UNIVERSITA' DEGLI STUDI DI NAPOLI FEDERICO II

FACOLTA' DI SCIENZE MATEMATICHE FISICHE E NATURALI

DOTTORATO DI RICERCA IN SCIENZE CHIMICHE XX CICLO

2004-2007

SYNTHESIS AND REACTIVITY OF NATURAL AND UNNATURAL CARBOHYDRATES

DANIELE D'ALONZO

TUTORE: PROF. GIOVANNI PALUMBO

COORDINATORE: PROF. ALDO VITAGLIANO

alla mia famiglia

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II FACOLTÀ DI SCIENZE MM.FF.NN.
DOTTORATO DI RICERCA IN SCIENZE CHIMICHE (XX CICLO)

Tutore: **Ch. mo Prof. Giovanni Palumbo**

Candidato: **Dr Daniele D'Alonzo**

Coordinatore: **Ch. mo Prof. Aldo Vitagliano**

SYNTHESIS AND REACTIVITY OF NATURAL AND UNNATURAL CARBOHYDRATES

The role of chirality at the chemistry-biology interface is a topic of enormous fascination and importance.[1] Organic structures have an intrinsic capacity for chiral discrimination, but the relationships between molecular stereochemistry and biological function are not always predictable. Particularly, while it is often assumed that biomolecular recognition processes are stereospecific, there are many notable exceptions. For instance, the mammalian taste bud receptor recognizes D-sugars **1** and L-sugars **2** with equal avidity[2] (**Figure 1**). In an other context, the human deoxycytidine kinase (dCK), the enzyme responsible for the phosphorylation of cytidine nucleosides, discovered as a target enzyme for HIV, HSV and HBV inhibition, recognizes and therefore phosphorylates D-series nucleoside analogues as well as their L-enantiomers (as reported[3] in the case of BCH 189 **3** and 3TC **4**, **Figure 1**). Furthermore, although glycosides belonging to D-series are the natural substrates for all glycosidases (except for fucosidases), deoxy-L-allonojirimycin (**5**) is a much better inhibitor (IC_{50} = 30 μM) of α-D-mannosidase than deoxy-D-mannojirimycin (**6**, IC_{50} = 840 μM).[4]

Such a kind of data demonstrates fairly well that enantiospecificity, although important in many cases, could not be a universal trait of biomolecular recognition. To further probe this feature, a number of research groups have engaged in the synthesis and evaluation of mirror-image molecules.[5] With regards to L-sugar related molecules, their synthesis represents a challenging area of investigation, since efficient methodologies are required to have a rapid access to mirror-image sugars.

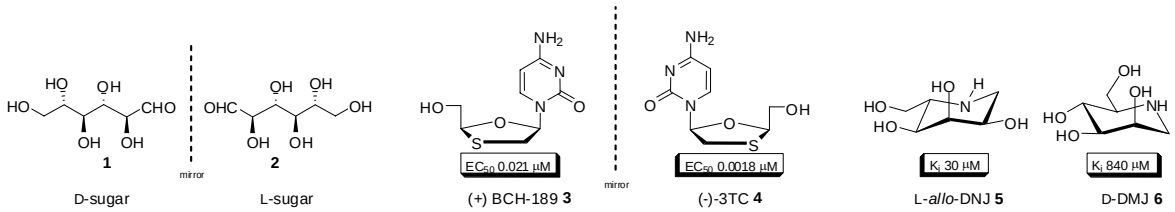
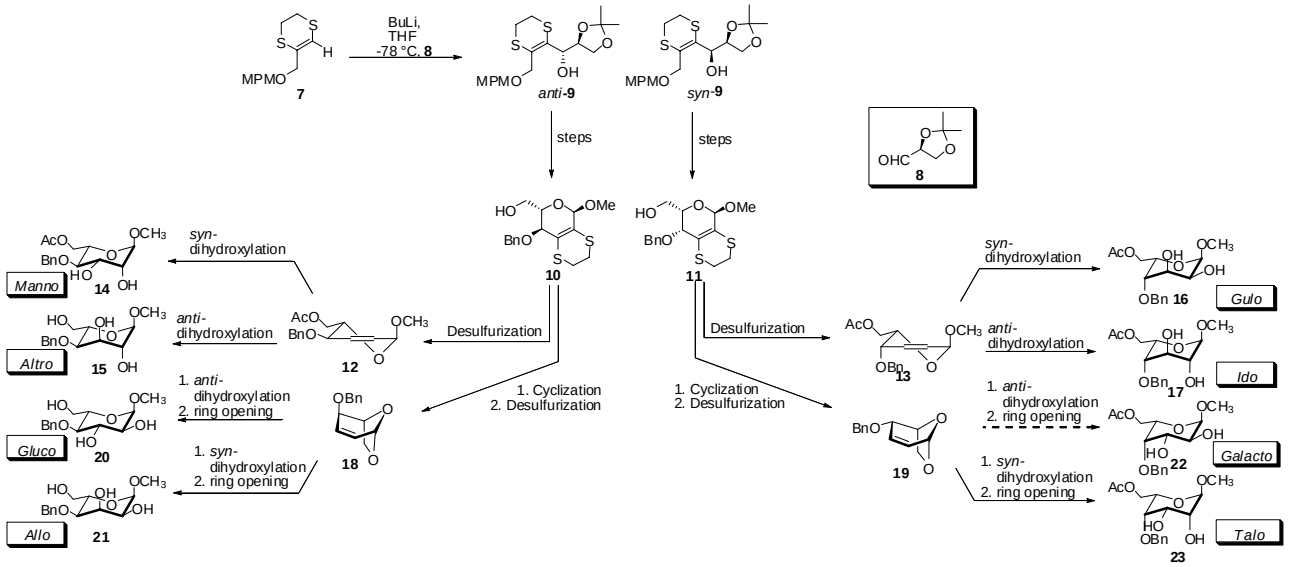


Figure 1

Inspired by these findings, the current PhD project has been based on the achievement of a general and stereoselective procedure for the synthesis of enantiopure sugar derivatives. Particular attention has been devoted to a strategy for the synthesis of L-series molecules, which has been profitably employed for the preparation of L-hexoses, L-iminosugars, L-series anomeric and non-anomeric nucleosides, and enantiopure six-membered oligonucleotides.

A VERSATILE ROUTE TO L-HEXOSES: STEREOSELECTIVE SYNTHESIS OF RARE SUGARS

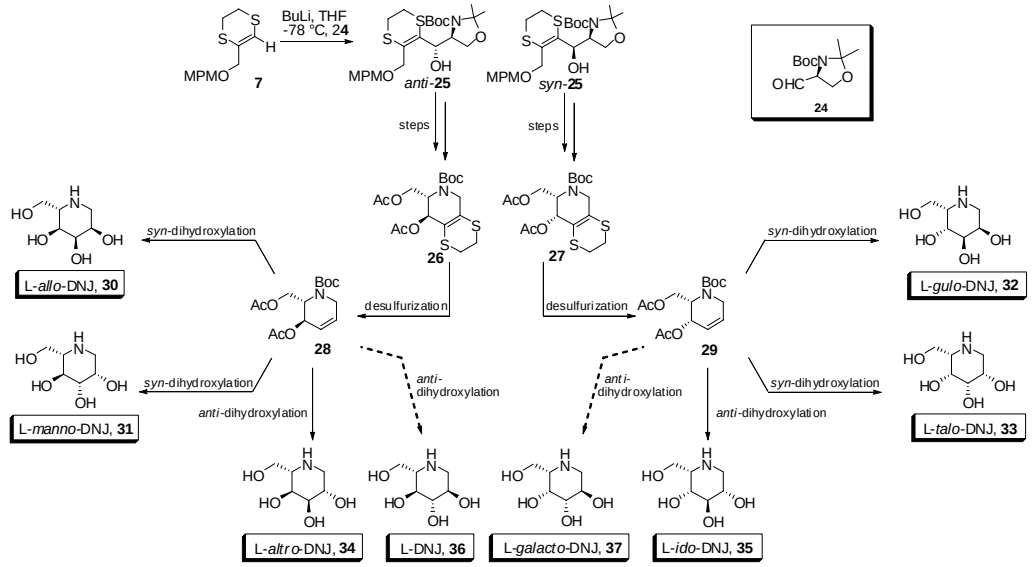
As part of our efforts working toward the synthesis of polyhydroxylated compounds,[6] in the first year of my PhD programme we have explored a general and efficient route for the preparation of L-hexoses (as well as their D-enantiomers) starting from the three-carbon homologating agent **7** (**Scheme 1**). Particularly, the employ of **7** and of the protected L-glyceraldehyde **8** has enabled the preparation of key intermediates **10** and **11** in a few steps. Hence, seven of eight L-epimers have been so far obtained, with a high degree of stereoselectivity, through suitable manipulations of the double bond in olefins **12** and **13** (**Scheme 1**).[7] In detail, it has been exploited the bulky benzyl group on 4-OH that let exposed only the *anti*-face of the olefins **12** and **13** for dihydroxylation and epoxidation reactions, affording protected L-sugars **14-17**. On the other hand, compounds **20-21** and **23** have been prepared through *syn*- and *anti*- dihydroxylation of the 1,6-anhydro derivatives **18** and **19**, prepared by a further cyclization of the intermediates **10-11**. In this case, in fact, it has been exploited a useful feature of 1,6-anhydro derivatives, according to which all stereocenters are in opposite orientation with respect to the corresponding classical pyranosides.[8] Therefore, an inverse stereoselectivity has been achieved in the dihydroxylation and epoxidation reactions, affording remaining L-sugars **20, 21** and **23** (**Scheme 1**).



Scheme 1 – *Total synthesis of L-hexopyranosides.*

A GENERAL APPROACH TO THE SYNTHESIS OF 1-DEOXY-L-IMINOSUGARS

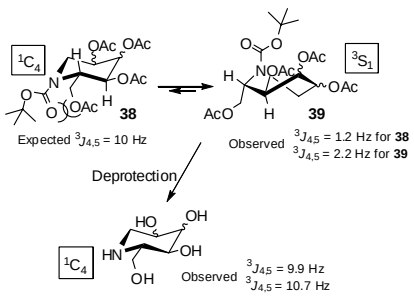
On the basis of the remarkable results reported in literature about the therapeutic relevance of 1-deoxy-L-iminosugars, a versatile strategy for their synthesis has been developed in the second year of my PhD programme.



Scheme 2 – *Total synthesis of 1-deoxy-L-iminosugars.*

The synthesis has involved the use of the homologating system **7** and the well known Garner's aldehyde **24**, which has led, after a few steps, to the iminosugar precursors **26-27**, in which the configuration of the starting aldehyde is preserved (**Scheme 2**). Starting from **26-27**, dithiodimethylene bridge removal and suitable protecting groups manipulation, followed by *syn*- or *anti*-dihydroxylation reactions, have enabled the achievement of L-iminosugars **30-35** in stereoselective manner.[9]

It is noteworthy that the *N*-Boc intermediates **38-39** do not conform to the expected $^1C_4$ chair conformation, typical of L-sugars; conversely they adopt a conformation close to $^3S_1$ (as observed from NMR analysis, **Scheme 3**).[10] This finding will be farther on exploited for the preparation of 2,3-*anti* di-equatorial substituted iminosugars *gluco*- and *galacto*-L-DNJ **36** and **37**, otherwise not easily achievable (**Scheme 2**).



Scheme 3 - *Preferred conformations for compounds **38-39.***

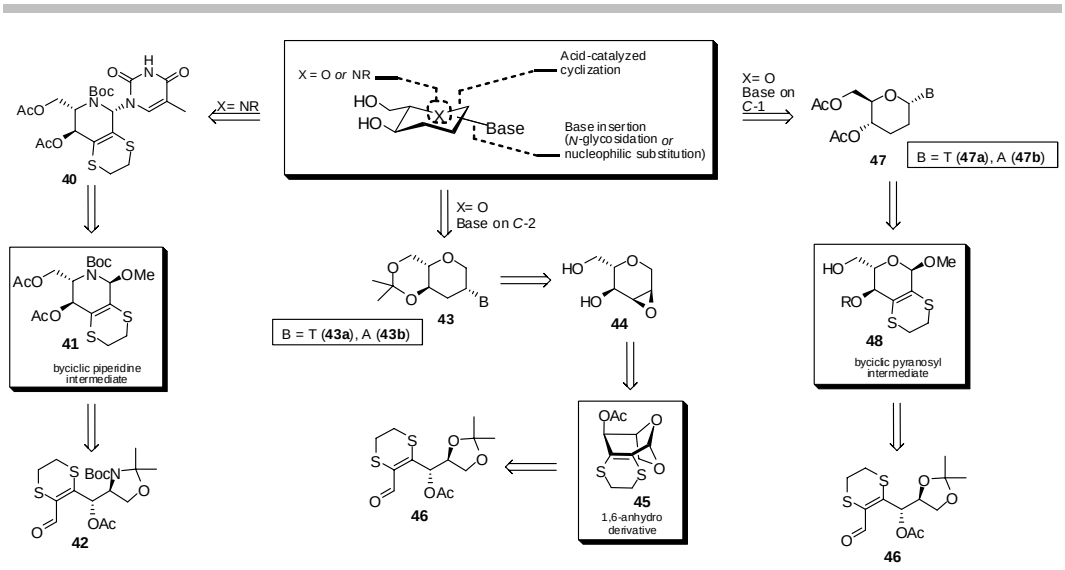
A GENERAL ROUTE TO ENANTIOPURE SIX-MEMBERED NUCLEOSIDE ANALOGUES AND OLIGONUCLEOTIDES

In the frame of a project joined in collaboration with the group of prof. Herdewijn from the Rega Institute for Medicinal Chemistry (Leuven, Belgium), the successful application of our methodology for the preparation of L-hexoses and L-iminosugars has been in the third year extended to the synthesis of azanucleosides and anomeric and non-anomeric hexopyranosyl nucleosides. Moreover, hexopyranosyl nucleoside analogues have been inserted in several oligonucleotide sequences; for such molecules it has been evaluated their aptitude to hybridize with natural deoxyribo-oligonucleotide (DNA) complements, therefore testing their feasibility for antisense purposes.

a) Synthesis of azapyranosyl and hexopyranosyl nucleoside analogues. Driven by the discovery according to which a number of human enzymes are able to phosphorylate nucleosides belonging to unnatural L-series,[3] a general route for the synthesis of six membered L-nucleosides has been opened up. As depicted in Scheme 4, all nucleoside analogues can be prepared starting from the homologating agent **7** and a suitable chiral electrophile (to confer the desired D- or L-configuration to the target molecules). Depending on the nature of the "x" group (**Scheme 4**), hexopyranosyl- or azapyranosyl- purine and pyrimidine nucleosides **40**, **43** and **47** have been synthesized. Within the synthesis of each nucleoside analogue, particular attention has been given to the stereochemical outcome of *N*-glycosidation reactions, since target compounds need a β-installation of the base. To test the breadth of our methodology, insertion of thymine (T) and adenine (A) bases (as pyrimidine and purine base models, respectively) has been preliminarily studied (**Scheme 4**).

With regards to azanucleosides, the current synthesis must be considered the first entry to the preparation of such molecules, whether they belong to D or L series.

b) Synthesis of six-membered oligonucleotide analogues. Enantiopure six-membered nucleosides 1-(2',3'-dideoxy-β-L-*ribo*-hexopyranosyl) thymine (**47a**), 1-(2',3'-dideoxy-β-L-*ribo*-hexopyranosyl) adenine (**47b**), 2'-(2',3'-dideoxy-1',5'-anhydro-L-*arabino*-hexitol) thymine (**43a**) and 2'-(2',3'-dideoxy-1',5'-anhydro-L-*arabino*-hexitol) adenine (**43b**), prepared as above reported, have been employed for the preparation of oligonucleotide sequences: L-Hexitol Nucleic Acids (L-HNA) and 2,3-dideoxy-L-Hexopyranosyl Nucleic Acids (L-Homo-DNA).



Scheme 4 – *A general route to enantiopure six-membered nucleoside analogues.*

Once obtained, the oligonucleotide sequences have been used for different purposes. Particularly, sequences **49-56** have been studied considering their stability towards natural DNA-type oligonucleotide sequences (to determine whether the unnatural nucleosides **A*** and **T*** may perturb the structure of a short DNA duplex).[11] On the other hand, sequences **57-59** were studied considering their aptitude to give hybridization towards unnatural oligonucleotide sequences, in the frame of a project focused towards the understanding of the conformational and chiral selection of oligonucleotides.[12]

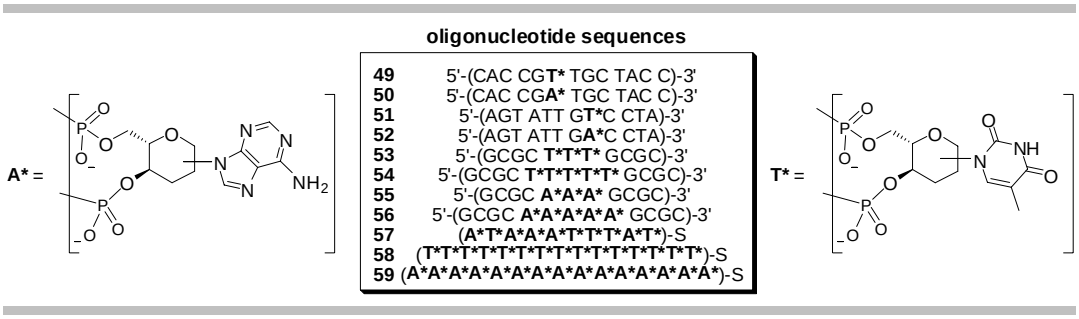


Figure 2 – *Oligonucleotide sequences of L-HNA and L-Homo-DNA.*

REFERENCES

[1] (a) Joyce, G. F.; Visser, G. M.; van Boeckel, C. A. A.; van Boom, J. H.; Orgel, L. E.; van Westrenen, J. *Nature* **1984**, *310*, 602. (b) Takats, Z.; Nanita, S. C.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2003**, *42*, 3521.
[2] Shallenberger, R. S.; Acree, T. E.; Lee, C. Y. *Nature* **1969**, *221*, 555.
[3] Sabini, E.; Hazra, S.; Konrad, M.; Lavie, A. *J. Med. Chem.* **2007**, *50*, 3004.
[4] Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J. Med. Chem.* **2005**, *48*, 2036.
[5] Boulineau, F. P.; Wei, A. *J. Org. Chem.* **2004**, *69*, 3391.
[6] Caputo, R; De Nisco, M.; Festa, P.; Guaragna, A; Palumbo, G.; Pedatella, S. *J. Org. Chem* **2004**, *69*, 7033.
[7] Guaragna, A.; Napolitano, C.; D'Alonzo, D.; Pedatella S.; Palumbo, G. *Org. Lett.* **2006**, *8*, 4863.
[8] Kulkarnia, S. S.; Leeb, J.-C.; Hung, S.-C. *Curr. Org. Chem.* **2004**, *8*, 475.
[9] Guaragna, A.; D'Errico, S.; D'Alonzo, D.; Pedatella S.; Palumbo, G. *Org. Lett.* **2007**, *9*, 3473.
[10] (a) Kilonda, A.; Compernolle, F.; Hoornaert, G. J. *J. Org. Chem.* **1995**, *60*, 5820. (b) Kazmaier, U.; Grandel, R. *Eur. J. Org. Chem.* **1998**, 1833.
[11] Hendrix, C.; Rosemeyer, H.; Verheggen, I.; Seela, F.; Van Aerschot, A.; Herdewijn, P. *Chem. Eur. J.* **1997**, *3*, 110.
[12] Froeyen, M.; Nielsen, P.; Van Aerschot, A.; Rosemeyer, H.; Herdewijn, P. *Chem. Biodiv.* **2007**, *4*, 804.

LIST OF ABBREVIATIONS

A	adenine
Ac	acetyl group
Ac_2O	acetic anhydride
AcOH	acetic acid
ACV	acyclovir
AE	asymmetric epoxidation
Ag_2O	silver oxide
All	allyl group
AMP-DNJ	*N*-(5-adamantane-1-yl-methoxypentyl)-DNJ
ANA	altritol nucleic acid
Ar	aryl group
AZT	3'-azidothymidine
Bn	benzyl group
BnBr	benzyl bromide
Boc	*t*-butoxycarbonyl group
BSA	*N*,*N*-*bis*-dimethylsilylacetamide
BuLi	butyllithium
Bz	benzoyl group
BzCl	benzoyl chloride
BCH-189	2'-deoxy-3'-thiacytidine
t-BuOH	*tert*-butanol
BVdU	5-bromovinyl-2'-deoxyuridine
C	cytosine
CD	circular dichroism
CE	capillary electrophoresis
CeNA	cyclohexenyl nucleic acid
CF_3COCH_3	trifluoroacetone
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
CH_3CN	acetonitrile

CH_3OH	methanol
CNA	cyclohexyl nucleic acid
COSY	correlation spectroscopy
Cp	cyclopentadienyl group
m-CPBA	*m*-chloroperbenzoic acid
CyA	cyclosporin A
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
dCK	deoxycytidine kinase
dCTP	2'-deoxycytidine 5'-triphosphate
ddC	2',3'-dideoxycytidine
ddI	2',3'-dideoxyinosine
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DGJ	deoxygalactonojirimycin
dGK	deoxyguanosine kinase
DIPEA	diisopropylethylamine
DMAP	4-*N*,*N*-dimethylaminopyridine
DMDO	dimethyldioxirane
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DMT	dimethoxytrityl
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNJ	1-deoxy-nojirimycin
dNTP	2´-deoxynucleoside 5´-triphosphate
dr	diastereomeric ratio
ds	double stranded
dT	deoxythymidine
EC_{50}	half maximal effective concentration
EDTA	ethylenediaminetetraacetic acid
ee	enantiomeric excess

Et_2O	diethyl ether
EtOAc	ethyl acetate
Et_3SiH	triethylsilane
FDA	food and drug administration
FMAU	1-(2'-deoxy-2'-fluoro-beta-D-arabinofuranosyl)-5-methyluracil
FTC	5-fluoro- β-L-thiocytidine
G	guanine
GCV	ganciclovir
GSL	glycosphingolipid
HBV	hepatitis B virus
HCl	hydrogen chloride
$HClO_4$	perchloric acid
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HMBC	heteronuclear multiple bond correlation experiment
HMDS	hexamethyldisilazane
HNA	hexitol nucleic acid
Homo-DNA	hexopyranosyl-(4'→6') DNA
H_2SO_4	sulphuric acid
HSQC	heteronuclear single quantum coherence
HSV	herpes simplex virus
I_2	iodine
IC_{50}	half maximal inhibitory concentration
J	coupling constant
K_i	inhibition constant
KOH	potassium hydroxide
LAH	lithium aluminium hydride
MeONa	sodium methoxide
$MgBr_2$	magnesium bromide
MMTCl	(4-methoxyphenyl)diphenylmethyl chloride
MNA	mannitol nucleic acid
MPMCl	*p*-methoxybenzyl chloride

*m*RNA	messenger ribonucleic acid
MsCl	mesyl chloride
NaH	sodium hydride
NaOBz	sodium benzoate
NaOH	sodium hydroxide
*N*B-DGJ	*N*-butyl-deoxygalactonojirimycin
*N*B-DNJ	*N*-butyl-deoxynojirimycin
NBS	*N*-bromosuccinimide
NH_3	ammonia
NH_4Cl	ammonium chloride
$(NH_4)_2SO_4$	ammonium sulfate
Ni/Ra	raney Nickel
NJ	nojirimycin
NMO	4-methylmorpholine *N*-oxide
NMR	nuclear magnetic resonance
*N*N-DNJ	*N*-nonyl-DNJ
NRTIs	nucleoside reverse transcriptase inhibitors
L-OddC	β-L-dioxolane cytosine
OsO_4	osmium tetroxide
Oxone	potassium peroxomonosulfate
PCC	pyridinium chlorochromate
Pd/C	palladium on actived carbon
$Pd(OH)_2/C$	palladium hydroxide on actived carbon
Ph_3P	triphenylphosphine
PNA	peptide nucleic acid
PTSA	*p*-toluenesulfonic acid
Py	pyridine
RNA	ribonucleic acid
RT	reverse transcriptase
$SnCl_4$	tin chloride
T	thymine
TBAF	tetrabutylammonium fluoride

TBDPS	*t*-butyldiphenylsilyl group
3TC	β-L-thiocytidine
TEA	triethylamine
Tf	trifluoromethanesulfonyl group
TFA	triflouroacetic acid
TfOTMS	trimethylsilyltrifluoromethansulfonate
THF	tetrahydrofuran
$TiCl_4$	titanium chloride
$Ti(O\text{-}Pr^i)_4$	titanium tetraisopropoxide
TK	thymidine kinase
T_m	melting temperature
TMEDA	*N*,*N*,*N*,*N*-tetramethylenediamine
TMSCl	chlorotrimethylsilane
TMSI	iodotrimethylsilane
TMST	2-(trimethylsilyl) thiazole
TPP	triphenylphosphine
TsCl	*p*-toluenesulfonyl chloride
TsOH	*p*-toluenesulfonic acid
UDP	uridine diphosphate
VR	virus rating
VZV	varicella zoster virus

TABLE OF CONTENTS

Chapter 1

From Natural Glycans to Synthetic Glycomimetics: Emerging Roles of Carbohydrates and their Analogues in Modern Drug Discovery

1. FROM NATURAL GLYCANS TO SYNTHETIC GLYCOMIMETICS: EMERGING ROLES OF CARBOHYDRATES AND THEIR ANALOGUES IN MODERN DRUG DISCOVERY

In the last two decades a fundamental new role of carbohydrates in living organisms has emerged: glycoconjugates of oligosaccharides with proteins or lipids proved to be the major information carriers between cells and their surroundings. This finding has rapidly brought to the renaissance of several aspects of carbohydrate chemistry. The need for specific oligosaccharide sequences in quantities larger than those isolable from biological sources (in order to thoroughly understand their biological functions) has inspired new synthetic approaches. An increasing demand for compounds which can mimic the above natural substances by way of similar structure and/or biological action (the so-called glycomimetics) has in parallel arisen and stimulated elaboration of novel preparative procedures in carbohydrate synthesis. The need for analogues of mono- and oligosaccharides resistant to hydrolytic/enzymatic cleavage has been carried out as well. All these efforts in chemistry and biology have converged in establishing a new discipline, today called glycoscience.[1]

Especially with respect to investigations about mono- and oligosaccharide analogues, an emerging interest concerning mirror-image carbohydrates is occurring as an extremely intriguing topic, since they may be useful for investigating the role of molecular chirality in carbohydrate recognition. This suggests that procedures which enable access to mirror-image mono-, oligosaccharides and glycoconjugates may present untapped opportunities for enhancing our understanding of biologically relevant sugar interactions and may provide new approaches for the development of improved therapeutic agents.

1.1 *INTRODUCTION*

Carbohydrates are unrivalled in the density of information that they can supply. Only considering the degree of molecular diversity that can be generated from glycosidic linkage assembly of two monomeric pyranose units (e.g. 1-2, 1-3, 1-4, or 1-6 linkages), a significant contrast with the linear nature of proteins and nucleic acids emerges. Besides, whereas DNA (with a basis set of 4 nucleobases) and amino acids (with a basis set of 20 units) may construct a biological language for information transfer of about 10^7 “words”, carbohydrates (with a basis

set of 6 sugars common in mammalian carbohydrates) have access to more than 10^{12} variations.[2] In addition, considering the further variety afforded by anomeric stereochemistry, ring size and sub-unit modification (e.g., sulfation, phosphorylation or acylation), it can be quickly seen that this greater variety of possible combinations gives the language of carbohydrates exquisite eloquence.

1.2 *CARBOHYDRATES IN BIOLOGICAL EVENTS: GLYCOMICS*

Because of their enormous structural and functional diversity, carbohydrates have been found to play crucial roles in numerous contexts, whether they are in the form of monomers, oligomers, polymers, or as components of biopolymers and other naturally occurring substances.[3] Although up to few decades ago most of them were thought to be able to play roles just as structural and food storage elements, whereas the control of the organism functioning was left to the other main natural polymers, such as DNA and proteins, nowadays the increased awareness of the vital biological roles exerted by oligosaccharides has led to an renewed interest in the carbohydrate field, concerning the elucidation of their structural complexity, their structure-function relationships and the exploitment of their vast potential in drug development. With a re-evaluation of the importance of carbohydrates, analogously to Proteomics and Genomics, the term "Glycomics"[4] (or "Glycobiology"[5] or "Glycoscience"[6]) has been coined to refer to the role of carbohydrates in biological events,[7] including carbohydrate-carbohydrate, carbohydrate-protein, and carbohydrate-nucleic acid interactions (**Figure 1**).

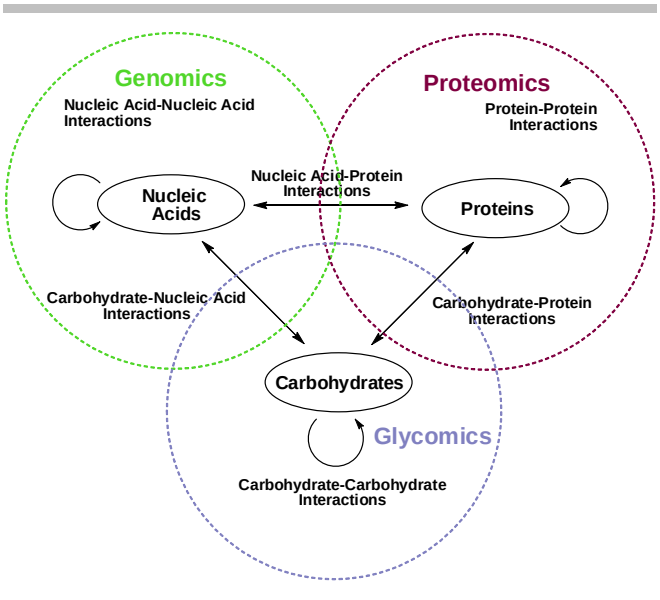


Figure 1 - *Biopolymer interactions (reproduced from Ref 4c).*

In the form of glycopeptides, glycolipids, glycosaminoglycans, proteoglyans, or other glycoconjugates, carbohydrates have been found on cell surfaces to coordinate biological events (see **Figure 2**). These ones include signal transduction,[8] inflammation processes,[9] cell-cell interactions,[10] bacteria-host interactions,[11] viral entry,[12] fertility and development.[13]

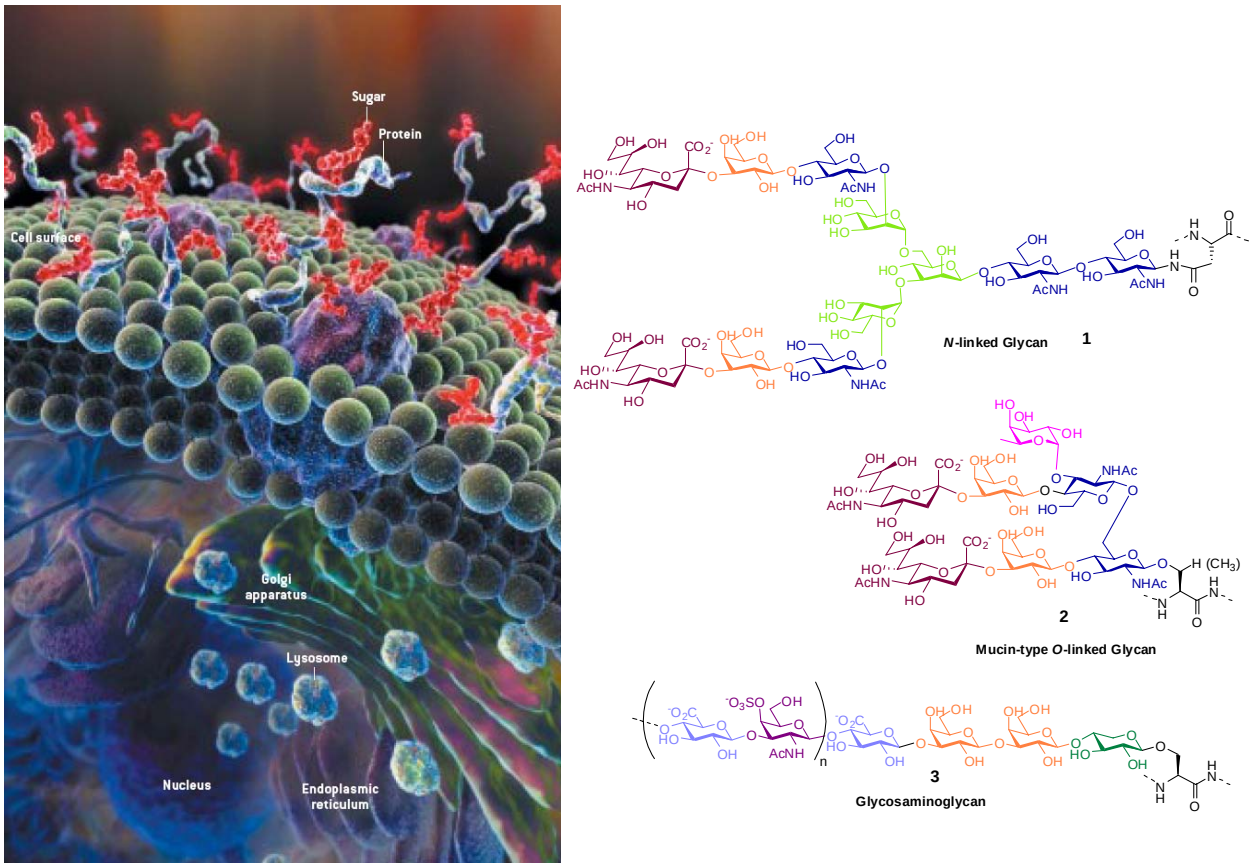


Figure 2 - *The structure and conformation of complex carbohydrates are important in their function; they affect protein binding, targeting, folding, storing signalling molecules, mediating cell-surface binding and signalling events, or directing intracellular-transport events. The picture is reproduced from ref. 4a.*

Particularly, depending on the nature of glycoconjugates, i.e. whether they are constituted by linear (end-to-end linked repeating monosaccharide units) or branched (multiply linked repeating monosaccharide units) glycans, biological roles in different contexts are ascribed. Branched glycans (such as **1** and **2**, **Figure 2**), attached to proteins, lipids and other biomolecules as glycoconjugates represent the most extensive and diverse form of post-translational modification. The structure and conformation of branched glycans are important in their function; they affect protein binding, targeting and folding. On the other hand, linear polysaccharides (such as **3**, **Figure 2**), whether attached to a protein core or free, display, depending on their location and sequence, functions like storing signalling molecules, mediating cell-surface binding and signalling. In view of the various biochemical pathways and disease

processes in which glycoconjugates are crucially engaged - for example angiogenesis,[14] cancer,[15] tissue repair, cardiovascular disease,[16] immune-system function,[17] microbial and viral pathogenesis[18] - the possibilities for their employ as therapeutics and diagnostics are numerous and exciting.

1.3 *CARBOHYDRATES ANALOGUES AS THERAPEUTIC AGENTS: GLYCOMIMETICS*

As our understanding of carbohydrate-mediated biological recognition processes advances to the molecular level, new carbohydrate-based therapies are becoming readily available and widely utilized. In search for chemical tools that are able to block glycoconjugate formation and/or function, carbohydrate-derived therapeutics include synthetic carbohydrate vaccines[19] and glycomimetics, which have been used whether as antagonists of carbohydrate-protein interaction[20] or as inhibitors of carbohydrate processing enzymes.[21]

The term "glycomimetics" refers to the creation of molecules that mimic the activity of carbohydrates. The need to synthesize glycomimetics derives from the fact that the carbohydrates themselves are often degraded too rapidly *in vivo* and because they have relatively weak binding to proteins. Therefore, a line of research is focused on rationally designed carbohydrate analogues, with shapes and polarities resembling natural sugars, which are able to mimic, alter or replace native carbohydrate moieties, for improving their biological, pharmacological or drug-like properties.[22]

Glycomimetic research has so far progressed in numerous fields of investigation, developing structures alternative to native carbohydrate buildings for the treatment of a broad range of inflammatory and infectious diseases[19a] (**Figure 3**).

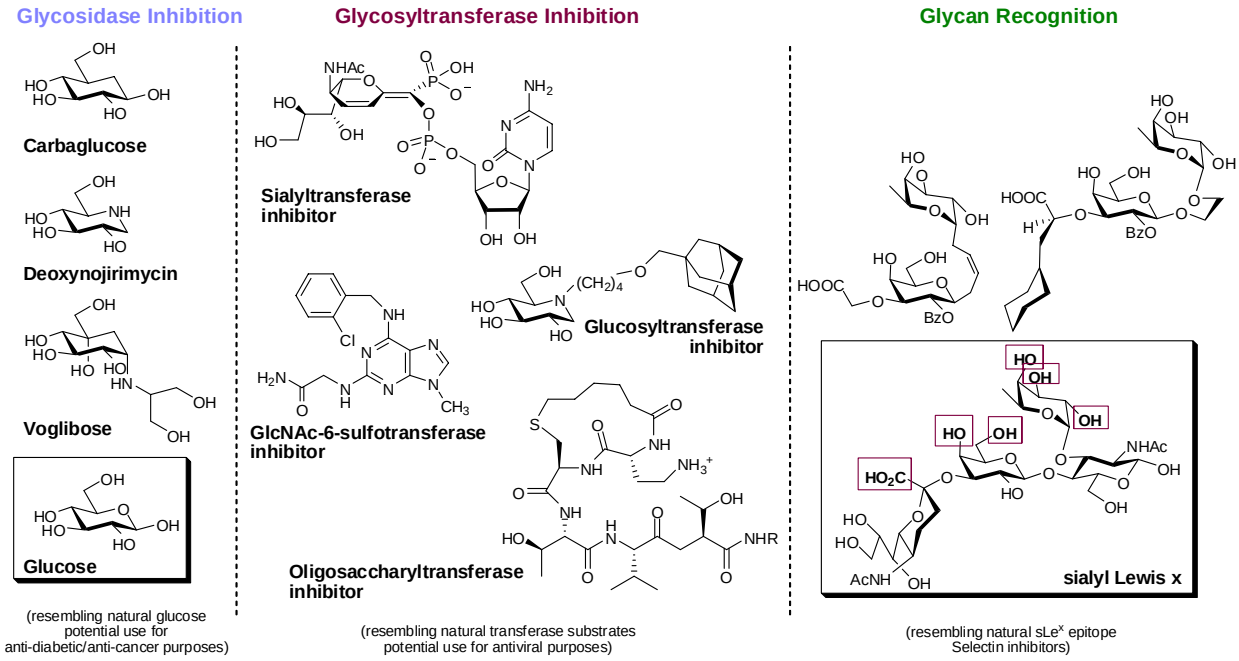


Figure 3 – *Glycomimetics used for several therapeutic purposes.*

As above mentioned, at least two general types of inhibitors are being sought: those that block glycoconjugate synthetic pathways (biosynthesis or degradation) and those that interfere with glycoconjugate recognition. Concerning glycoconjugate biosynthesis or degradation, many efforts have been pursued to generate antagonists either of glycosyltransferases,[23] that mediate the formation of glycosidic bonds, or of glycosidases,[24] that hydrolyze glycosidic bonds. Natural sugar targets being identified, numerous antagonists, inspired by such molecules, have been synthesized (**Figure 3**).

On the other hand, a big challenge is still represented by the creation of compounds that block glycoconjugate recognition. In this field of investigation, the identification of specific glycan structures that contribute to leukocyte-endothelial cell adhesion,[20] viral infection, bacterial colonization and immunological recognition,[25] has inspired innovative strategies for the design of inhibitors of the carbohydrate-receptor interactions. Particularly, current glycomimetic design has been focused to the identification of structural elements on the native oligosaccharides that are wholly indispensable for recognition, these latter ones having been replaced with simplified scaffolds[26] (**Figure 3**).

1.4 *UNNATURAL CARBOHYDRATES AS TOOLS FOR GLYCOBIOLOGY: REVISITING THE ROLE OF CHIRALITY AT THE CHEMISTRY-BIOLOGY INTERFACE*

As latest approach in the field of glycomimetics, quite a few innovative lines of research are focusing to the employ of unnatural mono- and oligosaccharides, driven by the possibility to explore the function of molecular chirality in carbohydrate recognition.[27]

The role of chirality at the chemistry-biology interface is a topic of enormous fascination and importance, in glycobiology as well as in other fields of investigation.[28] Optically active molecules, among which carbohydrates are of course included, often possess specific conformational and high order structures associated with their chiral features; their structures are deeply connected with their properties and functions, including the ability for chiral recognition. Nevertheless, the relationships between stereochemistry of a potentially active molecule and its effective biological function are not always predictable. Particularly, even if it is often assumed that biomolecular recognition processes are stereospecific, there are many notable exceptions. For instance, the mammalian taste bud receptor recognizes D-sugars **4** and L-sugars *ent*-**4** with equal avidity[29] (**Figure 4**). In an other context, the human deoxycytidine kinase (dCK), the enzyme responsible for the phosphorylation of cytidine nucleosides, identified as a target enzyme for HIV, HSV and HBV inhibition, recognizes and therefore phosphorylates D-series nucleoside analogues as well as their L-enantiomers (as reported[30] in the case of BCH 189, **5** and 3TC *ent*-**5**, **Figure 4**). Furthermore, although glycosides belonging to D-series are the natural substrates for all glycosidases (made exception for L-fucosidases), deoxy-L-allonojirimycin (**6**) is a much better inhibitor (IC_{50} = 30 μM) of α-D-mannosidase than deoxy-D-mannojirimycin (**7**, IC_{50} = 840 μM).[31]

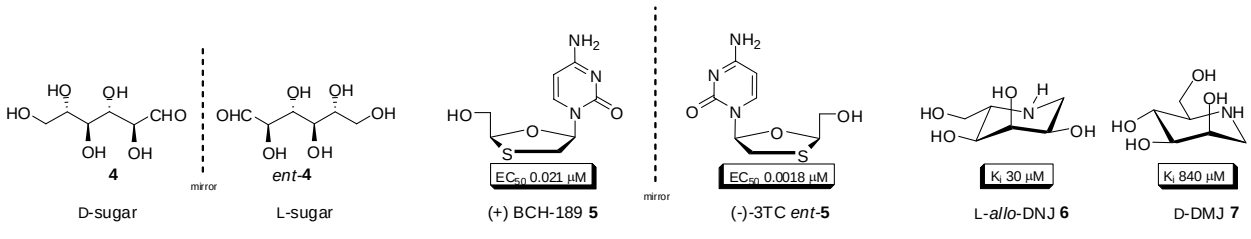


Figure 4 - *The relationships between molecular stereochemistry and biological function are not always predictable.*

Such a kind of data demonstrates fairly well that enantiospecificity, although important in many cases, could not be a universal trait related to biological function. To further probe this fundamental aspect, a number of research groups have engaged in the synthesis and evaluation of

mirror-image biopolymers. Proteins and polypeptides comprised solely of D-amino acids have been synthesized and shown to be intriguing compounds in mirror-image receptor binding, as these are likely to resist enzymatic degradation.[32] A similar line of thinking has been applied to nucleic acids: oligomers composed of 2-deoxy-L-ribofuranose based nucleotides have been synthesized and successfully assembled into mirror-image DNA duplexes.[33]

In this context, mirror-image carbohydrates may be useful for addressing the significance of molecular chirality in glycobiology, particularly in situations where the biomolecular interactions are not well defined.[34] In fact, the interference with carbohydrate biosynthetic pathways and therefore the alteration of glycoconjugate structures expressed on cell surfaces by means of unnatural metabolic substrates might drive to important insights into the understanding of their biological functions.[35] In principle, the use of mirror-image sugars can produce several outcomes on the cell surface (**Figure 5**). For example, oligosaccharide elaboration might divert away from endogenous scaffolds destined for the cell surface (**Figure 5/A**), resulting in a reduction in the amount of mature structures expressed by the cell. Alternatively, addition of unnatural substrates might produce an incorporation into cell surface glycoconjugates (**Figure 5/B**), resulting in the creation of an unnatural epitope that might display different receptor binding properties than its native counterpart.

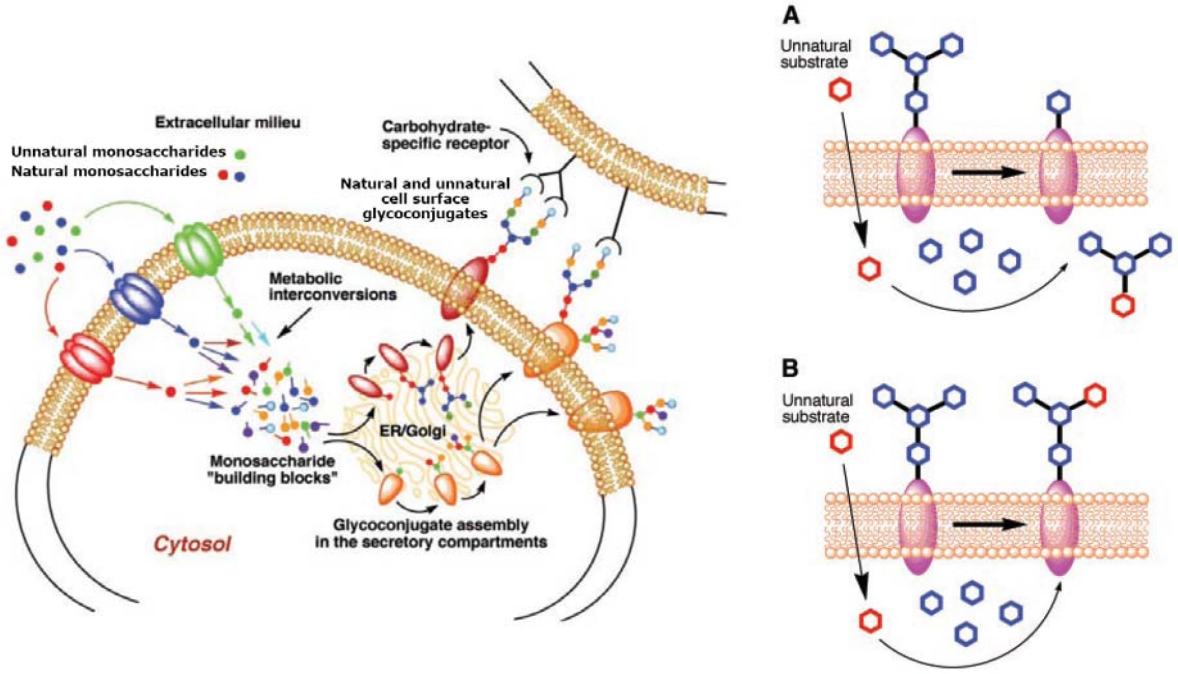


Figure 5 – (**Left**). *Glycoconjugate biosynthesis and cell surface recognition. Exogenously supplied monosaccharides are taken up by cells and converted to monosaccharide "building blocks" (typically nucleoside sugars) inside the cell. The building blocks are imported in the cytosol where they are assembled by glycosyltransferases into oligosaccharides bound to a protein (or lipid) scaffold. Once expressed in fully mature form on the cell surface, the glycoconjugates can serve as ligands for receptors on other cells or pathogens. Chemical tools can be used to inhibit or control any stage of this process.Particularly, the employ of unnatural sugars may be useful for achieving alterations in glycan biosynthetic pathways.* (**Right**). *As result of their introduction in cellular compartiments, unnatural sugars can divert oligosaccharide biosynthesis away from endogenous scaffolds, reducing the expression of specific carbohydrate structures (**A**); on the other hand, unnatural substrates can be used in biosynthetic pathways and incorporated into cell surface glycoconjugates (**B**).*

These findings suggest that synthesis of mirror-image oligosaccharides and glycoconjugates may present untapped opportunities for the discovery and development of compounds with potent biological activity. However, oligosaccharides and glycoconjugates based on mirror-image pyranosides have received so far a few attention. This is evidently due to the synthetic challenge and high cost of using L-pyranosides as synthetic precursors. Therefore, the search for new and efficient methodologies which can enable rapid access to L-carbohydrates for investigating the role of chirality in glycobiology represents a challenging issue.

1.5 *Concluding Remarks and Future Perspectives*

In the last years, advances in oligosaccharide and glycoprotein synthesis have yielded an abundance of relevant information for biological investigations. Access to oligosaccharides and

glycoconjugates in molecular detail has provided an essential contribution for unravelling their function. On this purpose, chemical tools have proven to be indispensable as well for studies in glycobiology. Synthetic oligosaccharides and glycoconjugates have supplied materials for correlating structure with function. Synthetic mimics of the complex constructions that are found on cell surfaces can modulate cellular interactions and are currently under development as therapeutic agents. Small molecule inhibitors of carbohydrate biosynthetic and processing enzymes can block the assembly of specific oligosaccharide structures. Inhibitors of carbohydrate recognition and biosynthesis can reveal the biological functions of the carbohydrate epitope and its related receptors. Carbohydrate biosynthetic pathways are often subjected to interception with synthetic unnatural substrates. Such metabolic interference can block the expression of oligosaccharides or alter the structures of the sugars present on cells. Collectively, these chemical approaches, together with explorations of biochemical pathways, are providing new methodologies for modulating the expression of oligosaccharide epitopes on cell surfaces, contributing to create an exciting platform from which to tackle problems in glycobiology.

1.6 *REFERENCES*

[1] Bertozzi, C. R.; Kiessling, L. L. Chemical glycobiology. *Science* **2001**, *291*, 2357.

[2] Laine, R. A. A calculation of all possible oligosaccharide isomers both branched and linear yields 1.05 x 10^{12} structures for a reducing hexasaccharide: the isomer barrier to development of single-method saccharide sequencing for synthesis systems. *Glycobiology* **1994**, *4*, 759.

[3] Varki, A. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* **1993**, *3*, 97.

[4] (a) Maeder, T Sweet medicines. *Scientific American* **2002**, 40. (b) Shriver, Z.; Raguram, S.; Sasisekharan, R. Glycomics: a pathway to a class of new and improved therapeutics. *Nat. Rev.* **2004**, *3*, 863. (c) P. H. Seeberger, Automated carbohydrate synthesis to drive chemical glycomics. *Chem. Commun.* **2003**, *10*, 1115.

[5] Dwek, R. A. Glycobiology: toward understanding the function of sugars. *Chem. Rev.* **1996**, *96*, 683.

[6] *Glycoscience*, B. O. Fraser-Reid, K. Tatsuta, J. Thiem, Eds., Springer: Berlin, **2001**.

[7] (a) Hirabayashi, J.; Kasai, K.-I. Glycomics, coming of age! *Trends Glycosci. Glycotechnol.* **2000**, *12*, 1. (b) Feizi, T.; Mulloy. B. Carbohydrates and glycoconjugates. Glycomics: the new era of carbohydrate biology. *Curr. Opin. Struc. Biol.* **2003**, *13*, 602.

[8] Sacchettini, J. C.; Baum, L. G.; Brewer, C. F. Multivalent protein-carbohydrate interactions. A new paradigm for supramolecular assembly and signal transduction. *Biochemistry* **2001**, *40*, 3009.

[9] Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. Selectin-carbohydrate interactions: from natural ligands to designed mimics. *Chem. Rev.* **1998**, *98,* 833.

[10] Geijtenbeck, T.; Torensama, R.; van Vliet, S.; van Duijnhoven, G.; Adema, G.; van Kooyk, Y.; Figdor. C. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune response. *Cell* **2000**, *100*, 575.

[11] Karlsson, K. A. Bacterium-host protein-carbohydrate interactions and pathogenicity. *Biochem. Soc. Trans.* **1999**, *27*, 471.

[12] Feizi, T. Glycobiology of AIDS. *Carbohydr. Chem. Biol.* **2000**, *4*, 851.

[13] Tiemeyer, M.; Goodman, C. S. Gliolectin is a novel carbohydrate-binding protein expressed by a subset of glia in the embryonic Drosophila nervous system. *Development* **1996**, *122*, 925.

[14] Liu, D.; Shriver, Z.; Venkataraman, G.; El Shabrawi, Y.; Sasisekharan, R. Tumor cell surface heparan sulfate as cryptic promoters or inhibitors of tumor growth and metastasis. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 568.

[15] (a) Smorenburg, S. M.; Van Noorden, C. J. The complex effects of heparin on cancer progression and metastasis in experimental studies. *Pharmacol. Rev.* **2001**, 53, 93. (b) Liu, D.; Shriver, Z.; Qi, Y.; Venkataraman, G.; Sasisekharan, R. Dynamic regulation of tumor growth and metastasis by heparan sulfate glycosaminoglycans. *Semin. Thromb. Hemost.* **2002**, *28*, 67.

[16] Shriver, Z.; Liu, D.; Sasisekharan, R. Emerging views of heparan sulfate glycosaminoglycan structure/activity relationships modulating dynamic biological functions. *Trends Cardiovasc. Med.* **2002**, *12*, 71.

[17] Crocker, P. R. SIGLECS: sialic-acid-binding immunoglobulinlike lectins in cell-cell interactions and signalling. *Curr. Opin. Struct. Biol.* **2002**, *12*, 609.

[18] Fu, X. Antibiotic optimization via *in vitro* glycorandomization. *Nat. Biotech.* **2003**, *21*, 1467.

[19] (a) Koeller, K. M.; Wong, C.-H. Emerging themes in medicinal glycoscience. *Nat. Biotech.* **2000**, *18*, 835. (b) Bencomo, V. V. *et al.* A synthetic conjugate polysaccharide vaccine against haemophilus influenzae type b. *Science* **2004**, *305*, 522.

[20] Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong. C.-H. Selectin-carbohydrate interactions: from natural ligands to designed mimics. *Chem. Rev.* **1998**, *98*, 833.

[21] Lillehund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. Recent developments of transition-state analogue glycosidase inhibitors of non-natural product origin. *Chem. Rev.* **2002**, *102*, 515.

[22] Bach, P.; Bols, M.; Damsgård, A.; Hansen, S. U.; Lohse, A. Introduction to glycomimetics, in *Glycoscience*, B. O. Fraser-Reid, K. Tatsuta, J. Thiem, Eds., Springer: Berlin, **2001**, Chapter 10.2, 2542.

[23] Butters, T. D.; Dwek, R. A.; Platt, F. M. Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorders. *Chem. Rev.* **2000**, *100*, 4683.

[24] Asano, N. Glycosidase inhibitors: update and perspectives on practical use. Glycobiology **2003**, *13*, 93R.

[25] Rudd, P. M.; Elliott, T.; Cresswell, P.; Wilson, I. A.; Dwek, R. A. Glycosylation and the immune system. *Science* **2001**, *291*, 2370.

[26] Kaila, N.; Thomas IV, B. E. Design and synthesis of Sialyl Lewis X mimics as E- and P-selectin inhibitors. *Med. Res. Rev.* **2002**, *22*, 566.

[27] For an example, see: Boulineau, F. P.; Wei, A. Mirror-image carbohydrates: synthesis of the unnatural enantiomer of a blood group trisaccharide. *J. Org. Chem.* **2004**, *69*, 3391.

[28] (a) Joyce, G. F.; Visser, G. M.; van Boeckel, C. A. A.; van Boom, J. H.; Orgel, L. E.; van Westrenen, J. Chiral selection in poly(C)-directed synthesis of oligo(G). *Nature* **1984**, *310*, 602. (b) Takats, Z.; Nanita, S. C.; Cooks, R. G. Serine octamer reactions: indicators of prebiotic relevance. *Angew. Chem., Int. Ed.* **2003**, *42*, 3521.

[29] Shallenberger, R. S.; Acree, T. E.; Lee, C. Y. Sweet taste of D- and L- sugars and amino acids and the steric nature of their chemo-receptor site. *Nature* **1969**, *221*, 555.

[30] Sabini, E.; Hazra, S.; Konrad, M.; Lavie, A. Nonenantioselectivity property of human deoxycytidine kinase explained by structures of the enzyme in complex with L- and D-nucleosides. *J. Med. Chem.* **2007**, *50*, 3004.

[31] Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. Biological Properties of D- and L-1-deoxyazasugars. *J. Med. Chem.* **2005**, *48*, 2036.

[32] (a) Milton, R. C. D.; Milton, S. C. F.; Kent, S. B. H. Total chemical synthesis of a D-enzyme: the enantiomers of HIV-1 protease show reciprocal chiral substrate specificity. *Science* **1992**, *256*, 1445. (b) Chalifour, R. J.; McLaughlin, R. W.; Lavoie, L.; Morissette, C.; Tremblay, N.; Boule, M.; Sarazin, P.; Stea, D.; Lacombe, D.; P., T.; Gervais, F. Stereoselective interactions of peptide inhibitors with the β-amyloid peptide. *J. Biol. Chem.* **2003**, *278*, 34874. (c) Schumacher, T. N. M.; Mayr, L. M.; Minor, D. L. J.; Milhollen, M. A.; Burgess, M. W.; Kim, P. S. identification of D-peptide ligands through mirror-image phage display. *Science* **1996**, *271*, 1854.

[33] (a) Urata, H.; Shinohara, K.; Ogura, E.; Ueda, Y.; Akagi, M. Mirror-image DNA. *J. Am. Chem. Soc.* **1991**, *113*, 8174. (b) Williams, K. P.; Liu, X.-H.; Schumacher,T. N. M.; Lin, H. Y.; Ausiello, D. A.; Kim, P. S.; Bartel, D. P. Bioactive and nuclease-resistant L-DNA ligand of vasopressin. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11285. (c) Negi, S.; Dhanasekaran, M.; Hirata, T.; Urata, H.; Sugiura, Y. Biomolecular mirror-image recognition: reciprocal chiral-specific DNA binding of synthetic enantiomers of zinc finger domain from gaga factor. *Chirality* **2006**, *18*, 254.

[34] (20) (a) Rojo, J.; Morales, J. C.; Penade´s, S. Host-Guest Chemistry, in *Topics in Currrent Chemistry*, Penade´s, S., Ed.; Vol. 218; Springer: Berlin, **2002**.

[35] Yarema, K. J.; Goon, S.; Bertozzi, C. R. Metabolic selection of glycosylation defects in human cells. *Nat. Biotech.* **2001**, *19*, 553.

Chapter 2

Rare Sugars: a Chemically Available Source of Building Blocks for the Synthesis of Bioactive Molecules

A Brief Introduction to L-Hexose Synthesis

2. RARE SUGARS: A CHEMICALLY AVAILABLE SOURCE OF BUILDING BLOCKS FOR THE SYNTHESIS OF BIOACTIVE MOLECULES

A BRIEF INTRODUCTION TO L-HEXOSE SYNTHESIS

Due to their importance as building blocks, synthetic targets, biological tools and drug candidates, an enormous body of investigations on carbohydrates has been carried out in the last decades. But while D-series sugars are often available in large amount from natural sources, their rare L-enantiomers result to be much less available; nonetheless they have been often recognized as key components of numerous bioactive molecules. Given their importance for such purposes, approaches to the synthesis of rare sugars have represented a challenging issue.

2.1 *INTRODUCTION*

Given their abundance in nature and their diverse roles in biological systems, carbohydrates represent attractive tools for many different purposes. Besides their significance as energy reservoirs in living cells, they are also important from a biological standpoint, owing to their involvement in cell-cell recognition processes and signal transduction.[1] Moreover, the use of carbohydrates as chiral building blocks[2] in natural product and drug synthesis[3] is an increasingly challenging area of contemporary synthetic organic chemistry.

However, while numerous common D-sugars are available in kilogram quantities from natural products and therefore constitute a convenient source of starting materials for organic synthesis, their L-enantiomers[4] are the minor nonetheless very remarkable components of bioactive compounds. In fact, although much less common in nature than natural D-counterparts, L-hexoses (especially in their pyranosidic form) are key constituents of numerous bioactive[5] oligosaccharides, antibiotics, glycopeptides and terpene glycosides, as well as steroid glycosides and other clinically useful agents.[6] Some significant examples are represented by L-gulopyranoside-containing compounds such as the antitumor drug Bleomycin A_2[7] and the nucleoside antibiotic Adenomycin[8] (**Figure 1**). Moreover, L-altrose has been found to be a typical component of the extracellular polysaccharides from *Butyrivibrio fibrisolvens* strain CF3.[9] On the other hand, L-mannose has been discovered in some steroid glycosides,[10] and its

phenol derivatives are potent substrates for measuring the α-L-mannosidase activity of commercial naringinase.[11]

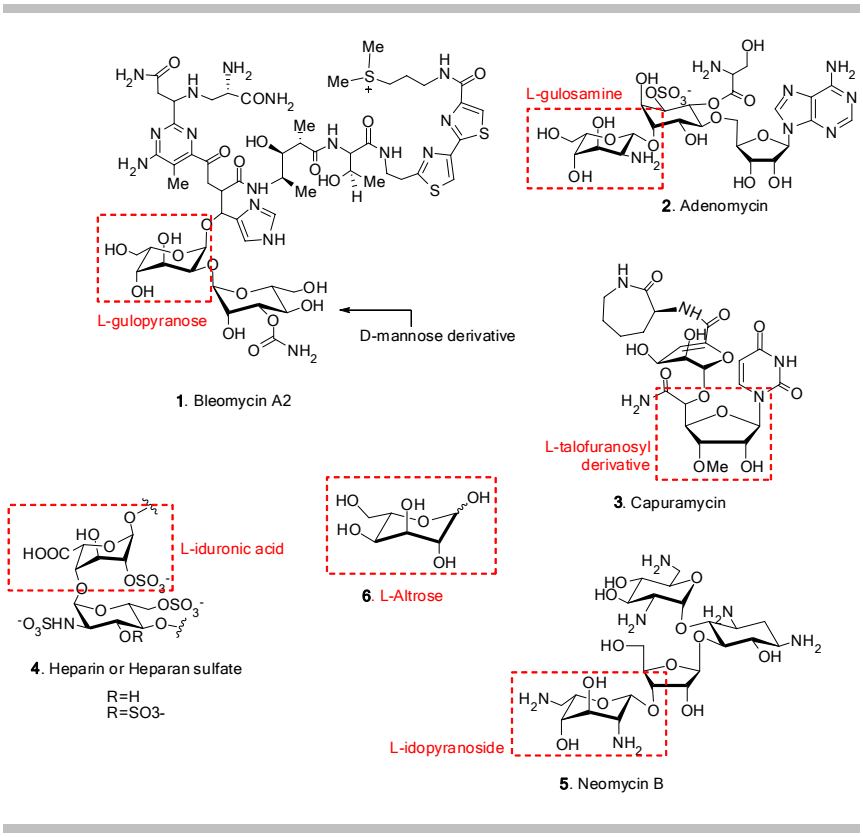


Figure 1 - *Biologically potent and rare L-hexoses and their related biomolecules*

With the heightened biological and medicinal interest in carbohydrates in recent years, many methodologies for their construction have been described.[12] In fact, not all L-hexoses are commercially available; this feature, together with the practical difficulties in obtaining these compounds from natural sources has led chemists to develop new and convenient methods for their production. However, in spite of the number of protocols reported, just a few of them can claim to have fulfilled a general synthetic procedure. The L-hexose strategies farther on described represent a few of the innumerable examples so far present in literature:[13] still they stand out just for their general character, besides their indeed scientific value.

2.2 *CARBON CHAIN ELONGATION: SOME SELECTED EXAMPLES OF L-HEXOSE SYNTHESIS*

a) One-carbon chain elongation: the DONDONI*'s thiazole-based approach*

The one-carbon chain elongation methodology by means of 2-(trimethylsilyl) thiazole (**Figure 2**, *upper side*), already successfully applied for many stereoselective syntheses,[14] has been also devoted to the preparation of the rare hexoses.[15] Through a linear iterative homologation procedure (consisting of two main operations, i.e. thiazole addition and thiazole to formyl unmasking) of chiral aldehydes (in this context, L-glyceraldehyde) it's possible to efficiently transform them into higher term aldehydes having one more carbon atom (**Figure 2**). Although the thiazole addition usually occurs with high stereoselectivity (favouring the formation of the *anti*-product), the strategy results to be general, considering that the configuration of each alcohol obtained can be inverted after a simple oxidation/reduction procedure. For instance, L-gulose has been obtained by *anti*-addition of 2-(trimethylsilyl) thiazole to aldehydo-L-xylose, followed by thiazole/formyl conversion. On the other hand, inversion of the addition product by an oxidation/reduction sequence followed by thiazole/formyl conversion furnishes L-idose (**Figure 2**, *red square*).[16]

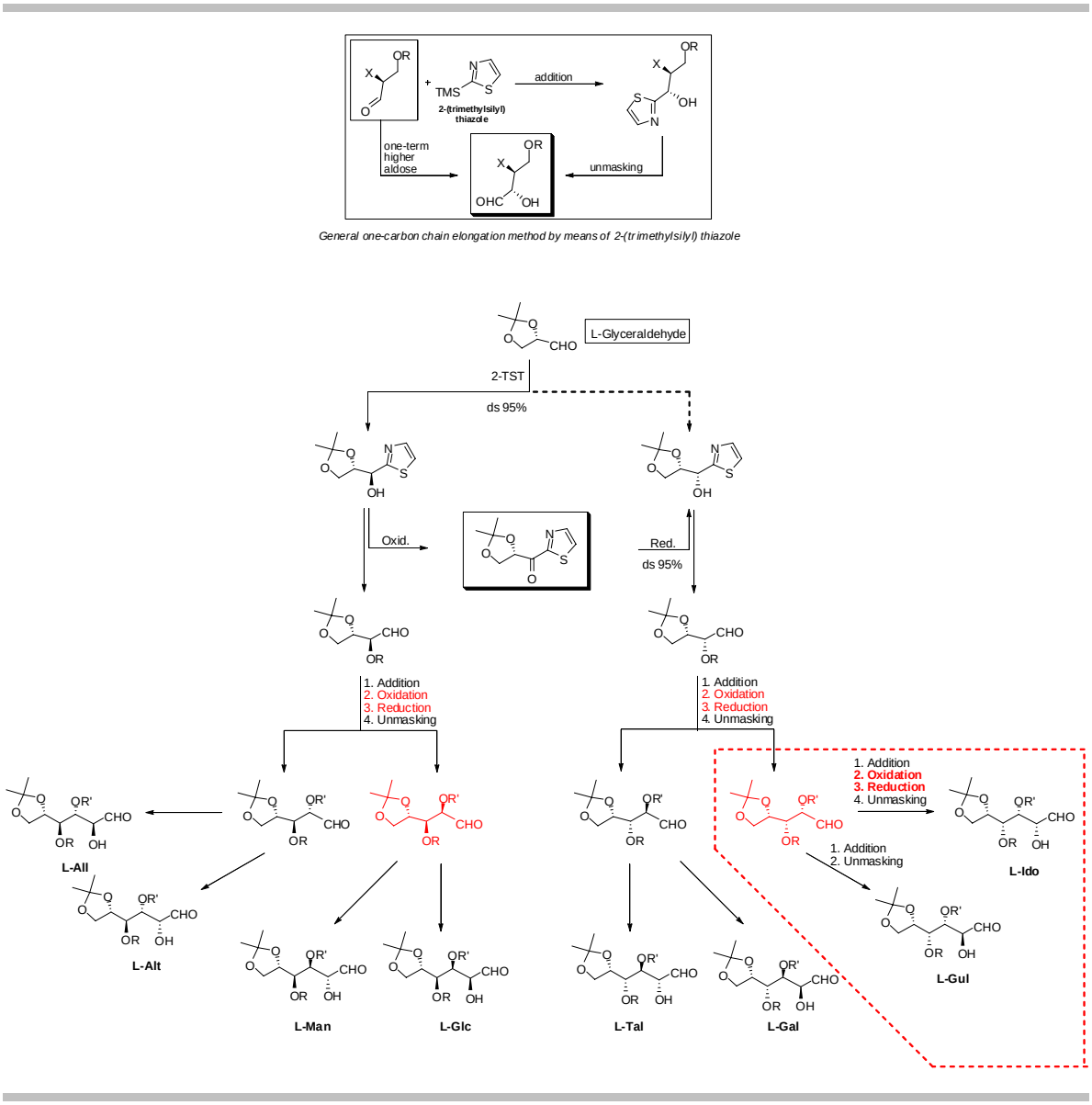


Figure 2 - *One-carbon chain elongation: the Dondoni's approach.*

b) Two-carbon chain elongation with asymmetric oxidations: the SHARPLESS *approach*

Masamune and Sharpless have provided a seminal and brilliant illustration of the potential of catalyst-induced double stereodifferentiation in their stereocontrolled synthesis of the L-hexoses.[17] The subsequently developed titanium tartrate epoxidation catalyst[18] has been firstly used to generate the key chiral starting material and subsequently for the diastereoselective elaboration of a late-stage intermediate. Ultimately, all the four contiguous stereocenters have been controlled independently, providing access to each of the eight hexose diastereomers by essentially the same route[19] (**Figure 3**).

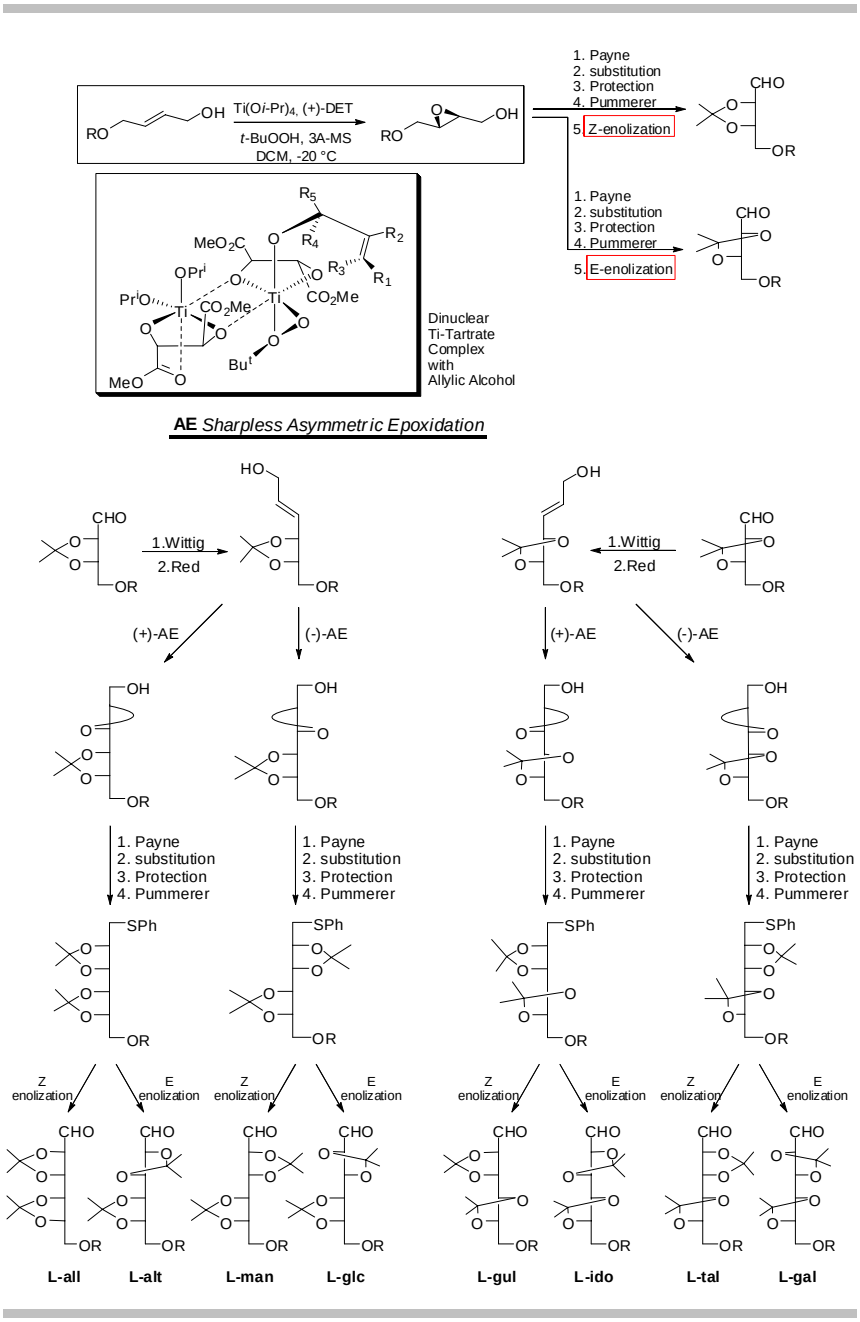


Figure 3 - *Two-carbon chain elongation: the Sharpless approach.*

2.3 *AN ALTERNATIVE STRATEGY FOR CARBOHYDRATE CONSTRUCTION: THE PROLINE-MEDIATED ENANTIOSELECTIVE ALDOL REACTIONS*

Recently, a completely different approach to enantiopure monosaccharide synthesis has emerged, based on the outstanding results obtained by the use of amino acids as enantioselective catalysts in several coupling reactions.[20] For example, enantiopure proline (and their derivatives) has been found to catalyze aldol reactions between non-chiral enolizable aldehydes

in a highly enantioselective way (**Figure 4**), mediating asymmetric *de novo* syntheses of L- as well as D-hexoses and their analogues with excellent overall stereoselectivity (in some cases, the new stereocenters have been assembled with almost absolute stereocontrol).

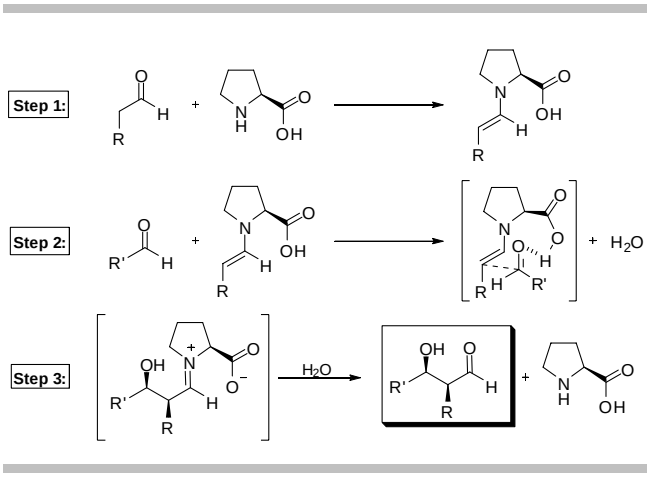


Figure 4 –*Proline-catalyzed enantioselective aldol reaction.*

From a conceptual standpoint, monosaccharide synthesis can be envisioned based on a two-step iterative aldol sequence using simple α-oxyaldehydes. In the first practical applications of this concept,[21] *O*-protected glycol aldehydes were converted, in the presence of (*S*)-proline, into the corresponding aldol products with high *ee* values (**Figure 5**, *left side*). The diastereoselective ratios varied in the range of 87:13-95:5 in favour of the *anti*-product. Interestingly, a further reaction of the aldol products with the starting material has not been observed. Hence, the aldol product was subsequently subjected to a second aldol reaction (with an other substrate): following this route, partially protected carbohydrates have been obtained from the reaction of L-tetrose with protected silyl enol ethers (**Figure 5**). At this stage, the stereochemical outcome of the reaction was under the control of the reaction conditions used. For instance, the employ of $MgBr_2$ in Et_2O led to glucose derivative, whereas the corresponding mannose derivative was formed in CH_2Cl_2. In the presence of $TiCl_4$, the corresponding allose was obtained.[22] Similar results could be obtained until affording the whole L-series.

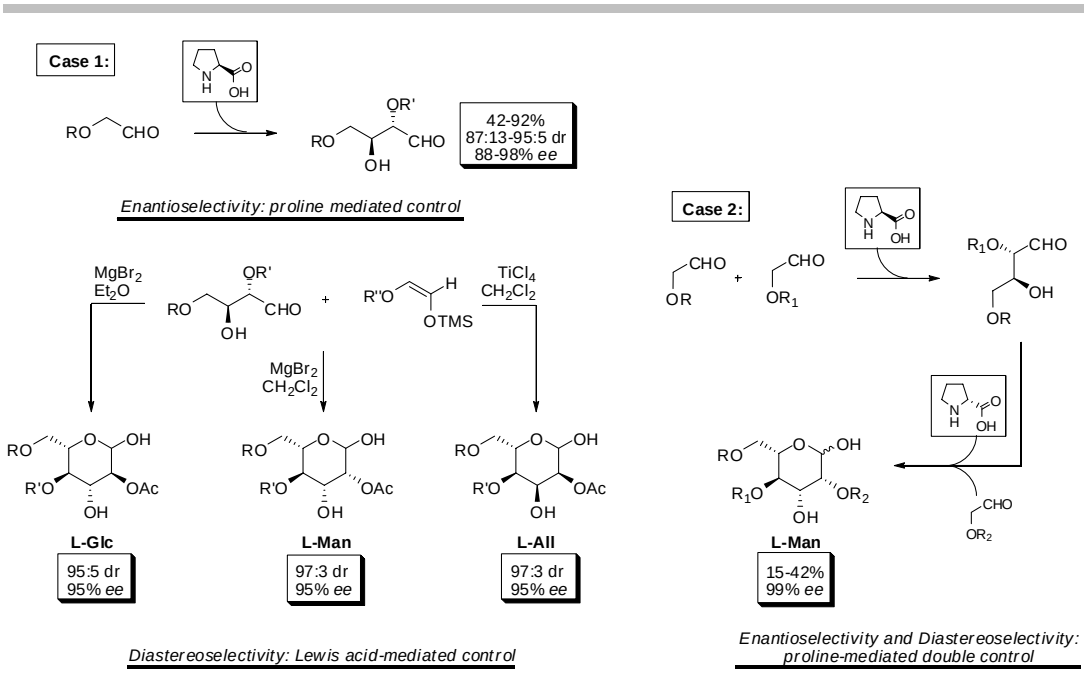


Figure 5 – Case 1: *Proline-catalyzed carbohydrate synthesis: AA-mediated enantioselectivity and Lewis acid-mediated diastereoselectivity (MacMillan and co-workers).* Case 2: *Proline-catalyzed carbohydrate synthesis: AA-mediated enantio and diastereocontrol (Cordova and co-workers).*

Very recently, such two-step concept has been realized under complete proline catalysis. The aldol product obtained in an (*S*)-proline-catalyzed reaction reacted in a second aldol reaction, now catalyzed by (*R*)-proline, to directly give a carbohydrate derivative (**Figure 5**, *right side, example with* L*-mannose*). Independently by the aldehydes used, the enantioselectivities were excellent (>99% *ee*), even if the overall yields were consistently low (from 15 to 42%). If the catalysts were used in the opposite sequence, the enantiomeric products were formed.[23] Incidentally, because of the inherent simplicity of this catalytic process, it has been suggested that a catalytic prebiotic "gluconeogenesis" may occur, in which amino acids transfer their stereochemical information to sugars.[24]

2.4 *FUTURE PERSPECTIVES: APPLICATION OF* DE NOVO *SYNTHETIC METHODS FOR THE ASSEMBLY OF BIOLOGICALLY RELEVANT OLIGOSACCHARIDES*

As further progress in monosaccharide synthesis, *de novo* protocols focused to the construction of carbohydrate-like building blocks have been employed for the synthesis of complex oligosaccharide sequences, often demonstrating to represent a better alternative in comparison to the traditional synthetic approaches beginning from natural monosaccharides. In fact, in the

conventional approaches up to five chemically similar hydroxyl groups need to be differentiated, requiring temporary protecting groups as well as selective masking of the anomeric hydroxyl group: this frequently results in a time consuming and expensive process. Conversely, *de novo* strategies are convergent and minimize the number of synthetic steps. As matter of fact, dissection of carbohydrate building blocks into linear fragments reduces the number of hydroxyl groups and avoids temporary protection of the anomeric hydroxyl. Moreover, it is possible to prepare several structurally related carbohydrate building blocks from one common linear precursor by varying the conditions for stereoselective construction of the carbon skeleton.

As proof of such findings, several noteworthy examples can be found in literature, demonstrating the feasibility of *de novo* strategies for oligosaccharide synthesis. One of the most explanatory examples has been recently reported[25] (**Figure 7**), in which the successful approach to the natural product Anthrax tetrasaccharide has been described by a highly stereocontrolled 25 step synthesis (longest linear, 39 total steps) with a 13% overall yield from achiral acetylfuran.

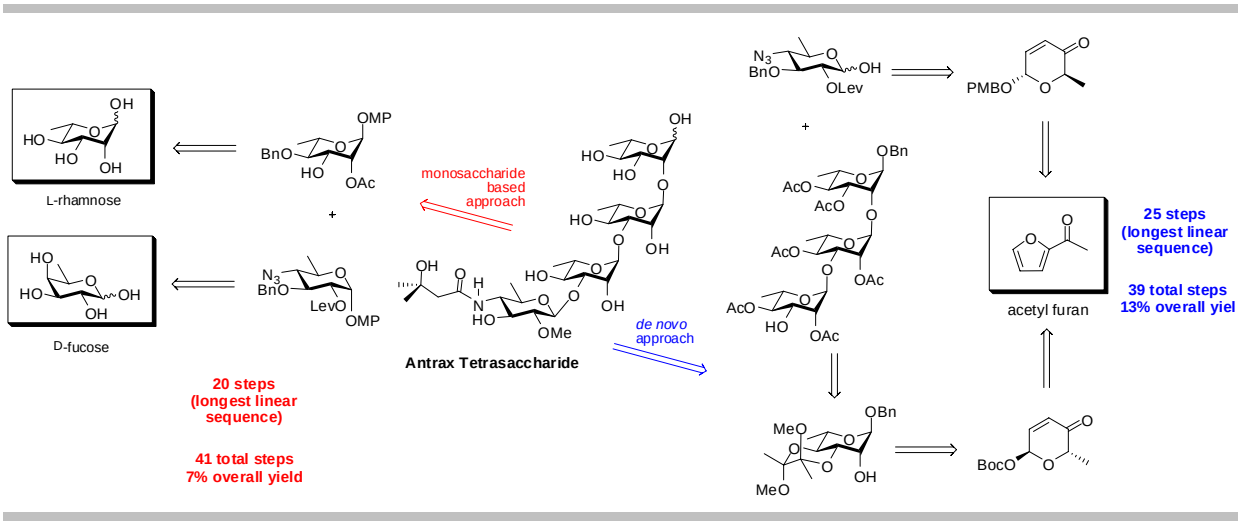


Figure 7 – *Monosaccharide-based and* de novo *approaches to the synthesis of Antrax tetrasaccharide.*

The comparison with a previous monosaccharide-based approach[26] (attained as from L-rhamnose and the rare D-fucose) demonstrates the practicability of this methodology: while the *de novo* synthesis is longer in terms of longest linear sequence (see text in **Figure 7**), it is shorter in terms of total steps.

Although the search for approaches to the synthesis of carbohydrates is now over one hundred years old,[27] the field of investigation is still dynamic, because of new technologies, new ideas

and the development of structured and general approaches. As shown above, since the degree of advancement reached in organic synthesis by *de novo* synthetic methods allows the preparation of almost any kind of polyfunctional systems with high stereoselectivity, high versatility, and sometimes in large amount, further progresses should be further addressed to the use of such knowledge in providing the suitable building blocks for the creation of more complex bioactive molecules, thus supporting and facilitating chemical biology and drug discovery endeavours.

2.5 *REFERENCES*

[1] Nicolaou, K. C.; Mitchell, H. J. Adventures in carbohydrate chemistry: new synthetic technologies, chemical synthesis, molecular design, and chemical biology. *Angew. Chem. Int. Ed.* **2001**, *40*, 1576.

[2] Bols, M. *Carbohydrate Building Blocks*; Wiley-VCH, Weinheim, **1996**.

[3] *Carbohydrate-based Drug Discovery* (Eds.: C. H. Wong), Wiley-VCH, Weinheim, **2003**.

[4] McNaught, A. D. Nomenclature of carbohydrates. *Pur. Appl. Chem.* **1996**, *68*, 1919.

[5] (a) Collins, P. M. *Dictionary of Carbohydrates*; Chapman and Hall: London, **1987**. (b) Lee, J.-C.; Chang, S.-W.; Liao, C.-C.; Chi, F.-C.; Chen, C.-S.; Wen, Y.-S.; Wang, C.-C.; Kulkarni, S. S.; Puranik, R.; Liu, Y.-H.; Hung, S.-C. From
D-Glucose to biologically potent L-hexose derivatives: synthesis of α-L-iduronidase fluorogenic detector and the disaccharide moieties of Bleomycin A_2 and heparan sulfate. *Chem. Eur. J.* **2004**, *10*, 399.

[6] (a) Lane, D. A.; Lindahl, U. *Heparin-Chemical and Biological Properties Clinical Applications*; Edward Arnold: London, 1989. (b) Lindahl, U. Heparan sulphate – a polyanion with multiple messages. *Pure Appl. Chem.* **1997**, *69*, 1897. (c) Iozzo, R. V. Matrix proteoglycans: from molecular design to cellular function. *Annu. Rev. Biochem.* **1998**, *67*, 609. (d) Rabenstein, D. L. *Nat. Prod. Rep.* **2002**, *19*, 312.

[7] (a) Burger, R. M. Cleavage of nucleic acid by Bleomycin. *Chem. Rev.* **1998**, *98*, 1153. (b) Boger, D. L.; Ramsey, T. M.; Cai, H.; Hoehn, S. T.; Stubbe, J. A systematic evaluation of the Bleomycin A_2 L-threonine side chain: its role in preorganization of a compact conformation implicated in sequence-selective DNA cleavage. *J. Am. Chem. Soc.* **1998**, *120*, 9139. (c) Katano, K.; An, H.; Aoyagi, Y.; Overhand, M.; Sucheck, S. J.; Stevens, W. C.; Hess, C. D.; Zhou, X.; Hecht, S. M. Total synthesis of Bleomycin group antibiotics. Total syntheses of Bleomycin Demethyl A_2, Bleomycin A_2, and decarbamoyl Bleomycin Demethyl A_2. *J. Am. Chem. Soc.* **1998**, *120*, 11285.

[8] Ogita, T.; Otake, N.; Miyazaki, Y.; Yonehara, H.; Macfarlane, R. D.; McNeal, C. J. The structure of Adenomycin ($C_{19\text{-}97}$ substance). *Tetrahedron Lett.* **1980**, *21*, 3203.

[9] Stack, R. J. Identification of L-altrose in the extracellular polysaccharide from *Butyrivibriofibrisolvens* strain CF3. *FEMS Microbiol. Lett.* **1987**, *48*, 83.

[10] Kubelka, W.; Kopp, B.; Jentsch, K. Zur biogenese von strophanthidin-glykosiden: convallatoxol als vorstufe von convallatoxin in *Convallaria Majalis*. *Phytochemistry* **1974**, *13*, 1805.

[11] Esaki, S.; Ohishi, A.; Katsumata, A.; Sugiyama, N.; Kamiya, S. Synthesis of α-L-mannopyranosyl-containing disaccharides and phenols as substrates for the α-L-mannosidase activity of commercial naringinase. *Biosci. Biotech. Biochem.* **1993**, *57*, 2099.

[12] Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. Modern methods of monosaccharide synthesis from non-carbohydrate sources. *Chem. Rev.* **1996**, *96*, 1195.

[13] For further selected examples concerning this issue, see: (a) Babu, R. S.; O'Doherty, G. A. Palladium-catalyzed glycosylation reaction: the *de novo* synthesis of natural and unnatural glycosides. *J. Am. Chem. Soc*. **2003**, *125*, 12406. (b) Ermolenko, L.; Sasaki, N. A. Diastereoselective synthesis of all eight L-hexoses from L-ascorbic acid. *J. Org. Chem.* **2006**, *71*, 693. (c) Takahashi, H.; Shida, T.; Hitomi, Y.; Iwai, Y.; Miyama, N.; Nishiyama, K.; Sawada, D.; Ikegami, S. Divergent synthesis of L-sugars and L-iminosugars from D-sugars. *Chem. Eur. J.* **2006**, *12*, 5868.

[14] Dondoni, A. Formylation of carbohydrates and the evolution of synthetic routes to artificial oligosaccharides and glycoconjugates. *Pure Appl. Chem.* **2000**, *72*, 1577.

[15] (a) Dondoni, A. New organometallic reagents. Use of 2-(trimethylsilyl) thiazole in acyclic stereoselective strategies. *Pure & Appl. Chem.* **1990**, *62*, 643. (b) Dondoni, A.; Marra, A. Thiazole-mediated synthetic methodology, *Chem. Rev.* **2004**, *104*, 2557.

[16] Dondoni, A.; Marra, A.; Massi, A. Carbohydrate homologation by the use of 2-(Trimethylsilyl) thiazole. Preparative scale synthesis of rare sugars: L-gulose, L-idose, and the disaccharide subunit of Bleomycin A_2. *J. Org. Chem.* **1997**, *62*, 6261.

[17] Ko, S. Y.; Lee, A. W. M.; Masamune, S.; Reed III, L. A.; Sharpless, K. B.; Walker, F.J. Total synthesis of L-hexoses. *Tetrahedron* **1990**, *46*, 245.

[18] (a) Johnson, R. A.; Sharpless, K. B. Addition reactions with formation of carbon-oxygen bonds: asymmetric methods of epoxidation, "*Comprehensive Organic Synthesis*" , Vol.7, Trost B. M. Ed.; Pergamon Press, **1991**. (b) Johnson, R. A.; Sharpless, K. B. *Catalytic asymmetric epoxidation,* Ojima I. Ed.; VCH Publishers, **1993**.

[19] Ko, S. Y.; Lee, A. W. M.; Masamune, S.; Reed III, L. A.; Sharpless, K. B. Total synthesis of L-hexoses. *Science* **1983**, *220*, 949.

[20] (a) Córdova, A. The direct catalytic asymmetric Mannich reaction. *Acc. Chem. Res.* **2004**, *37*, 102. (b) Kazmaier, U. Amino acids-valuable organocatalysts in carbohydrate synthesis. *Angew. Chem. Int. Ed.* **2005**, *44*, 2186.

[21] Mangion, I. K.; Northrup, A. B.; MacMillan, D. W. C. The importance of iminium geometry control in enamine catalysis: identification of a new catalyst architecture for aldehyde-aldehyde couplings. *Angew. Chem. Int. Ed.* **2004**, *43*, 6722.
[22] Northrup, A. B.; MacMillan, D. W. C. Two-step synthesis of carbohydrates by selective aldol reactions. *Science* **2004**, *305*, 1752.
[23] Córdova, A.; Ibrahem, I.; Casas, J.; Sundén, H.; Engqvist, M.; Reyes, E. Amino acid catalyzed neogenesis of carbohydrates: a plausible ancient transformation. *Chem. Eur. J.* **2005**, *11*, 4772.
[24] Córdova, A.; Engqvist, M.; Ibrahem, I.; Casas, J.; Sundèn, H. Plausibile origins of homochirality in the amino acid catalyzed neogenesis of carbohydrate. *Chem. Commun.* **2005**, 2047.
[25] Guo, H.; O'Doherty, G. A. *De Novo* Asymmetric synthesis of the anthrax tetrasaccharide by a palladium-catalyzed glycosylation reaction. *Angew. Chem. Int. Ed.* **2007**, *46*, 5206.
[26] Werz, D. B.; Seeberger, P. H. Total synthesis of antigen *Bacillus Anthracis* tetrasaccharide – creation of an Antrax vaccine candidate. *Angew. Chem. Int. Ed.* **2005**, *44*, 6315.
[27] Since the classical 1861's formose synthesis from formaldehyde: Boutlerow, M. A. *Compt. Rend.* **1861**, *53*, 145.

Chapter 3

THE HETEROCYCLE-FUNCTIONAL GROUP EQUIVALENCE AS A POWERFUL TOOL FOR THE SYNTHESIS OF ORGANIC MOLECULES

3. THE HETEROCYCLE-FUNCTIONAL GROUP EQUIVALENCE AS A POWERFUL TOOL FOR THE SYNTHESIS OF ENANTIOPURE ORGANIC MOLECULES

The synthesis of both natural and unnatural organic compounds in optically active form is a central challenge in chemistry, especially in relation to the study of biologically active compounds. A feature common to many such compounds is the presence of multiple stereocenters, giving rise to the possibility of large numbers of stereoisomers (2^n, where n is the number of stereocenters). An essential element for the synthesis of many complex targets of interest is represented by the control of the relationship between such stereocenters, whether they are already present in the starting materials, or they need to be further introduced. Meaningful applications of such a kind of stereocontrol in organic synthesis regard the achievement of enantiopure compounds containing a polyhydroxylated chain embedded in their structural framework, such as carbohydrates and related bioactive molecules, which synthesis always represents a topic of great interest. In fact, thanks to the continuous efforts in the search of new reagents for assembling molecular building blocks via efficient and stereoselective carbon-carbon bond-forming reactions, many elegant solutions have been developed in the last decades for the synthesis of structural domains containing contiguous oxygen-bearing stereocenters.

*In this context, among the numerous examples of homologating agents so far reported, the possibility of introducing an allylic alcohol residue on a chiral substrate, which has been demonstrated to have a great potential utility for synthetic purposes, is nowadays restricted only to a few examples. For this aim, the 3-C-lithiated (5,6-dihydro-1,4-dithiin-2-yl)[(4-methoxybenzyl) oxy]methane (**1**), which versatility has been already displayed in many examples, has been herein exploited for the synthesis of numerous enantiopure polyhydroxylated building blocks, which features and importance for the synthesis of bioactive compounds will be farther on discussed.*

3.1 *INTRODUCTION*

The second half of the last century has witnessed to an enormous progress in organic synthesis as a consequence of the introduction of innovative concepts and the development of new strategies

and technologies, culminating in the preparation of numerous natural and unnatural products of great complexity.[1] Formidable targets have been achieved owing to the continuous efforts in the search of new reagents and methods, particularly those allowing to assemble building blocks *via* chemically efficient and stereoselective carbon-carbon bond-forming reactions. At the present, research in this field is even more active, because of the interplay of organic chemistry and various disciplines of life science such as biology, pharmacology and medicine, that are posing a pressing demand for natural products and synthetic analogues in considerable amount and high purity.[2] Because of their utility for such purposes, a great deal of new reagents and catalysts have been constructed as the result of profitable studies in organometallic chemistry.[3] In addition to the fact that they have demonstrated to be effective synthetic auxiliaries, a peculiar importance is recognized to be due to the heterocycle-functional group equivalence, which is a powerful tool in numerous synthetic approaches[4] (**Figure 1**). With this aim, reviews and articles dealing with the use of readily available heterocycles have been reported, including 1,3-dithianes,[5] oxazolines,[6] oxazolinones,[7] 2-silyloxyfurans and thiophenes,[8] isoxazoles,[9] thiazoles[10] and many other examples.[11]

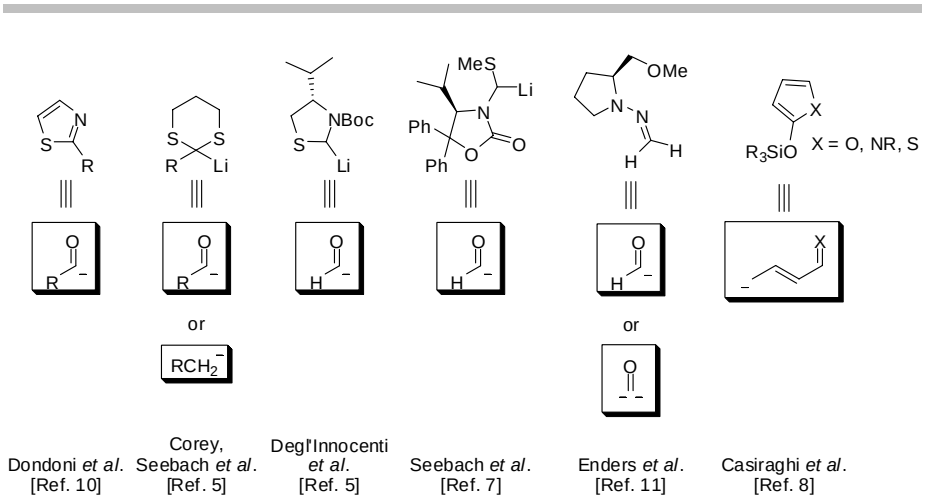


Figure 1 - *Readily available heterocycles employed as functional group equivalents.*

In such context, the discovery of the protected (5,6-dihydro-1,4-dithiin-2-yl)methanol (such as **1** or **2**, **Figure 2**), an allylic alcohol anion or acyl *β*-anion equivalent, as a reagent capable of three-carbon homologation[12] of organic molecules by introduction of a fully protected allylic alcohol at the new terminus, has represented a useful and versatile building block. As a result, it has been already successfully employed for the elongation of a number of different substrates, which has

brought to the synthesis of quite a few enantiopure compounds, among which 1,6-dioxaspiro[4.5]dec-3-ene[13] and 1,7-dioxaspiro[5,5]undec-4-ene[14] derivatives of sugars (**3**-**4**), 4-deoxy-hexoses[15] and 4-deoxy-pyranosyl nucleosides,[16] *C*-glycosides[17] and sLex mimetics (such as **5**, **6**, **7** and **8**, **Figure 2**). On the basis of these data, the three-carbon homologation of aldehyde-based chiral electrophiles 2,3-*O*-isopropylidene-L-glyceraldehyde (**9**) and 1,1-dimethylethyl-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (**10**), otherwise called Garner's aldehyde, has been herein envisaged. The control of the stereochemical concerns for such a kind of reactions has enabled the application of this methodology for starting an efficient and elegant approach to the synthesis of a broad number of enantiopure polyhydroxylated molecules, which potential as bioactive compounds will be discussed in the next chapters.

