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Monrad, Rune Nygaard; Madsen, Robert

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Methods for Shortening and Extending the Carbon Chain in Carbohydrates

Ph.D. Thesis

By

Rune Nygaard Monrad

December 2008

Department of Chemistry

Technical University of Denmark

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Preface

This thesis describes the work carried out during my three years as a Ph.D. student in Center for Sustainable and Green Chemistry at the Technical University of Denmark. In addition to research within the fields of organometallic and carbohydrate chemistry at the Technical University of Denmark, I had the opportunity to become acquainted with chemical biology during a six months research stay at University of Oxford. My period as a Ph.D. student has been very fruitful for me not only by means of education and achieving academic and technical qualifications, but also with respect to personal development as an individual and as a scientist. Many people have contributed to the present work, and most of all, I would like to express my gratitude to professor Robert Madsen. During the last three years of Ph.D. study, my theoretical and experimental skills have improved considerably. In particular, Robert Madsen's support and guidance through critical decisions and his ability to suggest improvements of both overall strategies and specific reaction conditions have had a huge impact on the success of the projects I have been involved in. The decarbonylation team: Mike Kreis and Esben Taarning are thanked for good discussions, and Charlotte B. Pipper and Mette Fanefjord are acknowledged for collaborations on the calystegine and gabosine projects. A special thanks goes to Lars Linderoth for lots of fun and great company in the lab. The Department of Chemistry building 201, in particular the Madsen group, are gratefully acknowledged for invaluable help and for always maintaining an enthusiastic and positive spirit. I would like to thank professor Benjamin G. Davis for giving me the opportunity to work within such an interesting field of research in an interdisciplinary and highly dynamic group. The entire Davis group, in particular James, Conor, Justin and Nicola are thanked for invaluable help and good times in the lab. I am grateful to professor Andrew V. Stachulski for providing acyl glucuronide samples. Furthermore, Katja Rohr-Gaubert and Thomas Jensen are thanked for proofreading parts of this thesis. Last but not least, the Technical University of Denmark, Center for Sustainable and Green Chemistry, Danish Chemical Society, Civilingeniør Frants Allings Legat, Vera & Carl Johan Michaelsens Legat, Ulla & Mogens Folmer Andersens Fond, Krista & Viggo Petersens Fond, Fabrikant P. A. Fiskers Fond, Knud Højgaards Fond, Otto Mønsteds Fond and Oticon Fonden are gratefully acknowledged for financial support.

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Rune Nygaard Monrad

Lyngby, December 2008

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Abstract

Carbohydrates play a central role in a variety of physiological and pathological processes such as HIV, cancer and diabetes. The understanding of these processes and the development of specific therapeutic agents is relying on the ability to chemically synthesize unnatural sugars, glycoconjugates and carbohydrate mimetics. Such polyhydroxylated compounds are conveniently synthesized from carbohydrates, however, due to the scarcity of many sugars from nature, efficient methods for transformation of readily available carbohydrates into valuable chiral building blocks are required. The work presented in this thesis focuses on the development and application of transition metal mediated methods for shortening and extending the carbon chain in carbohydrates thereby providing access to lower and higher sugars.

A new catalytic procedure for shortening unprotected sugars by one carbon atom has been developed. By means of a rhodium-catalyzed decarbonylation of the aldehyde functionality, aldoses are converted into their corresponding lower alditols in yields around 70%. The reaction is performed with 8% of the catalyst $Rh(dppp)_2Cl$ in the presence of small amounts of pyridine to facilitate mutarotation. The procedure has been employed as the key step in a short five-step synthesis of the unnatural sugar L-threose in 74% overall yield from D-glucose.
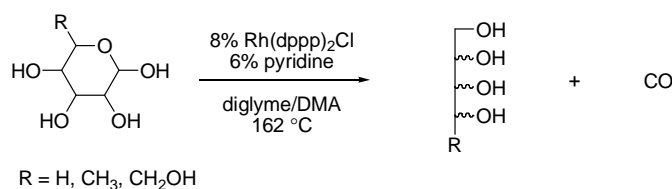
A zinc-mediated one-pot fragmentation-allylation reaction has been used to elongate D-glucose and D-ribose by three carbon atoms thereby producing carbohydrate-derived α,ω-dienes, which have been converted into the natural products calystegine A_3 and gabosine A. The glycosidase inhibitor calystegine A_3 was produced by two similar routes from commercially available methyl α-D-glucopyranoside in 13 and 14 steps with 8.3 and 5.3% overall yield, respectively. The present work thereby constitutes the shortest synthesis of enantiomerically pure calystegine A_3, and furthermore, it enables the absolute configuration of the natural product to be determined. Gabosine A has been prepared in nine steps and 13.9% overall yield from D-ribose, and this synthesis provides the first route to gabosine A from an abundant carbohydrate precursor.
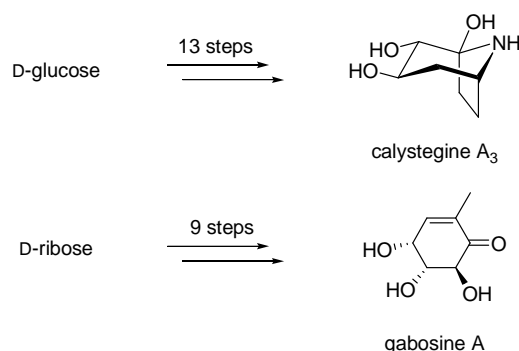
D-glucose — 13 steps → calystegine A$_3$

D-ribose — 9 steps → gabosine A

During an external stay at University of Oxford, the metabolism of nonsteroidal anti-inflammatory drugs (NSAIDs) has been investigated. It was found that known acyl glucuronide metabolites of ibuprofen and several analogues modify human plasma protein under conditions encountered in therapy. Two different kinds of protein modification occur depending on the structure of the parent drug. The obtained results strongly suggest that irreversible modification of human proteins takes place during treatment with carboxylic acid containing drugs such as NSAIDs. Furthermore, the observed reactivity of these metabolites with respect to protein modification may provide an explanation for the severe toxicity that has led to the withdrawal of certain carboxylate drugs.
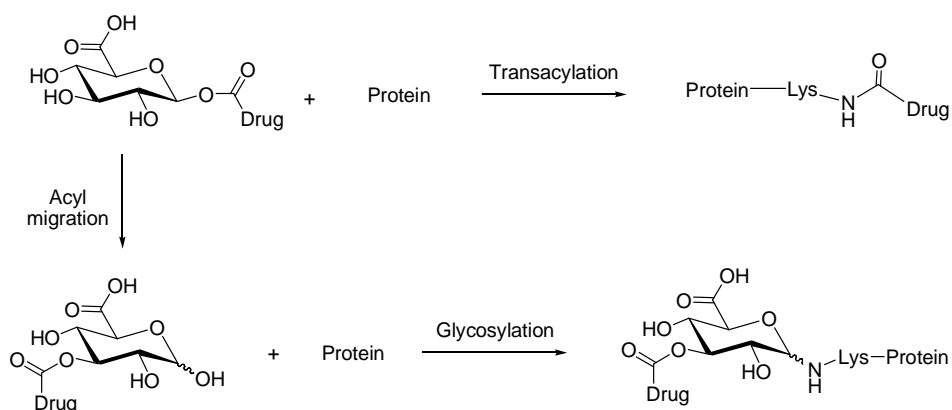
Resumé

Kulhydrater spiller en central rolle i mange forskellige fysiologiske og patologiske processer såsom HIV, cancer og diabetes. Forståelsen af disse processer samt udviklingen af specifikke lægemidler afhænger i høj grad af kemisk at kunne syntetisere unaturlige sukkerstoffer samt stoffer, der imiterer kulhydrater. Ideelt set fremstilles sådanne polyhydroxylerede forbindelser fra kulhydrater, men på grund af meget lav tilgængelighed af mange sukkerstoffer fra naturens side, er der behov for effektive metoder til at omdanne tilgængelige kulhydrater til værdifulde kemiske byggeblokke. Det arbejde, der præsenteres i denne afhandling, fokuserer på udvikling og anvendelse af metoder, hvor overgangsmetaller benyttes til at forkorte og forlænge sukkerstoffers kulstofkæde og dermed giver adgang til ellers utilgængelige kulhydrater.

En ny katalytisk metode til at forkorte ubeskyttede kulhydrater med ét kulstofatom er blevet udviklet. Ved hjælp af en rhodium-katalyseret decarbonylering af aldehyd-gruppen kan monosakkarider omdannes til de tilsvarende forkortede polyoler i udbytter omkring 70%. Reaktionen udføres med rhodium-katalysatoren Rh(dppp)$_2$Cl i tilstedeværelse af en lille smule pyridin, der katalyserer mutarotation mellem kulhydratets hemiacetal- og aldehydform. Den udviklede metode er blevet anvendt som nøgletrin i en kort syntese af det unaturlige sukkerstof L-threose i 74% samlet udbytte i fem trin fra D-glukose.
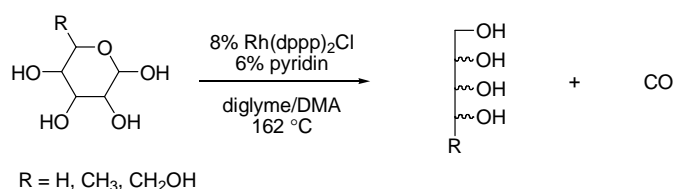
Som en del af fremstillingen af naturstofferne calystegin A$_3$ og gabosin A er D-glukose og D-ribose blevet forlænget med tre kulstofatomer ved hjælp af en zink-medieret fragmentering-allyleringsreaktion. Calystegin A$_3$ blev fremstillet på to lidt forskellige måder i 13 og 14 trin med henholdsvis 8,3 og 5,3% overordnet udbytte fra D-glukose. Herved er det lykkedes at udvikle den hidtil korteste syntese af naturligt forekommende calystegin A$_3$ i enantiomerisk ren form, hvilket blandt andet har muliggjort, at den absolutte konfiguration af naturstoffet er blevet bestemt. Fremstillingen af gabosin A blev gennemført i ni trin med 13,9% samlet udbytte fra D-ribose og

udgør den første synteserute til gabosin A, der gør brug af et let tilgængeligt kulhydrat som startmateriale.
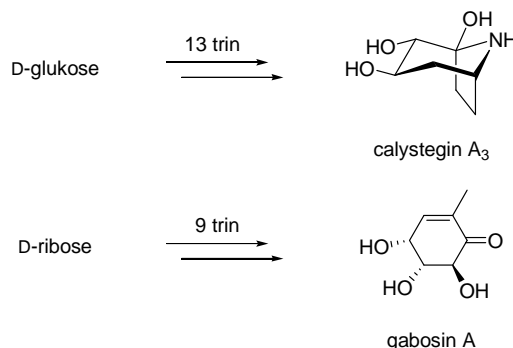
I løbet af et eksternt ophold ved Oxford Universitet er metabolismen af nonsteroidale anti-inflammatoriske lægemidler (NSAID'er) blevet undersøgt. Under forsøgsbetingelser, som forventes at kunne forekomme ved behandling med ibuprofen, blev det observeret, at kendte acylglukuronid-metabolitter af ibuprofen og flere analoger reagerer med et humant plasmaprotein. Afhængig af strukturen af lægemidlet blev der observeret to forskellige former for protein-modifikation, og de her opnåede resultater indikerer kraftigt, at der foregår irreversibel modifikation af proteiner i mennesker, når der indtages lægemidler, der indeholder en carboxylsyre-gruppe (f.eks. NSAID'er). Endvidere kan disse metabolitters reaktivitet med hensyn til modifikation af proteiner give en mulig forklaring på den toxicitet, der har været skyld i, at visse lægemidler indeholdende en carboxylsyre-gruppe er blevet trukket tilbage fra markedet.
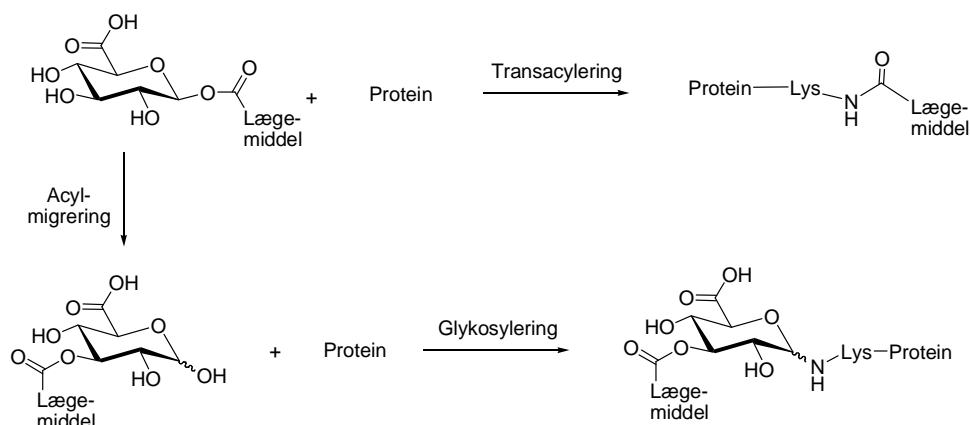
<u>Publications</u>

At the time for submission of this thesis, the research had resulted in the following scientific publications. Copies of the manuscripts are included in Appendix IV at the end of this thesis. In addition to these publications, work is in progress to convert the literature described in chapter 2 and the research presented in chapter 4 into a review and a full paper, respectively.

1) <u>Rune Nygaard Monrad</u>, Robert Madsen, **Rhodium-Catalyzed Decarbonylation of Aldoses**, *Journal of Organic Chemistry* **2007**, *72*, 9782-9785.

2) <u>Rune Nygaard Monrad</u>, Mette Fanefjord, Flemming Gundorph Hansen, N. Michael E. Jensen, and Robert Madsen, **Synthesis of Gabosine A and N from Ribose by the Use of Ring-Closing Metathesis**, *European Journal of Organic Chemistry* **2009**, 396.

3) <u>Rune Nygaard Monrad</u>, James C. Errey, Mazhar Iqbal, Xiaoli Meng, Lisa Iddon, John R. Harding, Ian D. Wilson, Andrew V. Stachulski, Benjamin G. Davis, **Dissecting the Reaction of Phase II Metabolites of Ibuprofen and Other NSAIDS with Human Plasma Protein**, *Nature Medicine*, submitted.

Abbreviations

)))	sonication	DMA	*N,N*-dimethylacetamide
A, Ala	alanine	DMAP	4-dimethylaminopyridine
Ac	acetyl	DMDO	dimethyldioxirane
AG	1-β-*O*-acyl glucuronide	DMF	*N,N*-dimethylformamide
AIBN	2,2'-azo bisisobutyronitrile	DMP	Dess-Martin Periodinane
aq.	aqueous	DMSO	dimethylsulfoxide
Asp	asparagine	DPPA	diphenylphosphoryl azide
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl	dppb	1,4-bis(diphenylphosphino)butane
		dppe	1,2-bis(diphenylphosphino)ethane
Bis-Tris	1,3-bis[tris(hydroxymethyl)-amino]propane	dpph	1,6-bis(diphenylphosphino)hexane
		dppm	bis(diphenylphosphino)methane
Bn	benzyl	dppp	1,3-bis(diphenylphosphino)propane
Boc	*tert*-butoxycarbonyl	DTT	1,4-dithiothreitol
bp	boiling point	E	glutamate
BSA	bovine serum albumin	EDTA	ethylenediamine tetraacetic acid
Bu	butyl	*ee*	enantiomeric excess
Bz	benzoyl	*ent*	enantiomer
C	cytidine	eq.	equivalent(s)
C, Cys	cysteine	ESI	electron spray ionization
CAN	cerium(IV) ammonium nitrate	Et	ethyl
Cbz	carboxybenzyl	EWG	electron-withdrawing group
CMP	cytidine 5'-monophosphate	F	phenylalanine
COD	1,5-cyclooctadiene	G	glycine
COE	cyclooctene	Gal	galactose
Cp*	η^5-pentamethylcyclopentadienyl	GalT	galactosyl transferase
CSA	camphorsulfonic acid	GlcNAc	*N*-acetylglucosamine
CstII	sialyl transferase	Grubbs' 2nd generation catalyst	
Cy	cyclohexyl		$(PCy_3)(C_3H_4N_2Mes_2)Cl_2Ru=CHPh$
D	aspartate	H, His	histidine
d	days	h	hour(s)
d	doublet (NMR)	HATU	*o*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
Da	Dalton		
DABCO	1,4-diazabicyclo[2.2.2]octane		
DAH	3-deoxy-D-*arabino*-hept-2-ulosonic acid	HEPES	4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene	HPLC	high performance liquid chromatography
DCC	dicyclohexylcarbodiimide	HSA	human serum albumin
DCM	dichloromethane	I	isoleucine
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone	IBX	2-iodoxybenzoic acid
		Im	imidazolyl
de	diastereomeric excess	IME	2-imino-2-methoxyethyl
DHP	3,4-dihydro-2*H*-pyran	IR	infrared
DIAD	diisopropyl azodicarboxylate	K	lysine
DIB	(diacetoxyiodo)benzene	**K**(+)	modified lysine
diglyme	diethyleneglycoldimethylether		

KDG	(4*S*,5*R*)-D-2-keto-3-deoxy-gluconic acid		PG	protective group
KDN	3-deoxy-D-*glycero*-D-*galacto*-non-2-ulosonic acid		Ph	phenyl
			Piv	pivaloyl
KDO	3-deoxy-D-*manno*-oct-2-ulosonic acid		PMB	*p*-methoxybenzyl
			PPTS	pyridinium *p*-toluenesulfonate
K_i	inhibition constant		Pr	propyl
L, Leu	leucine		Q	glutamine
LacNAc	*N*-acetyllactosamine		q	quartet (NMR)
LC	liquid chromatography		R	arginine
LiHMDS	lithium bis(trimethylsilyl)-amide		*rac*	racemic
Lys	lysine		RDS	rate-determining step
M	methionine		rt	room temperature
m	multiplet (NMR)		S	serine
MALDI	matrix-assisted laser desorption and ionization		s	singlet (NMR)
			sat.	saturated
Me	methyl		SDS	sodium dodecylsulfate
Mes	mesityl		sec	seconds
min	minutes		SiaT	sialyl transferase
MOM	methoxymethyl		T	threonine
MOPS	3-(*N*-morpholino)propane-sulfonic acid		t	triplet (NMR)
			TBDMS	*tert*-butyldimethylsilyl
mp	melting point		TBDPS	*tert*-butyldiphenylsilyl
MS	mass spectrometry		Tf	trifluoromethanesulfonyl
MS	molecular sieves		TFA	trifluoroacetic acid
N	asparagine		THF	tetrahydrofuran
n.d.	not determined		THP	2-tetrahydropyranyl
NBS	*N*-bromosuccinimide		TLC	thin layer chromatography
NHS	*N*-hydroxysuccinimide		TMS	trimethylsilyl
NMM	*N*-methylmorpholine		TOF	time of flight
NMO	*N*-methylmorpholine oxide		Tol	toluene
NMP	*N*-methyl-2-pyrrolidinone		TPPTS	triphenylphosphane-3,3',3''-trisulfonic acid trisodium salt
NMR	nuclear magnetic resonance		Tr	trityl
NPht	*N*-phthalimidyl		triphos	bis(2-diphenylphosphinoethyl)-phenylphosphine
NSAID	nonsteroidal anti-inflammatory drug		Tris	tris(hydroxymethyl)aminomethane
Oxone	potassium peroxymonosulfate		Ts	toluenesulfonyl
p	pentet (NMR)		TS	transition state
P	proline		UDP	uridine 5'-diphosphate
PAGE	polyacrylamide gel electrophoresis		UV	ultraviolet
			V	valine
PBS	phosphate-buffered saline		W	tryptophan
PCC	pyridinium chlorochromate		Y	tyrosin
PDC	pyridinium dichromate			

Contents

1 The diverse nature of carbohydrates

Carbohydrates are the most abundant biomolecules in nature, and they are responsible for two thirds of the carbon found in the biosphere.[1,2] In the form of mono-, di-, oligo- and polysaccharides, carbohydrates constitute our primary nutrient. In addition, various processed sugars, primarily polyols, are used in the food industry as reduced-calorie sweeteners and sugar substitutes.[3]

Biochemically, carbohydrates are some of the most crucial molecules for life.[1,4] Every cell is coated with carbohydrates in the form of glycoproteins and glycolipids.[5] These glycoconjugates take part in a number of different processes including cell adhesion, cell growth and intercellular communication.[6,7] The most frequent post-translational modification of proteins is glycosylation, and around 50% of all proteins found in nature are glycosylated.[2,8] On proteins, carbohydrates ensure a correct folding, they increase the stability against proteolytic degradation and provide epitopes for recognition.[2,5,7,9] In particular oligosaccharides are important in signal recognition events, where they act as information carriers. In such processes, lectins (sugar binding proteins) are capable of recognizing complex oligosaccharides thereby triggering a variety of cellular responses.[1,2,6,10]

Glycosidases are enzymes, which are responsible for the cleavage of glycosidic bonds in saccharides and glycoconjugates.[1,11] Carbohydrate-dependant processes are relying on the activity of specific glycosidases, and by interfering with such glycosidases, it is possible to inhibit resulting physiological or pathological effects induced by the enzymes. By chemical synthesis of glycosidase inhibitors, this concept has been used therapeutically in the treatment of influenza, viral infections (e.g. HIV), cancer and diabetes.[1,2,10,11]

Contrary to inhibiting biological processes, certain desirable therapeutic effects may be gained by *inducing* such processes. This has been used in the development of carbohydrate-based cancer and HIV vaccines, where oligosaccharides conjugated to proteins and peptides have been used to induce an immune response leading to the production of specific antibodies.[4,12]

Carbohydrates also play a central role in the metabolism of xenobiotics, where D-glucuronic acid is coupled to hydrophobic drugs thereby increasing the water solubility of the drug and enabling its excretion.[13,14] In analogy, enhanced anti-cancer activity has recently been achieved by coupling of a monosaccharide to an anti-cancer agent to increase its water solubility thereby improving the anti-cancer activity of the drug.[15]

In chemistry, carbohydrates represent cheap and readily available densely functionalized, chiral starting materials.[16] Since many biologically active natural products are glycosylated or contain polyhydroxylated carbohydrate motifs as part of their structure, carbohydrates are well-suited enantiomerically pure precursors.[10,17,18]

With the great potential of sugars, glycoconjugates and carbohydrate mimetics in biochemistry and medicine, the development of new methodologies for controlled formation of glycosidic bonds,[19] synthesis of homogeneous glycoproteins[20] and preparation of glycosidase inhibitors[10,18] is important to understand biological processes, and to be able to modulate or alter biosynthetic pathways e.g. to obtain desired therapeutic effects.[2,4,7]

From another perspective, concerns about the depletion of the fossil fuel reserves demand new technologies for producing energy from renewable resources.[21] In this respect carbohydrates, most efficiently as polyols, are promising substrates for the production of hydrogen by steam reforming.[22,23] Also the generation of liquid fuels from biomass is increasingly important. Bio-ethanol and liquid hydrocarbons can be produced from carbohydrates by fermentation of glucose[24] and by various reforming, dehydration and hydrogenation processes, respectively.[25]

All together, carbohydrates are highly important biomolecules, and from a synthetic point of view, it is important to develop new synthetic methodologies to convert these inexpensive compounds into valuable synthetic building blocks and biologically relevant targets.

2 Methods for shortening and extending the carbon chain in carbohydrates

As mentioned in chapter 1, lower sugars like pentose and tetrose derivatives are important for example as sweeteners in food ingredients and as building blocks in organic synthesis. Higher sugars are often employed as intermediates for the synthesis of biologically active, polyhydroxylated compounds, and due to the scarcity of many lower and higher sugars from nature, the development of efficient protocols for shortening and extending readily available carbohydrates is important. Shortening and extending the carbon chain in carbohydrates has been a subject in carbohydrate chemistry for more than a century, and the literature up to 1997 is covered in the book 'Monosaccharide Sugars: Chemical Synthesis by Chain Elongation, Degradation, and Epimerization' by Györgydeák and Pelyvás.[26] The scope of the present chapter is to give an overview of the advances within the field since then.

C-Glycosides are an important class of carbohydrates, which are potential inhibitors of carbohydrate processing enzymes due to their increased stability as compared to *O*- and *N*-glycosides. The formation of *C*-glycosides from monosaccharides can be considered a chain elongating process, which affords higher carbon *anhydro* sugars, and the purpose of the present review is only to include the recent advances in *C*-glycoside formation, which have particular relevance to chain elongation.

2.1 Methods for shortening the carbon chain in carbohydrates

2.1.1 Ruff degradation

The available methods for shortening the chain in unprotected sugars are sparse. The Ruff degradation, which has been known since 1898,[27] converts salts of aldonic acids into aldoses with loss of one carbon atom. The reaction is performed with hydrogen peroxide in alkaline solution in the presence of Fe(III) or Cu(II)-salts, the latter being the most efficient.[28] Due to its importance in the preparation of pentose sugars e.g. industrial production of xylitol,[3] the Ruff degradation has received considerable interest in recent years.

The reaction generally occurs in moderate yields,[26] and since one of the major disadvantages in the Ruff degradation is the separation of the product from large quantities of metal salts, work has been done to cleave carbon dioxide from the aldonate electrochemically or by the use of catalytic

amounts of metal. Jiricny and Stanek recently used a fluidized bed electrode cell for the production of D-arabinose in approximately 70% yield from sodium D-gluconate without addition of any chemical oxidants.[29] The production of D-arabinose from calcium D-gluconate has been achieved catalytically by Germain and co-workers using Cu(II)-exchanged zeolites.[30] During the reaction, copper was found to leach from the zeolite, and once the aldonic acid was consumed, copper precipitated on the zeolite again. The catalyst could be recycled twice thereby achieving the advantages of heterogeneous catalysis, although copper was in solution during the reaction.[30]
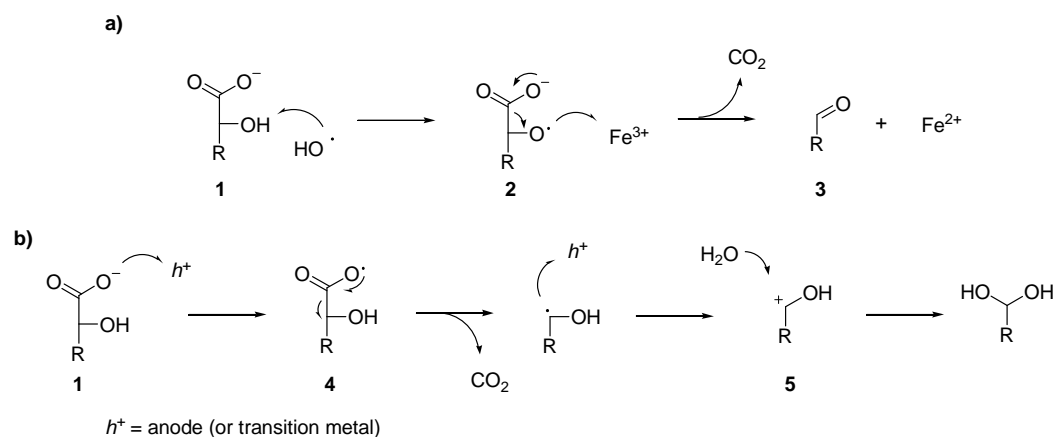
Scheme 1. Proposed mechanisms for the Ruff degradation suggested by a) Isbell and Salam[31] and b) Stapley and BeMiller.[28]

Several different mechanisms for the Ruff degradation have been proposed over the years, and these have recently been critically reviewed.[28] The generally accepted mechanism suggested by Isbell and Salam[31] (Scheme 1a) starting with H-abstraction to generate an α-alkoxy radical (**2**) followed by oxidation by Fe(III) to produce the lower aldose **3** with loss of carbon dioxide has been questioned by Stapley and BeMiller.[28] Instead, a Hofer-Moest-type reaction mechanism with two successive one-electron oxidations has been proposed (Scheme 1b). The aldonic acid **1** is oxidized to an acyloxy radical (**4**), which upon loss of carbon dioxide and subsequent oxidation produces a carbocation (**5**) that is captured by the solvent. This mechanism is believed to be valid both in the electrochemical Ruff degradation and in the classical versions, where the anode is replaced by a transition metal, which is regenerated by oxidation with hydrogen peroxide.[28]

In transition metal mediated Ruff degradations the carboxylate and the α-hydroxy group of the aldonate **1** are believed to coordinate to the transition metal during the initial oxidation.[28] As a

result, uronic acids are not decarboxylated efficiently by iron or copper due to the α-hydroxy group being part of a hemiacetal group thereby disabling coordination to the metal, when the uronic acid is on the pyranose form.[32] However, recently sodium D-glucuronate and methyl D-glucuronopyranoside have been found to undergo *electrochemical* degradation yielding the corresponding *xylo*-configured pentodialdose,[32] thus enabling degradation of uronic acids by electrochemical methods.

2.1.2 Periodate cleavage

Cleavage of 1,2-diols and α-hydroxy carbonyl compounds with periodates or lead tetraacetate to yield the corresponding aldehydes is a well-known and widely applied method for shortening the carbohydrate chain.[33-36] Oxidative cleavage with sodium periodate is usually performed on partly protected sugars because the oxidation of unprotected sugars cannot be controlled and over-oxidation otherwise occurs.
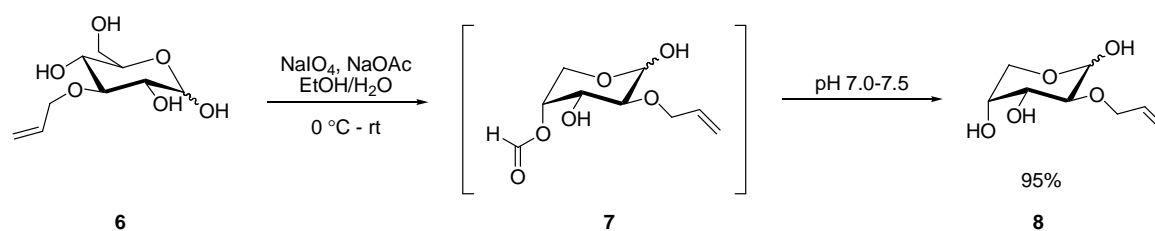
Scheme 2. Periodate cleavage of monoprotected D-glucopyranose.[37]

Recently, Storz and Vasella applied the periodate oxidation on *mono*-protected 3-*O*-allyl-D-glucopyranose (**6**) easily available in three steps from diacetone D-glucose (Scheme 2).[37] D-Glucose and D-galactose are known to react with sodium periodate primarily in their pyranose form,[38] and by careful control of the pH only the C1-C2 bond was cleaved by sodium periodate leaving the formyl group as a 'protective group' on the C4 alcohol in the resulting D-arabinopyranose **7** thereby preventing further periodate cleavage. Adjustment of the pH after quenching with ethylene glycol and removal of inorganic salts by filtration effected hydrolysis of the intermediate formyl ester. Loss of the formyl group during the reaction with periodate or isomerization to the furanose would lead to failure producing a mixture of pentose, tetrose and trioses, but under mild conditions this was elegantly avoided producing the interesting chiral pentose building block **8** in 95% yield.[37]

2.1.3 Alkoxy radical fragmentation

The alkoxy radical fragmentation of anomeric alcohols was first reported in 1992,[39,40] and has since then been further developed by Suárez and co-workers to become a useful tool for the synthesis of a variety of one carbon atom shortened sugar-derived chiral building blocks.
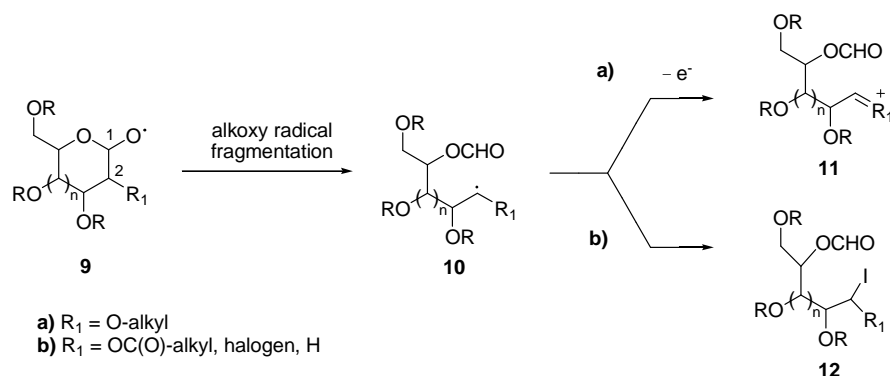
Scheme 3. Alkoxy radical fragmentation.[39,41]

Under oxidative conditions employing the hypervalent iodine reagents (diacetoxyiodo)benzene (DIB) or iodosylbenzene in the presence of iodine, carbohydrate anomeric alcohols (**9**) undergo alkoxy radical fragmentation cleaving the C1-C2 bond to produce a C2 radical **10**, which can react in two different ways depending on the nature of the substituents at C2 (Scheme 3). The presence of an ether functionality at C2 leads to oxidation of **10** to an oxonium ion (**11**) which can be inter- or intramolecularly trapped by nucleophiles (path a). Electron-withdrawing groups decrease the electron density at C2 thereby preventing oxidation of **10**, which is then trapped by iodine leading to 1-iodoalditols (**12**) with one less carbon atom (path b). 2-Deoxy- and 2-deoxy-2-haloaldoses also lead to iodine incorporation following path b, and mono-, di- and trihalo-1-deoxyalditols can be achieved from the corresponding 2-deoxy-,[39] 2-deoxy-2-halo-[41-43] and 2-deoxy-2,2-dihalosugars.[44-46] Instead of trapping the intermediate radical **10** by nucleophiles (Scheme 3a and b), one carbon atom shortened alditols possessing a terminal alkene (ald-1-enitols) can be formed by radical fragmentation of 2,3-dideoxy-3-(phenylsulfonyl)-aldoses, which leads to the corresponding 1,2-dideoxy-2-(phenylsulfonyl)-ald-1-enitol derivatives with loss of a carbon atom.[47]

Fragmentation of 2,3,5,6-tetra-*O*-methyl-D-galactofuranoside (Table 1, entry 1) and subsequent nucleophilic attack of acetate from DIB leads to the corresponding D-arabinose derivative in 85% yield as the mixed acetal. The presence of a benzoate at C2 results in incorporation of iodine

producing the corresponding 1-iodo-D-arabinitol instead (entry 2). Such iodoalditols can be reduced to the corresponding alditols by treatment with Bu_3SnH and AIBN, or they can be elongated for example by radical allylation using allyltri-*n*-butyltin and AIBN (see also section 2.2.7).[48]

Table 1. Oxidative alkoxy radical fragmentation using DIB or PhIO and I_2.

Entry	Substrate	Substituents	Product	Yield (%)
1 2		R = Me, R_1 = OAc R = Bz, R_1 = I		85 (1:1)[48] 63 (1:1)[48]
3 4 5		X = O, R = H, H X = R = O X = NBoc, R = H, H		36[49] 43[50,51] 73[52]
6 7		R = Ac R = β-D-Gal		88[53,54] 97[54]
8				70[a,55]
9 10		R = Bn, R_1 = OAc R = Piv, R_1 = NHAc[b]		28 (1:0)[56] 81 (15:66)[56]

[a] DIB was replaced by $(CF_3CO_2)_2IPh$ and H_2O (1 eq.) [b] The reaction was performed in MeCN.

Intramolecular capture of oxonium ions (**11**) by attack of alcohols, carboxylic acids or amines occur in moderate yield leading to the corresponding cyclized aldoses, alduronic acid lactones and azasugars (Table 1, entries 3-5). The presence of an azide at C2 leads to aldononitriles with loss of one carbon in excellent yield, and the methodology can even be applied on disaccharides (entries 6-7). When 2-ketoses are subjected to alkoxy radical fragmentation using the $(CF_3CO_2)_2IPh/I_2$ system, the sugar chain is shortened by two carbon atoms, and in the presence of water, free *aldehydo* sugars can be obtained.[55] Alkoxy radical fragmentation of benzyl protected L-tagatose (entry 8), which is readily available by Meerwein-Ponndorf-Verley/Oppenauer oxidation of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose,[57] gave a L-threose derivative with conveniently differentiated protective groups at C2/C3 and C4 enabling further synthetic manipulations.

In contrast to anomeric alcohols, fragmentation of *primary* alcohols by alkoxy radical fragmentation is not completely reliable due to competing intramolecular hydrogen atom transfer leading to various side-products.[56] When intramolecular hydrogen atom transfer from the anomeric protective group is possible, the alkoxy radical fragmentation is disfavored as illustrated with the benzyl and pivaloyl anomeric protective groups (Table 1, entries 9-10). Performing the reaction in acetonitrile led to fragmentation in 81% yield producing the shortened 4-acetamido derivative by a Ritter reaction between the intermediate oxonium ion and the solvent (entry 10).

Under *reductive* conditions, the alkoxy radical fragmentation can be achieved by treatment of anomeric nitrates or *N*-phthalimido glycosides with Bu_3SnH and AIBN to produce alditols shortened by one carbon atom (Table 2).

Table 2. Reductive alkoxy radical fragmentation using Bu_3SnH and AIBN.

Entry	Substrate	Substituents	Product	Yield (%) R = CHO	Yield (%) R = H
1		$R_1 = NO_2$, $R_2 = TBDMSO$		79	16[58,59]
2		$R_1 = NPht$, $R_2 = TBDMSO$		42	39[59,60]
3		$R_1 = NO_2$, $R_2 = CN$		-	83[59]
4				-	47[59,60]
5				78[a,59,60]	-
6		$R_1 = NO_2$		33	18[59]
7		$R_1 = NPht$		64	31[59,60]
8		$R_1 = H$			89[61]
9		$R_1 = Me$			95[61]

[a] After hydrolysis of the formate and silyl protection.

To avoid the formation of toxic tin byproducts during the fragmentation, $(Me_3Si)_3SiH$ can replace Bu_3SnH, however in slightly lower yields.[61] Introduction of the *N*-phthalimido group and the nitrate ester can easily be accomplished from the corresponding alcohol under Mitsunobu conditions or by employing $LiNO_3/(CF_3CO)_2O$,[62] respectively.

Partial hydrolysis of the resulting formate is often observed, and under reductive conditions, the degree of hydrolysis depends on the substrate and the reaction time (Table 2, entry 1 and 2). The methodology requires fully protected carbohydrates, but tolerates functional groups like nitriles and acetamido groups, the latter however in moderate yield (entry 3 and 4). 2-Deoxy substrates are easily fragmented giving 1-deoxyalditols (entry 5). In general, *N*-phthalimido derivatives react faster than the corresponding nitrate esters, and due to instability of the latter compounds in some cases (compare entries 6 and 7), the two different approaches complement each other well.

Recently, the reductive alkoxy radical fragmentation was used to prepare 1,2-*O*-isopropylidene-β-L-threose in 55% overall yield in three steps from readily available 1,2:5,6-di-*O*-isopropylidene-D-glucofuranose thereby providing a very efficient approach to an otherwise inaccessible sugar.[59]
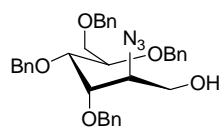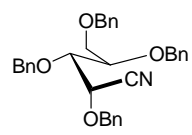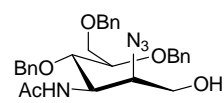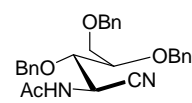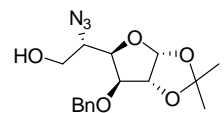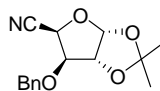
In the case of *primary N*-phthalimido glycosides, the alkoxy radical fragmentation competes with intramolecular hydrogen atom transfer, and unexpectedly, Sartillo-Piscil and co-workers[61] found that formation of internal hydrogen bonds may have a drastic effect on the fragmentation of primary alkoxy radicals. The primary alkoxy radical derived from the *N*-phthalimido derivative in Table 2, entry 8 can achieve a stabilizing internal six-membered hydrogen bond interaction with the free C3 alcohol leading to fragmentation, whereas in the C3 *methoxy* substrate (entry 9) no such six-membered interaction is possible resulting in intramolecular hydrogen atom transfer followed by reduction regenerating the parent alcohol. In good hydrogen bond accepting solvents like THF, increased amounts of the hydrogen atom transfer products were observed at the expense of the fragmentation products.[61]

2.1.4 PCC-induced shortening of β-azido alcohols

In addition to the alkoxy radical fragmentation (Table 1, entry 6 and 7), aldononitriles can also be produced by a recently developed PCC-induced oxidative degradation of primary β-azido alcohols (Table 3).[63] The oxidation is performed under very mild conditions using two equivalents of PCC in

DCM at room temperature. Good yields are obtained for protected 2-azido-2-deoxyalditols, and in contrast to alkoxy radical fragmentation, the acetamido functionality is tolerated without reducing the yield (Table 3, entries 1-2). Also furanose derivatives with terminal β-azido alcohol side-chains can be oxidized to their corresponding nitriles (entry 3), whereas *secondary* β-azido alcohols are inert, and β-azido *hemiacetals* are oxidized to their 2-azidolactones instead of being shortened.[63] The incompatibility of the PCC-induced degradation of β-azido alcohols with anomeric substrates renders the oxidative alkoxy radical fragmentation (section 2.1.3) a more widely applicable approach to aldononitriles.

Table 3. Oxidative degradation of β-azido alcohols using PCC.[63]

Entry	Substrate	Product	Yield (%)
1			64
2			67
3			75

The mechanism for the PCC-mediated degradation of β-azido alcohols is believed to proceed *via* a 2-azidoaldehyde generated by PCC-oxidation followed by intramolecular attack of the azide on the carbonyl group giving a hydroxy-triazole derivative, which is subsequently oxidized by PCC affording the aldononitrile with loss of carbon monoxide and nitrogen.[63]
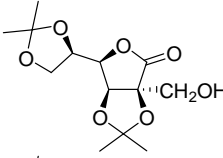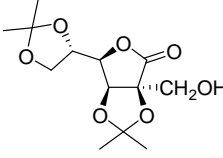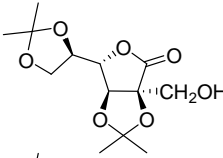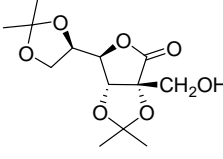
2.2 Methods for extending the carbon chain in carbohydrates

2.2.1 The Kiliani ascension

The Kiliani ascension is one of the longest known tools to elongate carbohydrates,[26] but it has primarily been used on aldoses due to low accessibility of ketoses and difficulties separating the formed epimers. The Fleet group has recently applied the Kiliani ascension on different ketoses thereby accessing a range of different branched carbohydrate building blocks (Table 4).[64-66] After

subjecting ketoses to aqueous sodium cyanide at room temperature, hydrolysis of the nitrile functionality by heating to reflux afforded epimeric pairs of chain elongated lactones. Treatment of the crude product with sulfuric acid and acetone gave the corresponding diisopropylidene derivatives, which could easily be separated by crystallization. Although the yields are moderate, the procedure constitutes a very easy and convenient approach to 2-*C*-branched sugars in high purity. Further manipulation of these *via* reduction to the corresponding alditols gives access to several 5-*C*-branched carbohydrate scaffolds by microbial oxidation and isomerization.[67]

Table 4. One-pot Kiliani ascension and diisopropylidene protection of unprotected ketoses.

Entry	Ketose	2-*C*-Branched aldonolactone	Yield (%)
1	D-fructose		51[64]
2	L-sorbose		17[64]
3	D-tagatose		44[66]
4	D-psicose		38[66]

The one-pot Kiliani ascension and diisopropylidene protection of D-fructose gave 51% yield of 2-*C*-hydroxymethyl-2,3:5,6-di-*O*-isopropylidene-D-mannono-1,4-lactone (Table 4, entry 1), which could be converted into the formal Kiliani product of the inaccessible sugar L-psicose in 42% overall yield in only three steps by inversion of two stereocenters.[64] With this Kiliani ascension of D-fructose, a range of different 2-*C*-branched derivatives of D-mannose has become available after only a few synthetic transformations.[68]

The diacetonide of 2-*C*-hydroxymethyl L-gulonolactone (Table 4, entry 2) could be obtained from L-sorbose in 17% yield *without* chromatography, which despite the low yield illustrates the simplicity of the procedure even on a moderate scale. D-Tagatose and D-psicose can similarly be converted into the corresponding 2-*C*-branched acetonides and crystallized in 44 and 38% yield, respectively (entry 3 and 4). The one-step Kiliani ascension and isopropylidene protection of aldoses[69] and ketoses followed by isolation of the major products by simple crystallization is a convenient improvement of the classic procedure.[26]

The problems associated with separation of epimers have recently been addressed by Herbert and co-workers by applying Sm^{3+}-ion exchange column chromatography. Separation of the epimeric mixture of D-ribose and D-arabinose produced from the Kiliani ascension of D-erythrose could readily be achieved on a preparative scale by this methodology, and the column could be used repeatedly without recharging or cleaning.[70]

13: L-*glycero*
15: D-*glycero*

14: L-*threo*   99% (82% de)
16: D-*erythro* 85% (60% de)
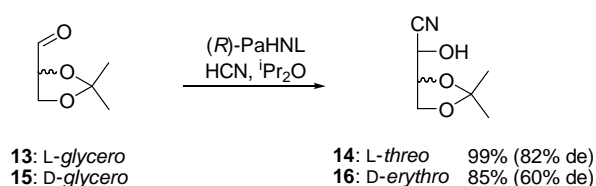
Scheme 4. Enzymatic Kiliani ascension of isopropylidene protected glyceraldehydes **13** and **15**.[71]

Recently, the first stereoselective, *enzymatic* Kiliani ascension of a sugar aldehyde was reported.[71] 2,3-*O*-Isopropylidene-glyceraldehydes **13** and **15** were converted into tetrononitriles **14** and **16** in excellent yield and moderate to good stereoselectivity by treatment with (*R*)-hydroxynitrile lyase from *Prunus amygdalus* ((*R*)-PaHNL) (Scheme 4). (*R*)-PaHNL afforded 99% yield of 3,4-*O*-isopropylidene-L-threononitrile (**14**) in 82% *de* from 2,3-*O*-isopropylidene-L-glyceraldehyde (**13**), whereas 3,4-*O*-isopropylidene-D-erythrononitrile (**16**) was produced in 85% yield and 60% *de* from the corresponding D-glyceraldehyde derivative **15**. The enzymatic Kiliani ascension is a new possibility for enantioselective synthesis of desired cyanohydrins in high yield thereby circumventing tedious separation of epimers.

2.2.2 The Sowden homologation
Together with the Kiliani ascension, the Sowden homologation[72] is one of the classical ways to obtain one-carbon elongated sugars. Both methodologies suffer from moderate selectivities and

difficulties separating the formed epimers. The Kiliani ascension is usually preferred, but the Sowden protocol is often used when the latter procedure is impeded by tedious separation or fails to give the desired epimer.[26] Recently, Dromowicz and Köll reported an improved synthesis of D-idose from D-xylose by means of the Sowden homologation (Scheme 5).[73] The separation of the epimeric 1-deoxy-1-nitro-alditols produced by the nitroaldol condensation (Henry reaction) was improved by multiple fractional crystallizations, and the desired isomer **17** could be isolated in 75% yield. By performing the subsequent Nef reaction[74] under an argon atmosphere, an increased yield compared to the literature procedure was obtained,[75] and D-idose could thereby be isolated in 51% overall yield over two steps.[73]

Scheme 5. Improved Sowden homologation of D-xylose by Dromowicz and Köll.[73]

2.2.3 Chain elongation by means of the Baylis-Hillman reaction

Over the last five years, the application of the Baylis-Hillman reaction in carbohydrate chemistry has received considerable interest in particularly from the group of Krishna, who has recently reviewed the field.[76] The chirality of carbohydrates enables stereoselective transformations, and sugars have lately been applied in the Baylis-Hillman reaction as electrophilic aldehydes,[77-87] activated alkenes[88,89] and chiral auxiliaries.[80,90] Different applications of the Baylis-Hillman reaction as a tool to elongate carbohydrates are shown in Table 5. The reaction of ethyl acrylate, methyl vinyl ketone or acrylonitrile with protected and partially protected sugar-derived aldehydes occurs in good yield with low to moderate *de* (entries 1-4).[77,78] Double asymmetric induction using both a sugar-based acrylate and a sugar aldehyde can lead to excellent stereoselectivities, however often in moderate yields (entry 5). The existence of matched and unmatched pairs of chiral aldehydes and acrylates leads to observed *de*'s ranging from excellent to poor as illustrated in entries 6 and 7. Proper selection of the sugar acrylate can improve the stereoselectivity significantly, but finding suitably matched pairs may be time consuming. As observed for 1-*aldehydo*-2,3:4,6-di-*O*-isopropylidene-L-sorbose (entries 8 and 9), very good yields and *de*'s can be achieved with non-chiral activated alkenes in some cases. Of the available activated alkenes, ketones and

nitriles generally give higher yields than the corresponding esters, whereas the best stereoselectivities are achieved with nitriles followed in turn by ketones and esters.[77,78]

Table 5. Chain elongation of carbohydrates by the Baylis-Hillman reaction.

Entry	Aldehyde	Substituents	EWG	Product	Yield (%)	*de* (%)
1		R_1 = OMe, R_2 = H	CO_2Et		73[77-79]	36
2		R_1 = OMe, R_2 = H	COMe		82[77,78]	53
3		R_1 = OMe, R_2 = H	CN		76[77,78]	60
4		R_1 = H, R_2 = OH	CN		80[78]	76
5		R_1 = OMe, R_2 = H	CO-Sug		43[80]	>95
6		2*R*,3*S*	CO-Sug		73[80]	>95
7		2*S*,3*R*	CO-Sug		78[80]	33
8			CN		85[78]	90
9			CO_2Et		80[78]	>95
10			COMe		55[a,81,82]	
11			COMe		70[b,81,82]	

[a] DABCO was replaced by Me_2S–$TiCl_4$ at 0 °C for 15 min. [b] DABCO was replaced by Me_2S–$TiCl_4$ at 0 °C for 6-9 h.

α,β-Unsaturated aldehydes derived from sugars cannot easily be used in the Baylis-Hillman reaction, however, in the presence of Me_2S–$TiCl_4$ at 0 °C, moderate yields can be obtained (Table 5, entry 10). Side-reactions leading to chloro-substituted byproducts is a major problem (entry 11), and the reaction has to be stopped before full conversion to afford the initial Baylis-Hillman product.[81,82]

Even stereoselective, *intramolecular* Baylis-Hillman reactions giving rise to the corresponding chain elongated lactones have been reported (Scheme 6). The intramolecular Baylis-Hillman reaction of **18** occured with >95% *de* giving the desired lactone **19** as a single isomer in 71% yield together with small amounts of 3-*O*-alkyl derivatives as byproducts (8% of **20**), when alcohols were present as co-solvents.[91]
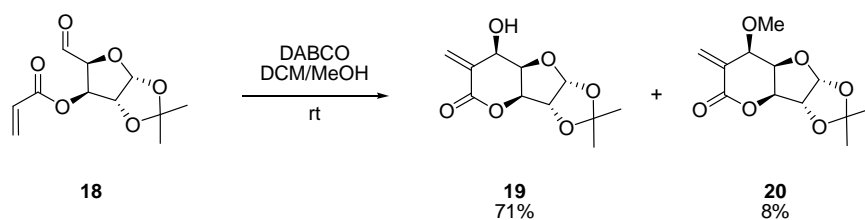
Scheme 6. Intramolecular Baylis-Hillman reaction.[91]

Recently, sugar-derived activated alkenes have also been used in the Baylis-Hillman reaction as a synthetic route to 2- or 3-*C*-branched carbohydrates (Table 6). Of a range of aromatic and aliphatic aldehydes, the best results were achieved with electron poor aldehydes, however, excellent stereoselectivities were achieved in most cases independently of the nature of the aldehyde (entries 1-4).

Table 6. Baylis-Hillman reaction of sugar acrylates.

Entry	Acrylate	Aldehyde R-CHO	Product	Yield (%)	*de* (%)
1		R = *p*-NO$_2$-Ph		75[89]	82
2		R = Ph		26[88]	>98
3		R = *p*-NO$_2$-Ph		82[88]	>98
4		R = CH$_3$(CH$_2$)$_2$		85[88]	>98

If the Baylis-Hillman reaction is combined with ozonolysis, fully hydroxylated higher carbon sugars can be obtained in relatively few steps. The D-*glycero*-D-*altro*-heptose derivative **24** was isolated in 13% overall yield as the major isomer in six steps from D-ribose as illustrated in Scheme

7. A few synthetic manipulations of 2,3-*O*-isopropylidene-D-ribose (**21**) afforded the suitably protected aldehyde **22**, which was reacted with ethyl acrylate in the Baylis-Hillman reaction giving **23** in 30% *de*. The diastereomers could easily be separated and subsequent ozonolysis and reduction afforded the fully hydroxylated heptose derivative **24** in 60% *de*. Similarly, diacetone D-mannose could be converted into four diastereomeric octose derivatives in nine steps in a 7:3:2:1 ratio.[83]
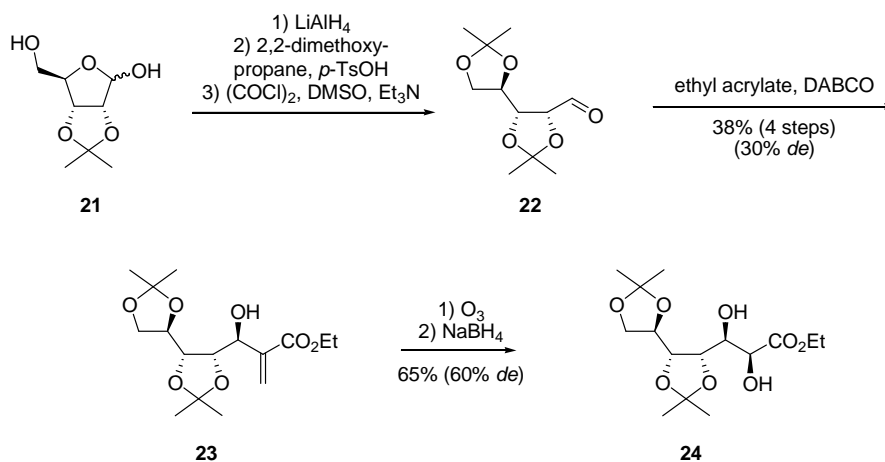
Scheme 7. Synthesis of higher sugars by the Baylis-Hillman reaction.[83]

2.2.4 Chain extension based on the aldol reaction

The aldol reaction is one of the most frequently employed methods for C-C bond formation in carbohydrate chemistry.[92] Aldol reactions can be performed on protected as well as unprotected sugars, and the reaction is particularly well-suited for the synthesis of ketoses, aldonic acid esters and ulosonic acid derivatives.[26,93] Diastereoselective aldol reactions have been extensively studied, and under non-chelating conditions, several models for asymmetric induction have been proposed.[94,95] Under chelating conditions, thermodynamic control leads to *threo* products exclusively, whereas under kinetic control, *E* and *Z* enolates predominantly give *threo* and *erythro* products, respectively.[92,93]

During the last 10 years, stereoselective, *organocatalytic* aldol reactions of 1,3-dihydroxyacetone derivatives have appeared as a powerful tool to construct polyhydroxylated compounds.[96,97] The methodology has recently been used in the synthesis of a range of ketopentoses and -hexoses from C_2 and C_3 building blocks.[98-102] Very recently, the organocatalyzed aldol reaction was applied on protected D-ribose[102] and D-arabinose[101] to give fully hydroxylated 2-octuloses (Scheme 8). By the use of (*S*)-proline and 2-*tert*-butyl-2-methyl-1,3-dioxan-5-one (**25**), the protected D-ribose **26** could

be elongated by three carbon atoms to give the corresponding D-*glycero*-D-*manno*-2-octulose **27** in good yield and excellent *de* together with small amounts of the dehydrated product **28**.
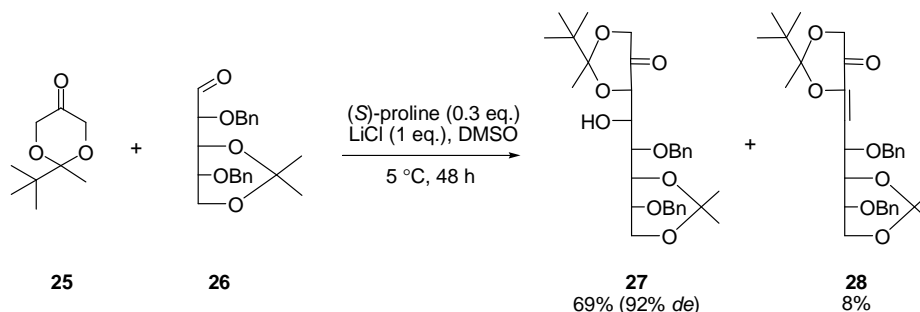
Scheme 8. Organocatalytic aldol reaction on protected D-ribose.[102]

More importantly, MacMillan and co-workers have developed a procedure for enantioselective assembly of aldohexoses in only two steps from α-hydroxy acetaldehydes by employing two subsequent aldol reactions.[103,104] Organocatalyzed dimerization of protected α-hydroxy acetaldehydes affords fully hydroxylated *aldehydo* tetroses, which after Lewis acid catalyzed Mukaiyama aldol reaction with a second protected α-hydroxy acetaldehyde afford hexoses in good yields and excellent stereoselectivities. Furthermore, the preparation of hexoses from two different C_2 building blocks conveniently enables orthogonal protection of the hydroxy groups, which is particularly useful for further synthetic manipulations.

Enzymatic aldol reactions have also been extensively studied and are emerging as attractive reactions even on a preparative scale.[105] By the reaction of pyruvate with various aldoses, aldolases have been utilized to produce a range of 3-deoxy-2-ulosonic acids without the requirement of protective groups.[105-107] This has for example been achieved by chain elongation of L-threose to give 3-deoxy-L-*lyxo*-hept-2-ulosonate (**29**) in 70% yield and >98% *de* (Scheme 9).[108]
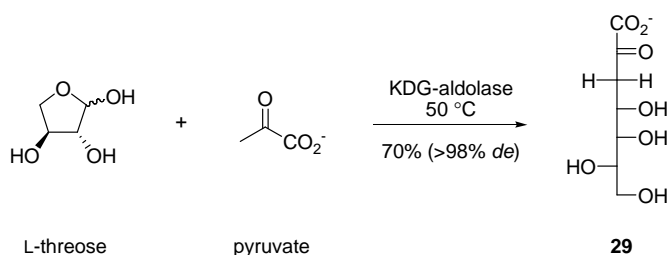
Scheme 9. Enzymatic aldol reaction on unprotected L-threose.[108]

2.2.5 Organometallic addition to sugar aldehydes and hemiacetals

The addition of organometallic reagents to various protected carbohydrates in their hemiacetal or *aldehydo* form is one of the most studied methods for extending the carbohydrate chain.[92] The organometallic addition to C1 or C6 *aldehydo* sugars generally follows the known models for asymmetric induction during nucleophilic attack on carbonyl groups e.g. the Felkin-Anh and the Cram chelate models,[109-111] whereas organometallic addition to free hemiacetals is more complex and dependant on the substrate and the reaction conditions.[26,112] Addition of vinylic reagents to sugar hemiacetals or aldehydes followed by either ozonolysis[113,114] or dihydroxylation[115,116] is a widely applied methodology to obtain higher carbon sugars possessing a fully hydroxylated carbon skeleton.

Most organometallic additions require fully protected carbohydrate substrates, but in the early 1990's tin[117] and, more efficiently, indium[118] were found to mediate allylation of unprotected sugars in aqueous media. With unprotected carbohydrates, the reaction most often occurs with chelation favoring the *threo* configuration between the α-hydroxy group and the newly formed stereocenter.[119] Indium-mediated allylation of unprotected sugars has received considerable interest in particular in combination with ozonolysis and dihydroxylation to produce chain elongated carbohydrates like the 3-deoxy-2-ulosonic acids KDN, KDO and *N*-acetyl neuraminic acid.[116,120-122]

The methodology has been extended to carbohydrate-derived allylic bromides by Lubineau and co-workers.[123,124] As shown in Table 7, 2-*C*- and 4-*C*-branched sugars can be formed this way, and by subsequent dihydroxylation, the saturated sugars can be accessed in good yield. Unprotected hydroxy groups in the allylic bromides are tolerated, but so far only protected sugar aldehydes have been employed in the reaction.
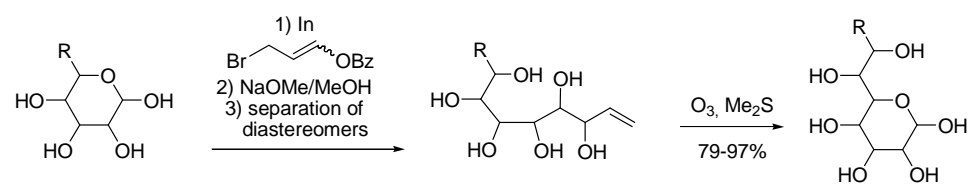
Table 7. Indium-mediated allylation of aldehydes in aqueous solution using sugar-derived allylic bromides.
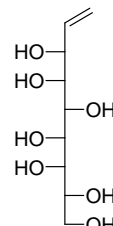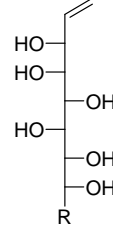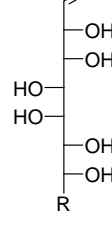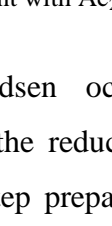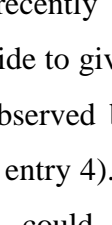
Entry	Allylic bromide	Aldehyde R-CHO	Product	Yield (%)	*R/S*
1		R = Ph		95[124]	0:1
2		R =		94[124]	1:0
3		R =		55[124]	1:1
4		R = Ph		45[123]	1:0
5		R = Ph		57[a,123]	0:1

[a] Together with 13% of the 2-*C*-branched product.

Excellent yields and diastereoselctivities can be obtained (Table 7, entries 1-2), but with slower reacting aldehydes (entry 3), indium-promoted elimination of the ethoxy group by Vasella-type fragmentation[125-128] of the allylic bromide becomes an increasing problem reducing the yield of the desired product.[124] With the β-anomer of the allylic bromide (entry 4), the yield is considerably lower, and the stereoselectivity is reversed due to steric repulsion between indium and the anomeric ethoxy group. With the bromide in a pseudo-equatorial position, the α-anomer led to the 4-*C*-branched product in 57% yield together with small amounts of the 2-*C*-branched product (13%) (entry 5), whereas the β-anomer primarily gave the 2-*C*-branched product, however in low yield (not shown).[123]

Recently, Palmelund and Madsen employed 3-bromopropenyl acetate and benzoate in indium-mediated allylation of unprotected sugars to obtain fully hydroxylated carbohydrates elongated with two carbon atoms (Table 8).[129] Following allylation, deesterification of the crude reaction mixture gave two diastereomers, which were separated and subjected to ozonolysis to afford heptoses and octoses in good overall yield.

Table 8. Sequential indium-mediated allylation and ozonolysis of unprotected sugars.[129]

Entry	Substrate	Substituents	Major diastereomer	Yield (%)	Selectivity
1	D-galactose			90[a]	3.5:1
2	D-xylose	R = H		75[a]	4.5:1
3	D-glucose	R = CH$_2$OH		71[a]	3.5:1
4	D-lyxose	R = H		60	8.5:1
5	D-mannose	R = CH$_2$OH		71[a]	3:1

[a] Isolated as the peracetate after treatment with Ac$_2$O/pyridine.

The allylation developed by Palmelund and Madsen occurs with moderate to good diastereoselectivity favoring the *lyxo* configuration at the reducing end, and the procedure is a convenient improvement of previously reported multistep preparations of heptoses based on the indium-mediated allylation.[130] Kosma and co-workers recently allylated unprotected L-lyxose in aqueous ethanol in the presence of indium and allylbromide to give an 8:1 *threo/erythro* mixture.[131] This result is in accordance with the stereselectivity observed by Palmelund and Madsen when using D-lyxose and 3-bromopropenyl benzoate (Table 8, entry 4). Following allylation of L-lyxose, the corresponding 1,2,3-trideoxy-L-*galacto*-oct-1-enitol could be isolated in 75% yield after peracetylation, and it was subsequently converted into 3,4,5,6,7-penta-*O*-acetyl-2-deoxy-L-*galacto*-heptose in 47% overall yield from L-lyxose after dihydroxylation of the double bond and oxidative cleavage of the diol with sodium periodate.[131]

Reductive fragmentation of ω-haloglycosides developed by Bernet and Vasella[125-128] produces *aldehydo* sugars containing a terminal alkene, which can be elongated by organometallic addition to the carbonyl group. Since the development of efficient ring-closing olefin metathesis catalysts,[132-134] the Vasella-fragmentation has been extensively studied in combination with elongation by organometallic addition to produce carbohydrate-derived *α,ω*-dienes. This has elegantly been accomplished by Madsen and co-workers in a zinc-mediated tandem reaction where ω-iodoglycosides (**30**) are converted into *α,ω*-dienes (**32**) in one pot (Scheme 10).[135,136] Zinc-mediated reductive fragmentation of a protected ω-iodoglycoside **30** generates an aldehyde **31**, which can be alkylated immediately by an *in situ* formed organozinc species thereby extending the carbohydrate chain by one, two or three carbon atoms. One carbon atom elongations can be achieved with diiodomethane in the presence of a Lewis acid and catalytic $PbCl_2$. Introduction of two carbon atoms can be effected by vinylation, however, divinyl zinc cannot be formed *in situ* and has to be preformed, whereas the addition of allylbromides facilitates three-carbon homologations. The resulting dienes **32** (α,ω-dienitols) can be subjected to ring-closing olefin metathesis, and by choosing either a pentose or a hexose in combination with the above mentioned homologations, carbocycles **33** with different ring sizes can easily be accessed. The methodology has recently been reviewed[18] and has successfully been applied in the synthesis of a number of natural products from readily available carbohydrate precursors.
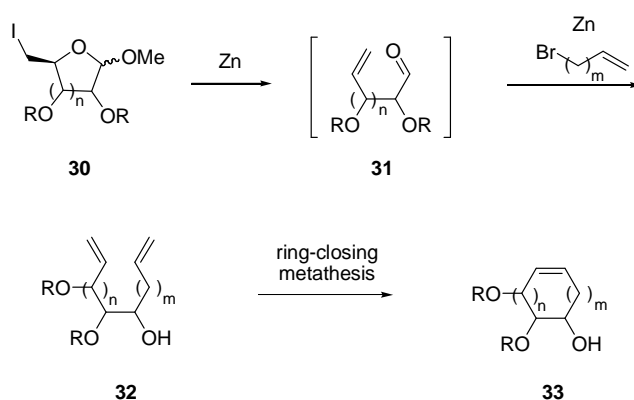
Scheme 10. Zinc-mediated tandem fragmentation-alkylation of ω-iodoglycosides **30** and subsequent ring-closing metathesis to produce carbocycles **33**.[135,136]

2.2.6 Chain elongation by olefination

Both phosphorane (Wittig olefination) and phosphonate ylides (i.e. the Horner-Emmons olefination) have found numerous applications in the chain extension of carbohydrate substrates.[26] Although the

Wittig chain extension has been known on unprotected carbohydrates for more than 40 years,[137] the formation of complex product mixtures results in moderate yields of the desired chain elongated products. With stabilized ylides (**34**), both open chain (**35**) and different cyclized products are produced due to spontaneous intramolecular Michael addition giving *C*-glycosides (**36** and **37**), or addition to the carbonyl group giving lactones (**38**) (Scheme 11).[26]
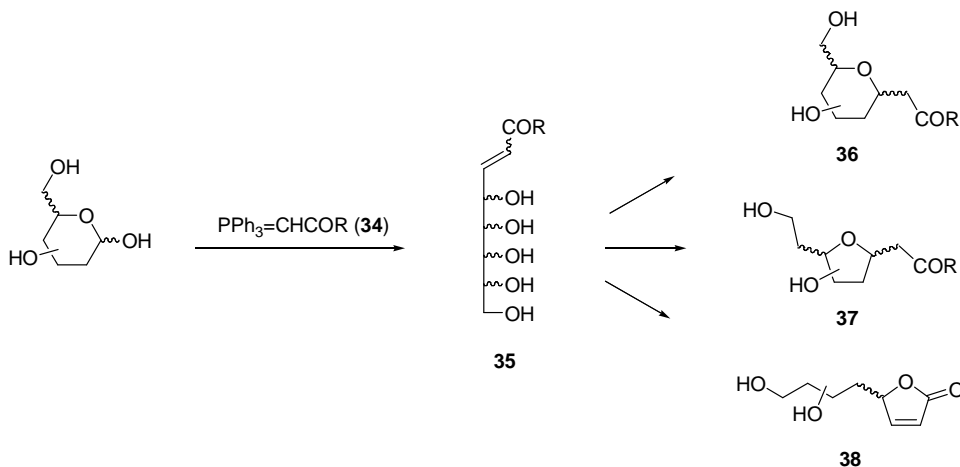
Scheme 11. Wittig reactions on unprotected sugars usually give complex product mixtures.[26]

As a result, the Wittig and the Horner-Emmons approaches to higher carbon sugars are primarily performed on protected sugars possessing a free aldehyde or hemiacetal functionality. Dihydroxylation of the resulting double bond is a common way to produce fully hydroxylated sugars, and this methodology has recently been employed by Ohira and co-workers to synthesize the naturally occurring 2-ulosonic acid KDO from D-glucose.[138]

With unprotected sugars, the formation of the Michael adducts **36** and **37** can be partially suppressed by addition of cupric acetate.[139] Furthermore, Railton and Clive recently found that by using bulky ester-stabilized phosphoranes, the Michael addition can be completely suppressed giving open chain α,β-unsaturated esters **35** with high *E* selectivities.[140]

Table 9. Sequential Wittig reaction and dihydroxylation to produce elongated sugars.

$$\text{aldose} \xrightarrow{\text{PPh}_3=\text{CHCO}_2{}^t\text{Bu}} \text{unsaturated ester} \xrightarrow{\text{OsO}_4,\ \text{NMO}} \text{higher sugar}$$

Entry	Aldose	Unsaturated ester	Substituents	Yield (%)	E/Z selectivity	Higher sugar	Yield (%)	Selectivity
1	D-glucose		R = CH$_2$OH	66[140]	1:0		76[141]	5:1
2	D-glucose		R = CH$_2$OH				68[b,141]	
3	D-xylose		R = H	94[141]	1:0		53[141]	6:1
4	D-xylose		R = H				63[b,141]	
5	D-arabinose			92[140]	1:0		69[141]	7:1
6	D-arabinose						73[b,141]	
7	D-galactose		R = H	76[140]	1:0		85[141]	8:1
8	D-galactose		R = H				77[b,141]	
9	D-*glycero*-D-*galacto*-heptose		R = CH$_2$OH	52[141]	1:0		54[a,141]	5:1

[a] Isolated as the 1,4-lactone, [b] Isolated yield of the major isomer after one-pot Wittig-dihydroxylation reaction.

As depicted in Table 9, Wittig reactions of unprotected sugars using stabilized *tert*-butyl ester phosphoranes are more efficient for pentoses (entry 3 and 5) than for hexoses (entry 1 and 7) and heptoses (entry 9). However, in all cases the *E* unsaturated ester is formed exclusively. Subsequent dihydroxylation gives fully hydroxylated sugars elongated with two carbon atoms in good yield. The dihydroxylation follows Kishi's rule[142] giving good diastereoselectivities for 2,3-*threo* configured sugars, and the Wittig-dihydroxylation sequence is therefore most efficient for formation of higher sugars containing the *galacto* configuration at the reducing end. The Wittig-dihydroxylation sequence can even be carried out as a one-pot procedure (entries 2, 4, 6 and 8), which is more convenient and give higher yields than the corresponding two-step procedure.[141]

Railton and Clive's *E*-selective procedure has recently been used by Chang and Paquette in an approach towards the highly hydroxylated polyketide amphidinol 3. In this case, the Wittig reaction between the *tert*-butyl-stabilized phosphonium ylide $PPh_3=CHCO_2{}^tBu$ and the partially protected 3,4-*O*-isopropylidene-β-D-ribopyranose afforded the *E* isomer exclusively in 90% yield.[143]

Recently, Sasaki and co-workers synthesized all eight L-hexoses by elongation of each of the two protected L-tetroses **39**, which are available in six and seven steps from L-ascorbic acid (Scheme 12).[144,145] Wittig reactions on tetroses **39** using the stabilized phosphorane ylide $PPh_3=CHCO_2Et$ gave somewhat poor *E/Z* selectivities ranging from 2:1 to 10:1, and instead the Horner-Emmons approach using the corresponding stabilized phosphonate ylide afforded (*E*)-α,β-unsaturated esters **40** in excellent stereoselectivity. By applying the Still modification[146] of the Horner-Emmons reaction, the two (*Z*)-configured unsaturated esters **42** were obtained exclusively. Subsequent asymmetric dihydroxylation of each of the four unsaturated esters **40** and **42** afforded the eight L-hexose ester derivatives **41** and **43**, which were further manipulated to produce the corresponding L-aldoses.[145]
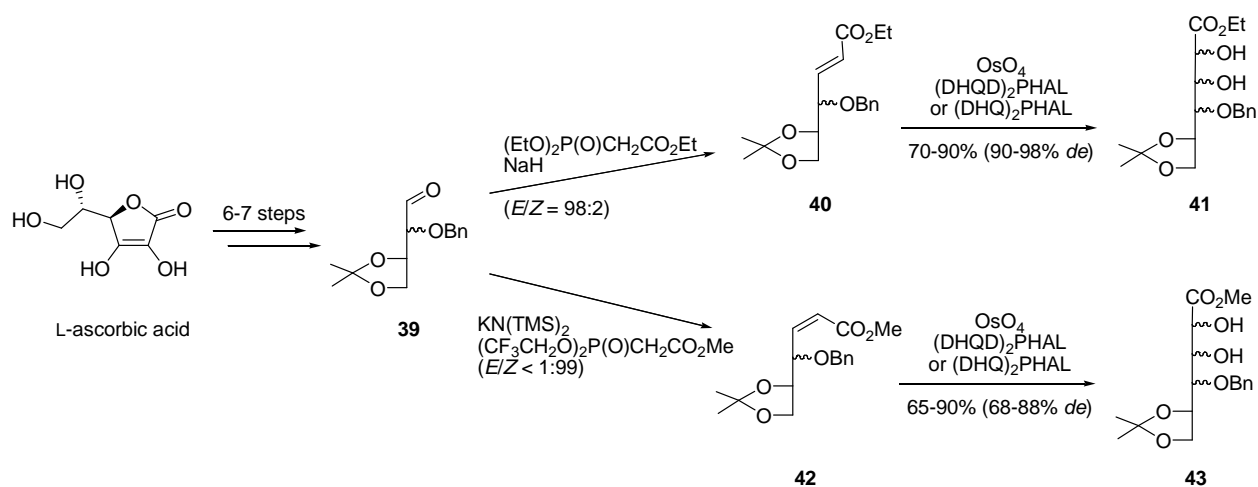
Scheme 12. Schematic representation of Sasaki and co-workers' route to all eight L-hexoses from L-ascorbic acid.[145]

One-carbon homologation of sugars by formation of ketene dithioacetals *via* Peterson and Horner-Emmons olefination has been achieved by Mlynarski and Banaszek (Table 10). Transformation of sugar lactones (**44**) into ketene dithioacetals (**45**) using the Peterson olefination only works for *lyxo* configured aldonolactones (Table 10, entry 1). Due to the basicity of the reagent, there is a great tendency to β-eliminate an alkoxy substituent,[147] and application of the Peterson olefination on

arabino and *ribo* configured aldonolactones either resulted in decomposition, or led to formation of unsaturated lactones, which upon exposure to excess reagent gave the corresponding α,β-unsaturated ketene dithioacetals in low yield.[148] On the other hand, the Horner-Emmons approach is more generally applicable (entries 2-6), however, due to the competing β-elimination, the yields of the desired ketene dithioacetals **45** are strongly dependant on the configuration of the aldonolactone (**44**).

