Georg Schitter

Pharmaceutical Chaperones for GM1-Gangliosidosis and Morbus Morquio B: Synthesis of Lipophilic and Fluorophilic 1-Deoxy- D-galactonojirimycin Derivatives

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Diese Arbeit wurde in Zusammenarbeit mit Prof. Stephen G. Withers (University of British Columbia, Vancouver, Canada), Prof. Michael B. Tropak (Hospital for Sick Children, Toronto, Canada) und Prof. Eduard Paschke (Medizinische Universität Graz) durchgeführt.

Meinen Eltern und Sonja

The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny...'

- Isaac Asimov -

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Abstract

A novel concept for the treatment of lysosomal storage diseases is the so-called chemical chaperone therapy in which glycosidase inhibitors are administered in subinhibitory concentrations, based on the effect that active-site specific inhibitors can aid the folding of proteins, thus protecting them from premature degradation. In this work, the synthesis of putative pharmaceutical chaperones for the D-galactosidase deficiency related diseases GM1-gangliosidosis, Morbus Morqiuo B, and Morbus Fabry is described. The substances are based on the powerful D-galactosidase inhibitor 1-deoxygalactonojirimycin (DGJ), which is derivatized by N-substitution. The choice of the substituents was made based on the observation that large, lipophilic moieties improve inhibitor as well as chaperone potential. In this work, substances with lipophilic as well as amphiphilic N-substituents have been prepared. Another approach of this work is the synthesis of fluorophilically derivatized DGJ derivatives to exploit the further increase of lipophilicity as well as making the inhibitors available for fluorous methodologies. The scaling-up of DGJ-lysine-hybrids comprises the final part of the work, since larger amounts of these substances are needed to conduct biological experiments. All newly synthesised substances were tested for inhibitor activity which was performed by collaboration with partners at the University of British Columbia. Investigations toward the chaperone potential of the inhibitors were carried out at the Medical University Graz (patient cell-lines) as well as at the Hospital for Sick Children in Toronto (patient and feline fibroblasts). Preliminary results of these studies are discussed in the work and support the working hypothesis.

Kurzfassung

Die Erforschung der Therapie lysosomaler Speichererkrankungen mit pharmazeutischen Chaperonen gewann in den letzten Jahren zunehmend an Bedeutung. In diesem Ansatz werden Glycosidaseinhibitoren in subinhibitorischen Konzentrationen verabreicht, um die Faltung von Proteinmutanten zu erleichtern und diese vor dem vorzeitigen Abbau zu schützen. Die Arbeit behandelt die Synthese pharmazeutischer Chaperone für die Therapie der lysosomalen Speicherkrankheiten GM1-Gangliosidose, Morbus Morquio B und Morbus Fabry, bei denen lysosomale Galactosidasen von Mutationen betroffen sind. Die synthetisierten Substanzen sind Derivate des D-Galactosidaseinhibitors 1-Desoxygalactonojirimycin, deren Aktivität durch geeignete Substitution des Ringstickstoffes moduliert und die biologische Aktivität verbessert wird. Schwerpunkt ist die Einführung großer, unpolarer N-Substituenten, da gezeigt werden konnte, dass eine hydrophobe Substitution die Aktivität der Inhibitoren steigern kann. Der Einfluss ionisierbarer Seitenketten wird in der Arbeit ebenfalls behandelt. Ein weiterer Schwerpunkt der Arbeit ist die Synthese fluorophil substituierter Iminozucker, um die Hydrophobie des Systems weiter zu steigern und die Chaperone der „Fluorous Chemistry“ zugänglich zu machen. Weiters wurde ein Scale-Up von Iminozucker-Lysin-Hybriden durchgeführt, um größere Mengen dieser Substanzen für biologische Tests zur Verfügung zu stellen. Die neuartigen 1-Desoxygalactonojirimycinderivate wurden von Kooperationspartnern der University of British Columbia auf ihre Inhibitoraktivität und von Kooperationspartnern der MedUni Graz (Patientenzellinien)und des Hospital for Sick Children in Toronto (feline und humane Fibroblasten) auf ihre Chaperonaktiviät getestet. Erste Ergebnisse dieser Untersuchungen sind vielversprechend und unterstützen die Arbeitshypothese.

Contents

Abbreviations

(F)-SPE	(fluorous) solid phase extraction
(p)TLC	(preparative) thin layer chromatography
ABG	*Agrobacterium sp*. β-glycosidase
$[\alpha]_D^{20}$	specific optical rotation
α-Gal A	lysosomal α-galactosidase
α-Gal GCB	α-galactosidase from green coffee beans
ASSC	active-site specific chaperone
BBB	blood-brain barrier
β-Gal EC	β-galactosidase from *E. coli*.
BMT	bone marrow transplantation
BOC	tert-butoxycarbonyl
CBz / Z	benzyloxycarbonyl
Cy	cyclohexane
DAB	1,4-dideoxy-1,4-imino-D-arabinitol
DEAD	diethyl azodicarboxylate
DGJ	1-deoxygalactonojirimycin
DMAP	4-dimethylaminopyridine
DMDP	2,5-dideoxy-2,5-imino-D-mannitol
DMF	*N,N*-dimethyl formamide
DMJ	1-deoxymannojirimycin
DNA	deoxyribonucleic acid
DNJ	1-deoxynojirimycin
DNS	dansyl-
ER	endoplasmatic reticulum
ERAD	endoplasmatic reticulum associated degradation
ERT	enzyme replacement therapy
Et_2O	diethyl ether
Et_3N	triethylamine
EtOAc	ethyl acetate
GlcCer	glucosyl ceramide
GSL	glycosphingolipid
HBTU	*O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluoro-phosphate
HGJ	homogalactonojirimycin
HNJ	homonojirimycin
IFG	isofagomine
iPr_2NEt	diisopropyl ethyl amine
KOtBu	potassium tert-butoxide
LSD	lysosomal storage disease
Lys	lysine
m.p.	melting point
mCPBA	3-chloroperbenzoic acid
MeOD	methanol-d_4
MeOH	methanol
MW	molecular weight
NaOMe	sodium methoxide
NB-DNJ	*N*-butyl-1-deoxynojirimycin
NJ	nojirimycin
NMR	nuclear magnetic resonance
NOEV	*N*-octyl-4-*epi*-valienamine
PCT	pharmaceutical chaperone therapy
PC	pharmaceutical chaperone
PDMP	D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol
Ph_3P	triphenylphosphine
SGC	silica gel chromatography
SRT	substrate reduction therapy
TBTU	*O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate
THF	tetrahydrofurane
UDP	uridine diphosphate
Ts	4-toluenesulfonyl-

1 Introduction

1.1 Carbohydrates

Carbohydrates, seen from a historical point of view, are organic compounds with the molecular formula $C_n(H_2O)_n$, leading to the name by formally being hydrates of carbon. Commonly, the term carbohydrate is used as a synonym of saccharide, or sugar.

This relatively strict definition of carbohydrates has been changed over the decades, since on the one hand not all saccharides (*e.g.*, deoxy- or aminosugars, sugar acids) fulfil this rule, and on the other hand, molecules not considered being carbohydrates (*e.g.* formaldehyde, inositols) however do.

A more applied definition of carbohydrates includes polyhydroxyaldehydes or –ketones (saccharides), as well as the derivatives that can be obtained by either reduction (alditols) or oxidation (sugar acids) of saccharides as well as deoxygenated derivatives and molecules with heteroatoms such as nitrogen or sulphur. This definition also includes oligo- or polymeric saccharides that can be converted to the respective monomers by hydrolysis.

The monomers of oligo- and polysaccharides are so-called monosaccharides, being the simplest group of saccharide compounds: Polyhydroxylated aldehydes (aldoses) or- ketons (ketoses).

Besides the position of the carbonyl functionality, monosaccharides can be classified by the number of carbon atoms, starting from glycerol aldehyde (C_3), forming the groups of tetroses (C_4), pentoses (C_5), hexoses (C_6) etc.

Di- and oligosaccharides consist of two up to 10 monosaccharide units linked together by so-called glycosidic linkages. If a saccharide is linked by two anomeric hydroxyl functions, the resulting disaccharide is called non-reducing (*e.g.*, trehalose, sucrose), whereas the linkage between an anomeric and an alcoholic hydroxyl function yields a reducing sugar (*e.g.*, lactose, maltose).

Polysaccharides are biomacromolecules of immense biological importance as energy storage or structural components. For example, cellulose is the most abundant organic compound concerning its annual production. Other polysaccharides such as chitin are important constituents of the exo-skeleton of anthropods and insects.

Oligo- and polysaccharides can be built up from a single monomer forming the group of homooligo- or polysaccharides (*e.g.*, chitin, cellulose). Heterooligo- or polysaccharides show different monomers as structural elements (lipopolysaccharides, glycans).

Although carbohydrates are one of the most abundant classes of biomolecules, considerably little attention has been drawn to carbohydrate chemistry over the last decades, due to a number of reasons [1].

The chemical complexity of carbohydrates exacerbates their synthesis as well as the structural identification compared to proteins and nucleic acids. Due to the high functionalisation of the saccharides, a vast variety of structural and stereoisomerisms is possible already at low molecular weights.

