Synthesis of Glucosamine Mimics as Antibiotics

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Abbreviations

5'UTR	5'-Untranslated region (of the mRNA)
AllN6P	D-Allosamine-6-phosphate
ABNO	9-Azabicyclo[3.3.1]nonane N-oxyl
Ac	Acetyl
AD-mix	Asymmetric dihydroxylation-mix
AIBN	Azobisisobutyronitrile
atm	Atmosphere (pressure)
B. cereus	*Bacillus cereus*
BMS	Borane dimethylsulfide
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
B. subtilis	*Bacillus subtilis*
B. thuringiensis	*Bacillus thuringiensis*
Bz	Benzoyl
CBS	Corey-Bakshi-Shibata
Cbz	Carbobenzoxy
COD	1,5-Cyclooctadiene
Coll	Collidine
COSY	Correlation spectroscopy
Cp	Cyclopentadiene
CSA	Camphorsulfonic acid
Cm	Chloramphenicol
DAST	Diethylaminosulfur trifluoride
DCM	Dichloromethane

DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTMP	2,6-Di-*tert*-butyl-4-methylpyridine
E. coli	*Escherichia coli*
FA	Formic acid
FC	Flash column chromatography
FMN	Flavin mononucleotide
FMDP	N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid
Fru6P	D-Frucotse-6-phosphate
GalN6P	D-Galactosamine-6-phosphate
Glc6P	D-Glucose-6-phosphate
GlcN	D-Glucosamine
GlcNAc	*N*-Acetyl-D-glucosamine
GlcNAc1P	*N*-Acetyl-D-glucosamine-1-phosphate
GlcN6P	D-Glucosamine-6-phosphate
glmS	(Gene segment of) glucosamine-6-phosphate-synthase
GlmS	Glucosamine-6-phosphate-synthase (enzyme)
GlmU	*N*-Acetylglucosamine-1-phosphate uridyltransferase
Gln	Glutamine
Glu	Glutamic acid
HILIC	Hydrophilic interaction liquid chromatography
HMBC	Heteronuclear multiple bond correlation
HMDS	Hexamethyldisilazan
HOESY	Heteronuclear Overhauser Effect Spectroscopy

HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
$IpcBH_2$	monoisopinocampheylborane
J	Coupling constant
LAH	Lithium aluminum hydride
LCMS	Liquid chromatography-mass spectrometry
LDA	Lithium diisopropylamide
*m*CPBA	meta-Chloroperoxybenzoic acid
ManN6P	D-Mannosamine-6-phosphate
MeCN	Acetonitrile
MeOH	Methanol
MIC	Minimal inhibitory concentration
Ms	Mesyl
MTBA	α-methoxy-α-trifluoromethylphenylacetic acid
MurA	UDP-GlcNAc-3-enolpyruvyl transferase
MurNAc	*N*-Acetylmuramic acid
NMO	*N*-Methylmorpholine N-oxide
NMR	Nuclear magnetic resonance spectroscopy
NOESY	Nuclear Overhauser Enhancement Spectroscopy
NFSI	*N*-Fluorobenzenesulfonimide
ORF	open reading frame
PCC	Pyridinium chlorochromate
PDB	Protein Data Bank
Pd/C	Palladium on charcoal

ppm	Parts per million
PTFE	Polytetrafluoroethylene
PTPP	pyrithiamine pyrophosphate
PTS	Phosphoenolpyruvate-dependent phosphotransferase system
RCM	Ringclosing-metathesis reaction
R_f	Retardation factor
rt	Room temperature
SAR	Structure-activity relationship
S_NAr	Nucleophilic aromatic substitution
TBDPS	*Tert*-butyl(chloro)diphenylsilyl
TEAB	Triethylammonium bicarbonate
TBAF	Tetra-*n*-butylammonium fluoride
TBAI	Tetra-*n*-butylammonium iodide
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
Tf	Triflyl
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TPAP	Tetrapropylammonium perruthenate
TPP	Thiamine pyrophosphate
Ts	Tosyl
UDP-GlcNAc	UDP-*N*-acetylglucosamine
UTP	Uridine triphosphate
WHO	World Health Organization

Z	Carbobenzoxy
δ	Chemical shift

1 Introduction

In 2014 the then UK Prime Minister presented the study “Review on Antimicrobial Resistance”[1] and he commented in clear words: "If we fail to act, we are looking at an almost unthinkable scenario where antibiotics no longer work and we are cast back into the dark ages of medicine". The report suggests that in 2014 at least 700,000 people died from drug resistant bacterial infection, stating that this number is likely an underestimation. These casualties are believed to rise to 10 million annual deaths by 2050.[1] Bacteria that are resistant to at least first line antibiotic treatment are estimated to cost the US health system alone 20 billion US dollar every year.[1,2] Bacteria have long been deemed largely vanquished by the golden age of antibiotic drug discovery in 1950s and 60s. However, the tranquility and safety provided by theses antibiotics are being undermined by an ever increasing number of multidrug resistant bacteria. One particular statement made in the study “Review on Antimicrobial Resistance” underlines the urgency of the issue. In the past the antibiotic colistin was not given due to the possibility of resulting kidney failures. However, today it has reentered service as a last resort for the treatment of otherwise resistant bacterial infections.[1,3] The development of resistances is hard to stop as some pathogens have incredibly short generation times allowing them to double their biomass every 20 to 30 minutes. Under the selection pressure of antibiotics, pathogens can acquire resistances by random DNA mutations and rapidly outgrow the wild type. Therefore, we need to be vigilant and develop new antibiotic in order to be prepared for these pathogens.

One potential target for these envisioned drugs are riboswitches. Riboswitches are RNA fragments that regulate gene expression in dependency of a metabolite concentration. Up to 4 % of the bacterial genome consist of riboswitches, rendering them interesting for drug development. The *glmS* riboswitch is promising as a drug target as it is found in a multitude of human pathogens.[4] It is predominantly found in the 5’-untranslated regions (5’UTR) of the mRNA of gram-positive bacteria. The *glmS* riboswitch is unique compared to the other classes of riboswitches because it does not simply control the expression of the downstream genes in a metabolite-

dependent manner, but it is able to catalyze its own self-cleavage upon metabolite binding. The gene, controlled by the *glmS*-ribozyme encodes for the glucosamine-6-phosphatesynthase (GlmS). This enzyme catalyzes the formation of glucoseamine-6-phosphate (GlcN6P) from fructose-6-phosphate and glutamine. GlcN6P in return is the metabolite, which is able to induce the self-cleavage reaction of the *glmS*-ribozyme. Therefore, the concentration of GlcN6P is self-regulated by controlling the amount of cleaved *glmS* riboswitch and hence the amount of GlmS. GlcN6P is metabolized to UDP-*N*-acetylglucosamine (UDP-GlcNAc) which is important for the biosynthesis of peptidoglycan. This polymer is also called murein and is an essential part of the bacterial cell wall.

A compound that is able to activate the *glmS* riboswitch without fulfilling the other biological function of GlcN6P would represent a promising antibiotic because it would allow to starve the bacterium of UDP-GlcNAc and therefore interfere with the bacterial ability to replicate. The structural requirements for such an activator were intensively investigated and narrowed down to two different sites. The ring oxygen of GlcN6P can be exchanged to a carbon atom, giving 5a-carba-glucosamine-6-phosphate (CGlcN6P). The introduction of phosphate surrogates to GlcN6P has also been proposed as a potential modification, but requires further investigation.

In this thesis these two sites of potential variation were investigated in detail. This was achieved by:

- Extending the exchange of the ring oxygen with sulfur giving thia-glucosamine-6-phosphate.
- Synthesizing a library of new phosphonate analogues of GlcN6P. This allows the adjustment of the pK_a of the phosphonate to the naturally occurring phosphate.
- Providing a viable synthetic route to 5a modified derivatives of carba-glucosamine, allowing a late stage modification. This resulted in a large library of new glucosamine mimics.

2 State of Knowledge

2.1 Antibiotics and Multidrug Resistant Bacteria

The development of resistances against antibiotics can be expected due to the rapid evolution of bacteria characterized by their fast growth and short doubling time as well as a general instability of the genome compared to eukaryotes.[5] The alarmingly quick development was enabled by human factors such as overuse and misuse of antibiotics in human medicine as well as for infection prevention measures in animal breeding and agriculture.[6] After the golden age of antibiotic development from 1940 to 1960, bacterial infections were considered as a problem of the past.[7,8] Unfortunately, the current development is teaching us that this can be considered a misconception and that the fight against bacterial pathogens is still an urgent one.[1] Most of the currently marketed antibiotics act via one of the following four modes of action: Interference with bacterial gene expression, inhibition of cell wall formation, hindrance of the activity of specific enzymes or the direct intervention in DNA replication. At the same time, antibiotic resistances can be categorized in three main modes of action:[9] Antibiotic modification signifies a modification or breakdown of the active antibiotic into an inactive derivative or decomposition product.[10] Antibiotic efflux or decreased influx is a second common form of developed resistance. The antibiotic is either excreted more effectively by the bacterium or up take is downregulated for example by mutations in active transporters.[11] The third commonly observed mode of resistance is the target modification. In this case, the target normally affected by the antibiotic is mutated resulting in an inert target, that is still able to fulfill its biological function in the presence of the antibiotic.[12] The exploitation of new modes of actions is highly desired in order to provide new approaches for antibacterial treatments and to slow down the generation of multidrug resistant pathogens.

Fortunately, the importance of this issue was recognized by the World Health Organization (WHO) which has published a list of pathogens deemed to require immediate attention, in the form of novel drug development.[13] In order to stay vigilant in the fight against resistant pathogens, not only derivatives of already existing antibiotics, utilizing the already exploited targets in the bacteria but also

molecules with new targets and novel modes of action are required.[14] This shift in paradigm is underlined by antibiotic drug discovery programs aiming to target proteins as well as underexplored nucleic acid targets.[15-17] One of these currently unexploited nucleic acid targets recognized as promising drug targets are riboswitches.[17-21]

2.2 Riboswitches

In contrast to eukaryotic gene regulation, multiple bacterial genes are controlled by mRNA structures, which are called riboswitches.[18,22-24] By 2019, around 40 distinct riboswitch classes have been discovered.[25-28] Certain estimations, based on the abundance and the distribution of these known riboswitch classes, propose that there are thousands of additional riboswitches left to be discovered.[25,26,29,30] Most of these structures are found in the 5'-untranslated regions (5'-UTR) of the mRNA. Riboswitches can form tertiary structures capable to bind a metabolite and can undergo a structural change upon binding of the activating metabolite.[18] The riboswitch does not interfere with the translation of downstream located gene in the absence of the metabolite. When a sufficient concentration of the metabolite is reached, binding of this metabolite to the riboswitch either provokes a cleavage of the riboswitch or a conformational change blocking the adjacent open reading frame (ORF).[18] Both cases lead to an interference with the expression of downstream located genes. Most of the genes or group of genes that are controlled by a riboswitch are responsible for the expression of proteins for the uptake or synthesis of the activating metabolite. Therefore, riboswitches are regulators of the intracellular concentration of associated metabolites.[18,22] In light of this important function for bacterial homeostasis some riboswitches represent promising drug targets. Especially those riboswitches that regulate the expression of genes, that influence, via their corresponding metabolite, the survival or virulence of a bacterial pathogen, are interesting for the development of new drugs.[31] These drugs can be mimics of the natural metabolite activating the riboswitch, and thereby, interfering with gene expression. In order to be successful in this application, the mimic of the natural metabolite must not be able to replace other functions of the natural

activator while preserving the ability to bind to the riboswitch.[18] Keeping these requirements in mind, several mimics of natural riboswitch activators have been shown to possess antimicrobial properties. One example is the flavin mononucleotide (FMN) riboswitch, the natural ligand FMN (**1**) is mimicked by the antibacterial compound roseoflavin (**2**). **2** has been shown to bind the FMN riboswitch because of its structural similarity to **1** (Figure. 1).[32] For the thiamine pyrophosphate (TPP) riboswitch an artificial activator was found by rational design, investigating the 3D-structur of the TPP (**3**) bound riboswitch.[33-35] Exchanging the thiazole by a pyridine resulted in pyrithiamine pyrophosphate (**4,** PTPP), which led to similar binding affinity and the ability to turn off the expression of the downstream genes.[18,35] Moreover, the lysine riboswitch has been targeted by artificial mimics. Thioether **5**, ether **6**, sulfone **7** and alkene **8** have been shown to be recognized by the riboswitch and to activate the former.[18,20]

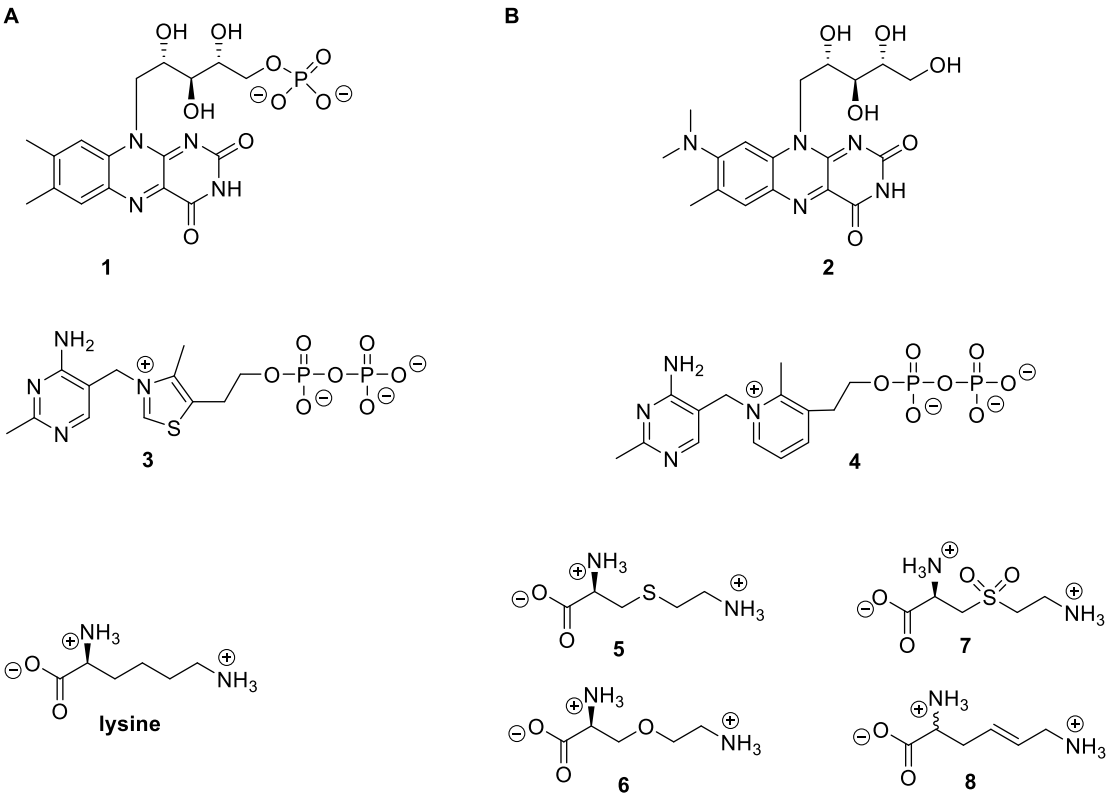


Figure 1: **A** Natural riboswitch activators, thiamine pyrophosphate **1**, lysine and Flavin mononucleotide **3**. **B** mimics of the natural metabolite shown to activate the riboswitch. Roseoflavin **2** pyrithiamine pyrophosphate **4**, lysine derivatives **5**, **6**, **7** and **8**.

2.2.1 *glmS* Riboswitch

The riboswitch of special interest for this work is the *glmS* riboswitch. In 2004, it was discovered by Breaker[36] and it is mainly found in the 5'-UTR of gram positive bacteria. The *glmS* riboswitch controls the gene encoding for the enzyme glucosamine-6-phosphatesynthase (GlmS), which catalyzes the reaction of glutamine and fructose-6-phosphate (Fru6P) to glutamate and glucosamine-6-phosphate (GlcN6P). GlcN6P in return is the activator for the riboswitch (Figure 2).[37-41]

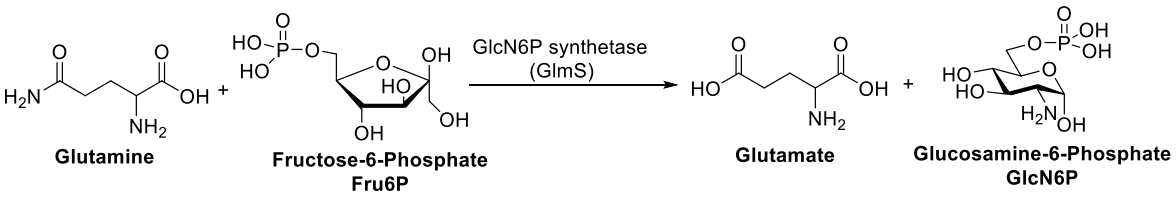


Figure 2: Reaction catalyzed by GlmS. Glutamine and Fru6P are converted to glutamate and GlcN6P.[42,43]

GlcN6P can bind to the *glmS* riboswitch via a binding pocket. As a result, a self-cleavage reaction is induced (Figure 3). The cleavage reaction, catalyzed by the metabolite as coenzyme is a unique feature of this class of riboswitches. Most of the other known riboswitches undergo a conformational change after binding of the corresponding metabolite, but not a chemical reaction. For this property the *glmS* riboswitch is also classified as a ribozyme.[36,44] The cleavage of the RNA leads to the recruitment of RNase J1 and subsequent degradation of the downstream coding RNA.[45]

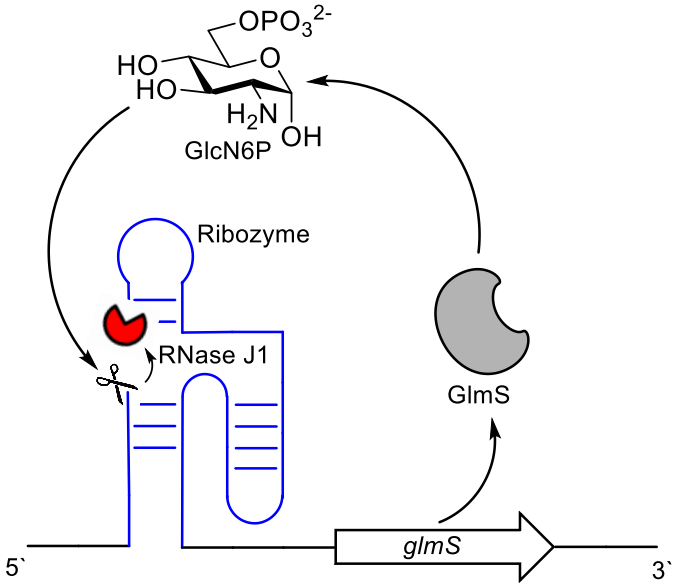


Figure 3: The *glmS* ribozyme is controlling the *glmS* gene. This gene is encoding for the enzyme GlmS which is catalyzing the formation of GlcN6P. The ribozyme can sense the concentration of GlcN6P and undergo a self-cleavage reaction upon binding of GlcN6P. The cleaved RNA is recognized by RNase J1, which degrading the RNA. [45-47]

Chemically, the cleavage of the *glmS* riboswitch is the fission of a 3',5'-phosphodiester (Figure 4). This results in a 2',3' cyclic phosphate as well as the 5'-OH group at the resulting 3' fragment.[48] The resulting cleavage product is recognized by the enzyme RNase J1 which degrades the cleaved RNA.[45,49] At physiological pH, GlcN6P acts as an acid base catalyst, as it accepts a proton at the dianionic phosphate and donates a proton from the protonated amine.[48,50] The exact base involved in the cleavage reaction is still disputed. It is unclear whether the phosphate, a metal bound hydroxide ion or another external base is involved in the deprotonation initiating the cleavage reaction.[51,52]

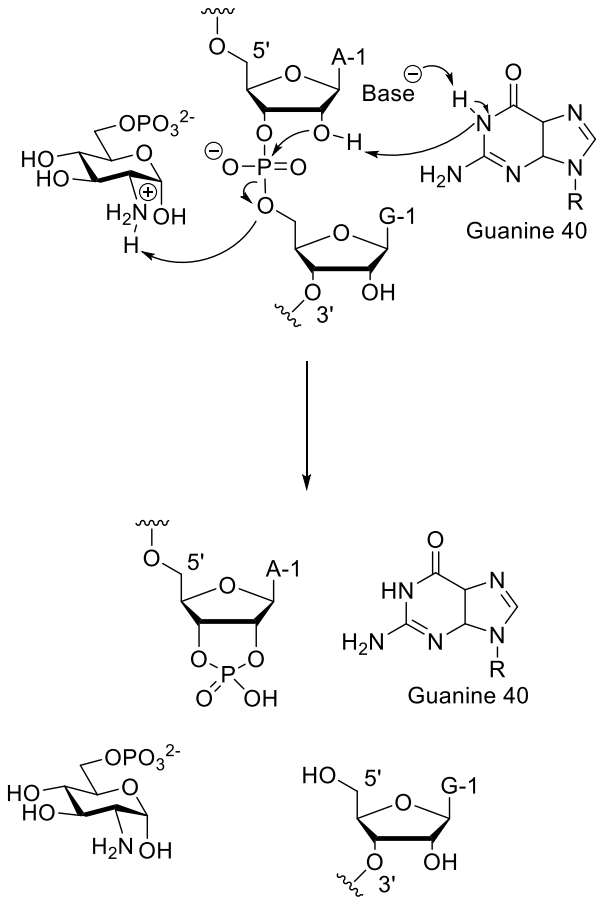


Figure 4: Self cleavage mechanism of the *glmS* riboswitch as found in *Thermoanaerobacter tengcongensis*. The N1 of the guanine 40 is deprotonated acting as a base for the following ribose deprotonation, leading to the formation of a 2',3' cyclic phosphate. This results in the cleavage of the RNA into two fragments with the 3' cleavage product containing a free 5' OH group [48,52,53]

Depending on the bacterial strain, slight differences in the structure of the *glmS* riboswitch exists. One consequence of this is a variation in half-life towards hydrolysis. The ribozyme present in *B. subtilis* has an inherent half-life of 4 h towards background hydrolysis, this is reduced to 15 s upon exposition to a saturating concentrations of GlcN6P.[36] This does signify an acceleration by a factor of 1,000. In other gram positive bacteria this acceleration can reach a factor of up to 100,000. This is the case in the *B. cereus glmS* ribozyme. Here, the half-life is reduced from 48 days to below 1 min upon saturation with GlcN6P.[40]

2.2.2 *glmS* Riboswitch Activators

Ligand recognition of the *glmS* riboswitch occurs via six nucleotides which form the binding pocket of this riboswitch. These nucleotides are highly conserved across bacteria carrying the *glmS* riboswitch and recognize the ligand by hydrogen bonding (Figure 5).[54,55]

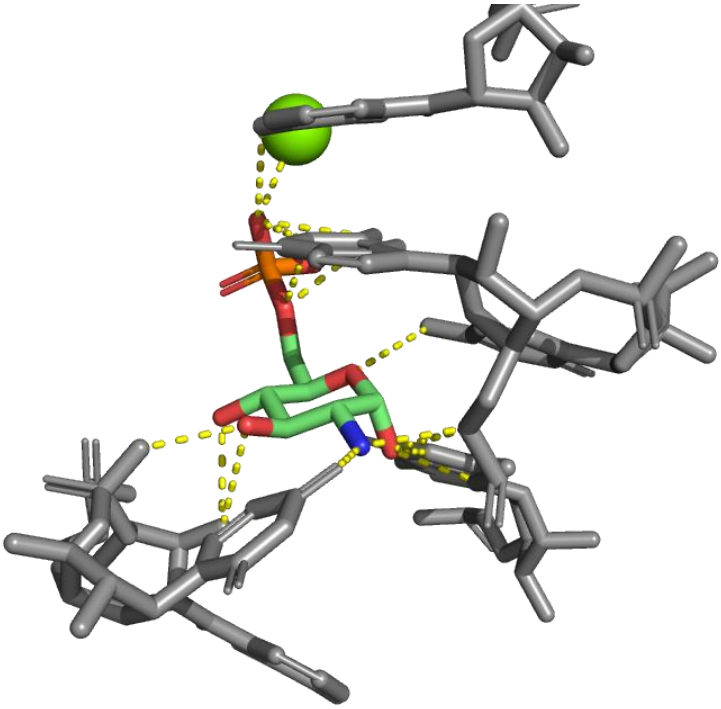


Figure 5: Crystal structure of the *Bacillus anthracis glmS* riboswitch bound to GlcN6P. Important hydrogen bonds to the binding pocket are visualized in yellow. The phosphate of GlcN6P binds to Mg^{2+}, shown as a green dot. (PDB: 2NZ4)[55] The riboswitch binding pocket is depicted in grey. Carbon atoms of GlcN6P are green, oxygen is red, nitrogen blue and phosphorous orange. Hydrogen is not shown.

There have been attempts to identify artificial activators by a highthroughput screening approach of a large library of commercially available and partly FDA approved compounds. This approach did not result in the discovery of activators apart from the previously known glucosamine (GlcN) .[56,57] This hinted to a high selectivity of the binding pocket towards the natural ligand GlcN6P. In order to better understand the requirements for successful activation of this riboswitch several mimics of GlcN6P were synthesized and investigated for their ability to induce the self-cleavage of the *glmS* riboswitch. This allowed the discovery of

several unnatural riboswitch activators demonstrating the utility of rational designed artificial activators.[37,39,42,58-60]

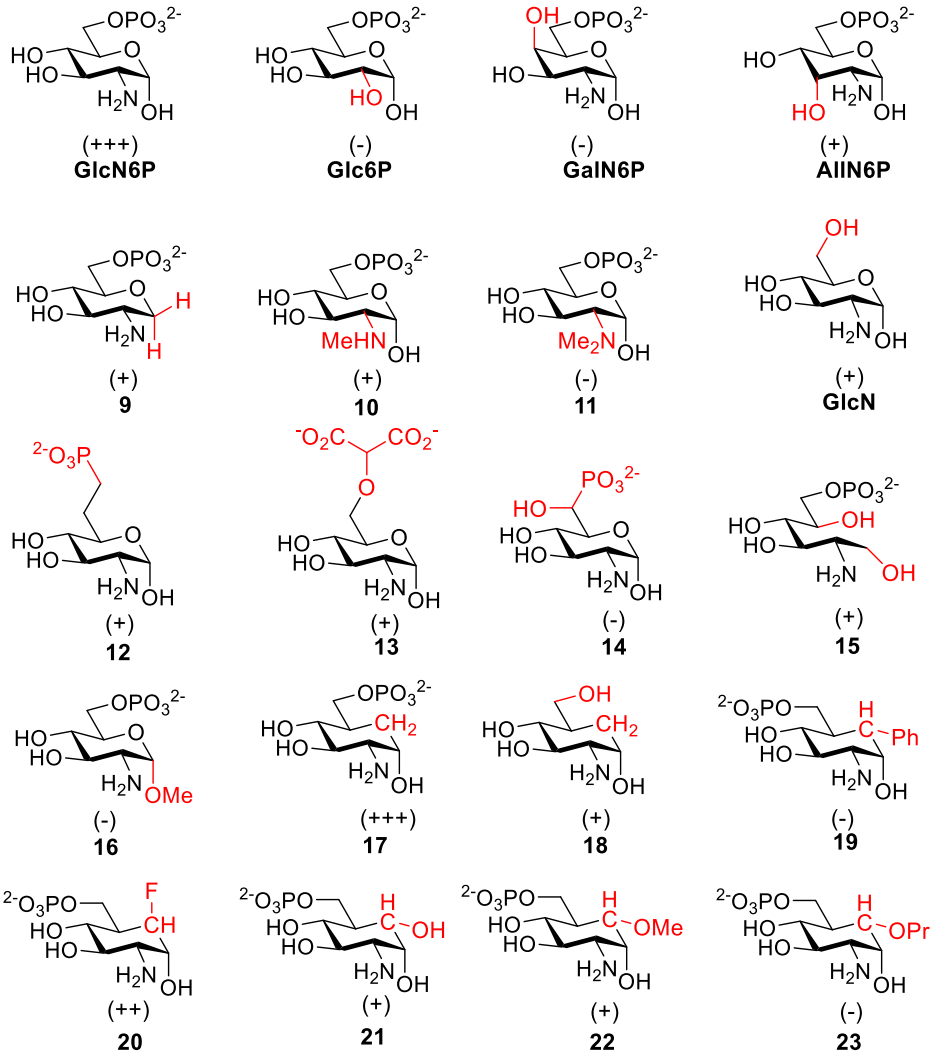


Figure 6: Natural occurring and synthesized GlcN6P mimics. Tested for their ability to activate the self-cleavage of the *glmS* riboswitch. A rough rating of their activity is given as follows: (+++) strong, (++) moderate to good, (+) weak, (-) no activation.[37,39,42,56,58-60]

These experiments also revealed several structural necessities, an activator has to possess (Figure 6). The cyclic structure, the equatorial amino group in the 2-position and the equatorial hydroxy group in the 4-position are necessary for a successful activation.[53,59,61] An alpha hydroxy group in the 1-position improves the binding to the riboswitch.[61] The 1-hydroxygroup is further of importance for the cleavage of

the *glmS* riboswitch as the 1-deoxy derivative **9** of GlcN6P shows a 70-fold decreased k_{obs} of the cleavage reaction compared to the natural ligand GlcN6P.[59] The inversion of the stereochemistry in the 3-position, as in allosamine-6-Phosphate (AllN6P), is less critical for successful activation than the inversion of the 4-position, as in galactosamine-6-phosphate (GalN6P).[50,62,63] Usage of AllN6P instead of GlcN6P as an activator resulted in a 3.5-fold decrease whereas GalN6P showed nearly a total loss of activation.[59]

When methylation of the amino group was performed it was observed that the naturally present primary amine GlcN6P performs best. In comparison to the amine GlcN6P, the secondary **10** and tertiary amine **11** showed an increasing loss in activity with increasing degree of alkylation.[59]

The phosphate group in the 6-position was also shown to be important for a successful activation. This was demonstrated by the significantly decreased activity of GlcN.[40,59]It was also investigated, if phosphate mimics can be introduced in the 6-position. The methylene phosphonate **12** and malonic acid derivative **13** were able to activate the riboswitch. However, they were suffering from decreased activity compared to the phosphate GlcN6P.[39] The hydroxy phosphonate **14** showed greatly decreased activation of the riboswitch, most likely due to the missing 6-position resulting in a shorter chain length.[60] Reduction of the hemiacetal to the sugar alcohol **15** as well as modification of GlcN6P to methyl glycoside **16** also resulted in the inability to induce the self-cleavage reaction efficiently. This signifies that a cyclic hemiacetal is necessary in order to activate the *glmS* riboswitch.

These experiments demonstrate that the design space for exploration of new artificial activators of the *glmS* riboswitch is very limited.[40,64] The most promising mimics for GlcN6P found so far are carba-sugars. In this type of compound, the ring oxygen is replaced by a methylene group. Within this thesis, numbering of all C-atoms is kept the same as in the parent sugar and the new position is referred to as the 5a-position. The methylene carba-derivative **17** of GlcN6P shows an activity comparable to the natural ligand.[37] Whereas the unphosphorylated derivative **18** shows a reduced activation of the *glmS* riboswitch. This is analogous to the reported relationship of GlcN6P and GlcN. The good mimetic properties of **17** are signified by

its ability to activate the riboswitch effectively. This inspired further work to introduce substituents in the 5a-position. Introduction of a phenyl moiety at the 5a-position resulted in compound **19** which did not activate the riboswitch efficiently.[47] Fluoro carba-sugar **20** on the other hand was able to induce the self-cleavage of the riboswitch. However, to a lesser degree than **17**.[47,58] *David Stängle* from the *Wittmann* group started the development of 5a-alkoxy and hydroxy modified carba-sugars. The new derivatives **21**, **22** and **23** showed some degree of activation.[64]

2.2.3 Biosynthesis of Peptidoglycan and the *glmS* Riboswitch as a Drug Target

Peptidoglycan, also called murein, is a major component of the cell wall of almost all bacteria.[65,66] This heteropolymer contains glycans that are cross linked by short peptides.[65] Glycans that are found in peptidoglycan are composed of *N*-acetyl glucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) (Figure 7). The lactic acid residue of MurNAc serves as anchor point for a short peptide containing a diaminopimelic acid (A_2pm) This residue allows for cross-linking by substitution of a D-Ala residue from a second chain.[67]

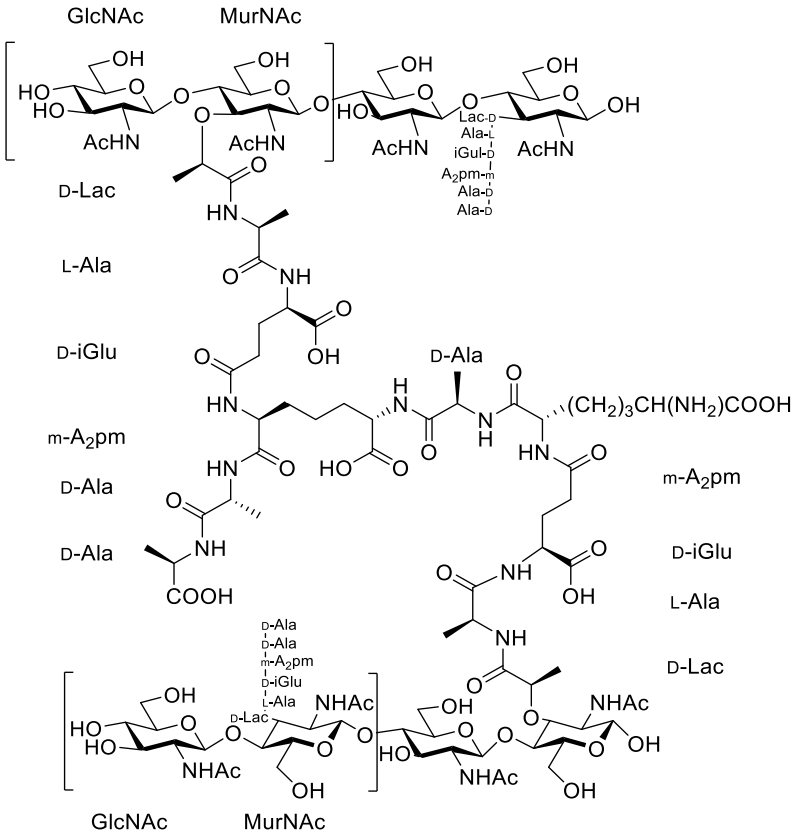


Figure 7: General structure of peptidoglycan.[67] Glycans containing GlcNAc and MurNAc are cross-linked by A_2pm containing peptides.

The peptidoglycan biosynthesis is necessary for the bacterium to build up its cell wall, which in return is needed to maintain its shape and to grow.[68] Inhibition of the bacterial synthesis of peptidoglycans effectively hinders a bacterium from growing. Therefore a substance that interferes with this process can be a promising antibiotic.[66] The peptidoglycan biosynthesis will be summarized by using *Bacillus subtilis (B. subitilis*) as an example organism . One important intermediate during the peptidoglycan biosynthesis is uridine diphosphate *N*-acetylglucosamine (UDP-D-GlcNAc), which is synthesized from *N*-acetyl-D-glucosamine-1-phosphate (D-GlcNAc1P) and uridine-5′-triphosphate (UTP) by the enzyme *N*-acetylglucosamine-1-phosphate uridyltransferase (GlmU) (Figure 8). D-GlcNAc1P is either synthesized from *N*-acetyl-D-glucosamine 6-phosphate (D-GlcNAc6P) or D-glucosamine-1-phosphate (D-GlcN1P). D-GlcN1P is synthesized from D-GlcN6P, which can be obtained from phosphorylation of D-GlcN provided by external sources. D-GlcN1P can also be synthesized from fructose 6-phosphate (D-Fru6P) and

glutamine (L-Gln) by the enzyme GlmS.[66,69-72] As described above, the expression of this enzyme is controlled by the *glmS*-riboswitch in multiple gram positive bacteria.[36] Therefore, activation of the *glmS* riboswitch by an activator that cannot be used for the synthesis of peptidoglycan would help to interfere at an early stage of the bacterial peptidoglycan biosynthesis. It has to be considered that two alternative sources for amino sugars are known to contribute to the synthesis of peptidoglycan. GlcN and GlcNAc bypass GlmS, the are also transported by the phosphoenolpyruvate-dependent phosphotransferase system (PTS), a system of several transports responsible for the uptake and phosphorylation of multiple hexoses.[73] GlcN and GlcNAc are transported by the PTS transporters $EIICBA^{GlcN}$ and EIICB respectively.[66,68,74] The two proteins also phosphorylate GlcN to GlcN6P and GlcNAc to GlcNAc6P. If GlcN and GlcNAc are not available in sufficient amounts to support the biosynthesis of peptidoglycan, GlmS becomes an essential enzyme for the survival of the bacterium.[43,75,76] It has been shown that GlmS mutants cannot sustain growth in human body fluids. [77-79] This can be explained by insufficient concentrations of GlcN and GlcNAc. Therefore, the bacterium relies on GlmS in the biosynthesis pathway of peptidoglycan. This makes GlmS an essential enzyme.[77-79]

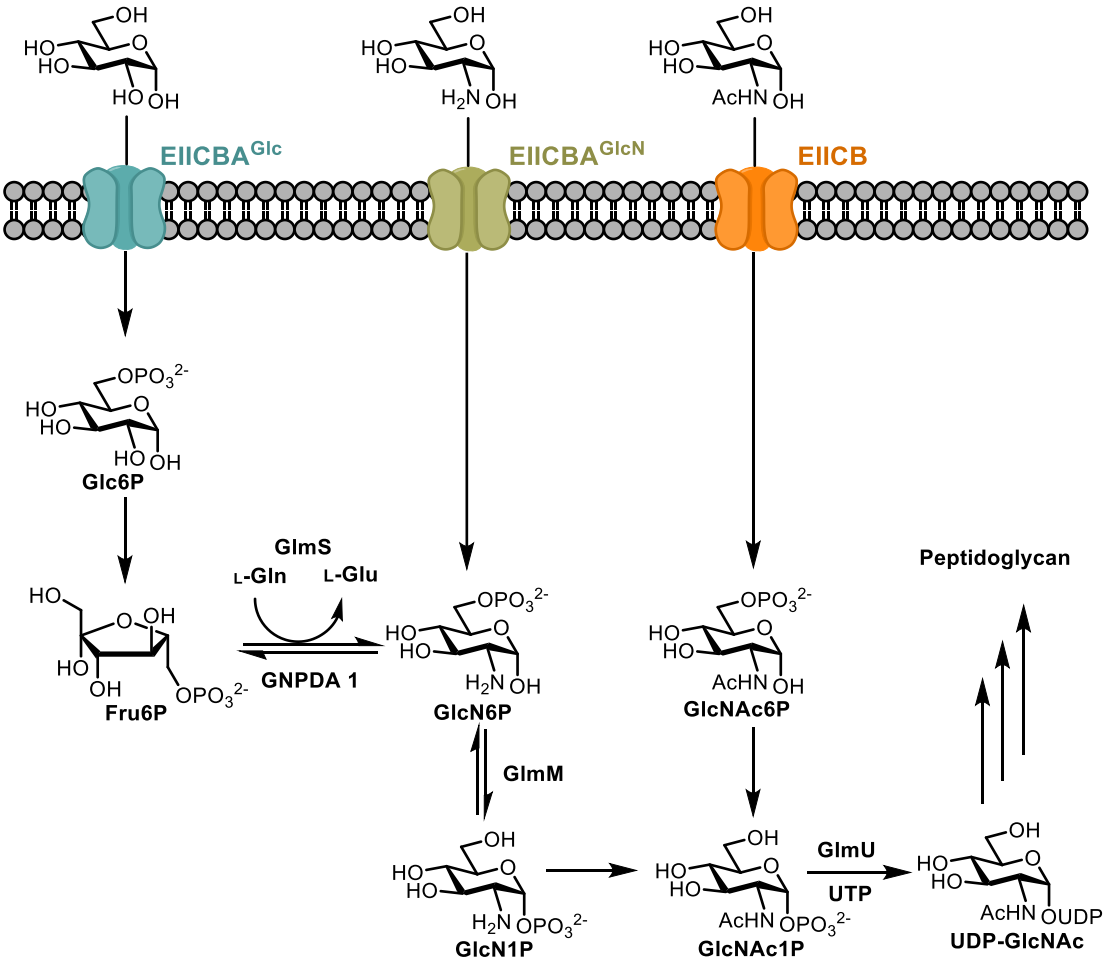


Figure 8: Schematic representation of UDP- GlcNAc synthesis in bacteria. UDP- GlcNAc is needed for the biosynthesis of peptidoglycan. Three amino sugar sources can be used for the synthesis. GlcNAc can be converted to GlcNAc6P upon active transport. This can be converted to GlcNAc1P. GlcNAc1P is the substrate for the enzyme GlmU to form UDP-D-GlcNAc. GlcN can be actively transported and phosphorylated by EIICBA, yielding GlcN6P. GlmM converts this to GlcN1P, which serves as precursor for GlcNAc1P. The final source is glucose giving Glc6P after active transport and phosphorylation by EIICBA^{Glc}. This can be converted to Fru6P serving together with Gln as the substrate of GlmS to give GlcN6P and glutamic acid (Glu).[47,69-72]

Depriving bacteria of UDP-GlcNAc by interfering with the biosynthesis of GlcN6P was demonstrated as a feasible approach a study two naturally produced antibiotics Bacilysin (**24**) and compound A 19009 (**25**) (Figure 9).[80,81] Bacilysin is produced in *Bacillus subtilis* and was shown to inhibit GlmS in *Escherichia coli* and *Salmonella*.[43,82,83] Compound A 19009 is produced by *Streptomyces collinus*.[80] The compound and artificial N^3-(4-methoxyfumaroyl)-L-2,3-diaminopropanoic acid (FMDP) peptides also inhibit GlmS by irreversible binding the glutamine binding domain of the enzyme.[84,85] The most effective of these artificial compounds is the

dipeptide antibiotic Nva-FMDP **26**.[86,87]. Compounds **24** and **25** are produced by gram positive bacteria and target mainly gram negative bacteria.[43,80-83]

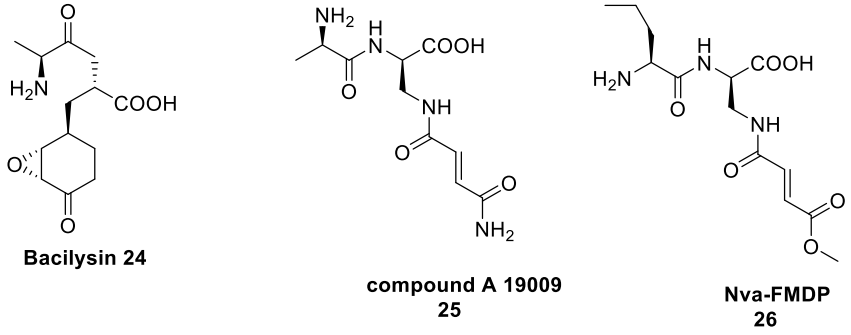


Figure 9: Antibiotics targeting GlmS. Bacilysin **24**, compound A 19009 **25** and Nva-FMDP **26** have been demonstrated to inhibit the GlmS by irreversible binding to the glutamine biding domain and thereby inhibiting bacterial growth. [84-87]

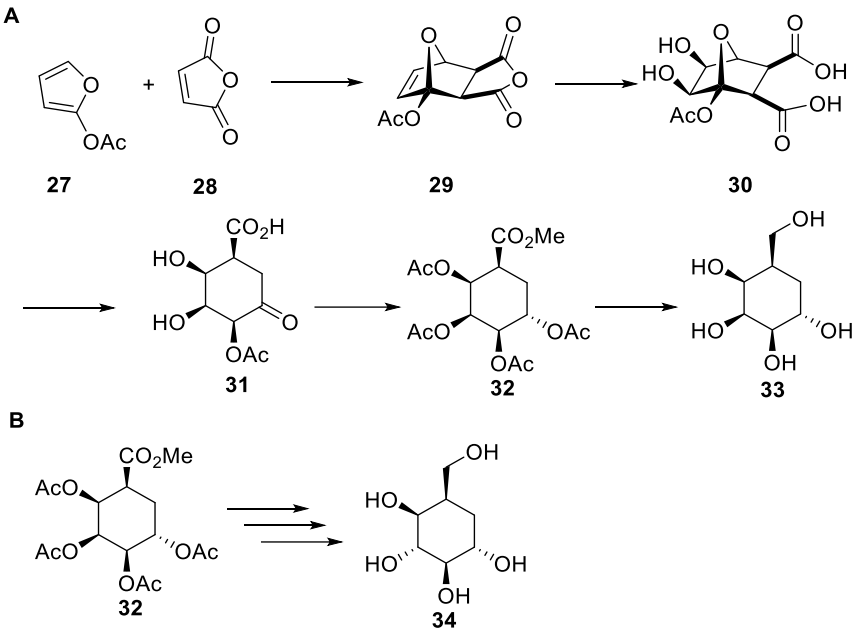
Targeting GlmS indirectly by activating the *glmS* riboswitch, would expend the interference with GlcN6P synthesis to gram positive bacteria which carry the riboswitch.[88,89] It has been shown that carba-mimics of GlcN6P possess the ability to activate the *glmS* riboswitch. These carbohydrate mimics are promising targets for the development of antibiotics that interfere with the GlcN6P synthesis. One class of the mentioned carbohydrate mimics are synthetic carba-pyranoses.

2.3 Synthesis of Carba-Pyranoses

2.3.1 Using Non-Carbohydrate Precursors for the Synthesis of Carba-Pyranoses

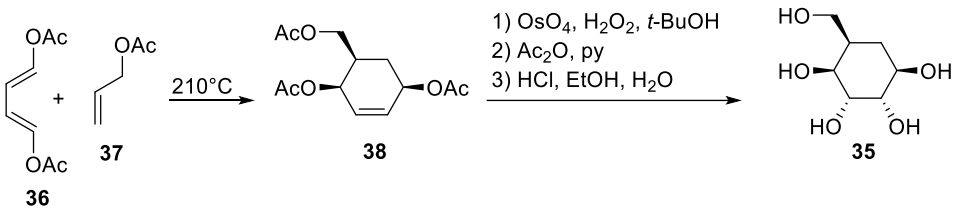
The synthesis of carba-pyranoses has been subject of extensive research and several general strategies to gain access to this compound class have been developed. A selection of these strategies will be presented in the following chapter

The first synthesis of a carba-sugar was performed by *McCasland* in 1966[90]. The synthesis started with a sequence reported before by *Daniels,*[91] namely with a Diels-Alder reaction using 2-acetofuran (**27**) and maleic anhydride (**28**) yielding racemic compound **29**(Scheme 1). After dihydroxylation and hydrolysis of the reaction product, compound **30** was obtained. This intermediate was reacted further with water achieving the transformation to ketoacid **31**. The ketone was reduced, the acid esterified and a peracetylation was performed, yielding compound **32**. Reduction of the esters gave the first carba-pyranose 5a-carba-α-DL-talopyranose **33**.



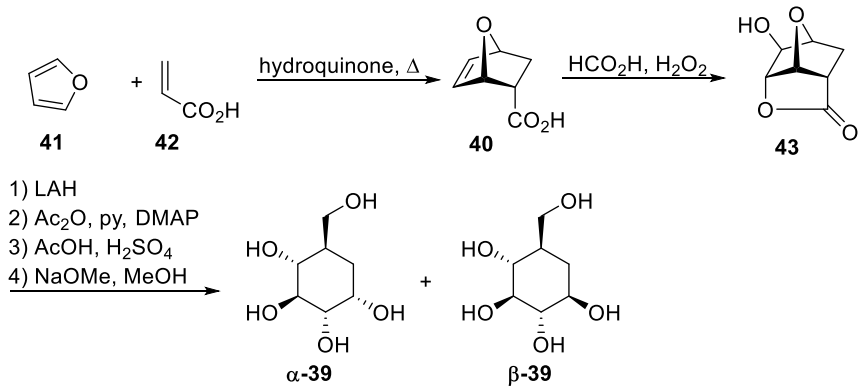
Scheme 1: **A** Synthesis of 5a-carba-α-DL-talopyranose **33**. Utilizing Diels-Alder chemistry and modification of the resulting 7-Oxanorbornene **29**.[90,91] Only the D-enantiomer is shown **B** Epimerization of **32** allows for the synthesis of 5a-carba-α-DL-galactopyranose **34.**[92] Only the D-enantiomer is shown

In the following years, several other sugar epimers were synthesized either by epimerization of compound **32**, as for example 5a-carba-α-DL-galactopyranose **34**[92] or by utilizing different starting materials in the Diels-Alder reaction yielding 5a-carba-β-DL-gulopyranose **35** (Scheme 2). In this synthesis, 1,4-diacetoxy-1,3-butadiene **36** was used as the diene and reacted with allyl acetate **37** to give compound **38** as product. Dihydroxylation followed by deacetylation yielded compound **35**.[93]



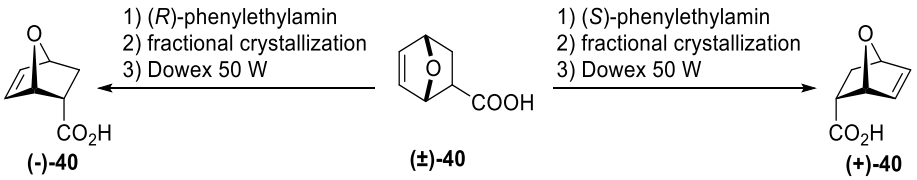
Scheme 2: Synthesis of 5a-carba-β-DL-gulopyranose **35**. Involving a Diels Alder reaction followed by dihydroxylation. [93] Only one enantiomer is shown.

The synthesis of these carbohydrate mimics by *McCasland* inspired other researchers to synthesize further epimers of these carba-sugars. Especially noteworthy is the synthesis of 5a-carba-α/β-DL-glucopyranose **39** by *Ogawa* who established not only the synthesis of compound **39** but also a plethora of different carba-analogues. His strategy uses the common intermediate 7-oxanorbornene **40** from which several carba-sugars can be accessed (Scheme 3).[94,95] The key intermediate **40** was prepared by reacting furan (**41**) with acrylic acid (**42**). Reaction of **40** with hydrogen peroxide and formic acid yielded lactone **43**, which was further converted to compound **39** by reduction with lithium aluminum hydride (LAH), acetylation and hydrolysis of the bridging ether followed by acetyl deprotection.



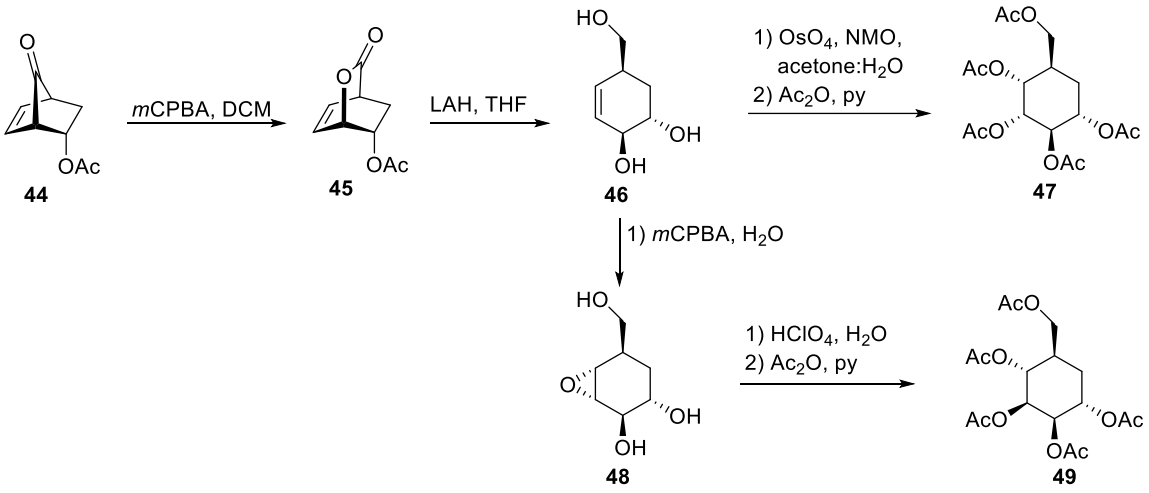
Scheme 3: Synthesis of carba-sugars by *Ogawa*. Using the 7-Oxanorbornene **40** as intermediate giving access to a multiple racemic structures. [94,95] Only one enantiomer is shown.

However, one major drawback of this approach is the lack of enatioselectivity. All of the syntheses described above give a racemic mixture of the D- and L- carba-sugar. This issue was addressed by *Ogawa* by providing optically pure **40** via enantiomeric resolution by fractional crystallization with either (*R*)- or (*S*)-phenylethylamine. This provides a suitable route to the enantiopure carba-derivatives of glucose as well as galactose (Scheme 4).[96,97]



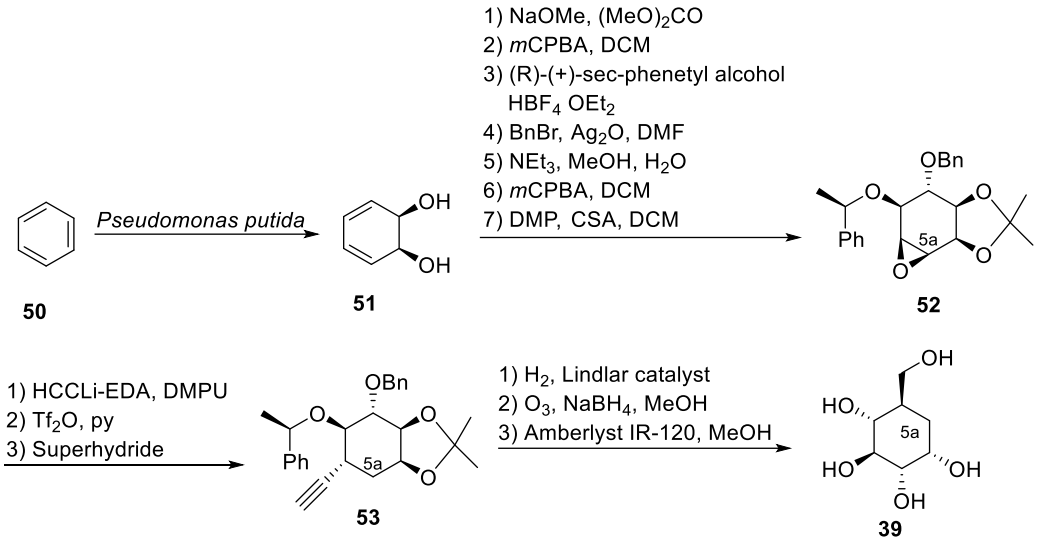
Scheme 4: Enantiomeric resolution of the 7-oxanorbornene **40**. Allowing for the synthesis of enantiomerically pure carba-sugars.[96,97]

The group of *Metha* pursued an approach via a different intermediate. They used endo-2-acetoxy-7 norbornenones **44** and Bayer-Villiger oxidation conditions to gain access to lactone **45** (Scheme 5). When performing a reduction of compound **45,** they accessed the carbocycle **46**, which was further decorated by dihydroxylation followed by peracetylation to give access 5a-carba-α-DL-altropyranose pentaacetate **47**. Treatment of **46** with *m*CPBA resulted in the formation of epoxide **48** which was converted to 5a-carba-α-DL-mannopyranose pentaacetate **49** by peracetylation.[98]



Scheme 5: 7-Norbornenones serve as precursors for 5a-carba-α-DL-altropyranose pentaacetate **47** and 5a-carba-α-DL-mannopyranose pentaacetate **49**. [98] Only one enantiomer is shown.

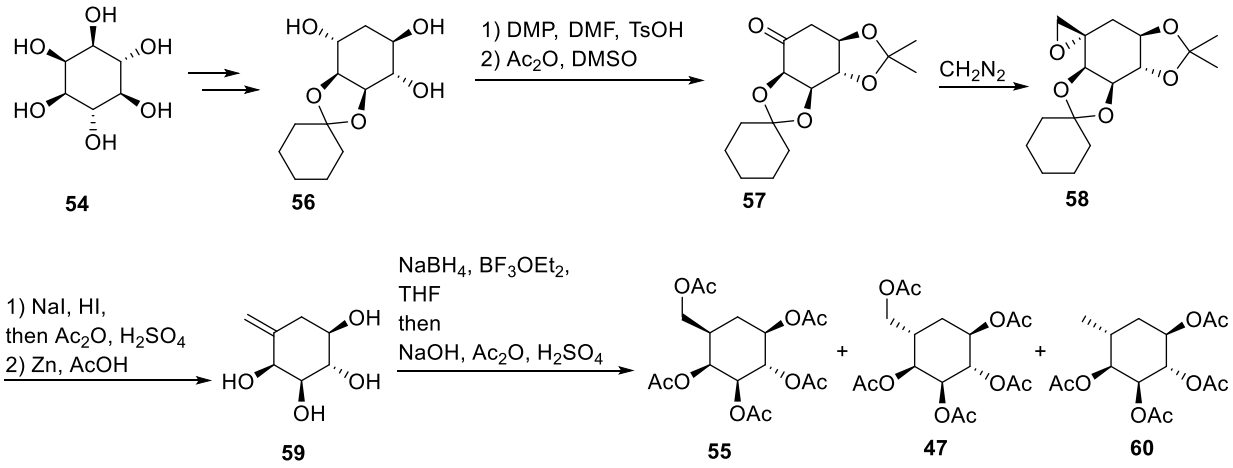
Another interesting approach to access carba-sugars starts from an aromatic precursor. *Ley* reported a synthesis of 5a-carba-α-D-glucopyranose **39** starting with an oxidation of benzene **50** to meso-cyclohexa-3,5-diene-1,2-diol **51** by using *Pseudomonas putida* (Scheme 6).[99] **51** was then converted to the intermediate epoxide **52**, which can be isolated as one enantiomer after several steps. The epoxide of **52** was attacked by lithium acetylide ethylene diamine complex to give alkyne **53**. This transformation was followed by a deoxygenation and Lindlar reduction with subsequent ozonolysis yielding the enantiopure 5a-carba-α-D-glucopyranose **39**.



Scheme 6: Conversion of benzene to meso-cyclohexa-3,5-diene-1,2-diol **51** followed by enantioselective transformation to the epoxide **52** allowes for the synthesis of enantiomerically pure 5a-carba-α-D-glucopyranose **39**. [99]

2.3.2 Using Inositols as Precursors for the Synthesis of Carba-Pyranoses

At a first glance, inositols appear like the ideal choice for the synthesis of carba-sugars. All that is missing is the 6 position to yield a 5a-hydroxy modified carba-sugar. However, the differentiation of the six hydroxy groups makes this starting material less appealing. Despite these challenges synthetic routs have been reported. Myo-Inositol **54** is the most abundant natural occurring cyclitol and was used as starting material in the synthesis of 5a-carba-β-DL-galactopyranose pentaacetate **55** and 5a-carba-α-DL-altropyranose pentaacetate **47** (Scheme 7).[100,101] Myo-inositol was converted to isoproylidene protected anhydroinositol **56** and oxidized to the ketone **57** using Albright-Goldman conditions. A Büchner–Curtius–Schlotterbeck reaction with diazomethane yielded epoxide **58**. Opening of this epoxide and elimination gave alkene **59**. Hydroboration of **59** afforded a mixture of 5a-carba-β-DL-galactopyranose pentaacetate **55**, 5a-carba-α-DL-altropyranose pentaacetate **47** and 6-deoxy 5a-carba-α-DL-altropyranose tetraacetate **60**. This relatively complicated mixture of three different racemic carba-sugars demonstrates the complications associated with this kind of synthetic strategy.



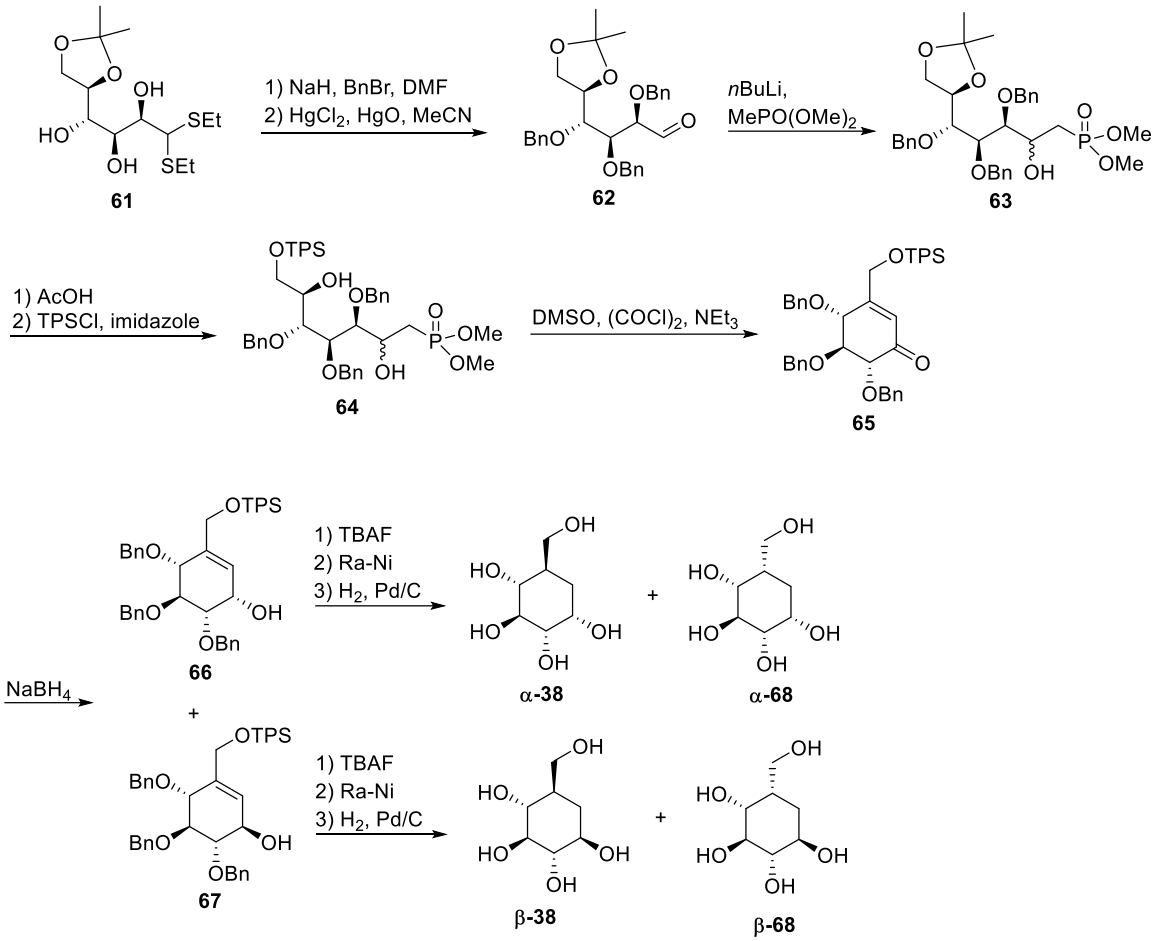
Scheme 7: Myo-inositol **54** was used as starting material for the synthesis of a mixture of 5a-carba-β-DL-galactopyranose pentaacetate **55**, 5a-carba-α-DL-altropyranose pentaacetate **47** and 6-deoxy 5a-carba-α-DL-altropyranose tetraacetate **60**.[100,101] Only one enantiomer is shown.

2.3.3 Using Carbohydrate Precursors for the Synthesis of Carba-Pyranoses

Another alternative to the above described synthetic strategies is the use of carbohydrates as precursors for the synthesis of carba-mimics. This has two main advantages. No new hydroxy groups have to be introduced and there is no concern for enantiomeric purity because it is delivered by the starting material. Two major challenges arise from the use of this kind of starting material. The introduction of the additional carbon atom to the parent carbohydrate and the cyclization towards the all carbon ring is often challenging and several strategies have been developed.[100]

Paulsen established a synthetic approach towards carba-pyranoses utilizing the Horner–Wadsworth–Emmons reaction (Scheme 8).[102] D-Glucose diethyl dithioacetal **61** was reacted to aldehyde **62,** which was converted to phosphonate **63** by reaction with diethyl methyl phosphonate. After protection group manipulation the resulting alcohol **64** was subjected to Swern oxidation conditions. This allowed an in situ Horner–Wadsworth–Emmons reaction to take place. The resulting allyl alcohol further reacted under the oxidative conditions present to yield enone **65**. Reduction of **65** gave a mixture of allyl alcohol **66** and **67**. When subjecting **66** to desilylation, hydrogenation and acetylation conditions a mixture of 5a-carba-α-D-glucopyranose α-**38** and 5a-carba-α-L-idopyranose α-**68**

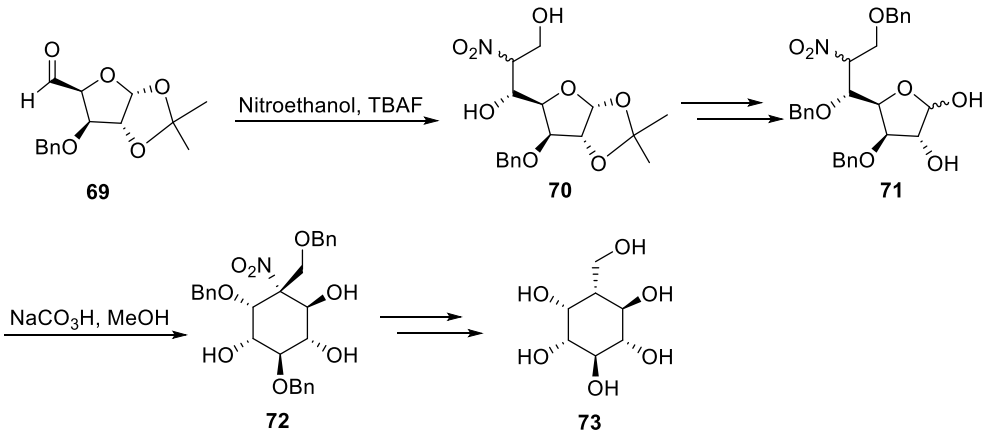
was obtained. Upon reacting **67** under the same conditions, a mixture of 5a-carba-β-D-glucopyranose β-**38** and 5a-carba-β-L-idopyranose β-68 was obtained.[102] J



Scheme 8: Synthesis of 5a-carba-D-glucopyranoses and 5a-carba-L-idopyranoses from a carbohydrate precursor. Benzyl protection and cleavage of the thioacetal **61** to the aldehyde **62** allowed for the synthesis of the phosphonate **63**. Protecting group manipulation gave compound that reacted in an intramolecular Horner-Emmons olefination under Swern reaction conditions. The resulting two compounds **66** and **67** were further modified to yield 5a-carba-α-D-glucopyranose **38**, 5a-carba-α-L-idopyranose **68** and 5a-carba-β-D-glucopyranose **38**, 5a-carba-β-L-idopyranose **68**. [102]

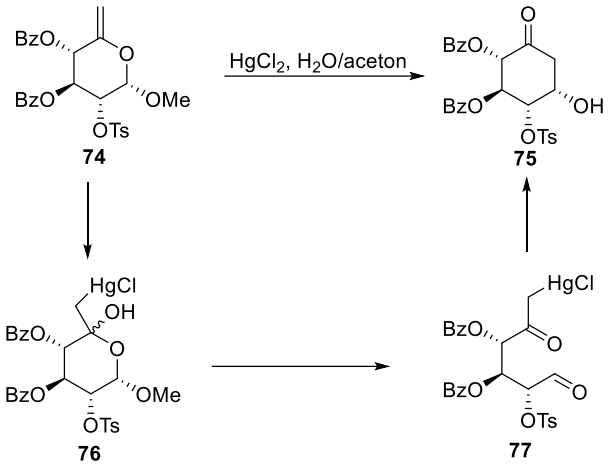
Another interesting strategy, which is relevant for this thesis is the approach chosen by *Estévez*.[103] The authors performed a stereoselective intramolecular nitroaldol condensation leading to 5a-hydroxycarba-sugar **73** (Scheme 9).[103] This represents one of the few examples for the synthesis of a 5a-modified carba-sugar. They synthesized aldehyde **69**, derived from D-glucose and subjected it to a mixture of nitroethanol and TBAF resulting in a nitroaldol reaction. The resulting compound **70** was subjected to protecting group manipulation and with the obtained

hemiacetal **71** a second, intramolecular nitroaldol reaction was performed giving carbocycle **72** as a single diastereomer. Removal of the nitro group and deprotection yielded the 5a-hydroxy carba-sugar **73**.



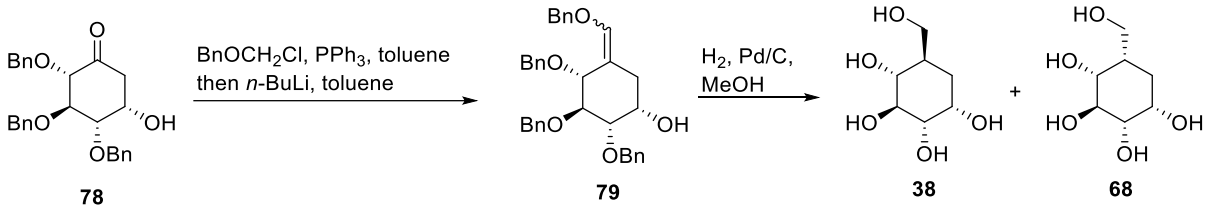
Scheme 9: Usage of nitroaldol reaction for the synthesis of carba-sugar **73** by *Estévez.*[103] 5-Hydroxycarba-sugars **73** was synthesized utilizing one intermolecular Henry reaction resulting in compound **70** followed by a second intramolecular Henry reaction giving compound **72**.

One of the most commonly used reactions to archive the carbacyclization is the Ferrier II reaction (also called Ferrier carbacyclization) (Scheme 10).[104] This reaction allows for the transformation of a hex-5-enopyranoside such as compound **74** into a cyclohexanone such as compound **75**. The reaction proceeds via an oxymercuration of the alkene resulting in an unstable hemiacetal **76.** After loss of methanol a dicarbonyl compound **77** was obtained. The carbacycle itself is formed in an aldol type reaction.[100]



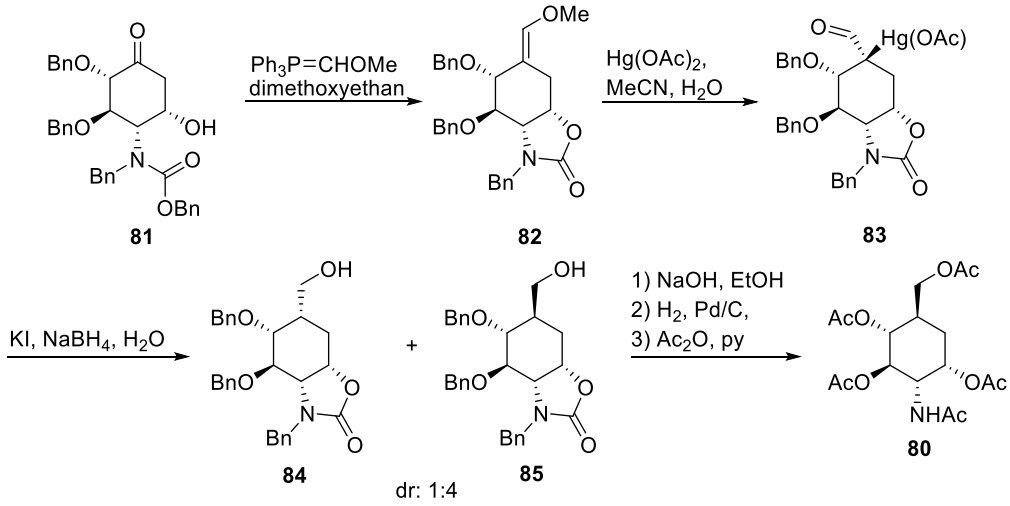
Scheme 10: Mechanism of the Ferrier carbacyclization. Alkene **74** reacts in an oxymercuration reaction to give compound **76**. This compound can loss methanol giving the dicarbonyl **77** that reacts in an aldol type reaction to the cyclohexanone **75**.

Several research groups relied on the Ferrier carbacyclization for their synthetic strategy, including past approaches in our own group.[37,58,105-107] This strategy has drawbacks that have partially been addressed in past research. Initial reaction conditions required stoichiometric amounts of mercury, but modifications of the synthetic protocol have been published that require only catalytic amounts of mercury sulfate.[108] Furthermore variants of the Ferrier carbacyclization using palladium chloride instead of mercury salts have been published.[109] Another drawback is the reintroduction of the 6 position, which is lost during cyclisation. This introduction of the additional carbon can be achieved by a Wittig olefination. When using benzyloxymethylenetriphenylphosphorane as Wittig salt, the obtained vinyl ether can be cleaved to the alcohol (Scheme 11). This approach was followed by *Barton et al.* in his synthesis of carba-sugars. In his approached a Wittig reaction is performed on the Ferrier carbacyclization product **78** to obtain vinyl ether **79**.[110,111] His synthesis also demonstrated the problem of this approach yielding a mixture of the gluco carba-sugar **38** and the ido carba-sugar **68**.



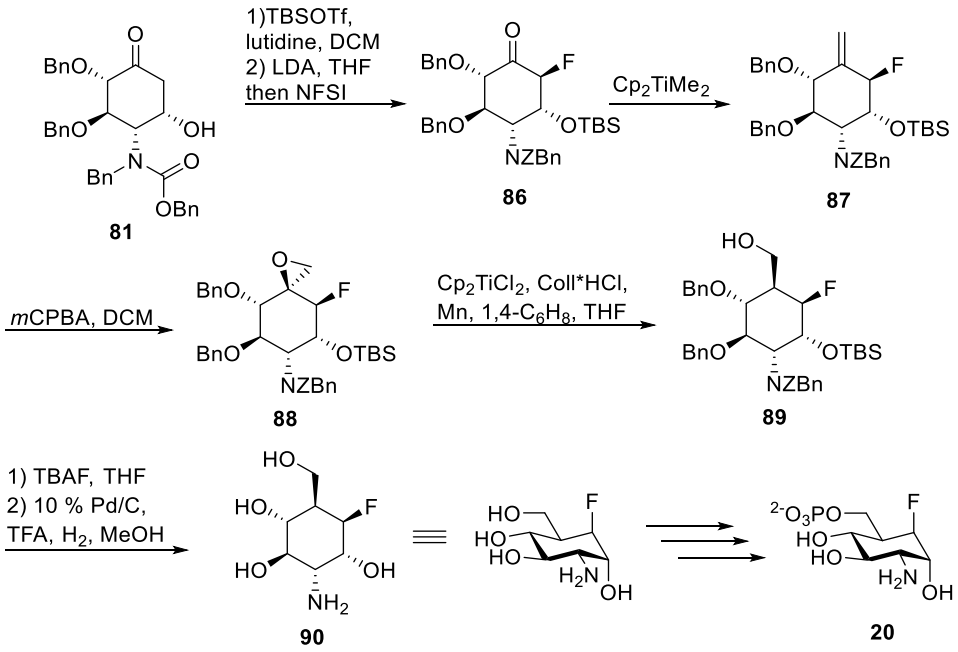
Scheme 11: Synthesis of gluco carba-sugar **38** and ido carba-sugar **68.** A Wittig reaction was performed with the Ferrier carbacyclization product **78** yielding compound **79**. Hydrogenation yielded a diastereomeric mixture of **38** and **39**.

Of particular relevance for this work is that the Ferrier carbacyclization was used for the first synthesis of carba-glucosamine pentaacetate **80**, which was reported by *Quiclet-Sire* (Scheme 12).[105] The Ferrier carbacyclization product **81** reacted in a Wittig reaction giving vinyl ether **82**, which was cleaved by employing mercury acetate. Organomercury compound **83** was converted under reductive conditions to the protected carba-idosamine derivative **84** and protected carba-glucosamine derivative **85.** After deprotection and peracetylation carba-glucosamine pentaacetate **80** was obtained. However, also their approach yielded the carba-idosamine derivative in significant amounts.[105]



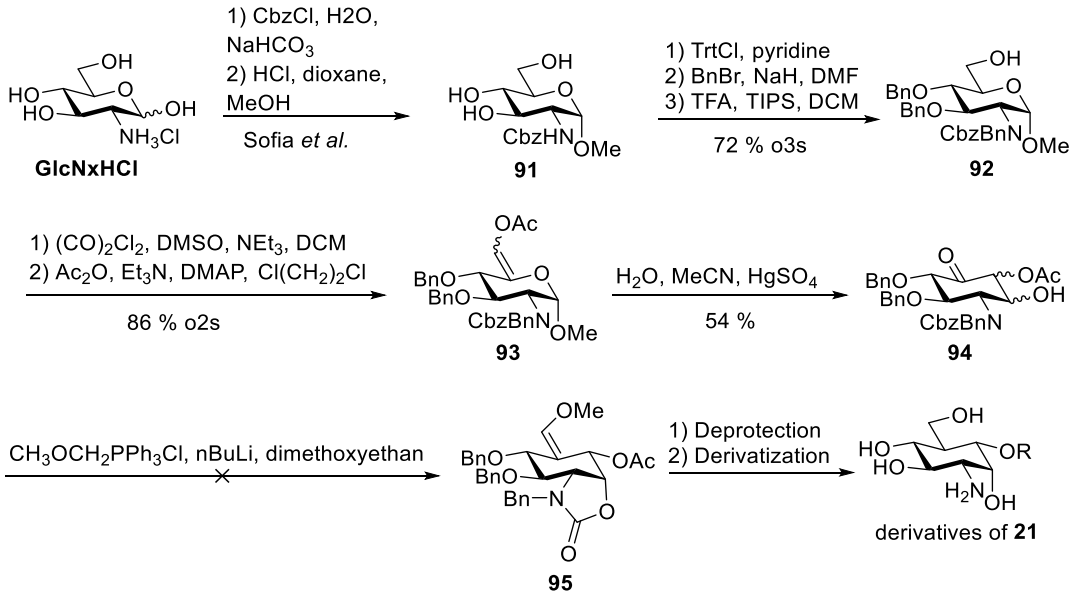
Scheme 12: Synthesis of carba-glucosamine pentaacetate **80**. Ferrier product **81** was reacted in a Wittig reaction introducing the 6 position. Cleavage of the vinyl ether **82** gave a mixture of the idosamine derivative **84** and glucosamine derivative **85**.[105]

Daniel Matzner from the research group of *Mayer* together with the research group of *Wittmann* developed a synthesis of 5a-fluoro-carba-α-D-glucosamine-6-phosphate **20** based on a Ferrier carbacyclisation (Scheme 13).[47,58] This derivative represents the first 5a modified carba-glucosamine derivative. Compound **20** proved to be a potent riboswitch activator and is a promising lead compound for the development of future antibiotics. The synthesis utilizes cyclohexanone **81**, obtained from a Ferrier II reaction. After protection of the 1-hydroxygroup, an axial fluorine was introduced using enol chemistry and *N*-fluorobenzenesulfonimide (NFSI) as an electrophilic fluorine source. This yielded in fluoride **86**. Homologation was achieved using the Petasis reagent to give allyl fluoride **87**. This compound was transformed to epoxide **88**, which can be opened in a high dia- and regioselective manner to give compound **89**. After deprotection 5a-fluoro-carba-α-D-glucosamine **90** was isolated. This compound was phosphorylated to give the *glmS* riboswitch activator **20**.



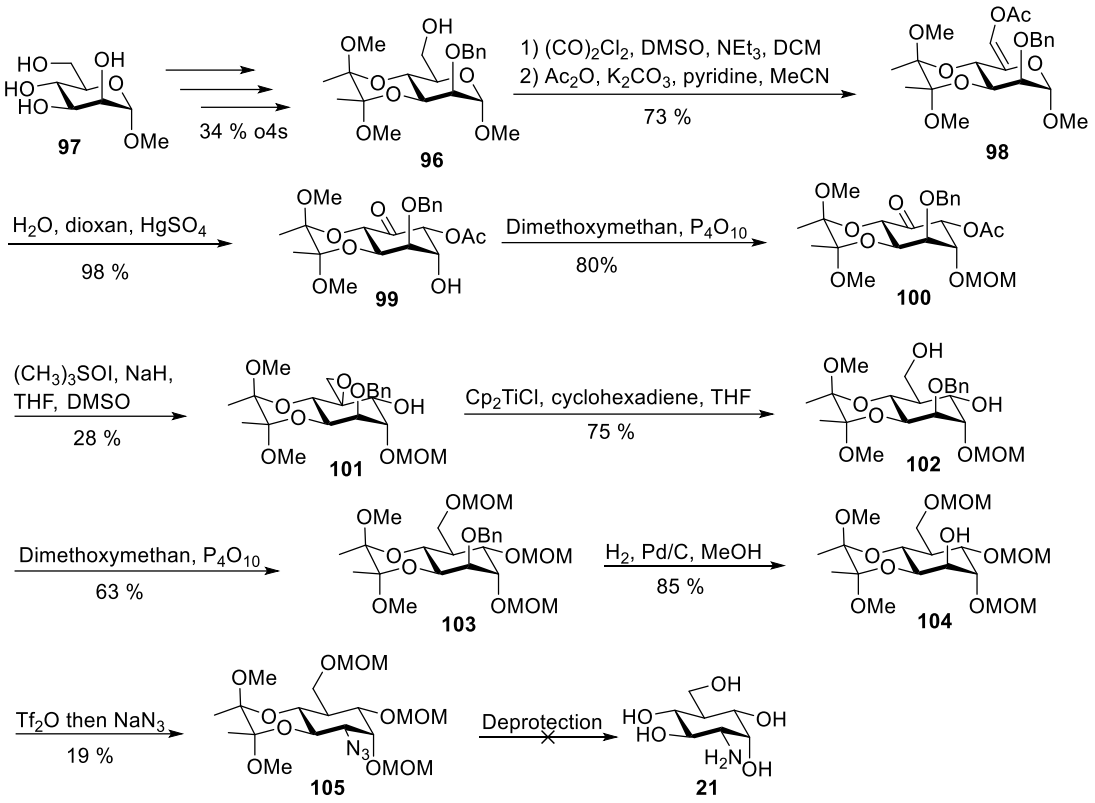
Scheme 13: Synthesis of 5a-fluoro-carba-α-D-glucosamine **20**. The fluoride was introduced via the enolate of Ferrier product **81**. Petasis olefination gave compound **87**. The introduction of the 6-hydroxy group was achieved by epoxidation giving compound **88.** Regioselective epoxide opening resulted in **89**. Deprotection yielded 5a-fluoro-carba-α-D-glucosamine **90**, which was phosphorylated to give the riboswitch activator **20**.[58]

In the Wittmann group, an intense interested in the synthesis of 5a modified carba-α-D-glucosamine resulted from the promising properties of **19** and **20** as *glmS* activators and as antibiotics. *Torben Seitz* investigated a strategy towards 5a-hydroxy-carba-α-D-glucosamines (Scheme 14).[112] His initial synthesis started with GlcN, which was converted in a literature known procedure to the Cbz protected methylacetal **91**.[113] This compound was selectively protected in the 6-position followed by benzylation of the 2-, 3- and 4-position and deprotection of the 6-OH group was performed. To obtain compound **92**. Swern oxidation of **92** followed by treatment with acetic anhydride and triethylamine resulted in the formation of the vinyl acetate **93**. This compound served as the starting material for a Ferrier carbacyclisation giving compound **94**. A Wittig reaction with (methoxymethyl)triphenylphosphonium chloride was expected to give compound **95**. However, *Seitz* did not observe any product formation. Therefore, he did not continue his planed synthesis which involved deprotection and derivatization of the 5a-position, followed by a phosphorylation and deprotection protocol previously established by *Lünse et al.*.[37]



Scheme 14: First attempted synthetic strategy for 5a-hydroxy-carba-α-D-glucosamines by *Seitz*. He relied on a Ferrier carbacyclisation to build up the carba-sugar. The C1 homologation, which should achieve the synthesis of 9**5** was unsuccessful. This jeopardized the synthetic strategy of 5a modified carbaglucosamines.[112]

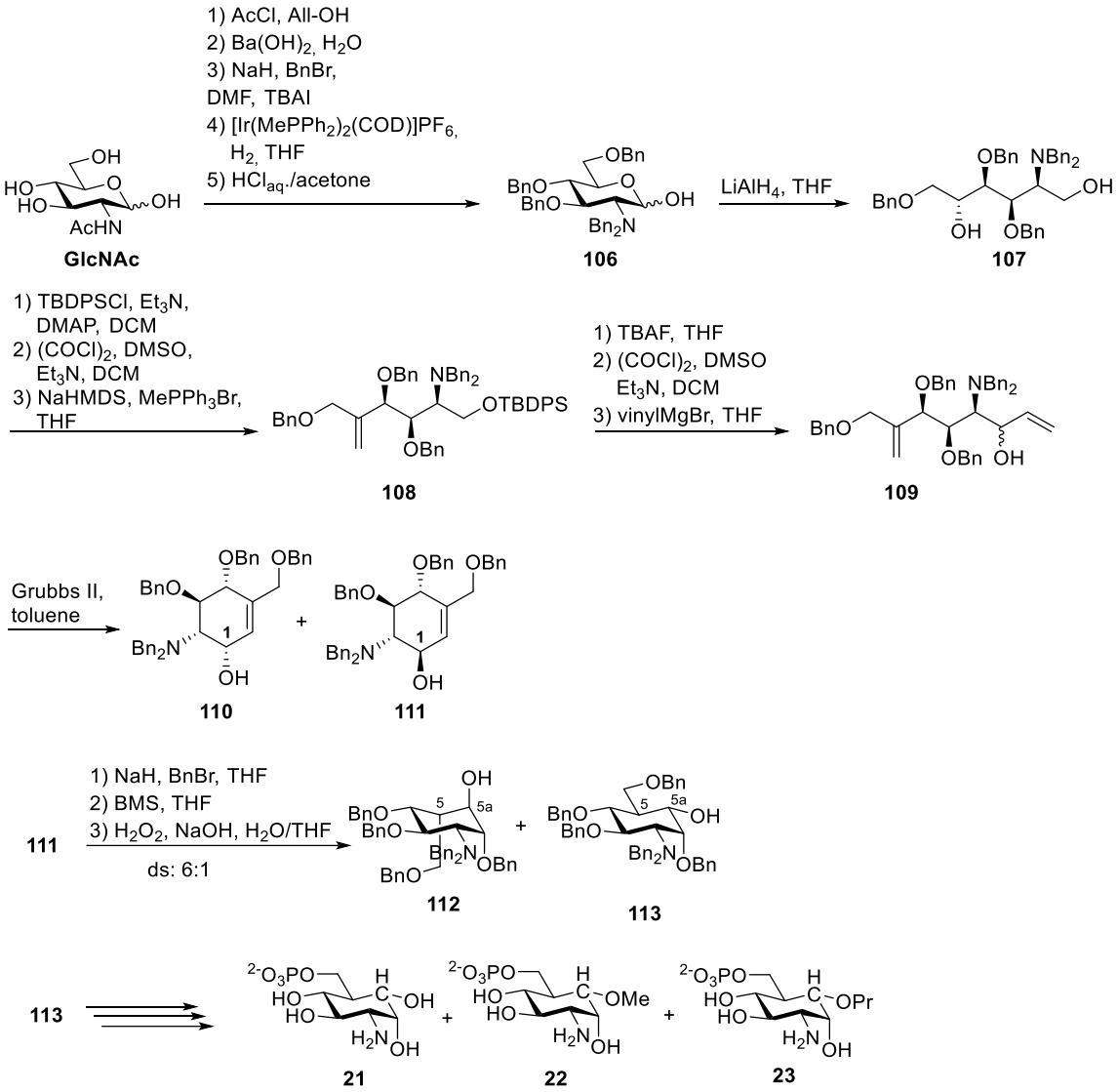
To circumvent this problem, he decided to try another synthetic strategy relying on the literature known synthesis of mannose derivative **96** starting from methyl mannopyranoside **97** (Scheme 15).[114] This compound was converted to vinyl acetate **98**, which is similar to the previous attempted synthesis. After Ferrier carbacyclization the acetate **99** was isolated. This was followed by MOM-protection yielding **100**. This compound was subjected to a Corey-Chaykovsky reaction.[115,116] The resulting epoxide **101** was opened using conditions established by *Matzner* in his synthesis of **90** (Scheme 13) giving compound **102**. The two unprotected hydroxy groups of **102** were MOM-protected and compound **103** was isolated. After hydrogenation of the axial benzyl ether alcohol **104** was isolated. This compound was converted in two steps in to azide **105**. This transformation suffered from a low yield of only 19 % creating a bottle neck in the synthesis. In his work *Seitz* was unable to remove the protecting groups of compound **105** and therefore, he did not proceed with the derivatization of the 5a-position. The synthesis of the desired 5a-hydroxy-carba-glucosamine **21** was not achieved.



Scheme 15: Second attempted synthetic strategy for 5a-hydroxy-carba-α-D-glucosamines by *Seitz*. This strategy used mannose derivative **96.** Introduction of the amine was performed at a late stage suffering from low yields. The strategy failed because deprotection of **105** to give the target compound **21** was not successful.

The application of metathesis catalysts allowed an alternative approach for the construction of the carbacycle of the desired carba-pyranoses.[117] Compared to the previous described methods three instead of one new carbon atoms have to be introduced. Two carbon atoms are lost during the metathesis as ethylene. The utility of this reaction for the construction of unsaturated carba-analogues was demonstrated by *Kim* and later by *Jung* in their synthesis of valienamine.[118,119] *David Stängle* from the *Wittmann* group developed a synthetic approach towards 5a-modified carba-α-D-glucosamines using the ring closing metathesis (Scheme 16). He started his synthesis with *N*-acetylglucosamine which was converted to lactol **106**. This compound can be reduced to sugar alcohol **107**. Selective TBDPS protection of the primary alcohol allowed a Swern oxidation at the secondary alcohol. The obtained ketone was converted to the 1,1-disustituted alkene **108**. After TBDPS deprotection, the obtained alcohol was oxidized to an aldehyde and the missing two carbon atoms were introduced by using vinylmagnesium bromide. The

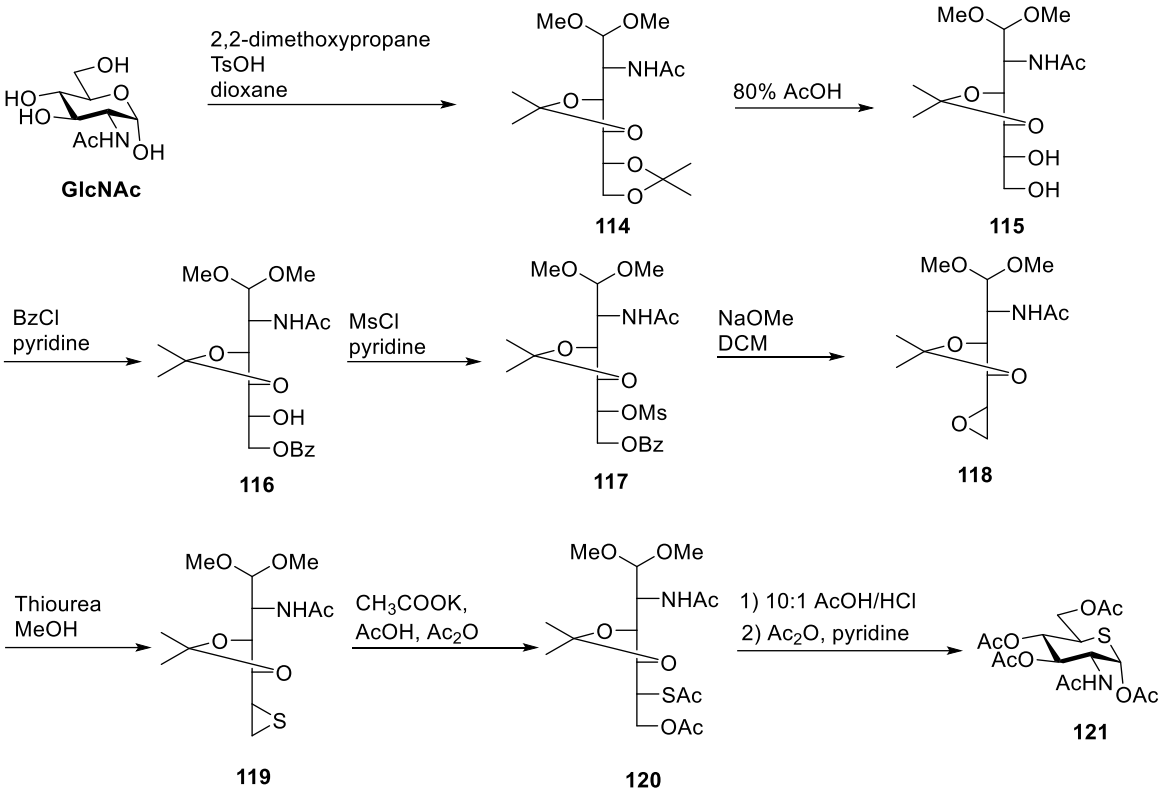
obtained diastereomeric dienes **109** were reacted in a ring closing metathesis yielding the cyclic allyl alcohols **110** and **111**. When **110** was benzyl protected and subjected to hydroboration conditions, the major product corresponded to the 5a-hydroxy-carba-β-L-idosamine derivative **112** and only the minor product was identified as the 5a-hydroxy-carba-α-D-glucosamine derivative **113. 112** and **113** were obtained in a diastereomeric ratio of 6:1. This proved to be detrimental for the planned synthesis of a large library of 5a-hydroxy-carba-α-D-glucosamine derivatives. This synthetic challenged remained unanswered leaving room for further studies to access the desired library.



Scheme 16: Synthesis of 5a modified carba-glucosamine by *Stängle.* GlcNAc was converted to the hemiacetal **106**, which yielded the diol 107 upon reduction. Regioselective protection and olefination afforded **108**, which was further converted to the diene **109**, allowing for RCM using Grubbs II catalyst. Protection of the allyl alcohol and hydroboration yielded an unfavorable mixture of the undesired 5a-carba-idosamine **112** and the desired 5a-carba-glucosamine **113** in a ratio of 6:1. [120] The compound **113** was further converted to the carba-GlcN6P derivatives **21**, **22** and **23.**

2.4 Sulfur and Nitrogen at the 5a-position – Thia- and Imino-Sugars

Besides carba-sugars, two main groups of carbohydrate mimics based on manipulation of the 5a-position have been developed. Especially nitrogen and sulfur as replacement of the ring oxygen have been investigated in depth.[121-124] Sulfur has been an interesting replacement because its position in the periodic table, below oxygen, gives it a generally similar reactivity, allowing for the synthesis of thiohemiacetals[125], thioethers[126] and thioacetals.[127] The most common method for the introduction of the sulfur features an epoxidation between the 5 and 6 position followed by an exchange to the thiirane by thiourea. This has been used by the *Whistler* group in their synthesis of 5-thio-α-D-glucose.[125] *Hasegawa* developed the synthesis of 5-thio α-D-N-acetyl-glucosamine (Scheme 17).[128,129] This synthesis is important for this work, it features an acetal protection of GlcNAc to give the triacetal **114**. Selective deprotection of the acetal involving the primary alcohol gave diacetal **115**. Benzoyl protection of the primary alcohol gave compound **116**. Then, this compound was mesylated giving the activated compound **117**. Debenzoylation under basic condition afforded epoxide **118**. This compound was converted to the thiirane **119** by using thiourea, followed by a ring opening using potassium acetate to give thioacetate **120**. Deacetylation allows the formation of the hemithioacetal followed by peracetylation to give 5-thio α-D-glucosamine pentaacetate **121**.



Scheme 17: Synthesis of **121** published by *Hasegawa*.

5-Amino-5-deoxy-D-glycopyranose **122**, was the first iminosugar discovered, it was found in extracts from *Streptomyces nojiriensis* (Figure 10).[130] It was given the name nojirimycin **122**, after the organism producing it.[131,132] It was found to be a potent antibiotic, likely due to its glucosidases inhibiting properties.[133,134] *Yagi et al.* isolated the compound moranoline **123** from *Morus* (Mulberry tree). This compound was fund to be similar to nojirimycin **122**, missing the anomeric hydroxy group, and therefore, containing an amine and not an N,O-hemiacetal. Accordingly, It was named 1-deoxynojirimycin **123**.[134-136] The hemiaminal renders nojirimycin **122** unstable under basic and neutral conditions.[137] This has deterred its application as a drug. In contrast, 1-deoxynojirimycin **123** is stable and derivatives of it have been marketed as the drugs Miglitol **124**[138] and Miglustat **125**.[139] Although, the missing 1-hydroxygroup is beneficial for the stability of **123**, the development of GlcN6P mimics, with application as *glmS* riboswitch activators, require a 1-OH group in order to induce efficient cleavage of the *glmS* riboswitch. For this reason, amino sugars were not considered for this application within this work.

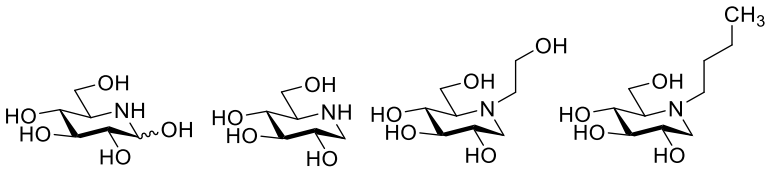


Figure 10: Iminosugars of biological and pharmacological importance. Nojirimycin **122** was the first iminosugar to be discovered. Deoxynojirimycin **123** is missing the hemiaminal of **122** rendering it more stable. Miglitol **123** and Miglustat **125** are derivatives of **123** having earned their importance due to their medical application.

3 Objectives

With the threat posed by multiple drug-resistant bacteria, new antibiotics are needed to remain vigilant in the fight against infectious diseases. Given the sophisticated mechanisms that bacteria have developed to evade antibiotic effects of established antibiotics, compounds with untapped mechanisms of action are desirable because resistances to these new drugs have not yet developed.

The *glmS* riboswitch, a 5'-UTR of RNA, mainly found in gram positive bacteria, is considered to be a promising target for new antibiotics. This riboswitch controls the gene, encoding for the enzyme GlmS. GlmS catalyzes the formation of GlcN6P. The riboswitch undergoes a catalytic self-cleavage reaction, induced by GlcN6P binding. This represents a negative feedback loop, which controls GlcN6P concentration. GlcN6P is an intermediate for bacterial cell wall synthesis and consequently important for bacterial growth. GlcN6P mimics, which are able to activate the riboswitch and thereby inhibit further synthesis of GlcN6P could disturb bacterial growth.

The structural requirements for *glmS* riboswitch activators have been studied in the past. Modification of the 1-, 2-, 3-, or 4-position of GlcN6P resulted in decreased activity as *glmS* riboswitch activator, or even outright inefficacy of the mimic. It was discovered that viable points of derivatization are limited to the replacement of the ring oxygen and modification of the 6-phosphate. Exchange of the ring oxygen to carbon resulted in carba-mimics of GlcN6P, have been shown to activate the *glmS* riboswitch as well as to inhibit bacterial growth.[37,140] A first aim of this thesis was to extend this exchange to another atom (Figure 11). For this purpose, sulfur was planned to be introduced at the 5a-position resulting in thia-GlcN6P. The introduction of another chalcogen was expected to yield a promising new activator of the *glmS* riboswitch and therefore a potential new antibiotic.

Modification of the phosphate to a phosphonate, resulting in phosphatase-inert *glmS* riboswitch activators, has been shown to be possible in literature.[39] These activators showed a diminished activity compared to GlcN6P. In the literature this has been explained by the differing p*K*a values between the phosphonate and the phosphate. Therefore, I was interested in synthesizing phosphonates mimics with a

pK_a value comparable to the natural phosphate. With these derivatives it was envisioned to further elucidate the structural requirements of the *glmS* riboswitch activation to address the question of the 6-position being a viable modification site (Figure 11).

As mentioned above, carba-GlcN6P mimics have been shown to be the best synthetic *glmS* riboswitch activators known so far. The Wittmann group had a longstanding interest in 5a-modified carba-glucosamines in order to further investigate and fine tune the antibiotic activity of these compounds. *Stängle* established a synthetic strategy towards 5a-alkoxy modified carba-glucosamines. However, his synthetic route suffered from poor yields and allowed only the synthesis of a limited quantity of mimics instead of the desired ambitious library. I was aiming to improve the synthetic route and thereby making the desired library available. Thereby establishing synthetic access to the new compound class of 5a modified carba-glucosamines (Figure 11). It was envisioned to contribute to the challenge of finding new antibiotics by establishing a reliable synthetic route to these glucosamine mimics as well as to assess the potential of these derivatives as new novel antimicrobial agents.

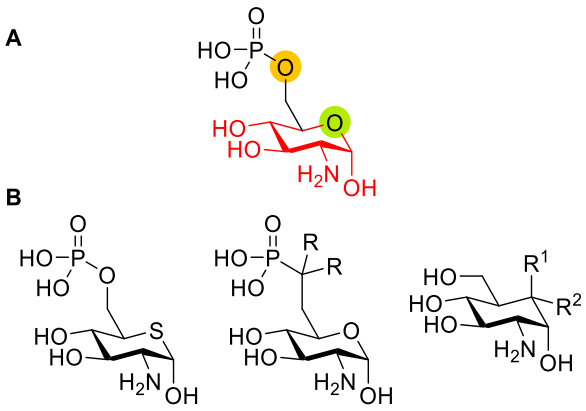
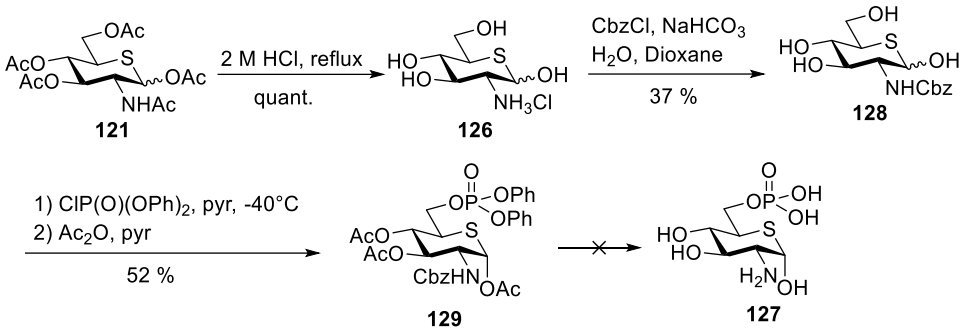


Figure 11: **A**: Structural requirements for GlcN6P derivatives acting as activators of the *glmS* riboswitch. Red: The cyclic structure and the equatorial substituents in the 2-, 3- and 4-position are essential for riboswitch binding. Yellow: Modification of the 6-phosphate have been shown to result in activators with diminished activity. Green: Variation of the ring oxygen to carbon is tolerated. **B**: Target compounds of the three projects of this thesis. Left: Thia-GlcN6P, expanding the 5a-mimics to other atoms. Middle: Phosphonates with different substituents in the 7-position. Right: Various 5a-modified-carba-glucosamines, a large library of these compounds is desired to estimate the antimicrobial potential of these compounds.

4 Results and Discussion

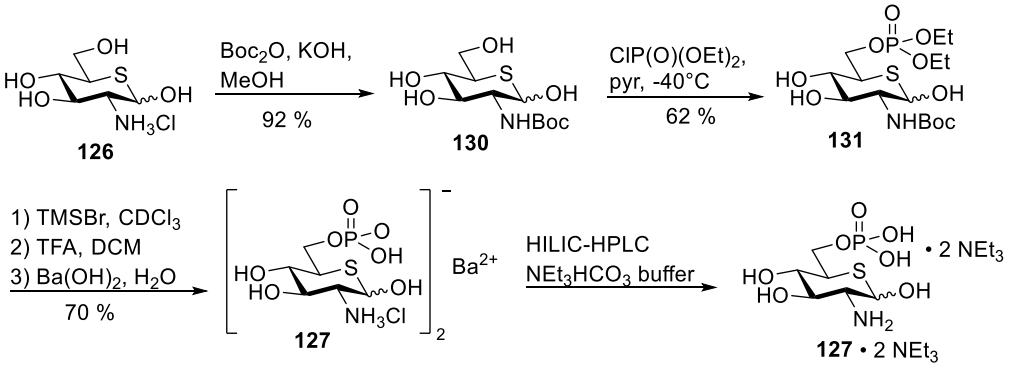
4.1 Synthesis of Thia-GlcN6P

Previously, carba-sugar derivatives of GlcN6P have been shown to be effective GlcN6P mimics, in regard of their ability to activate the *glmS* riboswitch. Other carbohydrate mimics with the ring oxygen exchanged by another atom, have not yet been synthesized and tested for their ability to activate the *glm*S riboswitch. This sparked the interested in synthesizing a thia-mimic of GlcN6P. It was postulated that the oxygen can also be replaced by another chalcogen, such as sulfur, while retaining the ability to initiate the self-cleavage reaction. In chapter 2.4 the synthesis of 5-thio α-D-*N*-acetyl-glucosamine pentaacetate **121** by *Hasegawa* is described[128] This route served as a starting point for the synthesis of thia-GlcN6P, which is not yet described in literature. Following the synthetic procedure published by *Hasegawa*, I synthesized 5-thio α-D-*N*-acetyl-glucosamine pentaacetate **121** in a yield of 31 % over nine steps. This compound can be converted to the hydrochloride **126** in quantitative yields by treating it with 2 M aqueous HCl.[141,142] *Schmidhäuser* from the *Wittmann* group tried to convert compound **126** to the desired thia-GlcN6P **127** in her master thesis (Scheme 18). She pursued a synthetic strategy relying on Cbz protection of the amine giving compound **128** and introducing the phosphate via diphenyl chlorophosphate giving the phosphate ester **129**. This strategy necessitated the removal of the Cbz group by hydrogenation with Pd/C as catalyst as well as removal of the phenyl groups by PtO_2. This was unsuccessful presumably due to catalyst poisoning by the introduced hemithioacetal.[143,144] Therefore, it was ultimately not possible to bring the above described synthesis of **127** to an end.



Scheme 18: Initial attempt to synthesize thia-glucosamine-6-phosphate **127** by *Schmidhäuser*.[145] The free thia-GlN was Cbz protected and the phosphate was introduced by diphenylchloro phosphate giving **129.** Deprotection to give **127** was unsuccessful.

With these results in mind, I developed a synthetic strategy, which did not rely on the use of metal catalysts (Scheme 19). This was achieved by performing a Boc-protection of the amine giving compound **130** in a yield of 92 % followed by introducing the phosphate by using diethyl chlorophosphate resulting in phosphate ester **131** in a yield of 62 %. Total deprotection was carried out without the use of a metal catalyst and was easily achieved by treatment of the compound with TMSBr. Thereby, the ethyl groups were cleaved and subsequent addition of trifluoroacetic acid led to Boc-deprotection. The crude phosphate was precipitated as barium salt **127,** which was obtained in a yield of 70 %. This salt was purified by Hydrophilic interaction chromatography (HILIC) HPLC using a triethylammonium bicarbonate buffer giving pure triethylammonium salt **127**•2 NEt_3. This provided access to a new potential *glmS* riboswitch activator. The biological activity of this compound was tested by riboswitch cleavage assays. These experiments are presented in chapter 4.3.



Scheme 19: Synthesis of triethylammonium thia-glucosamine-6-phosphate **127**•2 NEt_3.

4.2 Phosphonates as Phosphatase Inert GlcN6P Mimics

4.2.1 Phosphate Mimics

Studies by *Soukup* revealed that 6-phosphate of GlcN6P can be replaced in principle by phosphate surrogates while retaining the ability to activate the *glmS* riboswitch.[39] Methylene phosphonate **12** is the most promising derivative studied by *Soukup* (Figure 12). However, this compound did only show a fraction of the induced *glmS* riboswitch cleavage when compared to GlcN6P. One possible reason for this discrepancy might be the different pK_a values between the phosphate and the phosphonate. The phosphate has two acidic protons. Under physiological conditions the dissociation of the second proton is of relevance. For GlcN6P a second acid dissociation constant (pK_{a2}) of 6.2 has been published *Soukup* determined the pK_{a2} value of **12** to be 7.4.[39] This means that, at physiological pH, there is significantly less compound **12** in the dianionic form than the natural ligand GlcN6P. The dianionic form is necessary for Mg^{2+} binding which is in return important for successful activation of the *glmS* riboswitch. Therefore, I wanted to introduce several phosphonates at the C-6 position with different pK_{a2} values spanning a large range including one derivative matching the pK_{a2} of GlcN6P. The research group of *Ye* synthesized hydroxy phosphonate **14** and observed that this compound did not activate the *glmS* riboswitch (Figure 12). Compound **14** features a shorter chain length than the natural ligand GlcN6P. Consequently, phosphonates with the same chain length as GlcN6P were planned to be synthesized.

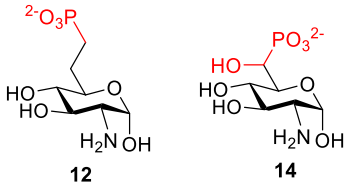
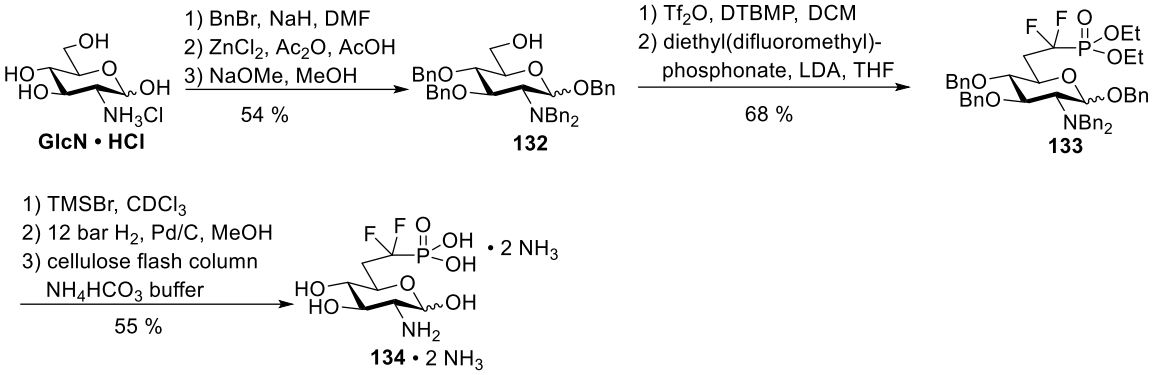


Figure 12: Literature known phosphate surrogates as potential *glmS* activators. Methylenphosphonate **12** has been synthesized by *Soukup* and hydroxyphosphonate **14** has been synthesized by *Ye.*

4.2.1.1 Synthesis of a α-Difluoro-Phosphonate as Phosphate Mimics

Introduction of fluorine in alpha position of the phosphonate was deemed promising to lower the pK_a value of the phosphate mimic.[146] Fluorine is a strongly electron withdrawing substituent and introduction of these substituent alpha to organic acids is a well-established principle for the creation of stronger organic acids. One prominent example is trifluoroacetic acid with a pK_a of 0.5 compared to a pK_a value of 4.8 for acetic acid.[147] Fluorine is furthermore a very small electron withdrawing group. This small size is desirable because it limits the influence on the structure caused by the introduction of the substituents.[148,149] For this reason, the first phosphate mimic aimed for was an (α-difluoro) phosphonate.

In order to introduce a phosphate mimic in the 6-position of glucosamine, the primary hydroxy group needs to be selectively accessed (Scheme 20). I decided to achieve this by using a protection group strategy that leaves this group unprotected. Glucosamine hydrochloride was perbenzylated followed by acetolysis using zinc chloride in acetic anhydride and acetic acid to convert the benzyl ether in the 6-position into an acetate. De-*O*-acetylation with sodium methoxide gave the primary alcohol **132** in a yield of 54 % over 3 steps. The synthesis of **132** is reported in the literature by *Ye*.[60] In this synthesis, the acetolysis was carried out with sulfuric acid, which gave inconsistent results in my hands resulting in the use of zinc chloride for this reaction resulting in higher and more consistent yields in this thesis. The alcohol **132** was converted to the corresponding triflate with 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) as base. This activated compound was directly converted to the difluoro phosphonate **133** by deprotonating diethyl (difluoromethyl) phosphonate with LDA and the attack of the resulting anion at the triflate. Compound **133** was obtained in a yield of 68 % over two steps. The deprotection of the ethyl groups was achieved using trimethylsilyl bromide (TMSBr) in $CDCl_3$ in order to monitor the reaction by NMR. The benzyl groups were cleaved by hydrogenation at 12 atm hydrogen under palladium catalysis. The obtained difluoro phosphonate was purified by cellulose flash column chromatography using ammonium bicarbonate buffer as eluent to give the diammonium salt **134**•2 NH_3 in a yield of 55 %.

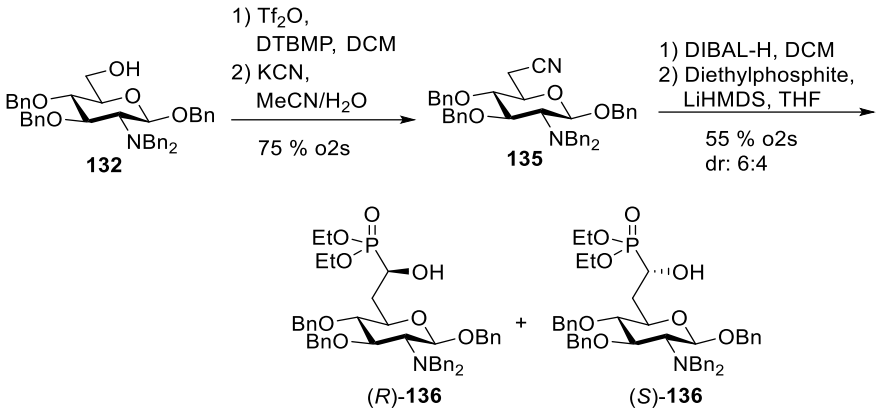


Scheme 20: Synthesis of **134** starting from glucosamine hydrochloride via the selectively deprotected alcohol **132** which was activated by triflation allowing for the introduction of the difluoro phosphonate. Deprotection was achieved by ester cleavage with TMSBr and benzyl ether cleavage was performed by hydrogenation.

4.2.1.2 Synthesis of α-Hydroxy-Phosphonates as Phosphate Mimics

To study phosphate mimics with a wide range of pK_a values, different substituents alpha to the phosphate were desired. For this reason, (α-hydroxy) phosphonates were synthesized. The hydroxy group is less electron withdrawing than the fluorine. Therefore, giving less acidic mimics then compound **134** but more acidic than methylene phosphonate **12.**

In order to introduce an α-hydroxy-phosphonate, alcohol **132** was activated by triflation, and homologation was achieved by reaction with potassium cyanide giving nitrile **135** in a yield of 75 % over 2 steps (Scheme 21). This nitrile was reduced to the corresponding aldehyde by reaction with DIBAL-H and the aldehyde was attacked by diethyl phosphite using lithium bis(trimethylsilyl) amide (LiHMDS) as base. This resulted in separable mixture of the two diastereomers (*R*)-**136** and (*S*)-**136** in a ratio of 6:4 and a combined yield of 55 %.



Scheme 21: Synthesis of the two (α-hydroxy)phosphonates (*R*)-**136** and (*S*)-**136**.

Assignment of the stereochemistry alpha to the phosphorus proved to be challenging. Crystallization of the compounds (*R*)-**136** and (*S*)-**136** was unsuccessful and NOESY NMR did not deliver the necessary information due to the free rotation of the carbon chain. Therefore, a different way of determining the stereochemistry was required. A technique for the determination of the absolute configuration of secondary alcohols by NMR was developed by Mosher. [150-153] In this method, the alcohol is first reacted with the two enantiomers of α-methoxy-α-trifluoromethyl-α-phenylacetic acid (MTPA) to give two diastereomeric esters. MTBA acts as a chiral auxiliary and the phenyl group of this ester exerts an anisotropic effect on the two substituents (L_1 and L_2) of the secondary alcohol (Figure 13).[153] This means that in the NMR spectrum one of the two substituents experiences more shielding, resulting in an upfield shift in the spectrum corresponding to a smaller δ value, while the other one experiences less shielding, which substituent experiences which effects depends on the applied MTBA enantiomer. A correlation of the spatial position of L_1 and L_2, with respect to the phenyl group of the MTBA moiety can be gained from the sign of $\Delta\delta^{SR}$ of the substituents.[153] Mosher assumed that the proton geminal to the alcohol, the carbonyl group and the CF_3 group of the MTBA ester are situated in the same plane.[153] Consequently all protons of the substituent L_2 are more shielded in the (*R*)-MTBA ester (Figure 13 A). In the (*S*)-MTBA ester the protons of L_1 are more shielded (Figure 13 B).

The shielding in these Mosher-esters is selective which can be expressed by the parameter $\Delta\delta^{SR}$, which was calculated by the equation:[153]

$$\Delta\delta^{SR} = \delta^{S} - \delta^{R}$$

All protons shielded by the (*R*)-MTBA ester will show a positive $\Delta\delta^{SR}$ value. Whereas all protons shielded by the (*S*)-MTBA ester will show a negative $\Delta\delta^{SR}$ value.[153] This allows for the determination of the absolute configuration.

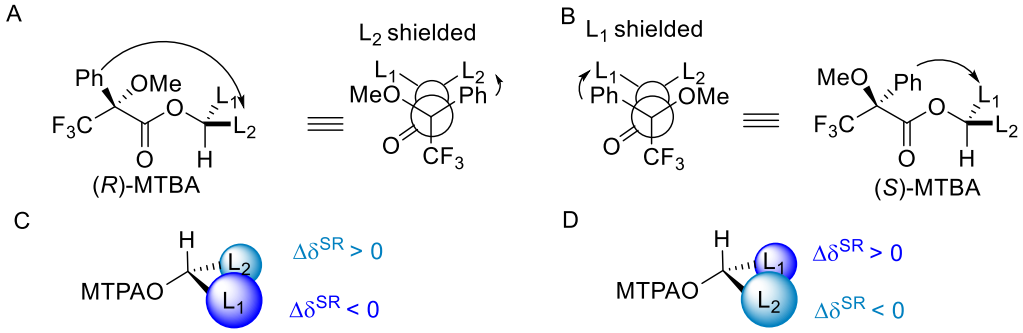


Figure 13: A and B: Moshers model for the assignment of the absolute configuration by ^{1}H-NMR. C and D: The expected sign of $\Delta\delta^{SR}$ depends on the absolute configuration of the secondary alcohol. This figure was adapted from *Riguera.*[153]

To determine the absolute configuration of **136** using the method developed by *Mosher*, the (*S*)-and (*R*)-MTBA ester of (*R*)-**136** and (*S*)-**136** were synthesized (Figure 14). After assignment of all proton signals in the ^{1}H NMR and ^{31}P spectra, I determined the chemical shift differences $\Delta\delta^{SR}$ value of all signals for the (S)- and the (*R*)-MTPA ester (Table 1). For the major isomer, all $\Delta\delta^{SR}$ values of the sugar resonances were negative and all $\Delta\delta^{SR}$ values of the phosphonate resonances (ethyl groups as well as ^{31}P resonances) were positive. Accordingly, the major isomer was assigned to be (*R*)-**136**. Similarly, for the minor isomer, all $\Delta\delta^{SR}$ values had opposite signs, and this isomer was assigned to be (*S*)-**136**.

Table 1: Table of chemical shifts observed for the synthesized Mosher-esters The difference between the (*S*)-MTBA and the corresponding (*R*)-MTBA ester was determined and with this value empiric assignment of absolute configuration was possible

136 major isomer	**δ S-MTPA ester [ppm]**	**δ R-MTPA ester [ppm]**	**ΔδSR =δS −δR [ppm]**	**136 minor isomer**	**δ S-MTPA ester [ppm]**	**δ R--MTPA ester [ppm]**	**ΔδSR =δS −δR [ppm]**
H6	1.78 2.43	1.80 2.51	-0.02 -0.08	H6	1.94 2.41	1.86 2.3	0.08 0.11
H5	3.54	3.64	-0.1	H5	3.63	3.54	0.09
H4	3.14	3.22	-0.08	H4	3.23	3.16	0.07
H3	3.67	3.73	-0.06	H3	n.d.	n.d.	n.d.
H2	2.92	2.95	-0.03	H2	2.95	2.91	0.04
H1	4.63	4.71	-0.08	H1	4.53	4.48	0.05
C$\underline{H_2}$-P-Ester	4.08	4.06	0.02	C$\underline{H_2}$-P-Ester	4.04	4.09	-0.05
C$\underline{H_3}$-P-Ester	1.25	1.21	0.04	C$\underline{H_3}$-P-Ester	1.24	1.26	-0.02
^{31}P-NMR	19.41	18.73	0.68	^{31}P-NMR	19.36	19.62	-0.26

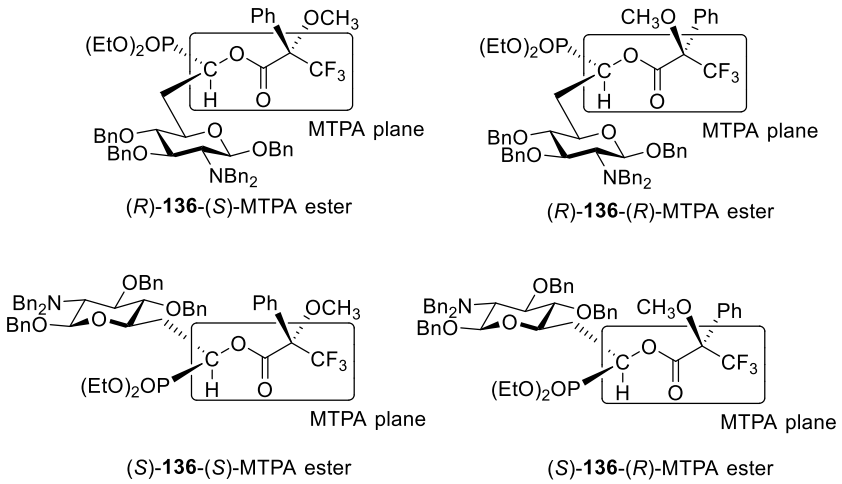
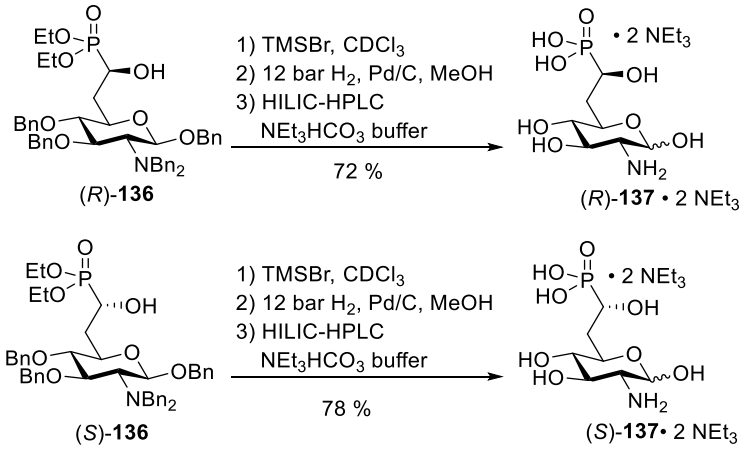


Figure 14: Synthesized Mosher esters of (*R*)-**136** and (*S*)-**136** used for the determination of the stereochemistry at C-7.

Final deprotection of (*R*)-**136** and (*S*)-**136** was achieved in two steps for each isomer (Scheme 22). The ethyl groups were cleaved with TMSBr. Subsequent hydrogenation at 12 atm H_2 with palladium on charcoal resulted in benzyl deprotection. After purification by HILIC HPLC, the two diastereomers were obtained as triethylammonium salts (*R*)-**137**•2 NEt_3 and (*S*)-**137**•2 NEt_3 in a yield of 72 % and 78 %, respectively.

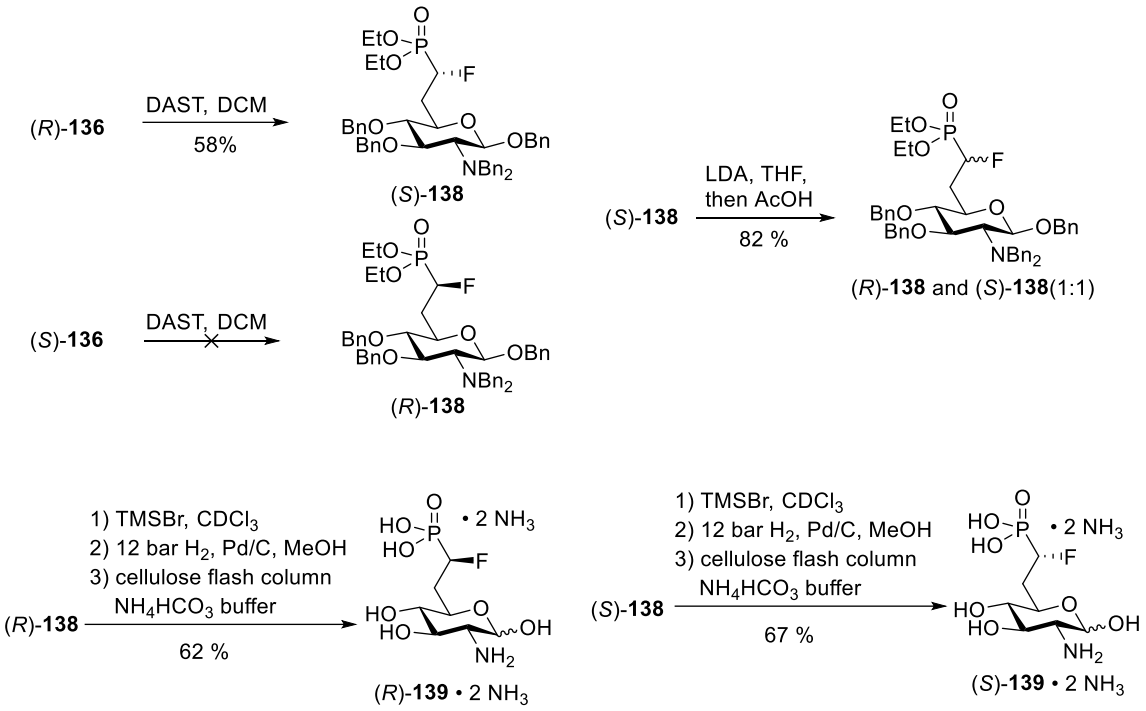


Scheme 22: Deprotection of (*R*)-**136** and (*S*)-**136** to yield triethylammonium hydroxyphosphonates (*R*)-**137**•2 NEt_3 and (*S*)-**137**•2 NEt_3

4.2.1.3 Synthesis of α-Fluoro-Phosphonates as Phosphate Mimics

Having synthesized α-difluoro-phosphonate **134** and (α-hydroxy) phosphonates (*R*)-**137** and (*S*)-**137**, a derivative with less electron withdraw from the phosphorous than **134** and more than **137** was desired to complete the library. For this reason, the synthesis of (α-monofluoro) phosphonates was conducted.

Monofluoro phosphonates are accessible from the corresponding hydroxyphosphonates by deoxyfluorination (Scheme 23).[146] A typical reagent to substitute a hydroxy group with a fluoride under inversion of configuration is diethylaminosulfur trifluoride (DAST).[154] During the synthesis of fluorophosphonates of glucose as substrate mimics for glucose 6-phosphate dehydrogenase, the *Berkowitz* group observed that only one diastereomer of tetra-*O*-benzylated glucose- hydroxyphosphonate reacted smoothly to the fluoride under inversion of stereochemistry. The other diastereomer mainly decomposed during the reaction.[146] When these conditions were applied to the diastereomeric hydroxyphosphonates (*R*)-**136** and (*S*)-**136**, I observed only (*R*)-**136** reacted with DAST to the corresponding fluoride while (*S*)-**136** decomposed during the reaction. Given the similarity of the two isomers of **136** to the hydroxyphosphonates investigated by *Berkowitz*, I assumed that also in case of (*R*)-**136** an inversion of configuration takes place. Accordingly, the reaction product obtained from (*R*)-**136** in a yield of 58 % is expected to be fluorophosphonate (*S*)-**138** (Scheme 23). We also investigated a large variety of deoxyfluorination reagents to achieve the conversion of (*S*)-**136** to (*R*)-**138** including PyFluor®[155], pentafluorobenzene sulfonyl fluoride[155], Deoxo-Fluor®[156], Xtal-Fluor-M®[157] and Xtal-Fluor-E®[157]. However, in all cases either no reaction or a decomposition similar to the reaction with DAST occurred. To gain access to compound (*R*)-**138**, an isomerization of (*S*)-**138** was performed by treatment with LDA giving a 1:1 mixture of (*R*)-**138** and (*S*)-**138.** After an acidic workup using acetic acid, the isomers were separated by flash chromatography. Deprotection of (*R*)-**138** and (*S*)-**138** was achieved as described above for (*R*)-**136** and (*S*)-**136.** The diastereomeric fluorophosphonates were purified by cellulose flash column chromatography using ammonium bicarbonate buffer and obtained as diammonium salts (*R*)-**139**•2 NH_3 and (*S*)-**139**•2 NH_3 in a yield of 62 % and 67 %, respectively.



Scheme 23: Synthesis of fluoro phosphonate (*R*)-**139** and (*S*)-**139**. (*R*)-**136** did react in a deoxyfluorination with DAST to give (*S*)-**138**. The synthesis of (*R*)-**138** was achieved by isomerization of (*S*)-**138** using LDA and acidic work up giving a separable mixture of (*R*)-**138** and (*S*)-**138**. The isomers were deprotected separately to give (R)-**139**•2 NH_3 and (S)-**139**•2 NH_3.

4.2.2 p*K*a Values of the Newly Synthesized Phosphonates

In order to compare the amount of dianion present in the newly synthesized phosphonates at physiological pH to the natural *glmS* activator GlcN6P, the pK_{a2} value had to be determined. This is of interest because the phosphate binds effectively to magnesium during the catalysis of the self-cleavage reaction in its dianionic form. Several methods are available to determine the pK_{a2} value of phosphates or phosphonates. Potentiometric acid base titration has the disadvantage that the pK_a value of the amine of glucosamine and modified phosphonates can have similar pK_a values. Therefore, they might be difficult or even impossible to distinguish by potentiometric titration. With this concern in mind it was decided that a determination of the pK_{a2} value of the phosphonate via titration in a ^{31}P-NMR experiment seemed most promising.[158] For this cause, a 50 µM solution of the compound of interest was prepared in a 10:1 mixture of H_2O and D_2O. The pH of this solution was adjusted to around 2.5 and a ^{31}P spectrum was recorded. Then, the pH was raised in intervals of approximately 0.5 followed by recording a

new ^{31}P spectrum. After raising the pH to approximately 10.5 the ^{31}P shifts were plotted against the pH value (Figure 15). This allowed fitting of a sigmoidal function. The point of inflection of the function represents the pK_a value. Two literature known compounds were tested and used as control. The pK_a value of GlcN6P was determined to be 6.2±0.1, which is in accordance with the literature value of 6.1 reported by *Strobel.*[159] The pK_a value of methylene phosphonate **12** was determined to be 7.5±0.1 and is also comparable to the reported value of 7.4 by *Soukup*.[39]

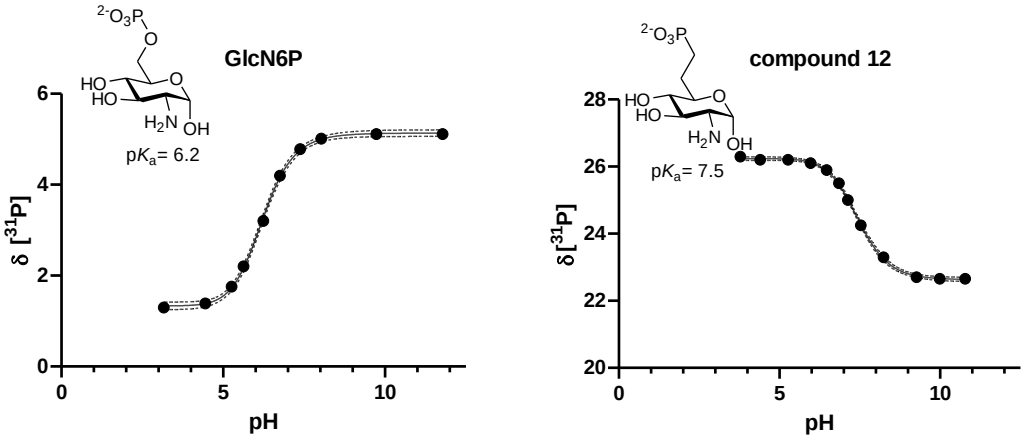


Figure 15: Titration curve of GlcN6P as well as the literature known methylene phosphonate **12**. The chemical shifts of the phosphorus in the ^{31}P NMR are plotted against the pH. The point of inflection of the fitted sigmoid function represents the pK_a value. For GlcN6 it was found to be 6.2±0.1 and for **12** to be 7.5±0.1. The 95 % confidence interval is shown.

Having shown the accuracy of the NMR experiment, the difluoro phosphonate **134** the monohydroxy phosphonate (R)-**137**, and the monofluoro phosphonate (*S*)-**139** were also subjected to titration in the described NMR experiment (Figure 16). It was assumed that the stereochemistry alpha to the phosphonate has no influence on the pK_a value. The pK_a value of **134** was determined to be 5.4±0.1, the pK_a value of **137** 7.2±0.1 and pK_a value of **139** to be 6.3±0.1. This experiment demonstrated that phosphate mimics spanning a wide range of pK_a values are represented in the synthesized library. With **134** this includes a mimic that is more acidic then GlcN6P. Mimics that are less acidic as **137** and **12,** are included as well. When comparing the monofluoro phosphonate **139** to GlcN6P, **139** showed a pK_a value that matches the

natural ligand GlcN6P nearly perfectly with 6.3 in comparison to 6.2. Consequently, this library of phosphonates is well suited to study the effect of the pK_a value of phosphonate mimics on the *glmS* riboswitch activation

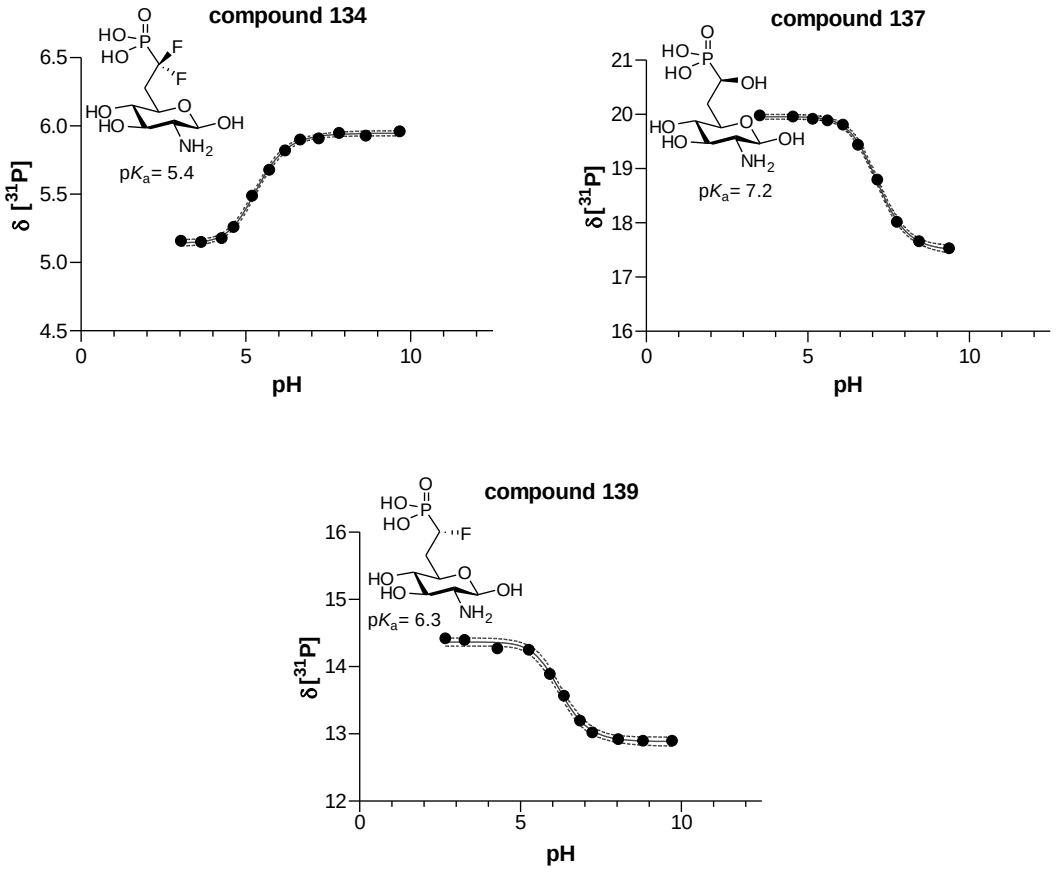


Figure 16: Titration curve of **134**, **137,** and **139**. The chemical shifts of the phosphorus in the 31P NMR are plotted against the pH. The point of inflection of the function represents the pK_a value. For **134** it was found to be 5.4±0.1 and for **137** to be 7.2±0.1 and for **139** to be 6.3. ±0.1.

4.3 Self-Cleavage Assay and Assessment of Antimicrobial Potential of the GlcN6P Mimics

Experimental work described in this chapter has been performed by Dennis Kläge from the research group of Prof. Hartig with compounds synthesized by me.

In order to investigate the ability of the newly synthesized compounds to induce the self-cleavage reaction of the *glmS* ribozyme, ligand dependent self-cleavage assays[37] with the 5'-^{32}P labeled *B. subtilis* ribozyme sequence were performed (Figure 17).

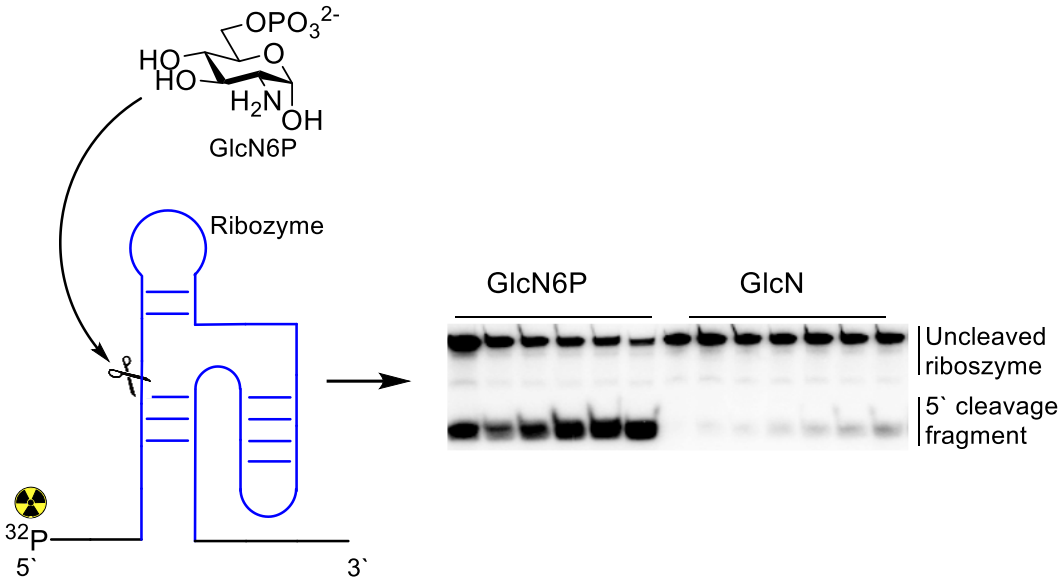


Figure 17: Schematic overview of the self-cleavage assay. An activator (here GlcN6P) is incubated with the 5'-^{32}P labeled *B. subtilis glmS* ribozyme sequence. The amount of cleaved ribozyme can be determined by PAGE-Gel followed by radioactive signal readout.

For an initial assessment of the activity, compounds **134**, (*R*)&(*S*)-**137**, (*R*)&(*S*)-**139** and **127** were incubated with the *glmS* ribozyme in presence of 10 mM $MgCl_2$ (Figure 18). These experiments revealed that the compounds (*R*)-**137**, (*S*)-**137** and **127** cleaved the riboswitch efficiently under these conditions. Compound (*R*)-**139** and (*S*)-**139** showed slightly diminished activity and difluoro phosphonate **134** only exhibited minor induction of the self-cleavage reaction.

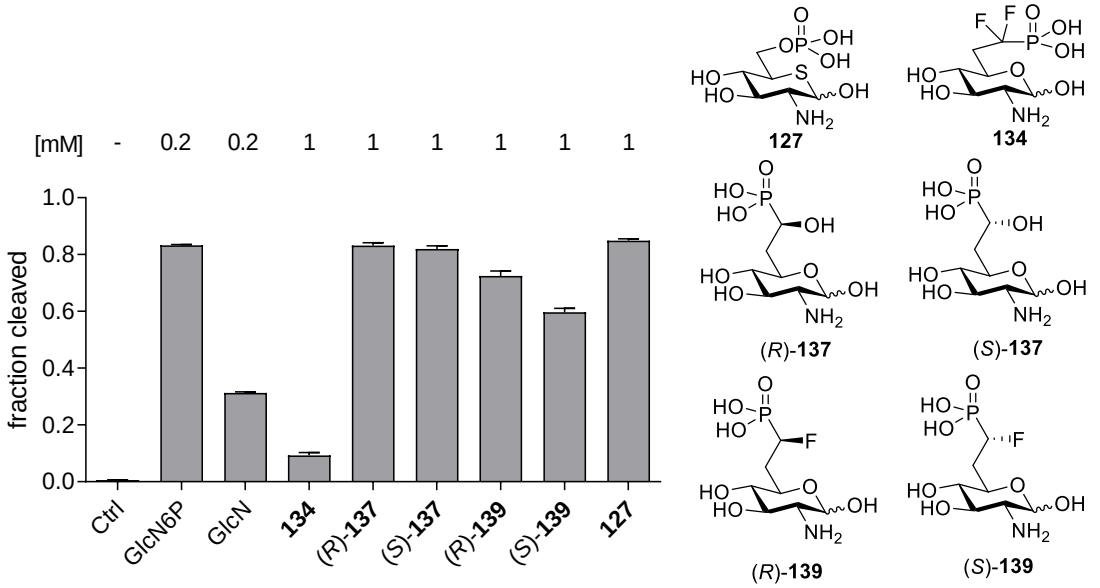


Figure 18: Initial assessment of the cleavage induction of *B. subtilis glmS* ribozyme by the newly synthesized compounds **134** ,(*R*)&(*S*)-**137**, (*R*)&(S)-**139** and **127**. 5'-^{32}P labeled *B. subtilis* ribozyme sequence was incubated with 0.2 mM GlcN6P or GlcN. The artificial activators were used in a concentration of 1 mM. Ribozyme and activator and 10mM magnesium chloride were incubated for 30 min at 37 °C. The control sample contained the ribozyme and Mg^{2+} only. The cleavage product was detected after gel electrophoresis by radioactive signal readout.

4.3.1 Kinetic Measurements of the Phosphonates

As the phosphonates (*R*)&(*S*)-**137**, (*R*)&(*S*)-**139**, seemed to be promising *glmS* activators it was planned to obtain further insights into the cleavage efficiency. *Soukup* showed that phosphonates in principle can active the *glmS* riboswitch although significantly slower in comparison to the native activator.[39] We hypothesized that this can be explained by the higher pK_a value of the phosphonate in comparison to the phosphate of GlcN6P. To test, whether the pK_a values of the phosphonates influences the riboswitch activation, literature known methylene phosphonate **12** was synthesized and compared to the compounds (*R*)-**137**, (*S*)-**137** and (*R*)-**139** by determining the rate constant k_{obs} of the cleavage reaction. For the kinetic measurements 5' ^{32}P labeled *B. subtilis* ribozyme was incubated with different concentrations of the activators and fraction cleavage was detected over a period of 300 seconds. The methylene phosphonate **12** showed the highest rate constants (*R*)&(*S*)-**137** was slower. (*R*)&(*S*)-**139** was even slower then (*R*)&(*S*)-**137** slower (Table 2, Figure 19). For hydroxy phosphonate (*S*)-**137** a k_{obs} of 0.078 min^{-1} was determined at 200 µM. The k_{obs} increased with increasing concentrations

(0.158 min^{-1} at 500 µM, 0.362 min^{-1} at 1 mM). For hydroxy phosphonate (*R*)-**137** a k_{obs} value of 0.069 min^{-1} was determined at 200 µM. The k_{obs} value increased with increasing concentrations (0.211 min^{-1} at 500 µM, 0.269 min^{-1} at 1 mM). The slower fluoro phosphonates did show less of a concentration dependency. For (*S*)-**139** the k_{obs} value at 500 µM was 0.091 min^{-1} and at 1 mM 0.055 min^{-1}, signifying a limited activation of the riboswitch. For hydroxy phosphonate (*R*)-**139** a k_{obs} value of 0.049 min^{-1} was determined at 200 µM. The k_{obs} value increased to 0.174 min^{-1} at 500 µM but stayed in the same range for a concentration of 1 mM (0.173 min^{-1} at 1 mM). The methylene phosphonate **12** showed the highest k_{obs} values. A k_{obs} value of 0.103 min^{-1} was determined at 200 µM. The k_{obs} value increased with increasing concentrations (0.333 min^{-1} at 500 µM, 0.439 min^{-1} at 1 mM).

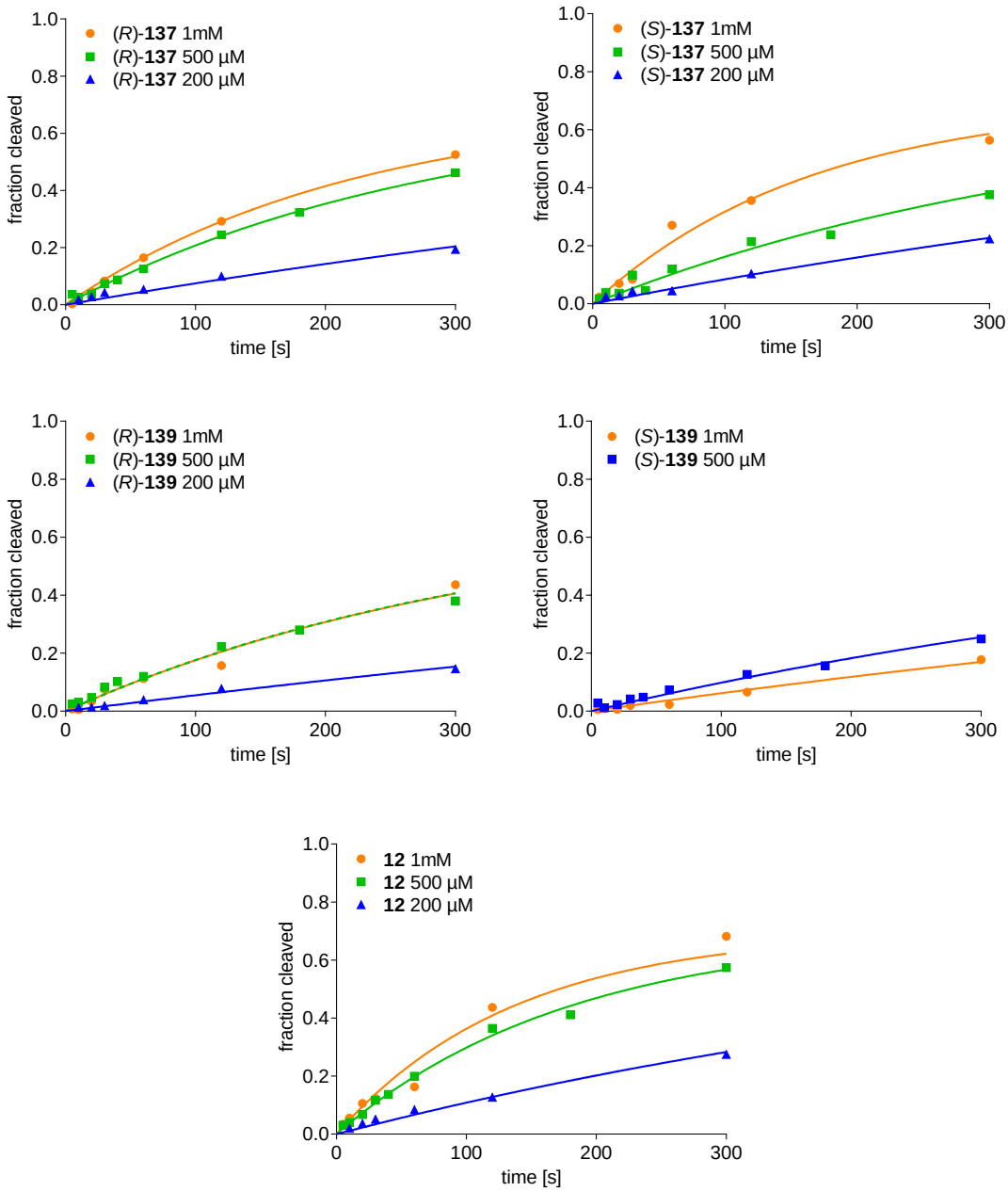


Figure 19: Kinetic measurements of the self-cleavage of 5' ^{32}P labeled B. subtilis *glmS* ribozyme induced by (Top), Hydroxyphosphonates (R)-**137** and (S)-**137**. (Middle) Fluorophosphonat**e** (R) **139** and (S)-**139**. (Bottom) Methylene phosphonate **12** was measured as comparison. All measurements performed at the same concentration are represented in one color. When two curves overlapped one is shown as dotted.