Table 10. One-carbon homologation of sugar lactones *via* ketene dithioacetals.
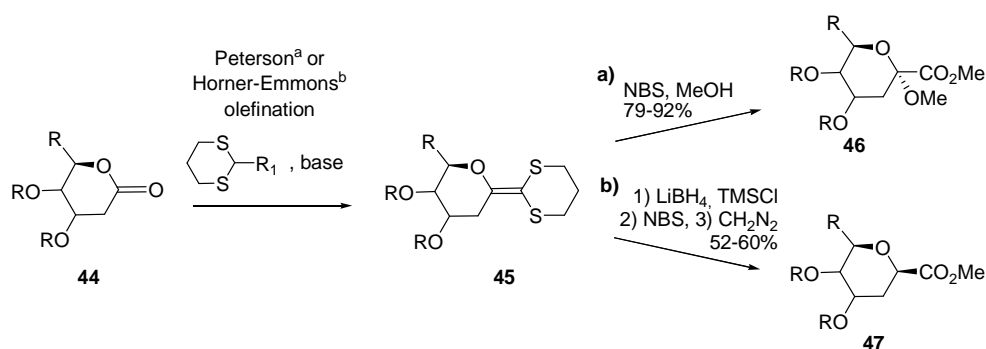
Entry	Sugar lactone 44	Substituents	Yield of ketene dithioacetal 45 (%)
1		R_2 = TBS, R_3 = H	78[a,148]
2		R_2 = TBS, R_3 = H	80[b,148]
3		R_2 = Bn, R_3 = H	72-82[b,148,149]
4		R_2 = Bn, R_3 = CH$_2$OBn	51[b,150]
5		R_2 = OBn, R_3 = H	62[b,148,149]
6		R_2 = H, R_3 = OBn	37[b,148]

[a] Peterson olefination was carried out with BuLi and R_1 = TMS, [b] Horner-Emmons olefination was carried out with KHMDS and R_1 = P(O)(OCH$_2$CF$_3$)$_2$.

As with the Peterson olefination, the Horner-Emmons reaction gave best results for *lyxo* and *manno* configured aldonolactones (Table 10, entries 2-4). Conversion of the ketene dithioacetals into 2-ulosonic acid derivatives has been accomplished by oxidation and hydrolysis of **45** in methanolic solution giving 3-deoxy-2-ulosonic acids **46** (Table 10, path a), or by reduction of the double bond followed by hydrolysis of the dithiane affording the *C*-glycosidic 2,3-dideoxy ulosonic acids **47** (Table 10, path b).[148,149] By this procedure KDO and several 2-*S*-, 2-*N*- and 2-*O*-glycosides of KDO

have been prepared by hydrolysis of ketene dithioacetals **45** in the presence of thiols, azides and sugar-derived primary alcohols, respectively.[147,150,151]

In an efficient two-step sequence, protected D-*manno* and D-*arabino* hemiacetals **48** and **51** have been converted into one carbon atom elongated 2-deoxy-aldonolactones **50** and **53** *via* ketene dithioacetals by Peterson olefination (Scheme 13). The key step in the protocol is the cyclization of the ketene dithioacetal functionality of **49** and **52** with the free hydroxy group, and by subsequent deprotection of the resulting thioacetal, chain elongated lactones are produced. This procedure constitutes an improved route to the 2-ulosonic acids KDO and DAH since the produced D-*manno* and D-*arabino* configured lactones **50** and **53** are known intermediates in their synthesis.[152]
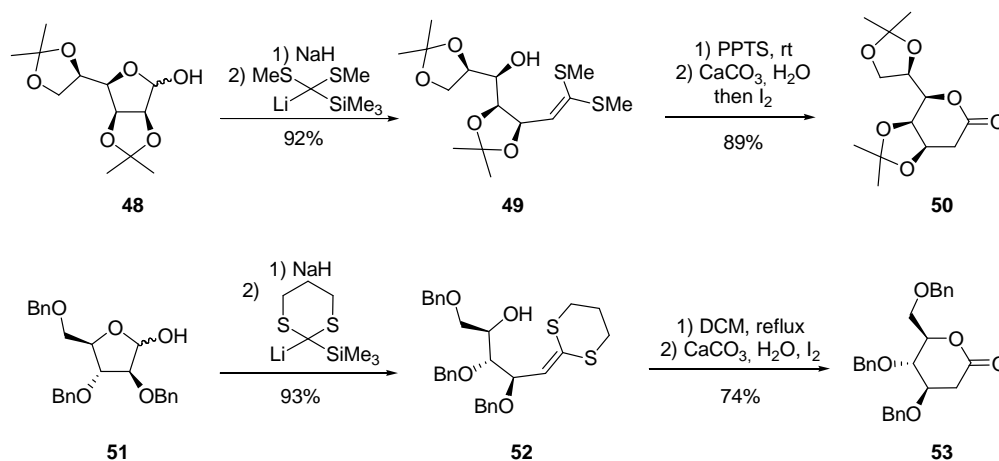
Scheme 13. One carbon atom homologation *via* ketene dithioacetals using the Peterson olefination.[152]

2.2.7 Radical based approaches to chain elongated sugars

The alkoxy radical fragmentation, which is usually a chain shortening process (see section 2.1.3), can be coupled to a three carbon atom extension by a tandem radical fragmentation-allylation reaction. Alkoxy radical fragmentation of *N*-phthalimido glycosides **54** and **57** in the presence of allyltri-*n*-butyltin and AIBN generates an intermediate radical (**55** or **58**), which is intermolecularly trapped by allyltri-*n*-butyltin to produce the corresponding hept-1-enitols **56** and **59** (Scheme 14). The presence of a carbamate at C2 (**54**) decreases the electron density at this position and enables a faster and higher yielding reaction with the electron-rich allyltri-*n*-butyltin species (2 h, 81% yield) compared to the diisopropylidene ether **57** (3.5 h, 65%).[153] By this one-pot reaction, aldoses are first shortened by one carbon atom followed by a three carbon atom extension thereby producing two-

carbon elongated ald-1-enitols, which may be useful intermediates in the synthesis of polyhydroxylated compounds.

54: R = O
57: R = Me, Me

55: R = O
58: R = Me, Me

56: R = O   81%[a]
59: R = Me, Me   65%[a]
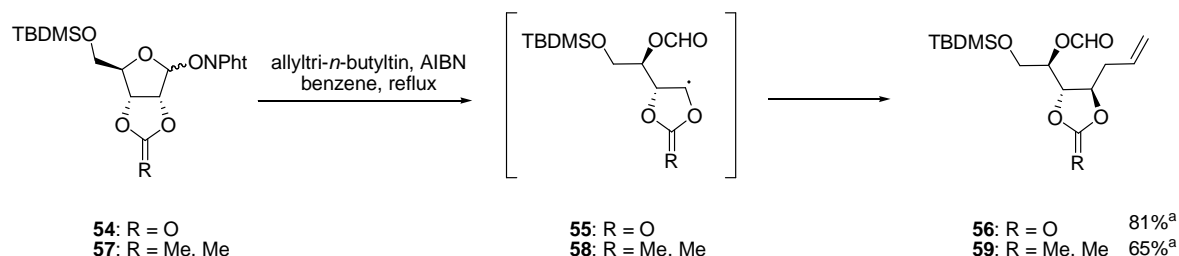
Scheme 14. One-pot alkoxy radical fragmentation and allylation of *N*-phthalimido glycosides. [a] Partial hydrolysis of the formate occurs during the reaction (~10%).[153]

Synthesis of γ-butyrolactones by SmI$_2$-mediated radical coupling of aldehydes and acrylates is well-known in organic chemistry.[154,155] Recently, this reaction was successfully applied on a sugar aldehyde for the first time. The samarium(II)-induced reductive coupling of diisopropylidene D-arabinose **60** and ethyl acrylate was achieved in THF in the presence of small amount of ^{t}BuOH to give a 68:32 epimeric mixture of *gluco* and *manno* configured 2,3-dideoxy-octono-1,4-lactones **61** in 74% yield (Scheme 15). Only moderate stereoselectivity was obtained, but the octose derivatives **61**, which are intermediates in previously reported syntheses of *gluco-* and *manno*-KDO, could easily be separated by chromatography.[156]

60   **61**
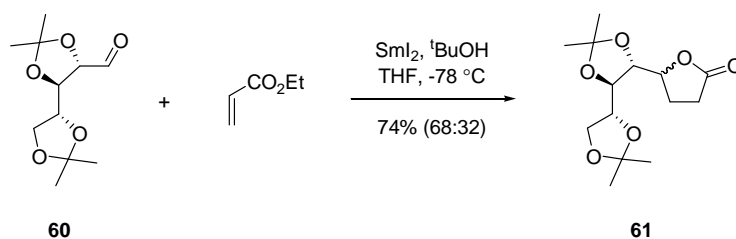
Scheme 15. Samarium(II)-mediated coupling of protected D-arabinose (**60**) and ethyl acrylate.[156]

The formation of 2-*C*-branched sugars by transition metal mediated radical addition of malonates to glycals has been achieved by Linker and co-workers (Table 11).[157] By treatment of malonates **63** with either Mn(OAc)$_3$ or CAN, malonyl radicals are formed. These can react with glycals (**62**) in a highly regioselective process to give an anomeric radical that is oxidized to a glycosyl cation, which is finally trapped by the solvent to produce 2-*C*-branched sugars (**64**). The reaction is sensitive to sterics, and the malonyl radicals predominantly attack *trans* to the 3-*O*-acetyl substituent. Similarly,

steric effects, and probably also neighboring group participation of the malonyl substituent, leads to a stereoselective attack of the solvent *trans* to the newly incorporated malonyl substituent. Attack of the malonyl radical *cis* to the 3-*O*-acetyl group leads to small amounts of the 2-epimeric byproducts (0-14% yield), and in the CAN-mediated reactions, capture of the glycosyl cation by nitrate from the oxidizing agent instead of by the solvent is a competing side-reaction.[157,158]

Table 11. Transition metal mediated radical addition of malonates to glycals.

Entry	Starting material	Substituents	Products A: glycoside	B: ortho ester	A Yield (%)	B Yield (%)	α/β ratio
1		$R_1 = {}^iPr$, $R_2 = Ac$, $R_3 = OAc$			$57^{a,158}$		8:92
2		$R_1 = {}^iPr$, $R_2 = Me$, $R_3 = OAc$			$68^{b,158}$		0:1
3		$R_1 = {}^iPr$, $R_2 = Me$, $R_3 = H$			$75^{b,158}$		3:97
4		$R_1 = Me$, $R_2 = Me$, $R_3 = H$			$81^{b,158}$		5:95
5		$R_1 = Me$			$89^{b,158}$		92:8
6		$R_1 = Me$, $R_3 = H$			$78\text{-}86^{b,158,159}$	0	0:1
7		$R_1 = Me$, $R_3 = CONH_2$			$70^{b,159}$	0	
8		$R_1 = Me$, $R_3 = CO_2Me$			7	$70^{b,159}$	
9		$R_1 = Me$, $R_3 = CN$			0	$80^{b,159}$	

^{a} Mn(OAc)$_3$ (2-4 eq.), AcOH, KOAc, 95 °C, ^{b} CAN (2-6 eq.), MeOH, 0 °C.

With Mn(III), 95 °C are required for the reaction to proceed (Table 11, entry 1), and the 2-*C*-branched *gluco* derivative can be obtained in good yield and diastereoselectivity. CAN, on the other hand, is capable of mediating the reaction under mild conditions (0 °C), which leads to higher yields and increased diastereoselectivities as compared to Mn(OAc)$_3$ (entry 2). Pentals give higher yields than the corresponding hexals (entries 2 and 3), and by using the more sterically demanding isopropyl malonate (as compared to the methyl malonate), slightly higher diastereoselectivities can be obtained at the expense of the yield (compare entries 3 and 4). Arabinal and galactal (entry 5 and 6), which only have substituents on one side of the pyranoid ring, react with malonyl radicals exclusively from the β- and α-face of the ring, respectively, and no *cis* byproducts with respect to

the 3-*O*-acetyl group are observed.[158] With electron-withdrawing substituents at the anomeric center (entries 7-9), the oxidation potential of the produced anomeric radicals change,[159] and with esters and nitriles this leads to cyclic ortho esters as the main products due to intramolecular capture of the anomeric radical by the malonyl carbonyl oxygen atom.

This transition metal mediated radical reaction is a highly efficient way to obtain 2-*C*-branched sugars **64** from easily available glycals **62**, and the methodology has recently been used in the synthesis of *C*-glycosylated glycine derivatives in few synthetic steps.[160]

Also ald-1-enitols react with malonyl radicals in the presence of Mn(OAc)$_3$. In this case the malonyl group is attached to C1 of the ald-1-enitol **65**, however in low yield (<25%).[156,161] Instead, it has been found that in the absence of dimethyl malonate, acetate can be added to the double bond to produce γ-butyrolactones in excellent yield (Scheme 16). After heating to 90 ºC for 60 h, the epimeric two-carbon extended 2,3-dideoxy-*gluco*- and *manno*-octono-1,4-lactones **66** were formed with moderate stereoselectivity in favor of the *manno* isomer.[161]
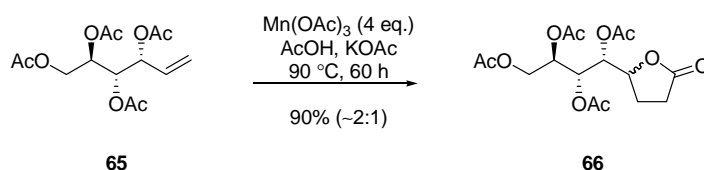
Scheme 16. Manganese-mediated radical addition of acetate to a protected D-gluc-1-enitol (**65**).[161]
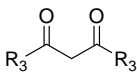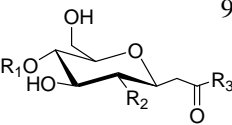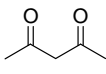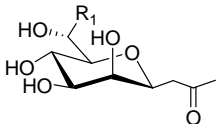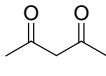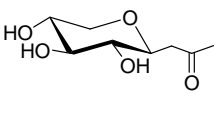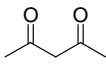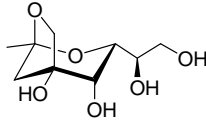
2.2.8 Formation of C-glycosides by the Knoevenagel condensation

Chain elongation of protected or unprotected sugars with malonester derivatives by a non-radical mechanism, i.e. the so-called Knoevenagel condensation of aldehydes and ketones has been used to elongate carbohydrates during the last 40 years.[26] Recently, Lubineau and co-workers found that condensation of unprotected carbohydrates with penta-2,4-dione in alkaline aqueous solution resulted in formation of β-glycosidic ketones in excellent yield (Table 12).[162] As a result of thermodynamic control, the β-*C*-glycosides are formed exclusively in most cases.

D-Glucose, D-mannose and the disaccharide D-cellobiose are cleanly converted into their corresponding β-*C*-glycosides (Table 12, entries 1, 2 and 5) using penta-2,4-dione. Lipophilic β-diketones result in lower yields due to decreased solubility (entry 3), whereas unsymmetrical

β-diketones give 1:1 mixtures of the corresponding β-*C*-glycosidic ketones with no selectivity in the elimination step (not shown).[163] In the case of *N*-acetyl-D-glucosamine (entry 4), equilibration at C2 occurs to produce a 2:3 epimeric mixture of the corresponding 1-β-*C*-D-glucose and 1-β-*C*-D-mannose derivatives in 83% combined yield. Epimerization is also observed in the case of *N*-acetyl-D-mannosamine, whereas *N*-acetyl-D-galactosamine gives a single isomer.[164] D-*Glycero*-D-*manno*-heptose gives a majority of the α-glycosidic ketone (entry 6), but after equilibration of the 2:1 α/β-mixture, 65% of the desired β-isomer could be isolated as its peracetylated derivative.[165] D-Xylose gives a 2:5 α/β-mixture in 97% yield (entry 7), whereas D-ribose predominantly gives the furanoid product as a 1:2 anomeric mixture (not shown).[166] Surprisingly, a bicyclic branched 2-octulose was isolated in 27% yield when the reaction conditions were applied to D-fructose (entry 8).

Table 12. One-step Knoevenagel condensation of unprotected sugars with β-diketones in aqueous $NaHCO_3$.

Entry	Substrate	Diketone	Substituents	Product	Yield (%)	β/α ratio
1	D-glucose		R_1 = H, R_2 = OH, R_3 = CH_3		96-99[162,166]	10:1-1:0
2	D-cellobiose		R_1 = β-D-glc, R_2 = OH, R_3 = CH_3		93[162]	1:0
3	D-glucose		R_1 = H, R_2 = OH, R_3 = $(CH_2)_5CH_3$		75[163]	1:0
4	*N*-acetyl-D-glucosamine		R_1 = H, R_2 = NHAc, R_3 = CH_3		83[a,164]	1:0
5	D-mannose		R_1 = H		95-97[162,166]	20:1-1:0
6	D-*glycero*-D-*manno*-heptose		R_1 = CH_2OH		(65% β)[b,165]	1:2
7	D-xylose				97[166]	5:2
8	D-fructose				27[167]	

[a] Formed as a 2:3 mixture with the C2 epimer, [b] 65% of the β-isomer was isolated as the pentaacetate after equilibration of the initially formed α/β-mixture with NaOMe/MeOH and subsequent treatment with Ac_2O/pyridine.

The applicability of this one-step procedure on unprotected carbohydrates in water with excellent stereocontrol is a major improvement of previous chain elongation procedures based on the Knoevenagel condensation, and the formed β-*C*-glycosidic ketones can easily be transformed into

other functionalities e.g. conversion into the corresponding 1-β-*C*-formyl-glycosides in few steps (Scheme 17). By this procedure acetyl protected 2,6-anhydro-D-*glycero*-D-*gulo*-heptose **68** has been prepared in five steps in 59% overall yield from D-glucose.[168,169] Both the β-*C*-glycosidic ketone **67** and the 2,6-anhydroheptose **68** are conveniently set up for further chain elongation reactions e.g. the aldol condensation.[170]
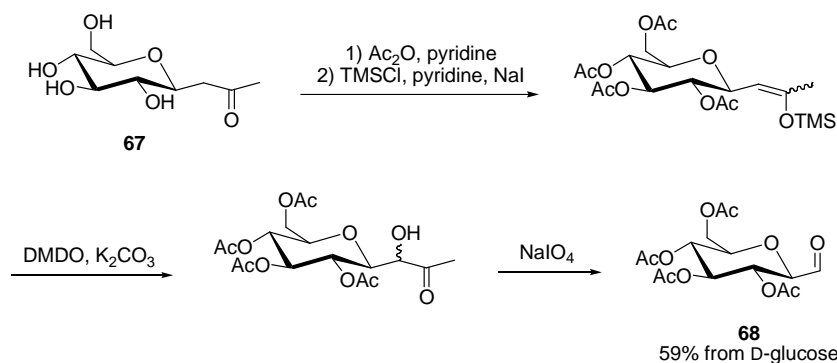
Scheme 17. Transformation of 1-β-*C*-glycosidic ketone **67** into 2,6-anhydro-D-*glycero*-D-*gulo*-heptose **68**.[168]

The condensation of unprotected sugars with β-dicarbonyl compounds to produce polyhydroxylated furans is known as the Garcia-González reaction, and it is usually performed under relatively harsch reaction conditions.[171] As a result of increased attention on the preparation of β-*C*-glycosidic ketones from sugars *via* the Knoevenagel cndensation, the Garcia-González reaction has also been improved. As shown in Scheme 18, D-glucose and penta-2,4-dione can be converted into a polyhydroxylated furan derivative **69** in 95% yield under solvent free conditions using catalytic amounts of CeCl$_3$ and NaI.[171,172]
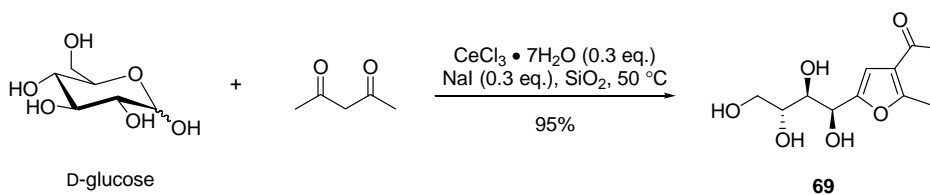
Scheme 18. Improved protocol for the Garcia-González reaction between D-glucose and penta-2,4-dione.[172]

2.2.9 Synthesis of exo glycals

C-Glycosides having an *exo* double bond at the anomeric center (*exo* glycals) are interesting synthetic intermediates towards various *C*-glycosides. The presence of the ring oxygen next to the double bond enables transformation of these enol ethers into a variety of useful functionalities. The synthesis of *exo* glycals has recently been described in two reviews, to which the reader is referred for a more thorough discussion.[93,173]

Exo glycals are most frequently accessed from sugar lactones, although efficient transformation of sugar lactones into *exo* glycals mainly has been limited to the Tebbe reagent.[26] By this procedure only disubstituted *exo* glycals can be produced, and tri- and tetrasubstituted *exo* glycals are harder to access. Olefination reactions based on the Wittig reaction suffer from poor reactivity of sugar lactones, and quite harsh reaction conditions (110-140 ºC) are required giving almost no *E*/*Z* selectivity.[173] Recently, Xie and co-workers found that perbenzylated 1,5-lactones **70** and **72** react with stabilized phosphonium ylides to give exclusively the *Z* isomers **71** and **73** in good yield (Scheme 19).[174,175]

70: D-*gluco*
72: D-*galacto*

71: D-*gluco*   87%
73: D-*galacto*   90%
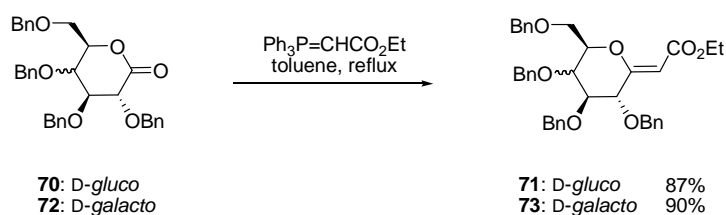
Scheme 19. Perbenzoylated six-membered sugar lactones give exclusively the *Z exo* glycals by Wittig reaction with stabilized ylides.[174]

Alternatively, carbohydrates carrying a phosphonium ylide at the anomeric carbon can be employed to produce tri- and tetrasubstituted *exo* glycals.[173] An improved protocol for the formation of anomeric phosphonium ylides has recently been reported by Lieberknecht and co-workers, and by refluxing methyl glycosides with PPh_3 and HBF_4 in acetonitrile, the corresponding anomeric phosphonium salts are formed in excellent yield in a single step.[176]

Also the Ramberg-Bäcklund rearrangement of anomeric sulfoxides can be employed to produce *exo* glycals.[177-179] Base-mediated rearrangement of anomeric sulfoxides (**75**), which are easily available from thioglycosides (**74**) by oxidation, predominantly gives *Z exo* glycals (**76**) (Scheme 20). This

procedure has recently been employed in the synthesis of *C*-disaccharides[180,181] and a *C*-glycoside of an amino acid.[182]
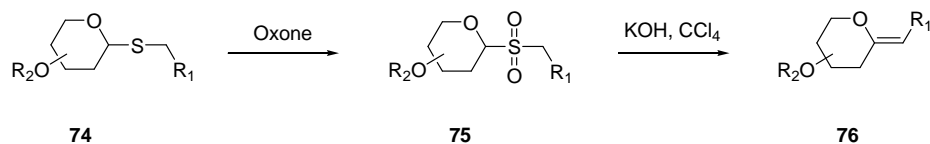
Scheme 20. The Ramberg-Bäcklund approach to *exo* glycals.[177]

Very recently, Gueyrard and co-workers applied the Julia olefination on sugar lactones producing a number of di-, tri- and tetrasubstituted *exo* glycals (Scheme 21).[183,184] For trisubstituted *exo* glycals, the *E*/*Z* selectivity is good for *arabino* configured lactones (e.g. **77**, which favors the *E exo* glycal **78**), whereas *gluco* configured lactones are less selective giving 1:1 mixtures.
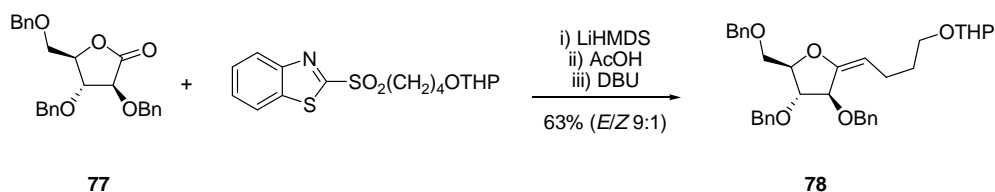
Scheme 21. Formation of *exo* glycals by the Julia olefination.[184]

2.2.10 Chain extension by coupling of two sugars

Several strategies for extending the carbohydrate chain by coupling of two suitably derivatized sugars have recently been exploited. A detailed discussion of such approaches is outside the scope of the present chapter, but the available methods include nitrile oxide cycloaddition,[185] Baylis-Hillman type condensation,[186,187] cross metathesis,[188] aldol condensation,[189-191] hetero Diels-Alder reaction,[192] Wittig and Horner-Emmons reactions,[193-195] and tin-mediated allylation of aldehydes.[196,197]

2.3 Concluding remarks

Several different methods for shortening and extending the carbon chain in sugars exist, each with its own specific advantages and drawbacks. The most noteworthy advantage is the ability to modulate sugars without protective groups e.g. the recent advances within indium-mediated allylation, the Wittig reaction and the Knoevenagel condensation. In addition, the fact that the

stereochemical outcome can be predicted and controlled makes such processes extremely attractive routes for elongation of carbohydrates. Together, all the available methods constitute a diverse tool box for construction of carbohydrate-based polyhydroxylated compounds, which is particularly useful in synthetic chemistry given the importance of sugars in chemistry and biology as discussed in chapter 1.

3 Chain shortening of aldoses by rhodium-catalyzed decarbonylation

3.1 Introduction

As mentioned in chapter 2, a number of different methods for shortening protected carbohydrates are available,[26] although not many of these are applicable to *unprotected* sugars. The most notable methods for shortening unprotected carbohydrates are the Ruff degradation (section 2.1.1), which converts salts of aldonic acids into aldoses with one less carbon,[27,31] and the Humphlett degradation, which produces aldonic acids from the corresponding higher aldoses.[198,199] Recently, homogeneous transition metal mediated transformations have grown to become an extremely powerful tool in synthetic organic chemistry,[200] and transition metals have also found numerous applications in carbohydrate chemistry.[16] In 1988, Andrews and Klaeren[201] used Wilkinson's reagent[202] (RhCl(PPh$_3$)$_3$) to shorten unprotected aldoses by one carbon atom by means of a decarbonylation of the aldehyde functionality to produce the corresponding lower alditol (Scheme 22).
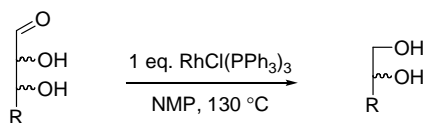
Scheme 22. Decarbonylation of aldoses using stoichiometric amounts of Wilkinson's reagent.[201]

However, the decarbonylation reaction developed by Andrews and Klaeren requires stoichiometric amounts of rhodium, and as a result, the procedure is not convenient except for small scale synthesis. As we shall see in section 3.2.2 below, the rhodium-mediated decarbonylation of simple alkyl and aryl aldehydes has improved considerably during the last few years, and it can now be performed with catalytic amounts of rhodium. Based on these recent developments, the objective of this project is to improve the decarbonylation of unprotected carbohydrates to become catalytic with regards to rhodium thereby providing a procedure to shorten unprotected sugars, which is complementary to other existing methods.

3.2 Literature background

3.2.1 Rhodium-mediated decarbonylation

The rhodium-mediated decarbonylation of aldehydes was initially discovered by Tsuji and Ohno[203] in an attempt to find a more efficient decarbonylation catalyst than metallic palladium. At that time

palladium on carbon was known to decarbonylate both aldehydes and acyl halides catalytically at temperatures exceeding 200 ºC (Scheme 23).[204]

X = H, halide
R = alkyl, aryl
M = transition metal species

Scheme 23. Transition metal mediated decarbonylation of aldehydes and acyl halides.

It was found that square-planar $RhCl(PPh_3)_3$ could mediate the decarbonylation of a range of aliphatic, α,β-unsaturated, and aromatic aldehydes under mild conditions at room temperature or in refluxing solvents like benzene and toluene.[203] The decarbonylation was stoichiometric in rhodium due to formation of $RhCl(CO)(PPh_3)_2$, which is very stable[205] and cannot lose carbon monoxide to regenerate the active decarbonylation species.[206] A couple of years later, Tsuji and Ohno reported that catalytic decarbonylations with $RhCl(CO)(PPh_3)_2$[207,208] or $RhCl(PPh_3)_3$[206] could be achieved at higher temperatures. In fact, temperatures above 200 ºC were required in order for catalytic decarbonylation to occur.[208] Due to increasing decomposition and unwanted side-reactions of aliphatic aldehydes (aldol condensations) at elevated temperatures, the catalytic version was only applicable to aromatic aldehydes possessing no other functional groups.[206,208,209]

Tsuji and Ohno also discovered that acyl halides could be catalytically decarbonylated using Wilkinson's reagent.[205,206,208] With acyl halides, the corresponding shortened olefins are formed as a result of facile β-hydrogen elimination, whereas in the absence of β-hydrogens, alkyl or aryl halides are formed instead.[208,210] Later it was found that also ketones could be decarbonylated in specialized cases. There are only a few examples in the literature, but α- or β-diketones[211] as well as biarylketones[212] can be decarbonylated catalytically employing Wilkinson's reagent, however in low to moderate yields with relatively high catalyst loadings. For example acetylacetone is converted into methyl ethyl ketone,[211] and 4,4'-dimethylbenzophenone is converted into 4,4'-dimethylbiphenyl[212] by this procedure. Also various carboxylic acid derivatives like acid anhydrides[213,214] and acyl nitriles[215] can be decarbonylated by Rh(I)-species.

Other transition metals than rhodium have also been found to facilitate the decarbonylation of aldehydes. In addition to Wilkinson's reagent, stoichiometric amounts of $[RuCl_2(PPhEt_2)_3]_2$ can be

applied in homogeneous decarbonylation at 80-90 °C,[206,209] whereas catalytic decarbonylation can be achieved homogeneously with [IrCl(COD)]$_2$ in the presence of phosphine ligands at 101-120 °C,[♦,216,217] or heterogeneously with metallic Pd, Pt, Rh and Ni at temperatures above 200 °C.[209] In contrast to Wilkinson's reagent, the above mentioned metals generally produce the corresponding decarbonylated olefins as the major products, and alkene-alkane mixtures are often observed. Furthermore, Ir gives rise to alkene isomerization even at low temperature.[217] In any case, until recently, RhCl(PPh$_3$)$_3$ was the most efficient reagent for decarbonylation of aldehydes.[206,209]

The rhodium-mediated decarbonylation of aldehydes is sensitive to steric hindrance, and for aliphatic aldehydes the order of reactivity is primary > secondary > tertiary.[207,208,218] Primary aldehydes are readily decarbonylated at room temperature, whereas secondary aldehydes generally require heating.[208] Sterically hindered secondary and tertiary aldehydes are not easily decarbonylated, but when excess of Wilkinson's reagent is used more sterically demanding substrates can be decarbonylated in good yields (Scheme 24).[219,220]
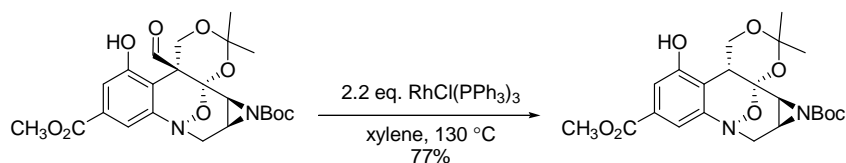
Scheme 24. The decarbonylation reaction is sensitive to steric hindrance, and bulky tertiary aldehydes require an excess of Wilkinson's reagent.[219]

Rhodium-mediated decarbonylation is stereospecific and both alkene geometry and the configuration of a stereocenter next to the aldehyde moiety are rigorously retained.[207-209,218] However, under catalytic decarbonylation at forcing conditions (160-260 °C), alkene isomerization can be observed. Walborsky and Allen found that during decarbonylation of 2-substituted cinnamaldehydes, up to 26% double bond isomerization to the more stable (*E*)-isomers occurred.[218] The alkene isomerization is rather thermal than rhodium-mediated,[218] but as a result, substituted α,β-unsaturated aldehydes cannot conveniently be decarbonylated catalytically, and instead stoichiometric amounts of Wilkinson's reagent at lower temperatures are required.

♦ During the preparation of this thesis, an efficient procedure for decarbonylation of various aldehydes in good yield (63-94%) using commercially available [IrCl(COD)]$_2$ (5%) and PPh$_3$ (5%) for 24-48 h in refluxing dioxane (101 °C) was reported (Iwai, T.; Fujihara, T.; Tsuji, Y. *Chem. Commun.* **2008**, 6215). Compare with the catalytic decarbonylation mediated by rhodium(I), which will be described in section 3.2.2.

3.2.2 Catalytic decarbonylation

Due to the inconvenience of using stoichiometric amounts of costly rhodium complexes, considerable effort has been put into making the decarbonylation catalytic with regards to rhodium. In 1978, Doughty and Pignolet speculated that cationic rhodium(I) complexes containing chelating phosphine ligands should bind carbon monoxide less strongly. This would enable decarbonylation and subsequent loss of carbon monoxide at lower temperatures than required with Wilkinson's reagent.[221] Cationic metal carbonyl complexes are less electron rich than their corresponding neutral metal complexes, and this results in a decreased Rh-CO π back-donation making carbon monoxide more weakly bound to the metal.[222] Doughty and Pignolet rationalized that the lower electron density on the metal complex combined with a *trans* rhodium-phosphorous stereochemistry would bind carbon monoxide less strongly and place carbon monoxide in an equatorial position in a distorted trigonal bipyramidal structure as depicted in Figure 1.[221] This structural composition had earlier been firmly determined by X-ray crystallography of the analogues iridium complex [Ir(CO)(dppe)$_2$]$^+$Cl$^-$,[223] which is known to reversibly bind carbon monoxide in solution.[221]
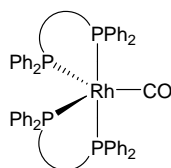
Figure 1. Proposed distorted trigonal bipyramidal structure of cationic Rh(I)-complexes containing two bidentate phosphine ligands and carbon monoxide.[221,223]

Doughty and Pignolet screened the rhodium(I)-complexes of four different bidentate phosphine ligands: dppm, dppe, dppp and dppb (Figure 2). Catalytic decarbonylation was achieved at 115-178 °C for all four rhodium(I) complexes, but the reaction rate was very dependant on the chelate ring size and the bite angle.[224] The reaction rate increases when going from four- to six-membered chelate rings obtaining the best results with Rh(dppp)$_2$Cl. Further increase to the seven-membered chelate ring of Rh(dppb)$_2$Cl results in much lower catalytic activity.[225]
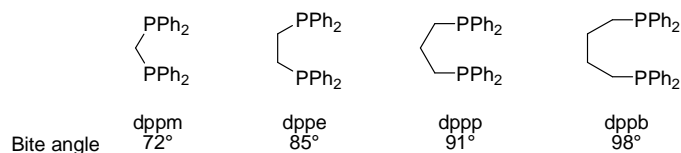
Figure 2. Structures and bite angles[224] of bidentate phosphine ligands tested by Doughty and Pignolet.[225]

The catalytic activity was found to be independant of the identity of the counterion, and identical results could be obtained using Cl^- and BF_4^- metal complexes. Several different aliphatic, α,β-unsaturated and aromatic aldehydes could be decarbonylated catalytically with $Rh(dppp)_2Cl$ at more useful temperatures.[221,225] However, $Rh(dppp)_2Cl$ is not commercially available thereby limiting the utility of the procedure compared to Wilkinson's reagent. In an attempt to address this problem, Meyer and Kruse formed $Rh(dppp)_2Cl$ *in situ* by heating Wilkinson's reagent together with 2.2 equivalents of dppp in xylene at 80 ºC for 15 min. Subsequent addition of different aromatic aldehydes followed by heating to reflux for 18-24 h gave the decarbonylated products in 82-95% yields with 1-4% *in situ* formed $Rh(dppp)_2Cl$.[226] Boeckman and co-workers later successfully formed the catalyst *in situ* from dppp and another rhodium(I)-complex, $[RhCl(COD)]_2$.[227] However, since the introduction of $Rh(dppp)_2Cl$ by Doughty and Pignolet, its use in the decarbonylation reaction has remained somewhat limited. In fact, it may not be completely reliable as experienced by McCague and co-workers, who failed decarbonylating a dialdehyde with catalytic $Rh(dppe)_2Cl$ and $Rh(dppp)_2Cl$, and had to turn to stoichiometric amounts of Wilkinson's reagent instead.[228] Also Hansson and Wickberg unsuccessfully applied catalytic $Rh(dppp)_2Cl$ to the decarbonylation of a vinylic aldehyde, and just like McCague and co-workers, they had to use Wilkinson's reagent instead.[229] As a result of these unsuccessful attempts to use $Rh(dppp)_2Cl$, most applications of rhodium-mediated decarbonylation in synthesis have employed the more reliable Wilkinson's reagent in stoichiometric amounts.[219,220,230,231]

In a search for a more widely applicable mild catalytic decarbonylation procedure, O'Connor and Ma realized that additives capable of abstracting carbon monoxide from rhodium would enable catalysis at low temperature.[232] They found that stoichiometric amounts of diphenylphosphoryl azide (DPPA) together with catalytic $RhCl(PPh_3)_3$ in THF decarbonylated a range of different primary aldehydes in excellent yield at room temperature. By addition of DPPA, carbon monoxide is abstracted from the initially formed $RhCl(CO)(PPh_3)_2$ by formation of diphenylphosphoryl isocyanate with loss of nitrogen gas. However, the application of DPPA to abstract carbon

monoxide is limited to primary aldehydes,[232] and attempts to apply the procedure to aromatic aldehydes failed.[233]

At the same time, it was found that catalytic decarbonylation could be achieved using the dimeric rhodium(I)-species [RhCl(CO)(PMe₃)]₂ during continuous purging with argon.[234] The rate of decarbonylation with [RhCl(CO)(PMe₃)]₂ at 100 ºC was comparable to the rate of Rh(dppp)₂⁺ developed by Doughty and Pignolet.[221,234] Crabtree and co-workers later used a tridentate phosphine ligand in the metal complex [Rh(CO)(triphos)]SbF₆, which reversibly binds a second molecule of carbon monoxide.[235] In this way, they successfully decarbonylated 4-biphenylcarboxaldehyde in refluxing dioxane at 100 ºC, but the reaction was very slow. After 94 h, only 34% yield was achieved with 5% catalyst loading. However, increasing the temperature to 162 ºC in refluxing diglyme enabled simple primary and aromatic aldehydes to be decarbonylated in 43-100% yield after 45-48 h, whereas more sterically demanding aliphatic aldehydes gave low conversion. In addition to reasonably slow conversion, the catalyst requires several steps to prepare, and as a result, it has only found very little practical use.

A major improvement was provided by our group when Anders Palmelund found that Rh(dppp)₂Cl could be prepared *in situ* form commercially available RhCl₃ • 3H₂O and dppp.[236] Testing a number of different solvents showed that the polyether solvent diglyme with a boiling point of 162 ºC was very suitable for the decarbonylation. A screening of numerous bi- and tridentate phosphine ligands revealed that metal complexes containing ligands with bite angles[224] between 91 and 96º were very reactive giving quantitive conversion of 2-naphthaldehyde into naphthalene within 3 h.[236]
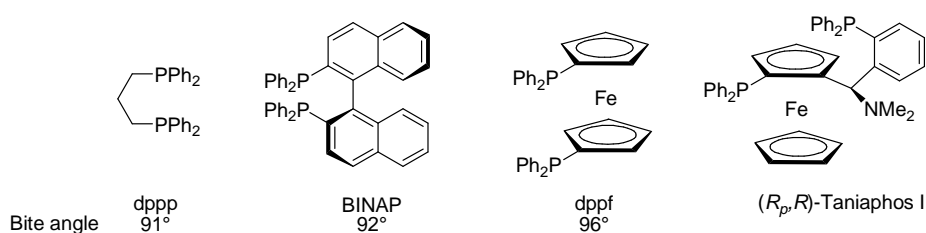
Figure 3. The four most reactive metal complexes employed bidentate phosphine ligands with bite angles between 91 and 96º.[224,236]

Metal complexes of BINAP and dppp were found to be the most reactive (Figure 3), and due to lower cost, dppp was chosen for further optimization. Best results were obtained with two

equivalents of dppp compared to RhCl$_3$ • 3H$_2$O, and a range of aromatic aldehydes were smoothly decarbonylated with 0.4-10% catalyst in 74-94% isolated yield. Also primary and secondary aliphatic aldehydes were easily decarbonylated, however, with sterically demanding tertiary substrates, no decarbonylation was achieved. The procedure tolerates many functional groups such as ethers, esters, nitriles, chlorides, substituted amines and amides as well as alkenes, and it constitutes a very general and convenient procedure for catalytic decarbonylation of aldehydes.[236]

3.2.3 Mechanism

In 1982, Doughty and Pignolet suggested a mechanism for the rhodium-catalyzed decarbonylation of aldehydes using bidentate phosphine ligands based on deuterium labeling, kinetic isotope effect, reaction kinetics and analysis of reaction intermediates (Scheme 25a).[225] They believed that the square-planar Rh(dppp)$_2$$^+$ (**79**) was the catalytically active species and their proposed mechanism consisted of five elementary steps: coordination of the aldehyde to **79**, oxidative addition to give **81**, migratory extrusion of carbon monoxide giving **82**, liberation of the product by reductive elimination and finally loss of carbon monoxide to regenerate **79**.

Scheme 25. Proposed catalytic cycles for the rhodium(I)-catalyzed decarbonylation of aldehydes. a) Doughty and Pignolet's mechanism consists of five elementary steps: coordination of the aldehyde, oxidative addition, migratory extrusion, reductive elimination and loss of carbon monoxide.[225] b) The mechanism proposed by Fristrup *et* al. consists of four elementary steps: coordination of the aldehyde, oxidative addition, migratory extrusion and reductive elimination.[237]

However, mechanistic studies in our group by Fristrup *et* al. by means of experimental Hammett studies and kinetic isotope effect as well as computational DFT (density functional theory) calculations suggest a catalytic cycle based on the catalytically active species Rh(dppp)(CO)L$^+$ (**83**),

where L is a spectator ligand (Scheme 25b).[237] The identity of the ligand L may be either a second molecule of carbon monoxide or another dppp coordinating as a monodentate ligand as suggested by Doughty and Pignolet,[225] but calculations showed that coordination of an aldehyde to the square-planar Rh(dppp)$_2^+$ (**79**) is highly unfavorable.[237] As a result, the square-planar Rh(dppp)(CO)L$^+$ (**83**) is more likely to be the catalytically active species.

The mechanism proposed by Fristrup *et* al. is based on experimental and computational studies with benzaldehyde and phenylacetaldehyde, and it consists of four elementary steps: coordination of the aldehyde to **83** with loss of the carbon monoxide ligand, oxidative addition producing **84**, migratory extrusion of carbon monoxide to give **85** and finally reductive elimination. The experimentally determined kinetic isotope effects were in excellent agreement with computational values suggesting that the reaction mechanism is the same for both substrates, and that migratory extrusion (**84** → **85**) is the rate-determining step.[237] This is in contrast to Doughty and Pignolet's proposed mechanism where oxidative addition (**80** → **81**) was believed to be rate-determining,[225] but the comprehensive experimental study supported by computational calculations performed by Fristrup and co-workers strongly suggests a mechanism according to Scheme 25b.

3.2.4 Recent synthetic applications

The impact of the decarbonylation of aldehydes in synthetic organic chemistry can be illustrated by the following recent applications. In these examples the rhodium-mediated decarbonylation of an aldehyde has been used in combination with a number of other reactions to achieve more than one transformation in a single pot.

Intramolecular trapping of aryl-rhodium species produced from their corresponding aldehydes have been achieved by Kampmeier and co-workers to perform carbocyclizations (Scheme 26).[238] Insertion of rhodium into the aldehydic C-H bond of 2-allylbenzaldehyde (**86**) followed by decarbonylation and intramolecular capture by the alkene led to indane (**87**) in a 4:1 ratio with the decarbonylation product.

Scheme 26. Intramolecular capture of rhodium-aryl species to produce carbocycles.[238]

Tandem Oppenauer-decarbonylation reactions can be achieved using *in situ* generated Rh(dppp)$_2$Cl at 170 ºC thereby removing a hydroxymethyl group from a primary alcohol (Scheme 27).[236] Applying 2% of the hydrogen transfer catalyst [Cp*IrCl$_2$]$_2$ in the presence of K$_2$CO$_3$ together with 4% of the *in situ* generated decarbonylation catalyst afforded 73% yield of *p*-xylene from 2-(4-methylphenyl)ethanol (**88**).

Scheme 27. Tandem Oppenauer-decarbonylation reaction.[236]

Aldehydes can be used as alkene equivalents in the Diels-Alder reaction.[239] Performing a BF$_3$ • Et$_2$O catalyzed Diels-Alder reaction followed by quenching of the Lewis acid, addition of Rh(dppp)$_2$Cl and heating to 162 ºC lead to good yields of cyclohexene products, which are not available by a direct Diels-Alder reaction between the diene and ethylene (Scheme 28). In this way, the carbonyl group can be used as a removable handle controlling both reactivity and regioselectivity.

Scheme 28. Tandem Diels-Alder – decarbonylation sequence.[239]

This concept of using the carbonyl group as a removable steering group to enable specific chemical reactivity has also been achieved very recently by a one-pot 1,4-addition of a boronic acid to the α,β-unsaturated aldehyde **89** followed by decarbonylation to produce enantiomerically pure

1,1-diarylethane **90** (Scheme 29).[240] The one-pot reaction could be achieved with dimeric [Rh(*epi*-(–)-dolefin)Cl]₂ complexes in low yield, but addition of an additional rhodium(I)-catalyst (1.5% each) to facilitate the decarbonylation was necessary to achieve a reasonable yield.

Scheme 29. Sequential conjugate addition – decarbonylation sequence.[240]

Application of aldehydes as a source of carbon monoxide in different *carbonylation* reactions was first envisioned by Morimoto and co-workers.[241] The fact that carbonylation reactions are usually carried out by the same kind of transition metal catalysts as the decarbonylation reaction has been utilized in a number of rhodium-catalyzed Phauson-Khand reactions employing aldehydes as the source of carbon monoxide (Scheme 30).[241-243] Catalytic Phauson-Khand reactions using aldehydes as the source of carbon monoxide has also recently been achieved with 5% of [IrCl(COD)]₂ and 10% of BINAP by using a five-fold excess of the aldehyde.[216]

Scheme 30. Phauson-Khand reaction employing 2-naphthaldehyde as carbon monoxide donor.[241]

The Pauson-Khand reaction of enyne **91** can even be performed with formaldehyde as the carbon monoxide donor.[244] In this case, the reaction is performed in aqueous formaldehyde in the presence of the surfactant SDS together with dppp and the water soluble analogue TPPTS producing the desired product **92** in 97% yield with 5% catalyst loading.

3.2.5 Application on carbohydrate substrates

In carbohydrate chemistry, decarbonylation has not received much attention, and only a few decarbonylations of fully protected sugars using stoichiometric amounts of Wilkinson's reagent have been reported.[245,246] Transition metal mediated decarbonylation of *unprotected* sugars was first addressed by Kruse and Wright[247] in 1978 and Kuriacose and co-workers[248] in 1983. In two studies investigating the hydrogenation of D-glucose catalyzed by $RuCl_2(PPh_3)_3$ in DMA, it was found that the desired reaction was inhibited by a decarbonylation reaction. No carbohydrate products were isolated, but three metal complexes were characterized as $RuHCl(CO)(PPh_3)_2$,[247] $RuCl_2(CO)(PPh_3)_2(DMA)$ and *cis*-$RuCl_2(CO)_2(PPh_3)_2$,[248] and it was realized that D-glucose had underwent a decarbonylation reaction.[248] However, in 1988 Andrews and Klaeren reported the first useful transition metal mediated decarbonylation of unprotected sugars.[201] The reactions were performed on a small scale (150 μmol) with stoichiometric amounts of Wilkinson's reagent in NMP at 130 ℃ producing the corresponding lower alditols. The following year the procedure was extended to a range of different aldoses including deoxyaldoses, *N*-acetylaldoses and disaccharides, which were synthesized in 37-87% isolated yields.[249] As with simple aldehydes, the decarbonylation occurred with complete retention of stereochemistry. All attempts to make the reaction catalytic in rhodium employing $Rh(dppe)_2^+$ or $Rh(dppp)_2^+$ were unsuccessful.[249]

Andrews also applied the procedure to ketoses and found that in the case of D-fructose a complex dehydration-isomerization mechanism occurred leading to intermediate 5-hydroxymethyl furfural (**93**), which was decarbonylated to give furfuryl alcohol (**94**) (Scheme 31).[201,250] As with the aldoses, the reaction was stoichiometric in rhodium and **94** was formed in 80% GC-yield.

Scheme 31. Dehydration and decarbonylation of D-fructose gives furfuryl alcohol (**94**).[201,250]

Similar to O'Connor and Ma's observations, Beck and co-workers found that the decarbonylation of D-glucose could be achieved catalytically in the presence of additives.[251] Performing the reaction with DPPA, sodium azide, urea or the bidentate phosphine ligands dppe, dppb or dpph together with 5-10% of $RhCl(PPh_3)_3$ in NMP gave 30-52% HPLC-yield of D-arabinitol after 24 h at

50-130 °C. The decarbonylations were rather slow, and in all cases full conversion was not achieved resulting in considerable amounts of residual D-glucose,[251] which may be difficult to remove from D-arabinitol. Based on these results, the decarbonylation of aldoses is not feasible on a preparative scale emphasizing the need for a convenient catalytic procedure.

3.3 Previous work on the decarbonylation project in the group

As a part of the studies on the decarbonylation of aldehydes in our group,[236,237,239] Anders Palmelund discovered that unprotected D-arabinose could be decarbonylated catalytically with preformed Rh(dppp)$_2$Cl in a closed vial in a microwave oven (Scheme 32). Attempts to generate Rh(dppp)$_2$Cl *in situ* from RhCl$_3$ • 3H$_2$O or perform the reaction in an open system failed.[252]

Scheme 32. Rhodium(I)-catalyzed decarbonylation of D-arabinose by Anders Palmelund employing microwave heating.[252]

3.4 Results and discussion

Because the evolution of a molecule of carbon monoxide has been found to inhibit decarbonylation in a closed system,[239] and the application of microwave heating is less convenient for general synthetic use, it is desirable to be able to decarbonylate aldoses in an open system. As a result of this, the initial decarbonylation experiments were carried out in an open system. Due to Anders' failed attempts to use the *in situ* generated catalyst on unprotected sugars, *preformed* Rh(dppp)$_2$Cl was used for the initial experiments on D-glucose. (For experiments with *in situ* generated catalysts, see section 3.4.3).

Rh(dppp)$_2$Cl was synthesized in two steps in excellent yield using a slightly modified literature procedure (Scheme 33).[253,254] Reduction of Rh(III) to Rh(I) with 2-propanol followed by exchange of the dummy ligand COE with dppp gave Rh(dppp)$_2$Cl in 91% yield over two steps. The metal complex is not sensitive to air when stored at room temperature, but in solution at elevated temperatures, a strict inert atmosphere is necessary to obtain constant catalytic activity.[236]

$$RhCl_3 \bullet 3H_2O \xrightarrow[\text{92\%}]{\substack{\text{COE, H}_2\text{O} \\ \text{2-propanol} \\ \text{rt, 15 d}}} Rh(COE)_2Cl \xrightarrow[\text{99\%}]{\substack{\text{dppp, toluene} \\ \text{reflux, 1.5 h}}} Rh(dppp)_2Cl$$

Scheme 33. Synthesis of Rh(dppp)$_2$Cl from RhCl$_3 \bullet$ 3H$_2$O.

3.4.1 Solvent system

Not many solvents can be used in the decarbonylation reaction due to the hydrophobic nature of Rh(dppp)$_2$Cl in contrast to the hydrophilic carbohydrate substrates. In addition, a sufficiently high boiling solvent system is required in order for the reaction to proceed at a reasonable rate. Andrews and co-workers employed NMP as the solvent for stoichiometric decarbonylation of sugars,[201,249] and such amide solvents are uniquely capable of dissolving both metal complexes and unprotected carbohydrates. However, the disadvantage of using NMP is the difficulties associated with its removal in the purification step. From the results on simple aryl and alkyl aldehydes,[236] diglyme, which might have a stabilizing effect on the positively charged metal complex, was found to be a suitable solvent, but since carbohydrates are not soluble in pure diglyme, a number of different solvent systems were screened (Table 13).

Table 13. Decarbonylation of D-glucose into D-arabinitol using different solvent systems.

Entry	Solvent	Reflux temperature (°C)	Conversion[a] (%)
1	diglyme	162	decomposition
2	H$_2$O	100	0[b]
3	diglyme/H$_2$O (40:1)	152	100
4	diethyleneglycolmonoethylether	202	100
5	diglyme/NMP	162	100
6	diglyme/DMA	162	100

[a] Conversions are based on TLC analysis. [b] No conversion was observed within 64 h.

The use of pure diglyme led to decomposition of D-glucose due to poor solubility (Table 13, entry 1). Performing the reaction in water at 100 °C gave no conversion within 64 h (entry 2), whereas diglyme/water mixtures were found to produce a homogeneous reaction mixture. However, diglyme and water forms an azeotrope (bp 99 °C) resulting in no conversion. With low amounts of water (diglyme/water = 40:1 – 20:1), a high reaction temperature (152 °C) could be maintained

leading to full conversion in 18 h producing D-arabinitol as the main product (entry 3). However, by reducing the amount of water, decomposition became an increasing problem. A diglyme/water ratio of 40:1 was found to be optimal on a small scale (100 mg D-glucose), but on a larger scale this led to poor conversion due to the decreased reaction temperature. Diethyleneglycolmonoethylether dissolves both the metal complex and the carbohydrate, but the solvent is very difficult to remove after the reaction (entry 4). Extraction of the product into an aqueous phase is not possible, and due to a high boiling point (bp 202 ℃), the solvent cannot be removed by evaporation. Additionally, glycosylation with the solvent thereby deactivating the substrate towards decarbonylation might take place during the harsh reaction conditions. Using NMP (bp 202 ℃) or DMA (bp 165 ℃) as co-solvents enabled full conversion without substantial decomposition (entries 5 and 6). The reaction can easily be performed in pure DMA, but although it is easier to remove than NMP, the amount of DMA should be minimized. Using a 7:1 diglyme/DMA ratio gave consistently good results. Initially, the purification was performed by reverse phase column chromatography eluting with water, but residual DMA was not efficiently removed due to co-elution with D-arabinitol. Other workup procedures were therefore attempted. Bulb-to-bulb distillation at 50-90 ℃ neither removed DMA nor diglyme, but the latter could be completely removed as an azeotrope with water by steam distillation. However, only traces of DMA could be removed by steam distillation for 2 h. Instead, it was found that residual DMA could be removed effectively by normal column chromatography eluting with DCM/MeOH/H_2O = 65:25:4. A control experiment revealed that no silica gel was dissolved by the polar eluent during the column chromatography.

3.4.2 Formation of 1,4-anhydro-D-arabinitol

Decarbonylation of D-glucose in refluxing diglyme/DMA with 10% of Rh(dppp)$_2$Cl gave the desired product D-arabinitol in 71% yield in 9 h. To our surprise, 20% of 1,4-anhydro-D-arabinitol (**95**) was isolated as a byproduct in addition to the desired product (Scheme 34).

Scheme 34. Rhodium(I)-catalyzed decarbonylation of D-glucose produces D-arabinitol together with the byproduct 1,4-anhydro-D-arabinitol (**95**).

1,4-Anhydro-D-arabinitol **95** is formed to a much larger extent than 2,5-anhydro-D-arabinitol **96** (1,4-anhydro-D-lyxitol) (Scheme 35). Although **95** was isolated in 20% yield,[♦] **96** could only be detected in trace amounts by NMR spectroscopy. As we will see later in section 3.4.5 (Table 17), the exclusive formation of 1,4-anhydroalditols is general for the decarbonylation of aldoses. In addition, only 1,4-anhydroalditols with retention of stereochemistry at C4 are observed, which suggests an activation of the C1 hydroxy group followed by displacement by the secondary alcohol at C4 (Scheme 35, green). The formation of 2,5-anhydroalditols would require displacement of the C5 hydroxy group by the secondary alcohol at C2 (Scheme 35, red).

Scheme 35. 1,4-Anhydro-D-arabinitol (**95**) is formed exclusively (green), whereas only a trace of 2,5-anhydro-D-arabinitol (**96**) can be observed (red). The formation of **95** requires activation of the C1 hydroxy group followed by nucleophilic displacement by the C4 hydroxy group.

[♦] The identity of **95** was rigorously established by conversion into the corresponding triacetate (see section 8.2 for experimental details).

To test if rhodium is responsible for the formation of 1,4-anhydroalditols, D-glucose was heated in refluxing diglyme and DMA for 24 h without any rhodium present, and, as expected, no anhydroarabinitols were formed. In addition, D-arabinitol was heated to reflux in diglyme/DMA together with 5% of Rh(dppp)$_2$Cl for 32 h, and neither 1,4- nor 2,5-anhydro-D-arabinitol were formed. This clearly indicates that the formation of 1,4-anhydroalditols is rather mediated by rhodium than a result of a thermal dehydration. In addition, since no 2,5-anhydroalditols are formed, the formation of 1,4-anhydroproducts most likely occurs while rhodium is still bound to the C1 of the alditol. We tried to avoid the formation of **95** by reducing the reaction temperature and by adding a small amount of water to the reaction mixture, but it was still formed in 20% yield. Since it was formed in quite large amounts, it was attempted to direct the decarbonylation process towards this product, because it might be an interesting chiral building block for organic synthesis. However, treatment of D-glucose with 1% of the catalyst at 162 ºC for 74 h failed to produce the anhydroproduct in a reasonable yield. Instead, D-arabinitol and **95** were isolated in less than 30% combined yield indicating that the degree of decomposition is more pronounced for prolonged reaction times.

3.4.3 Catalyst system

After the successful decarbonylation of D-glucose in diglyme/DMA with preformed Rh(dppp)$_2$Cl (Table 14, entry 1), other catalysts were screened as well. As mentioned in section 3.2.2, rhodium(I)-complexes of BINAP and dppp were found to be equally reactive in the decarbonylation of simple aryl and alkyl aldehydes (Figure 3),[236] and it was decided to test Rh(BINAP)$_2$Cl in the decarbonylation of sugars. Rh(BINAP)$_2$Cl was synthesized by ligand exchange of Rh(COE)$_2$Cl in 82% yield, but in the decarbonylation of D-glucose, it reacted considerably slower than Rh(dppp)$_2$Cl (entry 2).

Rh(COE)$_2$Cl is known to decarbonylate 2-naphthalene catalytically, but it reacts much slower than Rh(dppp)$_2$Cl.[252] Therefore, it was attempted to generate Rh(dppp)$_2$Cl *in situ* from Rh(COE)$_2$Cl and dppp (Table 14, entry 3). However, the decarbonylation occurred slowly and full conversion was observed only after more than 17 h using 7.5% catalyst loading.

After testing these stable Rh(I)-complexes (Table 14, entries 1-3), it was attempted to develop a simple procedure to generate the catalytic species directly from Rh(III). Preparation of Rh(dppp)$_2$Cl

in two steps from RhCl$_3 \cdot$ 3H$_2$O and dppp (Scheme 33) is not difficult, but due to the time-consuming precipitation of the intermediate Rh(COE)$_2$Cl, it would be more convenient to be able to prepare the catalyst *in situ* from commercially available precursors.

Table 14. Decarbonylation of D-glucose using different rhodium catalysts.

Entry	Catalyst	Additive	Time (h)	Yield
1	Rh(dppp)$_2$Cl	–	9	71%
2	Rh(BINAP)$_2$Cl	–	6	<10% conversion
3	Rh(COE)$_2$Cl + 2 dppp	–	17	>90% conversion[a]
4	RhCl$_3 \cdot$ 3H$_2$O + 2 dppp	–	16	decomposition[b]
5	RhCl$_3 \cdot$ 3H$_2$O + 2 dppp	20% PhCH$_2$CHO	16	trace[b]
6	crude Rh(dppp)$_2$Cl	–	2	decomposition[b]
7	crude Rh(dppp)$_2$Cl	20% PhCH$_2$CHO	3	9 %[b]

[a] 7.5% catalyst loading was used. [b] D-Glucose dissolved in DMA was added after 20 min.

It was attempted to generate the catalyst *in situ* by heating RhCl$_3 \cdot$ 3H$_2$O, dppp and D-glucose in diglyme,[236] however, the reaction mixture turned black and a rhodium mirror could be observed. The same result was achieved in diglyme/DMA. Although dppp is usually responsible for reduction of Rh(III) to Rh(I), it was realized that D-glucose also acts as a reducing agent resulting in formation of Rh(0). This means that D-glucose has to be added *after* formation of the catalytically active species. However, addition of D-glucose in crystalline form or dissolved in H$_2$O or DMA to a preheated solution of RhCl$_3 \cdot$ 3H$_2$O, dppp and diglyme only resulted in decomposition (Table 14, entry 4).

In another approach, the catalyst was formed *in situ* using 20% of phenylacetaldehyde as an additive together with RhCl$_3 \cdot$ 3H$_2$O and dppp followed by addition of D-glucose dissolved in DMA. In this case, traces of D-arabinitol were observed (Table 14, entry 5). Most likely not all the Rh(III) is reduced to Rh(I) when the catalyst is generated *in situ*, and the presence of Rh(III) together with D-glucose in the reaction mixture probably leads to further reduction to Rh(0) and decomposition.

Since Wilkinson's reagent can be formed in one step from $RhCl_3 \cdot 3H_2O$ in the presence of PPh_3 by reduction in ethanolic solution,[202] it was anticipated that $Rh(dppp)_2Cl$ could be prepared in the same way. When refluxing $RhCl_3 \cdot 3H_2O$ and dppp in a solution of 96% EtOH for 30 min, a color change from dark red to orange could be observed indicating reduction of Rh(III) to Rh(I). Removal of EtOH *in vacuo* to prevent possible sugar glycosylation gave *crude* $Rh(dppp)_2Cl$. The crude $Rh(dppp)_2Cl$ has been successfully used in a one-pot Diels-Alder – decarbonylation reaction in our group by Esben Taarning.[239] Crude $Rh(dppp)_2Cl$ readily dissolves in cold diglyme whereas *preformed* $Rh(dppp)_2Cl$ is completely non-soluble in cold diglyme. In addition, subjecting D-glucose to the crude catalyst at 162 ºC leads to complete decomposition within 2 h (Table 14, entry 6). These observations suggest that crude $Rh(dppp)_2Cl$ might not have the same composition as preformed $Rh(dppp)_2Cl$. Analysis of the crude catalyst by IR spectroscopy revealed a weak band at 2168 cm^{-1}, which might indicate the presence of a carbonyl ligand[222] arising from the decarbonylation of acetaldehyde produced by oxidation of EtOH during formation of crude $Rh(dppp)_2Cl$. Benzaldehyde, on the other hand, could readily be decarbonylated using crude $Rh(dppp)_2Cl$,[239] and after the reaction, the catalyst was precipitated and analyzed by IR spectroscopy. In this case, strong bands were observed at 2101, 1971 and 1718 cm^{-1}, indicating that carbon monoxide and benzaldehyde are present as ligands. Based on these data, the crude $Rh(dppp)_2Cl$ most likely only contains very small amounts of carbonyl ligands, and the difference in reactivity between the crude and the preformed catalysts is probably a matter of purity rather than identity.

Using the crude $Rh(dppp)_2Cl$ together with 20% of phenylacetaldehyde as an additive followed by addition of D-glucose dissolved in DMA leads to full conversion within 3 h giving 9% of D-arabinitol (Table 14, entry 7). This result can probably be improved, but it would require a lot of work with no foreseeable success in the near future. The preformed catalyst was therefore selected for further optimizations.

3.4.4 Optimization of the decarbonylation procedure

A series of experiments were performed to optimize the conditions as shown in Table 15. Reducing the catalyst loading from 10 to 1% (entries 1-3) resulted in considerably longer reaction times and lower yields. With 5% catalyst, 40% of D-arabinitol could be isolated (entry 2), whereas with 1% catalyst full conversion could not be achieved within 16 h (entry 3).

Table 15. Optimization of the decarbonylation procedure.

Entry	Catalyst loading	Additive	Time (h)	Isolated Yield	Reflux temperature (ºC)
1	10%	–	9	71%[a]	162
2	5%	–	14	40%[b]	162
3	1%	–	16	<20% conversion	162
4	10%	20% dppp	16	16%	162
5	5%	10 eq. H_2O	18	50%	152
6	5%	Ar-bubbling[c]	13	52%	155
7	5%	15% AcOH	9	51%	162
8	5%	13% pyridine	9.5	58%	162

[a] 20% of 1,4-anhydro-D-arabinitol (**95**) is formed as well. The amount of **95** has not been determined for the other entries. [b] A slightly higher yield (44%) could be achieved by stopping the reaction before full conversion (11 h), however, this was inconvenient due to the tedious separation of D-glucose and D-arabinitol. [c] The reaction was performed by bubbling a continous stream of argon through the reaction mixture.

With longer reaction times decomposition becomes an increasing problem, and in order to reduce the degree of decomposition, the evolution of carbon monoxide during the course of the reaction was measured (Figure 4). In this way, the progress of the decarbonylation can be monitored, and the reaction can be stopped when no more carbon monoxide is produced. From Figure 4 it is clear that the decarbonylation of D-glucose proceeds faster with 10% catalyst (green) than with 5% catalyst (black).

During decarbonylation of D-glucose, Beck and co-workers found that the addition of dppp together with Wilkinson's reagent had a favorable effect possibly forming Rh(dppp)$_2$Cl *in situ*.[251] When D-glucose was decarbonylated in the presence of dppp and Rh(dppp)$_2$Cl, only 16% of D-arabinitol was formed (Table 15, entry 4) showing that the presence of phosphines is not desirable when the preformed catalyst is used. Addition of small amounts of water (diglyme/H_2O = 40:1) (entry 5) improved the yield of D-arabinitol, but required 18 h to reach full conversion due to a lower reflux temperature. When argon was bubbled through the reaction mixture in an attempt to remove carbon monoxide,[234] 52% yield could be obtained (entry 6).

Figure 4. Carbon monoxide evolution during the course of the decarbonylation. The figure shows carbon monoxide evolution curves for decarbonylation of 2.78 mmol aldose in a diglyme/DMA solution with different Rh(dppp)$_2$Cl loading and additives. At room temperature (25 °C), full conversion corresponds to formation of 68 mL CO. See section 8.2 for further experimental details.

The reaction rate for the decarbonylation of carbohydrates is much lower than for simple aldehydes. D-Glucose requires 9 h to reach full conversion using 10% preformed Rh(dppp)$_2$Cl, whereas only 3 h are needed to decarbonylate 2-naphthaldehyde using 5% *in situ* generated catalyst. The reaction of sugars is limited by an unfavorable hemiacetal-aldehyde equilibrium (Scheme 36), and in the case of D-glucose, less than 0.02%♦ of the sugar is present as the free aldehyde in aqueous solution.[255,256] Both acids and bases are known to catalyze the mutarotation *via* the open-chain *aldehydo* form,[257] and small amounts of acetic acid and pyridine were therefore added to the reaction mixture (Table 15, entries 7 and 8). Both additives were found to decrease the reaction time, and as a result, increased yields were obtained.

♦ In aqueous solution at 82 °C and pH 4.8, D-glucose exists as a mixture of the free aldehyde (0.019%), the hydrated aldehyde (0.022%), two furanose forms (1.29%) and two pyranose forms (98.6%) (Maple, S. R.; Allerhand, A. *J. Am. Chem. Soc.* **1987**, *109*, 3168).

Scheme 36. Hemiacetal – aldehyde equilibrium for D-glucose.

A series of experiments with acetic acid and pyridine were performed, and it was found that pyridine gave slightly better results than acetic acid (Table 16, compare entries 1-3 with 4-6). The effect of added pyridine can be visualized in the carbon monoxide evolution curve (Figure 4, red). Using an excess of pyridine resulted in a considerably lower yield (Table 16, entry 7). A catalyst loading of 8 or 10% together with pyridine (6%) led to full conversion in 8 and 7.5 h, respectively (entries 8 and 9), and it was decided to reduce the catalyst loading to 8% using 6% of pyridine as an additive.

Table 16. Optimization of the decarbonylation procedure with acid and base.

Entry	Catalyst loading	Additive	Time (h)	Isolated yield
1	5%	15% AcOH	9	39%
2	5%	7% AcOH	9.5	51%
3	5%	3% AcOH	9.5	39%
4	5%	13% pyridine	9.5	58%
5	5%	6% pyridine	9.5	55%
6	5%	1% pyridine	10.5	40%
7	5%	4 eq. pyridine	18	18%
8	8%	6% pyridine	8	71%
9	10%	6% pyridine	7.5	67%

3.4.5 Extension of the procedure to other aldoses

Having successfully developed a catalytic decarbonylation procedure, it was applied on a range of aldoses (Table 17). Of the hexoses, D-glucose and D-mannose gave similar results (entries 1-4), whereas D-galactose was decarbonylated in lower yield (entries 5-6). In addition to the desired D-arabinitol, D-galactono-1,4-lactone (**97**) and 1,4-anhydro-D-lyxitol **96** (2,5-anhydro-D-arabinitol) were formed as an inseparable mixture in 34% yield during decarbonylation of D-galactose

(entry 5). The reason for oxidation of D-galactose to produce **97** in approximately 19% yield cannot be rationalized. D-Galactose is less soluble in DMA than D-glucose and D-mannose, and decarbonylation of D-galactose in pure DMA gave 27% of D-arabinitol accompanied by significant decomposition.

The pentoses (Table 17, entries 7-12) react slightly faster than the hexoses, and the evolution of carbon monoxide from the decarbonylation of D-xylose can be seen in Figure 4 (Figure 4, blue). Although it is more soluble in DMA, the 6-deoxy sugar, L-rhamnose, (Table 17, entries 13-14) requires slightly longer reaction times than the other hexoses. However, the desired product, 5-deoxy-L-arabinitol, is isolated in comparable yields. As mentioned earlier in section 3.4.2, 1,4-anhydroalditols are formed as the only byproducts in the decarbonylation reaction, and the amount of 1,4-anhydroalditols produced from each carbohydrate is shown in Table 17. 1,4-Anhydroalditols are formed both in the absence and in the presence of pyridine.

Table 17. Application of the procedure on different aldoses.

$$C_n \text{ aldose} \xrightarrow[\text{reflux}]{\substack{Rh(dppp)_2Cl \\ \text{diglyme/DMA}}} C_{n-1} \text{ alditol} + C_{n-1} \text{ 1,4-anhydroalditol}$$
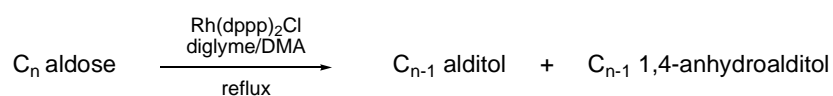
Entry	Aldose	Method[a]	Time (h)	Alditol	Yield (%)	1,4-Anhydroalditol	Yield (%)
1	D-glucose	A	9	D-arabinitol	71	1,4-anhydro-D-arabinitol (**95**)	20
2	D-glucose	B	8	D-arabinitol	71	n.d.	
3	D-mannose	A	9	D-arabinitol	69	n.d.	
4	D-mannose	B	8	D-arabinitol	72	1,4-anhydro-D-arabinitol (**95**)	20
5	D-galactose	A	9	D-arabinitol	39	1,4-anhydro-D-lyxitol (**96**)	15[b]
6	D-galactose	B	8	D-arabinitol	56	n.d.	
7	D-arabinose	A	9	erythritol	68	n.d.	
8	D-arabinose	B	6.5	erythritol	70	n.d.	
9	D-ribose	A	8	erythritol	71	n.d.	
10	D-ribose	B	6.5	erythritol	76	n.d.	
11	D-xylose	A	8	D-threitol	70	1,4-anhydro-D-threitol (**98**)	13
12	D-xylose	B	7.5	D-threitol	74	n.d.	
13	L-rhamnose	A	11	5-deoxy-L-arabinitol	66	n.d.	
14	L-rhamnose	B	10	5-deoxy-L-arabinitol	71	1,4-anhydro-5-deoxy-L-arabinitol (**99**)	17

[a] The experiments were performed according to the general decarbonylation procedure A (10% $Rh(dppp)_2Cl$) or B (8% $Rh(dppp)_2Cl$ and 6% pyridine), see section 8.2. [b] Formed as an inseparable 3:4 mixture with D-galactono-1,4-lactone (**97**) (19%).

Decarbonylation of *N*-acetyl-D-glucosamine required reaction times of around 16 h leading to 28 and 34% yield of 1-acetylamino-1-deoxy-D-arabinitol (**100**) in the absence and in the presence of pyridine, respectively (Table 18, entries 1 and 2). The slow decarbonylation of *N*-acetyl-D-glucosamine was also observed by Andrews and co-workers,[249] and can be explained by coordination to rhodium with the *N*-acetyl group. In addition to the desired product **100**, a complex mixture of unidentified byproducts was formed. Both the addition of small amounts of water and increasing the amount of pyridine were found to be favorable, however, the decarbonylation still occurred in low yield (entries 3-4).

Table 18. Decarbonylation of *N*-acetyl-D-glucosamine to produce 1-acetylamino-1-deoxy-D-arabinitol (**100**).

Entry	Method[a]	Additive	Time (h)	Yield (%)
1	A	–	16	28
2	B	–	15.5	34
3	A	10 eq. H_2O	16	42
4	B	–	14.5	40[b]

[a] The experiments were performed according to the general decarbonylation procedure A (10% Rh(dppp)$_2$Cl) or B (8% Rh(dppp)$_2$Cl and 6% pyridine), see section 8.2. [b] The amount of pyridine was increased to 15%.

3.4.6 Decarbonylation of other substrates

Attempts to decarbonylate D-glucurono-6,3-lactone (**101**) which would produce the rare and expensive sugar D-lyxose by subsequent reduction were unsuccessful (Scheme 37). The lactone **101** was consumed very rapidly, and only 14% of the desired D-lyxono-1,4-lactone **102** was produced. In addition, 5% of L-gulono-1,4-lactone **103** (formed by reduction of **101**) was isolated as a byproduct. The reaction mixture turns completely black upon heating and the development of gas occurs very quickly (Figure 4, orange). Performing the reaction at 125 °C resulted in recovery of 72% of the starting material after 7 h, and neither addition of small amounts of water or pyridine nor changing the substrate to free D-glucuronic acid improved the yield.
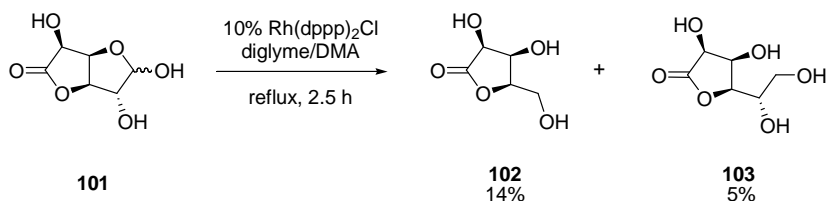
Scheme 37. Decarbonylation of D-glucurono-6,3-lactone (**101**).