From the eight most relevant monosaccharides found in mammals, already over one million of tetrasaccharides can be formed.

Thus, the synthesis and analysis of oligosaccharides is a time and resource intensive area of research, which is on the other hand of crucial importance for the understanding of physiological processes.

Pharmaceutical industries were sceptical about carbohydrate based drugs in the last decades, not only because of the complexity, but also because of their often non-ideal physicochemical behaviour, such as the problematic instability of glycosidic linkages or the poor ability of passing biological membranes due to their high polarity. Additionally, carbohydrate based drugs often show dissociation constants in the millimolar range, which is relatively high compared to other drug candidates [2].

This situation has changed significantly with the discovery of the impact of carbohydrates to cancer biology, cell-cell adhesion in inflammation and metastasis, or diabetes. In the field of glycobiology, the discovery, isolation, and study of glycoproteins, glycolipids and oligosaccharides has led to a deeper understanding of the importance of carbohydrates in physiological processes other than energy supply and scaffold formation.

1.2 Glycosidases and Glycosidase Inhibitors

In living organisms, carbohydrates are processed mainly by glycosidases and glycosyltransferases. These enzymes are involved in many biological processes ranging from intestinal digestion, lysosomal degradation of glycoconjugates, post-translational modification of glycoproteins to endoplasmatic reticulum associated degradation pathways.

The synthesis of glycosides is carried out by glycosyltransferases, using nucleotide activated glycosyl donors for the glycosylation of proteins, lipids or growing oligosaccharide chains.

Glycosidases are hydrolases cleaving monosaccharidic (exo-glycosidases) or oligosaccharidic (endo-glycosidases) units from glycoproteins, glycolipids or oligo- and polysaccharides. They are divided into two subgroups: Inverting and retaining glycosidases, depending on the stereochemical outcome of the cleavage reaction regarding the anomeric centre.

Inverting glycosidases cleave the aglycon under inversion of the anomeric configuration, which is in accordance with a direct attack of the nucleophile. A schematic representation of the S_N2-like mechanism [3] is depicted in Figure 1-1.

Kinetic isotope effects, measured on inverting glycosidases showed the same order of magnitude like retaining amylases indicating that a glycosyl-cation-like transition state is likely [4].

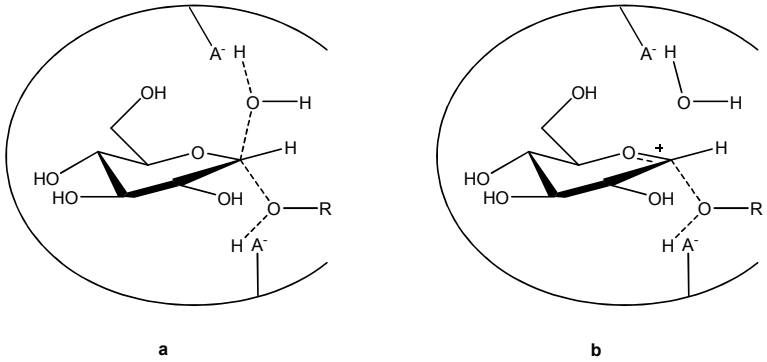


Figure 1-1: Models of inverting α-glucosidase mechanisms. a) S_N2-like transition state. b) Proton assisted aglycon departure as starting step**.**

The proposed mechanism of retaining glycosidases proceeds via two consecutive inversions on the anomeric centre [3]. In the first step, the aglycon is released by the attack of an active site nucleophile, leading to an adduct with inverted configuration. The second step of the mechanism is the cleavage of the intermediate by the attack of an activated water molecule.

Thus, for this mechanism, at least two transition states must exist (Figure 1-2). Experimental indications for this mechanism could be gained by the measurement of secondary kinetic isotope effects [5]. Common to the molecular mechanism of both subtypes, retaining and inverting glycosidases, is the generally accepted oxocarbenium ion-like transition state which shows an uncommon half-chair conformation and sp^2-hybridisation of the anomeric carbon [6-16].

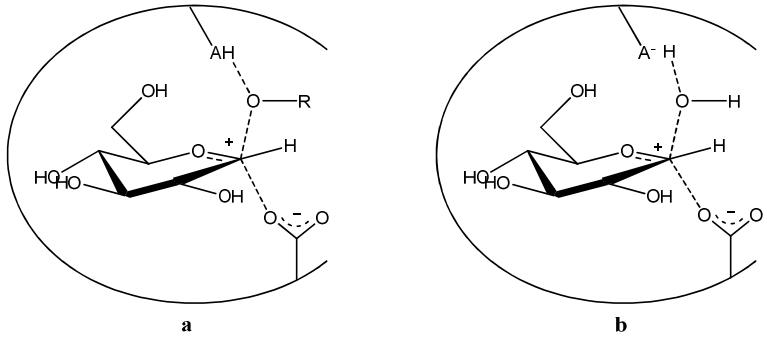


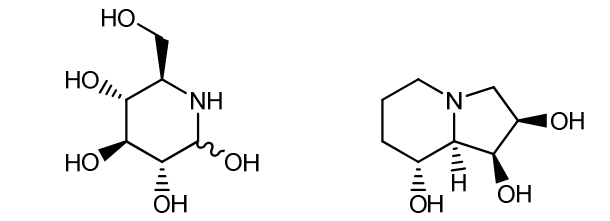
Figure 1-2: Models of the first and second transition states of a retaining β-glucosidase. In this double displacement mechanism, the first step is the release of the aglycon (a), followed by the attack of an activated water molecule (b).

As already mentioned above, glycosidases fill many roles in living organisms. Thus, a profound interest in substances selectively inhibiting glycosidases exists.

A variety of naturally occurring glycosidase inhibitors from microbial or plant sources are known and have been already used in Western as well as traditional Chinese medicine, *e.g.*, for the treatment of diabetes [17].

The biological activity of most reversible competitive glycosidase inhibitors relies on the structural analogy to the natural substrate. The inhibitor is able to bind to the enzymes active-site, however, it cannot be converted like the natural substrate.

Examples of naturally occurring reversible glycosidase inhibitors can be found in Figure 1-3.

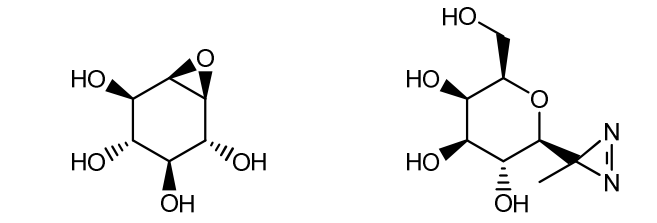


Nojirimycin
α- and β-glucosidase inhibitor

Swainsonine
α-mannosidase inhibitor

Figure 1-3: Examples of reversible glycosidase inhibitors**.**

Irreversible glycosidase inhibitors inactivate the enzyme by the formation of a covalent bond between the inhibitor and an amino acid residue of the glycosidase. This type of glycosidase inhibitor, of which selected structures are shown in Figure 1-4, are powerful tools for the investigation of enzyme mechanisms and the labelling of enzymes [18], but are however less suitable for the reversible modulation of enzyme activity.



Conduritol B-epoxide
α- and β-glucosidase inactivator

Diaziridine galactoside
β-galactosidase inactivator
photolabel

Figure 1-4: Examples of irreversible glycosidase inhibitors

1.3 Iminosugars as Glycosidase Inhibitors

Iminosugars are structural analogues of carbohydrates containing a basic nitrogen atom instead of an oxygen atom in the ring. There exist several classes of iminosugars which are differentiated by the oxidation state of the formal anomeric carbon, the size of the ring and the number of fused rings. Upon these prerequisites, the groups of “true” iminosugars, where the carbonyl group is preserved and iminoalditols, where no carbonyl group is present are defined. The classification concerning the ring size leads to the groups of pyrrolidines, piperidines, and bicyclic base structures such as pyrrolizidines and indolizidines. There exist also various *N*-substituted and *O*-glycosylated derivatives of the described basic structure types (Figure 1-5). Besides the classification of iminosugars by structural features, a classification by their stereochemical configuration is done in analogy to monosaccharide characterisation.