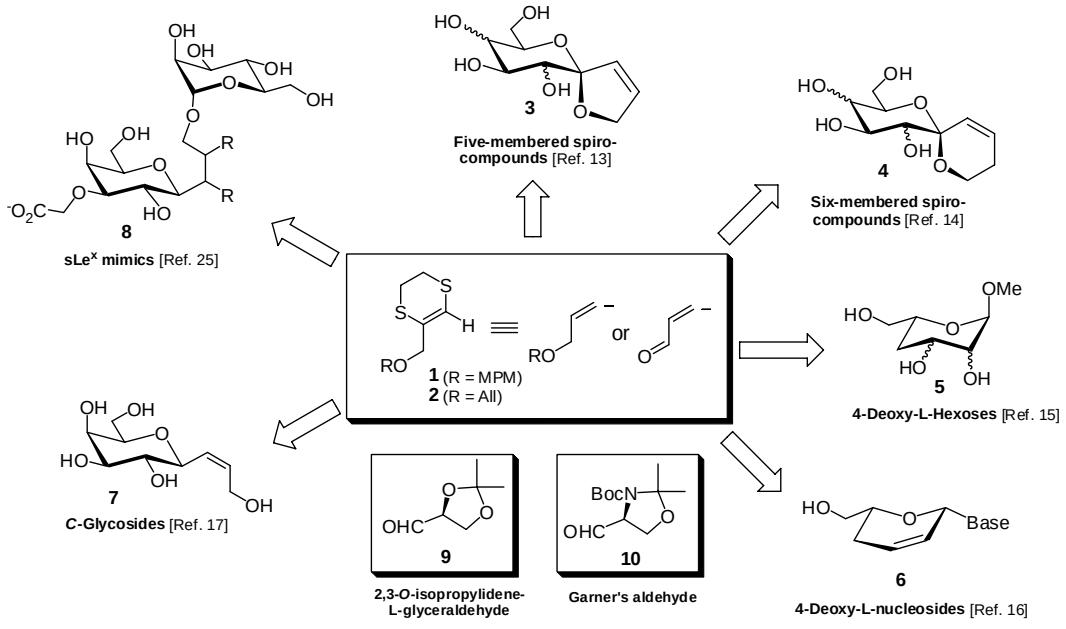
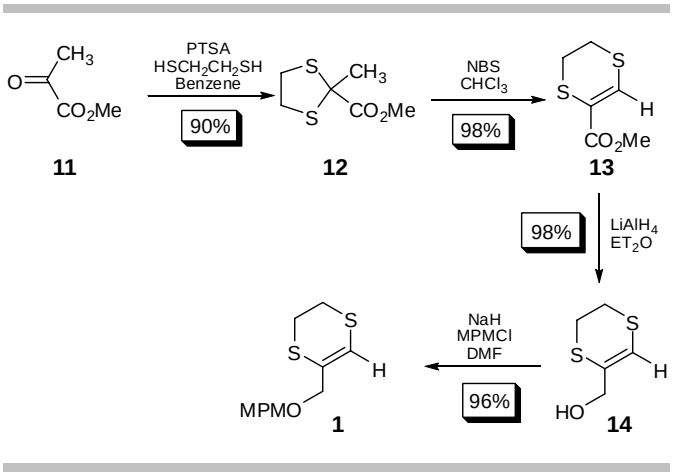


Figure 2 - *(5,6-dihydro-1,4-dithiin-2-yl)methanol as versatile homologating agent.*

The preparation of the 5,6-dihydro-1,4-dithiin ring is of straightforward access.[12] For example, the reagent **1** can be easily prepared in four steps from methyl pyruvate (83% overall yield) as follows: *thioacetalation* of methyl pyruvate (**11**) by means of ethandithiol ($HSCH_2CH_2SH$) and catalytic *p*-toluenesulfonic acid (PTSA) in refluxing benzene; *ring expansion*[18] of methyl pyruvate 1,3-dithiolane **12** by *N*-bromosuccinimide in anhydrous $CHCl_3$; *reduction* of the methyl 5,6-dihydro-1,4-dithiine-2-carboxylate (**13**) with lithium aluminum hydride in anhydrous Et_2O; *MPM protection*[19] of the alcohol **14** in anhydrous DMF using NaH and *p*-methoxybenzyl chloride (MPMCl) affording, after chromatographic purification, the pure title compound **1**[20]

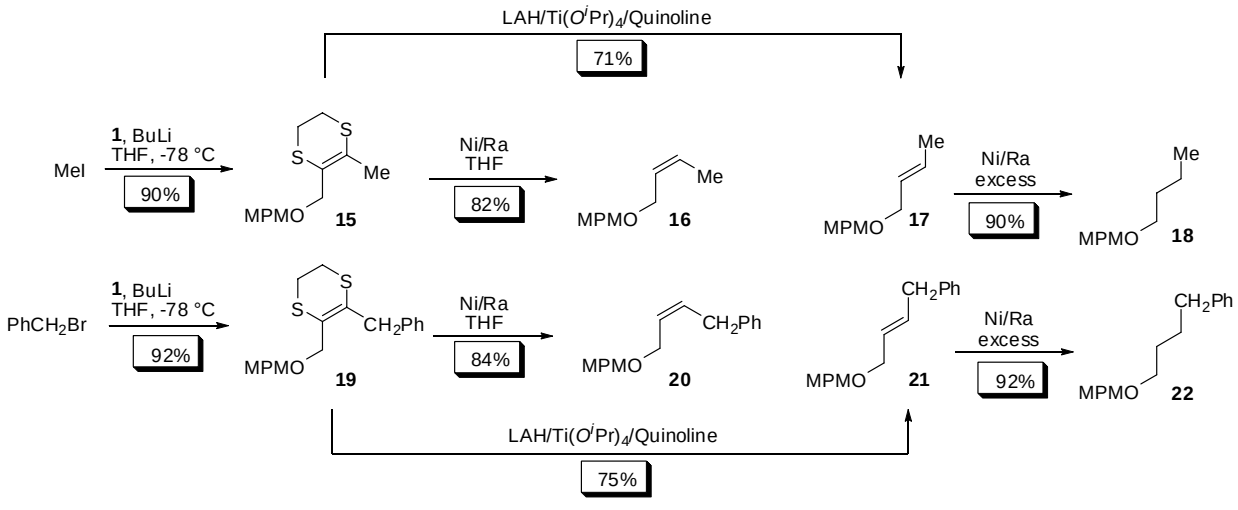
(**Scheme 1**). Morevover, once obtained **1** is stable and can be stored in the refrigerator for months.



Scheme 1 - *5,6-Dihydro-1,4-dithiin-2-yl[(4-methoxybenzyl)oxy] methane (**1**) synthesis from methyl pyruvate.*

3.2 *THREE-CARBON HOMOLOGATION*

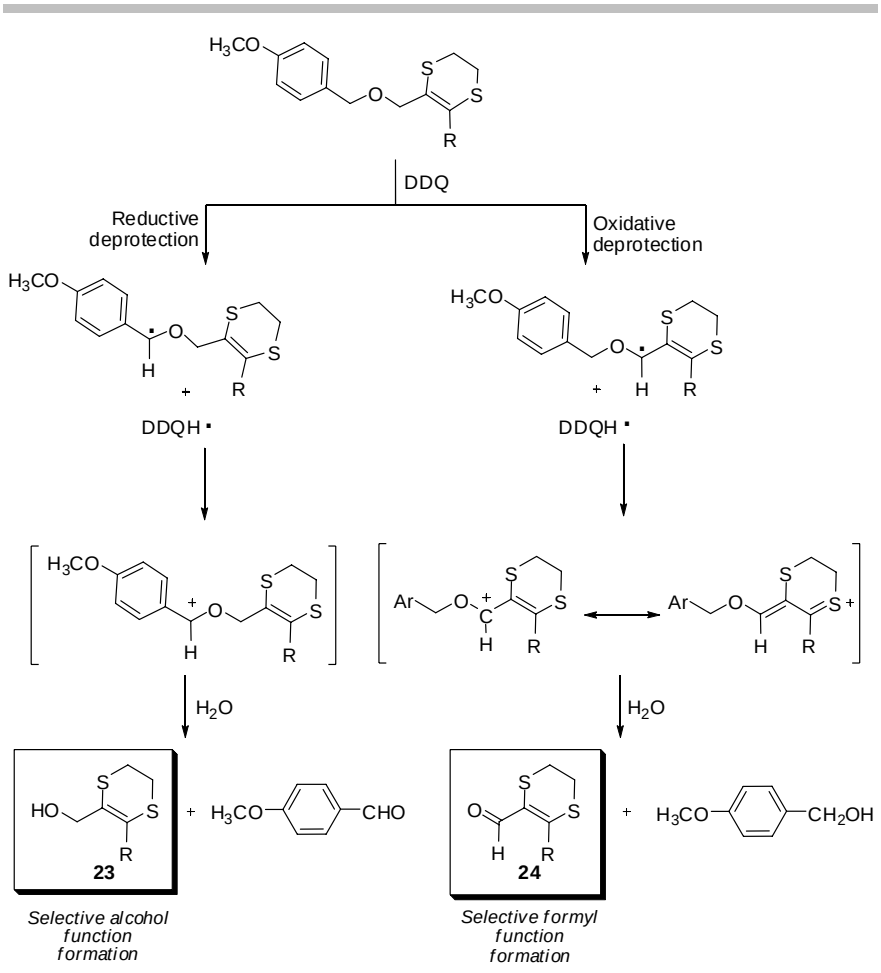
The corresponding 3-*C*-lithiated derivative of **1** is generated by treating this latter with *n*-butyllithium at -78 °C in anhydrous THF or Et_2O. It leads, by introduction of a three carbon moiety, to elongations of various electrophiles (such as in compounds **15** and **19**, derived from methyl iodide or benzyl bromide, respectively; **Scheme 2**) in good yields.



Scheme 2 – *Examples of three-carbon homologation from the 3-C-lithiated derivative of **1**.*

The synthetic relevance of these coupling reactions lies in the fact that the products can be stereoselectively desulfurized[21] either with Raney Nickel, affording a *cis*-configurated MPM propenyl ethers (like compounds **16** and **20**), or with lithium aluminium hydride/titanium tetraisopropoxide/quinoline,[22] yielding the *trans* isomers (**17** and **21**). Obviously, extended reaction times or use of reagent excess afford the over-reduction product (**18** and **22**, **Scheme 2**).

The major advantage of MPM hydroxyl protecting group is represented by being selectively removed also in the presence of other common protecting groups including benzyl ethers.[23] In particular, depending on the conditions used, the cleavage of the 4-methoxybenzyl ether function with 2,3-dihydro-5,6-dicyano-1,4-benzoquinone (DDQ) selectively affords (**Scheme 3**) the expected primary alcohol (such as **23**), or a formyl function[12,15] (like **24**). Both of them can be interesting starting materials for further chemical manipulations.



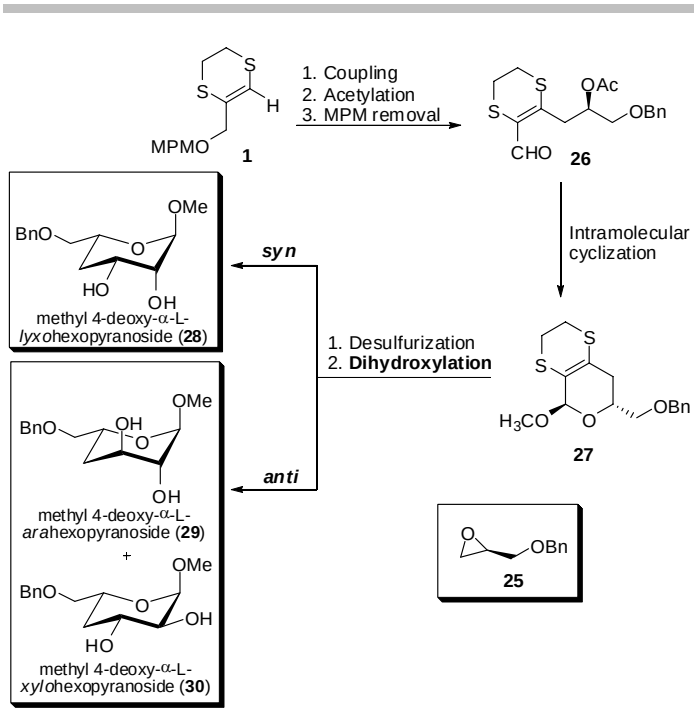
Scheme 3 – *DDQ mediated MPM group removal: conjectured mechanism for the selective achievement of a hydroxyl function or a formyl one.*

3.3 *HOMOLOGATION OF CHIRAL ELECTROPHILES*

The reaction of **1** with chiral electrophiles represents a powerful tool for designing new synthetic strategies in the area of biologically relevant polyhydroxylated products. To better illustrate the potential of this methodology for synthetic purposes, many diverse target molecules have been synthesized, depending on the substrates (such as epoxides, lactones, aldehydes) and on the conditions employed throughout the synthetic routes.

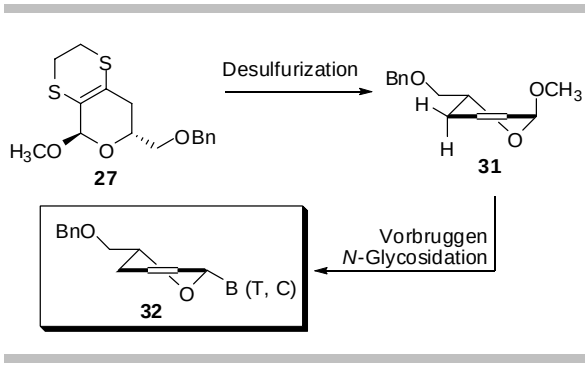
3.3.1 *HOMOLOGATION OF CHIRAL ELECTROPHILES: ADDITION TO EPOXIDES*

In a previous example of synthesis of enantiopure substrates for the preparation of potentially bioactive molecules,[15] (*R*)-benzyl glycidyl ether (**25**) reacted with **1**, providing 4-deoxy-L-hexoses (**Scheme 4**) and 4-deoxy-L-pyranosyl nucleosides[16] (**Scheme 5**). In both cases, six-membered ring closure by means of trimethylsilyl trifluoromethansulfonate (TfOTMS) and triethylamine (TEA) in methanol occurred *via* a *domino* reaction consisting of a rapid hydrolysis of the acetoxy group in intermediate **26** and an intramolecular transacetalation, to afford **27**. Then, in order to fulfil desired 4-deoxy-L-hexoses **28**-**30**, subsequent dithiodimethylene bridge removal and double bond dihydroxylation was attained (**Scheme 4**).



Scheme 4 - *Homologation of chiral epoxides: synthesis of 4-deoxy sugars.*

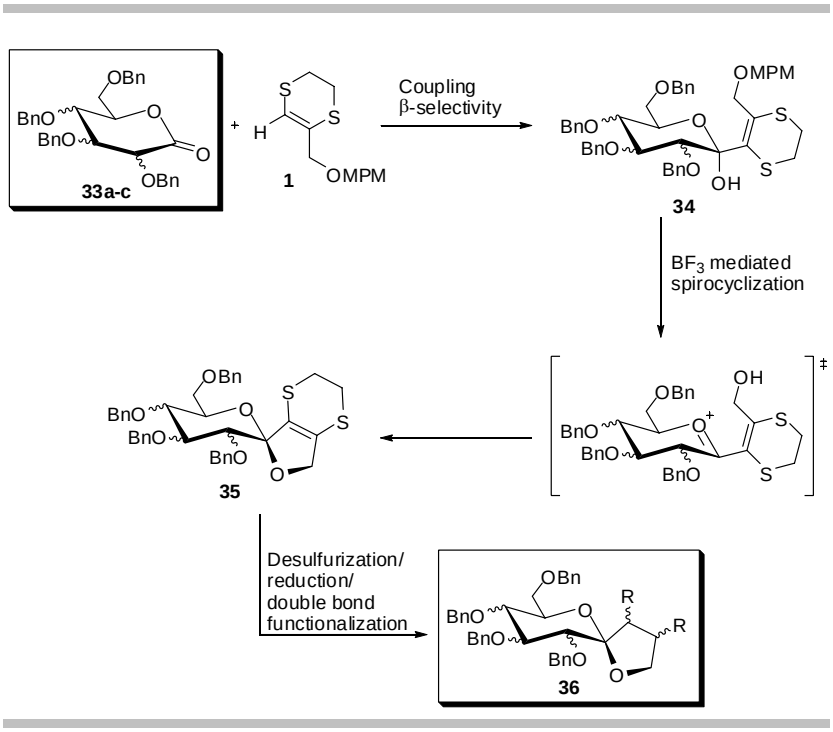
Otherwise, *N*-glycosylation reaction on the olefin **31** with silylated bases, under common Vorbrüggen conditions,[24] led to unnatural pyranosyl nucleosides (**Scheme 5**).



Scheme 5 – *Synthesis of unnatural pyranosyl nucleosides.*

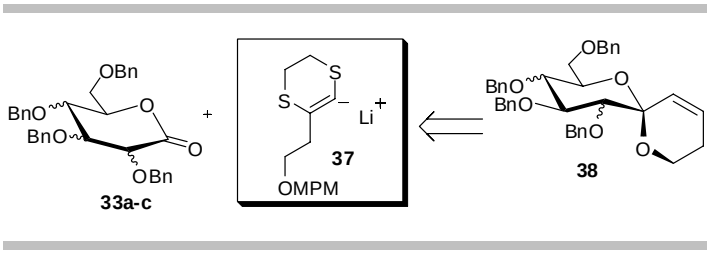
3.3.2 *HOMOLOGATION OF CHIRAL ELECTROPHILES: ADDITION TO LACTONES*

In the context of an ongoing program directed towards the achievement of new glycomimetic molecules,[13,14,17] the study of the coupling reaction between the 3-*C*-lithiated **1** with sugar-lactone derivatives **33a-c** (**Schemes 6-9**) has enabled to develop a practical methodology for *C*-glycoside synthesis. For such purposes, it has been noteworthy the finding according to which this coupling reaction is carried out by a selective attack on the *β*-face of the sugar ring, affording the sole thermodynamically more stable hemiacetal **34**. Following this path, intriguing spiro-compounds have been obtained by the coupling of **1** with protected glycono-1,5-lactones **33a-c** to give 1,6-dioxaspiro[4.5]dec-3-ene derivatives of sugars[13] (**Scheme 6**). Spirocyclization of **34** is accomplished using boron trifluoride etherate ($BF_3{\cdot}OEt_2$) as catalyst, through free hemiacetal hydroxyl group attack on the allylic position (**Scheme 6**). The double bond in the sulfur-free unsaturated spiroacetals **35** can be then easily reduced, or hydroxylated under stereocontrolled conditions, to afford more complex dioxaspiroacetal-based compounds **36**.



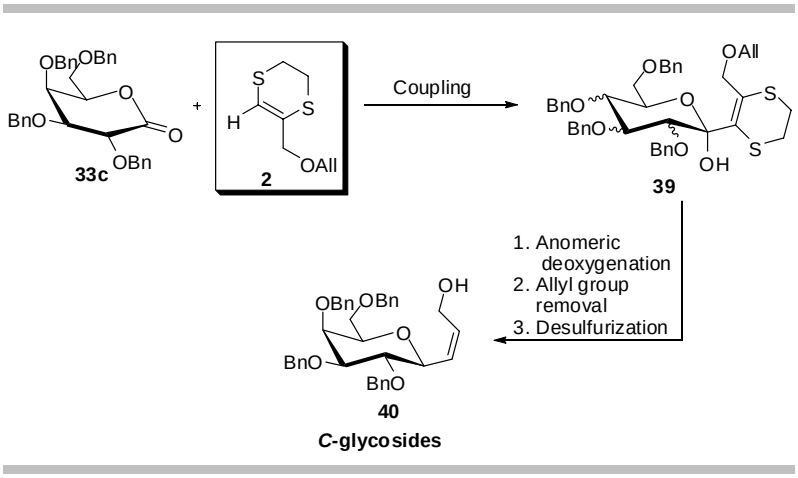
Scheme 6 – *Synthesis of 1,6-dioxaspiro[4.5]dec-3-ene derivatives of sugars.*

A similar procedure enables the synthesis of 1,7-dioxaspiro[5,5]undec-4-ene derivatives of sugars,[14] using a homologue of the heterocyclic system **1** (**37**, **Scheme 7**).



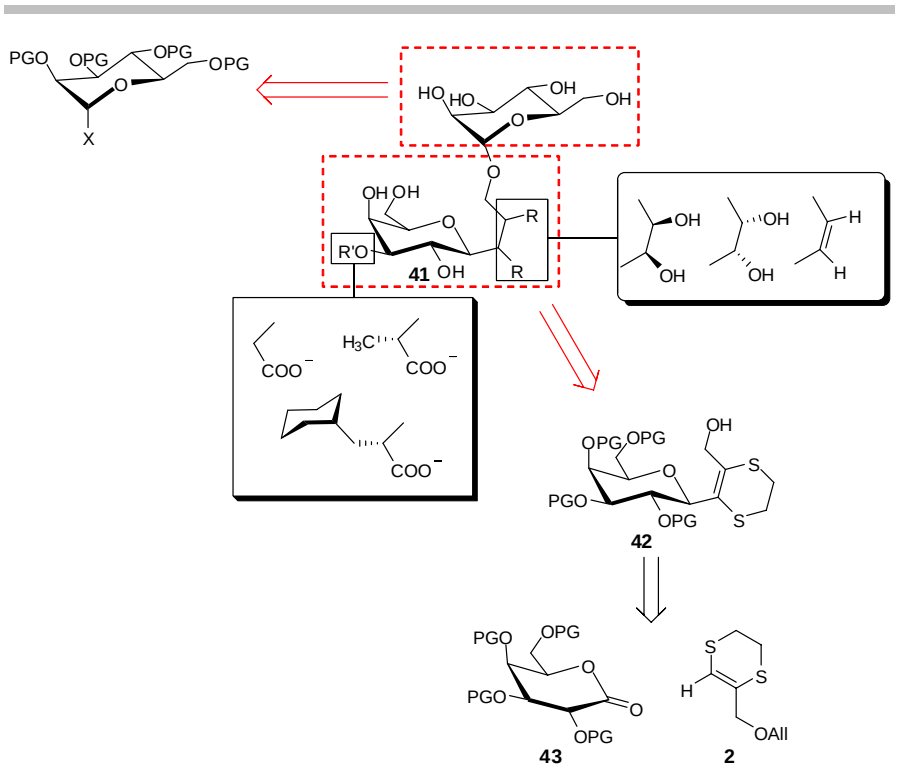
Scheme 7 - *1,7-dioxaspiro[5,5]undec-4-ene derivatives of sugars.*

On the other hand, the replacement of the MPM group with an allyl one (leading to **2**) allowed the formation of *C*-glycosidic skeleton, affording the coupling product **39** and, after a few steps, the *C*-glycoside[17] **40** in high yields and with full *β*-selectivity (**Scheme 8**).



Scheme 8 – *C-glycoside synthesis.*

The presence of a non-hydrolyzable *C*-glycosidic linkage in compound **40** has also set the basis for the preparation of a wide class of mimetics **41**, in the frame of a project focused to the achievement of a new family of enzyme-stable E- and P-Selectin inhibitors[25] (**Scheme 9**).

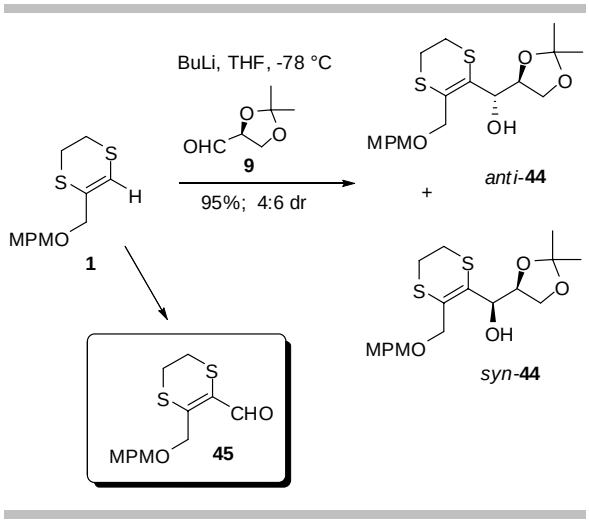


Scheme 9 – *Studies for the synthesis of Selectin inhibitors are in progress starting from C-glycoside* ***42****.*

3.3.3 *HOMOLOGATION OF CHIRAL ELECTROPHILES: ADDITION TO ALDEHYDES*

The study of three-carbon homologations of chiral aldehydes 2,3-*O*-isopropylidene-L-glyceraldehyde (**9**, **Scheme 10**) and Garner's aldehyde (**10**, **Scheme 14**) has represented one of the main subjects of this thesis. In particular, such a kind of homologations have been focused towards the construction of densely functionalized carbon chains with orthogonal protection at the functional groups, allowing selective transformations leading to products with a well-defined structure.

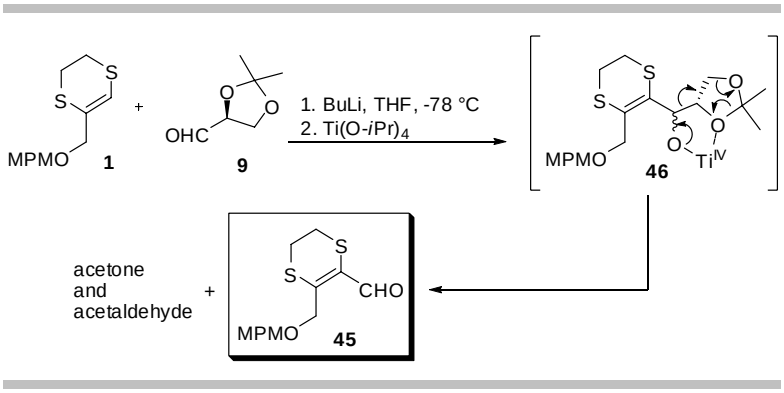
The coupling reaction of **1** with 2,3-*O*-isopropylidene-L-glyceraldehyde generates a diastereoisomeric mixture of secondary alcohols **44** (**Scheme 10**) with a slight preference for the *syn* compound (6:4 dr).*



Scheme 10. - *Three-Carbon Homologation with chiral electrophile **9**.*

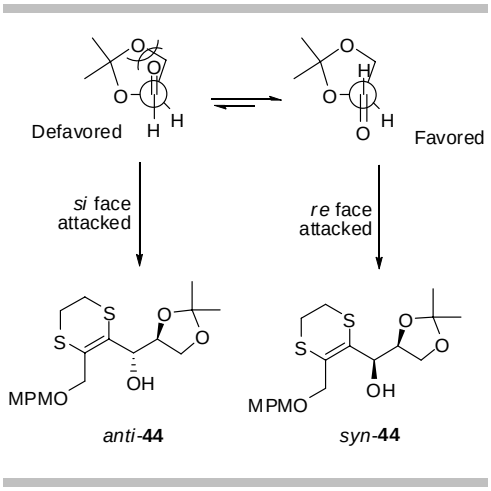
If the reaction was carried out through the use of a promoter such as titanium tetraisopropoxide (Ti(*O*-*i*-Pr)$_4$) the coupling products **44**, once formed, was readily changed into the unsaturated aldehyde **45**; it has been assumed that this reaction proceeds *via* the titanium complex **46** by a *pseudo*-retroaldolic reaction of the coupling product (**Scheme 11**).

* The *C*-4 absolute stereochemistry was clearly established in the course of our synthesis on the basis of the $^3J_{4,5}$ of the following cyclic compounds.



Scheme 11 – *Titanium-mediated synthesis of the unsaturated aldehyde **45**.*

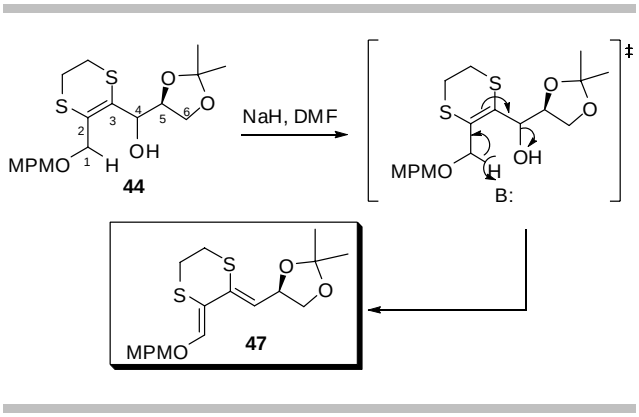
On the contrary, in the absence of catalysts this side-reaction proceeded much more slowly and the alcohols **44** were obtained in an excellent yield (95%). The slight preference for the *syn* compound is consistent with a non-chelation controlled reaction* according to Felkin-Anh model prediction (**Scheme 12**).



Scheme 12 - *Felkin-Ahn models for the aldehyde **9**.*

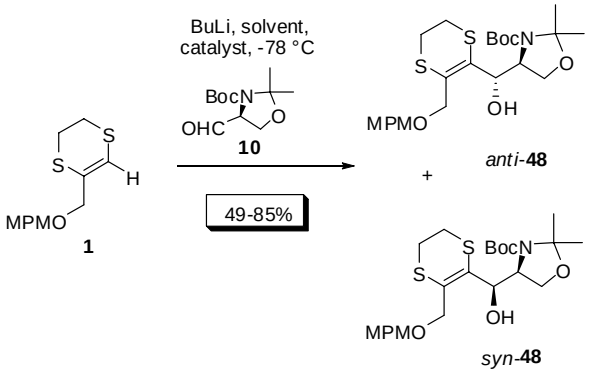
In the same context, the coupling products **44** displayed an unusual behaviour in the presence of basic conditions. For example, if the reaction was carried out by treatment with an excess of NaH, the formation of an unexpected byproduct **47** was observed. This latter could presumably be generated by an allylic *C*-1 proton abstraction with subsequent electronic shift, to give the thermodynamically stable diene (**Scheme 13**).

* As recently reported [Badorrey, R.; Cativiela, C.; Díaz-de-Villegas, M. D.; Díez, R.; Gálvez, J. A. *Eur. J. Org. Chem.* **2003**, 2268-2275] steric and stereoelectronic interactions between the chiral aldehyde **2** and the nucleophile across the two diastereotopic faces of carbonyl group do not play a significant role in determining the stereochemical outcome of the reaction, a situation that allows the nucleophilic attack on the more stable conformer leading to a slight preference for the *syn* compound.



Scheme 13 – *Undesired diene **47** formation from the coupling products **44**.*

Under similar conditions above reported about the coupling reaction of **1** with the protected L-glyceraldehyde **9**, the reaction between the *in situ* prepared *C*-3 lithiated carbanion of **1** and the Garner[26] aldehyde **10** afforded a *syn/anti* diastereomeric mixture of alcohols **48** (**Scheme 14**). As highlighted (table in **Scheme 14**), the best stereoselectivity was achieved by the use of Et_2O without catalyst, providing *anti*-**48** in a 91:9 dr. Interestingly, the stereochemical outcome of the reaction seemed to be mainly influenced by the nature of the solvent, whereas any significant induction was not observed in the presence of the catalysts.* In particular, this solvent-dependent stereoselective effect could be related to the nature of the organolithium intermediate: as already reported,[27] a "nude" and more reactive ionic couple prevails in THF, while a less reactive non-ionized species is formed in Et_2O, driving the reaction towards a better stereoselective outcome (**Scheme 14**).

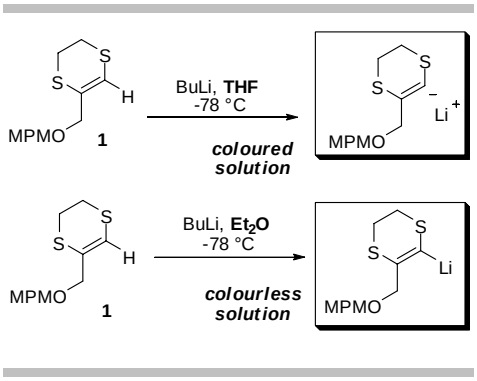


Solvent	*Catalyst (20%)*	*(anti/syn) dr*	*yield(%)*
THF	None	60:40	83
THF	$Ti(O\text{-}i\text{-}Pr)_4$	60:40	80
THF	Cp_2TiCl_2	60:40	85
Et_2O	Cp_2TiCl_2	70:30	49
Et_2O	$ZnBr_2$	82:18	73
Et_2O	None	91:9	72

Scheme 14. - *Three-carbon homologation with chiral electrophile **10**.*

* In all experiments the reported *syn*-stereoselection [Liang, X.; Andersch, J.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2136-2157] was never observed. Because of the chemical characteristics of compound **1**, such discrepancy could be rationalized assuming that in the reaction medium the catalyst does not complex Garner aldehyde, but it can be sequestrated by the negatively-charged heterocyclic system, without induction of stereoselectivity.

As double-check of this finding, an intensely colored solution occurred when **1** was treated with BuLi in THF; conversely, any colour change was not observed when the same experiment is carried out in Et_2O (**Scheme 15**).



Scheme 15 – *Treatment of **1** with Buli in THF or Et_2O.*

3.4 *FUTURE PERSPECTIVES*

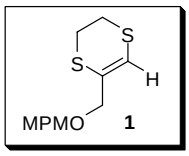
The synthetic examples based upon the use of the 5,6-dihydro-1,4-dithiin ring may serve to illustrate the potential of this approach in synthetic programs toward the construction of complex systems or special molecular fragments. In addition to the examples above illustrated about the stereoselective homologations of chiral electrophiles, this system has been also found to be a convenient mean in the preparation of dithiin-containing agrochemically interesting compounds,[28] as well as of synthetically useful chiral redox active ligands.[29] The type and variety of the classes of compounds which are accessible by this methodology demonstrate its wide scope and utility. This is mainly due to the easy entry to dithiin-based reagents and to their efficacy in carbon-carbon bond-forming reactions. However, one of the most significant aspects that emerge from the examples illustrated in this chapter is the fact that the dithiin ring is able to overcome potential compatibility problems with contiguous chemical systems. More specifically, the dithiin ring is able to withstand the most chemistry handled throughout the manipulation of substrates in which it has been introduced, but it can be very easily removed by a procedure which is orthogonal to most functional groups and does not affect existing stereocenters.

The inherent utility of the products derived from this synthetic approach carries the attendant expectation of further creative chemistry in this field. Considering the new results preliminarily obtained (see above) by the homologations of 2,3-*O*-isopropylidene-L-glyceraldehyde (**9**) and

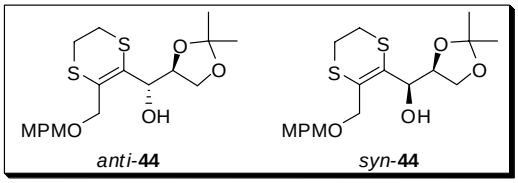
1,1-dimethylethyl-4-formyl-2,2-dimethyloxazolidine-3-carboxylate (**10**), significant attention will be given in this thesis on the possibility to exploit such findings for the construction of intriguing oxygen- and nitrogen-containing polyhydroxylated molecules, i.e. L-hexopyranosides and L-iminopyranosides, provided with orthogonal protection of the functional groups and therefore utilizable as such or as building blocks for further synthetic manipulations.

3.5 *EXPERIMENTAL SECTION*

All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Solvents were dried over standard drying agents and freshly distilled prior to use. Reactions were monitored by TLC (precoated silica gel plate F_{254}, Merck). Column chromatography: Merck Kieselgel 60 (70-230 mesh); flash chromatography: Merck Kieselgel 60 (230-400 mesh). Melting points are uncorrected and were determined with a capillary apparatus. Optical rotations were measured at 25±2 °C in the stated solvent. ^{1}H and ^{13}C NMR spectra were recorded on NMR spectrometers operating at 200, 300, 400 or 500 MHz and 50, 75, 100 or 125 MHz, respectively. Wherever necessary, two-dimensional H-H COSY experiments were carried out for complete signal assignments. Combustion analyses were performed using CHNS analyzer.



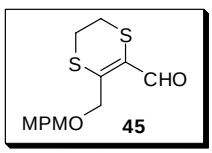
(5,6-Dihydro-1,4-dithiin-2-yl)[(4-methoxybenzyl)oxy]-methane (1). 4-Methoxybenzyl chloride (1.3 g, 8.0 mmol) dissolved in dry DMF (20 mL) was added dropwise to a solution of the pure **14** (1.0 g, 6.7 mmol) and NaH (0.2 g, 8.3 mmol) in the same solvent (20 mL) that had been kept under magnetic stirring and N_2 atmosphere for 30 min at room temperature. The stirring was continued for 15 h, and the reaction mixture was diluted with brine and extracted with Et_2O. The combined organic layers, after drying (Na_2SO_4) and evaporating under reduced pressure, gave a crude product which chromatography on silica gel (light petroleum-Et_2O, 9:1) and afforded the pure oily **1** (6.4 g, 96%). ^{1}H NMR (400 MHz): δ 3.13-3.23 (m, 4H), 3.83 (s, 3H), 4.00 (s, 2H), 4.43 (s, 2H), 6.20 (s, 1H), 6.88 (d, 2H, J = 8.5), 7.26 (d, 2H, J = 8.0). MS: m/z 268 (M^+). Anal. Calcd for $C_{13}H_{16}O_2S_2$: C, 58.18; H, 6.00. Found: C, 57.97; H, 6.08.



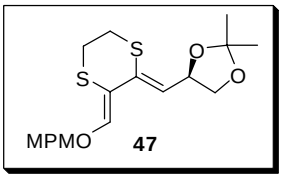
Compounds *syn*-44 and *anti*-44. BuLi (1.6 M in hexane, 2.82 mL) was added dropwise to a stirred solution of **1** (1.01 g, 3.76 mmol) in anhydrous THF (5 mL) at -78° C and under nitrogen atmosphere. After 10 min a solution of (*S*)-glyceraldehyde acetonide **9** (0.64 g, 4.89 mmol) in the same solvent (3 mL) was added. The reaction mixture was stirred for 30 min at -78° C, then carefully quenched with 10% aq NH_4Cl. The mixture was extracted with EtOAc, the combined organic phases washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. Flash chromatography of the crude residue over silica gel (hexane/Et_2O = 7:3) gave the two separated diastereomers *syn*-**44** and *anti*-**44** (1.42 g, 95% overall yield, 6:4 dr).

Data for *syn*-**44** (0.86 g, 57% yield): oily, $[\alpha]_D^{25}$ +11.6 (*c* 0.64, acetone). ^{1}H NMR (500 MHz): δ 1.38 (s, 3H), 1.44 (s, 3H), 1.60 (bs, 1H), 3.10-3.15 (m, 2H), 3.18-3.28 (m, 2H), 3.78 (dd, *J* = 8.6, *J* = 5.9 Hz, 1H), 3.80 (s, 3H), 3.97 (dd, *J* = 8.6, *J* = 6.5 Hz, 1H), 4.00 (d, *J* = 12.0 Hz, 1H), 4.15 (d, *J* = 12.0 Hz, 1H), 4.33 (ddd, *J* = 7.4, *J* = 6.5, *J* = 5.9 Hz, 1H), 4.46 (d, *J* = 11.4 Hz, 1H), 4.50 (d, *J* = 11.4 Hz, 1H), 4.56 (d, *J* = 7.4 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 2H), 7.28 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (100 MHz): δ 25.2, 26.6, 27.3, 29.7, 55.3, 66.6, 70.2, 71.6, 73.1, 76.5, 109.3, 113.9, 125.9, 129.4, 129.7, 130.2, 159.7. Anal. calcd for $C_{19}H_{26}O_5S_2$: C, 57.26; H, 6.58. Found: C, 57.40; H, 6.61.

Data for *anti*-**44** (0.56 g, 38% yield): oily, $[\alpha]_D^{25}$ -5.9 (*c* 0.49, $CHCl_3$). ^{1}H NMR (500 MHz): δ 1.35 (s, 3H), 1.38 (s, 3H), 3.06-3.18 (m, 2H), 3.20-3.23 (m, 2H), 3.80 (s, 3H), 3.90 (d, *J* = 11.7 Hz, 1H), 4.03 (dd, *J* = 8.8, *J* = 5.9 Hz, 1H), 4.06 (dd, *J* = 8.8, *J* = 5.8 Hz, 1H), 4.22 (ddd, *J* = 6.8, *J* = 5.9, *J* = 5.8 Hz, 1H), 4.29 (d, *J* = 11.7 Hz, 1H), 4.45 (d, *J* = 11.7 Hz, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.66 (d, *J* = 6.8 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 2H), 7.28 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (125 MHz): δ 25.2, 26.6, 27.3, 29.8, 55.3, 66.6, 70.2, 71.6, 72.1, 76.6, 109.3, 113.9, 126.0, 129.5, 129.7, 130.1, 159.5. Anal. calcd for $C_{19}H_{26}O_5S_2$: C, 57.26; H, 6.58. Found: C, 57.42; H, 6.56.

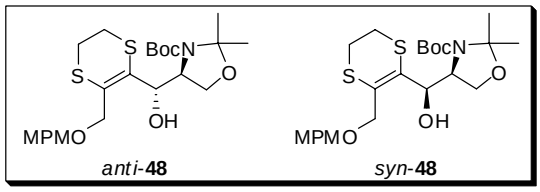


Compound 45. Under similar conditions reported above, treatment of **1** (1.01 g, 3.76 mmol) with BuLi (1.6 M in hexane, 2.82 mL) and **9** (0.64 g, 4.89 mmol) in the presence of a 20% amount of Ti(O-*i*-Pr)$_4$ led, after usual work-up and purification procedures, to the pure oil **45** (0.96 g, 86 % yield), besides traces of alcohols **44**. ^{1}H NMR (500 MHz): δ 3.10-3.18 (m, 2H), 3.28-3.32 (m, 2H), 3.81 (s, 3H), 4.41 (s, 2H), 4.52 (s, 2H), 6.89 (d, *J* = 8.3 Hz, 2H), 7.26 (d, *J* = 8.3 Hz, 2H), 9.82 (s, 1H). ^{13}C NMR (125 MHz): δ 25.5, 30.2, 55.3, 68.9, 72.2, 114.0, 129.0, 129.6, 148.8, 159.6, 183.5. Anal.calcd for $C_{14}H_{16}O_3S_2$: C, 56.73; H, 5.44. Found: C, 56.90; H, 5.42.



47

Compound 47. Treatment of *anti*- or *syn*-**44** (0.30 g, 0.75 mmol) with an excess of NaH (1.5 mmol) in anhydrous DMF (6 mL) afforded, after common work-up and purification procedures, the pure **47** (0.06 g, 20% yield): oily, $[\alpha]_D^{25}$ -61.8 (*c* 0.43, $CHCl_3$). ^{1}H NMR (500 MHz): δ 1.38 (s, 3H), 1.41 (s, 3H), 3.00-3.15 (m, 4H), 3.54 (t, *J* = 6.8 Hz, 1H), 3.81 (s, 3H), 4.11 (t, *J* = 6.8 Hz, 1H), 4.86-4.94 (m, 3H), 5.56 (d, *J* = 8.3 Hz, 1H), 6.64 (s, 1H), 6.90 (d, *J* = 8.3 Hz, 2H), 7.28 (d, *J* = 8.3 Hz). ^{13}C NMR (125 MHz): δ 25.8, 26.7, 29.7, 30.0, 55.3, 68.9, 73.1, 74.4, 108.7, 109.2, 114.0, 122.7, 128.6, 129.2, 135.7, 144.3, 159.7. Anal. calcd for $C_{19}H_{24}O_4S_2$: C, 59.97; H, 6.36. Found: C, 59.85; H, 6.34.



anti-**48** *syn*-**48**

Compounds *syn*-48 and *anti*-48. BuLi (1.6 M in hexane, 4.7 mL) was added dropwise to a stirred solution of **1** (1.7 g, 6.3 mmol) in anhydrous Et_2O (5 mL), at -78 °C and under argon atmosphere. After 10 min a solution of Garner aldehyde **10** (1.9 mL, 8.3 mmol) in the same solvent (11 mL) was added. The reaction mixture was stirred for 2 h at -78 °C and then quenched carefully with 10% aqueous NH_4Cl (10 mL). The mixture was extracted with EtOAc, the combined organic phases washed with brine, dried (Na_2SO_4) and the solvents evaporated under reduced pressure. Flash chromatography of the crude residue on silica gel (hexane/EtOAc = 8:2) gave the two separated diastereoisomers, *syn*-**48** and *anti*-**48** (9:91 dr) (2.2 g, 72% overall yield).

Data for *syn*-**48** (0.2 g): oily, $[\alpha]_D^{25}$ -44.2 (*c* 1.9, $CHCl_3$). ^1H NMR (500 MHz): δ 1.46 (s, 6H), 1.53 (s, 9H), 3.07-3.13 (m, 1H), 3.15-3.24 (m, 2H), 3.25-3.32 (m, 1H), 3.82 (s, 3H), 3.95 (m, 2H), 4.05 (d, *J* = 11.9 Hz, 1H), 4.20 (d, *J* = 11.9 Hz, 1H), 4.36-4.40 (m, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.53 (d, *J* = 11.6 Hz, 1H), 4.77-4.78 (bd, *J* = 8.9 Hz, 1H), 5.27 (bs, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 7.31 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR (50 MHz): δ 26.9 (2C), 27.0, 28.0 (3C), 29.0, 54.9, 58.5, 64.0, 69.4, 71.7, 72.2, 79.9, 94.0, 113.4 (2C), 125.0, 129.4 (2C), 129.5, 129.7, 158.9, 169.4. Anal. calcd for $C_{24}H_{35}NO_6S_2$: C 57.92, H 7.09, N 2.81. Found: C 57.75, H 7.12, N 2.82.

Data for *anti*-**48** (2.0 g): mp 112.5-113.6 °C (from hexane/Et_2O = 9:1), $[\alpha]_D^{25}$ + 46.1 (*c* 2.0, $CHCl_3$). ^1H NMR (500 MHz): δ 1.48 (s, 9H), 1.58 (s, 3H), 1.61 (s, 3H), 2.71 (bs, 1H, exchange with D_2O), 3.10-3.26 (m, 4H), 3.80 (s, 3H), 3.90 (dd, *J* = 9.3, *J* = 6.4 Hz, 1H), 4.05 (d, *J* = 12.2 Hz, 1H), 4.15 (bs, 1H), 4.37-4.44 (m, 2H), 4.48 (d, *J* = 11.4 Hz, 1H), 4.52 (d, *J* = 11.4 Hz, 1H), 4.96 (bs, 1H), 6.87 (d, *J* = 8.2 Hz, 2H), 7.30 (d, *J* = 8.2 Hz, 2H). ^{13}C NMR (50 MHz): δ 26.9 (2C), 27.7, 28.3 (3C), 29.1, 55.1, 60.9, 63.7, 69.5, 70.8, 71.6, 80.4, 93.8, 113.7 (2C), 125.0, 129.6 (2C), 129.8, 130.0, 152.7, 159.2. Anal. calcd for $C_{24}H_{35}NO_6S_2$: C 57.92, H 7.09, N 2.81. Found: C 57.82, H 7.11, N 2.82.

3.6 *REFERENCES*

[1] (a) Hanessian, S. *Total Synthesis of Natural Products. The Chiron Approach*; Pergamon Press: Oxford, **1983**. (b) Nicolaou, K. C.; Sorensen, E. J. *Classics in Total Synthesis*; VCH: Weinheim, **1996**. (c) Smit, W. A.; Bochkov, A. F.; Caple, R. *Organic Synthesis. The Science behind the Art*; The Royal Society of Chemistry: Cambridge, **1998**.

[2] Dwek, R. A. Glycobiology: toward understanding the function of sugars. *Chem. Rev.* **1996**, *96*, 683.

[3] Metz, P. *Topics in Current Chemistry*; Springer-Verlag: Berlin Heidelberg, **2001**.

[4] Lin, G.-Q.; Li, Y.-M.; Chan, A. S. C. *Principles and Applications of Asymmetric Synthesis*; John Wiley & Sons, Inc.: New York, **2001**.

[5] (a) Corey, E. J.; Seebach, D. Carbanionen der 1,3-dithiane, reagentien zur *C*-*C*-verknüpfung durch nucleophile substitution oder carbonyl-addition. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 1075. (b) Corey, E. J.; Seebach, D. Synthese von 1,n-Dicarbonylverbindungen mit Carbanionen der 1,3-Dithiane. *Angew. Chem., Int. Ed. Engl.* **1965**, *4*, 1077. (c) Yus, M.; Najera, C.; Foubelo, F. The role of 1,3-dithianes in natural product synthesis. *Tetrahedron* **2003**, *59*, 9255. (d) Degl'Innocenti, A.; Pollicino, S.; Capperucci, A. Synthesis and stereoselective functionalization of silylated heterocycles as a new class of formyl anion equivalents. *Chem. Commun.* **2006**, 4881.

[6] Cardillo, G.; Gentilucci, L.; Tolomelli, A. Aziridines and oxazolines: valuable intermediates in the synthesis of unusual amino acids. *Aldrichim. Acta* **2003**, *36*, 39.

[7] Gaul, C.; Seebach, D. A valine-derived lithiated 3-methylthiomethyl-1,3-oxazolidin-2-one for enantioselective necleophilic hydroxymethylation, formylation and alkoxycarbonylation of aldehydes. *Org. Lett.* **2000**, *2*, 1501.

[8] Rassu, G.; Zanardi, F.; Battistini, L.; Casiraghi, G. The synthetic utility of furan-, pyrrole- and thiophene-based 2-silyloxy dienes. *Chem. Soc. Rev.* **2000**, *29*, 109.

[9] Baraldi, P. G.; Barco, A.; Benetti, S.; Pollini, G. P. Simoni, D. Synthesis of natural products via isoxazoles. *Synthesis* **1987**, 857.

[10] Dondoni, A.; Marra, A. Thiazole-mediated synthetic methodology. *Chem. Rev.* **2004,** *104*, 2557.

[11] (a) Lipshutz, B. H. Five-membered heteroaromatic rings as intermediates in organic synthesis. *Chem. Rev.* **1986**, *86*, 795. (b) Kirschning, A.; Kujat, C.; Luiken, S.; Schaumann, E. Small and versatile – formyl anion and dianion equivalents. *Eur. J. Org. Chem.* **2007**, 2387.

[12] Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S. A new and versatile allylic alcohol anion and acyl β-anion equivalent for three-carbon homologations. *J. Org. Chem.* **1997**, *62*, 9369.

[13] Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S.; Solla, F. Mild stereoselective synthesis of fully protected 1,6-dioxaspiro[4.5]dec-3-ene derivatives of sugars. *Eur. J. Org. Chem.* **2002**, 534.

[14] Caputo, R.; Ciriello, U.; Festa, P.; Guaragna, A.; Palumbo, G.; Pedatella, S. Stereoselective synthesis of fully protected (*S*)-1,7-dioxaspiro[5,5]undec-4-ene derivatives of sugars. *Eur. J. Org. Chem.* **2003**, 2617.

[15] (a) Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S.; Solla, F. A new approach to the synthesis of enantiomerically pure 4-deoxy sugars. *J. Carbohydr. Chem.* **2000**, *19*, 631. (b) Caputo, R.; De Nisco, M.; Festa, P.; Guaragna, A.; Palumbo, G.; Pedatella, S. Synthesis of 4-deoxy-L-(and D-) hexoses from chiral noncarbohydrate building blocks. *J. Org. Chem.* **2004**, *69*, 7033.

[16] Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S. A versatile synthesis of enantiomerically pure D- and L-pyranosyl nucleoside analogues. *Nucleos. Nucleot.* **1999**, *18*, 651.

[17] Caputo, R.; Festa, P.; Guaragna, A.; Palumbo, G.; Pedatella, S. A novel approach to the stereocontrolled synthesis of *C*-vinyl β-D-galactopyranosides. *Carbohydr. Res.* **2003**, *338*, 1877.

[18] Caputo, R.; Ferreri, C.; Palumbo, G. The reaction of ethanediyl *S*,*S*-acetals with halogens. *Tetrahedron* **1986**, *42*, 2369.

[19] Takaku, H.; Kamaike, K.; Tsuchiya, H. Oligonucleotide synthesis. Part 21. Synthesis of ribooligonucleotides using the 4-methoxybenzyl group as a new protecting group for the 2'-hydroxyl group. *J. Org. Chem.* **1984**, *49*, 51.

[20] Guaragna, A.; Pedatella, S.; Palumbo, G. 5,6-Dihydro-1,4-dithiin-2-yl[(4-methoxybenzyl)oxy]methane. *e-Enciclopedia of Reagents for Organic Synthesis* **2008**, in press.

[21] Becker, S.; Fort, Y.; Caubère, P. New desulfurizations by nickel-containing complex reducing agents. *J. Org. Chem.* **1990**, *55*, 6194.

[22] Caputo, R.; Palumbo, G.; Pedatella, S. Diastereoselective desulfurization of 5,6-dihydro-1,4-dithiins. Synthesis of muscalure from *Musca domestica L*. *Tetrahedron* **1994**, *50*, 7265 and literature therein cited.

[23] Horita, K.; Yoshioka, T.; Tanaka, T.; Oikawa, Y.; Yonemitsu, O. On the selectivity of deprotection of benzyl, MPM (4-methoxybenzyl) and DMPM (3,4-dimethoxybenzyl) protecting groups for hydroxy functions. *Tetrahedron* **1986**, *42*, 3021.

[24] (a) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. Nucleoside synthesis with trimethylsylyl triflate and perchlorate as catalyst. *Chem. Ber.* **1981**, *114*, 1234. (b) Vorbrüggen H.; Bennua, B. A new simplified nucleoside synthesis. *Chem. Ber* **1981**, *114*, 1279.

[25] Pedatella, S.; D'Alonzo, D.; Guaragna, A.; De Nisco, M.; Palumbo, G. *manuscript in preparation*.

[26] Garner, P.; Park, J. M. 1,1,-dimethylethyl (*S*)- or (*R*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate: a useful serinal derivative. *Org. Synth. Vol. IX* **1998**, 300.

[27] (a) Seyferth, D.; King, R. B. *Annual Surveys of Organometallic Chemistry Vol. 1-3*; Elsevier Publishing Co.: Amsterdam, 1965-1967. (b) Maercker, A.; Roberts, J. D. Small-ring compounds. XLVI. Stabilized cyclopropylcarbinyl anions. A retro cyclopropylcarbinyl-allylcarbinyl rearrangement. *J. Am Chem. Soc.* **1966**, *88*, 1742.

[28] Caputo, R.; Ferreri, C.; Guaragna, A.; Palumbo, G. Pedatella, S. New synthesis of carboxin and oxocarboxin pesticides: application to the preparation of their new analogues substituted at the C-2 methyl group. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1971.

[29] Rèthorè, C.; Suisse, I.; Agbossou-Niedercorn, F.; Guillamòn, E.; Llusar, R.; Fourmiguè, M.; Avarvari, N. Chiral tetrathiafulvalene based phosphine- and thiomethyloxazoline ligands. Evaluation in palladium catalysed asymmetric allylic alkylation. *Tetrahedron* **2006**, *62*, 11942.

Chapter 4

A Versatile Route to L-Hexoses: Stereoselective Synthesis of Rare Sugars

4. A VERSATILE ROUTE TO L-HEXOSES: STEREOSELECTIVE SYNTHESIS OF RARE SUGARS

*An efficient route for the synthesis of orthogonally protected L-sugars has been described, starting from the heterocyclic homologating agent **1** and 2,3-O-isopropylidene-L-glyceraldehyde (**2**). The synthetic path enables the synthesis of 2,3-unsaturated-α-L-pyranoside diastereomers **12** and 1,6-anhydro-β-L-pyranoside derivatives **31**, which can be suitably dihydroxylated in stereoselective manner to afford L-hexoses. This strategy has to be considered of general applicability, since it allows to prepare all eight sugars in enantiomerically pure form.*

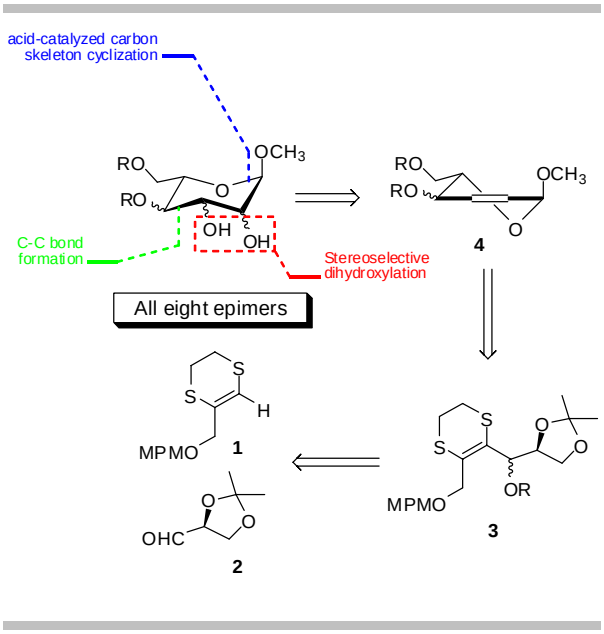
4.1 *INTRODUCTION*

While D-sugars are widely available in nature and frequently used as chiral resources in the synthesis of complex natural products, L-sugars are rare and have been overlooked in synthetic organic chemistry for a long time. However, L-sugars play important roles in the microbial world.* As the requirement for L-sugars has therefore increased in scientific fields, it has resulted necessary to develop efficient methodologies that make them readily available; thus, quite a few approaches to L-pyranose preparation have been reported, including homologation of shorter-chain sugars,[1] epimerization of readily available D-sugars,[2] and *de novo* syntheses.[3]

As part of our efforts working toward the synthesis of bioactive polyhydroxylated compounds, we have explored[4] a general and efficient route for the preparation of L-hexoses (as well as their D-enantiomers) starting from the three-carbon homologating agent **1** (**Scheme 1**) and a chiral building block (the 2,3-*O*-isopropylidene-L-glyceraldehyde, **2**) which provides the inherent chirality at the *C*-5 stereocenter of final products.[5]

As shown in the retrosynthetic path (**Scheme 1**), our strategy comprises the following key steps: (i) preparation of **3** by three-carbon homologation reaction, employing the heterocyclic system **1** and the well-known[6] 2,3-*O*-isopropylidene-L-glyceraldehyde (**2**); (ii) synthesis of the 2,3-unsaturated pyranoside **4** by carbon skeleton cyclization; (iii) suitable double bond functionalization by stereoselective dihydroxylation of **4**.

* See Chapter 2, "Rare Sugars: a Chemically Available Source of Building Blocks for the Synthesis of Bioactive Molecules".



Scheme 1 - *Retrosynthetic path.*

4.2 *RESULTS AND DISCUSSION*

4.2.1 *SYNTHESIS OF THE L-HEXOSE FRAMEWORK*

At the beginning, as the coupling reaction was afforded under previously reported conditions,* the coupling product *anti*-**5** diastereoisomer was chosen as a model to test the whole synthetic path (**Scheme 2**). Benzylation of the secondary hydroxyl function, treating *anti*-**5** with NaH and BnBr, afforded **6** in almost quantitative yield. Interestingly, if the reaction was carried out in the presence of an excess of NaH, the formation of the unexpected diene **7**, besides the benzylated alcohol **6**, was observed.*

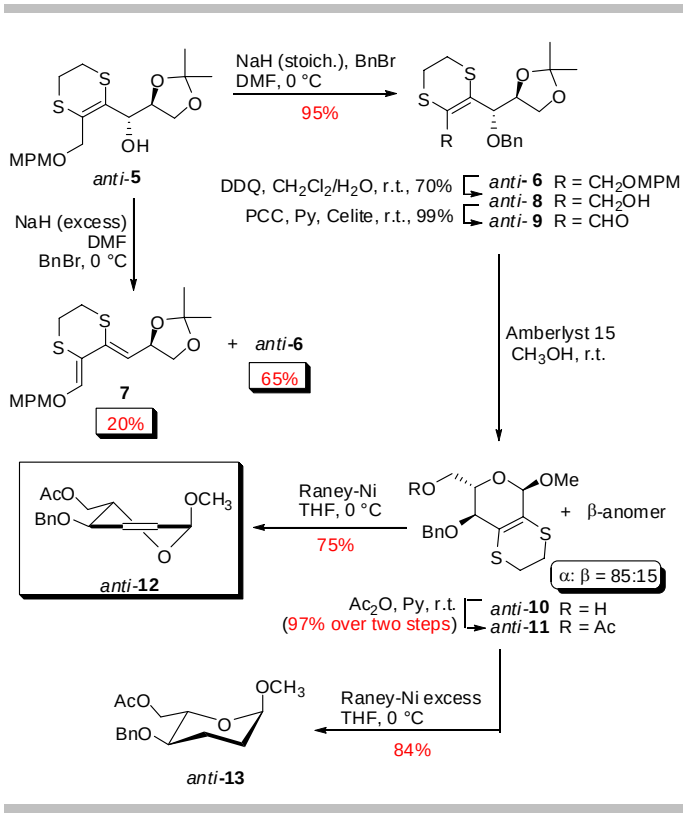
4-Methoxybenzyl protecting group removal was next attempted by treatment of **6** with DDQ (1.2 eq) in 18:1 CH_2Cl_2/H_2O emulsion (**Scheme 2**). As described in Chapter 3, with similar substrates such conditions led quantitatively to the formation of a formyl function, rather than the expected primary alcohol. To our regret, under the same conditions **6** was converted both into aldehyde **9** and into the corresponding alcohol **8** with an unsatisfactory overall yield (48%) and in 4:6 ratio.† Therefore, a two-step reaction sequence was preferred, firstly converting **6** into **8**

* See Chapter 3, "The Heterocycle-Functional Group Equivalence as a Powerful Tool for the Synthesis of Organic Molecules".

† Attempts to obtain only the aldehyde **9** in high yield were tried carrying the reaction mixture out for more prolonged times and with larger amounts of DDQ. Results of such efforts will be mentioned farther on in this

and then oxidizing **8** to **9** (**Scheme 2**). The complete conversion of **6** into **8** (70% yield) was accomplished using DDQ in the presence of a higher water percentage; then, oxidation of the primary hydroxyl function of **8** was easily performed by treatment with PCC and Celite in pyridine to afford quantitatively **9**, which was directly employed in the next cyclization step.

Treatment of the aldehyde **9** in the presence of Amberlyst 15 in methanol allowed, in a *domino* simple procedure, the conversion of the formyl group into its di-*O*-methyl acetal,[5] the acetonide deprotection, and then the intramolecular transacetalation to give the bicyclic compound **10** (**Scheme 2**). After subsequent acetylation of the crude residue, an α:β diastereomeric mixture (85:15 dr) was obtained in 97% overall yield; then, recrystallization by methanol allows the separation of the major *α*-anomer **11** from its *β*-form.



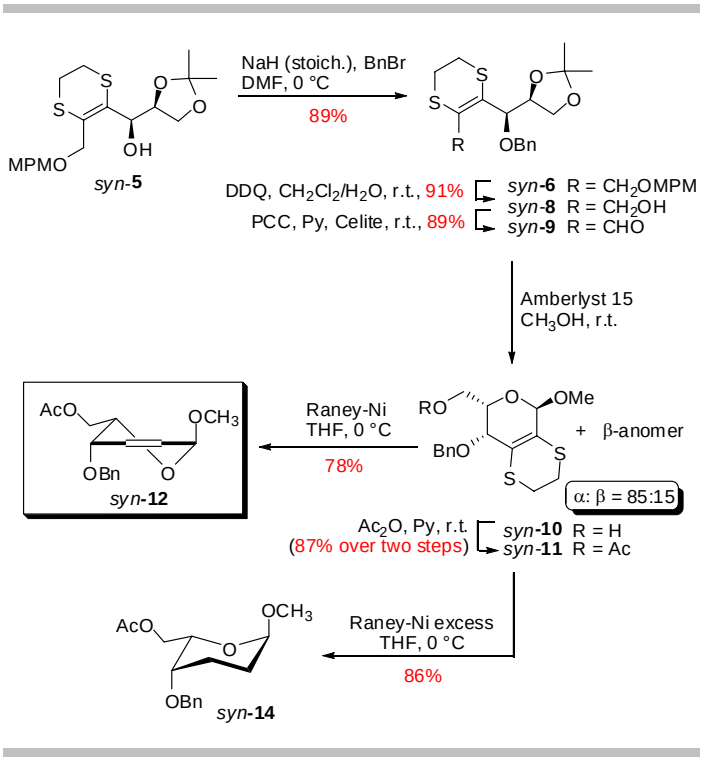
Scheme 2 - *Cyclization of the carbon skeleton on the* anti-**5** *diastereomer.*

Hence, desulfurization of the α-anomer **11** by Raney-Ni in THF at 0 °C for 2h led to the unsaturated pyranosyl derivative **12** (75% yield). Moreover, when the dithiodimethylene bridge removal was carried out with a Raney-Ni excess the over-reduction product was obtained with

chapter, since they enabled the formation of an intriguing byproduct, which was profitably used during the synthesis.

satisfactory yield (84%), affording[7] the interesting* 2,3-dideoxy-L-hexopyranoside **13** (**Scheme 2**).

To keep results consistent along with all the series, the same synthetic path was repeated on the *syn*-**5** diastereomer, obtaining the desulfurized olefin *syn*-**12** (**Scheme 3**). This latter, together with the intermediate *anti*-**12**, underwent the final steps of the synthetic route, represented by the stereoselective double bond dihydroxylation reactions.



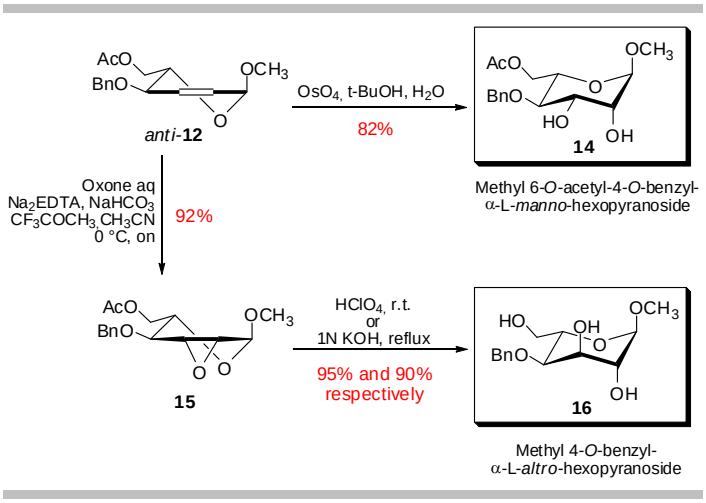
Scheme 3 - *Cyclization of the carbon skeleton on* syn-***5*** *diastereomer.*

4.2.2 *STEREOSELECTIVE DOUBLE BOND DIHYDROXYLATIONS: L-HEXOSE SYNTHESIS*

Methyl L*-manno- *and L*-altro-*hexopyranosides (**14** and **16**)**.** In order to access the desired L-*manno*- and L-*altro*pyranosides, we next explored the stereoselective dihydroxylation of olefin *anti*-**12** (**Scheme 4**). Under common Upjohn conditions (OsO_4/NMO), the 6-*O*-acetyl-4-*O*-benzyl-α-L-*manno*-pyranoside **14** was obtained as a single diastereomer in 82% yield. This results concurs with earlier investigations[3d,8] into the dihydroxylation of allylic alcohol derivatives: the osmylation reaction occurred *anti* to the pseudoequatorial benzyloxy group.

* The over-reduction intermediate **13** has been later successfully employed as the sugar moiety of nucleosides and oligonucleotide analogues, see Chapters 8 and 10.

Having successfully synthesized the protected L-mannose **14**, the preparation of the L-altrose derivative was attempted by introducing an oxirane functionality,* treating olefin **12** with *in situ*[9] generated DMDO (Oxone/trifluoroacetone). The *anti*-epoxide **15** was exclusively obtained[10] in 92% yield (**Scheme 4**). Subsequent ring opening of the 2,3-anhydro derivative **15** either by acid[11] or base[12] catalyzed hydrolysis afforded methyl 4-*O*-benzyl-α-L-*altro*pyranoside **16** (95% and 90% yield, respectively), with *C*-6 *O*-deacetylation under both conditions.



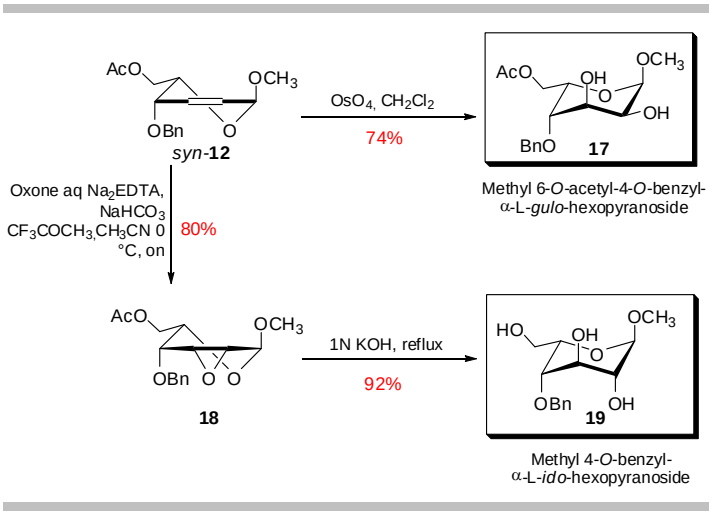
Scheme 4 – Syn- *and* anti-*dihydroxylation of the unsaturated derivative* anti-***12***.

Methyl L-gulo- and L-ido-hexopyranosides (**17** and **19**). Entry to L-*gulo*- and L-*ido*-hexopyranosides implied for the use of olefin *syn*-**12** (**Scheme 5**). In major detail, *syn*-dihydroxylation was achieved by treatment of *syn*-**12** with OsO_4/NMO in CH_2Cl_2, furnishing methyl 6-*O*-acetyl-4-*O*-benzyl-α-L-gulopyranoside **17** in good yield (74%) and in high selectivity (9:1 dr). It is noteworthy to underline that the catalytic dihydroxylation, carried out using the usual *t*-BuOH/acetone rather than CH_2Cl_2 as reaction mixture, did not furnish the same results in terms of stereoselectivity.

On the other hand, *anti*-hydroxylation of *anti*-**12** was attained by epoxidation of the double bond followed by alkaline hydrolysis of the resulting epoxide (**Scheme 5**). Under our conditions, *anti*-**12** was treated with *in situ* generated DMDO affording a single epoxide **18** (80%), as expected[9] in consideration of the steric hindrance by the *C*-4 benzyl group. Then, epoxide ring

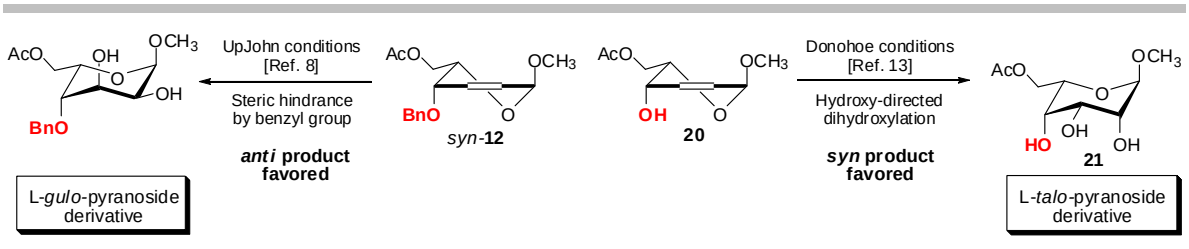
* In an initial experiment, the oxidation of *anti*-**12** with *m*-CPBA resulted in lower yield (70%) and with a 10:1 *anti/syn* dr.

opening by means of aqueous $HClO_4$ afforded methyl 6-*O*-acetyl-4-*O*-benzyl-α-L-*ido*pyranoside **19** in 92% yield.



Scheme 5 – Syn- *and* anti-*dihydroxylation reaction on the unsaturated derivative* syn-***12***.

Methyl L*-talo-*hexopyranoside (**27**). Methyl 6-*O*-acetyl-4-*O*-benzyl-α-L-*talo*pyranoside was already obtained as minor component next to *syn*-dihydroxylation of the olefin *syn*-**12**. In the effort to selectively achieve it, the possibility to drive the osmylation reaction towards the *syn* adduct **21** by exploiting the hydrogen bonding ability of the allylic alcohol **20** with the OsO_4 (according to Donohoe's conditions,[13] **Scheme 6**) was examined.

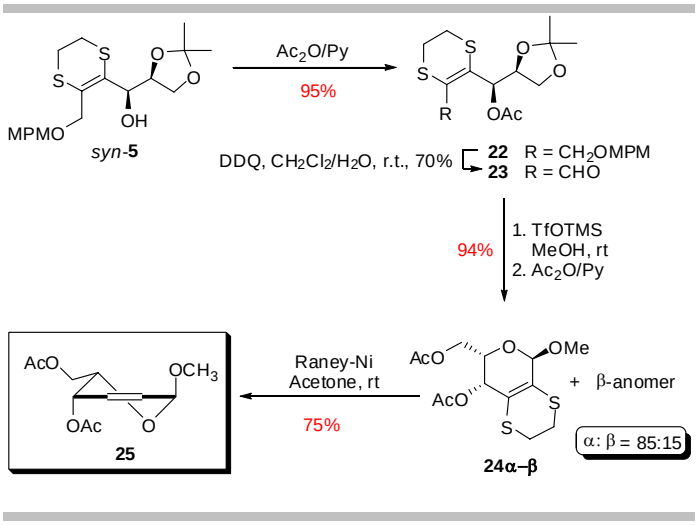


Scheme 6 - *Dihydroxylation reaction can be selectively driven towards the* syn *or the* anti *adduct, depending on the conditions and the substrates used.*

With this aim, efficient *O*-benzyl group removal was attempted. Unfortunately, neither treatment of *syn*-**12** with palladium-based reagents[14] (Pd/C; $Pd(OH)_2/C$) or with TMSI[15] afforded the desired free-hydroxy intermediate **20**, because of the observed chemical inactivity of the

substrate.* Therefore, since an easily achievement of **20** was not possible from the benzylated derivative **12**, an alternative route was forcedly examined. More specifically, considering that an acetyl group should be able to withstand all the conditions foreseen during the synthesis, the opportunity to replace the benzyl group with the acetyl one was investigated.

Following this novel route, acetylation of the secondary hydroxyl function in *syn*-**5** under usual conditions afforded **22** in almost quantitative yield (**Scheme 7**). Then, 4-methoxybenzyl group removal was achieved treating **22** with DDQ: interestingly, under usual conditions, only aldehyde **23** was obtained in good yield (70%). Hence, exposure of the aldehyde **23** to Amberlyst 15 in methanol did not give the expected product of ring closure. Conversely, better results were achieved by means of TfOTMS in MeOH, which gave the anomeric mixture of the byciclic compounds **24** (α/β = 85:15), the major α component **24α** being isolated by recrystallization (MeOH). Finally, careful desulfurization with Ni/Ra afforded the desired key intermediate **25** (**Scheme 7**).

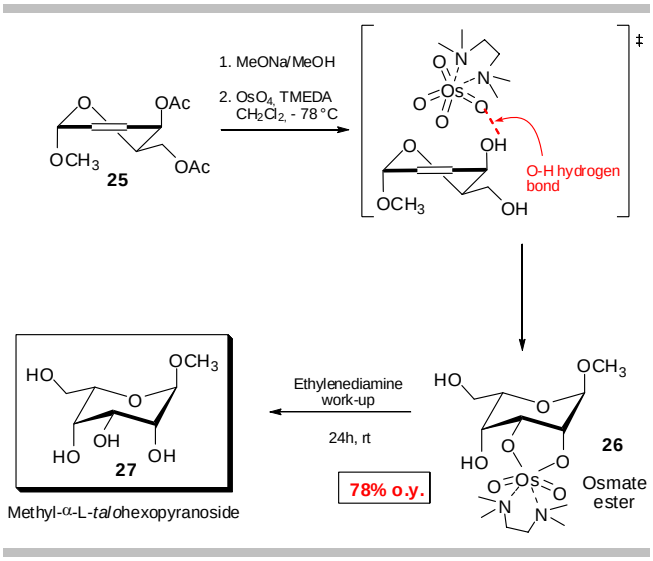


Scheme 7 – *Alternative path for* syn-***5*** *diastereomer.*

Dihydroxylation reaction under Donohoe's conditions was finally examined (**Scheme 8**). Intermediate **25** was first deacetylated under usual Zemplèn conditions, then subsequent treatment of the latter with stoichiometric OsO_4 and *N*,*N*,*N*,*N*-tetramethylenediamine (TMEDA) afforded the dihydroxylated **26** with complete *syn*-selectivity. Importantly, hydrolysis of the osmate ester **26** to liberate methyl-α-L-*talo*-hexopyranoside **27** was successfully achieved by

* Further addition of numerous equivalents of reagents to the reaction mixture brought to the formation of several and not easily isolable byproducts.

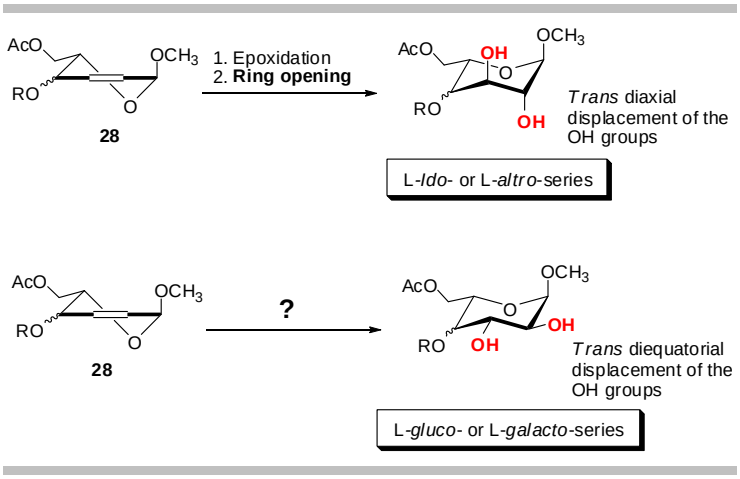
means of ethylenediamine,[13] since treatment with sodium sulfite or with HCl/MeOH resulted in the formation of a complex mixture of byproducts.



Scheme 8 - *Methyl-α-L-talo-hexopyranoside* ***27*** *synthesis.*

4.2.3 *L*-GLUCO-, *L*-ALLO- *AND L*-GALACTO-*HEXOPYRANOSIDE SYNTHESIS: ACCESS FROM β-L-1,6-ANHYDRO DERIVATIVES*

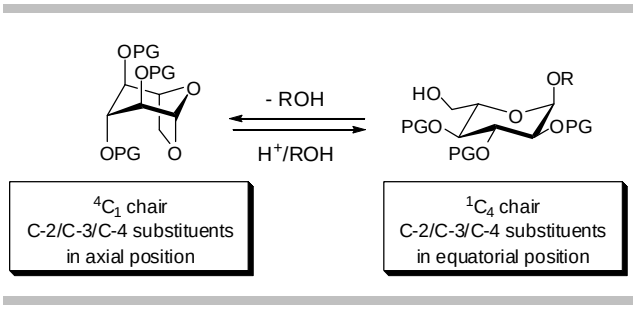
In the effort to synthesize L-*gluco*- and L-*galacto*-hexoses a major problem occurred, since the *trans* diequatorial OH groups located on *C*-2, *C*-3 positions cannot be easily installed under common chemical manipulations as from olefin intermediates **28** (**Scheme 9**). In fact, as previously described with regards to the preparation of L-idose and L-altrose (**Schemes 4-5**), only *trans*-diaxial products of oxirane ring opening can be obtained.



Scheme 9 - *A major problem occurred about the synthesis of hexoses belonging to* L*-gluco and* L*-galacto-series.*

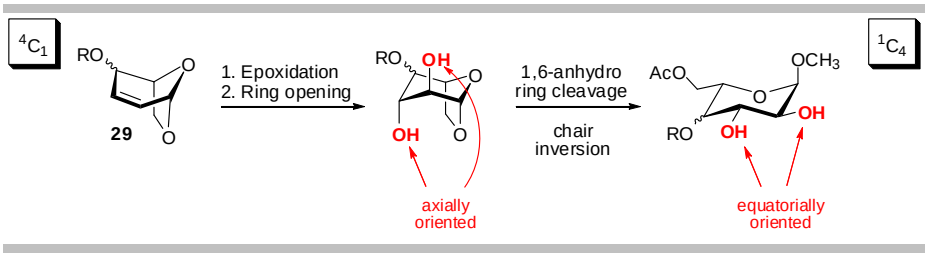
As it will be discussed below, this problem was solved through the use of 1,6-anhydrosugar derivatives.

1,6-Anhydrosugars are stable intermediates with considerable synthetic potential, which employ in organic synthesis has been widely exploited in the past years.[16] Indeed their bicyclic skeleton, which enables high regio- and stereocontrolled reactions, makes them versatile building blocks for a wide range of synthetic purposes.[17]



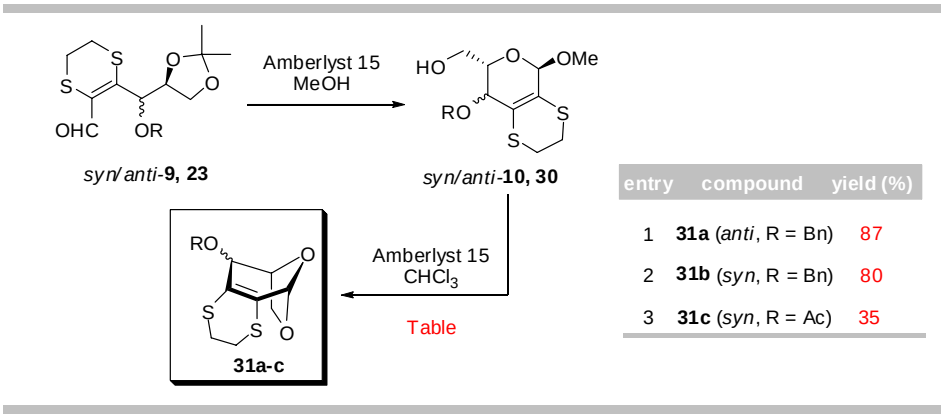
Scheme 10 – *Inverted chair conformation of the 1,6-anhydro ring.*

The 1,6-anhydro sugar skeleton involves a locked conformation of the pyranose ring, in which the stereocenters are in opposite orientation with respect to the corresponding classical pyranosides (**Scheme 10**). As it will be discussed below in major detail, the peculiar behaviour of such a kind of molecules has been herein exploited for our purposes. In fact, because of the equatorial-axial conversion of OH groups at *C*-2, *C*-3, and *C*-4 positions, *syn*- and *anti*-dihydroxylation reactions carried out on 1,6-anhydro olefins **29** will enable, after the anhydro-ring cleavage, to obtain the desired L-epimers (**Scheme 11**).



Scheme 11 – *Access to* L-gluco- *and* L-galacto-*epimers by exploitment of 1,6-anhydrosugar chemistry.*

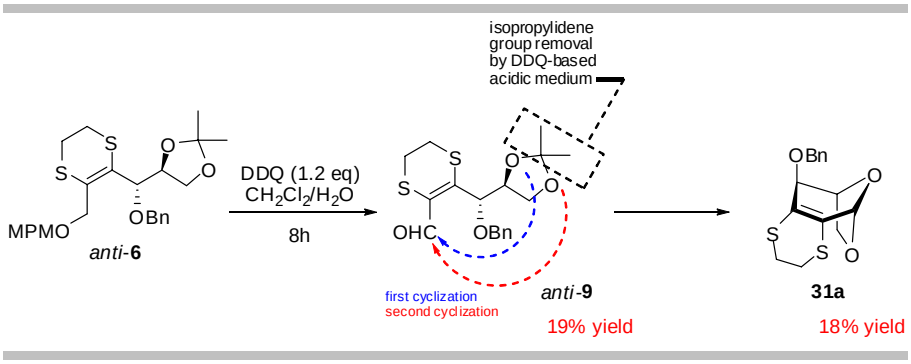
A first entry for the construction of 1,6-anhydro moiety was carried out as from bicycle intermediates *syn/anti*-**10** and **30**. Treatment of *anti*-**10** with Amberlyst 15 using chloroform as solvent smoothly furnished the desired 1,6-anhydro-*β*-derivative **31a** through a 1,6-cyclization reaction *via* oxonium ion formation (**Scheme 12**). Farther on, both diastereomers *syn/anti*-**10** underwent the same reaction: an analogous trend was reported. Moreover, since acetyl aldehyde **23** was in our hands as well, it was subjected to the same conditions, obtaining 1,6-anhydro derivative **31c** even if in worst yields (**Scheme 12**).



entry	compound	yield (%)
1	**31a** (*anti*, R = Bn)	87
2	**31b** (*syn*, R = Bn)	80
3	**31c** (*syn*, R = Ac)	35

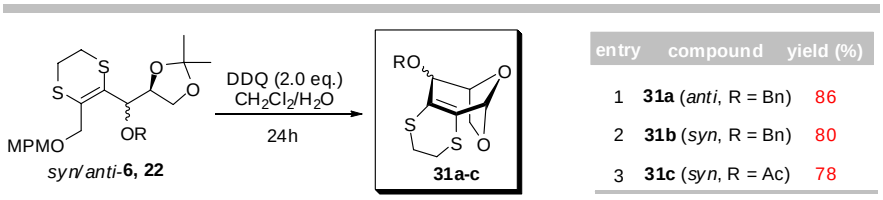
Scheme 12 – *First entry to 1,6-anhydro derivatives* ***31a-c.***

Later on, a more profitable and intriguing route as from alcohols *anti/syn*-**6** and **22** through a *domino* approach was considered. More specifically, going back to the DDQ-mediated synthetic step to afford unsaturated aldehyde *anti*-**6** (**Scheme 2**), it was noted that the reaction medium was acidic enough (pH ≈ 3) to allow removal of isopropylidene protection as well, hence creating the suitable conditions for a double cyclization (**Scheme 13**). As a matter of fact, in such a kind of step direct transformation into the 1,6-anhydro derivative **31a** was just detected, since it represented the major byproduct isolated (18%).



Scheme 13 - *1,6-Anhydro derivative **31a** as the main byproduct in the DDQ-mediated synthetic step to unsaturated aldehyde* anti-***9.***

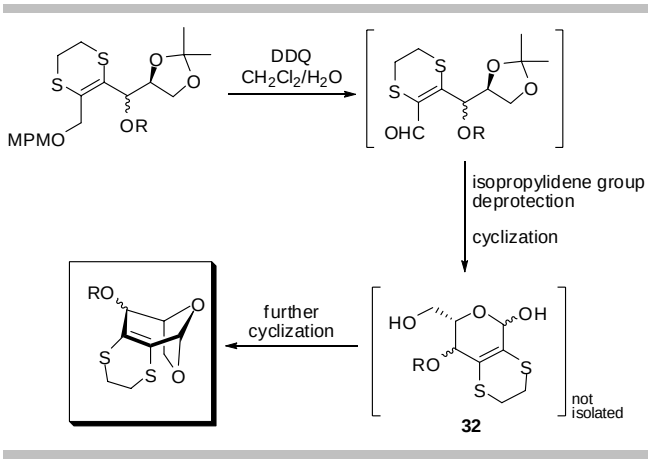
Therefore, efforts were carried out for finely tuning the reaction, so allowing complete conversion of the alcohols *anti/syn*-**6** and **22** into the desired **31**. As a result, a slight eccess of DDQ (2.0 eq.), and a higher temperature (DCM reflux) were required to afford **31a-b** in remarkable yield (80-86%). Attempts were achieved on the substrate **22** as well, obtaining analogous results (**Scheme 14**).



entry	compound	yield (%)
1	**31a** (*anti*, R = Bn)	86
2	**31b** (*syn*, R = Bn)	80
3	**31c** (*syn*, R = Ac)	78

Scheme 14 – *Direct conversion of derivatives* anti/syn-***6*** *and **22** into 1,6-anhydro compounds **31a-c**.*

It's noteworthy to underline that this reaction formally includes five synthetic steps, i.e. MPM group removal, oxidation, isopropylidene group removal, cyclization by the hydroxyl group on *C*-5 (giving most probably rise to the emiacetal **32** not detected in the reaction mixture) and cyclization by the hydroxyl group on *C*-6 (**Scheme 15**).

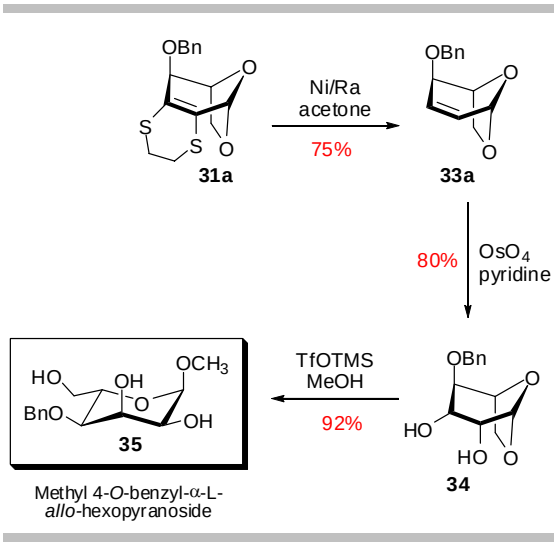


Scheme 15 – *Conjectured mechanism for the synthesis of 1,6-anhydro derivative **31a-c** by domino reaction.*

Once 1,6-anhydro derivatives **31a-b** were obtained, desulfurization of this latter with Ni/Ra at 0 °C furnished the unsaturated pyranosyl derivatives **33a-b** (**Schemes 16-18**, 75-76% yield).* These latter ones were then dihydroxylated to afford the remaining L-hexoses.

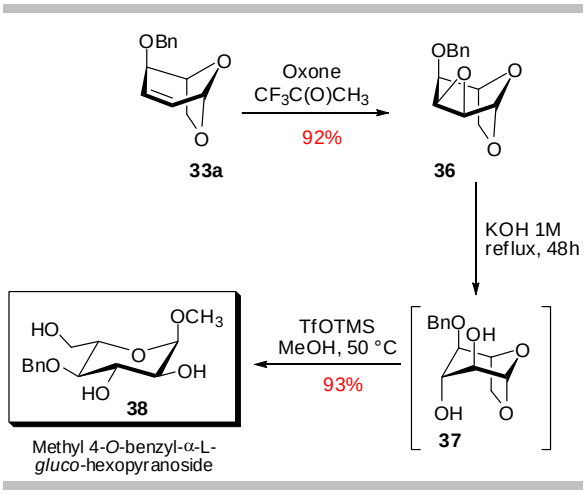
Methyl L*-allo-** ***and L*-gluco-*hexopyranosides (**35** and **38**). To access the desired L-*gluco*- and L-*allo*-pyranosides, we first explored the stereoselective dihydroxylation of olefin **33a**. Treatment of **33a** with OsO_4 in pyridine afforded the 1,6-anhydro derivative **34** with complete selectivity, because of the attack on the less hyndered face of the olefin. Hence, careful ring opening was studied. Since 1,6-anhydropyranosides have been described to be definitively stable in alkaline media but rather amenable to acidic conditions,[18] a few acidic agents (such as HCl or TFA) were examined, with the purpose to yield the corresponding hexoses with a good stereoselectivity. At the end, best results were attained exposing **34** to catalytic TfOTMS in MeOH, allowing smooth 1,6-ring opening which led to methyl 4-*O*-benzyl-α-L-*allo*-pyranoside **35**. This latter was obtained with a complete α-selectivity at the anomeric center (most probably owing to the direct S_N2 displacement of the methoxy group) and in almost quantitative yield.

* Moreover, as already reported for previous substrates, when the dithiodimethylene bridge removal was carried out with an excess of Ni/Ra, the over-reduction product was obtained with satisfactory yield (84%), affording the 2,3-dideoxy-1,6-anhydro-β-L-hexopyranose.



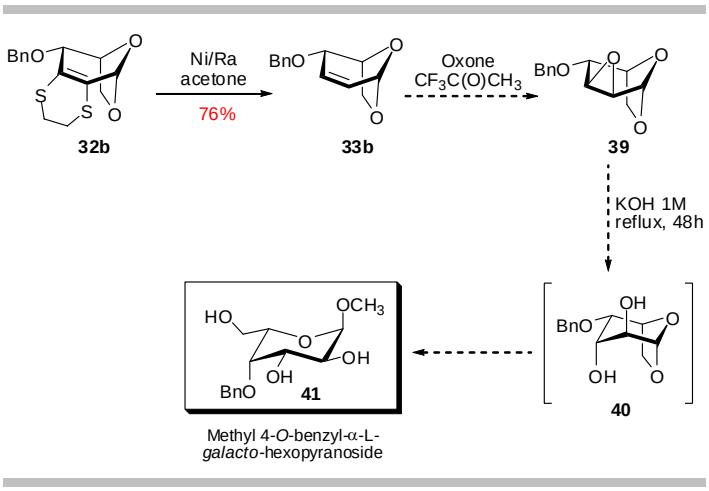
Scheme 16 – *Synthesis of methyl 4-O-benzyl-α-L-allo-pyranoside* ***35*** *from 1,6-anhydro derivative* ***31a.***

Stereoselective *anti* dihydroxylation was then considered (**Scheme 17**). The allylic ether **33a** was oxidized with *in situ* generated DMDO to yield the 1,6:2,3-dianhydro-β-L-hexopyranoside **36** in 92% yield.[19] Hence, the 2,3 oxirane ring was opened with refluxing KOH solution in a predictable regio- and stereoselectivity, providing the dihydroxylated product **37** with the OH groups aligned in a *trans*-diaxial manner. Finally, treatment of the crude **37** with catalytic TfOTMS in MeOH easily opened the 1,6-anhydro ring, leading to methyl 4-*O*-benzyl-α-L-*gluco*-hexopyranoside (**38**, **Scheme 17**) as single anomer and in excellent yield (93%).



Scheme 17 - *methyl 4-O-benzyl-α-L-gluco-pyranoside* ***38*** *from 1,6-anhydro derivative* ***31a.***

4-O-Benzyl-α-L-*galacto*-hexopyranoside (**41**). In a very similar fashion, methyl 4-*O*-benzyl-α-L-*galacto*-pyranoside (**41**) will be obtained as from 1,6-anhydro derivative **33b**, next to Ni/Ra-mediated desulfurization, epoxidation (DMDO) and opening of both rings (KOH and TfOTMS, **Scheme 18**).

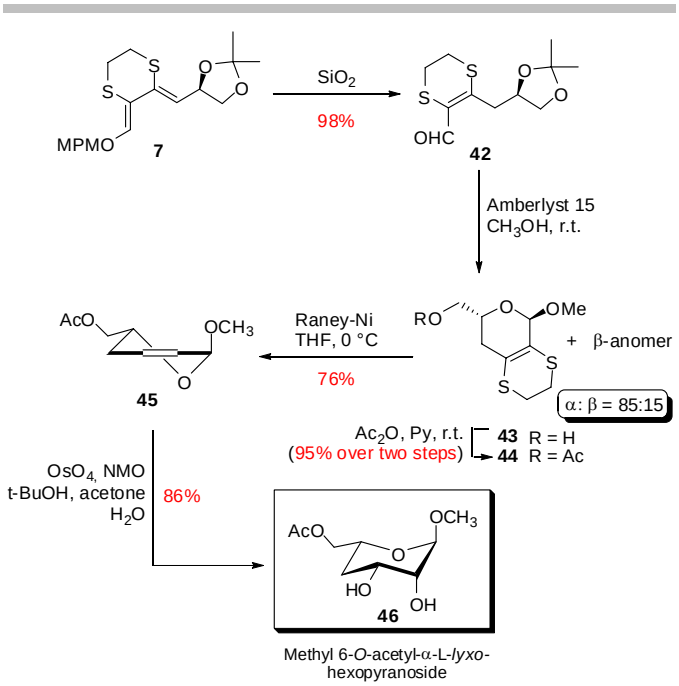


Scheme 18 - *methyl 4-O-benzyl-α-L-galacto-pyranoside **41** from 1,6-anhydro derivative **31b.***

In summary, most of the eight epimers belonging to L-series were synthesized in high yields (15-24% o.y.) and in highly stereoselective manner.

