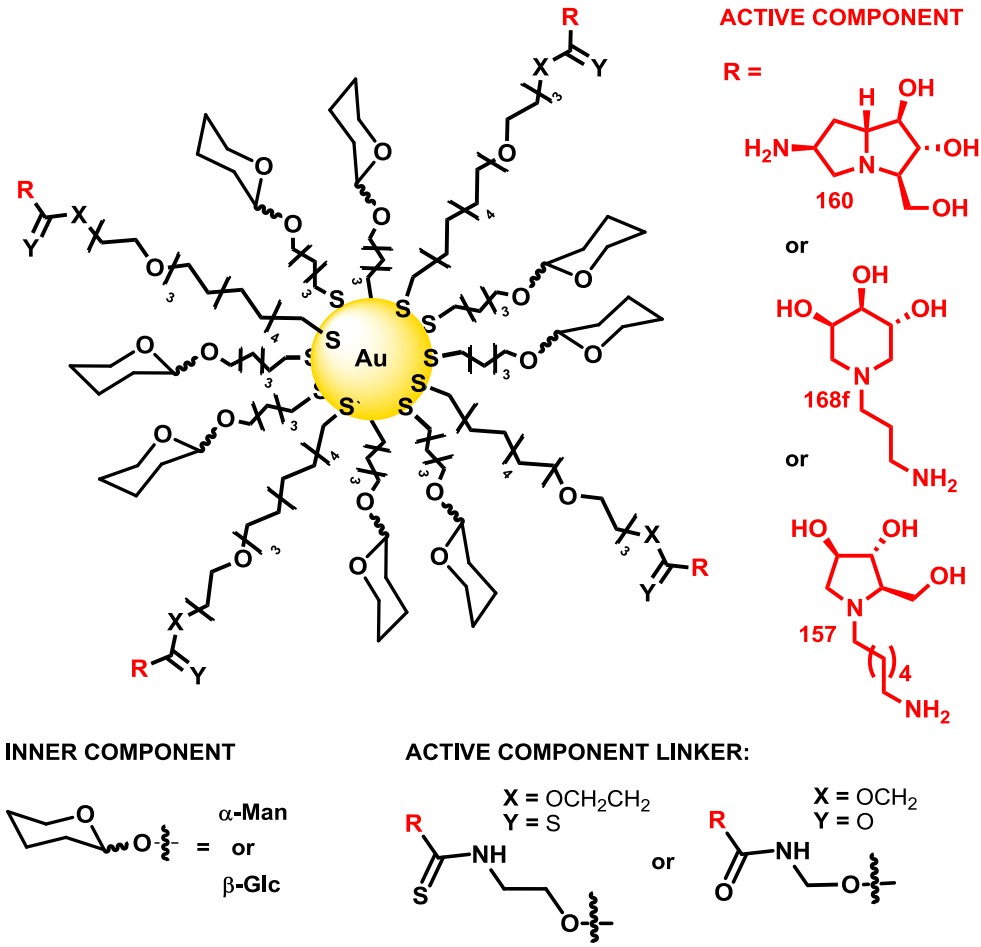


Figure 4.5: Schematic representation of Au-GNPs coated with iminosugars. The GNPs consist of: 1) a nanometric gold core; 2) an *active component* R (in red), which is an iminosugar among **160**, **168f** and **157**, linked to gold core through an amphiphilic linker. The coupling with the linker could be achieved by thiourea or amide formation (bottom right); 3) an *inner component* which is a simple monosaccharide (β-glucose α-mannose) linked to gold core through a short aliphatic linker (bottom left).

[186] O. Martínez-Ávila, K. Hijazi, M. Marradi, C. Clevel, C. Campion, C. Kelly, S. Penadés, *Chem. Eur. J.* **2009**, *15*, 9874 –9888.

Three biologically active iminosugars (compounds **157**, **160** and **168f**, Figure 4.6) with structures belonging to the classes of polyhydroxylated pyrrolizidines, piperidines and pyrrolidines, were selected to be incorporated as *active components* of the gold nanoparticles. These molecules are slight modified analogues of compounds **114**, **168b** and **197** (Figure 4.6), in turn obtained through efficient and selective strategies starting from carbohydrates, during our studies on the synthesis of polyhydroxylated alkaloids and their unnatural analogs (see *Chapter 2* and *3*).

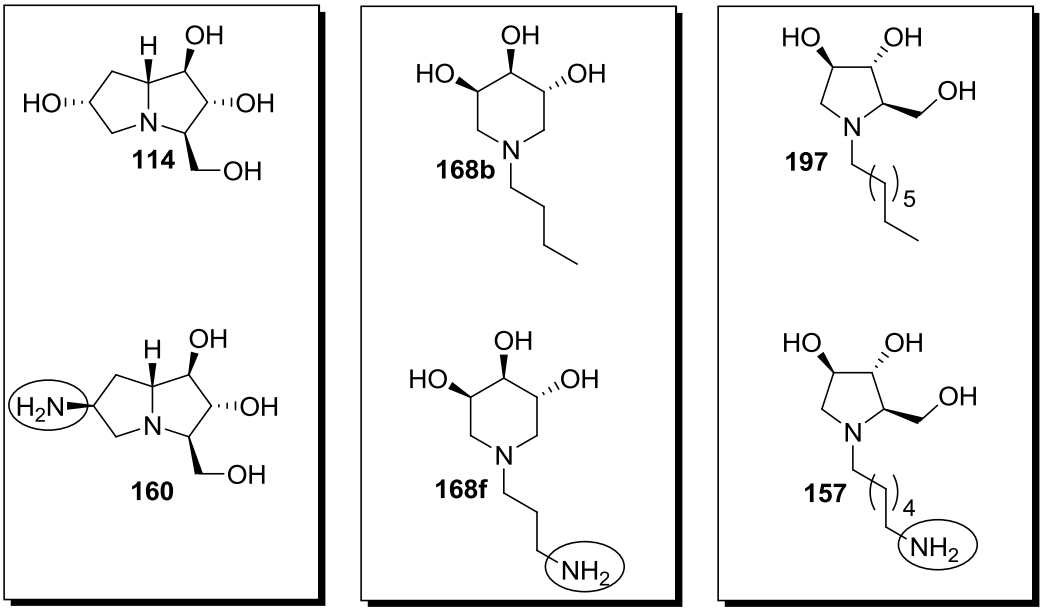


Figure 4.6: Biologically active iminosugars (top) and their corresponding amino-derivative analogues (bottom). From left to right, polyhydroxylated pyrrolizidines, piperidines and pyrrolidines compounds.

In particular, casuarine analogue **114** mimics glucose and is able to inhibit commercial and human glucosidases.[87] Piperidine **168b** showed to be a selective inhibitor of commercial and human α-L-fucosidase, in agreement with literature data reported for the non-alkylated analogue.[187] The *N*-octyl DAB-1 (1,4-dideoxy-1,4-imino D-arabinitol) derivative **197**, obtained by reductive amination of DAB-1 with the suitable aliphatic aldehyde[106] resulted to inhibit not only commercial α- and β-glucosidases but also human α-mannosidase, according to preliminary not yet published biological evaluations.

In order to attach biologically active compounds **114**, **168b** and **197** to gold nanoparticles, their structures were modified by introducing an amino moiety (synthesis reported in *Chapter 2* and *Chapter 3*) for further functionalization with long and amphiphilic thiol-ending linkers (Figure 4.6). The choice of this kind of linkers was made on the basis of

[187]G. Legler, A. E. Stütz,H. Immich, *Carbohydr. Res.* **1995**, *272*, 17–30.

previous works[186,188]. In particular, the amphiphilic nature of mixed aliphatic/polyethylene glycol linker imparts flexibility and assists the solubility of whole nanoparticle. Furthermore, these long linkers allow the *active component* (iminosugars) to protrude above the gold surface with respect to the *inner component* (monosaccharides glucose and mannose). It should be noticed that the structure modifications of the iminosugars left unchanged the sugar mimic moiety of compound, which is responsible for the recognition with the glycosidase binding site.

On the other hand, two different natural hexoses, β-glucopyranose and α-mannopyranose, were selected as *inner component*. In this case, a short (C_5), aliphatic linker was chosen to impart rigidity to the GNPs. The preparation of GNPs with two different sugars has been planned to evaluate if the *inner component* could play any role in the glycosidases inhibition process. Moreover the GNPs incorporating only β-glucose and only α-mannose have been prepared as controls in the biological tests.

4.2.1 Synthesis of Thiol-Ending Ligands

In order to prepare GNPs, suitable thiol-ending linkers have to be synthesized to exploit the gold–sulphur affinity in attaching the ligands to the gold nanocluster surface.[189]

To this aim, sugar-based ligands **198** ($GlcC_5S$) and **199** ($ManC_5S$) were employed as *inner components.* These neoglycoconjugates have been prepared following a well-established protocol, while the conjugation of iminosugars **157**, **160** and **168f** to different amphiphilic linkers to obtain *active component* ligands has been investigated and reported herein for the first time.

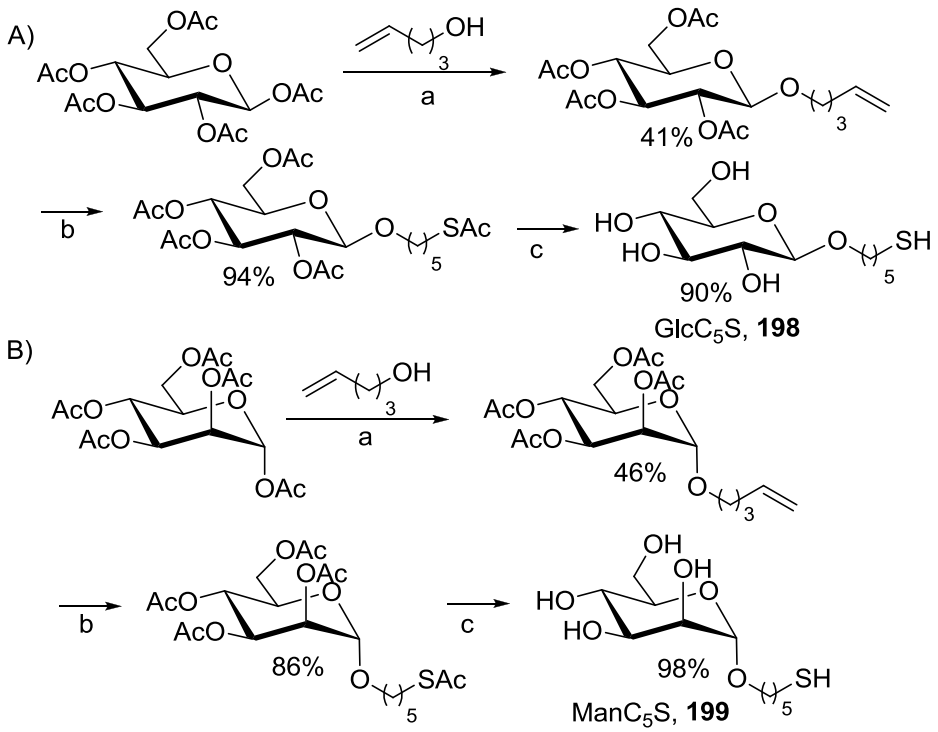
Sugar-based ligands: $GlcC_5S$ and $ManC_5S$ conjugates **198** and **199** were easily prepared starting from the peracetylated sugars which are commercially available, according to published procedures (Scheme 4.1).[186,190]

Briefly, the pentacetate glycoside was glycosylated with 4-penten-1-ol in Fisher conditions, followed by free radical mediated tioacetilation with thioacetic acid; the subsequent deprotection with sodium methoxide in MeOH afforded desired derivative in good overall yields.