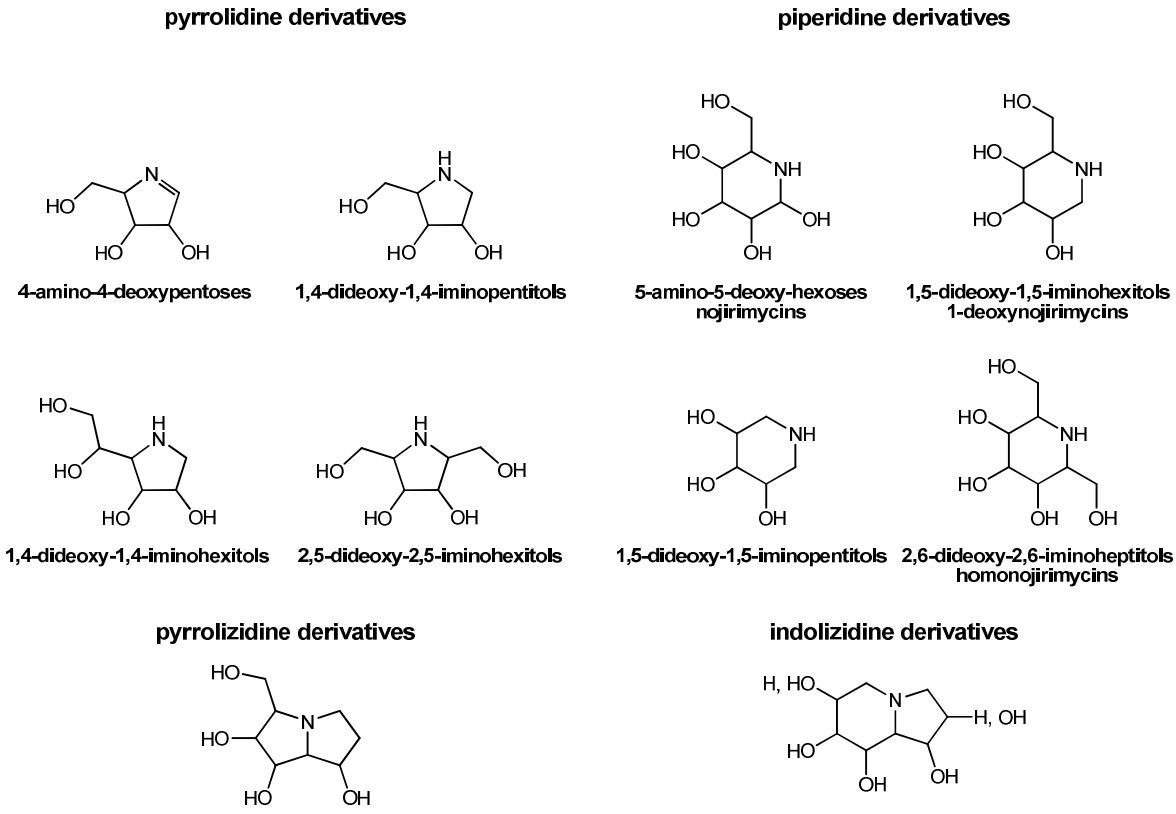


Figure 1-5: Structural types of iminosugars.

The origin of the therapeutic use of iminosugars dates back to traditional Chinese medicine. In Europe, Haarlem oil, the first pharmaceutical product manufactured on a large scale in the 17th century was applied in the treatment of diabetes and was also used as a skin whitening agent. The

major component of this oil was an extract of *Morus alba* (mulberry) leaves, which is a rich source of iminosugars. [17]

Figure 1-6: Iminosugar producing plants. From left to right: *Morus alba, Castanospermum australe, Swainsona formosa.*

The scientific rise of iminosugars began in the early 1960s with the report of the synthesis of sugar derivatives with a nitrogen atom in the ring. [19-21]

In 1966, a synthesis for 1-deoxynojirimycin (DNJ) was published by Paulsen [22], prior to its discovery in nature. At the same time the group of Inouye was able to isolate nojirimycin (NJ, 5-amino-5-deoxy-D-glucose) from *Streptomyces* sp. and to investigate the antibiotic power of the substance [23].

With improved separation techniques, a variety of iminosugars and iminoalditols could be isolated from the *Morus* spp. (*Moraceae*), among them fagomine (1,2-dideoxynojirimycin), *N*-methyl-1-deoxynojirimycin [24,25] and 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), which was originally isolated from *Angylocalyx boutiqueanus* (*Leguminosae*).

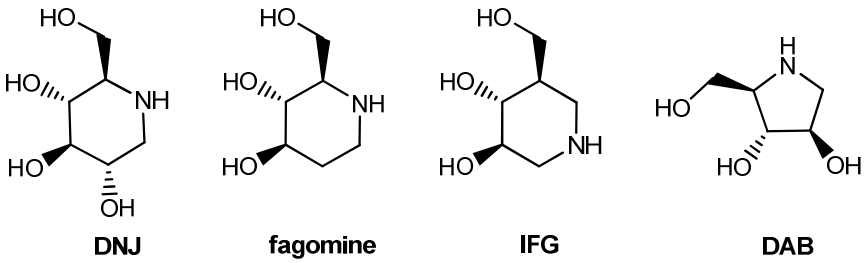


Figure 1-7

From the tropical liana *Omphalia diandra (Euphiorbiceae*), α-homonojirimycin (α-HNJ) could be isolated as first naturally occurring nojirimycin derivative bearing a carbon substituent at C-1 [26]. Again, a glucosylated derivative of α-HNJ has been synthesised to be evaluated as a pharmaceutical against diabetes prior to the discovery of the natural product, [27,28].

Further iminoalditols, such as β-homonojirimycin (β-HNJ) and β-homomannojirimycin (β-HMJ) as well as a glucosylated derivative of α-HNJ could be isolated from from *Aglaonema treubii* (*Araceae*), a popular indoor plant, originating from south-east asian rainforests.

Castanospermum australe was investigated because of its toxicity for livestock and within the alkaloid fraction castanospermine and australine could be identified as lead toxic substances disturbing glycogen catabolism [29,30].

Casuarine, a pyrrolizidine type iminosugar, as well as its 6-*O*-α-D-glucoside could be isolated from the bark of *Casuarina equisetifolia* (*Casuarinaceae*) and was found to be a competitive inhibitor of porcine kidney trehalase [31-33]. 2,5-Dideoxy-2,5-imino-D-mannitol (DMDP) was found in the leaves of *Derris elliptica.*

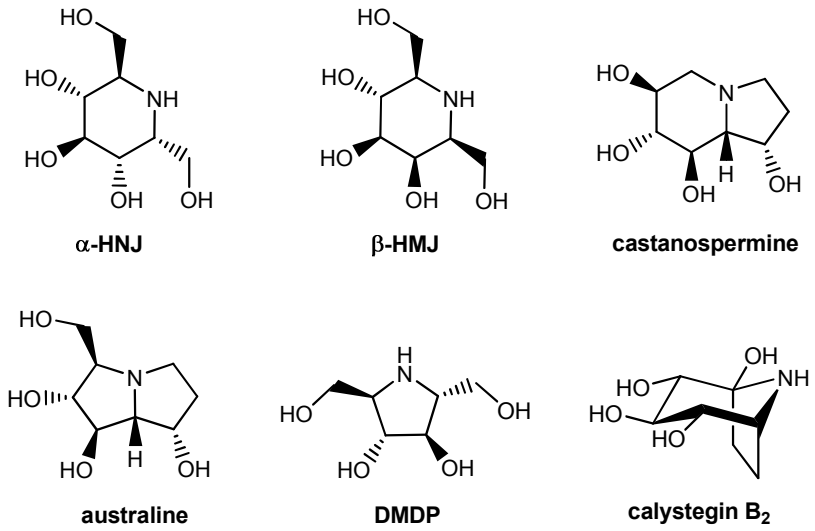


Figure 1-8

Calystegines, polyhydroxylated nortropane alkaloids, were isolated from the families of *Solanaceae* and *Convolvulaceae*. Besides the bridged ring system, calystegines feature 2-4 secondary hydroxyl functions and a tertiary hydroxyl group generated by the formation of an aminoketal. The members of the calystegine family known to date have been assigned into three subgroups, calystegines A (3 OH groups), B (4 OH groups) and C (5 OH groups) [34,35]

Galactonojirimycin (galactostatin) was isolated from the fermentation broth of *Streptomyces lydicus* [36]. 1-Deoxygalactonojirimycin (DGJ) has not been found in nature as yet, however, β-1-*C*-butyl-DGJ has been isolated from *Adenophora* sp. (*Campanulaceae*). The chain-elongated homogalactonojirimycin (HGJ) has not been discovered in nature thus far, whereas its 2-epimer α-homoallonojirimycin (α-*allo*-HNJ) which also shows galactosidase sensitivity is a natural product [37].

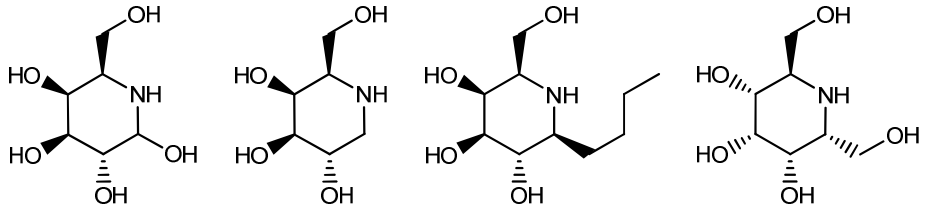


Figure 1-9

Consumption of the Australian legumes, *Swainsona* as well as of the closely related *Astralagus* and *Oxytropis* species, which occur in many parts of the world causes symptoms in animals that are called “locoism” or “pea-struck” [38-40]. These phenomena originate from the accumulation of mannose-rich oligosaccharides in the lysosomes of neuronal cells upon inhibition of endoplasmatic reticulum and Golgi mannosidases [41] by swainsonine, a trihydroxyindolizidine alkaloid. [39,42]. *Astragalus lentiginosus* produces another alkaloid of this type, 2-*epi*-lentiginosine, which is believed to be an intermediate in swainsonine biosynthesis [43,44]. Indolizidine iminosugars are also produced in a variety of other plants, including *Rhizoctonia leguminicola* and *Ipomoea carnea*.