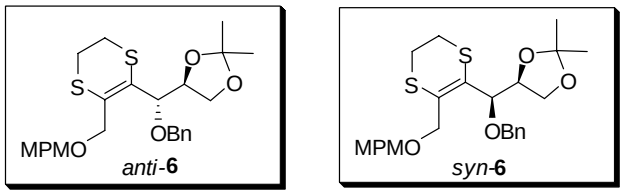
4.3 *GOING BACK TO DIENE: 4-DEOXY-L-HEXOPYRANOSIDE SYNTHESIS*

It is noteworthy to recall the value of diene **7**, obtained as byproduct in Scheme 2, as useful intermediate with the purpose to prepare 4-deoxy-L-hexopyranosides. In fact, following chromatographic purification the compound **7** afforded quantitatively the aldehyde **42** (**Scheme 19**). When this was submitted to the synthetic steps described above, it gave the intermediates **43-44**, which after desulfurization (76% yield) and double-bond osmylation (86% yield) led, according to our previous results,[5] to methyl 6-*O*-acetyl-4-deoxy-α-L-*lyxo*-hexopyranoside **46** as a single diastereomer. Further studies are currently in progress in our laboratory with the aim to improve the yield of compound **7**.



Scheme 19 – *Synthesis of methyl 6-O-acetyl-4-deoxy-α-L-lyxo-hexopyranoside* ***46*** *starting from diene* ***7****.*

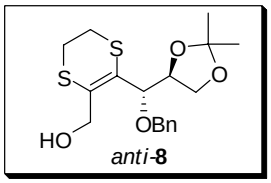
4.4 *EXPERIMENTAL SECTION*



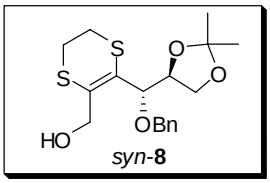
Compounds **anti-*6* *and*** **syn-*6***. NaH (0.07 g, 1.83 mmol) was added to a solution of *anti*-**5** (0.56 g, 1.41 mmol) in anhydrous DMF (12 mL) at 0 °C under nitrogen atmosphere. After 10 min benzyl bromide (0.23 mL, 1.93 mmol) was added in one portion. The reaction mixture was warmed to room temperature, stirred for 2h, then carefully quenched with 10% aq NH_4Cl. The mixture was extracted with EtOAc, the combined organic phases washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/Et_2O = 8:2) afforded the pure *anti*-**6** (0.66 g, 95% yield): oily, $[\alpha]_D^{25}$ -26.1 (*c* 0.54, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 1.31 (s, 6H), 3.08-3.14 (m, 1H), 3.17-3.23 (m, 2H), 3.27-3.34 (m, 1H), 3.78 (d, *J* = 11.7 Hz, 1H), 3.80 (s, 3H), 3.86 (dd, *J* = 8.8, *J* = 5.8 Hz, 1H), 4.09 (dd, *J* = 8.8, *J* = 6.8 Hz, 1H), 4.24-4.30 (m, 1H), 4.31 (d, *J* = 11.6 Hz, 1H), 4.40 (d, *J* = 7.8 Hz, 1H), 4.41 (d, *J* = 11.7 Hz, 1H), 4.44 (d, *J* = 11.7 Hz, 1H), 4.48 (d, *J* = 11.7 Hz, 1H),

4.64 (d, *J* = 11.7 Hz, 1H), 6.86 (d, *J* = 8.6 Hz, 2H), 7.24-7.33 (m, 7H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.2, 25.9, 27.4, 29.7, 55.4, 67.5, 69.8, 70.5, 72.1, 76.2, 79.2, 109.3, 114.0, 127.3, 127.9, 128.1, 128.3, 129.7, 130.0, 137.9, 159.2. Anal. calcd for $C_{26}H_{32}O_5S_2$: C, 63.90; H, 6.60. Found: C, 64.12; H, 6.57.

Under analogous conditions, the pure *syn*-**6** was obtained (89% yield) as from *syn*-**5**: oily, $[\alpha]_D^{25}$= +33.8 (*c* 0.47, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.33 (s, 3H), 1.36 (s, 3H), 3.08-3.12 (m, 1H), 3.14-3.18 (m, 2H), 3.22-3.25 (m, 1H), 3.80 (s, 3H), 3.83 (dd, *J* = 6.5 Hz, *J* = 8.6 Hz, 1H), 3.86 (d, *J* = 11.7 Hz, 1H), 3.93-3.97 (m, 2H), 4.31-4.48 (m, 5H), 4.71 (d, *J* = 12.3 Hz, 1H), 6.84-6.86 (d, *J* = 8.7 Hz, 2H), 7.21 (d, *J* = 8.7 Hz, 2H), 7.26-7.39 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.0, 27.1, 27.9, 29.6, 55.7, 66.2, 70.2, 70.3, 72.7, 78.4, 79.7, 110.3, 114.2, 127.0, 127.6, 127.9, 128.3, 128.6, 129.0, 130.0, 138.4, 159.7. Anal. calcd for $C_{26}H_{32}O_5S_2$: C 63.90, H 6.60. Found: C 64.02, H 6.57.



anti-**8**

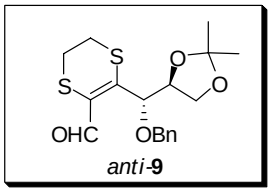


syn-**8**

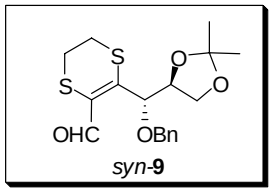
Compounds* anti-*8* *and* syn-*8. Method A: to a stirred 18:1 CH_2Cl_2/H_2O emulsion (5 mL) containing the MPM ether *anti*-**6** (0.54 g, 1.10 mmol), DDQ (0.38 g, 1.68 mmol) was added in one portion at room temperature. After 12 h H_2O was added to the reaction and the mixture extracted with CH_2Cl_2; the organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 95:5) gave the pure *anti*-**8** (0.12 g, 29% yield) beside a remarkable amount of *anti*-**9** (0.08 g, 19% yield). Method B: to a stirred 9:1 CH_2Cl_2/H_2O emulsion (50 mL) containing the MPM ether *anti*-**6** (0.54 g, 1.10 mmol), DDQ (0.38 g, 1.68 mmol) was added in one portion at room temperature. After 3h, H_2O was added to the reaction and the mixture extracted with CH_2Cl_2; the organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 95:5) gave the pure *anti*-**8** (0.28 g; 70% yield): oily, $[\alpha]_D^{25}$-61.8 (*c* 0.43, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (s, 3H), 1.40 (s, 3H), 3.04-3.11 (m, 1H), 3.19-3.26 (m, 2H), 3.31-3.36 (m, 1H), 3.71 (d, *J* = 13.2 Hz, 1H), 3.76-3.79 (m, 1H), 4.17-4.22 (m, 1H), 4.28 (d, *J* = 11.7 Hz, 1H), 4.32-4.34 (m, 2H), 4.36 (d, *J* = 13.2 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 7.28-7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.9, 25.9,

26.7, 29.9, 62.8, 68.1, 70.3, 74.7, 78.4, 109.9, 127.1, 128.0, 128.2, 128.4, 131.2, 137.0. Anal. calcd for $C_{18}H_{24}O_4S_2$: C, 58.67; H, 6.56. Found: C, 58.55; H 6.58.

Under analogous conditions, the pure *syn*-**8** was obtained (91% yield) as from *syn*-**6**: oily, $[\alpha]_D^{25}$ +62.3 (*c* 0.33, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.33 (s, 3H), 1.40 (s, 3H), 2.70 (bs, 1H), 3.09-3.27 (m, 4H), 3.95 (dd, *J* = 6.3 Hz, *J* = 8.5 Hz, 1H), 4.07 (d, *J* = 12.9 Hz, 1H), 4.08 (dd, *J* = 6.3 Hz, *J* = 8.5 Hz, 1H), 4.13 (d, *J* = 12.9 Hz, 1H), 4.37-4.44 (m, 3H), 4.73 (d, *J* = 12.2 Hz, 1H), 7.22-7.42 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 25.7, 26.8, 28.0, 29.5, 63.2, 65.9, 70.8, 77.5, 78.8, 110.2, 125.7, 128.1, 128.2, 128.6, 130.7, 137.8. Anal. calcd for $C_{18}H_{24}O_4S_2$: C 58.67, H 6.56, Found: C 58.71, H 6.60.



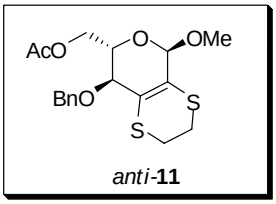
anti-**9**



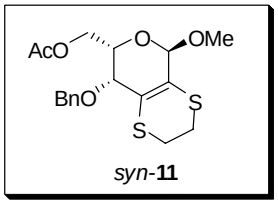
syn-**9**

Compounds* anti-*9* *and* syn-*9. A solution of alcohol *anti*-**8** (0.28 g, 0.75 mmol) in pyridine (2 mL) was added in one portion to a stirred suspension of PCC (0.22 g, 1.02 mmol) and Celite (0.22 g) in Py (7 mL) at room temperature. The resulting mixture was stirred for 8 h and then diluted with 10 mL of anhydrous Et_2O, kept in ultrasound bath for 30 min and filtered on a Celite pad. After solvent removal under reduced pressure, chromatography of the crude residue over silica gel (CH_2Cl_2) gave the pure *anti*-**9** (0.27 g, 99% yield): white solid, mp 91.7-93.3 °C (from MeOH), $[\alpha]_D^{25}$ +35.5 (*c* 0.53, C_6H_6). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30 (s, 3H), 1.34 (s, 3H), 3.15-3.22 (m, 2H), 3.23-3.38 (m, 2H), 3.92 (dd, *J* = 8.8, *J* = 4.8 Hz, 1H), 4.18 (dd, *J* = 8.8, *J* = 6.8 Hz, 1H), 4.26 (ddd, *J* = 7.8, *J* = 6.8, *J* = 4.8 Hz, 1H), 4.37 (d, *J* = 11.3 Hz, 1H), 4.71 (d, *J* = 11.3 Hz, 1H), 4.80 (d, *J* = 7.8 Hz, 1H), 7.30-7.40 (m, 5H), 9.84 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.9, 26.1, 27.3, 29.1, 67.6, 71.5, 76.4, 78.4, 110.1, 128.3, 128.6, 131.1, 136.5, 151.5, 182.5. Anal. Calcd for $C_{18}H_{22}O_4S_2$: C, 58.99; H, 6.05. Found: C, 58.75; H 6.08.

Under analogous conditions, the pure *syn*-**9** was obtained (89% yield) as from *syn*-**8**: white crystals, mp 83.1-84.5 °C, MeOH; $[\alpha]_D^{25}$ +45.8 (*c* 0.52, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (s, 3H), 1.34 (s, 3H), 3.04-3.10 (m, 1H), 3.15-3.21 (m, 1H), 3.27-3.30 (m, 2H), 3.95-4.01 (m, 2H), 4.40 (dd, *J* = 6.1 Hz, *J* = 12.2 Hz, 1H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.70 (m, 1H), 4.78 (d, *J* = 12.0 Hz, 1H), 7.28-7.36 (m, 5H), 9.86 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.7, 26.3, 26.7, 30.1, 65.9, 72.1, 77.6, 78.1, 110.8, 127.3, 128.6, 128.9, 131,2, 137.1, 184.1. Anal. Calcd for $C_{18}H_{22}O_4S_2$: C 58.99, H 6.05. Found: C 59.03, H 6.00.



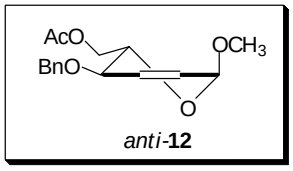
anti-**11**



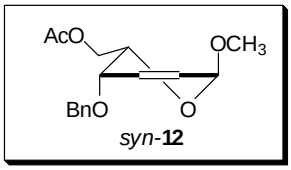
syn-**11**

***Compounds* anti-*9 and* syn-*9*.** Amberlyst 15 (1.2 g, previously washed with anhydrous MeOH), was added in one portion to a stirred solution of aldehyde *anti*-**9** (0.12 g, 0.32 mmol) in methanol (10 mL) at 0 °C. After 10 min, the suspension was warmed to room temperature and stirred for 1h. Then the solid was filtered off and washed with MeOH; Py was added until pH 8. MeOH was evaporated under reduced pressure and replaced by Py (3 mL); to the solution was added Ac_2O at room temperature (0.04 mL, 0.40 mmol). After 3 h, solvent removal under reduced pressure and chromatography of the crude residue over silica gel (CH_2Cl_2) afforded the pure *anti*-**11**, beside to a minor amount of its β-anomer (0.12 g, 97% overall yield; 85:15 dr): white solid, mp 70.3-71.8 °C (from MeOH), $[\alpha]_D^{25}$ -12.0 (*c* 0.48, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.10 (s, 3H), 3.07-3.14 (m, 1H), 3.20-3.30 (m, 2H), 3.32-3.38 (m, 1H), 3.42 (s, 3H), 4.19 (d, *J* = 9.8 Hz, 1H), 4.25 (dd, *J* = 11.7, *J* = 4.9 Hz, 1H), 4.28-4.34 (m, 1H), 4.36 (dd, *J* = 11.7, *J* = 2.0 Hz, 1H), 4.59 (d, *J* = 11.2 Hz, 1H), 4.70 (d, *J* = 11.2 Hz, 1H), 4.73 (s, 1H), 7.30-7.43 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.6, 29.3, 30.0, 55.8, 63.3, 68.0, 71.9, 74.3, 98.1, 122.7, 126.7, 128.0, 128.4, 130.9, 137.5, 170.7. Anal. calcd for $C_{18}H_{22}O_4S_2$: C, 58.99; H, 6.05. Found: C, 59.15; H, 6.07.

Under analogous conditions, the pure *syn*-**11** (α-anomer) was obtained (87% yield) as from *syn*-**9**: mp 56.4-58.3 °C, MeOH; $[\alpha]_D^{25}$ +74.6 (*c* 0.93, C_6H_6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.08 (s, 3H), 3.11-3.38 (m, 4H), 3.44 (s, 3H), 3.71 (d, *J* = 1.6 Hz, 1H), 4.26-4.37 (m, 3H), 4.63 (d, *J* = 11.0 Hz, 1H), 4.76 (d, *J* = 11.0 Hz, 1H), 4.82 (s, 1H), 7.25-7.40 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7, 27.7, 27.9, 55.3, 63.2, 69.4, 71.4, 71.9, 97.7, 123.3, 125.0, 127.7, 128.1, 128.2, 137.7, 170.4. Anal. calcd for $C_{18}H_{22}O_5S_2$: C 56.52, H 5.80. Found: C 56.57, H 6.02.



anti-**12**

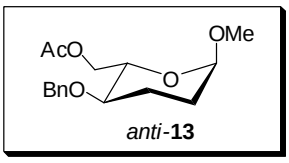


syn-**12**

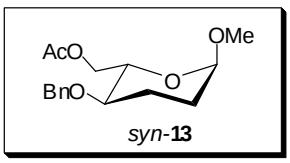
***Compounds* anti-*12 and* syn-*12*.** A solution of *anti*-**11** (0.1 g, 0.26 mmol) in THF (3 mL) was added in one portion to a stirred suspension of Raney-Ni (W2) (0.9 g, wet) in the same solvent (3 mL) at 0 °C and under nitrogen atmosphere. The suspension was stirred for 2h, then the solid

was filtered off and washed with THF. The filtrate was evaporated under reduced pressure to afford a crude residue which chromatography over silica gel (CH_2Cl_2) gave the pure *anti*-**12** (0.06 g, 75% yield) beside a small amount of *anti*-**13** (0.01 g, 11% yield). Data for compound *anti*-**12**: oily, $[\alpha]_D^{25}$ -156.1 (*c* 0.14, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.04 (s, 3H), 3.43 (s, 3H), 4.00 (bs, 2H), 4.30 (bs, 2H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 1H), 4.88 (d, *J* = 2.4 Hz, 1H), 5.79 (dd, *J* = 10.7, *J* = 2.4 Hz, 1H), 6.10 (d, *J* = 10.2 Hz, 1H), 7.28-7.40 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7, 55.7, 63.4, 67.6, 69.9, 70.6, 95.5, 126.6, 127.8, 127.9, 128.4, 130.0, 137.5, 170.7. Anal. calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C 65.91; H 6.88.

Under analogous conditions, the pure *syn*-**12** was obtained (78% yield) as from *syn*-**11**: oily, $[\alpha]_D^{25}$ +80.9 (*c* 0.093, MeOH). ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.05 (s, 3H), 3.43 (s, 3H), 3.69 (dd, *J* = 2.5 Hz, *J* = 4.8 Hz, 1H), 4.18-4.24 (m, 1H), 4.33-4.40 (m, 2H), 4.53 (d, *J* = 11.9 Hz, 1H), 4.65 (d, *J* = 11.9 Hz, 1H), 4.96 (d, *J* = 2.7 Hz, 1H), 6.01 (dd, *J* = 2.7 Hz, *J* = 10.2 Hz, 1H), 6.14 (dd, *J* = 4.8 Hz, *J* = 10.2 Hz, 1H), 7.27-7.38 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.8, 63.8, 66.9, 68.6, 70.8, 95.0, 126.6, 127.8, 128.4, 129.8, 138.1, 170.7. Anal. calcd for $C_{16}H_{20}O_5$: C 65.74, H 6.90. Found: C 65.79, H 6.94.



anti-**13**

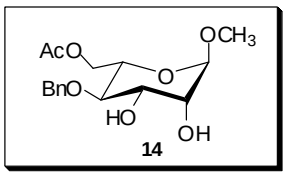


syn-**13**

***Compounds* anti-*13 and* syn-*13*.** Under similar conditions reported above, treatment of *anti*-**11** (0.1 g, 0.26 mmol) with an excess of Raney-Ni (W2) (1.8 g, wet) afforded, after common work-up and purification procedures, the pure *anti*-**13** (0.06 g, 84% yield): oily, $[\alpha]_D^{25}$ -36.8 (*c* 0.078, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.66-1.88 (m, 4H), 2.04 (s, 3H), 3.27-3.40 (m, 4H), 3.83 (ddd, *J* = 9.5 Hz, *J* = 4.5 Hz, *J* = 3.1 Hz, 1H), 4.29 (dd, *J* = 12.0 Hz, *J* = 3.0 Hz, 1H), 4.31 (dd, *J* = 12.0 Hz, *J* = 5.0 Hz, 1H), 4.43 (d, *J* = 11.7 Hz, 1H), 4.63 (d, *J* = 11.7 Hz, 1H), 4.68-4.71 (m, 1H), 7.24-7.40 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7, 23.5, 28.6, 54.2, 64.0, 69.6, 70.3, 72.4, 97.3, 127.6, 127.7, 127.8, 128.3, 128.4, 138.2, 170.2. Anal. calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.13; H, 7.51.

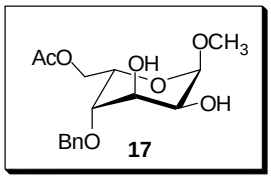
Under analogous conditions, the pure *syn*-**13** was obtained (86% yield) as from *syn*-**11**: oily, $[\alpha]_D^{25}$ -10.7 (*c* 0.14, MeOH). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.78-2.10 (m, 7H), 3.37 (s, 3H), 3.46-3.49 (m, 1H), 3.96 (m, 1H), 4.17-4.24 (m, 2H), 4.40 (d, *J* = 12.2 Hz, 1H), 4.67 (d, *J* = 12.2 Hz, 1H), 4.77 (d, *J* = 3.4 Hz, 1H), 7.25-7.38 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 22.2,

23.9, 54.5, 64.6, 68.4, 70.5, 76.6, 97.9, 127.6, 127.8, 128.3, 138.1, 164.5. Anal. calcd for $C_{16}H_{22}O_5$: C 65.29, H 7.53. Found: C 65.34, H 7.49.



14

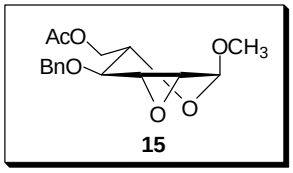
***Methyl 6*-O-*acetyl*-4-O-*benzyl*-α-*L*-manno-*pyranoside* (14).** To a solution of *anti*-**12** (0.06 g, 0.21 mmol) in 1:1 (v/v) *tert*-butyl alcohol/acetone (2.4 mL), cooled at 0 °C in ice bath, an excess of a chilled 1:1 (w/w) solution of 4-methylmorpholine-*N*-oxide (0.06 g, 0.42 mmol) in water was added in one portion. After few minutes, a catalytic amount (0.04 mmol) of OsO_4 was added, and the resulting mixture was stirred overnight at room temperature; then the reaction was quenched with saturated aq Na_2SO_3 and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/ acetone, 1:1) afforded the diol **14** (0.06 g, 82% yield) as a single diastereomer: oily, $[\alpha]_D^{25}$ -41.0 (*c* 0.29, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (s, 3H), 2.28 (bs, 2H, exchange with D_2O), 3.37 (s, 3H), 3.62 (dd, *J* = 9.8 Hz, *J* = 8.8 Hz, 1H), 3.80 (ddd, *J* = 9.8 Hz, *J* = 5.1 Hz, *J* = 2.2 Hz, 1H), 3.91-3.97 (m, 2H), 4.30 (dd, *J* = 11.8 Hz, *J* = 5.1 Hz, 1H), 4.38 (dd, *J* = 11.8 Hz, *J* = 2.2 Hz, 1H), 4.69 (d, *J* = 11.3 Hz, 1H), 4.73 (d, *J* = 1.6 Hz, 1H), 4.76 (d, *J* = 11.3 Hz, 1H), 7.27-7.40 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9, 55.0, 63.4, 69.0, 71.0, 71.8, 74.8, 75.7, 100.5, 124.5, 128.1, 128.7, 137.9, 170.8. Anal. calcd for $C_{16}H_{22}O_7$: C, 58.89; H, 6.79. Found: C, 59.05; H, 6.77.



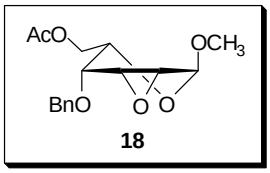
17

***Methyl 6*-O-*acetyl*-4-O-*benzyl*-α-*L*-gulo-*pyranoside* (17).** To a solution of *syn*-**12** (0.026 g, 0.0896 mmol) in CH_2Cl_2 (1.5 mL), cooled at 0 °C in ice bath, 4-methylmorpholine-*N*-oxide (0.063 g, 0.467 mmol) was added in one portion. After few minutes, a catalytic amount (0.3 mL) of a 49 mM OsO_4 solution in CH_2Cl_2 was added, the resulting mixture being stirred overnight at room temperature; then the reaction was quenched with saturated aq Na_2SO_3 and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (CH_2Cl_2) afforded the diol **17** (0.068 mmol, 74% yield) as a single diastereomer: oily, $[\alpha]_D^{25}$ -13.8 (*c* 0.093, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.01 (s, 3H), 2.48 (bs, 1H), 3.17 (bs, 1H), 3.46 (s, 3H), 3.65 (bd,

J = 3.8 Hz, 1H), 3.96-4.01 (m, 1H), 4.05-4.10 (m, 1H), 4.11-4.16 (m, 2H), 4.28 (dd, *J* = 6.6 Hz, *J* = 10.9 Hz, 1H), 4.51 (d, *J* = 11.5 Hz, 1H), 4.67 (d, *J* = 11.5 Hz, 1H), 4.82 (d, *J* = 3.8 Hz, 1H), 7.28-7.39 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.0, 56.3, 63.4, 65.6, 69.1, 73.0, 76.7, 77.4, 101.2, 128.3, 128.8, 137.8, 168.4. Anal. calcd for $C_{16}H_{22}O_7$: C 58.89, H 6.79. Found: C 58.93, H 6.82.



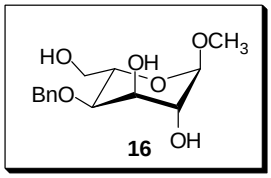
15



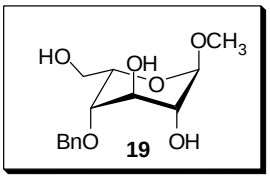
18

Compounds 15 and 18. Na_2EDTA (4.0 x 10^{-4} M, 0.60 mL) and CF_3COCH_3 (0.11 mL) were added to a solution of *anti*-**12** (0.03 g, 0.12 mmol) in CH_3CN (1.25 mL) at 0 °C. After a few minutes a mixture of $NaHCO_3$ (0.08 g) and Oxone® (0.37 g) was added over 1 h and the whole resulting mixture was stirred for 30 min at the same temperature. Then the reaction was diluted with H_2O and extracted with CH_2Cl_2. The extracts were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 8:2) afforded the pure **15** (0.03 g, 92% yield) as a single diastereoisomer: oily, $[\alpha]_D^{25}$ -107.3 (*c* 0.33, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.00 (s, 3H), 3.10 (d, *J* = 3.9 Hz, 1H), 3.37 (d, *J* = 3.9 Hz, 1H), 3.45 (s, 3H), 3.54 (d, *J* = 9.8 Hz, 1H), 3.76 (ddd, *J* = 9.8 Hz, *J* = 5.4 Hz, *J* = 2.5 Hz, 1H), 4.14 (dd, *J* = 11.7 Hz, *J* = 5.4 Hz, 1H), 4.19 (dd, *J* = 11.7 Hz, *J* = 2.5 Hz, 1H), 4.55 (d, *J* = 11.7 Hz, 1H), 4.78 (d, *J*=11.7 Hz, 1H), 4.89 (s, 1H), 7.27-7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 49.5, 53.0, 55.4, 63.2, 65.5, 68.3, 71.7, 96.0, 128.1, 128.5, 128.6, 136.8, 171.0. Anal. calcd for $C_{16}H_{20}O_6$: C, 62.33; H, 6.54. Found: C, 62.12; H, 6.56.

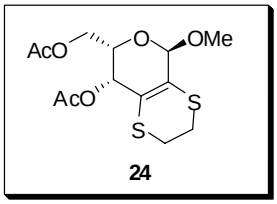
Under analogous conditions, the pure **18** was obtained (80% yield) as from *syn*-**12**: oily, $[\alpha]_D^{25}$ 11.1 (*c* 0.18, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65 (s, 3H), 2.76-2.82 (m, 2H), 3.13 (s, 3H), 3.18 (dt, *J* = 1.3 Hz, *J* = 4.4 Hz, 1H), 3.79-3.85 (m, 1H), 4.26 (d, *J* = 12.0 Hz, 1H), 4.37 (dd, *J* = 4.5 Hz, *J* = 11.7 Hz, 1H), 4.42 (dd, *J* = 7.7 Hz, *J* = 11.6 Hz, 1H), 4.63 (d, *J* = 11.8 Hz, 1H), 4.70 (s, 1H), 7.10-7.20 (m, 5H). ^{13}C NMR (100 MHz, C_6D_6): δ 29.9, 43.9, 48.7, 49.6, 63.1, 66.2, 67.8, 70.2, 95.9, 127.5, 128.2, 128.3, 134.8, 171.2. Anal. calcd for $C_{16}H_{20}O_6$: C, 62.33; H, 6.54. Found: C, 62.30; H, 6.58.



Methyl-4*-O-*benzyl*-α-*L*-altro-*pyranoside (**16**)**.** Method A: the epoxide **15** (0.03 g, 0.09 mmol) was refluxed for 5 days in a 0.1 M aq solution of KOH (1 ml). After solvent removal under reduced pressure, chromatography of the crude residue over silica gel ($CHCl_3$/MeOH = 95/5) afforded the pure **16** (0.02 g, 90% yield). Method B: a solution of the epoxide **15** (0.03 g, 0.09 mmol) in an aqueous solution of 6% $HClO_4$ (0.6 mL) was stirred at reflux temperature for 16 h. Solid Na_2CO_3 was added until pH 8, then solvent removal under reduced pressure afforded **16** (0.02 g, 95% yield): oily, $[\alpha]_D^{25}$ -21.1 (*c* 2.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.90 (bs, 3H, D_2O exchange), 3.42 (s, 3H), 3.78-3.84 (m, 2H), 3.87 (dd, *J* = 12.2, *J* = 3.2 Hz, 1H), 3.92-3.99 (m, 2H), 4.12 (bs, 1H), 4.56 (d, *J* = 11.2 Hz, 1H), 4.68 (s, 1H), 4.71 (d, *J* = 11.7 Hz, 1H), 7.28-7.40 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 55.9, 62.7, 67.3, 68.4, 70.0, 71.3, 71.6, 101.7, 128.3, 128.8, 128.9, 137.8. Anal. calcd for $C_{14}H_{20}O_6$: C, 59.14; H, 7.09. Found: C, 59.01; H, 7.07.

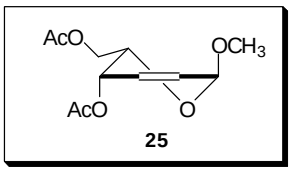


Methyl-4*-O-*benzyl*-α-*L*-ido-*pyranoside (**19**)**.** A solution of the epoxide **18** (0.03 g, 0.10 mmol) in an aqueous solution of 6% $HClO_4$ (0.6 mL) was stirred for 12 h at room temperature. Solid Na_2CO_3 was added until pH 8, then solvent removal under reduced pressure afforded **19** (0.03 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.16 (s, 3H), 3.35 (dd, *J* = 1.1 Hz, *J* = 3.8 Hz, 1H), 3.40 (t, *J* = 3.5 Hz, 1H), 3.76-3.80 (m, 2H), 3.81-3.83 (m, 1H), 4.22 (t, *J* = 5.8 Hz, 1H), 4.65 (d, *J* = 11.8 Hz, 1H), 4.73 (d, *J* = 11.9 Hz, 1H), 5.04 (d, *J* = 3.1 Hz, 1H), 7.25-7.38 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.2, 58.7, 62.4, 67.6, 68.6, 71.1, 73.3, 101.1, 128.3, 128.4, 128.7, 128.9, 130.9. Anal. calcd for $C_{16}H_{22}O_7$: C, 58.89; H, 6.79. Found: C, 59.05; H, 6.77.



24

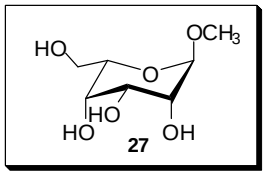
Compound 24. Similar conditions observed for cyclization of benzyl aldehydes *anti*-**9** and *syn*-**9** were employed for the cyclization of acetyl aldehyde **23**. More specifically, amberlyst 15 (3.9 g, previously washed with anhydrous MeOH) was added in one portion to a stirred solution of aldehyde **23** (0.39 g, 1.08 mmol) in anhydrous methanol (30 mL) at 0 °C. After 10 min, the suspension was warmed to room temperature and stirred for 1h. Then the solid was filtered off and washed with MeOH; pyridine was added until pH 8. MeOH was evaporated under reduced pressure and replaced by further pyridine (15 mL); to the solution was added Ac_2O at room temperature (0.12 mL, 1.2 mmol). After 3 h, solvent removal under reduced pressure and chromatography of the crude residue over silica gel (CH_2Cl_2) afforded the pure **24**α, beside to a minor amount of its β-anomer (0.39 g, 94% overall yield; 85:15 dr). Data for the pure **24**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.06 (s, 3H), 2.14 (s, 3H), 3.17-3.29 (m, 4H), 3.45 (s, 3H), 4.11 (dd, J = 7.6 Hz, J = 11.6 Hz, 1H), 4.18 (dd, J = 5.5 Hz, J = 11.3 Hz, 1H), 4.46 (m, 1H), 4.82 (s, 1H), 5.19 (d, J = 2.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.6, 27.6, 27.9, 29.5, 55.6, 62.0, 65.0, 67.3, 97.6, 122.2, 125.9, 170.3, 170.4. Anal. calcd for $C_{13}H_{18}O_6S_2$: C 46.69, H 5.43, S 19.18. Found: C 46.71, H 5.47, S 19.19.



25

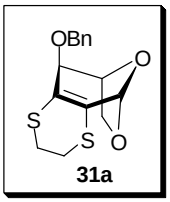
Compound 25. A solution of **24** (0.1 g, 0.29 mmol) in THF (5 mL) was added in one portion to a stirred suspension of Raney-Ni (W2) (1.0 g, wet) in the same solvent (5 mL) at 0 °C and under nitrogen atmosphere. The suspension was stirred for 2h, then the solid was filtered off and washed with THF. The filtrate was evaporated under reduced pressure to afford a crude residue which chromatography over silica gel (CH_2Cl_2) gave the pure **25** (0.05 g, 75% yield): white solid, mp 60.5-61.5, MeOH; $[\alpha]_D^{25}$ +174.3 (*c* 0.8, $CHCl_3$). ^{1}H NMR (200 MHz, C_6D_6): δ 1.75 (s, 3H), 1.81 (s, 3H), 4.23-4.28 (m, 3H), 4.75 (d, J = 2.3 Hz, 1H), 4.95 (d, J = 10.0 Hz, 1H), 5.80 (dd, J = 5.3 Hz, J = 10.0 Hz, 1H), 5.99 (dd, J = 1.3 Hz, J = 5.3 Hz, 1H). ^{13}C NMR (75 MHz,

$CDCl_3$): δ 21.4, 55.4, 62.7, 66.6, 78.4, 94.3, 125.2, 130.4, 171.0, 172.3. Anal. calcd for $C_{11}H_{16}O_6$: C, 54.11; H, 6.61. Found: C, 54.13; H, 6.64.

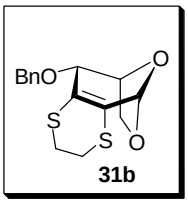


27

***Methyl α-L-talo-pyranoside* (27).** Zemplén deacetylation was accomplished by treatment of **25** (0.05 g, 0.22 mmol) in MeOH (5 ml) with MeONa (41 mg, 0.76 mmol) for 4h at room temperature. Then the mixture was neutralized with few drops of acetic acid and the solvents evaporated under reduced pressure. The crude residue was dissolved in anhydrous DCM (8 ml), the temperature kept at -78 °C, then TMEDA (0.23 mmol) and an OsO_4 solution in DCM (0.22 mmol) were added. The reaction mixture was stirred at -78 °C for 3h, after which the solvent was removed and ethylenediamine (1.0 mmol) was added. The resulting solution was stirred for 48 h during which time a brown precipitate formed. The solution was then concentrated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = (8/2) afforded the pure **27** as single epimer (0.03 g, 78% overall yield): $[\alpha]_D^{25}$ -101.0 (*c* 0.9, H_2O). ^{1}H NMR (400 MHz, D_2O): δ 3.49 (s, 3H), 3.84 (dd, *J* = 3.7 Hz, *J* = 11.0 Hz, 1H), 3.87-3.96 (m, 4H), 3.97-4.00 (m, 1H), 4.94 (s, 1H). ^{13}C NMR (100 MHz, D_2O): δ 58.0, 64.7, 68.5, 72.7, 73.0, 74.6, 104.6. Anal. calcd for $C_7H_{10}O_6$: C 43.30, H 7.27. Found: C 43.33, H 7.30.



31a

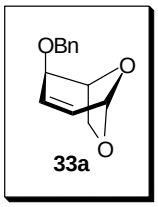


31b

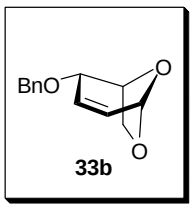
Compounds 31a and 31b. Method A: amberlyst 15 (1.7 g, previously washed with anhydrous $CHCl_3$) was added in one portion to a stirred solution of bicycle *anti*-**10** (0.12 g, 0.39 mmol) in $CHCl_3$ (20 mL) at 0 °C. After 10 min, the suspension was warmed to room temperature and further stirred for 1h. Then the solid was filtered off, washed with $CHCl_3$ (100 mL) and the resulting solution washed with saturated $NaHCO_3$ solution (2 x 80 mL) and brine (2 x 100 mL). The organic layers were dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 95:5) gave the pure **31a** (0.10 g, 80% yield). Method B: to a stirred 18:1 CH_2Cl_2/H_2O emulsion (10 mL) containing the

MPM ether *anti*-**6** (1.08 g, 2.2 mmol), DDQ (0.76 g, 3.36 mmol) was added in one portion at room temperature. The resulting mixture was warmed to a gentle reflux and further stirred for 24 h. Then H_2O was added to the reaction and the mixture extracted with CH_2Cl_2; the organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 95:5) gave the pure **31a** (0.59 g, 87% yield): white solid, mp 132.3-134.4 °C, MeOH; $[\alpha]_D^{25}$ +6.5 (*c* 0.22, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.16-3.29 (m, 4H), 3.56 (dd, *J* = 2.0 Hz, *J* = 7.7 Hz, 1H), 3.58 (d, *J* = 1.0 Hz, 1H), 3.98 (dd, *J* = 6.8 Hz, *J* = 7.7 Hz, 1H), 4.72 (s, 2H), 4.80-4.82 (m, 1H), 5.24 (s, 1H), 7.29-7.44 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.1, 28.2, 64.5, 70.7, 75.8, 77.3, 99.3, 119.4, 127.3, 128.2, 128.5, 128.8, 138.4. Anal. calcd for $C_{15}H_{16}O_3S_2$: C 58.41, H 5.23. Found: C 58.45, H 5.28.

Under analogous conditions, the pure **31b** was obtained (80% yield) as from *syn*-**6**: oily, $[\alpha]_D^{25}$ +22.2 (*c* 0.83, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.07-3.31 (m, 4H), 3.78-3.81 (m, 1H), 4.27 (dd, *J* = 1.7 Hz, *J* = 7.8 Hz, 1H), 4.45-4.51 (m, 2H), 4.58 (d, *J* = 11.9 Hz, 1H), 4.72 (d, *J* = 11.9 Hz, 1H), 5.14 (s, 1H), 7.30-7.40 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 26.3, 27.7, 62.6, 73.2, 73.4, 84.9, 99.1, 121.6, 124.4, 127.9, 128.5, 131.0. Anal. calcd for $C_{15}H_{16}O_3S_2$: C 58.41, H 5.23. Found: C 58.46, H 5.20.



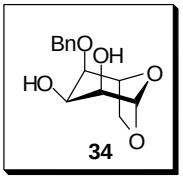
33a



33b

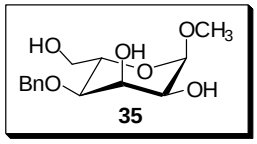
Compounds 33a and33b. A solution of **31a** (0.15 g, 0.49 mmol) in acetone (6 mL) was added in one portion to a stirred suspension of Raney-Ni (W2) (1.5 g, wet) in the same solvent (5 mL) at 0 °C and under nitrogen atmosphere. The suspension was stirred for 2h, then the solid was filtered off and washed with further acetone. The filtrate was evaporated under reduced pressure to afford a crude residue which chromatography over silica gel (CH_2Cl_2) gave the pure **33a** (0.08 g, 75% yield): oily; $[\alpha]_D^{25}$ -107.0 (*c* 0.01, $CHCl_3$). ^{1}H NMR (500 MHz, CDCl3): δ 3.40 (dd, *J* =1.9 Hz, *J* =7.8 Hz, 1H), 3.53 (d, *J* =4.4 Hz, 1H), 3.91-3.95 (m, 1H), 4.68 (d, *J* =12.2 Hz, 1H), 4.70 (d, *J* =12.2 Hz, 1H), 4.78-4.82 (m, 1H), 5.57 (d, *J* = 3.4 Hz, 1H), 5.85 (ddd, *J* =1.9 Hz, *J* = 3.9 Hz, *J* =9.8 Hz, 1H), 6.14 (dd, *J* =3.4 Hz, *J* = 9.8 Hz, 1H), 7.25-7.40 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 63.0, 70.6, 73.0, 74.0, 95.5, 124.0, 126.8, 127.7, 128.4, 131.3, 138.1. Anal. calcd for $C_{13}H_{14}O_3$: C 71.54, H 6.47. Found: C 71.62, H 6.51.

Under analogous conditions, the pure **33b** was obtained (76% yield) as from **31b**: oily, $[\alpha]_D^{25}$ +11.0 (*c* 0.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.87 (ddd, *J* = 7.9Hz, *J* = 6.3 Hz, *J* = 1.4 Hz, 1H), 4.15 (dt, *J* = 1.6 Hz, *J* = 0.5 Hz, 1H), 4.25-4.65 (m, 4H), 5.38 (dd, *J* = 3.1 Hz, *J* = 0.7 Hz, 1H), 5.65 (ddd, *J* = 9.9 Hz, *J* = 2.0 Hz, 1H), 5.86 (d, *J* = 1.4 Hz, 1H), 7.20-7.40 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 64.5, 69.5, 73.9, 78.2, 95.0, 127.2, 128.6, 121.0, 126.3, 128.6, 131.0. Anal. calcd for $C_{13}H_{14}O_3$: C 71.54, H 6.47. Found: C 71.21, H 6.34.



34

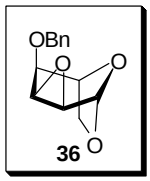
Compound 34. To a solution of **33a** (0.04 g, 0.18 mmol) in 1:1 (v/v) *tert*-butyl alcohol/acetone (1.0 mL), cooled at 0 °C in ice bath, an excess of a chilled 1:1 (w/w) solution of 4-methylmorpholine-*N*-oxide (0.03 g, 0.23 mmol) in water was added in one portion. After few minutes, a catalytic amount (2% mol) of OsO_4 was added and the resulting mixture was stirred overnight at room temperature; then the reaction was quenched with a saturated solution of Na_2SO_3 and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = 9/1) afforded the pure **34** (0.04 g, 82% yield) as a single diastereomer: white solid, mp 110 °C, $[\alpha]_D^{25}$ +71.1 (*c* 0.6, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.64-3.01 (bs, 2H), 3.60 (bt, *J* = 1.8 Hz, 1H), 3.72 (dd, *J* = 1.9 Hz, *J* = 5.4 Hz, 1H), 4.02-4.08 (m, 1H), 4.12 (d, *J* = 7.4 Hz, 1H), 4.57 (bd, *J* = 5.8 Hz, 1H), 4.62 (d, *J* = 12.4 Hz, 1H), 4.64 (d, *J* = 12.4 Hz, 1H), 5.69 (bt, *J* = 1.9 Hz, 1H), 7.28-7.42 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 64.8, 66.6, 68.8, 71.5, 74.0, 78.1, 101.4, 127.7, 127.9, 128.4, 137.4. Anal. calcd for $C_{13}H_{16}O_5$: C 61.90, H 6.39. Found: C 61.97, H 6.33.



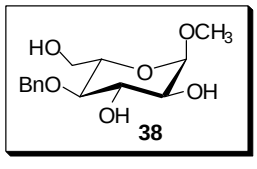
35

Methyl 4*-O-*benzyl*-α-*L*-allo-*pyranoside **(35).** To a solution of **34** (0.04 g, 0.16 mmol) in MeOH (4 mL) a catalytic amount of trimethylsilyl trifluoromethansulfonate (TfOTMS, 0.02 mmol) was added and the resulting reaction mixture was stirred at 50 °C for 48 h. Then the reaction was quenched with solid $NaHCO_3$ and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = 8/2) gave the pure **35** (0.04

g, 90% yield). ^{1}H NMR (500 MHz, CD_3OD): δ 3.39 (s, 3H), 3.42-3.47 (m, 1H), 3.58 (t, *J* = 9.7 Hz, 1H), 3.62 (dd, *J* = 5.4 Hz, *J* = 12.2 Hz, 1H), 3.68-3.74 (m, 2H), 3.77 (d, *J* = 3.4 Hz, *J* = 9.2 Hz, 1H), 4.55 (d, *J* = 10.7 Hz, 1H), 4.58 (s, 1H), 4.84 (d, *J* = 10.7 Hz, 1H), 7.19 (t, *J* = 6.8 Hz, 1H), 7.25 (t, *J* = 7.3 Hz, 2H), 7.30 (d, *J* = 7.9 Hz, 2H). ^{3}C NMR (50 MHz, $CDCl_3$): δ 53.8, 61.3, 71.2, 71.6, 72.1, 74.5, 75.4, 101.3, 127.2, 127.7, 127.9, 138.7. Anal. calcd for $C_{14}H_{20}O_6$: C 59.14, H 7.09. Found: C 59.10, H 7.09.

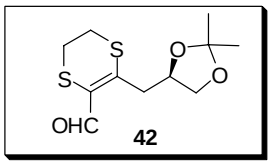


Compound 36. Na_2EDTA (4.0 x 10^{-4} M, 0.8 mL) and CF_3COCH_3 (0.14 mL) were added to a solution of **33a** (0.03 g, 0.16 mmol) in CH_3CN (1.6 mL) at 0 °C. After a few minutes a mixture of $NaHCO_3$ (0.1 g) and Oxone® (0.4 g) was added over 1 h and the whole resulting mixture was stirred for 30 min at the same temperature. Then the reaction was diluted with H_2O and extracted with CH_2Cl_2. The extracts were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 8:2) afforded the pure **36** (0.03 g, 92% yield) as a single diastereoisomer. M.p. 74-76 °C, MeOH; $[\alpha]_D^{25}$= -120 (c 2.8, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.09 (dd, *J* = 0.9 Hz, *J* = 4.4 Hz, 1H), 3.31 (ddd, *J* = 1.9 Hz, *J* = 4.4 Hz, *J* = 4.4 Hz, 1H), 3.45 (d, *J* = 4.4 Hz, 1H), 3.60 (dd, *J* = 1.9 Hz, *J* = 7.8 Hz, 1H), 3.88 (appt, *J* = 7.3 Hz, *J* = 7.8 Hz, 1H), 4.47-4.53 (m, 1H), 4.74 (d, *J* = 12.4 Hz, 1H), 4.87 (d, *J* =12.4 Hz, 1H), 5.65 (bs, 1H), 7.41-7.56 (m, 5H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 47.5, 47.8, 65.4, 70.7, 72.3, 75.4, 97.1, 127.8, 127.9, 128.4, 137.6. Anal. calcd for $C_{13}H_{14}O_4$: C 66.66, H 6.02. Found C 66.74, H 6.10.



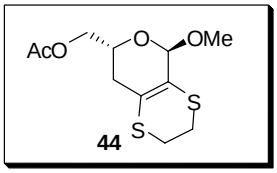
***Methyl 4-O-benzyl-α-L-gluco-pyranoside* (38).** The epoxide **36** (0.04 g, 0.17 mmol) was refluxed for 3 days in a 1M aqueous solution of KOH (4 ml). Then 1N HCl was careful added at 0 °C until neutrality. After solvent removal under reduced pressure, the crude residue was dissolved in MeOH (5 mL) and a catalytic amount of trimethylsilyl trifluoromethansulfonate (TfOTMS, 0.02 mmol) was added. The resulting reaction mixture was stirred at 50 °C for 48 h;

then the reaction was quenched with solid $NaHCO_3$ and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = 8/2) gave the pure **38** (0.04 g, 93% yield): $[\alpha]_D^{25}$ –140.2 (c 1.2, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 3.33-3.36 (m, 1H), 3.39 (s, 3H), 3.42 (dd, *J* = 3.8 Hz, *J* = 9.7 Hz, 1H), 3.54-3.58 (m, 1H), 3.65 (dd, *J* = 4.9 Hz, *J* = 11.8 Hz, 1H), 3.76 (dd, *J* = 2.1 Hz, *J* = 11.6 Hz, 1H), 3.80 (t, *J* = 8.8 Hz, 1H), 4.63 (d, *J* = 11.1 Hz, 1H), 4.67 (d, *J* = 3.7 Hz, 1H), 4.93 (d, *J* = 11.1 Hz, 1H), 7.22-7.38 (m, 5H). ^{13}C NMR (100 MHz, CD_3OD): δ 54.0, 60.8, 61.3, 72.3, 74.0, 74.3, 78.0, 99.7, 127.1, 127.5, 127.7, 138.5. Anal. calcd for $C_{14}H_{20}O_6$: C 59.14, H 7.09. Found: C 59.19, H 7.01.



42

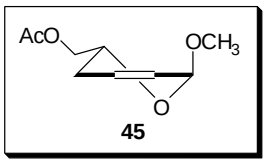
Compound 42. As result of a purification procedure of compound **7** (0.11 g, 0.29 mmol), chromatography over silica gel (1.5 g, CH_2Cl_2) afforded the pure **42** (0.08 g, 98% yield): oily, $[\alpha]_D^{25}$ +30.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35 (s, 3H), 1.42 (s, 3H), 2.72 (dd, *J* = 14.8, *J* = 4.7 Hz, 1H), 3.08-3.16 (m, 2H), 3.21 (dd, *J* = 14.9, *J* = 7.3 Hz, 1H), 3.25-3.38 (m, 2H), 3.76 (dd, *J* = 8.3, *J* = 6.5 Hz, 1H), 4.13 (dd, *J* = 8.3, *J* = 5.8 Hz, 1H), 4.28-4.38 (m, 1H), 9.90 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 25.2, 26.5, 29.1, 29.5, 38.7, 68.4, 78.9, 109.7, 131.9, 159.0, 183.4. Anal. calcd for $C_{11}H_{16}O_3S_2$: C, 50.74; H, 6.19. Found: C, 50.88; H, 6.21.



44

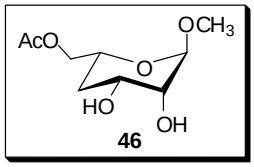
Compound 44. Amberlyst 15 (1.0 g), was added in one portion to a stirred solution of aldehyde **42** (0.10 g, 0.37 mmol) in methanol (9.0 mL) at 0 °C. After 10 min, the suspension was warmed to room temperature and stirred for 1h. Then the solid was filtered off and washed with MeOH; Py was added until pH 8. MeOH was evaporated under reduced pressure and replaced by Py (3 mL); to the solution was then added Ac_2O (0.04 mL, 0.45 mmol). After 3 h, solvent removal and chromatography of the crude residue over silica gel (CH_2Cl_2) afforded the pure **44**, beside to a minor amount of its β-anomer (0.10 g, 95% overall yield; 85:15 dr): oily, $[\alpha]_D^{25}$ +20.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.95 (dd, *J* = 16.1, *J* = 3.4 Hz, 1H), 2.10 (s, 3H), 2.34 (dd, *J* = 16.1, *J* = 11.2 Hz, 1H), 3.13-3.20 (m, 2H), 3.21-3.30 (m, 2H), 3.43 (s, 3H), 4.14 (dd, *J* =

11.7, *J* = 3.9 Hz, 1H), 4.18 (dd, *J* = 11.7, *J* = 5.9 Hz, 1H), 4.29-4.35 (m, 1H), 4.74 (m, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.7, 27.3, 28.5, 32.8, 55.2, 66.3, 71.6, 98.2, 127.3, 128.1, 170.7. Anal. calcd for $C_{11}H_{16}O_4S_2$: C, 47.80; H, 5.84. Found: C, 47.95; H, 5.82.



45

Compound 45. A solution of **44** (0.08 g, 0.29 mmol) in THF (2.5 mL) was added in one portion to a stirred suspension of Ra-Ni (W2) (0.7 g, wet) in the same solvent (2.5 mL) at 0 °C and under nitrogen atmosphere. The suspension was stirred for 1h, then the solid was filtered off and washed with THF. The filtrate was evaporated to afford a crude residue which chromatography over silica gel (CH_2Cl_2) gave the pure **45** (0.04 g, 76% yield): oily. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.92-195 (m, 1H), 2.08-2.15 (m, 4H), 3.43 (s, 3H), 4.25-4.32 (m, 3H), 4.88 (d, *J* = 2.5 Hz, 1H), 5.80 (dd, *J* = 2.5 Hz, *J* = 10.3 Hz, 1H), 6.10 (d, *J* = 10.3 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7, 27.1, 55.2, 65.3, 66.8, 95.6, 126.8, 128.3, 170.7. Anal. calcd for $C_9H_{14}O_4$: C, 58.05; H, 7.58. Found: C, 58.30; H, 7.55.



46

Methyl 6-O-acetyl-4-deoxy-α-L-lyxo-hexopyranoside (**46**). To a solution of **45** (0.04 g, 0.22 mmol) in 1:1 (v/v) *tert*-butyl alcohol/acetone (2.5 mL), cooled at 0 °C in ice bath, an excess of a chilled 1:1 (w/w) solution of 4-methylmorpholine-*N*-oxide (0.06 g, 0.44 mmol) in water was added in one portion. After few minutes, a catalytic amount (0.04 mmol) of OsO_4 was added, and the resulting mixture was stirred overnight at room temperature; then the reaction was quenched with saturated aq Na_2SO_3 and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/ acetone, 6:4) afforded the diol **46** (0.04 g, 86% yield) as a single diastereomer: oily. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.58-1.68 (m, 1H), 1.73-1.78 (m, 1H), 2.11 (s, 3H), 2.40 (bs, 2H, exchange with D_2O), 3.37 (s, 3H), 3.72-3.79 (m, 1H), 3.93-4.01 (m, 2H), 4.26 (dd, *J* = 4.4 Hz, *J* = 10.2 Hz, 1H), 4.29 (dd, *J* = 5.4 Hz, *J* = 10.2 Hz, 1H), 4.78 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 20.7, 30.7, 55.1, 63.6, 68.1, 69.8, 101.3, 170.7. Anal. calcd for $C_9H_{16}O_6$: C, 49.09; H, 7.32. Found: C, 49.31; H, 7.30.

4.5 *REFERENCES*

[1] Some recent examples of homologation of shorter-chain sugars: (a) Takahashi, S.; Kuzuhara, H. Simple syntheses of L-fucopyranose and fucosidase inhibitors utilizing the highly stereoselective methylation of an arabinofuranoside 5-urose derivative. *J. Chem. Soc., Perkin Trans. 1* **1997**, 607. (b) Dondoni, A.; Marra, A.; Massi, A. Carbohydrate homologation by the use of 2-(trimethylsilyl)thiazole. Preparative scale synthesis of rare sugars: L-gulose, L-idose, and the disaccharide subunit of bleomycin A_2. *J. Org. Chem.* **1997**, *62*, 6261. (c) Lubineau, A.; Gavard, O.; Alais, J.; Bonnaffé, D. New accesses to L-iduronyl synthons. *Tetrahedron Lett.* **2000**, *41*, 307. (d) Ermolenko, L.; Sasaki, N. A. Diastereoselective synthesis of all eight L-hexoses from L-ascorbic acid. *J. Org. Chem.* **2006**, *71*, 693.

[2] Some recent examples of *C*-5 epimerization of sugars: (a) Ojeda, R.; de Paz, J. L.; Martín-Lomas, M.; Lassaletta, J. M.A new route to L-iduronate building blocks for the synthesis of heparin-like oligosaccharides. *Synlett* **1999**, *8*, 1316. (b) Adinolfi, M.; Barone, G.; De Lorenzo, F.; Iadonisi, A. Intramolecular Tishchemnko reactions of protected hexos-5-uloses: a novel and efficient synthesis of L-idose and L-altrose. *Synlett* **1999**, *3*, 336. (c) Takahashi, H.; Hitomi, Y.; Iwai, Y.; Ikegami, S. A Novel and practical synthesis of L-hexoses from D-glycono-1,5-lactones. *J. Am. Chem. Soc*. **2000**, *122*, 2995. (d) Hung, S.-C.; Wang, C.-C.; Thopate, S. R. Efficient synthesis of L-altrose and L-mannose. *Tetrahedron Lett*. **2000**, *41*, 3119. (e) Boulineau, F. P.; Wei, A. Synthesis of L-sugars from 4-deoxypentenosides. *Org. Lett.* **2002**, *4*, 2281.

[3] Some recent examples of *de novo* syntheses: (a) Ko, S. Y.; Lee, A. W. M.; Masamune, S.; Reed III, L. A.; Sharpless, K. B.; Walker, F.J. Total synthesis of L-hexoses. *Tetrahedron* **1990**, *46*, 245. (b) Harris, J. M.; Keränen, M. D.; Nguyen, H.; Yong, V. G.; O'Doherty, G. A. Synthesis of four D- and L-hexoses via diastereoselective and enantioselective dihydroxylation reactions. *Carbohydr. Res.* **2000**, *328*, 17. (c) Honzumi, M.; Taniguchi, T.; Ogasawara, K. A new convergent route to aldohexoses from a common chiral building block. *Org. Lett.* **2001**, *3*, 1355. (d) Hodgston, R.; Majid, T.; Nelson, A. Towards complete stereochemical control: complementary methods for the synthesis of six diastereomeric monosaccharide mimetics. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1444. (e) Northrup, A. B.; MacMillan, D. W. C. Two-step synthesis of carbohydrates by selective aldol reactions. *Science* **2004**, *305*, 1752. (f) Córdova, A.; Ibrahem, I.; Casas, J.; Sundén, H.; Engqvist, M.; Reyes, E. Amino acid catalyzed neogenesis of carbohydrates: a plausible ancient transformation. *Chem. Eur. J.* **2005**, *11*, 4772.

[4] Guaragna, A.; Napolitano, C.; D'Alonzo, D.; Pedatella, S.; Palumbo, G. A Versatile route to L-hexoses: synthesis of L-mannose and L-altrose. *Org. Lett.* **2006**, *8*, 4863.

[5] In a similar fashion, the homologating system **1** has also been recently employed to prepare 4-deoxy-hexopyranoses belonging to D- or L-series: Caputo, R.; De Nisco, M.; Festa, P.; Guaragna, A.; Palumbo, G.; Pedatella, S. Synthesis of 4-deoxy-L-(and D-) hexoses from chiral noncarbohydrate building blocks. *J. Org. Chem.* **2004**, *69*, 7033.

[6] Hubschwerlen, C.; Specklin, J.-L.; Higelin, J. L-(*S*)-Glyceraldehyde acetonide. *Org. Synth.* **1995**, *72*, 1.

[7] For examples of bioactive 2,3-dideoxy-L-hexopyranoside based compounds see: Guppi, S. R.; Zhou, M.; O' Doherty, G. A. *De novo* asymmetric synthesis of homoadenosine *via* a palladium-catalyzed *N*-glycosylation. *Org. Lett.* **2006**, *8*, 293. For their enantiomers see: Bohringer, M.; Roth, H. J.; Hunziker, J.; Gobel, M.; Krishnan, R.; Giger, A.; Schweizer, B.; Schreiber, J.; Leumann, C.; Eschenmoser, A. Warum Pentose- und nicht Hexose-Nucleinsauren? Teil II) Oligonucleotide aus 2',3'-Dideoxy-β-D-glucopyranosyl-Bausteinen (`Homo-DNS'): Herstellung. *Helv. Chim. Acta* **1992**, *75*, 1416.

[8] Cha, J. K.; Christ, W. J.; Kishi, Y. On stereochemistry of osmium tetraoxide oxidation of allylic alcohol systems. Empirical rule. *Tetrahedron* **1984**, *40*, 2247.

[9] Yang, D.; Wong, M.-K.; Yip, Y.-C Epoxidation of olefins using methyl(trifluoromethyl)dioxirane generated *in situ*.
J. Org. Chem. **1995**, *60*, 3887.

[10] For similar results see: Kim, K. S.; Moon, C. W.; Park II, J.; Han, S.-H. Enantiopure synthesis of carbohydrates mediated by oxyselenylation of 3,4-dihydro-2*H*-pyran. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1341.

[11] Fieser L. F.; Goto, T. Oxido alcohols and ketoxides. *J. Am. Chem. Soc.* **1960**, *82*, 1693.

[12] Barili, P. L.; Berti, G.; Catelani, G.; Colonna, F. Mastrorilli, E. Alkaline and enzymatic hydrolysis of isobutyl 3,4-anhydro-2,6-dideoxy-DL-hexopyranosides. Preparation of enantiomeric boivinopyranosides through a highly efficient kinetic resolution. *J. Org. Chem.* **1987**, *52*, 2886.

[13] Donohoe, T. J.; Blades, K.; Moore, P. R.; Waring, M. J.; Winter, J. J. G.; Helliwell, M.; Newcombe, N. J.; Stemp, G. Directed dihydroxylation of cyclic allylic alcohols and trichloroacetamides using OsO_4/TMEDA. *J. Org. Chem.* **2002**, *67*, 7946.

[14] Dondoni, A.; Massi, A.; Minghini, E. Dihydropyridine *C*-glycoconjugates by *Hantzsch* cyclocondensation. Synthesis of a *C*(6)-glycosylated nifedipine analogue. *Helv. Chim. Acta* **2002**, *85*, 3331.

[15] (a) Jung, M. E.; Martinelli, M. J. Iodotrimethylsilane, in *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. Ed., John Wiley & Sons, Ltd., New York, **1998**. (b) Jung, M. E.; Lyster, M. A. Quantitative dealkylation of alkyl ethers via treatment with trimethylsilyl iodide. A new method for ether hydrolysis. *J. Org. Chem.* **1977**, *42*, 3761.

[16] (a) Bols, M. 1,6-Anhydro sugars, in *Carbohydrate building blocks.* Wiley, New York, **1996**. (b) Kulkarnia, S. S.; Leeb, J.-C.; Hung, S.-C. Recent advances in the applications of D- and L-form 1,6-anhydrohexopyranoses for the synthesis of oligosaccharides and nature products. *Curr. Org. Chem.* **2004**, *8*, 475.

[17] For an example of 1,6-anhydrosugars as useful synthons in organic synthesis, see: Lee, J.-C.; Chang, S.-W.; Liao, C.-C.; Chi, F.-C.; Chen, C.-S.; Wen, Y.-S.; Wang, C.-C.; Kulkarni, S. S.; Puranik, R.; Liu, Y.-H.; Hung, S.-C. From D-glucose to biologically potent L-hexose derivatives: synthesis of α-L-iduronidase fluorogenic detector and the disaccharide moieties of bleomycin A_2 and heparan sulfate. *Chem. Eur. J.* **2004**, *10*, 399.

[18] Černý, M.; Stanek, J. 1,6-anhydro derivatives of aldohexoses. *Adv. Carb. Chem. Biochem.* **1977**, *34*, 23.

[19] (a) Lauer, G.; Oberdorfer, F. A simple route from glucal to Cerny epoxides. *Angew. Chem. Int. Ed.* **1993**, *32*, 273. (b) Sharma, G. V. M.; Venkataramanaiah, K. C.; Krishnadu, K. Còlay montmorillonite in carbohydrates: use of "claysil" as an efficient heterogeneous catalyst for the intramolecular Ferrier reactions leading to 1,6-anhydro rare saccharides. *Tetrahedron: Asymmetry* **1994**, *5*, 1905-1908.

Chapter 5

IMINOSUGARS: HIGH POTENTIAL MULTI-TARGETED INHIBITORS OF CARBOHYDRATE PROCESSING ENZYMES

5. IMINOSUGARS: HIGH POTENTIAL MULTI-TARGETED INHIBITORS OF CARBOHYDRATE PROCESSING ENZYMES

The essential role of carbohydrates in biology has made the control of carbohydrate biochemistry an important target for pharmaceutical research. One of the focuses of this research is usually the search for enzyme inhibitors of carbohydrate metabolism, since typically carbohydrates themselves are degraded too rapidly in vivo. *Therefore, scientists are focusing their research on the design and synthesis of compounds with improved enzyme stability, to use as carbohydrate analogues. To meet this demand, the term* glycomimetics *has emerged to describe a group of artificially or naturally derived compounds which have shapes and polarities resembling sugars. Among them, iminosugars (monosaccharide mimetics) have readily become an interesting subject of investigation, because of their deep interfering with the function of carbohydrate handling enzymes and therefore utilizable as targets for several and diverse therapeutical purposes.*

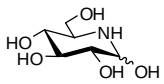
5.1 *INTRODUCTION*

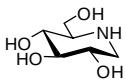
The possible exploitations of enzymes acting on carbohydrate biochemistry are so wide that the study of such enzymes has become one of the major research areas over the past decades.[1] Given their ubiquitous significance, inhibitors of carbohydrate relating enzymes are expected to play an important role every time their activity needs to be regulated. Among them, several reversible inhibitors, such as iminosugars, have emerged as versatile tools for biochemists and cell biologists, especially those in the quest for new therapeutic agents.[2] Some representatives of this class of compounds are already marketed as pharmaceuticals against certain forms of diabetes;[3] quite a few others exhibit promising anti-infective properties.[4] Actually, the large variety of structures and their remarkable biological activities have posed pertinent challenges for scientists in many areas.

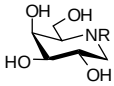
Iminosugars (also known as azasugars) represent sugar analogues with a nitrogen atom in place of the ring oxygen of the corresponding carbohydrate.[5] Strictly speaking, iminosugars belong to the polyhydroxylated alkaloid family: in fact, the iminosugar motif can be recognised in five classes, based on their structures: polyhydroxylated pyrrolidines, piperidines, indolizidines, pyrrolizidines, and nortropanes (**Figure 1**).

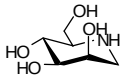
Piperidine Iminosugars

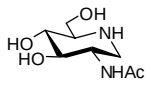
a) polyhydroxylated piperidines


Nojirimycin, NJ (**1**)

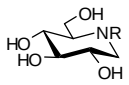

Deoxynojirimycin, DNJ (**2**)

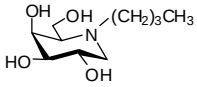

Galacto-DNJ (DGJ, **3**)

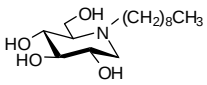

Manno-DNJ (DMJ, **4**)

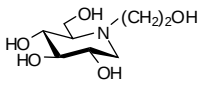

2-acetylamino-2-deoxy-DNJ (**5**)

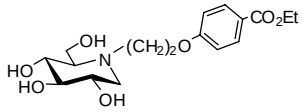
b) *N*-substituted piperidines


R = Me-, *N*-Methyl-DNJ (*N*M-DNJ, **6**)
R = *n*Bu-, *N*B-DNJ (OGT 918, Zavesca®, **7**)

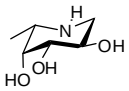

*N*B-DGJ (**8**)

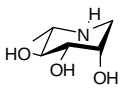

N-nonyl-DNJ (*N*N-DNJ, **9**)

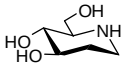

Miglitol® (**10**)

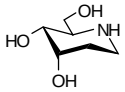

Miglitate (**11**)

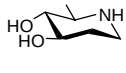
c) deoxy iminosugar analogues


Fuco-DNJ (**12**)

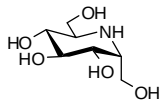

Rhamno-DNJ (**13**)

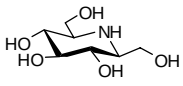

Fagomine (**14**)

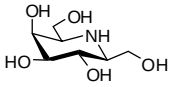

3-*epi*-fagomine (**15**)

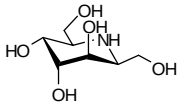

6-deoxy-fagomine (**16**)

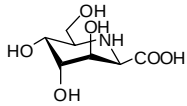
d) piperidine homologues


α-homo-nojirimycin (α-HNJ, **17**)

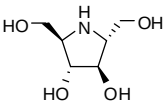

β-homo-nojirimycin (β-HNJ, **18**)

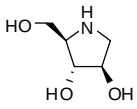

β-homo-*galacto*-NJ (**19**)

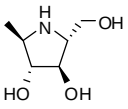

β-homo-*altro*-NJ (**20**)

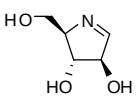

1-carboxy-β-homo-*altro*-NJ (**21**)

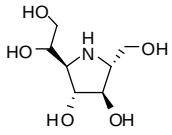
Pyrrolidine Iminosugars


2,5-dideoxy-2,5-imino-D-mannitol (DMDP, **22**)

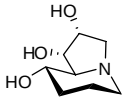

D-AB-1 (**23**)

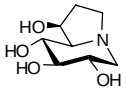

6-deoxy-DMDP (**24**)

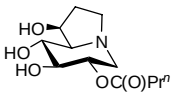

Nectrisine (**25**)

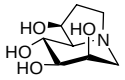

Homo-DMDP (**26**)

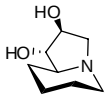
Indolizidine Iminosugars


Swainsonine (**27**)

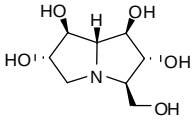

Castanospermine (**28**)

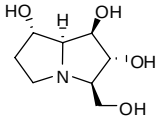

6-O-Butanoyl-castanospermine (MDL 28574, **29**)

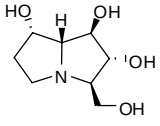

6-*epi*-castanospermine (**30**)

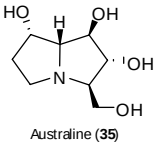

Lentiginosine (**31**)

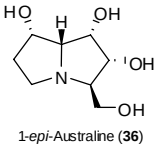
Pyrrolizidine Iminosugars


Casuarine (**32**)

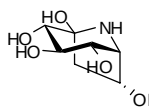

Alexine (**33**)

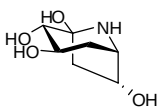

7a-*epi*-alexine (**34**)

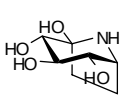

Australine (**35**)

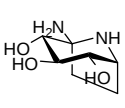

1-*epi*-Australine (**36**)

Nortropane Iminosugars


Calystegine C_1 (**37**)


Calystegine B_1 (**38**)


Calystegine B_2 (**39**)


Calystegine N_1 (**40**)

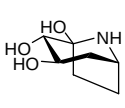

Calystegine A_3 (**41**)

Figure 1 – *The five classes of naturally occurring iminosugars.*

The great deal of attention around iminosugars lies in the fact that they are powerful inhibitors of glycosidases and glycosyltransferases. From a pharmaceutical point of view, as alterations in biosynthesis and function of these enzymes are implicated in a wide variety of diseases,[2,4-5] the significant inhibitory properties of iminosugars make them excellent targets for medical intervention.

5.2 *CARBOHYDRATE PROCESSING ENZYMES: BIOLOGICAL SIGNIFICANCE AND RELATED DISEASES*

Current scientific evidence demonstrates that Nature abounds with enzymes that act on glycosidic linkage. Virtually, all organisms need them to break down polysaccharides to give smaller molecules, so providing the energy for survival; quite a few others need them to promote glycosylations, affording the highly complex glycolipid chains, necessary to recognise and interact with the external environment. Depending on the kind of reaction involved in the anomeric centre of the sugars, such enzymes can be classified as:

a. Glycosidases (*glycoside hydrolases*), responsible for the hydrolysis of oligo- and/or polysaccharides. Glycosidases are involved in the metabolism of the oligosaccharide chains and in the control mechanisms of the *N*-linked glycoproteins, which role, depending on their nature and collocation in the cell, may range from cell-cell communication to tissue organization, cell growth, etc.[6]
b. Glycosyltransferases (*glycoside synthases*), responsible for the transfer of a glycosyl unit from an oxygen atom to another one; although less available than glycosidases, hundreds of glycosyltransferases are estimated to be implicated in the assembly of the many existing saccharide structures. Most of them have been found to be involved in glycoprotein construction.[7] Therefore, drug-induced alterations in the activity of these enzymes can have profound effects on control, maturation, transport, and secretion of glycoproteins and can alter cell-cell (or cell-virus) recognition processes.

While the hydrolysis/assembly of glycosides is highly regulated by the healthy body, the unregulated activity of such enzymes may be the cause for numerous severe diseases.

5.2.1 *GLYCOSIDASE INHIBITION: ENZYME DYSFUNCTIONS AND IMINOSUGAR-BASED THERAPEUTIC AGENTS*

a) Intestinal glycosidases and Diabetes Mellitus treatment. Intestinal glycosidases are integrated into the cell membranes of the brush border region in the small intestine, where they break down dietary oligosaccharides to give monosaccharides. Already in the late 1970s it was realized that inhibition of all or some intestinal glycosidases (as well as pancreatic α-amylase) could regulate the absorption of carbohydrates and could be used therapeutically in the oral treatment of the non-insulin-dependent diabetes mellitus (type II diabetes).[8] In this context, several iminosugars were found to inhibit various α-glucosidase specific disaccharidases involved in mammalian digestion (sucrase, maltase, isomaltase, etc.).[2] One of the first candidates was DNJ (**2**): it showed excellent α-glucosidase inhibitory activity *in vitro*, but its efficacy *in vivo* was only moderate.[9] Therefore, a large number of DNJ derivatives was prepared with the aim to increase the *in vivo* activity. For instance, fagomine (2-deoxy-DNJ, **14**) was found to significantly reduce blood glucose levels; castanospermine (**28**) showed strong activity as well, but its toxicity was too great. Out of a large number of *in vitro* active agents, *N*-substituted iminosugars were found as the most favourable inhibitors. In fact, both *N*-hydroxyethyl-DNJ (**10**, Miglitol®) and the analogous Miglitate (**11**) gave very good and long-term inhibition *in vivo*.[10] In 1996, Miglitol was approved by the U.S. Food and Drug Administration (FDA) and in 1999 it was introduced into the market as a potent "last-generation" α-glucosidase inhibitor with few gastrointestinal side effects.

b) Processing α-glucosidase I regulation: iminosugars as antiviral agents. Many mammalian viruses have a heavily glycosylated lipoprotein envelope. The viral envelope of glycoproteins is often essential for virion assembly, secretion and/or infectivity (carbohydrates are used to recognise and interact with preferred hosts). Therefore, compounds that interfere with glycoside assembly of viral glycoproteins can be expected to show antiviral activity.[11] For instance, α-glucosidase inhibitors such as DNJ (**2**), *N*B-DNJ (Zavesca®, **7**), castanospermine (**28**) and 6-*O*-butanoyl castanospermine (**29**, MDL 28574) are able to inhibit human immunodeficiency virus (HIV) replication[12] and HIV-mediated syncytium formation[13] *in vitro,* although these compounds are not as potent as AZT (respective EC_{50} values: 560, 56, 29 and 1.1 μM against 0.1 μM). On another side, the treatment of glycosidase inhibitors against Hepatitis B Virus (HBV) had the result to hinder the transport of the virus out of the host cell: for instance, *in vitro* assay of *N*B-DNJ (**7**) led to a high percentage of virus particles being retained inside the cells.[14]

Moreover, *N*-nonyl-DNJ (*N*N-DNJ, **9**) showed to be 100-200 times more potent than *N*B-DNJ in inhibiting HBV in cell-based assays. *N*N-DNJ, compared with *N*B-DNJ, exhibited also a prolonged hepatic retention of bovine viral diarrhea virus, a tissue culture surrogate of human Hepatitis C virus.[15] Therefore, although not preferable against HIV in comparison with the current clinically used drugs, the employ of iminosugars against HBV and HCV could be of great therapeutic value.

c) Aberrant glycosylation and cancer treatment. There is an increasing evidence that glycosylation plays a key role in the formation of tumour cells.[16] Particularly, oligosaccharides on the surface of tumor cells are believed to have an important function in malignant phenotype and tumor growth. It has been also shown that high levels of many glycosidases are present in some tumour cells and interstitial fluids. It's not yet understood why tumour cells should show abnormal glycosidation, but it has been assumed it could occur next to an alteration of the glycosyltransferase structures.[17] Therefore, a line of treatment has involved the inhibition of such a kind of enzymes associated with cancer. Castanospermine (**28**) and *N*-methyl-DNJ (**6**) demonstrated to have anti-metastatic activity by inhibiting platelet aggregation as well as reducing vascular endothelium adhesion of tumour cells.[18] To date, swainsonine (**27**) has attracted the most attention as it inhibits tumor growth and stimulates the immune response.*,[19] This latter was found to be an excellent inhibitor of Golgi α-mannosidase II (K_i = 40 nM) with low toxicity and good oral accessibility.[20] Given such hopeful results, **27** was also evaluated in human patients as chemotherapeutic agent. However, results have so far been not so promising, since patients suffered adverse side effects. In fact, due to co-inhibition of lysosomal mannosidase, swainsonine caused the accumulation of high-mannose containing oligosaccharides in tissues (swainsonine induced mannosidosis).[21] Therefore, synthetic analogues of swainsonine are currently under investigation.

5.2.2 *INSIGHT INTO THE MECHANISM FOR GLYCOSIDASE INHIBITION: A CLOSE RELATIONSHIP BETWEEN IMINOSUGARS AND THE TRANSITION STATE OF GLYCOSIDE HYDROLYSIS*

At first sight, a simple explanation for the strong affinity of iminosugars for glycosidases lies in the similarity between the proposed transition state of glycoside hydrolysis and the iminosugar

* Early studies indicated that swainsonine activate natural anti-tumor immunity and enhance the production of T-cells [Ref. 19].

scaffold[22] (**Figure 2**). The break of glycosidic bond results in a developing positive charge on the anomeric carbon; this charge is shared with the endocyclic oxygen, originating an oxocarbenium-ion-like character. This fact results in a double bond character between the endocyclic oxygen and the anomeric carbon, distorting the ring to a half-chair conformation. Glycosidases stabilize this positive charge with anionic active-site residues (such as aspartate and glutamate).[23]

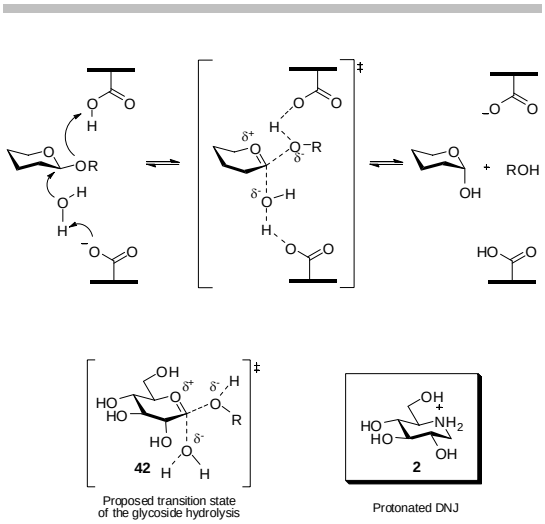


Figure 2 - *Comparison of the transition state of glycoside hydrolysis with DNJ (**2**). The picture depicts the most accredited mechanism for glycoside hydrolysis, the one which occurs with inversion of configuration.*

As this mechanism occurs in enzymatic glycoside cleavage, compounds that are able to resemble the oxocarbenium ion should be inhibitors of glycosidases. In fact, when the amine function of the iminosugar is protonated under physiological conditions, the resulting ammonium ion would be expected to mimic the partial positive charge developing on the endocyclic oxygen and bind to the anionic residues.[24]

Although there are strong parallels between iminosugars and the proposed transition state of glycoside hydrolysis, some controversies are associated with this argument.[25] For example, it could be noticed that DNJ (**2**) and its analogues lack the sp^2 character of *C*-1 and ring-oxygen present in ion **42**, and thus they are imperfect analogues of the transition state.* This may be true, but as these derivatives bind 10^3-10^4 times more strongly to glycosidases than glucose, the

* Actually, quite a few number of iminosugars provided of the suitable structural distortion to better resemble the transition state has been synthesized; however, the reported glycosidase inhibition for such compounds was weaker than DNJ [Ref. 26].

nitrogen must play an important role in the interaction. Therefore, the strong and general inhibition of glycosidases by DNJ and derivatives seems best explained by the nitrogen being protonated in the active site, so that the compounds resemble transition state ion **42** in terms of charge.[26]

5.2.3 *GLYCOSYLTRANSFERASE INHIBITION: LYSOSOMAL STORAGE DISORDERS*

Lysosomal diseases (also known as glycosphingolipid storage diseases) are relatively rare hereditary disorders (that are severe in nature and frequently fatal) due to a lack in the activity of specific enzymes involved in the catabolism of glycosphingolipids in lysosomes.[27]

Glycosphingolipids (GSLs) are ubiquitous components of the cell membranes, having several critical functions including cell adhesion, cell growth regulation and differentiation. GSLs are derived from a common biosynthetic pathway, that takes place in the cytosol, endoplasmic reticulum and Golgi apparatus; it starts with the condensation reaction of palmitoyl-CoA and serine (to produce ceramide farther on) and ends with the synthesis of Ganglio-,[28] Globo- and Lacto-[29] series oligosaccharides (**Figure 3**).

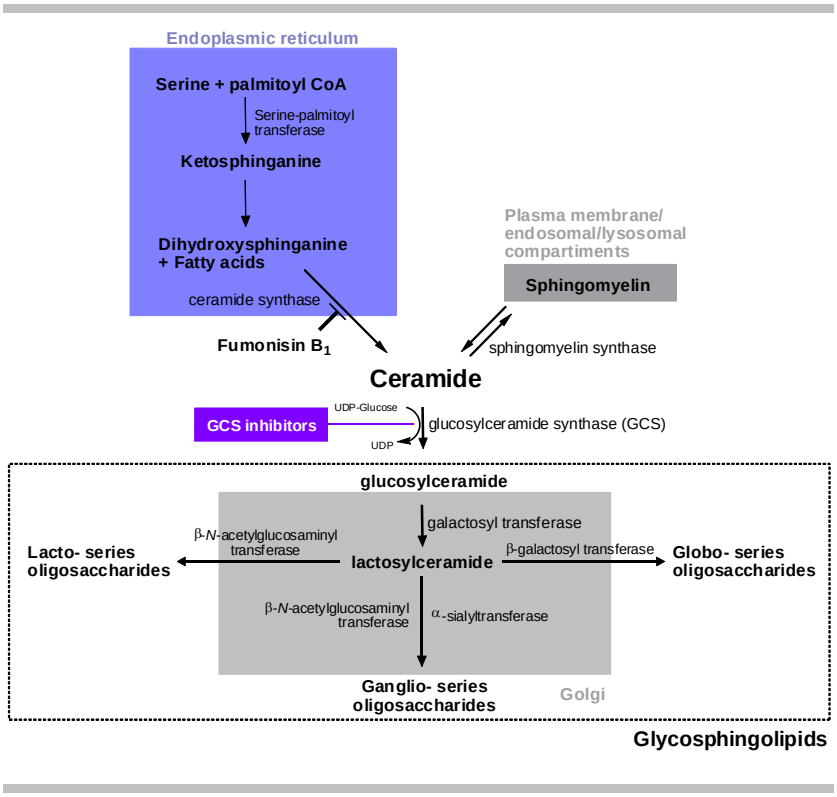


Figure 3 – *Different cellular compartments involved in the GSL biosynthetic pathway.*

Catabolism is essentially the reverse path, even if, in this case, specialized proteins are needed to assist the catalysis. Reactions take place in a specialized organelle, the lysosome, where glycon-specific acid glycosidases hydrolyze glycosphingolipids from the nonreducing terminus (**Figure 4**). Both the monosaccharides and the ceramide moiety can be scavenged for re-utilization in the cytosol.

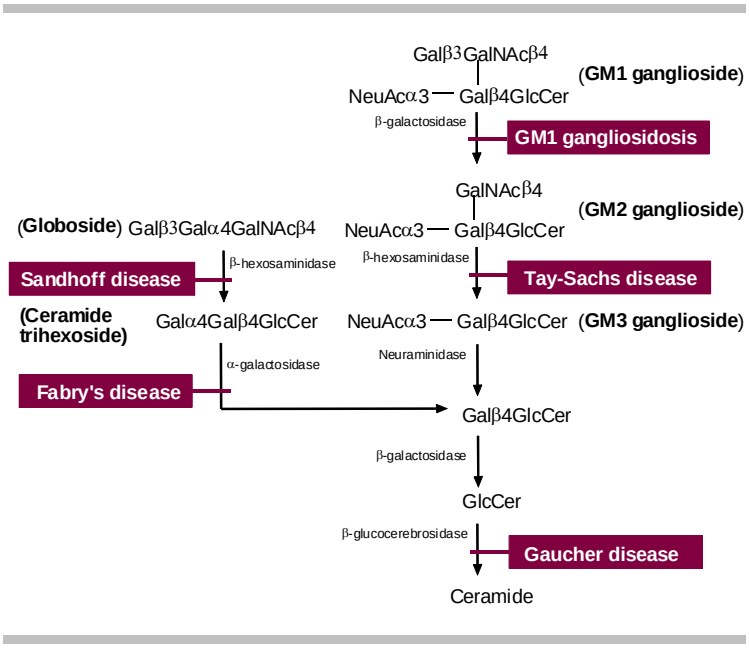


Figure 4 – *Lysosome located GSL catabolic pathway.*

As depicted in Figure 4, each GSL storage disease is associated with the deficiency of a specific enzyme. For example, among the most severe and studied pathologies, Gaucher's disease implies for the accumulation of lysosomal glucosylceramide due to deficiencies in the activity of ceramide α-glucosidase;[30] conversely, Fabry's disease results from a deficiency in the activity of lysosomal α-galactosidase, accumulating those glycosphingolipids containing a terminal α-galactose moiety, such as ceramide trihexoside or di-galactosylceramide, in the lysosomes of several tissues including heart, kidney, spleen and liver.[31]

The existing strategies for overcoming the deficit in enzyme capacity provide for: **a)** endogenous supply of fully functional enzyme (by direct infusion); **b)** cellular replacement with cells capable of secreting enzyme (bone marrow replacement); **c)** gene delivery. Besides these, an alternative route is to reduce the influx of substrate to the lysosome by inhibiting the synthesis of glycosphingolipids. This strategy has been called *substrate deprivation*.[32] By balancing the rate of GSL synthesis with the impaired rate of GSL breakdown, it should be

possible to regulate substrate influx/efflux to rates that do not lead to storage. In other words, if the storage and disease phenotype is caused by an accumulation of substrate, then a partial inhibition of substrate synthesis by an inhibitor should be an effective strategy.[33]

*N*B-DNJ (**7**) and *N*B-DGJ (**8**) were discovered to be specific and powerful inhibitors of glycosyltransferase-catalyzed biosynthesis of glucosylceramide[34] and galactosylceramide.[35] With particular regard to *N*B-DNJ, the results were so impressive that the route towards the approval for its clinical use was relatively fast: in 1999-2000, Oxford Glycosciences performed multiple clinical studies using *N*B-DNJ in type 1 Gaucher disease patients in Europe. The result of clinical trials indicated that *N*B-DNJ (**7**) was effective in depleting GSLs and led to an improvement in clinical parameters measuring disease proceeding.[36] Few years later (2002-2003), Zavesca® became one of the first iminosugar medicines to receive approval, both in the European Union and in the United States, for its use in patients with mild to moderate type 1 Gaucher disease.

5.2.4 *INSIGHT INTO GSL BIOSYNTHESIS REGULATION: STRUCTURAL RESEMBLANCE OF* N-*ALKYLATED IMINOSUGARS WITH A GSL METABOLITE*

*N*B-DNJ (**7**) and *N*B-DGJ (**8**) are inhibitors of ceramide-specific glucosyltransferase in tissue-cultured cells and in *in vitro* assays (K_i = 7.4 μM for *N*B-DNJ, K_i = 10.6 μM for *N*B-DGJ). The type of inhibition is reversible and competitive for ceramide and noncompetitive for UDP-glucose.[37]

As observed, the molecular requirements of iminosugars provided with transferase inhibitory properties are the *N*-alkyl chain length and ring stereochemistry. Moreover, some of the molecular features that contribute to the action mechanism of *N*-alkylated iminosugar inhibitors of ceramide specific glucosyltransferases have been recently determined.[37] The presence of an *N*-alkyl chain is obligatory for transferase inhibition and an increase in alkyl chain length (such as in *N*N-DNJ, **9**) provides an increase in inhibitory potency, perhaps reflecting a greater ceramide mimicry.[38] Through ring substitutions it was identified that *C*-4 hydroxyl group was critical for both enzymes, furthermore also *C*-1 and *C*-6 modifications led to a loss of transferase inhibition. Attempts to rationalize these data for transferase inhibition have been realized by studying energy-minimized molecular models of *N*B-DNJ (**7**) and ceramide (**43**, **Figure 5**). Such study showed a structural homology of three chiral centers and the *N*-alkyl chain of *N*B-DNJ and *N*B-DGJ, with the *trans*-alkenyl and *N*-acyl chain of ceramide.[37]

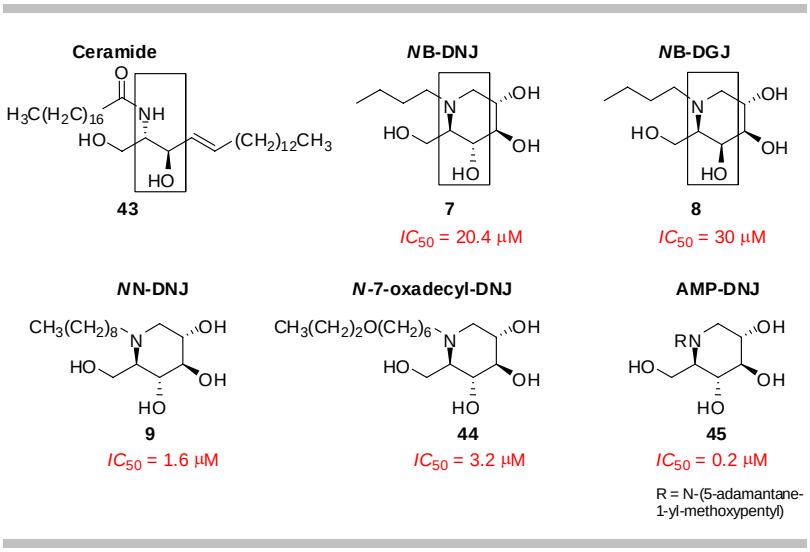


Figure 5 - *Possible overlay of DNJ derivatives and ceramide (**43**).*

On the basis of these studies, modifications on iminosugars have been suggested to improve mimicry and thus potency. As mentioned above, these ones include alterations of the alkyl chain (chain length, saturation, and hydroxylation) and modifications of hydroxyl group residues (i.e. their removal or alkylation). Early modifications to **7** and **8** led to even higher-activity molecules, as reported for *N*-nonyl-DNJ (**9**), *N*-oxadecyl-DNJ (**44**) and *N*-(5-adamantane-1-yl-methoxypentyl)-DNJ (AMP-DNJ, **45**), some of the most potent target compounds so far available.[39]

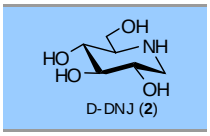
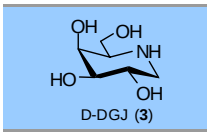
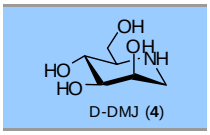
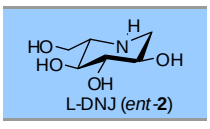
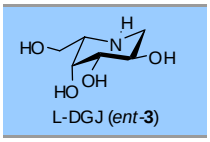
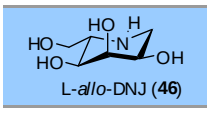
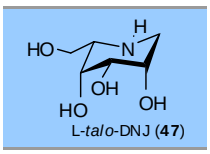
Because of the above discussed broad inhibitory properties and their wide applications for several medical purposes, an enormous body of literature is appeared in the last years, focused on the synthesis, isolation, and biological evaluation of iminosugars.[40] As reported, most syntheses have fixed attention on the preparation of iminosugars with D-configuration, whereas few routes are available for the synthesis of their corresponding L-analogues.[41] This fact is evidently due to the larger commercial availability of D-series sugars as starting materials, as well as to the fact that glycosides belonging to D-series are the natural substrates of almost all enzymes (glycosidases and glycosyl transferases are obviously included). However, in recent times several interesting early reports on the biological activities of L-iminosugars are appeared,[42] turning attention on an unpredicted and underestimated class of potential drug candidates.

5.3 *L-IMINOSUGARS: AN UNEXPECTED NEW SOURCE OF ENZYME INHIBITORS*

The enzymes tipically distinguish between the desired substrate and its mirror image. This fact can be easily explained considering that each enzyme has an active site that only accepts one of the enantiomers but rejects the other one. This may be the possible reason why it has been so difficult to attract attention on the L-iminosugars: it has been simply assumed that optically active compounds resembling natural carbohydrates should be invariably more active than the "unnatural" L-enantiomers. However, the recent reports about the biological activity exhibited by L-iminosugars in the same "investigation field" of their D-counterparts (i.e. as glycosidase inhibitors) don't mean that the enzymes implicated lack in enantioselectivity (as far as it should not represent a surprise, see Chapter 7), but probably that L-iminosugars can inhibit them with a different, but still efficient, mechanism of action.

a) L-Iminosugars as glycosidase inhibitors. Although D-DNJ (**2**) and D-*galacto*-DNJ (**3**) are known to be powerful competitive inhibitors of α-glucosidase (IC_{50} = 0.03 μM) and α-galactosidase (IC_{50} = 0.003 μM), respectively, L-DNJ (***ent*-2**) and L-*galacto*-DNJ (***ent*-3**) demonstrated to be inhibitors of α-glucosidase (IC_{50} = 4.3 μM) and α-galactosidase (IC_{50} = 13 μM) as well (**Table 1**). However, iminosugars mimicking the sugar moiety structure of the natural substrate are not always inhibitors of the corresponding glycosidase. D-*manno*-DNJ (D-DMJ, **4**) is known as a better inhibitor of α-L-fucosidase (IC_{50} = 39 μM) than α-D-mannosidase (IC_{50} = 840 μM); on the other hand L-*allo*-DNJ (**46**), is a much better inhibitor (IC_{50} = 30 μM) of α-D-mannosidase (from *Jack Bean*) than D-DMJ (IC_{50} = 840 μM).[43] As recently shown, an explanation of this behaviour could be found considering that D-enantiomers are competitive inhibitors of D-glycosidases, whereas their L-enantiomers are non-competitive inhibitors of the same enzymes.

Table 1 - *Glycosidase inhibition by several enantiopure iminosugar derivatives*

Iminosugar derivatives	IC_{50}(μM) α-glucosidase: -rice -rat intestinal maltase	β-glucosidase: almond	α/β-mannosidase: -Jack bean -rat epididymis	α/β-galactosidase: -coffee bean -*Aspergillus Niger* -bovine liver	α-fucosidase: bovine epididymis
 D-DNJ (**2**)	0.03-0.65	80	/	880 (α)	/
 D-DGJ (**3**)	/	/	/	0.003-1.8 (α) 24 (β)	/
 D-DMJ (**4**)	110	/	560-840 (α)	/	39
 L-DNJ (*ent*-**2**)	4.3-28	980	/	560 (α)	/
 L-DGJ (*ent*-**3**)	/	/	/	13 (α) 560 (β)	0.63
 L-*allo*-DNJ (**46**)	/	/	30-59 (α)	290(α)	/
 L-*talo*-DNJ (**47**)	10^{-3}*	/	/	/	$1.6 \cdot 10^{-3}$*

*: K_i value (μM).

b) L-Iminosugar derivatives as new potential immunosuppressive agents. Very recently a further pharmacological application of iminosugars is emerged because of the exhibited inhibition effects on immune system.[44] Particularly, first data demonstrated that a selected class of iminosugars (**48, 49** and *ent*-**21, Figure 6**) displayed comparable activity to cyclosporin A (CyA), which is a well-known immunosuppressive drug.[45] Compared with D-iminosugars **19**

and **21**, the L-analogues **48, 49** and *ent*-**21** had stronger reduction effects on the immune system (the compound *ent*-**21** having the strongest inhibition effect, holding reduction efficiency to both humoral response and cell-mediated immune system). Moreover, it has been found these L-iminosugars are much less toxic than CyA. Overally, all data converge to confirm the high potential that L-iminosugars hold as a new class of drug candidates possessing immunosuppressive activity.

