CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES

Javier Castilla López

Dipòsit Legal: T.1214-2012

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

JAVIER CASTILLA LÓPEZ

CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES

DOCTORAL THESIS

Supervised by

Dra. Yolanda Díaz Giménez and Dr. Sergio Castillón Miranda

Departament de Química Analítica i Química Orgànica

UNIVERSITAT ROVIRA I VIRGILI

Tarragona, 2012

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

UNIVERSITAT ROVIRA I VIRGILI

Departament de Química Analítica i Química Orgànica
Facultat de Química
c/ Marcel·lí Domingo, s/n
43007, Tarragona

Els sotasignants Sergio Castillón Miranda, Catedràtic de Química Orgànica, i Yolanda Díaz Giménez, Professora Titular de Química Orgànica, del Departament de Química Analítica i Química Orgànica de la Universitat Rovira i Virgili,

FEM CONSTAR que aquesta memòria, titulada *Carbohydrate manipulations towards high-mannose oligosaccharides*, que presenta en Javier Castilla López per optar al grau de Doctor en Química per la Universitat Rovira i Virgili, ha estat realitzada sota la nostra direcció al Departament de Química Analítica i Química Orgànica d'aquesta universitat, així com en d'altres laboratoris universitaris en el marc d'una sèrie de col·laboracions científiques i que, a més, compleix els requeriments per poder optar a la Menció Europea.

Tarragona, 14 de juny de 2012

Dr. Sergio Castillón Miranda

Dra. Yolanda Díaz Giménez

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

La present memòria de Tesi Doctoral s'ha dut a terme gràcies al *Programa de Formación del Profesorado Universitario* (referència: AP2007-03782), finançat pel Ministerio de Ciencia e Innovación. La realització d'aquesta Tesi Doctoral ha estat possible gràcies al finançament dels següents projectes d'investigació:

- *Síntesis de monómeros, oligómeros, dendrímeros y polímeros derivados de carbohidratos con propiedades antibióticas y antivirales* (CTQ2005-03124-BQU)

- *New synthetic methods in carbohydrate chemistry* (CTQ2008-01569-BQU)

- *Diseño de Catalizadores para una Química Sostenible: Una Aproximación Integrada. Consolider Ingenio 2010 - Proyecto INTECAT* (CSD2006-0003)

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

CONTRIBUCIONS A CONGRESSOS

Castilla, J.; Cruz, D.; Rísquez, R.; Ortiz Mellet, C.; García Fernández, J. Díaz, Y.; Castillón, S. **Carbohydrate-based 1,3-oxazoline-2-thiones: from synthesis to biological applications**. 26th International Carbohydrate Symposium. Comunicació flash F61. Madrid, **2012**.

Castilla, J. **Synthesis and biological evaluations of 1,3-oxazoline-2-thione-sugar derivatives.** CEICS Nobel Campus - Chemistry for life. Comunicació oral. Tarragona, **2012**.

Castilla, J.; Cruz, D.; Rísquez, R.; Ortiz Mellet, C.; García Fernández, J. Díaz, Y.; Castillón, S. **Bicyclic pyranose—2-alkylsulfanyl-1,3-oxazoline glycomimetics: Conformationally-locked *N*-glycosides with selective β-glucosidase inhibitory activity**. CEICS Nobel Campus - Chemistry for life. Pòster. Tarragona, **2012**.

Castilla, J.; Cruz, D.; Rísquez, R.; Ortiz Mellet, C.; García Fernández, J. Díaz, Y.; Castillón, S. **Synthesis, derivatization and biological evaluation of 1,3-oxathiolan-2-imine-carbohydrate derivatives**. 16th European Carbohydrate Symposium. Pòster PO 53. Sorrento-Nàpols, **2011**.

Díaz, Y.; Marín, I.; Castilla, J.; Matheu, M. I.; Castillón, S. **Synthetic transformations from gycal epoxides**. X Jornadas de Carbohidratos. Reunión del Grupo Especializado de Hidratos de Carbono de la RSEQ. Comunicació convidada IL 6. Granada, **2010**.

Castilla, J.; Cruz, D.; Matheu, M. I.; Díaz, Y.; Castillón, S. **Synthesis and derivatization of 1,2-oxycarbonimidoylthio-sugar derivatives**. X Jornadas de Carbohidratos. Reunión del Grupo Especializado de Hidratos de Carbono de la RSEQ. Pòster P8. Granada, **2010**.

Castilla, J.; Marín, I.; Matheu, M. I.; Díaz, Y.; Castillón, S. **Síntesi i reaccions de 1,2-anhidrosucres. Síntesi de carbohidrats 1,3-oxatiolan-, 1,3-oxaselenolan- i 1,3-oxazolidin-2-derivats**. VI Trobada de Joves Investigadors dels Països Catalans. Comunicació oral 2.3.1. València, **2010**.

Castilla, J.; Matheu, M. I.; Díaz, Y.; Castillón, S. **Synthesis of 1,2-oxycarbonimidoylthio-sugar derivatives**. 15th European Carbohydrate Symposium. Pòster PA 100. Viena, **2009**.

Castilla, J.; Matheu, M. I.; Díaz, Y.; Castillón, S. **Towards the synthesis of glycal-1,2-episulfides. Synthesis of 1-deoxy-1,2-oxycarbonimidoylthio-sugar derivatives**. IV Reunión Química-Orgánica del Mediterráneo, REQOMED. Pòster P-15. Girona, **2008**.

Castilla, J.; Matheu, M. I.; Díaz, Y.; Castillón, S. **An approach to the synthesis of glycal-1,2-episulfides**. IV Iberian Carbohydrate Meeting. IX Jornadas de Carbohidratos. Pòster P04. Santiago de Compostela, **2008**.

PUBLICACIONS

Castilla, J.; Rísquez, R.; Cruz, D.; Higaki, K.; Nanba, E.; Ohno, K.; Suzuki, Y.; Díaz, Y.; Ortiz Mellet, C.; García Fernández, J. M., Castillón, S. **Conformationally-locked *N*-glycosides with selective β-glucosidase inhibitory activity: identification of a new pharmacological chaperone for Gaucher disease.** *J. Med. Chem.* **2012**, *55*, 6857-6865.

Marín, I.; Castilla, J.; Matheu, M. I.; Díaz, Y.; Castillón, S. **Sequential directed epoxydation-acidolysis from glycals with MCPBA. A flexible approach to protected glycosyl donors.** *J. Org. Chem.* **2011**, *76*, 9622-9629.

Castilla, J.; Marín, I.; Matheu, M. I.; Díaz, Y.; Castillón, S. **Short and general procedure for synthesizing cis-1,2-fused 1,3-oxazolidine-2-thione and 2-selone carbohydrate derivatives.** *J. Org. Chem.* **2010**, *75*, 514-517. Corrigendum *J. Org. Chem.* **2012**, *77*, 3687.

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

AGRAÏMENTS

Desitjo començar la memòria dedicant unes paraules d'agraïment a la Dra. Yolanda Díaz i al Dr. Sergio Castillón, directors de la tesi doctoral. Sense la seva ajuda, confiança, dedicació i tenacitat no hauria estat possible l'elaboració d'aquest treball. Agraeixo que m'hagin donat l'oportunitat de formar part del seu grup de recerca.

Voldria fer extensiu el meu agraïment a la Dra. María Isabel Matheu, qui sempre ha trobat temps per escoltar-me i aconsellar-me durant la realització d'aquest estudi.

Expreso también mi agradecimiento al Dr. Eddy Maerten y, en especial, al Dr. Antoine Baceiredo por brindarme la oportunidad de realizar una estancia de seis meses en el Laboratoire Hétérochimie Fondamentale et Appliquée de la Université Paul Sabatier de Toulouse. Gracias también a todos los compañeros con los que compartí aquellos meses (Fanny P., Juliette B., Jordi A., Noel N. y Ricardo R.). No puc deixar de recordar aquells amics fora del laboratori, en Marcos O. i la Raquel M. Un tout grand merci à vous tous pour avoir rendu mon séjour en France inoublidable.

Merecen una mención especial aquí la Dra. Carmen Ortiz Mellet y el Dr. José M. García Fernández, por la colaboración y el interés prestados en el último capítulo de la memoria. También en este apartado deseo poner de relieve la labor de Deysi Cruz y Rocío Rísquez. Gracias por vuestras aportaciones.

Vull agrair també a totes aquelles persones que conformen el departament de Química Analítica i Química Orgànica la seva ajuda i amabilitat en els moments de dificultats.

Durant aquests quatre anys com a becari he treballat i conviscut amb un grup de persones excepcionals, persones que són gairebé com una segona familia per a mi. El meu agraïment va dirigit a tots ells, tant els que ja han marxat (David B., Patri. V., Josep Ll., Isidro C. i Irene M.), com els que s'han incorporat recentment (Sébastien S., Joan G., Emma B., Omar B. i Míriam S.). En aquest sentit tampoc no puc deixar de mencionar els veterans que es troben gairebé en la mateixa situació que jo (Míriam D., Mariam A. i Ismael A.). Gràcies a tots pels consells, les pauses i tots els moments que hem viscut, de debò: moltes gràcies.

Arribat aquest moment, no voldria oblidar-me dels bons amics que m'han acompanyat durant aquesta tesi, com ara l'Eugènia M., la Sílvia S., la Gemma B., la Neus B., la Lucía T., la Maria A., la Gemma S. i la Laura R. Gràcies pel suport i l'ajuda que sempre m'heu proporcionat. També vull recordar aquí aquelles persones que han patit com jo aquest procés i han volgut compartir-ne un tram: la Dolores G. i la Mireia M. Moltes gràcies a les dues.

Me siento muy afortunado de haber podido trabajar codo con codo con Irene. Gracias por iluminar el laboratorio con tu forma de ser y ahuyentar los fantasmas de esta tesis doctoral.

Gràcies, Cristina, per haver cregut sempre en mi, fins i tot quan jo mateix no hi creia. Encara que els nostres camins arribin a separar-se, sempre trobarem cinc minuts per tocar un *Le Monsieur* plegats.

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

A tu, Eduard, per fer-me costat sempre de forma incondicional en els bons i en els mals moments, per fer-me creure en cada moment que podia amb aquest projecte, per tots els esforços que has fet per mi i per fer-me feliç. Moltes gràcies per formar part de la meva vida.
Finalmente me queda dar las gracias a mi familia, especialmente a mis hermanos Sergio y Toni. Muchísimas gracias por tener la puerta siempre abierta para mí. El agradecimiento más profundo y sentido va dirigido a mi madre, a quien dedico esta tesis doctoral de todo corazón por su capacidad de encajar, resistir y superar todas las adversidades de la vida.

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

«L'originalitat consisteix en el retorn a l'origen;
així doncs, original és allò que torna a la
simplicitat de les primeres solucions»

Antoni Gaudí i Cornet

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

TABLE OF CONTENTS

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

ABBREVIATIONS AND ACRONYMS

A

Abs	absorbance
ABX	ambroxol
Ac	acetyl
acac	acetylacetonate
AIBN	azobisisobutyronitrile
aq	aqueous
ATR	attenuated total reflectance

B

Bn	benzyl
Boc	*tert*-butyl carbonate
bs	broad singlet
BSP	1-benzenesulfinyl piperidine
Bz	benzoyl

C

Calcd	calculated
CAZy	Carbohydrate-Active Enzyme classification
Conv.	Conversion
CS	(+)-castanospermine

D

d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCE	1,2-dichloroethane
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIX	1,5-dideoxy-1,5-imino-D-xylitol
DMAP	4-dimethylaminopyridine
DMDP	(*2R, 3R, 4R, 5R*)-2,5-bis(hydroxymethyl)-3,4-dihydropyrrolidine
DMEM	Dulbecco/Vogt modified Eagle's minimal essential medium
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNJ	1-deoxynojirimycin
DPn	number-average degree of polymerization
dtc	dithiocarbamate

dtp	dithiophosphate

E

E	enzyme
El	electrophile
EO	ethylene oxide
ER	endoplasmatic reticulum
ES	enzyme-substrate complex
ESI	electrospray ionization
Et	ethyl

F

FBS	fetal bovine serum
FCC	flash column chromatography
FT	Fourier transform

G

g	gram(s)
GCase	β-glucocerebrosidase
gCOSY	gradient correlation spectroscopy
GH	glycoside hydrolases
gHMBC	gradient heteronuclear multiple bond correlation
gHSQC	gradient heteronuclear single quantum coherence
Glc	glucose
GlcCer	glucosyl ceramide
GlcNAc	*N*-acetyl-D-glucosamine

H

h	hour(s)
HCV	human hepatitis C
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
HSAB	Hard and soft (Lewis) acids and bases
Hz	hertz(s)

I

I	inhibitor
IC_{50}	half maximal inhibitory concentration
IDipp	1,3-di-*tert*-butylimidazol-2-ylidene
imid	imidazole

In	initiator
^{i}Pr	isopropyl
IR	infrared
I^{t}Bu	1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene

J

J	coupling constant

K

K_{app}	apparent dissociation constant
K_{cat}	constant of proportionality
K_{i}	inhibition constant
K_{m}	Michaelis constant

L

L	litre(s)
LA	Lewis acid
LC/MSD	liquid chromatography/mass spectrometry detector
LDH	lactate dehydrogenase
LSD	lysosomal storage disease

M

m	meter(s)
m (in NMR)	multiplet
M	molar
m/z	mass under charge
MALDI-TOF	matrix-assisted laser desorption/ionisation time-of-flight
Man	mannose
MBCG	methyl 4,6-*O*-benzylidene-2,3-*O*-carbonyl-α-D-glucopyranoside
Me	methyl
min	minute(s)
Mp	melting point
MS	mass spectrometry

N

n	number of repeat units
NDP	nucleotide diphosphosugar
NHC	*N*-heterocyclic carbene
NI	no inhibition
NIS	*N*-bromosuccinimide
NMR	nuclear magnetic resonance

NNDNJ	*N*-(n-nonyl)-1-deoxynojirimycin
NOESY	nuclear Overhauser effect spectroscopy
Nu	nucleophile

O

OAT	1,3-oxazolidine-2-thione

P

P	product
pClBn	para-chlorobenzyl
PEEC	poly(ethylene ether carbonate)
PEO	poly(ethylene oxide)
PG	protecting group
Ph	phenyl
ppm	parts per million
PSO	pyranose —2-alkylsulfanyl-1,3-oxazoline
PTSA	*p*-toluenesulfonic acid

Q

q	quadruplet

R

R	radical
Rf	retention factor
ROP	ring-opening polymerization

S

s	singlet
S	substrate
sat.	saturated
Select.	selectivity
SEM	standard error of the mean

T

t	time
t (in NMR)	triplet
TBDMS	tert-butyldimethylsilyl
TCA	trichloroacetimidate
TES	triethyl silyl
TFMA	trifluoromethane sulfonic acid
Tf	trifluoromethane sulfonyl

THF	tetrahydrofuran
TLC	thin layer chromatography
TOF	time of flight
Tol	tolyl
TBD	1,5,7-triazabicyclo[4.4.0]dec-5-ene

U

UV	ultra-violet

V

v	reaction rate
v_{max}	maximum reaction rate
VNMR	Varian nuclear magnetic resonance

#

[I]	concentration of inhibitor
[S]	concentration of substrate
δ (in IR)	bending
δ (in NMR)	chemical shift
ΔGp	Variation of the Gibbs free energy during a polymerization process
ΔHp	Variation of the enthalpy during a polymerization process
ΔSp	Variation of the entropy during a polymerization process
υ (in IR)	stretching
υ (in IR, neat)	wave number

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

SUMMARY

The final goal of this thesis is the development of strategic methods for the synthesis of well-defined 1,2-linked mannose oligosaccharides and *S*-linked thio-analogues presenting low polydispersity indexes through different polymerization techniques. In this context, the present work aims to develop new procedures in carbohydrate chemistry, focusing not only in the oligomerization reactions, but also in the development of efficient syntheses for accessing the suitable monomers. With this purpose, the objectives of the present work are the following:

1. Synthesis of 1,2-linked mannose oligosaccharides. The specific aims for the synthesis of *manno*-oligosaccharides are the synthesis and oligomerization of different carbohydrate-based monomers, including epoxides and carbonates.

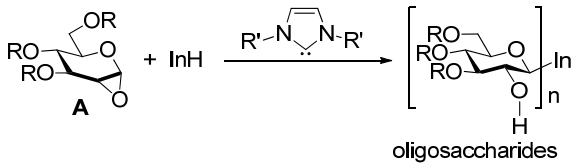
2. Synthesis of *S*-1,2-linked *manno*-thiooligosaccharides. The work presented in this section has as a final objective the development of new methods for preparing *manno*-thiooligosaccharides of interest starting from *manno*-epithiosugars and analogous monomers. To achieve this objective it is necessary to establish efficient protocols for accessing the starting materials.

Synthesis of 1,2-linked mannose oligosaccharides

Chapter 3 investigated *N*-heterocyclic carbenes (NHCs) in order to bring about the ring-opening polymerization (ROP) of 1,2-anhydro-carbohydrates **A** with the objective to achieve oligosaccharides (Scheme I). This methodology affords exclusively linear polymers in a very versatile fashion with simple conventional epoxides, combining excellent control of molar masses, exhibiting narrow polydispersities, and quantitative chain-end functionalization.[1]

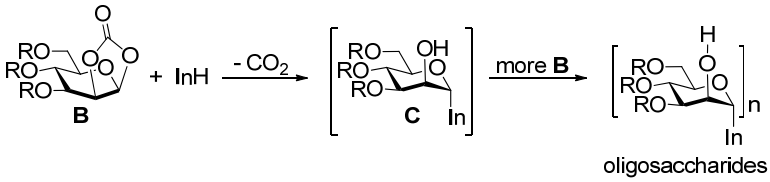
However, application of this organocatalytic procedure to the synthesis of carbohydrate-derived oligomers was totally unsuccessful because of the particular reactivity of 1,2-anhydro-carbohydrates.

This study has been carried out in collaboration with Dr. Antoine Baceiredo in the Laboratoire Hétérochimie Fondamentale et Appliquée from Université Paul Sabatier (Toulouse).



Scheme I. NHC-catalyzed ring-opening oligomerization of 1,2-*gluco*-epoxides

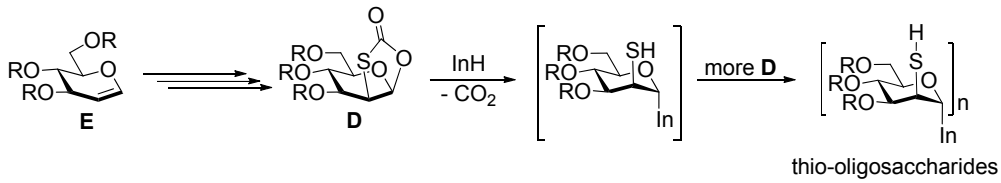
Chapter 4 studied the ring-opening oligomerization of 1,2-*O*-carbonyl-α-D-mannoses (**B**) to the straightforward synthesis of oligo-α-mannosides (Scheme II). These kinds of monomers are known to undergo ring-opening polymerization that partly proceeds with elimination of carbon dioxide to give copolymers formed by carbonate and ether linkages.[2] The synthesis and the reaction of mannose derived cyclic carbonates **B** with several nucleophiles was studied under different polymeration conditions. Mannopyranoside **C** was synthesized employing soft nucleophiles as initiators. Unfortunately, this compound was not reactive enough to attach the starting material in excess and therefore the oligomerization reaction did not further evolve.



Scheme II. ROP and consecutive decarboxylation of 1,2-*O*-carbonyl-α-D-mannoses

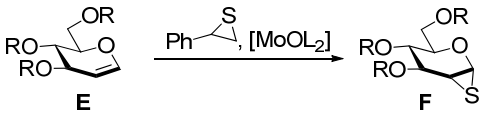
Synthesis of *S*-1,2-linked manno-thiooligosaccharides

Chapter 4 also focused on the synthesis of 2-thio-(1→2)-mannopyranosides by the ring-opening oligomerization of 1-*O*,2-*S*-thiocarbonyl-α-D-mannoses **D** with several nucleophiles under different polymeration conditions. This approach followed the same proposed methodology for the obtention of 1,2-linked mannose oligosaccharides (Scheme II). In connection with this purpose, the chemical synthesis of these compounds starting from glycals **E** was undertaken (Scheme III). Application of this methodology to the ROP of thiocarbonyl-α-D-mannose **D** failed and complex mixtures were always obtained.



Scheme III. ROP and consecutive decarboxylation of 1-*O*, 2-*S*-thiocarbonyl-α-D-mannoses

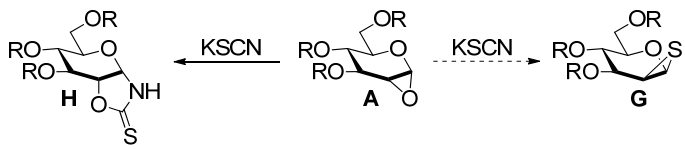
Chapter 5 explored the feasibility of molybdenum oxo complexes as sulfur-transfer agents for the episulfidation of glycals (**E**) in order to synthesize glycal-1,2-episulfides (**F**) by employing phenylthiirane as a cheap and easily accessible sulfur source (Scheme IV). Although this reaction is considered one of the most efficient methods for thiirane preparation,[3] application of this methodology to unsaturated carbohydrates was unsuccessful.



Scheme IV. Molydenum-catalyzed episulfidation reaction of glycals

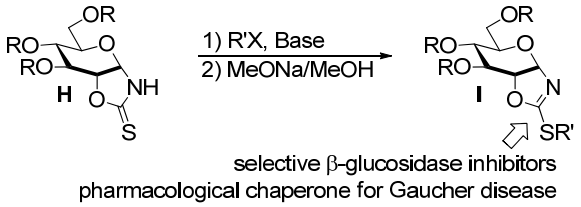
Chapter 6 has also studied the synthesis of *manno*-1,2-epithiosugars (**G**) by conversion of epoxides **A** with inorganic thiocyanates. Although this methodology has been used onto the saccharidic scaffold,[4] it has not been applied to 1,2-epoxy sugars. Unexpectely, employment of alkali thiocyanates afforded exclusively glyco-1,3-oxazolidine-2-thione **H** (Scheme V). On account of this unexpected reactivity, a new methodology was developed for the construction of carbohydrate-based *cis*-1,2-fused 1,3-oxazolidine-2-thione and 1,3-oxazolidine-2-selone derivatives.

The procedure was compatible with several protecting groups such as acyl, benzyl and silyl, and also with sugars of different configurations. The structure of these oxazolidine-2-thione derivatives was confirmed by X-ray crystallography.



Scheme V. Unexpected reactivity of 1,2-anhydrocarbohydrates with potassium thiocyanate

Chapter 7 made account of this unexpected reactivity and it focused on the synthesis of several *cis*-1,2-fused pyranose—1,3-oxazoline-2-thione derivatives (**I**) bearing different substituents at the exocyclic sulfur as new enzyme inhibitors. The polyhydroxylated bicyclic system was built in only two steps by S-alkylation and acetyl deprotection from the corresponding glyco-1,3-oxazolidine-2-thione **H** (Scheme VI). A biological screening against several glycosidase enzymes showed highly specific inhibition of mammalian β-glucosidase with a marked dependence of the potency upon the nature of the exocyclic substituent. The most potent representative, bearing an *S*-(ω-hydroxyhexadecyl) substituent, was further assayed as inhibitor of the human lysosomal β-glucocerebrosidase and as pharmacological chaperone in Gaucher disease fibroblasts. Activity enhancements in N370S/N370 mutants analogous to those achieved with the reference compound ambroxol were attained, with a more favourable chaperone/inhibitor balance.



Scheme VI. Synthesis of 2-*S*-alkylsulfanyl-1,3-oxazoline D-glucopyranose derivatives

References

1. a) Raynaud, J.; Absalon, C.; Gnanou, Y.; Taton, D. *Macromolecules*, **2010**, *43*, 2814-2823. b) Raynaud, J.; Absalon, C.; Gnanou, Y.; Taton, D. *J. Am. Chem. Soc.* **2009**, *131*, 3201-3209.
2. a) Clements, J. H. *Ind. Eng. Chem. Res.* **2003**, *42*, 663-674. b) Rokicki, G. *Prog. Polym. Sci.* **2000**, *25*, 259-342.
3. Adam, W.; Bargon, R. M.; Schenk, W. A. *J. Am. Chem. Soc.* **2003**, *125*, 3871-3876.
4. Repetto, E.; Manzano, V. E.; Uhrig, M. L.; Varela, O. *J. Org. Chem.* **2011**, *77*, 253-265.

CHAPTER 1

GENERAL INTRODUCTION

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

1.1. Synthesis and properties of oligosaccharides

Oligosaccharides form a complex group of biomolecules with an unsurpassed structural diversity, performing a variety of biological functions. They play thereby a fundamental role in the development, growth, and functioning of cells and living organisms.[1] Their almost omnipresent occurrence has generated great interest in biology and chemistry. Thus, development of carbohydrate-based therapies has increased the demand for plenty quantities of pure oligosaccharides. However, their isolation from natural sources is very difficult, involving tedious purifications, and it only provides small quantities of material that often lacks the required degree of purity for detailed biochemical studies. Therefore, the synthesis of polysaccharides having defined structures has become an important field of research.[2]

1.1.1. Natural occurrence and importance of high-mannose oligosaccharides

High-mannose type oligosaccharides are a subset of the *N*-glycan polymeric chains, containing from five to nine mannose residues. They can be found attached to

proteins as glycoproteins and proteoglycans,[3] where they have an important role in many biological events such as cell-cell adhesion, immune system modulation, and signal transduction. In most cases, the role of the carbohydrates in these functions is still obscure. However, there is evidence that carbohydrates are involved in stabilization of glycoproteins and in recognition phenomena.[4] Furthermore, the modes of molecular recognition toward high-mannose oligosaccharides have been intensively studied for mannose-binding protein which belongs to different pathogens,[5] including retroviruses.[6]

For example, most of the envelope surface of human immunodeficiency virus (HIV) is covered by high-mannose-type *N*-glycans attached to the viral envelope protein, gp120 (Figure 1.1).[7] This glycoprotein is essential in HIV infection and therefore high-mannose type oligosaccharides present in the gp120 molecule are essentially critical in viral attachment and initiation of infection.[8]

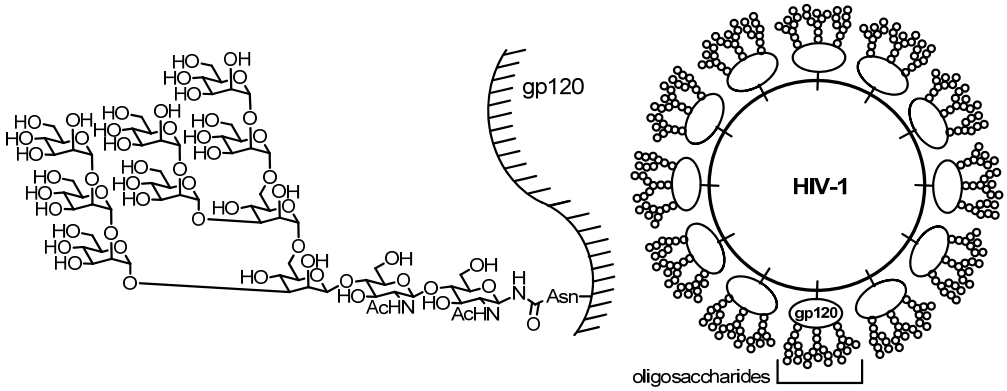


Figure 1.1. Chemical structure of $Man_9GlcNAc_2$, one of the oligosaccharides contained in glycoprotein gp120 (left)[9] and schematic representation of HIV-1, emphasizing the envelope gp120 and the oligosaccharides (right)[10]

Investigation on the oligosaccharides of gp120 not only clarified the role of carbohydrates in HIV-infection, but also indicated a strategy for designing antiviral agents and novel vaccines, by targeting the cluster presentation of the oligomannosides on the virus surface.[9-11]

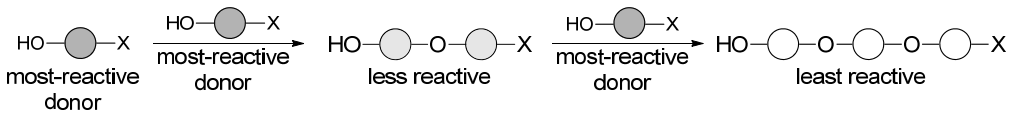
These circumstances have stimulated the chemical synthesis of high-mannose type cell surface glycans, which are found throughout nature as *N*-linked glycoconjugates, as a method to provide sufficient quantities for further research. Achievements have been made in glycosidic bond formation over the last decades;[12] however, new processes for forming this linkage in a regio- and stereoselective manner are still needed.

1.1.2. Synthesis of *manno*-oligomers

To access to pure and well-defined oligosaccharides and glycoconjugate structures, one must typically resort to chemical synthesis, reliying on custom-tailored strategies for each target compound.

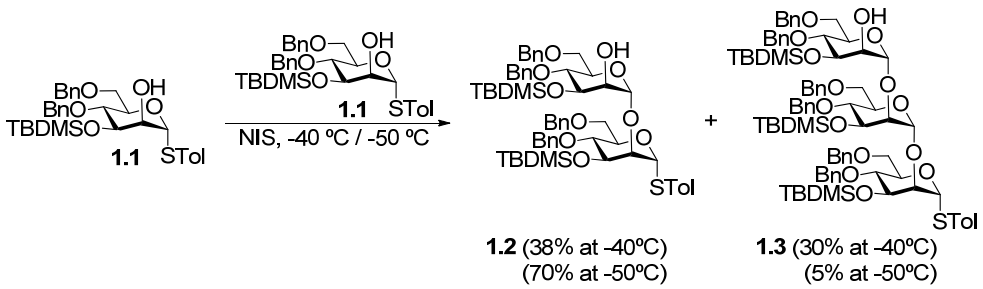
Classical solution syntheses allow efficient homogeneous conditions. However, there are not only problems with loading capacity, but also with product losses during purification.[13] Recently, fluorous chemistry, employing fluorous tags for purification, has been studied intensively for the synthesis of oligosaccharides.[14]

In 2004 Wong developed a new methodology to produce biologically relevant 1,2-*manno*-oligosaccharides based on one-pot self-condensation reactions. In this strategy, the most reactive monomer undergoes self-condensation to give a less-reactive dimer. The disaccharide then serves as an acceptor for another molecule, which leads to formation of the trimer. The reactivity of the monomer has to be tuned by selecting the appropriate protecting groups (Scheme 1.1).[9]



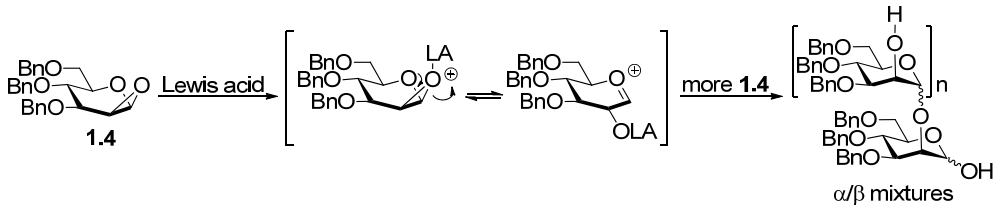
Scheme 1.1. Strategy for one-pot self-condensation synthesis

This approach has been successfully applied to carbohydrate **1.1** for the synthesis of several biologically significant oligosaccharides, which include dissacharide **1.2** and Manα1→2Manα1→2Man **1.3** (Scheme 1.2). The main problem is that the reactivity of the glycosylation is not fully controlled and mixtures were always obtained.



Scheme 1.2. Synthesis of building blocks **1.2** and **1.3** by one-pot self-condensation reaction

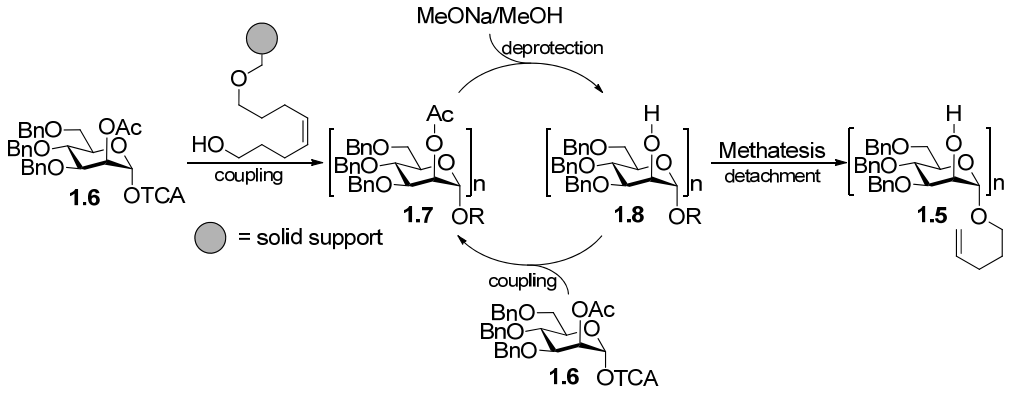
Chemical synthesis of 1,2-*manno*-oligomers has also been achieved by polymerization of 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannopyranose (**1.4**) in the presence of Lewis acids, cationic coordination catalysts, and strong bases.[15]
Under acid catalysis, this technique affords a series of polymers varying in anomeric configuration (Scheme 1.3, from 90% α to 70% β), whereas polymerization by means of potassium alkoxide, complexed with crown ethers, leads to essentially stereoregular (1→2)-α-D-mannopyranan.[15a]



Scheme 1.3. Oligomerization of epoxide **1.4** in the presence of Lewis acids (n = 1-4)

An alternative to traditional chemical synthesis for the procurement of oligosaccharides is to use the highly successful solid-phase synthesis.[16] However, this technique has various drawbacks, amongst others the heterogeneous reaction conditions employed, as well as the stepwise characterization. Recent advancements in solid-phase synthesis have made possible the construction of complex oligosaccharides with the invention of the Automated Oligosaccharide Synthesizer.[16b,c; 17] This general and automated method for oligosaccharide assembly allows the rapid preparation of structures of interest.
The automated synthesis of oligo-α-mannoside **1.5** was carried out with the cycle described in Scheme 1.4. Carbohydrate **1.6** was coupled with the hydroxyl group of the octenediol functionalized polystyrene resin by means of catalytic TMSOTf, through activation of the anomeric trichloroacetimidate. This donor, which bears an acetate group on position C-2, was chosen for conferring excellent α-selectivity during glycosylation due to the anchimeric assistance. After polymer anchoration, saponification of the ester group in **1.7** allows rapid obtention of glycosyl acceptor **1.8** that is subsequent glycosylated with building block **1.6**. The coupling/deprotection cycle was repeated until the desired chain length. Finally, cleavage from the support by cross methatesis (in the case of the octenediol linker) affords the desired *manno*-oligosaccharide (**1.5**).
Linear heptamannoside (**1.5**, n=7) was constructed in only 20 hours. HPLC analysis of the crude reaction mixture after cleavage from the support indicated a 42% overall yield.[17] In comparison to the manual solid-support synthesis, the same

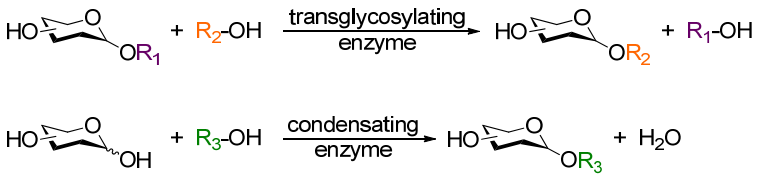
heptamannoside was synthesized in 14 days with 9% overall yield.[18] These results highlight the important advance of automated solid phase syntheses toward streamlining the synthesis of oligosaccharides.



Scheme 1.4. Automated coupling cycle showing coupling, deprotection, and final detachment from the solid support employed for the synthesis of an oligo-α-mannoside bearing a pentenyl linker on the reducing end[19]

During the past decades, the field of glycochemistry has stablished new techniques employing the enzyme machinery involved in the biosynthesis of carbohydrates. The enzymes employed can be usually divided into two major groups: glycosidases and glycosyltransferases.

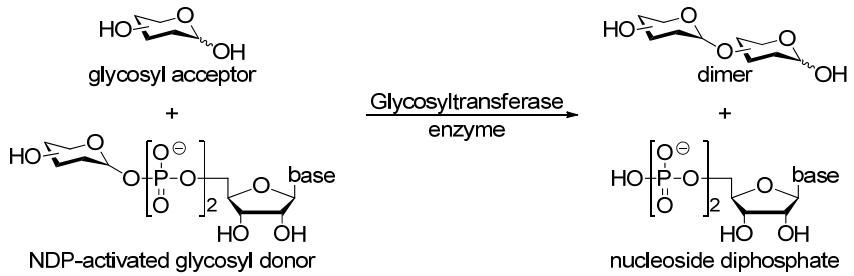
The glycosidase family catalyzes the oligosaccharide assembly of underivatised sugars by transglycosylation reactions[20], as well as by condensation reactions (Scheme 1.5).[21] Although glycosidases have the advantatge that both enzymes and substrates are inexpensive and readily available, the regioselectivity of the process is rarely absolute and, at equilibrium, several different oligosaccharides are obtained.



Scheme 1.5. Glycosidase-catalyzed transglycosylation (up) and condensation (down)[22]

In this context, the use of α 1,2-mannosidase isolated from *Aspergillus phoenicis* to synthesize mannobiose, mannotriose, and other highly regioselective *manno*-oligosaccharides has been reported. This enzyme gave exclusively α1→2 linkage.[23]

Glycosyltransferases, which catalyze the efficient and specific transfer of a saccharide from a sugar nucleotide donor to an acceptor (Scheme 1.6), are also a powerful tool for the practical synthesis of oligosaccharides.[24] The obvious drawback is the high cost associated with the nucleotide diphosphosugars (NDPs).



Scheme 1.6. Enzymatic synthesis of oligosaccharides

Additionally, the narrow scope of transferase-mediated glycosylations necessitates the isolation and purification of multiple enzymes to synthesize diverse structures. This field of enzymatic synthesis is currently ongoing through considerable research and should lead to new, more efficient and flexible enzymatic methods of attaining well-defined oligomers in sufficient amounts.[25]

1.2. Thiooligosaccharides: achievements and perspectives

The emerging use of specific techniques for quick access to oligosaccharides has not only contributed to biological, biochemical and biophysical investigations, but also to the development of carbohydrate-based therapies.[26] This class of compounds presents both potential and problems: although their biological relevance has been recognized, problems with the lability of the glycosidic bond by enzymatic degradation inside organisms rendered them as not desirable for drug discovery.

Adressing this issue, different methods are currently investigated in order to obtain carbohydrate mimic structures with improved stability profile.[27] In this context, modifications of the *O*-glycosidic linkage by substitution of the oxygen glycosidic atom by other heteroatoms, like sulfur, have been carried out.[28]

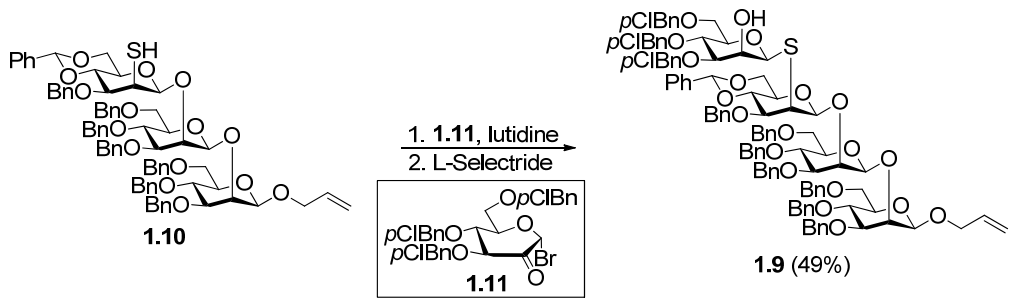
The thioglycosidic linkage is usually stable to enzymatic processes and is less susceptible to acid/base hydrolysis.[29] Thus, thiooligosaccharides are tolerated by most biologicals systems, displaying inhibitory activity against glycosidases.[30] Since this heteroatom is larger than oxygen, it provides a high degree of flexibility between

glycosyl units and these molecules possess more conformers than their natural analogues. Hence they enable a better fit in the catalytic site of enzymes.[31] Besides, the electronic structure of sulfur makes it compatible with hydrogen-bonding interactions.

1.2.1. Synthesis of *S*-linked *manno*-thiooligosaccharides

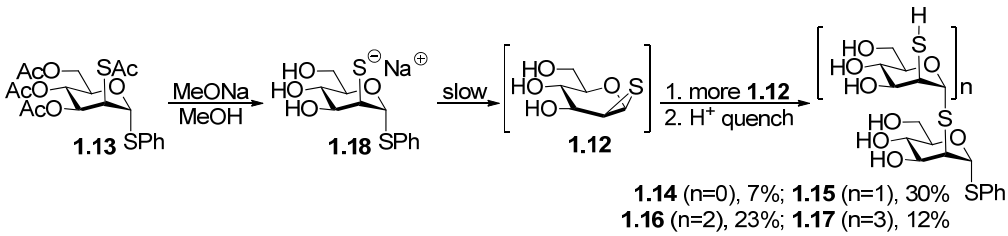
One of the most commonly applied procedures for synthesizing 1-thioglycosides consists in the S_N2 displacement reaction of halogenoses with high-nucleophilic thiolate groups.[28] This approach affords equatorial thioglycoside linkages because the halogen atom on the starting halogenoses generally adopts the axial position. Alternatively, the synthesis of the axial epimer can be achieved by using *O*-glycosyl trichloroacetimidates and other typical glycosyl donors under acid-promoted reaction conditions.[28] In this case, the reaction is prone to undergo a S_N1 mechanism. It is worth pointing out that the formation of disulfide bridges, formed from the air oxidation of sulfhydryl groups, is usually the main side-reaction.

In this context, several 1,2-linked β-mannopyranan oligomers, containing a terminal *S*-linked (1→2)-β-D-mannopyranosyl residue, have been synthesized in order to study the unique immunological properties of the cell wall mannan of *Candida albicans*.[32] The synthesis of tetrasaccharide **1.9** was accomplished as outlined in Scheme 1.7. Thiol **1.10** was condensed with ulosyl bromide **1.11** under basic conditions to give, after reduction of the 2-keto functionality employing L-selectride, the desired tetrasaccharide, **1.9**, in 49% yield. The corresponding 1-thio-α-*gluco* epimer was also isolated from the reaction (~12%). This compound may be formed from the *in situ* epimerization of the α-ulosyl bromide via halide exchange. S_N1 reaction with **1.11** and even base catalyzed isomerisation of the ulosyl thioglycoside cannot be discarded.



Scheme 1.7. Synthesis of the thioglyoside mimetic of (1→2)-β-D-mannopyranotetraose

As a variation of classical anomeric S-alkylations, Knap and Malolanarasimhan have reported a new method for synthesizing thio-oligo-α-D-mannopyranosides via ring-opening oligomerization of 1,2-epithiosugar **1.12** (Scheme 1.8).[33] Deacetylation of **1.13** under Zemplen conditions gave rise not only to the expected mercaptotriol **1.14**, but also to a mixture of oligomeric thioglycosides (**1.15**-**1.17**) still bearing the 2-mercapto substituent.



Scheme 1.8. Synthesis of 2-thio-(1→2)-mannopyranosides

Under these conditions, transient thiirane **1.12** is slowly formed through the ring closure reaction of intermediate **1.18**. The autors attributed this reaction to a favourable S_N2 trajectory and to the softness match between the participating thiolate at position C-2 and the *trans-anti* anomeric leaving group. The stability of phenylthiolate as leaving group relative to alkylthiolate was also considered. Once the epithiosugar is formed, it is trapped by the more reactive thiolates present in the medium, thus leading to thioglycosides (**1.14**) and related oligomers (**1.15**-**1.17**).

1.3. References

1. Varki, A. *Glycobiology,* **1993**, *3*, 97-130.
2. a) Weijers, C. A. G. M.; Franssen, M. C. R.; Visser, G. M. *Biotech. Adv.* **2008**, *26*, 436-456. b) Pukin, A. V.; Weijers, C. A. G. M.; van Lagen, B.; Wechselberger, R.; Sun, B.; Gilbert, M.; Karwaski, M. F.; Florack, D. E. A.; Jacobs, B. C.; Tio-Gillen, A. P.; van Belkum, A. F.; Endtz, H. P.; Visser, G. M.; Zuilhof, H. *Carbohydr. Res.* **2008**, *343*, 636-650. c) Hölemann, A.; Seeberger P. H. *Curr. Opin. Biotechnol.* **2004**, *15*, 615-622. d) Koeller, K. M.; Wong. C. H. *Glycobiology,* **2000**, *10*, 1157-1169.
3. a) Dorland, L.; van Halbeek, H.; Vliegenthart, J. F. G.; Lis, H.; Sharon, N. *J. Biol. Chem.* **1981**, *256*, 7708-7711. b) Li, E.; Kornfeld, S. *J. Biol. Chem.* **1979**, *254*, 1600-1605. c) Lis, H.; Sharon, N. *J. Biol. Chem.* **1978**, *253*, 3468-3476.
4. Schachter, H. *Clin. Biochem.* **1984**, *17*, 3-14.
5. Thielens, N. M.; Tacnet-Delorme, P.; Arlaud, G. J. *Immunobiol.* **2002**, *205*, 563-574.

6. Lee, H.-K.; Scanlan, C. N.; Huang, C.-Y.; Chang, A. Y.; Calarese, D. A.; Dwek, R. A.; Rudd, P. M.; Burton, D. R.; Wilson, I. A.; Wong, C.-W. *Angew. Chem. Int. Ed.* **2004**, *43*, 1000-1003 and references therein.
7. a) Kwong, P. D.; Wyatt, R.; Robinson, J.; Sweet, R. W.; Sodroski, J.; Hendrickson, W. A. *Nature*, **1998**, *393*, 648-659. b) Rizzuto, C. D.; Wyatt, R.; Hernández-Ramos, N.; Sun, Y.; Kwong, P. D.; Hendrickson, W. A.; Sodroski, J. *Science*, **1998**, *280*, 1833-1834. c) Montagnier, L.; Clavel, F.; Kurst, B.; Chamaret, S.; Rey, F.; Barre-Sinoussi, F.; Chermann, J. C. *Virology* **1985**, *144*, 283-289.
8. a) Lasky, L. A.; Nakamura, G.; Smith, D. H.; Fennie, C.; Shimasaki, C.; Patzer, E.; Berman, P.; Gregory, T.; Capon, D. J. *Cell* **1987**, *50*, 975-985. b) McDougal, J. S.; Kennedy, M. S.; Sligh, J. M.; Cort, S. P.; Mawle, A.; Nicholson, J. K. A. *Science* **1986**, *231*, 382-385.
9. Lee, H.-K.; Scanlan, C. N.; Huang, C.-Y.; Chang, A. Y.; Calarese, D. A.; Dwek, R. A.; Rudd, P. M.; Burton, D. R.; Wilson, I. A.; Wong, C. H. *Angew. Chem.*, *Int. Ed.* **2004***, 43,* 1000-1003.
10. Mizuochi, T.; Nakata, M. *J. Infect. Chemother.* **1999**, *5*, 190-195.
11. a) Scanlan, C. N.; Offer, J.; Zitzmann, N.; Dwek, R. A. *Nature* **2007**, *446*, 1038-1045. b) McReynolds, K. D.; Gervay-Hague, J. *Chem. Rev.* **2007**, *107*, 1533-1552.
12. a) Ley, S. V.; Baeschlin, D. K.; Dixon, D. J.; Foster, A. C.; Ince, S. J.; Priepke, H. W. M.; Reynolds, D. *J. Chem. Rev.* **2001**, *101*, 53-80. b) Merritt, J. R.; Naisang, E.; Fraser-Reid, B. *J. Org. Chem.* **1994**, *59*, 4443-4449. c) Ogawa, T.; Nukada, T. *Carbohydr. Res.* **1985**, *136*, 135-152.
13. Demchenko, A.V. *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*. Wiley-VCH; Weinheim, **2008** and refrences therein.
14. Zhang, W. *Chem Rev.* **2009**, *109*, 749-795.
15. a) Trumbo, D. L.; Schuerch, C. *Carbohydr. Res.* **1985**, *135*, 195-202. b) Yamaguchi, H.; Schuerch, C. *Biopolymers* **1980**, *19*, 297-309.
16. a) Fréchet, J. M.; Schuerch, C. J. *J. Am. Chem. Soc.* **1971**, *93*, 492-496. b) Seeberger P. H. *Chem. Soc. Rev.* **2008**, *37*, 19-28. c) Seeberger P. H. *Solid Support oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries*. John Wiley, New York, **2001** and refrences therein.
17. Plante, O. J.; Palmacci, E.R.; Seeberger, P.H. *Science* **2001**, *291*, *1523*-1527.
18. Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett*. **1999**, *1*, 1811-1814.
19. Stallforth, P.; Lepenies, B.; Adibekian, A.; Seeberg, P. H. *J. Med. Chem.* **2009**, *52*, 5561-5577.
20. a) Murata, T.; Usui, T. *Trends Glycosci. Glycotechnol.* **2000**, *65*, 161-174. b) Murata, T.; Usui, T. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1059-1066.

21. a) van Rantwijk, F.; Woudenberg-van Oosterom, M.; Sheldon, R. A. *J. Mol. Cat. B: Enzymatic*, **1999**, *6*, 511-532. b) Crout, D. H.; Vic, G. *Curr. Opin. Chem. Biol.* **1998**, *2*, 98-111.
22. Murata, T.; Usui, T. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 1049-1059.
23. a) Suwasono, S.; Rastall, R. A. *Biotech. Lett.* **1998**, *20*, 15-17. b) Suwasono, S.; Rastall, R. A. *Biotech. Lett.* **1996**, *18*, 851-856.
24. Koeller, K.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465-4493.
25. Jiang, J.; Biggins, J. B.; Thorson, J. S. *J. Am. Chem. Soc.* **2000**, *122*, 6803–6804.
26. Seeberger, P. H.; Werz, D. B. *Nature Rev. Drug. Disc*. **2005***, 4,* 751-763.
27. Koester, D. C.; Holkenbrink, A.; Werz, D. B. *Synthesis*, **2010**, *19*, 3217-3242.
28. a) Pachamuthu, K.; Schmidt, R. R. *Chem. Rev.* **2006**, *106*, 160-187. b) Szilágyi, L.; Varela, O. *Curr. Org. Chem.* **2006**, *10*, 1745-1770.
29. Witczak, Z. J.; Chhabra, R.; Chen, H.; Xie, X.-Q. *Carbohydr. Res.* **1997**, *301*, 167-175.
30. Witczak, Z. J.; Boryczewski, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3265-3268 and references therein.
31. a) Driguez, H. *ChemBioChem* **2001**, *2*, 311-318. b) Driguez, H. *Top. Curr. Chem.* **1997**, *187*, 85-116.
32. a) Nitz, M.; Ling, C.-C.; Otter, A.; Cutler, J. E.; Bundle, D. R. *J. Biol. Chem.* **2002**, *277*, 3440-3446. b) Nitz, M.; Bundle, D. R. *J. Org. Chem.* **2001**, *66*, 8411-8423.
33. Knapp, S.; Malolanarasimhan, K. *Org. Lett.* **1999**, *1*, 611-613.

CHAPTER 2

OBJECTIVES

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

The final goal of this thesis is the development of strategic methods for the synthesis of well-defined 1,2-linked mannose oligosaccharides and *S*-linked thio-analogues presenting low polydispersity indexes through different polymerization techniques. In this context, the present work aims to develop new procedures in carbohydrate chemistry, focusing not only in the oligomerization reactions, but also in the development of efficient syntheses for accessing the suitable monomers. In connection with this purpose, the specific objectives of this thesis are the following:

1. Synthesis of 1,2-linked mannose oligosaccharides. The research described in this part aims to develop new methods for the synthesis of *manno*-oligosaccharides through the oligomerization of different carbohydrate based monomers:
    - Ring-opening oligomerization of 1,2-anhydro carbohydrates through catalytic polymerization using *N*-heterocyclic carbenes (Chapter 3).
    - Ring-opening oligomerization of 1,2-*O*-carbonyl-α-D-mannoses under different polymeration conditions (Chapter 4).

2. Synthesis of *S*-1,2-linked *manno*-thiooligosaccharides. The work presented in this section has as a final objective the development of new methods for preparing *S*-1,2-linked *manno*-thiooligosaccharides of interest starting from

manno-epithiosugars or analogous monomers. To achieve this objective it is necessary to establish efficient protocols for accessing the starting materials. The following issues will be analyzed:

- Synthesis and ring-opening oligomerization of 1-*O*,2-*S*-thiocarbonyl-α-D-mannoses following the same proposed methodology applied to 1,2-*O*-carbonyl-α-D-mannoses to the straightforward synthesis of oligo-α-mannosides (Chapter 4).
- Synthesis of glycal-1,2-episulfides by the direct molybdenum-catalyzed episulfidation of glycals with phenylthiirane as sulfur source (Chapter 5).
- Synthesis of glycal-1,2-episulfides by conversion of 1,2-anhydro carbohydrates with inorganic thiocyanates (Chapter 6).