[188] D. Safari, M. Marradi, F. Chiodo, H. A. Th Dekker, Y. Shan, R. Adamo, S. Oscarson, G. T. Rijkers, M. Lahmann, J. P. Kamerling, S. Penadés, H. Snippe, *Nanomedicine* **2012**, 7, 651-662.

[189] J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo, G. M. Whitesides, *Chem. Rev.* **2005**, *105*, 1103-1169.

[190] T. Buskas, E. Sçderberg, P. Konradsson, B. Fraser-Reid, J. *Org. Chem*. **2000**, *65*, 958 – 963.



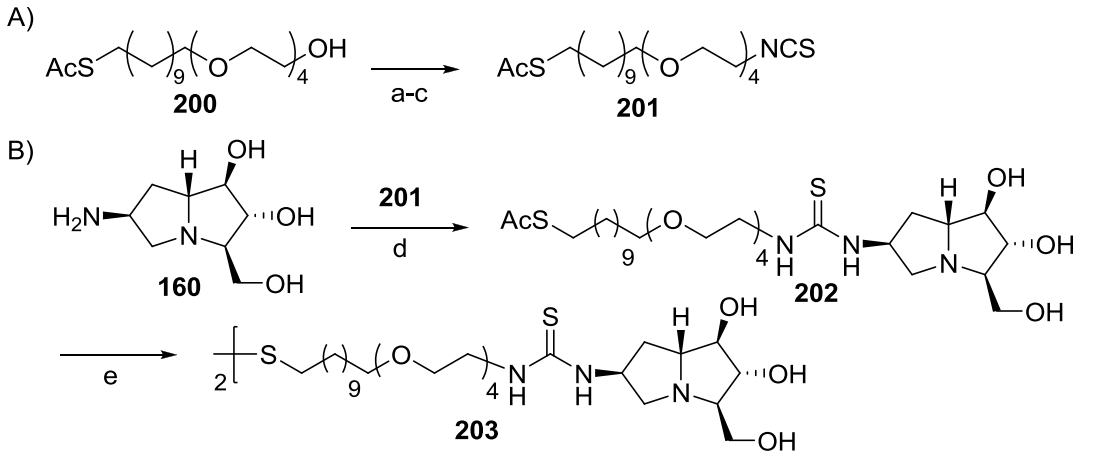
Scheme 4.1: a) CH_2Cl_2, $BF_3 \cdot Et_2O$, rt, 22h; b) CH_3COSH, AIBN, THF, reflux, 2h; c) NaOMe, MeOH, Ar, rt, 2h.

Iminosugar-based ligands: the conjugation of amino-functionalized iminosugars **157**, **160** and **168f** to a bifunctional linker bearing a thiol-protected ending group and a carboxylic or an isothiocyanate group at the other terminus was reported herein for the first time and was performed by modification of a procedure reported for amino-sugars.[186]

As a first attempt, we investigated the conjugation of the amino derivative **160** with the linker **201** bearing an isothiocyanate group (Scheme 4.2), since the formation of a thiourea linkage is a well-established reaction in bioconjugation. The isothiocyanate linker **201** was obtained in 68% yield from the corresponding alcohol **200**[191] by a straightforward conversion of the alcohol group to azide and subsequent conversion of the azido group into the isothiocyanate functionality by treating the crude intermediate with carbon disulfide (Scheme 4.2A). Both linker **201** and iminosugar derivative **160** resulted well soluble in MeOH and could be coupled at room temperature without any additional reagent. The reaction afforded compound **202** in good yield after purification by column chromatography. The thioacetyl derivative **202** was treated with sodium methoxide (2.0 equiv.) in MeOH, under Ar atmosphere, in order to liberate the thiol group. The reaction

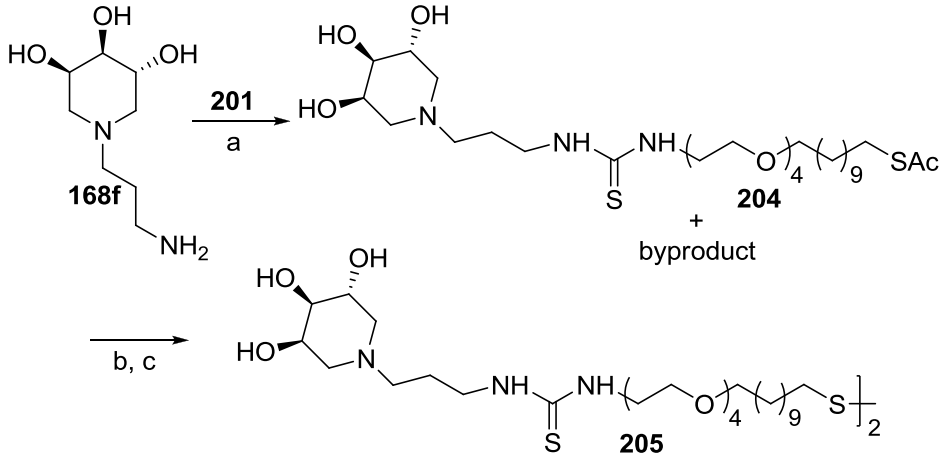
[191] C. Pale-Grosdemange, E. S. Simon, K. L. Prime, G. M. Whitesides, *J. Am. Chem. Soc*. **1991**, *113*, 12–20.

proceeded in eight hours affording quantitatively disulfide derivative **203**, after purification by size exclusion (Sephadex LH-20) chromatography (Scheme 4.2B).



Scheme 4.2: Synthesis of A) isothiocyanate linker **201** and B) pyrrolizidine conjugate **203** by thiourea formation. Reagents and conditions: a) PPh_3, CBr_4, CH_2Cl_2, rt, 10 min, 80%; b) NaN_3, DMF, rt, 14 h; iii) PPh_3, CS_2, toluene, 50 °C, 16 h, 68%; c) MeOH, rt, 18 h, 72%; d) NaOMe, MeOH, rt, 8 h, 100%.

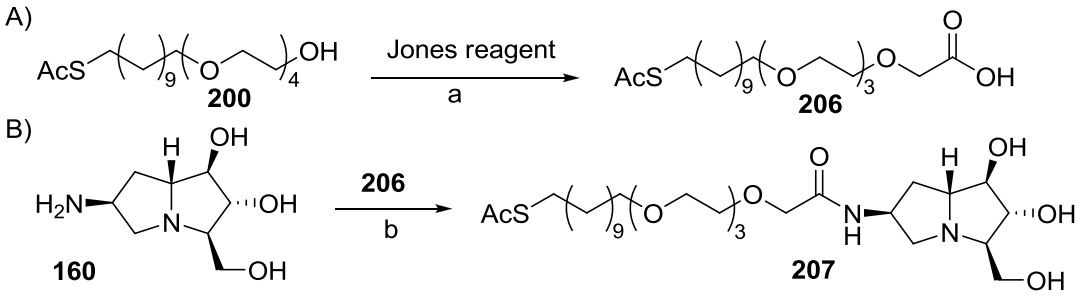
The same protocol was applied to compound **168f** and afforded, after Sephadex LH-20 purification, the conjugate **205** as a 1:1 mixture of thiol and disulfide. In this case, an inseparable by-product, presumably coming from the rearrangement of linker **201,** was unfortunately present. Then, the mixture was reduced with PPh_3 and further purified by column chromatography (eluent: CH_2Cl_2/MeOH/6% NH_4OH 10:1:0.1), affording pure **205** in 56% yield (Scheme 4.3).



Scheme 4.3: Synthesis of piperidine conjugate **205** by thiourea formation. Reagents and conditions: a) MeOH, rt, 18 h, b) NaOMe, MeOH, rt, 8 h, 100%, c) PPh_3, CH_2Cl_2/ MeOH 1:1, rt, 60h, 56% over 3 steps.

Since the GNPs synthesized with these conjugates were not soluble in water (see *Chapter 4.2.2*), the conjugation of compound **157** with linker **201** was not performed. In order to overcome this problem, amidic coupling was used as an alternative. The coupling of

iminosugar derivatives **157**, **160** and **168f** with carboxylated linker **206** was thus performed. In this case, the linker was easily synthesized by Jones oxidation of the alcohol **200**[192] (Scheme 4.4A) and the conjugates **207-209** were prepared employing different coupling conditions (Scheme 4.4B and 4.5).



Scheme 4.4: Synthesis of A) carboxylated linker **206** and B) pyrrolizidine conjugate **207** by amidic bond formation. Reagents and conditions:a) Acetone, Jones reagent, rt, 20 min, 69%; b) HBTU, HOBt, DIPEA, DMF, rt, 15 h, 51%.

The reaction with amino-pyrrolizidine derivative **160** was performed in dimethylformamide, employing 1–hydroxybenzotriazole (HOBt) and *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU, 97.5 mg, 257 µmol) as coupling agents. The crude mixture was first triturated with *n*-hexane to remove the unreacted linker and subsequently purified over silica gel, affording pure **207** in 51% yield.