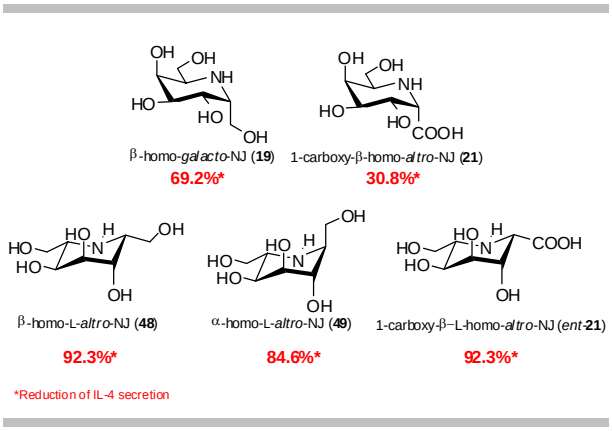


Figure 6 – *Comparison between* D- *and* L-*iminosugars as immunosuppressive agents.*

5.4 *CONCLUDING REMARKS AND FUTURE PERSPECTIVES*

The alteration of glycosidase and glycosyltransferase activity has become a process of widespread scientific interest, because of their involvement in a broad range of anabolic and catabolic events. Among the numerous inhibitors in use for medical purposes, polyhydroxylated alkaloids such as piperidine iminosugars have demonstrated to be some of the most successful and intriguing molecules, due to their structural relationship to natural carbohydrates. As a result, isolation of such inhibitors from natural sources or their chemical synthesis has provided biochemical tools for the elucidation of enzyme mechanistic activity through the use of kinetic data combined with variations in potential inhibitor structural information. In parallel, a rational design towards novel iminosugar-based compounds has been required by the current knowledge of structure/activity relationships.

In addition to such valuable approaches, an emerging role of L-series iminosugars is occurring in many fields of investigation. These range from glycosidase inhibition, in which a noncompetitive mode of action has been recognized, to the cellular immune system regulation,

where a very promising role as immunosuppressive agents is arising. Because of the overlooked interest in L-iminosugars for many years, much remains to be discovered, in consideration of their perspective use for therapeutic applications. Such findings may therefore lead to further insights into carbohydrate related processes in health and disease, opening new avenues in the development of a new class of drug candidates.

5.5 *REFERENCES*

[1] Dwek, R. A. Glycobiology: toward understanding the function of sugars. *Chem. Rev.* **1996,** *96*, 683.
[2] Asano, N. Glycosidase inhibitors: update and perspectives on practical use. Glycobiology **2003**, *13*, 93R.
[3] Schmidt, D. D., Frommer, W., Müller, L., Truscheit, E. Glucosidase inhibitors from bacilli. *Naturwissenshaften* **1979**, *66*, 584.
[4] Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. Polyhydroxylated alkaloids – natural occurrence and therapeutic applications. *Phytochemistry* **2001**, *56*, 265.
[5] (a) Stütz, A., Ed. *Iminosugars as Glycosidase Inhibitors*; *Nojirimycin and Beyond*; Wiley-VCH: Weinheim, Germany, **1999**. (b) Asano, N.; Hashimoto, H. Azaglycomimetics: synthesis and chemical biology, in *Glycoscience*, B. O. Fraser-Reid, K. Tatsuta, J. Thiem, Eds. (Springer, Berlin, **2001**), Chapter 10.2, 2542.
[6] Sears, P.; Wong, C.-H. Enzyme action in glycoprotein synthesis. *Cell. Mol. Life Sci.* **1998**, *54*, 223.
[7] Varki, A. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* **1993**, *3*, 97.
[8] It deals with the use of a carbocyclic analogue of glucose, Acarbose: Schmidt, D. D.; Frommer, W.; Müller, L.; Junge, B.; Wingender, W.; Truscheit, E. α-Glucosidase inhibitors, new complex oligosaccharides of microbial origin. *Naturwissenshaften* **1977**, *64*, 535.
[9] Junge, B.; Matzke, M.; Stltefuss, J. Chemistry and structure-activity relationships of glucosidase inhibitors, in *Handbook of experimental pharmacology*, Kuhlmann, J. and Puls, W. (Eds.), Springer-Verlag, New York, **1996**, 411.
[10] (a) Joubert, P. H.; Foukaridis, G. N.; Bopape, M. L. Miglitol may have a blood glucose lowering effect unrelated to inhibition of α-glucosidase. *Eur. J. Clin. Pharmacol.* **1987**, *31*, 723. (b) Joubert, P. H.; Venter, H. L.; Foukaridis, G. N. The effect of miglitol and acarbose after an oral glucose load: a novel hypoglycaemic mechanism? *Br. J. Clin. Pharmacol.* **1990**, *30*, 391.
[11] Robina, I.; Moreno-Vargas, A. J.; Carmona, A. T.; Vogel, P. Glycosidase inhibitors as potential HIV entry inhibitors? *Curr. Drug Met.* **2004**, *5*, 329.
[12] Ficher, P. B.; Collin, M.; Karlsson, G. B.; Lames, W.; Butters, T. D.; Davis, S. J.; Gordon, S.; Dwek, R. A.; Platt, F. M. The α-glucosidase inhibitor *N*-butyldeoxynojirimycin inhibits Human Immunodeficiency Virus entry at the level of post-CD4 binding. *J. Virol.* **1995**, *69*, 5791.
[13] Fu, Y.-K.; Hart, T. K.; Jonak, Z. L.; Bugelski, P. J. Physicochemical dissociation of CD4-mediated syncytium formation and shedding of Human Immunodeficiency Virus type 1 gp120. *J. Virol.* **1993**, *67*, 3818.
[14] Block, T. M.; Lu, X.; Platt, F. M.; Foster, G. R.; Gerlich, W. H.; Blumberg, B. S.; Dwek, R. A. Secretion of human Hepatitis B Virus is inhibited by the iminosugar *N*-butyldeoxynojirimycin. *Proc. Natl Acad. Sci. USA* **1994**, *91*, 2235.
[15] Zitzmann, N.; Mehta, A. S.; Carrouée, S.; Butters, T. D.; Platt, F. M.; McCauley, J.; Blumberg, B. S.; Dwek, R. A.; Block, T. M. Iminosugars inhibit the formation and secretion of bovine viral diarrhoea virus, a pestivirus model of Hepatitis C Virus: implications for the development of broad spectrum anti-hepatitis virus agents. *Proc. Natl Acad. Sci. USA* **1999**, *96*, 11878.
[16] Nishimura, Y. *Gem*-diamine 1-*N*-iminosugars and related iminosugars, candidate of therapeutic agents for tumor metastasis. *Curr. Top. Med. Chem.* **2003**, *3*, 575.
[17] Goss, P. E.;Baker, M. A.; Carver, J. P.; Dennis, J. W. Inhibitors of carbohydrate processing: a new class of anticancer agents. *Clin. Canc. Res.* **1995**, *1*, 935.
[18] Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and prospective therapeutic application. *Tetrahedron: Asymmetry* **2000**, *11*, 1645.
[19] Hino, M.; Nakayama, O.; Tsurumi, Y.; Adachi, K.; Shibata, T.; Terano, H.; Kohsaka, M.; Aoki, H.; Imanaka, H. *J. Antibiot.* **1985,** *38,* 926.
[20] Dorling, P. R.; Huxtable, C. R.; Colegate, S. M. Inhibition of lysosomal alpha-mannosidase by swainsonine, an indolizidine alkaloid isolated from *Swainsona canescens*. *Biochem. J.* **1980**, *191:* 649.
[21] Al Daher, S.; De Gasperi, R., Daniel, P.; Hall, N.; Warrern, C. D.; Winchester, B. The substrate-specificity of human lysosomal alpha-D-mannosidase in relation to genetic alpha-mannosidosis. *Biochem. J.* **1991**, *277:* 743.
[22] Withers, S. G.; Namchuk, M.; Mosi, R. Potent glycoside inhibitors: transition state mimics or simply fortuitous binders? in *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Stütz, A. E., Ed.; Wiley-VCH: Weinheim, **1999**; Chapter 9, 188.
[23] Whalen, L. J.; Wong, C.-H. Enzymes in organic synthesis: aldolase-mediated synthesis of iminocyclitols and novel heterocycles. Aldrichimica Acta **2006**, *39*, 63.
[24] (a) Vasella, A.; Davies, G. J.; Böhm, M. Glycosidase mechanisms. *Curr. Opin. Chem. Biol.* **2002**, *6*, 619. (b) Rye, C. S.; Withers, S. G. *Curr. Opin. Chem. Biol.* **2000,** *4,* 573.

[25] Bols, M. 1-Aza sugars, apparent transition state analogues of equatorial glycoside formation/cleavage. *Acc. Chem. Res.* **1998**, *31*, 1.
[26] Lillehund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. Recent developments of transition-state analogue glycosidase inhibitors of non-natural product origin. *Chem. Rev.* **2002**, *102*, 515-553.
[27] Neufeld, E.F. Lysosome storage diseases. *Ann. Rev. Biochem.* **1991**, *60*, 257.
[28] van Echten, G.; Sandhoff, K. Ganglioside metabolism. Enzimology, topology and regulation. *J. Biol. Chem.* **1993**, *268*, 5341.
[29] Chou, D. K.; Jungalwala, F. B. *N*-acetylglucosaminyltransferase regulates the expression of neolactoglycolipids including sulfoglucuronylglycolipids in the developing nervous system. *J. Biol. Chem.* **1993**, *268*, 21727.
[30] Kolter, T. *Angew. Chem., Int. Ed.* **1997**, *36*, 1955.
[31] Butters, T. D.; Dwek, R. A.; Platt, F. M. Therapeutic applications of imino sugars in lysosomal storage disorders. *Curr. Top. Med. Chem.* **2003**, *3*, 561.
[32] (a) Beutler, E. Gaucher disease as a paradigm of current issues regarding single gene mutations of humans. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5384. (b) Platt, F. M.; Butters, T. D. Inhibitors of glycosphingolipid biosynthesis. *TIGG* **1995**, *7*, 495.
[33] Butters, T. D.; Dwek, R. A.; Platt, F. M. Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorders. *Chem. Rev.* **2000,** *100,* 4683.
[34] Platt, F. M.; Neises, G. R.; Dwek, R. A.; Butters, T. D. *N*-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. *J. Biol. Chem.* **1994**, *269*, 8362.
[35] Tominaga, L., Ogawa, Y., Taniguchi, M., Ohno, K., Matsuda, J., Oshima, A., Suzuki, Y., and Nanba, E. Galactonojirimycin derivatives restore mutant human β-galactosidase activities expressed in fibroblasts from enzyme-deficient knockout mouse. *Brain Dev.* **2001**, *23*, 284.
[36] Cox, T.; Lachmann, R.; Hollak, C.; Aerts, J.; van Weely, S.; Hrebicek, M.; Platt, F.; Butters, T.; Dwek, R.; Moyses, C. *et al.* Novel oral treatment of Gaucher's disease with *N*-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. *Lancet* **2000**, *355*, 1481.
[37] Butters, T. D.; van den Broek, L. A. G. M.; Fleet, G. W. J.; Krulle, T. M.; Wormald, M. R.; Dwek, R. A.; Platt, F. M. Molecular requirements of imino sugars for the selective control of *N*-linked glycosylation and glycosphingolypid synthesis. *Tetrahedron: Asymmetry* **2000**, *11*, 113.
[38] Mellor, H. R.; Neville, D. C. A.; Harvey, D. J.; Platt, F. M.; Dwek, R. A.; Butters, T. D. Cellular effects of deoxynojirimycin analogues: inhibition of *N*-linked oligosaccharide processing and generation of free glycosylated oligosaccharides. *Biochem. J.* **2004**, *381*, 867.
[39] Wennekes, T.; van den Berg, R. J. B. H. N.; Donker, W.; van der Marel, G. A.; Strijland, A.; J. M. F. G.; Overkleeft, H. S. Development of adamantan-1-yl-methoxy-functionalized 1-deoxynojirimycin derivatives as selective inhibitors of glucosylceramide metabolism in man. *J. Org. Chem.* **2006**, *72*, 1088.
[40] For recent comprehensive reviews see: (a) Afarinkia, K.; Bahar, A. Recent advances in the chemistry of azapyranose sugars. *Tetrahedron: Asymmetry* **2005**, *16*, 1239. (b) Pearson, M. S. M.; Allaimat, M. M.; Fargeas, V.; Lebreton, J. Recent advances in the total synthesis of piperidine azasugars. *Eur. J. Org. Chem.* **2005**, 2159.
[41] Meyers, A. I.; Andres, C. J.; Resek, J. E.; Woddall, C. C.; McLaughlin, M. A.; Lee, P. H.; Price, D. A. Asymmetric route to azasugars from chiral bicyclic lactams. Synthesis of 1,4-dideoxy-imino-D-lyxitol; L-deoxy-mannojirimycin; rhamno-1-deoxynojirimycin and 1-deoxy-6-*epi*castanospermine. *Tetrahedron* **1999**, *55*, 8931.
[42] Asano, N.; Ikeda, K.; Yu, L.; Kato, A.; Takebayashi, K.; Adachi, I.; Kato, I.; Ouchi, H.; Takahata, H.; Fleet, G. The L-enantiomers of D-sugar-mimicking iminosugars are noncompetitive inhibitors of D-glycohydrolase? *Tetrahedron: Asymmetry* **2005**, *16*, 223 and literature therein cited.
[43] Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. Biological properties of D- and L-1-deoxyazasugars. *J. Med. Chem.* **2005**, *48*, 2036.
[44] Ye, X.-S.; Sun, F.; Liu, M.; Li, Q.; Wang, Y.; Zhang, G.; Zhang, L.-H.; Zhang, X. L. Synthetic iminosugar derivatives as new potential immunosuppressive agents. *J. Med. Chem.* **2005,** *48,* 3688.
[45] Myers, B. D.; Sibley, R.; Newton, L.; Tomlanovich, S. J.; Boshkos, C.; Stinson, E.; Luetscher, J. A.; Whitney, D. J.; Krasny, D.; Coplon, N. S. et al. The long-term course of cyclosporine-associated chronic nephropathy. *Kidney Int.* **1988**, *33*, 590.

Chapter 6

A General Approach to the Synthesis of 1-Deoxy-L-Iminosugars

6. A GENERAL APPROACH TO THE SYNTHESIS OF 1-DEOXY-L-IMINOSUGARS

A stereoselective procedure for the preparation of unnatural deoxy iminosugars belonging to L*-series has been developed. The synthesis involves the construction of the key intermediate olefins* ***7****, available in few steps by the coupling reaction of the heterocyclic synthon* ***1*** *and the readily available Garner aldehyde* ***2****. Through suitable* syn- *and* anti-*dihydroxylation reactions deoxy-iminosugars have been synthesized in stereoselective manner. The possibility to have a strategy of general applicability derives from a peculiar feature of protected iminosugar derivatives, such as* ***10-11****, not conforming to the expected* $^{1}C_{4}$ *chair, typical of* L*-sugars, conversely adopting a conformation close to* $^{3}S_{1}$*. By exploiting this characteristic, access to all eight epimers belonging to* L*-series has been opened.*

6.1 *INTRODUCTION*

Since their first discovery over 40 years ago, iminosugars have gained a great deal of attention as inhibitors of carbohydrate-processing enzymes glycosidases and glycosyltransferases. As alterations in biosynthesis and function of these enzymes are implicated in a wide variety of diseases, the significant inhibitory properties of iminosugars make them excellent targets for medical intervention. Their prospective therapeutical uses range from diabetes[1] through cancer[2] and viral diseases[3] to metabolic and neurological disorders.[4]

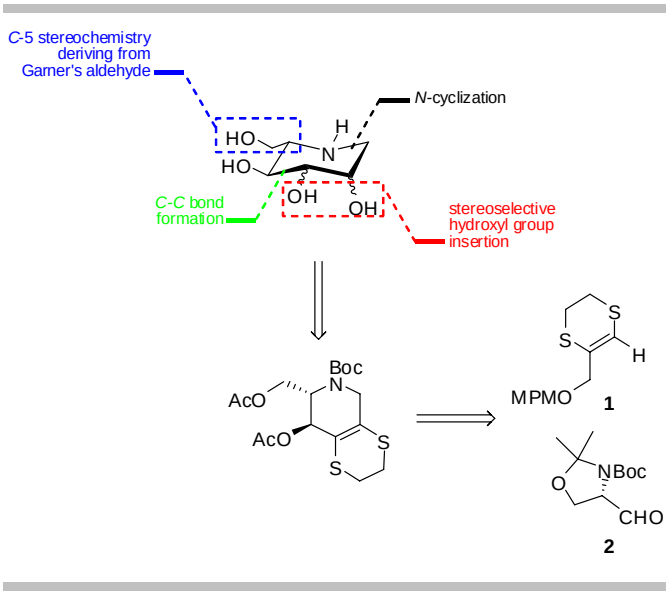
From a synthetic point of view, most syntheses of piperidine iminosugars so far reported[5] have focused attention to the preparation of iminosugars with D-configuration, whereas few routes are available for the synthesis of their corresponding L-analogues.[6] However, interest on L-iminosugars is nowadays arising, since late intriguing reports have shown that L-iminosugars could act as glycosidase inhibitors as well *via* a non-competitive mode of action.[7]

6.2 *RESULTS AND DISCUSSION*

6.2.1 *SYNTHESIS OF THE PIPERIDINE SKELETON*

In the context of an ongoing program directed towards the achievement of a new synthetic methodology for the preparation of polyhydroxylated molecules,* we have developed a versatile strategy for the synthesis of non-naturally occurring deoxy-iminopyranoses belonging to L-series, through a non-carbohydrate based route.[8]

As outlined in Scheme 1, the synthesis involves the use[9] of the versatile heterocyclic synthon **1** and of the well known 1,1-dimethylethyl-(*S*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate (**2**), otherwise called Garner's aldehyde.[10,†] By exploiting the inherent chirality of this latter, the L-configuration at the *C*-5 stereocenter of final products is guaranteed.

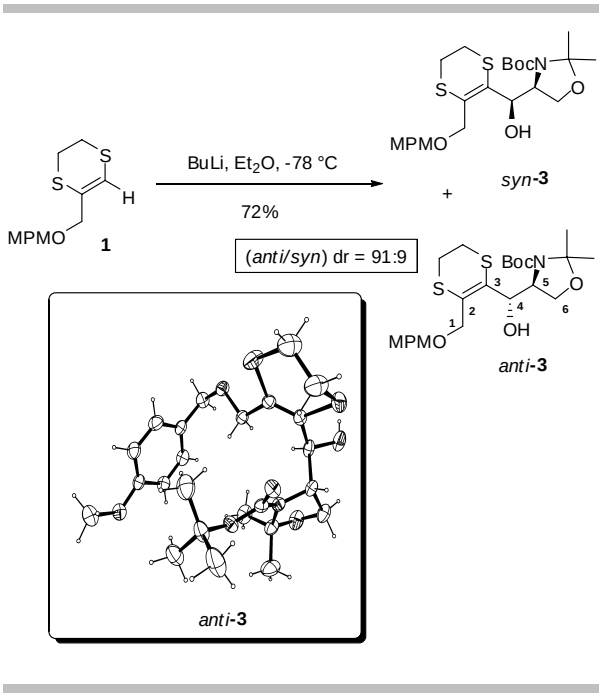


Scheme 1 - *Retrosynthetic path.*

The synthesis began with the coupling of the *in situ* prepared *C*-3 lithiated carbanion of **1** with the Garner aldehyde **2** to afford a *syn/anti* diastereomeric mixture of alcohols **3** (**Scheme 2**). As previously highlighted, the best stereoselectivity was achieved by the use of Et_2O without catalyst, providing *anti*-**3** in good stereoselectivity (91:9 dr).‡

* See also Chapter 4, "A Versatile Route to L-Hexoses: Stereoselective Synthesis of Rare Sugars".
† The same procedure, starting from enantiomeric (*R*)-Garner's aldehyde, allows the preparation of their D-deoxy iminosugars.
‡ See Chapter 3, "The Heterocycle-Functional Group Equivalence as a Powerful Tool for the Synthesis of Organic Molecules".

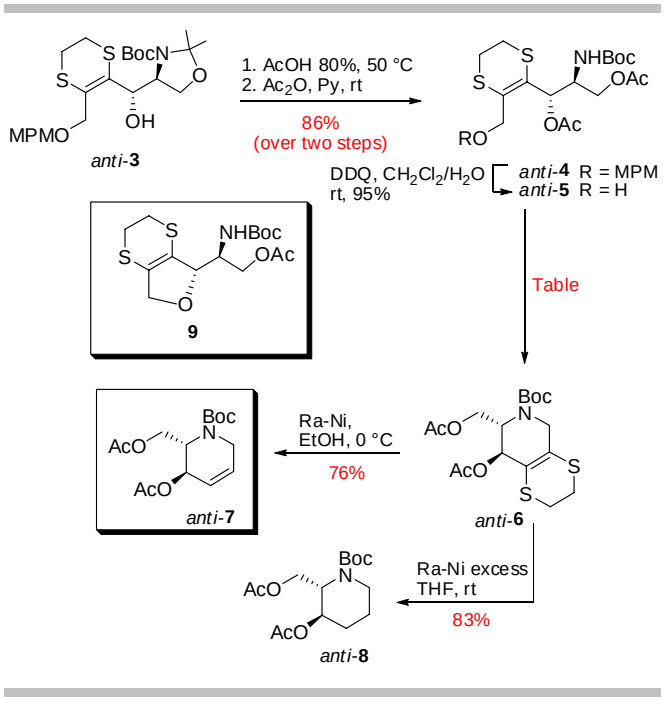
The secondary alcohol* *anti*-**3**, obtained by the coupling reaction, was separated from its diastereomer by flash chromatography; the stereochemical assignment at the newly generated *C*-4 was clearly deduced by X-ray analysis (**Scheme 2**).



Scheme 2 – *Three-carbon homologation and X-ray analysis of* anti-***3***.

With the adduct **3** in our hand, interest was focused to the achievement of key intermediate **4** (**Scheme 3**). To this purpose, the alcohol **3** was converted in its diacetate **4** by deprotection of oxazolidine ring and acetylation of the crude residue (86% overall yield). Removal of MPM group by treatment of **4** with DDQ in a CH_2Cl_2/H_2O (9:1) emulsion gave the primary alcohol **5** with an excellent yield (95%).

* An alternative numbering reported on the carbon skeleton has been employed to identify carbon atoms that will belong to the carbohydrate-like ring.



Scheme 3 - *Synthesis of key intermediate* anti-*7*.

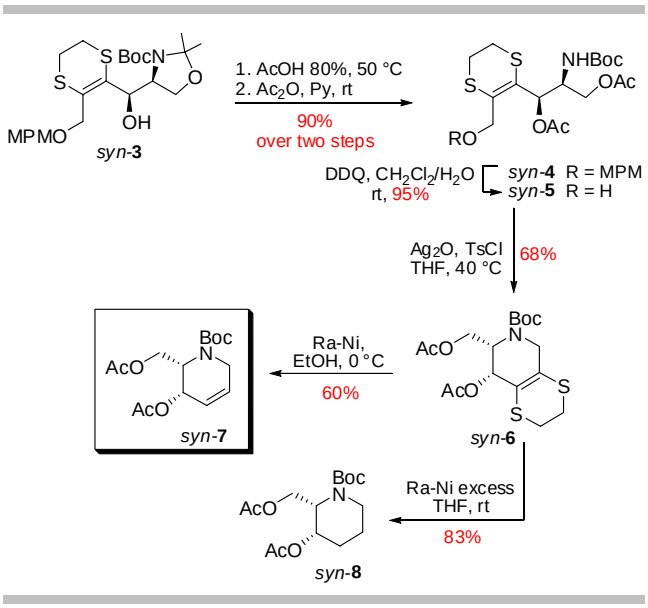
Intramolecular cyclization was then attempted. Among a wide range of choices for piperidine ring closing by means of nitrogen atom (**Table 1**), best results were provided by treatment of **5** with Ag_2O/TsCl in THF at 40 °C (*entry 3*) affording the bicycle piperidine **6** with a good yield (85% yield). The reaction proceeds through an S_N2 pathway *via* a very reactive tosylate ester intermediate. Conversely, the use of strongly basic conditions (*entry 6*) led both to the desired product **6** (25%) and to the attractive five-membered ring compound **9** (56%, **Table 1** and **Scheme 3**).

Table 1 - *Attempts for ring-closing reaction of* anti-***5***.

		isolated yield (%)	
Entry	***Conditions***	**6**	**9**
1	DEAD/TPP, THF, 25 °C	/	/
2	TPP/I_2/Im, CH_3CN, 25 °C	/	/
3	Ag_2O/TsCl, THF, 40 °C	85	11
4	TEA/DMAP/MsCl, CH_2Cl_2, -20→0 °C	75	/
5	TsCl/Py, 25 °C	/	/
6	NaH/TsCl,THF, 0 °C	25	56

Subsequently, removal of the dithioethylene bridge on intermediate **6** was attained by treatment with Raney-Ni in ethanol at 0 °C for 2h, leading to the olefin *anti*-**7** (76% yield). As already observed for similar substrates,* when the reaction was carried out with a Raney-Ni excess in THF at room temperature, the over-reduction product was obtained with a satisfactory yield (83%), affording the 1,2,3-trideoxy-L-iminosugar **8** (**Scheme 3**).

The same synthetic path was repeated on the *syn*-**3** diastereomer (as minor component from the coupling reaction using THF as solvent, see Chapter 3), obtaining the desulfurized olefin *syn*-**7**, formally the *C*-4 epimer of *anti*-**7**, in a similar fashion (**Scheme 4**). This latter, together with the intermediate *anti*-**7**, underwent the final steps of the synthetic route by the stereoselective double bond dihydroxylation reactions.



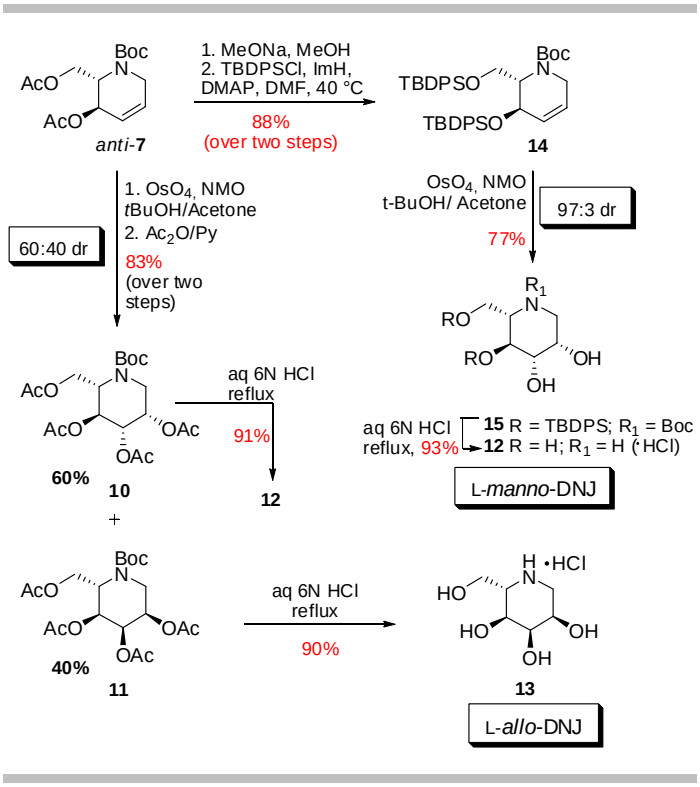
Scheme 4 - *Synthesis of key intermediate* syn-*7*.

6.2.2 *STEREOSELECTIVE DOUBLE BOND DIHYDROXYLATIONS: L-IMINOSUGAR SYNTHESIS*

***1-Deoxy-L-*manno*-nojirimycin* (12) *and 1-deoxy-L-*allo*-nojirimycin* (13).** With the olefins **7** in hands, interest was turned to the stereoselective double bond dihydroxylation reaction (**Scheme 5**). Exposure of *anti*-**7** to common Upjohn conditions[11] (OsO_4/NMO) followed by acetylation of the crude residue yielded a fully separable mixture of the protected L-*manno*-DNJ **10** and L-*allo*-

* See Chapter 3, Paragraph 3.2, "Three-Carbon Homologation".

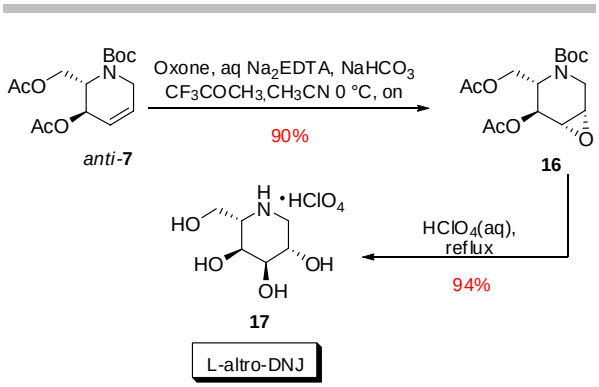
DNJ **11** in low diastereomeric ratio (6:4). Both diastereomers were deprotected by means of refluxing aq 6N HCl solution, obtaining 1-deoxy-L-mannojirimycin (**12**) and 1-deoxy-L-*allo*-nojirimycin (**13**) in remarkable yields (91% and 90%, respectively). Further attempts to improve the stereoselectivity in the dihydroxylation reaction (for example using the Sharpless catalysts[12]) showed no significant effects.[13] The observed low selectivity in the above dihydroxylation reactions might be attributed to the relatively small size of the *C*-4 acetyl group and thus both faces of the double bond were almost equally hindered. As a matter of fact, the replacement of the acetyl groups of *anti*-**7** with the much bigger TBDPS ethers (**14**, 88% overall yield, **Scheme 5**), afforded, after dihydroxylation of **14** under Upjohn conditions, the protected L-*manno*-DNJ **15** with a high stereoselectivity (97:3 dr). Then, treatment with aq 6N HCl allowed removal of all protective groups to obtain deoxy-L-mannojirimycin (**10**) in 93% yield.



Scheme 5 - Syn-*dihydroxylation of* anti-*7*.

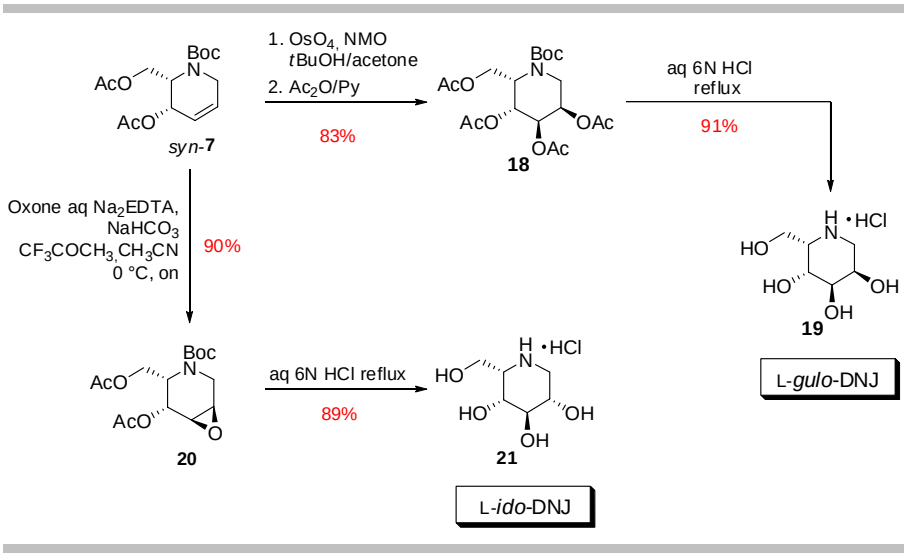
1-Deoxy-L-altro-nojirimycin (**17**). The above successes led us to consider the *anti*-dihydroxylation of the key olefin *anti*-**7**. Under our conditions, *syn*-**4** was firstly treated with *m*-chloro-peroxybenzoic acid (*m*-CPBA), but very long reaction times were required, because of the chemical inactivity of this latter towards the substrate. Better results were achieved by means of *in situ* generated[14] DMDO (Oxone®/trifluoroacetone) afforded exclusively the *anti*-epoxide **16**

in 90% yield (**Scheme 6**). Ring opening of the 2,3-anhydro derivative **16** along with the removal of all protecting groups by means of refluxing $HClO_4$ gave the 1-deoxy-L-*altro*-nojirimycin (**17**) as single epimer and in an amazing overall yield (94%).



Scheme 6 - *Anti-dihydroxylation of* anti-*7***.**

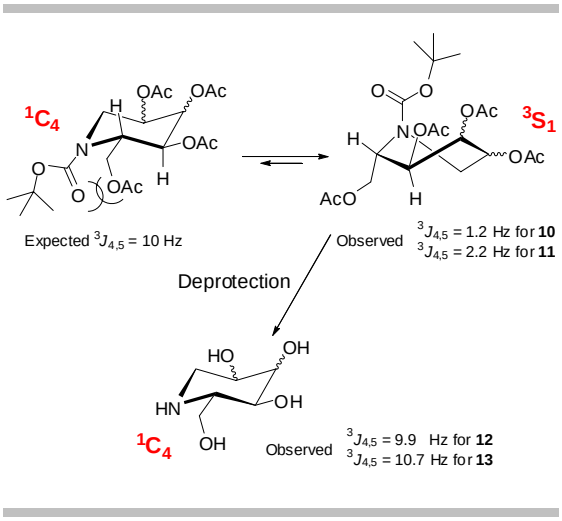
1-Deoxy-L*-gulo-*nojirimycin **(19)** ***and 1-deoxy-L*-ido-*nojirimycin*** **(21).** Entry to 1-deoxy-L-*gulo*- and 1-deoxy-L-*ido*-nojirimycin (**19** and **21**) provide for the use of olefin *syn*-**7** (**Scheme 7**). More specifically, *syn*-dihydroxylation achieved by treatment of *syn*-**7** with OsO_4/NMO in *t*-BuOH/acetone and then acetylation of the crude residue afforded exclusively the protected gulo-DNJ derivative **18**. Hence, exposure to usual acidic conditions (conc. HCl) furnished the pure deoxy-L-*gulo*-nojirimycin (**19**) in an excellent 91% yield. On the other hand, *anti*-dihydroxylation of *syn*-**7** was attained by epoxidation of the double bond followed by epoxide hydrolysis (**Scheme 7**). Treatment of this latter with *in situ* generated DMDO afforded the single epoxide **20** (90%). Simultaneous ring opening and protective group removal (HCl) afforded the pure 1-deoxy-L-*ido*-nojirimycin (**21**, **Scheme 7**).



Scheme 7 – *Stereoselective dihydroxylation of* syn-*7*.

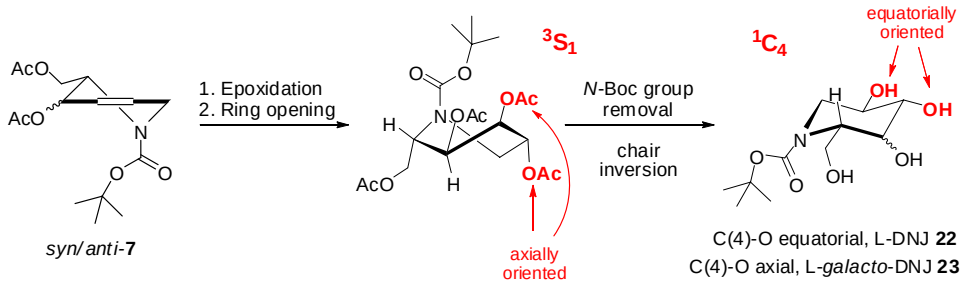
6.2.3 *Insight into the Conformations of* N*-Boc Iminosugar Derivatives: Access to Remaining DNJ Epimers*

At this stage of the synthesis it is noteworthy to discuss about some significant findings related to compounds **10**, **11**, **12** and **13** that will be useful for the preparation of remaining epimers, the ones with *gluco*- as well as those with *galacto*- configuration. Particularly, although the stereochemistry of compounds **10**, **11**, **12** and **13** is consistent with X-ray analysis of the *anti*-**7** compound, observing the coupling constant values in the ^{1}H NMR spectra ($^3J_{4,5}$ = 1.2 Hz for **10**; $^3J_{4,5}$ = 2.2 Hz for **11**), it is fairly evident that the *N*-Boc compounds **10** and **11** do not conform to the expected ^{1}C$_4$ chair conformation, typical of L-sugars.[15] In this chair form a downward orientation is imposed on the *N*-Boc substituent due to the trigonal nature of the sp^2 ring nitrogen atom, resulting in a strong repulsive interaction with the nearly coplanar *C*-6 methylene group (**Scheme 8**). This repulsion can be removed by an upward movement of the ring nitrogen, to obtain a conformation close to ^{3}S$_1$. The divergent torsional angles associated with the ^{3}S$_1$ and ^{1}C$_4$ conformations are demonstrated most clearly by comparing the *J* values for *H*-4, *H*-5 in **10** and **11** to those for the corresponding free piperidines **12** and **13**. Indeed, the coupling constant values indicate a dihedral angle of ca. 70° ($^3J \sim 2$ Hz) for the trans-disposed *H*-4 and *H*-5 protons of compounds **10** and **11** in clear opposition to the values of $^3J \sim 10$ Hz, expected for a diaxial orientation. Actually, when the *N*-Boc protection is removed (**Scheme 8**) the coupling constant values in ^{1}H NMR are consistent with a structure in which *H*-4 and *H*-5 are in a trans-diaxial orientation ($^3J_{4,5}$ = 9.9 Hz and $^3J_{4,5}$ = 10.7 Hz for compounds **12** and **13**, respectively).



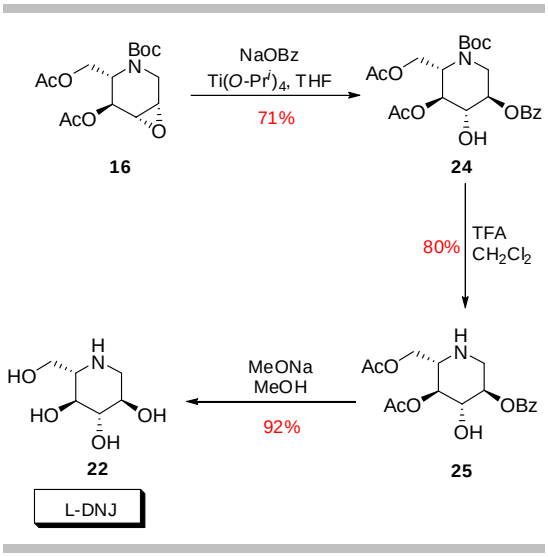
Scheme 8 - *Preferred conformations for compounds **10-11**.*

1-Deoxy-L-*gluco*-nojirimycin **(22)** ***and 1-deoxy-L-*galacto*-nojirimycin*** **(23).** As mentioned above, this peculiar feature of *N*-Boc iminosugar derivatives **10-11** was exploited for our synthetic purposes. Particularly, endeavours were attempted to synthesize L-*gluco*- and L-*galacto*-DNJ (**22** and **23**) by *anti*-dihydroxylation of the olefins *anti*-**7** and *syn*-**7**, followed by suitable chair inversion next to *N*-Boc group removal (**Scheme 9**).



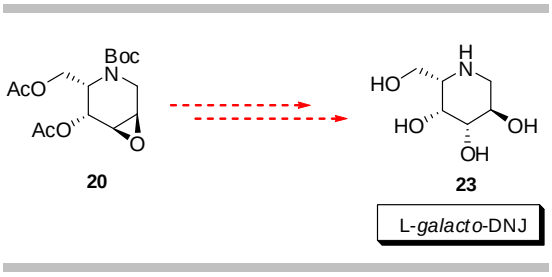
Scheme 9 - L-gluco- *and* L-galacto-*DNJ **22** and **23** can be synthesized as from* anti-*7 and* syn-*7 by chair inversion next to* N-*Boc group removal.*

The synthetic route which led to L-DNJ (**22**) was depicted in Scheme 10. Particularly, treatment of epoxide **16** with sodium benzoate (NaOBz) and stoichiometric Ti(*O*-Pr^i)$_4$ in THF allowed smooth oxirane ring opening,[16] affording protected iminosugar derivative **24** in good yield (71%). Then, *N*-*t*-butoxycarbonyl protective group removal was achieved by means of trifluoroacetic acid (TFA) in CH_2Cl_2, leading to protected L-DNJ **25** (80% yield). Finally, basic condition amenable acetyl and benzoyl groups were removed by treatment with NaOH in MeOH, obtaining 1-deoxy-L-nojirimycin (**22**) in almost quantitative yield (**Scheme 10**).



Scheme 10 – *L-DNJ (**22**) synthesis.*

In a similar fashion, deoxy-L-*galacto*-nojirimycin (**23**) will be synthesized as from diastereomeric epoxide **20** (**Scheme 11**).

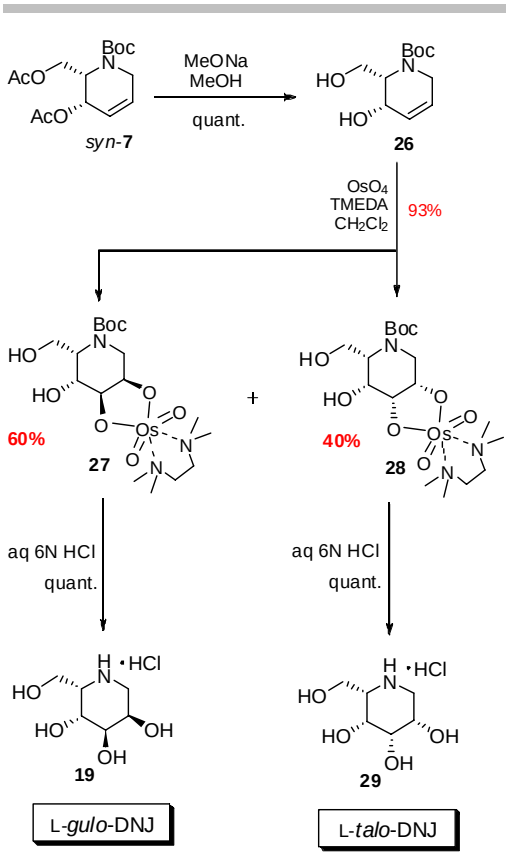


Scheme 11 – *L-Galacto-DNJ (**23**) synthesis.*

1-Deoxy-L-talo-nojirimycin (**29**). Driven by the same considerations made above with regard to methyl-α-L-*talo*-pyranoside synthesis,* the approach to L-*talo*-DNJ (**29**) was attempted by *syn*-dihydroxylation using Donohoe's conditions[17] as from the olefin *syn*-**7** (**Scheme 12**). With this aim, acetyl groups on intermediate *syn*-**7** were removed by use of common Zemplén conditions,[18] quantitatively affording the partly deprotected olefin **26**. Then, osmylation under Donohoe's conditions was examined (**Scheme 12**). Treatment of **26** with stoichiometric OsO_4 and *N,N,N,N*-tetramethylenediamine (TMEDA) in CH_2Cl_2 at -78 °C afforded a mixture of L-*gulo*- and L-*talo*-DNJ derivatives **27** and **28** in a very good overall yield (93%) but without a significant stereoselectivity (6:4 dr). Further hydrolysis of the osmate esters **27** and **28** along with

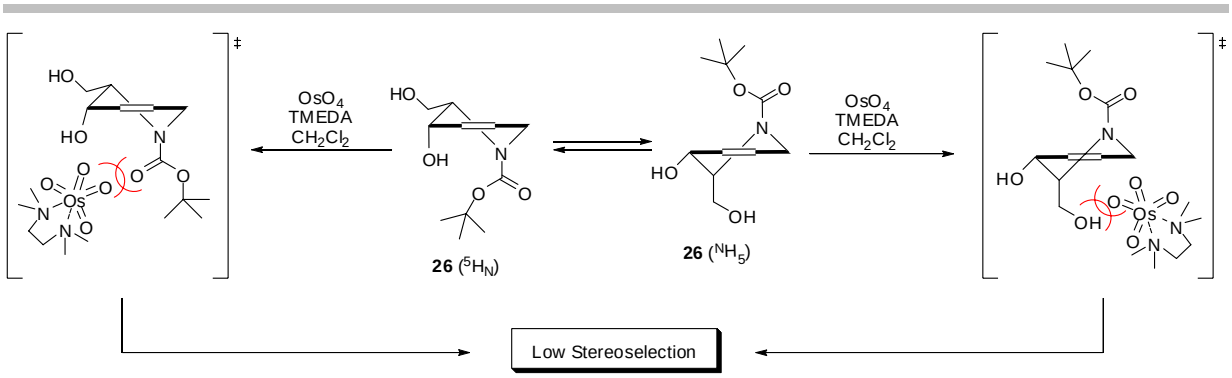
* See Chapter 4, Paragraph 4.2.2, "Stereoselective Double Bond Dihydroxylations: L-Hexose Synthesis".

removal of *N*-Boc groups by means of concentrated HCl furnished deoxy-L-*gulo*-nojirimycin (**19**) and deoxy-L-*talo*-nojirimycin (**29**) in almost quantitative yield.



Scheme 12 - *Synthesis of deoxy-L-talo-nojirimycin (**29**) from olefin* syn-*7 by osmylation reaction using Donohoe's conditions.*

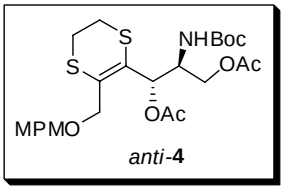
The unusual behaviour in this osmylation reaction could be conjectured to be due once more to the presence of the *N*-Boc substituent. In fact, whether the olefin **26** exists in a conformation close to $^{5}H_{N}$ or $^{N}H_{5}$ (^{1}H NMR data do not allow an unambiguous assignment), addition of OsO_4/TMEDA to the double bond occurs with steric hindrance to the *syn* face (the one from the *O*-4 side required for the hydrogen bond-forming, **Scheme 13**). In major detail, if the conformation close to $^{5}H_{N}$ prevails, entry to OsO_4/TMEDA complex is hampered by *t*-butoxycarbonyl group; conversely, if the $^{N}H_{5}$ chair predominates, the axially oriented *C*-6 methylene group obstructs the access. In both cases, addition to the olefin **26** brings to a low stereoselective outcome of the overall process (**Scheme 13**).



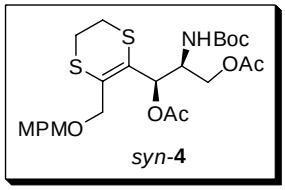
Scheme 13 - *Conjecturing on the low stereoselectivity observed for the synthesis of* L-talo-*DNJ (**29**) by Donohoe's osmylation.*

Driven by these hypotheses, further efforts will be focused to the achievement of L-*talo*-DNJ **29** with a better selectivity: particularly, early *N*-Boc group removal of the olefin **26** before the osmylation step could create the appropriate conditions for driving the Donohoe's *syn* dihydroxylation towards improved stereoselective outcomes.

6.3 *EXPERIMENTAL SECTION*



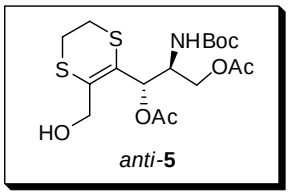
anti-**4**



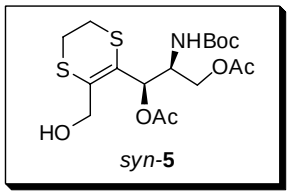
syn-**4**

Compounds* anti-*4 and* syn-*4. 80% Aqueous acetic acid (40.0 mL) was added to *anti*-**3** (2.0 g, 4.0 mmol) and the mixture warmed to 50 °C and stirred for 1 h at the same temperature; after cooling to rt, the reaction was quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. The crude residue was directly acetylated by treatment with Ac_2O in pyridine overnight at room temperature. Then solvent removal under reduced pressure and chromatography of the crude residue on silica gel (hexane/EtOAc = 7:3) gave the pure *anti*-**4** (1.86 g, 86% overall yield): oily, $[\alpha]_D^{25}$ +30.4 (*c* 0.8, acetone). ^{1}H NMR (500 MHz): δ 1.41 (s, 9H), 1.98 (s, 3H), 2.07 (s, 3H), 2.91-2.98 (m, 1H), 3.10-3.18 (m, 2H), 3.20-3.29 (m, 1H), 3.72 (d, *J* = 11.8 Hz, 1H), 3.81 (s, 3H), 4.14 (dd, *J* = 11.8, *J* = 2.6 Hz, 1H), 4.25 (dd, *J* = 11.8, *J* = 3.1 Hz, 1H), 4.32-4.38 (m, 1H), 4.55 (s, 2H), 4.66 (d, *J* = 11.8 Hz, 1H), 5.58 (d, *J* = 9.4 Hz, 1H, exchange with D_2O), 5.79 (d, *J* = 10.2 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (50 MHz): δ 20.6, 20.7, 26.9, 28.2 (3C), 30.3, 50.2, 55.1, 63.4, 70.4, 70.5, 72.2, 79.1, 113.7 (2C), 126.5 (2C), 127.9, 129.2 (2C), 155.0, 159.2, 169.6, 170.6. Anal. calcd for $C_{25}H_{35}NO_8S_2$: C 55.43, H 6.51, N 2.59. Found: C 55.52, H 6.48, N 2.61.

Under analogous conditions, the pure *syn*-**4** was obtained (90% yield) as from *syn*-**3**: oily; $[\alpha]_D^{25}$ = -38.0 (*c* 0.9, acetone). ^{1}H NMR (500 MHz): δ 1.42 (s, 9H), 2.02 (s, 3H), 2.10 (s, 3H), 3.04-3.26 (m, 4H), 3.80 (s, 3H), 3.98-4.10 (m, 3H), 4.32 (d, *J* = 12.7 Hz, 1H), 4.35-4.42 (m, 1H), 4.45 (d, *J* = 11.5 Hz, 1H), 4.52 (d, *J* = 11.5 Hz, 1H), 4.77 (d, *J* = 9.3 Hz, 1H), 5.82 (d, *J* = 7.2 Hz, 1H), 6.85 (d, *J* = 8.7 Hz, 2H), 7.25 (d, *J* = 8.7 Hz, 2H); ^{13}C NMR (50 MHz): δ 20.5, 20.7, 26.9, 28.2, 29.5, 50.2, 55.1, 63.4, 70.3, 70.4, 72.1, 79.1, 113.7, 126.5, 127.9, 129.2, 155.0, 159.2, 169.5, 170.6.



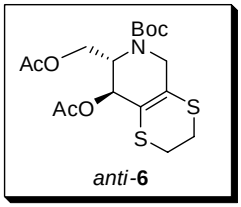
anti-**5**



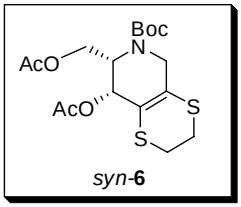
syn-**5**

Compounds* anti-*5* *and* syn-*5. To a stirred CH_2Cl_2-H_2O (9:1) emulsion (100 mL) containing the MPM ether *anti*-**4** (1.8 g, 3.4 mmol), DDQ (1.0 g, 4.4 mmol) was added in one portion at room temperature. After 5 h H_2O was added to the reaction and the mixture was extracted with CH_2Cl_2; the organic layers were dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/EtOAc = 6:4) gave the pure *anti*-**5** (1.4 g, 95% yield): oily, $[\alpha]_D^{25}$ + 72.0 (*c* 0.4, $CHCl_3$). ^{1}H NMR (500 MHz) δ 1.46 (s, 9H), 2.10 (s, 6H), 2.95-3.09 (m, 1H), 3.10-3.18 (m, 2H), 3.20-3.29 (m, 1H), 4.16 (d, *J* = 8.8 Hz, 1H), 4.21-4.40 (m, 4H), 5.30 (bd, *J* = 8.3 Hz, 1H, exchange with D_2O), 5.75 (bd, *J* = 8.7 Hz, 1H). ^{13}C NMR (50 MHz) δ 20.7 (2C), 27.2, 28.3 (3C), 30.0, 50.9, 63.5 (2C), 71.4, 80.1, 124.5, 131.5, 155.1, 170.0, 170.8. Anal. calcd for $C_{17}H_{27}NO_7S_2$: C 48.44, H 6.46, N 3.32. Found: C 48.52, H 6.49, N 3.34.

Under analogous conditions, the pure *syn*-**5** was obtained (95% yield) as from *syn*-**4**: oily; $[\alpha]_D^{25}$ = -23.4 (*c* 0.8, acetone). ^{1}H NMR (500 MHz): δ 1.45 (s, 9H), 2.08 (s, 3H), 2.09 (s, 3H), 3.02-3.13 (m, 2H), 3.14-3.26 (m, 2H), 3.98 (d, *J* = 13.2 Hz, 1H), 4.06 (dd, *J* = 4.1 Hz, *J* = 11.6 Hz , 1H), 4.17 (dd, *J* = 4.1 Hz, *J* = 11.6 Hz, 1H), 4.40-4.45 (m, 2H), 4.80 (bd, *J* = 9.5 Hz, 1H), 5.85 (d, *J* = 8.6 Hz, 1H). ^{13}C NMR (50 MHz): δ 20.7, 20.8, 27.1, 28.2, 29.5, 51.2, 62.9, 63.5, 72.5, 80.0, 123.2, 131.9, 155.2, 170.5, 171.0.



anti-**6**

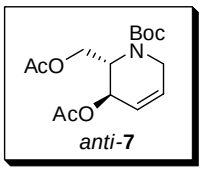


syn-**6**

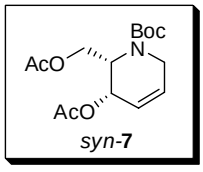
Compounds* anti-*6* *and* syn-*6. Silver oxide (1.1 g, 4.8 mmol) was added in one portion to a solution of alcohol *anti*-**5** (1.3 g, 3.1 mmol) in anhydrous THF (15 mL) at room temperature under nitrogen stream; after 10 min anhydrous TsCl (0.6 g, 3.1 mmol) was added and the suspension was stirred overnight at 40 °C. Then the solid was filtered off and washed with $CHCl_3$; solvent removal under reduced pressure afforded a crude residue, which chromatography over silica gel (hexane/EtOAc = 9:1) gave the pure *anti*-**6** (1.06 g, 85% yield): oily, $[\alpha]_D^{25}$ -42.9 (*c* 1.8, acetone). ^{1}H NMR (500 MHz, 25 °C): δ 1.47 (s, 5.4H), 1.48 (s, 3.6H), 2.01-2.12 (m, 6H),

3.16-3.28 (m, 4H), 3.52 (d, *J* = 17.8 Hz, 0.6H), 3.60 (d, *J* = 17.8 Hz, 0.4H), 4.02-4.10 (m, 1H), 4.12-4.20 (m, 1H), 4.25 (d, *J* = 17.6 Hz, 0.4H), 4.42 (d, *J* = 17.6 Hz, 0.6H), 4.61-4.68 (m, 0.6H), 4.69-4.76 (m, 0.4H), 4.98 (s, 0.6H), 5.09 (s, 0.4H). ^{1}H NMR (400 MHz, 60 °C): δ 1.49 (s, 9H), 2.05 (s, 3H), 2.08 (s, 3H), 3.11-3.27 (m, 4H), 3.60 (bd, *J* = 17.2 Hz, 1H), 4.09 (dd, *J* = 11.3, *J* = 6.2 Hz, 1H), 4.21 (dd, *J* = 11.3, *J* = 7.8 Hz, 1H), 4.40 (bs, 1H), 4.68 (bs, 1H), 5.05 (bs, 1H). ^{13}C NMR (100 MHz, 60 °C): δ 20.6, 20.8, 27.8, 28.1 (3C), 29.5, 43.3, 53.2, 60.8, 69.2, 80.5, 115.1, 125.7, 154.1, 170.3 (2C). Anal. calcd for $C_{17}H_{25}NO_6S_2$: C 50.60, H 6.24, N 3.47. Found: C 50.74, H 6.27, N 3.48.

Under analogous conditions, the pure *syn*-**7** was obtained (68% yield) as from *syn*-**6**: oily; $[\alpha]_D^{25}$ = -17.5 (*c* 1.3, $CHCl_3$). ^{1}H NMR (200 MHz): δ 1.47 (s, 9H), 2.02 (s, 3H), 2.12 (s, 3H), 2.98-3.16 (m, 2H), 2.99-3.36 (m, 2H), 4.14-4.36 (m, 3H), 4.85 (bs, 1H), 5.64 (bd, *J* = 6.0 Hz, 1H). ^{13}C NMR (125 MHz): δ 20.7, 20.8, 27.0, 28.2, 28.6, 43.7, 50.5, 59.7, 69.1, 80.9, 117.3, 121.7, 154.0, 169.7, 170.6.



anti-**7**

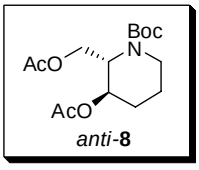


syn-**7**

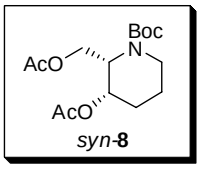
Compounds* anti-*7 and* syn-*7. A solution of bicycle piperidine *anti*-**6** (0.9 g 2.2 mmol) in EtOH (25 mL) was added in one portion to a stirred suspension of Raney-Ni (W2) (8.2 g, wet) in the same solvent (5 mL) at 0 °C. The suspension was stirred for 2h, then the solid was filtered off and washed with EtOH. The filtrate was evaporated under reduced pressure to afford a crude residue which chromatography on silica gel (hexane/acetone = 9/1) gave the pure *anti*-**7** (0.53 g, 76% yield) beside a small amount of *anti*-**8** (0.08 g, 12% yield). Data for compound *anti*-**7**: oily, $[\alpha]_D^{25}$ - 84.0 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz): δ 1.50 (s, 9H), 2.06 (s, 3H), 2.09 (s, 3H), 3.61 (bd, *J* = 18.2 Hz, 1H), 4.04 (dd, *J* = 11.3, *J* = 6.5 Hz, 1H), 4.13 (dd *J* = 11.2, *J* = 8.0 Hz, 1H), 4.40 (bd, *J* = 18.2 Hz, 1H), 4.62-4.78 (m, 1H), 5.14 (bd, *J* = 4.4 Hz, 1H), 5.86-5.96 (m, 1H), 6.05-6.15 (m, 1H). ^{13}C NMR (100 MHz): δ 21.0, 21.2, 28.6 (3C), 41.2, 52.7, 61.4, 65.8, 80.5, 120.6, 131.4, 155.2, 170.7, 170.9. Anal. calcd for $C_{15}H_{23}NO_6$: C 57.50, H 7.40, N 4.47. Found: C 57.65, H 7.38, N 4.46.

Under analogous conditions, the pure *syn*-**7** was obtained (60% yield) as from *syn*-**6**: oily; $[\alpha]_D^{25}$ = -21.6 (*c* 0.075, $CHCl_3$); ^{1}H NMR (400 MHz): δ 1.47 (s, 9H), 2.02 (s, 3H), 2.12 (s, 3H), 3.40-3.60 (m, 1H), 4.10-4.30 (m, 3H), 4.80-5.0 (m, 1H), 5.50 (s, 1H), 5.60 (bd, 1H), 5.70-5.90

(m, 1H). ^{13}C NMR (500 MHz): δ 20.7, 20.8, 28.2, 39.5, 59.6, 66.9, 77.2, 80.3, 123.7, 126.2, 154.6, 170.7.



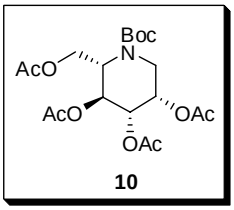
anti-**8**



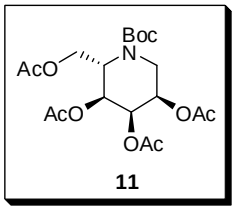
syn-**8**

Compounds* anti-*8 and* syn-*8. Under similar conditions reported above, treatment of *anti*-**6** (0.9 g, 2.2 mmol) in THF with an excess of Raney-Ni (W2) (16.5 g, wet) afforded, after common work-up and purification procedures, the pure *anti*-**8** (0.57 g, 83% yield): oily, $[\alpha]_D^{25}$-4.3 (*c* 2.0, $CHCl_3$). ^{1}H NMR (500 MHz): δ 1.42-1.52 (m, 10H), 1.68-1.90 (m, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.84 (bt, *J* = 12.7 Hz, 1H), 3.98-4.18 (m, 2H), 4.23 (dd, *J* = 11.2, *J* = 1.7 Hz, 1H), 4.55 (bs, 1H), 4.92 (bs, 1H). ^{13}C NMR (125 MHz): δ 21.2, 21.3, 23.9, 25.7, 28.6 (3C), 29.9, 51.5, 59.4, 70.0, 80.3, 155.0, 170.2, 171.1. Anal. calcd for $C_{15}H_{25}NO_6$: C 57.13, H 7.99, N 4.44. Found: C 56.98, H 8.01, N 4.43.

In an analogous manner, *syn*-**8** was obtained (83% yield) as from *syn*-**6**: ^{1}H NMR (500 MHz): δ 1.43 (s, 9H), 1.47-1.62 (m, 2H), 1.68-1.90 (m, 2H), 2.05 (s, 3H,), 2.06 (s, 3H), 2.56 (bs, 1H), 3.90-4.10 (m, 1H), 4.20 (dd, *J* = 11.3 Hz, *J* = 3.9 Hz, 1H), 4.30-4.50 (m, 1H), 4.70 (bs, 1H), 4.88-4.92 (m, 1H). ^{13}C NMR (125 MHz): δ 21.2, 21.3, 28.6, 51.2, 59.4, 70.0, 80.3, 155.0, 170.2, 171.1.



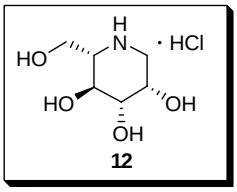
10



11

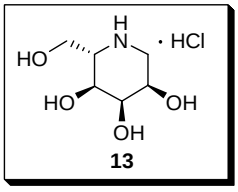
Compounds 10 and 11. To a solution of *anti*-**7** (0.24 g, 0.76 mmol) in 1:1 (v/v) *tert*-butyl alcohol/acetone (6 mL) cooled at 0 °C in ice bath an excess of a chilled 1:1 (w/w) solution of 4-methylmorpholine-*N*-oxide (0.18 g; 1.5 mmol) in water was added in one portion. After few minutes, a catalytic amount (0.03 mmol) of OsO_4 was added, and the reaction mixture was stirred overnight at room temperature; then the reaction was quenched with saturated aqueous Na_2SO_3 and evaporated under reduced pressure. The crude residue was directly acetylated by treatment with Ac_2O in pyridine; after solvent removal under reduced pressure, the chromatography of the crude residue over silica gel (hexane/acetone = 9:1) gave the two

separated diastereoisomers **10** and **11** (6:4 dr) (0.27 g, 83% overall yield). Data for protected L- *manno*-DNJ **10** (0.162 g): oily, $[\alpha]_D^{25}$ + 29.0 (*c* 2.0, $CHCl_3$). ^1H NMR (200 MHz): δ 1.48 (s, 9H), 2.02 (s, 3H), 2.05 (s, 3H), 2.09 (s, 3H), 2.15 (s, 3H), 3.14 (bt, *J* = 12.0 Hz, 1H), 4.00-4.34 (m, 2H), 4.48 (dd, *J* = 11.0, *J* = 9.0 Hz, 1H), 4.54-4.72 (m, 1H), 4.95 (dd, *J* = 3.0, *J* = 1.2 Hz, 1H), 5.08 (ddd, *J* = 11.4, *J* = 5.3, *J* = 3.1 Hz, 1H), 5.30 (bt, *J* = 3.0 Hz, 1H). ^{13}C NMR (50 MHz): δ 21.0 (2C), 28.4 (2C), 29.9, 52.8, 60.2, 65.4, 67.4, 68.4, 81.1, 155.0, 169.2, 169.4, 169.9, 170.8. Anal. calcd for $C_{19}H_{29}NO_{10}$: C 52.89, H 6.78, N 3.25. Found: C 52.75, H 6.80, N 3.24. Data for protected L-*allo*-DNJ **11** (0.108 g): oily, $[\alpha]_D^{25}$ + 1.2 (*c* 2.0, $CHCl_3$). ^1H NMR (200 MHz): δ 1.49 (s, 9H), 2.03 (s, 3H), 2.09 (bs, 6H), 2.10 (s, 3H), 3.18 (bd, *J* = 15.5 Hz, 1H), 4.16 (dd, *J* = 11.4, *J* = 6.0 Hz, 1H), 4.34 (dd, *J* = 11.4, *J* = 7.6 Hz, 1H), 4.41 (bd, *J* = 15.5 Hz, 1H), 4.78 (bt, *J* = 6.3 Hz, 1H), 5.06-5.14 (m, 2H), 5.19 (bt, *J* = 2.2 Hz, 1H). ^{13}C NMR (125 MHz): δ 21.0 (2C), 21.2 (2C), 28.5 (3C), 29.9, 54.2, 61.3, 67.0, 67.1, 67.3, 80.9, 155.4, 170.1 (2C), 170.3 (2C). Anal. calcd for $C_{19}H_{29}NO_{10}$: C 52.89, H 6.78, N 3.25. Found: C 52.98, H 6.80, N 3.24.



12

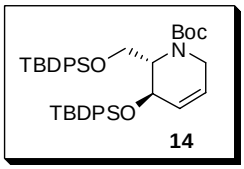
1-deoxy-L-mannojirimycin hydrochloride (**12**) [*from* **10**]. A mixture of **10** (0.15 g, 0.35 mmol) and 6N aq HCl (10 mL) was refluxed for 4h. Solvent removal afforded **12** (63 mg, 91%) as a white solid: mp (dec.) 183-184 °C, $[\alpha]_D^{25}$ + 41.0 (c 0.40, H_2O). ^1H NMR (400 MHz, D_2O): δ 3.15 (ddd, *J* = 10.3, *J* = 6.7, *J* = 3.2 Hz, 1H), 3.29 (dd, *J* = 13.6, *J* = 1.4 Hz, 1H), 3.32 (dd, *J* = 13.6, *J* = 3.2 Hz, 1H), 3.60 (dd, *J* = 9.5, *J* = 3.0 Hz, 1H), 3.77 (dd, *J* = 12.6, *J* = 6.8 Hz, 1H), 3.78 (bt, *J* = 9.9 Hz, 1H), 3.91 (dd, *J* = 12.6, *J* = 3.3 Hz, 1H), 4.12-4.18 (m, 1H). ^{13}C NMR (125 MHz, D_2O): δ 47.4, 58.2, 60.2, 65.8, 66.0, 72.5. Anal. calcd for $C_6H_{14}ClNO_4$: C 36.10, H 7.07, N 7.02. Found: C 36.15, H 7.05, N 7.00.



13

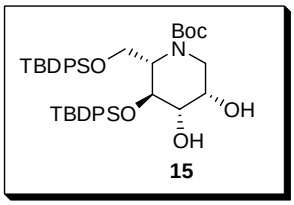
1-deoxy-L-allonojirimycin hydrochloride (**13**). A mixture of **11** (0.10 g, 0.23 mmol) and 6N aq HCl (7 mL) was refluxed for 4h. Solvent removal afforded **13** (41 mg, 90%) as a white solid: mp

166-167 °C, $[\alpha]_D^{25}$ - 37.7 (c 1.00, MeOH). ^{1}H NMR (500 MHz, D_2O): δ 3.15 (t, *J* = 12.0 Hz, 1H), 3.29 (dd, *J* = 12.2, *J* = 4.9 Hz, 1H), 3.32-3.39 (m, 1H), 3.85 (dd, *J* = 10.7, *J* = 2.5 Hz, 1H), 3.88 (dd, *J* = 12.7, *J* = 5.4 Hz, 1H), 3.96 (dd, *J* = 12.7, *J* = 3.0 Hz, 1H), 4.02 (ddd, *J* = 11.9, *J* = 4.9, *J* = 2.5 Hz, 1H), 4.20 (bs, 1H). ^{13}C NMR (125 MHz D_2O): δ 44.5, 57.4, 60.4, 67.3, 68.0, 72.6. Anal. calcd for $C_6H_{14}ClNO_4$: C 36.10, H 7.07, N 7.02. Found: C 36.18, H 7.04, N 7.05.



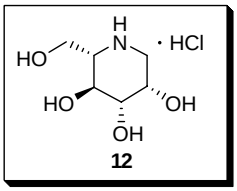
14

Compound 14. Zemplén deacetylation was accomplished by treatment of *anti*-**7** (0.24 g, 0.76 mmol) in MeOH (5 mL) with MeONa (41 mg, 0.76 mmol) for 4h at room temperature. Then, the mixture was neutralized with few drops of acetic acid, the solvents evaporated under reduced pressure. The crude residue was dissolved in $CHCl_3$ and filtered through a short pad of silica gel, the resulting filtrate was concentrated to dryness. The residue was dissolved in anhydrous DMF (5 mL), then TBDPSCl (0.49 mL, 1.9 mmol), DMAP (4.6 mg, 0.04 mmol) and imidazole (0.26 g, 3.8 mmol) were added at room temperature. The resulting mixture was kept at 40 °C and stirred for 8 h. The reaction was then cooled to room temperature, quenched with aq NH_4Cl and extracted with CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 95:5) gave the pure **14** (0.47 g, 88% overall yield) as a mixture of two conformers: oily, $[\alpha]_D^{25}$ -47.8 (*c* 0.1, $CHCl_3$). ^{1}H NMR (500 MHz) δ 0.95 (s, 2.25 H), 0.96 (s, 6.75H), 1.05 (s, 2.25H), 1.07 (s, 6.75H), 1.49 (s, 4.5H), 1.52 (s, 1.5H), 3.26-3.44 (m, 2H), 4.17-4.23 (m, 0.75H), 4.24-4.28 (m, 0.25H), 4.35 (t, *J* = 2.9, 0.6H) 4.40 (t, *J* = 3.1, 0.4H), 4.60 (t, *J* = 7.4 Hz, 0.75H), 4.82 (t, *J* = 7.4 Hz, 0.25H), 5.37-5.48 (m, 1H), 5.54-5.63 (m, 0.25H), 5.66-5.75 (m, 0.75H), 7.29-7.66 (m, 20H). ^{3}C NMR (75 MHz $CDCl_3$): δ 18.9, 19.1, 26.6 26.8, 28.3, 40.0, 41.1, 56.5, 58.2, 61.9, 64.6, 79.2, 79.4, 124.3, 127.5, 129.5, 133.2, 135.3, 135.6, 155.1, 155.4. Anal. calcd for $C_{43}H_{55}NO_4Si_2$: C 73.15, H 7.85, N 1.98. Found: C 73.28, H 7.89, N 1.99.



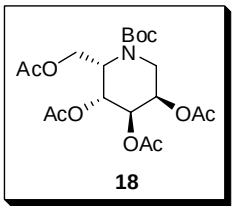
15

Compound 15. To a solution of **14** (0.30 g, 0.42 mmol) in 1:1 (v/v) *tert*-butyl alcohol/acetone (4 mL) cooled at 0 °C in ice bath an excess of a chilled 1:1 (w/w) solution of 4-methylmorpholine-*N*-oxide (0.91 g; 0.84 mmol) in water was added in one portion. After few minutes, a catalytic amount (0.02 mmol) of OsO_4 was added, and the reaction mixture was stirred overnight at room temperature; then the reaction was quenched with saturated aqueous Na_2SO_3 and evaporated under reduced pressure to afford a diastereomeric mixture (0.240 g, 77% yield; 97:3 dr) which chromatography over silica gel (hexane/acetone = 8:2) gave the pure **15** (0.232 g) as a mixture of two conformers: oily, $[\alpha]_D^{25}$ +18.2 (*c* 0.11, $CHCl_3$). ^{1}H NMR (500 MHz): δ 0.96 (s, 5.9H), 0.98 (s, 3.1H), 1.05 (s, 3.1H), 1.08 (s, 5.9H), 2.17 (d, *J* = 9.0 Hz, 0.66H), 2.26 (d, *J* = 9.8 Hz, 0.34H), 2.88 (t, d, *J* = 12.2 Hz, 0.66H), 2.97 (t, d, *J* = 12.2 Hz, 0.34H), 3.26 (dd, *J* = 10.5 Hz, *J* = 4.5 Hz, 0.66H), 3.31 (dd, *J* = 10.9 Hz, *J* = 4.1 Hz, 0.34H), 3.35 (d, *J* = 6.9 Hz, 0.66H), 3.60-3.65 (m, 1H), 3.69-3.77 (m, 0.68H), 3.79-3.84 (m, 0.66H), 4.01-4.15 (m, 2H), 4.28 (dd, *J* = 12.9 Hz, *J* = 5.5 Hz, 0.66H), 4.31-4.34 (m, 0.34H), 7.29-7.66 (m, 20H). ^{13}C NMR (100 MHz): δ 18.9, 19.22, 26.7, 26.9, 28.3, 40.7, 42.4, 56.5, 58.0, 63.9, 64.6, 64.8, 70.3, 70.5, 72.0, 72.4, 79.8, 80.0, 127.8, 129.9, 132.0, 132.2, 133.1, 133.3,135.4, 135.8, 136.0, 154.8, 155.1. Anal. calcd for $C_{43}H_{57}NO_6Si_2$: C 69.78, H 7.76, N 1.89. Found: C 69.93, H 7.74, N 1.90.



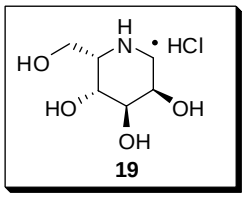
12

1-deoxy-L-mannojirimycin hydrochloride (**12**) [*from* **15**]. A mixture of **15** (0.20 g, 0.27 mmol) and 6N aq HCl (8 mL) was refluxed for 4h. Solvent removal afforded **12** (50 mg, 93%). Physical and spectroscopic data were identical to those reported above. Anal. calcd for $C_6H_{14}ClNO_4$: C 36.10, H 7.07, N 7.02. Found: C 36.16, H 7.09, N 7.04.



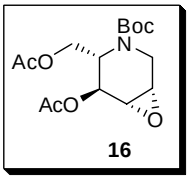
18

Compound 18. To a solution of *syn*-**7** (0.12 g, 0.38 mmol) in 1:1 (v/v) *tert*-butyl alcohol/acetone (3 ml) cooled at 0 °C in ice bath an excess of a chilled 1:1 (w/w) solution of 4-methylmorpholine-*N*-oxide (0.09 g; 0.8 mmol) in water was added in one portion. After few minutes, a catalytic amount (0.02 mmol) of OsO_4 was added, and the reaction mixture was stirred overnight at room temperature; then the reaction was quenched with saturated aqueous Na_2SO_3 and evaporated under reduced pressure. The crude residue was directly acetylated by treatment with Ac_2O in pyridine; after solvent removal under reduced pressure, the chromatography of the crude residue over silica gel (hexane/acetone = 9:1) gave the pure **18** (0.18 g, 83%): oily, ^{1}H NMR (500 MHz): δ 1.47 (s, 9H), 2.02 (s, 3H), 2.12 (s, 3H), 3.10-3.35 (m, 1H), 4.10-4.50 (m, 3H), 4.80-5.0 (m, 1H, 2-H), 5.20 (bd, 1H), 5.30-5.50 (m, 1H). ^{13}C NMR (100 MHz): δ 21.0, 21.2, 28.5, 45.2, 53.7, 62.8, 71.1, 72.3, 79.9, 83.9, 155.4, 173.0.

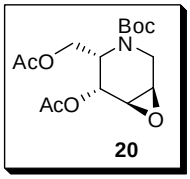


19

***1-deoxy-L-*gulo-*nojirimycin hydrochloride* (19)**. A mixture of **18** (0.10 g, 0.18 mmol) and 6N aq HCl (4 mL) was refluxed for 4h. Solvent removal afforded **19** (25 mg, 91%): white crystals, $[\alpha]_D^{25}$ = +45 (MeOH). ^{1}H NMR (500 MHz, D_2O): δ 3.7 (t, *J* = 11.7 Hz, 1H), 3.30 (dd, *J* = 12.2 Hz, *J* = 4.8 Hz, 1H), 3.54-360 (m, 1H), 3.82 (dd, *J* = 9.3 Hz, *J* = 12.2 Hz, 1H), 3.90 (dd, *J* = 12.2Hz, *J*= 4.9 Hz, 1H), 4.02-4.15(m, 2H), 4.20-4.30 (m, 1H). ^{13}C NMR (100 MHz D_2O): δ 42.5, 55.4, 58.9, 62.6, 67.1, 68.5.



16

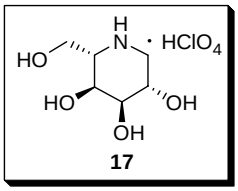


20

Compound 16 and 20. CF_3COCH_3 (0.7 mL, 0.008 mmol) and 4 mmol/L of aqueous Na_2EDTA and were added to a solution of *anti*-**7** (0.24 g, 0.76 mmol) in CH_3CN (7 mL) at 0 °C. After a

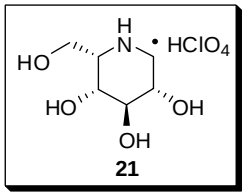
few minutes a mixture of $NaHCO_3$ (447 mg, 5.32 mmol) and Oxone (2.1 g, 3.5 mmol) was added slowly over 1 h at 0 °C. After being stirred at 0 °C overnight the reaction was quenched with H_2O and extracted with CH_2Cl_2. The extracts were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 8:2) afforded the pure **16** (0.22 g, 90% yield) as a single diastereoisomer: oily, $[\alpha]_D^{25}$ -11.7 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz $CDCl_3$): δ 1.47 (s, 9H), 2.07 (s, 3H), 2.15 (s, 3H), 3.21 (bd, *J* = 15.3 Hz, 1H), 3.32 (d, *J* = 3.7 Hz, 1H), 3.51 (bt, *J* = 4.3 Hz, 1H), 4.08-4.16 (m, 2H), 4.38-4.58 (m, 2H), 4.93 (d, *J* = 4.3 Hz, 1H). ^{13}C NMR (100 MHz): δ 20.8 (2C), 28.3 (3C), 37.5, 48.4, 50.0, 61.6, 65.5, 77.5, 80.8, 155.2, 170.4, 170.6. Anal. calcd for $C_{15}H_{23}NO_7$: C 54.70, H 7.04, N 4.25. Found: C 54.82, H 7.02, N 4.22.

In an analogous manner, **20** was obtained (90% yield) as from *syn*-**7**: oily; $[\alpha]_D^{25}$ -8.6, (*c* 0.2, $CHCl_3$). ^{1}H NMR (400 MHz): δ 1.47 (s, 9H), 2.02 (s, 3H), 2.12 (s, 3H), 3.15-3.18 (m, 1H), 3.24 (s, 1H), 4.16-4.23 (m, 3H), 4.40-4.50 (m, 1H), 4.70-5.15 (m, 2H). ^{13}C NMR (500 MHz): δ 20.7, 20.8, 28.2, 39.5, 49.5, 51.6, 59.4, 65.2, 77.0, 80.6, 153.8, 170.7.



17

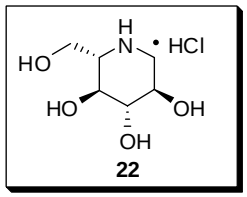
***1-deoxy-L*-altro-*nojirimycin perchlorate* (17)**. A solution of the epoxide **16** (0.20 g, 0.61 mmol) in an aqueous solution of 6% $HClO_4$ (10.0 mL) was stirred for 4 h at room temperature. The solvent was evaporated to dryness and washed with ether to afford **17** (0.15 mg, 94 % yield): oily, $[\alpha]_D^{25}$ -20.8 (*c* 1.0, H_2O). ^{1}H NMR (500 MHz, D_2O): δ 3.21 (d, *J* = 13.4 Hz, 1H), 3.32-3.40 (m, 2H), 3.86 (dd, *J* = 12.5 Hz, *J* = 6.7 Hz, 1H), 3.98 (dd, *J* = 12.5 Hz, *J* = 3.4 Hz, 1H), 4.02-4.07 (m, 2H), 4.14-4.18 (m, 1H). ^{13}C NMR (125 MHz D_2O): δ 44.0, 55.8, 56.7, 64.1, 66.8, 68.9. Anal. calcd for $C_6H_{14}ClNO_8$: C, 27.34; H, 5.35; N, 5.31. Found: C 27.38, H 5.38, N 5.28.



21

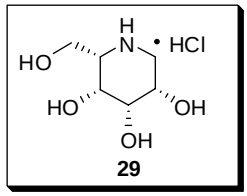
***1-deoxy-L*-ido-*nojirimycin perchlorate* (21)**. Compound **21** was obtained in 90% yield as from epoxide **20** by using analogous conditions reported above for the preparation of **17**: oily,

$[\alpha]_D^{25}$ + 8.7° (*c* 0.56, MeOH). ^{1}H NMR (500 MHz, D_2O): δ 2.77 (dd, *J* = 8.2 Hz, *J* = 12.8 Hz, 1H), 2.99 (dd, *J* = 4.1 Hz, *J* = 12.8 Hz, 1H), 3.18 (ddd, *J* = 4.6 Hz, *J* = 5.5 Hz, *J* = 8.3 Hz, 1H), 3.58-3.63 (m, 2H), 3.75 (dd, *J* = 4.6 Hz, *J* = 8.3 Hz, 1H), 3.77 (dd, *J* = 5.5 Hz, *J* = 11.9 Hz, 1H), 3.79 (dd, *J* = 8.3 Hz, *J* = 11.9 Hz, 1H). ^{13}C NMR (125 MHz D_2O): δ 46.7, 59.1, 60.4, 72.9, 73.4, 75.2.



22

***1-deoxy-L-nojirimycin hydrochloride* (22)**. To a solution of the epoxide **16** (0.12 g, 0.38 mmol) in anhydrous THF (3 ml) was added Ti(*O*-*i*Pr)$_4$ (0.04 ml, 0.57 mmol) and NaOBz (0.08 g, 0.57 mmol). The resulting heterogeneous mixture was stirred for 12 h at room temperature. Then EtOAc (20 ml) was added and the solution was washed with brine (3 x 20 ml). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The crude residue was diluted with 6N aq HCl (5 mL) and the mixture was refluxed for 4h. The solvent was then evaporated to dryness and the residue washed with ether (2 x 5 ml), acetone (2 x 5 ml) and toluene (2 x 5 ml) to afford **22** (0.03 g, 87% yield): $[\alpha]_D^{25}$ -40.1° (*c* 1.42, H_2O). ^{1}H NMR (D_2O): δ 2.42 (dd, *J* = 11.0 Hz, *J* = 12.3 Hz, 1H), 2.56 (ddd, *J* = 3.2 Hz, *J* = 6.4 Hz, *J* = 9.2 Hz, 1H), 3.13 (dd, *J* = 5.0 Hz, *J* = 12.3 Hz, 1H), 3.25 (t, *J* = 9.2 Hz, 1H), 3.34 (t, *J* = 9.2 Hz, 1H), 3.51 (ddd, *J* = 5.0 Hz, *J* = 9.2 Hz, *J* = 11.0 Hz, 1H), 3.64 (dd, *J* = 6.4 Hz, *J* = 11.4 Hz, 1H), 3.85 (dd, *J* = 3.2 Hz, *J* = 11.4 Hz, 1H). ^{13}C NMR (D_2O): δ 51.4, 63.2, 64., 73.6, 74.3, 81.1. Anal. Calcd for $C_6H_{13}NO_4$: C 44.16, H 8.03, N 8.58. Found: C 44.23, H 7.81, N 8.54.



29

***1-deoxy-L-talo-nojirimycin hydrochloride* (29)**. Zemplén deacetylation was accomplished by treatment of *syn*-**7** (0.08 g, 0.262 mmol) in MeOH (2 ml) with MeONa (0.04 g, 0.790 mmol) for 4h at room temperature. Then the mixture was neutralized with few drops of acetic acid, the solvents evaporated under reduced pressure. The crude residue was dissolved in anhydrous DCM (8 ml), the temperature kept at -78 °C, then TMEDA (3.5 μl) and an OsO_4 solution in DCM (0.5

ml) were added. The reaction mixture was stirred at -78 °C for 3h, after which the solvent was replaced with CH_3OH (3 ml) and few drops of conc. HCl were added. The mixture was stirred for 2h more, then the solvent was evaporated. The ratio between 1-deoxy-L-*gulo*-nojirimycin hydrochloride (**19**) and 1-deoxy-L-*talo*-nojirimycin hydrochloride (**29**) was determined by ^{1}H NMR (6:4 dr) Data for 1-deoxy-L-*talo*-nojirimycin hydrochloride (**29**, 0.03 g, 45% yield): white powder, $[\alpha]_D^{25}$ + 20.9 (CH_3OH). ^{1}H NMR (D_2O, 300 MHz): δ 3.20-3.34 (m, 2H), 3.45-3.79 (m, 3H), 3.83 (s, 1H), 4.10 (m, 1H), 4.18 (m, 1H). ^{13}C NMR (D_2O 75 MHz): δ 50.7, 61.4, 62.8, 69.0, 69.5, 70.0.

Single Crystal X-ray Diffraction.[*] Data collection was performed on a Enraf-Nonius MACH3 automated single crystal diffractometer, using graphite monochromated MoKα radiation (λ=0.71069 Å) in the ω scan mode. Cell parameters were obtained from least-squares fit of θ angles of 25 reflections in the range 10.24°≤θ≤13.87°.

Crystal data for $C_{24}H_{35}NO_6S_2$ (*anti*-**7**): M = 497.65, colourless prism 0.59 x 0.13 x 0.10 mm, monoclinic, $P2_1$, Z=2, a=11.014 (7), b=10.562 (5), c = 11.34(2)Å, β = 100.35(5)°, V = 1298(3) Å^3, ρ_{calc} = 1.274 g/cm^3, μ = 0.243 mm^{-1}. 6899 reflections collected (± *h*, ± *k*, ± *l*), max. θ = 27.98°, 6256 independent reflections (R_{int} = 0.0246). Structure was solved by direct method and refined by the full matrix least-squares method on F^2 against all independent measured reflections (SHELX97). All non-H atoms were anisotropically refined, H atoms were geometrically positioned and allowed to ride on carriers atoms. The absolute configuration was established by anomalous dispersion effects, Flack =0.08(9). 304 refined parameters, R1= 0.0483 wR2=0.1166 (on reflections with I>2σ(I)), R1= 0.1266 wR2=0.1403 (on all reflections). Max. and min. residual electron density (e Å^{-3}): +0.241 and –0.195.

[*] All crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (CCDC). Deposition number is CCDC 608202. These data can be obtained at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (internat.) +44-1223/336-033; Email: deposit@ccdc.cam.ac.uk] free of charge.

6.4 *REFERENCES*

[1] Somsak, L.; Nagya, V.; Hadady, Z.; Docsa, T.; Gergely, P. Glucose analog inhibitors of glycogen phosphorylases as potential antidiabetic agents: recent developments. *Curr. Pharm. Des.* **2003**, *9*, 1177.

[2] (a) Goss, P. E.; Baker, M. A.; Carver, J. P.; Dennis, J. W. Inhibitors of carbohydrate processing: a new class of anticancer agents. *Clin. Canc. Res.* **1995**, *1*, 935. (b) Weiss, M.; Hettmer, S.; Smith, P.; Ladish, S. Inhibition of melanoma tumor growth by a novel inhibitor of glucosylceramide synthase. *Cancer Res.* **2003**, *63*, 3654.

[3] Greimel, P.; Spreitz, J.; Stütz, A .E.; Wrodnigg, T. M. Iminosugars and relatives as antiviral and potential anti-infective agents. *Curr. Topics Med. Chem.* **2003**, *3*, 513.

[4] Butters, T. D.; Dwek, R. A.; Platt, F. M. Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorders. *Chem. Rev.* **2000,** *100*, 4683.

[5] (a) Afarinkia, K.; Bahar, A. Recent advances in the chemistry of azapyranose sugars. *Tetrahedron: Asymmetry* **2005**, *16*, 1239. (b) Pearson, M. S. M.; Allaimat, M. M.; Fargeas, V.; Lebreton, J. Recent advances in the total synthesis of piperidine azasugars. *Eur. J. Org. Chem.* **2005**, 2159. Carbohydrate-based routes to DNJ and congeners: (c) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. Nitrogen-in-the-ring pyranoses and furanoses: structural basis of inhibition of mammalian glycosidases. *J. Med. Chem.* **1994**, *37*, 3701. (d) O' Brien, J. L.; Tosin, M.; Murphy, P. V. Novel Synthesis of the glycosidase inhibitor deoxymannojirimycin and of a synthetic precursor D-*lyxo*-hexos-5-ulose. *Org. Lett.* **2001**, *3*, 3353. (e) Spreidz, J. S.; Stütz, A. E.; Wrodnigg, T. M. A simple access to the D-mannosidase inhibitor, 1-deoxymannojirimycin. *Carbohydr. Res.* **2002**, *337*, 183 and references cited therein. Non-carbohydrate based routes to DNJ and congeners: (f) Haukaas, M. H.; O'Doherty, G. A. Synthesis of D- and L-deoxymannojirimycin via an asymmetric aminohydroxylation of vinylfuran. *Org. Lett.* **2001**, *3*, 401. (g) Ruiz, M.; Ojea, V.; Ruanova, T. M.; Quintela, J. M. Access to iminosugars by aldol additions of metalated bis-lactim ethers to L-erythrose derivatives. *Tetrahedron: Asymmetry* **2002**, *13*, 795. (h) Takahata, H.; Banba, Y.; Sasatani, M.; Nemoto, H.; Kato, A.; Adachi, I. Asymmetric synthesis of 1-deoxynojirimycin and its congeners from a common chiral building block. *Tetrahedron* **2004**, *60*, 8199 and literature cited therein.

[6] Meyers, A. I.; Andres, C. J.; Resek, J. E.; Woddall, C. C.; McLaughlin, M. A.; Lee, P. H.; Price, D. A. Asymmetric routes to azasugars from chiral bicyclic lactams. Synthesis of 1,4-dideoxy-l,4-imino-D-lyxitol; l-deoxymannojirimycin; rhamno-L-deoxynojirimycin and 1-deoxy-6-*epi*castanospermine. *Tetrahedron* **1999**, *55*, 8931.

[7] Asano, N.; Ikeda, K.; Yu, L.; Kato, A.; Takebayashi, K.; Adachi, I.; Kato, I.; Ouchi, H.; Takahata, H.; Fleet, G. The L-enantiomers of D-sugar-mimicking iminosugars are noncompetitive inhibitors of D-glycohydrolase? *Tetrahedron: Asymmetry* **2005**, *16*, 223.

[8] Guaragna, A.; D'errico, S.; D'Alonzo, D.; Pedatella, S.; Palumbo, G. A general procedure for the synthesis of 1-deoxy-L-iminosugars. *Org. Lett.* **2007**, *9*, 3473.

[9] Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S. A new and versatile allylic alcohol anion and acyl β-anion equivalent for three-carbon homologations. *J. Org. Chem.* **1997**, *62*, 9369.

[10] Garner, P.; Park, J. M. 1,1,-dimethylethyl (*S*)- or (*R*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate: a useful serinal derivative. *Org. Synth., Collected Vol. IX* **1998**, 300.

[11] Van Rheenen, V.; Kelly, R.C.; Cha, D.Y. An improved catalytic OsO_4 oxidation of olefins to *cis*-1,2-glycols using tertiary amine oxides as the oxidant. *Tetrahedron Lett.* **1976**, 1973.

[12] Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. Catalytic asymmetric dihydroxylation. *Chem. Rev.* **1994**, *94*, 2483.

[13] Wu, X.-D.; Khim, S.-K.; Zhang, X.; Cederstrom, E. M.; Mariano, P. S. An oxidative mannich cyclization methodology for the stereocontrolled synthesis of highly functionalized piperidines. *J. Org. Chem.* **1998**, *63*, 841.

[14] Yang, D.; Wong, M.-K.; Yip, Y.-C. Epoxidation of olefins using methyl(trifluoromethyl)dioxirane generated *in situ*. *J. Org. Chem.* **1995**, *60*, 3887.

[15] For similar results see: (a) Kilonda, A.; Compernolle, F.; Hoornaert, G. J. *J. Org. Chem.* **1995**, *60*, 5820. (b) Kazmaier, U.; Grandel, R. A short synthesis of polyhydroxylated piperidines by aldol reaction of chelated amino acid ester enolates. *Eur. J. Org. Chem.* **1998**, 1833.

[16] Caron, M.; Sharpless, K. B. Titanium isopropoxide-mediated nucleophilic openings of 2,3-epoxy alcohols. A mild procedure for regioselective ring-opening. *J. Org. Chem.* **1985**, *1557*.

[17] Donohoe, T. J.; Blades, K.; Moore, P. R.; Waring, M. J.; Winter, J. J. G.; Helliwell, M.; Newcombe, N. J.; Stemp, G. Directed dihydroxylation of cyclic allylic alcohols and trichloroacetamides using OsO_4/TMEDA. *J. Org. Chem.* **2002**, *67*, 7946.

[18] Zemplen, G.; Kuntz, A. Amygdalin. IV. Synthesis of natural L-amygdalin. *Chem. Ber.* **1924**, *57B*, 1357.

Chapter 7

Nucleoside Analogues in Antiviral Drug Discovery: Old Agents and New Candidates for Chemotherapeutic Intervention

7. NUCLEOSIDE ANALOGUES IN ANTIVIRAL DRUG DISCOVERY: OLD AGENTS AND NEW CANDIDATES FOR CHEMOTHERAPEUTIC INTERVENTION

Nucleoside analogues have been the cornerstone of antiviral and anticancer therapy over the past thirty years. In the effort to discover effective, selective and non-toxic agents, a variety of strategies and a number of nucleoside analogues have been synthesized and evaluated. Among the great amount of nucleosides studied, L-nucleosides and six-membered nucleosides have represented a significant and unpredicted breakthrough, their efficacy being based on the surprising finding according to which they can be recognized by host enzymes and can, therefore, exhibit biological activity.

7.1 *INTRODUCTION*

The aim of antiviral therapy is to achieve substantial and prolonged suppression of viral replication. This goal is usually attained by interfering with the viral life cycle in one of its key events, such as the binding to host human cells,[1] the transcription of genomic RNA,[2] or the gene expression.[3] Depending on the molecular features, different classes of drugs have been thought and designed to focus their activity against at least one of these targets.[4]

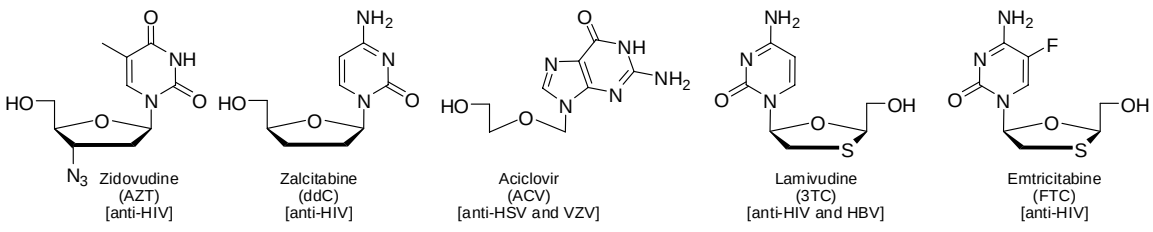
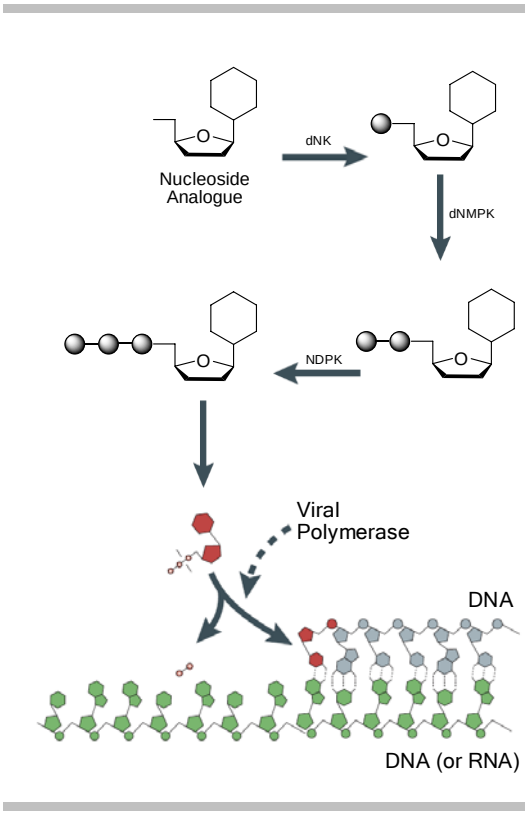


Figure 1 – *Some nucleoside analogues currently used in antiviral therapy.*

Among antiviral agents, nucleoside analogues occupy an important position. Because of their structural relationship to natural nucleosides, they have demonstrated to hold some of the most intriguing properties as antiviral compounds, since they are able to inhibit the viral replication, at

the transcription level, by blocking the information flow enclosed in the viral genome.[5] As a result of the progresses made over the years in the understanding of viral life cycles, prompted in particular by the need to combat human immunodeficiency virus (HIV), near twenty nucleosides are currently in use for therapeutic intervention; besides, some of them represent the lead compounds for the treatment of HIV and HSV infections[6] (**Figure 1**).