CHAPTER 3

N-HETEROCYCLIC CARBENE-INDUCED RING OPENING OLIGOMERIZATION

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

3.1. Background

3.1.1. General properties/chemistry of *N*-heterocyclic carbenes

After Bertrand et al.[1] and Arduengo et al.[2] described the first stable carbenes, their utilization as synthetic tools in organic chemistry has outreached all expectations.[3] The reason for their immense catalytic potential has to be found in their structural diversity, which challenge most active and selective metal-based or enzymatic catalysts.[1-3]

Since their isolation in 1991,[2] *N*-heterocyclic carbenes (NHC) have been widely studied in order to achieve greater understanding of their chemical and electronic properties. Differing from traditional carbenes, cyclic diaminocarbenes are stabilized thermodynamically by two π-donating groups which compensate the electronic deficiency of the vacant p-orbital.[4] Figure 3.1, left shows how the nitrogen lone pairs donate electron density into the carbene centre, allowing allylic π stabilization. This "push-push" effect enlarges the singlet-triplet energy gaps (67-90 kcal/mol) and, therefore, NHCs exhibit poorly the typical carbene reactivity. Thus, they are considered as strong nucleophiles and very poor electrophiles.[5] Moreover, the

carbene undergoes a σ-withdrawing effect induced by the amino substituents, and therefore stabilizing it (Figure 3.1, center). Although the main contribution to the stability of the NHCs comes from the combination of these two electronic effects, the presence of a carbon-carbon double bond in the backbone provides partial aromatic character, which contributes to additional thermodynamic stabilisation (Figure 3.1, right).[6] Steric hindrance contributes to kinetic stability. It is remarkable that both electronic and steric factors are essential.[7]

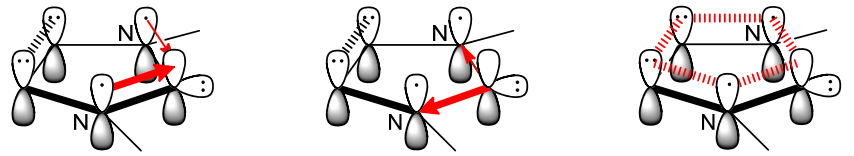
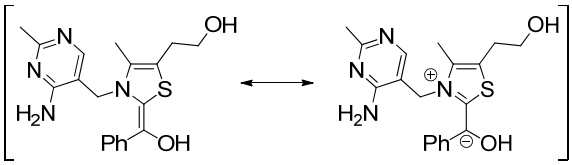


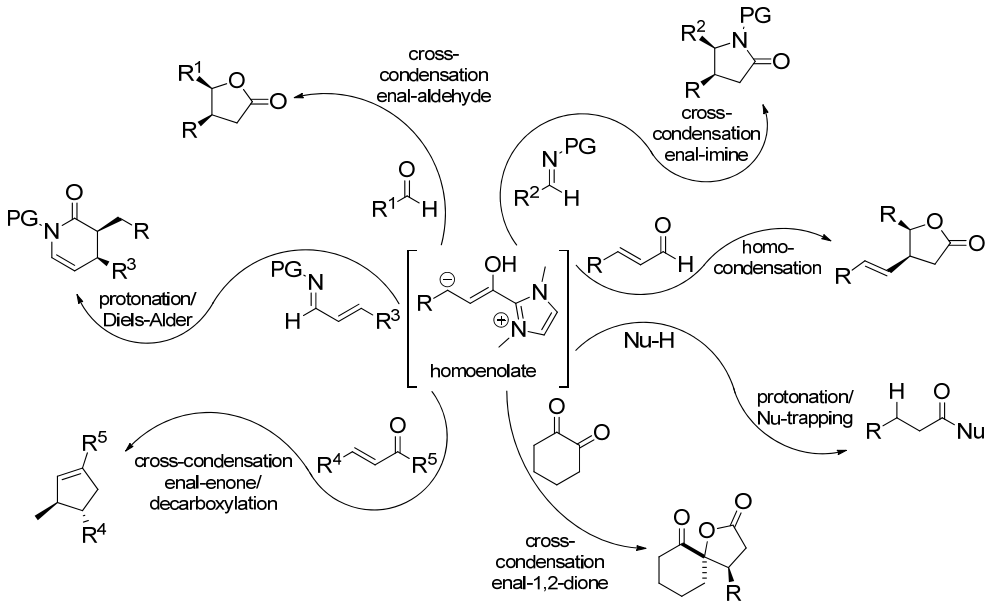
Figure 3.1. Electronic stabilization of NHCs

NHCs behave as σ-donor ligands, stronger than phosphines, with little π-backbonding character.[3a, 8] These attractive features are moving away organophosphanes as efficient ligands for transition metal catalyst. NHC-metal complexes have been extensively exploited in organometallic synthesis, outperforming the analogous phosphine-metal complexes.[9]

Apart from valuable ligands for transition metals, their own use as potent organocatalysts has attracted considerable attention in recent years. NHCs have been widely used to catalyze organic reactions such as condensations, including benzoin and stetter-type reactions, nucleophilic substitutions, transesterifications and acylations reactions, 1,2-additions, Diels-Alder cycloadditions, and redox processes.[10] Most of these synthetic transformations involve initial activation of the diaminocarbene with aldehydes, forming homoenolates analogous to the Breslow intermediate[11] (Scheme 3.1 and Scheme 3.2). Reactions involving acyl groups instead of aldehydes have also hypothesized equivalent NHC-acyl intermediates.[12]



Scheme 3.1. Breslow intermediate



Scheme 3.2. Applications of NHC-generated homoenolates (PG = protecting group)[10b]

3.1.2. *N*-heterocyclic carbenes for the organocatalytic ROP of epoxides

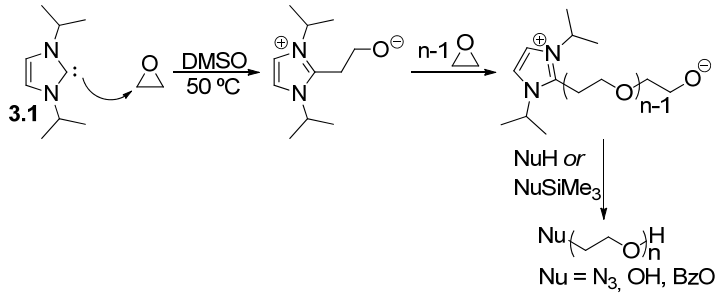
The high reactivity of *N*-heterocyclic carbenes for activation of carbonyl moieties is manifested in their ability to catalyze the ring-opening polymerization (ROP) of cyclic esters, mainly lactide and ε-caprolactone.[13] The use of NHCs as organocatalysts initiators for the ROP of epoxides has been briefly described by Taton et al. (e.g. ethylene oxide, EO).[14]

Diaminocarbenes, in absence of any other reagents, can efficiently catalyze ethylene oxide and trigger living ring-opening polymerizations. Living polymerization is a process where not only irreversible chain termination reactions, but also irreversible chain transfer reactions are absent.[15]

This new methodology achieves exclusively linear polymers in a very versatile fashion. It combines excellent control of molar masses, exhibits narrow polydispersities, and includes quantitative chain-end functionalization.

From a mechanistic point of view, the growth of poly(ethylene oxide) (PEO) chains occurs under zwitterionic ring-opening conditions (Scheme 3.3).[14c] The reaction starts via nucleophilic attack of organocatalyst **3.1** onto one molecule of EO to generate the corresponding zwitterionic 1,3-(diisopropyl)imidazol-2-ylidinium alkoxide. The latter species is nucleophilic enough to propagate chain-growth until complete conversion of EO. The imidazolium moiety plays here the role of the

counterion, allowing further propagation. The appropriate choice of the terminating agent serves as a chain stopper, which gives a variety of α,ω-difunctionalized PEOs.[14c] Analogous mechanisms have been already proposed and demonstrated in the ROP of lactones, specifically lactide.[16]

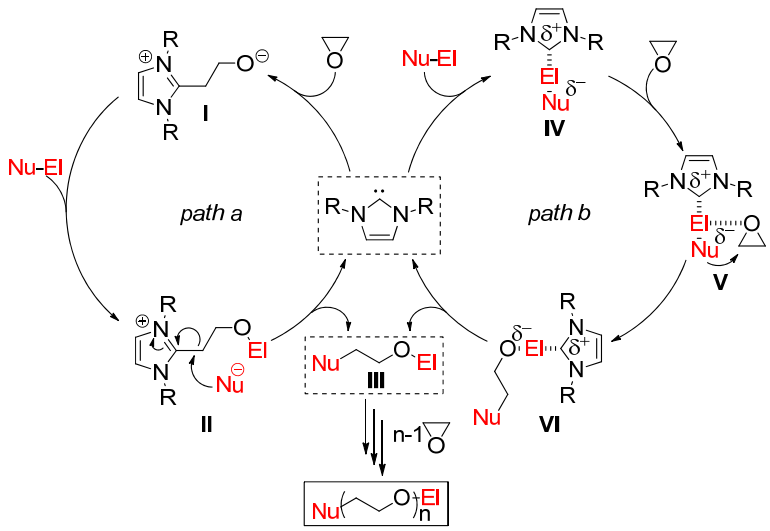


Scheme 3.3. Carbene promoted polymerization of ethylene oxide (EO)

Alternatively to the strategy previously reported, NHCs can be employed as real catalysts in conjunction with an initiator, introduced at the beginning, that play the role of chain regulators during the ROP of either ethylene or propylene oxide.[14a,b 17] This synthetic approach also offers α,ω-heterodifunctionalized polymers with several advantages, like optimal polymer chain-end fidelity. Moreover, molar masses and dispersities can be controlled excellently.

The mechanisms for initiation and chain growth can be envisaged as a monomer-activated mechanism mediated by the nucleophilic attack of the carbene (Scheme 3.4, path a). The key feature is the formation of transient zwitterionic **I**, obtained from the direct nucleophilic attack by the NHCs catalyst onto the monomer. This intermediate is capable to react with the electrophilic moiety (El) of the chain regulator (NuEl), forming species **II**. Addition of the resulting nucleophile fragment (Nu) to the activated azolium (**II**) generates a new adduct having incorporated a monomer unit (**III**) that can serve for subsequent chain propagation. In the meantime, the NHC catalyst is released and can activate another EO molecule.[14b]

An alternative mechanism can be conceived via chain end activation (Scheme 3.4, path b). In this case, ROP starts with the activation of the chain regulator by hydrogen-bonding to the carbene (**IV**) that concomitantly induces an attack onto ethylene oxide (**V**-**VI**). Such pathway provides the monoadduct (**III**) with the subsequently free carbene to sustain the catalytic cycle.[14b, 18] Indeed, background literature clearly indicates that silylated and hydroxylated groups are prone to activation by NHC.[18, 19]



Scheme 3.4. Proposed ROP mechanisms catalyzed by *N*-heterocyclic carbenes: monomer activation (path a) and chain-end activation (path b)

In the presence of initiators, NHC-catalyzed ROP is likely to operate by a combination of both mechanisms, depending on the interaction of the chain regulator with NHC catalyst against that of the latter with the monomer.

3.2. Results and discussion

3.2.1. Oligomerization using *N*-heterocyclic carbenes as initiators

Two *N*-heterocyclic carbenes were investigated for the ring-opening polymerization of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose[20] (**3.2**). Both were selected for their easy obtention as free molecules, with no need to generate them *in situ*. While 1,3-di-*tert*-butylimidazol-2-ylidene[21] (I^tBu, **3.3**) is considered better σ-donor with great steric demands presented by the nitrogen substituents, commercially available 1,3-bis(2,6-diisopropylphenyl)imidazol-2-ylidene (IDipp, **3.4**) is less electron-donating by means of the less sterically encumbered arenes (Figure 3.2).[6a, 22]

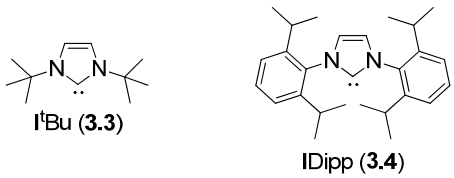
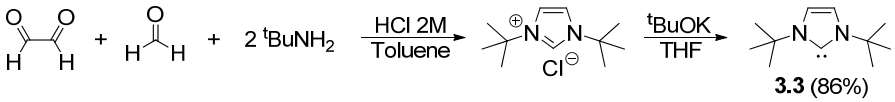


Figure 3.2. Chemical structures of carbenes I^tBu (**3.3**) and IDipp (**3.4**)

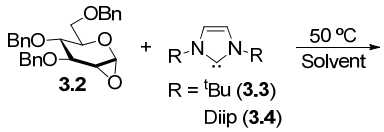
Carbene **3.3** was synthesised in two steps using the assembly route to create the imidazole ring starting from glyoxal, formaldehyde and *tert*-butylamine in the presence of hydrochloric acid. The free carbene was generated from the precursor by reaction with potassium *tert*-butoxide. Sublimation delivers the stable and pure catalyst I^{t}Bu in 86% global yield (Scheme 3.5).[23]



Scheme 3.5. Synthesis of symmetrically substituted carbene **3.3**

N-heterocyclic carbene-initiated ring-opening oligomerization of anhydrosugar **3.2** was investigated with both carbenes in the concentration of 20 mol % at 50 ºC. Polymerization data are summarized in Table 3.1. No reaction was observed with any catalysts after stirring seven days using deuterated benzene as a solvent (Table 3.1, entries 1 and 5). The employment of more polar solvents triggered the reaction. To discontinue the oligomerization, few drops of methanol were added after all the starting material had reacted. The products obtained were analyzed by NMR spectroscopy and MALDI-TOF mass spectrometry.

Table 3.1. Carbene promoted ring-opening oligomerization of epoxide **3.2**[a]



Entry	NHC	Solvent	Conv. (%)[c]	Stopper	Select. (%)[d]
1	**3.3**	C_6D_6	< 2		-
2[b]	**3.3**	THF-d8	> 98	MeOH	product decomposition
3[b]	**3.3**	DMF-d6	> 98	MeOH	product decomposition
4	**3.3**	DMSO-d6	< 2	-	-
5	**3.4**	C_6D_6	< 2	-	-
6	**3.4**	THF-d8	< 2	-	-
7	**3.4**	DMF-d6	57	MeOH	product decomposition
8[b]	**3.4**	DMSO-d6	> 98	MeOH	product decomposition

[a] Conditions: 0.07 mmol glucal, 20 mol % NHC, and 1 mL solvent for 7 days. [b] Reaction time: 4 days. [c] Determined by ^{1}H NMR integration of characteristic signals on the reaction crude. [d] Data obtained by MALDI-TOF MS spectra.

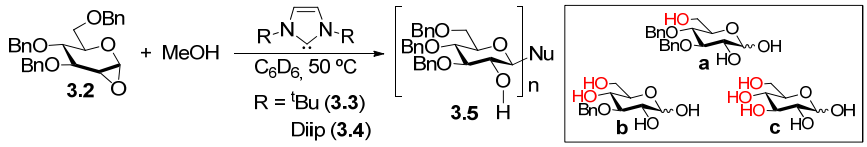
The more electron-rich carbene **3.3** reacts in THF and DMF which leads to complex product decomposition (Table 3.1, entries 2 and 3). It is worth mentioning that benzyl alcohol is the main byproduct always obtained. When DMSO was used as a solvent, no conversion took place (Table 3.1, entry 4). I^{t}Bu is known for reacting rapidly with DMSO-d6 under hydrogen-deuterium exchange of the ring protons.[24] Probably these interactions deactivate the catalyst.
Reaction of catalyst **3.4** with THF did not proceed, while in DMF provided moderate conversion towards a complex mixture (Table 3.1, entries 6 and 7, respectively). Longer reaction times did not further improve overall conversion, suggesting catalyst deactivation. Finally, fully conversion was observed using DMSO, but again starting material decay took place towards the release of benzyl alcohol, just like happened with the other carbene.
After these tests, it can be concluded that NHCs produce the deprotection of benzyl alcohol with the consequent destruction of the saccharidic backbone.

3.2.2. Oligomerization using *N*-heterocyclic carbenes as catalyst

At this point, the use of *N*-heterocyclic carbenes as real catalysts was studied in conjunction with an initiator, introduced at the beginning of the reaction. Initial investigations focused on the use of methanol as a chain regulator. This metoxy group is anchored into the α-position of the oligomeric chains and it can be easily revealed by ^{1}H NMR spectroscopy.
In order to assess the feasibility of the reaction and identify the nature of the possible oligomers formed, the reaction mixtures were analysed regarding their distribution by standard matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry. The selection of a suitable matrix makes possible the specific identification of oligomers triggered by different initiators by means of their corresponding mass peaks. MALDI-TOF MS is not the best technique for quantitation because the intensity of the mass peaks does not necessarily reflect the real quantity of the different products in the crude mixtures.[25]
The number of structural units in the average oligomer molecule (number-average degree of polymerization, DPn) was estimated from the relative intensity of the pertinent signals. The DPn is equal to the average number of monomer units in a polymer molecule.
Carbene-catalyzed ring-opening oligomerization of anhydrocarbohydrate **3.2** initiated with methanol was investigated at 50 ºC in the presence of NHC **3.3** or **3.4**

(20 mol % relative to **3.2**) using benzene as a solvent (Table 3.2). When the reaction was carried out in an excess of methanol as chain regulator with both catalysts, the ring-opening reaction took place, mainly affording the expected methyl glycosides (Table 3.2, entries 1 and 4). However, undesired products generated by water were also obtained.

Table 3.2. Catalyst screening on the oligomerization reaction of epoxide **3.2** (note that there are three possible structural isomers of species **a** and **b**)[a]



Entry	NHC	MeOH (eq)	Conv. (%)[b]	NuH	DPn[c]	Select. (%)[c]
1	**3.3**	excess	80	MeOH	1.1	24.9
				H_2O	2.6	5.7
2	**3.3**	0.50	> 98	BnOH	2.2	1.0
				H_2O	2.5	2.2
				a	3.4	0.5
				b	2.3	0.9
3	**3.3**	0.25	> 98	H_2O	2.8	7.4
				a	2.5	2.7
				b	2.5	1.2
				c	4.8	0.6
4	**3.4**	excess	> 98	MeOH	1.0	14.2
				H_2O	1.9	1.4
5	**3.4**	0.50	< 2	-	-	-
6	**3.4**	0.25	< 2	-	-	-

[a] Conditions: 0.07 mmol glucal, 20 mol % NHC, and 1 mL C_6D_6. [b] Determined by ^1H NMR of the reaction crude. [c] Data obtained by MALDI-TOF MS spectra.

In order to control the oligosaccharide size, substoichiometric amounts of methanol were employed using both NHC catalysts. Addition of half equivalent relative to the epoxide in the presence of sterically hindered carbene **3.3** led to a complex mixture of oligosaccharides **3.5** which could not be separated by column chromatography.

MALDI-TOF analyses and oligomer assignments revealed up to four families of different products (Figure 3.3 and Figure 3.4). As shown in the MALDI-TOF spectrum of the reaction mixture, the corresponding peaks of all detected compounds had low intensities. Each series of oligomers had incorporated a different initial

group: BnOH, H_2O, carbohydrate **a**, and carbohydrate **b**. (Table 3.2, entry 2). Since the structures of compounds **a** and **b** were elucidated by their mass, it is possible that they may exist as regioisomers (Figure 3.4).

Looking at the peak distribution of the benzyloxy-derived oligomers (**3.5-BnOH**) in Figure 3.3, this family of oligomers has incorporated from one to five units of saccharide **3.2**. As seen from the peak intensities of this family, the saccharide, the disaccharide and the trisaccharide showed the highest intensity values. However, this family has included, in average, 2 units of saccharide **3.2** (DPn=2.2; Table 3.2, entry 2). Oligomers with initial groups of carbohydrates **a** and **b** show a similar pattern.

In the case of hydroxy-derived oligomers (**3.5-H_2O**), oligomeric chains which contain from two to five carbohydrate repeating units were identified with low intensity, being the dissacharide the main compound (Figure 3.3). Still, they can be considered in general terms as a mixture of disaccharides and trisaccharides because of the DPn achieved (DPn=2.5; Table 3.2, entry 2).

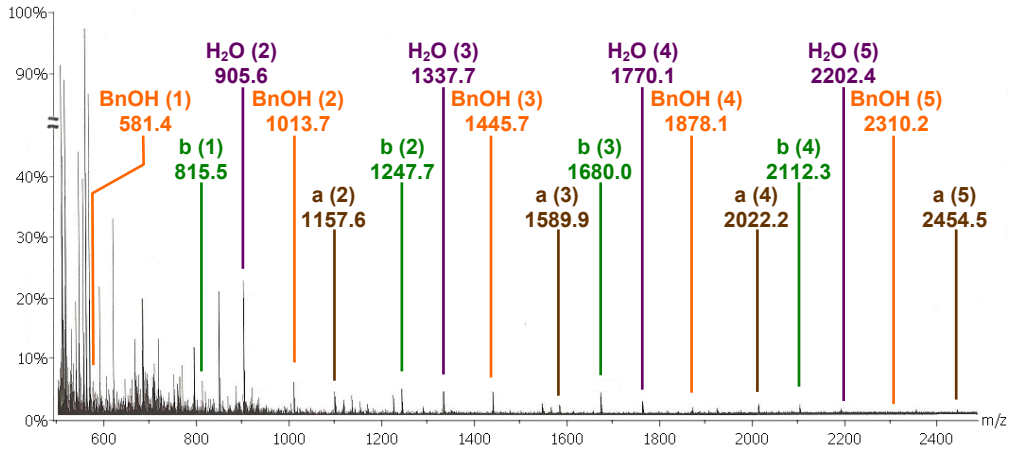


Figure 3.3. MALDI TOF mass spectrum of Table 3.2, entry 2 showing the filtered oligomer assignments (while first text line specifies the initial group, brackets indicate the polymerization degree; the digit below shows the mass value)

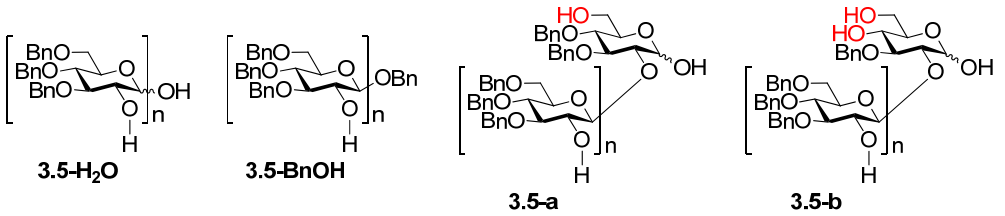
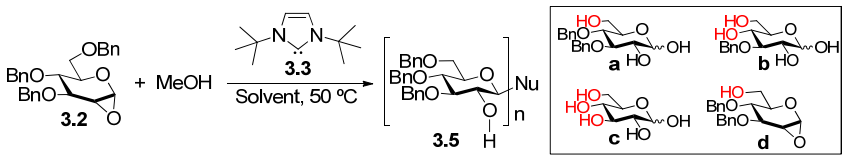


Figure 3.4. Oligomers formed during the experiment of Table 3.2, entry 2 (note that there are three possible structural isomers of species **a** and **b**)

It seems that cleavage of benzyl ether protecting groups in **3.2** takes place, delivering benzyl alcohol and the corresponding partial deprotected carbohydrates (**a** and **b**). Consecutively, these *in situ* generated compounds can compete in the ROP process.
Reaction with 0.25 equivalents of methanol relative to **3.2** did not improve the results previously obtained (Table 3.2, entry 3). Under these conditions, the same oligomers **3.5** were identified (except for the series derived from BnOH). Hence, a new family of oligomers with fully deprotected **c** as initial group was also observed. This last family, with DPn = 4.8, is formed mostly by pentasaccharides and hexasaccharides.
Reaction with less hindered IDipp with substoichiometric quantities of methanol was then tested, but no reaction took place (Table 3.2, entries 5 and 6).
Water, which seems necessary for all these side reactions, might come from the unstable 1,2-anhydro-sugar **3.2** since it cannot be well dryed.
In order to assess the factors influencing the reactivity of the catalyst, the nature of the solvent was explored. For this purpose, substrate **3.2**, NHC **3.3**, and 0.50 equivalents of methanol were reacted in different solvents (Table 3.3). Carbene **3.4** was not tested because of its poor performance in the previous reactions. THF allowed carrying out the reaction without benzyl ether cleavage (Table 3.3, entry 2).

Table 3.3. Solvent screening on the oligomerization reaction of epoxide **3.2** (note that there are three possible structural isomers of species **a**, **b**, and **d**)[a]



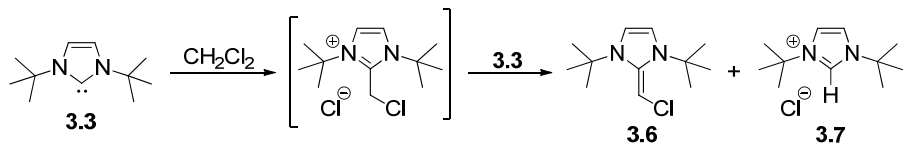
Entry	Solvent	Conv. (%)[b]	NuH	DPn[c]	Select. (%)[c]
1	C_6D_6	> 98	H_2O	2.8	7.4
			a	2.5	2.7
			b	2.5	1.2
			c	4.8	0.6
2	THF-d8	> 98	H_2O	1.2	2.4
3	DMSO-d6	> 98	H_2O	1.3	2.5
			d	1.1	0.4
4	$CDCl_3$	< 2	-	-	-

[a] Conditions: 0.12 mmol glucal, 0.06 mmol methanol, 20 mol % NHC, and 1 mL solvent. [b] Determined by ^{1}H NMR integration of reaction crude. [c] Data obtained by MALDI-TOF MS spectra.

Other aprotic polar solvents, like DMSO, performed similar results to that obtained with C_6D_6 (Table 3.3, entry 1 vs entry 3). Benzyl alcohol cleavage was partially avoided, although oligomers **3.5** with the monodeprotected epoxide **d** were detected.

In all cases, water-derived oligomers were always identified. Water is reactive enough to interfere within the process, so it does not allow the poor nucleophilic methanol to react. Low selectivities may give rise to other side-reactions as well as product decomposition.

Finally, reaction in dichloromethane led to instant catalyst decomposition (Table 3.3, entry 4). Presumably, reaction of the carbene with methylene chloride proceeds rapidly through cleavage of the C-Cl bond, producing a 2-(chloromethyl) imidazolinium ion (Scheme 3.6). Another free molecule of carbene is sufficiently basic to deprotonate this intermediate and, therein, methyleneimidazolidine **3.6** is formed altogether with imidazolium salt **3.7**.[26]

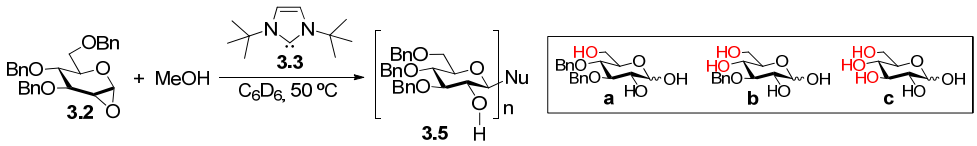


Scheme 3.6. Side-reaction of I^{t}Bu with CH_2Cl_2

At this point, the concentration of catalyst was studied. Table 3.4 displays all results obtained on the oligomerization of epoxide **3.2** with 0.50 equivalents, relative to **3.2**, of methanol as initiator. When 10 mol % of carbene were tested, results were similar to those obtained with the concentration 20 mol % (Table 3.4, entry 1) and the corresponding oligomers **3.5** were obtained in similar yields (Table 3.4, entry 2). The use of lower concentrations such as 1 mol %, did not afford any oligomer but fully product decomposition was instead observed (Table 3.4, entry 3). Thus, decreasing the concentration of the catalyst down to 0.1 mol % did not activate the reaction, even after stirring seven days (Table 3.4, entry 4).

Then, different initiators were studied in order to avoid the benzyl ether deprotection and water-derived oligomers **3.5** (Table 3.5). First oligomerization attempts were carried out with 0.50 equivalents of benzyl alcohol relative to **3.2**, under similar reported conditions (Table 3.5, entry 1). Apart from a variety of undesired oligomers, benzyloxy-derived oligomers (**3.5-BnOH**) containing from one to five carbohydrate units were identified with 18 % selectively. A detailed analysis of this family revealed that the benzyl glycoside was mainly obtained (DPn = 1.2). The use of thiophenol mainly afforded the phenyl 1-thio-glucopyranoside (Table 3.5, entry 2).

Table 3.4. Influence of the concentration of NHC on the oligomerization reaction (note that there are three possible structural isomers of species **a** and **b**)[a]

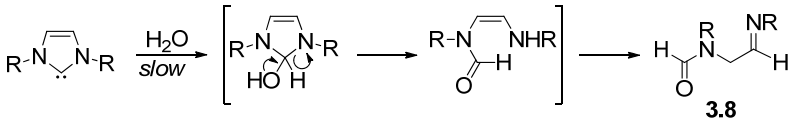


Entry	NHC (mol %)	Conv. (%)[b]	NuH	DPn[c]	Select. (%)[c]
1	20	> 98	H_2O	2.8	7.4
			a	2.5	2.7
			b	2.5	1.2
			c	4.8	0.6
2	10	> 98	H_2O	2.2	2.7
			a	2.0	1.5
			b	2.4	0.5
			c	2.2	0.3
3[d]	1	> 98	-	-	-
4[e]	0.1	< 2	-	-	-

[a] Conditions: 0.12 mmol glucal, 0.06 mmol methanol, and 1 mL solvent. [b] Determined by ^{1}H NMR integration of characteristic signals against starting materials of reaction crude. [c] Data obtained by MALDI-TOF MS spectra. [d] No oligomers were detected. [e] Reaction time: 7 days.

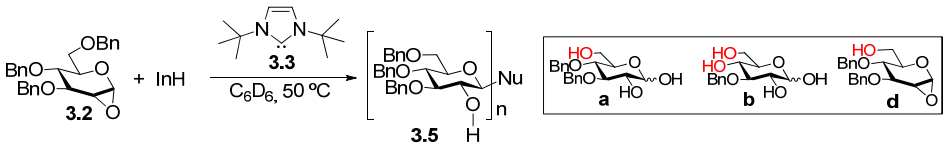
Alternatively, aniline and hexamethyldisiloxane were also used for the *N*-heterocyclic carbene-induced ring-opening oligomerization of epoxide **3.2** with catalyst **3.3**, but the corresponding reactions did not proceed at all, which leaded to benzyl ether clevage accompanied by several side-products (Table 3.5, entries 3 and 4). Furthermore, dimethyl hydroxylamine undergone oligomerization, but the reaction did not took place toward the desired oligomers (Table 3.5, entry 5).

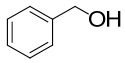
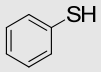
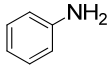
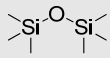
Since water-derived oligomers are always formed, water was then tested as initiator to perform the catalysis (Table 3.6). In all cases the sluggish decomposition of the *N*-heterocyclic carbene was observed by the end of the reaction. The drawback of this approach is that carbenes slowly hydrolyze to formamides **3.8** (Scheme 3.7).[27]



Scheme 3.7. Hydrolysis of stable *N*-heterocyclic carbenes to formamide **3.8**

Table 3.5. Initiator screening on the oligomerization reaction of epoxide **3.2** (note that there are three possible structural isomers of species **a**, **b**, and **d**)[a]



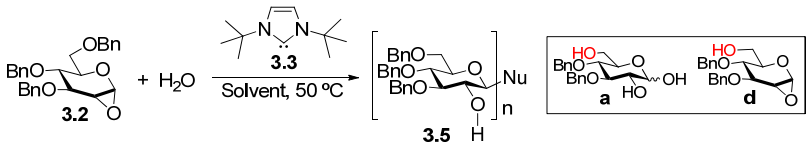
entry	InH	Conv. (%)[b]	NuH	DPn[c,d]	Select. (%)[c]
1		> 98	BnOH	1.3	18.4
			H_2O	2.9	18.1
			a	2.9	2.0
			b	2.1	1.4
2		> 98	PhSH	1.0	12.2
			H_2O	2.3	4.4
			a	2.0	1.0
3		> 98	H_2O	2.5	14.4
			a	2.4	1.6
4		> 98	H_2O	2.9	10.7
			a	2.9	1.8
			d	2.2	1.1
5		> 98	H_2O	2.6	8.3
			a	2.3	1.5
			b	2.2	0.4
			d	1.5	0.8

[a] Conditions: 0.12 mmol glucal, 0.06 mmol initiator, 20 mol % NHC, and 1 mL solvent. [b] Determined by ^{1}H NMR integration of characteristic signals of reaction crude. [c] Data obtained by MALDI-TOF MS spectra.

The best results were obtained using benzene as solvent since it furnished the water-derived oligomers (**3.5-H_2O**) as the major products (Table 3.6, entry 1). As usual, the molar masses of the oligomers could not be controlled, showing wide dispersities. Moreover, decreasing the quantity of initiator seems to increase the yield of the side-products (Table 3.6, entry 2). In order to control the chain length of the oligomer, the reaction was repeated with polar THF employing different amounts of H_2O (Table 3.6, entries 3 and 4). None of these experiments gave better results than the previously obtained with benzene (Table 3.6, entries 1 and 2 vs 3 and 4).

On the basis of all preceding experiments and thinking about the reaction mechanism for the deprotection of benzyl ether, the ability of NHC **3.3** for cleaving this protecting group was investigated with a range of differently benzylic compounds involving primary, secondary and tertiary alcohol-protected groups (Table 3.7).

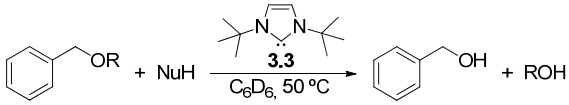
Table 3.6. Water oligomerization tests of epoxide **3.2** (note that there are three possible structural isomers of species **a** and **d**)[a]

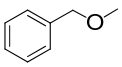
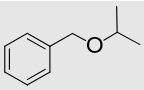
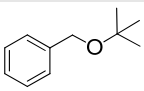
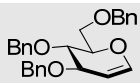


Entry	Solvent	H_2O (eq)	Conv. (%)[b]	NuH	DPn[c]	Select. (%)[c]
1	C_6D_6	0.33	> 98	H_2O	2.3	38.5
				a	2.1	2.5
				BnOH	1.1	7.3
2	C_6D_6	0.20	> 98	H_2O	2.3	9.0
				a	1.7	0.6
				d	2.9	0.9
3	THF-d8	0.33	> 98	H_2O	2.4	29.6
				a	2.1	3.8
				d	2.2	2.3
4	THF-d8	0.20	> 98	H_2O	2.5	15.3
				a	2.1	1.6
				d	2.0	1.7

[a] Conditions: 0.12 mmol glucal, 20 mol % NHC, and 1 mL solvent. [b] Determined by ^{1}H NMR integration of characteristic signals against starting materials of reaction crude. [c] Data obtained by MALDI-TOF MS spectra.

Table 3.7. Deprotection assays of different benzyl ethers with carbene **3.3**[a]



Entry	BnOR	NuH	Conv. (%)[b]	Select. (%)[b]
1		MeOH	< 2	< 2
2		BnOH	< 2	< 2
3		MeOH	< 2	< 2
4		BnOH	< 2	< 2
5		MeOH	< 2	< 2
6		BnOH	< 2	< 2
7		MeOH	< 2	< 2
8		BnOH	< 2	< 2

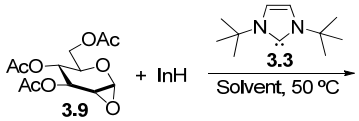
[a] Conditions: 0.12 mmol glucal, 0.12 mmol nucleophile, 20 mol % NHC, and 1 mL solvent. Reaction was carried out at 50 ºC for seven days. [b] Determined by ^{1}H NMR integration of characteristic signals against starting materials of reaction crude.

Reaction of benzyl methyl ether with methanol or benzyl alcohol in the presence of 20 mol % of catalyst **3.3** at 50 º C for seven days did not lead to any conversion, which did not improve by heating at reflux for further three days (Table 3.7, entries 1 and 2). Hence, the carbene was decomposed and all the starting material was thus recovered. Isobutyl benzyl ether was then treated with MeOH or BnOH in the presence of the catalyst, but no perceptible conversion took place (Table 3.7, entries 3 and 4). Moreover, deprotection of *tert*-butyl benzyl ether and more complex benzyl compounds, such as tri-*O*-benzyl-D-glucal, did not proceed (Table 3.7, entries 5-8). Since benzyl ethers were not cleaved in all the above cases, deprotection of benzyl ethers in carbohydrate **3.2** might arise from its particular reactivity.

Problems to obtain the desired oligomers with 1,2-anhydrocarbohydrate **3.2** led to a change in the protecting group of the saccharidic scaffold. Consequently 1,2-anhydro-3,4,6-tri-*O*-acetyl-α-D-glucopyranose[20] (**3.9**) was chosen as a suitable substrate for the NHC-catalyzed ring-opening polymerization.

Firsts attempts consisted in the reaction of **3.9** with half equivalent of methanol relative to the epoxide, in THF, but complex product decomposition was observed after addition of the carbene (Table 3.8, entry 1). Analysis of the ^{1}H-NMR spectrum showed a downfield proton resonance at approximately 10 ppm, characteristic of imidazolium salts.

Table 3.8. NHC-catalyzed ring-opening oligomerization of epoxide **3.9**[a]

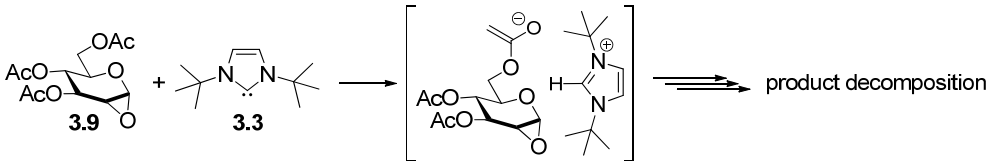


Entry	InH	Solvent	Conv. (%)[b]	NuH	DPn[c]	Select. (%)[c]
1[a]	MeOH	THF-d8	> 98[d]	-	-	-
2[a]	BnOH	THF-d8	> 98[d]	-	-	-
3	MeOH	C_6D_6	> 98[d]	-	-	-
4	BnOH	C_6D_6	> 98[d]	-	-	-

[a] Conditions: 0.18 mmol glucal, 0.09 mmol initiator, 20 mol % NHC, and 1 mL solvent. [b] Determined by ^{1}H NMR integration of characteristic signals on the reaction crude. [c] Data obtained by MALDI-TOF MS spectra. [d] No oligomers were detected.

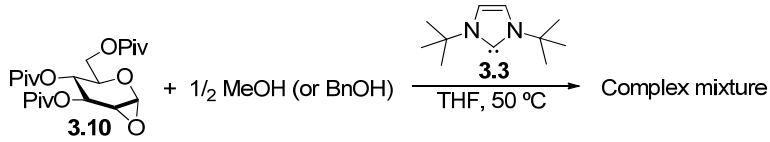
This unexpected result could be explained by an acid-base reaction between **3.3** and the acetyl protecting group of substrate **3.9**. NHC is known to behave as a strong base, abstracting a weakly acidic proton from methyl acetate. This process affords enolate species, which have been used in transesterification reactions and also in

claisen condensations.[28] Once the enolate derived from substrate **3.9** has been formed, the intermediate created is capable of reacting by several sites of the carbohydrate. As a consequence, the starting material is decomposed (Scheme 3.8).
In order to avoid this undesired acid-base reaction, other conditions were tested. Neither the use of benzyl alcohol as an initiator (Table 3.8, entry 2), nor switching to an apolar solvent as benzene (Table 3.8, entries 3 and 4) gave traces of any oligomer. Instead, extensive product decomposition was always produced.



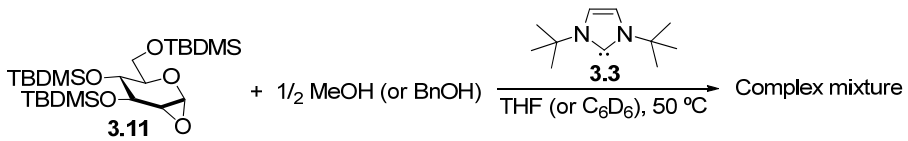
Scheme 3.8. Speculated reaction pathway of epoxide **3.9** with I^{t}Bu

It seems that the suitable protecting group needs to be compatible with the basicity of the carbene. Since methyl ester groups are not compatible with highly-basic catalyst **3.3**, the derivative 1,2-anhydro-3,4,6-tri-*O*-pivaloyl-α-D-glucopyranose (**3.10**), holding non-enolizable carbonyl groups, was synthesized as a substrate following a published procedure.[29]
The oligomerization reactions were then carried out in THF employing half equivalent of MeOH or BnOH relative to epoxide **3.10** in the presence of 10 mol % of catalyst I^{t}Bu. After stirring for 7 days at 50 ºC, a complex mixture was obtained whereas the formation of oligomers was not observed (Scheme 3.9). Analogous results were obtained when benzene was used as a solvent using the same reaction conditions.



Scheme 3.9. Ring-opening oligomerization of epoxide **3.10** catalyzed by NHC

At this point, it has become clear that the choice of the proper protecting group is not a trivial task. *Tert*-butyl-dimethyl silyl group was next tested, using anhydrosugar **3.11**[30] as a starting material. Reaction of **3.11** in different solvents (THF or C_6D_6) and the use of different initiators (MeOH or BnOH) afforded complex mixtures and no oligomers were detected (Scheme 3.10).



Scheme 3.10. Ring-opening oligomerization of epoxide **3.11** catalyzed by NHC

3.3. Conclusions

N-heterocyclic carbene-mediated ring-opening oligomerizations, including the use of NHC as initiators as well as catalysts, of several 1,2-anhydrocarbohydrates have been studied with two different carbenes (**3.3** and **3.4**). Although these organocatalytic procedures have been proved to be an efficient method for polymerizing structurally simple epoxides, its application to the synthesis of carbohydrate-derived oligomers was totally unsuccessful.

The precedent information obtained from per-*O*-benzylated protected carbohydrate **3.2** clearly indicates that water competes with the initiator during the whole process. In addition, it also cleaves the protecting groups of the monomer.

Furthermore, oligomerization tests of other monomers with several protecting groups including acetate ester (**3.9**) as well as pivaloate ester (**3.10**) and even *t*-butyldimethylsilyl ether (**3.11**) did not occur either, which suggests extensive product decomposition.

3.4. Experimental section

3.4.1. General methods

All chemicals used were reagent grade and were employed as supplied unless otherwise specified. Solvents were purified using standard procedures.[31] Reactions involving water-sensitive catalysts **3.3** and **3.4** were carried out under argon-gas atmosphere with solvents dried and distilled under argon gas prior to use.

^{1}H and ^{13}C NMR spectra were recorded using two spectrometers (Varian® Mercury VX 400 and Varian® 400-MR), both operating at frequency of 400 MHz for proton and 100.6 MHz for carbon. C_6D_6, $CDCl_3$, THF-d8, DMF-d6, and DMSO-d6 were used as solvents. Chemical shifts (δ) were referenced to internal standards (C_6D_6 (7.16 ppm ^{1}H, 128.06 ppm ^{13}C), $CDCl_3$ (7.27 ppm ^{1}H, 77.23 ppm ^{13}C), THF-d8 (1.73 ppm ^{1}H, 25.37 ppm ^{13}C), DMF-d6 (8.03 ppm ^{1}H, 163.15 ppm ^{13}C), and DMSO-d6

(3.33 ppm ^{1}H, 39.52 ppm ^{13}C)) or Me_4Si as an internal reference (0.00 ppm ^{1}H). 2D correlation spectra (gCOSY, NOESY, gHSQC and gHMBC) were visualized using the VNMR program (Varian®). MALDI-TOF mass spectrometry was performed using an applied Biosystems Voyager System 4243. All mass spectra were obtained through the use of dithranol matrixes and sodium iodide salt as cationization reagent. Reactions were monitored by TLC carried out on 0.25 mm E. Merck® silica gel 60 F_{254} glass or aluminium plates. Developed TLC plates were visualized under a short-wave UV lamp (254 nm) or dipping the plate in a suitable developing solution.[32] Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka® or Merck® silica gel 60 (230-400 mesh). Radial chromatography was performed on a Harrison® chromatotron model 7924T in 1 or 2 mm plates of Kieselgel 60 PF_{254} silica gel, depending on the amount of product. Flash column chromatography (FCC) was performed using flash silica gel (32–63 μm) and employing a polarity elution system correlated to TLC mobility.

3.4.2. General procedures

General procedure for NHC-induced zwitterionic ring-opening oligomerization: in a NMR tube was placed a solution of 1,2-anhydrosugar (0.07 mmol) and ylide (20 mol %) in 1.0 mL of deuterated solvent. The NMR tube was sealed with a rubber septum and Parafilm®, then heated to 50 ºC. After cooling to room temperature (ca. 20 ºC) it was analyzed by ^{1}H- and C^{13}-NMR spectroscopy. Conversion was determined by means of their characteristic ^{1}H-NMR against starting materials. After all the starting material has disappeared, methanol was added and heated again at 50 ºC for 2 h in order to quench the reaction. The NMR tube was allowed to cool to room temperature (ca. 20 ºC), opened, and the solvent was removed. The resulting residue was submitted to MALDI-TOF analysis. The number-average degree of polymerization (DPn), and the selectivity were determined by the MALDI-TOF MS spectra.

General procedure for NHC-induced catalytic ring-opening oligomerization: in a NMR tube was placed a solution of 1,2-anhydrosugar (0.18 mmol), initiator (0.09 mmol, aiming for the dimer), and ylide (20 mol %) in 1.0 mL of deuterated solvent. The NMR tube was sealed with rubber septum and Parafilm®, then heated to 50 ºC. After cooling to room temperature (ca. 20 ºC), it was analyzed by ^{1}H- and C^{13}-NMR spectroscopy. Conversion was determined by means of their characteristic ^{1}H-NMR

against starting materials. After all the starting material has disappeared, the NMR tube was opened and the solvent was finally removed. The resulting residue was submitted to MALDI-TOF analysis. The number-average degree of polymerization (DPn), and the selectivity were determined by the MALDI-TOF MS spectra.

3.4.3. Compound characterization

All products synthesized in this study had been already reported in the literature and the spectroscopic data obtained matched with those reported. The oligomers formed could not be purified due the high complex mixtures obtained in their preparation and they were identified by MALDI-TOF MS analysis.

3.5. References

1. Igau, A.; Grutzmacher, H.; Baceiredo, A.; Bertrand, G. *J. Am. Chem. Soc.* **1988**, *110*, 6463-6466.
2. Arduengo, A. J.; Harlow, R. L.; Kline, M. *J. Am. Chem. Soc.* **1991**, *113*, 361-363.
3. a) Enders, D. ; Niemeier, O. ; Henseler, A. *Chem. Rev.* **2007**, *107*, 5606-5655. b) Bourissou, D.; Guerret, O.; Gabbai, F. P.; Bertrand, G. *Chem. Rev.* **2000**, *100*, 39-92.
4. Kato, T.; Maerten, E.; Baceiredo, A. *Transition Metal Complexes of Neutral Metal η^1-Carbon Ligands* (in Topics in Organometallic Chemistry; 30), Springer, Heidelberg/Dordrecht/London/New York, **2010**, *30*, 131-147.
5. a) Heinemann, C.; Thiel, W. *Chem. Phys. Lett.* **1994**, *217*, 11-16. b) Olsson, M. H. M.; Borowski, P.; Roos, B. O. *Theor. Chim. Acta* **1993**, *93*, 17-33.
6. a) Dorta, R.; Stevens, E. D.; Scott, N. M.; Costabile, C.; Cavallo, L.; Hoff, C. D.; Nolan, S. P. *J. Am. Chem. Soc.* **2005**, *127*, 2485-2495. b) Heinemann, C.; Müller, T.; Apeloig, Y.; Schwarz, H. *J. Am. Chem Soc.* **1996**, *118*, 2023-2038.
7. a) Bourissou, D.; Guerret, O.; Gabbaie, F. P.; Bertrand, G. *Chem. Rev.* **2000**, *100*, 39-91. b) Nemirowski, A.; Schreiner, P. R. *J. Org. Chem.* **2007**, *72*, 9533-9540.
8. Kelly, R. A. III; Clavier, H.; Giudice, S.; Scott, N. M.; Stevens, E. D.; Bordner, J.; Samardjivev, I.; Hoff, C. D.; Cavallo, L.; Nolan, S. P. *Organometallics* **2008**, *27*, 202-210.
9. a) Herrmann, W. A. *Angew.Chem.Int. Ed.* **2002**, *41*, 1290-1309. b) Herrmann, W. A.; Weskamp, T. Bihm, V. P. W. *Adv. Organomet. Chem.* **2001**, *48*, 1-69. c) Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18-29.
10. a) Moore, J. L.; Rovis, T. *Top. Curr. Chem.* **2010**, *291*, 77-144. b) Marion, N.; Díez-González, S.; Nolan, S. P. *Angew. Chem. Int. Ed.* **2007**, *46*, 2988-3000.

11. Breslow, R. *J. Am. Chem. Soc.* **1958**, *80*, 3719-3726.
12. a) Grasa, G. A.; Guveli, T.; Singh, R.; Nolan, S. P. *J. Org. Chem.* **2003**, *68*, 2812-2819. b) Singh, R.; Kissling, R. M.; Letellier, M.-A.; Nolan, S. P. *J. Org. Chem.* **2004**, *69*, 209-212. c) Nyce, G. W.; Glauser, T.; Connor, E. F.; Mock, A.; Waymouth, R. M.; Hedrick, J. L. *J. Am. Chem. Soc.* **2003**, *125*, 3046-3056.
13. a) Kiesewetter, M. K.; Shin, E. J.; Hedrick, J. L.; Waymouth, R. M. *Macromolecules*, **2010**, *43*, 2093-2107. b) Kamber, N. E.; Lohmeijer, G. G.; Hedrick R. M. *Chem. Rev.* **2007**, *107*, 5813-5840.
14. a) Raynaud, J.; Ottou, W. N.; Gnanou, Y.; Taton, D. *Chem. Commun.* **2010**, *46*, 3203-3205. b) Raynaud, J.; Absalon, C.; Gnanou, Y.; Taton, D. *Macromolecules***, 2010**, *43*, 2814-2823. c) Raynaud, J.; Absalon, C.; Gnanou, Y.; Taton, D. *J. Am. Chem. Soc.* **2009**, *131*, 3201-3209.
15. Moad, G.; Solomon, D. H. *The Chemistry of Radical Polymerization - Second fully revised edition*, Elsevier, Amsterdam, **2006**, 451-452.
16. Culkin, D. A.; Jeong, W.; Csihony, S. ; Gomez, E. D.; Balsara, N. P.; Hedrick, J. L.; Waymouth, R. M. *Angew. Chem. Int. Ed.* **2007**, *46*, 2627-2630.
17. Mason, A. F.; Lohmeijer, B. G. G.; Li, H.; Pratt, R. C.; Liao, Q.; Frank, C. W.; Kingsbury, K.; Waymouth, R. M.; Hedrick, J. L. *ACS Polym. Prep.* **2006**, *47*, 99-100.
18. Movassagui, M.; Schimdt, M. A. *Org. Lett.* **2005**, *7*, 2453-2456.
19. a) Kano, T.; Sasaki, K.; Konishi, T.; Mii, H.; Maruoka, K. *Tetrahedron Lett.* **2006**, *47*, 4615-4618. b) Suzuki, Y.; Bakar, M. D. A.; Muramatsu, K.; Sato, M. *Tetrahedron* **2006**, *62*, 4227-4231.
20. Cheshev, P.; Marra, A.; Dondoni, A. *Carbohydr. Res.* **2006**, *341*, 2714-2716.
21. Denk, M. K.; Rodezno, J. M.; Gupta, S.; Lough, A. J. *J. Organomet. Chem.* **2001**, *617*, 242-253.
22. Chase, P. A.; Stephan, D. W. *Angew. Chem. Int. Ed.* **2008**, *47*, 7433-7437.
23. Gojon, S.; Kato, T.; Baceiredo, A.; Maliverney, C.; Saint-James, L. International patent WO2011/083146 A1, **2011**.
24. Denk, M. K.; Rodezno, J. M. *J. Organomet. Chem.* **2000**, *608*, 122-125.
25. Tholey, A.; Heinzle, E. *Anal. Bioanal. Chem.* 2006, *386*, 24-37.
26. a) Liu, Q.-X.; Song, H.-B.; Xu, F.-B.; Li, Q.-S.; Zeng, X.-S.; Leng, X.-B.; Zhang, Z.-Z. *Polyhedron*, **2003**, *22,* 1515-1521. b) Arduengo, III, A. J.; Davidson, F.; Dias, H. V. R.; Georlich J. R.; Khasnis, D.; Marshall, W. J.; Prakasha, T. K. *J. Am. Chem. Soc.* **1997**, *119*, 12742-12749.
27. Denk, M. K.; Rodezno, J. M.; Gupta, S.; Lough, A. J. *J. Organometall. Chem.* **2001**, *617-618*, 242-253.
28. Grasa, G. A.; Sing, R.; Scott, N. M.; Stevens, E. D.; Nolan, S. P. *Chem. Commun.* **2004**, 2890-2891 and references therein.

29. Matsushita, Y.-i.; Sugamoto, K.; Kita, Y.; Matsui, T. *Tetrahedron Lett.* **1997**, *38*, 8709-8712.
30. Chow, K.; Danishesky, S. *J. Org. Chem.* **1990**, *55*, 4211-4214.
31. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3er ed., Pergamon Press, Oxford, **1989**.
32. Reactivos Merck *Reactivos de coloración para cromatografía en capa fina y en papel*, E. Merck. Darsmtadt, **1980**.

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

CHAPTER 4

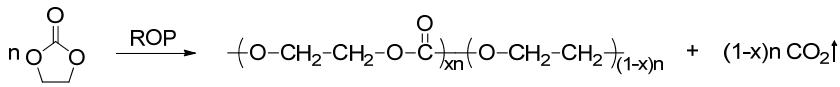
PERSPECTIVES OF SUGAR-DERIVED CARBONATES AND THIO-ANALOGUES IN OLIGOSACCHARIDE SYNTHESIS

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

4.1. Background

4.1.1. Ring-opening polymerization of cyclic alkylene carbonates

Polycarbonates represent a versatile class of polymers which are used in a broad range of applications due to their excellent biocompatibility and their good mechanical properties.[1] In general, six- and higher membered cyclic carbonates undergo ring-opening polymerization rapidly and selectively,[2] whereas the five-membered alkylene derivatives are polymerized with difficulty. Thus, the polymerization of these unique compounds to afford polycarbonates is thermodynamically unfavored and therefore it results in the production of polycarbonates with significant amounts of ether linkages that are the direct consequence of decarboxylation (Scheme 4.1).

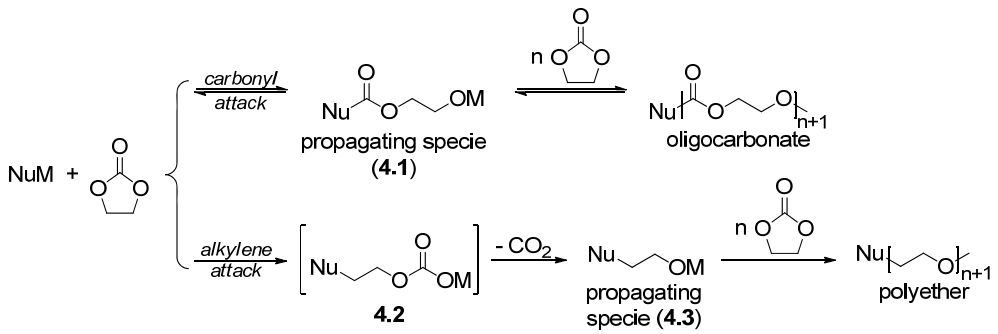


Scheme 4.1. ROP of five-membered cyclic carbonates (x stands for the mole fraction of carbonate units)

This loss of carbon dioxide increases the entropy of the whole process, and hence, the polymerization becomes thermodynamically possible.[3]

In general the ROP of five-membered cyclic carbonates is conducted with anionic initiators at temperatures exceeding 100 ºC. The reaction mechanism has not been fully elucidated although a few hypotheses have been proposed.[3] As far as cationic polymerization is concerned, these compounds do not react with acid catalysts.[4]

Scheme 4.2 ilustrates a possible ROP mechanism under basic conditions for the obtention of poly(ethylene ether carbonate)s, PEECs. In this proposal, the polymerization mechanism is a combination of two different routes for the reaction of the initiator with the monomer (Scheme 4.2).[5] The first pathway is the classical attack at the carbonyl carbon of the monomer, yielding compound **4.1**. This propagating specie can add to more monomer, resulting in the formation of a polymer with pure carbonate linkages. However, this attack is reversible and therefore only oligocarbonates are formed. The second route proceeds by the irreversible alkylene attack, accompanied by decarboxylation of intermediate **4.2**. By repetition of this attack, the generated metal alcoholate (**4.3**) would produce a polyether. As a result of combining these two attacks, a copolymer with random distribution of both linkage types is formed. Experimentally, the ethylene carbonate content in the polymer strongly depends on the catalyst chosen. Indeed, the degree of CO_2 retained varies from 10 to 50 mol %.



Scheme 4.2. Proposed anionic ROP mechanism

The labile carbonate linkages can easily be hydrolised and, therefore, the resulting degradation products can be employed to elucidate the structural composition of the parent polymer. Sequence analyses of these residues by gel permeation chromatography carried out by Vogdanis et al. showed that the major hydrolysis product was diethylene glycol, when organotin compounds (such as dibutyltin dimethoxide) were used as catalyst.[6] This fact indicates that the PEECs formed are

actually alternating copolymers with alternating ether and carbonate units (Figure 4.1.). This regular structure revealed that the polymerization process must be different from the one illustrated in Scheme 4.2.

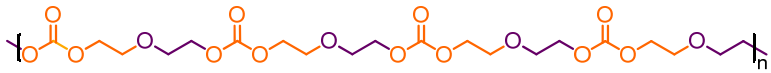


Figure 4.1. Proposed structure for PEEC with ethylene carbonate content of 50 mol % (carbonate linkages are highlighted in orange, while ether linkages are in purple)

Thus, calorimetric investigations showed that poly(ethylene carbonate)s are not accessible from ethylene carbonate because the process is thermodynamically forbidden, $\Delta G_p > 0$ (Equation 4.1). The reason is the entropy loss during the polymerization, $\Delta S_p < 0$,[7] along with the positive enthalpy of the reaction, ΔH_p = 125.6 kJ/mol at 25 ºC.[8] This last value was calculated indirectly from the enthalpies of combustion and the heat capacity data of pure poly(ethylene carbonate)s.