Although D-fructose and L-ascorbic acid do not possess an aldehyde functionality, they were subjected to the decarbonylation conditions to see if interesting products could be formed from these cheap and readily available carbohydrates. As mentioned in section 3.2.5, Andrews and co-workers reported that furfuryl alcohol (**10**) was formed as the main product following treatment of D-fructose with Wilkinson's reagent (Scheme 31).[201,249] However, treatment of D-fructose with 10% of Rh(dppp)$_2$Cl surprisingly afforded 21% of 1,4-anhydro-D-arabinitol (**95**) after 5.5 h. When L-ascorbic acid was subjected to the decarbonylation conditions at 162 ºC, decomposition was a major problem, and it was not possible to detect any carbohydrate products. At lower temperatures L-ascorbic acid was not consumed as rapidly, and 29% of the starting material could be recovered after 23 h at 125 ºC. No other products could be isolated.

3.4.7 Decarbonylation of unprotected cyclodextrins

To test the scope of the rhodium-catalyzed decarbonylation reaction, it was attempted to decarbonylate aldehydes derived from cyclodextrins. Cyclodextrins are composed of D-glucopyranose residues connected in a ring structure with α(1,4)-glycosidic linkages.[258] The α-, β- and γ-cyclodextrins contain six, seven and eight glucose residues, respectively,[258] and their structure can be represented by a 'bucket' with the hydroxy groups positioned on the outside (Figure 5). As a result, cyclodextrins have a hydrophilic surface and a hydrophobic cavity.[259] The latter is known to form complexes with small organic and inorganic compounds thereby resembling the pocket of an enzyme.[258]

In fact, Bols and co-workers have recently used modified α- and β-cyclodextrins[260] as artificial enzymes catalyzing the oxidation of anilines to nitrobenzenes[261] and benzylic alcohols to aldehydes[262] in the presence of hydrogen peroxide. The inner diameter varies from 5.7 to 9.5 Å when going from α- to γ-cyclodextrin,[259] and by choosing different cyclodextrins as template, one can design artificial enzymes with different properties. The ability to bind organic molecules in the

hydrophobic cavity combined with a high solubility in water make cyclodextrins well suited for biologically important applications like drug delivery or artificial enzymes.[259] Due to an increasing interest in cyclodextrins, it is important to be able to perform selective modifications on cyclodextrins without protective group manipulations. However, despite their well-defined structure, the selective modification of cyclodextrins is not an easy task due to the presence of a large number of hydroxy groups.[259]
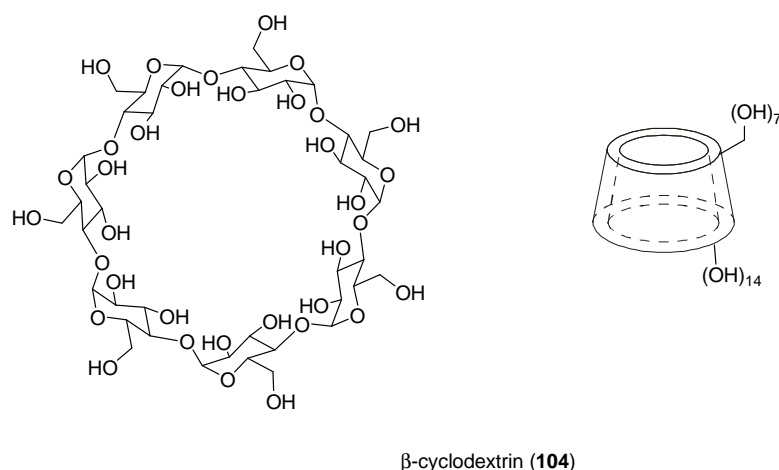
β-cyclodextrin (**104**)

Figure 5. Two representations of β-cyclodextrin (**104**). The primary hydroxy groups are positioned on the upper part of the 'bucket' (right), whereas the secondary hydroxy groups are on the bottom.[259]

In the following it was attempted to remove a hydroxymethyl group from unprotected β-cyclodextrin (**104**) using an oxidation – decarbonylation sequence (Scheme 38). Following the procedure of Bieniarz and co-workers,[263] **104** was oxidized to the monoaldehyde **105** using two equivalents of Dess-Martin Periodinane (DMP) in d_6-DMSO. As described by Bieniarz,[263] the reaction can be followed by ^{1}H NMR spectroscopy by comparing the developing signal of a new anomeric proton at 4.93 ppm with the remaining anomeric protons at 4.82 ppm. A 1:6 ratio between these signals indicates full conversion to the monoaldehyde **105**, and depending on the quality of the DMP reagent, the reaction requires 2-4 h at room temperature. However, analysis of the product by MALDI TOF MS revealed the presence of unreacted **104** as well as trace of the di- and tri-aldehydes. If the reaction was performed with longer reaction times or with a larger excess of DMP, considerable amounts of the di- and tri-aldehydes were formed. Using IBX[264] for the oxidation instead of DMP also led to **105** contaminated by the di- and tri-aldehydes as well as unreacted **104**. The problems of obtaining a pure product in this simple oxidation illustrates the difficulties

associated with selective reactions on cyclodextrins,[259] and as a result, slightly impure samples of **105** were subjected to the decarbonylation conditions.
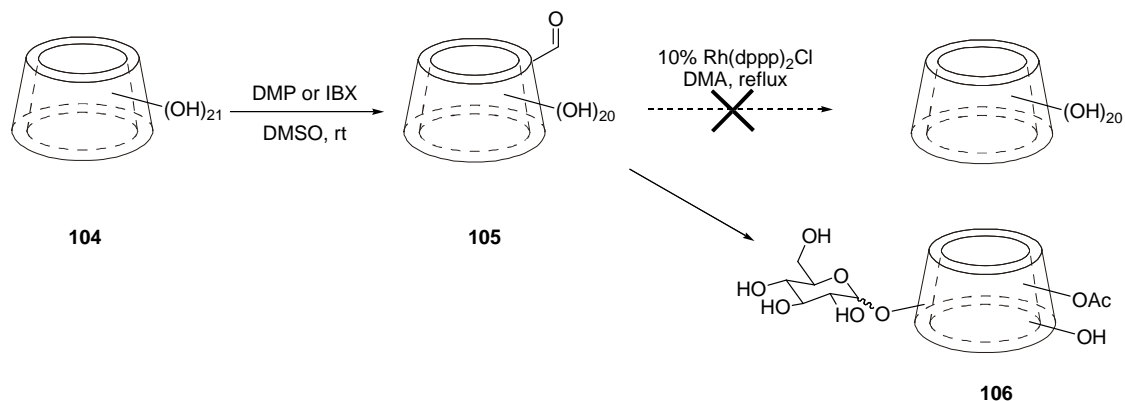
Scheme 38. Attempted decarbonylation of β-cyclodextrin monoaldehyde (**105**). The desired product was not formed, but instead a complex mixture presumably consisting of glycosylated and acetylated β-cyclodextrins **106** was observed.

Using 10% of Rh(dppp)$_2$Cl in pure DMA$^\blacklozenge$ led to significant decomposition. Only approximately 20% of the material could be isolated presumably as a mixture of glycosylated and acetylated β-cyclodextrins **106**. The reaction mixture turned black within 5 min, and almost no gas was developed during the reaction. It was impossible to follow the reation by TLC or ^{1}H NMR spectroscopy due to formation of product mixtures with very similar structure and polarity. Analysis of the crude product by MALDI TOF MS showed a mixture of four major compounds separated by 162 Da, which is indicative of a glycosylation of β-cyclodextrin with up to three glucose residues. In addition, minor compounds corresponding to the masses of the before-mentioned glucosylated products plus 42 and 2 x 42 Da were observed. The observation of (M + 42 Da) and (M + 2 x 42 Da) indicates that acetyl groups are incorporated on the glycosylated β-cyclodextrins (see Table 34 in Appendix I for further details).

The crude product was peracetylated[265] using acetic anhydride, DMAP and pyridine, and a complex mixture of glucosylated β-cyclodextrins were obtained after flash column chromatography. Analysis of these peracetylated products by MALDI TOF MS revealed a mixture of 13 compounds separated by 288 Da indicating peracetylated β-cyclodextrins containing up to 12 peracetylated glucose residues (see Table 35 in Appendix I for further details). Surprisingly, in both cases the MS data fits very well with derivatives of β-cyclodextrin (**104**) rather than β-cyclodextrin

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$^\blacklozenge$ β-Cyclodextrin is completely insoluble in diglyme, but dissolves readily in DMA.

monoaldehyde (**105**). This suggests that **105** may not be stable to the reaction conditions, and upon decomposition of **105**, glucose residues or smaller fragments might be formed, which glycosylate the more stable **104**. The observation of acetyl groups in the *crude* product can only be rationalized by decomposition of the solvent DMA during the reaction leading to acetylation. Based on these results, it was decided not to attempt further decarbonylations on cyclodextrins.

3.4.8 Hydroacylation of alkenes

Intramolecular hydroacylation of 4-alkenals **107** to produce cyclopentanones **108** can be catalyzed by rhodium(I)-complexes (Scheme 39).[266] The procedure has been limited to the synthesis of five-membered rings due to competing decarbonylation, which reduces the yield of the hydroacylation product.[267] This was experienced by Gable and Benz[268] obtaining a moderate yield of the desired cyclohexanone in a rhodium-catalyzed intramolecular hydroacylation of a carbohydrate-derived 5-alkenal. On the other hand, Kampmeier and co-workers[238] *utilized* the competing decarbonylation to convert the 5-alkenal 2-allylbenzaldehyde (**86**) into indane (**87**) rather than the corresponding cyclohexanone produced by hydroacylation (see Scheme 26).
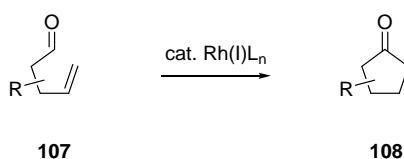
Scheme 39. Rhodium(I)-catalyzed intramolecular hydroacylation.[266,267]

Recently, several research groups have attempted to extend the method to *intermolecular* hydroacylation of alkenes. However, this has proven difficult, and it can only be achieved by addition of amines to generate an imine with the aldehyde *in situ*,[269] or when either the aldehyde[270] or the alkene[271] possess a functional group capable of chelating to rhodium thereby preventing decarbonylation. Although several examples of hydroacylation of alkenes have been reported recently, there is no precedence for direct hydroacylation with carbohydrates in the literature. The presence of several hydroxy groups might enable carbohydrates to react as chelating aldehydes, and it was therefore attempted to hydroacylate styrene with D-glucose using Rh(dppp)$_2$Cl. The formation of a carbon-carbon bond at the anomeric center by direct hydroacylation would thereby constitute a new route to chain elongated carbohydrates.

When D-glucose was subjected to Rh(dppp)$_2$Cl in the presence of five equivalents of styrene, no coupling product **109** could be isolated within 23 h. Instead of hydroacylation, decarbonylation occurred to give 45% of D-arabinitol and 28% of 1,4-anhydro-D-arabinitol (**95**) (Scheme 40). The development of carbon monoxide during decarbonylation of D-glucose in the presence of styrene can be seen in Figure 4 (purple). The formation of the usual decarbonylation products indicated that direct hydroacylation of alkenes with carbohydrates would require considerable experimentation with the reaction conditions, and as a result, the hydroacylation was not pursued further.
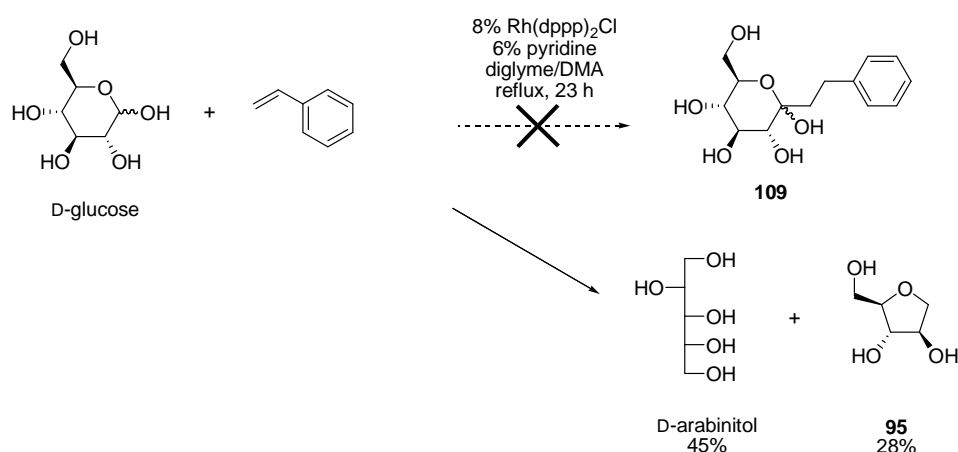
Scheme 40. Attempted intermolecular hydroacylation of styrene with D-glucose.

3.4.9 Synthesis of L-threose by chain shortening of D-glucose

L-Threose is often employed as a chiral C$_4$ building block in synthesis,[95,272-276] but it is not available from nature. By using two consecutive chain shortening reactions, it is possible to synthesize L-threose in a few steps from D-glucose by employing the rhodium-catalyzed decarbonylation reaction as the key step (Scheme 41). L-Threose can be formed by decarbonylation of a protected D-*xylo*-dialdose **110**, which is readily available from diactoneglucose **111** by periodate cleavage.
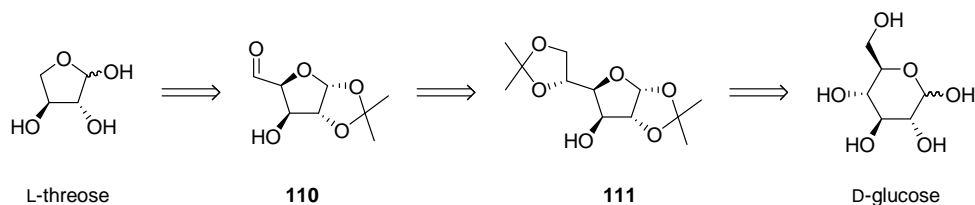
Scheme 41. Retrosynthetic analysis of L-threose.

Diisopropylidene protected D-glucose **111**, which is readily available from D-glucose in 96% yield,[277] was selectively deprotected and oxidatively cleaved using H_5IO_6 following the one-pot procedure developed by Wu and Wu[34] (Scheme 42). Unfortunately, only 42-50% yield of **110** could be achieved using 1.5-3 equivalents of H_5IO_6 in dry ether at 25-40 ℃. Instead, the terminal isopropylidene protective group of **111** was removed to produce **112** in quantitative yield using aqueous acetic acid.[33] Subsequent sodium periodate cleavage[33] gave the one carbon atom shortened aldehyde **110** in 91% yield. The aldehyde **110** crystallizes as the dimer **114** (Scheme 43), but slowly equilibrates to the monomer **110** in aqueous solution.
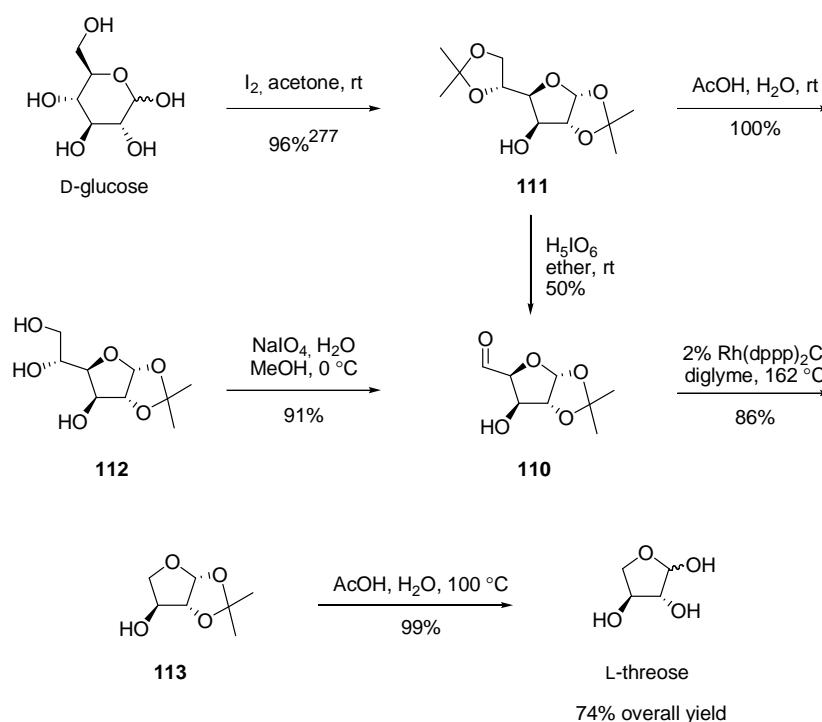
Scheme 42. Synthesis of L-threose from D-glucose by employing the rhodium(I)-catalyzed decarbonylation reaction as the key step.

The decarbonylation of **110** with $Rh(dppp)_2Cl$ proceeded smoothly in 86% yield using only 2% catalyst loading (Scheme 42). Attempts to use the *in situ* generated catalyst were unsuccessful and led to decomposition of the carbohydrate and precipitation of the catalyst. In contrast to unprotected monosaccharides, the *xylo*-pentodialdose **110** is not in equilibrium with cyclic hemiacetals, and the addition of pyridine is unnecessary. In addition, the partially protected **110** is readily soluble in organic solvents and the reaction can be performed in pure diglyme.
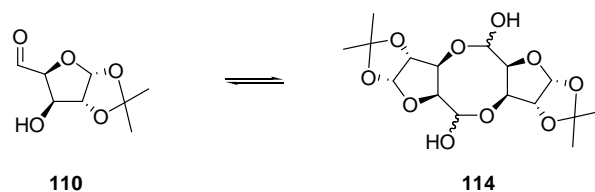
Scheme 43. The aldehyde **110** crystallizes as the dimer **114**, but slowly equilibrates to the monomer **110** in solution.

Final deprotection of **113** using aqueous acetic acid[278] afforded L-threose in an overall yield of 74% for the five-step sequence from D-glucose. The current synthesis of L-threose employing rhodium-catalyzed decarbonylation is the shortest and most high-yielding route to L-threose from D-glucose to date. Synthesis of L-threose from D-glucose can also be accomplished by employing the alkoxy radical fragmentation of a nitrate ester developed by Suaréz and co-workers as the key step (see section 2.1.3), however in this case, L-threose is produced in lower yield than in the present synthesis.[59]

3.5 Concluding remarks

A catalytic procedure for decarbonylation of unprotected carbohydrates using $Rh(dppp)_2Cl$ has been developed (Scheme 44). In contrast to simple aryl and alkyl aldehydes, the aldehyde functionality of unprotected carbohydrates is masked as a hemiacetal making it less available. Prolonged heating at 162 ℃ is therefore required for the reaction to occur, and decomposition of the carbohydrate substrate becomes an increasing problem. However, the addition of small amounts of pyridine facilitates mutarotation making the aldehyde functionality more available. As a result, slightly shorter reaction times and lower catalyst loadings are possible in the presence of pyridine. The procedure has successfully been used to convert a range of different aldoses into their corresponding shortened alditols in 70-75% yield. In addition to the desired alditols, 1,4-anhydroalditols are also formed as byproducts in 10-20% yield.
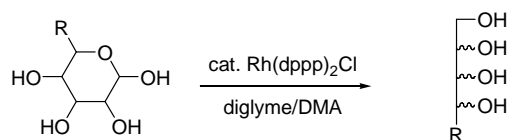
Scheme 44. Rhodium-catalyzed decarbonylation of aldoses.

Requiring only catalytic amounts of expensive rhodium complexes, the developed decarbonylation procedure is a major improvement compared to the previously reported stoichiometric decarbonylation using Wilkinson's reagent.[249,251] Application of the rhodium-catalyzed decarbonylation as the key step in a short and efficient synthesis of L-threose from D-glucose illustrates that the catalytic decarbonylation constitutes a general procedure for shortening unprotected and protected carbohydrates by one carbon atom.

3.6 Outlook

In an industrial view, carbohydrates are a renewable source of energy, and as a result, considerable effort has recently been invested in the conversion of biomass into hydrogen.[22,23,25,279,280] Production of hydrogen can be performed by steam reforming of sugars like glucose, sorbitol and glycerol,[22,23,280] and since alditols are more easily converted into hydrogen than aldoses,[22] the decarbonylation might become useful in combination with steam reforming. Furthermore, ruthenium-mediated dehydrogenation of secondary alcohols to produce ketones and hydrogen has recently been reported,[281,282] and based on these results, further development of the decarbonylation reaction in combination with such dehydrogenation catalysts might enable direct degradation of sugars into hydrogen and carbon monoxide. In that case carbohydrates might even be used for asymmetric hydrogenation of double bonds, since rhodium(I)-species are well-known hydrogenation catalysts.[200] At present the efficiency of the rhodium-catalyzed decarbonylation of carbohydrates is not sufficient to enable such applications, but it might become relevant once more potent decarbonylation catalysts are developed.

4 Synthesis of calystegine A$_3$ by chain elongation of D-glucose

4.1 The calystegine alkaloids

The calystegine alkaloids are a family of naturally occurring iminosugar mimetics.[283] They were isolated from *Calystegia sepium* in 1988,[284] and the first structure elucidations were reported in 1990.[285,286] Since then a total of 14 different calystegines have been isolated from various plants (Scheme 45).[287]
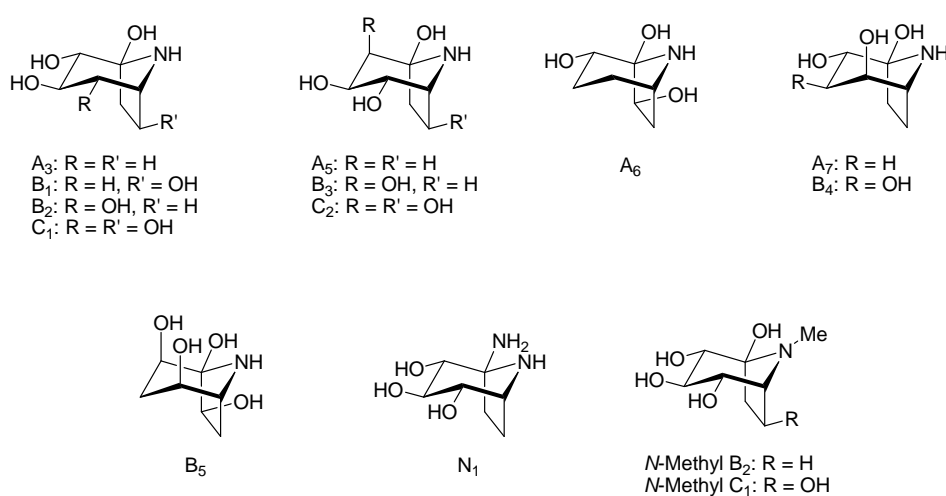
Scheme 45. Naturally occurring calystegine alkaloids.[287]

The calystegines all share a polyhydroxylated, bicyclic nortropane ring system possessing a tertiary hydroxy group as part of an interesting bridgehead aminoketal functionality. They are divided into four groups according to the number of hydroxy groups. The A calystegines contain three hydroxy groups, whereas four and five hydroxy groups are present in the B and C calystegines, respectively. The bridgehead hydroxy group has been exchanged with an amino group in calystegine N$_1$, which is the only discovered calystegine with an aminal functionality.[287]

Many of the calystegine alkaloids have been found to be potent glycosidase inhibitors[288-294] making them interesting pharmaceutical lead compounds especially in relation to cancer, viral infection and diabetes.[283] Calystegine A$_3$ is a moderately potent inhibitor of various glycosidases. Calystegine A$_3$ inhibits pig kidney trehalase, *Caldocellum saccharolyticum* β-glucosidase, almond β-glucosidase and green coffee bean α-galactosidase with K_i values of 5.3, 12, 20 and 20 μM, respectively.[289,294]

Due to scarcity of calystegines from natural sources, efficient chemical syntheses are necessary in order to establish their full potential as drug candidates.[287]

The synthetic efforts towards the calystegine alkaloids were pioneered by Lallemand and co-workers in the early 1990's,[295-298] but since then several syntheses have been reported. Of the naturally occurring calystegines only A_3,[296,299] A_7,[300] B_2,[297,298,301-306] B_3[304,306] and B_4[304,306] have been made by chemical synthesis, but also a number of analogues have been synthesized and tested for glycosidase activity.[293,307-313]
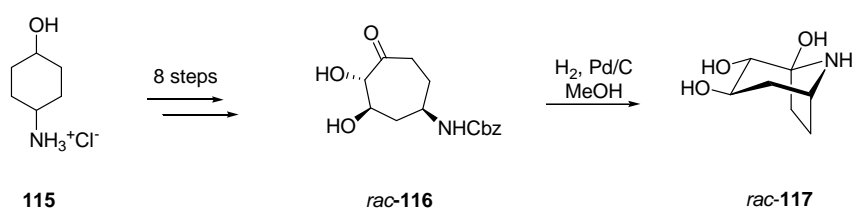
Scheme 46. The synthesis of racemic calystegine A_3 (**117**) by Lallemand and co-workers.[296]

The first synthesis of calystegine A_3 (**117**) was reported in 1992 by Lallemand and co-workers and gave racemic **117** in nine steps from the hydrochloride of 4-aminocyclohexanol (**115**) (Scheme 46).[296] In 1995 Johnson and Bis synthesized both enantiomers of calystegine A_3 (**117**) in 18 and 20 steps respectively from cycloheptatriene (**118**) (Scheme 47).[299] However, the identity of each enantiomer was not established, and as a result, the absolute configuration of naturally occurring calystegine A_3 is still not known, although it is believed to exhibit the same configuration as naturally occurring calystegine B_2 (see Scheme 45 above).[287]
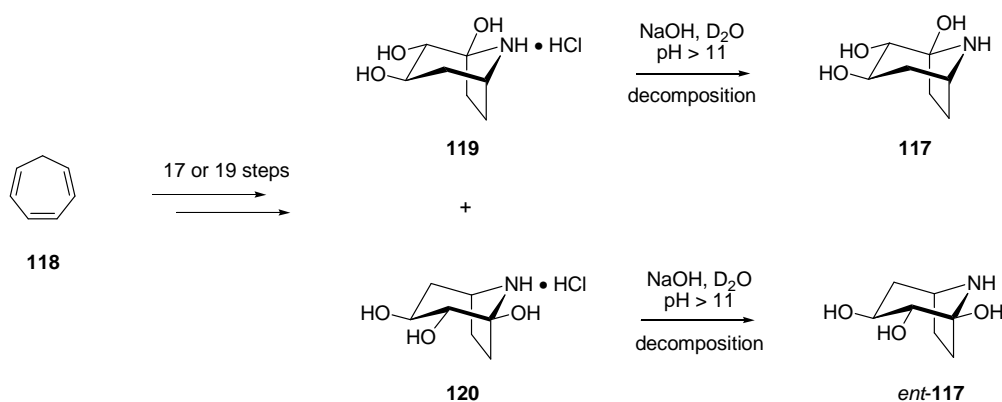
Scheme 47. Synthesis of both enantiomers of calystegine A_3 (**117**) by Johnson and Bis.[299] Due to decomposition of the product in the final step, the absolute configuration of each enantiomer was not determined.

In addition, the synthesis by Johnson and Bis was performed *via* the hydrochloride of calystegine A₃ (**119** and **120**), and upon conversion into the natural product using strong base (pH > 11), the compound could not be recovered, and only NMR data were reported (Scheme 47).[299] As a result, it is still necessary to establish a short and reliable synthesis of calystegine A₃ for further biological evaluation.

The objective of this project is therefore to apply the fragmentation-allylation-metathesis methodology developed by our group to the synthesis of calystegine A₃. This methodology might provide the shortest synthetic route to enantiomerically pure calystegine A₃, which would enable the absolute configuration of calystegine A₃ to be determined. In addition to this, the aim of the project is also to determine the stability of calystegine A₃ in basic solution.

4.2 Synthesis of natural products by chain elongation of ω-iodoglycosides

As mentioned earlier (section 2.2.5), a variation of the chain elongation of carbohydrates is the reductive elimination (fragmentation) of a methyl ω-iodoglycoside followed by a one-, two- or three-carbon elongation of the carbohydrate chain by either olefination (CH_2I_2), vinylation or allylation, respectively.[136] This sequence gives an α,ω-diene, which is conveniently set up for ring-closing olefin metathesis[18] to produce carbocycles. The strategy has successfully been used by our group to synthesize a number of natural products: cyclophellitol,[314] 7-deoxy pancratistatin,[315] conduritols,[136,316,317] inositols[317] and quercitols[136] as well as the calystegines B₂, B₃ and B₄.[304,306] Other research groups have also used this methodology to synthesize natural products,[18] e.g. the recent synthesis of calystegine A₇ by Csuk's laboratory.[300]
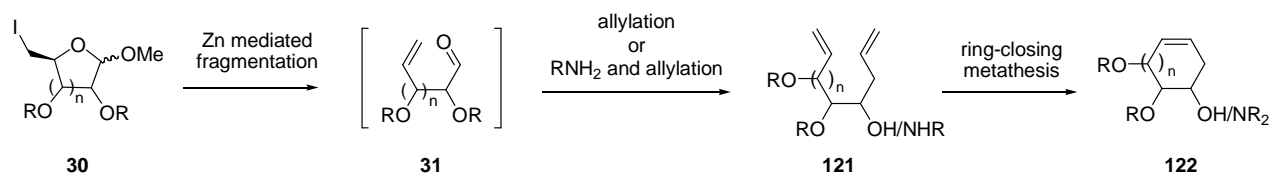
Scheme 48. Formation of carbocycles from ω-iodoglycosides.

The synthetic methodology is well described in the literature,[18,135,136] and the overall strategy will only be discussed briefly (Scheme 48). The key steps include the conversion of a protected or unprotected methyl ω-iodoglycoside (**30**) into an unsaturated aldehyde **31** by Vasella fragmentation[125-128] employing zinc as the metal of choice. Other metals like indium and manganese

have also been found to facilitate the reductive elimination, however, the use of indium is limited,[136,314,318,319] and manganese requires a co-catalyst/oxidant.[320] The subsequent Barbier type[321] allylation of the aldehyde **31** or the corresponding imine can either be conducted as a one-pot fragmentation-allylation with zinc, or by allylation of the aldehyde or imine with other metals like indium or magnesium.[136,306,314,316] The resulting diene **121** can subsequently be subjected to ring-closing olefin metathesis[18,136] to produce a carbocycle **122** which can be used for further manipulations.

4.3 Retrosynthetic analysis of calystegine A$_3$

Retrosynthetically (Scheme 49), the bicyclic aminoketal of calystegine A$_3$ is expected to be formed by cyclization of aminoketone **123** during hydrogenolysis similar to the racemic synthesis of calystegine A$_3$ by Lallemand and co-workers (see the conversion of **116** to **117** in Scheme 46). The aminoketone **123** may be formed by hydroboration and oxidation of a suitably protected cycloheptene **124**, which is available from the corresponding diene **125** by ring-closing metathesis. The required diene **125** can be produced from the protected ω-iodoglucopyranoside **126** by zinc-mediated fragmentation and allylation. Removal of the secondary alcohol in the 2-position of the D-glucopyranoside can either be performed before or after the fragmentation-allylation sequence.
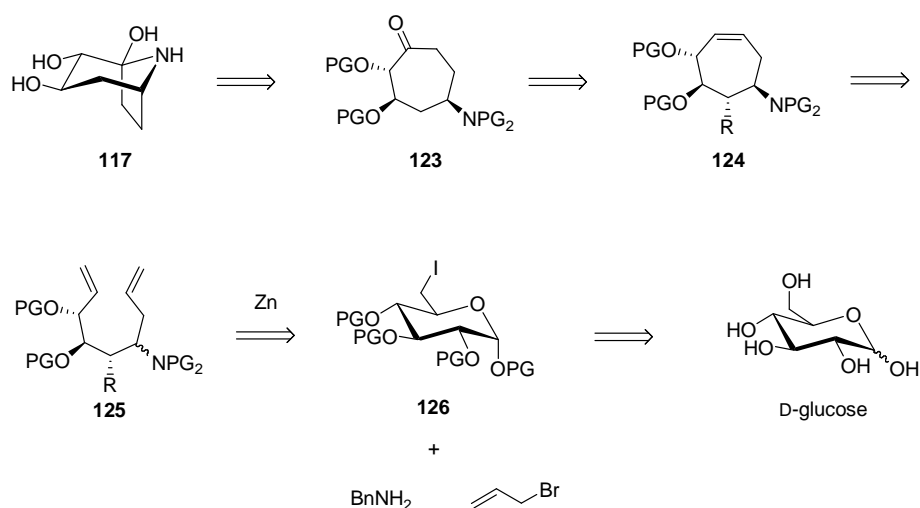
Scheme 49. Retrosynthetic analysis of calystegine A$_3$.

4.4 Previous work on the calystegine project in the group

The current project is based on previous work on the calystegine alkaloids in our group. Philip R. Skaanderup's synthesis of calystegine B_2 is shown in Scheme 50,[304,306] whereas the previous synthetic efforts towards calystegine A_3 by Charlotte B. Pipper are summarized in Scheme 51.[322]
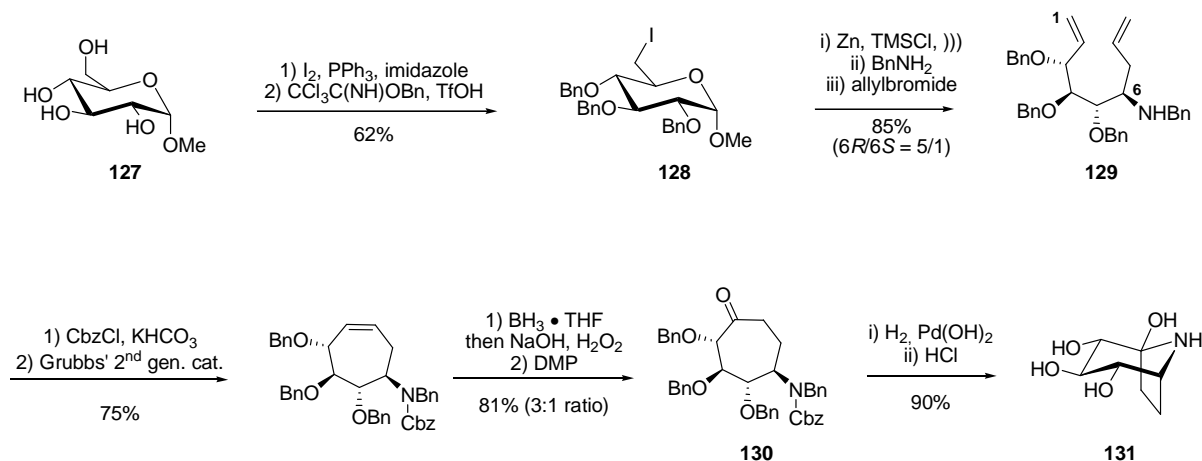
Scheme 50. Synthesis of calystegine B_2 (**131**) by Philip R. Skaanderup.[306,323]

In Philip's synthesis of calystegine B_2, iodination and benzyl protection of methyl α-D-glucopyranoside **127** furnished the protected methyl 5-deoxy-5-iodo-α-D-glucopyranoside **128** for the key tandem sequence involving zinc-mediated fragmentation, imine formation and allylation. The diene **129** was isolated in good diastereoselectivity (5:1) in favor of the desired (6R)-isomer. Noteworthy, the stereochemistry could be further improved if the imine allylation was carried out as a separate step replacing zinc with magnesium (16:1) or indium (1:0). After protection of the amine, ring-closing metathesis and oxidation, the final deprotection-cyclization step was performed by hydrogenolysis of **130** using Pearlman's catalyst in acidic THF/water to give the natural product **131** in excellent yield.[306]

In Charlotte's synthetic efforts towards calystegine A_3, she was following the same strategy as Philip, but using different protecting groups in order to selectively deoxygenate D-glucose in the 2-position after performing the fragmentation-allylation reaction (Scheme 51, pathway A). Methyl α-D-glucopyranoside (**127**) was therefore selectively trityl- and PMB-protected in the 6- and 2-positions, respectively, which after benzylation enabled selective transformations at these postitions. The removal of the trityl group and iodination to give **133** was followed by the zinc-

mediated fragmentation and subsequent imine allylation affording **134** in excellent yield and good diastereoselectivity (5.3:1) in accordance with Philip's observations during the calystegine B$_2$ synthesis.
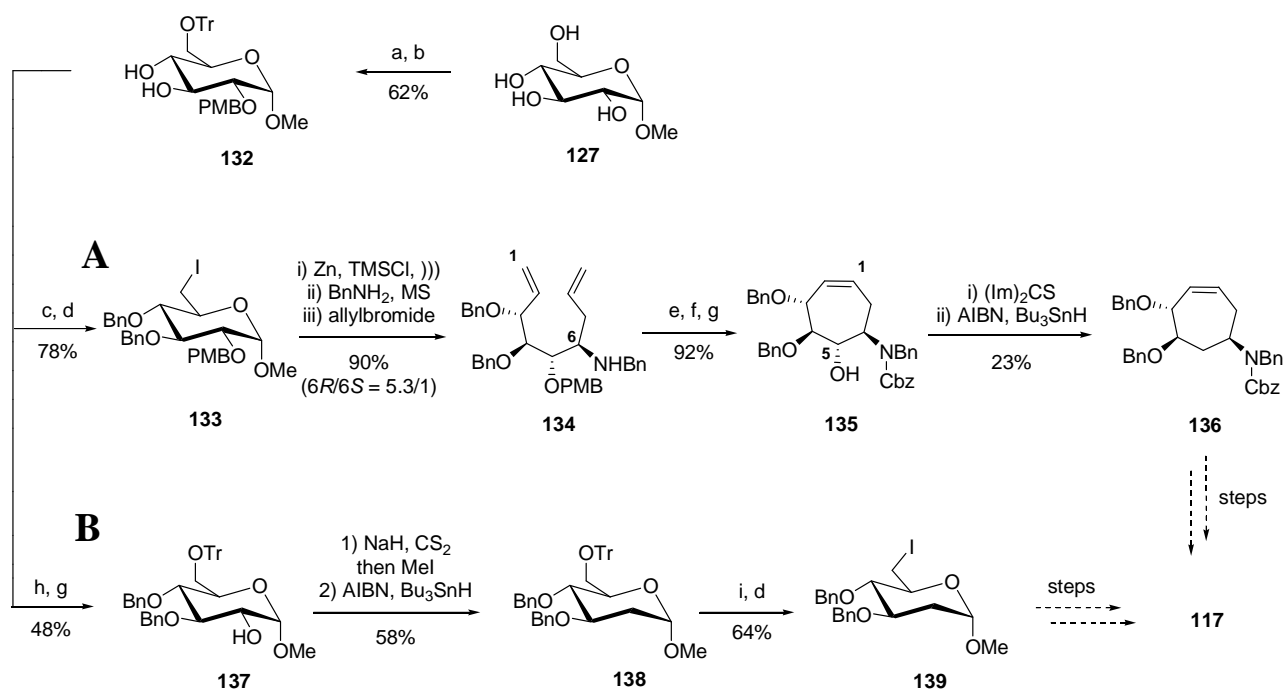
Scheme 51. Synthetic efforts towards calystegine A$_3$ (**117**) by Charlotte B. Pipper.[322] Reagents: a) TrCl, pyridine, b) Bu$_2$SnO, Bu$_4$NI, PMBCl, c) NaH, Bu$_4$NI, BnBr then H$_2$SO$_4$, d) I$_2$, PPh$_3$, imidazole, e) CbzCl, KHCO$_3$, f) Grubbs' 2nd gen. cat., g) DDQ, h) NaH, Bu$_4$NI, BnBr, i) H$_2$SO$_4$.

However, after Cbz-protection, ring-closing metathesis and removal of the PMB-group, Charlotte encountered unexpected problems regarding the subsequent removal of the C5 secondary alcohol of **135**. After numerous attempts to deoxygenate **135** including hydride displacement of a triflate and several variations of the Barton-McCombie radical deoxygenation,[324-326] the desired cycloheptene **136** could be isolated in only 23% yield over the two steps. Charlotte therefore decided to try the deoxygenation of D-glucopyranoside **137** in the 2-position *before* the fragmentation-allylation sequence (Scheme 51, pathway B). The standard Barton-McCombie radical deoxygenation worked well on this substrate producing the desired 2-deoxy D-glucose derivative **138**, which was transformed into the ω-iodoglucopyranoside **139** for fragmentation and allylation.[322] At this point, the project was handed over to the author of this thesis.

4.5 Results and discussion

4.5.1 Initial strategy towards calystegine A₃

Initially, the work was directed towards removal of the C2 hydroxy group of D-glucose *before* fragmentation and allylation (Scheme 51, pathway B). Starting from the trityl- and PMB-protected methyl α-D-glucopyranoside **132**, the C3 and C4 hydroxy groups were benzyl protected[327] in good yield followed by removal of the 2-*O*-PMB-group with DDQ to give **137** in excellent yield applying standard conditions[328] (Scheme 52).
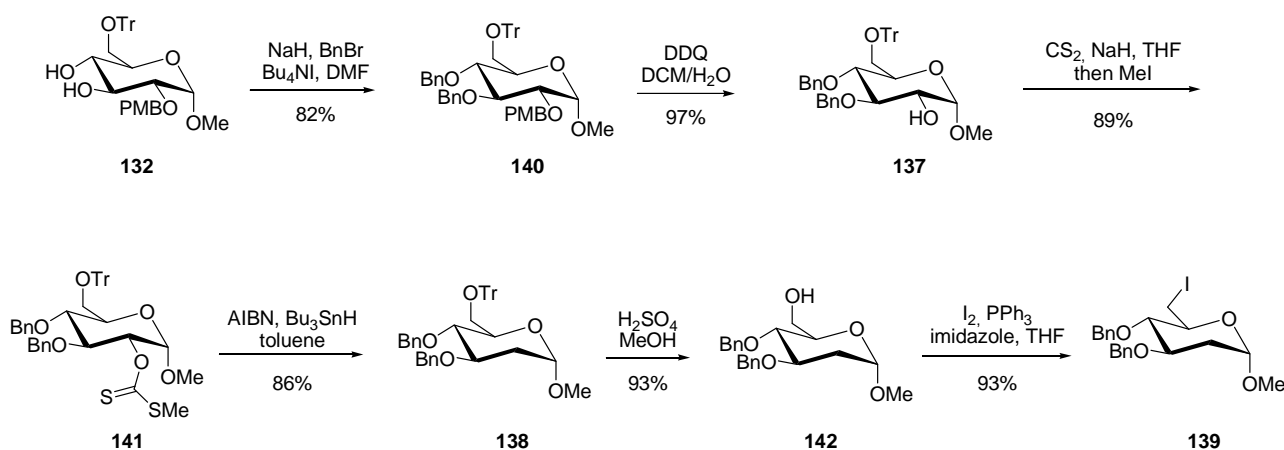
Scheme 52. Synthesis of the protected 2-deoxy-6-iodoglucopyranoside **139** for zinc-mediated fragmentation.

The Barton-McCombie radical deoxygenation[324] using NaH, imidazole and CS₂ produced the intermediate methyl xanthate **141** in 89% yield, and the following AIBN-initiated Bu₃SnH reduction of **141** to **138** occurred in 86% yield. The removal of the trityl group using sulfuric acid in methanol and the introduction of iodine occurred in excellent yield producing the protected methyl 2,6-dideoxy-6-iodo-α-D-glucopyranoside **139** for the fragmentation-allylation sequence. Notably, the more sensitive glycosidic linkage in the 2-deoxy glucoside **138** was not affected by the reaction conditions used for removal of the trityl group.

4.5.2 Fragmentation-imine formation-allylation

Subjecting **139** and freshly activated zinc[136] to sonication at 40 ℃ in THF resulted in fragmentation to produce the desired aldehyde **143** (Table 19). Addition of benzylamine to trap **143** as the imine **144** followed by drowise addition of allylbromide gave a diastereomeric mixture of the two dienes **145** and **146** in 94% yield (Table 19, entry 1). In contrast to Philip and Charlotte's results employing the 2-*O*-Bn and the 2-*O*-PMB substituted imines **147** and **148** (Table 19, entries 2

and 3), almost no stereoselectivity was observed, and **145** and **146** were formed in a 4,6-syn/4,6-anti (6*R*/6*S*) ratio of 1:1.13 favoring the undesired 4,6-anti product **146** (Table 19, entry 1). The identity of each diastereomer was later established by Cbz-protection and ring-closing metathesis followed by comparison with material synthesized *via* strategy A (see section 4.5.7 below).

Table 19. Zinc-mediated tandem fragmentation-allylation sequence.
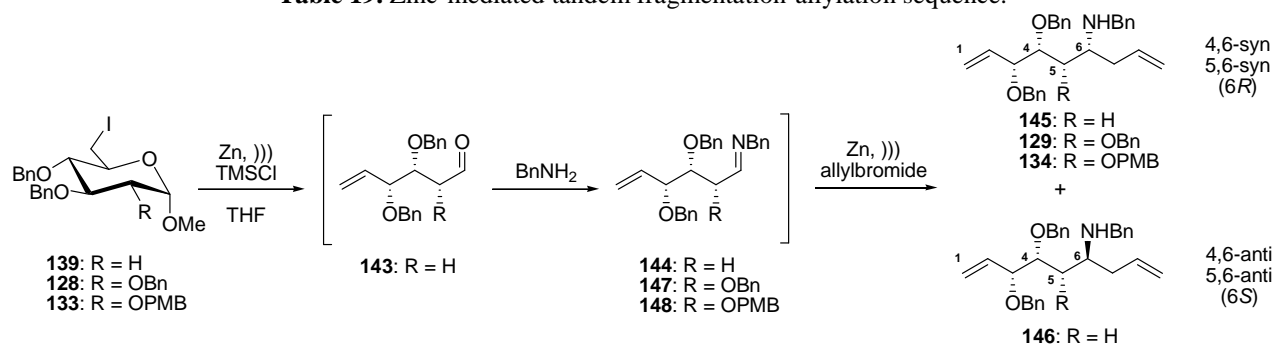
Entry	R	Yield (%)	Selectivity (6*R*/6*S*)
1	H	94	1 : 1.13
2[306]	OBn	85	5 : 1
3[329]	OPMB	90	5.3 : 1

Diastereomer ratios were determined by ^{13}C NMR spectroscopy.

It was attempted to improve the selectivity of the allylation by varying the metal and the reaction conditions (Table 20). The fragmentation of **139** was performed with zinc metal in THF under sonication followed by addition of benzylamine to trap aldehyde **143** as imine **144**. Zinc salts were removed by filtration followed by evaporation of the solvent, and the crude imine **144** was subsequently redissolved in different solvents and allylated with allylbromide and the metals zinc, indium and magnesium.

Both indium and magnesium are known to allylate by chelation to α-alkoxy substituents,[119,306,330] and in such cases a non-polar solvent might improve the degree of chelation of the metal in the transition state thereby enhancing the stereoselectivity. Like-wise addition of excess metal salts in a polar solvent can be used to inhibit the organometallic species from coordinating to α- or β-alkoxy substituents thereby reversing the stereochemistry by favoring non-coordinating pathways.[317] Both a polar (THF) and a non-polar solvent (toluene/DCM = 4:1) were therefore used, however, no

significant improvement of the diastereoselectivity was observed, although indium and magnesium seem to be slightly more selective towards the undesired 4,6-anti isomer **146** than zinc (Table 20).

Table 20. Imine allylation with different metals.
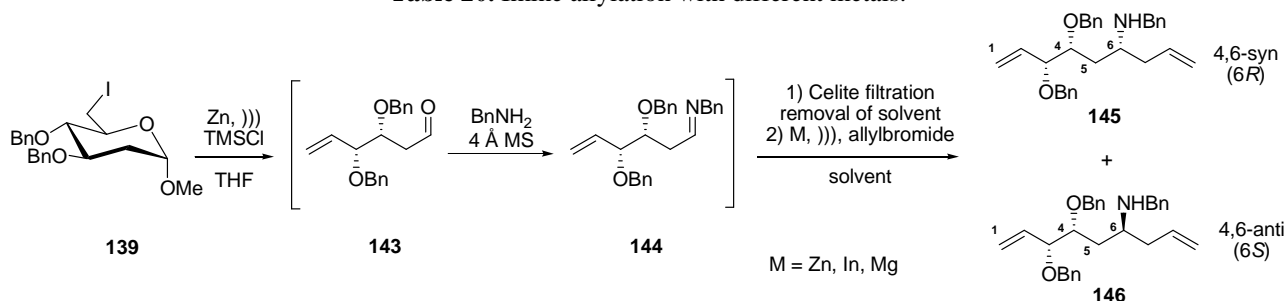
Metal	Yield (%)	Selectivity (6*R*/6*S*) THF	Selectivity (6*R*/6*S*) Tol/DCM = 4:1
Zinc	85	1 : 1.13	1 : 1.27
Indium	73	1 : 1.38	1 : 1.44
Magnesium	71	1 : 1.33	1 : 1.38

Diastereomer ratios were determined by ^{13}C NMR spectroscopy.

Initial 1:1 diastereoselectivities for organometallic addition to aldehydes have previously successfully been improved in our group by variation of solvent polarity, additives such as metal salts as well as the identity of the metal counterion.[314,317] But with no indication of success after these initial variations of metal and solvent (Table 20), it was decided to abandon this strategy.

4.5.3 Stereochemical considerations

Usually the Felkin-Anh model[109,110] and Cram's chelate model[111] are used to predict the π-facial selectivity of nucleophilic addition to α-alkoxy aldehydes. By analogy these models can also be used for α-substituted imines,[330] and by applying these models to the allylation of the 2-*O*-Bn and the 2-*O*-PMB protected imines **147** and **148** (Table 19, entries 2 and 3), the 5,6-anti product would be expected under non-chelating conditions (the Felkin-Anh model, Scheme 53a), whereas the 5,6-syn isomer would be produced under chelation control (Cram's chelate model, Scheme 53b). Good diastereoselectivities (5,6-syn/5,6-anti = 5:1), which may be explained by chelation, were observed by Philip and Charlotte with the 2-alkoxy substrates **147** and **148** (Table 19 , entries 2 and 3).
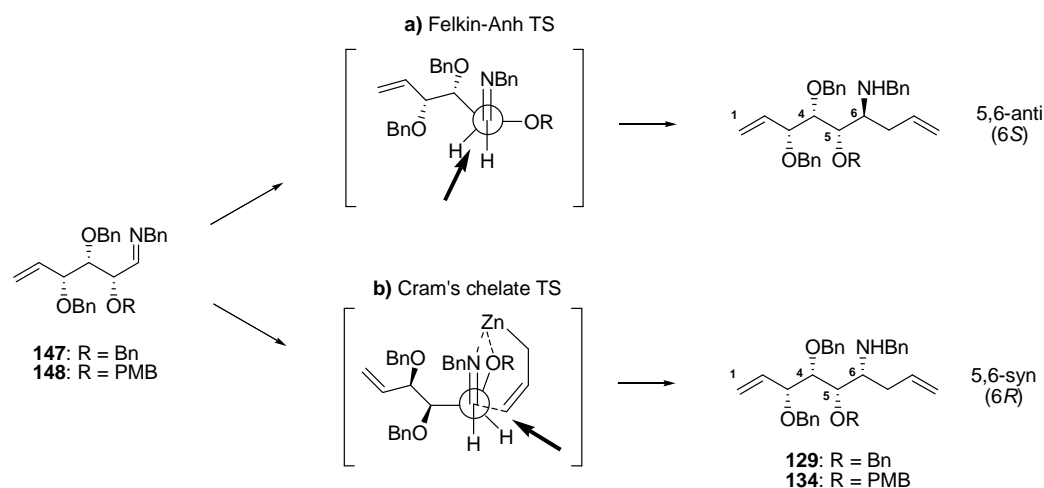
Scheme 53. Predominant transition states during allylation of α-alkoxy imines **147** and **148** according to a) the Felkin-Anh model[109,110] and b) Cram's chelate model.[111]

With β-alkoxy aldehydes and imines lacking an α-alkoxy substituent, the Felkin-Anh and Cram chelate models can no longer be used. Such β-alkoxy aldehydes are known to preferentially afford the anti products regardless of chelating or non-chelating conditions (Scheme 54a and b), however, product mixtures with low selectivity are most often observed.[94,119,331] The anti relationship between the β-alkoxy substituent and the newly formed stereocenter can either be rationalized by chelation[119,331] (Scheme 54a) or by minimizing steric and electrostatic interactions with the β-alkyl and -alkoxy substituents as depicted for the 2-deoxy imine **144** in Scheme 54b in a transition state model proposed by Evans and co-workers.[94]
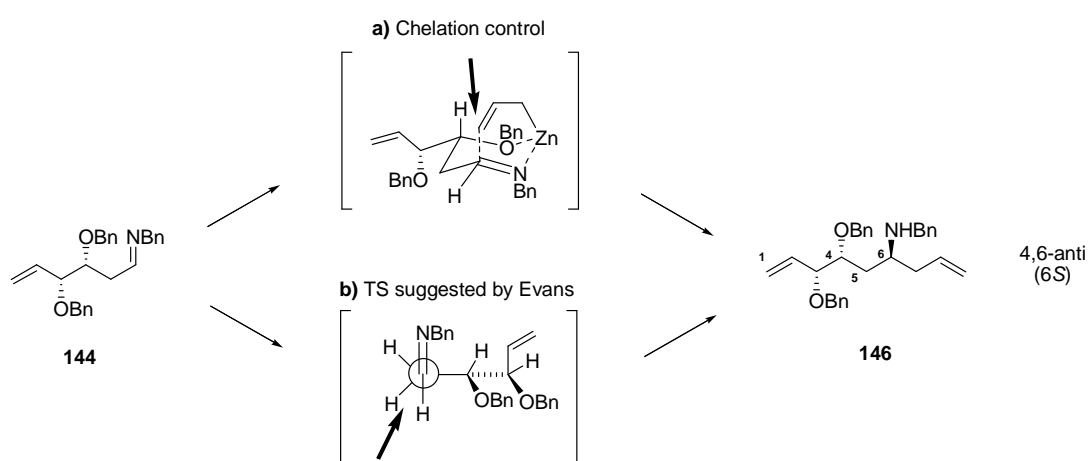
Scheme 54. Predominant transition states during allylation of β-alkoxy imine **144** a) under chelation control[119,331] and b) as recently suggested by Evans and co-workers.[94]

As shown in Table 19 (entry 1), the 2-deoxy substrate **139**, gave a 4,6-syn/4,6-anti ratio of 1:1.13 when zinc was used for the allylation. With indium and magnesium, which are known to react by chelation,[119,306,330] the 4,6-anti isomer **146** was obtained as the major product in slightly higher diastereoselectivity than with zinc (Table 20). Although the general trend in reactivity from Table 20 is consistent with the reported models for asymmetric induction in β-alkoxy aldehydes (Scheme 54),[94,119,331] the stereoselectivity is poor, and the 4,6-syn and the 4,6-anti isomers **145** and **146** are formed in almost equal amounts.

Evans and co-workers recently reported that nucleophilic attack on α,β-bisalkoxy aldehydes under non-chelating conditions is more complex than what can be explained by the Felkin-Anh model.[95] α,β-Anti bisalkoxy aldehydes seem to give good diastereoselectivities favoring an anti relationship between the α-alkoxy substituent and the newly formed stereocenter, whereas for α,β-syn bisalkoxy aldehydes, which are less selective, it is difficult to predict whether the anti or the syn product will dominate.[95] These trends suggested by Evans are in accordance with previous results from our group in the synthesis of calystegine B$_2$, B$_3$ and B$_4$, where D-glucose and D-galactose derived imines (α,β-syn configuration) gave lower stereoselectivities than the corresponding D-mannose derived imine (α,β-anti configuration).[306] As a consequence of these recent observations, it is not completely clear, whether the observed 5:1 5,6-syn/5,6-anti ratio for allylation of the 2-alkoxy substituted imines **147** and **148** (Table 19, entries 2 and 3) is a result of chelation or a complex relationship between the nature of the alkoxy substituents and the organometallic reagent.

4.5.4 Formation of diastereomeric carbocycles

The completion of pathway B with both isomers **145** and **146** required Cbz-protection of the amine followed by ring-closing metathesis using Grubbs' 2nd generation catalyst[133] (Scheme 55). Protection of the amine is necessary because unprotected amines are known to coordinate to ruthenium thereby hampering the ring-closing metathesis reaction.[18,332] The Cbz-protective group was chosen since it is readily removed by hydrogenation in the final step.[333] With the introduction of the Cbz-group, a broadening of the peaks in both ^{1}H and ^{13}C NMR spectroscopy could be observed due to the presence of rotamers thereby complicating analysis by NMR spectroscopy. Protection of the diastereomeric amines **145** and **146** with CbzCl to produce **149** and **150** occurred in 91 and 93% yield, respectively, and the subsequent ring-closing metatheses both gave 97% yield of **136** and **151**, respectively.
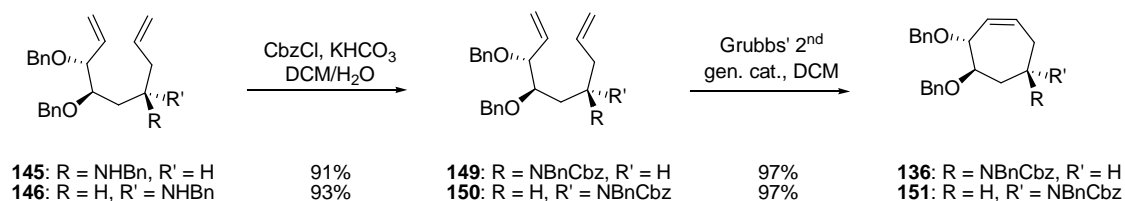
Scheme 55. Formation of seven-membered carbocycles **136** and **151** from the diastereomeric nonadienes **145** and **146**.

The conversion of the diastereomeric nonadienes **149** and **150** into the corresponding cycloheptenes **136** and **151** enabled direct comparison with the cycloheptene **136** synthesized by pathway A (see section 4.5.7 below), thereby determining the identity of each diastereomer. The lack of stereocontrol in the fragmentation-allylation sequence produced the desired nonadiene **145** as the minor isomer in less than 50% yield in the key step, and this disfavors pathway B as a synthetic route to calystegine A_3. As a result, the synthetic efforts towards calystegine A_3 were focused on optimization of the low-yielding Barton-McCombie deoxygenation of the 5-hydroxy-cycloheptene **135** (Scheme 51, pathway A).

4.5.5 Revised strategy towards calystegine A_3

Since the difficult deoxygenation of **135** is late in the synthesis, it was necessary to carry out the first steps on a large scale (20-30 grams). The fully protected intermediate **140** was deprotected at the 6-position using sulfuric acid in MeOH/toluene followed by iodination with iodine, PPh_3 and imidazole in THF in 98 and 93% yield, respectively (Scheme 56). This gave access to the 2,3,4-O-protected methyl 6-deoxy-6-iodo-α-D-glucopyranoside **133** for the fragmentation-allylation sequence.
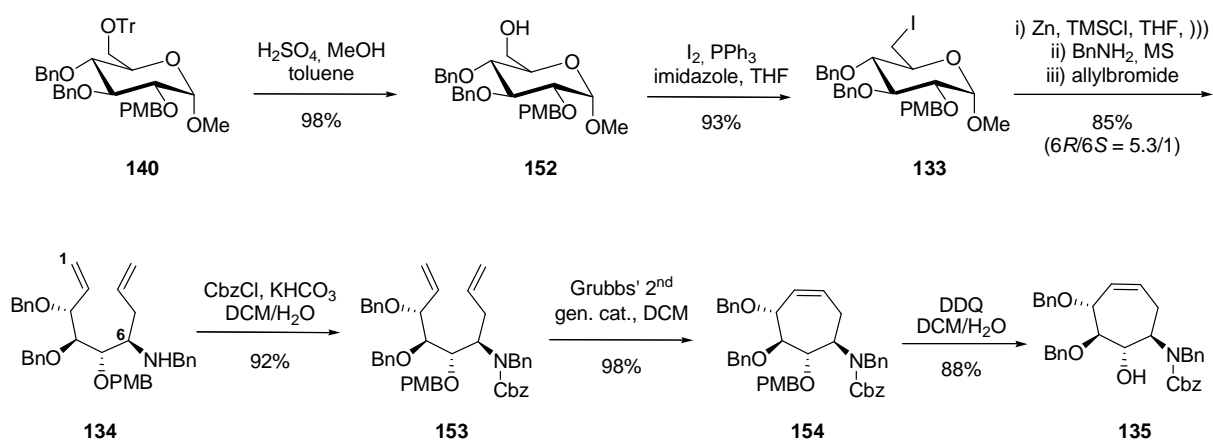
Scheme 56. Synthesis of cycloheptene **135** *via* pathway A.

Applying the conditions previously used on this 2-*O*-PMB protected substrate **133** gave two diastereomeric dienes in 85% yield in a 5.3:1 ratio favoring the desired (6*R*)-isomer **134**[♦] (Scheme 56). This result was in accordance with Philip and Charlotte's observations (Table 19, entries 2 and 3), and the stereochemical outcome was later confirmed by completing the synthesis of calystegine A$_3$ (see section 4.5.7).

The major (6*R*)-isomer **134** was subsequently Cbz-protected affording the desired diene **153** for ring-closing metathesis. The diene **153** was successfully cyclized with 5% of Grubbs' 2nd generation catalyst[133] in DCM to produce the seven-membered carbocyclic skeleton of calystegine A$_3$ in almost quantitative yield. Subsequent deprotection of the PMB-protected secondary alcohol **154** to give **135** occurred in 88% yield.

4.5.6 Deoxygenation

Due to the previous difficulties with the deoxygenation, a number of different deoxygenation strategies were considered. Barton-McCombie deoxygenations[324,325] are usually performed by reduction with Bu$_3$SnH, but also other hydrogen sources like H$_3$PO$_2$[334] and a range of different silyl hydrides including (Me$_3$Si)$_3$SiH[335] and Ph$_2$SiH$_2$[336] have been developed primarily due to lower toxicity. Displacement of leaving groups like sulfonates with hydrides was also considered. Mesylates and tosylates of primary alcohols can readily be displaced by various hydride reagents, but with secondary substrates, the reaction often fail due to competing attack on sulfur thereby regenerating the starting alcohol.[337,338] This problem can be avoided using Bu$_4$NBH$_4$, which is a useful reagent for the reduction of triflates of secondary alcohols.[339] However, since Charlotte had no success with such triflate displacements, the two classical Barton-McCombie deoxygenation variations using 1,1'-thiocarbodiimidazole[325] or CS$_2$[324] together with Bu$_3$SnH and AIBN were attempted (Scheme 57).

[♦] The desired (6*R*)-isomer **134** is the 5,6-syn product according to the nomenclature of Table 19.
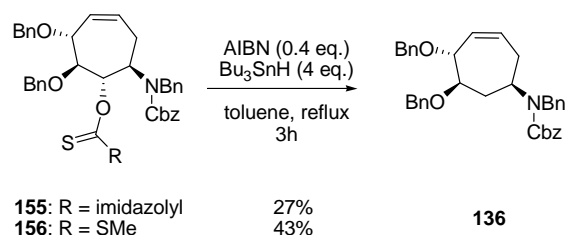
Scheme 57. Initial Barton-McCombie radical deoxygenation. The thiocarbonyl derivative (**155** or **156**) (40 mg) was degassed and heated to reflux in toluene. To the solution was added a mixture of AIBN and Bu$_3$SnH in toluene over a period of 30 min.

Of the available thiocarbonyl derivatives, methyl xanthates are known to be the most reactive species with respect to the deoxygenation step,[340] and indeed the methyl xanthate **156** gave an encouraging yield of 43% in the initial deoxygenation experiments. Based on this result, it was decided to optimize the deoxygenation using the methyl xanthate approach. However, having decided to use the CS$_2$-protocol, the formation of methyl xanthate **156** proved difficult, because a bicyclic side-product **157** was formed as the major product in 62% yield using 20 equivalents of CS$_2$ as depicted in Table 21 (entry 1). The side-product **157** results from intramolecular attack of the alkoxy anion on the carbonyl functionality of the Cbz-group instead of intermolecular attack on CS$_2$.

Table 21. Formation of methyl xanthate **156**.

Entry	NaH (eq.)	CS$_2$ (eq.)	Yield of 156 (%)	Yield of 157 (%)
1	3	20	22	62
2	3	200	64	26
3	3	500	66	19
4	3	1000[a]	39	34
5	5	500	72	16
6	4	1000	78	13

The reactions were performed by dropwise addition of the alcohol **135** dissolved in THF/CS$_2$ to a suspension of NaH and imidazole in CS$_2$. [a] No THF was used.

Increasing the amount of CS$_2$ to 200 equivalents gave the desired methyl xanthate **156** as the major product in 64% yield (Table 21, entry 2). Only a slight improvement was observed using 500 equivalents of CS$_2$, and performing the reaction neat resulted in a considerably lower yield

(entries 3 and 4). Changing the amount of NaH from three to five equivalents was found to have a favorable effect (entry 5), and combining this finding with an even larger excess of CS$_2$ (1000 eq.), gave **156** in 78% yield (entry 6).

Thorough degassing of both the reaction mixture and the reagents to exclude oxygen is of vital importance in order to prevent side reactions in highly functionalized substrates during the deoxygenation step.[341] The thiocarbonyl derivative is usually added to a refluxing solution of Bu$_3$SnH in toluene to ensure an excess of Bu$_3$SnH compared to the thiocarbonyl derivative,[324,325] and in contrast to the earliest reported radical deoxygenations,[324,325] a catalytic amount of AIBN is often used to increase the reaction rate and reproducibility.[341,342] In addition, it seems advantageous to add the thiocarbonyl derivative together with AIBN to a refluxing solution of Bu$_3$SnH to maintain a constant amount of radicals throughout the reaction. Taking the above parameters into consideration, an initial screening of the reaction conditions was performed according to Table 22.

Table 22. Deoxygenation of methyl xanthate **156** – screening of conditions.
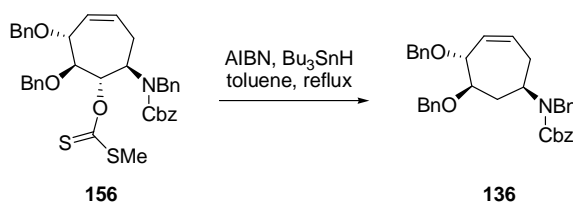
Entry	AIBN (eq.)	Bu$_3$SnH (eq.)	Conditions	Conversion[a] after 90 min (%)
1	0	3		~ 40
2	0.2	3	**156** was added to Bu$_3$SnH and AIBN	~ 75
3	0.2	3	AIBN and **156** were added to Bu$_3$SnH	>95
4	0.2	1.5	AIBN and **156** were added to Bu$_3$SnH	~ 90

The deoxygenations were performed by dropwise addition of **156** (100 mg) in toluene to freshly distilled Bu$_3$SnH in refluxing toluene. [a] Conversions are based on TLC analysis.

As can be seen from Table 22, the highest conversion within 90 min was achieved by adding **156** together with AIBN (0.2 eq.) to a refluxing solution of Bu$_3$SnH (3 eq.) in toluene (entry 3). Notably, when no AIBN is used (entry 1), the conversion is much lower than in the presence of AIBN. This illustrates that AIBN efficiently generates radicals, and in order for the deoxygenation to occur without AIBN, a much longer reaction time is required.

Choosing the conditions described in Table 22, entry 3, the reaction was scaled up and optimized with respect to reaction time as can be seen in Table 23 below.

Table 23. Deoxygenation of methyl xanthate **156** – optimization of conditions.

Entry	AIBN (eq.)	Bu$_3$SnH (eq.)	Scale (mg)	Time (min)	Yield of 136 (%)
1[a]	0.4	4	40	180	43
2	0.2	3	100	90	56
3	0.2	3	200	60	74
4	0.2	3	500	50	72

The deoxygenations were performed by dropwise addition of **156** and AIBN in toluene to Bu$_3$SnH in refluxing toluene. [a] Bu$_3$SnH and AIBN in toluene were added dropwise to a refluxing solution of **156** in toluene.

Increasing the scale and at the same time reducing the reaction time gave a gratifying 74% yield (Table 23, entry 3) in the light of previous failed attempts to optimize this reaction. From Table 23 it is also obvious that radical reactions are sensitive to the scale. On a small scale it is more difficult to produce a sufficient amount of radicals for the reaction to proceed, and small scale reactions therefore constitute a higher risk of failure.

4.5.7 Calystegine A$_3$ end game

The completion of the deoxygenation sequence produced material to establish the identity of the two cycloheptenes **136** and **151** synthesized *via* strategy B, and as mentioned previously, the minor isomer **136** from strategy B was identical in all respects to the cycloheptene **136** produced by strategy A.

Having successfully optimized the Barton-McCombie radical deoxygenation of **135**, we looked forward towards the end game of the calystegine A$_3$ synthesis. The next step was the hydroboration-oxidation sequence[306] (Scheme 58), which was performed with borane-THF complex followed by oxidation with alkaline hydrogen peroxide to produce an isomeric mixture of four alcohols. The crude alcohols were directly oxidized using DMP to produce a 2:1 mixture of the isomeric ketones **158** and **159** in 76% yield. The selectivity in the hydroboration was a bit lower than the 3:1 ratio observed by Philip in the similar calystegine B$_2$ system,[306] but the desired isomer was still favored.
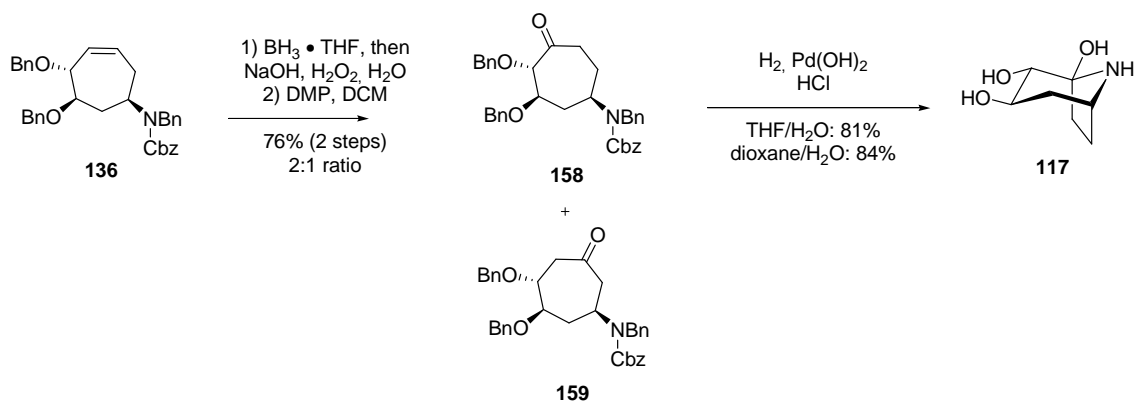
Scheme 58. Synthesis of calystegine A₃ (**117**).

The final deprotection and cyclization to the bicyclic aminoketal was performed by hydrogenation using Pearlman's catalyst followed by acidification with hydrochloric acid to facilitate the ring closure as developed by Philip.[306] Initially, THF/H₂O was used as the solvent, but after leaving the reaction mixture for a prolonged time (36 h) under acidic conditions, small amounts of 1,4-butanediol resulting from unexpected ring opening of THF could be observed. The diol was difficult to remove from the natural product by Sephadex LH-20 column chromatography,[343] and it was found that by performing the reaction in dioxane/H₂O, the side-reaction could be avoided. In addition, dioxane/H₂O gave a slight improvement of the yield from 81 to 84% as compared to THF/H₂O. However, these differences are subtle on such a small scale and are probably within experimental error.
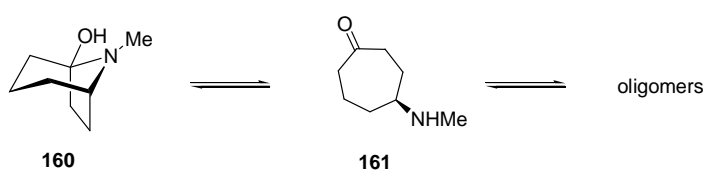
Scheme 59. Aminoketal – aminoketone equilibrium of physoperuvine (**160**).[344]

Physoperuvine (**160**), which represents the general nortropane skeleton, exists as an equilibrium mixture between its bicyclic aminoketal **160** and the corresponding aminocycloheptanone **161** (Scheme 59).[344] Even though the naturally occurring calystegines are found exclusively in their bicyclic form in nature, it is reasonable to assume that the calystegine alkaloids also exist in equilibria between their aminoketal and aminoketone forms in which the number and position of oxygenated substituents are decisive for the position of the equilibrium.[295,296] The formation of

oligomers of certain unnatural calystegines *via* the monocyclic aminoketone as experienced by Lallemand and co-workers under acidic conditions[296,298] was therefore a concern prior to the final deprotection-cyclization step producing calystegine A_3. This concern was amplified by difficulties producing calystegine A_3 from its hydrochloric salts as experienced by Johnson and Bis[299] (Scheme 47) and by Philip's observation that epimers of B_2, B_3 and B_4 were not well-defined compounds.[306] However, despite those concerns, calystegine A_3 was formed in good yield in accordance with the data reported for the natural product.[289] Our work-up procedure[306] utilized ion exchange resin IRA 400 OH⁻ thereby avoiding the problems associated with the hydrochloric salt of the natural product, and it is therefore a major improvement to the procedure by Johnson and Bis.[299]
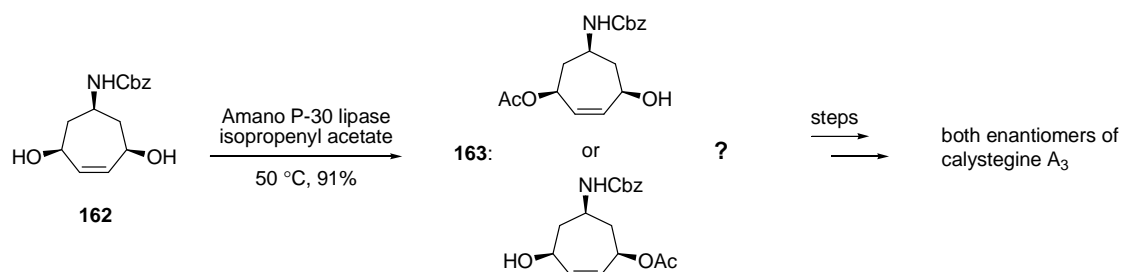
Scheme 60. The desymmetrization step in the synthesis of calystegine A_3 by Johnson and Bis was performed enzymatically, but the absolute configuration of the produced acetate **163** was not established.[287,299]

The structure of calystegine A_3 was reported in 1990,[285,286] however, no specific rotation was given in the literature until 1995. At that time Asano and co-workers reported $[\alpha]_D$ –17.3.[289] Even though calystegine A_3 had already been synthesized twice; racemic by Lallemand and co-workers in 1992[296] (Scheme 46) and both enantiomers by Johnson and Bis in 1995[299] (Scheme 47), the absolute configuration has remained unknown until now. Since both enantiomers of calystegine A_3 synthesized by Johnson and Bis were produced by enzymatic acetylation of a single hydroxy group in the prochiral diol **162**, the absolute configuration of the resulting acetate **163** could not be determined (Scheme 60). As a result of this, the identity of each enantiomer of calystegine A_3 was not established leaving the absolute configuration of calystegine A_3 unknown.[287,299]

With the current synthesis of calystegine A_3 from D-glucose, the absolute configuration can finally be determined. The specific rotation was measured to $[\alpha]_D^{25}$ –13.6, which is in accordance with the value reported by Asano and co-workers,[289] and as expected, the absolute configuration of naturally occurring calystegine A_3 resembles that of calystegine B_2.