Table 2: Rate constants (k_{obs}) of B.subtilis Ribozyme Cleavage induced by the compounds (*R*)-**137**, (*S*)-**137**, (*R*)-**139,** (*S*)-**139,** Methylene phosphonate **12.**

compound	k_{obs} **[min^{-1}]** **200 µM**	k_{obs} **[min^{-1}]** **500 µM**	k_{obs} **[min^{-1}]** **1 mM**
(*S*)-**137**	0.078± 0.004	0.158± 0.010	0.362± 0.027
(*R*)-**137**	0.069± 0.004	0.211± 0.005	0.269± 0.007
(*S*)-**139**	n.d	0.091±.0.004	0.055± 0.003
(*R*)-**139**	0.049± 0.002	0.174± 0.008	0.173± 0.013
12	0.103± 0.004	0.333± 0.010	0.439± 0.049

The pK_a value of **137** (pK_a = 6.2) is comparable to the pK_a value of the natural activator GlcN6P (pK_a = 6.3), whereas the pK_a value of the methylene phosphonate **12** is higher (pK_a = 7.5). Kinetic measurements indicate that the difference in activity, when comparing phosphates to phosphonates cannot be explained by the difference in the pK_a value. A possible explanation for the difference in activity of the synthesized phosphonates and the natural occurring phosphate might be explained by analyzing the crystal structure of the *glmS* riboswitch bound to GlcN6P (Figure 20). Here it is visible that the oxygen between the 6-position of the sugar core and the phosphate is involved in hydrogen bonding to a guanine in the binding pocket of the *glmS* riboswitch. For the phosphonates this bonding is not possible. Therefore, the interaction of the oxygen in the 7-position might be more important than initially thought.

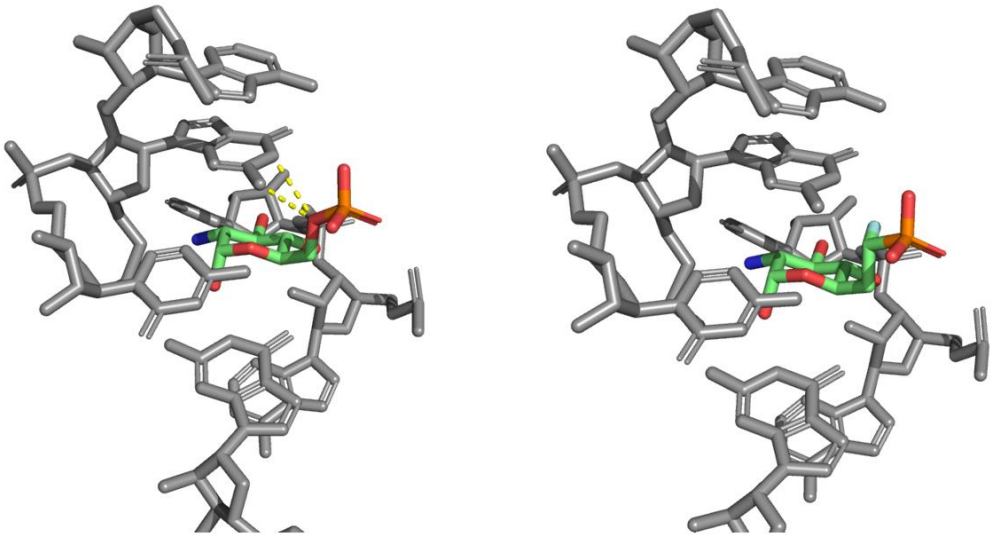


Figure 20: (Left) Crystal structure of the *glmS* bound to the GlcN6P. (PDB: 2nz4)[55] Two Hydrogen bonds between a guanidine and the oxygen connecting the carbohydrate core and the phosphate are highlighted in yellow. (Right) When compound **139** is modeled in the same binding pocket these interactions are lost.

4.3.2 Kinetic Measurements of Thia-GlcN6P 127 and Comparison to GlcN6P

Another promising candidate was **127** which was also tested in this kinetic assay. Thia-GlcN6P **127** and natural ligand GlcN6P were incubated with 5' ^{32}P labeled *B. subtilis* ribozyme as described above. Both compounds showed a concentration depend activation of the ribozyme and have identical k_{obs} values for the measured concentrations (500 µM, 200µM, 10µM) (Table 3, Figure 21). For GlcN6P a k_{obs} value of 0.82 min^{-1} was determined at 10 µM. The k_{obs} value increased to 2.21 min^{-1} at 200 µM and remained in the same range at 500 µM (2.49 min^{-1} at 500 µM). For thia-GlcN6P a k_{obs} value of 1.09 min^{-1} was determined at 10 µM. The k_{obs} value increased to 2.44 min^{-1} at 200 µM and remained in the same range at 500 µM (2.36 min^{-1} at 500 µM. These results signify that thia-GlcN6P **127** is an effective activator of the *glmS* riboswitch with kinetic properties, rivaling the natural ligand GlcN6P. The only other carbohydrate mimetic described in literature with comparable properties is carba-GlcN6P **17**.

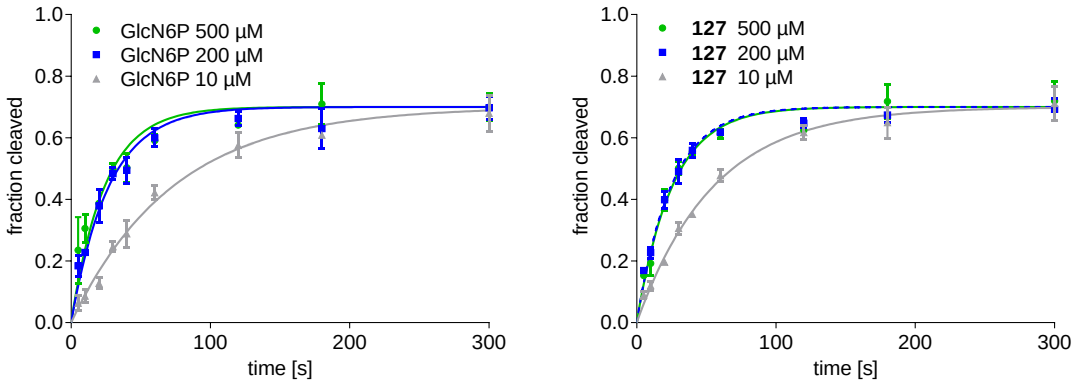


Figure 21: Kinetic measurements of the self-cleavage of 5' ^{32}P labeled B. subtilis *glmS* ribozyme induced by (left) GlcN6P and (right) Thia-GlcN6P **127**. Data show means of three independent experiments. Error bars are shown as standard deviation (SD). When two curves overlapped one is shown as dotted.

Table 3: Rate constants (k_{obs}) of *B. subtilis* ribozyme cleavage induced by GlcN6P and thia-GlcN6P **127**.

compound	k_{obs} **[min^{-1}]**	k_{obs} **[min^{-1}]**	k_{obs} **[min^{-1}]**
	10 µM	**200 µM**	**500 µM**
GlcN6P	0.82± 0.04	2.21± 0.18	2.49± 0.28
127	1.09± 0.04	2.44± 0.12	2.36± 0.15

4.3.3 Antimicrobial Assays

Antimicrobial assays were performed by *Dennis Kläge* from the research group of Prof. *Hartig.*

Having shown that a number of synthesized GlcN6P mimics activate the *glmS* riboswitch, we were interested whether these derivatives are able to interfere with bacterial growth. In order to exhibit antimicrobial properties these derivatives need to enter the bacterium. This is known to be difficult for polar compounds such as the phosphonates and phosphates described in this work. Previously, we investigated the antimicrobial properties of carba-GlcN in its unphosphorylated form.[160] It is assumed that this compound is taken up actively into the bacterium by phosphotransferase (PTS) transporters with concomitant phosphorylation to the 6-phosphate. This cannot be an applied to the phosphonates **134**, **137** and **139**. We still tested these compounds as the free phosphonates, knowing that this might impede uptake. In contrast, thia-GlcN **126** might be a substrate for the PTS resulting in efficient uptake and phosphorylation to **127**. Accordingly, the thia-mimic of glucosamine **126** was used in its non-phosphorylated form for the following experiments.

Initial tests were performed via a filter disk assay to estimate the antimicrobial potential. In these tests chloramphenicol was used as a positive control and GlcN was applied as a negative control. In these assays a substance is applied on a filter disk. This substance diffuses through the agar plate. If an antimicrobial effect is exhibited by the substance a zone of inhibited bacterial grow is visible around the filter disk. For thia-glucosamine **126** an antibiotic effect against *B. subtilis* and *B. thuringensis* was observed (Figure 22). Against *E. coli*. no antimicrobial effect was detected. The two bacillus strains are known to carry the *glmS* ribozyme and *E. coli* is known not to possess it. It is also noteworthy that when thia-glucose was tested instead of thia-glucosamine **126** no inhibitory effect was observed.

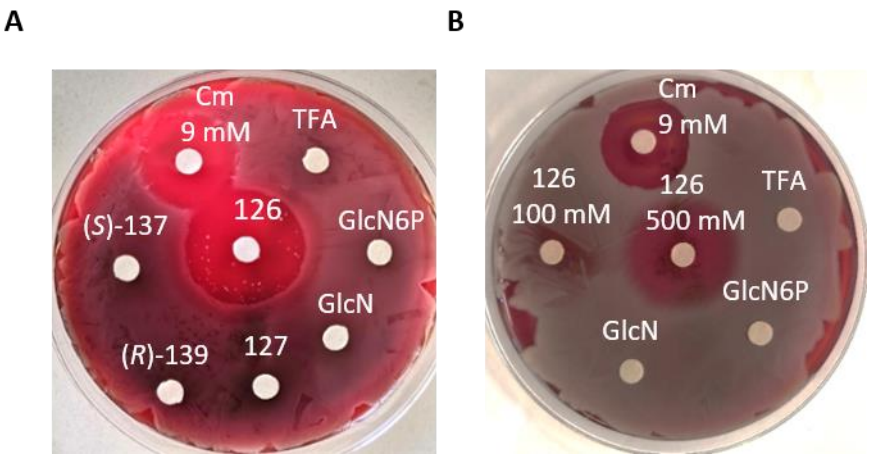


Figure 22: Filter disk assay of selected glucosamine mimics. Compound **126** shows an inhibitory effect against **A** *B. subtilis* and **B** *B. thuringensis.* Chloramphenicol (Cm) was applied as a positive control. If an antimicrobial effect is exhibited a zone of inhibition is visible around the filter disk with the applied substance.

After the assessment of antibiotic activity, the minimal inhibitory concentration (MIC) of thia-glucosamine was determined, by cultivating the bacillus strains on agar plates with increasing concentrations of **126**. The MIC was determined to be the lowest concentration that did inhibit the bacterial growth. The MIC of compound **126** was determined to be around 2 mM for *B. subtilis* and around 5 mM for *B. thuringensis.* These concentrations are relatively high to be considered an effective antibiotic. Nevertheless, the observation of an antibiotic effect of this highly polar thia-glucosamine mimic acts as a prove of principle that the exchange of the 5a-position of GlcN6P yields an active *glmS* riboswitch activator and a potential antibiotic.

To gain further insight in the mode of action of the compound **126**, thia-glucosamine resistant strains were created. This was achieved by cultivation of *B. subtilis* at concentration inferior to the MIC value allowing bacterial growth. The concentration of **126** was elevated with every cycle favoring growth of resistant bacteria. Genome analysis of resistant strains revealed that they carry a mutation in *ptsH* gene. The *ptsH* gene encodes for a phosphocarrier protein of the PTS. Therefore, the resistant *B. subtilis* strain is unable to take up thia-glucoamine **126** and to phosphorylate it to 6-phosphate **127**. For this reason, the *glmS* riboswitch cannot be activated in the resistant *B. subtilis* strain. Even though the mutation is present in a protein that is involved in the uptake of **126** and metabolism to the

active *glmS* riboswitch activator **127** it was not possible to prove a link between the observed antibiotic effect and the *glmS* ribozyme cleavage with this experiment. It is possible that hindering the uptake of **126** can block interactions with other targets in the bacterium, and thus, explain the resistance. To identify the main target of the antimicrobial effect of novel antibiotic **126** further studies are necessary.

4.4 5a-Modified Carba-Glucosamine Mimics as Potential New Antibiotics

Parts of the results described in this chapter have been published in Chemistry a European Journal[64]

The carba-analogue of glucosamine **18** has been shown to exhibit an antibiotic effect in previous studies (Figure 23).[140] This encouraged the synthesis of 5a-modified carba-mimics of glucosamine. The first example of this compound class was the 5a-fluoro-carba-α-D-glucosamine **90**, which also showed antimicrobial potential.[47,58] These results raised the question whether introduction of substituents in the 5a-position could further improve these antibiotic properties. When I started my investigations, *Stängle* already developed a synthetic route in order to access 5a-hydroxy modified carba-mimics of glucosamine. He aimed for a synthetic route that would allow a late stage derivatization of the 5a-position, to investigate the influence of 5a-substituents on the antibiotic properties of carba-mimics of glucosamine. However, the synthetic route suffered from low yields, which ultimately jeopardized the ambitious plans for a library of 5a-modified carba-mimics of glucosamines. This strategy only gave access to the alkoxy derivatives **21**, **22**, **23** in limited quantities. Therefore, I set out to develop a synthetic strategy solving the problems faced by *Stängle* providing access to initially desired library of 5a-modified carba-mimics of glucosamine.

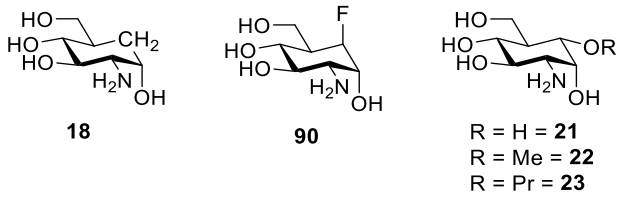
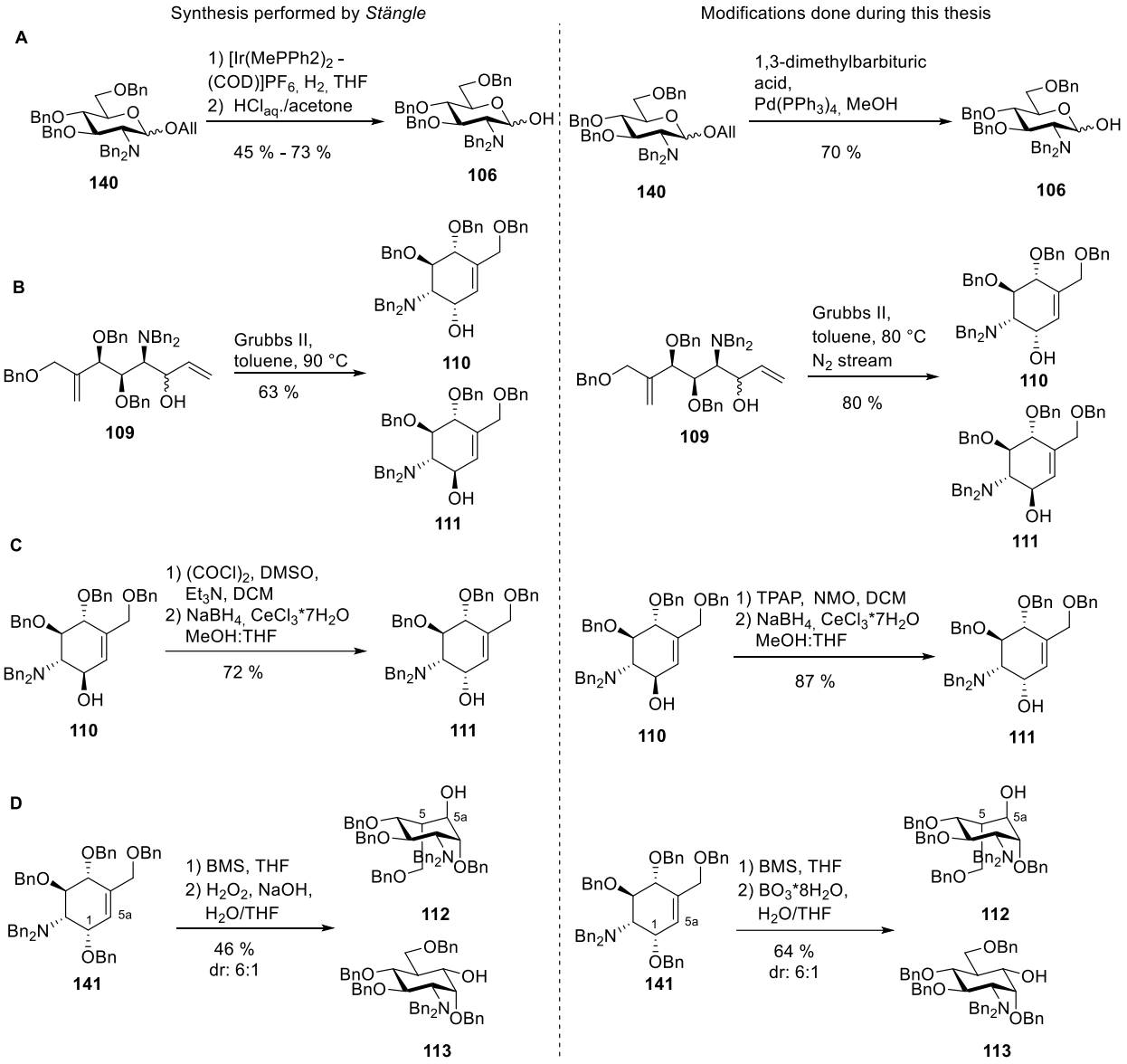


Figure 23: Carba-mimics of glucosamine reported before the beginning of this thesis.

4.4.1 Problems Faced During Previous Synthesis of 5a-Modified Carba-Glucosamine Mimics

As described in chapter 2.3 *Stängle* synthesized 5a-modified carba-mimics of glucosamine. The bottleneck of his synthesis was the preparation of a sufficient quantity of the desired key intermediate **113**. This compound possesses the necessary equatorial stereochemistry at the 5 position in order to mimic the natural configuration of glucosamine in addition to an equatorial hydroxy group at the 5a-position. To introduce a variety of substituents in the 5a-position large amount of this compound was desired, requiring an efficient and robust synthesis route. *Stängle* encountered a multitude of difficulties during his synthesis and serval of theses remained unanswered in his thesis. I optimized the synthesis developed by *Stängle* in order to allow the preparation of a larger library of modified carba-analogues of glucosamine. A comparison of the conditions used by Stängle and the changes made during this thesis is shown in Scheme 24. The first change undertaken during my work was the change of the allyl deprotection step (Scheme 24). *Stängle* used $[Ir(MePPh_2)_2(COD)]PF_6$ as catalyst in order to rearrange the allyl group into a vinyl group, followed by cleavage of said group under acidic conditions.[161] Using this chemistry, it was possible to convert the allyl protected sugar **140** into the free hemiacetal **106** in a yield of 73 %. Even though the yield itself looks sufficient the reaction suffered from a lack of robust reproducibility giving a range of yields from 45 % to 73 %. This was aggravated by a limited commercial supply of $[Ir(MePPh_2)_2(COD)]PF_6$ manifesting in long delivery times. Therefore, the conditions were changed to $Pd(PPh_3)_4$ and 1,3-dimethylbarbituric acid in order to synthesize compound **106** in a Tsuji-Trost reaction.[162] This allowed the synthesis of compound **106** in a yield of 70 % in a highly reliable reaction (Scheme 24 A). The second change made was to perform the ring closing metathesis (RCM) used for the construction of the carbacycle under a constant stream of nitrogen. This allowed for the removal of the forming ethylene during the reaction resulting in a longer lifetime of the Grubbs catalyst manifesting in the complete conversion of the starting material **109**.[163] During previous attempts incomplete conversion was observed and this minor change resulted in an improvement of the combined yield of compound **110** and **111** from 63 % to 80 %.[164] The next step was also target of an

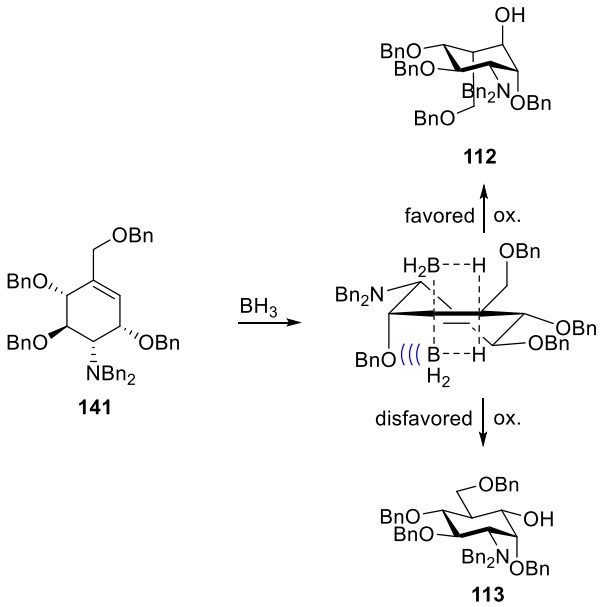
adjustment. *Stängle* used Swern conditions[165] to convert compound **111** into the corresponding enone. A following Luche reduction[166] resulted in the interconversion of compound **111** to **110** in a yield of 72 % over two steps (Scheme 24 B). Changing the oxidation step to a Ley Griffith[167] oxidation using TPAP and NMO resulted in an improvement of the yield to 87 % over two steps (Scheme 24 C). The desired hydroxy group was introduced using a hydroboration reaction. *Stängle* reported a combined yield of 46 % for the compounds **112** and **113** (Scheme 24 D). This yield can be explained by the formation of side products under the harsh oxidative conditions used. Changing hydrogen peroxide to sodium perborate in the oxidation step minimized the observed side reactions and resulted in an improved combined yield of 64 %.



Scheme 24: Left: Synthesis performed by *Stängle* Right: Modifications described in this thesis.

The main problem encountered during the synthesis of **113** was the stereo selectivity during the hydroboration favoring the ido derivative **112** over the glucose derivative **113** in a ratio of around 6:1 (Scheme 25). This preference can be explained by the steric hindrance exhibited by the benzyl ether in the 1-position. This group shields one side of the alkene from the addition of the borane during the hydroboration and favors the formation of the 5,5a-diaxial hydroboration product **112** over the 5,5a-diequatorial one **113**. Circumventing this selectivity proved to be more challenging than the comparable small improvements done to the previously

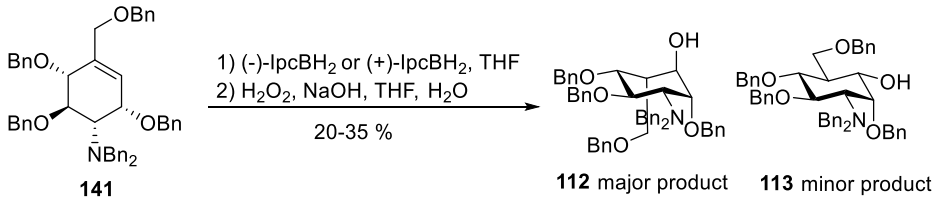
mentioned steps. The main strategies explored to improve the overall yield of a 5a-modified carba-analogue of glucosamine are discussed in the following chapters.



Scheme 25 *Syn*-addition of BH_3 to the olefin **111**. Steric clash of the benzyl group in the 1-position favors the formation of **112** over the desired key intermediate **113**. The scheme was adapted from *Stängle.* [120]

4.4.2 Chiral Organoboranes for the Introduction of the 5a-Hydroxygroup

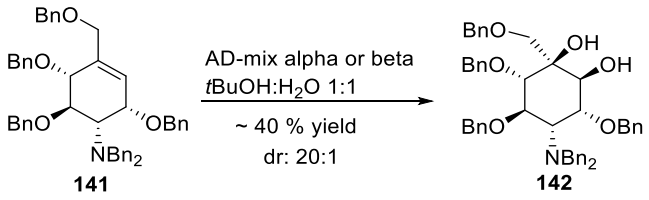
Initial attempts to solve the problem described in scheme 25 focused on using chiral boranes instead of borane dimethyl sulfide for the hydroboration of olefin **141**. To this end, both enantiomers of monoisopinocampheylborane ($IpcBH_2$)[168] were synthesized as both enantiomers. However, when performing the hydroboration with either (-)-$IpcBH_2$ or (+)-$IpcBH_2$, **112** was still the major product in both cases after oxidation. Additionally, the obtained yield was significantly lower than when using borane dimethyl sulfide (Scheme 26). The substrate control by the starting material **141** was concluded to be higher than the impact of this class of chiral boranes. Consequently, another strategy was pursued to increase the overall yield of **113**.



Scheme 26: The use of chiral boranes did not lead to a higher yield of compound **113**.

4.4.3 Dihydroxylation as an Alternative Way of Carba-Sugar Derivatization

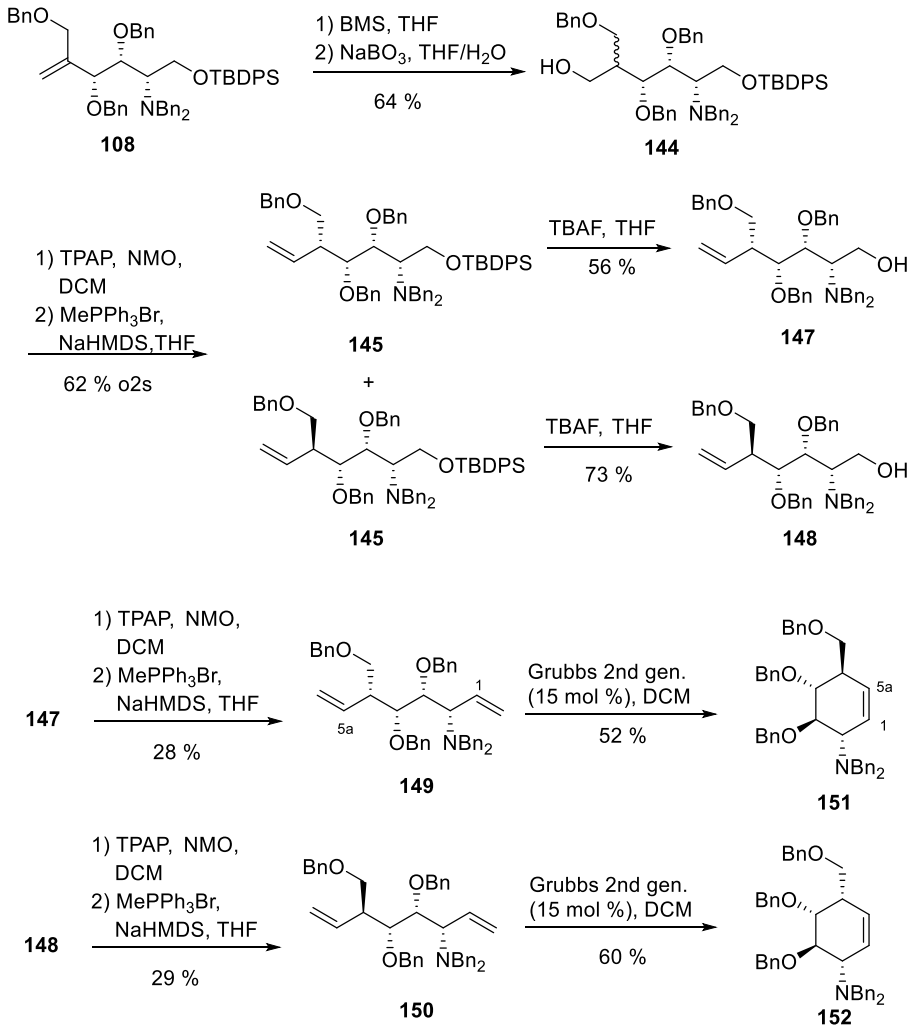
One additional strategy pursued to circumvent the selectivity problem encountered during the hydroboration was to perform a dihydroxylation instead of a hydroboration. This would allow to introduce the additional hydroxy group in the 5a-position by another class of chemical reaction. It was assumed that when using Sharpless asymmetric dihydroxylation[169] conditions it would be possible to control the diastereoselectivity of the reaction, by the chiral ligands applied during this reaction (Scheme 27). Unfortunately, it was observed that the main product of the dihydroxylation was the ido-derivative **142** independent of the use of AD-mix alpha or beta, hinting again to a high substrate control of the stereochemistry.



Scheme 27: Dihydroxylation of compound **141** giving the compound **142** as the main product independent of the AD-mix used.

Therefore, I designed a new synthetic route towards a cyclic carba-sugar precursor bearing an alkene between the 5a- and 1-position. In this compound the stereochemistry at the 5-position would already be adjusted in the desired glucose-like configuration and the two missing hydroxy group could be introduced by dihydroxylation. The synthesis of the cyclic precursor **151** was performed by the bachelor student Fiona Waschbüsch under my supervision (Scheme 28). We used the 1,1 substituted alkene intermediate **108** and performed a hydroboration

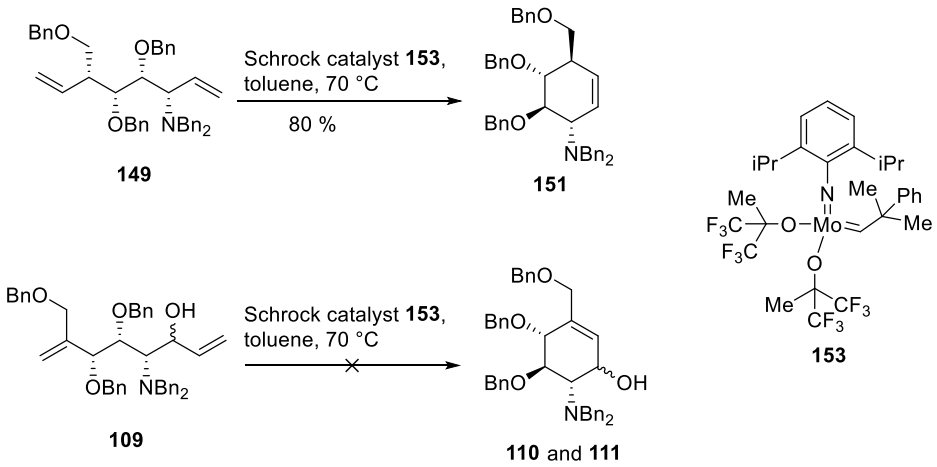
reaction giving the primary alcohol **144** in a yield of 64 % as a mixture of two diastereomers. They were oxidized to the corresponding aldehydes using Ley Griffith conditions and the crude aldehydes were reacted in a Wittig reaction with methyltriphenylphosphonium bromide giving the two separable terminal alkenes **145** and **146** in a yield of 66 % over two steps. The two alkenes were separately deprotected using TBAF giving the free alcohols **147** and **148** in 56% and 73 % yield. They were oxidized again using Ley Griffith conditions and reacted in a Wittig reaction with methyltriphenylphosphonium bromide to give dialkenes **149** and **150** in 28 % and 29 % over two steps. Ring closing metathesis was performed using Grubbs 2nd generation[170] catalyst giving the two cyclic alkenes **151** and **152** in a yield of 52 % and 60 %.



Scheme 28: Synthesis of alkene **151** and **152**, bearing a double bond between the 1- and 5a-position. Both diastereomers were synthesized for easier determination of the stereochemistry at the 5 position.

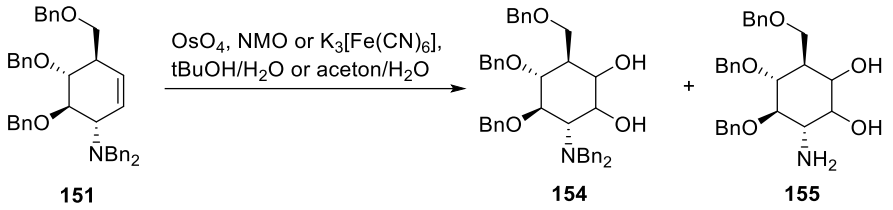
After obtaining the compounds **151** and **152** it was possible to assign the stereochemistry of the 5-position by NOESY NMR allowing for the retrospective assignment of the stereochemistry of compounds **145**, **146**, **147**, **148**, **149** and **150**.

It is noteworthy that for the RCM towards **151** Schrock catalyst was also tested for RCM and performed better than the Grubbs catalyst giving yields of up to 80 % (Scheme 29). Schrock catalyst **153**[171] was not applicable for RCM of diene **109** which has an allylic alcohol and was therefore used only for the compounds **151**.



Scheme 29: RCM using Schrock catalyst **153** is possible for the diene **149.** This yields the alkene **151** in higher yields than the Grubbs II catalyst. For the allylic alcohol **109** no RMC was observed.

Then, compound **151** was reacted in a dihydroxylation reaction with OsO_4 and either NMO or potassium ferricyanide as co oxidants (Scheme 30). The desired product of this reaction was diol **154**. It was observed that independent of the temperature (0-60 °C), the amount of OsO_4 (0.1 to 1 Eq.), and the amount of co oxidant (0.8-50 Eq.) deprotection of the benzyl groups at the amine in addition to dihydroxylation was observed. This resulted in a complex reaction mixture. With enough co oxidant it was possible to fully convert the compound **151** to the diol with a free amine **155**. However, stopping at the protected amine **154** was only achieved in low yields (~10 %).

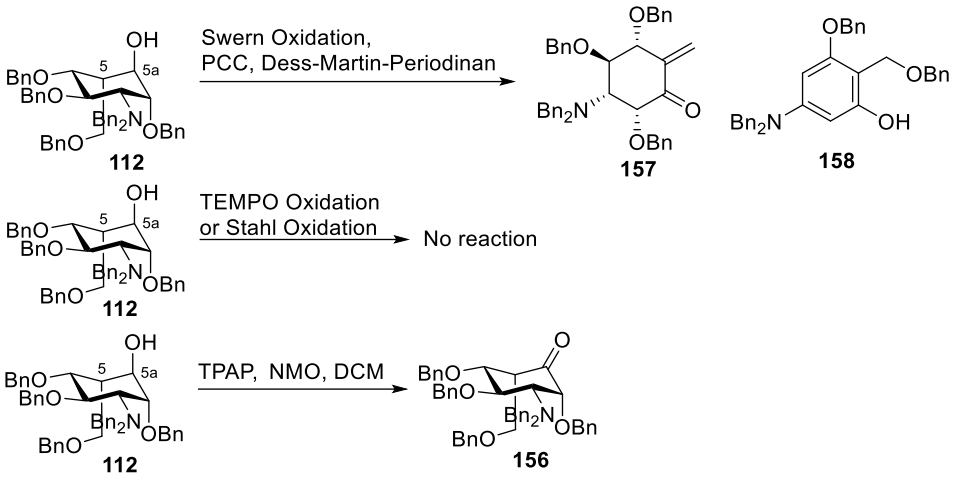


Scheme 30: Dihydroxylation of alkene **151** resulted in a mixture of **154** and **155**.

The unavoidable formation of significant amounts of **155** complicated further derivatization. Therefore, it was decided to pursue a different strategy to obtain compound **113** in higher yields.

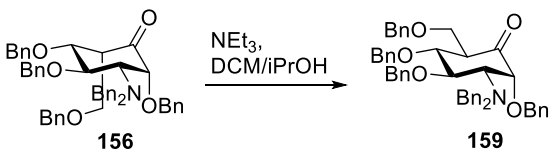
4.4.4 Isomerization of the 5-Position – Converting the Ido-Carba-Sugar to the Desired Gluco-Isomer

The next strategy relied on the assumption that the configuration at the 5 position present in compound **113** is thermodynamically more stable than the ido form present in compound **112**. It was postulated that the configuration can be adjusted if the system is subjected to conditions that allow an equilibrium to be established. It was intended to reach this equilibrium via deprotonation at the 5-position. However, this was not possible for compound **112** directly but after oxidation of the alcohol in the 5a-position via enolate formation. Therefore, different conditions for the oxidation of the alcohol **112** to the ketone **156** were tested. Oxidation with PCC, Swern oxidation and oxidation with Dess-Martin periodinane[172] provided only traces of the target ketone **156.** The reaction mostly led to decomposition yielding in the exocyclic alkene **157** and the aromatized compound **158** (Scheme 31). Oxidation with nitroxides such as TEMPO and ABNO[173] showed no reaction at all. Stahl[174,175] conditions also did not give the desired ketone. Synthesis of the ketone **156** was finally achieved by oxidation with TPAP and NMO. Under theses mild conditions the side reactions were reduced to a minimum (Scheme 31).



Scheme 31: Oxidation of compound **112** to ketone **156**. Swern oxidation, PCC or oxidation with Dess-Martin periodinane did lead to decomposition to compound **157** and **158**. TEMPO oxidation or ABNO oxidation did not proceed. Ley-Griffith oxidation was successful giving ketone **156**.

Ketone **156** proved to be very sensitive to treatment with acid or strong base resulting in decomposition. Consequently, isomerization of the 5-position, which is alpha to the ketone was more difficult than initially estimated. For example, treatment with sodium methoxide resulted in the elimination of a benzyl ether yielding an enone. Moreover, treatment with pyridine resulted in decomposition as well. Formation of the TMS enol ether followed by acidic workup also resulted in decomposition. However, after intensive screening, isomerization was achieved in a 1:1 mixture of dry isopropanol and dry DCM with 10 equivalents of triethylamine in a concentration of 35 mM (Scheme 32). The reaction was carefully monitored by LC-MS because prolonged exposure to these conditions also resulted in decomposition (Figure 24).



Scheme 32: Successful isomerization of ketone **156** to give **159.**

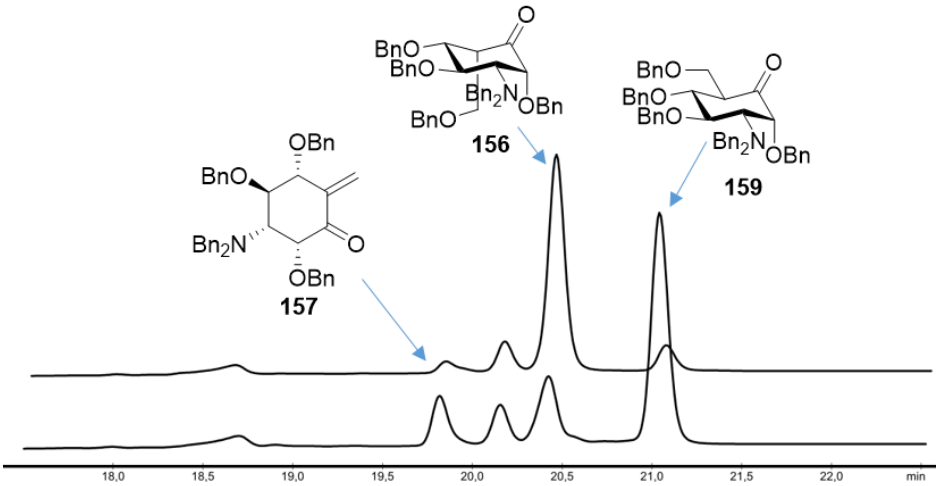
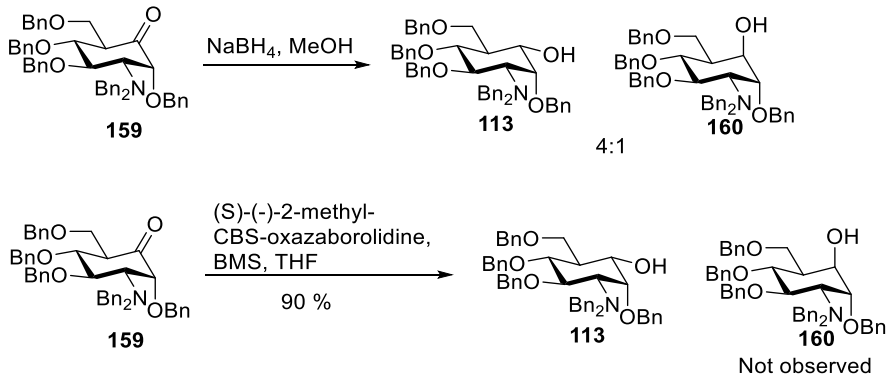


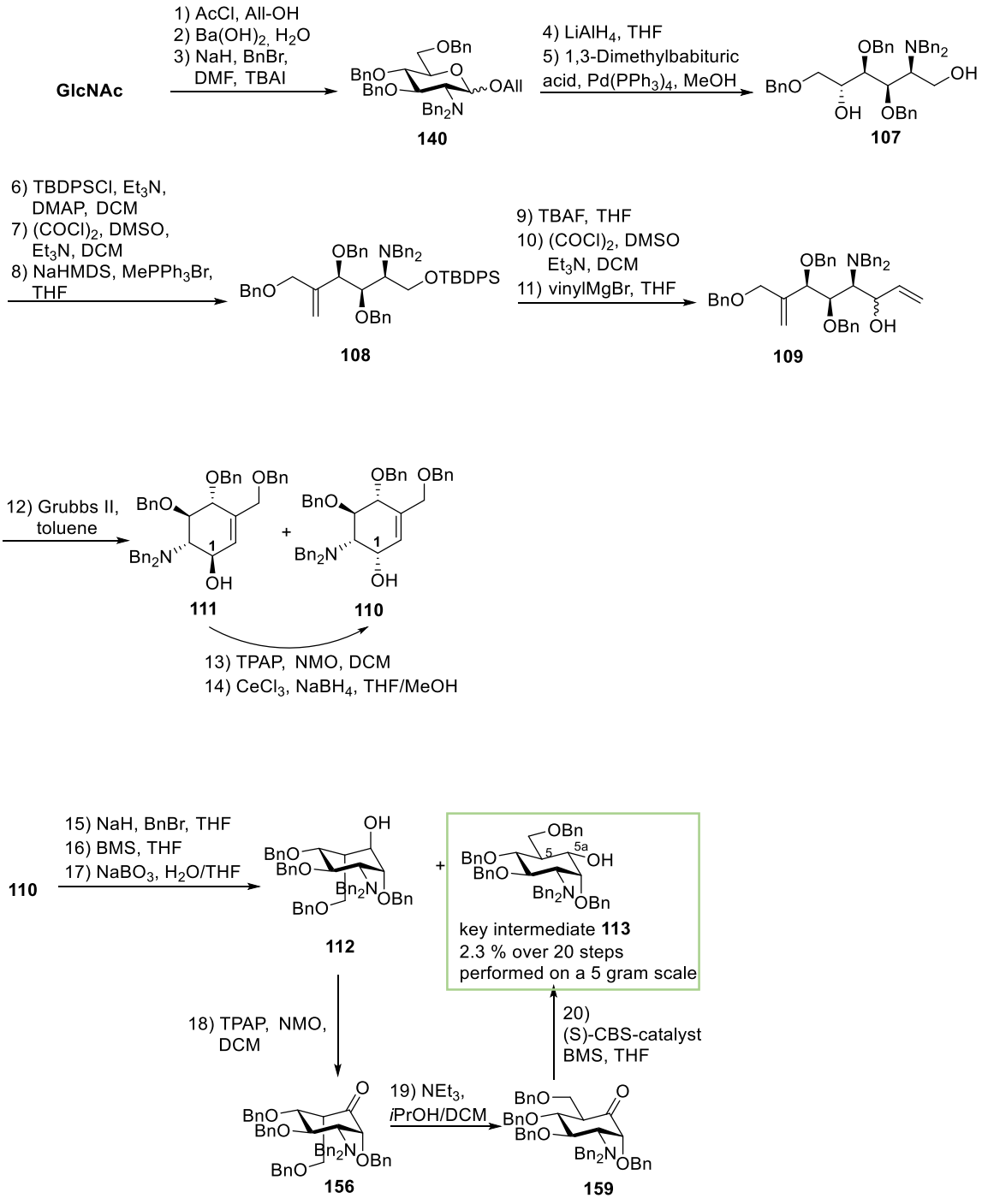
Figure 24: LC-MS chromatogram of the isomerization of ketone **156** to ketone **159** (UV: 220 nm). In the upper chromatogram the crude ketone after oxidation is shown (20.5 min). Slight isomerization visible (21.1 min) presumably due to the slightly basic conditions of the Ley Griffith oxidation. During isomerization with NEt_3 in DCM/*i*PrOH, an equilibrium is reached (lower chromatogram). An increase in decomposition product is visible (19.8 min) The oxidation had therefore to be monitored carefully. Gradient: 50 % to 100 % MeCN in 15 min then 10 min 100 % MeCN, 8 mL/min.

The ketone **159** was still sensitive and column chromatography resulted in partial decomposition. However, it could be isolated by HPLC in and characterized. Treating ketone **159** with $NaBH_4$ resulted in the formation of the desired alcohol **113** as the main product. Nevertheless, the axial isomer **160** was also observed (ratio **113**:**160** = 4:1) (Scheme 33). The diastereoselectivity was drastically improved when applying CBS-reduction conditions with (*S*)-(-)-2-methyl-CBS-oxazaborolidine as catalyst.[176] In this case the formation of axial alcohol **160** was not observed. This allowed interconversion of the ido derivative **112** into gluco derivative **113** in a yield of 74 % over 3 steps solving the main problem in the synthesis of 5a-modified carba-derivatives of glucosamine.



Scheme 33: Reduction of ketone **159** with $NaBH_4$ resulted in the formation of **113** as the main product but also **160** as a side product The selectivity was greatly improved by applying CBS reduction conditions which led to total conversion to the desired product **113**.

With this sequence of oxidation, isomerization and diastereoselective reduction, this work provides an efficient and reliable synthetic route towards the key intermediate **113** with a yield of 2.3 % in 20 steps starting from GlcNAc (Scheme 34). This represents an average yield of over 80 % per step and allowed the preparation of **113** on a multigram scale in one batch. Previously only milligram amounts of 113 were available. This ultimately paved the way for the multitude of novel derivatizations described in the following chapters.



Scheme 34: Optimized synthesis of key intermediate **113** with the new synthesis route **113** is accessible in a yield of 2.3 % over 20 steps. It was possible to obtain **113** on a gram scale in one batch.

4.5 Synthesis of a Carba-Glucosamine Library – Derivatization of the 5a-Position

4.5.1 S_NAr Reaction Allows Access to 5a-Aryloxy Derivatives

Having provided reliable access to key intermediate **113,** the extension of the substitution spectrum from alkoxy substituents towards aryloxy substituents was considered. These derivatives are particularly interesting because it was postulated that the aryl moiety can intercalate in nucleobases forming the binding pocket of the *glmS* riboswitch (Figure 25). Another benefit of these less polar compounds is that they might be lipophilic enough to diffuse over the bacterial cellular barriers, therefore not relying on active transport over the cell wall. In this thesis, I wanted therefore to develop a synthesis of 5a-aryl modified carba-glucosamines to test these compounds for their antibacterial potential.

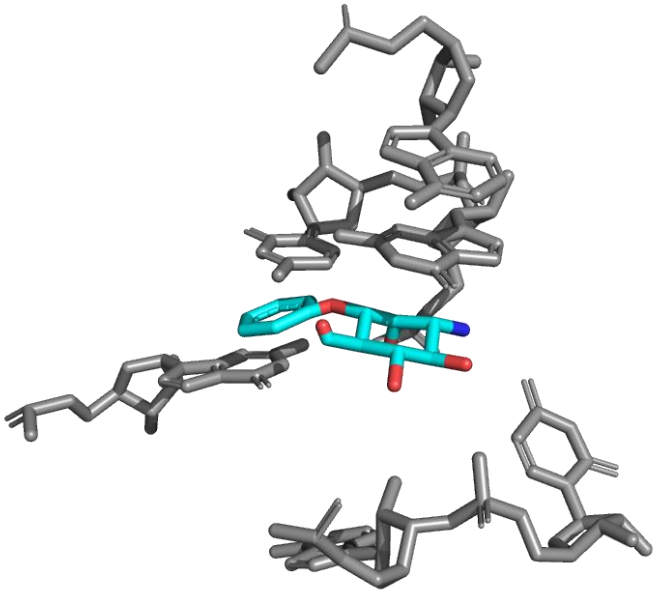
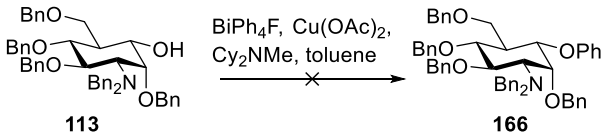


Figure 25: Envisioned binding of 5a-phenoxy modified carba-derivatives of GlcN to the *glmS* riboswitch. (PDB: 2NZ4)[55] The riboswitch binding pocket is depicted in grey. Carbon atoms of GlcN6P are lightblue, oxygen is red, nitrogen blue and. Hydrogen is not shown.

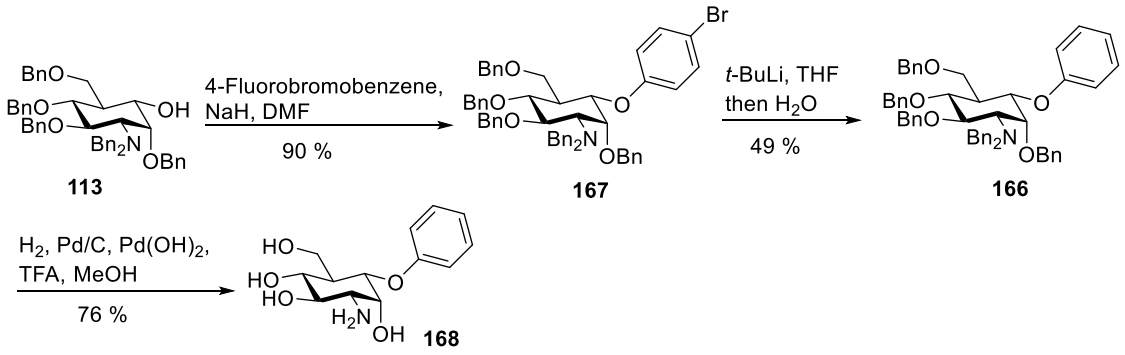
The direct synthesis of 5a-phenoxy modified carba-glucosamines directly from **113** is rather difficult because few reactions are known to convert an alcohol into a phenyl ether. *Kazuhiro* reported use of tetraphenylbismuth fluoride for

O-phenylation of cyclohexanol under cupper catalysis.[177] When applying the reported conditions to alcohol **113,** only traces of the desired phenoxy carba-sugar **166** were obtained (Scheme 35). The original publication reported the reaction only on very simple alcohols. The reaction might therefore not be applicable to the highly decorated alcohol **113.**



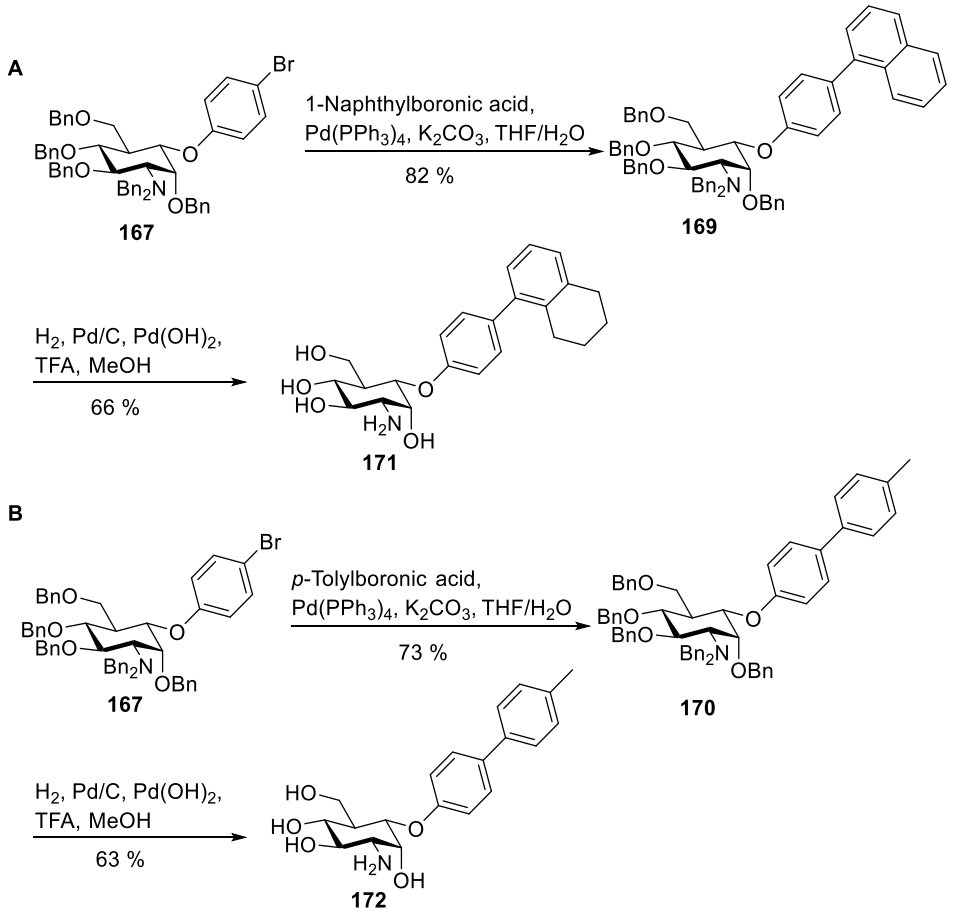
Scheme 35: Attempted derivatization of **113** using conditions reported by *Kazuhiro* for simpler alcohols.[177]

After this initial setback, another strategy was applied. **113** was reacted in an aromatic nucleophile substitution with 4-fluorobromobenzene to give compound **167** in a yield of 90 % (Scheme 36). This compound can be dehalogenated by treatment with *t*-butyl lithium followed by aqueous work up to give the phenoxy modified glucosamine mimic **166** in a yield of 49 %. After deprotection using Pd(0) on carbon and $Pd(OH)_2$ on carbon as catalysts in presence of trifluoroacetic acid, compound **168** was obtained in a yield of 76 %. This was achieved without affecting the aryl moiety in the 5a-position.



Scheme 36: S_NAr reaction of **113** with 4-fluorobromobenzene to give the compound **167**. Dehalogenation and hydrogenation resulted in the synthesis of the phenoxy modified carba-glucosamine **168**.

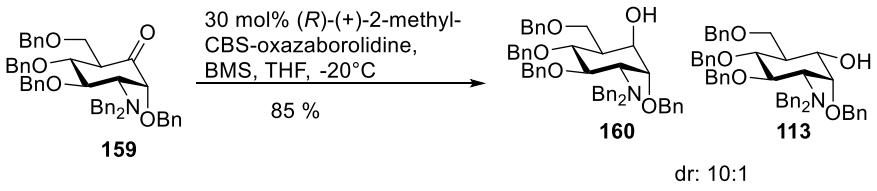
In addition to the synthesis of 5a-phenoxy modified carba-glucosamine, bromide **167** proved to be a valuable intermediate in the synthesis of even larger aromatic substituents. **167** reacted readily in a Suzuki-Miyaura cross coupling[178] with 1-naphthylboronic acid as well as *p*-tolylboronic acid (Scheme 37). The compounds **169** and **170** were obtained in yields of 82 % and 73 %, respectively. When these compounds were subjected to hydrogenation, compound **169** reacted to the tetrahydronaphthyl compound **171** in a yield of 66 %. Compound **170** was deprotected to obtain compound **172** in a yield of 63 %.



Scheme 37: **A:** Bromide **167** was converted by Suzuki-Miyaura cross coupling to the compound **169**. Hydrogenation afforded the aryl modified carba-glucosamine **171 B:** Bromide **167** was converted by Suzuki-Miyaura cross coupling to the compound **170**. Hydrogenation afforded the aryl modified carba-glucosamine **172**

4.5.2 Selective Access to Axial 5a-Hydroxy and 5a-Alkoxy Derivatives of Carba-glucosamine

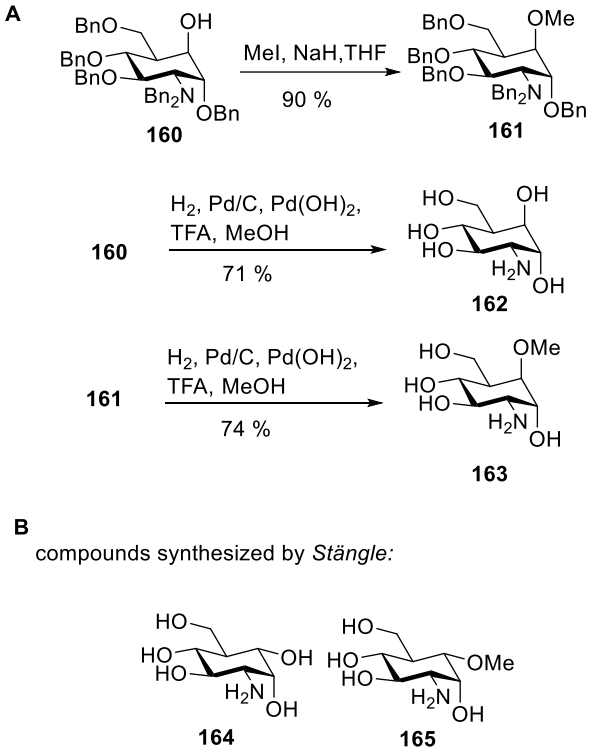
In addition to 5a-equatorial alcohol **113**, I was interested in selectively synthesizing axial alcohol **160** as well. This would allow a better understanding of the effect that the substituent in the 5a-position can have. This is especially interesting in regard to the antimicrobial properties of these compounds as well as the ability to activate the *glmS* riboswitch. The use of 10 mol% (*R*)-(+)-2-methyl-CBS-oxazaborolidine as catalyst to reduce the ketone **159** at room temperature led to compound **160** as the major isomer, however, with significant amounts of the equatorial isomer **113** in a ratio of 5:1. These two compounds were particularly difficult to separate chromatographically, which motivated further optimization of the diastereoselectivity. Finally, a diastereomeric ratio of 10:1 of **160/113** was achieved by using 30 mol% of (*R*)-(+)-2-methyl-CBS-oxazaborolidine[176] and performing the reduction at -20 °C in THF (Scheme 38). These conditions differ from the conditions applied for the selective synthesis of the equatorial alcohol **113**, here 10 mol% (*S*)-(-)-2-methyl-CBS-oxazaborolidine[176] were applied at room temperature (Scheme 33).



Scheme 38: Optimized conditions for the selective synthesis of **160.** 30 mol% of (*R*)-(+)-2-methyl-CBS-oxazaborolidine and reaction at -20 °C resulted in a 10:1 ratio of **160** to **113.**

Compound **160** was alkylated with methyl iodide to give the methyl ether **161** in a yield of 90 % (Scheme 39 A). The compounds **160** and **161** were subjected to debenzylation conditions using Pd/C and $Pd(OH)_2/C$ as catalyst under hydrogen atmosphere in methanol containing TFA. The deprotected 5a-axial hydroxy modified carba-glucosamine **162** as well as the 5a-axial methoxy modified carba-glucosamine **163** were obtained in a yield of 71 % and 74 %, respectively. These

compound are the 5a-epimers to the compound **164** and **165** synthesized by *Stängle* in his work (Scheme 39 B).[120]

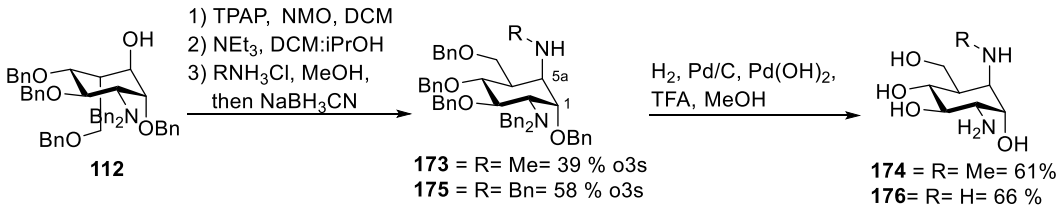


Scheme 39: **A**. Methylation of **160** resulted in methyl ether **161** which was also subjected to hydrogenation to give **163**. Hydrogenation of compound **160** gave **162 B** compound synthesized by *Stängle* in his thesis. **162** and **163** are the 5a-axial epimers of **164** and **165**.

4.5.3 Reductive Amination – Introducing Amines in the 5a-Position to Yield Carba-Glucose Diamines

After successfully establishing an efficient synthesis to hydroxy modified carba-glucosamines it was speculated if other protic group could also be introduced in the 5a-position. It was decided that amines are particularly of interest in this regard for their ability to form hydrogen bonds as well as the possibility to be involved in the acid base reaction that leads to the cleavage of the *glmS* riboswitch described in chapter 2.2. The isomerized ketone **159** offered an ideal starting material for a reductive amination. It was observed that the best results were achieved when not performing intermediary chromatographic work ups but instead performing the Ley Griffith oxidation of compound **112**, followed by isomerization to give **159.** The

desired amine hydrochloride, which was pre-dissolved in methanol, was added directly to the isomerization reaction after LC-MS analysis indicated that isomerization has reached the equilibrium. Addition of sodium cyanoborohydride afforded the desired amine in an axial orientation (Scheme 40). Remarkably, the bond between the 5-position and 6-position was solely found to be in the desired equatorial form. It was concluded that this configuration reacts faster with the amine leading to an equilibrium shift favoring the more reactive glucose form. The axially oriented amine can be explained by complexation of the soft reducing agent ($NaCNBH_4$) by the neighboring benzyl ether in the 1-position, leading to a directed reduction. It is notable that when amines were used instead of amine hydrochlorides, the yields were dramatically reduced. Moreover, application of other Brønsted acids or Lewis acids such as formic acids, acetic acid, titanium tetrachloride or titanium isopropoxide also led to decomposition of **159**. Using this strategy with methyl ammonium hydrochloride gave methylamine **173** in a yield of 39 % over 3 steps. This allowed the synthesis of the secondary amine **174** upon hydrogenation in a yield of 61 % (Scheme 40). Amination with benzyl ammonium hydrochloride gave compound **175** in a yield of 58 % over 3 steps. This compound was deprotected to the primary amine **176** upon hydrogenation in a yield of 66 %.

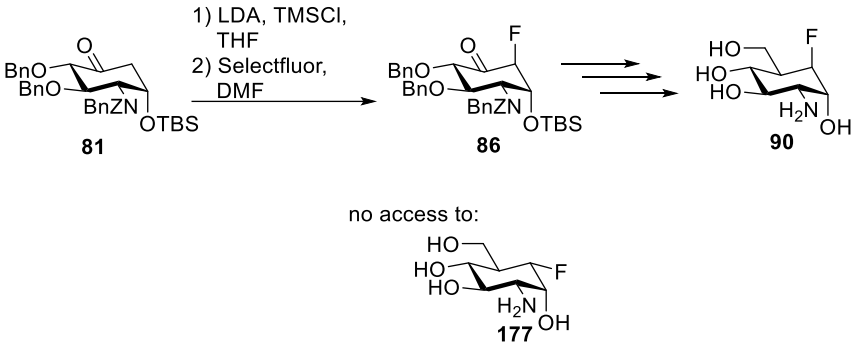


Scheme 40: Oxidation, isomerization and reductive amination of **112** gave the two protected amines **173** and **175**. They were deprotected to give the 5a-amine modified carba-glucose amines **174** and **176**.

4.5.4 Fluorination at the 5a-Position – Withdrawing Electrons from the Carba-Position

4.5.4.1 Monofluorination

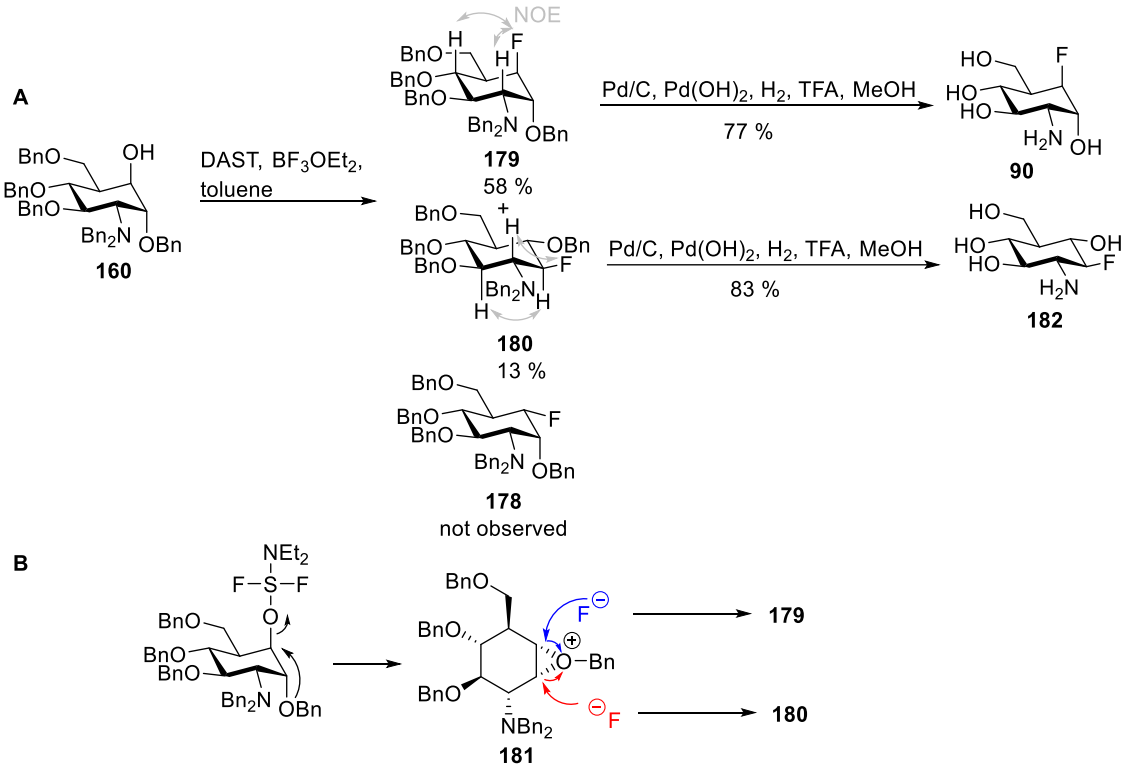
Matzner synthesized 5a-fluoro-carba-α-D-glucosamine **90** in his work (Scheme 41).[47,58] This compound showed promising activities as an antimicrobial agent. The corresponding 6-phosphate **90** was also shown to activate the *glmS* riboswitch. He was only able to develop a synthetic route to the axial fluoride **90**. The equatorial fluoride **117** was not accessed during his work. *Matzner* chose a route different from the approach presented in this work. He relied on the introduction of the fluoride via enol chemistry and Selectfluor® as an electrophilic fluoride source.[179] The synthetic route is described in more detail in chapter 2.3.



Scheme 41: Synthesis of **90** by *Matzner.* **177** was not accessible via the strategy used by *Matzner*.

Encouraged by the promising properties of **90**, I envisioned the synthesis of the complementary diastereomer **177** starting from the axial alcohol **160** via deoxyfluorination (Scheme 42). It was postulated that the deoxyfluorination would proceed, at least partly in an S_N2 fashion resulting the in equatorial fluoride **178**. Upon treatment of **160** with DAST in DCM, the formation of two compounds of equal mass was observed by LC-MS. The crude ^{19}F-NMR did also show two distinct signals. After HPLC separation of the two compounds, following NMR analysis using NOESY and ^{19}F-HOESY spectra revealed that none of the isolated compounds was the desired compound **178**. Instead, the major compound was identified as axial fluoride **179** and the minor compound as rearranged compound **180**. The formation

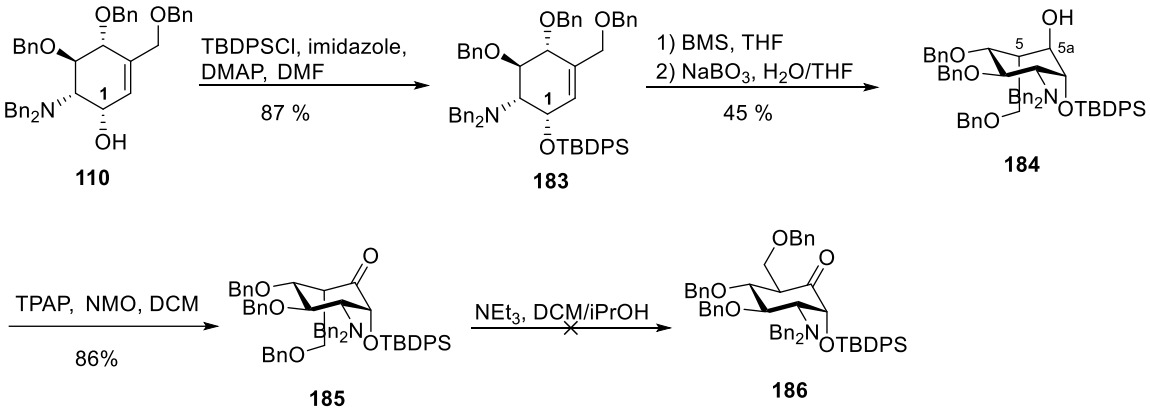
of these two compounds hints to the formation of oxonium ion **181** during the reaction as an intermediate, resulting in either retention of stereochemistry at the 5a-position (compound **180**, yield: 58 %) or migration of the benzyl ether (compound **181**, yield: 13 %) (Scheme 42). Both compounds were isolated and deprotected via hydrogenation to yield the compounds **90** and **182**.



Scheme 42: **A** Deoxyfluorination of compound **160** did not result the formation of compound **178** instead the axial fluoride **179** was observed as the major product and **180** as the minor product. **B** proposed mechanistic explanation for this diastereomeric outcome, the oxonium ion **181** can be opened from two direction giving either **179** or **180**.

It was tried to circumvent the formation of the oxonium ion by using different deoxygenation agents instead of DAST. None of the tested agents including, PyFluor®[155], pentafluorobenzene sulfonyl fluoride[155], Deoxo-Fluor®[156], Xtal-Fluor-M®[157], Xtal-Fluor-E®[157], and perfluoro-1-butanesulfonyl fluoride[180], did lead to formation of equatorial fluoride **178**. Another strategy used to circumvent this problem was the introduction of a different protecting group at the 1-position. It was decided to use TBDPS because of its relative stability towards DAST and its very bulky character. In order to test, whether the change of the

protecting group in the 1-position allows a deoxyfluorination without neighboring group participation, allylic alcohol **110** was converted to TPDPS ether **183** in a yield of 87 % (Scheme 43). With this compound a hydroboration was performed giving **184** as the only isolated product in a yield of 45 %. After oxidation to ketone **185** in a yield of 86 %, an isomerization at the 5-position, as previous established for benzyl ether **156**, was attempted. However, this strategy did not give isomerization of compound **185** to compound **186** (Scheme 43). Even though ultimately the synthesis of **178** was unsuccessful, this endeavor allowed for discovery of a new synthetic route towards the known antibiotic 5a-fluoro-carba-α-D-glucosamine **90** with a yield around 25 times higher than previously reported (synthesis starting from GlcNAc in this approach and from GlcN•HCl in literature[58]).

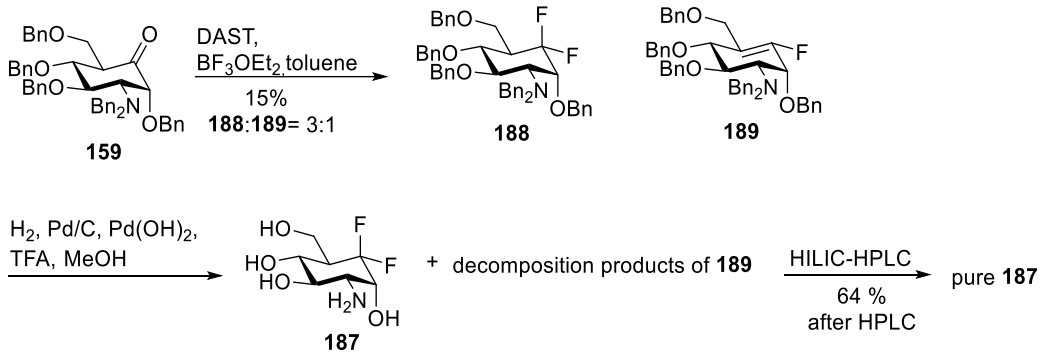


Scheme 43: Attempt to synthesize the isomerized TPDPS protected ketone **186.** The allyl alcohol was TBDPS protected to yield compound **183**. Hydroboration yielded the carba-ido derivative **184.** Oxidation yielded ketone **185**. Isomerization of the ketone to the gluco form **186** was unsuccessful.

4.5.4.2 Difluorination

In order to mimic the naturally occurring hemiacetal in glucosamine, introduction of a difluoro moiety in the 5a-position was envisioned. The electron withdrawing effect of the fluorine is reported to approach the properties of the 5a-CF_2 group to the natural occurring ring oxygen.[181] This functionality can be introduced by reacting a ketone **159** with DAST (scheme 44). For ketones, which have a trisubstituted alpha carbon this kind of conversion is reported to be difficult, suffering from rearrangement and elimination reaction as side reactions.[181]

Indeed, when treating ketone **156** with DAST, only traces of the difluoride were observed by ^{19}F NMR and LC-MS analysis in a very complex mixture. *Deleuze et al.* reported the synthesis of gem-difluorocarba-D-glucose via a triisobutylaluminium-promoted rearrangement, here the CF_2 group is initially introduced as the 1-*gem*-difluoroalkene[182] the compound undergoes a rearrangement to the 5a-*gem*-difluorocarba-sugar. It was found that in *gem*-difluorocarba-disaccharides the anomeric effect is restored.[183] This implied that gem-difluorocarba-D-glucosamine **187** might be a very promising mimic of glucosamine and therefore I was convinced that a synthesis of this derivative is desirable even with the above described complications. After extensive optimization of the conditions, ten equivalents of DAST, one equivalent of boron diethyl etherate in toluene in a PTFE reaction vessel gave 15 % of an inseparable 3:1 mixture of difluoride **188** and the elimination product **189** (Scheme 44). After hydrogenation of the benzyl protecting groups and the alkene in the side product **189**, pure difluoride **187** was isolated after HILIC-HPLC.

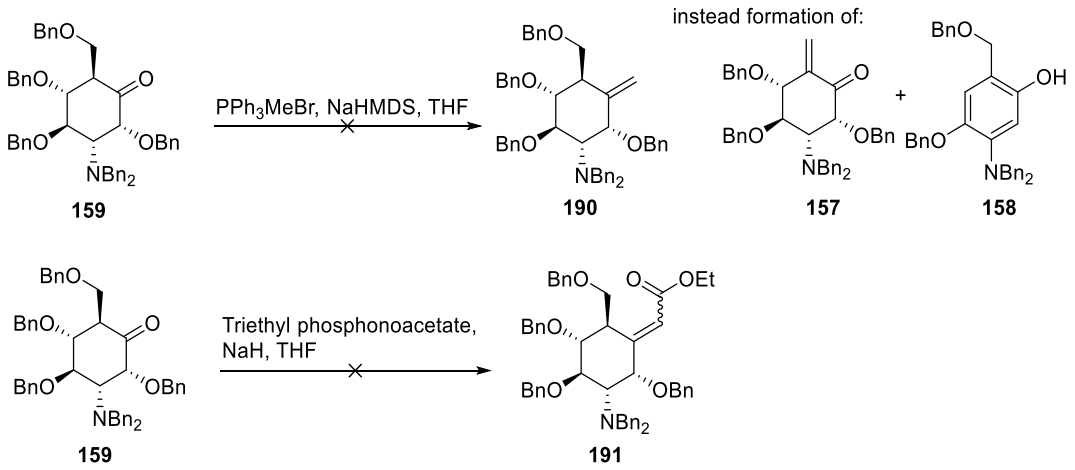


Scheme 44: Reaction of ketone **156** with DAST gave an inseparable 3:1 mixture of difluoride **188** and the elimination product **189**. Hydrogenation of the mixture of **188** and **189** gave **187** and decomposition products of **189.** Isolation of pure unprotected difluoride **187** was possible after hydrogenation and HILIC-HPLC.

4.5.5 Carbon-Carbon Bonds at the 5a-Position

4.5.5.1 Initial attempts using the Wittig and Horner–Wadsworth–Emmons reaction for Olefination in the 5a-Position

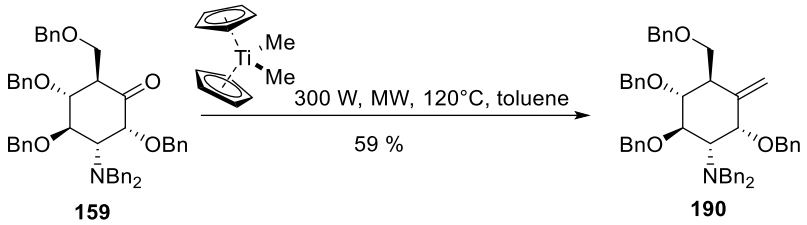
Another kind of modification that was envisioned was a C-C modification at the 5a-position. This would allow further modifications directly at the carba-position without needing a heteroatom as connecting atom. Initial attempts were made by reacting ketone **159** in a Wittig reaction with methyltriphenylphosphonium bromide (Scheme 45). However, only decomposition was observed. The formation of the elimination products **157** and **158** was observed by LC-MS. This is most likely the result of the high basicity of the formed ylide. No desired product **190** was isolated. The same outcome was observed when ketone **159** was reacted in a Horner-Wadsworth-Emmons reaction with triethyl phosphonoacetate.[184] Also in this case, the product **191** was not detected (Scheme 45). It was concluded that olefination reactions using basic conditions are not compatible with ketone **159**.



Scheme 45: Attempts at the formation of a carbon carbon bond at the 5a-position. Wittig conditions as well as Horner-Wadsworth-Emmons conditions lead to decomposition of the ketone **156**.

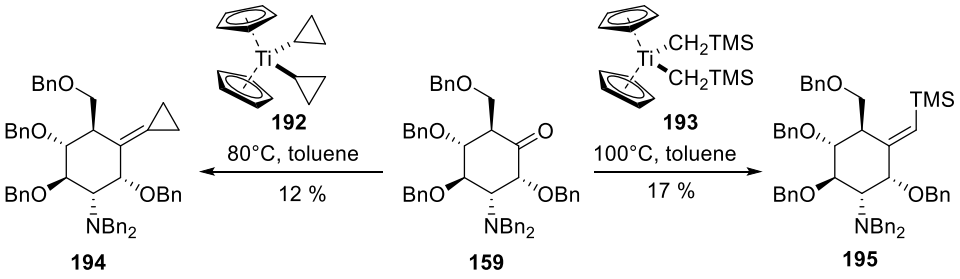
4.5.5.2 Petasis Reaction for 5a Olefin Synthesis

In order to circumvent the elimination during the olefination reaction, non-basic olefination conditions were desired. The Petasis reaction[185] was deemed promising for this endeavor. Indeed, after optimization of the reaction conditions, alkene **159** was obtained in a yield of 59 % using dimethyltitanocene and microwave irradiation (Scheme 46).



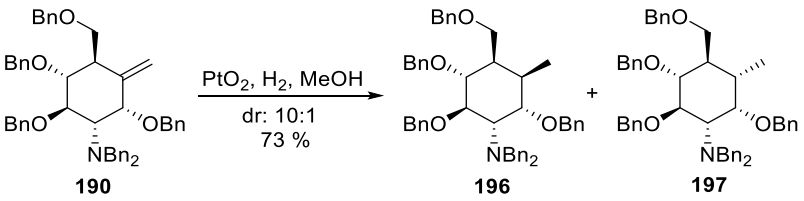
Scheme 46: Dimethyltitanocene was used for the olefination of **159** to the alkene **190** in a yield of 59 % using microwave irradiation.

In order to test the scope of the Petasis olefination[185] with ketone **159**, the two literature known reagents, biscyclopropyl titanocene **192** [186] and di(TMS-methyl) titanocene **193**[187] were synthesized. Like dimethyltitanocene, these reagents form a carbene upon heating and allow the introduction of more complex alkenes. With these reagents it was possible to access the two alkenes **194** and **195** (Scheme 47). However, it was only possible to isolate the desired product in diminished yields compared to the synthesis of compound **190**.



Scheme 47: Use of the more complex Petasis reagents bis cyclopropyl titanocene **192** and di(TMS-methyl) titanocene **193**. Synthesis of **194** and **195** was possible.

A major problem was encountered when compound **190** was subjected to hydrogenation conditions, which resulted in both, the cleavage of the benzyl ether, and reduction of the alkene. The resulting mixture of diastereomers was not separable by HILIC chromatography. To overcome this problem, it was attempted to reduce the alkene selectively in the presence of the benzyl protecting groups. Reductions using Crabtree catalyst,[188] Wilkinson catalyst[189] or Schrock-Osborn catalyst[190] did not proceed even at high hydrogen pressures of up to 90 bar. In literature poisoning of a Pd/C catalyst is reported, in order to suppress benzyl ether cleavage while retaining the potential to reduce alkenes. This was done by addition of triethylamine, pyridine or various ammonium salts.[191] However, it appeared difficult for substrate **190**, possibly because of the multitude of benzyl ethers. These conditions resulted in a complex reaction mixture and the desired compounds comprising an exocyclic methyl group was not isolated. Finally, it was possible to reduce the alkene using PtO_2 under hydrogen atmosphere while the benzyl ethers remained mostly intact (Scheme 48). This resulted in an inseparable mixture of the two protected diastereomers **196** and **197** in a ratio of 10:1 and a yield of 73 %.

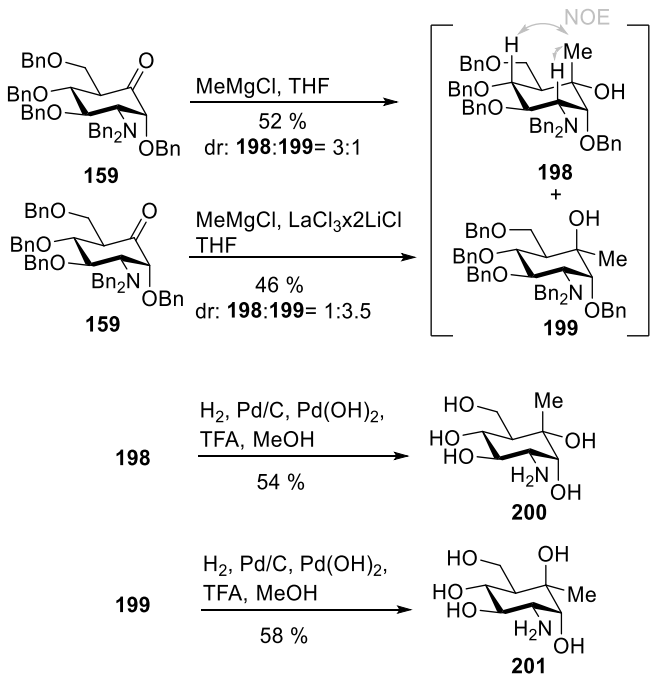


Scheme 48: Chemoselective hydrogenation of the exomethylen group in the presence of benzyl ether protective group resulting in a 10:1 mixture of **196** and **197** in a yield of 73 %

4.5.5.3 Nucleophilic Attack at the 5a-Carbonyl – Grignard and Ruppert-Prakash Reaction with Subsequent Deoxygenation

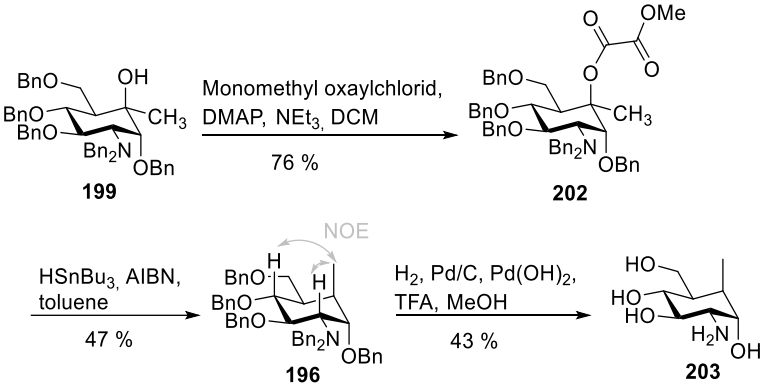
Parts of the synthetic work described in this chapter were performed by bachelor student *Philip Eppelin* under my supervision.

In parallel to the above described approach to C-C modified carba-sugars, I was interested in synthesizing disubstituted 5a-carba-glucosamines. To achieve this, methyl magnesium chloride was used to attack the carbonyl moiety in compound **159**, resulting in a separable 3:1 mixture of compound **198** and **199** in a yield of 52 % (Scheme 49). Adding $LaCl_3x2LiCl$ to ketone **159** followed by the addition of the Grignard reagent MeMgCl resulted in an inversion of the stereochemical outcome and a 1:3.5 mixture of **198** and **199** was obtained in a yield of 46 %. Without the addition of $LaCl_3x2LiCl$, the Grignard reagent attacks from the sterically less hindered side resulting in **198** as the major product. The unprotected disubstituted carba-sugars **200** and **201** were obtained in a yield of 54 % and 58 % respectively upon hydrogenation (Scheme 49).



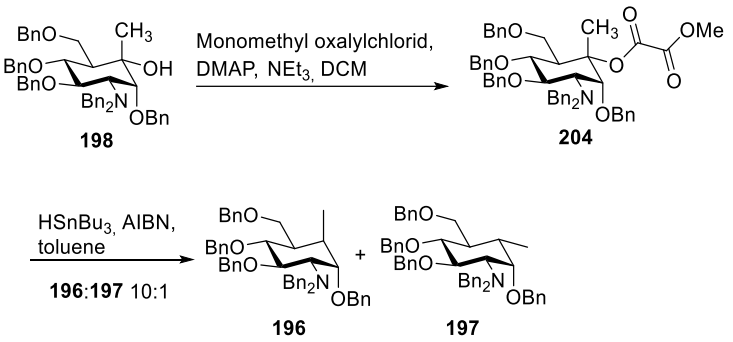
Scheme 49: Grignard reaction of ketone **159** with MeMgCl gave **198** and **199**. The diastereomeric ratio depended on addition of $LaCl_3x2LiCl$. Hydrogenation gave the two disubstituted carba-sugars **200** and **201**.

Compound **198** and **199** were considered a promising starting material for deoxygenation to obtain an 5a-alkyl-modified glucosamine mimetic, which represents an alternative to the synthetic strategy described in chapter 4.5.5.2. This proved difficult as only traces of the desired product were observed by LC-MS analysis when xanthogenates were used for Barton-McCombie deoxygenation.[192] Other deoxygenation methods, reported by *Jang et al*. (using the trifluoroactetate and diphenylsilane)[193] and *Yasuda et al.* (using chlorodiphenylsilane and catalytic amounts of $InCl_3$)[194] did not provide access to methyl carba-glucosamine **196** either. After intensive screening, successful deoxygenation conditions were established. Converting the tertiary alcohol to methyl oxalic acid ester **202** in a yield of 76 % followed by a radical deoxygenation employing $HSnBu_3$ and AIBN, enable the synthesis of the axially methyl modified compound **196** in a yield of 47 % (Scheme 50). Surprisingly, this synthesis only provided one diastereomer, solving the previously described chromatography problem for compounds **196** and **197**. Unprotected compound **203** was obtained after hydrogenation in a yield of 43 %.



Scheme 50: Axial alcohol **199** was converted to the oxalic acid ester **202** and a reductive desoxygenation was performed giving **196** as a single diastereomer in a yield of 47 %. Deprotection via hydrogenation gave the free methyl modified carba-sugar **203** in a yield of 43 %.

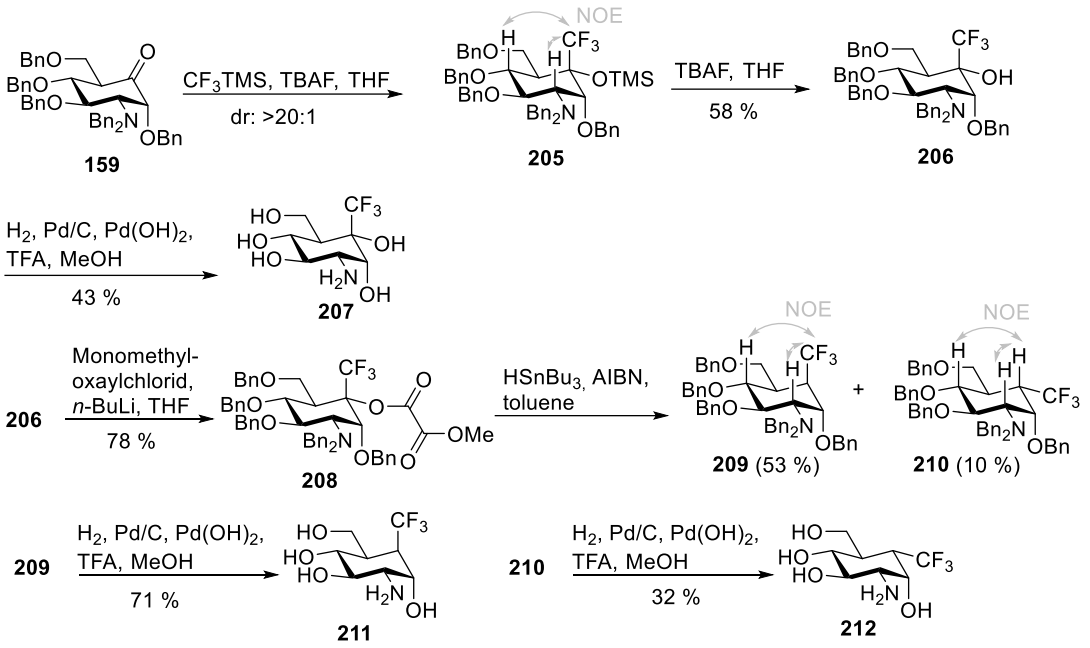
Conversion of the equatorial alcohol **198** to the corresponding methyl oxalic acid ester **202** followed by deoxygenation conditions resulted in a 10:1 mixture of **196** and **197** (scheme 51). This resulted in the same chromatographic problems as described for the hydrogenation of alkene **190**. Therefore, this approach was not further pursued (Scheme 51).



Scheme 51: Equatorial alcohol **198** was converted to the oxalic acid ester **204** and a reductive deoxygenation was performed giving a mixture of **196** and **197**

In order to introduce electron withdrawing groups other than fluorine, I was interested in the introduction of electron withdrawing groups in the 5a-position. A trifluoromethyl group was deemed to be an interesting target to compare it to the methyl group present in compound **199**. The introduction of the trifluoromethyl functionality was achieved by treatment of ketone **159** with CF_3TMS (Ruppert-Prakash reagent)[195] and catalytic amounts of TBAF (Scheme 52). These conditions gave TMS-ether **205** in situ, this compound can be isolated but best result were obtained by direct conversion to the alcohol **206**. TMS deprotection with an excess of TBAF yielded trifluoromethyl alcohol **206** in a yield of 58 %. The benzyl protecting groups were cleaved upon hydrogenation yielding compound **207** in a yield of 43 %.

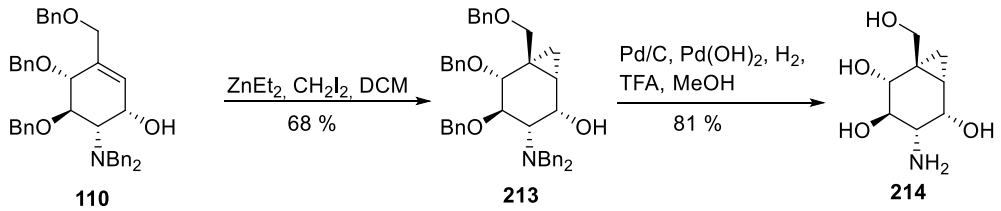
In a manner analogous to the deoxygenation described for the methyl alcohol **199**, compound **206** was converted to methyl oxalic acid ester **208** in 78 % yield (Scheme 52). Deoxygenation with $HSnBu_3$ and AIBN successfully provided diastereomer **209** in a yield of 53 % and diastereomer **210** in a yield of 10 %. The diastereomeric ratio of **209** and **210** was 5:1. The stereochemistry was determined for both derivatives by NOESY and ^1H-^{19}F-HOESY NMR spectroscopy. These compounds were benzyl deprotected and the 5a-trifluoromethyl substituted carba-glucosamines **211** & **212** were isolated in a yield of 71 % and 32 %, respectively.



Scheme 52: Trifluoromethylation was successfully achieved by treatment of the ketone **159** with CF_3TMS and catalytically amounts of TBAF. Hydrogenation afforded the free CF_3-carba-glucosamine **207**. The trifluoromethyl alcohol **206** was converted to the methyl oxalic acid ester and reductive deoxygenation yielded the compounds **209** and **210**. These compounds were deprotected, resulting in the free trifluoromethyl carba-sugars **211** and **212**.

4.5.6 Cyclopropanation – Bicyclic Carba-Glucosamine Mimics

Most of the derivatives synthesized in this thesis used ketone **159** or alcohols **113** and **160** as the starting material. However, these compounds are not the only possible precursors for novel carba-glucosamine mimics. To demonstrate this, I decided to directly modify the alkene present in precursor **110** (Scheme 53). This allylic alcohol is an ideal substrate for a Simmons Smith cyclopropanation.[196] It was postulated that the hydroxy group would lead to a precomplexation of the cyclopropanation agent, giving the stereochemistry as in a glucose at the 5-position. Indeed, upon reaction of **110** with diethylzinc and diiodomethane, cyclopropane **213** was synthesized as the only isolated product in a yield of 68 %. The cyclopropane ring was stable under hydrogenation conditions for benzyl deprotection. Thus, compound **214** was isolated in a yield of 81 %.

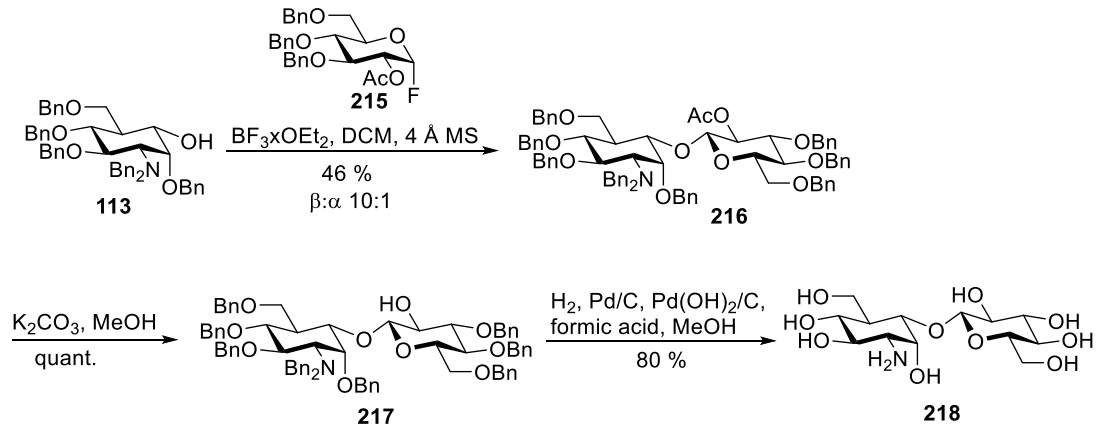


Scheme 53: The previously described allyl alcohol **110** was transformed in the cyclopropane **211**. After hydrogenation the bicyclic carba-glucosamine **212** was obtained.

4.5.7 1,5a-Pseudo-Disaccharide – Glycosylation at the 5a Position

To fully show the scope of possible 5a-modifications, glycosylation of carba-glucosamine to a pseudo-disaccharide was desired. This expands the known glyosidic bonds by new 1,5a-disaccharides, giving access to interesting new structural motives. To this end, the equatorial 5a-alcohol **113** was used to screen different glycosylation methods. The glycosylation reaction turned out to be very challenging. Reaction with anomeric acetates as glycosyl donors under Lewis acid catalysis gave only trace amounts of disaccharide. The same was observed when anomeric bromides were used as glycosyl donors under Koenigs-Knorr conditions.[197] Thioglycosides activated by *N*-iodosuccinimide yielded also only traces of a 1,5a-disaccharide. Trichloroacetimidates [198] were screened as donors as

well but only up to 10 % of 1,5a-pseudo-disaccharide formation was observed by LC-MS analysis. Finally, the use of glycosyl fluorides gave satisfying results (Scheme 54).[199] Glycosyl fluoride **215** was reacted with alcohol **113** and boron trifluoride etherate as activator. This gave the β-1,5a-pseudo-glycoside **216** in a yield of 46 %. The reaction showed a high β-selectivity due to the neighboring acetate, namely a diastereomeric ratio of α:β 1:10. The 1,5a-pseudo-disaccharide was deacetylated with potassium carbonate in MeOH to give compound **217** in quantitative yields. Subsequent hydrogenation of benzyl protecting groups gave the unprotected β-1,5a-pseudo-disaccharide **218** in a yield of 80 %.



Scheme 54: Preparation of β-1,5a-glycoside **216.** Equatorial 5a-alcohol **113** was reacted with **213**. Subsequent deprotection steps yielded free carba-disaccharide **216**.

4.5.8 Assessment of Antimicrobial Properties of the New 5a-Derivatives

Antimicrobial assays were performed by *Dennis Kläge* from the research group of Prof. *Hartig.*

Carba-glucosamine mimics, **90**, **162**, **163**, **168**, **171**, **172**, **174**, **176**, **182**, **187**, **198**, **201**, **203**, **207** and **214** were tested for their antibiotic properties in a filter disk assay against *B. subtilis* (Results for selected compounds are shown in Figure 26). These tests were performed to estimate the antimicrobial potential. In these assays a substance is applied on a filter disk. This substance diffuses through the agar plate. If an antimicrobial effect is exhibited by the substance a zone of inhibited bacterial grow is visible around the filter disk. Glucosamine was used as a negative control

and Chloramphenicol (9.3 mM) was used as a positive control. For the mono-fluoride **90** an inhibitory effect was observed, which is in line with the preciously published results.[58] For the 5a-aryloxy substituted carba-glucosamine mimics **171** and **172** an inhibitory effect was observed as well. This growth inhibition is an encouraging sign that this class of compounds can represent a lead structure for further development of new antibiotics. For the other tested compounds, no inhibition was observed.

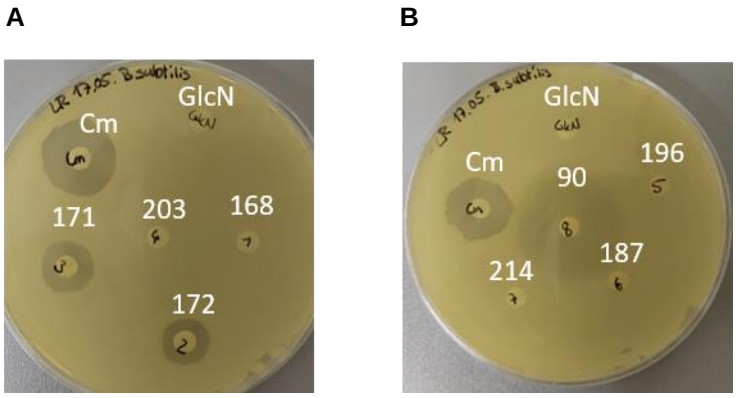


Figure 26: Filter disk assay of selected glucosamine mimics. Compounds **90**, **171** and **172** show an inhibitory effect against *B. subtilis.* Chloramphenicol (Cm) was applied as a positive control. If an antimicrobial effect is exhibited a zone of inhibition is visible around the filter disk with the applied substance.

5 Summary

The synthesis of GlcN6P mimics is of interest because these artificial compounds are potential *glmS* riboswitch activators and are to be considered as potential antibiotics with a novel mode of action. The available design space for new *glmS* activators is very limited, as only two positions for modification have been shown to be viable. Exchange of the ring oxygen as well as introduction of phosphonates as phosphatase-inert mimics are interesting modifications. The high density of functional groups and stereocenters within these compounds renders the selective access to these positions into a synthetic challenge. Within the scope of this work, a total of 23 new and several literatures known GlcN and GlcN6P derivatives have been synthesized These mimics are potential riboswitch activators and have been tested for their antibiotic properties. The diverse library includes three classes of derivatives, which allow the investigation of the structure-activity relationship of these mimics.

Thia-glucosamine-6-phosphate **127** explores the substitution of the ring oxygen present in GlcN6P with a sulfur giving a hemithioacetal (Figure 27 A). The synthesis of thia-glucosamine-6-phosphate **127** was already subject of a study performed in the *Wittmann* group. It turned out that established procedures for the introduction and subsequent deprotection of 6-phosphates were not applicable to the hemithioacetal, as this functionality acts as a catalyst poison for the transition metal catalysts required for the deprotection. Therefore, I established a procedure for the introduction and subsequent deprotection without the need of metal catalysts. This resulted in a reliable 4 step procedure for the conversion of the unprotected thia-sugar **126** to thia-GlcN6P **127** in a yield of 43 %. Thia-GlcN6P **127** turned out to be a very potent activator of the self-cleavage reaction of the *glmS* riboswitch (Figure 27). Kinetic studies were performed in cooperation with *Dennis Kläge* (research group of Prof. *J. Hartig,* University of Konstanz). These studies revealed that Thia-GlcN6P **127** is an activator as potent and as the natural ligand GlcN6P. Moreover, the unphosphorylated derivative **126** was shown to inhibit the growth of *B. subtilis* and *B. thuringiensis.* Although activation of the riboswitch is only one explanation for the antibiotic properties of **126**, the fact that the exchange of the ring oxygen to

sulfur in GlcN6P yields a new antibiotic is of interest for the development of a novel class of antimicrobial agents.

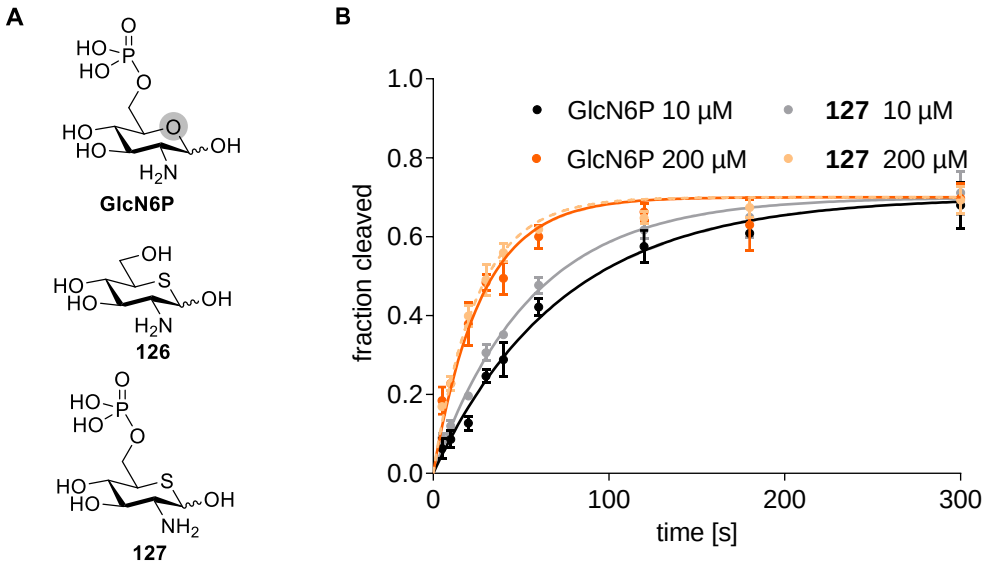


Figure 27: **A** Exchange of the ring oxygen to another chalcogen resulted in thia-GlcN **126**, which was synthesized and converted to thia-GlcN6P **127**. **B** Compound **127** was shown to be an activator of the *glmS* riboswitch, as potent as GlcN6P.

The next class of derivatives that were investigated included novel phosphate surrogates of GlcN6P (Figure 28). Methylene phosphonate **12** is a literature known phosphate mimic of GlcN6P. The ability of this compound to induce self-cleavage of the *glmS* riboswitch is diminished when compared to the natural ligand GlcN6P. In the literature, this discrepancy is explained by the different pK_{a2} values of the phosphonate present in compound **12** and the phosphate in GlcN6P. Therefore, I decided to synthesize a library of modified phosphonates, in order to mimic the pK_{a2} value of GlcN6P and investigate the scope of phosphate surrogates as *glmS* activators. I synthesized the difluoro phosphonate **134**, as well as the two diastereomeric hydroxy phosphonates (*S*)-**137** and (*R*)-**137.** Providing access to the two diastereomeric monofluoro phosphonates (*S*)-**139** and (*R*)-**139** turned out to be challenging, as one of the precursors decomposed during the deoxyfluorination. However, the proton alpha to the phosphonate proved to be acidic enough to allow for an isomerization under basic conditions followed by acidic work up. This procedure gave access to both diastereomers of the monofluoro phosphonate, (*S*)-**139** and (*R*)-**139.** The pK_{a2} values of these compounds have been determined by

^{31}P NMR titration (Figure 28). The difluoro phosphonate **134** was shown to be more acidic than GlcN6P, monohydroxy phosphonate (*R*)-**137**, and presumably its isomer (*S*)-**137,** are less acidic, and the monofluoro phosphonate (*S*)-**139**, and presumably its isomer (*R*)-**139**, have a pK_{a2} value comparable to the one of GlcN6P. When cleavage assays with the *glmS* riboswitch were performed, difluoro phosphonate **134** was observed to not induce self-cleavage of the riboswitch. Monohydroxy phosphonates (*S*)-**137** and (*R*)-**137** and monofluoro phosphonates (*S*)-**139** and (*R*)-**139** are capable to activate the riboswitch. However, none of them show faster reaction kinetics than the methylene phosphonate **12.** Consequently, adjustment of the pK_a value of the phosphonate to the pK_a value of the natural ligand GlcN6P does not result in an activator of the *glmS* riboswitch as potent as the natural ligand. The diminished activity of the phosphonates can therefore not be explained by their pK_a value. The hydrogen bond towards the oxygen connecting the phosphate to C-6 of the carbohydrate might therefore be more important than previously estimated. Absence of these hydrogen bonds in the phosphonates might contribute to their diminished ability to induce the self-cleavage of the *glmS* riboswitch when compared to GlcN6P. This insight into the binding requirements of the *glmS* riboswitch is important to keep in mind for future design of *glmS* riboswitch activators.

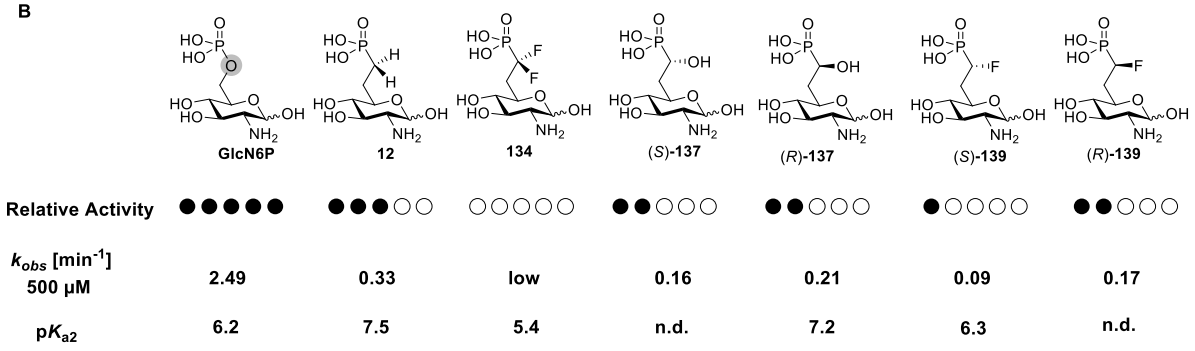


Figure 28: Synthesized phosphonate mimics of GlcN6P with varying pK_a values to investigate the influence of the pK_{a2} on the ability to induce the self-cleavage of the *glmS* riboswitch.

Previous studies performed in the *Wittmann* group established access to 5a-hydroxy-carba-α-D-glucosamine and 5a-alkoxy-carba-α-D-glucosamines However, the amount of synthesized derivatives was limited to only three compounds. The synthesis of compounds with more complex substitutions in the

5a-position was impeded by a low yielding synthetic route. Within this work, the deficiencies of the existing synthesis strategy were overcome, resulting in an improved and robust synthesis route giving access to a large library of 5a-modfied-carba-α-D-glucosamines (Figure 29).

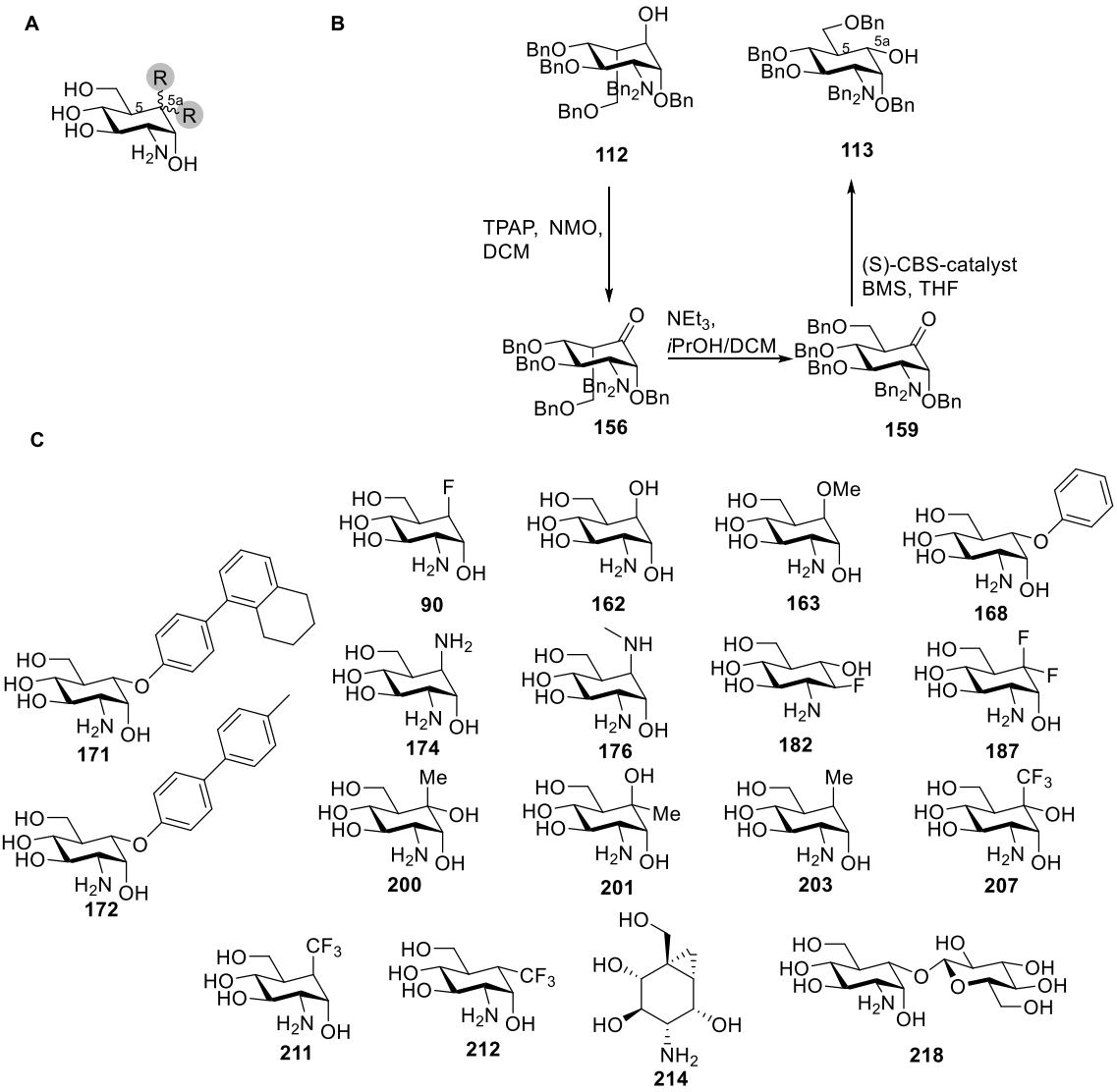


Figure 29: **A** The 5a-position of carba-sugars offers unexploited room for derivatization. **B** Preparation of large amounts of the key intermediate **113** was achieved by a developed isomerization procedure. **C** Library of 5a-modified carba-glucosamines synthesized in this work.

One main drawback of the previous synthesis was a hydroboration step that yielded the wrong diastereomer. Ido derivative **112** was obtained as the main derivative and the desired gluco derivative **113** was only obtained as a side product. This problem was addressed by establishing a reliable and efficient way to convert the undesired compound **112** into key intermediate **113** in a yield of 74 % over 3 steps.

This was achieved by realizing that compound **113** represents the thermodynamically more stable form, allowing equilibration to the equatorially oriented 5-position in equilibrium (Figure 29 B). After oxidation of the 5a-alcohol **112** to the ketone **156**, mildly basic conditions resulted in the isomerization of the 5-position favoring the gluco form **159**. Stereoselective reduction of the ketone gave the key intermediate **113**. After addressing some, in comparison smaller issues, the synthesis route was converted into a highly reliable and high yielding synthesis of key intermediate **113** in a yield of 2.3 % over 20 steps corresponding to an average yield of over 80 % per step. The route can be performed on a scale that delivers 5 g of the desired compound **113** in one batch. Furthermore, the provided abundance of key intermediate **113** made the synthesis of a large library of 5a-modified-carba-α-D-glucosamines possible (Figure 29 C). This library offers the largest variation of 5a-modified carba-sugars reported to date, with the majority of 5a-decorations being introduced for the first time to any kind of a carba-sugar. Apart from the literature known fluoro carba-sugar **90**, the library includes 5a-axially substituted hydroxy and alkoxy derivatives **162** and **163**. Phenoxy derivatives and larger aromatic substituents as **168**, **171** and **172** have been synthesized by an S_NAr reaction followed by Suzuki-Miyaura coupling or dehalogenation. Diamine derivatives **174** and **176** are also included in the library. To better mimic the electronic properties of the naturally occurring hemiacetal, difluorination at the 5a-position was performed, resulting in **187.** The 5a-position was further decorated with carbon-carbon bonds resulting in 5a-modified-carba-glucosamine mimics **200**, **201**, **203** as well as the trifluoromethyl derivatives **207**, **211**, **212,** and the bicyclic compound **214.** To show the potential of these 5a-modified-carba-glucosamines, they were used as glycosyl acceptors and with compound **218** the first ever synthesized 1,5a-pseudo-disaccharide is included in this library All compounds were screened for their antibacterial potential. The 5a-aryloxy-carba-α-D-glucosamines **170** and **171** showed growth inhibition against *B. subtilis.* Therefore, **170** and **171** can be considered as promising lead compounds for further development of GlcN mimics as antibiotics.

6 Zusammenfassung

Die Synthese von GlcN6P-Mimetika ist von Interesse, da diese Verbindungen potenzielle *glmS*-Riboswitch-Aktivatoren sind und als potentielle Antibiotika mit neuen Wirkungsmodus in Betracht gezogen werden sollten. Der verfügbare Spielraum für Modifizierungen für neue *glmS*-Riboswitch Aktivatoren ist sehr begrenzt, da sich nur zwei Positionen für Veränderungen als zielführend herausgestellt haben. Der Austausch des Ringsauerstoff sowie die Einführung von Phosphonaten als Phosphatase-inerte Mimetika sind interessante Modifikationen. Die hohe Dichte an funktionellen Gruppen und Stereozentren innerhalb dieser Verbindungen macht den selektiven Zugang zu diesen Positionen zu einer synthetischen Herausforderung. Im Rahmen dieser Arbeit wurden insgesamt 23 neue und mehrere literaturbekannte GlcN- und GlcN6P-Derivate synthetisiert. Diese Mimetika sind potenzielle *glmS*-Riboswitch-Aktivatoren und wurden auf ihre antibiotischen Eigenschaften getestet. Die Bibliothek umfasst drei Klassen von Derivaten, die es ermöglichen, die Struktur-Wirkungs-Beziehung dieser Mimetika zu untersuchen.

Thiaglucosamin-6-phosphat **127** zeigt die Substitution des im GlcN6P vorhandenen Ringsauerstoffes durch Schwefel auf, wodurch ein Hemithioacetal entsteht (Abbildung 30 A). Die Synthese von Thiaglucosamin-6-Phosphat **127** war bereits Gegenstand einer Studie in der Arbeitsgruppe Wittmann. Es stellte sich heraus, dass etablierte Verfahren zur Einführung und anschließenden Entschützung von 6-Phosphaten für das Hemithioacetal behindert wurden, da diese Funktionalität als Katalysatorgift für die Metallkatalysatoren fungiert, die für die Entschützung erforderlich sind. Daher habe ich ein Verfahren zur Einführung und anschließenden Deprotection ohne die Notwendigkeit von Metallkatalysatoren etabliert. Dies führte zu einem zuverlässigen 4-Schritt-Verfahren zur Umwandlung des ungeschützten Thiasugars **126** in Thia-GlcN6P **127**, in einer Ausbeute von 43%. Thia-GlcN6P **127** stellte sich als sehr potenter Aktivator der Selbstspaltungsreaktion des *glm*S-Riboswitches heraus (Figure 30). Kinetische Studien wurden in Zusammenarbeit mit Dennis Kläge (Forschungsgruppe von Prof. J. Hartig, Universität Konstanz) durchgeführt. Diese Studien zeigten, dass Thia-GlcN6P **127** ein ebenso potenter

Aktivator des *glmS*-Riboswitches ist, wie der natürliche Ligand GlcN6P. Darüber hinaus wurde gezeigt, dass das unphosphorylierte Derivat **126** das Wachstum von *B. subtilis* und *B. thuringiensis* hemmt. Obwohl die Aktivierung des Riboswitches nur eine Erklärung für die antibiotischen Eigenschaften von **126** ist, ist die Tatsache, dass der Austausch des Ringsauerstoffes durch Schwefel ein neues Antibiotikum ergibt, von Interesse für die Entwicklung einer neuen Klasse antimikrobieller Wirkstoffe.

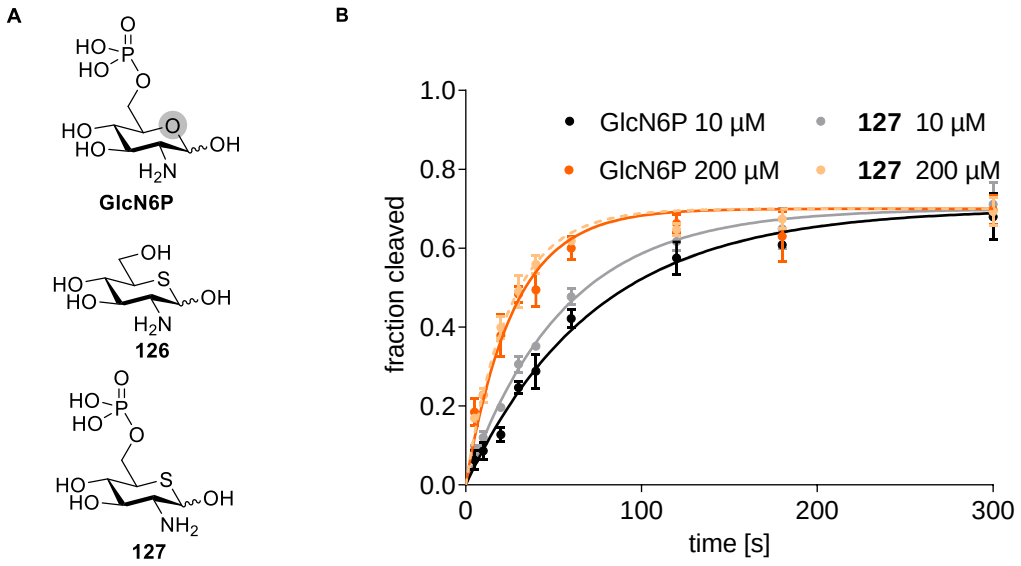


Figure 30: **A** Ein Austausch des Ringsauerstoffs zu einem anderen Chalkogene führte zu Thia-GlcN **126**, das synthetisiert und in Thia-GlcN6P **127** umgewandelt wurde. **B** Die Verbindung **127** zeigte sich als ein ebenso guter *glmS* Riboswitchaktivator wie der natürliche Ligand GlcN6P.

Die nächste Klasse von Derivaten, die untersucht wurden, umfasste neue Phosphat-Mimetika von GlcN6P (Abbildung 29). Methylenphosphonat **12** ist ein aus der Literatur bekanntes Phosphat-Mimetikum von GlcN6P. Die Fähigkeit dieser Verbindung, die Selbstspaltung des *glmS*-Riboswitchs zu induzieren, ist im Vergleich zum natürlichen Liganden GlcN6P vermindert. In der Literatur wird diese Diskrepanz durch die unterschiedlichen pK_{S2}-Werte des Phosphonats in Verbindung **12** und des Phosphats in GlcN6P erklärt. Daher entschied ich mich, eine Bibliothek modifizierter Phosphonate zu synthetisieren, um den pK_{S2}-Wert von GlcN6P nachzuahmen und die Anwendbarkeit von Phosphonaten als *glmS*-Aktivatoren zu untersuchen. Ich synthetisierte das Difluorphosphonat **134** sowie die zwei diastereomeren Hydroxyphosphonate (*S*)-**137** und (*R*)-**137**. Der Zugang zu den

beiden diastereomeren Monofluorphosphonaten (*S*)-**139** und (*R*)-**139** stellte sich schwierig heraus, da eines der Edukte bei der Deoxyfluorierung zersetzte. Allerdings erwies sich der Proton alpha zum Phosphonat als sauer genug, um unter basischen Bedingungen eine Isomerisierung zu ermöglichen. Dieser Prozess ermöglichte den Zugang zu beiden Diastereomeren des Monofluorphosphonats, (*S*)-**139** und (*R*)-**139**. Die pK_{S2}-Werte dieser Verbindungen wurden mittels ^{31}P-NMR-Titration bestimmt (Abbildung 29 B). Das Difluorphosphonate **134** war saurer als GlcN6P, Monohydroxyphosphonat (*R*)-**137** und vermutlich sein Isomer (*S*)-**137** waren weniger sauer und das Monofluorphosphonate (*S*)-**139** und vermutlich sein Isomer (*R*)-**139** hatten einen pK_{S2}-Wert der mit GlcN6P vergleichbar ist. Bei den Spaltungsassays mit dem *glmS*-Riboswitch wurde festgestellt, dass das Difluorphosphonate **134** nicht die Selbstspaltung des Riboswitchs induziert. Monohydroxyphosphonate (*S*)-**137** und (*R*)-**137** sowie Monofluorphosphonate (*S*)-**139** und (*R*)-**139** sind in der Lage, den Riboswitch zu aktivieren. Allerdings zeigt keiner von ihnen schnellere Reaktionskinetiken als das Methylenphosphonate **12**. Daher führte die Anpassung des pK_{S2}-Werts des Phosphonates an den pK_{S2}-Wert des natürlichen Liganden GlcN6P nicht zu einem so leistungsfähigen Aktivator des *glmS*-Riboswitchs wie der natürliche Ligand. Die verminderte Aktivität der Phosphonate kann daher nicht auf ihren pK_{S2}-Wert zurückgeführt werden. Die Wasserstoffbrückenbindung zu dem Sauerstoff zwischen Phosphat und C-6 des Kohlenhydrats, könnte daher wichtiger sein, als zuvor angenommen. Das Fehlen dieser Wasserstoffbrückenbindungen in den Phosphonaten könnte zu ihrer verminderten Fähigkeit beitragen, die Selbstspaltung des *glmS*-Riboswitchs zu induzieren. Diese Erkenntnisse in die Bindungsanforderungen des *glmS*-Riboswitchs müssen bei der zukünftigen Entwicklung von *glmS*-Riboswitch-Aktivatoren berücksichtigt werden.

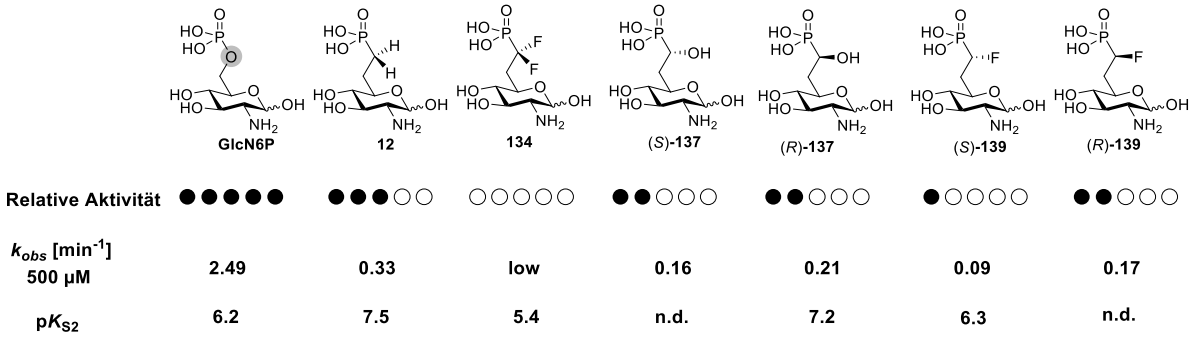


Figure 31: Synthetisierte Phosphonat-Mimetika von GlcN6P mit unterschiedlichen pK_S-Werten, um den Einfluss des pK_{S2} auf die Fähigkeit zur Selbstspaltungsreaktion des *glmS*-Riboswitches zu induzieren zu untersuchen.

Frühere Studien, die in der Forschungsgruppe von Prof. Wittmann durchgeführt wurden, etablierten den Zugang zu 5a-Hydroxy-Carba-α-D-Glucosamin und 5a-Alkoxy-Carba-α-D-Glucosaminen. Allerdings war die Menge der synthetisierten Derivate auf nur drei Verbindungen begrenzt. Die Synthese von Verbindungen mit komplexeren Substitutionen in der 5a-Position wurde durch eine synthetische Route mit niedrigen ausbeuten erschwert. Innerhalb dieser Arbeit wurden die Defizite der bestehenden Synthesestrategie überwunden, wodurch ein verbesserter und robusterer Syntheseweg entstand. Dieser liefert den Zugang zu einer großen Bibliothek von 5a-modifizierten Carba-α-D-Glucosaminen (Abbildung 30).

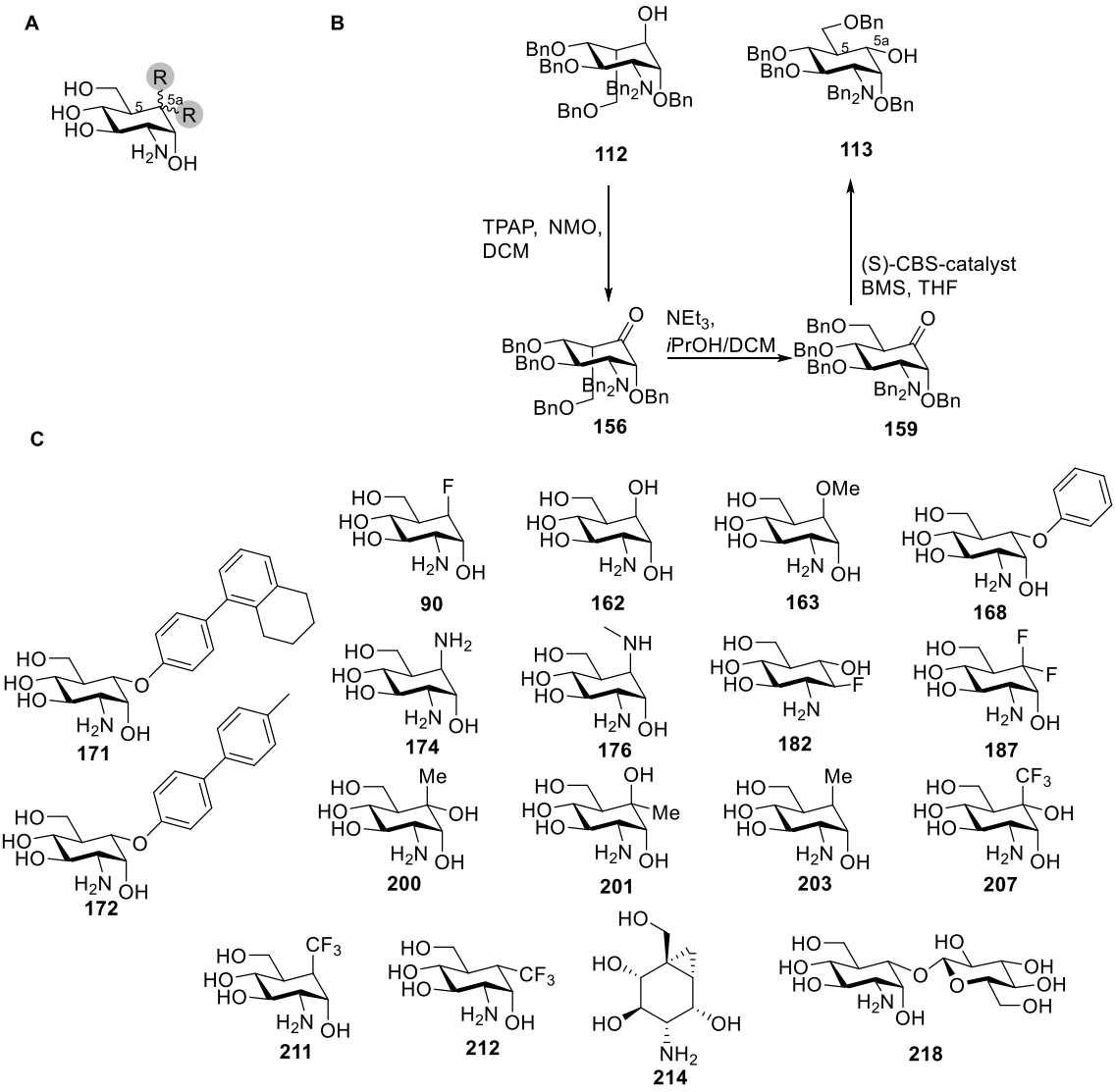


Figure 32: **A** Die 5a-Position von Carbazuckern bietet ungenutzte Raum für Derivatisierung. **B** Die Synthese großer Mengen des Schlüsselintermediats **113** wurde durch ein entwickeltes Isomerisierungsverfahren erreicht. **C** Bibliothek von 5a-modifizierten Carbaglucosaminen, die in dieser Arbeit synthetisiert wurden.

Ein wesentlicher Nachteil der vorherigen Synthese war ein Hydroborierungsschritt, der das falschen Diastereomer ergab. Das Ido-Derivat **112** wurde als Hauptprodukt erhalten und das gewünschte Gluco-Derivat **113** wurde nur als Nebenprodukt erhalten. Dieses Problem wurde dadurch gelöst, dass ein zuverlässiger und effizienter Weg gefunden wurde, um die unerwünschte Verbindung **112** in das Schlüsselintermediate **113** in einer Ausbeute von 74% über 3 Schritte zu überführen. Dies wurde dadurch ermöglicht, dass festgestellt wurde, dass das Derivat **113** die thermodynamisch stabilere Form darstellt, das äquatoriale Produkt

konnte so im Equilibrium erhalten werden (Abbildung 29 B). Nach der Oxidation des 5a Alkohols **112** zum Keton **156** führten milde basische Bedingungen zu einer Isomerisierung der 5-Position, hier war die Gluco-Form **159** bevorzugt. Die stereoselektive Reduktion des Ketons ergab das Schlüsselintermediate **113**. Nachdem weitere kleinere Probleme behoben wurden, wurde die Syntheseroute in eine hochzuverlässige und ergiebige Synthese des Schlüsselintermediates **113** in einer Ausbeute von 2,3 % über 20 Schritte, dies entspricht einer durchschnittlichen Ausbeute von über 80 % pro Schritt, überführt. Der Weg kann auf einer Skala durchgeführt werden, die 5 g des gewünschten Derivats **113** in einem Durchlauf liefert. Darüber hinaus ermöglichte die Verfügbarkeit von Schlüsselintermediate **113** die Synthese einer großen Bibliothek von 5a modifizierten Carba-α-D-Glucosaminen (Abbildung 29 C). Diese Bibliothek bietet die größte Variation von 5a modifizierten Carbasugars, wobei der Großteil der 5a-Dekorationen zum ersten Mal an Carbasugar eingeführt wurde. Neben dem aus der Literatur bekannten fluorierten Carbazucker **90** umfasst die Bibliothek 5a axial substituierte Hydroxy- und Alkoxy-Derivate **162** und **163**. Phenoxy-Derivate und größere aromatische Substituenten wie **168**, **171** und **172** wurden durch eine S_NAr-Reaktion gefolgt von einer Suzuki-Miyaura-Kupplung oder Dehalogenierung synthetisiert. Diaminderivate **174** und **176** sind ebenfalls in der Bibliothek enthalten. Um die elektronischen Eigenschaften des natürlich vorkommenden Hemiacetals besser zu nachzuahmen, wurde eine Difluorierung an der 5a-Position durchgeführt, was zu **187** führte. An der 5a-Position wurde weiterhin mit Kohlenstoff-Kohlenstoff-Bindungen dekoriert, was zu den 5a modifizierten Carba-Glucosamin-Mimetikan **200**, **201**, **203** sowie den Trifluormethyl-Derivaten **207**, **211**, **212** und der bicyclischen Verbindung **214** führte. Um das Potenzial dieser 5a modifizierten Carba-Glucosamine zu demonstrieren, wurden sie als Glykosyl-Akzeptoren verwendet und mit Verbindung **218** wurde das erste je synthetisierte 1,5a-Pseudodisaccharid in diese Bibliothek aufgenommen. Alle Verbindungen wurden auf ihr antibakterielles Potential hin untersucht. Die 5a-Aryloxy-Carba-α-D-Glucosamine **170** und **171** zeigten eine Wachstumshemmung gegenüber B subtilis. Daher können **170** und **171** als vielversprechende Leitverbindungen für weitere Entwicklungen von GlcN-Mimetika als Antibiotika angesehen werden.

7 Experimental

7.1 General Methods

7.1.1 Solvents and Reagents

Technical solvents were distilled prior to use. Dry solvents were either purchased from *Sigma Aldrich*, *Acros* or were dried and distilled. For degassing of dry solvents, the freeze-pump-thaw method was applied. Deuterated solvents for NMR spectroscopy were purchased from *Deutero*.

Chemicals were purchased from Acros Organics, Sigma-Aldrich, TCI Chemicals Europe or abcr. The chemicals were used without further purification. Chromatography

7.1.1.1 Thin Layer Chromatography (TLC)

All reactions were monitored by TLC with silica gel 60 F_{254} coated on aluminum sheets from *Merck*. UV active compounds were detected at λ = 254 nm. Additionally, different staining solutions followed by gentle heating were used for the visualization of the reactants:

- Anisaldehyde solution: EtOH (150 mL), acetic acid (15 mL), (5 mL), *p*-methoxybenzaldehyde (3.7 mL).
- Ninhydrin solution: EtOH (200 mL), acetic acid (3 mL), ninhydrin (0.2 g).
- Potassium permanganate solution: 0.1 % $KMnO_4$ in 1 M NaOH
- Mostein: Phosphomolybdic acid (25 g), 10 Cer(IV)sulfat (10 g), conc. H_2SO_4 (60 mL), water (1 L)

The composition of the solvents is stated as a ratio of volumes (v/v).

7.1.1.2 Flash Chromatography (FC)

For the preparative FC silica gel 60 (Geduran®Si 60, 0.040-0.063 mm particle size) from *Merck* was used. Solvent mixtures are specified as volume ratio (v/v) and all solvents were distilled prior to usage. Additionally, FC was performed on a MPLC-Reveleris® X2 system from *Grace*.

7.1.1.3 High Pressure Liquid Chromatography with Mass Detection (HPLC-MS)

LC-MS analysis was performed with LCMS2020 by *Shimadzu* (pumps: LC-20 AD, auto sampler: SIL-20A HT, UV-Vis detector: SPD-20A, oven: CTO-20AC, communications bus module: CBM-20A, ESI detector, software LCMS-Solution) with an EC 125/4 C18, 3 µM column (*Machery Nagel*) and a binary gradient of acetonitrile and water supplemented with 0.1 % formic acid. The flow rate was 0.4 mL min^{-1}.

7.1.1.4 High-Performance Liquid Chromatography (HPLC)

Preparative high-performance liquid chromatography (HPLC) was performed on a LC-20A device from *Shimadzu* or a LC-20AP device The device contains the following components: degaser: DGU-20A3, auto sampler: SIL-20A, pumps: LC-20AT or LC-20AP, column oven: CTO-20AC, controller: CMB-20A, photodiode array detector: SPD-M20A, columns that were used are Phenomenex® KINETEX® C18 5µ 100Å, 250 x 21.20 mm, Phenomenex® KINETEX® C8 5µ 100Å, 250 x 21.20 mm and Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm.

As eluent a gradient of water with 0.1 % formic acid and acetonitrile with 0.1 % formic acid was used. For HILIC chromatography a 10 to 15 mM triethylammonium buffer with a pH of 7 was used as the aqueous phase. Data regarding eluents are given in the syntheses procedures as a volume ratio of acetonitrile in water (v/v). The Data analysis was performed with LCsolution v. 1.25 from *Shimadzu*.

7.1.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear magnetic resonance (NMR) spectra were recorded on the following spectrometers Avance III 600 MHz, Avance III 400 MHz, Avance Neo 800 from *Bruker*, or a Lambda 400 or a Lambda 500 spectrometer from JEOL at room temperature The resonance signals of different deuterated solvents were used as internal standards: $CDCl_3$ (δ_H = 7.26 ppm, δ_C = 77.16 ppm), DMSO-d_6 (δ_H = 2.50 ppm, δ_C = 39.5 ppm), D_2O (δ_H = 4.79 ppm). In addition to first-order analysis, $^{1}H,^{1}H$ homo- and $^{1}H,^{13}C$-, $^{1}H,^{31}P$- hetero nuclear two-dimensional correlation spectra like HSQC, COSY, TOCSY and HMBC were recorded for the assignment of signals. For stereochemistry determination NOESY and ^{19}F HOESY

spectra were recorded. The multiplicities of the resonances are abbreviated as followed: s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), t (triplet), dt (doublet of triplets), td (triplet of doublets), m (multiplet), pt (pseudo triplet). The recorded NMR spectra were analyzed by using the software *MestReNova v14.2.0-26256* by *Mestrelab Research*.

7.1.3 High Resolution Mass Spectrometry (HRMS)

High resolution masses were measured on a micrOTOF II instrument from *Bruker* in positive mode. Electrospray was used as ionization method (ESI) and the time of flight (TOF) method was used for detection. The recorded mass spectra were analyzed by using the software *Xcalibur v3.0* by *Thermo Fischer Scientific.*

7.1.4 Thin Layer Chromatography/Mass Spectrometry (TLC-MS)

Thin layer chromatography/mass spectrometry was performed on an *Advion Expression CMS* mass spectrometer attached to a *Plate express* TLC plate reader. As ionization source, either an ESI or APCI probe was used, depending on the polarity of the compound. As a mobile phase MeCN with 0.1% FA was used. The TLC plates were not stained prior to the TLCMS measurement. If the product exhibited UV activity, the spot on the TLC was marked with a pencil. If not, two TLC plates were prepared and were applied to a TLC measurement at the same time with the same mobile phase. Afterwards, one plate was stained with an appropriate staining mixture. After determination of the R_f-value, the unstained plate was marked at that R_f-value with a pencil and afterwards applied to the TLCMS measurement.

In some cases, staining of the TLC plate in an iodine chamber, prior to the TLCMS measurement and removal of the iodine via heating, was successful.

7.2 Experimental procedures

Numbering of protons for NMR spectroscopy

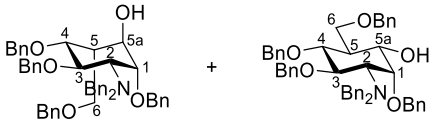
The numbering of protons and carbons for the NMR analysis is in accordance with the general indexing for carba-sugars from Suami and Ogawa.[200] The numbering is analogous to that of natural monosaccharides, except for the ring oxygen position, which is called the 5a-position in case of carba-hexoses.

General Method A:

The reaction was stirred overnight. The volatiles were removed under reduced pressure and the residue was dissolved in MeOH and the solvent was removed under reduced pressure. This step was repeated three times. The crude free phosphonate was dissolved in MeOH (20 mL/1.0 mmol) and 10% Pd/C (waterwet, 35% w/w% of sugar) was added. The reaction was placed into a laboratory autoclave and was stirred under 10 bar hydrogen pressure until HPLC monitoring showed complete consumption of the starting material. Afterwards, the catalyst was removed by filtration over a plug of celite followed by filtration through a regenerated cellulose syringe filter. The solvent was removed under reduced pressure to give the target compound.

The compound was purified either by HILIC HPLC or FC using a cellulose-stationary phase. Specifics are given for each compound in the experimental section.

(1*R*,2*R*,3*S*,4*R*,5*R*,6*R*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)cyclohexan-1-ol (112) and (1*S*,2*R*,3*S*,4*R*,5*R*,6*S*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)cyclohexan-1-ol (113)



To a stirred solution of **141** (8.5 g, 11.9 mmol) in anhydrous THF (120 mL), BH_3•SMe_2 (28.5 mL, 142.5 mmol, 5 M in Et_2O) was added dropwise, and the reaction was stirred overnight at room temperature. The reaction was quenched carefully by addition of H_2O (120 mL). Sodium perborate tetrahydrate (27.4 g, 178.1 mmol) was added carefully and the reaction stirred overnight. Then, the reaction was diluted with H_2O (100 mL) and EtOAc (200 mL) and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 x 100 mL), washed with brine (50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 12:1 – 5:1) to give **112** (4.8 g, 6.54 mmol, 55 %) and **113** (810 mg, 1.1 mmol, 9 %) as colorless syrups. **112**: R_f = 0.23 (petroleum ether/EtOAc = 5:1), vanillin; ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.31 – 7.09 (30H, arenes), 4.81 (t, *J* = 9.8 Hz, 1H, O-CH<u>H</u>'Ph), 4.58 (d, *J* = 11.1 Hz, 2H, O-C<u>H$_2$</u>Ph), 4.53 (d, *J* = 10.9 Hz, 1H, O-C<u>H</u>H'Ph), 4.45 (d, *J* = 10.9 Hz, 2H, O-C<u>H$_2$</u>Ph), 4.31 (m, 2H, O-CH<u>H</u>'Ph, H-5a), 4.24 (d, *J* = 11.8 Hz, 1H, O-C<u>H</u>H'Ph), 4.06 (dd, *J* = 9.3 Hz, 7.4 Hz, 1H, H-3), 3.97 (d, *J* = 14.0 Hz, 2H, N-C<u>H$_2$</u>Ph), 3.89 (d, *J* = 13.9 Hz, 2H, N-C<u>H$_2$</u>Ph), 3.83 (m, 1H, H-4), 3.80 (d, *J* = 9.6 Hz, 1H, H-6b), 3.73 (t, *J* = 3.8 Hz, 1H, H-1), 3.67 (dd, *J* = 9.1 Hz, 5.3 Hz, 1H, H-6a), 3.21 (dd, *J* = 9.4 Hz, 3.7 Hz, 1H, H-2), 2.41 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$, 101 MHz): δ [ppm] = 141.0, 139.2, 138.34, 138.31, 138.0 ($C_{quart.}$), 128.5, 128.4, 128.28, 128.24, 128.15, 128.10, 128.0, 127.96, 127.73, 127.69, 127.44, 127.42, 127.39, 127.3, 127.2, 127.1, 127.0, (arenes), 84.2 (C-1), 80.3 (C-4), 77.4 (C-3), 73.0, 72.8, 72.5, 72.0 (4 x O-<u>C</u>H$_2$Ph), 68.1 (C-6), 67.1 (C-5a), 57.3 (C-2), 56.5 (2 x N-<u>C</u>H$_2$Ph), 43.9 (C-5); HRMS (ESI) *m*/*z* calcd for $C_{49}H_{51}NO_5$: 734.3840 [*M*+H]$^+$ found: 734.3835. **113**: R_f = 0.56 (petroleum ether/EtOAc = 5:1), vanillin; ^{1}H NMR (600 MHz,

$CDCl_3$): δ [ppm] = 7.34-7.04 (m, 30H, arenes), 4.93 (t, *J* = 17.3 Hz, 11.3 Hz, 2H, O-C$\underline{H}_2$Ph), 4.82 (d, *J* = 11.6 Hz, 1H, O-CH$\underline{H}$'Ph), 4.70 (d, *J* = 18.9 Hz, *J* = 10.9 Hz, 2H, O-C$\underline{H}_2$Ph), 4.38 (m, 3H, O-C$\underline{H}_2$Ph, O-C$\underline{H}$H'Ph), 4.17 (dd, *J* = 11.0 Hz, 8.6 Hz, 1H, H-3), 4.04 (t, *J* = 2.3 Hz, 1H, H-1), 3.98 (m, 4H, 2 x N-C$\underline{H}_2$-Ph), 3.73 (dd, *J* = 9.1 Hz, 2.7 Hz, 1H, H-6b), 3.55 (dd, *J* = 9.1 Hz, 5.8 Hz, 1H, H-6a), 3.46 (ddd, *J* = 10.8 Hz, 5.3 Hz, 2.4 Hz, 1H, H-5a), 3.28 (dd, *J* = 11.0 Hz, 8.6 Hz, 1H, H-4), 2.74 (d, *J* = 5.3 Hz, 1H, OH), 2.64 (dd, *J* = 11.1 Hz, 2.1 Hz, 1H, H-2), 2.21 (dddd, *J* = 10.8 Hz, 10.8 Hz, 5.8 Hz, 2.7 Hz, 1H, H-5); ^{13}C NMR ($CDCl_3$, 101 MHz): δ [ppm] = 139.6, 138.3, 138.8, 137.2, 136.7 ($C_{quart.}$), 127.5, 127.4, 127.3, 127.2, 127.1, 126.81, 126.80, 126.77, 126.60, 126.55, 126.4, 126.1, 125.9, 125.6 (arenes), 81.3 (C-1), 80.0 (C-4), 79.5 (C-3), 74.2, 73.9, 72.3, 72.0 (4 x O-$\underline{C}H_2$Ph), 71.7 (C-5a), 62.7 (C-6), 57.7 (C-2), 54.9 (2 x N-$\underline{C}H_2$Ph), 42.8 (C-5); HRMS (ESI) *m/z* calcd for $C_{49}H_{51}NO_5$: 734.3840 $[M+H]^+$ found: 734.3824.