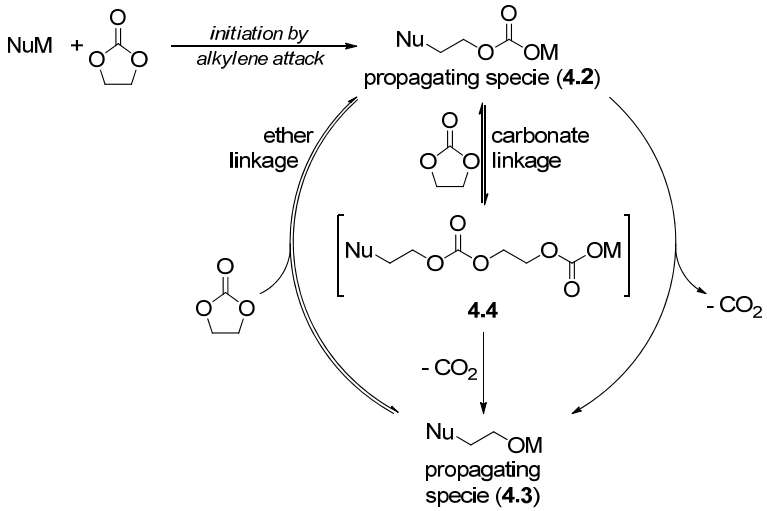
$$\Delta G_p = \Delta H_p - T\Delta S_p$$

Equation 4.1. Gibbs free energy equation

Vogdanis et al. proposed a different mechanism where the metal carbonate produced in Scheme 4.2 is also an active chain-propagating species (Scheme 4.3).[6] Carbonate **4.2** not only undergoes decarboxylation to afford species **4.3**, but can also propagate the polymerization by reaction with one molecule of monomer. Due to the thermodynamic reasons previously stated, this addition must be reversible and only a small population of intermediate **4.4** can be generated before decarboxylation and subsequent formation of alkoxide **4.3**. This chain intermediate can proceed to add another molecule of monomer, and form thereby an ether linkage.

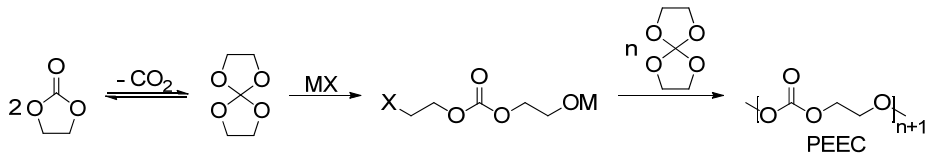
The amount of CO_2 retained in the copolymer is governed by the relative rates of both decarboxylation processes. If the decarboxylation rate of **4.2** is small compared to the decarboxylation of **4.4**, the ethylene carbonate content will approach the ideal 50 mol % and an alternating polymer will be formed. Since kinetics also depends on the catalyst used, the PEEC composition would also rely on the catalytic system.

Besides this aspect, mechanisms that proceed via orthocarbonate species cannot be discarded.[9] Indeed, Soga et al. already proposed the formation of spiro-orthocarbonates for propylene carbonate polymerization with metal alcoxides. This key intermediate resulted from the dimerization of the cyclic carbonate with the loss of CO_2 (Scheme 4.4).[10]



Scheme 4.3. Alternative anionic ROP mechanism proposed by Vogdanis

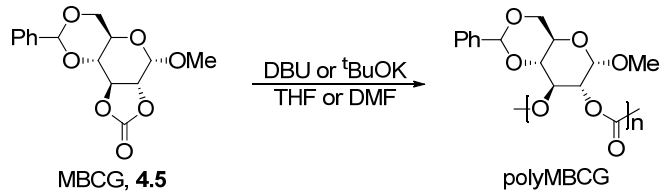
The plausibility of such mechanisms is ascertained by the polymerization of spiro-orthocarbonates with zinc-based coordination catalysts. These processes involve zinc alcoholate propagating species, which yields poly(propylene ether carbonate)s.[11]



Scheme 4.4. Dimerization of propylene carbonate and consecutive polymerization

Interestingly, none of the mechanisms discuss the occurrence of chain cleavages, intramolecular transesterification (back-biting reactions) and other possible side-reactions. All carbonate linkages are likely to undergo such reactions since they have been discussed to happen.[12] Thus, Harris et al. used GC analysis in conjunction with ^{13}C NMR spectroscopy to elucidate the structure of certain intermediates produced during the ROP of ethylene carbonate. These authors found that diethylene glycol was an important reaction intermediate and was present in steady-state concentrations.[13] To account for these observations, Hoffman et at. proposed a complex mechanism with the presence of diethylene glycol and other higher poly(ethylene glycols) as reaction intermediates.[14] Furthermore, Rokicki et al. proposed an alternative reaction mechanism that involves the formation of an alkoxide group from cyclic carbonate and alkali metal salts.[15]

In this context, there is only one example of a sugar-derived polycarbonate produced via the ROP of a five-membered cyclic carbonate. Interestingly, the anionic polymerization of methyl 4,6-*O*-benzylidene-2,3-*O*-carbonyl-α-D-glucopyranoside (MBCG, **4.5**) proceeded in good yields, when 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) or ^{t}BuOK were used as initiators, without the occurrence of CO_2 elimination (Scheme 4.5).[16] This unusual result may be explained by the rigid strain of **4.5**, in which the carbonate ring is fused *trans* to the rigid bicyclic *trans*-decalin fragment.



MBCG, **4.5** polyMBCG

Scheme 4.5. Synthesis of polyMBCG starting from monomer **4.5**

4.1.2. Ring-opening polymerization of cyclic alkylene thiocarbonates

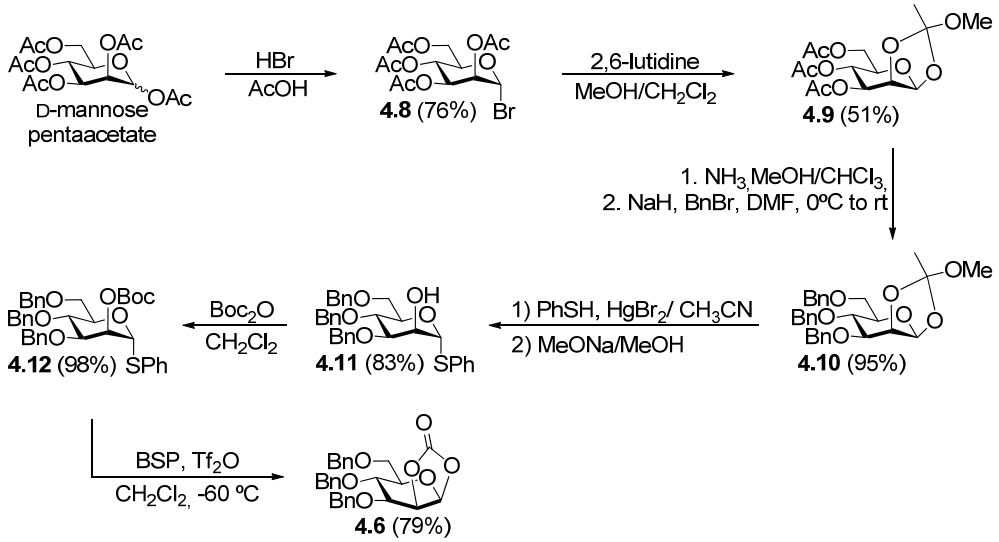
As compared with the polymerization of five-membered ring carbonates, cyclic alkylene thiocarbonates undergo polymerization easier with less tendency to carbon dioxide elimination, and so they afford poly(ethylene sulfide-monothiocarbonate)s rich in carbonate linkages. The reaction has been carried out with different initiators, including metal alkyls, such as $ZnEt_2$ and $CdEt_2$, as well as metal alkoxides like $Mg(OMe)_2$, $Al(O\textit{n}Bu)_3$, and $Ti(O\textit{n}Bu)_4$.[17] Thus, polymerization run with $Ti(O\textit{n}Bu)_4$ at 40 ºC as catalyst lead to a polymer with a content of monothiocarbonate units up to 77 mol %.

4.2. Results and discussion

4.2.1. Synthesis of the mannosyl-derived monomers

Prior to investigate the viability of the ROP of carbohydrate-derived cyclic carbonates and thiocarbonates, monomers, **4.6** and **4.7**, which are not commercially available, were synthesised.

1,2-*O*-carbonyl-α-D-mannose **4.6**[18] was prepared according to reported methods in seven reaction steps and starting from ready-available D-mannose pentaacetate (Scheme 4.6).

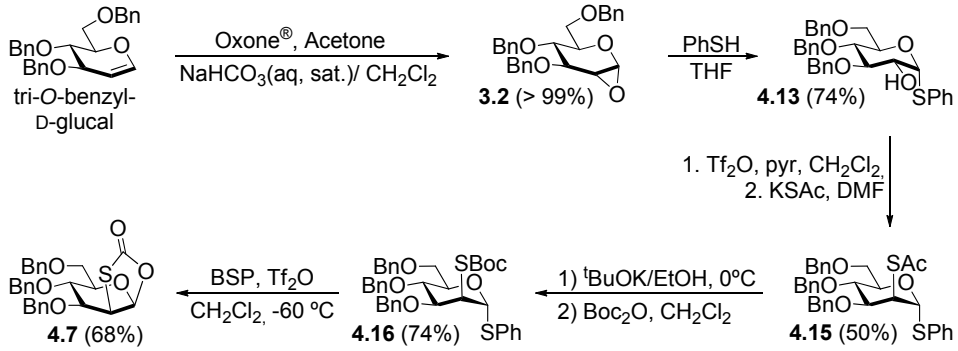


Scheme 4.6. Preparation of 3,4,6,-tri-*O*-benzyl-1,2-*O*-carbonyl-α-D-mannopyranoside (**4.6**)

Thus, mannopyranosyl bromide **4.8** was obtained from the starting material by bromination with a dilution of hydrogen bromide in acetic acid.[19] Treatment of **4.8** with 2,6-lutidine and methanol as a solvent yielded the mannopyranose-derived methyl 1,2-orthoacetate (**4.9**) as a 9:1 *exo*/*endo* diastereomeric mixture in 51% yield.[20] Deacetylation of compound **4.9** by aminolysis followed by reaction with benzyl bromide in the presence of sodium hydride afforded crystalline **4.10** in 95% yield.[21] Sugar orthoester **4.10** was allowed to react with thiophenol and a catalytic amount of mercury (II) bromide. Consecutive removal of the acetyl protecting group at the C-2 position under Zemplen conditions gave rise to D-mannose derivative **4.11** with a 83% yield.[22] This compound was readily treated with a solution of di-*tert*-butyl dicarbonate (Boc_2O) in CH_2Cl_2 to give key intermediate **4.12** in excellent yield.[18] Finally, 2-*O*-mannosyl carbonate **4.12** gave the desired cyclic carbonate **4.6** in 74% yield on treatment with trifluoromethanesulfonic anhydride (Tf_2O) and 1-benzenesulfinyl piperidine (BSP)[23] in dichloromethane at -60 ºC.[18]

Novel 1-*O*-2-*S*-carbonyl-α-D-mannose **4.7** was prepared in seven steps from commercial tri-*O*-benzyl-D-glucal (Scheme 4.7). Carbohydrate **3.2**[i] was obtained in quantitative yield from the starting glucal by reaction with dimethyldioxirane, generated *in situ* from Oxone®/acetone in a biphasic system (CH_2Cl_2/$NaHCO_3$ (aq, sat.).[24] Reaction of epoxide **3.2** with thiophenol under Danishefsky's conditions[25] led to the formation of the α-phenylthioglycoside (**4.13**) in 74% yield.

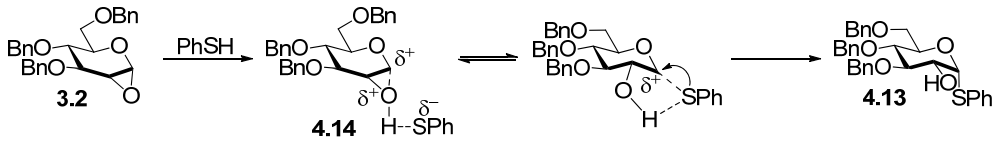
[i] This compound also appears in Chapter 3, thus same codification will be employed.



Scheme 4.7. Synthesis of 3,4,6,-tri-*O*-benzyl-1-*O*-2-*S*-carbonyl-α-D-mannopyranoside (**4.7**)

The stereoselectivity observed can be rationalized on the basis of hydrogen-bonding interactions between thiophenol and the epoxide. Under these conditions, the nucleophile is acidic enough to interact with the oxirane oxygen, forming an intramolecular intimate ion dipole pair (**4.14**). Subsequent nucleophilic internal attack on C-1 affords mainly the α-anomer, as admitted in the case of selenoglycosides (Scheme 4.8).[26]

Inversion of configuration of the equatorial hydroxyl at posicion C-2 in glucoside **4.13** to provide thiomannoside **4.15** was achieved in 50% yield through triflation of position C-2, followed by S_N2 displacement with KSAc. Hydrolysis of **4.15** with potassium *tert*-butoxide in ethanol at 0 ºC and subsequent treatment with Boc_2O in dichloromethane furnished compound **4.16** in 74% yield. Activation of the 2-*O*-Boc protected thiomannoside with a mixture of Tf_2O and BSP at -60 ºC in dichoromethane in the absence of an external nucleophile led to the isolation of the desired cyclic thiocarbonate (**4.7**) in 68% yield.



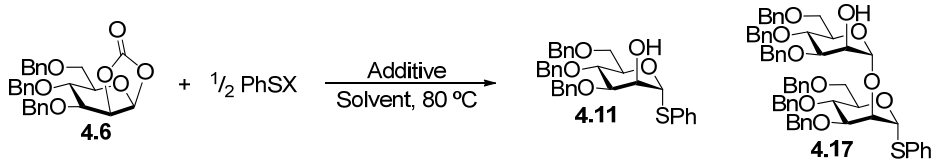
Scheme 4.8. Rationalizarion for the estereoselective α-thioglycosylation of epoxide **3.2**

4.2.2. Oligomerization tests

Initial investigations focused on the anionic ring-opening process of carbohydrate **4.6** with half equivalent of different aromatic thiols relative to carbonate **4.6** in order to obtain the disaccharide. Table 4.1 lists the results of the different assays with the

purpose of dimerization. The reaction was firstly assayed with sodium thiophenolate using THF as a solvent. However, no perceptible conversion took place after 24 h, even when 15-crown-5 ether was employed as the solvating agent of the ion pair (Table 4.1, entries 1 and 2). Besides, the reaction did not proceed at all when more polar solvents, like DMF, were employed (Table 4.1, entries 3 and 4).

Table 4.1. Dimerization tests with sugar carbonate **4.6**[a]

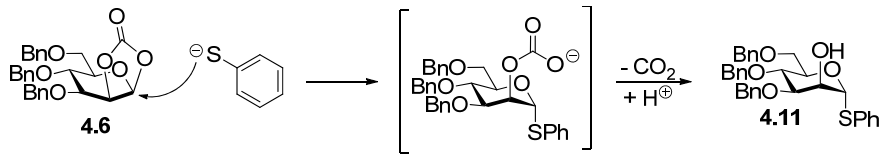


Entry	PhSX	Additive (eq)	Solvent	T (ºC)	Conv. (%)[b]	Yield (%)
1	PhSNa	-	THF	reflux	< 2	-
2	PhSNa	15-crown-5 (0.55)	THF	reflux	< 2	-
3	PhSNa	-	DMF	80	< 2	-
4	PhSNa	15-crown-5 (0.55)	DMF	80	< 2	-
5	PhSK	-	THF	reflux	64[c]	**4.11** (23)
6	PhSK	18-crown-6 (0.55)	THF	reflux	30[c]	**4.11** (40)
7	PhSK	-	DMF	80	39[c]	**4.11** (41)
8	PhSK	18-crown-6 (0.55)	DMF	80	35[c]	**4.11** (45)
9	PhSH	K_2CO_3 (0.10)	DMF	80	25[c]	**4.11** (39)
10	PhSH	DBU (0.10)	DMF	80	37[c]	**4.11** (36)
11	PhSH	TBD (0.10)	DMF	80	42[c]	**4.11** (45)
12	PhSH	I^{t}Bu (0.10)	DMF	80	> 98	degradation
13	PhSH	-	DMF	80	< 2	-
14	PhSH	PTSA (0.10)	DMF	80	< 2	-
15	PhSH	TFMA (0.10)	DMF	80	< 2	-
16	PhSH	$BF_3{\cdot}OEt_2$ (0.10)	DMF	80	< 2	-
17	PhSH	$Yb(OTf)_3$ (0.10)	DMF	80	< 2	-
18	PhSH	$ZnCl_2$ (0.10)	DMF	80	< 2	-

[a] Conditions: 0.08 mmol carbonate, 0.04 mmol NuH, 10 mol % additive, and 1 mL of solvent for 24 h. [b] Determination by ^{1}H NMR integration of characteristic signals on the reaction crude. [c] Yield recovered of **4.6** after purification by chromatography on silica gel.

On the basis of these preliminary experiments, potassium thiophenolate was investigated as a means to enhance the reactivity of the nucleophile. The dimerization reaction of carbonate **4.6** in THF led to compound **4.11** in 23% yield(Table 4.1, entry 5). Five-membered alkylene carbonates are known to react with aromatic nucleophiles at alkylene positions. In this case, the reaction proceeds

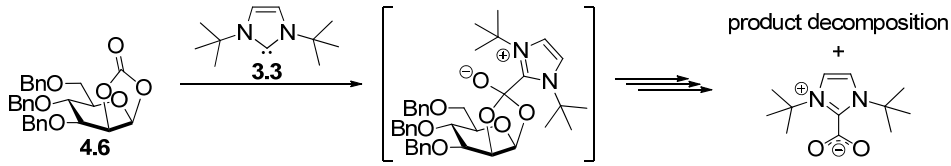
by nucheophilic attack at the anomeric position. This process is followed by the loss of carbon dioxide to give thiomannoside **4.11** (Scheme 4.9).[3a] Unfortunately, this compound is not reactive enough to attack the starting material in excess and therefore the reaction does not evolve, even when 16-crown-6 ether was used to complex the counterion of the nucleophile (Table 4.1, entry 6).



Scheme 4.9. Reaction of 1,2-carbonyl carbohydrate **4.6** with potassium thiophenolate

Since no dissacharide **4.17** was obtained, DMF was used as polar aprotic solvent to increase the nucleophilicity of both potassium thiophenolate and thiomannoside **4.11**. Under these conditions, PhSK was consumed in 3 h to afford carbohydrate **4.11** in 41% yield. However, neither longer reaction periods nor adding 18-crown-6 ether led to any conversion of **4.11** to dimer **4.17** (Table 4.1, entries 7 and 8). Alternatively, thiomannoside **4.11** can also be obtained in virtually the same yield employing thiophenol in the presence of alkali catalysts, such as K_2CO_3, DBU, and 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD). In all cases the reactions did not further evolve and starting materials were recovered (Table 4.1, entries 9-11).

Interestingly, **4.6** was found to react irreversibly with *N*-heterocyclic carbene **3.3**.[ii] In this process it provided full carbohydrate decomposition altogether with the obtention of the stable zwitterionic NHC carboxylate adduct (Table 4.1, entry 12). Decarboxylation of carbonate **4.6** after possible initial nucleophilic attack of the catalyst at the electropositive carbonyl carbon atom may trigger the degradation of the material (Scheme 4.10).

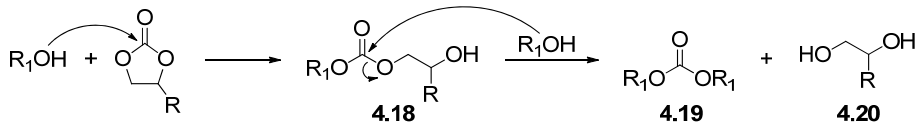


Scheme 4.10. Postulated reaction of carbonate **4.6** with carbene **3.3**

[ii] This compound also appears in Chapter 3, thus same codification will be employed.

The ring-opening oligomerization of saccharide **4.6** was then tested with thiophenol, but no reaction took place (Table 4.1, entries 13). In order to activate the electrophilic carbonate group, different cationic catalysts, including not only protic acids like *p*-toluenesulfonic acid (PTSA) and trifluoromethane sulfonic acid (TFMA), but also Lewis acids such as $BF_3{\cdot}OEt_2$, $Yb(OTf)_3$, $ZnCl_2$ were employed. However, the reactions did not lead to any perceptible conversion (Table 4.1, entries 14-18).

In the light of the previous unsuccessful experiments, the attention was turned to aliphatic nucleophiles. This kind of nucleophiles has been reported to behave with five-membered alkylene carbonates differently than their aromatic analogues.[3a] Hence, they undergo attack at the carbonyl position via the generalized route shown in Scheme 4.11. In such a process, the resulting mixed carbonate (**4.18**) can further be attacked by a second molecule of nucleophile to give dialkyl carbonate **4.19** and ethylene glycol **4.20**. This methodology has been applied to the synthesis of useful reactive intermediates such as dimethyl carbonate (R_1= Me).[27]



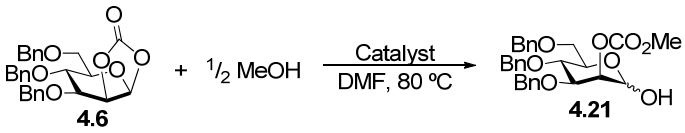
Scheme 4.11. Reactions of five-membered alkylene carbonates with aliphatic nucleophiles (note that there are two possible regioisomers of species **4.18**)

The oligomerization of carbonate **4.6** was then investigated with half equivalent of methanol relative to **4.6** at 80 ºC in DMF employing different bases and catalysts (Table 4.2). Thus, methanol by itself is not reactive enough to interact with the substrate after stirring 24 h (Table 4.2, entry 1). When the reaction was carried out in the presence of catalytic DBU, the starting material partially reacted and carbonate **4.21** was obtained in 24% yield (Table 4.2, entry 2). Just as it happened with thiomannoside **4.11**, saccharide **4.21** is not reactive enough to attack the carbonyl group of carbohydrate **4.6** and, therefore, no disaccharide was observed. Moreover, the TBD catalyst proved to be an efficient base for the ring-opening of carbonate **4.6** with methanol, since derivative **4.21** was synthesized in 40% yield (Table 4.2, entry 3). Attemps to use any acid catalyst (including protic acids as well as Lewis acids) failed since starting materials have always been recovered (Table 4.2, entries 4-8).

Alternatively, compound **4.6** was tested for its oligomerization potential employing 10 mol % of different basic initiators (DBU and TBD) in DMF (Table 4.3). Thus, all

reactions led to complex product mixtures (Table 4.3, entries 2 and 5). It is worth mentioning that benzyl alcohol was always obtained.

Table 4.2. Oligomerization attempts of compound **4.6** with MeOH and different additives[a]



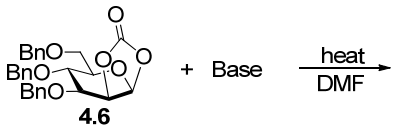
Entry	Catalyst	Conv. (%)[b]	Yield (%)
1	-	< 2	-
2	DBU	30[c]	**4.21** (24)
3	TBD	45[c]	**4.21** (40)
4	PTSA	< 2	-
5	TFMA	< 2	-
6	$BF_3{\cdot}OEt_2$	< 2	-
7	$Yb(OTf)_3$	< 2	-
8	$ZnCl_2$	< 2	-

[a] Conditions: 0.08 mmol carbonate, 0.04 mmol MeOH, 10 mol % catalyst, and 1 mL of DMF for 24 h. [b] Determination by ^1H NMR integration of characteristic signals on the reaction crude. [c] Yield recovered of **4.6** after purification by chromatography on silica gel.

The cleavage of benzyl ether protecting groups may arise from the basic conditions in conjunction with the high temperatures employed. In this context, attempts to decrease the reaction temperature in order to minimize this undesired side-reaction failed. No reaction was observed with any base at lower temperatures after 24 h stirring (Table 4.3, entries 1, 3, and 4).

Finally, the oligomerization reaction was tested on 1,2-monothiocarbonyl carbohydrate **4.7** under the best conditions already found for substrate **4.6**. Due to the high nucleophilicity of the propagating species (thiolates, good nucleophiles and weak bases) the thioglycoside bond formation can be readily based on an S_N2 displacement. Hence, the mild character of thiolates makes no necessary the use of extremely anhydrous conditions (just like the one used for the ROP of carbonate **4.6**). Reaction on substrate **4.7** was investigated with half equivalent of thiophenol and 10 mol % of TBD as a basic catalyst in DMF. After stirring the solution for 5 h at 80 ºC, all starting material disappeared but, however, a complex mixture was obtained (Scheme 4.12).

Table 4.3. Oligomerization attempts of compound **4.6** with different bases[a]



4.6

Entry	Base	T (ºC)	Conv. (%)[b]	Yield (%)
1	DBU	80	< 2	-
2[c]	DBU	100	> 98	degradation
3	TBD	80	< 2	-
4	TBD	100	< 2	-
5[c]	TBD	120	> 98	degradation

[a] Conditions: 0.08 mmol carbonate, 10 mol % base, and 1 mL of DMF for 24 h. [b] Determination by ^{1}H NMR integration on the reaction crude. [c] Reaction time: 10 h.

In order to examine the influence of the catalyst in the reaction, thiocarbonate **4.7** was then allowed to react with potassium thiophenolate in DMF, but a complex mixture was again obtained. ^{1}H and ^{13}C NMR spectroscopy, combined with MALDI-TOF mass spectrometry of both reaction crudes showed traces of carbohydrate **4.22** altogether with its disulfide derivative. Disaccharide **4.23**, among other related possible oligomers, was not found.

4.7 **4.22** **4.23**

Scheme 4.12. Reaction of substrate **4.7** with thiophenol in the presence of TBD

To sum up, reaction of **4.7** with 0.50 eq. of MeOH, respectively to the substrate, and 10 mol % of TBD in DMF at 80 ºC also afforded a complex mixture. After purification, the starting material was partially recovered (19% yield).

4.3. Conclusions

In summary, the ring-opening oligomerization of sugar carbonate **4.6** and its 2-thioanalogue (**4.7**) has been explored. With this purpose, 1,2-*O*-carbonyl pyranose **4.6** in 24% total yield has been prepared over seven synthetic steps from D-mannose

pentaacetate. Novel 1-*O*-2-*S*-thiocarbonyl pyranose **4.7** has also been synthesized from commercial tri-*O*-benzyl-D-glucal in seven steps (18% global yield).

The ring-opening oligomerization of cyclic carbonate **4.6** has been preliminary studied with several nucleophiles under different oligomerization conditions. Thiomannopyranoside **4.11** was synthesized with good conversion and selectivity employing PhSK as well as PhSH in conjuction with a catalytic base. Hence, the use of MeOH in the presence of a base such as TBD proceeds through nucleophilic attack at the carbonyl position to afford methoxycarbonyl derivative **4.21**. Both compounds are not reactive enough and therefore the oligomerization reaction does not further evolve.

Application of these optimized conditions to **4.7** was unsuccessful and complex mixtures were always obtained. Further comprehensive studies will need to be carried out in order to investigate the potential of **4.7** for oligosaccharide synthesis.

4.4. Experimental section

4.4.1. General methods

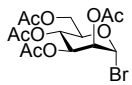
All chemicals used were reagent grade and were employed as supplied unless otherwise specified. HPLC grade dichloromethane and diethyl ether were dried using a solvent purification system (Pure SOLV system-4®). Solvents were purified using standard procedures.[28] Catalyst **3.3** was previously prepared in Chapter 3. ^{1}H and ^{13}C NMR spectra were recorded using two spectrometers (Varian® Mercury VX 400 and Varian® 400-MR) both operating at frequency of 400 MHz for proton and 100.6 MHz for carbon. $CDCl_3$ was used as solvents. Chemical shifts (δ) were referenced to internal standards (7.27 ppm ^{1}H, 77.23 ppm ^{13}C) or Me_4Si as an internal reference (0.00 ppm ^{1}H). 2D correlation spectra (gCOSY, NOESY, gHSQC and gHMBC) were visualized using the VNMR program (Varian®). Melting points (Mp) were measured on a Griffin melting point apparatus and were uncorrected. Optical rotations were measured at 598 nm at room temperature in a Perkin-Elmer® 241 MC apparatus with 10 cm cells. ESI-MS were run on an Agilent® 1100 Series LC/MSD instrument. IR spectra were recorded on a JASCO FT/IR-600 plus Fourier Transform Infrared Spectrometer ATR Specac Golden Gate. Reactions were monitored by TLC, carried out on 0.25 mm E. Merck® silica gel 60 F_{254} glass or aluminium plates. Developed TLC plates were visualized under a short-wave UV lamp (254 nm) and by heating plates that were dipped in ethanol/H_2SO_4. Flash column chromatography

was carried out using forced flow of the indicated solvent on Fluka® or Merck® silica gel 60 (230-400 mesh). Radial chromatography was performed on a Harrison® chromatotron model 7924T in 1 or 2 mm plates of Kieselgel 60 PF_{254} silica gel, depending on the amount of product. Flash column chromatography (FCC) was performed using flash silica gel (32–63 μm) and employing a polarity elution system correlated to TLC mobility.

4.4.2. General procedures

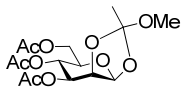
General Procedure for ring-opening oligomerization of (thio)carbonates: to a solution of (thio)carbonate (0.08 mmol) in THF or DMF (1 mL), nucleophile (0.04 mmol, aiming for the dimmer) and the additive (10 mol % when needed) were added. The resulting solution was heated up (80 ºC, if not indicated). After stirring for 24 h, the mixture was cooled to room temperature and brine (10 mL) was added. The resultant mixture was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography on silica gel gave the products in the yields shown.

4.4.3. Compound characterization

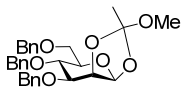


2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (4.8):[19] to D-mannose pentaacetate (21.50 g, 55.51 mmol), 130 mL of HBr in AcOH (33% solution) was added at room temperature. The reaction was stirred for 40 minutes and then diluted with CH_2Cl_2. The solution was transferred to a separatory funnel containing ice and the organic layer was washed with ice water until neutral pH. The organic extract was dried over $MgSO_4$, filtered and concentrated to afford product (17.30 g, 76%) from D-mannose. The crude product was crystallized from diethyl ether to form white powder. Data: R_f (3:1 AcOEt/Hexane): 0.37. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 6.28 (d, 1H, $J_{1,2}$=1.3 Hz, H-1); 5.71 (dd, 1H, $J_{3,2}$= 3.5 Hz, $J_{3,4}$= 10.5 Hz, H-3); 5.44 (dd, 1H, $J_{2,1}$= 1.3 Hz, $J_{2,3}$= 3.5 Hz, H-2); 5.36 (t, 1H, $J_{4,3}$= $J_{4,5}$= 10.1 Hz, H-4); 4.35-4.32 (m, 1H, $J_{6,6'}$= 12.5 Hz, H-6); 4.24-4.19 (m, 1H, 5-H); 4.15-4,10 (m, 1H, $J_{6',6}$= 12.5 Hz, H-6'); 2.17 (s, 3H, AcO); 2.10 (s, 3H, AcO); 2.07 (s, 3H, AcO); 2.00 (s, 3H, AcO). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 170.3, 169.5, 169.4 (C(O),

AcO); 83.0 (C-1); 72.7 (C-5); 72.0 (C-2); 67.8 (C-3); 65.1 (C-4); 61.3 (C-6); 20.6 (CH_3O, AcO); 20.5 (CH_3O, AcO); 20.5 (CH_3O, AcO); 20.4 (CH_3O, AcO).

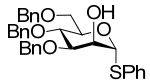


3,4,6-tri-*O*-acetyl-β-D-mannopyranose 1,2-(methyl orthoacetate) (4.9):[20] to a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (17.30 g, 47.62 mmol) in CH_2Cl_2 (93 mL) was treated with a mixture of MeOH (93 mL) and 2,6-lutidine (13 mL) for 14 h at room temperature. The mixture was diluted with CH_2Cl_2 (100 mL), washed with $NaHCO_3$ (aq, s) solution, water and then concentrated. After coevaporation of toluene, the residue was crystallized from MeOH/H_2O to afford the product 7.86 g, 51%) as a 9:1 *exo/endo* diastereomeric mixture. Data: R_f (1:1 AcOEt/Hexane): 0.43. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm (*exo* isomer): 5.50 (d, 1H, $J_{1,2}$=2.6 Hz, H-1); 5.30 (t, 1H, $J_{4,3}$= $J_{4,5}$= 9.6 Hz, H-4); 5.15 (dd, 1H, $J_{3,2}$= 3.9 Hz, $J_{3,4}$= 9.8 Hz, H-3); 4.62 (dd, 1H, $J_{2,1}$= 2.6 Hz, $J_{2,3}$= 3.9 Hz, H-2); 4.25 (dd, 1H, $J_{6',5}$= 2.8 Hz $J_{6',6}$= 12.5 Hz, H-6'); 4.14 (dd, 1H, $J_{6,5}$ =4.8 Hz, $J_{6,6'}$= 12.5 Hz, H-6); 3.69 (ddd, 1H, , $J_{5,4}$ =9.6 Hz, $J_{5,6}$ =4.8 Hz $J_{5',6}$= 2.8 Hz, H-5); 3.28 (s, 3H, OCH_3); 2.12 (s, 3H, AcO); 2.08 (s, 3H, AcO); 2.06 (s, 3H, AcO); 1.74 (s, 3H, $OCCH_3$). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm (*exo* isomer): 170.7, 170.4, 169.4 (C(O), AcO); 124,5 (C_{quat}); 97.1 (C-1); 76.6 (C-3); 71.3 (C-2); 70.6 (C-5); 65.5 (C-4); 62.3 (C-6); 49.9 (CH_3O); 24.3 (CCH_3); 20.8 (CH_3O, AcO); 20.7 (CH_3O, AcO); 20.6 (CH_3O, AcO).



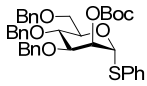
3,4,6-tri-*O*-benzyl-β-D-mannopyranose 1,2-(methyl orthoacetate) (4.10):[21] 3,4,6-tri-*O*-acetyl 1,2-*O*-(methoxyethylidene)-β-D-mannopyranose (4,95g, 13.66 mmol) was deacetylated in MeOH (40 mL) by the addition of ammonia-saturated methanol (8.2 mL) at room temperature overnight. The solution was concentrated to chromatographically pure syrup. The product was dissolved in DMF (66 mL) and NaH (2.97 g, 123.72 mmol) was added and the reaction mixture was stirred for 0.5 h. Benzyl bromide (8.24 mL, 69.69 mmol) was dropped into the reaction mixture for a period of 0.5 h at 15ºC and the stirring was continued for 5 h. Water (200 mL) was added and the mixture was extracted with chloroform, washed with water, 1 M solution of tartaric acid and water. The extract was dried, concentrated and the residue was purified by crystallization from hexane to yield pure desired compound (6.57 g, 95%). Data: R_f (1:1 AcOEt/Hexane): 0.55. ^{1}H NMR (400 MHz, $CDCl_3$) δ in

ppm (*exo* isomer): 7.40-7.15 (stack, 15H, Ar); 5.34 (d, 1H, $J_{1,2}$= 2.5 Hz, H-1); 4.88 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.77 (s, 2H, CH_2Ph); 4.61 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.60 (d, 1H, J_{AB}= 12.0 Hz, CH_2Ph); 4.52 (d, 1H, J_{AB}= 12.0 Hz, CH_2Ph); 4.40 (dd, 1H, $J_{2,1}$= 2.5 Hz, $J_{2,3}$= 4.5 Hz H-2); 3.91 (t, 1H, $J_{4,3}$= $J_{4,5}$= 9.3 Hz, H-4); 3.76-3.68 (stack, 3H, H-3, H-6 and H-6'); 3.42 (ddd, 1H, $J_{5,4}$= 9.0 Hz, $J_{5,6}$= 4.0 Hz, $J_{5,6'}$= 2.5 Hz, H-5); 3.28 (s, 3H, OCH_3); 1.73 (s, 3H, CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm (*exo* isomer): 138.3, 138.1, 137.9 (C, Ph); 128.5, 128.4, 128.3, 128.0, 127.8, 127.5 (CH, Ph); 124.0 (C_{quat}); 97.6 (C-1); 79.1 (C-3); 77.1 (C-2); 75.2 (CH_2Ph); 74.3 (C-4, C-5); 73.4 (CH_2Ph); 72.4 (CH_2Ph); 69.1 (C-6); 49.8 (CH_3O); 24.4 (CCH_3).

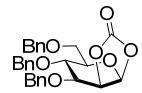


Phenyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (4.11):[22] activated molecular sieves (4 Å, 323 mg and 5 Å, 323 mg) were added to a solution of sugar orthoester **4.10** (6.44 g, 11.93 mmol) and thiophenol (13.1 mL, 128.15 mmol) in CH_3CN (26 mL) and the resulting suspension was stirred at R.T. for 30 min before adding $HgBr_2$ (461 mg, 1.28 mmol). The reaction was stirred for 1h and the slurry then filtered into $NaHCO_3$ solution (40 mL). The liquid was extracted with EtOAc (3 x 25 mL) and the combined organic extracts were washed with H_2O (25 mL), brine (20 mL), dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting dark syrup was dissolved in MeOH (130 mL) and MeONa (1.38 g, 24.27 mmol) was added at R.T. The solution was stirred for 6 h before adding Amberlite® IR-120(plus) ion exchange resin (1.3 g). The solution was filtered and the solid residue washed with MeOH (25 mL). The filtrate was then concentrated under reduced pressure. EtOAc (50 mL) was added and the solution was washed with H_2O (13 mL), brine (10 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to leave a dark oil which was purified by flash column chromatography (3:7 AcOEt/Hexane) to afford the desired alcohol as a colorless oil (5.84 g, 83%). Data: R_f (3:7 AcOEt/Hexane): 0.20. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.51-7.44 (m, 2H, Ar); 7.40-7.17 (stack, 18H, Ar); 5.62 (d, 1H, $J_{1,2}$= 1.2 Hz, H-1); 4.85 (d, 1H, J_{AB}= 10.7 Hz, CH_2Ph); 4.73 (s, 2H, CH_2Ph); 4.63 (d, 1H, J_{AB}= 11.9 Hz, CH_2Ph); 4.53 (d, 1H, J_{AB}= 10.7 Hz, CH_2Ph); 4.46 (d, 1H, J_{AB}= 11.9 Hz, CH_2Ph); 4.31 (ddd, 1H, $J_{5,4}$= 9.0 Hz, $J_{5,6}$= 4.4 Hz, $J_{5,6'}$= 1.6 Hz, H-5); 4.27 (m, 1H, H-2); 3.95 (t, 1H, $J_{4,3}$= $J_{4,5}$= 9.0 Hz, H-4); 3.88 (dd, 1H, $J_{3,2}$= 3.2 Hz, $J_{3,4}$= 9.0 Hz, H-3); 3.81 (dd, 1H, $J_{6,5}$= 4.4 Hz, $J_{6,6'}$= 10.8 Hz, H-6); 3.69 (dd, 1H, $J_{6',5}$= 1.6 Hz, $J_{6',5}$= 10.8 Hz, H-6'); 2.68 (d, 1H, $J_{OH,2}$= 2.4 Hz OH). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 138.2, 138.1, 137.6, 133.8 (C, Ph); 128.5, 128.3, 128.2, 128.0, 127.9, 127.8 127.7, 127.6, 127.5,

127.3 (CH, Ph); 87.3 (C-1); 80.2 (C-3); 75.1 (CH_2Ph); 74.4 (C-4); 73.3 (CH_2Ph); 72.2 (C-5); 72.0 (CH_2Ph); 69.8 (C-2); 68.8 (C-6).

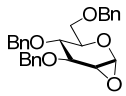


Phenyl 2-*O*-tert-butxoycarbonyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (4.12):[18] to a solution of phenyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (448.3 mg, 0.83 mmol) in CH_2Cl_2 (12.4 mL) were added Boc_2O (706.7 mg, 3.3 mmol), Et_3N (149 μL, 1.07 mmol) and DMAP (10.2 mg, 0.08 mmol) followed by stirring at room temperature overnight. The mixture was washed with saturated $NaHCO_3$ and brine, dried, and concentrated. Chromatographic purification (AcOEt/Hexane 1:5) afforded the Boc protected sugar as a colorless oil (519.7 mg, 98%). Data: R_f (3:7 AcOEt/Hexane): 0.50. ^1H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.51-7.49 (m, 2H, Ar); 7.38-7.19 (stack, 18H, Ar); 5.61 (d, 1H, $J_{1,2}$= 1.5 Hz, H-1); 5.37 (t, 1H, $J_{2,1}$= $J_{2,3}$= 1.5 Hz H-2); 4.90 (d, 1H, J_{AB}= 10.8 Hz, CH_2Ph); 4.76 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.65 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.61 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.52 (d, 1H, J_{AB}= 10.8 Hz, CH_2Ph); 4.47 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.35 (m, 1H, H-5); 3.97-3.92 (m, 2H, H-3 and H-4); 3.84 (dd, 1H, $J_{6,5}$= 5.0 Hz, $J_{6,6'}$= 10.5 Hz, H-6); 3.74 (dd, 1H, $J_{6',5}$= 2.0 Hz, $J_{6',5}$= 10.5 Hz, H-6'); 1.47 (s, 9H, Boc). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 153.1 (C(O), Boc); 138.4, 138.3, 137.8, 133.8 (C, Ph); 131.9, 129.0, 128.4, 128.3, 128.1, 128.0 127.9, 127.8, 127.7, 127.6, 127.5 (CH, Ph); 86.1 (C-1); 82.7 (C_{quat}, Boc) 78.6 (C-3); 75.3 (CH_2Ph); 74.7 (C-4); 73.4 (CH_2Ph); 73.0 (C-2); 72.5 (C-5); 71.9 (CH_2Ph); 69.0 (C-6); 27.8 (CH_3, Boc).

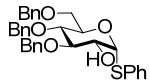


3,4,6-Tri-*O*-benzyl-1,2-*O*-carbonyl-α-D-mannopyranose (4.6):[18] phenyl 2-*O*-tert-butoxycarbonyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (520 mg, 8.08 mmol), 1-benzenesulfinyl piperidine (209 mg, 9.70 mmol), 2,4,6-tri-tertbutylpyrimidine (311 mg, 12.12 mmol) and activated 4 Å powdered molecular sieves (800 mg) were dissolved in dry CH_2Cl_2 (8 mL) and stirred at -60 ºC under N_2 atmosphere. After stirring 10 min, trifluoromethanesulfonic anhydride (0.26 mL, 12.12 mmol) was added dropwise and maintained at -60 ºC for 2 h. The reaction mixture was then allowed to warm to room temperature before it was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phase was washed with brine, dried, and concentrated. The crude reaction

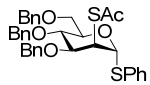
mixture was purified by chromatography on silica gel (from 3:7 to 1:1 AcOEt/Hexane), affording the carbonate (302 mg, 79% yield) as a white solid. Data: R_f (3:7 AcOEt/Hexane): 0.20. Mp: 107.3 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.38-7.29 (stack, 13, Ar); 7.21-7.19 (m, 2H, Ar); 5.79 (d, 1H, $J_{1,2}$= 5.0 Hz, H-1); 4.80 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.79 (m, 1H, $J_{2,3}$= 3.7 Hz, H-2); 4.78 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.75 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.55 (2H, CH_2Ph); 4.53 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 3.93 (dd, $J_{4,3}$= 8.6 Hz, $J_{4,5}$= 7.6 Hz, 1H, H-4), 3.90 (dd, 1H, $J_{3,2}$= 3.7 Hz, $J_{3,4}$= 8.6 Hz, H-3); 3.83 (m, $J_{4,5}$= 7.6 Hz, 1H, H-5); 3.61-3.56 (m, 2H, H-6 and H-6'). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 153.0 (C(O)); 137.7, 137.6, 137.2 (C, Ph); 128.7, 128.5, 128.4, 128.2, 128.1, 128.0 127.9, 127.8 (CH, Ph); 96.4 (C-1); 76.8 (C-5); 75.9 (C-3); 75.3 (C-2); 74.7 (CH_2Ph); 73.6 (CH_2Ph); 73.0 (C-4); 72.9 (CH_2Ph); 69.4(C-6).



1,2-Anhydro-3,4,6-Tri-*O*-benzyl-α-D-glucopyranose (3.2):[24] tri-*O*-benzyl-D-glucal (510 mg, 1.22 mmol) was dissolved in an ice bath cooled biphasic solution of CH_2Cl_2 (5 mL), acetone (0.5 mL) and saturated aqueous $NaHCO_3$ (8.4 mL). The mixture was vigorously stirred and a solution of Oxone® (752 mg, 2.44 mmol) in H_2O (5.9 mL) was added dropwise over 30 min. The crude reaction was vigorously stirred at 0º C for 30 min and was then allowed to warm to room temperature until TLC indicated complete consumption of the glycal. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3x 15 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated to afford the 1,2-anhydro-pyranose in a quantitative yield (516 mg) as a white solid. The crude could not be purified due the decomposition of the 1,2-anhydro-sugar. Data: R_f (1:1 AcOEt/Hexane): 0.18. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.40-7.13 (stack, 15H, Ar); 4.96 (d, 1H, $J_{1,2}$= 2.4 Hz, H-1); 4.81 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.78 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.66 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.61 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.59 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.51 (d, 1H, J_{AB}= 12.2. Hz, CH_2Ph); 3.96 (d, 1H, $J_{3,4}$= 8.0 Hz, H-3); 3.80-3.69 (m, 2H, H-5 and H-6'); 3.68-3.60 (m, 2H, H-4 and H-6); 3.02 (d, 1H, $J_{2,1}$= 2.4 Hz, H-2).^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 138.3, 138.0, 137.6 (C, Ph); 128.7, 128.6, 128.5, 128.4, 128.1, 128.0 127.9, 127.8 (CH, Ph); 79.0 (C-3); 77.6 (C-1); 74.7 (CH_2Ph); 74.3 (C-4); 73.6, 72.3 (CH_2Ph); 69.5 (C-5); 68.3 (C-6); 52.6 (C-2).

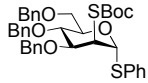


Phenyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-glucopyranose (4.13):[29] following the procedure reported by Crotti et al,[30] 1,2-Anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (3.04 g, 7.03 mmol) was disolved in anhydrous THF (117 mL) and cooled at -40 ºC. Then, thiophenol (1.49 mL, 21.09 mmol) was added dropwise. After 30 min of stirring, the solution was warmed at room temperature for 12 h. The reaction was diluted with CH_2Cl_2 (100 mL) and washed with $NaHCO_3$ (aq, s) (2 x 50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated to afford a anomeric mixture of phenyl 3,4,6-tri-*O*-benzyl-1-thio-D-glucopyranose (10:1 α/β). The crude reaction mixture was purified by chromatography on silica gel (from 1:3 to 1:1 AcOEt/Hexane), affording pure phenyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-glucopyranose (2.82 g, 74% yield) as a colorless syrup. Data: R_f (1:1 AcOEt/Hexane): 0.52. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.55-7.16 (stack, 20H, Ar); 5.65 (d, 1H, $J_{1,2}$= 5.8 Hz, H-1); 4.92 (d, 1H, J_{AB}= 11.2 Hz, CH_2Ph); 4.88 (d, 1H, J_{AB}= 11.2 Hz, CH_2Ph); 4.83 (d, 1H, J_{AB}= 10.6 Hz, CH_2Ph); 4.65 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.54 (d, 1H, J_{AB}= 10.6 Hz, CH_2Ph); 4.48 (d, 1H, J_{AB}= 12.2. Hz, CH_2Ph); 4.32 (ddd, 1H, $J_{5,4}$ = 9.2 Hz, $J_{5,6}$ = 3.7 Hz, $J_{5,6'}$ = 1.8 Hz, H-5); 4.03 (dt, $J_{2,1}$= $J_{2,OH}$ = 5.8 Hz, $J_{2,3}$= 9.0 Hz, H-2); 3.84 (dd, 1H, $J_{6,5}$= 3.7 Hz, $J_{6,6'}$= 10.4 Hz, H-6); 3.77 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 9.2 Hz, H-4); 3.73-3.62 (m, 2H, H-3 and H-6'), 2.40 (1H, $J_{OH,2}$ = 5.8 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 138.5, 138.1, 138.0, 134.2 (C, Ph); 129.2, 128.8, 128.7, 128.6, 128.2, 128.1, 128.0, 127.9, 127.7 (CH, Ph); 90.2 (C-1); 83.6 (C-3); 77.7 (C-4); 75.6, 75.2, 73.7 (CH_2Ph); 72.5 (C-2); 72.1 (C-5); 68.5 (C-6).

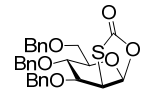


Phenyl 2-*S*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranose (4.15): phenyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-glucopyranose (3.28 g, 6.04 mmol) was dissolved in CH_2Cl_2 (20 mL) and the mixture was cooled to 0 ºC under argon. Dry pyridine (1.22 mL, 15.0 mmol) and trifluoromethanesulfonic anhydride (1.22 mL, 7.6 mmol) were added. After 2 h of stirring at 0 ºC, thin layer chromatograpy (1:3 AcOEt/Hexane) showed complete conversion of the staring material into a single product. The mixture was poured into ice-water (100 mL) and extracted with CH_2Cl_2 (3x50 mL). The organic extracts were dried over MgSO4, filtered and concentrated in vacuo. To a solution of the crude triflate in dry DMF (25 mL) at room temperature was added potassium thioacetate (1.78 g, 14.5 mmol). After 19 h, the crude mixture was poured

into diethyl ether (200 mL) and it was washed with brine (200 mL). The aqueous phase was re-extracted with more ether (2x60 mL) and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (from 1:10 to 1:3 AcOEt/Hexane), affording the desired compound (1.52 g, 50% yield) as a yellowish syrup. Data for the intermediate sugar triflate: R_f (1:3 AcOEt/Hexane): 0.55. $[\alpha]_D$ + 1.4 (*c* 1.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.52-7.49 (m, 2H, Ar); 7.37-7.27 (stack, 16H, Ar); 7.17-7.13 (m, 2H, Ar); 5.72 (d, 1H, $J_{1,2}$= 5.6 Hz, H-1); 5.03 (dd, 1H, $J_{2,1}$= 5.6 Hz, $J_{2,3}$= 9.8 Hz, H-2); 4.90 (d, 1H, J_{AB}= 10.4 Hz, CH_2Ph); 4.82 (d, 1H, J_{AB}= 10.4 Hz, CH_2Ph); 4.81 (d, 1H, J_{AB}= 10.4 Hz, CH_2Ph); 4.62 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.52 (d, 1H, J_{AB}= 10.4 Hz, CH_2Ph); 4.46 (d, 1H, J_{AB}= 12.2. Hz, CH_2Ph); 4.42 (ddd, 1H, $J_{5,4}$ = 9.8 Hz, $J_{5,6}$ = 3.6 Hz, $J_{5,6'}$ = 1.8 Hz, H-5); 3.98 (t, 1H, $J_{3,2}$= $J_{3,4}$= 9.8 Hz, H-3); 3.82 (pt, 1H, $J_{4,3}$= $J_{4,3}$= 9.8 Hz, H-4); 3.82 (dd, 1H, $J_{6,5}$= 3.6 Hz, $J_{6,6'}$= 10.8 Hz, H-6); 3.68 (dd, 1H, $J_{6',5}$= 1.8 Hz, $J_{6',6}$= 10.8 Hz, H-6); ^{19}F NMR (372.5 MHz, $CDCl_3$) δ in ppm: -74.8. ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 137.8, 137.7, 137.4 (C, CH_2Ph); 133.0 (C, SPh); 129.5 (CH, SPh); 128.7,128.6 (CH, CH_2Ph); 125.5 (CH, SPh); 128.3, 128.2, 128.1, 128.0 (CH, CH_2Ph); 118.6 (q, $J_{C,F}$= 319 Hz; CF_3); 86.9 (C-1); 83.7 (C-2); 79.9 (C-3); 78.2 (C-4); 73.3, 75.5, 73.7 (CH_2Ph); 71.7 (C-5); 68.0 (C-6). +TOF MS Calcd for $C_{34}H_{33}F_3O_7S_2$ *m/z* [M-NH_4]+: 692,1958, found: 692.1954; [M-Na]+: 697.1512, found: 697.1508; [M-K]+: 713.1251, found: 713.1275. Data for the final product: R_f (3:7 AcOEt/Hexane): 0.71. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.41-7.38 (m, 2H, Ar); 7.28-7.16 (stack, 16H, Ar); 7.11-7.08 (m, 2H, Ar); 5.50 (s, 1H, H-1); 4.80 (d, 1H, J_{AB}= 10.4 Hz, CH_2Ph); 4.62 (d, 1H, J_{AB}= 11.4 Hz, CH_2Ph); 4.58 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 5.57 (m, 1H, H-2); 4.46 (d, 1H, J_{AB}= 11.4 Hz, CH_2Ph); 4.39 (d, 1H, J_{AB}= 10.4 Hz, CH_2Ph); 4.36 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.28 (ddd, 1H, $J_{5,4}$ = 9.6 Hz, $J_{5,6}$ = 4.2 Hz, $J_{5,6'}$ = 1.6 Hz, H-5); 4.16 (dd, 1H, $J_{3,2}$= 4.6 Hz, $J_{3,4}$= 9.0 Hz, H-3); 3.74 (dd, 1H, $J_{6,5}$= 4.2 Hz, $J_{6,6'}$= 10.8 Hz, H-6); 3.58 (pt, 1H, $J_{4,3}$= $J_{4,3}$= 9.6 Hz, H-4); 3.59 (m, 1H, H-6); 2.31 (s, 3H, AcS). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 194.7 (C(O), SAc); 138.4, 138.3, 137.6, 134.3 (C, Ph); 132.0, 129.2, 128.6, 128.5, 128.4, 128.3 128.1, 128.0, 127.9, 127.8, 127.7 (CH, Ph); 88.6 (C-1); 77.9 (C-3); 75.9 (C-4); 75.4, 73.5 (CH_2Ph); 72.7 (C-5); 71.7 (CH_2Ph); 68.9 (C-6); 49.2 (C-2); 30.9 (CH_3, SAc).

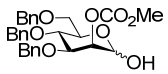


Phenyl 2-*S*-tert-butoxycarbonyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (4.16): to a stirred solution of phenyl 2-*S*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranose (1.52 g, 2.53 mmol) in a mixture 2:1 of absolute ethanol/CH_2Cl_2 (30 mL) at O ºC was added a solution of potassium tert-butoxide (0.31 g, 2.77 mmol) in absolute ethanol (27 mL) dropwise. After 20 min, a solution of Boc_2O (0.62 g, 2.77 mmol) in CH_2Cl_2 (10mL) was added and maintained at 0 ºC for 2h. The mixture was allowed to warm to room temperature and then brine (100 mL) was added. The resultant mixture was extracted with CH_2Cl_2 (3x 50 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (1:25 AcOEt/Hexane) to give the desired product as a yellowish syrup (1.24 g, 74%). R_f (1:15 AcOEt/Hexane): 0.22. $[\alpha]_D$ + 0.4 (*c* 1.27, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 1715, 1700, 1124, 1094. ^1H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.52-7.49 (m, 2H, Ar); 7.43-7.37 (m, 2H, Ar); 7.34-7.24 (stack, 14H, Ar); 7.21-7.17 (m, 2H, Ar); 5.74 (d, 1H, $J_{1,2}$= 1.2 Hz, H-1); 4.90 (d, 1H, J_{AB}= 10.8 Hz, CH_2Ph); 4.79 (d, 1H, J_{AB}= 11.2 Hz, CH_2Ph); 4.65 (d, 1H, J_{AB}= 12.0 Hz, CH_2Ph); 4.58 (d, 1H, J_{AB}= 11.2 Hz, CH_2Ph); 4.48 (d, 1H, J_{AB}= 10.8 Hz, CH_2Ph); 4.46 (d, 1H, J_{AB}= 12.0 Hz, CH_2Ph); 4.41 (dd, 1H, $J_{2,1}$= 1.6 Hz, $J_{2,3}$= 4.6 Hz, H-2); 4.38 (ddd, 1H, $J_{5,4}$= 9.2 Hz, $J_{5,6}$= 4.6 Hz, $J_{5,6'}$= 1.8 Hz, H-5); 4.22 (dd, 1H, $J_{3,2}$= 4.6 Hz, $J_{3,4}$= 9.2 Hz, H-3); 3.84 (dd, 1H, $J_{6,5}$= 4.6 Hz, $J_{6,6'}$= 10.8 Hz, H-6); 3.69 (t, 1H, $J_{4,3}$= $J_{4,5}$= 9.2 Hz, H-4); 3.67 (dd, 1H, $J_{6',5}$= 1.8 Hz, $J_{6',5}$= 10.8 Hz, H-6'); 1.49 (s, 9H, Boc). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 168.5 (C(O), Boc); 138.4, 137.9, 134.5 (C, Ph); 131.8, 129.2, 128.6, 128.5, 128.4, 128.4 128.2, 128.0, 127.9, 127.7, 127.6 (CH, Ph); 88.7 (C-1); 85.7 (C_{quat}, Boc) 78.3 (C-3); 75.7 (C-4); 75.5, 73.5 (CH_2Ph); 72.7 (C-5); 71.7 (CH_2Ph); 69.0 (C-6); 51.2 (C-2); 28.4 (CH_3, Boc). +TOF MS Calcd for $C_{38}H_{42}O_6S_2$ *m/z* [M-NH_4]+: 676.2661, found: 676.2635; [M-Na]+: 681.2315 found: 681.2288; [M-K]+: 697.2054, found: 697.2006.