4.5.8 Isomerization of calystegine A₃ to A₆

Dräger has previously suggested that calystegine A_3 (**117**) might be involved in the biosynthesis of calystegine A_6 (**166**) by a base-mediated isomerization *via* **164** and **165** as depicted in Scheme 61.[287] Calystegine A_6 has been isolated from *Hyoscyamus niger*[290] and *Lycium chinense*,[294] and in both cases together with calystegine A_3.[287] It is therefore reasonable to suspect that calystegine A_6 might be derived from calystegine A_3 by simple isomerization. Based on the instability of calystegine A_3 observed by Johnson and Bis,[299] it was therefore decided to investigate the stability of calystegine A_3 in basic solution with focus on possible isomerization to calystegine A_6.
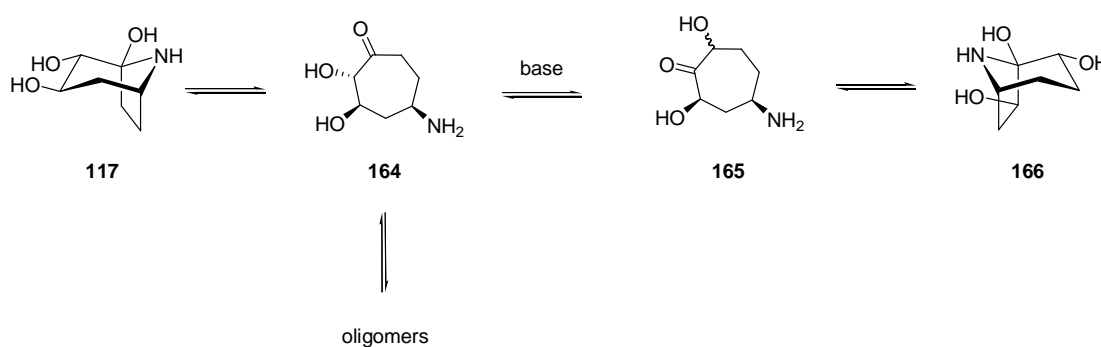
Scheme 61. Proposed base-mediated isomerization of calystegine A_3 (**117**) to A_6 (**166**).[287]

Base-mediated isomerization of sugars is most often performed with bases like KOH, $Ca(OH)_2$, Et_3N or pyridine.[26] Usually, isomerization and anomerization of carbohydrates is achieved under slightly basic conditions,[26] and refluxing pyridine is frequently used.[345,346] However, also stronger alkaline media like aqueous KOH at pH > 11 has been studied in detail and facilitates a range of isomerizations.[346] Since the isomerization of carbohydrates is dependant on pH,[26] both a weak (pyridine-d_5) and a strong ($Ca(OH)_2$) base were chosen for the attempted isomerization of calystegine A_3 to calystegine A_6 (Table 24).

Initially, calystegine A_3 was dissolved in pyridine-d_5 and left at room temperature for 14 days to investigate its stability in weak base (Table 24, entry 1). Surprisingly, no conversion could be observed neither by TLC nor ^1H NMR, and calystegine A_3 could be recovered in >80% yield. Calystegine A_3 was even stable in pyridine-d_5 at 100 ºC for up to 24 h, after which slow degradation was indicated by TLC analysis (entries 2-4).

Table 24. Attempted base-mediated isomerization of calystegine A_3 (**117**) to A_6 (**166**).

Entry	Base	Temperature (ºC)	Time	Observations
1	pyridine-d_5	25	14 days	No conversion. Recovery of **117**
2	pyridine-d_5	100	1 h	No conversion. Recovery of **117**
3	pyridine-d_5	100	4 h	No conversion. Recovery of **117**
4	pyridine-d_5	100	24 h	No conversion. Recovery of **117**[a]
5[b]	1 M aq. Ca(OH)$_2$	25	14 days	Slow degradation.[c] More than one spot[d]
6[b]	1 M aq. Ca(OH)$_2$	100	1 h	Residue of **117**
7[b]	1 M aq. Ca(OH)$_2$	100	4 h	Trace of **117**. More than one spot[d]
8[b]	1 M aq. Ca(OH)$_2$	100	24 h	More than one spot[d]

[a] Slow degradation. [b] The reaction mixtures were inhomogeneous and stirring was applied. [c] Slow broadening of the peaks in ^{1}H NMR when the reaction was performed in D$_2$O. [d] After workup TLC analysis revealed more than one spot (R_f 0.13 for the most intense new spot), a complex product mixture was observed by ^{1}H NMR, and no calystegine A_3 could be isolated. TLCs were eluted with 1-propanol/AcOH/H$_2$O = 4:1:1 (R_f 0.42 for calystegine A_3).

In stronger base however, calystegine A_3 proved to be less stable. When calystegine A_3 was dissolved in 1 M aqueous Ca(OH)$_2$ (pH >12) at room temperature, slow degradation was observed accompanied by development of a weak spot with a smaller R_f than calystegine A_3 (Table 24, entry 5). Performing the same reaction in 1 M Ca(OH)$_2$ in D$_2$O resulted in broadening followed by slow disappearance of the signals in ^{1}H NMR spectroscopy. The broadening of the signals in ^{1}H NMR spectroscopy can either be explained by a beginning epimerization of the stereocenters or by deuterium exchange with the solvent due to the highly basic conditions. In addition, it may also be a result of partial oligomerization,[296,298] which would give a less well-defined spectrum. After stirring for 14 days at room temperature (entry 5), no calystegine A_3 could be recovered just as experienced by Johnson and Bis treating calystegine A_3 with 2 M NaOH in D$_2$O.[299] Heating calystegine A_3 to 100 ºC in the presence of 1 M aqueous Ca(OH)$_2$ revealed almost complete degradation of calystegine A_3 within 4 h (entries 6-8). However, a new spot could be seen by TLC analysis, but after workup and Sephadex LH-20 column chromatography, a complex product mixture was observed by ^{1}H NMR spectroscopy.

In summary, calystegine A_3 is stable to weak base even at elevated temperatures, but it is quickly degraded under strong alkaline conditions. Furthermore, on the basis of the failed attempts to isomerize calystegine A_3 to A_6 under several different conditions, it is reasonable to conclude that calystegine A_6 is in fact an individual natural product and not just a base-mediated isomerization product of calystegine A_3 as speculated by Dräger.[287]

4.6 Concluding remarks

Enantiomerically pure calystegine A$_3$ has been synthesized by elongation of the carbon chain of commercially available methyl α-D-glucopyranoside (**127**) by two different routes. By deoxygenating D-glucose in the 2-position before the key zinc-mediated tandem fragmentation-allylation, almost no diastereoselectivity was obtained in the allylation reaction, and calystegine A$_3$ was produced in 14 steps and 5.3% overall yield. Removal of the secondary C2-hydroxy group of D-glucose *after* the fragmentation-allylation sequence gave rise to 6.4% yield of calystegine A$_3$ in 14 steps. However, combined with the work performed by Charlotte B. Pipper,[322] we have developed a 13 step synthesis of calystegine A$_3$ in 8.3% overall yield. To close this project, the two parallel routes to calystegine A$_3$ (**117**) developed in the group are shown in their entirety in Scheme 62.

The current work constitutes the shortest synthesis of enantiomerically pure calystegine A$_3$ to date and proves the absolute configuration of naturally occurring calystegine A$_3$. Calystegine A$_3$ is stable in weak base, but is quickly degraded in strong base. It was not possible to isomerize calystegine A$_3$ to A$_6$, which indicates that calystegine A$_6$ is a natural product itself in contrast to an isomerization product of calystegine A$_3$.
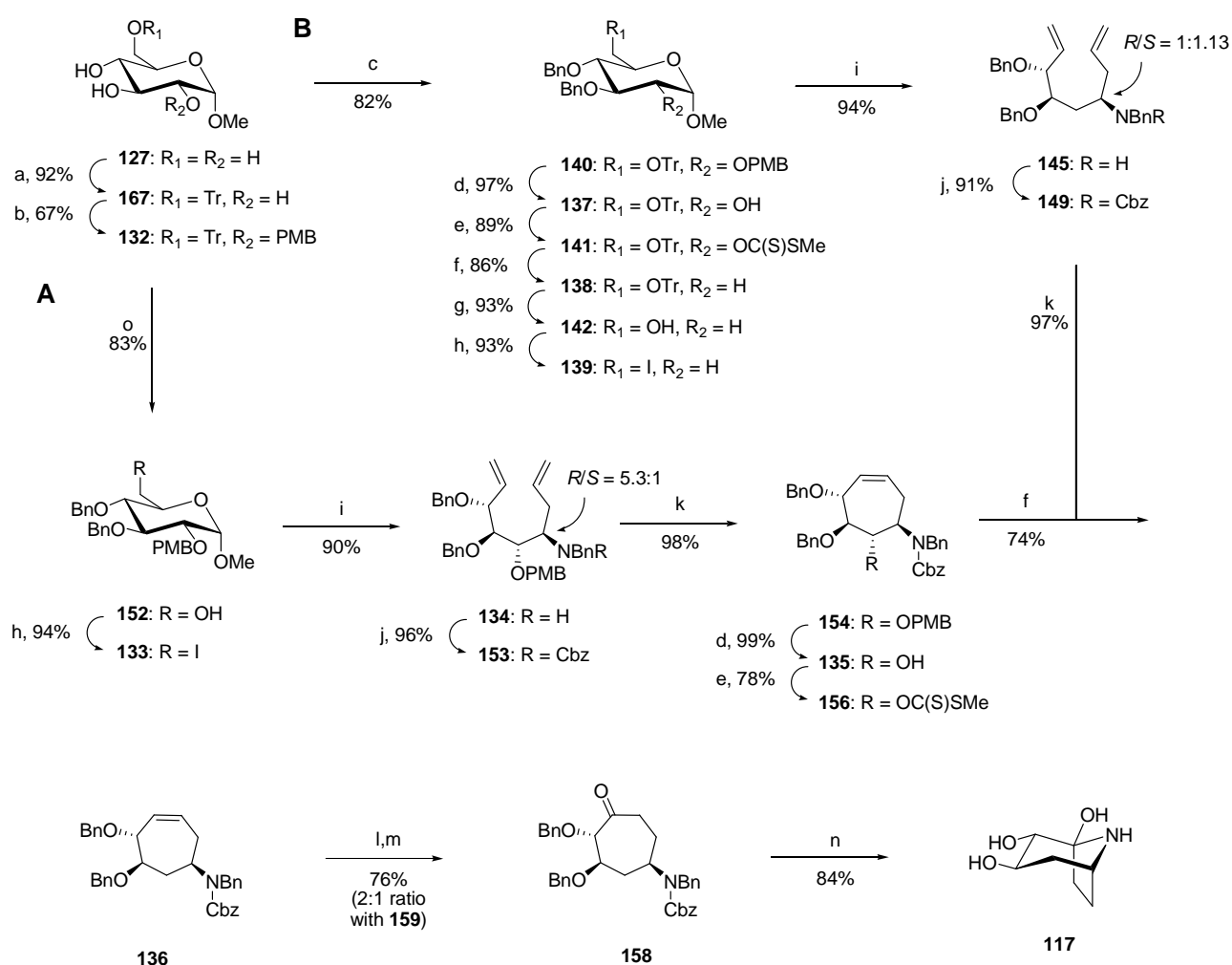
Scheme 62. Overview of the two routes to calystegine A_3 (**117**) developed by our group. Reagents and conditions: a) TrCl, pyridine, 90 ℃, b) Bu$_2$SnO, Bu$_4$NI, PMBCl, MeCN, reflux, c) NaH, Bu$_4$NI, BnBr, DMF, 0 ℃ → rt, d) DDQ, DCM, H$_2$O, rt, e) NaH, CS$_2$, imidazole, THF, rt, then MeI, f) Bu$_3$SnH, AIBN, toluene, reflux, g) H$_2$SO$_4$, MeOH, H$_2$O, rt, h) I$_2$, PPh$_3$, imidazole, THF, 40 ℃, i) Zn, TMSCl,))), THF, 40 ℃, then BnNH$_2$, then allylbromide, j) CbzCl, KHCO$_3$, DCM, H$_2$O, 0 ℃ → rt, k) 5% Grubbs' 2nd gen. cat., DCM, rt, l) BH$_3$, THF, -40 ℃ → rt, then NaOH, H$_2$O$_2$, H$_2$O, rt, m) DMP, DCM, rt, n) H$_2$, Pd(OH)$_2$/C, dioxane, H$_2$O, rt, then HCl, o) NaH, Bu$_4$NI, BnBr, DMF, 0 ℃ → rt, then H$_2$SO$_4$, MeOH, H$_2$O, rt.

5 Synthesis of gabosine A by chain elongation of D-ribose

5.1 The gabosines

The gabosines are a family of polyhydroxylated carbocycles possessing a cyclohexanone/cyclohexenone skeleton (Figure 6). They were first isolated in 1974[347] as secondary metabolites from *Streptomyces* strains. Since then a total of 14 different gabosines have been isolated.[348-350] The gabosines do not possess any significant biological activity, but Thiericke and co-workers found that many of the gabosines exhibit weak DNA binding properties.[349]
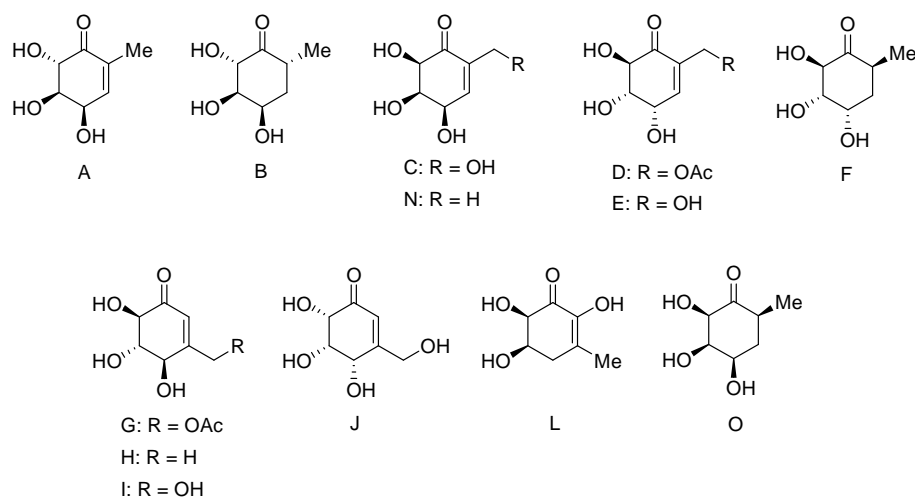
Figure 6. Naturally occurring gabosines. The structure of gabosine K is at present unknown since the initially proposed structure[348] turned out to be wrong.[351]

The biosynthesis of the gabosines occurs *via* a pentose phosphate pathway by cyclization of sedoheptulose 7-phosphate by an aldol condensation.[352] Chemical syntheses have been reported for gabosines A,[353,354] B,[351,354] C,[355-360] D,[354] E,[354,356] G,[361] I,[361,362] N[363] and O.[363,364] Gabosine A has been synthesized by Banwell and co-workers in 2001[353] and Shinada and co-workers in 2002.[354,365] The shortest synthesis was provided by Banwell accessing gabosine A in six steps from *cis*-1,2-dihydroxycatechol **168** (Scheme 63), which is available in a single step by enzymatic dihydroxylation of iodobenzene.[353]
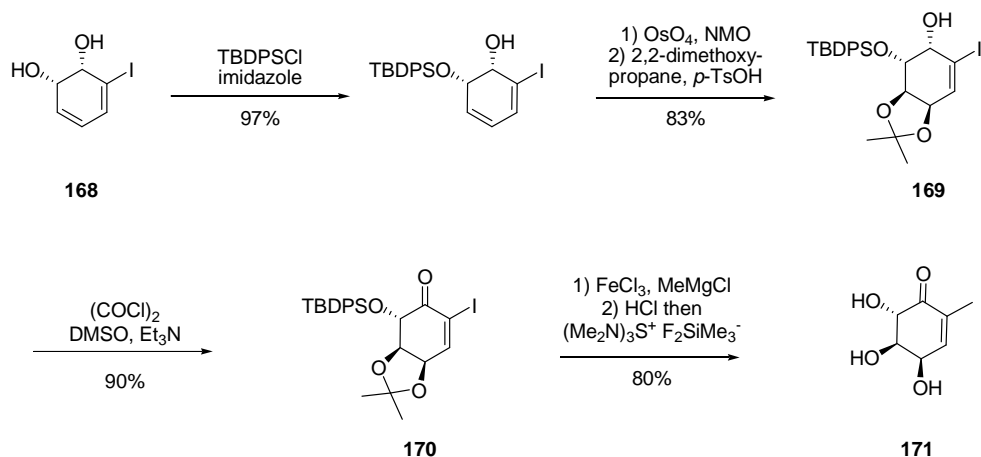
Scheme 63. Synthesis of gabosine A (**171**) by Banwell and co-workers.[353]

Selective silyl protection of a single hydroxy group of **168** followed by dihydroxylation and isopropylidene protection afforded **169**, which was subsequently oxidized to cyclohexenone **170**. Displacement of the iodide by a methyl group and deprotection gave gabosine A (**171**) in 58% overall yield from **168**. The synthesis by Shinada and co-workers, on the other hand, required 12 steps from (-)-quinic acid (**172**) affording gabosine A in approximately 8% overall yield (Scheme 64).[354,365]
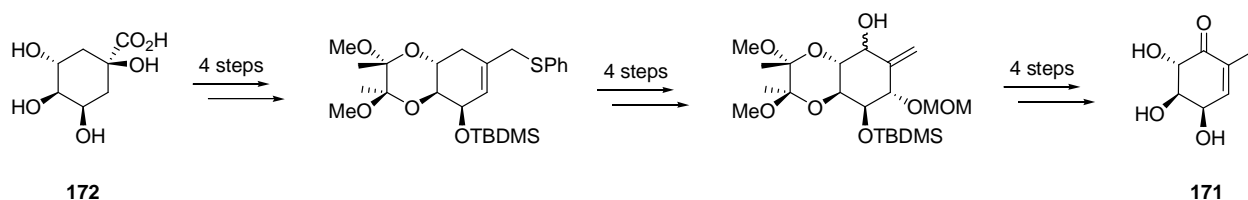
Scheme 64. Synthesis of gabosine A (**171**) by Shinada and co-workers.[354,365]

None of the above mentioned syntheses take advantage of the convenient stereochemical configuration of abundant carbohydrates, and the aim of the current project is to apply the well-established fragmentation-allylation-metathesis methodology (section 4.2) to the synthesis of gabosine A from D-ribose.

5.2 Retrosynthetic analysis of gabosine A

The synthetic strategy towards gabosine A is very similar to the synthesis of calystegine A$_3$, and the retrosynthesis of gabosine A is shown in Scheme 65. The cyclohexenone skeleton can be accessed

by ring-closing metathesis of diene **174**. The required diene **174** could be provided by chain elongation of a suitably protected ω-iodoribofuranoside **175** by zinc-mediated fragmentation and allylation.[135,136] By employing D-ribose as the starting material, two of the stereocenters in gabosine A are conveniently set from chiral pool, and the strategy enables preparation of both gabosine A and its epimer, gabosine N.
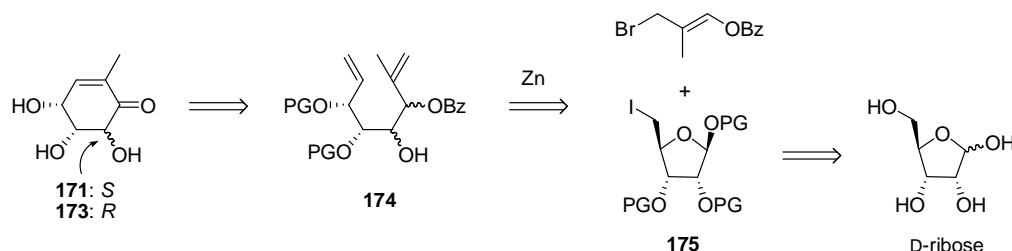
Scheme 65. Retrosynthetic analysis of gabosines A (**171**) and N (**173**).

5.3 Previous work on the gabosine project in the group

Several people from the group have been working on the synthesis of the gabosines. Mette Fanefjord successfully completed the synthesis of gabosine N,[366] whereas Flemming Gundorph Hansen prepared small amounts of gabosine A.[367] The work towards gabosine A and N performed by Mette and Flemming is summarized in Scheme 66.[366-368]
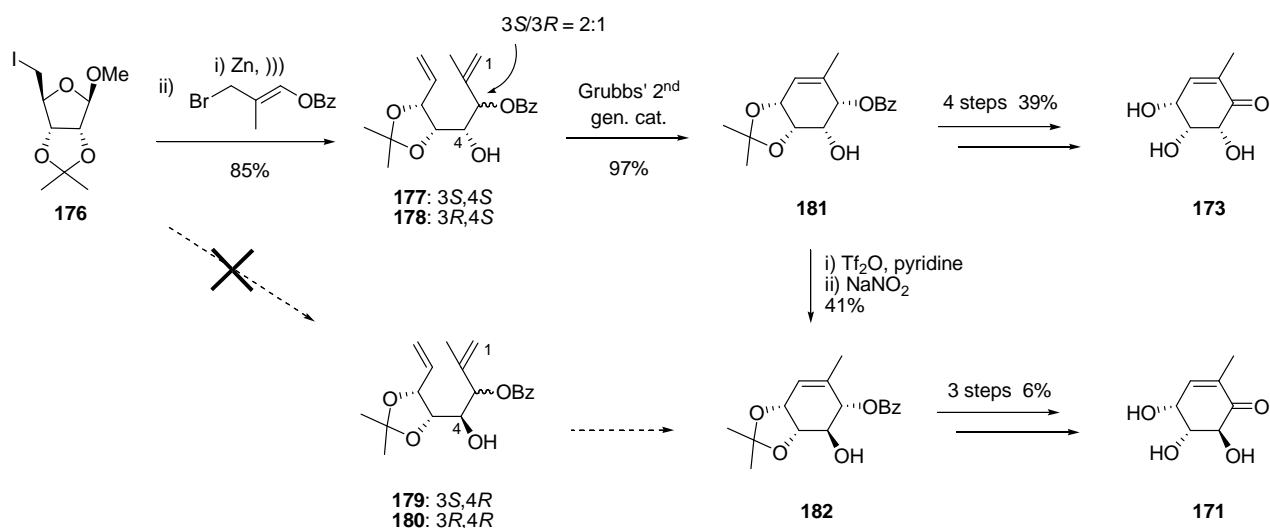
Scheme 66. Synthesis of gabosine N (**173**) by Mette Fanefjord[366,368] and synthetic efforts towards gabosine A (**171**) by Flemming Gundorph Hansen and Mette Fanefjord.[367,368]

The initial strategy was to use the known methyl 2,3-*O*-isopropylidene-5-deoxy-5-iodo-β-D-ribofuranoside **176**[369] as a common intermediate. The iodide **176** is available from D-ribose in two steps in 77% yield,[370] and metal-mediated fragmentation-allylation would produce the diastereomeric (4*S*)- and (4*R*)-dienes (**177**-**180**) conveniently set up for the synthesis of both gabosines A and N. However, zinc-mediated fragmentation-allylation only produced the diastereomeric (4*S*)-dienes **177** and **178**, and after several failed attempts to synthesize the (4*R*)-dienes **179** and **180** for gabosine A by zinc and indium mediated allylation,[367] it was decided to invert the C4 secondary alcohol of **181** instead. However, this proved difficult and in addition to this, the following steps occurred in very low yield leaving the synthesis of gabosine A unfinished.

5.4 Results and discussion

5.4.1 Stereochemical considerations

The synthesis of gabosine N by Mette Fanefjord was performed with the major (3*S*,4*S*)-isomer **177** from the allylation as shown in Scheme 67. The stereochemistry at C4 was rigorously established by completing the synthesis, but the configuration at C3 has not been unambiguously established.
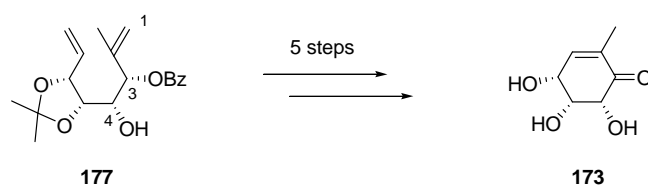
Scheme 67. Mette's synthesis of gabosine N employed only the major (3*S*,4*S*)-isomer **177**.[366]

In order to determine the absolute configuration of the allylation product **177** at C3 (Scheme 67), the diol **183** derived from the major isomer **177** was subjected to 2,2-dimethoxypropane and catalytic amounts of CSA at room temperature (Scheme 68).
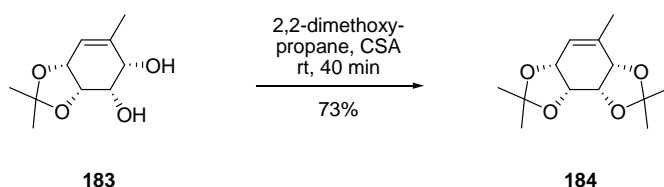
Scheme 68.

The diol **183** reacted smoothly to produce the diisopropylidene derivative **184** in 73% yield within 40 min, and this indicates a *cis* relationship between the hydroxy groups in question. However, since a *trans* fused bicyclic system is not impossible (especially not when the six-membered ring is slightly flattened by the double bond), the reactivity of the minor isomer **178** was also investigated (Scheme 69).
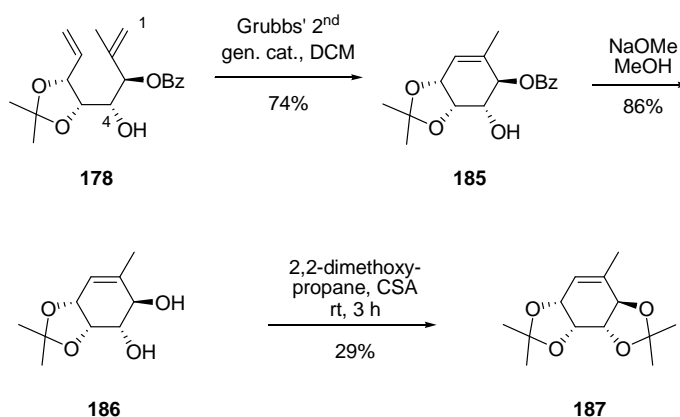
Scheme 69.

After ring-closing metathesis of **178** (which will be discussed in section 5.4.2 below) and deprotection, the *trans* diol **186** was subjected to 2,2-dimethoxypropane and CSA. Under identical conditions as with the *cis* isomer **183**, no conversion was observed, and only after 3 h using a larger excess of 2,2-dimethoxypropane, any conversion was achieved. The diisopropylidene protected compound **187** could be isolated in 29% yield together with 35% of unreacted starting material. Although these data strongly support the proposed stereochemical configurations, ^{13}C NMR data for the acetal carbons were compared for the two diisopropylidene compounds. According to Buchanan and co-workers[371] *cis* fused isopropylidene acetals have a slightly lower chemical shift than *trans* fused isopropylidene acetals.♦ The *cis* fused substrate **184** showed acetal ^{13}C chemical shifts of 110.4 and 110.3, whereas the *trans* fused derivative **187** showed acetal ^{13}C chemical shifts of 111.2 and 109.9 for the two isopropylidene groups. Together, the reactivities of the two isomeric diols **183** and **186** with 2,2-dimethoxypropane and the ^{13}C NMR data of the corresponding diisopropylidene

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♦ Isopropylidene acetals which are *cis* fused to a pyranoid or cyclohexane rings are reported to have a ^{13}C chemical shift of 108.1-111.4 ppm for the acetal carbon, whereas *trans* fused isopropylidene acetals have ^{13}C chemical shifts of 111.8-112.3 ppm for the acetal carbon. (Buchanan, J. G.; Edgar, A. R.; Rawson, D. I.; Shahidi, P.; Wightman, R. H. *Carbohydr. Res.* **1982**, *100*, 75).

derivatives **184** and **187** provide evidence for the proposed stereochemistry in the allylation reaction (Scheme 66).

The obtained (*S*)-configuration at C4 can be rationalized by the Felkin-Anh model[109,110] (Scheme 70), and it is in accordance with previous observations in a similar allylation in the synthesis of conduritol D.[316] In contrast to allylation of α,β-syn bisalkoxy imines **147** and **148** in the synthesis of calystegine A$_3$ (see section 4.5.2 and 4.5.3), the stereochemical outcome from allylation of the α,β-anti bisalkoxy aldehyde **188** (generated by zinc-mediated fragmentation of **176**) can be explained by the Felkin-Anh model although the reactions were performed under identical conditions.
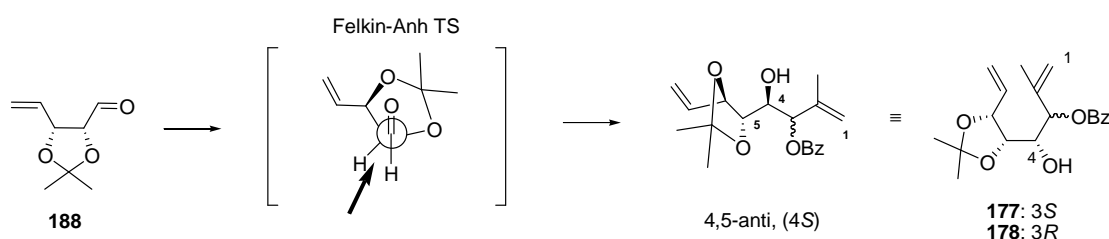
Scheme 70. The observed stereochemical outcome in the allylation of aldehyde **188** can be rationalized by the Felkin-Anh model.[109,110]

The observation that the α,β-anti bisalkoxy acetonide **188** reacts according to the Felkin-Anh model and produces the 4,5-anti (4*S*) product (**177** and **178**) is in accordance with recent results from the Evans group, which suggest that acetonide protected α,β-bisalkoxy aldehydes predominantly afford anti relationships between the α-alkoxy substituent and the newly formed stereocenter irrespective of the configuration of the β-alkoxy substituent.[95] The decreased conformational flexibility of the isopropylidene protected aldehyde **188** decreases steric and electrostatic interactions between the β-substituent and the incoming nucleophile, and the π-facial stereoselectivity can thus be predicted by the Felkin-Anh model.

5.4.2 Ring-closing metathesis

As shown in Scheme 69, ring-closing metathesis of **178** using Grubbs' 2nd generation catalyst[133] occurred in 74% yield. Earlier, Mette only obtained a maximum of 47% yield for ring-closing metathesis of **178** using P(CH$_2$OH)$_3$ at 40 °C for 24 h to quench the catalyst.[366] Although P(CH$_2$OH)$_3$ has been reported to remove ruthenium byproducts efficiently,[372] the product may not

be stable to these conditions. Instead, it was found that by purifying the product directly by flash column chromatography, a considerably higher yield of **185** could be achieved. The 74% yield for the ring-closing metathesis of **178** is in contrast to Mette's nearly quantitative yield of 97%[♦] for the diastereomeric diene **177**.[368] Generally, ring-closing metathesis of 2,2-disubstituted alkenes occur in slightly lower yield than monosubstituted alkenes,[373] but since both substrates **177** and **178** contain a disubstituted double bond, an explanation for their different reactivity could not be rationalized. However, significant differences in the reactivity of diastereomeric dienes in ring-closing metathesis reactions have previously been observed in the literature,[305,374,375] and it can therefore be concluded that the reactivity of dienes in ring-closing metathesis is highly dependant on the nature and the configuration of the substituents.

5.4.3 Synthesis of gabosine A

In order to synthesize gabosine A, the unprotected hydroxy group of **181** needs to be inverted. Usually the Mitsunobu reaction[376] would be employed to invert secondary alcohols, but in this case the substrate is probably too sterically hindered, and instead triflic anhydride in pyridine followed by treatment with sodium nitrite[377] in DMF was used (Table 25, entry 1). Triflates can be formed in almost quantitative yield, whereas the displacement by nitrite may often be accompanied by significant decomposition or byproduct formation leading to moderate overall yields.[315] In this case, **182** was isolated in 52% yield from **181**, and competing E2 elimination of the triflate **189** might be responsible for the moderate yield. No single byproduct could be identified, since a complex mixture of byproducts was observed by ^{1}H NMR spectroscopy.

In Table 25, the observed 52% yield of **182** is compared with Mette and Flemming's previous attempts to invert the secondary alcohol of **181** (entries 2-5).[367,368] From these results it seems important to keep the reaction time of the nitrate-mediated triflate displacement low to prevent decomposition and side reactions.

[♦] In this case the catalyst was removed by treatment with activated carbon. (Cho, J. H.; Kim, B. M. *Org. Lett.* **2003**, *5*, 531).

Table 25. Inversion of the secondary alcohol of **181**.
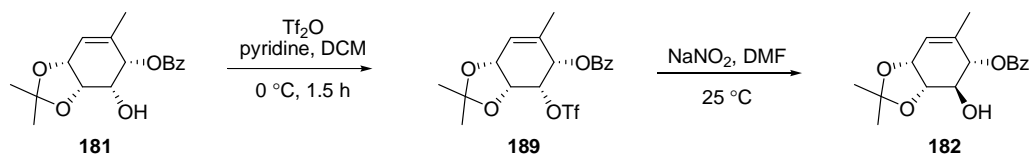
Entry	Base	Tf$_2$O (eq.)	NaNO$_2$ (eq.)	Time (h)	Yield of 182 (%)
1	pyridine	1.5	4	5.5	52
2	pyridine	1.4	4	16	33
3	pyridine	5	10	16	41
4	Et$_3$N	2	5	16	13
5	pyridine	1.4	7 [a]	16	40

Entries 2-5 were performed by Mette and Flemming.[367,368]
[a] NaNO$_2$/DMF was replaced by Bu$_4$NNO$_2$/toluene.

Completion of the synthesis of gabosine A was achieved by protective group manipulations and allylic oxidation as shown in Scheme 71. Direct allylic oxidation with DDQ, PDC or MnO$_2$ in the presence of an unprotected homoallylic alcohol has previously been found to occur in low yield,[366,367] and a protective group for the homoallylic alcohol of **182** is therefore necessary. The tetrahydropyranyl (THP) group[378] was chosen since it can be removed together with the acetonide under acidic conditions. However, analysis by NMR spectroscopy was complicated by the introduction of the THP group, since the additional stereocenter affords two diastereomers.
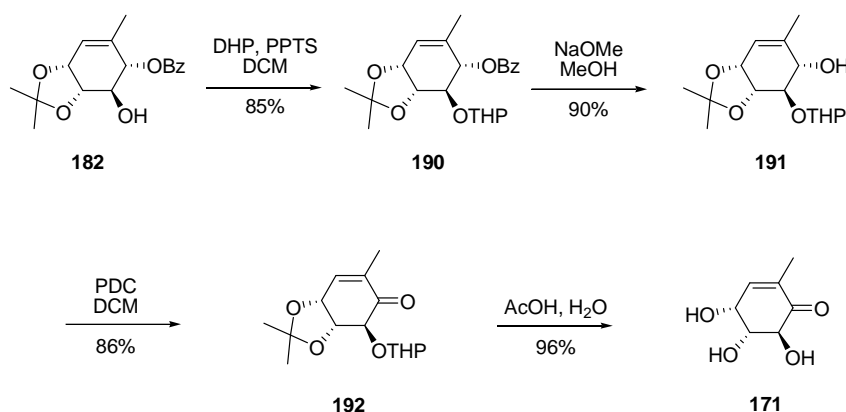
Scheme 71. Synthesis of gabosine A (**171**).

The THP group was introduced in 85% yield, and subsequent deprotection of **190** under Zémplen conditions[129] afforded **191** in 90% yield. Oxidation of the allylic alcohol **191** with PDC and final deprotection of **192** with acetic acid afforded gabosine A with analytical data in accordance with the natural product.[348,353]

5.5 Concluding remarks

To conclude the gabosine project, gabosine N (**173**) and A (**171**) have been synthesized from D-ribose in eight and nine steps, respectively together with Mette Fanefjord and Flemming Gundorph Hansen (Scheme 72). The key steps in the syntheses are the chain elongation of 2,3-*O*-isopropylidene-5-deoxy-5-iodo-β-D-ribofuranoside **176** by zinc-mediated fragmentation-allylation and subsequent ring-closing olefin metathesis to produce **181**. Due to unsuccessful attempts to control the stereochemical outcome of the allylation reaction, an additional step was required to invert the homoallylic alcohol of **181** to obtain **182** for the synthesis of gabosine A. Gabosine N was prepared in 16.5% yield, whereas gabosine A was synthesized in 13.9% overall yield from D-ribose. The work has very recently been published in *European Journal of Organic Chemistry*.
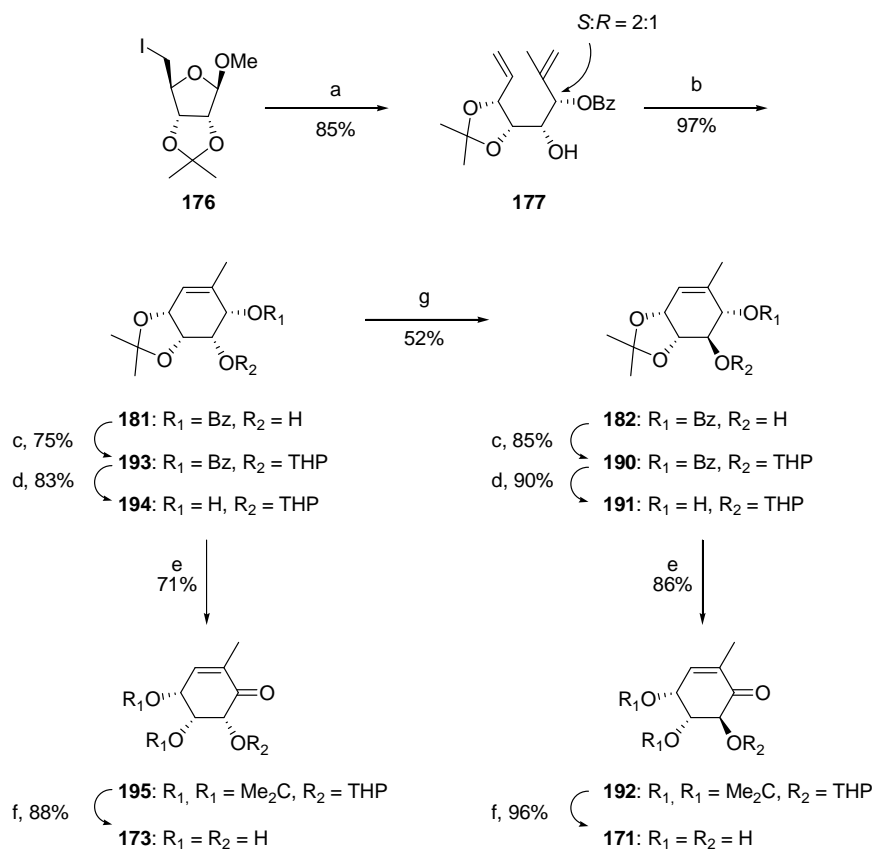
Scheme 72. Overview of the syntheses of gabosine N (**173**) and A (**171**) from our group. Reagents and conditions: a) Zn,))), THF, H$_2$O, 40 ºC, then (*E*)-BrCH$_2$C(CH$_3$)=CHOBz, b) 10% Grubbs' 2nd gen. cat., DCM, 40 ºC, c) DHP, PPTS, DCM, rt, d) NaOMe, MeOH, rt, e) PDC, DCM, rt, f) AcOH, H$_2$O, 40 ºC, g) Tf$_2$O, pyridine, DCM, -20 ºC → rt, then NaNO$_2$, DMF, rt.

The present synthesis of gabosine A cannot compete with the synthesis developed by Banwell and co-workers,[353] but together with the synthesis of calystegine A_3 (chapter 4) and previous reports from our group,[136,304,306,314-317] it illustrates that carbocyclic natural products are easily available from carbohydrates by chain elongation and subsequent cyclization.

6 Interaction between plasma protein and acyl glucuronide drug metabolites

This part of the thesis describes the work carried out during a six months stay at the Chemistry Research Laboratory at University of Oxford. The research was performed under the supervision of professor Benjamin G. Davis in collaboration with the group of professor Andrew V. Stachulski at University of Liverpool. The project involves the metabolism of nonsteroidal anti-inflammatory drugs (NSAIDs) with particular focus on their acyl glucuronide (AG) metabolites and the interaction of these with human plasma protein.

6.1 Introduction

The NSAIDs are among the most widely used drugs on the market, and include drugs like acetylsalicylic acid, ibuprofen and diclofenac. In humans, the NSAIDs function by inhibiting cyclooxygenase 1 and 2, which are enzymes responsible for the production of prostaglandins from arachidonic acid. Whenever the body is 'threatened' by a trauma, an infection or introduction of a foreign object, the synthesis of prostaglandins is stimulated. The liberation of prostaglandins induce pain, inflammation and fever, and the inhibition of cyclooxygenase 1 and 2 by NSAIDs lead to analgesic, anti-inflammatory and fever reducing effects.[13,14]

The metabolism of relatively non-polar drugs like NSAIDs primarily takes place in the liver, whereas polar drugs, on the other hand, are excreted in the kidney. Non-polar drugs are metabolized by phase I and II reactions in the liver to increase their water solubility and enable subsequent urinary excretion *via* the kidney (Figure 7).[13,14] The phase I reactions include oxidation, hydroxylation, dealkylation, deamination and hydrolysis to make the drug more polar. Some drugs can be excreted after phase I metabolism, but many drugs including the NSAIDs require further conjugation in phase II metabolism to be excreted. One of the most important phase II reactions is glucuronidation, in which UDP-glucuronosyltransferases couple D-glucuronic acid to the drug.[13] Other phase II reactions include sulfonation and coupling of the drug to glutathione. As a result of increased water solubility, the metabolites are transported to the kidney or the gallbladder and excreted in urine or faeces, respectively.[13,14]
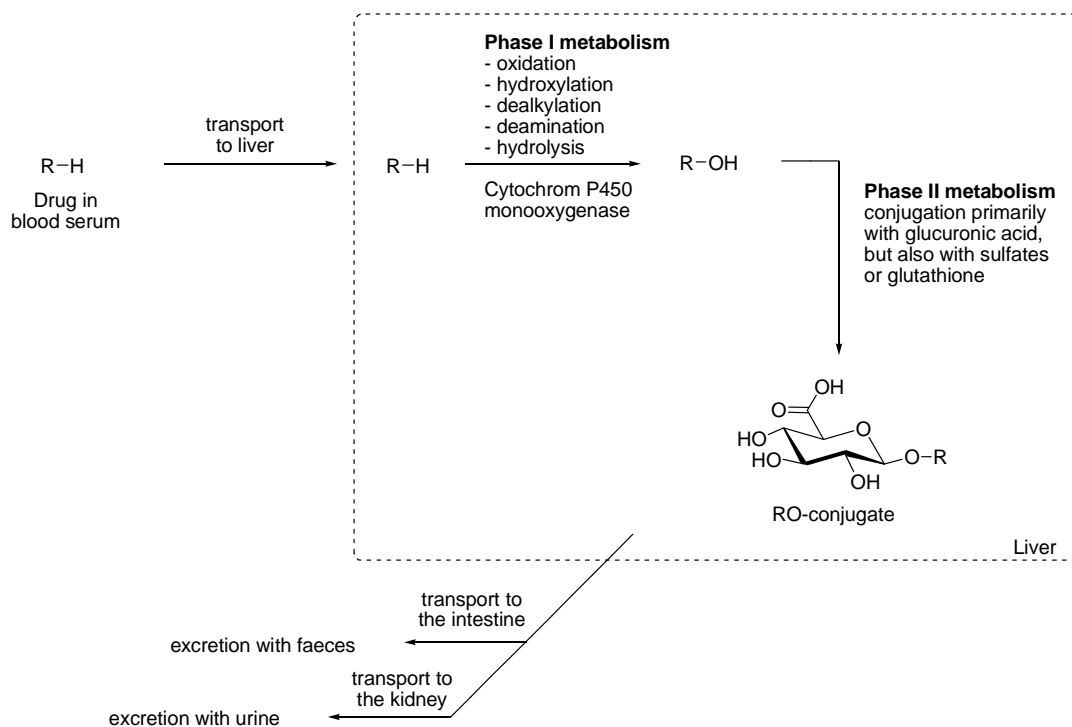
Figure 7. Phase I and II drug metabolism in the liver.[13,14]

Phase I metabolites are often more reactive and may be more toxic than their parent drugs, whereas the phase II metabolites are generally inactive.[13] The NSAIDs are extensively metabolized to their 1-β-*O*-acyl glucuronides (AGs),[379] but in contrast to the unreactive glucuronides of alcohol and phenolic drugs (**196**), the glucuronides of carboxylate drugs (**197**) are highly reactive metabolites (Scheme 73).[380] The increased reactivity of the AGs **197** as compared to their parent drugs enables reaction with nucleophiles, and such processes have the potential to covalently modify endogenous macromolecules like proteins or DNA (Scheme 73b). Hydrolysis to reform the parent drug and transacylation to produce **198** occur by direct displacement of glucuronic acid by attack of nucleophiles. Acyl migration producing 2-, 3- or 4-*O*-AGs (e.g. **199**), on the other hand, leads to various *N*-glycosylation adducts such as **200**.[381] 1-β-*O*-Acyl glucuronides are stable to acid, but under basic or neutral conditions, migration occurs to produce 2-, 3- or 4-*O*-AGs.[382,383] After migration, the acyl group is less prone to hydrolysis or transacylation, and stable 2-, 3- or 4-*O*-AGs can be isolated.[384] Both the transacylation and the glycosylation pathways are capable of covalently modifying hepatic and plasma proteins *in vivo*, however, the resulting modified proteins (**198** and **200**) have not been fully characterized.[381]
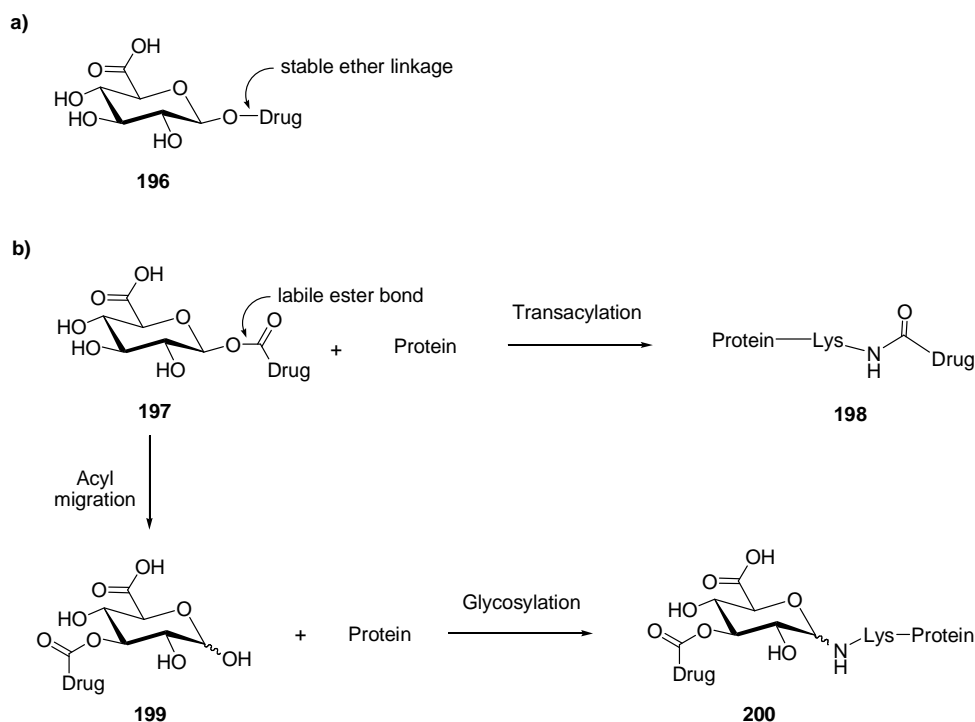
Scheme 73. Reactivity of glucuronides. a) Glucuronides of alcohol and phenolic drugs (**196**) are stable, whereas b) glucuronides of carboxylate drugs (**197**) may react directly with proteins *via* transacylation, or by initial acyl migration to produce 2-, 3- or 4-*O*-acyl glucuronides such as **199** (only shown for the 3 position), which can subsequently modifiy proteins by *N*-glycosylation. Evidence for the glycosylation pathway has been made by trapping experiments with a reducing agent like sodium cyanoborohydride.[381]

Around 25% of all drugs withdrawn due to severe toxicity are carboxylic acid containing drugs,[385-387] and one of the mechanisms underlying the observed toxicity of NSAIDs is believed to be the AG-derived covalent modification of endogenous proteins (Scheme 73b).[388] Such protein modification might be associated with immune responses and cellular dysfunction, and acyl glucuronides constitute a major concern in drug discovery and development.[381,388,389] Evidence for AG-induced toxicity is still lacking,[379,381,389,390] but it has been shown that reversible binding of other compounds (like diazepam or warfarin) to plasma protein is severely altered, when plasma protein is modified by AGs.[391]

AGs can be synthesized chemically in a number of ways. Classical approaches mainly rely on the coupling of fully protected glucuronic acids, which are typically produced in several steps from glucose[392,393] or levoglucosan,[394,395] to carboxylic acids under Mitsunobu conditions.[396-398] In 1997 Juteau and co-workers found that 6-*O*-allyl-D-glucuronate (**201**) could be conveniently coupled to carboxylic acids under Mitsunobu conditions without any other protective groups (Scheme 74),[399] however this strategy suffers from poor α/β-selectivity.[383,400]
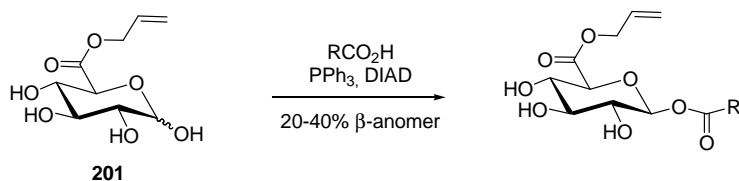
Scheme 74. Synthesis of AGs under Mitsunobu conditions.[399]

Very recently Stachulski and co-workers reported a selective acylation strategy, which exclusively produces acyl glucuronides as their β-anomers. In a three-step procedure HATU and NMM are used to couple partially protected glucuronic acids **202** to carboxylic acids to produce the desired AGs **203** in good yield (Scheme 75).[401,402]
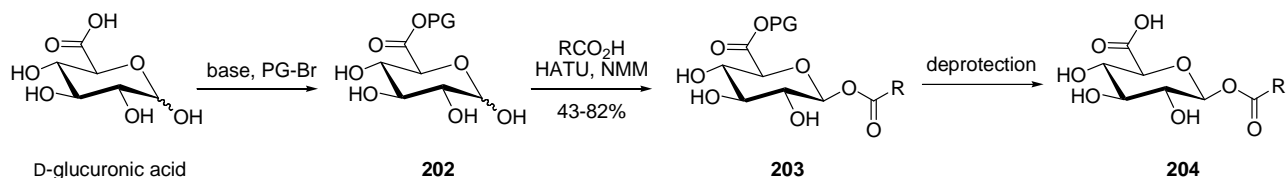
PG = allyl, Bn or PMB

Scheme 75. Synthesis of 1-β-*O*-acyl glucuronides by selective acylation of allyl, benzyl or *p*-methoxybenzyl protected glucuronic acid **202**.[401,402]

1-β-*O*-Acyl glucuronides **211**-**216** derived from *p*-bromobenzoic acid **205**, ponalrestat **206** and four ibuprofen analogues **207-210** (Figure 8) were available in small quantities from the Stachulski group in Liverpool *via* the above mentioned acylation strategy (Scheme 75). *p*-Bromobenzoic acid (**205**) resembles a model NSAID, whereas ponalrestat (**206**) is a known aldose reductase inhibitor, which has been used in the treatment of diabetes.[403-405] In contrast to (*R*)- and (*S*)-ibuprofen (**208** and **209**), which are used as an NSAID worldwide, ibufenac (**207**) was withdrawn from the U.K. market in 1968 due to hepatotoxicity.[406]
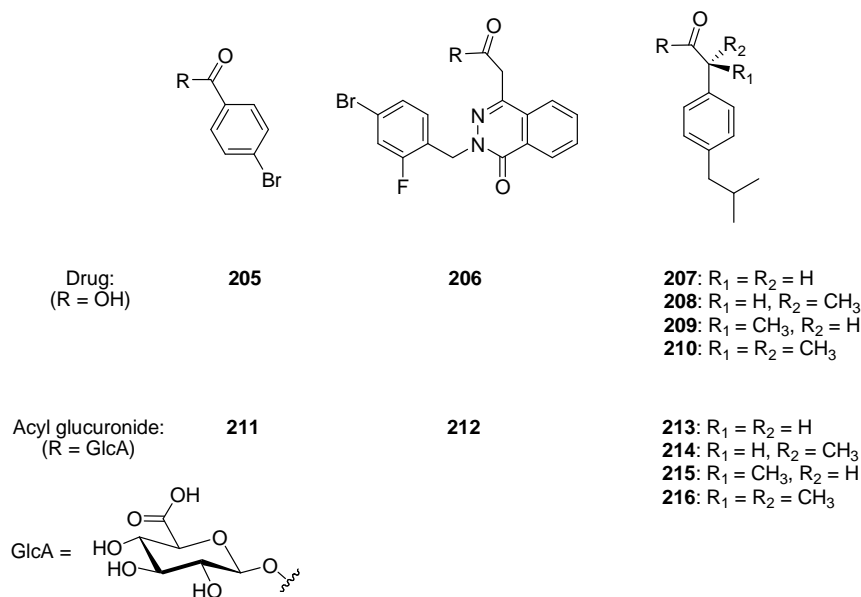
Figure 8. Available AGs (**211**-**216**) of carboxylate drugs and analogues (**205**-**210**) from the Stachulski group.

As a result of the potential toxicity of acyl glucuronides, the assessment of the degree of protein modification by NSAID-derived acyl glucuronides is important in both drug monitoring and development. The objective of the present project is to investigate, if such drug-derived acyl glucuronide metabolites modify plasma protein under conditions encountered in therapy.

6.2 Results and discussion

6.2.1 Purification of HSA

With plasma concentrations in the range of 30-50 g/L, human serum albumin (HSA) is the most abundant protein in blood plasma.[407,408] It is monomeric and consists of 585 amino acids of which 59 are lysines.[409-411] HSA has a molecular mass of 66438 Da and the protein is held together by 17 disulfides.[409,412] The protein is produced in the liver, and it has several different roles such as buffering of pH and transport of various compounds in blood plasma.[13,14] In addition to transport of drugs and other compounds in serum, HSA has been reported to participate in drug transport into the brain across the blood-brain barrier.[413,414] It has also shown esterase activity and has been found to convert acetyl salicylic acid into salicylic acid, thereby increasing the activity of the drug.[415]

The first objective of the project was to obtain HSA as a single protein species. Albumin isolated from humans contains several post-translational modifications of which glycosylation (lysines 199, 281, 439 and 525),[416-418] oxidation of Cys34 by binding to NO or a free cysteine, as well as loss of

Asp-Ala from the N-terminus and loss of Leu from the C-terminus are the most common.[412] MS analysis of commercial HSA isolated from humans revealed an approximately 1:1 mixture of unmodified HSA and Cys34-cysteine modified HSA.♦ Separation of these by preparative HPLC proved very difficult. Some purification could be achieved, but it was very dependant on the amount of protein injected, and in most cases no purification was obtained. Instead, purification of the protein was achieved by reduction of the Cys34-cysteine disulfide bond with 1,4-dithiothreitol (DTT).[419,420] The disulfides were reduced by treatment of commercial HSA with 13 mM DTT at pH 8. Excess reducing agent and free cysteine were removed by size-exclusion chromatography, and the protein was refolded[420] in atmospheric air in the presence of 0.02 mM DTT. Refolding to a different secondary structure than the native HSA structure is not believed to occur.[420] This purification process gave a single protein species contaminated with trace amounts of glycosylated and acetylated HSA. Attempts to perform the disulfide reduction on a large scale (500 mg) resulted in denaturation and precipitation of the protein, and only 5% of the reduced protein could be recovered. As a result, the DTT reduction was performed batch-wise on a smaller scale (50-100 mg) to minimize denaturation of the disulfide-reduced protein. Concentration of the protein solution was either achieved using Vivaspin or Amicon filtration. The latter method gave the best results, since protein material was lost by adhesion to the Vivaspin filter. When Amicon filtration was employed, more than 95% of the protein material could be recovered.

6.2.2 Methods for modification of lysines

The reactivity of acyl glucuronides is expected to be between an activated ester and an anhydride, and they are expected to react with amino groups of lysine residues by transacylation. Because of low availability of the acyl glucuronides **211**-**216**, model studies with other lysine modifying reagents were performed.

Several different techniques for modification of lysine residues exist. Some of the most widely used protein glycosylation methods are the direct reductive amination of an aldehyde and a lysine amino group with sodium cyanoborohydride,[421] and the reaction of 2-imino-2-methoxyethyl thioglycosides (IME reagents, **217**) with lysine amino groups.[422] The sequential attack of a primary amine and a lysine residue on diethyl squarate[423,424] as well as the reaction between a lysine amino group and acyl azides,[425] mixed anhydrides[426] or activated esters like *N*-hydroxysuccinimide esters

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♦ Protein solutions were analyzed by LC/MS and/or MALDI TOF MS (see section 9.2 for experimental details).

(NHS esters, **218**),[427] are all well-established methods for protein modification. Most of the above mentioned methods are applicable to protein glycosylation as well as modification with non-carbohydrate substrates, but they are all unselective and the lysine residues are more or less randomly modified. To be able to obtain uniformly modified protein species, the development of site-selective protein modification techniques (mainly relying on the presence of a single cysteine residue) has received increasing interest in recent years.[9,428-430]

Acyl glucuronides (**204**) are expected to react with lysine residues in a *non-controlled* manner, and the non-selective IME reagents **217** and NHS esters **218** were therefore chosen as model compounds to get a feel for the biochemical techniques before incubating the acyl glucuronides with HSA (Figure 9).
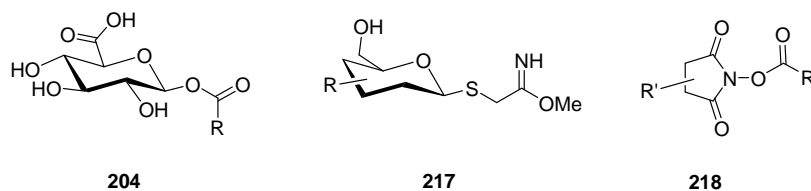
Figure 9. General structures of acyl glucuronides (**204**) and model compounds (**217** and **218**).

6.2.3 Modification of HSA with IME reagents

The IME reagents (**217**) were developed by Lee and co-workers[422,431] more than 20 years ago. Since then IME reagents have been widely used to couple sugars to proteins,[432,433] and the Davis group have shown their selectivity for lysine side chains on smaller peptides[434] and used them for virus modification[435] and drug delivery.[436]

It was chosen to synthesize the D-galactose (Gal-) and *N*-acetyl-D-glucosamine (GlcNAc-) IME reagents **223** and **229**, since Gal- and GlcNAc-modified proteins (the latter when converted into LacNAc) are suitable starting materials for enzymatic sialylation. Following the route developed by Stowell and Lee,[431] Gal- and GlcNAc-IME reagents **223** and **229** were synthesized in five steps according to Scheme 76 and Scheme 77, respectively.
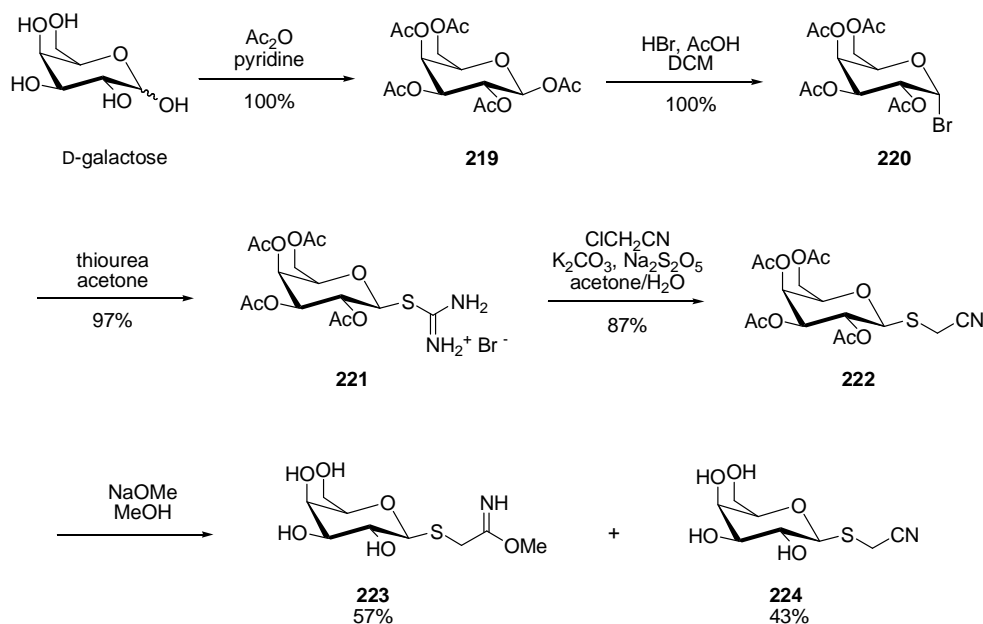
Scheme 76. Synthesis of Gal-IME (**223**) following the procedure developed by Stowell and Lee.[431]

Peracetylation of D-galactose, introduction of bromine at the anomeric position and subsequent displacement of bromine by thiourea proceeded in excellent yield to give **221**. Attachment of the cyanomethyl group occurred in 87% yield, and the final deprotection of **222** and nucleophilic attack by methoxide on the nitrile gave a 4:3 mixture of Gal-IME (**223**) and the corresponding deacetylated cyano-compound **224**. Gal-IME **223** was thereby synthesized in 48% overall yield, and the mixture of **223** and **224** was used directly for the coupling reactions with HSA.

Similarly, GlcNAc-IME **229** was synthesized in 19% overall yield (Scheme 77). The bromination of peracetylated *N*-acetyl-D-glucosamine (**225**) only occurred in 47% yield, which is probably due to loss of material during the workup, and a direct one-step acetylation and chlorination using acetylchloride might therefore be a more convenient approach. Displacement of bromine and introduction of the cyanomethyl group proceeded in good yield, and GlcNAc-IME **229** was formed as a 5:3 mixture with the corresponding deacetylated cyano-compound **230**.
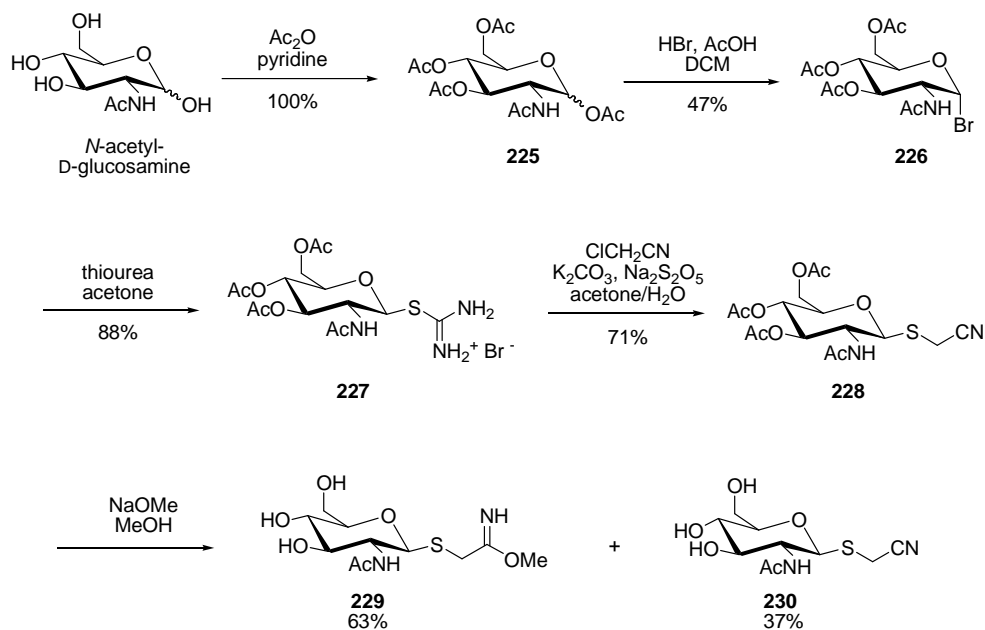
Scheme 77. Synthesis of GlcNAc-IME (**229**) following the procedure developed by Stowell and Lee.[431]

When IME reagents are used for protein modification, hydrolysis by water is a competing side reaction, and the choice of pH is important to avoid hydrolysis of the coupling reagent. With smaller peptides, it was found that IME reagents are most reactive at pH > 9,[434] and in order to remain close to physiological pH, HSA was incubated with the Gal- and GlcNAc-IME reagents **223** and **229** at pH 9 (Table 26).

Table 26. Modification of HSA with Gal- and GlcNAc-IME reagents **223** and **229**.
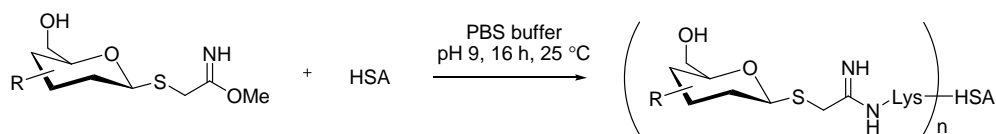
Eq. per lysine	c_{lysine} (mM)	Average number of modifications	
		Gal-IME	GlcNAc-IME
1	4	26.0	15.9
2.5	4	37.4	26.4
5	4	43.0	35.1
16	4	48.9	44.9
25	4	49.4	46.7
16	29	50.3	47.5

As can be seen in Figure 10, the degree of modification increases rapidly with the number of equivalents of IME reagent per lysine residue, and 45-50 lysines can easily be glycosylated by using a 16-25 fold excess of the IME reagent. However, with more than 45-50 modified lysines, the

conversion cannot be further improved by increasing the number of equivalents or the lysine concentration. The protein is probably very crowded, and the remaining lysine side chains are therefore less reactive due to decreased surface availability.
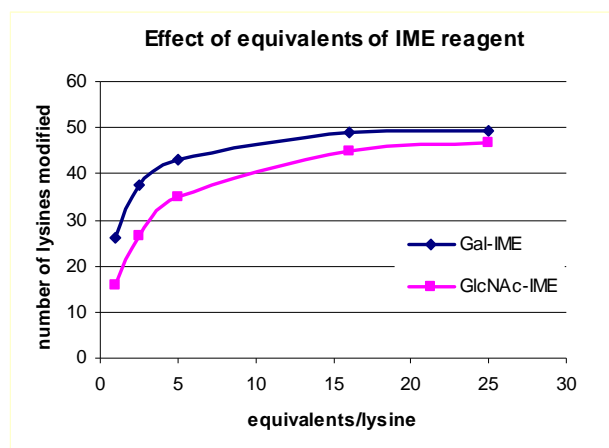
Figure 10. Degree of HSA-modification with Gal- and GlcNAc-IME reagents **223** and **229** at pH 9 by varying the number of equivalents (see Table 26 for further details).

After modification of HSA with the Gal-IME reagent **223**, it was attempted to sialylate the modified protein by the use of various enzymes. Glycoproteins containing a terminal sialic acid residue as well as polysialylated cell surfaces are found in several mammals including humans.[437,438] The protein bound, linear α(2,8)-polysialic acid polymer has been found to be up-regulated in leukemia and breast cancer cells,[439] and polysialylated cells are receiving increased attention as potential markers in cancer diagnostics.[440,441] As a result of this, the synthesis and structural investigation of polysialylated surfaces is an important research area.

Gal-modified HSA **231** (76.4 kDa, 42.5 galactose units on average) was incubated with CMP-sialic acid and α(2,3)-SiaT[442] at 37 ℃, but even though additional reagents were added, almost no conversion to **232** (approximately 5%) was achieved within 48 h (Scheme 78). The same result (<10% conversion) was obtained with two other sialylating enzymes, CstII and *Trypanosoma cruzi* (*T. cruzi*) transsialidase.[443,444] α(2,3)-SiaT and CstII employ CMP-sialic acid as the glycosyl donor, whereas *T. cruzi* transsialidase transfers sialic acid from one protein to another. Sialic acid is thereby transferred to the desired carbohydrate from fetuin, which is a protein covered with

tetrasaccharides in which sialic acid is the terminal residue.[444] The activity of both α(2,3)-SiaT and *T. cruzi* transsialidase was confirmed by simple sialylations of di- and tetrasaccharides in solution.♦
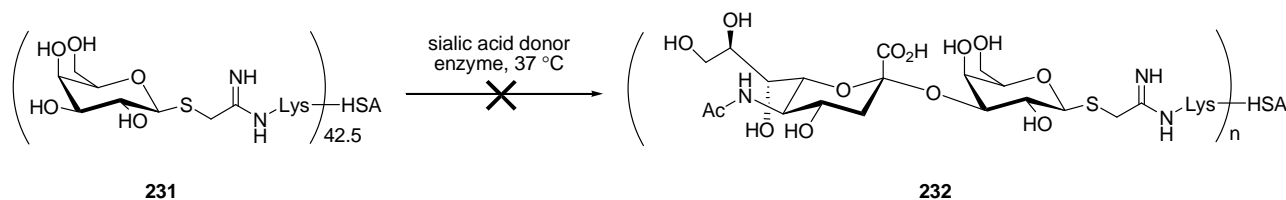
Scheme 78. Attempted enzymatic sialylation of Gal-modified HSA **231**. The enzymes α(2,3)-SiaT and CstII were used together with CMP-sialic acid, whereas *T. cruzi* transsialidase employed the protein fetuin as the sialic acid donor. Less than 10% conversion was achieved in each case.

α(2,3)-SiaT and other sialyl transferases are usually inhibited by the liberation of CMP, and alkaline phosphatase[442] can therefore be added to the reaction mixture to cleave CMP to phosphate and cytidine thereby pushing the reaction forward. Although no alkaline phosphatase was used in these enzymatic reactions, this is not the reason for the failed sialylations since the enzymes are usually not inhibited until a reasonable concentration of CMP is achieved. Because all three enzymes usually transfer sialic acid to LacNAc (as opposed to D-galactose), the low conversion might instead be explained by a high degree of substrate specificity. It may also simply be a matter of steric effects since the glycosyl acceptor is more flexible and available when it is bound to HSA as the *disaccharide* LacNAc.
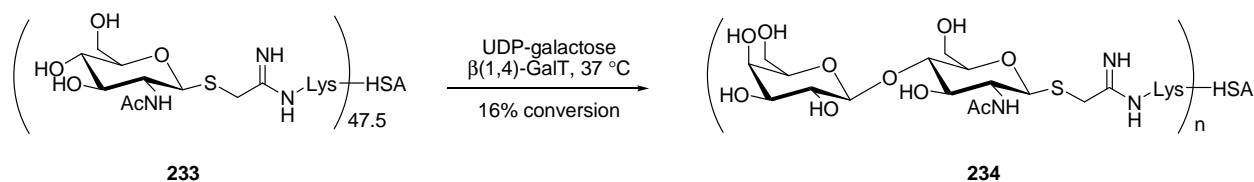
Scheme 79. Attempted enzymatic synthesis of LacNAc-modified HSA from GlcNAc-modified HSA. Only 16% conversion could be achieved.

Instead of coupling sialic acid to D-galactose, it was attempted to synthesize LacNAc-modified HSA **234** by attaching D-galactose to GlcNAc-modified HSA **233** (79.5 kDa, 47.5 *N*-acetyl-D-glucosamine units on average) by using β(1,4)-GalT[445] as shown in Scheme 79. The reaction was very slow, and addition of more β(1,4)-GalT as well as alkaline phosphatase to prevent UDP

♦ The activity assays of (2,3)-SiaT, *T. cruzi* transsialidase and β(1,4)-GalT (the latter will be discussed below) were performed by Wei-Chun Liu, Sarah Allman and Sung You Hong, respectively.

inhibiting the galactosyltransferase did not improve the conversion, and only 16% conversion could be achieved within 48 h. The enzymatic synthesis of LacNAc-modified HSA **234** and subsequent sialylation would have been attempted more thoroughly, but due to lack of time, the focus was concentrated on the acyl glucuronide incubations, which will be discussed in section 6.2.5.

6.2.4 Modification of HSA with NHS esters

N-Hydroxysuccinimide activated esters (NHS esters, **218**) are well-known to react with primary amines to produce amides with liberation of *N*-hydroxysuccinimide.[446] NHS esters react readily with lysine residues in peptides[447,448] and proteins,[427] and they constitute a simple and convenient way to form amides in aqueous solution at physiological pH. The reactivity of NHS esters is expected to be similar to acyl glucuronides (transacylation), and the NHS esters are therefore believed to be a better AG model system than the IME reagents (**217**). *p*-Bromobenzoic acid (**205**) was chosen as a model NSAID, and *p*-bromobenzoic acid NHS ester **236** was synthesized by a DCC coupling[446,449] between *N*-hydroxysuccinimide (**235**) and **205** in both DCM (81%) and DMF (94%) (Scheme 80). In DCM, column chromatography was required to remove the byproduct, dicyclohexylurea, whereas in DMF it could readily be removed by filtration.
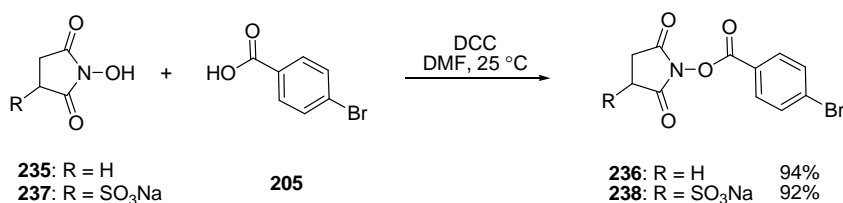
Scheme 80. Synthesis of NHS esters by DCC coupling.

Initial incubation of HSA with 50 equivalents of **236** per lysine showed that only a few lysines could be modified (Table 27, entries 1-5). As opposed to the IME reagents, a slightly acidic pH is optimal for NHS esters (Figure 11a). Hydrolysis of NHS esters is more facile under neutral or basic conditions, and as a result, no protein modification could be obtained at pH 9. In order to mimic physiological conditions (pH 7.4) and still get a reasonable degree of protein modification, pH 6 was chosen for the remaining experiments.

Table 27. Modification of HSA with **236** at 5.0 mM lysine concentration.
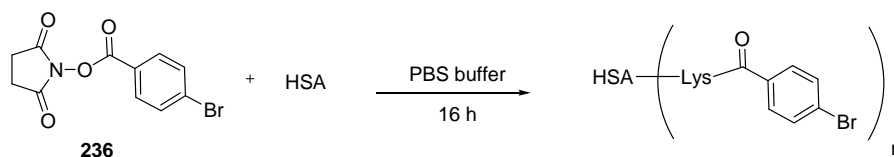
Entry	Eq. per lysine	pH	Temperature (°C)	Average number of modifications
1	50	9	25	0
2	50	7.4	25	2.6
3	50	6	25	4.3
4	50	5	25	6.5
5	50	4	25	5.5
6	0.02	6	25	0.6
7	1	6	25	2.0
8	16	6	25	3.3
9	1	6	37	3.0
10	16	6	37	4.3
11	50	6	37	10.7
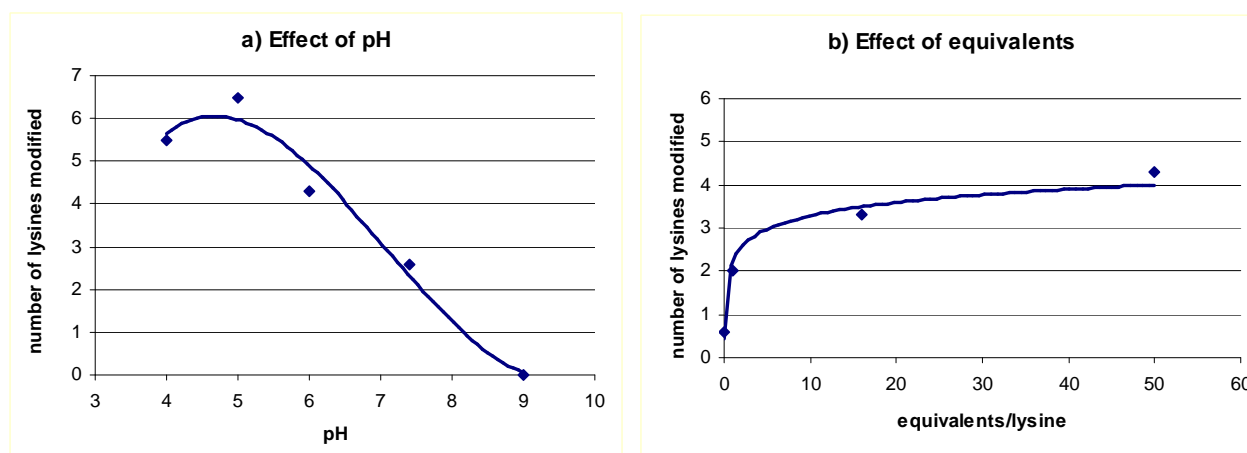
Figure 11. Degree of HSA-modification with **236** by varying a) the pH and b) the number of equivalents (see Table 27 for further details).