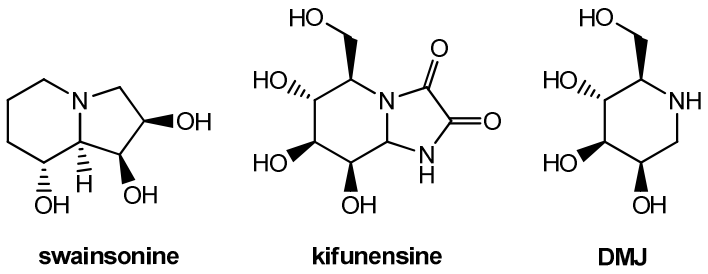


Figure 1-10

1-Deoxymannojirimycin (DMJ) was first isolated from the seeds of the tropical plant *Lonchocarpus sericeus* (*Leguminosae*) and was also shown to be present in a variety of different plants and microorganisms [45,46]. Another *manno*-type iminosugar, kifunensine, was isolated from the fermentation broth of *Kitasatosporia kifunense* and found to be a moderate mannosidase inhibitor [47].

Basic sugar analogues have early been recognised as powerful glycosidase inhibitors. Besides iminosugars, such as nojirimycin, azasugars [48-50], sugar derivatives where the anomeric carbon has been replaced by nitrogen (*e.g.*, isofagomine, Figure 8), and glycosyl amines [51] are strongly inhibiting compounds.

Not only the position of the nitrogen has an effect on the inhibitory power but also the basicity, the geometry and the charge distribution at the anomeric position as well as the hydrogen bond formation with the catalytic acid and interactions with the aglycon binding site have to be

considered. The selectivity of a glycosidase inhibitor is also strongly dependent on the ring size, the hydroxylation pattern including stereochemistry and the configurational flexibility of the molecule [52].

The role of the position of the nitrogen can be made obvious by the comparison of the inhibitory power of 1-deoxynojirimycin, fagomine, and isofagomine (Figure 1-7).

Whereas isofagomine is a 3 times weaker inhibitor for α-glucosidase from yeast, it inhibits β-glucosidase from almonds almost 500-fold better than DNJ [48-50]. This is a surprising result, since the shift of the nitrogen position seems to easily compensate for 2-deoxygenation which leads to almost 280 times increase of K_I in comparison of fagomine and DNJ [53].

Nojirimycin type inhibitors are of moderate basicity in a pK_a range between 5 and 6, whereas deoxynojirimycins show pK_a values from 6 to 8.2.

Looking at the basicity of basic glycosidase inhibitors, it has to be considered in which state the inhibitor is tackling the enzymes active site. When the inhibitor equilibrates with the solvent and binds to the negatively charged active site of the enzyme, stronger bases are supposed to be better inhibitors than weaker bases. But if the inhibitor has to enter the active site as a neutral molecule and gets protonated by a catalytic acid, weaker bases are supposed to be better inhibitors.

Many of the above mentioned iminosugars and iminoalditols are glycosidase inhibitors, exhibiting biological activities with K_I values ranging from the micromolar to the subnanomolar range. Naturally occurring as well as synthetic iminosugars are promising lead substances for pharmaceutical and diagnostical research against a variety of symptoms and diseases.

Iminosugars have been evaluated as drugs for the therapy of various diseases including cancer, viral diseases and diabetes. For general reviews of the therapeutic applications see references [54,55].

1.4 Synthesis of Iminosugars

The increasing interest in the chemistry of iminosugars combined with the attractive physiological properties has stimulated the research of synthetic chemists in this area. Many iminosugars known from natural sources as well as synthetic derivatives have been prepared in the last decades, thus, a variety of synthetic approaches has been developed over the years. Chemical as well as chemo-enzymatical methods employing starting materials ranging from saccharides to pyridine derivatives or amino acids have been established. Comprehensive review articles on this topic are available [56-61] and fundamental concepts will be described briefly within this section.

1.4.1 Iminosugars from Saccharides

The use of carbohydrates as starting materials in the syntheses of iminosugars is suggested by the structural resemblance of the two classes of molecules. In analogy to the biosynthesis of iminosugars, two synthetic transformations, namely the introduction of an amine and the subsequent cyclisation are necessary.

Figure 1-11 gives an overview of different procedures to obtain DNJ by the cyclisation of aminated intermediates.

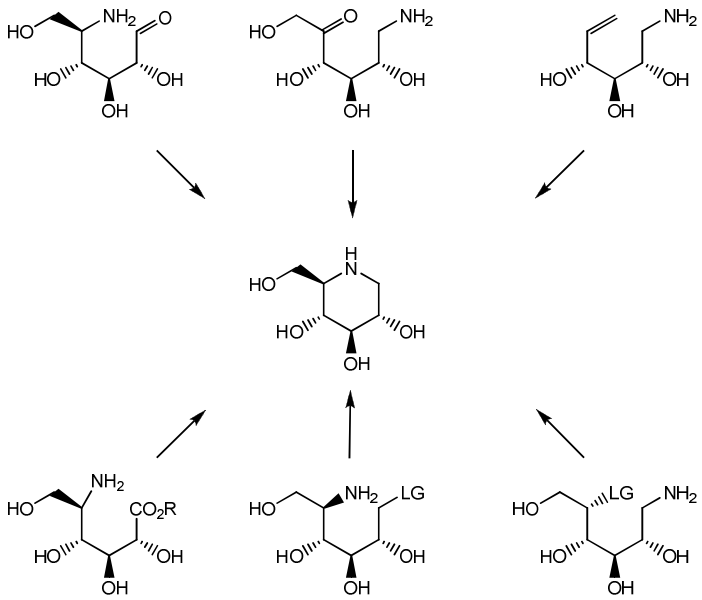


Figure 1-11: General strategies for the synthesis of DNJ by the cyclisation of aminated saccharide derivatives (LG = leaving group).

Iminoalditols lack the carbonyl functional group of sugars, hence, the two carbon atoms that are linked to the ring nitrogen do not differ in terms of functional groups. Thus, in the synthesis of iminoalditols, the amine functionality can be introduced at either of the 2 atoms, provided that a suitable cyclisation method is available.

Iminosugars from “true” sugars and aminosugars.

In 1967, Paulsen and co-workers reported the first synthesis of iminosugars by reductive amination [62]. This effective approach for the formation of an N-heterocyclic ring structure bases on the intramolecular Schiff-base formation of a carbonyl group with an amine. The subsequent reduction of this labile imine with a complex metal hydride or a supported hydrogenation catalyst and H_2 is an elegant method for the synthesis of iminoalditols.

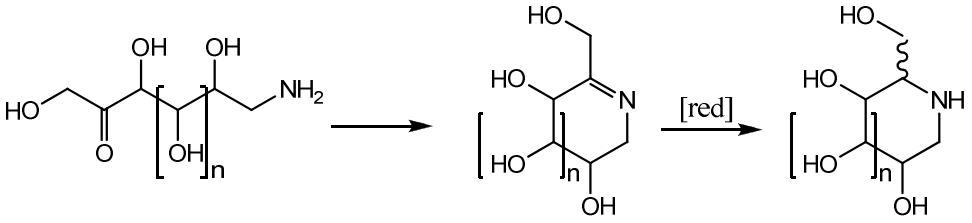


Figure 1-12: Synthesis of iminoalditols via reductive amination.

If the reacting carbonyl functionality is a ketone (Figure 1-12), a new stereogenic centre is created during the reduction procedure. Generally both possible diastereomers can be formed, however, the stereochemical outcome of the reaction can be controlled by the reaction conditions.

When heterogeneous hydrogenation catalysts are applied, a control of stereochemistry can be observed disregarding the respective configuration of the starting material as well as the absence or presence of protecting groups. This phenomenon, known as the “empirical rule” of intramolecular catalytic reductive amination, suggests that the side chain at the newly formed chiral centre and the functional group (methyl, hydroxyl, alkoxy or acetal) in β-position to this carbon are practically always *cis*-oriented. This stereochemical outcome is presumably controlled by the sterically hindered access of the hydrogenation catalyst to one of the ring faces [63,64].

The amine group in naturally occurring aminosugars can be employed as amine component for the synthesis of aminosugars. The synthesis of 3-hydroxymethyl-6-*epi*-castanospermine from *N*-CBz-neuraminic acid [65] is an interesting example for this approach (Figure 1-13). In this case, an imine is formed after the hydrogenolytic removal of the N-protecting group, which is further hydrogenated to give a pyrrolidine derivative. Subsequent reprotection of the amine and selective activation of the primary hydroxyl function leads to the indolizidine-type aminosugar after final N-deprotection.