3,4,6-Tri-*O*-benzyl-1-*O*-2-*S*-carbonyl-α-D-mannopyranose (4.7): following the procedure reported by Crich et al.[18], phenyl 2-*S*-tert-butxoycarbonyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (1.07 g, 1.62 mmol), 1-benzenesulfinyl piperidine (0.42 g, 1.94 mmol), 2,4,6-tri-tertbutylpyrimidine (0.63 g, 2.43 mmol) and activated 4 Å powdered molecular sieves (1.62 g) were dissolved in dry CH_2Cl_2 (20

mL) and stirred at -60 ºC under N_2 atmosphere. After stirring 10 min, trifluoromethanesulfonic anhydride (0.40 mL, 2.43 mmol) was added dropwise and maintained at -60 ºC for 2 h. The reaction mixture was then allowed to warm to room temperature before it was quenched with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phase was washed with brine, dried, and concentrated. The crude reaction mixture was purified by chromatography on silica gel (from 3:7 to 1:1 AcOEt/Hexane), affording the carbonate (0.54 g, 68% yield) as a white solid. Data: R_f (3:7 AcOEt/Hexane): 0.50. Mp: 92 – 93 ºC. $[\alpha]_D$ + 0.24 (*c* 5.45, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 1736, 1454, 1359, 1110, 1087, 1060, 1024, 1012, 696. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.34-7.29 (stack, 13, Ar); 7.21-7.19 (m, 2H, Ar); 5.56 (d, 1H, $J_{1,2}$= 2.8 Hz, H-1); 4.87 (d, 1H, J_{AB}= 10.8 Hz, CH_2Ph); 4.74 (d, 1H, J_{AB}= 11.4 Hz, CH_2Ph); 4.71 (d, 1H, J_{AB}= 11.4 Hz, CH_2Ph); 4.67 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.66 (m, 1H, H-2); 4.64 (d, 1H, J_{AB}= 10.8 Hz, CH_2Ph); 4.60 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.00 (m, 2H, H-3 and H-4); 3.78 (m, 2H, H-6 and H-6'); 3.52 (m, 1H, H-5). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 171.7 (C(O)); 138.0, 137.9, 137.1 (C, Ph); 128.8, 128.6, 128.5, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8 (CH, Ph); 97.3 (C-1); 78.3 (C-3); 75.4 (CH_2Ph); 74.6 (C-5); 73.7 (CH_2Ph); 73.6 (C-4); 72.7 (CH_2Ph); 68.5 (C-6); 54.3 (C-2). +TOF MS Calcd for $C_{28}H_{28}O_6S$ *m/z* [M-NH_4]+: 510.1945, found: 510.1967; [M-Na]+: 515.1499, found: 515.1534; [M-K]+: 531.1238, found: 531.1251.



3,4,6-Tri-*O*-benzyl-2-*O*-methoxycarbonyl-D-mannopyranose (4.21): according to the general procedure for ring-opening oligomerization of (thio)carbonates, the title compound was synthesized by reaction of **4.6** (53.9 mg, 0.11 mmol), methanol (2.3 µL, 0.06 mmol) and TBD (1.7 mg, 0.01 mmol) in DMF (1.4 mL) at 80 ºC for 24 h. The reaction mixture was then allowed to cool to room temperature before it was quenched with brine and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phase was washed with more brine, dried, and concentrated. The crude reaction mixture was purified by chromatography on silica gel (from 1:9 to 1:3 AcOEt/Hexane), affording **4.21** (22.4 mg, 40% yield) as a colourless syrup. Data for the α anomer: R_f (1:3 AcOEt/Hexane): 0.80. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.40-7.25 (stack, 13, Ar); 7.20-7.15 (m, 2H, Ar); 5.28 (d, 1H, $J_{1,2}$= 1.8 Hz, H-1); 5.17 (dd, 1H, $J_{2,1}$= 1.8, $J_{2,3}$= 3.4 Hz, H-1); 4.84 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.74 (d, 1H, J_{AB}= 11.2 Hz, CH_2Ph); 4.56 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.55 (d, 1H, J_{AB}= 11.2 Hz, CH_2Ph); 4.49 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.4 (d, 1H, J_{AB}= 11.0 Hz,

CH_2Ph); 4.10 (ddd, 1H, $J_{5,4}$= 9.6 Hz, $J_{5,6}$= 6.2 Hz, $J_{5,6'}$= 2.4 Hz, H-5); 4.05 (dd, 1H, $J_{3,2}$= 3.4 Hz, $J_{3,4}$= 9.6 Hz, H-3); 3.77 (s, 3H, CH_3O); 3.69 (t, 1H, $J_{4,3}$= $J_{4,5}$= 9.6 Hz, H-4); 3.68 (dd, 1H, $J_{6',5}$= 2.4 Hz, $J_{6',6}$= 10.6 Hz, H-6'); 3.64 (dd, 1H, $J_{6,5}$= 6.2 Hz, $J_{6,6'}$= 10.6 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 155.5 (C(O)); 138.3, 138.1, 137.7 (C, Ph); 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8 (CH, Ph); 92.2 (C-1); 77.8 (C-3); 75.3 (CH_2Ph); 74.9 (C-4); 73.4 (CH_2Ph); 73.1 (C-2); 71.9 (CH_2Ph);70.9 (C-5); 69.5 (C-6); 55.1 (CH_3O).

4.5. References

1. a) Webster, D. C. *Progress Org. Coat.* **2003**, *47*, 77-86 and references therein. b) Parrish, J. P.; Salvatore, R. N.; Jung, K. W. *Tetrahedron* **2000**, *56*, 8207-8237 and references therein.
2. a) Albertsson, A.-C.; Sjoeling M. *J. Macromol Sci. Pure Appl. Chem.* **1992**, *29*, 43-54. b) Kühling, S.; Keul, H.; Höcker, H.; Buysch, H.-J.; Shön, N.; Leitz, E. N. *Macromolecules* **1991**, *24*, 4229-4235.
3. a) Clements, J. H. *Ind. Eng. Chem. Res.* **2003**, *42*, 663-674. b) Rokicki, G. *Prog. Polym. Sci.* **2000**, *25*, 259-342.
4. a) Kricheldorf, H. R.; Jenssen, J. J. *Macromol. Sci. Pure Appl. Chem.* **1989**, *A26*, 631-644. b) Kricheldorf, H. R.; Dunsing, R.; Serra i Albet, A. *Makromol. Chem.* **1987**, *188*, 2453-2466.
5. a) Kéki, S.; Török, J.; Deák, G.; Miklos, Z. *Macromolecules* **2001**, *34*, 6850-6857. b) Lee, J.-C.; Morton, H. L. *Macromolecules* **2000**, *33*, 1618-1627. c) Soós, L.; Deák, G.; Kéki, S.; Zsuga, M. *J. Polym. Sci. Part A: Polym. Chem.* **1999**, *37*, 545-550.
6. Vogdanis, L.; Heitz, W. *Makromol. Chem., Rapid Commun.* **1986**, *7*, 543-547.
7. Kricheldorf, H. R.; Jonte, J. M.; Berl, M. *Makromol. Chem., Suppl.* **1985**, *12*, 25-38.
8. Vogdanis, L.; Heitz, W. *Makromol. Chem.* **1990**, *191*, 465-472.
9. a) Rokicki, G. *Prog. Polym. Sci.* **2000**, *25*, 259-342. b) Kuran, W.; *Prog. Polym. Sci.* **1998**, *23*, 919-992.
10. a) Soga, K.; Hosoda, S.; Tazuke, Y.; Ikeda, S. *J. Polym. Sci., Polym. Chem. Ed.* **1977**, *15*, 219-229. b) Soga, K.; Hosoda, S.; Tazuke, Y.; Ikeda, S. *J. Polym. Sci., Polym. Lett. Ed.* **1976**, *14*, 161-165.
11. a) Rokicki, G.; Nguyen, X. T. *J. Macromol. Sci., Part A: Pure Appl. Chem.* **1995**, *32*, 265-274. b) Sakai, S.; Fujinami, T.; Sakurai, S. *J. Polym. Sci., Polym. Lett. Ed.* **1973**, *11*, 631-633.
12. Lee, J.-C.; Litt, M. H. *Macromolecules* **2000**, *33*, 1618-1627.
13. Harris, R. F.; McDonald, L. A. *J. Appl. Polym. Sci.* **1989**, *37*, 1491-1511.
14. Storey, R. F.; Hoffman, D. C. *Macromolecules* **1992**, *25*, 5369-5382.

15. Pawlowski, P.; Rokicki, G. *Polymer* **2004**, *45*, 3125-3137.
16. Haba, O.; Tomizuka, H.; Endo, T. *Macromolecules* **2005**, *38*, 3562-3563.
17. Soga, K.; Imamura, H.; Ikeda, S. *Makromol. Chem.* **1975**, *176*, 807-811.
18. Crich, D.; Hu, T.; Cai, F. *J. Org. Chem.* **2008**, *73*, 8942-8953.
19. Czechura, P.; Tam, R. Y.; Dimitrijevic, E.; Murphy, A. V.; Ben R. N. *J. Am. Chem. Soc.* **2008**, *130*, 2928-2929.
20. Ziegler, T.; Lemanski, G. *Eur. J. Org. Chem.* **1998**, *1,* 163-170.
21. Garegg, P. J.; Maron, L. *Acta Chem. Scand. B.* **1979**, *33,* 39-41.
22. Beignet, J.; Tiernan, J.; Woo, C. H.; Kariuki, B. M. Cox, L. R. *J. Org. Chem.* **2004**, *69*, 6341-6356.
23. Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015-9020.
24. Cheshev, P.; Marra, A.; Dondoni, A. *Carbohydr. Res.* **2006**, *341*, 2714-2716.
25. a) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem., Int. Ed.* **1996**, *35*, 1380-1419. b) Gordon, D. M.; Danishefsky, S. *J. Carbohydr. Res.* **1990**, *206*, 361-366.
26. Di Bussolo, V.; Fiasella, A.; Balzano, F.; Uccello Barretta, G.; Crotti, P. *J. Org. Chem.* **2010**, *75*, 4284-4287.
27. a) Hironori, M.; Shinsuke, F.; Hiroshi, H. WO2007/69462 A1, **2008**. b) Shinsuke, F.; Hironori, M.; Hiroshi, H.; Kazuhiko, M. International patent WO2007/34669 A1, **2007**.
28. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed., Pergamon Press, Oxford, **1989**.
29. Carpintero, M.; Nieto, I.; Fernández-Mayoralas, A. *J. Org. Chem.* **2001**, *5*, 1768-1774.
30. Di Bussolo, V.; Fiasella, A.; Balzano, F.; Barretta, G. U.; Crotti, P. *J. Org. Chem.* **2010**, *75*, 4284-4287.

CHAPTER 5

MOLYBDENUM-CATALYZED EPISULFIDATION REACTIONS

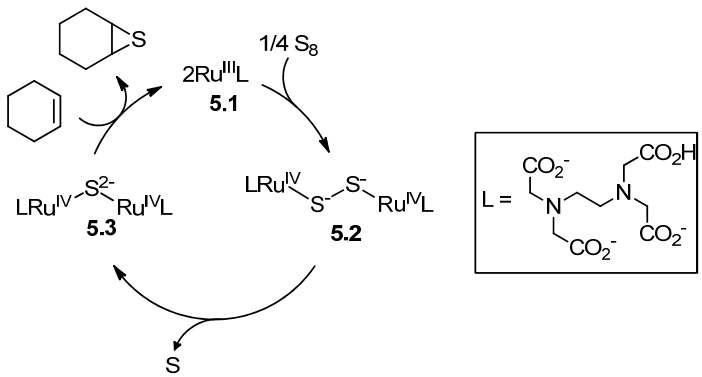
UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

5.1. Background

Despite the fact that metal peroxo complexes are among the most active and efficient catalysts for alkene epoxidation,[1] little is know about the analogous episulfidation reaction.[2] The most significant feature of sulfur versus oxygen transfer is its much lower electronegativity, which limits severely the electrophilic character of the sulfur donor: thus, the unreactive sulfur source must either be activated or a reactive sulfur donor must be generated *in situ*. Another disadvantage of sulfur versus oxygen transfer is the persistence of the S-S bond as compared to the labile O-O (peroxo) bond, which provides sulfur the propensity to catenate; consequently, instead of sulfur transfer, the reactive sulfur donor prefers to extrude elemental sulfur. Futhermore, unlike oxygen, sulfur may exist in higher oxidation states, which implies that the divalent sulfur donor necessary for the sulfur transfer may be readily oxidized, especially under the condition of oxidative activation

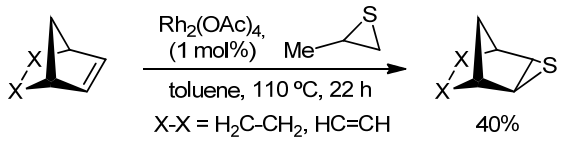
Metal-catalyzed sulfur transfer is still a relatively unexplored field of sulfur chemistry and offers promising opportunities for the direct sulfidation of organic substrates. Indeed, few metal catalysts showing direct sulfur-atom transfer have been published. Specifically, Taqui Khan and Siddiqui[3] claimed the very first catalytic

sulfur atom transfer reaction to cyclohexene by persulfidorhuthenium (IV) complexes. Reactions employing protic media (ethanol/water) takes place in a stoichiometric manner with the formation of cyclohexene sulfide and complex **5.1** (Scheme 5.1). Thus, in the presence of an excess of sulfur the reaction becomes catalytic. The proposed mechanism for the sulfur atom transfer to cyclohexene is shown in Scheme 5.1.[3] The initial [$Ru^{III}L$] complex (**5.1**) activates elemental sulfur (S_8) to form a μ-η^1 disulfide complex **5.2** that disproportionates to form the μ-η^1 sulfide complex (**5.3**). This active species transfers the sulfur atom to the substrate. It affords thereby cyclohexene sulfide and regenerates initial [$Ru^{III}L$] complex **5.1**. However, attempts to reproduce this report have failed, even when a stoichiometric amount of sulfur-activated ruthenium complex **5.3** was employed.[2, 5, 7]



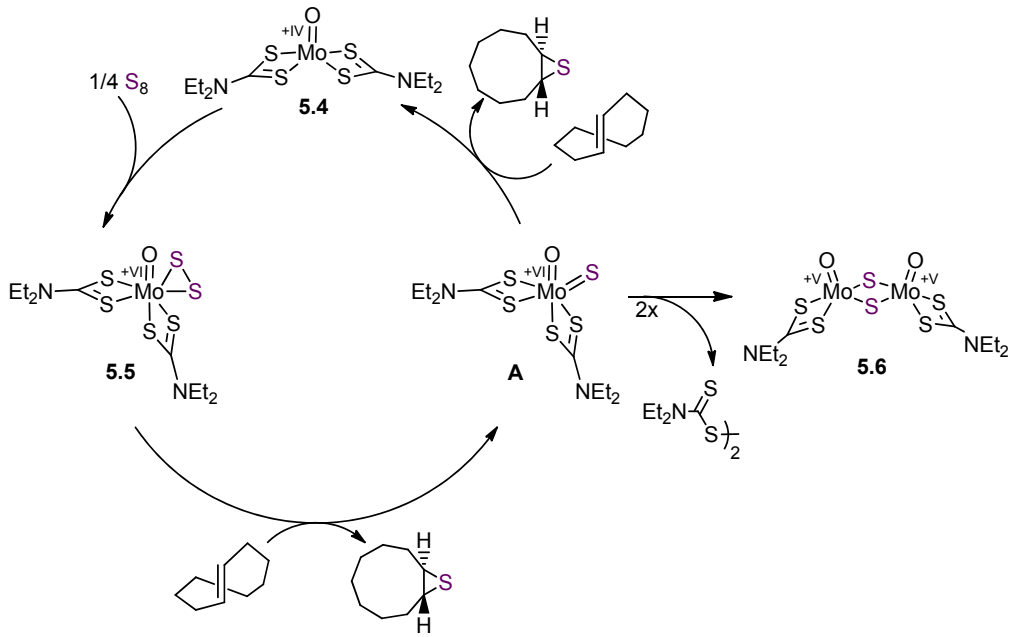
Scheme 5.1. Catalytic cycle for the ruthenium-catalyzed sulfur transfer

Later, Kendall and Simpkins[4] reported a rhodium-catalyzed episulfidation of norbornene and norbornadiene with methylthiirane as a sulfur source (Scheme 5.2). Both episulfides were isolated in 40% yield after heating the substrate at 110 ºC for 22 h with 1 mol % of rhodium acetate and propylene sulfide. The mechanism of the sulfur transfer pathway still remains unknown. Although this attractive methatesis reaction offers a direct and effective method of episulfidation, it is ineffective with *Z*-cyclooctene and dicyclopentadiene.



Scheme 5.2. Rhodium-catalyzed sulfur transfer to norbornene and norbornadiene

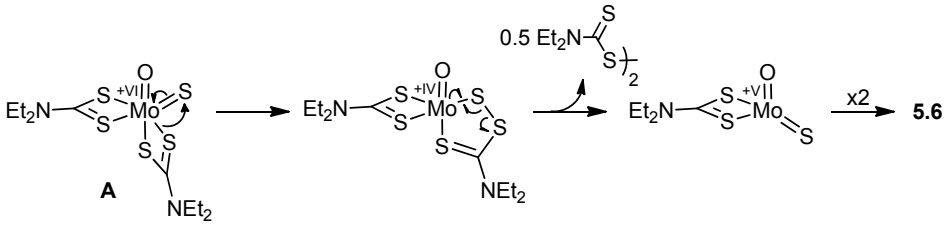
In 2001, Adam and Bargon published a novel sulfur-transfer catalysis mediated by oxomolybdenum complex **5.4**.[5] In Scheme 5.3, a mechanism is proposed for this catalytic transfer, where the molybdenum persulfido complex **5.5** is obtained from the molybdenum oxo complex (**5.4**) and S_8, as sulfur source. Disulfur bridge **5.5** extrudes successively both sulfur atoms to two akene molecules. This occurs by regenerating the initial complex and sustaining the catalytic cycle.



Scheme 5.3. Catalytic cycle for the molybdenum-catalyzed sulfur transfer to alkenes

During this sulfur transfer, sulfido complex **A** is formed as intermediate.[5, 6] Under catalytic conditions, *E*-cycloalkenes were episulfidated in good yields, but less reactive alkenes (e.g. *Z*-cyclooctene) and other less strained olefins failed to react.[5] These unreactive alkenes cannot sustain the catalysis since they do not accept the sulfur atom from oxo-sulfido intermediate **A**. Instead, this complex dimerizes irreversibly to dimer **5.6**, which is inactive for sulfur transfer.[7] Detailed speculation concerning its formation is unwarranted, but the likely sequence of steps is shown in Scheme 5.4. The mechanism would involve an internal thio-group insertion into the dithiocarbamate (dtc) ligand, which promotes the reduction of Mo (VI) center to Mo (IV). After simultaneosly occurring homolysis of the disulfur bridge and electron back-transfer from the metal centre, the resulting Mo (V) compound dimerizes to complex **5.6**.[7,8] This redox flexibility in the coordination sphere has also been proposed in the mechanism of action of nitrogenase enzymes.[9] Accordingly, all the

substrates were transformed into the corresponding episulfides in moderate yields with stoichiometrically amounts of the active disulfur-species **5.5**.[5]

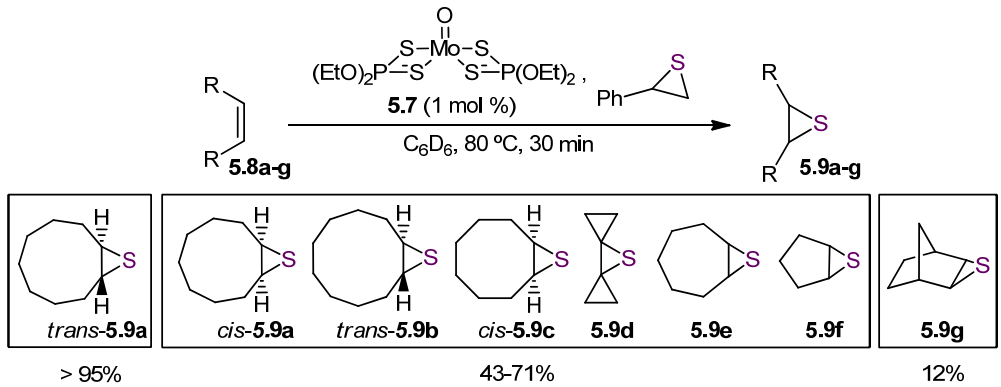


Scheme 5.4. Internal ligand redox reaction of intermediate **A**

These encouraging results undertook an intense search for a cheap, reactive and easily accessible sulfur source. It also stimulated a proper design of the metal with the objective of preventing the accumulation of the corresponding sulfur-transferring active species to avoid competitive reactions.

In this context, the best metal-catalyzed sulfur-transfer system ever achieved so far involves the combination of molybdenum oxo complex **5.7** with styrene sulfide as sulfur donor.[7] This new episulfidation catalyst performs sulfur transfer to a variety of alkenes smoothly, even the less reactive *Z*-cycloalkenes, more efficiently than when stoichiometric amounts of molybdenum oxo complex **5.4** are utilized (Scheme 5.5).

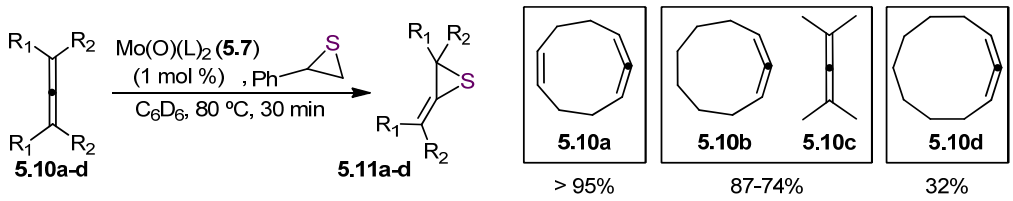
Also allenes are effectively episulfidated to the respective methylenethiiranes (Scheme 5.6).[7] Such a catalytic process was to date unprecedented.



Scheme 5.5. Episulfidation of cycloalkenes, mediated by the molybdenum oxo complex **5.7**

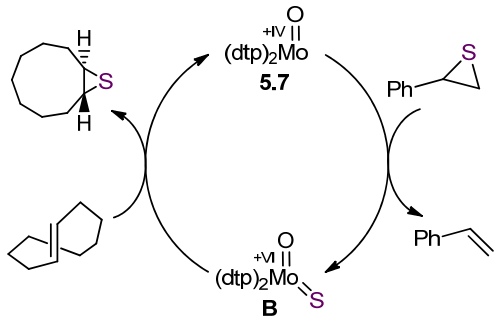
Treatment of the alkene **5.8** with a catalytic amount (1 mol %) of complex **5.7** and a stoichiometric quantity of phenylthiirane in benzene for 30 min afforded the episulfide *E*-cyclononene (*E*-**5.9a**) quantitatively; the thiiranes of *Z*-cyclononene (*Z*-**5.9a**), *E*-cyclodecene (*E*-**5.9b**), and even *Z*-cyclooctene (*Z*-**5.9c**), were isolated in

good yields; whereas those of bicyclopropylidene (**5.9d**), cycloheptene (**5.9e**), and cyclopentene (**5.9f**), however, in moderate yields.[7] Norbornene (**5.9g**) although known as a relatively good substrate for episulfidation,[10] exhibited only a low reactivity possibly by steric interferences between the dithiophosphate ligands and the methylene bridge.[7] Cyclohexene, with a great steric hindrance caused by the rigid chair conformation, was also resistant towards episulfidation.[7]



Scheme 5.6. Molybdenum-catalyzed episulfidation of allenes

The results in Scheme 5.6 demonstrate that the thermally stable methylenethiiranes **5.10a-d** were efficiently converted to their episulfides **5.11a-d** with this catalytic molybdenum-based system developed.[7] The catalysis operates with a similar catalytic cycle as the one proposed for catalyst **5.4**, where the dithiophosphate (dtp) complex **5.7** is converted to the corresponding oxo-thio complex **B**. Apparently, this intermediate is more reactive than the analogous derived from precursor **5.4** and it transfers sulfur to an alkene, instead of being further deactived into dinuclear complexes (Scheme 5.7).[7]



Scheme 5.7. Sulfur transfer mediated by the molybdenum oxo complex **5.7**

Although all attempts to isolate active episulfidation species **B** have failed, it would explain the higher efficiency of catalyst **5.7** compared to **5.4** for sulfur transfer process to alkenes.[7]

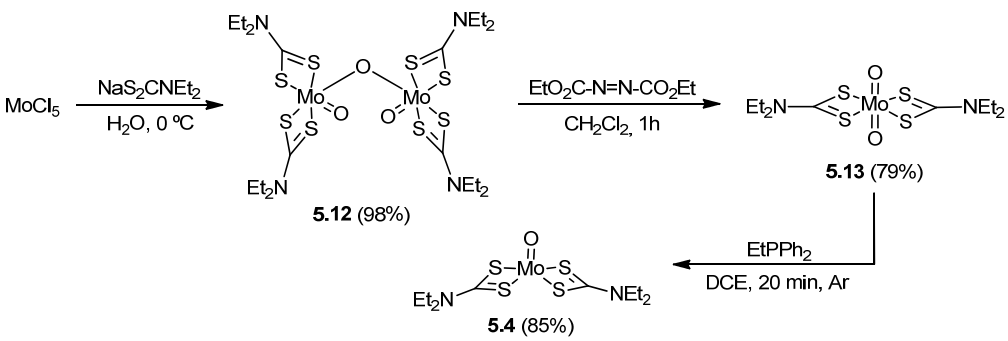
5.2. Results and discussion

5.2.1. Synthesis of molybdenum-catalysts and sulfur donor

Prior to investigate the feasibility of the molybdenum-catalyzed episulfidation with glycals, molybdenum catalysts **5.4** and **5.7**, which are not commercially available, were synthesised following reported methodologies.

Molybdenum oxo complex **5.4** was obtained in three reaction steps from molybdenum (V) chloride (Scheme 5.8).[11] In the first step, the dimeric complex **5.12** is prepared in 98% yield by reaction of molybdenum pentachloride and sodium diethyl dithiocarbamate in water. The structure of the compound was confirmed by IR spectroscopy. Apart from the infrared bands assignable to the ligands, terminal molybdenum-oxygen stretching vibration was observed as a strong band at 934 cm^{-1}. The Mo-O-Mo moiety was confirmed by weak to medium absorptions at 470 and 750 cm^{-1}, assigned to the symmetric and antisymmetric bridge bending vibrations, respectively. Compound **5.12** was impurified with a second molybdenum species, identified as $Mo_2O_4(dtc)_4$ which exhibited infrared bands at ~980 cm^{-1} (υ(Mo=O)) and bridge Mo-O_2-Mo vibrations at ~740 cm^{-1} (antisymmetric) and ~480 cm^{-1} (symmetric).[11a]

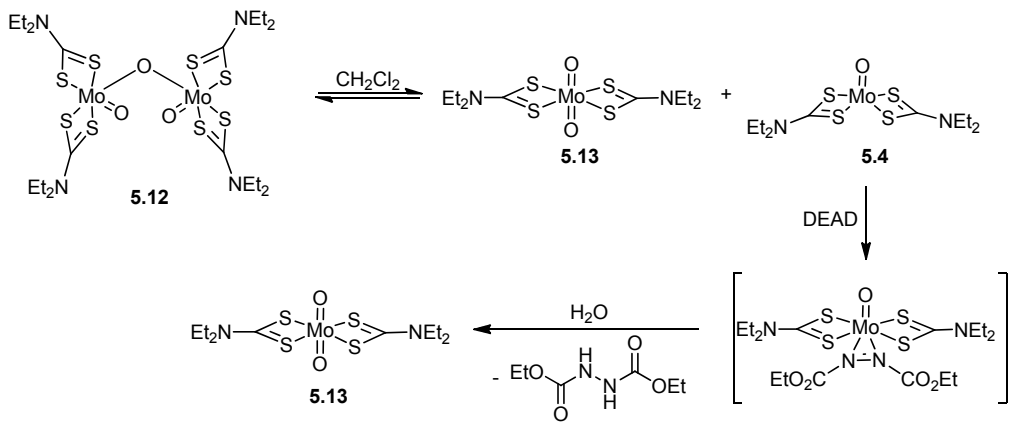
Oxidative cleavage of impurified dimer **5.12**, promoted by diethyl azodicarboxylate (DEAD), affords dioxo complex **5.13** in 79% yield. It seems that the reaction involves an equilibrium rather than an oxidative addition to one of the metal centers.



Scheme 5.8. Synthesis of the molybdenum oxo complex **5.4**

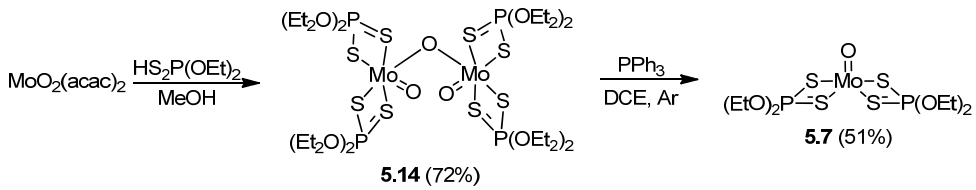
This reaction may be rationalized by the disproportionation of molybdenum (V) into molybdenum (VI) and molybdenum (IV) (Scheme 5.9).[11a] DEAD is able to trap the molybdenum (IV) as its 1:1 adduct by shifting the equilibrium completely to product formation. The resulting compound is easily hydrolyzed and releases thus

1,2-bis(ethoxycarbonyl)hydrazine and the appropriate *cis*-dioxobis(*N*,*N*-diethyldithiocarbamato)molybdenum (IV) (**5.13**).[12] IR spectrum signals at 910 cm^{-1} and 873 cm^{-1} (υ(Mo=O)) established the obtention of desired complex **5.13**. Absence of characteristic signals at 1725 and 1710 cm^{-1} (υ(C=O)) and 933 cm^{-1} (υ(Mo=O)) confirmed that there is not the symmetrically coordinated azo group adduct.
Deoxygenation by basic ethyldiphenylphosphine in 1,2-dichoroethane (DCE) gave the air-sensitive complex **5.4** in 85% yield. The disappearance of one υ(Mo=O) and the increase of the remaining from 910 cm^{-1} to 962 cm^{-1} supports the reduction in the formal oxidation state of the metal.[13]



Scheme 5.9. Dismutation equilibrium of dimolybdenum complex **5.13**

Catalyst **5.7** was prepared in two steps from commercial molybdenum (VI) dioxide bis-(acetylacetonate) ($MoO_2(acac)_2$, Scheme 5.10).[11b] Molybdenum anhydride **5.14** was obtained directly from molybdenyl acetylacetonate in 72% yield utilizing the reducing ability of diethyl dithiophosphoric acid in methanol.

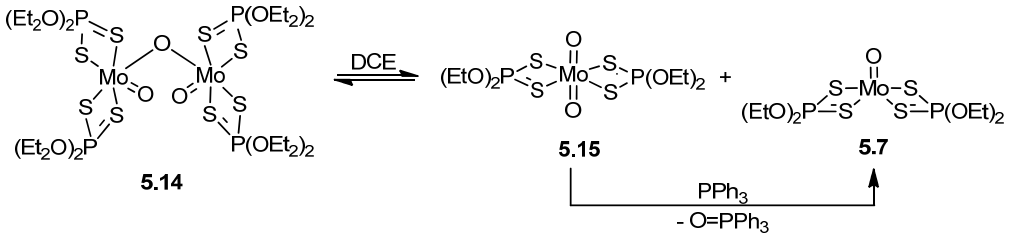


Scheme 5.10. Synthesis of the molybdenum oxo complex **5.7**

Ligand coordination was confirmed by the strong characteristic vibrations on IR spectrum (1003 and 810 cm^{-1}) assigned to P-O-C deformation. The structure of the complex was elucidated through the intense band at 962 cm^{-1}, characteristic of the

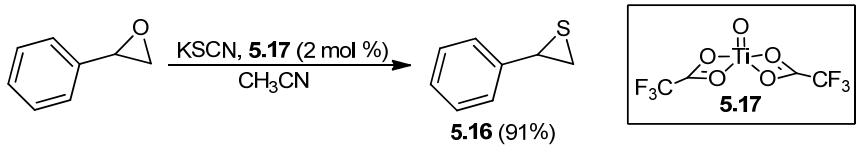
Mo=O stretching, and the molybdenum bridge bending vibrations at 775 and 430 cm^{-1}. Unexpected absorptions at ~980 cm^{-1} and ~740 cm^{-1} suggested the presence of $Mo_2O_4(dtp)_4$.

The synthesis of desired catalyst **5.7** was achieved by addition of excess of triphenylphosphine in 51% yield. This reaction mechanism is entirely analogous to the one proposed for the formation of compound 5.13 and is shown in Scheme 5.11. In this case, however, it is the rapid deoxygenation of intermediate **5.15** what shifts the characteristic equilibrium. Formation of **5.13** was confirmed by the characteristical IR peak at 972 cm^{-1} (υ(Mo=O)) and by signal absence in region for bridging oxygen deformations, between 700 and 800 cm^{-1}.



Scheme 5.11. Dismutation equilibrium of dimolybdenum complex **5.14**

Next step was the synthesis of phenylthiirane (**5.16**) as a sulfur source. It was obtained from styrene oxide with potassium thiocyanate in the presence of Lewis acid **5.17**, according to a literature-reported protocol (Scheme 5.12).[14]



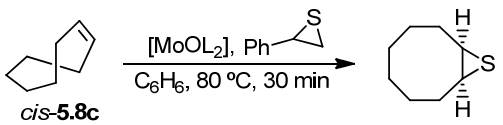
Scheme 5.12. Synthesis of styrene thioepoxide by stoichiometric episulfidation

Catalyst **5.17** was prepared according to the literature procedure.[15] The corresponding titano-complex was obtained in 66% yield by mixing titanium tetrachloride with trifluoroacetic acid at reflux for 250 h. The structure was also confirmed by IR spectroscopy. Apart from the Ti=O band (1615 cm^{-1}), there are strong absorptions bands at 1475 and 1152 cm^{-1} assigned to the bending of CF_3 group and coordinated carboxylate respectively.

5.2.2. Catalytic experiments

In order to test the catalytic activity for sulfur transfer of catalysts **5.4** and **5.7** previously synthesised, a control experiment was performed with the objective to reproduce the reaction described by Adam and Bargon.[7] With this purpose, both complexes were tested as catalysts under identical reaction conditions (80 ºC for 30 min under magnetic stirring) by employing *Z*-cyclooctene as a model substrate and phenylthiirane as a sulfur donor. These assays revealed that both complexes are active for the metal-catalyzed episulfidation-reaction (Table 5.1.). Regarding efficiency, whereas catalyst **5.7** selectively transformed olefin *cis*-**5.8c** into episulfide *cis*-**5.9c** with 87% conversion (Table 5.1., entry 2), catalyst **5.4** afforded **5.9c** in a poor 12% yield (Table 5.1., entry 1).

Table 5.1. Episulfidation of *Z*-cyclooctene (*cis*-**5.8c**) catalyzed by complex **5.4** or **5.7**[a]



cis-**5.8c** *cis*-**5.9c**

Entry	L	[Mo]	Conv. 5.8c (%)[b]	Select. 5.9c (%)[b]
1	dtc	**5.4**	12	> 98
2	dtp	**5.7**	87	> 98

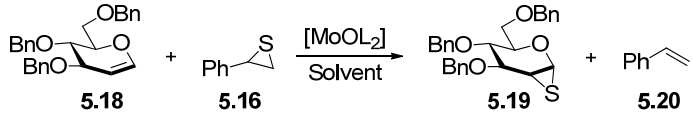
[a] Conditions: 0.25 mmol alkene, 0.28 mmol phenylthiirane, 1 mol % catalyst and 1.9 mL benzene. [b] Determined by ^{1}H NMR analysis by integration of characteristic signals against starting materials of the reaction crude.

The superior performance of catalyst **5.7** was herein confirmed. It is worth pointing out that *Z*-cyclooctene is a relatively unreactive substrate and might not be the best model alkene.

Based on these preliminary experiments, the episulfidation reaction was applied to glucal **5.18** using stoichiometric amounts of phenylthiirane in the presence of the oxomolybdenum catalysts (**5.4** or **5.7**) in refluxing toluene; however, the desired episulfide **5.19** was not observed (Table 5.2, entries 1 and 2). In fact, ^{1}H-NMR analysis showed that all the starting material was transformed into a complex sugar mixture and no evidence of the episulfide was observed. Lowering the temperature to 60 ºC did not improve the results already obtained since decomposition also took place again in slower rate (Table 5.2, entries 3 and 4). Thus, no successful result was

achieved with catalyst **5.4** and **5.7** in benzene under reflux or at 60 ºC, although benzene was used as solvent (Table 5.2, entries 5, 6, 7 and 8).

Table 5.2. Episulfidation of glucal **5.18** with oxo-molybdenum catalysts



5.18 **5.16** **5.19** **5.20**

Entry	L	[Mo]	Solvent	T (º C)	t (h)	Conv. (%)[c]		Select. (%)[c]	
						5.18	**5.16**	**5.19**	**5.20**
1[a]	dtc	**5.4**	toluene	reflux	6	> 98	> 98	< 2	> 98
2[a]	dtp	**5.7**	toluene	reflux	7	> 98	> 98	< 2	> 98
3[a]	dtc	**5.4**	toluene	60	24	> 98	> 98	< 2	> 98
4[a]	dtp	**5.7**	toluene	60	24	> 98	> 98	< 2	> 98
5[a]	dtc	**5.4**	benzene	reflux	5	> 98	> 98	< 2	> 98
6[a]	dtp	**5.7**	benzene	reflux	6	> 98	> 98	< 2	> 98
7[a]	dtc	**5.4**	benzene	60	24	> 98	> 98	< 2	> 98
8[a]	dtp	**5.7**	benzene	60	24	> 98	> 98	< 2	> 98
9[b]	dtc	**5.4**	benzene	40	24	< 2	65	< 2	> 98
10[b]	dtp	**5.7**	benzene	40	24	< 2	> 98	< 2	> 98
11[b]	dtc	**5.4**	benzene	rt	24	< 2	3	< 2	> 98
12[b]	dtp	**5.7**	benzene	rt	24	< 2	> 98	< 2	> 98

[a] Conditions: 0.24 mmol glucal, 0.26 mmol phenylthiirane, 1 mol % catalyst, and 1.8 mL solvent. [b] Conditions: 0.12 mmol glucal, 0.13 mmol phenylthiirane, 1 mol % catalyst, and 0.5 mL deuterated solvent. [c] Determined by ^{1}H NMR integration of characteristic signals against starting materials of the reaction crude.

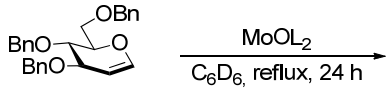
New attempts were made to diminish degradation of starting material by carrying out the reactions at lower temperatures. Two parallel tests were then run at 40 ºC with different catalysts, but in both cases only starting material (**5.18**) and styrene (**5.20**) were recovered after 24 h (Table 5.2, entries 9 and 10). The same negative results were obtained when the reactions were performed at room temperature (Table 5.2, entries 11 and 12).

It is also worth mentioning that in almost all mentioned cases desulfurization of episulfide **5.16** to styrene by both molybdenum oxo complexes was observed (Table 5.2, entries 1-8). Therefore, catalyst **5.7** reacts smoothly with the sulfur donor even at room temperature (Table 5.2, entries 10 and 12) in contrast with complex **5.4**, which does not catalyze the episulfidation at temperatures below 60 ºC (Table 5.2, entries 9 and 11). Consumption of **5.18** requires the same threeshold of temperature. At 60 ºC the glucal might react to afford episulfide **5.19**, which would undergo ring-opening

degradation. Sugar 1,2-episulfides are known to be quite reactive and undergo spontaneously oligomerization to afford thiooligosaccharides.[16] However, any attempts to identify products derived from the sugar 1,2-episulfide by HRMS failed. At lower temperatures, alkene **5.18** is not reactive enough as a sulfur acceptor, and consumption of phenylthiirane with extrusion of polymeric sulfur is particularly prone to take place.[7]

Consumption of the starting material can also be explained by thermal decomposition of glucal **5.18**. Therefore, thermal stress of tri-*O*-benzyl-D-glucal was then tested. When the reaction was refluxed for 24 h in the presence of either molybdenum catalyst without phenylthiirane, no degradation was observed (Table 5.3, entries 1 and 2).

Table 5.3. Thermal degradation tests of glucal **5.18**[a]



5.18

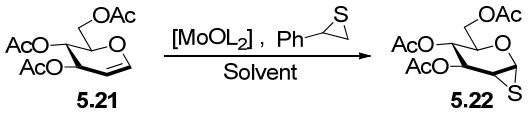
Entry	L	[Mo]	Conv. (%)[b]
1	dtc	**5.4**	< 2
2	dtp	**5.7**	< 2
3	-	-	< 2

[a] Conditions: 0.12 mmol glycal, 1 mol % catalyst, and 0.5 mL deuterated benzene at reflux for 24 h. [b] Determined by ^{1}H NMR integration of characteristic signals of thereaction crude.

Heating an equimolar relation between thiirane **5.16** and alkene **5.18** by using the same temperature conditions also afforded the same negative results (Table 5.3, entry 3). However, unexpected formation of styrene was observed. From these last experiments, it can be concluded that compound **5.18** is really thermally stable under the reaction conditions and it might be converted to the corresponding episulfide which could lead *in situ* to subsequent degradation processes.

In the light of the previous experiments, tri-*O*-acetyl-D-glucal (**5.21**) was then assayed as a substrate. Catalytic assays in toluene gave no conversion of **5.21** after 48 h and the starting material was recovered unaltered (Table 5.4, entries 1 and 2). Catalytic reactions of **5.4** or **5.7** in refluxing benzene did not proceed after 24 h (Table 5.4, entries 3 and 4). Increasing catalyst loading up to 10 mol % did not improve the results obtained before (Table 5.4, entries 5 and 6). Styrene was always obtained as a main product without any traces of the corresponding episulfide **5.22**.

Table 5.4. Episulfidation of glucal **5.21** with oxo-molybdenum catalysts[a]



5.21 5.22

Entry	L	[Mo]	Solvent	Conv. (%)[c]	Select. (%)[c]
1	dtc	**5.4**	toluene	< 2	< 2
2	dtp	**5.7**	toluene	< 2	< 2
3	dtc	**5.4**	benzene	< 2	< 2
4	dtp	**5.7**	benzene	< 2	< 2
5[b]	dtc	**5.4**	benzene	< 2	< 2
6[b]	dtp	**5.7**	benzene	< 2	< 2

[a] Conditions: 0.24 mmol glucal, 0.26 mmol phenylthiirane, 1 mol % catalyst, and 1.8 mL solvent at reflux for 24 h. [b] Reaction carried out with 10 mol % catalyst. [c] Determined by ^{1}H NMR integration of characteristic signals against starting materials of the reaction crude.

It can be concluded that glucals **5.18** and **5.21** are not appropriate sulfur acceptors for catalytic-episulfidation reaction with oxo-molybdenum catalysts **5.4** and **5.7**. Both protected sugars have a great resemblance to the cyclohexene in terms of conformational rigidity and steric hindrance. This fact may explain their reluctance towards episulfidation. Besides, the different reactivity between sugars **5.18** and **5.21** can be explained by the so-called "armed-disarmed approach" developed by Fraser-Reid,[17] that nowadays can be applied to nearly any class of glycosyl donor, including glycals.[18] Indeed, carbohydrate **5.21**, with electron-withdrawing ester protecting groups, has a diminished reactivity because the electron density of the vinyl ether moiety decreases (Figure 5.1).[19]

Figure 5.1. Armed/disarmed effect on glucals **5.18** and **5.21**

5.3. Conclusions

In this chapter, the synthesis of sugar episulfides by molybdenum-catalyzed sulphur transfer to glycals has been explored. In particular, two molybdenum complexes (**5.4** and **5.7**) have been prepared following discussed methods and used as catalysts for

the metal-catalyzed episulfidation of glycals by employing phenylthiirane as a sulfur donor. Although this procedure has been proved to be an efficient and general method for alkene episulfidation, like low reactive *Z*-cyclooctene, its application to unsaturated carbohydrates was unsuccessful.

Episulfidation of glucal **5.18** proceeds with general degradation of the starting material. Since this compound is thermally stable under the conditions carried out, this unexpected process might be rationalized by the formation of the high unstable 1,2-episulfide that degrades *in situ*.

Furthermore, application of this methodology to glycal **5.21** also failed because none of the reactions took place. This fact may be explained probably due to a disarming effect by the presence of electron-withdrawing acetyl protecting groups.

As a result, this approach has highlighted the requirement for an alternative route for the efficient synthesis of carbohydrate thiiranes.

5.4. Experimental section

5.4.1. General methods

All chemicals used were reagent grade and were employed as supplied unless otherwise specified. HPLC grade dichloromethane and diethyl ether were dried using a solvent purification system (Pure SOLV system-4®). Solvents were purified using standard procedures.[20] Reactions involving air-sensitive catalysts **5.4** and **5.7** were carried out under argon-gas atmosphere and with solvents dried and distilled under argon gas prior to use.

^{1}H and ^{13}C NMR spectra were recorded using two spectrometers (Varian® Mercury VX 400 and Varian® 400-MR) both operating at frequency of 400 MHz for proton and 100.6 MHz for carbon. $CDCl_3$ and C_6D_6 were used as solvents. Chemical shifts (δ) were referenced to internal standards ($CDCl_3$ (7.27 ppm ^{1}H, 77.23 ppm ^{13}C) and C_6D_6 (7.16 ppm ^{1}H, 128.06 ppm ^{13}C)) or Me_4Si as an internal reference (0.00 ppm ^{1}H). 2D correlation spectra (gCOSY, NOESY, gHSQC and gHMBC) were visualized using the VNMR program (Varian®). ESI-MS were run on an Agilent® 1100 Series LC/MSD instrument. IR spectra were recorded on a JASCO FT/IR-600 plus Fourier Transform Infrared Spectrometer ATR Specac Golden Gate.

Reactions were monitored by TLC carried out on 0.25 mm E. Merck® silica gel 60 F_{254} glass or aluminium plates. Developed TLC plates were visualized under a short-wave UV lamp (254 nm) or dipping the plate in a suitable developing solution.[21]

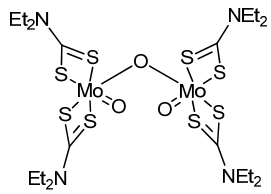
Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka® or Merck® silica gel 60 (230-400 mesh). Radial chromatography was performed on a Harrison® chromatotron model 7924T in 1 or 2 mm plates of Kieselgel 60 PF_{254} silica gel, depending on the amount of product. Flash column chromatography (FCC) was performed using flash silica gel (32–63 μm) and employing a polarity elution system correlated to TLC mobility.

5.4.2. General procedures

General procedure for catalytic alkene episulfidation on analytical scale:[7] in a NMR tube was placed a solution of molybdenum oxo complex (2.0 μmol), alkene (0.20 mmol) and phenylthiirane (0.21 mmol) in 0.8 mL of deuterobenzene. The NMR tube was sealed with a rubber septum and Parafilm®, then heated to 80 ºC. After cooling to room temperature (ca. 20 ºC) it was analyzed by ^{1}H- and C^{13}-NMR spectroscopy. Conversion and selectivity were determined by means of their characteristic ^{1}H-NMR against starting materials.

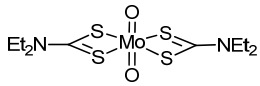
General procedure for catalytic alkene episulfidation on preparative scale:[7] schlenck was charged with a solution of molybdenum complex (20.0 μmol), alkene (2.00 mmol) and phenylthiirane (2.10 mmol) in 15 mL of benzene. After sealing the tube, the solution was magnetically stirred and heated to 80 ºC in an oil bath. The schlenk was allowed to cool to room temperature (ca. 20 ºC), opened, and the solvent was removed. The resulting residue was submitted to NMR analysis. Conversion and selectivity were determined by means of their characteristic ^{1}H-NMR against starting materials.

5.4.3. Compound characterization

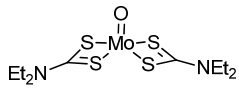


***μ*-Oxo-bis[oxobis(*N,N*-diethyldithiocarbamato)molybdenum (V)] (5.12):**[11a] to a solution of molybdenum pentachloride (1 g, 3.60 mmol) in water (100 mL) was slowly added to an ice-cold solution of *N,N*-diethyldithiocarbamate sodium salt (8.34

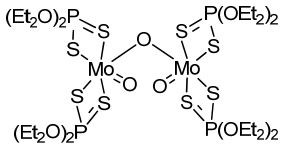
g, 36 mmol) in water (100 mL). The dark purple precipitate which formed immediately, was collected by filtration, washed thoroughly with water and dried *in vacuo* to obtain the desired compound (1.52 g, 98% yield) as a dark purple solid. Data: IR (KBr) cm^{-1}: 1500 (υ(CN)); 1200 (δ(NC_2)); 970 (δ(CS_2)); 934 (υ(Mo=O)); 750 (δ(MoOMo)); 470 (δ(MoOMo)); 370 (υ(MoS)).



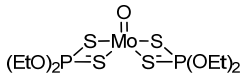
Dioxo-bis(*N,N*-diethyldithiocarbamato)molybdenum (VI) (5.13):[11a] to a stirred suspension of **5.12** (500 mg, 0.6 mmol) in dichloromethane (225 mL) was added diethyl azodicarboxylate (360 mg, 1.2 mmol). The purple colour was discharged and all the solid dissolved. After 1 h, the solution was evaporated to dryness. The oily yellow residue was washed with dry hexane to remove excess of the diethyl azodicarboxilate and then dried *in vacuo*. Extractions with dry benzene gave a residue of the desired compound (204 mg, 43% yield) as a yellow solid. Evaporation of the benzene extract gave the 1:1 diethyl azodicarboxylate-oxobis(*N,N*-diethyldithiocarbamato)molybdenum (IV) adduct that was redissolved in moist chloroform (10 mL) and stirred for 3 h. Evaporation of the solution and consecutive hexane washing gave also **5.13** (171 mg, 36% yield) also as a yellow solid. Total yield: 379 mg, 79%. Data: IR (KBr) cm^{-1}: 1515 (υ(CN)); 1200 (δ(NC_2)); 980 (δ(CS_2)); 910 (υ(Mo=O)); 870 (υ(Mo=O)); 390 (υ(MoS)).



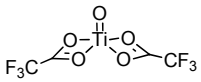
Oxo-bis(*N,N*-diethyldithiocarbamato)molybdenum (IV) (5.4):[11b] ethyldiphenylphosphine (214 mg, 1 mmol) was added to a solution of complex **5.13** (200 mg, 0.5 mmol) in dichloroethane (10 mL). After refluxing for 20 min, the red reaction mixture was filtered and the filtrate was evaporated to dryness under vacumm. Trituration of the residue with deoxygenated ethanol yielded pink solid **5.4** which was isolated by filtration, then washed with more ethanol and degasified ether and finally dried in vacuo (160 mg, 85% yield). Data: IR (KBr) cm^{-1}: 1536 (υ(CN)); 1196 (δ(NC_2)); 980 (δ(CS_2)); 962 (υ(Mo=O)); 380 (υ(MoS)).



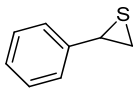
***μ*-Oxo-bis[oxobis(*O,O'*-diethyldithiophosphato)molybdenum (V)] (5.14):**[11b] dithio-phosphoric acid (1.4 mL, 8 mmol) was added to a solution of $MoO_2(acac)_2$ (818 mg, 2.5 mmol) in methanol (16 mL). After stirring for 45 min, the dark purple suspension was filtrated, washed with methanol and ether and dried *in vacuo* affording the purple solid **5.14** (817 mg, 72% yield). Data: IR (KBr) cm^{-1}: 1003 (δ(POC)); 962 (υ(Mo=O)); 810 (δ(POC)); 775 (δ(MoOMo)); 640 (υ(PS)); 528 (υ(PS)); 430 (δ(MoOMo)); 340 (υ(MoS)).



Oxo-bis(*O,O'*-diethyldithiophosphato)molybdenum (IV) (5.7):[11b] triphenyl-phosphine (184 mg, 0.7 mmol) was added to a solution of **5.14** (460 mg, 0.46 mmol) in dichloroethane (20 mL) and the reaction mixture was heated at reflux for 10 min. The pink solution was filtered and the filtrate was evaporated to dryness yielding a pink oil which was extracted with hexane. Evaporation of the extract to dryness under vacuum gave pink solid **5.7** (230 mg, 51% yield). Data: IR (KBr) cm^{-1}: 1005 (δ(POC)); 972 (υ(Mo=O)); 812 (δ(POC)); 635 (δ(MoOMo)); 640 (υ(PS)); 337 (υ(MoS)).

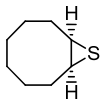


Oxo-bis(trifluoroacetato)titanium (IV) (5.17):[15] trifluoroacetic acid (8.4 mL, 108 mmol) was added dropwise to titanium (IV) tetrachloride (2 mL, 18 mmol). The mixture was heated at 70 ºC. After stirring for 250 h, the grey suspension was filtrated, the solid was washed with trifluoroacetic acid and dried *in vacuo*. Sublimation affords **5.17** as a white solid (3.50 g, 66%). Data: IR (neat) cm^{-1}: 1615 (υ(C=O)); 1475 (δ(CF_3)); 1152 (υ(Ti=O).



Phenylthiirane (5.16):[22] a mixture of styrene oxide (2 g, 16.65 mmol), potassium thiocyanate (4.86 g, 50 mmol) and titano-complex **5.17** (0.10 g, 0.33 mmol) in acetonitrile (80 mL) was heated at reflux for 4 h. The crude was purified by column

chromatography (hexane/EtOAc 9:1) to afford **5.16** as a colourless dense liquid (2.05 g, 91%). Data: NMR ^{1}H ($CDCl_3$, 400 MHz) δ in ppm: 7.25-7.10 (m, 5H, Har); 3.75 (t, 1H, $J_{cis} = J_{trans} = 6.0$ Hz, CHS); 2.71 (d, 1H, $J_{cis} = 6.0$ Hz, CH_2S); 2.51 (d, 1H, $J_{trans} = 6.0$ Hz, CH_2S). NMR ^{13}C ($CDCl_3$, 100.6 MHz) δ in ppm: 139.0 (Car); 128.5, 127.5, 126.6 (CHar); 36.1 (CHS); 27.4 (CH_2S).



***Cis*-9-thiabicyclo[7.1.0]nonane (5.9c):**[23] according to the general procedure for catalytic alkene episulfidation on preparative scale, the title compound was synthesized by reaction of *Z*-cyclooctene (28.0 mg, 0.25 mmol), phenylthiirane (37.5 mg, 0.28 mmol) and molybdenum oxo complex **5.7** (0.8 mg, 2 μmol) in benzene (1.9 mL). The solvent was evaporated and the resulting residue was submitted to ^{1}H-NMR analysis, revealing that about 87% of the phenylthiirane was converted to **5.9c** as the main product (selectivity > 98%). Data: NMR ^{1}H ($CDCl_3$, 400 MHz) δ in ppm: 2.93 (m, 2H, H-1, H-8); 2.34 (m, 2H); 1.80-1.20 (m, 10H). NMR ^{13}C ($CDCl_3$, 100.6 MHz) δ in ppm: 41.2 (CHS); 29.5, 29.3, 26.3 (CH_2).