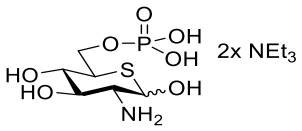
Conversion of 112 to 113

Compound **112** (200 mg, 0.27 mmol) was dissolved in anhydrous DCM (5 mL). TPAP (10 mg, 27 µmol) and NMO (634 mg, 0.55 mmol) were added, and the solution was stirred for 1 h at room temperature. The reaction mixture was filtered over a short plug of silica which was washed with petroleum ether/EtOAc 1:1 (10 mL), and the solvent was removed under reduced pressure. The crude ketone **156** was dissolved in a 1:1 mixture of anhydrous DCM and anhydrous isopropanol (8 mL) to which NEt_3 (376 µL, 2.7 mmol) was added. The reaction was stirred for 6 h, and the solvent was removed under reduced pressure. The crude isomerized ketone **159** was dissolved in anhydrous THF (5 mL) and (*S*)-(–)-2-methyl-CBS-oxazaborolidine (1 M in THF, 27 µL, 27 µM) was added. After stirring for 5 min, BH_3•SMe_2 (5 M in THF, 54 µL, 272 µmol) was added slowly. After stirring for 30 min, the reaction was quenched by the addition of water (2 mL), and extracted with DCM (3 x 4 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by FC (petroleum ether/EtOAc = 10:1 – 5:1) to yield **113** (148 mg, 202 µmol, 74 %). Although intermediate ketone **159** was immediately used for the CBS reduction, a small sample was purified by FC (petroleum ether/EtOAc = 14:1 – 10:1) and characterized.

159: R_f: 0.34 (petroleum ether/EtOAc = 6:1); ^{1}H NMR (500 MHz, CDCl3): δ [ppm] = 7.46 – 7.07 (m, 30H, arenes), 4.92 (d, 1H, *J* = 11.3 Hz, O-C$\underline{H}$HPh), 4.84 (d, 1H,

J = 11.3 Hz, O-C<u>H</u>HPh), 4.77 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.53 – 4.35 (m, 5H, 2 x O-CH_2Ph, H-3), 4.14 (d, 1H, *J* = 4.3 Hz, H-1), 3.99 – 3.92 (m, 4H, 2 x N- CH_2Ph), 3.80 – 3.68 (m, 3H, H-6a, H-6b, H-4), 3.07 (dd, 1H, *J* = 9.2 Hz, 4.3 Hz, H-2), 3.00 (ddd, 1H, *J* = 11.1 Hz, 4.4 Hz, 2.3 Hz, H-5); ^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 207.0 (C-5a), 140.1, 138.9, 138.2, 137.1 (arenes Cquart.), 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.5, 127.3, 126.9 (arenes), 84.9 (C-3), 81.6 (C-1), 78.8 (C-4), 75.2, 73.7, 73.4, 72.4 (4 x O-CH_2Ph), 64.9 (C-6), 59.5 (C-2), 56.0 (2 x N-CH_2Ph), 52.1 (C-5); HRMS (ESI) *m/z* calcd for $C_{49}H_{49}NO_5$: 732.3684 $[M+H]^+$ found: 732.3690.

<u>((2*R*,3*S*,4*R*,5*R*)-5-amino-3,4,6-trihydroxytetrahydro-2H-thiopyran-2-yl)methyl dihydrogen phosphate (127)</u>



Ethyl phosphate **131** (130 mg, 301 µmol) was dissolved in $CDCl_3$ (3 mL). TMSBr (1 mL) was added and the reaction was stirred for 1h. TFA (1 mL) was added to the reaction mixture and the reaction was stirred for 5 min. The volatiles were removed under reduced pressure and the residue was dissolved in H_2O (2 ml) which was removed under reduced pressure. The crude residue was dissolved in 0.1 M aqueous HCl (1 mL). The pH of the solution was adjusted to 4-5 using $Ba(OH)_2$. EtOH was added until a precipitate formed. The mixture was centrifuged and the solvent was removed by decantation. The precipitation process was repeated three times. The precipitate was purified by HILIC-HPLC to yield the di-NEt_3-Phosphate **127** (101 mg, 211 µmol, 70 %) as a colourless solid.

Alpha: For better assignment the 800 MHz ^{1}H spectrum with suppressed NEt_3 is shown. In the appendix a 400 MHz spectrum without NEt_3 suppression is also available.

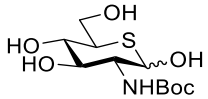
Alpha isomer:

^{1}H NMR (D_2O, 800 MHz) δ [ppm] = 5.13 (d, 1H, *J* = 3.1 Hz, H-1), 4.18 (ddd, 1H, *J* = 11.5, 6.8, 4.8 Hz, H-6), 3.90 (ddd, 1H, *J* = 11.5, 5.6, 2.5 Hz, H-6), 3.76 (pt, 1H, *J* = 9.8 Hz, H-3), 3.71 (pt, 1H, *J* = 9.8 Hz, H-4), 3.55 (dd, 1H, *J* = 9.8, 3.1 Hz, H-2), 3.27 (pdt, 1H, *J* = 10.4, 3.6 Hz, H-5). 13C NMR (D_2O ,201 MHz) δ [ppm] = 72.9 (C-4), 70.3 (C-3), 70.0 (C-1), 62.6 (C-6), 58.6 (C-2), 42.0 (C-5). ^{31}P NMR (162 MHz, D_2O) δ [ppm] = 3.29.

Beta isomer:

^{1}H NMR (D_2O, 800 MHz) δ [ppm] = 4.97 (d, 1H *J* = 9.8 Hz, H-1), 4.10 (m, 1H, H-6), 3.95 (m, 1H, H-6), 3.66 (m, 1H, H-4 under NEt_3), 3.42 (pt, 1H, *J* = 9.7 Hz, H-3), 3.32 (m, 1H, H-2), 3.02 (m, 1H, H-5). ^{13}C NMR (D_2O, 201 MHz) δ [ppm] = 72.7 (C-3), 72.6 (C-4), 70.5 (C-1), 62.5 (C-6), 60.8 (C-2), 44.4 (C-5). ^{31}P NMR (D_2O, 162 MHz) δ [ppm] = 3.29. HPLC: t_R = 6.6 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 40% isocrat. MeCN in 15 mM TEAB buffer pH = 6.99 in 15 min, 10.0 mL/min, ELSD) HRMS (ESI) *m*/*z* calcd for $C_6H_{14}NO_7PS$ 274.0156; [*M*-H^+] found: 274.0157.

tert-butyl ((3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-thiopyran-3-yl)carbamate (130)

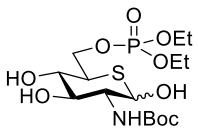


The free thia-sugar **126** (190 mg, 820 µmol) was dissolved in MeOH (3 mL). Boc_2O (215 mg, 215 µmol) was added followed by the addition of KOH (96 mg, 1.7 mmol). The resulting solution was stirred for 4 h. The solution was diluted with H_2O (4 mL) and the solution was carefully neutralized by addition of 0.1 M HCl. The solvent was removed under reduced pressure und the residue was purified by FC (DCM/MeOH = 2% MeOH to 15 %) to give the compound **130** (188 mg, 637 µmol, 78 %) as a colorless solid.

^{1}H NMR (MeOD, 400 MHz) δ [ppm] = 6.32 (d, 1H, *J* = 8.6 Hz, H-1-beta), 4.90 (d, 1H, *J* = 2.8 Hz, H-1 alpha), 4.00 – 3.70 (m, 3H,), 3.66 – 3.51 (m, 2H), 3.26 (m, 1H), 1.47 (s, 10H, CH_3). ^{13}C NMR (MeOD, 101 MHz) δ [ppm] = 80.4, 76.8, 73.8, 73.6, 62.6, 61.2,

44.8, 28.7. HRMS (ESI) m/z calcd for $C_{11}H_{21}NO_6S$ 318.0982 ; [M+Na^+] found: 318.0978.

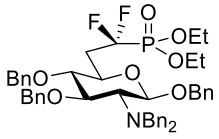
tert-butyl ((3*R*,4*R*,5*S*,6*R*)-6-(((diethoxyphosphoryl)oxy)methyl)-2,4,5-trihydroxytetrahydro-2H-thiopyran-3-yl)carbamate (131)



Boc-protected thia-sugar **130** (180 mg, 609 µmol) was dissolved in pyridine (5 mL) and cooled to -50 °C. Diethylchlorophosphate (124 µL, 853 µmol) was slowly added. The reaction was stopped after 1.5 h by the addition of MeOH (4 mL). Volatiles were removed under reduced pressure. The crude product was purified by FC (DCM/MeOH = 0% MeOH to 10 %) to give the product **131** (163 mg, 377 µmol, 62 %) as a colorless solid.

^{1}H NMR (MeOD, 500 MHz) δ [ppm] = 4.91 (d, 1H J = 2.6 Hz, 1H, H-1), 4.41 (ddd, 1H J = 10.9, 4.8, 5.0 Hz, H-6), 4.30 (ddd, 1H, J = 10.9, 5.5, 2.2 Hz, H-6), 4.15 (m, 4H, CH_2OP), 3.79 – 3.71 (m, 1H, H-2), 3.62 – 3.53 (m, 2H, H-3, H-4), 3.36 (ddd, 1H, J = 9.9, 4.8, 2.2 Hz, H-5), 1.45 (s, 9H, CH_3-Boc), 1.35 (m, 6H, CH_3). ^{13}C NMR (MeOD, 126 MHz) δ [ppm] = 157.6 (C-Carbonyl), 79.9 (C-quart), 75.0 (C-4), 73.5 (C-3), 73.2 (C-1), 67.2 (C-6), 65.1 (CH_2OP), 60.8 (C-2), 42.5 (C-5), 28.3 (CH_3-Boc), 16.0 (CH_3-Ethyl). ^{31}P (MeOD, 202 MHz,) δ [ppm] = -0.98. HRMS (ESI) m/z calcd for $C_{15}H_{30}NO_9PS$ 454.1271; [M+Na^+] found: 454.1270.

diethyl (1,1-difluoro-2-((2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,6-tris(benzyloxy)-5-(dibenzylamino)tetrahydro-2H-pyran-2-yl)ethyl)phosphonate (133)



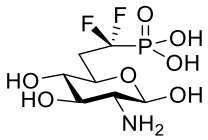
Alcohol **132** (950 mg, 1.5 mmol) was dissolved in DCM (20 mL) The solution was cooled to -40 °C and 2,6-di-tert-butyl-4-methylpyridine (464 mg, 2.3 mmol) was added. Trifluoromethanesulfonic anhydride (380 µL, 2.3 mmol) was slowly added. The solution was stirred for 1.5 h. The reaction was stopped by addition of 1 M $NaHSO_4$ (150 mL). The aqueous layer was extracted with DCM (2 x 50 mL) and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was dissolved in a mixture of petroleum ether/EtOAc and filtered over a short plug of silica. The solvent was removed under reduced pressure and the resulting crude triflate was used without further purification. A 2 M solution of lithium diisopropylamide (3.8 mL, 7.5 mmol) was cooled to -78 °C. Diethyl (difluoromethyl) phosphonate (1.2 mL, 7.5 mmol) was dissolved in THF (2 mL) and cooled to -78°C. The LDA solution was slowly added to the phosphonate solution. The resulting solution was stirred for 10 min at -78 °C. The crude triflate (1.2 g, 1.5 mmol) was dissolved in THF (3 mL) and cooled to -78 °C. To this solution the solution of the deprotonated phosphonate was slowly added. The reaction was stopped by the addition of concentrated aqueous NH_4Cl-solution. The resulting slurry was allowed to warm to room temperature and was extracted with Et_2O (3x 25 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 5:1) to give **133** (0.8 g, 1.0 mmol, 68 % o2s) as a colorless oil.

R_f = 0.37 (petroleum ether/EtOAc = 5:1)

^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] = 7.55 – 7.09 (m, 25H), 5.06 (d, 1H *J* = 11.1 Hz, O-C<u>H</u>HPh), 4.96 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.80 (m, 2H, O-C<u>H</u>HPh), 4.72 – 4.63 (m, 2H, O-C<u>H</u>HPh, H-1), 4.51 (d, *J* = 11.1 Hz, 1H, O-C<u>H</u>HPh), 4.24 (m, 4H, PC<u>H$_2$</u>), 3.91 (d, 2H, *J* = 13.7 Hz, N-C<u>H$_2$</u>Ph,), 3.83 – 3.65 (m, 4H, N-C<u>H$_2$</u>Ph, H-3, H-5), 3.24 (dd, 1H,

J = 9.8, 8.3 Hz, H-4), 3.00 (dd, 1H, *J* = 10.1, 8.3 Hz, H-2), 2.62 – 2.41 (m, 1H, H-6), 2.16 (m, 1H, H-6), 1.34 (m, 6 H, CH_3). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] = 139.8, 139.0, 138.0, 137.5, 129.1, 128.4, 128.3, 128.1, 128.0, 127.5, 127.4, 126.9, 100.1 (C-1), 82.1 (C-4), 81.5 (C-3), 75.0 (O-$\underline{C}H_2Ph$), 74.7(O-$\underline{C}H_2Ph$), 70.3(O-$\underline{C}H_2Ph$), 69.0 (C-5), 64.7(2x PCH_2), 63.3 (C-2), 54.7 (2 x N-$\underline{C}H_2Ph$), 35.7 (C-6), 16.5(CH_3). ^{19}F NMR ($CDCl_3$, 377 MHz) δ [ppm] = -108.9 (dddd, *J* = 299.0, 107.6, 29.7, 10.7 Hz), -111.9 (dddd, *J* = 299.0, 107.6, 26.1, 12.2 Hz). ^{31}P NMR ($CDCl_3$, 202 MHz,) δ [ppm] = 7.28 (t, *J* = 107.6 Hz). HRMS (ESI) *m/z* calcd for $C_{46}H_{52}F_2NO_7P$: 800.3522; [*M*+H^+] found: 800.3516.

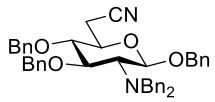
(2-((2*R*,3*S*,4*R*,5*R*,6*R*)-5-amino-3,4,6-trihydroxytetrahydro-2H-pyran-2-yl)-1,1-difluoroethyl)phosphonic acid (134)



Ethylphophonate **133** was treated according to the general procedure A to yield the di-NH_3-Phosphonate **134** as a colorless solid.

^1H NMR (D_2O, 600 MHz) δ [ppm] = 5.37 (d, 1H, *J* = 3.6 Hz, H-1 alpha), 4.90 (d, 1H, *J* = 8.4 Hz, H-1 beta), 4.29 (pt, 1H, *J* = 9.5 Hz, H-5 alpha), 3.89 (pt, 1H, *J* = 9.4 Hz, H-5 beta), 3.84 (pt, 1H, *J* = 9.8 Hz, H-3 alpha), 3.62 (pt, 1H, *J* = 9.7 Hz, H-3 beta), 3.31 (m, 2H, H-4 alpha and beta), 3.27 (dd, 1H, *J* = 10.6, 3.6 Hz, H-2 alpha), 2.96 (dd, 1H, *J* = 10.6, 8.4 Hz, H-2 beta), 2.67 – 2.50 (m, 2H, H-6), 2.30 – 2.08 (m, 2H, H-6). ^{13}C NMR (D_2O,151 MHz) δ [ppm] = 93.0 (C-1 beta), 89.1 (C-1 alpha), 72.9 (C-4 alpha and beta), 72.2(C-3 beta), 70.7 (C-5 beta), 69.7 (C-3 alpha), 66.1 (C-5 alpha), 56.8 (C-2 beta), 54.3 (C-2 alpha), 35.0 (C-6). ^{19}F (D_2O, 377 MHz) δ [ppm] = -109.2, -112.41. ^{31}P NMR (D_2O, 202 MHz) δ [ppm] = 5.9. HRMS (ESI) *m/z* calcd for $C_7H_{14}F_2NO_7P$: 292.0403; [*M*-H^+] found: 292.0405.

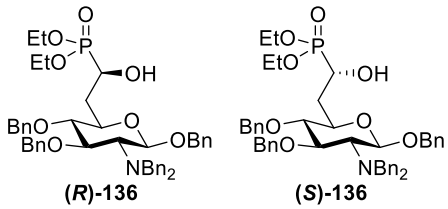
2-((2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,6-tris(benzyloxy)-5-(dibenzylamino)tetrahydro-2H-pyran-2-yl)acetonitrile (135)



Alcohol **132** (5.7 g, 9.1 mmol) was dissolved in DCM (40 mL) and cooled to -40 °C. Lutidine (1.56 mL, 13.5 mmol) and trifluoromethanesulfonic anhydride (2.3 mL, 13.6 mmol) were slowly added. The resulting mixture was stirred for 45 min. The reaction was stopped by addition of 1 M $NaHSO_4$ (150 mL). The aqueous layer was extracted with DCM (2 x 50 mL) and the combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was used without further purification in the next step. Crude triflate (6.9 g, 9.1 mmol) was dissolved in MeCN (90 mL) at room temperature. KCN (5.9 g, 91mmol) was suspended in water (15 mL) and was added to the reaction mixture. The reaction was stirred overnight. The solvent was removed under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 8:1 to 5:1) to give **135** (4.3 g, 6.8 mmol, 75 % o2s) as a colorless solid.

Rf = 0.59 (petroleum ether/EtOAc = 5:1) ^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] = 7.57-7.07 (m, 25H, arenes), 5.05 (d, 1H, *J* = 11.1 Hz, O-C<u>H</u>HPh), 4.97 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.81 (m, 2H, 2x O-C<u>H</u>HPh), 4.69 (d, 1H *J* = 7.9 Hz, H-1), 4.66 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.51 (d, 1H, *J* = 11.1 Hz, O-C<u>H</u>HPh), 3.92 (d, 2H, *J* = 13.7 Hz, N-C<u>H</u>$_2$Ph), 3.77 (d, 2H, *J* = 13.7 Hz, N-C<u>H</u>$_2$Ph), 3.73 (dd, 1H, *J* = 9.9, 8.4 Hz, H-3), 3.47 (ddd, 1H, *J* = 9.6, 8.6, 3.1 Hz, H-5), 3.32 (dd, 1H, *J* = 9.6, 8.4 Hz, H-4), 3.03 (dd, 1H, *J* = 9.9, 7.9 Hz, H-2), 2.66 (dd, 1H, J = 16.8, 3.1 Hz,H-6), 2.36 (dd, 1H, *J* = 16.8, 8.6 Hz, H-6). ^{13}C NMR ($CDCl_3$,126 MHz) δ [ppm] = 139.5, 138.8, 137.6, 137.0, 129.0, 128.9, 128.8, 128.7, 128.5, 128.4, 128.3, 128.2, 127.6, 127.4, 127.0, 117.4 (C-Nitrile), 100.3 (C-1), 81.5 (C-4), 81.1 (C-3), 75.2 (O-<u>C</u>H$_2$Ph), 74.6 (O-<u>C</u>H$_2$Ph), 70.9 (O-<u>C</u>H$_2$Ph), 70.7 (C-5), 63.5 (C-2), 54.9 (2 x N-<u>C</u>H$_2$Ph), 21.5 (C-6). HRMS (ESI) *m*/*z* calcd for $C_{42}H_{42}N_2O_4$: 639.3217; [*M*+H$^+$] found: 639.3212.

diethyl ((*R*)-1-hydroxy-2-((2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,6-tris(benzyloxy)-5-(dibenzylamino)tetrahydro-2H-pyran-2-yl)ethyl)phosphonate ((*R*)-136) & diethyl ((*S*)-1-hydroxy-2-((2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,6-tris(benzyloxy)-5-(dibenzylamino)tetrahydro-2H-pyran-2-yl)ethyl)phosphonate ((*S*)-136)



Nitrile **135** (2.8 g, 4.4 mmol) was dissolved in DCM (60 mL) and cooled to -78 °C. DIBAL-H (1 M in toluene) (13.2 mmol, 13.2 mL) was added slowly. The resulting mixture was stirred for 1 h and the reaction was stopped by the addition of 1 M HCL (60 mL). The organic phase was washed with 1 M HCl (2x 50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Crude aldehyde was used without further purification in the next step.

Crude aldehyde (2.8 g, 4.4 mmol) was dissolved in THF (60 mL) and cooled to -78 °C. In another flask diethylphosphite (1.1 g, 7.8 mmol) was dissolved in THF (60 mL) and cooled to -78 °C. LiHMDS (1 M in THF) (5.6 mL, 5.5 mmol) was added to the phosphite. The solution was stirred for 15 min. The cooled aldehyde was added slowly to the resulting mixture. The reaction was stirred for 30 min. The reaction was stopped by the addition of saturated NH_4Cl solution. The aqueous layer was extracted with Et_2O (3 x 50 mL) and the combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 1:1 to 1:3) to give **136** (1.9 g, 2.42 mmol, 80 % o2s) as a colorless oil as a 3:2 separable mixture of diastereomers.

R-Diastereomer:

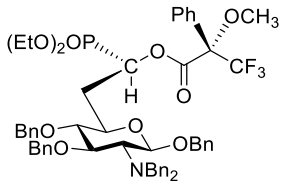
R_f = 0.52 (petroleum ether/EtOAc = 1:2) ^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] = 7.48 – 7.16 (m, 25H), 5.02 (d, 1H, *J* = 11.8 Hz, O-C<u>H</u>HPh), 4.88 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.83 (d, 1H *J* = 11.8 Hz, O-C<u>H</u>HPh), 4.79 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.69 (d, 1H, *J* = 8.3 Hz, H-1), 4.58 (m, 2H, 2x O-C<u>H</u>HPh), 4.17 (m, 5H, 2x P<u>CH$_2$</u>, C<u>H</u>OH), 3.93 (d, 2H, *J* = 13.7 Hz, N-C<u>H$_2$</u>Ph), 3.78 (d, 2H *J* = 13.7 Hz, N-C<u>H$_2$</u>Ph), 3.74 (dd, 1H, *J* = 10.1, 8.4 Hz,

H-3), 3.55 (td, 1H, *J* = 9.4, 3.0 Hz, H-5), 3.34 (dd, 1H, *J* = 9.6, 8.4 Hz, H-4), 3.06 (dd, 1H, *J* = 16.7, 3.1 Hz, OH), 3.00 (dd,1H, *J* = 10.0, 8.2 Hz, H-2), 2.39 (m, 1H. H-6), 1.91 (m, 1H, H-6), 1.32 (td, 6H, *J* = 7.1, 5.5 Hz, 2x CH_3). ^{13}C NMR ($CDCl_3$,126 MHz) δ [ppm] = 139.7, 139.0, 138.0, 137.2, 129.0, 128.7, 128.6, 128.6, 128.5, 128.3, 128.3, 128.1, 128.0, 127.5, 127.4, 127.0, 101.2 (C-1), 83.2 (C-4), 81.2 (C-3), 75.3 (C-5) 75.2 (O-$\underline{C}H_2Ph$), 74.6 (O-$\underline{C}H_2Ph$), 71.1 (O-$\underline{C}H_2Ph$), 63.4 (C-2), 62.8 (2xCH_2P), 55.1 (2 x N-$\underline{C}H_2Ph$), 33.5 (C-6), 16.7 (CH_3). ^{31}P NMR (CDCl3, 202 MHz) δ [ppm] = 23.68. HRMS (ESI) *m/z* calcd for $C_{46}H_{54}NO_8P$: 780.3660; [*M*+H^+] found: 780.3659

S-Diastereomer:

R_f = 0.45 (petroleum ether/EtOAc = 1:2) ^1H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.55 - 7.12 (m, 25H), 5.03 (d, 1H, *J* = 11.2 Hz, O-C$\underline{H}$HPh), 4.94 (d, 1H, *J* = 11.8 Hz, O-C$\underline{H}$HPh), 4.84 (d, 1H, *J* = 11.2 Hz, O-C$\underline{H}$HPh), 4.80 – 4.67 (m, 3H, O-C$\underline{H}$HPh, H-1), 4.54 (d, 1H *J* = 11.2 Hz, O-C$\underline{H}$HPh), 4.23 – 4.08 (m, 5H, 2x P$C\underline{H_2}$, C$\underline{H}$OH), 3.94 (d, 2H, *J* = 13.8 Hz, N-$C\underline{H_2}$Ph), 3.85 – 3.67 (m, 4H, H-4, H-5, N-$C\underline{H_2}$Ph), 3.34 (m, 1H, OH), 3.28 (dd, 1H, *J* = 9.7, 8.3 Hz, H-3), 3.00 (dd, 1H, *J* = 10.1, 8.3 Hz, H-2), 2.24 (m, 1H, H-6), 1.83 (m, 1H, H-6), 1.31 (td, 6H, *J* = 7.1, 3.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$,126 MHz) δ [ppm] = 139.8, 139.1, 138.2, 137.5, 129.00, 128.8, 128.6, 128.46, 128.4, 128.2, 128.1, 128.0, 127.8, 127.4, 126.8, 101.1 (C-1), 83.2 (C-3), 81.4 (C-4), 75.0 (O-$\underline{C}H_2Ph$), 74.7 (O-$\underline{C}H_2Ph$), 70.94 (O-$\underline{C}H_2Ph$), 70.86 (C-5), 64.6 (d, *J* = 163.6 Hz), 63.4 (C-2), 62.8 (2x PCH_2), 54.9 (2 x N-$\underline{C}H_2Ph$), 33.3 (C-6), 16.6 (CH_3). ^{31}P NMR (CDCl3, 202 MHz) δ [ppm] = 25.33. HRMS (ESI) *m/z* calcd for $C_{46}H_{54}NO_8P$: 780.3660; [*M*+H^+] found: 780.3653.

(*R*)-1-(diethoxyphosphoryl)-2-((2R,3R,4R,5R,6R)-3,4,6-tris(benzyloxy)-5-(dibenzylamino)tetrahydro-2H-pyran-2-yl)ethyl (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (*R*)-136-(*S*) MTPA ester

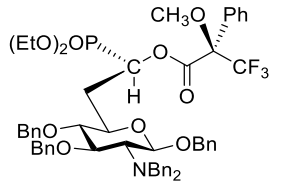


(*R*)-**136**-(*S*)-MTPA ester

Alcohol (*R*)-**136** (20.0 mg. 25.6 µmol), DMAP (4.7 mg, 38.5 µmol), EDC*HCl (11.9 mg, 76.9 µmol) and (*S*)-MTPA acid (18.0 mg, 76.9 µmol) were dissolved in dry DCM (1 mL) and stirred at room temperature for two hours. The reaction was stopped by addition of aq. 0.2 M HCl solution (8 mL) and extracted with DCM (3x10 mL). The mixture was dried over MgSO4 and the solvents were evaporated under reduced pressure. The crude product was purified using HPLC and isolated as a colorless oil (14.4 mg, 56%).

R_f = 0.35 (EA/PE, 1:2); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.60-7.00 (m, 25H), 5.74 (dt, 1H, *J* = 8.5, 6.5 Hz, H-7), 5.29 (s, 1H), 4.99 (d, 1H, *J* = 11.2 Hz, CH_2-OBn), 4.95 (d, 1H, *J* = 11.7 Hz, CH_2-OBn), 4.77 (d, 1H, *J* = 11.2 Hz, CH_2-OBn), 4.69 (d, 1H, *J* = 11.2 Hz, CH_2-OBn), 4.65 (d, 1H, *J* = 4.6 Hz, CH2-OBn), 4.63 (d, 1H, *J* = 8.1 Hz, H1), 4.46 (d, 1H, *J* = 11.2 Hz, CH_2-OBn), 4.18-4.02 (m, 4H, CH_2-OEt), 3.91 (d, 2H, *J* = 13.8 Hz, CH_2-NBn), 3.75 (d, 2H, *J* = 13.8 Hz, CH2-NBn), 3.68 (dd, 1H, *J* = 10.0 Hz, J = 8.4 Hz, H-3), 3.58 (s, 3H, OMe), 3.54 (1H, m, H-5), 3.14 (dd, 1H, *J* = 9.7, 8.4 Hz, H-4), 2.93 (dd, 1H, *J* = 10.0, 8.1 Hz, H-2), 2.42 (m, 1H, H-6), 1.84- 1.72 (m, 1H, H-6), 1.25 (td, 6H, *J* = 7.0, 1.4 Hz); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 101.1 (C-1), 83.4 (C-4), 81.0 (C-3), 71.0 (C-5), 70.9 (Bn), 74.9 (Bn), 74.5 (Bn), 66.8 (C-7), 63.1 (C-2), 62.7 (C-10), 62.7 (C-8), 55.8 (Methoxy-Me), 54.6 (NBn), 32.6 (C-6), 16.5 (Me-OEt), 16.3 (Me-OEt); ^{19}F NMR (471 MHz, $CDCl_3$) δ = - 71.28; ^{31}P NMR (162 MHz, $CDCl_3$) δ = 19.29 HPLC: rt = 4.4 min (Kinetex® C-18, 21.2 mm, 30 mL/min, 100% MeCN in 30 min)

(*R*)-1-(diethoxyphosphoryl)-2-((2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,6-tris(benzyloxy)-5-(dibenzylamino)tetrahydro-2H-pyran-2-yl)ethyl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate (*R*)-136-(*R*)-MTPA ester

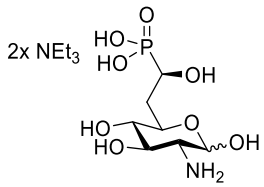


(*R*)-**136**-(*R*)-MTPA ester

Alcohol (*R*)-**136** (20.0 mg. 25.6 µmol), DMAP (4.7 mg, 38.5 µmol, 1.5 eq.), EDC*HCl (11.9 mg, 76.9 µmol) and (*R*)-MTPA acid (18.0 mg, 76.9 µmol) were dissolved in dry DCM (1 mL) and stirred at room temperature for two hours. The reaction was stopped by addition of aq. 0.2 M HCl solution (8 mL) and extracted with DCM (3x10 mL). The mixture was dried over MgSO4 and the solvents were evaporated under reduced pressure. The crude product was purified using HPLC and isolated as a colorless oil (12.4 mg, 47 %).

R_f = 0.30 (EA/PE, 1:2); ^{1}H NMR (400 MHz, $CDCl_3$,) δ = 7.60-7.12 (m, 25H), 5.72 (dt, 1H, *J* = 8.3, 6.1 Hz, H-7), 5.03 (d, 1H, *J* = 11.2 Hz, CH_2-OBn), 4.95 (d, 1H, *J* = 11.7 Hz, CH_2-OBn), 4.80 (d, 1H, *J* = 11.2 Hz, CH_2-OBn), 4.76 (d, 1H, *J* = 11.2 Hz, CH2-OBn), 4.72 (d, 1H, *J* = 8.2 Hz, H-1), 4.66 (d, 1H, *J* = 11.7 Hz, CH2-OBn), 4.51 (d, 1H, *J* = 11.3 Hz, CH2-OBn), 4.08 (m, 4H, NBn), 4.02-3.89 (m, 4H, CH2-OEt), 3.73 (d, 1H, *J* = 13.9 Hz, H-3), 3.64 (d, 1H, *J* = 14.0 Hz, H-5), 3.49 (s, 3H, OMe), 3.23 (dd, 1H, *J* = 9.5 Hz, 8.3 Hz, H-4), 2.96 (dd, 1H, *J* = 10.1 Hz, 8.2 Hz, H-2), 2.56-2.43 (m, 1H, H-6), 1.90-1.72 (m, 1H, H-6), 1.26 (t, 3H, *J* = 7.1 Hz, Me-OEt), 1.19 (t, 3H, *J* = 7.1 Hz, Me-OEt); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 101.2 (C-1), 83.0 (C-4), 81.0 (C-3) 74.7 (OBn), 74.4 (OBn), 71.2 (OBn), 71.3 (C-5), 70.6 (OBn), 66.4 (C-7), 63.1 (CH_2-OEt), 62.8 (C-2), 54.7 (NBn), 33.0 (C-6), 16.7 (Me-OEt). ^{19}F NMR (377 MHz, CDCl3,) δ = -71.53; ^{31}P NMR (162 MHz, $CDCl_3$) δ = 18.78. HPLC: rt = 4.5 min (Kinetex C-18, 30 mL/min, 100% MeCN in 30 min)

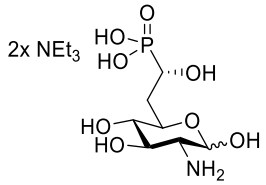
(2-((2*R*,3*S*,4*R*,5*R*)-5-amino-3,4,6-trihydroxytetrahydro-2H-pyran-2-yl)-1-hydroxyethyl)phosphonic acid (*R*)-137



Ethyl phosphonate (*R*)-**136** was treated according to the general procedure A to yield the di NEt_3 Phosphonate (*R*)-**137** as a colorless solid.

^{1}H NMR (D_2O, 500 MHz) δ [ppm] = 5.42 (d, 1H, *J* = 3.7 Hz, H-1 alpha), 4.91 (d, 1H, *J* = 8.4 Hz, H-1 alpha), 4.09 (ddd, 1H *J* = 10.4, 7.8, 3.4 Hz, 1x H-5), 3.99 – 3.89 (m, 2H, HC-P), 3.86 (dd, 1H *J* = 10.6, 9.1 Hz, H-3-alpha), 3.70 (ddd, 1H, *J* = 10.7, 7.6, 3.1 Hz, 1x H-5), 3.66 – 3.59 (m, 1H, H-3 beta), 3.40 (m, 2H, 2x H-4), 3.34 – 3.28 (dd, 1H, *J* = 10.6, 3.7 Hz, H-2 alpha), 3.22 (q, 24H, *J* = 7.4 Hz, CH_2N), 3.04 – 2.95 (dd, 1H, *J* = 10.4, 8.4 Hz, H-2beta), 2.34 (m, 2H, H-6), 1.91 – 1.72 (m, 2H, H-6), 1.30 (t, *J* = 7.3 Hz, 36H, CH_3). ^{13}C NMR (D_2O, 126 MHz) δ [ppm] = 93.1 (C-1 beta), 89.3 (C-1 alpha), 74.7 (1x C-5), 74.0 (C-4), 72.3(C-3 beta), 70.7 (1x C-5), 69.7 (C-3 alpha), 57.0 (C-2 beta), 54.5 (C-2 alpha), 46.8 (CH_2N), 34.3 (C-6), 8.3 (CH_3). ^{31}P NMR (D_2O, 202 MHz) δ [ppm] = 19.33 HPLC: t_R = 6.5 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 50% MeCN to 40 % MeCN in 15 mM TEAB buffer in 15 min pH = 7.00, 10.0 mL/min, ELSD) HRMS (ESI) *m*/*z* calcd for $C_7H_{16}NO_8P$:272.0541; [*M*-H^+] found: 272.0543.

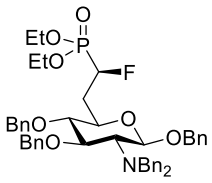
(2-((2*R*,3*S*,4*R*,5*R*)-5-amino-3,4,6-trihydroxytetrahydro-2H-pyran-2-yl)-1-hydroxyethyl)phosphonic acid (*S*)-137



Ethyl phosphonate (*S*)-**136** was treated according to the general procedure A to yield the Di NEt_3 phosphonate (*S*)-**137** as a colorless solid.

^{1}H NMR (D_2O, 500 MHz) δ [ppm] = 5.31 (d,1 H, *J* = 3.7 Hz, H-1 alpha), 4.80 (d, 1H, *J* = 8.4 Hz, H-1 beta), 3.94 (t, 1H *J* = 10.1 Hz, 1H, 1x H-5), 3.77 (m, 4H, 2x H-3, 2x OC<u>H</u>P), 3.60 – 3.47 (m, 3H, 2x H-3, 1x-H-5), 3.27-3.17 (m, 3H, H-2 beta, 2x H-4), 3.14-3.05 (q, 24H, *J* = 7.3 Hz, CH_2N) 2.90 (m, 1H, *J* = 10.6, 8.4 Hz, H-2 alpha), 2.02 (m, 2H, 2x H-6), 1.73 (m, 2H, 2x H-6), 1.24-1.08 (t, 36H, *J* = 7.3 Hz, CH_3). ^{13}C NMR (D_2O, 126 MHz) δ [ppm] = 93.0 (C-1 beta), 89.2 (C-1-alpha), 73.6 (C-4), 72.2 (C-3), 71.9 (1x C-6), 69.8, 67.4 (1x-C-6), 63.9 (C-P), 57.1 (C-2 alpha), 54.6 (C-2 beta), 47.30 (CH_2N), 33.5 (C-6), 7.6 (CH_3). ^{31}P NMR (D_2O,202 MHz) δ [ppm] = 20.10. HPLC: t_R = 7.5 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 40% isocrat. MeCN in 15 mM TEAB buffer pH = 7.00 in 15 min, 10.0 mL/min, ELSD) HRMS (ESI) *m*/*z* calcd for $C_7H_{16}NO_8P$: 272.0541 ; [*M*-H^+] found: 272.0544

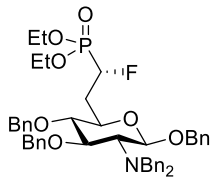
diethyl ((*R*)-1-fluoro-2-((2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,6-tris(benzyloxy)-5-(dibenzylamino)tetrahydro-2H-pyran-2-yl)ethyl)phosphonate (*R*)-138



(*S*)-**137** (180 mg, 230 µmol) was dissolved in THF (3 mL) and cooled to -78 °C. A freshly prepared 2 M solution of lithium diisopropylamide (1.73 mL, 3.45 mmol) in THF was cooled to -78 °C and added slowly to the solution of compound (*S*)-**137**. After stirring for 1 h at -78 °C a solution of AcOH (395 µL, 6.1 mmol) in 1 mL THF was slowly added and the resulting mixture was allowed to reach room temperature. DCM (3 mL) was added and the organic phase was washed with water (2x 1 mL) and brine (2 mL). The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 3:1 to 1:1) to give (*R*)-**138** (76 mg, 97 µmol, 42 %) and (*S*)-**138** (82 mg, 104 µmol, 45 %) as a colorless oils.

Rf = 0.62 (petroleum ether/EtOAc = 1:2) ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.55 – 7.10 (m, 25H), 5.15 – 4.96 (m, 2H, O-C<u>H</u>HPh, PC<u>H</u>F), 4.93 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.86 – 4.74 (m, 2H, O-C<u>H</u>HPh), 4.69 (d, 1H, *J* = 8.3 Hz, H-1), 4.64 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.56 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.22 – 4.12 (m, 4H, PC<u>H</u>$_2$), 3.93 (d, 2H, *J* = 13.7 Hz, N-C<u>H</u>$_2$Ph), 3.79 (d, 2H, *J* = 13.8 Hz, N-C<u>H</u>$_2$Ph), 3.72 (dd, 1H, *J* = 10.1, 8.3 Hz, H-3), 3.60 – 3.45 (m, 1H, H-5), 3.37 (pt, 1H, *J* = 9.0 Hz, H-4), 2.99 (dd, 1H, *J* = 10.1, 8.3 Hz, H-2), 2.49 – 2.28 (m, 1H, H-6), 2.31 – 2.12 (m, 1H, H-6), 1.33 (m, 6H, 2x CH_3). ^{13}C NMR ($CDCl_3$,101 MHz) δ [ppm] = 139.8, 139.0, 138.2, 137.6, 129.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.5, 127.4, 126.9, 100.9 (C-1), 83.0 (C-4), 81.5 (C-3), 75.0 (O-<u>C</u>H$_2$Ph), 74.6 (O-<u>C</u>H$_2$Ph), 70.8 (O-<u>C</u>H$_2$Ph), 63.4 (2x PCH$_2$), 54.9 (2 x N-<u>C</u>H$_2$Ph), 32.6 (C-6), 16.7 (CH_3). ^{31}P NMR (CDCl3, 162 MHz) δ [ppm] = 17.5 (d, *J* = 73.2 Hz). ^{19}F NMR (CDCl3, 377 MHz) δ [ppm] =-205.9 (d, *J* = 73.1 Hz). HRMS (ESI) *m/z* calcd for $C_{46}H_{53}FNO_7P$: 782.3616 ; [*M*+H^+] found: 782.3613.

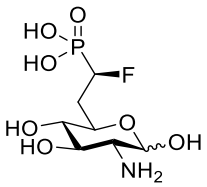
diethyl ((*S*)-1-fluoro-2-((2*R*,3*R*,4*R*,5*R*,6*R*)-3,4,6-tris(benzyloxy)-5-(dibenzylamino)tetrahydro-2H-pyran-2-yl)ethyl)phosphonate (*S*)-138



Diethylaminosulfur trifluoride (88.0 µL, 667 µmol) was dissolved with DCM (7 mL) and cooled to -78 °C. The alcohol (*R*)-**137** (260 mg, 333 µmol) was dissolved in DCM (3 mL) and cooled to -78 °C. The solution of the alcohol was slowly added to the DAST solution. The reaction was allowed to warm to room temperature and was stirred for 1.5 h. The reaction was stopped by addition of a saturated aqueous $NaHCO_3$-solution (10 mL). The aqueous layer was extracted with DCM (3x10 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 2:1 to 1:1) to give (*S*)-**138** (152 mg, 194 µmol, 58 %) as a colorless oil.

Rf = 0.54 (petroleum ether/EtOAc = 1:2) ^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm] = 7.55 – 7.12 (m, 25H), 5.10 – 4.95 (m, 2H, O-C<u>H</u>HPh, PC<u>H</u>F), 4.94 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.85 (d, 1H, *J* = 11.1 Hz, O-C<u>H</u>HPh), 4.80 (d, 1H, *J* = 11.0 Hz, O-C<u>H</u>HPh), 4.73 – 4.61 (m, 2H, H-1, O-C<u>H</u>HPh), 4.56 (d, 1H *J* = 10.9 Hz, O-C<u>H</u>HPh), 4.20 (qd, 3H, *J* = 7.1, 6.5 Hz, PC<u>H$_2$</u>), 3.95 (d, 2H, *J* = 13.6 Hz, N-C<u>H$_2$</u>Ph), 3.83 – 3.75 (m, 3H, N-C<u>H$_2$</u>Ph, H-3), 3.50 (pt, 1H, *J* = 10.0 Hz, H-5), 3.28 (pt, 1H *J* = 9.4 Hz, H-4), 3.02 (dd, 1H, *J* = 10.1, 8.2 Hz, H-2), 2.51-2.42 (m,1H, H-6), 1.90 – 1.69 (m, 1H, H-6). 1.36 (td, 6H, *J* = 7.1, 4.3 Hz, 2x CH_3) ^{13}C NMR ($CDCl_3$,126 MHz) δ [ppm] = 139.6, 138.9, 137.9, 137.1, 128.9, 128.5, 128.4, 128.3, 128.1, 128.1, 127.9, 127.4, 127.3, 126.8, 100.7 (C-1), 84.8 (C-F), 83.1 (C-4), 81.3 (C-3), 75.0 (O-<u>C</u>H$_2$Ph), 74.6 (O-<u>C</u>H$_2$Ph), 70.8(O-<u>C</u>H$_2$Ph), 69.2 (C-5), 63.3 (C-2), 63.1 (2x PCH$_2$), 54.7 (2 x N-<u>C</u>H$_2$Ph), 32.6 (C-6), 16.5 (CH_3). ^{31}P NMR ($CDCl_3$, 162 MHz) δ [ppm] = 18.3. ^{19}F NMR (CDCl3, 377 MHz) δ [ppm] = -213.9. HRMS (ESI) m/z calcd for $C_{46}H_{53}FNO_7P$: 782.3616 ; [M+H$^+$] found: 782.3614.

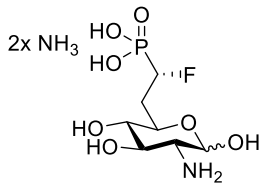
((*R*)-2-((2*R*,3*S*,4*R*,5*R*)-5-amino-3,4,6-trihydroxytetrahydro-2H-pyran-2-yl)-1-fluoroethyl)phosphonic acid (*R*)-(139)



Ethylphophonate (*R*)-**138** was treated according to the general procedure A to yield the di-NH_3-Phosphonate (*R*)-**139** as a colorless solid.

^{1}H NMR (D_2O, 500 MHz) δ [ppm] = 5.30 (d, *J* = 3.6 Hz, 1H), 4.80 (d, *J* = 8.2 Hz, 1H), 3.95 (ddd, *J* = 10.3, 7.1, 3.7 Hz, 1H), 3.73 (dd, *J* = 10.6, 9.1 Hz, 1H), 3.56 (ddd, *J* = 10.2, 6.9, 3.8 Hz, 1H), 3.50 (dd, *J* = 10.5, 8.8 Hz, 1H), 3.32 (td, *J* = 9.4, 5.1 Hz, 2H), 3.20 (dd, *J* = 10.6, 3.7 Hz, 1H), 2.88 (dd, *J* = 10.5, 8.4 Hz, 1H), 2.35 – 2.15 (m, 2H), 1.98 (dtt, *J* = 16.0, 12.3, 8.2 Hz, 1H). ^{13}C NMR (D_2O, 151 MHz) δ [ppm] = δ 93.0 (C-1 beta), 89.1 (C-1 alpha), 74.2, 73.4, 72.2, 70.1, 69.6 (C-3 alpha), 56.8 (C-2 beta), 54.4 (C-2 alpha), 32.5 (C-6). ^{19}F NMR (D_2O, 471 MHz) δ [ppm] = -199.13. ^{31}P NMR (D_2O, 202 MHz) δ [ppm] = 12.83 (dd, *J* = 65.4, 14.1 Hz). HRMS (ESI) m/z calcd for $C_7H_{15}FNO_7P$: 274.0497; [*M*-H^+] found: 274.0499.

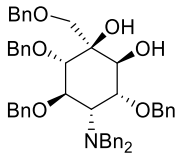
((*S*)-2-((2*R*,3*S*,4*R*,5*R*)-5-amino-3,4,6-trihydroxytetrahydro-2H-pyran-2-yl)-1-fluoroethyl)phosphonic acid (*S*)-(139)



Ethylphophonate (*S*)-**138** was treated according to the general procedure A to yield the si-NH_3-Phosphonate (*S*)-**139** as a colorless solid.

^{1}H NMR (D_2O, 500 MHz) δ [ppm] = 5.43 (d, 1H, *J* = 3.7 Hz, H-1 alpha), 4.93 (d, 1H, *J* = 8.4 Hz, H-1 beta), 4.84-4.65 (m, 2H, HCF), 4.03 (ptd, 1H, *J* = 10.2, 1.9 Hz, 1x H-5), 3.87 (dd, 1H, *J* = 10.5, 9.1 Hz, H-3 alpha), 3.66 – 3.58 (m, 2H, H-3 beta, 1x H-5), 3.37 (pt, 2H, *J* = 9.4 Hz, 2x H-4), 3.33 (dd, 1H, *J* = 10.6, 3.7 Hz, H-2 alpha), 3.01 (dd, 1H, *J* = 10.6, 8.4 Hz, H-1 beta), 2.44– 2.31 (m, 2H, H-6), 1.97 – 1.79 (m, 2H, H-6). ^{13}C NMR (D_2O, 126 MHz) δ [ppm] = 93.2 (C-1 beta), 89.2 (C-1 alpha), 73.4 (C-4), 72.4, (C-3 beta) 71.8 (1x C-5), 69.9 (C-3 alpha), 67.2 (1x C-5), 57.1 (C-2 beta), 54.6 (C-2 alpha), 32.8 (C-6). ^{19}F NMR (D_2O, 471 MHz) δ [ppm] = -205.01. ^{31}P NMR (D_2O, 202 MHz) δ [ppm] = 12.96 (dd, *J* = 63.1, 26.8 Hz). HRMS (ESI) *m*/*z* calcd for $C_7H_{15}FNO_7P$: 274.0497; [*M*-H^+] found:274.0499.

(1*S*,2*S*,3*R*,4*S*,5*R*,6*S*)-3,5,6-tris(benzyloxy)-1-((benzyloxy)methyl)-4-(dibenzylamino)cyclohexane-1,2-diol (142)

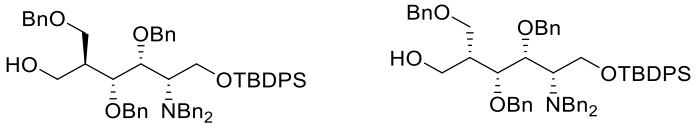


Alkene **141** (200 mg, 279 µmol) was dissolved in *t*BuOH:H_2O (1:1, 10 mL). AD-mix beta (0.53 g) was added. Methansulfonamid (10 mg) was added. The reaction was stirred for 3 days at room temperature after which the reaction was stopped by addition of aqueous sodium sulfite solution. The reaction was extracted with DCM

(3x10 mL). The combined organic phases were dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (PE:EE 5:1 to 1:1) and the product **142** was isolated as a colorless oil (80.0 mg, 107 µmol, 38 %). The remaining starting material could be isolated.

^{1}H NMR (400 MHz, $CDCl_3$) δ =7.51 – 6.99 (m, 30H, arenes), 5.01 (d, 1H, *J* = 11.3 Hz, O-C<u>H</u>HPh), 4.84 (d, 1H, *J* = 10.9 Hz, O-C<u>H</u>HPh), 4.78 (d, 1H, *J* = 11.3 Hz, O-C<u>H</u>HPh), 4.70 – 4.64 (m, 2H, 2x O-C<u>H</u>HPh), 4.60 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.39 (d, 1H, *J* = 11.8 Hz, O-C<u>H</u>HPh), 4.29 (d, 1H, *J* = 11.8 Hz, O-C<u>H</u>HPh), 4.17 (d, 1H, *J* = 3.2 Hz, H-5a), 4.09 (d, 2H, *J* = 14.1 Hz, 2 x N-C<u>H$_2$</u>Ph), 4.05 (m, 1H, H-1), 4.04 – 3.97 (m, 4H, 2 x N-C<u>H$_2$</u>Ph, H-3, 1x H-6), 3.93 (d, 1H, *J* = 9.3 Hz, 1x H-6), 3.83 (d, 1H, *J* = 9.4 Hz, H-4), 3.36 (dd, 1H, *J* = 11.0, 3.2 Hz, H-2), 2.90 (s, 1H, OH-5), 2.65 (s, 1H, OH-5a). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 141.1, 139.6, 139.0, 138.0, 128.6, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.3, 126.7 (arenes), 84.9 (C-4), 81.9 (C-1), 78.1 (C-5), 77.8 (C-3), 76.0 (O-<u>C</u>H$_2$Ph), 73.5 (O-<u>C</u>H$_2$Ph), 73.2 (O-<u>C</u>H$_2$Ph), 70.4 (O-<u>C</u>H$_2$Ph), 68.0 (C-5a), 57.0 (C-2), 56.7 (2x N-<u>C</u>H$_2$Ph).

(2*S*,3*R*,4*R*,5*S*)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-6-((tert-butyldiphenylsilyl)oxy)-5-(dibenzylamino)hexan-1-ol & (2*R*,3*R*,4*R*,5*S*)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-6-((tert-butyldiphenylsilyl)oxy)-5-(dibenzylamino)hexan-1-ol (144)



Method A:

To a stirred solution of compound **108** (3.32 g, 3.8 mmol) in anhydrous THF (100 mL) borane dimethylsulfide complex (2.3 mL, 5 M in Et_2O, 11.5 mmol) was added under inert gas atmosphere. After stirring over night at room temperature the reaction was slowly stopped by the addition of water (100 mL) at 0 °C. Afterwards, sodium perborate (6.59 g, 42.8 mmol) was added to the mixture and stirred vigorously for 70 h. The reaction mixture was diluted with water and the aqueous layer was extracted with EtOAc (3 x 150 mL), dried over MgSO4 and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 15:1 - 8:1) to give compound **144** as diastereomer **144a** (1.63 g, 1.8 mmol, 48 %) and diastereomer **144b** (0.55 g, 0.6 mmol, 16 %) as colorless crystals.

Method B:

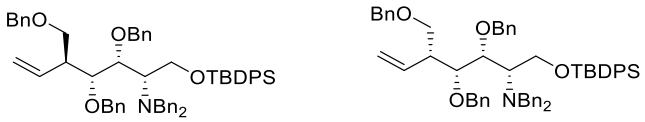
To a stirred solution of compound **108** (1.0 g, 1.2 mmol) in anhydrous THF (30 mL) 9-BBN (6.9 mL, 500 mM in Et_2O, 3.46 mmol) was added under inert gas atmosphere. After stirring over night at 50 °C another portion of 9-BBN (3.5mL, 500mM in Et_2O, 1.7 mmol) was added and stirred again over 1 h. Afterwards, the reaction was slowly stopped by the addition of water (30 mL) at 0 °C. Thereafter, sodium perborate (1.15 g, 7.4 mmol) was added to the mixture and stirred vigorously. After 1.5 h another portion of sodium perborate (0.85 g, 5.5 mmol) was added and the reaction mixture was stirred for 16 h. The reaction mixture was diluted with water and the aqueous layer was extracted with EtOAc (3 x 100 mL), dried over MgSO4 and concentrated under reduced pressure. The residue was purified by FC (petroleum

ether/EtOAc = 15:1 - 8:1) to give compound **144** as one diastereomer (0.96 g, 1.1 mmol, 80 %) as colorless crystals.

144a: R_f = 0.26 (petroleum ether/EtOAc = 10:1), vanillin; ^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] = 7.75-7.69 (m, 2H, arenes), 7.68-7.62 (m, 2H, arenes), 7.54-7.08 (m, 26H, arenes), 4.89 (d, 1H, *J* = 11.4 Hz, O-CH_2Ph), 4.74 (t, 2H, *J* = 11.7 Hz, O-CH_2Ph), 4.55-4.47 (m, 3H,O-CH_2Ph), 4.41-4.35 (m, 1H, H-4), 4.25-4.10 (m, 5H, 2 x H-1 & H-3 & N-CH_2Ph), 3.67 (d, 1H, *J* = 11.4 Hz, H-5a), 3.52 (t, 1H, *J* = 8.6 Hz, H-6), 3.42 (t, 1H, *J* = 8.6 Hz, H-6), 3.39-3.34 (m, 1H, H-5a), 3.31 (d, 2H, *J* = 13.4 Hz, N-CH_2Ph), 2.79 (m, 1H, H-2), 2.69 (m, 1H, OH), 1.32-1.20 (m, 1H, H-5), 1.13-1.04 (m, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$,101 MHz) δ [ppm] = 140.2, 139.4, 138.7, 138.7, 136.0, 135.8, 133.5, 133.4, 130.1, 129.6, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 127.2, 127.1, 127.0 (arenes), 81.9 (C-3), 81.7 (C-4), 76.1 (O-CH2Ph), 75.9 (O-CH_2Ph), 73.7 (O-CH_2Ph), 70.0 (C-6), 61.4 (C-5a), 58.9 (C-2), 57.8 (C-1), 55.6 (N-(CH_2Ph), 40.3 (C-5), 27.1 (Si-C-$(CH_3)_3$), 19.2 (Si-C-$(CH_3)_3$); HRMS(ESI): HRMS (ESI) *m*/*z* calcd for $C_{58}H_{65}NO_5Si$ 884.4705, [*M*+H^+] found: 884.4670.

144b: Rf = 0.22 (petroleum ether/EtOAc = 10:1), vanillin; ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] =7.75-7.69 (m, 2H, arenes), 7.67-7.62 (m, 2H, arenes), 7.52-7.11 (m, 32H, arenes), 4.80 (dd, 2H, *J* = 11.3 Hz, 8.5 Hz, O-CH_2Ph), 4.59 (d, 1H, *J* = 11.2 Hz, O-CH_2Ph), 4.53 (d, 1H, *J* = 11.2 Hz, O-CH_2Ph), 4.25 (m, 2H, O-CH_2Ph), 4.22-4.10 (m, 5H, 2 x H-1 & H-4 & N-CH_2Ph), 4.07-4.02 (m, 1H, H-3), 3.62-3.48 (m, 4H, H-5a & 2 x H-6), 3.42 (m, 2H, N-CH_2Ph), 3.39-3.31 (m, 1H, H-5a), 3.05-2.97 (m, 1H, H-2), 1.89-1.80 (m, 1H, OH), 1.24-1.16 (m, 1H, H-5), 1.07 (s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$,126 MHz) δ [ppm] = 140.6, 139.3, 138.9, 138.6, 135.9, 135.8, 133.5, 130.1, 129.9, 129.6, 128.4, 128.4, 128.0, 128.0, 127.9, 127.7,127.6, 127.5, 127.3, 127.1 (arenes), 81.6 (C-3), 80.8 (C-4), 75.7 (O-CH_2Ph), 75.2 (O-CH_2Ph), 73.0 (C-6), 69.1 (OCH_2Ph), 64.9 (C-5a), 59.0 (C-2), 59.0 (C-1), 55.9 (N-(CH_2Ph), 41.5 (C-5), 27.1 (Si-C-$(CH_3)_3$), 19.3 (Si-C-$(CH_3)_3$); HRMS(ESI): HRMS (ESI) m/z calcd for $C_{58}H_{65}NO_5Si$ 884.4705, [*M*+H+] found: 884.4671.

(2*S*,3*R*,4*R*,5*S*)-N,N-dibenzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-1-((tert-butyldiphenylsilyl)oxy)hept-6-en-2-amine & (2*S*,3*R*,4*R*,5*R*)-N,N-dibenzyl-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-1-((tert-butyldiphenylsilyl)oxy)hept-6-en-2-amine (145 & 146)



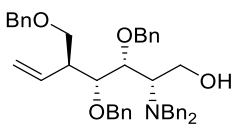
To a stirred suspension of methyltriphenylphosphonium bromide (3.26 g, 9.1 mmol) in anhydrous tetrahydrofuran (THF, 50mL) sodium bis(trimethylsilyl)amide (NaHMDS, 6.8 mL, 1 M in THF, 6.8 mmol) was added at 0 °C and stirred for 30 min. Compound **144** was dissolved in THF (30 mL) and added to the mixture. After stirring for 20 minutes at room temperature the reaction was stopped by the addition of water. The aqueous phase was extracted with EtOAc (3 x 100 mL), dried over MgSO4 and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 20:1 - 5:1) to give compound **145** (0.68 g, 0.78 mmol, 34 % over 2 steps) and the diastereomere **146** (0.56 g, 0.4 mmol, 28 % over 2 steps) as colorless oils.

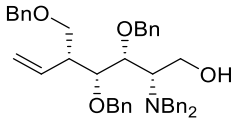
145: Rf = 0.5 (petroleum ether/EtOAc = 8:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.75–7.67 (m, 2H, arenes), 7.65–7.59 (m, 2H, arenes), 7.55–7.10 (m, 33H, arenes), 5.73 (dt, 1H, *J* = 17.3 Hz, 10.1 Hz, R-C(H)=CH_2), 4.92 (d, 1H, *J* = 11.5 Hz, O-CH_2Ph), 4.91 (dd, 1H, *J* = 10.1 Hz, 2.3 Hz, R-C(H)=CH_2), 4.80 (d, 1H, *J* = 11.3 Hz, O-CH_2Ph), 4.69 (d, 1H, *J* = 11.6 Hz, O-CH_2Ph), 4.62 (d, 1H, *J* = 11.4 Hz, O-CH_2Ph), 4.52 (dd, 2H, *J* = 15.7 Hz, 11.8 Hz, O-CH_2Ph), 4.45 (dd, 1H, *J* = 11.8 Hz, 9.9 Hz, H-4), 4.25 (t, 2H, *J* = 9.4 Hz, H-1), 4.22 (dd, 1H, *J* = 17.3 Hz, 2.4 Hz, R-C(H)=CH_2), 4.14 (dd, 2H, *J* = 9.5 Hz, 4.3 Hz, N-CH_2Ph), 3.96 (dd, 1H, *J* = 9.9 Hz, 3.6 Hz, H-3), 3.53 (dd, 1H, *J* = 9.9 Hz, 8.6 Hz, H-6), 3.28 (d, 2H, *J* = 13.4 Hz, N-CH_2Ph), 3.12 (dd, 1H, *J* = 8.4 Hz, 5.7 Hz, H-6), 2.89-2.81 (m, 1H, H-2), 2.00-1.91 (m, 1H, H-5), 1.02 (s, 9H, *t*Bu); ^{13}C NMR (101 MHz, CDCl3, 300 K) δ [ppm] = 139.9, 139.8, 139.0, 136.0, 135.8, 134.8 (C-5), 133.8, 133.6, 130.0, 129.8, 128.6, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.6, 127.3, 126.9 (arenes), 118.2 (R-C(H)=CH_2), 82.7 (C-3), 79.7 (C-4), 75.9 (O-CH_2Ph), 75.3 (O-CH_2Ph), 73.5 (O-CH_2Ph), 71.1 (C-6), 57.9 (C-2), 57.2 (C-1), 55.5

(N-CH_2Ph), 45.2 (C-5), 27.1 (Si-C-$(CH_3)_3$), 19.2 (Si-C-$(CH_3)_3$); HRMS (ESI) m/z calcd for $C_{59}H_{65}NO_4Si$ 880.4756, [*M*+H+] found: 880.4747.

146: Rf = 0.5 (petroleum ether/EtOAc = 8:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.74–7.69 (m, 2H, arenes), 7.67–7.62 (m, 2H, arenes), 7.53–7.36 (m, 5H, arenes), 7.34–7.10 (m, 31H, arenes), 5.75–5.61 (m, 1H, R-C(H)=CH_2), 5.09–5.00 (m, 2H, R-C(H)=CH_2), 4.80 (d, 1H, *J* = 11.4 Hz, O-CH_2Ph), 4.71 (d, 1H, *J* = 11.2 Hz, O-CH_2Ph), 4.60 (d, 1H, *J* = 11.5 Hz, O-CH_2Ph), 4.52 (d, 1H, *J* = 11.3 Hz, O-CH_2Ph), 4.24–4.13 (m, 6H, 2 x O-CH_2Ph & 2 x N-CH_2Ph & 2 x H-1), 4.12-4.04 (m, 2H, H-3 & H-4), 3.61-3.54 (m, 1H, H-6), 3.51 – 3.42 (m, 3H, H-6 & 2 x N-CH_2Ph), 3.14 – 3.06 (m, 1H, H-2), 2.30 – 2.19 (m, 1H, H-5), 1.05 (s, 9H, *t*Bu); ^{13}C NMR ($CDCl_3$, 101MHz) δ [ppm] = 140.4, 139.6, 139.3, 139.0, 136.0, 135.9, 133.5, 130.0, 129.9, 129.4, 128.4, 128.3, 128.3, 128.0, 127.9, 127.6, 127.5, 127.4, 127.2, 127.1, 127.0 (arenes), 116.2 (R-C(H)=CH_2), 82.4 (C-3), 81.4 (C-4), 75.5 (O-CH_2Ph), 75.1 (O-CH_2Ph), 72.4 (O-CH_2Ph), 69.5 (C-6), 59.3 (C-2), 58.8 (C-1), 55.8 (N-CH_2Ph), 44.6 (C-5), 27.1 (Si-C-$(CH_3)_3$), 19.3 (Si-C-$(CH_3)_3$); HRMS (ESI) m/z calcd for $C_{59}H_{65}NO_4Si$ 880.4756, [*M*+H+] found: 880.4747.

(2*S*,3*R*,4*R*,5*S*)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-2-(dibenzylamino)hept-6-en-1-ol & (2*S*,3*R*,4*R*,5*R*)-3,4-bis(benzyloxy)-5-((benzyloxy)methyl)-2-(dibenzylamino)hept-6-en-1-ol (147 & 148)



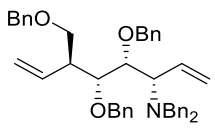


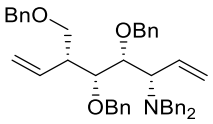
To a stirred solution of compound **146** (268 mg, 0.32 mmol) in anhydrous THF, TBAF (0.6 mL, 1 M in THF, 0.6 mmol) was added. After stirring for 70 h the reaction was stopped by the addition of water. The aqueous phase was extracted with EtOAc (3 x 30 mL), dried over MgSO4 and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 20:1 - 3:1) to give compound **148** (143 mg, 0.22 mmol, 73 %).

147: Rf = 0.7 (petroleum ether/EtOAc = 6:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.48-7.19 (m, 31H, arenes), 5.74 (dt ,1H, *J* = 17.3 Hz, 10.0 Hz, R-C(H)=CH_2), 4.89 (dd, 1H, *J* = 10.2 Hz, 2.0 Hz, R-CH=CH_2), 4.87 (d, 1H, *J* = 11.3 Hz, O-CH_2Ph), 4.79 (d, 1H, *J* = 11.4 Hz, O-CH_2Ph), 4.61 (d, 1H, *J* = 11.3 Hz, O-CH_2Ph), 4.55 (d, 1H, *J* = 11.5 Hz, O-CH_2Ph), 4.52 (dd, 2H, *J* =16.9 Hz, 11.9 Hz, O-CH_2Ph), 4.39 (dd, 1H, *J* = 8.9 Hz, 1.9 Hz), 4.30 (dd, 1H, *J* = 17.5 Hz, 2.1 Hz, R-CH=CH_2), 4.17 (d, 2H, *J* = 13.3 Hz, N-CH_2Ph), 4.02-3.85 (m, 2H, H-1), 3.78-3.69 (m, 1H, H-3), 3.66 (d, 2H, *J* = 14.0 Hz, N-CH_2Ph), 3.51 (dd, 1H, *J* = 9.7 Hz, 8.6 Hz, H-6), 3.21 (dd, 1H, *J* = 8.6 Hz, 5.6 Hz, H-6), 2.80 (dt, 1H, *J* = 6.9 Hz, 5.1 Hz), 2.27-2.15 (m, 1H, H-5), 1.97 (bs, 1H, OH); ^{13}C NMR ($CDCl_3$, 101MHz) δ [ppm] = 140.2, 139.4, 138.9, 138.8, 134.9 (R-C(H)=CH2), 129.7, 128.6, 128.5, 128.4, 128.0, 127.9, 127.7, 127.4, 127.1 (arenes), 118.4 (R-C(H)=CH2), 83.0 (C-3), 78.7 (C-4), 75.4 (O-CH2Ph), 75.0 (O-CH2Ph), 73.3 (O-CH2Ph), 71.1 (C-6), 58.7 (C-1), 57.7 (C-2), 55.5 (N-CH2Ph), 45.4 (C-5); HRMS (ESI) m/z calcd for $C_{43}H_{47}NO_4$ 642.3578, [*M*+H^+] found: 642.3567.

148: R_f = 0.7 (petroleum ether/EtOAc = 6:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.44-7.16 (m, 32H, arenes), 5.83 (ddd, 1H, *J* = 17.3 Hz, 10.5 Hz, 8.5 Hz, R-C(H)=CH_2), 5.11-5.00 (m, 2H, R-CH=CH_2), 4.80 (d, 1H, *J* = 11.4 Hz, O-CH_2Ph), 4.68 (d, 1H, *J* = 11.3 Hz, O-CH_2Ph), 4.56-4.48 (m, 2H, 2 x O-CH_2Ph), 4.40-4.29 (m, 2H, 2 x O-CH_2Ph), 3.99-3.90 (m, 4H, H-3 & H-4 & N-CH_2Ph), 3.78-3.67 (m, 3H, H-1 & N-CH_2Ph), 3.66-3.52 (m, 3H, H-1 & 2 x H-6), 3.11 (q, 1H, *J* = 6.5 Hz, H-2), 2.49-2.41 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$,101MHz) δ [ppm] =140.1, 138.8, 138.7, 138.6, 138.1 (R-C(H)=CH_2), 129.7, 129.4,128.5, 128.5, 128.4, 127.8, 127.8, 127.7, 127.6, 127.2 (arenes), 116.5 (R-C(H)=CH_2), 81.2 (C-3), 79.5 (C-4), 77.2 (O-CH_2Ph), 72.3 (O-CH_2Ph), 73.9 (O-CH_2Ph), 70.9 (C-6), 59.5 (C-2), 59.1 (C-1), 55.1 (N-CH_2Ph), 45.6 (C-5); HRMS (ESI) m/z calcd for $C_{43}H_{47}NO_4$ 642.3578, [*M*+H^+] found: 642.3568.

(3*S*,4*R*,5*R*,6*S*)-N,N-dibenzyl-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)octa-1,7-dien-3-amine & (3*S*,4*R*,5*R*,6*R*)-N,N-dibenzyl-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)octa-1,7-dien-3-amine (149 & 150)





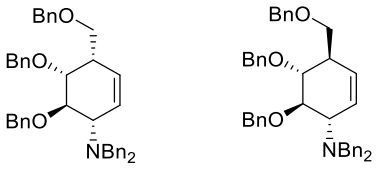
Compound **148** (120 mg, 0.2 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL). After addition of TPAP (8 mg, 0.02 mmol) and NMO (40 mg, 0.34 mmol) the reaction mixture was stirred at room temperature overnight. After 21 h TLC analysis (petroleum ether/EtOAc = 6:1) showed complete conversion of the starting material. The mixture was washed with distilled water (3 x 25 mL), dried over magnesium sulfate (MgSO4) and concentrated under reduced pressure. The crude product was used in the next step without further purification. Rf = 0.5 (petroleum ether/EtOAc = 8:1). To a stirred suspension of methyltriphenylphosphonium bromide (320 mg, 0.9 mmol) in anhydrous THF (5 mL), NaHMDS (0.67 mL, 1 M in THF, 0.67 mmol) was added at 0 °C and stirred for 1 h. Compound XXX was dissolved in THF (2 mL) and added to the mixture. After stirring for 30 minutes at room temperature the reaction was stopped by the addition of water. The aqueous phase was extracted with EtOAc (3 x 70 mL), dried over MgSO4 and concentrated under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 15:1 - 5:1) to give compound **150** (42 mg, 0.07 mmol, 29 % over 2 steps). Additionally, compound **148** (25 mg, 0.04 mmol, 21 %) could be extracted.

149: R_f = 0.7 (petroleum ether/EtOAc = 8:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.57-7.17 (m, 29H, arenes), 6.36 (dt, 1H, H-1), 5.73 (m, 1H, H-5a), 5.44 (m, 1H, 1'C-C(H)=CH2), 5.21 (dd, 1H, 1'C-C(H)=CH_2), 4.90-4.76 (m, 3H, 2x O-CH_2Ph, 1x 5a'C-C(H)=CH_2), 4.64 (d, 1H, *J* = 11.3 Hz, O-CH_2Ph), 4.62 (d, 1H, *J* = 11.7 Hz, O-CH_2Ph), 4.57 (d, 1H, *J* = 11.1 Hz, O-CH_2Ph), 4.54 (d, 1H, *J* = 11.4 Hz, O-CH_2Ph), 4.48 (dd, 1H, *J* = 9.9 Hz, 1.6 Hz, H-4), 4.33 (d, 2H, *J* = 13.3 Hz, N-CH_2Ph), 4.07 (dd, 1H, *J* = 17.8 Hz, 2.3 Hz, 5a'C-C(H)=CH_2), 3.59 (t, 1H, *J* = 9.4 Hz, H-6), 3.51 (dd, 1H,

J = 9.4 Hz, 4.0 Hz, H-3), 3.38 (d, 2H, *J* = 13.1 Hz, N-CH_2Ph), 3.21 (dd, 1H, *J* = 8.7 Hz, 5.6 Hz, H-6), 3.16 (dd, 1H, *J* = 9.5 Hz, 4.2 Hz, H-2), 2.34-2.24 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$,101MHz) δ [ppm] = 140.3, 139.7, 139.5, 139.0, 135.1 (R-C(H)=CH2), 133.5 (R-C(H)=CH2), 129.8, 128.6, 128.3, 128.3, 128.2, 128.0, 127.7, 127.7, 127.2, 127.2, 127.2, 127.0 (arenes), 119.3 (R-C(H)=CH_2), 118.1 (R-C(H)=CH_2), 87.9 (C-3), 79.2 (C-4), 76.2 (O-CH_2Ph), 75.3 (O-CH_2Ph), 73.4 (O-CH_2Ph), 71.0 (C-6), 61.0 (C-2), 55.4 (N-CH_2Ph), 45.3 (C-5); HRMS (ESI) m/z calcd for $C_{44}H_{47}NO_3$ 638.3629, [*M*+H^+] found: 638.3614.

150: R_f = 0.7 (petroleum ether/EtOAc = 8:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.44-7.36 (m, 4H, arenes), 7.35-7.16 (m, 17H, arenes), 7.12-7.06 (m, 2H, arenes), 6.13 (dt, 1H, *J* = 17.3 Hz, 9.7 Hz, H-1), 5.79 (ddd, 1H, *J* = 17.3 Hz, 10.4 Hz, 8.5 Hz, H-5a), 5.32 (dd, 1H, *J* = 10.3 Hz, 2.1 Hz, R-1'C(H)=CH_2), 5.16 (dd, 1H, *J* = 17.3 Hz, 1.3 Hz, 5a'C-C(H)=CH_2), 5.10 (dd, 1H, *J* = 10.4 Hz, 2.0 Hz, 5a'C-C(H)=CH_2), 4.96 (dd, 1H, *J* = 17.3 Hz, 2.1 Hz, R-1'C(H)=CH_2), 4.71 (t, 2H, *J* = 10.8 Hz, 2 x O-CH_2Ph), 4.59 (d, 1H, *J* = 11.3 Hz, O-CH_2Ph), 4.50 (d, 1H, *J* = 11.3 Hz, O-CH_2Ph), 4.28 – 4.14 (m, 4H, 2 x O-CH_2Ph, 2 x N-CH_2Ph), 4.03 (dd, 1H, *J* = 7.4 Hz, 4.0 Hz, H-4), 3.85 (dd, 1H, *J* = 7.5 Hz, 5.4 Hz, H-3), 3.68 (dd, 1H, *J* = 9.4 Hz, 6.9 Hz, H-6), 3.45 (dd, 1H, *J* = 9.5 Hz, 5.3 Hz, H-2), 3.40 – 3.32 (m, 3H, H-6 & N-CH_2Ph), 2.53-2.43 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$,101MHz) δ [ppm] = 140.3, 139.6, 139.3, 138.8, 138.8 (C-5a), 133.3 (C-1), 129.4, 128.3, 128.3, 128.3, 127.6, 127.6, 127.4, 127.2, 127.1, 127.0 (arenes), 119.7 (R-1'C(H)=CH_2), 116.3 (R-C(H)=CH_2), 84.4 (C-3), 82.2 (C-4), 75.0 (OCH_2Ph), 75.0 (O-CH_2Ph), 72.6 (O-CH_2Ph), 70.0 (C-6), 61.9 (C-2), 55.1 (N-CH_2Ph), 44.5 (C-5); HRMS (ESI) m/z calcd for $C_{44}H_{47}NO_3$ 638.3629, [*M*+H^+] found: 638.3612.

(1*S*,4*R*,5*R*,6*R*)-N,N-dibenzyl-5,6-bis(benzyloxy)-4-((benzyloxy)methyl)cyclohex-2-en-1-amine & (1*S*,4*S*,5*R*,6*R*)-N,N-dibenzyl-5,6-bis(benzyloxy)-4-((benzyloxy)methyl)cyclohex-2-en-1-amine (151 & 152)



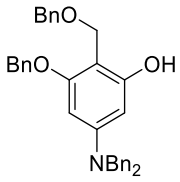
To a stirred solution of **150** (42 mg, 0.7 mmol) in anhydrous toluene (2 mL), Grubbs 2nd generation catalyst (2.8 mg, 3 µmol, 0.05 equiv.) was added. After 2 h TLC showed remaining starting material. Another portion of Grubbs 2nd generation (2.8 mg, 3 µmol, 5 mol%) was added. After stirring for further 1 h another portion of Grubbs 2nd generation (2.8 mg, 3 µmol, 5 mol%) was added and stirred for 1 h. Afterwards the solvent was removed under reduced pressure. The residue was purified by FC (petroleum ether/EtOAc = 20:1) to give compound **152** (20.8 mg, 0.03 mmol, 52 %)

151: R_f = 0.5 (petroleum ether/EtOAc = 8:1); ^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm] = 7.40-7.17 (m, 28H, arenes), 5.86-5.82 (m, 1H, H-5a), 5.81-5.77 (m, 1H, H-1), 4.94 (d, 1H, *J* = 10.5 Hz, O-CH_2Ph), 4.78 (d, 1H, *J* = 10.5 Hz, O-CH_2Ph), 4.60 (s, 2H, O-CH_2Ph), 4.51 (dd, 2H, *J* = 12.2 Hz, 4.8 Hz, O-CH_2Ph), 4.04 (dd, 1H, *J* = 9.4 Hz, 7.4 Hz, H-3), 3.78 (d, 2H, *J* = 14.0 Hz, N-CH_2Ph), 3.75-3.72 (m, 1H, H-4), 3.71 (dd, 1H, *J* = 8.9 Hz, 4.5 Hz, H-6), 3.67 (dd, 1H, *J* = 8.9 Hz, 6.3 Hz, H-6), 3.61 (d, 2H, *J* = 14.0 Hz, N-CH_2Ph), 3.53-3.50 (m, 1H, H-2), 2.78-2.73 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$,151 MHz) δ [ppm] = 140.5, 139.5, 138.9,138.5, 128.8, 128.8, 128.4 (C-5a), 128.4 (C-1), 128.3, 128.2, 128.1, 127.8, 127.8, 127.6, 127.4, 126.7 (arenes), 80.2 (C-4), 78.1 (C-3), 74.4 (O-CH_2Ph), 73.4 (O-CH_2Ph), 72.5 (O-CH_2Ph), 69.9 (C-6), 61.4 (C-2), 54.8 (N-CH_2Ph), 39.7 (C-5); HRMS (ESI) m/z calcd for $C_{42}H_{43}NO$ 610.3316, [*M*+H^+] found: 610.3300.

152: R_f = 0.5 (petroleum ether/EtOAc = 8:1); ^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm] = 7.41-7.35 (m, 8H, arenes), 7.34-7.26 (m, 8H, arenes), 7.26-7.16 (m, 9H, arenes),

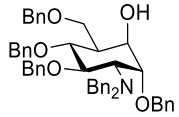
7.13-7.09 (m, 2H, arenes), 5.81 – 5.74 (m, 2H, H-1 & H-5a), 4.98 (q, 2H, *J* = 11.1 Hz, O-CH_2Ph), 4.81 (d, 1H, *J* = 10.8 Hz, O-CH_2Ph), 4.47 (d, 1H, *J* = 12.2 Hz, O-CH_2Ph), 4.43 (d, 1H, *J* = 12.2 Hz, O-CH_2Ph), 4.39 (d, 1H, *J* = 10.8 Hz, O-CH_2Ph), 3.85 (dd, 1H, *J* = 9.6 Hz, 8.2 Hz, H-3), 3.85 (d, 2H, *J* = 14.0 Hz, N-CH_2Ph), 3.70-3.77 (m, 1H, H-2), 3.63 (d, 2H, *J* = 14.0 Hz, N-CH_2Ph), 3.60 (t, 1H. *J* = 9.7 Hz, H-4), 3.56 (dd, 1 H, *J* = 9.0 Hz, 3.2 Hz, H-6), 3.51 (dd, 1 H, *J* = 9.0 Hz, 5.3 Hz, H-6), 2.51 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$,151 MHz) δ [ppm] = 140.2, 139.4, 138.7, 138.4, 129.9 (C-1), 128.8, 128.5, 128.4, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.4, 127.3 (C-5a), 126.9 (arenes), 82.6 (C-3), 80.0 (C-4), 75.4 (O-CH_2Ph), 74.7 (O-CH_2Ph), 73.3 (O-CH_2Ph), 69.5 (C-6), 62.1 (C-2), 54.7 (N-CH_2Ph), 43.9 (C-5); HRMS (ESI) m/z calcd for $C_{42}H_{43}NO$ 610.3316, [*M*+H^+] found: 610.3305.

Characterization of decomposition product 158



^{1}H NMR (600 MHz, CDCl3) δ = 7.52 – 7.09 (m, 20H), 6.62 (d, 1H, *J* = 2.7 Hz), 6.59 (d, 1H, *J* = 2.7 Hz), 5.01 (d, 4H, *J* = 11.1 Hz,), 4.51 (d, 4H, *J* = 5.6 Hz), 4.11 (s, 2H) ^{13}C NMR (151 MHz, $CDCl_3$) δ = 154.4, 152.5, 140.9, 138.3, 137.3, 137.0, 136.6, 131.3, 128.7, 128.7, 128.6, 128.5, 128.3, 128.1, 128.1, 127.8, 127.8, 127.6, 127.1, 106.9, 101.4, 72.3, 70.8, 70.6, 69.5, 54.0.

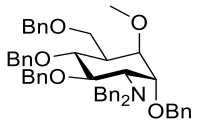
(1*R*,2*R*,3*S*,4*R*,5*R*,6*S*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)cyclohexan-1-ol (160)



The ketone **159** (130 mg, 177.8 µmol) was dissolved in anhydrous THF (5 mL) cooled to -20 °C and (*R*)-(+)-2-methyl-CBS-oxazaborolidine (1 M in THF, 53 µL, 53 µM) was added. After stirring for 5 min, BH_3•SMe_2 (5 M in THF, 35 µL, 177.6 µmol) was added slowly. After slowly warming to room temperature, the reaction was stopped by the addition of water (2 mL), and extracted with DCM (3 x 3 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by HPLC to yield **160** (98 mg, 133.5 µmol, 75 %) with minor contamination of **113**.

^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] =7.55 – 7.10 (m, 30H, H-arenes), 5.03 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.92 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.81 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.62 (d, *J* = 10.8 Hz, 1H, O-C<u>H</u>HPh), 4.58 – 4.46 (m, 2H, 2x O-C<u>H</u>HPh), 4.44 (m, 2H, 2x O-C<u>H</u>HPh), 4.21 – 4.13 (m, 2H, H-3, H-5a), 4.05 (m, 5H, 4x N-C<u>H</u>HPh, 1x H-6), 4.00 – 3.87 (m, 2H, H-4, H-1), 3.65 (m, OH, 1x H-6), 3.37 (dd, 1H, *J* = 11.0, 2.6 Hz, H-2), 2.09 (m, 1H, H-5a). ^{13}C NMR ($CDCl_3$, 201 MHz) δ [ppm] =141.2, 139.8, 138.8, 138.4, 137.3 ($C_{quart.}$ arenes), 128.8, 128.6, 128.5, 128.4, 128.4, 128.2, 128.2, 128.2, 127.9, 127.7, 127.7, 127.2, 127.1, 126.6 (C_{arenes}), 81.9 (C-1), 81.5 (C-3), 79.4 (C-4), 75.3 (O-<u>C</u>H_2Ph), 73.9 (O-<u>C</u>H_2Ph), 73.0 (O-<u>C</u>H_2Ph), 72.8 (O-<u>C</u>H_2Ph), 71.1 (C-5a), 70.6 (C-6), 57.4 (C-2), 56.5 (2x N-<u>C</u>H_2Ph), 41.8 (C-5). HRMS (ESI) *m/z* calcd for $C_{49}H_{51}NO_5$ 734.3840; [*M*+H^+] found: 734.3830. HPLC: t_R = 3.8 min (Kinetex® 5 µm C18 100Å, 250 x 21.2 mm 100 % MeCN, 30mL/min)

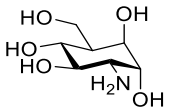
(1*S*,2*R*,3*R*,4*S*,5*R*,6*R*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-methoxycyclohexan-1-amine (161)



Alcohol **160** (80.0 mg, 109 µmol) was dissolved in THF (4 mL) and NaH (60 wt%, 13.0 mg, 327.0 µmol) was added. After stirring for 10 min MeI (27.1 mL, 436.0 µmol) was added. After 3h MeOH (3 mL) was added. Water (5 mL) was added and the aqueous phase was extracted with DCM (3x 10 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (EA:PE 1:10) and the product **161** was isolated as a yellow oil (57.0 mg, 76.2 µmol, 90 %).

^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] =7.45 – 7.10 (m, 30H, H-arenes), 5.02 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.90 (d, 1H *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.70 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.60 – 4.54 (m, 2H, 2x O-C<u>H</u>HPh), 4.49 (d,1H, *J* = 12.2 Hz, O-C<u>H</u>HPh), 4.43 (d, 1H, *J* = 12.2 Hz, O-C<u>H</u>HPh), 4.38 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.17 (dd, 1H, *J* = 11.0, 8.5 Hz, H-3), 4.08 (d, 2H, *J* = 13.8 Hz, 2x N-C<u>H</u>HPh), 3.98 (d, 2H, *J* = 13.8 Hz, N-C<u>H</u>HPh), 3.96 (pt, 1H, *J* = 3.1 Hz, H-1), 3.69 (dd, 1H, *J* = 9.0, 4.0 Hz, 1x H-6), 3.62 (pt, 1H, *J* = 3.3 Hz, H-5a), 3.57 (dd, 1H, *J* = 10.4, 9.0 Hz, 1x H-6), 3.45 (dd, 1H, *J* = 11.5, 8.5 Hz, H-4), 3.13 (dd, 1H, *J* = 11.0, 2.5 Hz, H-2), 2.97 (s, 3H, CH_3), 2.42 (ptpt, 1H, *J* = 10.9, 3.4 Hz, H-5). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] =141.3, 139.8, 138.9, 138.5, 138.2 ($C_{quart.\ arenes}$), 128.8, 128.5, 128.4, 128.2, 128.1, 127.9, 127.7, 127.9, 127.5, 127.2, 127.0, 126.6 (C_{arenes}), 81.9 (C-4), 81.4 (C-3), 79.3 (C-1), 74.9 (C-5a), 74.7 (O-<u>C</u>H_2Ph), 73.0 (O-<u>C</u>H_2Ph), 72.9 (O-<u>C</u>H_2Ph), 72.8 (O-<u>C</u>H_2Ph), 67.1 (C-6), 57.4 (CH_3), 56.7 (C-2), 56.6 (2x N-<u>C</u>H_2Ph), 41.9 (C-5). HRMS (ESI) m/z calcd for $C_{50}H_{53}NO_5$ 748.3997; [M+H^+] found: 748.3990.

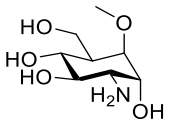
(1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-3-amino-6-(hydroxymethyl)cyclohexane-1,2,4,5-tetraol (162)



To a stirred solution of **160** (8.0 mg, 10.9 µmol) in MeOH abs. (3 mL) was added Pd/C (10% waterwet, 8.0 mg), $Pd(OH)_2/C$ (20% waterwet, 8.0 mg) and TFA (8.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24 h under hydrogen atmosphere. The reaction mixture was filtered over celite. The crude product was purified by HILIC-HPLC to give compound **162** (1.50 mg, 10.9 µmol, 71 %) as a white powder

^{1}H NMR (D_2O, 500 MHz) δ [ppm] = 4.13 (pt, 1H, *J* = 3.2 Hz, H-5a), 4.07 (pt, 1H, *J* = 3.5 Hz, H-1), 3.96 (dd, 1H *J* = 11.1, 4.4 Hz, 1x H-6), 3.81 (dd, 1H, *J* = 11.1, 8.7 Hz, 1x H-6), 3.65 (dd, 1H, *J* = 10.6, 9.1 Hz, H-3), 3.55 (dd, 1H, *J* = 11.3, 9.1 Hz, H-4), 3.30 (dd, 1H, *J* = 10.6, 3.1 Hz, H-2), 2.03 (ptdd, 1H, *J* = 11.3, 5.6, 3.5 Hz, H-5). ^{13}C NMR (126 MHz, D_2O) δ 72.6 (C-3), 70.3 (C-4), 69.8 (C-1), 69.2 (C-5a), 59.4 (C-6), 52.9 (C-2), 42.5 (C-5). HRMS (ESI) *m/z* calcd for $C_7H_{15}NO_5$ 194.1023; [*M*+H^+] found: 194.1027. HPLC: t_R = 17.4 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 30% isocrat. MeCN in 12 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

(1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-3-amino-6-(hydroxymethyl)-5-methoxycyclohexane-1,2,4-triol (163)

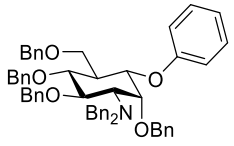


To a stirred solution of **161** (18.0 mg, 24.1 µmol) in MeOH abs. (3 mL) was added Pd/C (10% waterwet, 18.0 mg), $Pd(OH)_2/C$ (20% waterwet,18.0 mg) and TFA (18.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24 h under hydrogen atmosphere. The reaction mixture was filtered over celite. The

crude product was purified by HILIC-HPLC to give compound **163** (3.70 mg, 17.9 µmol, 74 %) as a white powder after lyophilization.

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm] = 4.29 (pt, 1H *J* = 3.2 Hz, H-1), 3.90 (dd, 1H, *J* = 11.0, 4.2 Hz, 1x H-6), 3.74 – 3.66 (m, 2 H, 1x H-6, H-5a), 3.59 (dd, 1H, *J* = 10.7, 9.1 Hz, H-3), 3.42 (s, 3H, CH_3), 3.41 – 3.37 (m, 1H, H-4), 3.18 (dd, 1H, *J* = 10.7, 3.2 Hz, H-2) 2.03 (dddd, 1H, *J* = 11.9, 9.2, 4.2, 2.9 Hz, H-5). ^{13}C NMR (151 MHz, D_2O): δ [ppm] =78.7 (C-5a), 72.0 (C-3), 70.4 (C-4), 65.4 (C-1), 58.9 (C-6), 58.1 (CH_3, 52.9 (C-2), 42.5 (C-5). HRMS (ESI) *m/z* calcd for $C_8H_{17}NO_5$ 208.1179; [*M*+H^+] found: 208.1177. HPLC: t_R = 16.7 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 30 % isocrat. MeCN in 12 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

(1*R*,2*R*,3*R*,4*R*,5*S*,6*R*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)cyclohexan-1-amine (166)

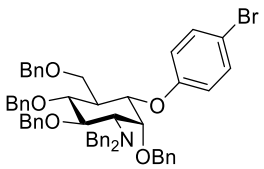


Compound **167** (77.0 mg, 86.6 µmol) was dissolved in dry THF (2 mL) and cooled to -78 °C. *t*-BuLi (1.7 M in hexanes, 203.8 µL, 346.5 µmol) was added and the mixture was stirred for 5 minutes. Water (2 mL) was added and the aqueous phase was extracted with DCM (3x5 mL). The solvents were evaporated under reduced pressure. The crude product was purified by HPLC and **166** was isolated as an oil (32.9 mg, 40.62 µmol, 49%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.53 – 6.97 (m, 33H, H-arenes), 6.82 (d, 2H, *J* = 8.2 Hz, H-arenes), 5.08 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.94 – 4.85 (m, 2H 2x O-C<u>H</u>HPh), 4.82 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.61 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.54 (d, 1H, *J* = 10.5 Hz, O-C<u>H</u>HPh), 4.40 – 4.23 (m, 5H, 2x O-C<u>H</u>HPh, H-5a, H-3, H-1), 4.11 -3.94 (m, 4H, 2x N-C<u>HH</u>Ph), 3.82 (dd, 1H, *J* = 9.2, 2.0 Hz, 1x H-6), 3.78 – 3.64 (m, 2H, H-4, 1x H-6), 2.80 (dd, 1H, *J* = 11.0, 1.9 Hz, H-2), 2.56 (m, H-5). ^{13}C NMR ($CDCl_3$,

126 MHz) δ [ppm] =158.0, 140.9, 139.7, 138.8, 138.7, 138.2 ($C_{quart.\ arenes}$), 129.9, 128.5, 128.4, 128.4, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.5, 127.3, 127.1, 126.8, 121.0, 115.3 (C_{arenes}), 80.8 (C-4), 80.7 (C-3), 78.7 (C-1), 75.4 (O-$\underline{C}H_2$Ph), 74.7 (C-5a), 73.2 (O-$\underline{C}H_2$Ph), 73.1 (O-$\underline{C}H_2$Ph), 64.6 (C-6), 58.8 (C-2), 56.1 (2x N-$\underline{C}H_2$Ph), 43.1 (C-5), 27.1 (CH_3). HRMS (ESI) *m/z* calcd for $C_{55}H_{55}NO_5$ 810.4153; [M+H^+] found: 810.4144. HPLC: t_R = 3.3 min (Kinetex® 5 µm C8 100Å, 250 x 21.2 mm 100 % MeCN, 30 mL/min)

(1*R*,2*R*,3*R*,4*R*,5*S*,6*R*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)cyclohexan-1-amine (167)

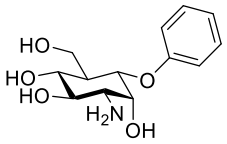


Alcohol **113** (200.0 mg, 275.5 µmol, 1.00 eq.) was dissolved in dry DMF (1 mL). NaH (60 wt%, 14.2 mg, 354.35 µmol) and 4-bromofluorobenzene (33.0 µL, 52.5 mg) were added and the mixture was stirred at 50 °C. After 90 min another equivalent of NaH (10.9 mg) and of the 4-bromofluorobenzene (29.99 µL) was added. The reaction was stopped by addition of saturated aq. NH_4Cl solution (10 mL) and extracted with DCM (3x10 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (EA:PE 1:10) and the product **167** was isolated as a yellow oil (218.2 mg, 0.25 mmol, 90%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] = 7.48 – 7.11 (m, 32H, H-arenes), 7.09 – 7.01 (m, 2H, H-arenes), 6.67 – 6.63 (m, 2H, H-arenes), 5.07 (d, 1H, *J* = 11.6 Hz, O-C$\underline{H}$HPh), 4.89 (d, 1H *J* = 11.6 Hz, O-C$\underline{H}$HPh), 4.80 (m, 2H, 2x O-C$\underline{H}$HPh), 4.58 (d, 1H, *J* = 10.7 Hz, O-C$\underline{H}$HPh), 4.53 (d, 1H, *J* = 10.5 Hz, O-C$\underline{H}$HPh), 4.36 (d, 1H, *J* = 12.1 Hz, O-C$\underline{H}$HPh), 4.31 – 4.20 (m, 3H, O-C$\underline{H}$HPh, H-1, H-3), 4.17 (dd, 1H, *J* = 11.1, 1.9 Hz, H-5a), 4.02 (m, 4H, 2x N-C$\underline{HH}$Ph), 3.79 (dd, 1H, *J* = 9.2, 2.0 Hz,1x H-6), 3.70 (dd, 1H, *J* = 11.1, 8.7 Hz,

H-4), 3.63 (dd, 1H, *J* = 9.2, 2.3 Hz, 1x H-6), 2.76 (d, 1H, *J* =11.1, 2.0 Hz, H-2), 2.52 (ptpt, 1H, *J* = 11.1, 2.2 Hz, H-5). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] =157.4, 141.1, 139.8, 138.9, 138.3, 132.9, 128.8, 128.7, 128.9, 128.6, 128.2, 128.2, 128.0, 127.9, 127.6, 127.3, 127.1, 117.3, 113.4 (C_{arenes}), 81.1 (C-4), 80.9 (C-3), 79.1 (C-1), 75.7 (O-$\underline{C}H_2$Ph), 75.6 (C-5a), 75.1 (O-$\underline{C}H_2$Ph), 73.5 (O-$\underline{C}H_2$Ph), 64.7 (C-6), 59.0 (C-2), 56.4 (2x N-$\underline{C}H_2$Ph), 43.3 (C-5). HRMS (ESI) *m/z* calcd for $C_{55}H_{54}NBrO_5$ 888.3258; [*M*+H$^+$] found: 888.3251.

(1*R*,2*R*,3*R*,4*R*,5*S*,6*R*)-3-amino-6-(hydroxymethyl)-5-phenoxycyclohexane-1,2,4-triol (168)

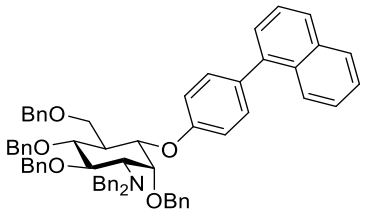


Compound **166** (33.0 mg, 40.7 µmol) was dissolved in methanol p.a. (5 mL). $Pd(OH)_2$/C and Pd/C (each 35 wt%) and TFA (31.2 µL, 407.4 µmol) were added to the mixture. Hydrogen was added to the reaction continuously via a balloon. The reaction was monitored by LC-MS. The catalyst was filtrated off by using a syringe filter, which was washed with 20 mL of methanol. The solvent was evaporated under reduced pressure. The crude product was purified using HPLC and was isolated as a colorless solid (9.4 mg, 31.4 µmol, 76 %) as the formic acid salt **168**.

^{1}H NMR (D_2O, 500 MHz) δ [ppm] = 7.47 – 7.35 (m, 2H, H-ortho), 7.21 – 7.08 (m, 3H, H-meta, H-para), 4.53 (dd, 1H, *J* = 11.3, 2.7 Hz, H-5a), 4.46 (pt, 1H, *J* = 2.7 Hz, H-1), 4.02 (dd, 1H, *J* = 11.6, 2.3 Hz, 1x H-6), 3.89 (dd, 1H, *J* = 11.6, 2.6 Hz, 1x H-6), 3.85 (dd, 1H, *J* = 10.8, 9.2 Hz, H-3), 3.59 (dd, 1H, *J* = 11.0, 9.2 Hz, H-4), 3.30 (dd, 1H, *J* = 10.8, 2.7 Hz, H-2), 2.15 (ptt, 1H, *J* = 11.2, 2.5 Hz, H-5). ^{13}C NMR (D_2O, 126 MHz) δ [ppm] =159.4 (O-C phenyl), 132.7 (C-ortho), 125.1 (C-para), 119.2 (C-meta), 76.2 (C-5a), 73.5 (C-3), 72.0 (C-4), 68.2 (C-1), 58.7 (C-6), 56.1 (C-2), 45.6 (C-5). HRMS (ESI) *m/z* calcd for $C_{13}H_{19}NO_5$ 270.1336; [*M*+H$^+$] found: 270.1334. HPLC: t_R = 8.7 min

(Kinetex® 5 µm C18 100Å, 250 x 21.2 mm, 10-100 % MeCN in 8 min then 100 % MeCN, 0.1 % FA, 8 mL/min)

(*1R*,2*R*,3*R*,4*R*,5*S*,6*R*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-(4-(naphthalen-1-yl)phenoxy)cyclohexan-1-amine (169)

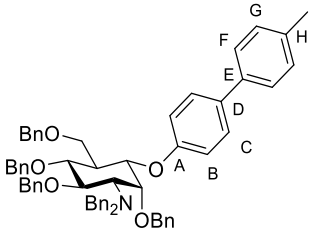


Compound **167** (15.0 mg, 16.9 µmol) was dissolved in degasified THF/water (2:1, 3 mL) in a pressure tube. 1-Naphthylboronic acid (8.7 mg, 50.6 µmol), $Pd(PPh_3)_4$ (1.95 mg, 1.7 µmol) and K_2CO_3 (35.0 mg, 253.1 µmol) were added, the tube was sealed and the mixture was stirred for two hours at 80 °C. Water (5 mL) was added and the mixture extracted with DCM (3x5 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (PE with 1% EA) and the product **169** was isolated as a yellow oil (13.0 mg, 13.9 µmol, 82 %).

^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] =8.01 (d, 1H *J* = 8.2 Hz, H-arenes), 7.94 (dd, 1H, *J* = 8.0, 1.5 Hz), 7.88 (d, 1H, *J* = 8.2 Hz, H-arenes), 7.61 – 7.09 (m, 36 H, H-arenes), 6.94 – 6.88 (m, 2H, H-arenes) , 5.09 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.96 (d, 1H, *J* = 10.5 Hz, O-C<u>H</u>HPh), 4.91 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.83 (d, 1H, *J* = 10.5 Hz, O-C<u>H</u>HPh), 4.63 (m, 2H, 2x O-C<u>H</u>HPh), 4.47 – 4.39 (m, 2H, H-1, O-C<u>H</u>HPh), 4.37 – 4.27 (m, 3H, H-3, H-5a, O-C<u>H</u>HPh), 4.05 (s, 4H, 2x N-C<u>H</u>HPh), 3.85 (dd, 1H, *J* = 9.2, 2.1 Hz, 1x H-6), 3.81 – 3.70 (m, 2H, 1x H-6)), 2.83 (dd, 1H, *J* = 11.0, 2.0 Hz, H-2), 2.60 (m, 1H, H-5). ^{13}C NMR ($CDCl_3$, 101 MHz) δ [ppm] =157.5, 140.9, 140.0, 139.6, 138.8, 138.7, 138.3, 134.1, 133.5, 132.0, 131.5, 128.6, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 128.0, 127.8, 127.7, 127.6, 127.3, 127.1, 126.8, 126.2, 125.9, 125.6, 115.1 (C_{arenes}),

80.9 (C-3), 80.8 (C-4), 78.9 (C-1), 75.4 (C-5a), 75.2 (O-$\underline{C}H_2Ph$), ,74.9 (O-$\underline{C}H_2Ph$), 73.2(O-$\underline{C}H_2Ph$), 73.2 (O-$\underline{C}H_2Ph$), 64.7 (C-6), 58.9 (C-2), 56.2 (2x N-$\underline{C}H_2Ph$) 43.2 (C-5). HRMS (ESI) *m/z* calcd for $C_{65}H_{61}NO_5$ 936.4623; [*M*+H^+] found: 936.4611.

(1*R*,2*R*,3*R*,4*R*,5*S*,6*R*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)cyclohexan-1-amine (170)

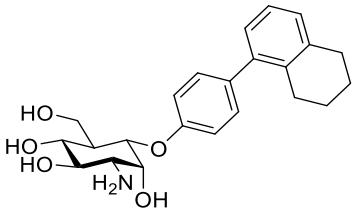


Compound **167** (50.0 mg, 56.3 µmol) was dissolved in degasified THF/water (2:1, 3 mL) in a pressure tube. *p*-Tolylboronic acid (30.6 mg, 225.0 µmol), $Pd(PPh_3)_4$ (6.5 mg, 5.6 µmol) and K_2CO_3 (116.6 mg, 843.7 µmol) were added, the tube was sealed and the mixture was stirred for two hours at 80 °C. Water (5 mL) was added and the mixture extracted with DCM (3x5 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (PE with 1% EA) and the product **170** was isolated as a yellow oil (36.9 mg, 41.0 µmol, 73 %).

^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] = 7.58 – 7.49 (m, 4H, H-arenes), 7.49 – 7.38 (m, 8H, H-arenes), 7.36 – 7.06 (m, 24H, H-arenes), 6.86 (d, *J* = 8.6 Hz, 2H, H-arenes), 5.08 (d, 1H, *J* = 11.6 Hz, O-C$\underline{H}$HPh), 4.91 (m, 2H, 2x O-C$\underline{H}$HPh), 4.83 (d, 1H, *J* = 10.6 Hz, OC$\underline{H}$HPh), 4.64 – 4.55 (m, 2H, 2x O-C$\underline{H}$HPh), 4.41 – 4.35 (m, 2H, H-1, 1x O-C$\underline{H}$HPh), 4.30 (m, 3H, H-5a, H-3, 1x O-C$\underline{HH}$Ph), 4.11 – 3.98 (m, 4H, 2x N-C$\underline{HH}$Ph), 3.83 (dd, 1H *J* = 9.3, 2.0 Hz, 1x H-6), 3.78 – 3.69 (m, 2H, 1x H-6, H-4), 2.82 (dd, 1H, *J* = 11.0, 2.0 Hz, H-2), 2.57 (pt, 1H, *J* = 11.0 Hz, H-5), 2.43 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] =157.4, 140.9, 139.6, 138.8, 138.7, 138.2, 138.0, 136.7, 134.1 ($C_{quart.\ arenes}$), 129.7, 128.6, 128.4, 128.4, 128.4, 128.3, 128.0, 128.0, 128.0, 127.7, 127.7, 127.5,

127.3, 127.1, 126.8, 126.75, 115.5 (C_{arenes}), 80.8 (C-3), 80.7 (C-4), 78.7 (C-1), 75.4 (O-$\underline{C}H_2$Ph), 75.0 (O-$\underline{C}H_2$Ph), 74.8 (C-5a), 73.2 (O-$\underline{C}H_2$Ph), 73.2 (O-$\underline{C}H_2$Ph), 64.6 (C-6), 58.8 (C-2), 56.1 (2x N-$\underline{C}H_2$Ph), 43.1 (C-5), 21.2 (CH_3). HRMS (ESI) *m/z* calcd for $C_{62}H_{61}NO_5$ 900.4623; [*M*+H^+] found: 900.4611.

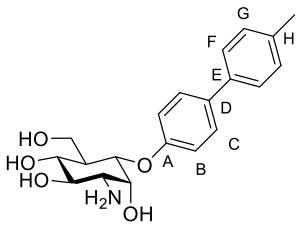
(1*R*,2*R*,3*R*,4*R*,5*S*,6*R*)-3-amino-6-(hydroxymethyl)-5-(4-(5,6,7,8-tetrahydronaphthalen-1-yl)phenoxy)cyclohexane-1,2,4-triol (171)



Compound **169** (23 mg, 24.6 µmol) was dissolved in methanol p.a. (5 mL). $Pd(OH)_2$/C and Pd/C (each 35 wt%) and TFA (18.9 µL, 245.8 µmol) were added to the mixture. Hydrogen was added to the reaction continuously via a balloon. The reaction was monitored by LC-MS. The catalyst was filtrated off by using a syringe filter, which was washed with 20 mL of methanol. The solvent was evaporated under reduced pressure. The crude product was purified using HPLC and was isolated as a colorless solid (7.1 mg, 16.5 µmol, 66 %) as the formic acid salt **171**.

^{1}H NMR (D_2O, 400 MHz) δ [ppm] = 8.46 (s, 1H, FA), 7.37 – 7.00 (m, 7H, arenes), 4.57 (d, 1H *J* = 11.2 Hz, H-5a), 4.51 (m, 1H, H-1), 4.03 (m, 1H, 1x H-6), 3.94 – 3.87 (m, 1H, 1x H-6), 3.88 – 3.78 (m, 1H, H-3), 3.68 – 3.52 (m, 1H, H-4), 3.36 – 3.22 (m, 1H, H-2), 2.86 (t, 2H, *J* = 6.2 Hz, 1x CH_2), 2.59 (t, 2H, *J* = 6.2 Hz, 1x CH_2), 2.17 (m,1H, H-5), 1.81 – 1.63 (m, 4H, 2x CH_2). ^{13}C NMR (D_2O, 151 MHz) δ [ppm] =170.8 (FA), 155.5, 154.7, 141.1, 138.0, 135.2, 134.9, 130.3, 129.9, 128.9, 128.5, 126.8, 125.5, 115.7 (C_{arenes}), 73.1 (C-5a), 70.7 (C-3), 68.9 (C-4), 65.3 (C-2), 55.5 (C-6), 53.1 (C-2), 42.7 (C-5), 29.0 (CH_2), 27.5 (CH_2), 22.6 (CH_2), 22.1 (CH_2). HRMS (ESI) *m/z* calcd for $C_{23}H_{30}NO_5$ 400.2118; [*M*+H^+] found: 400.2114. HPLC: t_R = 9.7 min (Kinetex® 5 µm C18 100Å, 250 x 21.2 mm, 10-100 % MeCN in 8 min then 100 % MeCN, 0.1 % FA, 8 mL/min)

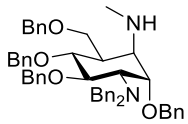
(1*R*,2*R*,3*R*,4*R*,5*S*,6*R*)-3-amino-6-(hydroxymethyl)-5-((4'-methyl-[1,1'-biphenyl]-4-yl)oxy)cyclohexane-1,2,4-triol (172)



Compound **170** (24.0 mg, 26.7 µmol) was dissolved in methanol p.a. (5 mL). $Pd(OH)_2$ and Pd/C (each 35 wt%) and TFA (20.4 µL, 266.6 µmol) were added to the mixture. Hydrogen was added to the reaction continuously via a balloon. The reaction was monitored by LC-MS. The catalyst was filtrated off by using a syringe filter, which was washed with 20 mL of methanol. The solvent was evaporated under reduced pressure. The crude product was purified using HPLC and was isolated as a colorless solid (6.0 mg, 17.7 µmol, 63%) as the formic acid salt **172**.

^{1}H NMR (D_2O, 800 MHz) δ [ppm] =8.32 (s, 1H, FA), 7.54 (d, *J* = 8.2 Hz, 2H, H-C), 7.45 (d, *J* = 7.7 Hz, 2H, H-F), 7.21 (dd, *J* = 7.7 Hz, 2H, H-G), 7.06 (d, *J* = 8.2 Hz, 2H, H-B), 4.43 – 4.37 (m, 1H, H-5a), 4.32 (pt, 1H, *J* = 2.8 Hz, H-1), 3.89 (d, 1H, *J* = 11.5 Hz, 1x H-6), 3.78 – 3.71 (m, 1H, 1x H-6), 3.67 (pt, 1H *J* = 10.0 Hz, H-3), 3.45 (pt, 1H *J* = 10.1 Hz, H-4), 3.13 – 3.07 (m, 1H, H-2), 2.25 (s, 3H, CH_3), 2.03 (pt, 1H, *J* = 11.2 Hz, H-5). ^{13}C NMR (D_2O, 201 MHz) δ [ppm] =171.0 (C-FA), 156.3 (C-A), 137.7 (C-H), 136.9 (C-E), 134.5 (C-D), 129.7 (C-G), 128.1 (C-F), 126.5 (C-C), 117.0 (C-B), 73.7 (C-5a), 71.4 (C-3), 69.4 (C-4), 65.9 (C-1), 56.2 (C-6), 53.4 (C-2), 43.1 (C-5), 20.0 (CH_3). HRMS (ESI) *m*/*z* calcd for $C_{20}H_{25}NO_5$ 360.1805; [*M*+H^+] found: 360.1804. HPLC: t_R = 9.6 min (Kinetex® 5 µm C18 100Å, 250 x 21.2 mm, 10-100 % MeCN in 8 min then 100 % MeCN, 0.1 % FA, 8 mL/min)

(1*S*,2*S*,3*R*,4*R*,5*R*,6*R*)-N1,N1-dibenzyl-2,5,6-tris(benzyloxy)-4-((benzyloxy)methyl)-N3-methylcyclohexane-1,3-diamine (173)

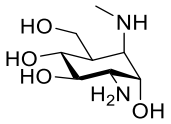


Compound **112** (171 mg, 23.3µmol) was dissolved in anhydrous DCM (4 mL). TPAP (8 mg, 23 µmol) and NMO (54 mg, 466.0 µmol) were added, and the solution was stirred for 1 h at room temperature. The reaction mixture was filtered over a short plug of silica which was washed with petroleum ether/EtOAc 1:1 (10 mL), and the solvent was removed under reduced pressure. The crude ketone **156** was dissolved in a 1:1 mixture of anhydrous DCM and anhydrous isopropanol (4 mL) to which NEt_3 (193.1 µL, 1.39 mmol) was added. The reaction was stirred for 6 h. Methylammonium chloride (1.1 g, 16.26 µmol) was suspended in dry methanol (8 mL) and the reaction mixture containing the ketone **159** was added. The mixture was stirred for 10 min and $NaBH_3CN$ (24.8 mg, 394.8 µmol) was added. The reaction was stirred overnight. Saturated $NaHCO_3$ solution (10 mL) was added. The aqueous phase was extracted with DCM (3x 15 mL). The crude product was purified by FC (petroleum ether/EtOAc = 10:1 – 2:1) to yield **173** (67 mg, 89.7 µmol, 39 % o3s starting from alcohol **112**).

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm] = 7.51 – 7.06 (m, 30H, arenes), 5.08 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.94 (d, 1H *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.75 (d, 1H *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.63 – 4.56 (m, 2H, O-C<u>HH</u>Ph), 4.54 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.46 (d, 1H, *J* = 12.0 Hz, O-C<u>H</u>HPh), 4.43 (d, 1H, *J* = 12.0 Hz, O-C<u>H</u>HPh), 4.18 (dd, 1H, *J* = 11.0, 8.5 Hz, H-3), 4.10 (d, 2H, *J* = 13.9 Hz, 2x N-C<u>H</u>HPh), 4.00 (d, 2H, *J* = 13.9 Hz, N-C<u>HH</u>Ph), 3.96 – 3.89 (m, 2H, H-4, H-1), 3.85 (dd, 1H *J* = 9.5, 6.0 Hz, 1x H-6), 3.59 (dd, 1H *J* = 9.5, 2.6 Hz, 1x H-6), 3.43 (dd, 1H, *J* = 11.0, 2.5 Hz, H-2), 2.92 (pt, 1H, *J* = 3.2 Hz, H-5a), 2.30 (ddpt, 1H, *J* = 11.6, 5.8, 2.8 Hz, H-5), 2.00 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 151 MHz) δ [ppm] = 141.6, 140.0, 138.9, 138.6, 138.2 ($C_{quart.}$), 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.8, 127.6, 127.6, 127.1, 127.0, 126.5, (C_{arenes}) 82.3 (C-4), 81.0 (C-3), 80.9 (C-1), 74.8 (O-<u>C</u>H_2Ph), 73.4 (O-<u>C</u>H_2Ph), 72.9

(O-$\underline{C}H_2Ph$), 72.5 (O-$\underline{C}H_2Ph$), 69.9 (C-6), 60.3 (C-5a), 56.8 (2x N-$\underline{C}H_2Ph$), 40.9 (C-5), 34.5 (CH_3). HRMS (ESI) *m/z* calcd for $C_{50}H_{54}N_2O_4$ 747.4156; [*M*+H^+] found: 747.4138.

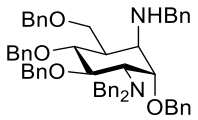
(1*R*,2*R*,3*S*,4*R*,5*R*,6*R*)-3-amino-6-(hydroxymethyl)-5-(methylamino)cyclohexane-1,2,4-triol (174)



To a stirred solution of **173** (25.0 mg, 33.5 µmol) in MeOH abs. (3 mL) was added Pd/C (10% waterwet, 25.0 mg), $Pd(OH)_2$/C (20% waterwet, 25.0 mg) and TFA (20.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24 h under hydrogen atmosphere. The reaction mixture was centrifuged. The supernatant was purified by HILIC-HPLC to give compound **174** (4.2 mg, 20.4 µmol, 61 %) as a white powder after lyophilization.

^{1}H NMR (D_2O, 600 MHz) δ [ppm] = 4.12 (pt, 1H, *J* = 3.2 Hz, H-1), 3.90 (dd, 1H, *J* = 11.5, 4.1 Hz, 1x H-6), 3.81 (dd, 1H, *J* = 11.5, 7.6 Hz, 1x H-6), 3.51 – 3.44 (m, 2H, H-3 & H-4), 2.99 (dd, 1H, *J* = 10.1, 3.2 Hz, H-2), 2.97 (pt, 1H, *J* = 3.5 Hz, H-5a), 2.36 (s, 3H, CH_3), 2.06 (ddpt, 1H, *J* = 11.2, 7.6, 3.5 Hz, H-5). ^{13}C NMR (D_2O, 151 MHz) δ [ppm] = 74.2 (C-3), 70.2 (C-4), 67.6 (C-1), 61.6 (C-5a), 59.8 (C-6), 52.5 (C-2), 41.2 (C-5), 34.3 (CH_3). HRMS (ESI) *m/z* calcd for $C_8H_{18}N_2O_4$ 207.1339; [*M*+H^+] found: 207.1342. HPLC: t_R = 13.7 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 40% isocrat. MeCN in 12 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

(1*S*,2*S*,3*R*,4*R*,5*R*,6*R*)-N1,N1,N3-tribenzyl-2,5,6-tris(benzyloxy)-4-((benzyloxy)methyl)cyclohexane-1,3-diamine (175)

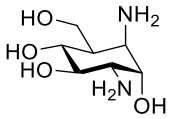


Compound **112** (171 mg, 23.3µmol) was dissolved in anhydrous DCM (4 mL). TPAP (8 mg, 23 µmol) and NMO (54 mg, 466.0 µmol) were added, and the solution was stirred for 1 h at room temperature. The reaction mixture was filtered over a short plug of silica which was washed with petroleum ether/EtOAc 1:1 (10 mL), and the solvent was removed under reduced pressure. The crude ketone **156** was dissolved in a 1:1 mixture of anhydrous DCM and anhydrous isopropanol (4 mL) to which NEt_3 (193.1 µL, 1.39 mmol) was added. The reaction was stirred for 6 h. Benzylamin hydrochloride (2.3 g, 16.26 µmol) was suspended in dry methanol (8 mL) and the reaction mixture containing the ketone **159** was added. The mixture was stirred for 10 min and $NaBH_3CN$ (24.8 mg, 394.8 µmol) was added. The reaction was stirred overnight. Saturated $NaHCO_3$ solution (10 mL) was added. The aqueous phase was extracted with DCM (3x 15 mL). The crude product was purified by FC (petroleum ether/EtOAc = 10:1 – 3:1) to yield **175** (110 mg, 133.6 µmol, 58 % o3s starting from alcohol **112**).

^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.56 – 7.10 (m, 33H, arenes), 6.99 – 6.89 (m, 2H, arenes), 5.11 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.98 (d, 1H, *J* = 11.7 Hz, 1H, O-C<u>H</u>HPh), 4.78 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.61 – 4.53 (m, 2H, 2x O-C<u>H</u>HPh), 4.50 (d, 1H, *J* = 11.1 Hz, O-C<u>H</u>HPh), 4.45 – 4.34 (m, 2H, 2x O-C<u>H</u>HPh), 4.21 (dd, 1H, *J* = 10.9, 8.5 Hz, H-3), 4.14 (d, 2H, *J* = 14.0 Hz, 2x N-C<u>H</u>HPh), 4.08 – 3.97 (m, 3H, 2x N-C<u>H</u>HPh, H-4), 3.97 – 3.93 (m, 1H, H-1), 3.94 – 3.88 (m, 1H, 1x H-6), 3.59 (dd, 1H, *J* = 10.9, 2.6 Hz, H-2), 3.55 (dd, 1H, *J* = 9.3, 2.3 Hz, 1x H-6), 3.45 (d, 1H, *J* = 12.6 Hz, 1x NH-C<u>H</u>HPh), 3.30 (d, 1H, *J* = 12.6 Hz, 1x NH-C<u>H</u>HPh), 3.10 (pt, 1H, *J* = 3.2 Hz, H-5a), 2.31 (ddpt, 1H, *J* = 11.3, 5.5, 2.6 Hz, H-5), 2.19 (s, 1H, N<u>H</u>). ^{13}C NMR ($CDCl_3$, 101 MHz) δ [ppm] = 141.6, 140.6, 140.00, 139.1, 138.7, 138.0 ($C_{quart.}$), 128.7, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 127.8, 127.7, 127.6, 127.2, 127.1, 126.9, 126.6 (C_{arenes}),

82.5 (C-3), 81.6 (C-1), 80.9 (C-4), 74.9 (O-$\underline{C}H_2Ph$), 73.6 (O-$\underline{C}H_2Ph$), 73.3 (O-$\underline{C}H_2Ph$), 72.5 (O-$\underline{C}H_2Ph$), 70.3 (C-6), 58.0 (C-5a), 57.1 (C-2), 56.6 (2x N-$\underline{C}H_2Ph$), 52.4 (NH-CH_2Ph), 40.9 (C-5). HRMS (ESI) *m/z* calcd. for $C_{56}H_{58}N_2O_4$ 823.4469; [*M*+H^+] found: 823.4465.

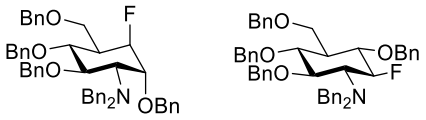
(1*R*,2*R*,3*S*,4*S*,5*R*,6*R*)-3,5-diamino-6-(hydroxymethyl)cyclohexane-1,2,4-triol (176)



To a stirred solution of **173** (20.0 mg, 24.3 µmol) in MeOH abs. (3 mL) was added Pd/C (10% waterwet, 20.0 mg), $Pd(OH)_2$-C (20% waterwet, 20.0 mg) and TFA (20.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24h under hydrogen atmosphere. The reaction mixture was centrifuged. The supernatant was purified by HILIC-HPLC to give compound **176** (3.1 mg, 16.1 µmol, 66 %) as a white powder after lyophilization.

^{1}H NMR (D_2O, 600 MHz) δ [ppm] = 4.00 (pt, 1H, *J* = 3.5 Hz, H-1), 3.93 (dd, 1H, *J* = 11.6, 4.7 Hz, 1x H-6), 3.76 (dd, 1H, *J* = 11.6, 8.6 Hz, 1x H-6), 3.62 – 3.58 (m, 1H, H-3), 3.58 – 3.54 (m, 1H, H-4), 3.34 (pt, *J* = 3.8 Hz, H-5a), 3.24 (dd, 1H *J* = 10.6, 3.3 Hz, H-2), 2.06 (ddpt, 1H, *J* = 11.5, 8.5, 4.1 Hz, H-5). ^{13}C NMR (D_2O, 151 MHz) δ [ppm] = 72.7 (C-3), 70.6 (C-1), 70.0 (C-4), 59.5 (C-6), 52.7 (C-2), 51.7 (C-5a), 41.3 (C-5). HRMS (ESI) *m/z* calcd for $C_7H_{16}N_2O_4$ 193.1183; [*M*+H^+] found: 193.1185. HPLC: t_R = 12.2 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 40 % isocrat. MeCN in 12 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

(1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-fluorocyclohexan-1-amine &(1*R*,2*R*,3*R*,4*R*,5*S*,6*S*)-N,N-dibenzyl-2,3,5-tris(benzyloxy)-4-((benzyloxy)methyl)-6-fluorocyclohexan-1-amine (179 & 180)

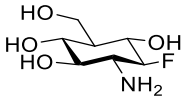


A boron trifluoride diethyl etherate solution (1 % in toluene, 4.5 mL, 258.9 µmol) was cooled to 0 °C and DAST (342 µL, 2.6 mmol) was added. The mixture was stirred for 1 h and then cooled to -78 °C. Alcohol **160** (190 mg, 258.9 µmol) was dissolved in dry toluene (1 mL) and slowly added to the DAST solution. The reaction was allowed to warm up to room temperature overnight. The reaction mixture was slowly added into 1 M aqueous NaOH solution (5 mL) and extracted with DCM (3x 2 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. HPLC purification resulted in axial fluoride **179** (110 mg, 149.5 µmol, 58 %) as well as the rearrangement product **180** (25 mg, 34.0 µmol, 13 %).

179: ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.52 – 7.07 (m, 30H), 5.03 – 4.84 (m, 3H, 2x O-C<u>H</u>HPh, H-5a), 4.74 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.61 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.55 (d, 1H, J = 10.8 Hz, O-C<u>H</u>HPh), 4.47 (m, 2H, 2x O-C<u>H</u>HPh), 4.40 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.20 (dd, 1H, *J* = 11.1, 8.6 Hz, H-3), 4.10 – 3.98 (m, 5H, 4x N-C<u>H</u>HPh, H-1), 3.71 (dd, 1H, *J* = 9.1, 4.0 Hz, 1x H-6), 3.49 (m, 2H, 1x H-6, H-4), 3.12 (dt, 1H, *J* = 11.1, 3.2 Hz, H-1), 2.50 – 2.30 (m, 1H, H-5).^{13}C NMR ($CDCl_3$, 101 MHz) δ [ppm] =140.9, 139.5, 138.6, 138.3, 137.8 ($C_{quart.}$), 128.6, 128.5, 128.5, 128.5, 128.3, 128.1, 128.0, 127.9, 127.8, 127.6, 127.3, 127.1, 126.8 (C_{arenes}), 86.9 (d, *J* = 177.5 Hz, C-5a), 80.8 (C-3, C-4), 79.6 (d, J = 25.5 Hz, C-1) , 75.2 (O-<u>C</u>H_2Ph), 73.4 (O-<u>C</u>H_2Ph), 73.2 (O-<u>C</u>H_2Ph), 73.1 (O-<u>C</u>H_2Ph), 66.6 (C-6), 57.3 (C-2), 56.6 (C-2), 42.1 (d, *J* = 25.5 Hz, C-5). ^{19}F NMR (377 MHz, $CDCl_3$) δ [ppm] = -203.63 (dd, J = 46.9, 37.0 Hz). HPLC: t_R = 4.1 min (Kinetex® 5 µm C18 100Å, 250 x 21.2 mm 100 % MeCN, 30mL/min)

180: ^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] = 7.82 – 6.79 (m, 30H, H-arenes), 4.99 (m, 2H, 2x O-C<u>H</u>HPh), 4.89 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.82 – 4.67 (ddd, 1H, *J* = 51.4, 10.4, 8.7 Hz, H -1), 4.51 (m, 2H, 2x O-C<u>H</u>HPh), 4.43 – 4.34 (m, 2H, 2x O-C<u>H</u>HPh), 3.98 (d, 2H, *J* = 14.0 Hz, 2x N-C<u>H</u>HPh), 3.92 (d, 2H, *J* = 14.0 Hz, 2x N-C<u>H</u>HPh), 3.79 – 3.73 (m, 2H, 2x H-6), 3.69 (ddd, 1H, *J* = 12.6, 11.4, 8.7 Hz, H-5a), 3.65 – 3.55 (m, 2H, H-3, H-4), 3.07 (m, 1H, H-2), 1.57 (m, 1H, H-5). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] =139.9, 139.2, 138.6, 138.5, 138.1 ($C_{quart.}$), 128.9, 128.6, 128.5, 128.5, 128.3, 128.3, 128.2, 128.0, 127.9, 127.9, 127.8, 127.4, 127.2, 126.9 (C_{arenes}), 96.4 (d, *J* = 182.9 Hz, C-1), 81.8 (d, *J* = 9.7 Hz, C-3), 79.1 (C-4), 76.7 (d, *J* = 17.2 Hz, C-5a), 75.5 (O-<u>C</u>H_2Ph), 75.3 (O-<u>C</u>H_2Ph),74.4 (O-<u>C</u>H_2Ph), 73.4 (O-<u>C</u>H_2Ph), 64.3 (C-6), 60.9 (d, *J* = 15.7 Hz, C-2), 55.0 (2x N-<u>C</u>H_2Ph), 44.4 (d, *J* = 9.7 Hz, C-5). ^{19}F NMR (377 MHz, $CDCl_3$) δ [ppm] = -186.3 (dpt, *J* = 51.4, 11.4 Hz). HRMS (ESI) *m/z* calcd for $C_{49}H_{50}FNO_4$ 736.3797; [*M*+H^+] found: 736.3787. HPLC: t_R = 5.1 min (Kinetex® 5 µm C18 100Å, 250 x 21.2 mm 100 % MeCN, 30mL/min)

<u>(1*R*,2*R*,3*R*,4*S*,5*S*,6*R*)-6-amino-5-fluoro-3-(hydroxymethyl)cyclohexane-1,2,4-triol (182)</u>

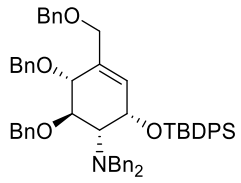


To a stirred solution of **180** (5.0 mg, 6.8 µmol) in MeOH abs. (1.5 mL) was added Pd/C (10% waterwet, 5.0 mg), $Pd(OH)_2$-C (20 % waterwet, 5.0 mg) and TFA (7.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 12 h under hydrogen atmosphere. The reaction mixture was centrifuged. The supernatant was purified by HILIC-HPLC to give compound **182** (1.1 mg, 5.6 µmol, 83 %) as a white powder after lyophilization.

^{1}H NMR (D_2O, 500 MHz) δ [ppm] =4.65 – 4.50 (ddd, 1H, *J* = 51.8, 10.3, 8.9 Hz, H-1), 3.93-3.91 (m, 2H, H-6), 3.88 (ddd, 1H, *J* = 13.4, 11.2, 8.9 Hz, H-5a), 3.62 – 3.53 (m, 2H, H-3, H-4), 3.37 (pq, 1H, *J* = 10.1 Hz, H-2), 1.58 – 1.52 (m, 1H, H-5). ^{13}C NMR (D_2O,

151 MHz, ^{19}F-coupled) δ [ppm] =93.1 (d, *J* = 180.8 Hz, C-1), 71.8 (d, *J* = 8.9 Hz, C-3), 69.1 (C-4), 67.4 (d, *J* = 17.8 Hz, C-5a), 55.9 (C-6), 54.0 (d, *J* = 17.1 Hz, C- 2), 45.3 (d, *J* = 7.2 Hz, C-5). ^{13}C NMR (D_2O, 126 MHz, ^{19}F-decoupled) δ [ppm] =93.2 (C-1), 71.8 (C-3), 69.1 (C-4), 67.4 (C-5a), 55.9 (C-6), 54.0 (C-2), 45.3(C-5) ^{19}F NMR (471 MHz, D_2O) δ [ppm] = -192.7 (dpt, *J* = 51.8, 11.7 Hz). HRMS (ESI) *m/z* calcd for $C_7H_{14}FNO_4$ 196.0980; [*M*+H$^+$] found: 196.0979. HPLC: t_R = 12.5 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 30 % isocrat. MeCN in 12 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

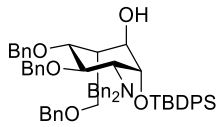
(1*R*,2*S*,5*R*,6*R*)-N,N-dibenzyl-5,6-bis(benzyloxy)-4-((benzyloxy)methyl)-2-((tert-butyldiphenylsilyl)oxy)cyclohex-3-en-1-amine (183)



Alcohol **110** (200.00 mg, 319.59 µmol), DMAP (108.79 mg, 1.60 mmol), imidazole (11.71 mg, 95.88 µmol.) and TBDPSCl (250.98 mmL, 263.53 mg) was dissolved in dry DMF (0.4 mL). The mixture was stirred for 3 days at 50 °C. The reaction was quenches with water (10 mL) and extracted with DCM (3 x 10 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (EA:PE, 1:1) and **183** was isolated as a colorless oil (240.00 mg, 143.5 µmol, 87 %)

^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ = 7.84 (dt, 2H, *J* = 6.8 Hz, *J* = 1.5 Hz), 7.77-7.69 (m, 7H), 7.69-7.66 (m, 2H), 7.52-7.07 (m, 25H), 5.37 (dd, 1H, *J* = 5.4 Hz, *J* = 1.5 Hz), 4.96 (d, 1H, *J* = 11.5 Hz), 4.76 (d, 1H, *J* = 11.5 Hz), 4.70 (s, 1H), 4.67-4.63 (m, 1H), 4.24-4.05 (m, 1H), 3.98 (d, 1H, *J* = 12.2 Hz), 3.48 (d, 1H, *J* = 12.2 Hz), 2.86 (dd, 1H, *J* = 10.7 , 3.2 Hz), 2.21 (s, 2H), 2.04 (s, 6H), 1.26 (t, 3H, *J* = 7.1 Hz), 1.06 (d, 3H, *J* = 3.9 Hz).

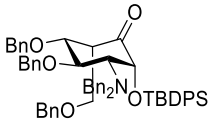
(3*R*,4*R*,5*R*,6*R*)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-6-((tert-butyldiphenylsilyl)oxy)-5-(dibenzylamino)cyclohexan-1-ol (184)



TBDPS-ether **183** (164.0 mg, 189.8 µmol) was dissolved in dry THF (2 mL). SMe_2BH_3 (569.3 µL, 2.9 mmol) was added and the mixture was stirred at room temperature. The mixture was quenched with water (20 mL) and $NaBO_3$*4 H_2O (437 mg) was added and the mixture was stirred for two hours. The mixture was extracted with EA (3x10 mL) dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (EA:PE, 1:10). The product **184** was isolated as a colorless oil (74.0 mg, 85.4 µmol, 45 %).

^{1}H NMR (600 MHz, $CDCl_3$, 300 K) δ = 7.78-7.75 (m, 2H, OTBDPS), 7.72-7.70 (m, 2H, OTBDPS), 7.29-7.06 (m, 25H, arenes), 4.57 (d, 1H, *J* = 11.4 Hz, CH_2-OBn), 4.37 (d, *J* = 6.2 Hz, 1H, CH_2-OBn), 4.35 (d, *J* = 6.6 Hz, 1H, CH_2-OBn), 4.32-4.37 (m, 1H, H-1), 4.22 (d, 1H, *J* = 11.6 Hz, CH_2-OBn), 4.16 (d, 1H, *J* = 11.6 Hz, CH_2-OBn), 4.01 (d, 1H, *J* = 14.2 Hz, H-5a), 3.93 (dd, 1H, *J* = 6.3, 3.3 Hz, H-3), 3.72 (d, 1H, *J* = 14.0 Hz, H-4), *J* = 3.68 (dd, 1H, *J* = 9.0 Hz, *J* = 7.1 Hz, C-6), 3.58 (d, 1H, *J* = 8.9, 7.0 Hz, H-6), 3.22 (dd, *J* = 6.3, 4.3 Hz, H-2), 1.97 (qd, 1H, *J* = 9.0 Hz, *J* = 7.1 Hz, H-5), 1.03 (s, 9H, Me-OTBDPS).
^{13}C NMR (151 MHz, $CDCl_3$, 300K) δ = 140.8, 138.3, 136.3, 134.8, 134.6, 133.3, 129.7, 128.8, 128.4, 128.2, 128.0, 137.7, 127.6, 127.3, 126.5 (C_{arenes}), 77.8 (C-4), 77.3 (C-3), 76.8 (C-1), 73.3 (OBn), 72.5 (OBn), 72.0 (OBn), 71.9 (C-5a), 69.8 (C-6), 60.4 (C-2), 55.9 (NBn), 43.4 (C-5), 29.6 (Me), 27.8 (Me), 26.4 (Me).

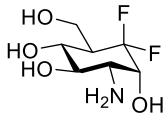
(2*R*,3*R*,4*R*,5*R*,6*R*)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-6-((tert-butyldiphenylsilyl)oxy)-5-(dibenzylamino)cyclohexan-1-one (185)



Alcohol **184** (16.00 mg, 18.14 µmol) was dissolved in dry DCM (2 mL). TPAP (2.00 mg, 1.81 mmol, 0.10 eq.) and NMO (4.25 mg, 36.27 mmol, 2.00 eq.) were added and the mixture was stirred for one hours. The mixture was filtrated with a 1:1 (EA:PE) mixture and the solvents were evaporated. The crude ketone was purified by HPLC and **185** was obtained as a clear oil (13.5 mg, 15.6 µmol, 86 %)

^{1}H NMR (400 MHz, $CDCl_3$, 300 K) δ = 7.64-7.59 (d, 2H, *J* = 7.25 Hz, OTBDPS), 7.58-7.52 (d,2H, *J* = 7.25 Hz, OTBDPS), 7.41-7.30 (m, 1H, OTBDPS), 7.25 (t, 3H, *J* = 7.8 Hz, OTBDPS), 7.20-7.03 (m, 25H, arenes), 7.00-6.95 (m, 2H), 6.86 (dd. 2H, *J* = 6.6, 3.0 Hz, OTBDPS), 4.79 (d, 1H, *J* = 1.4 Hz, H-1), 4.49 (d, 1H, *J* = 11.9 Hz, CH_2-OBn), 4.29-4.16 (m, 3H, CH_2-OBn), 3.97-3.91 (m, 4H, NBn), 3.98 (d, 1H, *J* = 4.0 Hz, H-4), 3.85 (d, 1H, *J* = 2.3 Hz, CH_2-OBn), 3.80 (d, 1H, *J* = 7.25 Hz, C-2), 3.69 (m, 1H, H-3), 3.53 (m, 2H, H-6), 2.69 (dt, 1H, *J* = 9.7, 4.8 Hz, H-5), 1.47 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$, 300K) δ = 207.0, 141.1,138.1, 137.3, 136.3, 135.8, 134.16, 133.1, 129.8, 128.2, 128.1, 128.0, 127.6, 127.5, 126.5 (C_{arenes}), 79.7 (C-4), 78.5 (C-3), 77.2 (C-1), 73.2 (CH_2-OBn), 73.1 (CH_2-OBn), 71.6 (CH_2-OBn), 66.0 (C-2), 65.0 (C-6), 56.5 (CH_2-NBn), 49.9 (C-5), 27.3 (Me). HPLC: t_R = 3.8 min (Kinetex® 5 µm C8 100Å, 250 x 21.2 mm, 100 % MeCN, 30 mL/min)

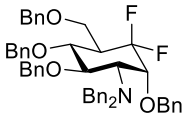
(1*R*,2*R*,3*R*,4*R*,6*R*)-3-amino-5,5-difluoro-6-(hydroxymethyl)cyclohexane-1,2,4-triol (187)



To a stirred solution of the 3:1 mixture **188 & 189** (30.0 mg, 6.8 µmol) in MeOH abs. (4 mL) was added Pd/C (10% waterwet, 30.0 mg), $Pd(OH)_2/C$ (20% waterwet, 30.0 mg) and TFA (20.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24 h under hydrogen atmosphere. The catalyst was filtrated off by using a syringe filter, which was washed with 20 mL of methanol. The solvent was evaporated under reduced pressure. The crude product was purified by HILIC-HPLC to give compound **187** (5.4 mg, 25.3 µmol, 64 %) as a white powder after lyophilization.

^{1}H NMR (D_2O, 500 MHz) δ [ppm] =4.27 (m,1H, H-1), 4.08 – 4.01 (m, 2H, H-6), 3.86 (dd, 1H, *J* = 10.9, 9.2 Hz, H-3), 3.61 (dd, 1H, *J* = 11.3, 9.2 Hz, H-4), 3.41 (m, 1H, H-2), 2.43 – 2.32 (m, 1H, H-5). ^{13}C NMR (D_2O, 151 MHz, ^{19}F-coupled) δ [ppm] =121.7 (dd, *J* = 252.8, 244.6 Hz, C-5a), 70.3 (C-3), 68.8 (d, *J* = 10.8 Hz, C-4), 67.7 (dd, *J* = 34.3, 26.8 Hz, C-1), 55.4 (d, *J* = 2.8 Hz, C-6), 52.2 (d, *J* = 9.7 Hz, C-2), 44.0 (t, *J* = 19.6 Hz, C-5). ^{13}C NMR (D_2O, 126 MHz, ^{19}F-decoupled) δ [ppm] =121.9 (C-5a), 70.4 (C-3), 69.0 (C-4), 68.0 (C-1), 55.6 (C-6), 52.3 (C-2), 44.2 (C-5). ^{19}F NMR (471 MHz, D_2O) δ [ppm] = -109.90 (d, *J* = 261.4 Hz), -114.53 (dd, *J* = 261.4, 30.4 Hz). HRMS (ESI) *m/z* calcd for $C_7H_{13}F_2NO_4$ 214.0885; [*M*+H^+] found: 214.0888. HPLC: t_R = 9.2 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 30 % isocrat. MeCN in 10 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

(1*R*,2*R*,4*R*,5*R*,6*R*)-N,N-dibenzyl-2,5,6-tris(benzyloxy)-4-((benzyloxy)methyl)-3,3-difluorocyclohexan-1-amine (188)

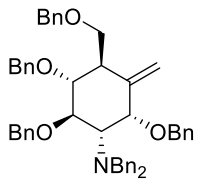


HPLC-purified ketone **159** (300 mg, 409.9 µmol) was dissolved in dry toluene (2 mL) in a Teflon reaction flask. DAST (541 µL, 4.1 mmol) was added followed by the addition of boron trifluoride diethyl etherate (67 µL, 409.9 µmol). The reaction flask was sealed and stirred overnight. The reaction mixture was slowly added into 1M aqueous NaOH solution (5 mL) and extracted with DCM (3x 2 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. HPLC purification resulted in a 3:1 mixture of the desired compound **188** and the elimination product **189** (47 mg, approximately 62 µmol, 15 %). In order to get purer spectra 10 mg of this mixture were dissolved in in MeOH abs. (1 mL). Pd/C (10% waterwet, 5.0 mg) was added. The reaction was purged with hydrogen. The reaction was stirred for 8 h under hydrogen atmosphere. The reaction mixture was filtered. The crude product was purified by HPLC and the compound **188** was obtained with a small amount (approximately 10 % based on ^{19}F NMR) of the by-product **189** still remaining. The spectra after hydrogenation are shown.

^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] = 7.46 – 7.05 (m, 30H), 5.01 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.86 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.76 (d, 1H, *J* = 10.6 Hz, O-C<u>H</u>HPh), 4.62 (m, 2H, 2x O-C<u>H</u>HPh), 4.53 (d, 1H, *J* = 12.3 Hz, O-C<u>H</u>HPh), 4.49 (d, 1H, *J* = 12.3 Hz, O-C<u>H</u>HPh), 4.23 (dd, 1H, *J* = 11.2, 8.6 Hz, H-3), 4.07 – 3.94 (m, 5H, H-1, 4x N-C<u>H</u>HPh), 3.84 (dd, 1H, *J* = 10.2, 4.3 Hz, 1x H-6), 3.80 (dd, 1H, *J* = 10.1, 2.1 Hz, 1x H-6), 3.74 (dd, 1H, *J* = 11.2, 8.6 Hz, H-4), 3.05 (d, 1H, *J* = 11.2 Hz, H-2), 2.61 – 2.47 (m, 1H, H-5). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] =140.3, 139.3, 138.4, 138.2, 137.5 ($C_{quart.\ arenes}$), 128.5, 128.5, 128.2, 128.0, 127.9, 127.8, 127.7, 127.4, 127.0, 126.9 (C_{arenes}), 80.7 (C-1), 80.2 (C-3), 79.4 (C-4), 75.3 (O-<u>C</u>H_2Ph), 75.0 (O-<u>C</u>H_2Ph), 73.4 (O-<u>C</u>H_2Ph), 73.1(O-<u>C</u>H_2Ph), 63.7 (C-6), 57.1 (C-2), 56.2 (2x N-<u>C</u>H_2Ph), 44.1 (C-5). ^{19}F NMR (471 MHz, $CDCl_3$) δ [ppm] = -108.9 (d, *J* = 256.3 Hz), -109.8 (dd, *J* = 256.3, 28.3 Hz).

HPLC: t_R = 3.6 min (Kinetex® 5 µm C18 100Å, 250 x 21.2 mm 100 % MeCN, 30 mL/min)

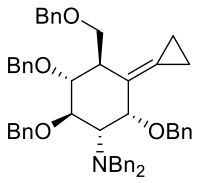
(1*S*,2*R*,3*R*,4*R*,6*S*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-methylenecyclohexan-1-amine (189)



Ketone **159** (269 mg, 367 µmol) was dissolved in dry toluene (3 mL) and dimethyl titanocene (5 % in toluene, 2.6 mL, 735.04 µmol) was added. The reaction was put in a microwave reactor for 1.5 h at 300 W and a limit of 120 °C. After cooling to rt the reaction as diluted with DCM (15 mL) and washed with saturated NH_4Cl_{aq} (3x 5 mL) and water (2x 10 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified using flash column chromatography on silica gel (PE:EE 10:1 to 8:1) and the product **189** was isolated as a colorless oil (159.0 mg, 217.2 µmol, 59 %).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.50 – 7.02 (m, 30H, arenes), 5.15 (m, H alkene), 5.06 – 4.98 (m, 2H, H alkene, O-C<u>H</u>HPh), 4.91 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.79 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.57 (d,1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.54 (d, 1H, *J* = 12.0 Hz, O-C<u>H</u>HPh), 4.47 (d, 1H, *J* = 12.0 Hz, O-C<u>H</u>HPh), 4.45 – 4.39 (m, 2H, H-3), 4.31 (m, 1H, O-C<u>H</u>HPh), 4.19 (d, 1H, *J* = 2.5 Hz, H-1), 4.10 – 4.00 (m, 4H, 2 x N-C<u>H</u>$_2$Ph), 3.88 (dd, 1H, *J* = 9.5, 5.0 Hz, H-6), 3.76 (dd, 1H, *J* = 9.5, 1.9 Hz, H-6), 3.55 (dd, 1H, *J* = 11.1, 8.4 Hz, H-4), 2.82 (dd, 1H, *J* = 10.9, 2.5 Hz, H-2), 2.75 – 2.65 (m, 1H, H-5). ^{13}C NMR (121 MHz, $CDCl_3$) δ = 143.3 (C-alkene), 141.1, 139.7, 138.8, 138.4, 138.1, 128.6, 128.5, 128.5, 128.4, 128.2, 128.2, 128.1, 127.9, 127.9, 127.6, 127.5, 127.2, 127.0, 126.6 (C_{arenes}), 113.6 (CH_2-alkene), 84.2 (C-1), 82.6 (C-4), 81.0 (C-3), 74.9 (O-<u>C</u>H$_2$Ph), 73.5 (O-<u>C</u>H$_2$Ph), 72.8 (O-<u>C</u>H$_2$Ph), 69.8 (O-<u>C</u>H$_2$Ph), 66.9(C-6), 61.8 (C-2), 56.3 (2 x N-<u>C</u>H$_2$Ph), 42.9 (C-5).