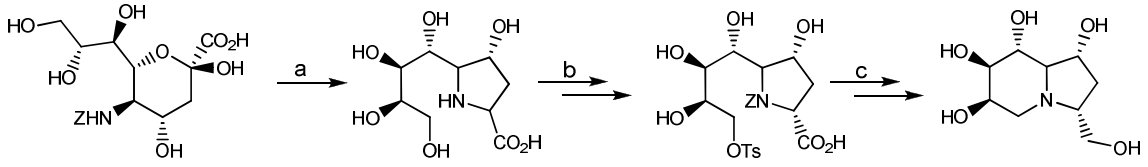


Figure 1-13: Synthesis of 3-hydroxymethyl-6-*epi*-castanospermin from *N*-CBz-neuraminic acid. Reagents and conditions: a) H_2, Pd/C, b) 1) CBzCl, 2) MeOH, HCl, 3) TsCl; c) 1) H_2, Pd/C, 2) $NaBH_4$.

When “true” sugars are used as starting materials in iminosugar syntheses, an amine functionality has to be introduced to the molecule. One approach is the introduction of an amine group by reductive amination of the anomeric carbon with suitable amines.

An example for this strategy is given by the synthesis of 1-deoxynojirimycin by the reductive amination of 2,3,4-tri-*O*-benzylglucopyranose with benzylamine (Figure 1-14). N-Acylation, 5,6-

epoxide formation and subsequent N-deprotection lead to DNJ and the corresponding 7-membered ring iminosugar [66].

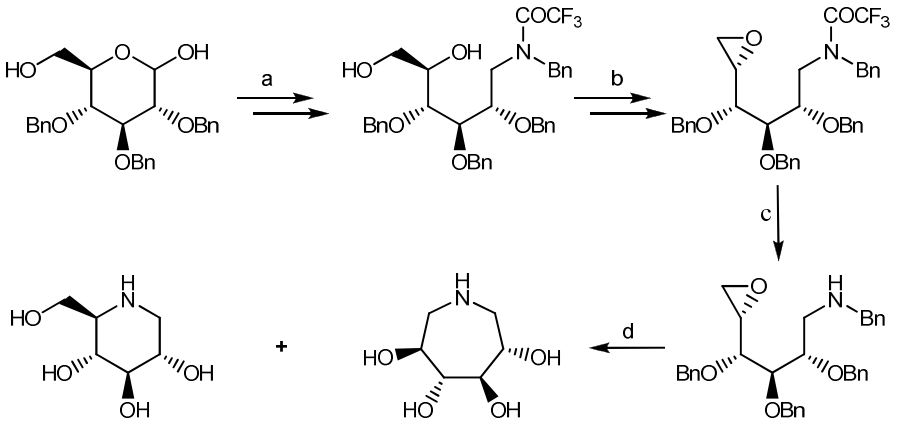


Figure 1-14: Synthesis of deoxynojirimycin and 1,6-dideoxy-1,6-imino-D-glucitol. Reagents and conditions: a) 1) $BnNH_2$, 2) $LiAlH_4$, 3) $(CF_3CO)_2O$; b) $tBuMe_2SiCl$, imidazole, 2) MsCl, 3) Bu_4NF-THF 4) MeONa, MeOH; d) $NaBH_4$, EtOH;

Another example for this approach is the chemo-enzymatic synthesis of *N*-butyl-DNJ (NB-DNJ) (Figure 1-15). In the first step, D-glucose is reductively aminated employing butylamine under hydrogenation conditions to yield 1-butylamino-1-deoxy-D-glucitol, followed by enzymatic oxidation of C-5, to the corresponding L-sorbose derivative. Subsequent intramolecular reductive amination leads to the formation of NB-DNJ [67].

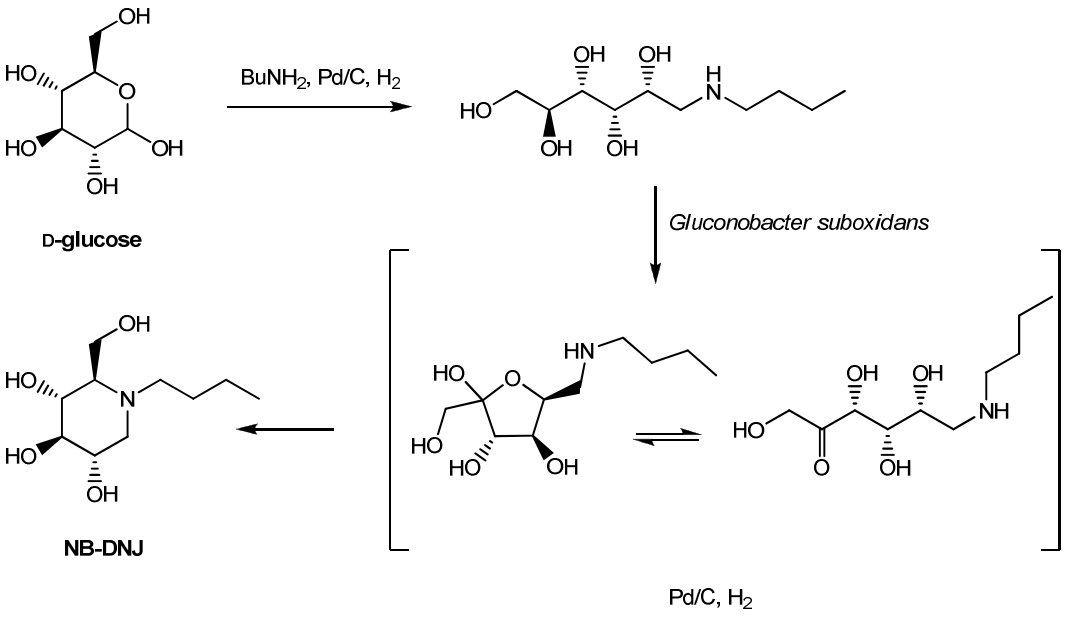


Figure 1-15: Chemo-enzymatic synthesis of NB-DNJ.

When the anomeric carbonyl function cannot be used for the introduction of an amine, a synthetic alternative to introduce an amine is the “chain amination” of sugars. A demonstrative example of this strategy is the synthesis of 1-deoxymannojirimycin (Figure 1-16) from 6,6’-dichloro-6,6’-dideoxysucrose [68]. In a first step, the dichlorosugar is converted to the corresponding diazide. Cleavage of the glycosidic bond leads to 6-azido-6-deoxy-D-fructose and 6-azido-6-deoxy-D-glucose, the latter which can be converted to the fructose-derivative by treatment with glucose isomerase. Catalytic hydrogenation of 6-azido-6-deoxy-D-fructose finally yields 1-deoxy-mannojirimycin.

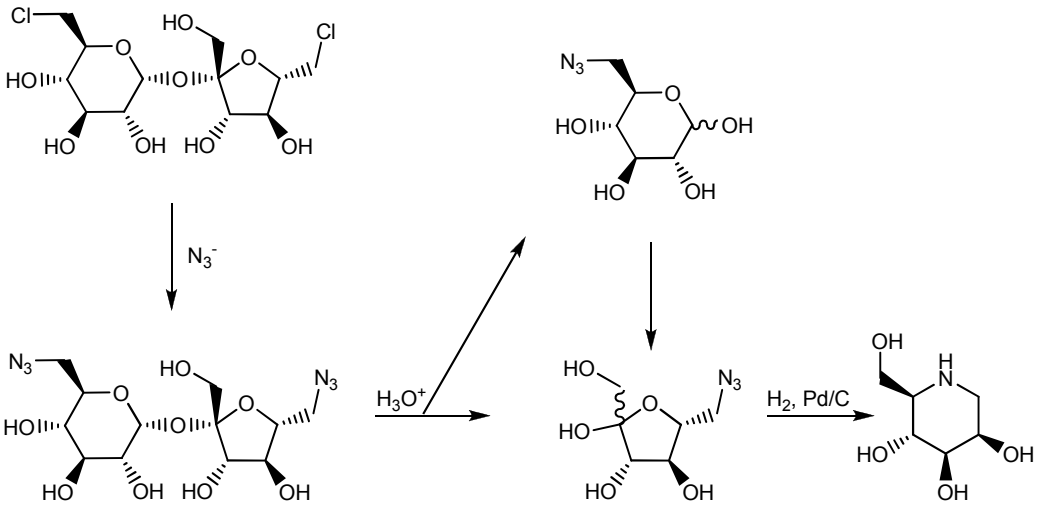


Figure 1-16: Synthesis of DMJ from dichlorosucrose by “chain amination”.

In contrast to the described methods, the synthesis of iminoalditols can also be carried out by the double reductive amination of suitable 1,5-dicarbonyl sugars. The introduction of the additional carbonyl function can be achieved either by enzymatic conversion as shown, or by the synthetic transformation of suitably protected sugars.

The synthesis of L-*arabino*-hexos-5-ulose (Figure 1-17) is an example for this strategy [69]. Starting from methyl β-D-galactopyranoside, 3,4-*O*-isopropylideneation and subsequent 2,6-*O*-protection followed by elimination of the isopropylidene group yield a 4,5-unsaturated sugar derivative that can be epoxidised employing *m*CPBA. The epoxide is cleaved during the reaction by benzyl alcohol which yields a partially protected L-*arabino*-hexos-5-uloside that can be used directly in a catalytic reductive amination for the synthesis of 1-deoxygalactonojrimycin.