5.5. References

1. Sharples, K. B.; Verhoeven, T. R. *Aldrichim. Acta* **1979**, *12*, 63-74.
2. Adam, W.; Bargon, R. M. *Chem. Rev.* **2004**, *104*, 251-261 and references therein.
3. Taqui Khan, M. M.; Siddiqui, M. R. H. *Inorg. Chem.* **1991**, *68*, 6049-6052.
4. Kendall, J. D.; Simpkins, N. S. *Synlett* **1998**, 391-392.
5. Adam, W.; Bargon, R. M. *Chem. Commun.* **2001**, 1910-1911.
6. Yan, X. F.; Young, C. G. *Aust. J. Chem.* **1991**, *44*, 361-367.
7. Adam, W.; Bargon, R. M.; Schenk, W. A. *J. Am. Chem. Soc.* **2003**, *125*, 3871-3876.
8. Smirth, P. D.; Slizys, D. A.; George, G. N.; Young. C. G. *J. Am. Chem. Soc.* **2000**, *122*, 2946-2947.
9. Stiefel, E. I. *Pure Appl. Chem.* **1998**, *70*, 889-896.
10. Adam, W.; Bargon, R. M. *Eur. J. Org. Chem.* **2001**, 1959-1962.
11. a) Newton, W. E.; Corbin, J. L.; Bravard, D. C.; Searles, J. E.; McDonald, J. W. *Inorg. Chem.* **1974**, *5*, 1100-1104. b) Chen, G. J.-J.; McDonald, J. W.; Newton, W. E. *Inorg. Chem.* **1976**, *15*, 2612-2615.
12. Schneider, P. W.; Bravard, D. C.; McDonald, J. W.; Newton, W. E. *J. Am. Chem. Soc.* **1972**, *94*, 8640.

13. Jowitt, R. N.; Mitchell, P. C. H.; *J. Chem. Soc. A* **1970**, 1702-1708.
14. Iranpoor, N.; Zeynizadeh, B. *Synth. Commun.* **1998**, *28*, 3913-3918.
15. Sartori, P.; Weidenbruch, M. *Angew. Chem.* **1964**, *76*, 376-377.
16. Knapp, S.; Malolanarasimhan, K. *Org. Lett.* **1999**, *1*, 611−613.
17. Fraser-Reid, B.; Wu, Z.; Udodong U. E.; Ottosson, H. *J. Org. Chem.* **1990**, *55*, 6068-6070.
18. Friesen, R. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6656-6660.
19. a) Premathilake, H. D.; Demchenko, A. V. *Top Curr. Chem.* **2011**, *301*, 189-221. b) Crich, D.; Vinogradova, O. *J. Am. Chem. Soc.* **2007**, *129*, 11756-11765. c) Kamat, M. N.; Demchenko, A. V. *Org. Lett.* **2005**, *7*, 3215-3218. d) Clausen, M. H.; Madsen, R. *Chem. Eur. J.* **2003**, *9*, 3821-3832.
20. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3er ed., Pergamon Press, Oxford, **1989**.
21. Reactivos Merck *Reactivos de coloración para cromatografía en capa fina y en papel*, E. Merck. Darsmtadt, **1980**.
22. Iranpoor, N.; Kazemi, F. *Synthesis*, **1996**, 821-822.
23. Bombala, M. U.; Ley, S. V. *J. Chem. Soc., Perkin Trans. 1*, **1979**, 3013-3016.

CHAPTER 6

REACTION OF SUGAR EPOXIDES WITH THIOCYANATE EQUIVALENTS: SYNTHESIS OF THIONOCARBAMATES

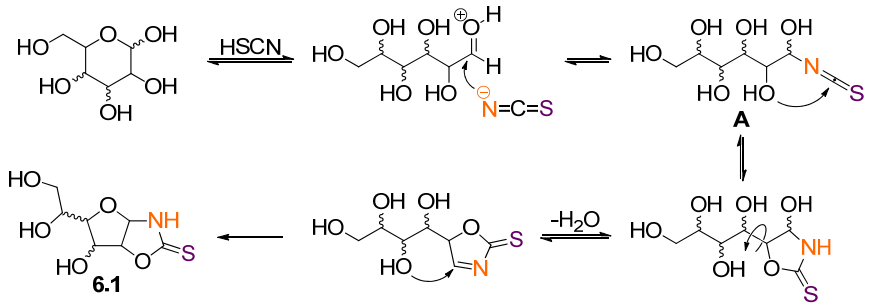
UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

6.1. Background

6.1.1. Synthesis of fused thionocarbamates by thiocyanic acid

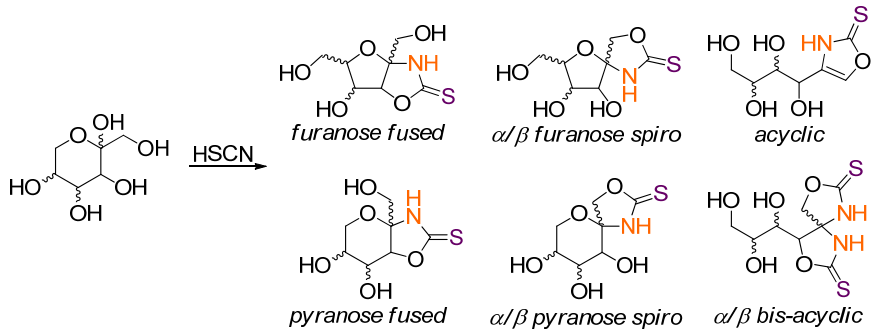
The first syntheses of fused thionocarbamates on carbohydrate scaffolds consisted of the treatment of unprotected reducing aldoses with potassium thiocyanate in acidic media.[1] Little is known about the reaction mechanism but it could involve nucleophilic addition of the *in situ*-generated thiocyanic acid to the aldehyde group of the fleeting open-chain form of the sugar. The generated transient isothiocyanate **A**, subsequently undergoes intramolecular cyclization. Under this thermodynamic conditions, the main product is the more stable 1,2-fused 1,3-oxazolidine-2-thione furanose derivative **6.1** (Scheme 6.1).[2]

Ketoses also undergo condensation with thiocyanic acid. Indeed, under these acid conditions complex product mixtures — up to 9 different thionocarbamates — are frequently obtained (Scheme 6.2).[3] Moreover, reaction of ketoses with potassium cyanate does afford plenty 1,3-oxazolidin-2-one analogues.[4]



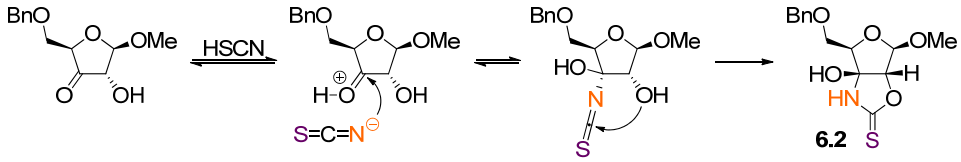
Scheme 6.1. Condensation mechanism of reducing carbohydrates with thiocyanic acid

This lack of selectivity may be explained by the complex tautomeric equilibria and their tendency to form tertiary oxocarbenium ions under acidic conditions. In order to control the selectivity of the reaction, different hydroxyl protections have been performed.[5] While spiro-thionocarbamates can be achieved by protection of the alcohol in position C-3,[6] locking the anomeric carbon induces limitation to fused structures.[2]



Scheme 6.2. Products postulated when ketoses are confronted to thiocyanic acid

Anchoring thionocarbamates moieties on saccharidic backbones can also be achieved using thiocyanic acid onto ulosides.[7] In this case, the condensation affords carbohydrate-fused hemiaminal 1,3-oxazolidine-2-thiones. This original approach starts with protonation of the keto group followed by isothiocyanate addition. This intermediate undergoes spontaneous attack by the alcohol in position C-2 to produce the desired bicycle **6.2** in a *cis* relationship to the hydroxyl group involved (Scheme 6.3.). Ulopyranosides can also react in the same way but with lower yields.

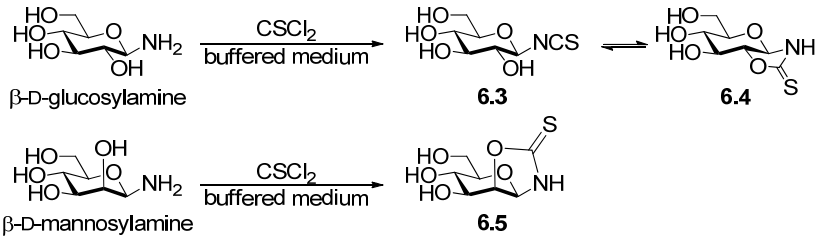


Scheme 6.3. Mechanism for the condensation of ulofuranosides with thiocyanic acid

6.1.2. Synthesis by CS_2, $CSCl_2$ and other thiocarbonyl sources

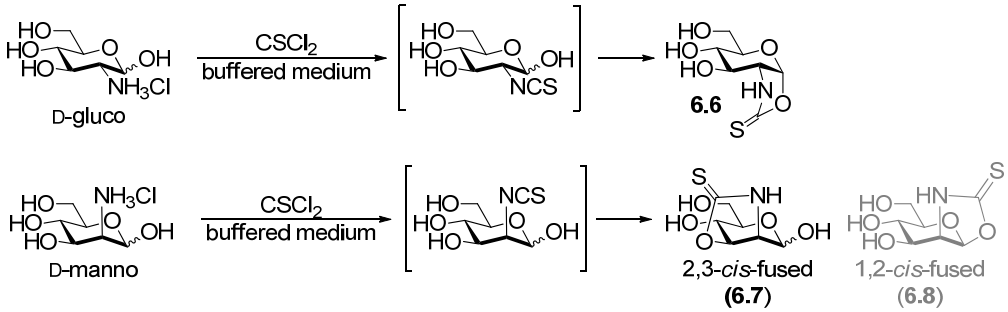
Fused glycofuranosyl and glycopyranosyl thionocarbamates are mainly prepared by reaction of a β-aminoalcohol carbohydrate derivative with a thiocarbonyl-source under basic conditions. The reaction generally proceeds through an isothiocyanate intermediate, formed from carbon disulfide or thiophosgene, followed by spontaneous or base-induced cyclization. This methodology has been applied to aldohexoses differing in the position of the amino group.[5]

In this context, *O*-deprotected glycosylamines condense with thiophosgene in buffered medium:[8] Whereas β-D-glucosylamine affords an equilibrium between the anomeric isothiocyanate **6.3** and the corresponding *trans*-fused 1,3-oxazolidine-2-thione (OAT) **6.4**, the *cis*-fused thionocarbamate **6.5** is obtained from β-D-mannosylamine (Scheme 6.4). *Trans*-diaxial-arranged OATs are quite strained and their formation is not so favourable, which explains the observed equilibrium.



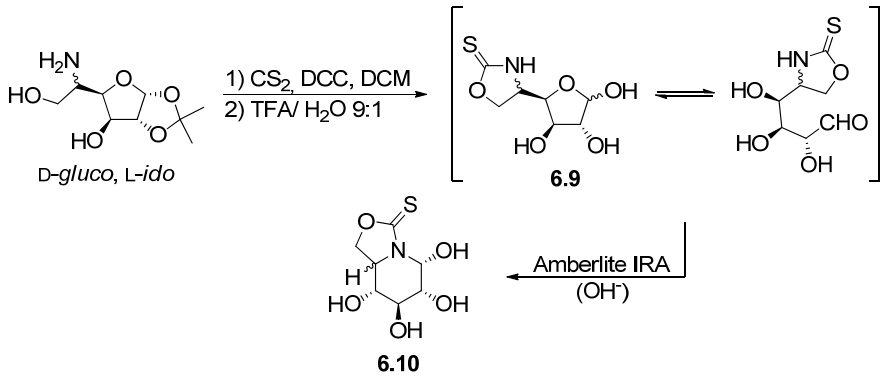
Scheme 6.4. Condensation of glycopyranosylamines with thiophosgene in buffered medium

2-Amino-2-deoxy-D-hexose hydrochlorides also react analogously affording *cis*-fused OAT when the previously described conditions are applied (compounds **6.6** and **6.7**, Scheme 6.5).[9] Exclusively formation of 2,3-*cis*-fused 1,3-oxazolidine-2-thione-*manno*-derivative **6.7** rather than the 1,2-*cis* regioisomer **6.8** is explained by the reduced nucleophilicity of the anomeric hydroxyl group.



Scheme 6.5. Reaction of 2-amino-2-deoxy aldoses with thiophosgene in buffered medium

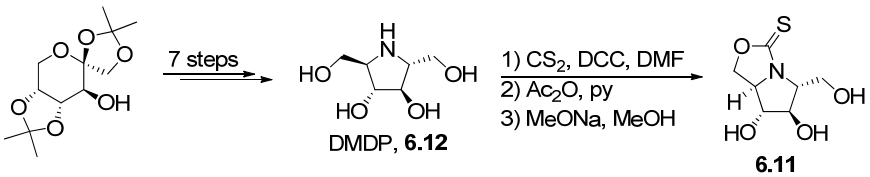
Ortiz Mellet and co-workers reported a similar process for the synthesis of 5,6-fused 1,3-oxazolidine-2-thiones by treating the corresponding 5-Amino-5-deoxy-1,2-*O*-isopropylidene-hexofuranoses with carbon disulfide and dicyclohexylcarbodiimide with excellent yield.[10] Acid-catalyzed hydrolysis of the acetal protecting group led to a mixture of α- and β-furanoses **6.9**. After neutralisation, they rearranged to the fused indolizidine-type byciclic compounds **6.10** via intramolecular nucleophilic addition of the nitrogen to the masked aldehyde group of the monosaccharide (Scheme 6.6). These derivatives are structurally related to azasugars-glycosidase inhibitors family. NMR analysis showed the presence of a single tautomeric form at the pseudoanomeric centre having *R*- or *S*-configuration (for D-*gluco* and L-*ido*-derivatives, respectively).



Scheme 6.6. Synthesis of 5,6-fused 1,3-oxazolidine-2-thiones **6.10**

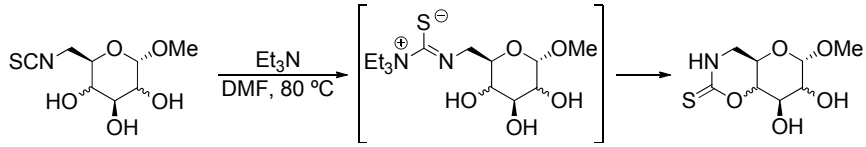
The synthesis of indolizine-type thionocarbamate **6.11** has also been accomplished in a straightforward manner by direct thiocarbonylation of (*2R,3R,4R,5R*)-2,5-bis(hydroxymethyl)-3,4-dihydroxypyrrolidine (DMDP, **6.12**) with carbon

disulfide/DCC (Scheme 6.7). This intermediate is obtained in seven steps from 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose.[11]



Scheme 6.7. Preparation of indolizine derivative **6.11** by direct thiocarbonylation of DMDP

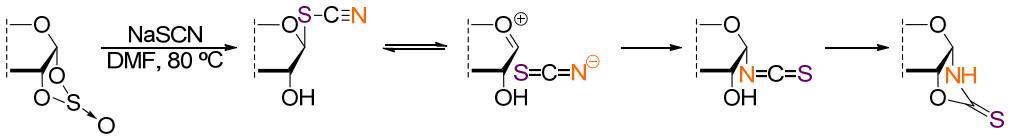
Similarly to the precedent approaches, Ortiz Mellet et al. have also studied the preparation of fused tetrahydro-1,3-oxazine-2-thiones arrays employing 6-deoxy-6-isothiocyanate **6.13** intermediates (Scheme 6.8).[12] The procedure involves the reaction of a methyl 6-amino-6-deoxyaldopyranoside with thiophosgene, which leads to the corresponding 6-isothiocyanate. In the presence of a catalytic amount of Et_3N, compound **6.13** readily underwent intramolecular cyclization to give the fused oxazine-2-thione **6.14**. This result may be explained by the formation of a zwitterionic complex between the amine and the isothiocyanate which undergoes nucleophilic displacement by the γ-located hydroxyl on C-4 of the glycopyranose ring. The resulting *cis*- or *trans*-decalin-type core depends on the configuration of the sugar precursor: while 1,3-oxazine-2-thione cycle is fused *trans* in the cases of D-*gluco* and D-*manno* carbohydrates **6.14**, a *cis* fusion is obtained with the D-*galacto* derivative.



Scheme 6.8. Synthesis of 6-isothiocyanates-deoxy pyranoses by base-promoted condensation

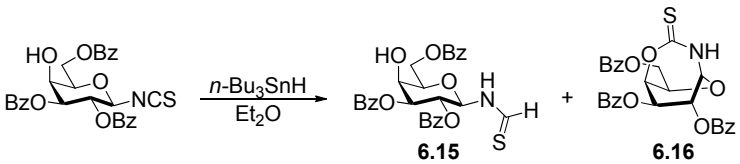
6.1.3. Synthesis by alternative methods

An alternative route to 1,2-fused carbohydrate-derived OAT has been reported employing 1,2-*O*-sulfinyl carbohydrates.[13] The key step is the epimerisation of the β-thiocyanate derivative into the α-isothiocyanate anomer, which undergoes spontaneous cyclisation into the fused 1,3-oxazolidine-2-thione (Scheme 6.9).



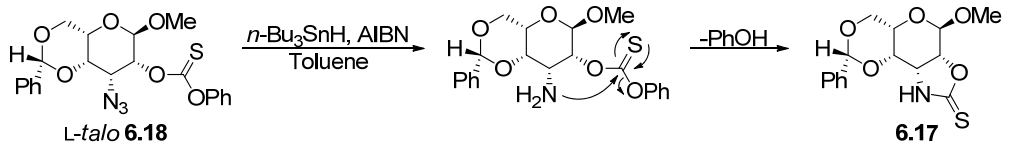
Scheme 6.9. Synthesis of OAT by treatment of 1,2-carbohydrate sulfites with NaSCN

Similarly, reduction of a partially protected β-D-galactopyranosyl isothiocyanate with tri-*n*-butyltin hydride afforded the expected glycosyl thioformamide (**6.15**) with seven-membered thionocarbamate **6.16** as a minor product.[14]



Scheme 6.10. Preparation of thioformarmide from isothiocyanate with tri-*n*-butyltin hydride

Carbohydrate-derived thionocarbamate **6.17** was also achieved by reaction of compound **6.18** with tri-*n*-butyltin hydride.[15] This transformation can be explained by the rapid reduction of the azido-group and subsequent nucleophilic attack of the nitrogen onto the vicinal *cis* thiocarbonyl. This leads to the formation of **6.17** with subsequent loss of a phenol molecule (Scheme 6.11).



Scheme 6.11. Cyclization triggered by reduction of the azido group with Bu_3SnH/AIBN

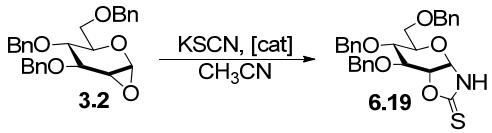
6.2. Results and discussion

6.2.1. Reactions with potassium thiocyanate

As a consequence of the results obtained in the previous chapter, an alternative synthetic route towards the synthesis of 1,2-epithiocarbohydrates was contemplated. In this context, the reaction of 1,2-anhydrocarbohydrates with reagents typically used for transforming epoxides into episulfides was explored.

Initially, reaction of 1,2-anhydro-3,4,6-tri-O-benzyl-α-D-glucopyranose[16] (**3.2**[i]) with typically episulfidation reagents such as thiourea[17] (result not shown in Table 6.1) or KSCN (Table 6.1, entry 1) afforded complex mixtures. Different Lewis acid catalysts were then used. Thus, the use of BF_3•OEt_2 did not improve the results and a complex mixture was also obtained (Table 6.1, entry 2). When the reaction was conducted in the presence of trimetylsilyl triflate, no thiirane was detected; instead, compound **6.19** was isolated in 40% yield (Table 6.1, entry 3). The use of titanium catalysts, such as titanium isopropoxide, afforded **6.19** with low conversion but with almost complete selectivity (Table 1, entry 4). The highest conversions and selectivities were obtained when $TiCl_4$ and $TiO(CF_3CO_2)_2$ were used as catalysts (Table 6.1, entries 5 and 6), and with $TiO(CF_3CO_2)_2$[18] the yield was quantitative. $TiO(CF_3CO_2)_2$ is considered an efficient catalyst for the conversion of epoxides to thiiranes with thiocyanate under aprotic conditions. [19] It seems that a strong oxophile catalyst is necessary in order to activate the epoxide.

Table 6.1 Lewis acid-catalyzed reaction of 1,2-anhydro-glucopyranose **3.2** with KSCN[a]



Entry	[cat]	Conv. (%)[b]	Select. (%)[c]
1	-	> 98	mixture
2	BF_3·OEt_2	> 98	mixture
3	TMSOTf	> 98	40
4	$Ti(OCH(CH_3)_2)_4$	40	95
5	$TiCl_4$	> 98	95
6	$TiO(CF_3CO_2)_2$	> 98	> 98

[a] Conditions: 1 mmol of **3.2**, 3mmol of KSCN, 2 mol % of catalyst, and 5 mL of CH_3CN at reflux for 3 h. [b] Determined by ^{1}H NMR integration of characteristic signals against starting materials of the reaction crude.

The structure of compound **6.19**, incorporating an unexpected 1,3-oxazolidine-2-thione moiety, was determined by NMR and IR spectroscopy, as well as by exact mass spectrometry. The presence of the thionocarbonyl group was determined by ^{13}C NMR (C=S appears at roughly 189 ppm), and by the presence of two strong signals at 1495 and 1453 cm^{-1} in the IR spectrum, which are characteristic of this thionocarbonyl functional group. The presence of a broad singlet at 8 ppm in the ^{1}H

[i] This compound also appears in Chapter 3, thus same codification will be employed.

NMR spectrum is assigned to the amino hydrogen, which completes the characterization of this group.

Figure 6.1 illustrates the ^{1}H NMR spectrum showing a doublet at 5.66 ppm ($J_{1,2}$ = 6.4 Hz) that correlated with a carbon at 82.2 ppm (C-1), which was assigned to an unshielded anomeric hydrogen. The chemical shift for C-1 indicated that it is bonded to nitrogen and oxygen and not to two oxygens (δ ~ 100 ppm). Vicinal coupling constants $J_{2,3}$ (3.2 Hz) and $J_{3,4}$ (3.2 Hz) have unexpected small values, which suggests a distorted pyranose ring with a preferential $^0S_2$ pyranose conformation, as previously reported for structurally related structures in solution (Figure 6.2).[20]

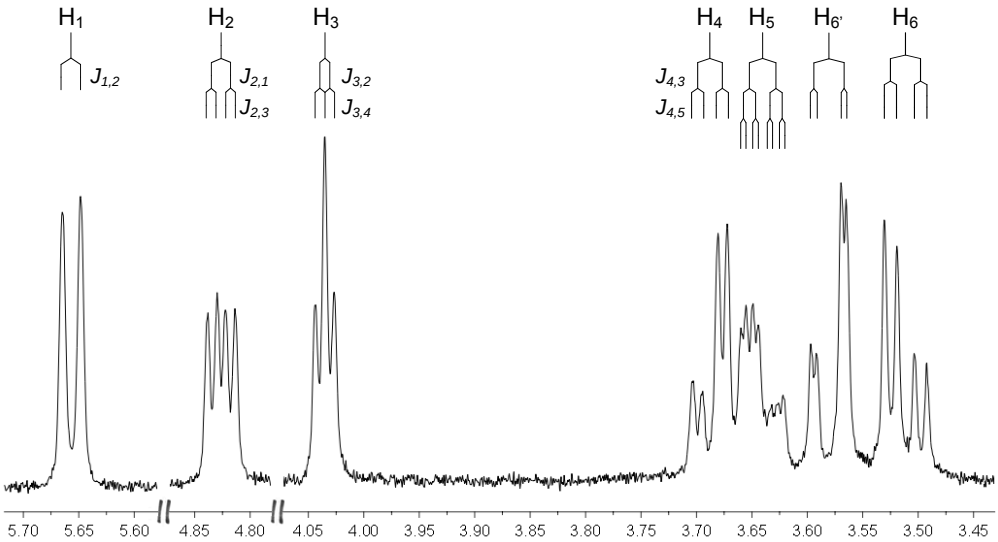


Figure 6.1. ^{1}H NMR spectrum, in $CDCl_3$, of the pyranose ring protons of **6.19**

In structurally related systems, coupling constant $J_{1,2}$ between 6 Hz and 7 Hz is characteristic for H-1 in an equatorial position.[21] This indicates that the oxazolidine ring is fused *cis* with the carbohydrate (Figure 6.2). A *trans* fusion is described to provide larger coupling constants (~9 Hz).[21f, 22]

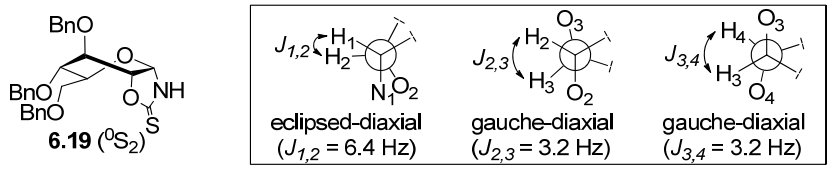
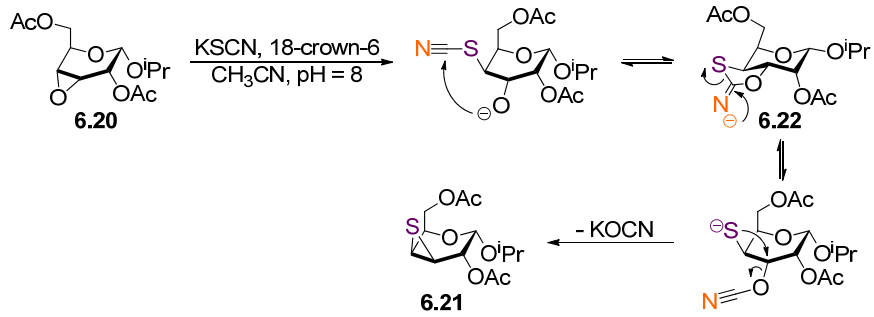


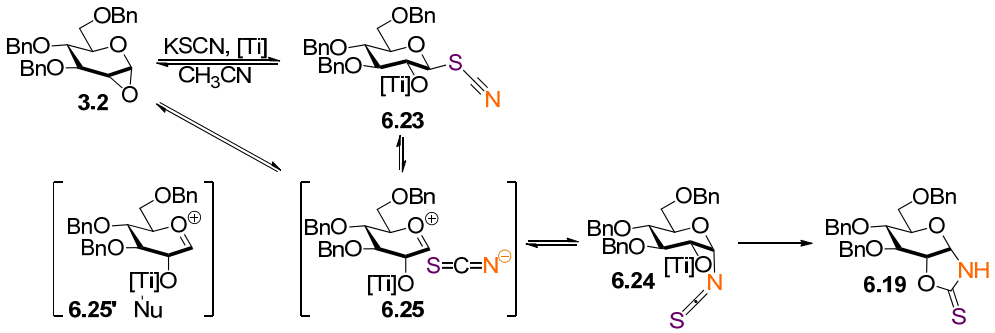
Figure 6.2. Newman projections of **6.19** showing the values of the coupling constants

According to bibliography, this unexpected result does not fit with other published syntheses of epithiosugars from the corresponding epoxide precursors by conversion with alkali thiocyanates.[23] For instance, conversion of 3,4-anhydro-pyranoside **6.20** into the corresponding episulfide (**6.21**[23a]) by means of KSCN involves the initial ring-opening attack onto position C-4, which is followed by alkoxy-promoted intramolecular cyclization. This process builds up the *trans*-fused imino-oxazolidine **6.22**. This strained intermediate collapses to the formation of a cyanate β-sulfide thiolate. The displacement of cyanate by the thiolate ion is the final step of the mechanism.[24] The entire reaction is accompanied by Walden inversion at both of carbon atoms of the ring, so that 3,4-epithio-α-D-galactopyranoside results from the 3,4-anhydro-α-D-altropyranoside.



Scheme 6.12. Mechanism of the reaction of anhydro-sugars with KSCN

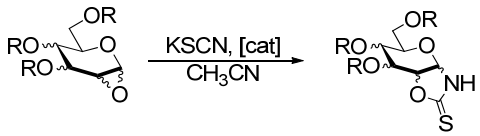
A proposed mechanism for the transformation of **3.2** into **6.19** is shown in Scheme 6.13. The reaction is presumably initiated by the coordination of titanium catalyst to epoxide **3.2** with concomitant opening of the epoxide by KSCN to give **6.23**.
Since substituents at positions C-1 and C-2 in compound **6.19** have a *cis* relationship, compound **6.23** must isomerize to **6.24** through cation **6.25**.[13] Further attack of the alcoholate to the isothiocyanate group in **6.24** would render the final product. Alternatively, activation of **3.2** with the Lewis acid could directly give **6.25** as an intermediate. The different behaviour of anhydro-sugar **3.2** compared to other epoxysugars may be ascribed to the stability of the oxocarbenium ion.
It has been reported that stoichiometric reactions with organometallic reagents (Zr, Zn or Al) afforded compounds resulting from a *cis* opening.[25] Intermediates type **6.25'**, which have been postulated in these cases, can not be discarded. There are few examples of epoxides where their opening proceeds in a *cis* fashion.[26]

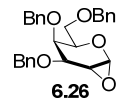
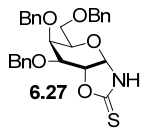
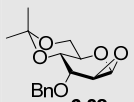
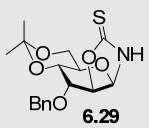
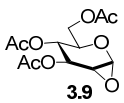
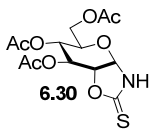


Scheme 6.13. Possible mechanism for product **6.19** formation

The reaction was then extended to other 1,2-anhydropyranoses, such as the *galacto* derivative **6.26**,[16] which afforded compound **6.27** in excellent yield when treated with KSCN under the optimized conditions (Table 6.2, entry 1). With the aim to investigate the possibility of obtaining compounds with opposite configuration at the 1,2-positions, the *altro* derivative **6.28**[27] was also treated with KSCN in the presence of $TiO(CF_3COO)_2$ to afford the expected product **6.29** in 75% yield (Table 6.2, entry 2).

Table 6.2. Reaction of 1,2-anhydro-pyranoses with KSCN in the presence of $TiO(CF_3CO_2)_2$[a]



Entry	Reactant	Product	t (h)	Yield (%)
1	 **6.26**	 **6.27**	3	87
2	 **6.28**	 **6.29**	3	75
3	 **3.9**	 **6.30**	2	87

[a] Conditions: All reactions were carried out at reflux under argon with 1.0 equiv of substrate, 3.0 equiv of KSCN, 2 mol % of $TiO(CF_3CO_2)_2$, and CH_3CN.

On the one hand, the altropyranose configuration was deduced from the very small value of $J_{1,2}$ (3.2 Hz), indicative of the *cis* disposition of the oxazolidine cycle as found for analogous systems.[28] On the other hand, small $J_{2,3}$ values (2.8 Hz) combined with $J_{1,2}$ (3.2 Hz) seem to agree with a regular $^4C_1$ conformation, probably due to the fixation of the 4,6-*O*-benzylidene protecting group. This result confirms the observation that the final thionocarbamate retains the relative configuration of the epoxide of the parent sugar.

The procedure is compatible with other protecting groups such as acetates. Thus, reaction of 1,2-anhydro-3,4,6-tri-*O*-acetyl-α-D-glucopyranose (**3.9**[ii])[16] following the optimized conditions to affords **6.30** in 87% yield (Table 6.2, entry 3).

A single crystal of the acetylated oxazolidine-2-thione **6.30** suitable for an X-ray diffraction analysis allowed the confirmation of the proposed structure (Figure 6.3). As clearly inferable from the dihedral angles listed out in Table 6.3, the pyranoid ring exhibits a $^0S_2$ skew conformation, the same conformation adopted in solution.

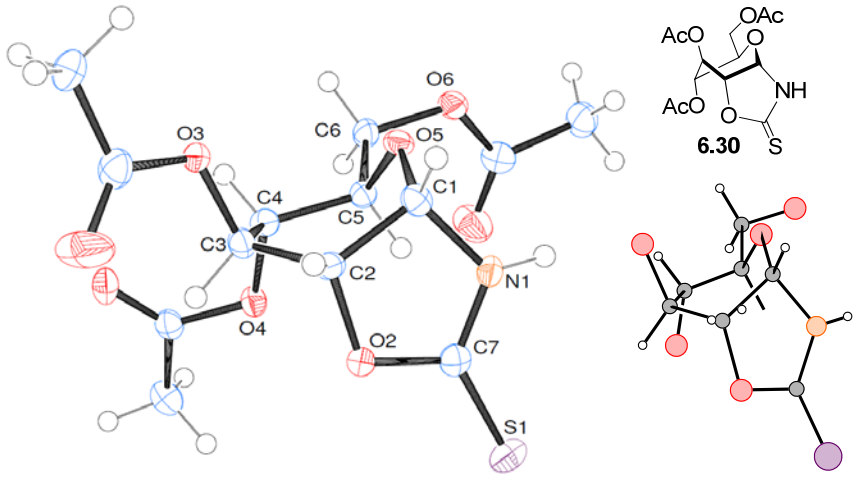


Figure 6.3. ORTEP (ellipsoids at 50% probability) view of the X-ray structure of **6.30** and numbering system (to facilitate visualization of the $^0S_2$ conformation, a second view is given in which acetyl groups are omitted for clarity)

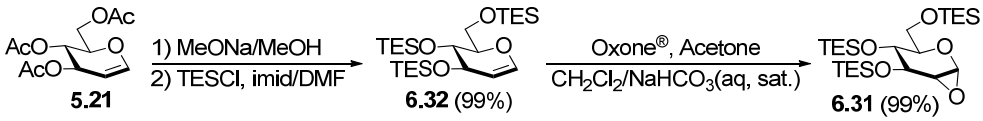
A more lucid substantiation of this conformation is provided by the Cremer-Pople ring puckering parameters[29] (Table 6.3): the puckering angles φ = 337.0º, θ = 80.7º, and the amplitude Q = 0.655 Å are very close to the values postulated for the $^0S_2$ skew conformation (phase magnitudes of φ = 330.0º, θ = 90.0º).

[ii] This compound also appears in Chapter 3, thus same codification will be employed.

Table 6.3. Torsion angles in **6.30** and calculated ring puckering parameters

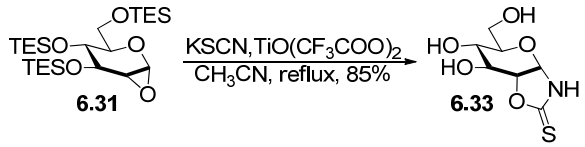
Pyranoid ring	(º)	Substituents	(º)	Puckering parameters
C1-C2-C3-C4	+41.7	C1-C2-O2-C7	-8.8	φ = 337.0º
C2-C3-C4-C5	-21.0	N1-C1-C2-O2	+12.0	θ = 80.7º
C3-C4-C5-O5	-32.0	O2-C2-C3-O3	+168.1	
C4-C5-O5-C1	+70.7	O2-C2-C3-C4	-73.6	Q = 0.655 Å
C5-O5-C1-C2	-49.2	O3-C3-C4-O4	-144.3	
O5-C1-C2-C3	+0.8	O4-C4-C5-C6	+89.2	

Continuing the investigations on the influence of the protecting groups, 1,2-anhydro-3,4,6-tri-*O*-(triethylsilyl)-α-D-glucopyranose (**6.31**) was synthesized in three reaction steps from commercially available tri-*O*-acetyl-D-glucal (Scheme 6.14) in quantitative yield (99%). Transformation of glucal **5.21**[iii] into the persilylated derivative was best carried out through a two-step sequence: saponification of the aceyl protecting groups and then silylation of the latter employing classical Hanessian conditions[30] yielded **6.32**. Formation of **6.31** was accomplished by reaction with dimethyldioxirane generated *in situ* from Oxone®/acetone in a biphasic system.



Scheme 6.14. 1,2-Anhydro-3,4,6-tri-*O*-(triethylsilyl)-α-D-glucopyranose (**6.31**)

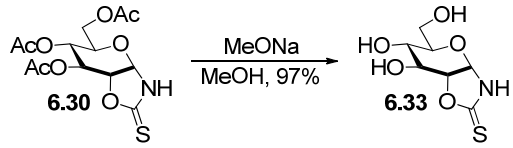
Reaction of derivative **6.31** with KSCN/TiO(CF$_3$COO)$_2$ in the optimized conditions afforded the unprotected 1,3-oxazolidine-2-thione derivative **6.33**, directly recovered from the work-up in a 85% yield (Scheme 6.15). The silylated product was not present in the organic phase. TLC control indicated that silyl ether deprotection takes place during the reaction and not in the workup.



Scheme 6.15. Synthesis of compound **6.33** from **6.31**

[iii] This compound also appears in Chapter 5, thus same codification will be employed.

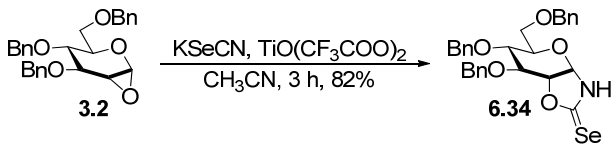
Deprotection of the silyl groups in the presence of a strong oxophile catalyst can explain the direct formation of the unprotected compound **6.33**. Deprotection test of compound **6.30** with Zemplen conditions was successful. This leds also to the unprotected 1,3-oxazolidine-2-thione **6.33** in an excellent 97% yield (Scheme 6.16).
In order to check the viability of the benzyl ether deprotection, palladium-catalyzed hydrogenation was performed over thionocarbamate **6.19**, but the reaction proceeded with decomposition of the starting material.



Scheme 6.16. Deprotection of 1,3-oxazolidine-2-thione **6.30**

6.2.2. Reactions with other analogous agents

The generality of the reaction has also been studied using different cyanate reagents. Reaction of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (**3.2**) with potassium selenocyanate gave after 3 hours the light-sensitive 1,3-oxazolidine-2-selone derivative **6.34** in very good yield (Scheme 6.17). The structure was again confirmed by mass spectrometry and by NMR spectroscopy. ^{1}H NMR signals and coupling constants are in accordance with the thione analog **6.19** (Table 6.4). Signal at 190.9 ppm in ^{13}C-NMR spectrum confirmed the oxazolidinselone scaffold (δ ~190 ppm) and discarded also its selenocarboimidate isomer (δ ~160 ppm).[31]



Scheme 6.17. Synthesis of selone derivative **6.34**

In order to obtain the deprotected seleno derivative, acetyl derivative **3.9** and silyl derivative **6.31** were also treated with potassium selenocyanate under similar reactions conditions but both reactions led to decomposition products.

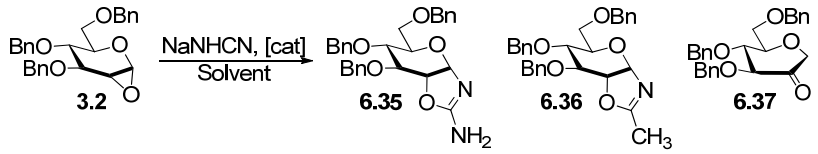
Table 6.4. Selected ^{1}H and ^{13}C NMR data[a] for the compounds **6.19** and **6.34**[a]

Compound	Chemical shifts (δ) / Coupling constants (Hz)						
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	C-1	C-2	C-3	NC(X)O
6.19	5.66 (6.4)	4.83 (3.2)	4.04 (3.2)	82.2	81.5	76.0	188.9
6.34	5.60 (7.0)	4.78 (3.4)	4.05 (3.4)	83.0	82.4	75.2	190.9

[a] Recorded in $CDCl_3$ at 400 and 100.6 MHz for ^{1}H and ^{13}C, respectively.

Sodium cyanamide was also used as a cyclating reagent (Table 6.5). Preliminary test working in the optimal conditions did afford a complex mixture instead of the expected 1,3-oxazol-2-amine **6.35** (Table 6.5, entry 1); and so did happen after increasing the catalyst quantity loading up to 10 mol % (Table 6.5, entry 2). Then, a variety of solvents were employed. A complex mixture was always obtained in all cases (Table 6.5, entries 3-6). The use of similar titano-complexes that also catalyzed this kind of transformation, such as $Ti(^{i}PrO)_4$ and $TiCl_4$, did not improve the results already obtained (Table 6.5, entries 7 and 8).

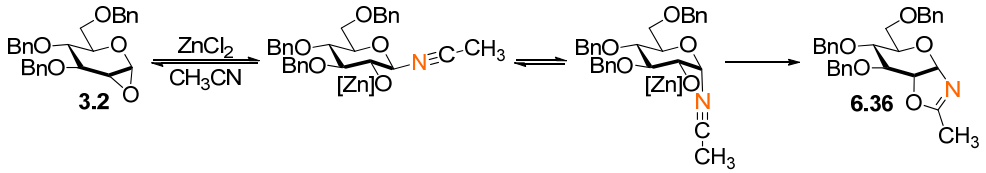
Table 6.5. Cyanamide tests with anhydrosugar **3.2**[a]



Entry	[cat] (eq)	Solvent	T (ºC)	Conv. (%)[b]	Yield (%)
1	$TiO(CF_3CO_2)_2$ (0.02)	CH_3CN	reflux	Complex mixture	
2	$TiO(CF_3CO_2)_2$ (0.10)	CH_3CN	reflux	Complex mixture	
3	$TiO(CF_3CO_2)_2$ (0.10)	THF	reflux	Complex mixture	
4	$TiO(CF_3CO_2)_2$ (0.10)	DMF	80	Complex mixture	
5	$TiO(CF_3CO_2)_2$ (0.10)	acetone	reflux	Complex mixture	
6	$TiO(CF_3CO_2)_2$ (0.10)	CH_3NO_2	reflux	Complex mixture	
7	$Ti(^{i}PrO)_4$ (0.10)	CH_3CN	reflux	Complex mixture	
8	$TiCl_4$ (0.10)	CH_3CN	reflux	Complex mixture	
9	$ZnCl_2$ (0.10)	CH_3CN	reflux	> 98	93 (**6.36**)
10	$ZnCl_2$ (0.10)	THF	reflux	> 98	92 (**6.37**)
11	$ZnCl_2$ (0.10)	DMF	80	> 98	90 (**6.37**)

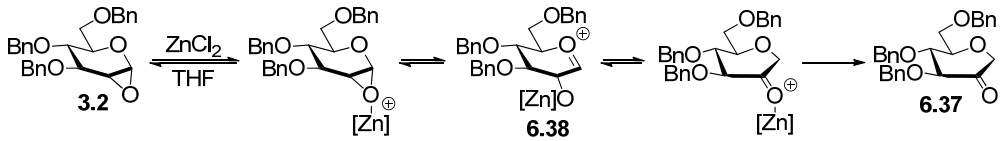
[a] Conditions: 0.12 mmol epoxide, 0.36 mmol NaNHCN, catalyst, and 5 mL solvent for 3h.
[b] Determined by ^{1}H NMR integration of characteristic signals against starting materials of the reaction crude.

In order to seek for a more appropriate catalyst, reactions of **3.2** using zinc (II) chloride were next explored. Reaction with this Lewis acid in acetonitrile took place with complete conversion towards oxazoline **6.36** in a 93% yield (Table 6.5, entry 9). Formation of this compound starting from **3.2** under similar conditions was already reported by Danishefsky.[32] It is suggested that the transformation proceeds through the usual *trans* opening of the epoxide by a solvent molecule. Epimerization of this intermediate produces the axial anomer, where the acetonitrile is captured by the vicinal hydroxyl group at position C-2. This process leads to the formation of oxazoline **6.36** (Scheme 6.18). Alternatively, S_N1 type opening of the oxirane ring might also produce the axial anomer.



Scheme 6.18. Possible mechanism for product **6.36** formation

Interestingly, the stereoselectivity of the reaction was observed to depend on the solvent. Thus the use of non-reacting solvents, like THF or DMF, allowed the exclusively obtention of uloside **6.37** (Table 6.5, entries 10 and 11). Rearrangements of epoxides to ketones can be facilitated by protic or Lewis acids, involving migration of a proton from one carbon of the epoxide ring to the other.[33] A mechanism of $ZnCl_2$-catalyzed is shown in Scheme 6.19. Coordination of the Lewis acid to the electron lone pairs of epoxide **3.2** provides electrophilic assistance for opening of the epoxide ring. Cleavage of the epoxide C-O bond can proceed through a short lived carbocation intermediate **6.38**, where the hydrogen on position C-2 can migrate to give ketone **6.37**.



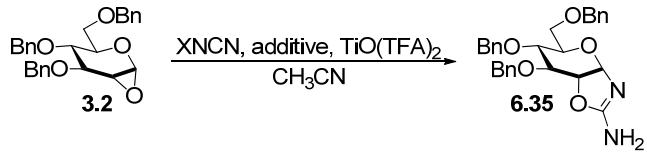
Scheme 6.19. Possible mechanism for product **6.37** formation

These results suggest that cyanamide is not reactive enough to open the epoxide from the carbohydrate. In order to assess the factors influencing the activity of this reagent, different cyanamides were prepared using standard literature procedures and

used for this heterocyclation reaction. Table 6.6 summarizes the results obtained. Thus, no successful result was achieved attempting to increase the nucleophilicity of sodium cyanamide by solvation of the ion pair with 15-crown-5 (Table 6.6, entry 1). The reaction was attempted with a more soluble reagent, NH_2CN, but it also afforded a complex mixture (Table 6.6, entry 2). Treatment of this compound with different bases, like K_2CO_3 or ^tBuOK, to generate *in situ* the cyanamide ion did not improve the results already obtained (Table 6.6, entries 3-4). The use of synthesised potassium cyanamide was then considered, but there were not significant differences with the previously test, even if a crown ether was used (Table 6.6, entries 5-6).

At this point, the hardness of the nucleophile was "softened" by the formation of the corresponding cesium and silver salts. In the latter cases, complex mixtures were also obtained (Table 6.6, entries 7 and 8). It seems that the nature of ion-pairing does not influence significantly the heterocyclation reaction with cyanamide. Final tests with cyanamide were carried out with the electron-withdrawing benzoyl derivative. Unfortunately, none of the experiments gave satisfactory results and the synthesis of compound **6.35** was not achieved (Table 6.6, entries 9-11).

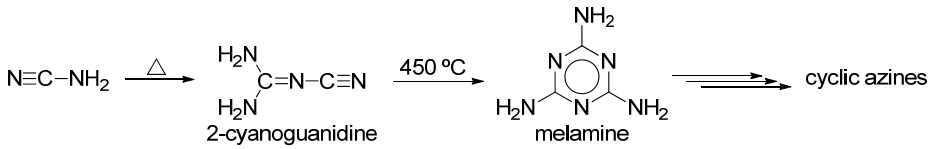
Table 6.6. Heterocyclation attempts of **3.2** with different cyanamide reagents[a]



Entry	XNCN	Additive (eq)	Conv. (%)[b]	Select. (%)[b]
1	NaNHCN	15-crown-5	Complex mixture	
2	NH_2CN	-	Complex mixture	
3	NH_2CN	K_2CO_3	Complex mixture	
4	NH_2CN	^tBuOK	Complex mixture	
5	KHCN	-	Complex mixture	
6	KNHCN	18-crown-6	Complex mixture	
7	NH_2CN	Cs_2CO_3	Complex mixture	
8	AgNHCN	-	Complex mixture	
9	BzNHCN/	K_2CO_3	Complex mixture	
10	BzNHCN	^tBuOK	Complex mixture	
11	BzNHCN/	Cs_2CO_3	Complex mixture	
12	KOCN	-	Complex mixture	

[a] Conditions: 0.12 mmol epoxide, 0.36 mmol XNCN, additive, $TiO(CF_3CO_2)_2$ (0.02), and 5 mL solvent for 3h. [b] Determined by ^1H NMR integration of characteristic signals against starting materials of the reaction crude.

The different reactivity of cyanamide may be explained through a self-side reaction of the nucleophile. This compound is known to dimerize on mild heating to 2-cyanoguanidine, and then to form higher molecular weight cyclic azines on further heating (Scheme 6.20).[34] 2-Cyanoguanidine would then be anticipated to be formed under the reaction conditions, which could then undergo a variety of undesired side-reactions.



Scheme 6.20. Dimerization of cyanamide to give 2-cyanoguanidine

Finally, the generality of the reaction was also explored using potassium cyanate. Reaction of **3.2** with this reagent gave another complex mixture of products (Table 6.6, entry 12). Since cyanate is an ambident nucleophile where the two electron-rich centers show similar hard base characters, both nitrogen and oxygen atoms can attack to the epoxide, affording two or more products.

6.3. Conclusions

To conclude, 1,3-oxazolidine-2-thione heterocycles fused to the 1,2 positions of different pyranoses were efficiently prepared by reaction of 1,2-anhydrosugars with KSCN using $TiO(CF_3COO)_2$ as catalyst. The structure was elucidated by NMR, MS, and IR spectroscopy and then confirmed by X-ray crystallography. The reaction is general and therefore 1,2-anhydrosugars of *gluco*, *galacto* and *altro* configurations afforded compounds with the same relative configurations, although the configuration of the anomeric position for the two first cases was α, and for the altro derivative was β.

Fully deprotected thionocarbamates were obtained either in a one-pot process starting from triethylsilyl protected 1,2-anhydrosugars or by deprotection of peracetilated oxazolidine-2-thione derivative.

Although the procedure can also be extended to the preparation of seleno derivatives, such as compound **6.34**, its application to cyanamide was unsuccessful. Furthermore, heterocyclation reaction with potassium cyanate also failed.

The use of Zn (II) chloride as Lewis catalyst in acetonitrile produce the insertion of one solvent molecule toward the *cis* 1,2-fused oxazoline. Hence, the use of $ZnCl_2$ as Lewis acid catalyst in the presence of solvents such as THF or DMF, proceed through rearrangement of the anhydrocarbohydrate to the 2-uloside derivatives.

6.4. Experimental section

6.4.1. General methods

All chemicals used were reagent grade and were employed as supplied unless otherwise specified. HPLC grade dichloromethane and diethyl ether were dried using a solvent purification system (Pure SOLV system-4®). Solvents were purified using standard procedures.[35] 1,2-Anhydrosugars used as starting materials were synthesized following the epoxidation methodology reported by Dondoni et al.[16] The $TiO(CF_3COO)_2$ catalyst was prepared in Chapter 5 as compound **5.17** following also a previously reported procedure.[36] Potassium hydrogen cyanamide,[37] silver hydrogen cyanamide,[38] and benzoyl cyanamide[39] were synthesized using literature procedures.
^1H and ^{13}C NMR spectra were recorded using two spectrometers (Varian® Mercury VX 400 and Varian® 400-MR) both operating at frequency of 400 MHz for proton and 100.6 MHz for carbon. $CDCl_3$ and CD_3OD were used as solvents. Chemical shifts (δ) were referenced to internal standards ($CDCl_3$ (7.27 ppm ^1H, 77.23 ppm ^{13}C) and CD_3OD (3.31 ppm ^1H, 49.14 ppm ^{13}C)) or Me_4Si as an internal reference (0.00 ppm ^1H). 2D correlation spectra (gCOSY, NOESY, gHSQC and gHMBC) were visualized using the VNMR program (Varian®). Melting points (Mp) were measured on a Griffin melting point apparatus and were uncorrected. Optical rotations were measured at 598 nm at room temperature in a Perkin-Elmer® 241 MC apparatus with 10 cm cells. ESI-MS were run on an Agilent® 1100 Series LC/MSD instrument. IR spectra were recorded on a JASCO FT/IR-600 plus Fourier Transform Infrared Spectrometer ATR Specac Golden Gate.
Suitable crystals of compound **6.30** were mounted on glass fibers and these samples were used for data collection. Data collection: *APEX2* v2011.4-1 (Bruker AXS, 2011); cell refinement: *APEX2* v2011.4-1 (Bruker AXS, 2011); data reduction: *APEX2* v2011.4-1 (Bruker AXS, 2011); program(s) used to solve structure: *SIR97*(Altomare et al., 1999); program(s) used to refine structure: *SHELXL97* (Sheldrick, 2008); molecular graphics: *ORTEP-3 for Windows* (Farrugia, 1997);

software used to prepare material for publication: *WinGX* publication routines (Farrugia, 1999).

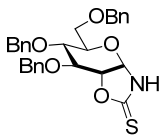
Reactions were monitored by TLC carried out on 0.25 mm E. Merck® silica gel 60 F_{254} glass or aluminium plates. Developed TLC plates were visualized under a short-wave UV lamp (254 nm) and by heating plates that were dipped in ethanol/H_2SO_4. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka® or Merck® silica gel 60 (230-400 mesh). Radial chromatography was performed on a Harrison® chromatotron model 7924T in 1 or 2 mm plates of Kieselgel 60 PF_{254} silica gel, depending on the amount of product. Flash column chromatography (FCC) was performed using flash silica gel (32–63 μm) and employing a polarity elution system correlated to TLC mobility.

6.4.2. General procedures

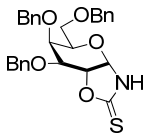
General Procedure for glycal epoxydation:[16] the glycal (1.00 mmol) was dissolved in an ice bath cooled biphasic solution of CH_2Cl_2 (4 mL), acetone (0.4 mL), and saturated aqueous $NaHCO_3$ (6.5 mL). The mixture was vigorously stirred and a solution of Oxone® (1.23 g, 2.00 mmol) in H_2O (5 mL) was added dropwise over 15 min. The crude reaction was vigorously stirred at 0º C for 30 minutes and was then allowed to warm to room temperature until TLC indicated complete consumption of the glycal. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2x 4 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated to afford the 1,2-anhydro-pyranoses in the yields shown. The crude could not be purified due the decomposition of the 1,2-anhydro-sugar.

General Procedure for the synthesis of *cis*-1,2-fused 1,3-oxazolidine-2-thione and 1,3-oxazolidine-2-selone carbohydrate derivatives: to a stirred solution of the 1,2-anhydro-sugar (1.00 mmol) and XCN (X=KS, KSe) (3.00 mmol) in dry CH_3CN (5 mL), finely powdered $TiO(CF_3COO)_2$ (0.02 mmol) was added under an argon atmosphere, and the mixture was heated to boiling for appropriate time. After completion of the reaction, followed by TLC, the mixture was cooled to room temperature and H_2O (10 mL) was added. The resultant mixture was extracted with EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography on silica gel gave the products in the yields shown.

6.4.3. Compound characterization

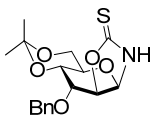


3,4,6-tri-*O*-benzyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-1,3-oxazolidine-2-thione (6.19): the title compound was prepared following the general procedure for 1,3-oxazolidine-2-thione formation starting from 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (**3.2**) (51.6 mg, 0.12 mmol), KSCN (34.8 mg, 0.36 mmol) and $TiO(CF_3COO)_2$ (0.5 mg, 2.4 μmol) in dry CH_3CN (5 mL). The reaction mixture was stirred under reflux for 16 h. After standard workup, the crude was purified by radial chromatography (from hexane to 4:1 AcOEt/hexane) to afford **6.19** (52 mg, 89% yield) as a beige syrup. Data: R_f (4:1 AcOEt/hexane): 0.75. $[\alpha]_D$ + 25.7 (*c* 1, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 3228, 2921, 2867, 1495, 1453, 1266, 1163, 1069, 1027. ^1H NMR (400 MHz, $CDCl_3$) δ in ppm: 8.06 (bs, 1H, NH); 7.40-7.10 (m, 15H, Ar); 5.66 (d, 1H, $J_{1,2}$= 6.4 Hz, H-1); 4.83 (dd, 1H, $J_{1,2}$= 6.4 Hz, $J_{2,3}$= 3.2 Hz, H-2); 4.65 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.57 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.51 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.50 (d, 1H, J_{AB}= 12 Hz, CH_2Ph); 4.41 (d, 1H, J_{AB}= 12 Hz, CH_2Ph); 4.28 (d, 1H, J_{AB}= 11.8 Hz, CH_2Ph); 4.04 (t, 1H, $J_{2,3}$= $J_{3,4}$= 3.2Hz, H-3); 3.69 (dd, 1H, $J_{3,4}$= 3.2 Hz, $J_{4,5}$= 9.2 Hz, H-4); 3.58 (ddd, 1H, $J_{4,5}$= 9.2 Hz, $J_{5,6}$= 1.8 Hz, $J_{5,6'}$= 4.6 Hz, H-5); 3.52 (dd, 1H, $J_{5,6}$= 1.8 Hz, $J_{6,6'}$= 10.8 Hz, H-6); 3.45 (dd, 1H, $J_{5,6'}$= 4.6 Hz, $J_{6,6'}$= 10.8, H-6'). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 188.9 (C=S); 137.8, 137.6, 137.1 (C, Ar); 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0 (CH, Ar); 82.2 (C-1); 81.5 (C-2); 76.0 (C-3); 74.2 (C-4); 73.6, 72.7, 72.6 (CH_2Ph); 71.2 (C-5); 69.6 (C-6). +TOF MS Calcd for $C_{28}H_{29}NO_5S$ *m/z* [M-H]+: 492.1845, found: 492.1804; [M-Na]+: 514.1664, found: 514.1617.