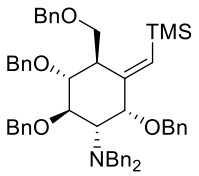
(1*S*,2*R*,3*R*,4*R*,6*S*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-cyclopropylidenecyclohexan-1-amine (194)



Ketone **159** (100 mg, 136.6 µmol) was dissolved in dry toluene (5 ml). Biscyclopropyl titanocene (142.2 mg, 546.5 µmol) was added and the reaction mixture was heated to 80 °C for five hours. After cooling to room temperature, the solution was diluted with PE and filtered through Celite and silica. The crude product was purified by column chromatography on silica (PE/EE, 50:1) and HPLC, respectively. Compound **194** was obtained as a colorless solid (12.4 mg 16.4 µmol, 12 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.45-7.09 (m, 30H, HAr), 5.03 (d, 1H, *J* = 11.60 Hz, HCH_2Bn), 4.89 (d, 1H, *J* = 11.52 Hz, O-C<u>H</u>HPh), 4.75 (d, 1H, *J* = 10.56 Hz, O-C<u>H</u>HPh), 4.56 (d, 1H, *J* = 11.63 Hz, O-C<u>H</u>HPh), 4.51-4.43 (m, 4H, H1, H4, CH_2Bn), 4.37 (d, 1H, *J* = 11.45 Hz, O-C<u>H</u>HPh), 4.30 (d, 1H, *J* = 11.44 Hz, O-C<u>H</u>HPh), 4.06 (d, 2H, *J* = 14.53 Hz, $N(CH_2Ph)_2$), 4.02 (d, 2H, *J* = 14.53 Hz, $N(CH_2Ph)_2$), 3.95 (dd, 1H, *J* = 9.0 Hz, 5.3 Hz, H6/H6'), 3.84 (dd, 1H, *J* = 8.99 Hz, 1.93 Hz, H6/H6'), 3.56 (dd, 1H, *J* = 11.06 Hz, 8.48 Hz, H3),2.85-2.80 (m, 1H, H5), 2.78 (dd, 1H, *J* = 10.94 Hz, 2.57 Hz, H2), 1.30-1.24 (m, 1H, Hcypr),1.17-1.10 (m, 1H, Hcypr), 0.96-0.89 (m, 1H, Hcypr), 0.77-0.72 (m, 1H, Hcypr). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 141.3-123.1 (CBn), 120.5 (C5a), 83.1 (C4), 82.7 (C3), 81.1 (C1), 74.9-69.8 (CBn), 67.1 (C6), 61.6 (C2), 56.3 (CBn), 43.0 (C5), 27.1-14.2 (CBn), 4.1 (CCypr), 0.2 (CCypr). HRMS: *m*/*z* calcd for $C_{52}H_{53}NO_4$ = 756.4047 g/mol, $[M+H^+]_{found}$ = 756.4042 g/mol. HPLC t_R = 4.1 min (Kinetex® 5 µm C8 100Å, 250 x 21.2 mm, 90 % - 100 % MeCN in 1 min followed by 100 % MeCN)

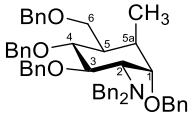
(1*S*,2*R*,3*R*,4*R*,6*S*,*E*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-((trimethylsilyl)methylene)cyclohexan-1-amine (195)



Di(TMS-methyl) titanocene (192.9 mg, 546.5 µmol) was dissolved in dry toluene (1.5 ml) and Ketone **159** (100.0 mg, 136.6 µmol) in dry toluene (0.5 ml) was added. The reaction mixture was heated to 100 °C for one hour. The reaction progress was monitored by LC-MS. After completion of the reaction, the mixture was diluted with PE and filtered through Celite and silica. The crude product was purified by column chromatography on silica (PE/EE, 50:1) and HPLC, respectively. Compound **195** was obtained as a colorless oil (18.0 mg, 22.4 µmol, 17 %)

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.54-7.10 (m, 30H, HAr), 5.95 (s, 1H, Hα'), 4.79 (d, 1H, *J* = 11.12 Hz, O-C<u>H</u>HPh), 4.75 (dd, 2H, *J* = 11.35 Hz, 11.15 Hz, O-C<u>H</u>HPh), 4.65 (d, 1H, *J* = 11.12 Hz, O-C<u>H</u>HPh), 4.43 (d, 1H, *J* = 11.33 Hz, O-C<u>H</u>HPh), 4.35-4.25 (m, 4H, H1, O-C<u>H</u>HPh), 4.08 (d, 2H, *J* = 14.30 Hz, $N(CH_2Ph)_2$), 4.00 (d, 2H, *J* = 14.3 Hz, $N(CH_2Ph)_2$), 3.88 (dd, 1H, *J* = 9.37 Hz, 7.97 Hz, H4), 3.74 (dd, 1H, *J* = 9.37 Hz, 6.72 Hz, H3), 3.58 (dd, 1H, *J* = 8.52 Hz, 3.40 Hz, H6/H6'), 3.51 (dd, 1H, *J* = 8.52 Hz, 4.25 Hz, H6/H6'), 3.38 (dd, 1H, *J* = 6.73 Hz, 0.90 Hz, H2), 2.75 (ddd, 1H, *J* = 7.82 Hz, 3.64 Hz, 4.46 Hz, H5), 0.10 (s, 9H, HTMS). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 154.5 (C5a), 141.2-126.6 (CBn), 122.3 (Cα'), 83.4 (C3), 81.8 (C1), 77.7 (C4), 74.6 (CBn); 74.4 (CBn), 73.3 (CBn), 72.7 (C6), 71.8-56.1 (CBn), 46.6 (C5), 0.5 (CTMS). HRMS: *m/z* calcd for $C_{52}H_{59}NO_4Si$ = 806.4286 g/mol, $[M+H^+]_{found}$ = 806.4262g/mol. HPLC t_R = 7.9 min (Kinetex® 5 µm C8 100Å, 250 x 21.2 mm, 90 % - 100 % MeCN in 1 min followed by 100 % MeCN)

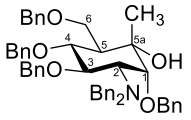
(1*S*,2*R*,3*R*,4*R*,5*R*,6*S*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-methylcyclohexan-1-amine (196)



To a stirred solution of **202** (60.0 mg, 71.9 µmol) in toluene abs. (2 mL) was added AIBN (71.9 µL, 14.4 µmol, 0.2 M in toluene) and tributyltin hydride (38.8 µL, 143.8 µmol). The reaction mixture was heated to 100 °C and stirred for 2 h. The crude product was purified using column chromatography (petroleum ether / EtOAc = 18:1 – 14:1) to give compound **196** (25 mg, 34.2 µmol, 47%) as a colourless oil.

R_f: 0.43 (petroleum ether / EtOAc = 10:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.50 – 7.15 (m, 30H, arenes), 5.03 (d, 1H, *J* = 11.5 Hz, O-C<u>H</u>HPh), 4.93 (d, 1H, *J* = 11.5 Hz, O-C<u>H</u>HPh), 4.76 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.62 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.52 – 4.41 (m, 4H, 2 x O-C<u>H</u>$_2$Ph), 4.25 (t, 1H, *J* = 9.6 Hz, H-3), 4.06 (q, 4H, *J* = 13.9 Hz, 2 x N-C<u>H</u>$_2$Ph), 3.75 – 3.66 (m, 2H, H-1, H-6a), 3.56 – 3.47 (m, 2H, H-4, H-6b), 2.99 (d, 1H, *J* = 11.0 Hz, H-2), 2.53 – 2.36 (m, 2H, H-5, H-5a), 0.61 (d, 3H, *J* = 7.1 Hz, C-C<u>H</u>$_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 141.3, 139.8, 138.7, 138.7 ($C_{quart.}$), 128.7, 128.5, 128.4, 128.4, 128.3, 128.2, 127.9, 127.9, 127.7, 127.7, 127.6, 127.5, 127.2, 127.2, 126.6 (arenes), 85.1 (C-1), 81.9, (C-3), 81.9 (C-4), 74.8, 73.3, 73.0, 71.3 (O-<u>C</u>H$_2$Ph), 69.2 (C-6), 57.1 (C-2), 56.5 (2 x N-<u>C</u>H$_2$Ph), 39.7 (C-5), 31.3 (C-5a), 12.2 (C-<u>C</u>H$_3$); HRMS (ESI) *m*/*z* calcd for $C_{50}H_{53}NO_4$ 732.4047; [*M*+H$^+$] found: 732.4043.

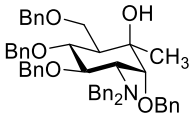
(1*S*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)-1-methylcyclohexan-1-ol (198)



To a stirred solution of **159** (280.0 mg, 382.6 µmol) in THF abs. (5 mL) at 0 °C was added methyl magnesium chloride (30.04 mg, 401.7 µmol, 133.9 µL, 3 M in THF). The solution was stirred for 30 min. The reaction was stopped by adding sat. NH_4Cl_{aq} (3 mL) and water (2 mL). The aqueous layer was washed with CH_2Cl_2 (3 x 3 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether / EtOAc = 12:1 – 8:1) to give compound **198** (160.0 mg, 213.9 µmol, 56 % over 3 steps) as a colourless oil.

R_f: 0.73 (petroleum ether : EtOAc = 5:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.55 - 7.20 (m, 30H, arenes), 5.09 (d, 2H, *J* = 11.3 Hz, O-C$\underline{H_2}$Ph), 4.97 (d, 1H, *J* = 11.6 Hz, O-C$\underline{H}$HPh), 4.89 – 4.79 (m, 2H, O-C$\underline{H_2}$Ph), 4.60 – 4.54 (m, 2H, O-C$\underline{H_2}$Ph), 4.48 (d, 1H, *J* = 11.6 Hz, O-C$\underline{H}$HPh), 4.34 (dd, 1H, *J* = 11.2 Hz, 8.6 Hz, H-3), 4.14 - 4.03 (m, 4H, 2 x N- C$\underline{H_2}$Ph), 3.95 (dd, 1H, *J* = 9.3 Hz, 3.0 Hz, H-6a), 3.75 - 3.69 (m, 2H, H-6b, H-1), 3.47 - 3.39 (m, 2H, H-4, O-$\underline{H}$), 2.98 (dd,1H, *J* = 11.2 Hz, 2.0 Hz, H-2), 2.51 (ddd, 1H, *J* = 11.1 Hz, 8.0 Hz, 3.0 Hz, H-5), 0.95 (s, 3H, C-C$\underline{H_3}$); ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 140.7, 139.4, 138.9, 138.5, 137.8 (arenes, $C_{quart.}$), 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.5, 127.5, 127.2, 127.0, 126.7 (arenes), 88.2 (C-1), 82.0 (C-4), 81.4 (C-3), 75.6 (O-$\underline{C}H_2$Ph), 74.9 (C-5a), 74.7, 73.5, 73.1 (3 x O-$\underline{C}H_2$Ph), 68.0 (C-6), 57.6 (C-2), 56.1 (2 x N-$\underline{C}H_2$Ph), 46.2 (C-5), 22.3 (C-$\underline{C}H_3$); HRMS: $[M+H^+]_{calculated}$ = 748.3997 g/mol, $[M+H^+]_{found}$ = 784.4012 g/mol.

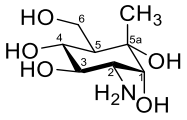
(1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)-1-methylcyclohexan-1-ol (199)



To a stirred solution of **159** (84.0 mg, 114.8 µmol) in THF abs. (3 mL) at 0 °C was added $LaCl_3$ x LiCl (37.9 mg, 114.8 µmol, 191.3 µL, 0.6 M in THF) and the reaction was stirred for 10 min. To the reaction mixture was added methyl magnesium chloride (9.0 mg, 120.5 µmol, 40.2 µmol, 3 M in THF) and the reaction was stirred for 30 min. The reaction was stopped by adding sat. NH_4Cl_{aq} (2 mL) and water (1 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 3 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified using column chromatography (petroleum ether / EtOAc = 12:1 – 8:1) to give compound **199** (39.6 mg, 52.8 µmol, 46 %, over 3 steps) as a colourless oil.

R_f: 0.79 (petroleum ether / EtOAc = 5:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.49 - 7.04 (m, 30H, arenes), 5.11 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.94 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.85 (d, 2H, *J* = 10.8 Hz, O-C<u>H$_2$</u>Ph), 4.69 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.53 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.44 (q, 2H, *J* = 11.6 Hz, O-C<u>H$_2$</u>Ph), 4.15 (dd, 1H, *J* = 11.0 Hz, 8.6 Hz, H-3), 4.09 (d, 3H, *J* = 4.4 Hz, N-C<u>H$_2$</u>Ph, N-C<u>H</u>HPh), 4.05 - 3.95 (m, 3H, H-6a, H-4, N-C<u>H</u>HPh), 3.75 (dd, 1H, *J* = 9.5 Hz, 2.7 Hz, H-6b), 3.68 (s, 1H, O-<u>H</u>), 3.62 (d, 1H, *J* = 2.6 Hz, H-1), 3.53 (dd, 1H, *J* = 11.0 Hz, 2.6 Hz, H-2), 1.98 – 1.93 (m, 1H, H-5), 1.31 (s, 3H, C-C<u>H$_3$</u>); ^{13}C NMR (101 MHz, $CDCl_3$): δ [ppm] = 141.0, 139.7, 138.6, 138.4, 137.1 (arenes, $C_{quart.}$), 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 127.7, 127.5, 127.3, 127.1, 127.0, 126.9, 126.4 (arenes), 87.8 (C-1), 81.8 (C-3), 80.5 (C-4), 75.1 (C-5a), 75.0, 73.9, 72.6 (O-<u>C</u>H$_2$Ph), 67.2 (C-6), 57.4 (C-2), 56.3 (2 x N-<u>C</u>H$_2$Ph), 45.0 (C-5), 25.7 (C-<u>C</u>H$_3$); HRMS: $[M+H^+]_{calculated}$ = 748.3997 g/mol, $[M+H^+]_{found}$ = 748.4012 g/mol.

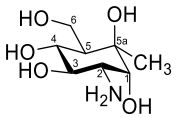
(1*S*,2*R*,3*R*,4*R*,5*R*,6*R*)-3-amino-6-(hydroxymethyl)-1-methylcyclohexane-1,2,4,5-tetraol (200)



To a stirred solution of **198** (20.0 mg, 26.7 µmol) in MeOH abs. (3 mL) was added Pd/C (10% waterwet, 20.0 mg), $Pd(OH)_2/C$ (10 % waterwet, 20.0 mg) and TFA (10.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24 h under hydrogen atmosphere. The reaction mixture was centrifuged. The supernatant was purified by HILIC-HPLC to give compound **200** (3.0 mg, 14.5 µmol, 54 %) as a white powder after lyophilization.

^{1}H NMR (600 MHz, D_2O): δ [ppm] = 3.84 (dd, 2H, *J* = 5.0 Hz, 1.7 Hz, H-6a, H-6b), 3.52 (d, 1H, *J* = 2.8 Hz, H-1), 3.44 (dd, 1H, *J* = 10.7 Hz, 8.9 Hz, H-4), 3.36 (dd, 1H, *J* = 11.5 Hz, 8.9 Hz, H-3), 2.83 – 2.79 (m, 1H, H-2), 1.86 (dt, 1H, *J* = 10.7 Hz, 5.0 Hz, H-5), 1.08 (s, 3H, C$\underline{H_3}$); ^{13}C NMR (151 MHz, D_2O): δ [ppm] = 76.3 (C-1), 74.1 (C-4), 73.1 (C-5a), 72.1 (C-3), 62.4, 59.5 (C-6), 52.8 (C-2), 46.0 (C-5), 20.1 ($\underline{C}H_3$); HRMS: $[M+H^+]_{calculated}$ = 208.1179 g/mol, $[M+H^+]_{found}$ = 208.1183 g/mol. HPLC: t_R = 11.2 min (Phenomenex® Luna 5µ HILIC 200Å Pa, 250 x 21.20 mm, 30 % MeCN isocrat. in 12 mM TEAB buffer pH = 7.0, 8 mL/min, ELSD)

(1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-3-amino-6-(hydroxymethyl)-1-methylcyclohexane-1,2,4,5-tetraol (201)

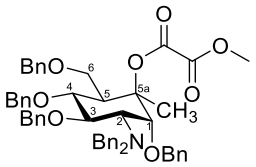


To a stirred solution of **199** (20.0 mg, 26.7 µmol) in MeOH abs. (3 mL) was added Pd/C (10% waterwet, 20.0 mg), $Pd(OH)_2/C$ (10% waterwet, 20.0 mg) and TFA (10.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24h

under hydrogen atmosphere. The reaction mixture was filtered over celite. The crude product was purified by HILIC-HPLC to give compound **201** (3.2 mg, 15.4 µmol, 58 %) as a white powder.

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 3.99 – 3.84 (m, 2H, H-6a, H-6b), 3.58 (dd, 1H, *J* = 11.8 Hz, 9.1 Hz, H-4), 3.46 (dd, 1H, *J* = 3.1 Hz, 1.1 Hz, H-1), 3.37 (ddd, 1H, *J* = 10.4 Hz, 9.1 Hz, 1.1 Hz, H-3), 3.09 – 3.03 (m, 1H, H-2), 1.56 – 1.50 (m, 1H, H-5), 1.30 (s, 3H, C-C$\underline{H_3}$);^{13}C NMR (126 MHz, D_2O): δ [ppm] = 75.9 (C-1), 75.0 (C-5a), 74.2 (C-3), 70.7 (C-4), 58.9 (C-6), 52.5 (C-2), 45.6 (C-5), 24.4 (C-$\underline{C}H_3$); HRMS: $[M+H^+]_{calculated}$ = 208.1179 g/mol, $[M+H^+]_{found}$ = 208.1181 g/mol. HPLC: t_R = 10.5 min (Phenomenex® Luna 5µ HILIC 200Å Pa, 250 x 21.20 mm, 30 % MeCN isocrat. in 12 mM TEAB buffer pH = 7.0, 8 mL/min, ELSD)

<u>methyl ((1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)-1-methylcyclohexyl) oxalate (202)</u>

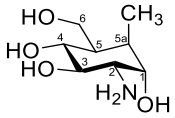


To a stirred solution of **201** (22.0 mg, 29.4 µmol) in CH_2Cl_2 abs. (3 mL) was added 4-dimethyl amino pyridine (2.16 mg, 17.65 µmol) and methyl oxalyl chloride (10.8 mg, 88.2 µmol, 8.1 µL). The reaction mixture was stirred for 24 h. The reaction was stopped by adding NH_4Cl_{aq} (2 mL) and water (2 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 2 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified using column chromatography (petroleum ether / EtOAc = 20:1 – 16:1) to give compound **202** (18.6 mg, 22.4 µmol, 76 %) as a colourless oil.

R_f: 0.58 (petroleum ether / EtOAc = 8:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 7.44 – 7.02 (m, 30H), 5.06 (d, 1H, *J* = 11.6 Hz), 4.92 (d, 1H, *J* = 11.6 Hz), 4.84 – 4.76 (m, 3H), 4.60 – 4.52 (m, 2H), 4.40 (s, 2H), 4.25 (dd, 1H, *J* = 11.1 Hz, 8.7 Hz), 4.12 (dq, 2H, *J* = 14.3 Hz, 4.8 Hz), 4.01 (d, 2H, 14.3 Hz), 3.76 (dd, 1H, *J* = 10.2 Hz, 6.0 Hz),

3.71 – 3.62 (m, 5H), 3.00 (dd, 1H, *J* = 11.0 Hz, 2.2 Hz), 2.31 (ddd, 1H, *J* = 11.0 Hz, 6.0 Hz, 2.2 Hz), 1.80 (s, 3H), 1.25 (s, 1H); ^{13}C NMR (151 MHz, D_2O): δ [ppm] = 157.5, 155.3 (C=O), 140,3, 139.3, 138.5, 138.5, 137.8 ($C_{quart.}$), 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.2, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.2, 126.7 (arenes), 83.3 (C-1), 81.2 (C-4), 81.1 (C-3), 75.6, 75.2, 74.2, 73.4 (O-CH_2Ph), 66.4 (C-6), 57.0 (C-2), 56.5 (2 x N-CH_2Ph), 53.3 (O-CH_3), 47.3 (C-5), 22.7 (C-CH_3); HRMS: $[M+H^+]_{calculated}$ = 834.4000 g/mol, $[M+H^+]_{found}$ = 834.4016 g/mol.

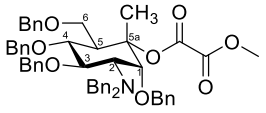
(1*R*,2*R*,3*S*,4*S*,5*R*,6*R*)-3-amino-6-(hydroxymethyl)-5-methylcyclohexane-1,2,4-triol (203)



A stirred solution of **194** (18.00 mg, 24.59 µmol) in MeOH abs. (3 mL) Pd/C (10 % waterwet, 20.00 mg), $Pd(OH)_2$/C (10 % waterwet, 20.00 mg) and TFA (14.9 µg, 0.13 µmol, 10.0 µL) was put under H_2 atmosphere. The reaction was stirred for 24 h. The reaction mixture was centrifugated. The supernatant was purified by HILIC-HPLC to give compound **203** (2.00 mg, 10.46 µmol, 43 %) as a white powder after lyophilization.

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 3.83 (ddd, 1H, *J* = 1.1 Hz, 5.1 Hz, 11.2 Hz, H-6a), 3.93 - 3.90 (m, 1H, H-1), 3.52 (ddd, 1H, *J* = 1.2 Hz, 9.5 Hz, 11.0 Hz, H-6b), 3.42 – 3.33 (m, 2H, H-3, H-4), 2.97 (dd, *J* = 1.4 Hz, 10.1 Hz, H-2), 2.14 – 2.07 (m, 1H, H-5a), 2.03 – 1.96 (m, 1H, H-5), 0.92 (d, 3H, *J* = 7.5 Hz, C-CH_3); ^{13}C NMR (126 MHz, D_2O): δ [ppm] = 75.0 (C-3), 73.4 (C-1), 71.4 (C-4), 60.9 (C-6), 52.7 (C-2), 40.7 (C-5), 35.1 (C-5a), 10.8 (C-CH_3); HRMS: $[M+H^+]_{calculated}$ = 192.1230 g/mol, $[M+H^+]_{found}$ = 192.1229 g/mol. HPLC: t_R = 12.8 min (Phenomenex® Luna 5µ HILIC 200Å Pa, 250 x 21.20 mm, 30 % MeCN isocrat. in 12 mM TEAB buffer pH = 7.0, 8 mL/min, ELSD)

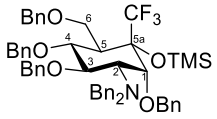
methyl ((1S,2R,3R,4R,5R,6R)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)-1-methylcyclohexyl) oxalate (204)



To a stirred solution of **198** (100.0 mg, 133.7 mmol) in THF abs. (4 mL) at -78 °C was added *n*-butyl-lithium (11.13 mg, 173.8 mmol, 69.52 µL, 2.5 M in hexane). After stirring for 10 min methyl oxalyl chloride (49.13 mg, 401.1 mmol, 36.94 µL) was added to the solution. The reaction mixture was allowed to reach room temperature and was stirred for 72 h. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified using column chromatography (petroleum ether / EtOAc = 16:1 – 12:1) to give compound **204** (64.9 mg, 77.8 µmol, 58 %) as a colourless oil.

R_f: 0.49 (petroleum ether / EtOAc = 8:1); ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm] = 7.39 – 6.98 (m, 30H, arenes), 4.97 (d, 1H, *J* = 11.5 Hz, O-C<u>H</u>HPh), 4.80 (d, 1H, *J* = 11.5 Hz, O-C<u>H</u>HPh), 4.68 (d, 1H, *J* = 11.3 Hz, O-C<u>H</u>HPh), 4.62 (t, 2H, *J* = 11.0 Hz, O-C<u>H$_2$</u>Ph), 4.55 (d, 1H, *J* = 1.6 Hz, H-1), 4.48 (d, 1H, *J* = 11.4 Hz, O-C<u>H</u>HPh), 4.34 (q, 2H, *J* = 11.3 Hz, O-C<u>H$_2$</u>Ph), 4.18 (dd, 1H, *J* = 8.5 Hz, 11.2 Hz, H-3), 4.00 – 3.90 (m, 4H, 2 x N-C<u>H$_2$</u>Ph), 3.86 – 3.83 (m, 1H, H-6a), 3.77 (dd, 1H, *J* = 1.0 Hz, 11.4 Hz, H-6b), 3.67 - 3.70 (m, 4H, H-4, O-C<u>H$_3$</u>), 2.78 (dd, 1H, *J* = 1.6 Hz, 11.2 Hz, H-2), 2.50 (dd, 1H, *J* = 3.0 Hz, 11.5 Hz, H-5), 1.24 – 1.20 (m, 3H, C- C<u>H$_3$</u>); ^{13}C NMR (151 MHz, $CDCl_3$): δ [ppm] = 158.4, 157.0 (<u>C</u>=O), 140.4, 139.4, 138.7, 138.3, 138.2 ($C_{quart.}$), 128.6, 128.5, 128.5, 128.4, 128.3, 127.8, 127.7, 127.7, 127.7, 127.5, 127.5, 127.4, 127.1, 126.9 (arenes), 84.2 (C-1), 81.3 (C-3), 80.3 (C-4), 75.0, 74.9 (O-<u>C</u>H$_2$Ph), 73.3, 73.3 (O-<u>C</u>H$_2$Ph), 65.6 (C-6), 57.6 (C-2), 56.2 (2 x N-<u>C</u>H$_2$Ph), 53.5 (O-<u>C</u>H$_3$), 45.6 (C-5), 20.6 (C-<u>C</u>H$_3$); HRMS: $[M+H^+]_{calculated}$ = 834.4000 g/mol, $[M+H^+]_{found}$ = 834.4013 g/mol.

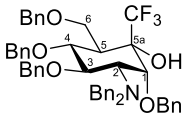
(1*R*,2*R*,3*S*,4*R*,5*R*,6*R*)-N,N-dibenzyl-2,5,6-tris(benzyloxy)-4-((benzyloxy)methyl)-3-(trifluoromethyl)-3-((trimethylsilyl)oxy)cyclohexan-1-amine (205)



To a stirred solution of **159** (152.0 mg, 207.7 µmol) in THF abs. (3 mL) at 0 °C was added trifluoromethyl trimethyl silane (59.1 mg, 415.3 µmol, 61.5 µL) and TBAF (5.4 mg, 20.8 µmol, 20.8 µL, 1 M in THF). The reaction mixture was allowed to reach rt and was stirred for 15 min. The reaction was stopped by adding water. The aqueous layer was extracted with CH_2Cl_2 (3 x 4 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by HPLC to give compound **205** (41.2mg, 47.1 µmol, 23 %, over 3 steps) as a colourless oil.

R_f: 0.46 (petroleum ether / EtOAc = 8:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.46 – 7.08 (m, 30H, arenes), 5.07 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.99 (d, 1H, *J* = 10.6 Hz, O-C<u>H</u>HPh), 4.90 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.72 (d, 3H, *J* = 10.6 Hz, O-C<u>H</u>HPh, O-C<u>H$_2$</u>Ph), 4.41 – 4.26 (m, 2H, O-C<u>H$_2$</u>Ph), 4.31 (dd, 1H, *J* = 11.1 Hz, 8.5 Hz, H-3), 4.10 – 3.91 (m, 7H, H-4, H-6a, H-1, 2 x N- C<u>H$_2$</u>Ph), 3.72 (dd, 1H, *J* = 9.8 Hz, 1.4 Hz, H-6b), 3.09 (dd, 1H, *J* = 11.1 Hz, 1.9 Hz, H-2), 2.53 (dd, 1H, *J* = 11.1 Hz, 4.1 Hz, H-5), 0.16 (s, 9H, Si(C<u>H$_3$</u>)$_3$); ^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 140.5, 139.5, 138.9, 138.2, 138.2 (arenes, $C_{quart.}$), 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.1, 127.9, 127.8, 127.6, 127.6, 127.5, 127.3, 127.2, 127.0, 126.8 (arenes), 124.1 (<u>C</u>F$_3$), 83.7 (C-1), 81.1 (C-3), 80.3 (C-5a), 80.1 (C-4), 75.6, 74.9, 73.3, 73.0 (O-<u>C</u>H$_2$Ph), 64.5 (C-6), 56.5, (C-2), 56.3 (2 x N-<u>C</u>H$_2$Ph), 46.3 (C-5), 2.6 (Si(<u>C</u>H$_3$)$_3$); ^{19}F NMR (471 MHz, $CDCl_3$): δ [ppm] = -68.81 (C<u>F$_3$</u>); HPLC: t_R = 4.7 min (Kinetex® 5 µm C8 100Å, 250 x 21.2 mm, 90 % - 100 % MeCN in 1 min followed by 100 % MeCN for 7 min); HRMS: $[M+H^+]_{calculated}$ = 874.4109 g/mol, $[M+H^+]_{found}$ = 874.4122 g/mol.

(1*S*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)-1-(trifluoromethyl) cyclohexan-1-ol (206)



Method A:

To a stirred solution of **159** (150.0 mg, 204.9 µmol) in THF abs. (3 mL) at 0 °C was added trifluoromethyl trimethyl silane (50.3 mg, 415.3 µmol, 60.5 µL) and TBAF (5.4 mg, 20.8 µmol, 20.4 µL, 1 M in THF). The reaction mixture was allowed to reach rt and was stirred for 15 min. The reaction was stopped by adding water. The aqueous layer was extracted with CH_2Cl_2 (3 x 4 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in THF and TBAF (58.9.4 mg, 225.3 µmol, 225.3 µL, 1 M in THF) was added the reaction was stirred for 1 h. The reaction was stopped by adding water The aqueous layer was extracted with CH_2Cl_2 (3 x 4 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (petroleum ether / EtOAc = 10:1 – 5:1) to give compound **206** (95.0 mg, 118.5 µmol, 58 %) as a colourless oil.

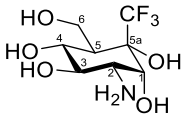
Method B:

To a stirred solution of **205** (41.2 mg, 47.1 µmol) in THF abs. (5 mL) was added TBAF (12.6 mg, 48.2 µmol, 48.2 µL, 1 M in THF). The reaction mixture was stirred for 1.5 h. The reaction was stopped by adding water. The aqueous layer was extracted with CH_2Cl_2 (3 x 3 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified using column chromatography (petroleum ether / EtOAc = 12:1 – 8:1) to give compound **206** (22.8 mg, 28.4 µmol, 60 %) as a colourless oil.

R_f:0.46 (petroleum ether / EtOAc = 6:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.44 – 7.05 (m, 30H, arenes), 5.15 (d, 1H, *J* = 10.3 Hz, O-C<u>H</u>HPh), 4.96 – 4.86 (m, 2H, O-C<u>H$_2$</u>Ph), 4.72 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.64 (d, 1H, *J* = 10.3 Hz, O-C<u>H</u>HPh), 4.53 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.39 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.33 (d, 1H,

10.8 Hz, O-C<u>H</u>HPh), 4.31 – 4.26 (m, 1H, H-3), 4.07 (d, 1H, *J* = 1.9 Hz, H-1), 4.04 – 3.97 (m, 5H, H-6a, 2 x N- C<u>H</u>$_2$Ph), 3.62 – 3.54 (m, 1H, H-6b), 3.45 (dd, 1H, *J* = 11.8 Hz, 8.3 Hz, H-4), 3.01 (dd, 1H, *J* = 11.2 Hz, 1.9 Hz, H-2), 2.93 – 2.84 (m, 1H, H-5), 1.44 (s, 1H, O-<u>H</u>); ^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 140.3, 139.2, 138.6, 137.8, 136.8 (arenes, $C_{quart.}$), 128.7, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.8, 127.6, 127.3, 127.0, 126.9, 126.8 (arenes), 125.6, 126.6 (<u>C</u>F$_3$), 80.5 (C-1), 80.3 (C-3, C-4), 80.0, 79.8 (C-5a), 75.9, 75.1, 74.0, 73.0 (O-<u>C</u>H$_2$Ph), 69.2 (C-6), 56.5 (C-2), 56.1 (2 x N-<u>C</u>H$_2$Ph), 43.6 (C-5); ^{19}F NMR (377 MHz, $CDCl_3$): δ [ppm] = -70.95 (C<u>F$_3$</u>); HRMS: $[M+H^+]_{calculated}$ = 802.3714 g/mol, $[M+H^+]_{found}$ = 802.3724 g/mol.

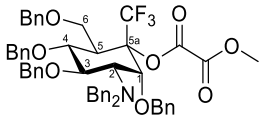
(1*S*,2*R*,3*R*,4*R*,5*R*,6*R*)-3-amino-6-(hydroxymethyl)-1-(trifluoromethyl) cyclohexane-1,2,4,5-tetraol (207)



To a stirred solution of **206** (20.0 mg, 26.7 µmol) in MeOH abs. (3 mL) was added Pd/C (10 % waterwet, 20.0 mg), Pd(OH)$_2$/C (10 % waterwet, 20.0 mg) and TFA (10.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24h under hydrogen atmosphere. The reaction mixture was centrifuged. The supernatant was purified by HILIC-HPLC to give compound **207** (2.8 mg, 10.7 µmol, 43 %) as a white powder after lyophilization.

^{1}H NMR (600 MHz, D_2O): δ [ppm] = 3.95 – 3.82 (m, 3H, H-1, H-6a, H-6b), 3.57 – 3.50 (m, 1H, H-4), 3.46 – 3.32 (m, 1H, H-3), 2.69 – 2.58 (m, 1H, H-2), 2.19 – 2.08 (m, 1H, H-5); ^{13}C NMR (151 MHz, D_2O): δ [ppm] = 126.3 (CF_3), 76.6 (C-5a), 75.1 (C-3), 71.2 (C-1), 70.9 (C-4), 58.6 (C-6), 51.9 (C-2), 45.1 (C-5); ^{19}F NMR (376 MHz, D_2O): δ [ppm] = -71.0 (s, 1H, C<u>F$_3$</u>); HRMS: $[M+H^+]_{calculated}$ = 262.0897 g/mol, $[M+H^+]_{found}$ = 262.0901 g/mol. HPLC: t_R = 9.7 min (Phenomenex® Luna 5µ HILIC 200Å Pa, 250 x 21.20 mm, 30 % MeCN isocrat. in 12 mM TEAB buffer pH = 7.0, 8 mL/min, ELSD)

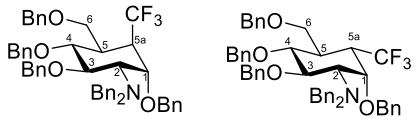
Methyl ((1*S*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)-1-(trifluoromethyl)cyclohexyl) oxalate (206)



To a stirred solution of **206** (930 mg, 115.9µmol) in THF abs. (3 mL) at -78 °C was added *n*-butyllithium (139.2 µL, 2.5 M in hexane, 347.0 µmol) and methyl oxalyl chloride (21.3 mg, 173.9 µmol, 16.0 µL) The reaction mixture was stirred for 2 h at -78 °C. The reaction was stopped by adding NH_4Cl_{aq} (2 mL) and water (2 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 2 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified using column chromatography (petroleum ether / EtOAc = 15:1 – 10:1) to give compound **208** (80.0 mg, 90.0 µmol, 78 %) as a colourless oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.47 – 6.95 (m, 30H, arenes), 5.10 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh),), 4.93 (m, 2H, O-C<u>H</u>HPh, H-1), 4.78 (d, 1H, *J* = 11.4 Hz, O-C<u>H</u>HPh), 4.73 (m, 3H, O-C<u>H</u>HPh),), 4.53 (d, 1H *J* = 11.4 Hz, O-C<u>H</u>HPh), 4.48 (d, 1H, *J* = 11.5 Hz, O-C<u>H</u>HPh), 4.39 – 4.34 (m, 1H, O-C<u>H</u>HPh), 4.31 (m, 1H, H-3), 4.12 – 4.02 (m, 3H, H-4, N-C<u>H$_2$</u>Ph), 4.02 – 3.93 (m, 3 H, N- C<u>H$_2$</u>Ph, H-6), 3.80 (dd, 1H, *J* = 10.4, 1.6 Hz, H-6), 3.75 (s, 3H, OMe), 3.03 (m, 1H,H-2), 2.83 (m, 1H, H-5). ^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = δ 157.2 (CO), 155.1(CO), 139.9, 139.2, 138.6, 138.1, 137.8, 128.6 (arenes, $C_{quart.}$), 128.5, 128.4, 128.4, 127.7, 127.6, 127.5, 127.2, 127.1, 127.0, 126.9 (arenes), 124.6 (CF_3), 86.5(C-5a), 81.0(C-1), 80.9 (C-3), 80.0 (C-4), 75.7 (O-<u>C</u>H$_2$Ph), 74.9 (O-<u>C</u>H$_2$Ph), 73.6 (O-<u>C</u>H$_2$Ph), 73.4 (O-<u>C</u>H$_2$Ph), 64.1 (C-6), 56.8 (C-2), 56.4 (2 x N-<u>C</u>H$_2$Ph), 53.7(OMe), 45.3(C-5). ^{19}F NMR (471 MHz, $CDCl_3$): δ [ppm] = -64.45(CF_3); HRMS (ESI) *m*/*z* calcd for $C_{53}H_{52}F_3NO_8$ 888.3718; [*M*+H$^+$] found: 888.3708

(1*S*,2*R*,3*R*,4*R*,5*R*,6*S*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-(trifluoromethyl)cyclohexan-1-amine& (1*S*,2*R*,3*R*,4*R*,5*S*,6*S*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-(trifluoromethyl)cyclohexan-1-amine (209 & 210)

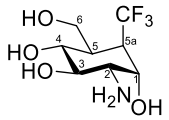


To a stirred solution of **208** (80.0 mg, 90.1 µmol) in degassed toluene abs. (3 mL) was added AIBN (18.02 3 mg, 18.0 µmol,) and tributyltin hydride (48.6 µL, 180.2 µmol). The reaction mixture was heated to 100 °C and stirred for 15 h. The reaction mixture was concentrated. The crude product was purified using HPLC to give compound **209** (38 mg, 48.4 µmol, 54%) and **210** (7 mg, 8.9 µmol, 10 %) as a colourless oils.

209: ^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.47 – 7.07 (m, 30H, arenes), 5.01 (d, 1H, *J* = 11.5 Hz, O-C<u>H</u>HPh), 4.91 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.73 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.52 (m, 2H, O-C<u>H</u>HPh), 4.49 – 4.44 (m, 2H, O-C<u>H</u>HPh), 4.44 – 4.37 (m, 1H, O-C<u>H</u>HPh), 4.20 (dd, 1H, *J* = 11.1, 8.3 Hz, H-3), 4.15 (pt, 1H, *J* = 2.5 Hz, H-1), 4.05 (d, 2H, *J* = 13.9 Hz, N- C<u>H$_2$</u>Ph), 3.97 (d, 2H, *J* = 13.9 Hz, N- C<u>H$_2$</u>Ph), 3.77 (dd, 1H, *J* = 9.2, 3.9 Hz, H-6), 3.68 (dd, 1H, *J* = 11.9, 8.3 Hz, H-4), 3.52 (pt, 1H, *J* = 9.7 Hz, H-6), 3.02 (dd, 1H, *J* = 11.1, 2.4 Hz, H-2), 2.99 – 2.88 (m, 1H, H-5a), 2.63 (m,1H, H-5). ^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 140.6, 139.4, 138.4, 138.2, 137.4 (arenes, $C_{quart.}$), 128.6, 128.5, 128.5, 128.4, 128.4, 128.2, 128.2, 127.9, 127.9, 127.8, 127.7, 127.6, 127.3 (arenes), 127.1(CF_3), 126.8, 126.6 (arenes), 81.5 (C-3), 81.1 (C-4), 78.5 (C-1), 75.1 (O-<u>C</u>H$_2$Ph), 73.3 (O-<u>C</u>H$_2$Ph), 73.1 (O-<u>C</u>H$_2$Ph), 72.3 (O-<u>C</u>H$_2$Ph), 67.4 (C-6), 58.1 (C-2), 56.5 (2 x N-<u>C</u>H$_2$Ph), 41.8 (C-5a), 38.4 (C-5). ^{19}F NMR (377 MHz, $CDCl_3$): δ [ppm] = -60.29 (d, *J* = 10.9 Hz). HRMS (ESI) *m/z* calcd for $C_{50}H_{50}F_3NO_4$ 786.3765; [*M*+H$^+$] found: 786.3758. HPLC: t_R = 13.6 min (Kinetex® 5 µm C8 100Å, 250 x 21.2 mm 100 % MeCN, 10 mL/min)

210: ^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 7.58 – 6.87 (m, 30H, arenes), 5.07 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.87 (d, 1H, *J* = 11.7 Hz, O-C<u>H</u>HPh), 4.76 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.68 (s, 2H, O-C<u>H</u>HPh), 4.52 (d, 1H, *J* = 10.8 Hz, O-C<u>H</u>HPh), 4.44 (d, 1H, *J*- = 11.6 Hz,), 4.38 – 4.31 (m, 1H, O-C<u>H</u>HPh), 4.29 (m, 1H, H-1), 4.22 (t, 1H, *J* = 9.7 Hz, H-3), 4.12 (d, 2H, *J* = 14.7 Hz, N- C<u>H</u>$_2$Ph), 4.01 (d, 2H, *J* = 14.6 Hz, N- C<u>H</u>$_2$Ph), 3.95 (d, 1H, *J* = 9.7 Hz, H-6), 3.63 (pt, 1H, *J* = 9.6 Hz, H-4), 3.48 (d, 1H, *J* = 9.7 Hz, H-6), 2.68 (d, 1H, *J* = 10.8 Hz, H-2), 2.33 (m, 1H, H-5), 2.29 – 2.16 (m, 1H, H-5a). ^{13}C NMR (126 MHz, $CDCl_3$): δ [ppm] = 140.5, 139.4, 138.4, 138.0 (arenes, $C_{quart.}$), 128.5, 128.5, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.3, 127.0, 126.8 (arenes), 82.2 (C-4), 81.1 (C-3), 79.0 (C-1), 75.3 (O-<u>C</u>H$_2$Ph), 73.9 (O-<u>C</u>H$_2$Ph), 73.4 (O-<u>C</u>H$_2$Ph), 73.3 (O-<u>C</u>H$_2$Ph), 65.6 (C-6), 61.4 (C-2), 56.3 (2 x N-<u>C</u>H$_2$Ph), 43.8 (C-5a), 38.1 (C-5). ^{19}F NMR (377 MHz, $CDCl_3$): δ [ppm] = -62.57 (d, *J* = 8.5 Hz). HRMS (ESI) *m*/*z* calcd for $C_{50}H_{50}F_3NO_4$ 786.37647; [*M*+H$^+$] found: 786.3764. HPLC: t_R = 17.1 min (Kinetex® 5 µm C8 100Å, 250 x 21.2 mm 100 % MeCN, 10 mL/min)

(1*S*,2*R*,3*R*,4*R*,5*R*,6*S*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-(trifluoromethyl)cyclohexan-1-amine (211)

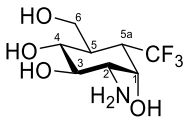


To a stirred solution of **209** (20.0 mg, 25.5 µmol) in MeOH abs. (2 mL) was added Pd/C (10 % waterwet, 20.0 mg), Pd(OH)$_2$/C (20 % waterwet, 20.0 mg) and TFA (20.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 12 h under hydrogen atmosphere. The reaction mixture was centrifuged. The supernatant was purified by HILIC-HPLC to give compound **211** (4.4 mg, 17.9 µmol, 71 %) as a white powder after lyophilization with minor impurities.

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 4.32 (t, 1H, *J* = 2.9 Hz, H-1), 4.05 (dd, 1H, *J* = 11.7, 5.3 Hz, H-6), 3.77 (m, 1H, H-6), 3.70 – 3.58 (m, 1H, H-4), 3.45 (m, 1H, H-3), 2.99 (m, 1H, H-2), 2.77 (m, 1H, H-5a), 2.35 (m, 1H, H-5). ^{13}C NMR (126 MHz, D_2O): δ [ppm] =

75.6 (C-3), 71.5 (C-4), 67.4 (C-1), 59.9 (C-6), 53.3 (C-2), 45.4 (C-5a), 39.4 (C-5). ^{19}F NMR (377 MHz, $CDCl_3$): δ [ppm] = -60.27 (d, *J* = 12.4 Hz). HRMS (ESI) *m*/*z* calcd for $C_8H_{14}F_3NO_4$ 246.0948; [*M*+H^+] found: 246.0945. HPLC: t_R = 14.7 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 40% isocrat. MeCN in 12 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

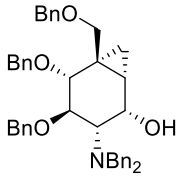
(1*S*,2*R*,3*R*,4*R*,5*S*,6*S*)-N,N-dibenzyl-2,3,6-tris(benzyloxy)-4-((benzyloxy)methyl)-5-(trifluoromethyl)cyclohexan-1-amine (212)



To a stirred solution of **210** (5.0 mg, 6.4 µmol) in MeOH abs. (0.8 mL) was added Pd/C (10% waterwet, 5.0 mg), $Pd(OH)_2$/C (20% waterwet, 5.0 mg) and TFA (5.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 12 h under hydrogen atmosphere. The reaction mixture was centrifuged. The supernatant was purified by HILIC-HPLC to give compound **212** (0.5 mg, 2.0 µmol, 32 %) as a white powder after lyophilization with minor impurities. Because of the limited amount of product only a ^{1}H and ^{19}F NMR are reported.

^{1}H NMR (500 MHz, D_2O): δ [ppm] = 4.26 (pt, 1H, *J* = 2.6 Hz, H-1), 3.98 (d, 1H, *J* = 12.0 Hz, H-6), 3.62 (d, 1H, *J* = 12.0 Hz, H-6), 3.44 – 3.34 (m, 2H, H-3, H-4), 2.63 (m, 1H, H-2), 2.58 – 2.47 (m, 1H, H-5a), 1.94 – 1.83 (m, 1H, H-5). ^{19}F NMR (377 MHz, D_2O): δ [ppm] = -63.64 (d, *J* = 11.0 Hz). HRMS (ESI) *m*/*z* calcd for $C_8H_{14}F_3NO_4$ 246.0948; [*M*+H^+] found: 246.0944 HPLC: t_R = 15.2 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 40% isocrat. MeCN in 12 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

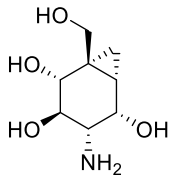
(1*S*,2*S*,3*S*,4*R*,5*R*,6*R*)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)bicyclo[4.1.0]heptan-2-ol (213)



Alcohol **110** (60 mg, 95.9 µmol) was dissolved in DCM (1.5 mL) and the solution was cooled to 0 °C. $ZnEt_2$ solution (958.8 µL, 958.8 µmol, 1 M in hexanes) was added to DCM (1.5 mL), the solution was cooled to 0 °C and diiodomethan (61.9 µL, 767 µmol) was added to the solution. After 5 min this solution was added to the solution containing the alcohol **210**. The resulting mixture was allowed to warm to room temperature overnight. Water (2 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (3 x 2 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified using column chromatography (petroleum ether / EtOAc = 5:1 – 3:1) to give compound **213** (42.0 mg, 65.6 µmol, 68 %) as a colourless oil.

^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] =7.42 – 7.15 (m, 25H, H-arenes), 5.00 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.83 – 4.70 (m, 2H, 2x O-C<u>H</u>HPh), 4.67 (d, 1H *J* = 11.2 Hz, O-C<u>H</u>HPh), 4.39 – 4.30 (m, 2H, 2x O-C<u>H</u>HPh), 4.24 (dd, 1H, *J* = 7.8, 5.0 Hz, H-1), 4.16 (d, 1H, *J* = 7.2 Hz, H-4), 4.00 (d, 2H, *J* = 14.0 Hz, 2x N-C<u>H</u>HPh), 3.91 (d, 2H,*J* = 14.0 Hz, 2x N-C<u>H</u>HPh), 3.81 (dd, 1H, *J* = 10.4, 7.2 Hz, H-3), 3.72 (d, 1H, *J* = 10.4 Hz, 1x H-6), 2.81 (d, 1H, *J* = 10.4 Hz, 1x H-6), 2.78 (dd, 1H, *J* = 10.4, 5.0 Hz, H-2), 2.57 (s, 1H, OH), 1.30 – 1.19 (m, 1H, H-5a), 0.97 (pt, 1H, *J* = 5.5 Hz, 1x $CH_{cyclopropan}$), 0.47 (dd, 1H, *J* = 9.4, 5.5 Hz, 1x $CH_{cyclopropan}$). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] =140.7, 139.2, 138.6, 138.3 ($C_{quart.\ arenes}$), 128.6, 128.5, 128.4, 127.9, 127.7, 127.7, 127.5, 127.3, 126.9 (C_{arenes}), 81.8 (C-4), 78.5(C-3), 74.6 (C-6), 74.2 (O-<u>C</u>H_2Ph), 73.1 (O-<u>C</u>H_2Ph), 72.6 (O-<u>C</u>H_2Ph), 68.6 (C-1), 62.9 (C-2), 57.1 (2x N-<u>C</u>H_2Ph), 27.2(C-5), 24.4 (C-5a), 8.5 (CH_2cyclopropan). HRMS (ESI) *m*/*z* calcd for $C_{43}H_{45}NO_4$ 640.3421; [*M*+H$^+$] found: 640.3416.

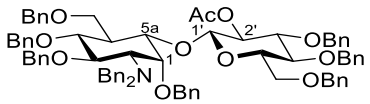
(1*R*,2*R*,3*R*,4*S*,5*S*,6*S*)-4-amino-1-(hydroxymethyl)bicyclo[4.1.0]heptane-2,3,5-triol (214)



To a stirred solution of **213** (10.0 mg, 15.6 µmol) in MeOH abs. (2 mL) was added Pd/C (10 % waterwet, 10.0 mg), $Pd(OH)_2/C$ (20 % waterwet, 10.0 mg) and TFA (10.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 12 h under hydrogen atmosphere. The reaction mixture was filtered over celite. The crude product was purified by HILIC-HPLC to give compound **214** (2.40 mg, 12.7 µmol, 81 %) as a white powder after lyophilization.

^{1}H NMR (CD_3OD, 400 MHz) δ [ppm] = 4.45 (dd, 1H, *J* = 8.0, 4.7 Hz, H-1), 4.06 – 3.91 (m, 2H, H-4, 1x H-6), 3.41 (dd, 1H, *J* = 11.3, 8.5 Hz, H-3), 2.93 – 2.84 (m, 2H, H-2, 1x H-6), 1.47 (m, 1H, H-5a), 0.94 (pt, 1H *J* = 5.6 Hz, $CH_{cyclopropan}$), 0.53 (dd, 1H, *J* = 6.6, 5.6 Hz, $CH_{cyclopropan}$). ^{13}C NMR (CD_3OD, 126 MHz) δ [ppm] =73.9 (C-4), 68.7 (C-3), 67.0 (C-6), 64.2 (C-1), 56.5 (C-2), 32.3 (C-5), 25.0 (C-5a), 9.6 (CH_2). HRMS (ESI) *m*/*z* calcd for $C_8H_{15}NO_4$ 190.1074; [*M*+H^+] found: 190.1073. HPLC: t_R = 10.2 min (Phenomenex® Luna 5µ HILIC 200Å, AXIA Pa, 250 x 21.20 mm, 30% isocrat. MeCN in 10 mM TEAB buffer pH = 7.00, 8.0 mL/min, ELSD)

(2*S*,3*S*,4*R*,5*S*,6*S*)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((1*S*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)cyclohexyl)oxy)tetrahydro-2H-pyran-3-yl acetate (215)

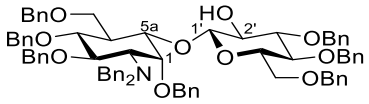


Carba-sugar **113** (50.0 mg, 68.1 µmol) and fluoride **216** (102 mg, 204.4 µmol) were dissolved in DCM (2 mL). 4 Å molecular sieve was added. The mixture was cooled to 0 °C. Boron trifluoride diethyl etherate (20 µL, 136 µmol) was added. The reaction allowed to reach room temperature and was stirred overnight. The reaction was stopped by addition of saturated $NaHCO_3$ solution (4 mL) and extracted with DCM (4x 3 mL). The organic layer was dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. The crude product was purified by HPLC. The product **215** was isolated as a colorless oil (38.0 mg, 31.4 µmol, 46 %). The alpha product was also isolated (4 mg, 3.31 µmol, 5 %).

^{1}H NMR ($CDCl_3$, 500 MHz) δ [ppm] =7.49 – 6.97 (m, 45H, H-arenes), 5.07 (dd, 1H, *J* = 9.5, 7.9 Hz, H-2'), 5.00 (d, 1H, *J* = 11.6 Hz, O-C<u>H</u>HPh), 4.91 (d, 1H, *J* = 10.2 Hz, O-C<u>H</u>HPh), 4.83 (m, 2H, *J* = 11.5, 8.2 Hz, O-C<u>H</u>HPh), 4.76 (d, 1H, *J* = 10.7 Hz, O-C<u>H</u>HPh), 4.70 (d, 1H, *J* = 10.4 Hz, O-C<u>H</u>HPh), 4.64 (d, 1H, *J* = 11.4 Hz, O-C<u>H</u>HPh), 4.59 – 4.53 (m, 3H, O-C<u>H</u>HPh, H-1'), 4.48 (m, 2H, O-C<u>H</u>HPh), 4.35 (d, 1H, *J* = 11.9 Hz, O-C<u>H</u>HPh), 4.32 – 4.28 (m, 2H, O-C<u>H</u>HPh), 4.20 (dd, 1H, *J* = 10.9, 8.7 Hz, H-3), 4.08 (d, *J* = 1.9 Hz, 1H, H-1), 4.01 (s, 4H, N-C<u>H</u>HPh), 3.83 (m, 2H, 1x H-6, H-4'), 3.77 – 3.71 (m, 2H, 1x H-6, H-5a), 3.66 (m, 4H, 2x H-6', H-4,H-3'), 3.41 (dt, 1H, *J* = 9.8, 3.0 Hz, H-5'), 2.66 (dd, 1H, *J* = 10.9, 1.9 Hz, H-2), 2.36 – 2.26 (m, 1H, H-5), 1.67 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] =169.6 (C=O), 141.0, 139.7, 139.0, 138.7, 138.3, 138.1, 138.0 ($C_{quart.\ arenes}$), 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.5, 127.2, 127.1, 126.7 (C_{arenes}), 99.6 (C-1'), 83.6 (C-3'), 80.9 (C-4), 80.8 (C-3), 79.52 (C-1), 78.2 (C-4'), 77.1 (C-5a), 75.3 (O-<u>C</u>H_2Ph), 75.3 (O-<u>C</u>H_2Ph), 75.2 (O-<u>C</u>H_2Ph), 75.1 (C-5'), 74.6 (O-<u>C</u>H_2Ph), 73.7 (C-2'), 73.4 (O-<u>C</u>H_2Ph), 73.3 (O-<u>C</u>H_2Ph), 73.0 (O-<u>C</u>H_2Ph), 68.7 (C-6'), 64.5 (C-6), 59.3 (C-2), 56.2

(2x N-<u>C</u>H$_2$Ph), 43.3 (C-5), 21.1 (CH$_3$). HRMS (ESI) m/z calcd for $C_{78}H_{81}NO_{11}$ 1208.5882; [M+H$^+$] found: 1208.5856 HPLC: t_R = 10.4 min (Kinetex® 5 µm C8 100Å, 250 x 21.2 mm 100 % MeCN, 10 mL/min)

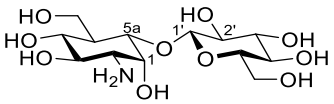
<u>(2*S*,3*S*,4*S*,5*S*,6*S*)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((1*S*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)-3-(dibenzylamino)cyclohexyl)oxy)tetrahydro-2H-pyran-3-ol (217)</u>



Compound **216** (10 mg, 8.27 µmol) was dissolved in MeOH (3 mL). K_2CO_3 (11.5 mg, 83 µmol) was added. The solution was stirred for 4 h. The solvent was removed under reduced pressure and the residue was taken up with DCM (3 mL) and water (3 mL). The organic phase was separated and was dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The crude compound **217** was obtained in quantitative yield and used without further purification.

^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm] = 7.50 – 6.97 (m, 45H, H-arenes), 5.15 (d, 1H, J = 11.1 Hz, O-C<u>H</u>HPh , 4.99 (d, 1H, J = 11.5 Hz, O-C<u>H</u>HPh), 4.88 – 4.81 (m, 4H, O-C<u>H</u>HPh), 4.74 – 4.66 (m, 2H, O-C<u>H</u>HPh), 4.61 – 4.52 (m, 2H), 4.50 – 4.43 (m, 3H, O-C<u>H</u>HPh, H-1'), 4.41 – 4.32 (m, 3H, O-C<u>H</u>HPh, H-1), 4.25 (dd, 1H, J = 11.0, 8.6 Hz, H-3), 4.07 (d, 2H, J = 14.6 Hz, N-C<u>H</u>HPh), 3.95 (d, 2H, J = 14.6 Hz, N-C<u>H</u>HPh), 3.86 – 3.54 (m, 9H, H-6, H-6', H-5a, H-4, H-4', H-3, H-2'), 3.43 (ddd, 1H, J = 9.8, 3.6, 2.1 Hz, H-5'), 2.75 (d,1H, J = 2.0 Hz, OH), 2.66 (dd, 1H, J = 11.0, 1.8 Hz, H-2), 2.36 (dd, 1H, J = 12.1, 10.0 Hz, H-5). ^{13}C NMR ($CDCl_3$, 126 MHz) δ [ppm] = 141.0, 139.9, 139.6, 139.1, 138.6, 138.4 ($C_{quart.\ arenes}$), 128.6, 128.5, 128.5, 128.4, 128.4, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.5, 127.4, 127.2, 126.8 (C_{arenes}), 102.0 (H-1'), 85.3, 80.8, 80.6, 79.4, 78.0, 75.7, 75.4, 75.3, 75.0, 75.0, 74.5, 73.8, 73.7, 73.5, 72.9, 69.4 (C-6'), 65.2 (C-6), 59.4 (C-2), 56.1 (2x N-<u>C</u>H$_2$Ph), 43.3 (C-5). HRMS (ESI) m/z calcd for $C_{76}H_{79}NO_{10}$ 1166.5777; [M+H$^+$] found: 1166.5743

(2*S*,3*S*,4*R*,5*R*,6*S*)-2-(((1*S*,2*R*,3*R*,4*R*,5*R*,6*R*)-3-amino-2,4,5-trihydroxy-6-(hydroxymethyl)cyclohexyl)oxy)-6-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol (218)



To a stirred solution of **217** (8.0 mg, 6.9 µmol) in MeOH abs. (3 mL) was added Pd/C (10% waterwet, 8.0 mg), $Pd(OH)_2/C$ (20% waterwet,8.0 mg) and FA (10.0 µL). The reaction was purged with hydrogen. The reaction was stirred for 24 h under hydrogen atmosphere. The reaction mixture was filtered over celite. The crude product was taken up in D_2O and the product **218** (1.0 mg, 4.8 µmol, 70 %) was obtained as a white powder as the formic acid salt after lyophilization.

^{1}H NMR (D_2O, 500 MHz) δ [ppm] = 8.47 (s, 1H, FA), 4.61 (d, 1H, *J* = 7.8 Hz, H-1'), 4.41 (pt, 1H, *J* = 2.7 Hz, H-1), 3.96-3.91 (m, 4H, 3x H-6, H-5a), 3.78 (dd, 1H, *J* = 10.8, 9.2 Hz, H-3), 3.73 (dd, 1H, *J* = 12.1, 6.6 Hz, 1x H-6'), 3.55 – 3.46 (m, 3H,), 3.45 – 3.37 (m, 2H,), 3.19 (dd, 1H, *J* = 10.8, 2.8 Hz, H-2), 1.94 (ptpt, 1H, *J* = 11.3, 2.4 Hz, H-5). ^{13}C NMR (D_2O, 126 MHz) δ [ppm] = 171.1 (C=O, FA), 100.8 (C-1'), 75.9 (C-4), 75.8, 74.3 (C-5a), 72.9 (C-2'), 71.0, 69.8, 69.3, 66.7 (C-1), 60.9 (C-6), 56.3 (C-6), 53.4 (C-2), 42.7 (C-5). HRMS (ESI) *m*/*z* calcd for $C_{13}H_{25}NO_{10}$ 356.1551; [*M*+H^+] found: 356.1547

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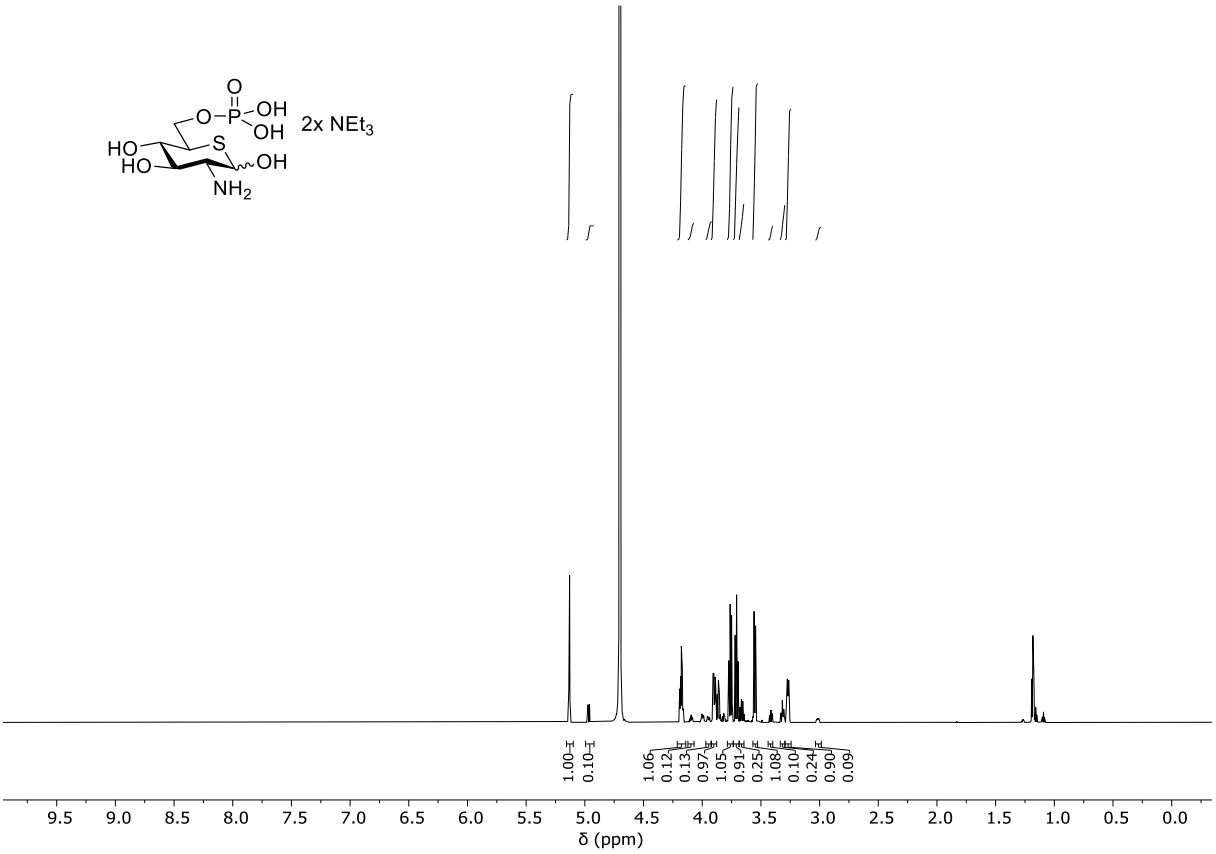
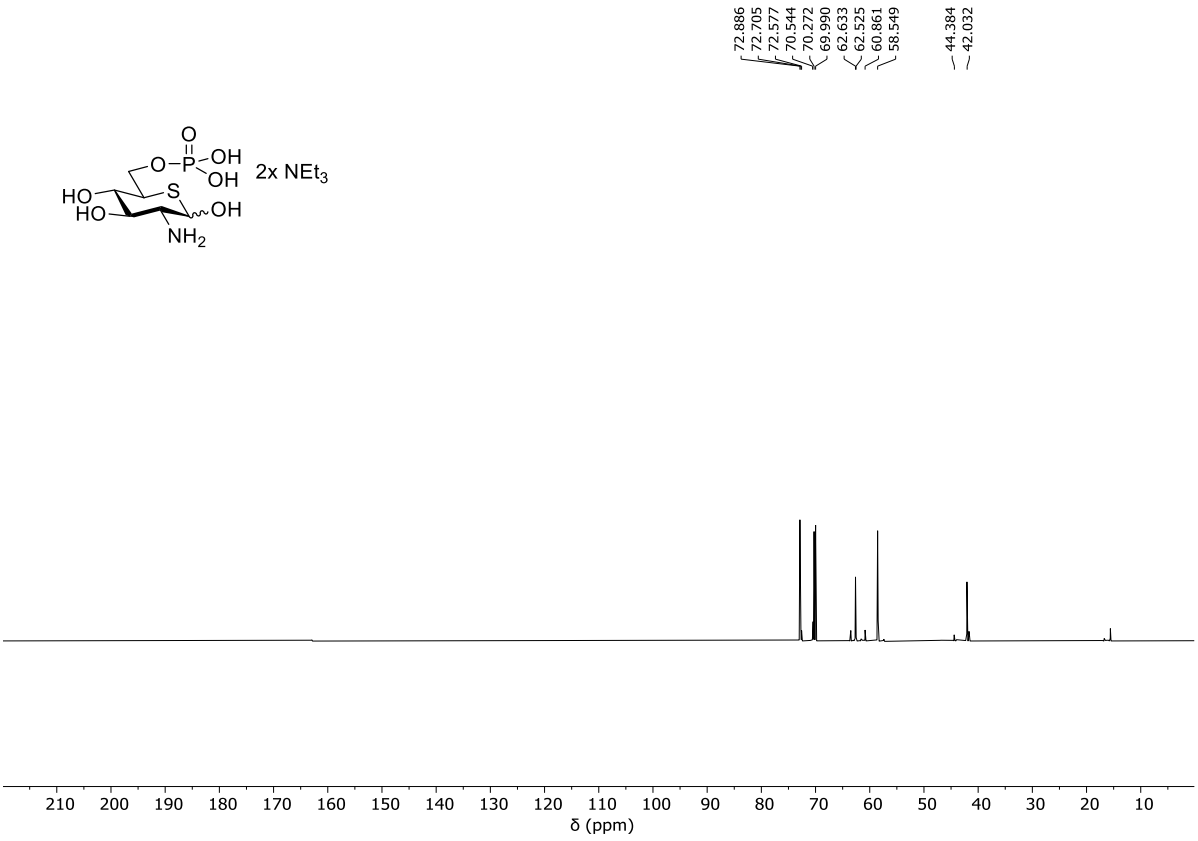
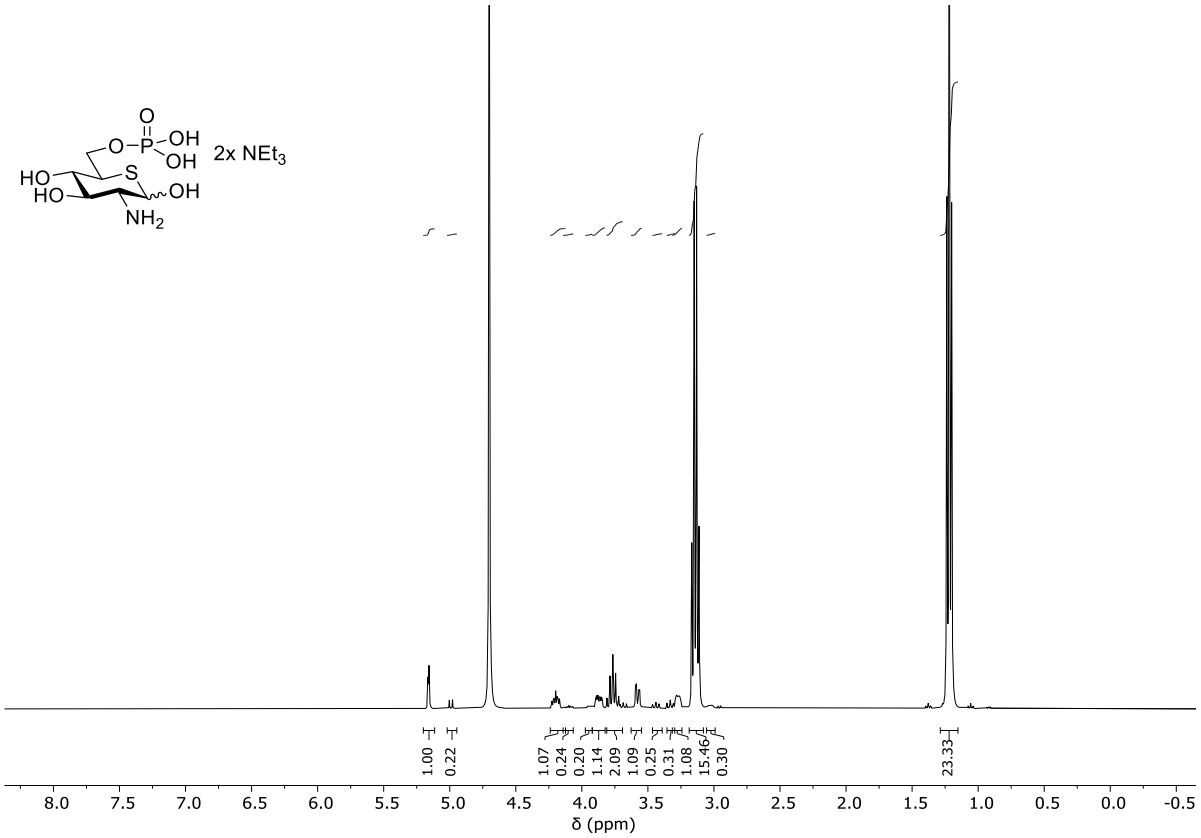
9 NMR



^{1}H NMR spectrum (800 MHz, D_2O) of **127** with NEt_3 supression



^{13}C NMR spectrum (201 MHz, D_2O) of **127** with NEt_3 supression



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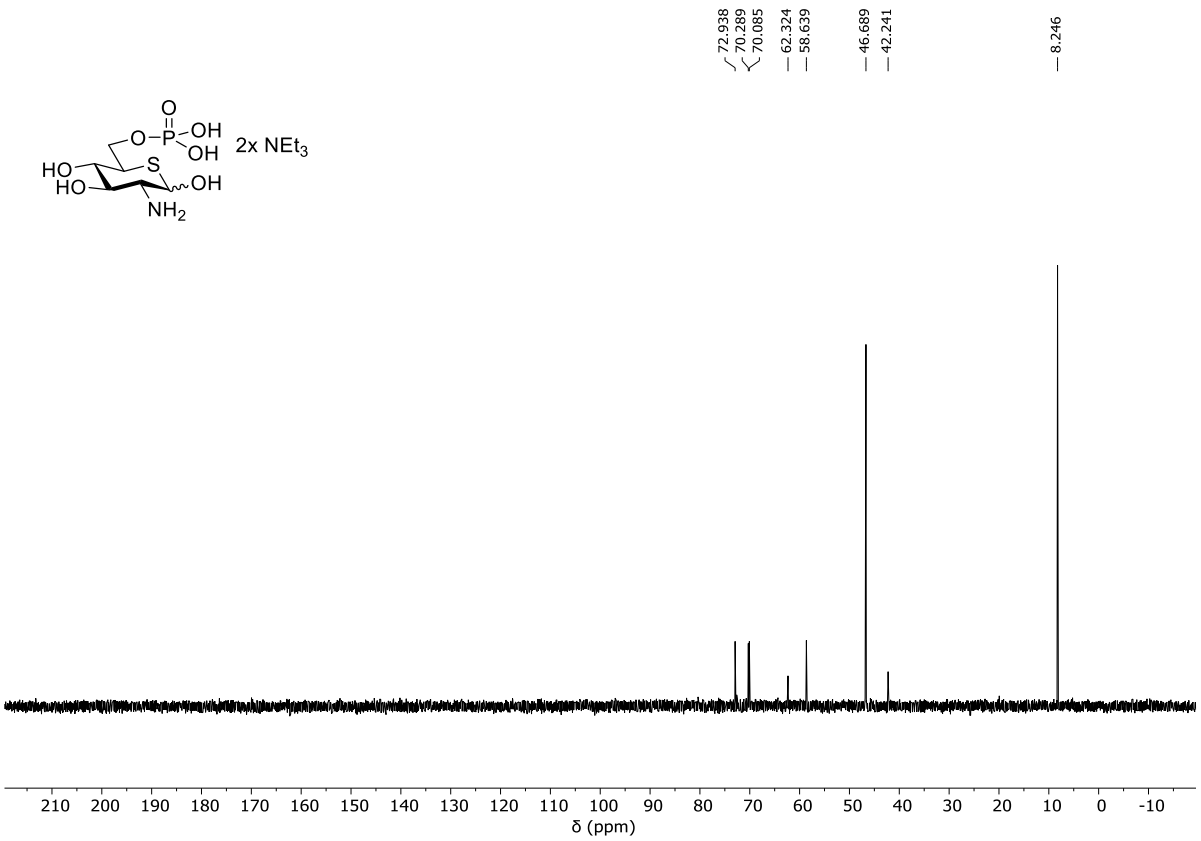
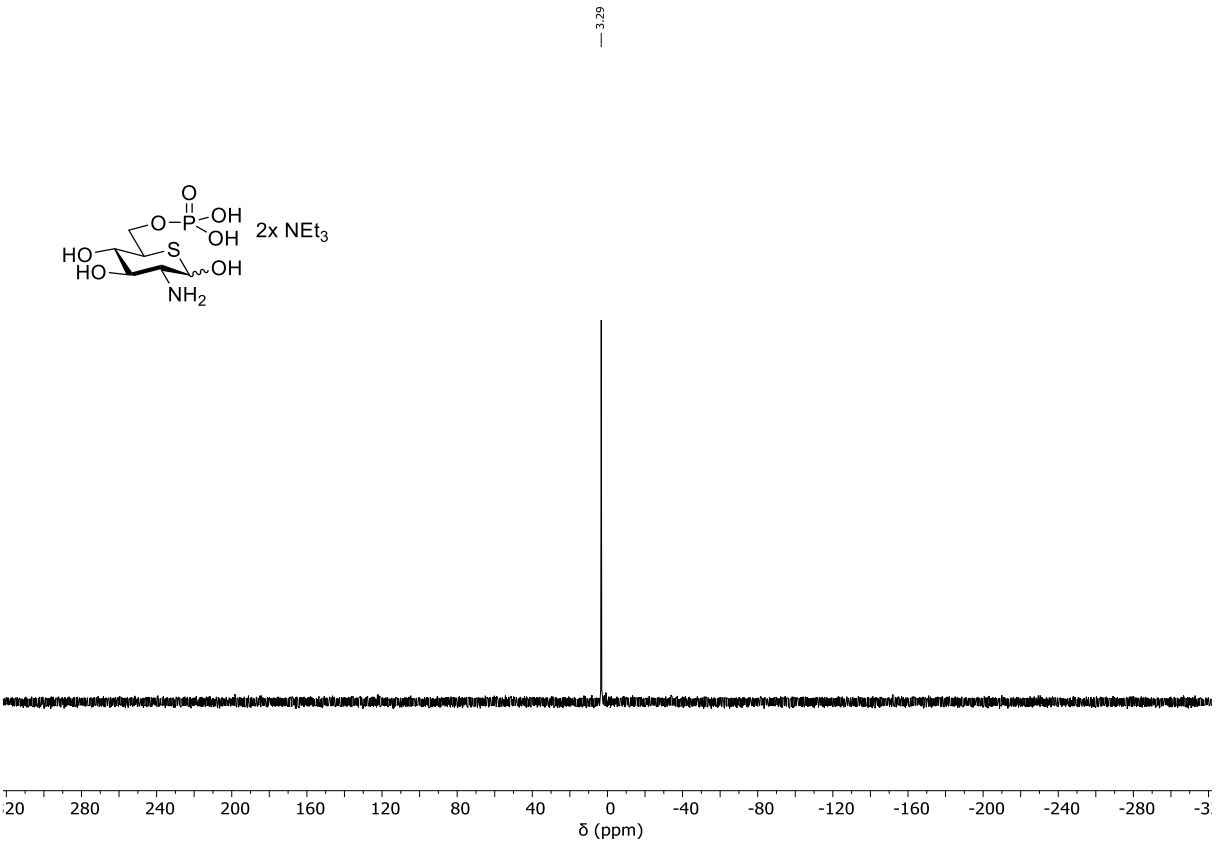
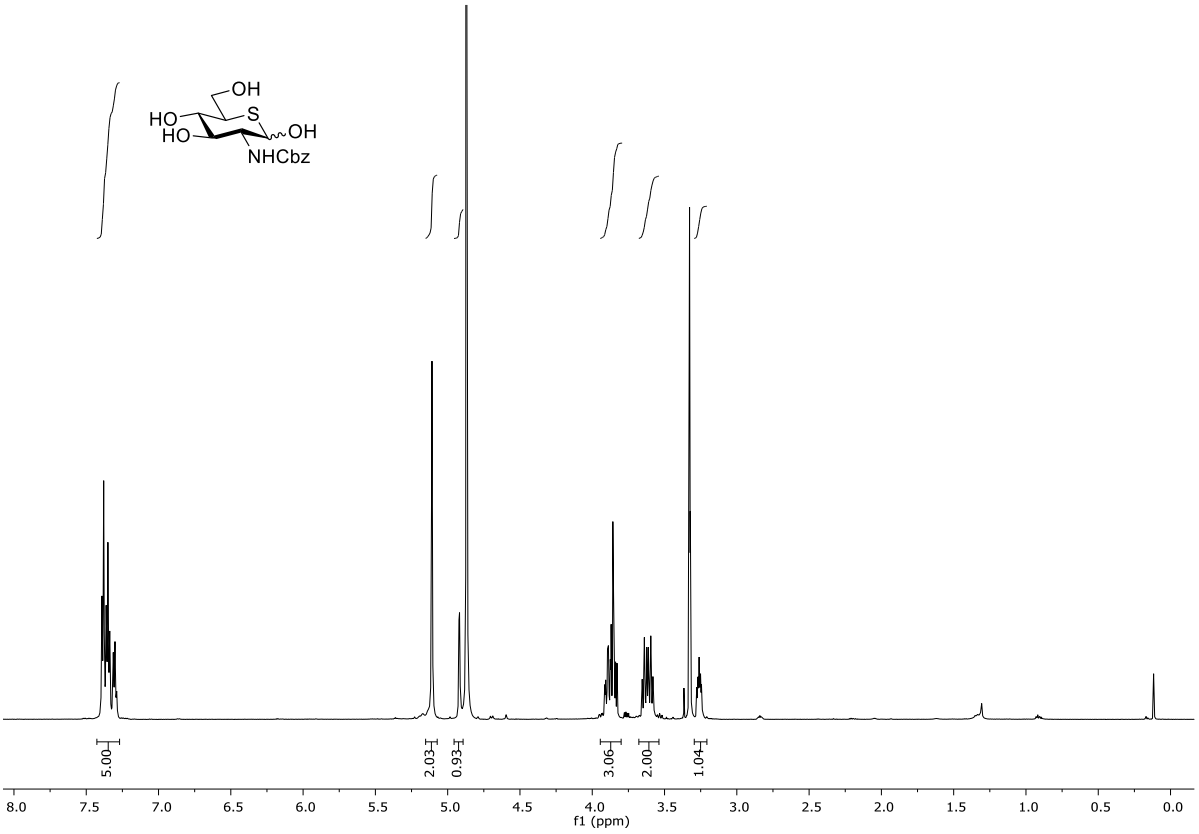


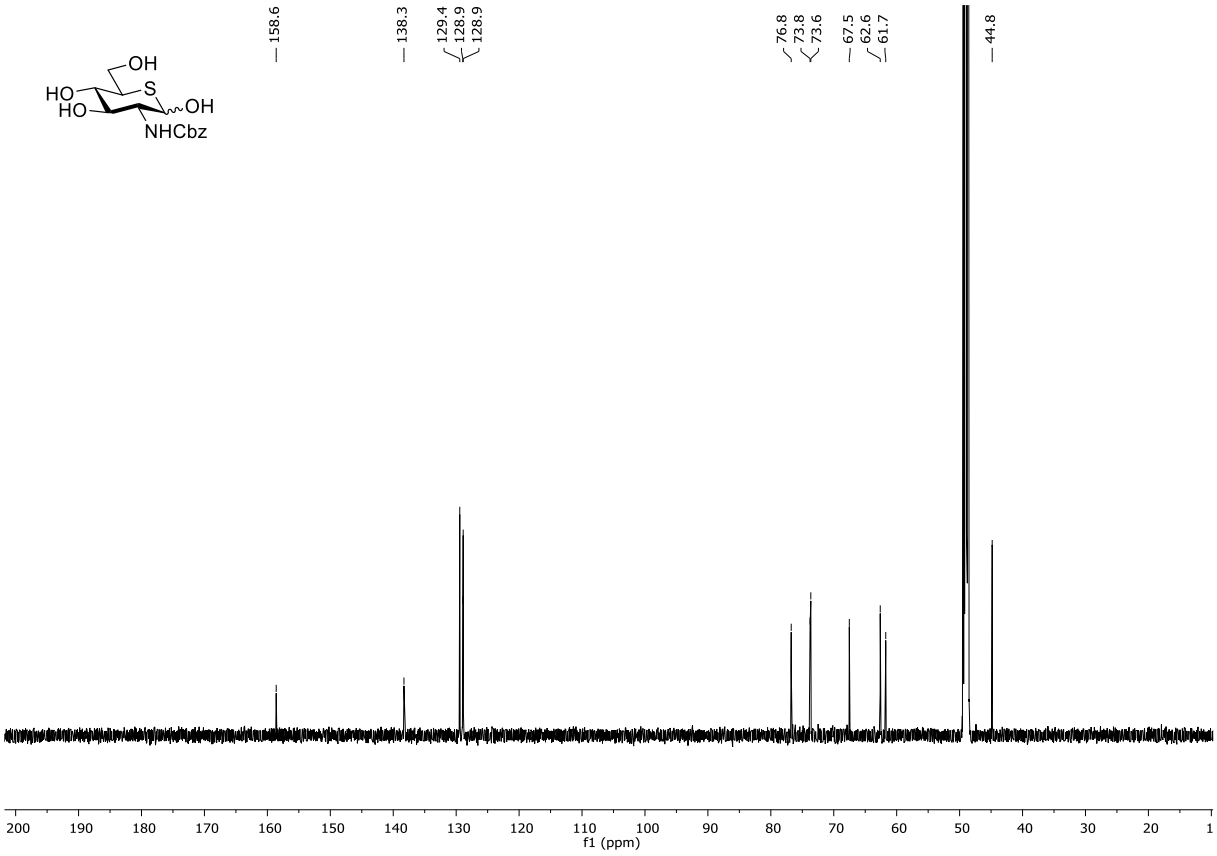
^{13}C NMR spectrum (101 MHz, D_2O) of **127** without NEt_3 supression



^{31}P NMR spectrum (162MHz, D_2O) of **127**



^{1}H NMR spectrum (600 MHz, MeOD) of **128**



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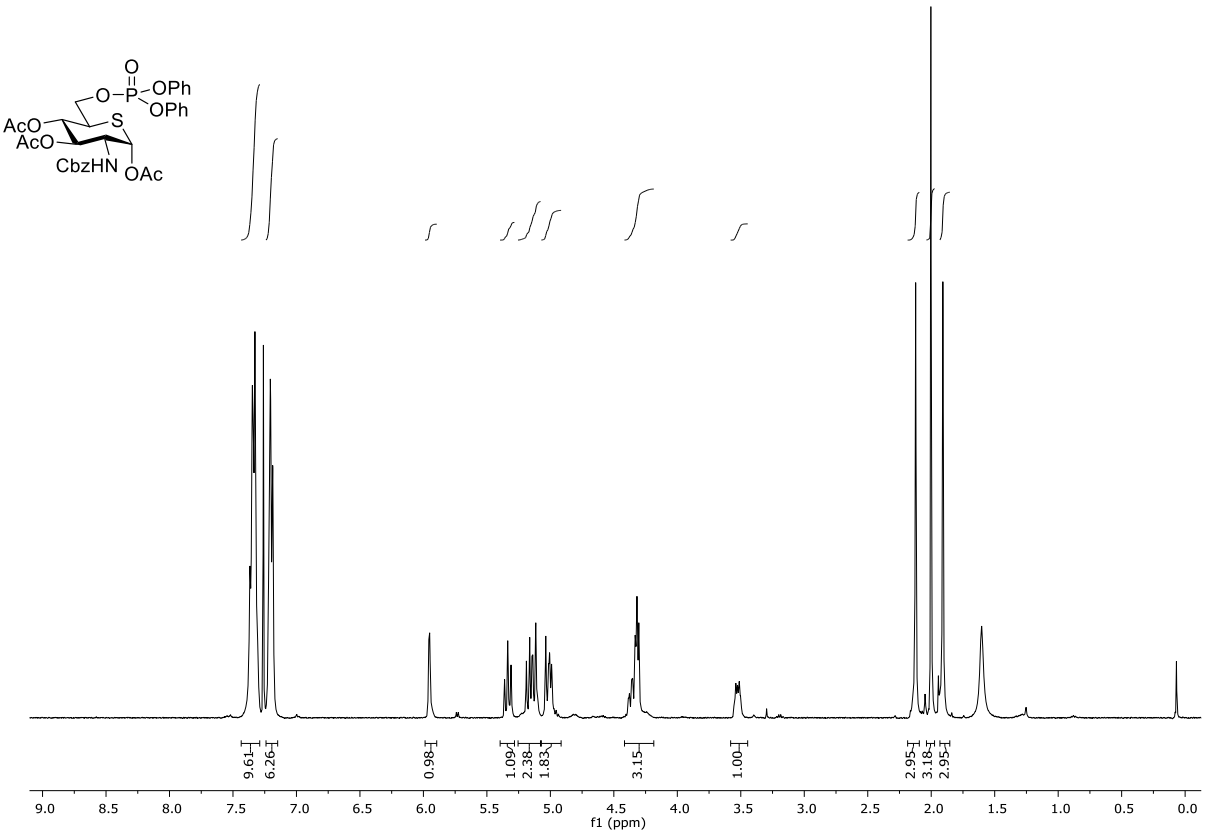

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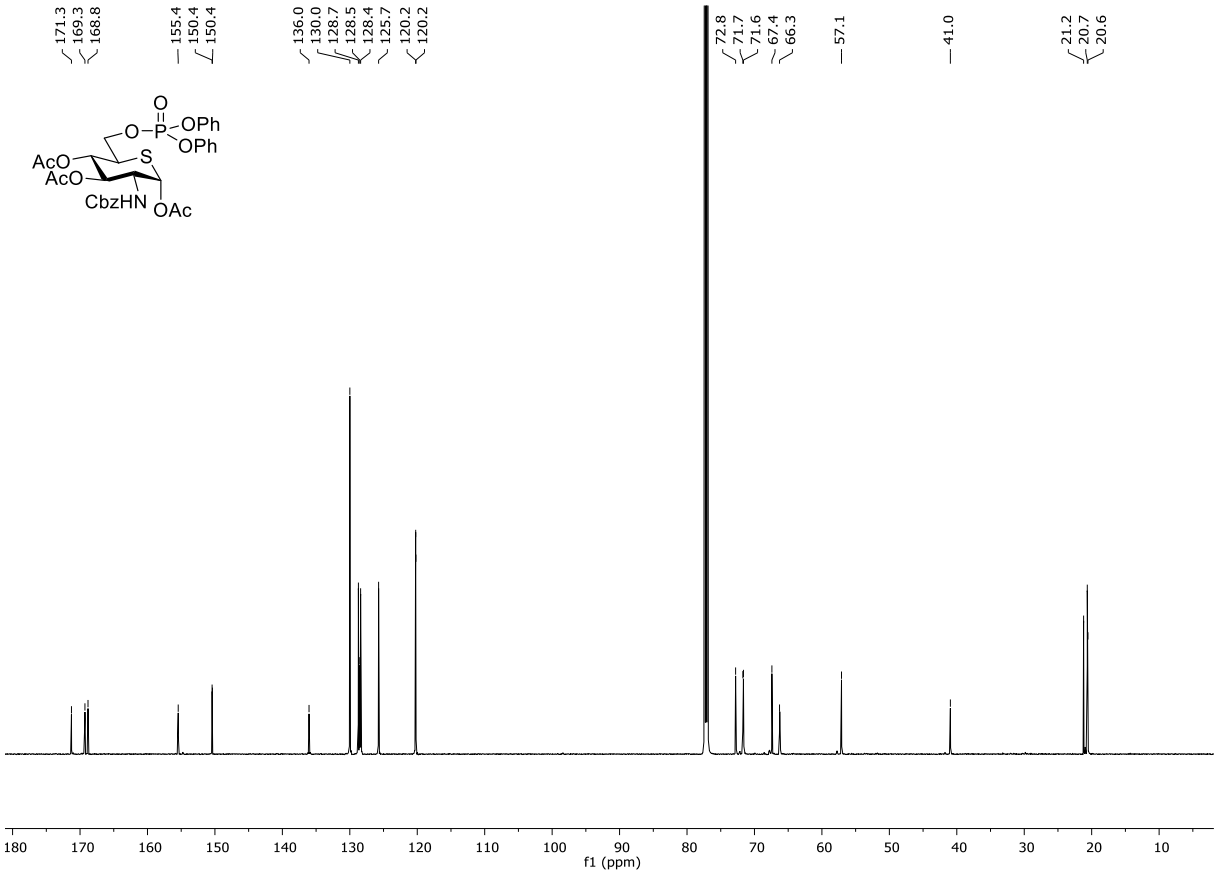

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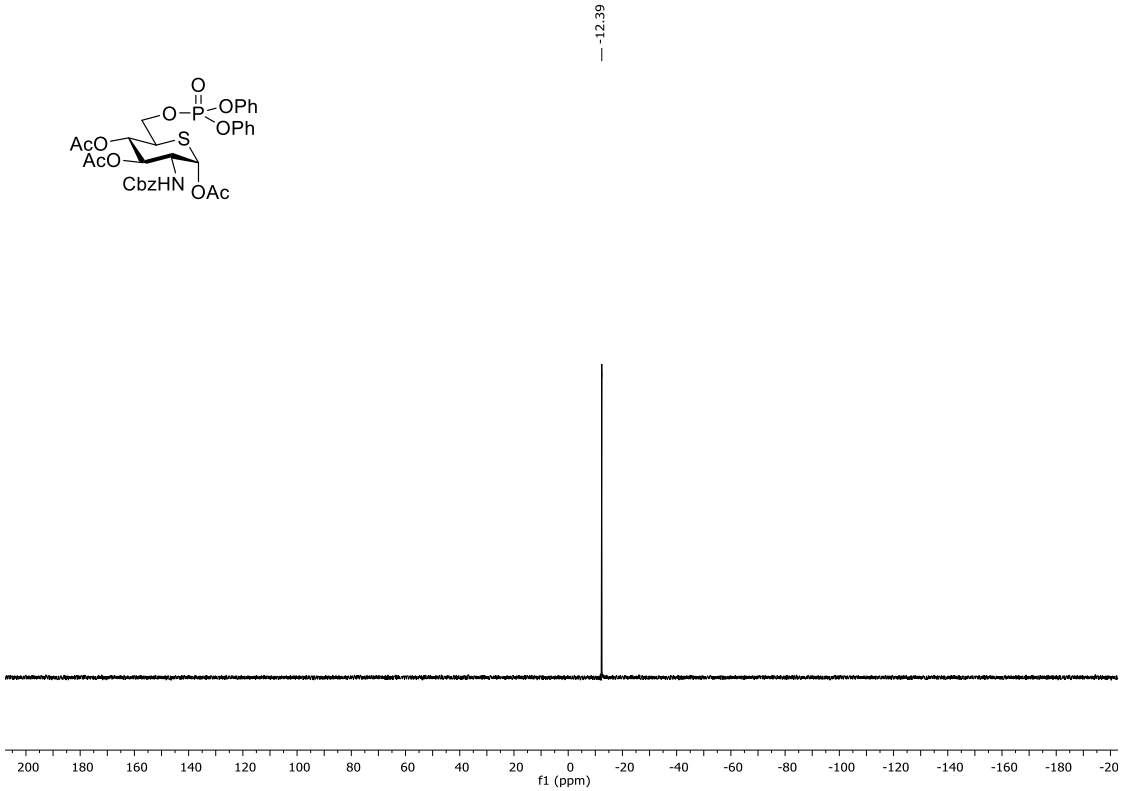

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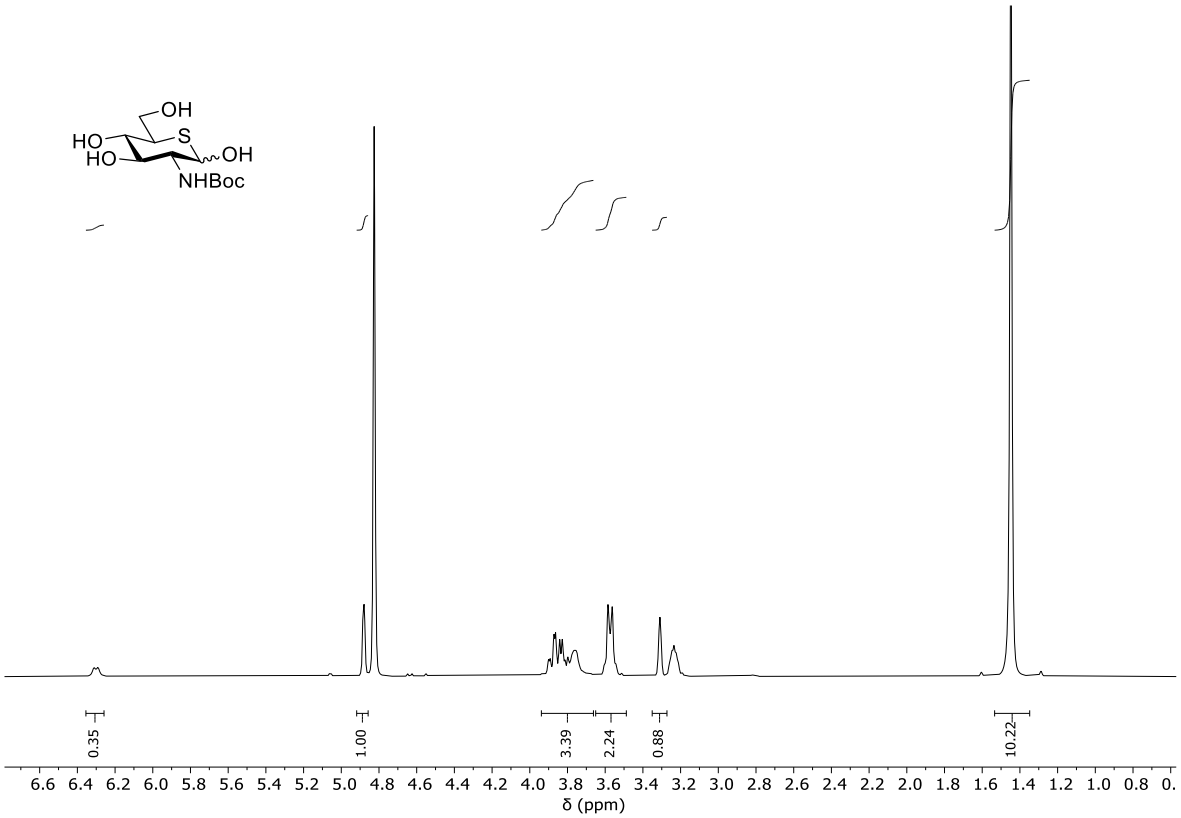

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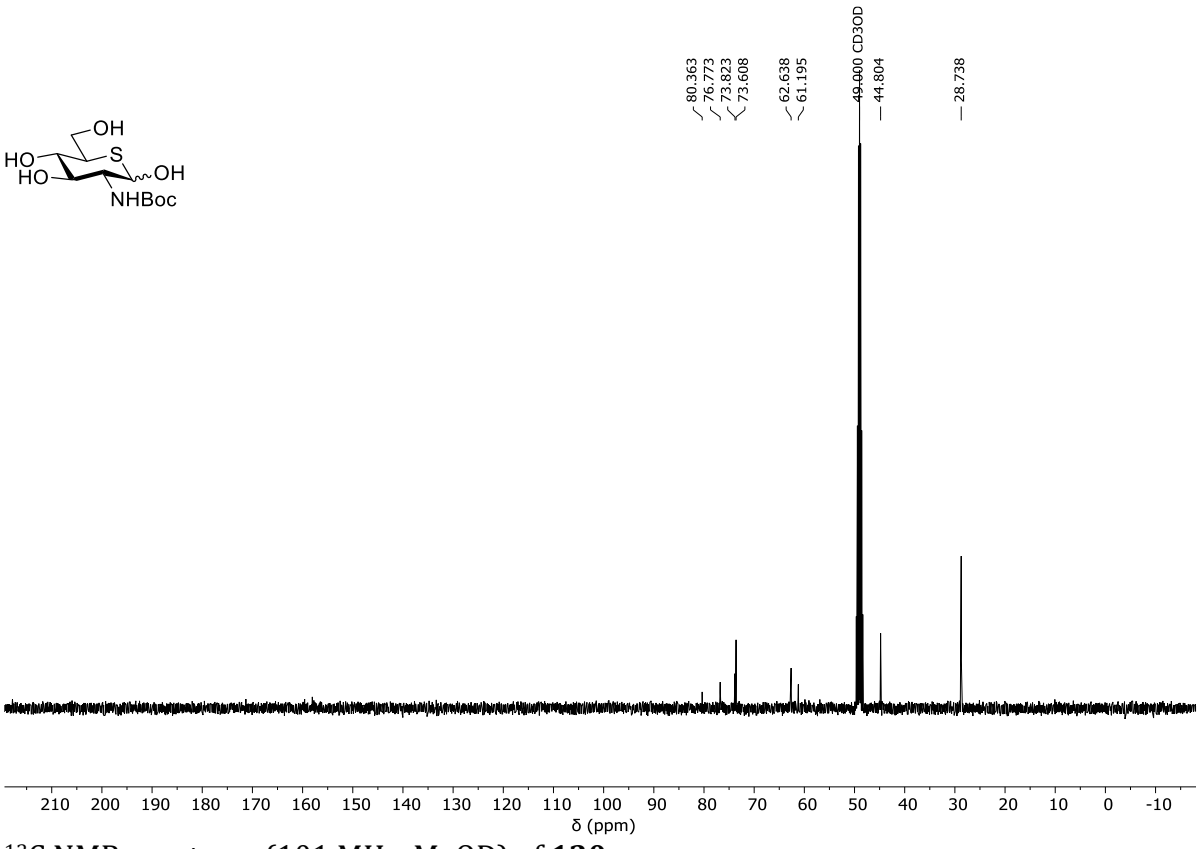

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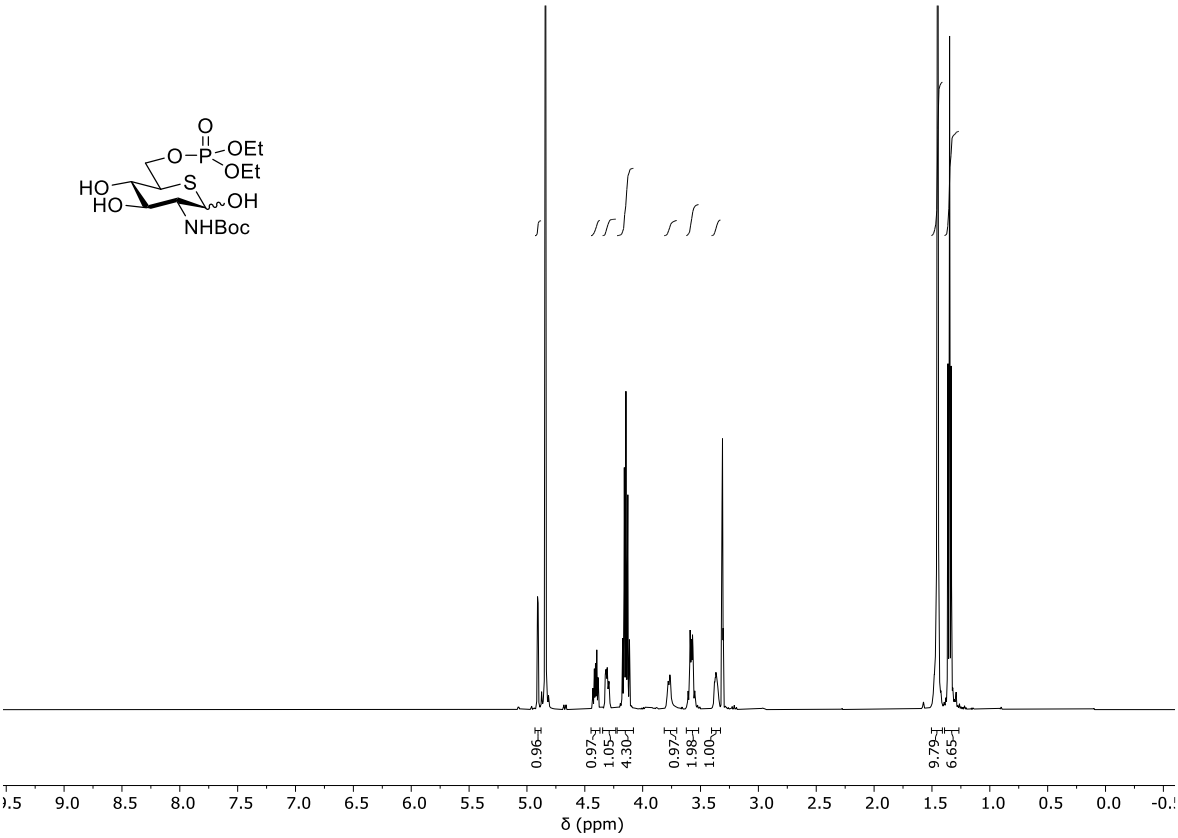

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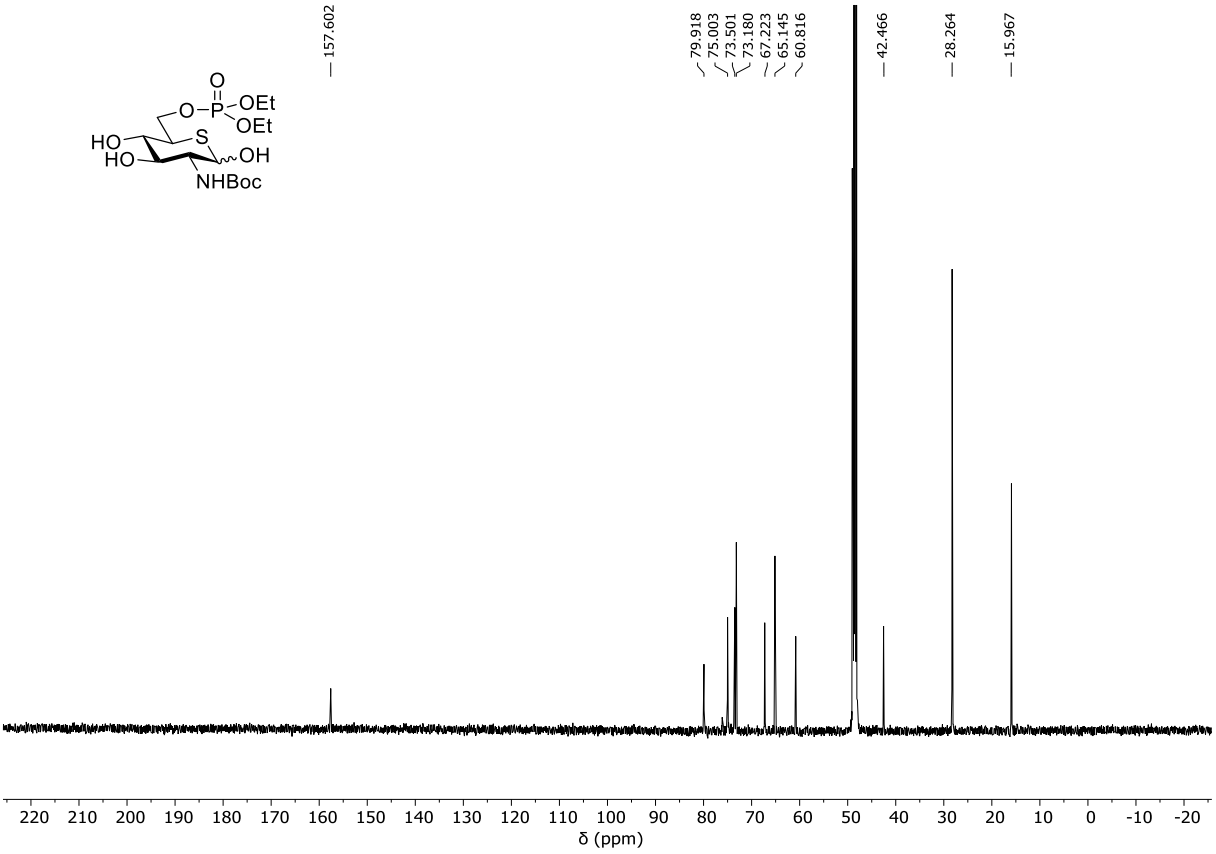

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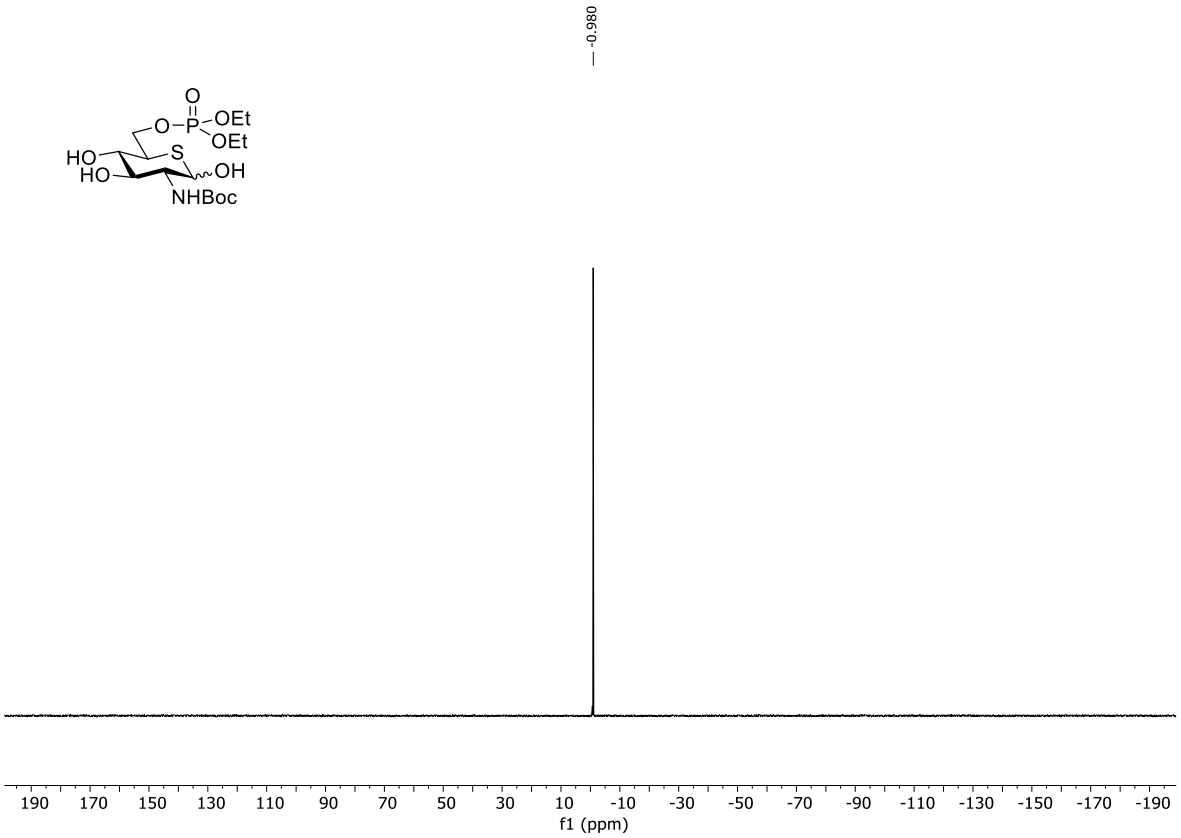

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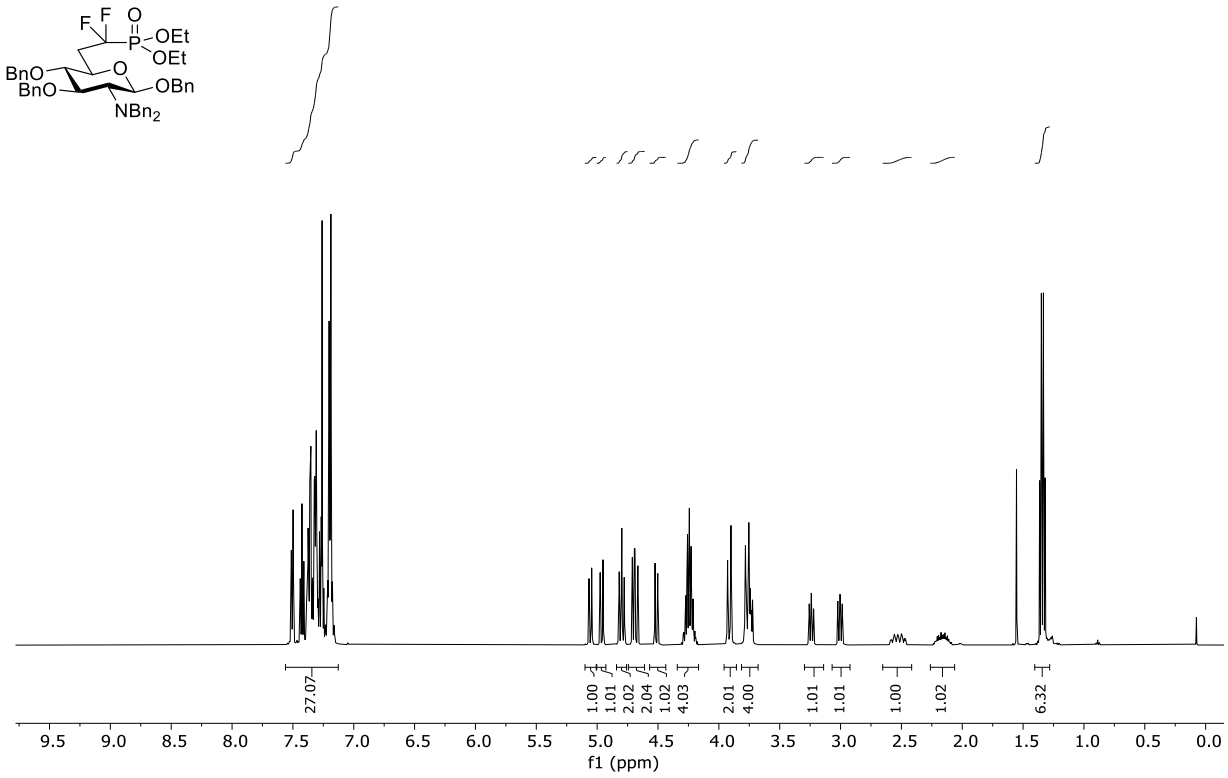

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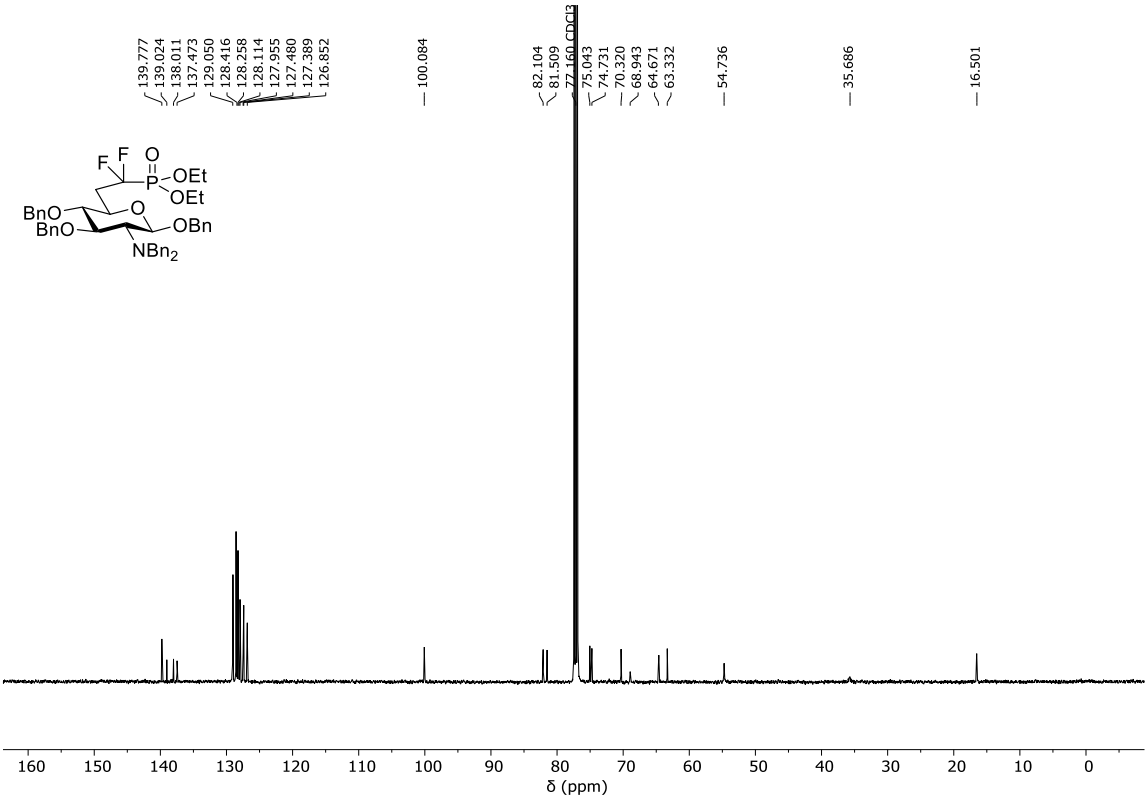

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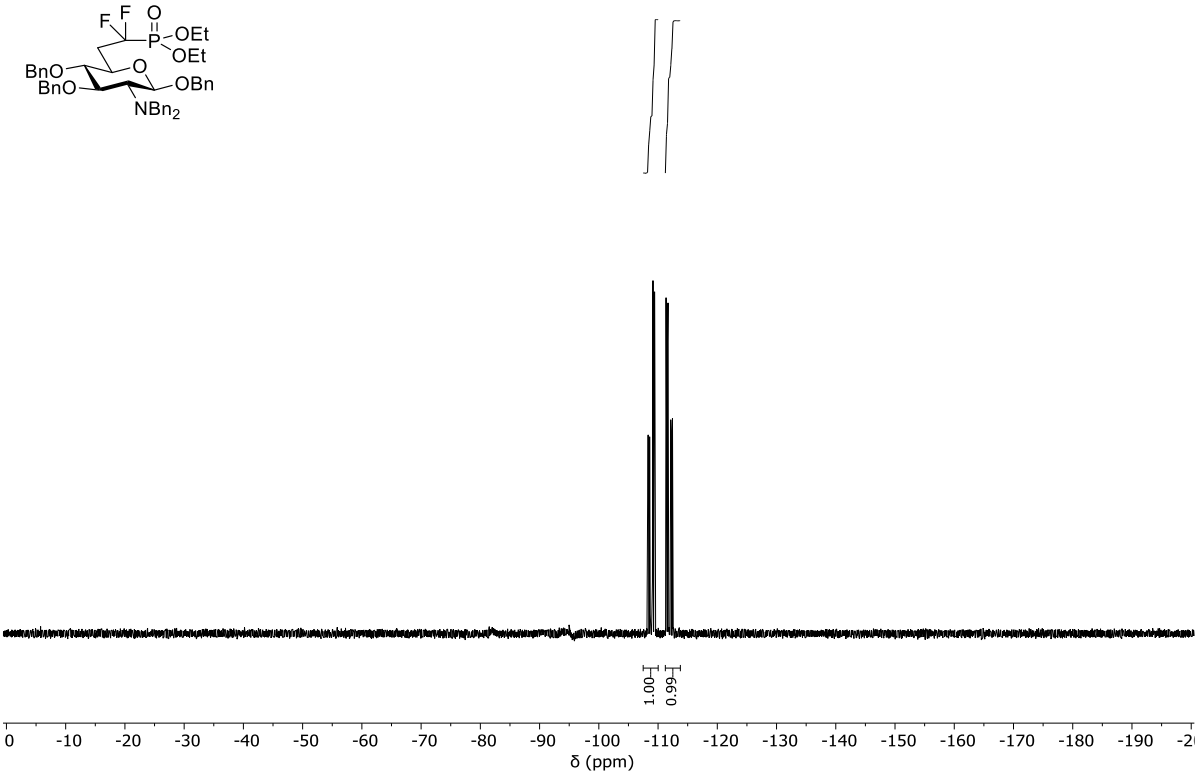
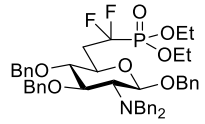
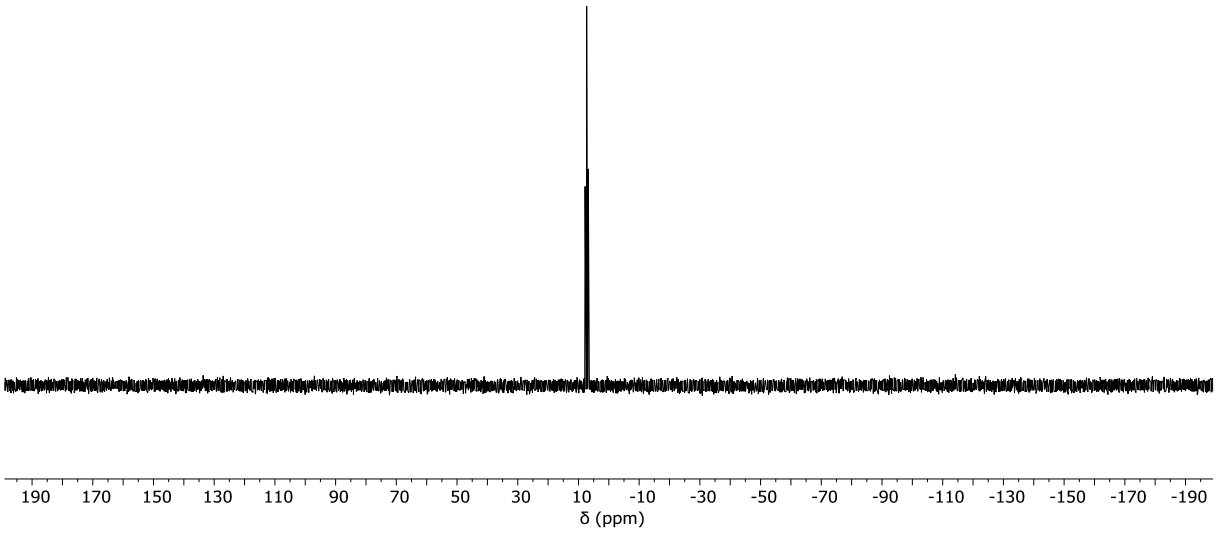
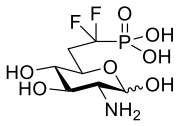


^{19}F NMR spectrum (377MHz, $CDCl_3$) of **133**

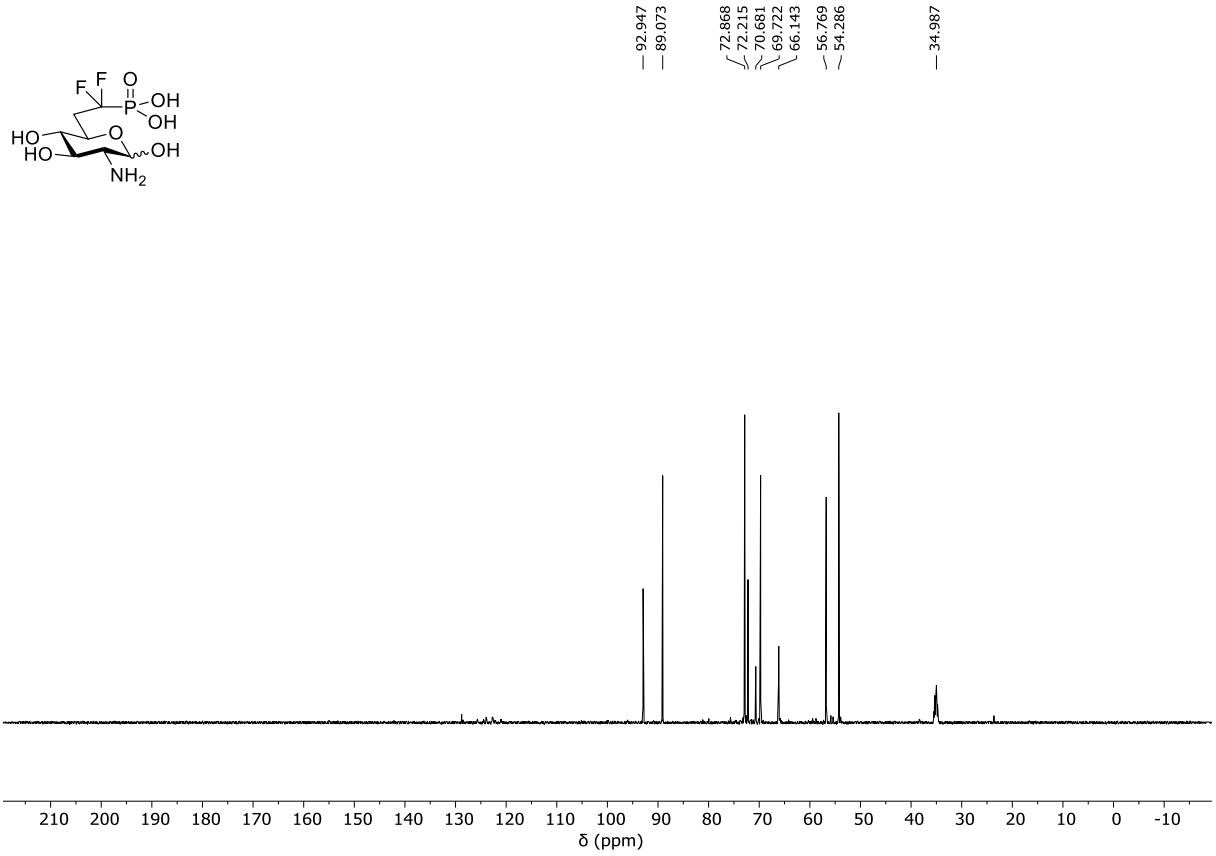




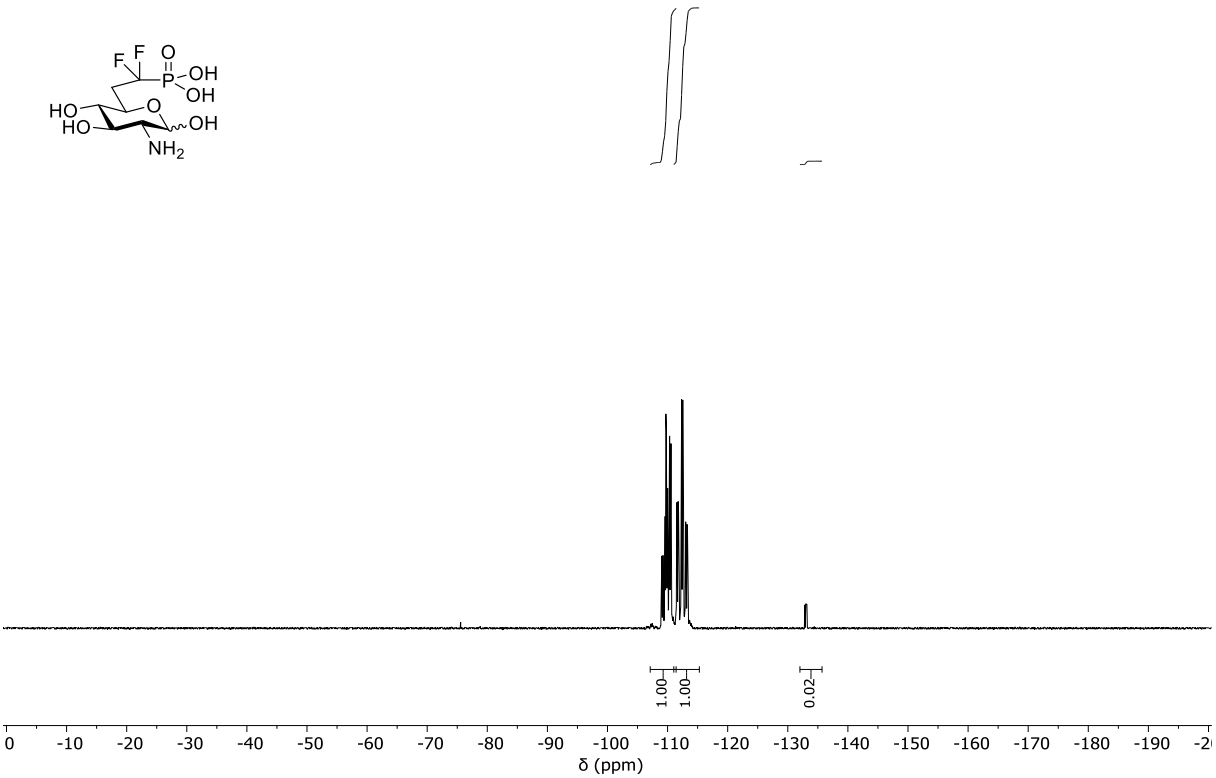
^{31}P NMR spectrum (202 MHz, $CDCl_3$) of **133**



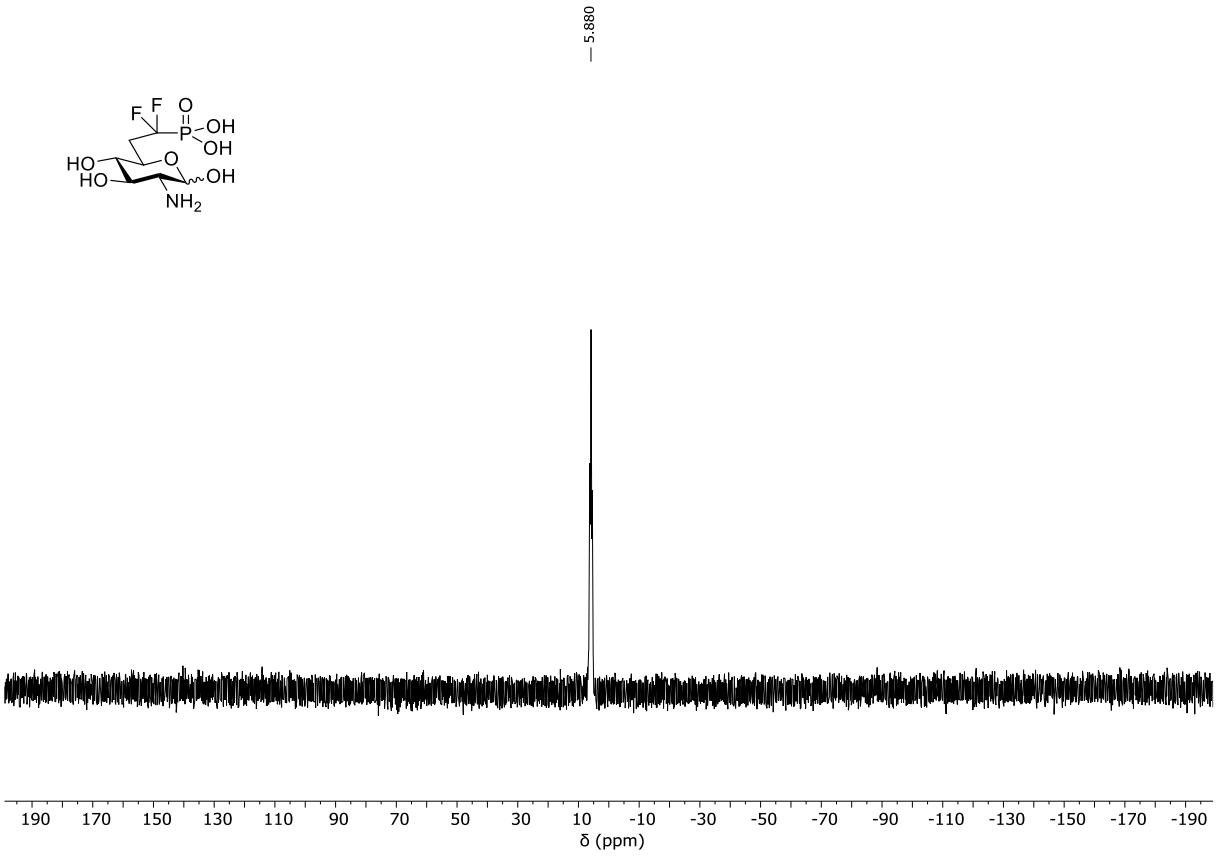
^{1}H NMR spectrum (600 MHz, D_2O) of **134**



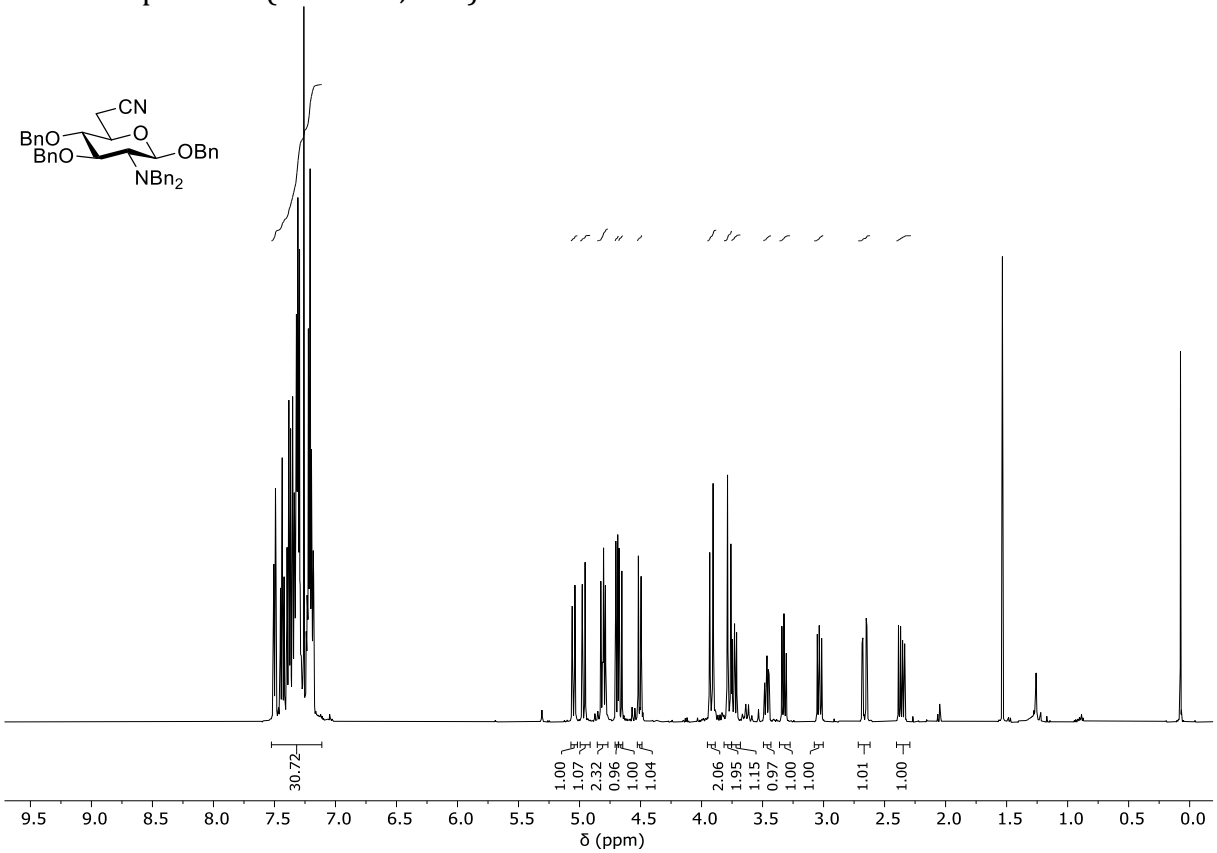
^{13}C NMR spectrum (151 MHz, D_2O) of **134**



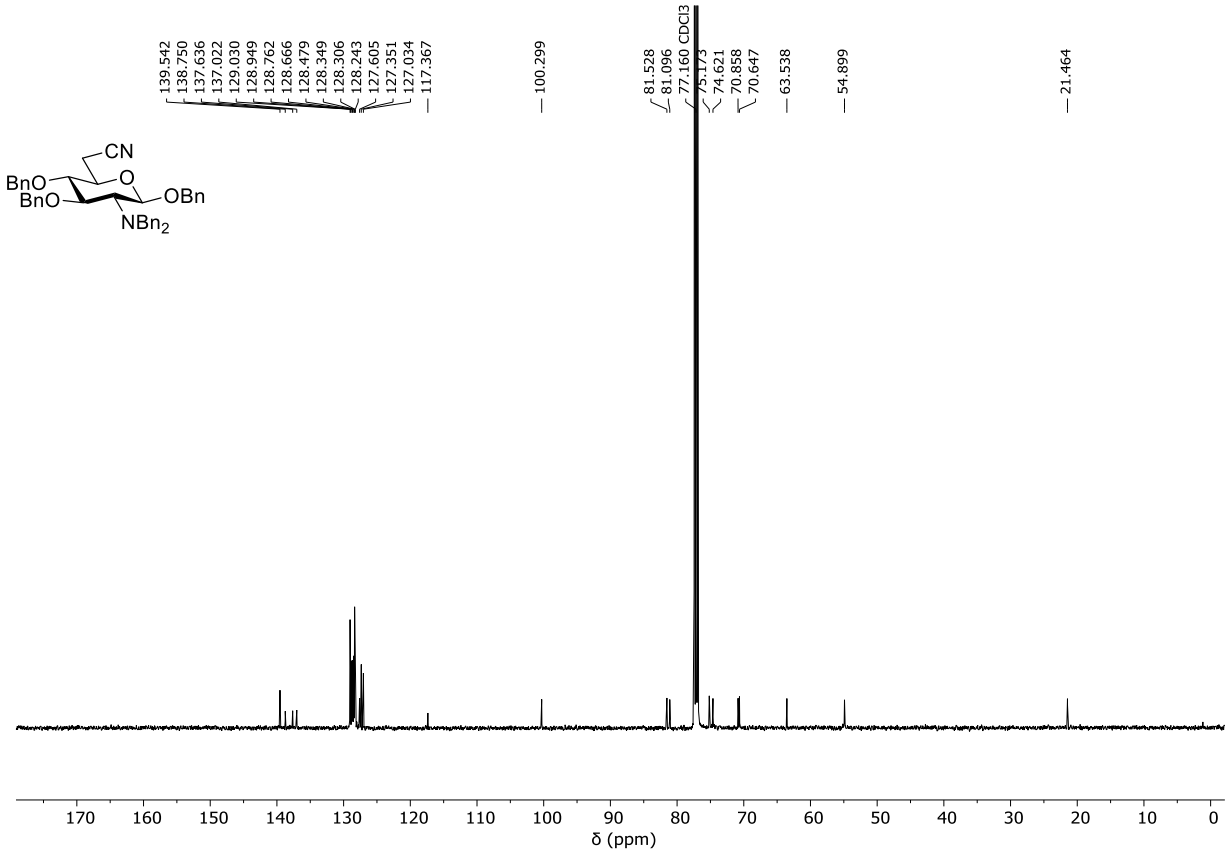
^{19}F NMR spectrum (377MHz, $CDCl_3$) of **134**



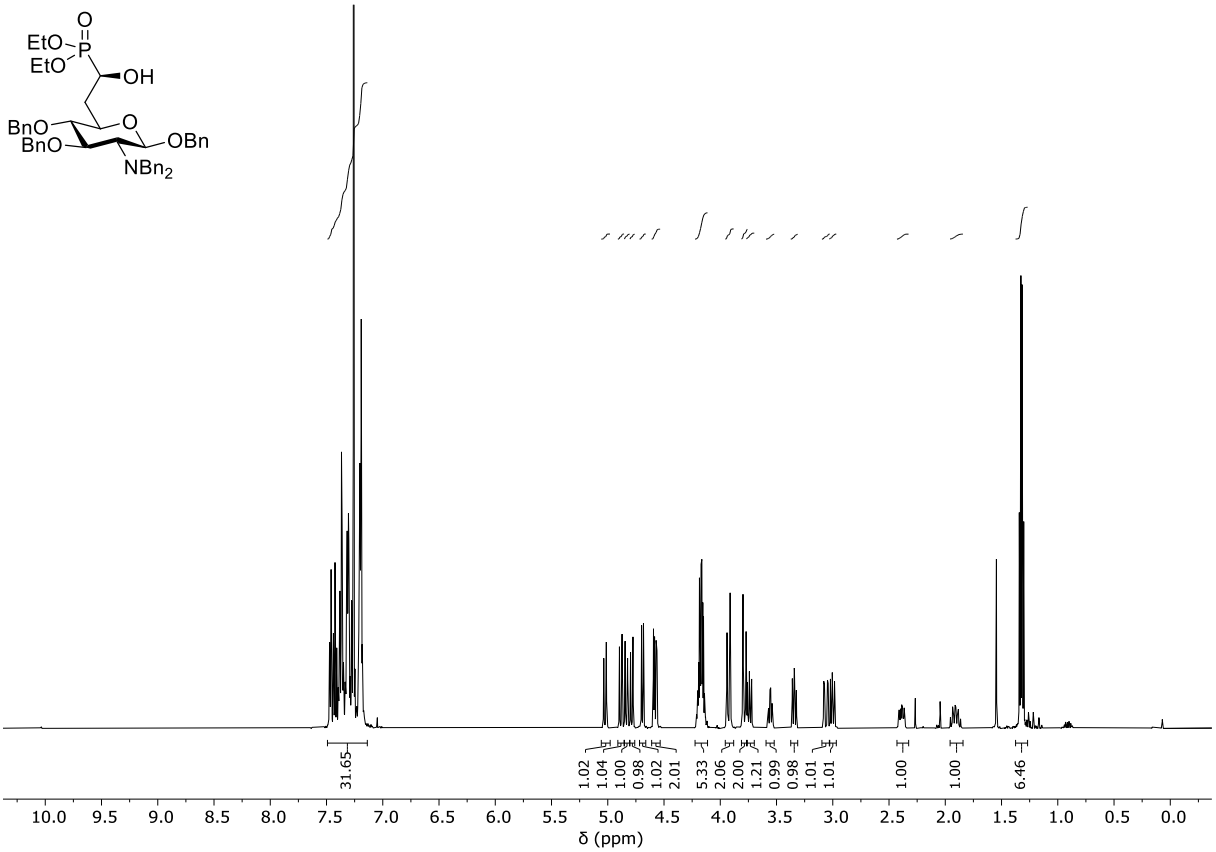
^{31}P NMR spectrum (202 MHz, D_2O) of **134**



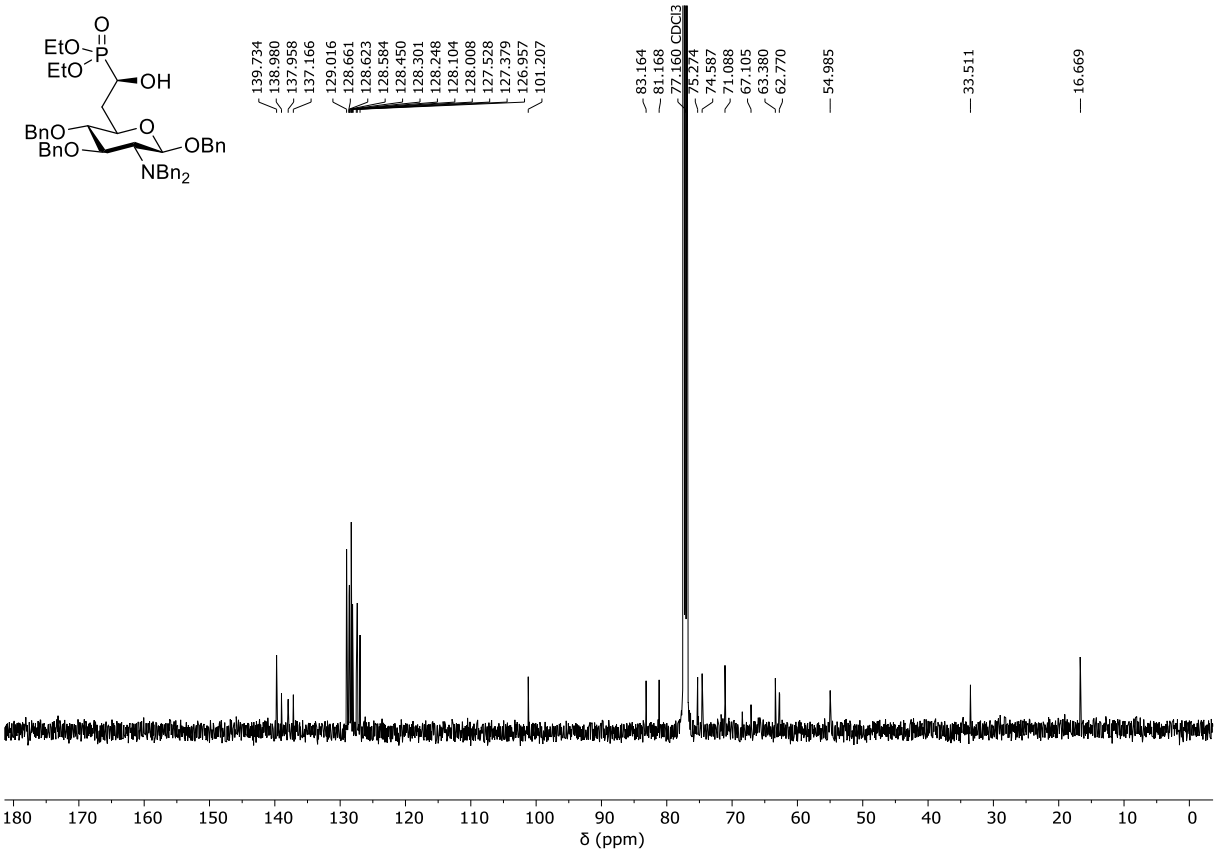
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **135**



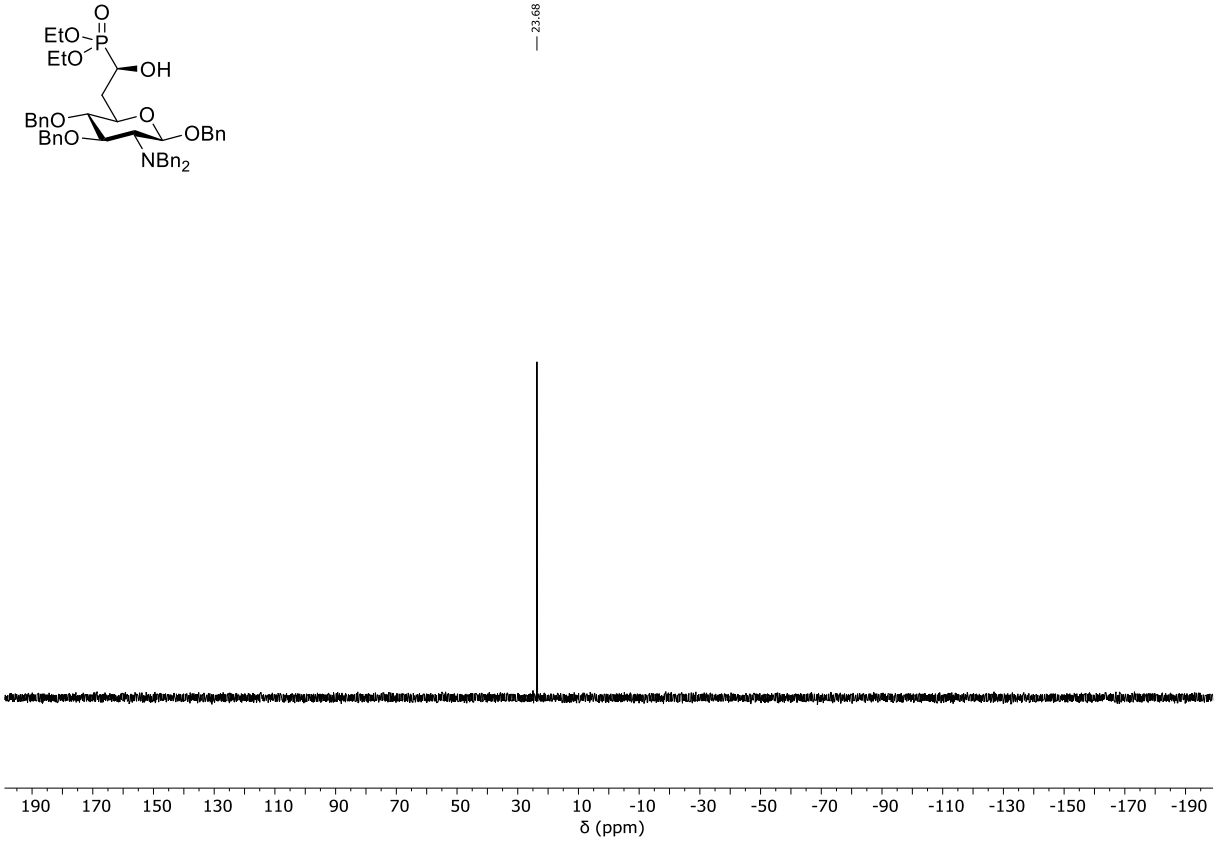
^{13}C NMR spectrum (128 MHz, $CDCl_3$) of **135**



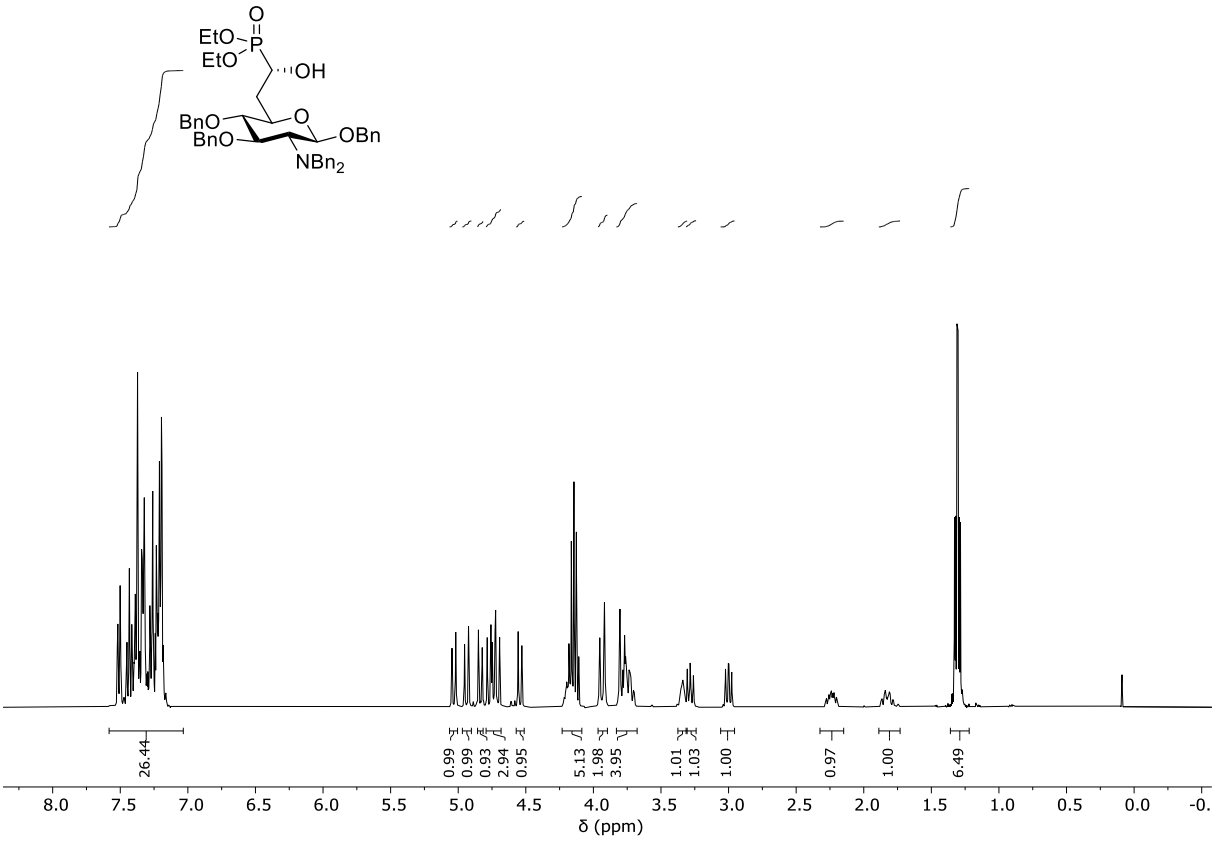
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of (*R*)-**136**



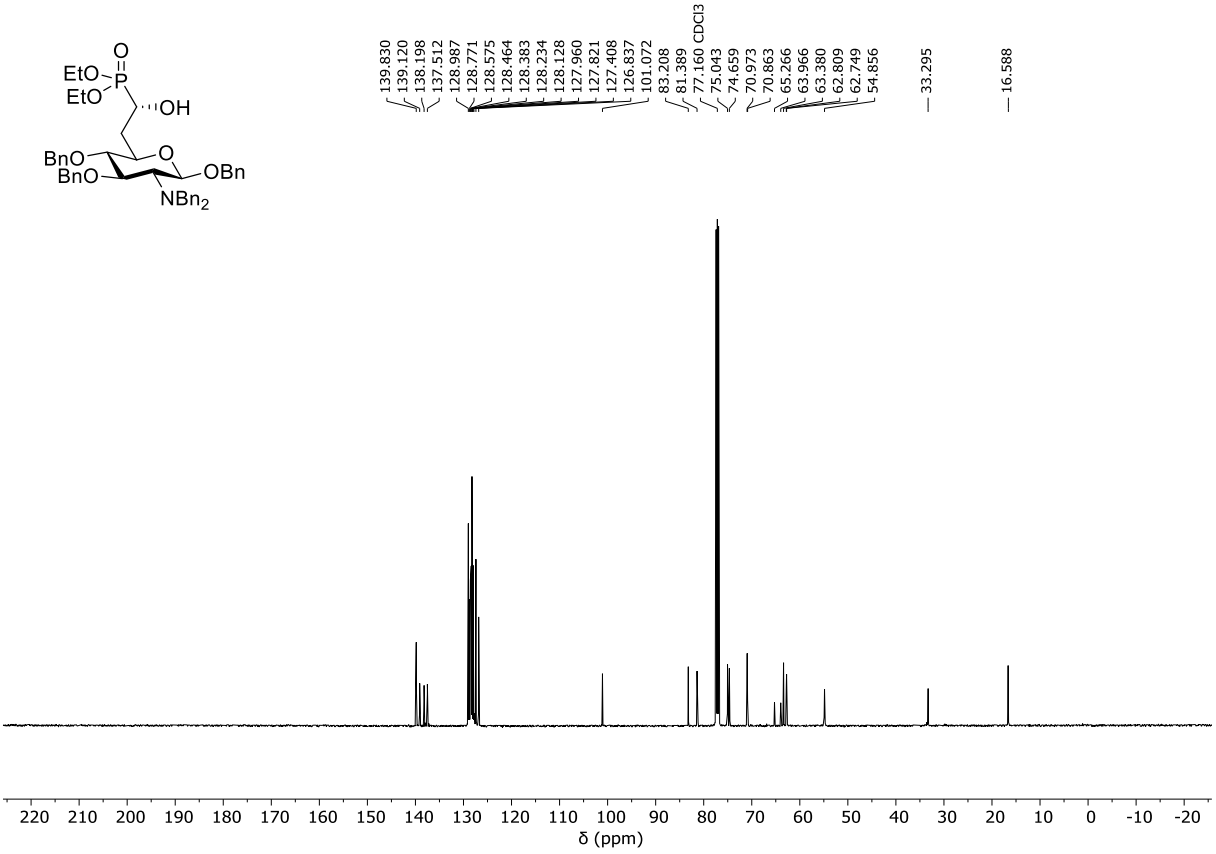
^{13}C NMR spectrum (128 MHz, $CDCl_3$) of (*R*)-**136**



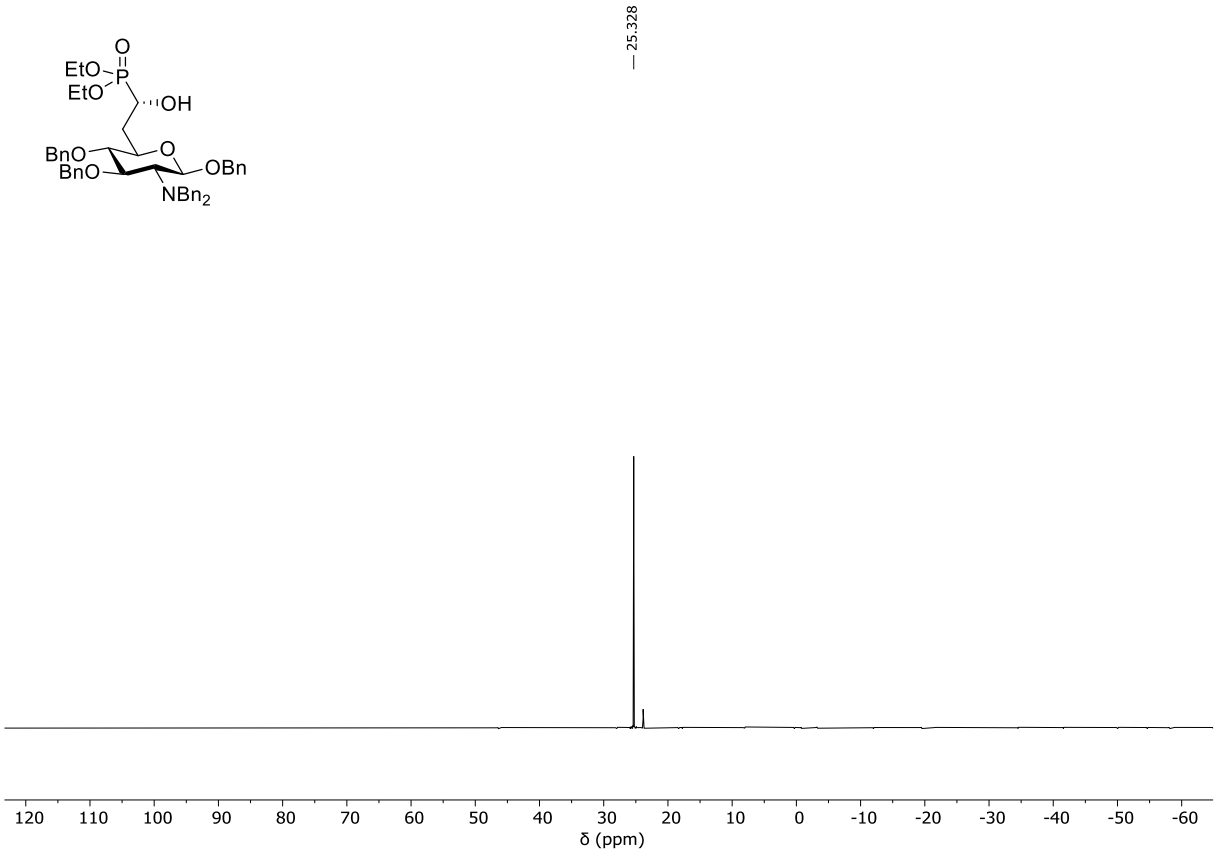
^{31}P NMR spectrum (202 MHz, $CDCl_3$) of (*R*)-**136**



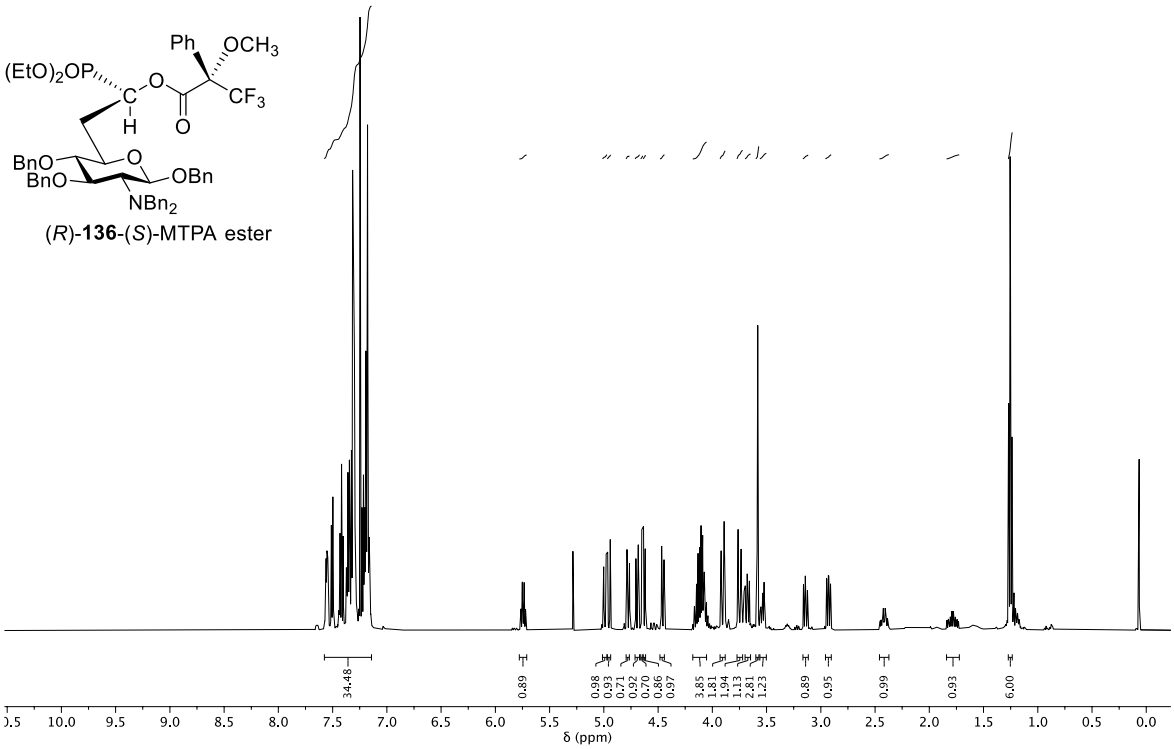
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of (*S*)-**136**



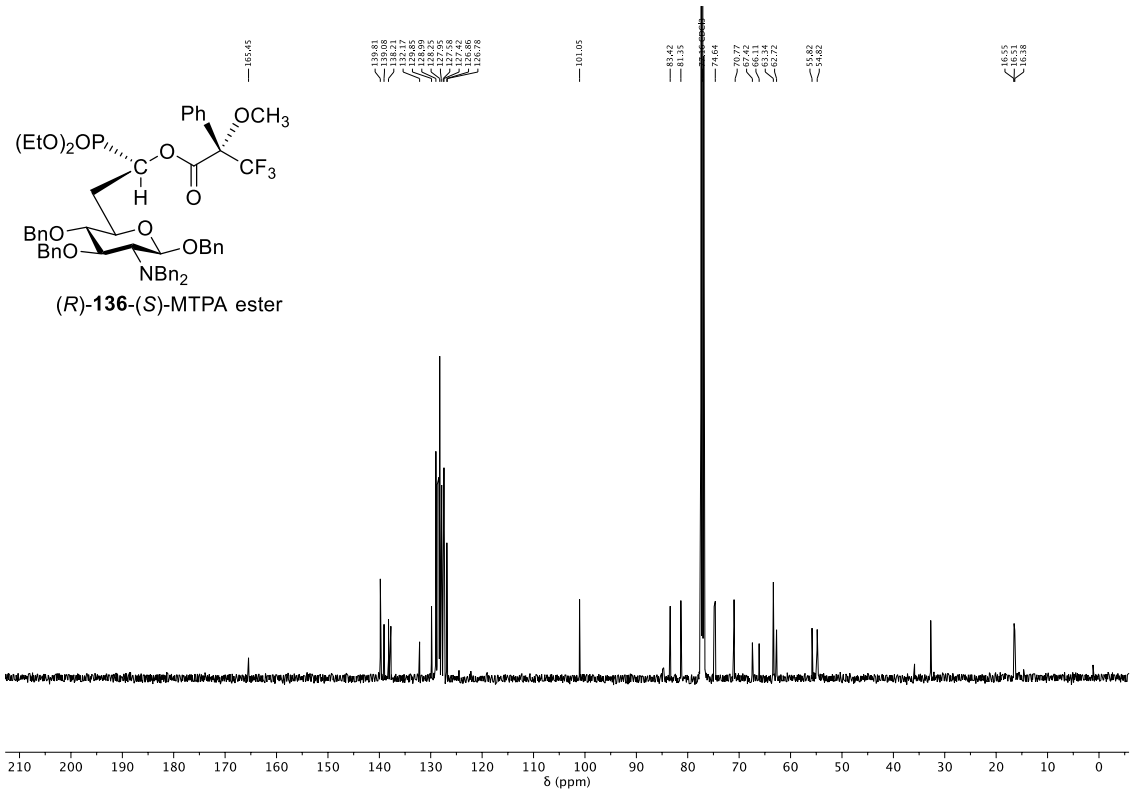
^{13}C NMR spectrum (128 MHz, $CDCl_3$) of (*S*)-**136**



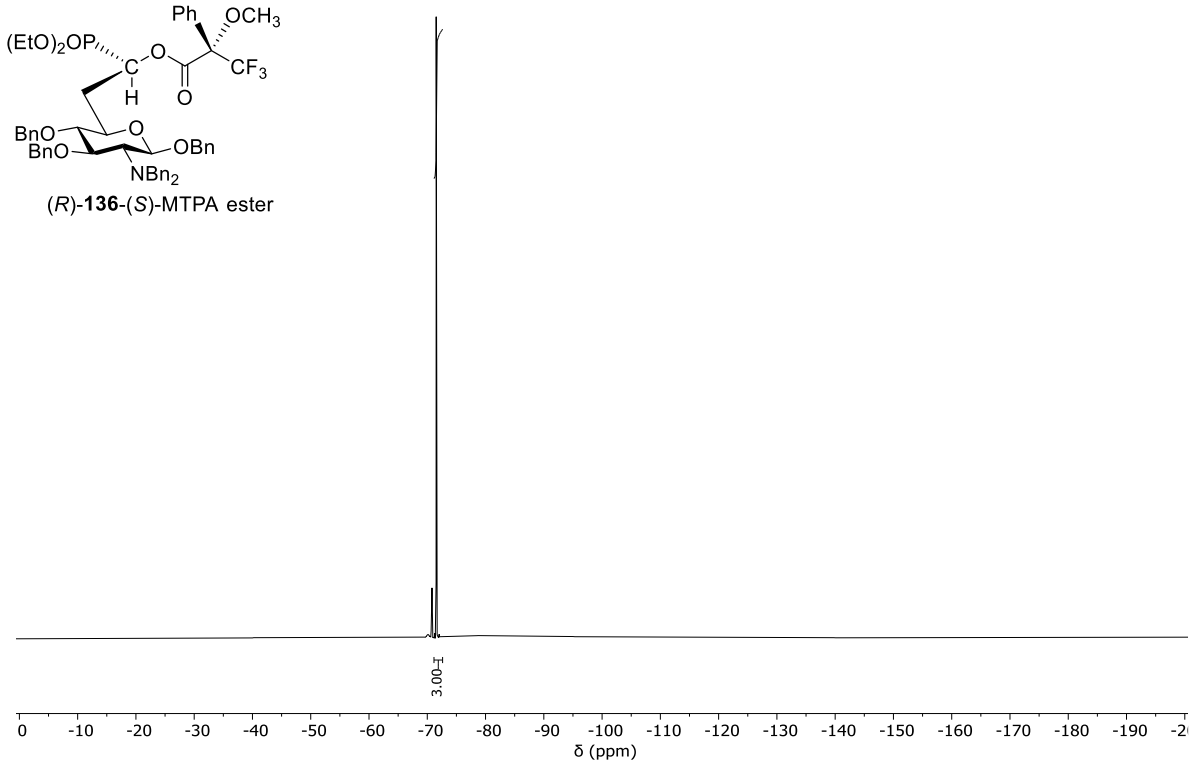
^{31}P NMR spectrum (202MHz, $CDCl_3$) of (*S*)-**136**



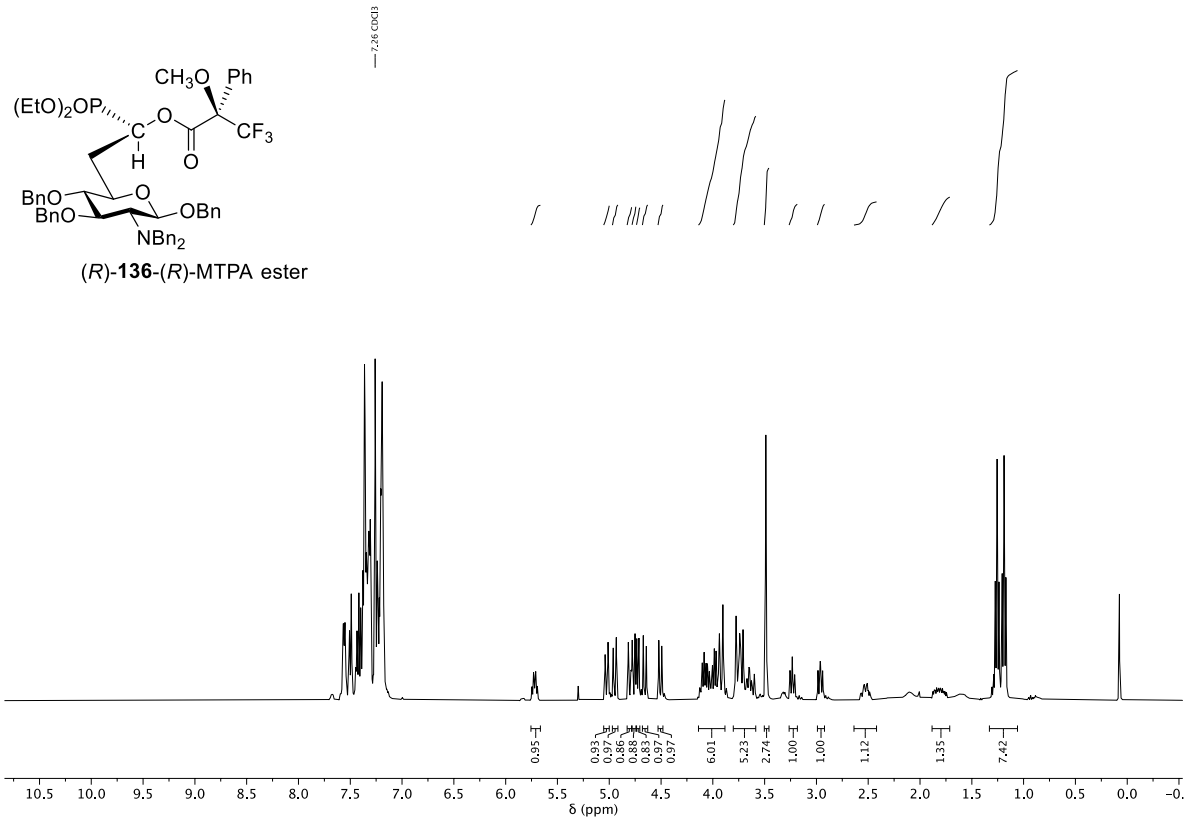
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **(*R*)-136-(*S*)-MTPA ester**



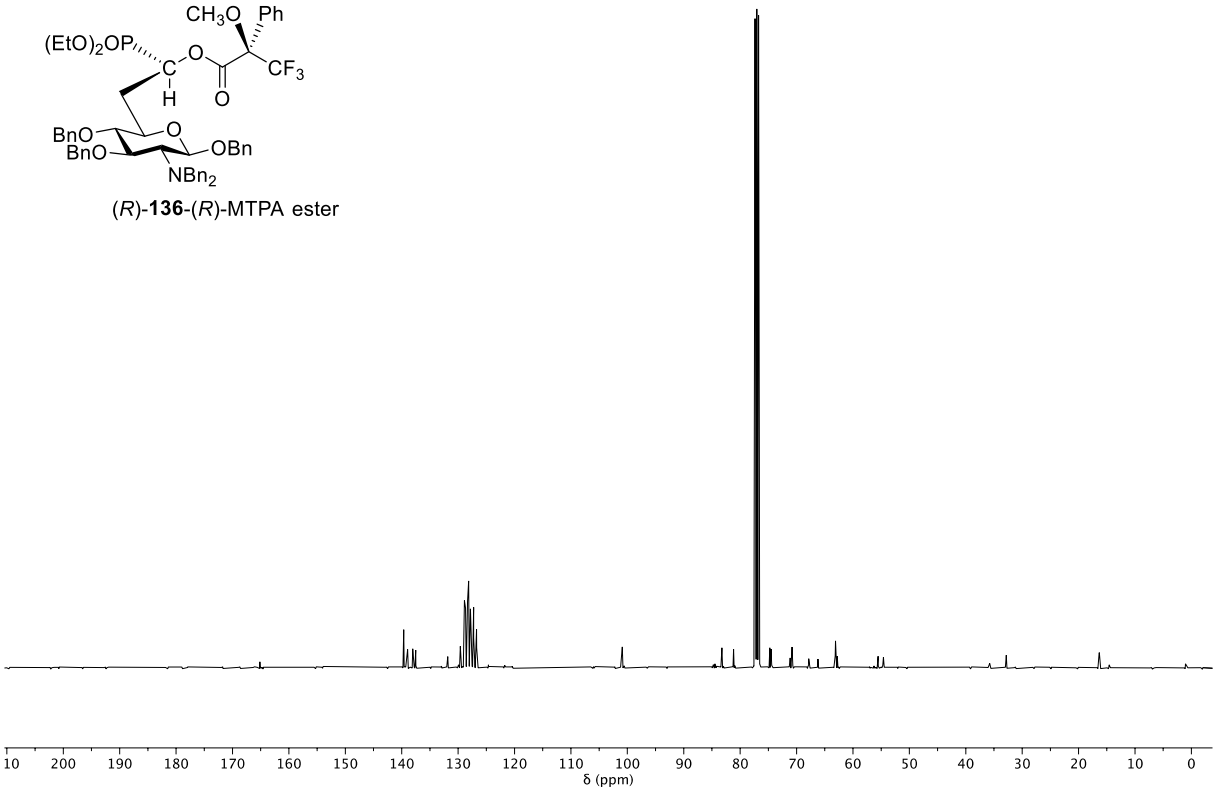
^{13}C-NMR (377 MHz, $CDCl_3$) spectrum of **(*R*)-136-(*S*)-MTPA ester**.