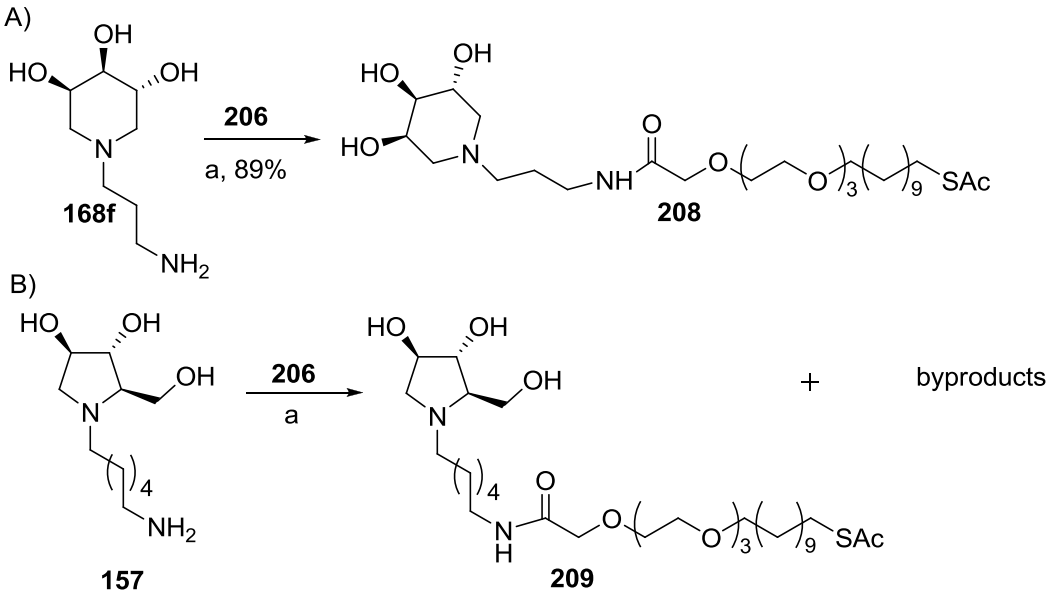
Unfortunately, these coupling conditions failed in the synthesis of piperidine analogue **208**, but a change to (1-ethyl-3-(3-dimethylaminopropyl)carbodimide (EDC) as coupling agent and dimethyl sulfoxide as solvent allowed to obtain compound **208** in very good yield after trituration and column chromatography (Scheme 4.5A).

Employing these latter conditions (EDC in DMSO), the pyrrolidine conjugate **209** (Scheme 4.5B) was obtained as a major product of an unseparable mixture of compounds. Attempts of purification by trituration and flash column chromatography were in fact unsuccessful. A small amount of the triturated crude was subjected to semi-preparative reverse LC (gradient from H_2O/MeOH 30/70 to MeOH in 45 min; retention time of the product: 10 minutes). After evaporation of the solvents, the pure product was obtained and characterized. The low amount of pure product recovered was not enough to prepare the corresponding GNPs.

The amidic linker conjugates **207** and **208** were completely characterized since the corresponding de-acetylated compounds were directly employed in the GNPs synthesis

[192] S. He, I. Garcia, J. Gallo, S. Penadés, *CrystEngComm* **2009**, *11*, 2605-2607.

without further purification in order to reduce the disulfide formation (see "General Procedure for the *in situ* deprotection of *S*-acetyl conjugates", *Chapter 4.5*).



Scheme 4.5: Synthesis of A) piperidine conjugate **208** and B) pyrrolidine conjugate **209** by amidic bond formation. Reagents and conditions a) EDC, HOBt, DIPEA, DMSO, Ar, rt, 65 h.

The iminosugar-based ligands synthesized by thiourea (**203** and **205**) or amide (deacetylated **207** and **208**) linkage were then employed in the preparation of GNPs, as described in the next section.

4. 2.2 Preparation of Glyconanoparticles

There are several methods to prepare gold nanoparticles functionalized with carbohydrates.[183] In this PhD Thesis, the so-called direct *in situ* formation of glyconanoparticles based on a modification of Brust's method[193] and developed by the group of Penadés[177a] was used (Figure 4.7). The protocol consists in treating a water solution of a gold(III) salt with a reductive agent in the presence of an excess of thiol-ending neoglycoconjugates.

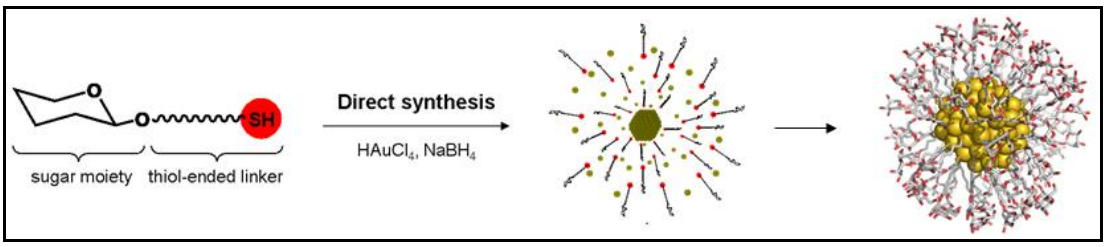
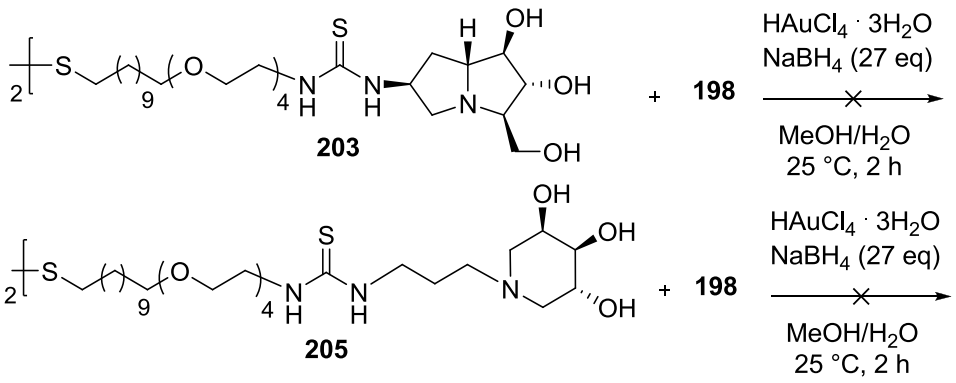


Figure 4.7: Schematic representation of the direct (*in situ*) synthesis of gold glyconanoparticles.

[193] M. Brust, M. Walker, D. Bethell, D. J. Schiffrin, R. Whyman, *J. Chem. Soc. Chem. Commun.* **1994,** 7**,** 801–802.

This *in situ* synthesis permits control of the growth of the metal cluster by varying the organic materials/Au(III) molar ratio and the simultaneous insertion of different ligands. Water-soluble and stable gold GNPs of 2-nm average diameter were obtained by adding an aqueous solution of tetrachloroauric acid ($HAuCl_4$, 1 equiv.) to a methanolic solution of a mixture of thiol-derivatized conjugates (*active* iminosugar conjugate and *inner* sugar component, 3 equiv.) in the desired proportion. The resulting mixture was reduced with an excess of $NaBH_4$ (27 equiv.) as reducing agent and the suspension was vigorously shaken for 2 h at 25 °C. The supernatant was removed, the nanoparticles were washed with methanol and the residue was dissolved in milliQ water, purified by dialysis and characterized by ^{1}H NMR spectrometry, transmission electron microscopy (TEM), infrared spectroscopy (IR), and ultraviolet spectroscopy (UV). Glyconanoparticles with different density of *active component* were obtained by changing the ratio of iminosugar conjugate respect to sugar derivative as *inner component* ($GlcC_5S$ or $ManC_5S$) in the initial mixture before GNP formation. The ratio of the components was controlled by ^{1}H NMR. The proportion of the ligands on the gold surface was also examined by ^{1}H NMR after cluster formation (analysis of the supernatant and washings) as well as performing qNMR of GNPs in deuterium oxide with an internal reference. The hybrid GNPs reported in this Chapter can be classified according to the iminosugar-based ligand employed as *active component*: pyrrolizidine alkaloid (PA), piperidine alkaloid (PIPA) and pyrrolidine alkaloid (PYRRA).

In a first attempt, hybrid GNPs employing 20% of PA thiourea linker conjugate **203** and 80% of $GlcC_5S$ in the starting ligand mixture were synthesized. Unfortunately, the nanoparticles formed in the above described conditions resulted insoluble in water even under vigorous stirring, or after adding growing amounts of different acids (HCl, AcOH, TFA) in order to protonate the amine moiety of iminosugar residues. Also GNPs employing 13% of PIPA thiourea linker conjugate **205** and 87% of $GlCC_5S$ showed similar solubility problems: the formed GNPs could be solubilized in water under vigorous stirring, but they precipitated during the dialysis purification (Scheme 4.6). These results suggested that insolubility in water of the nanoparticles did not depend either on the kind, the density or protonation state of the iminosugar derivatives. Curiously these GNPs resulted also completely insoluble in all the solvents subsequently tested (including iPrOH, DMF, DMSO), hampering the purification and characterization of these systems. The dispersibility of nanoparticles in water is an important feature both from a synthetic point of view (since their purification could be easily performed by dialysis) and for any further potential applications in biological fields. Thus, we reasoned to change the coupling strategy of the iminosugar to the linker (see *Chapter 4.2.1*).



Scheme 4.6: Failed synthesis of GNPs employing thiourea linker conjugates.

We switched from the thiourea linkage to an amidic bond in order to evaluate if the nature of the coupling moiety (amide instead of thiourea) could affect the solubility of these systems in water. To our delight, this protocol afforded water soluble GNPs both when 20% and 40% of iminosugar ligands were used in the starting mixture. After purification by dialysis, GNPs with two different densities of the *active component* (Figure 4.8-4.9) were thus obtained.

For the preparation of these new hybrid GNPs, iminosugar conjugates **207-208** were deacetylated and, without purification, mixed *in situ* with $GlCC_5S$ or $ManC_5S$ in the proper ratio, before adding the gold(III) solution and the reductive agent. When conjugates **207-208** were deacetylated and purified by Sephadex, a complete oxidation to the corresponding disulfide occurred. This resulted in a lower incorporation of the iminosugar ligands respect to the sugar ligands on the nanoparticle surface, as attested by the ^1H NMR spectra performed before and after cluster formation. Indeed, sugar derivatives $GlCC_5S$ or $ManC_5S$ were used as thiols because their oxygen-mediated oxidation to the corresponding disulfide was 20% at most, indicating a lower tendency to pass from thiol to disulfide respect to the iminosugar derivatives.

On the contrary, when conjugates **207-208** were deacetylated under Ar atmosphere and immediately employed in the preparation of GNPs without further purification, the ratio between iminosugar ligands and sugar ligands was preserved from the starting mixture to the gold cluster surface.

In particular, the PA-Au GNPs were prepared by using β-$GlcC_5S$ (PA-Au-Glc) or α-$ManC_5S$ (PA-Au-Man) as inner component, since an additive or synergetic effect of β-Glc (inner component) and PA (active component) could potentially be observed in the inhibition towards β-glucosidases (Figure 46). On the contrary, due to the extreme selectivity of PIPA towards α-L-fucosidases, only β-$GlcC_5S$ was employed as inner component.