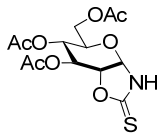
3,4,6-tri-*O*-benzyl-1,2-dideoxy-α-D-galactopyranoside[1,2-d]-1,3-oxazolidine-2-thione (6.27): the title compound was prepared following the general procedure for 1,3-oxazolidine-2-thione formation starting from 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-galactopyranose (**6.26**) (30.5 mg, 0.07 mmol), KSCN (20.0 mg, 0.21 mmol) and $TiO(CF_3COO)_2$ (0.28 mg, 1.4 μmol) in dry CH_3CN (3 mL). The reaction mixture was stirred under reflux for 12 h. After standard workup, the crude was purified by radial

chromatography (from hexane to 4:1 AcOEt/hexane) to afford **6.27** (30.3 mg, 87% yield) as a beige syrup. Data for **6.27**: R_f (4:1 AcOEt/hexane): 0.75. $[\alpha]_D$ + 24.6 (*c* 0.51, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 3252, 2923, 2854, 1495, 1453, 1055, 1026. ^1H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.77 (bs, 1H, NH); 7.40-7.2 (m, 15H, Har); 5.45 (d, 1H, $J_{1,2}$= 5.6 Hz, H-1); 4.94 (t, 1H, $J_{1,2}$= 5.6 Hz, $J_{2,3}$= 5.8 Hz, H-2); 4.89 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.86 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.68 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.58 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.51 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.46 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.16-4.12 (m, 1H, H-5); 4.05 (dd, 1H, $J_{3,4}$= 2.0 Hz, $J_{4,5}$= 3.6 Hz, H-4); 3.90 (dd, 1H, $J_{2,3}$= 5.8 Hz, $J_{3,4}$= 2.0 Hz, H-3); 3.62-3.55 (m, 1H, H-6); 3.53-3.49 (m, 1H, H-6'). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 190.5 (C=S); 137.7, 137.5, 137.4 (Car); 128.6, 128.5, 128.4, 128.1, 128.0, 127.3, 127.6 (CHar); 86.7 (C-2); 81.8 (C-1); 78.8 (C-3); 74.8 (CH_2Ph); 74.7 (C-5); 73.6 (CH_2Ph); 73.5 (C-4); 72.2 (CH_2Ph); 68.1 (C-6). +TOF MS Calcd for $C_{28}H_{29}NO_5S$ *m/z* [M-H]+: 492.1845, found: 492.1822; [M-Na]+: 514.1664, found: 514.1683.

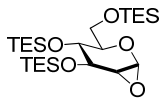


3-O-benzyl-1,2-dideoxy-4,6-*O*-isopropylidene-β-D-altropyranoside[1,2-d]-1,3-oxazolidine-2-thione (6.29): the title compound was prepared following the general procedure for 1,3-oxazolidine-2-thione formation starting from 1,2-anhydro-3-*O*-benzyl-1,2-dideoxy-4,6-*O*-isopropylidene-β-D-allopyranose (**6.28**) (49.6 mg, 0.17 mmol), KSCN (38.7 mg, 0.50 mmol) and $TiO(CF_3COO)_2$ (1 mg, 3.4 μmol) in dry CH_3CN (5 mL). The reaction mixture was stirred under reflux for 6 h. After standard workup, the crude was purified by flash chromatography (from 1:3 AcOEt/hexane to 4:1 AcOEt/hexane) to afford **6.29** (45 mg, 75% yield) as a beige syrup. Data for **6.29**: R_f (4:1 AcOEt/hexane): 0.72. $[\alpha]_D$ -66.7 (*c* 0.17, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 3313, 3260, 2922, 1725, 1495, 1468, 1072, 1053. ^1H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.3-7.24 (m, 5H, Har); 7.11 (bs, 1H, NH); 5.22 (d, 1H, $J_{1,2}$= 3.2 Hz, H-1); 4.88 (d, 1H, J_{AB}= 11.4 Hz, CH_2Ph); 4.59 (d, 1H, J_{AB}= 11.4 Hz, CH_2Ph); 4.53 (dd, 1H, $J_{1,2}$= 3.2 Hz, $J_{2,3}$= 2.8 Hz, H-2); 4.11 (m, 1H, H-3); 3.90-3.79 (m, 3H, H4, H-5 and H-6); 3.62 (dt, 1H, $J_{5,6}$= $J_{6,6'}$= 9.6 Hz, $J_{4,6'}$= 1.6 Hz, H-6'); 1.45 (s, 3H, CH_3); 1.40 (s, 3H, CH_3).^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 190.8 (C=S); 137.5 (Car); 128.5, 128.2, 128.0 (CHar); 99.7 ($C(CH_3)_2$); 83.7 (C-2); 81.0 (C-1); 74.7 (CH_2Ph); 70.4 (C-3); 69.9 (C-5); 61.8 (C-6); 61.2 (C-4); 29.0 (CH_3); 18.8 (CH_3). +TOF MS Calcd

for $C_{17}H_{21}NO_5S$ *m/z* [M-H]+: 352.1219, found: 352.1202; [M-Na]+: 374.1038 found: 374.1036.

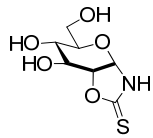


3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-1,3-oxazolidine-2-thione (6.30): the title compound was prepared following the general procedure for 1,3-oxazolidine-2-thione formation starting from 1,2-anhydro-3,4,6-tri-*O*-acetyl-α-D-glucopyranose (**3.9**) (mixture *gluco/manno* 7:1) (503 mg, 1.74 mmol), KSCN (508 mg, 5.23 mmol) and $TiO(CF_3COO)_2$ (10.1 mg, 0.03 mmol) in dry CH_3CN (50 mL). The reaction mixture was stirred under reflux for 2 h. After standard workup, the crude was purified by flash chromatography (2:1 AcOEt/hexane) to afford **6.30** (530.0 mg, 87% yield) as a beige syrup. Data for **6.30**: R_f (4:1 AcOEt/hexane): 0.65. Mp: 135 – 136 ºC. $[\alpha]_D$ + 48.2 (*c* 1.53, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 3297, 1737, 1491, 1367, 1214, 1159, 1035. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.46 (bs, 1H, NH); 5.70 (d, 1H, $J_{1,2}$= 6.8 Hz, H-1); 5.31 (bdd, 1H, $J_{2,3}$= 2.8 Hz, $J_{3,4}$= 2.0 Hz, H-3); 4.98 (ddd, 1H, $J_{2,4}$=1.2 Hz, $J_{3,4}$ = 2.0 Hz, $J_{4,5}$= 8.8 Hz, H-4); 4.89 (ddd, 1H, $J_{1,2}$= 6.8 Hz, $J_{2,3}$= 2.8 Hz, $J_{2,4}$=1.2 Hz, H-2); 4.23-4.19 (m, 2H, H-6 and H-6'); 3.85 (ddd, $J_{4,5}$= 8.8 Hz, $J_{5,6}$= 5.0 Hz, $J_{5,6'}$ =3.8 Hz, 1H, H-5); 2.16 (s, 3H, AcO); 2.13 (s, 3H, AcO); 2.11 (s, 3H, AcO). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 188.9 (C=S); 170.6, 169.7, 168.8 (C(O), AcO); 81.4 (C-1); 78.2 (C-2); 68.1 (C-3); 67.8 (C-5); 67.4 (C-4); 63.0 (C-6); 20.8, 20.7, 20.6 (CH_3, AcO). +TOF MS Calcd for $C_{13}H_{17}NO_8S$ *m/z* [M-Na]+: 370.0573, found: 370.0574.

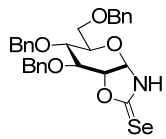


1,2-Anhydro-3,4,6-tri-*O*-(triethylsilyl)-α-D-glucopyranose (6.31): the title compound was prepared following the general procedure for the glycal epoxydation starting from 3,4,6-tri-*O*-(triethylsilyl)-D-glucal[30] (**6.32**) (73 mg, 0.14 mmol) in a mixture of CH_2Cl_2 (0.65 mL), acetone (0.06 mL) and saturated aqueous $NaHCO_3$ (1.03 mL) and Oxone® (92 mg, 0.29 mmol) in H_2O (0.72 mL). The reaction mixture was stirred for 6 h. Standard workup afforded **6.31** (74.6 mg, 99% yield) as a white syrup. Data for **6.31**: R_f (1:1 AcOEt/hexane): 0.71. $[\alpha]_D$ + 29.5 (*c* 1.50, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 2953, 2912, 2876, 1458, 1415, 1104, 1004, 721. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 4.80 (d, 1H, $J_{1,2}$= 2.0 Hz, H-1); 3.86 (dd, 1H, $J_{3,4}$= 7.8 Hz,

$J_{3,5}$= 1.0 Hz, H-3); 3.73-3.64 (m, 2H, H-6 and H-6'); 3.50 (dd, 1H, $J_{3,4}$= 7.8 Hz, $J_{4,5}$= 9.5 Hz, H-4); 3.36 (ddt, 1H, $J_{3,5}$= 1.0 Hz, $J_{4,5}$= 9.5; $J_{5,6}$= 3.0 Hz; H-5); 2.82 (d, 1H, $J_{1,2}$= 2.0 Hz, H-2); 0.96-0.86 (m, 27H, CH_2Si); 0.67-0.50 (m, 18H, CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 77.6 (C-1); 73.7 (C-3); 72.4 (C-5); 70.1 (C-4); 61.8 (C-6); 55.7 (C-2); 7.3, 7.2, 7.1, 6.9 (CH_2Si); 5.5, 5.4, 5.3, 5.3, 5.2 (CH_3). +TOF MS Calcd for $C_{24}H_{52}O_5Si_3$ *m/z* [M-Na]+: 527.3020, found: 4527.3011.

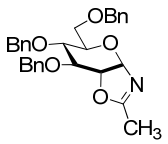


1,2-dideoxy-α-D-glucopyranoside[1,2-d]-1,3-oxazolidine-2-thione (6.33) a) By one-pot cyclation-silyl hydrolysis reaction: 1,2-Anhydro-3,4,6-tri-*O*-(triethylsilyl)-α-D-glucopyranose (**6.31**) (85 mg, 0.17 mmol) was treated, as reported for the general procedure for heterocyclic ring formation, with KSCN (38.4 mg, 0.5 mmol) and $TiO(CF_3COO)_2$ (1 mg, 3.4 μmol) in dry CH_3CN (5 mL) at reflux for 4h. After standard workup the aqueous phase was concentrated to give the unprotected cyclic-sugar, which was purified by silica gel chromatography (1:9 MeOH/CH_2Cl_2) to afford **6.33** (32 mg, 85% yield) as a colourless syrup. **b) By acetate saponification:** To a solution of **6.30** (100 mg, 0.29 mmol) in methanol (5.8 mL) was added MeONa (0.75 mg, 14 μmol). The reaction mixture was stirred at rt for 30 min. After standard workup, the crude was purified by flash chromatography (10:90 MeOH/CH2Cl2) to afford the desired compound (61.2 mg, 97% yield) as colourless syrup. Data for **6.33**: $[\alpha]_D$ + 23.3 (*c* 1.02, MeOH). FT-IR (neat) υ in cm^{-1}: 3376, 2924, 2553, 2361, 2339, 2066, 1635, 1507. ^1H NMR (400 MHz, MeOH) δ in ppm: 5.67 (d, 1H, $J_{1,2}$= 6.6 Hz, H-1); 4.74 (ddd, 1H, $J_{1,2}$= 6.6 Hz, $J_{2,3}$= 4.0 Hz, $J_{2,4}$= 0.8. Hz, H-2); 3.96 (dd, 1H, $J_{2,3}$= 4.0 Hz, $J_{3,4}$= 4.3 Hz, H-3); 3.78 (dd, 1H, $J_{5,6}$= 2.7 Hz, $J_{6,6'}$= 12.1 Hz, H-6); 3.66 (dd, 1H, $J_{5,6'}$= 5.9 Hz, $J_{6,6'}$= 12.1, H-6'); 3.61 (dd, 1H, $J_{3,4}$= 4.3 Hz, $J_{4,5}$= 9.0 Hz, H-4); 3.40 (ddd, 1H, $J_{4,5}$= 9.0 Hz, $J_{5,6}$= 2.7 Hz, $J_{5,6'}$= 5.9 Hz, H-5).^{13}C NMR (100.6 MHz, MeOH) δ in ppm: 190.2 (C=S); 83.0 (C-2); 82.1 (C-1); 74.1 (C-5); 71.4 (C-3); 68.5 (C-4); 61.8 (C-6).



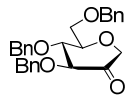
3,4,6-tri-*O*-benzyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-1,3-oxazolidine-2-selone (6.34): to a stirred solution of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-

glucopyranose (**3.2**) (30.5 mg, 0.07 mmol) and KSeCN (30.2 mg, 0.21 mmol) in dry CH_3CN (3 mL) was added finely powdered $TiO(CF_3COO)_2$ (0.28 mg, 1.4 μmol) under argon atmosphere, and the mixture was heated to boiling for appropriate time. After consumption of the substrate, followed by TLC, the mixture was cooled to room temperature and H_2O (10 mL) was added and extracted with AcOEt. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (from hexane to 4:1 AcOEt/hexane) afforded **6.34** (30.7 mg, 82% yield) as a beige syrup. Data for **6.34**: R_f (4:1 AcOEt/hexane): 0.70. $[\alpha]_D$ + 67.2 (*c* 1.52, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 2916, 2849, 1494, 1453, 1260, 1070, 1025. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 8.04 (bs, 1H. NH); 7.40-7.1 (m, 15H, Ar); 5.60 (d, 1H, $J_{1,2}$= 7.0 Hz, H-1); 4.78 (ddd, 1H, $J_{1,2}$= 7.0 Hz, $J_{2,3}$= 3.4. Hz, $J_{2,4}$= 1.0. Hz, H-2); 4.57 (d, 1H, J_{AB}= 12.0 Hz, CH_2Ph); 4.51 (d, 1H, J_{AB}= 12.0 Hz, CH_2Ph); 4.43 (d, 2H, J_{AB}= 11.6 Hz, CH_2Ph); 4.32 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.20 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.05 (t, 1H, $J_{2,3}$= $J_{3,4}$= 3.4Hz, H-3); 3.68 (ddd, 1H, $J_{2,4}$= 1.0 Hz, $J_{3,4}$= 3.4 Hz, $J_{4,5}$= 9.2 Hz, H-4); 3.58 (ddd, 1H, $J_{4,5}$= 9.2 Hz, $J_{5,6}$= 2.2 Hz, $J_{5,6'}$= 4.8 Hz, H-5); 3.56 (dd, 1H, $J_{5,6}$= 2.2 Hz, $J_{6,6'}$= 10.8 Hz, H-6); 3.52 (dd, 1H, $J_{5,6'}$= 4.8 Hz, $J_{6,6'}$= 10.8, H-6'). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 190.9 (C=Se); 137.8, 137.5, 137.0 (Car); 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0 (CHar); 83.0 (C-1); 82.4 (C-2); 75.2 (C-3); 74.2 (C-4); 73.5, 72.6 (CH_2Ph); 71.2 (C-5); 69.6 (C-6); 60.6 (CH_2Ph). +TOF MS Calcd for $C_{28}H_{29}NO_5Se$ *m/z* [M-Na]+: 560.1117 and 562.1109, found: 560.1122 and 562.1106.



3,4,6-tri-*O*-benzyl-1,2-dideoxy-α-D-glucopyranoside[2,3-d]-2-methyl-1,3-oxazoline (6.36):[32] to a stirred solution of **3.2** in (53.0 mg, 0.12 mmol) in dry CH_3CN (5 mL) was added finely powdered $ZnCl_2$ (1.4 mg, 0.01 mmol) under argon atmosphere, and the mixture was heated at reflux. After 3h, the mixture was cooled to room temperature and H_2O (5 mL) was added and extracted with AcOEt. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (4:1 AcOEt/hexane) afforded **6.36** (55.8 mg, 93% yield) as a syrup. Data for **6.36**: ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.36-7.06 (m, 15H, Ar); 5.78 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 4.68 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.62 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.57 (d, 1H, J_{AB}= 11.6 Hz, CH_2Ph); 4.51 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.39 (d, 1H, J_{AB}= 11.0 Hz, CH_2Ph); 4.38 (d, 1H, J_{AB}= 12.2 Hz,

CH_2Ph); 4.37 (dd, 1H $J_{2,3}$= 7.6 Hz, $J_{2,3}$= 4.4 Hz, Hz, H-2); 3.72 (dd, 1H, $J_{2,3}$= 4.4 Hz, $J_{3,4}$= 6.6 Hz, H-3); 3.67 (dd, 1H, $J_{4,3}$= 6.6 Hz, $J_{4,5}$= 8.6 Hz, H-4); 3.59 (d, 2H, $J_{5,6}$= 3.0 Hz, H-6); 3.47 (dt, 1H, $J_{5,4}$= 8.6 Hz, $J_{5,6}$= 3.0 Hz, H-5); 2.04 (s, 3 H, CH_3). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 168.3 (C=N); 138.2, 138.1, 137.9 (Car); 128.6, 128.5, 128.2, 128.1, 128.0, 127.9, 127.8 (CHar); 93.7 (C-1); 80.4 (C-3); 80.0 (C-2); 74.6 (C-4); 73.8, 73.6, 72.5 (CH_2Ph); 71.6 (C-5); 69.7 (C-6); 14.5 (CH_3).



3,4,6-tri-*O*-benzyl-1,5-anhydro-D-fructose (6.37):[40] to a stirred solution of **3.2** in (55.2 mg, 0.13 mmol) in dry THF (5 mL) was added finely powdered $ZnCl_2$ (1.4 mg, 0.01 mmol) under argon atmosphere, and the mixture was heated at reflux. After 3h, the mixture was cooled to room temperature and H_2O (5 mL) was added and extracted with AcOEt. The combined organic extracts were dried over $MgSO_4$, filtered and concentrated. Flash chromatography on silica gel (from hexane to 4:1 AcOEt/hexane) afforded **6.37** (51.3 mg, 92% yield) as a syrup. Data for **6.37**: ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.41-7.14 (m, 15H, Ar); 5.02 (d, 1H, J_{AB}= 11.2 Hz, CH_2Ph); 4.82 (d, 1H, J_{AB}= 10.8 Hz, CH_2Ph); 4.63 (d, 1H, J_{AB}= 11.2 Hz, CH_2Ph); 4.57 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.52 (d, 1H, J_{AB}= 10.8 Hz, CH_2Ph); 4.51 (d, 1H, J_{AB}= 12.2 Hz, CH_2Ph); 4.18 (d, 1H, $J_{3,4}$= 9.0 Hz, H-3); 4.15 (d, 1H, $J_{A'B'}$= 14.6 Hz, H-1); 3.96 (d, 1H, $J_{B'A'}$= 14.6 Hz, H-1); 3.89 (t, 1H, $J_{4,3}$= $J_{4,5}$= 9.0 Hz, H-4); 3.74 (ddd, $J_{5,4}$= 9.0 Hz, $J_{5,6}$= 4.8 Hz, $J_{5,6'}$= 2.0 Hz, H-5); 3.73 (dd, 1H, $J_{6',5}$= 2.0 Hz, $J_{6',6}$= 11.0 Hz, H-6'); 3.67 (dd, 1H, $J_{6,5}$= 2.0 Hz, $J_{6,'6}$= 11.0 Hz, H-6'). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 203.3 (C-2); 137.9, 137.8, 137.6 (Car); 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8 (CHar); 86.2 (C-3); 79.4 (C-4); 75.1, 74.2 (CH_2Ph); 73.8 (C-1); 73.3 (CH_2Ph); 69.1 (C-6).

6.4.4. Crystallographic data of **6.30**

Table 6.7. Crystal data and structure refinement

Crystallographic data	
Identification code	compound **6.30**
Empirical formula	$C_{13}H_{17}NO_8S$
Formula weight	347.35
Temperature	100(2) K
Wavelength	0.71073 Å

Continuation of Table 6.7

Crystallographic data		
Crystal system	Orthorhombic	
Space group	$P2_12_12_1$	
Unit cell dimensions	a = 8.1051(8) Å	α = 90°
	b = 9.4473(11) Å	β = 90°
	c = 21.079(3) Å	γ = 90°
Volume	1614.1(3) $Å^3$	
Z	4	
Density (calculated)	1.429 Mg/m^3	
Absorption coefficient	0.241 mm^{-1}	
F(000)	728	
Crystal size	0.28 x 0.13 x 0.12 mm^3	
Theta range for data collection	1.93 to 26.83°	
Index ranges	-9<=h<=9, -11<=k<=12, -26<=l<=26	
Reflections collected	18510	
Independent reflections	3424 [R(int) = 0.0852]	
Completeness to theta = 26.83°	98.9%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.9711 and 0.7105	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3424 / 0 / 214	
Goodness-of-fit on F^2	0.962	
Final R indices [I>2sigma(I)]	R1 = 0.0428, wR2 = 0.0858	
R indices (all data)	R1 = 0.0582, wR2 = 0.0908	
Absolute structure parameter	0.00(9)	
Largest diff. peak and hole	0.252 and -0.262 e.$Å^{-3}$	

Table 6.8. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3; U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor)

	x	y	z	U(eq)
S(1)	-727(1)	4754(1)	1112(1)	27(1)
C(2)	-97(3)	3637(3)	1658(1)	19(1)
O(3)	1463(2)	3694(2)	1885(1)	20(1)
C(4)	1694(3)	2614(3)	2367(1)	18(1)
C(5)	3262(3)	1812(3)	2193(1)	19(1)
C(6)	3082(3)	815(3)	1626(1)	19(1)
C(7)	1286(3)	391(3)	1489(1)	18(1)
O(8)	399(2)	344(2)	2074(1)	18(1)

Continuation of Table 6.8

	x	y	z	U(eq)
C(9)	126(3)	1703(3)	2338(1)	20(1)
N(10)	-910(2)	2586(2)	1942(1)	19(1)
O(11)	3710(2)	945(2)	2726(1)	19(1)
C(12)	4875(3)	1453(3)	3112(1)	27(1)
O(13)	5477(3)	2594(3)	3041(1)	70(1)
C(14)	5270(3)	455(3)	3634(1)	26(1)
O(21)	3646(2)	1484(2)	1043(1)	20(1)
C(22)	5305(3)	1608(3)	980(1)	21(1)
O(23)	6242(2)	1237(2)	1393(1)	26(1)
C(24)	5771(3)	2249(3)	365(1)	28(1)
C(30)	1209(3)	-1084(3)	1213(1)	21(1)
O(31)	-474(2)	-1518(2)	1103(1)	22(1)
C(32)	-1148(3)	-1125(3)	550(1)	25(1)
O(33)	-441(2)	-415(2)	165(1)	40(1)
C(34)	-2860(3)	-1711(3)	478(1)	31(1)

Table 6.9. Bond lengths [Å]

Bond lengths (Å)			
S(1)-C(2)	1.643(3)	C(12)-O(13)	1.192(3)
C(2)-N(10)	1.334(3)	C(12)-C(14)	1.484(4)
C(2)-O(3)	1.353(3)	C(14)-H(14A)	0.9800
O(3)-C(4)	1.452(3)	C(14)-H(14B)	0.9800
C(4)-C(5)	1.525(3)	C(14)-H(14C)	0.9800
C(4)-C(9)	1.536(3)	O(21)-C(22)	1.357(3)
C(4)-H(4)	10.000	C(22)-O(23)	1.206(3)
C(5)-O(11)	1.436(3)	C(22)-C(24)	1.481(4)
C(5)-C(6)	1.529(3)	C(24)-H(24A)	0.9800
C(5)-H(5)	10.000	C(24)-H(24B)	0.9800
C(6)-O(21)	1.456(3)	C(24)-H(24C)	0.9800
C(6)-C(7)	1.537(3)	C(30)-O(31)	1.443(3)
C(6)-H(6)	10.000	C(30)-H(30A)	0.9900
C(7)-O(8)	1.428(3)	C(30)-H(30B)	0.9900
C(7)-C(30)	1.511(3)	O(31)-C(32)	1.340(3)
C(7)-H(7)	10.000	C(32)-O(33)	1.199(3)
O(8)-C(9)	1.417(3)	C(32)-C(34)	1.502(4)
C(9)-N(10)	1.449(3)	C(34)-H(34A)	0.9800
C(9)-H(9)	10.000	C(34)-H(34B)	0.9800

Continuation of Table 6.9

Bond lengths (Å)			
N(10)-H(10)	0.93(3)	C(34)-H(34C)	0.9800
O(11)-C(12)	1.335(3)		

Table 6.10. Bond angles [°]

Bond angles (º)			
N(10)-C(2)-O(3)	109.4(2)	C(9)-N(10)-H(10)	121.6(17)
N(10)-C(2)-S(1)	129.74(19)	C(12)-O(11)-C(5)	116.8(2)
O(3)-C(2)-S(1)	120.85(19)	O(13)-C(12)-O(11)	122.5(3)
C(2)-O(3)-C(4)	109.89(18)	O(13)-C(12)-C(14)	125.4(3)
O(3)-C(4)-C(5)	106.8(2)	O(11)-C(12)-C(14)	112.1(2)
O(3)-C(4)-C(9)	105.02(19)	C(12)-C(14)-H(14A)	109.5
C(5)-C(4)-C(9)	113.7(2)	C(12)-C(14)-H(14B)	109.5
O(3)-C(4)-H(4)	110.4	H(14A)-C(14)-H(14B)	109.5
C(5)-C(4)-H(4)	110.4	C(12)-C(14)-H(14C)	109.5
C(9)-C(4)-H(4)	110.4	H(14A)-C(14)-H(14C)	109.5
O(11)-C(5)-C(4)	107.8(2)	H(14B)-C(14)-H(14C)	109.5
O(11)-C(5)-C(6)	106.5(2)	C(22)-O(21)-C(6)	115.52(19)
C(4)-C(5)-C(6)	114.5(2)	O(23)-C(22)-O(21)	121.9(2)
O(11)-C(5)-H(5)	109.3	O(23)-C(22)-C(24)	126.2(2)
C(4)-C(5)-H(5)	109.3	O(21)-C(22)-C(24)	111.9(2)
C(6)-C(5)-H(5)	109.3	C(22)-C(24)-H(24A)	109.5
O(21)-C(6)-C(5)	111.3(2)	C(22)-C(24)-H(24B)	109.5
O(21)-C(6)-C(7)	104.60(19)	H(24A)-C(24)-H(24B)	109.5
C(5)-C(6)-C(7)	113.5(2)	C(22)-C(24)-H(24C)	109.5
O(21)-C(6)-H(6)	109.1	H(24A)-C(24)-H(24C)	109.5
C(5)-C(6)-H(6)	109.1	H(24B)-C(24)-H(24C)	109.5
C(7)-C(6)-H(6)	109.1	O(31)-C(30)-C(7)	111.27(19)
O(8)-C(7)-C(30)	106.5(2)	O(31)-C(30)-H(30A)	109.4
O(8)-C(7)-C(6)	108.8(2)	C(7)-C(30)-H(30A)	109.4
C(30)-C(7)-C(6)	110.6(2)	O(31)-C(30)-H(30B)	109.4
O(8)-C(7)-H(7)	110.3	C(7)-C(30)-H(30B)	109.4
C(30)-C(7)-H(7)	110.3	H(30A)-C(30)-H(30B)	108.0
C(6)-C(7)-H(7)	110.3	C(32)-O(31)-C(30)	116.5(2)
C(9)-O(8)-C(7)	112.96(18)	O(33)-C(32)-O(31)	123.3(2)
O(8)-C(9)-N(10)	112.7(2)	O(33)-C(32)-C(34)	125.4(3)
O(8)-C(9)-C(4)	113.23(19)	O(31)-C(32)-C(34)	111.3(2)
N(10)-C(9)-C(4)	100.4(2)	C(32)-C(34)-H(34A)	109.5

Continuation of Table 6.10

Bond angles (º)			
O(8)-C(9)-H(9)	110.1	C(32)-C(34)-H(34B)	109.5
N(10)-C(9)-H(9)	110.1	H(34A)-C(34)-H(34B)	109.5
C(4)-C(9)-H(9)	110.1	C(32)-C(34)-H(34C)	109.5
C(2)-N(10)-C(9)	113.62(19)	H(34A)-C(34)-H(34C)	109.5
C(2)-N(10)-H(10)	118.5(18)	H(34B)-C(34)-H(34C)	109.5

Table 6.11. Anisotropic displacement parameters ($Å^2$x 10^3; the anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2}U^{11} + ... + 2 h k a^* b^* U^{12}]$)

	U^{11}	**U^{22}**	**U^{33}**	**U^{23}**	**U^{13}**	**U^{12}**	**U^{11}**
S(1)	31(1)	23(1)	27(1)	3(1)	0(1)	8(1)	S(1)
C(2)	20(1)	18(1)	19(1)	-4(1)	2(1)	3(1)	C(2)
O(3)	16(1)	18(1)	24(1)	4(1)	-1(1)	0(1)	O(3)
C(4)	20(1)	15(1)	19(2)	2(1)	-4(1)	-1(1)	C(4)
C(5)	16(1)	21(1)	19(2)	1(1)	-2(1)	-2(1)	C(5)
C(6)	21(1)	17(1)	17(2)	0(1)	-1(1)	-1(1)	C(6)
C(7)	17(1)	16(1)	19(1)	0(1)	0(1)	0(1)	C(7)
O(8)	19(1)	13(1)	22(1)	1(1)	2(1)	-1(1)	O(8)
C(9)	19(1)	20(1)	21(2)	-2(1)	3(1)	0(1)	C(9)
N(10)	14(1)	20(1)	23(1)	1(1)	-4(1)	1(1)	N(10)
O(11)	17(1)	19(1)	21(1)	3(1)	-5(1)	-2(1)	O(11)
C(12)	26(1)	27(2)	28(2)	-3(1)	-8(1)	0(1)	C(12)
O(13)	98(2)	41(2)	73(2)	26(1)	-61(2)	-41(2)	O(13)
C(14)	24(1)	31(2)	22(2)	-2(1)	-5(1)	4(1)	C(14)
O(21)	15(1)	23(1)	20(1)	0(1)	1(1)	0(1)	O(21)
C(22)	19(1)	17(1)	26(2)	-4(1)	0(1)	-1(1)	C(22)
O(23)	17(1)	31(1)	30(1)	-1(1)	-4(1)	0(1)	O(23)
C(24)	25(1)	31(2)	26(2)	-2(1)	3(1)	-5(1)	C(24)
C(30)	18(1)	18(1)	27(2)	-1(1)	-4(1)	0(1)	C(30)
O(31)	22(1)	19(1)	24(1)	1(1)	-3(1)	-4(1)	O(31)
C(32)	31(2)	22(2)	22(2)	-6(1)	0(1)	-3(1)	C(32)
O(33)	40(1)	54(1)	27(1)	9(1)	-4(1)	-14(1)	O(33)
C(34)	26(1)	27(2)	39(2)	-3(2)	-6(1)	-3(1)	C(34)

Table 6.12. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3)

	x	**y**	**z**	**U (eq)**
H(4)	1809	3059	2795	22
H(5)	4168	2504	2107	23

Continuation of Table 6.12

	x	y	z	U (eq)
H(6)	3748	-59	1704	22
H(7)	764	1085	1193	21
H(9)	-368	1612	2771	24
H(10)	-1860(30)	2230(30)	1748(13)	23
H(14A)	6210	-136	3511	38
H(14B)	4311	-149	3718	38
H(14C)	5549	990	4018	38
H(24A)	6934	2533	377	41
H(24B)	5082	3083	285	41
H(24C)	5606	1557	24	41
H(30A)	1825	-1106	807	25
H(30B)	1744	-1757	1509	25
H(34A)	-3379	-1299	101	46
H(34B)	-3514	-1475	854	46
H(34C)	-2803	-2742	431	46
H(4)	1809	3059	2795	22
H(5)	4168	2504	2107	23
H(6)	3748	-59	1704	22
H(7)	764	1085	1193	21
H(9)	-368	1612	2771	24
H(10)	-1860(30)	2230(30)	1748(13)	23

Table 6.13. Torsion angles [°]

Torsion angles (º)			
N(10)-C(2)-O(3)-C(4)	-1.2(3)	C(5)-C(4)-C(9)-O(8)	-8.0(3)
S(1)-C(2)-O(3)-C(4)	179.07(18)	O(3)-C(4)-C(9)-N(10)	-12.0(2)
C(2)-O(3)-C(4)-C(5)	129.8(2)	C(5)-C(4)-C(9)-N(10)	-128.3(2)
C(2)-O(3)-C(4)-C(9)	8.8(3)	O(3)-C(2)-N(10)-C(9)	-7.7(3)
O(3)-C(4)-C(5)-O(11)	168.08(18)	S(1)-C(2)-N(10)-C(9)	172.0(2)
C(9)-C(4)-C(5)-O(11)	-76.6(3)	O(8)-C(9)-N(10)-C(2)	-108.4(2)
O(3)-C(4)-C(5)-C(6)	-73.6(2)	C(4)-C(9)-N(10)-C(2)	12.4(3)
C(9)-C(4)-C(5)-C(6)	41.7(3)	C(4)-C(5)-O(11)-C(12)	-98.6(2)
O(11)-C(5)-C(6)-O(21)	-144.26(18)	C(6)-C(5)-O(11)-C(12)	138.1(2)
C(4)-C(5)-C(6)-O(21)	96.7(2)	C(5)-O(11)-C(12)-O(13)	3.2(4)
O(11)-C(5)-C(6)-C(7)	98.1(2)	C(5)-O(11)-C(12)-C(14)	-178.4(2)
C(4)-C(5)-C(6)-C(7)	-21.0(3)	C(5)-C(6)-O(21)-C(22)	74.2(3)

Continuation of Table 6.13

Torsion angles (º)			
O(21)-C(6)-C(7)-O(8)	-153.41(19)	C(7)-C(6)-O(21)-C(22)	-162.9(2)
C(5)-C(6)-C(7)-O(8)	-32.0(3)	C(6)-O(21)-C(22)-O(23)	-2.8(4)
O(21)-C(6)-C(7)-C(30)	89.9(2)	C(6)-O(21)-C(22)-C(24)	177.9(2)
C(5)-C(6)-C(7)-C(30)	-148.6(2)	O(8)-C(7)-C(30)-O(31)	59.8(3)
C(30)-C(7)-O(8)-C(9)	-170.07(18)	C(6)-C(7)-C(30)-O(31)	177.9(2)
C(6)-C(7)-O(8)-C(9)	70.7(2)	C(7)-C(30)-O(31)-C(32)	85.5(3)
C(7)-O(8)-C(9)-N(10)	63.9(2)	C(30)-O(31)-C(32)-O(33)	-2.1(4)
C(7)-O(8)-C(9)-C(4)	-49.2(3)	C(30)-O(31)-C(32)-C(34)	176.7(2)
O(3)-C(4)-C(9)-O(8)	108.4(2)		

6.5. References

1. a) Zemplén, G.; Gerecs, A.; Rados, M. *Ber. Dtsch. Chem. Ges.* **1936**, *39*, 748-754. b) Zemplén, G.; Gerecs, A.; Illés, E. *Ber. Dtsch. Chem. Ges.* **1938**, *71*, 590-596. c) Gosselin, G.; Bergogne, M. C.; Rudder, J.; Clerq, E.; Imbach, J. L. *J. Med. Chem.* **1986**, *29*, 203-213. d) Ranganathan, R. Tetrahedron Lett. **1975**, *13*, 1185-1188.
2. Sandrina Isabel Ribeiro Martins da Silva, Doctoral Dissertation, Universidade de Lisboa and Université d'Orléans, **2008**.
3. a) Mota, J. F.; Blanco, J. L. J.; Ortiz Mellet, C.; García Fernández, J. M. *Carbohydr. Res.* **1994**, *257*, 127-135. b) Bolanos, J. G. F.; López, A. B.; Mota, J. F. *Carbohydr. Res.* **1990**, *199*, 239-242. c) Grouiller, A.; Mackenzie, G.; Najib, B.; Shaw, G.; Ewig, D. *J. Chem. Soc., Chem. Commun.* **1988**, 671-672.
4. Lichtenthaler, F. W.; Klotz, J.; Flath, F.-J. *Liebigs Ann.* **1995**, 2069-2080.
5. Simão, A. C.; Rousseau, J. ; Silva, S. ; Rauter, A. P.; Tatibouët, A. ; Rollin, P. *Thionocarbamates on carbohydrate scaffolds—from synthesis to bioactivity* (in Carbohydrate Chemistry. Chemical and Biological Approaches, 35), RSC Publishing, Dorchester, **2009**, *35*, 127-172.
6. Tatibouët, A.; Lawrence, S.; Rollin, P.; Holman, G. D. *Synlett* **2004**, 1945-1948.
7. Silva, S.; Simão, A. C.; Tatibouët, A.; Rollin, P.; Rauter, A. P. *Tetrahedron Lett.* **2008**, *49*, 682-686.
8. a) Fernández-Bolaños, J. G.; Zafra, E.; Robina, I.; Fuentes J. *Carbohydr. Lett.* **1999**, *3*, 239-246. b) Maya, I.; López, O.; Fernández-Bolaños, J. G.; Robina, I.; Fuentes, J. *Tetrahedron Lett.* **2001**, *42*, 5413-5416. c) López, O.; Maya, I.; Fuentes, J.; Fernández-Bolaños, J. G. *Tetrahedron* **2004**, *60*, 61-72.

9. López, O.; Zafra, E.; Maya, I; Fuentes, J.; Diánez, M. J.; Estrada, M. D.; Pérez-Garrido, S.; Fernández-Bolaños, J. G. *Tetrahedron* **2008**, *64*, 11789.
10. a) Díaz Pérez, V. M.; García Moreno, I.; Ortiz Mellet, C.; Fuentes, J.; Díaz Arribas, J. C.; Cañada, F. J.; García Fernández, J. M. *J. Org. Chem.* **2000**, *65*, 136-143. b) Jiménez Blanco, J. L.; Díaz Pérez, V. M.; Ortiz Mellet, C.; Fuentes, J.; García Fernández, J. M.; Díaz Arribas, J. C.; Cañada, F. J. *J. Chem. Soc., Chem. Commun.* **1997**, 1960-1970.
11. García-Moreno, M. I.; Rodríguez-Lucena, D.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2004**, *69*, 3578–3581.
12. a) García Fernández, J. M.; Ortiz Mellet, C.; Fuentes, J. *Tetrahedron Lett.* **1992**, *33*, 3931-3934. b) García Fernández, J. M.; Ortiz Mellet, C.; Fuentes J. *J. Org. Chem.* **1993**, *58*, 5192-5199.
13. Beaupère, D.; El Meslouti, A.; Lelièvre, P.; Uzan, R. *Tetrahedron Lett.* **1995**, *36*, 5347-5348.
14. Ávalos, M.; Babiano, R.; García-Verdugo, C.; Jiménez, J. L.; Palacios, J. C. *Tetrahedron Lett.* **1990**, *31*, 2467-2470.
15. a) Daley, L.; Roger, P.; Monneret, C. *J. Carbohydr. Chem.* **1997**, *16*, 25-48. b) Zehl, A.; Cech, D. *Liebigs. Ann. Chem.* **1997**, 595-600
16. Cheshev, P.; Marra, A.; Dondoni, A. *Carbohydr. Res.* **2006**, *341*, 2714-2716.
17. Dachlauer, K.; Jackel, L. *Chem Abstr.* **1936***, 30*, 7122.
18. Sartori, P.; Weidenbruch, M. *Angew. Chem.* **1964**, *76*, 376-377.
19. Iranpoor, N.; Zeynizadeh, B. *Synth. Commun.* **1998**, *28*, 3913-3918.
20. a) Ávalos, M.; Babiano, R.; Cintas, P.; Hursthouse, M. B.; Jiménez, J. L.; Light, M. E., Palacios, J. C.; Perez, E. M. S. *Eur. J. Org. Chem.* **2006**, *3*, 657-671. b) Santoyo-González, F.; García-Calvo-Flores, F.; Isac-García, J.; Hernández-Mateo, F.; García-Mendoza, P.; Robles-Díaz, R. *Tetrahedron* **1994**, *50*, 2877-2894. c) Nashed, M. A.; Slife, C. W.; Kiso, M.; Anderson, L.; *Carbohydr. Res.* **1980**, *82*, 237-252.
21. a) López, O.; Zafra, E.; Maya, I.; Fuentes, J.; Diánez, M. J.; Estrada, M. D.; Pérez-Garrido, S.; Fernández-Bolaños, J. G. *Tetrahedron* **2008**, *64*, 11789-11796. b) Ávalos, M.; Babiano, R.; Cintas, P.; Hursthouse, M. B.; Jiménez, J. L.; Light, M. E., Palacios, J. C.; Perez, E. M. S. *Eur. J. Org. Chem.* **2006**, *3*, 657-671. c) Isac-García, J.; Hernández-Mateo, F.; Calvo-Flores, F. G.; Santoyo-González, F. *J. Org. Chem.* **2004**, *69*, 202-205. d) Rück-Braun, K.; Stamm, A.; Engel, S.; Kunze, H. *J. Org. Chem.* **1997**, *62*, 967. e) Santoyo-González, F.; García-Calvo-Flores, F.; Isac-García, J.; Hernández-Mateo, F.; García-Mendoza, P.; Robles-Díaz, R. *Tetrahedron* **1994**, *50*, 2877-2894. f) Kovács, J.; Pintér, I.; Tóth, G.; Györgydeák, Z.; Köll, P. *Carbohydr. Res.* **1993**, *239*, 95-106.
22. a) López, O.; Maya, I.; Fuentes, J.; Fernández-Bolaños, J. G. *Tetrahedron* **2004**, *60*, 61-72. b) Kovács, J.; Pintér, I.; Messmer, A.; Tóth, G. *Carbohydr. Res.* **1985**, *141*, 57-65.
23. a) Repetto, E.; Manzano, V. E.; Uhrig, M. L.; Varela, O. *J. Org. Chem.* **2012**, *77*, 253-265. b) Gurjar, M. K.; Hough, L.; Richardson. A. C.; Sincharoenkul, L. V. *Carbohydr.*

Res. **1986**, *150*, 53-61. c) Boigegrain, R.-A.; Gross, B. *Carbohydr. Res.* **1975**, *41*, 135-142.

24. Sander, M. *Chem. Rev.* **1966**, *66*, 297-339.
25. a) Rainier, J. D.; Cox, J. M. *Org. Lett.* **2000**, *2*, 2707-2709. b) Wipf, P.; Pierce, J. G.; Zhuang, N. *Org. Lett.* **2005**, *7*, 483-485. c) Xue, S.; Han, K.-Z.; He, L.; Guo, Q.-X. *Synlett* **2003**, 870-872.
26. a) Arif, J. M; Sawant, S. S.; El-Sayed, K. A.; Kunhi, M.; Subramanian, M. P.; Siddiqui, Y. M.; Youssef, D. A.; Al-Hussain, K.; Al-Ahdal, M. N; Al-Khodairy, F. *J. Nat. Med.* **2007**, *61*, 154-158. b) El-Sayed, K. A.; Laphookhiero, S.; Yousaf, M.; Prestrifge, J. A.; Shirode, A. B.; Wali, V. B.; Sylvester, P. W. *J. Nat. Prod.* **2008**, *71*, 1117-122.
27. Goo Soo, L.; Hye Kung, M.; Bong Young, C.; *Tetrahedron Lett.* **1999**, *40*, 543-544.
28. Kóvacs, J.; Pintér, I.; Köll, P. *Carbohydr. Res.* **1995**, *272*, 255-262.
29. a) Cremer, D.; Pople, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 1354-1358. b) Jeffrey, G. A.; Yates, J. H. *Carbohydr. Res.* **1979**, *74*, 319-322.
30. Lellouche, J.-P.; Koeller, S. *J. Org. Chem.* **2001** , *66*, 693-696.
31. Asanuma, Y.; Fujiwara, S.; Shinike, T.; Kambe, N. *J Org. Chem.* **2004**, *69*, 4845-4848.
32. Gordon, D. M.; Danishefsky, S. J. *J. Org. Chem.* **1991** , *56*, 3713 - 3715.
33. a) Blackett, B. N.; Coxon, l M.; Hartshorn, M. P.; Richards, K. E. *Aust. J. Chem.* **1970**, *23*, 2077-2084. b) Blackett, B. N.; Coxon, l M.; Hartshorn, M. P.; Jackson, B. L. J.; Muir, C. N. *Tetrahedron **1969**, 25*,1479-1487.
34. a) Dronskowski, R.; Liu, X.-H. *Acta Crystal. C.* **2003**, *59*, 243-245. b) Belsky, A. J.; Li T.-J.; Brill T.B. *J. Supercrit. Fluids* **1997**, *10*, 201-208 and references therein.
35. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed., Pergamon Press, Oxford, **1989**.
36. Sartori, P.; Weidenbruch, M. *Angew. Chem.* **1964**, *76*, 376-377.
37. Breugst, M.; Tokuyasu, T.; Mayr, H. *J. Org. Chem* . **2010**, *75*, 5250-5258.
38. Klapotke, T. M.; Krumm, B.; Mayer, P.; Piotroski, H.; Schwab, I.; Vogt, M. *Eur. J. Inorg. Chem.* **2002**, 2701-2709.
39. Hegarty, A. F.; Hegarty C. N.; Scott, F. L. *J. Chem. Soc. Perkin Trans 2* **1973**, 2054-2060.
40. Brehm, M.; Goeckel, V. H.; Jarglis, P.; Lichtenthaler, F. W. *Tetrahedron: Asym.* **2008**, *19*, 358-373.

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

CHAPTER 7

ENZYMATIC EVALUATION OF CARBOHYDRATE-DERIVED 1,3-OXAZOLIDINE-2-THIONES

UNIVERSITAT ROVIRA I VIRGILI
CARBOHYDRATE MANIPULATIONS TOWARDS HIGH-MANNOSE OLIGOSACCHARIDES
Javier Castilla López
Dipòsit Legal: T.1214-2012

7.1. Background

7.1.1. Bioapplications of 1,3-oxazolidine-2-thiones

Carbohydrate-fused thionocarbamates form a diverse family of compounds that exhibit interesting biological effects,[1] including fructose transport inhibition,[2] as well as antitumor[3] and clinically useful antibacterial activity.[4] Since the heterocyclic moiety is directly involved in the binding process in the enzyme pocket,[5] much effort in recent years has focused on the synthesis of new and more potent analogous compounds.[6] In addition, these OAT saccharidic derivatives can be useful as chiral intermediates for asymmetric syntheses[7] as well as potential nucleoside precursors.[8]
In this context, there are two main families of compounds which have been chemically synthesized using sugar-derived 1,3-oxazolidine-2-thiones: nucleoside analogues (showing antiviral and anticancer activity)[9] and enzyme-inhibitor iminosugars.[7a,b]
In summary, saccharidic OAT derivatives have proved to be quite attractive due to the wide range of potential biological application. However, these applications still remain rather unexploited and they need to be further developed.

7.1.2. Glycoside hydrolase inhibitors: current relevance of imino sugars

Given the broad range of biological and pathological processes where glycosidases are involved (from sugar catabolism to the biosynthesis of complex oligosaccharides in glycoproteins and glycolipids), specific inhibitors of these enzymes bear a strong therapeutic potential for the development of new pharmaceuticals. Examples include the treatment of viral infections (such as human immunodeficiency virus (HIV), human hepatitis C (HCV) or dengue virus),[10] cancer,[11] diabetes,[12] tuberculosis,[13] and lysosomal storage diseases (LSDs).[14] This finding has strongly stimulated research in glycobiology, namely glycomics.[15] With few exceptions,[16] the glycosidase inhibitors mimic the glyconic moiety of the putative substrate, which is shared within a series of isoenzymes and enzymes acting on anomeric substrates.

Figure 7.1 illustrates a recompilation of sugar-derived inhibitors that can be regarded as stereochemical mimic of D-glucose (e.g. the piperidine-type iminosugar 1-deoxynojirimycin, DNJ).[17] This observation is consistent with the behaviour of all these compounds as potent inhibitors of mammalian and plant α- and β-glucosidases.

The indolizidine-type iminosugar (+)-castanospermine (CS), with an analogous hydroxylation profile, exhibits higher enzyme specificity as compared with monocyclic analog DNJ. This phenomenom is ascribed to the conformational restriction imposed by the rigid bicyclic structure (Figure 7.1).[18] In any case, the clinical application of both compounds represents serious drawbacks because they can simultaneously inhibit several human α- as well as β-glucosidases.[19]

In connection with the design of more fine-tuned inhibitors, numerous syntheses of DNJ and CS derivatives have been reported (Figure 7.1).[20]

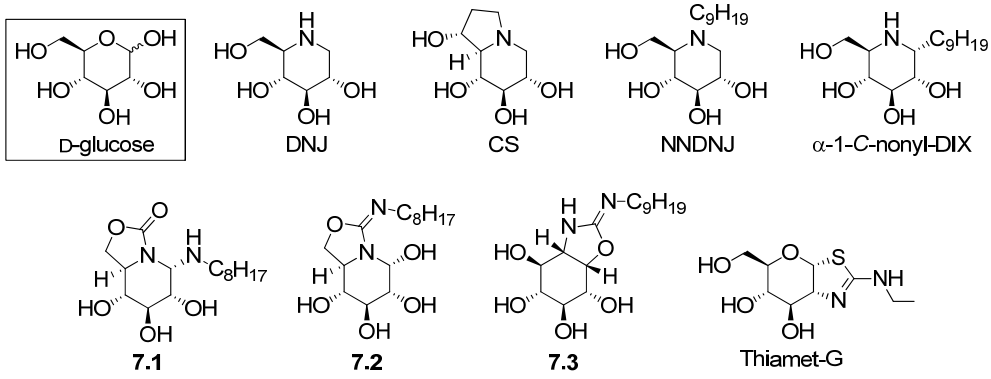


Figure 7.1. Recopilation of sugar inhibitors for α- and β-glucosidases exhibiting a structural resemblance to D-glucose

Incorporation of alkyl substituents at the nitrogen atom or at its vicinity in monocyclic iminosugar frameworks (e.g. as in *N*-(*n*-nonyl)-1-deoxynojirimycin (NNDNJ)[21] or α-1-*C*-nonyl-1-deoxynojirimycin (α-1-*C*-nonyl-DIX)[22]) has been shown to improve the affinity towards certain glycosidases.

Further chemical and structural evidence indicated that once the azaheterocycle ring occupies the glyconic site of the enzyme, the alkyl substituent can favorably interact with other regions of the protein.[23] This result strongly suggests that exploiting non-glyconic interactions has the potential to become a general strategy to elaborate selective glycosidase inhibitors. Implementing molecular diversity-oriented strategies in conformationally locked bicyclic glycomimetics is particularly attractive. Several polyhydroxylated bicyclic cores armed with anchoring functionalities compatible with library generation schemes have been proposed, among which the so-called sp^2-iminosugars have proven particularly useful (Figure 7.1).[24]

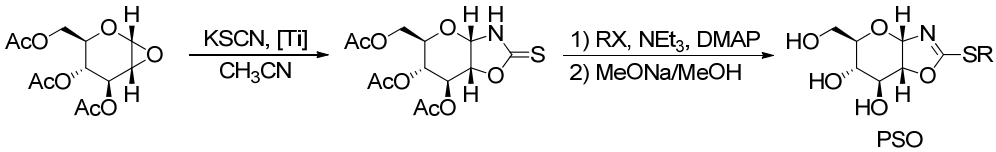
This family of sugar mimics incorporates a carbamate group in their structure that facilitates the installation of substituents at either the pseudoanomeric position (e.g. in the carbamate-type bicyclic nojirimycin derivative **7.1,** Figure 7.1)[25] or at an exocyclic nitrogen (e.g. in the isourea-type bicyclic nojirimycin derivative **7.2**, Figure 7.1).[26] Total discrimination between α- and β-glucosidase enzymes, and even between closely related α-glucosidase isoenzymes, could be achieved in this manner. This discrimination can be translated into the development of promising drug candidates for the treatment of breast cancer[25b] and Gaucher disease (the LSD with the highest prevalence).[27] In the first case, the biological activity was ascribed to selective inhibition of the neutral α-glucosidases at the endoplasmic reticulum (ER). In the second case, binding of the glycomimetic to the active site of mutant β-glucocerebrosidase (GCase) in the ER restored trafficking to the lysosome, acting as a pharmacological chaperone. Similarly, *N'*-alkylated bicyclic isoureas derived from aminocyclitol scaffolds (e.g. **7.3**, Figure 7.1) turned out to behave as very selective GCase inhibitors with strong pharmacological chaperone potential.[28]

All the examples mentioned are compiled in Figure 7.1. It illustrates the suitability of incorporating substituents susceptible of participating in non-glyconic interactions to achieve strong and selective glycosidase inhibition. These bicyclic glycomimetics also demonstrate that the presence of a basic amine-type nitrogen in the six-membered ring is not a requisite for strong enzyme affinity. Actually, protonation under physiological conditions is probably responsible for the broad-range activity of iminosugars rather than its low selectivity.[29]

To account for the glycone specificity of a target glycosidase, the pyranose ring present in the natural glycoside substrate could be directly incorporated into the

molecular design of bicyclic competitive inhibitors. This would greatly simplify the synthetic scheme. In this context, Vocadlo and coworkers[30] recently developed Thiamet-G, a fused pyranose-thiazoline derivative which behaves a potent inhibitor for the hydrolysis of *O*-linked *N*-acetyl-2-amino-2-deoxy-β-D-glucopyranosides. This inhibitor is being investigated as a potential therapeutic target that could hinder progression of Alzheimer's disease.[31]

To further test this hypothesis, a new family of glycomimetics bearing a *cis*-1,2-fused pyranose—2-alkylsulfanyl-1,3-oxazoline structure (PSO) has been prepared taking into account the unexpected reactivity of 1,2-anhydro-carbohydrates, previously explained in Chapter 6 (Scheme 7.1).



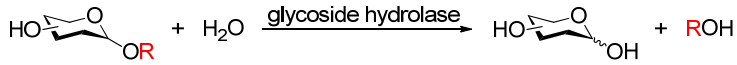
Scheme 7.1. Preparation of a library of PSO derivatives with potential inhibitor effect

PSO derivatives share with compound **7.1** the *N*-glycoside-type character. Actually, they can be formally considered as conformationally-locked *N*-glycoside derivatives, which should warrant chemical and enzymatic stability and, at the same time, impart selectivity.[32] Similarly to the imine-type nitrogen-bearing compounds **7.2** and **7.3**, PSO is also very well suited for the incorporation of a broad battery of substituents on the exocyclic heteroatom. In connection with this purpose, the assessment of the affinity and selectivity of the final compounds against a panel of commercial glycosidases have been studied. Moreover, the evaluation of a selected candidate as a pharmacological chaperone in human Gaucher disease fibroblasts will be discussed.

7.1.3. Glycoside hydrolases: biochemical principles underlying enzyme inhibition

Glycoside hydrolases, also known as glycosidases and sometimes referred as glycosyl hydrolases, are important enzymes in a number of essential biological processes.[33] Their function or dysfunction has been therefore implicated in a number of different diseases.[34] In this context, inhibition of these glycosidases can have profound effects in a variety of processes, including viral infection, cancer, and genetic disorders.[35] Moreover, they form a widespread group of enzymes which hydrolyse the glycosidic linkage of glycosides (Scheme 7.2). These complex proteins

can be classified in many different ways, where the Carbohydrate-Active Enzyme (CAZy) classification is widely used by the scientific community.[36] This categorization is based on experimentally characterized proteins, reflecting the structural features of these enzymes. When a group of families possesses significant similarity in the tertiary structure, the catalytic residues, and the mechanism, they can be grouped in clans.