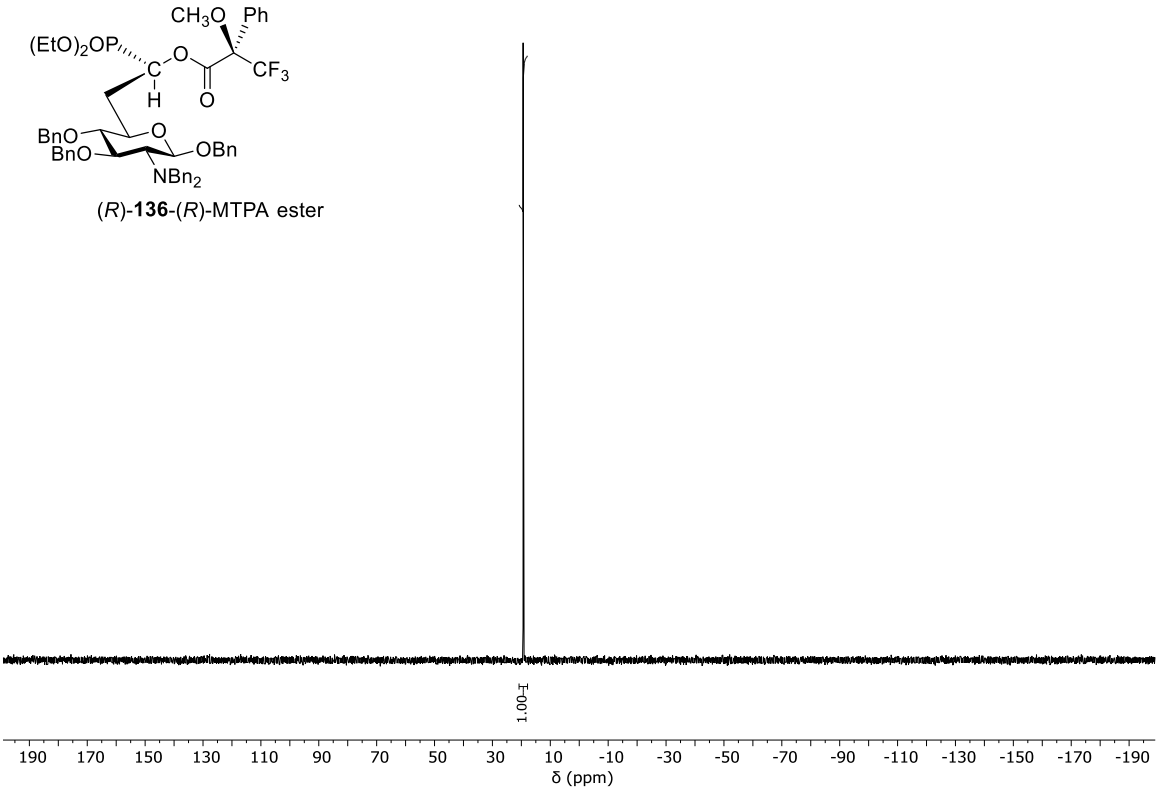
^{19}F NMR spectrum (376 MHz, $CDCl_3$) of **(*R*)-136-(*S*)-MTPA ester**



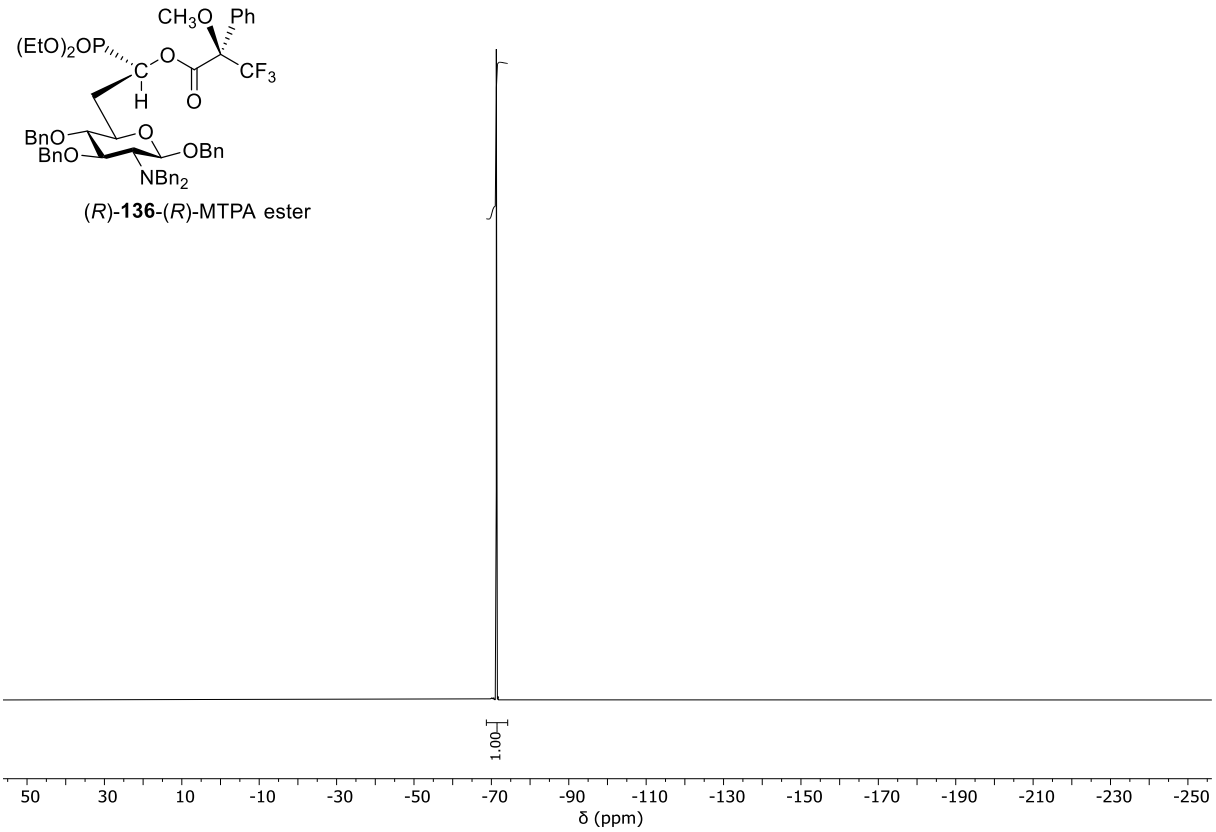
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **(*R*)-136-(*R*)-MTPA ester**



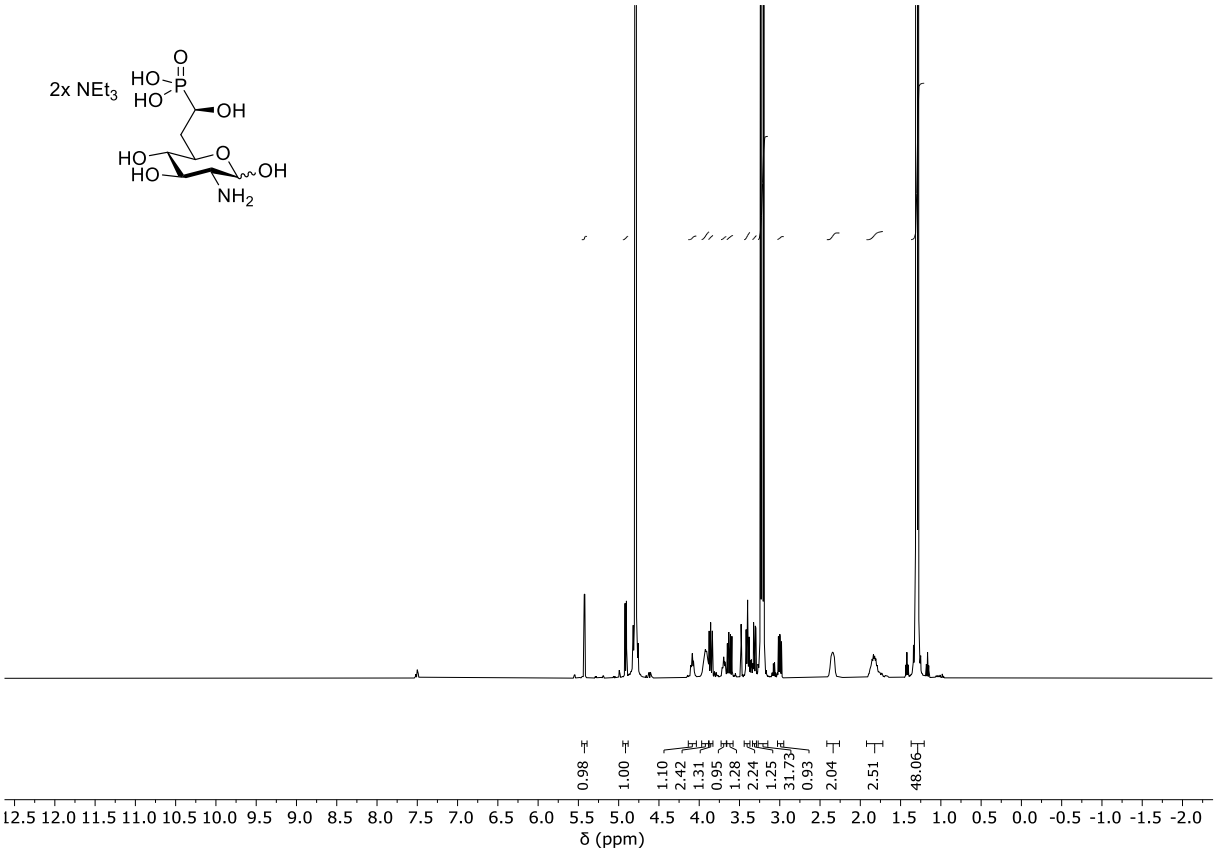
^{13}C-NMR (101 MHz, $CDCl_3$) spectrum of **(*R*)-136-(*R*)-MTPA ester**



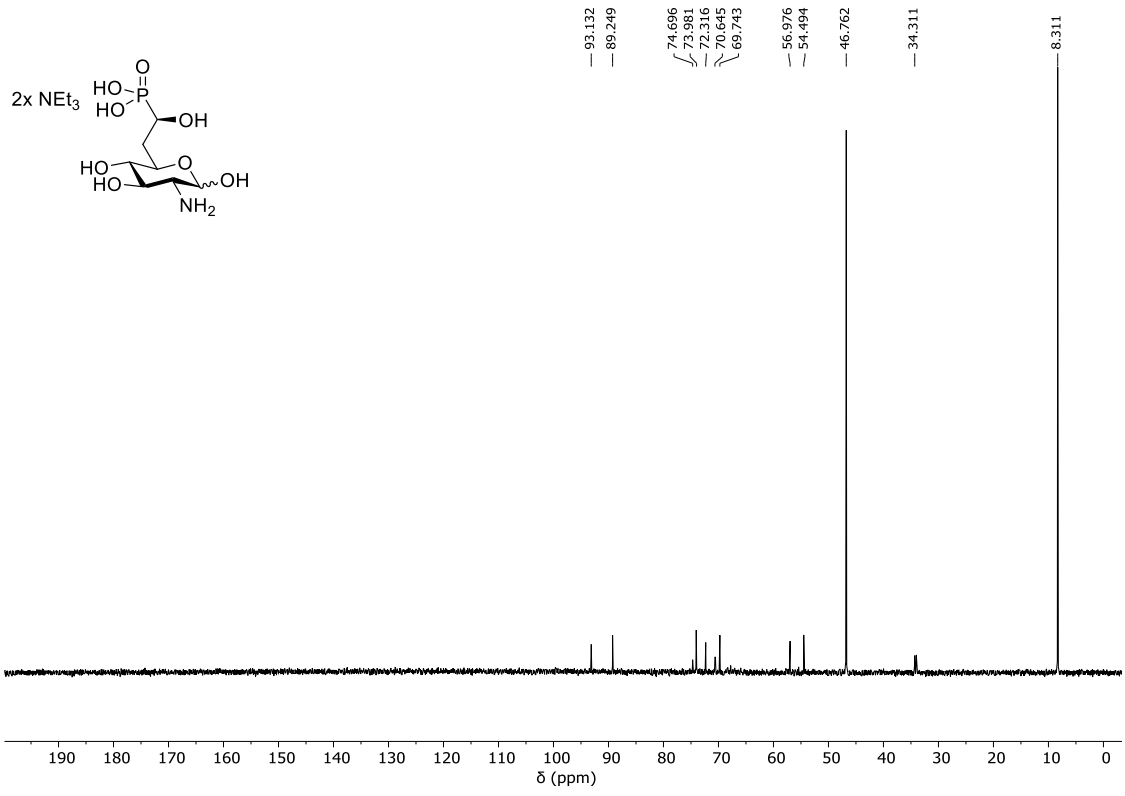
^{19}F NMR spectrum (471 MHz, $CDCl_3$) of **(*R*)-136-(*R*)-MTPA ester**



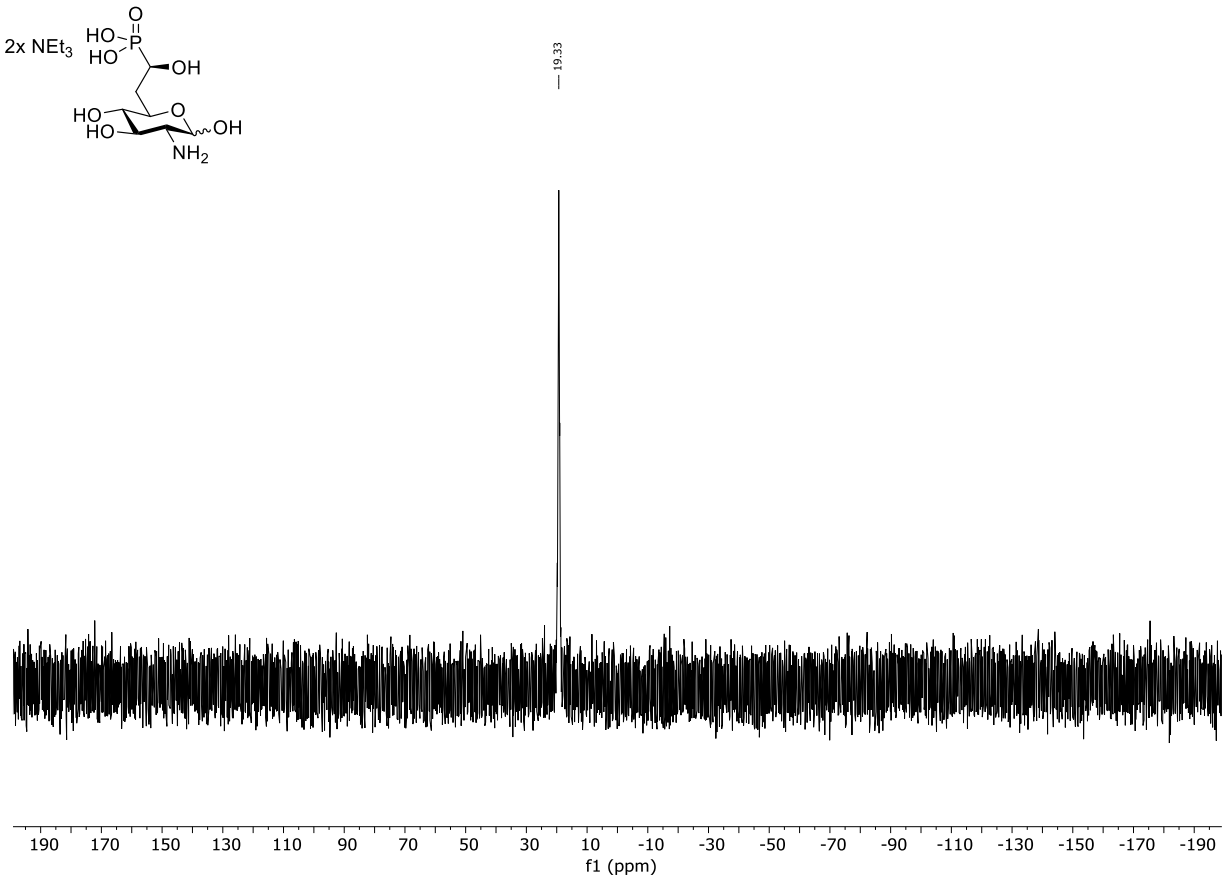
^{31}P NMR spectrum (202MHz, $CDCl_3$) of **(*R*)-136-(*R*)-MTPA ester**



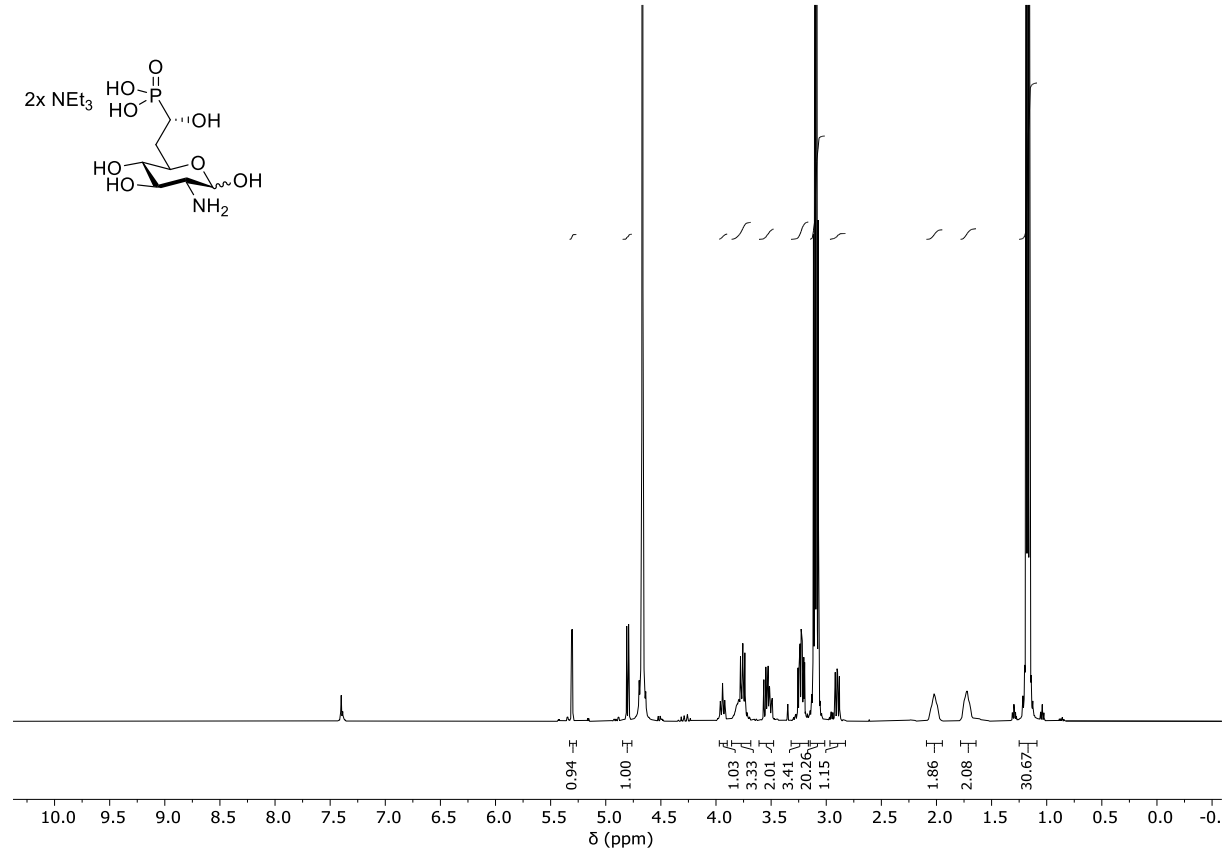
^{1}H NMR spectrum (500 MHz, D_2O) of (*R*)-**137**



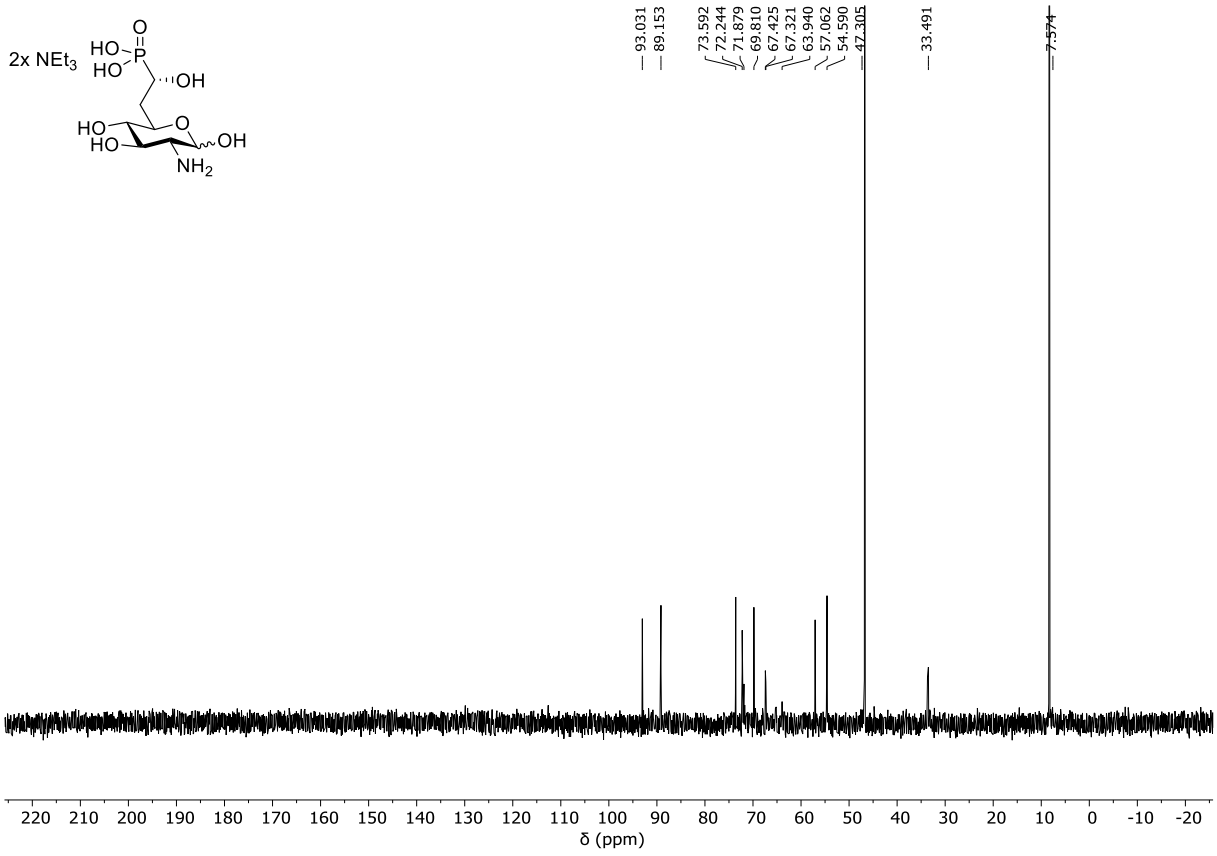
^{13}C NMR spectrum (126 MHz, D_2O) of (*R*)-**137**



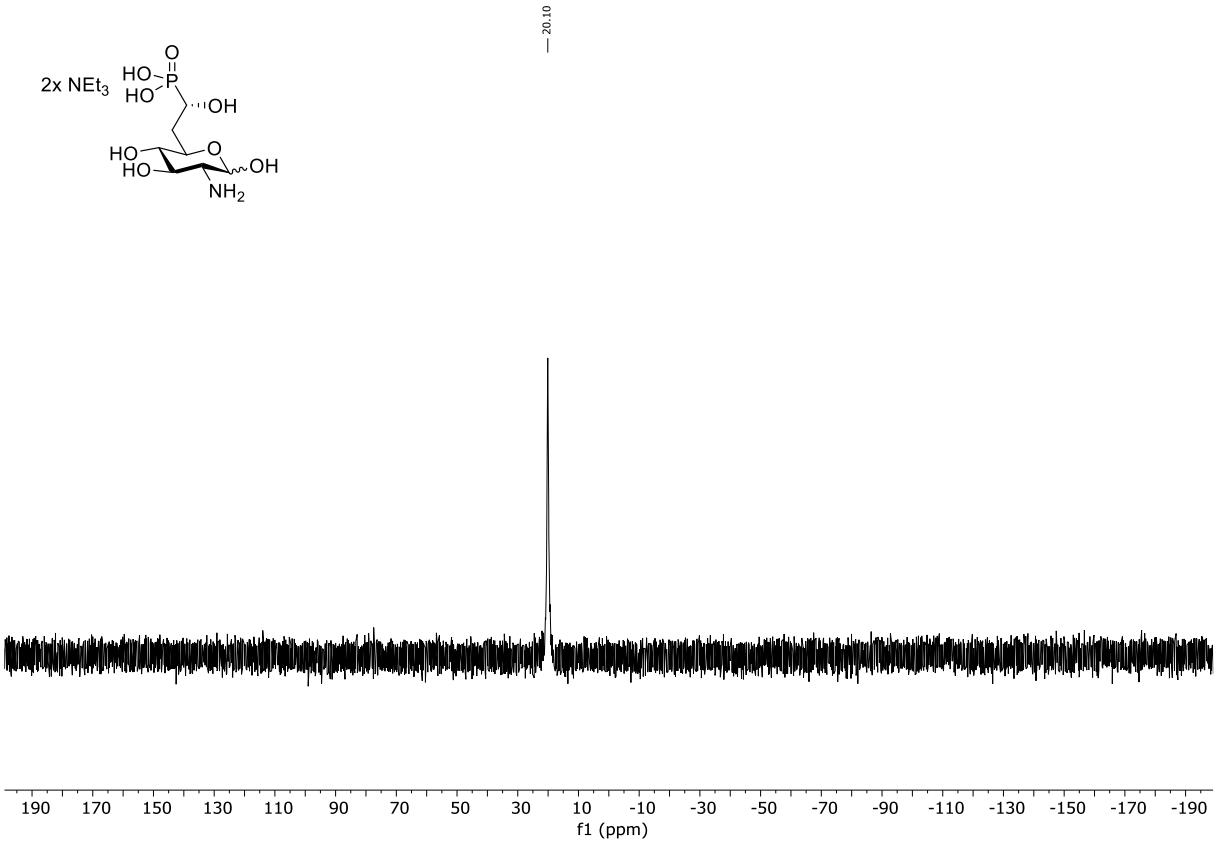
^{31}P NMR spectrum (202 MHz, D_2O) of (*R*)-**137**



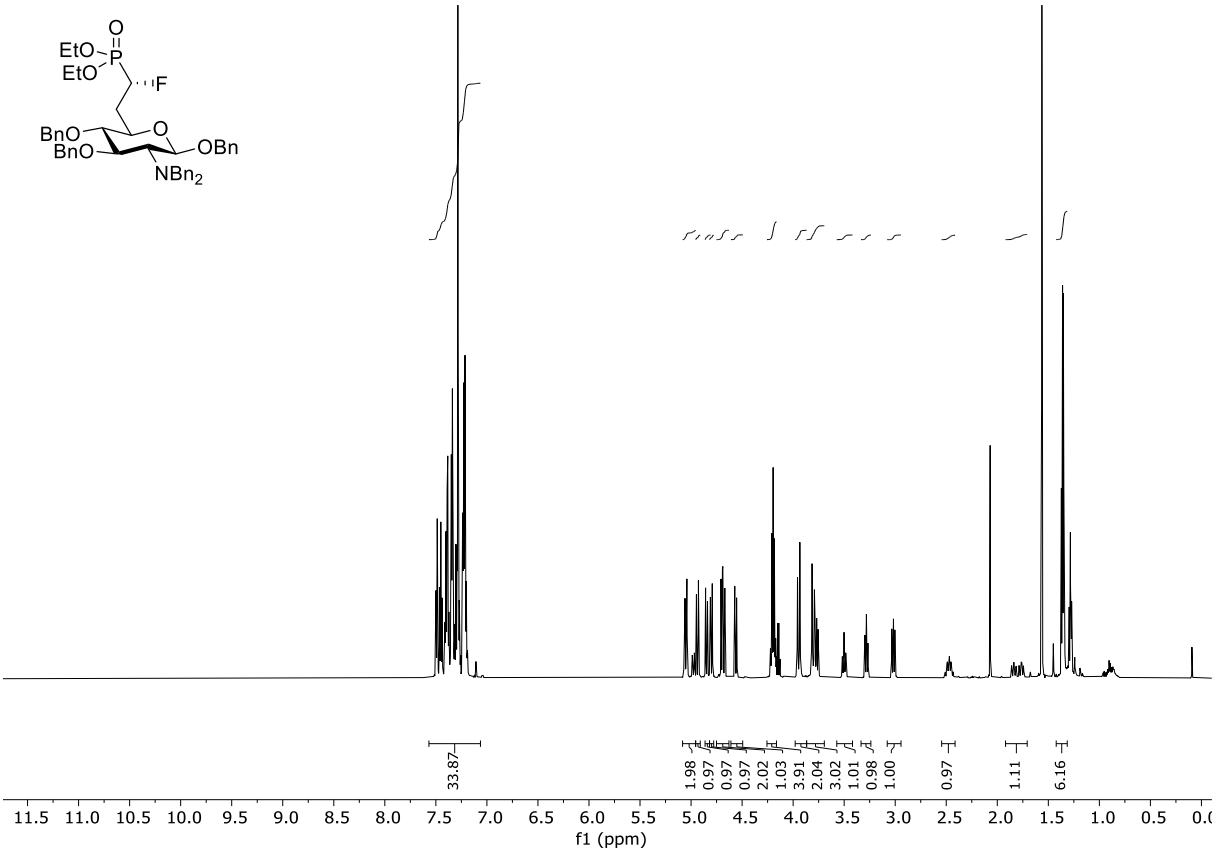
^{1}H NMR spectrum (500 MHz, D_2O) of (*S*)-**137**



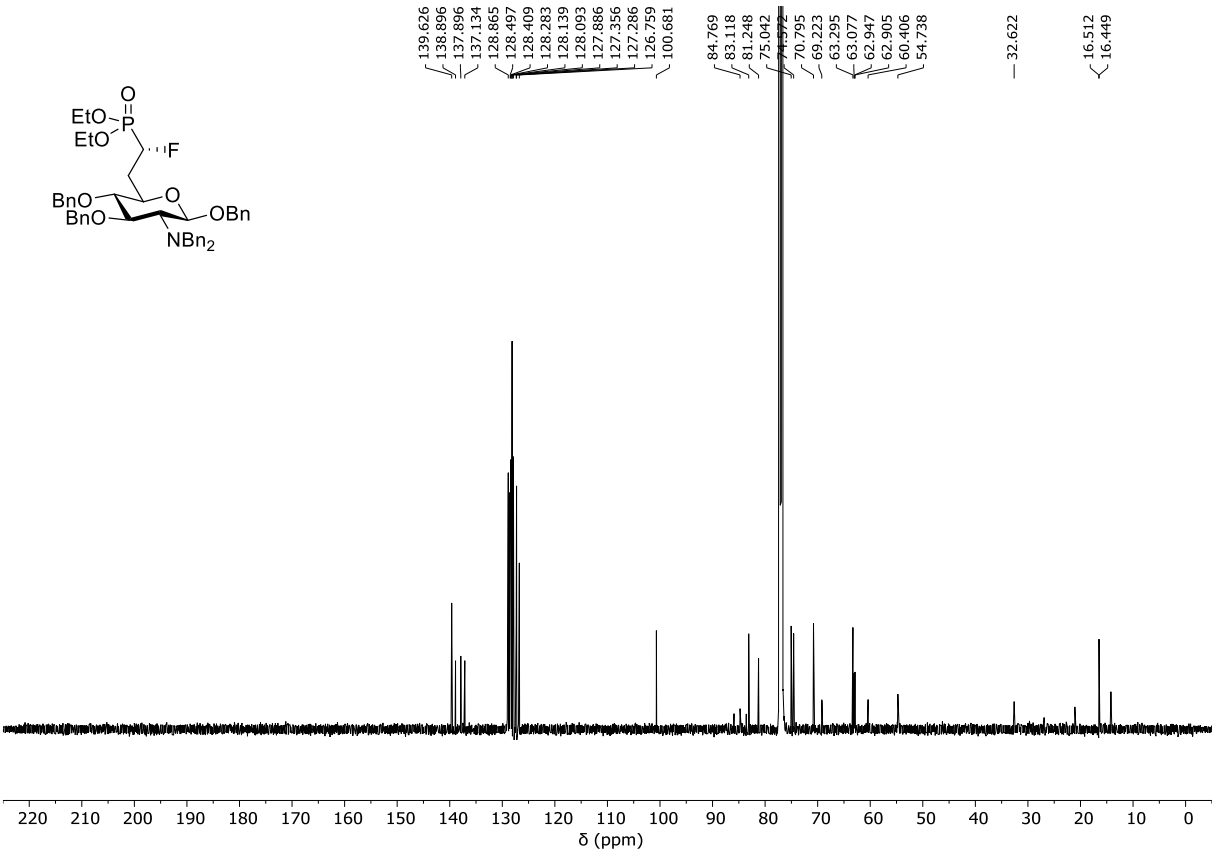
^{13}C NMR spectrum (126 MHz, D_2O) of(*S*)-**137**



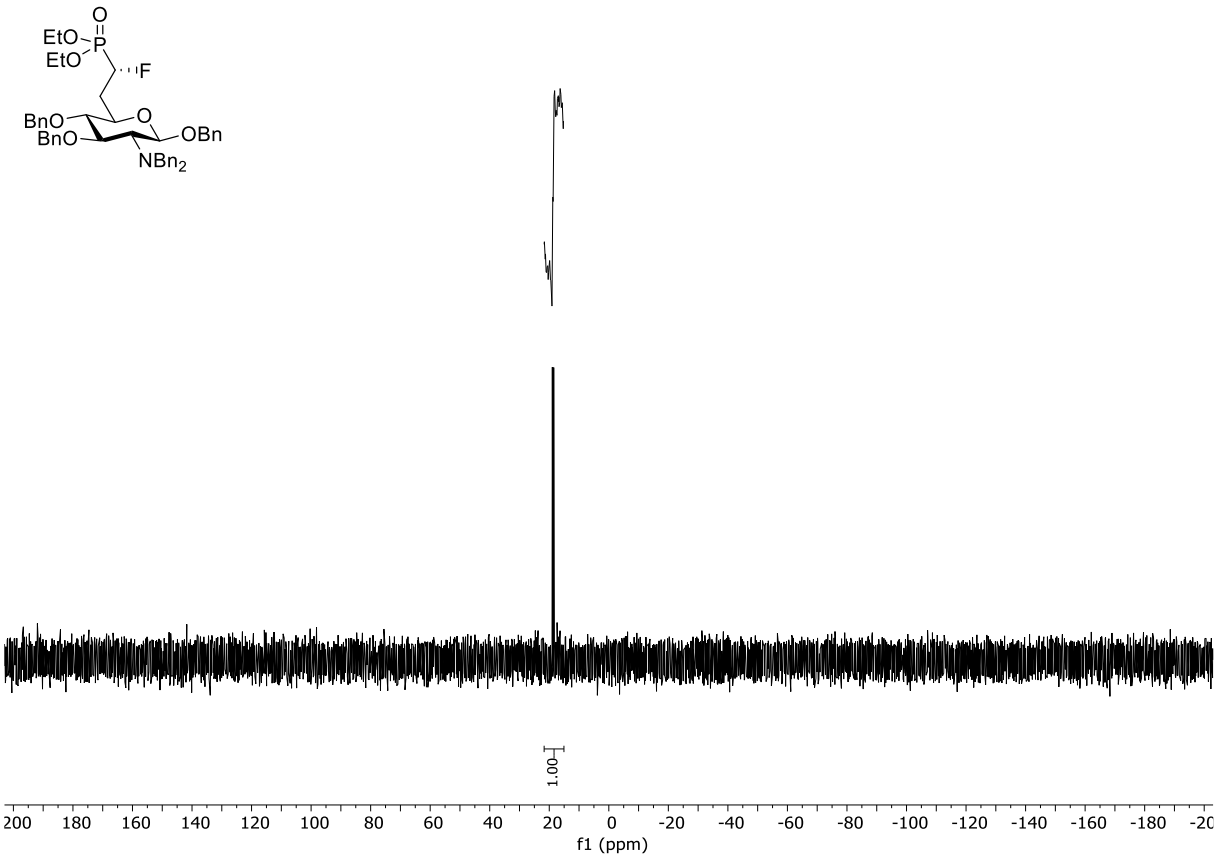
^{31}P NMR spectrum (202 MHz, D_2O) of (*S*)-**137**



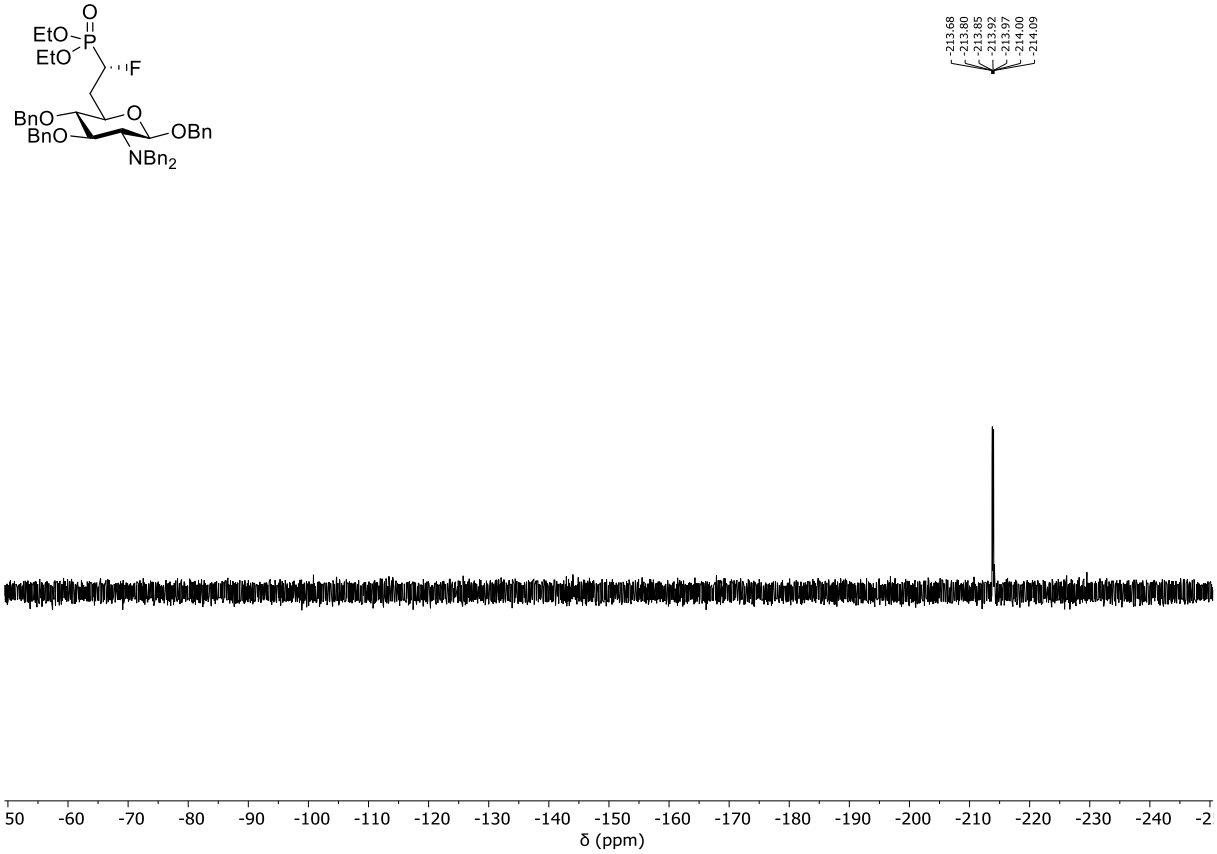
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of (*S*)-**138**



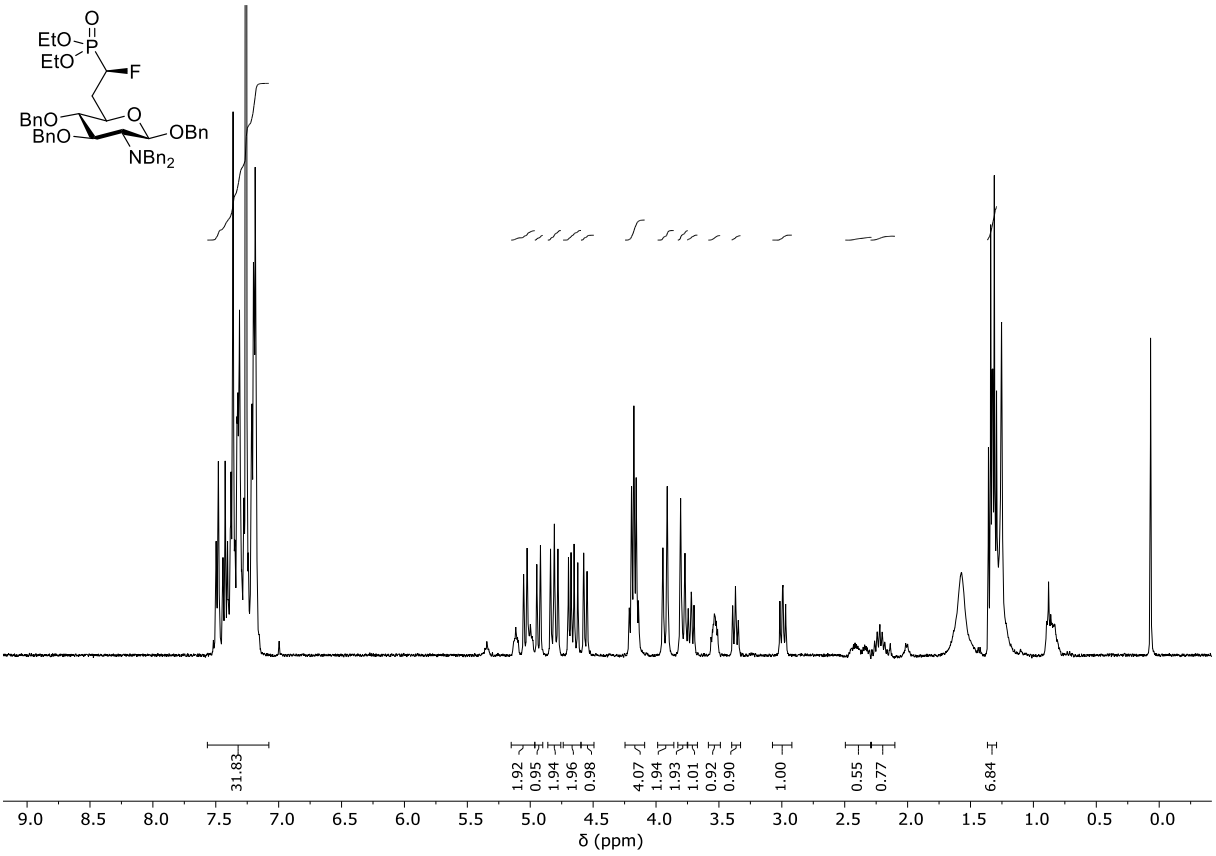
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of (*S*)-**138**



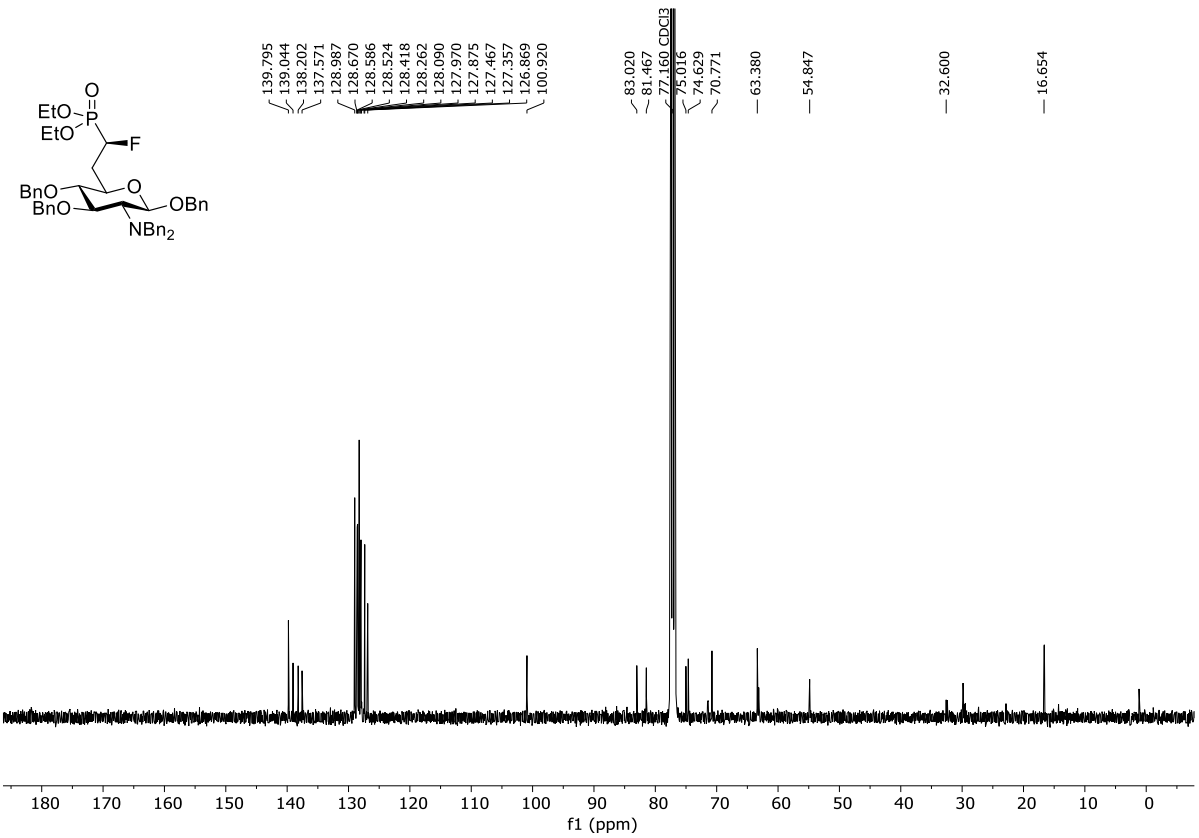
^{31}P NMR spectrum (162 MHz, $CDCl_3$) of (*S*)-**138**



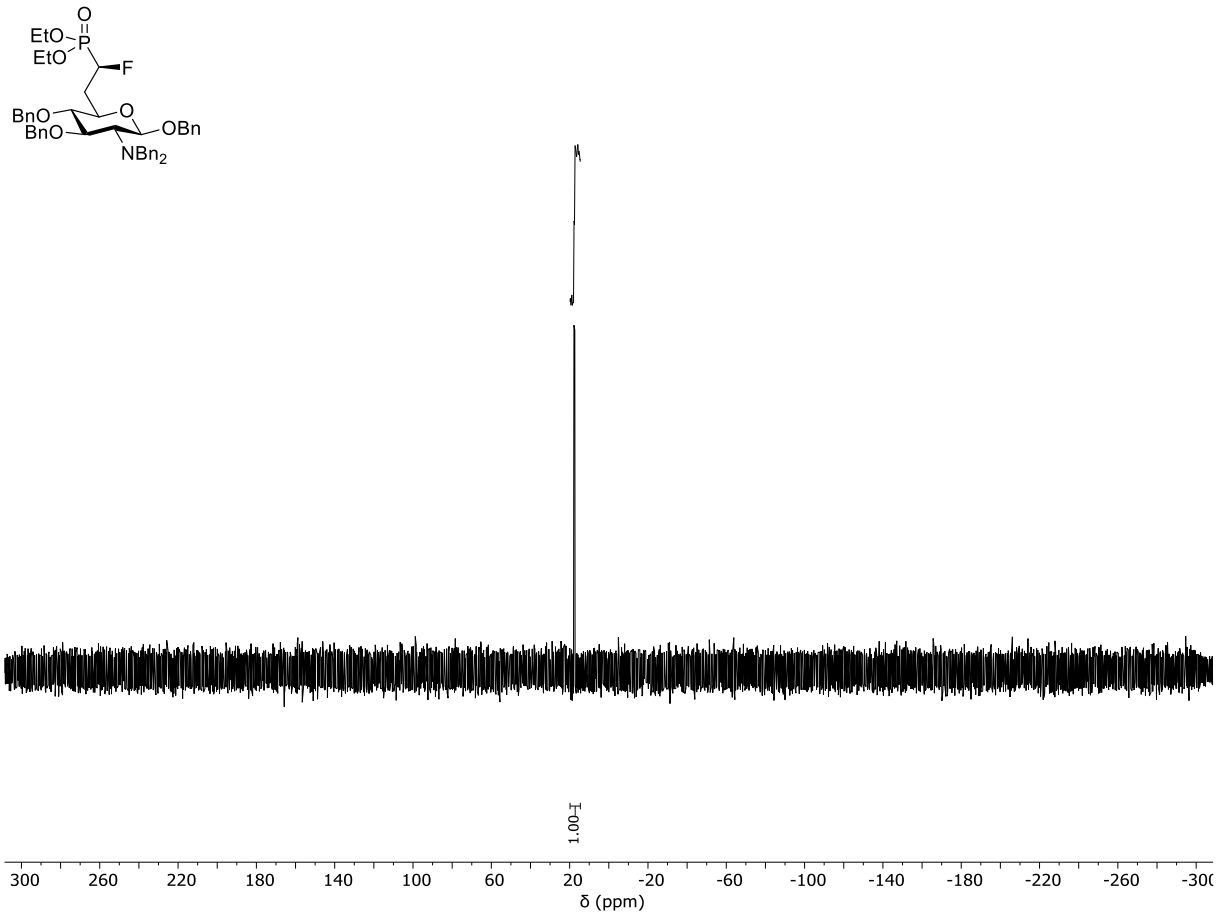
^{19}F NMR spectrum (377MHz, $CDCl_3$) of (*S*)-**138**



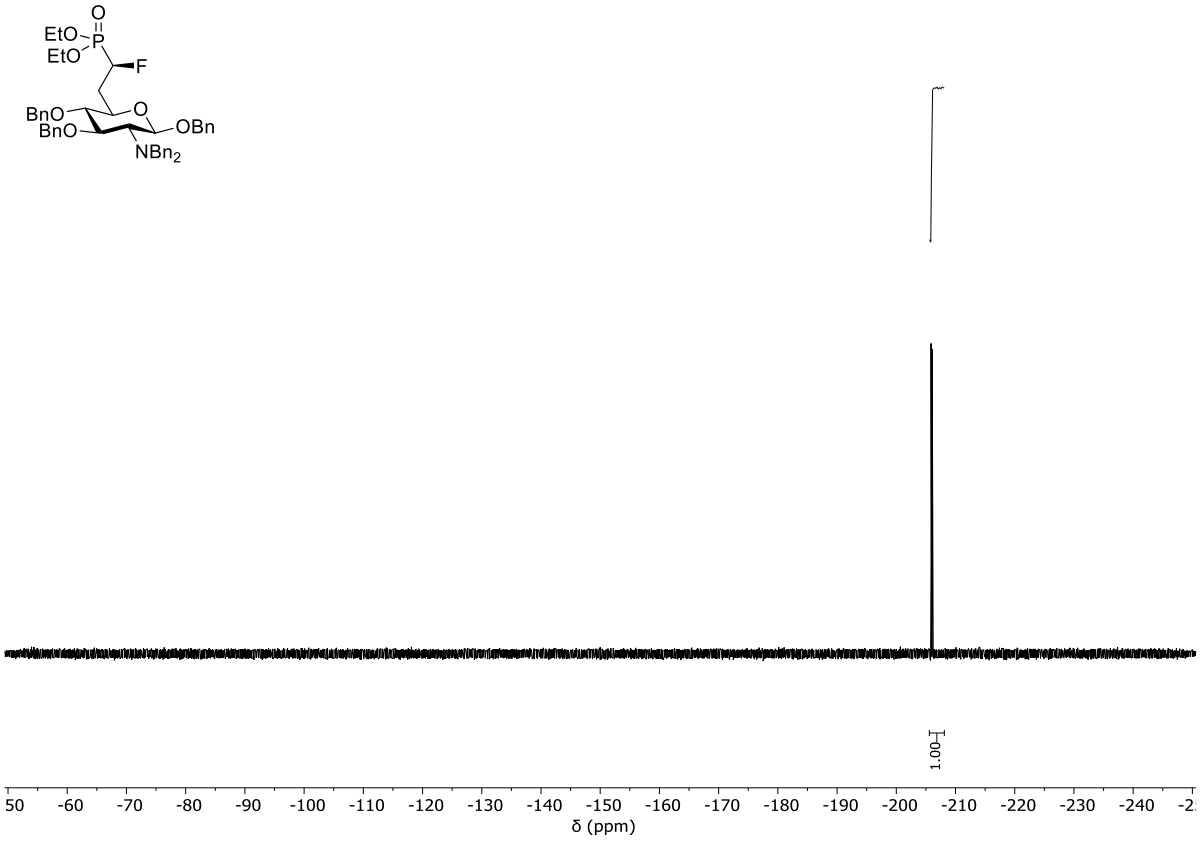
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of (*R*)-**138**



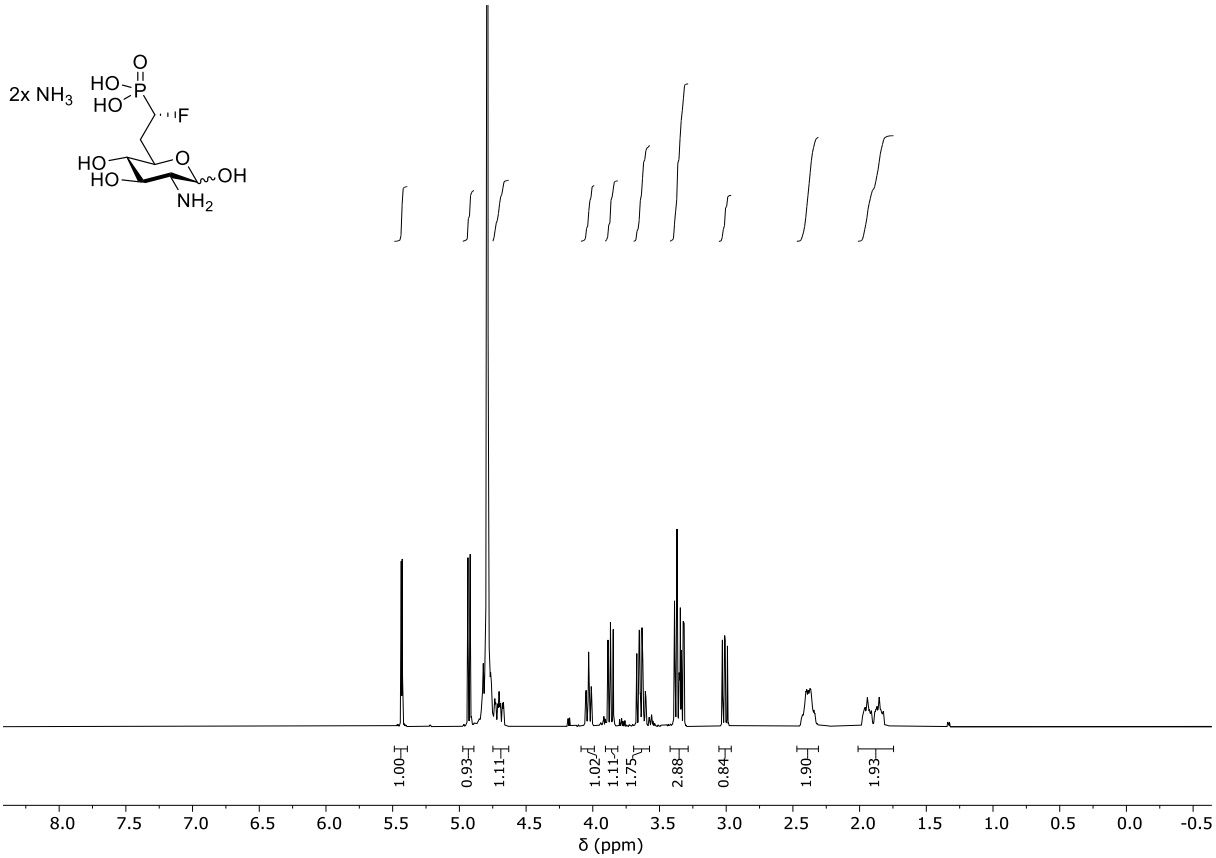
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of (*R*)-**138**



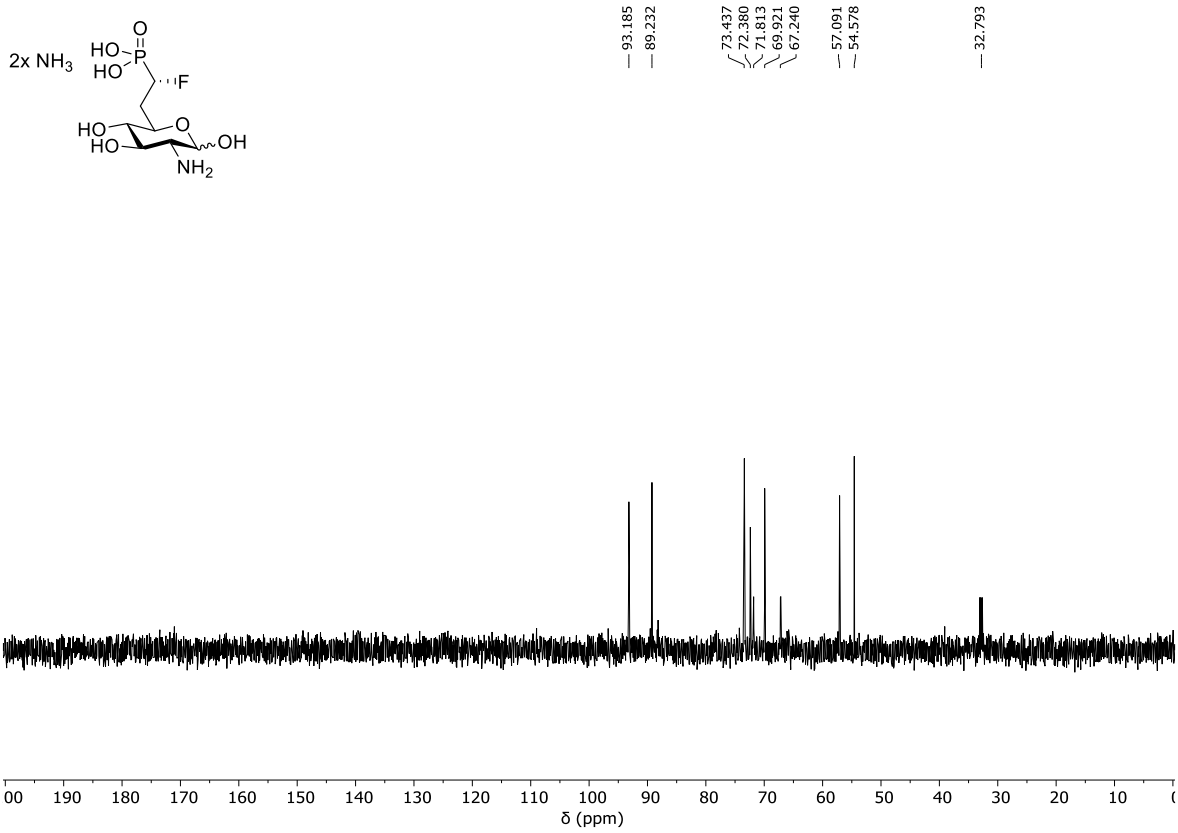
^{31}P NMR spectrum (162 MHz, $CDCl_3$) of (*R*)-**138**



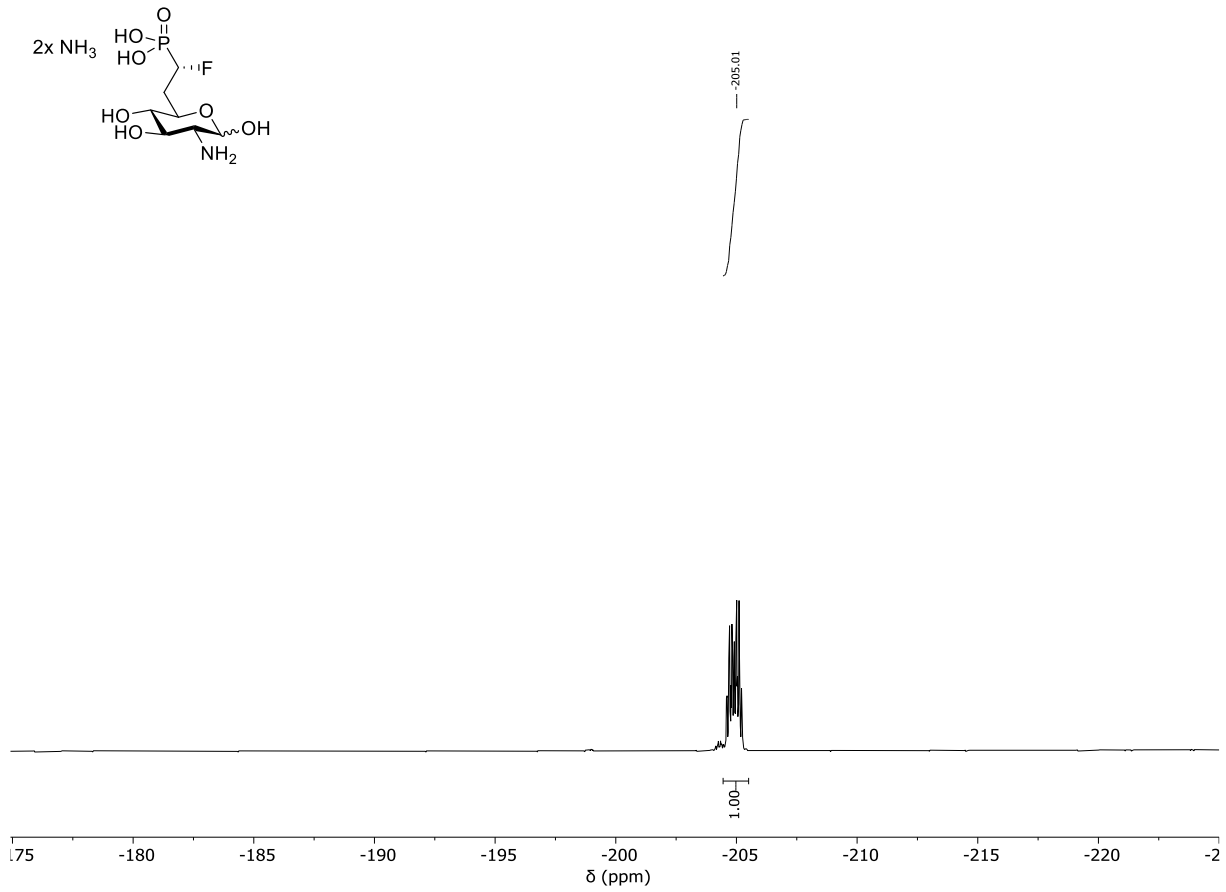
^{19}F NMR spectrum (377MHz, $CDCl_3$) of (*R*)-**138**



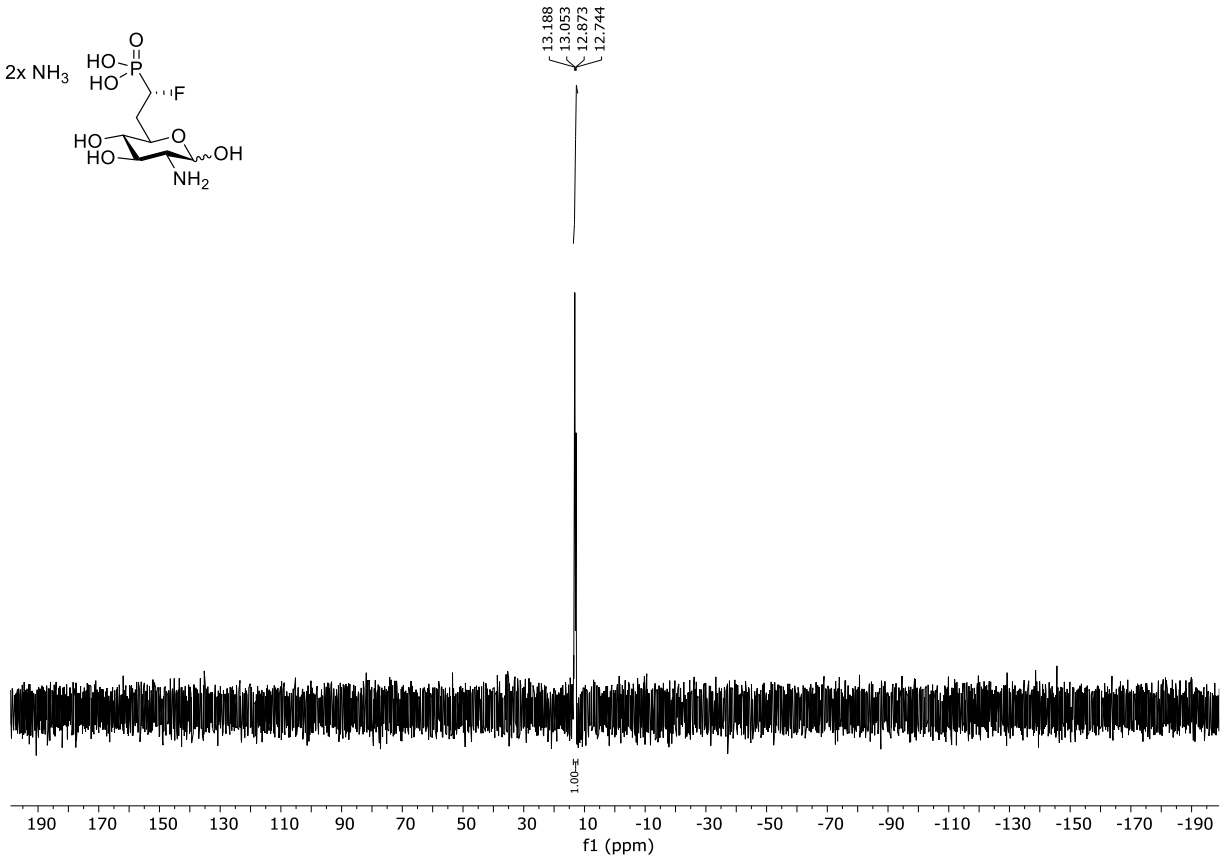
^{1}H NMR spectrum (500 MHz, D_2O) of (*S*)-**139**



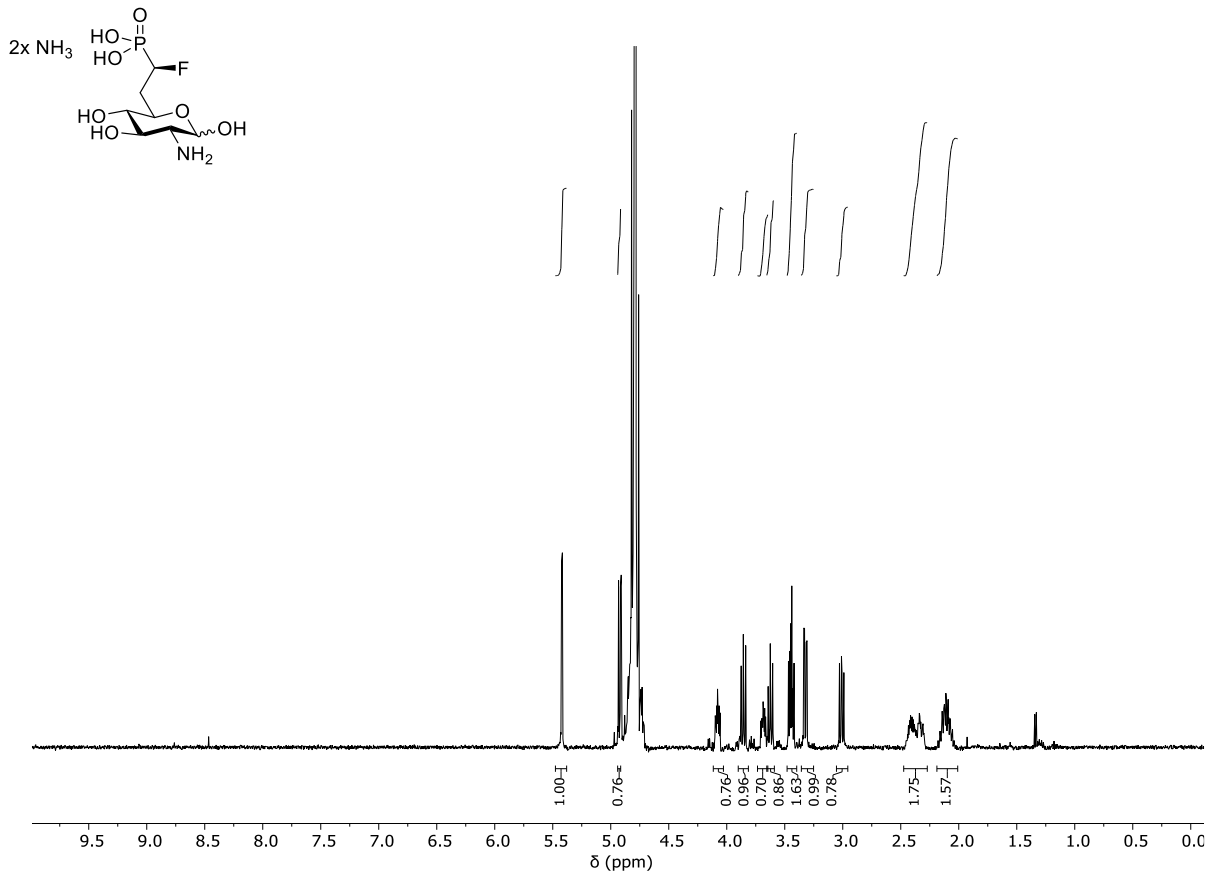
^{13}C NMR spectrum (121 MHz, D_2O) of (*S*)-**139**



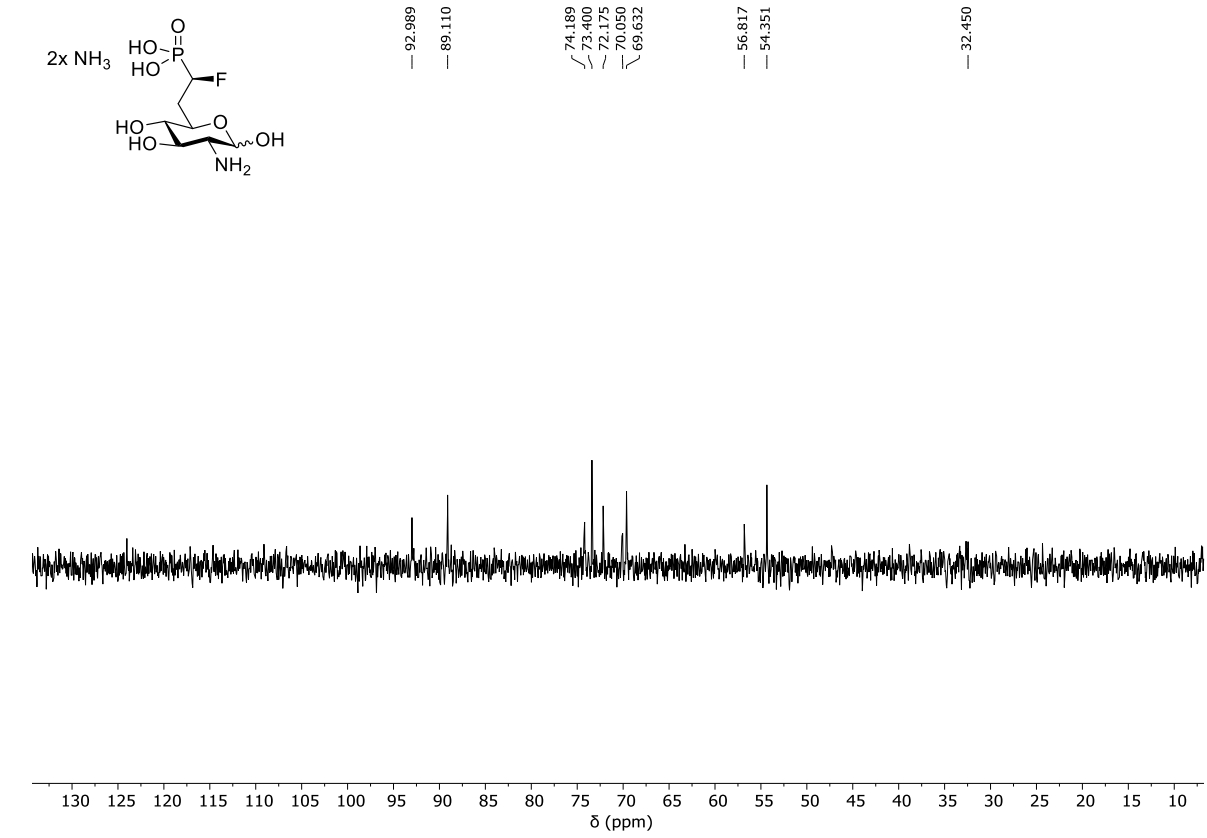
^{19}F NMR spectrum (471 MHz, D_2O) of (*S*)-**139**



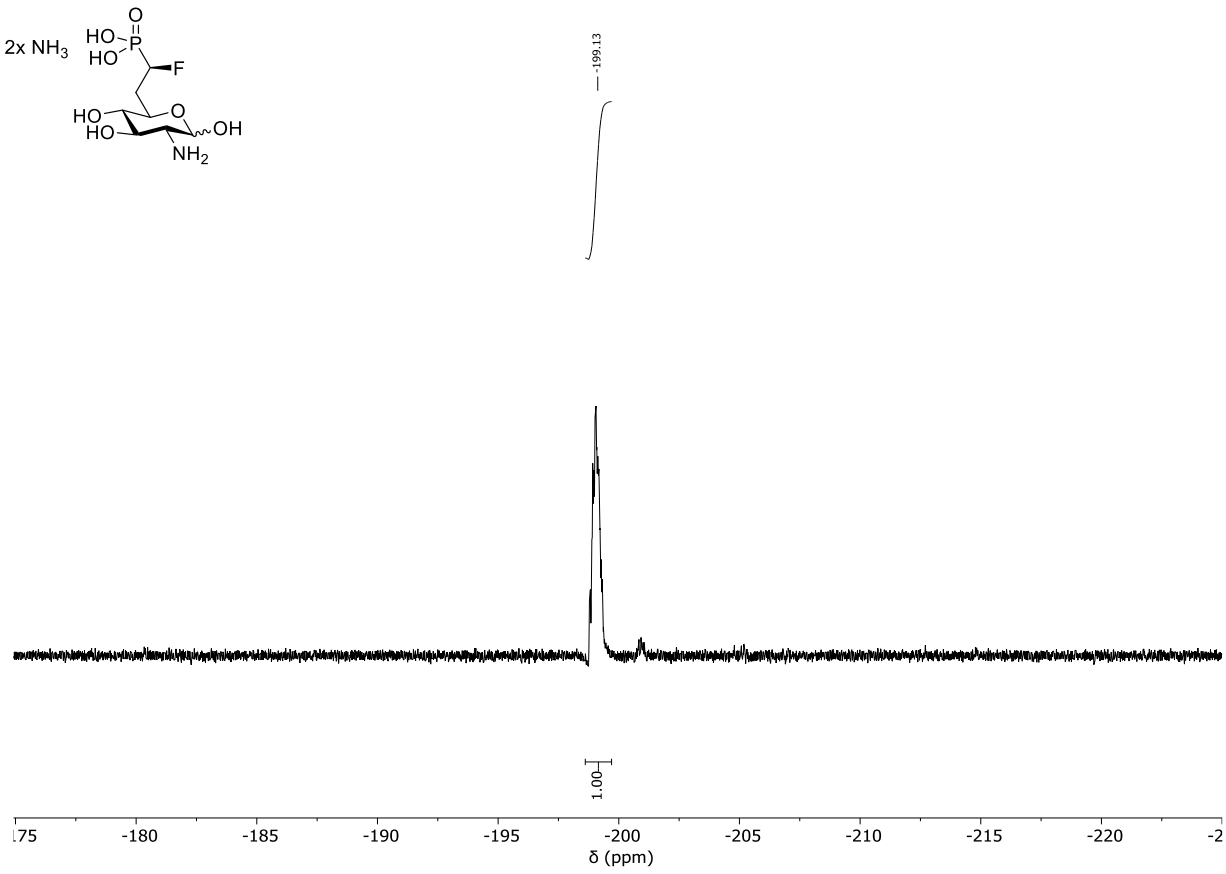
^{31}P NMR spectrum (202 MHz, D_2O) of (*S*)-**139**



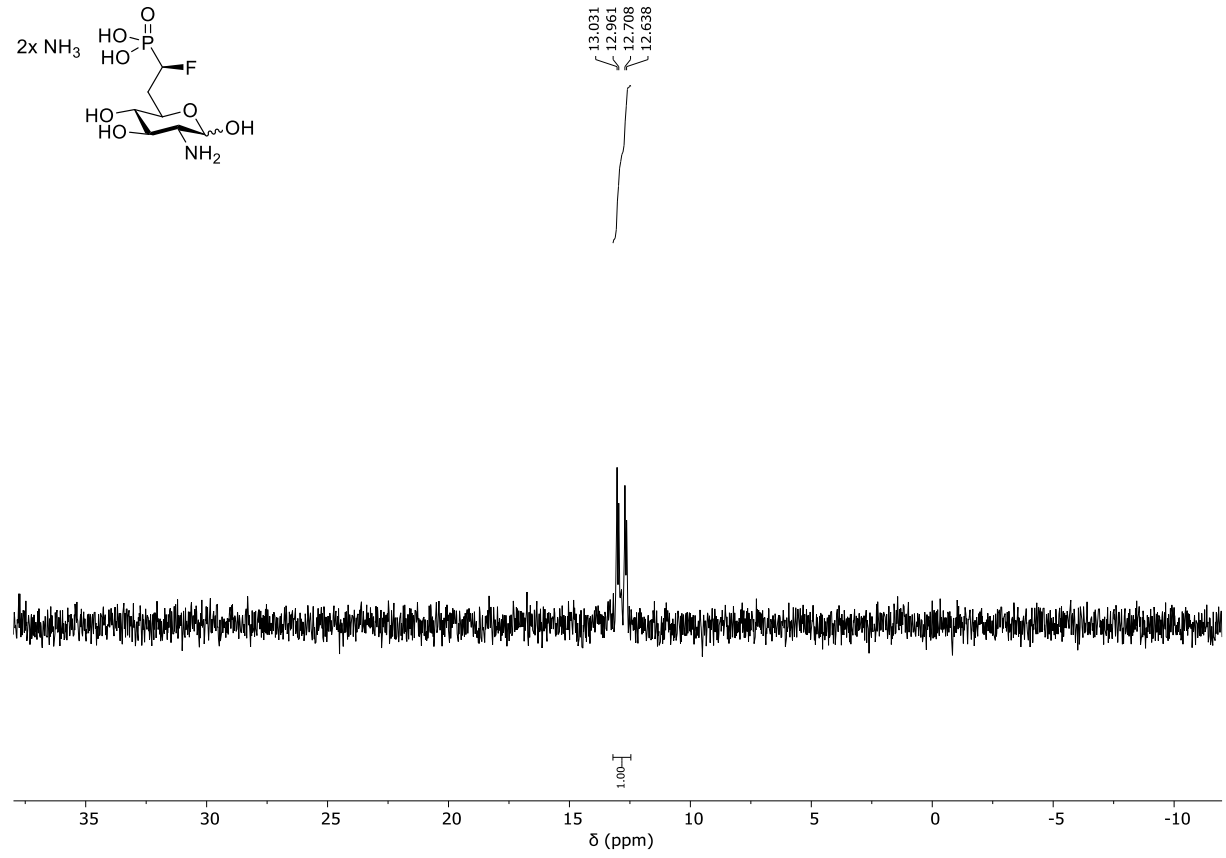
^{1}H NMR spectrum (500 MHz, D_2O) of (*R*)-**139**



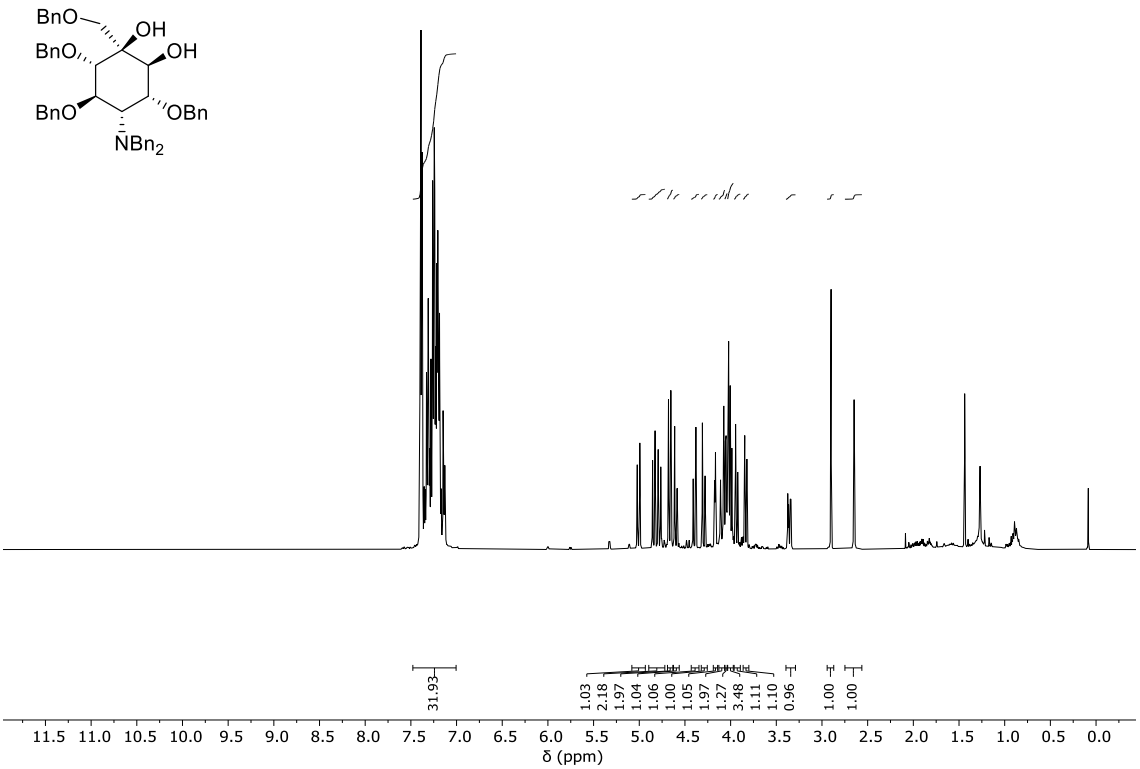
^{13}C NMR spectrum (151 MHz, D_2O) of (*R*)-**139**



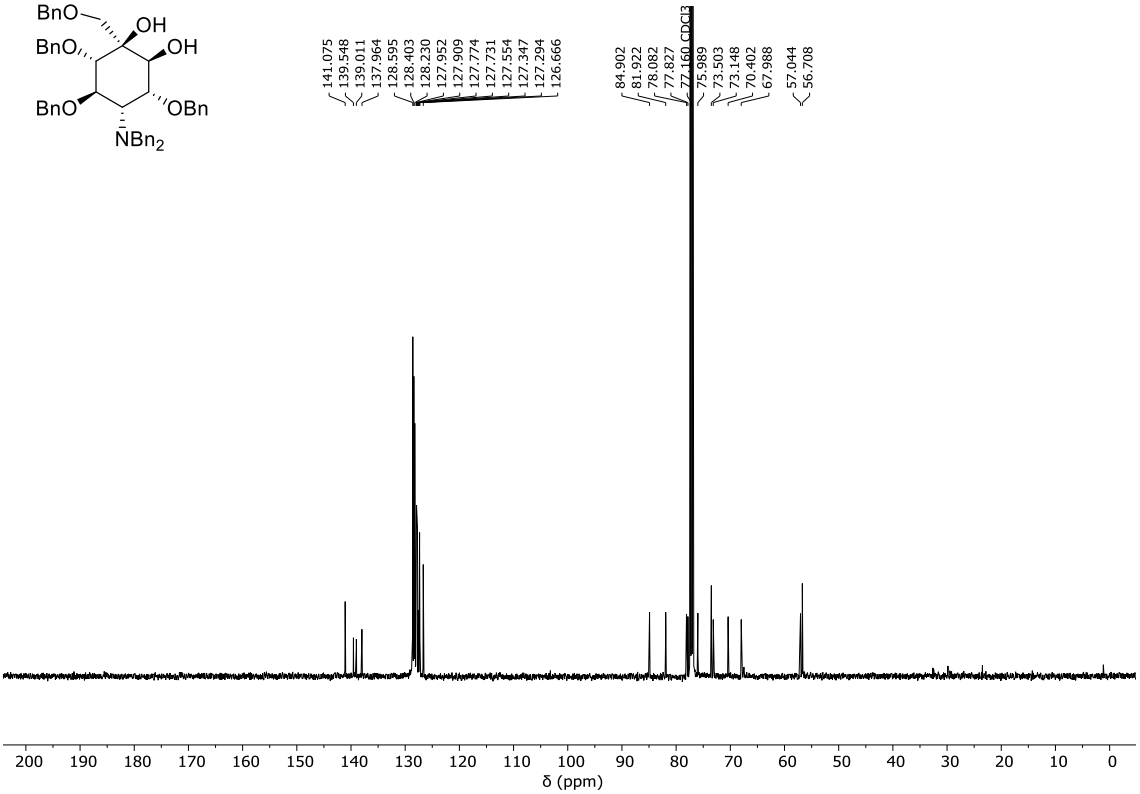
^{19}F NMR spectrum (471 MHz, D_2O) of (*R*)-**139**



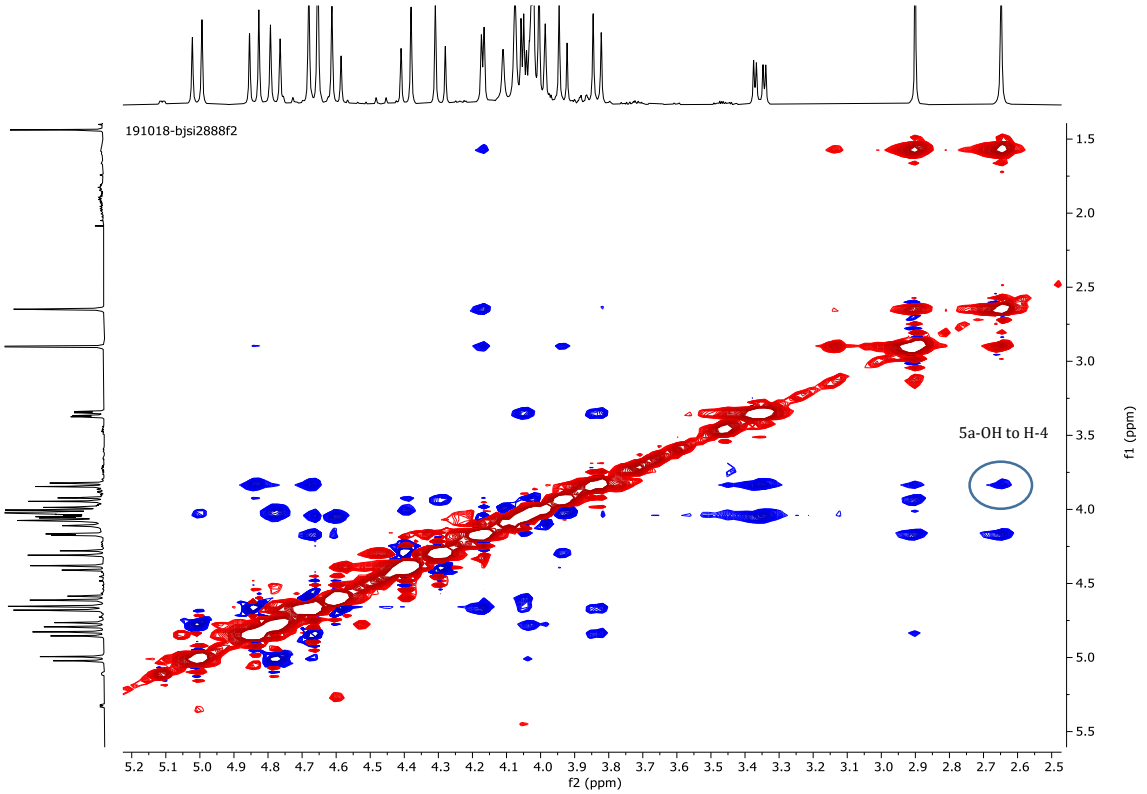
^{31}P NMR spectrum (202 MHz, D_2O) of (*R*)-**139**



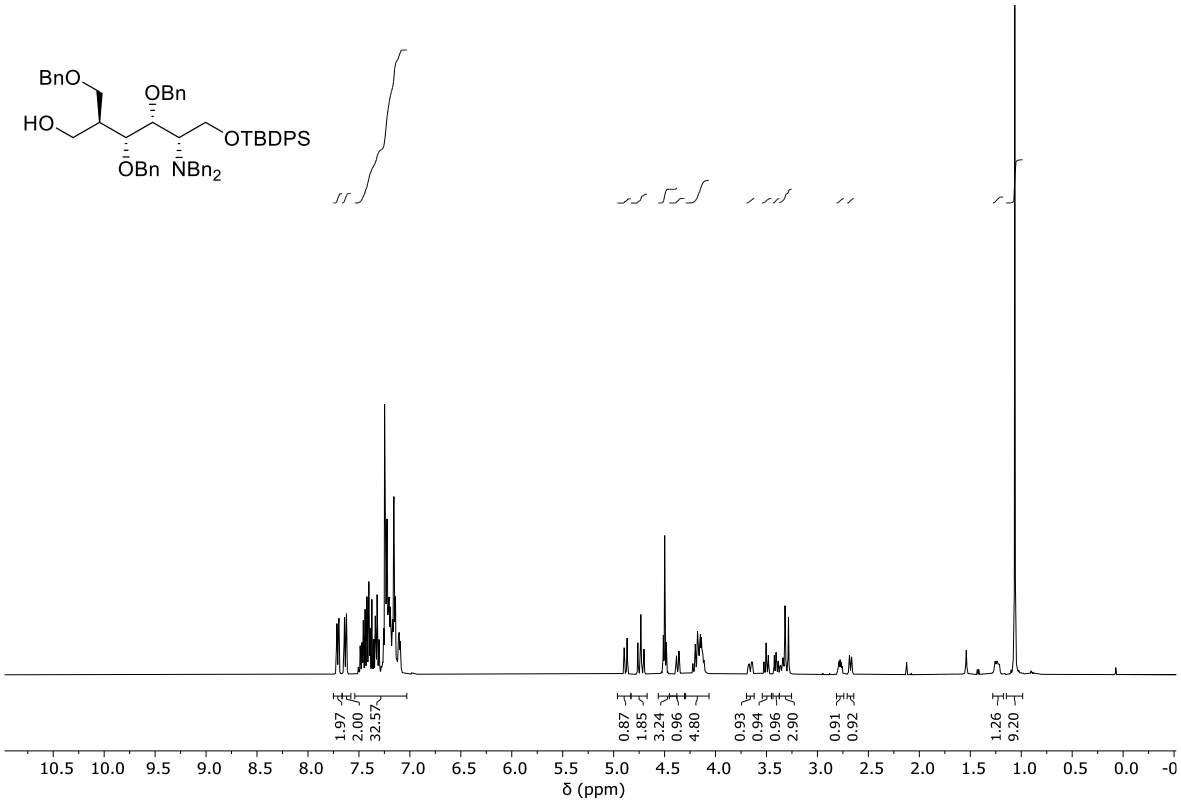
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **142**.



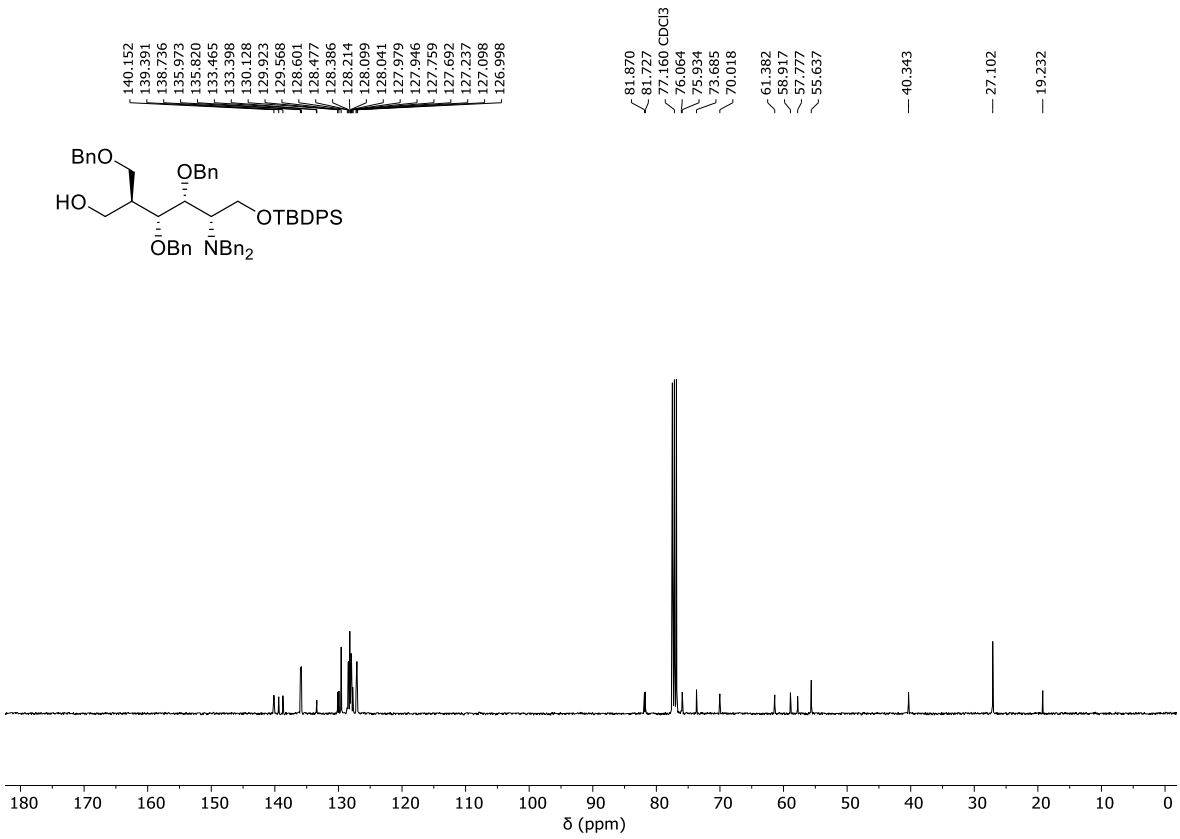
^{13}C-NMR (121 MHz, $CDCl_3$) spectrum of **142**.



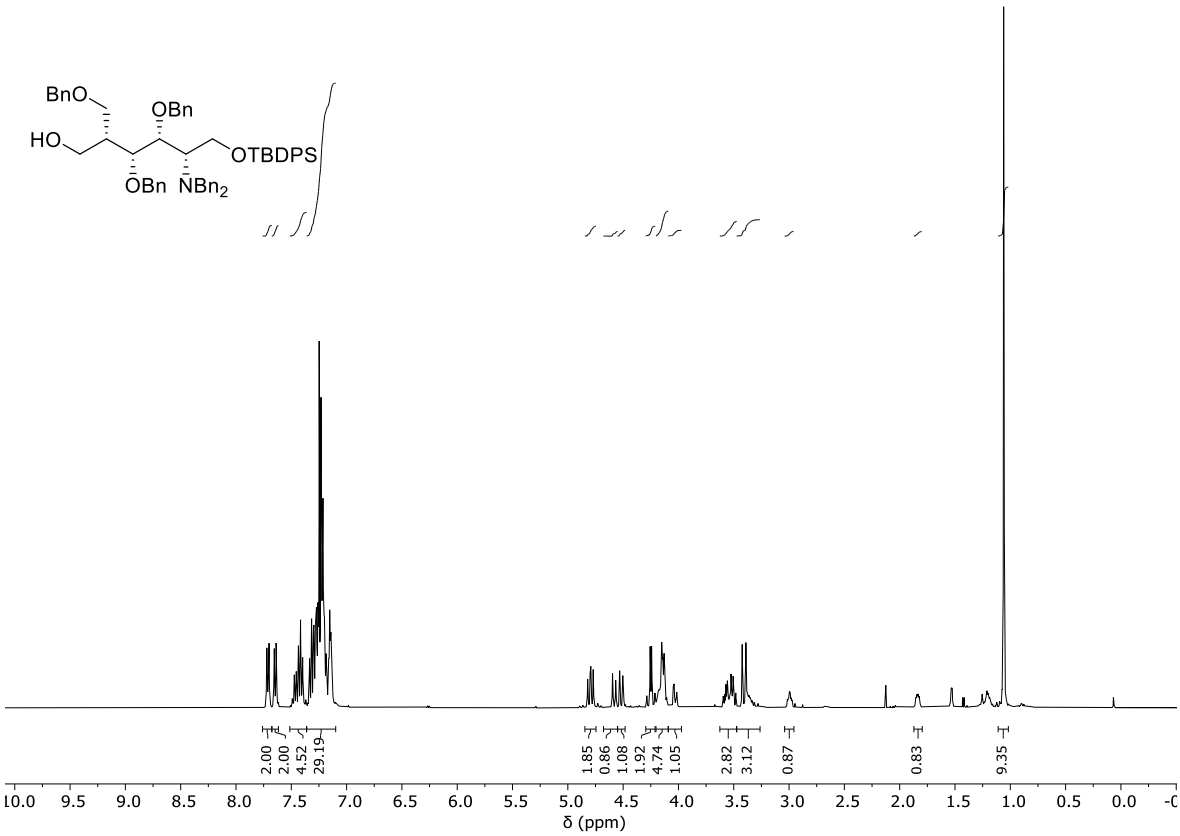
NOESY spectrum of **142**



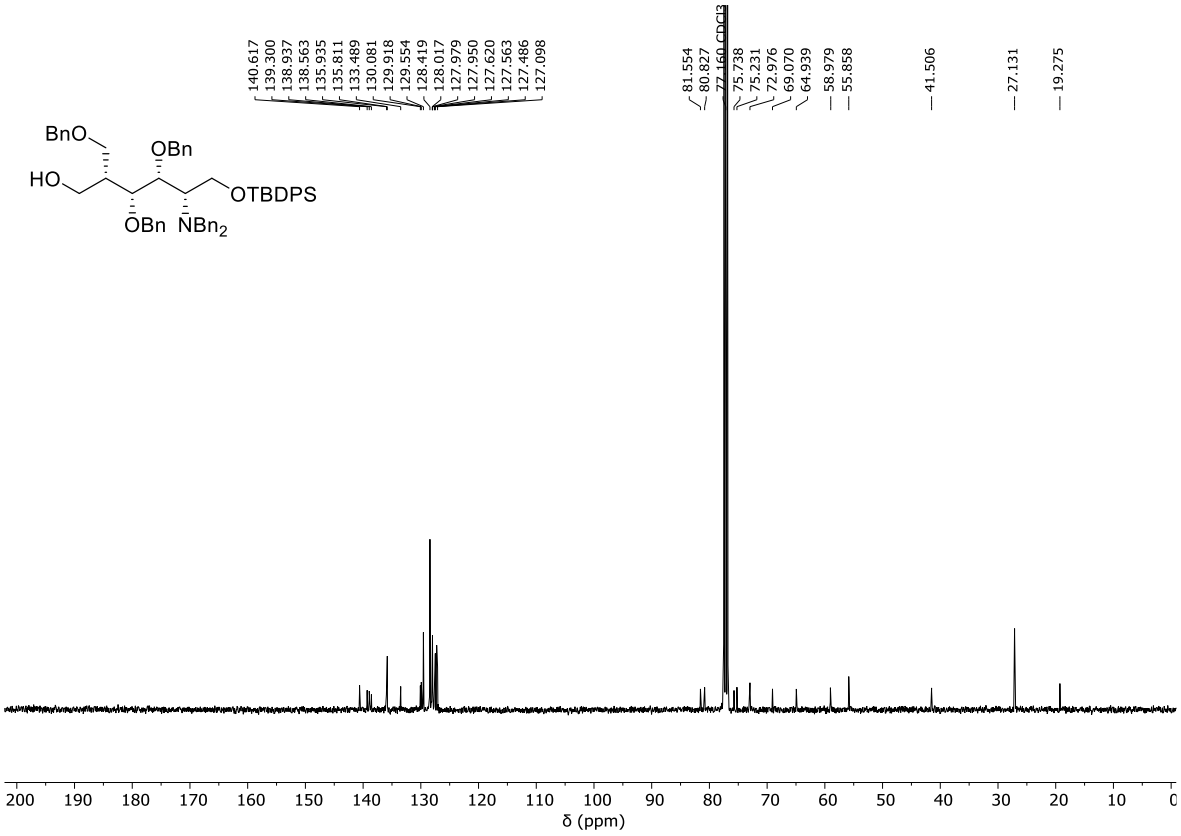
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **144**



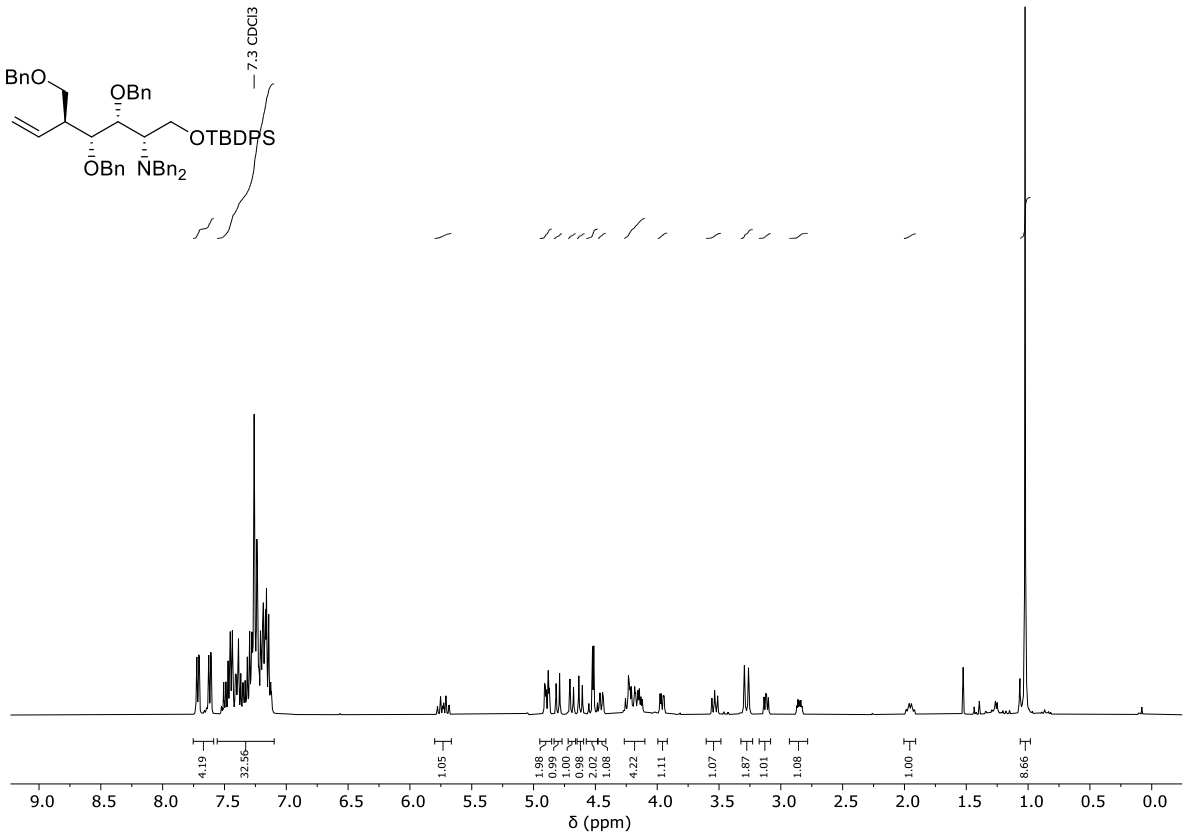
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **144**



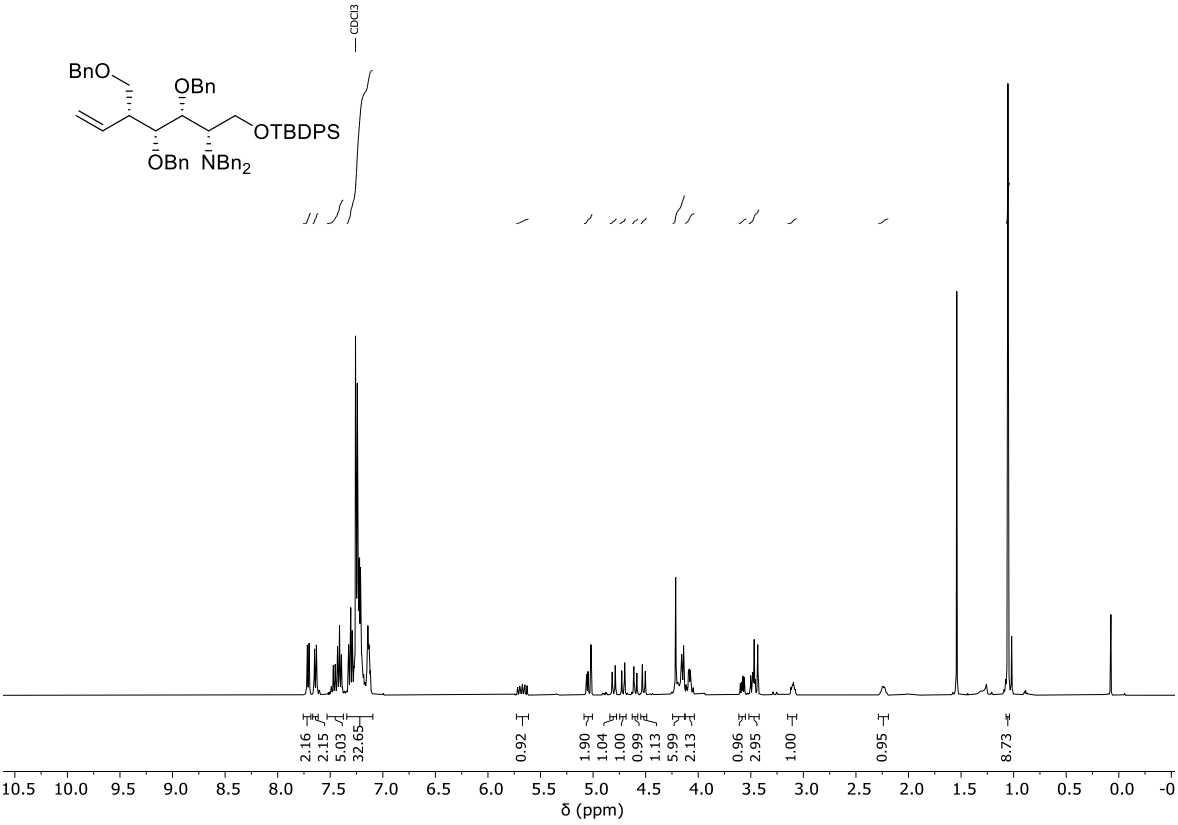
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **144**



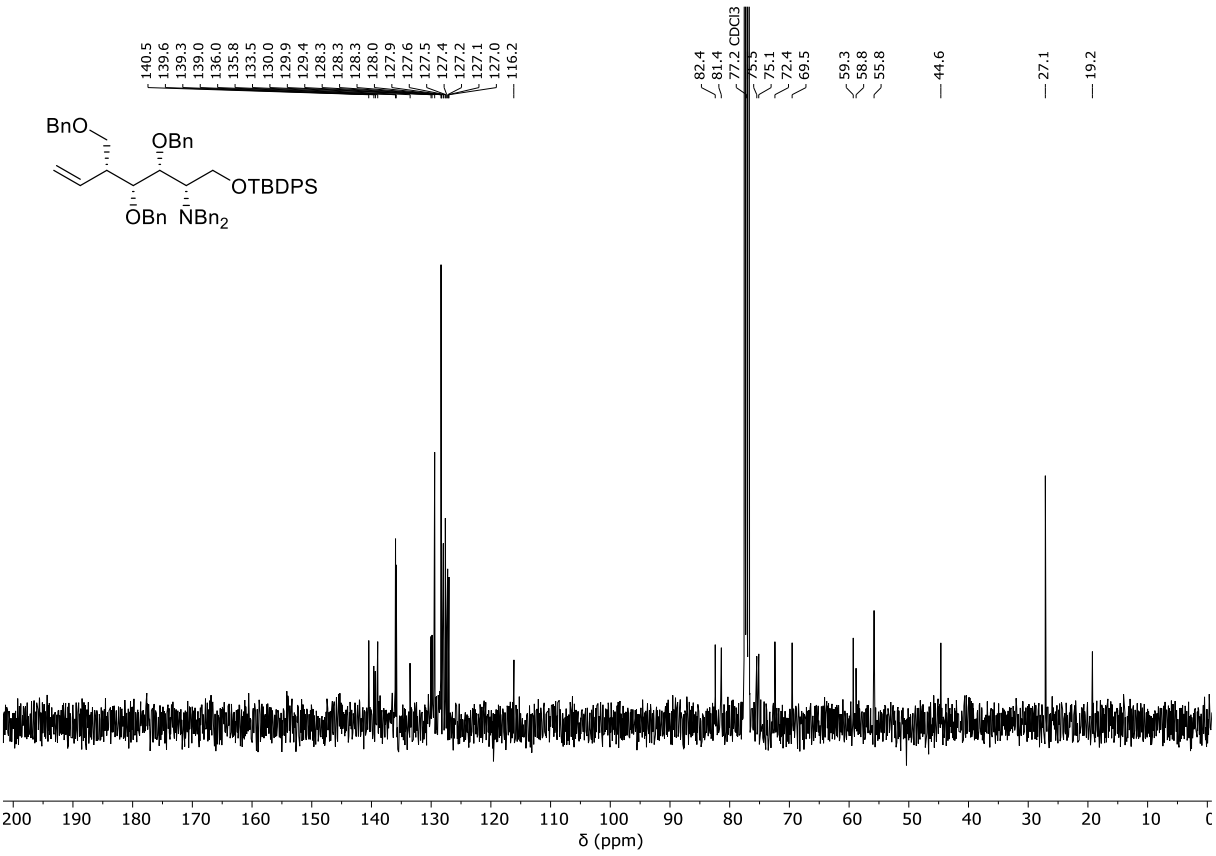
^{13}C NMR spectrum (101MHz, $CDCl_3$) of **144**



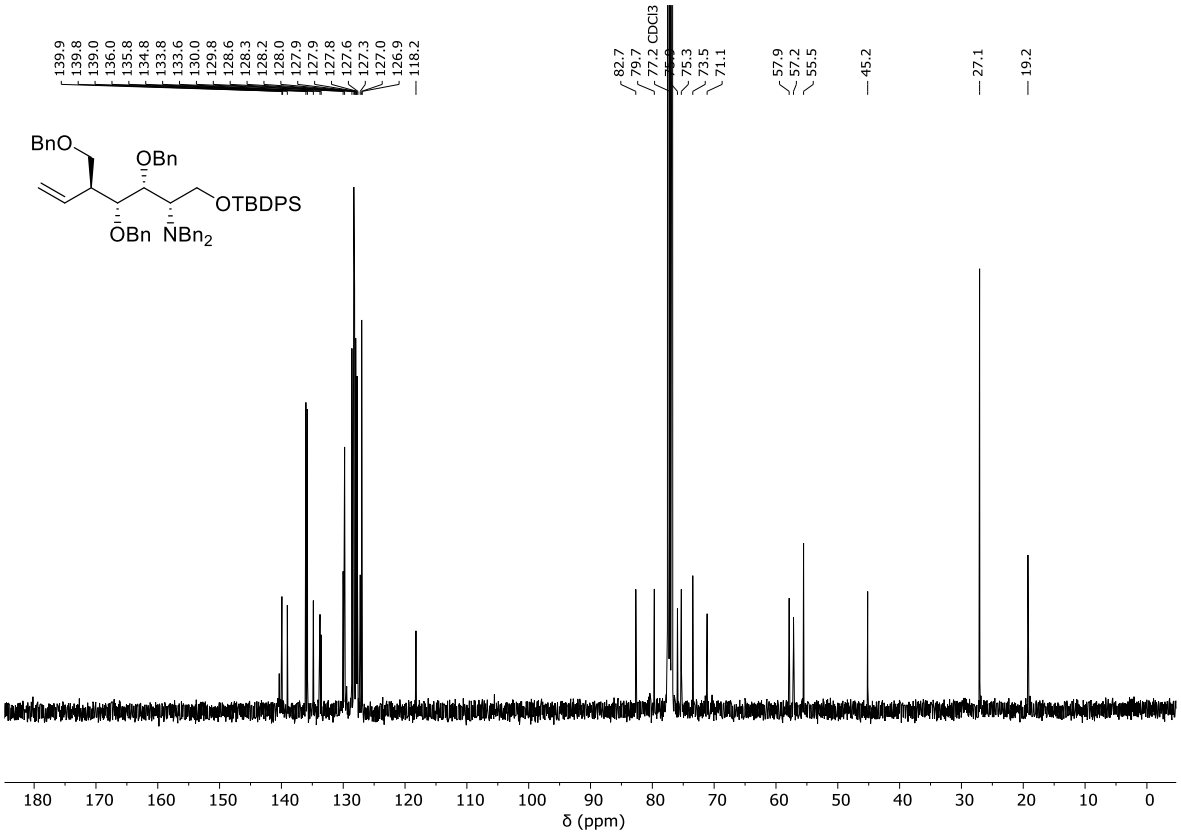
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **146**



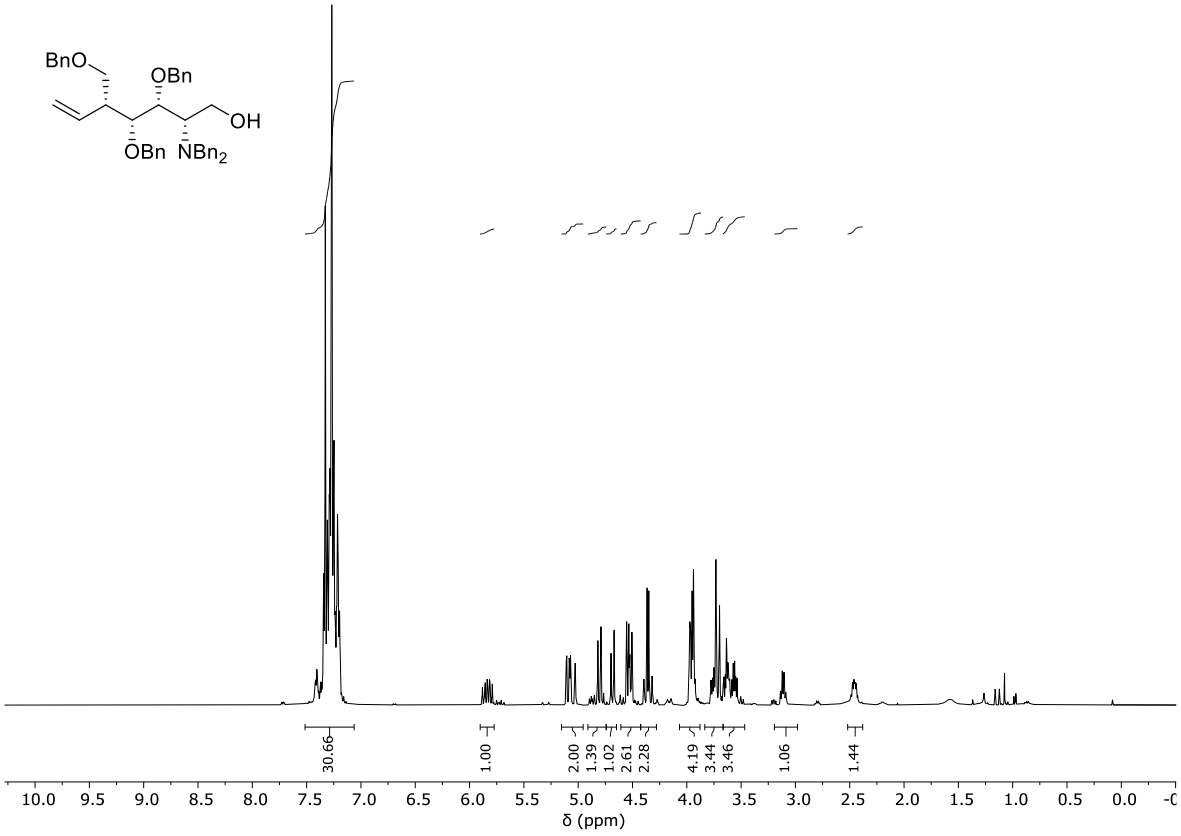
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **145**



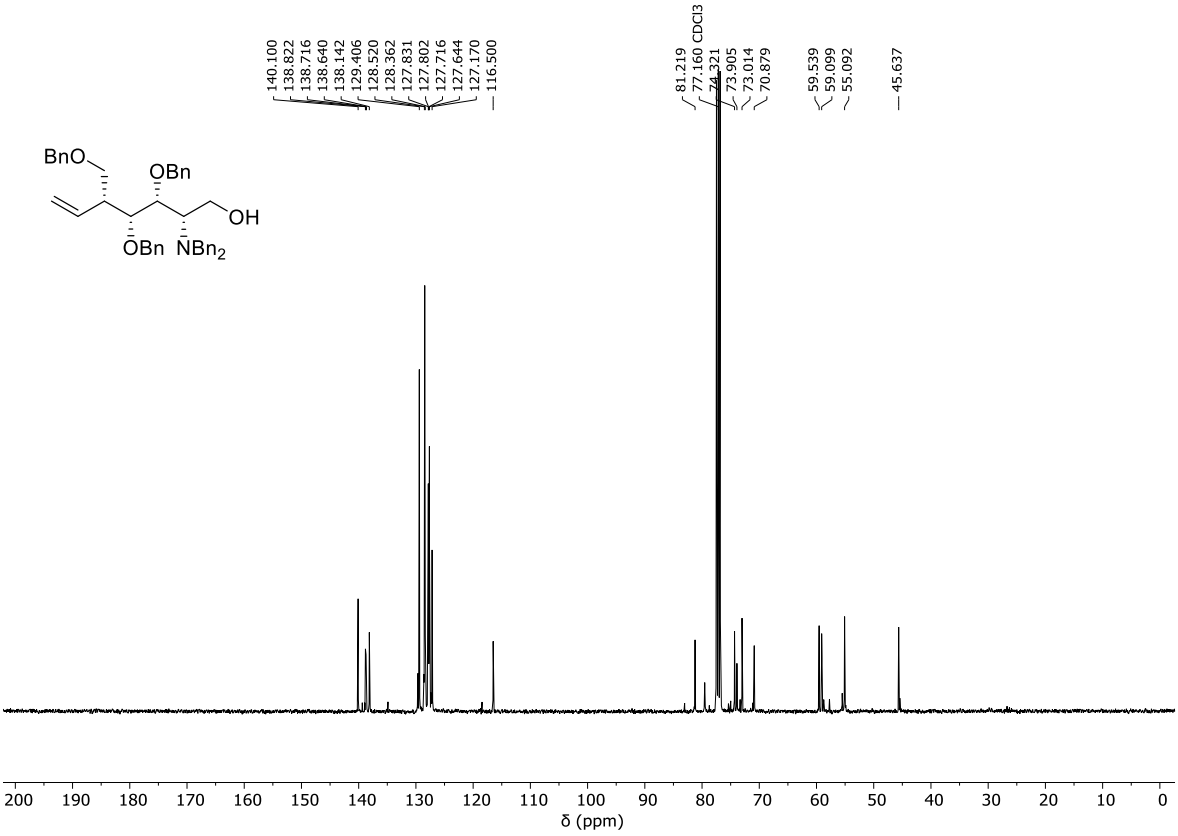
^{13}C NMR spectrum (101MHz, $CDCl_3$) of **145**



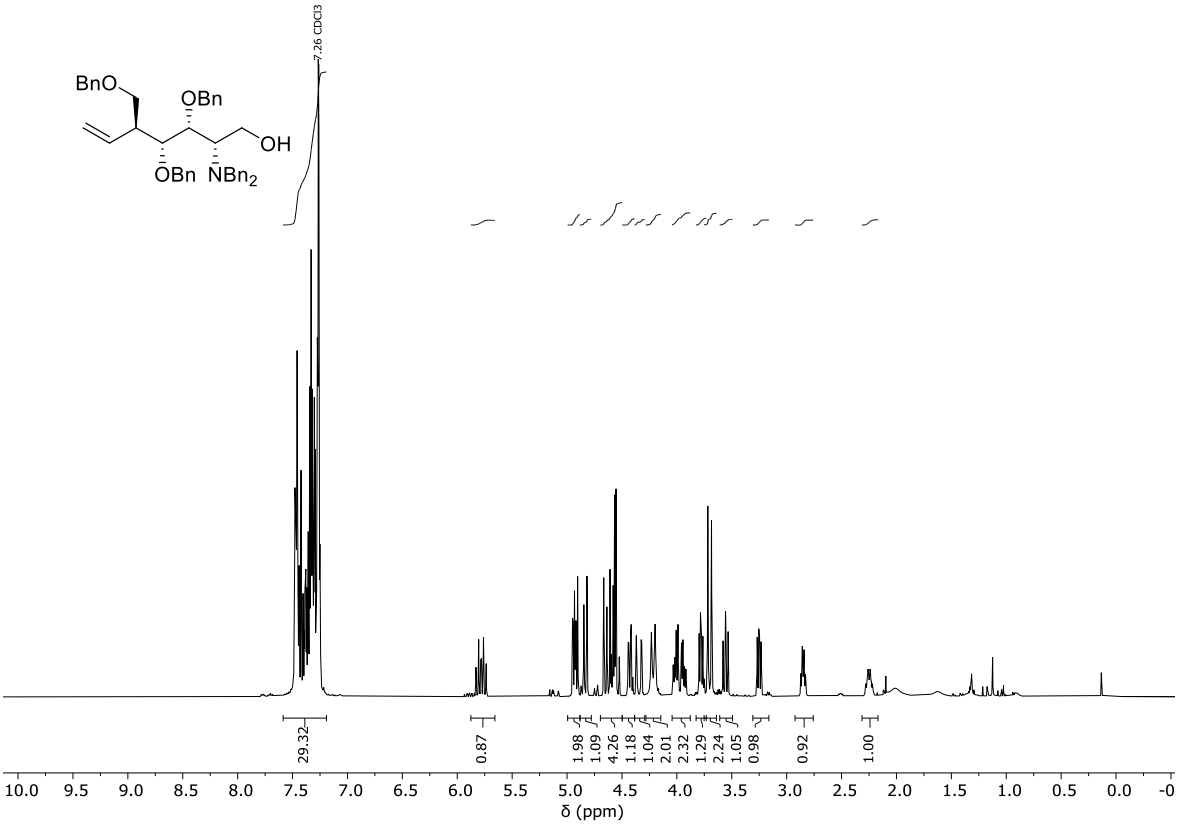
^{13}C NMR spectrum (101MHz, $CDCl_3$) of **146**



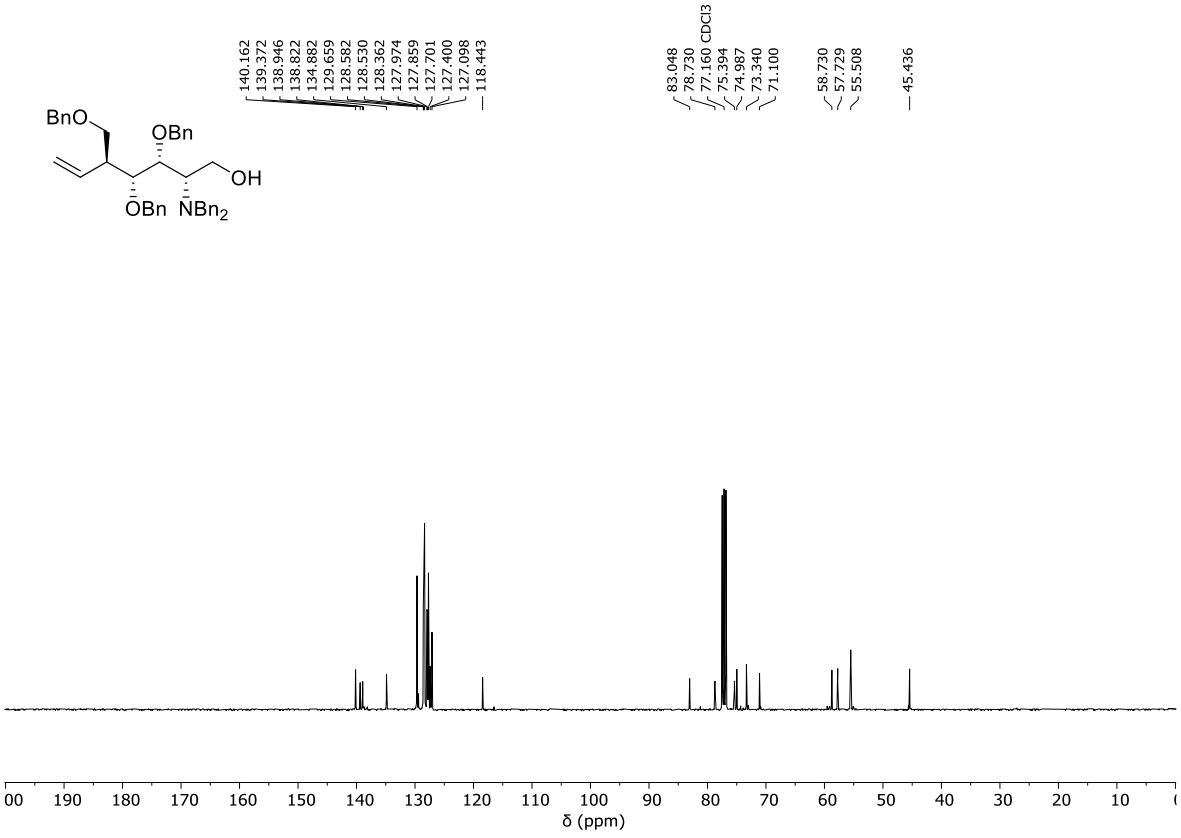
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **147**