Using this strategy or modifications thereof, a variety of *N*-L-lysyl-1-DGJ derivatives have been prepared [70,71].

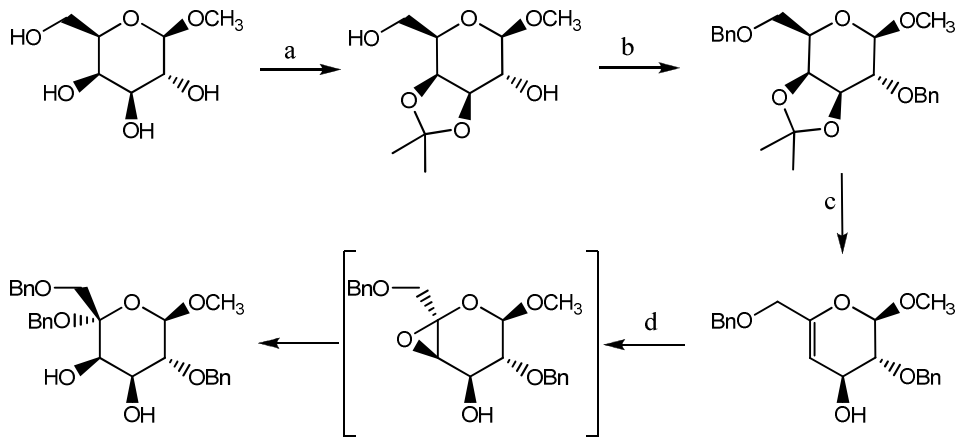


Figure 1-17: Synthesis of an L-*arabino*-hexos-5-ulosid derivative. Reagents and conditions: a) 2,2-dimethoxypropane, H^+; b) BnBr, NaH; c) KO^tBu, THF; d) *m*CPBA, BnOH;

Iminosugars from alditols.

Another strategy for the synthesis of iminosugars from carbohydrate starting materials is the cyclisation of activated alditols. An example of this method is given by McCaig and co-workers, where the primary hydroxyl functions of alditols are selectively activated and treated with a nucleophilic amine under S_N2-conditions [72].

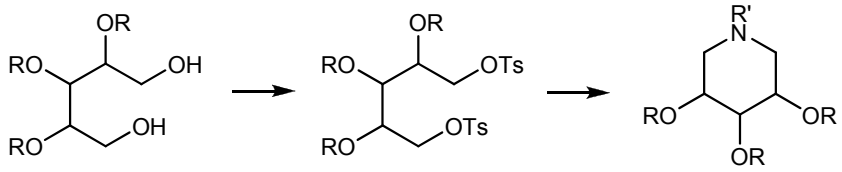


Figure 1-18: Cyclisation of activated alditols**.**

The treatment of 2,7-dihalogenated aldonolactones with aqueous ammonia leads to iminoheptonic acids via epoxide intermediates. Alternatively, the lactones can be reduced to the corresponding 2,7-dibromoalditols prior to cyclisation to iminoalditols (Figure 1-19) [73].

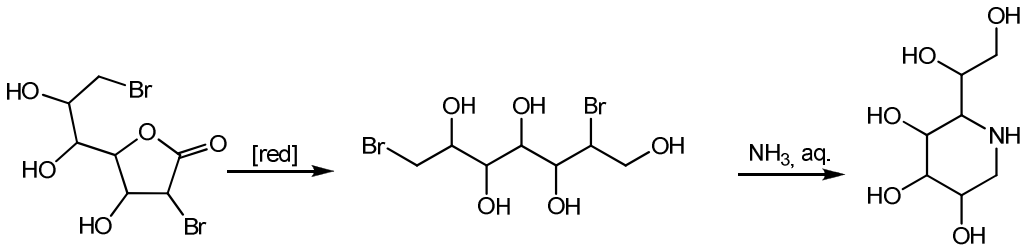


Figure 1-19: Iminoalditols from dihaloaldonolactones

The oxidation of 1,2:3,4-di-*O*-benzylidene-D-mannitol to the corresponding 2,5-dicarbonyl compound and subsequent reductive amination has been shown to be a convenient method for the synthesis of 2,5-dideoxy-2,5-imino-L-iditol[74].

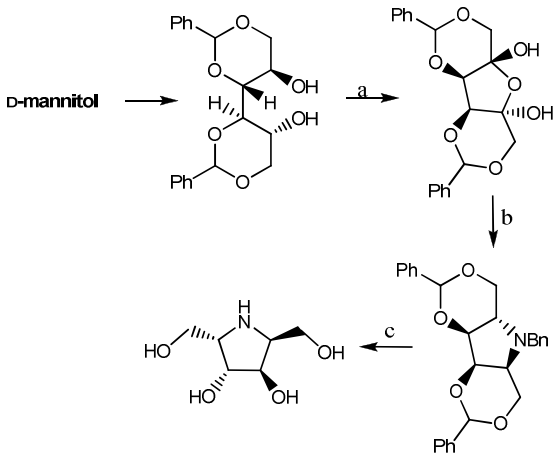


Figure 1-20: Synthesis of 2,5-dideoxy-2,5-imino-L-iditol. Reagents and conditions: a) PCC; b) $BnNH_2$, $NaB(CN)H_3$,; c) H_2, Pd/C;

1.4.2 Iminosugars from Non-Carbohydrate Starting Materials

The use of carbohydrate starting materials is not always the most efficient approach towards the synthesis of iminosugars. Strategies for the synthesis of iminosugars from starting materials other than carbohydrates have been investigated extensively. In these methods, chiral centres are built up by either chemo-enzymatic approaches or the use of smaller, easily available starting materials such as amino acids paired with stereoselective hydroxylation reactions. An example of an iminosugar synthesis using a chiral amino aldehyde derived from L-serine [75] is given in Figure 1.21.

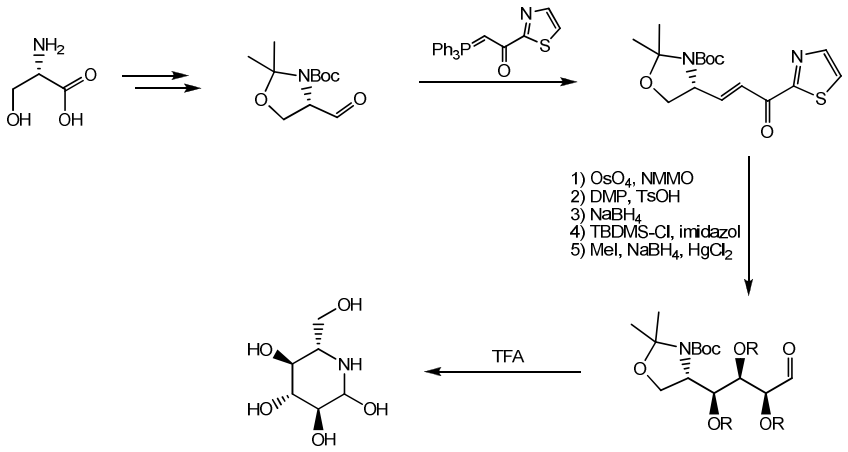


Figure 1-21: Nojirimycin from L-serine

An example for the synthesis of D/L-1-deoxymannojirimycin [76] by employment of stereoselective reactions is given in Figure 1-22. The key step of this synthesis is the chemo- and stereoselective amination of a silylated butendiol dicarbonate. After conversion to an epoxaldehyde, cyclisation by intramolecular aldolisation yields a silylated piperidine derivative, which is oxidised and subsequently deprotected to give D/L-DMJ.

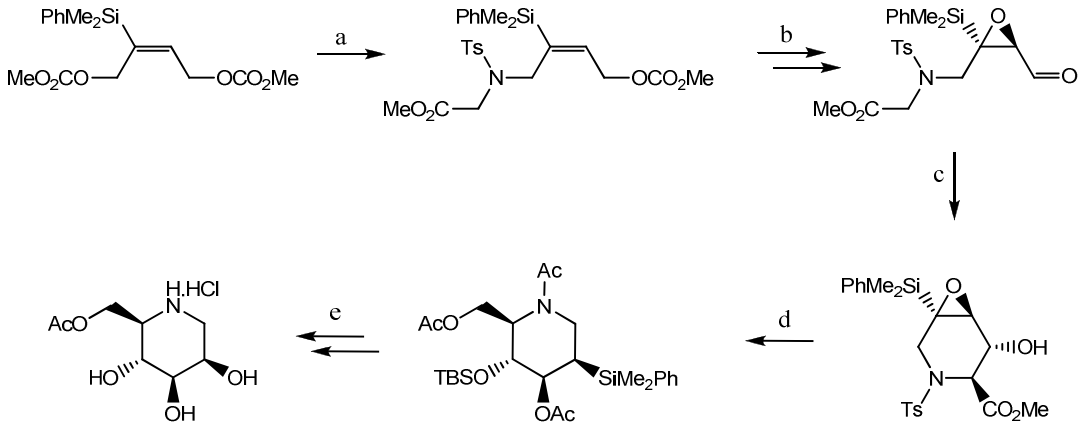


Figure 1-22: DMJ from an butendicarbonate derivative. Reagents and conditions: a) Ts-Gly-OMe, NEt_3, $Pd(OAc)_2$, dppe; b) 1) K_2CO_3, MeOH, 2) *m*CPBA, 3) IBX, DMSO; c) DBU; d) 1) $LiAlH_4$, Et_2O, 2) Ac_2O, NEt_3, DMAP; e) 1) $Hg(OAc)_2$, AcOOH/AcOH, 2) TBAF, 3) Ac_2O, 4) HCl 6N; only D-enantiomer shown.