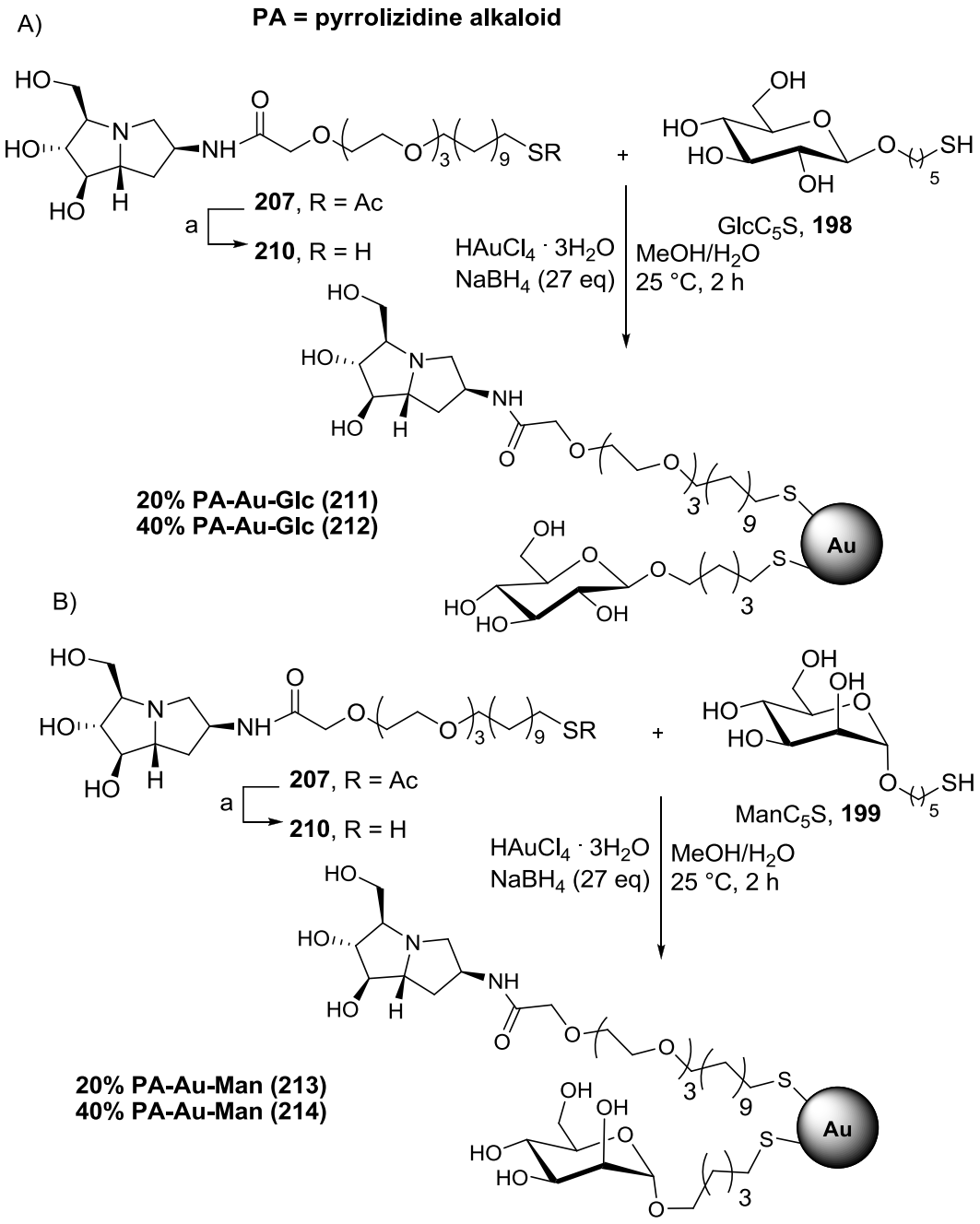


Figure 4.8: Complete scheme of synthesized hybrid PA-GNPs A) with β-GlCC$_5$S as *inner componen*t and B) with α-ManC$_5$SH (PA-Au-Man) as *inner component*. Reagents and conditions a) CH_3ONa, MeOH, Ar, rt, 2 h. The percentage is referred to the ligand ratio in the starting mixture, which is maintained in the mixture after the nanoparticle formation.

All the resulting GNPs showed an exceptionally small core (1–2 nm), as demonstrated by TEM analysis. TEM micro-graphs showed uniform dispersion of the GNPs and no aggregation was evident. The UV/Vis spectra were often characterized by a surface plasmon band at around 520 nm, except in the case of the smallest core-sized GNPs, for which the plasmon was scarcely visible. The ^{1}H NMR spectra of the GNPs featured broader peaks compared to those of the corresponding ligands.

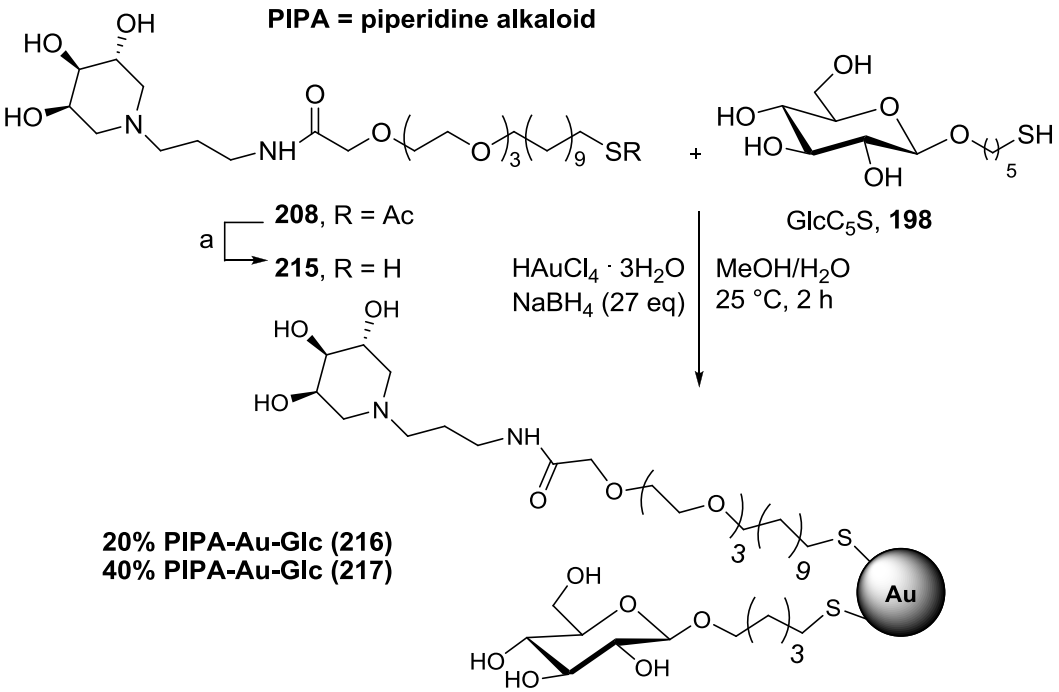


Figure 4.9: Complete scheme of synthesized hybrid PIPA-GNPs with β-GlcC_5S as *inner component*. Reagents and conditions a) CH_3ONa, MeOH, Ar, rt, 2 h.

The ligands loading on the PA-GNPs and PIPA-GNPs was also evaluated by quantitative NMR (qNMR) using 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid (TSP-*d*$_4$) as an internal standard in the D_2O solution of the PA-GNPs or PIPA-GNPs. An example of GNP characterization is shown in Figure 4.10.

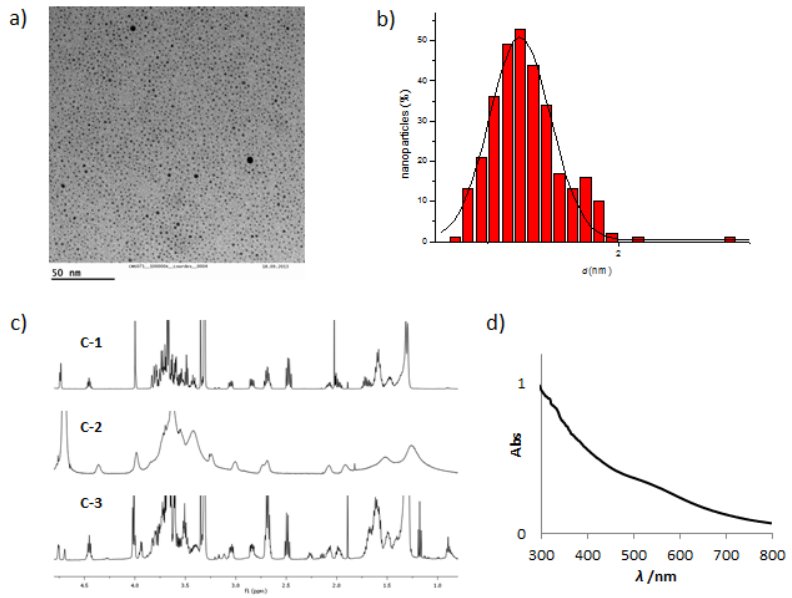
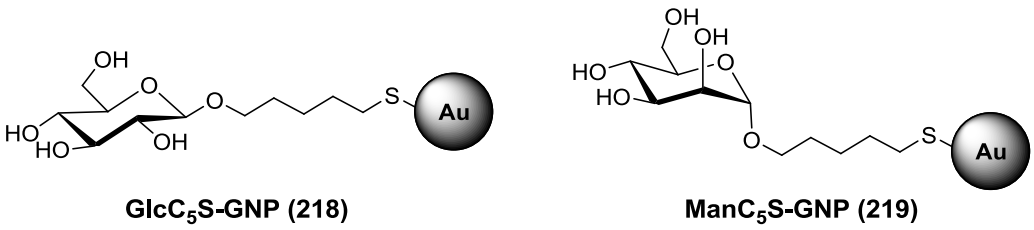


Figure 4.10: Characterization of glyconanoparticles **214** (40% PA-Au-Man): a) TEM micrograph in H_2O; b) size-distribution histogram obtained by measuring 300 nanoparticles (average diameter 1.2 ± 0.2 nm); c) ^{1}H NMR spectra of mixture of ManC_5S and derivative **210** before (C-1) and after (C-3) GNP formation, and of the corresponding GNP **214** (C-2); D) UV/Vis spectrum.

As mentioned above, all of the GNPs were found to be water-soluble and were stable for months under physiological conditions without flocculation. Based on the gold core size (determined by TEM) an average molecular formula and the corresponding molecular weights were estimated comparing the literature[194] and the values obtained from qNMR.

GNPs 100%-functionalized with glucose ($GlcC_5$-Au) or mannose ($ManC_5$-Au) were also prepared as control systems, following a reported procedure[186] (Figure 4.11).