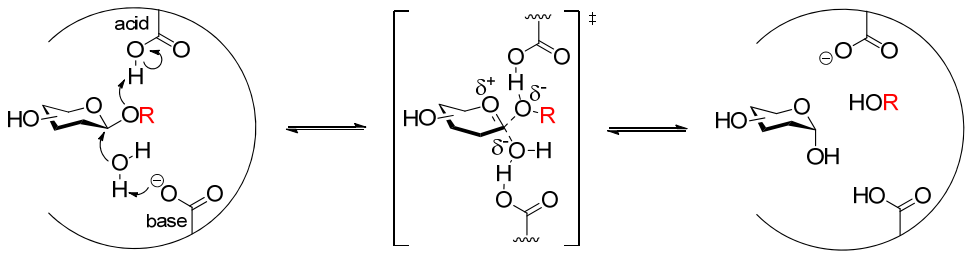


Scheme 7.2. Glycosidase-catalyzed hydrolysis of the glycosidic bond

In most cases, hydrolysis of the glycosidic bond is catalyzed by two residues of the enzyme: a general acid (proton donor) and a base/nucleophile.[37] The reaction usually proceeds through transition states with significant oxocarbenium ion-like character.[38] Hence, from all compounds that are known to inhibit the action of glycoside hydrolases, the ones that mimic this glycosidase "oxocarbenium-ion-like" transition state are actually notable.[29a, 39]

Depending on the spatial position of the amino acid side chains, the hydrolysis can occur via two pathways: retention or inversion of the anomeric configuration.

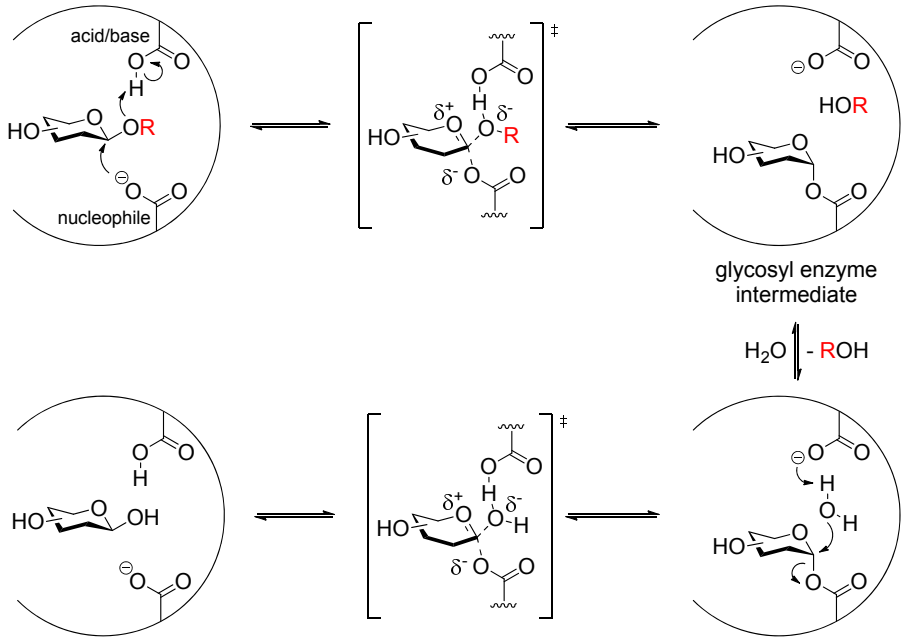
On the one hand, inverting enzymes achieve the hydrolysis with inversion of configuration via a single-displacement mechanism passing through oxocarbenium ion-like transition state (Scheme 7.3).



Scheme 7.3. Inverting mechanism for a β-glycosidase

On the other hand, retaining glycosidases operate via double-displacement Koshland mechanism, involving a covalent glycosyl-enzyme intermediate, as shown in Scheme 7.4. The reaction starts with the nucleophilic attack at the anomeric position of one enzyme-borne carboxylate. This residue displaces the aglycon and leads to the covalent α-glycosyl enzyme intermediate. This complex is subsequently hydrolized

by water and gives thus the hydrolyzed product with retained configuration at the anomeric centre and regenerating the enzyme.



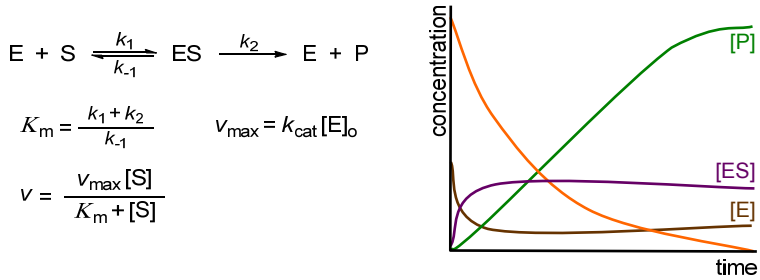
Scheme 7.4. Double-displacement Koshland retaining mechanism for a β-glycosidase

Both steps proceed through acid/base assistance provided by a carboxylic acid. Moreover, each displacement involves a transition state with significant oxocarbenium ion-like character. As said before, compounds with chemical structures resembling these transition states can inhibit the enzyme.

Like all other catalysts, enzymes are characterized by increasing the reaction rates not neither without altering the chemical equilibrium between reactants and products, nor without being consumed by the reaction itself. In a typical enzyme-catalysed reaction, a substrate S is converted to products P catalysed by an enzyme E (Scheme 7.5, left upper-side). In principle, the reaction rate depends linearly on the enzyme concentration. However, when there is an abundance of substrate molecules, the number of substrate binding sites in the catalyst is limited, thus causing saturation. Under these conditions, the reaction remains essentially constant at maximum rate as it proceeds (Scheme 7.5, right). This phenomenon is characteristic of catalytic reactions and is explained by the substrate binding at a fixed ratio.

In these cases, the biochemical reaction follows the Michaelis-Mentes kinetics. This mathematical model describes the rate of enzymatic reactions by relating the reaction

rate (v) to the concentration of substrate ([S]) and assuming that the formation of the enzyme-substrate complex equals its breakdown (Scheme 7.5, left bottom-side).



Scheme 7.5. Enzyme-catalyzed reaction exhibiting saturation kinetics: reaction and rate equation during the steady state (left) and graphical representation of all concentrations over time (right)

Variable v_{max}, which is found to be linearly proportional to the total enzyme concentration, is the maximum rate. Hence, the constant of proportionality k_{cat} is known as the turnover number, and represents the maximum number of molecules of substrate that an enzyme can convert to product per catalytic site per unit of time. The constant K_m is called the Michaelis constant, and can be determined as the substrate concentration at which 1/2 v_{max} is achieved. This kinetic parameter defines the affinity of the enzyme for the substrate.

The velocity of enzyme-catalysed reaction depends on the substrate concentration in a non-linear way. This makes it difficult to estimate K_m and v_{max} accurately (Figure 7.2, left). Therefore, linearisation of the Michaelis–Menten equation overcomes this problem and affords the Lineweaver–Burk plot (Figure 7.2, right). This graphical representation is widely used for the determination of different kinetic parameters. Therefore, the x/intercept of this graph represents -1/K_m while the y/intercept corresponds to 1/v_{max}.

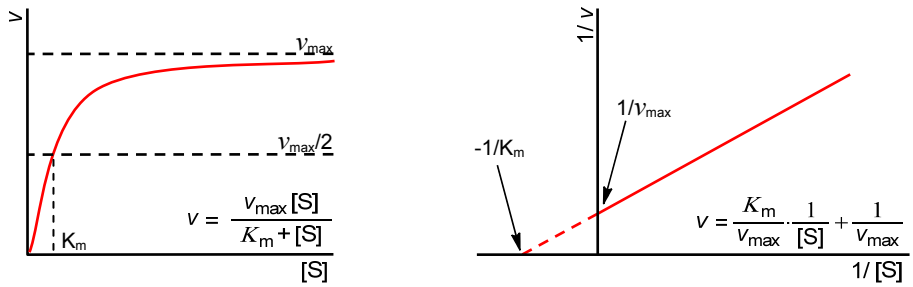


Figure 7.2. Representation of the reaction rate depending on the substrate concentration (left) and Lineweaver–Burk plot showing the significance of the axis intercepts and gradient (right)

The Lineweaver–Burk plot is also used for determining the potential of molecules as enzyme inhibitors. Hence, the binding of an inhibitor can be either irreversible or reversible. While the first class of inhibitors irreversibly inactivates enzymes by changing chemically the active site residues, the other family binds to enzymes within non-covalent interactions. In this context, four types of reversible inhibitors can be differenced by the Michaelis–Menten equation and the Lineweaver–Burk graphical representation. Thus, all competitive inhibitions are categorized based on their kinetic characteristics:

- Competitive, where both substrate and inhibitor compete for binding the enzyme. This means that v_{max} can still be reached if sufficient substrate is available. However, a higher concentration of substrate will be required and thus K_m will be larger. The Lineweaver-Burk double reciprocal plot for this kind of inhibition shows a series of lines crossing the y (1/v) axis at the same point (Figure 7.3, left upper-side).
- Uncompetitive, where the inhibitor only binds to the substrate-enzyme complex. Uncompetitive inhibitor enhances the binding of substrate, reducing K_m. Thus, the resultant enzyme-inhibitor-substrate complex reacts slowly, so v_{max} is also reduced. The Lineweaver-Burk double reciprocal plot for this set shows a series of parallel lines (Figure 7.3, right upper-side).
- Mixed, where the inhibitor can bind to the enzyme at the same time as the substrate-enzyme complex. Conceptually it is a combination of the two previous modes of inhibition. Mixed inhibitors do not bind directly in the active site, and therefore do not block substrate binding. However, they distort the active site and therefore, the affinity of the substrate for the active site is reduced. This inhibition decreases v_{max} and increases K_m. The Lineweaver-Burk double reciprocal shows a series of lines crossing at the same point (Figure 7.3, left bottom-side).
- Noncompetitive, a special case of mixed inhibition. The affinity of the inhibitor for the enzyme and the enzyme-substrate complex is the same. This inhibition reduces the enzyme activity but does not affect the binding of substrate. Thus, v_{max} is reduced while K_m remains unchanged. The Lineweaver-Burk double reciprocal plot shows a series of lines converging on the same point on the X (1/S) axis (Figure 7.3, right bottom-side).

Enzyme inhibition is one of the most important phenomenona in biochemistry where molecules showing inhibiting properties are potential drugs in medicine. These compounds are qualified by their specificity (degree of interaction with only one particular enzyme) as well as their potency (inhibition constant, K_i).

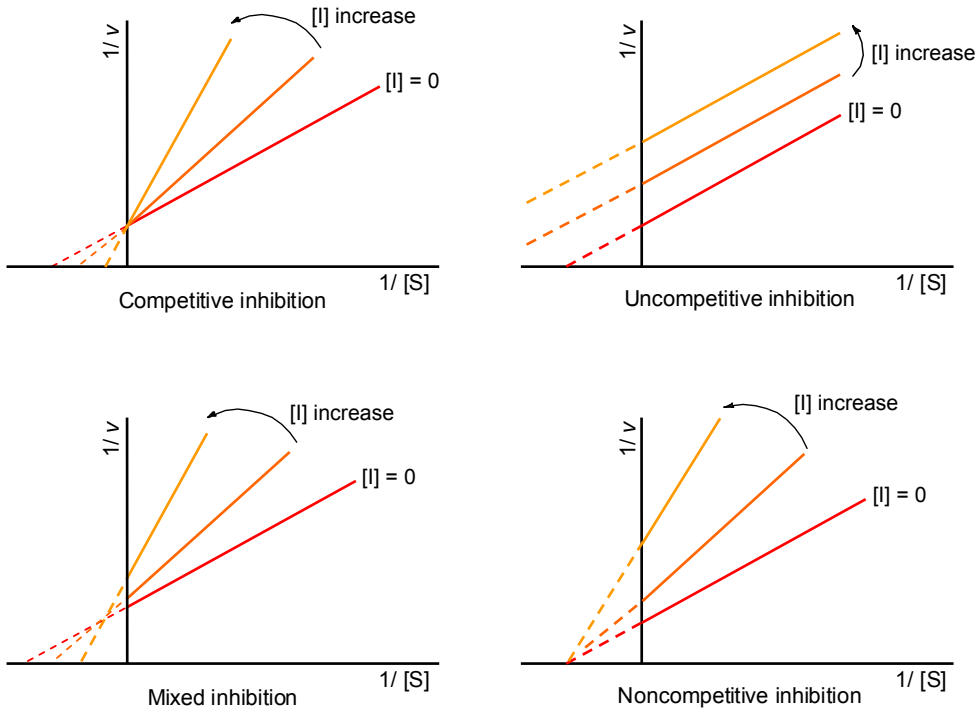


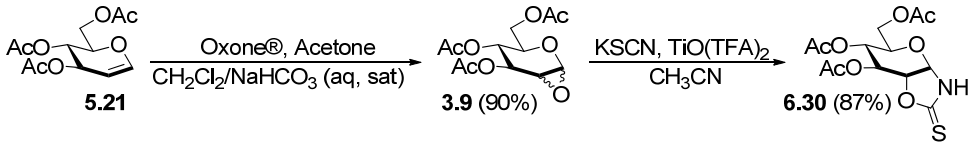
Figure 7.3. Lineweaver–Burk plots for inhibitors ([I] stands for concentration of inhibitor)

7.2. Results and discussion

7.2.1. Synthesis of novel cis-1,2-fused pyranose—1,3-oxazoline-2-thione derivatives

The initial synthetic objective of this research was the preparation of *S*-alkyl *cis*-1,2-fused pyranose–(2-alkylsulfany-1,3-oxazoline) carbohydrate derivatives bearing different substituents at the exocyclic sulfur atom. The methodology developed for the construction of the heteroatomic bicycle system exploits the reactivity of sugar epoxides explained in the previous chapter. The reaction sequence started with the conventional epoxidation of commercial tri-*O*-acetyl-D-glucal (**5.21**[i]) to afford a mixture of the corresponding tri-*O*-acetyl-1,2-anhydrosugars (**3.9**[ii], D-*gluco*/ D-*manno* ratio 7:1) in 90% yield.[40] Treatment of **3.9** with potassium thiocyanate and catalytic amounts of $TiO(CH_3CO_2)_2$ led to the key thionocarbamate **6.30**[iii] in 87% yield (Scheme 7.6).[41]

[i] This compound also appears in Chapter 5, thus same codification will be employed.
[ii] This compound also appears in Chapter 3, thus same codification will be employed.
[iii] This compound also appears in Chapter 6, thus same codification will be employed.



Scheme 7.6. Synthesis of 3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-1,3-oxazolidine-2-thione (**6.30**)

Thionocarbamates display ambident functionality while offering diverse reactivity.[1,42] The different properties of both N and S electron-rich centers is explained by Pearson's hard−soft acid−base (HSAB) theory,[43] where the nitrogen atom acts as a hard basic center while the sulfur atom shows a soft base character. This reactivity has been extensively investigated by Rollin et al.[44] Reagents of R-X type are mostly considered as soft electrophilic species as these provide high-yielding S-alkylation.[45]

Reaction of **6.30** with a series of different alkyl halides in basic medium led to the expected 2-alkylsulfanyl-1,3-oxazoline derivatives **7.4-7.12** in 69-91% yield (Table 1). Structures of the *S*-alkylated compounds were ascertained by ^{13}C NMR spectroscopy in comparison with analogously synthesized thionocarbamates.[44]

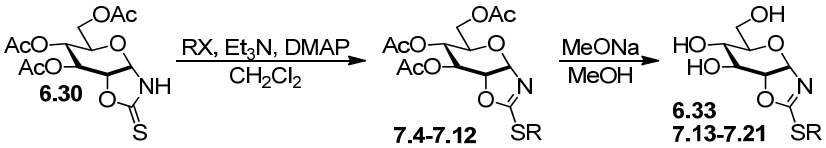
Chemical shifts for the quaternary sp^2 carbon atom at position 2 of the five-membered heterocycle varied from roughly 190 ppm (N-C=S in 1,3-oxazolidine-2-thione **6.30**) to approximately 170 ppm (N=C-SR in 2-alkylsulfanyl-1,3-oxazoline-derivatives **7.4-7.12**).

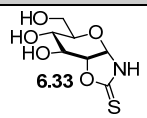
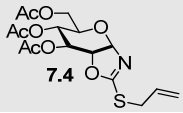
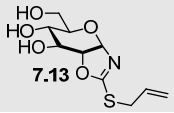
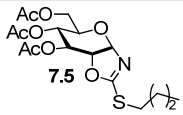
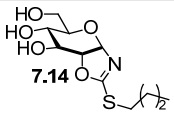
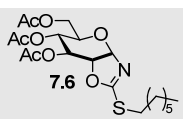
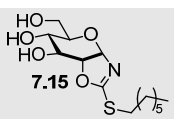
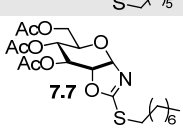
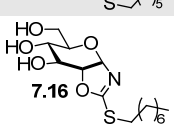
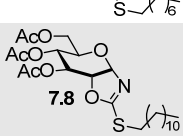
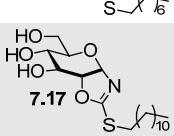
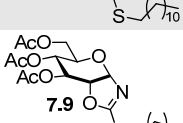
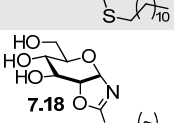
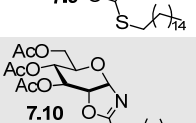
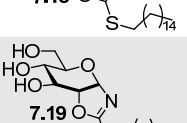
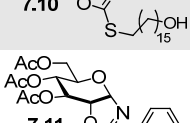
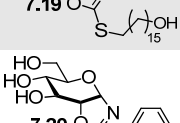
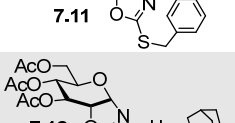
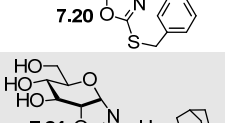
Final removal of the acetyl protecting groups using methanol under standard NaOMe-catalyzed conditions provided the requested PSO-glucomimetics **6.**33**,** 7**.13-7.21** in 89-99% yields (Table 7.1). Both alkylation and deprotection steps requested longer reaction times for the bulkier alkyl groups of the series (Table 7.1, entries 6-8 and 10). The unprotected *S*-alkylated derivatives **6.**33**,** 7**.13-7.21** maintain the skewed boat conformation already observed for their corresponding acetylated precursors **6.**33**,** 7**.13-7.21** and the parent compound **6.30**.

7.2.2. Evaluation of the glycosidase inhibitory activity and chaperone effect

All these new *cis*-1,2-fused D-glucopyranose-(2-alkylsulfanyl-1,3-oxazoline) derivatives have been preliminary evaluated at the Universidad de Sevilla against a panel of commercial glycosidases.

Table 7.1. Synthesis of 2-*S*-alkylsulfanyl-1,3-oxazoline D-glucopyranose derivatives[a]



E.	Alkyl Bromide	S-alkylation[b]			O-deprotection[c]		
		Product	t (h)	Y (%)	Product	t (h)	Y (%)
1	-	-	-	-		0.5	97
2			3	91		0.5	98
3			3	69		0.5	90
4			3	82		0.5	89
5			4	77		3	96
6			6	90		3	97
7			24	90		20	92
8			24	86		20	89
9			3	75		0.5	98
10			24	78		5	99

[a] Entry (E), Yield (Y). [b] Conditions: rt with 1.0 eq. substrate, 3.0 eq. alkyl bromide, 3.0 eq. Et_3N, and 2 mol % DMAP in CH_2Cl_2. [c] Conditions: rt with 1.0 eq. of *S*-alkylated substrate and 5 mol% MeONa in MeOH.

This panel of commercial glycosidases includes α-glucosidase (yeast), β-glucosidase (almonds and bovine liver, cytosolic), α-mannosidase (Jack bean), β-mannosidase (*Helix pomatia*), trehalase (pig kidney), amyloglucosidase (*Aspergillus niger*), naringinase (β-glucosidase/α-l-rhamnosidase; *Penicillium decumbens*), α-galactosidase (green coffee beans), β-galactosidase (*E. coli*), and isomaltase (yeast). The corresponding inhibition constants (K_i) are collected in Table 7.2.

The *S*-unsubstituted PSO derivative **6.33** did not inhibit any of the assayed glycosidases at concentrations up to 2 mM. Interestingly, the *S*-substituted derivatives **7.13**-**7.21** behaved as specific, though modest, competitive inhibitors of the bovine liver β-glucosidase among the 11 glycosidases tested. The capacity to discriminate between the mammalian and the plant β-glucosidases is particularly remarkable. Both the enzyme from almonds and the enzyme from bovine liver belong to the same glycosyl hydrolase family GH1 in the CAZy classification.[36] This indicates that the enzymes just mentioned bear considerable homology within their active sites. This family of enzymes is found across a broad spectrum of life forms, acting on a large variety of substrates. Moreover, they are retaining enzymes and follow a classical Koshland double-displacement mechanism.

The previous results indicate that subtle differences must exist in areas close to the active site and further substantiate that non-glyconic interactions are better suited that glyconic interactions to attain high levels of selectivity among isoenzymes.

Table 7.2. K_i values (μM) for compounds **6.**33, 7**.13**-7.**21** against glycosidases[a]

Enzyme	**6.33**	**7.13**	**7.14**	**7.15**	**7.16**	**7.17**	**7.18**	**7.19**	**7.20**	**7.21**
α-glucosidase (yeast)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-glucosidase (almonds)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-glucosidase (bovine liver)	NI	220	580	310	280	70	50	12	210	230
α-mannosidase (Jack bean)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-mannosidase (*Helix pomatia*)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
trehalase (pig kidney)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
amyloglucosidase (*Aspergillus niger)*	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
naringinase (*Penicillum decumbens*)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
β-galactosidase (*E. coli*)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
α-galactosidase (green coffe beans)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
isomaltase (yeast)	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
GCase (*Homo sapiens*)[b]	99	97	92	91	91	87	86	36	88	91

[a] Inhibition was competitive in all cases. NI, no inhibition observed at 2 mM concentration. [b] Relative to control (%).

A structure-activity relationship analysis within the PSO series **7.13**-**7.21** evidenced a notable influence of the nature of the exocyclic substituent at the sulfur functionality on the inhibitory potency.

For linear alkyl substituents, a progressive decrease of the corresponding K_i values, indicative of increased binding affinity, was observed with the chain length, going from 580 μM for the *n*-butyl derivative **7.14** to 50 μM for the *n*-hexadecyl derivative **7.18**. This observation is compatible with accommodation of the aliphatic chain into a hydrophobic pocket of the protein. No significant improvement was observed when aromatic (**7.20**) or adamantyl residues (**7.21**) were present. Noteworthy, installation of a terminal hydroxyl group at the hexadecyl chain (**7.19**) further improved binding by a factor of 4.3-fold (K_i = 12 μM), probably due to adventitious hydrogen-bonding interactions. Figure 7.4 shows the Lineweaver-Burk Plot for K_i determination of **7.19** against bovine liver β-glucosidase (pH 7.3).

The molecular basis for the unprecedented specificity of the PSO family towards the mammalian β-glucosidase is still unknown. The presence of the glycosidic nitrogen atom anchored in the α-configuration might seem to mismatch the β-anomeric selectivity of this enzyme. However, recent X-ray evidence on human GCase-inhibitor complexes has shown that α-configured glycomimetics can be accommodated at the active site in a skew-boat conformation in which the pseudoanomeric substituent adopts a pseudoequatorial disposition.[46]

PSO derivatives are preorganized in such a binding conformation, which could be at the origin of the observed specificity.

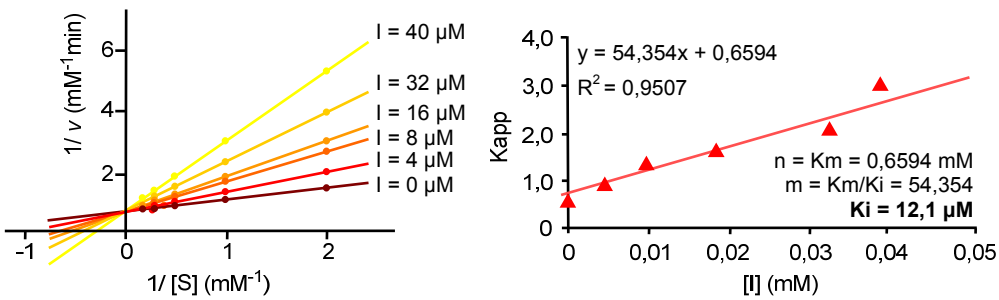


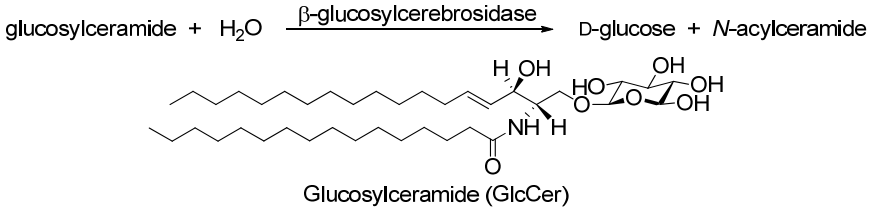
Figure 7.4. Effect of inhibitor **7.19** at different concentrations on bovine liver β-glucosidase (left) and K_i determinations from the slope of Lineweaver-Burk plots and double reciprocal analysis (right)

In any case, the results here reported represent a proof of concept for the utmost importance of implementing non-glyconic interactions in the design of potent and specific glycosidase inhibitors.

Inhibition of bovine liver β-glucosidase is often used as a preliminary parameter to select candidates as pharmacological chaperones for mutant human β-glucocerebrosidase (GCase) associated to Gaucher disease, since both enzymes belong to the same clan GHA in the CAZy classification.

Gaucher disease is a lipid storage disease resulted from the hereditary deficiency of β-glucocerebrosidase. This protein is responsible for the catabolism of β-glucocerebrosides, mainly glucosylceramide (GlcCer, Scheme 7.7). When the enzyme is defective, β-glucocerebrosides accumulate, particularly in white blood cells. The disease is caused by a recessive mutation, which prevents the β-glucocerebrosidase from assuming its correct conformation. Although the mutant molecule retains the proper glycosylase function, it is recognized by the quality-control system of the cell and retained (and often destroyed).

The significant inhibitory potential and total selectivity encountered for compound **7.19** against the commercial enzymes warranted further evaluation in this sense towards β-glucocerebrosidase by the Tottori University (Japan).



Scheme 7.7. Metabolic degradation of glucosylceramide (GlcCer)

Determination of the inhibitor activity on human GCase, relative to control, for the whole set of compounds at concentration 100 μM confirmed this point: only carbohydrate **7.19** within all PSO derivatives overpassed inhibition values of 50 % at this concentration (Table 7.2, last entry).

In further studies, ambroxol (ABX), a non-glycomimetic-type GCase inhibitor under investigaction as pharmacological chaperone for Gaucher disease,[47] was assayed in parallel as a reference. Pharmacological chaperones are small molecules that are able to fold a misfolded mutant protein. As a result, the macromolecule is stabilized against thermal denaturation and proteolytic degradation. Once the protein has successfully been intracellulary transported to the lysosome in the cells, the correct folding induces expression of its catalyitic activity.[48] Chaperones work only with certain mutations.

Inhibition studies in cell lysates from healthy fibroblasts indicated that **7.19** inhibited lysosomal GCase slightly less potently as compared to ABX (IC_{50} value 11.4 versus

4.1 μM, Figure 7.5). Enzyme activities in normal cell lysates were determined in the absence or presence of increasing concentrations of chaperones. Each point represents means of triplicate determinations obtained in a single experiment. Values were expressed relative to the activity in the absence of compounds (100%).

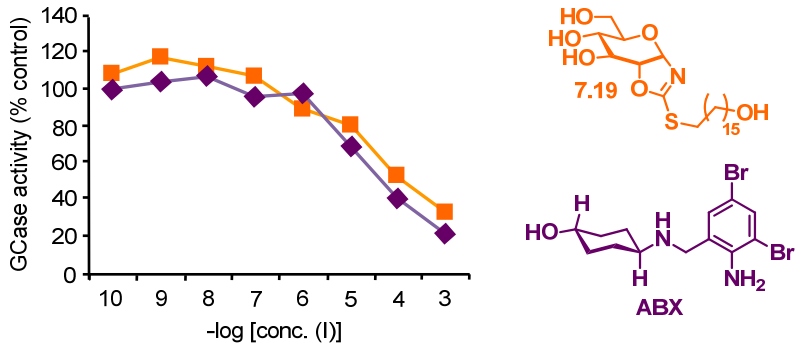


Figure 7.5. Effects of ambroxol (ABX) and PSO **7.19** on lysosomal enzyme activities in lysate from human normal fibroblasts

No inhibition of other lysosomal enzymes, such as α-glucosidase, α-galactosidase, β-galactosidase and β-hexosaminidase, was observed, reproducing the selectivity pattern already found for commercial enzymes (Table 7.3).

Table 7.3. IC_{50} values (μM) for compound **7.19** against lysosomal enzymes[a]

Compound	α-glucosidase	β-gluco cerebrosidase	α-galactosidase	β-galactosidase	β-hexosaminidase
7.19	NI	11.4	NI	NI	NI

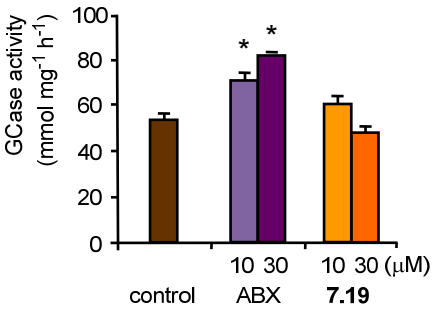
[a] NI, no half maximal inhibitory concentration (IC_{50}) observed at 2 mM concentration.

Further comparative enzyme activity enhancement assays were conducted in healthy as well as Gaucher fibroblasts from patients having the N370S/370S or the L444P/L444P mutations. The first one, the most common mutation among Gaucher patients, is located in the catalytic domain of the enzyme, while the second one is located in a non-catalytic domain. The cells were cultured for 5 days in the absence and in the presence of various concentrations of **7.19** or ABX, then lysed and the β-glucocerebrosidase activity determined using 4-methylumbelliferyl β-D-glucopyranoside as substrate. All values were calculated by doing three determinations each done by triplicate. Each bar of the associated histogram represents the mean ± SEM. The asterisks indicate 'highly significantly statistically differences ($p<0.01$) from the values in the absence of the compound (t test)'.

In normal cells, **7.19** had no effect on GCase activity, whereas ABX induced a statistically significant activity enhancement as compared to the control at 10 and 30 μM concentration (Table 7.4).

Table 7.4. Influence of ABX and PSO **7.19** in GCase activity in normal fibroblasts

Inhibitor	[I] (μM)	GCase activity (mmol mg^{-1} h^{-1})	SEM
Control	-	52.90	2,94
ABX	10	70,60	6,50
ABX	30	80,90	0,88
7.19	10	59,00	3,63
7.19	30	48,35	2,33

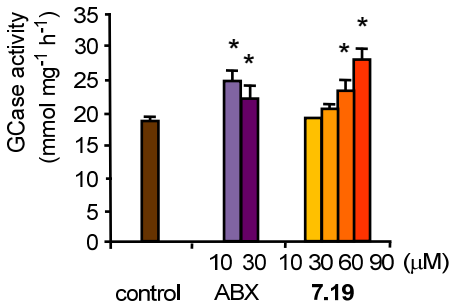


In N370S/370S Gaucher fibroblasts both PSO **7.19** and ABX significantly up-regulated the activity of the mutant enzyme. In the case of ABX a decrease in the relative activity increase occurs from 30 μM concentration. This means, however, that the inhibitory activity overcomes the chaperone effect. In contrast, the mutant enzyme activity enhancement induced by **7.19** steadily increased in a dose dependent manner in the range 30 to 90 μM, evidencing a more favourable chaperone/inhibitor balance (Table 7.5).

Neither **7.19** nor ABX were effective at increasing the activity in the case of the L444P/L444P mutant GCase. Neither ABX nor **7.19** had toxic effect on any of the normal or mutant cell lines assayed for 5 days incubation (Table 7.6).

Table 7.5. Influence of ABX and PSO **7.19** in GCase activity in N370S/N370S fibroblasts

Inhibitor	[I] (μM)	GCase activity (mmol mg^{-1} h^{-1})	SEM
Control	-	18,30	0,56
ABX	10	24,40	0,97
ABX	30	21,90	1,98
7.19	10	18,70	1,80
7.19	30	20,30	0,63
7.19	60	23,00	1,52
7.19	90	27,90	1,61

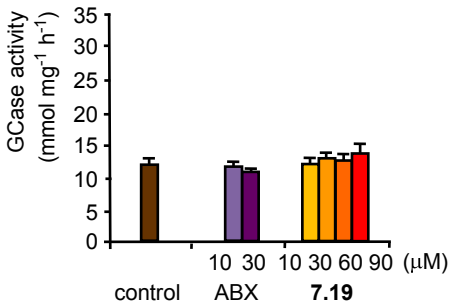


Altogether, the above data support that compound **7.19** behaves, similarly to the reference compound ABX, as an active site-directed pharmacological chaperone. However, the observed activity increase (1.8-fold) does not surpass the values

reported for the most effective iminosugar-type chaperones. For instance, NNDNJ elicit up to 2.3-fold activity enhancement in the N370S mutant and α-1-*C*-nonyl-DIX, developed by Compain and coworkers, reaches 1.8-fold increase already at 10 nM concentration.[49]

Table 7.6. Influence of ABX and PSO **7.19** in GCase activity in L444P/L444P fibroblasts

Inhibitor	[I] (μM)	GCase activity (mmol mg^{-1} h^{-1})	SEM
Control	-	7,87	0,86
ABX	10	7,89	0,53
ABX	30	7,21	0,17
7.19	10	7,90	0,69
7.19	30	8,70	0,53
7.19	60	8,20	0,70
7.19	90	9,02	1,28



7.3. Conclusions

In summary, carbohydrate-based OAT can easily undergo regioselective S-alkylation, producing 2-*S*-alkylsulfanyl-1,3-oxazoline D-glycopyranose derivatives. The use of these compounds as highly-specific, though modest, competitive inhibitors of the bovine liver β-glucosidase has been discovered. These results also provide a proof of concept that non-glyconic interactions can be advantageously exploited to endow a rigid pyranoid glyconic moiety with high binding affinity and selectivity towards a given glycosidase.

Among all derivatives tested, compound **7.19** has attracted great interest thanks to its significant inhibitory potential (K_i = 12.1 μM). Moreover, inhibition studies of human lysosomal enzymes in cell lysates showed total selectivity encountered towards β-glucocerebrosidase. Most importantly, the above data support that compound **7.19** behave similarly to the reference compound ABX as an active site-directed pharmacological chaperone. Furthermore, the synthetic methodology developed is very well adapted to molecular diversity-oriented strategies and compatible with lead identification and optimization of pharmacological chaperones.

7.4. Experimental section

7.4.1. General methods

All chemicals used were reagent grade and were employed as supplied unless otherwise specified. Compounds **6.30** and **6.33** were prepared in Chapter 6. ^{1}H and ^{13}C NMR spectra were recorded on a Varian® Mercury VX 400 (400 MHz and 100.6 MHz respectively) and Varian 400-MR spectrometer in $CDCl_3$ or CD_3OD as solvents, with chemical shifts (δ) referenced to internal standards $CDCl_3$ (7.26 ppm ^{1}H, 77.23 ppm ^{13}C), CD_3OD (3.31 ppm ^{1}H, 49.14 ppm ^{13}C) or Me_4Si as an internal reference (0.00 ppm). 2D correlation spectra (gCOSY, NOESY, gHSQC, gHMBC) were visualized using the VNMR program (Varian®). ESI-MS were run on an Agilent® 1100 Series LC/MSD instrument. Melting points (Mp) were measured on a Griffin melting point apparatus and were uncorrected. Optical rotations were measured at 598 nm at room temperature in a Perkin-Elmer® 241 MC apparatus with 10 cm cells. IR spectra were recorded on a JASCO FT/IR-600 plus Fourier Transform Infrared Spectrometer ATR Specac Golden Gate in the Servei de Recursos Científics (SRCiT-URV). Reactions were monitored by TLC carried out on 0.25 mm E. Merck® silica gel 60 F254 glass or aluminium plates. Developed TLC plates were visualized under a short-wave UV lamp (250 nm) or dipping the plate in a suitale developing solution.[50] Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka® or Merck® silica gel 60 (230-400 mesh). Radial chromatography was performed on 1 or 2 mm plates of Kieselgel 60 PF254 silica gel, depending on the amount of product. Flash column chromatography (FCC) was performed using flash silica gel (32–63 μm) and employing a solvent polarity correlated with TLC mobility.

7.4.2. General procedures

General procedure for the S-alkylation of *cis*-1,2-fused D-glucopyranose—1,3-oxazolidine-2-thione derivatives: to a solution of **6.30** (1.00 mmol) in CH_2Cl_2 (3.5 mL), the appropiate alkyl bromide (3.00 mmol), Et_3N (3.00 mmol) and DMAP (0.2 mmol) were added followed by stirring at room temperature. After completion of the reaction, the mixture was washed with saturated $NaHCO_3$ and brine, dried, and concentrated. Chromatographic purification afforded the *S*-alkylated compounds in the yields shown.

General procedure for acetyl deprotection of *cis*-1,2-fused D-glucopyranose—2-alkylsulfanyl-1,3-oxazoline derivatives: sodium methoxide (0.05 mmol) was added to a solution of protected 1,3-oxazoline carbohydrate (1.00 mmol) in methanol (20 mL), followed by stirring at room temperature. Upon completion of the reaction, the solvent was removed *in vacuo*, and the crude product was purified by flash chromatography on silica gel to afford the deprotected compounds in the yields shown.

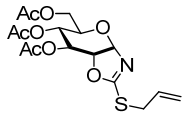
Inhibition studies with commercial enzymes: inhibition constant (K_i) values were determined by spectrophotometrically measuring the residual hydrolytic activities of the glycosidases against the respective *p*-nitrophenyl α- or β-D-glycopyranoside, *o*-nitrophenyl β-D-galactopyranoside (for β-galactosidase from *E. coli*), or α , α'-trehalose (for trehalase) in the presence of compounds **6.33, 7.13-7.21**. Each essay was performed in phosphate buffer or phosphate-citrate buffer (for α- or β-mannosidase and amyloglucosidase) at the optimal pH of the enzyme for the enzymes. The reactions were initiated by addition of enzyme to a solution of the substrate in the absence or presence of various concentrations of inhibitor. The mixture was incubated for 10-30 min at 37 ºC or 55 ºC (for amyloglucosidase) and the reaction was quenched by addition of 1M Na_2CO_3 or by heating and subsequent addition of a solution of Glc-Trinder (Sigma, for trehalase). Reaction times were appropiated to obtain 10-20% conversion of the substrate in order to achieve linear rates. The absorbance of the resulting mixture was determined at 405 nm or 492 nm (for trehalase). Approximate values of K_i were determined using a fixed concentration of substrate (around the K_m value for the different glycosidases) and various concentrations of inhibitor. Full K_i determinations and enzyme inhibition mode were determined from the slope of Lineweaver-Burk plots and double reciprocal analysis.

Lysosomal enzyme activity assay: lysosomal enzyme activities in cell lysates were determined as described previously.[51] Briefly, cells were scraped in ice-cold 0.1% Triton X-100 in water. After centrifugation (6,000 rpm for 15 min at 4 ºC) to remove insoluble materials, protein concentrations were determined using Protein Assay Rapid Kit (Wako, Tokyo, Japan). The lysates were incubated at 37 ºC with the corresponding 4-methylumbelliferyl β-D-glycopyranoside solution in 0.1 M citrate buffer (pH 4). The librated 4-methylumbelliferone was measured with a fluorescence plate reader (exitation 340 nm; emission 460 nm; Infinite F500, TECAN Japan, Kawasaki, Japan). For enzyme inhibition assay, cell lysates from normal skin

fibroblasts were mixed with the 4-methylumbelliferyl β-D-glycopyranoside substrates in the absence or presence of increasing concentrations of ABX or PSO **7.19**.

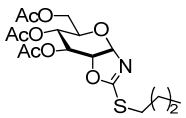
Cell culture and GCase activity enhancement assay: human skin fibroblasts from a healthy and two Gaucher disease patients (with N370S/N370S and L444P/L444P mutations) were maintained in our laboratory with DMEM supplemented with 10% FBS as the culture medium. For enzyme activity enhancement assay, cells were cultured in the presence of different concentrations of ABX or PSO **7.19**, or DMSO alone (as a control) for 5 days and harvested by scraping.[32] Cytotoxicity of the compounds was monitored by measuring the lactate dehydrogenase activities in the cultured supernatants (LDH assay kit, Wako, Tokyo, Japan).

7.4.3. Compound characterization

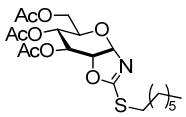


3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-2-allylsulfanyl-1,3-oxazoline (7.4): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazolidine carbohydrate derivatives starting from **6.30** (98.5 mg, 0.28 mmol), allyl bromide (73 μL, 0.84 mmol), Et_3N (117 μL, 0.84 mmol), DMAP (6.8 mg, 0.06 mmol) and CH_2Cl_2 (1 mL). The reaction mixture was stirred at rt for 3 h. After standard workup, the crude was purified by flash chromatography (from 1:9 to 1:6 AcOEt/hexane) to afford the desired compound (100.0 mg, 91% yield) as colourless syrup. Data: Rf (4:6 AcOEt/hexane x2): 0.45. $[\alpha]_D$ + 40.0 (*c* 5.00, $CHCl_3$). FT-IR (neat) in υ cm^{-1}: 2955, 1739, 1591, 1425, 1367, 1214, 1144, 1109, 1035, 996, 913 ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.94 (ddt, 1H, $J_{1',2'}$= 10.4 Hz, $J_{1',3'}$= 16.8 Hz, $J_{1',4'}$= 6.8 Hz, H-1' allyl); 5.86 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 5.33 (dd, 1H, $J_{3',1'}$= 16.8, $J_{3',2'}$= 0.8, H-3' allyl); 5.19-5.16 (m, 2H, H-3 and H-2' allyl); 4.91 (dd, 1H, $J_{3,4}$ = 4.4 Hz, $J_{4,5}$= 8.4 Hz, H-4); 4.52 (dd, 1H, $J_{1,2}$= 7.6 Hz, $J_{2,3}$= 3.6 Hz, H-2); 4.26 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 4.17 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.75-3.65 (m, 3H, 2xH4' allyl and H-5); 2.11 (s, 3H, AcO); 2.07 (s, 3H, AcO); 2.06 (s, 3H, AcO). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 170.8 (C=N); 170.3, 169.8, 169.5 (C(O), AcO); 132.4 (=CH, allyl); 119.2 (=CH_2, allyl); 92.5 (C-1); 77.2 (C-2); 70.1 (C-3); 68.0 (C-5); 67.4 (C-4); 63.5 (C-6);

34.8 (CH_2-N, allyl); 21.0, 20.98, 20.94 (CH_3, AcO). +TOF MS Calcd for $C_{16}H_{21}NO_8S$ *m/z* $[M-H]^+$: 388.1066, found: 388.1051.

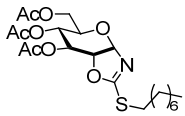


3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-butylsulfanyl-1,3-oxazoline (7.5): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **6.30** (50.0 mg, 0.14 mmol), 1-bromobutane (47 μL, 0.43 mmol), Et_3N (74 μL, 0.43 mmol), DMAP (3.5 mg, 0.03 mmol) and CH_2Cl_2 (0.5 mL). The reaction mixture was stirred at rt for 3 h. After standard workup, the crude was purified by flash chromatography (4:6 AcOEt/hexane) to afford the desired compound (40.1 mg, 69% yield) as colourless syrup. Data: R_f (4:6 AcOEt/hexane x2): 0.48. $[\alpha]_D$ + 72.4 (*c* 1.43, $CHCl_3$). FT-IR (neat) in υ cm^{-1}: 2959, 2919, 1741, 1590, 1367, 1215, 1144, 1108, 1035. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.86 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 5.18 (t, 1H, $J_{3,2}$= $J_{3,4}$= 4.4 Hz, H-3); 4.9 (ddd, 1H, $J_{4,2}$=0.8 Hz, $J_{4,3}$= 4.4 Hz, $J_{4,5}$= 8.4 Hz, H-4); 4.51 (ddd, 1H, $J_{2,1}$= 7.6 Hz, $J_{2,3}$= 4.4 Hz, $J_{2,4}$= 0.8 Hz, H-2); 4.27 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 4.16 (dd, 1H, $J_{6',6}$= 12 Hz, $J_{6',5}$= 2.8 Hz, H-6'); 3.69 (ddd, 1H, $J_{5,4}$= 8.4 Hz, $J_{5,6}$= 5.2 Hz, $J_{5,6'}$= 2.8 Hz, H-5); 3.06 (t, 2H, *J*= 7.2 Hz, CH_2 aliph); 2.11 (s, 3H, AcO); 2.07 (s, 3H, AcO); 2.06 (s, 3H, AcO); 1.74-1.66 (m, 2H, CH_2 aliph); 1.52-1.38 (m, 2H, CH_2 aliph); 0.92 (t, 3H, *J*= 7.6 Hz, CH_3 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 171.1 (C=N); 170.8, 169.8, 169.5 (C(O), AcO); 92.5 (C-1); 77.1 (C-2); 70.2 (C-3); 68.0 (C-5); 67.4 (C-4); 63.5 (C-6); 32.0 (CH_2, aliph); 31.5 (CH_2, aliph), 22.0 (CH_2, aliph); 22.0, 21.9, 20.0 (CH_3, AcO), 13.7 (CH_3, aliph). +TOF MS Calcd for $C_{17}H_{25}NO_8S$ *m/z* $[M-H]^+$: 404.1379, found: 404.1345.



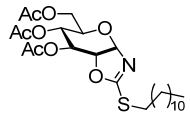
3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-heptylsulfanyl-1,3-oxazoline (7.6): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **6.30** (50.0 mg, 0.14 mmol), 1-bromoheptane (69 μL, 0.43 mmol), Et_3N (74 μL, 0.43 mmol), DMAP (3.5 mg, 0.03 mmol) and CH_2Cl_2 (0.5 mL). The reaction mixture was stirred at rt for 3 h. After standard workup, the crude was purified by flash chromatography (4:6 AcOEt/hexane) to afford the desired compound (52.7 mg, 82% yield) as colourless syrup. Data: R_f (1:3 AcOEt/hexane): 0.75. $[\alpha]_D$ + 66.3 (*c* 1.49,

$CHCl_3$). FT-IR (neat) in υ cm^{-1}: 2926, 2856, 1742, 1591, 1367, 1216, 1144, 1108, 1036, 1000. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.86 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 5.19 (t, 1H, $J_{3,2}$= $J_{3,4}$= 4.4 Hz, H-3); 4.91 (ddd, 1H, $J_{4,2}$= 0.8 Hz, $J_{4,3}$= 4.4 Hz, $J_{4,5}$= 8.8 Hz, H-4); 4.52 (ddd, 1H, $J_{2,1}$= 7.6 Hz, $J_{2,3}$= 4.4 Hz, $J_{2,4}$= 0.8 Hz, H-2'); 4.28 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 4.17 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.69 (ddd, 1H, $J_{5,4}$= 8.8 Hz, $J_{5',6}$= 5.2 Hz, $J_{5',6'}$=2.8 Hz, H-5); 3.06 (t, 2H, $J_{1,2}$=7.2 Hz, CH_2 aliph); 2.11 (s, 3H, AcO); 2.08 (s, 3H, AcO); 2.06 (s, 3H, AcO); 1.74-1.66 (m, 2H, CH_2 aliph); 1.52-1.38 (m, 8H, 4xCH_2 aliph); 0.86 (t, 3H, J= 7.6 Hz, CH_3 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 171.1 (C=N); 170.8, 169.8, 169.5 (C(O), AcO); 92.5 (C-1); 77.1 (C-2); 70.2 (C-3); 68.0 (C-5); 67.4 (C-4); 63.5 (C-6); 32.4 (CH_2, aliph); 29.4, 31.8, 28.9, 28.8, 22.8 (CH_2, aliph); 21.0, 21,0 20.9 (CH_3, AcO), 14.3 (CH_3, aliph). +TOF MS Calcd for $C_{20}H_{31}NO_8S$ *m/z* $[M-H]^+$: 446.1849, found: 446.1831.

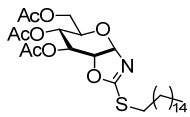


3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-octylsulfanyl-1,3-oxazoline (7.7): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **6.30** (98.2 mg, 0.28 mmol), 1-bromooctane (150 μL, 0.86 mmol), Et_3N (148 μL, 0.86 mmol), DMAP (7.0 mg, 0.06 mmol) and CH_2Cl_2 (1.0 mL). The reaction mixture was stirred at rt for 4 h. After standard workup, the crude was purified by flash chromatography (4:6 AcOEt/hexane) to afford the desired compound (100.0 mg, 77% yield) as colourless syrup. Data: R_f (4:6 AcOEt/hexane): 0.79. $[\alpha]_D$ + 32.8 (*c* 1.36, $CHCl_3$). FT-IR (neat) in υ cm^{-1}: 2926, 2855, 1744, 1592, 1367, 1219, 1145, 1109, 1038. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.86 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 5.19 (t, 1H, $J_{3,2}$= $J_{3,4}$= 4.4 Hz, H-3); 4.91 (ddd, 1H, $J_{4,2}$=0.8 Hz, $J_{4,3}$ = 4.4 Hz, $J_{4,5}$= 8.4 Hz, H-4); 4.52 (ddd, 1H, $J_{2,1}$= 7.6 Hz, $J_{2,3}$= 4.4 Hz, $J_{2,4}$= 0.8 Hz, H-2); 4.28 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 4.17 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.69 (ddd, 1H, $J_{5,4}$= 8.4 Hz, $J_{5,6}$= 5.2 Hz, $J_{5,6'}$= 2.8 Hz, H-5); 3.05 (t, 2H, J= 7.2 Hz, CH_2 aliph); 2.11 (s, 3H, AcO); 2.08 (s, 3H, AcO); 2.06 (s, 3H, AcO); 1.74-1.66 (m, 2H, CH_2 aliph); 1.52-1.38 (m, 10H, 5xCH_2 aliph); 0.87 (t, 3H, J= 6.8 Hz, CH_3 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 171.1 (C=N); 170.8, 169.8, 169.5 (C(O), AcO); 92.5 (C-1); 77.1 (C-2); 70.2 (C-3); 68.0 (C-5); 67.4 (C-4); 63.5 (C-6); 32.4, 32.0,

29.4, 29.3, 29.2, 28.9, 22.8 (CH_2, aliph); 21.0, 21.0, 20.9 (CH_3, AcO), 14.3 (CH_3, aliph). +TOF MS Calcd for $C_{21}H_{33}NO_8S$ *m/z* $[M-H]^+$: 460.2005, found: 460.1994.

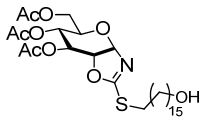


3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-dodecylsulfanyl-1,3-oxazoline (7.8): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **6.30** (50.0 mg, 0.14 mmol), 1-bromododecane (101 μL, 0.42 mmol), Et_3N (72 μL, 0.42 mmol), DMAP (3.4 mg, 0.03 mmol) and CH_2Cl_2 (0.5 mL). The reaction mixture was stirred at rt for 6 h. After standard workup, the crude was purified by flash chromatography (1:6 AcOEt/hexane) to afford the desired compound (100.0 mg, 77% yield) as colourless syrup. Data: R_f (1:6 AcOEt/hexane x2): 0.74. $[\alpha]_D$ + 45.9 (*c* 3.62, $CHCl_3$). FT-IR (neat) in υ cm^{-1}: 2922, 2852, 1744, 1592, 1366, 1217, 1145,1108, 1037. ^1H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.88 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 5.20 (t, 1H, $J_{3,2}$= $J_{3,4}$= 4.0 Hz, H-3); 4.93 (ddd, 1H, $J_{4,2}$= 0.8 Hz, $J_{4,3}$ = 4.0 Hz, $J_{4,5}$= 8.4 Hz, H-4); 4.51 (ddd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 4 Hz, $J_{2,4}$=0.8 Hz, H-2); 4.29 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 4.18 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.70 (ddd, 1H, $J_{5,4}$= 8.0 Hz, $J_{5,6}$= 5.2 Hz, $J_{5,6'}$= 2.8 Hz, H-5); 3.06 (t, 2H, *J*= 7.6 Hz, CH_2 aliph); 2.12 (s, 3H, AcO); 2.09 (s, 3H, AcO); 2.07 (s, 3H, AcO); 1.73-1.65 (m, 2H, CH_2 aliph); 1.42-1.23 (m, 18H, 9xCH_2 aliph); 0.92 (t, 3H, *J*= 7.6 Hz, CH_3 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 171.1 (C=N); 170.8, 169.8, 169.5 (C(O), AcO); 92.5 (C-1); 77.1 (C-2); 70.2 (C-3); 68.0 (C-5); 67.4 (C-4); 63.4 (C-6); 32.3; 32.0, 29.81, 29.8, 29.7, 29.6, 29.5, 29.4, 29.2, 28.8, 22.8 (CH_2, aliph) 21.0, 20.99, 20.95 (CH_3, AcO), 14.3 (CH_3, aliph). +TOF MS Calcd for $C_{25}H_{41}NO_8S$ *m/z* $[M-H]^+$: 516.2431, found: 516.2412.



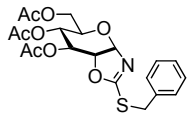
3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-hexadecylsulfanyl-1,3-oxazoline (7.9): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **6.30** (81.2 mg, 0.23 mmol), $C_{16}H_{33}Br$ (0.22 mL, 0.70 mmol), Et_3N (98 μL, 0.70 mmol), DMAP (5.7 mg, 0.05 mmol) and CH_2Cl_2 (0.8 mL). The reaction mixture was stirred at rt for 24 h. After standard workup, the crude was

purified by flash chromatography (from 2:8 to 1:1 AcOEt/hexane) to afford the desired compound (120.5 mg, 90% yield) as a white solid. Data: Rf (1:1 AcOEt/hexane): 0.45. Mp: 46-48 ºC. $[\alpha]_D$ + 0.6 (*c* 0.87, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 2917, 2848, 1743, 1589, 1365, 1241, 1220, 1119, 955. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.88 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 5.20 (t, 1H, $J_{3,2}$= $J_{3,4}$= 4.0 Hz, H-3); 4.98 (dd, 1H, $J_{4,3}$= 4.0 Hz, $J_{4,5}$= 8.4 Hz, H-4); 4.52 (ddd, 1H, $J_{2,1}$= 7.6 Hz, $J_{2,3}$= 4.0 Hz, $J_{2,4}$=0.8 Hz, H-2); 4.29 (dd, 1H, $J_{6,5}$= 5.4 Hz, $J_{6,6'}$= 12.2 Hz, H-6); 4.17 (dd, 1H, $J_{6',5}$= 3.0 Hz, $J_{6',6}$=12.2 Hz, H-6'); 3.69 (ddd, $J_{5,4}$= 8.4 Hz, $J_{5,6}$= 5.4 Hz, $J_{5,6'}$ = 3.0 Hz, 1H, H-5); 3.06 (t, 2H, *J*= 7.4 Hz, CH_2-N aliph); 2.12 (s, 3H, AcO); 2.09 (s, 3H, AcO); 2.07 (s, 3H, AcO); 1.72 (q, 2H, *J*= 7.6 Hz, aliph); 1.43-1.24 (m, 26H, aliph); 0.87 (t, 3H, *J*= 7.0, CH_3 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 171.2 (C=N); 170.9, 169.8, 168.6 (C(O), AcO); 92.6 (C-1); 71.1 (C-2); 70.2 (C-3); 68.1 (C-4); 67.5 (C-5); 63.5 (C-6); 32.4, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.3, 28.9, 22.9 (CH_2 aliph); 21.1, 21.0, 20.9 (CH_3, AcO); 14.4 (CH_3 aliph). +TOF MS Calcd for $C_{29}H_{49}NO_8S$ *m/z* [M-H]+: 572.3257, found: 572.3247.