The photoinduced transformation of pyridine perchlorate to 3,5-dihydroxy-4-aminocyclopentene followed by acetylation and enzymatical desymmetrisation of the *meso*-compound yields a chiral building block which can be used in the synthesis of swainsonine and 2-*epi*-swainsonine[77]. Allylation and ring rearrangement metathesis lead to a tetrahydropyridine derivative that can be cyclised to yield the indolizidine iminosugars after chemoselective dihydroxylation (Figure 1-23)

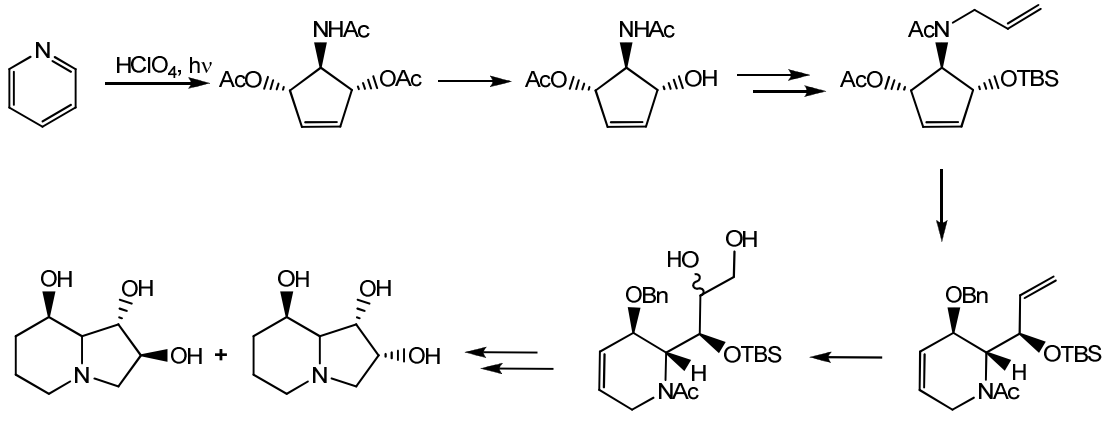


Figure 1-23: Synthesis of swainsonine and 2-*epi*-swainsonine**.**

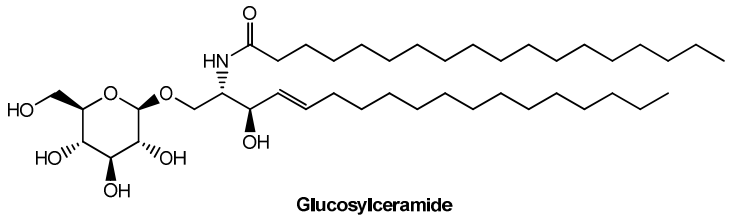
1.5 Iminosugars as Therapeutics in Lysosomal Storage Diseases

1.5.1 Glycosphingolipid Metabolism and Lysosomal Storage Disorders

The surface of eukaryotic cells is covered by a dense layer of carbohydrates, the so-called glycocalix. It consists of glycoproteins, glycosaminoglycans and glycosphingolipids.

Glycosphingolipids (GSLs) are anchored to the cell by ceramide, a substance consisting of the long chain amino alcohol sphingosine, the amino group of which is acylated with a long chain fatty acid [78-80]. The carbohydrate chain is attached to ceramide by an *O*-glycosidic linkage to the hydroxyl function of sphingosine.

GSLs form cell-specific patterns on the surface which change with the state of differentiation, oncogenic or viral transformations [81]. GSLs on the cell-surface are involved in adhesion processes [82] where they can function as binding site for viruses, toxins and bacteria [83,84]. In the GSLs of vertebrates, a D-glucose or D-galactose residue is attached by β-glycosidic linkage to the 1-position of ceramide (Figure 1-24). Most vertebrate GSLs do not require galactosylation but glucosylation of ceramide, which is accomplished by glucosylceramide (GlcCer) synthase that transfers a glucosyl residue from UDP-glucose to ceramide. Subsequently, glycosyltransferases catalyse the linkage of nucleotide activated individual glycosyl donors to the glucosyl or galactosyl ceramide.



Glucosylceramide

Figure 1-24

The catabolism of GSLs takes place in the acidic compartments of the cell, in the late endosomes and predominantly in the lysosomes [85,86].

The individual sugar residues are cleaved from the non-reducing end of GSLs by lysosomal exo-glycosidases, which are generally specific for the cleavage of a particular terminal residue, but are non-specific regarding the aglycon. Finally, ceramide is degraded to sphingosine and a long chain fatty acid. A schematic representation of this pathway can be found in Figure 1-25.

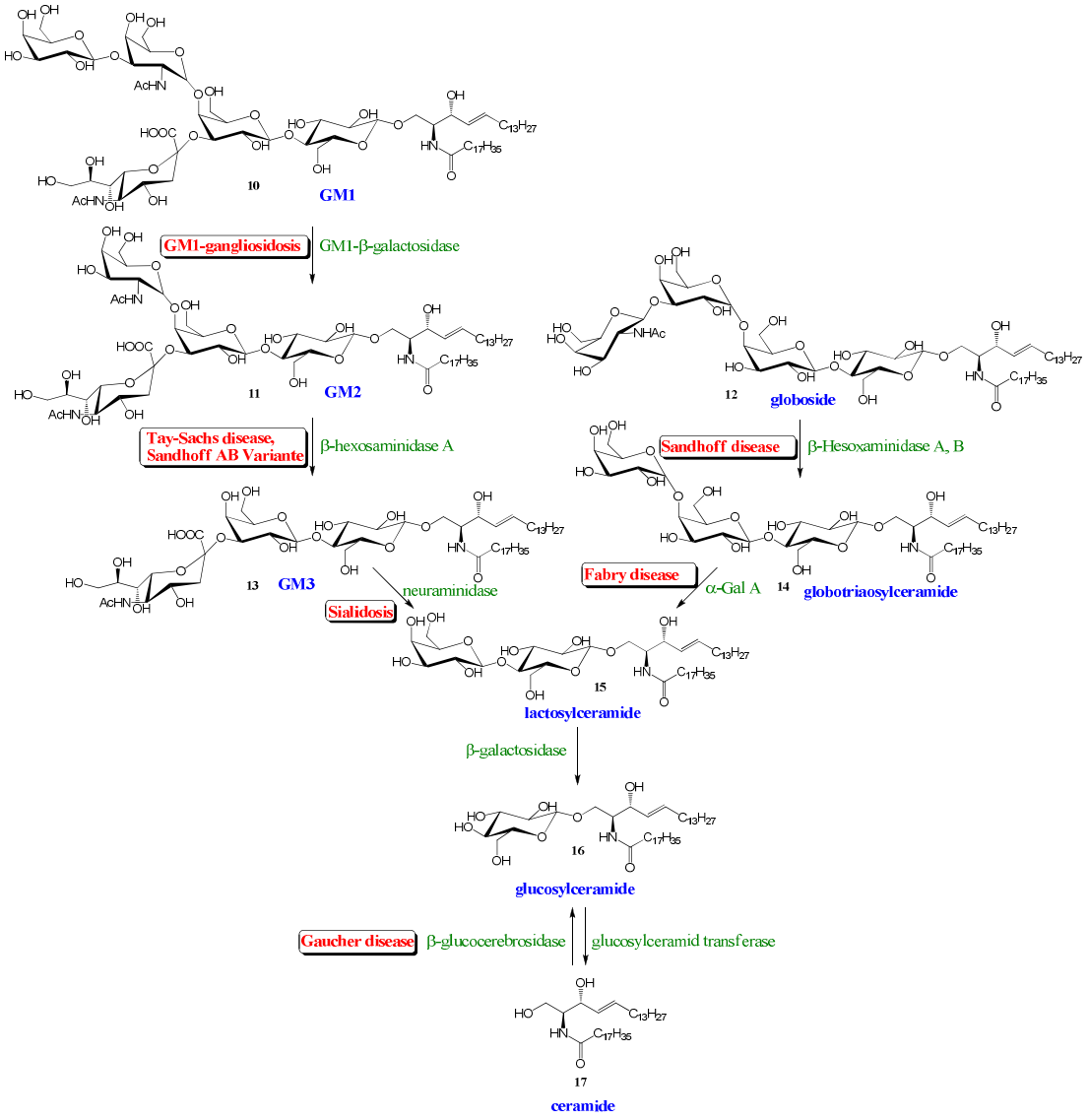


Figure 1-25: Catabolic pathways of glycosphingolipids. Adapted from Ref. [87].