$GlcC_5$S-GNP (218) **$ManC_5$S-GNP (219)**

Figure 4.11: 100%-glucose gold nanoparticles $GlcC_5$-Au and 100%-mannose gold nanoparticles $ManC_5$-Au.

All GNPs prepared in this project together with their main characteristics have been summarized in Table 4.1, while their complete characterization is reported in *Chapter 4.5.*

[194] M. J. Hostetler, J. E. Wingate, C.-J. Zhong, J. E. Harris, R. W. Vachet, M. R. Clark, J. D. Londono, S. J. Green, J. J. Stokes, G. D. Wignall, G. L. Glish, M. D. Porter, N. D. Evans, R. W. Murray, *Langmuir* **1998**, *14*, 17– 30.

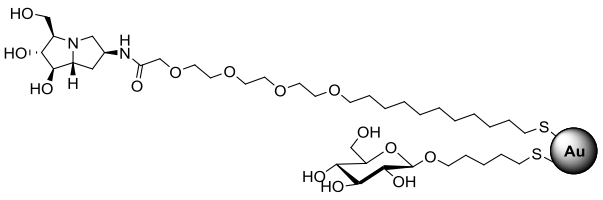
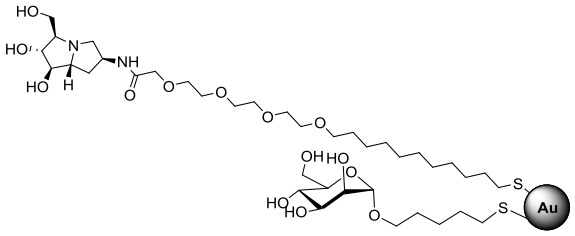
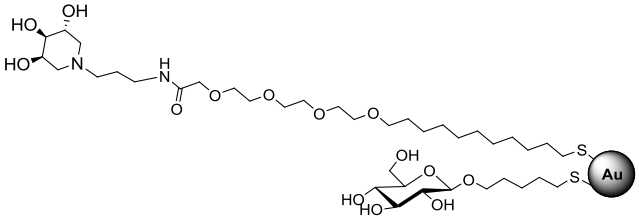
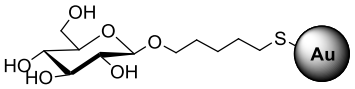
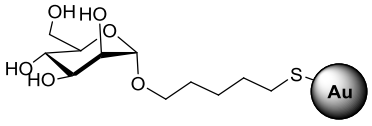
GNP	Diameter (nm)	Iminosugar loading	Scheme
211	1.3±0.3	100 µg/mL = 6.6 nmol/mL (6.6 µM) of pyrrolizidine	 **211**: 20% PA 80% β-GlcC_5S **212**: 40% PA 60% β-GlcC_5S
212	1.4±0.4	100 µg/mL = 18 nmol/mL (18 µM) of pyrrolizidine	
213	1.9±0.4	100 µg/mL = 6.3 nmol/mL (6.3 µM) of pyrrolizidine	 **213**: 20% PA 80% α-ManC_5S **214**: 40% PA60% α-ManC_5S
214	1.2±0.2	100 µg/mL = 24 nmol/mL (24 µM) of pyrrolizidine	
216	1.6±0.3	100 µg/mL = 12 nmol/mL (12 µM) of piperidine	 **216**: 20% PIPA 80% β-GlcC_5S **217**: 40% PIPA 60% β-GlcC_5S
217	1.7±0.4	100 µg/mL = 21.5 nmol/mL (21.5 µM) of piperidine	
218	1.7±0.4	–	 **218**: 100% βGlcC_5S
219	1.1±0.2	–	 **219**: 100% αManC_5S

Table 4. 1: Hybrid gold GNPs functionalized with iminosugars, structure and main characteristics.

4. 3 Biological Evaluation

Thanks to a collaboration with the group of Prof. I. Robina (University of Seville), the GNPs **212**, **214**, **217**, **218**, **219** and were preliminary assayed towards a panel of eleven commercially available glycosidases (Table 4.2). Since we were searching for a remarkable enhancement of the GNPs inhibitory activity respect to the monovalent compounds (which are usually evaluated at 1mM concentration), a first trial was carried out using low concentrations (around 10 μM) of iminosugars on the GNPs.

GNPs / ENZYME	**212** 40% PA 60% Glc	**214** 40% PA 60% Man	**217** 40% PIPA 60% Glc	**218** Glc	**219** Man
α-L-fucosidase EC 3.2.1.51 1-bovine kidney	n.i.	n.i.	n.i.	n.i.	n.i.
α-galactosidase EC 3.2.1.22 2-coffee beans	n.i.	n.i.	n.i.	n.i.	n.i.
β-galactosidase EC 3.2.1.23 5-*Escherichia coli* 8-*Aspergillus orizae*	n.i. n.i.	n.i. n.i.	n.i. n.i.	n.i. n.i.	n.i. n.i.
α-glucosidase EC 3.2.1.20 10-yeast 11-rice	n.i. n.i.	n.i. n.i.	n.i. n.i.	n.i. n.i.	n.i. n.i.
amyloglucosidase EC 3.2.1.3 13-*Aspergillus niger*	7%	7%	n.i.	n.i.	n.i.
β-glucosidase EC 3.2.1.21 15-almonds	n.i.	n.i.	n.i.	n.i.	n.i.
α-mannosidase EC 3.2.1.24 16-jack beans	n.i.	n.i.	n.i.	n.i.	n.i.
β-mannosidase EC 3.2.1.25 18-snail	n.i.	n.i.	n.i.	n.i.	n.i.
β-*N*-acetylglucosaminidase EC 3.2.1.30 21-jack beans	n.i.	n.i.	n.i.	n.i.	n.i.

Table 4.2: Inhibitory activities of GNPs **212, 214**, **217**, **218**, **219** toward glycosidases. Percentage of inhibition at 0.016 mg/mL of GNP. Optimal pH, 35 ºC (a-b). a) For conditions of measurements see [Ref111b]) n.i.: no inhibition was detected at 0.016 mg/mL concentration of the corresponding compound.

Unfortunately, at the tested concentration (0.016 mg/mL in terms of GNP), none of the GNPs showed inhibition to the screened enzymes, except a no relevant 7% of inhibition towards amyloglucosidases from *Aspergillus niger* in the cases of compounds **212** and **214**.

It should be noticed that also the control Glc-GNPs and Man-GNPs did not inhibit any screened enzyme.

These preliminary results seem to indicate that multimerization of PA and PIPA iminosugars onto gold nanoparticles do not lead to significant multivalent effects towards the tested enzymes. However, experiment with higher concentration of nanoparticles are still needed before excluding the employing of these systems as glycosidases inhibitors. Furthermore,, experiments with cellular systems, instead of purified enzymes, are currently underway to check wheter the capacity of easy internalization of GNPs onto cells can help in reducing the dosis of iminosugar required to reach a significant inhibition of selected enzymes. In particular, the inhibitory activity of PIPA-Au-GNPs towards human α-L-fucosidase is also currently under evaluation in monocyte cells.

4. 4 Conclusions

In this chapter the first example of gold GNPs coated with iminosugars has been reported. Hybrid Au-GNPs consisting of a nanometric gold core and bearing different percentage of selected iminosugar derivatives as *active components* and simple monosaccharides as *inner components* were prepared by direct synthesis and characterized by different techniques. The sugar based thiol-ending ligands ($GlcC_5S$ and $ManC_5S$) were synthesized as *inner components* following a well-established protocol. Then, pyrrolizidine (PA) and piperidine (PIPA) iminosugar-based ligands were synthesized as *active components* by coupling amino-funtionalized PA or PIPA to a protected thiol-ending linker through thiourea or amide linkage. Only in the latter case, the GNPs subsequently prepared resulted dispersible and well stable in water. GNPs with two different densities (20% and 40%) of PA and PIPA amide-linked iminosugars were prepared, purified and characterized by TEM analysis, UV/Vis spectrophotometry, IR, ^{1}H NMR and quantitative NMR (qNMR).

Until now, only three of these GNPs were assayed towards a panel of commercial glycosidase, together with the relative control GNPs (100% $GlcC_5S$ and 100% $ManC_5S$). The preliminary inhibition results were not promising in terms of implication of multivalent effect, but other experiments are currently underway in order to get more information on the potential use of GNPs as multivalent systems for glycosidases inhibition.

4.5 Experimental Section

Laboratory of GlycoNanotechnology, Biofunctional Nanomaterials Unit, CIC biomaGUNE, Paseo Miramón 182, 20009, San Sebastián, Spain

General methods: All chemicals were purchased as reagent grade from Sigma–Aldrich, except chloroauric acid (Strem Chemicals), and were used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminium-backed sheets (Merck) with visualization under UV (254 nm) and/or by staining with *p*-anisaldehyde solution [anisaldehyde (25 mL), H_2SO_4(25 mL), EtOH (450 mL), and CH_3COOH (1 mL)], 10% H_2SO_4 solution in EtOH, ninhydrin solution [ninhydrin (0.25 mL), EtOH (100 mL)] followed by heating at over 200 °C. Size-exclusion column chromatography was performed on Sephadex LH-20 (GE Healthcare). Flash column chromatography (FCC) was performed on silica gel 60 (0.063–0.200 mm; Merck). UV/Vis spectra were measured with Beckman Coulter DU 800 UV/Vis spectrophotometer. Infrared (IR) spectra were recorded from 4000 to 750 cm^{-1} with a Thermo Nicolet 6700 FT-IR model spectrometer; solids were pressed into KBr pellets and oils were subjected to attenuated total reflection (ATR). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz (high resolution) spectrometer. Chemical shifts (δ) are given in ppm relative to the residual signal of the solvent used. Coupling constants (J) are reported in Hz. Splitting patterns are described by using the following abbreviations: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet. Mass spectra were measured with an Esquire 6000 ESI-Ion Trap spectrometer from Bruker Daltonics. High-resolution mass spectra (HRMS) were obtained using the MALDI technique with a 4700 Proteomics Analyzer (Applied Biosystems) operated in MALDI-TOF-TOF configuration. Optical rotations were determined with a Perkin–Elmer 341 polarimeter. For transmission electron microscopy (TEM) examinations, a single drop (2 µL) of an aqueous solution (ca. 0.05 $mgmL^{-1}$ in Milli-Qwater) of the gold glyconanoparticles (GNPs) was placed on a copper grid coated with a carbon film (Electron Microscopy Sciences). The grid was left to dry in air for several hours at room temperature. TEM analysis was performed with a JEOL JEM-2100Fmicroscope, both operating at 200 kV. The average diameters and numbers of gold atoms of the GNPs were deduced as described in a previous study. [186]Laboratory distilled water was further purified using a Milli-Qreagent grade water system (Millipore).