As depicted in Figure 11b, the conversion does not improve significantly by increasing the number of equivalents. The low conversion might be a result of low solubility of **236** in aqueous solution, and to improve the solubility, 10-20% of DMSO, MeCN and DMF were used as co-solvents. However, HSA only tolerates small amounts of organic solvents and precipitation of the protein was observed. Instead, it was decided to synthesize a more water soluble coupling reagent thereby mimicking the high polarity of the acyl glucuronides and at the same time avoiding the use of co-solvents. The sulfonated NHS ester **238** was therefore synthesized by a DCC coupling[448] in 92% yield (Scheme 80).

Due to a greater solubility in aqueous solution, the sulfonated NHS ester **238** is more reactive than **236**, and a high degree of modification can be achieved with relatively few equivalents (Table 28). The degree of modification increases almost linearly with increasing equivalents (Figure 12a),[♦] whereas a much smaller effect can be seen when varying the lysine concentration (Figure 12b).

Table 28. Modification of HSA with **238** at pH 6.
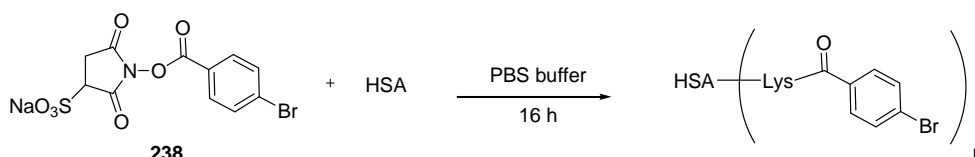
Entry	Eq. per lysine	c_{lysine} (mM)	Temperature (ºC)	Average number of modifications
1	0.02	5	25	1.4
2	0.17	5	25	7.7
3	0.51	5	25	22.0
4	1	5	25	33.4
5	50	5	25	precipitation
6	0.17	1	25	6.6
7	0.17	4	25	7.1
8	0.17	9	25	8.2
9	0.17	27	25	9.3
10	0.17	5	37	7.5
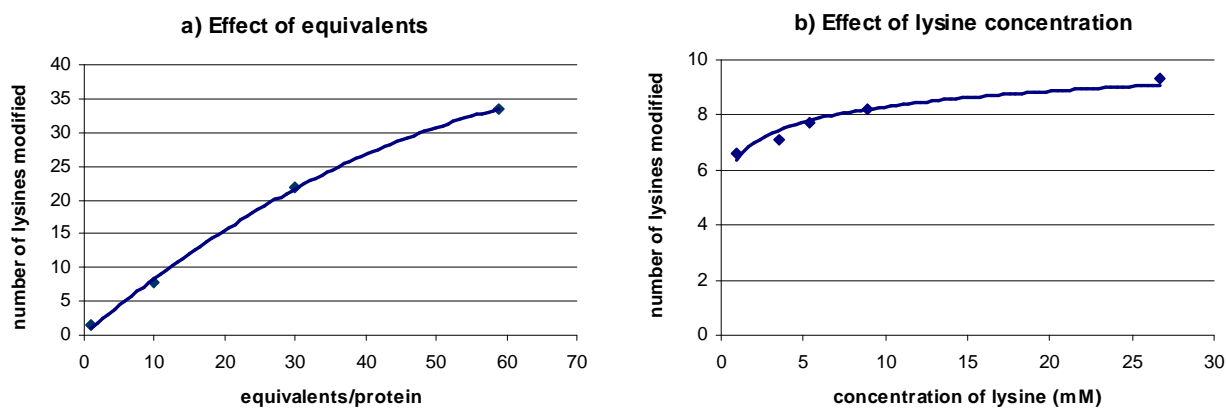
Figure 12. Degree of HSA-modification with **238** at pH 6 by varying a) the number of equivalents and b) the lysine concentrations (see Table 28 for further details).

With more than one equivalent of **238** per lysine (Table 28, entry 5), the modified protein precipitates out during the reaction presumably as a result of the increasing number of hydrophobic

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[♦] Note, the X-axis in Figure 12a has a different unit than in Figure 11b.

aromatic groups on the surface. The degree of protein modification with **236** and **238** seems to increase slightly when the temperature is increased from 25 to 37 ºC, however, the results are not unambiguous (compare Table 27, entries 7 and 9, 8 and 10, 3 and 11 and Table 28, entries 2 and 10).

To map the sites of modification, the modified HSA was digested with trypsin and the resulting tryptic peptides were analyzed by LC-coupled MS/MS analysis. Two lysines (Lys137 and Lys212) could be identified as selective sites for modification with **236** (see Appendix III for further details).

6.2.5 Incubation of HSA with acyl glucuronides

After the successful reaction of HSA with the sulfonated NHS ester **238** using a relatively low number of equivalents, the six drug-derived acyl glucuronides **211**-**216** (Figure 8) were incubated with HSA. The incubations of AGs with HSA were performed at 37 ºC in phosphate buffered saline at pH 7.4, and the concentration of HSA was chosen to be within the level of albumin in human serum (33 g/L).[407] AGs are generally thought to be rapidly excreted, but substantial plasma concentrations, sometimes exceeding their parent drug, are often found.[450,451] In order to mimic conditions encountered in therapy, an AG concentration of 0.5 mM resembling the peak plasma concentration of drug achieved in chronic cystic fibrosis patients treated with ibuprofen was used.[452] In addition, high-concentration experiments using 5.0 mM of AG were also performed. The results of the incubations of acyl glucuronides **211**-**216** with HSA are shown in Table 29 (see Scheme 73b for a generalized reaction scheme).

In the case of the acyl glucuronide of *p*-bromobenzoic acid (**211**), a *N*-glycosylation adduct was observed (together with unmodified HSA) at both 0.5 and 5.0 mM of AG in 58 and 59% conversion, respectively (Table 29, entries 1 and 2).♦ Incubation of D-glucuronic acid with HSA as a control experiment gave no conversion under identical conditions. Although HSA is *not* modified by D-glucuronic acid alone, acyl glucuronides are sufficiently activated to cause extensive protein modification as shown in Table 29.

♦ The observed mass of the glycosylation adduct of **211** and HSA (found 66798, expected 66797) could *in principle* be a result of double transacylation (expected 66804). However, this is very unlikely since no adducts corresponding to single or triple transacylation could be observed.

Previously, evidence for the formation of such *N*-glycosylation adducts from proteins and AGs have only been achieved by trapping the adducts with a reducing agent like sodium cyanoborohydride (reduction of the formed imine),[383,391,453] or at very high concentrations of the AG compared to the protein.[383,453,454] As a result, this is the first time a stable glycosylation adduct has been isolated *in vitro* under conditions encountered in therapy. The formation of stable glycosylation adducts in the absence of a reducing agent was surprising, but the high conversion even at 0.5 mM clearly indicates that irreversible glycosylation of endogenous proteins can occur during treatment with AG metabolizing drugs.

Table 29. Selectivity and conversion after incubation of HSA with AGs.

Entry	AG	Concentration (mM)	Selectivity (Transacylation/ Glycosylation)	Conversion (%)
1	**211**	0.5	<5:95	58
2	**211**	5.0	<5:95	59
3	**212**	0.5	55:45	4
4	**212**	5.0	72:28	24
5	**213**	0.5	60:40	16
6	**213**	5.0	61:39	44
7	**214**	0.5	64:36	5
8	**214**	5.0	63:37	23
9	**215**	0.5	44:56	9
10	**215**	5.0	55:45	24
11	**216**	0.5	-	0
12	**216**	5.0	34:66	8

Incubation of HSA (33 g/L) with AGs **211**-**216** for 16 h at 37 °C and pH 7.4. Selectivities between the transacylation and glycosylation products and conversions (% modified HSA) are based on peak heights in respective deconvoluted mass spectra.

As mentioned earlier, the acyl group of 2-, 3- or 4-*O*-acyl glucuronides is less prone to direct hydrolysis or displacement by nucleophiles as well as further migration due to the lack of a neighboring electron withdrawing ring oxygen.[388] Since the migration is catalyzed more readily by base than acid, it was attempted to perform the incubation of **211** with HSA at pH 6 to observe whether the acyl migration pathway was suppressed or not. As expected, the glycosylation was in fact inhibited, and no glycosylation occurred, however, no transacylation occurred either (0% conversion).
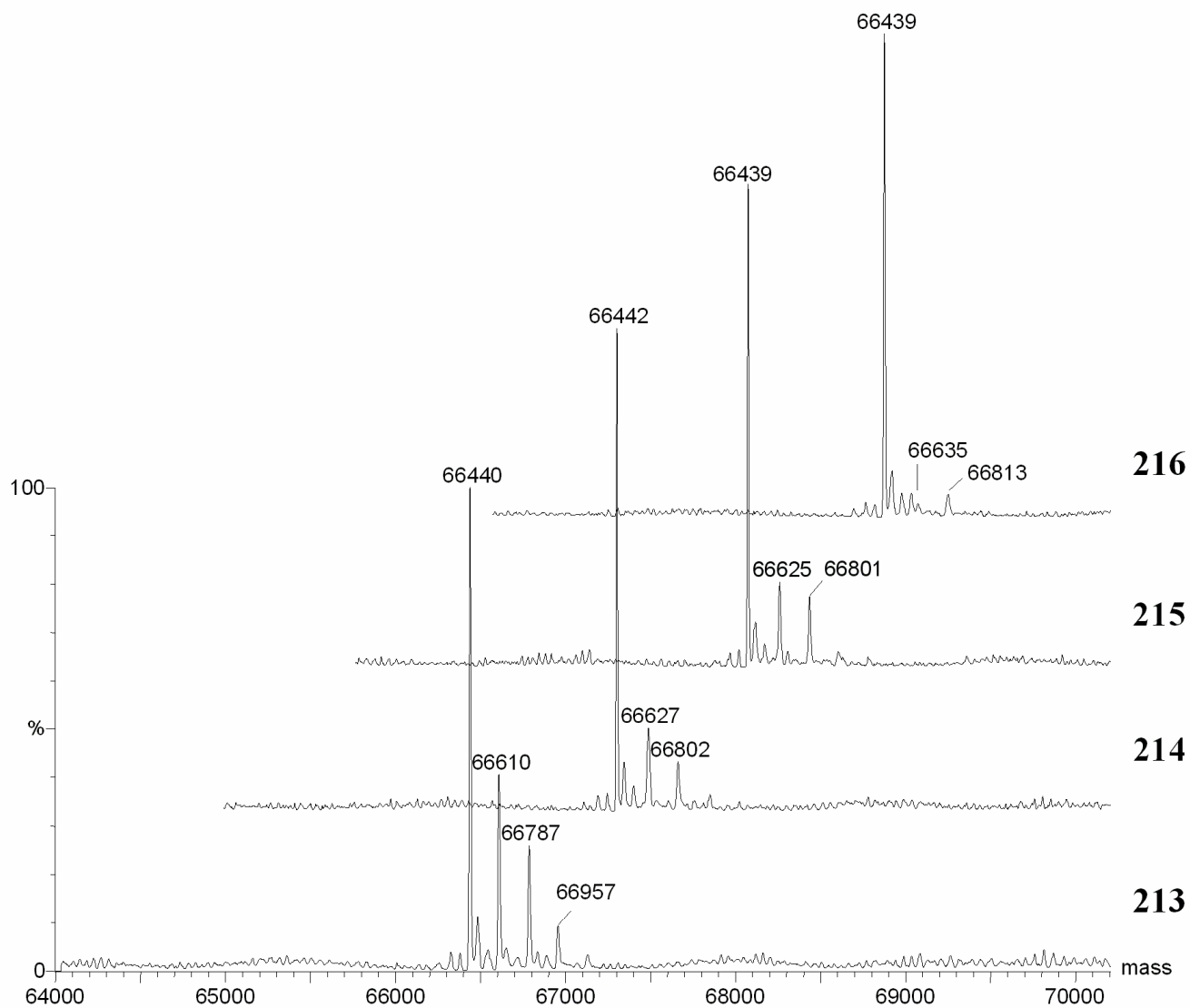
AG	Transacylation		Glycosylation	
	Found (Da)	Expected (Da)	Found (Da)	Expected (Da)
213	66610	66612	66787	66788
214	66627	66626	66802	66802
215	66625	66626	66801	66802
216	66635	66640	66813	66816

Figure 13. Mass spectra (LC/MS) of HSA (33 g/L) incubated with ibuprofen analoques **213**-**216** at 5.0 mM for 16 h at 37 ºC and pH 7.4. Unmodified HSA is the major peak in all cases (found 66440, 66442, 66439 and 66439 Da; expected 66438 Da).

With the acyl glucuronide of ponalrestat (**212**), only 5.0 mM AG concentration (Table 29, entry 4) led to formation of adducts (24% conversion). In this case both transacylation and glycosylation could be observed in a 72:28 ratio. The ibuprofen analogues (entries 5-12) also gave mixtures of transacylation and glycosylation adducts,♦ and the degree of modification can be visualized in the combined mass spectrum of **213-216** (Figure 13).

Ibufenac, (*R*)- and (*S*)-ibuprofen AGs (**213**-**215**) are reasonably reactive with conversions of 44, 23 and 24% respectively, whereas the dimethyl analogue **216** (only 8% conversion) is much less reactive (Table 29, entries 6, 8, 10 and 12). In addition to the individual transacylation and glycosylation adducts, small amounts of dual adducts (produced by simultaneous transacylation and glycosylation) could be observed for **212-215**. The type (transacylation or glycosylation) and site of modification was further analyzed by tryptic digest followed by MS/MS analysis (Table 30).

In the case of incubation of HSA with ibufenac AG **213**, seven different lysines were found to be modified (Table 30).♦♦ As expected from the data in Table 29, both transacylation (lysines 195, 436, 525 and 534) and glycosylation (lysines 137, 199, 205, 525) were observed. (For a sequence map of HSA, see Figure 14 in Appendix II). Lys525 is the only residue, which exhibits both transacylation and glycosylation reactivity. The dual reactivity of this residue is in accordance with previously reported results, stating that Lys525 is one of the major non-enzymatic glycosylation sites of HSA responsible for approximately 33% of overall non-enzymatic glycosylation *in vivo*.[416,417] In order to investigate the correlation between modification sites and accessibility, relative solvent accessibilities for each individual amino acid residue were calculated using the computer program Naccess.[455] However, a direct correlation between AG-modification site and calculated accessibility could not be found. (For a thorough discussion of surface accessibilities and site of modification, see Appendix II).

♦ The observed mass of the *N*-glycosylation adduct of ibufenac AG **213** (observed 66787, expected 66788) could *in principle* be a result of double transacylation (expected 66786). However, the observed dual reactivity of the structurally similar ponalrestat AG **212** (72:28 ratio) as well as the presence of *both* transacylation and *N*-glycosylation adducts in the MS/MS analysis of tryptic peptides from ibufenac-modified HSA (see Table 30) renders double transacylation in place of glycosylation extremely unlikely.
♦♦ Data for tryptic peptides of HSA modified with **211** and **212** can be found in Appendix III.

Table 30. Tryptic peptides of HSA modified with ibufenac AG **213**.

Retention time (min)	*m/z* predicted	*m/z* observed	Residues	Sequence	Modified lysine	Modification
33.49	704.1	704.1	137 – 144	**K**(+)YLYEIAR	K-137	Glycosylation
24.36	921.8	921.4	191 – 197	ASSA**K**(+)QR	K-195	Transacylation
27.80	621.6	621.6	198 – 205	L**K**(+)CASLQK	K-199	Glycosylation
27.80	621.6	621.6	198 – 205	LKCASLQ**K**(+)	K-205	Glycosylation
24.16	450.5	451.2	433 – 439	VGS**K**(+)CCK	K-436	Transacylation
22.07	652.7	651.4	525 – 534	**K**(+)QTALVELVK	K-525	Transacylation
18.86	740.7	741.1	525 – 534	**K**(+)QTALVELVK	K-525	Glycosylation
22.07	652.7	651.4	525 – 534	KQTALVELV**K**(+)	K-534	Transacylation

Modified protein was hydrolyzed with trypsin followed by LC-coupled MS/MS analysis. The amino acid sequence of HSA was derived from the RCSB protein data bank: DOI 10.2210/pdb1bm0/pdb.[411] Residues are numbered for this sequence of 585 amino acids.

Based on the results in Table 29, it can be concluded that in all cases except *p*-bromobenzoic acid AG **211**, the amount of adduct formed is considerably lower than unmodified HSA indicating a much lower reactivity of *alkyl* as compared to *aryl* AGs. Furthermore, both the nature and extent of protein modification with alkyl AGs are highly dependant on the degree of α-substitution. When the α-substitution is varied from unsubstituted (**212** and **213**) through monosubstituted (**214** and **215**) to disubstituted AGs (**216**), the preference for formation of transacylation adducts slowly changes to a preference for glycosylation. This is in fact a logical observation, because a higher degree of α-substitution constitutes an increased steric hindrance disfavoring direct displacement following the transacylation pathway. Acyl migration, on the other hand, is not affected by increased α-substitution to the same extent, and glycosylation adducts are therefore predominantly formed for highly α-substituted AGs like the dimethyl analogue **216** (Table 29, entry 12). In addition to the different type of reactivity (transacylation or glycosylation), alkyl AGs with no α-substitution are more reactive than mono- and disubstituted alkyl AGs, and AGs of drugs without α-substitution are therefore more likely to modify endogenous proteins *in vivo* than AGs of α-substituted drugs. The observed reactivity of alkyl AGs with respect to α-substitution is also reflected in their half-lives. Half-lives of less than 1 h for unsubstituted (zomepirac 0.45 h, diclofenac 0.51 h), 1-4 h for monosubstituted ((*S*)-naproxen 1.8 h, (*S*)-ibuprofen 3.3 h) and from 7 to more than 40 h for disubstituted (clofibric acid 7.3 h, gemfibril 44 h) alkyl AGs have previously been found.[381] As indicated by short half-lives (probenecid 0.40 h, diflunisal 0.67 h)[381] and supported by the reactivity of **211**, *aryl* AGs are very reactive with respect to protein modification, but they seem to be too hindered for transacylation to occur, and glycosylation adducts are therefore formed exclusively.

6.3 Concluding remarks

In the present study, HSA has initially been modified by the well-known IME reagents (**223** and **229**) and NHS esters (**236** and **238**). The dependency of pH, the number of equivalents of the coupling reagent, the lysine concentration and the temperature has been determined for the NHS esters. The techniques have been used to incubate 1-β-O-acyl glucuronides **211**-**216** with HSA to investigate the reactivity of phase II metabolites of NSAIDs with respect to potential modification of plasma protein in humans. For the first time, it has been found that aryl 1-β-O-acyl glucuronides form stable glycosylation adducts with HSA at conditions encountered in therapy. In contrast to aryl AGs, *alkyl* AGs, which are slightly less reactive, exhibit both transacylation and glycosylation reactivity. A rationalization of AG-reactivity based on structure has been established, and this may become a useful tool for development of new carboxylic acid containing drugs as well as in monitoring and assessment of potential toxicity of existing drugs. The increased reactivity of ibufenac AG **213** compared to (R)- and (S)-ibuprofen AGs **214** and **215** due to the lacking α-methyl substituent is striking, when keeping in mind that hepatotoxicity led to withdrawal of ibufenac (**207**) from the U.K. market 40 years ago and (R)- and (S)-ibuprofen (**208** and **209**) are still extensively used. The current work strongly supports that AG-induced modification of plasma protein during treatment with carboxylic acid containing drugs is likely to take place thereby potentially initiating adverse drug reactions.

7 General concluding remarks

The importance of carbohydrates in glycobiology, as chiral building blocks in organic chemistry, and as renewable resources cannot be over-emphasized. The investigation of their roles in biological processes and the development of new synthetic methodologies, which can be applied to carbohydrates are therefore key research areas for understanding and utilizing nature's most abundant biomolecules in chemistry, biology and medicine. The present research has dealt with different areas of carbohydrate chemistry mainly focusing on the development and application of transition metal mediated transformations for shortening and extending the carbohydrate chain.

A new rhodium-catalyzed procedure for shortening unprotected aldoses by one carbon atom has been developed. Aldoses are decarbonylated to their corresponding shortened alditols by employing Rh(dppp)$_2$Cl as the catalyst. The developed catalytic decarbonylation reaction is an improvement of a previously reported stoichiometric procedure, and it constitutes an interesting alternative to classical methods for shortening unprotected carbohydrates. The general applicability of the procedure has been illustrated by a concise synthesis of the unnatural sugar L-threose in 74% overall yield in five steps from D-glucose. At present, the method is limited to aldoses, and more challenging substrates like uronic acid lactones and unprotected cyclodextrins led to disappointing results. Initially, the rhodium-catalyzed decarbonylation of carbohydrates was envisioned to be potentially relevant to the production of hydrogen from renewable resources, but the catalyst is not yet efficient enough for such applications.

Carbocyclic natural products often possess interesting biological activities, which make them potential pharmaceutical lead compounds. A zinc-mediated fragmentation-allylation sequence has been used to elongate D-glucose and D-ribose by three carbon atoms, and by subsequent ring-closing metathesis, they have been converted into the carbocyclic natural products calystegine A$_3$ and gabosine A. The synthesis of the glycosidase inhibitor calystegine A$_3$ constitutes the shortest enantioselective route to the natural product, and it firmly establishes the absolute configuration of naturally occurring calystegine A$_3$. The synthetic route to gabosine A represents the first synthesis from a carbohydrate precursor. Together with previous syntheses of carbocyclic natural products developed in the group, the present syntheses of calystegine A$_3$ and gabosine A illustrate that such compounds are conveniently synthesized from carbohydrates.

During an external stay at University of Oxford, the metabolism of NSAIDs has been investigated. Carboxylate drugs like NSAIDs are extensively metabolized to 1-β-O-acyl glucuronides, and it has been found that such metabolites are highly reactive and may lead to potentially toxic protein adducts in man. By incubation of human serum albumin with acyl glucuronides of several ibuprofen analogues, it has been found that transacylation and glycosylation adducts are formed. The product ratios and the degree of protein modification can be rationalized by the degree of α-substitution in the acyl group, and this information may provide a useful tool in the monitoring, interpretation and assessment of the potential toxicity of existing carboxylic acid containing drugs.

8 Experimental work performed at DTU

8.1 General experimental methods

Diglyme, DMA and pyridine were distilled before use. DCM was dried by distillation from CaH_2 while MeOH and DMF were dried over 4 Å molecular sieves. All reagents were obtained from commercial sources and used without further purification. All sonications were performed in a Branson 1210 sonic bath. For reproducibility, sonications were performed in flat-bottomed conical flasks using 2% liquid detergent in water as bath media.[456] Reactions were monitored by TLC using aluminum plates precoated with 0.25 mm silica gel 60. Compounds were visualized by dipping in a solution of $(NH_4)_6Mo_7O_{24} \bullet 4H_2O$ (25 g/L) and $Ce(SO_4)_2$ (10 g/L) in 10% aqueous H_2SO_4 followed by heating. Flash column chromatography was performed on silica gel 60 (particle size 0.035 – 0.070 mm). Machery-Nagel Silica gel 60 C_{18} (particle size 0.055 – 0.105 mm) was used for reverse phase column chromatography. NMR spectra were recorded on a Varian Mercury 300 instrument. Chemical shifts (δ) are given in ppm employing residual solvent peaks as internal references according to literature.[457] High resolution mass spectra were recorded at the Department of Physics and Chemistry, University of Southern Denmark. Microanalyses were performed at the Mikroanalytisches Laboratorium at Universität Wien. Optical rotations were measured on a Perkin-Elmer 241 polarimeter while IR spectra were recorded on a Bruker Alpha FT-IR spectrometer. IR absorptions are given in cm^{-1}. Melting points are uncorrected.

8.2 Compounds referred to in chapter 3

Rhodium(I)bis(cyclooctene)chloride

Following a literature procedure,[254] degassed solutions of 2-propanol (50 mL), H_2O (12 mL) and cyclooctene (8.6 mL, 67 mmol) were added to $RhCl_3 \bullet 3H_2O$ (2.52 g, 9.55 mmol) under an argon atmosphere. The suspension was stirred at room temperature for 20 min and then left without stirring for 15 days under an argon atmosphere. The reaction mixture was filtered, and the crystals were washed with EtOH (150 mL) and dried *in vacuo* to give orange-red crystals (2.95 g). Additional crystals (0.19 g) could be obtained by further crystallization of the remaining liquids

followed by filtration, washing with EtOH and drying *in vacuo*. This gave Rh(COE)$_2$Cl (3.14 g, 92%) as orange-red crystals. IR (KBr): 3448, 3000, 2977, 2920, 2845, 1465, 1448, 1355, 952, 899.

Rhodium(I)bis(1,3-diphenylphosphinopropane)chloride

Following a modified literature procedure,[253] a suspension of Rh(COE)$_2$Cl (2.95 g, 8.2 mmol) and dppp (7.80 g, 18.9 mmol) in freshly distilled toluene (150 mL) was thoroughly degassed under high vacuum and then stirred under an argon atmosphere at reflux for 1.5 h. The solution was cooled to room temperature, and the reaction mixture was filtered. The solids were washed with toluene (150 mL) and dried *in vacuo* to give Rh(dppp)$_2$Cl (7.83 g, 99%) as yellow crystals. IR (KBr): 3410, 3047, 2909, 2848, 1477, 1433, 1095, 743, 700, 652.

Rhodium(I)bis(BINAP)chloride

A suspension of Rh(COE)$_2$Cl (108 mg, 0.30 mmol) and *rac*-BINAP (467 mg, 0.75 mmol) in freshly distilled toluene (10 mL) was thoroughly degassed under high vacuum and then stirred under a nitrogen atmosphere at reflux for 1 h. The solution was cooled to room temperature, and the reaction mixture was filtered. The solids were washed with toluene (20 mL) and dried *in vacuo* to give Rh(BINAP)$_2$Cl (343 mg, 82%) as pale, brown crystals. IR (KBr): 3447, 3050, 1481, 1434, 1116, 818, 740, 695.

General procedures for decarbonylation of unprotected aldoses

General decarbonylation procedure A: To the aldose (400-650 mg, 2.78 mmol) were added Rh(dppp)$_2$Cl (267 mg, 0.28 mmol), DMA (3 mL) and diglyme (20 mL). The mixture was thoroughly degassed under high vacuum and then stirred at reflux (162 ºC) under a nitrogen atmosphere until TLC showed full conversion to the corresponding alditol (8-16 h). The solution was cooled to room temperature followed by addition of H$_2$O (50 mL). The mixture was washed with DCM (4 × 50 mL) and the combined organic phases were extracted with H$_2$O (2 × 10 mL). The combined aqueous phases were concentrated and the residue co-concentrated with EtOH. The resulting residue was purified by either flash column chromatography (DCM/MeOH/H$_2$O = 4:1:0 – 65:25:4) or reverse phase column chromatography (H$_2$O).

General decarbonylation procedure B: To the aldose (400-650 mg, 2.78 mmol) were added Rh(dppp)$_2$Cl (214 mg, 0.22 mmol), DMA (3 mL), diglyme (20 mL) and freshly distilled pyridine (14.5 µL, 0.18 mmol). The mixture was thoroughly degassed under high vacuum and then stirred at reflux (162 ºC) under a nitrogen atmosphere until TLC showed full conversion to the corresponding alditol (6-15 h). The solution was cooled to room temperature and the product was isolated as described in general decarbonylation procedure A.

General procedure for measurement of CO evolution: A flask containing aldose (2.78 mmol), Rh(dppp)$_2$Cl (0.14 – 0.28 mmol), pyridine (0 – 0.14 mmol), diglyme (20 mL) and DMA (3 mL) was equipped with a condenser connected to a burette filled with water. The bottom of the burette was further connected to a water reservoir with a large surface area. A three-way valve was placed between the condenser and the burette enabling evacuation and purge with nitrogen. The reaction mixture was thoroughly degassed and then stirred at reflux (162 ºC) in a preheated oil bath. The measurement of developing carbon monoxide was started 15 min after the reaction flask was placed in the oil bath. The amount of carbon monoxide produced was measured as the amount of water depleted from the burette. At room temperature (25 ºC), full conversion of the aldose corresponds to formation of 68 mL of carbon monoxide.

Attempted decarbonylation of D-glucose using *in situ* generated Rh(dppp)$_2$Cl

Following the literature procedure for decarbonylation of simple aldehydes,[236] a mixture of RhCl$_3$ • 3H$_2$O (15 mg, 0.06 mmol), dppp (47-59 mg, 0.12-0.15 mmol), diglyme (10 mL) and phenylacetaldehyde (14 µL, 0.12 mmol) was thoroughly degassed under high vacuum and heated to reflux (162 ºC). The solution quickly turned yellow and homogeneous. A degassed solution of D-glucose (100 mg, 0.56 mmol) in DMA (1 mL) was added to the reaction mixture after 20 min at reflux. The color of the reaction mixture slowly changed from yellow-orange to black. The reaction was followed by TLC analysis but only traces of D-arabinitol were formed within 16 h.

Decarbonylation of D-glucose using *crude* Rh(dppp)$_2$Cl[239]

RhCl$_3$ • 3H$_2$O (73 mg, 0.28 mmol), dppp (229 mg, 0.56 mmol) and EtOH (96 % aq., 12 mL) were mixed, and the solution was thoroughly degassed under high vacuum and then stirred at reflux under a nitrogen atmosphere for 30 min. The reaction mixture was cooled to room temperature and the solvent was removed under high vacuum to give fine yellow-orange crystals. IR (KBr): 3448,

3052, 2917, 2168 (weak), 1483, 1434, 1097, 742, 696, 647. The crude Rh(dppp)$_2$Cl was used directly for decarbonylation of D-glucose. To the crude product were added phenylacetaldehyde (46 μL, 0.42 mmol) and diglyme (20 mL), and the solution was thoroughly degassed under high vacuum. The solution was heated to reflux under a nitrogen atmosphere and after 20 min, a degassed solution of D-glucose (500 mg, 2.78 mmol) dissolved in DMA (3 mL) was added. After 3 h at reflux, the reaction mixture was cooled to room temperature, diluted with H$_2$O (20 mL), and washed with DCM (5 x 30 mL). The aqueous phase was co-concentrated with EtOH, and the residue was purified by flash column chromatography (DCM/MeOH/H$_2$O = 65:25:4) to give D-arabinitol (39 mg, 9%) as a colorless oil. Spectroscopic data are given below.

When crude Rh(dppp)$_2$Cl was used for decarbonylation of benzaldehyde according to the procedure developed by Esben Taarning,[239] a crystalline metal complex could be isolated after the reaction and an IR-spectrum of the crystals was recorded. IR (KBr): 3421, 3043, 2938, 2101 (strong), 1971 (strong), 1718 (strong), 1434, 1270, 1097, 752, 703.

D-Arabinitol
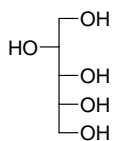
White crystals. R_f 0.49 (acetone/BuOH/H$_2$O = 5:4:1). $[\alpha]_D^{22}$ –10.3 (c 0.2, MeOH) (lit.[458] $[\alpha]_D^{19}$ –12 (c 1, MeOH)). mp 98 – 99 °C (MeOH) (lit.[459] mp 101 – 102 °C (EtOH)). ^{1}H NMR (300 MHz, D$_2$O): δ 3.86 (ddd, J = 2.0, 5.3, 7.3 Hz, 1H), 3.77 (dd, J = 2.7, 11.5 Hz, 1H), 3.68 (ddd, J = 2.7, 6.2, 8.8 Hz, 1H), 3.58 (m, 3H), 3.50 (dd, J = 2.0, 8.3 Hz, 1H). ^{13}C NMR (75 MHz, D$_2$O): δ 72.3, 71.8, 71.7, 64.5, 64.4. NMR data are in accordance with literature values.[460,461] Anal. calcd for C$_5$H$_{12}$O$_5$: C, 39.47; H, 7.95. Found: C, 39.55; H, 7.65.

1,4-Anhydro-D-arabinitol (95) and 2,3,5-tri-O-acetyl-1,4-anhydro-D-arabinitol (239)
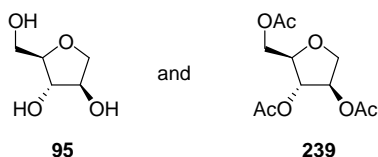
1,4-Anhydro-D-arabinitol (95) was isolated as a byproduct from decarbonylation of D-glucose or D-mannose as a slightly yellow oil. R_f 0.68 (acetone/BuOH/H$_2$O = 5:4:1). ^{13}C NMR (75 MHz, D$_2$O): δ

86.5, 78.9, 77.8, 73.8, 62.6. ^{13}C NMR data are in accordance with literature values.[461] The anhydro compound **95** was peracetylated[462] by addition of freshly distilled pyridine, acetic anhydride and a catalytic amount of DMAP followed by stirring at room temperature under an argon atmosphere overnight. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (EtOAc/heptane = 1:9 – 1:4) to give **239** as a slightly yellow oil. R_f 0.14 (EtOAc/heptane = 1:4). $[\alpha]_D^{25}$ +13.7 (*c* 0.5, MeOH). ^{1}H NMR (300 MHz, D$_2$O): δ 5.27-5.24 (m, 1H), 5.08 (d, *J* = 3.7 Hz, 1H), 4.40-4.06 (m, 5H), 2.12 (s, 3H), 2.11 (s, 6H). ^{13}C NMR (75 MHz, D$_2$O): δ 174.0, 173.1, 173.1, 81.5, 78.1, 77.9, 71.6, 63.6, 20.5, 20.3, 20.3. NMR data are in accordance with literature values.[463] Anal. calcd for C$_{11}$H$_{16}$O$_7$: C, 50.77; H, 6.20. Found: C, 50.59; H, 6.34.

1,4-Anhydro-D-lyxitol (96) and D-galactono-1,4-lactone (97)
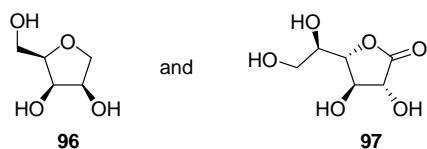
An inseparable 3:4 mixture of **96** and **97** was isolated in 34% combined yield as byproducts from decarbonylation of D-galactose. R_f 0.68 (acetone/BuOH/H$_2$O = 5:4:1). ^{13}C NMR (75 MHz, D$_2$O): **96**: δ 81.6, 72.1, 72.1, 71.8, 61.3; **97**: δ 172.7, 80.5, 74.9, 73.8, 69.6, 62.9. ^{13}C NMR data are in accordance with literature values.[461]

Erythritol
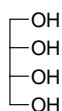
White crystals. R_f 0.47 (acetone/BuOH/H$_2$O = 5:4:1). mp 116 – 117 °C (MeOH/heptane) (lit.[464] mp 120 – 121 °C). ^{1}H NMR (300 MHz, D$_2$O): δ 3.74-3.65 (m, 2H), 3.62-3.49 (m, 4H). ^{13}C NMR (75 MHz, D$_2$O): δ 73.3, 64.0. ^{13}C NMR data are in accordance with literature values.[461] Anal. calcd for C$_4$H$_{10}$O$_4$: C, 39.34; H, 8.25. Found: C, 39.05; H, 8.00.

D-Threitol
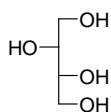
White crystals. R_f 0.52 (acetone/BuOH/H_2O = 5:4:1). $[\alpha]_D^{22}$ –7.5 (c 0.5, MeOH) (lit.[465] $[\alpha]_D^{23}$ –7.0 (c 0.9, MeOH)). mp 89 – 91 °C (MeOH) (lit.[466] mp 90 – 91 °C (BuOH)). ^{1}H NMR (300 MHz, D_2O): δ 3.69-3.51 (m, 6H). ^{13}C NMR (75 MHz, D_2O): δ 72.9, 63.9. NMR data are in accordance with literature values.[460,461] Anal. calcd for $C_4H_{10}O_4$: C, 39.34; H, 8.25. Found: C, 39.19; H, 8.05.

1,4-Anhydro-D-threitol (98) and 2,3-di-*O*-benzoyl-1,4-anhydro-D-threitol (240)
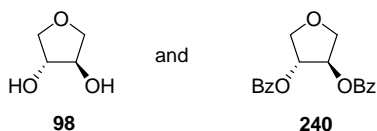
1,4-Anhydro-D-threitol (**98**) was isolated as a byproduct from decarbonylation of D-xylose as a slightly yellow oil. R_f 0.76 (acetone/BuOH/H_2O = 5:4:1), ^{1}H NMR (300 MHz, D_2O): δ 4.24 (d, J = 3.5 Hz, 2H), 4.00 (dd, J = 3.5, 10.5 Hz, 2H), 3.74 (d, J = 10.4 Hz, 2H). ^{13}C NMR (75 MHz, D_2O): δ 77.2, 73.8. ^{13}C NMR data are in accordance with literature values.[461] The anhydro compound **98** was cooled to 0 °C and perbenzoylated by addition of freshly distilled pyridine and benzoylchloride followed by stirring at room temperature under an argon atmosphere overnight. The reaction was quenched by addition of EtOH, diluted with DCM and washed with sat. aq. $NaHCO_3$ and brine. The organic phase was dried (MgSO$_4$), filtered and concentrated *in vacuo* to give a residue, which was purified by flash column chromatography (EtOAc/heptane = 1:9 → 2:8) to afford **240** as a white crystalline material. R_f 0.31 (EtOAc/heptane = 1:4). ^{1}H NMR (300 MHz, CD$_3$OD): δ 8.06-7.98 (m, 4H), 7.58-7.48 (m, 2H), 7.46-7.36 (m, 4H), 5.54 (d, J = 4.2 Hz, 2H), 4.27 (dd, J = 4.3, 10.6 Hz, 2H), 3.97 (d, J = 10.7 Hz, 2H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 166.9, 134.6, 131.7, 129.6, 79.2, 72.8. *m/z* (ESI$^+$) $C_{18}H_{16}O_5$ 190 ([M – $C_6H_5CO_2H$]).

5-Deoxy-L-arabinitol
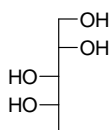
Colorless syrup. R_f 0.68 (acetone/BuOH/H_2O = 5:4:1). $[\alpha]_D^{22}$ +11.7 (c 3.8, MeOH). $[\alpha]_D^{22}$ +13.1 (c 0.5, H_2O) (reported for the enantiomer[467] $[\alpha]_D^{23}$ –10.6 (H_2O)). ^{1}H NMR (300 MHz, D_2O): δ 3.85-

3.74 (m, 2H), 3.64-3.53 (m, 2H), 3.38-3.28 (m, 1H), 1.17 (d, J = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, D_2O): δ 74.6, 70.7, 67.1, 63.1, 18.3. NMR data are in accordance with literature values.[249] Anal. calcd for $C_5H_{12}O_4$: C, 44.11; H, 8.88. Found: C, 43.85; H, 8.59.

1,4-Anhydro-5-deoxy-L-arabinitol (99)

and 2,3-di-*O*-acetyl-1,4-anhydro-5-deoxy-L-arabinitol (241)
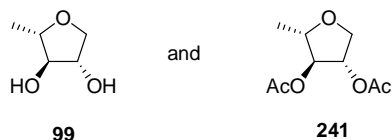
1,4-Anhydro-5-deoxy-L-arabinitol (**99**) was formed as a byproduct from decarbonylation of L-rhamnose. R_f 0.76 (acetone/BuOH/H_2O = 5:4:1). ^{13}C NMR (75 MHz, D_2O): δ 82.6, 81.6, 77.6, 72.7, 18.1. The anhydro compound **99** was peracetylated[462] by addition of freshly distilled pyridine, acetic anhydride and a catalytic amount of DMAP followed by stirring at room temperature under an argon atmosphere overnight. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (EtOAc/heptane = 1:9 – 1:4) to afford **241** as a colorless oil. R_f 0.17 (EtOAc/heptane = 1:4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.13 (dt, J = 1.6, 4.4 Hz, 1H), 4.85 (d, J = 3.4 Hz, 1H), 4.05-3.84 (s, 3H), 2.09 (s, 6H), 1.38 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.4, 170.2, 82.4, 80.2, 78.7, 71.8, 21.1, 21.0, 18.6. HRMS calcd for $C_9H_{14}O_5Na$ [M + Na]$^+$ *m/z* 225.0739, found *m/z* 225.0733.

1-Acetylamino-1-deoxy-D-arabinitol (100)
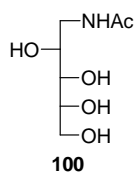
White crystals. R_f 0.49 (acetone/BuOH/H_2O = 5:4:1). $[\alpha]_D^{22}$ +23.5 (*c* 0.5, H_2O) (lit.[468] $[\alpha]_D^{23}$ +23 (H_2O)). mp 142 – 143 °C (MeOH) (lit.[468] mp 146.5 – 147.5 °C). ^{1}H NMR (300 MHz, D_2O): δ 3.81 (t, J = 6.7 Hz, 1H), 3.67 (dd, J = 2.6, 11.4 Hz, 1H), 3.57 (ddd, J = 4.4, 7.8, 7.7 Hz, 1H), 3.48 (dd, J = 6.2, 11.5 Hz, 1H), 3.32 (d, J = 8.5 Hz, 1H), 3.25-3.11 (m, 2H), 1.85 (s, 3H). ^{13}C NMR (75 MHz, D_2O): δ 175.4, 71.7, 71.7, 69.1, 63.9, 43.3, 22.8. NMR data are in accordance with literature values.[249] Anal. calcd for $C_7H_{15}NO_5$: C, 43.52; H, 7.83; N, 7.25. Found: C, 43.67; H, 7.56; N, 7.18.

D-Lyxono-1,4-lactone (102)

102
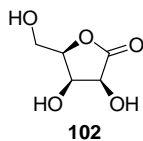
Following general decarbonylation procedure A, D-glucurono-6,3-lactone (**101**) (489 mg, 2.78 mmol) was decarbonylated. The solution turned black within 45 min. Based on TLC analysis and the amount of carbon monoxide developed, full conversion was achieved within 2.5 h. Workup and flash column chromatography (DCM/MeOH = 4:1) gave **102** (58 mg, 14%) as a colorless oil. R_f 0.75 (acetone/BuOH/H$_2$O = 5:4:1). ^{1}H NMR (300 MHz, D$_2$O): δ 4.72-4.68 (m, 1H), 4.62-4.56 (m, 1H), 4.55-4.51 (m, 1H), 3.90-3.85 (m, 2H). ^{13}C NMR (75 MHz, D$_2$O): δ 179.0, 82.4, 71.3, 70.3, 60.6. ^{13}C NMR data are in accordance with literature values.[461]

L-Gulono-1,4-lactone (103)

103
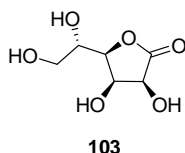
L-Gulono-1,4-lactone (**103**) was isolated as a byproduct from decarbonylation of D-glucurono-6,3-lactone (**101**) (25 mg, 5%) as a colorless oil. R_f 0.66 (acetone/BuOH/H$_2$O = 5:4:1). ^{1}H NMR (300 MHz, D$_2$O): δ 4.58-4.50 (m, 1H), 4.11-4.02 (m, 1H), 3.92-3.60 (m, 4H). ^{13}C NMR (75 MHz, D$_2$O): δ 178.8, 82.2, 71.7, 70.9, 70.4, 62.3. ^{13}C NMR data are in accordance with literature values.[461]

β-Cyclodextrin monoaldehyde (105)

Following a literature procedure,[263] a solution of Dess Martin Periodinane (1.12 g, 2.6 mmol) in anhydrous d_6-DMSO (9 mL) was added to a solution of β-cyclodextrin (**104**) (1.5 g, 1.3 mmol) in anhydrous d_6-DMSO (15 mL) and stirred at room temperature. The reaction was monitored by ^{1}H NMR spectroscopy and after 3.5 h, the reaction mixture was poured into a beaker and the product was precipitated by addition of acetone (400 mL) and cooling to -10 ℃. The solution was filtered, washed with cold acetone (100 mL) and dried *in vacuo* to give **105** (1.5 g, 100%) as an inseparable mixture with the di- and tri-aldehydes as well as unreacted **104**. White crystals. R_f 0.76 (MeOH/H$_2$O = 10:1). IR (KBr): 3331, 2926, 1734 (weak), 1617, 1420, 1339, 1136, 1020, 947. ^{1}H NMR (300 MHz, d_6-DMSO): δ 9.69 (s, **C**HO), 4.93 (s, H1 of residue containing CHO), 4.83 (s, 6 x H1). MALDI TOF MS calcd for C$_{42}$H$_{68}$O$_{35}$Na [M + Na]$^+$ *m/z*: 1155.4, found *m/z* 1155.6.

β-Cyclodextrin monoaldehyde (105)

Following a literature procedure,[264] IBX (320 mg, 1.1 mmol) was added to a solution of β-cyclodextrin (**104**) (1.0 g, 0.88 mmol) in anhydrous DMSO (29 mL) and stirred at room temperature. After 3 h, the solution was poured into a beaker, and acetone (350 mL) was added to facilitate precipitation. The solids were washed with acetone (150 mL), redissolved in H_2O (90 mL) and stirred at 50 ºC for 1 h to remove complexed DMSO. After cooling to room temperature, the product was precipitated by addition of acetone (300 mL) and cooling on ice. The solution was filtered, washed with acetone (100 mL) and dried *in vacuo* to give **105** (764 mg, 77%) as an inseparable mixture with the di- and tri-aldehydes as well as unreacted **104**. Spectroscopic data are given above.

Attempted decarbonylation of β-cyclodextrin monoaldehyde

Following general decarbonylation procedure A, a solution of **105** (1.5 g, 1.3 mmol) and Rh(dppp)$_2$Cl (128 mg, 0.13 mmol) in DMA (10 mL) was heated to reflux. The solution turned black within 5 min. After 11 h the reaction mixture was cooled to room temperature (neutral pH) and the solvents were evaporated at 70 ºC using high vacuum. The resulting black syrup was dissolved in DMSO, cooled to -10 ºC and precipitated by addition of acetone (350 mL). The black crystals were filtered, washed with acetone (100 mL) and dried *in vacuo*. The crude product (640 mg) was peracetylated[265] by addition of freshly distilled pyridine (10 mL), Ac$_2$O (10 mL) and a catalytic amount of DMAP followed by stirring at 60 ºC overnight. The reaction was quenched by addition of ice (40 g). The mixture was filtered, and the black crystals were washed with cold H_2O and dried *in vacuo*. The residue was purified by flash column chromatography (acetone/heptane = 2:3 → 4:1) to give a mixture of glycosylated β-cyclodextrin products (approximately 540 mg) as white crystals. For MALDI TOF MS data of both crude and peracetylated products see Table 34 and Table 35 in Appendix I.

Attempted hydroacylation of styrene with D-glucose

Following general decarbonylation procedure B, D-glucose (500 mg, 2.78 mmol) was decarbonylated in the presence of styrene (1.6 mL, 13.9 mmol). Full conversion was achieved within 23 h, and workup and flash column chromatography (DCM/MeOH/H_2O = 65:25:0 → 65:25:2) gave D-arabinitol (188 mg, 45%) and 1,4-anhydro-D-arabinitol (**95**) (103 mg, 28%). Spectroscopic data are given above.

1,2-*O*-Isopropylidene-α-D-glucofuranose (112)

112
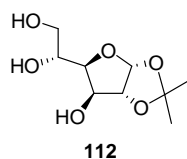
1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**111**) (10.0 g, 38.4 mmol) was dissolved in 60% aqueous AcOH (150 mL) and stirred at room temperature for 21 h.[33] The liquids were evaporated *in vacuo* and remaining AcOH was removed by co-evaporation with H_2O to give **112** (8.44 g, 100%) as white crystals. R_f 0.18 (EtOAc/heptane = 4:1). $[\alpha]_D^{22}$ –12.0 (*c* 1.0, H_2O) (lit.[469] $[\alpha]_D^{25}$ –12.2 (H_2O)). mp 153 – 154 ºC (H_2O) (lit.[470] mp 159 – 160 ºC). ^1H NMR (300 MHz, D_2O): δ 5.90 (d, *J* = 3.7 Hz, 1H), 4.58 (d, *J* = 3.7 Hz, 1H), 4.20 (d, *J* = 1.9 Hz, 1H), 3.98 (dd, *J* = 2.5, 9.0 Hz, 1H), 3.80 (t, *J* = 6.1 Hz, 1H), 3.70 (ddd, *J* = 1.3, 2.7, 12.0 Hz, 1H), 3.53 (ddd, *J* = 1.3, 6.0, 12.1 Hz, 1H), 1.41 (s, 3H), 1.26 (s, 3H). ^{13}C NMR (75 MHz, D_2O): δ 113.5, 105.6, 85.3, 80.6, 74.5, 69.3, 64.4, 26.4, 26.0. NMR data are in accordance with literature values.[471,472] Anal. calcd for $C_9H_{16}O_6$: C, 49.09; H, 7.32. Found: C, 48.87; H, 7.09.

1,2-*O*-Isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (110)

110
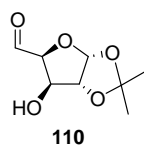
To a solution of diol **112** (1.00 g, 4.54 mmol) in MeOH (30 mL) at 0 ºC was added a cooled solution of $NaIO_4$ (0.97 g, 4.54 mmol) in H_2O (10 mL) dropwise over 5 min.[33] The solution was stirred at 0 ºC for 1 h and then filtered. The filtercake was washed with DCM (3 × 20 mL) and the combined liquids were evaporated *in vacuo* to give the crude aldehyde as a semi-crystalline residue. Further purification was achieved by dissolving the residue in H_2O (20 mL) and extracting with DCM (8 × 30 mL). The combined organic phases were dried ($MgSO_4$), filtered and concentrated *in vacuo* to give a residue, which was purified by flash column chromatography (EtOAc/heptane = 1:3 – 3:1). This afforded **110** (780 mg, 91%) as a mixture of the free aldehyde (**110**) and its dimer **114** as white crystals. R_f 0.35 (EtOAc/heptane = 4:1). $[\alpha]_D^{22}$ +13.1 (immediately) → –22.3 (after 24h) (*c* 4.0, H_2O) (lit.[473] $[\alpha]_D^{23}$ –25.7 (monomer, *c* 0.5, H_2O)). $[\alpha]_D^{22}$ +17.5 (*c* 3.3, $CHCl_3$) (lit.[474] $[\alpha]_D^{25}$ +27.8 (dimer, *c* 0.3 $CHCl_3$)). mp 78 – 86 ºC (EtOAc/heptane) (lit.[473] mp 162 – 164 ºC (monomer, H_2O)) (lit.[474] mp 181 – 182 ºC (dimer, EtOAc/hexane)). IR (KBr): 3440, 2988, 2938, 1735 (weak),

1636 (weak), 1457, 1377, 1219, 1165, 1074, 1017, 858. ^{1}H NMR (300 MHz, D$_2$O): δ 6.00 (d, J = 3.6 Hz, 1H), 5.09 (d, J = 7.5 Hz, 1H), 4.66 (d, J = 3.6 Hz, 1H), 4.25 (d, J = 2.6 Hz, 1H), 4.00 (dd, J = 2.7, 7.5 Hz, 1H), 1.49 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (75 MHz, D$_2$O): δ 113.8, 105.6, 89.0, 85.6, 83.7, 74.5, 26.6, 26.1. Anal. calcd for C$_8$H$_{12}$O$_5$/C$_{16}$H$_{24}$O$_{10}$: C, 51.06; H, 6.43. Found: C, 50.77; H, 6.43. HRMS calcd for C$_{16}$H$_{24}$O$_{10}$Na [M + Na]$^+$ m/z 399.1267, found m/z 399.1284.

1,2-*O*-Isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (110)

Following a modified literature procedure,[34] 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**111**) (2.00 g, 7.68 mmol) was added to a solution of H$_5$IO$_6$ (2.63 g, 11.5 mmol) in freshly distilled ether (30 mL) under an argon atmosphere. The solution was stirred at room temperature for 20 h. The reaction mixture was filtered and the sticky precipitate was washed with dry ether (3 x 20 mL). The combined liquids were evaporated *in vacuo* and the residue was purified by flash column chromatography (EtOAc/heptane = 1:1) to give **110** (716 mg, 50%) as a mixture of the free aldehyde (**110**) and its dimer **114** as white crystals. Spectroscopic data are given above.

1,2-*O*-Isopropylidene-β-L-threofuranose (113)

113
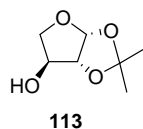
To aldehyde **110** (500 mg, 2.66 mmol) were added Rh(dppp)$_2$Cl (51 mg, 0.053 mmol) and a degassed solution of diglyme (10 mL). The mixture was thoroughly degassed and then stirred at reflux (162 °C) in a preheated oil bath for 26 h. The solvent was removed under high vacuum at 70 °C to give a black residue, which was purified by flash column chromatography eluting with ether/pentane = 2:3 → 4:1 to afford **113** (366 mg, 86%) as white crystals. R_f 0.35 (EtOAc/heptane = 3:2). $[\alpha]_D^{22}$ +13.1 (*c* 0.8, acetone) (lit.[278] $[\alpha]_D$ +13 (*c* 1, acetone)). mp 80 – 81 °C (ether) (lit.[59,278] mp 84 – 85 °C (ether/hexane)). ^{1}H NMR (300 MHz, CD$_3$OD): δ 5.78 (d, J = 3.7 Hz, 1H), 4.35 (d, J = 3.7 Hz, 1H), 4.04 (d, J = 2.8 Hz, 1H), 3.92 (dd, J = 2.9, 9.8 Hz, 1H), 3.68 (dd, J = 1.0, 9.8 Hz, 1H), 1.32 (s, 3H), 1.19 (s, 3H). ^{13}C NMR (50 MHz, CD$_3$OD): δ 112.7, 106.7, 86.4, 75.9, 73.9, 27.1, 26.4. NMR data are in accordance with literature values.[59] Anal. calcd for C$_7$H$_{12}$O$_4$: C, 52.49; H, 7.55. Found: C, 52.79; H, 7.47.
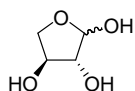
L-Threose

Compound **113** (100 mg, 0.62 mmol) was dissolved in 30% aqueous AcOH (10 mL) and heated to reflux for 4 h.[278] The liquids were removed *in vacuo* and the residue was purified by reverse phase column chromatography eluting with H_2O to give L-threose (74 mg, 99%) as a colorless oil consisting of a 14:11:5 mixture of the α- and β-furanose forms and the hydrate. R_f 0.57 (acetone/BuOH/H_2O = 5:4:1). $[\alpha]_D^{22}$ +12.3 (*c* 2.0, H_2O) (lit.[278] $[\alpha]_D$ +12 (*c* 1, H_2O)). ^{1}H NMR (300 MHz, D_2O): δ 5.33 (d, *J* = 4.2 Hz, H1 β-anomer), 5.17 (d, *J* = 1.1 Hz, H1 α-anomer), 4.94 (d, *J* = 6.3 Hz, H1 hydrate), 4.50-3.36 (m, 4H). ^{13}C NMR (75 MHz, D_2O): α-anomer: δ 103.4, 81.9, 76.4, 74.3; β-anomer: δ 97.9, 77.4, 76.1, 71.8; hydrate: δ 91.0, 74.5, 72.1, 64.2. NMR data are in accordance with literature values.[461,475,476] Anal. calcd for $C_4H_8O_4$: C, 40.00; H, 6.71. Found: C, 40.74; H, 6.72. HRMS calcd for $C_4H_8O_4Na$ $[M + Na]^+$ *m/z* 143.0320, found *m/z* 143.0327.

8.3 Compounds referred to in chapter 4[♦]

Activation of Zn[136]

Zn dust (<10 micron, 50 g) was suspended in H_2O (100 mL) and 4 M aqueous HCl (100 mL) was added. After stirring under an argon atmosphere at room temperature for 15 min, the suspension was filtered and washed with H_2O (2 x 20 mL), MeOH (20 mL) and Et_2O (20 mL). The finely dispersed Zn powder was dried *in vacuo* by heating with a heat gun for 10 min.

General procedure for imine allylation using different metals

A suspension of activated Zn (140 mg, 2.14 mmol) and **139** (100 mg, 0.214 mmol) in freshly distilled THF (4 mL) was sonicated at 40 °C under an argon atmosphere in a flat-bottomed conical flask. TMSCl (13.5 μL, 0.107 mmol) was added in two portions, after 10 and 20 min of sonication. When NMR revealed full conversion to the aldehyde (1.5 h), flame-dried, powdered 4 Å molecular sieves (50 mg) were added followed by dropwise addition of $BnNH_2$ (117 μL, 1.07 mmol). When NMR revealed full conversion of the aldehyde to the imine (1 h), the solution was cooled to room

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[♦] A small part of the characterization of the intermediates towards calystegine A_3 was performed by Charlotte B. Pipper.

temperature, filtered through a plug of Celite, washed with anhydrous Et_2O (20 mL) and concentrated *in vacuo*.

The crude imine (**144**) was redissolved in freshly distilled solvent (THF or toluene/DCM = 4:1), the metal (Zn, In or Mg, 0.64 mmol) was added and the solution was sonicated at 40 ^{o}C under an argon atmosphere. Allylbromide (59.1 μL, 0.68 mmol) was added dropwise to the solution, and after an additional 2-3 h of sonication, the solution was cooled to room temperature, diluted with Et_2O (60 mL) and H_2O (20 mL) and filtered through a plug of Celite. The organic phase was washed with H_2O (3 x 20 mL) and brine (20 mL), dried (K_2CO_3), filtered and concentrated *in vacuo* to give a colorless oil, which was purified by flash column chromatography (EtOAc/heptane 1:6 → 1:2) to afford a diastereomeric mixture of **145** and **146**.

Methyl 3,4-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-6-*O*-trityl-α-D-glucopyranoside (140)

140
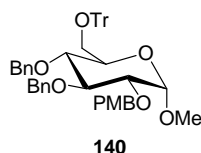
To a solution of diol **132** (31.5 g, 56.6 mmol) and NaH (19 mg, 50%, 0.40 mmol, prewashed with heptane) in anhydrous DMF (600 mL) at 0 ^{o}C was added Bu_4NI (2.1 g, 5.7 mmol) followed by dropwise addition of BnBr (21.7 mL, 183 mmol). The reaction mixture was left at ambient temperature overnight while it warmed to room temperature. The reaction mixture was quenched by addition of MeOH (60 mL), diluted with Et_2O (600 mL) and washed with H_2O (700 mL). The aqueous phase was extracted with Et_2O (2 x 500 mL), and the combined organic phases were dried ($MgSO_4$), filtered and evaporated on Celite. Dry column vacuum chromatography[477] (EtOAc/heptane = 1:5) afforded **140** (34.4 g, 82%) as a white foam. R_f 0.47 (EtOAc/heptane = 1:2). $[\alpha]_D^{25}$ +10.9 (*c* 1.0, $CHCl_3$). IR (KBr): 3061, 3030, 2926, 1612, 1513, 1449, 1249, 1159, 1072, 1036, 1028, 755, 703. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41-7.35 (m, 6H), 7.30-7.06 (m, 19H), 6.83-6.74 (m, 4H), 4.86 (d, *J* = 10.7 Hz, 1H), 4.71 (d, *J* = 10.7 Hz, 1H), 4.69 (d, *J* = 11.8 Hz, 1H), 4.62 (d, *J* = 3.7 Hz, 1H), 4.57 (d, *J* = 11.1 Hz, 1H), 4.61 (d, *J* = 9.0 Hz, 1H), 4.20 (d, *J* = 10.4 Hz, 1H), 3.86 (t, *J* = 8.8 Hz, 1H), 3.76-3.68 (m, 1H), 3.73 (s, 3H), 3.57-3.49 (m, 2H), 3.41 (d, *J* = 10.0 Hz, 1H), 3.36 (s, 3H), 3.10 (dd, *J* = 4.7, 9.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.5, 144.1, 138.8, 138.0, 130.5, 129.8, 128.9, 128.6, 128.3, 128.3, 128.2, 127.9, 127.8, 127.7, 127.0, 114.0, 98.1, 86.4, 82.4, 79.9, 78.2, 76.1, 75.1, 73.1, 70.3, 62.7, 55.4, 55.1. HRMS calcd for $C_{48}H_{48}O_7Na$ [M+Na]$^+$ *m/z* 759.3292, found *m/z* 759.3257.

Methyl 3,4-di-*O*-benzyl-6-*O*-trityl-α-D-glucopyranoside (137)

137
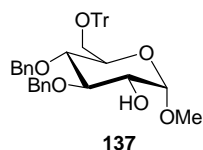
To a solution of fully protected **140** (14.8 g, 20.1 mmol) in DCM/H_2O (19:1, 350 mL) was added DDQ (6.84 g, 30.1 mmol). The atmosphere was exchanged with argon and the reaction mixture was stirred at room temperature for 2.5 h. The solution was diluted with DCM (700 mL) and quenched by stirring with sat. aqueous $NaHCO_3$ (500 mL) for 1.5 h. The aqueous phase was extracted with DCM (2 x 500 mL) and the combined organic phases were washed with brine (200 mL), dried (Na_2SO_4), filtered and evaporated on Celite. Purification by dry column vacuum chromatography (EtOAc/heptane = 2:1) gave **137** (12.0 g, 97%) as a colorless oil. R_f 0.49 (EtOAc/heptane = 1:1). $[\alpha]_D^{25}$ +71.5 (*c* 1.0, $CHCl_3$). IR (KBr): 3448, 3060, 3029, 2929, 1490, 1449, 1156, 1125, 1047, 745, 698. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51-7.45 (m, 6H), 7.41-7.15 (m, 17H), 6.87 (dd, *J* = 1.8, 7.7 Hz, 2H), 4.91-4.81 (m, 3H), 4.67 (d, *J* = 10.4 Hz, 1H), 4.29 (d, *J* = 10.4 Hz, 1H), 3.84-3.62 (m, 4H), 3.53 (dd, *J* = 1.8, 10.1 Hz, 1H), 3.47 (s, 3H), 3.22 (dd, *J* = 4.6, 10.0 Hz, 1H), 2.17 (d, *J* = 7.4 Hz, OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 144.1, 138.7, 137.9, 128.9, 128.6, 128.3, 128.2, 128.1, 127.9, 127.7, 127.1, 99.3, 86.5, 83.6, 78.0, 75.7, 75.0, 73.2, 70.7, 62.6, 55.1. HRMS calcd for $C_{40}H_{40}O_6Na$ [M+Na]$^+$ *m/z* 639.2717, found *m/z* 639.2734.

Methyl 3,4-di-*O*-benzyl-2-*O*-(methylsulfanylthiocarbonyl)-6-*O*-trityl-α-D-glucopyranoside (141)

141
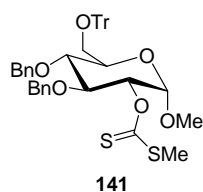
To NaH (1.65 g, 50%, 34.4 mmol, prewashed with heptane) were added imidazole (374 mg, 5.5 mmol) and a solution of alcohol **137** (7.06 g, 11.4 mmol) in CS_2 (13.8 mL, 22.8 mmol) and freshly distilled THF (140 mL) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3.5 h after which MeI (3.6 mL, 57.8 mmol) was added. After an additional 1.5 h at room temperature, the reaction mixture was evaporated, redissolved in DCM (300 mL), and washed with H_2O (2 x 100 mL). The aqueous phase was extracted with DCM (50 mL) and the combined organic phases were dried (Na_2SO_4), filtered, evaporated on Celite followed by dry column vacuum

chromatography (heptane → EtOAc/heptane = 1:19) to afford **141** (7.2 g, 89%) as a yellow solid. R_f 0.71 (EtOAc/heptane = 1:1). $[\alpha]_D^{25}$ +52.9 (*c* 1.0, CHCl$_3$). IR (KBr): 3058, 3030, 2926, 1491, 1449, 1359, 1207, 1171, 1046, 740, 688, 630. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.51-7.46 (m, 6H), 7.32-7.16 (m, 17H), 6.87 (dd, *J* = 1.7, 7.7 Hz, 2H), 5.80 (dd, *J* = 3.7, 9.9 Hz, 1H), 5.17 (d, *J* = 3.7 Hz, 1H), 4.78 (d, *J* = 10.7 Hz, 1H), 4.72 (d, *J* = 10.7 Hz, 1H), 4.68 (d, *J* = 10.4 Hz, 1H), 4.30 (d, *J* = 10.4 Hz, 1H), 4.16 (dd, *J* = 8.7, 9.9 Hz, 1H), 3.90-3.83 (m, 1H), 3.76 (dd, *J* = 8.7, 10.1 Hz, 1H), 3.53 (dd, *J* = 1.5, 9.9 Hz, 1H), 3.43 (s, 3H), 3.24 (dd, *J* = 4.5, 10.1 Hz, 1H), 2.60 (s, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 216.1, 144.0, 138.2, 137.8, 128.9, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.1, 96.2, 86.5, 81.6, 80.4, 78.2, 75.8, 75.2, 70.4, 62.5, 55.1, 19.6. HRMS calcd for C$_{42}$H$_{42}$O$_6$S$_2$Na [M+Na]$^+$ *m/z* 729.2315, found *m/z* 729.2330.

Methyl 3,4-di-*O*-benzyl-2-deoxy-6-*O*-trityl-α-D-glucopyranoside (138)

138
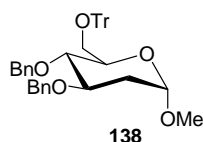
The methyl xanthate **141** (10.3 g, 14.6 mmol) was dissolved in anhydrous toluene (190 mL) under a nitrogen atmosphere and heated to reflux. A solution of AIBN (239 mg, 1.46 mmol) and Bu$_3$SnH (8.9 mL, 33.6 mmol) in anhydrous toluene (60 mL) was added dropwise to the reaction mixture over a period of 1 h. The solution was stirred at reflux for 6 h, cooled to room temperature and evaporated on Celite. The product was purified by dry column vacuum chromatography (heptane → EtOAc/heptane = 1:19) to afford **138** (7.53 g, 86%) as a white solid. R_f 0.55 (EtOAc/heptane = 1:2). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.49-7.37 (m, 6H), 7.39-7.07 (m, 17H), 6.83 (dd, *J* = 2.0, 7.2 Hz, 2H), 4.84 (d, *J* = 2.5 Hz, 1H), 4.67 (d, *J* = 10.5 Hz, 1H), 4.59-4.56 (m, 2H), 4.26 (d, *J* = 10.5 Hz, 1H), 3.92-3.81 (m, 1H), 3.71 (dd, *J* = 3.3, 9.8 Hz, 1H), 3.57-3.48 (m, 1H), 3.47-3.41 (m, 1H), 3.30 (s, 3H), 3.17 (dd, *J* = 4.8, 9.9 Hz, 1H), 2.25 (dd, *J* = 5.0, 12.9 Hz, 1H), 1.70 (ddd, *J* = 3.6, 11.7, 13.0 Hz, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 144.2, 138.8, 138.3, 129.0, 128.5, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.0, 98.4, 86.4, 78.8, 77.9, 75.0, 72.2, 70.9, 63.1, 54.5, 35.8. HRMS calcd for C$_{40}$H$_{40}$O$_5$Na [M+Na]$^+$ *m/z* 623.2768, found *m/z* 623.2764.

Methyl 3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (142)
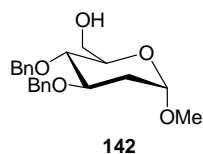
A suspension of **138** (7.5 g, 12.5 mmol) in 1% H_2SO_4 in MeOH (550 mL) was stirred at room temperature under an argon atmosphere for 2.5 h. The reaction mixture was neutralized by stirring with Na_2CO_3 (10 g), filtered and evaporated on Celite. Purification by dry column vacuum chromatography (EtOAc/heptane = 1:9 → 1:4) gave **142** (4.15 g, 93%) as a colorless oil. R_f 0.38 (EtOAc/heptane = 1:1). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.39-7.24 (m, 10H), 4.95 (d, J = 11.1 Hz, 1H), 4.80 (d, J = 3.2 Hz, 1H), 4.72-4.60 (m, 3H), 4.05-3.94 (m, 1H), 3.85-3.72 (m, 2H), 3.68-3.60 (m, 1H), 3.55-3.47 (m, 1H), 3.30 (s, 3H), 2.29 (dd, J = 5.0, 13.1 Hz, 1H), 1.95 (s, OH), 1.66 (ddd, J = 3.6, 11.5, 13.0 Hz, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 138.7, 138.5, 128.5, 128.5, 128.1, 127.9, 127.7, 127.7, 98.6, 78.2, 77.6, 75.0, 71.9, 71.2, 62.3, 54.7, 35.6. HRMS calcd for $C_{21}H_{26}O_5Na$ [M+Na]$^+$ m/z 381.1672, found m/z 381.1693.

Methyl 3,4-di-*O*-benzyl-2,6-dideoxy-6-iodo-α-D-glucopyranoside (139)
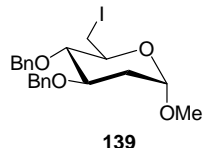
Alcohol **142** (4.4 g, 12.2 mmol), PPh$_3$ (5.1 g, 19.4 mmol) and imidazole (2.0 g, 29.4 mmol) were co-evaporated with toluene (2 x 125 mL) and then dissolved in freshly distilled THF (250 mL) under an argon atmosphere. The solution was heated to reflux and a solution of I$_2$ in freshly distilled THF (0.17 mmol/mL) was added dropwise to the reaction mixture until a permanent color change was achieved (79.4 mL, 13.3 mmol). Full conversion was confirmed by TLC analysis, and the reaction mixture was cooled to room temperature, filtered, washed with a little THF and evaporated on Celite. The product was purified by dry column vacuum chromatography (EtOAc/heptane = 1:49 → 1:9) to afford **139** (5.33 g, 93%) as a colorless oil. R_f 0.53 (EtOAc/heptane = 1:2). IR (film): 3030, 2933, 2901, 1496, 1453, 1366, 1213, 1130, 1100, 1047, 955, 741, 699. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.32-7.15 (m, 10H), 4.93 (d, J = 11.0 Hz, 1H), 4.75 (d, J = 3.0 Hz, 1H), 4.64 (d, J = 11.0 Hz, 1H), 4.58 (d, J = 11.5 Hz, 1H), 4.51 (d, J = 11.5 Hz, 1H), 3.93 (ddd, J = 5.1, 8.3, 11.5 Hz, 1H), 3.52-3.20 (m, 4H), 3.28 (s, 3H), 2.23 (dd, J = 5.1, 13.1 Hz, 1H), 1.62 (ddd, J = 3.6, 11.6, 13.1 Hz, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 138.5, 138.4, 128.6, 128.5, 128.1, 127.9, 127.8, 98.6, 82.2,

136

77.3, 75.4, 71.8, 69.9, 55.1, 35.5, 8.7. HRMS calcd for $C_{21}H_{25}IO_4Na$ [M+Na]$^+$ *m/z* 491.0690, found *m/z* 491.0698.

(3*R*,4*R*,6*R*)- and (3*R*,4*R*,6*S*)-6-[(*N*-Benzyl)-amino]-3,4-bis(benzyloxy)-nona-1,8-diene (145 and 146)
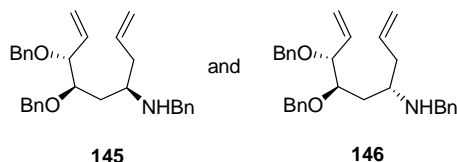
A suspension of activated Zn (140 mg, 2.14 mmol) and iodide **139** (100 mg, 0.214 mmol) in freshly distilled THF (4 mL) was sonicated at 40 °C under an argon atmosphere in a flat-bottomed conical flask. TMSCl (13.5 μL, 0.107 mmol) was added in two portions, after 15 and 25 min of sonication. When NMR revealed full conversion to the aldehyde (1 h 15 min), BnNH$_2$ (117 μL, 1.07 mmol) was added dropwise to the solution. When NMR revealed full conversion of the aldehyde to the imine (45 min), allylbromide (55.4 μL, 0.64 mmol) was added dropwise to the solution. After an additional 2 h of sonication, the solution was cooled to room temperature, diluted with Et$_2$O (60 mL) and H$_2$O (20 mL) and filtered through a plug of Celite. The organic phase was washed with H$_2$O (3 x 20 mL) and brine (20 mL), dried (K$_2$CO$_3$), filtered and concentrated *in vacuo* to give a colorless oil, which was purified by flash column chromatography (EtOAc/heptane 1:4) to afford a separable 1:1.13 diastereomeric mixture of **145** and **146** (89 mg, 94%) as colorless oils.

Data for the minor isomer **145**: R_f 0.20 (EtOAc/heptane = 1:2). $[\alpha]_D^{23}$ +6.7 (*c* 2.0, CDCl$_3$). IR (film): 3064, 3029, 2925, 2861, 1496, 1454, 1353, 1206, 1092, 1069, 1027, 995, 918, 735, 697. ^{1}H NMR (300 MHz, CDCl$_3$): 7.28-7.09 (m, 15H), 5.74 (ddd, *J* = 7.5, 10.6, 17.1 Hz, 1H), 5.68-5.53 (m, 1H), 5.27-5.17 (m, 2H), 5.01-4.92 (m, 2H), 4.66 (d, *J* = 11.5 Hz, 1H), 4.55 (d, *J* = 12.0 Hz, 1H), 4.41 (d, *J* = 11.5 Hz, 1H), 4.29 (d, *J* = 12.0 Hz, 1H), 3.83 (dd, *J* = 5.6 Hz, 7.4 Hz, 1H), 3.61 (s, 2H), 3.58-3.50 (m, 1H), 2.62 (p, *J* = 6.3 Hz, 1H), 2.18-2.06 (m, 1H), 2.04-1.93 (m, 1H), 1.62-1.54 (m, 2H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 140.8, 138.7, 138.6, 135.5, 135.3, 128.4, 128.3, 128.1, 127.9, 127.6, 127.6, 126.8, 119.0, 117.5, 82.2, 79.0, 72.9, 70.6, 53.8, 51.1, 38.3, 35.3. HRMS calcd for $C_{30}H_{36}NO_2$ [M+H]$^+$ *m/z* 442.2741, found *m/z* 442.2733.

Data for the major isomer **146**: R_f 0.27 (EtOAc/heptane = 1:2). $[\alpha]_D^{23}$ +32.5 (*c* 1.0, CDCl$_3$). IR (film): 3064, 3028, 2918, 2859, 1495, 1453, 1355, 1261, 1207, 1089, 1065, 1027, 993, 913, 733,

696. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.44-7.27 (m, 15H), 5.94-5.75 (m, 2H), 5.41-5.31 (m, 2H), 5.19-5.10 (m, 2H), 4.82-4.64 (m, 2H), 4.49 (d, J = 11.4, 1H), 4.46 (d, J = 12.0, 1H), 3.96 (t, J = 6.6 Hz, 1H), 3.90-3.80 (m, 2H), 3.65 (d, J = 12.9 Hz, 1H), 2.96-2.86 (m, 1H), 2.32 (dd, J = 6.9, 14.5 Hz, 2H), 2.04 (bs, NH), 1.70-1.62 (m, 2H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 140.8, 138.8, 138.6, 135.4, 135.3, 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.5, 127.4, 126.9, 118.9, 117.4, 82.6, 78.6, 73.4, 70.6, 52.9, 50.5, 38.7, 35.6. HRMS calcd for C$_{30}$H$_{36}$NO$_2$ [M+H]$^+$ m/z 442.2741, found m/z 442.2728.