The mechanism of action of nucleoside analogues is based upon the intracellular phosphorylation to their 5′-triphosphate form, which can interact with virus-specific polymerases, acting as a competitive inhibitor or an alternative substrate for these target enzymes, usually preventing further viral nucleic acid chain elongation* (**Scheme 1**). The efficiency of nucleoside analogue inhibition of viral replication relies on four major factors: a) cellular uptake of the analogue; b) phosphorylation of the analogue by cellular kinases; c) level of competitive inhibition enacted; d) its incorporation in the viral DNA.[2]



Scheme 1 - *Antiviral nucleoside analogues acting by a chain termination mechanism.*

* The absence of a 3′-hydroxyl group in most analogues (see **Figure 1**) obviously confirms such a mechanism of chain termination of DNA elongation; nonetheless, a clear comprehension about this issue is far to be completely attained, since quite often the efficiency of deoxy-nucleosides is greater than that of dideoxy-nucleosides [see Ref. 2.]

Over the years, a variety of strategies have been devised with the aim to design nucleoside analogues as effective, selective and non-toxic antiviral agents. These strategies have involved several formal modifications of the naturally occurring nucleosides: especially, modifications can be divided into three categories: 1) alterations on the phosphate group,[7] 2) alterations on the nucleobase[8] and 3) alterations on the sugar moiety[9] (**Figure 2**). Particularly, modification in the sugar moiety of nucleosides has led to the development of several nucleoside analogues which are able to exert biological activity. Well-known examples are acyclic nucleosides (Acyclovir, ACV),[10] *C*-nucleosides (Showdomycin)[11] and carbocyclic nucleosides (Carbovir).[12]

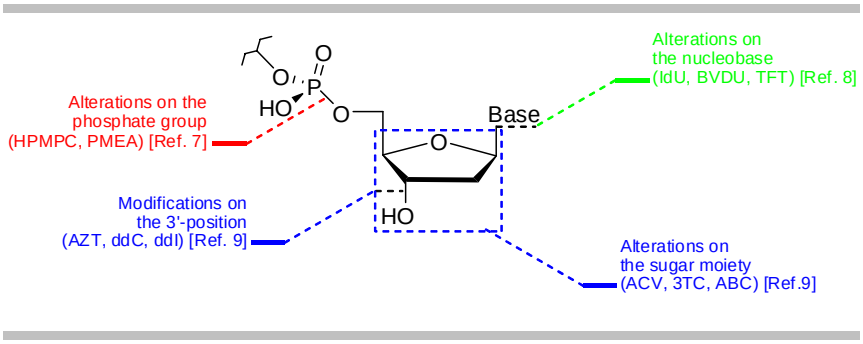


Figure 2 – *The broad spectrum of the possible modifications on the natural deoxyribofuranosyl nucleoside.*

Among the great number of sugar-modified nucleosides, a special attention has been deserved to two specific classes, i.e. L-nucleoside and six-membered nucleoside analogues. Although for a long time a little attention has been given to such compounds, because it was assumed they did not resemble the natural nucleosides, later they have been re-evaluated as new classes of antiviral agents, since the very promising bioactive features.

7.2 *SUGAR MODIFICATIONS IN NUCLEOSIDE ANALOGUES: L-NUCLEOSIDES*

Compared to natural nucleosides, their L-enantiomers have inverted configuration at all chiral centers.[13] By analogy with natural D-nucleosides, the β-anomers bring the base moiety in a *cis* orientation, with regards to the 4'-hydroxymethyl group (**Figure 3**).

Although the first synthesis of L-nucleosides was reported in the 1960s, little consideration has been given to this class of molecules until the appearance of 3TC (the first effective L-nucleoside analogue with strong antiviral power, **Figure 4**).

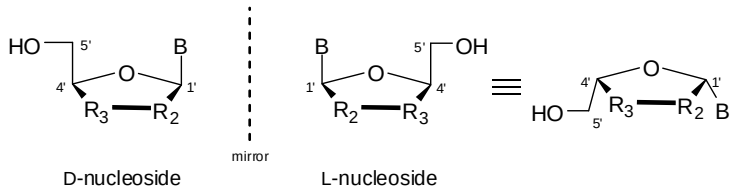


Figure 3 – *D- and L- nucleosides.*

The molecular world is supposed to be in a completely asymmetric environment and most biologically active compounds have a defined D- or L-configuration. Enzyme catalyzed reactions are usually highly stereoselective (nearly stereospecific) and act mainly on one enantiomer, such as D-nucleosides (as well as D-mono- and oligosaccharides or L-amino acids). This may be the possible reason why it took so long to pay attention on L-nucleosides: it was simply assumed that optically active compounds resembling the natural nucleosides should be invariably more active than the "unnatural" L-enantiomers. On the basis of these concerns, it appeared rather surprisingly the discovery[14] according to which in the racemic potent anti-HIV (EC_{50} = 0.02-0.06 μM) nucleoside BCH-189 ((±)-2',3'-dideoxy-3'-thiacytidine **1**) the L-isomer (later called 3TC, lamivudine, **2**) was more potent (EC_{50} = 0.0018 μM) than its D-isomer* (**Figure 4**). In addition, compared to **1**, 3TC included more favorable toxicological profiles and a major metabolic stability. Since this first discovery, a number of L-nucleoside analogues have been synthesized and biologically evaluated and the importance of chirality and the influence on antiviral activity of the L-nucleosides has been recognized and studied.

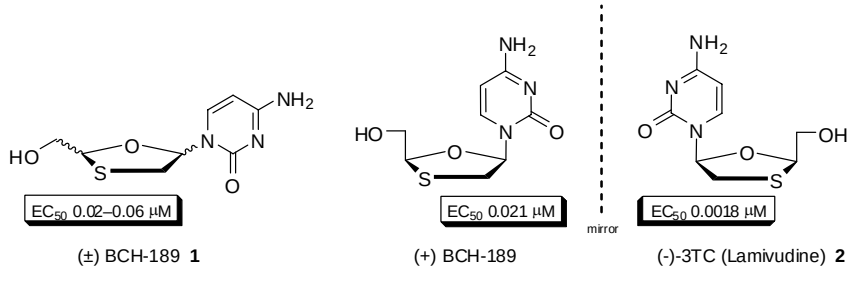


Figure 4 – *(+) BCH-189 and 3TC.*

* 3TC has been later approved by FDA as inhibitor of HIV and HBV replication and currently it is clinically used either alone or in combination with other inhibitors.

Following the example of 3TC, the 5-fluoro analogue of 3TC, (-)-2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC or Emtricitabine, **Figure 1**) showed potent antiretroviral activity against HIV-1 and HIV-2 in various cell cultures. Compared to its (+) enantiomer, FTC exhibited 20-fold more potent anti-HIV-1 activity (EC_{50} = 0.008 μM, the (+) counterpart having an EC_{50} = 0.84 μM) and a 40-fold more potent anti-HIV-2 activity.*,[15]

In addition to 3TC and FTC, among the L-nucleoside analogues currently at the stage of clinical trials a special attention must be deserved to the L-nucleoside analogue L-(-)-dioxolane cytidine (β-L-(-)-2',3'-dideoxy-3'-oxacytidine) (L-OddC, **Figure 5**). Besides having potent anti-HBV and anti-HIV activity, it demonstrated to hold significant antileukemic properties, to the extent that it represents one of the most promising antitumor drug candidates (**Figure 5**). When evaluated by *in vitro* and *in vivo* experiments, this compound also exhibited potent activity against various solid tumour cell lines, including prostate, renal, hepatoma and colon. On the other side, toxicity experiments indicated that very high doses of the drug were well tolerated by mice. All these factors contribute to a greater efficacy of this compound in treating some types of tumours, in comparison to the currently available anti-tumour chemotherapeutic agents.

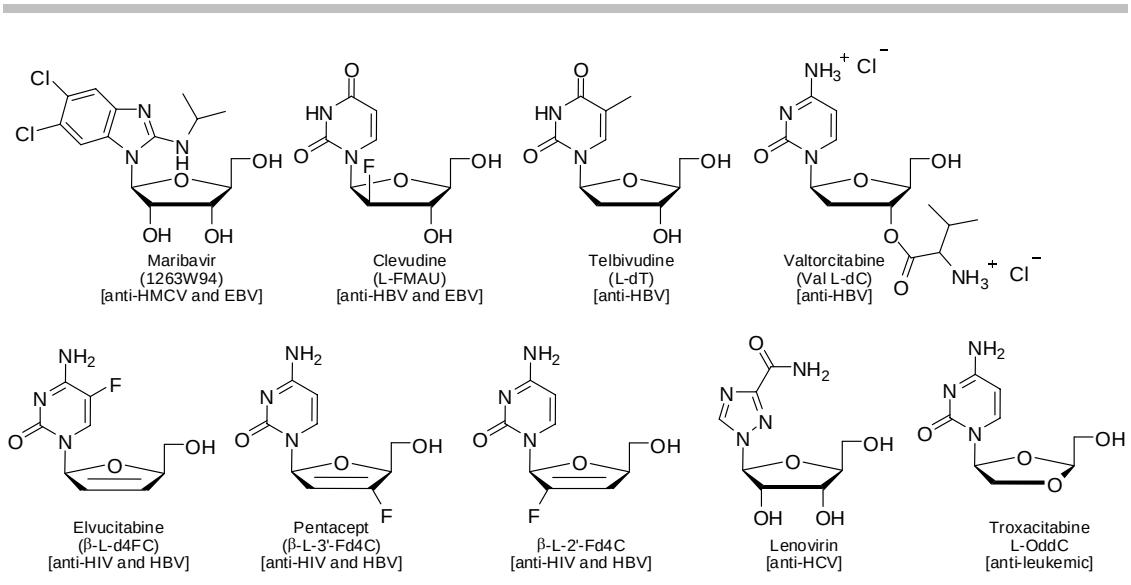


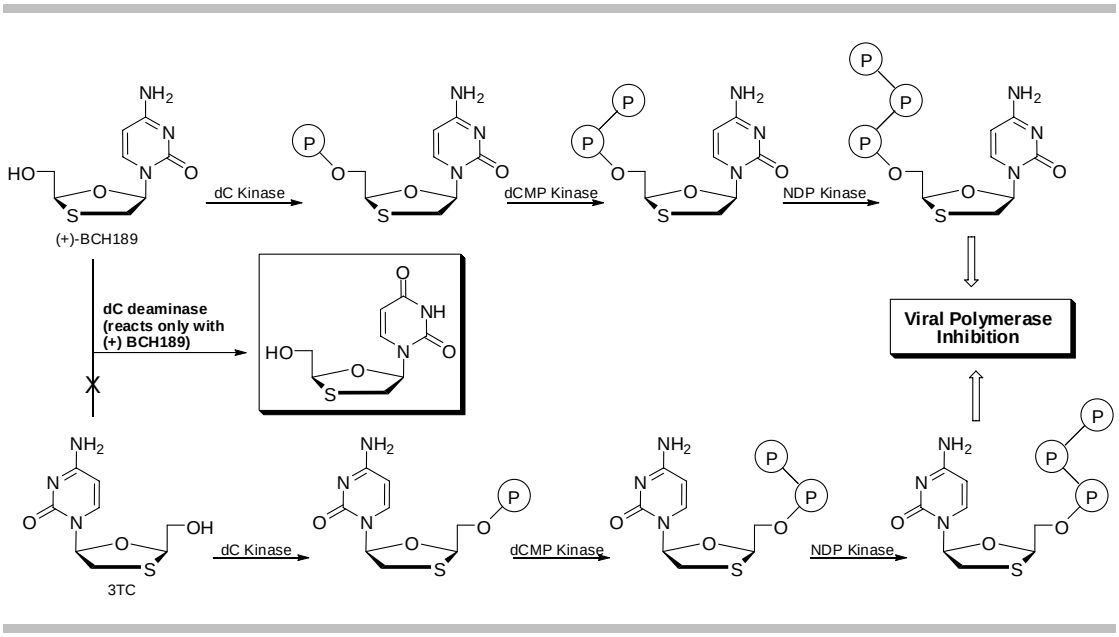
Figure 5 – *L-Nucleoside analogues currently at the stage of clinical trials.*

* Currently, FTC is used in combination with other antiretroviral agents for the treatment of HIV infection in adults; it is also in clinical trials in order to evaluate its potency for the treatment of chronic Hepatitis B [Ref 13a].

7.3 *INSIGHT INTO THE ANTIVIRAL ACTIVITY: L-NUCLEOSIDES AND THE LACK OF ENZYMATIC SELECTIVITY OF SOME NUCLEOSIDE KINASES*

The most unpredicted aspect in the activity of L-nucleoside analogues is the fact that certain enzymes are able to phosphorylate L-nucleosides to their tri-phosphate derivatives and thereby provide biological activity. In fact, as above mentioned (**Scheme 1**), nucleosides require a serial phosphorylation, *via* mono- and di-phosphate intermediates, to the tri-phosphorylated form, in order to gain pharmacological activity. Moreover, once phosphorylation is achieved, L-triphosphate nucleoside needs to successfully compete with physiological nucleoside triphosphates for polymerase inhibition and/or incorporation into the viral DNA. In human cells, the phosphorylation of nucleosides to the monophosphate form, which is often rate-limiting, is catalyzed by two cytosolic enzymes, thymidine kinase 1 (TK-1) and deoxycytidine kinase (dCK) and by two mitochondrial enzymes, thymidine kinase 2 (TK-2) and deoxyguanosine kinase (dGK). These enzymes play a crucial role in supplying triphosphate nucleoside precursors for DNA synthesis, as well as in activating therapeutically relevant nucleosides.

Biochemical studies[16] realized with regards to the enzymes involved in 3TC, FTC and L-OddC mono-phosphorylation showed that among these, human deoxycytidine kinase (dCK) lacked in stereoselectivity and could phosphorylate both enantiomers. Particularly, concerning 3TC, it was found that this one was a better substrate for dCK than the D-enantiomer (+)-BCH-189. Moreover, 3TC and FTC were resistant to deamination by deoxycytidine deaminase (that instead works in a strictly enantioselective manner), whereas the corresponding D-enantiomers are deaminated by the same enzyme (**Scheme 2**), so finding a reason why for 3TC and FTC a longer half-life than their D-counterparts is also reported.



Scheme 2 - *Metabolism of optical isomers of BCH-189.*

Moreover, human dCk is not the only enzyme that displayed non-enantioselective properties. Although L-thymidine is not recognized by human thymidine kinase (TK), it functions as a substrate for Herpes Simplex 1 Thymidine Kinase (HSV-1 TK). In fact, HSV-1 TK shows no stereospecificity as well and it phosphorylates both D- and L-thymidine with identical efficiency, the K_i of L-thymidine (2 μM) being almost identical to the K_i for the natural substrate thymidine (2.8 μM). In addition, TK-2 and dGk have shown an analogous trend.[17]

In summary, while human TK-1 is strictly enantioselective, human nucleoside kinases dCK, dGK, TK-2 and the herpes simplex virus type 1 thymidine kinase (HSV-1 TK) don't show stereoselectivity. This unusual behaviour so far remains not completely rationalized; however, some interesting structural similarities sharing such enzymes have been recently reviewed,[18] pointing to a common feature present in dCK-like enzymes that confers relaxed enantioselectivity.

7.4 *SUGAR MODIFICATIONS IN NUCLEOSIDE ANALOGUES: SIX-MEMBERED NUCLEOSIDES*

A discussion about the possible employ of six-membered nucleoside analogues as antiviral agents can take place beginning from the early finding according to which some pyranosyl nucleosides are effectively provided with biological activity, which mainly demonstrates that

six-membered sugar rings can be recognized by the binding cavity of enzymes. For instance, 1-(2′-deoxy-β-D-*arabino*-hexopyranosyl)-thymine (**3**) was identified to be an inhibitor of the pyrimidine nucleoside phosphorylase from Ehrlich ascites tumour.[19] The guanosine analogue 2-amino-9-(2′-deoxy-β-D-*ribo*-hexopyranosyl)-purin-6-one (**6**) was moderately active against HSV-2 virus (VR = 1.06).[20] The uridine analogue, 1-(2′-deoxy-6′-*O*-phosphono-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidine-dione (**4**) showed activity against HSV-2 and parainfluenza 3 virus and was also active against L1210 (ID_{50} = 39 μM) and P388 (ID_{50} = 33 μM) leukemic cell lines. 9-β-D-Fucopyranosyl adenine (**5**) has been reported to be an inhibitor of the development of leukemia cells L1210 as well[21] (**Figure 6**).

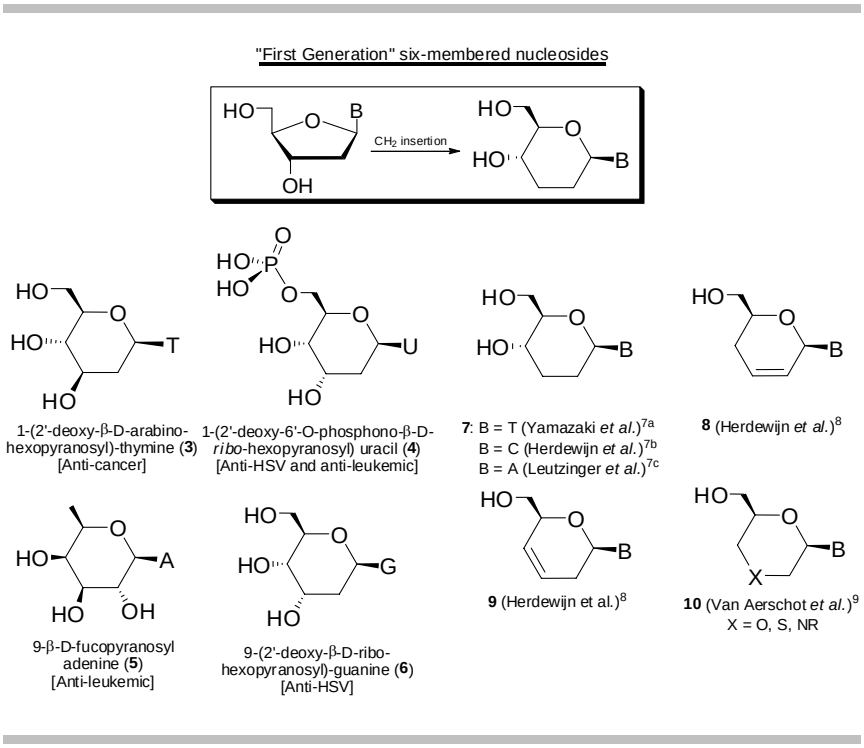


Figure 6 – *Early examples of pyranose nucleoside analogues.*

Inspired by these reports, further modifications were realized on hexose architecture, achieving at first a wide series of deoxy-pyranosyl nucleosides[22] (**7**), deoxy-D-*hex*-2-enopyranosyl nucleosides[23] (**8**, **9**) and 1,4-dioxane, 1,4-oxathiane, or 1,4-oxazine ring structures[24] (**10**). Compounds **7-10** didn't show significant antiviral activity (**Figure 6**). Conversely, more interesting results were obtained from the study of the 1,5-anhydrohexitol ring nucleosides like **11-13** (**Figure 7**).

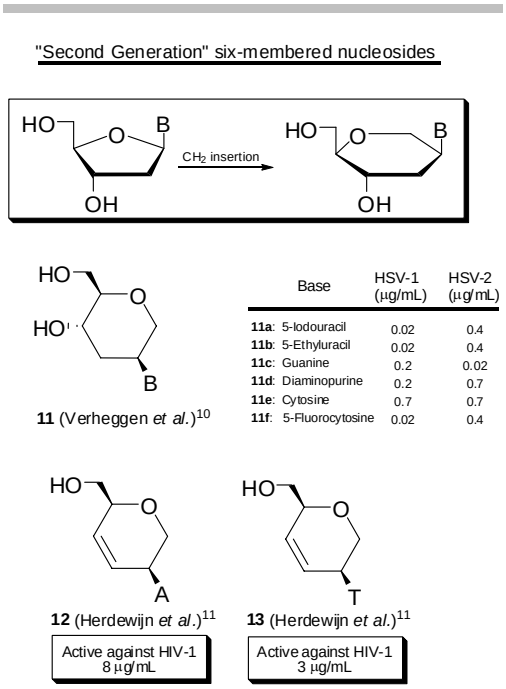


Figure 7 – *Early examples of pyranose nucleoside analogues.*

The concept behind hexitol nucleosides was the construction of molecules more stable against enzymatic degradation, without abolishing their substrate properties for viral kinases. With this aim, 2',3'-dideoxy-1',5'-anhydro-D-*arabino*-hexitol nucleosides and their derivatives (**11-13**), the base being axially oriented at the *C*-2' position, were synthesized[25] (**Figure 7**). As reported,[26] due to the axial orientation of the nucleobase, the six-membered ring **14** is able to mimic a furanose ring **15** frozen in a 2'-*exo*, 3'-*endo* conformation (**Figure 8**). It can be seen as an extended furanose, in which a CH_2 group is inserted between the endocyclic oxygen and the *C*-1' position.

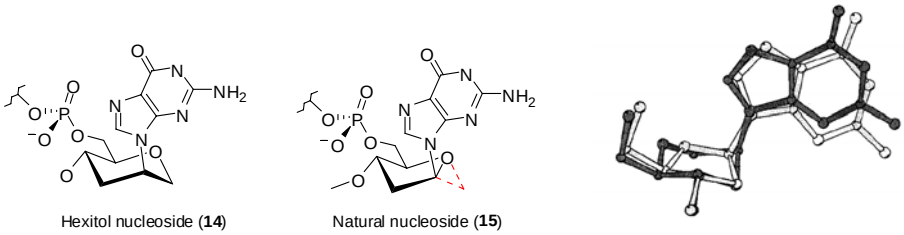


Figure 8 – *Hexitol nucleosides are mimetics of natural deoxyribofuranosyl nucleosides frozen in a 2'*-exo*, 3'*-endo *conformation.*

5-Iodouracil and 5-ethyluracil analogues of anhydrohexitol nucleosides (**11a-b**) displayed activity similar to acyclovir against HSV-1 and HSV-2 (see table in **Figure 7**). Moreover, no evidence of cytotoxicity to a variety of cells was reported.[27] The corresponding guanine and cytosine analogues **11c**, **11e** were also potent inhibitors of human cytomegalovirus (HCMV) *in vitro* (IC_{50} = 1.2 and 0.75 μM, respectively). In addition, also 1,5-unsaturated hexitol congeners **12** and **13** showed interesting antiviral properties, displaying low but significant activity against HIV-1.[27]

A more sophisticated modification in pyranose ring implied for the replacement of furanose oxygen atom by a double carbon-carbon bond, obtaining cyclohexenyl nucleosides (**Figure 9**). As carbocyclic nucleosides, cyclohexenyl nucleosides are stable against chemical and enzymatic degradation due to the absence of an anomeric center.[28]

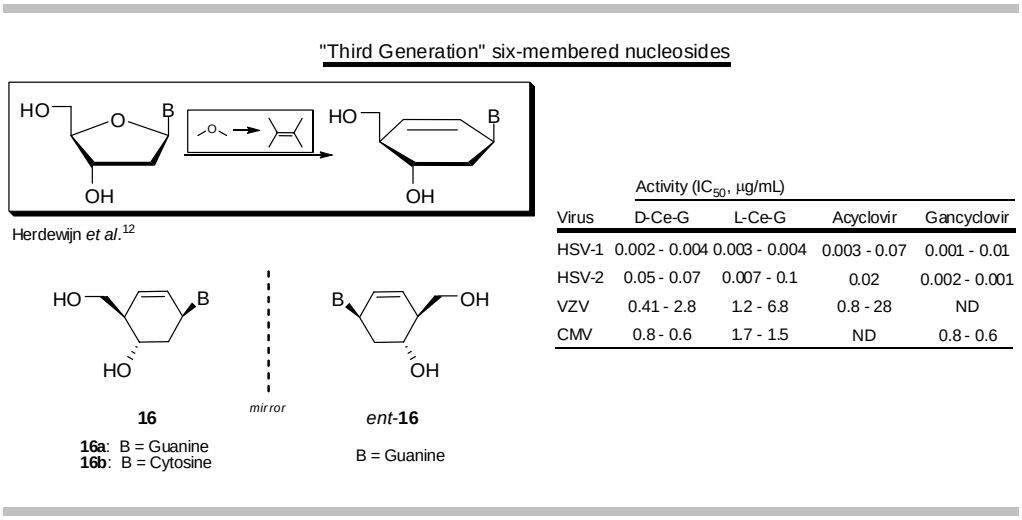


Figure 9 – *D-and L- cyclohexenyl nucleoside analogues.*

These compounds represent the most potent antiviral nucleosides with a six-membered carbohydrate-like moiety that has been reported to date. Moreover, D- and L-series nucleosides **16** and *ent*-**16** displayed similar significant antiviral activity (L-cyclohexenyl nucleosides showed a little less activity). D-Cyclohexenyl guanine (**16a**), in particular, was tested against a whole range of herpes viruses and its activity was comparable with those of Acyclovir (ACV) and Ganciclovir (GCV) (see table in **Figure 9**).[29] In addition, cyclohexenyl cytosine was found to be a potent anti-VZV compound.

Intrigued by such promising results as antivirals, both anhydrohexitol and cyclohexenyl nucleosides **11** and **16** were also used to construct oligonucleotide sequences: hexitol nucleic

acids (HNA) and cyclohexenyl nucleic acids (CeNA) showed strong hybridization with RNA and DNA.[30]

7.5 *CONCLUDING REMARKS*

Concerted efforts made worldwide by medicinal chemists, biologists and clinicians to eradicate the most advanced viral strains are providing valuable treatments in antiviral chemotherapy by supplying an ever-increasing number of molecules targeted at virtually any step of the viral replicative cycle. However, despite the enormous progresses made in this field, effective therapies for several viral infections still lack and established treatments are not always effective or well tolerated, highlighting the need for further refinement of antiviral drug design and development.

Within the class of nucleoside analogues, which represent some of the most successful lead compounds for chemotherapeutic intervention, L-nucleoside enantiomers have constituted an essential discovery. In fact, as a general feature, those compounds with an unnatural configuration confer in most cases lower toxicity and higher metabolic stability compared to the natural D-counterparts. As a result, several L-nucleoside analogues are currently used as antiviral agents and some of them are likely to be introduced into antiviral treatments in the next years. Moreover, it is noteworthy that the chemotherapeutic potential of L-nucleoside analogues is not limited to the antiviral field. The antileukemic properties of L-OddC have been already pointed out; several other L-nucleosides, like L-adenosine and L-thymidine, could find utility in new malaria chemotherapy.[31] As from such examples, the ever-increasing interest towards L-nucleosides appears fully justified and approaches to the synthesis of such molecules continue to represent a challenging area of investigation. In the search for new, safe and effective agents, further refinements along these lines could yield more selective and potent antiviral compounds.

7.6 *REFERENCES*

[1] Nair, V.; Chi, G. HIV integrase inhibitors as therapeutic agents in AIDS. *Rev. Med. Virol.* **2007**, *17*, 277.

[2] Arts, E. J.; Wainberg, M. A. Mechanisms of nucleoside analog antiviral activity and resistance during human immunodeficiency virus reverse transcription. *Antimicrob. Agents Chemother.* **1996**, *40*, 527.

[3] Tomasselli, A. G.; Heinrikson, R. L. Targeting the HIV-protease in AIDS therapy: a current clinical perspective. *Biochim. Biophys. Acta* **2000**, *1477*, 189.

[4] De Clercq, E. Strategies in the design of antiviral drugs. *Nat. Rev.* **2002**, *1*, 13.

[5] De Clercq, E. Antiviral drug discovery and development: where chemistry meets with biomedicine. *Antiviral Res.* **2005**, *67*, 56.

[6] (a) Stevens, M.; De Clercq, E.; Balzarini, J. The regulation of HIV-1 transcription: molecular targets for chemotherapeutic intervention. *Med. Res. Rev.* **2006**, *26*, 595. (b) Coen, D. M.; Schaffer, P. A. Anti-herpes virus drugs: a promising spectrum of new drugs and drug targets. *Nat. Rev.* **2003**, *2*, 278.

[7] De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A novel selective broad-spectrum anti-DNA virus agent. *Nature* **1986**, *323*, 464.

[8] Robins, R. K.; Revankar, G. R. Purine analogs and related nucleosides as antitumor agents. *Med. Res. Rev.* **1985**, *5*, 273.

[9] Ichikawa, E.; Kato, K. Sugar-modified nucleosides in past 10 years, a review. *Curr. Med. Chem.* **2001**, *8*, 385.

[10] Schaeffer, H. J.; Beauchamp, L.; de Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. 9-(2-hydroxyethoxymethyl) guanine Activity against Viruses of the Herpes Group. *Nature* **1978**, *272*, 683.

[11] *Nucleoside mimetics: their chemistry and biological properties* (Clare Simons); Gordon and Breach Science Publishers, Amsterdam, **2001**.

[12] Vince, R.; Hua, M.; Brownell, J.; Daluge, S.; Lee, F.; Shannon, W. M.; Lavelle, G. C.; Qualls, J.; Weislow, O. S.; Kiser, R.; Canonico, P. G.; Schultz, R. H.; Narayanan, V. L.; Mayo, J. G.; Shoemaker, R.H.;Boyd,M.R. Potent and selective activity of a new carbocyclic nucleoside analog (Carbovir) against human immunodeficiency virus in vitro. *Biochem. Biophys. Res. Commun.* **1988**, *156*, 1046.

[13] (a) Wang, P.; Hong, J.H.; Cooperwood, J. S.; Chu, C. K., Recent advances in L-nucleosides: chemistry and biology. *Antiviral Research* **1998**, *40*, 19. (b) Mathè, C.; Gosselin, G. L-Nucleoside enantiomers as antivirals drugs: a
mini-review. *Antivir. Res.* **2006**, *71*, 276.

[14] Pèlicano, H.; Pierra, C.; Eriksson, S.; Gosselin, G.; Imbach, J.-J.; Maury, G. Enzymatic properties of the unnatural β-L-enantiomers of 2',3'dideoxyadenosine and 2',3'-didehydro-2',3'dideoxyadenosine. *J. Med. Chem*. **1997**, *40*, 3969.

[15] Saag, M. S. Emtricitabine, a new antiretroviral agent with activity against HIV and Hepatitis B virus. *Clin. Infect. Dis.* **2006**, *42*, 126.

[16] (a) Van Draanen, N.A. *et al.* Influence of stereochemistry on antiviral activities and resistance profiles of dideoxycytidine nucleosides, *Antimicrob. Agents Chemother.* **1994**, *38*, 868. (b) Sabini, E.; Hazra, S.; Konrad, M.; Lavie, A., Nonenantioselectivity Property of Human Deoxycytidine Kinase Explained by Structures of the Enzyme in Complex with L- and D-Nucleosides, *J Med Chem* **2007**, *50*, 3004.

[17] Maury, G. The enantioselectivity of enzymes involved in current antiviral therapy using nucleoside analogues: a new strategy? *Anti*V*iral Chem. Chemother.* **2000**, *11*, 165.

[18] Gumina, G.; Chong, Y.; Choo, H.; Song, G.-Y.; Chu, C. L-Nucleosides: antiviral activity and molecular mechanism. *Curr. Top. Med. Chem.* **2002**, *2*, 1065.

[19] Laugen, P.; Etzold, G. *Biochem. J.* **1963**, *339*, 190.

[20] Nord, L. D.; Dalley, N. K.; McKernan, P. A.; Robins, R. K. Synthesis, structure, and biological activity of certain 2'-deoxy-β-D-*ribo*-hexopyranosyl nucleosides and nucleotides. *J. Med. Chem.* **1987**, *30*, 1044.

[21] (a) Lerner, L. Synthesis of 9-α and 9-β-D-fucopyranosyl adenine. *Carbohydr. Res.* **1971**, *2*, 255. (b) Hanessian, S.; Condet, J. J.; Khai, H. H.; Lou, B. The stereocontrolled synthesis of 1,2-*trans* hexopyranosyl nucleosides *via* a novel anomeric activation. *Tetrahedron* **1996**, 52, 10827.

[22] (a) Yamazaki, T.; Shiraishi, H.; Matsuda, K.; Sugiyama, H.; Seto, S. New synthetic route to 2',3'-unsaturated pyranosyl thymine from the corresponding *O*-2'nucleoside. *J. Chem. Soc., Chem. Commun.* **1975**, *13*, 518. (b) Van Aerschot, A.; Kerremans, L.; Balzarini, J.; De Clercq, E.; Herdewijn, P. Synthesis and anti-HIV Activity of dideoxycytidine analogues containing a pyranose carbohydrate moiety. *Nucleosides and Nucleotides* **1991**, *10*, 589. (c) Leutzinger, E. E.; Robins, R. K.; Townsend, L. B. The direct utilization of unsaturated sugars in nucleoside synthesis. An approach to the preparation of analogs of blasticidin S. *Tetrahedron Lett.* **1968**, *43*, 4475.

[23] (a) Herdewijn, P.; Van Aerschot, A. Synthesis of Trideoxy-hexopyranosylated Nucleoside Analogues as Potential Anti-HIV agents. *Bull. Soc. Chim. Belg.* **1990**, *99*, 895. (b) Herdewijn, P.; Van Aerschot, A.; Balzarini, J.; De Clercq, E. Synthesis of pyranosyl nucleosides. *Nucleosides Nucleotides* **1991**, *10*, 119.

[24] Van Aerschot, A.; Jansen, G.; Herdewijn, P. Nucleoside Analogues with a 1,4-Dioxane, 1,4-Oxathiane or 1,4-Oxazine Ring Structure as the Carbohydrate Fragment. *Bull. Soc. Chim. Belg.* **1990**, *99*, 769.

[25] Verheggen, I.; Van Aerschot, A.; Toppet, S.; Snoeck, R.; Janssen, G.; Balzarini, J.; De Clercq, E.; Herdewijn, P. Synthesis and Antiherpes Virus Activity of 1,5-Anhydrohexitol Nucleosides. *J. Med. Chem.* **1993**, *36*, 2033.

[26] Herdewijn, P. The interplay between antiviral activity, oligonucleotide hybridisation and nucleic acids incorporation studies. *Antivir. Res.* **2006**, *71*, 317.

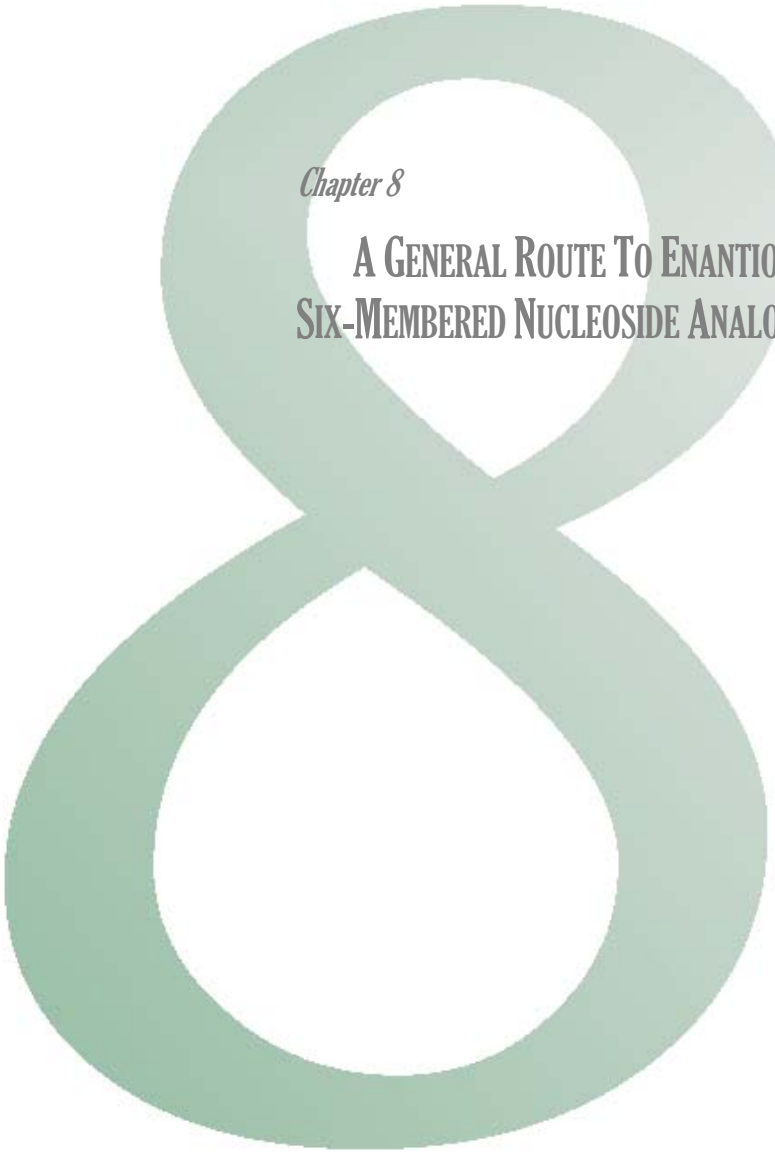
[27] Herdewijn, P., Nucleosides with a six-membered carbohydrate moiety: structural requirements for antiviral activity. In: Bentley, P.H., O'Hanlon, P.J. (Eds.), Anti-Infectives. *Recent Advances in Chemistry and Structure-Activity Relationships;* The Royal Society of Chemistry: Cambridge, **1997**.

[28] Herdewijn, P.; De Clercq, E. The cyclohexene ring as bioisostere of a furanose ring: synthesis and antiviral activity of cyclohexenyl nucleosides. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1591.

[29] Wang, J.; Froeyen, M.; Hendrix, C.; Andrei, G.; Snoeck, R.; De Clercq, E.; Herdewijn, P. The Cyclohexene Ring System as a Furanose Mimic: Synthesis and Antiviral Activity of Both Enantiomers of Cyclohexenylguanine. *J. Med. Chem.* **2000**, *43*, 736.

[30] (a) Hendrix, C.; Rosemeyer, H.; De Bouvere, B.; Van Aerschot, A.; Seela, F.; Herdewijn, P. 1,5-Anhydrohexitol oligonucleotides: hybridization and strand displacement with oligoribonucleotides, interaction with RNase H and HIV reverse transcriptase. *Chem. Eur. J.* **1997**, *3*, 1513. (b) Wang, J.; Verbeure, B.; Luyten, I.; Lescrinier, E.; Froeyen, M.; Hendrix, C.; Rosemeyer, H.; Seela, F.; Van Aerschot, A.; Herdewijn, P. Cyclohexene nucleic acids (CeNA): serum stable oligonucleotides that activate RNase H and increase duplex stability with complementary RNA. *J. Am. Chem. Soc.* **2000**, *122,* 8595. For a more accurate discussion about this issue see Chapter 9.

[31] Gero, A. M.; Dunn, C. G.; Brown, D. M.; Pulenthiran, K.; Gorovits, E. L.; Bakos, T.; Weis, A. L.. New malaria chemotherapy developed by utilization of a unique parasite transport system. *Curr. Pharm. Design* **2003**, *9*, 867



Chapter 8

A General Route To Enantiopure Six-Membered Nucleoside Analogues

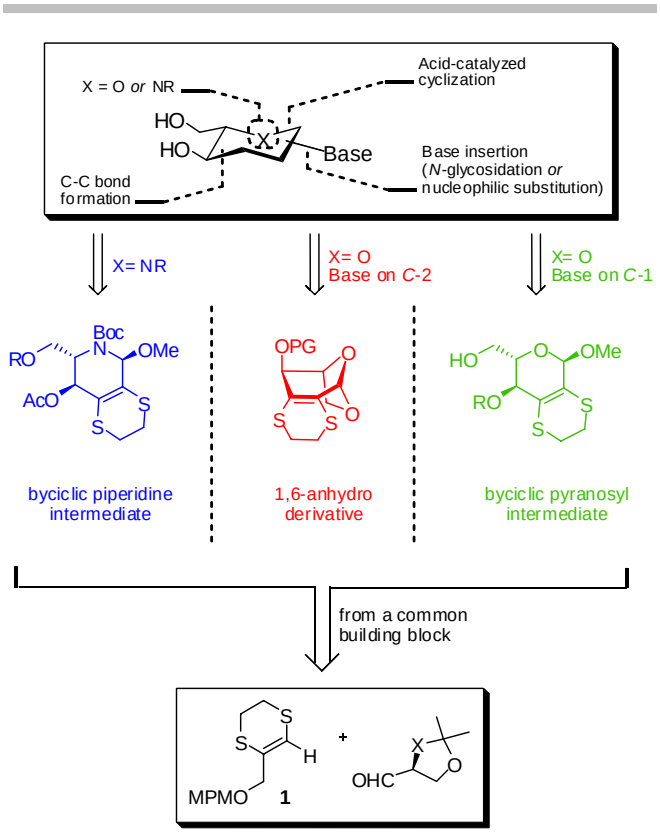
8. A GENERAL ROUTE TO ENANTIOPURE SIX-MEMBERED NUCLEOSIDE ANALOGUES

An efficient and stereoselective route for the synthesis of six membered nucleosides belonging to L*-series is herein reported. More specifically, the use of our three carbon homologating agent* ***1*** *coupled with suitable electrophiles (i.e. the Garner's aldehyde,* ***2*** *and the 2,3-*O*-isopropylidene-*L*-glyceraldehyde,* ***15****) enables the preparation of diverse nucleosides, such as iminonucleosides and hexopyranosyl nucleosides (the nucleobases being located in different positions of the ring). With regard to hexopyranosyl nucleoside synthesis,* L*-enantiomers of nucleosides provided with interesting biological activity have been prepared in high yields and stereoselectivity. On the other hand, the current route towards iminonucleosides must be considered as the first entry to such molecules, whether they belong to* D *or* L *series. Therefore, the preparation of compounds from both series could be farther on achieved.*

8.1 *INTRODUCTION*

The current arsenal of bioactive compounds in use for the treatment of viral infections is the result of several decades of research, including syntheses and biological evaluations of nucleoside analogues, which have involved several formal modifications of the naturally occurring nucleosides.[1] As already reported,* modifications in the sugar moiety leading to pyranosyl nucleosides has drawn much attention, because of the promising features of some of them.[2] As part of our efforts working toward the *de novo* synthesis of carbohydrates and their derivatives, we have herein explored a new and general approach to the synthesis of six-membered nucleoside analogues, belonging to both D- or L-series. As depicted in Scheme 1, such molecules can be prepared starting from a common building block, the three-carbon homologating agent **1**, and diverse enantiopure electrophiles: depending on the nature of the "X" group (see **Scheme 1**), hexopyranosyl- or iminopyranosyl- purine and pyrimidine nucleosides can be synthesized.

* See Chapter 7, "Nucleoside Analogues in Antiviral Drug Discovery: Old Agents and New Candidates for Chemotherapeutic Intervention".



Scheme 1 – *A common building block as starting material for the synthesis of diverse L-six-membered nucleoside analogues.*

8.2 *SIX-MEMBERED NUCLEOSIDE ANALOGUES, PART I: IMINOPYRANOSYL NUCLEOSIDE SYNTHESIS*

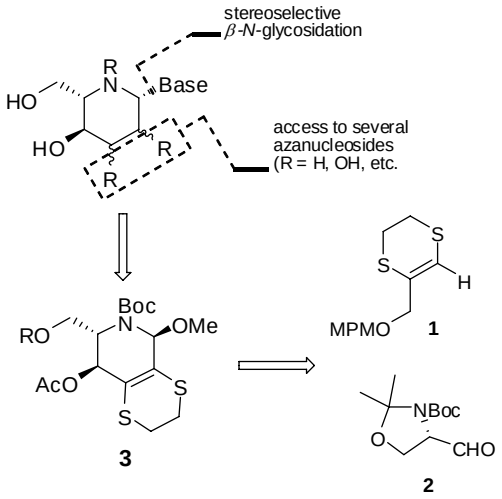
8.2.1 *RESULTS AND DISCUSSION*

Although the introduction of a nitrogen atom in nucleoside architectures is a fairly new concept, quite a few promising reports can be already found in literature about this issue.[3] However, all synthetic procedures so far described are limited to the preparation of five-membered iminonucleosides: conversely, no data have been collected about the six-membered ones, although their significance as monosaccharide mimetics could have some intriguing concerns also in antiviral research.

In this preliminary approach, the synthesis of enantiopure iminonucleosides by a non-carbohydrate based route is reported. Given the possibility to use the readily available Garner's aldehyde[4] **2**, already employed for the synthesis of a family of non-competitive glycosidase

inhibitors,* the current research has been focused to the preparation of L-series nucleosides; nonetheless, the employ of the same procedure, accomplished by simply replacing the chiral electrophile with its enantiomer, will easily enable the construction of the corresponding D-series nucleoside analogues.

As depicted in the retrosynthetic path, the strategy comprised a few major steps: after the three-carbon homologation of Garner's aldehyde **2** under usual conditions, six-membered ring closure was achieved with the aim to install a methoxy function on the *C*-1 in the iminosugar precursor **3**; then, suitable base insertion gave access to our six-membered iminonucleosides (**Scheme 2**).

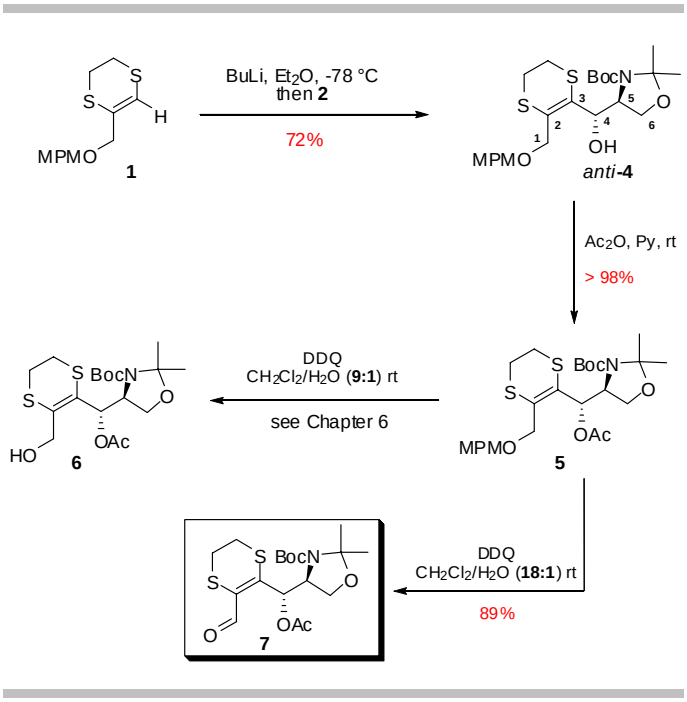


Scheme 2 – *Iminopyranosyl nucleoside synthesis: retrosynthetic path.*

The synthesis began with the coupling of **1** with aldehyde **2** (**Scheme 3**), which provided an *anti/syn* (9:1 dr) separable diastereomeric mixture of alcohols **4**† in 72% yield. After separation by SiO_2 flash chromatography, the more abundant *anti*-**4** diastereoisomer was chosen as a model to test the synthetic path. Acetylation of the secondary hydroxyl function, using Ac_2O in Py, afforded **5** in almost quantitative yield. Then, 4-methoxybenzyl protecting group removal was obtained (**Scheme 3**) by treating **5** with DDQ (1.5 eq) in CH_2Cl_2/H_2O (18:1). Although such reaction has already driven* to the formation of the alcohol **6**, conversely the use of a less water

* See Chapter 5, "A General Approach to the Synthesis of 1-Deoxy-L-Iminosugars".
† For a discussion on syn/anti selectivity see Charter 3, Paragraph 3.3.3: "Homologation of Chiral Electrophiles: Addition to Aldehydes".

percentage led quantitatively to the formation of a formyl function such as in **7**, as described with similar substrates.*



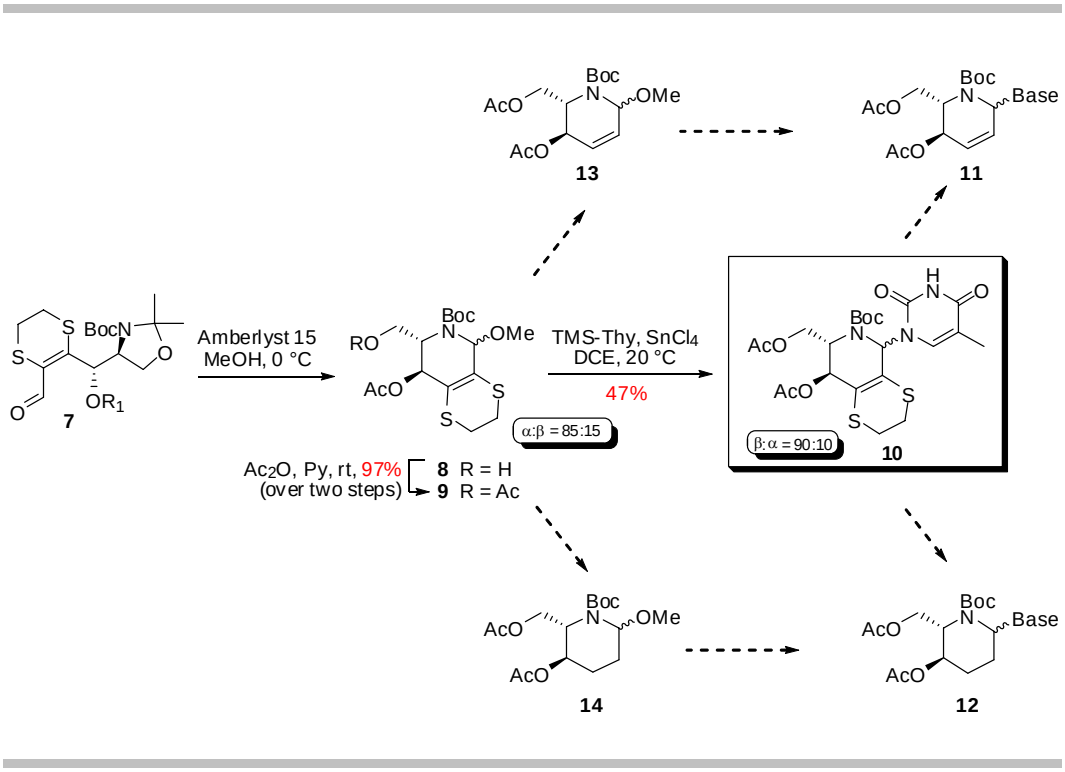
Scheme 3 - *Homologation reaction and MPM group removal.*

Cyclization in acidic conditions of aldehyde **7** was next examined. Treatment of **7** in the presence of acidic Amberlyst in methanol allowed the cleavage of oxazolidine ring and cyclization, to afford the bicyclic compound **8** (**Scheme 4**). After acetylation of the crude residue,†,[5] an α:β diastereomeric mixture of **9** (85:15 dr) was obtained in 97% overall yield. The key intermediate **9** was then coupled with the heterocyclic bases, under standard glycosidation conditions,[6,7] to afford an anomeric mixture of nucleoside derivatives **10** (β:α = 90:10).‡

As reported in Scheme 4, the versatility of **9** allows the preparation of unsaturated and saturated iminonucleosides **11** and **12**. Indeed, compatibly with each substrate, desulfurization will be performed prior or after base insertion by means of Raney-Ni (1:10 w/w) in THF at 0 °C, or using a large excess of Raney-Ni in order to obtain the over-reduction product. Suitable functionalizations at *C*-2/*C*-3 positions, carried out on compound **13**, will fulfil the wide class of six-membered iminonucleosides§. It's noteworthy to remind that the present route represents the

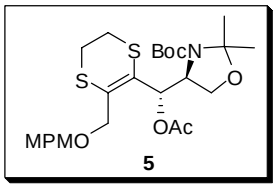
* See Chapter 3, Paragraph 3.2, "Three-Carbon Homologation".
† At this stage, as already observed for L-hexose synthesis, acetylation was required to avoid the formation of 1,6-anhydro derivative [Ref. 5].
‡ Anomeric ratios of *O*-methyl glycosides **9** and of nucleosides **10** have been easily determined by ^{1}H NMR analysis.
§ One of the most limiting problems regarding the synthesis of iminonucleoside analogues is the fact that, once all the protective groups will be removed, the endocyclic free amino group could render the molecule unstable, as

first example of anomeric iminopyranosyl nucleoside synthesis reported to date: therefore, the same approach can be performed using the D-series counterpart of the Garner's aldehyde (***ent*-2**), achieving a wide class of D-iminopyranosyl nucleosides as well, to be tested for antiviral purposes.



Scheme 4 - *Carbon skeleton cyclization and base insertion.*

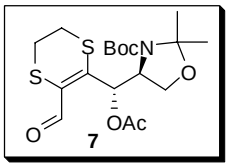
8.2.2 *EXPERIMENTAL SECTION*



***Compound* 5**. The diastereomer *anti*-**4** was acetylated by treatment with Ac_2O in pyridine overnight at room temperature. Then, solvent removal under reduced pressure and chromatography of the crude residue on silica gel (hexane/EtOAc = 7:3) gave the pure **5** (99%

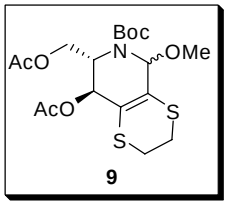
already reported for anomeric iminosugars (see Chapter 5). In our case, a possible synthetic solution could lie in the insertion of a small group on the nitrogen atom: for instance, a methyl group can be anytime obtained from the *t*-butoxycarbonyl (Boc) group under reducing conditions.

yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.47 (s, 9H), 2.10 (s, 3H), 3.02-3.27 (m, 4H), 3.76-3.85 (m, 4H), 4.08-4.18 (m, 3H), 4.20-4.40 (m, 1H), 4.46-4.58 (m, 2H), 5.98 (d, *J* = 5.4 Hz, 1H), 6.86 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.6 Hz, 2H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.5, 22.5, 23.7, 25.5, 28.0, 29.6, 60.2, 63.1, 63.7, 70.0, 70.7, 77.1, 81.2, 94.5, 123.1, 130.8, 139.5, 147.8, 152.8, 168.9, 182.4. Anal. calcd for $C_{26}H_{37}NO_7S_2$: C 57.86, H 6.91, N 2.60. Found: C 57.90, H 6.87, N 2.57.



7

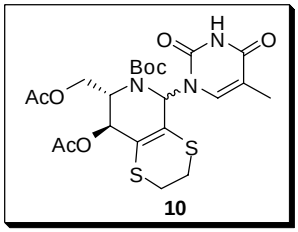
Compound 7. To a stirred 18:1 CH_2Cl_2/H_2O emulsion (5 mL) containing the MPM ether **5** (0.2 g, 0.37 mmol), DDQ (0.12 g, 0.56 mmol) was added in one portion at room temperature. After 18 h H_2O was added to the reaction and the mixture extracted with CH_2Cl_2; the organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 95:5) gave the pure **7** (0.14 g, 89% yield) ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.48 (s, 9H), 2.12 (s, 3H), 2.93-3.38 (m, 4H), 3.93 (dd, *J* = 5.9 Hz, *J* = 9.8 Hz, 1H), 4.16-4.48 (m, 2H), 6.38-6.60 (m, 1H), 9.80 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.8, 24.2, 25.5, 25.8, 27.5, 28.4, 29.5, 29.8, 60.4, 64.3, 71.5, 81.3, 94.5, 148.4, 153.1, 169.4, 182.6. Anal. calcd for $C_{18}H_{27}NO_6S_2$: C 51.78, H 6.52, N 3.35. Found: C 51.80, H 6.49, N 3.39.



9

Compound 9. Amberlyst 15 (1.0 g, previously washed with anhydrous MeOH), was added in one portion to a stirred solution of aldehyde **7** (0.1 g, 0.24 mmol) in methanol (8 mL) at 0 °C. After 10 min, the suspension was warmed to room temperature and stirred for 1h. Then the solid was filtered off and washed with MeOH; Py was added until pH 8. MeOH was evaporated under reduced pressure and replaced by Py (3 mL); to the solution was added Ac_2O at room temperature (0.04 mL, 0.40 mmol). After 3 h, solvent removal under reduced pressure and chromatography of the crude residue over silica gel (CH_2Cl_2) afforded **9** as a diastereomeric mixture (0.12 g, 97% overall yield; 85:15 dr). Data for the pure **9**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.47 (s, 9H), 2.10 (s, 3H), 3.19-3.38 (m, 4H), 3.41 (s, 3H), 3.72-3.78 (m, 1H), 3.89-4.05 (m, 1H), 4.22-4.38 (m, 1H), 5.06 (s, 1H), 5.40 (d, *J* = 5.4 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$):

δ 20.2, 23.0, 28.1, 28.7, 31.9, 46.7, 55.0, 60.5, 63.6, 73.9, 85.9, 115.1, 125.7, 154.1, 180.3, 183.6. Anal. calcd for $C_{18}H_{27}NO_7S_2$: C 49.87, H 6.28, N 3.23. Found: C 49.89, H 6.31, N 3.28.



10

Compound 10. To a stirring solution of **9** (0.1 g, 0.23 mmol) and silylated thymine (0.09 g, 0.34 mmol) in anhydrous $ClCH_2CH_2Cl$ (3 mL), kept at -20 °C and under nitrogen atmosphere, freshly distilled $SnCl_4$ (0.004 mL, 0.02 mmol) was added dropwise within 10 min. The resulting mixture was warmed to 0 °C and stirred for 1 h. Then pyridine (3 mL) was added, the solution was warmed to room temperature, diluted with CH_2Cl_2 and washed with a saturated $NaHCO_3$ solution (2 x 100 mL) and brine (2 x 100 mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = 95/5) gave the pure **10** as a diastereomeric mixture (0.06 g, 47% yield, α/β = 1/9). Data for the pure **10**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.48 (s, 9H), 1.95 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 3.20-3.40 (m, 4H), 3.75-3.80 (m, 1H), 3.95-4.05 (m, 1H), 4.26-4.34 (m, 1H), 5.96 (s, 1H), 5.44 (d, *J* = 5.4 Hz, 1H), 7.19 (s, 1H), 8.86 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.4, 23.2, 28.4, 29.2, 31.9, 46.7, 60.5, 65.6, 73.9, 80.2, 111.8, 115.1, 125.7, 135.2, 152.2, 154.1, 163.5, 170.3, 171.0. Anal. calcd for $C_{22}H_{29}N_3O_8S_2$: C 50.08, H 5.54, N 7.96. Found: C 50.11, H 5.57, N 7.99.

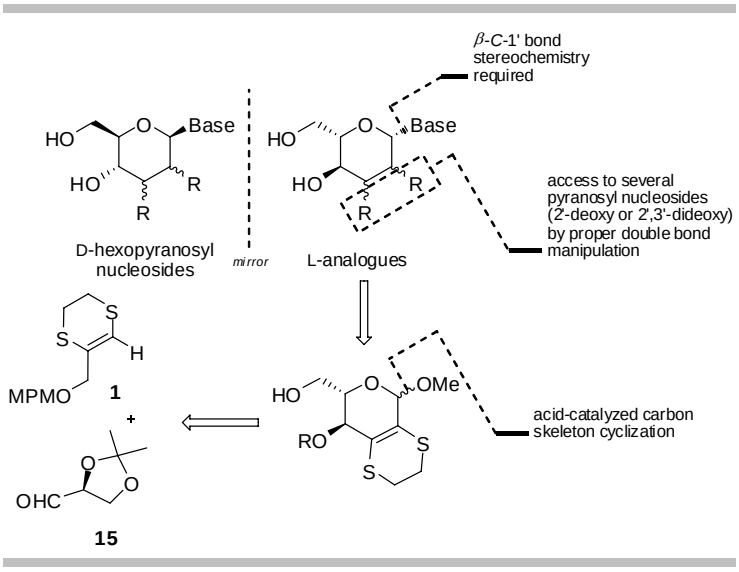
8.3 *SIX-MEMBERED NUCLEOSIDE ANALOGUES, PART II: ANOMERIC HEXOPYRANOSYL NUCLEOSIDE SYNTHESIS*

8.3.1 *RESULTS AND DISCUSSION*

Hexopyranosyl nucleosides containing 2-deoxy-*β*-D-*ribo*-hexopyranose as carbohydrate moiety possess obvious structural similarities to natural *ribo*- and 2-deoxy-*ribo*-furanosylnucleosides.[8] In this perspective, the biological activity of several D-hexopyranosyl nucleosides has been already highlighted in many examples.*

* See Charter 7, Paragraph 7.3, "Sugar Modifications in Nucleoside Analogues: Six-Membered Nucleosides".

Inspired by these reports, as well as by the fundamental knowledge according to which a number of human enzymes are able to phosphorylate nucleosides belonging to unnatural L-series,[9] a general route for the synthesis of L-hexopyranosyl nucleosides has been convincingly opened up, starting from the usual starting material* **1** and the chiral electrophile (i.e. the 2,3-*O*-isopropylidene-L-glyceraldehyde, **15**) to set the chirality of the molecules. Among a wide choice of target molecules to which this approach can have access (**Scheme 5**), the synthetic study of 2',3'-dideoxy-L-hexopyranosyl nucleosides was preliminarily undertaken. In addition, since target compounds need a stereoselective *β*-installation of the nucleobase moiety at *C*-1, a number of methods for the stereoselective *N*-glycosidation of deoxy sugars has been examined. Therefore, the route to enantiopure pyranosyl nucleosides has also become an investigation about the reactivity of 2,3-dideoxy monosaccharides.

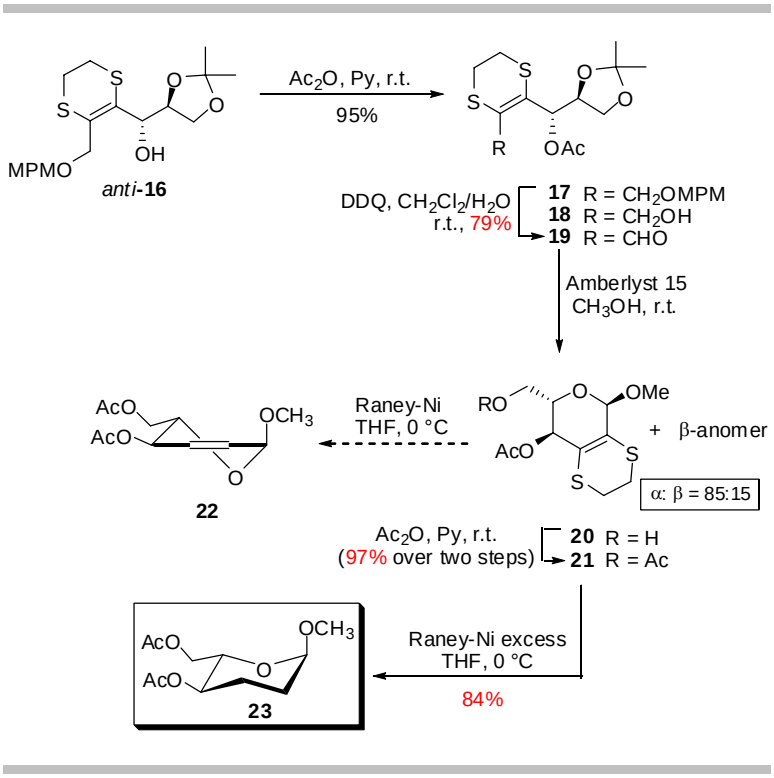


Scheme 5 – *Retrosynthetic path.*

The preparation of the sugar precursor **23** as from *anti*-**16** intermediate implied the use of the same synthetic route observed for the total synthesis of rare sugars† (**Scheme 6**). However, while earlier a benzyl group on the *O*-4 was installed, to give orthogonality to hydroxyl groups of L-sugars, herein an acetyl group was preferred, due to its better handiness. Therefore, once the coupling reaction was achieved, treatment of *anti*-**16** with Ac_2O/Py afforded the acetylated

* See Chapter 3, "The Heterocycle-Functional Group Equivalence as a Powerful Tool for the Synthesis of Organic Molecules".
† See Chapter 4, "A Versatile Route to L-Hexoses: Stereoselective Synthesis of Rare Sugars".

intermediate **17**. Subsequently, MPM removal by means of DDQ in a CH_2Cl_2/H_2O emulsion gave the aldehyde **19** (79%), together with a small amount of alcohol **18** (8%).



Scheme 6 – *Synthesis of the key intermediate **23**.*

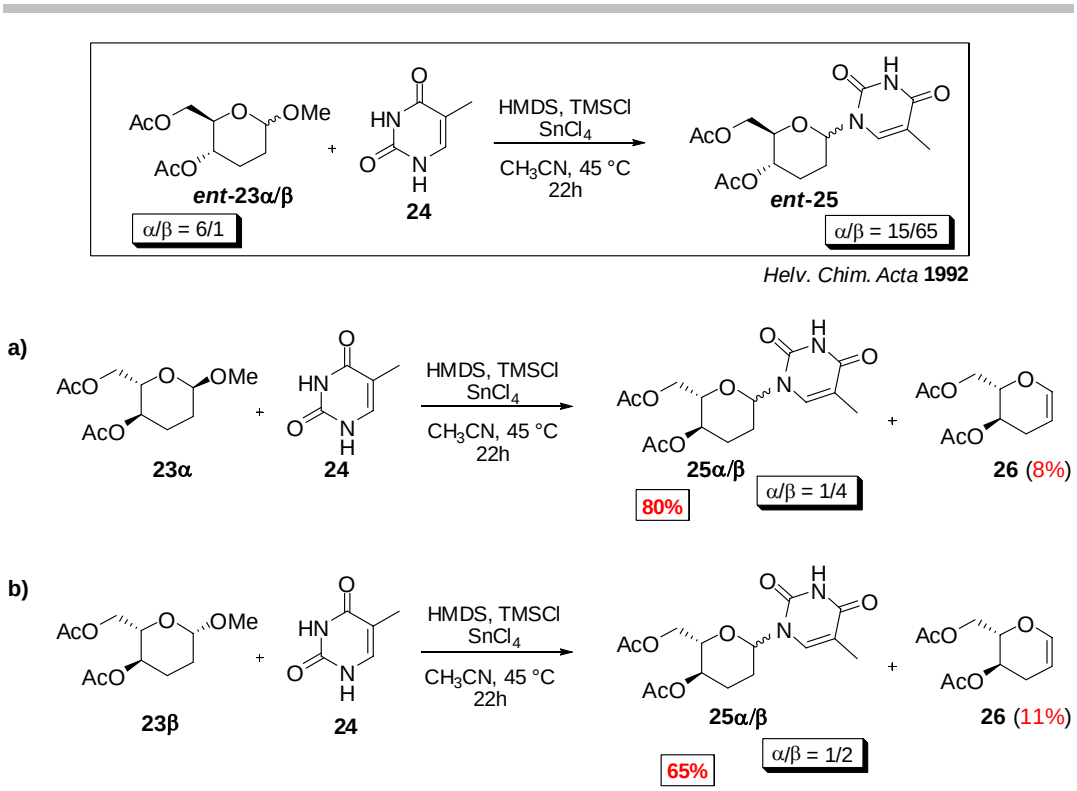
Cyclization under usual acidic conditions afforded the bicycle intermediate **20** as an anomeric mixture (α/β = 85/15), which crude residue was directly subjected to acetylation (to avoid the further undesired cyclization*), obtaining the compound **21**. Finally, complete dithioethylene bridge removal was considered. As reported earlier,† Ra-Ni treatment of **21** could give the olefin **22**. However, owing to the aim to synthesize 2',3'-dideoxy-L-hexopyranosyl nucleosides, a further hydrogenation on **22** was required. For this purpose, exposure of **21** to a Ra-Ni excess directly afforded the over-reduced key intermediate **23** (84%), the anomeric mixture being easily resolved through a common chromatographic procedure.

N-Glycosidation under acidic conditions of **23** was then studied. To test the breadth of our methodology, insertion of thymine (T) and adenine (A) bases (as pyrimidine and purine base models, respectively) was preliminarily studied.

* The formation of the 1,6-anhydro ring, coming from the cyclization of **20**, has been employed for the synthesis of enantiopure non anomeric nucleosides [see farther on in this chapter].
† See Chapter 4, Paragraph 4.2.1, "Synthesis of the L-Hexose Framework".

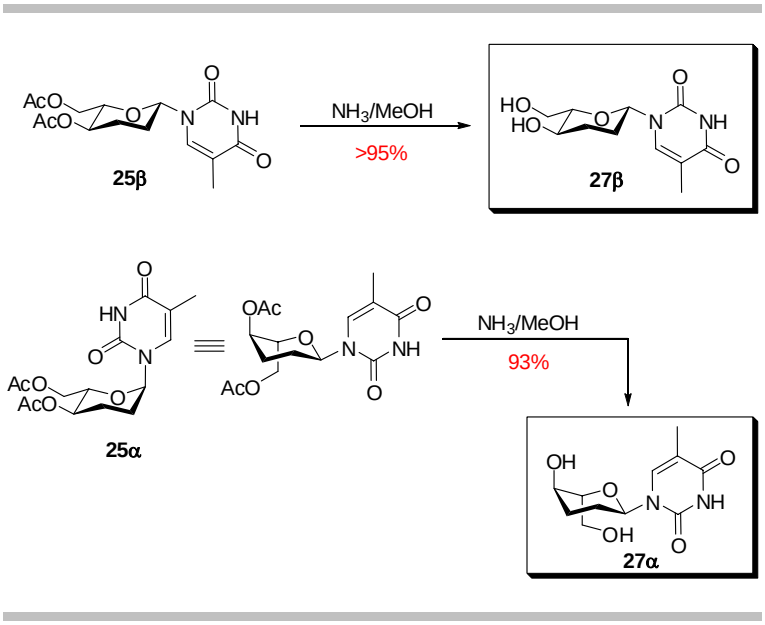
1-(Thymin-1-yl)-2,3-dideoxy-β-L-erythro-hexopyranoside and ***1-(thymin-1-yl)-2,3-dideoxy-α-L-erythro-hexopyranoside*** (**27β** and **27α**)**.** The L-enantiomer of the key intermediate **23** has been already employed in a previous investigation for the preparation of a wide class of pyranosyl nucleotides, with the aim to synthesize and study the biochemical behaviour of some pyranose oligonucleotides, better known as "Homo-DNA".[10] In these studies, the preparation of the homo-deoxyribonucleosides implied the use of the well-known Vorbrüggen one-pot protocol,[11] in which the glycosidic bond was achieved by Lewis acid-catalyzed *N*-glycosidation reaction of ***ent*-23α/β** with *in situ* silylated bases (**Scheme 7**). Considering the thymine nucleoside synthesis, a mixture of α/β anomers *ent*-**25** was formed and the components were separated by column chromatography, the thermodynamically more stable β-nucleoside being isolated as the major product.

Although the protocol reported in literature involved the use of an anomeric mixture (α:β = 6:1) of ***ent*-23**, the same reaction, carried out with our pure α-L-anomer **23** gave, as expected, nearly the same results. Particularly, treatment of **23α** with unprotected thymine (**24**), hexamethyldisilazane (HMDS), trimethylsilylchloride (TMSCl) and Tin chloride ($SnCl_4$) in acetonitrile (CH_3CN) at rt afforded a mixture of the two protected nucleosides **25α/β** (α:β = 1:4) in 80% overall yield, the β-anomer being isolated in 65% yield (**Scheme 7**). On the other hand, the pure β-anomer of **23**, separated from its α-component, was tested in the same reaction as well, affording **25α/β** with lesser selectivity (1/2) and in lower yield (65% o.y.). In both cases, a byproduct was formed in small amount (8-11%) during the course of the reaction, identified as the 3-deoxy-L-glucal derivative **26**.



Scheme 7 – *Approach to the synthesis of 1-(thymin-1-yl)-2,3-dideoxy-β-*L-erythro-*hexopyranosyl nucleoside.*

Once nucleobase insertion was achieved, nucleosides were then deprotected (**Scheme 8**): the reaction of *β*-anomer **25β** with saturated NH_3 in MeOH allowed an easy removal of acetyl groups in 18h at room temperature, affording the pure **27β** in almost quantitative yield. The minor component α-anomer **25α**, which preferred conformation was already established to be the one with a $^4C_1$ chair,[10] underwent the same reaction obtaining the pure **27α** in a similar yield (93%).

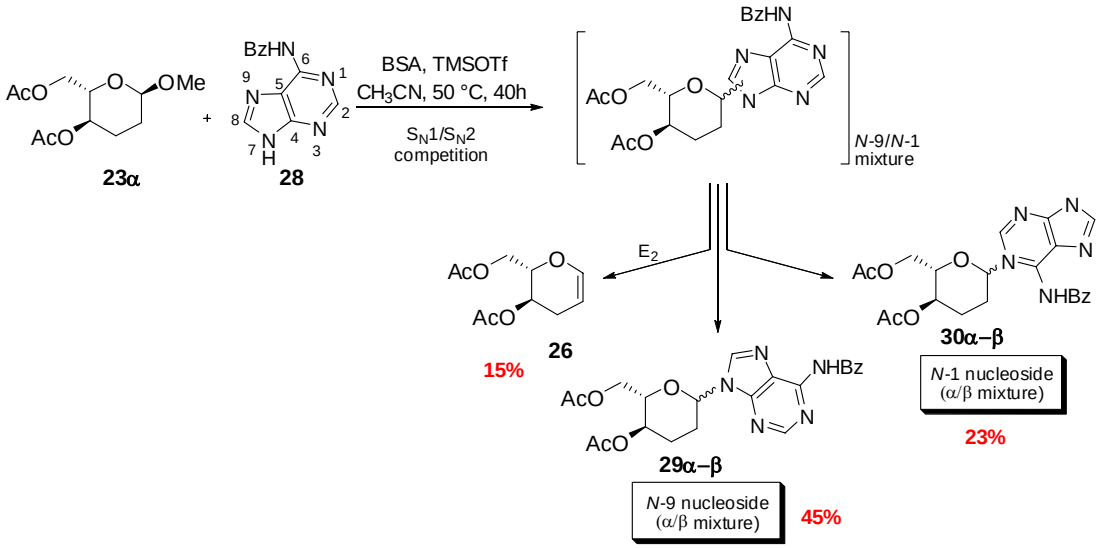


Scheme 8 – *Acetyl group removal: synthesis of α- and β- pyranosyl thymidine nucleosides* ***27α/β****.*

1-(Adenin-9-yl)-2,3-dideoxy-β-L-erythro-hexopyranoside (**33β**). The preparation of the L-pyranosyl purine nucleoside, adenine being selected as a purine model, was next investigated (**Scheme 9**). Compared to the easily separable anomeric mixture of protected thymine nucleosides **25α/β**, the main problem herein observed was represented by the failure of any chromatographic separation on the α/β mixture of adenine nucleosides. As a matter of fact, the use of the *one-pot* Vorbrüggen conditions[6] treating **23** with benzoyladenine **28** gave a α:β = 35:65 inseparable mixture **29α/β**[*] (45% yield). In addition, a consistent amount of byproducts was formed, i.e. the deoxy-glucal derivative **26** (15%) and the nucleoside isomer[†] **30α-β** (23%).[12] Driven by the need of improving the stereoselective outcome of the reaction, several alternative glycosyl donors were therefore synthesized from **23** and diverse reaction conditions were attempted.

[*] The reaction proceeded *via* oxocarbenium ion formation, as simply shown by TLC.

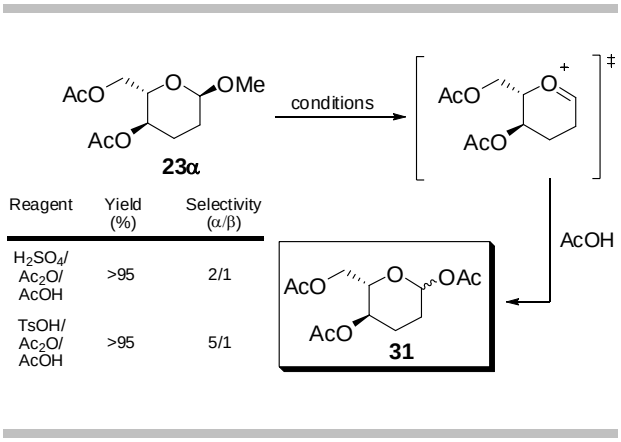
[†] Although the common knowledge on adenine-based *N*-glycosidation reactions suggests that this nucleoside isomer deals with an *N*-7 purine nucleoside [Ref. 11], a recent report claims that an *N*-1 nucleoside derivative (like **30**) is favourably formed if the adenine is *N*-6 benzoylated [Ref. 12]. Concerning our substrates, although 2D (COSY, HSQC, HMBC) NMR spectra did not unambiguously determine the structure of **30**, we are driven to believe that the formation of the *N*-1 nucleoside derivative is the most favourable one as well.



Scheme 9 – *Insight into the adenine moiety insertion.*

The results of these endeavours are summarized in Table 1. Glycosyl donors **31-32** were synthesized as reported below:

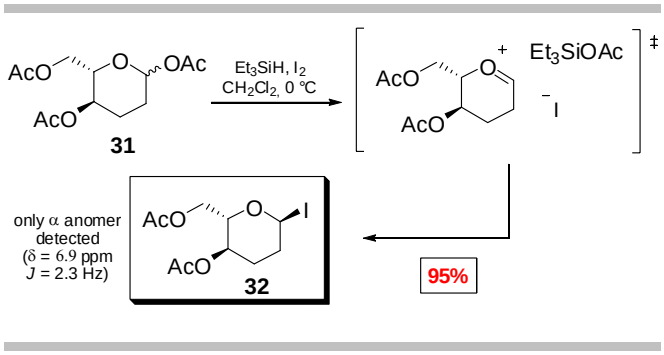
1. *Glycosyl acetate **31***. Compound **23** was directly converted, *via* acetolysis, in its peracetylated derivative **31**: while the standard H_2SO_4/Ac_2O furnished this latter in a unsatisfying α:β selectivity (2:1), a better result was achieved by using *p*-toluenesulfonic acid in a Ac_2O/AcOH solution, affording **31** in a α:β = 5:1 ratio (**Scheme 10**).



Scheme 10 – *Glycosyl acetate **31** preparation.*

2. *Glycosyl iodide **32***. The peracetate derivative **31** was stereoselectively converted in its iodide **32** by treatment with iodine (I_2) and triethylsilane (Et_3SiH) in CH_2Cl_2 at 0 °C for 30

min, obtaining the only α-anomer (^{1}H NMR), as already reported for similar substrates[13] (**Scheme 11**).



Scheme 11 – *Glycosyl iodide **32** from glycosyl acetate **31**.*

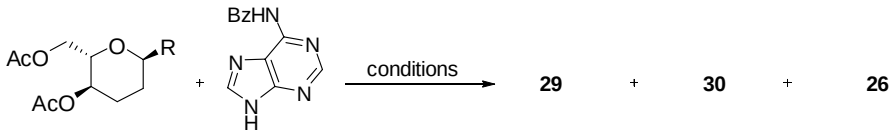
Once the preparation of glycosyl donors was achieved, reactions were carried out as follows:

1. Glycosyl acetate **31** was glycosylated with benzoyladenine (**28**) in CH_3CN under *one-pot* Vorbrüggen conditions, replacing HMDS with the better silylating agent *N*,*N*-*bis*-dimethylsilylacetamide (BSA),[14] whereas $SnCl_4$ was substituted with the more handy trimethylsilyl trifluoromethanesulfonate (TMSOTf). As mentioned for the previous glycosidation, this one also proceeded *via* the oxocarbenium ion formation; however, in this case, a somewhat major S_N2 contribution occurs, since the reaction, stopped after 40h at room temperature, afforded the anomeric mixture of the two nucleosides **29α/β** with a better selectivity (α:β = 1:3). In addition, when the reaction was warmed at 50 °C, an equilibrium occurred between several species: α/β mixture of *N*-1 nucleosides **30**,[13] 3-deoxy-glucal derivative **26** and obviously the desired α/β *N*-9 nucleoside anomers **29**. This equilibrium was gradually moved towards the mixture of **29** and towards the undesired glucal **26** as well. After a reaction time of 40h, a mixture of *N*-9 anomers **29** (α:β = 1:6) was afforded in 40% yield, while a few traces of regioisomer nucleosides was detected (**Table 1**). Regardless the good selectivity achieved, the glucal derivative **26** was formed in 47% yield, rendering this route inadequate for the purpose.

2. Glycosyl iodide **32** was glycosylated with benzoyladenine (**28**), using NaH in THF, with the aim to enable a direct S_N2 proceeding of the reaction.[13] However, carrying out the reaction at room temperature for 1h (iodide consumption), an unsatisfying selectivity was reached (α:β ≈ 1:1, 38% yield). The use of the *β*-addressing CH_3CN[15] in place of THF did not improve the selectivity. Moreover, since the reaction seemed to be too fast, a lower temperature (0 °C)

was also attempted, obtaining a selectivity for the α-anomer (kinetic product being favoured, α:β = 2:1; 41% yield). In both cases, regioisomer nucleosides **30** were observed (18-20%) and an even larger amount of glucal **26** was formed (44-52%), compared to the previous results, due to the stronger basic conditions.

3. Glycosyl acetate **31** was glycosylated under Vorbrüggen conditions upon the use of a previously silylated base.[11] In fact, although the *in situ* silylation and the use of an already silylated base are nowadays considered roughly equivalent procedures in an *N*-glycosidation reaction, this latter condition provides a more reactive nucleobase moiety, hence a lower temperature can be used, so minimizing the formation of **26** (which formation is supposed to arise with the relatively high temperatures so far employed). As a result, treatment of acetate **31** with N^6,N^9-bis-trimethylsilyl-N^6-benzoyladenine* [$(TMS)_2$-**28**] and TMSOTf in CH_3CN at room temperature for 3h afforded **29** in a satisfying yield (65%) with the most preferred anomeric ratio (α:β = 1:8.3). Beside this, the formation of **26** was remarkably reduced (11%), whereas no regioisomer nucleoside was detected.



entry	R	conditions	α/β ratio of **29**	yield of **29** (%)	yield of **30** (%)	yield of **26** (%)
1	OAc	BSA, TMSOTf CH_3CN, 50 °C, 40h	1/6	40	traces	47
2	I	NaH THF (or CH_3CN) rt, 10 min	1/1	38	18	44
3	I	NaH CH_3CN 0 °C, 30 min	2/1	41	20	52
4	OAc	TMSOTf§ CH_3CN, rt, 3h	1/8.3	65	/	11

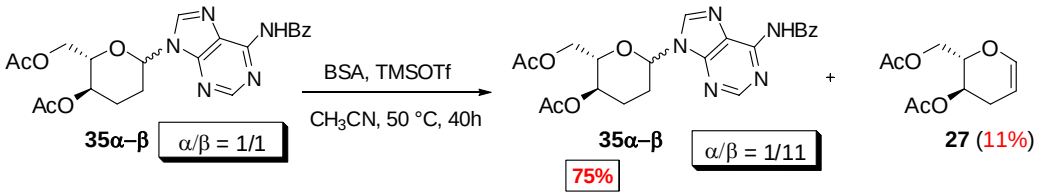
§: $(TMS)_2A^{Bz}$ was used in this case.

Table 1 – *Attempts for stereoselective introduction of the adenine moiety.*

The anomerization of the *N*-9 nucleoside anomers **29** was finally examined (**Scheme 12**). In fact, driven by the understanding that in acidic conditions an equilibrium occurs, which is shifted towards the termodinamically more stable β-anomer **29**, a α/β = 1:1 mixture of **29** was warmed

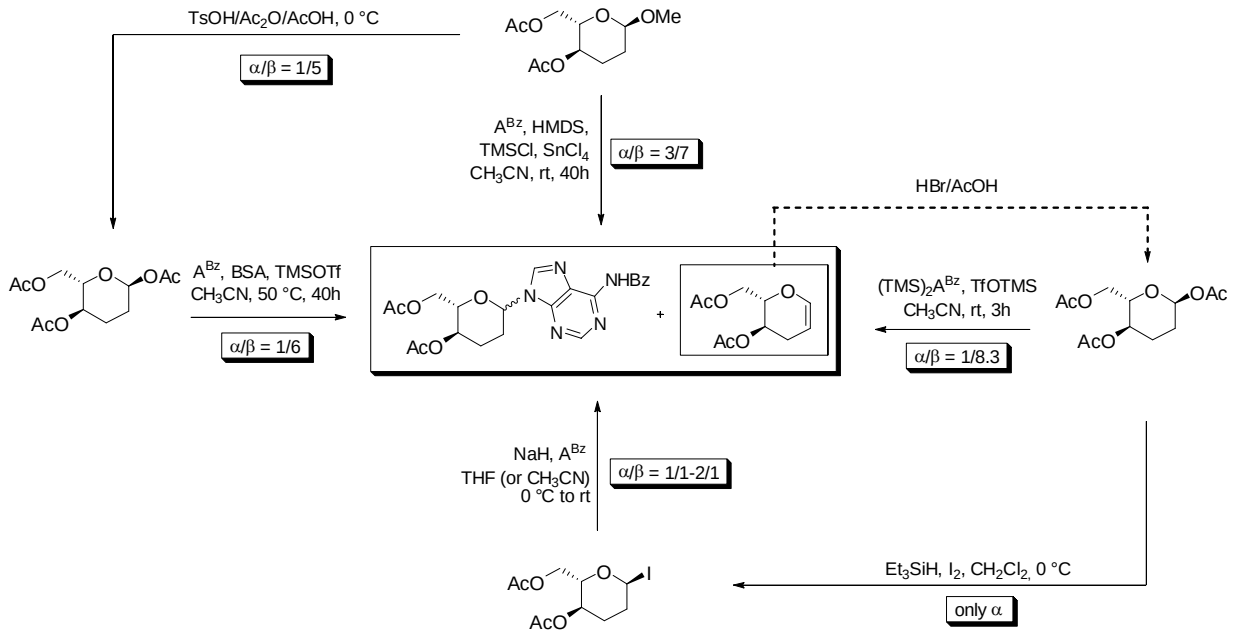
* Obtained under standard conditions from **28**, i.e. HMDS/TMSCl/$(NH_4)_2SO_4$ or BSA/$(NH_4)_2SO_4$ [see Ref. 11 and literature cited therein].

at 60 °C in CH_3CN with BSA and TMSOTf, obtaining an improvement of the β/α ratio (with a 75% yield) until 11:1 after 48h.*



Scheme 12 – *Acid-mediated anomerization of the nucleoside mixture **29.***

Overally, the results obtained for the synthesis of protected β-L-2',3'-dideoxypyranosyl adenine nucleoside are summarized as depicted below (**Scheme 13**):

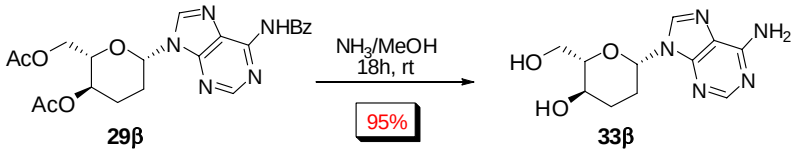


Scheme 13 – *Summary of nucleobase insertion attempts.*

* More prolonged times were avoided, since the formation of the glucal **26** was observed to be time-depending.

By the way, further efforts could be focused towards the possibility to directly convert the 3-deoxy glucal **26** into the peracetate **31**, under the acidic conditions (HBr/AcOH/Ac_2O) already reported on similar substrates:[12] in this way, **26** could be considered a useful byproduct.

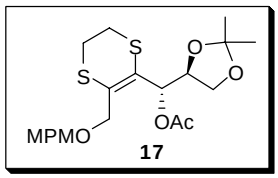
As the nucleoside **29** was obtained, it was easily deprotected (**Scheme 14**): treatment of **29** with a saturated methanolic NH_3 solution afforded the free nucleoside 1-(adenin-9-yl)-2,3-dideoxy-β-L-*erythro*-hexopyranoside **33β** (95% yield).



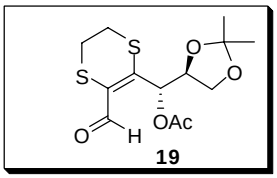
Scheme 14 – *Protective group removal: synthesis of 1-(2',3'-dideoxy-β-L-*ribo-*hexopyranosyl) adenine* ***33β***.

The nucleoside analogues described **27α**, **27β** and **33β** are currently undergoing biological evaluation as HIV and/or HSV agents.

8.3.2. *EXPERIMENTAL SECTION*

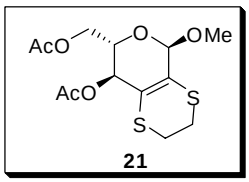


Compound 17. The *anti*-diastereomer was acetylated by treatment with Ac_2O in pyridine overnight at room temperature. Then solvent removal under reduced pressure and chromatography of the crude residue on silica gel (hexane/EtOAc = 7:3) gave the pure **17** (99% yield). ^{1}H NMR (400 MHz, C_6D_6): δ 1.27 (s, 3H), 1.35 (s, 3H), 1.71 (s, 3H), 2.38-2.49 (m, 3H), 2.55-2.61 (m, 1H), 3.29 (s, 3H), 3.99 (dd, *J* = 6.5 Hz, *J* = 8.5 Hz, 1H), 4.08 (dd, *J* = 6.5 Hz, *J* = 8.5 Hz, 1H), 4.32 (d, *J* = 12.2 Hz, 1H), 4.48 (s, 3H), 4.63 (d, *J* = 12.2 Hz, 1H), 6.26 (d, *J* = 5.9 Hz, 1H), 6.81 (d, *J* = 8.6 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H). ^{13}C NMR (50 MHz, C_6D_6): δ 20.9, 26.2, 27.1, 27.8, 30.1, 55.3, 66.6, 71.1, 71.5, 73.8, 77.3, 110.0, 114.6, 125.7, 130.5, 131.4, 160.4, 169.6. Anal. calcd for $C_{21}H_{28}O_6S_2$: C 57.25, H 6.41. Found: C 57.28, H 6.45.



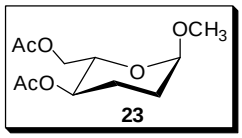
19

Compound 19. To a stirred 18:1 CH_2Cl_2/H_2O emulsion (10 mL) containing the MPM ether **17** (1.08 g, 2.2 mmol), DDQ (0.76 g, 3.36 mmol) was added in one portion at room temperature. After 48 h H_2O was added to the reaction and the mixture extracted with CH_2Cl_2; the organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (hexane/acetone = 95:5) gave the pure **19** (0.50 g, 79% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35 (s, 3H), 1.39 (s, 3H), 2.11 (s, 3H), 3.05-3.18 (m, 2H), 3.20-3.28 (m, 2H), 3.91 (dd, *J* = 4.9 Hz, *J* = 8.8 Hz, 1H), 4.14 (dd, *J* = 5.2 Hz, *J* = 10.8 Hz, 1H), 4.31-4.43 (m, 1H), 6.11 (d, *J* = 3.8 Hz, 1H), 10.0, (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 20.6, 25.0, 25.9, 26.4, 29.1, 66.8, 72.5, 75.7, 110.6, 130.6, 147.2, 169.2, 182.9. Anal. calcd for $C_{13}H_{18}O_5S_2$: C 49.04, H 5.70. Found: C 49.05, H 5.74.



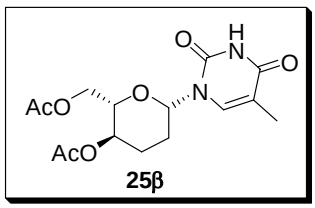
21

Compound 21. Amberlyst 15 (3.9 g, previously washed with anhydrous MeOH), was added in one portion to a stirred solution of aldehyde **19** (0.39 g, 1.08 mmol) in methanol (30 mL) at 0 °C. After 10 min, the suspension was warmed to room temperature and stirred for 1h. Then the solid was filtered off and washed with MeOH; Py was added until pH = 8. MeOH was evaporated under reduced pressure and replaced by Py (15 mL); to the solution was added Ac_2O at room temperature (0.12 mL, 1.2 mmol). After 3 h, solvent removal under reduced pressure and chromatography of the crude residue over silica gel (CH_2Cl_2) afforded the pure **21**, beside to a minor amount of its *β*-anomer (0.39 g, 94% overall yield; 85:15 dr), as a colourless oil. Data for the pure **21**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.07 (s, 3H), 2.12 (s, 3H), 2.95-3.02 (m, 1H), 3.13-3.32 (m, 3H), 3.48 (s, 3H), 3.96-4.09 (m, 2H), 4.13-4.22 (m, 1H), 4.22-4.26 (m, 1H), 4.71 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.8, 27.3, 28.2, 29.7, 55.9, 61.7, 65.2, 67.3, 97.9, 122.3, 126.3, 169.9, 170.3. Anal. calcd for $C_9H_{14}O_4S_2$: C 43.18, H 5.64. Found: C 43.18, H 5.68.



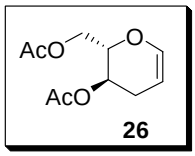
23

Compound 23. Treatment of **21** (0.1 g, 0.26 mmol) with an excess of Raney-Ni (W2) (1.8 g, wet) afforded, after common work-up and purification procedures, the pure **23** (0.06 g, 84% yield) as a colourless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.78-1.86 (m, 3H), 1.95-2.01 (m, 1H), 2.05 (s, 3H), 2.09 (s, 3H), 3.38 (s, 3H), 3.90 (ddd, *J* = 10.0 Hz, *J* = 5.3 Hz, *J* = 2.3 Hz, 1H), 4.10 (dd, *J* = 12.0 Hz, *J* = 2.3 Hz, 1H), 4.24 (dd, *J* = 12.0 Hz, *J* = 5.3 Hz, 1H), 4.72 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.8, 26.6, 29.0, 54.4, 63.9, 65.7, 71.8, 97.5, 170.0, 170.9. Anal. calcd for $C_7H_{14}O_4$: C 51.84, H 8.70. Found: C 51.86, H 8.78.

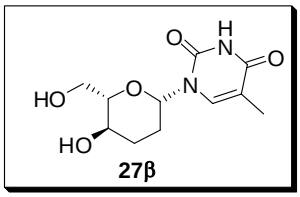


25β

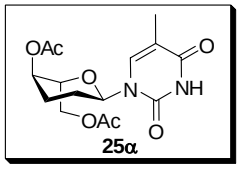
Compound 25β. To a solution of methyl glycoside **23** (0.15 g, 0.59 mmol) and thymine **24** (82 g, 0.65 mmol) in anhydrous CH_3CN (8 mL) and under nitrogen atmosphere, hexamethyldisilazane (HMDS, 0.14 mL, 0.65 mmol) and trimethylsilyl chloride (TMSCl, 0.09 mL, 0.71 mmol) were added and the resulting solution was stirred for 30 min at room temperature. Hence, $SnCl_4$ (0.09 mL, 0.72 mmol) was added dropwise in 10 min. The reaction mixture was further stirred for 40 h at 50 °C. Then few drops of anhydrous pyridine were added; the solution was diluted with AcOEt and washed with a saturated $NaHCO_3$ solution (2 x 100 mL) and brine (2 x 100 mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = 95/5) gave the pure **25β** (0.13 g, 65% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68-1.89 (m, 2H), 1,97 (s, 3H), 1.70-2.06 (m, 1H), 2.09 (s, 3H), 2.10 (s, 3H), 2.33-2.42 (m, 1H), 3.85 (ddd, *J* = 2.3 Hz, *J* = 6.4 Hz, *J* = 10.2 Hz, 1H), 4.17 (dd, *J* = 2.2 Hz, 12.2 Hz, 1H), 4.25 (dd, *J* = 6.4 Hz, *J* = 12.2 Hz, 1H), 4.75 (dt, *J* = 4.6 Hz, *J* = 10.3 Hz, 1H), 5.78 (dd, *J* = 2.5 Hz, *J* = 10.5 Hz, 1H), 7.19 (s, 1H), 8.82 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.6, 20.8, 21.0, 27.9, 29.3, 29.7, 62.9, 66.5, 77.9, 81.3, 111.5, 134.9, 150.0, 163.3, 170.0, 170.8. Anal. calcd for $C_{15}H_{20}N_2O_7$: C 52.94, H 5.92, N, 8.23. Found: C 52.95, H 5.97, N, 8.25.



Compound 26. 3-Deoxy glucal **26** was isolated as minor byproduct (8% yield) in the above reported reaction between dideoxypyranoside **23** and thymine **24**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04-2.13 (m, 1H), 2.08 (s, 3H), 2.09 (s, 3H), 2.48 (dddd, *J* = 2.0 Hz, *J* = 5.0 Hz, *J* = 6.1 Hz, *J* = 17.2 Hz, 1H), 4.04 (ddd, *J* = 2.7 Hz, *J* = 5.2 Hz, *J* = 8.2 Hz, 1H), 4.22 (dd, *J* = 3.1 Hz, *J* = 12.1 Hz, 1H), 4.31(dd, *J* = 5.6 Hz, *J* = 12.1 Hz, 1H), 4.67-4.72 (m, 1H), 4.95-5.02 (m, 1H), 6.32 (dt, *J* = 2.0 Hz, *J* = 6.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.8, 21.1, 25.6, 62.4, 65.7, 73.9, 97.8, 142.6, 170.1, 170.9. Anal. calcd for $C_6H_{10}O_3$: C 55.37, H 7.74. Found: C 55.41, H 7.79.