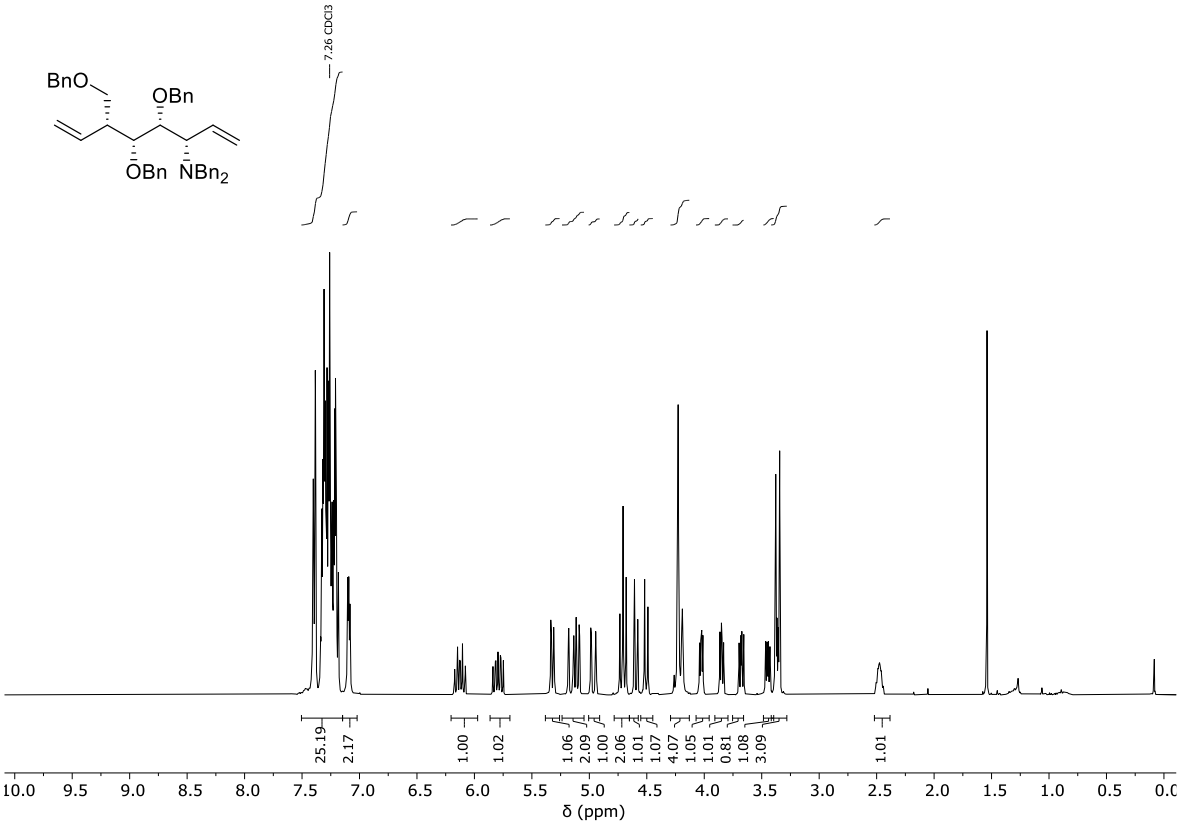
^{13}C NMR spectrum (101MHz, $CDCl_3$) of **147**



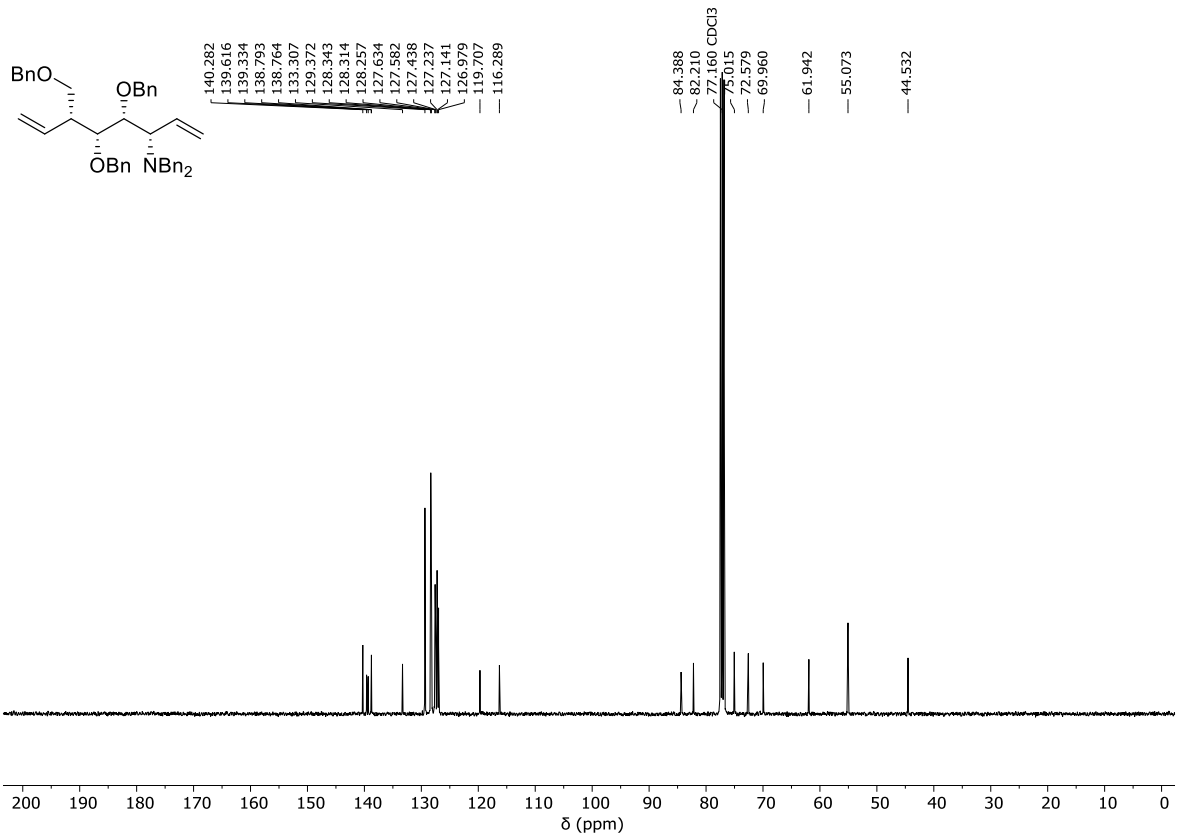
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **148**



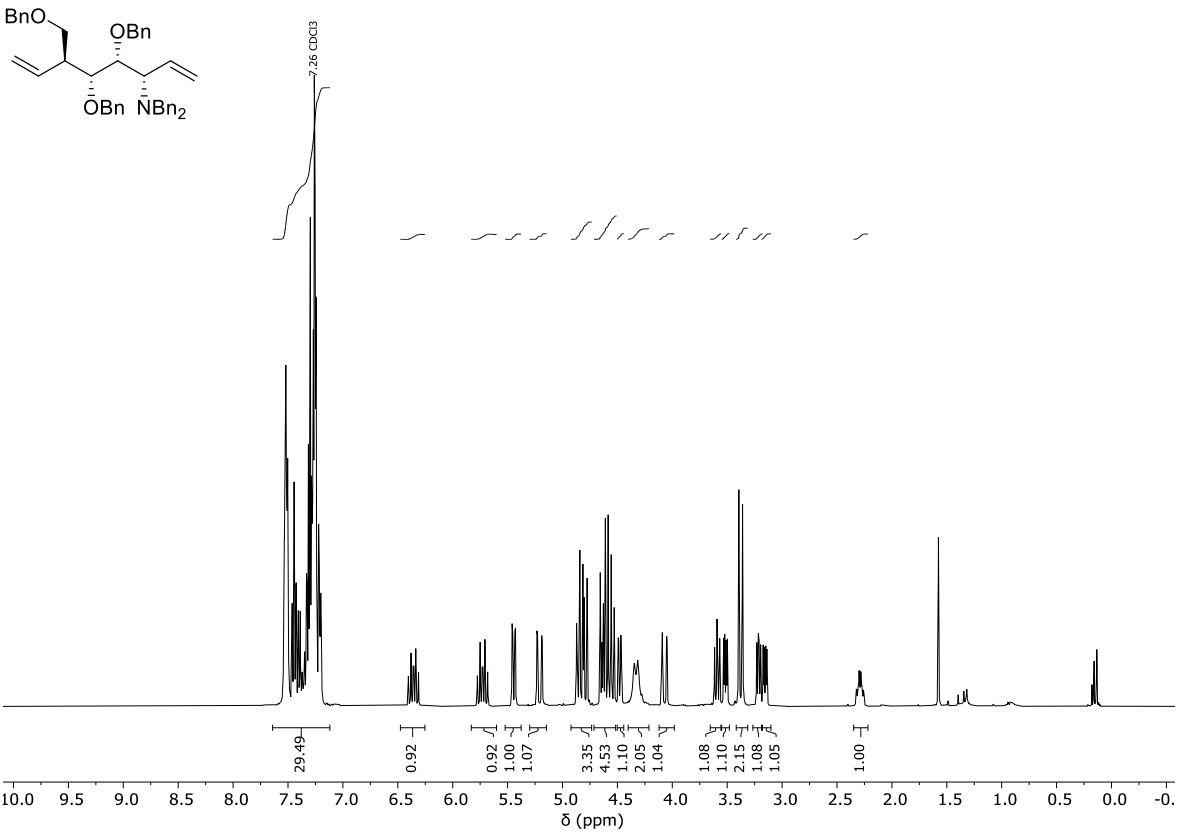
^{13}C NMR spectrum (101MHz, $CDCl_3$) of **148**



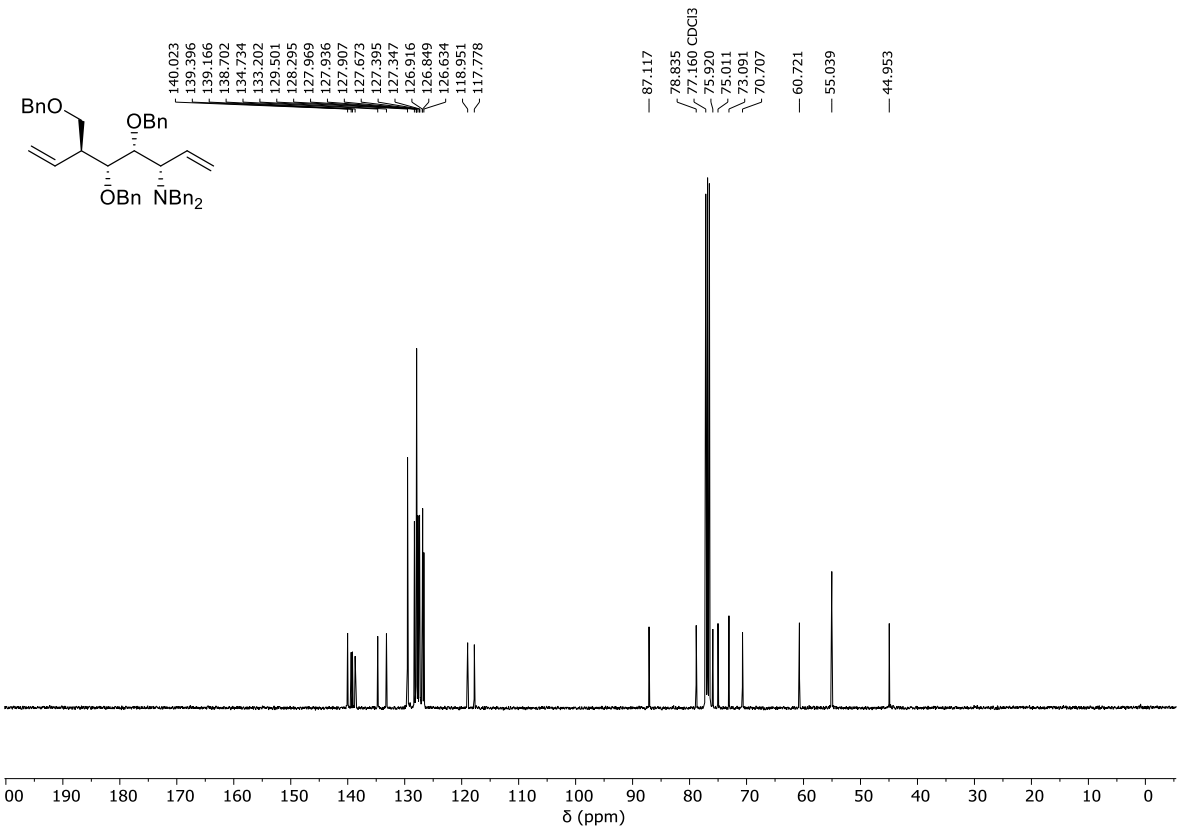
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **149**



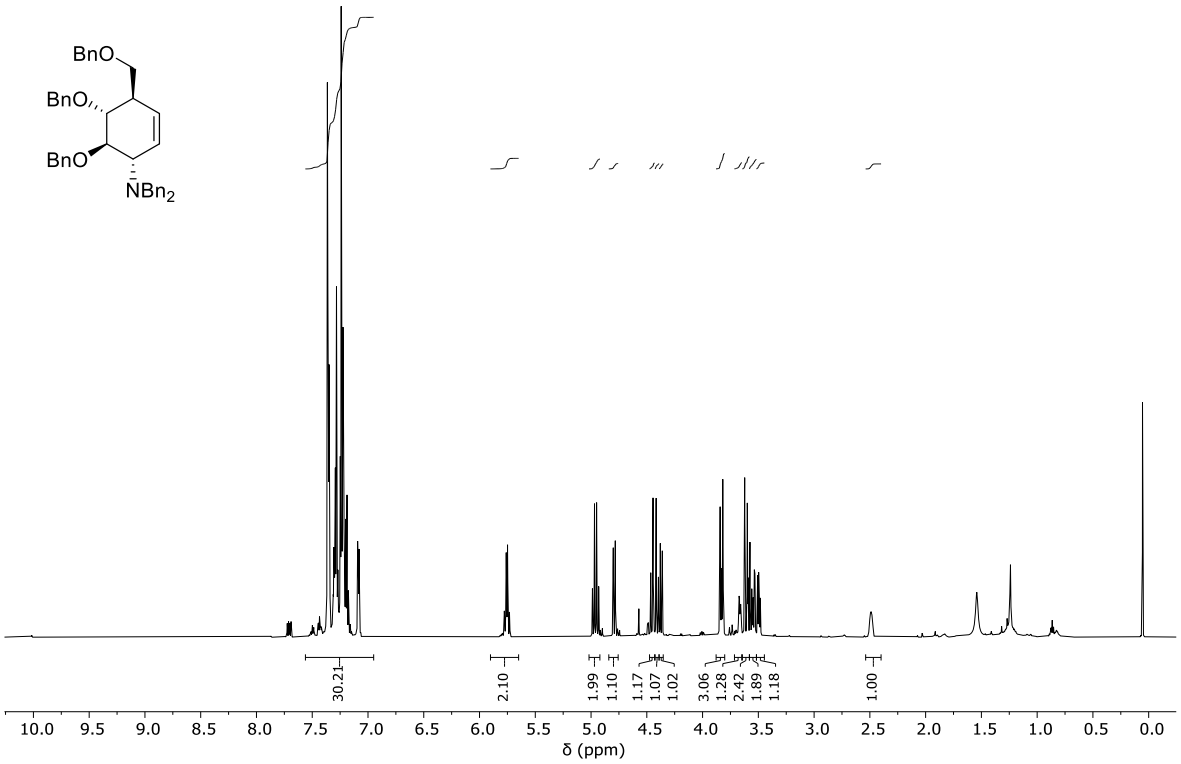
^{13}C NMR spectrum (101MHz, $CDCl_3$) of **149**



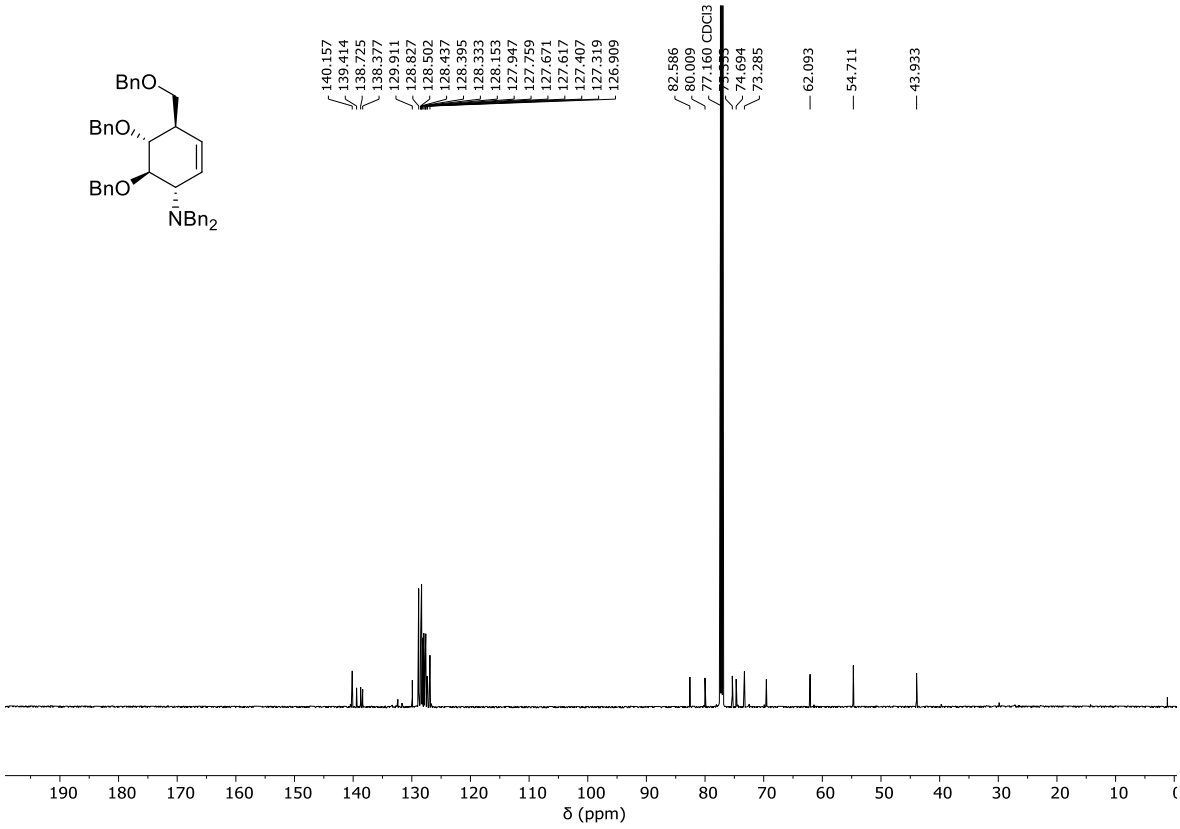
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **150**



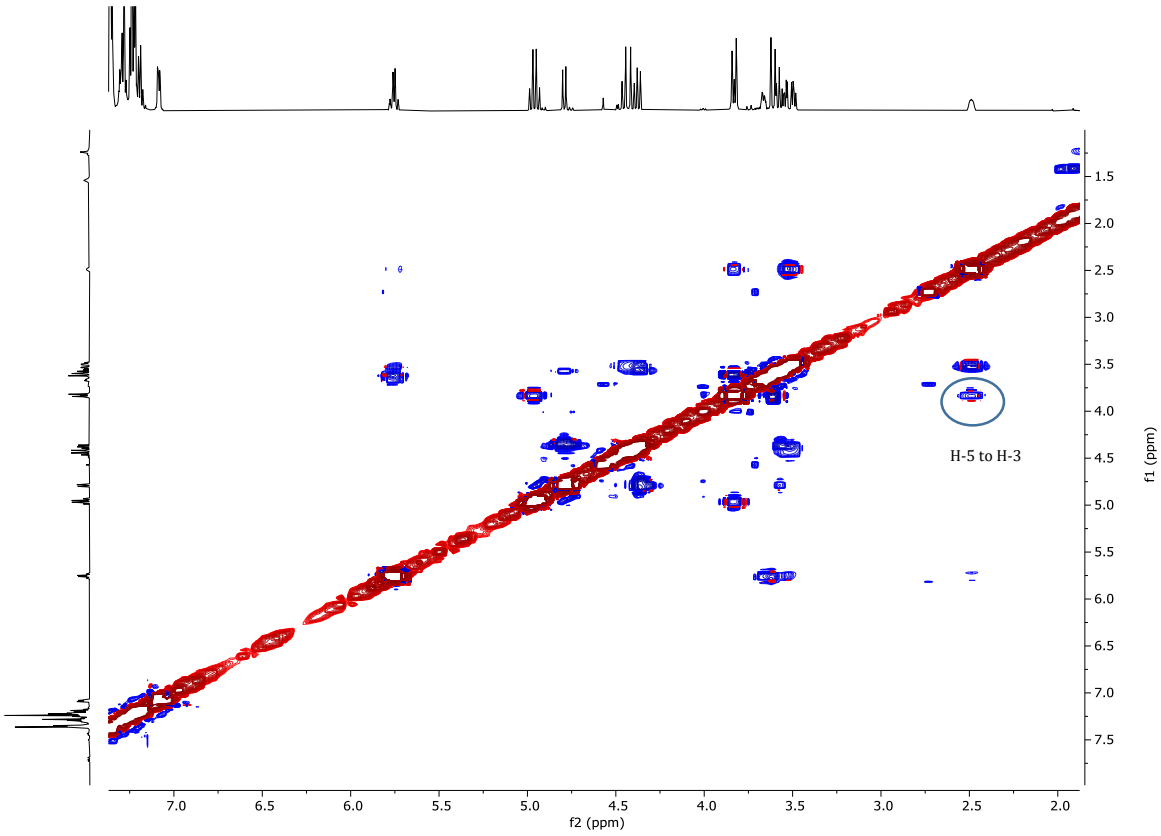
^{13}C NMR spectrum (101MHz, $CDCl_3$) of **150**



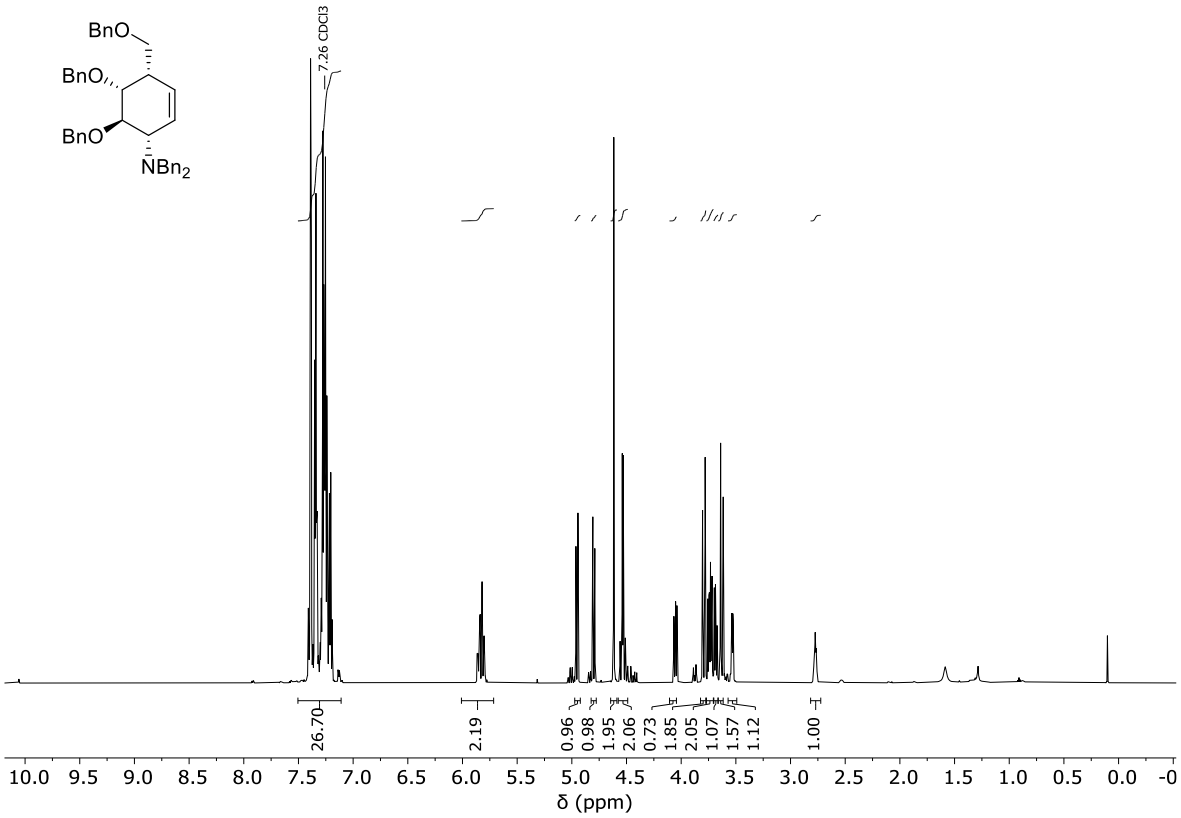
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **151**



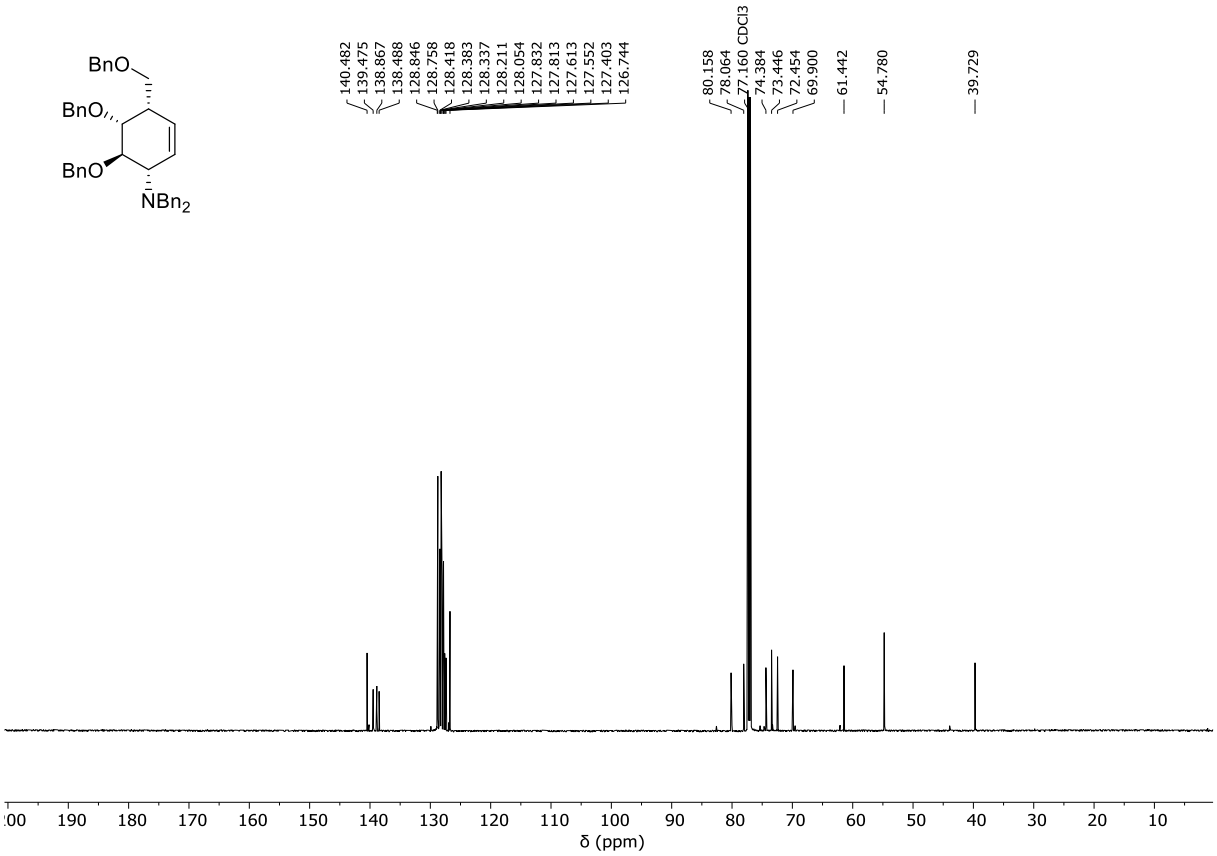
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **151**



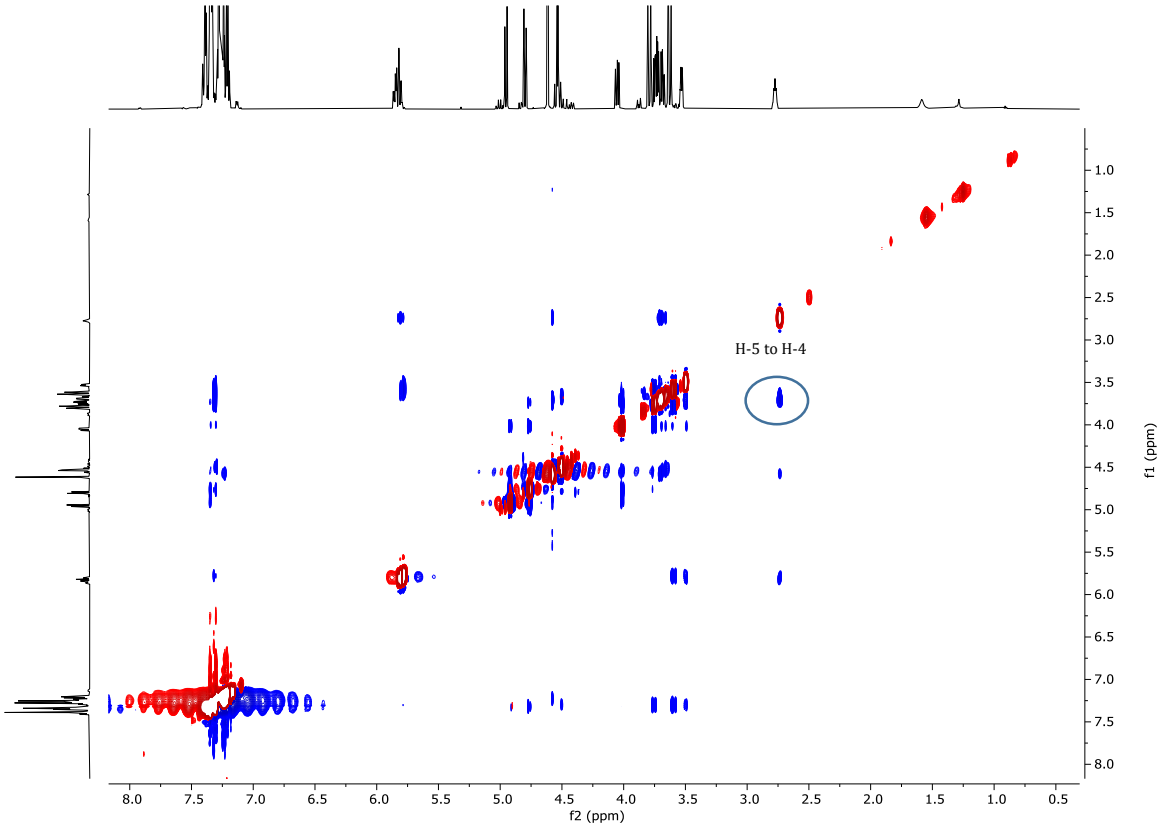
NOESY spectrum of **151**



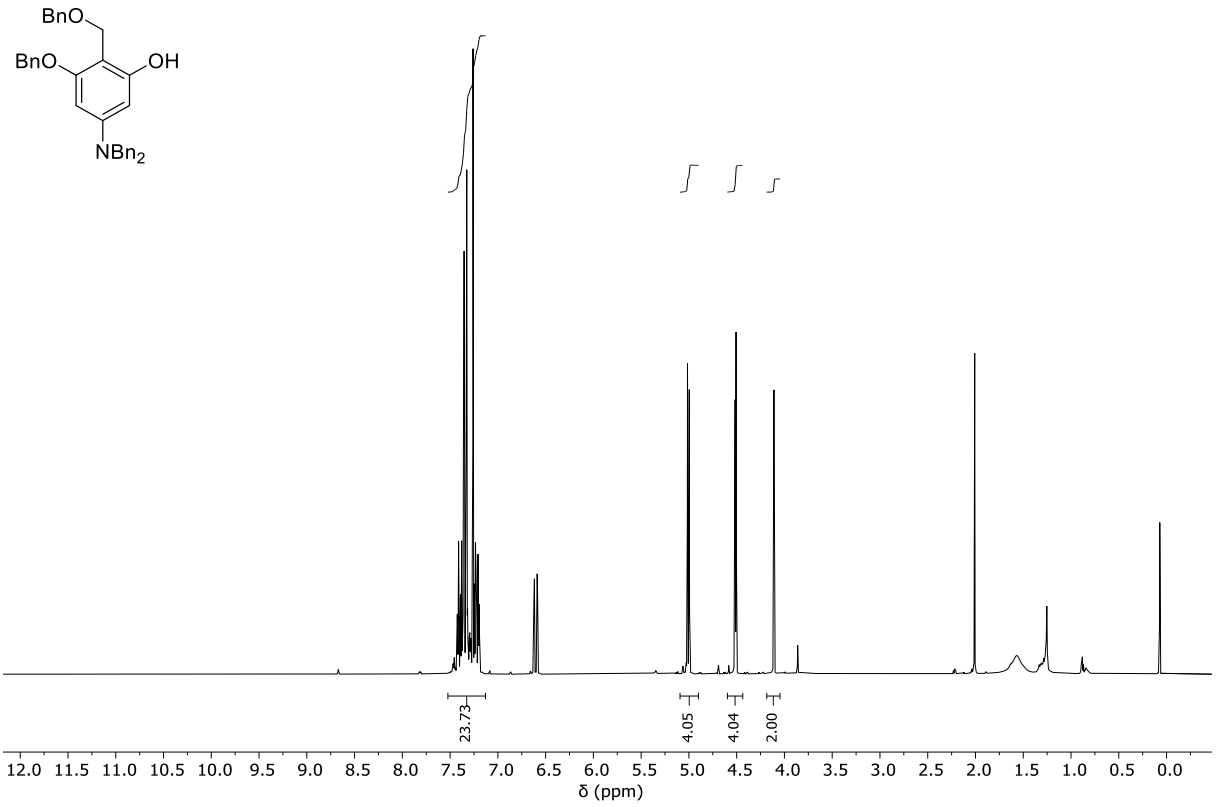
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **152**



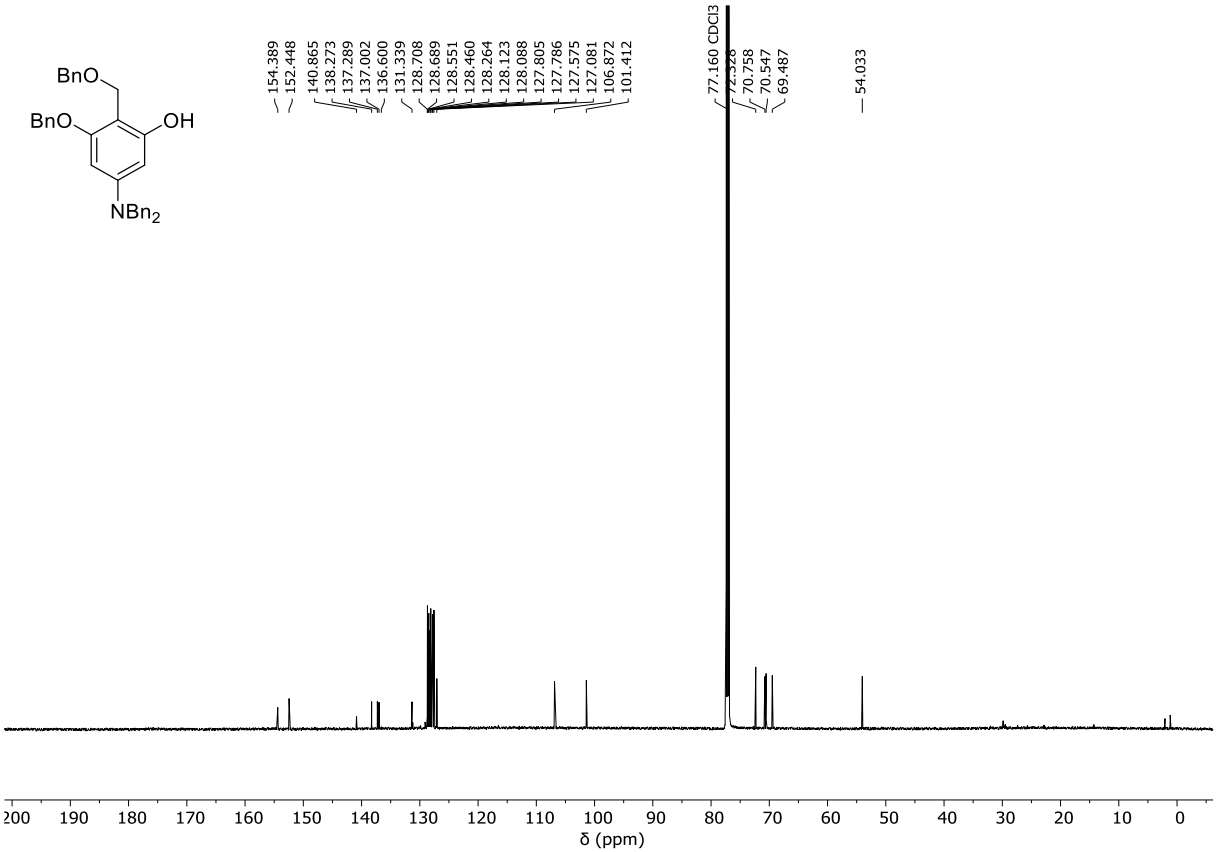
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **152**



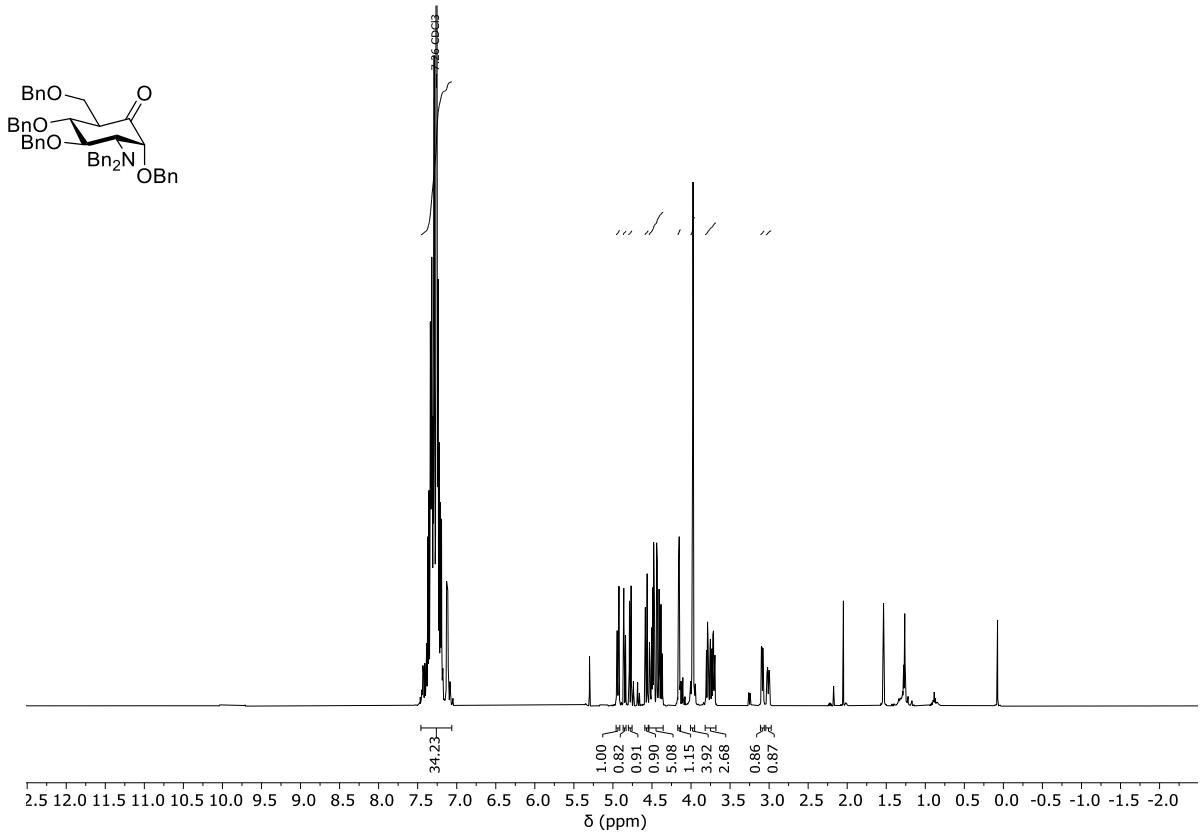
NOESY spectrum of **152**



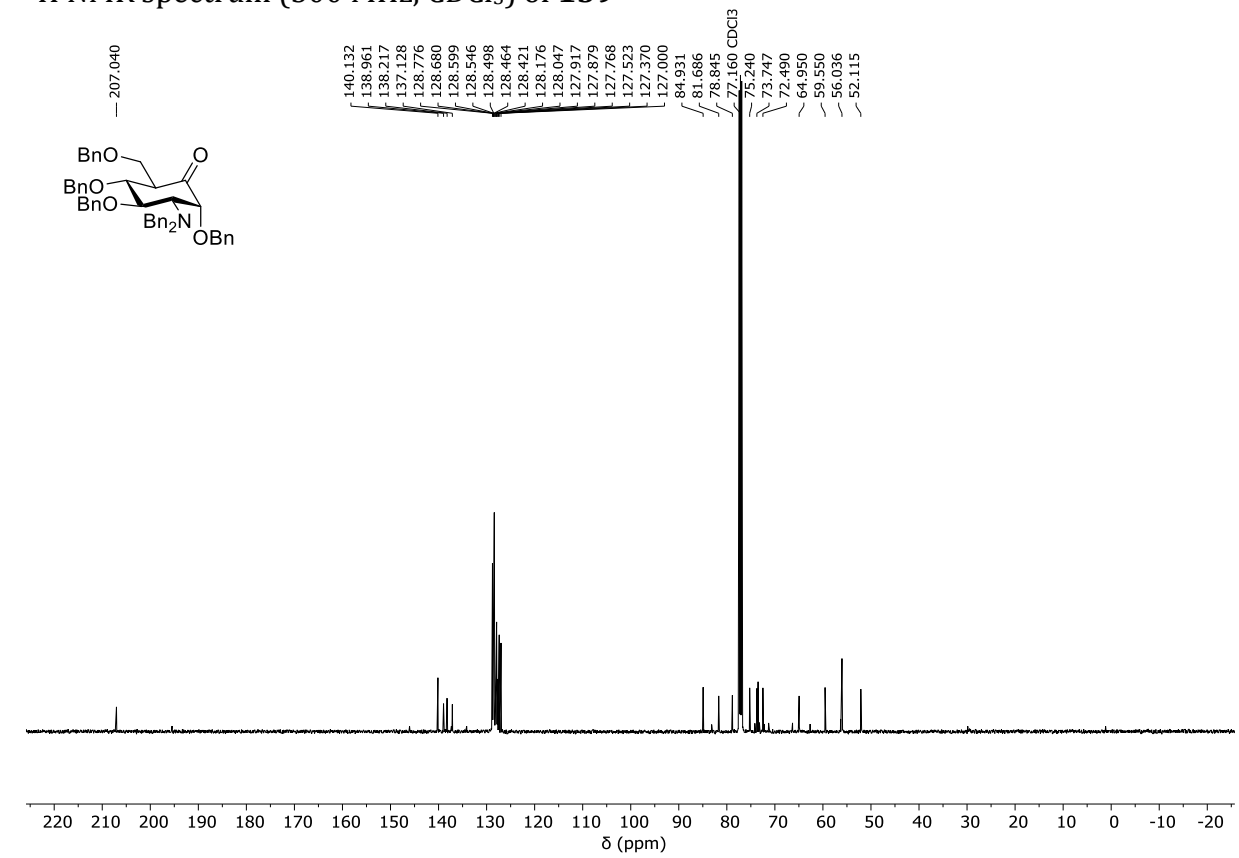
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **158**



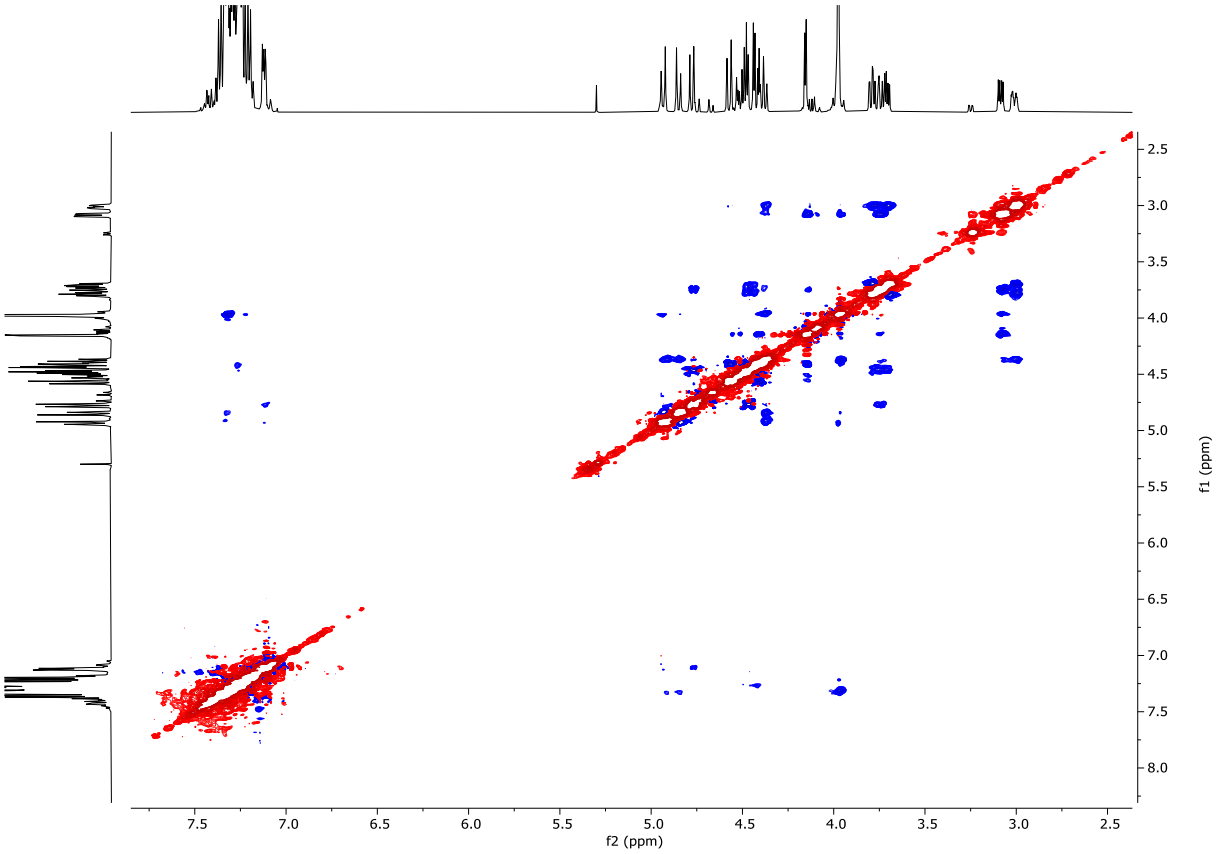
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **158**



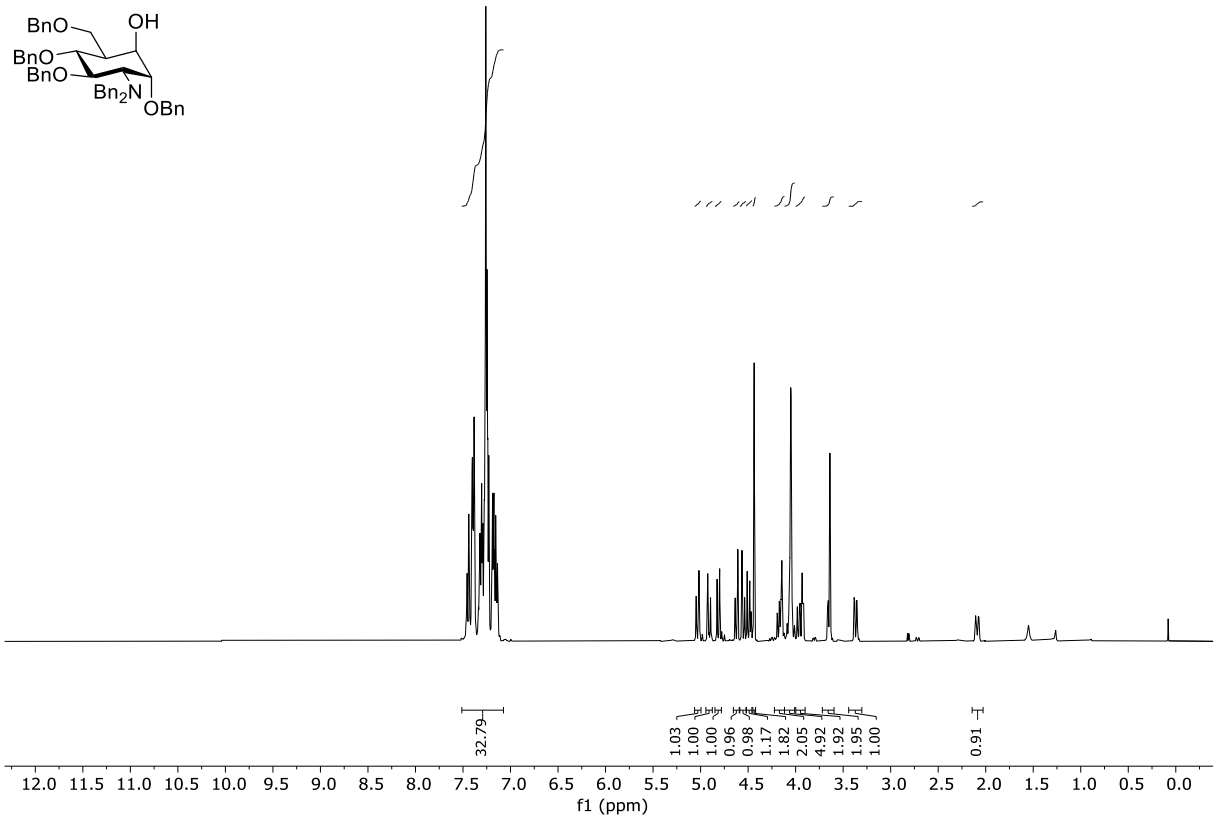
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **159**



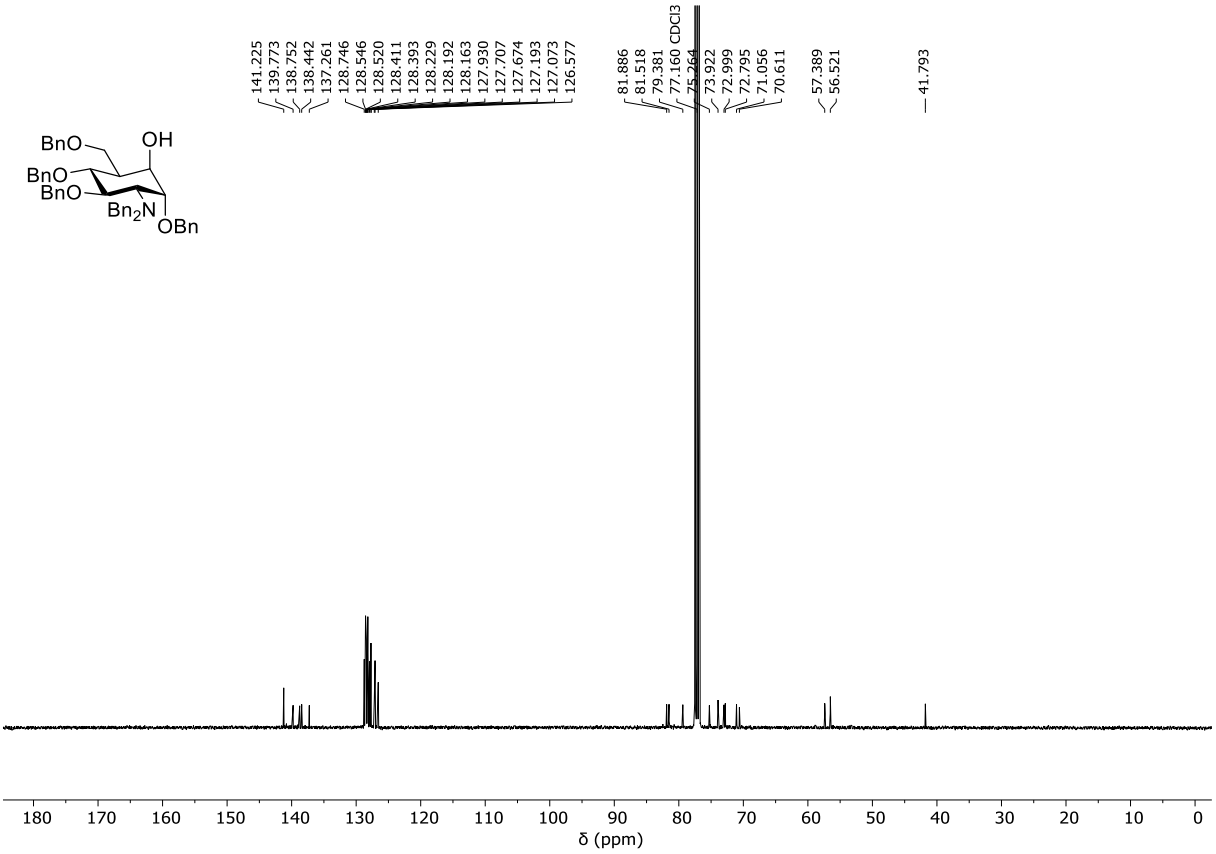
^{13}C NMR spectrum (126MHz, $CDCl_3$) of **159**



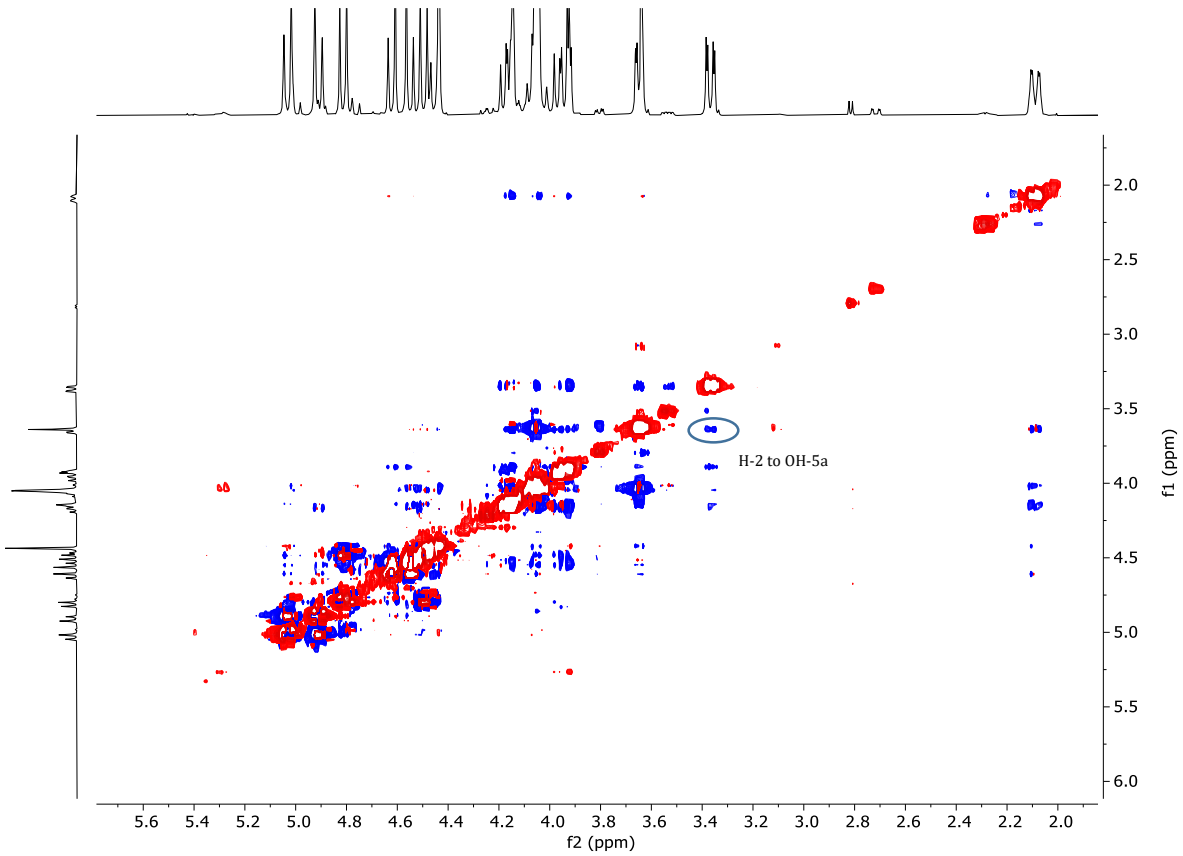
NOESY spectrum of **159**



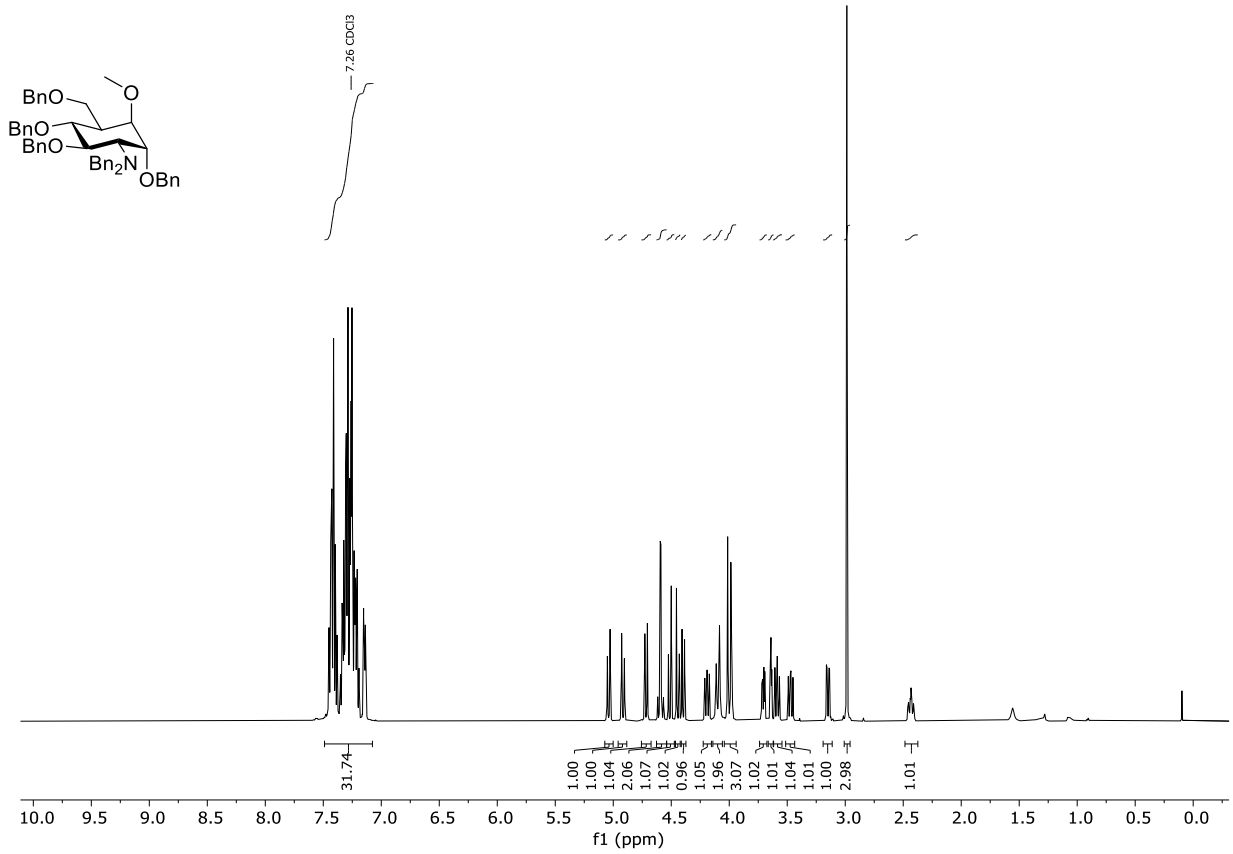
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **160**



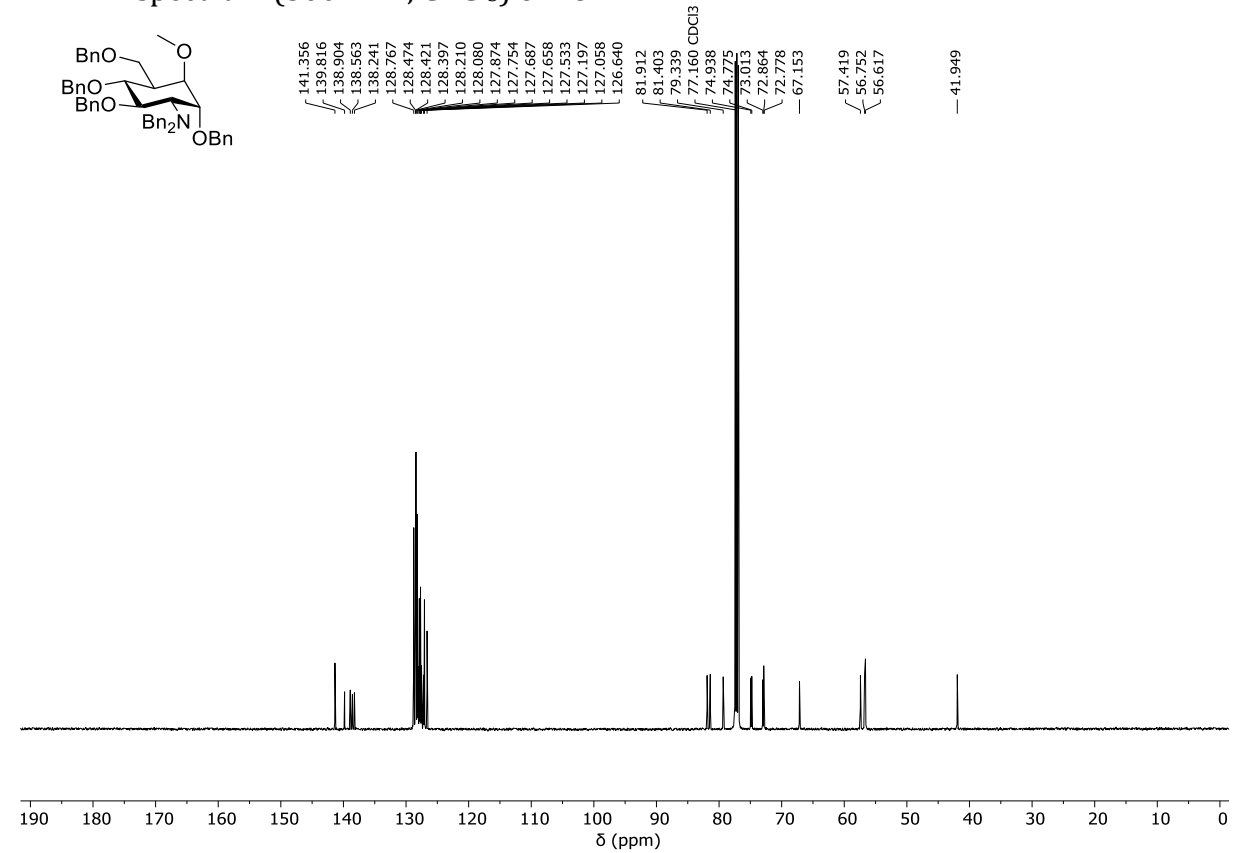
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **160**



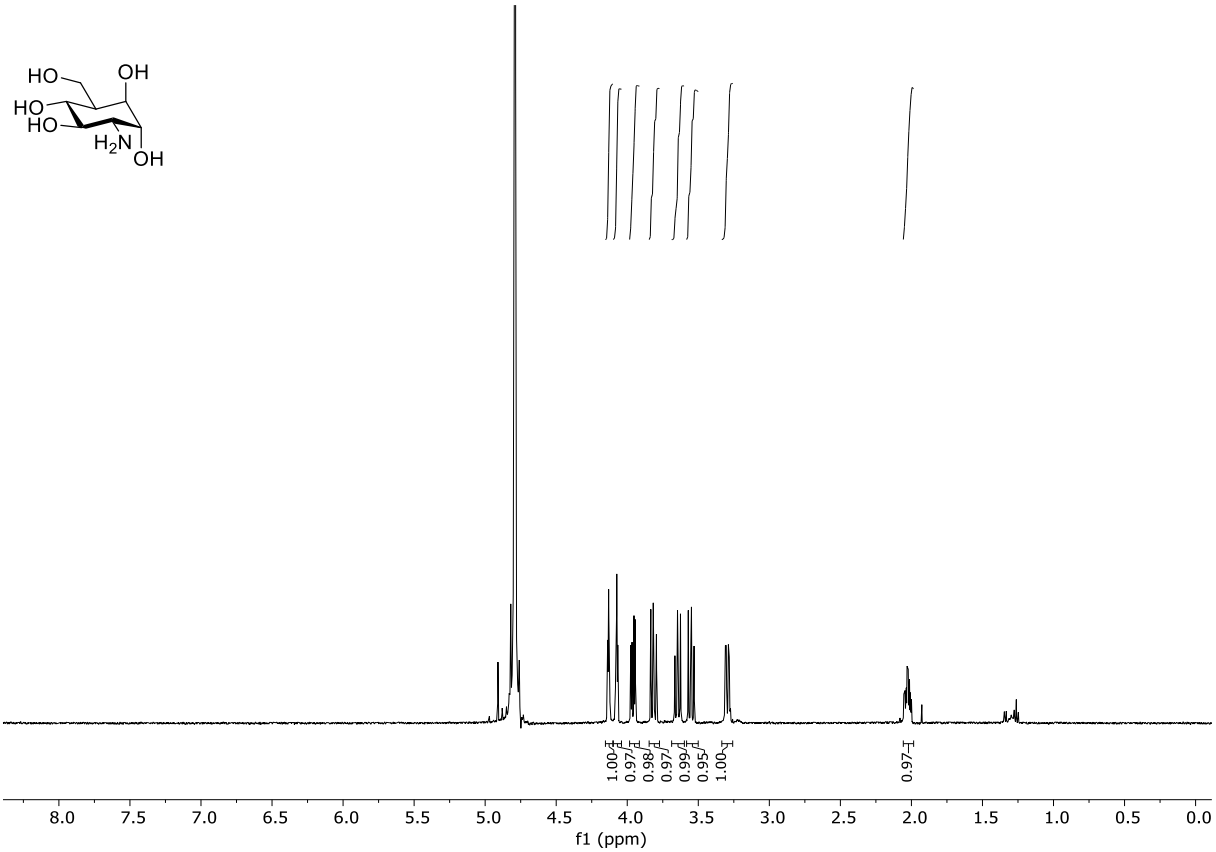
NOESY spectrum of **160**



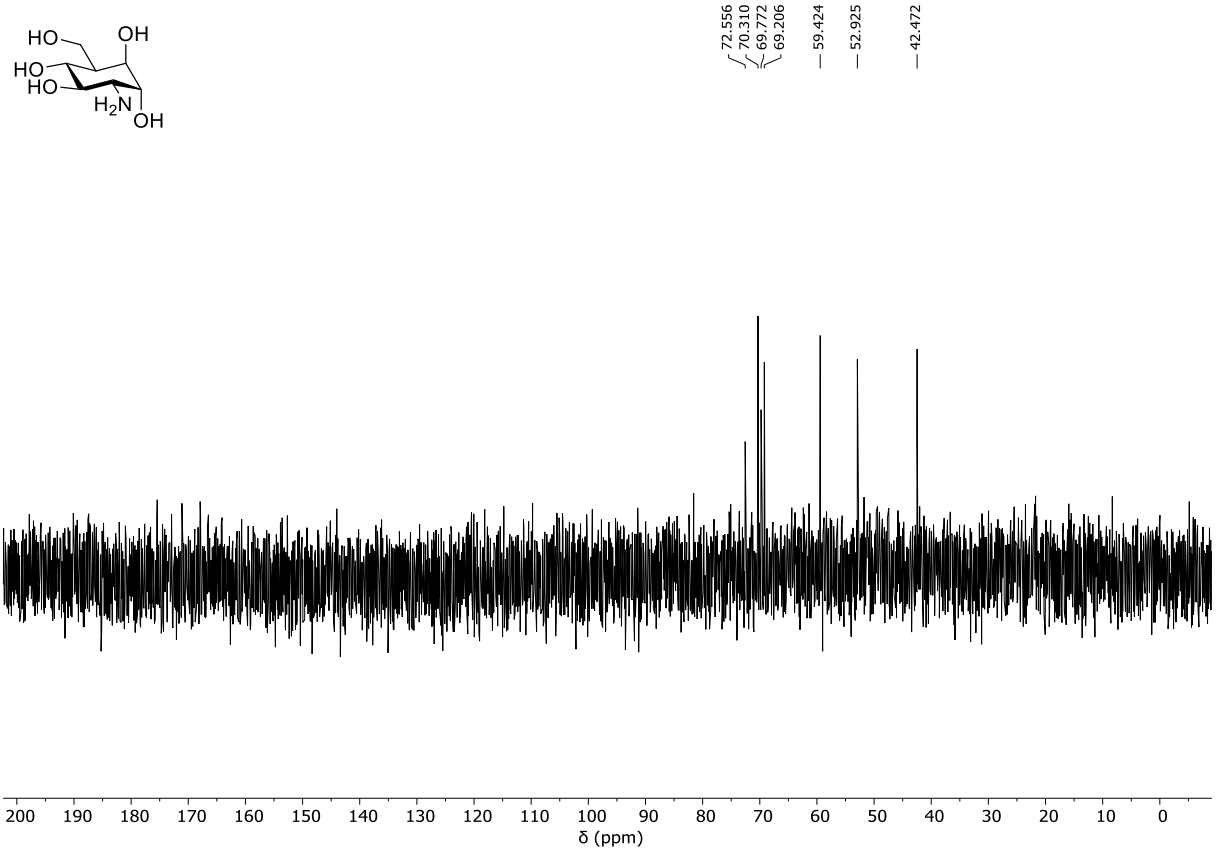
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **161**



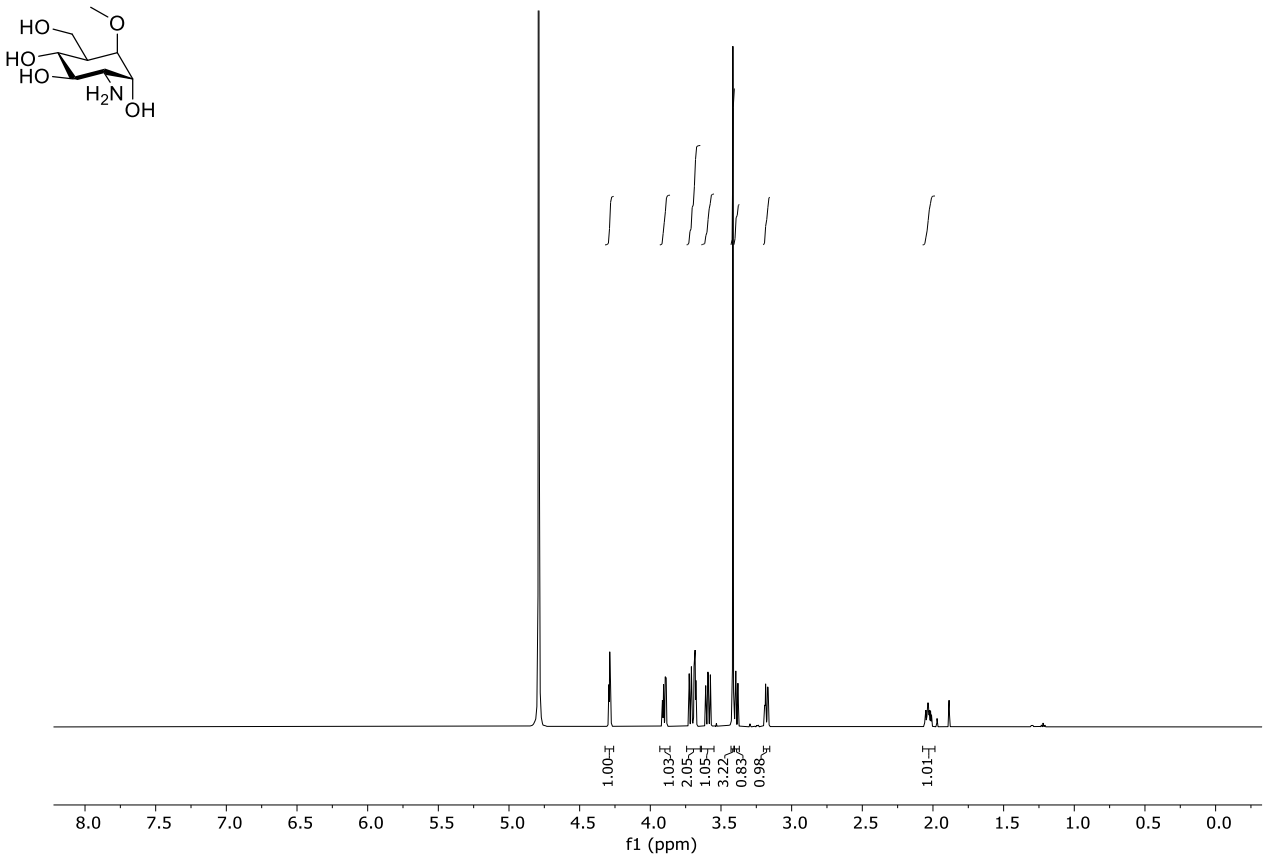
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **161**



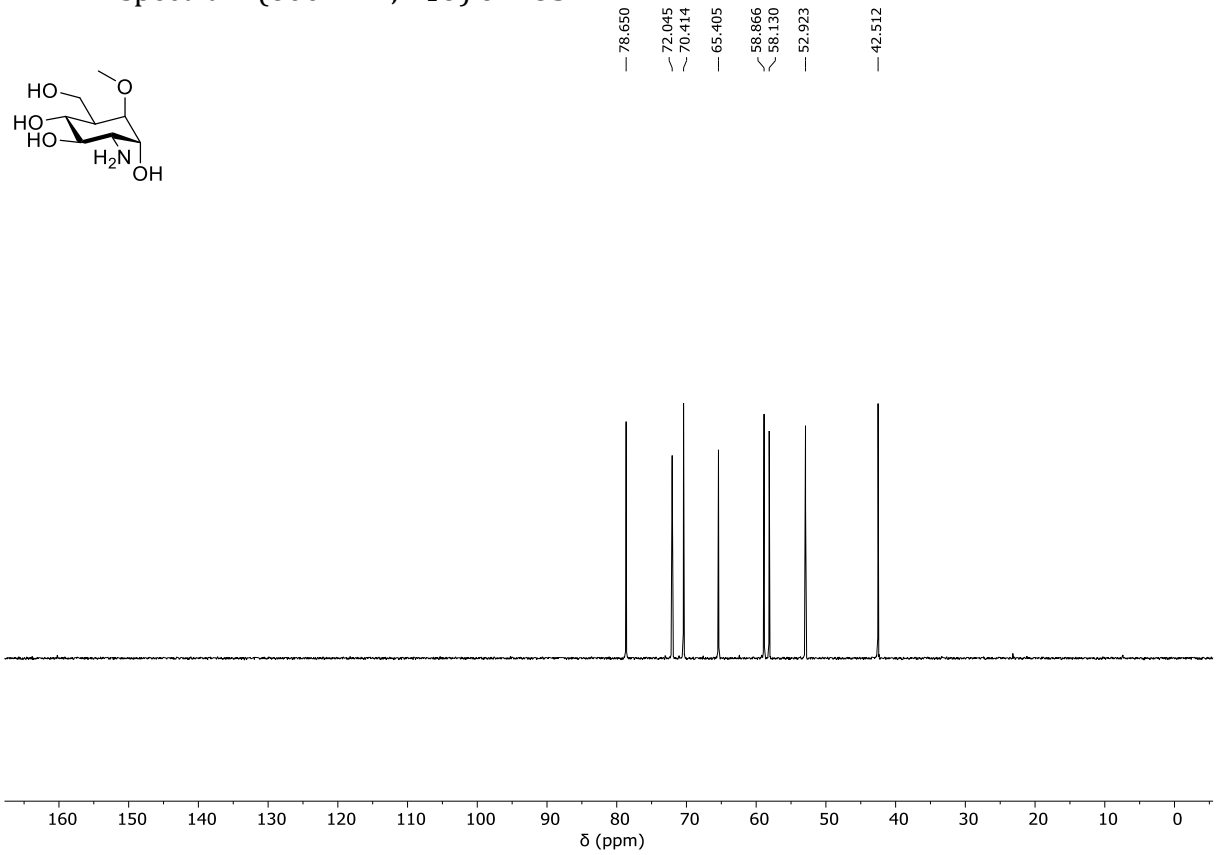
^{1}H NMR spectrum (500 MHz, D_2O) of **162**



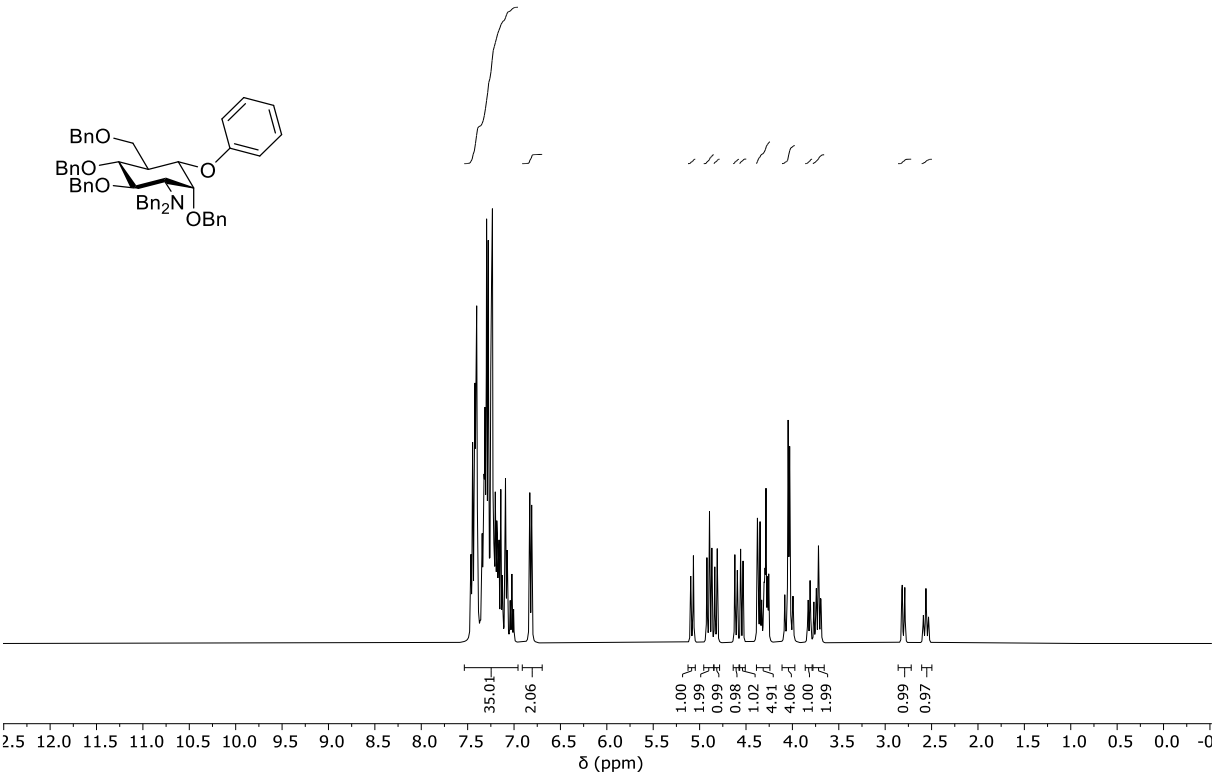
^{13}C NMR spectrum (126 MHz, D_2O) of **162**



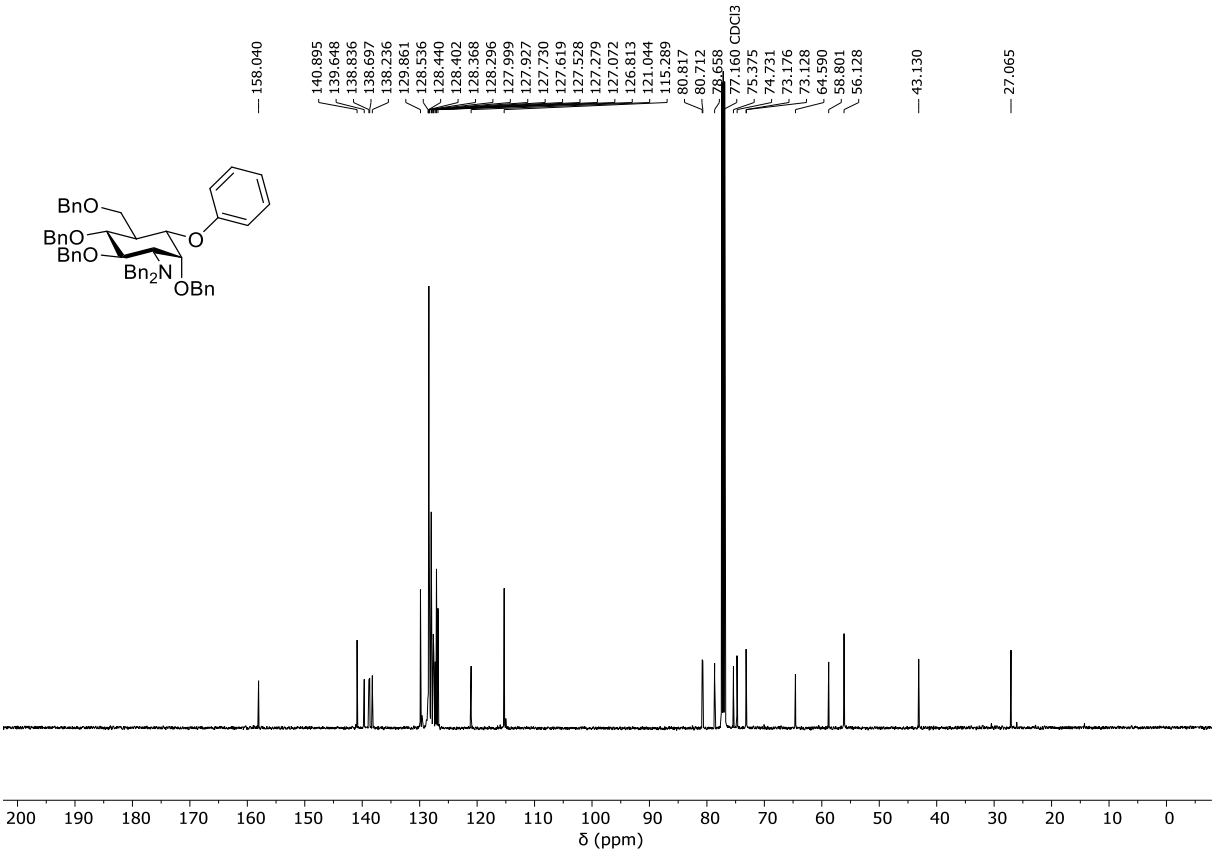
^{1}H NMR spectrum (600 MHz, D_2O) of **163**



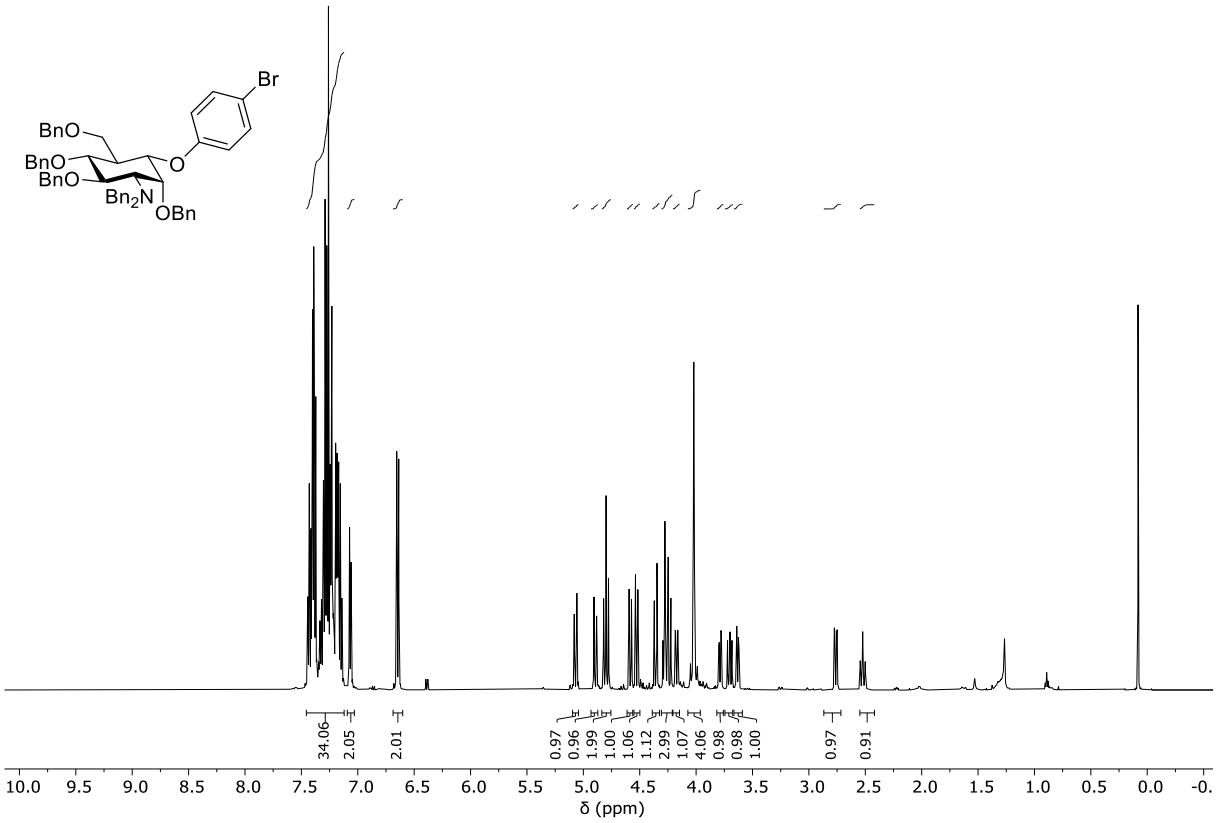
^{13}C NMR spectrum (150 MHz, D_2O) of **163**



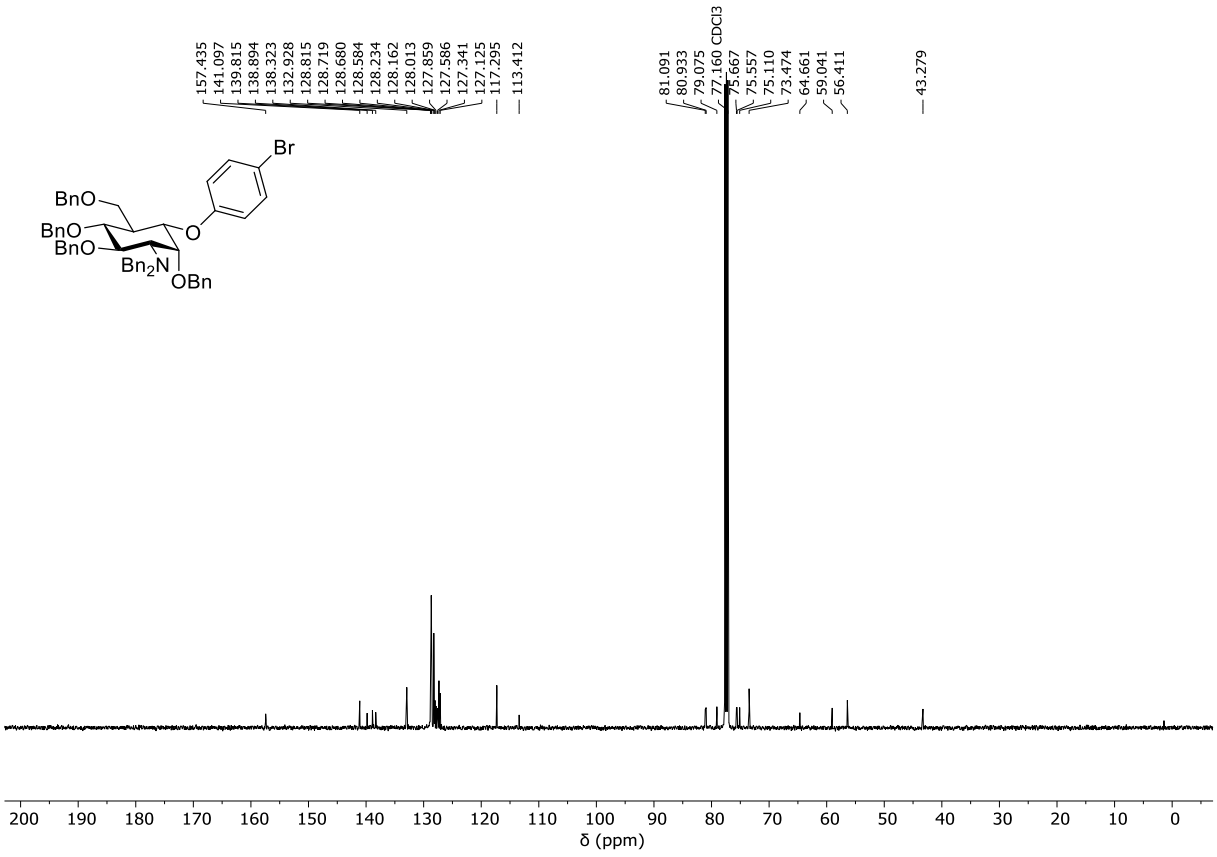
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **166**



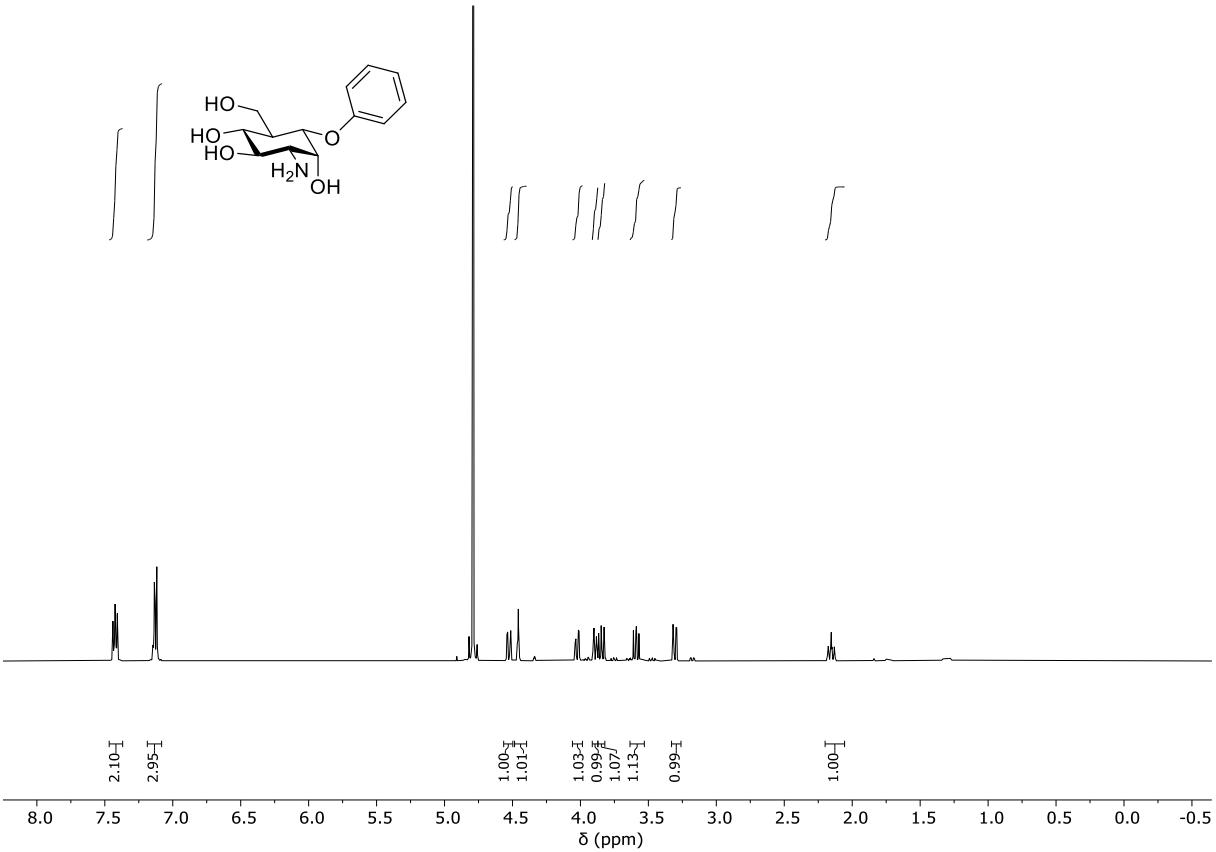
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **166**



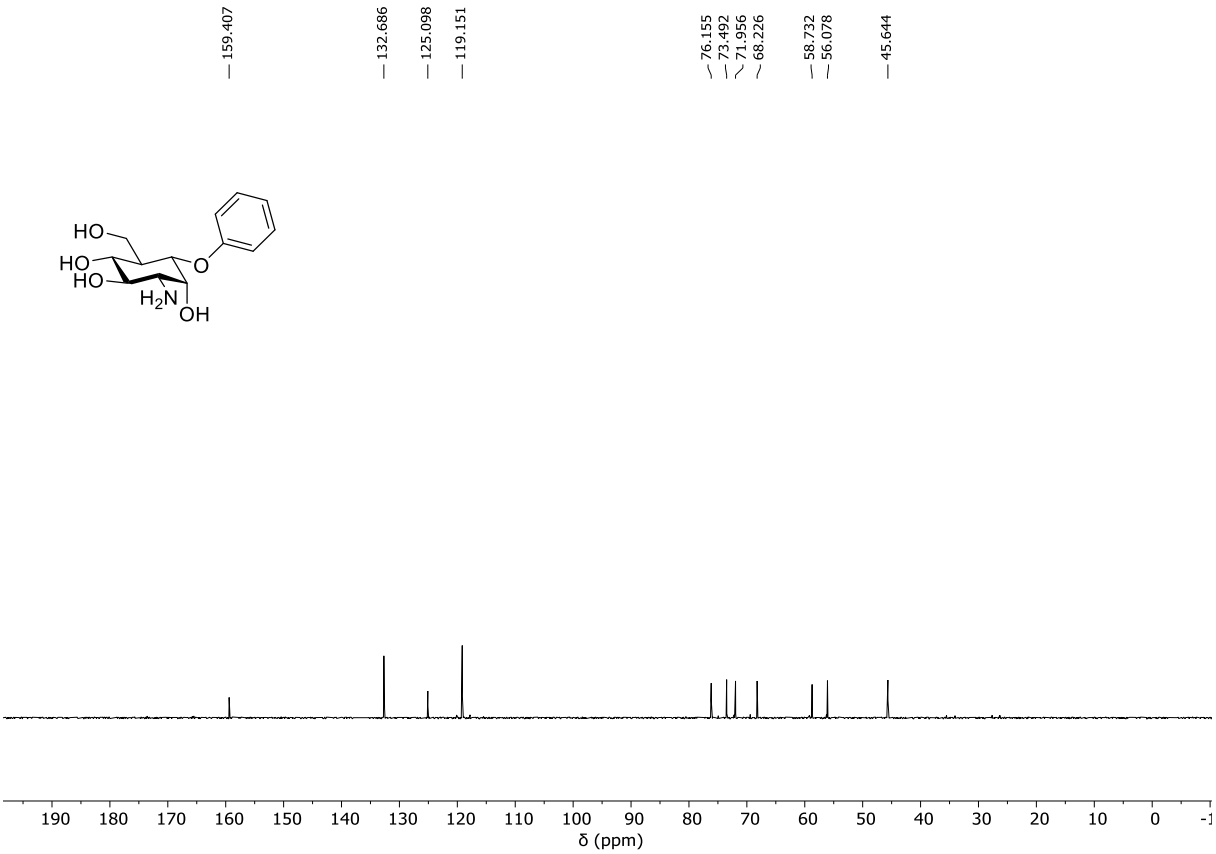
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **167**



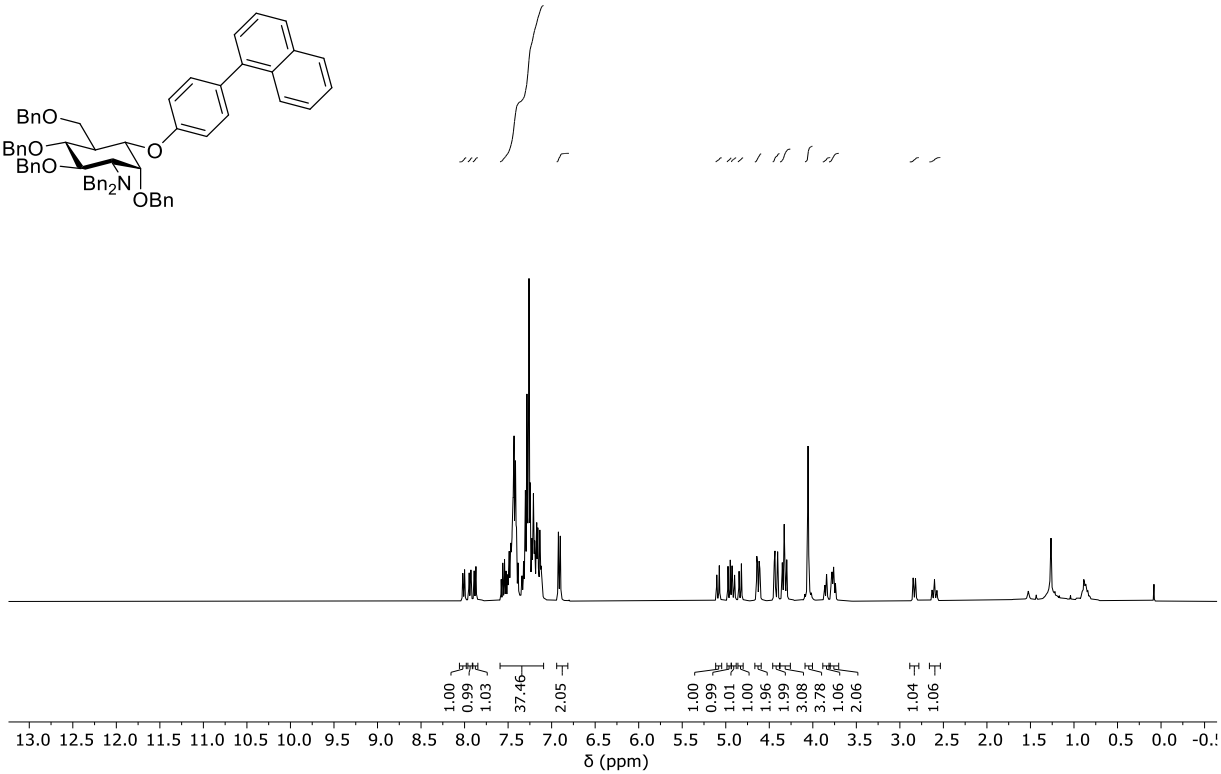
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **167**



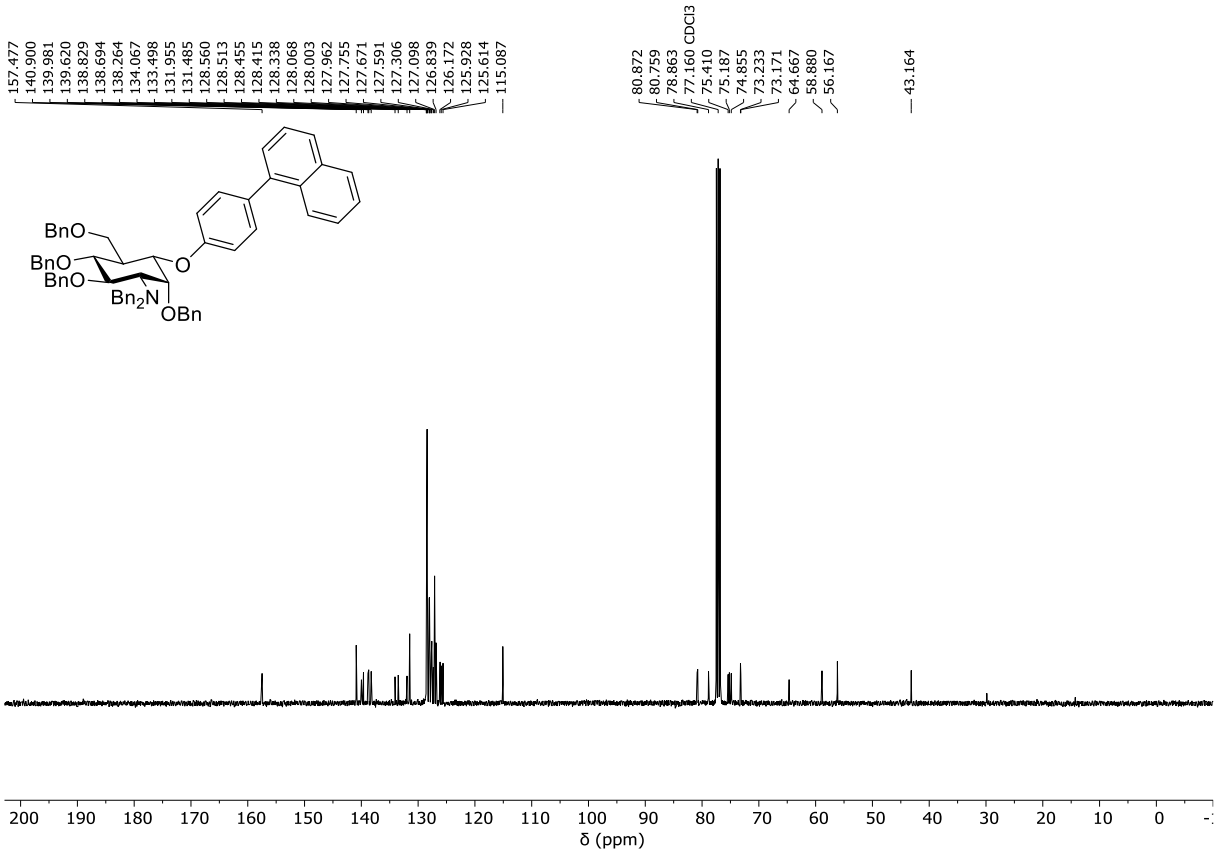
^{1}H NMR spectrum (500 MHz, D_2O) of **168**



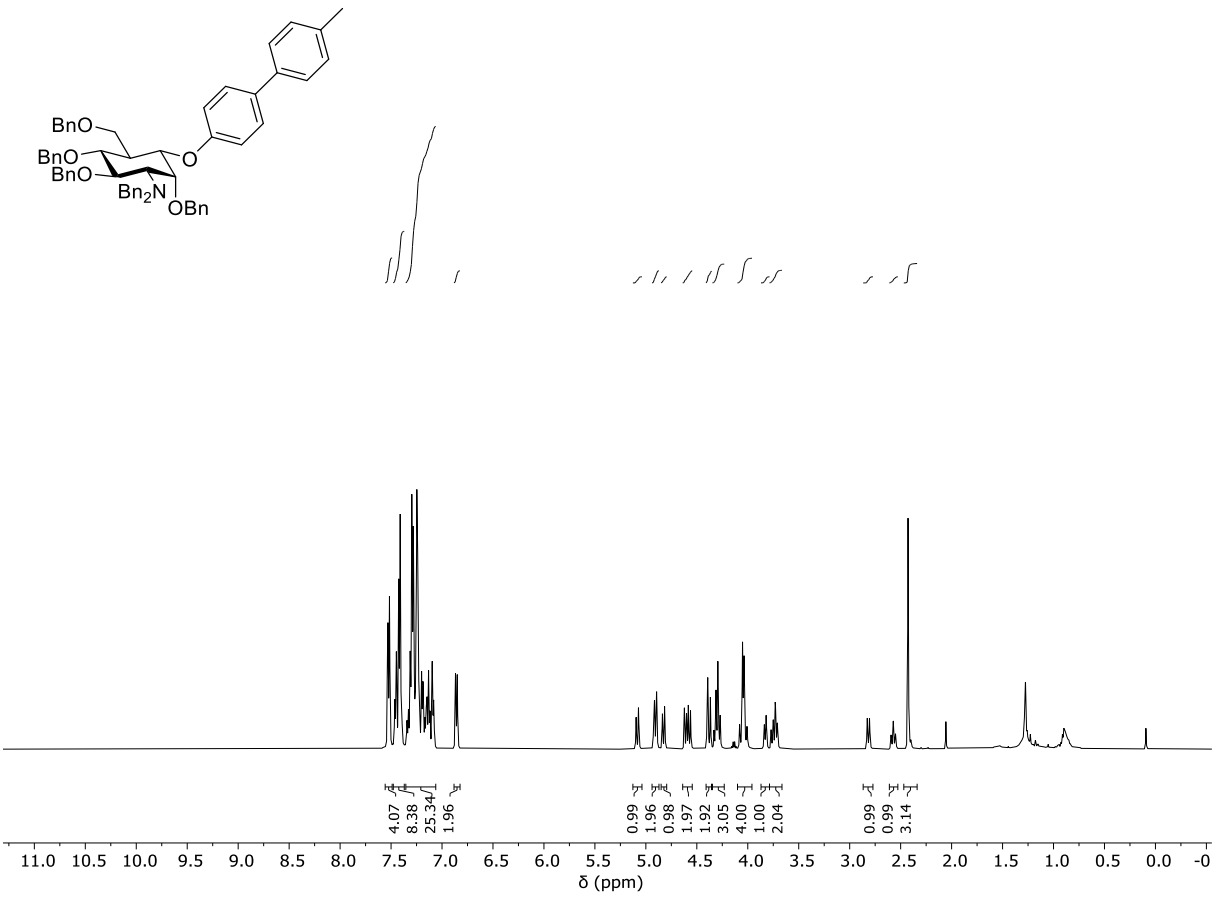
^{13}C NMR spectrum (126 MHz, D_2O) of **168**



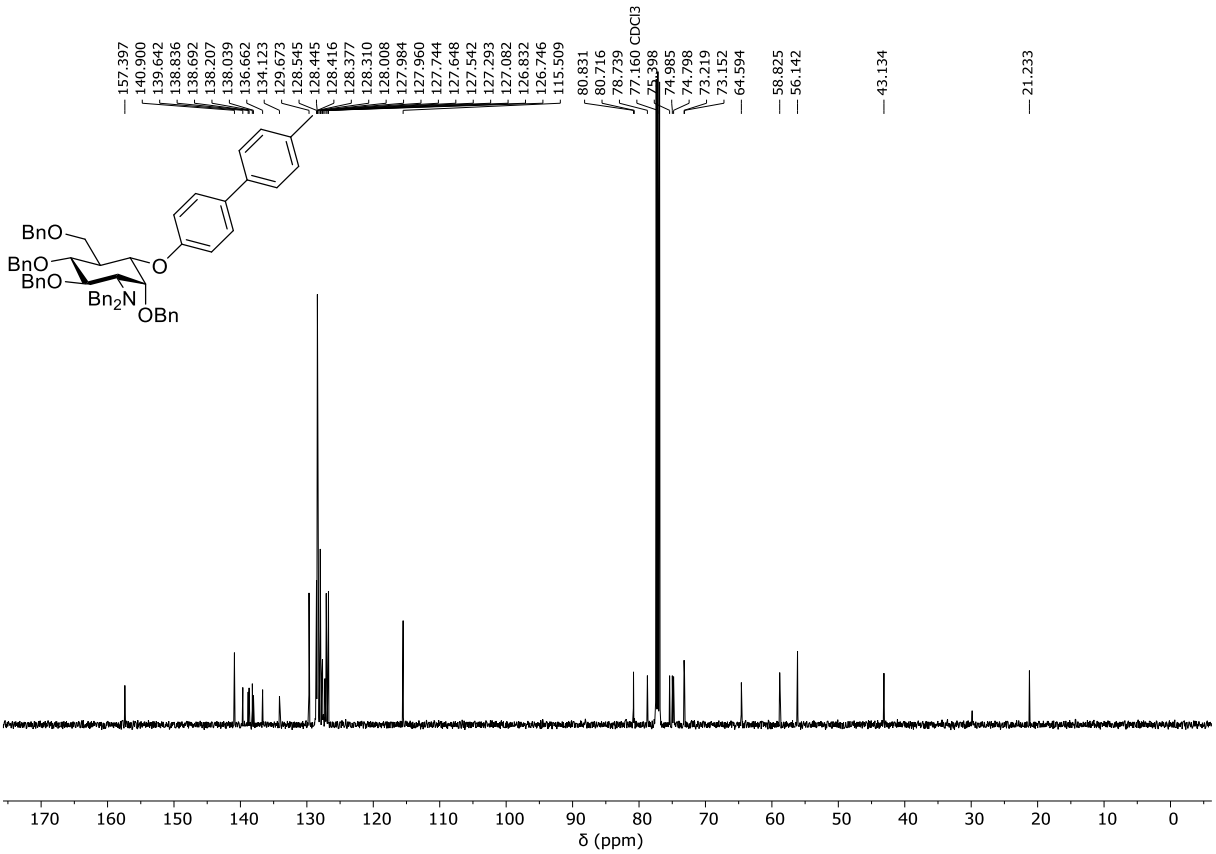
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **169**



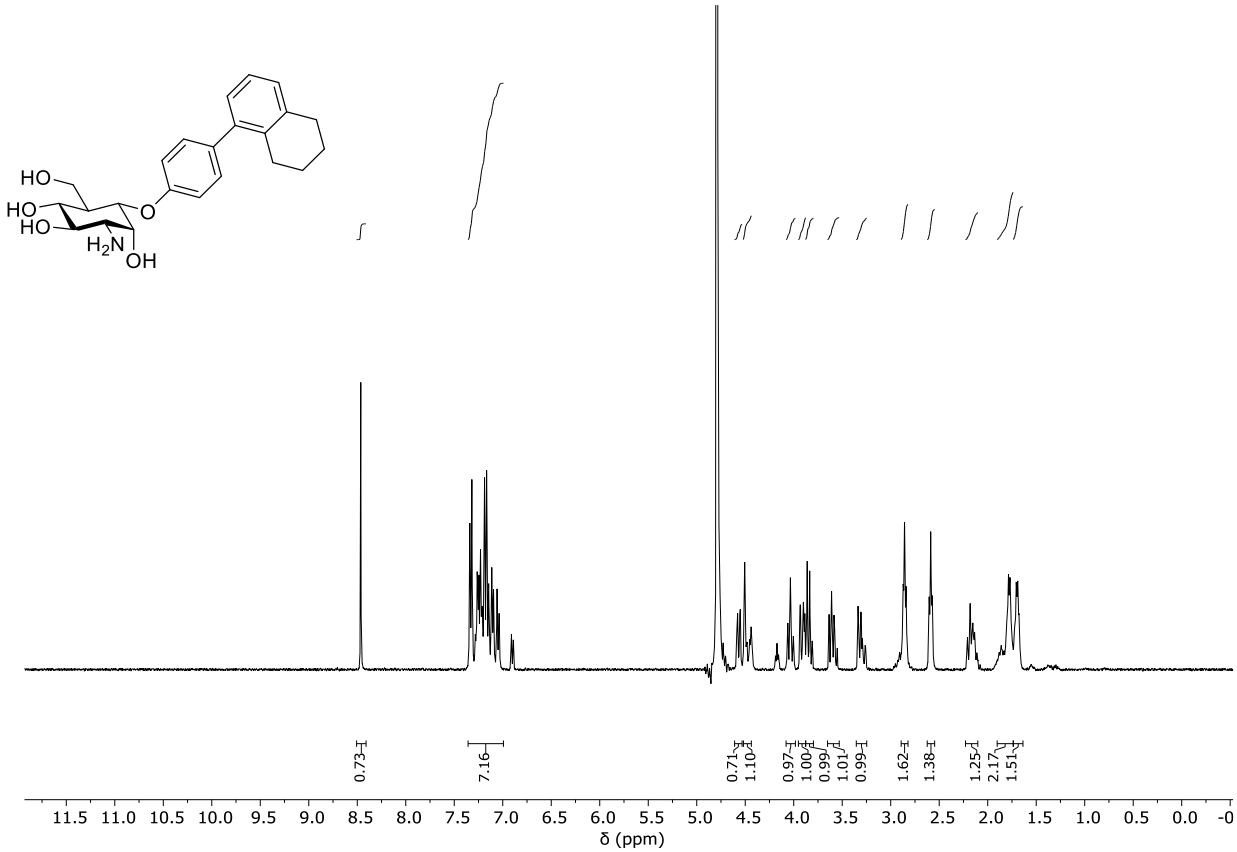
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **169**



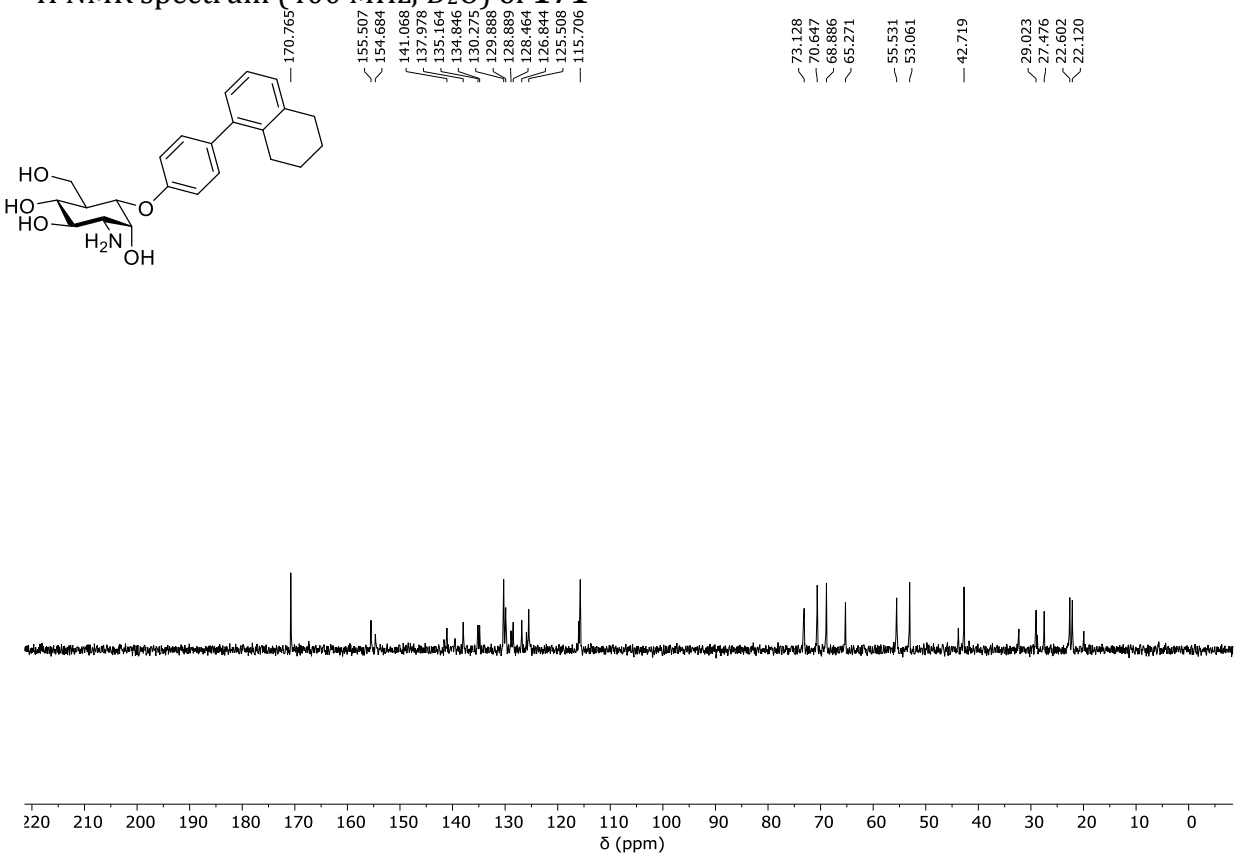
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **170**



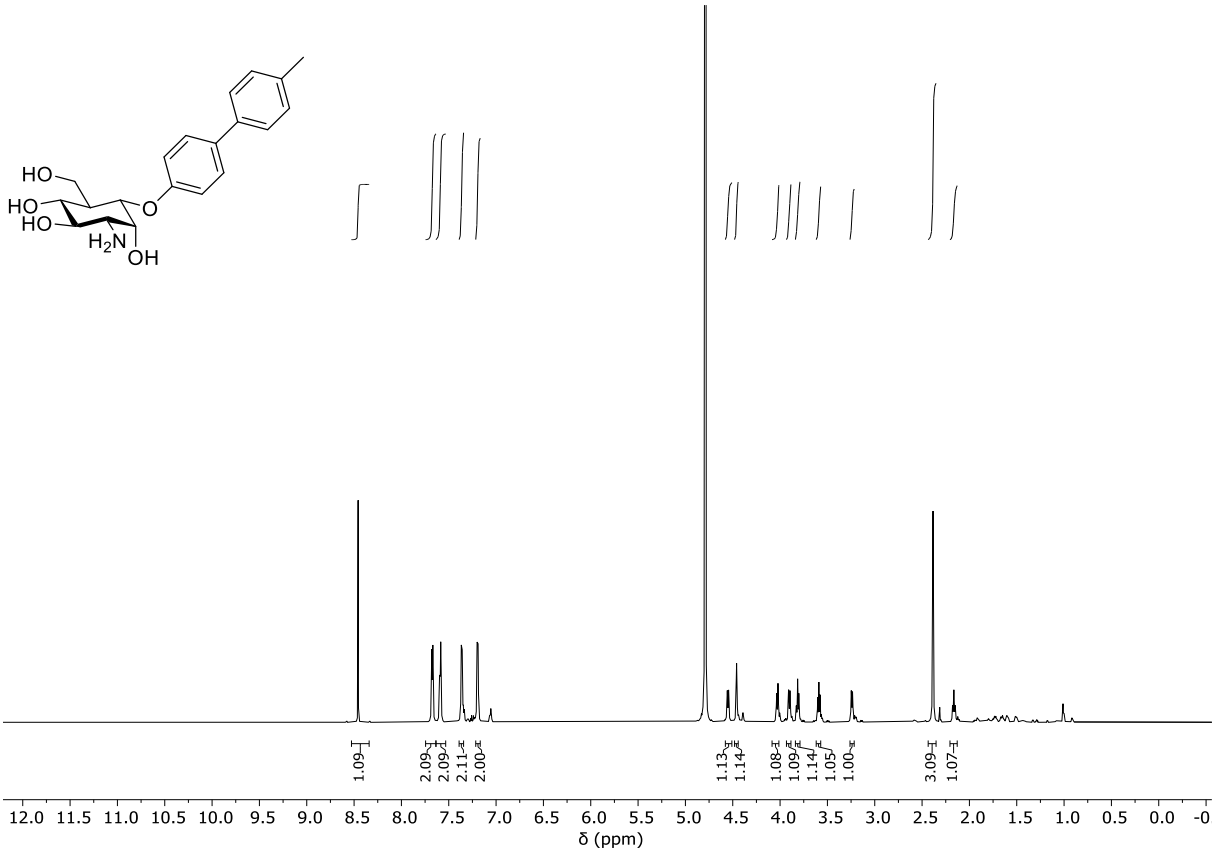
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **170**



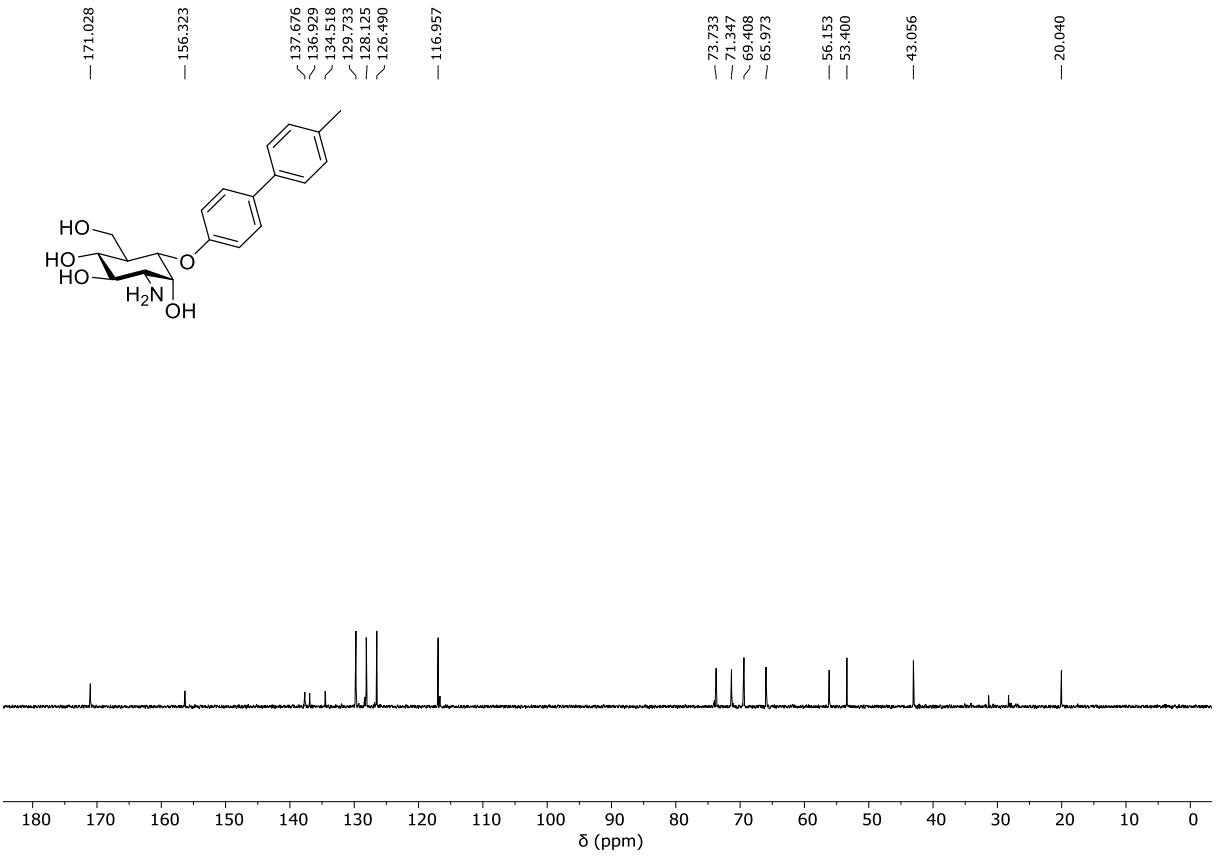
^{1}H NMR spectrum (400 MHz, D_2O) of **171**



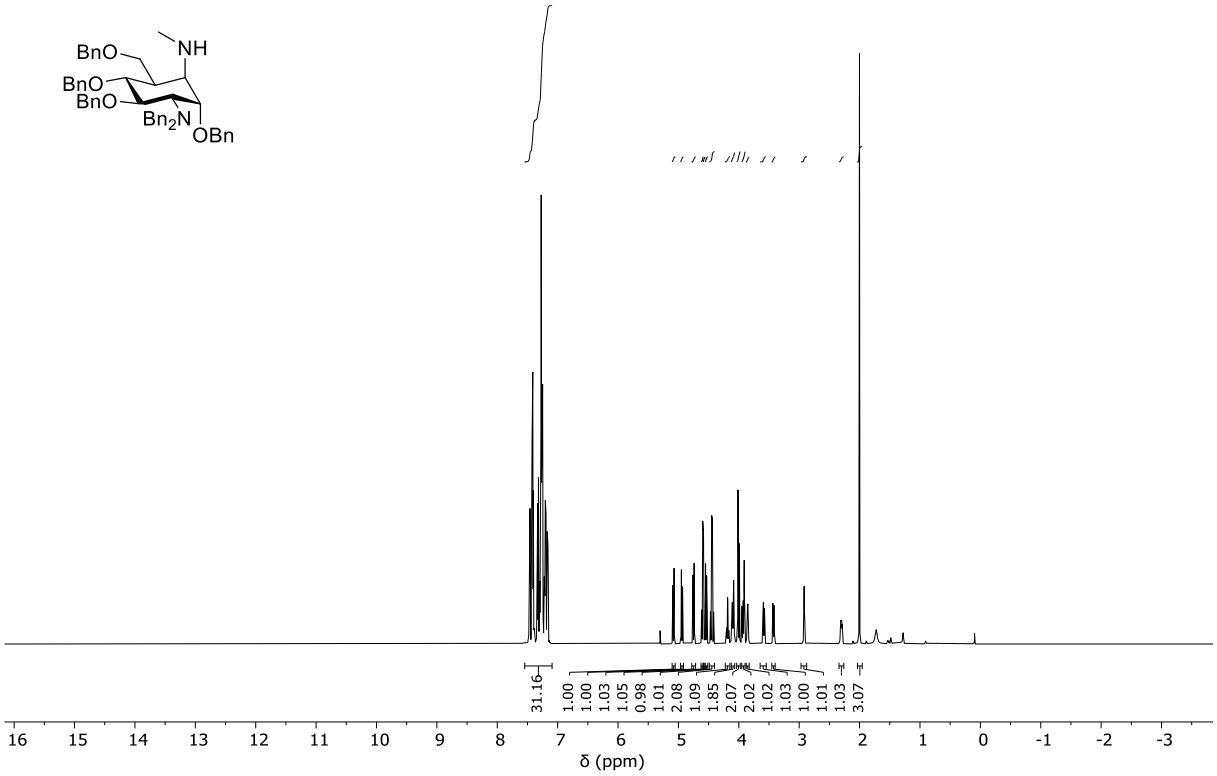
^{13}C NMR spectrum (151 MHz, D_2O) of **171**



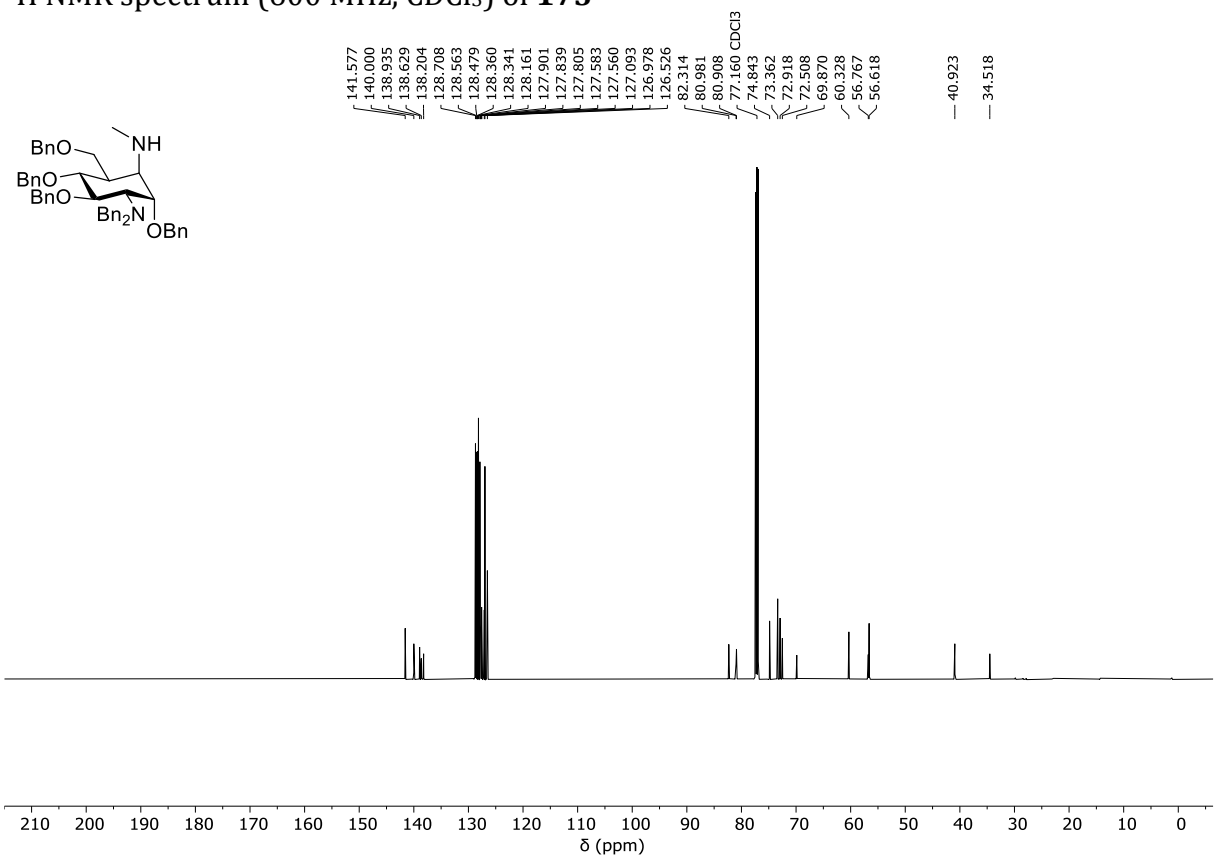
^{1}H NMR spectrum (800 MHz, D_2O) of **172**



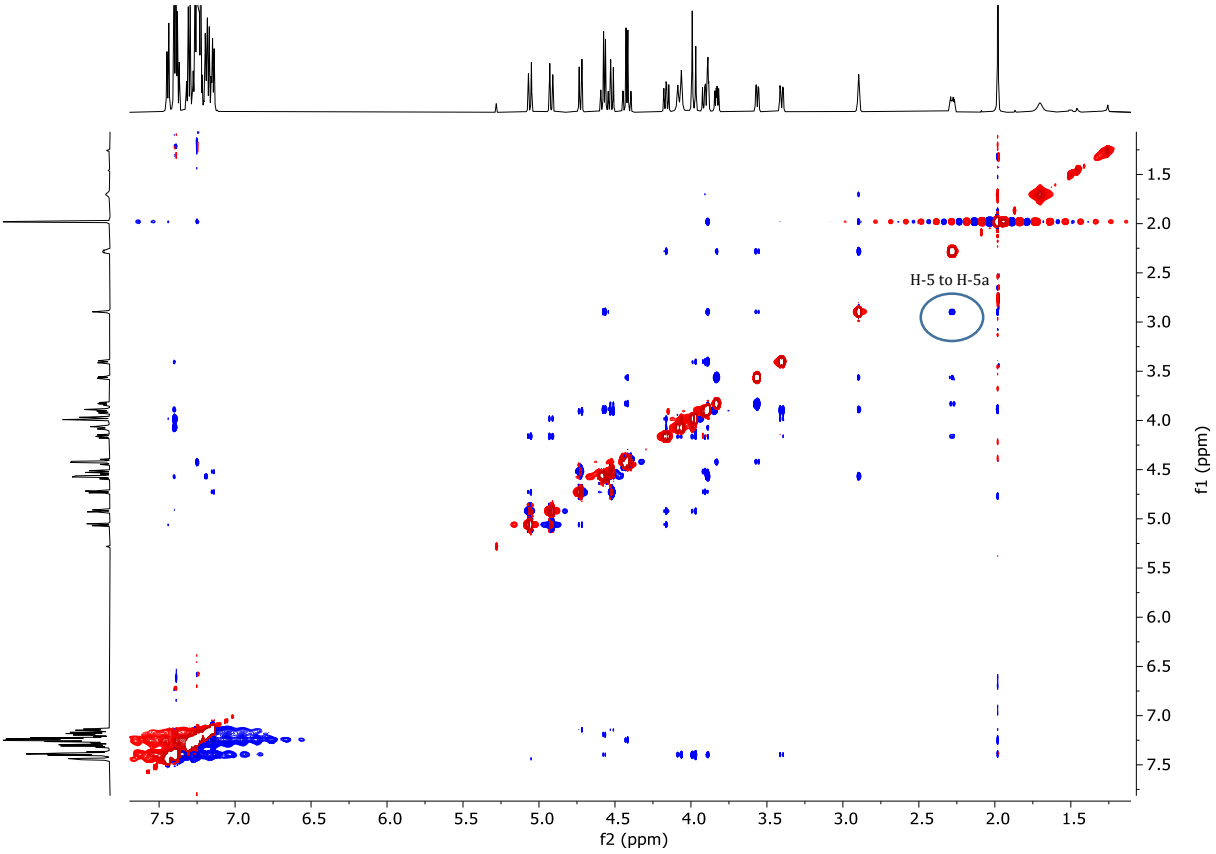
^{13}C NMR spectrum (201 MHz, D_2O) of **172**



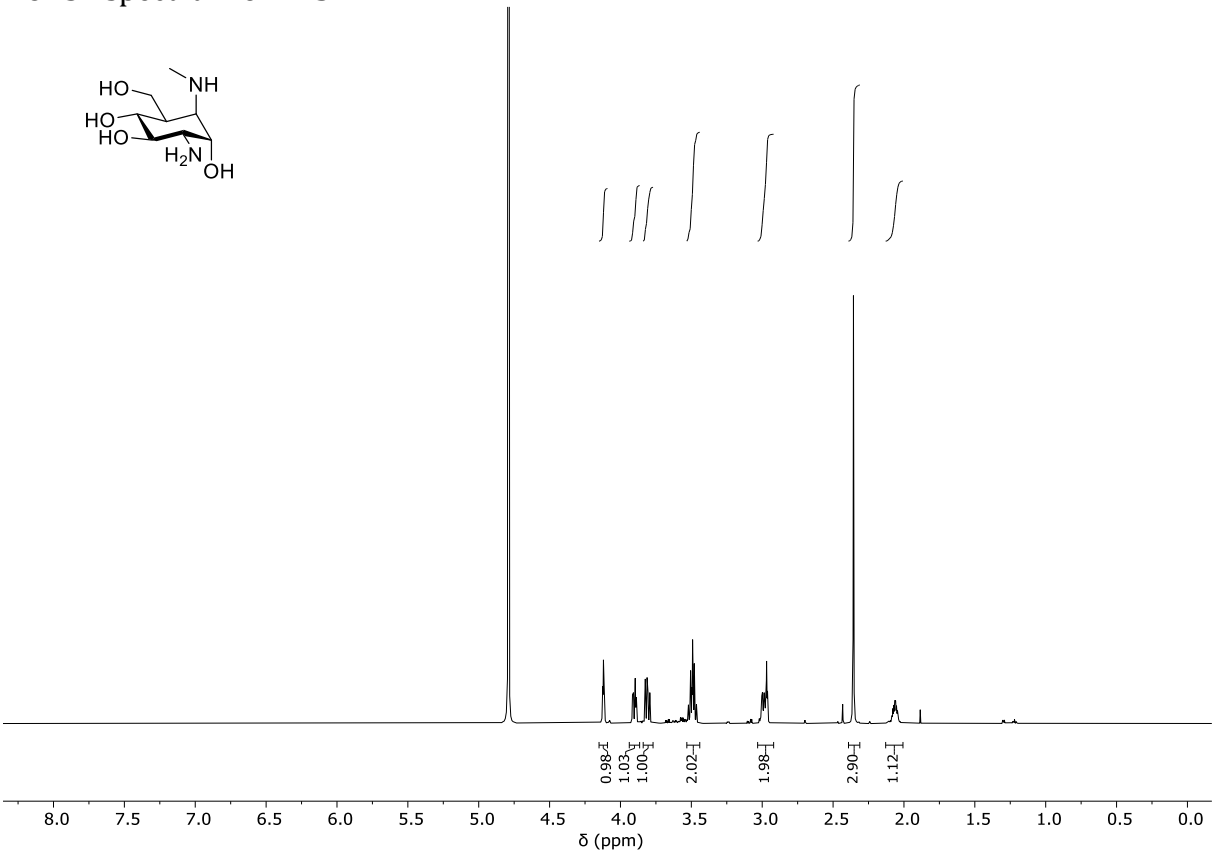
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **173**



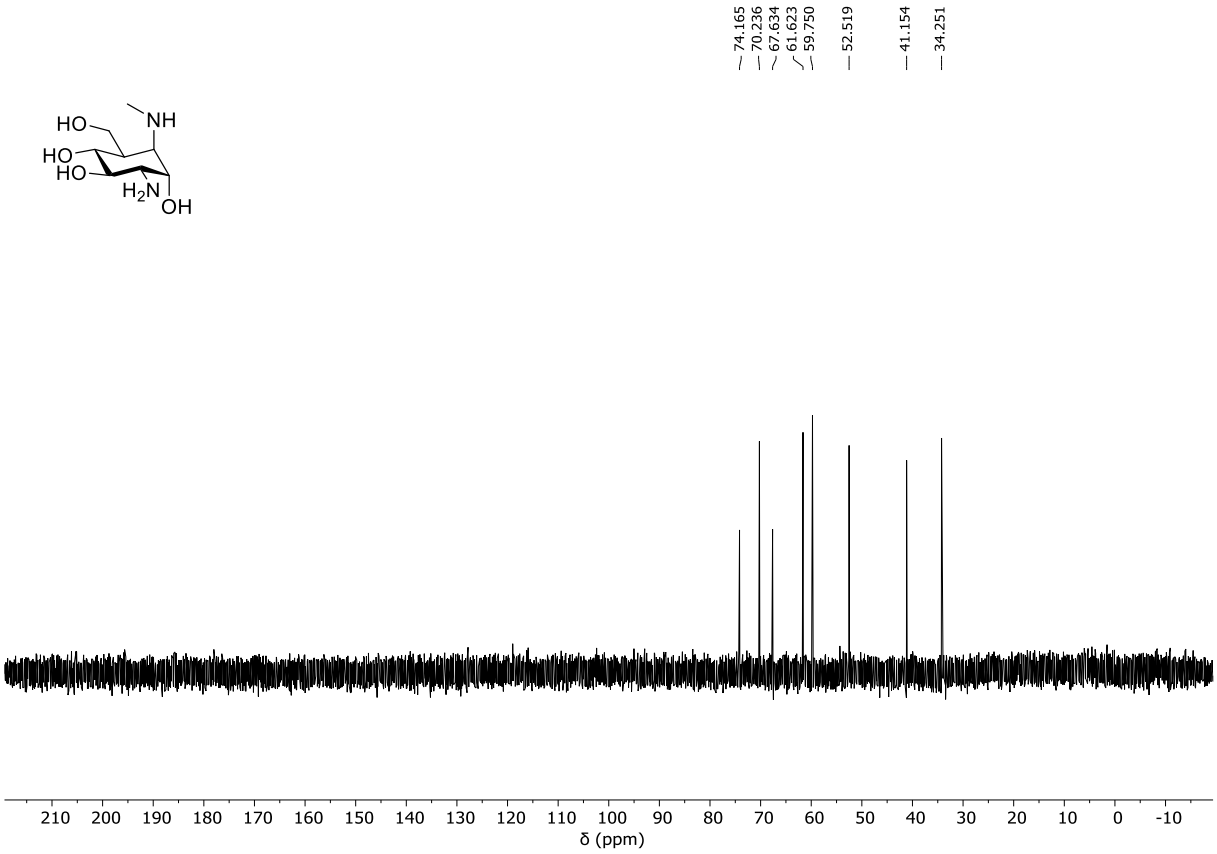
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **173**



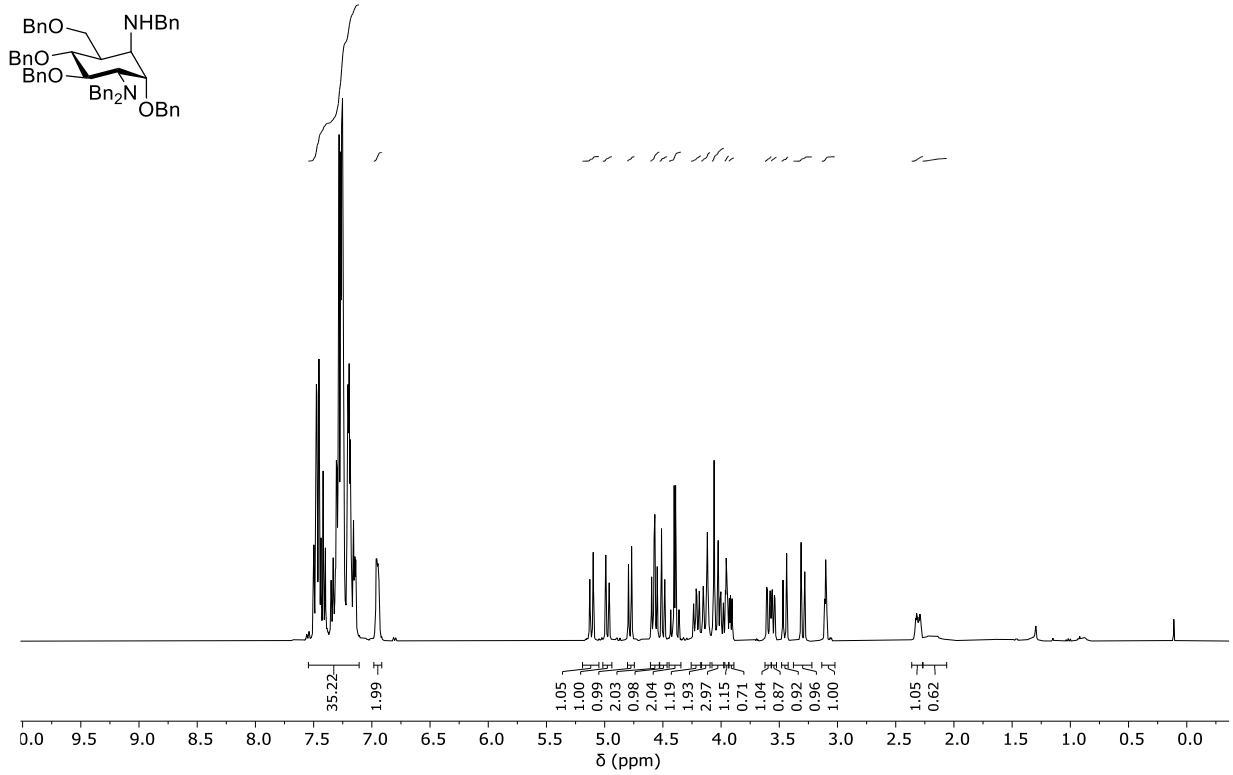
NOESY spectrum of **173**



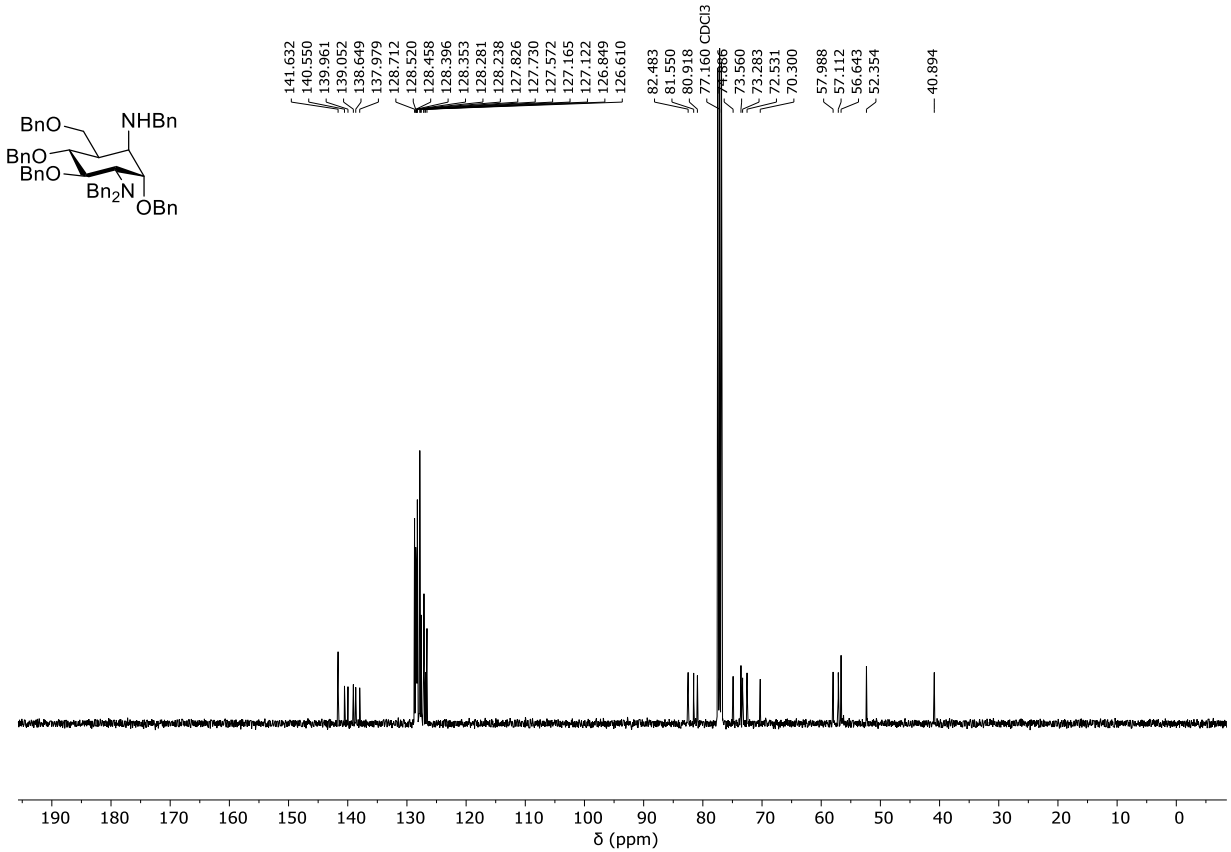
^{1}H NMR spectrum (600 MHz, D_2O) of **174**



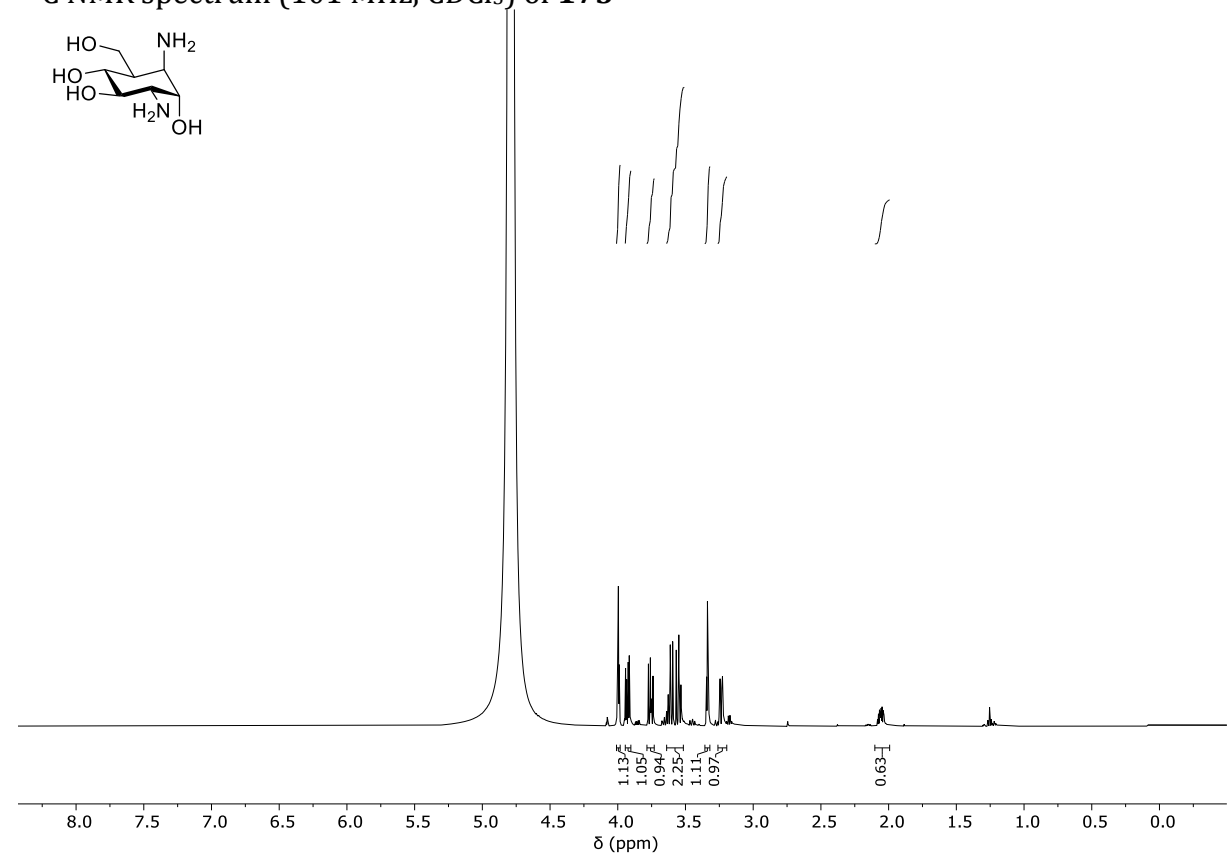
^{13}C NMR spectrum (151 MHz, D_2O) of **174**