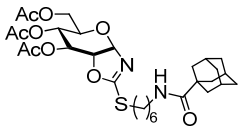


3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-(16-hydroxyhexadecyl)sulfanyl-1,3-oxazoline (7.10): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **6.30** (85.6 mg, 0.25 mmol), 16-bromohexadecanol (**7.22**, 237.7 mg, 0.75 mmol), Et_3N (106 µL, 0.75 mmol), DMAP (6.2 mg, 0.05 mmol) and CH_2Cl_2 (0.9 mL). The reaction mixture was stirred at rt for 24 h. After standard workup, the crude was purified by flash chromatography (from 1:10 to 1:3 AcOEt/hexane) to afford the desired compound (124.6 mg, 86% yield) as a colourless syrup. Data: Rf (4:6 AcOEt/hexane x6): 0.16. $[\alpha]_D$ + 0.5 (*c* 1.91, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 3352, 2916, 2848, 1745, 1591, 1369, 1241, 1219, 1165, 1041. ^{1}H NMR (400 MHz, $CDCl_3$) d in ppm: 5.84 (d, 1H, $J_{1,2}$= 7.4 Hz, H-1); 5.16 (t, 1H, $J_{3,2}$= $J_{3,4}$= 4.0 Hz, H-3); 4.88 (ddd, 1H, $J_{4,2}$= 0.8 Hz, $J_{4,3}$= 4.0 Hz, $J_{4,5}$= 8.4 Hz, H-4); 4.49 (ddd, 1H, $J_{2,1}$= 7.4 Hz, $J_{2,3}$= 4.0 Hz, $J_{2,4}$= 0.8 Hz, H-2); 4.24 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12.2 Hz, H-6); 4.13 (dd, 1H, $J_{6',5}$= 3.0 Hz, $J_{6',6}$= 12.2 Hz, H-6'); 3.65 (ddd, $J_{5,4}$= 8.4 Hz, $J_{5,6}$= 5.2 Hz, $J_{5,6'}$ =3.0 Hz, 1H, H-5); 3.58 (t, 2H, *J*= 6.6 Hz, CH_2-OH aliph); 3.02 (t, 2H, *J*= 7.4 Hz, CH_2-N aliph); 2.10 (s, 3H, AcO); 2.06 (s, 3H, AcO); 2.05 (s, 3H, AcO); 1.69 (q, 2H, *J*= 7.2 Hz, aliph); 1.53 (q, 2H, *J*= 7.2 Hz, aliph); 1.40-1.20 (m, 26H, aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 171.1 (C=N);

170.8, 169.7, 169.4 (C(O), AcO); 92.4 (C-1); 77.0 (C-2); 70.1 (C-3); 68.0 (C-4); 67.3 (C-5); 63.4 (C-6); 63.1 (CH_2-OH); 32.9, 32.3, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 28.8, 25.8 (CH_2 aliph); 21.0, 20.9, 20.8 (CH_3, AcO). +TOF MS Calcd for $C_{29}H_{49}NO_9S$ *m/z* [M-H]+: 588.3201, found: 588.3195.

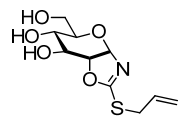


3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-benzylsulfanyl-1,3-oxazoline (7.11): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **6.30** (53.0 mg, 0.15 mmol), benzyl bromide (54 μL, 0.46 mmol), Et_3N (64 μL, 0.46 mmol), DMAP (3.4 mg, 0.03 mmol) and CH_2Cl_2 (0.5 mL). The reaction mixture was stirred at rt for 4 h. After standard workup, the crude was purified by flash chromatography (4:6 AcOEt/hexane) to afford the desired compound (90.0 mg, 75% yield) as colourless syrup. Data: R_f (4:6 AcOEt/hexane): 0.37. $[\alpha]_D$ + 35.9 (*c* 0.78, $CHCl_3$). FT-IR (neat) in υ cm^{-1}: 2923, 1739, 1593, 1496, 1454, 1367, 1215, 1143, 1107, 1035, 999. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.39-7.28 (stack, 5H, Ar); 5.88 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 5.18 (t, 1H, $J_{2,3}$= $J_{3,4}$= 4.0 Hz, H-3); 4.93 (ddd, 1H, $J_{4,2}$= 0.8 Hz, $J_{3,4}$= 4.0 Hz, $J_{4,5}$= 8.0 Hz, H-4); 4.53 (ddd, 1H, $J_{2,1}$= 7.6 Hz, $J_{2,3}$= 4.0 Hz, $J_{2,4}$= 0.8 Hz, H-2); 4.35 (d, 1H, J_{AB}= 13.6 Hz, CH_2Ph); 4.26 (d, 1H, J_{AB}= 13.6 Hz, CH_2Ph); 4.29 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 4.16 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.60 (ddd, 1H, $J_{5,4}$= 8.0 Hz, $J_{5,6}$= 5.2 Hz, $J_{5,6'}$= 2.8 Hz, H-5); 2.12 (s, 3H, AcO); 2.09 (s, 3H, AcO); 2.01 (s, 3H, AcO). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 170.9 (C=N); 170.4, 169.8, 169.5 (C(O), AcO); 136.32 (C, PhAr); 129.23, 128.88, 128.04 (CH, Ph); 92.5 (C-1); 77.2 (C-2); 70.0 (C-3); 68.0 (C-5); 67.3 (C-4); 63.4 (C-6); 36.6 (CH_2Ph); 21.0, 20.92, 20.91 (CH_3, AcO). +TOF MS Calcd for $C_{20}H_{23}NO_8S$ *m/z* $[M-H]^+$: 438.1223, found: 438.1208.



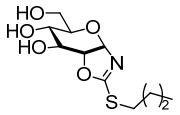
3,4,6-Tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoside[1,2-d]-(6-(1-adamantanecarboxamido)-hexyl)sulfanyl-1,3-oxazoline (7.12): the title compound was prepared following the general procedure for the S-alkylation of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **6.30** (61.8 mg, 0.18 mmol), *N*-(6-bromohexyl)-1-adamantanecarboxamide (**7.23**, 182.8 mg, 0.53 mmol), Et_3N

(75 μL, 0.53 mmol), DMAP (4.3 mg, 0.04 mmol) and CH_2Cl_2 (0.6 mL). The reaction mixture was stirred at rt for 24 hours. After standard workup, the crude was purified by flash chromatography (2:1 AcOEt/hexane) to afford the desired compound (84.6 mg, 78% yield) as colourless syrup. Data: Rf (2:1 AcOEt/hexane x6): 0.21. $[\alpha]_D$ + 0.5 (*c* 3.28, $CHCl_3$). FT-IR (neat) υ in cm^{-1}: 3353, 2907, 2851, 1743, 1638, 1593, 1522, 1367, 1219, 1038. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.86(d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 5.60 (m, 1H, NH); .18 (t, 1H, $J_{3,2}$= $J_{3,4}$= 4.0 Hz, H-3); 4.91 (ddd, 1H, $J_{4,2}$= 0.8 Hz, $J_{4,3}$= 4.0Hz, $J_{4,5}$= 8.4 Hz, H-4); 4.51 (ddd, 1H, $J_{2,1}$= 7.6 Hz, $J_{2,3}$= 4.0, $J_{2,4}$= 0.8 Hz, H-2); 4.27 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$=12.2Hz, H-6); 4.16 (dd, 1H, $J_{6',5}$= 3.0 Hz, $J_{6',6}$= 12.2 Hz, H-6'); 3.68 (ddd, $J_{5,4}$= 8.4 Hz, $J_{5,6}$= 5.2Hz, $J_{5,6'}$= 3.0 Hz, 1H, H-5); 3.21 (q, 2H, *J*= 7.0 Hz, CH_2-NH); 3.04 (td, 2H, *J*= 7.4 Hz, *J*= 1.8 Hz, CH_2-S); 2.11 (s, 3H, AcO); 2.07 (s, 3H, AcO); 2.06 (s, 3H, AcO); 2.02 (m, 3H, H-3' Adam); 1.82 (m, 6H, H-2' Adam); 1.75-1.65 (m, 8H, H-4' Adam, CH_2 aliph); 1.50-1.18 (m, 6H, 3xCH_2 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 178.1 (C(O)); 171.0 (C=N); 170.9, 169.8, 169.5 (C(O), AcO); 92.5 (C-1); 77.2 (C-2); 70.3 (C-3); 68.1 (C-4); 67.4 (C-5); 63.4 (C-6); 40.7 (C-1' Adam); 39.5 (3x C-2' Adam); 39.3 (CH_2-NH); 36.7 (3x C-4 Adam); 32.2 (CH_2-S); 29.7, 29.3, 28.5 (CH_2 aliph.); 28.3 (3x C-3 Adam); 26.5 (CH_2 aliph.) 21.0, 21.0, 20.9 (CH_3, AcO). +TOF MS Calcd for $C_{30}H_{44}N_2O_9S$ *m/z* [M-H]+: 609.2840, found: 609.2838; [M-Na]+: 631.2660, found: 631.2655.

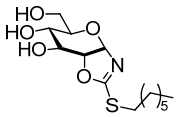


1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-allylsulfanyl-1,3-oxazoline (7.13): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.4** (100 mg, 0.26 mmol), MeONa (8 mg, 14 μmol) and MeOH (5.2 mL). The reaction mixture was stirred at rt for 30 min. After standard workup, the crude was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to afford the desired compound (66.0 mg, 98% yield) as colourless syrup. Data: R_f (1:9 MeOH/CH_2Cl_2): 0.45. $[\alpha]_D$ + 18.6 (*c* 1.00, MeOH). FT-IR (neat) in υ cm^{-1}: 3361, 1703, 1638, 1582, 1477, 1362, 1296, 1229, 1114, 1046, 992, 949. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 5.90 (ddt, 1H, $J_{1',2'}$= 10.4 Hz, $J_{1',3'}$= 17.2 Hz, $J_{1',4'}$= 6.8 Hz, H-1' allyl); 5.78 (d, 1H, $J_{1,2}$= 7.2 Hz, H-1); 5.28 (dd, 1H, $J_{3',1'}$= 17.2 Hz, $J_{3',2'}$= 0.8 Hz, H-3' allyl); 5.19 (dd, 1H, $J_{2',1'}$= 10.4 Hz, $J_{2',3'}$= 0.8 Hz, H-2' allyl); 4.48 (dd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 5.2 Hz, H-2); 3.74 (dd, 1H, $J_{6',5'}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.69 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, , H-6);

5.20 (t, 1H, $J_{3,2}$= $J_{3,4}$= 6.8 Hz, H-3); 3.46 (dd, 1H, $J_{4,3}$ = 6.8 Hz, $J_{4,5}$= 8.8 Hz, H-4); 3.51-3.30 (m, 3H, 2xH4' allyl and H-5); ^{13}C NMR (100.6 MHz, CD_3OD) δ in ppm: 171.8 (C=N); 134.5 (=CH allyl); 119.6 (=CH_2 allyl); 94.4 (C-1); 84.4 (C-2); 75.9 (C-5); 75.6 (C-3); 69.9 (C-4); 63.3 (C-6); 35.5 (CH_2-N, allyl). +TOF MS Calcd for $C_{10}H_{15}NO_5S$ *m/z* $[M-H]^+$: 262.0749, found: 262.0737.

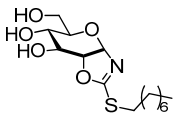


1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-butylsulfanyl-1,3-oxazoline (7.14): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.5** (50.1 mg, 0.12 mmol), MeONa (3.8 mg, 6 μmol) and MeOH (2.5 mL). The reaction mixture was stirred at rt for 30 min. After standard workup, the crude was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to afford the desired compound (31.0 mg, 90% yield) as colourless syrup. Data: R_f (1:9 MeOH/CH_2Cl_2): 0.16. $[\alpha]_D$ + 92.4 (*c* 2.50, MeOH). FT-IR (neat) in υ cm^{-1}: 3342, 2930, 2872, 1579, 1455, 1294, 1144, 1114, 952. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 5.77 (d, 1H, $J_{1,2}$= 7.2 Hz, H-1); 4.47 (dd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 5.2 Hz, H-2); 3.78 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.72 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 3.68 (dd, 1H, $J_{3,2}$= 5.2 Hz, $J_{3,4}$= 6.8 Hz, H-3); 3.47 (dd, 1H, $J_{4,3}$= 6.8 Hz, $J_{4,5}$= 9.2 Hz, H-4); 3.34-3.29 (m, 1H, H-5); 3.03 (t, 2H, *J*= 7.2 Hz, CH_2 aliph); 1.71-1.65 (m, 2H, CH_2 aliph); 1.47-1.41 (m, 2H, CH_2 aliph); 0.92 (t, 3H, *J*= 7.2 Hz, CH_3 aliph). ^{13}C NMR (100.6 MHz, CD_3OD) δ in ppm: 170.8 (C=N); 92.4 (C-1); 82.3 (C-2); 74.0 (C-5); 73.7 (C-3); 68.0 (C-4); 61.4 (C-6); 31.4, 30.7, 21.3 (CH_2, aliph); 12.5 (CH_3, aliph). +TOF MS Calcd for $C_{11}H_{19}NO_5S$ *m/z* $[M-H]^+$: 278.1062, found: 278.1054.

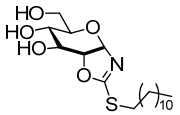


1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-heptylsulfanyl-1,3-oxazoline (7.15): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.6** (51.6 mg, 0.12 mmol), MeONa (3.8 mg, 6 μmol) and MeOH (2.5 mL). The reaction mixture was stirred at rt for 30 min. After standard workup, the crude was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to afford the desired compound (33.0 mg, 89% yield) as a white solid. Data: R_f (0.5:9.5 MeOH/CH_2Cl_2): 0.09. Mp: 48-50 ºC. $[\alpha]_D$ +

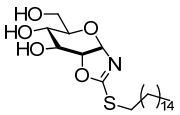
80.2 (*c* 5.50, MeOH). FT-IR (neat) in υ cm^{-1}: 3372, 2925, 2856, 1582, 1456, 1295, 1149, 1117, 954. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 5.75 (d, 1H, $J_{1,2}$= 7.2 Hz, H-1); 4.44 (dd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 5.2 Hz, H-2); 3.75 (dd, 1H, $J_{5,6'}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.66 (dd, 1H, $J_{6,6'}$= 12 Hz, $J_{6,5}$= 5.2 Hz, H-6); 3.66 (dd, 1H, $J_{3,2}$= 5.2 Hz, $J_{3,4}$= 6.8 Hz, H-3); 3.44 (dd, 1H, $J_{4,3}$= 6.8 Hz, $J_{4,5}$= 9.2 Hz, H-4); 3.32-3.27 (m, 1H, H-5); 3.0 (t, 2H, *J*= 7.2 Hz, CH_2 aliph); 1.72-1.65 (m, 2H, CH_2 aliph); 1.40-1.25 (m, 8H, 4x CH_2 aliph); 0.87 (t, 3H, *J*= 7.2 Hz, CH_3 aliph). ^{13}C NMR (100.6 MHz, CD_3OD) δ in ppm: 172.7 (C=N); 94.2 (C-1); 84.2 (C-2); 75.9 (C-5); 75.6 (C-3); 69.9 (C-4); 63.4 (C-6); 33.4, 32.9, 31.2, 30.4, 30.1, 24.1 (CH_2, aliph); 14.9 (CH_3, aliph). +TOF MS Calcd for $C_{14}H_{25}NO_5S$ *m/z* $[M-H]^+$: 320.1532, found: 320.1519.



1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-octylsulfanyl-1,3-oxazoline (7.16): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.7** (73.2 mg, 0.16 mmol), MeONa (5.1 mg, 8 μmol) and MeOH (3.3 mL). The reaction mixture was stirred at rt for 30 min. After standard workup, the crude was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to afford the desired compound (51.0 mg, 96% yield) as a white solid. Data: R_f (5:95 MeOH/CH_2Cl_2): 0.16. Mp: 56-58 ºC. $[\alpha]_D$ + 44.7 (*c* 5.60, MeOH). FT-IR (neat) in υ cm^{-1}: 3259, 2923, 2854, 1578, 1467, 1352, 1289, 1163, 1099, 1046, 957. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 5.75 (d, 1H, $J_{1,2}$= 7.2 Hz, H-1); 4.44 (dd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 5.2 Hz, H-2); 3.75 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.70 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 3.66 (dd, 1H, $J_{3,2}$= 5.2 Hz, $J_{3,4}$= 6.8 Hz, H-3); 3.44 (dd, 1H, $J_{4,3}$= 6.8 Hz, $J_{4,5}$= 9.2 Hz, H-4); 3.31-3.26 (m, 1H, H-5); 2.99 (t, 2H, $J_{1,2}$= 7.6 Hz, CH_2 aliph); 1.71-1.64 (m, 2H, CH_2 aliph); 1.40-1.27 (m, 10H, 5x CH_2 aliph); 0.87 (t, 3H, *J*= 7.2 Hz, CH_3 aliph). ^{13}C NMR (100.6 MHz, CD_3OD) δ in ppm: 171.7 (C=N); 93.3 (C-1); 83.2 (C-2); 74.7 (C-5); 73.6 (C-3); 68.9 (C-4); 62.4 (C-6); 32.5, 32.0, 30.2, 29.8, 29.7, 29.2, 23.2 (CH_2, aliph) 13.95 (CH_3, aliph). +TOF MS Calcd for $C_{15}H_{27}NO_5S$ *m/z* $[M-H]^+$: 334.1688, found: 334.1672.

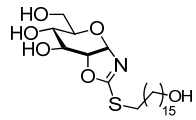


1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-dodecylsulfanyl-1,3-oxazoline (7.17): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.8** (49.17 mg, 0.10 mmol), MeONa (2.7 mg, 4 μmol) and MeOH (1.7 mL). The reaction mixture was stirred at rt for 30 min. After standard workup, the crude was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to afford the desired compound (36.0 mg, 97% yield) as colourless syrup. Data: R_f (5:95 MeOH/CH_2Cl_2): 0.26. $[\alpha]_D$ + 50.6 (*c* 1.00, MeOH). FT-IR (neat) in υ cm^{-1}: 3300, 2919, 2850, 1573, 1468, 1349, 1292, 1163, 959. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 5.8 (d, 1H, $J_{1,2}$= 7.2 Hz, H-1); 4.47 (dd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 5.2 Hz, H-2); 3.74 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.69 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 3.65 (dd, 1H, $J_{3,2}$= 5.2 Hz, $J_{4,3}$= 6.8 Hz, H-3); 3.43 (dd, 1H, $J_{4,3}$= 6.8 Hz, $J_{4,5}$= 8.8 Hz, H-4); 3.3-3.26 (m, 1H, H-5); 3.03 (t, 2H, *J*= 7.6 Hz, CH_2 aliph); 1.71-1.63 (m, 2H, CH_2 aliph) 1.39-1.25 (m, 18H, 9x CH_2 aliph); 0.92 (t, 3H, *J*= 7.6 Hz, CH_3 aliph). ^{13}C NMR (100.6 MHz, CD_3OD) δ in ppm: 172.7 (C=N); 94.3 (C-1); 84.3 (C-2); 75.9 (C-5); 75.6 (C-3); 69.9 (C-4); 63.4 (C-6); 33.6, 33.0, 31.3, 31.2, 31.1, 31.0, 30.7, 30.2, 24.2 (CH_2, aliph); 15.0 (CH_3, aliph). +TOF MS Calcd for $C_{19}H_{35}NO_5S$ *m/z* $[M-H]^+$: 390.2314, found: 390.2298.

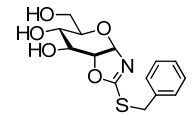


1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-hexadecylsulfanyl-1,3-oxazoline (7.18): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.9** (83.7 mg, 0.15 mmol), MeONa (0.5 mg, 9 μmol) and MeOH (3.0 mL). The reaction mixture was stirred at rt for 20 h. After standard workup, the crude was purified by flash chromatography (2:98 MeOH/CH_2Cl_2) to afford the desired compound (60.1 mg, 92% yield) as a white solid. Data: Rf (1:1 AcOEt/hexane): 0.00. Mp: 81-82 ºC. $[\alpha]_D$: +0.58 (*c* 1.22, CD_3OD). FT-IR (neat) υ in cm^{-1}: 3352, 2916, 2848, 1745, 1592, 1369, 1241, 1219, 1166, 1041. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 5.79 (d, 1H, $J_{1,2}$= 7.6 Hz, H-1); 4.47 (dd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 5.2 Hz, H-2); 3.78 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$= 12.0 Hz, H-6'); 3.73 (dd, 1H, $J_{6',5}$= 5.2 Hz, $J_{6',6}$=12.0 Hz, H-6); 3.69 (dd, 1H, $J_{3,2}$= 5.2 Hz, $J_{3,4}$= 6.8, H-3); 3.48 (dd, 1H, $J_{4,3}$= 6.8

Hz, $J_{4,5}$= 9.0 Hz, H-4); 3.32 (m, 1H, H-5); 3.03 (t, 2H, *J*= 7.2 Hz, CH_2-N aliph); 1.72 (q, 2H, *J*= 7.6 Hz, aliph); 1.44-1.22 (m, 26H, aliph); 0.90 (t, 3H, *J*= 7.0, CH_3 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 172.7 (C=N); 94.3 (C-1); 84.3 (C-2); 75.9 (C-5); 75.6 (C-3); 69.9 (C-4); 63.4 (C-6); 33.6, 33.0, 31.3, 31.2, 31.1, 31.0, 30.7, 30.2, 24.3 (CH_2 aliph); 15.0 (CH_3 aliph). +TOF MS Calcd for $C_{23}H_{43}NO_5S$ *m/z* [M-H]+: 446.2840, found: 446.2820.

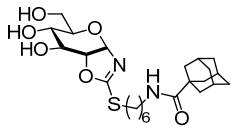


1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-(16-hydroxyhexadecyl)sulfanyl-1,3-oxazoline (7.19): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.10** (95.5 mg, 0.16 mmol), MeONa (0.6 mg, 10 μmol) and MeOH (3.2 mL). The reaction mixture was stirred at rt for 20 h. After standard workup, the crude was purified by flash chromatography (2:98 MeOH/CH_2Cl_2) to afford the desired compound (66.8 mg, 89% yield) as colourless syrup. Data: Rf (1:1 AcOEt/hexane): 0.00. $[\alpha]_D$: +0.67 (*c* 1.15, CD_3OD). FT-IR (neat) υ in cm^{-1}: 3397, 2918, 2849, 1745, 1587, 1464, 1158, 1219, 1039, 986. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 5.78 (d, 1H, $J_{1,2}$= 7.0 Hz, H-1); 4.47 (dd, 1H, $J_{2,1}$= 7.0 Hz, $J_{2,3}$= 5.0 Hz, H-2); 3.77 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$=11.6 Hz, H-6'); 3.72 (dd, 1H, $J_{6',5}$= 5.4 Hz, $J_{6',6}$=11.6 Hz, H-6); 3.69 (dd, 1H, $J_{3,2}$= 5.2 Hz, $J_{3,4}$= 6.4, H-3); 3.53 (t, 2H, *J*= 6.6 Hz, CH_2-OH aliph); 3.48 (dd, 1H, $J_{4,3}$= 6.4 Hz, $J_{4,5}$= 8.8 Hz, H-4); 3.31 (m, 1H, H-5); 3.03 (t, 2H, *J*= 7.4 Hz, CH_2-N aliph); 1.71 (q, 2H, *J*= 7.6 Hz, aliph); 1.53 (q, 2H, *J*= 6.8 Hz, aliph); 1.42-1.23 (m, 26H, aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 172.4 (C=N); 94.0 (C-1); 83.9 (C-2); 75.6 (C-5); 75.3 (C-3); 69.6 (C-4); 63.2 (C-6); 63.1 (CH_2-OH); 33.8, 32.6, 30.9, 30.9, 30.8, 30.8, 30.7, 30.4, 29.8, 27.1 (CH_2 aliph). +TOF MS Calcd for $C_{23}H_{43}NO_6S$ *m/z* [M-H]+: 462.2884, found: 463.2888; [M-Na]+: 484.2703, found: 484.2684.



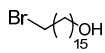
1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-benzylsulfanyl-1,3-oxazoline (7.20): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.11** (100 mg, 0.23 mmol), MeONa (6.8 mg, 12 μmol) and MeOH (4.6 mL). The reaction mixture

was stirred at rt for 30 min. After standard workup, the crude was purified by flash chromatography (1:9 MeOH/CH_2Cl_2) to afford the desired compound (70.0 mg, 98% yield) as colourless syrup. Data: R_f (1:9 MeOH/CH_2Cl_2): 0.16. $[\alpha]_D$ + 68.9 (*c* 1.00, MeOH). FT-IR (neat) in υ cm^{-1}: 3345, 2923, 1579, 1495, 1453, 1296, 1144, 1113, 1045, 993, 949. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 7.39-7.22 (stack, 5H, Ar); 5.78 (d, 1H, $J_{1,2}$= 7.2 Hz, H-1); 4.47 (dd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 5.2 Hz, H-2); 4.29 (d, 1H, J_{AB}= 13.4 Hz, CH_2Ph); 4.26 (d, 1H, J_{AB}= 13.4 Hz, CH_2Ph); 3.74 (dd, 1H, $J_{5,6'}$= 2.8 Hz, $J_{6',6}$= 12 Hz, H-6'); 3.69 (dd, 1H, $J_{6,5}$= 5.2 Hz, $J_{6,6'}$= 12 Hz, H-6); 3.65 (dd, 1H, $J_{3,2}$= 5.2 Hz, $J_{3,4}$= 6.8 Hz, H-3); 3.45 (dd, 1H, $J_{4,3}$= 6.8 Hz, $J_{4,5}$= 8.8 Hz, H-4); 3.30-3.26 (m, 1H, H-5). ^{13}C NMR (100.6 MHz, CD_3OD) δ in ppm: 171.6 (C=NH); 138.1 (C, Ph); 130.2, 129.9, 128.9 (CH, Ph); 94.1 (C-1); 84.2 (C-2); 75.6 (C-5); 75.3 (C-3); 69.6 (C-4); 63.0 (C-6); 36.8 (CH_2Ph). +TOF MS Calcd for $C_{14}H_{17}NO_5S$ *m/z* $[M-H]^+$: 312.0906, found: 312.0895.

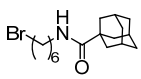


1,2-Dideoxy-α-D-glucopyranoside[1,2-d]-(6-(1-adamantanecarboxamido)-hexyl)sulfanyl-1,3-oxazoline (7.21): the title compound was prepared following the general procedure for acetyl deprotection of *cis*-1,2-fused 1,3-oxazoline carbohydrate derivatives starting from **7.12** (36.9 mg, 0.06 mmol), MeONa (0.2 mg, 3 μmol) and MeOH (1.2 mL). The reaction mixture was stirred at rt for 5 h. After standard workup, the crude was purified by flash chromatography (2:98 MeOH/CH_2Cl_2) to afford the desired compound (29.0 mg, 99% yield) as colourless syrup. Data: Rf (1:1 AcOEt/hexane): 0.00. $[\alpha]_D$: +0.54 (*c* 1.29, CD_3OD). FT-IR (neat) υ in cm^{-1}: 3334, 2904, 2850, 1630, 1587, 1530, 1451, 1367, 1289, 1097. ^{1}H NMR (400 MHz, CD_3OD) δ in ppm: 5.78 (d, 1H, $J_{1,2}$= 7.2 Hz, H-1); 4.47 (dd, 1H, $J_{2,1}$= 7.2 Hz, $J_{2,3}$= 5.2 Hz, H-2); 3.78 (dd, 1H, $J_{6',5}$= 2.8 Hz, $J_{6',6}$=12.0 Hz, H-6'); 3.73 (dd, 1H, $J_{6',5}$= 5.2 Hz, $J_{6',6}$=12.0 Hz, H-6); 3.69 (dd, 1H, $J_{3,2}$= 5.2 Hz, $J_{3,4}$= 6.8, H-3); 3.47 (dd, 1H, $J_{4,3}$= 6.8 Hz, $J_{4,5}$= 9.2 Hz, H-4); 3.32 (m, 1H, H-5); 3.15 (t, 2H, *J*= 7.0 Hz, CH_2-NH); 3.03 (t, 2H, *J*= 7.4 Hz, CH_2-S); 1.98 (m, 3H, H-3' Adam); 1.81 (m, 6H, H-2' Adam); 1.76-1.65 (m, 8H, H-4' Adam, CH_2 aliph); 1.50-1.24 (m, 6H, 3xCH_2 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 181.0 (C(O)); 172.3 (C=N); 94.0 (C-1); 83.9 (C-2); 75.6 (C-5); 75.2 (C-3); 69.6 (C-4); 63.1 (C-6); 41.9 (C-1' Adam); 40.4 (3x C-2' Adam); 40.3 (CH_2-NH); 37.8 (3x C-4 Adam); 32.5 (CH_2-S); 30.8, 30.5 (CH_2 aliph.);

29.8 (3x C-3 Adam); 29.4, 27.5 (CH_2 aliph.). +TOF MS Calcd for $C_{24}H_{38}NO_6S$ *m/z* [M-H]+: 483.2523, found: 483.2515; [M-Na]+: 505.2343, found: 505.2335.

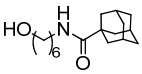


16-Bromohexadecanol (7.22):[52] a solution of 16-bromohexadecanoic acid (650 mg, 1.94 mmol) was dissolved in dry THF (3.5 mL) and cooled to 0 ºC. Borane 1 M in THF (2.60 mL, 2.58 mmol) was added dropwise via an addition funnel and the mixture was allowed to come to room temperature before refluxing for 2 h. Upon completion, the reaction was quenched dropwise with water at 0 ºC and the THF was removed by rotoevaporation. The crude was added to a separatory funnel with 5 mL of water and was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$ and filtered. The concentration afforded the desired compound as off-white crystals (592 mg, 95%). Data: R_f (4:6 AcOEt/Hexane): 0.43. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 3.62 (t, 2H, *J*= 6.8 Hz, CH_2-Br); 3.42 (t, 2H, *J*= 6.8 Hz, CH_2-OH); 2.34 (bs, 1H, OH); 1.82 (quint, 2H, *J*= 6.8 Hz, CH_2 aliph.); 1.56-1.17 (m, 26H, aliph.). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 63.1 (CH_2-OH); 34.1 (CH_2-Br); 32.9, 32.9, 29.8, 29.7, 29.6, 29.5, 28.9, 28.3, 25.9 (CH_2 aliph).



***N*-(6-bromohexyl)-1-adamantanecarboxamide (7.23):** to a 0 ºC refrigerated mixture of *N*-(6-hydroxyhexyl)-1-adamantanecarboxamide (**7.24**, 432 mg, 1.55 mmol) and carbon tetrabromide (1.380 g, 4.16 mmol) in methylene chloride (5.6 mL) was added triphenylphosphine (582 mg, 2.22 mmol). The reaction mixture was then allowed to warm to room temperature and stirred for 6 hours before it was concentrated under reduced pressure. The residue was purified by flash chromatography (2:1 AcOEt/hexane) to afford the desired compound (466 mg, 92% yield) as a white solid. Data: R_f (2:1 AcOEt/Hexane): 0.40. Mp: 57-58 ºC. FT-IR (neat) υ in cm^{-1}: 3306, 2914, 2899, 2849, 1620, 1325. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.73 (m, 1H, NH); 3.34 (t, 2H, *J* = 6.8 Hz, CH_2-Br); 3.13 (q, 2H, *J*= 7.2 Hz, CH_2-NH); 1.97 (m, 3H, H-3 Adam); 1.78 (m, 6H, H-2 Adam); 1.64 (m, 8H, H-4 Adam, CH_2 aliph); 1.49-1.35 (m, 4H, 2xCH_2 aliph.); 1.31-1.18 (m 2H, CH_2 aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 177.9 (C(O)); 40.5 (C-1 Adam); 39.3 (3x C-2 Adam); 39.1 (CH_2-NH); 36.5 (3x C-4 Adam); 33.9 (CH_2-Br); 32.6, 29.5, (CH_2

aliph.); 28.1 (3x C-3 Adam); 27.8, 26.4 (CH_2 aliph.). +TOF MS Calcd for $C_{17}H_{29}BrNO$ *m/z* [M-H]+: 342.1427, found: 342.1406.



***N*-(6-hydroxyhexyl)-1-adamantanecarboxamide (7.24):** to a solution of 1-adamantanecarbonyl chloride (108 mg, 0.54 mmol) in CH_2Cl_2 (10 mL) were added 6-aminohexan-1-ol (71 mg, 0.59 mmol) and Et_3N (247 μL, 1.64 mmol) followed by stirring at room temperature overnight. The mixture was washed with saturated $NaHCO_3$ and brine, dried, and concentrated to afford the desired compound quantitative as a white solid (149 mg, >99%). Data: R_f (4:6 AcOEt/Hexane): 0.38. Mp: 89-91 ºC. FT-IR (neat) υ in cm^{-1}: 3468, 3314, 2915, 2901, 2850, 2850, 1605, 1293. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 5.82 (m, 1H, NH); 3.51 (t, 1H, *J*= 6.6 Hz, CH_2-OH); 3.34 (bs, 1H, OH); 3.13 (q, 2H, *J*= 6.8 Hz, CH_2-NH); 1.94 (m, 3H, H-3 Adam); 1.75 (m, 6H, H-2 Adam); 1.62 (m, 6H, H-4 Adam); 1.51-1.36 (m, 4H, aliph.); 1.34-1.18 (m 4H, aliph). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ in ppm: 178.2 (C(O)); 62.1 (CH_2-OH); 40.5 (C-1 Adam); 39.2 (3x C-2 Adam); 39.0 (CH_2-NH); 36.4 (3x C-4 Adam); 32.4, 29.5 (CH_2 aliph.); 28.1 (3x C-3 Adam); 26.4, 35.5 (CH_2 aliph.). +TOF MS Calcd for $C_{17}H_{29}NO_2$ *m/z* [M-H]+: 280.2277, found: 280.2262.

7.4.4. K_i determination of **7.19** against bovine liver β-glucosidase

Table 7.7 Absorbances measured for **7.19** against bovine liver β-glucosidase[a]

[S] (mM)	1 /[S]	Concentration of inhibitor (μM)								
		0	**4**	**8**	**16**	**32**	**40**	**8[b]**	**16[b]**	**32[b]**
0,5	2,0000	1,3441	1,8232	1,9286	2,7064	3,5971	4,8426	-	2,5627	4,7339
1	1,0000	1,0616	1,3141	1,5163	2,2346	2,0471	2,8736	1,5753	2,0909	2,7649
2	0,5000	0,8496	0,9838	1,0272	1,3414	1,4327	1,8605	1,0862	1,1977	1,7518
4	0,2500	0,7345	0,8120	0,8547	0,9766	1,0588	1,2928	0,9137	0,8329	1,1841
6	0,1667	0,6741	0,7541	0,7660	0,8639	0,9398	1,0678	0,8250	0,7202	0,9591

[a] Values (1/Abs) determined spectrophotometrically at 405 nm measuring the residual hydrolytic activities of the glycosidases against the p-nitrophenyl β-D-glucopyranoside. [b] Corrections due temperature variation.

Table 7.8 Calculations of K_i for **7.19** against bovine liver β-glucosidase

[S]	[I] (mM)	n	Vmax = 1/n	m	Kapp = m*Vmax
1.6	0,000	0,6511	1,5359	0,3596	0,5523
0.8	0,004	0,6803	1,4699	0,5836	0,8579
0.4	0,008	0,6731	1,4857	0,8910	1,3237
0.2	0,016	0,6731	1,4857	1,0313	1,5322
0.1	0,032	0,6878	1,4539	1,4391	2,0923
-	0,040	0,6731	1,4857	2,0418	3,0334

7.5.5. IC_{50} determination against β-glucerebrosidase

Table 7.9 In vitro determination of of GCase activity of **7.19** and ABX against GCase[a]

Compound	-log ([I])							
	10	**9**	**8**	**7**	**6**	**5**	**4**	**3**
ABX	101	105	108	97	99	70	41	22
7.19	109	118	113	108	90	81	53	33

[a] Values determined spectrophotometrically at 460 nm measuring the residual hydrolytic activities of the glycosidases against the 4-methylumbelliferyl β-D-glycopyranoside.

7.5. References

1. Simão, A. C.; Rousseau, J. ; Silva, S. ; Rauter, A. P.; Tatibouët, A. ; Rollin, P. *Thionocarbamates on carbohydrate scaffolds—from synthesis to bioactivity* (in Carbohydrate Chemistry. Chemical and Biological Approaches, 35), RSC Publishing, Dorchester, **2009**, *35*, 127-172.
2. Girniene, J.; Tatibouët, A.; Sackus, A.; Yang, J.; Holman, G. D.; Rollin, P. *Carbohydr. Res.* **2003**, *338*, 711-719.
3. Dallemagne, P.; Khanh L. P.; Alsaïdi, A.; Varlet, I.; Collot, V.; Paillet, M.; Bureau, R.; Rault, S. *Bioorg. Med. Chem.* **2003**, *11*, 1161-1167.
4. Barbachyn M. R.; Ford, C. W. *Angew. Chem. Int. Ed.* **2003**, *42*, 2010-2023.
5. Bortone, K.; Monzigno, A. F.; Stephen, E.; Robertus, J. D. *J. Mol. Biol.* **2002**, *320*, 293-302.
6. a) López, O.; Zafra, E.; Maya, I.; Fuentes, J.; Diánez, M. J.; Estrada, M. D.; Pérez-Garrido, S.; Fernández-Bolaños, J. G. *Tetrahedron* **2008**, *64*, 11789-11796. b) Maya, I.; López, O.; Fernández-Bolaños J. G.; Robina, I.; Fuentes, J. *Tetrahedron Lett.* **2001**, *42*, 5413-5416. c) López, O.; Maya, I.; Fuentes, J.; Fernández-Bolaños, J. G. *Tetrahedron* **2004**, *60*, 61-72. d) Isac-García, J.; Hernández-Mateo, F.; Calvo-Flores, F. G.; Santoyo-González, F. *J. Org. Chem.* **2004**, *69*, 202-205.

7. a) Díaz Pérez, V. M.; García Moreno, M. I.; Órtiz, Mellet, C.; Fuentes, J.; Díaz Arribas, J. C.; Cañada, F. J.; García Fernández, J. M. *J. Org. Chem.* **2000**, *65*, 136-143. b) Jiménez Blanco, J. L.; Díaz Pérez, V. M.; Órtiz, Mellet, C.; Fuentes, J. M.; García Fernández, J. C.; Díaz Arribas, J. C.; Cañada, F. J. *J. Chem. Soc., Chem. Commun.* **1997**, 1969-1970.
8. a) Gosselin, G.; Bergogne, M.-C.; Imbach, J.-L. *Nucleosides Nucleotides* **1990**, *9*, 81-87. b) Grouiller, A.; Mackenzie, G.; Najib, B.; Shaw, G.; Ewig, D. *J. Chem. Soc., Chem. Commun,* **1988**, 671-672. c) Gosselin, G.; Bergogne, M.-C.; Rudder, J.; Clerq, E.; Imbach, J.-L. *J. Med. Chem.* **1986**, *29*, 203-213. d) Rayner, B.; Tapiero, C.; Imbach, J.-L. *J. Heterocycl. Chem.* **1982**, *19*, 593-596. e) Ranganathan, R. *Tetrahedron Lett.* **1977**, *15*, 1291-1294
9. Kifli, N.; Htar, T. T.; De Clercq, E.; Balzarini, J.; Simons, C. *Bioorg. Med. Chem.* **2004**, *12*, 3247-3257.
10. a) Durantel, D.; Alotte, C.; Zoulim, F. *Curr. Opin. Investig. Drugs* **2007**, *8*, 125-129. b) Greimel, P.; Spreitz, J.; Stütz, A. E.; Wrodnigg, T. M. *Curr. Top. Med. Chem.* **2003**, *3*, 513-523.
11. a) Wrodnigg, T. M.; Steiner, A. J.; Ueberbacher, B. J. *Anticancer Agents Med. Chem.* **2008**, *8*, 77-85. b) Sun, J.-Y.; Zhu, M.-Z.; Wang, S.-W.; Miao, S.; Xie, Y.-H.; Wang, J.-B. *Phytomedicine* **2007**, *14*, 353-359. c) Paulsen, H.; Brockhausen, I. *Glycoconjugate J.* **2001**, *18*, 867-870.
12. a) Anzeveno, P. B.; Creemer, L. J.; Daniel, J. K.; King, C.-H. R.; Liu, P. S. *J. Org. Chem*. **1989**, *54*, 2539-2542. b) Balfour, J. A.; McTavish, D. *Drugs* **1993**, *46*, 1025-1054. c) Tsujino, D; Nishimura, R; Taki, K.; Morimoto, A.; Tajima, N.; Utsunomiya, K. *Diabetes Technol. Ther.* **2011**, *13*, 303-308.
13. a) Cren, S.; Gurcha, S. S.; Blake, A. J.; Besra, G. S.; Thomas, N. R. *Org. Biomol. Chem*. **2004**, *2*, 2418-2420. b) Wrodnigg, T. M.; Sprenger, F. K. *Mini-Rev. Med. Chem*. **2004**, *4*, 437-59.
14. a) Benito, J. M.; García Fernández, J. M.; Ortiz Mellet, C. *Expert Opin. Ther. Patents* **2011**, *21*, 885-903. b) Parenti, G. *EMBO Mol. Med.* **2009**, *1*, 268-279. c) Wennekes, T.; van den Berg, R. J. B. H. N.; Boot, R. G.; van der Marel, G. A.; Overkleeft, H. S.; Aerts, J. M. F. G. *Angew. Chem. Int. Ed.* **2009**, 8848-8869. d) Fan, J. Q. *Biol. Chem.* **2007**, *389*, 1-11. e) Futerman, A. H.; van Meer G. *Nat. Rev. Mol. Cell. Biol.* **2004**, *5*, 554-565. f) Meikle, P. J.; Fietz, M. J.; Hopwood, J. J. *Expert. Rev. Mol. Diagn.* **2004**, *4*, 677-691.
15. a) Alonzi, D. S.; Butters, T. D. *Chimia* **2011**, *65*, 35-39. b) Butters, T. D.; Dwek, R. A.; Platt, F. M. *Chem. Rev.* **2000**, *100*, 4683-4696.
16. Zheng, W.; Padia, J.; Urban, D. J.; Jadhav, A.; Goker-Alpan, O.; Simeonov, A.; Goldin, E.; Auld, D.; LaMarca, M. E.; Inglese, J.; Austin, C. P.; Sidransky, E. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 13192-13197. b) Marugan, J. J.; Zheng, W.; Motabar, O.; Southall,

N.; Goldin, E.; Westbroek, W.; Stubblefield, B. K.; Sidransky, E.; Aungst, R. A.; Lea, W. A.; Simeonov, A.; Leister, W.;. Austin, C. P. *J. Med. Chem.* **2011**, *54*, 1033-1058.

17. Asano, N., Oseki, K.; Tomioka, E.; Kizu, H.; Matsui, K. *Carbohydr. Res*. **1994**, *259*, 243-255.
18. Winchester, B. G.; Cenci di Bello, I.; Richardson, A. C.; Nash, R. J.; Fellows, L. E.; Ramsden, N. G.; Fleet, G. W. J. *Biochem. J*. **1990**, *269*, 227-231.
19. Horne, G.; Wilson, F. C.; Tinsley, J.; Willians, D. H.; Storer, R. *Drug. Discov. Today* **2011**, *16*, 107-118.
20. For reviews, see: a) Dragutan, I.; Dragutan, V.; Mitan, C.; Vosloo, H. C. M.; Lionel Delaude, L.; Demonceau, A. *Beilstein J. Org. Chem.* **2011**, 7, 699-716. b) Smid, B. E.; Aerts, J. M. F. G.; Boot, R. G.; Linthorst, G. E.; Hollack, C. E. M. *Expert Opin. Invest. Drugs* **2010**, *19*, 1367-1379. c) Broges de Melo, E.; da Silveira Gome, A.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277-10302. d) Pearson, M. S. M.; Mathé-Allainmat, M.; Fargeas, V.; Lebreton, J. *Eur. J. Org. Chem.* **2005**, 2159-2191. e) Afarinkia, K.; Bahar, A. *Tetrahedron: Asymmetry* **2005**, *16*, 1239-1287. f) Germain, D. P. *Clin. Genet*. **2004**, *65*, 77-86. g) Cipolla, L.; La Ferla, B.; Nicotra, F. *Curr. Top. Med. Chem*. **2003**, *3*, 1349-1364. h) Compain, P.; Martin, O. R. *Curr. Top. Med. Chem*. **2003**, *3*, 541-560. i) Asano, N. *Curr. Top. Med. Chem*. **2003**, *3*, 471-484. j) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515-553. k) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **2000**, *11*, 1645-1680.
21. a) Sawkar, A. R.; Cheng, W. C.; Beutler, E.; Wong, C.-H.; Balch, W. E.; Kelly, J. W. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15428-15433. b) Sawkar, A. R.; Adamski-Werner, S. L.; Cheng, W. E.; Wong, C.-H.; Beutler, E.; Zimmer, K. P.; Kelly, J. W. *Chem. Biol.* **2005**, *12*, 1235-1244.
22. Compain, P.; Martin, O. R.; Boucheron, C.; Godin, G.; Yu, L.; Ikeda, K.; Asano, N. *ChemBioChem* **2006**, *7*, 1356-1359.
23. a) Goddard-Borger, E.D.; Tropak, M. B.; Yonekawa, S.; Tysoe, C.; Mahuran, D. J.; Withers, S. G. *J. Med. Chem.* **2012**, *55*, 2737-2745. b) Diot, J. D.; Moreno, I. G.; Twigg, G.; Mellet, C. O.; Haupt, K.; Butters, T. D.; Kovensky, J.; Gouin, S. G. *J. Org. Chem.* **2011**, *76*, 7757-7768. c) Brumshtein, B.; Greenblatt, H. M.; Butters, T. D.; Shaaltiel, Y.; Aviezer, D.; Silman, I.; Futerman, A. H.; Sussman, J. L. *J. Biol. Chem.* **2007**, *282*, 29052-29058.
24. a) Aguilar-Moncayo, M.; Takai, T.; Higaki, K.; Mena-Barragán, T.; Hirano, Y.; Yura, K.; Li, L.; Yu, Y.; Ninomiya, H.; García-Moreno, M. I.; Ishii, S.; Sakakibara, Y.; Ohno, K.; Nanba, E.; Ortiz Mellet, C.; García Fernández, J. M.; Suzuki, Y. *Chem. Commun.* **2012**, *48*, 6514-6516. b) Aguilar-Moncayo, M.; García-Moreno, M. I.; Trapero, A.; Egido-Gabás, M.; Llebaria, M.; García Fernández, J. M.; Ortiz Mellet, C. *Org. Biomol. Chem.* **2011**, *9*, 3698-3713. c) Brumshtein, B.; Aguilar-Moncayo, M.; Benito, J. M.; García Fernández, J. M.; Silman, I.; Shaaltiel, Y.; Aviezer, D.; Sussman, J. L.; Futerman

A. H.; Ortiz Mellet, C. *Org. Biomol. Chem.* **2011**, *9*, 4160-4167. d) Aguilar-Moncayo, M.; García-Moreno, M. I.; Stütz, A. E.; García Fernández, J. M.; Wrodnigg, T. M.; Ortiz Mellet, C. *Bioorg. Med. Chem.* **2010**, *18*, 7439-7345. e) Aguilar-Moncayo, M.; Gloster, T. M.; Turkenburg, J. P.; García-Moreno, M. I.; Ortiz Mellet, C.; Davies, G. J.; García Fernández, J. M. *Org. Biomol. Chem.* **2009**, *7*, 2738-2747. f) Aguilar, M.; Díaz-Pérez, P.; García-Moreno, M. I.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2008**, 73, 1995-1998. g) Jiménez Blanco, J. L.; Díaz Pérez, V. M.; Ortiz Mellet, C. García Fernández, J. M.; Fuentes, J.; Díaz Arriba, J. C.; Cañana, F. J. *Chem. Commun.* **1997**, 1969-1970.

25. a) Sánchez-Fernández, E. M.; Rísquez-Cuadro, R.; Aguilar-Moncayo, M.; García-Moreno, M. I.; Ortiz Mellet, C.; García Fernández, J. M. *Org. Lett.* **2009**, *11*, 3306-3309. b) Sánchez-Fernández, E. M.; Rísquez-Cuadro, R.; Chasseraud, M.; Ahidouch, A.; García-Moreno, M. I.; Ortiz Mellet, C.; Ouadid-Ahidouch, H.; García Ferández, J. M. *Chem. Commun.* **2010**, 46, 5328-5330.
26. a) García-Moreno, M. I.; Díaz-Pérez, P.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2003**, *68*, 8890-8891. b) García-Moreno, M. I.; Díaz-Pérez, P.; Ortiz Mellet, C.; García Fernández, J. M. *Chem. Commun.* **2002**, 848-849.
27. a) Luan, Z.; Higaki, K.; Aguilar-Moncayo, M.; Ninomiya, H.; Ohno, K.; García-Moreno, M. I.; Ortiz Mellet, C.; García Fernández, J. M.; Suzuki, Y. *ChemBioChem* **2009**, *10*, 2780-2792. b) Luan, Z.; Higaki, K.; Aguilar-Moncayo, M.; Li, L.; Ninomiya, H.; Namba, E.; Ohno, K.; García-Moreno, M. I.; Ortiz Mellet, C.; García Fernández, J. M.; Suzuki, Y. *ChemBioChem* **2010**, *11*, 2453-2464.
28. Trapero, A.; Alfonso, I.; Butters, T. D.; Llebaria, A. *J. Am. Chem. Soc.* **2011**, *133*, 5474-5484.
29. a) Withers, S. G.; Namchuk, M.; Mosi R. *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*, Wiley-VCH, Weinheim, **1999**. b) Compain, P.; Martin, O. R. *Iminosugars: from Synthesis to Therapeutic Applications*, Wiley-VCH, Weinheim, **2007**.
30. Yuzwa, S. A.; Macauley, M. S.; Heinonen, J. E.; Shan, X. Y.; Dennis, R. J.; He, Y. A.; Whitworth, G. E.; Stubbs, K. A.; McEachern, E. J.; Davies, G. J.; Vocadlo, D. J. *Nat. Chem. Biol.* **2008**, *4*, 483-490.
31. a) Yuzwa, S. A.; Shan, X. Y.; Macauley, M. S.; Clark, T.; Skorobogatko, Y.; Vosseller, K.; Vocadlo, D. J. *Nat. Chem. Biol.* **2012**, *8*, 393-399. b) Yu, Y.; Zhang, L.; Li, X.; Run, X.; Liang, Z.; Li, Y.; Liu, Y.; Lee, M. H.; Grundke-Iqbal, I.; Iqbal, K.; Vocadlo, D. J.; Liu, F. Gong, C.-X. *PLoS One* **2012**, *4*, e35277.
32. For a very recent example of the influence of conformational aspects in the inhibitory activity of aminoglycosides, see: Sánchez-Fernández, E. M.; Rísquez-Cuadro, R.; Ortiz Mellet, C.; García Fernández, J. M.; Nieto, P. M.; Angulo, J. *Chem. Eur. J.* **2012**, in press, DOI: 10.1002/chem.201200279.

33. a) Kobata, A. *Anal. Biochem.* **1979**, *100*, 1-14. b) Kornfeld, R.; Kornfeld, S. *Annu. Rev. Biochem.* **1976**, *45*, 217-237. c) Marshal, J. J. *Ad. Carbohydr. Chem. Biochem.* **1974**, *30*, 257-370.
34. Asano, N. *Glycobiology* **2003**, *13*, 93R-104R.
35. Rempel, B. P.; Withers, S. G. *Glycobiology* **2008**, *18,* 570-586.
36. Cantarel, B.L.; Coutinho, P.M.; Rancurel, C.; Bernard T.; Lombard, V; Henrissat, B. *Nucleic Acids Res.* **2009**, *37,* D233- D238.
37. Henrissat, B.; Bairoch, A. *Biochem J.* **1996**, *316*, 695-696.
38. Koshland, D. E. *Biol. Rev.* **1953**, *28*, 416-436.
39. M. Bols, *Acc. Chem. Res.* **1998**, *31*, 1-8.
40. Cheshev, P.; Marra, A.; Dondoni, A. *Carbohydr. Res.*, **2006**, *341*, 2714-2716.
41. Castilla, J.; Marín, I.; Matheu. M. I.; Díaz, Y.; Castillón, S. *J. Org. Chem.* **2010**, *75*, 514-517. Corrigendum *J. Org. Chem.* **2012**, *77*, 3687.
42. Silva, S.; Balla Sylla, Suzenet, F.; Tatibouët, A.; Rauter, A. P.; Rollin, P. *Org. Lett.* **2008**, *10*, 853-856.
43. Ho, T. L. *Chem. Rev.* **1975**, *75*, 1-20 and references therein.
44. a) Silva, S.; Simão, A. C.; Tatibouët, A.; Rollin, P.; Rauter, A. P. *Tetrahedron Lett.* **2008**, *49*, 682-686. b) Tardy, S.; Tatibouët, A.; Rollin, P.; Dujardin G. *Synlett*, **2006**, *108*, 1425-1427. c) Girniene, J.; Tardy, S.; Tatibouët, A.; Sackus, A.; Rollin, P. *Tetrahedron Lett.* **2004**, *45*, 6443-6446. d) Gueyrard, D.; Leoni, O.; Palmieri, S.; Rollin, P. *Tetrahedron: Asymm.* **2001**, *12*, 337-340. e) Gueyrard, D.; Grumel, V.; Leoni, O.; Palmieri, S.; Rollin, P. *Heteroycles* **2000**, *52*, 827-843.
45. a) Mészaros, P.; Pínter, I.; Kóvacs, J.; Tóth, G. *Carbohydr. Res.* **1994**, *258*, 287-291. b) Davidson, R. M.; Byrd, G. D.; White, E.; Margolis, S. A.; Coxon, B. *Magn. Res. Chem.* **1986**, *24*, 929-937. c) Pridgen, L. N.; Killmer, L. B.; Webb, R. L. *J. Org. Chem.* **1982**, *47*, 1985-1989.
46. Brumshtein, B.; Aguilar-Moncayo, M.; García-Moreno, M. I.; Ortiz Mellet, C.; García Fernández, J. M.; Silman, I.; Shaaltiel, Y.; Aviezer, D.; Sussman, J. L.; Futerman A. H. *ChemBioChem* **2009**, *10*, 1480-1485.
47. Maegawa, G. H. B.; Tropak, M. B.; Buttner, J. D.; Rigat, B. A.; Fuller, M.; Pandit, S.; Tang, L.; Kornhaber, G. J.; Hamuro, Y.; Joe T. R. Clarke, J. T. R.; Mahuran, D. J. *J. Biol. Chem.* **2009**, *284*, 23502-23516.
48. a) Leandro, P; Gomes, C. M. *Mini Rev. Med. Chem.* **2008**, *8*, 901-911. b)Arakawa, T.; Ejima, D; Kita, Y; Tsumoto, K; *Biochim Biophys Acta* **2006**, *1764*, 1677-1687 c) Ulloa-Aguirre, A; Janovick, J. A.; Brothers S. P.; Conn, P.M. *Traffic.* **2004**, *5,* 821-837.
49. a) Iwasaki, H; Watanabe, H.; Iida, M.; Ogawa, S.; Tabe, M.; Higaki, K.; Nanba, E.; Suzuki, Y. *Brain Dev.* **2006**, *28*, 482-486. b) Lin, H.; Sugimoto, Y.; Ohsaki, Y.; Ninomiya, H.; Oka, A.; Taniguchi, M.; Ida, H.; Eto, Y.; Ogawa, S.; Matsuzaki, Y.; Sawa,

M.; Inoue, T.; Higaki, K.; Nanba, E.; Ohno, K.; Suzuki, Y. *Biochim. Biophys. Acta* **2004**, *1689*, 219-228.

50. Reactivos Merck *Reactivos de coloración para cromatografía en capa fina y papel* E. Merck. Darsmtadt (RF Alemana) **1980**.
51. a) Liu, H.; Sugimoto, Y.; Ohsaki, Y.; Ninomiya, H.; Oka, A.; Taniguchi, M.; Ida, H.; Eto, Y.; Ogawa, S.; Matsuzaki, Y.; Sawa, M.; Inoue, T.; Higaki, K.; Nanba, E.; Ohno, K.; Suzuki, Y. *Biochim. Biophys. Acta* **2004**, *1689*, 219-228. b) Iwasaki, H.; Watanabe, H.; Iida, M.; Ogawa, S.; Tabe, M.; Higaki, K.; Nanba, E.; Suzuki, Y. *Brain Dev.* **2006**, *28*, 482-486.
52. Fisher, G. L.; Walker, A. V.; Hooper, A. E.; Tighe, T. B.; Bahnck, K. B.; Skriba, H. T.; Reinard, M. D.; Haynie, B. C.; Opila, R. L.; Winograd, N.; Allara, D. L. *J. Am. Chem. Soc.* **2002**, *124*, 5528-5541.