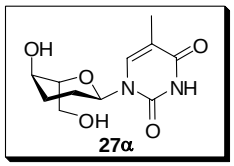


Compound 27β. Deacetylation of the thymine nucleoside **25β** (0.1 g, 0.29 mmol) was accomplished by treatment with a saturated metanolic NH_3 solution (4 mL). After stirring for 24 h at room temperature, the solvent was removed. Chromatography of the crude residue (DCM/MeOH = 8:2) gave the pure **27β** (97% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.50-1.60 (m, 1H), 1.71-1.83 (m, 5H), 2.00-2.08 (m, 1H), 3.23-3.30 (m, 2H), 3.45-3.50 (m, 1H), 3.67 (ddd, *J* = 11.9 Hz, *J* = 5.7 Hz, *J* = 1.8 Hz, 1H), 4.53 (t, *J* = 5.7 Hz, 1H), 4.89 (d, *J* = 5.1 Hz, 1H), 5.52 (dd, *J* = 2.6 Hz, *J* = 10.5 Hz, 1H), 7.57 (s, 1H), 11.33, (bs, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 10.9, 29.0, 31.0, 61.3, 64.3, 81.6, 83.1, 110.0, 136.8, 150.7, 164.9. Anal. calcd for $C_{11}H_{16}N_2O_5$: C 51.56, H 6.29, N 10.93. Found: C 51.61, H 6.39, N 10.94.



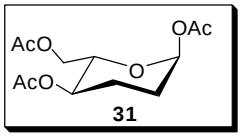
Compound 25α. Nucleoside **25α** was isolated as minor component (15% yield) in the above reported reaction between intermediate **23** and thymine (**24**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.81-1.94 (m, 2H), 1.95 (s, 3H), 2.05-2.08 (m, 2H), 2.10 (s, 3H), 2.16 (s, 3H), 4.25-4.29 (m,

2H), 4.39 (dd, *J* = 9.2 Hz, *J* = 13.7 Hz, 1H), 4.84-4.88 (m, 1H), 5.95 (dd, *J* = 3.5 Hz, *J* = 10.5 Hz, 1H), 7.25 (s, 1H), 9.19 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.6, 20.8, 21.2, 24.0, 24.9, 60.4, 65.8, 75.7, 76.7, 111.2, 135.2, 150.2, 163.9, 170.2, 170.5. Anal. calcd for $C_{15}H_{20}N_2O_7$: C 52.94, H 5.92, N, 8.23. Found: C 52.99, H 5.99, N, 8.27.



27α

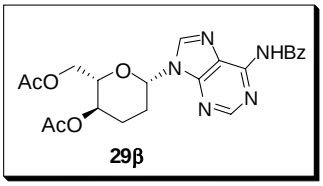
Compound 27α. Deacetylation of the thymine nucleoside **25α** (0.04 g, 0.12 mmol) was accomplished by treatment with a saturated metanolic NH_3 solution (3 mL). After stirring for 24 h at room temperature, the solvent was removed. Chromatography of the crude residue (DCM/MeOH = 8:2) gave the pure **27α** (93% yield) as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.67-1.77 (m, 1H), 1.85-1.97 (m, 5H), 1.99-2.12 (m, 1H), 3.71 (dd, *J* = 5.4 Hz, *J* = 11.7 Hz, 1H), 3.75-3.81 (m, 1H), 3.86 (dd, *J* = 7.2 Hz, *J* = 11.7 Hz, 1H), 3.93-4.01 (m, 1H), 5.90 (dd, *J* = 3.1 Hz, *J* = 9.7 Hz, 1H), 7.74 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 10.9, 23.8, 26.1, 59.8, 62.8, 77.3, 81.0, 110.0, 136.9, 150.9, 164.9. Anal. calcd for $C_{11}H_{16}N_2O_5$: C 51.56, H 6.29, N 10.93. Found: C 51.57, H 6.29, N 10.99.



31

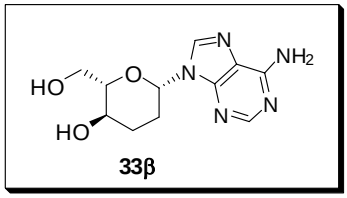
Compound 31. To a stirring solution of the methyl glycoside **23** (0.10 g, 0.41 mmol) in a Ac_2O/AcOH solution (2:1 v/v, 3 mL) at 0 °C, *p*-toluenesulfonic acid (TsOH, 0.15 g, 0.82 mmol) was added in one portion. The resulting mixture was stirred for 4 h, keeping the temperature at 0 °C. Then the solution was diluted with AcOEt, warmed to room temperature, washed with $NaHCO_3$ solution (2 x 100 mL) and brine (2 x 100 mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The crude acetyl glycoside **31** (0.11 g, 95% yield) was directly engaged in the next glycosidation reaction without further purification. α/β Anomeric ratio as determined by ^{1}H NMR was 5/1. Data for pure **31α**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.77-1.99 (m, 4H), 2.06 (s, 3H), 2.08 (s, 3H), 4.00 (ddd, *J* = 2.2 Hz, *J* = 4.7 Hz, *J* = 10.1 Hz, 1H), 4.10 (dd, *J* = 2.2 Hz, *J* = 12.2 Hz, 1H), 4.27 (dd, *J* = 4.8 Hz, *J* = 12.2 Hz, 1H), 4.80 (dt, *J* = 4.9 Hz, *J* = 10.5 Hz, 1H), 6.14 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.8, 21.0,

21.1, 23.6, 27.6, 62.7, 67.0, 70.8, 90.8, 169.3, 169.8, 170.8. Anal. calcd for $C_{12}H_{18}O_7$: C 52.55, H 6.62. Found: C 52.56, H 6.70.



29β

Compound 29β. To a stirring suspension of the acetyl glycoside **31** (0.15 g, 0.57 mmol) and benzoyladenine **28** (0.15 g, 0.68 mmol) in anhydrous CH_3CN (7.5 mL) and under nitrogen atmosphere, *N,N-bis*-trimethylsilyl acetamide (BSA, 0.51 mL, 2.0 mmol) was added and the resulting suspension was stirred for 30 min at 50 °C; after a few minutes the solution became homogeneous. Hence, TfOTMS (0.16 mL, 0.68 mmol) was added dropwise in 10 min. The reaction mixture was further stirred for 40 h at 60 °C. Then anhydrous pyridine (1 mL) was added; the solution was diluted with AcOEt and washed with a saturated $NaHCO_3$ solution (2 x 100 mL) and brine (2 x 100 mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = 95/5) gave the pure **29β** (0.17 g, 65% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.78-1.94 (m, 1H), 2.09 (s, 3H), 2.12 (s, 3H), 2.21-2.42 (m, 2H), 2.43-2.56 (m, 1H), 3.94-4.03 (m, 1H), 4.23 (dd, *J* = 2.1 Hz, *J* = 12.0 Hz, 1H), 4.31 (dd, *J* = 5.1 Hz, *J* = 12.0 Hz, 1H), 4.90 (dt, *J* = 4.8 Hz, *J* = 10.2 Hz, 1H), 5.97 (dd, *J* = 2.7 Hz, *J* = 10.5 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 2H), 7.64 (t, *J* = 7.0 Hz, 2H), 8.05 (d, *J* = 7.8 Hz, 1H), 8.24 (s, 1H), 8.84 (s, 1H), 9.03 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.8, 21.0, 28.1, 30.4, 62.7, 66.5, 77.8, 81.4, 123.0, 127.9, 128.9, 132.8, 133.6, 140.4, 141.7, 149.7, 152.8, 164.7, 169.9, 170.7. Anal. calcd for $C_{22}H_{23}N_5O_6$: C 58.27, H 5.11, N 15.44. Found: C 58.29, H 5.17, N 15.47.



33β

Compound 33β. Deacetylation of the thymine nucleoside **29β** (0.15 g, 0.32 mmol) was accomplished by treatment with a saturated metanolic NH_3 solution (5 mL). After stirring for 24 h at room temperature, the solvent was removed. Chromatography of the crude residue (DCM/MeOH = 8:2) gave the pure **33β** (95% yield) as a white powder. ^{1}H NMR (300 MHz,

DMSO-d_6): δ 1.62-1.71 (m, 1H), 1.99-2.13 (m, 1H), 2.28-2.43 (m, 2H), 3.35-3.53 (m, 3H), 3.70 (ddd, *J* = 11.8 Hz, *J* = 5.7 Hz, *J* = 1.6 Hz, 1H), 4.50 (t, *J* = 5.9 Hz, 1H), 4.92 (d, *J* = 4.9 Hz, 1H), 5.70 (dd, *J* = 11.1 Hz, *J* = 2.1 Hz, 1H), 7.23 (bs, 2H), 8.16 (s, 1H), 8.34 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 29.5, 31.8, 61.2, 64.3, 80.5, 83.4, 118.6, 138.7, 148.7, 152.1, 155.7. Anal. calcd for $C_{18}H_{19}N_5O_4$: C 58.53, H 5.18, N 18.96. Found: C 58.54, H 5.18, N 18.99.

8.4 *SIX-MEMBERED NUCLEOSIDE ANALOGUES, PART III: NON-ANOMERIC HEXOPYRANOSYL NUCLEOSIDE SYNTHESIS*

8.4.1 *RESULTS AND DISCUSSION*

The concept behind non-anomeric pyranosyl nucleosides is to create molecules stable against enzymatic degradation, without abolishing their substrate properties for viral kinases. With this aim, 2,3-dideoxy-1,5-anhydro-D-*arabino*-hexitol nucleosides and their derivatives, the base being axially positioned at the *C*-2', have been already synthesized (**Figure 1**). Hexitol nucleosides have shown to be selectively phosphorylated by herpes virus kinases (not by human kinases), displaying activity against HSV-1 and HSV-2, as well as against other viral infections.* As shown, due to the axial orientation of the nucleobase, the six-membered ring has been displayed to be a good mimic of the furanose ring frozen in a 2'-*exo*, 3'-*endo* conformation: it can be seen as an extended furanose, in which a CH_2 group is inserted between the endocyclic oxygen and the *C*-1.

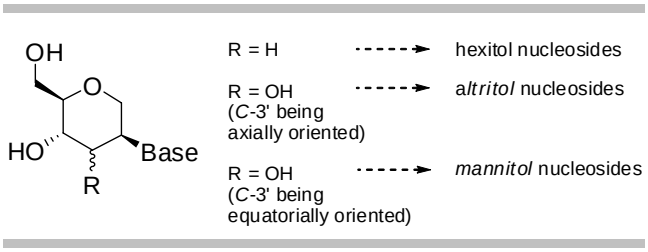


Figure 1 – *1,5-anhydro hexitol nucleosides.*

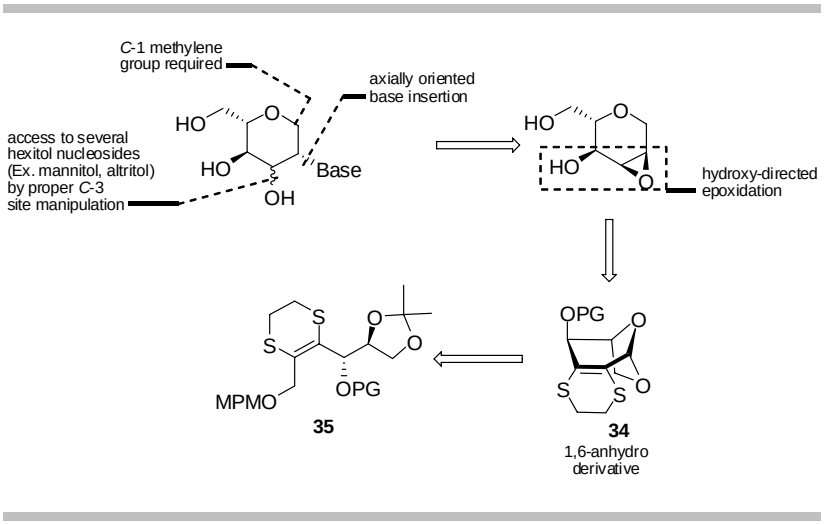
On the basis of these data, as well as by the knowledge regarding the lack of enantioselectivity of several kinases (included the herpes simplex virus type 1 thymidine kinase HSV-1 TK), a study directed to the achievement of a general strategy for the synthesis of non-anomeric pyranosyl

* See Chapter 7, Paragraph 5.3, "Sugar Modifications in Nucleoside Analogues: Six-Membered Nucleosides".

nucleosides has been undertaken. As already mentioned with regard to anomeric nucleoside analogues, nucleosides containing thymine and adenine bases (as pyrimidine and purine base models, respectively) have been initially studied, to test the breadth of our methodology.

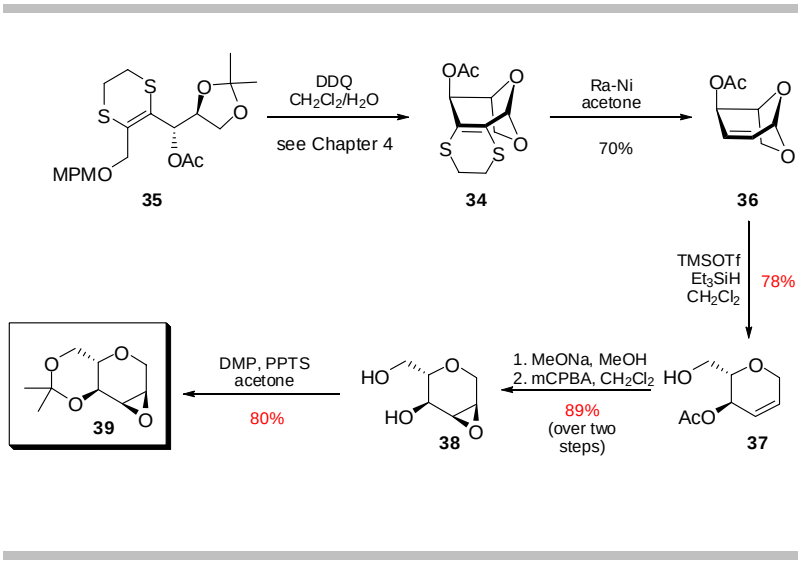
As depicted in the retrosynthetic path (**Scheme 15**), the current strategy diverges from the common route so far described, since a 1-deoxy functionality needs to be prepared. Driven by such purposes, a 1,6-anhydro function was thought to be prepared, since it can be cleaved anytime by hydride attack, to generate the *C*-1 methylene group.[16] Therefore, the strategy comprises the following key steps:

1. preparation of the 1,6-anhydro derivative **34** by double cyclization of the carbon skeleton as from the intermediate **35**;
2. dithioethylene bridge removal and ring cleavage of **34** by means of a hydride nucleophilic attack;
3. creation of *C*-2 electrophilic site and base insertion.



Scheme 15 – *Retrosynthetic path.*

The synthesis began with the preparation of the 1,6 anhydro derivative **34** as from the intermediate **35** by treatment with DDQ in a 18:1 CH_2Cl_2/MeOH emulsion (**Scheme 16**). Then, dithioethylene bridge removal on **34**, by means of Ra-Ni in acetone, afforded the olefin **36**. Hence, treatment of **36** with triethylsilane in acidic medium furnished the pseudo-glucal **37**. With regards to the latter reaction, boron trifluoride diethyl ether complex ($BF_3{\cdot}OEt_2$) and TMSOTf were attempted, the last one driving successfully the reaction in 2h at 0 °C (78% yield).



Scheme 16 – *Synthesis of the protected* syn*-epoxide* ***39****.*

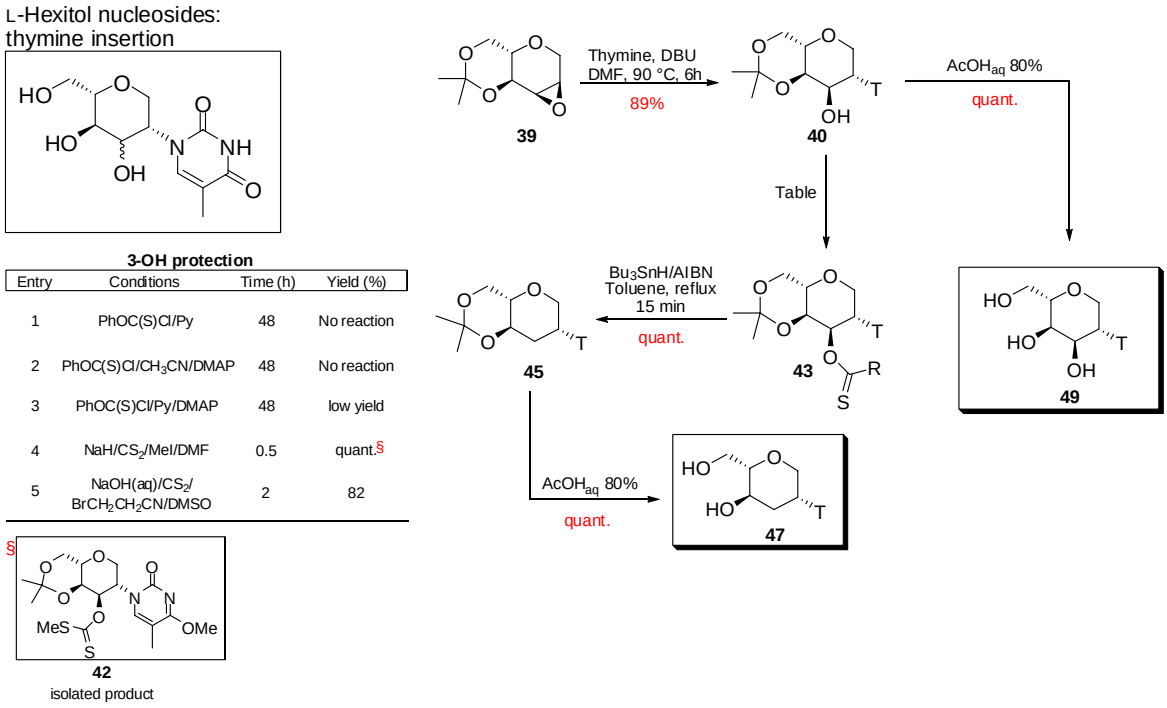
Once 1,6-anhydro ring cleavage was achieved, attention was turned to the construction of an electrophilic site on *C*-2 position, to allow insertion of an axially-oriented nucleobase moiety. With this aim, **37** was deacetylated under Zemplén conditions,[17] then the epoxide **38** (**Scheme 16**) was obtained by hydroxy-directed epoxidation of the olefin **37** by means of *m*-CPBA in CH_2Cl_2 at 0 °C; finally, mild isopropylidene protection[5] of **38** with PPTS and 2,2-dimethoxypropane in acetone afforded the protected epoxide **39** in 80% yield.*

Base insertion remained to be explored. To this concern, it's worthy to note that, once the nucleobase moiety will be installed by *trans*-diaxial nucleophilic attack on the epoxide **39**, an axially oriented 3-hydroxyl function will be present in the molecule. This allows us to have in hand a general procedure, as it will enable to get several hexitol nucleosides (such as *mannitol* or *altritol* nucleosides, **Scheme 17**) by appropriate manipulation of the 3- hydroxyl group. Particularly, when the hydroxyl group is deoxygenated, the desired 2,3-dideoxy-hexitol nucleosides are afforded. To this aim to find the best conditions for 3-OH removal, several deoxygenation procedures have been examined. Therefore, the synthesis of such molecules has also resulted in an investigation about the deoxygenation reaction[18] on anhydrohexitol nucleosides.

With the protected epoxide **39** in hand, the easiest way to obtain L-hexitol nucleosides was by epoxide ring opening, under basic conditions, using unprotected nucleobases, that will arrange into the required axial position. As already studied for similarly protected D-altritol

* At the beginning, two candidate protections were successfully installed on the epoxide **39**, i.e. the isopropylidene and the *t*butyldiphenylsilyl (TBDPS) groups: however, the route involving the use of TBDPS group was readily left due to very low yields observed in the further coupling step with the nucleobase (see text).

nucleosides,[19] the mildest conditions for the nucleophilic attack provided for the use of 1,8-diazabicylo[5.4.0]undec-7-ene (DBU) as the activating base.* Hence, treatment of **39** with thymine and DBU in *N*,*N*-dimethylformamide (DMF) at 90 °C for 8h afforded the protected L-altritol nucleoside **40** in 89% yield† (**Scheme 17**). Analogously, reaction of **39** with adenine under the same conditions gave **41** (**Scheme 18**) in a few lower yields (74%).

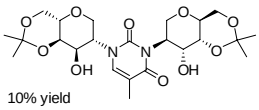


Scheme 17 - *1,5-Anhydro-2-(thymin-1-yl)-2,3-dideoxy-L-*arabino*-hexitol nucleoside synthesis.*

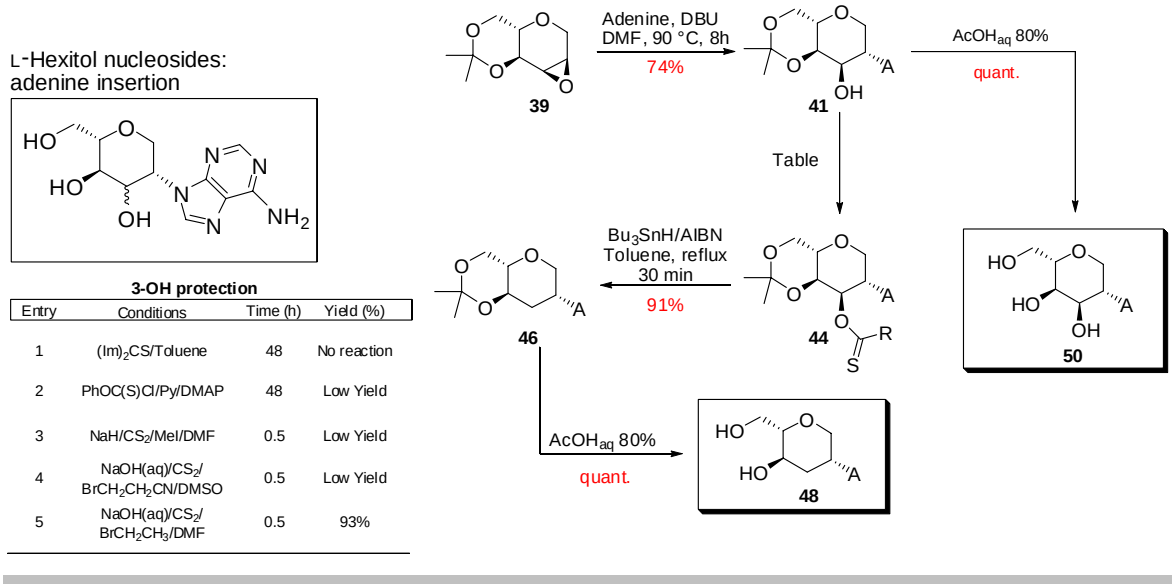
Then, 3-OH group removal by deoxygenation reaction was examined (**Schemes 17-18**). The alcohol **40**, treated with phenoxythiocarbonyl chloride (PhOC(S)Cl) under various conditions,[20] did not show almost any reactivity. Conversely, classic Barton-McCombie deoxygenation conditions,[21] i.e. sodium hydriide (NaH), carbon disulfide (CS_2), methyl iodide (MeI) in dimethylformamide (DMF) afforded the xanthate derivative **42** in almost quantitative yield; however, careful examination of this latter clarified that an extra-methylation on the nucleobase

* DBU was preferred to other activating bases commonly used, such as NaH or LiH, because lesser anhydrous conditions were required.

† During the course of the reaction, a UV detectable byproduct appeared: complete NMR characterization (COSY, HMBC, HSQC-TOCSY) unambiguously determined its structure, correspondent to the molecule reported below:



occurred (see table in **Scheme 17**). In search for a milder variant of this procedure, the combination of $NaOH_{aq}$, CS_2, bromoproprionitrile ($BrCH_2CH_2CN$) in DMSO furnished the nucleoside **43** in a satisfying yield (82%).[22] On the other hand, as far as it concerns the adenine nucleoside **41** (**Scheme 18**), the $NaOH_{aq}/CS_2/BrCH_2CH_2CN$/DMSO mixture did not give the expected results, the product **44** being obtained in low yields.* Analogously, the NaH/CS_2/MeI/DMF and PhOC(S)Cl/DMAP/Py mixtures failed. Reaction with thiocarbonyl diimidazole (Im_2CS)[20] in a number of solvents showed no reactivity at all. Finally, good results were found replacing the alkylating agents CH_3I and $BrCH_2CH_2CN$ with the less electrophilic (and less expensive) bromoethane ($BrCH_2CH_3$):[23] in fact, treatment of **41** with the $NaOH_{aq}/CS_2/BrCH_2CH_3$/DMF mixture gave, already after 30 min at 0 °C, the desired product **44** in 93% yield.



Scheme 18 - *1,5-Anhydro-2-(adenin-9-yl)-2,3-dideoxy-L-*arabino*-hexitol nucleoside synthesis.*

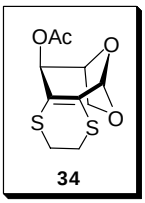
The remaining xanthate reduction and acetonide deprotection steps were then explored. Both thymine and adenine protected L-altritol nucleosides **43** and **44** were subjected to radical deoxygenation, by means of tributyltin hydride (Bu_3SnH) in refluxing toluene, using azobisisobutyronitrile (AIBN) as radical initiator. In both cases, immediate reduction occurred, the deoxygenated products **45** and **46** being obtained in excellent yields (98% and 91%,

* An inseparable mixture of two products was formed during the reaction (TLC). Although a clear identification of the structure was not possible, a second alkylation on the nucleobase moiety is assumed to be occurred, as already reported elsewhere [Ref. 22].

respectively). Furthermore, exposure of **45** and **46** to 80% aqueous acetic acid[24] at 60 °C for 4h gave, after purification procedures, 1,5-anhydro-2-(thymin-1-yl)-2,3-dideoxy-L-arabino-hexitol and 1,5-anhydro-2-(adenin-9-yl)-2,3-dideoxy-L-arabino-hexitol free nucleosides **47** and **48** in quantitative yields (**Schemes 17-18**). Compounds **40** and **41** were deprotected as well, under the same conditions, obtaining adenine and thymine L-altritol nucleosides **49** and **50** (**Schemes 17-18**).

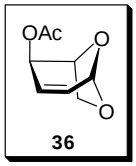
As already mentioned earlier with regard to anomeric hexopyranosyl nucleosides, also nucleoside analogues **47-50** are currently undergoing biological evaluation as HIV and/or HSV agents.

8.4.2 *EXPERIMENTAL SECTION*



34

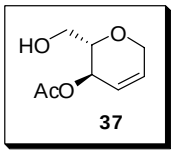
Compound 34. To a stirred 18:1 CH_2Cl_2/H_2O emulsion (50 mL) containing the ether **35** (4.4 g, 10 mmol), DDQ (3.4 g, 15 mmol) was added in one portion at room temperature. After 48 h H_2O was added and the mixture extracted with CH_2Cl_2; the organic layer was dried (Na_2SO_4) and the solvent evaporated. Chromatography of the crude residue (DCM) gave the pure **34** (2.1 g, 80% yield) as a colourless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.17 (s, 3H), 3.19-3.27 (m, 4H), 3.68 (dd, *J* = 1.9 Hz, *J* = 8.1 Hz, 1H), 3.98 (dd, *J* = 6.6 Hz, *J* = 8.0 Hz, 1H), 4.70-4.72 (m, 1H), 4.85 (d, *J* = 1.3 Hz, 1H), 5.34 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.5, 27.9, 29.7, 63.8, 70.5, 75.2, 98.9, 128.8, 130.9, 172.6. Anal. calcd for $C_{10}H_{12}O_4S_2$: C 46.14, H 4.65. Found: C 46.10, H 4.72.



36

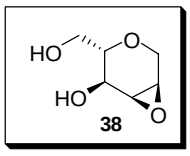
Compound 36. A solution of **34** (2.0 g, 10 mmol) in acetone (80 mL) was added in one portion to a stirred suspension of Raney-Ni (W2) (20 g, wet) in the same solvent (80 mL) at 0 °C and under nitrogen atmosphere. The suspension was stirred for 2h, then the solid was filtered off and washed with acetone. The filtrate was evaporated under reduced pressure to afford a crude

residue which chromatography over silica gel (CH_2Cl_2) gave the pure **36** (0.92 g, 75% yield) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.12 (s, 3H), 3.54 (dd, *J* = 2.1 Hz, *J* = 5.0 Hz, 1H), 3.97 (d, *J* = 6.7 Hz, *J* = 8.0 Hz, 1H), 4.71-4.74 (m, 1H), 4.80 (d, *J* = 4.6 Hz, 1H), 5.60 (d, *J* = 3.4 Hz, 1H), 5.78-5.82 (m, 1H), 6.20 (dd, *J* = 3.5 Hz, *J* = 9.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.6, 63.7, 68.5, 74.7, 96.0, 123.0, 133.2, 171.1. Anal. calcd for $C_8H_{10}O_4$: C 56.47, H 5.92. Found: C 56.40, H 5.99.



37

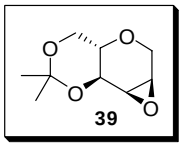
Compound 37. To a stirring solution of the 1,6-anhydro derivative **36** (0.82 g, 4.7 mmol) and triethylsilane (0.82 ml, 6.6 mmol) in CH_2Cl_2 (30 mL) at 0 °C, TfOTMS (0.2 ml, 0.9 mmol) was dropwise added. After 30 min, the reaction was quenched with anhydrous $NaHCO_3$, warmed to room temperature, diluted with CH_2Cl_2 (300 mL) and washed with brine (300 mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (CH_2Cl_2) gave the pure **37** (0.71 g; 89% yield) as a colourless oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.12 (s, 3H), 3.49-3.54 (m, 1H), 3.58-3.63 (m, 1H), 3.72-3.78 (m, 1H), 4.18-4.25 (m, 2H), 5.29 (bs, 1H), 5.76 (bd, *J* = 9.9 Hz, 1H), 5.93 (bd, *J* = 9.9 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 20.4, 63.5, 64.5, 65.6, 78.7, 127.8, 128.9, 181.3. Anal. calcd for $C_8H_{12}O_4$: C 55.81, H 7.02. Found: C 55.88, H 7.08.



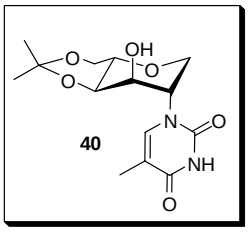
38

Compound 38. Zemplén deacetylation was accomplished by treatment of **37** (0.60 g, 3.49 mmol) in MeOH (50 mL) with MeONa (0.41 g, 7.6 mmol) for 4h at room temperature. Then, the mixture was neutralized with few drops of acetic acid, the solvents evaporated under reduced pressure. The crude residue was dissolved in $CHCl_3$ and filtered through a short pad of silica gel; the resulting filtrate was concentrated to dryness. The residue was dissolved in anhydrous DCM, hence *m*-CPBA (0.72 g, 4.18 mmol) was added at 0 °C. The resulting reaction mixture was stirred at room temperature overnight, then the insoluble materials were filtered off and the filtrate was evaporated. Chromatography of the crude residue over silica gel (DCM/MeOH = 9:1) gave the pure **38** (0.62 g; 96% yield) as a colourless oil. ^{1}H NMR (500 MHz, CD_3OD): δ 3.23 (ddd, *J* = 2.0 Hz, *J* = 5.9 Hz, *J* = 11.8 Hz, 1H), 3.29-3.34 (m, 1H), 3.40-3.43 (m, 1H), 3.51

(t, *J* = 4.2 Hz, 1H), 3.55 (dd, *J* = 4.4 Hz, *J* = 12.2 Hz, 1H), 3.77-3.82 (m, 2H), 4.08 (dd, *J* = 3.9 Hz, *J* = 13.2 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 54.2, 55.3, 61.9, 64.4, 65.6, 75.6. Anal. calcd for $C_6H_{10}O_4$: C 49.31, H 6.90. Found: C 49.30, H 6.96.

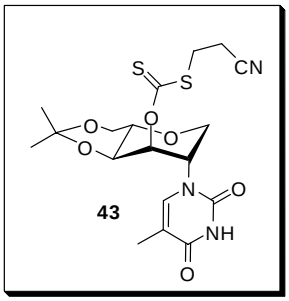


Compound 39. 2,2-Dimethoxypropane (DMP, 0.48 ml, 4.79 mmol) and pyridinium *p*-toluensulfonate (PPTS, 0.81 g, 3.19 mmol) were added to a solution of **38** (0.60 g, 3.19 mmol) in anhydrous acetone (25 ml) and the resulting reaction mixture was stirred for 12h at room temperature. Then the solvent was evaporated, the crude residue dissolved with EtOAc and washed with saturated $NaHCO_3$ (2 x 200 ml) and brine (2 x 150 ml). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM) gave the pure **39** (0.71 g; 80% yield) as a colourless oil. ^{1}H NMR (400 MHz, C_6D_6): δ 1.29 (s, 3H), 1.50 (s, 3H), 2.68 (bt, *J* = 3.7 Hz, 1H), 3.09 (bd, *J* = 4.4 Hz, 1H), 3.53 (dd, *J* = 3.5 Hz, *J* = 13.6 Hz, 1H), 3.58 (t, *J* = 10.3 Hz, 1H), 3.65 (d, *J* = 13.4 Hz, 1H), 3.71-3.78 (m, 1H), 3.79-3.85 (m, 2H). ^{13}C NMR (75 MHz, C_6D_6): δ 18.8, 29.3, 51.4, 52.6, 62.6, 64.2, 65.8, 71.2, 99.8. Anal. calcd for $C_9H_{14}O_4$: C 58.05, H 7.58. Found: C 58.09, H 7.50.

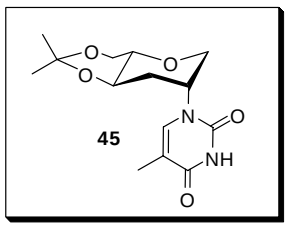


Compound 40. Thymine (0.78 g, 6.2 mmol) and the epoxide **39** (0.5 g, 2.7 mmol) were suspended in 7 ml of anhydrous DMF under nitrogen for 15 min at room temperature. Then 1,8-diazabicylo[5.4.0]undec-7-ene (DBU, 0.92 ml, 6.2 mmol) was added and the reaction mixture was heated at 90 °C for 8h, after which the reaction was cooled, quenched with NH_4Cl and concentrated. The residue was extracted with DCM and washed with brine (3 x 150 ml). The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = 98:2) gave the pure **40** (1.73 g; 89% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.44 (s, 3H), 1.50 (s, 3H), 1.97 (s, 3H), 3.67 (dd, *J* = 2.7 Hz, *J* = 9.7 Hz, 1H), 3.76 (t, *J* = 10.3 Hz, 1H), 3.90 (dd, *J* = 5.3 Hz, *J* = 10.0 Hz, 1H), 3.98 (dd, *J* = 5.3 Hz, *J* = 10.3 Hz, 1H), 4.03 (d, *J* = 13.7 Hz, 1H), 4.07 (bs, 1H), 4.34 (dd,

J = 4.3 Hz, *J* = 13.7 Hz, 1H), 4.49 (bt, *J* = 2.9 Hz, 1H), 7.84 (s, 1H), 8.43 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.7, 19.2, 28.9, 57.2, 62.3, 64.0, 66.0, 67.2, 69.2, 100.0, 111.4, 137.9, 151.3, 164.3. Anal. calcd for $C_{14}H_{20}N_2O_6$: C 53.84, H 6.45, N 8.97. Found: C 53.85, H 6.49, N 8.99.

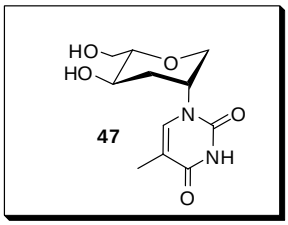


Compound 43. To a stirring solution of compound **40** (0.5 g, 1.6 mmol) in DMSO (10 ml) at 0 °C, a 5N aqueous NaOH solution (1.7 mL), CS_2 (1.7 mL) and then *β*-bromopropionitrile (4.1 mL) were added. After stirring for 30 min, the solvent was evaporated and the crude residue was extracted with DCM and washed with NH_4Cl solution. The organic layer was dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The crude residue was purified by chromatography over silica gel (DCM/MeOH = 95/5) to obtain the pure **43** (0.59 g, 82%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (s, 3H), 1.47 (s, 3H), 1.99 (d, *J* = 1.1 Hz, 3H), 2.88 (t, *J* = 7.1 Hz, 2H) 3.37-3.44 (m, 1H), 3.45-3.50 (m, 1H) 3.76 (t, *J* = 10.4 Hz, 1H) 3.85-3.92 (m, 2H), 4.00 (dd, *J* = 4.7 Hz, *J* = 10.4 Hz, 1H), 4.17 (d, *J* = 13.9 Hz, 1H), 4.28 (dd, *J* = 3.6 Hz, *J* = 13.9 Hz, 1H), 4.55 (bt, *J* = 2.9 Hz, 1H), 6.26 (s, 1H),7.84 (d, *J* = 1.1 Hz, 1H), 8.46 (bs, 1H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 12.9, 19.0, 28.8, 31.6, 41.0, 53.8, 62.1, 65.3, 68.5, 68.8, 74.8, 100.2, 111.8, 117.8, 136.9, 150.4, 163.0, 211.2. Anal. calcd for $C_{17}H_{21}N_3O_6S_2$: C 47.76, H 4.95, N 9.83. Found: C 47.79, H 4.99, N 9.88.

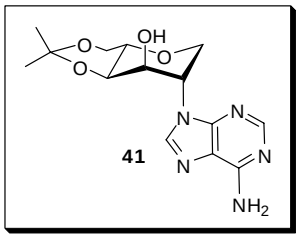


Compound 45. To a refluxing solution of compound **43** (0.3 g, 0.66 mmol) in anhydrous toluene (10 ml) kept under nitrogen flow, a solution of *n*-Bu_3SnH (0.43 mL, 0.69 mol) and AIBN (0.01 g, 0.03 mol) in anhydrous toluene (10 ml) was added dropwise over 1h. After the addition, the brown solution was stirred for additional 30 min under reflux. The reaction mixture was then concentrated under reduced pressure and the resulting residue was purified by column

chromatography on silica gel (DCM/MeOH = 95/5) to give the pure **45** (0.19 g, 0.66 mmol, quantitative yield) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 3H), 1.50 (s, 3H), 1.92 (ddd, *J* = 4.7 Hz, *J* = 13.3 Hz, *J* = 17.6 Hz, 1H), 1.99 (s, 3H), 2.28 (bd, *J* = 13.3 Hz, 1H), 3.35 (ddd, *J* = 5.3 Hz, *J* = 9.7 Hz, *J* = 14.9 Hz, 1H), 3.68-3.82 (m, 2H), 3.92-4.02 (m, 2H), 4.20 (bd, *J* = 14.0 Hz, 1H), 4.62 (bs, 1H), 7.84 (s, 1H), 8.65 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.8, 19.1, 29.1, 33.4, 51.5, 62.3, 66.0, 68.9, 75.5, 99.8, 110.6, 138.0, 150.7, 163.4. Anal. calcd for $C_{14}H_{20}N_2O_5$: C 56.75, H 6.80, N 9.45. Found: C 56.75, H 6.83, N 9.49.

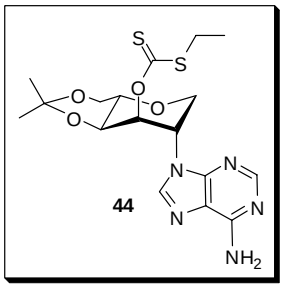


1,5-anhydro-2-(thymin-1-yl)-2,3-dideoxy-L*-arabino-*hexitol **(47).** A solution of nucleoside **45** (0.12 g, 0.42 mmol) in 80% AcOH (6 mL) was heated at 65 °C for 2h. The reaction mixture was then concentrated to dryness under reduced pressure and the residue was coevaporated with a mixture of toluene and EtOH (1:1 v/v, 5×30 ml). The residue was purified by column chromatography (DCM/MeOH = 9/1) to yield thymine nucleoside **47** (0.10 g, 99% yield) as white crystals. ^{1}H NMR (300 MHz, DMSO-d_6)**:** δ 1.77 (s, 1H), 1.60-2.52 (m, 2H), 3.05-3.30 (m, 1H), 3.4-4.1 (m, 4H), 3.7-4.1 (m, 2H) 4.52 (m, 1H), 4.65 (t, *J* = 5.7 Hz, 1H), 4.89 (d, *J* = 5.0 Hz, 1H), 7.88 (s, 1H), 11.25 (bs, 1H). ^{13}C NMR (300 MHz, DMSO-d_6): δ 12.3, 35.2, 50.1, 60.3, 60.8, 66.9, 82.4, 108.3, 138.9, 150.9, 163.8. Anal. calcd for $C_{11}H_{16}N_2O_5$: C 51.56, H 6.29, N 10.93. Found: C 51.59, H 6.39, N 10.95.



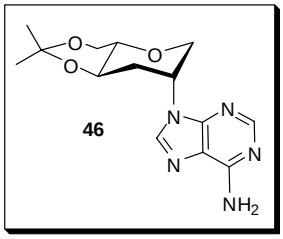
Compound 41. Adenine (0.50 g, 2.69 mmol) and the epoxide **39** (0.83 g, 6.19 mmol) were suspended in 6 ml of anhydrous DMF under nitrogen atmosphere for 15 min at room temperature. Then DBU (1.02 ml, 6.70 mmol) was added and the reaction mixture was heated at 90 °C for 8h, after which the reaction was cooled, quenched with NH_4Cl and concentrated. The residue was extracted with EtOAc and washed with brine (3 x 150 ml). The organic layer was

dried (Na_2SO_4) and the solvent evaporated under reduced pressure. Chromatography of the crude residue over silica gel (DCM/MeOH = 98:2) gave the pure **41** (0.64 g; 74% yield). ^{1}H NMR (300 MHz, DMSO): δ 1.27 (s, 3H), 1.33 (s, 3H), 3.52-3.60 (m, 1H), 3.72-3.90 (m, 3H), 4.03-4.08 (m, 1H), 4.18-4.29 (m, 2H), 4.52-4.56 (m, 1H), 5.58 (d, *J* = 4.3 Hz, 1H), 7.29 (bs, 2H), 8.17 (s, 1H), 8.29 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ 19.5, 29.4, 56.3, 62.0, 65.0, 65.8, 67.6, 69.4, 99.5, 118.6, 139.6, 150.0, 153.0, 156.5. Anal. calcd for $C_{14}H_{19}N_5O_4$: C 52.33, H 5.96, N 21.79. Found: C 52.30, H 5.97, N 21.82.



44

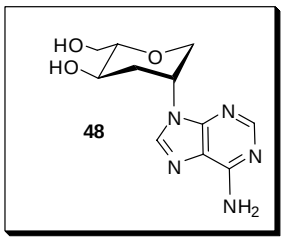
Compound 44. To a stirring solution of compound **41** (0.27 g, 0.84 mmol) in DMF (11 ml), 5N aqueous NaOH solution (0.93 mL), CS_2 (0.93 mL) and then bromoethane (0.27 mL) were added. After stirring for 30 min, the solvent was evaporated; the crude residue was extracted with DCM and washed with NH_4Cl solution. The organic layer was dried (Na_2SO_4) and the solvent evaporated. The crude residue was purified by chromatography over silica gel (DCM/MeOH = 95/5) to obtain the pure **44** (0.31 g, 91%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.36 (s, 3H), 1.41 (s, 3H), 1.43 (t, *J* = 7.2 Hz, 3H), 3.13-3.33 (m, 2H), 3.79-3.87 (m, 1H), 3.94-4.09 (m, 3H), 4.33 (d, *J* = 13.2 Hz, 1H), 4.39 (dd, *J* = 2.7 Hz, *J* = 13.2 Hz, 1H), 4.93-4.99 (m, 1H), 5.84 (bs, 2H), 6.32-6.38 (m, 1H), 8.38 (s, 1H), 8.42 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.4, 18.9, 28.9, 30.6, 52.6, 62.4, 66.5, 68.2, 69.5, 74.2, 100.2, 119.0, 139.2, 150.3, 153.5, 155.6, 213.4. Anal. calcd for $C_{17}H_{23}N_5O_4S_2$: C 47.98, H 5.45, N 16.46. Found: C 48.01, H 5.48, N 16.41.



46

Compound 46. To a refluxing solution of compound **44** (0.31 g, 0.76 mmol) in anhydrous toluene (20 ml), kept under nitrogen atmosphere, a solution of *n*-Bu_3SnH (0.28 mL, 1.14 mol)

and AIBN (0.02 g, 0.03 mol) in anhydrous toluene (20 ml) was dropwise added over 1h. After the addition, the brown solution was stirred for additional 30 min under reflux. The reaction mixture was then concentrated under reduced pressure and the resulting residue was purified by column chromatography on silica gel (DCM/MeOH = 95/5) to give the pure **46** (0.23 g, quantitative yield) as a white brilliant powder. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (ddd, *J* = 4.5 Hz, *J* = 11.5 Hz, 13.5 Hz, 1H), 3.44 (ddd, *J* = 5.2 Hz, *J* = 9.9 Hz, *J* = 14.9 Hz, 1H), 3.73, (ddd, *J* = 3.4 Hz, *J* = 7.9 Hz, *J* = 13.8 Hz, 1H), 3.82, (t, *J* = 10.4 Hz, 1H), 3.99 (dd, *J* = 5.2 Hz, *J* = 10.8 Hz, 1H), 4.12 (dd, *J* = 2.9 Hz, *J* = 13.0 Hz, 1H), 4.42(bd, *J* = 12.9 Hz, 1H), 4.94-5.00 (m, 1H), 5.60 (bs, 2H), 8.34 (s, 1H), 8.39 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.1, 29.1, 33.7, 50.8, 62.4, 66.3, 69.8, 75.8, 77.2, 99.8, 139.7, 153.1, 155.4. Anal. calcd for $C_{14}H_{19}N_5O_3$: C 55.07, H 6.27, N 22.94. Found: C 55.09, H 6.23, N 22.91.



48

1,5-anhydro-2-(adenin-9-yl)-2,3-dideoxy-L*-arabino-*hexitol **(48).** A stirring solution of nucleoside **46** (0.2 g, 0.65 mmol) in 80% AcOH (4 mL) was heated at 65 °C for 2h. Then the reaction mixture was concentrated to dryness and the residue purified by column chromatography [DCM/MeOH = 9/1] to yield adenine nucleoside **48** (0.18 g, 99%) as white crystals. ^{1}H NMR (300 MHz, DMSO-d_6)***:*** δ 1.89 (ddd, *J* = 13.0 Hz, *J* = 11 Hz, *J* = 4.0 Hz, 1H), 2.29 (d, *J* = 13.5 Hz, 1H), 3.20 (d, *J* = 9.0 Hz, 1H), 3.52 (d, *J* = 9.1 Hz, 1H), 3.59 (m, 1H), 3.70 (ddd, *J* = 12.0 Hz, *J* = 5.3 Hz, *J* = 2.5 Hz, 1H), 3.87 (dd, *J* = 12.5 Hz, *J* = 2.2 Hz, 1H), 4.21 (dt, *J* = 12.5 Hz, *J* = 2.5 Hz, *J* = 1.5 Hz, 1H), 4.67 (t, *J* = 5.5 Hz, 1H), 4.78 (d, *J* = 4.1 Hz,1H), 4.92 (d, *J* = 5.3 Hz, 1H), 7.25 (s, 2H), 8.15 (s, 1H), 8.30 (s, 1H). ^{1}H NMR (300 MHz, CD_3OD)***:*** δ 36.4, 50.5, 60.9, 61.1, 68.5, 83.5, 118.8, 140.0, 149.8, 152.8, 156.5. Anal. calcd for $C_{11}H_{15}N_5O_3$: C 49.81, H 5.70, N 26.40. Found: C 49.86, H 5.76, N 26.41.

8.5 *REFERENCES*

[1] De Clercq, E. Antiviral drug discovery and development: where chemistry meets biomedicine. *Antiviral Res.* **2005**, *67*, 56.

[2] Herdewijn, P. in *Recent Advances in Nucleosides: Chemistry and Chemotherapy*; Chu, C. K. Ed., Elsevier Science: Amsterdam, **2002**.

[3] (a) Yokoyama, M.; Momotake, A. Synthesis and biological activity of azanucleosides. *Synthesis* **1999**, *9*, 1541. (b) Schramm, V. L.; Tyler, P. C. Imino-sugar-based nucleosides. *Curr. Topics Med. Chem.* **2003**, *3*, 525.

[4] (a) Garner, P.; Park, J. M. 1,1,-Dimethylethyl (*s*)- or (*r*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate: a useful serinal derivative. *Org. Synth.* **1992**, *70*, 18. (b) Bols, M. Garner's aldehyde. *J. Chem. Soc*., *Perkin Trans. 1*, **2001**, 2136.

[5] Takahata, H.; Banba, Y.; Ouchi, H.; Nemoto, H. Concise and highly stereocontrolled synthesis of 1-deoxygalactonojirimycin and its congeners using dioxanylpiperidene, a promising chiral building block. *Org. Lett.* **2003**, *5*, 2527.

[6] (a) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. Nucleoside synthesis with trimethylsylyl triflate and perchlorate as catalyst. *Chem. Ber.* **1981**, *114*, 1234. (b) Vorbrüggen H.; Bennua, B. A new simplified nucleoside synthesis. *Chem. Ber* **1981**, *114*, 1279.

[7] Rassu, G.; Pinna, L.; Spanu, P.; Ulgheri, F.; Casiraghi, G. Total syntheses of *N*-Boc protected 3'-deoxy-4'-azathymidine and 4'azauridine. *Tetrahedron Letters* **1994**, *35*, 4019.

[8] Nord, L. D.; Dalley, N. K.; McKernan, P. A.; Robins, R. K. Synthesis, structure, and biological activity of certain 2'-deoxy-β-D-*ribo*-hexopyranosyl nucleosides and nucleotides. *J. Med. Chem.* **1987**, *30*, 1044.

[9] Sabini, E.; Hazra, S.; Konrad, M.; Lavie, A., Nonenantioselectivity Property of Human Deoxycytidine Kinase Explained by Structures of the Enzyme in Complex with L- and D-Nucleosides. *J. Med. Chem.* **2007**, *50*, 3004.

[10] Bohringer, M.; Roth, H. J.; Hunziker, J.; Gobel, M.; Krishnan, R.; Giger, A.; Schweizer, B.; Schreiber, J.; Leumann, C.; Eschenmoser, A. Warum Pentose- und nicht Hexose-Nucleinsauren? Teil II) Oligonucleotide aus 2',3'-Dideoxy-β-D-glucopyranosyl-Bausteinen (`Homo-DNS'): Herstellung. *Helv. Chim. Acta* **1992**, *75*, 1416.

[11] Vorbruggen, H.; Ruh-Polenz, C. *Handbook of Nucleoside Synthesis*; John Wiley & Sons Inc.: New York, **2001**.

[12] Framski, G.; Gdaniec, Z.; Gdaniec, M; Boryski, J. A reinvestigated mechanism of ribosylation of adenine under silylating conditions. *Tetrahedron* **2006**, *62*, 10123.

[13] Lam, S. L.; Gervay-Hague, J. Efficient route to 2-deoxy-β-*O*-aryl-D-glycosides via direct displacement of glycosyl iodides. *Org. Lett.* **2003**, *5*, 4219.

[14] Van Look, G.; Simchen, G.; Heberle, J. *Silylating agents* (2nd Ed.); Fluka, Buchs, **1995**.

[15] Kazimierczuk, Z.; Cottam, H. B.; Revankar, G. R.; Robins, R. K. Synthesis of 2'-deoxytubercidin, 2'-deoxyadenosine and related 2'-deoxynucleosides via a novel direct stereospecific sodium salt glycosylation procedure. *J. Am. Chem. Soc* **1984,** *106*, 6379.

[16] Yamamoto, H.; Maruoka, K. Regioselective carbonyl amination using diisobutylaluminium hydride. *J. Am. Chem. Soc.* **1981**, *103*, 4186.

[17] Zemplen, G.; Kuntz, A. Amygdalin. IV. Synthesis of natural L-amygdalin. *Chem. Ber.* **1924**, *57B*, 1357.

[18] For a survey of the deoxygenation methods, see: Chong, Y.; Chu, C. K. Efficient synthesis of 2-deoxy-L-*erythro*-pentose (2-deoxy-L-ribose) from L-arabinose. *Carbohydr. Res.* **2002**, *337*, 397.

[19] Allart, B.; Busson, R.; Rozenski, J.; Van Aerschot, A.; Herdewijn, P. Synthesis of protected D-altritol nucleosides as building blocks for oligonucleotide synthesis. *Tetrahedron* **1999**, *55*, 6527.

[20] Satoshi, T.; Satoshi, S.; Kunisuke, I. Process for producing nucleoside derivatives. *Eur. Pat. Appl.* **1999**, EP 0999219 A1.

[21] Barton, D. H. R.; McCombie, S. W. New method for the deoxygenation of secondary alcohols. *J. Chem. Soc., Perkin Trans. I* **1975**, 1574.

[22] Chu, C. K.; Bhadti, V. S.; Doboszewski, B.; Gu, Z. P.; Kosugi, Y.; Pullaiah, K. C.; Van Roey, P. General Syntheses of 2',3'-dideoxynucleosides and 2',3'-didehydrodideoxynucleosides. *J. Org. Chem.* **1989**, *54*, 2217.

[23] Zhao, S.; Ma, M.; Wen, K.; Sanghvi, Y. An improved synthesis of 2',3'-dideoxynucleosides. *Nucleos. Nucleot. Nucleic Acids* **2007**, *26*, 959.

[24] Babu, B. R.; Poopeiko, N. E.; Juhl, M.; Bond, A. D.; Parmar, V. S.; Wengel, J. XNA (xylose Nucleic Acid): a summary and new derivatives. *Eur. J. Org. Chem.* **2005**, 2297.

Chapter 9

Nucleic Acids with a Six-Membered Sugar Unit: New Concepts in Antisense Technology by Expanding the Sugar Ring size of Natural Oligonucleotides

9. NUCLEIC ACIDS WITH A SIX-MEMBERED SUGAR UNIT: NEW CONCEPTS IN ANTISENSE TECHNOLOGY BY EXPANDING THE SUGAR RING SIZE OF NATURAL OLIGONUCLEOTIDES

Mainly driven by the need of antisense research, during the last years considerable efforts have been devoted to the development of variously modified oligonucleotide analogues, to be employed as a source of information towards a deeper understanding of the pairing behaviour of DNA and RNA. The search for nucleic acid alternatives has inspired investigations on pyranosyl oligonucleotides, because of the much larger structural diversity of the six-membered moiety in the carbohydrate backbone, compared to natural furanosyl units. Due to an improved hybridization aptitude and a complete enzymatic stability, several examples of oligonucleotide analogues have been revealed excellent targets for antisense purposes.

9.1 *INTRODUCTION*

The notion that gene expression could be modified by exogenous nucleic acids derives from studies carried out over 25 years ago, since when Zamecnik and Stephenson demonstrated that (a) a single-stranded DNA could inhibit translation of a complementary RNA in a cell-free system and that (b) short oligomers (13-mer) of DNA could inhibit replication of Rous sarcoma virus gene sequences in a cellular system.[1] Pioneering papers like these set the basis of what is now called the "antisense approach" in human therapy and together with the thousands of papers that followed have stimulated the development of technologies that use nucleic acids to manipulate gene expression.

Basically, the concept behind the antisense approach is quite simple: inhibition of the expression of a specific gene at the RNA level (responsible for a certain disease) can be realized by using suitable oligonucleotides, that are complementary (antisense) with respect to the target RNA sequence, thereby blocking the expression of the protein encoded by the target RNA (**Figure 1**). Within this concept, inhibition has been demonstrated to occur in a great number of different mechanisms, depending on which kind of RNA is chosen as target.[2]

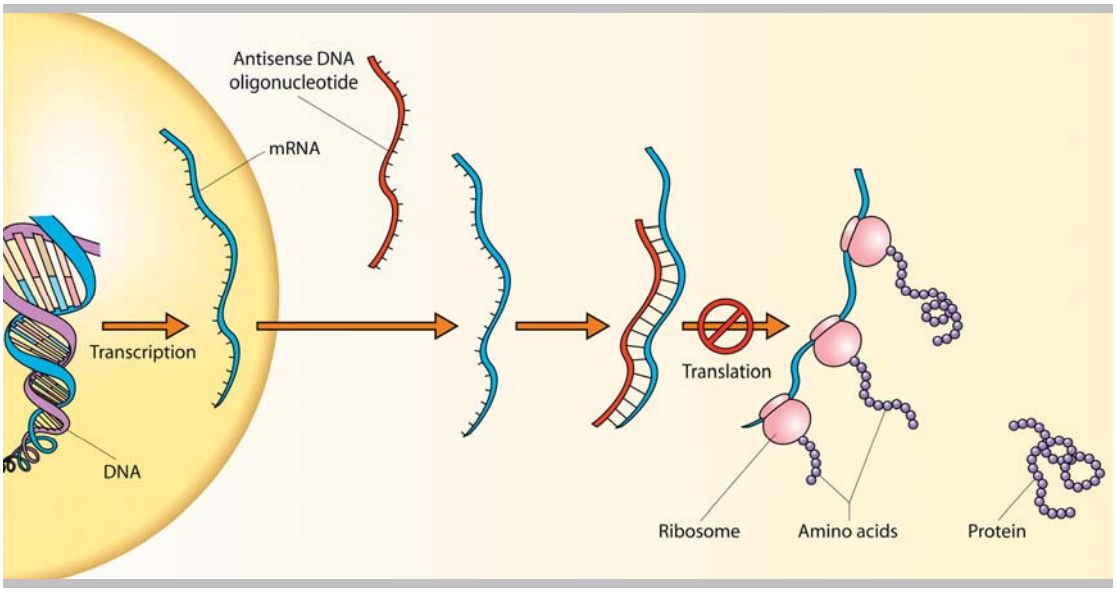
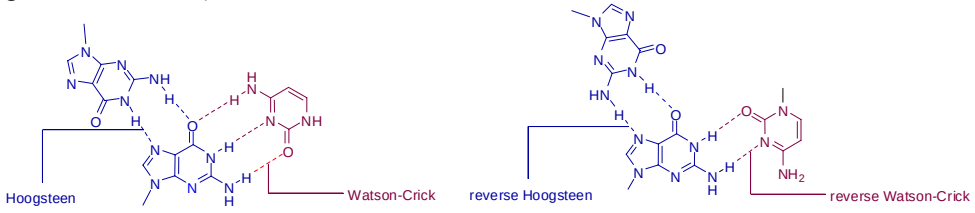


Figure 1 – *Schematic picture of the principle behind antisense technology.*

In principle, a therapeutic intervention at the level of nucleic acids offers a great number of advantages. In fact, on transcription, every gene gives rise to hundred copies of *m*RNA, which is translated into million copies of protein molecules. Therefore, inhibition of the gene expression has been thought to be more efficient than inhibition of the resulting proteins. As a matter of fact, driven by the same concept, a number of drugs (adriamycin, bleomycin, or cisplatin), whose activity is based on direct interaction with the ribonucleic chain, have been already put on the market. Such compounds, mainly used for chemotherapy, intercalate or bind specifically only to DNA. Unfortunately, they lack of complete specificity and thus do not act on particular genes. Conversely, the use of synthetic oligonucleotides that bind selectively to complementary nucleic acid strands* makes possible to achieve a sequence-specific recognition of nucleic acids. In fact, since, statistically, the base sequence of a 13-mer oligonucleotide occurs just once in the human genome, extremely selective intervention can be attained with antisense oligonucleotides of this length.

* Base pairing in oligonucleotides usually occurs through Watson-Crick type hydrogen bonds; however, the introduction of severe alterations in oligonucleotide structure to improve cellular uptake (see farther on) often drives to the formation of alternative hydrogen-bonding interactions (called "reverse Watson-Crick", "Hoogsteen" or "reverse Hoogsteen", see below).



Remarkably, oligonucleotide degradation by ubiquitous nucleases, especially while using unmodified oligonucleotides, is a major concern for the antisense research. Thus, driven by the need to construct stable molecules against enzymatic cleavages, many backbone modifications have been introduced and investigated.[3] Importantly, while increasing enzymatic stability, the hybridization capacity of such modified oligonucleotides with natural nucleic acids should be retained.

In the last years, a vast number of chemically modified nucleotides has been studied in antisense experiments. In general, three types of modifications can be distinguished: a) analogues with unnatural bases;[4] b) analogues with modified sugars;[5] c) analogues with altered phosphate backbones[6] (**Figure 2**). The replacement of natural furanose backbone with different chemical moieties has been developed in innumerable examples of modifications: among these, modifications that imply for the introduction of pyranose moieties hold a significant position. In fact, a fairly good number of pyranose oligonucleotides exhibiting hybridization aptitude as well as a complete resistance to nucleases has been discovered.[7]

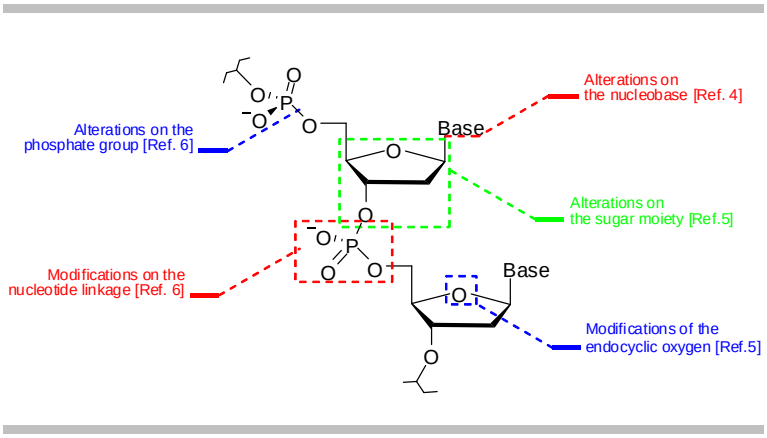


Figure 2 – *Chemical modifications in nucleotide backbone to improve cellular uptake and hybridization capacity.*

Generally speaking, such a kind of modifications in the sugar backbone should easily risk the hybridization potential, because changes for the sugar ring or steric constraints could hamper the normal Watson-Crick base pairing. Therefore, the study on pyranosyl oligonucleotides cannot leave out of consideration the changes of the backbone and the puckering in the carbohydrate moiety.

9.2 *CONFORMATIONAL DIVERSITY ARISING FROM THE RING SIZE IN THE SUGAR BACKBONE OF OLIGONUCLEOTIDES: FURANOSYL* VS *PYRANOSYL NUCLEIC ACIDS*

The replacement of a furanose ring with a pyranose one obviously involves differences in flexibility and structural diversity.[8] The conformational flexibility of the furanose ring is represented by its pseudorotation cycle (**Figure 3**). In oligonucleotides, the furanose ring fluctuates between the *C*-3'-*endo* and the *C*-2'-*endo* conformation, through the cross of an intermediate low energy barrier (ΔG = 20 kJ mol^{-1}). The preference for one conformation or for another one depends upon the nature of the *C*-2' group: if it deals with a withdrawing group, the *C*-3'-*endo* pucker is the only conformation observed (like in RNA); if it is absent, the *C*-2'-*endo* pucker is preferred to the *C*-3'-*endo* pucker (like in DNA).

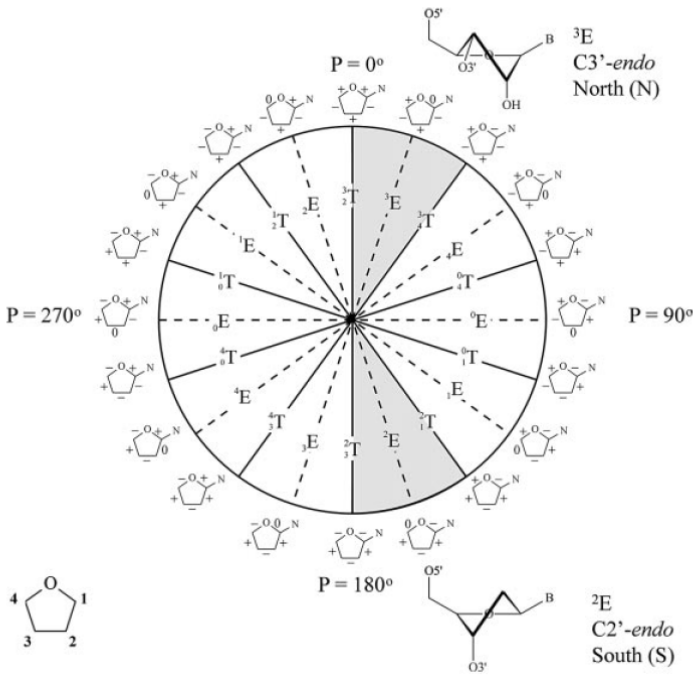


Figure 3 - *Pseudorotation cycle of furanose ring oligonucleotides.*

Conversely, in a saturated six-membered ring, the chair forms are the most stable conformations and a chair flip ($^4C_1 \leftrightarrow {}^1C_4$) requires considerable energy, since a high-energy barrier separates both low-energy states (**Figure 4**). This means that each single pyranose nucleotide monomer of an oligonucleotide sequence doesn't fluctuate between more conformations, but it is locked in the conformation it assumes for energetic requirements.

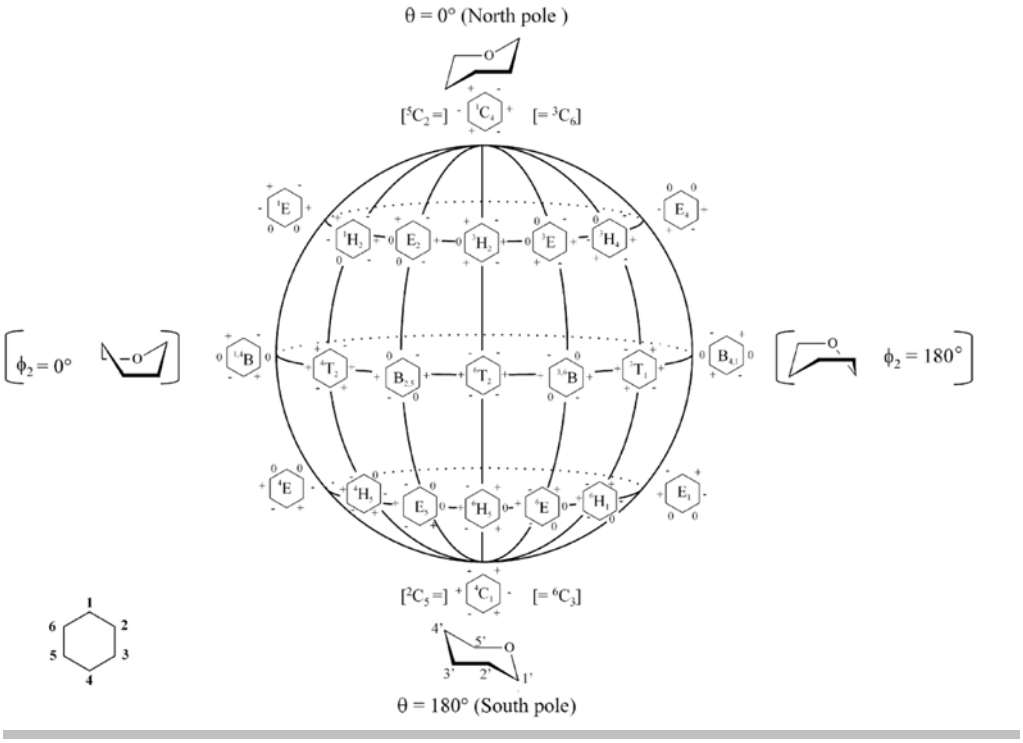


Figure 4 - *Pseudorotation cycle of pyranose ring oligonucleotides.*

On the other side, in comparison with a furanose moiety, pyranose rings have an extra carbon atom, which provides two more substitution sites. These additional substitution sites in a pyranosyl nucleoside lead to more isomers than a furanose one, because the possible locations of a nucleobase relative to the hydroxyl groups involved in phosphodiester bonding are increased. Overall, this results in a much larger structural diversity of pyranosyl versus furanosyl oligonucleotide sequences.

9.3 *PYRANOSE NUCLEIC ACIDS: α- AND β-HOMO-DNA*

9.3.1 *β-HOMO-DNA*

Early detailed investigation on pyranose nucleic acids was accomplished by Eschenmoser et al.[9] as an experimental entry towards the understanding of the evolutionary processes that led to the selection of RNA and DNA as today's genetic material.*

* Although these studies were completely driven by the question why nature selected DNA and RNA as the molecules of life, the results of these investigations have much influenced the design of new carbohydrate modified DNA analogues for antisense purposes (see farther on).

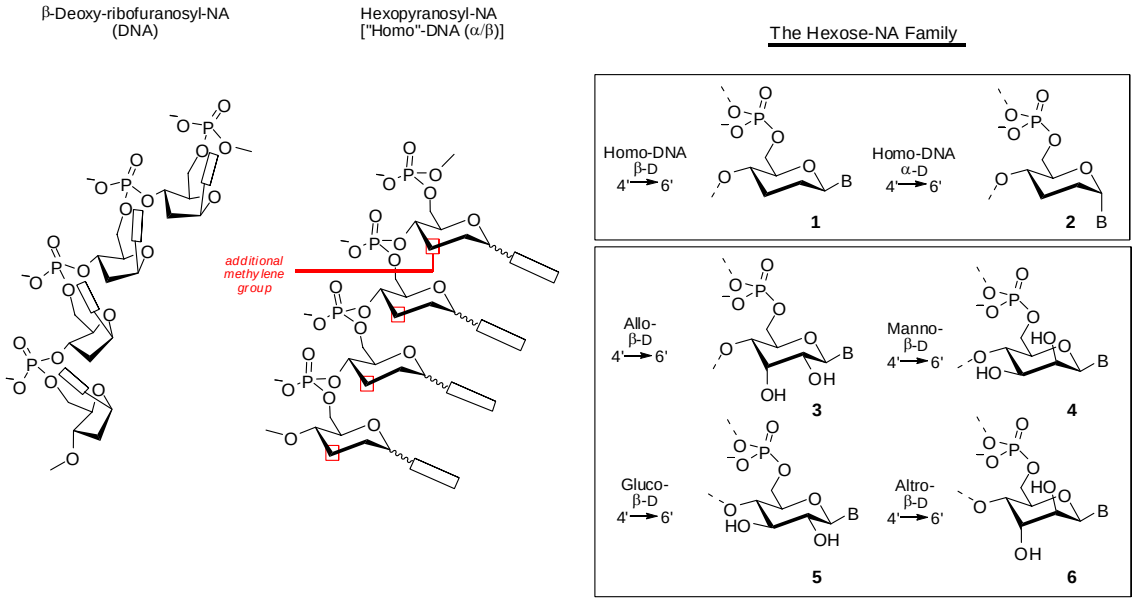


Figure 5 – *Hexopyranosyl-NA family.*

β-Homo-DNA (**1**, **Figure 5**) formally represents an analogue of natural DNA supplied with an additional methylene group in the carbohydrate backbone. It was the first hexose-nucleic acid system to be synthesized and its pairing behaviour the first to be analyzed.*,[10] In this construct, the nucleobase is positioned at the anomeric carbon atom of a pyranose, while the hydroxyl group and the hydroxymethylene group are situated at the *C*-4 and *C*-5 positions, respectively (**Figure 5**). The most stable structure of β-homo-DNA monomer is reached when all groups are equatorially oriented in a $^4C_1$ conformation. An accurate analysis of β-Homo-DNA oligomers revealed that:

- β-Homo-DNA had a much stronger Watson-Crick A-T and G-C base-pairing than natural DNA in self-pairing hybridization. The higher thermodynamic stability of Homo-DNA (compared to DNA duplexes) was not due to greater binding energy, but rather to a less negative entropy of duplex formation: it deals with the higher rigidity of the pyranose ring compared to the furanose one, resulting in a higher degree of pre-organization of the single strands towards duplex formation.[11]

* Together with Homo-DNA, other pyranose systems were analized: it deals with β-*allo*-, β-*manno*-, β-*gluco*-, and β-*altro*pyranosyl-NA (**3-6**, **Figure 5**). None of these ones exhibited efficient Watson-Crick and/or Hoogsteen self base pairing. This behaviour was interpreted as the consequence of intrastrand steric hindrance in the pairing conformation by the bulkier fully hydroxylated systems.

- In β-homo-DNA A and G pair strongly with themselves *via* Reverse Hoogsteen and Hoogsteen base pairing, respectively: therefore, the base pairing in homo-DNA is different from that operating in DNA (**Figure 6**).
- Analysis of a Watson-Crick β-homo-DNA duplex by NMR showed it to adopt an almost linear (non-helical) structure.

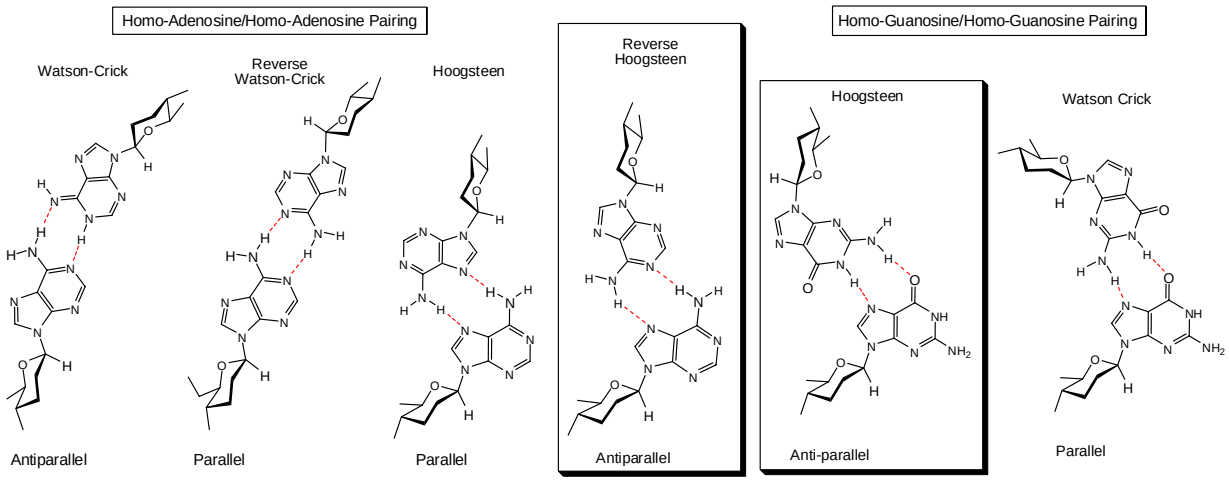


Figure 6 – *β-Homo-DNA: topologies of Homo-A/Homo-A and Homo-G/Homo-G pairing.*

- Complementary base sequences of β-homo-DNA and DNA do not pair: β-homo-DNA is an "autonomous" artificial pairing system.[12]
- Although β-homo-DNA was rewarding from a chemical point of view, it cannot be considered a potentially natural nucleic acid alternative.

9.3.2 *α-Homo-DNA*

The change of configuration at the *C*-1' position from β-homo-DNA to α-homo-DNA (**2**, **Figure 5**) causes modifications in the conformation of the pyranose moiety of the nucleotide monomer, therefore inducing a deep alteration in the conformation of the oligonucleotide analogue (and in the end leading to a different capacity to hybridize with natural nucleic acids). α-Homo-DNA is an axial (*C*(6)–*O*), axial (*C*(4)–*O*), equatorial (*C*(1)–*Base*) system in the thermodynamically

more stable $^1C_4$ conformation.* This chair inversion dramatically alters the torsion angle values in the minimized monomer backbone. In fact, such changes make possible to accommodate both RNA or α-homo-DNA strands to each other and allow cross-talk between these systems. As observed from analysis of α-homo-DNA oligomer:[13]

- α-Homo-DNA is a self-pairing system with properties of cross-pairing with its RNA complement (but less well with DNA complement), surprisingly by PARALLEL strand orientation. In the form of duplex, the base moieties in the α-homo-DNA strand are equatorially oriented (**Figure 7**).
- The geometry of the duplex is significantly different from those of existing double stranded nucleic acids with an average of 15 base pairs per turn (the value of base pairs per turn in natural nucleic acids is 8). The geometry of the duplex can be considered as intermediate between the natural A-type helix† (*ds*-RNA) and the almost linear β-homo-DNA structure.

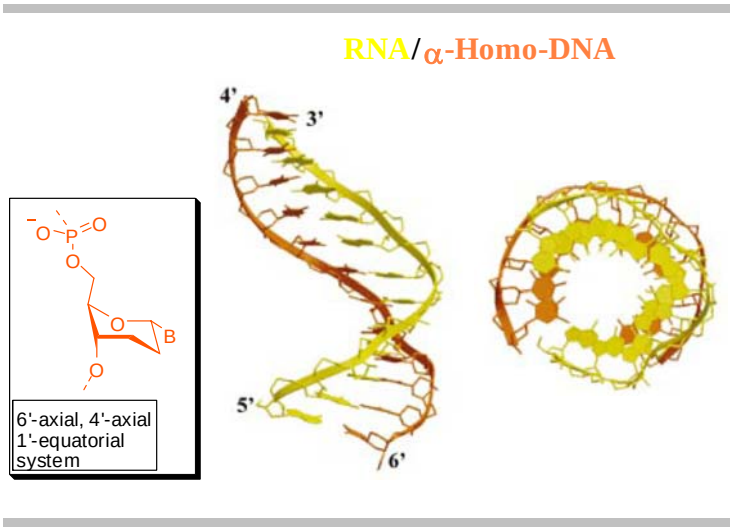


Figure 7 - *Side and top views of the RNA:α-homo-DNA duplex. The picture is reproduced from ref. 8.*

* As a proof of it, 1-(2,3-dideoxy-*erythro*-α-D-hexopyranosyl)thymine crystallizes in the $^1C_4$ conformation with an equatorially oriented heterocyclic base and axially oriented 4'-hydroxyl and 5'-hydroxymethyl moieties [Ref. 13a].

† Natural nucleic acids exist in several possible conformations, depending on the changing variables in the duplex structure (for example, they may depend by the nature of the sugar units: in fact, ribonucleic acids and deoxy-ribonucleic acids adopt different duplex conformations). The most frequent conformations so far identified in naturally occurring biological systems are: A-DNA, B-DNA and Z-DNA.

9.4 *THE HEXITOL NUCLEIC ACID FAMILY: HNA AND CONGENERS*

9.4.1 *HNA*

In an alternative strategy to allow cross-pairing with natural nucleic acids by means of a six-membered sugar moiety, it has been examined the possibility to insert the nucleobase at the *C*-2' axial position instead of the regular anomeric site.* This brings the extra methylene group of the six-membered ring in the minor groove site where it should not influence the conformational freedom of nucleobases in the neighboring residues.

HNA (that is the acronym for 1',5'-D-anhydrohexitol nucleic acids, **Figure 8**) represents one of the most prominent examples of conformationally restricted pyranose oligonucleotide analogues.[14] HNA has the sugar moiety in a $^4C_1$ conformation: it's an equatorial (*C*(6)–*O*), equatorial (*C*(4)–*O*), axial (*C*(1)–*Base*) system. As already observed for hexitol nucleosides,† such six-membered system mimics a furanose ring frozen in a 2'-*exo*, 3'-*endo* conformation (**Figure 9**). The conformational preference of hexitol nucleotides for the axial position of the nucleobase is a consequence of steric restraints caused by the unshared pairs of the hexitol oxygen. Due to this conformational strain, the bases stack efficiently, providing limited conformational freedom of the backbone.

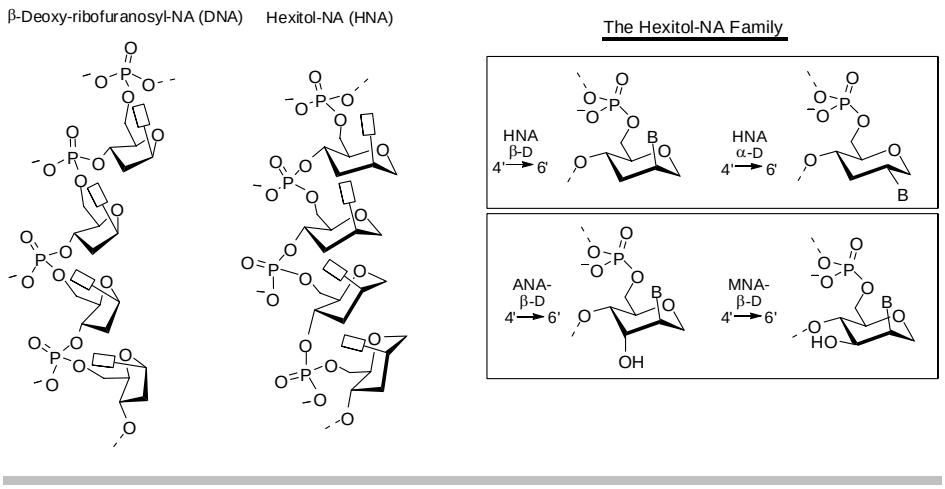


Figure 8 – *Anhydrohexitol-NA family.*

* The idea of inserting a nucleobase in the *C*-2' *equatorial* position has been tried as well with less promising results: actually, the axial base orientation seems necessary to achieve efficient base stacking in the double helix [Ref. 14b].
† See Chapter 7, Paragraph 7.3, "Sugar Modifications in Nucleoside Analogues: Six-Membered Nucleosides".

Hexitol nucleic acids represent an example of six-membered backbone modified oligonucleotides that can bind strongly and selectively to complementary RNA oligomers. The HNA:RNA complex forms an anti-parallel heteroduplex and adopts a helical conformation (**Figure 10**), the backbone torsion angles of the HNA strand of the duplex being very close to the A-type helix (the one observed in the *ds*-RNA). This means that torsion angles are about the same in the HNA and in the RNA strand.*

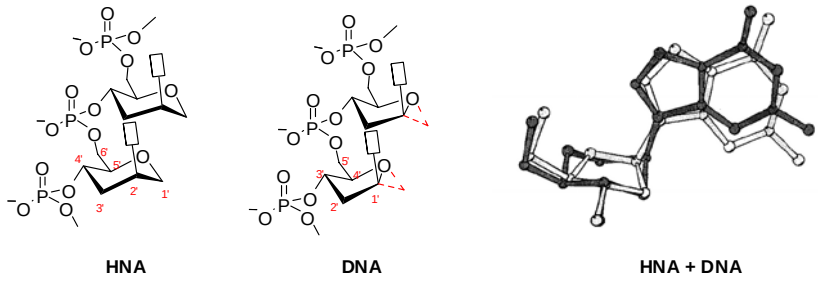


Figure 9 – *Superimposition HNA/DNA: anhydrohexitol unit is a good mimic of ribo(deoxy)furanosyl moiety*

The high stability of the HNA:RNA duplexes is assumed to be a consequence of the tendency of HNA to adopt A-form-like structures. This notion was not only based on CD measurements, but also supported by molecular dynamic simulations that predicted the formation of A-type helices for HNA:HNA as well as for HNA:RNA duplexes.[15]

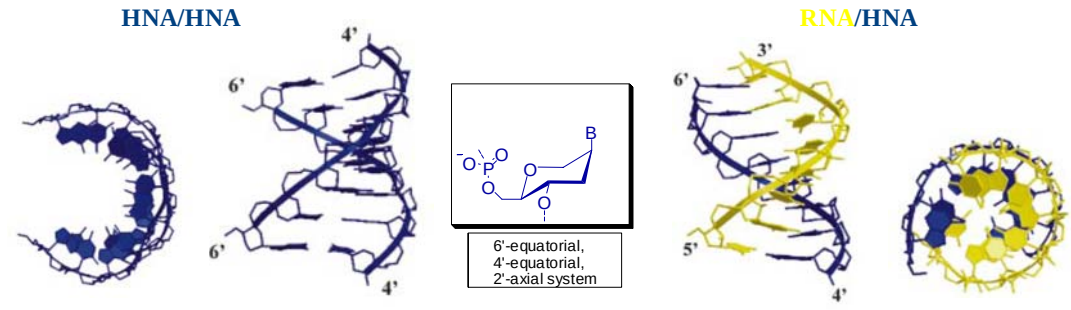


Figure 10 - *Comparison between dsHNA (left) and the helical structure of HNA:RNA duplex (right). The picture is reproduced from ref. 8.*

* Actually, a few torsion angles show somewhat larger differences, as obviously HNA is constrained by the conformation of a six-membered ring system instead of the usual five-membered furanose one.

Therefore, despite the fact that HNA has a rigid 1′,5′-anhydrohexitol sugar moiety whereas the RNA backbone is built from phosphorylated ribofuranose units, studies on HNA have demonstrated that the ring size is not determinative for the helicality of nucleic acids duplexes. Moreover, the A-form character of a nucleic acid with a six-membered ring, which is capable of binding to RNA, gives further support to the notion that somewhere during prebiotic molecular evolution nucleic acids with a six-membered ring could have existed, but somehow became extinct due to their rigidity, incompatible with processes where flexibility is needed.

9.4.2 *ANA (ALTRITOL NUCLEIC ACIDS) AND MNA (MANNITOL NUCLEIC ACIDS)*

The two hydroxylated versions of HNA, namely ANA (Altritol Nucleic Acids)[16] and MNA (Mannitol Nucleic Acids) (**Figure 8**) have also been prepared and their base-pairing properties with DNA and RNA studied. Complexes between ANA and RNA or DNA are reported to be even more stable than between HNA and natural oligonucleotides.[17] Interestingly, with the introduction of the axially oriented hydroxy group, affinity for DNA increases more than for RNA (in comparison with HNA). Conversely, the diastereoisomer MNA displays considerably less affinity to RNA as compared to ANA or HNA.[18] Molecular modelling suggested the reason for the reduced RNA affinity, lying in intrastrand inter-residue H-bond formation between the equatorial *C*-3 positioned hydroxyl function and the *O*-6 of the phosphodiester group of the following nucleotide unit, tying the MNA single strand into a non-pairing conformation.

9.5 *CONCLUDING REMARKS AND FUTURE PERSPECTIVES*

Expanding the sugar ring size of nucleic acids (but keeping the potential for communication *via* base pairing) has increased the structural diversity of nucleic acids beyond the classical A-, B- and Z-forms of DNA. With the appearance of “six-membered” nucleic acids, the double helix structure of Watson and Crick has lost its monopoly position; the classical forms of oligonucleotide duplexes have expanded into a whole family of new structures with the potential for selective cross-communication in a parallel or antiparallel orientation, opening up a new world for information storage and for molecular recognition-directed self-organization.

The concept behind six-membered oligonucleotides has relied on the fact that the high rigidity of the pyranose ring (and therefore the high degree of preorganization of pyranose

nucleotide strands towards duplex formation) has resulted in a much stronger base pairing towards natural complements, compared to what natural DNA sequences usually get. Driven by this finding, good candidates for antisense research have been expected to be found within this class of molecules, as already observed for HNA, ANA and α-Homo-DNA, where the suitable conformations adopted by the sugar moieties allowed getting hybridization with both RNA and DNA strands. Moreover, since an even wider structural and conformational diversity could be achieved, several hexose oligonucleotide analogues could be further designed and synthesized.

As a matter of fact, although six-membered oligonucleotides behave as a programmed system, it is still rather difficult to accurately predict the three-dimensional structure of a macromolecule based on the constitutional and conformational properties of its repeating monomers, even if some rules need to be respected (such as helicity or Watson-Crick base pairing). Hence, progresses in this field still need to be done, in order to attain a clear and comprehensive understanding of all the structural and functional features of such systems and, therefore, with the aim to create more sophisticated base-pairing systems and more promising candidates for the control of gene expression.

9.6 *REFERENCES*

[1] Zamecnik, P. C.; Stephenson, M. L. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 280. (b) Stephenson, M. L.; Zamecnik, P. C. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. *Proc. Natl. Acad. Sci.U.S.A.* **1978**, *75*, 285.
[2] For recent reviews concerning this argument, see: (a) Opalinska, J. B.; Gewirtz, A. M. Nucleic-acid therapeutics: basic principles and recent applications. *Nat. Rev.* **2002**, *1*, 503. (b) Aboul-Fadl, T. Antisense oligonucleotides: the state of the art. *Curr. Med. Chem.* **2005**, *12*, 2193.
[3] Leumann, C. J. DNA analogues: from supramolecular principles to biological properties. *Bioorg. Med. Chem.* **2002**, *10*, 841.
[4] Benner, S. A. *et al.* Redisigning Nucleic Acids. *Pure Appl. Chem.* **1998**, *70*, 263.
[5] Leumann, C. J. Tuning molecular recognition of RNA and DNA via sugar modification. *Chimia* **2005**, *11*, 776.
[6] (a) Uhlmann, E. Recent advances in the medicinal chemistry of antisense oligonucleotides. *Curr. Opin. Drug Disc. Devel.* **2000**, 3. (b) Hunziker, J.; Gèrald, M. DNA with artificial base pairs. *Chimia* **2005**, *11*, 780.
[7] Eschenmoser, A. Searching for nucleic acid alternatives. *Chimia* **2005**, *11*, 836.
[8] Lescrinier, E.; Froeyen, M.; Herdewijn, P. Difference in conformational diversity between nucleic acids with a six-membered `sugar' unit and natural `furanose' nucleic acids. *Nucleic Acids Res.* **2003**, *31*, 2975.
[9] Eschenmoser, A. Chemical etiology of nucleic acid structure. *Science* **1999**, *284*, 2118.
[10] Bohringer, M.; Roth, H. J.; Hunziker, J.; Gobel, M.; Krishnan, R.; Giger, A.; Schweizer, B.; Schreiber, J.; Leumann, C.; Eschenmoser, A. Warum Pentose- und nicht Hexose-Nucleinsauren? Teil II) Oligonucleotide aus 2',3'-dideoxy-β-D-glucopyranosyl-bausteinen (`homo-DNS'): herstellung. *Helv. Chim. Acta* **1992**, *75*, 1416.
[11] (a) Hunziker, J.; Roth, H.-J.; Böhringer, M.; Giger, A.; Diederichsen, U.; Göbel, M.; Krishnan, R.; Jaun, B.; Leumann, C.; Eschenmoser, A. Warum Pentose- und nicht Hexose-Nucleinsäuren?, Teil III, Oligo-(2',3'dideoxy-β-D-glucopyranosyl)-nucleotide (Homo-DNS): Paarungseigenschaften *Helv. Chim. Acta* **1993**, *76*, 259. (b) Groebke, K.; Hunziker, J.; Fraser, W.; Peng, L.; Diederichsen, U.; Zimmermann, K.; Holzner, A.; Leumann, C.; Eschenmoser, A. Why pentose- and not hexose-nucleic acids?, part V, purine-purine pairing in homo-DNA: guanine, isoguanine, 2,6-diaminopurine and xanthine. *Helv. Chim. Acta* **1998**, *81*, 375.
[12] Eschenmoser, A. Hexose nucleic acids. *Pure Appl. Chem.* **1993**, *65*, 1179.
[13] (a) De Winter, H.; Blaton, N. M.; Peeters, O. M.; De Ranter, C. J.; Van Aerschot, A.; Herdewijn, P. Structure of nucleoside analogue 1-(2,3-dideoxy-*erythro*-α-D-hexopyranosyl)thymine. *Acta Crystallogr. Sect. C* **1991**, *47*, 838. (b) Froeyen, M.; Lescrinier, E.; Kerremans, L.; Rosemeyer, H.; Seela, F.; Verbeure, B.; Lagoja, I.; Rozenski, J.; Van Aerschot, A.; Busson, R.; Herdewijn, P. α-Homo-DNA and RNA form a parallel oriented non-A, non-B-type double helical structure. *Chem. Eur. J.* **2001**, *7*, 5183.
[14] (a) Lescrinier, E.; Esnouf, R.; Schraml, J.; Busson, R.; Heus, H. A.; Hilbers, C. W.; Herdewijn, P. Solution structure of a HNA:RNA hybrid. *Chemistry & Biology* **2000**, *7*, 719. (b) Hybridization between "six-membered" nucleic acids: RNA as a universal information system. *Org. Lett.* **2001**, *3*, 4129.
[15] De Winter, H.; Lescrinier, E.; Van Aerschot, A.; Herdewijn, P. Minor groove solvatation contributes to the relative stabilityof HNA/RNA hybrids as compared to HNA/DNA complexes. *J. Am. Chem. Soc.* **1998**, *120*, 5381.
[16] (a) Allart, B.; Busson, R.; Rozenski, J.; Van Aerschot, A.; Herdewijn, P. Synthesis of protected D-altritol nucleosides as building blocks for oligonucleotide synthesis. *Tetrahedron* **1999**, *55*, 6527. (b) Abramov, M.; Schepers, G.; Van Aerschot, A.; Herdewijn, P. *Fmoc*-protected altritol phosphoramidite building blocks and their application in the synthesis of Altritol Nucleic Acids (ANAs). *Eur. J. Org. Chem.* **2007**, 1446.
[17] Allart, B.; Khan, K.; Rosemeyer, H.; Schepers, G.; Hendrix, C.; Rothenbacher, K.; Seela, F.; Van Aerschot, A.; Herdewijn, P. D-Altritol Nucleic Acids (ANA): hybridisation properties, stability, and initial structural analysis. *Chem. Eur. J.* **1999**, *5*, 2424.
[18] Froeyen, M.; Wroblowski, B.; Esnouf, R.; De Winter, H.; Allart, B.; Lescrinier, E.; Herdewijn, P. Molecular-dynamics studies of single stranded Hexitol, Altritol, Mannitol, and Ribose Nucleic Acids (HNA, MNA, ANA, and RNA) and of the stability of HNA:RNA, ANA:RNA and MNA:RNA duplexes. *Helv. Chim. Acta* **2000**, *83*, 2153.

Chapter 10

L-Hexoses as Tools for the Synthesis of L-Hexitol Nucleic Acids (L-HNA) and 2,3-Dideoxy-L-Hexopyranosyl Nucleic Acids (L-Homo-DNA)

10. L-HEXOSES AS TOOLS FOR THE SYNTHESIS OF L-HEXITOL NUCLEIC ACIDS (L-HNA) AND 2,3-DIDEOXY-L-HEXO-PYRANOSYL NUCLEIC ACIDS (L-HOMO-DNA)

*Enantiopure six-membered nucleosides 1,5-anhydro-2-(thymin-1-yl)-2,3-dideoxy-L-*arabino-*hexitol* (***1***)*, 1,5-anhydro-2-(adenin-9-yl)-2,3-dideoxy-L-*arabino-*hexitol* (***2***)*, 1-(thymin-1-yl)-2,3-dideoxy-β-L-*erythro-*hexopyranoside* (***3***) *and 1-(adenin-9-yl)-2,3-dideoxy-β-L-*erythro-*hexopyranoside* (***4***) *are herein employed for the preparation of L-shaped oligonucleotide analogues L-hexitol nucleic acids (L-HNA) and 2,3-dideoxy-L-hexopyranosyl nucleic acids (L-homo-DNA). Using the common phosphoramidite protocol, several oligonucleotide sequences have been synthesized, for each one of them having examined the capacity to hybridize with natural DNA strands as well as with unnatural ones, in the frame of a project focused towards the understanding of the conformational and chiral selection of oligonucleotides.*

10.1 *INTRODUCTION*

The high-order structure observed in natural nucleic acids is mainly determined by the chirality of the sugar-backbone units. At molecular level organisms utilize only D-nucleoside monomers, but not their enantiomers, probably for reasons closely related to the origin and the evolution of life. Nevertheless, molecular modelling studies have suggested that an L-sugar backbone could locate nucleobases appropriately for complementary base-pairing with natural nucleic acids by Watson-Crick or Hoogsteen base-pairing.[1] This has raised the possibility that L-sugar-containing nucleic acids might function as pseudo-DNA/pseudo-RNA, having the additional advantage to be more resistant against enzymatic degradation. To this aim, *enantio*-DNAs (i.e. pure L-sugar-containing nucleic acids) and *meso*-DNAs (i.e. DNAs having an alternating sequence of L-sugars and D-sugars) have been synthesized, showing high resistance to phosphodiesterases and displaying to recognize their complementary natural nucleic acids (with selectivity for RNA rather than DNA).[2] Such data have demonstrated that mirror-image oligonucleotides could be of significant value, mainly in a perspective use as antisense drug candidates.