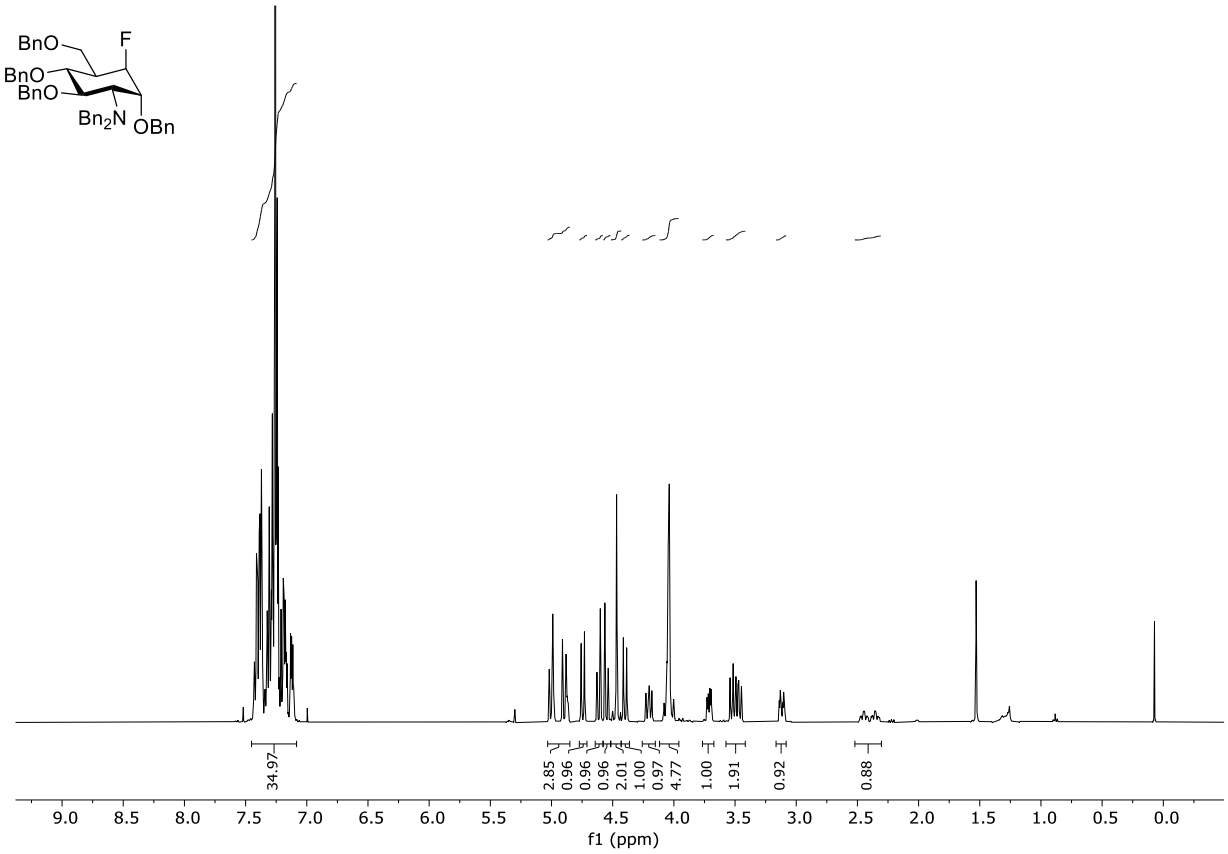
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **175**



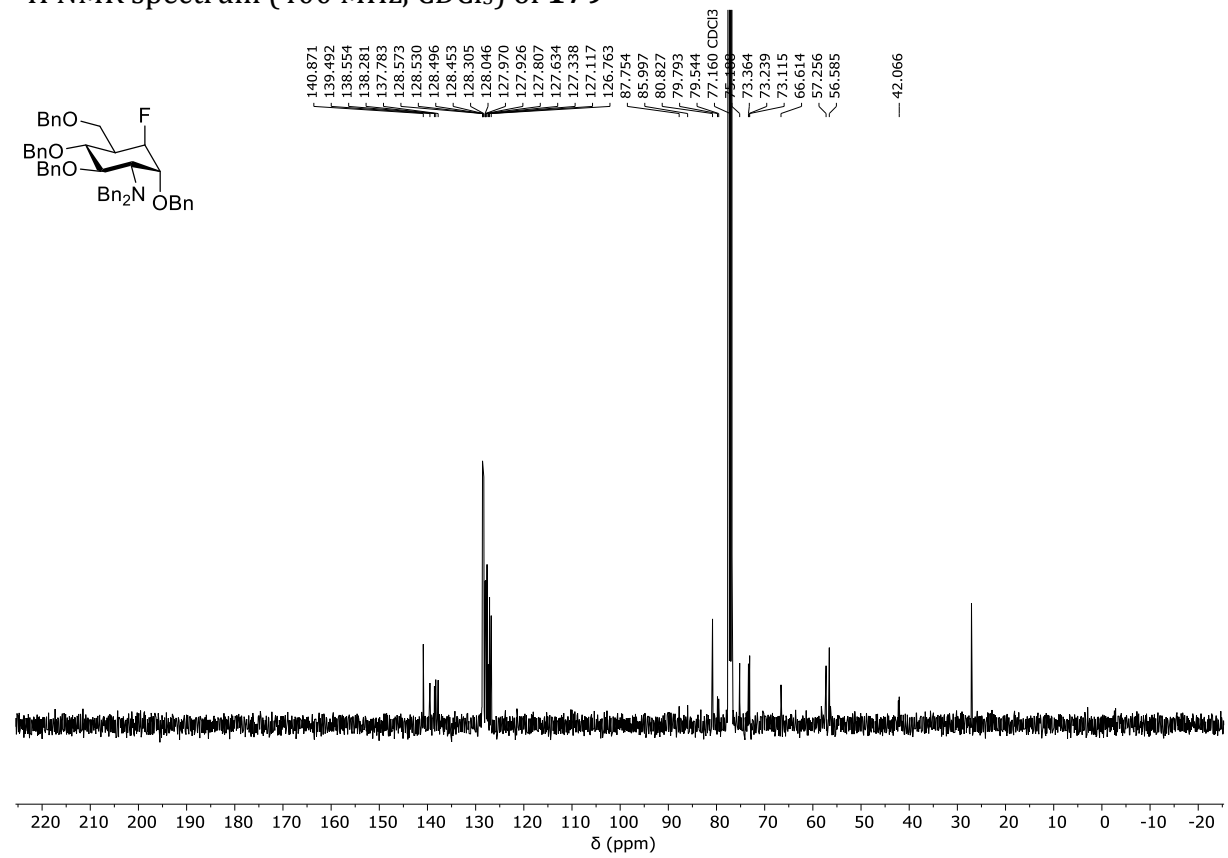
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **175**



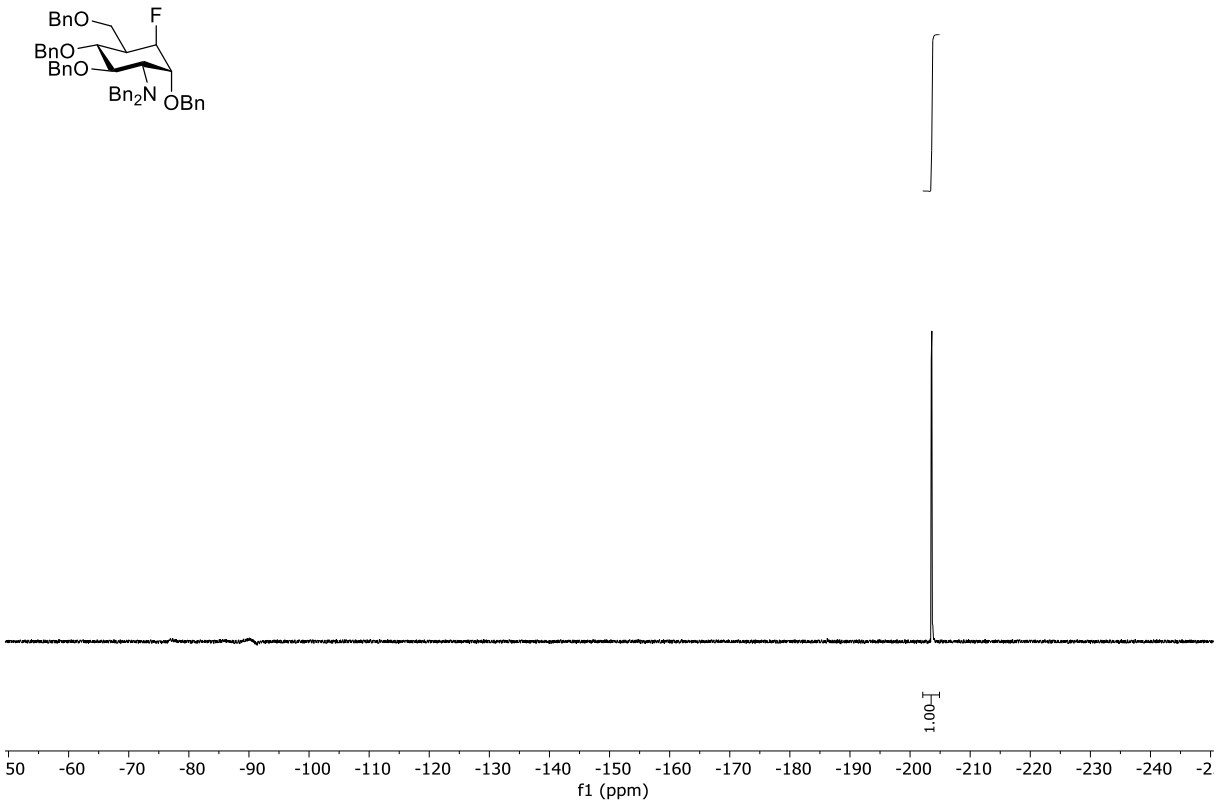
^{1}H NMR spectrum (600 MHz, D_2O) of **176**



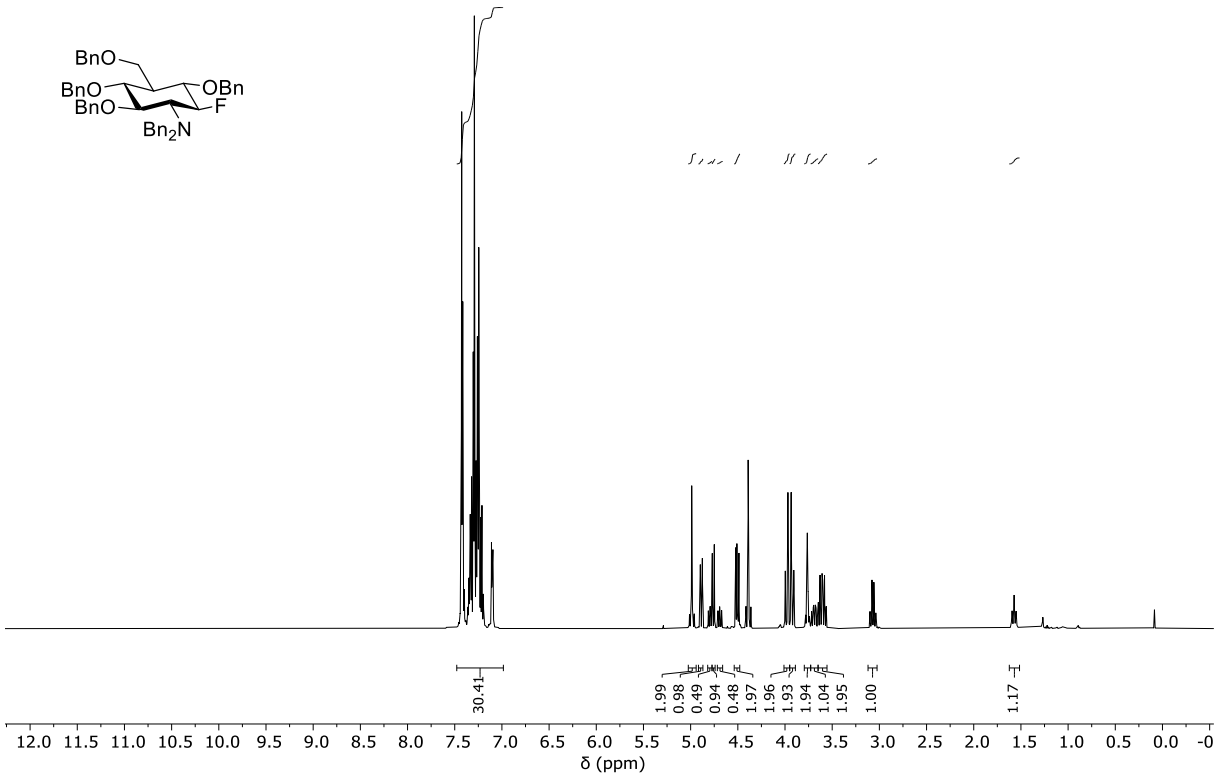
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **179**



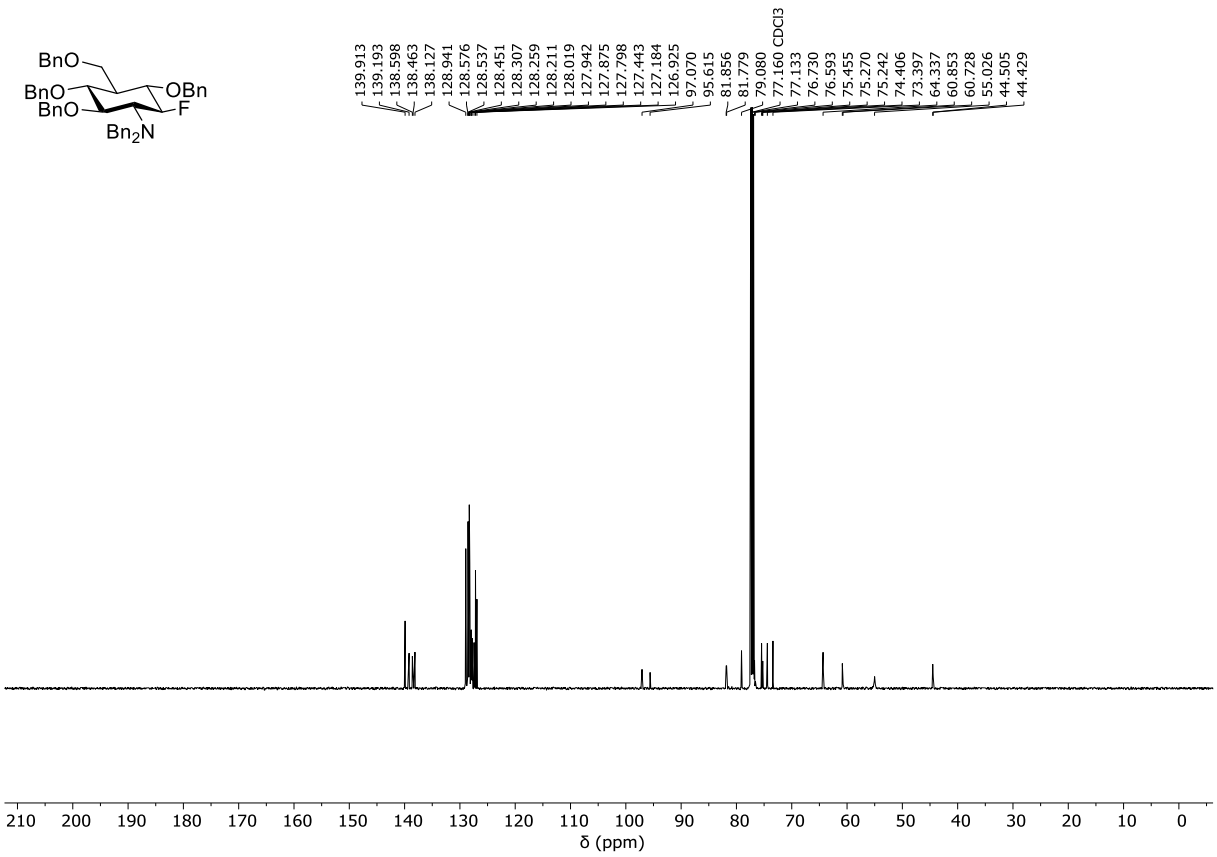
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **179**



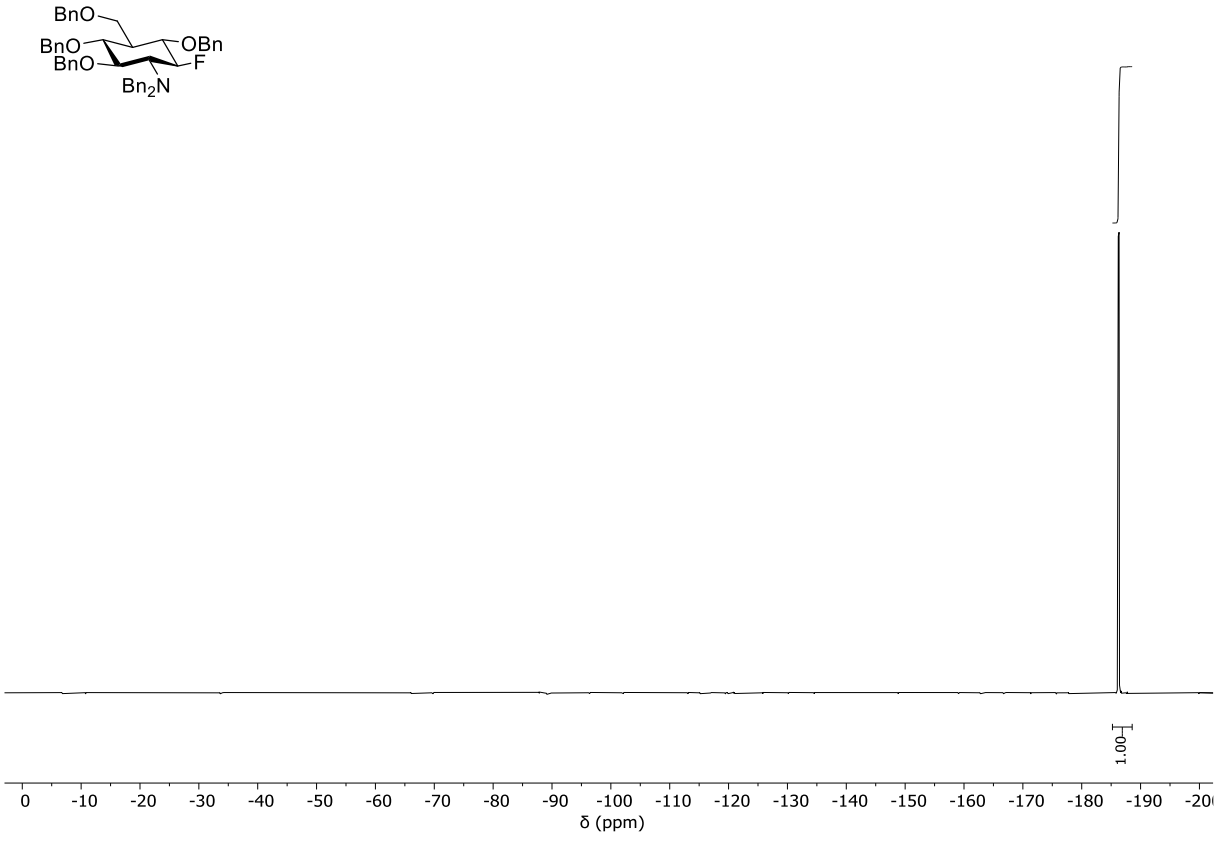
^{19}F NMR spectrum (377 MHz, $CDCl_3$) of **180**



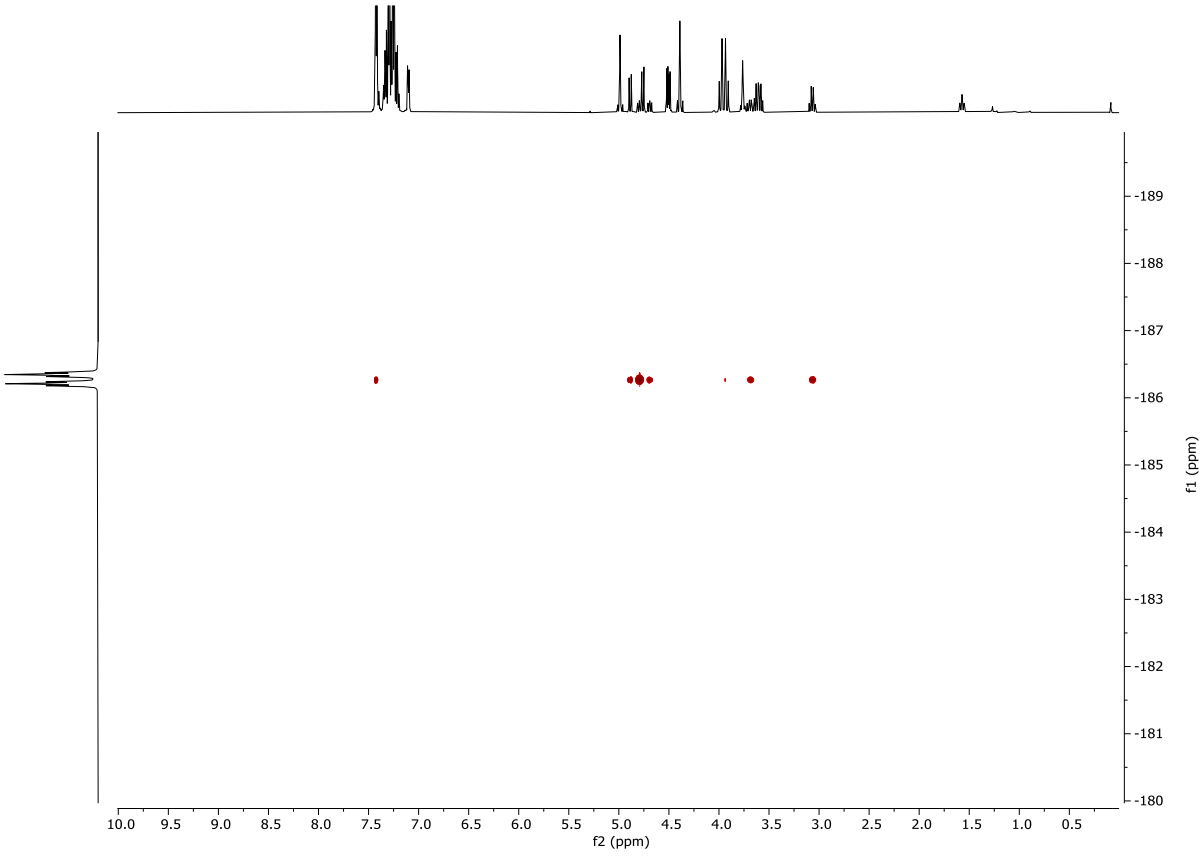
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **180**



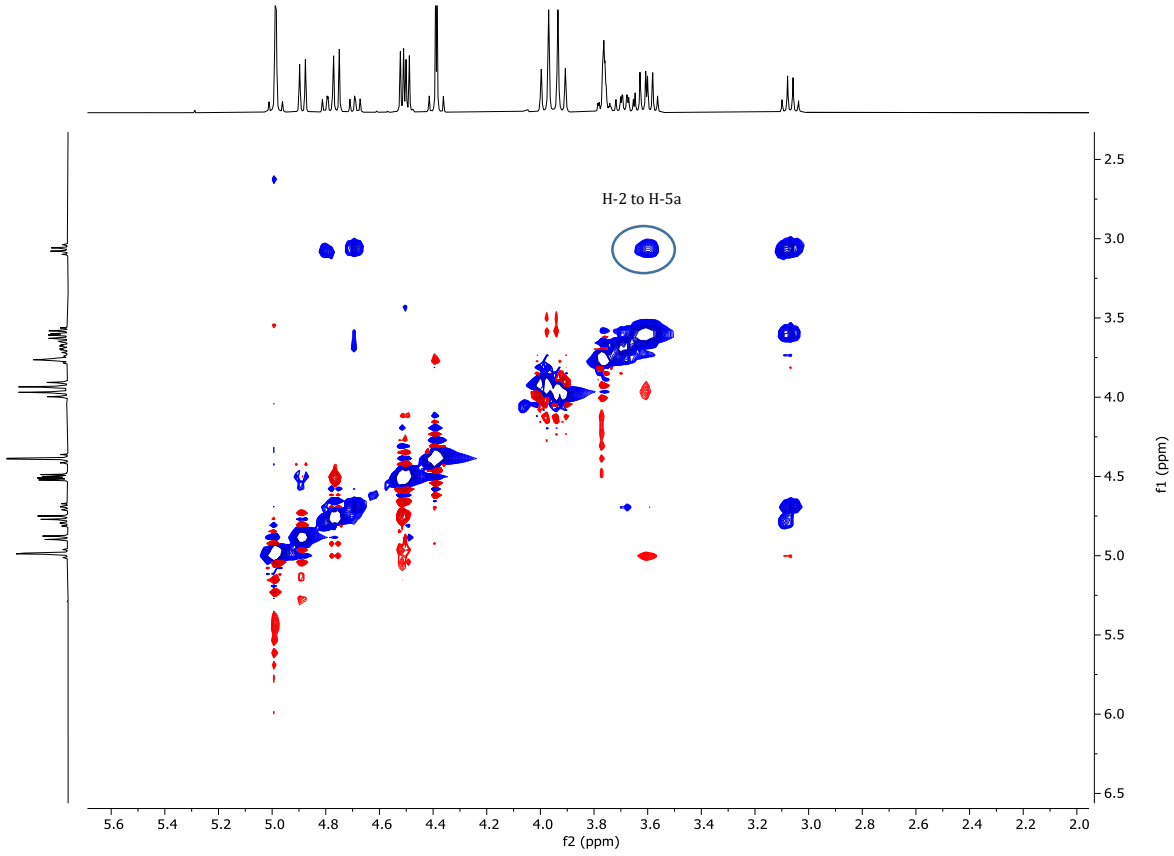
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **180**



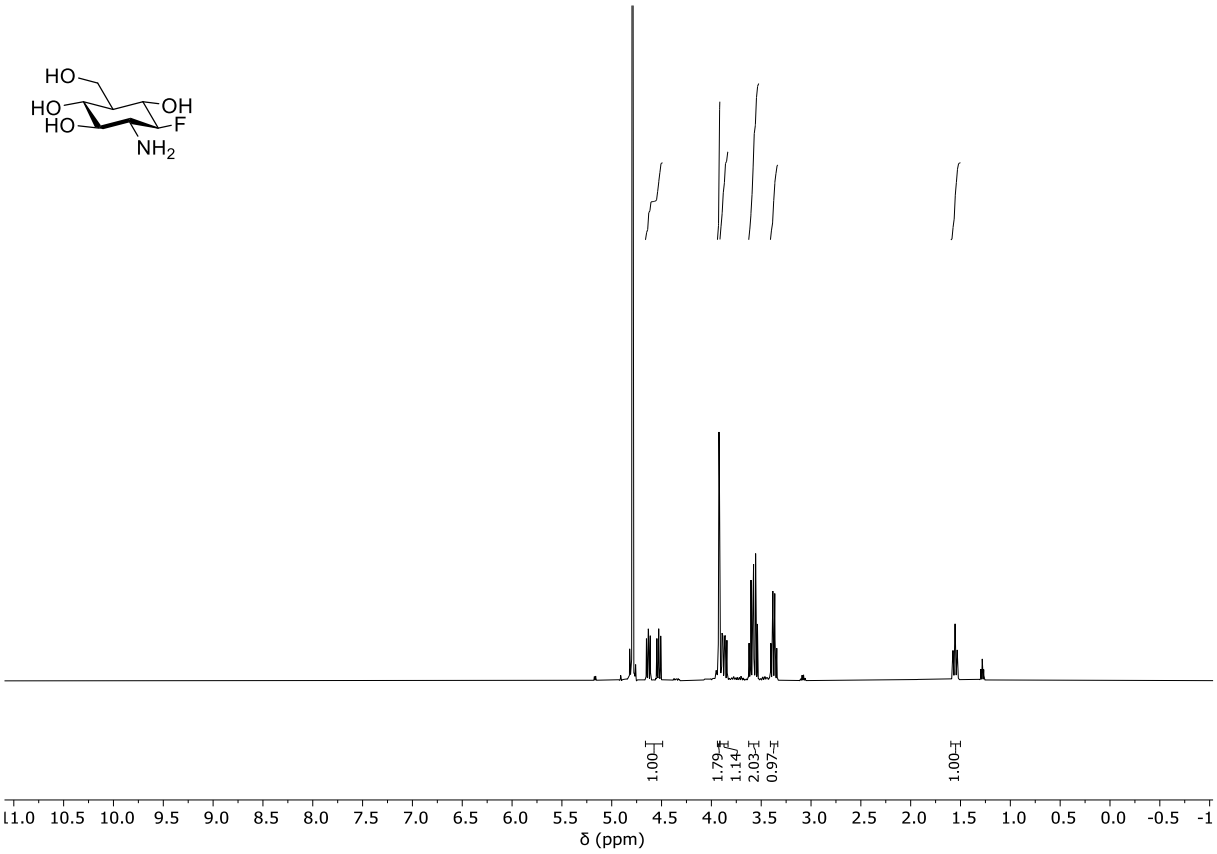
^{19}F NMR spectrum (377 MHz, $CDCl_3$) of **180**



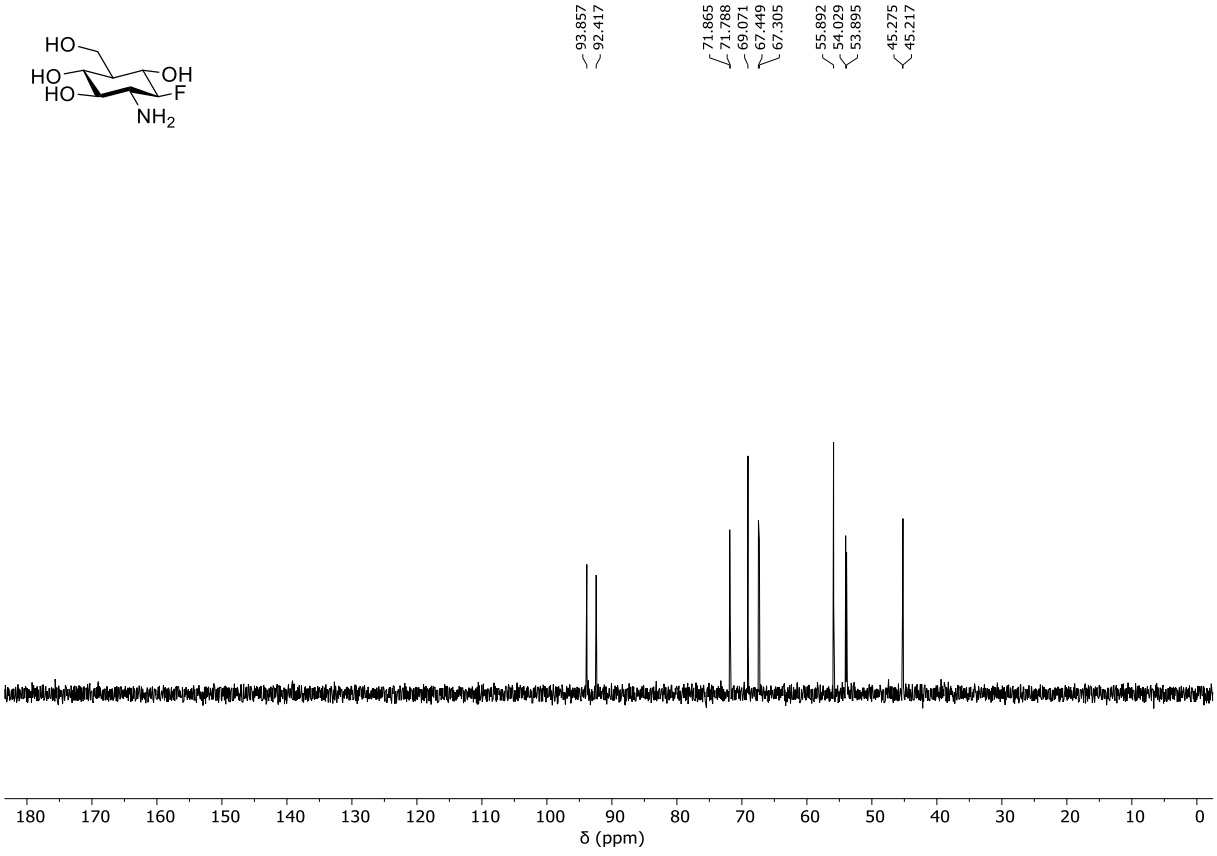
^{19}F-^{1}H HOESY spectrum of **180**



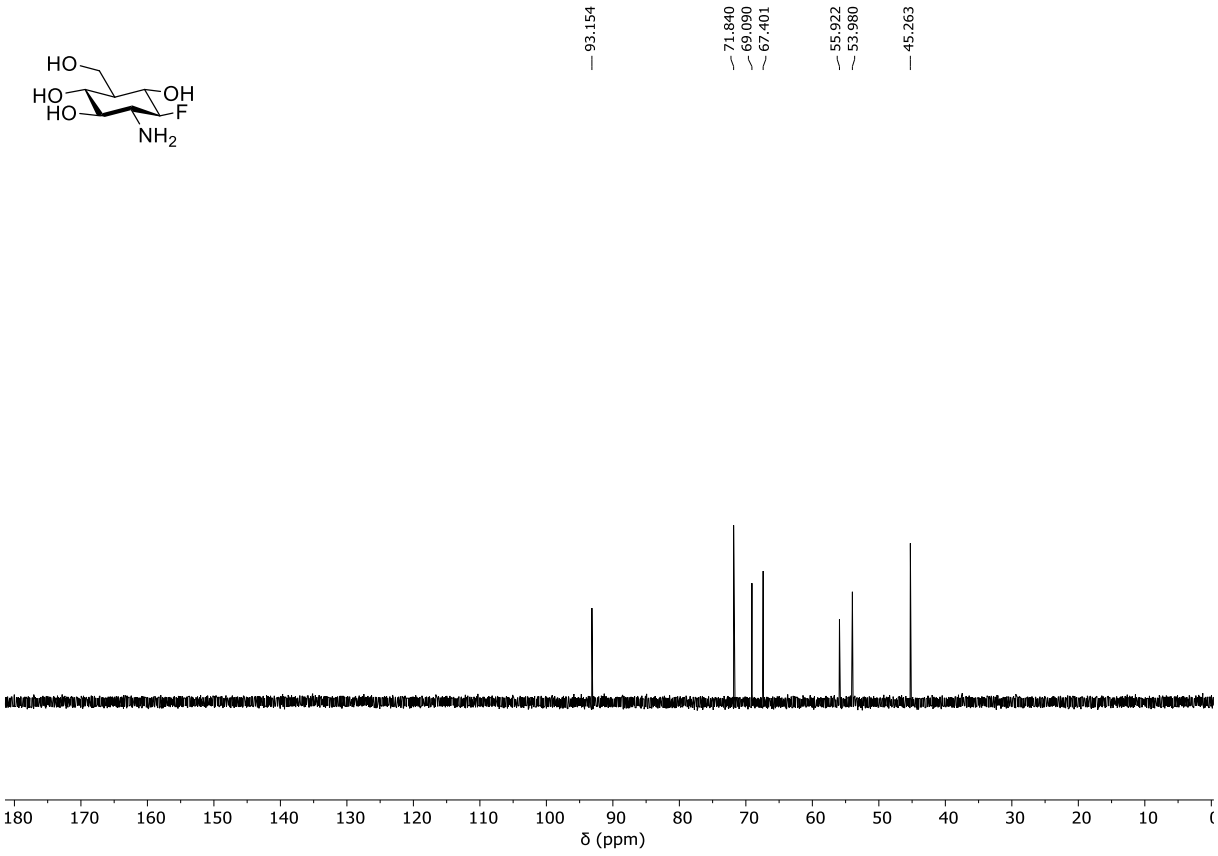
ROESY spectrum of **180**



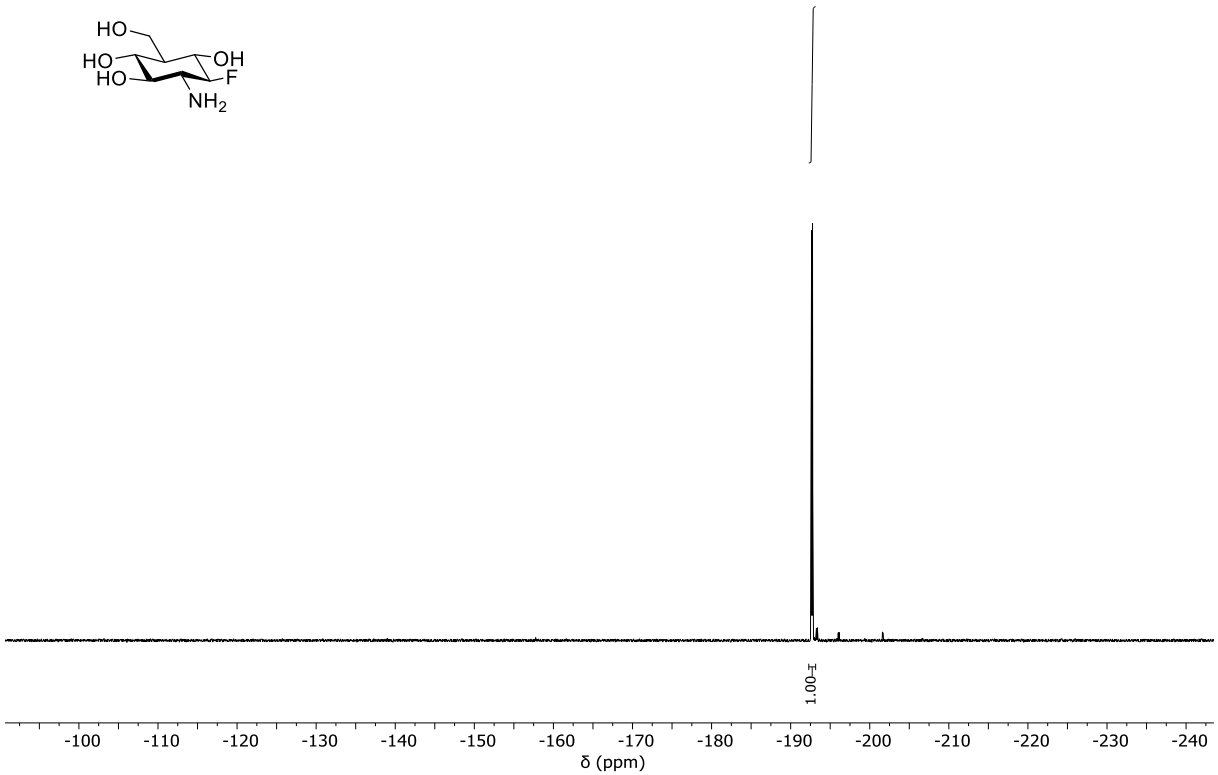
^{1}H NMR spectrum (500 MHz, D_2O) of **182**



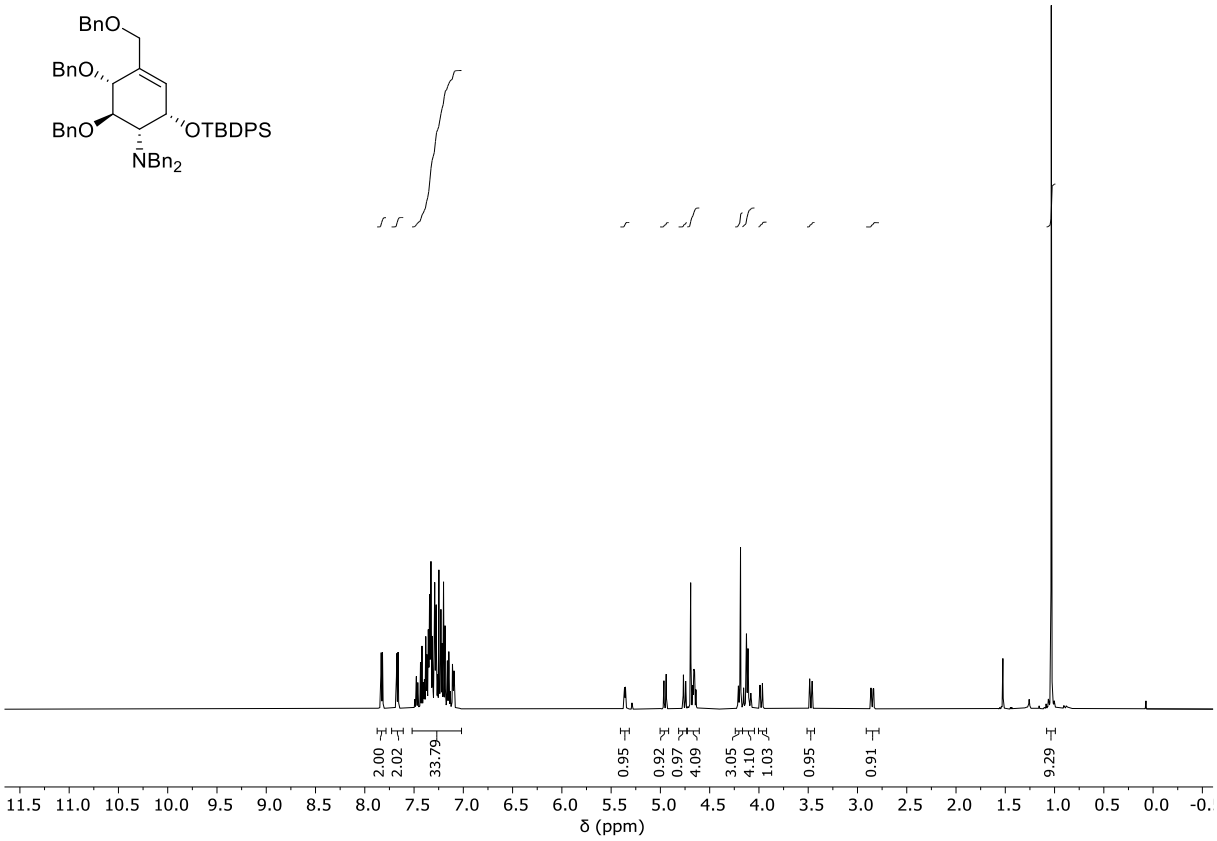
^{13}C NMR spectrum (151 MHz, D_2O, ^{19}F-coupled) of **182**



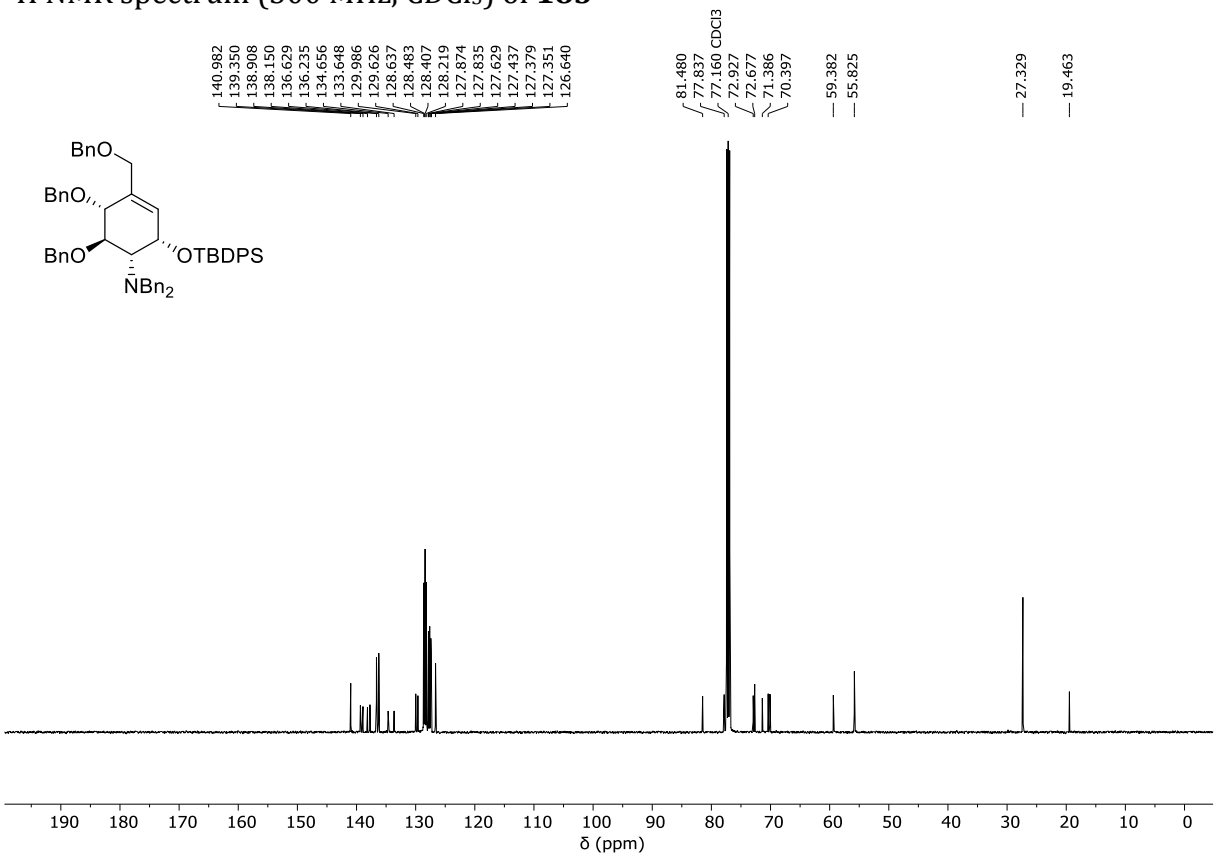
^{13}C NMR spectrum (126 MHz, D_2O, ^{19}F-decoupled) of **182**



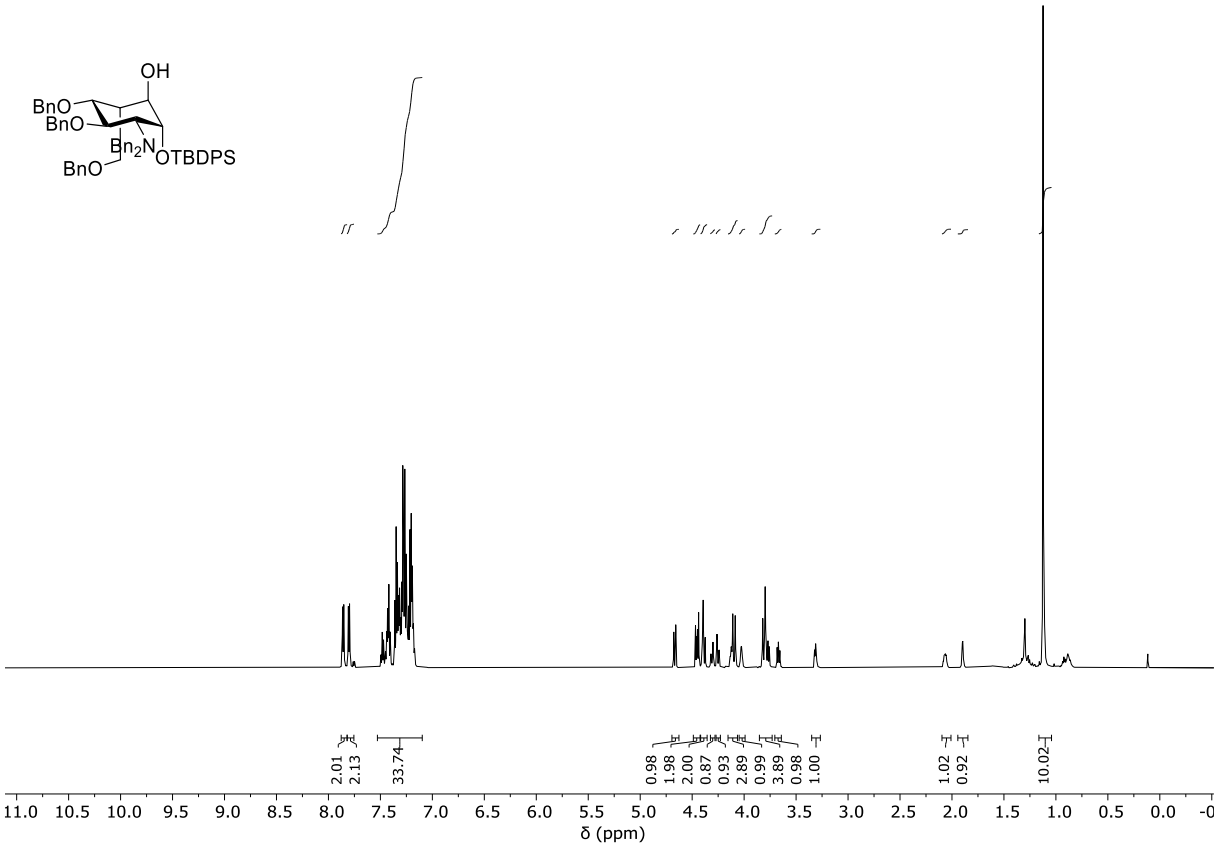
^{19}F NMR spectrum (471 MHz, D_2O) of **182**



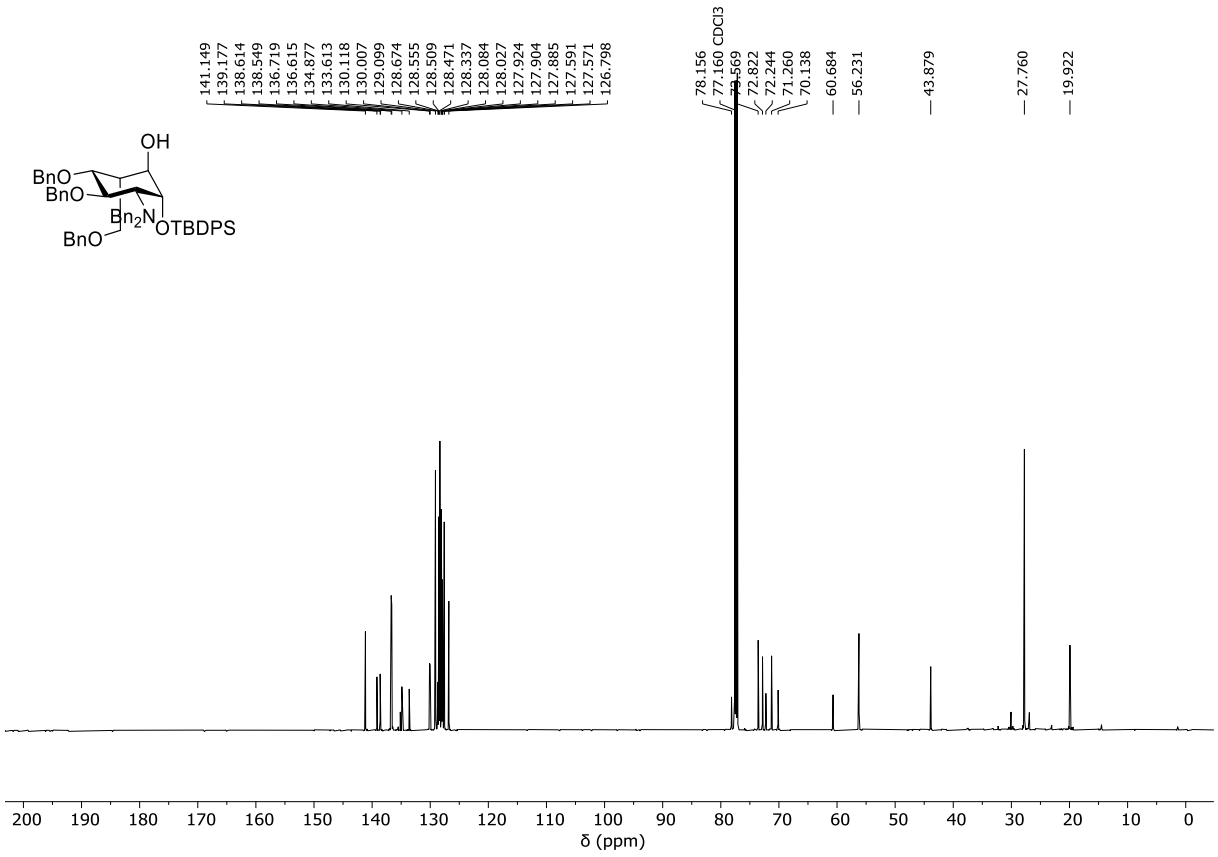
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **183**



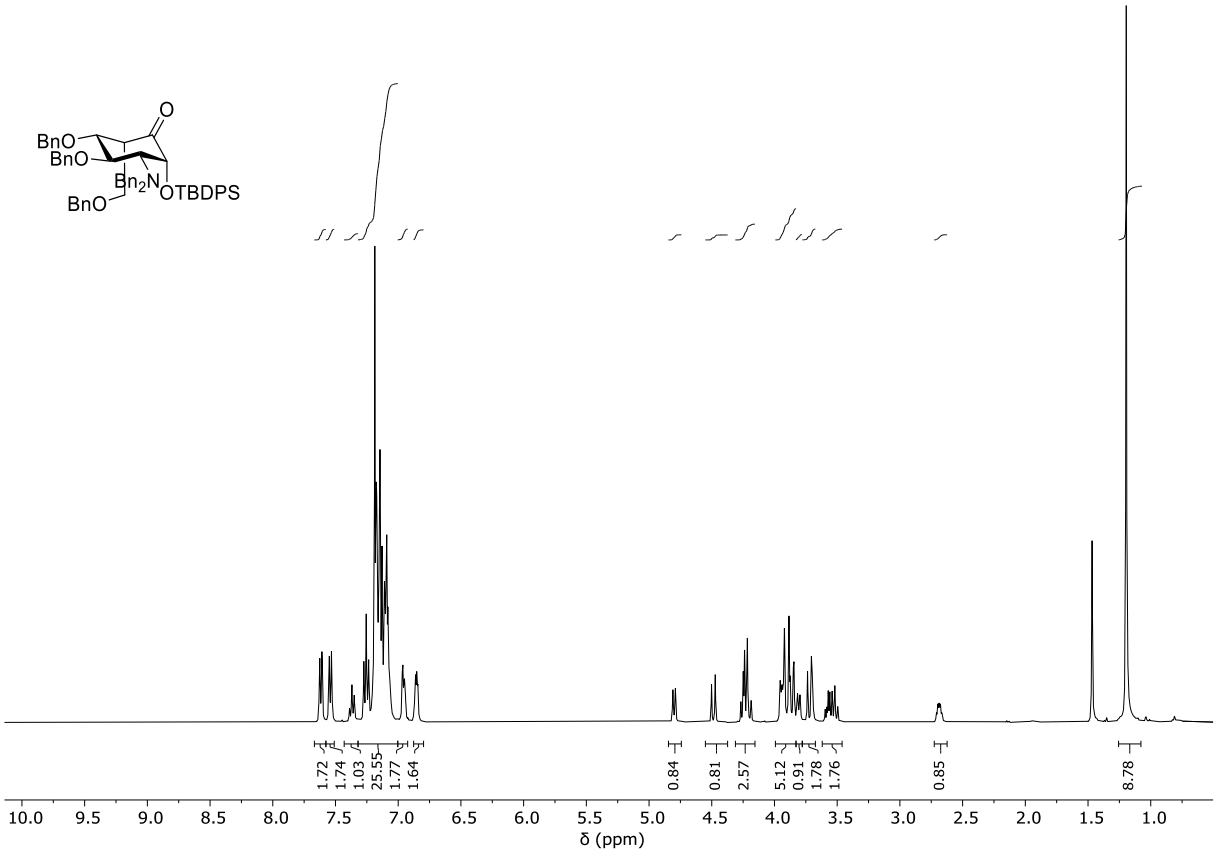
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **183**



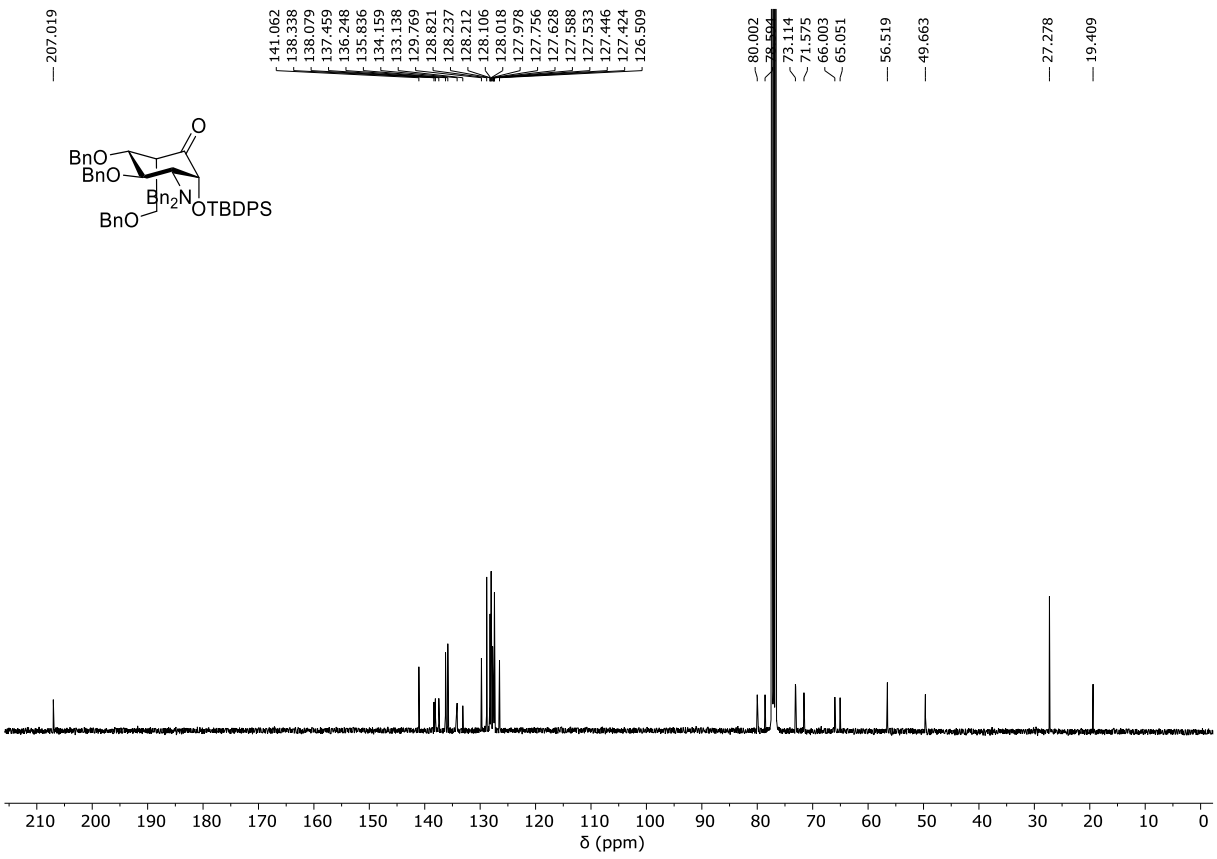
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **184**



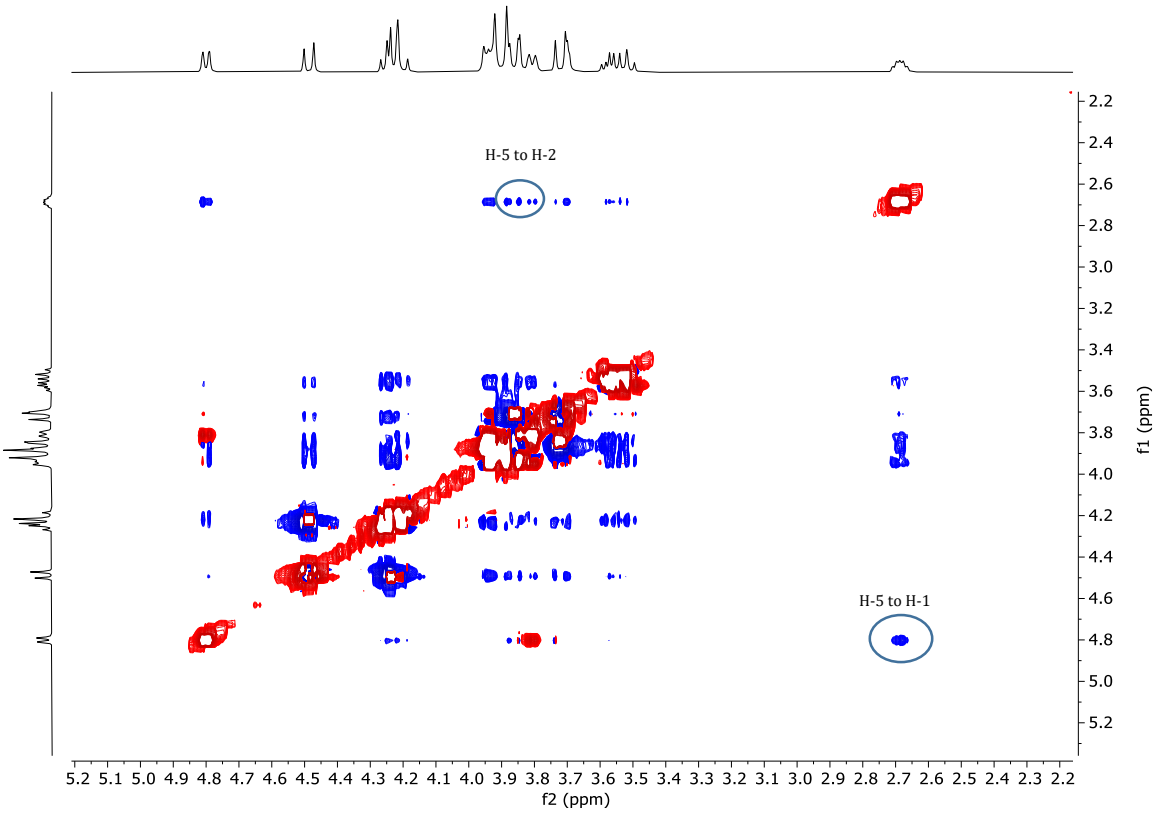
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **184**



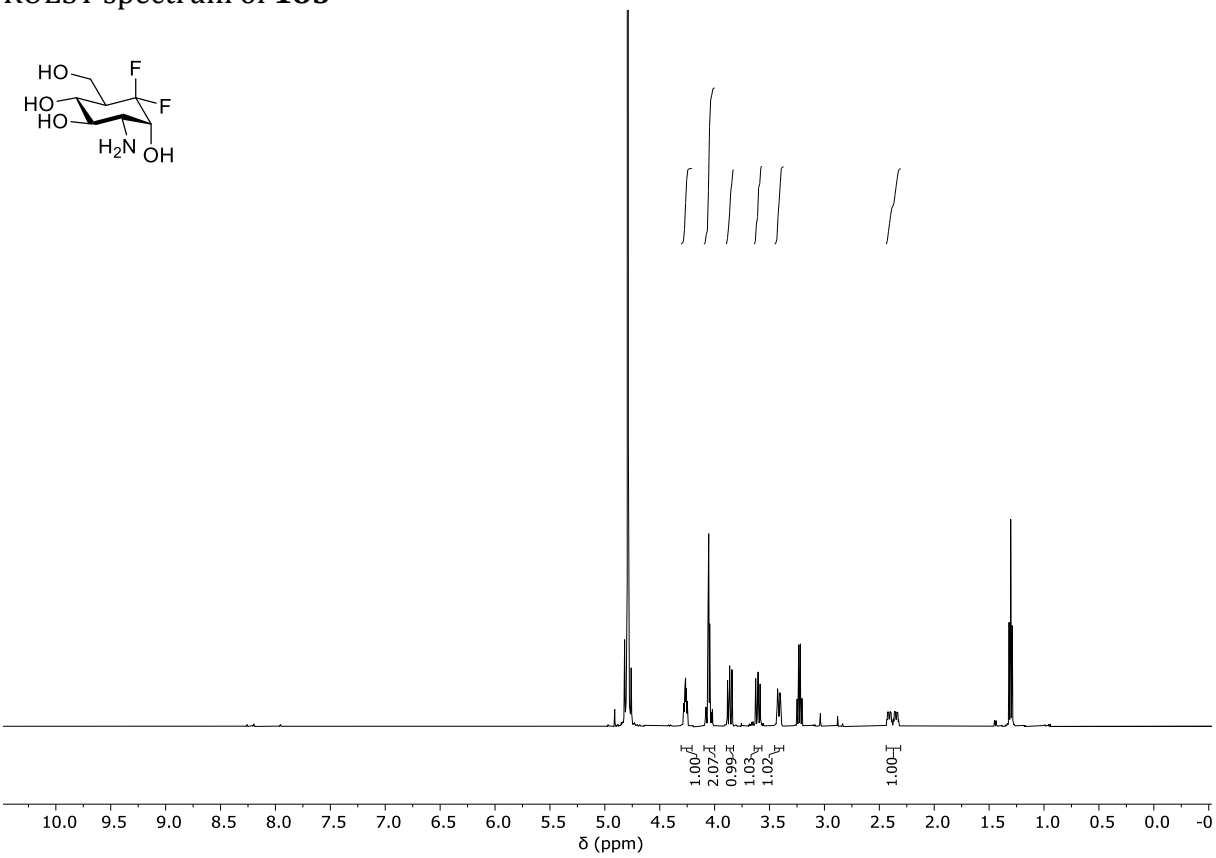
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **185**



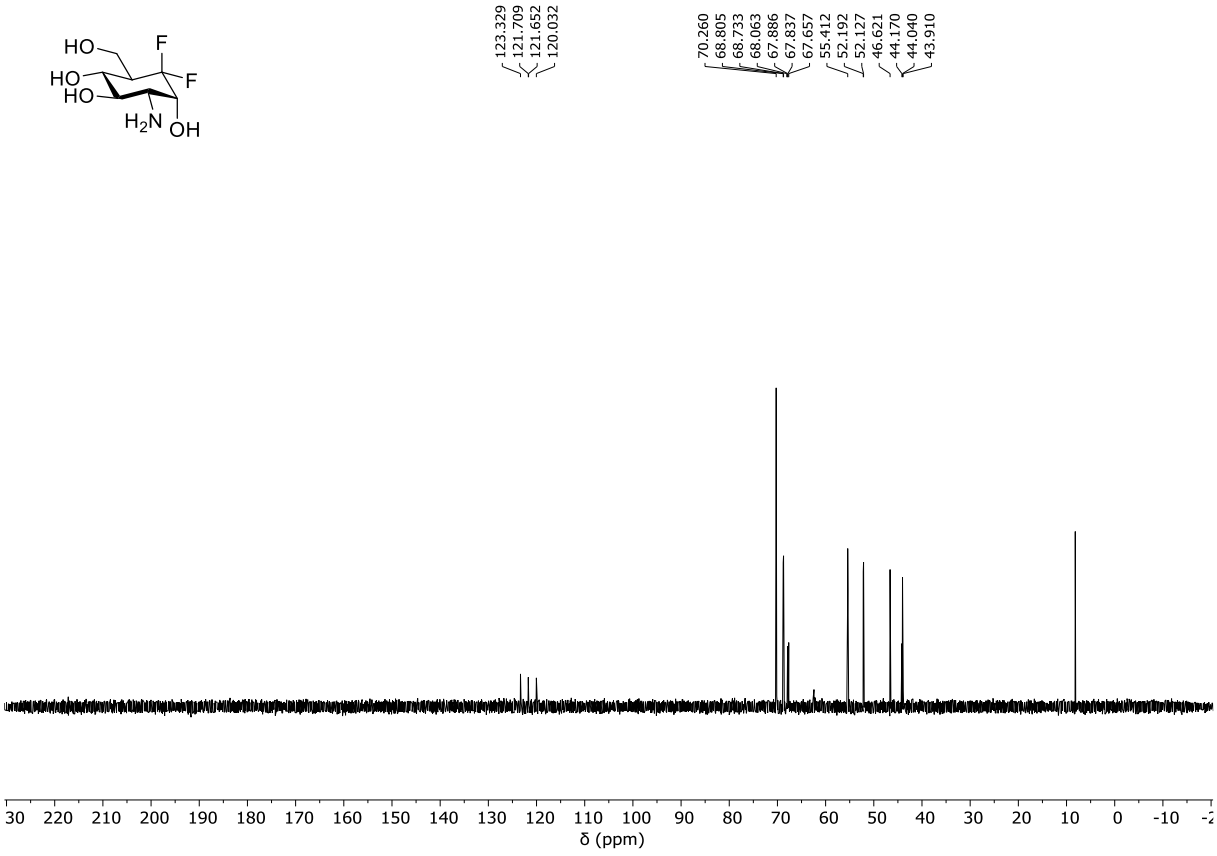
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **185**



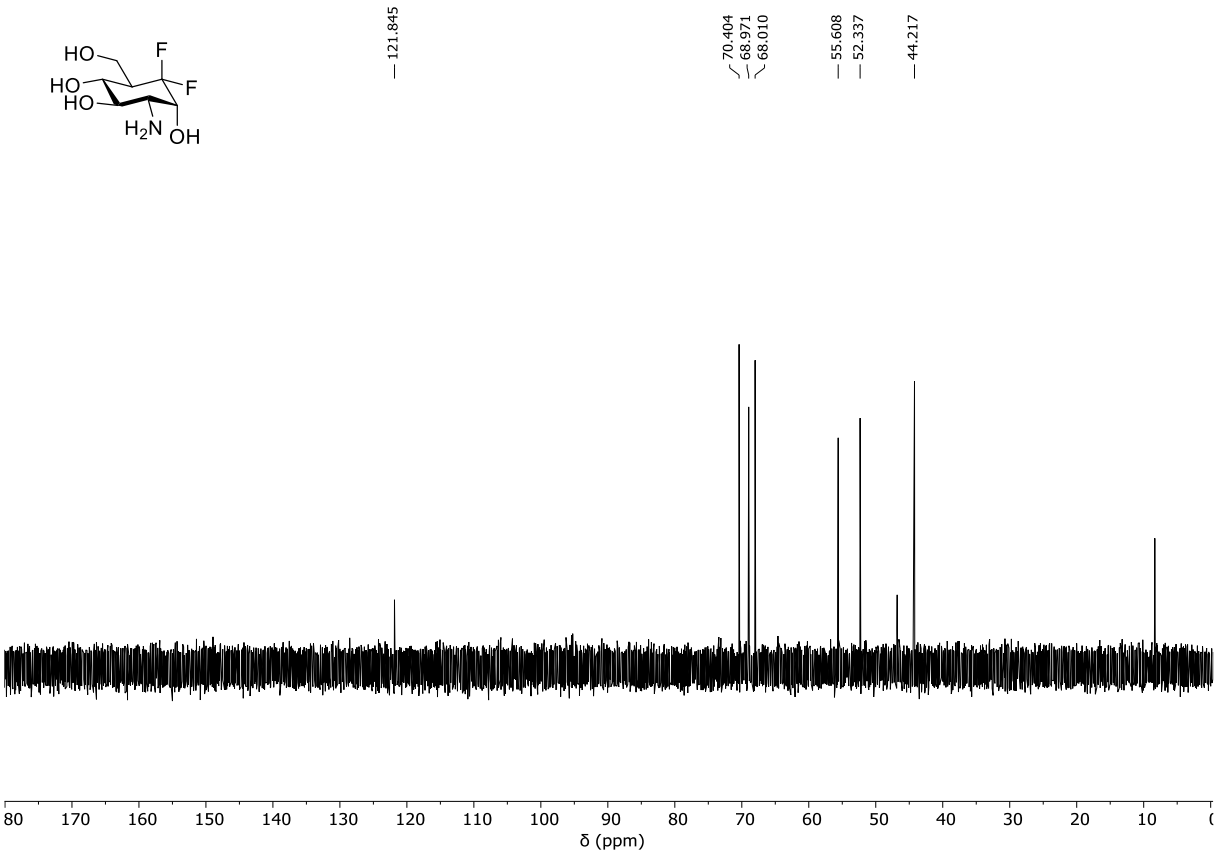
ROESY spectrum of **185**



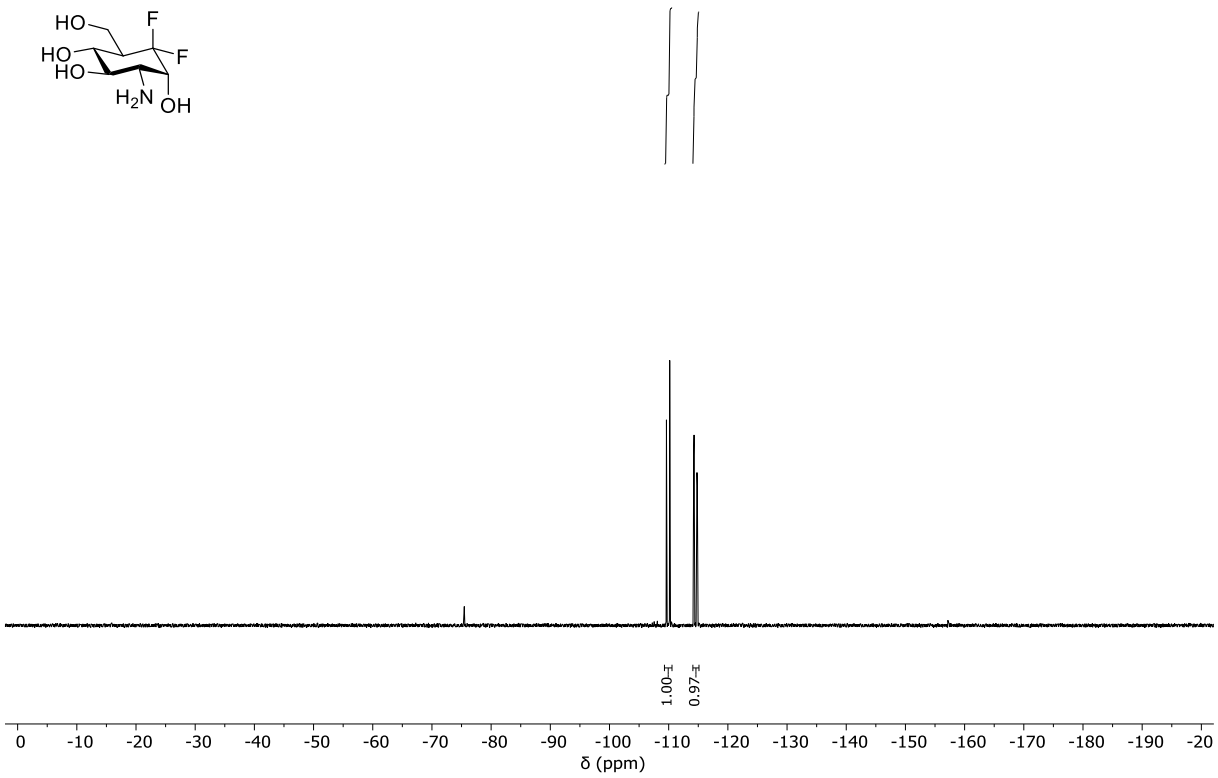
^{1}H NMR spectrum (500 MHz, D_2O) of **187**



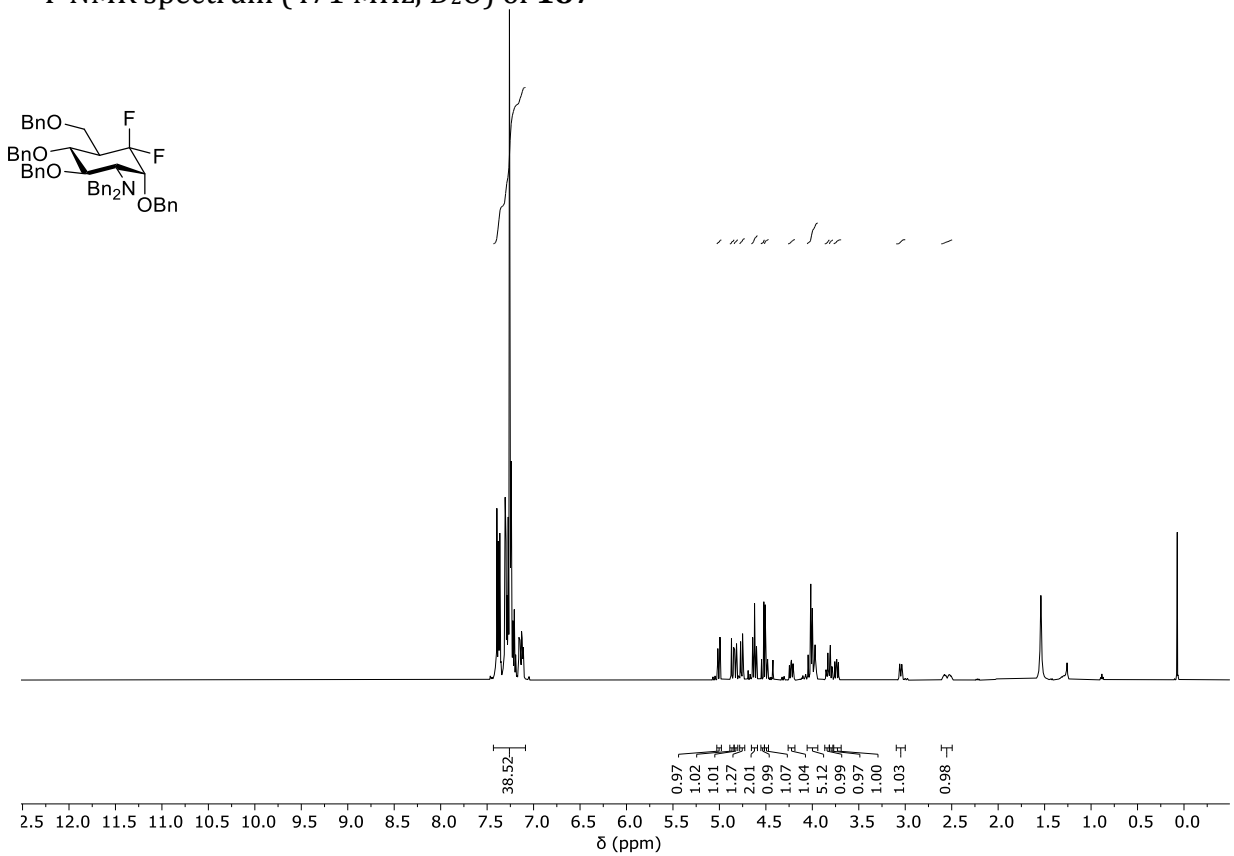
^{13}C NMR spectrum (151 MHz, D_2O, ^{19}F-coupled) of **187**



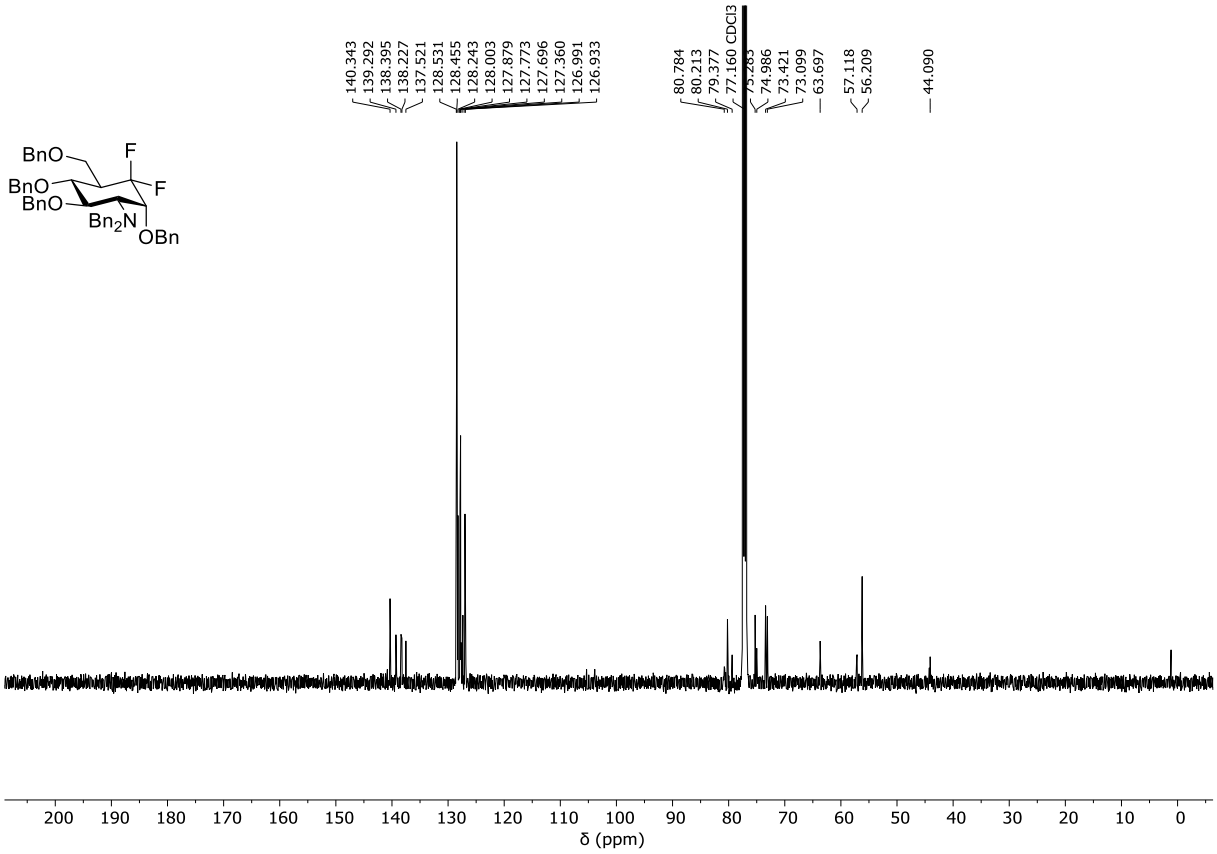
^{13}C NMR spectrum (126 MHz, D_2O, ^{19}F-decoupled) of **187**



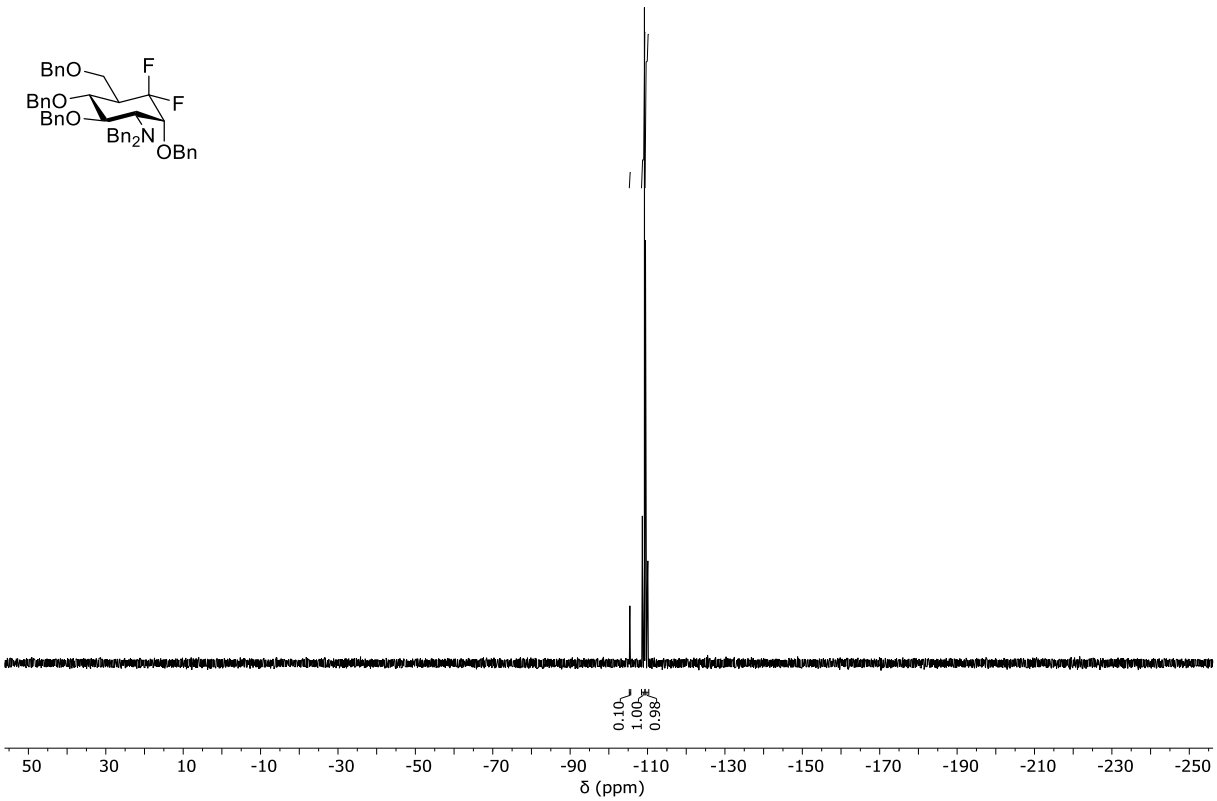
^{19}F NMR spectrum (471 MHz, D_2O) of **187**



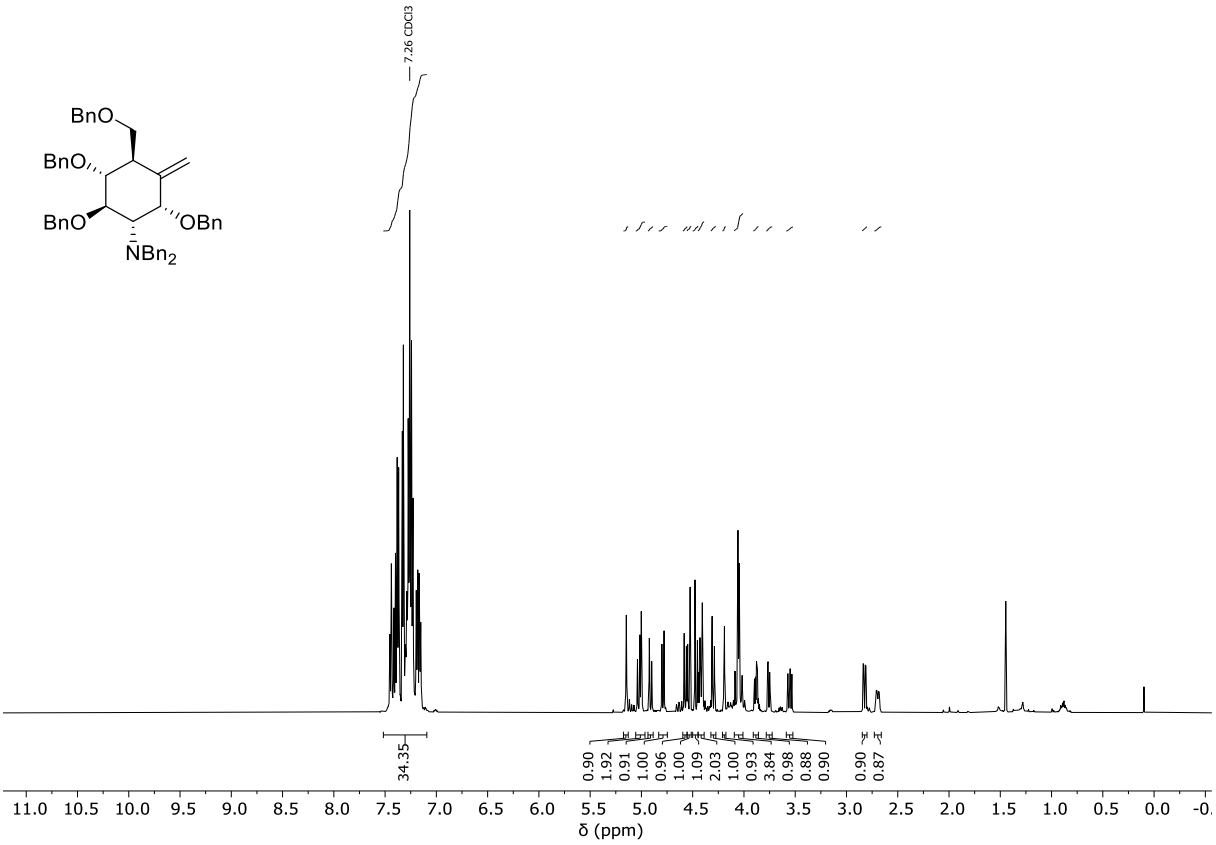
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **188**



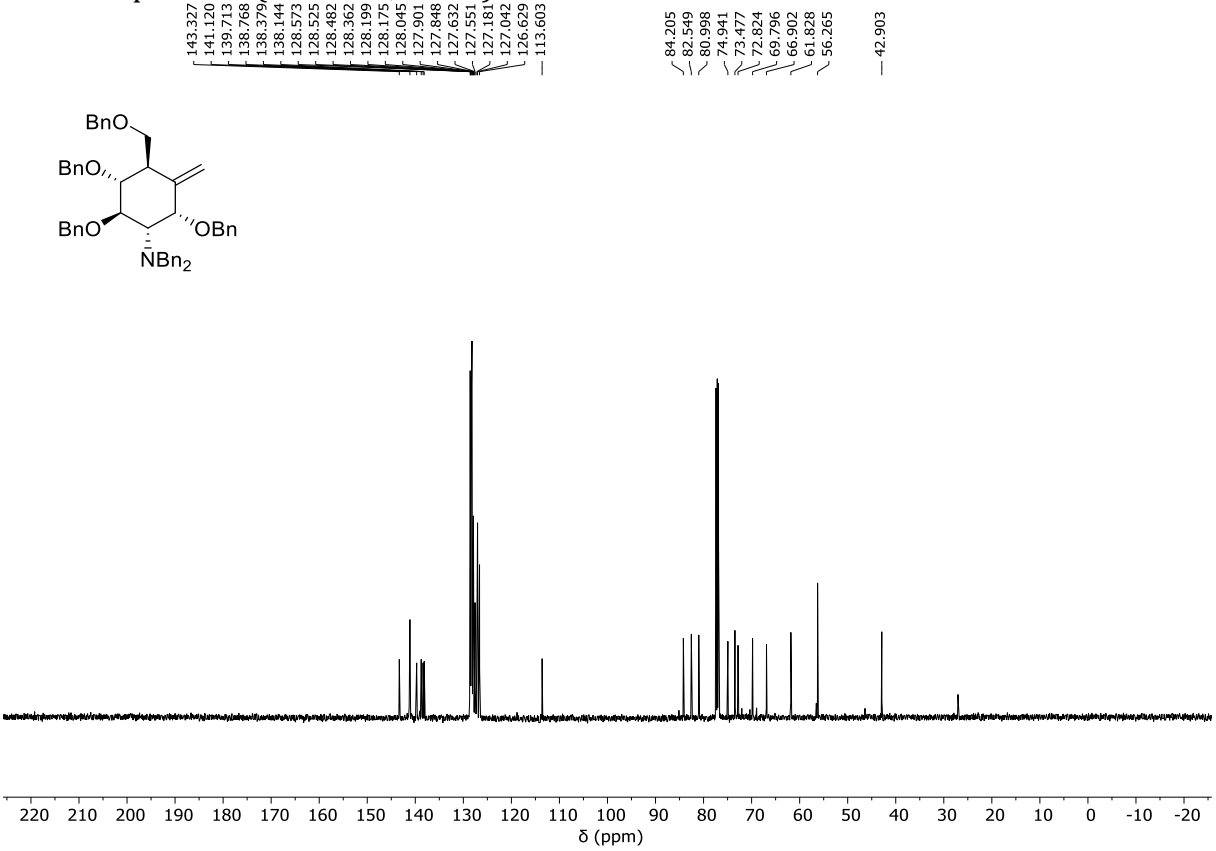
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **188**



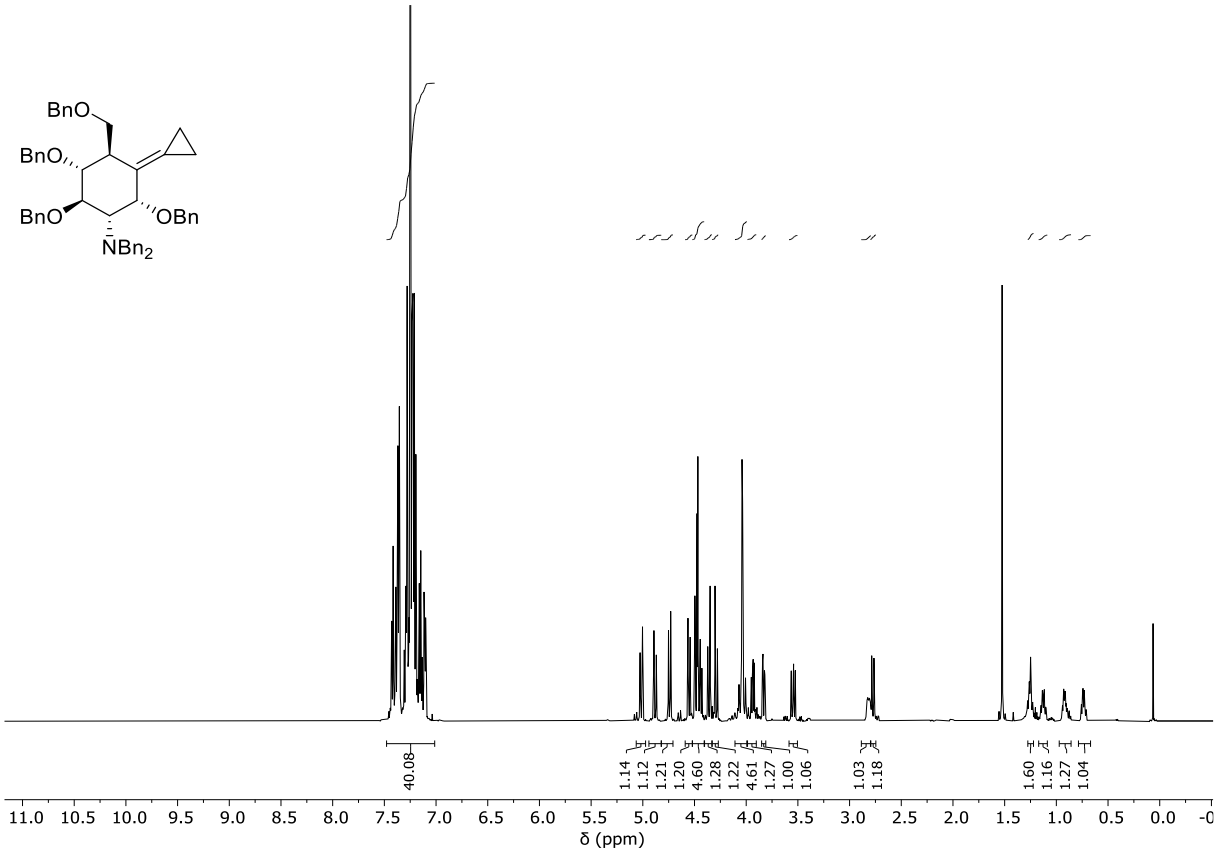
^{19}F NMR spectrum (471 MHz, $CDCl_3$) of **188**



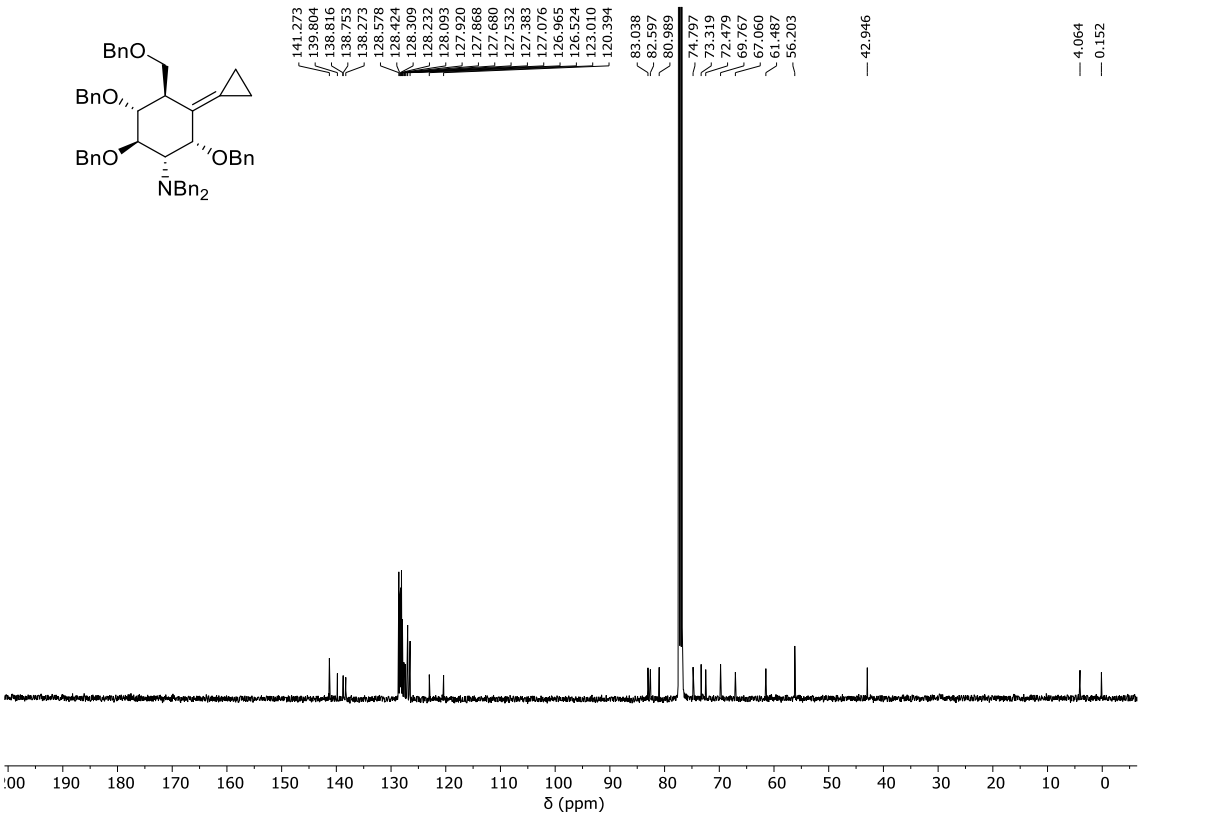
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **190**



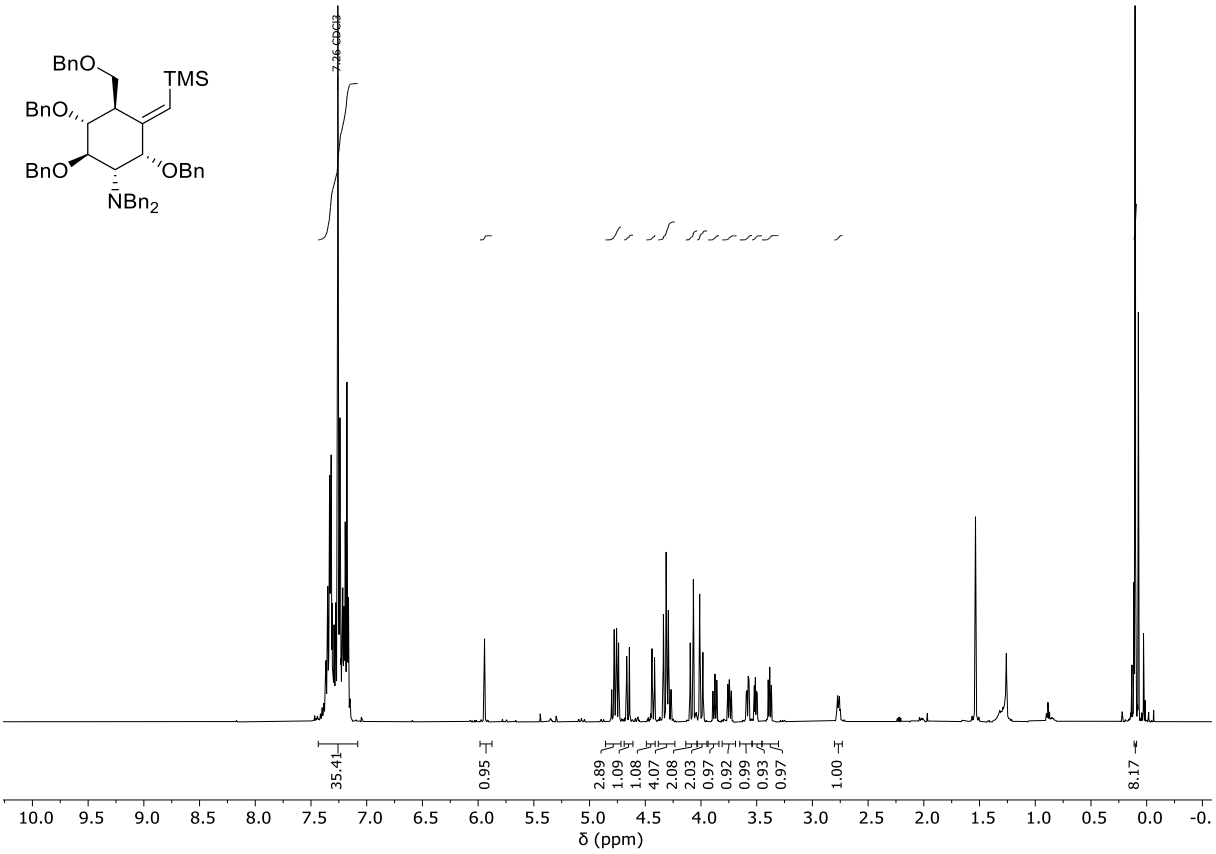
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **190**



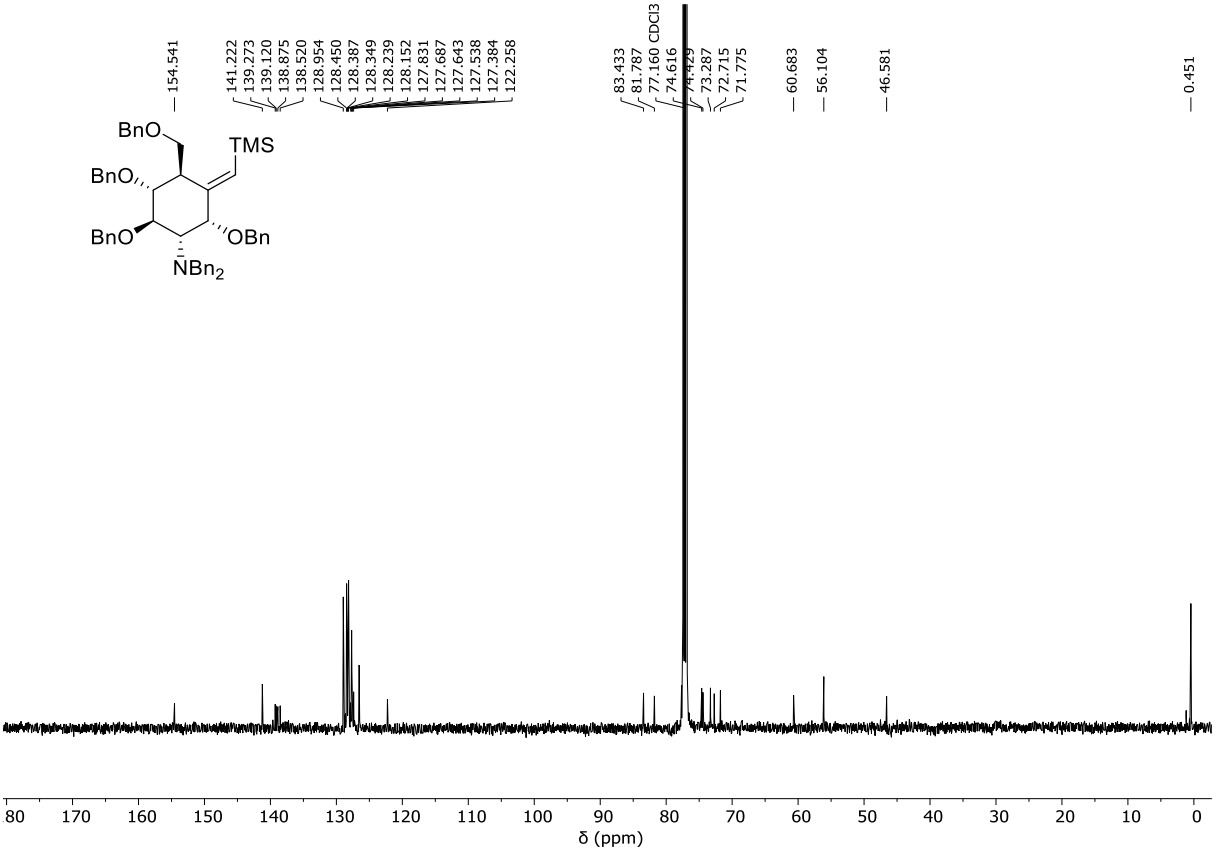
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **194**



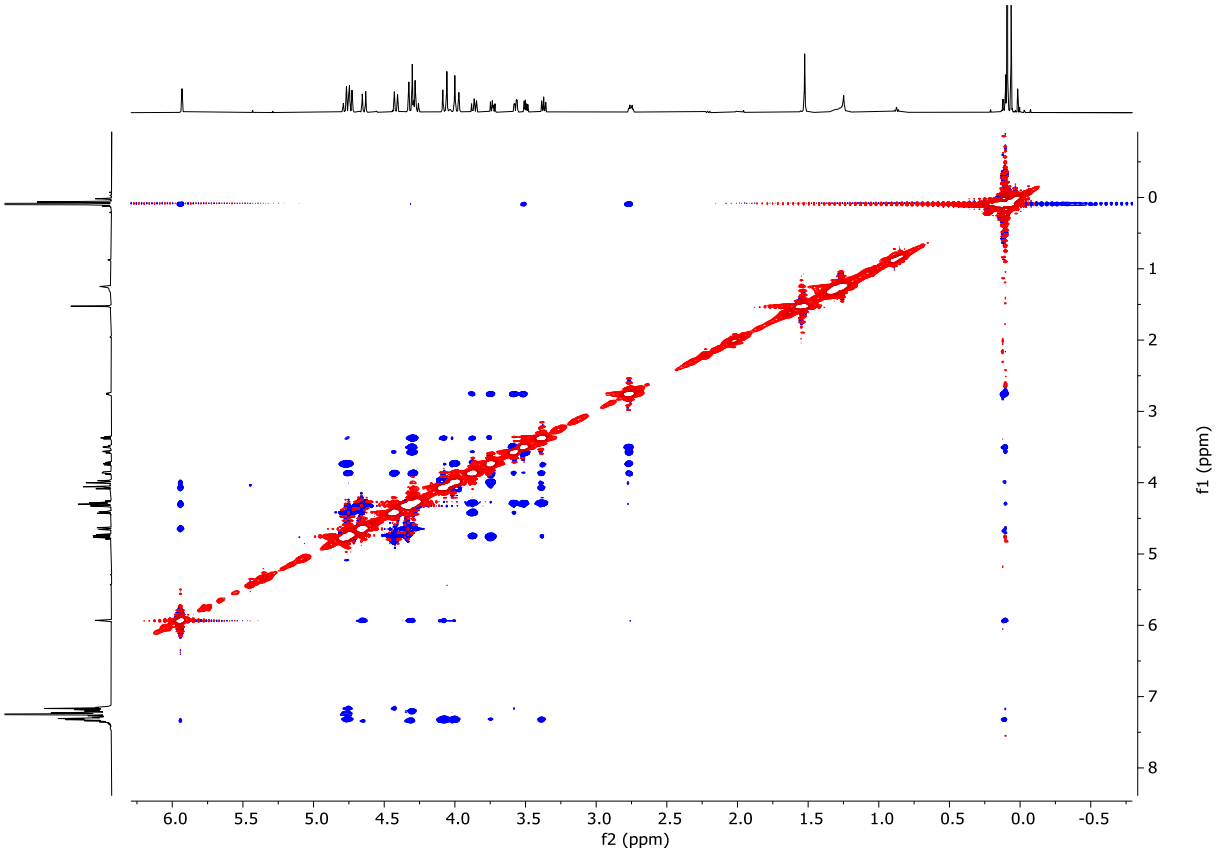
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **194**



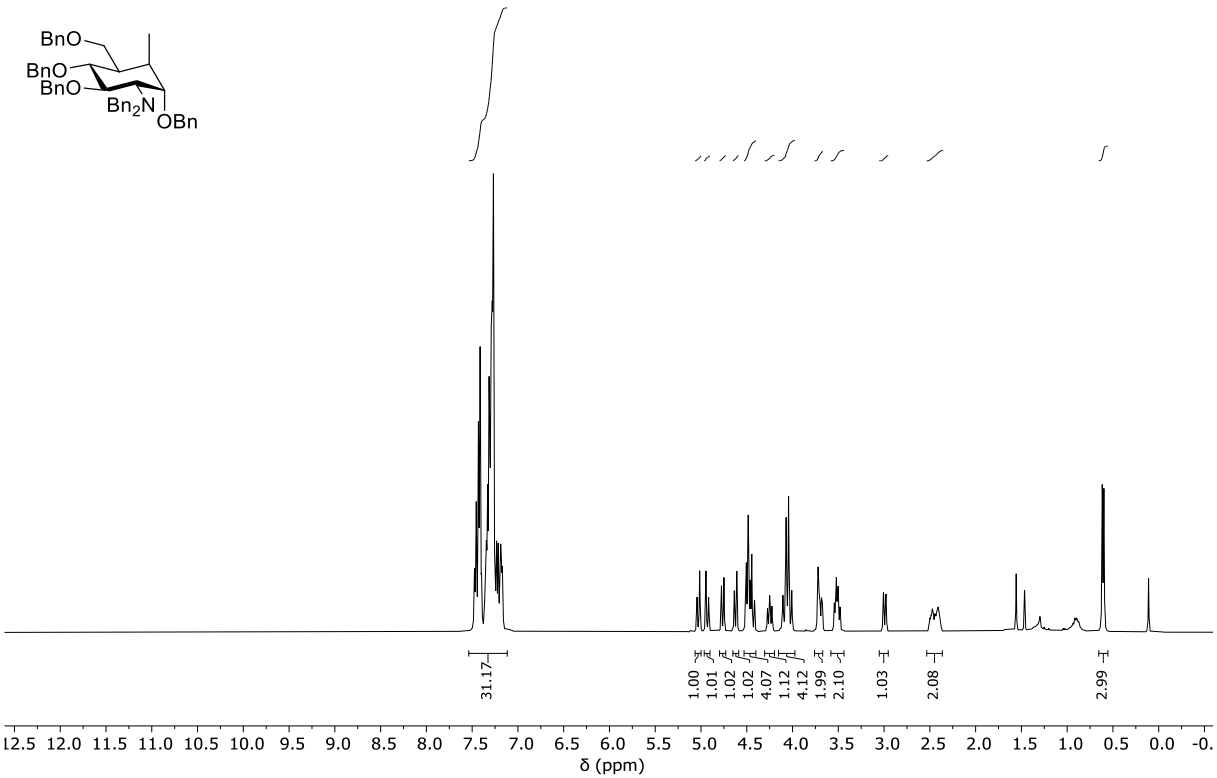
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **195**



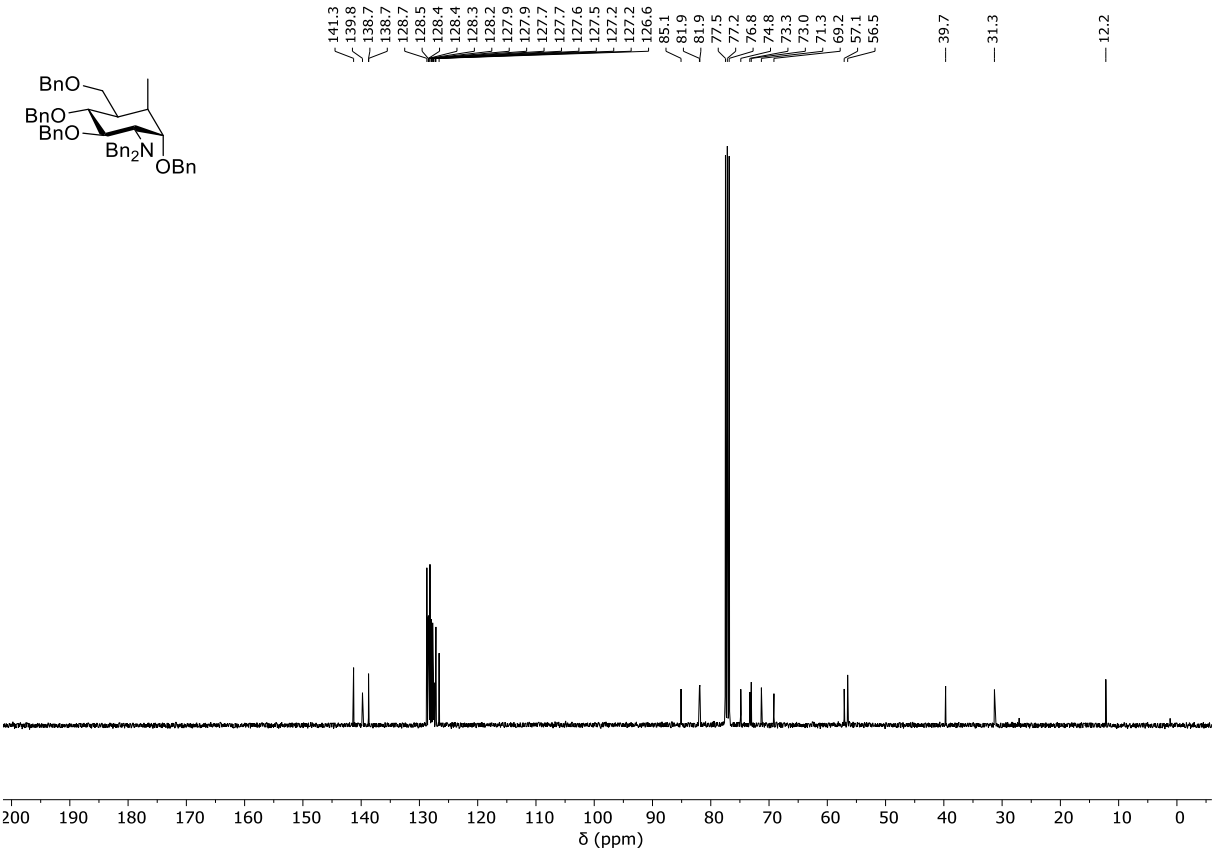
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **195**



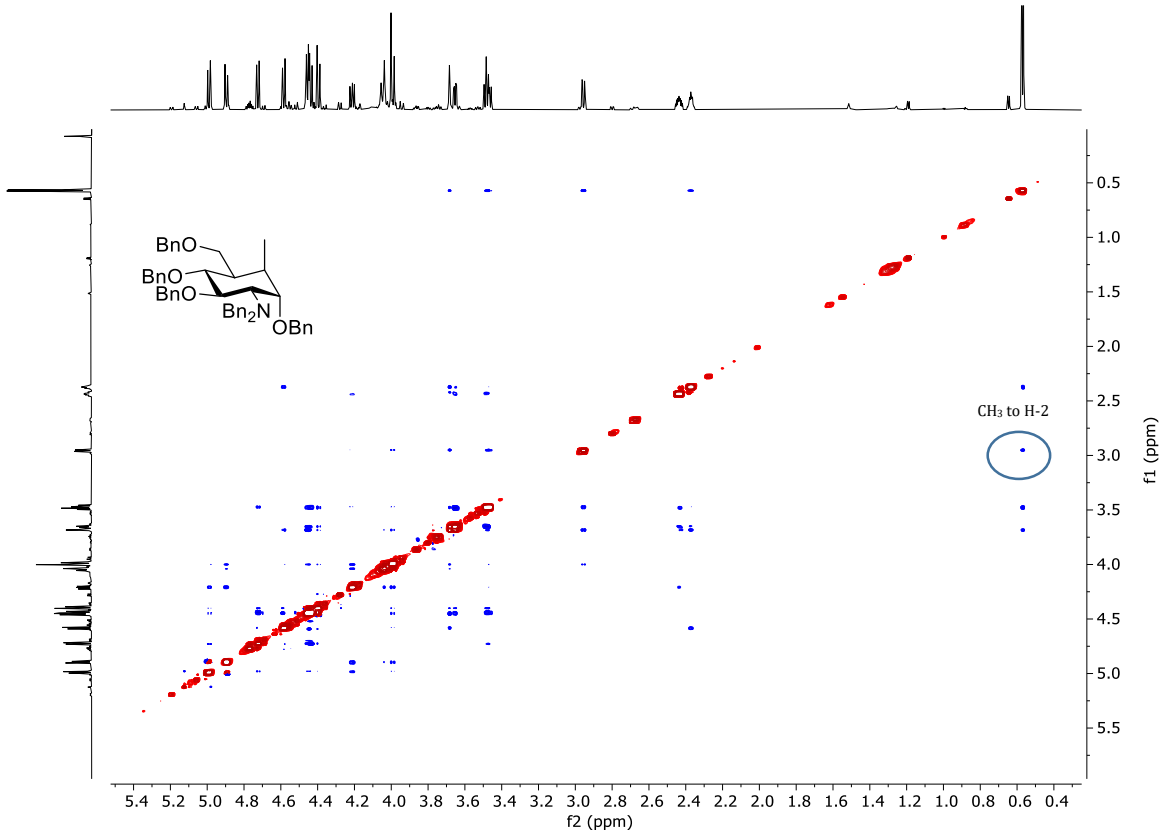
NOESY spectrum of **195**



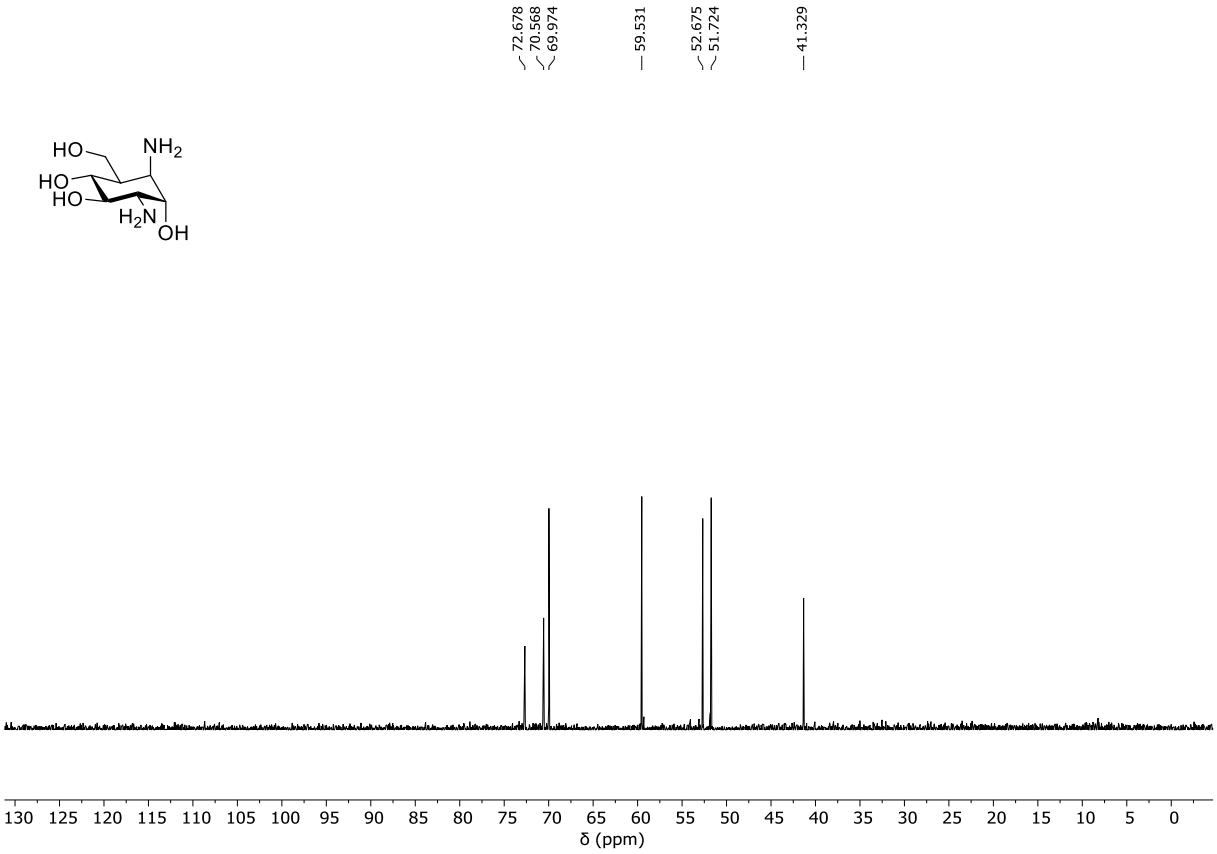
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **196**



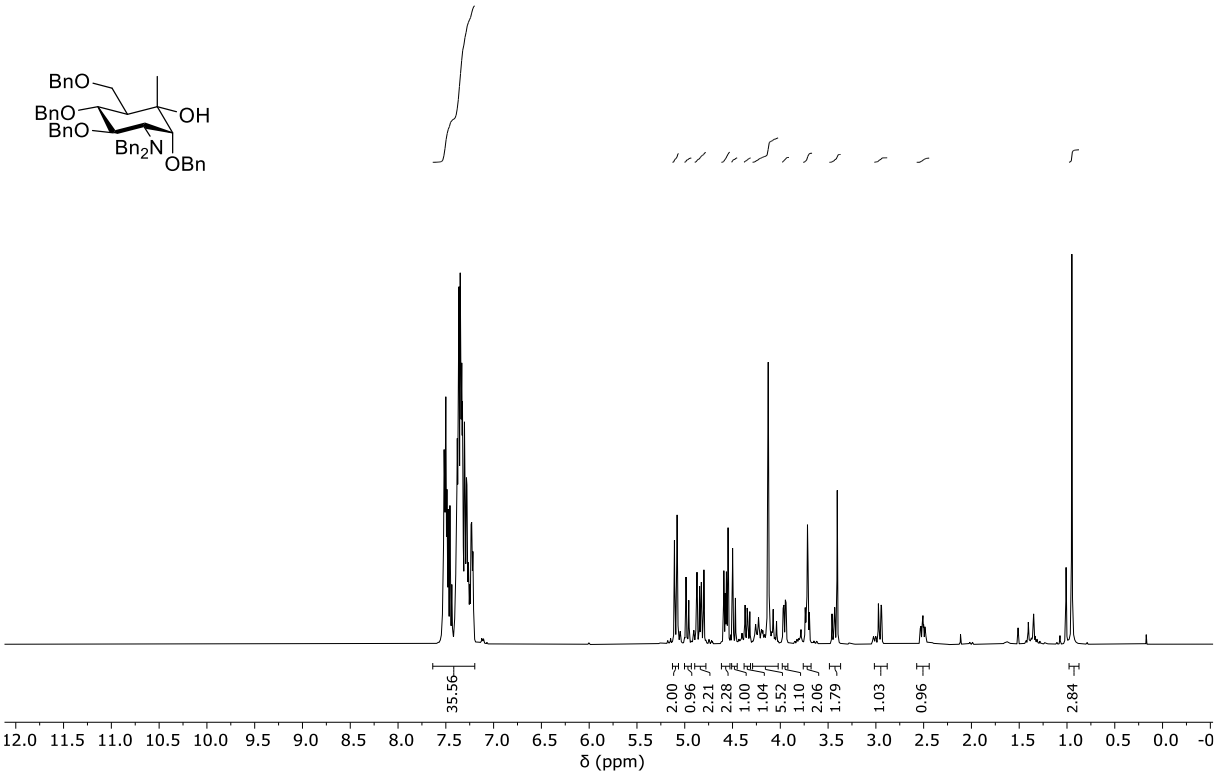
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **196**



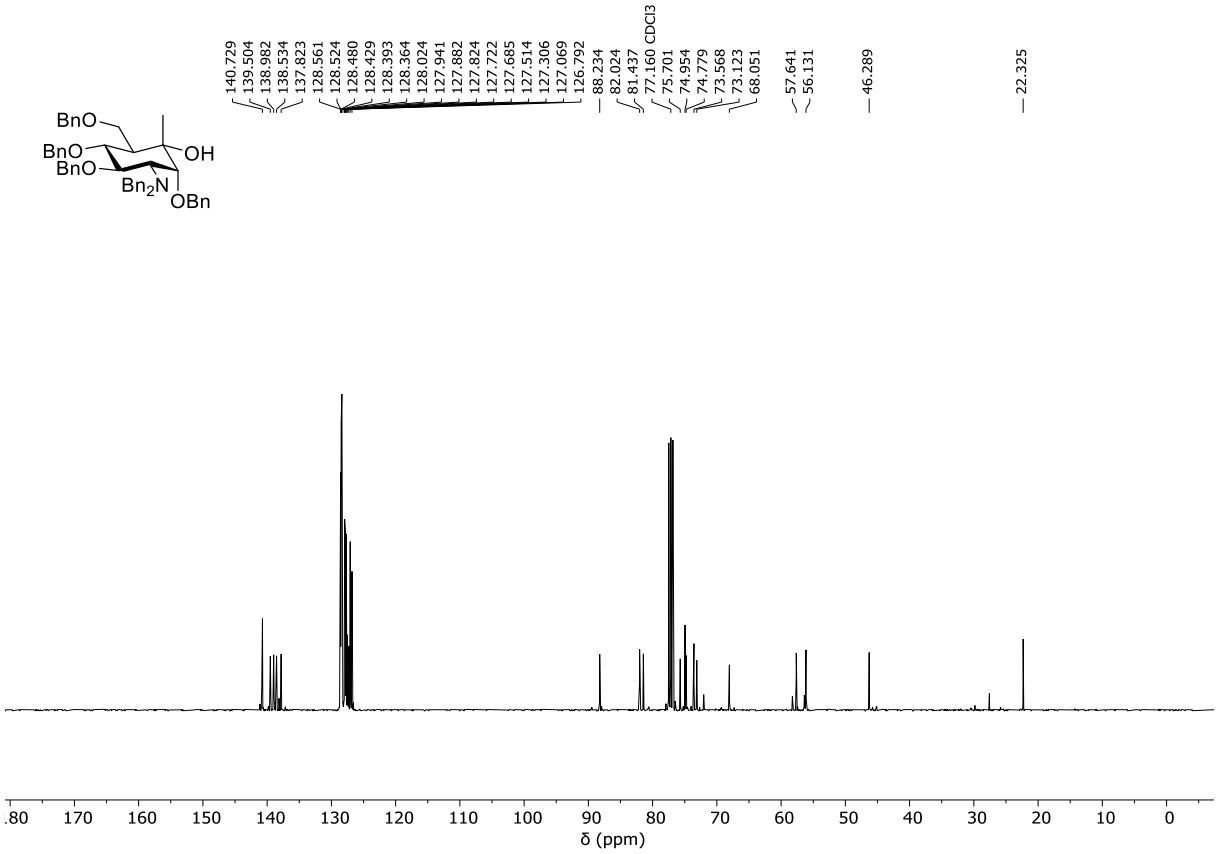
NOESY spectrum of **196**



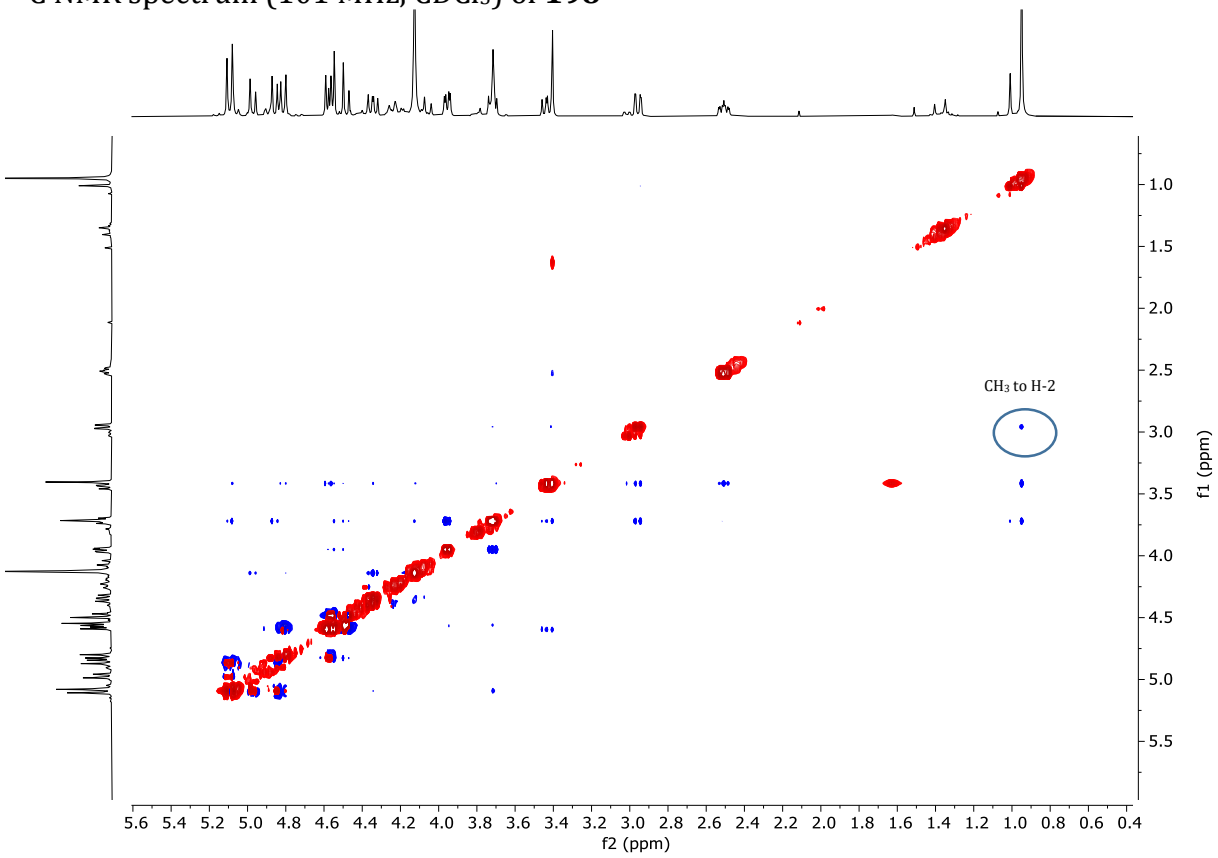
^{13}C NMR spectrum (151 MHz, D_2O) of **176**



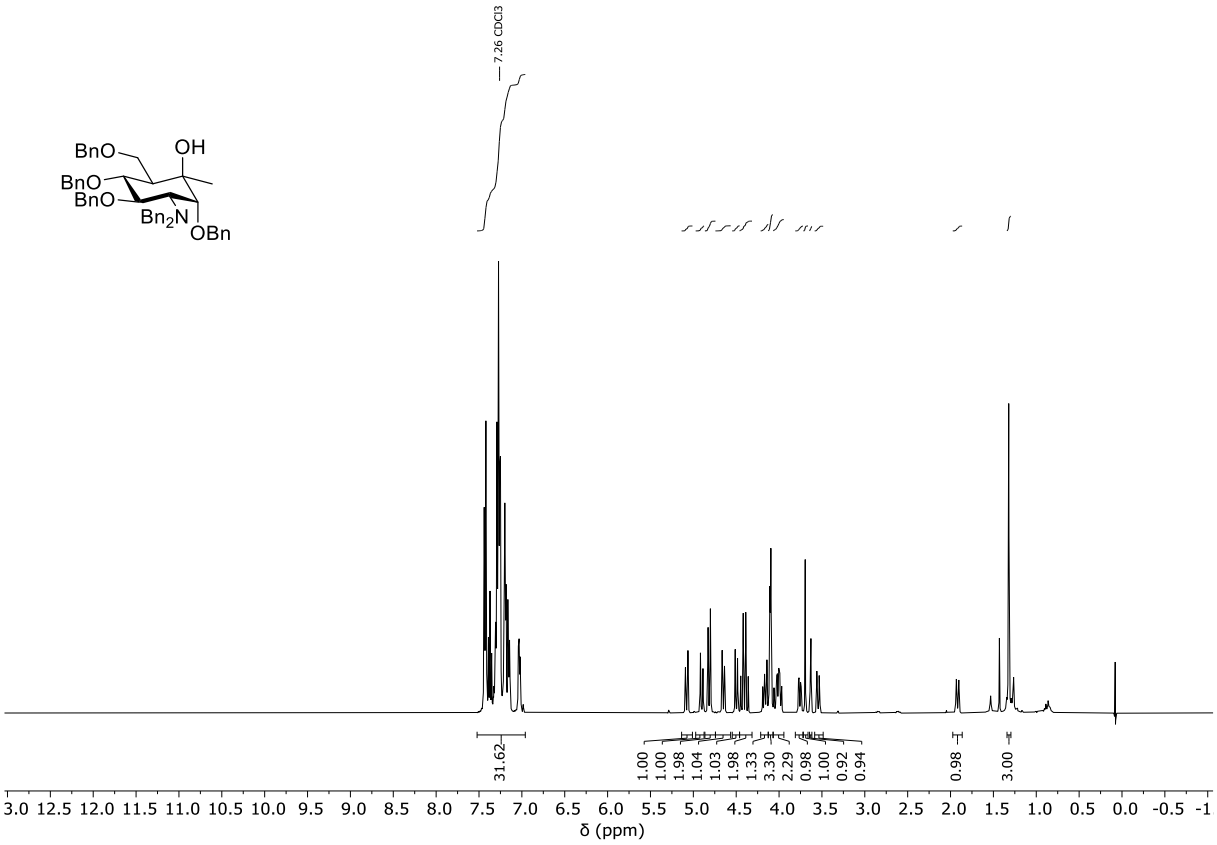
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **198**



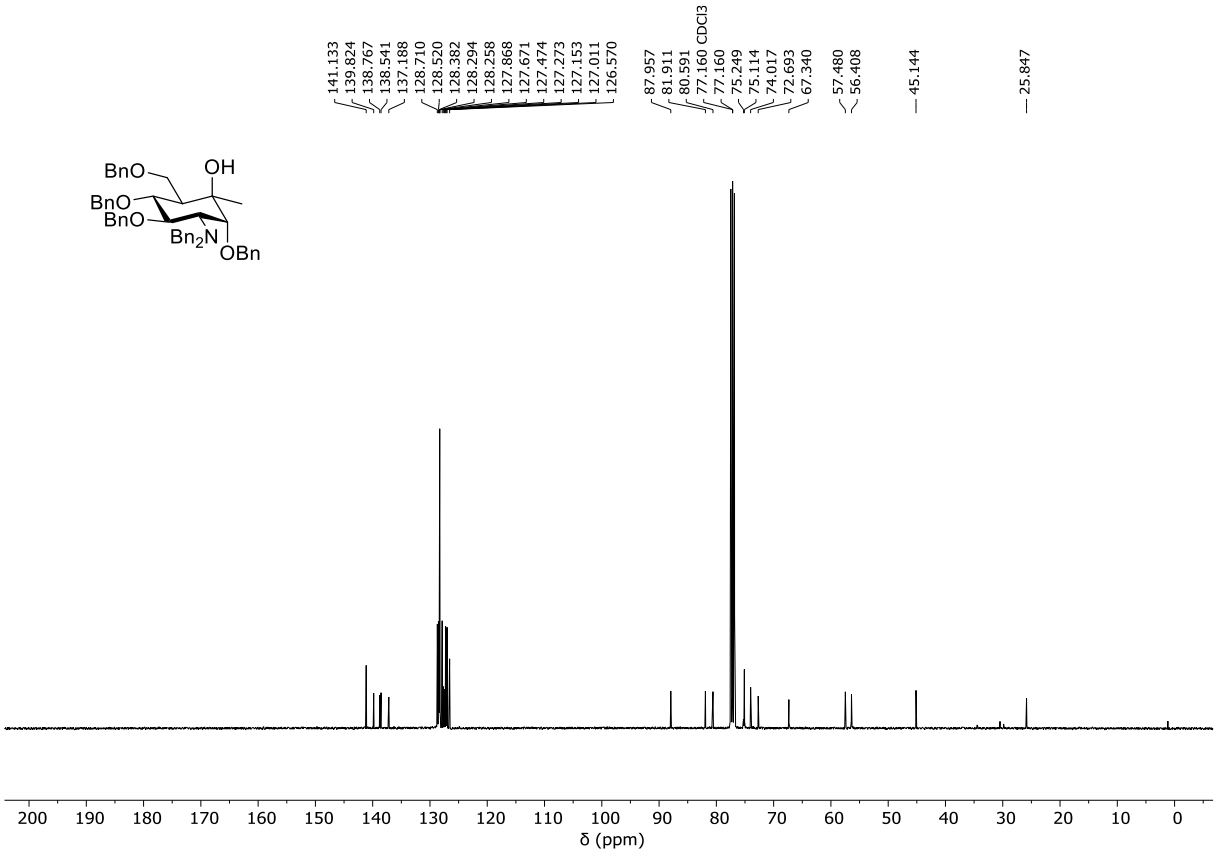
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **198**



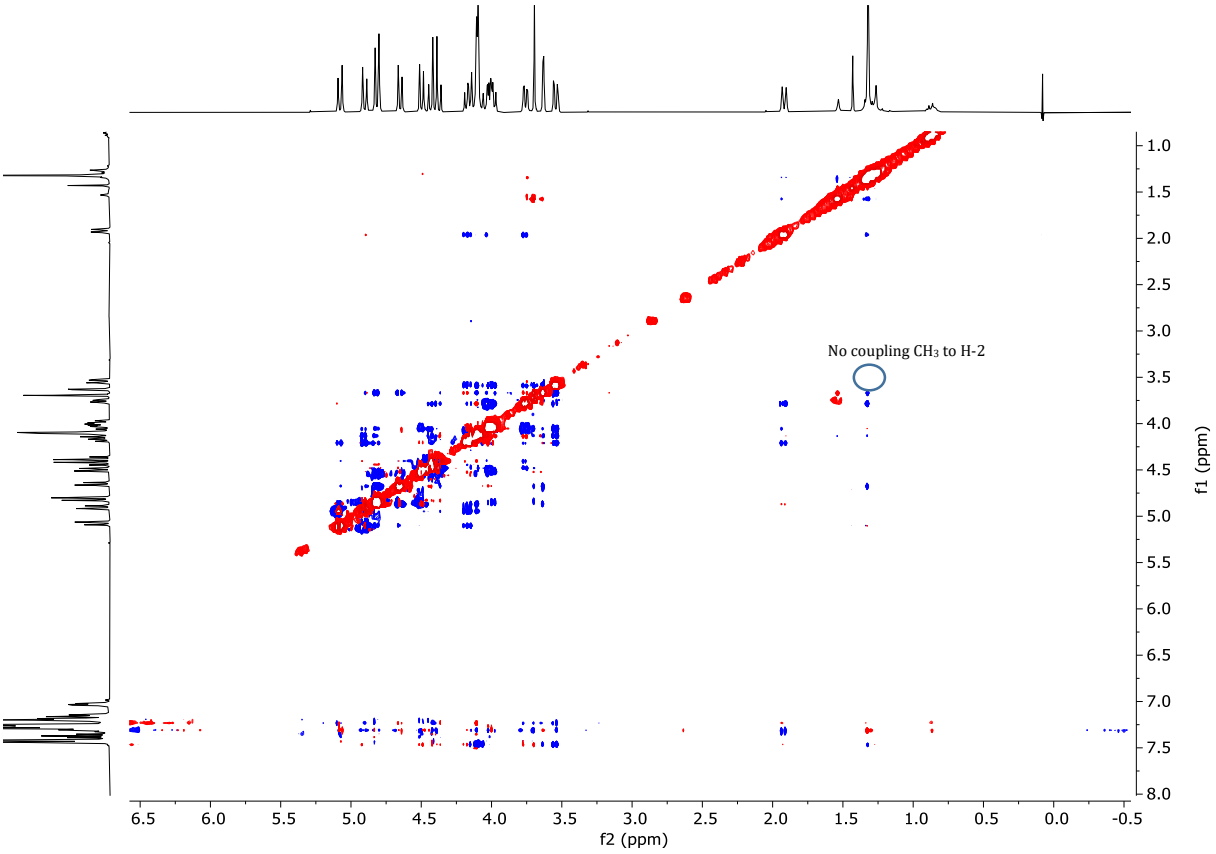
NOESY spectrum of **198**



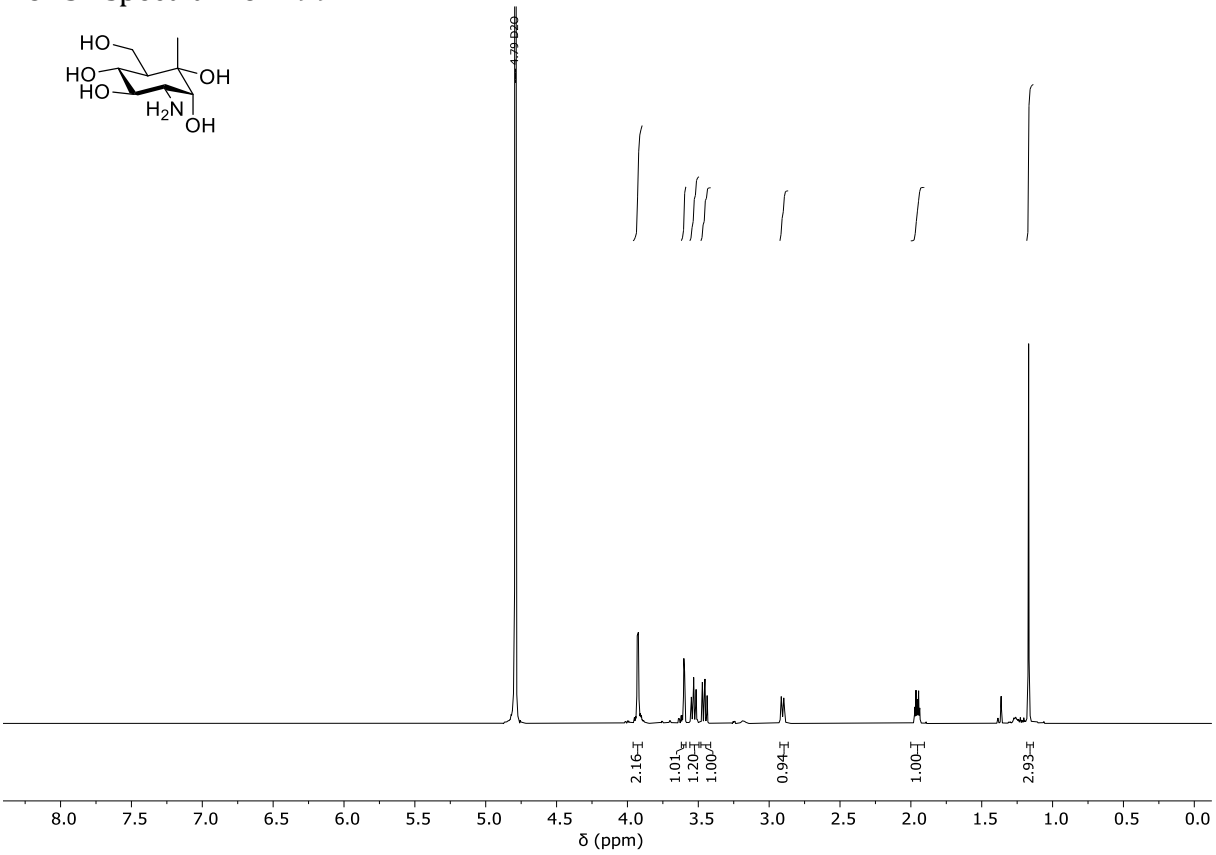
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **199**



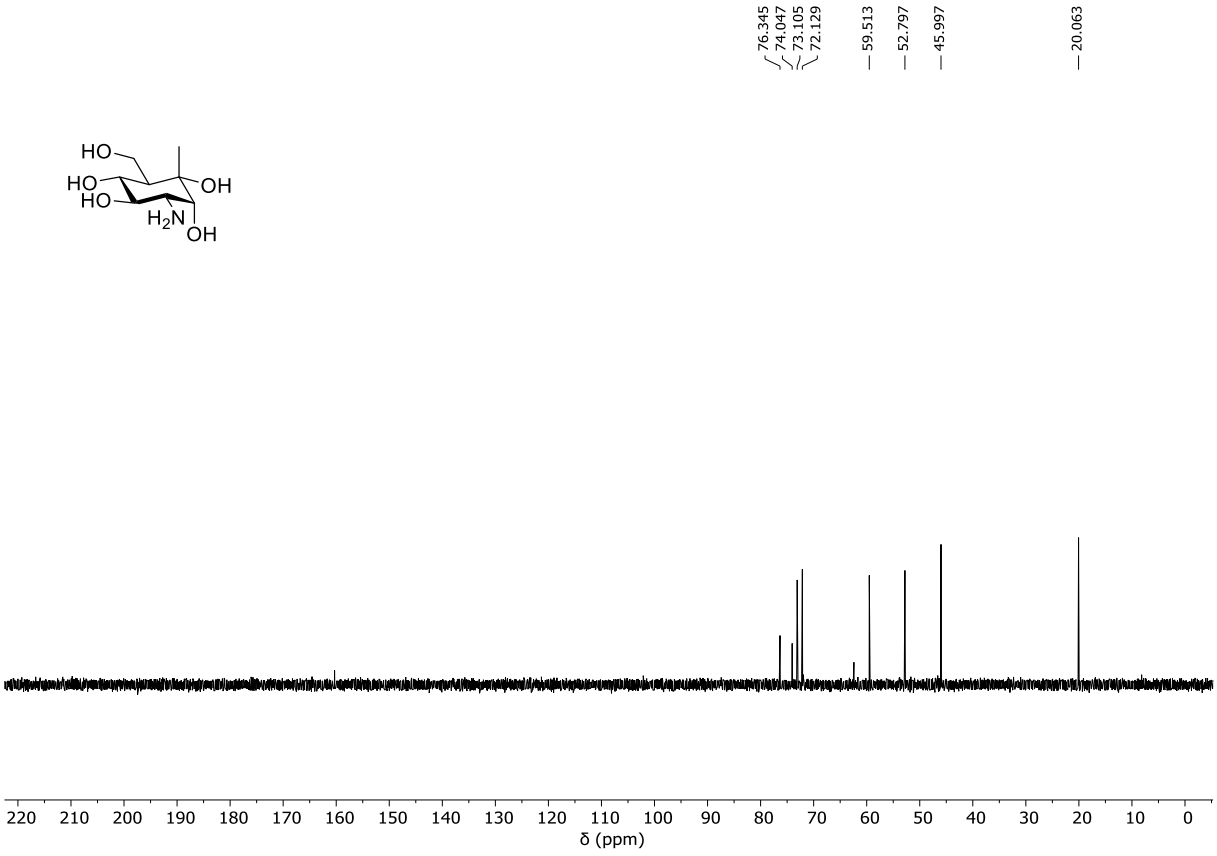
^{13}C NMR spectrum (151 MHz, CDCl3) of **199**