Preparation and characterization of linker and iminosugar conjugates.

Synthesis of 22-(thioacetyl)-2,5,8,11-tetraoxadocosan-1-oic acid (206):[192] [11-(methylcarbonylthio) undecyl]tetra(ethylene glycol) (289 mg, 0.68 mmol) was dissolved in acetone (2.0 mL) and 0.65 mL of Jones reagent (6.68 g CrO_3, 5.75 mL H_2SO_4, and H_2O to 25 mL) was added dropwise. The reaction mixture was left stirring for 20 minutes at 25 °C and then stopped by addition of 2-propanol (8-10 drops). Water (5 mL) was added to solubilize the chromium salts and the mixture was extracted with CH_2Cl_2(4 x 10 mL). The combined organic layers were washed with water (2 x 20 mL), brine (1 x 20 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuum. The crude compound was purified by gradient column chromatography (from DCM to DCM/MeOH 9 : 1), affording **206** (205 mg, 0.47 mmol) as a colorless oil in a 69% yield. R_f = 0.33 (DCM/MeOH 9 : 1). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.15 (s, 2H), 3.76-3.61 (m, 12H), 3.48 (t, *J* = 6.9 Hz, 1H), 2.87 (t, *J* = 7.4 Hz, 1H), 2.33 (s, 3H), 1.62-1.54 (m, 4H), 1.39-1.27 (m, 14H).

Synthesis of pyrrolizidine alkaloid (PA) derivative (207): A solution of compound **206** (74.5 mg, 171 µmol), 1–hydroxybenzotriazole (HOBt, 34.7 mg, 257 µmol) and *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU, 97.5 mg, 257 µmol) in DMF (2 mL) was left stirring for 30 min and then added to a solution of amino casuarine derivative **160** (33.5 mg, 178 µmol) and *N*,*N*-diisopropylethylamine (47 µL, 267 µmol) in DMF (1.8 mL). The reaction mixture was left stirring at room temperature for 15 hours, then diluted with AcOEt (10 mL) and washed with H_2O (5 x 4 mL). The organic layer was then washed with a saturated solution of $NaHCO_3$ (3 x 10mL), water (2 x 10 mL) and brine (1 x 8 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. Purification through gradient column chromatography (DCM/MeOH from 10:1 to 5 : 1) afforded **207** (53 mg, 87 µmol) in a 51% yield. R_f = 0.30 (DCM/MeOH 7 : 1). $[\alpha]_D^{29}$ **=** + 9.6 (c = 0.78, MeOH). ^{1}H NMR (500 MHz, CD_3OD) *δ* = 4.54 (quin, *J* = 6.4 Hz, 1H, 6-H), 4.02 (s, 2H, HNCOC*H*$_2$-), 3.84-3.77 (m, 3H, 1-H, 2-H, 8-Ha), 3.72-3.59 (m, 13H, OC*H*$_2$, 8-Hb), 3.53 (q, *J* = 6.5 Hz, 1H, 7a-H), 3.49 (t, *J* = 6.7 Hz, 2H, OC*H*$_2$(CH_2)$_{11}$SAc), 3.22 (dd, *J* = 11.6, 5.9 Hz, 1H, 5-Ha), 3.05 (dd, *J* = 11.6, 6.3 Hz, 1H, 5-Hb), 2.88 (t, *J* = 7.3 Hz, 3H, -C*H*$_2$SAc, 3-H), 2.32 (s, 3H, SAc), 2.22 (dt, *J* = 12.5, 6.0 Hz, 1H, 7-Ha), 2.09 (dt, *J* = 12.5, 7.5 Hz, 1H, 7-Hb), 1.62-1.55 (m, 4H, -C*H*$_2$$CH_2$SAc, -OC$H_2$C*H*$_2$), 1.40-1.29 (m, 14H, -C*H*$_2$-) ppm. ^{13}C NMR (125 MHz, CD_3OD) *δ* = 193.3 (s, S*C*OCH_3), 171.3 (s, *C*ONH_2), 80.8 (d, C-1), 77.2 (d, C-2), 70.1 (d, C-3), 70.0-69.8 (t, 8C, O*C*H_2), 67.1 (d, C-7a), 61.6 (t, C-8), 58.7 (t, C-5), 47.8 (d, C-6), 35.4 (t, C-7), 29.3-25.8 (11C, t,-*C*H_2-, q, SCO*C*H_3) ppm. IR (KBr): ν = 3351, 2925, 2854, 1691, 1660, 1542, 1465, 1108 cm^{-1}. HRMS (ESI): *m/z* calcd for $C_{29}H_{54}N_2O_9S$: 629.36. $[M+Na]^+$; found: 629.34.

Synthesis of piperidine alkaloid (PIPA) derivative (208): A solution of EDC·HCl (1-Ethyl-3-(3-dimethylaminopropyl)carbodimide hydrochloride (32.0 mg, 168 µmol), 1–hydroxybenzotriazole (HOBt, 21.0 mg, 158 µmol) and **206** (47.0 mg, 108 µmol) in DMSO (0.4 mL) was left stirring for 10 min. and then added to a solution of piperidine derivative **168f** (20.0 mg, 105 µmol) and *N*,*N*-diisopropylethylamine (33 µL, 189 µmol) in DMSO (0.3 mL). The reaction mixture was left stirring at room temperature, under Ar atmosphere, for 65 hours, then diluted with AcOEt (10 mL) and washed with H_2O (5 x 4 mL). The organic layer was then washed with water (2 x 6 mL) and brine (1 x 4 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude was triturated with *n*-Hexane (5 x 2 mL) and then purified by column chromatography (DCM/MeOH 10:1) affording 56 mg of **208** (92 µmol, 89% yield). R_f = 0.52 (DCM/MeOH 7 : 1). $[\alpha]_D^{29}$ **=** − 12.5 (c = 0.80, MeOH). ^{1}H NMR (500 MHz, CD_3OD) δ = 4.03 (s, 2H, HNCOCH_2-), 3.99 (*br* s, 1H, 3-H), 3.88 (td, *J* = 7.1, 3.7 Hz, 1H, 5-H), 3.77-3.48 (m, 17H, 1-H, OCH_2, 4-H, 3'-H), 2.97-2.95 (m, 1H, 6-Ha), 2.88 (t, *J* = 7.4 Hz, 3H, -CH_2SAc, 2-Ha), 2.68-2.60 (m, 2H, 1'-H), 2.58-2.48 (m, 1H, 2-Hb), 2.32 (s, 4H, Sac, 6-Hb), 1.82 (quin, 6.8 Hz, 2H, 2'-H), 1.64-1.55 (m, 4H, -CH_2CH$_2$SAc, -OCH$_2$CH_2), 1.43-1.29 (m, 14H, -CH_2-) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ = 196.2 (s, S*C*OCH$_3$), 171.5 (s, *C*ONH$_2$), 71.1 (d, C-4), 71.0-69.8 (8 C, t, NHCO*C*H$_2$O, *C*H$_2$O), 69.7 (d, C-3), 68.3 (d, C-5), 54.8 (3C, t, C-2, C-6, C-1'), 36.7 (t, C-3'), 29.4-29.1 (7C, t, -*C*H$_2$-), 28.8 (q, SAc), 28.5 (t, -*C*H$_2$-), 25.4 (t, *C*H$_2$SAc), 25.8 (t, -*C*H$_2$-), 25.7 (t, C-2').ppm. IR (KBr): ν = 3319, 2924, 2854, 1691, 1663, 1541, 1456, 1352, 1112 cm^{-1}. HRMS (ESI): *m/z* calcd for $C_{29}H_{56}N_2O_9S$: 610.37. $[M+2H]^+$, found: 610.16.

Synthesis of pyrrolidine alkaloid (PYRRA) derivative (209): A solution of EDC·HCl (1-Ethyl-3-(3-dimethylaminopropyl)carbodimide hydrochloride (13.5 mg, 70 µmol), 1–hydroxybenzotriazole (HOBt, 8.9 mg, 66 µmol) and **206** (19.6 mg, 44.9 µmol) in DMSO (0.3 mL) was left stirring for 10 min. and then added to a solution of pyrrolidine derivative **157** (10.2 mg, 44 µmol) and *N*,*N*-diisopropylethylamine (13.8 µL, 79 µmol) in DMSO (0.2 mL). The reaction mixture was left stirring at room temperature, under Ar atmosphere, for 64 hours, then diluted with AcOEt (4 mL) and washed with H_2O (3 x 2 mL). The organic layer was then washed with water brine (2 x 4 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. Trituration with *n*-Hexane (5 x 2 mL) afforded **209** impure of an unknown byproduct. A small amount of crude **209** was purified by semi-preparative reverse LC (gradient from H_2O/MeOH 30/70 to MeOH in 45 min; retention time of the product: 10 minutes) and characterized. ^{1}H NMR (500 MHz, CD_3OD) δ = 4.00 (s, 2H, HNCOCH_2-), 3.98-3.95 (m, 1H, 3-H), 3.93-3.91 (m, 1H, 4-H), 3.75-3.59 (m, 14H, OCH_2, 6-H), 3.50 (t, *J* = 6.6 Hz, 2H, -OCH_2(CH$_2$)$_{11}$SAc), 3.26 (t, *J* = 7.2 Hz, 2H, 6'-H), 3.07 (d, *J* = 10.4 Hz, 1H,

2-Ha), 2.88 (t, *J* = 7.3 Hz, 3H, -C*H*$_2$SAc, 1'-Ha), 2.72-2.70 (m, 1H, 2-Hb), 2.52-2.45 (m, 1H, 5-H), 2.41-2.34 (m, 1H, 1'-Hb), 2.32 (s, 3H, SAc), 1.63-1.50 (m, 8H, 2'-H, 5'-H, -C*H*$_2$CH$_2$SAc, -OCH$_2$C*H*$_2$), 1.45-1.28 (m, 18H, 3'-H, 4'-H, -C*H*$_2$-) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ = 196.4 (s, S*C*OCH$_3$), 171.2 (s, *C*ONH$_2$), 79.3 (d, C-4), 75.7 (d, C-3), 73.2 (d, C-5), 71.0 (t, -O*C*H$_2$(CH$_2$)$_{11}$SAc), 70.2-69.8 (6C,t, O*C*H$_2$), 69.7 (t, O*C*H$_2$CONH$_2$), 61.0 (t, C-6), 58.7 (t, C-2), 55.1 (t, C-1'), 38.5 (t, C-6'), 29.9-25.8 (15C, t ,*C*H$_2$SAc, C-2'-C-5', q, SCO*C*H$_3$) ppm. HRMS (ESI): *m/z* calcd for $C_{32}H_{62}N_2O_9S$: 651.42 $[M+H]^+$, 673.42. $[M+Na]^+$, 689.42. $[M+K]^+$, found: 651. 54, 673.53, 689.52.