Driven by the such findings, as well as by the successful application of the D-series Hexitol Nucleic Acids (D-HNA)[3] and 2,3-dideoxyhexopyranosyl Nucleic Acids (D-homo-DNA)[4] constructs in the formation of duplexes with natural nucleic acid complements, we have herein engaged a study on the characterization and analysis of the interaction with complementary nucleic acids of *enantio*- and *meso*-homo-DNA as well as *enantio*- and *meso*-HNA, by incorporation of thymine and adenine nucleosides **7**, **10** (**Scheme 1**) and **30**, **33** (**Scheme 3**) into suitable oligonucleotide sequences. For each kind of oligonucleotides, studies will be undertaken examining their aptitude to hybridize both with natural nucleic acids and with the unnatural ones.

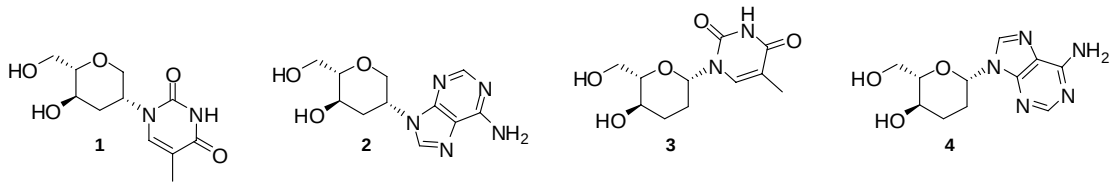


Figure 1 – *Adenine and thymine nucleoside analogues **1-4** as starting materials for oligonucleotide synthesis.*

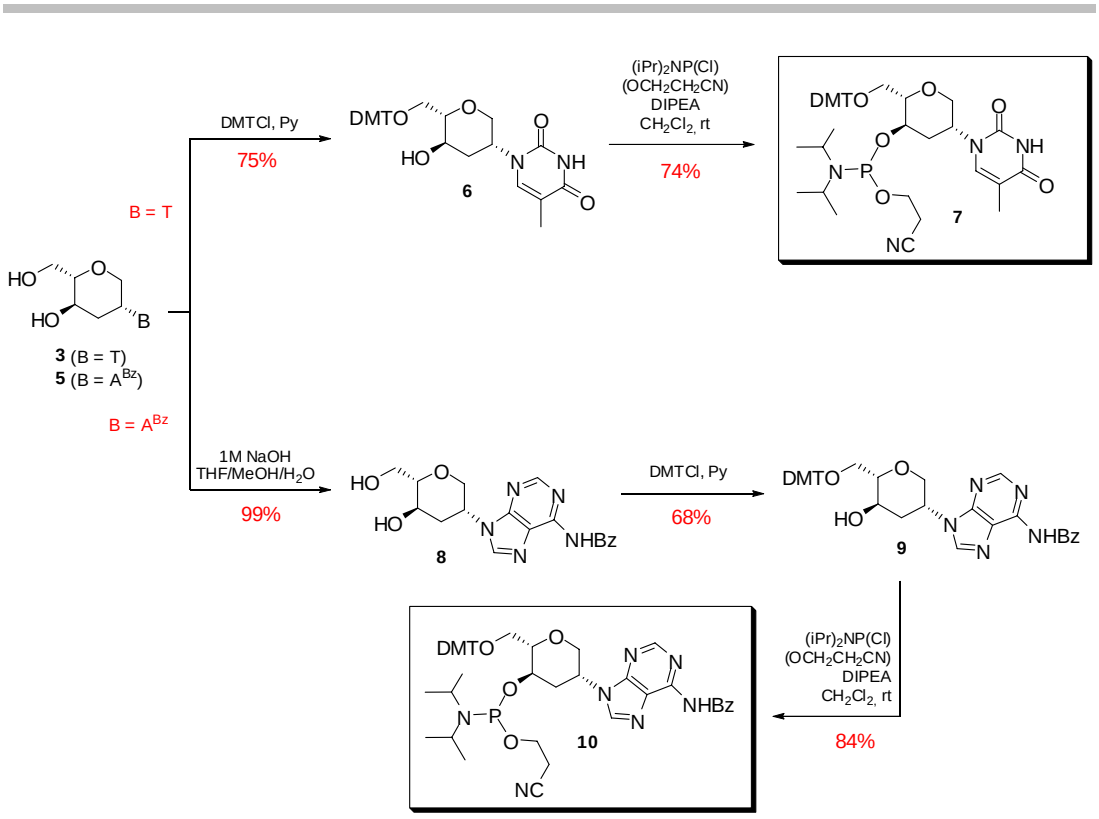
10.2 *SIX-MEMBERED OLIGONUCLEOTIDES, PART I: L-HEXITOL NUCLEIC ACIDS (L-HNA)*

10.2.1 *CHEMICAL SYNTHESIS OF PHOSPHORAMIDITE HEXITOL NUCLEOTIDE BUILDING BLOCKS*

The preparation of **T*** and **A*** nucleosides [**T*** being 1,5-anhydro-2,3-dideoxy-2-(thymin-1-yl)-L-*arabino*-hexitol nucleoside (**1**) and **A*** being 1,5-anhydro-2-(adenine-9-yl)-2,3-dideoxy-L-*arabino*-hexitol nucleoside (**2**, **Figure 1**)], which were used to synthesize the nucleotide building blocks **7**/**10** (**Scheme 1**) have been described previously.* Concerning thymine nucleoside **3** (B = T), the primary hydroxyl function was protected with a dimethoxytrityl group using dimethoxytrityl chloride in pyridine, affording the protected nucleoside **6**. Then, conversion into its phosphoramidite derivative **7** was carried out by means of freshly dried diisopropylethylamine and 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite. On the other hand, concerning adenine nucleoside **5** (B = A^{Bz}), suitable benzoyl group insertion on N^6-position of the base moiety was necessary (giving **8**, **Scheme 1**). Hence, dimethoxytrityl

* See Chapter 8, Paragraph 8.3.2 "Six-Membered Nucleoside Analogues, Part III: Non-Anomeric Hexopyranosyl Nucleoside Synthesis".

group protection and standard phosphoramidite installation afforded the suitable building block **10** for incorporation into several oligonucleotide strands.



Scheme 1 – *Preparation of adenine and thymine nucleotide building blocks **7** and **10** for oligonucleotide synthesis.*

10.2.2 *OLIGONUCLEOTIDE SYNTHESIS AND HYBRIDIZATION EXPERIMENTS*

The assembly in a DNA synthesizer of oligonucleotide **A***- and **T***-HNA strands was accomplished using the common phosphoramidite method[5] (see "Experimental Section" farther on). By means of this route, eleven oligonucleotide strands, which sequences are depicted in Table 1, were synthesized.

Table 1. - *ESI-MS monoisotopic data of oligonucleotides containing modified nucleosides.*

Entry	*DNA sequences*	*MS calcd.*	*MS found*
1	5'-(CAC CG**T*** TGC TAC C)-3’	3882.7	3882.7
2	5'-(CAC CG**A*** TGC TAC C)-3’	3891.7	3891.7
3	5’-(AGT ATT G**T***C CTA)-3’	3647.7	3647.7
4	5’-(AGT ATT G**A***C CTA)-3’	3656.7	3656.7
5	5’-(GCGC **T*T*T*** GCGC)-3’	3364.6	3364.6
6	5’-(GCGC **T*T*T*T*T*** GCGC)-3’	4000.7	4000.8
7	5’-(GCGC **A*A*A*** GCGC)-3’	3391.7	3391.8
8	5’-(GCGC **A*A*A*A*A*** GCGC)-3’	4045.8	4046.0
9	**A*T*A*A*A*T*T*T*A*T***-S	3301.7	3301.9
10	(**T*T*T*T*T*T*T*T*T*T*T*T*T*T*T***)-S	4210.9	4211.1
11	(**A*A*A*A*A*A*A*A*A*A*A*A*A*A*A***)-S	4328.0	4328.3

Such oligonucleotide sequences were used for different purposes. Sequences reported in entries 1-8 (**Table 1**) were studied considering their stability towards natural DNA-type strands; conversely, the ones reported in entries 9-11 were analyzed considering their aptitude to give hybridization towards unnatural oligonucleotide strands.

a) Hybridization with natural DNA sequences. To determine whether unnatural thymine and adenine nucleosides **1** and **2** may perturb the structure of a short DNA duplex, each unnatural nucleotide **7**/**10** was incorporated at the **X** position of the 5'-(CACCG**X**TGCTACC)-3' sequence. The potential of these nucleosides to hybridize with a natural base was studied in thermal denaturation experiments with the respective complementary strands. In major detail, the physicochemical base pairing properties of the analogues were examined by hybridizing the modified oligomers with natural DNA strands carrying one of the four natural bases in the complementary position to the modification. The strength of the hybridization was determined by temperature-dependent UV spectroscopy measurements.*

* For multiple copies of DNA molecules, the **melting temperature** (T_m) is defined as the temperature at which half of the DNA strands are in the double-helical state and half are in the "random-coil" states. In principle, an increase in the T_m values as a result of the incorporation of a modified nucleoside should not necessarily lead to a promising antisense construct. Other factors, such as RNase H activation, selectivity of hybridization, cellular uptake, nuclease stability and metabolic behavior are at least important as the hybridization strength. However, it seems obvious that

Table 2 displays T_m data obtained with sequence 5'-(CACCG**X**TGCTACC)-3' hybridized to oligomers, each one containing one of the four natural bases at the complement **Y** position. The T_m measurements were determined at 260 and 270 nm* in NaCl (0.1 M) buffer with KH_2PO_4 (20 mM, pH 7.5) and EDTA (0.1 mM), having a 4 μM concentration of each strand.

Compared to the DNA reference[6] (T_m = 44-50 °C), introduction of each nucleoside **1** and **2** led to moderately reduced thermal stability of the resulting duplex (**Table 2**). In fact, the introduction of L-hexitol thymine nucleoside **1** in the 5'-(CACCG**X**TGCTACC)-3' sequence led to T_m values in the range of 40-46 °C. Similarly, the introduction of the corresponding adenine nucleoside moiety (**2**) led to T_m values in the range of 41-45 °C. This proved that, with regard to this sequence, the incorporation of unnatural L-hexitol nucleosides doesn't perturb the structure of the whole sequence, resulting in a duplex which stability is reasonably close to that one of the natural DNA.

Table 2. *Stability studies of the oligonucleotide 5'-(CACCG**X**TGCTACC)-3' and its complement 3'-(GTGGC**Y**ACGATGG)-5' (4 μM concentration of each oligonucleotide; 0.1 M NaCl melting point buffer).*

Entry	*Oligonucleotide sequences*	*T_m Up*	*T_m Down*	*%*	*nm*
1	3'-(CCA TCG T**T*G** CCA C)-5'	40.0	39.8	17	**260**
	5'-(GGT AGC ATC GGT G)-3'	39.8	40.0	21	270
2	3'-(CCA TCG T**T*G** CCA C)-5'	46.0	46.2	19	**260**
	5'-(GGT AGC AAC GGT G)-3'	46.0	46.0	25	270
3	3'-(CCA TCG T**T*G** CCA C)-5'	37.6	38.2	16	**260**
	5'-(GGT AGC ACC GGT G)-3'	37.6	37.6	21	270
4	3'-(CCA TCG T**T*G** CCA C)-5'	43.8	43.4	18	**260**
	5'-(GGT AGC AGC GGT G)-3'	43.4	43.4	24	270
5	3'-(CCA TCG T**A*G** CCA C)-5'	45.2	45.6	21	**260**
	5'-(GGT AGC ATC GGT G)-3'	45.4	45.6	27	270
6	3'-(CCA TCG T**A*G** CCA C)-5'	43.0	43.0	16	**260**
	5'-(GGT AGC AAC GGT G)-3'	43.0	42.8	22	270
7	3'-(CCA TCG T**A*G** CCA C)-5'	41.2	41.0	18	**260**
	5'-(GGT AGC ACC GGT G)-3'	41.2	41.0	23	270
8	3'-(CCA TCG T**A*G** CCA C)-5'	43.2	43.0	18	**260**
	5'-(GGT AGC AGC GGT G)-3'	43.2	43.0	24	270

the higher is the stability of the duplex formed between natural nucleic acid and antisense oligonucleotide, the greater is the possibility of blockage of *m*RNA target and therefore its employ for inhibiting translational processes.

* 260 and 270 nm represent the λ values corresponding to the UV absorbances of purine and pyrimidine base moieties, respectively.

A further experiment of hybridization with natural DNA sequences was performed under the same experimental conditions previously described, but using the sequence 5'-(AGTATTG**X**CCTA)-3' hybridized with each of the four theoretical complements. Table 3 shows the T_m data obtained for the thermal denaturation experiments. All duplexes containing the **A*** and **T*** modifications in the sequence showed markedly lower T_m values compared to reference duplex (which melts at 43 °C), with reductions in rank until 20 degrees (**Table 3**). Therefore, in this latter sequence the structure perturbation of the natural DNA duplex appears to be more consistent and the unnatural synthesized duplexes result to be in a rather unstable organization.

Table 3. *Stability studies of the oligonucleotide 5'-(AGTATTG**X**CCTA)-3' and its complement 3'-(TCATAAC**Y**GGAT)-5' (4μM oligonucleotides concentration; 0.1 M NaCl melting point buffer).*

Entry	*Oligonucleotide sequences*	*T_m Up*	*T_m Down*	*%*	*nm*
1	3’-(ATC C**T***G TTA TGA)-5’	21.8	21.2	23	**260**
	5'-(TAG GTC AAT ACT)-3’	21.4	21.2	24	270
2	3’-(ATC C**T***G TTA TGA)-5’	20.6	20.0	19	**260**
	5'-(TAG GCC AAT ACT)-3’	20.5	20.2	21	270
3	3’-(ATC C**T***G TTA TGA)-5’	29.3	29.0	25	**260**
	5'-(TAG GAC AAT ACT)-3’	29.3	29.2	29	270
4	3’-(ATC C**T***G TTA TGA)-5’	30.1	30.1	22	**260**
	5'-(TAG GGC AAT ACT)-3’	29.7	29.9	27	270
5	3’-(ATC C**A***G TTA TGA)-5’	29.1	28.9	24	**260**
	5'-(TAG GTC AAT ACT)-3’	28.7	28.9	28	270
6	3’-(ATC C**A***G TTA TGA)-5’	25.0	24.7	22	**260**
	5'-(TAG GCC AAT ACT)-3’	24.8	24.8	25	270
7	3’-(ATC C**A***G TTA TGA)-5’	25.0	24.0	22	**260**
	5'-(TAG GAC AAT ACT)-3’	25.4	25.0	25	270
8	3’-(ATC C**A***G TTA TGA)-5’	25.8	26.6	22	**260**
	5'-(TAG GGC AAT ACT)-3’	26.1	26.4	23	270

a) Hybridization with unnatural oligonucleotide sequences. A not well-studied subject in the oligonucleotide research is the role that conformational diversity might play in the chiral selection of nucleic acid antipodes. As part of a wider study on the conformational and chiral selection of oligonucleotides,[7] it’s herein reported an investigation of the phenomenon

according to which oligomers of opposite chirality are recognized by different inclusion systems (based on conformational selection). Such a kind of chiral discrimination system can be best studied in the field of oligonucleotide research, because the physicochemical parameters involved in the inclusion-complex formation (base pairing as principle of molecular recognition) are well-understood. As regards to our molecules, fully modified L-hexitol oligonucleotide sequences were hybridized with several unnatural complementary strands, namely D- and L-cyclohexyl-NA[8] (**11-14**), D-hexopyranosyl-NA[9] (**15-18**), D-hexitol-NA[3] (**19-21**), D- and L-cyclohexenyl-NA[10,11] (**22-24**) and L-DNA[12] (**25**) (**Figure 2**), whose synthesis has been already elsewhere reported in major detail. In addition, hybridizations with D-DNA oligo-A (**26**) as well as other natural mixed sequences were attempted (see **Tables 4-5**).

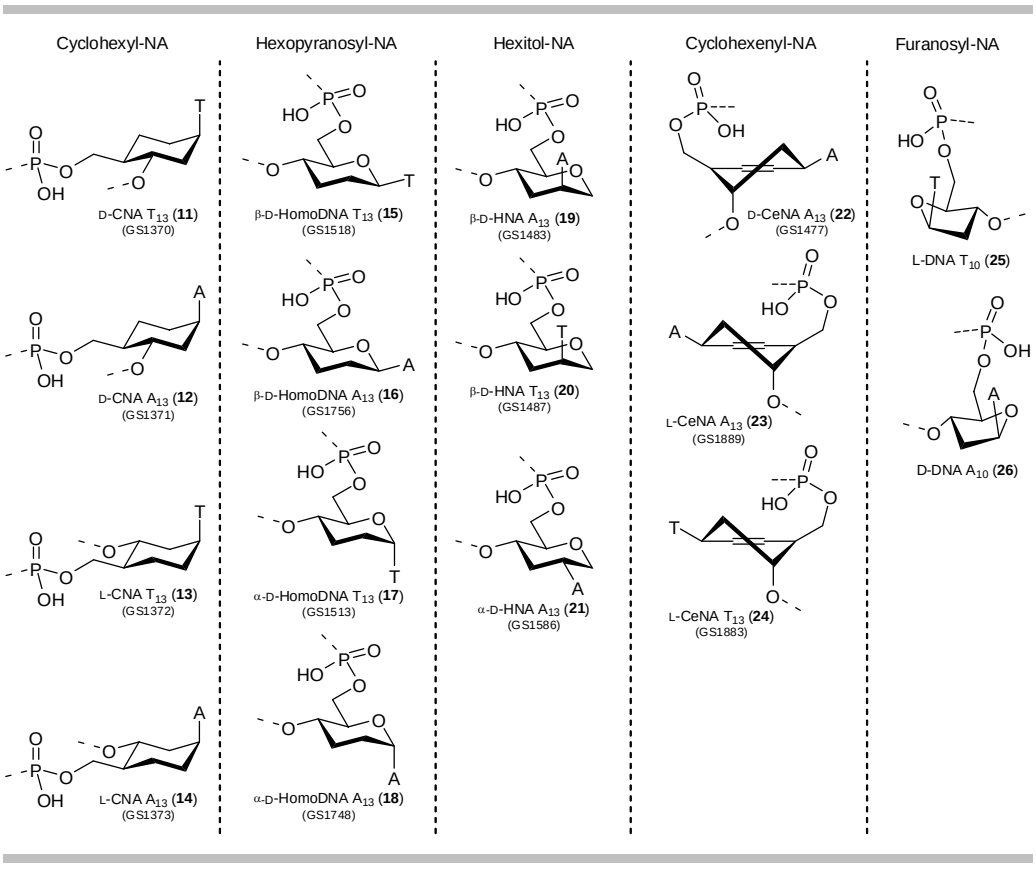


Figure 2 – *Selected unnatural oligomers for studying hybridization with L-HNA.*

Tables 4 and 5 display T_m data obtained with sequences $(\mathbf{X})_{13}$ (**X** = **A*** or **T***) hybridized to the complementary strands above mentioned. L-HNA strands showed hybridization capacity with the most oligomeric sequences (only exceptions are D-CNA and D-dA) with a preferable aptitude for L-oligomers: for example, while high T_m measurements were noticed in the L-HNA:L-HNA (77.3 °C) and L-HNA:L-CNA (72.2 °C) duplexes (**Table 4**, *entries 6 and 8*, respectively), weaker

hybridizations were observed for L-HNA:D-HNA (39.4 °C) duplex (**Table 4**, *entry 2)*; from weak to no stable duplexes were detected considering L-HNA:D-CNA hybridization (**Table 5**, *entry 4*

Table 4. *Stability studies of the oligonucleotide 6'-(**T***)$_{13}$-4' and its complements 4'-(Y)$_{13}$-6' (8 μM concentration of each oligonucleotide; 0.1 M NaCl melting point buffer).*

Entry	*Code*	*Oligonucleotide sequences*	T_m *Up*	T_m *Down*	*%*	*nm*
1	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5'	33.0	36.0	28	**260**
	L-HNA	5'-(**T*T*T*T*T*T*T*T*T*T*T***)-S-3'	32.4	33.8	43	270
2	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5	41.3	41.0	18	**260**
	D-HNA	6'-(**A*A*A*A*A*A*A*A*A*A*A***)-S-4'	37.4	37.6	42	270
3	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5	/	/	/	**260**
	D-DNA	5'-(AAAAAAAAAA)-3'	/	/	/	270
4	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5	/	/	/	**260**
	D-CNA	6'-(**A*A*A*A*A*A*A*A*A*A*A***)-S-4	/	/	/	270
5	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5	78.0	77.7	17	**260**
	β-Homo	6'-(**A*A*A*A*A*A*A*A*A*A*A***)-S-4'	77.4	77.4	71	270
6	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5	77.4	77.2	32	**260**
	L-HNA	5'-(**A*A*A*A*A*A*A*A*A*A*A***)-S-3'	77.5	77.6	40	270
7	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5	/	/	/	**260**
	D-DNA	5'-dTdTAAAAAAAAAAAAAAdTdT-3'	/	/	/	270
8	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5	72.4	72.0	34	**260**
	L-CNA	6'-(**A*A*A*A*A*A*A*A*A*A*A***)-S-4'	72.2	72.2	57	270
9	L-HNA	3'-S-(**T*T*T*T*T*T*T*T*T*T*T***)-5	60.5	51.5	30	**260**
	α-Homo	6'-(**A*A*A*A*A*A*A*A*A*A*A***)-S-4'	60.6	52.1	43	270

Table 5. *Stability studies of the oligonucleotide 5′-(**A***)$_{13}$-3′ and its complements 3′-(Y)$_{13}$-5′ (8 μM concentration of each oligonucleotide; 0.1 M NaCl melting point buffer).*

Entry	*Code*	*Oligonucleotide sequences*	*T_m Up*	*T_m Down*	*%*	*nm*
1	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	31.6	34.4	36	**260**
	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	29.7	30.9	71	270
2	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	30.0	37.1	19	**260**
	D-HNA	6'-(**T*T*T*T*T*T*T*T*T*T*T*T*T***)-S-4'	39.4	36.1	40	270
3	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	31.3	30.4	16	**260**
	LdT_{10}	5'-(TTTTTTTTTTTT)-3'	32.2	31.1	26	270
4	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	31.5	31.5	15	**260**
	D-CNA	6'-(**T*T*T*T*T*T*T*T*T*T*T*T*T***)-S-4	33.0	32.5	24	270
5	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	31.2	28.9	22	**260**
	LdT_{10}	5'-(TTTTTTTTTT)-3'	32.5	32.2	35	270
6	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	36.4	33.9	25	**260**
	β-Homo	6'-(**T*T*T*T*T*T*T*T*T*T*T*T*T***)-S-4'	37.5	35.9	17	270
7	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	27.8	28.6	17	**260**
	D-RNA	5'-dTdT-r(UUUUUUUUUUUU)dTdT-S-3'	27.3	29.7	26	270
8	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	60.8	60.7	29	**260**
	L-CNA	6'-(**T*T*T*T*T*T*T*T*T*T*T*T*T***)-S-4'	61.0	60.9	26	270
9	L-HNA	3'-S-(**A*A*A*A*A*A*A*A*A*A*A*A*A***)-5'	29.5	31.2	17	**260**
	α-Homo	6'-(**T*T*T*T*T*T*T*T*T*T*T*T*T***)-S-4'	32.0	31.5	27	270

and **Table 4**, *entry 4*, respectively). Interestingly, remarkable T_m values were noticed considering the L-HNA:α-D-Homo-DNA (56.2 °C) hybridization (**Table 4**, *entry 9)*; moreover, a very high T_m measure was detected in the L-HNA:β-D-Homo-DNA (77.5 °C) duplex (**Table 4**, *entry 5)*.

The selectivity of hybridization with unnatural nucleic acids could be explained in terms of sugar conformation assumed by the L-hexitol nucleoside monomers. In fact, the hexitol moiety of HNA may exist in two different conformations, **27**/**I** and **27**/**II** (**Figure 2**). As already demonstrated by previous experiments on the D-enantiomer of the HNA:DNA duplex,[3] the hexitol moieties of L-HNA adopt the conformation **27**/**I** when hybridized with L-DNA (T_m = 31.7 °C; **Table 5**, *entry 3*). Analogously, the same conformation could be assumed by L-HNA monomers when hybridized with L-CNA (since both oligomers adopt left-handed helices). In addition, L-CNA has a pretty close similarity to L-HNA structure, **Scheme 2**).

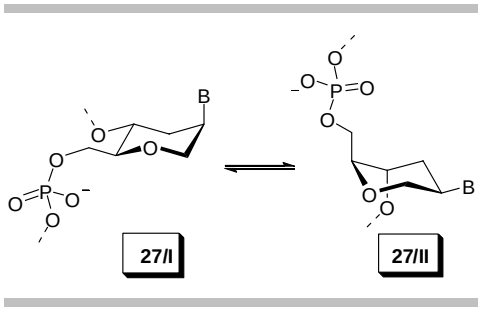
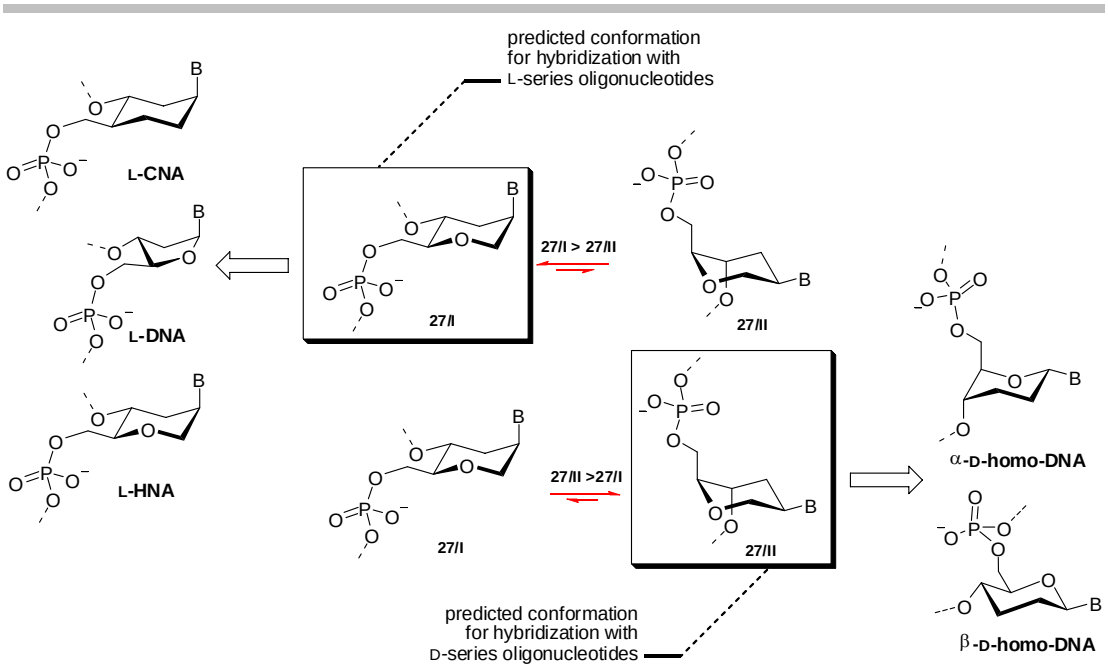


Figure 2 - *Two chair conformations of* L*-hexitol nucleoside monomers.*

Conversely, different considerations need to be argued for what concerns the L-HNA:β-D-Homo-DNA and L-HNA:α-D-Homo-DNA hybridizations, since the former (L-HNA) and the latter (α/β-D-Homo-DNA) strands are supposed to form helices of opposite handiness.[9] This discrepancy could be cleared up assuming that one of the two oligomers provide an inverted conformation of the corresponding monomers. In major detail, as the α/β-Homo-DNA backbones hold a fairly rigid structure, while hexitol nucleosides are reported to have a more flexible sugar skeleton,[13] it could be assumed* that L-HNA adopts the conformation **27**/**II** when hybridized with α- and β-D-Homo-DNA (**Scheme 2**).†

* Similarly to what has been already established for CNA:β-D-Homo-DNA duplex [Ref. 7].
† This finding obviously needs to be supported by further experiments, such as CD measurements, which could show if the helix is right- or left-handed, or by molecular modelling experiments.



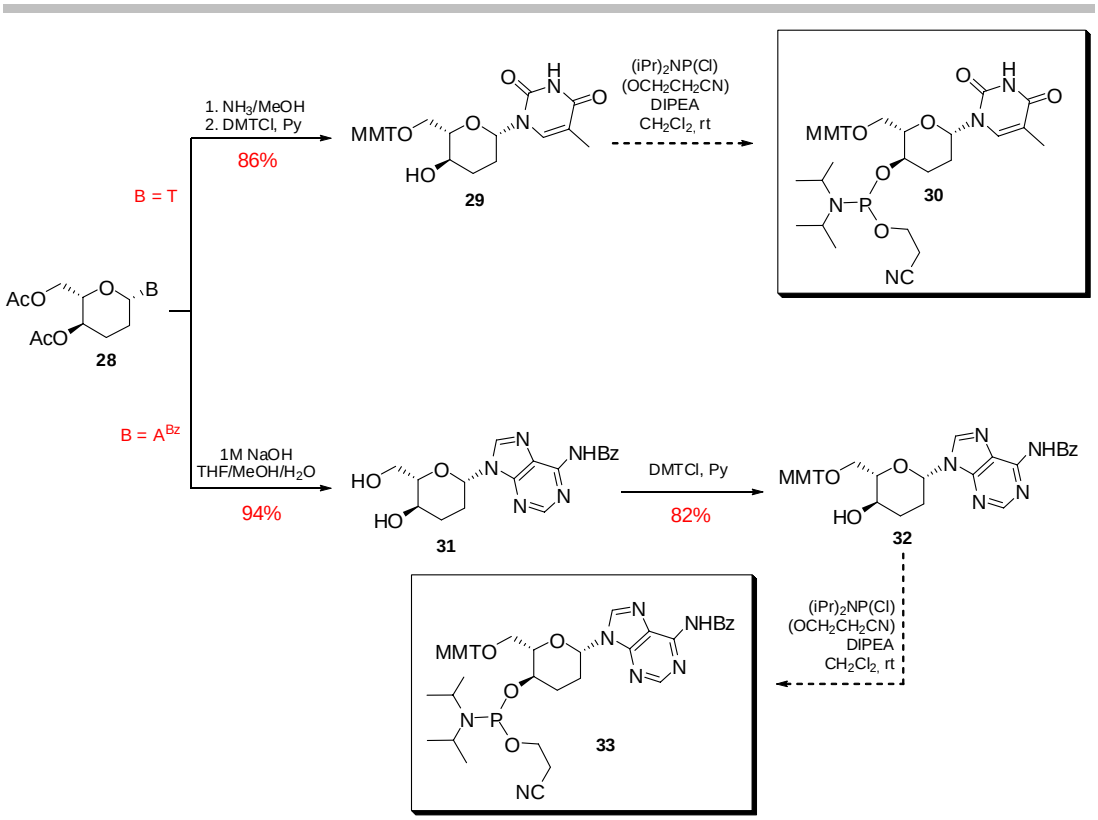
Scheme 2 – *Chiral recognition of oligonucleotide analogues based on conformational selection of L-HNA.*

10.3 *SIX-MEMBERED OLIGONUCLEOTIDES, PART II: 2,3-DIDEOXY-β-L-HEXOPYRANOSYL NUCLEIC ACIDS (L-HOMO-DNA)*

As already above mentioned regarding unnatural hexitol nucleosides **1** and **2**, the preparation of $\mathbf{T}^{\S}$ and $\mathbf{A}^{\S}$ nucleosides [$\mathbf{T}^{\S}$ being 1-(thymin-1-yl)-2,3-dideoxy-β-L-*erythro*-hexopyranoside (**3**) and $\mathbf{A}^{\S}$ being 1-(adenin-9-yl)-2,3-dideoxy-β-L-*erythro*-hexopyranoside nucleoside (**4**, **Figure 1**)], which were used to synthesize the nucleotide building blocks **30** and **33** (**Scheme 3**) have been described in a previous section.* Concerning thymine nucleoside **28** (B = T), the primary hydroxyl function of the nucleoside was protected with a 4-methoxytrityl group using 4-methoxytrityl chloride in pyridine, affording the nucleoside **29** in a good yield (86%). On the other hand, benzoylated adenine nucleoside **31** was obtained from partial deprotection of nucleoside **28** (B = A^{Bz}, see Chapter 8) by means of 1M NaOH in a THF/MeOH/H_2O emulsion (94% yield). Hence, protected adenine nucleoside analogue **32** was afforded by common monomethoxytrityl group insertion (**Scheme 3**). Both nucleosides **29** and **32** will be farther on protected at the *O*-4 function by common conversions into their phosphoramidite derivatives. Once obtained, 1-(6-*O*-monomethoxytrityl-4-*O*-[*N*,*N*-diisopropyl(2-cyanoethyl)

* See Chapter 8, Paragraph 8.3.1 "Six-Membered Nucleoside Analogues, Part III: Anomeric Hexopyranosyl Nucleoside Synthesis".

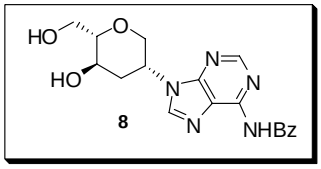
phosphoramidite]-2,3-dideoxy-β-L-*erythro*-hexopyranosyl)-thymine (**30**) and 1-(6-*O*-monomethoxytrityl-4-*O*-[*N*,*N*-diisopropyl(2-cyanoethyl) phosphoramidite]-2,3-dideoxy-β-L-*erythro*-hexopyranosyl)-N^6-benzoyladenine (**33**) will be ready to be used as nucleotide building blocks for incorporation into oligonucleotide sequences.



Scheme 3 – *Preparation of adenine and thymine nucleotide building blocks* ***30*** *and* ***33*** *for oligonucleotide synthesis.*

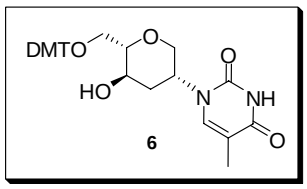
10.4 *EXPERIMENTAL SECTION*

10.4.1 *L-HEXITOL NUCLEIC ACIDS (L-HNA)*

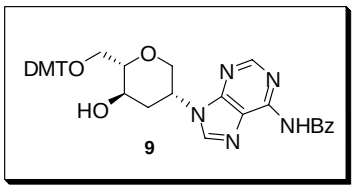


8

1,5-Anhydro-2-(N^6-benzoyladenin-9-yl)-2,3-dideoxy-L-*arabino-*hexitol (**8**). To a stirring solution of the nucleoside **5** (0.4 g, 1.51 mmol, see Chapter 8) in anhydrous pyridine (30 mL) and under nitrogen atmosphere, TMSCl (1.8 mL, 15.1 mmol) was added. After 30 min, BzCl (0.42 mL, 3.75 mmol) was added at 0 °C. The resulting reaction mixture was warmed to room temperature and further stirred for 16 h. Hence, H_2O (3 mL) was added at 0 °C and then NH_4OH (3 mL) after 10 min. The reaction mixture was stirred at the same temperature for 1 h, afterwards the solvent was removed under reduced pressure. Chromatography of the crude residue (DCM/MeOH = 9/1) afforded the pure benzoylated nucleoside **8** (0.56 g, 99% yield) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.96-2.15 (m, 1H), 2.49-2.62 (m, 1H), 3.59-3.73 (m, 1H), 3.80 (dd, J = 4.9 Hz, J = 12.0 Hz, 1H), 3.91 (dd, J = 2.2 Hz, J = 12.0 Hz, 1H), 4.08 (dd, J = 2.3 Hz, J = 12.9 Hz, 1H), 4.42 (d, J = 12.9 Hz, 1H), 5.05 (bs, 1H), 7.57 (t, J = 7.5 Hz, 1H), 7.66 (t, J = 7.4 Hz, 1H), 8.10 (d, J = 7.3 Hz, 1H), 8.72 (s, 1H), 8.78 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 35.8, 51.6, 61.0, 61.2, 68.3, 83.2, 123.0, 128.0, 128.4, 132.6, 133.6, 143.6, 149.6, 151.6, 152.3, 166.8. Exact mass calcd for $C_{18}H_{19}N_5O_4$ [M + H]$^+$: 370.1509, found 370.1511.



6

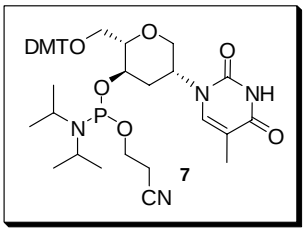


9

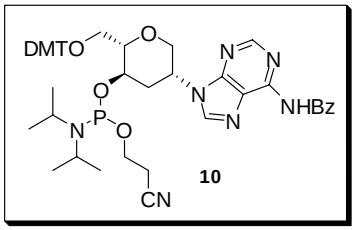
1,5-Anhydro-2,3-dideoxy-6-O-dimethoxytrityl-2-(thymin-1-yl)-L-*arabino-*hexitol (**6**) ***and 1,5-anhydro-2-(N^6-benzoyladenin-9-yl)-2,3-dideoxy-6-O-dimethoxytrityl-L-*arabino-*hexitol*** (**8**)**.** To a solution of the thymine nucleoside **3** (0.5 g, 1.95 mmol) in anhydrous pyridine (10 mL) and under nitrogen atmosphere, dimethoxytrityl chloride (DMTCl, 0.79 g, 2.34 mmol) was added at room temperature. After stirring at room temperature for 3 h, saturated $NaHCO_3$ solution (1 mL) was added, the reaction solvent was evaporated, diluted with CH_2Cl_2 (50 mL) and washed with brine (3 x 50 mL). The organic layer was dried (Na_2SO_4), evaporated and the crude residue was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) affording the dimethoxytritylated

nucleoside **6** (0.84 g, 75% yield) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60-2.46 (m, 2H), 1.93 (s, 3H), 3.15-3.59 (m, 2H), 3.77 (s, 6H), 3.65-4.15 (m, 4H), 4.55 (s, 1H), 4.89 (s, 1H), 6.81 (d, *J* = 8.7, 4H), 7.10-7.50 (m, 9H), 8.09 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.6, 35.5, 50.9, 54.7, 62.4, 63.1, 68.5, 81.0, 86.0, 110.2, 112.9, 126.9, 127.6, 127.9, 135.8, 138.5, 144.8, 151.2, 158.5, 163.8. Exact mass calcd for $C_{32}H_{34}N_2O_7$ $[M + H]^+$: 559.2444, found 559.2433.

Under the same conditions, the dimethoxytritylated nucleoside **9** (0.68 g, 68% yield) was synthesized as from adenine nucleoside **8**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.76-2.50 (m, 2H), 3.18-4.20 (m, 12H), 4.90-5.00 (m, 2H), 6.65-6.90 (m, 4H), 7.20-7.29 (m, 9H), 7.45-7.69 (m, 3H), 8.01-8.08 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 35.9, 50.9, 55.4, 60.6, 60.7, 67.9, 82.9, 87.0, 113.4, 122.9, 126.7, 127.9, 128.0, 129.1, 129.9, 132.6, 133.8, 136.0, 136.1, 143.6, 144.5, 149.6, 152.4, 152.7, 158.5, 165.7. Exact mass calcd for $C_{39}H_{37}N_5O_6$ $[M + H]^+$: 672.2822, found 672.2788.



7



10

1,5-Anhydro-2,3-dideoxy-6-O-dimethoxytrityl-4-O-[*N,N*-diisopropyl(2-cyanoethyl) phosphoramidite]-2-(thymin-1-yl)-L-*arabino*-hexitol** (**7**) ***and 1,5-anhydro-2-(*N^6*-benzoyladenin-9-yl)-2,3-dideoxy-6-O-dimethoxytrityl-4-O-[*N,N*-diisopropyl(2-cyanoethyl) phosphoramidite]-L-*arabino*-hexitol (**10**)**.** Phosphitylation was carried out on derivative **6** (0.62 g, 1.1 mmol) using freshly dried diisopropylethylamine (0.57 mL, 3.3 mmol) and 2-cyanoethyl-*N*,*N*-diisopropyl chlorophosphoramidite (0.37 mL, 1.6 mmol) in anhydrous CH_2Cl_2 (6 mL) at 0°C and under argon atmosphere. The reaction mixture was stirred at 0°C for 90 min afterwards the completeness of the reaction was indicated by TLC. Saturated $NaHCO_3$ solution (2 mL) was added, the solution was stirred for another 10 minutes and partitioned between CH_2Cl_2 (50 mL) and aqueous $NaHCO_3$ (30 mL). The organic layer was washed with brine (3 x 30 mL) and the aqueous phases were back extracted with CH_2Cl_2 (30 ml). After solvent evaporation, the resulting oil was purified by flash chromatography (hexane/acetone/TEA = 62/36/2). The yellow solid was then dissolved in CH_2Cl_2 (3 ml) and precipitated twice in cold hexane (160 mL, -60°C) to afford the desired compound **7** (0.62 g, 74% yield) as a white powder. The obtained product was dried *in vacuo* and stored overnight under nitrogen at -20°C.

1-(6-O-dimethoxytrityl-4-O-[N,N-diisopropyl(2-cyanoethyl)phosphoramidite]-2,3-dideoxy-β-L-glucopyranosyl)-thymine (**7**)*:* exact mass calcd for $C_{41}H_{51}N_4O_8P$ $[M + H]^+$: 759.3522, found 759.3510; ^{31}P NMR: δ = 148.41, 148.87.

Under the same conditions, phosphoramidite nucleoside **10** (0.70 g, 84% yield) was synthesized as from nucleoside **9** (0.64 g, 0.95 mmol).

9-(6-O-dimethoxytrityl-4-O-[N,N-diisopropyl(2-cyanoethyl)phosphoramidite]-2,3-dideoxy-β-L-glucopyranosyl)-N_6-benzoyladenine (**10**)*:* exact mass calcd for $C_{48}H_{54}N_7O_7P$: $[M + H]^+$: 872.3900, found 872.3887; ^{31}P NMR: δ = 148.40, 148.67.

Oligonucleotides synthesis and characterization. Oligonucleotide assembly was performed on an Expedite™ DNA synthesizer (Applied Biosystems) by using the phosphoramidite approach. The standard DNA assembly protocol was adjusted to double coupling and oxidation. The coupling time was prolonged to 3 min. The oligomers were deprotected and cleaved from the solid support by treatment with methylamine (40% in water) and concentrated aqueous ammonia (1:1, 30°C). After gel filtration on a NAP-10® column (Sephadex G25-DNA grade; Pharmacia) with water as eluent, the crude mixture was analyzed on a Mono-Q® HR 5/5 anion exchange column, after which purification was achieved on a Mono-Q® HR 10/10 column (Pharmacia) with the following gradient system (A=10 mM NaOH, pH 12.0, 0.1 M NaCl; B=10 mM NaOH, pH 12.0, 0.9 M NaCl).

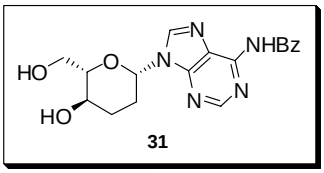
The low-pressure liquid chromatography system consisted of a Merck-Hitachi L 6200 A intelligent pump, a Mono Q® -HR 10/10 column (Pharmacia), a Uvicord SII 2138 UV detector (Pharmacia-LKB) and a recorder. The product-containing fraction was desalted on a NAP-10® column and lyophilized. Oligonucleotides were purified by RP HPLC on a *C*-18 column prior to mass spectrometric analysis. A linear gradient of A: ammonium bicarbonate (25 mM in H_2O, pH 7.0) and B: acetonitrile (80% in H_2O) was applied.

Mass spectrometric analysis of oligonucleotides. The purity of oligonucleotides was checked by HPLC/MS on a capillary chromatograph (CapLC, Waters, Milford, MA). Columns of 150 mm x 0.3 mm length (LC Packings, San Francisco, CA) were used. Oligonucleotides were eluted with a triethylammonium/1,1,1,3,3,3-hexafluoro-2-propanol/ acetonitrile solvent system. Flow rate was 5 μL/min. Electrospray spectra were acquired on an orthogonal acceleration/time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) in negative ion mode. Scan time used was 2 sec. The combined spectra from a chromatographic peak were deconvoluted using the

MaxEnt algorithm of the software (Masslynx 3.4, Micromass, Manchester, UK). Theoretical oligonucleotide masses were calculated by using the monoisotopic element masses.

Melting temperatures. Oligomers were dissolved in NaCl (0.1 M, unless otherwise stated), potassium phosphate (0.02M, pH 7.5), EDTA (0.1 mM). The concentration was determined by measuring the absorbance in MilliQ water at 260 nm at 80°C (assuming that the hexitol nucleoside analogues had the same extinction coefficients per base moiety in the denatured state as the natural nucleosides). The concentration for each strand was 4 μM in all experiments unless otherwise stated. Melting curves were determined with a Varian Cary 300 BIO spectrophotometer. Cuvettes were maintained at constant temperature by water circulation through the cuvette holder. The temperature of the solution was measured with a thermistor that was directly immersed in the cuvette. Temperature control and data acquisition were carried out automatically with an IBM-compatible computer by using Cary WinUV thermal application software. A quick heating and cooling cycle was carried out to allow proper annealing of both strands. The samples were then heated from 10°C to 80°C at a rate of 0.2°Cmin^{-}1, and were cooled again at the same speed. Melting temperatures were determined by plotting the first derivative of the absorbance as a function of temperature; data plotted were the average of two runs. Up and down curves in general showed identical T_m values.

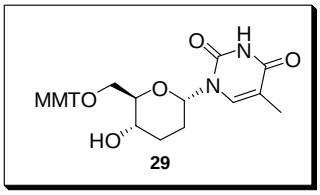
10.4.2 *2,3-DIDEOXY-β-L-HEXOPYRANOSYL NUCLEIC ACIDS (L-HOMO-DNA)*



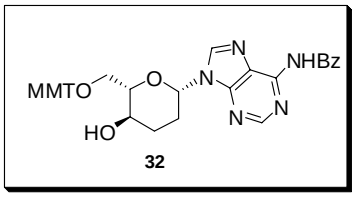
31

1-(N_6-benzoyladenin-9-yl)-2,3-dideoxy-β-L*-erythro-*hexopyranoside (**31**). A solution of the nucleoside **28** (B = A^{Bz}, 0.45 g, 0.78 mmol, see Chapter 8) in a 5/4/1 THF/MeOH/H_2O mixture (45 mL) was stirred for 10 min at 0 °C. Hence, solid NH_4Cl (0.80 g) was slowly added, the resulting mixture was further stirred for 30 min at the same temperature; then the solvent was evaporated under reduced pressure. Chromatography of the crude residue (DCM/MeOH = 8:2) afforded the pure **31** (0.62 g, 94% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.78-1.95 (m, 1H), 2.12-2.43 (m, 2H), 2.44-2.53 (m, 1H), 3.93-4.02 (m, 1H), 4.24 (dd, J = 2.2 Hz, J = 6.2 Hz, 1H), 4.31 (dd, J = 5.4 Hz, J = 12.3 Hz, 1H), 4.91 (dt, J = 3.8 Hz, J = 10.5 Hz, 1H),

5.98 (dd, *J* = 2.6 Hz, *J* = 10.8 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 2H), 7.64 (t, *J* = 7.0 Hz, 2H), 8.05 (d, *J* = 7.8 Hz, 1H), 8.25 (s, 1H), 8.83 (s, 1H), 9.03 (bs, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 29.3, 31.2, 60.8, 64.5, 80.7, 83.5, 125.7, 128.5, 132.8, 133.0, 142.9, 150.0, 151.9, 151.9, 165.8. Anal. calcd for $C_{18}H_{19}N_5O_4$: C 58.53, H 5.18, N 18.96. Found: C 58.49, H 5.19, N 18.99.



29



32

1-(6′-O-Monomethoxytrityl-2′,3′-dideoxy-β-L-erythro-hexopyranosyl)-thymine (**29**) ***and 1-(6′-O-monomethoxytrityl-2′,3′-dideoxy-β-L-erythro-hexopyranosyl)-N_6-benzoyladenine*** (**32**)**.** To a stirring solution of the thymine nucleoside **28** (0.5 g, 1.95 mmol) in anhydrous pyridine (25 mL) and under nitrogen atmosphere, monomethoxytrityl chloride (MMTCl, 0.72 g, 2.34 mmol) was added at room temperature. The resulting reaction mixture was stirred overnight at the same temperature. Then, saturated aqueous $NaHCO_3$ solution (1 mL) was added, the reaction solvent was evaporated, diluted with CH_2Cl_2 (50 mL) and washed with brine (3 x 50 mL). The organic layer was dried (Na_2SO_4), evaporated and the crude residue was purified by column chromatography (CH_2Cl_2/MeOH 98:2) affording the monomethoxytritylated nucleoside **29** (1.03 g, 86% yield) as a white foam. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.52-1.64 (m, 1H), 1.80-1.91 (m, 5H), 2.13-2.22 (m, 1H), 2.86-2.93 (bs, 1H), 3.25 (dd, *J* = 6.0 Hz, *J* = 9.5 Hz, 1H), 3.44 (dd, *J* = 5.8 Hz, *J* = 9.5 Hz, 1H), 3.51-3.63 (m, 2H), 3.75 (s, 3H), 5.61 (d, *J* = 10.3 Hz, 1H), 6.80 (d, *J* = 8.9 Hz, 2H), 7.03-7.19 (m, 12H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 12.4, 29.4, 30.6, 55.2, 65.3, 68.4, 79.5, 81.4, 87.3, 110.9, 113.2, 125.2, 127.1, 127.8-128.9, 130.2, 134.7, 135.1, 143.6, 143.8, 149.6, 158.7, 163.0. Exact mass calcd for $C_{31}H_{32}N_2O_6Na$ $[M + Na]^+$: 551.2158, found 551.2150.

Under the same conditions, monomethoxytritylated nucleoside **32** (0.82 g, 82% yield) was synthesized as from adenine nucleoside **31**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.71-1.82 (m, 1H), 2.00-2.16 (m, 1H), 2.21-2.34 (m, 2H), 2.96 (d, *J* = 2.6 Hz, 1H), 3.31 (dd, *J* = 6.2 Hz, *J* = 9.5 Hz, 1H), 3.47 (dd, *J* = 4.8 Hz, *J* = 9.6 Hz, 1H), 3.62-3.71 (m, 1H), 3.72-3.81 (m, 1H), 5.82 (dd, *J* = 1.8 Hz, *J* = 10.6 Hz, 1H), 6.79 (d, *J* = 8.9Hz, 1H), 7.04-7.20 (m, 12H), 7.57 (t, *J* = 7.8 Hz, 2H), 7.67 (t, *J* = 7.0 Hz, 2H), 8.05 (d, *J* = 7.8 Hz, 1H), 8.26 (s, 1H), 8.85 (s, 1H), 9.05 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.6, 30.8, 55.2, 65.2, 68.4, 79.8, 81.6, 87.4, 113.4, 125.3, 127.2-128.2, 129.2, 130.3, 132.8, 134.8, 140.5, 143.5, 143.7, 143.8, 149.5, 151.3, 152.8, 158.9, 164.5. Exact mass calcd $C_{38}H_{36}N_5O_5$ $[M + H]^+$: 642.2716, found 642.2702.

10.5 *REFERENCES*

[1] (a) Fujimori, S.; Shudo, K.; Hashimoto, Y. Enantio-DNA recognizes complementary RNA but not complementary DNA. *J. Am. Chem. Soc.* **1990**, *112*, 7436**.** (b) Fujimori, S.; Shudo, K.; Hashimoto, Y. Enantio-oligodeoxyribonucleotides. *Nucleic Acids Symp. Ser.* **1990**, *22*, 97. (c) Fujimori, S.; Iwanami, N.; Shudo, K.; Hashimoto, Y. Enantio- and meso-oligodeoxyribonucleotides. *Nucleic Acids Symp. Ser.* **1991**, *25*, 143. (d) Ashley, G. W. Modeling, synthesis, and hybridization properties of (L)-ribonucleic acid. *J. Am. Chem. Soc.* **1992**, *114*, 9731.

[2] Hashimoto, Y.; Iwanami, N.; Fujimori, S.; Shudo, K. *Enantio*- and *Meso*-DNAs: preparation, characterization, and interaction with complementary nucleic acids. *J. Am. Chem. Soc.* **1993**, *115*, 9883.

[3] Hendrix, C.; Rosemeyer, H.; Verheggen, I.; Seela, F.; Van Aerschot, A.; Herdewijn, P. 1′,5′-Anhhydrohexitol oligonucleotides: synthesis, base pairing and recognition by regular oligodeoxyribonucleotides and oligoribonucleotides. *Chem. Eur. J.* **1997**, *3*, 110.

[4] (a) Bohringer, M.; Roth, H. J.; Hunziker, J.; Gobel, M.; Krishnan, R.; Giger, A.; Schweizer, B.; Schreiber, J.; Leumann, C.; Eschenmoser, A. Warum Pentose- und nicht Hexose-Nucleinsauren? Teil II) Oligonucleotide aus 2',3'-Dideoxy-β-D-glucopyranosyl-Bausteinen (`Homo-DNS'): Herstellung. *Helv. Chim. Acta* **1992**, *75*, 1416. (b) Froeyen, M.; Lescrinier, E.; Kerremans, L.; Rosemeyer, H.; Seela, F.; Verbeure, B.; Lagoja, I.; Rozenski, J.; Van Aerschot, A.; Busson, R.; Herdewijn, P. α-Homo-DNA and RNA form a parallel oriented non-A, non-B-type double helical structure. *Chem. Eur. J.* **2001**, *7*, 5183.

[5] Gait, M. *Oligonucleotides: a practical approach*; IRL: Oxford, **1984**.

[6] Zhou D.; Lagoia, I. M.; Rozenski, J.; Busson, R.; Van Aerschot, A.; Herdewijn, P. Synthesis and properties of aminopropyl nucleic acids. *ChemBioChem* **2005**, 6, 2298.

[7] Froeyen, M.; Morvan, F.; Vasseur, J.-J.; Nielsen, P., Van Aerschot, A.; Rosemeyer, H.; Herdewijn, P. Conformational and chiral selection of oligonucleotides. *Chem. Biodiv.* **2007**, *4*, 803.

[8] Maurinsh, Y.; Rosemeyer, H.; Esnouf, R.; Medvedovici, A.; Wang, J.; Ceulemans, G.; Lescrinier, E.; Hendrix, C.; Busson, R.; Sandra, P.; Seela, F.; Van Aerschot, A.; Herdewijn, P. Synthesis and pairing properties of oligonucleotides containing 3-hydroxy-4-hydroxymethyl-1-cyclohexanyl nucleosides. *Chem. Eur. J.* **1999**, *5*, 2139.

[9] Groebke, K.; Hunziker, J.; Fraser, W.; Peng, L.; Diederichsen, U.; Zimmermann, K.; Holzner, A.; Leumann, C.; Eschenmoser, A. Why pentose- and not hexose-nucleic acids?, part V, purine-purine pairing in homo-DNA: guanine, isoguanine, 2,6-diaminopurine and xanthine. *Helv. Chim. Acta* **1998**, *81*, 375.

[10] Nauwelaerts, K.; Lescrinier, E.; Sclep, G.; Herdewijn, P. Cyclohexenyl nucleic acids: conformationally flexible oligonucleotides. *Nucleic Acid Res.* **2005**, *33*, 2452.

[11] Wang, J.; Verbeure, B.; Luyten, I.; Lescrinier, E.; Froeyen, M.; Hendrix, C.; Rosemeyer, H.; Seela, F.; Van Aerschot, A.; Herdewijn, P. Cyclohexene nucleic acids (CeNA): serum stable oligonucleotides that activate RNase H and increase duplex stability with complementary RNA. *J. Am. Chem. Soc.* **2000,** *122*, 8595.

[12] *Indenix* is kindly acknowledged for the generous gift of L-DNA T_{10}.

[13] Ostrowski, T.; Wroblowski, B.; Busson, R.; Rozenski, J.; De Clercq, E.; Bennett, M. S.; Champness, J. N.; Summers, W. C.; Sanderson, M. R.; Herdewijn, P. 5-Substituted pyrimidines with a 1,5-anhydro-2,3-dideoxy-D-*arabino*-hexitol moiety at *N*-1: synthesis, antiviral activity, conformational analysis and interaction with viral thymidine kinase. *J. Med. Chem.* **1998**, *41*, 4343.

Chapter 11

Development of a New and Enviromentally Friendly Synthetic Protocol for Acetonation of Carbohydrate Derivatives

11. DEVELOPMENT OF A NEW AND ENVIROMENTALLY FRIENDLY SYNTHETIC PROTOCOL FOR ACETONATION OF CARBOHYDRATE DERIVATIVES

As chemists need to synthesize more and more complex structures, the development of innovative and mild methodologies for the formation and cleavage of protected compounds is required. Contextually, the need to drive reactions under more environmentally friendly conditions is quickly growing, in carbohydrate synthesis as well as in all fields of organic and inorganic chemistry. In this perspective, a new methodology for the O*-isopropylidene group insertion in sugar derivatives has been herein developed by using the dehydrating agent triphenylphosphine polymer-bound/I_2 complex. This method is characterized by smooth, non-equilibrating reaction conditions and a very clean, simple work-up, making it particularly suitable for* O*-isopropylidenation reactions of sugars under smooth conditions and with low environmental impact.*

11.1 *INTRODUCTION*

In spite of the major improvements in selectivity and invention of new reactions, our dependence upon the use of protecting groups has not yet ceased, especially with reference to preparative carbohydrate chemistry, in which monosaccharide assembly for creating complex oligosaccharide structures still requires suitable hydroxyl group protections.[1] With special regard to this purpose, numerous innovative methodologies in carbohydrate synthesis have been developed during the years,[2] with the aim to find procedures as mild as possible for overcoming compatibility problems with contiguous chemical systems.

In this perspective, the search for new synthetic protocols for the isopropylidenation reaction of sugars hold a significant place, since this latter represents one of the most widely used and reliable groups to protect hydroxyl functions in carbohydrate chemistry.[3]

The conventional method for the preparation of such sugar derivatives consists in the condensation of a vicinal diol with acetone, in the presence of an acidic catalyst, under anhydrous conditions. Several different agents have been used as catalysts, including mineral acids,[4] anhydrous zinc chloride together with phosphoric acid,[5] ion exchange resins,[6]

anhydrous copper(II) sulphate,[7] iodine,[8] anhydrous ferric chloride,[9] boron trifluoride etherate,[10] anhydrous aluminium chloride[11] and, more recently, zeolites.[12]

Under the equilibrating conditions, water is formed which must be removed from the reaction mixture using either physical or chemical methods.[13] Generally, the major products obtained from these reactions are those which are the thermodynamically favoured. Otherwise, kinetic control can be obtained using other reagents for condensation, namely 2-methoxypropene in presence of *p*-toluenesulfonic acid.[14]

11.2 *RESULTS AND DISCUSSION*

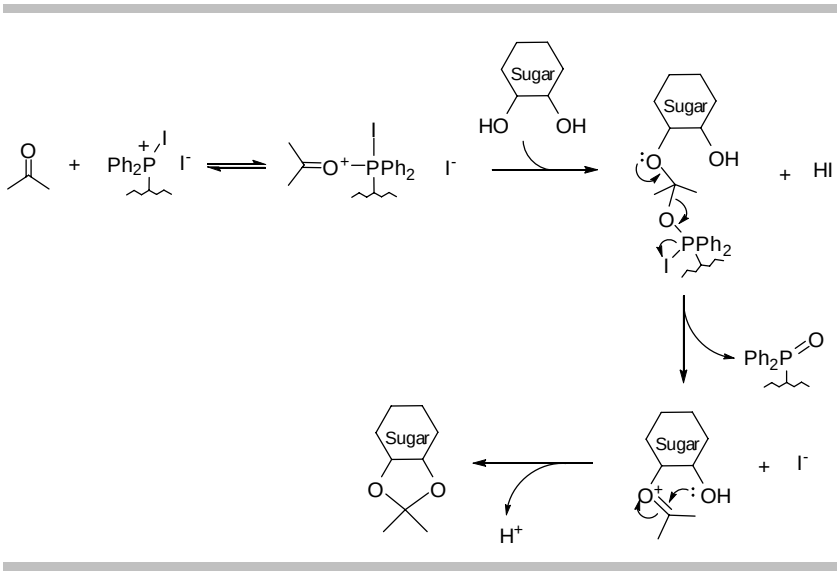
As part of ongoing efforts working toward the achievement of new synthetic methodologies in organic synthesis which provide the employ of environmentally friendly chemical agents, it is herein reported a new protocol based upon the use of triphenylphosphine polymer-bound/iodine complex for the thermodynamical acetonation of carbohydrate derivatives in high yields, in anhydrous medium, avoiding water formation.[15]

Reagents bound on polymeric supports offer a number of advantages over traditional solution-phase chemistry, hence supporting the recent interest in the development of polymer-bound catalysts and reagents for organic synthesis, mainly with reference to environmentally benign synthetic protocols.[16] In major detail, the triphenylphosphine polymer-bound/halogen complex acts as a Lewis acid and a dehydrating agent: it has already been widely used in several reactions[17] with low environmental impact. In fact, it avoids contamination from byproducts and use of solvents, which are not environmentally friendly, in the purification processes. Triphenylphosphine polymer-bound/iodine complex is an easy to prepare,[18] handy, semicrystalline solid, reasonably stable at room temperature. When dried and kept properly, it can be stored for weeks at room temperature, under nitrogen atmosphere. The somewhat high cost of the starting triphenylphosphine polymer-bound does not actually represent a limitation of this procedure, if one considers that the polymer-linked phosphine oxide generally obtained from the reaction can be readily reduced to the original phosphine form with trichlorosilane.[19]

Under our conditions, the sugar is added to a suspension of triphenylphosphine polymer-bound/iodine complex (polystyryl diphenyl iodophosphonium iodide) in anhydrous acetone.* An adduct is first formed, due to the presence of the positively charged phosphorous atom in the

* Triphenylphosphine-iodine complex showed the same reactivity of the polymer bound/iodine complex towards all the tested sugars.

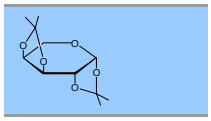
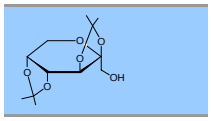
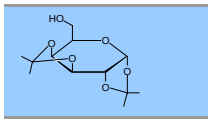
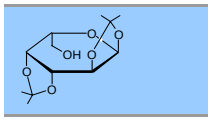
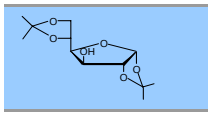
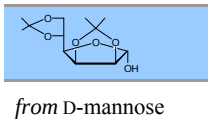
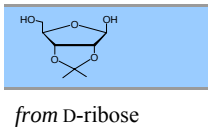
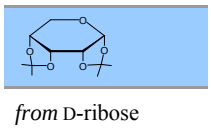
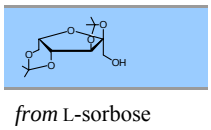
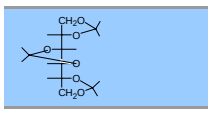
complex and the electron-rich carbonyl oxygen of acetone, which expose the carbonyl carbon atom to undergo nucleophilic attack by a first hydroxyl group of the sugar molecule (**Scheme 1**). The subsequent nonequilibrium step of the reaction is represented by the loss of polymer-linked phosphine oxide and thereby the formation of an oxygen-stabilized carbocation, known to be intermediate in the acetalation reaction.



Scheme 1 – *General scheme for the O-isopropylidenation reaction with diol sugars.*

This latter can undergo the intramolecular attack by a second hydroxyl group present in the sugar molecule to afford the final *O*-isopropylidene derivative. The reaction is carried out under mild conditions at room temperature and is generally fast and high-yielding affording the thermodynamically more stable isopropylidene derivatives. Results obtained from the acetonation of miscellaneous sugars, such as L-arabinose, D-fructose, D- and L-galactose, D-glucose, D-mannose, D-ribose, L-sorbose, D-glucitol, using a triphenyl phosphine polymerbound/iodine complex/sugar ratio of 2:1 are shown below (**Table 1**). Each acetonation has been compared with the best one reported in literature.[2,3,6,7,20]

Table 1 *O-Isopropylidene derivatives of miscellaneous sugars (literature data within parentheses)*

Isopropylidene derivatives	Yield %	M.p./°C	$[\alpha]_D^{25}$	^{1}H NMR ($CDCl_3$) Elemental Analysis
 *from* L-arabinose	95 (85)[6]	40-41 (40-41)[18]	+6.1 (*c* 1.5, H_2O) +5.8 (*c unknown*, H_2O)[7]	1.32 (s, 3 H), 1.41 (s, 3 H), 1.47 (s, 3 H), 1.52 (s, 3 H), 3.64 (d, *J* = 12.5 Hz, 1 H), 3.82 (d, *J* = 12.5 Hz, 1 H), 4.23 (br d, *J* = 7.5 Hz, 1 H), 4.30 (dd, *J* = 5.3 Hz, *J* = 2.3 Hz, 1 H), 4.54 (dd, *J* = 7.5 Hz, *J* = 2.3 Hz, 1 H), 5.48 (d, *J* = 5.3 Hz, 1 H) Anal. Calcd for $C_{11}H_{18}O_5$: C 57.38, H 7.88. Found: C 57.47, H 7.90.
 *from* D-fructose	90 (55)[19]	95-96 (97)[19]	-32.9 (*c* 1.5, H_2O) -24.7 (*c* 1.1, $CHCl_3$)[19]	1.28 (s, 3 H), 1.32 (s, 3 H), 1.35 (s, 3 H), 1.38 (s, 3 H), 2.28 (br s, 1 H), 3.62 (d, *J* = 10.7 Hz, 1 H), 3.66 (d, *J* = 10.7 Hz, 1 H), 3.79 (dd, *J* = 13.0 Hz, *J* = 0.8 Hz, 1 H), 3.88 (dd, *J* = 13.0 Hz, *J* = 2.0 Hz, 1 H), 4.21 (ddd, *J* = 7.7 Hz, *J* = 2.0 Hz, *J* = 0.8 Hz, 1 H), 4.32 (dd, *J* = 2.9 Hz, 1 H), 4.57 (dd, *J* = 7.7 Hz, *J* = 2.9 Hz, 1 H). Anal. Calcd for $C_{12}H_{20}O_6$: C 55.37, H 7.74. Found: C 55.45, H 7.69.
 *from* D-galactose	95 (76-92)[3]	oil (oil)[3]	-59.5 (*c* 1.5, $CHCl_3$) -55.0 (*c* 3.6, $CHCl_3$)[3]	1.32 (s, 6 H), 1.44 (s, 3 H), 1.55 (s, 3 H), 3.62-3.81 (m, 1 H), 3.84-3.95 (m, 2 H), 4.28 (dd, *J* = 8.0 Hz, *J* = 1.5 Hz 1 H), 4.34 (dd, *J* = 5.0 Hz, *J* = 2.0 Hz, 1 H), 4.62 (dd, *J* = 8.1 Hz, *J* = 2.0 Hz, 1 H), 5.57 (d, *J* = 5.0 Hz, 1 H). Anal. Calcd for $C_{12}H_{20}O_6$: C 55.37, H 7.74. Found: C 55.29, H 7.71.
 *from* L-galactose	97 (58)[20]	syrup (syrup)[20]	+57.0 (*c* 0.9, $CHCl_3$) *(not reported)*	Superimposable to that of the D-enantiomer Anal. Calcd for $C_{12}H_{20}O_6$: C 55.37, H, 7.74. Found: C 55.48, H 7.76.
 *from* D-glucose	95 (91)[3]	108-109 (110-111)[3]	-18.5 (*c* 5.0, H_2O) -18.5 (*c* 5.0, H_2O)[3]	1.30 (s, 3 H), 1.35 (s, 3 H), 1.43 (s, 3 H), 1.48 (s, 3 H), 3.97 (dd, *J* = 8.7 Hz, *J* = 5.1 Hz, 1 H), 4.04 (dd, *J* = 8.0 Hz, *J* = 2.9 Hz, 1 H), 4.15 (dd, *J* = 8.7 Hz, *J* = 6.6 Hz, 1 H), 4.25-4.38 (m, 2 H), 4.51 (d, *J* = 3.7 Hz, 1 H), 5.92 (d, *J* = 3.7 Hz, 1 H). Anal. Calcd for $C_{12}H_{20}O_6$: C 55.37, H 7.74. Found: C 55.43, H 7.72.
 *from* D-mannose	95 (92)[3]	121-122 (122-123)[3]	+16.6 (*c* 2.5, EtOH) +16.0 (*c* 2.6, EtOH)[3]	1.32 (s, 3 H), 1.38 (s, 3 H), 1.46 (s, 3 H), 1.48 (s, 3 H), 4.03 (dd, *J* = 10.2 Hz, *J* = 6.1 Hz, 1 H), 4.08 (dd, *J* = 10.2 Hz, *J* = 6.8 Hz, 1 H), 4.20 (dd, *J* = 7.2 Hz, *J* = 3.7 Hz, 1 H), 4.38-4.44 (m, 1 H), 4.63 (d, *J* = 6.2 Hz, 1 H), 4.81 (dd, *J* = 7.2 Hz, *J* = 6.2 Hz, 1 H), 5.39 (s, 1 H). Anal. Calcd for $C_{12}H_{20}O_6$: C 55.37, H 7.74. Found: C 55.30, H 7.73.
 *from* D-ribose	95 (90-93)[21a,b]	oil (oil)[21c]	-24.7 (*c* 1.1, $CHCl_3$) -25.9 (*c* 1.1, $CHCl_3$)[21c]	1.32 (s, 3 H), 1.48 (s, 3 H), 3.55-3.83 (m, 2 H), 4.40 (br s, 1 H), 4.57 (d, *J* = 6.0 Hz, 1 H), 4.85 (d, *J* = 6.0 Hz, 1 H), 5.42 (s, 1 H). Anal. Calcd for $C_8H_{14}O_5$: C 50.52, H 7.42. Found: C 50.41, H 7.39.
 *from* D-ribose	92 *(as byproduct)*[2b]	70-71 (68-69)[2b]	-55.0 (*c* 1.1, $CHCl_3$) -51.0 (*c* 0.6, $CHCl_3$)[2b]	1.33 (s, 3 H), 1.37 (s, 3 H), 1.55 (s, 3 H), 1.62 (s, 3 H), 3.83 (dd, *J* = 12.1 Hz, *J* = 9.7 Hz, 1 H), 4.01 (dd, *J* = 12.1 Hz, *J* = 7.7 Hz, 1 H), 4.24 (dd, *J* = 7.9 Hz, *J* = 5.2 Hz, 1 H), 4.40-4.53 (m, 2 H), 5.43 (d, *J* = 5.2 Hz, 1 H). Anal. Calcd for $C_{11}H_{18}O_5$: C 57.38, H 7.88. Found: C 57.50, H 7.88.
 *from* L-sorbose	95 (96-99)[22]	80-81 (77-78)[22]	-14.3 (*c* 1.5, Me_2CO) -18.1 (*c* 1.5, Me_2CO)[22]	1.37 (s, 6 H), 1.44 (s, 3 H), 1.51 (s, 3 H), 2.31 (t, *J* = 7.5 Hz, 1 H), 3.78 (dd, *J* = 12.5 Hz, *J* = 7.5 Hz, 1 H), 3.87 (dd, *J* = 12.5 Hz, *J* = 7.5 Hz, 1 H), 3.92-4.92 (m, 3 H),4.32 (d, *J* = 1.6 Hz, 1 H), 4.48 (*J* = 1.6 Hz, 1 H). Anal. Calcd for $C_{12}H_{20}O_6$: C 55.37, H 7.74. Found: C 55.29, H 7.76.
 *from* D-glucitol	97 (53)[23]	45-46 (45-46)[23]	+12.7 (*c* 1.1; EtOH) +14.2 (*c unknown*; EtOH)[23]	1.37 (s, 3 H), 1.38 (s, 3 H), 1.42 (s, 12 H), 3.69-4.12 (m, 8 H). Anal. Calcd for $C_{15}H_{26}O_6$: C 59.58, H 8.67. Found: C 59.48, H 8.69.

1,2:3,4-Di-*O*-isopropylidene-D-ribopyranose is a known compound[2b] that usually accompanies 2,3-*O*-isopropylidene-D-ribofuranose when the latter is prepared from D-ribose. However, to the best of our knowlewdge no intentional preparations, and consequently yields, are reported for it in literature. On the other hand, using only one equivalent of triphenyl phosphine polymer-bound/iodine complex under our conditions D-ribose afforded the pure mono-*O*-isopropylidene derivative, as well as the sole di-*O*-isopropylidene derivative when treated with two equivalents.

All the acetonides reported in Table 1 were obtained within 30 min by simple filtration of polymer bound phosphine oxide, the only byproduct of the reaction, with high purity. All the physical data reported in Table 1 within parentheses were withdrawn from the literature. A comparison of the data shows that the yields of termodinamically more stable isopropylidene derivatives are often higher than those of other methods.

11.3 *EXPERIMENTAL SECTION*

Typical acetonation procedure (preparation of 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose). To a magnetically stirred suspension of dry polystyryl diphenyl phosphine (1.12 g, ≈ 3.34 phosphine units) in anhydrous acetone (10 mL) at r.t., a solution of I_2 (0.85 g, 3.34 mmol) in the same solvent (30 mL) was added dropwise in the dark and under dry nitrogen atmosphere. After 15 min, solid D-glucopyranose (0.33 g, 1.67 mmol) was added in one portion to the suspension. TLC monitoring ($CHCl_3/CH_3OH$, 9:1) showed that the starting sugar was completely consumed within 30 min. The reaction mixture was then filtered through a glass sinter funnel and washed with acetone. The solvent was removed under reduced pressure and the solid residue recrystallized from $CHCl_3$:hexane (1:2) to give the final product (0.41 g, 95% yield).

Under the same conditions, the *O*-isopropylidene derivatives shown in Table 1 were prepared using one equivalent of triphenyl phosphine polymer-bound/iodine complex per each acetonide group expected in the product.

11.4 *REFERENCES*

[1] Jarowicki, K.; Kocienski, P. Protecting groups. *J. Chem. Soc*., *Perkin Trans. 1* **1999**, 1589.
[2] *Preparative Carbohydrate Chemistry*; S. Hanessian Ed., Marcel Dekker Inc.: New York, **1997**.
[3] *The Organic Chemistry of Sugars;* Levy, D. E., Fügedi, P. Eds, Taylor & Francis Group, LLC: Boca Raton, **2006**.
[4] (a) Stevens, J.D. Two septanose diacetals of D-glucose. *Chem. Commun.* **1969**, 1140. (b) Hughes, N.A.; Speakman, P.R.H. New isopropylidene derivatives of D-ribose: 1,2:3,4-di-*O*-isopropylidene-D-ribopyranose and 1,2-*O*-isopropylidene-D-ribopyranose. *Carbohydr. Res.* **1965**, *1*, 171.
[5] Schmidt, O. T. Isopropylidene derivatives. *Methods Carbohydr. Chem.* **1963**, *2*, 318.
[6] (a) Nair, P.R.M.; Shah, P.M.; Sreenivasan, B. A Study on the condensation of sorbose with acetone: catalysis by macroporous ion exchange resins. *Starch* **1981**, *33*, 384.
[7] (a) Gorin, P. A. J. Acetonation of aldose diethyl dithioacetals. *Can. J. Chem.* **1965**, *43*, 2078. (b) Morgenlie, S. Reaction of some aldoses with anhydrous cupric sulphate-acetone. Preparation of 3,4-*O*-isopropylidene derivatives. *Carbohydr. Res.* **1975**, *41*, 77.
[8] Kartha, K.P.R. Iodine, a novel catalyst in carbohydrate reactions I. *O*-Isopropylidenation of carbohydrates. *Tetrahedron Lett.* **1986**, *27*, 3415.
[9] Singh, P.P.; Gharia, M.M.; Dasgupta, F.; Srivastava, H.C. Use of ferric chloride in carbohydrate chemistry. I. A quick method for the preparation of *O*-isopropylidene derivatives of carbohydrates. *Tetrahedron Lett.* **1977**, 439.
[10] Pfaff, P.K.; Paust, J.; Hartmann, H. (BASF A.-G.) Ger. Offen: DE 3, 505, 150 (CI. C07H9/04), 21 Aug. 1986, Appl. 15 Feb. 1985 (CA 106:18984r).
[11] Lal, B.; Gidwani, R.M.; Rupp, R.H. Aluminum chloride as a powerful catalyst for the preparation of *O*-isopropylidene and *O*-benzylidene derivatives of labdanes. *Synthesis* **1989**, 711.
[12] Rauter, A.P.; Ramoa-Ribeiro, F.; Fernandes, A.C.; Figueiredo, J.A. A new method of acetonation with the zeolite HY as catalyst. Synthesis of O-Isopropylidene sugar derivatives. *Tetrahedron* **1995**, *51*, 6529.
[13] Meskens, F.A.J. Methods for the preparation of acetals from alcohols or oxiranes and carbonyl compounds. *Synthesis* **1981**, 501.
[14] (a) Catelani, G; Colonna, F.; Marra, A. An improved preparation of 3,4-*O*-isopropylidene derivatives of α- and β-D-galactopyranosides. *Carbohydr. Res.* **1988**, *182*, 297. (b) Gelas, J.; Horton, D. acetonation of carbohydrates under kinetic control by use of 2-alkoxypropenes. *Heterocycles* **1981**, *16*, 1587.
[15] Pedatella S.; Guaragna, A.; D'Alonzo, D.; De Nisco, M.; Palumbo, G. Triphenylphosphine polymer-bound/iodine complex: a suitable reagent for preparation of *O*-isopropylidene sugar derivatives. *Synthesis* **2006**, *2*, 305.
[16] Leadbeater, N.E.; Marco, M. Preparation of polymer-supported ligands and metal complexes for use in catalysis. *Chem. Rev.* **2002**, *102*, 3217.
[17] Dewry, D.H.; Coe, D.M.; Poon, S. Solid-supported reagents in organic synthesis. *Med. Res. Rev.* **1999**, *19*, 97.
[18] Caputo, R.; Ferreri, C.; Noviello, S.; Palumbo, G. Use of polymeric phosphine-halogen complexes in the conversion of epoxides to halohydrins. *Synthesis* **1986**, 499.
[19] (a) Bernard M.; Ford, W.T. Wittig reagents bound to crosslinked polystyrenes. *J. Org. Chem.* **1983**, *48*, 326. (b) Regen, S.L.; Lee, D.P. Solid phase phosphorus reagents. Conversion of alcohols to alkyl chlorides. *J. Org. Chem.* **1975**, *40*, 1669.
[20] (a) Stacey, B.E.; Tierney, B. The use of boric and benzeneboronic acids in the partial acetonation of monosaccharides. *Carbohydr. Res.* **1976**, *49*, 129. (b) Martin, O.R.; Korppi-Tommola, S.-L.; Szarek, W.A. Structure-sweetness relationships for fructose analogs. Part I. Synthesis and evaluation of sweetness of 5-deoxy-D-threo-hexulose *Can. J. Chem.* **1982**, *60*, 1857. (c) May, Jr, J.A.; Sartorelli, A.C. Synthesis and biological activity of potential antimetabolites of L-fucose. *J. Med. Chem*. **1979**, *22*, 971 and references cited therein. (d) Shaikh, N.S.; Bhor, S.S.; Gajare, A.S.; Deshpande, V.H.; Wakharkar, R.D. Mild and facile procedure for clay-catalyzed acetonide protection and deprotection of *N*(Boc)-amino alcohols and protection of 1,2-diols. *Tetrahedron Lett.* **2004**, *45*, 5395. (e) Moon, H.R.; Choi, W.J.; Kim, H.O.; Jeong, L.S. Improved and alternative synthesis of D- and L-cyclopentenone derivatives, the versatile intermediates for the synthesis of carbocyclic nucleosides. *Tetrahedron: Asymmetry* **2002**, *13*, 1189. (f) Asakura, J.; Matsubara, Y.; Yoshihara, M. Clay catalyzed acetonation: a simple method for the preparation of isopropylidene carbohydrates. *J. Carbohydr. Chem.* **1996**, *15*, 231. (g) Von Reichstein, T.; Grüssner, A. a high-yield synthesis of L-ascorbic acid (Vitamin C). *Helv. Chim. Acta* **1934**, *17*, 311. (h) Pressman, B.C.; Anderson, L.; Lardy, H.A. 1,2-Isopropylidene-D-glucitol, 1,2:5,6-diisopropylidene-D-glucitol and a new synthesis of D,L-glyceraldehyde dimmer. *J. Am. Chem. Soc.* **1950**, *72*, 2404 and references cited therein.

12

Chapter 12

General Conclusions and Future Perspectives

12. GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

Over a century has passed since Emil Fisher introduced his pioneering concepts about stereochemistry and selective functional group transformations into modern mechanistic and synthetic carbohydrate chemistry. Since then, our understanding of carbohydrate-related chemical processes has reached a considerably high degree of development, as it basically allows disclosing every hidden aspect referred to stereochemical, conformational or electronic features of sugar structures. In parallel, countless new roles of carbohydrates have emerged in diverse fields, ranging from chemical biology (as glycoconjugates or glycomimetics) through asymmetric catalysis (as chiral auxiliaries) to natural product synthesis (as chiral starting materials), rendering sugars attractive tools for further different purposes.

In spite of the number of investigations carried out in carbohydrate chemistry, quite a few topics still remain to be uncovered. To this concern, a subject of great fascination and importance, dealing with the role of unnatural carbohydrates at the chemistry-biology interface, has been studied during this PhD thesis. Particularly, the development of efficient methodologies for the synthesis of unnatural carbohydrates and their analogues has provided the suitable tools to open up an investigation about the behaviour of such a kind of unnatural molecules towards biological systems.

The thesis has been initially centred (**Chapter 3**) on the versatile synthetic applications deriving from the use of the homologating agent 5,6-dihydro-1,4-dithiin-2-yl[(4-methoxybenzyl) oxy]methane (**1**), a reagent capable of three-carbon homologation of organic molecules by introduction of a fully protected allylic alcohol at the new terminus. On the basis of the interesting results already attained from the elongation of different substrates, which has brought to the synthesis of quite a few enantiopure compounds [spiro-compounds (**2-3**), 4-deoxy-sugar derivatives (**4-5**) and *C*-glycoside derivatives (**6-7**)], in Chapter 3 the three-carbon homologation of chiral electrophiles such as 2,3-*O*-isopropylidene-L-glyceraldehyde (**8**) and 1,1-dimethylethyl 4-formyl-2,2-dimethyl oxazolidine-3-carboxylate (**9**) has been examined. More specifically, the reaction between the *in situ* prepared *C*-3 lithiated carbanion of the homologating agent **1** and the Garner aldehyde **9** has afforded a *syn/anti* diastereomeric mixture of alcohols **36**; the stereochemical outcome of this reaction has seemed to be mainly influenced by the nature of the solvent (Et_2O vs THF), whereas any significant induction has not been observed in the presence

of the catalysts [Ti(*O*-Pr^i)$_4$, $TiCp_2Cl_2$, $ZnBr_2$]. Such effect could be related to the nature of the organolithium intermediate: in fact, a "nude" and more reactive ionic couple prevails in THF, while a less reactive non-ionized species is formed in Et_2O, driving the reaction towards a better stereoselectivity.

Such a kind of homologations have been focused towards the construction of oxygen- and nitrogen-containing densely hydroxylated molecules, i.e. L-hexopyranosides and L-iminopyranoses, provided with orthogonal protection of the functional groups and therefore utilizable as such or as building blocks for further synthetic manipulations. In fact, the homologation between the agent **1** and the 2,3-*O*-isopropylidene-L-glyceraldehyde (**9**) has been explored (**Chapter 4**) to study a general and efficient route for the preparation of L-hexopyranosides. Following this route, most of the L-epimers have been so far synthesized, with a high degree of stereoselectivity, by suitable *syn*- and *anti*-dihydroxylation reactions on the double bond in olefins *syn/anti*-**12**. The possibility to have a strategy of general applicability derives from the employ of 1,6-anhydro derivatives **31a-b**, obtained by a further cyclization of the intermediates *syn/anti*-**10**. In major detail, we have exploited a useful feature of 1,6-anhydro derivatives, according to which all stereocenters are in opposite orientation with respect to the corresponding classical pyranosides. Therefore, an inverse stereoselectivity has been achieved in the *syn*- and *anti*-dihydroxylation reactions, affording remaining L-sugars.

Encouraged by the results reported in Chapter 4, a versatile pathway for the synthesis of non-competitive glycosidase inhibitors 1-deoxy-L-iminosugars has been herein opened up as well (**Chapter 6**). The required stereochemistry of the final compounds has been guaranteed by the introduction of the chiral electrophile 1,1-dimethylethyl-(*S*)-4-formyl-2,2-dimethyl-3-oxazolidine-carboxylate (the Garner's aldehyde) within the polyhydroxylated chain. Essential has been the finding according to which the protected iminosugar derivatives, such as **10-11**, do not conform to the expected $^1C_4$ chair, typical of L-sugars, conversely adopting a conformation close to $^3S_1$. By exploiting this characteristic, access to almost all eight epimers belonging to L-series has been opened; endeavours will be made farther on to complete the series. Moreover, considering that the double bond in olefins *anti/syn*-**7** may be also functionalized with groups diverse from -OH (for example, -NH_2 or -F), numerous compounds may be prepared as well, to be tested as glycosidase and glycosyltransferase inhibitors, thereby further illustrating the high synthetic utility of this methodology.

Subsequently, inspired by the literature reports describing numerous bioactive six-membered nucleoside analogues, as well as by the fundamental knowledge according to which a number of

human enzymes are able to phosphorylate nucleosides belonging to unnatural L-series, a general route for the synthesis of L-series six-membered nucleosides has been finely tuned (**Chapter 8**). Approaches have been mainly focused to three directions: (a) synthesis of L-iminosugar nucleosides; (b) synthesis of anomeric hexopyranosyl nucleosides; (c) synthesis of non-anomeric hexopyranosyl nucleosides.

(a) The preparation of imino-pyranosyl nucleosides like **10** has to be considered one of the first approaches to such a kind of molecules, whether they belong to D- or L-series. Therefore, special attention will be given to the biological assays, which will furnish early information about possible further modifications to further attain.

(b) With regard to the synthesis of anomeric hexopyranosyl nucleosides, the study of 2',3'-dideoxy-L-hexopyranosyl nucleosides **27** and **33** has been preliminarily undertaken. Since target compounds needed a stereoselective *β*-installation of the nucleobase moiety at *C*-1, a number of methods for the stereoselective *N*-glycosidation of deoxy sugars has been examined. Therefore, the route to enantiopure pyranosyl nucleosides has also become an investigation about the reactivity of 2,3-dideoxy monosaccharides.

(c) Concerning non-anomeric hexopyranosyl nucleosides, the synthesis of the L-enantiomers of the widely bioactive 1,5-anhydrohexitol nucleosides has been attained. Approach to such molecules has been accomplished by using the 1,6-anhydro derivative **36**, through hydride-mediated ring opening, stereoselective double bond epoxydation and nucleobase-mediated oxirane ring-opening. Then, removal of the 3'-hydroxyl group in compounds **40-41** has been examined, in order to afford the desired 1',3'-dideoxy-hexitol nucleosides. Since several deoxygenation procedures have been examined with the purpose to find the best conditions for 3'-OH removal, the route towards the synthesis of hexitol nucleosides has also resulted in a survey on the deoxygenation reaction on nucleoside derivatives.

The above reported approach to the preparation of carbohydrate-like building blocks belonging to L-series represents a scalable methodology, which has been further demonstrated (**Chapter 10**) by the synthesis of L-shaped pyranosyl oligonucleotides L-hexitol nucleic acids (L-HNA) and 2,3-dideoxy-L-hexopyranosyl nucleic acids (L-homo-DNA). Particularly, several oligonucleotide sequences have been synthesized as from their related phosphoramidite derivatives **7**/**10** and **30**/**33**; for each sequence has been examined the capacity to hybridize with natural DNA oligonucleotides, as well as with unnatural strands.

(1) Thymine- and adenine-L-anhydrohexitol nucleosides have been incorporated at the **X** position of the 5'-(CACCG**X**TGCTACC)-3' and 5'-(CACCG**X**TGCTACC)-3' sequences. The

potential of such nucleosides to hybridize with natural bases was studied in thermal denaturation experiments with the respective complements. With regard to the former strand, incorporation of unnatural L-hexitol nucleosides has not shown to perturb the structure of the whole sequence, resulting in a duplex which stability is reasonably close to that one of the natural DNA. Conversely, in the latter sequence the perturbation of the natural DNA duplex has appeared to be more consistent and the unnatural synthesized duplexes have resulted to be in a rather unstable organization.

(2) Fully modified L-hexitol oligonucleotide sequences $(\mathbf{X})_{13}$ (**X** = **A*** or **T***) have been hybridized with several unnatural complements, in the frame of a project focused towards the understanding of the conformational and chiral selection of oligonucleotides. Although the data in our hands need to be supported by further measurements, early experiments have suggested that L-HNA is able to hybridize both with L-series oligonucleotides, such as L-DNA, L-CNA and L-HNA itself, by adopting the natural $^{1}C_{4}$ chair conformation, and with D-series strands, such as β-D-homo-DNA and α-D-homo-DNA, by inverting its conformation into a $^{4}C_{1}$ chair.

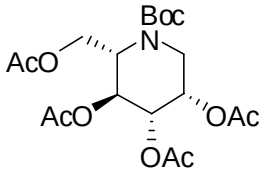
In **Chapter 11**, with the perspective to develop innovative and mild synthetic protocols for the insertion of protective groups in organic compounds and inspired by the need to drive reactions under more environmentally friendly conditions, a new methodology for *O*-isopropylidene group protection of carbohydrate derivatives has been developed, by using the dehydrating agent triphenylphosphine polymer-bound/I_2 complex. This method has been characterized by smooth, non-equilibrating reaction conditions and a very clean, simple work-up, making the reaction conditions particularly mild and with low environmental impact. We hopefully believe that this readily available protocol can be considered as a useful alternative for carbohydrate protection in oligosaccharide and natural product synthesis.

As above shown, the chemistry presented here has offered a stereoselective, high-yielding and scalable route for the preparation of a wide range of suitably protected building blocks related to hexopyranosides and iminopyranoses. Because of the *de novo* approach of such synthetic methodology, the strategy has demonstrated to be highly convergent as well, minimizing the number of synthetic steps. As a matter of fact, dissection of carbohydrate building blocks into linear fragments has reduced the number of hydroxyl groups, avoiding temporary protections, moreover rendering possible to prepare several structurally related building blocks from one common linear precursor by varying the conditions for the stereoselective construction of the carbohydrate skeleton.

Given the possibility to have easy access to such molecules in large amount, further endeavours could be addressed to their employ as scaffolds for the synthesis of more complex structures (oligosaccharides, glycoconjugates, glycomimetics) for enhancing our understanding of the role of carbohydrates in biological environments, for example investigating the role of molecular chirality in carbohydrate recognition and biological function, or with the aim to provide new substrates for the discovery and development of drug candidates with improved biological activity.