(3*R*,4*R*,6*R*)-6-[(*N*-Benzyl)-(*N*-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-nona-1,8-diene (149)

149
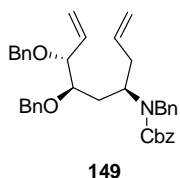
KHCO$_3$ (109 mg, 1.09 mmol) was added to a solution of amine **145** (80 mg, 0.18 mmol) in DCM/H$_2$O (1:1, 10 mL) at 0 ^{o}C. Then CbzCl (28 μL, 0.20 mmol) was added under vigorous stirring, and the reaction mixture was slowly allowed to reach room temperature. After 1.5 h, the solution was diluted with DCM (20 mL) and washed with H$_2$O (10 mL) and brine (10 mL). The combined aqueous phases were extracted with DCM (5 mL) and the combined organic phases were dried (K$_2$CO$_3$), filtered and concentrated *in vacuo*. Purification of the residue by flash column chromatography (EtOAc/heptane 1:9) gave **149** (95 mg, 91%) as a colorless oil. R_f 0.54 (EtOAc/heptane = 1:2). $[\alpha]_D^{25}$ –16.5 (c 3.0, CDCl$_3$). IR (film): 3086, 3064, 2926, 2864, 1692, 1496, 1453, 1414, 1338, 1309, 1232, 1208, 1093, 1069, 1028, 993, 918, 734, 697. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.34-7.02 (m, 20H), 5.77-5.53 (m, 1H), 5.49-4.96 (m, 5H), 4.85-4.66 (m, 2H), 4.59-4.09 (m, 6H), 4.01-3.67 (m, 2H), 3.38-3.20 (m, 1H), 2.26-1.55 (m, 4H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 157.3, 139.1, 138.7, 138.5, 136.8, 135.3, 135.1, 128.6, 128.4, 128.4, 128.2, 128.0, 127.9, 127.7, 127.2, 127.0, 118.9, 117.1, 81.4, 78.5, 72.7, 70.5, 67.0, 55.2, 48.7, 37.5, 33.9. Only the major rotamer reported. HRMS calcd for C$_{38}$H$_{41}$NO$_4$Na [M+Na]$^+$ m/z 598.2928, found m/z 598.2918.

(3*R*,4*R*,6*R*)-6-[(*N*-Benzyl)-(*N*-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-cycloheptene (136)

136
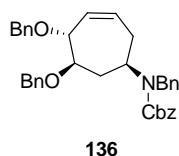
A solution of diene **149** (53 mg, 0.092 mmol) and Grubbs' 2nd generation catalyst (3.9 mg, 4.6 µmol) in freshly distilled DCM (15 mL) was degassed by bubbling nitrogen through the solution for 5 min. The solution was protected from sunlight and left stirring at room temperature for 48 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by flash column chromatography (heptane → EtOAc/heptane = 3:17) to give **136** (49 mg, 97%) as a colorless oil. R_f 0.45 (EtOAc/heptane = 1:2). $[\alpha]_D^{25}$ –10.6 (*c* 2.0, CDCl$_3$). IR (film): 3087, 3063, 3029, 2961, 2927, 2857, 1693, 1496, 1453, 1413, 1357, 1305, 1259, 1233, 1090, 1070, 1027, 801, 733, 695. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.32-7.00 (m, 20H), 5.64-5.51 (m, 2H), 5.18-4.97 (m, 2H), 4.66-4.19 (m, 6H), 4.00-3.80 (m, 2H), 3.43-3.18 (m, 1H), 2.35-1.83 (m, 4H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 155.7, 138.9, 138.7, 136.6, 133.0, 128.5, 128.3, 127.9, 127.8, 127.6, 127.5, 127.4, 127.1, 81.9, 79.2, 73.0, 72.6, 67.2, 53.9, 47.9, 39.6, 33.0. Only the major rotamer reported. HRMS calcd for C$_{36}$H$_{37}$NO$_4$Na [M+Na]$^+$ *m/z* 570.2615, found *m/z* 570.2633.

(3*R*,4*R*,6*S*)-6-[(*N*-Benzyl)-(*N*-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-nona-1,8-diene (150)

150
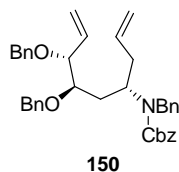
KHCO$_3$ (214 mg, 2.14 mmol) was added to a solution of amine **146** (157 mg, 0.36 mmol) in DCM/H$_2$O (1:1, 10 mL) at 0 ^{o}C. Then CbzCl (55 µL, 0.39 mmol) was added under vigorous stirring, and the reaction mixture was slowly allowed to reach room temperature. After 1.5 h, full conversion was not yet achieved and additional CbzCl (25 µL, 0.18 mmol) was added. After stirring at room temperature for 40 min, the phases were separated and the organic phase was washed with H$_2$O (20 mL) and brine (20 mL), dried (K$_2$CO$_3$), filtered and concentrated *in vacuo*. Purification of the residue by flash column chromatography (EtOAc/heptane 1:19 → 3:17) gave **150** (190 mg, 93%) as a colorless oil. R_f 0.56 (EtOAc/heptane = 1:2). $[\alpha]_D^{25}$ +30.6 (*c* 3.0, CDCl$_3$). IR (film): 3066,

3030, 2928, 2866, 1695, 1496, 1454, 1413, 1337, 1236, 1116, 1089, 1070, 1028, 994, 919, 735, 698. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.32-7.03 (m, 20H), 5.67-5.28 (m, 2H), 5.23-4.71 (m, 6H), 4.61-4.10 (m, 7H), 3.66-3.49 (m, 1H), 3.34-3.23 (m, 1H), 2.33-1.93 (m, 2H), 1.74 (t, J = 12.5 Hz, 1H), 1.44 (t, J = 12.6 Hz, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 157.9, 139.2, 138.9, 138.6, 136.8, 135.4, 135.0, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.2, 127.0, 119.0, 117.2, 82.5, 78.2, 73.8, 70.5, 67.2, 54.2, 54.2, 47.5, 38.7, 34.3. Only the major rotamer reported. HRMS calcd for C$_{38}$H$_{41}$NO$_4$Na [M+Na]$^+$ m/z 598.2928, found m/z 598.2906.

(3R,4R,6S)-6-[(N-Benzyl)-(N-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-cycloheptene (151)
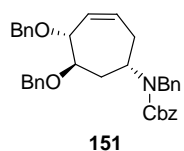
A solution of diene **150** (87 mg, 0.15 mmol) and Grubbs' 2nd generation catalyst (7 mg, 8.2 µmol) in freshly distilled DCM (10 mL) was degassed by bubbling argon through the solution for 10 min. The solution was protected from sunlight and left stirring at room temperature for 36 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by flash column chromatography (heptane → EtOAc/heptane = 3:17) to give **151** (80 mg, 97%) as a colorless oil. R_f 0.46 (EtOAc/heptane = 1:2). [α]$_D^{25}$ –106.1 (c 2.0, CDCl$_3$). IR (film): 3088, 3054, 3027, 2930, 2878, 2857, 1687, 1495, 1451, 1413, 1361, 1304, 1229, 1090, 1070, 1027, 969, 735, 696. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.32-7.06 (m, 20H), 5.77 (ddd, J = 4.5, 7.8, 11.6 Hz, 1H), 5.63 (ddd, J = 1.9, 5.5 11.4 Hz, 1H), 5.17-5.03 (m, 2H), 4.59-3.88 (m, 8H), 3.68 (d, J = 16.3 Hz, 1H), 2.69-2.57 (m, 1H), 2.36 (t, J = 12.5 Hz, 1H), 2.25-2.03 (m, 1H), 1.91 (t, J = 12.6 Hz, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 156.9, 139.1, 138.7, 136.8, 131.5, 130.7, 129.7, 128.5, 128.4, 128.3, 128.0, 127.6, 127.5, 127.1, 76.6, 76.2, 70.9, 70.8, 67.1, 50.7, 49.0, 34.4, 34.0. Only the major rotamer reported. HRMS calcd for C$_{36}$H$_{37}$NO$_4$Na [M+Na]$^+$ m/z 570.2615, found m/z 570.2602.

Methyl 3,4-di-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-glucopyranoside (152)

152
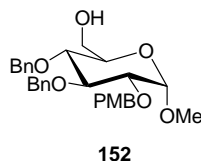
Compound **140** (33.9 g, 46.0 mmol) was dissolved in 1% H_2SO_4 in MeOH (1 L) and toluene (200 mL), and stirred at room temperature for 2 h 15 min. The reaction was stopped by stirring with Na_2CO_3 (63 g) until pH > 7 followed by removal of the solvent *in vacuo*. The residue was dissolved in DCM (500 mL), washed with H_2O (2 x 100 mL) and the combined aqueous phases were extracted with DCM (2 x 100 mL). The combined organic phases were dried ($MgSO_4$), filtered, concentrated *in vacuo* and purified by dry column vacuum chromatography (EtOAc/heptane = 1:9 → 3:1) to give **152** (22.3 g, 98%) as a slightly yellow oil. R_f 0.30 (EtOAc/heptane = 1:1). $[\alpha]_D^{25}$ +20.3 (*c* 2.2, $CHCl_3$). IR (film): 3475, 3063, 3030, 2925, 1700, 1612, 1586, 1512, 1496, 1456, 1359,1303, 1251, 1093, 913, 822, 735, 701. ^1H NMR (300 MHz, $CDCl_3$): δ 7.41-7.25 (m, 12H), 6.87 (d, *J* = 8.3 Hz, 2H), 4.99 (d, *J* = 10.8 Hz, 1H), 4.89 (d, *J* = 10.9 Hz, 1H), 4.83 (d, *J* = 10.9 Hz, 1H), 4.75 (d, *J* = 11.8 Hz, 1H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.60 (d, *J* = 11.7 Hz, 1H), 4.52 (d, *J* = 3.5 Hz, 1H), 4.00 (t, *J* = 9.2 Hz, 1H), 3.81 (s, 3H), 3.79-3.62 (m, 3H), 3.57-3.46 (m, 2H), 3.37 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.5, 138.9, 138.2, 130.3, 129.9, 128.6, 128.5, 128.2, 128.1, 128.0, 127.7, 114.0, 98.4, 82.1, 79.7, 77.5, 75.9, 75.2, 73.2, 70.7, 62.0, 55.4, 55.3. HRMS calcd for $C_{29}H_{34}O_7Na$ [M+Na]$^+$ *m/z* 517.2197, found *m/z* 517.2198.

Methyl 3,4-di-*O*-benzyl-6-deoxy-6-iodo-2-*O*-*p*-methoxybenzyl-α-D-glucopyranoside (133)

133
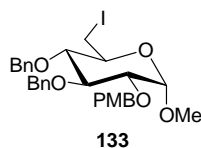
A mixture of alcohol **152** (21.7 g, 43.9 mmol), PPh_3 (18.4 g, 70.1 mmol) and imidazole (6.8 g, 99.9 mmol) was co-evaporated with toluene (2 x 300 mL) and dissolved in freshly distilled THF (500 mL). The solution was briefly evacuated and purged with argon 3 times. The solution was then heated to reflux and a solution of I_2 in freshly distilled THF (0.50 mmol/mL) was added dropwise to the reaction mixture until a permanent color change was achieved (113.0 mL, 56.5 mmol). Full conversion was confirmed by TLC analysis. The reaction mixture was cooled to room temperature, filtered, washed with a little THF and evaporated on Celite. The product was purified by dry column vacuum chromatography (EtOAc/heptane = 1:19 → 3:7) to afford **133** (24.7 g, 93%) as a

colorless oil, which slowly crystallized upon standing. R_f 0.68 (EtOAc/heptane = 1:1). $[\alpha]_D^{25}$ +27.2 (c 2.1, CHCl$_3$). IR (KBr): 3030, 2916, 2906, 2838, 1613, 1514, 1453, 1358, 1300, 1245, 1171, 1112, 1088, 1073, 1047, 1030, 1012, 735, 695. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.39-7.25 (m, 12H), 6.86 (d, J = 8.7 Hz, 2H), 4.99 (d, J = 10.8 Hz, 1H), 4.94 (d, J = 11.0 Hz, 1H), 4.79 (d, J = 10.8 Hz, 1H), 4.74 (d, J = 11.8 Hz, 1H), 4.68 (d, J = 10.9 Hz, 1H), 4.59 (d, J = 11.8 Hz, 1H), 4.55 (d, J = 3.6 Hz, 1H), 4.00 (dd, J = 8.8, 9.5 Hz, 1H), 3.81 (s, 3H), 3.54-3.42 (m, 3H), 3.41 (s, 3H), 3.37-3.25 (m, 2H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 159.4, 138.5, 137.9, 130.0, 129.7, 128.5, 128.4, 127.9, 127.8, 127.6, 113.8, 98.1, 81.5, 81.4, 79.6, 75.7, 75.3, 73.0, 69.2, 55.5, 55.2, 7.7. HRMS calcd for C$_{29}$H$_{33}$IO$_6$Na [M+Na]$^+$ m/z 627.1214, found m/z 627.1191.

(3R,4S,5S,6R)-6-[(N-Benzyl)-amino]-3,4-bis(benzyloxy)-5-p-methoxybenzyloxy-nona-1,8-diene (134)
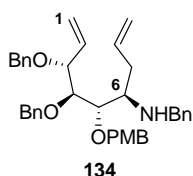
Freshly distilled THF (100 mL) was added to activated Zn (29.1 g, 0.45 mol) and iodide **133** (26.5 g, 0.044 mol) divided into five flat-bottomed conical flasks. The atmosphere was exchanged with argon and the solutions were sonicated at 40 ^{o}C. TMSCl (2.8 mL, 0.022 mol) was added in two portions, after 10 min and after 20 min of sonication. When NMR revealed full conversion to the aldehyde (2 h), BnNH$_2$ (24.3 mL, 0.22 mol) was added dropwise to the solutions. When NMR revealed full conversion of the aldehyde to the imine (1 h), allylbromide (11.6 mL, 0.13 mol) was very carefully added dropwise to the solutions. After an additional 4 h of sonication, the solutions were cooled to room temperature, combined and diluted with Et$_2$O (1 L). H$_2$O (500 mL) was added to precipitate the zinc salts followed by filtration through a plug of Celite. The organic phase was washed with brine (2 x 250 mL), which led to formation of a white precipitate which was removed by filtration. The combined aqueous phases were extracted with Et$_2$O (2 x 100 mL) and the combined organic phases were dried (K$_2$CO$_3$), filtered and evaporated on Celite. Dry column vacuum chromatography (EtOAc/heptane 1:4) gave a separable 5.3:1 diastereomeric mixture of **134** and its C6 epimer (21.5 g, 85%) as a colorless oil.

Data for the major isomer **134**: R_f 0.42 (EtOAc/heptane = 1:2). ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.38-7.15 (m, 17H), 6.82 (d, J = 8.6 Hz, 2H), 5.92 (ddd, J = 7.6, 10.4, 17.7 Hz, 1H), 5.69-5.54 (m, 1H), 5.25 (dd, J = 1.9, 10.4 Hz, 1H), 5.20 (dd, J = 1.4, 16.9 Hz, 1H), 5.06-4.96 (m, 2H), 4.78 (d, J = 10.9 Hz, 1H), 4.76 (d, J = 11.2 Hz, 1H), 4.66 (d, J = 11.2 Hz, 1H), 4.51 (d, J = 11.9 Hz, 1H), 4.50 (d, J = 10.8 Hz, 1H), 4.08 (d, J = 11.9 Hz, 1H), 3.93 (dd, J = 3.2, 7.6 Hz, 1H), 3.82 (d, J = 13.1 Hz, 1H), 3.79-3.75 (m, 2H), 3.78 (s, 3H), 3.69 (dd, J = 3.1, 7.7 Hz, 1H), 3.43 (d, J = 13.0 Hz, 1H), 2.46-2.35 (m, 2H), 2.32-2.16 (m, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 159.1, 141.1, 138.9, 138.2, 136.5, 136.4, 131.6, 129.4, 128.7, 128.6, 128.4, 128.3, 128.3, 128.2, 127.7, 127.5, 126.9, 118.4, 116.9, 113.7, 83.6, 80.6, 79.7, 75.4, 74.5, 70.2, 56.5, 55.4, 50.9, 35.2. HRMS calcd for C$_{38}$H$_{43}$NO$_4$Na [M+Na]$^+$ m/z 600.3084, found m/z 600.3112.

(3*R*,4*S*,5*S*,6*R*)-6-[(*N*-Benzyl)-(*N*-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-5-*p*-methoxybenzyloxy-nona-1,8-diene (153)
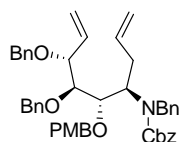
153

To a solution of amine **134** (19.9 g, 0.034 mol) in DCM/H$_2$O (1:1, 700 mL) at 0 ^{o}C was added KHCO$_3$ (26.0 g, 0.26 mol), and under vigorous stirring, CbzCl (6.7 mL, 0.048 mol) was added dropwise to the solution over a period of 20 min. The reaction mixture was allowed to reach room temperature, and after 2 h, the phases were separated and the organic phase was washed with H$_2$O (250 mL). The combined aqueous phases were extracted with DCM (150 mL) and the combined organic phases were dried (K$_2$CO$_3$), filtered and evaporated on Celite. Dry column vacuum chromatography (EtOAc/heptane 1:9) gave **153** (22.5 g, 92%) as a colorless oil. R_f 0.49 (EtOAc/heptane = 1:2). [α]$_D^{25}$ –4.9 (*c* 2.0, CHCl$_3$). IR (film): 3062, 3031, 2923, 1950, 1879, 1810, 1696, 1641, 1612, 1585, 1514, 1497, 1453, 1407, 1321, 1300, 1248, 1173, 1078, 924, 915, 823, 735, 700. ^{1}H NMR (300 MHz, d_6- DMSO, 60 ^{o}C): δ 7.36-7.16 (m, 20H), 7.12 (d, J = 8.5 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 5.85 (ddd, J = 7.6, 10.3, 17.5 Hz, 1H), 5.35-5.03 (m, 5H), 4.88-4.75 (m, 2H), 4.70-4.60 (m, 1H), 4.58-4.02 (m, 9H), 3.93-3.83 (m, 1H), 3.75 (s, 2.7H), 3.73 (s, 0.3H), 3.52-3.43 (m, 1H), 2.44-2.29 (m, 1H), 2.27-2.13 (m, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 159.1, 157.4, 138.6, 136.5, 135.9, 135.1, 134.8, 131.0, 129.7, 129.4, 129.2, 128.5, 128.4, 128.3, 128.1, 127.9, 127.6, 127.5, 126.7, 118.5, 117.5, 113.7, 83.1, 80.8, 79.9, 75.4, 75.2, 70.8, 67.2, 56.5, 55.4, 53.6,

35.2. Only the major rotamer reported. HRMS calcd for $C_{46}H_{50}NO_6$ [M+H]$^+$ m/z 712.3633, found m/z 712.3614. Anal. calcd for $C_{46}H_{49}NO_6$: C, 77.61; H, 6.94. Found: C, 77.56; H, 7.18.

(3R,4S,5S,6R)-6-[(N-Benzyl)-(N-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-5-p-methoxybenzyloxy-cycloheptene (154)
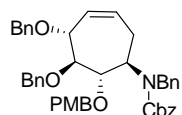
154

A solution of diene **153** (21.77 g, 30.6 mmol) in freshly distilled DCM (1.2 L) was degassed by bubbling nitrogen through the solution for 15 min. Grubbs' 2nd generation catalyst (1.3 g, 1.5 mmol) was added to the solution under a nitrogen atmosphere, and the solution was protected from sunlight and left stirring at room temperature for 48 h. The reaction mixture was evaporated on Celite and purified by dry column vacuum chromatography (heptane → EtOAc/heptane = 1:3) to give **154** (20.4 g, 98%) as a colorless syrup, which briefly turned into a white foam under vacuum. R_f 0.45 (EtOAc/heptane = 1:2). [α]$_D^{25}$ –19.1 (c 2.1, CHCl$_3$). IR (film): 3064, 3031, 2921, 1950, 1881, 1813, 1700, 1612, 1586, 1514, 1496, 1456, 1355, 1246, 1174, 1073, 824, 734, 701. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.42-7.03 (m, 22H), 6.86-6.74 (m, 2H), 5.73-5.54 (m, 2H), 5.25-5.06 (m, 2H), 4.98-4.56 (m, 6H), 4.42-4.00 (m, 5H), 3.76 (s, 1.2H), 3.73 (s, 1.8H), 3.62-3.52 (m, 1H), 3.31 (t, J = 10.1 Hz, 0.6H), 2.94-2.78 (m, 0.4H), 2.58 (t, J = 12.9 Hz, 0.4H), 2.02-1.82 (m, 0.6H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 159.1, 156.8, 139.0, 138.5, 138.2, 136.7, 132.8, 130.9, 129.7, 129.1, 128.5, 128.3, 128.1, 127.7, 127.7, 127.5, 127.3, 113.7, 84.7, 83.3, 77.9, 75.3, 75.0, 72.7, 66.9, 61.9, 55.3, 53.8, 30.0. Only the major rotamer reported. HRMS calcd for $C_{44}H_{46}NO_6$ [M+H]$^+$ m/z 684.3320, found m/z 684.3325.

(3R,4R,5S,6R)-6-[(N-Benzyl)-(N-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-5-hydroxy-cycloheptene (135)
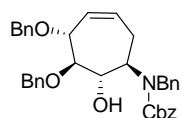
135

To a solution of **154** (187 mg, 0.27 mmol) in DCM/H$_2$O (19:1, 15 mL) was added DDQ (165 mg, 0.73 mmol) followed by stirring at room temperature under an argon atmosphere for 3 h. The reaction mixture was diluted with DCM (60 mL) and quenched by washing with sat. aqueous

NaHCO$_3$ (30 mL) for 30 min. The aqueous phase was extracted with DCM (2 x 30 mL) and the combined organic phases were washed with brine (20 mL). The aqueous phase from the brine washing was extracted with DCM (10 mL) and the combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo*. Purification of the residue by flash column chromatography (EtOAc/heptane = 1:4 → 1:3) afforded **135** (136 mg, 88%) as a colorless oil. R_f 0.39 (EtOAc/heptane = 1:2). $[\alpha]_D^{25}$ –13.1 (*c* 1.1, CHCl$_3$). IR (film): 3545, 3483, 3063, 3031, 2889, 1695, 1496, 1453, 1416, 1248, 1031, 734, 701. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.32-7.06 (m, 20H), 5.76-5.51 (m, 2H), 5.16-4.99 (m, 2H), 4.97-4.22 (m, 5H), 4.14-3.67 (m, 4H), 3.42 (t, *J* = 7.7 Hz, 1H), 2.52-2.18 (m, 1H), 2.01-1.80 (m, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 156.4 138.9, 138.2, 136.7, 132.6, 130.6, 129.5, 128.7, 128.6, 128.5, 128.4, 128.1, 127.9, 127.8, 127.4, 127.2, 84.0, 78.3, 75.3, 75.0, 72.3, 67.3, 60.0, 50.1, 30.2. Only the major rotamer reported. HRMS calcd for C$_{36}$H$_{38}$NO$_5$ [M+H]$^+$ *m/z* 564.2744, found *m/z* 564.2773.

(3*R*,4*S*,5*S*,6*R*)-6-[(*N*-Benzyl)-(*N*-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-5-(methylsulfanyl)thiocarbonyloxy-cycloheptene (156)

156
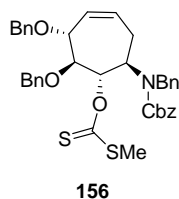
To NaH (267 mg, 50%, 5.56 mmol, prewashed with heptane) was added a solution of imidazole (47 mg, 0.69 mmol) in CS$_2$ (42 mL, 0.69 mol) under a nitrogen atmosphere. A solution of alcohol **135** (773 mg, 1.37 mmol) in CS$_2$ (42 mL, 0.69 mol) and freshly distilled THF (35 mL) was added dropwise to the reaction mixture under vigorous stirring over a period of 1 h 15 min. After 3 h, MeI (432 μL, 3.82 mmol) was added dropwise and the solution was stirred at room temperature overnight. The reaction mixture was evaporated on Celite and purified by flash column chromatography (EtOAc/heptane = 1:6) to afford **156** (700 mg, 78%) and the byproduct **157** (84 mg, 13%) as yellow oils. Data for **156**: R_f 0.50 (EtOAc/heptane = 1:2). $[\alpha]_D^{23}$ –34.7 (*c* 2.0, CDCl$_3$). IR (film): 3087, 3063, 3029, 2923, 2865, 1697, 1496, 1453, 1229, 1202, 1118, 1055, 734, 697. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.28-7.01 (m, 20H), 5.79-5.48 (m, 2H), 5.16-4.98 (m, 2H), 4.70 (q, *J* = 11.3 Hz, 1H), 4.57-3.91 (m, 8H), 3.82-3.65 (m, 1H), 2.83-2.52 (m, 1H), 2.38 (s, 1.5H), 2.32 (s, 1.5H), 2.09-1.84 (m, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 215.0, 156.5, 138.5, 138.4, 138.2, 138.1, 130.7, 130.2, 128.8, 128.6, 128.5, 128.4, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.2,

84.6, 79.9, 76.2, 74.2, 72.4, 67.3, 58.9, 54.5, 30.0, 19.2. Only the major rotamer reported. HRMS calcd for $C_{38}H_{39}NO_5S_2Na$ [M+Na]$^+$ m/z 676.2162, found m/z 676.2182.

(Z,3aR,7R,8S,8aS)-3-Benzyl-7,8-bis(benzyloxy)-3a,4,8,8a-tetrahydro-3H-cyclohepta[d]oxazol-2(7H)-one (157)
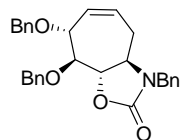
157

Compound **157** was isolated as a byproduct during formation of **156** from **135**. Data for **157**: R_f 0.27 (EtOAc/heptane = 1:2). $[\alpha]_D^{23}$ –96.4 (c 0.5, CDCl$_3$). IR (film): 3063, 3030, 2924, 2857, 1753, 1496, 1454, 1416, 1352, 1131, 1086, 1065, 1027, 737, 701. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.31-7.18 (m, 13H), 7.05-6.99 (m, 2H), 5.77 (ddt, J = 1.8, 6.1, 12.2 Hz, 1H), 5.67 (dt, J = 4.1, 12.2 Hz, 1H), 4.73 (d, J = 11.6 Hz, 1H), 4.56 (d, J = 11.6 Hz, 1H), 4.54 (d, J = 15.3 Hz, 1H), 4.41 (d, J = 11.6 Hz, 1H), 4.27 (d, J = 11.6 Hz, 1H), 4.20-4.13 (m, 3H), 3.92 (dd, J = 2.8, 6.3 Hz, 1H), 3.86 (dd, J = 2.8, 7.0 Hz, 1H), 2.62-2.51 (m, 1H), 2.05-1.92 (m, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 158.6, 137.9, 135.8, 129.9, 128.9, 128.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 127.2, 82.7, 82.6, 75.2, 72.9, 71.0, 52.6, 46.6, 33.4. HRMS calcd for $C_{29}H_{29}NO_4Na$ [M+Na]$^+$ m/z 478.1989, found m/z 478.1980.

(3R,4R,6R)-6-[(N-Benzyl)-(N-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-cycloheptene (136)

A solution of freshly distilled Bu$_3$SnH (243 μL, 0.92 mmol) in freshly distilled toluene (6 mL) was evacuated for 15 min, purged with nitrogen and heated to reflux. To this solution were added methyl xanthate **156** (200 mg, 0.31 mmol) and AIBN (10 mg, 0.061 mmol) in freshly distilled toluene (4 mL) dropwise under a nitrogen atmosphere over a period of 20 min. Additional toluene (1.5 mL) was used to transfer the material. Full conversion was achieved after 50 min and the solution was cooled to room temperature and concentrated *in vacuo*. The residue was purified by flash column chromatography (DCM/heptane = 4:1) to give **136** (124 mg, 74%) as a colorless oil. Spectroscopic data are given above.

(3*R*,4*R*,6*R*)-6-[(*N*-Benzyl)-(*N*-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-cycloheptene (136)

The (imidazolyl)thiocarbonyl derivative **155** (36 mg, 0.053 mmol) was co-evaporated with freshly distilled toluene (2 x 5 mL), evacuated, purged with argon and subsequently dissolved in freshly distilled toluene (3 mL). The reaction mixture was heated to reflux, and a solution of AIBN (4 mg, 0.024 mmol) and Bu$_3$SnH (57 μL, 0.22 mmol) in freshly distilled toluene (1 mL) was then added dropwise to the reaction mixture over a period of 30 min. After 3 h, the reaction mixture was allowed to reach room temperature and evaporated on Celite. Purification by flash column chromatography (EtOAc/heptane 1:9) afforded **136** (8 mg, 27%) as a colorless oil. Spectroscopic data are given above.

(2*S*,3*R*,5*R*)-5-[(*N*-Benzyl)-(*N*-benzyloxycarbonyl)-amino]-2,3-bis(benzyloxy)-cycloheptanone (158) and (3*R*,4*R*,6*S*)-6-[(*N*-benzyl)-(*N*-benzyloxycarbonyl)-amino]-3,4-bis(benzyloxy)-cycloheptanone (159)
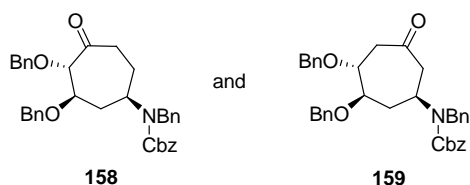
Cycloheptene **136** (600 mg, 1.1 mmol) was dissolved in freshly distilled THF (30 mL) under an argon atmosphere and cooled to -40 °C. BH$_3$ • THF (1 M in THF, 2.30 mL, 2.3 mmol) was added dropwise to the solution over a period of 20 min. After 3 h at -40 °C, the solution was allowed to reach room temperature, and after 4 h 2 M aqueous NaOH (2 mL) and 35% aqueous H$_2$O$_2$ (4 mL) were added to the reaction mixture. The solution was stirred at ambient temperature for 1 h, diluted with Et$_2$O (70 mL) and washed with H$_2$O (3 x 10 mL) and brine (10 mL). The organic phase was dried (K$_2$CO$_3$), filtered and concentrated *in vacuo* to give an isomeric mixture of alcohols, which were used directly in the next step.

The crude alcohols were dissolved in freshly distilled DCM (19 mL) and added to a solution of DMP (929 mg, 2.2 mmol) in freshly distilled DCM (15 mL). The reaction mixture was stirred at room temperature for 1 h, after which Et$_2$O (100 mL) was added. The resulting white suspension was stirred for 30 min and then gravity filtered. The filtrate was washed with sat. aqueous Na$_2$S$_2$O$_3$ (2 x 20 mL) and brine (30 mL), and the organic phase was dried (K$_2$CO$_3$), filtered and concentrated *in vacuo*. Flash column chromatography (EtOAc/heptane = 1:9) of the residue gave a separable 2:1

mixture of the isomeric cycloheptanones (468 mg, 76% combined yield) as colorless oils; **158** (308 mg, 50%) and **159** (160 mg, 26%).

Data for the major isomer **158**: R_f 0.38 (EtOAc/heptane = 1:2). $[\alpha]_D^{25}$ –4.5 (*c* 1.0, CDCl$_3$). IR (film): 3088, 3063, 3031, 2930, 2869, 1696, 1496, 1454, 1415, 1357, 1238, 1096, 1072, 1028, 736, 698. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.37-7.00 (m, 20H), 5.21-5.02 (m, 2H), 4.59-4.15 (m, 6H), 4.07-3.38 (m, 3H), 2.69-2.41 (m, 1H), 2.26-2.12 (m, 1H), 2.01-1.75 (m, 4H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 209.2, 155.7, 138.6, 138.0, 137.2, 136.4, 128.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 127.3, 126.9, 88.7, 79.4, 72.5, 72.2, 67.3, 55.4, 48.3, 37.3, 36.6, 30.5. Only the major rotamer reported. HRMS calcd for C$_{36}$H$_{37}$NO$_5$Na [M+Na]$^+$ *m/z* 586.2564, found *m/z* 586.2549.

Data for the minor isomer **159**: R_f 0.31 (EtOAc/heptane = 1:2). $[\alpha]_D^{25}$ –9.9 (*c* 2.0, CDCl$_3$). IR (film): 3087, 3063, 3030, 2930, 2871, 1695, 1496, 1454, 1415, 1352, 1246, 1095, 1071, 1028, 736, 698. ^{1}H NMR (300 MHz, CDCl$_3$): δ 7.30-7.04 (m, 20H), 5.18-5.00 (m, 2H), 4.62-3.95 (m, 7H), 3.57-3.28 (m, 2H), 2.86-2.31 (m, 4H), 2.14-1.74 (m, 2H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 207.2, 155.8, 138.4, 138.2, 138.0, 136.5, 128.9, 128.8, 128.7, 128.62, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.3, 81.2, 79.3, 72.3, 72.3, 67.6, 50.6, 48.3, 44.9, 36.9, 29.9. Only the major rotamer reported. HRMS calcd for C$_{36}$H$_{37}$NO$_5$Na [M+Na]$^+$ *m/z* 586.2564, found *m/z* 586.2582.

(-)-Calystegine A$_3$ (117)

117
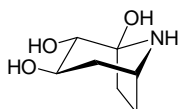
A solution of cycloheptanone **158** (39 mg, 0.069 mmol) in dioxane/H$_2$O (9:1, 3.3 mL) was degassed by bubbling nitrogen through the solution for 10 min. Pearlman's catalyst (8 mg, 7.5 μmol) was added to the solution and H$_2$ was bubbled through the solution for 10 min after which the solution was stirred at room temperature under 1 atmosphere of H$_2$ for 15 h. TLC analysis revealed disappearance of the protected cycloheptanone, and 1 M aqueous HCl (0.7 mL) was added to the solution, which was stirred at room temperature under 1 atmosphere of H$_2$ for an additional 32 h. The reaction mixture was neutralized by addition of Amberlite IRA-400 OH$^-$, filtered through a plug of Celite and thoroughly washed with H$_2$O (15 x 10 mL). The filtrate was co-evaporated with EtOH and purified by Sephadex LH-20 column chromatography (EtOAc/EtOH = 4:1) to give

(-)-calystegine A_3 (**117**) (9.2 mg, 84%). R_f 0.42 (1-propanol/AcOH/H_2O = 4:1:1). $[\alpha]_D^{25}$ –13.6 (*c* 0.3, D_2O) (lit.[289] $[\alpha]_D$ –17.3 (*c* 0.47, H_2O)). IR (film): 3382, 2954, 2929, 2857, 1635, 1399, 1387, 1259, 1088, 1056, 1026, 934. ^{1}H NMR (500 MHz, D_2O): δ 3.62 (ddd, *J* = 6.9, 8.5, 10.5 Hz, 1H), 3.45 (d, *J* = 6.6 Hz, 1H), 3.36 (d, *J* = 8.4 Hz, 1H), 2.06-1.90 (m, 3H), 1.53-1.43 (m, 3H). ^{13}C NMR (125 MHz, D_2O): δ 93.4, 82.5, 72.7, 54.2, 42.6, 31.8, 29.3. The chemical shift of C1 (93.4 ppm) was assigned by HSQC. NMR data are in accordance with literature values.[289] HRMS calcd for $C_7H_{13}NO_3Na$ [M+Na]$^+$ *m/z* 182.0788, found *m/z* 182.0793.

(-)-Calystegine A_3 (117)

A solution of cycloheptanone **158** (39 mg, 0.069 mmol) in THF/H_2O (9:1, 3.3 mL) was degassed by bubbling nitrogen through the solution for 10 min. Pearlman's catalyst (8 mg, 7.5 µmol) was added to the solution and H_2 was bubbled through the solution for 10 min after which the solution was stirred at room temperature under 1 atmosphere of H_2 for 15 h. TLC analysis revealed disappearance of the protected cycloheptanone, and 1 M aqueous HCl (0.7 mL) was added to the solution, which was stirred at room temperature under 1 atmosphere of H_2 for an additional 32 h. The reaction mixture was neutralized by addition of Amberlite IRA-400 OH$^-$, filtered through a plug of Celite and thoroughly washed with H_2O (15 x 10 mL). The filtrate was co-evaporated with EtOH and purified by Sephadex LH-20 column chromatography (EtOAc/EtOH = 4:1) to give (-)-calystegine A_3 (**117**) (8.9 mg, 81%). Spectroscopic data are given above.

Attempted isomerization of calystegine A_3 (117) to A_6 (166)

Calystegine A_3 (**117**) (2 mg, 0.013 mmol) was dissolved in pyridine-d_5 (0.7 mL, pH = 8-9) or 1 M aq. $Ca(OH)_2$ (0.7 mL, pH > 12). The reaction mixtures containing $Ca(OH)_2$ were inhomogeneous and stirring was applied. The reactions were performed at 25 or 100 ºC and were followed by TLC and ^{1}H NMR. Experiments in pyridine-d_5 could be evaporated and the product recovered directly, whereas experiments in aq. $Ca(OH)_2$ were acidified with 1 M aq. HCl, neutralized with IRA-400 OH$^-$ ion exchange resin, filtered through a plug of Celite and washed thoroughly with water. The filtrate was concentrated *in vacuo* and the residue was analyzed by TLC and ^{1}H NMR. When necessary, the crude product was purified on Sephadex LH-20 eluting with EtOAc/EtOH = 4:1.

8.4 Compounds referred to in chapter 5

(3*R*,4*R*,5*S*,6*S*)-3,4,5,6-Tetrahydroxy-3,4;5,6-di-*O*-isopropylidene-1-methylcyclohexene (184)
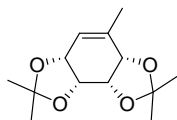
184

To a solution of diol **183** (100 mg, 0.50 mmol) in 2,2-dimethoxypropane (8 mL, 65 mmol) was added camphorsulfonic acid (7.3 mg, 0.31 mmol), and the reaction mixture was stirred at room temperature under an argon atmosphere. Full conversion was achieved after 40 min, and the reaction was quenched by addition of a few drops of Et$_3$N (pH > 7). The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (heptane/EtOAc = 3:1) furnishing **184** (88 mg, 73%) as white crystals. R_f 0.45 (EtOAc/heptane = 1:2). ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.54-5.49 (m, 1H), 4.53-4.47 (m, 1H), 4.38-4.29 (m, 3H), 1.84-1.81 (m, 3H), 1.45 (s, 3H), 1.42 (s, 3H), 1.36 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 135.1, 121.1, 110.4, 110.3, 74.7, 73.1, 72.6, 71.7, 27.2, 27.0, 26.6, 26.5, 20.0. ^{13}C NMR data are in accordance with two *cis* fused isopropylidene acetals.[371]

(1*S*,2*S*,3*R*,6*R*)-3-Benzoyloxy-2-hydroxy-4,8,8-trimethyl-7,9-dioxadicyclo[4.3.0]non-4-ene (185)
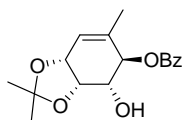
185

A degassed solution of diene **178** (34 mg, 0.10 mmol) and Grubbs' 2nd generation catalyst (7.4 mg, 0.0087 mmol) in freshly distilled DCM (24 mL) was protected from sunlight and stirred at reflux under an argon atmosphere for 3 days. The reaction mixture was evaporated on Celite and purified by flash column chromatography (heptane → heptane/EtOAc = 4:1) to give the target compound **185** (23 mg, 74%) as a white solid. R_f 0.29 (EtOAc/heptane = 2:1). $[\alpha]_D^{25}$ –40.4 (*c* 1.0, CDCl$_3$). IR (film): 3459, 3066, 3036, 2985, 2924, 2855, 1720, 1452, 1380, 1268, 1235, 1110, 1047, 1031, 712. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.11-8.06 (m, 2H), 7.59 (tt, *J* = 2.1, 7.4 Hz, 1H), 7.50-7.42 (m, 2H), 5.84 (dd, *J* = 1.0, 8.1 Hz, 1H), 5.57 (dq, *J* = 1.9, 3.3 Hz, 1H), 4.69-4.64 (m, 1H), 4.56 (ddd, *J* = 0.7, 2.9, 5.6 Hz, 1H), 3.97 (dt, *J* = 2.9, 8.1 Hz, 1H), 2.66 (d, *J* = 8.1 Hz, OH), 1.78 (dd, *J* = 1.3, 2.6 Hz, 3H), 1.43 (s, 3H), 1.40 (s, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 167.2, 134.6, 133.5, 130.0, 129.8,

128.6, 124.1, 109.9, 75.9, 74.1, 73.1, 71.8, 27.8, 26.5, 19.2. HRMS calcd for $C_{17}H_{20}O_5Na$ [M+Na]$^+$ *m/z* 327.1203, found *m/z* 327.1212.

(1*S*,2*S*,3*R*,6*R*)-2,3-Dihydroxy-4,8,8-trimethyl-7,9-dioxabicyclo[4.3.0]non-4-ene (186)

186
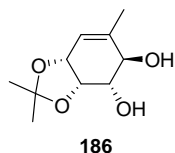
The benzoate **185** (15 mg, 0.049 mmol) was dissolved in 10% NaOMe in anhydrous MeOH (10 mL) and stirred at room temperature under an argon atmosphere for 3 h. The mixture was evaporated on Celite and purified by flash column chromatography (heptane/EtOAc = 1:2) to give **186** (8.5 mg, 86%) as a colorless oil. R_f 0.04 (EtOAc/heptane = 1:2). ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.44-5.40 (m, 1H), 4.65-4.58 (m, 1H), 4.50 (dd, *J* = 2.7, 5.8 Hz, 1H), 4.26 (bd, *J* = 5.6 Hz, 1H), 3.68-3.60 (m, 1H), 2.45-2.36 (m, OH), 2.25-2.17 (m, OH), 1.86-1.83 (m, 3H), 1.37 (s, 6H).

(3*R*,4*R*,5*S*,6*R*)-3,4,5,6-Tetrahydroxy-3,4;5,6-di-*O*-isopropylidene-1-methylcyclohexene (187)

187
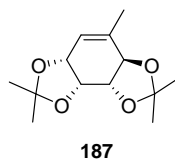
To a solution of diol **186** (8.5 mg, 0.042 mmol) in 2,2-dimethoxypropane (0.8 mL, 6.5 mmol) was added camphorsulfonic acid (0.7 mg, 0.03 mmol), and the reaction mixture was stirred at room temperature under an argon atmosphere. After 2 h, the starting material was still present as the major spot and additional 2,2-dimethoxypropane (1.0 mL, 8.2 mmol) was added. After stirring for an additional 1 h, the reaction was quenched by addition of a few drops of Et$_3$N (pH > 7). The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (heptane/EtOAc = 3:1 → 1:2) giving **187** (3 mg, 29%) as well as 35% recovered starting material (3 mg). R_f 0.51 (EtOAc/heptane = 1:2). ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.41-5.33 (m, 1H), 4.74-4.65 (m, 2H), 4.38 (bd, *J* = 8.3 Hz, 1H), 3.48 (dd, *J* = 1.9, 8.8 Hz, 1H), 1.84-1.81 (m, 3H), 1.51 (s, 3H), 1.46 (s, 3H), 1.42 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 140.3, 120.1, 111.2, 109.9, 80.2, 75.1, 73.6, 70.7, 29.9, 27.0, 26.7, 24.8, 17.7. ^{13}C NMR data are in accordance with one *cis* and one *trans* fused isopropylidene acetal.[371]

(1*S*,2*R*,3*S*,6*R*)-3-Benzoyloxy-2-hydroxy-4,8,8-trimethyl-7,9-dioxabicyclo[4.3.0]non-4-ene (182)

182
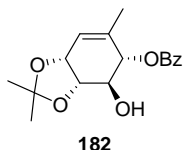
Following a modified literature procedure,[377] a solution of alcohol **181** (1.79 g, 5.9 mmol) in freshly distilled DCM (40 mL) under a nitrogen atmosphere was cooled to –20 ºC followed by addition of pyridine (2.14 mL, 26.5 mmol) and Tf$_2$O (1.48 mL, 8.8 mmol). The reaction mixture was slowly allowed to warm to room temperature and after 1.5 h the reaction was quenched with 2 M HCl (85 mL). The phases were separated and the aqueous phase was extracted with DCM (2 × 25 mL). The combined organic phases were washed with saturated aqueous NaHCO$_3$ (45 mL), dried (MgSO$_4$), filtered and concentrated *in vacuo* to give trifluoromethanesulfonate **189** (2.57 g, 5.9 mmol) as a black residue, which was used directly in the next step. The crude trifluoromethanesulfonate **189** was redissolved in anhydrous DMF under a nitrogen atmosphere, NaNO$_2$ (1.62 g, 23.5 mmol) was added and the mixture stirred at room temperature for 5.5 h. The reaction mixture was diluted with H$_2$O (120 mL) followed by extraction with Et$_2$O (5 × 50 mL). The combined organic phases were dried (MgSO$_4$), filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc/heptane = 1:5 → 1:1) to give alcohol **182** (933 mg, 52%) as a slightly yellow oil. R_f 0.49 (EtOAc/heptane = 1:1). $[\alpha]_D^{25}$ –12.0 (*c* 2.0, CD$_3$OD). IR (film): 3459, 3064, 3043, 2985, 2925, 2859, 1719, 1452, 1379, 1316, 1268, 1249, 1216, 1115, 1060, 1026, 979, 907, 710. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.11-8.05 (m, 2H), 7.59 (tt, *J* = 1.4, 7.4 Hz, 1H), 7.50-7.42 (m, 2H), 5.76-5.67 (m, 2H), 4.69-4.63 (m, 1H), 4.21 (dd, *J* = 6.3, 9.0 Hz, 1H), 3.98 (t, *J* = 8.9 Hz, 1H), 1.77 (dd, *J* = 1.3, 2.7 Hz, 3H), 1.55 (s, 3H), 1.41 (s, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 166.7, 139.1, 133.5, 130.0, 129.6, 128.6, 120.1, 110.6, 77.9, 74.7, 72.7, 72.3, 28.4, 26.1, 18.9. HRMS calcd for C$_{17}$H$_{20}$O$_5$Na [M+Na]$^+$ *m/z* 327.1203, found *m/z* 327.1216.

(1*R*,2*R*,3*S*,6*R*)-3-Benzoyloxy-4,8,8-trimethyl-2-tetrahydropyranyloxy-

7,9-dioxabicyclo[4.3.0]non-4-ene (190)

190
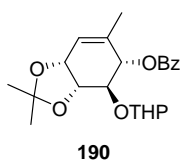
Following a modified literature procedure,[378] a solution of alcohol **182** (850 mg, 2.79 mmol) in freshly distilled DCM (60 mL) was treated with DHP (0.5 mL, 5.5 mmol) and PPTS (140 mg, 0.56

mmol). The mixture was stirred at room temperature under a nitrogen atmosphere overnight. The reaction was stopped by addition of saturated aqueous NaHCO$_3$ (100 mL) followed by extraction with DCM (3 × 50 mL). The combined organic phases were dried (MgSO$_4$), filtered, concentrated *in vacuo* and purified by flash column chromatography (heptane/EtOAc = 9:1 → 4:1) to give **190** (925 mg, 85%) as a colorless oil and a mixture of two diastereomers. R_f 0.72 (EtOAc/heptane = 1:1). IR (film): 3063, 2983, 2938, 2868, 1722, 1452, 1380, 1317, 1265, 1247, 1217, 1162, 1119, 1062, 1029, 986, 966, 869, 710. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.12-8.03 (m, 4H), 7.59-7.53 (m, 2H), 7.48-7.40 (m, 4H), 5.81-5.67 (m, 4H), 5.21 (t, J = 2.6 Hz, 1H), 4.83 (t, J = 3.3 Hz, 1H), 4.68-4.58 (m, 2H), 4.29 (dt, J = 6.1, 8.3 Hz, 2H), 4.19-4.08 (m, 3H), 3.64-3.54 (m, 1H), 3.46-3.37 (m, 1H), 3.23-3.14 (m, 1H), 1.78-1.70 (m, 8H), 1.62-1.58 (m, 4H), 1.55 (s, 9H), 1.40 (s, 3H), 1.39 (s, 6H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 166.4, 166.2, 139.3, 138.1, 133.4, 133.1, 130.2, 129.9, 129.8, 129.7, 128.7, 128.4, 120.9, 120.1, 110.2, 110.2, 99.0, 97.3, 78.5, 76.1, 75.6, 74.6, 73.0, 72.6, 72.6, 62.1, 61.3, 30.6, 30.5, 28.3, 28.1, 26.6, 26.5, 25.4, 25.3, 19.1, 19.0, 18.5. HRMS calcd for C$_{22}$H$_{28}$O$_6$Na [M+Na]$^+$ *m/z* 411.1778, found *m/z* 411.1796.

(1*R*,2*R*,3*S*,6*R*)-3-Hydroxy-4,8,8-trimethyl-2-tetrahydropyranyloxy-7,9-dioxabicyclo[4.3.0]non-4-ene (191)

191
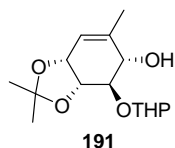
Fully protected **190** (595 mg, 1.53 mmol) was dissolved in 10% NaOMe in anhydrous MeOH (60 mL) and stirred at room temperature for 3 h. The mixture was concentrated *in vacuo* and purified by flash column chromatography (heptane/EtOAc = 4:1) to give alcohol **191** (393 mg, 90%) as a colorless oil and a mixture of two diastereomers. R_f 0.51 and 0.63 (EtOAc/heptane = 1:1). IR (film): 3442, 3037, 2982, 2936, 2860, 1453, 1441, 1372, 1243, 1215, 1135, 1072, 1047, 1022, 1007, 975, 890. ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.54-5.45 (m, 2H), 4.79 (dd, J = 2.6, 5.5 Hz, 1H), 4.62-4.50 (m, 2H), 4.40 (d, J = 1.7 Hz, 1H), 4.31 (t, J = 5.8 Hz, 1H), 4.14-4.07 (m, 2H), 4.04-3.91 (m, 3H), 3.87-3.80 (dd, J = 5.0, 8.5 Hz, 1H), 3.59 (t, J = 8.5 Hz, 1H), 3.56-3.47 (m, 2H), 2.99 (d, J = 8.5 Hz, OH), 1.96-1.77 (m, 3H), 1.87-1.85 (m, 3H), 1.84-1.83 (m, 3H), 1.61-1.47 (m, 9H), 1.47 (s, 3H), 1.43 (s, 3H), 1.36 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 142.1, 138.2, 120.4, 117.3, 110.0, 109.7, 102.7, 99.4, 84.3, 76.7, 75.9, 75.2, 72.7, 71.1, 70.5, 65.6, 64.0, 31.5, 30.9, 28.5, 28.2,

26.6, 26.1, 25.3, 25.1, 21.4, 20.4, 20.3, 19.0. HRMS calcd for $C_{15}H_{24}O_5Na$ [M+Na]$^+$ *m/z* 307.1516, found *m/z* 307.1521.

(1*R*,2*S*,6*R*)-4,8,8-Trimethyl-3-oxo-2-tetrahydropyranyloxy-7,9-dioxabicyclo[4.3.0]non-4-ene (192)

192
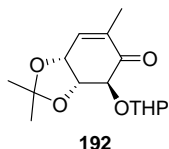
To a solution of alcohol **191** (389 mg, 1.37 mmol) in freshly distilled DCM (65 mL) were added Celite (1.8 g) and PDC (1.8 g, 4.78 mmol), and the reaction mixture was stirred at room temperature under an argon atmosphere for 26 h. The mixture was filtered through a plug of Celite, and concentrated *in vacuo* to give a slightly yellow oil, which was purified by flash column chromatography (heptane/EtOAc = 3:1) to afford **192** (334 mg, 86%) as a colorless oil and a mixture of two diastereomers. R_f 0.62 (EtOAc/heptane = 1:1). IR (film): 2985, 2938, 2886, 1698, 1453, 1380, 1371, 1240, 1219, 1167, 1125, 1063, 1031, 977, 966, 856. ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.54 (dq, *J* = 1.5, 4.2 Hz, 1H), 6.50-6.46 (m, 1H), 4.98 (t, *J* = 3.3 Hz, 1H), 4.87 (t, *J* = 3.2 Hz, 1H), 4.80-4.74 (m, 2H), 4.54-4.41 (m, 4H), 4.13-4.03 (m, 1H), 3.98-3.89 (m, 1H), 3.52-3.41 (m, 2H), 1.86 (t, *J* = 1.4 Hz, 3H), 1.85 (t, *J* = 1.4 Hz, 3H), 1.80-1.67 (m, 5H), 1.61-1.52 (m, 7H), 1.52 (s, 3H), 1.47 (s, 3H), 1.43 (s, 3H), 1.40 (s, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 197.2, 196.1, 137.5, 136.0, 135.8, 111.2, 111.1, 98.6, 97.8, 78.5, 77.7, 76.6, 71.7, 71.6, 62.2, 61.9, 30.4, 30.2, 28.1, 28.0, 26.7, 25.5, 25.4, 19.0, 18.7, 16.2, 15.9. HRMS calcd for $C_{15}H_{22}O_5Na$ [M+Na]$^+$ *m/z* 305.1359, found *m/z* 305.1368.

Gabosine A (171)

171
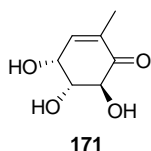
Ketone **192** (52 mg, 0.184 mmol) was dissolved in 80% acetic acid in H$_2$O (3.0 mL) and stirred under a nitrogen atmosphere for 9 h at 40 ºC. The reaction mixture was cooled to room temperature and co-concentrated with H$_2$O to give a residue, which was purified by flash column chromatography (EtOAc) to afford gabosine A (**171**) (28 mg, 96%) as a white crystalline material. R_f 0.16 (EtOAc). [α]$_D^{25}$ –125.4 (*c* 0.8, CD$_3$OD) (lit.[348] [α]$_D^{20}$ –132 (*c* 1, MeOH), lit.[353] [α]$_D$ –131 (*c*

0.27, MeOH). mp 56-60 ºC (MeOH). IR (film): 3354, 2955, 2924, 2862, 1684, 1448, 1236, 1139, 1092, 1028. ^{1}H NMR (300 MHz, CD$_3$OD): δ 6.75 (dq, J = 1.5, 5.6 Hz, 1H), 4.41-4.36 (m, 1H), 4.32 (d, J = 10.0 Hz, 1H), 3.73 (dd, J = 4.0, 10.0 Hz, 1H), 1.82 (dd, J = 0.9, 1.3 Hz, 3H). ^{13}C NMR (75 MHz, CD$_3$OD): δ 200.4, 143.0, 136.9, 75.0, 73.9, 67.4, 15.6. NMR data are in accordance with literature values.[348,353] HRMS calcd for C$_7$H$_{10}$O$_4$Na [M+Na]$^+$ *m/z* 181.0471, found *m/z* 181.0472.

9 Experimental work performed at University of Oxford

9.1 General experimental methods

All reagents were obtained from commercial sources without further purification. Water (H_2O) was purified using a Milli-Q purification system. Petrol refers to the fraction of petroleum ether in the boiling range of 40-60 ℃. TLC was carried out on aluminum plates precoated with 0.25 mm of silica gel 60. The plates were developed by dipping in a solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (25 g/L) and $Ce(SO_4)_2$ (10 g/L) in 10% aqueous H_2SO_4 followed by heating with a heat gun. Flash column chromatography was performed on silica gel 60 (particle size 0.035 – 0.070 mm). NMR spectra were recorded on a Bruker AV 400 instrument. Chemical shifts (δ) are given in ppm and residual solvents were used as internal references.[457] Low resolution mass spectra were recorded on a Micromass Platform 1 spectrometer using electron spray ionization with methanol as carrier solvent. Optical rotations were recorded on a Perkin-Elmer polarimeter. IR spectra were recorded on a Bruker Tensor 27 spectrophotometer and IR absorptions are given in cm^{-1}. Melting points are uncorrected.

9.2 Protein methods

Protein mass spectrometry

LC/MS. The protein solutions were analyzed under denaturing conditions by liquid chromatography (Waters, Milford, MA) coupled to positive electrospray ionization time of flight mass spectrometry (ESI$^+$ TOF MS, Micromass, UK) using a Phenomenex Jupiter 5u C4 300 Å 250 x 4.6 mm column. The protein solution was injected and eluted at 1 mL/min using a 35 min linear gradient method from solvent A (water/5% acetonitrile/0.1% formic acid) and solvent B (acetonitrile/0.1% formic acid), see Table 31. All solvents were degassed by sonication for 15 min prior to use. The output of the liquid chromatography was split 1:4 (mass spectrometer/waste) and injected into the mass spectrometer with a scan range of 300–1800 m/z, capillary voltage 2500 V, cone voltage of 30 V, source temperature of 80 °C, and desolvation temperature of 200 °C. Albumin elutes at 13.5 min and is visualized as a charge envelope from 950 to 1750 *m/z* representing +69 to +41 charges. The

spectrum was deconvolved to the uncharged parent mass using MaxEnt 1 (Micromass). Myoglobin (horse heart) was used as a calibration standard and to test the sensitivity of the system.

Table 31. Chromatographic conditions for LC/MS.

Time (min)	%A	%B	Flowrate mL/min
0.0	95	5	1
5.0	95	5	1
20.0	5	95	1
25.0	5	95	1
30.0	95	5	1
35.0	95	5	1

MALDI TOF MS. Analysis of proteins by MALDI TOF ESI$^+$ MS was performed on a MALDI micro MX (Micromass, UK). The sample was prepared by loading 2 μL of a solution of protein and matrix to the MALDI plate followed by drying under nitrogen. Sinapinic acid or cyano-4-hydroxycinnamic acid (10 mg/mL in 0.1% TFA in water/acetonitrile = 60:40) were used as matrices. The plate was analyzed using positive ionization in linear mode with a 30 kDa – 140 kDa scan range using the following parameters: source voltage of 12000 V, pulse voltage 3000 V and laser 150. BSA was used as a calibration standard and to test the sensitivity of the system.

Tryptic digestion and MS/MS

Trypsin (10 μg, 1 mg/mL in 50 mM NH$_4$HCO$_3$) was added to 50 μL of a solution of modified HSA (approximately 4 g/L) in NH$_4$HCO$_3$ (50 mM, pH 8.0) and incubated at 37 ℃ for 16 h. To the resultant solution was added 1% aq. formic acid (5 μL), and the mixture was analyzed by liquid chromatography (Agilent) coupled to ESI$^+$ TOF MS (LTQ FT Ultra, Micromass, UK) using a Phenomenex Jupiter 5u C18 300 Å 150 x 0.5 mm column. The tryptic peptides were injected and eluted at 15 μL/min using a 90 min linear gradient method from solvent A (water/0.1% formic acid) and solvent B (acetonitrile/0.1% formic acid), see Table 32. The output of the liquid chromatography was injected into the mass spectrometer with a scan range of 100–2800 *m/z*, capillary voltage 3000 V, cone voltage of 35 V, source temperature of 80 °C, and desolvation temperature of 200 °C.

Table 32. Chromatographic conditions for LC-coupled MS/MS.

Time (min)	%A	%B	Flowrate μL/min
0.0	95	5	15
5.0	95	5	15
30.0	60	40	15
60.0	5	95	15
70.0	95	5	15
90.0	95	5	15

Size-exclusion chromatography

Desalting and removal of excess reagents were carried out by PD10 size-exclusion chromatography (10 kDa molecular weight cutoff). The PD10 column was washed with buffer or water (25 mL), the protein sample (2.5 mL) was loaded and the column was eluted with buffer or water (3.5 mL).

Determination of protein concentration

Protein concentrations were measured by UV absorbance. The extinction coefficient for unmodified HSA at 280 nm ($\varepsilon_{280nm} = 28730$ M^{-1} cm^{-1}) was calculated from the amino acid sequence[410] using the following protein concentration calculater: http://www.mrc-lmb.cam.ac.uk/ms/methods/proteincalculator.html. Determination of protein concentration using Bradford assays with BSA as a standard agreed favorably with the concentration obtained by A_{280}. The concentrations of *modified* protein solutions were estimated using Bradford assay.

SDS-PAGE gel electrophoresis

To microcentrifuge tubes containing protein (15 μL) was added sample loading buffer [3 μL; Tris-HCl (1.52 g), glycerol (20 mL), SDS (2 g) and bromophenol blue (2 mg) dissolved in H_2O (90 mL), adjusted to pH 6.8 and made up to 100 mL H_2O]. The samples were heated to 100 ℃ for 5 min and together with protein marker (3 μL) they were loaded to a 10 well Invitrogen NuPAGE® Nowex 4-12% Bis-Tris gel, which was eluted for 45 min (200 V) with running buffer [20x NuPAGE® MOPS (40 mL), H_2O (760 mL)]. The gel was incubated in gel stain [Coomassie Brilliant Blue R-250 (2.5 g), methanol (500 mL), glacial acetic acid (400 mL), H_2O (100 mL)] on a rocking table for 30 min. The gel stain was then replaced with gel destain [methanol (400 mL), glacial acetic acid (70 mL), H_2O (530 mL)] and the gel was incubated on a rocking table overnight.

Preparative HPLC purification of human serum albumin

Commercial HSA was purified by preparative high-performance liquid chromatography using a Dionex Ultimate 3000 HPLC. 2 mL of a solution of HSA (25 mg/mL) in water was injected into a preparative HPLC column (Vydac, C18, 300 Å) at room temperature. A linear gradient method of solvent A (water/0.1% formic acid) and solvent B (acetonitrile/0.1% formic acid) was used (Table 33) and the fractions (Gilson FC204 fraction collector) were collected based on UV absorbance at 280 nm. The fractions were analyzed by LC/MS. The concentration of the purified protein solution was measured by UV absorption at 280 nm.

Table 33. Chromatographic conditions for preparative HPLC.

Time (min)	%A	%B	Flowrate mL/min
0.0	95	5	5
5.0	95	5	5
10.0	50	50	5
42.5	25	75	5
45.0	0	100	5
50.0	0	100	5
55.0	95	5	5
60.0	95	5	5

Purification of human serum albumin[419,420]

To commercial HSA (Lee BioSolutions, Inc., 50 mg, 0.75 µmol) and EDTA (1.5 mg, 5 µmol) in aq. phosphate buffered saline (5 mL, 20 mM, pH 8.5) were added DTT (10 mg, 65 µmol) in phosphate buffered saline (68 µL), and the reaction mixture was stirred at room temperature. The conversion was followed by mass spectrometry and after 2 h, full reduction of the Cys34-Cys disulfide was achieved. Cysteine and excess reducing agent was removed by PD10 size-exclusion chromatography eluting with a solution of EDTA (1 mM) and DTT (0.02 mM) in phosphate buffered saline (20 mM, ph 8.0). The solution was left for 48 h at 4 ℃ exposed to atmospheric air to refold. The protein solution was purified by PD10 size-exclusion chromatography eluting with phosphate buffered saline (20 mM, pH 7.4) to give Cys34-Cys reduced HSA in more than 95% yield. The protein solution was concentrated using a YM10 Amicon ultrafiltration membrane to a concentration of 42 g/L. The protein concentration was determined by UV absorbance. LC/MS: found 66437, expected 66438.

General protein modification methods

General procedure A: Modification of HSA with 2-imino-2-methoxyethyl reagents

Modifying the procedure developed by Stowell and Lee,[431] a solution of HSA (c_{lysine} = 4-30 mM) in phosphate buffered saline (20 mM, pH 9) was added to the IME reagent (**223** or **229**) (1-25 eq. per lysine) as an approximately 1:1 mixture with the corresponding cyanomethyl thioglycopyranoside **224** or **230**. The reaction mixture was stirred very vigorously for 30 sec and was then left under gentle stirring overnight at room temperature. After 16 h, the protein solution was cooled to 4 ºC and purified by PD10 size-exclusion chromatography eluting with water or phosphate buffered saline. The glycoprotein was analyzed by SDS-PAGE gel electrophoresis and/or mass spectrometry (MALDI TOF MS).

General procedure B: Modification of HSA with N-hydroxysuccinimide esters

To an aq. solution of HSA (c_{lysine} = 0.8-27 mM) in phosphate buffered saline (20 mM, pH 4-9) was added NHS ester **236** or **238** (0.017-250 eq. per lysine) either in crystalline form or dissolved in a co-solvent. The reaction mixture was stirred very vigorously for 30 sec and was then left under gentle stirring overnight at 25 or 37 ºC. After 16 h, the protein solution was cooled to 4 ºC and purified by PD10 size-exclusion chromatography eluting with water or phosphate buffered saline. The extent of modification was analyzed by SDS-PAGE gel electrophoresis and/or mass spectrometry (LC/MS or MALDI TOF MS).

General procedure C: Incubation of HSA with 1-β-O-acyl glucuronides

To an aq. solution of HSA (33 g/L) in phosphate buffered saline (20 mM, pH 7.4) were added acyl glucuronides **211**-**216** at a final concentration of 0.5 or 5.0 mM. The reaction mixture was mixed very vigorously for 30 sec and then gently shaken at 37 ºC for 16 h. The protein solution was then cooled to 4 ºC and purified by PD10 size-exclusion chromatography eluting with water to remove salts and excess reagent. The product was analyzed by mass spectrometry (LC/MS).

Incubation of HSA with D-glucuronic acid

As a control experiment, D-glucuronic acid (0.5 or 5.0 mM) was incubated with HSA (33 g/L) according to general procedure C. Workup by size-exclusion chromatography and subsequent analysis by mass spectrometry (LC/MS) revealed a completely unmodified spectrum of HSA.

Gal-modified HSA (231)

Following general procedure A, HSA (100 mg, 89 μmol lysines, c_{lysine} = 30 mM) was incubated with IME reagent **223** (0.4 g, 0.5 mmol) as an approximately 1:2 mixture with cyano compound **224** in PBS buffer (3 mL) at pH 9. Workup gave a solution of Gal-modified HSA **231** (42.5 Gal units on average, 3.5 mL, approximately 32 mg/mL). MALDI TOF MS: found 76.4 kDa.

Attempted α(2,3)-SiaT-catalyzed sialylation[442] of Gal-modified HSA

To a mixture of sialic acid (5.2 mg, 16.7 μmol) and CTP disodium salt (8.8 mg, 16.7 μmol) was added a solution of CMP-sialic acid synthetase (100 μL, 10 mg/mL, 0.04 μmol) in HEPES buffer (50 mM aq. HEPES, 10 mM MgCl$_2$, pH 7.8). The resulting solution was diluted with HEPES buffer (400 μL), and the reaction mixture was gently shaken in a fermentor at 37 °C.[442] After 1 h, solutions of Gal-modified HSA **231** (76.4 kDa, 42.5 Gal units on average, 529 μL, approximately 19 mg/mL, approximately 6 μmol galactose) and α(2,3)-SiaT (360 μL, 1 mg/mL in HEPES buffer, 0.009 μmol) in HEPES buffer were added to the mixture and the extent of reaction was followed by mass spectrometry (MALDI TOF MS). After 8 h, additional solutions of CMP-sialic acid synthetase (100 μL, 10 mg/mL, 0.04 μmol) and α(2,3)-SiaT (360 μL, 1 mg/mL, 0.009 μmol) in HEPES buffer were added to the reaction mixture, but after 24 h still almost no conversion was achieved. Precipitated enzymes and reagents were removed by centrifugation and to that solution was added a precipitate-free solution of α(2,3)-SiaT (0.5 mg, 0.013 μmol) and CMP-sialic acid [made from sialic acid (10.4 mg, 33.4 μmol), CTP disodium salt (20.0 mg, 37.9 μmol) and CMP-sialic acid synthetase (300 μL, 10 mg/mL, 0.12 μmol)] in HEPES buffer. After an additional 24 h at 37 °C approximately 5% conversion was achieved. MALDI TOF MS: found 77.0 kDa.

Attempted CstII-catalyzed sialylation of Gal-modified HSA

To sialic acid (5.2 mg, 16.7 μmol) and CTP disodium salt (8.8 mg, 16.7 μmol) was added CMP-sialic acid synthetase (100 μL, 10 mg/mL, 0.04 μmol) in HEPES buffer (50 mM aq. HEPES, 10 mM MgCl$_2$, pH 7.8). The resulting solution was diluted with HEPES buffer (400 μL) and was gently shaken in a fermentor at 37 °C.[442] After 1 h, solutions of Gal-modified HSA **231** (76.4 kDa, 42.5 Gal units on average, 529 μL, approximately 19 mg/mL, approximately 6 μmol galactose) and CstII (360 μL, 2 mg/mL, 0.01 μmol) in HEPES buffer were added and the extent of reaction was followed by mass spectrometry (MALDI TOF MS). After 8 h, additional solutions of CMP-sialic acid synthetase (100 μL, 10 mg/mL, 0.04 μmol) and CstII (360 μL, 2 mg/mL, 0.01 μmol) in HEPES

buffer were added to the reaction mixture, but after 24 h still almost no conversion was achieved. Precipitated enzymes and reagents were removed by centrifugation and to that solution was added a precipitate-free solution of CstII (1 mg, 0.014 μmol) and CMP-sialic acid [made from sialic acid (10.4 mg, 33.4 μmol), CTP disodium salt (20.0 mg, 37.9 μmol) and CMP-sialic acid synthetase (300 μL, 10 mg/mL, 0.12 μmol)] in HEPES buffer. After an additional 24 h at 37 ºC approximately 10% conversion was achieved. MALDI TOF MS: found 77.6 kDa.

Attempted *T. cruzi* transsialidase-catalyzed sialylation of Gal-modified HSA[443,444]

To a solution of Gal-modified HSA **231** (76.4 kDa, 42.5 Gal units on average, 500 μL, approximately 19 mg/mL, approximately 5 μmol galactose) and fetuin (100 mg, 16 μmol sialic acid) in HEPES buffer (50 mM aq. HEPES, 10 mM MgCl$_2$, pH 7.8) was added a crude batch of *T. cruzi* transsialidase (2.5 mL, approximately 0.05 μmol) in sodium cacodylate buffer (200 mM aq. sodium cacodylate, 20 mM MnCl$_2$, pH 7.7), and the reaction mixture was shaken gently in a fermentor at 37 ºC. The reaction was followed by mass spectrometry (MALDI TOF MS) and within 24 h approximately 6% conversion was achieved. MALDI TOF MS: found 77.2 kDa.

GlcNAc-modified HSA (233)

Following general procedure A, HSA (18 mg, 16 μmol lysines, c_{lysine} = 29 mM) was incubated with IME reagent **229** (156 mg, 0.25 mmol) as an approximately 1:1 mixture with cyanomethyl compound **230** in PBS buffer (550 μL) at pH 9. Workup gave a solution of GlcNAc-modified HSA **233** (47.5 GlcNAc units on average, 3.5 mL, approximately 6 mg/mL). MALDI TOF MS: found 79.5 kDa.

β(1,4)-GalT-catalyzed galactosylation[445] **of GlcNAc-modified HSA**

To a solution of UDP-galactose disodium salt (5.9 mg, 9.7 μmol) in HEPES buffer (100 μL, 50 mM aq. HEPES, 10 mM MgCl$_2$, pH 7.8) were added solutions of GlcNAc-modified HSA **233** (79.5 kDa, 47.5 GlcNAc units on average, 200 μL, approximately 4 mg/mL, 0.5 μmol *N*-acetyl-glucosamine) and β(1,4)-GalT (200 μL, 1 mg/mL, 0.006 μmol) in sodium cacodylate buffer (200 mM aq. sodium cacodylate, 20 mM MnCl$_2$, pH 7.4), and the reaction mixture was gently shaken in a fermentor at 37 ºC. After 24 h, a solution of additional UDP-galactose disodium salt (15.0 mg, 24.6 μmol) and β(1,4)-GalT (200 μL, 1 mg/mL, 0.006 μmol) in sodium cacodylate buffer as well as alkaline phosphatase (10 mg, 13 U/mg, 130 U) were added, and the reaction mixture was stirred for an

additional 24 h. The solution was centrifuged to remove precipitated enzymes and reagents, and the solution was analyzed by mass spectrometry (MALDI TOF MS) revealing 16% conversion (7.4 Gal units were incorporated on average). MALDI TOF MS: found 80.7 kDa.

9.3 Organic synthesis[♦]

1,2,3,4,6-Penta-*O*-acetyl-β-D-galactopyranose (219)[431]

219
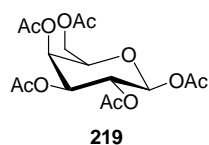
D-Galactose (1.0 g, 5.6 mmol) was added to dry pyridine (10 mL) and acetic anhydride (10 mL, 106 mmol) and stirred at room temperature under an argon atmosphere overnight. The liquids were removed *in vacuo* by co-evaporation with toluene to give **219** (2.2 g, 100%) as a semi-crystalline syrup. R_f 0.55 (EtOAc/petrol = 1:1); ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.72 (d, *J* = 8.3 Hz, 1H, H$_1$), 5.49-5.47 (m, 1H, H$_4$), 5.33-5.31 (m, 1H, H$_2$), 5.10-5.03 (m, 1H, H$_3$), 4.37-4.30 (m, 1H, H$_5$), 4.18-4.02 (m, 2H, H$_{6,6'}$), 2.15 (s, 3H), 2.14 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H) (CH$_3$ x 5); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.5, 170.3, 170.0, 169.1 (C=O x 5), 89.8 (C-1), 68.9, 67.5, 67.5, 66.5, 61.4 (C-2,3,4,5,6), 21.0, 20.9, 20.8, 20.7 (CH$_3$ x 5).

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (220)[431]

220
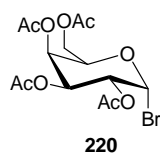
HBr (153 mL, 33% in acetic acid, 845 mmol) was added to a solution of **219** (30.0 g, 77 mmol) in DCM (80 mL) at 0 ºC, and the mixture was stirred at room temperature under an argon atmosphere for 4.75 h. The reaction mixture was quenched by addition of H$_2$O (1 L) followed by immediate extraction with DCM (3 x 250 mL). The combined organic phases were neutralized by washing with sat. aq. NaHCO$_3$ (1 L + 500 mL), dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to give **220** (31.7 g, 100%) as a brown oil. R_f 0.73 (EtOAc/petrol = 1:1); $[\alpha]_D^{22}$ +212 (CHCl$_3$, *c* 1.0), (lit.[478]

[♦] A small part of the characterization of the intermediates towards the Gal-IME reagent **223** was performed by Oliver Pearce.

$[\alpha]_D$ +227.4, CHCl$_3$, c 1.7); ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.70 (d, J = 4.0 Hz, 1H, H$_1$), 5.52 (d, J = 3.0 Hz, 1H, H$_4$), 5.41 (dd, J = 3.3, 10.6 Hz, 1H, H$_3$), 5.05 (dd, J = 3.9, 10.6 Hz, 1H, H$_2$), 4.49 (t, J = 6.6 Hz, 1H, H$_5$), 4.15 (dd, J = 6.6, 11.4 Hz, 2H, H$_{6,6'}$), 2.16 (s, 3H), 2.12 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H) (CH$_3$ x 4); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.5, 170.2, 170.0, 169.9 (C=O x 4), 88.2 (C-1), 71.2, 68.1, 67.9, 67.1, 61.1 (C-2,3,4,5,6), 20.9, 20.8, 20.7 (CH$_3$ x 4).

2-*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-2-thiouronium bromide (221)[431]

221
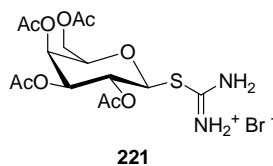
Bromide **220** (5.73 g, 13.9 mmol) and thiourea (1.59 g, 20.9 mmol) were dissolved in acetone (40 mL) and heated to reflux. After 3 h, the reaction mixture was cooled to room temperature and the product was crystallized by addition of petrol (100 mL). The solution was filtered and the solids were dried *in vacuo* to give **221** (6.62 g, 97%) as a sticky, white solid. R_f 0.03 (EtOAc/petrol = 1:1); mp 169 – 171 ºC (acetone/petrol) (lit.[479] mp 169.5 ºC); $[\alpha]_D^{22}$ +17 (EtOH, c 2.0) (lit.[479] $[\alpha]_D^{22}$ +16.0, EtOH, c 1.6); ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.34 (s, 2H), 9.13 (s, 2H) (NH$_2$ x 2), 5.72 (d, J = 9.9 Hz, 1H, H$_1$), 5.38 (d, J = 3.2 Hz, 1H, H$_4$), 5.23 (dd, J = 3.4, 9.9 Hz, 1H, H$_3$), 5.10 (t, J = 9.9 Hz, 1H, H$_2$), 4.45 (t, J = 6.2 Hz, 1H, H$_5$), 4.13-4.02 (m, 2H, H$_{6,6'}$), 2.13 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H) (CH$_3$ x 4); ^{13}C NMR (100 MHz, d_6-DMSO): δ 169.9, 169.8, 169.7, 169.4 (C=O x 4), 166.3 (C=NH), 80.1 (C-1), 74.4, 70.4, 67.1, 66.3, 61.2 (C-2,3,4,5,6), 20.5, 20.4, 20,3 (CH$_3$ x 4).