General Procedure for the "in situ" deprotection of *S*-acetyl conjugates 207 and 208: To a 0.03 M MeOH solution of iminosugar derivative (**207, 208)** solid CH_3OMe (10 equiv.) was added and the reaction mixture was left stirring for 2 hours at 25 °C under Ar. The complete disappearance of starting material was attested via ^{1}H NMR and the crude was directly used for the preparation of PA-GNPs and PIPA-GNPs.

Preparation and characterization of GNPs.

The hybrid gold NPs coated with iminosugars (PA or PIPA) and simple monosaccharide glucose or mannose (**PA-GNPs,** Figure 4.12 and **PIPA-GNPs**, Figure 4.13) were prepared by reduction of an Au(III) salt using sodium borohydride in the presence of a mixture of thiol-ending iminosugar conjugate (**210, 215**) and GlcC$_5$S **198** or ManC$_5$S **199**, as ligands, in different ratios following a reported procedure.[186] A 1:4 or a 2:3 ligands ratio was used to prepare 20% PA-GNPs and 40% PA-GNPs, respectively. For the analysis of the ratio between the iminosugar ligands and GlcC$_5$S (in the case of PA-Au-Glc or PIPA-Au-Glc) or ManC$_5$S (in the case of PA-Au-Man) ligands, ^{1}H NMR spectra of the initial mixture and of the supernatant after GNPs formation were recorded. The ligands loading on the PA-GNPs and PIPA-GNPs was also evaluated by quantitative NMR (qNMR) using 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid (TSP-*d*$_4$) as an internal standard in the D_2O solution of the PA-GNPs or PIPA-GNPs. The prepared PA-GNPs and PIPA-GNPs were freeze-dried and stored at 4 °C. In these conditions, the hybrid gold NPs can be stored for months maintaining their biophysical properties.

The GNPs coated only with simple monosaccharide (Figure 4.14) were also prepared as previously described[Errore. Il segnalibro non è definito.] and used as controls in this study: gold nanoparticles 100%-coated with 5-mercaptopentyl β-D-glucopyranoside **198** (**GlcC$_5$S-GNP, 218**) and gold nanoparticles 100%-coated with 5-mercaptopentyl α-D-mannopyranoside **199** (**ManC$_5$S-GNP, 219**).

General Procedure for the preparation of GNPs coated with iminosugars: An aqueous solution of $HAuCl_4$ (25 mM, 1 equiv.) was added to a 12 mM methanolic solution of a suitable mixture of thiol-ending iminosugar conjugate and β-Glc or α-Man conjugate (3 equiv.). An aqueous solution of $NaBH_4$ (1 M, 27 equiv.) was then added in four portions, with vigorous shaking. The black suspension formed was shaken for 2 hours at 25 °C. After that, the supernatant was removed and analysed by ^{1}H NMR to study the nanoparticle ligands composition. The residue was washed several times with MeOH. In order to well separate the nanoparticles from the supernatant a centrifugation (4000 xg, 10 °C, 2 min) is required in some cases. The residue was dissolved in a minimal volume of HPLC Gradient grade water and purified by dialysis (SnakeSkin® Pleated Dialysis Tubing, 10,000 MWCO). Iminosugar coated GNPs were obtained as a dark-brown powder after freeze-drying and characterized via ^{1}H NMR, UV-Vis Spectroscopy and TEM analysis. The average number of gold atoms was calculated on the basis of the average diameter obtained by TEM micrographs[194] and molecular formulas of the GNPs were estimated according to previous work.[186]

20% PA-Glc-GNPs (211): A 1:4 mixture of thiol-ending PA conjugate **210** (7.5 mg, 13.2 µmol) and $GlcC_5S$ **198** (14.6 mg, 51.7 µmol) in MeOH (5.4 mL) was used, to afford 5.6 mg of **211** (98% yield in Au). TEM (average diameter): 1.3 ±0.3 nm (main population, >85%). Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.58 mg of PA-GNPs were dissolved in 180 µL of D_2O and 40 µL of D_2O containing 0.05 wt.% TSP were added and 38 nmoles of PA conjugate were found.[195] Significant peaks: δ = 4.36 (br s, from $GlcC_5S$), 4.00 (s, NHCOC*H*$_2$- from PA conjugate), 3.84-3.22 (m), 3.05-2.97 (m, from PA conjugate), 2.78-2.66 (m, from PA conjugate), 2.15-1.14 (m); ratio between PA conjugate and $GlcC_5S$ signals ~1 to ~4.6. This result is in fair agreement with the molar ratio of conjugates per nanoparticle (20% of PA conjugate and 80% of $GlcC_5S$) as estimated by NMR analysis of the ligand mixture before and after nanoparticles formation. IR (KBr): ν ~ 3388, 2921, 2852, 1635 (amide), 1383, 1077 cm^{-1}. UV/Vis (H_2O, 0.05 and 0.10 mg/mL): λ =516 nm (gold surface plasmon band). Estimated average molecular weight for $(C_{27}H_{51}N_2O_8S)_3(C_{11}H_{21}O_6S)_{27}Au_{140}$: ~37.0 KDa.

40% PA-Glc-GNPs (212): A 2:3 mixture of thiol-ending PA conjugate **210** (3.7 mg, 6.6 µmol) and $GlcC_5S$ **198** (2.8 mg, 9.9 µmol)in MeOH (1.4 mL) was used, to afford 1.1 mg of **212** (77% yield in Au).TEM (average diameter): 1.4±0.4 nm. Quantitative ^{1}H NMR (500 MHz, D_2O

[195]In the quantitative NMR (qNMR) the **7-Ha** proton signal of PA-conjugate was selected for integration as it falls in a spectral region free of other signals.

containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.32 mg of PA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 56 nmoles of PA conjugate were found.195 Significant peaks: δ = 4.50-4.36 (m, from PA conjugate), 3.99 (s, NHCOC*H*$_2$- from PA conjugate), 3.84-3.14 (m), 3.07-2.97 (m, from PA conjugate), 2.77-2.65 (m, from PA conjugate), 2.15-2.03 (m, from PA conjugate), 1.97-1.87 (m, from PA conjugate), 1.81-1.01 (m); ratio between PA conjugate and $GlcC_5S$ signals is impossible to define since the $GlcC_5S$ anomeric signal disappeared from the spectrum; this phenomenon is in agreement with the literature[186] and it is probably due to the fact that at this densities the long active ligand folds and collapse on the glucose shell hampering a proper proton relaxation[196]. IR (KBr): ν ~3419, 2922, 2853, 1663, 1631, 1600 (amide), 1383, 1108 cm^{-1}. UV/Vis (H_2O, 0.05, 0.10 and 0.20 mg/mL): maximum absorption at 520 nm (gold surface plasmon band) not observed. Estimated average molecular weight for $(C_{27}H_{51}N_2O_8S)_9(C_{11}H_{21}O_6S)_{14}Au_{140}$: ~36.5 kDa.

20% PA-Man-GNPs (213): A 1:4 mixture of thiol-ending PA conjugate **210** (4.7 mg, 8.2 µmol) and $ManC_5S$ **199** (9.3 mg, 33.0 µmol) in MeOH (3.4 mL) was used, to afford 4.4 mg of **213** (96% yield in Au). TEM (average diameter): 1.9±0.4 nm. Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.60 mg of PA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 38 nmoles of PA conjugate were found.195 Significant peaks: δ = 4.41-4.32 (m, from PA conjugate), 4.00 (s, NHCOC*H*$_2$- from PA conjugate), 3.96-3.19 (m), 3.05-2.98 (m, from PA conjugate), 2.78-2.65 (m, from PA conjugate), 2.19-1.06 (m); ratio between PA conjugate and $ManC_5S$ signals is impossible to define since the Man anomeric signal is covered by the solvent residual peak. IR (KBr): ν ~3368, 2922, 2848, 1675, 1633 (amide), 1447, 1092 cm^{-1}. UV/Vis (H_2O, 0.05 and 0.10 mg/mL): λ=528 nm (gold surface plasmon band). Estimated average molecular weight for $(C_{27}H_{51}N_2O_8S)_4(C_{11}H_{21}O_6S)_{19}Au_{140}$**:** ~35 kDa.

40% PA-Man-GNPs (214): A 2:3 mixture of thiol-ending PA conjugate **210** (6.0 mg, 9.9 µmol) and $ManC_5S$ **199** (4.2 mg, 14.8 µmol) in MeOH (2.0 mL) was used, to afford 1.8 mg of **214**(84% yield in Au). TEM (average diameter): 1.2±0.2 nm. Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt as an internal standard): 0.33 mg of PA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 79 nmoles of PA conjugate were found.195

[196] M. Reynolds, M. Marradi, A. Imberty, S. Penades, S. Perez, *Chem. Eur. J.* **2012**, *18*, 4264-4273.

Significant peaks: δ = 4.43-4.29 (m, from PA conjugate), 3.99 (s, NHCOCH_2- from PA conjugate), 3.85-3.18 (m), 3.05-2.95 (m, from PA conjugate), 2.78-2.61 (m, from PA conjugate), 2.14-2.02 (m, from PA conjugate), 1.97-1.84 (m, from PA conjugate), 1.72-0.74 (m); ratio between PA conjugate and ManC_5S signals is impossible to define since the Man anomeric signal is covered by the solvent residual peak. IR (KBr): ν ~ 3418, 2924, 2856, 1660, 1628, 1437, 1383, 1093, 1064 cm^{-1}. UV/Vis (H_2O, 0.05 and 0.10 mg/mL): maximum absorption at 520 nm (gold surface plasmon band) not observed. Estimated average molecular weight for $(C_{27}H_{51}N_2O_8S)_9(C_{11}H_{21}O_6S)_{14}Au_{140}$: ~36.5 kDa.

20% PIPA-Glc-GNPs (215): A 1:4 mixture of thiol-ending PIPA conjugate **215** (3.1 mg, 5.5 µmol) and GlcC_5S **198** (6.0 mg, 21.3 µmol) in MeOH (5.4 mL) was used, to afford 1.6 mg of **215** (64%% yield in Au).