Appendix A

NMR SPECTRA



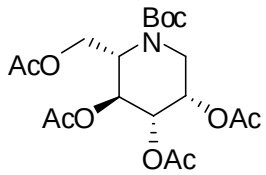
11

200 MHz ($CDCl_3$)

diene_MA

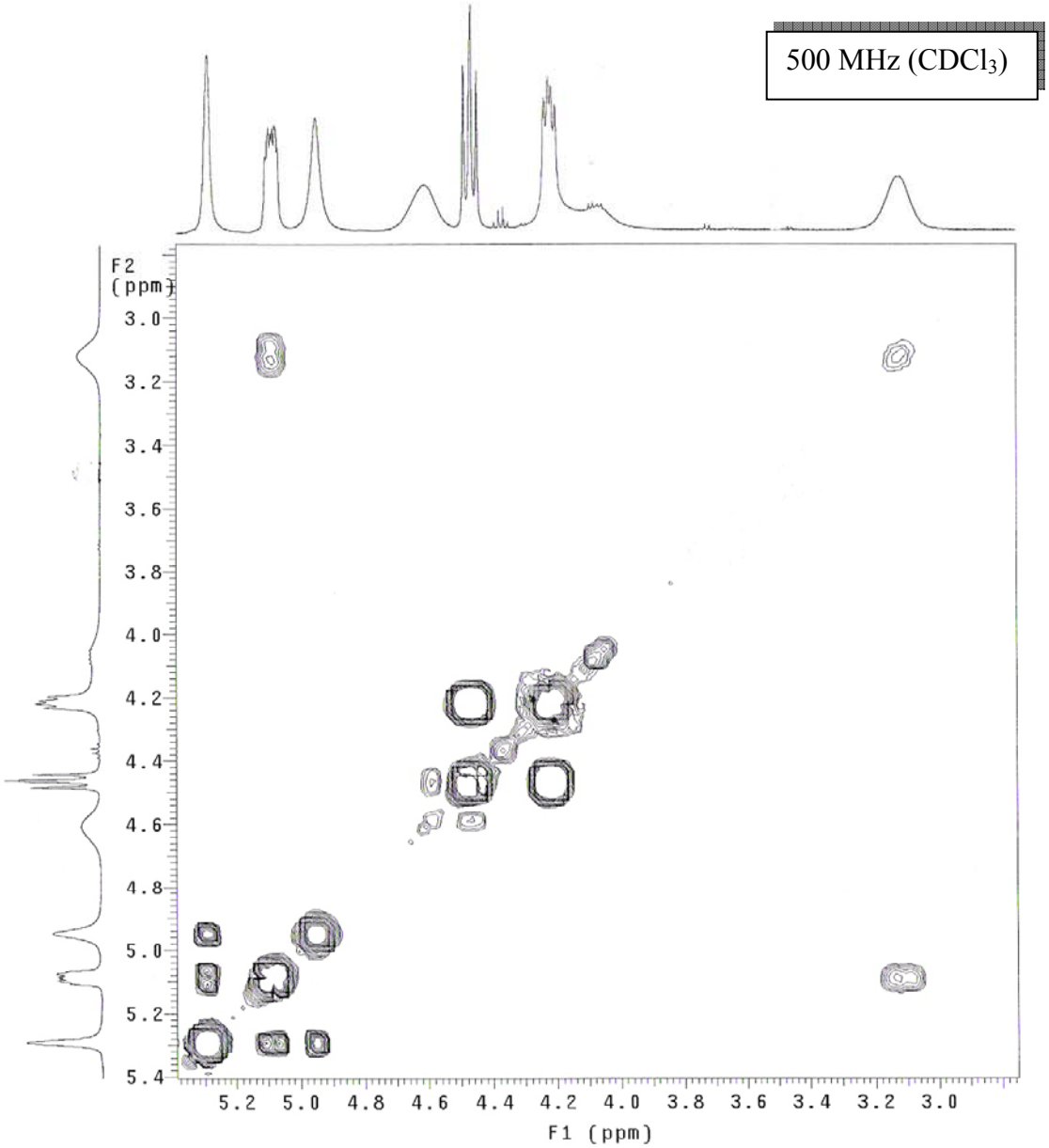
Pulse Sequence: gCOSY
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
File: MannoTetraAc_CO
INOVA-500 "nmr500"

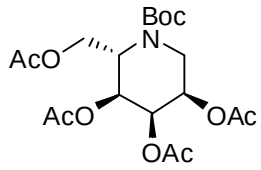
Relax. delay 1.000 sec
Acq. time 0.125 sec
Width 4089.4 Hz
2D Width 4089.4 Hz
16 repetitions
512 increments
OBSERVE H1, 499.7062174 MHz
DATA PROCESSING
Sq. sine bell 0.063 sec
F1 DATA PROCESSING
Sq. sine bell 0.031 sec
FT size 1024 x 1024
Total time 2 hr, 44 min, 14 sec



11

500 MHz ($CDCl_3$)





12

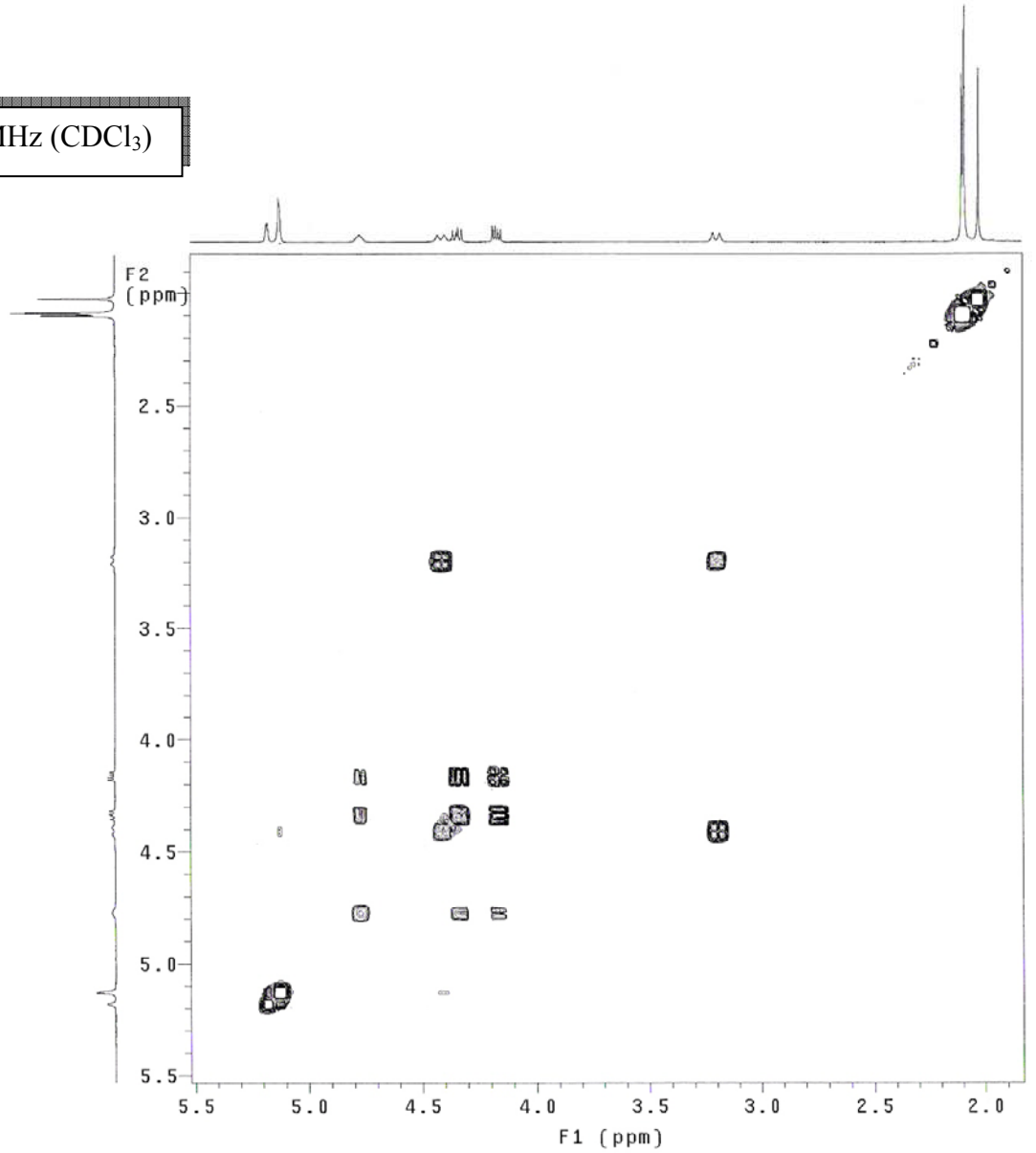
200 MHz ($CDCl_3$)

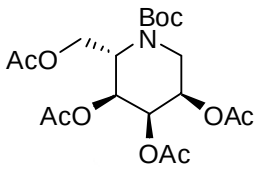
```
STANDARD PROTON PARAMETERS

Pulse Sequence: gCOSY
 Solvent: CDCl3
 Temp. 25.0 C / 298.1 K
File: AlloTetraAc_CO
INOVA-500  "nmr500"

 Relax. delay 1.000 sec
 Acq. time 0.214 sec
 Width     4790.1 Hz
 2D Width 4790.1 Hz
 8 repetitions
 512 increments
OBSERVE    H1, 499.7062174 MHz
DATA PROCESSING
 Gauss apodization 0.100 sec
 Sq. sine bell 0.107 sec
F1 DATA PROCESSING
 Sq. sine bell 0.027 sec
FT size 2048 x 2048
Total time 1 hr, 27 min, 42 sec
```

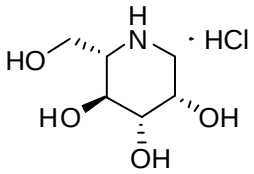
500 MHz ($CDCl_3$)



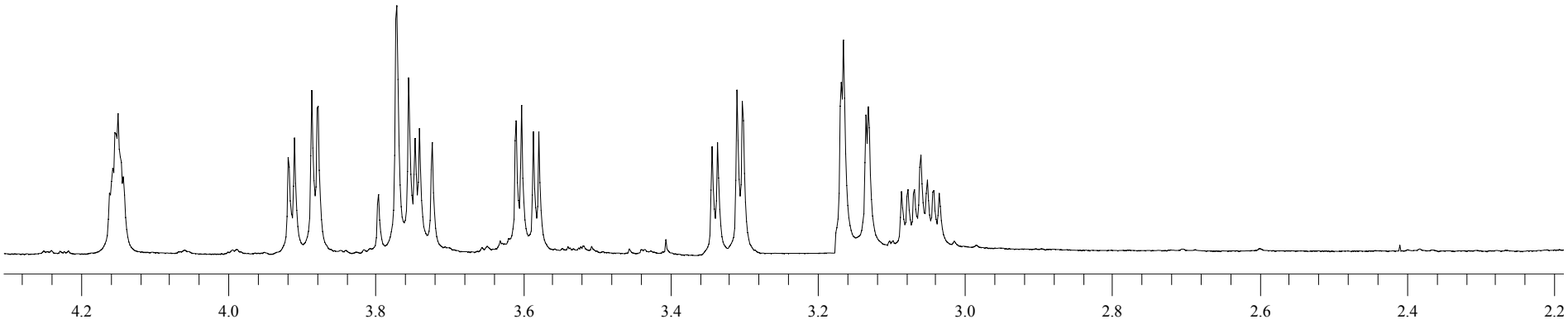


12

400 MHz (D_2O)



13

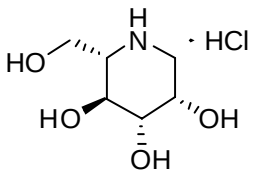


060714_LManno-DNJ_H

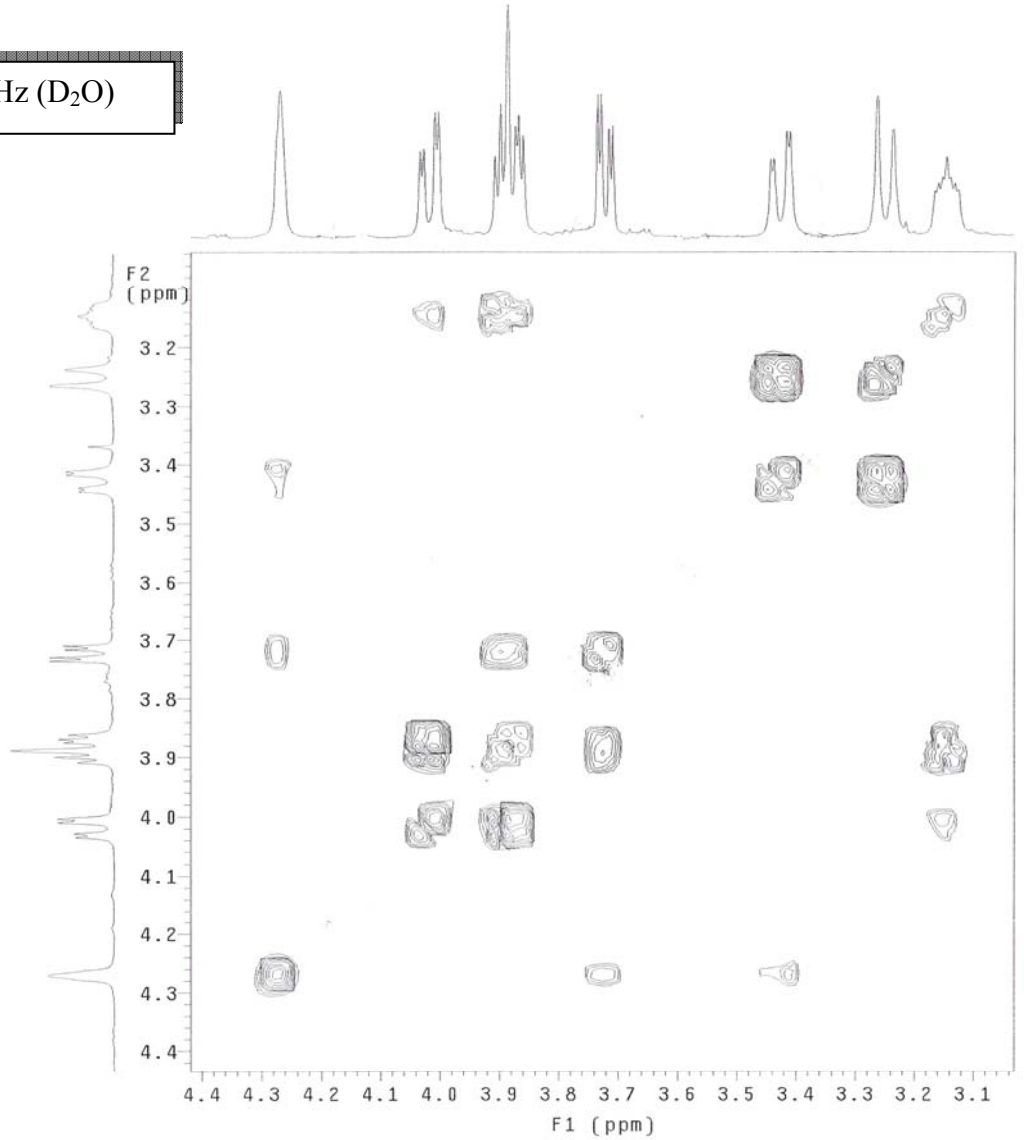
Pulse Sequence: gCOSY
Solvent: D2O
Temp. 25.0 C / 298.1 K
File: 060714_LManno-DNJ_CO
INOVA-500 "Inova500"

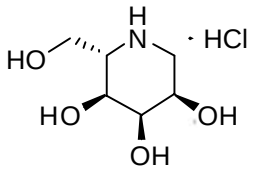
Relax. delay 1.000 sec
Acq. time 0.173 sec
Width 2955.8 Hz
2D Width 2955.8 Hz
8 repetitions
512 increments
OBSERVE H1, 499.7074350 MHz
DATA PROCESSING
Sq. sine bell 0.087 sec
F1 DATA PROCESSING
Sq. sine bell 0.043 sec
FT size 1024 x 1024
Total time 1 hr, 27 min, 11 sec

500 MHz (D_2O)

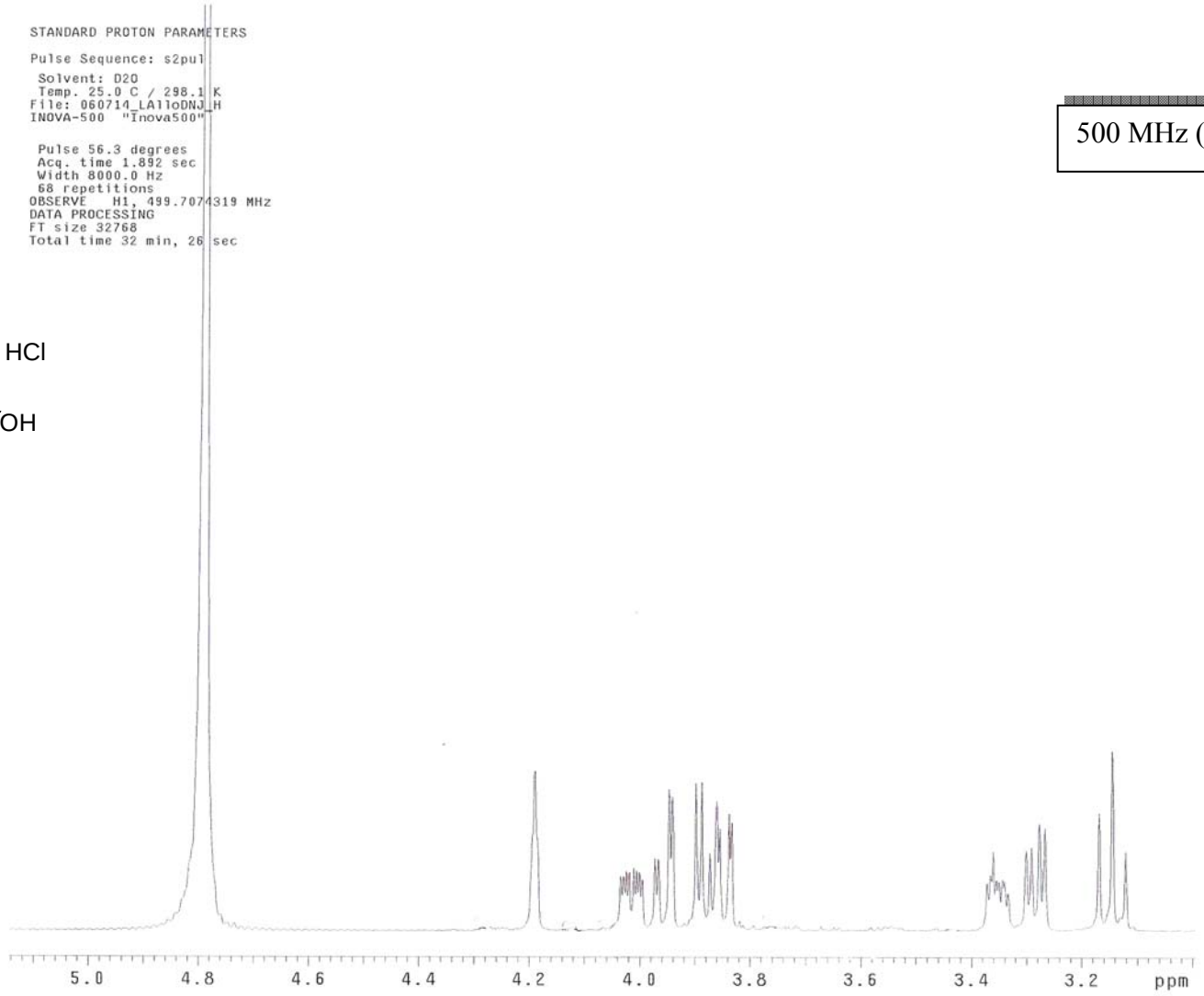


13





14



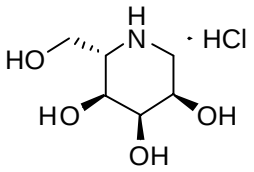
500 MHz (D_2O)

500 MHz (D_2O)

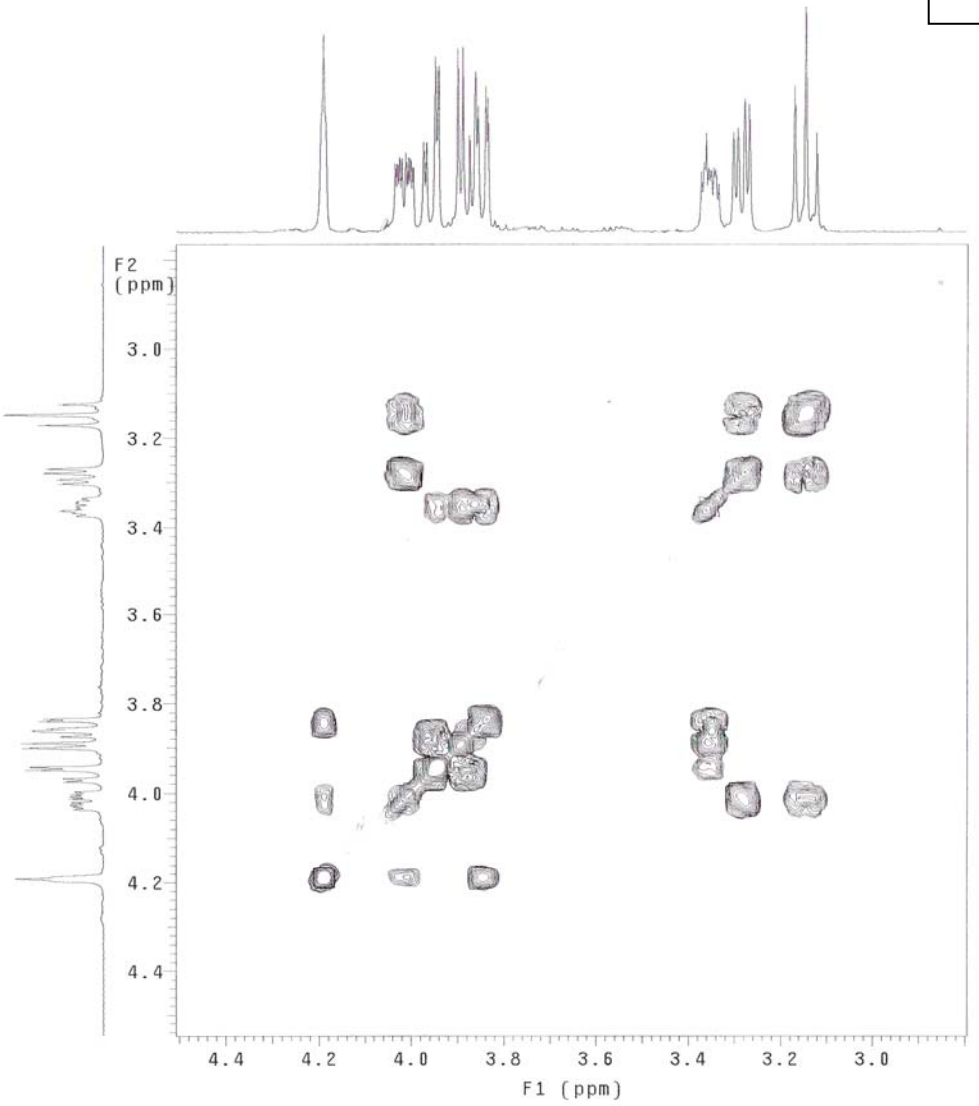
```
060714_LAlloDNJ_H

Pulse Sequence: gCOSY
 Solvent: D2O
 Temp. 25.0 C / 298.1 K
File: 060714_LAlloDNJ_CO
INOVA-500  "Inova500"

 Relax. delay 1.000 sec
 Acq. time 0.143 sec
 Width    1787.1 Hz
 2D Width 1787.1 Hz
 8 repetitions
 512 increments
OBSERVE    H1, 499.7074335 MHz
DATA PROCESSING
 Sq. sine bell 0.072 sec
F1 DATA PROCESSING
 Sq. sine bell 0.072 sec
FT size 1024 x 1024
Total time 1 hr, 29 min, 0 sec
```



14



Appendix B

PUBLICATIONS

A VERSATILE ROUTE TO L-HEXOSES: SYNTHESIS OF L-MANNOSE AND L-ALTROSE

Annalisa Guaragna, Carmela Napolitano, Daniele D'Alonzo, Silvana Pedatella and Giovanni Palumbo

Org. Lett. **2006**, *8*, 4863-4866.

ORGANIC LETTERS

2006
Vol. 8, No. 21
4863−4866

A Versatile Route to L-Hexoses: Synthesis of L-Mannose and L-Altrose

Annalisa Guaragna,* Carmela Napolitano, Daniele D'Alonzo, Silvana Pedatella, and Giovanni Palumbo

Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, via Cynthia, 4 I-80126 Napoli, Italy

guaragna@unina.it

Received August 2, 2006 *(Revised Manuscript Received September 15, 2006)*

ABSTRACT

An efficient route for the synthesis of orthogonally protected L-sugars has been opened up, starting from the heterocyclic homologating agent 1 and 2,3-*O*-isopropylidene-L-glyceraldehyde (2). Our synthetic path enables the synthesis of a 2,3-unsaturated-L-pyranoside, which can be suitably functionalized to afford the desired L-hexoses. In this paper, we report the synthesis of L-*manno*- and L-*altro*-pyranosides. Moreover, this strategy may be used to prepare all eight sugars and their derivatives in either enantiomeric form.

The rare L-sugars[1] are valuable compounds as precursors in the synthesis of various chemicals and also as agents in a wide range of crucial biological events. Although much less common in nature than their D-counterparts, L-hexoses (in their pyranosidic form) are key components of numerous bioactive[2] oligosaccharides, antibiotics, glycopeptides, and terpene glycosides, as well as of steroid glycosides and other clinically useful agents such as heparin.[3] Some remarkable examples are L-gulopyranoside-containing compounds such as the antitumor drug Bleomycin A_2[4] and the nucleoside antibiotic Adenomycin.[5] Moreover, L-altrose is a typical constituent of the extracellular polysaccharides from *Butyrivibrio fibrisolvens* strain CF3,[6] and L-mannose has been found in some steroidal glycosides.[7] Its phenolic derivatives are potent substrates for measuring the α-L-mannosidase activity of commercial naringinase[8] (Figure 1).

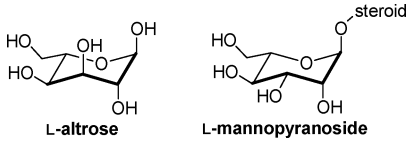


Figure 1. Bioactive L-sugars.

Not all L-hexoses are commercially available; this fact, together with the practical difficulties in obtaining these compounds from natural sources, has led chemists to develop new, general, and convenient methods for their production.

Numerous approaches to L-pyranose preparation have been reported, including homologation of shorter-chain sugars,[9] epimerization of readily available D-sugars,[10] and de novo syntheses.[11]

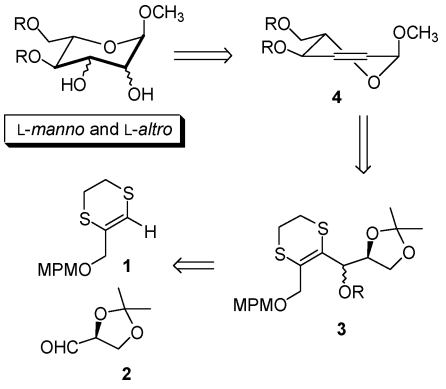
* Corresponding author. Phone: + 39 081 674 118. Fax: + 39 081 674 119.

(1) McNaught, A. D. *Pure Appl. Chem.* **1996**, *68*, 1919−2008.
(2) (a) Collins, P. M. *Dictionary of Carbohydrates*; Chapman and Hall: London, 1987. (b) Lee, J.-C.; Chang, S.-W.; Liao, C.-C.; Chi, F.-C.; Chen, C.-S.; Wen, Y.-S.; Wang, C.-C.; Kulkarni, S. S.; Puranik, R.; Liu, Y.-H.; Hung, S.-C. *Chem.−Eur. J.* **2004**, *10*, 399−415.
(3) (a) Lane, D. A.; Lindahl, U. *Heparin-Chemical and Biological Properties Clinical Applications*; Edward Arnold: London, 1989. (b) Lindahl, U. *Pure Appl. Chem.* **1997**, *69*, 1897−1902. (c) Iozzo, R. V. *Annu. Rev. Biochem.* **1998**, *67*, 609−652. (d) Rabenstein, D. L. *Nat. Prod. Rep.* **2002**, *19*, 312−331.
(4) (a) Burger, R. M. *Chem. Rev.* **1998**, *98*, 1153−1169. (b) Boger, D. L.; Ramsey, T. M.; Cai, H.; Hoehn, S. T.; Stubbe, J. *J. Am. Chem. Soc.* **1998**, *120*, 9139−9148. (c) Katano, K.; An, H.; Aoyagi, Y.; Overhand, M.; Sucheck, S. J.; Stevens, W. C.; Hess, C. D.; Zhou, X.; Hecht, S. M. *J. Am. Chem. Soc.* **1998**, *120*, 11285−11296.
(5) Ogita, T.; Otake, N.; Miyazaki, Y.; Yonehara, H.; Macfarlane, R. D.; McNeal, C. J. *Tetrahedron Lett.* **1980**, *21*, 3203−3206.
(6) Stack, R. J. *FEMS Microbiol. Lett.* **1987**, *48*, 83−87.
(7) Kubelka, W. *Phytochemistry* **1974**, *13*, 1805−1808.
(8) Esaki, S.; Ohishi, A.; Katsumata, A.; Sugiyama, N.; Kamiya, S. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 2099−2103.

10.1021/ol061916z CCC: $33.50
Published on Web 09/26/2006

As part of our efforts working toward the synthesis of bioactive polyhydroxylated compounds, we have explored a general and efficient route for the preparation of L-hexoses (as well as their D-enantiomers) starting from L-glyceraldehyde and the three-carbon homologating agent **1** (Scheme 1). The latter has recently been employed in a versatile

Scheme 1. Retrosynthetic Path



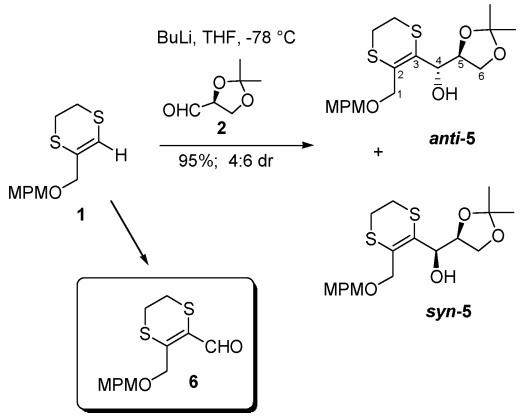
procedure to prepare both 4-deoxy-hexopyranoses[12] and 1-deoxy-iminosugars[13] belonging to the D- or L-series.

In this preliminary communication, we describe the preparation of orthogonally protected L-altro- and L-manno-pyranosides in enantiomerically pure form, testing, at the same time, the breadth of our methodology.

As shown in the retrosynthetic path (Scheme 1), our strategy comprises the following major steps: (i) preparation of **3** by a three-carbon homologation reaction, employing the heterocyclic system **1** and the well-known[14] 2,3-*O*-isopropylidene-L-glyceraldehyde (**2**); (ii) synthesis of the 2,3-unsaturated pyranoside **4** by carbon skeleton cyclization; (iii) suitable double-bond functionalization by stereoselective dihydroxylation of **4**.

The synthesis started with the coupling reaction of **1**, prepared in a few steps from methyl pyruvate,[15] with the protected aldehyde **2** to obtain a diastereoisomeric mixture of secondary alcohols **5** (Scheme 2). Oddly, our first attempts

Scheme 2. Three-Carbon Homologation



at using Ti(*O*-*i*-Pr)$_4$ as the catalyst[16] led only to the formation of a small amount of the desired alcohols; in fact, once formed, **5** readily changed, almost quantitatively and even at low temperature, into the unexpected aldehyde **6**.[17]

On the contrary, in the absence of catalysts, this side reaction proceeded much more slowly and the alcohols **5** were obtained in an excellent yield (95%) and in an anti/syn 4:6 diastereomeric ratio.[18] The slight preference for the syn compound is consistent with a nonchelation-controlled reaction[19] according to the Felkin−Anh model prediction (Figure 2).

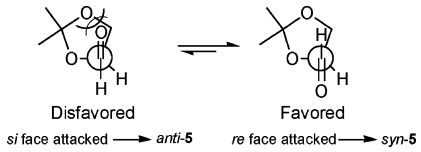


Figure 2. Felkin−Ahn models for the aldehyde **2**.

After mixture separation by SiO_2 flash chromatography, the *anti*-**5** diastereoisomer was chosen as a model to test the whole synthetic path. Benzylation of the secondary hydroxyl function, treating *anti*-**5** with NaH and BnBr, afforded **7** in almost quantitative yield (Scheme 3). Interestingly, if the reaction was carried out in the presence of an excess of NaH, the formation of an unexpected byproduct **8** in 20% yield[20] was observed besides the benzylated product **7** (65%).

4-Methoxybenzyl protecting group removal was next attempted by treating **7** with DDQ (1.2 equiv) in CH_2Cl_2/

(9) Some recent examples of homologation of shorter-chain sugars: (a) Takahashi, S.; Kuzuhara, H. *J. Chem. Soc., Perkin Trans. 1* **1997**, 607−612. (b) Dondoni, A.; Marra, A.; Massi, A. *J. Org. Chem.* **1997**, *62*, 6261−6267. (c) Lubineau, A.; Gavard, O.; Alais, J.; Bonnaffé, D. *Tetrahedron Lett.* **2000**, *41*, 307−311. (d) Ermolenko, L.; Sasaki, N. A. *J. Org. Chem.* **2006**, *71*, 693−703.

(10) Some recent examples of C-5 epimerization of sugars: (a) Ojeda, R.; de Paz, J. L.; Martín-Lomas, M.; Lassaletta, J. M. *Synlett* **1999**, *8*, 1316−1318. (b) Adinolfi, M.; Barone, G.; De Lorenzo, F.; Iadonisi, A. *Synlett* **1999**, *3*, 336−338. (c) Takahashi, H.; Hitomi, Y.; Iwai, Y.; Ikegami, S. *J. Am. Chem. Soc.* **2000**, *122*, 2995−3000. (d) Hung, S.-C.; Wang, C.-C.; Thopate, S. R. *Tetrahedron Lett.* **2000**, *41*, 3119−3122. (e) Boulineau, F. P.; Wie, A. *Org. Lett.* **2002**, *4*, 2281−2283.

(11) Some recent examples of de novo syntheses: (a) Ko, S. Y.; Lee, A. W. M.; Masamune, S.; Reed, L. A.; Sharpless, K. B.; Walker, F. J. *Tetrahedron* **1990**, *46*, 245−264. (b) Harris, J. M.; Keränen, M. D.; Nguyen, H.; Yong, V. G.; O'Doherty, G. A. *Carbohydr. Res.* **2000**, *328*, 17−36. (c) Honzumi, M.; Taniguchi, T.; Ogasawara, K. *Org. Lett.* **2001**, *3*, 1355−1358. (d) Hodgston, R.; Majid, T.; Nelson, A. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1444−1454. (e) Northrup, A. B.; Mangion, I. K.; Hettche, F.; MacMillan, D. W. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 2152−2154. (f) Córdova, A.; Ibrahem, I.; Casas, J.; Sundén, H.; Engqvist, M.; Reyes, E. *Chem.−Eur. J.* **2005**, *11*, 4772−4784.

(12) Caputo, R.; De Nisco, M.; Festa, P.; Guaragna, A; Palumbo, G.; Pedatella, S. *J. Org. Chem.* **2004**, *69*, 7033−7037.

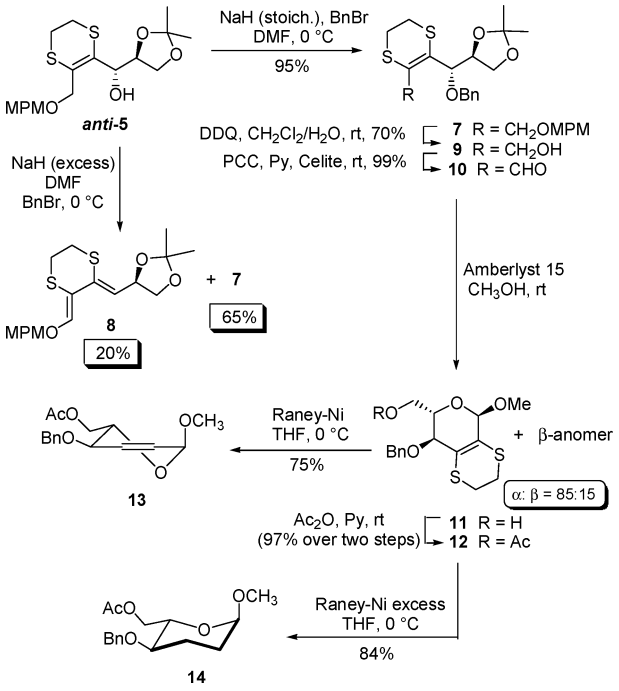
(13) Guaragna, A.; D'Errico, S.; D'Alonzo, D.; Pedatella, S.; Palumbo, G., manuscript in preparation.

(14) Hubschwerlen, C.; Specklin, J.-L.; Higelin, J. *Org. Synth.* **1995**, *72*, 1−3.

(15) Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S. *J. Org. Chem.* **1997**, *62*, 9369−9371.

(16) According to standard procedures carried out on the same chiral aldehyde **2**; see: Suzuki, K.; Yuki, Y.; Mukaiyama, T. *Chem. Lett.* **1981**, 1529−1532.

Scheme 3. Cyclization of the Carbon Skeleton



H_2O (18:1). As we have previously described,[12,15] with similar substrates, such removal conditions lead quantitatively to the formation of a formyl function rather than to the expected primary alcohol. To our regret, under the same conditions, **7** is converted both into **10** and into the corresponding alcohol **9** with an unsatisfactory overall yield (48%) and in a 4:6 ratio. All attempts to obtain quantitatively only the aldehyde **10** failed; therefore, a two-step reaction sequence was preferred, first converting **7** into **9** and then oxidizing **9** to **10**. The complete conversion of **7** into **9** (70% yield) was accomplished using DDQ in the presence of a higher-water percentage; on the other hand, oxidation of the primary hydroxyl function of **9** was easily performed by treatment with PCC and Celite in pyridine to afford quantitatively **10**, which was directly used in the next cyclization step.

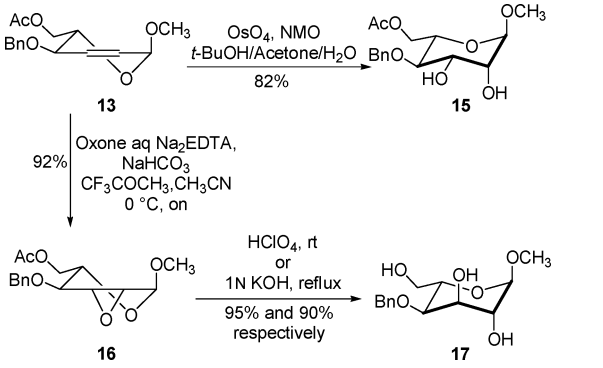
Treatment of the aldehyde **10** in the presence of Amberlyst 15 in methanol allowed, in a one-pot simple procedure, the conversion of the formyl group into its di-*O*-methyl acetal,[12] acetonide deprotection, and intramolecular transacetalation to give the unstable bicyclic compound **11**. After subsequent acetylation of the crude residue, an α/β diastereomeric mixture (85:15 dr) was obtained in 97% overall yield. Recrystallization from methanol allowed separation of the major α-anomer **12** from its β-form.

Desulfurization of the α-anomer **12** with Raney Ni in THF at 0 °C for 2 h led to the unsaturated pyranosyl derivative **13** (75% yield). Moreover, when the dithiodimethylene bridge removal was carried out with an excess of Raney Ni, the overreduction product was obtained with satisfactory yield (84%), affording the interesting 2,3-dideoxy-L-hexopyranoside **14**.[21]

To access the desired L-manno- and L-altropyranosides, we next explored the stereoselective dihydroxylation of olefin **13**. Under common Upjohn conditions (OsO_4/NMO), the L-mannopyranoside **15** was obtained as a single diastereomer in 82% yield. This result concurred with earlier investigations[11d,22] into the dihydroxylation of allylic alcohol derivatives: the osmylation reaction occurred anti to the pseudoequatorial benzyloxy group.

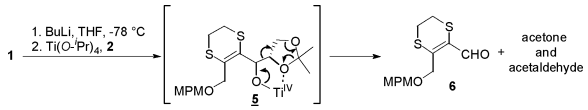
With our successful synthesis of the protected L-mannose **15**, we next attempted preparation of the L-altrose derivative by introducing an epoxy functionality.[23] For this, we treated olefin **13** with in situ[24] generated DMDO (Oxone/trifluoroacetone). The *anti*-epoxide **16** was obtained[25] exclusively in 92% yield (Scheme 4). Subsequent ring opening of the 2,3-

Scheme 4. Dihydroxylation of the Unsaturated Derivative **13**



anhydro derivative **16** either by acid-[26] or by base-catalyzed[27] hydrolysis afforded the L-altropyranoside **17** (95% and 90% yield, respectively), with C-6 O-deacetylation being observed under both conditions.

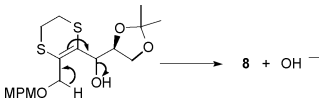
(17) This product, whose structure was unambiguously confirmed by spectroscopic data, seems to be formed by consumption of the coupling product **5** (TLC monitoring). The mechanism of such a reaction has to be proved, and it is still under investigation; nevertheless, we assume that it proceeds via the titanium complex **5** [see ref 11a]:



(18) The C-4 absolute stereochemistry was clearly established in the course of our synthesis on the basis of the $^3J_{4,5}$ of the cyclic compounds **12**, **15**, and **16**.

(19) As recently reported [Badorrey, R.; Cativiela, C.; Díaz-de-Villegas, M. D.; Díez, R.; Gálvez, J. A. *Eur. J. Org. Chem.* **2003**, 2268−2275], steric and stereoelectronic interactions between the chiral aldehyde **2** and the nucleophile across the two diastereotopic faces of the carbonyl group do not play a significant role in determining the stereochemical outcome of the reaction, a situation that allows the nucleophilic attack on the more stable conformer leading to a slight preference for the syn compound.

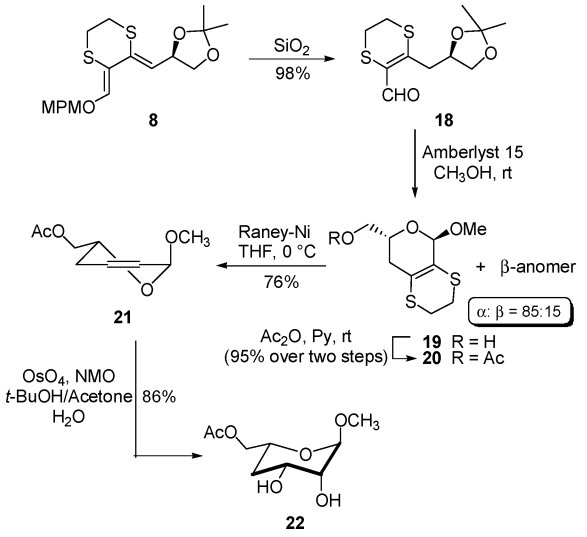
(20) The latter could presumably be generated by an allylic C-1 proton abstraction with a subsequent electronic shift, to give the thermodynamically stable **8**.



(21) For examples of bioactive 2,3-dideoxy-L-hexopyranoside-based compounds, see: Guppi, S. R.; Zhou, M.; O'Doherty, G. A. *Org. Lett.* **2006**, *8*, 293−296. For their enantiomers, see: Groebke, K.; Hunziker, J.; Fraser, W.; Peng, L.; Diederichsen, U.; Zimmermann, K.; Holzner, A.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1998**, 81, 375−474.

It is noteworthy to recall the value of diene **8**, obtained as byproduct in Scheme 3, as a useful intermediate with the purpose to prepare 4-deoxy-L-hexopyranosides. In fact, following chromatographic purification, the compound **8** afforded quantitatively the aldehyde **18** (Scheme 5). When

Scheme 5. 4-Deoxy-L-sugar Preparation Starting from **8**



this was submitted to the synthetic steps described above, it gave the intermediate **20**, which after desulfurization (76% yield) and double-bond osmylation (86% yield) led, according to our previous results,[12] to methyl 4-deoxy-L-*lyxo*-hexopyranoside **22** as a single diastereomer.

In summary, we have developed a pratical approach to the synthesis of orthogonally protected L-manno- and L-altropyranosides **15** and **17**. The versatility of our method lies in producing an intermediate bearing a double bond in C-2/C-3 positions (such as **13**), which can be suitably functionalized. We are currently investigating the appropriate conditions to achieve the remaining epimers belonging to the *gluco*-configuration. On the other hand, the use of a C-4 diastereomer of olefin **13** (coming from the *syn*-**5** intermediate) enables the preparation of all four *galacto*-epimers.

Obviously, it would be possible to synthesize D-analogues and their deoxy derivatives simply by replacing the chiral electrophile with its *ent*-**2**.

Acknowledgment. ^{1}H and ^{13}C NMR spectra were performed at Centro Interdipartimentale di Metodologie Chimico-Fisiche (CIMCF), Università di Napoli Federico II. The Varian Inova 500 MHz instrument is the property of Consorzio Interuniversitario Nazionale La Chimica per l'Ambiente (INCA) and was used in the frame of a project by INCA and M.I.U.R. (L. 488/92, Cluster 11-A).

Supporting Information Available: Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL061916Z

(22) Cha, J. K.; Christ, W. J.; Kishi, Y. *Tetrahedron* **1984**, *40*, 2247−2255.

(23) In an initial experiment, the oxidation of **13** with *m*-CPBA resulted in a lower yield (70%) and a 10:1 anti/syn dr.

(24) Yang, D.; Wong, M.-K.; Yip, Y.-C. *J. Org. Chem.* **1995**, *60*, 3887−3889.

(25) For similar results, see: Kim, K. S.; Moon, C. W.; Park, J., II; Han, S.-H. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1341−1343.

(26) Fieser, L. F.; Goto, T. *J. Am. Chem. Soc.* **1960**, *82*, 1693−1697.

(27) Barili, P. L.; Berti, G.; Catelani, G.; Colonna, F.; Mastrorilli, E. *J. Org. Chem.* **1987**, *52*, 2886−2892.

A General Approach to the Synthesis of 1-Deoxy-L-Iminosugars

Annalisa Guaragna, Stefano D'Errico, Daniele D'Alonzo, Silvana Pedatella and Giovanni Palumbo

Org. Lett. **2007**, *9*, 3473-3476.

ORGANIC LETTERS

2007
Vol. 9, No. 17
3473−3476

A General Approach to the Synthesis of 1-Deoxy-L-iminosugars

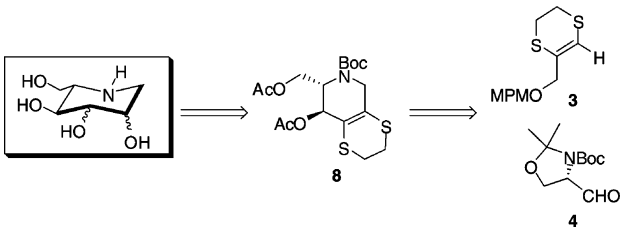
Annalisa Guaragna,* Stefano D'Errico, Daniele D'Alonzo, Silvana Pedatella, and Giovanni Palumbo

Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, via Cynthia, 4 I-80126 Napoli, Italy

guaragna@unina.it

Received June 21, 2007

ABSTRACT



A stereoselective procedure for the preparation of non-naturally occurring deoxy iminosugars belonging to L-series has been developed. The synthesis involves the construction of the key intermediate bicycle pyperidine 8, available in few steps by the coupling of the heterocyclic synthon 3 and the readily available Garner aldehyde 4.

Polyhydroxylated piperidines (commonly known as iminosugars or azasugars) represent sugar analogues with the nitrogen atom in place of the ring oxygen of the corresponding carbohydrate. Since their first discovery over 40 years ago, iminosugars have gained a great deal of attention as inhibitors of carbohydrate-processing enzymes glycosidases and glycosyltransferases. As extensively described,[1] their inhibitory aptitude has been linked with their structural resemblance to the glycone moiety of glycosides that interact with such enzymes.

As alterations in biosynthesis and function of these enzymes are implicated in a wide variety of diseases, the significant inhibitory properties of iminosugars make them excellent targets for medical intervention. Their prospective therapeutical uses range from diabetes[2] through cancer[3] and viral diseases[4] to metabolic and neurological disorders.[5] As a result, α-glucosidase inhibitors 1-deoxynojirimycin (DNJ, **1**) and *N*-butyl-1-deoxynojirimycin (*N*B-DNJ, Zavesca **2**) (Figure 1) have been shown to inhibit human immunodeficiency virus (HIV) replication and HIV-mediated syncytium formation in vitro.[6] Moreover, *N*B-DNJ has been the first iminosugar medicine to receive approval, in 2002 in the European Union and in 2003 in the United States, for use in patients with mild to moderate type 1 Gaucher disease.

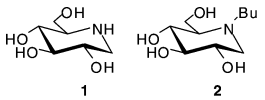


Figure 1. Bioactive iminosugars DNJ and *N*B-DNJ.

The principal advances in total and stereoselective syntheses of such compounds have recently been reviewed.[7] As reported, most syntheses have focused attention on the

* To whom correspondence should be addressed. Phone: + 39 081 674 118. Fax: + 39 081 674 119.

(1) (a) Stütz, A., Ed. *Iminosugars as Glycosidase Inhibitors*; Wiley-VCH: Weinheim, Germany, 1999. (b) Lillehund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515−553.

(2) Somsak, L.; Nagya, V.; Hadady, Z.; Docsa, T.; Gergely, P. *Curr. Pharm. Des.* **2003**, *9*, 1177−1189.

(3) Weiss, M.; Hettmer, S.; Smith, P.; Ladish, S. *Cancer Res.* **2003**, *63*, 3654−3658.

(4) Greimel, P.; Spreitz, J.; Stütz, A. E.; Wrodnigg, T. M. *Curr. Topics Med. Chem.* **2003**, *3*, 513−523.

(5) Butters, T. D.; Dwek, R. A.; Platt, F. M. *Chem. Rev.* **2000**, *100*, 4683−4696.

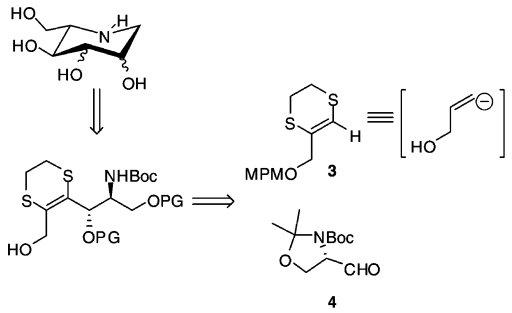
(6) Ficher, P. B.; Collin, M.; Karlsson, G. B.; Lames, W.; Butters, T. D.; Davis, S. J.; Gordon, S.; Dwek, R. A.; Platt, F. M. *J. Virol.* **1995**, *69*, 5791−5797.

10.1021/ol7014847 CCC: $37.00
Published on Web 07/20/2007

preparation of iminosugars with D-configuration, whereas few routes are available for the synthesis of their corresponding L-analogues.[8] This fact is evidently due to the larger commercial availability of D-series sugars as starting materials, as well as to the fact that glycosides belonging to D-series are the natural substrates of almost all glycosidases. However, it is worth recalling that iminosugars mimicking the sugar moiety structure of the natural substrate are not always inhibitors of the corresponding glycosidase. D-*manno*-DNJ (DMJ) is known as a much better inhibitor of α-L-fucosidase than α-D-mannosidase; on the other hand L-*allo*-DNJ is a better inhibitor of α-D-mannosidase than D-DMJ.[9] As recently shown,[10] an explanation of this behavior could be found considering that D-enantiomers are competitive inhibitors of D-glycosidases, whereas their L-enantiomers are noncompetitive inhibitors of the same enzymes.

In the context of our ongoing program directed toward the achievement of a new synthetic methodology for the preparation of polyhydroxylated molecules, we have developed a versatile strategy for the synthesis of non-naturally occurring deoxy-iminopyranoses belonging to L-series, through a non-carbohydrate based route.

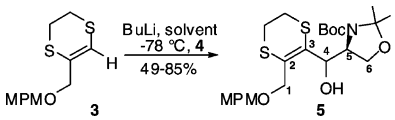
Scheme 1. Retrosynthetic Path



As outlined in Scheme 1, the synthesis involves the use of an heterocyclic synthon, the 5,6-dihydro-1,4-dithiin-2-yl-[(4-methoxybenzyl)oxy]methane[11] (**3**), a reagent capable of three-carbon homologation of electrophiles by the introduction of a fully protected allylic alcohol moiety, already devoted to the preparation of several polyhydroxylated compounds.[12]

The synthesis began with the coupling of the in situ prepared *C*-3 lithiated carbanion of **3** with the Garner[13] aldehyde **4** (Table 1) to afford a syn/anti diastereomeric

Table 1. Three-Carbon Homologation



solvent	catalyst (20%)	(anti/syn) dr	yield(%)
THF	none	60:40	83
THF	Ti(*O*-*i*-Pr)$_4$	60:40	80
THF	Cp_2TiCl_2	60:40	85
Et_2O	Cp_2TiCl_2	70:30	49
Et_2O	$ZnBr_2$	82:18	73
Et_2O	none	91:9	72

mixture of alcohols **5**. As highlighted in Table 1, the best stereoselectivity was achieved by the use of Et_2O without catalyst, providing *anti*-**5** in good stereoselectivity (91:9 dr). Interestingly, the stereochemical outcome of the reaction seemed to be mainly influenced by the nature of the solvent,[14] whereas any significant stereoselective induction was not observed in the presence of the catalysts.[15]

The secondary alcohol[16] *anti*-**5**, obtained by the coupling reaction, was separated from its diastereomer by flash chromatography; the stereochemical assignment at the newly generated *C*-4 was clearly deduced by X-ray analysis (Figure 2).

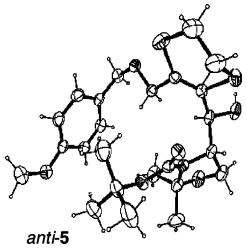


Figure 2. X-ray analysis of *anti*-**5**.

With the educt **5** in hand, our interest was focused on the achievement of key intermediate **9** (Scheme 2). To this purpose, we converted the alcohol **5** in its diacetate **6** by deprotection of the oxazolidine ring and acetylation of the

(7) For comprehensive reviews see: (a) Afarinkia, K.; Bahar, A. *Tetrahedron: Asymmetry* **2005**, *16*, 1239−1287. (b) Pearson, M. S. M.; Allaimat, M. M.; Fargeas, V.; Lebreton, J. *Eur. J. Org. Chem.* **2005**, 2159−2191. Carbohydrate-based routes to DNJ and congeners: (c) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. *J. Med. Chem.* **1994**, *37*, 3701−3706. (d) O'Brien, J. L.; Tosin, M.; Murphy, P. V. *Org. Lett.* **2001**, *3*, 3353−3356. (e) Spreidz, J. S.; Stütz, A. E.; Wrodnigg, T. M. *Carbohydr. Res.* **2002**, *337*, 183−191 and references cited therein. Non-carbohydrate based routes to DNJ and congeners: (f) Haukaas, M. H.; O'Doherty, G. A. *Org. Lett.* **2001**, *3*, 401−404. (g) Ruiz, M.; Ojea, V.; Ruanova, T. M.; Quintela, J. M. *Tetrahedron: Asymmetry* **2002**, *13*, 795−799. (h) Takahata, H.; Banba, Y.; Sasatani, M.; Nemoto, H.; Kato, A.; Adachi, I. *Tetrahedron* **2004**, *60*, 8199−8205 and literature cited therein.

(8) Meyers, A. I.; Andres, C. J.; Resek, J. E.; Woddall, C. C.; McLaughlin, M. A.; Lee, P. H.; Price, D. A. *Tetrahedron* **1999**, *55*, 8931−8952.

(9) Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J. Med. Chem.* **2005**, *48*, 2036−2044.

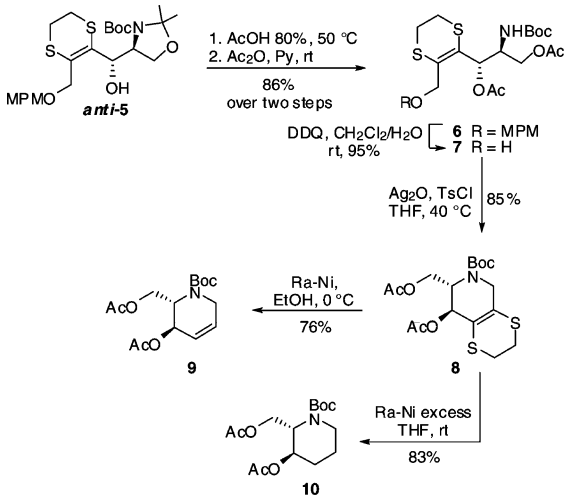
(10) Asano, N.; Ikeda, K.; Yu, L.; Kato, A.; Takebayashi, K.; Adachi, I.; Kato, I.; Ouchi, H.; Takahata, H.; Fleet, G. *Tetrahedron: Asymmetry* **2005**, *16*, 223−229.

(11) Guaragna, A.; Palumbo, G.; Pedatella, S. In *e-Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; John Wiley & Sons: New York, 2007. In press.

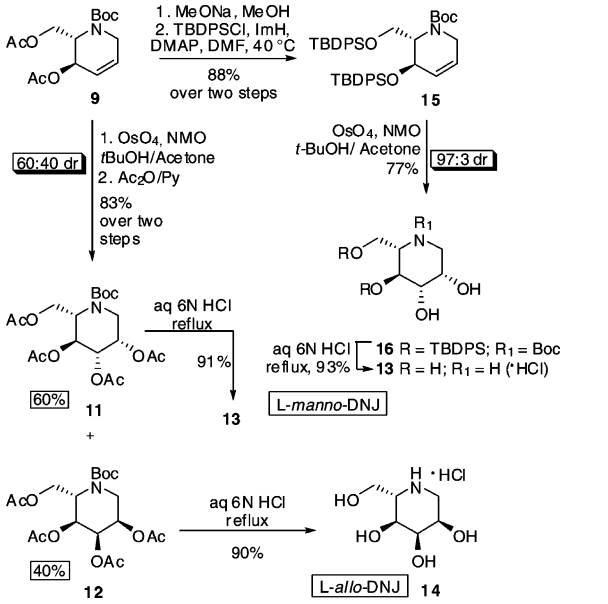
(12) (a) Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S. *J. Org. Chem.* **1997**, *62*, 9369−9371. (b) Caputo, R.; De Nisco, M.; Festa, P.; Guaragna, A.; Palumbo, G.; Pedatella, S. *J. Org. Chem.* **2004**, *69*, 7033−7037. (c) Guaragna, A.; Napolitano, C.; D'Alonzo, D.; Pedatella, S.; Palumbo, G. *Org. Lett.* **2006**, *8*, 4863−4866.

(13) Garner, P.; Park, J. M. *Org. Synth., Collected Vol. IX* **1998**, 300.

Scheme 2. Synthesis of the Key Intermediate **9**



Scheme 3. Syn Dihydroxylation of **9**

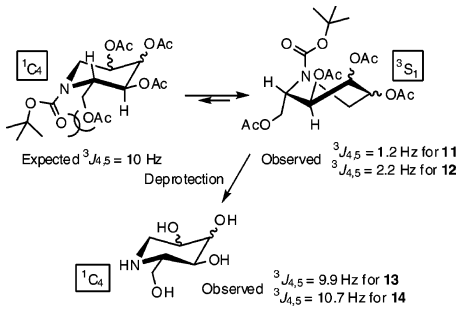


crude residue (86% overall yield). Removal of MPM group by treatment of **6** with DDQ in a CH_2Cl_2/H_2O (9:1) emulsion gave the primary alcohol **7** with an excellent yield (95%). Intramolecular cyclization was then carried out under mild conditions by treatment of **7** with Ag_2O/TsCl in THF at 40 °C (85%). Finally, removal of the dithioethylene bridge on intermediate **8** was achieved by treatment with Raney-Ni in ethanol at 0 °C for 2 h leading to the olefin **9**. Moreover, when the reaction was carried out with a Raney-Ni excess in THF, at room temperature, the over-reduction product was obtained with a satisfactory yield (83%), affording the 1,2,3-trideoxy-L-iminosugar **10**.

With the promising olefin **9** in hand our interest was directed to the stereoselective double-bond dihydroxylation (Scheme 3). Exposure of **9** to the common Upjohn conditions (OsO_4/NMO) followed by acetylation of the crude residue yielded a fully separable mixture of the protected L-*manno*-DNJ **11** and L-*allo*-DNJ **12** in low diastereomeric ratio (6:4). Both diastereomers were deprotected by means of refluxing aq 6 N HCl solution, obtaining deoxy-L-mannojirimycin (**13**) and deoxy-L-allonojirimycin (**14**) in remarkable yields (91% and 90%, respectively). Further attempts to improve the stereoselectivity of dihydroxylation reaction (i.e., using the bidentate complex OsO_4/TMEDA[17] and the Sharpless catalysts[18]) showed no significant effects.[19] The observed low selectivity in the above dihydroxylation reactions might be attributed to the relatively small size of the C-4 acetyl group and thus both faces of the double bond were almost equally hindered. As a matter of fact, the replacement of the Ac groups of **9** with the much bigger TBDPS ethers (**15**, 88% overall yield, Scheme 3), afforded after dihydroxylation of **15**, under Upjohn conditions, the protected L-*manno*-DNJ **16** with a high stereoselectivity (97:3 dr). Then, treatment with aq 6 N HCl allowed removal of all protective groups to obtain deoxy-L-mannojirimycin (**13**) in 93% yield.

It is noteworthy to recall that the stereochemistry of compounds **13** and **14** is consistent with X-ray analysis of the *anti*-**5** compound and with the spectroscopic data. However, observing the coupling constant values in the ^1H NMR spectra (Scheme 4), it is evident that the *N*-Boc compounds **11** and **12** do not conform to the expected $^1C_4$ chair conformation, typical of L-sugars adopting a conformation close to $^3S_1$.[20]

Scheme 4. Preferred Conformations for Compounds **11**, **12**



(14) The solvent-dependent stereoselective effect should be related to the nature of the organolithium intermediate: as already reported (see farther on), a "nude" and more reactive ionic couple prevails in THF, while a less reactive non-ionized species is formed in Et_2O, driving the reaction towards a better stereoselective outcome: (a) Seyferth, D.; King, R. B. *Annual Surveys of Organometallic Chemistry*, Vol. 1−3, Elsevier Publishing Co.: Amsterdam, 1965−1967. (b) Maercker, A.; Roberts, J. D. *J. Am Chem. Soc.* **1966**, *88*, 1742−1759.

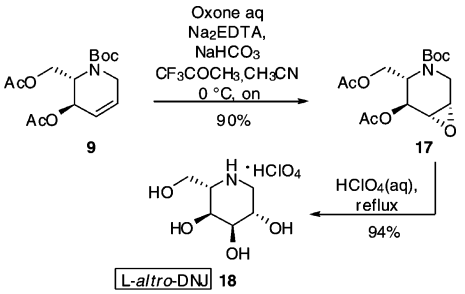
(15) In all our experiments the reported syn stereoselection [Liang, X.; Andersch, J.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2136−2157] was never observed. Because of the chemical characteristics of compound **5**, such discrepancy could be rationalized assuming that in the reaction medium the catalyst does not complex Garner aldehyde, but it can be sequestrated by the negatively-charged heterocyclic system, without induction of stereoselectivity.

(16) An alternative numbering reported on the carbon skeleton has been employed to identify carbon atoms that will belong to the carbohydrate-like ring (see Table 1).

(17) Donohoe, T. J.; Blades, K.; Moore, P. R.; Waring, M. J.; Winter, J. J. G.; Helliwell, M.; Newcombe, N. J.; Stemp, G. *J. Org. Chem.* **2002**, *67*, 7946−7956.

The above successes led us to consider the anti dihydroxylation of the key olefin **9**. Treatment of this latter with in situ generated[21] DMDO (oxone/trifluoroacetone) afforded exclusively the *anti*-epoxide **17** in 90% yield (Scheme 5).

Scheme 5. Anti Dihydroxylation of **9**



Ring opening[22] of the 2,3-anhydro derivative **17** along with the removal of all protecting groups by means of refluxing $HClO_4$ gave the deoxy-L-altronojirimycin (**18**) in 94% yield.

In summary, a versatile pathway for the synthesis of L-deoxyiminosugars belonging to L-series has been opened up in this paper. Together with L-*manno*-, L-*allo*-and L-*altro*-deoxyiminosugars **13**, **14**, and **18**, whose synthesis has been described, this path will be profitably employed for the synthesis of all the epimers with *galacto* configuration, simply applying the same procedure on the *syn*-**5** diastereomer. Furthermore, the whole synthetic procedure so far described, carried out from **3** and the *ent*-**4** (prepared from D-serine) enables the preparation of D-series iminosugars as well.

Acknowledgment. This research has been supported by a Regione Campania grant to A.G. (L.R. 5/2002). ^1H and ^{13}C NMR spectra and X-ray analysis were performed at Centro Interdipartimentale di Metodologie Chimico-Fisiche (CIMCF), Università di Napoli Federico II. Varian Inova 500 MHz instrument is property of Consorzio Interuniversitario Nazionale La Chimica per l'Ambiente (INCA) and was used in the frame of a project by INCA and M.I.U.R. (L. 488/92, Cluster 11-A). We are grateful to Dr Umberto Ciriello (ICI International Chemical Industry S.p.A.) for his collaboration to this work. We also thank Prof. Angela Tuzi (Dept. of Chemisty, University of Napoli) for X-ray analysis of *anti*-**5** compound.

Supporting Information Available: Experimental procedures, analytical data, X-ray crystallographyc data (cif file) for *anti*-**5**, ^1H and ^{13}C NMR spectra of all the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL7014847

(18) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483−2547.

(19) Wu, X.-D.; Khim, S.-K.; Zhang, X.; Cederstrom, E. M.; Mariano, P. S. *J. Org. Chem.* **1998**, *63*, 841−859.

(20) In the $^1C_4$ chair form, a downward orientation is imposed on the *N*-Boc substituent owing to the nature of the ring nitrogen atom, resulting in a strong repulsive interaction with the nearly coplanar *C*-6 methylene group. An upward movement of the ring nitrogen relieves such repulsion leading to a $^3S_1$ conformation. For similar results see: (a) Kilonda, A.; Compernolle, F.; Hoornaert, G. J. *J. Org. Chem.* **1995**, *60*, 5820−5824. (b) Kazmaier, U.; Grandel, R. *Eur. J. Org. Chem.* **1998**, 1833−1840.

(21) Yang, D.; Wong, M.-K.; Yip, Y.-C. *J. Org. Chem.* **1995**, *60*, 3887−3889.

(22) On the basis of ^1H NMR coupling constant values we established that also **17** essentially exists in a $^3S_1$ conformation and that the formation of *trans*-diaxial ring opening product **18** could be explained assuming that the $HClO_4$ first removes the *N*-Boc group, allowing the chair inversion from $^3S_1$ to $^1C_4$, and then leads to the epoxide cleavage.

A General Route to D- and L-Six-Membered Nucleoside Analogues

Annalisa Guaragna, Daniele D'Alonzo, Mauro De Nisco, Silvana Pedatella and Giovanni Palumbo

Nucleos. Nucleot. Nucl. Acids **2007**, *26*, 959-962.

Nucleosides, Nucleotides, and Nucleic Acids, 26:959–962, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701508166

Taylor & Francis
Taylor & Francis Group

A GENERAL ROUTE TO D- AND L-SIX-MEMBERED NUCLEOSIDE ANALOGUES

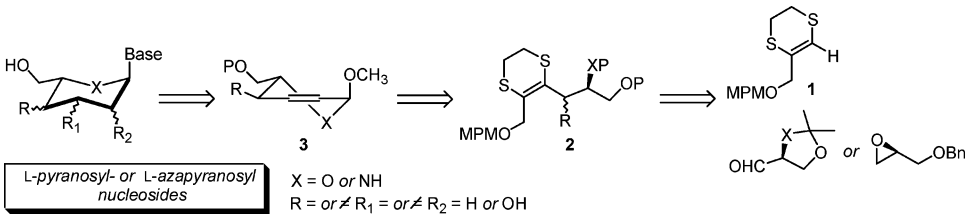
Annalisa Guaragna, Daniele D'Alonzo, Mauro De Nisco, Silvana Pedatella, and Giovanni Palumbo □ *Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, Napoli, Italy*

□ *A simple synthetic route for novel* L- *(as well as* D-*) six-membered nucleosides is described. Particularly, we have provided a general approach to the synthesis of azasugar-based nucleosides, which preparation has been easily achieved starting from the coupling of our three carbon homologating agent* ***1*** *with the well known Garner aldehyde* ***4****. Further suitable and stereocontrolled functionalizations of the intermediate* ***9*** *will provide, after the base insertion, a wide class of six membered modified azanucleosides to be tested as NRTIs.*

Keywords azasugar-based nucleosides; six-membered modified azanucleosides.

In the search for effective, selective and nontoxic antiviral agents, a variety of strategies have been devised to design nucleoside analogues. These strategies have involved several formal modifications of the naturally occurring nucleosides, especially alterations of the carbohydrate moiety.[1] Modifications[2] have concerned, for instance, the inversion of hydroxyl group configurations, their elimination leading to bioactive dideoxy- or didehydro-nucleosides (e.g., ddC and d4T, respectively) or the replacement of the endocyclic oxygen of the sugar moiety with an heteroatom (3TC). In addition, since the discovery of Lamivudine (3TC, β-L-(-)-2-deoxy-3-thiacytidine) as potent inhibitor of reverse transcriptase (NRTI), L-nucleoside enantiomers have been re-evaluated as an emerging class of antiviral agents[3] and a great deal of efforts have been focused towards the synthesis of new L-sugar-based nucleoside analogues as potential NRTIs. As part of our current interest in polyhydroxylated compounds,[4] we designed and set up a new general approach to the synthesis of azasugar based nucleoside analogues, as well as hexopyranosyl nucleosides, belonging to both D- or L-series.

Address correspondence to Annalisa Guaragna, Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, Via Cynthia, 4 I-80126 Napoli, Italy. E-mail: guaragna@unina.it.



SCHEME 1 Retrosynthetic path for L-six-membered nucleoside analogues.

As depicted in Scheme 1, L-nucleoside analogues easily can be prepared starting from our three-carbon homologating agent **1**[5] and chiral electrophiles such as 2,3-*O*-isopropylidene-L-glyceraldehyde, Garner aldehyde, as well as (*R*)-benzyl glycidyl ether.

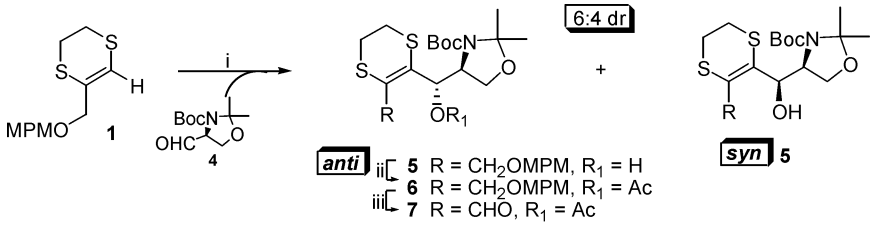
The current strategy comprises the following major steps: (i) preparation of **2** by three-carbon homologation; (ii) synthesis of the 2,3-unsaturated six-membered ring **3** by carbon skeleton cyclization; (iii) suitable double bond functionalization and base insertion.

Obviously, it would be possible to synthesize D-analogues simply by replacing the chiral electrophiles with their enantiomers.

In this preliminary communication, the synthesis of azanucleosides by a noncarbohydrate based route is reported.

The synthesis began with the coupling of **1**, prepared in a few steps from methyl pyruvate,[5] with Garner aldehyde[6] **4** (Scheme 2). Under our conditions, a solution of **4** and a catalytic amount of Ti(*O*-*i*-Pr)$_4$ in THF was added at low temperature to the in situ prepared *C*-3 lithiated carbanion of **1**, providing an *anti/syn* (6:4 dr) diastereomeric mixture of alcohols **5** in 83% yield. After mixture separation by SiO_2 flash chromatography, the more abundant *anti*-**5** diastereoisomer was chosen as a model to test the whole synthetic path. Acetylation of the secondary hydroxyl function, using Ac_2O in Py, afforded **6** in almost quantitative yield.

4-Methoxybenzyl protecting group removal was next attempted (Scheme 2) by treating **6** with DDQ (1.2 eq.) in CH_2Cl_2/H_2O (18:1). As we have previously described[5] with similar substrates, such removal



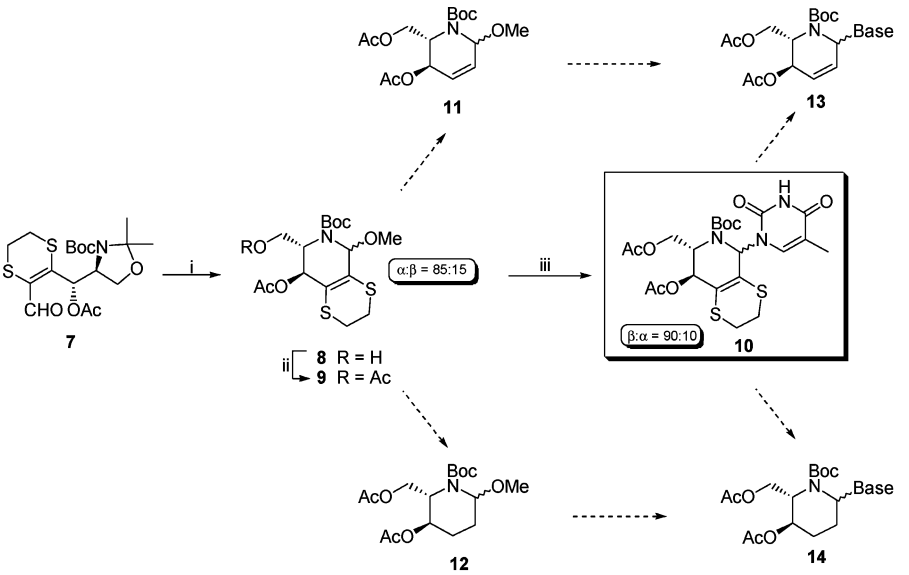
i: BuLi, THF, -78 °C, Ti (O^iPr)$_4$, 83%; ii: Ac_2O, Py, rt, 99%; iii: DDQ, CH_2Cl_2/H_2O, rt, 89%

SCHEME 2 Homologation reaction and MPM group removal.

conditions led quantitatively to the formation of a formyl function rather than the expected primary alcohol. Then, treatment of the aldehyde **7** in the presence of acidic Amberlyst in methanol allowed, in a one-pot simple procedure, the cleavage of the oxazolidine ring and the cyclization to afford the unstable bicyclic compound **8** (Scheme 3). After the acetylation of the crude residue, an α:β diastereomeric mixture of **9** (85:15 dr) was obtained in 97% overall yield. The key intermediate **9** was then coupled with the heterocyclic bases, under standard conditions,[7] to afford nucleoside derivatives **10** (β:α = 90:10). Diastereomeric ratio of the *O*-methyl glycoside **9** and of the nucleoside **10** has been determined by ^{1}HNMR analysis.

As reported in Scheme 3, the versatility of **9** allows the preparation of unsaturated and saturated azanucleosides **13** and **14**. Indeed, compatibly with each substrate, desulfurization will be performed prior or after the base insertion by means of Raney-Ni (1:10 w/w) in THF at 0°C, or using a large excess of Raney-Ni in order to obtain the over-reduction product. Suitable functionalizations at *C*-2/*C*-3 positions, carried out on the compound **13**, will fulfil the wide class of six-membered azanucleosides to be tested as NRTIs.

Works are still in progress concerning the synthesis of L-pyranosyl nucleosides, through the employment of different electrophiles (as shown in the retrosynthetic path), by means of a similar synthetic route to that so far described.



i: Amberlyst 15, MeOH, 0 °C to rt; ii: Ac_2O, Py, rt (97% over two steps); iii: Silylated thymine, $SnCl_4$, DCE, 0 to 20 °C, 72%

SCHEME 3 Carbon skeleton cyclization and base insertion.

In summary, a versatile and profitable approach to the synthesis of L-azapyranosyl nucleosides has been opened up. The versatility of such a method lies in producing intermediates bearing a double bond at *C*-2/*C*-3 positions (like **11**), which can be properly functionalized (or completely reduced) to afford a wide class of target molecules.

REFERENCES

1. Herdewijn, P. In *Recent Advances in Nucleosides: Chemistry and Chemotherapy*; Ed. Chu, C.K., Elsevier Science, Amsterdam, Netherlands, **2002**, pp. 239–290.
2. Ichikawa, E.; Kato, K. Sugar-modified nucleosides in past 10 years, a review. *Curr. Med. Chem.* **2001**, 8, 385–423.
3. Mathé, C.; Gosselin, G. L-Nucleoside enantiomers as antivirals drugs: A mini-review. *Antiviral Res.* **2006**, 71, 276–281.
4. Guaragna, A.; Napolitano, C.; D'Alonzo, D.; Pedatella, S.; Palumbo, G. A versatile route to L-hexoses: Synthesis of L-mannose and L-altrose. *Org. Lett.* **2006**, 8, 4863–4866.
5. a) Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S. A new and versatile allylic alcohol anion and acyl β-anion equivalent for three-carbon homologations. *J. Org. Chem.* **1997**, 62, 9369–9371; b) Caputo, R.; De Nisco, M.; Festa, P.; Guaragna, A.; Palumbo, G.; Pedatella, S. Synthesis of 4-deoxy-L-(and D-) hexoses from chiral noncarbohydrate building blocks. *J. Org. Chem.* **2004**, 69, 7033–7037.
6. Garner, P.; Park, J.M. 1,1,-Dimethylethyl (*S*)- or (*R*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate: A useful serinal derivative. *Org. Synth.* **1992**, 70, 18–26.
7. Vorbrüggen, H.; Höfle, G. On the mechanism of nucleoside synthesis. *Chem. Ber.* **1981**, 1256–1268.

TRIPHENYLPHOSPHINE POLYMER-BOUND/IODINE COMPLEX: SUITABLE REAGENT FOR PREPARATION OF *O*-ISOPROPYLIDENE SUGAR DERIVATIVES

Silvana Pedatella, Annalisa Guaragna, Daniele D'Alonzo,
Mauro De Nisco, Giovanni Palumbo

Synthesis **2006**, *2*, 305-308.

Triphenylphosphine Polymer-Bound/Iodine Complex: A Suitable Reagent for the Preparation of *O*-Isopropylidene Sugar Derivatives

Silvana Pedatella, Annalisa Guaragna, Daniele D'Alonzo, Mauro De Nisco, Giovanni Palumbo*

Università di Napoli Federico II, Dipartimento di Chimica Organica e Biochimica, Via Cynthia 4, 80126 Napoli, Italy
Fax +39(081)674393; E-mail: gipalumb@unina.it

Received 18 July 2005; revised 28 July 2005

Abstract: *O*-Isopropylidene derivatives of sugars are readily prepared by using the Lewis acid and dehydrating agent triphenylphosphine polymer-bound/I_2 complex. This new method is characterized by smooth, non-equilibrating reaction conditions and a very clean, simple work-up, making it particularly suitable for O-isopropylidenation of sugars under mild conditions and with low environmental impact.

Key words: acetals, carbohydrates, triarylphosphine–iodine complex, *O*-isopropylidene, acetonation

The condensation of acetone with aldoses and ketoses leading to the formation of isopropylidene derivatives has been widely used in synthesis to protect hydroxyl functions in carbohydrate chemistry. Furthermore, *O*-isopropylidene derivatives, for instance 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose derivatives, have shown anti-inflammatory and antipyretic activities as well as a very low toxicity.[1]

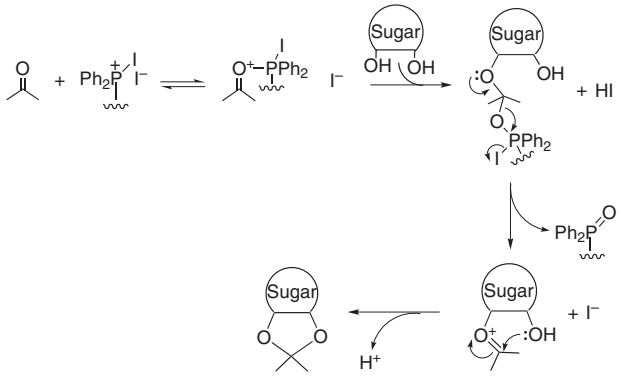
The conventional method for the preparation of such derivatives consists in the condensation of a vicinal diol with acetone, in the presence of an acidic catalyst, under anhydrous conditions. Many different agents have been used as catalysts, including mineral acids,[2] anhydrous zinc chloride together with phosphoric acid,[3] ion exchange resins,[4] anhydrous copper(II) sulfate,[5] iodine,[6] anhydrous ferric chloride,[7] $BF_3{\cdot}OEt_2$,[8] anhydrous $AlCl_3$[9] and, more recently, zeolites.[10]

Under the equilibrating conditions, water is formed which must be removed from the reaction mixture using either physical or chemical methods.[11] Generally, the major products obtained from these reactions are those which are thermodynamically favored. Otherwise, kinetic control can be obtained using other reagents for condensation, namely 2-methoxypropene in the presence of *p*-toluenesulfonic acid.[12]

We wish to report a new method based on the use of a triphenylphosphine polymer-bound/iodine complex for the thermodynamic acetonation of sugars in high yields, in anhydrous medium, avoiding water formation. Recent interest in the development of environmentally benign synthesis has led to a renewed interest in developing polymer-bound metal catalysts and reagents for organic synthesis that maintain high activity.[13] The reagents bound on polymeric supports offer a number of advantages over traditional solution-phase chemistry.

The triphenylphosphine polymer-bound/halogen complex is a Lewis acid and a dehydrating agent widely used in miscellaneous reactions[14] with low environmental impact. In fact, it avoids contamination from by-products and use of solvents, which are not environmentally friendly, in the purification processes. Triphenylphosphine polymer-bound/iodine complex is an easy to prepare,[15] handy, semi-crystalline solid, reasonably stable at room temperature. When dried and kept properly, it can be stored for weeks at room temperature, under N_2 atmosphere. The somewhat high cost of the starting triphenylphosphine polymer-bound does not actually represent a limitation of this procedure, if one considers that the polymer-linked phosphine oxide generally obtained from the reaction can be readily filtered off and reduced to the original phosphine form with trichlorosilane.[16]

Under our conditions, the sugar is added to a suspension of triphenyl phosphine polymer-bound/iodine complex (polystyryl diphenyl iodophosphonium iodide) in anhydrous acetone.[17] An adduct is first formed, due to the presence of the positively charged phosphorous atom in the complex and the electron-rich carbonyl oxygen of acetone, which exposes the carbonyl carbon atom to undergo nucleophilic attack by a first hydroxyl group of the sugar molecule (Scheme 1). The subsequent non-equilibrium step of the reaction is the loss of polymer-linked phosphine oxide and thereby formation of an oxygen-stabilized carbocation, known to be intermediate in the acetalization reaction.



Scheme 1

SYNTHESIS 2006, No. 2, pp 0305–0308
Advanced online publication: 21.12.2005
DOI: 10.1055/s-2005-918521; Art ID: Z14005SS

This latter can undergo the intramolecular attack by a second hydroxyl group present in the sugar molecule to afford the final *O*-isopropylidene derivative. The reaction is carried out under mild conditions, at room temperature, and is generally fast and high-yielding affording the thermodynamically more stable isopropylidene derivatives. Results obtained from the acetonation of miscellaneous sugars, such as L-arabinose, D-fructose, D- and L-galactose, D-glucose, D-mannose, D-ribose, L-sorbose, D-glucitol, using a triphenyl phosphine polymer-bound/iodine complex/sugar ratio of 2:1 are shown in Table 1. Each acetonation has been compared with the best one reported in literature.[2,3,6,7,18–23]

1,2:3,4-Di-*O*-isopropylidene-D-ribopyranose is a known compound[2b] that usually accompanies 2,3-*O*-isopropylidene-D-ribofuranose when the latter is prepared from D-ribose. However, to the best of our knowledge no intentional preparations, and consequently yields are reported for it in literature.

On the other hand, under our conditions using only one equivalent of triphenylphosphine polymer-bound/iodine complex D-ribose affords the pure mono-*O*-isopropylidene derivative, as well as, the sole di-*O*-isopropylidene derivative when treated with two equivalents.

All the acetonides reported in Table 1 were obtained within 30 minutes by simple filtration of polymer bound phosphine oxide, the only by-product of the reaction, with high purity. All the physical data reported in Table 1 within parentheses were withdrawn from the literature. A comparison of the data shows that the yields of thermodinamically more stable isopropylidene derivatives are often higher than those of other methods.

Table 1 *O*-Isopropylidene Derivatives of Miscellaneous Sugars[a]

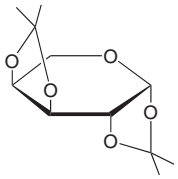
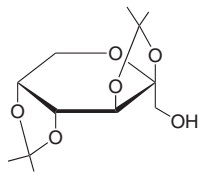
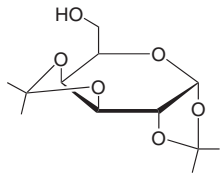
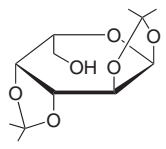
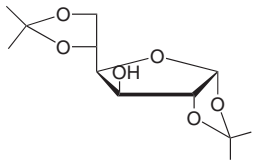
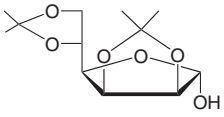
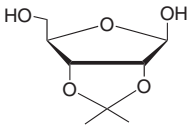
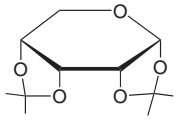
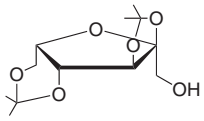
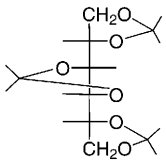
Isopropylidene derivatives from	Yield(%)	Mp (°C)	$[\alpha]_D^{25}$	^{1}H NMR ($CDCl_3$) Elemental Analysis
 L-Arabinose	95 (85)[6]	40–41 (40–41)[18]	+6.1 (*c* = 1.5, H_2O) +5.8 (*c* = *unknown*, H_2O)[7]	1.32 (s, 3 H, CH_3), 1.41 (s, 3 H, CH_3), 1.47 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 3.64 (d, $J_{5a,5b}$ = 12.5 Hz, 1 H, H_a-5), 3.82 (d, $J_{5b,5a}$ = 12.5 Hz, 1 H, H_b-5), 4.23 (br d, $J_{4,3}$ = 7.5 Hz, 1 H, H-4), 4.30 (dd, $J_{2,1}$ = 5.3 Hz, $J_{2,3}$ = 2.3 Hz, 1 H, H-2), 4.54 (dd, $J_{3,4}$ = 7.5 Hz, $J_{3,2}$ = 2.3 Hz, 1 H, H-3), 5.48 (d, $J_{1,2}$ = 5.3 Hz, 1 H, H-1). Anal. Calcd for $C_{11}H_{18}O_5$: C, 57.38; H, 7.88. Found: C, 57.47; H, 7.90.
 D-Fructose	90 (55)[19]	95–96 (97)[19]	–32.9 (*c* = 1.5, H_2O) –24.7 (*c* = 1.1, $CHCl_3$)[19]	1.28 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 2.28 (br s, 1 H, OH), 3.62 (d, $J_{1a,1b}$ = 10.7 Hz, 1 H, H_a-1), 3.66 (d, $J_{1b,1a}$ = 10.7 Hz, 1 H, H_b-1), 3.79 (dd, $J_{6a,6b}$ = 13.0 Hz, $J_{6a,5}$ = 0.8 Hz, 1 H, H_a-6), 3.88 (dd, $J_{6b,6a}$ = 13.0 Hz, $J_{6b,5}$ = 2.0 Hz, 1 H, H_b-6), 4.21 (ddd, $J_{5,4}$ = 7.7 Hz, $J_{5,6b}$ = 2.0 Hz, $J_{5,6a}$ = 0.8 Hz, 1 H, H-5), 4.32 (dd, $J_{3,4}$ = 2.9 Hz, 1 H, H-3), 4.57 (dd, $J_{4,5}$ = 7.7 Hz, $J_{4,3}$ = 2.9 Hz, 1 H, H-4). Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.45; H, 7.69.
 D-Galactose	95 (76–92)[3]	Oil (oil)[3]	–59.5 (*c* = 1.5, $CHCl_3$) –55.0 (*c* = 3.6, $CHCl_3$)[3]	1.32 (s, 6 H, CH_3), 1.44 (s, 3 H, CH_3), 1.55 (s, 3 H, CH_3), 3.62–3.81 (m, 1 H, H_a-6), 3.84–3.95 (m, 2 H, H_b-6, H-5), 4.28 (dd, $J_{4,3}$ = 8.0 Hz, $J_{4,5}$ = 1.5 Hz 1 H, H-4), 4.34 (dd, $J_{2,1}$ = 5.0 Hz, $J_{2,3}$ = 2.0 Hz, 1 H, H-2), 4.62 (dd, $J_{3,4}$ = 8.1 Hz, $J_{3,2}$ = 2.0 Hz, 1 H, H-3), 5.57 (d, $J_{1,2}$ = 5.0 Hz, 1 H, H-1) Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.29; H, 7.71.
 L-Galactose	97 (58)[20]	Syrup (syrup)[20]	+57.0 (*c* = 0.9, $CHCl_3$) *(not reported)*	Superimposable to that of the D-enantiomer Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.48; H, 7.76.

Table 1 *O*-Isopropylidene Derivatives of Miscellaneous Sugars[a] (continued)

Isopropylidene derivatives from	Yield(%)	Mp (°C)	$[\alpha]_D^{25}$	^{1}H NMR ($CDCl_3$) Elemental Analysis
 D-Glucose	95 (91)[3]	108–109 (110–111)[3]	–18.5 (*c* = 5.0, H_2O) –18.5 (*c* = 5.0, H_2O)[3]	1.30 (s, 3 H, CH_3), 1.35 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 3.97 (dd, $J_{6a,6b}$ = 8.7 Hz, $J_{6a,5}$ = 5.1 Hz, 1 H, H_a-6), 4.04 (dd, $J_{4,5}$ = 8.0 Hz, $J_{4,3}$ = 2.9 Hz, 1 H, H-4), 4.15 (dd, $J_{6b,6a}$ = 8.7 Hz, $J_{6b,5}$ = 6.6 Hz, 1 H, H_b-6), 4.25–4.38 (m, 2 H, H-3, H-5), 4.51 (d, $J_{2,1}$ = 3.7 Hz, 1 H, H-2), 5.92 (d, $J_{1,2}$ = 3.7 Hz, 1 H, H-1). Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.43; H, 7.72.
 D-Mannose	95 (92)[3]	121–122 (122–123)[3]	+16.6 (*c* = 2.5, EtOH) +16.0 (*c* = 2.6, EtOH)[3]	1.32 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 1.46 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 4.03 (dd, $J_{6a,6b}$ = 10.2 Hz, $J_{6a,5}$ = 6.1 Hz, 1 H, H_a-6), 4.08 (dd, $J_{6b,6a}$ = 10.2 Hz, $J_{6b,5}$ = 6.8 Hz, 1 H, H_b-6), 4.20 (dd, $J_{4,3}$ = 7.2 Hz, $J_{4,5}$ = 3.7 Hz, 1 H, H-4), 4.38–4.44 (m, 1 H, H-5), 4.63 (d, $J_{2,3}$ = 6.2 Hz, 1 H, H-2), 4.81 (dd, $J_{3,4}$ = 7.2 Hz, $J_{3,2}$ = 6.2 Hz, 1 H, H-3), 5.39 (s, 1 H, H-1). Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.30; H, 7.73.
 D-Ribose	95 (90–93)[21a,b]	Oil (oil)[21c]	–24.7 (*c* = 1.1, $CHCl_3$) –25.9 (*c* = 1.1, $CHCl_3$)[21c]	1.32 (s, 3 H, CH_3), 1.48 (s, 3 H, CH_3), 3.55–3.83 (m, 2 H, H-5), 4.40 (br s, 1 H, H-4), 4.57 (d, $J_{2,3}$ = 6.0 Hz, 1 H, H-2), 4.85 (d, $J_{3,2}$ = 6.0 Hz, 1 H, H-3), 5.42 (s, 1 H, H-1). Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.41; H, 7.39.
 D-Ribose	92 (as by-product) [2b]	70–71 (68–69)[2b]	–55.0 (*c* = 1.1, $CHCl_3$) –51.0 (*c* = 0.6, $CHCl_3$)[2b]	1.33 (s, 3 H, CH_3), 1.37 (s, 3 H, CH_3), 1.55 (s, 3 H, CH_3), 1.62 (s, 3 H, CH_3), 3.83 (dd, $J_{5a,5b}$ = 12.1 Hz, $J_{5a,4}$ = 9.7 Hz, 1 H, H_a-5), 4.01 (dd, $J_{5b,5a}$ = 12.1 Hz, $J_{5b,4}$ = 7.7 Hz, 1 H, H_b-5), 4.24 (dd, $J_{2,3}$ = 7.9 Hz, $J_{2,1}$ = 5.2 Hz, 1 H, H-2), 4.40–4.53 (m, 2 H, H-3, H-4), 5.43 (d, $J_{1,2}$ = 5.2 Hz, 1 H, H-1). Anal. Calcd for $C_{11}H_{18}O_5$: C, 57.38; H, 7.88. Found: C, 57.50; H, 7.88.
 L-Sorbose	95 (96–99)[22]	80–81 (77–78)[22]	–14.3 (*c* = 1.5, Me_2CO) –18.1 (*c* = 1.5, Me_2CO)[22]	1.37 (s, 6 H, CH_3), 1.44 (s, 3 H, CH_3), 1.51 (s, 3 H, CH_3), 2.31 (t, $J_{OH,1}$ = 7.5 Hz, 1 H, OH), 3.78 (dd, $J_{1a,1b}$ = 12.5 Hz, $J_{1a,OH}$ = 7.5 Hz, 1 H, H_a-1), 3.87 (dd, $J_{1b,1a}$ = 12.5 Hz, $J_{1b,OH}$ = 7.5 Hz, 1 H, H_b-1), 3.92–4.92 (m, 3 H, H-6, H-5),4.32 (d, $J_{4,3}$ = 1.6 Hz, 1 H, H-4), 4.48 ($J_{4,3}$ = 1.6 Hz, 1 H, H-3). Anal. Calcd for $C_{12}H_{20}O_6$: C, 55.37; H, 7.74. Found: C, 55.29; H, 7.76.
 D-Glucitol	97 (53)[23]	45–46 (45–46)[23]	+12.7 (*c* = 1.1; EtOH) +14.2 (*c* = *unknown*; EtOH)[23]	1.37 (s, 3 H, CH_3), 1.38 (s, 3 H, CH_3), 1.42 (s, 12 H, 4 × CH_3), 3.69–4.12 (m, 8 H, H-1, H-2, H-3, H-4, H-5, H-6). Anal. Calcd for $C_{15}H_{26}O_6$: C, 59.58; H, 8.67. Found: C, 59.48; H, 8.69.

[a] Literature data within parentheses.

^{1}H NMR spectra were taken on Bruker DRX-400 and Varian Gemini 200 spectrometers. Optical rotations were measured on a Jasco P-1010 instrument (1.0 dm cell). Combustion analyses were performed on Perkin-Elmer Series II 2400, CHNS analyzer. TLC analyses were carried out on silica gel Merck 60 F254 plates (0.2 mm layer thickness). Column chromatography was performed on Merck Kieselgel 60 (70–230 mesh). Anhyd acetone was distilled immediately before use. Triphenyl phosphine polymer-bound was purchased from Fluka Chemical Co.

Preparation of 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose; Typical Procedure

To a magnetically stirred suspension of anhyd polystyryl diphenyl phosphine (1.12 g, ca. 3.34 phosphine units) in anhyd acetone (10 mL) at r.t., a solution of I_2 (0.85 g, 3.34 mmol) in the same solvent (30 mL) was added dropwise in the dark and under dry N_2 atmosphere. After 15 min, solid D-glucopyranose (0.33 g, 1.67 mmol) was added in one portion to the suspension. TLC monitoring ($CHCl_3$–MeOH, 9:1) showed that the starting sugar was completely consumed within 30 min. The reaction mixture was then filtered

through a glass sinter funnel and washed with acetone. The solvent was removed under reduced pressure and the solid residue was recrystallized from $CHCl_3$–hexane (1:2) to give the final product (0.41 g, 95% yield).

Under the same conditions, the *O*-isopropylidene derivatives shown in Table 1 were prepared using one equivalent of triphenyl phosphine polymer-bound/iodine complex per acetonide group expected in the product.

Acknowledgment

^{1}H NMR spectra were performed at Centro Interdipartimentale di Metodologie Chimico-Fisiche, Università di Napoli Federico II.

References

(1) Goi, A.; Bruzzese, T.; Notarianni, A. F.; Riva, M.; Ronchini, A. *Arzneim. Forsch./Drug Res.* **1979**, *7*, 986.
(2) (a) Stevens, J. D. *Chem. Commun.* **1969**, 1140. (b) Hughes, N. A.; Speakman, P. R. H. *Carbohydr. Res.* **1965**, *1*, 171.
(3) Schmidt, O. T. *Meth. Carbohydr. Chem.* **1963**, *2*, 318.
(4) (a) Nair, P. R. M.; Shah, P. M.; Sreenivasan, B. *Starch* **1981**, *33*, 384. (b) Nikiforov, V. A.; Zarutsski, V. V.; Chapanov, I. D.; Vasil'eva, I. B.; Dolgushina, N. N. *Khim.-Farm. Zh.* **1982**, *16*, 1102; *Chem. Abstr.* **1982**, *97*, 215482x.
(5) (a) Mongenlie, S. *Acta Chem. Scand.* **1973**, *27*, 3609. (b) Mongenlie, S. *Acta Chem. Scand., Ser. B* **1975**, *297*, 367.
(6) Kartha, K. P. R. *Tetrahedron Lett.* **1986**, *27*, 3415.
(7) Singh, P. P.; Gharia, M. M.; Dasgupta, F.; Srivastava, H. C. *Tetrahedron Lett.* **1977**, 439.
(8) Pfaff, P. K.; Paust, J.; Hartmann, H. Ger. Offen, DE 3505150, **1986**; *Chem. Abstr.* **1985**, *106*, 18984r.
(9) Lal, B.; Gidwani, R. M.; Rupp, R. H. *Synthesis* **1989**, 711.
(10) Rauter, A. P.; Ramoa-Ribeiro, F.; Fernandes, A. C.; Figueiredo, J. A. *Tetrahedron* **1995**, *51*, 6529.
(11) Meskens, F. A. J. *Synthesis* **1981**, 501.
(12) (a) Catelani, G.; Colonna, F.; Marra, A. *Carbohydr. Res.* **1988**, *182*, 297. (b) Gelas, J.; Horton, D. *Heterocycles* **1981**, *16*, 1587.
(13) Leadbeater, N. E.; Marco, M. *Chem. Rev.* **2002**, *102*, 3217.
(14) Dewry, D. H.; Coe, D. M.; Poon, S. *Med. Res. Rev.* **1999**, *19*, 97.
(15) Caputo, R.; Ferreri, C.; Noviello, S.; Palumbo, G. *Synthesis* **1986**, 499.
(16) (a) Bernard, M.; Ford, W. T. *J. Org. Chem.* **1983**, *48*, 326. (b) Regen, S. L.; Lee, D. P. *J. Org. Chem.* **1975**, *40*, 1669.
(17) Triphenylphosphine–iodine complex showed the same reactivity of the polymer bound/iodine complex towards all the tested sugars.
(18) Stacey, B. E.; Tierney, B. *Carbohydr. Res.* **1976**, *49*, 129.
(19) Martin, O. R.; Korppi-Tommola, S.-L.; Szare, W. A. *Can. J. Chem.* **1982**, *60*, 1857.
(20) May, J. A. Jr.; Sartorelli, A. C. *J. Med. Chem.* **1979**, *22*, 971; and references cited therein.
(21) (a) Shaikh, N. S.; Bhor, S. S.; Gajare, A. S.; Deshpande, V. H.; Wakharkar, R. D. *Tetrahedron Lett.* **2004**, *45*, 5395. (b) Moon, H. R.; Choi, W. J.; Kim, H. O.; Jeong, L. S. *Tetrahedron: Asymmetry* **2002**, *13*, 1189. (c) Asakura, J.; Matsubara, Y.; Yoshihara, M. *J. Carbohydr. Chem.* **1996**, *15*, 231.
(22) von Reichstein, T.; Grüssner, A. *Helv. Chim. Acta* **1934**, *17*, 311.
(23) Pressman, B. C.; Anderson, L.; Lardy, H. A. *J. Am. Chem. Soc.* **1950**, *72*, 2404; references cited therein.