Cyanomethyl 2,3,4,6-tetra-*O*-acetyl-β-D-thiogalactopyranoside (222)[431]

222
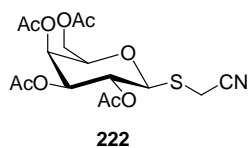
Thiouronium bromide **221** (6.56 g, 13.5 mmol), Na$_2$S$_2$O$_5$ (5.2 g, 27.5 mmol) and K$_2$CO$_3$ (2.0 g, 14.8 mmol) were dissolved in acetone/water (1:1, 80 mL). Chloroacetonitrile (3.4 mL, 54.0 mmol) was added to the solution and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated to give a yellow-white solid, to which ice-cold H$_2$O (200 mL) was added to dissolve the salts. The mixture was filtered and washed with water to give **222** (4.00 g) as yellow crystals. Additional crystals of the target compound (0.74 g) could be isolated by extraction of the combined filtrate and washings with DCM (100 mL). Recrystallization from hot MeOH gave **222**

(4.74 g, 87%) as white crystals. R_f 0.49 (EtOAc/petrol = 1:1); mp 92 – 94 ºC (MeOH) (lit.[422] mp 95 – 97 ºC); $[\alpha]_D^{23}$ -30 (CHCl$_3$, c 0.8) (lit.[422] $[\alpha]_D^{25}$ -30.0, MeOH, c 5.02); IR (film): 2974, 2937, 2310, 1745, 1369, 1222; ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.46 (d, J = 3.3 Hz, 1H, $\underline{H}_4$), 5.25 (t, J = 9.9 Hz, 1H, $\underline{H}_2$), 5.09 (dd, J = 3.3, 10.0 Hz, 1H, $\underline{H}_3$), 4.70 (d, J = 9.9 Hz, 1H, $\underline{H}_1$), 4.21-4.10 (m, 1H, $\underline{H}_{6,6'}$), 4.01 (t, J = 7.0 Hz, 1H, $\underline{H}_5$), 3.64 (d, J = 17.0 Hz, 1H, SC$\underline{H}$H'), 3.34 (d, J = 17.0 Hz, 1H, SCH$\underline{H}$'), 2.17 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H) (C$\underline{H}_3$ x 4); ^{13}C NMR (100 MHz, CDCl$_3$): δ 170.5, 170.2, 170.0, 169.9 ($\underline{C}$=O x 4), 115.9 ($\underline{C}$N), 82.4 ($\underline{C}$-1), 75.1, 71.6, 67.2, 66.9, 61.5 ($\underline{C}$-2,3,4,5,6), 20.8, 20.7 ($\underline{C}$H$_3$ x 4), 14.6 ($\underline{C}$H$_2$); m/z (ESI$^+$) C$_{16}$H$_{21}$NO$_9$SNa 426 ([M + Na] 94%), C$_{32}$H$_{42}$N$_2$O$_{18}$S$_2$Na 829 ([2M + Na] 100%).

2-Imino-2-methoxyethyl β-D-thiogalactopyranoside (223)[431]
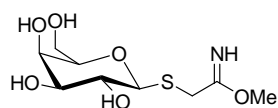
223

Cyano compound **222** (2.0 g, 5.0 mmol) was added to a solution of sodium (460 mg, 20.0 mmol) in dry MeOH (80 mL). The solution was stirred at room temperature under an argon atmosphere for 72 h. The pH of the mixture was adjusted to 7-8 by addition of Dowex H$^+$ followed by filtration and thorough wash of the filtercake with dry MeOH. Evaporation of the liquids afforded **223** in 57% yield (1.26 g, 100% conversion, as a 4:3 mixture with **224**) as a slightly orange gum, which turned into a fine crystalline product while kept under high vacuum. R_f 0.29 (EtOAc/MeOH = 4:1); mp: the crystals decompose at ~45 ºC; $[\alpha]_D^{23}$ -43 (MeOH, c 2.0); IR (film): 3357, 2929, 2349, 2164, 1652, 1593, 1407, 1353, 1086, 1060; ^{1}H NMR (400 MHz, CD$_3$OD): δ 8.54 (s, 1H, N$\underline{H}$), 4.54 (d, J = 9.5 Hz, 1H, $\underline{H}_1$-CN), 4.31 (d, J = 9.4 Hz, 1H, $\underline{H}_1$-IME), 4.04-3.38 (m, 6H, $\underline{H}_{2,3,4,5,6,6'}$), 3.35 (s, 3H, OC$\underline{H}_3$), 1.90 (s, 2H, SC$\underline{H}_2$); ^{13}C NMR (100 MHz, CD$_3$OD): δ 170.5 ($\underline{C}$=NH), 118.8 ($\underline{C}$N), 87.1, 85.9 ($\underline{C}$-1 x 2), 80.9, 80.7 ($\underline{C}$-2 x 2), 76.1, 76.0, 71.3, 71.2, 70.5, 70.4, 62.6, 62.5 ($\underline{C}$-3,4,5,6 x 2), 49.2 (O$\underline{C}$H$_3$), 24.2 (S$\underline{C}$H$_2$); m/z (ESI$^+$) **223**: C$_9$H$_{17}$NO$_6$SNa 290 ([M + Na] 100%), **224**: C$_8$H$_{13}$NO$_5$SNa 258 ([M + Na] 74%).

166

2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose (225)[431]

225
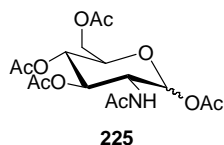
To *N*-acetyl-D-glucosamine (10.0 g, 45 mmol) was added dry pyridine (62.5 mL) and acetic anhydride (62.5 mL, 661 mmol), and the mixture was stirred at room temperature under an argon atmosphere overnight. The liquids were removed *in vacuo* by co-evaporation with toluene to give a 13:2 α:β anomeric mixture of **225** (17.6 g, 100%) as a white solid. R_f 0.38 (EtOAc/petrol = 1:1); mp 121 – 123 ºC (lit.[480] mp α-anomer 135 – 136 ºC, β-anomer 185 – 188 ºC (EtOH)); $[\alpha]_D^{22}$ +74 (CHCl$_3$, *c* 1.0) (lit.[480] $[\alpha]_D$ α-anomer +89.4, β-anomer +1.2, CHCl$_3$, *c* 1); ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.16-6.12 (m, 1H, N$\underline{H}$-α), 5.92 (d, *J* = 9.3 Hz, 1H, N$\underline{H}$-β), 5.70 (d, *J* = 8.2 Hz, 1H, $\underline{H}_1$-β), 5.67 (d, *J* = 2.6 Hz, 1H, $\underline{H}_1$-α), 5.26-5.06 (m, 5H), 4.46 (dd, *J* = 9.1, 10.6 Hz, 1H), 4.31-4.18 (m, 3H), 4.11-3.93 (m, 2H), 3.80 (dd, *J* = 2.2, 4.3 Hz, 1H) (α- and β-$\underline{H}_{2,3,4,5,6,6'}$), 2.17 (s, 3H), 2.09 (s, 3H), 2.06 (s, 6H), 2.02 (m, 12H), 1.91 (s, 6H) (C$\underline{H}_3$ x 10); ^{13}C NMR (100 MHz, CDCl$_3$): δ 171.7, 171.1, 170.7, 170.2, 170.0, 169.5, 169.3, 169.1, 168.7 ($\underline{C}$=O x 10), 92.5 ($\underline{C}$-1-β), 90.7 ($\underline{C}$-1-α), 72.8, 72.6, 70.6, 69.7, 67.7, 67.5, 61.6, 61.5, 52.9, 51.0 (α- and β-$\underline{C}$-2,3,4,5,6), 23.2, 23.0 (NHCO$\underline{C}$H$_3$ x 2), 21.0, 20.9, 20.7, 20.6, 20.5 ($\underline{C}$H$_3$ x 8).

2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (226)[431]


226
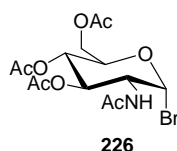
HBr (95 mL, 33% in acetic acid, 525 mmol) was added to a solution of **225** (17.5 g, 45 mmol) in DCM (50 mL) at 0 ºC, and the reaction mixture was stirred at room temperature under an argon atmosphere for 4 h. The reaction mixture was quenched by addition of H$_2$O (500 mL) followed by immediate extraction with DCM (3 x 200 mL). The combined organic layers were neutralized by washing with sat. aq. NaHCO$_3$ (3 x 250 mL). The combined aq. washings were extracted with DCM (100 mL) and the combined organic layers were dried (Na$_2$SO$_4$), filtered and concentrated *in vacuo* to give **226** (8.73 g, 47%) as a yellow oil, which solidified upon standing. R_f 0.58 (EtOAc/petrol = 1:1); mp 86 – 89 ºC (CH$_2$Cl$_2$) (lit.[481] mp 89 – 91 ºC); ^{1}H NMR (400 MHz, CDCl$_3$): δ 6.52 (d, *J* = 3.6 Hz, 1H, $\underline{H}_1$), 6.18 (dd, *J* = 6.0, 12.6 Hz, 1H, N$\underline{H}$), 5.45-5.04 (m, 3H), 4.39-4.04 (m, 3H) (H$_{2,3,4,5,6,6'}$), 2.09 (s, 3H), 2.07 (s, 6H), 2.04 (s, 3H) (C$\underline{H}_3$ x 4); ^{13}C NMR (100 MHz, CDCl$_3$): δ

176.3, 171.5, 170.6, 169.2 ($\underline{C}$=O x 4), 91.1 ($\underline{C}$-1), 72.6, 70.8, 66.7, 61.0, 53.5 ($\underline{C}$-2,3,4,5,6), 23.0 (NHCO$\underline{C}$H$_3$), 20.8, 20.7, 20.6 ($\underline{C}$H$_3$ x 3).

2-*S*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiouronium bromide (227)[431]
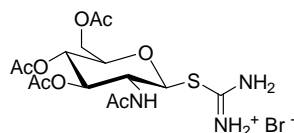
227

Bromide **226** (8.28 g, 20.2 mmol), thiourea (2.31 g, 30.3 mmol) and acetone (190 mL) were heated to reflux. After 4 h, the reaction mixture was cooled to room temperature and the product was crystallized by addition of petrol (650 mL). The solution was filtered and the solids were dried *in vacuo* to give **227** (8.62 g, 88%) as pale pink crystals. R_f 0.06 (EtOAc/petrol = 1:1); mp 145 – 147 ºC (acetone/petrol); $[\alpha]_D^{23}$ -8.1 (EtOH, *c* 1.0); IR (film): 3304, 3200, 1745, 1653, 1631, 1375, 1237, 1045; ^{1}H NMR (400 MHz, d_6-DMSO): δ 9.15 (s, 2H), 9.03 (s, 2H) (N$\underline{H}_2$ x 2), 6.18 (d, *J* = 3.6 Hz, 1H, N$\underline{H}$Ac), 5.56 (d, *J* = 10.3 Hz, 1H, $\underline{H}_1$), 5.47-4.71 (m, 3H), 4.21-3.96 (m, 3H) ($\underline{H}_{2,3,4,5,6,6}$), 2.01, 1.98, 1.94, 1.80 (CH$_3$ x 4); ^{13}C NMR (100 MHz, d_6-DMSO): δ 170.8, 170.7, 170.4, 170.1 ($\underline{C}$=O x 4), 168.0 ($\underline{C}$=NH), 81.5 ($\underline{C}$-1), 75.7, 73.4, 68.7, 62.4, 52.1 ($\underline{C}$-2,3,4,5,6), 23.4 (NHCO$\underline{C}$H$_3$), 21.4, 21.3, 21.2 ($\underline{C}$H$_3$ x 3).

Cyanomethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-thioglucopyranoside (228)[431]

228
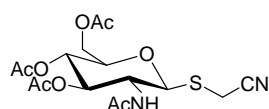
Thiouronium bromide **227** (8.3 g, 17.1 mmol), Na$_2$S$_2$O$_5$ (6.6 g, 34.7 mmol) and K$_2$CO$_3$ (2.7 g, 19.5 mmol) were dissolved in acetone/water (1:1, 80 mL). Chloroacetonitrile (4.3 mL, 67.8 mmol) was added to the solution and the reaction mixture was stirred at room temperature for 4.5 h. The product was precipitated by addition of ice (300 mL), filtered and recrystallized from dry MeOH to give **228** (4.86 g, 71%) as a white solid. R_f 0.13 (EtOAc/petrol = 1:1); mp 176 – 178 ºC (MeOH) (lit.[422] mp 181 – 182 ºC); $[\alpha]_D^{23}$ –76 (MeOH, *c* 0.7) (lit.[422] $[\alpha]_D^{22}$ –80.8, MeOH, *c* 5.05); IR (film): 3331, 2957, 2251, 1739, 1657, 1527, 1401, 1376, 1238, 1048; ^{1}H NMR (400 MHz, CDCl$_3$): δ 5.88 (d, *J* = 9.2 Hz, 1H, N$\underline{H}$), 5.23-5.09 (m, 2H, $\underline{H}_{3,4}$), 4.78 (d, *J* = 10.4 Hz, 1H, $\underline{H}_1$), 4.28-4.14 (m, 3H,

$\underline{H}_{2,6,6'}$), 3.77 (dd, J = 4.7, 9.6 Hz, 1H, $\underline{H}_5$), 3.67 (d, J = 17.0 Hz, 1H, SC$\underline{H}$H'), 3.32 (d, J = 17.0 Hz, 1H, SCH$\underline{H}$'), 2.10 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H) (C$\underline{H}_3$ x 4); ^{13}C NMR (100 MHz, CDCl$_3$): δ 171.4, 170.8, 170.6, 169.4 ($\underline{C}$=O x 4), 116.3 ($\underline{C}$N), 83.2 ($\underline{C}$-1), 76.4, 73.4, 68.1, 62.0, 52.9 ($\underline{C}$-2,3,4,5,6), 23.3 (NHCO$\underline{C}$H$_3$), 20.9, 20.8, 20.7 ($\underline{C}$H$_3$ x 3), 14.8 ($\underline{C}$H$_2$); m/z (ESI$^+$) C$_{16}$H$_{22}$N$_2$O$_8$SNa 425 ([M + Na] 45%), C$_{32}$H$_{44}$N$_4$O$_{16}$S$_2$Na 827 ([2M + Na] 100%).

2-Imino-2-methoxyethyl 2-acetamido-2-deoxy-β-D-thioglucopyranoside (229)[431]

229
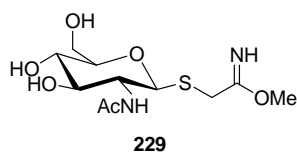
Cyano compound **228** (2.0 g, 5.0 mmol) was added to a solution of sodium (460 mg, 20.0 mmol) in dry MeOH (80 mL). The solution was stirred at room temperature under an argon atmosphere for 72 h. The pH of the mixture was adjusted to 7-8 by addition of Dowex H$^+$ followed by filtration and thorough wash of the filtercake with dry MeOH. Evaporation of the liquids *in vacuo* afforded **229** in 63% yield (1.5 g, 100% conversion, as a 5:3 mixture with **230**) as white crystals. When exposed to air, the crystals turned into a gum. R_f 0.28 (EtOAc/MeOH = 4:1); mp 136 – 139 °C (MeOH); $[\alpha]_D^{23}$ – 61 (MeOH, c 1.0); IR (film): 3282, 2927, 2250, 1651, 1563, 1446, 1375, 1313, 1063; ^{1}H NMR (400 MHz, CD$_3$OD): δ 4.66 (d, J = 10.4 Hz, 1H, $\underline{H}_1$-IME), 4.59 (d, J = 10.4 Hz, 1H, N$\underline{H}$COCH$_3$), 4.47 (d, J = 10.4 Hz, 1H, $\underline{H}_1$-CN), 3.94-3.38 (m, 6H, $\underline{H}_{2,3,4,5,6,6'}$), 3.35 (s, 3H, OC$\underline{H}_3$), 1.98 (s, 3H, COC$\underline{H}_3$), 1.90 (s, 2H, SC$\underline{H}_2$); ^{13}C NMR (100 MHz, CD$_3$OD): δ 173.7 ($\underline{C}$=NH), 109.5 ($\underline{C}$N), 82.4 ($\underline{C}$-1), 76.9 ($\underline{C}$-2), 71.9, 71.8, 62.9, 55.7 ($\underline{C}$-3,4,5,6), 49.9 (OC$\underline{H}_3$), 22.9, 22.8 (S$\underline{C}$H$_2$, CH$_3$); m/z (ESI$^+$) **229**: C$_{11}$H$_{20}$N$_2$O$_6$SNa 331 ([M + Na] 100%), **230**: C$_{10}$H$_{16}$N$_2$O$_5$SNa 299 ([M + Na] 66%).

p-Bromobenzoic acid *N*-hydroxysuccinimide ester (236)[446]

236
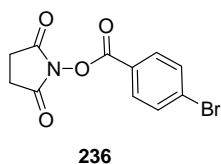
A solution of *p*-bromobenzoic acid (**205**) (500 mg, 2.5 mmol) and DCC (510 mg, 2.5 mmol) in DCM (10 mL) was stirred for 5 min at 0 °C. *N*-hydroxysuccinimide (**235**) (344 g, 3.0 mmol) was added to the solution, and after stirring under an argon atmosphere for 10 min, the ice bath was removed and the solution was allowed to reach room temperature. After stirring for 3.5 h, the solution was filtered through a plug of Celite, the solids were washed with DCM (50 mL) and the

combined liquids were concentrated *in vacuo* followed by purification by flash column chromatography (toluene/EtOAc = 9:1) to give **236** (603 mg, 81%) as white crystals. R_f 0.44 (toluene/EtOAc = 4:1); mp 228 – 230 ℃ (acetone) (lit.[449] mp 227 – 230 ℃ (acetone)); IR (KBr): 3080, 2988, 1772, 1727, 1589, 1402, 1377, 1234, 1211, 1078, 1066, 992, 847, 741; ^{1}H NMR (400 MHz, CDCl$_3$): δ 7.99 (d, J = 8.7 Hz, 2H, Ar $\underline{H}$ x 2), 7.67 (d, J = 8.7 Hz, 2H, Ar $\underline{H}$ x 2), 2.91 (s, 4H, C$\underline{H}_2$ x 2); ^{13}C NMR (100 MHz, CDCl$_3$): δ 169.2 ($\underline{C}$ON x 2), 161.4 ($\underline{C}$OO), 132.5, 132.1 ($\underline{C}$H-Ar x 4), 130.6, 124.2 ($\underline{C}$-Ar x 2), 25.8 ($\underline{C}$H$_2$ x 2); m/z (ESI⁻) C$_7$H$_4$O$_2$Br 199 ([M – C$_4$H$_4$O$_2$N] 96%) and 201 ([M+2 – C$_4$H$_4$O$_2$N] 100%).

p-Bromobenzoic acid *N*-hydroxysuccinimide ester (**236**)[449]

N-Hydroxysuccinimide (**235**) (286 mg, 2.5 mmol), *p*-bromobenzoic acid (**205**) (500 mg, 2.5 mmol) and DCC (501 mg, 2.5 mmol) in dry DMF (8 mL) were stirred at room temperature under an argon atmosphere overnight. The reaction mixture was filtered and the solids were washed with EtOAc. Concentration of the liquids *in vacuo* and recrystallization from acetone gave **236** (695 mg, 94%) as white crystals. Spectroscopic data are given above.

p-Bromobenzoic acid *N*-hydroxy-sulfosuccinimide ester sodium salt (**238**)[427,448]

238
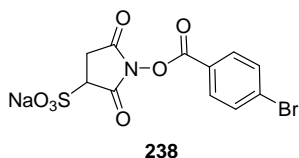
To *N*-hydroxy-sulfosuccinimide sodium salt (**237**) (434 mg, 2.0 mmol), *p*-bromobenzoic acid (**205**) (402 mg, 2.0 mmol) and DCC (411 mg, 2.2 mmol) was added dry DMF (5 mL), and the reaction mixture was stirred at room temperature under an argon atmosphere overnight. The mixture was cooled to 4 ℃ for 4 h, then filtered and the solids were washed with dry DMF (5 mL). The combined filtrate and washings were concentrated *in vacuo* to give a liquid residue, which was crystallized by addition of EtOAc (25 mL). Filtration and drying *in vacuo* afforded **238** (733 mg, 92%) as white crystals. Mp > 350 ℃ (decomposes slowly); IR (KBr): 3447, 2938, 1778, 1740, 1652, 1592, 1403, 1372, 1228, 1053, 989, 744; ^{1}H NMR (400 MHz, d_6-DMSO): δ 8.03 (d, J = 8.4 Hz, 2H, Ar $\underline{H}$ x 2), 7.87 (d, J = 8.5 Hz, 2H, Ar $\underline{H}$ x 2), 4.12-3.97 (m, 1H, O$_3$SC$\underline{H}$), 2.97-2.92 (m, 2H, C$\underline{H}_2$); ^{13}C NMR (100 MHz, d_6-DMSO): δ 168.8, 165.4 ($\underline{C}$ON x 2), 161.2 ($\underline{C}$OO), 132.8, 131.9 ($\underline{C}$H-Ar x 4), 130.0, 123.6 ($\underline{C}$-Ar x 2), 56.3 ($\underline{C}$H$_2$SO$_3$Na), 31.0 ($\underline{C}$H$_2$); m/z (ESI⁻) C$_{11}$H$_7$NO$_7$SBr 376 ([M – Na] 86%) and 378 ([M+2 – Na] 100%).

10 Appendices

10.1 Appendix I – MS data for cyclodextrin products

MALDI TOF MS data for the crude and peracetylated product mixtures formed by the attempted decarbonylation of β-cyclodextrin monoaldehyde (**105**) following general decarbonylation procedure A are shown in Table 34 and Table 35, respectively. See section 8.2 for further experimental details.

Table 34. MALDI TOF MS data of the crude product mixture from the attempted decarbonylation of β-cyclodextrin monoaldehyde.

Number of glucose residues incorporated	Observed Mass (Da)		
	zero Ac groups	one Ac group	two Ac groups
0	1157.6	1199.6	1241.7
1	1319.7	1361.7	1403.7
2	1481.8	1523.8	1565.8
3	1643.9	1685.9	1627.9

Expected mass of β-cyclodextrin (**104**): 1157.4 Da.

The data in Table 34 fits well with incorporation of up to three glucose residues (162 Da) and up to two acetyl groups (42 Da).

Table 35. MALDI TOF MS data of the peracetylated product mixture from the attempted decarbonylation of β-cyclodextrin monoaldehyde.

Number of glucose residues incorporated	Observed Mass (Da)
0	2040.4
1	2328.8
2	2618.2
3	2906.6
4	3195.4
5	3484.1
6	3772.8
7	4061.5

Expected mass of peracetylated β-cyclodextrin: 2040.1 Da.

The data in Table 35 fits well with incorporation of up to seven peracetylated glucose residues (288 Da). In addition to these major compounds, minor compounds corresponding to incorporation of up to 12 peracetylated glucose residues could also be observed.

10.2 Appendix II – Solvent accessibilities of HSA

Relative solvent accessibilities were calculated for each individual amino acid residue of HSA using the computer program Naccess.[455] Solvent accessibilities were calculated for the amino acids 5-582 in the A strain of dimeric HSA.[410] The B strain gave nearly identical values. The relative residue accessibility of an amino acid X was calculated as % total side-chain accessibility as compared to that residue in the corresponding Ala-X-Ala tripeptide. The solvent accessibility is thereby a measure of the accessibility of a given amino acid X relative to the same residue in the tripeptide Ala-X-Ala, and accessibilities greater than 100% is not unusual. In that case the amino acid X is just more accessible in the protein than in the Ala-X-Ala tripeptide. The calculations were performed using different probe sizes (1.0, 1.4 and 2.8 Å, respectively), and as can be seen from the reactive accessibility diagrams below, fewer amino acid side-chains become accessible as probe size increases.

Probe size: 1.0 Å

Probe size: 1.4 Å

Probe size: 2.8 Å
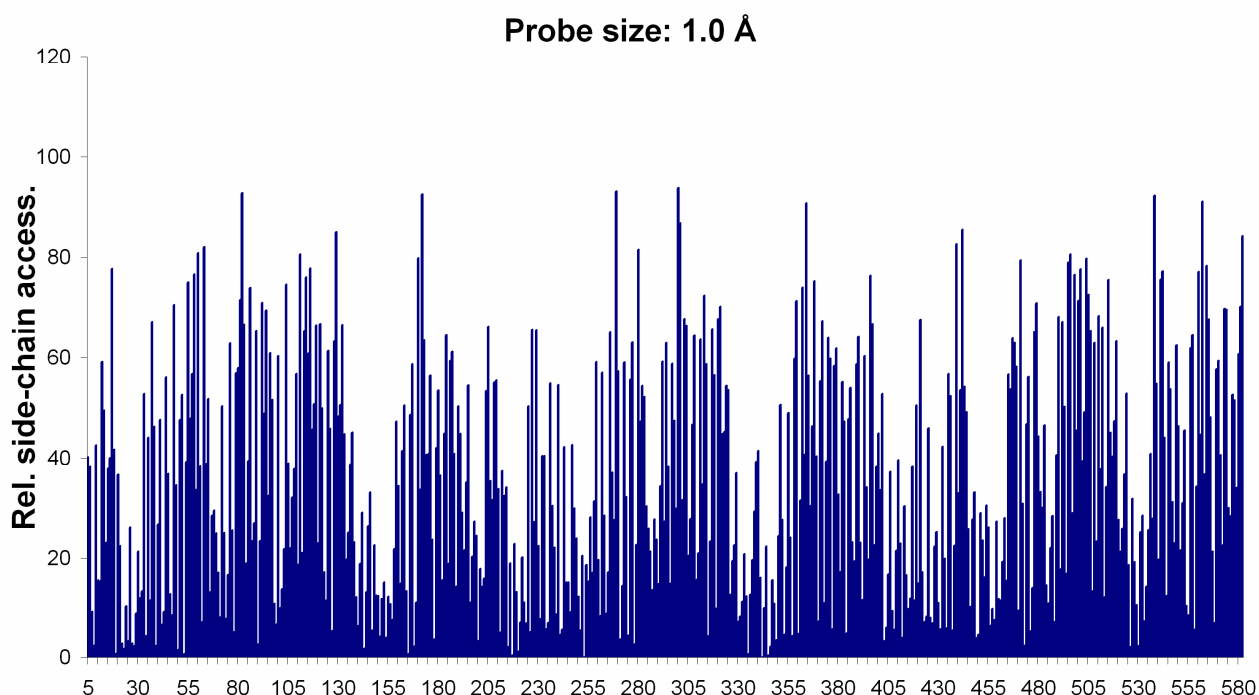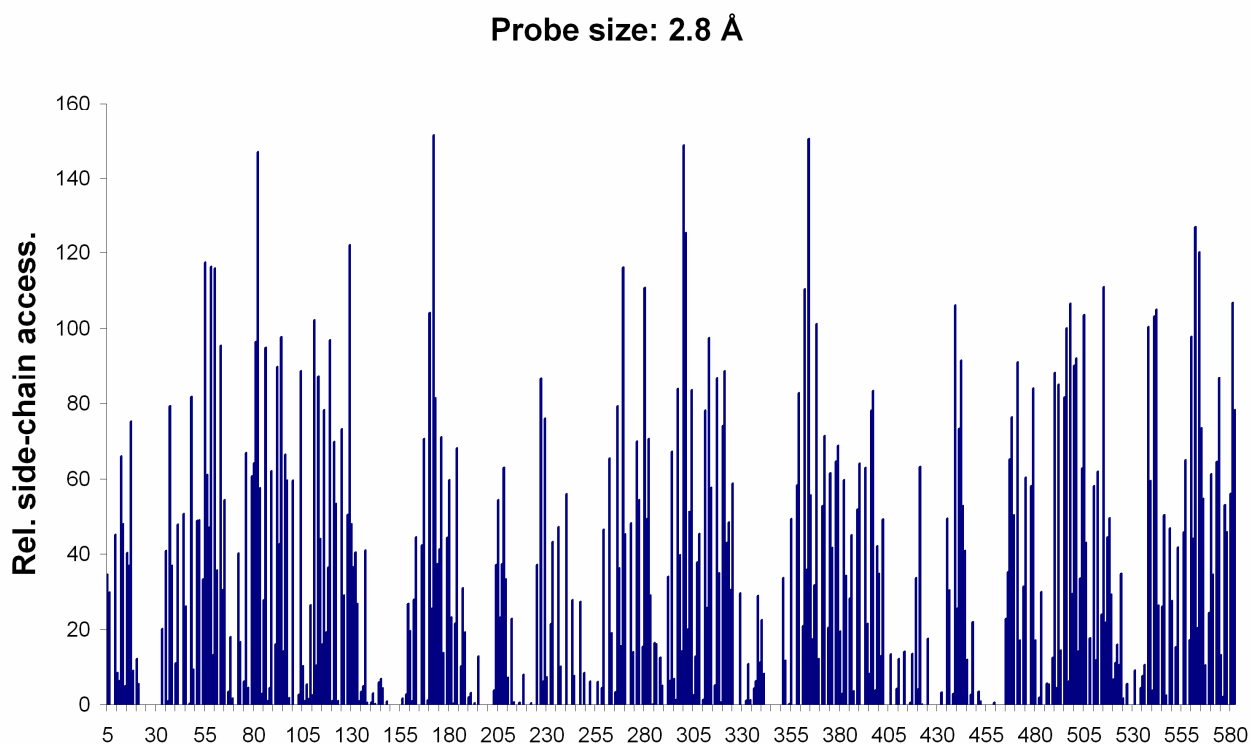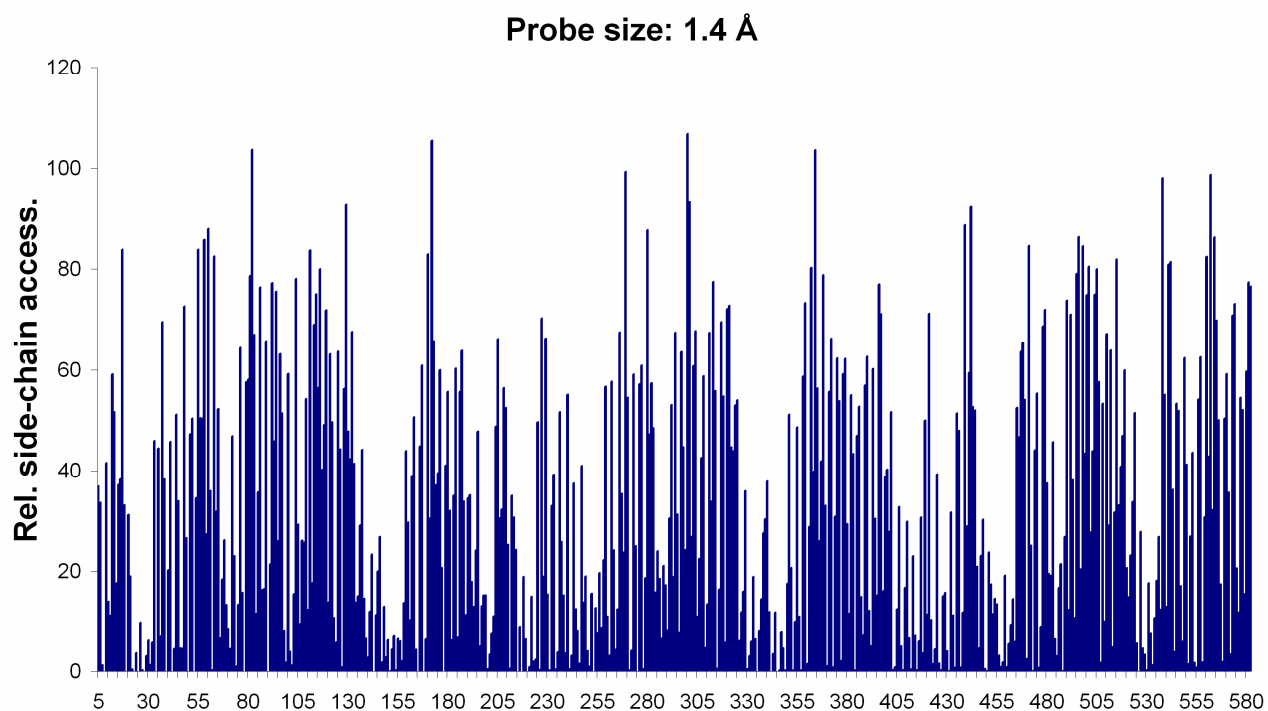
The relative accessibilities for the lysine residues in HSA are given in Table 36. Based on these data, it is anticipated that lysine residues with a higher relative accessibility are more exposed and

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will therefore be more susceptible to electrophilic attack, however, no direct correlation between AG-modification site and calculated accessibility could be observed. For example, the five most accessible lysine residues according to Table 36 (lysines 439, 538, 541, 560 and 564) are not found to be modified by ibufenac acyl glucuronide **213**.

Table 36. Total side-chain accessibilities (%) for lysine residues in HSA relative to the accessibility of the same amino acid in the corresponding Ala-X-Ala tripeptide at different probe sizes assessed by the computer program Naccess.[455]

Residue		Probe size			Residue		Probe size		
		1.0 Å	1.4 Å	2.8 Å			1.0 Å	1.4 Å	2.8 Å
Lys	12	59,10	59,10	66,10	Lys	323	45,30	44,00	48,40
Lys	20	36,60	31,20	12,10	Lys	351	50,60	51,20	33,70
Lys	41	47,70	45,80	47,80	Lys	359	71,30	73,30	82,70
Lys	51	47,70	47,30	48,80	Lys	372	67,40	66,30	71,40
Lys	64	38,90	31,90	30,50	Lys	378	58,40	59,20	64,70
Lys	73	25,00	23,00	16,80	Lys	389	58,70	57,00	52,10
Lys	93	48,90	45,90	42,70	Lys	402	52,80	51,70	49,10
Lys	106	22,00	9,40	1,00	Lys	413	30,30	23,00	14,00
Lys	136	38,80	29,10	4,90	Lys	414	16,60	7,10	0,00
Lys	137	45,10	44,20	41,00	Lys	432	42,40	31,70	3,20
Lys	159	47,30	44,00	26,90	Lys	436	52,40	48,00	30,40
Lys	162	41,50	39,00	28,00	Lys	439	82,60	88,70	106,10
Lys	174	40,80	37,20	37,40	Lys	444	49,20	52,00	40,90
Lys	181	36,50	32,10	23,30	Lys	466	53,70	46,70	35,20
Lys	190	50,40	34,60	2,00	Lys	475	56,20	55,40	60,40
Lys	195	54,50	47,80	12,70	Lys	500	71,40	74,90	90,30
Lys	199	24,40	15,20	0,00	Lys	519	63,20	60,00	29,30
Lys	205	66,30	66,20	54,50	Lys	524	52,90	51,50	34,80
Lys	212	37,40	35,10	22,90	Lys	525	18,60	5,60	1,60
Lys	225	50,40	49,70	37,20	Lys	534	14,30	10,50	4,40
Lys	233	40,50	39,30	43,20	Lys	536	40,90	26,90	10,50
Lys	240	54,60	55,10	56,10	Lys	538	92,40	98,10	100,40
Lys	262	57,00	57,70	65,50	Lys	541	75,60	80,90	103,20
Lys	274	32,20	25,00	13,90	Lys	545	59,00	53,40	26,20
Lys	276	55,70	57,20	70,00	Lys	557	64,40	62,60	65,00
Lys	281	47,30	47,30	49,20	Lys	560	77,10	82,40	97,90
Lys	286	21,30	18,50	16,20	Lys	564	78,30	86,30	120,10
Lys	313	72,40	77,50	97,50	Lys	573	69,80	70,90	64,60
Lys	317	65,80	69,50	86,70	Lys	574	69,60	73,10	86,80

As mentioned in chapter 6 (section 6.2.5), the following lysine residues were found to be glycosylated and/or transacylated by ibufenac acyl glucuronide **213**: Lysines 137, 195, 199, 205, 436, 525 and 534 (Figure 14).
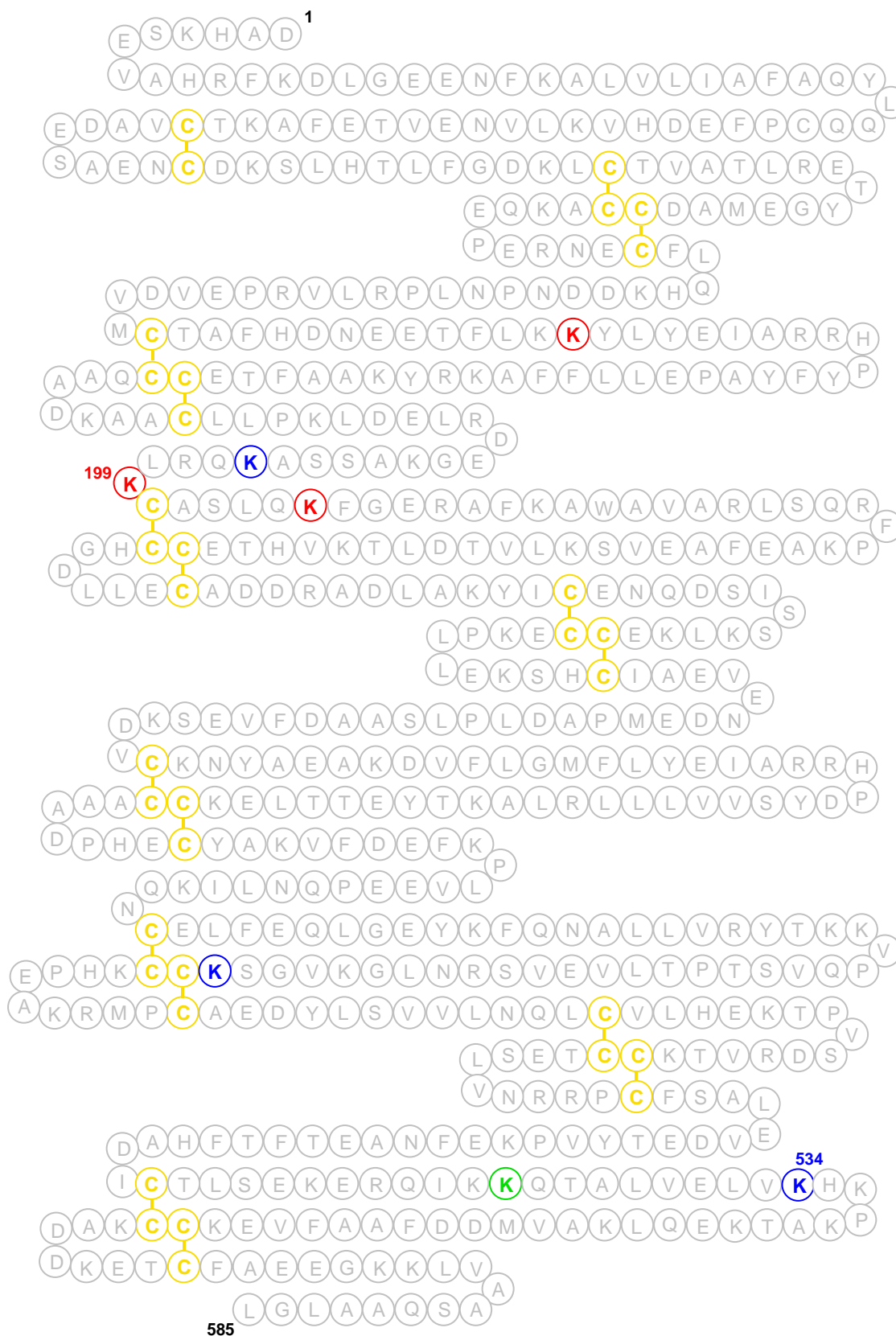
Figure 14. Sequence map of HSA indicating primary amino acid sequence including disulfide bonds (yellow).[408,410] Chemoselective reaction sites for glycosylation with ibufenac AG **213** are coded red; sites for transacylation are coded blue, whereas Lys525 exhibiting both glycosylation and transacylation reactivity is coded green.

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Especially Lys525, which exhibits both glycosylation and transacylation reactivity, has a very low accessibility. Also the low accessibility of Lys199 is in contrast to its observed reactivity, however, the reactivity of this amino acid can be rationalized by an unusually low pK_a of 7.9,[482] which may be explained by near proximity to His242.[408] In general, amino groups with low pK_a values are more reactive, and furthermore, it seems that glycosylation predominantly occurs at amino groups, which are close to other amino groups.[417] It has previously been suggested that amino acid residues, which are part of Lys-Lys, Lys-His or Lys-His-Lys sequences or which are close to disulfide bonds thereby enabling close interaction with a positively charged amino group from another part of the protein, are the most reactive sites with respect to non-enzymatic glycosylation.[417] Our results are in accordance with this assumption, since Lys137, Lys525 and Lys534 are neighbors to either Lys or His residues, whereas the amino acids Lys199 and Lys436 are placed next to a disulfide bond (Figure 14). The lack of a direct correlation between AG-modification site and accessibility is in accordance with a previous report on non-enzymatic glycosylation of HSA concluding that accessible surface areas cannot directly be used to predict major glycosylation sites.[418]

10.3 Appendix III – MS data for tryptic peptides

The analytical data for tryptic peptides of HSA modified with *p*-bromobenzoic acid AG **211**, ponalrestat AG **212** and *p*-bromobenzoic acid NHS ester **236** are shown in Table 37, Table 38 and Table 39, respectively. The tryptic peptides of HSA modified with acyl glucuronides **211** and **212** (Table 37 and Table 38) have only been analyzed by mass spectrometry, and no MS/MS analysis has been performed. Since these data are not supported by MS/MS analysis, they are not conclusive, but they give a good *indication* of the sites of modification in the case of ponalrestat and *p*-bromobenzoic acid AGs.

In general, the observed sites of modification of HSA by ibufenac AG (**213**) (Table 30), *p*-bromobenzoic acid AG (**211**) (Table 37) and ponalrestat AG (**212**) (Table 38) as well as p-bromobenzoic acid NHS ester **236** (Table 39) are in accordance with previously reported sites of modification of HSA *in vitro*[483] and *in vivo*.[416,417]

Table 37. Tryptic peptides of HSA modified with *p*-bromobenzoic acid AG **211**.

Retention time (min)	*m/z* predicted	*m/z* observed	Residues	Sequence	Modified lysine	Modification
22.80	708.6	707.6	137 – 144	**K**(+)YLYEIAR	K-137	Glycosylation
32.32	822.5	822.2	191 – 195	ASSA**K**(+)	K-195	Glycosylation
29.23	1106.8	1106.7	191 – 197	ASSA**K**(+)QR	K-195	Glycosylation
15.59	690.6	690.6	198 – 205	AF**K**(+)AWAVAR	K-212	Glycosylation
18.58	589.5	589.9	539 – 545	AT**K**(+)EQLK or ATKEQL**K**(+)	K-541/545	Glycosylation

Modified protein was hydrolyzed with trypsin and analyzed by LC/MS analysis. The amino acid sequence of HSA was derived from the RCSB protein data bank: DOI 10.2210/pdb1bm0/pdb.[411] Residues are numbered for this sequence of 585 amino acids.

Table 38. Tryptic peptides of HSA modified with ponalrestat AG **212**.

Retention time (min)	*m/z* predicted	*m/z* observed	Residues	Sequence	Modified lysine	Modification
30.48	715.6	714.9	137 – 144	**K**(+)YLYEIAR	K-137	Transacylation
34.47	817.4	817.1	146 – 160	HPYFYAPELLFF A**K**(+)R	K-159	Glycosylation
28.21	561.4	561.3	191 – 197	ASSA**K**(+)QR	K-195	Transacylation
21.35	721.1	720.4	198 - 205	L**K**(+)CASLQK or LKCASLQ**K**(+)	K-199/205	Glycosylation
24.22	633.1	632.9	198 – 205	L**K**(+)CASLQK or LKCASLQ**K**(+)	K-199/205	Transacylation
25.98	785.6	785.1	210 – 218	AF**K**(+)AWAVAR	K-212	Glycosylation
29.41	1008.0	1007.4	414 – 428	**K**(+)VPQVSTPTL VEVSR	K-414	Transacylation
29.35	825.7	826.2	415 – 432	VPQVSTPTLVEV SRNLG**K**(+)	K-432	Glycosylation
30.42	767.1	767.1	415 – 432	VPQVSTPTLVEV SRNLG**K**(+)	K-432	Transacylation
28.64	589.0	588.8	429 – 436	NLG**K**(+)VGSK or NLGKVGS**K**(+)	K-432/436	Transacylation
26.27	684.5	683.9	539 – 545	AT**K**(+)EQLK or ATKEQL**K**(+)	K-541/545	Glycosylation

Modified protein was hydrolyzed with trypsin and analyzed by LC/MS analysis. The amino acid sequence of HSA was derived from the RCSB protein data bank: DOI 10.2210/pdb1bm0/pdb.[411] Residues are numbered for this sequence of 585 amino acids.

Table 39. Tryptic peptides of HSA modified with *p*-bromobenzoic acid NHS ester **236**.

Retention time (min)	*m/z* predicted	*m/z* observed	Residues	Sequence	Modified lysine	Modification
29.56	1239.3	1237.8	137 – 144	**K**(+)YLYEIAR	K-137	Transacylation
30.51	1203.2	1201.8	210 – 218	AF**K**(+)AWAVAR	K-212	Transacylation

Modified protein was hydrolyzed with trypsin followed by LC-coupled MS/MS analysis. The amino acid sequence of HSA was derived from the RCSB protein data bank: DOI 10.2210/pdb1bm0/pdb.[411] Residues are numbered for this sequence of 585 amino acids.

10.4 Appendix IV – Publications

1) Rune Nygaard Monrad, Robert Madsen, **Rhodium-Catalyzed Decarbonylation of Aldoses**, *Journal of Organic Chemistry* **2007**, *72*, 9782-9785.

2) Rune Nygaard Monrad, Mette Fanefjord, Flemming Gundorph Hansen, N. Michael E. Jensen, and Robert Madsen, **Synthesis of Gabosine A and N from Ribose by the Use of Ring-Closing Metathesis**, *European Journal of Organic Chemistry* **2009**, 396.

3) Rune Nygaard Monrad, James C. Errey, Mazhar Iqbal, Xiaoli Meng, Lisa Iddon, John R. Harding, Ian D. Wilson, Andrew V. Stachulski, Benjamin G. Davis, **Dissecting the Reaction of Phase II Metabolites of Ibuprofen and Other NSAIDS with Human Plasma Protein**, *Nature Medicine*, submitted.

JOC*Note*

Rhodium-Catalyzed Decarbonylation of Aldoses

Rune Nygaard Monrad and Robert Madsen*

Center for Sustainable and Green Chemistry, Building 201, Department of Chemistry, Technical University of Denmark, DK-2800 Lyngby, Denmark

rm@kemi.dtu.dk
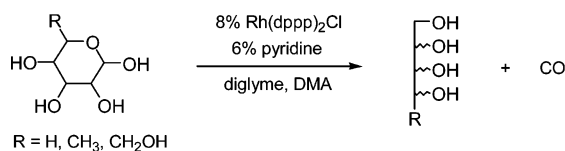
A catalytic procedure is described for decarbonylation of unprotected aldoses to afford alditols with one less carbon atom. The reaction is performed with the rhodium complex Rh(dppp)$_2$Cl in a refluxing diglyme−DMA solution. A slightly improved catalyst turnover is observed when a catalytic amount of pyridine is added. Under these conditions most hexoses and pentoses undergo decarbonylation into the corresponding pentitols and tetrols in isolated yields around 70%. The reaction has been applied as the key transformation in a five-step synthesis of L-threose from D-glucose.

Extending or shortening the carbon chain in unprotected aldoses has been a subject in carbohydrate chemistry for more than a century.[1] Although many methods are known for chain elongation of aldoses[2] there are only a few procedures for shortening the carbon chain. The Ruff degradation converts salts of aldonic acids into one carbon shorter aldoses.[3] The reaction is performed with hydrogen peroxide in the presence of iron-(III) or copper(II) salts and often occurs in a moderate yield. Another oxidative degradation reaction converts aldoses into salts of aldonic acids with loss of one carbon atom.[4] This reaction is carried out with molecular oxygen in an alkaline solution and gives rise to a good yield in most cases. Moreover, these procedures for shortening the carbon chain both involve stoichiometric amounts of inorganic salts.

Aldoses are aldehydes that can undergo a C−H insertion reaction with a metal followed by decarbonylation. This transformation converts C$_n$ aldoses into C$_{n−1}$ alditols. Andrews

and co-workers exploited this transformation with stoichiometric amounts of Wilkinson's catalyst (Rh(PPh$_3$)$_3$Cl).[5] The decarbonylation was performed in *N*-methyl-2-pyrrolidinone (NMP) at 130 °C and afforded alditols in isolated yields ranging from 37% to 87%.[5] The high temperature is due to the fact that only a minute amount of the aldose is present as the free aldehyde in solution. Ketohexoses underwent decarbonylation under the same conditions, but in this case the main product was furfuryl alcohol since ketohexoses are easily dehydrated into 5-hydroxymethylfurfural.[6] The reactions are stoichiometric since Rh-(PPh$_3$)$_3$Cl is converted into Rh(CO)(PPh$_3$)$_2$Cl, which will not perform the decarbonylation unless the temperature is raised to about 200 °C. However, if additives are added the decarbonylation of D-glucose can be achieved with 5−10% of Rh(PPh$_3$)$_3$-Cl in an NMP solution.[7] The additives are either diphenylphosphoryl azide,[8] sodium azide (both at 50 °C for 24 h), or a 1,ω-bis(diphenylphosphino)alkane (alkane = ethane, butane, hexane, at 130 °C for 24 h). In all cases, the conversion was rather slow and only a 30−49% HPLC yield of D-arabinitol was obtained in 24 h.[7]

Earlier work has shown that catalytic decarbonylations of aldehydes can be carried out with rhodium catalysts containing a bidentate phosphine ligand.[9] The complex Rh(dppp)$_2$Cl (dppp = 1,3-bis(diphenylphosphino)propane) has been shown to decarbonylate simple aldehydes in neat solution.[9] Unfortunately, Rh(dppp)$_2$Cl is not very soluble in organic solvents and has only found limited use as a decarbonylation catalyst. Recently, we reinvestigated the application of Rh(dppp)$_2$Cl in this reaction and found that the decarbonylation of a wide range of aldehydes could be effectively achieved in refluxing diglyme and that the active catalyst could be generated in situ from commercially available RhCl$_3$·3H$_2$O and dppp.[10]

Herein, we describe the catalytic decarbonylation of unprotected aldoses by the use of Rh(dppp)$_2$Cl. The reaction gives easy access to a number of chiral polyols which can be used as building blocks for further synthesis. Furthermore, the decarbonylation has been applied as the key step in a concise synthesis of L-threose from D-glucose.

The initial experiments were performed with D-glucose as the substrate. It soon became clear that the decarbonylation could not be achieved with an in situ generated catalyst. When glucose, RhCl$_3$·3H$_2$O, and dppp were mixed in refluxing diglyme the reaction immediately turned black due to precipitation of rhodium metal. Our earlier work has shown that Rh(III) is reduced to Rh(I) by dppp,[10] but glucose is also a reducing agent and is probably responsible for the further reduction to Rh(0). It was also attempted to form the active catalyst by mixing RhCl$_3$·3H$_2$O and dppp in refluxing diglyme and then adding glucose. However, these experiments mainly led to decomposition of the carbohydrate. As a result, it was decided to use a preformed catalyst, Rh(dppp)$_2$Cl, which can be prepared in two

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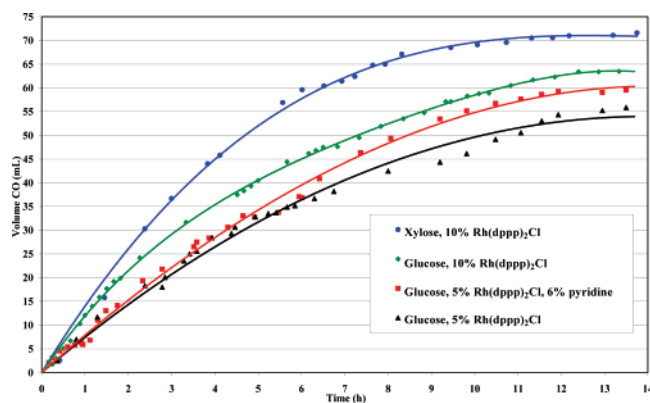
FIGURE 1. CO evolution during the course of the decarbonylation (see the Experimental Section for details).

TABLE 1. Decarbonylation of D-Glucose into D-Arabinitol

entry	amount of Rh(dppp)$_2$Cl, %	additive	reaction time, h	isolated yield, %
1	10	none	9	71
2	5	none	11	44
3	5	7% AcOH	9.5	39
4	5	15% AcOH	9	51
5	5	6% pyridine	9.5	55
6	5	13% pyridine	9.5	58
7	8	6% pyridine	8	71

TABLE 2. Decarbonylation of Pentoses and Hexoses

entry	aldose	method^a	reaction time, h	alditol	isolated yield, %
1	D-arabinose	A	9	erythritol	68
2	D-arabinose	B	6.5	erythritol	70
3	D-ribose	A	8	erythritol	71
4	D-ribose	B	6.5	erythritol	76
5	D-xylose	A	8	D-threitol	70
6	D-xylose	B	7.5	D-threitol	74
7	D-mannose	A	9	D-arabinitol	69
8	D-mannose	B	8	D-arabinitol	72
9	L-rhamnose	A	11	5-deoxy-L-arabinitol	66
10	L-rhamnose	B	10	5-deoxy-L-arabinitol	71
11	D-galactose	A	9	D-arabinitol	39
12	D-galactose	B	8	D-arabinitol	56
13	N-acetyl--D-glucosamine	A	16	1-acetylamino-1-deoxy-D-arabinitol	42
14	N-acetyl--D-glucosamine	B	14.5	1-acetylamino-1-deoxy-D-arabinitol	40^b

^{a} A: 10% Rh(dppp)$_2$Cl. B: 8% Rh(dppp)$_2$Cl, 6% pyridine. ^{b} 15% of pyridine was used.

steps from RhCl$_3$·3H$_2$O.[11] The reaction with this catalyst gave D-arabinitol as the main product, but the decarbonylation was still accompanied by significant decomposition due to the poor solubility of glucose in diglyme. Several cosolvents were investigated and it was found that smaller amounts of water, NMP, or *N,N*-dimethylacetamide (DMA) gave rise to a homogeneous reaction mixture. Unfortunately, water forms an azeotrope with diglyme with a boiling point of 99 °C, which is much lower than the boiling point of diglyme (162 °C). The decarbonylation requires a rather high temperature in order to proceed at a reasonable rate and the reactions in diglyme−water mixtures proceeded too slowly and were highly dependent on the diglyme−water ratio and the scale. NMP was used in the earlier decarbonylations with Rh(PPh$_3$)$_3$Cl[5−7] and we have shown that simple aldehydes could be decarbonylated in NMP with a catalyst generated from RhCl$_3$·3H$_2$O and dppp.[10] Glucose also underwent decarbonylation with Rh(dppp)$_2$Cl in a diglyme−NMP mixture, but it was difficult to remove the high-boiling NMP (bp 202 °C) in the workup. DMA (bp 165 °C), on the other hand, was easier to remove and gave similar results as NMP. We therefore selected a mixture of diglyme and DMA for the decarbonylations of monosaccharides. The progress of the reaction could be monitored by measuring the evolution of carbon monoxide (Figure 1). The solvents were removed in the workup by diluting the reaction with water and washing the mixture with dichloromethane. This afforded D-arabinitol in 71% isolated yield from D-glucose with 10% of Rh(dppp)$_2$Cl (Table 1, entry 1). The major byproduct was 1,4-anhydro-D-arabinitol, which was isolated in 20% yield and characterized as the corresponding triacetate. Only traces were observed of 2,5-anhydro-D-arabinitol (1,4-anhydro-D-lyxitol) and both anhydro sugars are probably formed from the parent arabinitol due to the high reaction temperature. It was not possible to obtain 1,4-anhydro-D-arabinitol as the major product by increasing the reaction time since these experiments were accompanied by significant decomposition.

The decarbonylation still required a rather high catalyst loading due to the tiny amount of the free aldehyde at equilibrium.[12] A lower catalyst loading gave a lower yield due

to incomplete conversion and decomposition (Table 1, entry 2 and Figure 1). It is known, however, that the mutarotation of aldoses can be accelerated by acid or base.[13] Therefore, several experiments were performed in the presence of acetic acid or pyridine (Table 1, entries 3−6). Both additives had a beneficial effect and made it possible to obtain higher yields with a shorter reaction time. Pyridine gave the best result and it was therefore decided to carry out the decarbonylation in the presence of 6% of pyridine (Figure 1). Under these conditions complete conversion of glucose was achieved with 8% of Rh(dppp)$_2$Cl in 8 h (Table 1, entry 7).

The reaction was then applied to a number of other monosaccharides (Table 2). The experiments were performed in the presence and in the absence of pyridine to illustrate the effect of the added base. The pentoses generally reacted slightly faster than the hexoses (Table 2 and Figure 1). Arabinose, ribose, xylose, mannose, and rhamnose gave similar yields as glucose

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SCHEME 1
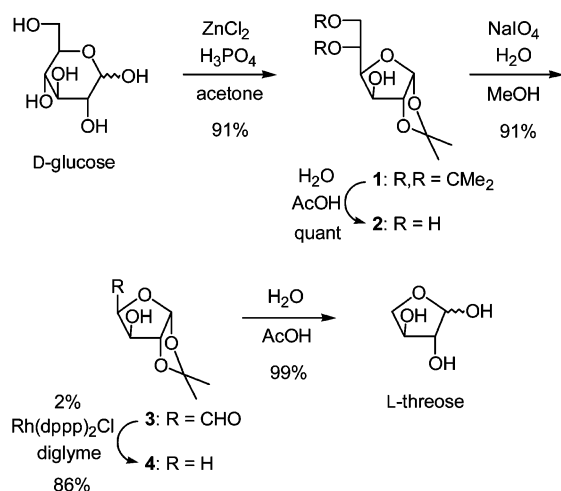
while the yields with galactose and *N*-acetylglucosamine were lower. Galactose is less soluble in diglyme−DMA than the other aldoses while *N*-acetylglucosamine is known to decarbonylate slowly due to coordination with the *N*-acetyl group.[5] The long reaction time and the high temperature did result in some decomposition of *N*-acetylglucosamine during the course of the reaction. In all cases, however, the addition of pyridine gave a faster transformation and made it possible to use slightly less of the rhodium catalyst. As in the case of the initial experiments with glucose, the major byproducts were C_{n-1} 1,4-anhydroalditols. For example, 1,4-anhydro-D-arabinitol was isolated in 20% yield from the experiment in entry 8 while 1,4-anhydro-5-deoxy-L-arabinitol was obtained in 17% yield from the reaction in entry 10.

The reaction can also be applied to partially protected carbohydrate substrates. We envisioned that the tetrose L-threose could be prepared from D-glucose in a few steps by using the decarbonylation as the key step. L-Threose is a useful chiral starting material,[14] but is not available from natural sources. It has previously been prepared by oxidative degradation of other carbohydrates[15] and from L-tartaric acid.[16] However, none of these routes takes advantage of the most abundant carbohydrate, D-glucose.

The synthesis of L-threose began by converting glucose into diisopropylidene glucofuranose 1[17] (Scheme 1). The more labile 5,6-*O*-isopropylidene acetal was selectively hydrolyzed in aqueous acetic acid followed by evaporation of the solvent. The crude triol 2 was then subjected to periodate cleavage to afford aldehyde 3.[18] Previously, periodic acid (H_5IO_6) in dry ether has been shown to affect acetal hydrolysis and glycol cleavage in

one step.[19] Unfortunately, when we applied this procedure to 1 we only obtained 3 in 40−50% yield, which is much lower than the 91% yield for the two-step procedure. Aldehyde 3 crystallizes as the dimer, but slowly equilibrates in aqueous solution to form the monomer. Contrary to the unprotected aldoses, aldehyde 3 is easy to dissolve in pure diglyme. Again, it was not possible to conduct the decarbonylation with an in situ generated catalyst from $RhCl_3\cdot3H_2O$ and dppp since this experiment only led to precipitation of rhodium metal. However, when aldehyde 3 was submitted to 2% of $Rh(dppp)_2Cl$, clean decarbonylation occurred into 1,2-*O*-isopropylidene-*β*-L-threo-furanose (4). Subsequent removal of the acetal then afforded L-threose in an overall yield of 71% from D-glucose. L-Threose is a syrup and exists as an almost equal mixture of the α- and the *β*-anomer together with a small amount of the hydrate of the aldehyde.

In conclusion, we have developed a catalytic procedure for decarbonylation of unprotected and partially protected carbohydrate aldehydes. This transformation will open new possibilities for using carbohydrates as chiral starting materials in synthetic chemistry.

Experimental Section

General Procedure for Decarbonylation of Unprotected Aldoses. To the aldose (400−650 mg, 2.78 mmol) were added Rh-$(dppp)_2Cl$ (214 mg, 0.22 mmol), DMA (3 mL), diglyme (20 mL), and freshly distilled pyridine (14.5 μL, 0.18 mmol). The mixture was thoroughly degassed under high vacuum and then stirred at reflux (162 °C) under a nitrogen atmosphere until TLC (acetone/BuOH/H$_2$O = 5:4:1) showed full conversion to the corresponding alditol (6−16 h). The solution was cooled to room temperature followed by addition of water (50 mL). The mixture was washed with CH$_2$Cl$_2$ (4 × 50 mL) and the combined organic phases were extracted with water (2 × 10 mL). The combined aqueous phases were concentrated and the residue co-concentrated with EtOH. The resulting residue was further purified by either flash column chromatography (CH$_2$Cl$_2$/MeOH/H$_2$O = 4:1:0 to 65:25:4) or reverse phase column chromatography (H$_2$O). The reaction could also be monitored by measuring the evolution of carbon monoxide. In this case, the reaction flask was connected to a burette filled with water. The bottom of the burette was further connected to a water reservoir with a large surface area. At rt (25 °C) full conversion of the aldose corresponds to 68 mL of carbon monoxide.

D-Arabinitol. White crystals. R_f 0.49 (acetone/BuOH/H$_2$O = 5:4:1). $[\alpha]^{22}_D$ −10.3 (*c* 0.2, MeOH) (lit.[20] $[\alpha]^{19}_D$ −12 (*c* 1, MeOH)). Mp 98−99 °C (MeOH) (lit.[21] mp 101−102 °C (EtOH)). ^{1}H NMR (300 MHz, D$_2$O) δ 3.86 (ddd, *J* = 2.0, 5.3, 7.3 Hz, 1H), 3.77 (dd, *J* = 2.7, 11.5 Hz, 1H), 3.68 (ddd, *J* = 2.7, 6.2, 8.8 Hz, 1H), 3.58 (m, 3H), 3.50 (dd, *J* = 2.0, 8.3 Hz, 1H). ^{13}C NMR (75 MHz, D$_2$O) δ 72.3, 71.8, 71.7, 64.5, 64.4. Anal. Calcd for C$_5$H$_{12}$O$_5$: C, 39.47; H, 7.95. Found: C, 39.55; H, 7.65.

Erythritol. White crystals. R_f 0.47 (acetone/BuOH/H$_2$O = 5:4:1). Mp 116−117 °C (MeOH/heptane) (lit.[22] mp 120−121 °C). ^{1}H NMR (300 MHz, D$_2$O) δ 3.74−3.65 (m, 2H), 3.62−3.49 (m, 4H). ^{13}C NMR (75 MHz, D$_2$O) δ 73.3, 64.0. Anal. Calcd for C$_4$H$_{10}$O$_4$: C, 39.34; H, 8.25. Found: C, 39.05; H, 8.00.

D-Threitol. White crystals. R_f 0.52 (acetone/BuOH/H$_2$O = 5:4:1). $[\alpha]^{22}_D$ −7.5 (*c* 0.5, MeOH) (lit.[23] $[\alpha]^{23}_D$ −7.0 (*c* 0.9, MeOH)). Mp 89−91 °C (MeOH) (lit.[24] mp 90−91 °C (BuOH)). ^{1}H NMR

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(300 MHz, D$_2$O) δ 3.69−3.51 (m, 6H). ^{13}C NMR (75 MHz, D$_2$O) δ 72.9, 63.9. Anal. Calcd for C$_4$H$_{10}$O$_4$: C, 39.34; H, 8.25. Found: C, 39.19; H, 8.05.

5-Deoxy-L-arabinitol. Colorless syrup. R_f 0.68 (acetone/BuOH/H$_2$O = 5:4:1). [α]$^{22}_D$ +11.7 (*c* 3.8, MeOH). [α]$^{22}_D$ +13.1 (*c* 0.5, H$_2$O) (reported for the enantiomer[25] [α]$^{23}_D$ −10.6 (H$_2$O)). ^{1}H NMR (300 MHz, D$_2$O) δ 3.85−3.74 (m, 2H), 3.64−3.53 (m, 2H), 3.38−3.28 (m, 1H), 1.17 (d, J = 6.4 Hz, 3H). ^{13}C NMR (75 MHz, D$_2$O) δ 74.6, 70.7, 67.1, 63.1, 18.3. Anal. Calcd for C$_5$H$_{12}$O$_4$: C, 44.11; H, 8.88. Found: C, 43.85; H, 8.59.

1-Acetylamino-1-deoxy-D-arabinitol. White crystals. R_f 0.49 (acetone/BuOH/H$_2$O = 5:4:1). [α]$^{22}_D$ +23.5 (*c* 0.5, H$_2$O) (lit.[26] [α]$^{22}_D$ +23 (H$_2$O)). Mp 142−143 °C (MeOH) (lit.[26] mp 146.5−147.5 °C). ^{1}H NMR (300 MHz, D$_2$O) δ 3.81 (t, J = 6.7 Hz, 1H), 3.67 (dd, J = 2.6, 11.4 Hz, 1H), 3.57 (ddd, J = 4.4, 7.8, 7.7 Hz, 1H), 3.48 (dd, J = 6.2, 11.5 Hz, 1H), 3.32 (d, J = 8.5 Hz, 1H), 3.25−3.11 (m, 2H), 1.85 (s, 3H). ^{13}C NMR (75 MHz, D$_2$O) δ 175.4, 71.7, 71.7, 69.1, 63.9, 43.3, 22.8. Anal. Calcd for C$_7$H$_{15}$NO$_5$: C, 43.52; H, 7.83; N, 7.25. Found: C, 43.67; H, 7.56; N, 7.18.

1,2-*O*-Isopropylidene-β-L-threofuranose (4). To 1,2-*O*-isopropylidene-α-D-*xylo*-pentodialdo-1,4-furanose (**3**) (500 mg, 2.66 mmol) were added Rh(dppp)$_2$Cl (51 mg, 0.053 mmol) and a degassed solution of diglyme (10 mL). The mixture was thoroughly degassed and then stirred at reflux (162 °C) in a preheated oil bath for 26 h. The solvent was removed under high vacuum at 70 °C to give a black residue, which was purified by flash column chromatography eluting with ether/pentane = 2:3 to 4:1 to afford **4** (366 mg, 86%) as white crystals. R_f 0.35 (EtOAc/heptane = 3:2). [α]$^{22}_D$ +13.1 (*c* 0.8, acetone) (lit.[15b] [α]$_D$ +13 (*c* 1, acetone)). Mp 80−81 °C (ether) (lit.[15b,27] mp 84−85 °C (ether/hexane)). ^{1}H NMR (300 MHz, CD$_3$OD) δ 5.78 (d, J = 3.7 Hz, 1H), 4.35 (d, J = 3.7 Hz,

1H), 4.04 (d, J = 2.8 Hz, 1H), 3.92 (dd, J = 2.9, 9.8 Hz, 1H), 3.68 (dd, J = 1.0, 9.8 Hz, 1H), 1.32 (s, 3H), 1.19 (s, 3H). ^{13}C NMR (50 MHz, CD$_3$OD) δ 112.7, 106.7, 86.4, 75.9, 73.9, 27.1, 26.4. Anal. Calcd for C$_7$H$_{12}$O$_4$: C, 52.49; H, 7.55. Found: C, 52.79; H, 7.47.

L-Threose. 1,2-*O*-Isopropylidene-β-L-threofuranose (**4**) (100 mg, 0.62 mmol) was dissolved in 30% aqueous AcOH (10 mL) and heated to reflux for 4 h. The liquids were removed in vacuo and the residue was purified by reverse-phase column chromatography eluting with H$_2$O to give L-threose (74 mg, 99%) as a colorless oil consisting of a 14:11:5 mixture of the α- and β-furanose forms and the hydrate. R_f 0.57 (acetone/BuOH/H$_2$O = 5:4:1). [α]$^{22}_D$ +12.3 (*c* 2.0, H$_2$O) (lit.[15b] [α]$_D$ +12 (*c* 1, H$_2$O)). ^{1}H NMR (300 MHz, D$_2$O) δ 5.33 (d, J = 4.2 Hz), 5.17 (d, J = 1.1 Hz), 4.94 (d, J = 6.3 Hz), 4.50−3.36 (m, 4H). ^{13}C NMR (75 MHz, D$_2$O), α-anomer: δ 103.4, 81.9, 76.4, 74.3; β-anomer: δ 97.9, 77.4, 76.1, 71.8; hydrate: δ 91.0, 74.5, 72.1, 64.2. Anal. Calcd for C$_4$H$_8$O$_4$: C, 40.00; H, 6.71. Found: C, 40.74; H, 6.72. HRMS calcd for C$_4$H$_8$O$_4$Na [M + Na]$^+$ *m/z* 143.0320, found *m/z* 143.0327. ^{1}H and ^{13}C data are in accordance with literature values.[28]

Supporting Information Available: General experimental methods, characterization of 1,4-anhydro-D-arabinitol and 1,4-anhydro-5-deoxy-L-arabinitol, synthesis of **2** and **3**, and copies of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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FULL PAPER

Synthesis of Gabosine A and N from Ribose by the Use of Ring-Closing Metathesis

Rune Nygaard Monrad,[a][‡] Mette Fanefjord,[a][‡] Flemming Gundorph Hansen,[a][‡] N. Michael E. Jensen,[a][‡] and Robert Madsen*[a]

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A concise synthetic route is described for the synthesis of gabosine A and N. The key step uses a zinc-mediated tandem reaction where methyl 5-deoxy-5-iodo-2,3-O-isopropylidene-β-D-ribofuranoside is fragmented to give an unsaturated aldehyde which is allylated in the same pot with 3-benzoyloxy-2-methylallyl bromide. The functionalized octa-1,7-diene, thus obtained, is converted into the six-membered ga-bosine skeleton by ring-closing olefin metathesis. Subsequent protective group manipulations and oxidation gives rise to gabosine N in a total of 8 steps from ribose while the synthesis of gabosine A employs an additional step for inverting a secondary hydroxy group.

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Introduction

The gabosines are a family of secondary metabolites iso-lated from *Streptomyces* strains which share a common tri-hydroxy(methyl)cyclohexenone/-cyclohexanone skeleton.[1] A total of 14 different gabosines have been identified[2] and the absolute configuration has been established for gabosine A–F, I, L, N, and O (Figure 1). None of the 14 compounds display any significant biological activity, but weak DNA-binding properties have been shown for several gabosi-nes.[1a] The biosynthesis of the gabosines occur through a pentose phosphate pathway in which sedoheptulose 7-phos-phate cyclizes by an aldol condensation.[3] The chemical synthesis of the gabosines has been achieved by several stra-tegies where the carbocyclic ring is either contained in the starting material [i.e. *p*-benzoquinone, (–)-quinine, or iodo-benzene][4] or is created by a Diels–Alder reaction[5] or by cyclization of a carbohydrate.[6] In the latter case, the car-bocyclization has been accomplished by an aldol condensa-tion, a nitrile oxide cycloaddition, a Nozaki–Hiyama–Kishi reaction, a Horner–Wadsworth–Emmons olefination and by ring-closing olefin metathesis.

We have described a zinc-mediated tandem reaction for converting carbohydrates into acyclic dienes that can be cy-clized by ring-closing metathesis.[7,8] In this reaction, methyl 5-iodopentofuranosides are treated with zinc metal and al-lylic bromides. First, a reductive fragmentation of the iodo-
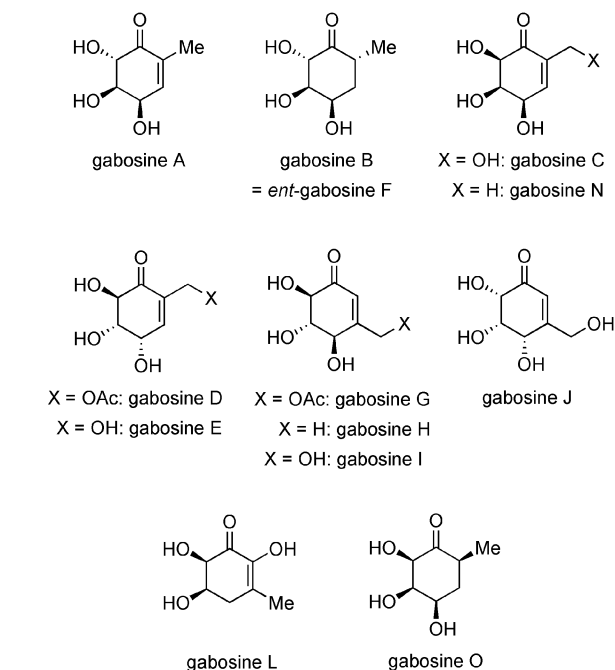
Figure 1. The gabosine family of secondary metabolites.

furanoside takes place to produce an unsaturated aldehyde which is subsequently allylated in the same pot by the al-lylzinc reagent. In this way, the zinc metal serves a dual purpose by promoting both the reductive fragmentation and the subsequent allylation reaction.[7] By using this pro-cedure, we have previously prepared several carbocyclic nat-ural products from carbohydrates including 7-deoxypancra-tistatin,[9] cyclophellitol,[10] and calystegine B$_2$, B$_3$ and B$_4$.[11]

We envisaged that the tandem reaction would also be an effective transformation for synthesis of the gabosines as illustrated with gabosine A and N in Figure 2. This would require allylic bromide **1** in order to install the methyl group in the product. Bromide **1** has not been described before, but the corresponding 3-benzoyloxyallyl bromide has been developed as an efficient α-hydroxyallylation agent of aldehydes in the presence of zinc or indium.[12] We have recently used 3-benzoyloxyallyl bromide in the tandem reaction with zinc for synthesis of the conduritols[13] and for chain elongation of aldoses in the presence of indium.[14] On the basis of these results we expected that the tandem reaction between **1** and the appropriate iodopentofuranoside could be achieved to give the desired octa-1,7-diene which upon ring-closure by metathesis would yield the gabosine skeleton.
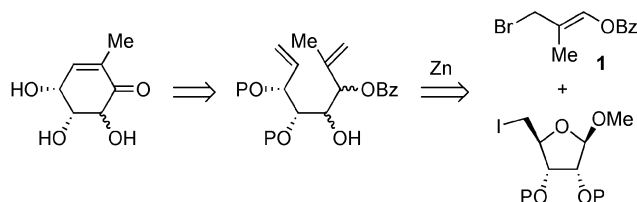
Figure 2. Retrosynthesis for gabosine A and N.

Herein, we describe a short synthesis of the two epimeric gabosines A and N by the use of a zinc-mediated tandem reaction and ring-closing olefin metathesis.