TEM (average diameter): 1.6 ±0.3 nm. Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d_4* acid, sodium salt as an internal standard): 0.32 mg of PIPA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 56 nmoles of PA conjugate were found.[197] Significant peaks: δ = 4.34 (br s, from GlcC_5S), 3.99 (s, NHCOCH_2- from PIPA conjugate), 3.94-2.98 (m), 2.94-2.75 (m, from PIPA conjugate), 2.52-2.22 (m, from PIPA conjugate), 2.18-1.99 (m, from PIPA conjugate), 1.82-0.72 (m). IR (KBr): ν ~ 3424, 2920, 2848, 1637 (amide), 1438, 1381, 1076 cm^{-1}. UV/Vis (H_2O, 0.05 and 0.10 mg/mL): λ =514 nm (gold surface plasmon band). Estimated average molecular weight for $(C_{26}H_{51}N_2O_8S)_7(C_{11}H_{21}O_6S)_{28}Au_{140}$: ~39.0 KDa. In this case a 20% of PIPA conjugate match perfectly with the quantitative analysis, albeit a 15% amount of PIPA conjugate was attested by the NMR analysis of the ligand mixture before and after nanoparticle formation.

40% PIPA-Glc-GNPs (217): A 2:3 mixture of thiol-ending PA conjugate **215** (3.7 mg, 6.5 µmol) and GlcC_5S **198** (2.8 mg, 9.8 µmol)in MeOH (1.4 mL) was used, to afford 1.1 mg of **217** (77% yield in Au).TEM (average diameter): 1.7±0.4 nm. Quantitative ^{1}H NMR (500 MHz, D_2O containing 0.05 wt. % of 3-(trimethylsilyl)propionic-*2,2,3,3-d_4* acid, sodium salt as an internal standard): 0.33 mg of PA-GNPs were dissolved in 180 µL of D_2O and 25 µL of D_2O containing 0.05 wt.% TSP were added and 71 nmoles of PA conjugate were found.197 Significant peaks: δ = 4.50-4.36 (m, from PA conjugate), 3.99 (s, NHCOCH_2- from PIPA conjugate), 3.91 (*br* s from PIPA conjugate), 3.80-3.30 (m), 3.56 (q, Et_2O), 3.27 (s, MeOH), 3.25-3.17 (m, from PIPA conjugate), 3.06-2.82 (m, from PIPA conjugate), 2.70-2.45 (m, from

[197] In the quantitative NMR (qNMR) the multiplet corresponding to **1'-H**, **2-Hb** and **6-Hb** proton signals (*δ* = 2.65-2.02 ppm, 4H) of PIPA-conjugate, was selected for integration as it falls in a spectral region free of other signals.

PIPA conjugate), 1.82-1.20 (m), 1.16 (t, Et_2O); ratio between PA conjugate and $GlcC_5S$ signals is impossible to define since the $GlcC_5S$ anomeric signal disappeared from the spectrum; see comment on **212**, Ref.[195]. IR (KBr): ν ~3436, 2920, 2856, 1631 (amide), 1392, 1364, 1033 cm^{-1}. UV/Vis (H_2O, 0.05, 0.10 and 0.20 mg/mL): maximum absorption at 520 nm (gold surface plasmon band) not observed. Estimated average molecular weight for $(C_{26}H_{51}N_2O_8S)_9(C_{11}H_{21}O_6S)_{14}Au_{140}$: ~36.5 kDa.

100% αManC5-coated gold nanoparticles (219, Man-Au): Reaction of a **199** (12.0 mg, 42.5 μmol) MeOH solution (3.54 mL) with $HAuCl_4$ (568 μL, 0.025 M) and $NaBH_4$ (383 μL, 1 N) gave **219** (3.0 mg) as a dark-brown powder. TEM (average diameter): 1.1±0.2 nm; ^1H NMR (500 MHz, D_2O): δ = 4.80 (s, 1H; 1-H), 4.05–3.00 (m, 8H), 2.15–1.06 ppm (m, 6H); UV/Vis (H_2O, 0.05 $mgmL^{-}1$): due to the exceptionally small core size of the GNPs, not a real surface plasmon resonance band was seen, but an absorption around λ = 520 nm (gold surface plasmon band); Estimated average molecular weight for $(C_{11}H_{21}O_6S)_{60}Au_{116}$: ~40 kDa).

100% βGlcC5-coated gold nanoparticles (218, Glc-Au): Reaction of a **198** (12.0 mg, 42.5 μmol) MeOH solution (3.54 mL) with $HAuCl_4$ (568 μL, 0.025 M) and $NaBH_4$ (383 μL, 1 N) gave **218** (2.4 mg) as a dark-brown powder. TEM (average diameter): 1.7±0.4 nm; ^1H NMR (500 MHz, D_2O): δ = 4.37 (s, 1H; 1-H), 4.04–2.83 (m, 8 H), 2.12–1.11 ppm (m, 6H); UV/Vis (H_2O, 0.05 $mgmL^{-1}$): λ = 519 nm (gold surface plasmon band); Estimated average molecular weight for $(C_{11}H_{21}O_6S)_{35}Au_{140}$:~37 kDa.

Part of this thesis has been the object of publications and communications at meeting.

PUBLICATIONS

D. Bini, M. Forcella, L. Cipolla, P. Fusi, C. Matassini and F. Cardona "Synthesis of Novel Iminosugar-Based Trehalase Inhibitors by Cross-Metathesis Reaction" *Eur. J. Org. Chem.* **2011**, 3995-4000**.**

C. Matassini, S.Mirabella, A. Goti, F. Cardona "Double Reductive Amination and Selective Strecker Reaction of a D-Lyxaric Aldehyde: Synthesis of Diversely Functionalized 3,4,5-Trihydroxypiperidines", *Eur. J. Org. Chem.* **2012**, 3920-3924.

C. Matassini, S. Mirabella, X. Ferhati, C. Faggi, I. Robina, A. Goti, E. Moreno-Clavijo, A. J. Moreno-Vargas, F. Cardona "Polyhydroxyamino piperidine iminosugars and pipecolic acid analogs from a D-mannose derived aldehyde: synthesis and biological evaluation as glycosidase inhibitors", Submitted.

CONFERENCE PRESENTATIONS AND SEMINARS

C. Matassini, F. Cardona, A. Goti, " Diversely Functionalized 3,4,5-Trihidroxy Piperidines as Potential Pharmacological Chaperones ", XXXVI "*A. Corbella"* Summer School*, Seminars in Organic Synthesis*, Palazzo Feltrinelli Gargnano del Garda (BS), 13-17 giugno **2011**, P14.

XIV CONVEGNO NAZIONALE SULLE REAZIONI PERICICLICHE E SINTESI DI ETERO E CARBOCICLI, Villa Ruspoli Firenze, 27-28 giugno **2011**.

C. Matassini, F. Cardona, A. Goti, "Double Reductive Amination and Selective Strecker Reaction of a D-Lyxaric Aldehyde: Synthesis of Diversely Functionalized 3,4,5-Trihydroxypiperidines", *XIII Convegno-scuola sulla Chimica dei Carboidrati*, Certosa di Pontignano, Siena, June 24^{th}-27^{th} **2012**, OC 7.

C. Matassini, **C. Parmeggiani**, F.Cardona, G. D'Adamio, A. Goti, "Iminosugars: Click-Chemistry For The Synthesis Of Multivalent Structures", *II International Symposium of the Collaborative Research Center (SFB) 765 about "Multivalency in Chemistry and Biochemistry",* Berlin, October 17^{th}-19^{th} **2012,** P32.

C. Matassini, F. Cardona, A. Goti, "Synthesis of Polyhydroxyamino Piperidine Iminosugars and Pipecolic Acid Analogs from a D-Mannose Derived Aldehyde", XXXVIII "*A. Corbella"* Summer School*, Seminars in Organic Synthesis*, Palazzo Feltrinelli Gargnano del Garda (BS), 17-21 giugno **2013**, O19.

CONFERENCE PRESENTATIONS AS CO-AUTHOR

F. Cardona, G. D'Adamio, C. Parmeggiani, C. Matassini, D. Martella and A. Goti, "Sugar-Derived Nitrones and Aldehydes: Key Building Blocks for the Synthesis of Glycosyl Hydrolases Inhibitors", *XVI European Carbohydrate Symposium*, Sorrento-Naples, July 3rd-7th, **2011**, OL 114.

D. Bini, M. Zappa, M. Forcella, F. Cardona, C. Matassini, L. Cipolla, P. Fusi, "Iminosugar-Based Trehalose Mimetics as Trehalase Inhibitors", *XVI European Carbohydrate Symposium*, Sorrento-Naples, July 3rd-7th, **2011**, PC 027.

G. D'Adamio, C. Matassini, A. Goti, **F. Cardona**, "Synthesis of multivalent pyrrolizidine and piperidine iminosugars", *XIII Convegno-scuola sulla Chimica dei Carboidrati*, Certosa di Pontignano, Siena, June 24th-27th **2012**, PC 11.

F. Cardona, C. Matassini, A. Goti, "Diversely functionalized 3,4,5-trihydroxypiperidines form a D-lyxaric aldehyde", *26th International Carbohydrate Symposium, ICS*, Madrid, July 22nd-27th **2012**, OC 132.

D. Bini, A. Sgambato, M. Forcella, F. Cardona, C. Matassini, P. Fusi, P. Parenti, L. Cipolla, "Iminosugar-based inhibitors of insect trehalase", *26th International Carbohydrate Symposium, ICS*, Madrid, July 22nd-27th **2012**, FC 154.

F. Cardona, G. D'Adamio, C. Matassini, A. Goti, "Synthesis of multivalent iminosugars", *26th International Carbohydrate Symposium, ICS*, Madrid, July 22nd-27th **2012**, PC 021.

Aknowledgements:

My heartfelt thanks to Prof. Andrea Goti and Dr. Francesca Cardona for this three years long PhD experience: thank you for your enthusiasm, your advices and for teaching me so much. It has been a pleasure to work with you.

My gratitude goes to Prof. Soledad Penadès, for gave me the opportunity to work in her Laboratory, encouraging me to extend my knowledge and to improve my competences.

I really want to thank Dr. Marco Marradi, for his help during my last PhD year, *inside* and *outside* the Lab.!

Many thanks also to Prof. Robina group for the biological evaluations of compounds, in particular to Dr. Antonio J. Moreno-Vargas for his help in the screening of GNPs.

Ringraziamenti:

Un grazie enorme a mamma, babbo e Enri, per avermi sempre sostenuto e per non avermi abbandonata in questo ultimo anno, in cui io ho abbandonato voi!

Grazie a Bado, che continua a esserci sempre, nonostante i periodi stressanti, la lontananza e le correzioni alla tesi, che ancora una volta ho scritto in Word!

Grazie alla Cami, perché è l'unica ad avere il superpotere di farmi passare l'ansia! Grazie di tutto, amica!

Grazie a Tele, perché con lui di ansia non si può parlare…

Grazie a tutti i compagni di "Polo" fiorentini y a todos los compañeros de biomaGUNE!

Grazie al *Malandrino*, che è stata la cosa più simile ad una famiglia che ho avuto in questi mesi a Donostia.

Thank you - Eskerrik asko - Muchas gracias - Grazie mille