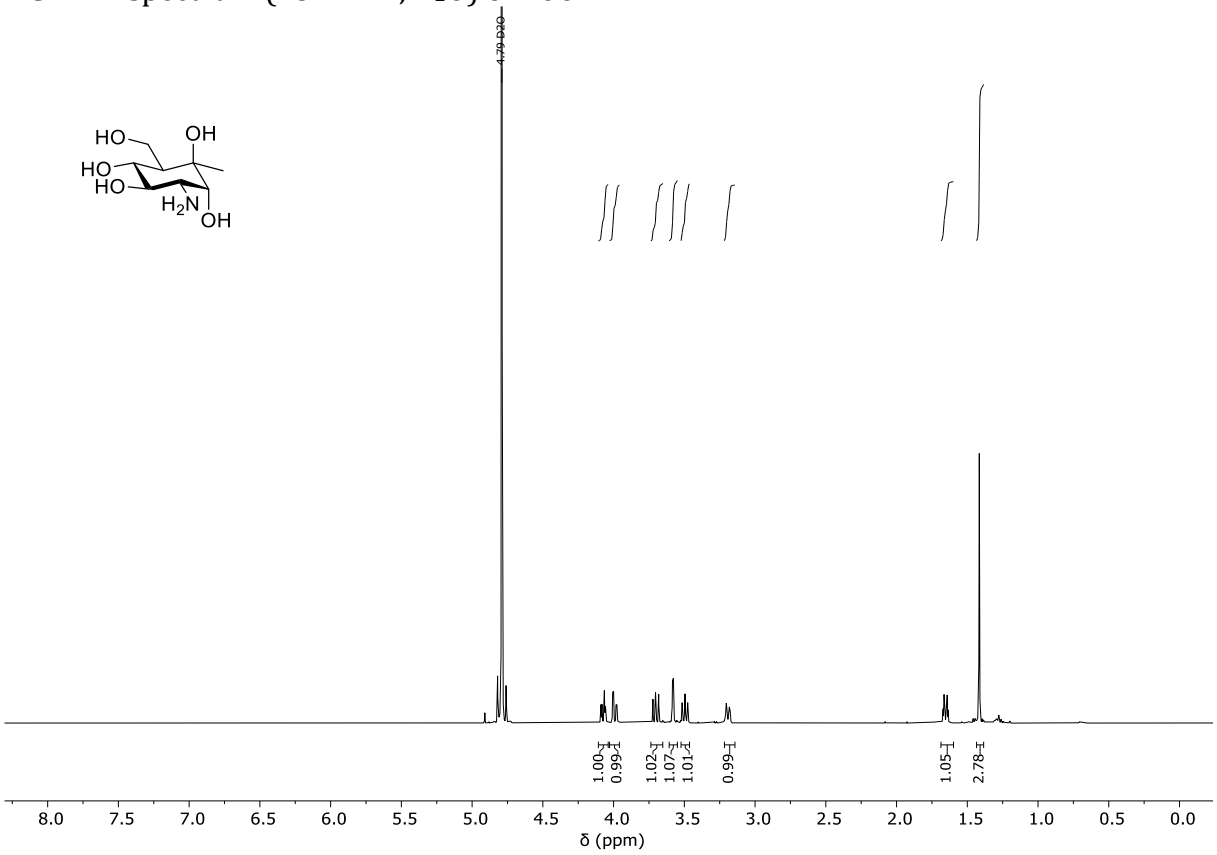
NOESY spectrum of **199**



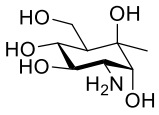
^{1}H NMR spectrum (600 MHz, D_2O) of **200**



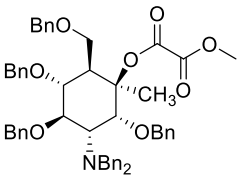
^{13}C NMR spectrum (151 MHz, D_2O) of **200**



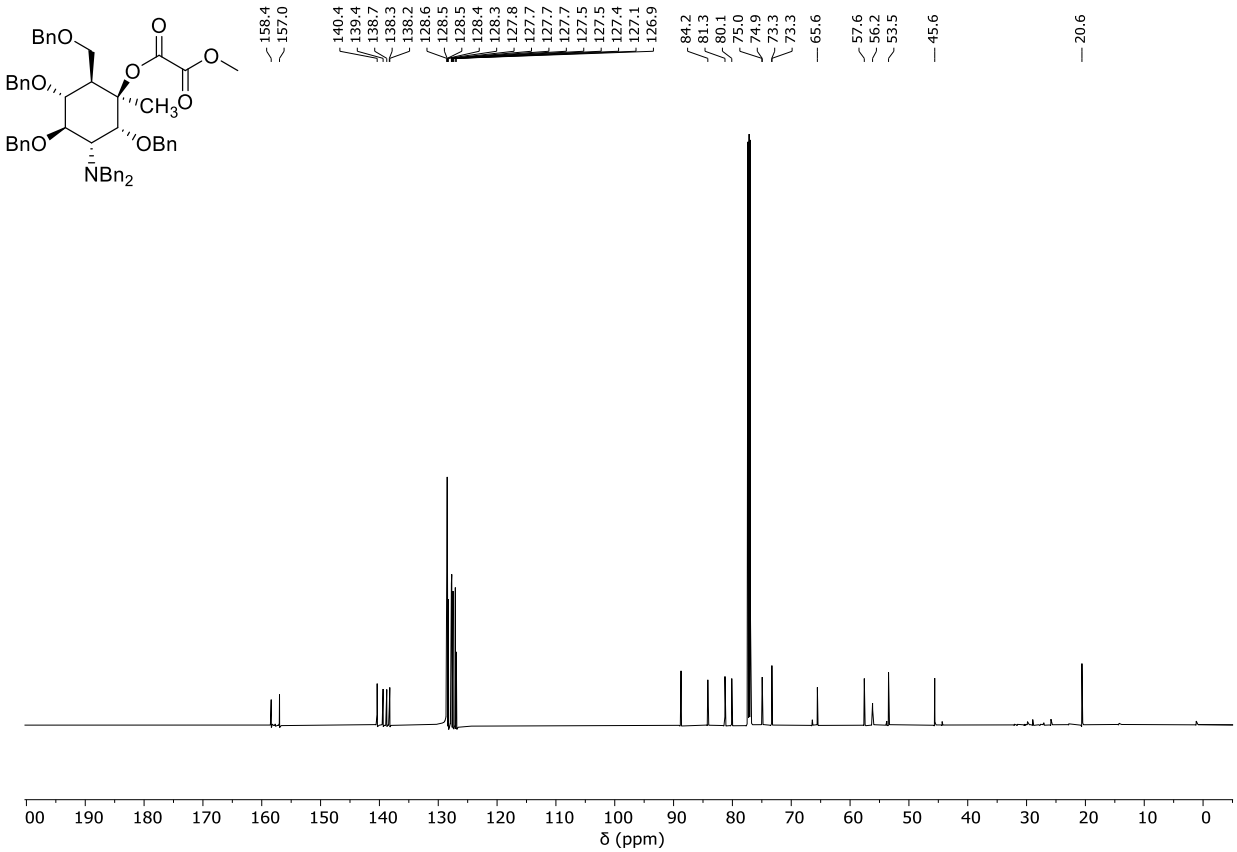
^{1}H NMR spectrum (500 MHz, D_2O) of **201**



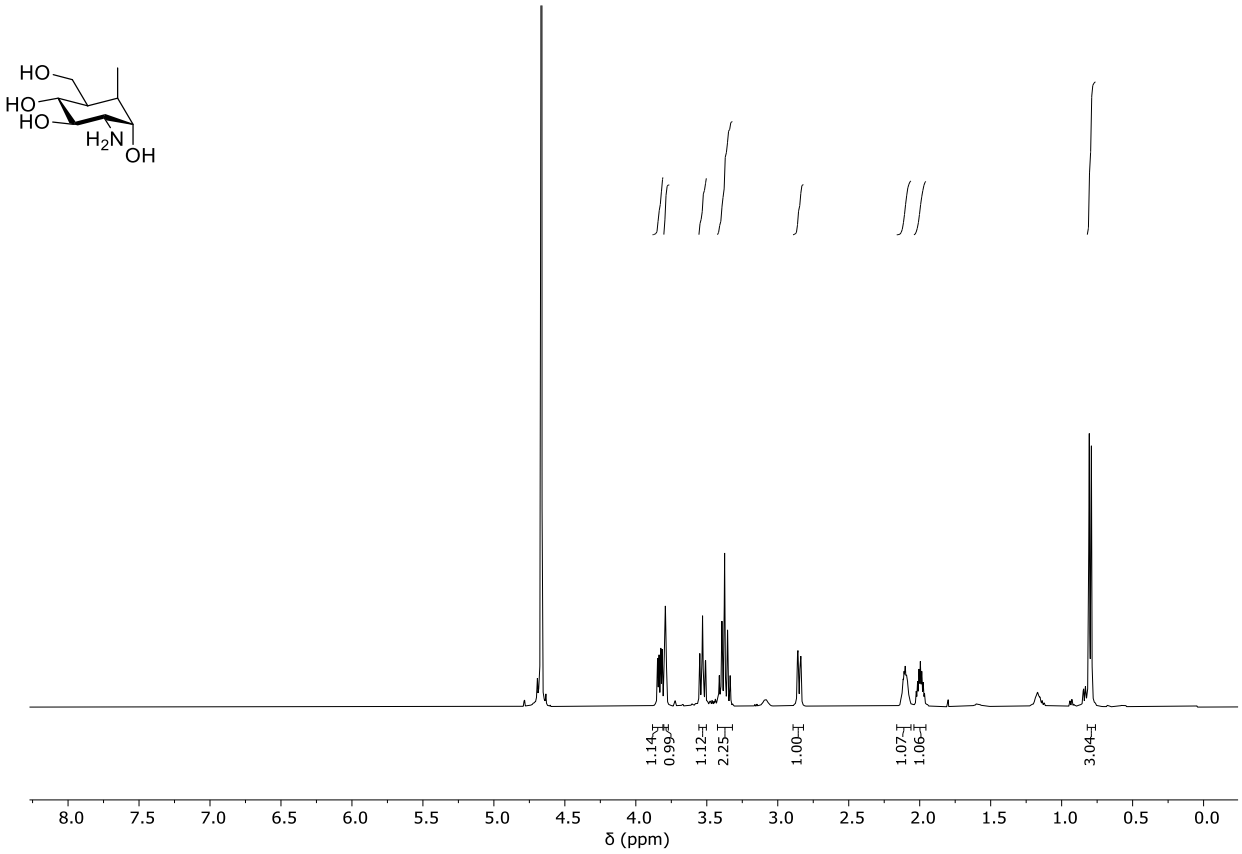
^{13}C NMR spectrum (126 MHz, D_2O) of **201**



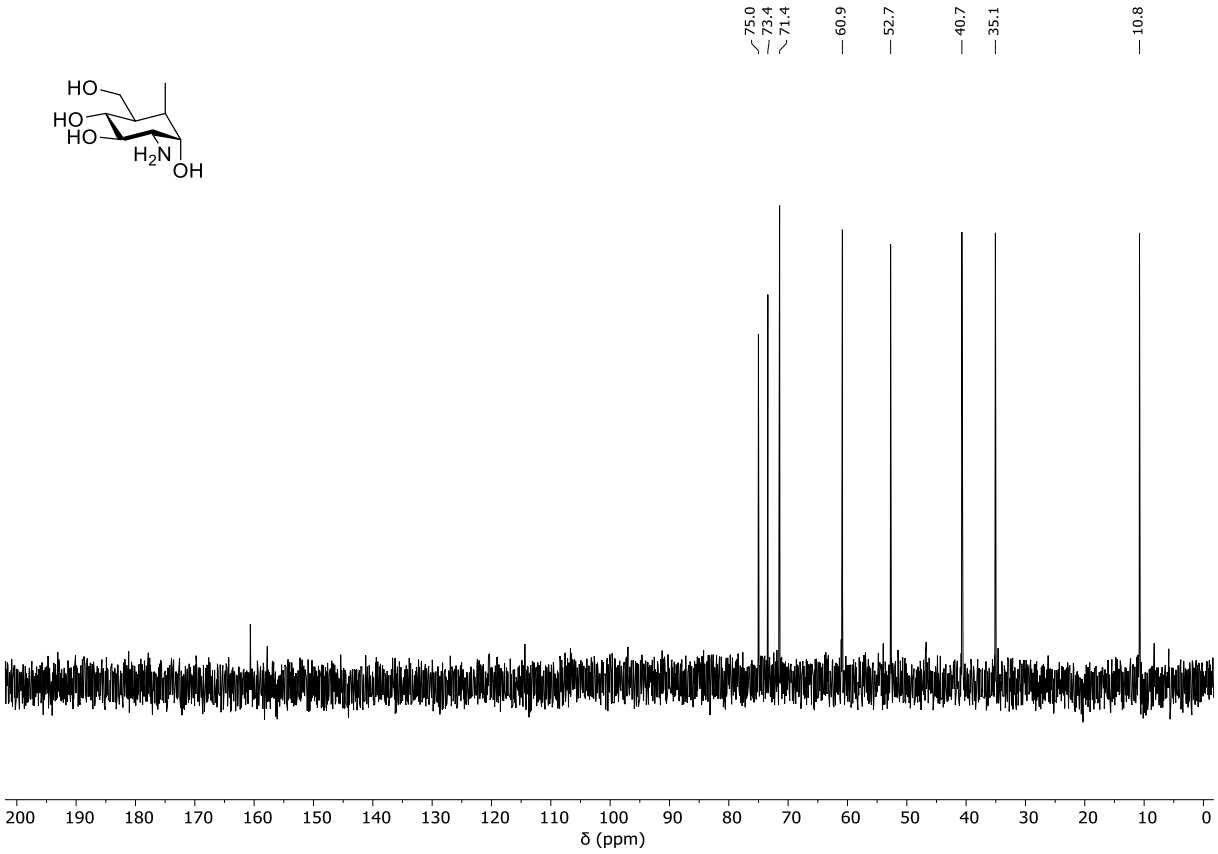
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **202**



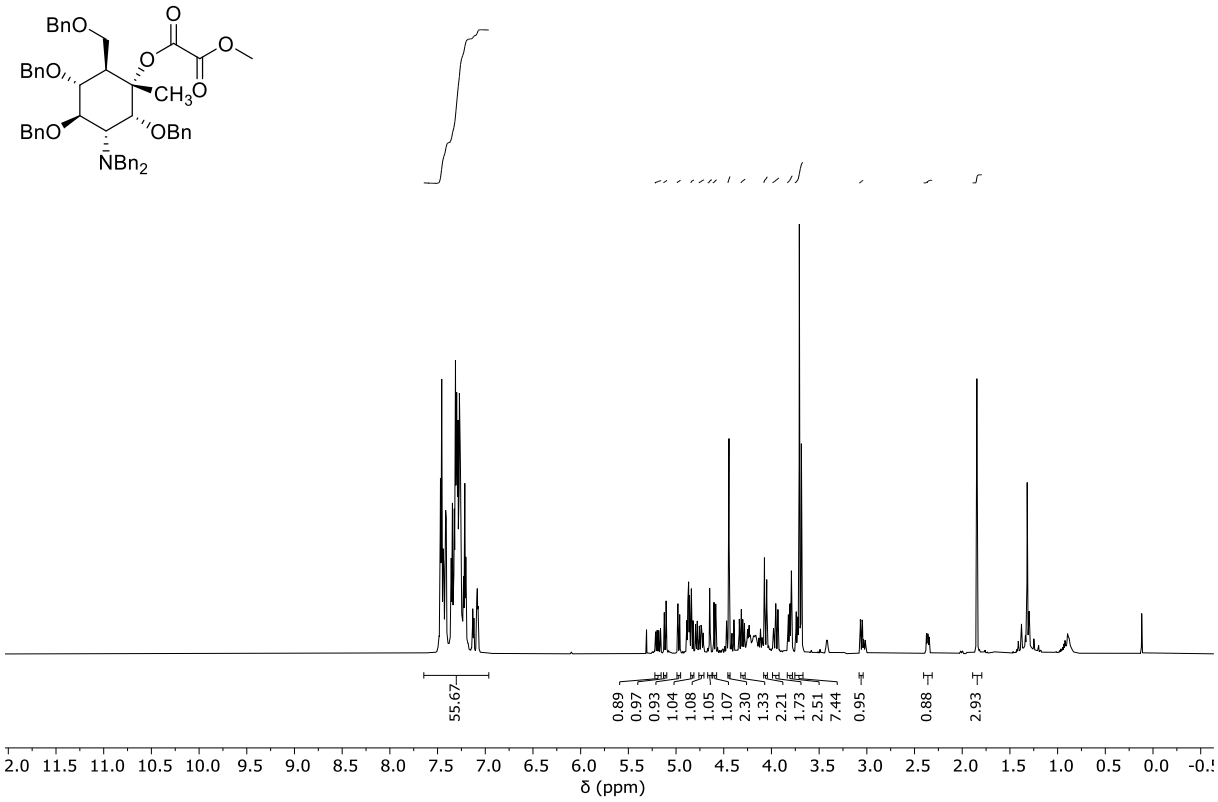
^{13}C NMR spectrum (150 MHz, $CDCl_3$) of **202**



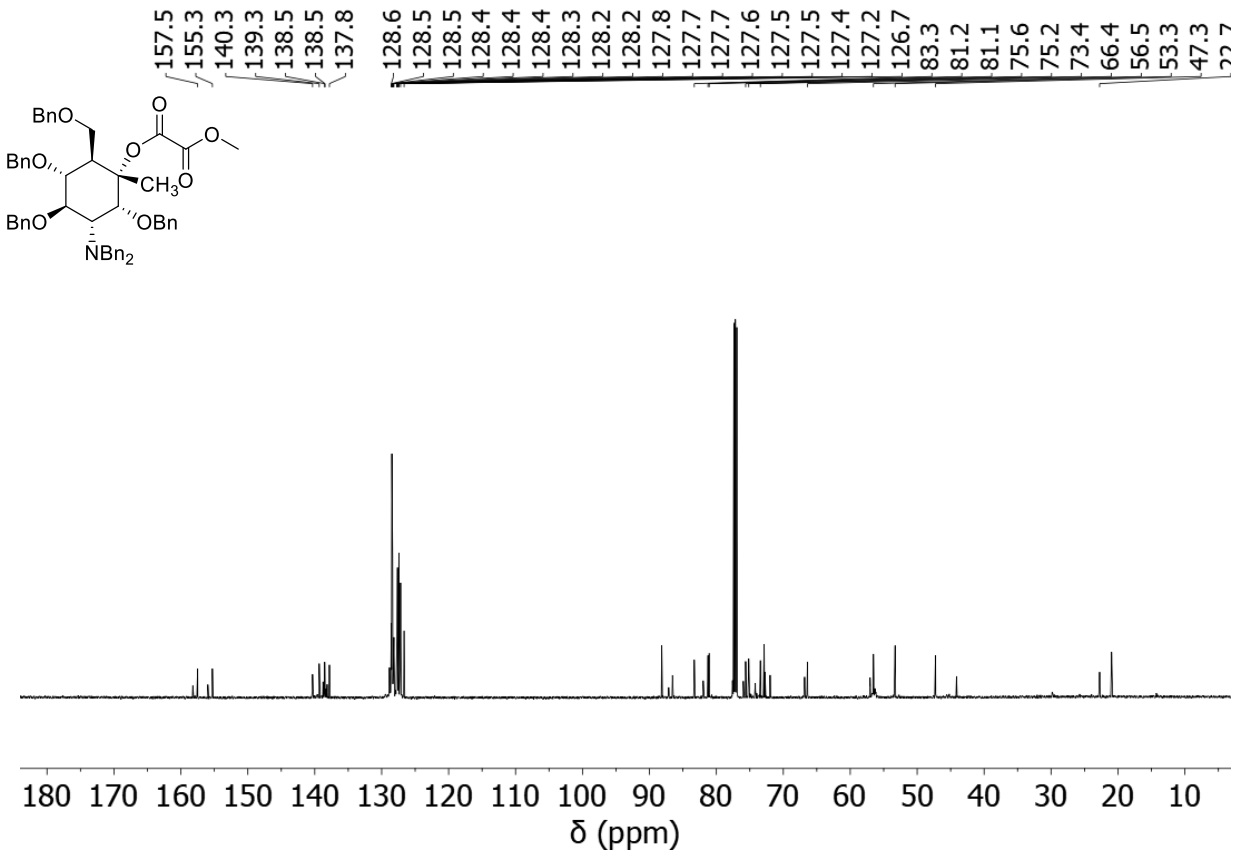
^{1}H NMR spectrum (500 MHz, D_2O) of **203**



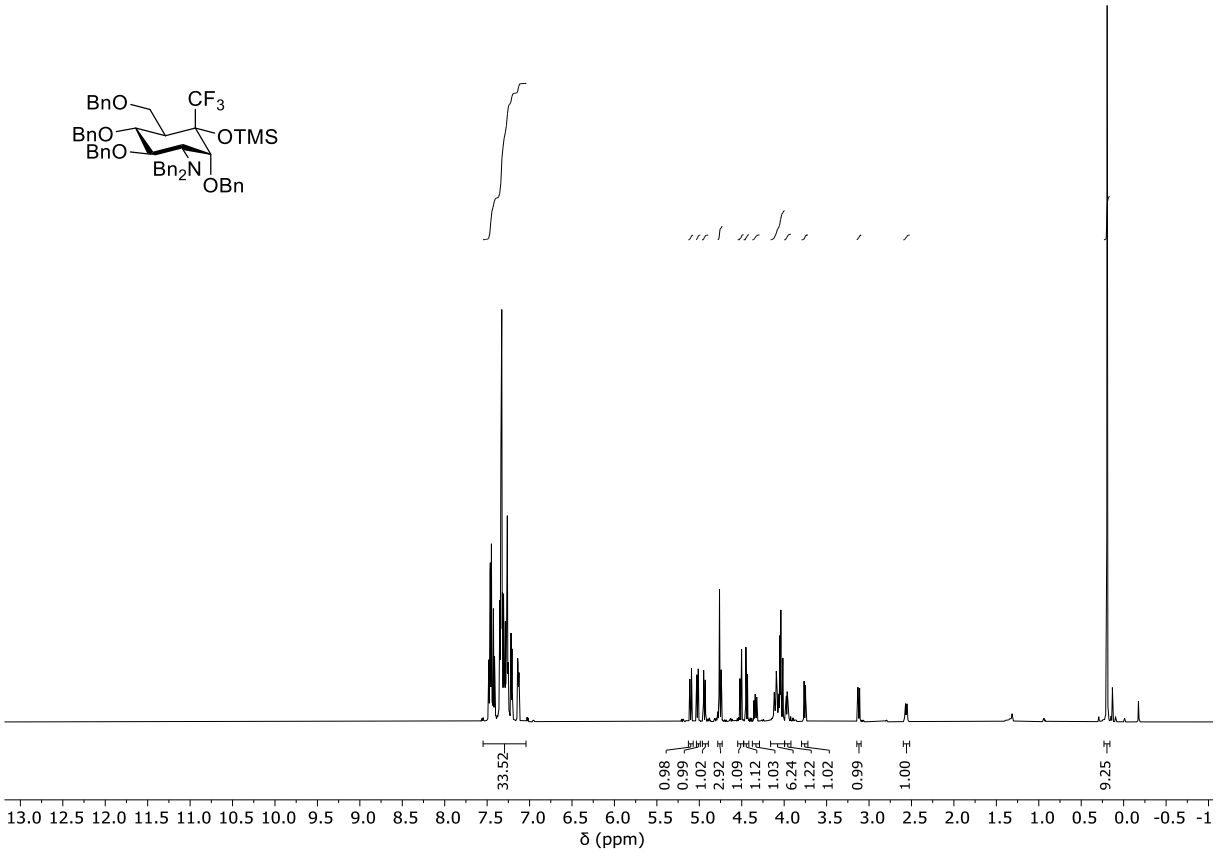
^{13}C NMR spectrum (126 MHz, D_2O) of **203**



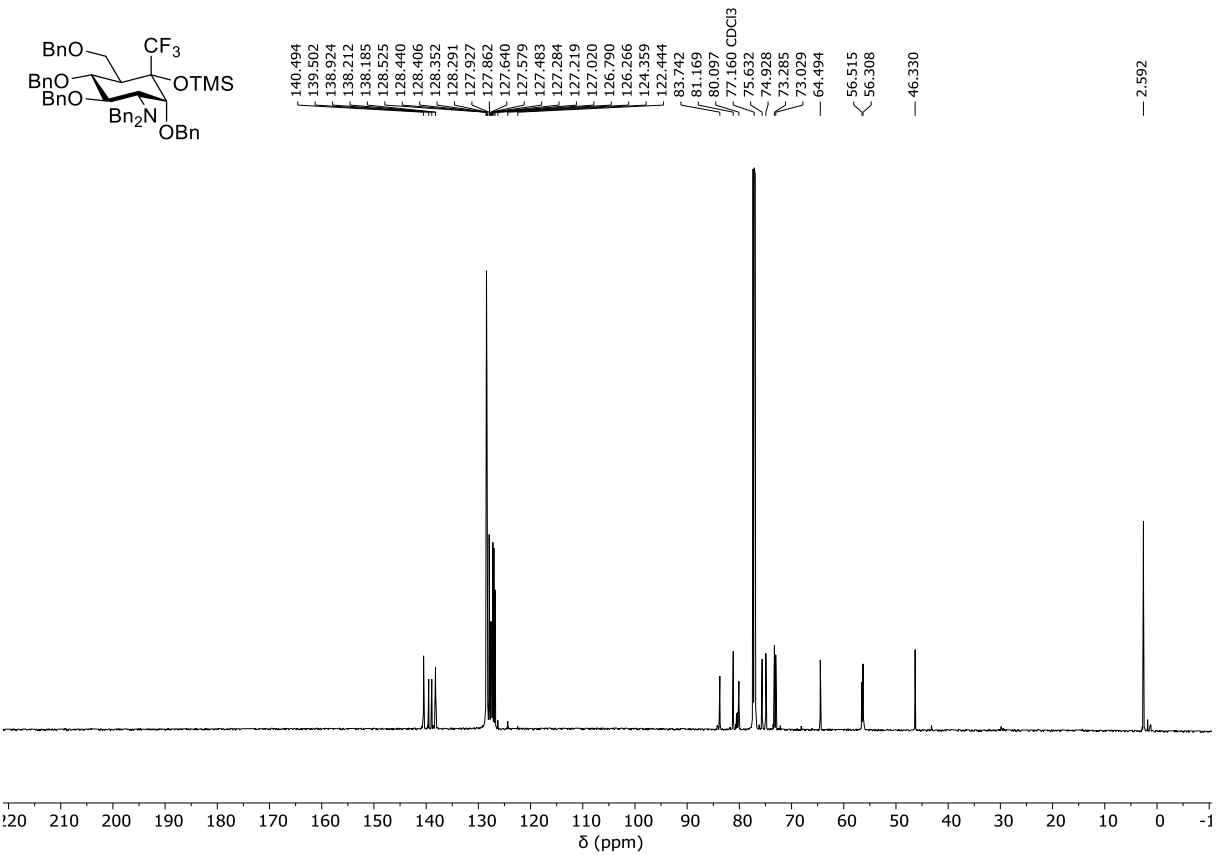
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **204**



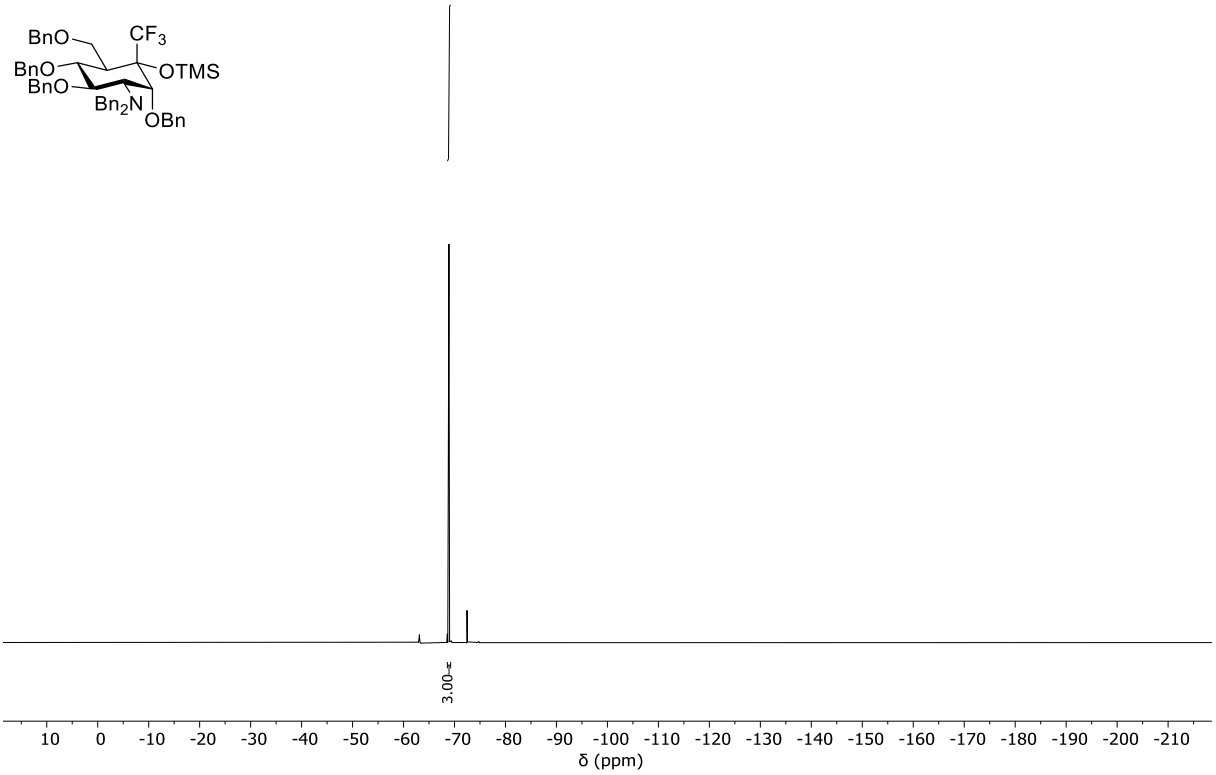
^{13}C NMR spectrum (150 MHz, $CDCl_3$) of **204**



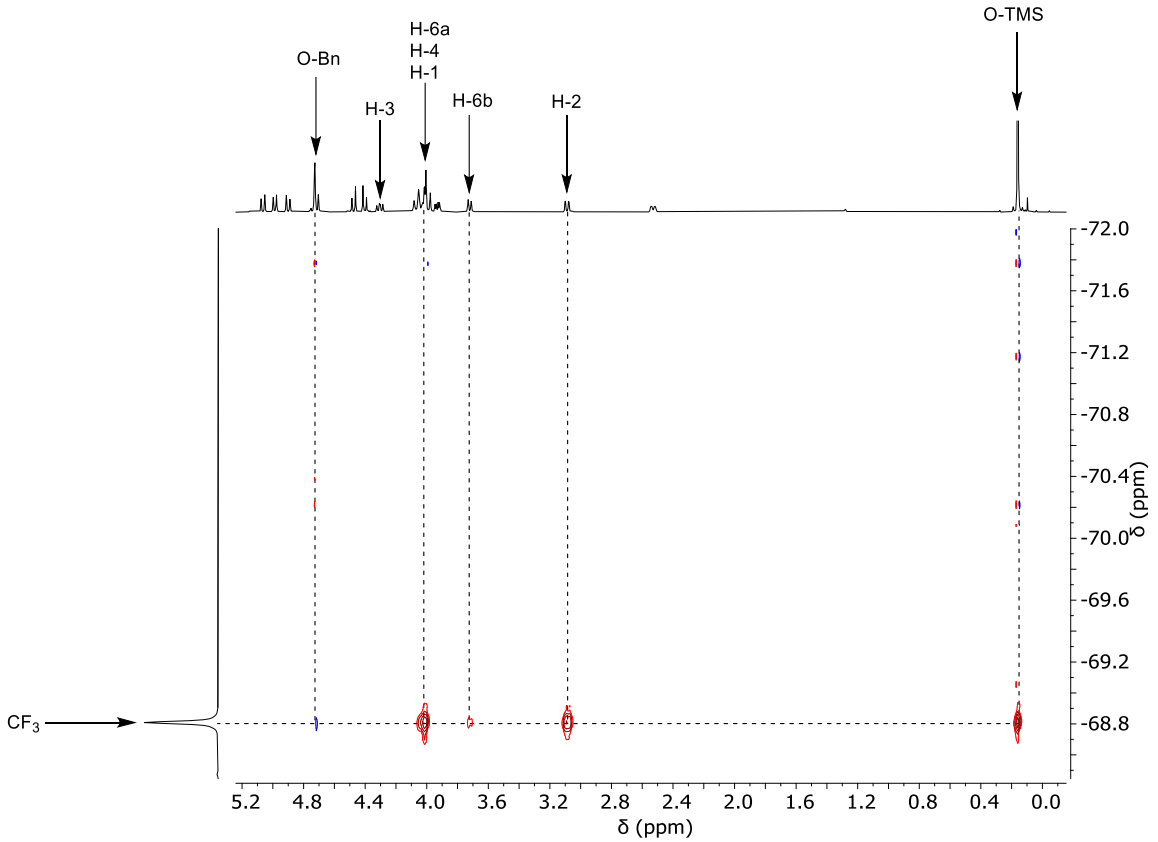
^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **205**



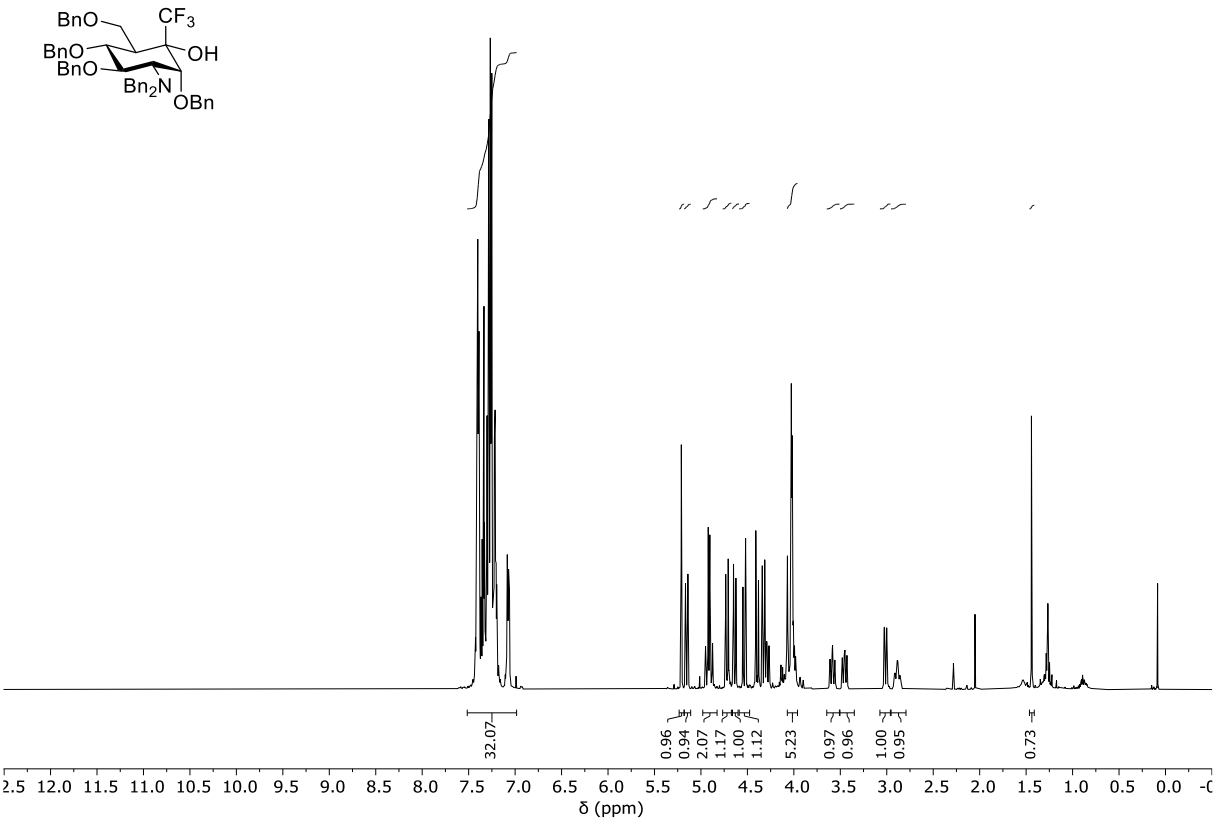
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **205**



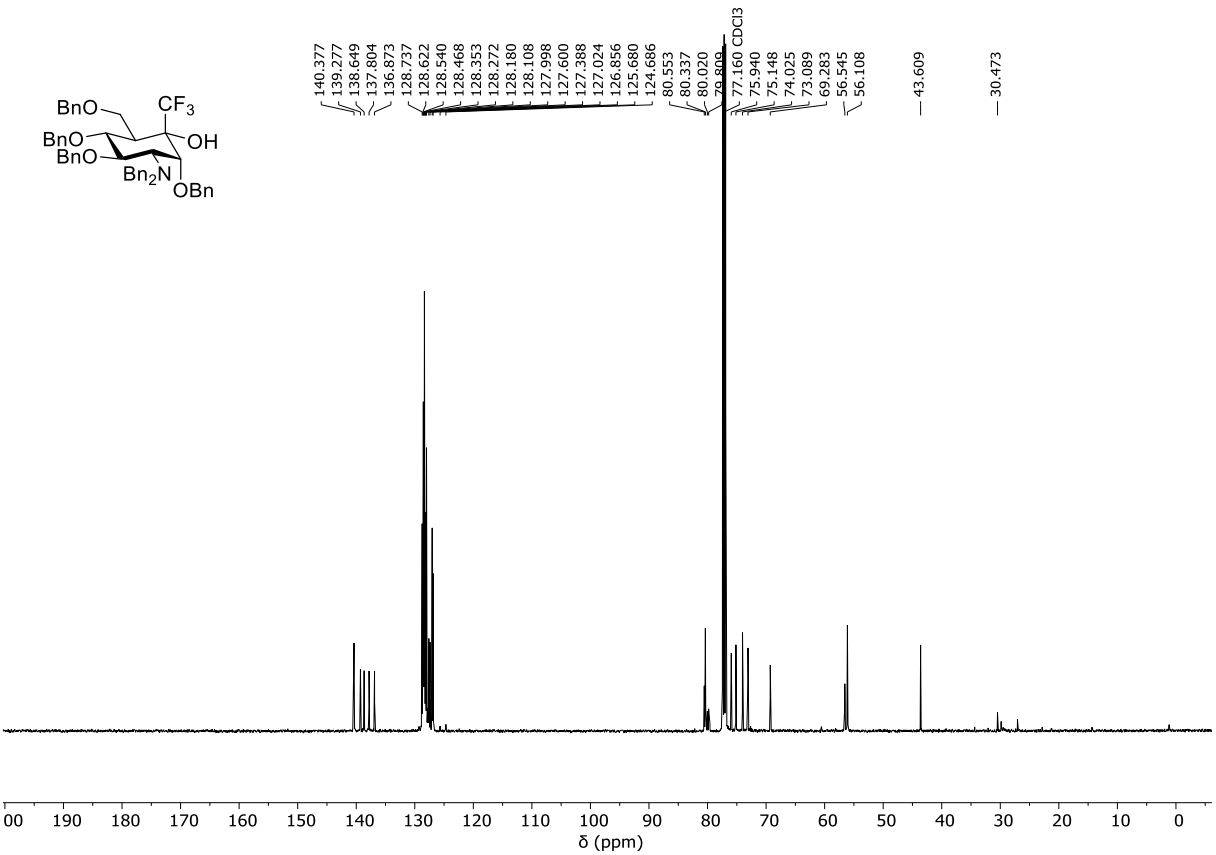
^{19}F NMR spectrum (377 MHz, $CDCl_3$) of **205**



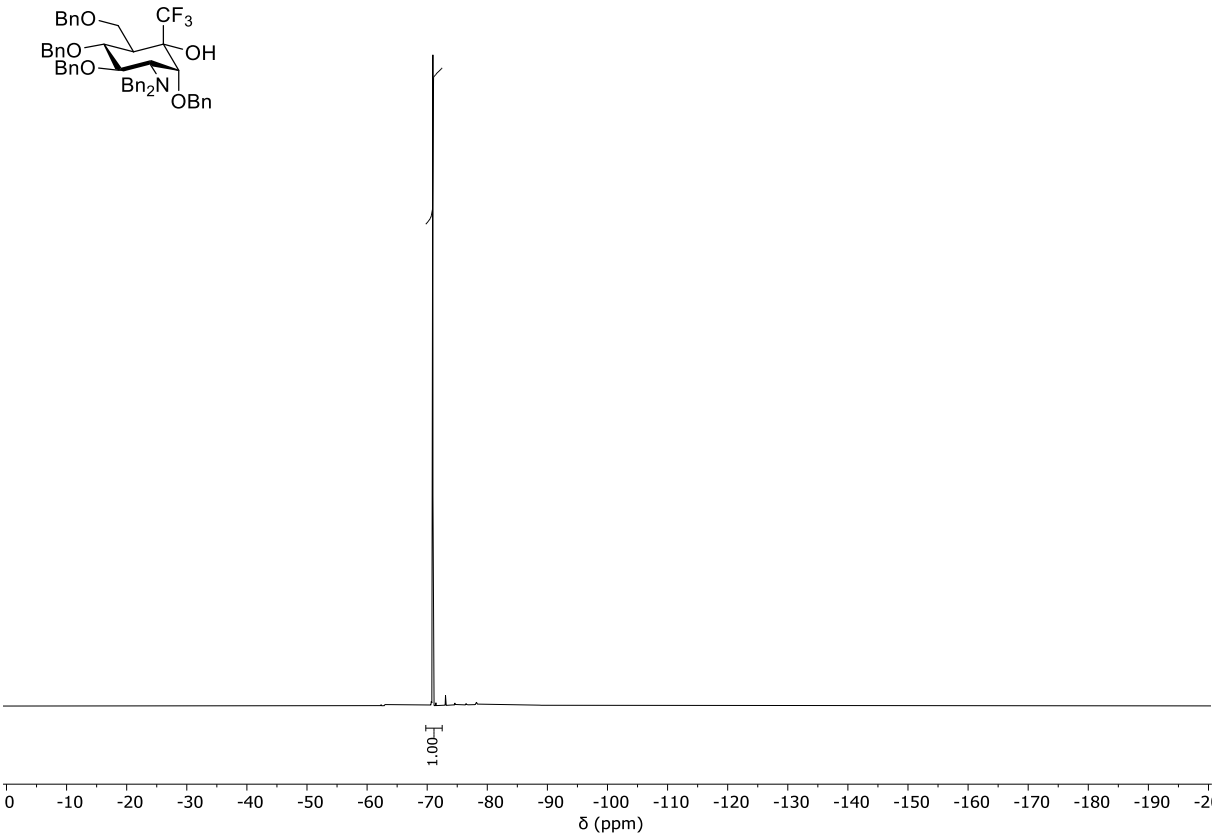
^{19}F-^{1}H HOESY spectrum of **205**



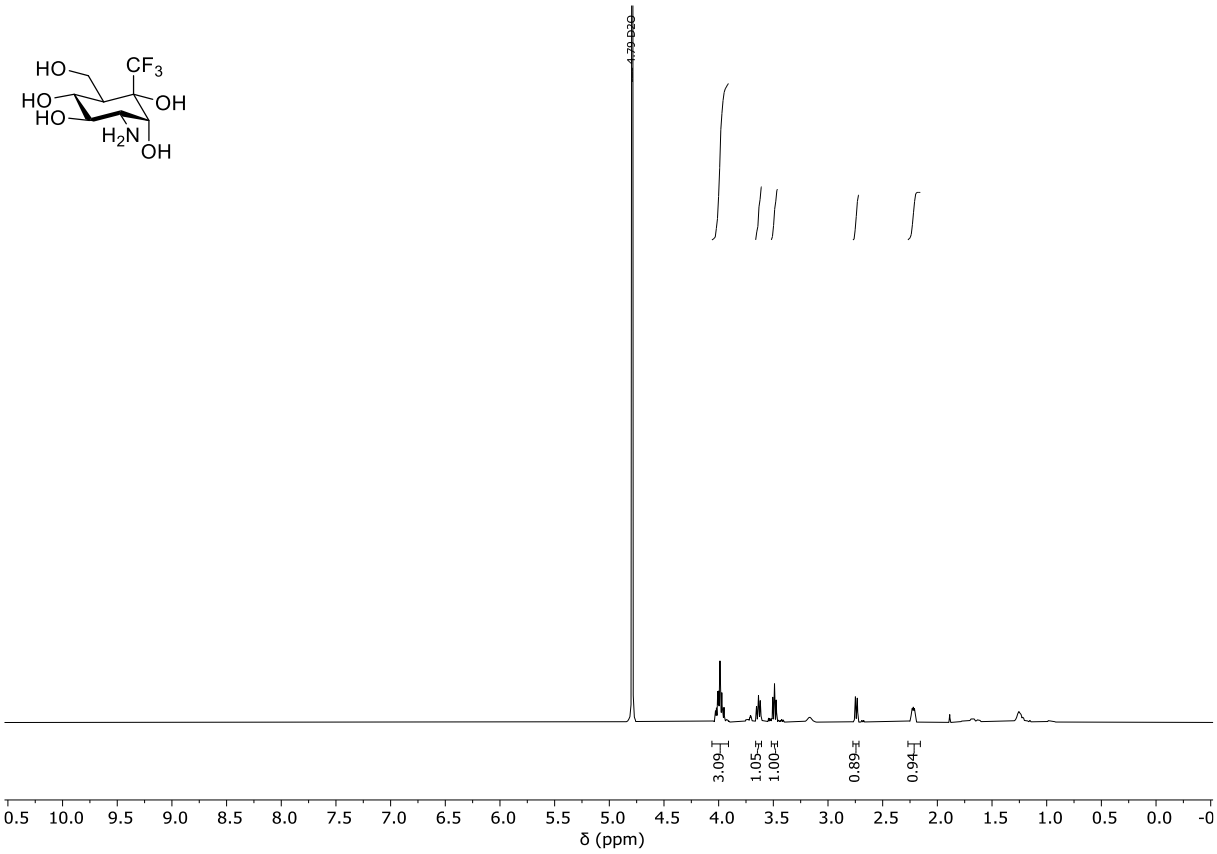
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **206**



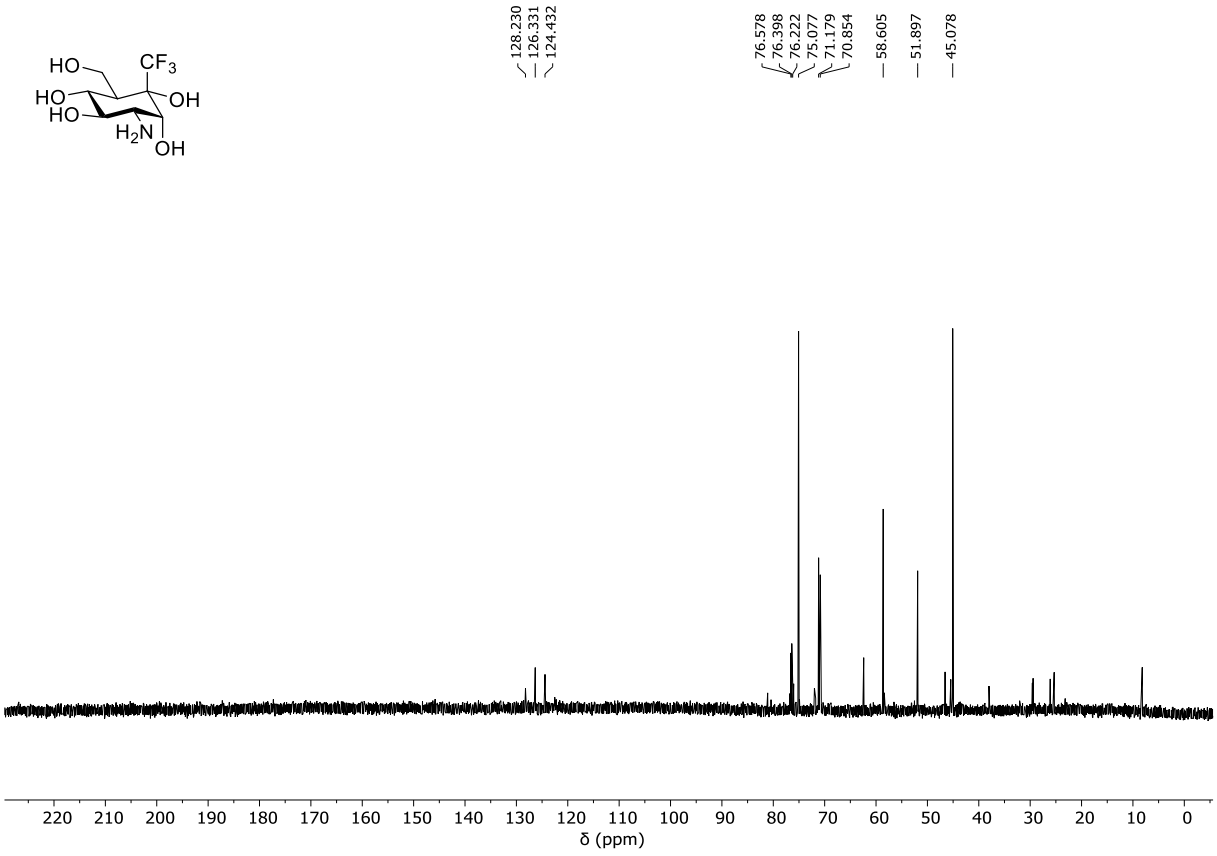
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **206**



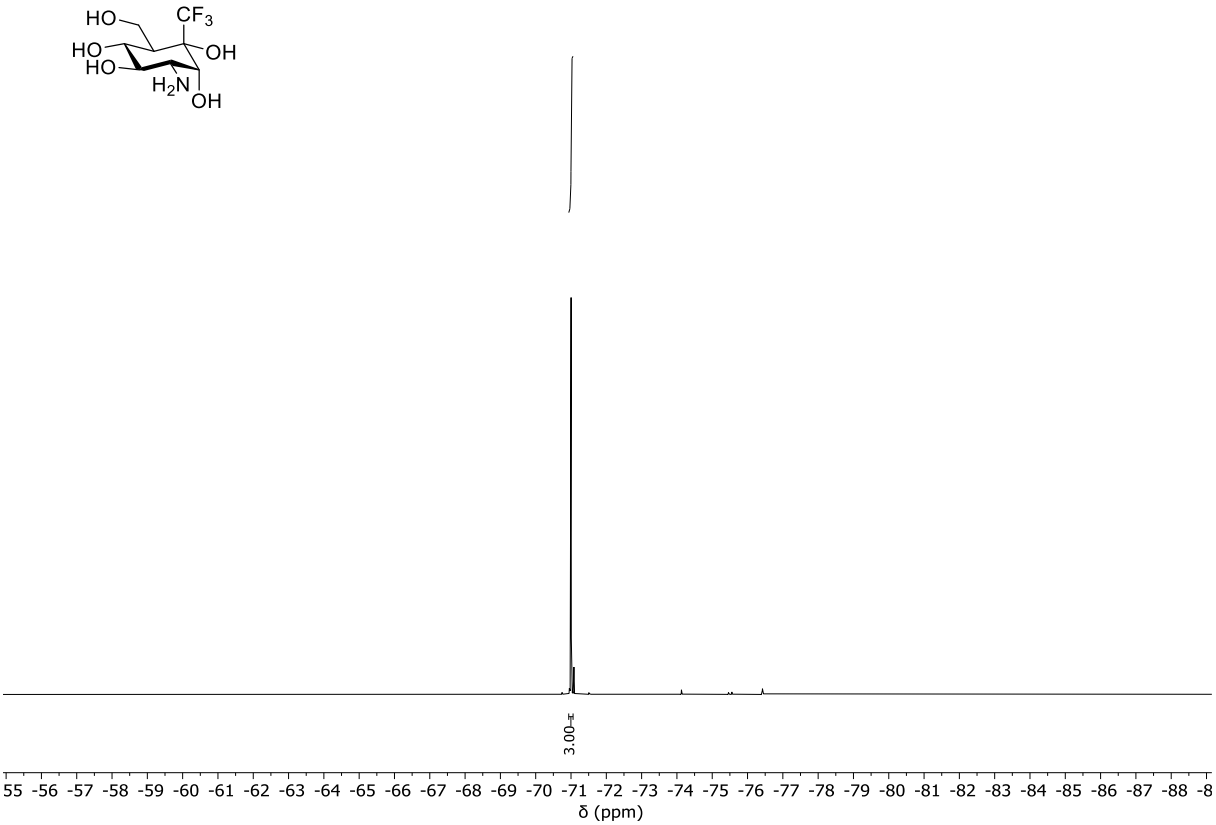
^{19}F NMR spectrum (377 MHz, $CDCl_3$) of **206**



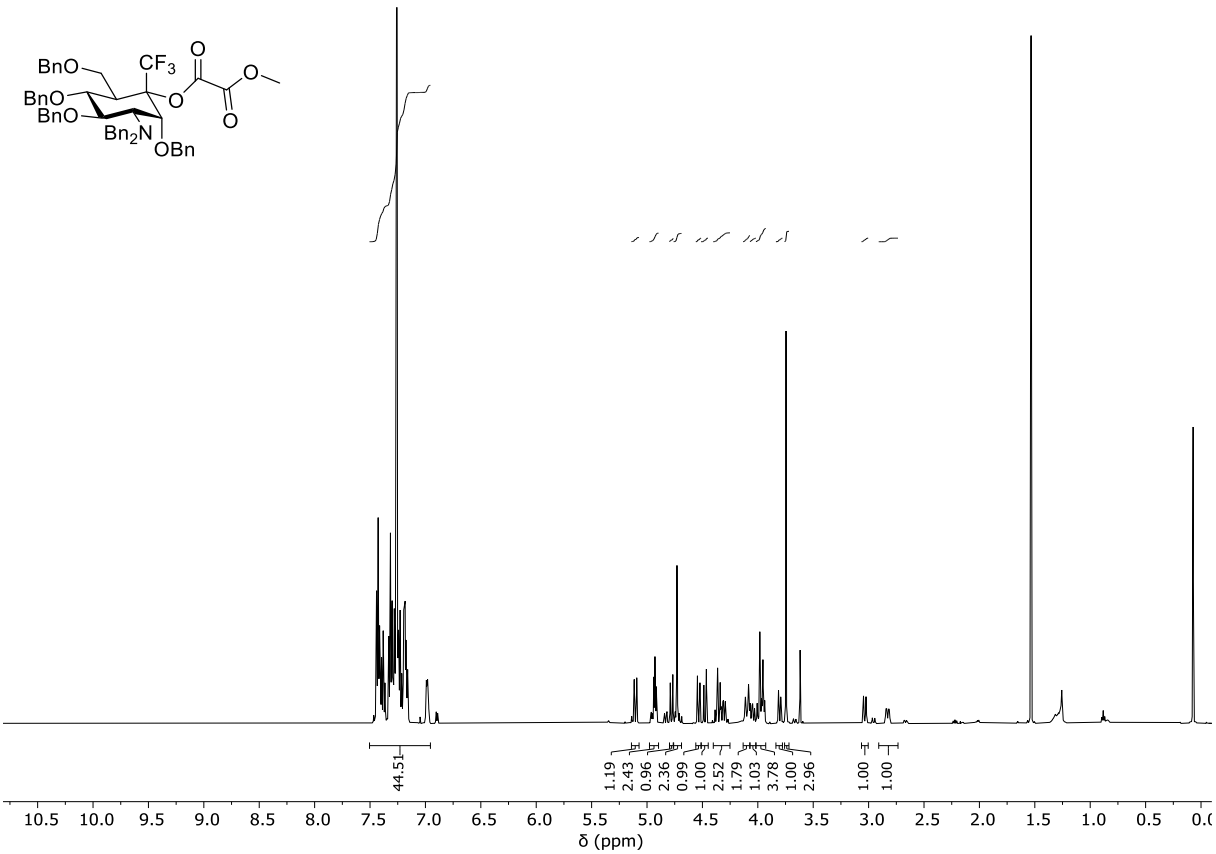
^{1}H NMR spectrum (600 MHz, D_2O) of **207**



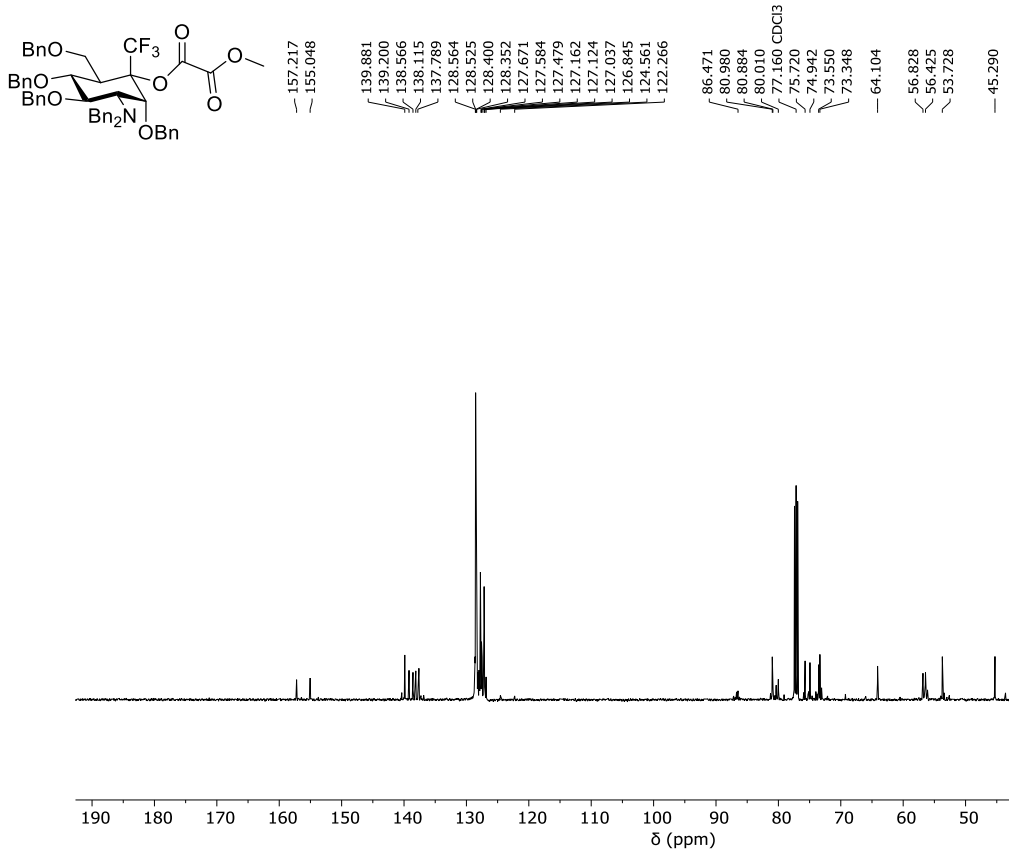
^{13}C NMR spectrum (151 MHz, D_2O) of **207**



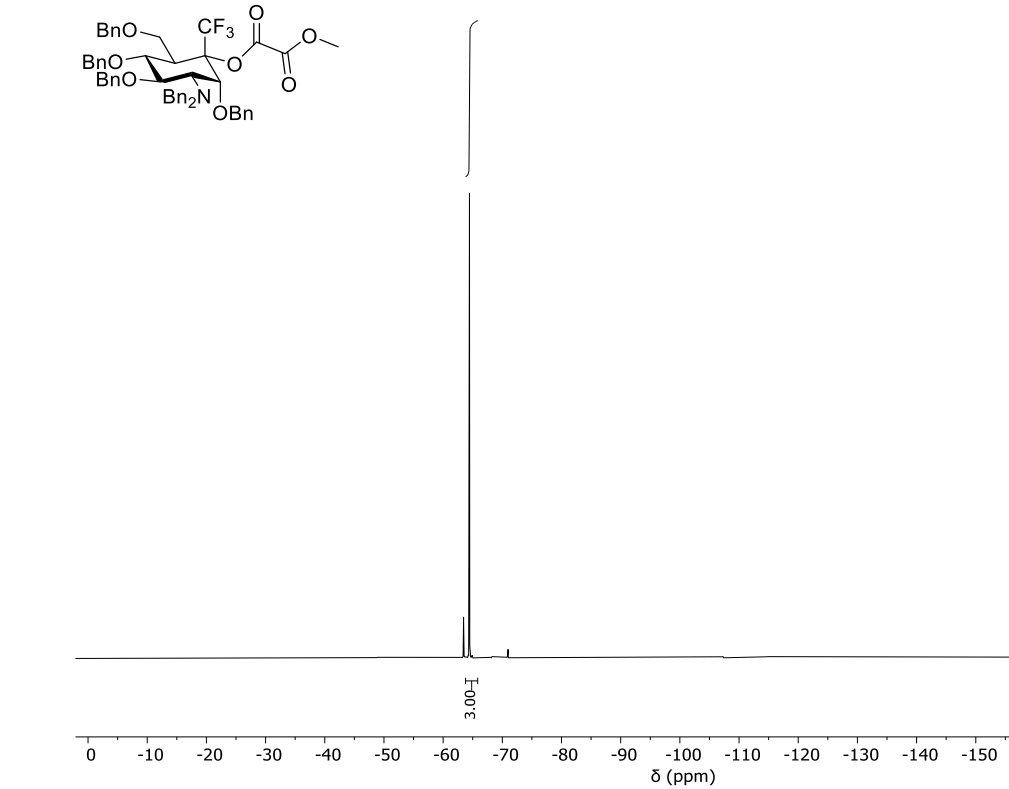
^{19}F NMR spectrum (377 MHz, D_2O) of **207**



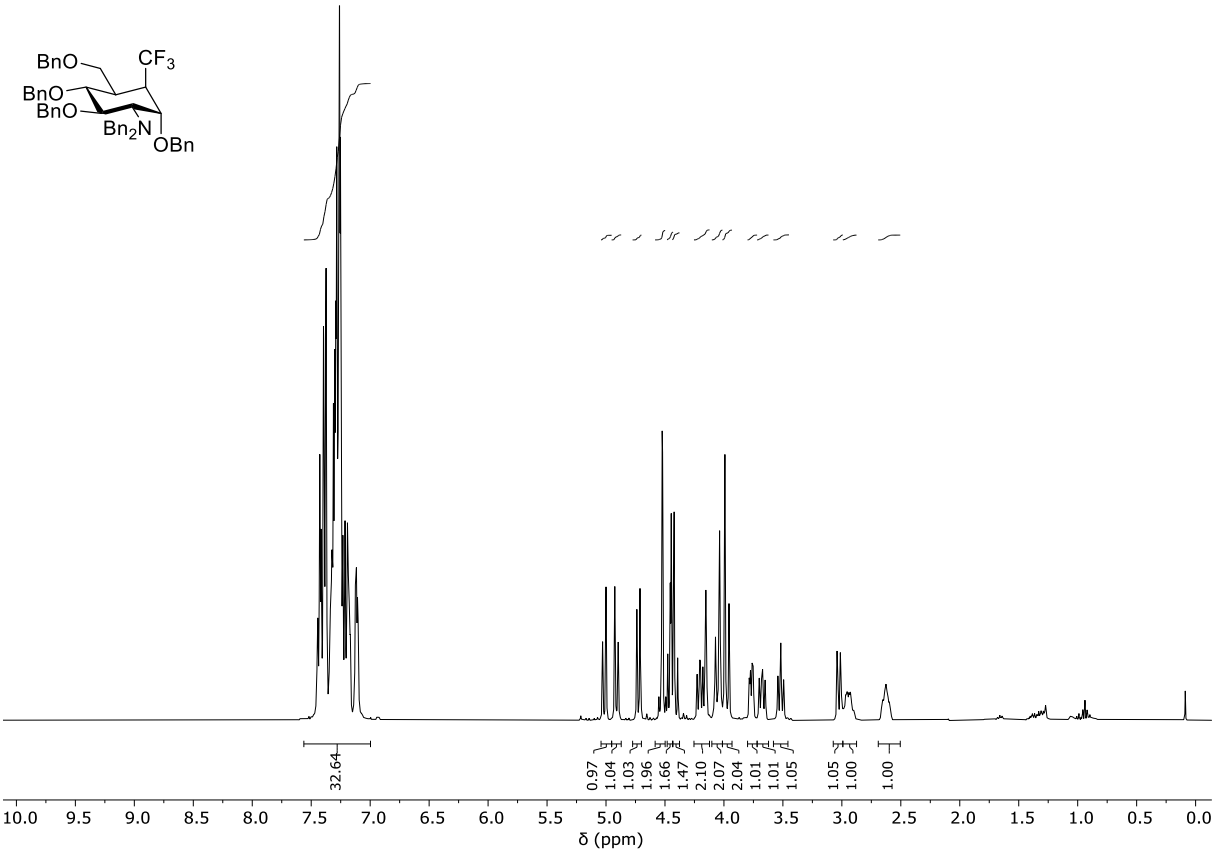
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **208**



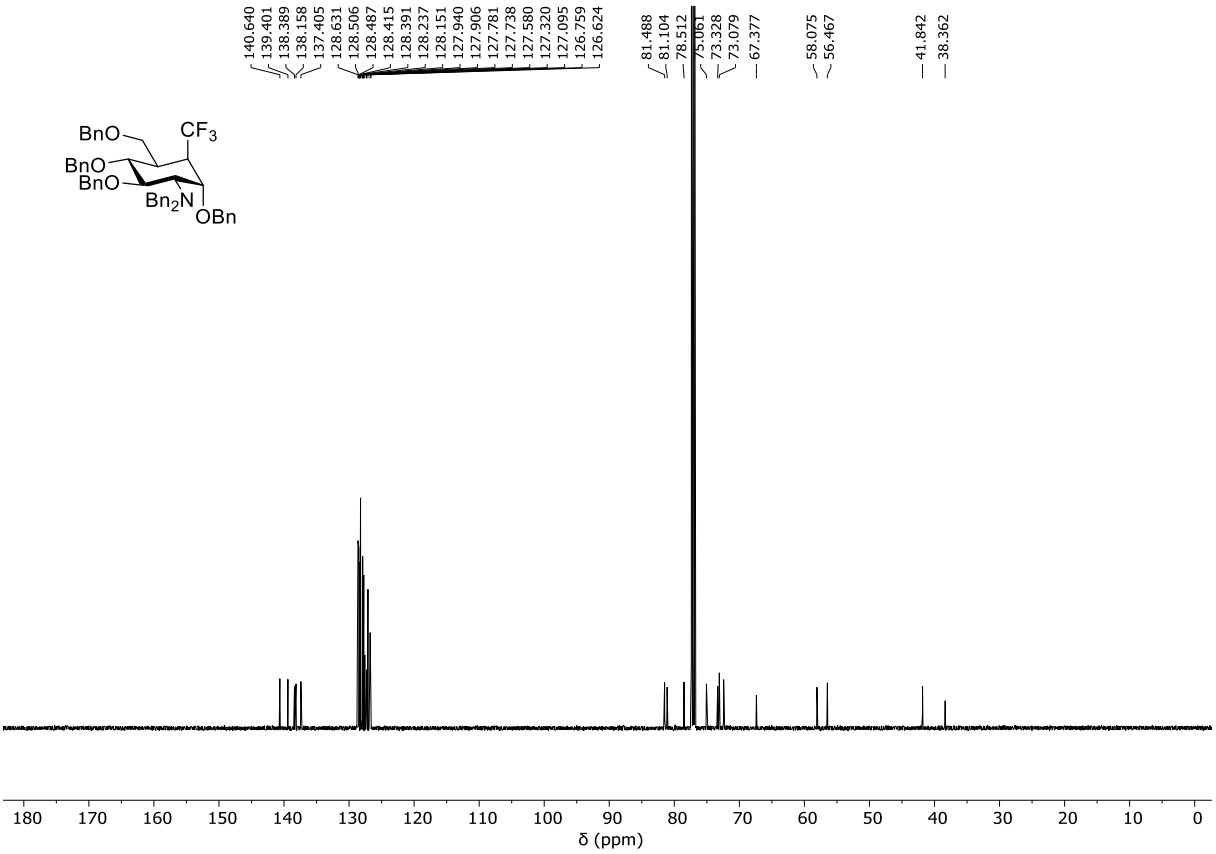
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **208**



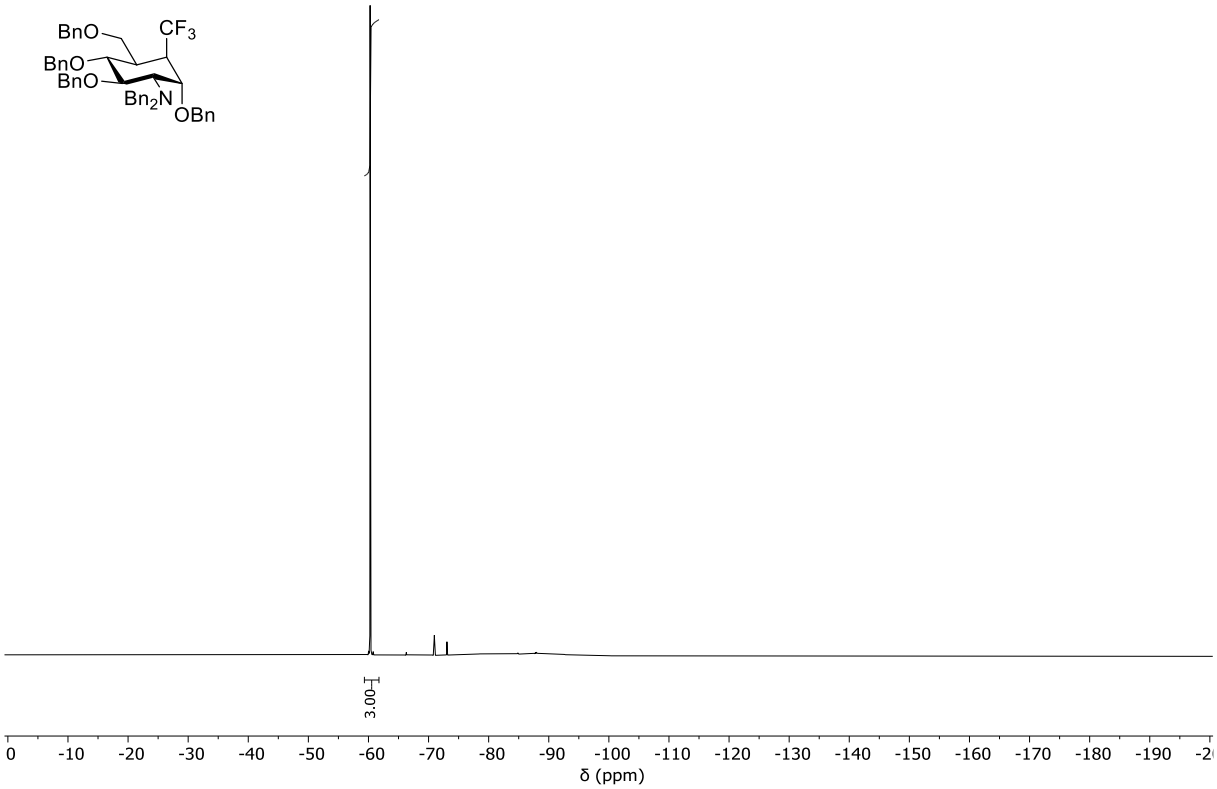
^{19}F NMR spectrum (471 MHz, $CDCl_3$) of **208**



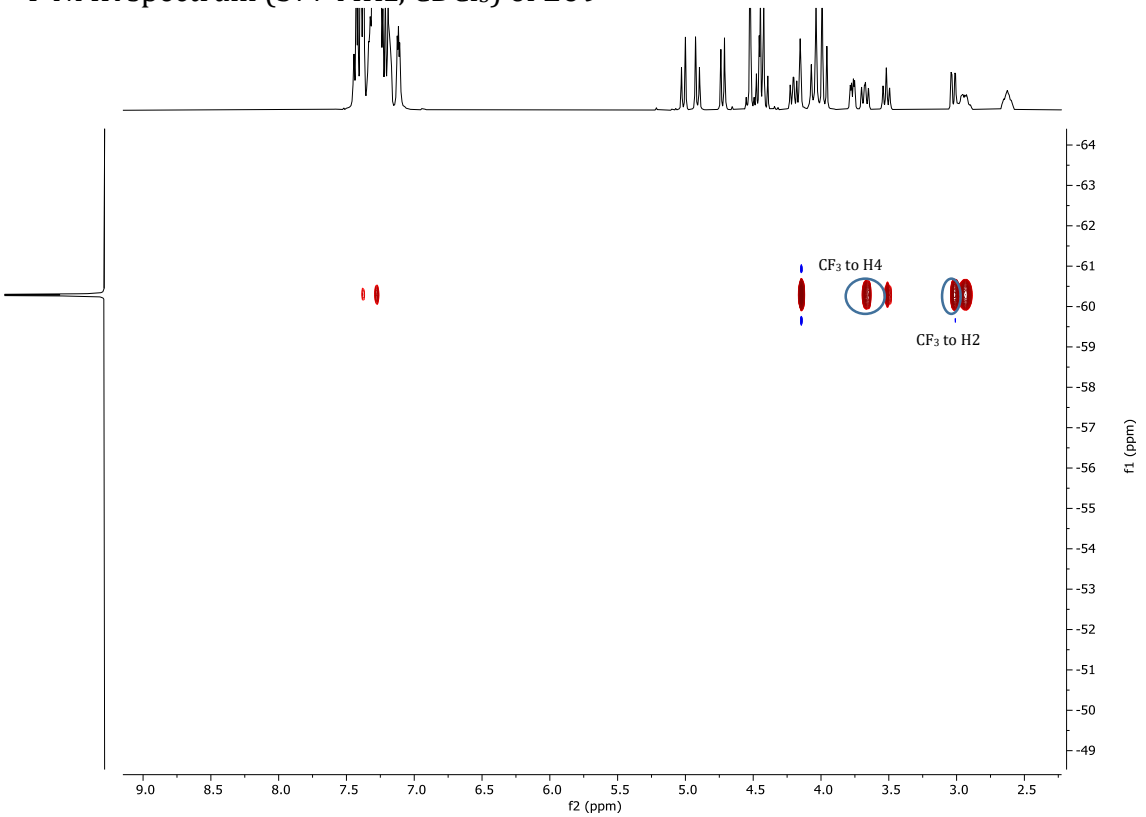
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **209**



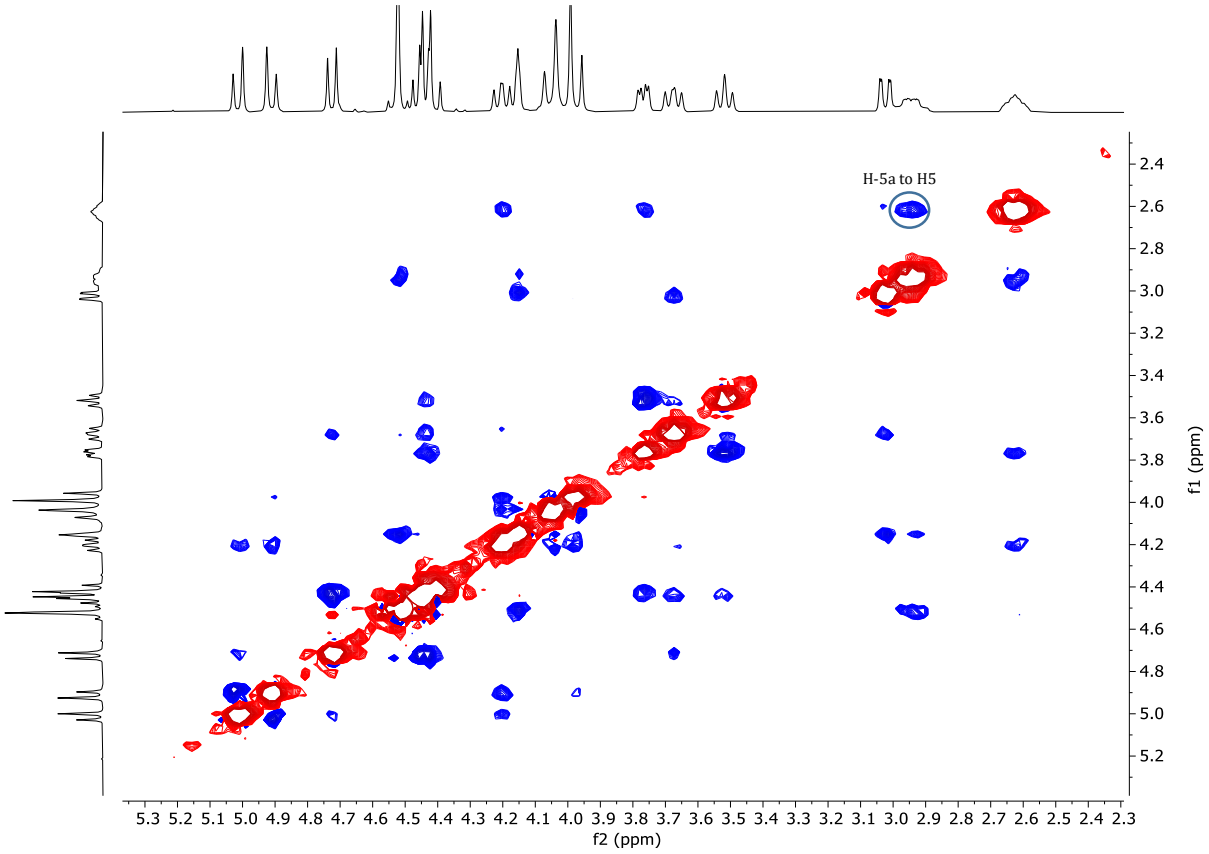
^{13}C NMR spectrum (121 MHz, $CDCl_3$) of **209**



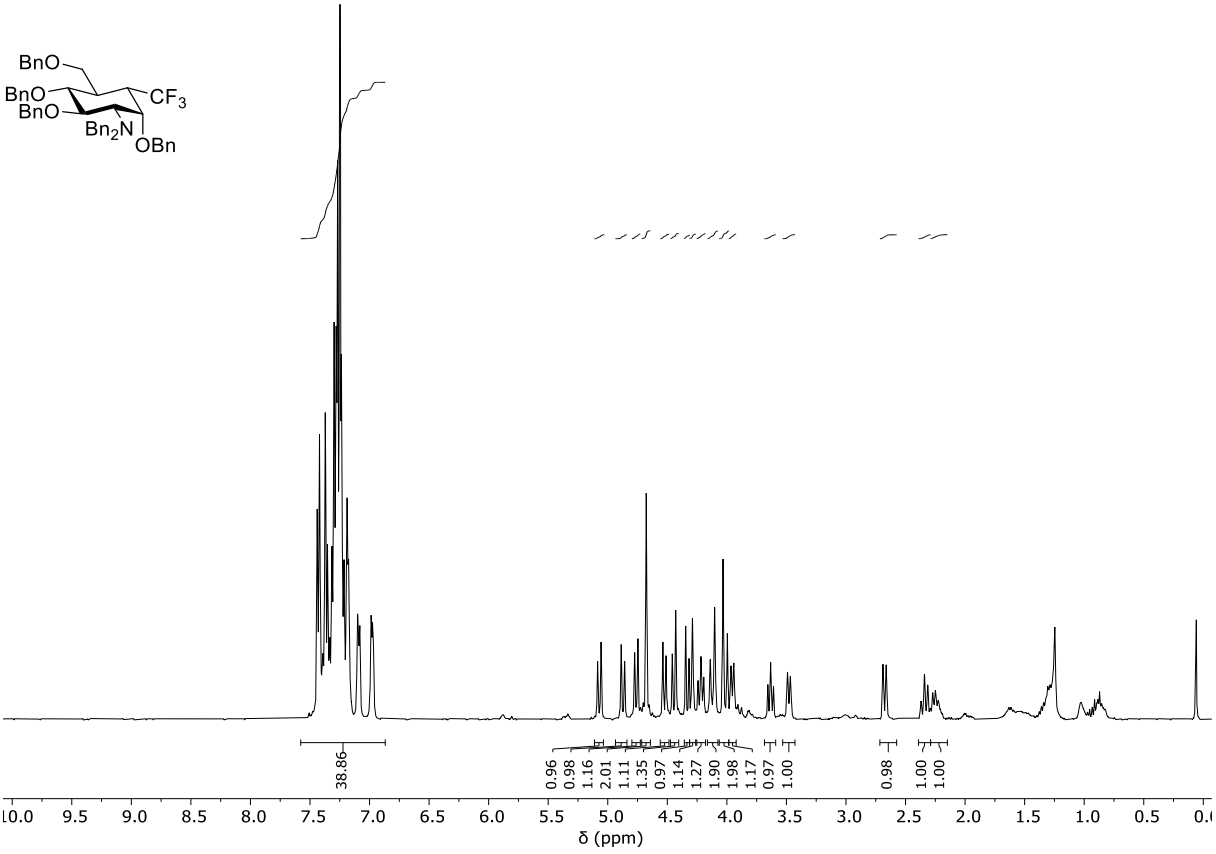
^{19}F NMR spectrum (377 MHz, $CDCl_3$) of **209**



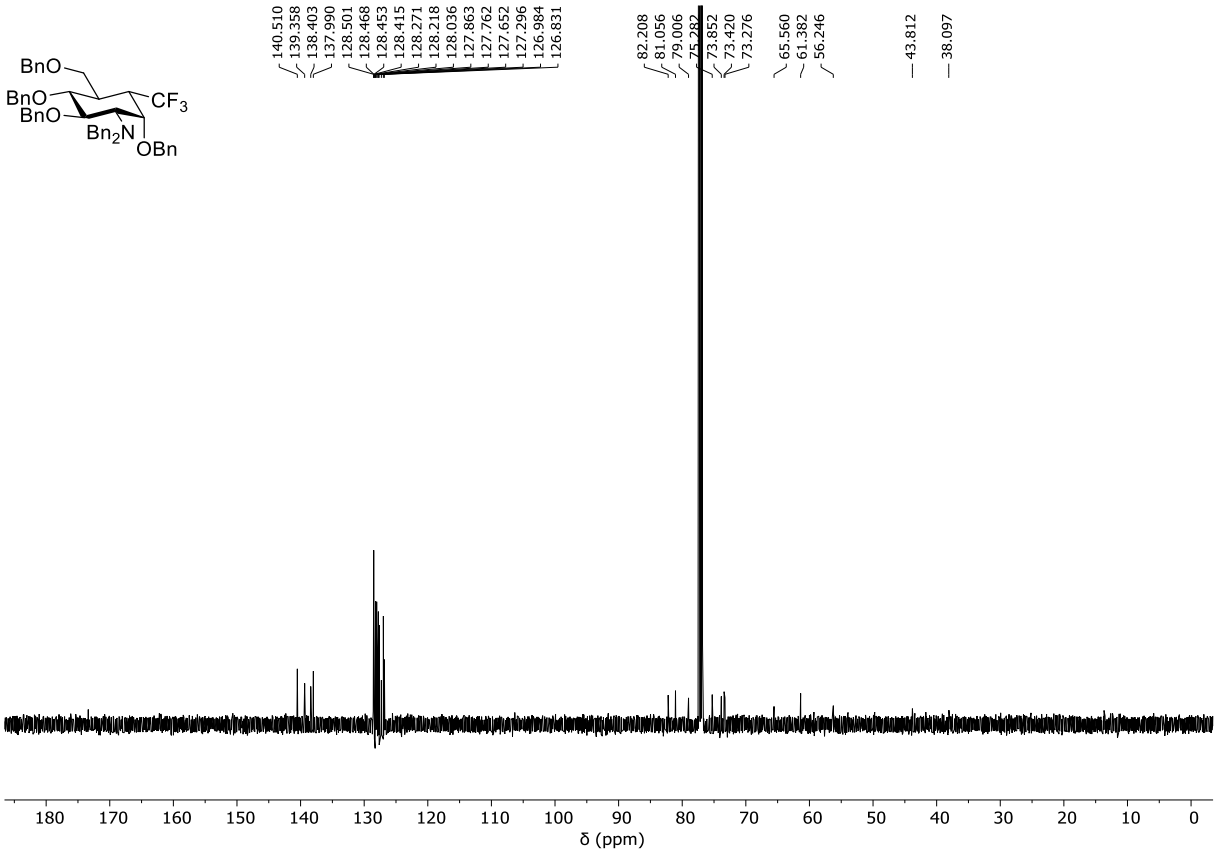
^{19}F-^{1}H HOESY spectrum of **209**



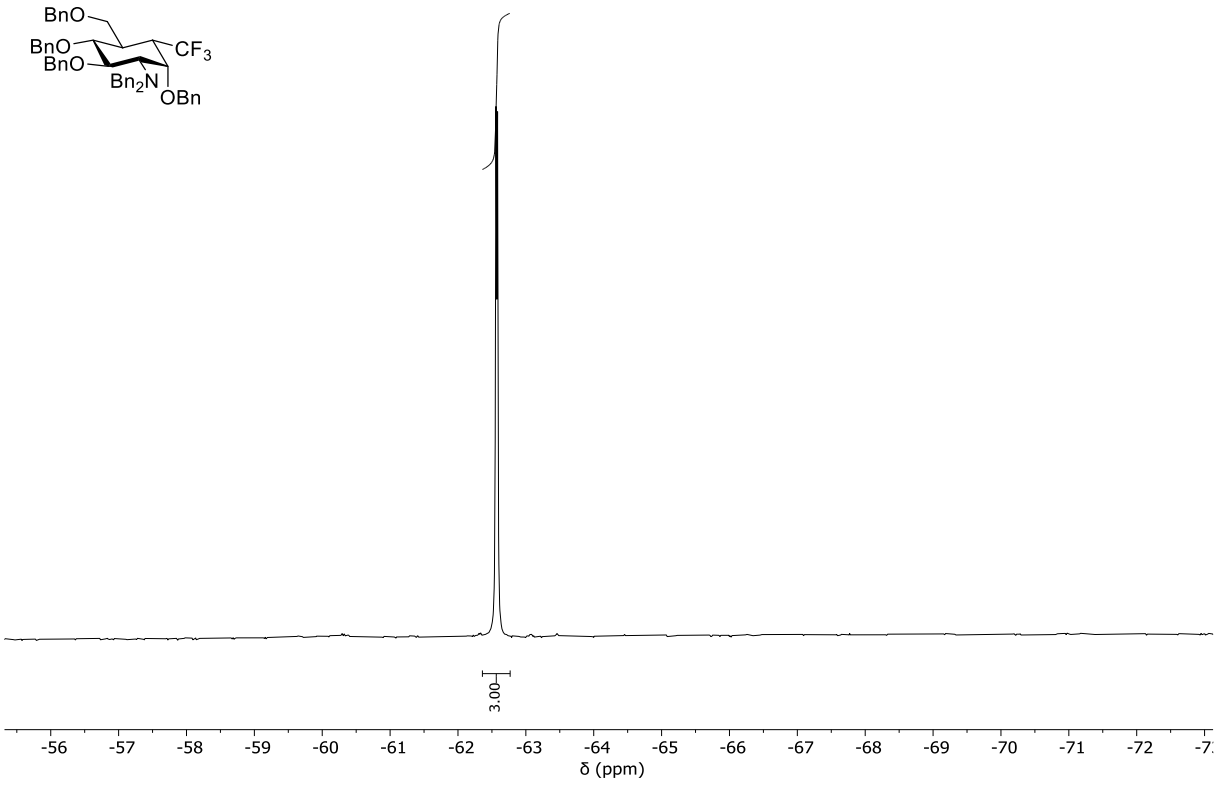
NOESY spectrum of **209**



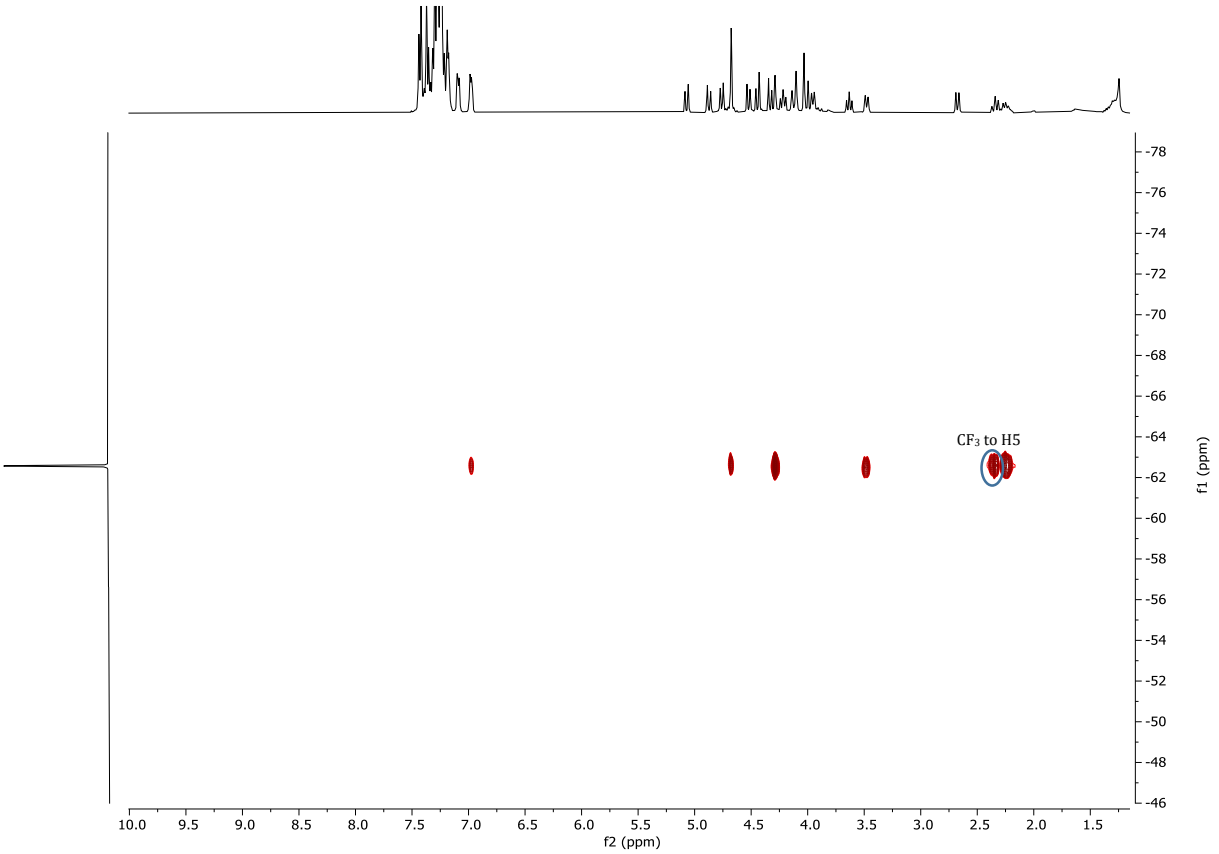
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **210**



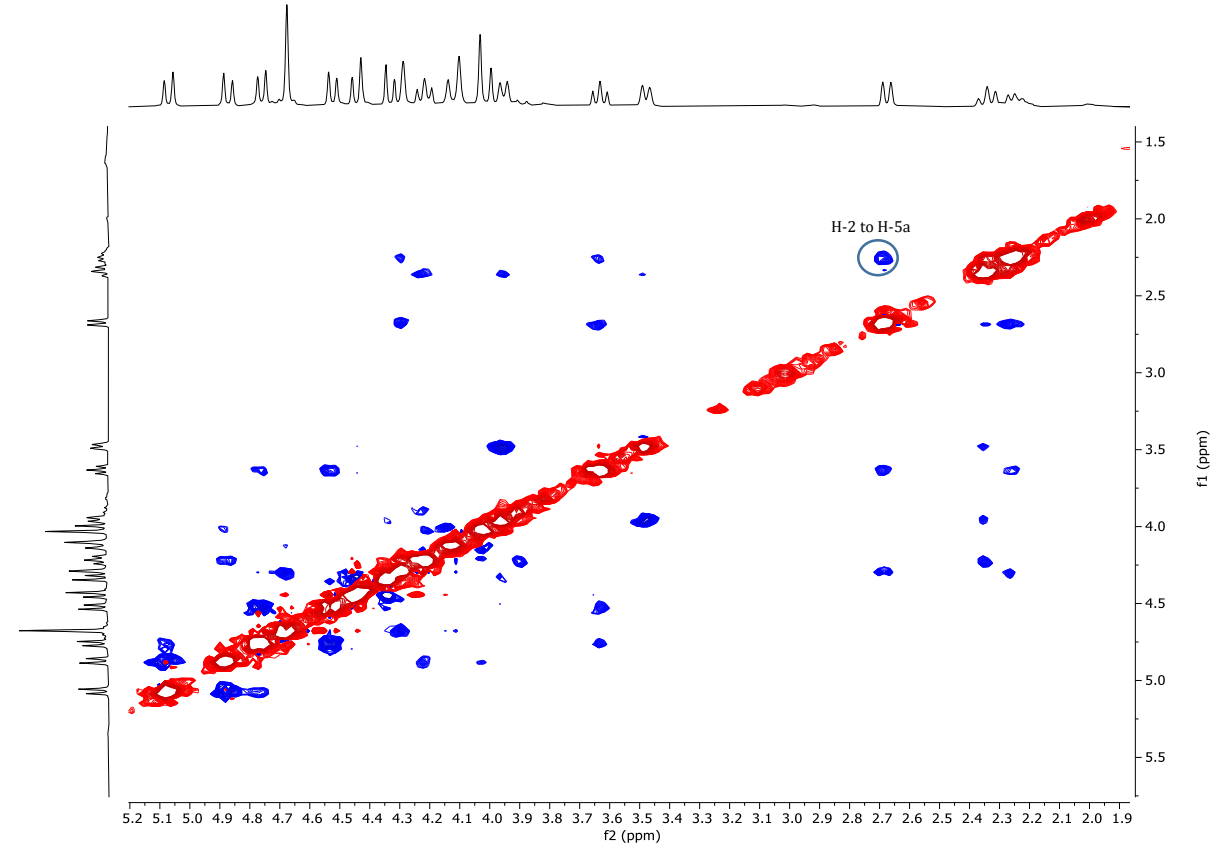
^{13}C NMR spectrum (121 MHz, $CDCl_3$) of **210**



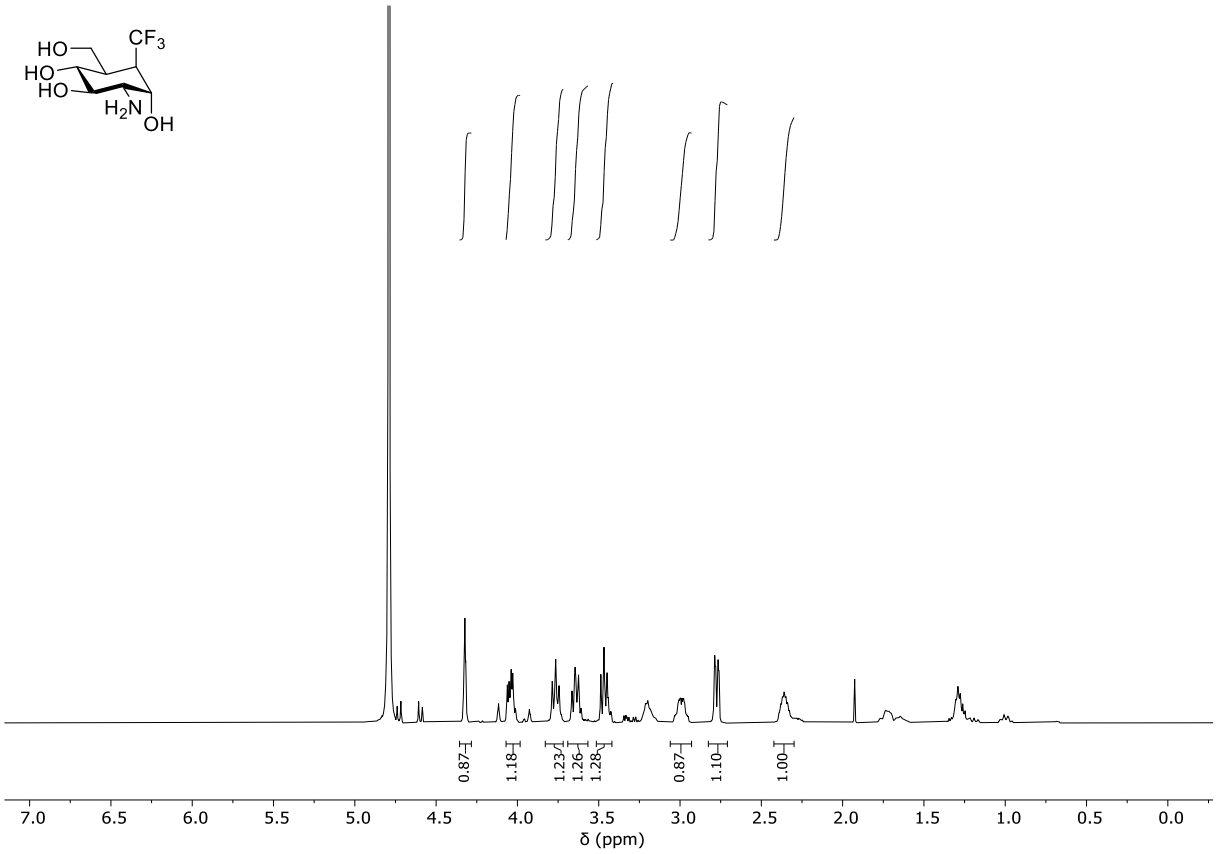
^{19}F NMR spectrum (377 MHz, $CDCl_3$) of **210**



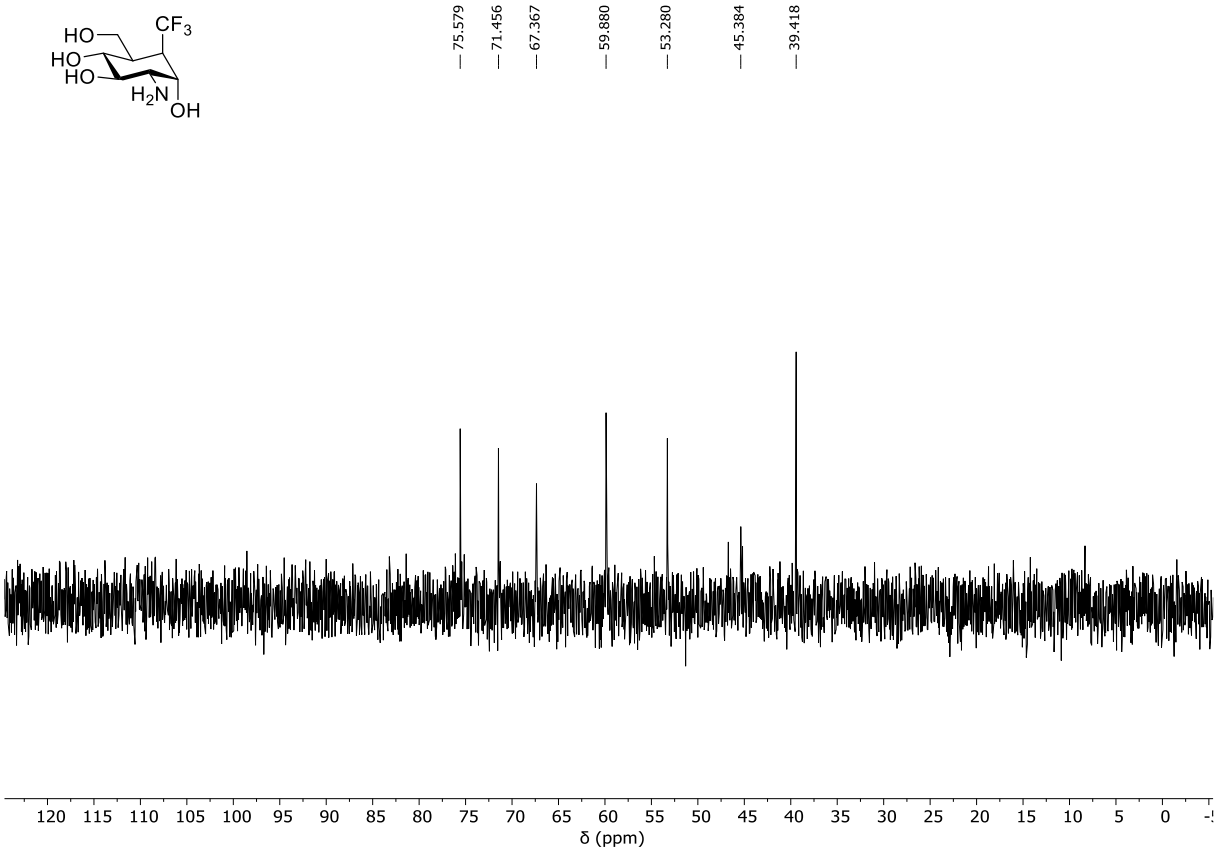
^{19}F-^{1}H HOESY spectrum of **210**



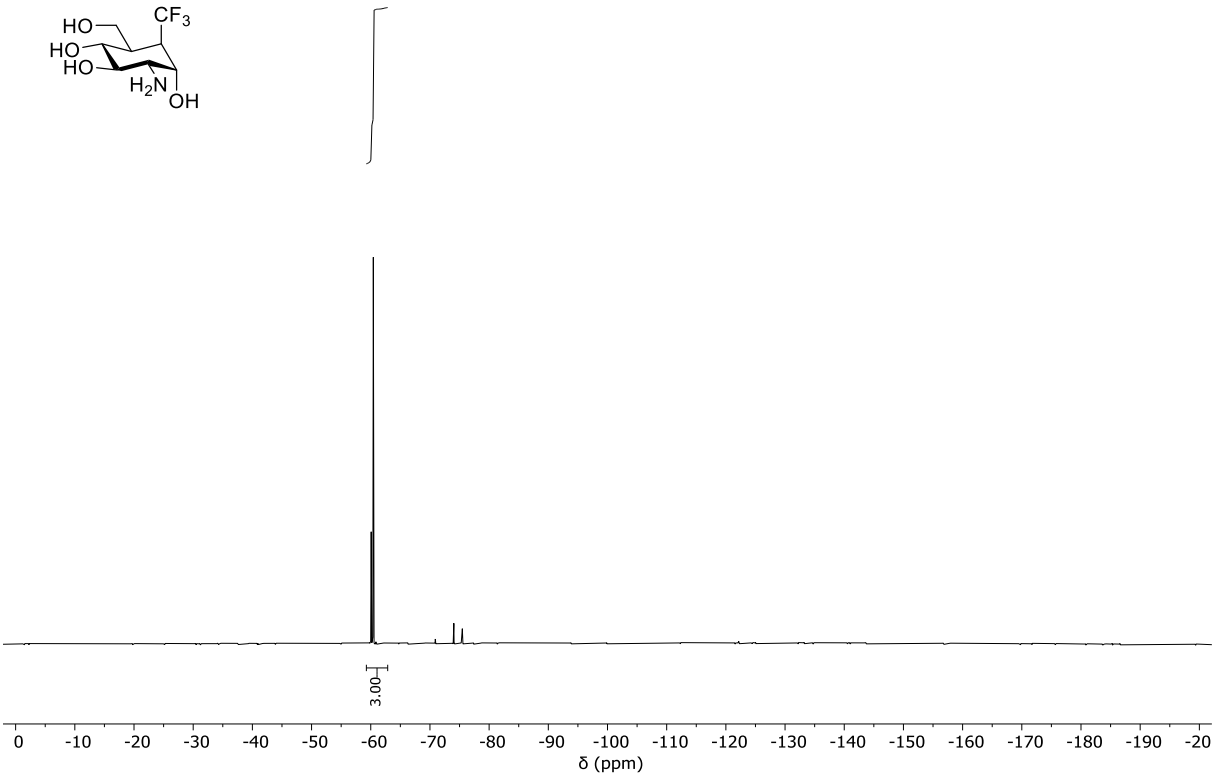
NOESY spectrum of **210**



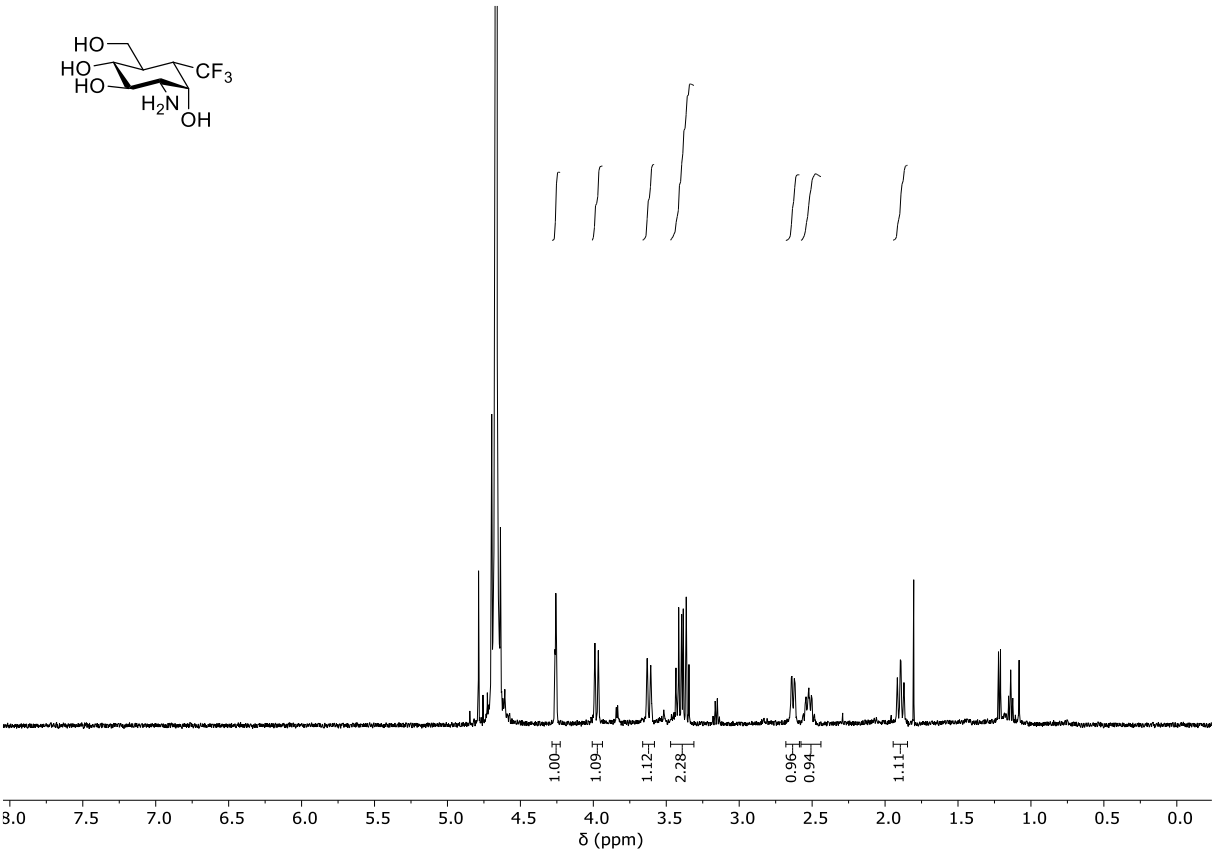
^{1}H NMR spectrum (500 MHz, D_2O) of **211**



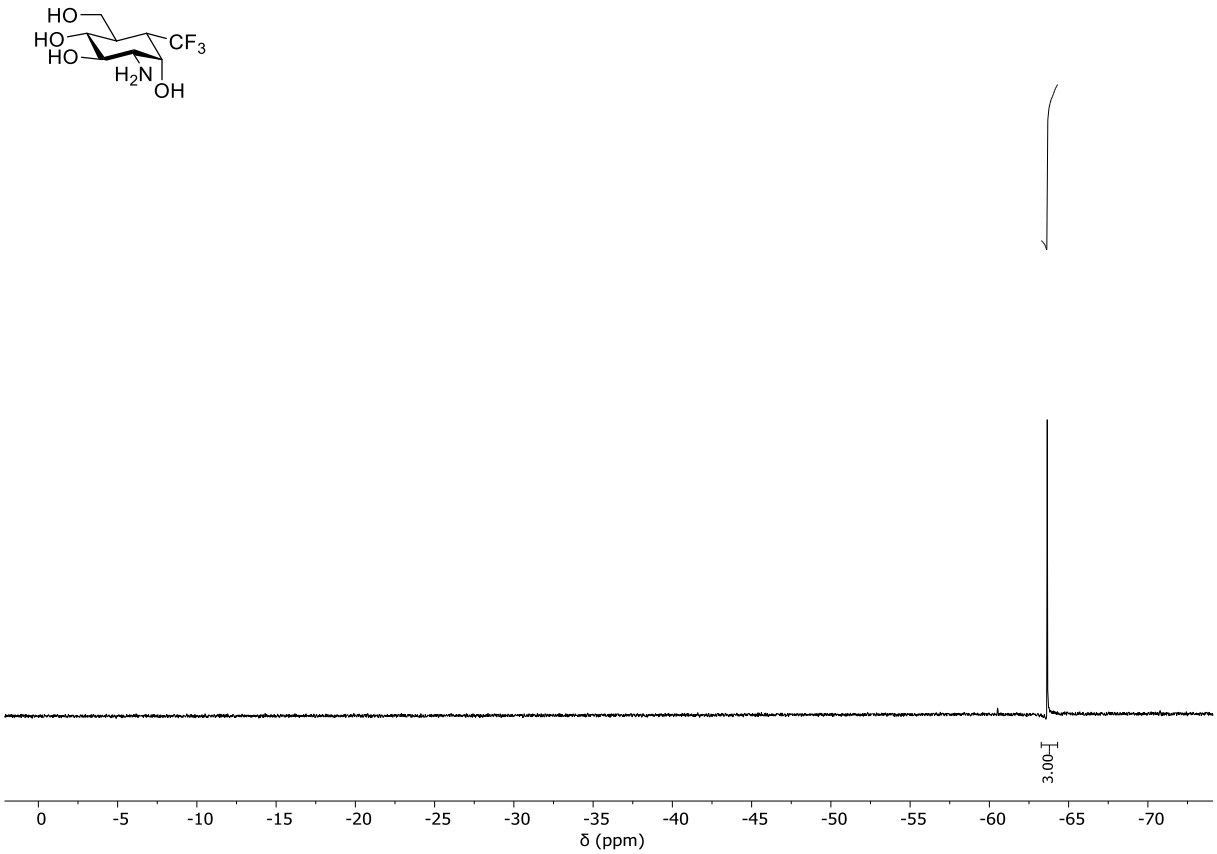
^{13}C NMR spectrum (126 MHz, D_2O) of **211**



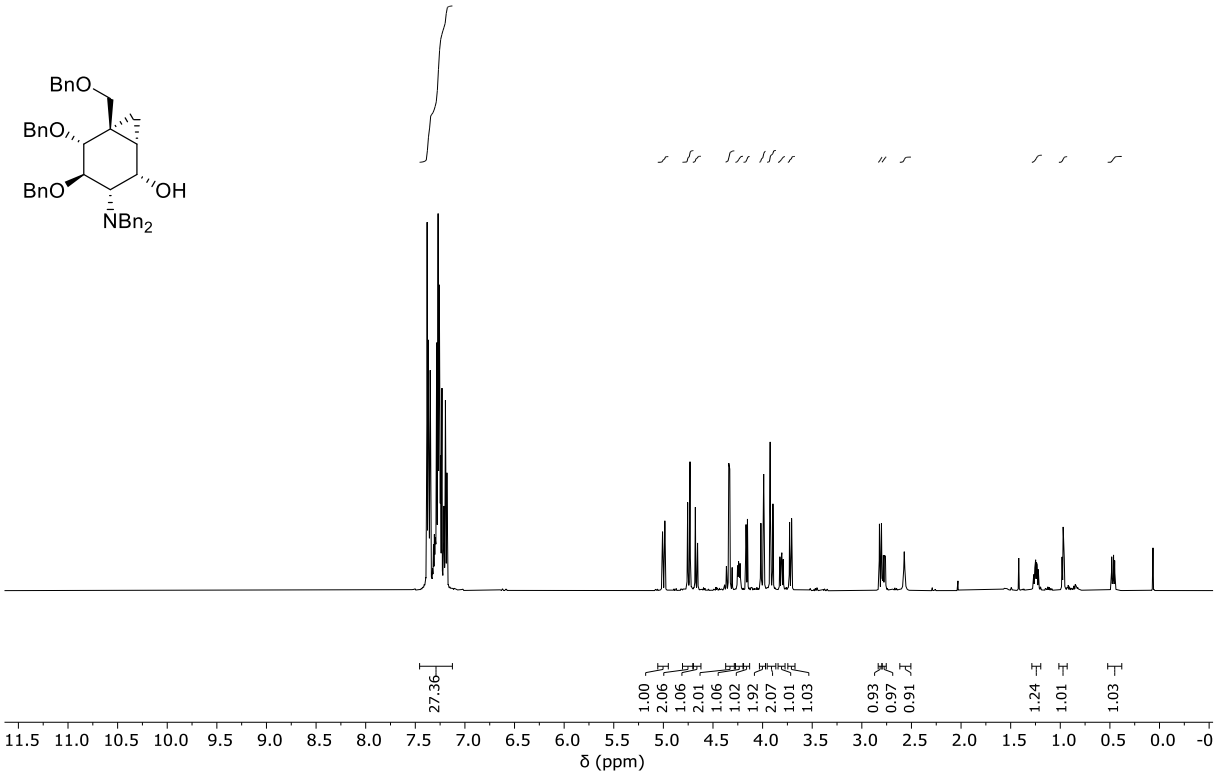
^{19}F NMR spectrum (471 MHz, D_2O) of **211**



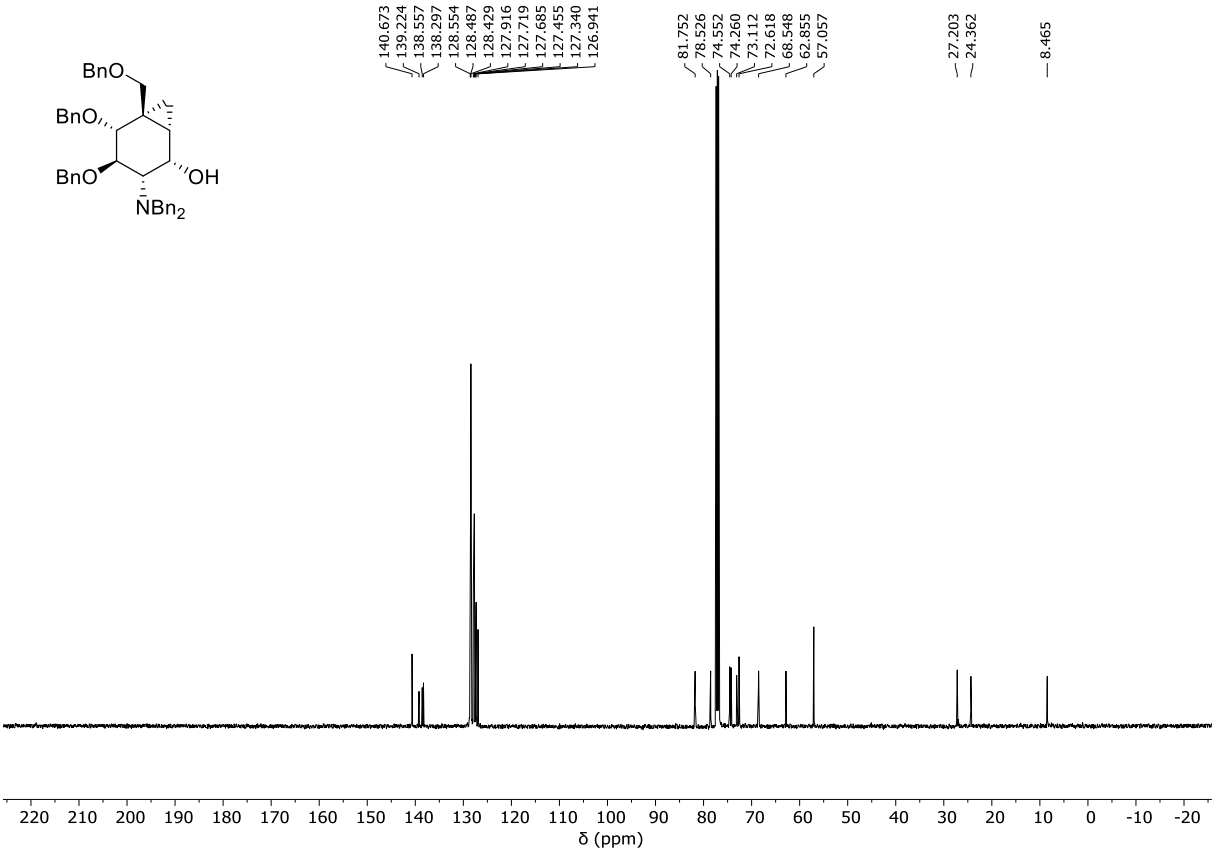
^{1}H NMR spectrum (500 MHz, D_2O) of **212**



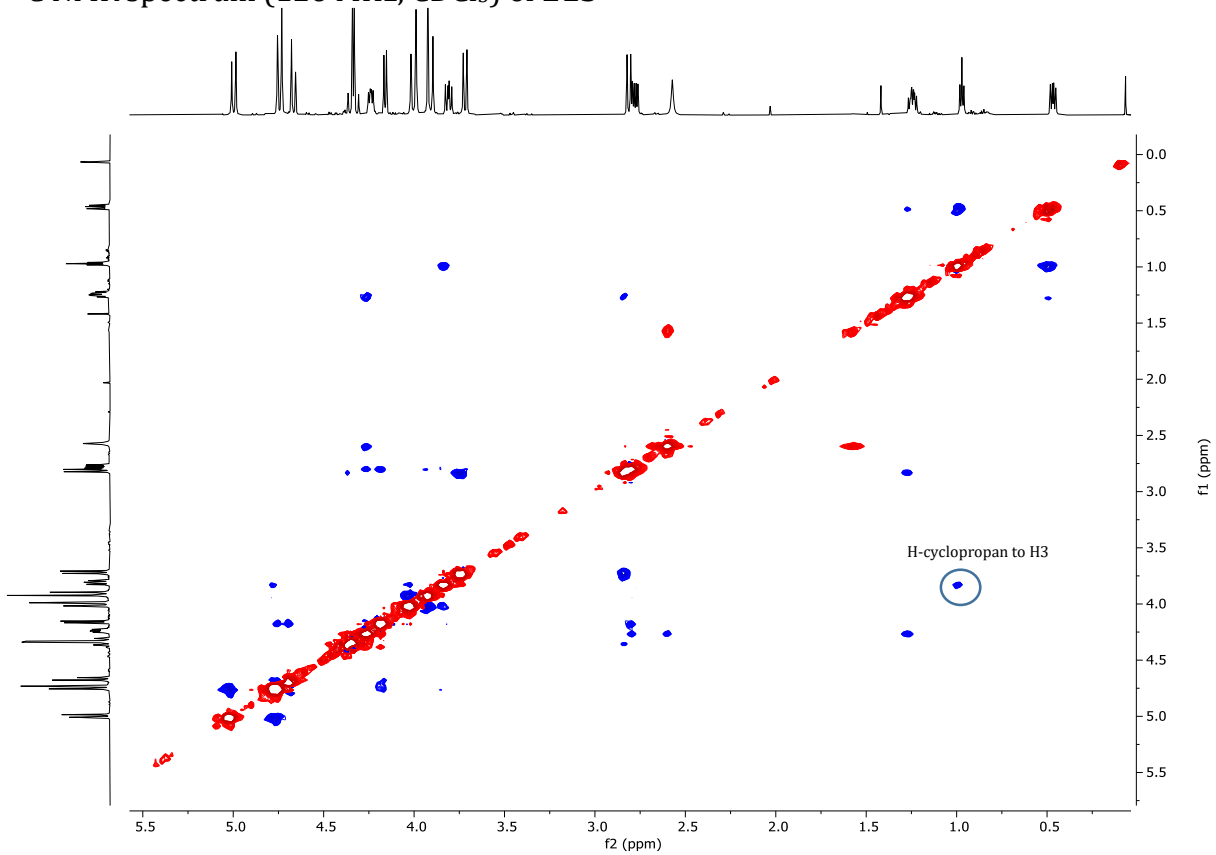
^{19}F NMR spectrum (471 MHz, D_2O) of **212**



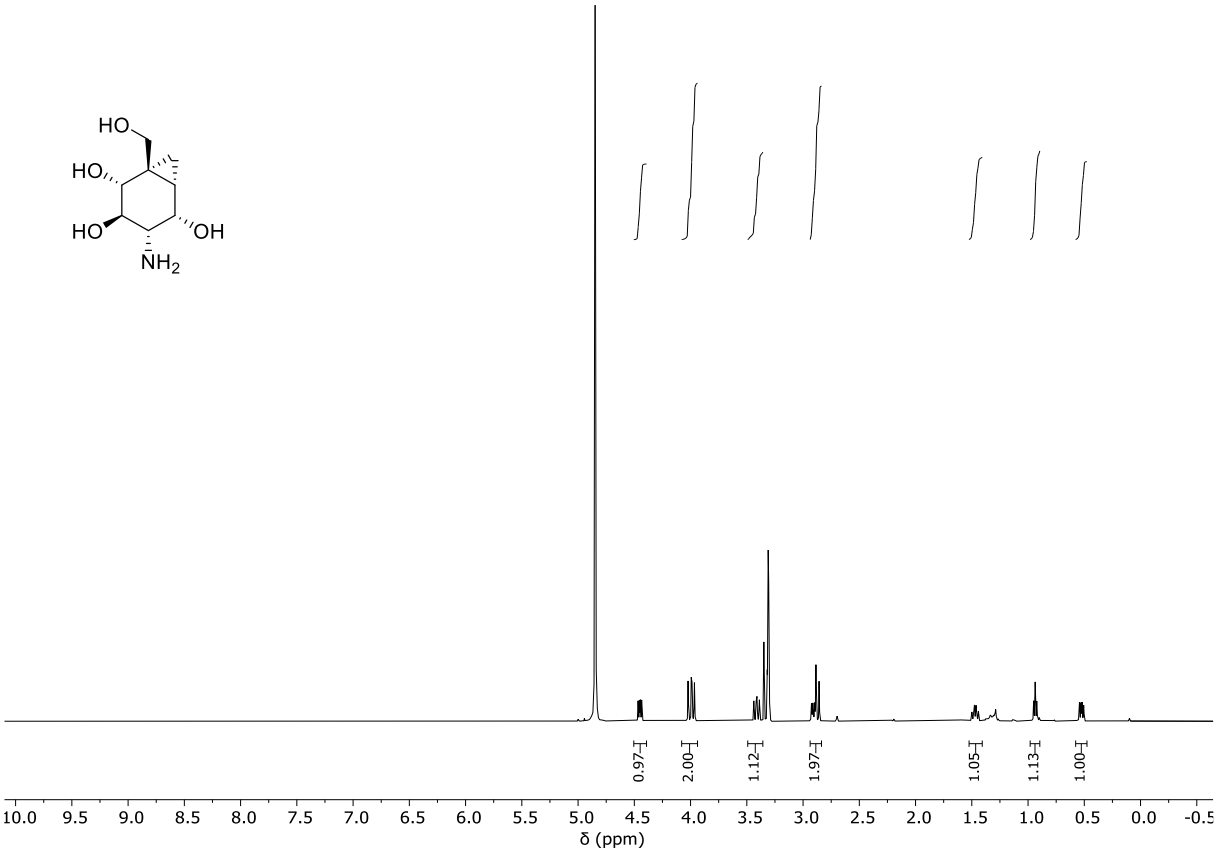
^{1}H NMR spectrum (500 MHz, $CDCl_3$) of **213**



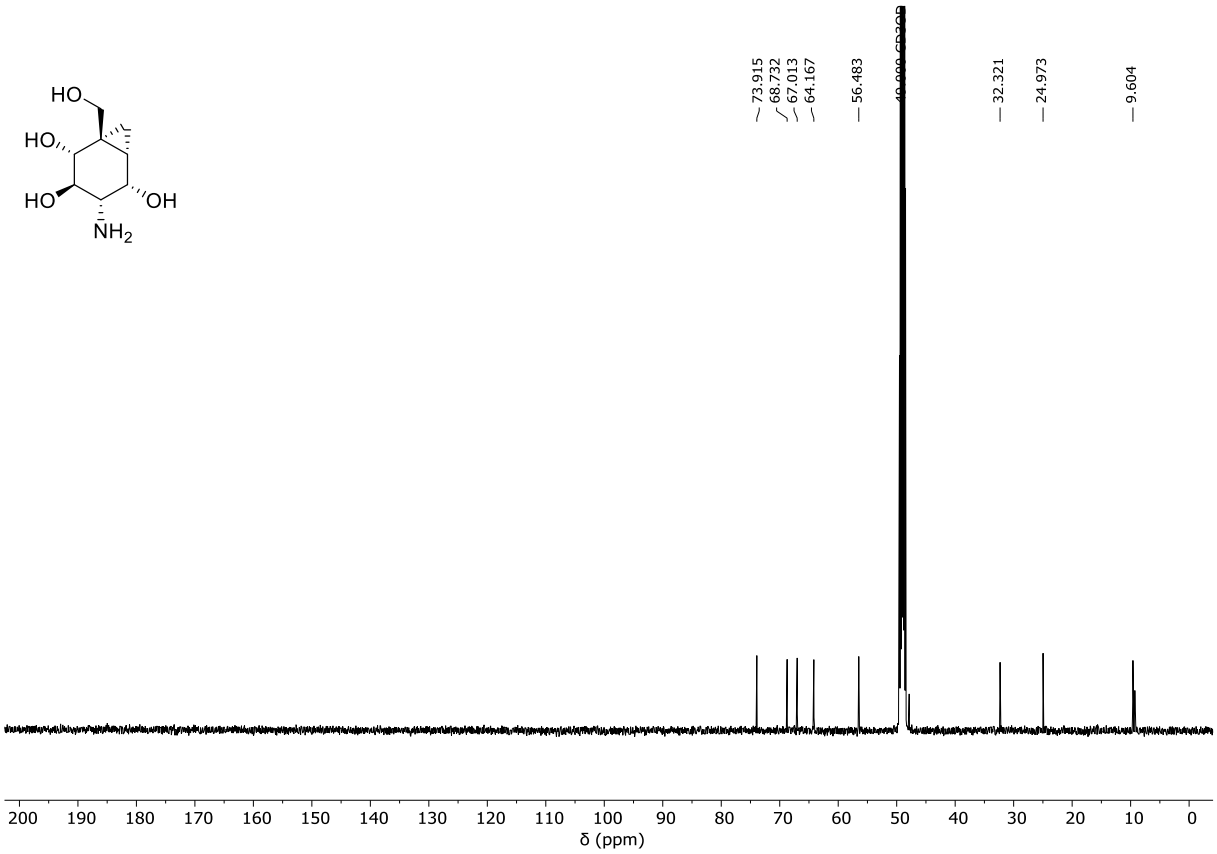
^{13}C NMR spectrum (126 MHz, $CDCl_3$) of **213**



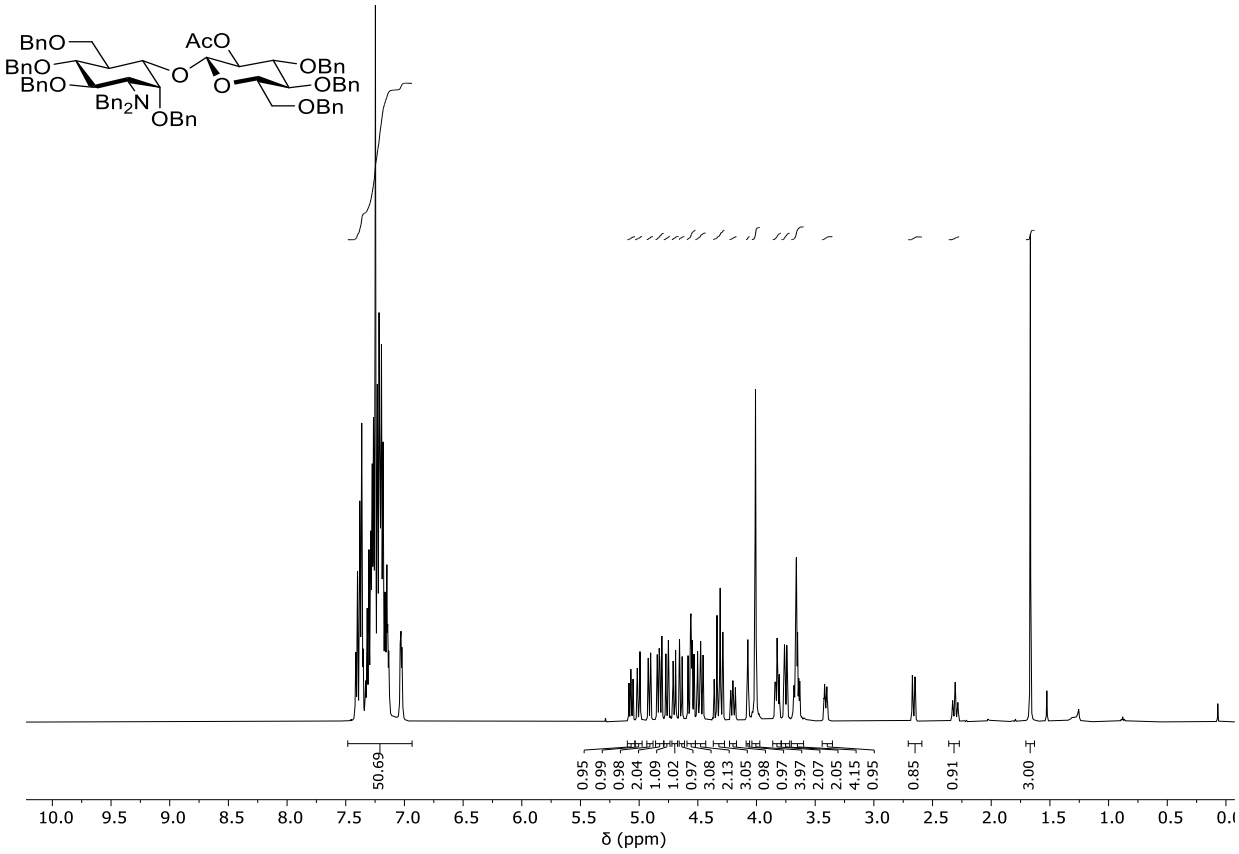
NOESY spectrum of **213**



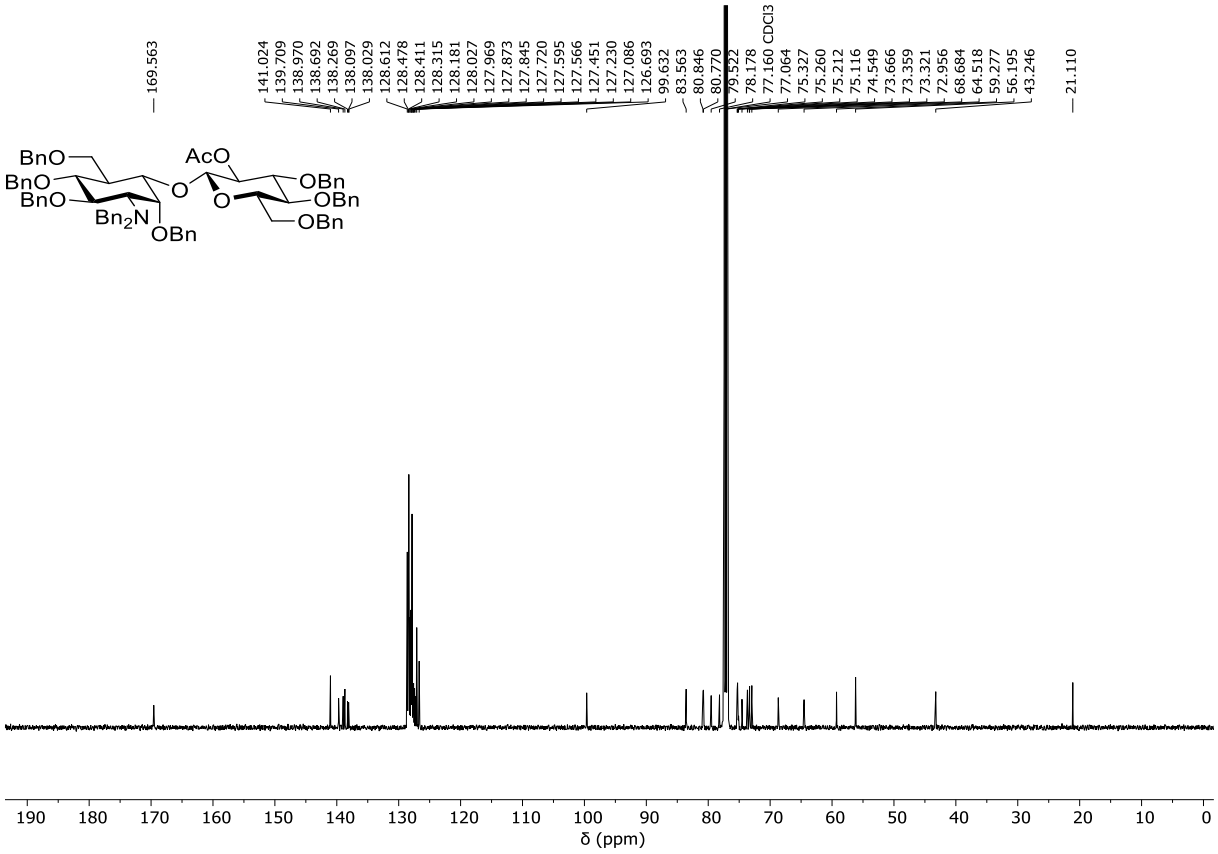
^{1}H NMR spectrum (500 MHz, D_2O) of **214**



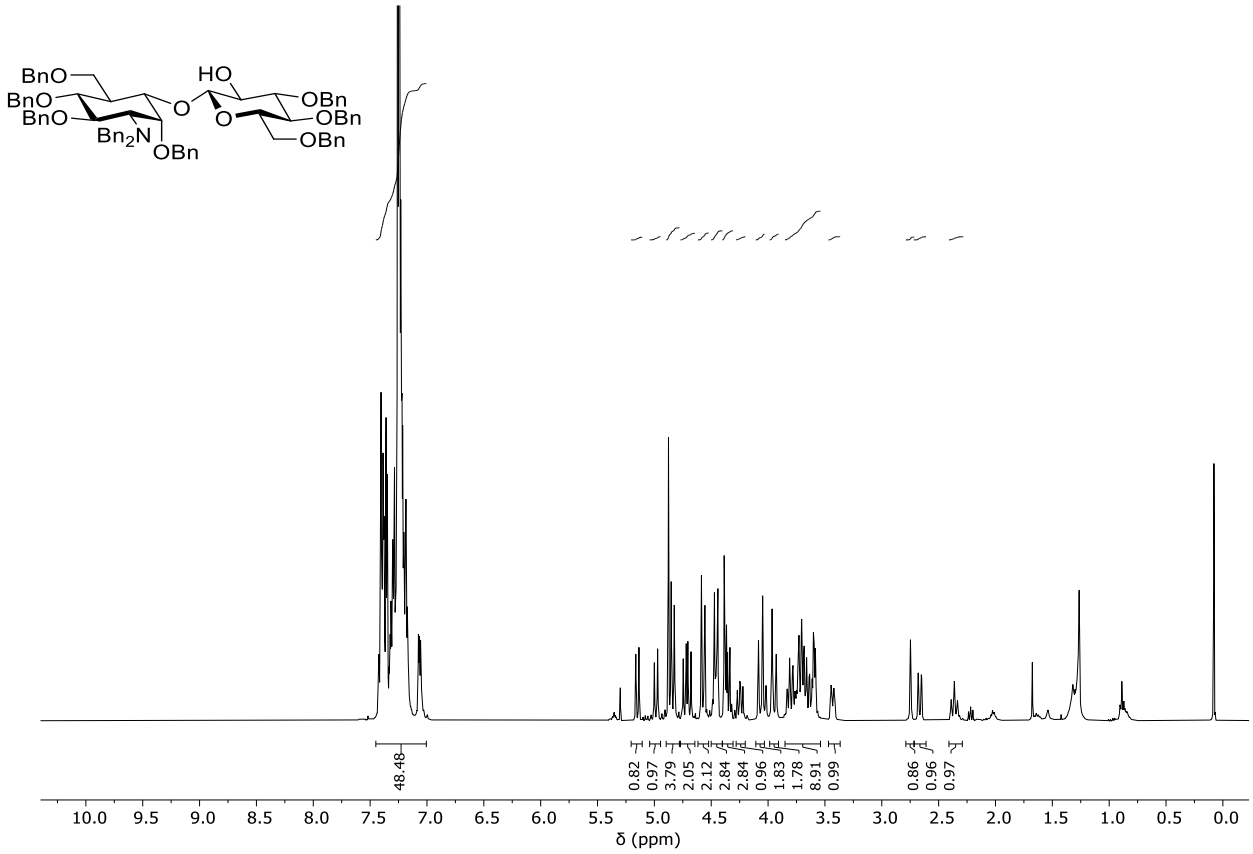
^{13}C NMR spectrum (126 MHz, CD_3OD) of **214**



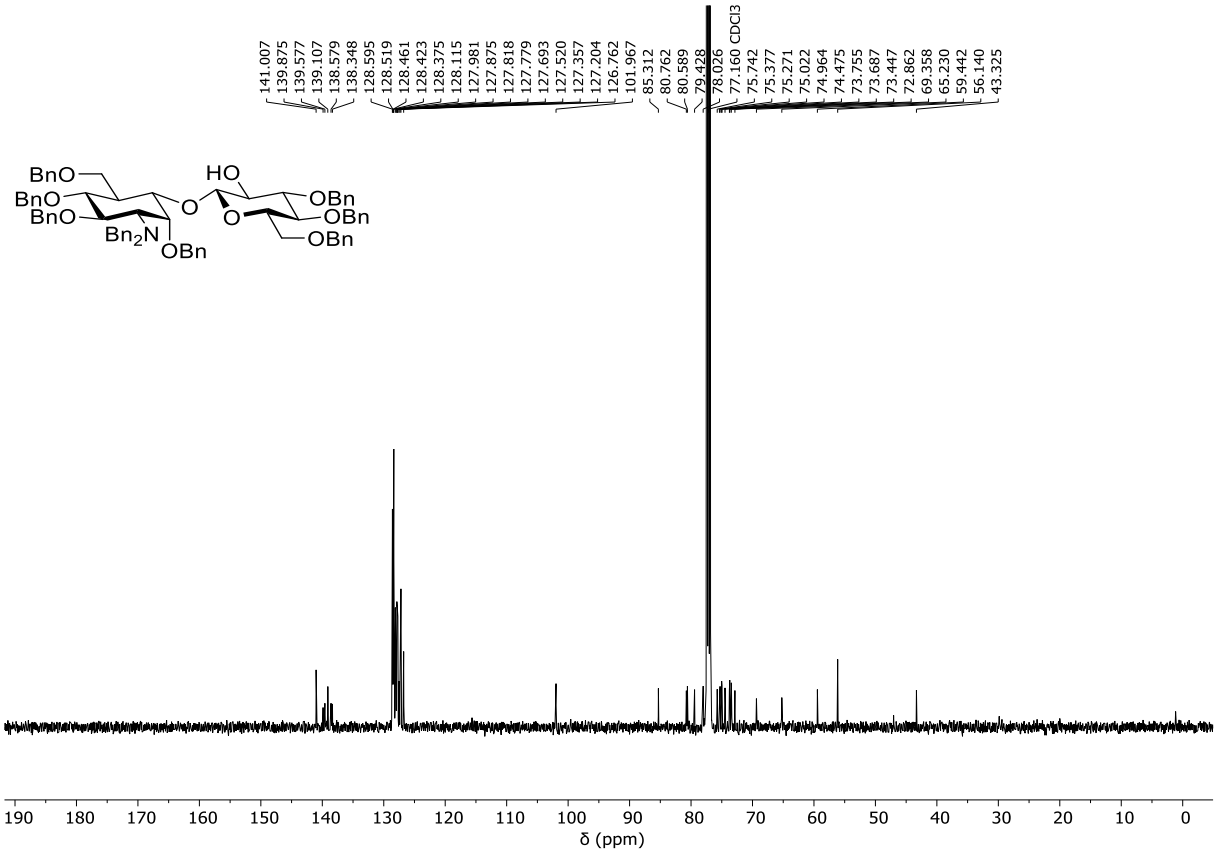
^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of **216**



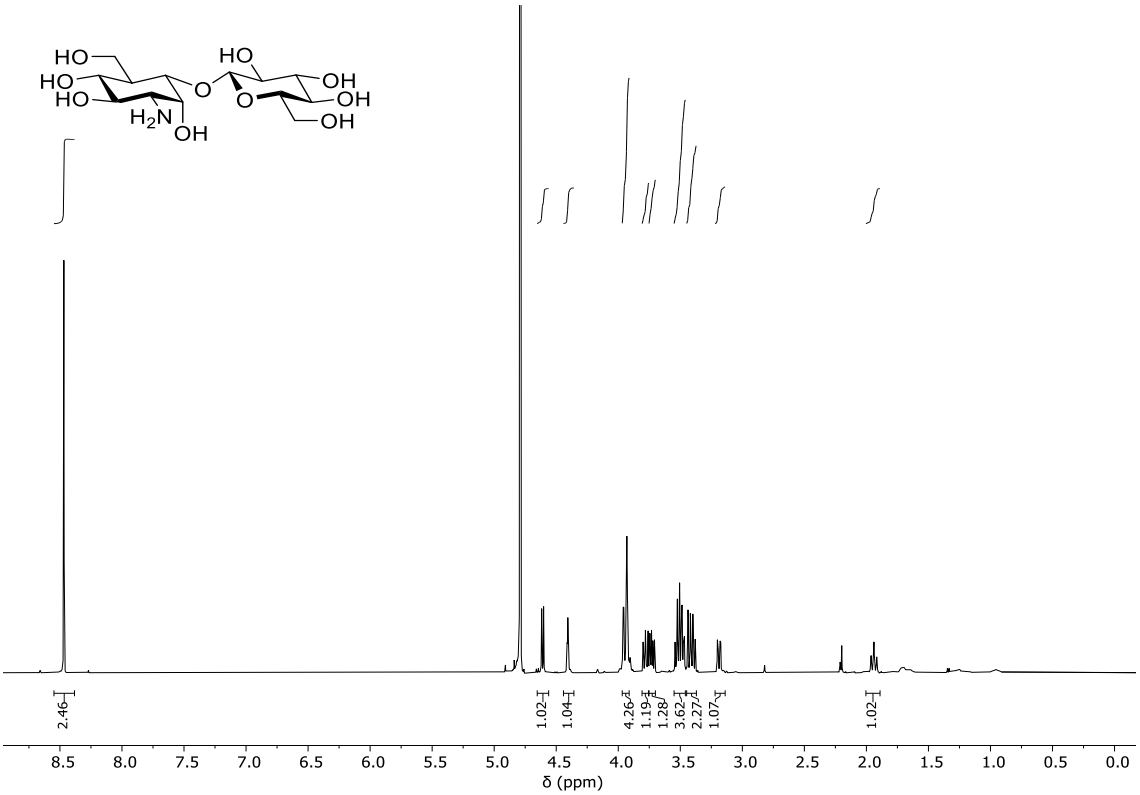
^{13}C-NMR (121 MHz, $CDCl_3$) spectrum of **216**



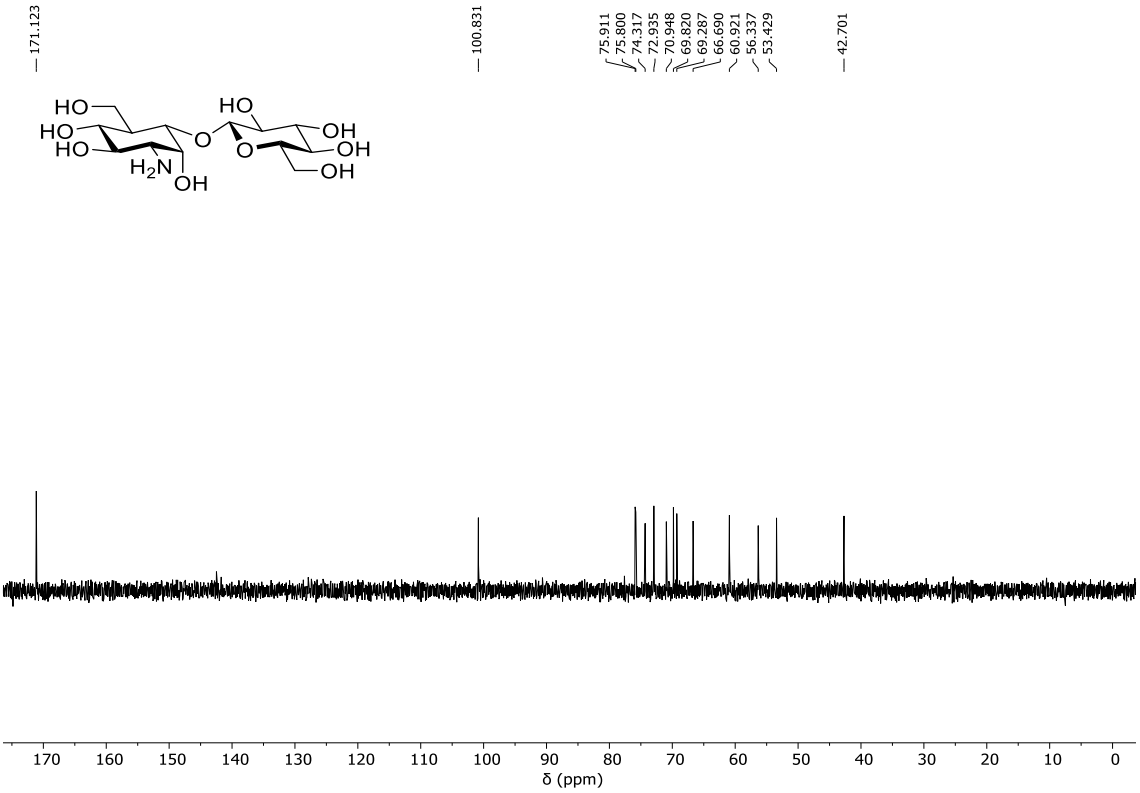
^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **217**



^{13}C-NMR (121 MHz, $CDCl_3$) spectrum of **217**



^{1}H-NMR (500 MHz, D_2O) spectrum of **218**



^{13}C-NMR (121 MHz, D_2O) spectrum of **218**