Results and Discussion

Gabosine A and N have the same stereochemistry at two hydroxy groups which will both originate from the pentose in the synthesis. The two natural products differ from each other at the third hydroxy group which will be installed in the tandem reaction (Figure 2). D-Ribose has the correct stereochemistry at C-2 and C-3 for both gabosines and the corresponding methyl furanoside **2a** is easy available in two steps from the parent pentose (Scheme 1).[15] Bromide **1** is prepared from methacrolein by haloacylation with benzoyl bromide. The synthesis is carried out in the same way as the preparation of 3-benzoyloxyallyl bromide from acrolein.[16] The kinetic product is initially formed by 1,2-addition to the aldehyde, but slowly equilibrates to the thermodynamic 1,4-addition product. The addition is rather slow, but the reaction can be accelerated by adding zinc(II) bromide as catalyst. Thus, treatment of methacrolein with benzoyl bromide and zinc(II) bromide gave the desired product in 76% yield after 2 h as a 4:3 mixture of the *E* and *Z* isomer which could not be separated by silica gel chromatography. When the reaction was performed in the absence of zinc(II) bromide the time for complete conversion increased significantly, but the yield was still acceptable and in this case only the *E* isomer was formed. Because this isomer is crystalline and completely stable at 5 °C we favored the latter synthesis of bromide **1**. Furthermore, the synthesis could be performed on a large scale and the reagent isolated without the use of flash chromatography.
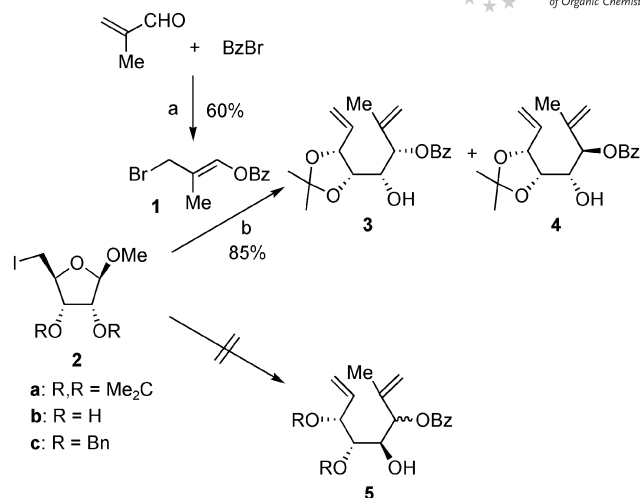
Scheme 1. Reagents and conditions: (a) CH₂Cl₂, room temp. (b) Zn, THF, H₂O, 40 °C, sonication.

The tandem reaction between **1** and **2a** was carried out in a THF/H₂O mixture under sonication at 40 °C. Treatment of furanoside **2a** with zinc for 2 h followed by addition of bromide **1** gave 85% yield of a 2:1 mixture of dienes **3** and **4** which could be separated by silica gel chromatography (Scheme 1). Notably, the stereochemistry at the hydroxy group is the same in **3** and **4** which make both compounds suitable for the synthesis of gabosine N. The structure of **3** and **4** was elucidated after ring-closing metathesis by ¹H NMR and by isopropylidene formation from the corresponding diol. The stereochemical outcome is in accordance with our earlier observations[13] and can be rationalized by the Felkin–Anh model.[17] The same mixture of **3** and **4** was obtained when the *E*:*Z* mixture of **1** was employed in the tandem reaction.

Because **3** and **4** both have the correct stereochemistry for gabosine N several experiments were carried out to invert the stereochemical outcome of the allylation in order to prepare gabosine A by the same reaction. We have shown earlier that the stereochemistry in the tandem reaction can be changed by using the unprotected ribofuranoside **2b**[18] or by using indium metal[10,18] where the latter is known to react by chelation control. However, treatment of **2b** with zinc in the presence of bromide **1** did not furnish the desired diene **5**. Instead, a complex mixture was obtained resulting from degradation of **2b** and self-coupling of **1**. When the same reaction was attempted with indium metal, self-coupling of **1** was the main product and very little fragmentation of **2b** occurred. It was therefore decided to carry out the fragmentation with zinc and isolate the intermediate aldehyde and then perform the subsequent allylation with indium. Only aldehydes that are prepared by fragmentation of protected ribofuranosides are sufficiently stable to be isolated. Hence, ribofuranosides **2a** and **2c** were fragmented with zinc followed by treatment of the corresponding aldehydes with **1** and indium. The allylations were carried out in the presence and in the absence of a Lewis acid[10] and in different solvent mixtures. Unfortunately, all attempts to

obtain a decent yield of diene **5** failed. In most cases, bromide **1** only underwent self-coupling and it appears that this reaction is faster than the allylation of the aldehydes. In other cases, small amounts of dienes were obtained as diastereomeric mixtures, but the major isomers had the same stereochemistry as in **3** and **4**. As a result, we decided to abandon the tandem reaction for synthesis of diene **5** and instead chose to invert the stereochemistry at the hydroxy group in **3** (vide infra).

The next step involved ring-closing olefin metathesis and was carried out with Grubbs' 2nd generation catalyst[19] in refluxing dichloromethane. Under these conditions the major diastereomer **3** cyclized cleanly to give cyclohexene **6** in 97% yield (Scheme 2). However, the minor isomer **4** reacted more sluggishly and afforded the corresponding cyclohexene **7** in 74% yield. The yield of **7** did not improve by using Grubbs' 1st generation catalyst, Hoveyda–Grubbs' 2nd generation catalyst or by changing the solvent to refluxing toluene.
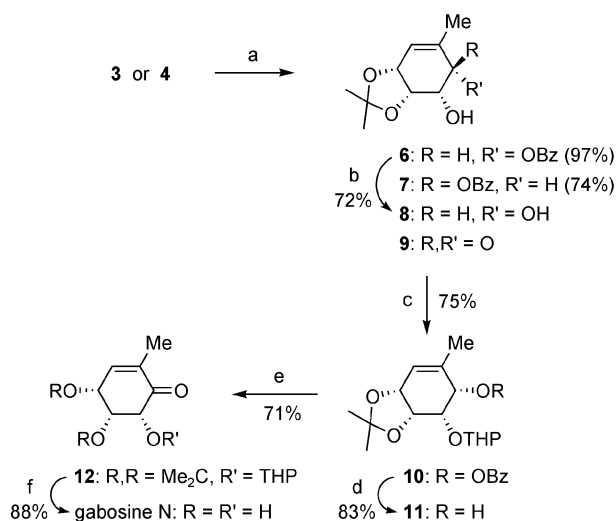
Scheme 2. Reagents and conditions: (a) 10% $(PCy_3)(C_3H_4N_2Mes_2)$-$Cl_2Ru=CHPh$, CH_2Cl_2, 40 °C. (b) K_2CO_3, MeOH, room temp. (c) DHP, PPTS, CH_2Cl_2, room temp. (d) NaOMe, MeOH, room temp. (e) PDC, CH_2Cl_2, room temp. (f) AcOH, H_2O, room temp.→40 °C.

To complete the synthesis of gabosine N the allylic position had to be oxidized to the ketone. These experiments were only performed with the major diastereomer **6**, but similar reactions can be envisioned from the minor isomer **7**. First, diol **8** was prepared by deprotection of **6** and it was attempted to carry out a selective allylic oxidation in the presence of PDC, MnO_2 or DDQ. However, these experiments only gave the desired ketone **9** in very low yield due to incomplete conversion or over-oxidation and it was therefore decided to protect the homoallylic hydroxy group in **6** prior to the oxidation. The THP group was chosen for this purpose since it can be removed under the same conditions as the isopropylidene acetal. Treatment of alcohol **6** with dihydropyran gave fully protected **10** in good yield which was followed by removal of the benzoate to give allylic alcohol **11**. The oxidation of **11** could now be ac-

complished in a satisfying yield with PDC to furnish protected gabosine **12**. Finally, deprotection under acidic conditions afforded gabosine N with spectral and physical data in excellent accordance with those reported for the natural product.[1a,4b]

The synthesis of gabosine A could be achieved by a similar sequence after inverting the hydroxy group in cyclohexene **6**. The inversion was carried out by initial conversion into the triflate followed by displacement with sodium nitrite in DMF to give alcohol **13** in 52% yield (Scheme 3). Attempts to improve the yield by using potassium nitrite in DMF or tetrabutylammonium nitrite in toluene[20] were not successful. Alcohol **13** was converted into gabosine A by using the same four steps as employed for gabosine N. THP protection gave acetal **14** and removal of the benzoate afforded alcohol **15** which was oxidized to the corresponding ketone **16** in 66% overall yield from **13**. Hydrolysis of the acetal protecting groups then furnished gabosine A with spectral and physical data in agreement with those reported for the natural product.[1b,4d]
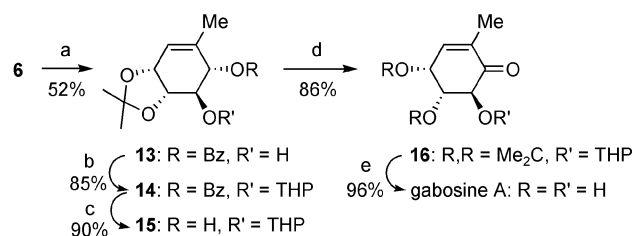
Scheme 3. Reagents and conditions: (a) Tf_2O, pyridine, CH_2Cl_2, –20 °C→room temp., then $NaNO_2$, DMF, room temp. (b) DHP, PPTS, CH_2Cl_2, room temp. (c) NaOMe, MeOH, room temp. (d) PDC, CH_2Cl_2, room temp. (e) AcOH, H_2O, 40 °C.

Conclusions

In summary, we have developed an 8-step synthesis of gabosine N and a 9-step synthesis of gabosine A. In both cases, D-ribose serves as the starting material and the cyclohexene skeleton is created by a zinc-mediated tandem reaction followed by ring-closing olefin metathesis. The results emphasize the utility of these two reactions in the preparation of polyhydroxylated carbocyclic natural products from carbohydrates.

Experimental Section

General: CH_2Cl_2 was dried by distillation from CaH_2 while MeOH and DMF were dried with 4-Å molecular sieves. Zinc dust (< 10 micron) was activated immediately before use: zinc (5 g) in 1 M HCl (50 mL) was stirred at room temperature for 20 min and then filtered, rinsed with water and Et_2O and finally dried at high vacuum with a heat gun. Zinc(II) bromide was dried at high vacuum with a heat gun. All other reagents were obtained from commercial sources and used without further purification. Reactions were monitored by TLC using aluminium plates precoated with silica gel 60. Compounds were visualized by dipping in a solution of $(NH_4)_6Mo_7O_{24}·4H_2O$ (25 g/L) and $Ce(SO_4)_2$ (10 g/L) in 10% aque-

ous H_2SO_4 followed by heating. Melting points are uncorrected. Flash column chromatography was performed with E. Merck silica gel 60 (particle size 0.040–0.063 mm). Optical rotations were measured with a Perkin–Elmer 241 polarimeter while IR spectra were recorded with a Bruker Alpha FT-IR spectrometer. NMR spectra were recorded with a Varian Mercury 300 instrument. Residual solvent peaks were used as internal reference in ^{1}H NMR (δ_{CHCl3} = 7.26 ppm and δ_{CD2HOD} = 3.31 ppm) while CDCl$_3$ (δ = 77.16 ppm) and CD$_3$OD (δ = 49.0 ppm) served as the internal standards in ^{13}C NMR spectroscopy. High-resolution mass spectra were recorded at the Department of Physics and Chemistry, University of Southern Denmark.

(E)-3-Bromo-2-methylprop-1-enyl Benzoate (1). Procedure with ZnBr$_2$: Benzoyl bromide (1.3 mL, 11.0 mmol) was added to a solution of freshly distilled methacrolein (770 mg, 11.0 mmol) in anhydrous CH$_2$Cl$_2$ and Et$_2$O (1:1, 18 mL) under nitrogen. The mixture was cooled to –20 °C followed by addition of anhydrous ZnBr$_2$ (25 mg, 0.11 mmol). The reaction was stirred at 0 °C for 2 h and then quenched by addition of saturated aqueous NaHCO$_3$ (15 mL). The aqueous solution was extracted with CH$_2$Cl$_2$ (3 × 10 mL) and the combined organic layers were dried (Na$_2$SO$_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/hexane, 1:20) to give 2.12 g (76%) of the desired compound as a white crystalline mass (4:3 mixture of the E and Z isomer).

Procedure without ZnBr$_2$: Freshly distilled methacrolein (15.0 mL, 181.7 mmol) was dissolved in anhydrous CH$_2$Cl$_2$ (200 mL) under nitrogen. Benzoyl bromide (22.0 mL, 183.5 mmol) was added at 0 °C and the solution was stirred at room temperature for 10 d. The mixture was concentrated in vacuo and the residue dissolved in pentane. Cooling to –20 °C gave white crystals which were isolated and recrystallized from pentane to afford **1** (27.8 g, 60%). R_f = 0.19 (EtOAc/heptane, 1:10). M.p. 61–62 °C. IR (KBr): ṽ = 3096, 2844, 2913, 1727, 1451, 1382, 1287, 1162, 1122, 704 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.14–8.08 (m, 2 H), 7.66–7.57 (m, 2 H), 7.52–7.45 (m, 2 H), 4.07 (s, 2 H), 1.98 (d, J = 1.4 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 163.2, 134.0, 133.9, 130.1, 128.9, 128.8, 119.2, 36.5, 13.2 ppm. HRMS: calcd. for C$_{11}$H$_{11}$BrO$_2$Na [M + Na]$^+$ 276.9835; found 276.9848.

3-Benzoyl-1,2,7,8-tetradeoxy-5,6-O-isopropylidene-2-C-methyl-D-$allo$- and -D-$altro$-octa-1,7-dienitol (3 and 4): Activated Zn (1.21 g, 18.5 mmol) was added to a degassed solution of ribofuranoside **2a**[15] (1.07 g, 3.4 mmol) in THF/H$_2$O (4:1, 30 mL). The mixture was sonicated at 40 °C under nitrogen until TLC revealed full conversion to the aldehyde (2 h). A deoxygenated solution of bromide **1** (1.28 g, 5.1 mmol) in THF (8.0 mL) was then added in two portions; one after 2 h and one after 3.5 h of sonication. After sonicating for an additional 2 h 45 min at 40 °C, the reaction mixture was filtered through a plug of Celite and the filter cake was rinsed thoroughly with CH$_2$Cl$_2$. The filtrate was washed with saturated aqueous NaHCO$_3$ (30 mL) and H$_2$O (2 × 30 mL). The combined aqueous phases were extracted with CH$_2$Cl$_2$ (2 × 30 mL) and the combined organic phases were dried (MgSO$_4$), filtered, and concentrated in vacuo. Purification by flash column chromatography (EtOAc/heptane, 1:5) gave dienes **3** and **4** (0.95 g, 85%) as a separable 2:1 mixture.

3: R_f = 0.33 (EtOAc/heptane, 1:2). $[a]_D^{30}$ = –5.0 (c = 1.0, CHCl$_3$). IR (film): ṽ = 3499, 3074, 2986 2925, 2855, 1720, 1650, 1611, 1452, 1373, 1318, 1270, 1218, 1110, 1070, 1027, 874, 712 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.10–8.05 (m, 2 H), 7.58 (tt, J = 1.4, 7.4 Hz, 1 H), 7.49–7.41 (m, 2 H), 6.05 (ddd, J = 7.2, 10.4, 17.4 Hz, 1 H), 5.68 (d, J = 3.1 Hz, 1 H), 5.43 (ddd, J = 1.2, 1.7, 17.2 Hz, 1 H),

5.30 (ddd, J = 1.1, 1.7, 10.4 Hz, 1 H), 5.20–5.18 (m, 1 H), 5.15 (p, J = 1.5 Hz, 1 H), 4.71–4.65 (m, 1 H), 4.09–4.05 (m, 2 H), 2.01 (d, J = 4.2 Hz, OH), 1.94 (s, 3 H), 1.49 (s, 3 H), 1.34 (s, 3 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 165.5, 140.6, 134.3, 133.3, 130.3 129.8, 128.6, 118.5, 116.1, 109.3, 79.1, 77.8, 77.5, 70.9, 28.0, 25.5, 20.3 ppm. HRMS: calcd. for C$_{19}$H$_{24}$O$_5$Na [M + Na]$^+$ 355.1516; found 355.1530.

4: R_f = 0.27 (EtOAc/heptane, 1:2). $[a]_D^{29}$ = –2.8 (c = 1.5, CHCl$_3$). IR (film): ṽ = 3490, 3091, 3071, 2987, 2937, 1725, 1654, 1602, 1452, 1379, 1316, 1270, 1218, 1116, 1070, 1027, 918, 875, 712 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.14–8.08 (m, 2 H), 7.58 (tt, J = 1.4, 7.4 Hz, 1 H), 7.50–7.42 (m, 2 H), 6.07 (ddd, J = 6.7, 10.4, 17.1 Hz, 1 H), 5.61 (s, 1 H), 5.46 (d, J = 17.1 Hz, 1 H), 5.32 (d, J = 10.8 Hz, 1 H), 5.07–5.02 (m, 2 H), 4.73 (t, J = 6.4 Hz, 1 H), 4.20 (dd, J = 6.1, 9.2 Hz, 1 H), 3.85 (ddd, J = 1.9, 4.7, 9.2 Hz, 1 H), 1.99 (d, J = 4.8 Hz, OH), 1.88 (s, 3 H), 1.48 (s, 3 H), 1.28 (s, 3 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 165.3, 141.1, 133.7, 133.3, 130.1, 129.8, 128.6, 118.2, 112.9, 109.0, 78.7, 77.1, 76.2, 69.0, 28.1, 25.5, 20.1 ppm. HRMS: calcd. for C$_{19}$H$_{24}$O$_5$Na [M + Na]$^+$ 355.1516; found 355.1521.

(1S,2S,3S,6R)-3-Benzoyloxy-2-hydroxy-4,8,8-trimethyl-7,9-dioxabi-cyclo[4.3.0]non-4-ene (6): Grubbs' 2nd generation catalyst (151 mg, 0.18 mmol) was added to a degassed solution of diene **3** (578 mg, 1.7 mmol) in anhydrous CH$_2$Cl$_2$ (54 mL) under nitrogen. The solution was protected from sunlight and stirred at reflux under nitrogen for 4 d. The reaction was stopped by addition of charcoal (5.52 g) and the mixture was filtered through a plug of Celite. The filtrate was concentrated in vacuo and purified by flash column chromatography (heptane/Et$_2$O, 3:1) to afford the target compound (511.5 mg, 97%) as a white solid. R_f = 0.52 (EtOAc/heptane, 1:1). $[a]_D^{29}$ = +65.0 (c = 1.1, CHCl$_3$). IR (film): ṽ = 3408, 3072, 2987, 2933, 2866, 1711, 1454, 1371, 1348, 1277, 1223, 1108, 1036, 1014, 712 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.14–8.09 (m, 2 H), 7.59 (tt, J = 2.1, 7.4 Hz, 1 H), 7.49–7.42 (m, 2 H), 5.79–5.74 (m, 1 H), 5.70 (bd, J = 3.8 Hz, 1 H), 4.67–4.61 (m, 1 H), 4.45 (dd, J = 3.2, 6.5 Hz, 1 H), 4.12 (dt, J = 3.5, 6.9 Hz, 1 H), 2.47 (d, J = 6.9 Hz, OH), 1.83 (d, J = 0.8 Hz, 3 H), 1.56 (s, 3 H), 1.43 (s, 3 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 166.8, 134.4, 133.5, 130.0, 129.8, 128.6 123.1, 110.6, 74.0, 72.0, 71.4, 67.3, 27.3, 25.9, 20.2 ppm. HRMS: calcd. for C$_{17}$H$_{20}$O$_5$Na [M + Na]$^+$ 327.1203; found 327.1212.

(1S,2S,3R,6R)-3-Benzyloxy-2-hydroxy-4,8,8-trimethyl-7,9-dioxadi-cyclo[4.3.0]non-4-ene (7): A degassed solution of diene **4** (34 mg, 0.10 mmol) and Grubbs' 2nd generation catalyst (7.4 mg, 0.0087 mmol) in freshly distilled CH$_2$Cl$_2$ (24 mL) was protected from sunlight and stirred at reflux under argon for 3 d. The reaction mixture was evaporated on Celite and purified by flash column chromatography (heptane → heptane/EtOAc, 4:1) to give the desired compound (23 mg, 74%) as a white solid. R_f = 0.29 (EtOAc/heptane, 2:1). $[a]_D^{25}$ = –40.4 (c = 1.0, CDCl$_3$). IR (film): ṽ = 3459, 3066, 3036, 2985, 2924, 2855, 1720, 1452, 1380, 1268, 1235, 1110, 1047, 1031, 712 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.11–8.06 (m, 2 H), 7.59 (tt, J = 2.1, 7.4 Hz, 1 H), 7.50–7.42 (m, 2 H), 5.84 (dd, J = 1.0, 8.1 Hz, 1 H), 5.57 (dq, J = 1.9, 3.3 Hz, 1 H), 4.69–4.64 (m, 1 H), 4.56 (ddd, J = 0.7, 2.9, 5.6 Hz, 1 H), 3.97 (dt, J = 2.9, 8.1 Hz, 1 H), 2.66 (d, J = 8.1 Hz, OH), 1.78 (dd, J = 1.3, 2.6 Hz, 3 H), 1.43 (s, 3 H), 1.40 (s, 3 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 167.2, 134.6, 133.5, 130.0, 129.8, 128.6, 124.1, 109.9, 75.9, 74.1, 73.1, 71.8, 27.8, 26.5, 19.2 ppm. HRMS: calcd. for C$_{17}$H$_{20}$O$_5$Na [M + Na]$^+$ 327.1203; found 327.1212. Deprotection with NaOMe in MeOH gave the corresponding diol as described below for **8**. No reaction occurred when the diol was treated with

2,2-dimethoxypropane and camphorsulfonic acid at room temperature for 1 h.

(1S,2S,3S,6R)-2,3-Dihydroxy-4,8,8-trimethyl-7,9-dioxabicyclo[4.3.0]-non-4-ene (8): K_2CO_3 (49 mg, 0.36 mmol) was added to a solution of benzoate **6** (0.107 g, 0.38 mmol) in anhydrous MeOH (10 mL) and the mixture was stirred at room temperature under nitrogen for 3 h. The reaction was quenched with 1 M HCl until neutral pH, followed by extraction with CH_2Cl_2 (3×15 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash column chromatography (EtOAc/heptane, 1:1) gave diol **8** (50 mg, 72%) as white crystals. R_f = 0.13 (EtOAc/heptane, 1:1). $[a]_D^{25}$ = +69.9 (c = 1.0, CHCl₃). IR (film): ṽ = 3447, 3002, 2931, 2858, 1370, 1231, 1177, 1083, 1038, 975 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ = 5.40 (dq, J = 1.4, 2.8 Hz, 1 H), 4.55–4.50 (m, 1 H), 4.48–4.44 (m, 1 H), 3.84 (dd, J = 4.1, 11.4 Hz, 1 H), 3.77–3.69 (m, 1 H), 3.34 (d, J = 9.3 Hz, OH), 2.74 (d, J = 11.5 Hz, OH), 1.84 (t, J = 1.5 Hz, 3 H), 1.38 (s, 3 H), 1.31 (s, 3 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 137.0, 122.2, 110.3, 76.7, 73.6, 70.5, 67.5, 28.2, 26.4, 21.0 ppm. HRMS: calcd. for $C_{10}H_{16}O_4Na$ [M + Na]⁺ 223.0941; found 223.0931. Reaction with 2,2-dimethoxypropane and camphorsulfonic acid at room temperature for 40 min gave 73% yield of the corresponding bis(isopropylidene) compound.

(1R,2S,3S,6R)-3-Benzoyloxy-4,8,8-trimethyl-2-tetrahydropyranyl-oxy-7,9-dioxabicyclo[4.3.0]non-4-ene (10): DHP (0.4 mL, 4.4 mmol) and PPTS (14 mg, 0.056 mmol) were added to a solution of alcohol **6** (657.7 mg, 2.16 mmol) in anhydrous CH_2Cl_2 (20 mL). The solution was stirred at room temperature under argon overnight. The reaction was stopped by addition of saturated aqueous $NaHCO_3$ until neutral pH. The phases were separated and the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (heptane/EtOAc, 5:1) to give **10** (627.0 mg, 75%) as a colorless oil and a mixture of two diastereomers. R_f = 0.44 (EtOAc/heptane, 1:1). IR (film): ṽ = 3070, 2981, 2939, 2873, 1715, 1452, 1369, 1270, 1232, 1109, 1069, 1054, 1016, 963, 919, 709 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ = 8.13–8.06 (m, 4 H), 7.52 (tt, J = 1.4, 7.4 Hz, 2 H), 7.44–7.36 (m, 4 H), 5.82–5.78 (m, 2 H), 5.62–5.58 (m, 2 H), 4.95–4.89 (m, 2 H), 4.66–4.58 (m, 2 H), 4.56–4.50 (m, 2 H), 4.15 (dt, J = 2.6, 4.7 Hz, 2 H), 4.00–3.84 (m, 2 H), 3.55–3.44 (m, 2 H), 1.78 (t, J = 1.3 Hz, 3 H), 1.76 (t, J = 1.4 Hz, 3 H), 1.70–1.59 (m, 8 H), 1.57 (s, 3 H), 1.55 (s, 3 H), 1.53–1.43 (m, 4 H), 1.41 (s, 3 H), 1.40 (s, 3 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 167.0, 166.9, 133.0, 132.8, 132.8, 131.5, 130.6, 130.3, 130.0, 128.4, 128.3, 126.4, 125.2, 111.0, 110.8, 97.2, 96.3, 74.5, 73.9, 73.6, 72.0, 69.8, 69.4, 69.3, 66.4, 63.0, 61.8, 30.1, 30.1, 28.3, 28.0, 26.8, 26.6, 25.5, 25.4, 20.8, 20.5, 19.5, 18.3 ppm. HRMS: calcd. for $C_{22}H_{28}O_6Na$ [M + Na]⁺ 411.1778; found 411.1774.

(1R,2S,3S,6R)-3-Hydroxy-4,8,8-trimethyl-2-tetrahydropyranyloxy-7,9-dioxabicyclo[4.3.0]non-4-ene (11): Fully protected **10** (150 mg, 0.386 mmol) was dissolved in 10% NaOMe in anhydrous MeOH (10 mL) and stirred at room temperature for 3 h. The mixture was evaporated in vacuo and purified by flash column chromatography (heptane/EtOAc, 3:1) to give **11** (91 mg, 83%) as a colorless oil and a mixture of two diastereomers. R_f = 0.28 and 0.33 (EtOAc/heptane, 1:1). IR (film): ṽ = 3529, 2983, 2937, 2874, 1440, 1371, 1227, 1133, 1120, 1075, 1051, 1029, 1012, 985, 970, 814 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ = 5.41–5.37 (m, 1 H), 5.34 (dq, J = 1.4, 2.8 Hz, 1 H), 4.92 (dd, J = 3.1, 4.3 Hz, 1 H), 4.77 (dd, J = 3.2, 4.5 Hz, 1 H), 4.58–4.50 (m, 2 H), 4.48–4.43 (m, 2 H), 4.02–3.92 (m, 4 H), 3.88 (dd, J = 1.9, 4.2 Hz, 1 H), 3.82 (dd, J = 2.4, 3.9 Hz, 1 H), 3.55–3.44 (m, 2 H), 1.96–185 (m, 2 H), 1.83 (s, 3 H), 1.82 (s, 3

H), 1.80–1.66 (m, 4 H), 1.57–1.46 (m, 6 H), 1.41 (s, 3 H), 1.39 (s, 3 H), 1.33 (s, 3 H), 1.32 (s, 3 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 138.6, 136.9, 121.9, 120.5, 110.8, 110.5, 99.2, 95.9, 76.4, 74.3, 74.3, 73.6, 73.6, 70.2, 69.9, 67.1, 63.1, 62.9, 30.7, 30.5, 28.2, 27.8, 26.8, 26.4, 25.4, 25.3, 21.2, 20.7, 19.7, 19.6 ppm. HRMS: calcd. for $C_{15}H_{24}O_5Na$ [M + Na]⁺ 307.1516; found 307.1529.

(1R,2R,6R)-4,8,8-Trimethyl-3-oxo-2-tetrahydropyranyloxy-7,9-dioxabicyclo[4.3.0]non-4-ene (12): PDC (0.235 g, 0.63 mmol) was added to a solution of alcohol **11** (51 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (15 mL) and the mixture was stirred at room temperature under nitrogen for 20 h. The solution was concentrated in vacuo and purified by flash column chromatography (heptane/EtOAc, 2:1) to give **12** (36 mg, 71%) as a colorless oil and a mixture of two diastereomers. R_f = 0.33 (EtOAc/heptane, 1:1). IR (film): ṽ = 2984, 2937, 2871, 1699, 1453, 1379, 1351, 1229, 1145, 1120, 1074, 1024, 971, 889 cm⁻¹. ¹H NMR (300 MHz, CDCl₃): δ = 6.32–6.28 (m, 1 H), 4.93–4.89 (m, 1 H), 4.82–4.76 (m, 2 H), 4.56 (d, J = 2.6 Hz, 1 H), 3.92–3.84 (m, 1 H), 3.55–3.46 (m, 1 H), 1.96–1.84 (m, 3 H), 1.81 (t, J = 1.4 Hz, 3 H), 1.62–1.50 (m, 3 H), 1.37 (s, 3 H), 1.32 (s, 3 H) ppm. ¹³C NMR (75 MHz, CDCl₃): δ = 196.0, 139.2, 134.5, 111.7, 99.5, 78.5, 74.9, 73.2, 63.1, 30.3, 28.0, 27.2, 25.6, 19.5, 15.6 ppm. Only NMR spectroscopic data for the major diastereomer are reported. HRMS: calcd. for $C_{15}H_{22}O_5Na$ [M + Na]⁺ 305.1359; found 305.1346.

Gabosine N: Ketone **12** (36 mg, 0.13 mmol) was dissolved in 80% acetic acid in H_2O (2.0 mL) and stirred under nitrogen for 23 h at room temperature followed by 16 h at 40 °C. The reaction mixture was cooled to room temperature and concentrated in vacuo to give a residue that was purified by flash column chromatography (EtOAc/MeOH, 99:1) to afford gabosine N (17.7 mg, 88%) as white crystals. R_f = 0.12 (EtOAc). $[a]_D^{25}$ = –150.5 (c = 0.3, CD₃OD) [lit.[1a] $[a]_D^{20}$ = –152.0 (c = 0.89, H_2O), lit.[4b] $[a]_D$ = –142 (c = 0.16, MeOH)]. M.p. 174–176 °C (MeOH) [lit.[4b] 174–175 °C (MeOH), lit.[1a] 182.5–183.3 °C]. IR (film): ṽ = 3427, 3308, 3244, 2943, 2925, 2851, 1680, 1408, 1343, 1197, 1132, 1044, 1022, 937, 854 cm⁻¹. ¹H NMR (300 MHz, CD₃OD): δ = 6.48 (dq, J = 1.5, 2.2 Hz, 1 H), 4.58–4.54 (m, 1 H), 4.34 (dt, J = 2.5, 3.4 Hz, 1 H), 4.23 (d, J = 2.5 Hz, 1 H), 1.81 (dd, J = 1.5, 2.2 Hz, 3 H) ppm. ¹³C NMR (75 MHz, CD₃OD): δ = 200.2, 145.9, 134.5, 77.7, 76.6, 69.2, 15.3 ppm. NMR spectroscopic data are in accordance with literature values.[1a,4b] HRMS: calcd. for $C_7H_{10}O_4Na$ [M + Na]⁺ 181.0471; found 181.0475.

(1S,2R,3S,6R)-3-Benzoyloxy-2-hydroxy-4,8,8-trimethyl-7,9-dioxabi-cyclo[4.3.0]non-4-ene (13): A solution of alcohol **6** (1.79 g, 5.9 mmol) in freshly distilled CH_2Cl_2 (40 mL) under nitrogen was cooled to –20 °C followed by addition of pyridine (2.14 mL, 26.5 mmol) and Tf₂O (1.48 mL, 8.8 mmol). The reaction mixture was slowly warmed to room temperature and after 1.5 h the reaction was quenched with 2 M HCl (85 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (2×25 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ (45 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo to give the crude trifluoromethanesulfonate (2.57 g, 5.9 mmol) as a black residue, which was used directly in the next step. The crude trifluoromethanesulfonate was redissolved in anhydrous DMF under nitrogen, $NaNO_2$ (1.62 g, 23.5 mmol) was added and the mixture stirred at room temperature for 5.5 h. The reaction mixture was diluted with H_2O (120 mL) followed by extraction with Et₂O (5×50 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/heptane, 1:5→1:1) to give alcohol **13** (933 mg, 52%) as a slightly yellow oil.

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R_f = 0.49 (EtOAc/heptane, 1:1). $[a]_D^{25}$ = –12.0 (*c* = 2.0, CD$_3$OD). IR (film): $\tilde{\nu}$ = 3459, 3064, 3043, 2985, 2925, 2859, 1719, 1452, 1379, 1316, 1268, 1249, 1216, 1115, 1060, 1026, 979, 907, 710 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.11–8.05 (m, 2 H), 7.59 (tt, *J* = 1.4, 7.4 Hz, 1 H), 7.50–7.42 (m, 2 H), 5.76–5.67 (m, 2 H), 4.69–4.63 (m, 1 H), 4.21 (dd, *J* = 6.3, 9.0 Hz, 1 H), 3.98 (t, *J* = 8.9 Hz, 1 H), 1.77 (dd, *J* = 1.3, 2.7 Hz, 3 H), 1.55 (s, 3 H), 1.41 (s, 3 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 166.7, 139.1, 133.5, 130.0, 129.6, 128.6, 120.1, 110.6, 77.9, 74.7, 72.7, 72.3, 28.4, 26.1, 18.9 ppm. HRMS: calcd. for C$_{17}$H$_{20}$O$_5$Na [M + Na]$^+$ 327.1203; found 327.1216.

(1*R*,2*R*,3*S*,6*R*)-3-Benzoyloxy-4,8,8-trimethyl-2-tetrahydropyranyloxy-7,9-dioxabicyclo[4.3.0]non-4-ene (14): DHP (0.5 mL, 5.5 mmol) and PPTS (140 mg, 0.56 mmol) were added to a solution of alcohol **13** (850 mg, 2.79 mmol) in freshly distilled CH$_2$Cl$_2$ (60 mL). The mixture was stirred at room temperature under nitrogen overnight. The reaction was stopped by addition of saturated aqueous NaHCO$_3$ (100 mL) followed by extraction with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic phases were dried (MgSO$_4$), filtered, concentrated in vacuo and purified by flash column chromatography (heptane/EtOAc, 9:1→4:1) to give **14** (925 mg, 85%) as a colorless oil and a mixture of two diastereomers. R_f = 0.72 (EtOAc/heptane, 1:1). IR (film): $\tilde{\nu}$ = 3063, 2983, 2938, 2868, 1722, 1452, 1380, 1317, 1265, 1247, 1217, 1162, 1119, 1062, 1029, 986, 966, 869, 710 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.12–8.03 (m, 4 H), 7.59–7.53 (m, 2 H), 7.48–7.40 (m, 4 H), 5.81–5.67 (m, 4 H), 5.21 (t, *J* = 2.6 Hz, 1 H), 4.83 (t, *J* = 3.3 Hz, 1 H), 4.68–4.58 (m, 2 H), 4.29 (dt, *J* = 6.1, 8.3 Hz, 2 H), 4.19–4.08 (m, 3 H), 3.64–3.54 (m, 1 H), 3.46–3.37 (m, 1 H), 3.23–3.14 (m, 1 H), 1.78–1.70 (m, 8 H), 1.62–1.58 (m, 4 H), 1.55 (s, 9 H), 1.40 (s, 3 H), 1.39 (s, 6 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 166.4, 166.2, 139.3, 138.1, 133.4, 133.1, 130.2, 129.9, 129.8, 129.7, 128.7, 128.4, 120.9, 120.1, 110.2, 110.2, 99.0, 97.3, 78.5, 76.1, 75.6, 74.6, 73.0, 72.6, 72.6, 62.1, 61.3, 30.6, 30.5, 28.3, 28.1, 26.6, 26.5, 25.4, 25.3, 19.1, 19.0, 18.5 ppm. HRMS: calcd. for C$_{22}$H$_{28}$O$_6$Na [M + Na]$^+$ 411.1778; found 411.1796.

(1*R*,2*R*,3*S*,6*R*)-3-Hydroxy-4,8,8-trimethyl-2-tetrahydropyranyloxy-7,9-dioxabicyclo[4.3.0]non-4-ene (15): Fully protected **14** (595 mg, 1.53 mmol) was dissolved in 10% NaOMe in anhydrous MeOH (60 mL) and stirred at room temperature for 3 h. The mixture was concentrated in vacuo and purified by flash column chromatography (heptane/EtOAc, 4:1) to give alcohol **15** (393 mg, 90%) as a colorless oil and a mixture of two diastereomers. R_f = 0.51 and 0.63 (EtOAc/heptane, 1:1). IR (film): $\tilde{\nu}$ = 3442, 3037, 2982, 2936, 2860, 1453, 1441, 1372, 1243, 1215, 1135, 1072, 1047, 1022, 1007, 975, 890 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 5.54–5.45 (m, 2 H), 4.79 (dd, *J* = 2.6, 5.5 Hz, 1 H), 4.62–4.50 (m, 2 H), 4.40 (d, *J* = 1.7 Hz, 1 H), 4.31 (t, *J* = 5.8 Hz, 1 H), 4.14–4.07 (m, 2 H), 4.04–3.91 (m, 3 H), 3.87–3.80 (dd, *J* = 5.0, 8.5 Hz, 1 H), 3.59 (t, *J* = 8.5 Hz, 1 H), 3.56–3.47 (m, 2 H), 2.99 (d, *J* = 8.5 Hz, OH), 1.96–1.77 (m, 3 H), 1.87–1.85 (m, 3 H), 1.84–1.83 (m, 3 H), 1.61–1.47 (m, 9 H), 1.47 (s, 3 H), 1.43 (s, 3 H), 1.36 (s, 3 H), 1.34 (s, 3 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 142.1, 138.2, 120.4, 117.3, 110.0, 109.7, 102.7, 99.4, 84.3, 76.7, 75.9, 75.2, 72.7, 71.1, 70.5, 65.6, 64.0, 31.5, 30.9, 28.5, 28.2, 26.6, 26.1, 25.3, 25.1, 21.4, 20.4, 20.3, 19.0 ppm. HRMS: calcd. for C$_{15}$H$_{24}$O$_5$Na [M + Na]$^+$ 307.1516; found 307.1521.

(1*R*,2*S*,6*R*)-4,8,8-Trimethyl-3-oxo-2-tetrahydropyranyloxy-7,9-dioxabicyclo[4.3.0]non-4-ene (16): Celite (1.8 g) and PDC (1.8 g, 4.78 mmol) were added to a solution of alcohol **15** (389 mg, 1.37 mmol) in freshly distilled CH$_2$Cl$_2$ (65 mL) and the reaction mixture was stirred at room temperature under argon for 26 h. The

mixture was filtered through a plug of Celite, and concentrated in vacuo to give a slightly yellow oil, which was purified by flash column chromatography (heptane/EtOAc, 3:1) to afford **16** (334 mg, 86%) as a colorless oil and a mixture of two diastereomers. R_f = 0.62 (EtOAc/heptane, 1:1). IR (film): $\tilde{\nu}$ = 2985, 2938, 2886, 1698, 1453, 1380, 1371, 1240, 1219, 1167, 1125, 1063, 1031, 977, 966, 856 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ = 6.54 (dq, *J* = 1.5, 4.2 Hz, 1 H), 6.50–6.46 (m, 1 H), 4.98 (t, *J* = 3.3 Hz, 1 H), 4.87 (t, *J* = 3.2 Hz, 1 H), 4.80–4.74 (m, 2 H), 4.54–4.41 (m, 4 H), 4.13–4.03 (m, 1 H), 3.98–3.89 (m, 1 H), 3.52–3.41 (m, 2 H), 1.86 (t, *J* = 1.4 Hz, 3 H), 1.85 (t, *J* = 1.4 Hz, 3 H), 1.80–1.67 (m, 5 H), 1.61–1.52 (m, 7 H), 1.52 (s, 3 H), 1.47 (s, 3 H), 1.43 (s, 3 H), 1.40 (s, 3 H) ppm. ^{13}C NMR (75 MHz, CDCl$_3$): δ = 197.2, 196.1, 137.5, 136.0, 135.8, 111.2, 111.1, 98.6, 97.8, 78.5, 77.7, 76.6, 71.7, 71.6, 62.2, 61.9, 30.4, 30.2, 28.1, 28.0, 26.7, 25.5, 25.4, 19.0, 18.7, 16.2, 15.9 ppm. HRMS: calcd. for C$_{15}$H$_{22}$O$_5$Na [M + Na]$^+$ 305.1359; found 305.1368.

Gabosine A: Ketone **16** (52 mg, 0.184 mmol) was dissolved in 80% acetic acid in H$_2$O (3.0 mL) and stirred under nitrogen for 9 h at 40 °C. The reaction mixture was cooled to room temperature, concentrated and co-concentrated with H$_2$O to give a residue that was purified by flash column chromatography (EtOAc) to afford gabosine A (28 mg, 96%) as a white crystalline material. R_f = 0.16 (EtOAc). $[a]_D^{25}$ = –125.4 (*c* = 0.8, CD$_3$OD) lit.[1b] $[a]_D^{20}$ = –132 (*c* = 1, MeOH), lit.[4d] $[a]_D$ = –131 (*c* = 0.27, MeOH). M.p. 56–60 °C (MeOH). IR (film): $\tilde{\nu}$ = 3354, 2955, 2924, 2862, 1684, 1448, 1236, 1139, 1092, 1028 cm^{-1}. ^{1}H NMR (300 MHz, CD$_3$OD): δ = 6.75 (dq, *J* = 1.5, 5.6 Hz, 1 H), 4.41–4.36 (m, 1 H), 4.32 (d, *J* = 10.0 Hz, 1 H), 3.73 (dd, *J* = 4.0, 10.0 Hz, 1 H), 1.82 (dd, *J* = 0.9, 1.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, CD$_3$OD): δ = 200.4, 143.0, 136.9, 75.0, 73.9, 67.4, 15.6 ppm. NMR spectroscopic data are in accordance with literature values.[1b,4d] HRMS: calcd. for C$_7$H$_{10}$O$_4$Na [M + Na]$^+$ 181.0471; found 181.0472.

Supporting Information (see also the footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra for compounds **1**, **3**, **4**, **6**–**8**, **10**–**16**, gabosine A and N.

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Dissecting the Reaction of Phase II Metabolites of Ibuprofen and Other NSAIDS with Human Plasma Protein

Rune Nygaard Monrad,[1] James C. Errey,[1] Mazhar Iqbal,[2] Xiaoli Meng,[2] Lisa Iddon,[2] John R. Harding,[3] Ian D. Wilson,[3] Andrew V. Stachulski,[2] Benjamin G. Davis[1]*

[1]Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, Oxford, OX1 3TA, United Kingdom,

[2]The Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, Liverpool, L69 7ZD, United Kingdom

[3]Department of Clinical Pharmacology, Drug Metabolism and Pharmacokinetics, Astra Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG UK

*Correspondence should be addressed to: Ben.Davis@chem.ox.ac.uk

2227 words excluding introductory paragraph, methods, references and legends.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs on the market. Whilst they are considered safe, several NSAIDs have been withdrawn from the market as a result of adverse drug reactions.[1] NSAIDs are extensively metabolised to their 1-β-*O*-acyl glucuronides (AGs),[2] and the risk of NSAID AGs covalently modifying biomacromolecules such as proteins or DNA, leading to immune responses and cellular dysfunction constitutes a major concern in drug discovery and development.[3-6] The assessment of the degree of protein modification and potential toxicity of individual NSAID AGs is therefore of importance in both drug monitoring and development. Herein, we report the covalent attachment of 1-β-*O*-acyl glucuronides of ibuprofen and several NSAID analogues to human serum albumin (HSA) protein *in vitro* under conditions encountered in therapy. Previously unobserved transacylation and glycosylation adducts are formed; the observed protein product ratios can be rationalised by the degree of α-substitution in the acyl group. Structure-based protein reactivity correlations of AGs, such as these, may prove a useful tool in distinguishing between carboxylic acid-containing drugs of similar structure that ultimately prove beneficial (e.g., Ibuprofen) from those that prove toxic (Ibufenac).[7]

Carboxylic acid containing drugs, most notably the NSAIDs are primarily metabolised to their 1-β-*O*-acyl glucuronides in the liver.[2] The glucuronidation of various drugs, which takes place during Phase II metabolism, is typically a

detoxification process that also increases water solubility thereby enabling excretion; the resulting products are typically deemed to be pharmacologically inactive.[6] However, in contrast to the unreactive glucuronides of alcohol and phenolic drugs, some of the resulting glucuronides of carboxylate drugs (acyl glucuronides, AGs) are highly reactive metabolites;[8] as a consequence AGs have very recently been deemed to be toxic by regulatory authorities.[6] Although, the increased reactivity of AGs compared with their parent drugs has almost exclusively been attributed to transacylation reactions with biological nucleophiles[4,9] (Figure 1b, e.g., formation of an amide bond with the amine group found at the terminus of the side-chain of lysine), alternative reaction pathways are potentially accessible to AGs (Figure 1c)[4] that might lead to alternative protein modification products. Surprisingly, these modification products have not been previously identified. Here we use AGs of the NSAIDs (Figure 2a) to show that not only do both pathways (Figure 1b and 1c) have the potential to covalently modify endogenous human proteins, but that the predominant pathway varies according to drug metabolite identity.

Once formed enzymatically during Phase II metabolism (Figure 1b) AGs may react via several competing pathways. Hydrolysis to reform the parent drug and transacylation by direct displacement by attack of nucleophiles (following Figure 1b) competes with acyl migration to produce 2-, 3- or 4-*O*-AGs followed by *N*-glycosylation and possibly also the Amadori rearrangement[10] (following Figure 1c). 1-β-*O*-Acyl glucuronides are stable in acid, but under basic or neutral conditions migration occurs to produce 2-, 3- or 4-*O*-AGs.[11,12] After migration, the

acyl group is less prone to hydrolysis or direct displacement by transacylation, and stable 2-, 3- or 4-*O*-AGs can be isolated.[13] Both the transacylation and the glycosylation pathways are theoretically capable of covalently modifying hepatic and plasma proteins *in vivo* however, the resulting modified proteins have not been fully characterised.[4]

Around 25% of all drugs that are withdrawn due to severe toxicity contain carboxylic acid groups and acyl glucuronide activity has been implicated as a possible mechanism.[1,14,15] Yet, the underlying chemical reactivity that might allow prediction of which drugs will show excellent efficacy leading to widespread use (e.g. **1b/c** Ibuprofen) or toxicity in man leading to withdrawal (e.g. **1a**, Ibufenac)[7,16] is not well understood; the similarity in structures such as **1b/c** and **1a** suggest that this is a subtle effect. It has been suggested, but not demonstrated, that one of the potential mechanisms underlying this observed idiosyncracy of toxicity of NSAIDs is AG-derived covalent modification of endogenous proteins.[3] Evidence for such an AG-induced toxicity mechanism is still lacking,[2,4,5,17] but, by way of circumstantial support, it has been shown that reversible binding of other compounds (like diazepam and warfarin) to plasma proteins, such as human serum albumin (HSA), is severely altered when plasma protein is modified by AGs.[18]

To investigate the nature and extent of AG binding to plasma proteins, a series of ibuprofen based 1-β-*O*-acyl glucuronides; ibufenac **1a**, (*R*)- and (*S*)-ibuprofen **1b-c** and a dimethyl analogue **1d**, as well as AGs of *p*-bromobenzoic acid **2**, as a model NSAID, and the aldose reductase inhibitor ponalrestat[19] **3**

were incubated with HSA. AGs were synthesised via a 3-step protection-acylation-deprotection strategy (Figure 2b) *via* allyl, benzyl or *para*-methoxybenzyl glucuronate esters.[20,21]

To obtain a representative single protein species suitable for precise reaction monitoring, abundant human serum protein HSA was reduced and re-purified. Commercially available and isolated HSA contains several post-translational modifications of which the disulfide oxidation of Cys34 by coupling to a single cysteine amino acid residue is the most abundant.[22] HSA was treated with 13 mM DTT at pH 8 to reduce the Cys34-Cys disulfide bond, followed by removal of cysteine and excess DTT by size-exclusion chromatography. The protein was then refolded in 0.02 mM DTT under exposure to atmospheric air to reform the internal disulfide bridges. Protein refoldng to a different secondary structure than native HSA is not believed to be occur.[22] This novel protein purification strategy allowed ready access to pure HSA suitable for precise AG protein reactivity assessment.

AGs **1-3** were incubated with pure HSA at physiological levels (33 g/L).[23] AGs are generally thought to be rapidly excreted, but substantial plasma concentrations, sometimes exceeding their parent drug, are often found.[24,25] In order to mimic conditions encountered in therapy, an AG concentration of 0.5 mM in phosphate buffered saline (20 mM, pH 7.4) at 37 °C, corresponding to the peak plasma concentration of drug achieved in chronic cystic fibrosis patients treated with ibuprofen was used.[26] In addition, high-concentration experiments using 5.0 mM AG were also performed. In all cases, identical conditions and

incubation times were used for direct comparison of the reactivity of different

AGs; this allows direct extrapolation of the levels of reactivity from the amount of

reaction product. The extent of coupling to HSA was analysed by LC-coupled

TOF MS (ES+) (Table 1 and Figure 3).

Table 1. Selectivity and conversion after incubation of HSA with AGs

Entry	AG	Conc. mM	Selectivity TA : Glyc	Conversion %
1	**2**	0.5	<5 : 95	58
2	**2**	5.0	<5 : 95	59
3	**3**	0.5	55 : 45	4
4	**3**	5.0	72 : 28	24
5	**1a**	0.5	60 : 40	16
6	**1a**	5.0	61 : 39	44
7	**1b**	0.5	64 : 36	5
8	**1b**	5.0	63 : 37	23
9	**1c**	0.5	44 : 56	9
10	**1c**	5.0	55 : 45	24
11	**1d**	0.5	-	0
12	**1d**	5.0	34 : 66	8

Incubation of 33 g/L HSA with AGs for 16 h at 37 °C and pH 7.4. Selectivities between the transacylation (TA) and glycosylation (Glyc) products and conversions (% modified HSA) were calculated based on peak heights in respective deconvoluted mass spectra.

At concentrations of both 0.5 and 5.0 mM of *p*-bromobenzoic acid AG **2** (entries 1

and 2) glycosylation adducts (Figure 1c) were observed following incubation for

16 h at a conversion of 58 and 59%, respectively. This is the first time a stable

glycosylation adduct have been isolated *in vitro* under conditions encountered in

therapy. Previously, such *N*-glycosylation (imine) adducts of proteins have only

been found by trapping the adducts with a reducing agent like sodium

cyanoborohydride[12,18,27] or at extreme high AG concentrations as compared to

HSA.[12,27,28] Furthermore, in most cases full characterisation of the formed

adducts was not performed.[12,18,28] Surprisingly, stable glycosylation adducts are formed without addition of a reducing agent, however this high conversion even at 0.5 mM of **2** clearly indicates that irreversible glycosylation of endogenous proteins can occur during treatment with drugs that are metabolised to AGs.

p-Bromobenzoic acid AG **2** reacts exclusively via the glycosylation pathway, and no transacylation product could be observed (Figure 1b). However, more subtle and intriguing results were observed following incubation with the AGs of ponalrestat **3** and the profens **1a-d** (entries 3-12, Table 1); both transacylation and glycosylation were unambiguously observed. These AGs were not as reactive as **2** with respect to protein modification. In most cases only trace modification occurred at 0.5 mM of AG, and significant amounts of coupling products could only be observed at relatively high AG concentrations (5.0 mM). In the case of ponalrestat AG **3** the transacylation and glycosylation adducts were formed in a 72:28 ratio at a combined yield of 24% at 5.0 mM **3** (entry 4). The ibuprofen analogues (entries 5-12) also gave a mixture of transacylation and glycosylation adducts, and the degree of modification can be visualised in the combined mass spectrum of **1a-d** (Figure 3). Ibufenac (**1a**), (*R*)- (**1b**) and (*S*)- (**1c**) Ibuprofen AGs showed clear reactivity with combined yields of 44, 23 and 24% respectively, whereas the α,α-disubstituted dimethyl analogue **1d** was significantly less reactive (entries 11,12, Table 1) showing no more than 8% reaction even at the higher concentrations and showing only trace products above background at lower concentrations. Interestingly, in addition to individual transacylation and glycosylation adducts, small amounts of dual adducts

resulting from concomitant transacylation and glycosylation were also seen for

1a-c.

These dual adducts highlighted that more than one reaction site was

present on HSA. To map these sites and to confirm the identities of the

glycosylation and transacylation products modified proteins were characterized

through 'peptide mapping' using tryptic digest followed by MS/MS sequencing

analysis. The products of reaction of Ibufenac-AG **1a** with HSA map to 7 different

lysine residues and revealed that different sites were selectively modified by

different reaction processes (Table 2 and Figure 4); these sites only correlate in

part with accessibility (see SI) and only Lys525 is reactive in both pathways.[29]

Table 2. Peptide Mapping of Reaction Sites in HSA when Modified with Ibufenac AG **1a**

Retention time (min)	*m/z* predicted	*m/z* observed	Peptide Residues	Peptide Sequence	Modified Amino Acid	Modification
33.49	704.1	704.1	137 – 144	**K**(+)YLYEIAR	K137	Glycosylation
24.36	921.8	921.4	191 – 197	ASSA**K**(+)QR	K195	Transacylation
27.80	621.6	621.6	198 – 205	L**K**(+)CASLQK	K199	Glycosylation
27.80	621.6	621.6	198 – 205	LKCASLQ**K**(+)	K205	Glycosylation
24.16	450.5	451.2	433 – 439	VGS**K**(+)CCK	K436	Transacylation
22.07	652.7	651.4	525 – 534	**K**(+)QTALVELVK	K525	Transacylation
18.86	740.7	741.1	525 – 534	**K**(+)QTALVELVK	K525	Glycosylation
22.07	652.7	651.4	525 – 534	KQTALVELV**K**(+)	K534	Transacylation

Modified protein was hydrolyzed with trypsin followed by LC-coupled MS/MS analysis. The amino acid sequence of HSA was derived from the RCSB protein data bank: DOI 10.2210/pdb1bm0/pdb.[30] Residues are numbered for this sequence of 585 amino acids.

As might be expected from the reactivity of α-unsubstituted ponalrestat AG **3**,

both transacylation (lysines 195, 436, 525 and 534) as well as glycosylation

(lysines 137, 199, 205 and 525) adducts could be observed for α-unsubstituted

ibufenac AG **1a** (Table 2); in all cases the mapping results correlated strongly

with the observed ratios determined by direct MS (Figure 3 and Table 1).

Alkyl AGs displayed (Table 1) lower reactivities than aryl AGs; with *p*-bromobenzoic acid AG **2** a majority of HSA was modified even at 0.5 mM. Furthermore, both the nature and extent of protein modification with alkyl AGs was highly dependant on the degree of α-substitution; variation of α-substitution from unsubstituted (**1a**, **3**) to monosubstituted (**1b-c**) to disubstituted (**1d**) AGs, moves reaction preference from transacylation to a preference for glycosylation. This is a logical observation consistent with $B_{Ac}2$ nucleophilic substitution at the carbonyl group of an AG; a higher degree of α-substitution constitutes an increased steric hindrance disfavouring addition during such a transacylation pathway. In contrast, acyl migration is not affected to the same extent by increased α-substitution,[4] and glycosylation adducts are therefore predominantly formed for highly α-substituted AGs as with the dimethyl analogue **1d**. In addition to different selectivity (transacylation versus glycosylation), alkyl AGs with no α-substitution are more reactive than mono- and disubstituted alkyl AGs, and AGs of drugs without α-substitution are therefore more likely to modify endogenous proteins *in vivo* than AGs of α-substituted drugs. The increased reactivity of ibufenac AG (**1a**) compared to (*R*)- (**1b**) and (*S*)-ibuprofen AG (**1c**) due to the lacking α-methyl substituent is striking considering that ibufenac was withdrawn from the U.K. market in 1968 as a result of hepatotoxicity,[7,31] whereas (*R/S*)-ibuprofen is still extensively used.

The observed reactivity of alkyl AGs with respect to α-substitution is also reflected in their half-lives. Unsubstituted alkyl AGs have been found to have half-lives of less than 1 h (zomepirac 0.45 h, diclofenac 0.51 h), while

monosubstituted ((*S*)-naproxen 1.8 h, (*S*)-ibuprofen 3.3 h) and disubstituted (clofibric acid 7.3 h, gemfibrozil 44 h) alkyl AGs have half-lives around 1 to 4 h and from 7 to more than 40 h, respectively.[4] As indicated by short half-lives (probenecid 0.40 h, diflunisal 0.67 h)[4] and supported by the reactivity of **2**, aromatic AGs are highly reactive with respect to protein modification, but they seem to be too hindered for transacylation to occur, thus glycosylation adducts are formed exclusively.

In summary, we have shown for the first time that multiple modes of reactivity exist for 1-β-*O*-acyl glucuronides of widely-used pharmaceuticals with an abundant human protein under concentrations representative of those found *in vivo*. Importantly, we reveal here the first direct observations of stable AG-glycosylation adducts, which is highlighted by contrasting human protein reaction selectivities determined by both site and drug-AG identity; these range from near exclusive glycosylation (>95%) to majority acylation (>70%). These demonstrate that althoughglucuronic acid alone cannot glycosylate human protein, unlike glucose,[29] AGs are sufficiently activated to cause extensive glycosylation. In this way we have established a rationalisation of AG reactivity based on structure, which we believe provide a useful tool in the informed development of new carboxylic acid containing drugs as well as in the monitoring, interpretation and assessment of the potential toxicity of existing drugs.[6] This work strongly indicates that AG-induced modification of plasma proteins during treatment with carboxylic acid containing drugs can take place in a number of ways that lead to different, and in some cases previously unidentified covalent adducts. This

seems of particular current relevance given recent decisions of certain drug development organizations to exclude all carboxylic acids, and in the light of regulatory opinions on AGs.[6] We are currently evaluating both the antigenic and immunogenic response of these unnatural protein motif structures as putative haptens, which may exist at high concentrations in patients chronically treated with e.g., NSAIDs.

Methods

Purification of human serum albumin. Commercial HSA (Lee BioSolutions, Inc., 50 mg, 0.75 µmol) and EDTA (1.5 mg, 5 µmol) in aq. phosphate buffered saline (5 mL, 20 mM, pH 8.5) were added dithiothreitol (10 mg, 65 µmol) in phosphate buffered saline (68 µL) and the reaction mixture was stirred at room temperature. The conversion was followed by mass spectrometry and after 2 h, full reduction of the Cys34-Cys disulfide was achieved. Cysteine and excess reducing agent was removed by PD10 size-exclusion chromatography eluting with a solution of EDTA (1 mM) and dithiothreitol (0.02 mM) in phosphate buffered saline (20 mM, pH 8.0). The solution was left for 48 h at 4 ºC exposed to atmospheric air to refold. The protein solution was purified by PD10 size-exclusion chromatography eluting with phosphate buffered saline (20 mM, pH 7.4) to give Cys34-Cys reduced HSA in more than 95% yield. The protein solution was concentrated using a YM10 Amicon ultrafiltration membrane to a

concentration of 42 g/L. The protein concentration was determined from $\varepsilon_{280nm} =$ 28730 M^{-1} cm^{-1}. ESI+ TOF MS: found: 66437, expected 66438.

General incubation of acyl glucuronides with HSA. To an aq. solution of HSA (33 g/L) in phosphate buffered saline (20 mM, pH 7.4) were added acyl glucuronides **1a-d**, **2** or **3** at a final concentration of 0.5 or 5.0 mM. The reaction mixture was mixed very vigorously for 30 sec and then gently shaken at 37 ºC for 16 h. The protein solution was then cooled to 4 ºC and purified by PD10 size-exclusion chromatography eluting with water to remove salts and excess reagent. The product was analysed by mass spectrometry (ESI+ TOF MS).

Supplementary information available: Additional discussion, experimental methods, raw and processed protein mass spectra as well as mass spectra of tryptic peptides.

Acknowledgements

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Supplementary information is linked to the online version of the paper at www.nature.com/XXXX.

Figure Legends

Figure 1: **The Reactions and Putative Products of Phase II Drug Metabolism**. **(a)** Typical glucuronidation of nucleophilic groups (**Nu**) in drugs during Phase II metabolism forms highly stable glucuronide glycosidic bonds and produces pharmacologically inactive metabolites **(b)** glucuronidation of carboxylic acid containing drugs forms acyl glucuronides (AGs) that can react with proteins through transacylation or **(c)** intramolecular acyl migration and subsequent reaction with proteins to form glycation and glycosylation products.

70 words

Figure 2: **AGs of NSAIDs and Analogues**. **(a)** Six representative, validated Phase II metabolites were used to study protein reactivity. These incorporated systematic variations in structure to probe variations in reactivity and reaction pathways **(b)** AGs were chemically synthesized through a novel partial protection strategy and made use of mild deprotection strategies that uniquely allowed access to these highly labile AG products (**1-3**); harsher conditions employed in other syntheses were incompatible.

71 words

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Figure 3. **Reaction of AGs with HSA.** Mass spectra of 33 g/L HSA incubated with ibuprofen analogues **1a-d** at 5.0 mM for 16 h at 37 ºC and pH 7.4. **1a**: transacylation (blue), found: 66610; expected: 66612; glycosylation (red), found: 66787; expected: 66788; dual addition (both transacylation and glycosylation, green), found: 66957; expected: 66962. **1b**: transacylation, found: 66627; expected: 66626; glycosylation, found: 66802; expected: 66802. **1c:** transacylation, found: 66625; expected: 66626; glycosylation, found: 66801; expected: 66802. **1d**: transacylation, found: 66635; expected: 66640; glycosylation, found: 66813; expected: 66816. Unmodified HSA is the major peak in all cases (found: 66440, 66442, 66439 and 66439 Da; expected: 66438 Da).

95 words

Figure 4: **Mapping the Sites and Types of Reactivity of Metabolites with Human Serum Protein**. Sequence map of HSA indicating primary amino acid sequence including disulfide bonds (yellow). Chemoselective reaction sites for glycosylation are coded red; sites for transacylation are coded blue, whereas Lys525 exhibiting both glycosylation and transacylation reactivity is coded green (see Table 2 for reactivity details).

59 words

18
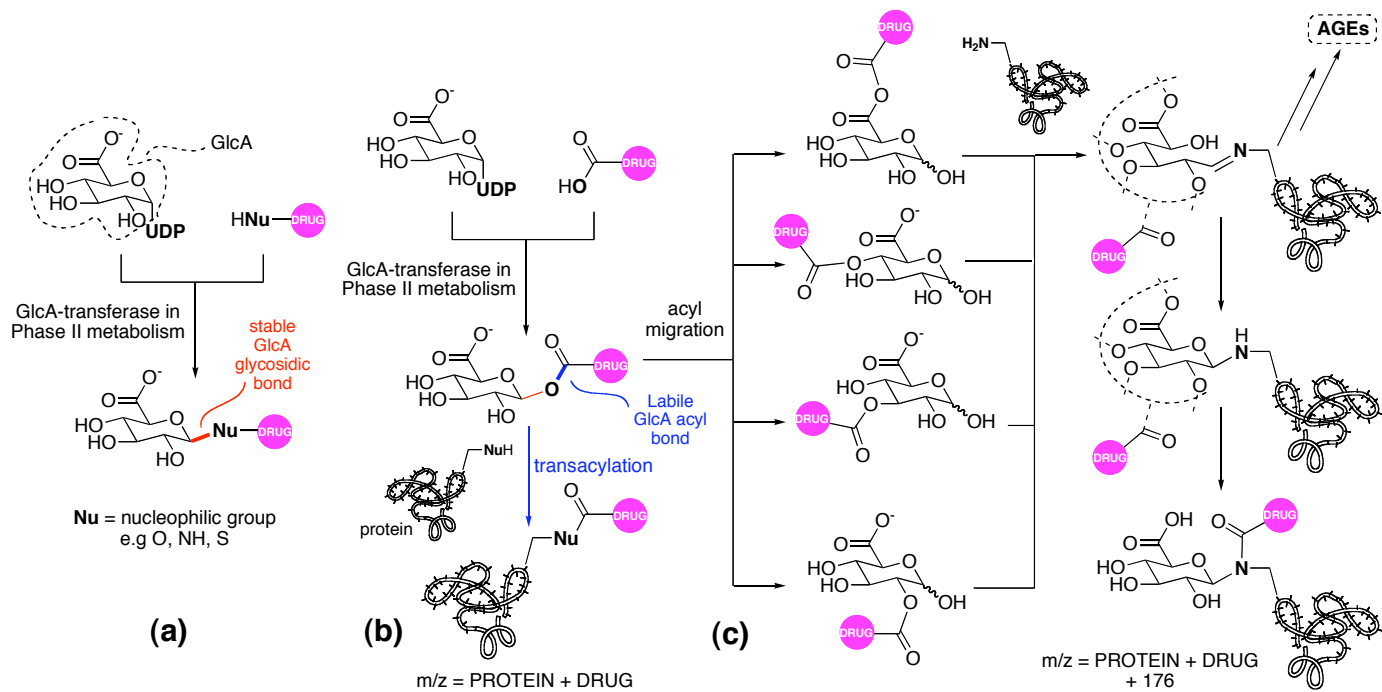
Figure 1 (width 89mm or 183mm or 120mm)

(a)

[P] = protecting group = allyl, Bn or PMBn

(b)
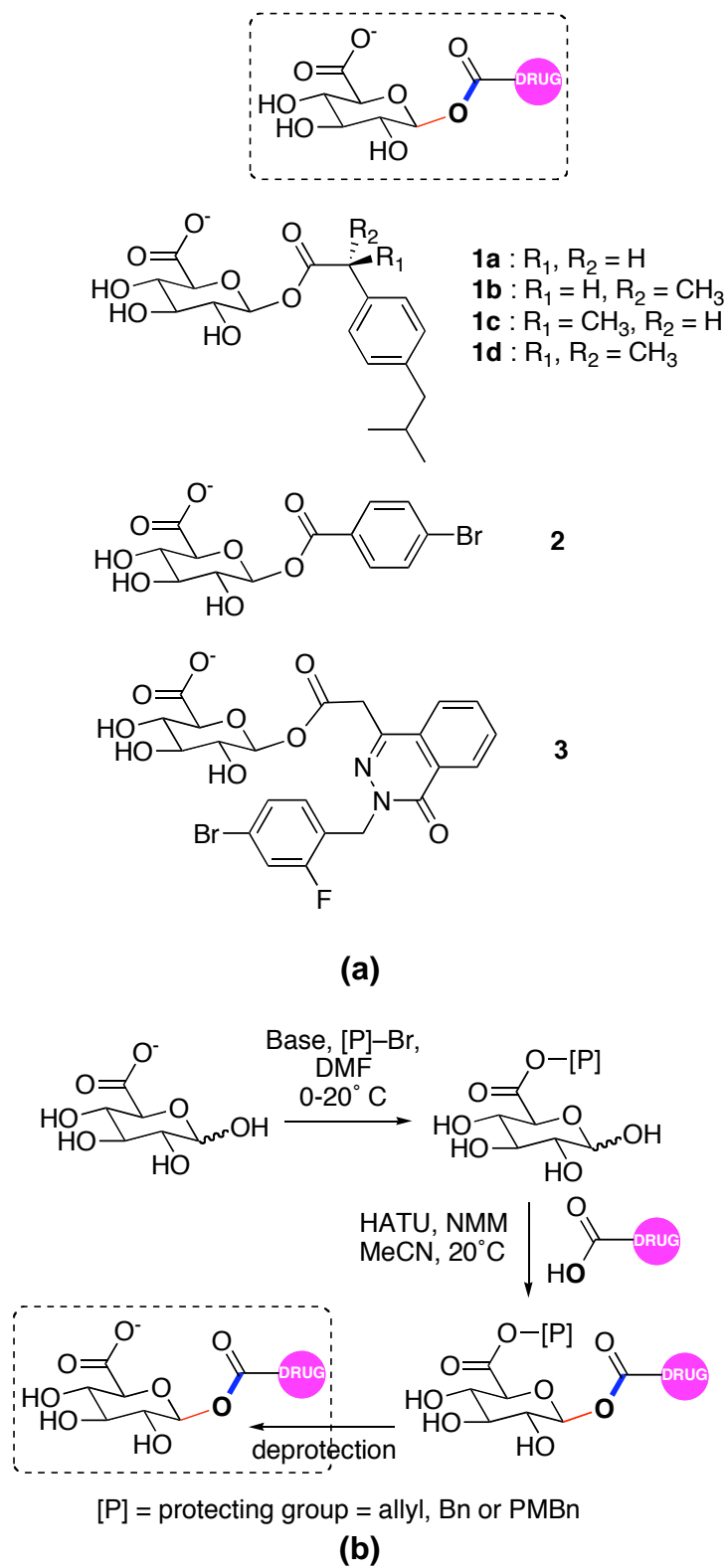
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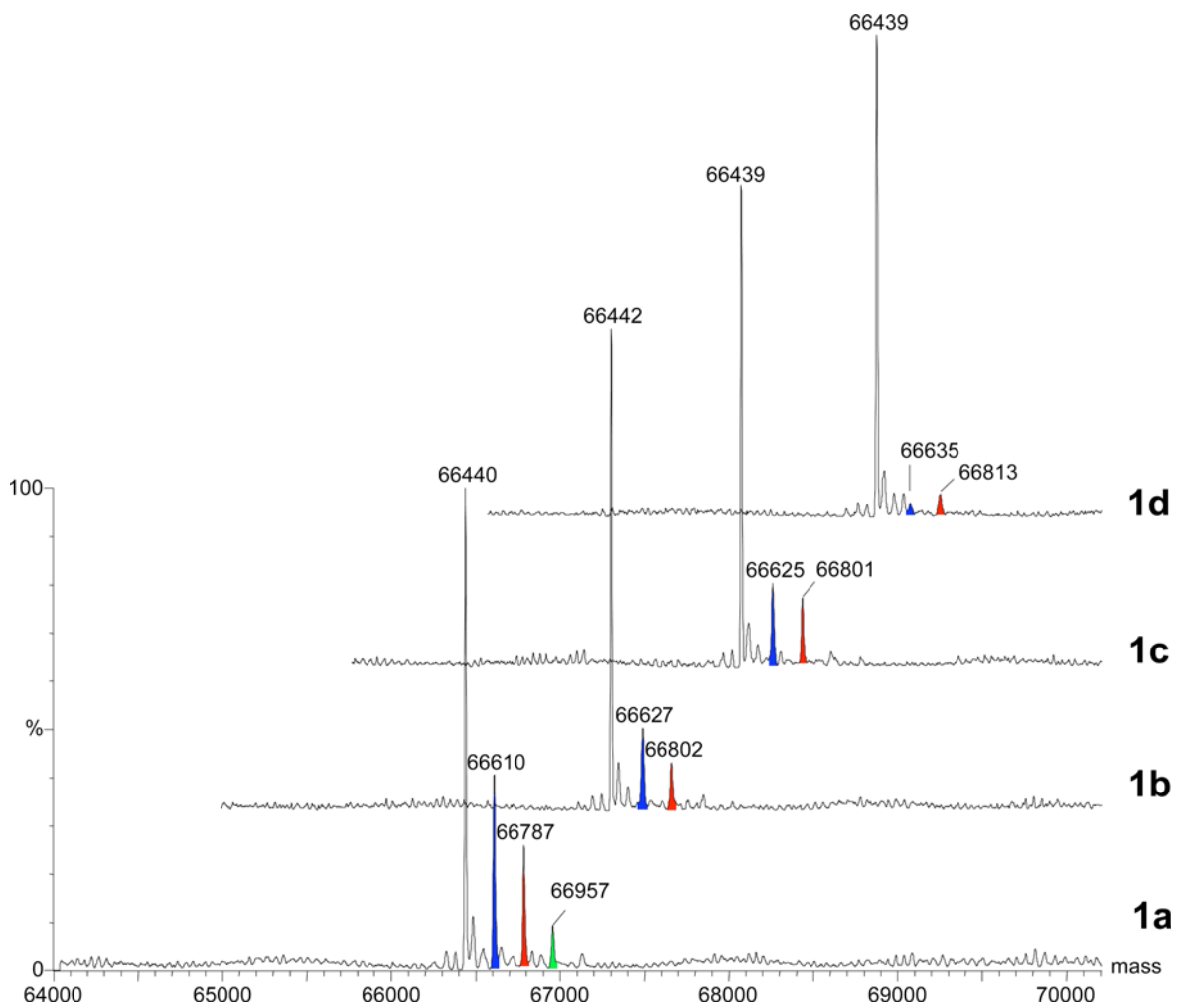
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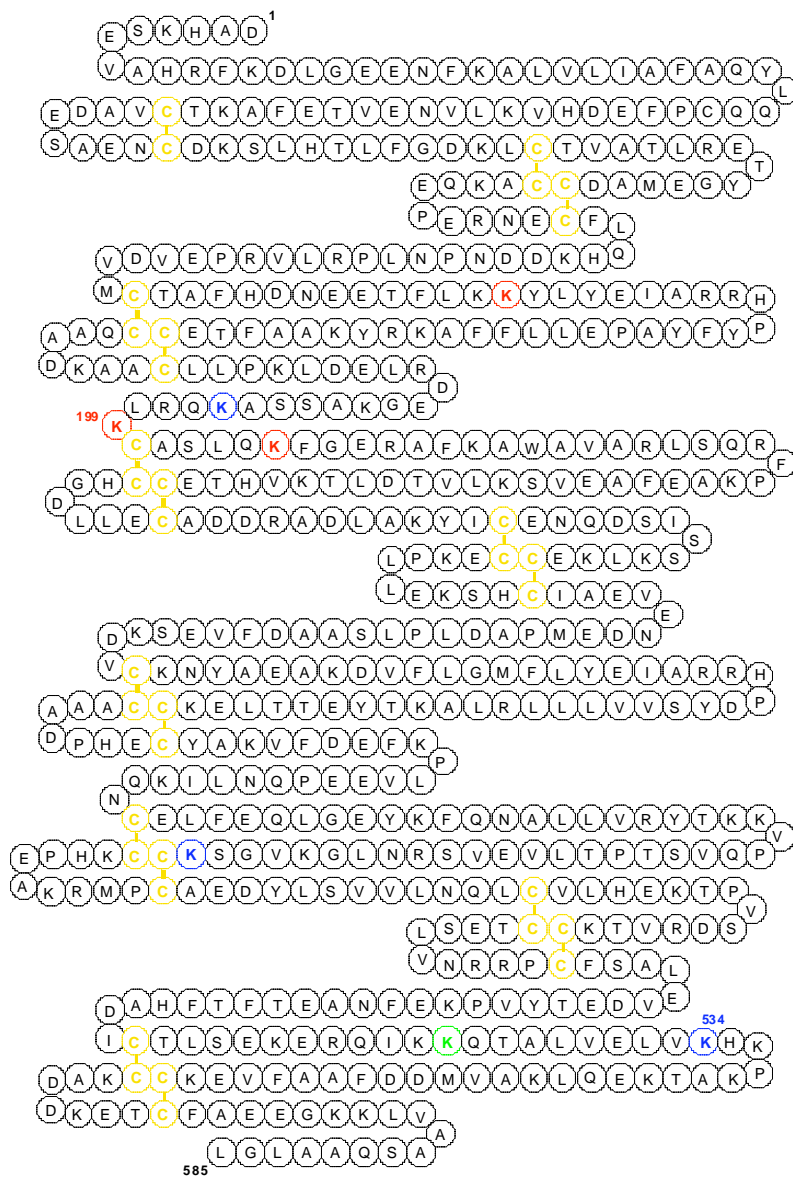
Figure 4 (width 89mm or 183mm or 120mm)

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