The released low molecular weight compounds, namely monosaccharides, sphingosine, and fatty acids are able to permeate through the lysosomal membrane and are recycled as building blocks in the cytosol. If one of the particular enzymes involved in the degradation of GSLs fails, non-degradable glycolipid intermediates accumulate in the lysosome. Those substances are poorly water soluble amphiphiles and tend to form solid aggregates in the cells [88]. The disorders caused by these phenomena belong to the larger group of lysosomal storage diseases.

Lysosomal storage diseases (LSDs) are a group of inherited metabolic disorders caused by the deficiency of lysosomal acid hydrolases, lysosomal membrane proteins, lysosomal enzyme trafficking related proteins or lysosomal activator proteins [89,90], leading to the impaired intracellular turnover and accumulation of a variety of substances including sphingolipids, glycosaminoglycans, glycoproteins and glycogen.

A number of those inherited diseases is closely associated with failures of the lysosomal degradation of GSLs [91,92]. A collection of prominent representatives of the class of sphingolipidoses can be found in Table 1-1.

Table 1-1: Common lysosomal storage diseases.

Disease	Affected enzyme
Gaucher disease	glucocerebrosidase
Fabry disease	α-galactosidase A
GM1-gangliosidosis	β-galactosidase
Morquio B disease	β-galactosidase
Tay-Sachs disease	β-hexosaminidase A
Sandhoff disease	β-hexosaminidase B

Although the individual disease incidence is rare, collectively, they form a significant group of disorders. The incidence of glycosphingolipid related lysosomal storage disorders has been estimated to occur at 1 in 8,000 – 10,000 live births world-wide [93], which makes the group of LSDs the most frequent cause of pediatric neurodegenerative disease.

Lysosomal storage disorders are characterised by involvement of multiple organs and tissues exhibiting complex phenotypes with the association of various visceral, skeletal and neurological manifestations. The most frequent lysosomal storage disease is Gaucher disease with an estimated prevalence of 1:200,000, meaning that more than 30,000 people are affected globally. Deficiencies in the activity of glucocerebrosidase, caused by more than 76 identified gene mutations, result in the accumulation of glucosylceramide in the lysosome, leading to symptoms such as hepatosplenomegaly, haematological disturbance and bone distruction.

Tay-Sachs disease is caused by the storage of ganglioside GM2 due to β-hexosamindase A deficiency. The earliest symptoms come up in the first months of life and are characterised by motoric disorders and progressing dementia. Macrocephaly and neuerological disorders worsen until death occurs.

The clinical and pathological pattern of Sandhoff's disease essentially corresponds to Tay-Sachs disease, additionally, hypertrophy of visceral organs and bone deformation occurs. Other to Tay-Sachs disease, the accumulation of oligosaccharides is caused by β-hexosaminidase B deficiency.

Fabry's disease is caused by a failure of lysosomal α-galactosidase A (α-Gal A). It leads to the accumulation of oligosaccharides carrying terminal α-glycosidically bound galactose. It is characterised by painful lesions of the skin, pain in the extremities and renal failure. The symptoms infrequently start in adolescence, but mostly, an adult form of the disease is observed. α-*N*-

acetylgalactosaminidase exhibits a slight overlap with α-Gal A substrate specificity, so little residual α-galactosidase A activity can be observed in Fabry's disease. Additionally, nerve cells synthesise the lipids affected in Fabry's disease only to a small extent, so usually the central nervous system is not affected, however, symptoms in the autonomic nervous system can be observed [94].

GM1-gangliosidosis and Morquio B syndrome are diseases caused by the lack of GM1 β-galactosidase. GM1-gangliosidosis is predominantly characterized by the accumulation of neuronal ganglioside GM1, whereas in Morquio B syndrome the accumulation of oligosaccharides in bones and inner organs is dominant. It is possible to differentiate 3 forms of GM1-gangliosidosis. In the infantile form (type 1), neurological symptoms occur within the first months of life and life expectancy is not better than 2 years. Characteristics of this form are cherry-red spots on the ocular fundus, facial dismorphia, liver and spleen hypertrophy and skeletal deformations. The juvenile form of the disease (type 2) progresses slower with a life expectancy of about 10 years. Symptoms like cerebral symptoms and skeletal disorders occur within the first year of life. The adult form (type 3) of the disease shows a comparatively mild progression of neurological disorders with only little involvement of the skeletal system.

Morquio B syndrome is also attributed to a defect in GM1-β-galactosidase. The symptoms include skeletal disorders, usually without involvement of the central nervous system [95]. Oligosaccharides with terminal galactose residues accumulate in the visceral organs and are secreted in the urine.

1.5.2 Therapy of Lysosomal Storage Diseases

The causal therapy of lysosomal storage disorders is currently not possible. For many LSDs, only supportive therapy, such as the administration of anti-inflammatory agents or anti-oxidants is available [96].

Moreover, the objective of most therapeutic approaches is the restoration of defective degradative activity in the lysosome. This can be achieved either directly by enzyme replacement therapy or indirectly by bone marrow or organ transplantation. Each of the introduced therapeutic options has limitations and is currently available with strict indications and criteria of applicability for individual LSDs or even for subsets of patients within the disease [97]. Substrate reduction therapy takes place at the metabolic level and attempts to reduce the level of substrate influx to the lysosome. Pharmaceutical chaperone therapy is a novel concept, where the premature degradation of misfolded proteins is tried to be avoided by the application of stabilising agents.

1.5.2.1 Enzyme Replacement Therapy

The discovery that lysosomal enzymes can be secreted from one cell and taken up by a neighbouring cell (cross-correction) opened up the possibility to exploit this process for therapeutical purposes [98]. The discovery of the mannose- [99] and mannose-6-phosphate- [100] recognition systems provided a framework by which recombinant lysosomal enzymes could be modified for targeted delivery.

The first successful trial of enzyme replacement therapy (ERT) was conducted in patients with type I Gaucher disease [101,102]. The therapy improves symptoms of the disease such as splenomegaly, skeletal abnormalities, and haematological parameters, respectively. Enzyme replacement therapy for Gaucher type I proved to be effective and is to date considered the standard-of-care [89].

After ERT in Gaucher disease has proven to be safe and efficient, efforts to develop ERT for other types of lysosomal storage diseases came up quickly.

Clinical trials were performed for a range of LSDs, such as mucopolysaccharidosis I and Fabry disease. Currently, ERT is the standard of care for the most prevalent of these diseases, such as Gaucher, Fabry, and Pompe disease as well as mucopolysaccharidoses I, II, and IV [103].

The common drawbacks of all ERT protocols are problems deriving from enzyme biodistribution. Recombinant lysosomal enzymes are large molecules that cannot freely diffuse across membranes but are depend on mannose- or mannose-6-phosphate transport pathways for efficient delivery [104]. Furthermore, those enzymes are not able to cross the blood-brain barrier (BBB), which makes ERT complicated for the treatment of LSDs with central nervous system participation. Efforts are made to chemically modify the recombinant enzymes to enable BBB crossing [105,106]. Also invasive methods such as intrathecal ERT are currently investigated [107].

Not only medical and physiolocial phenomena restrict the application of ERT, also economical aspects have to be considered. Costs related to the production of recombinant therapeutic enzymes and investments in research and development contribute to the high costs of ERT. The treatment costs for a single patient are as high as several hundred thousand US dollars per year [102,104] making ERT one of the most expensive therapies in the field.

Table 1-2: Enzyme replacement therapy for lysosomal storage disorders.

Disease	Enzyme replaced	Company	Status
Gaucher I and III	glucocerebrosidase	Genzyme	approved EU/US 1991
Fabry	α-galactosidase A	Genzyme	approved EU/US 2001/2003
Hurler	α-L-iduronidase	Transkaryotic Therapies	approved EU 2001
Maroteaux-Lamy	arylsulfatase B	BioMarin Pharmaceuticals	approved US 2005

Table 1-3 continued: Enzyme replacement therapy for lysosomal storage disorders.

Hunter	α-L-iduronate sulfatase	Transkaryotic Therapies	phase III clinical trial
Pompe	α-glucosidase	Genzyme	phase III clinical trial
Niemann-Pick B	acid sphingomyelase	Genzyme	preclinical
Metachromic leukodystrophy	arylsulfatase A	Zymenex	preclinical
α-Mannosidosis	1183 α-mannosidase	Zymenex	preclinical

1.5.2.2 Bone Marrow Transplantation and Gene Therapy

The aim of bone marrow transplantation is to avoid the obstacle of passing the blood-brain barrier. Bone marrow macrophages can cross the blood-brain barrier to a small extent and can serve as an enzyme source in the brain [108]. Although there have been reports of successful attempts, BMT does not seem to be a suited concept for the treatment of lysosomal storage disorders.

In animal models, BMT led to an improvement of neurological symptoms and also the life expectancy could be increased, however, no recovery of the testing animal could be observed [109-111].

Gene therapy approaches in the therapy of lysosomal storage diseases have the objective to insert a functional gene into the affected organism. One major problem is the efficient transfer of therapeutic DNA to the cells of the central nervous system. At the cell-culture level, experiments with galactosylceramidase *c*DNA have been conducted [112]. Adenovirus-mediated gene transfer in a mouse model of mucopolysaccharidosis VII could achieve a transient increase in β-glucuronidase activity in the brain of the experimental animals [113,114].

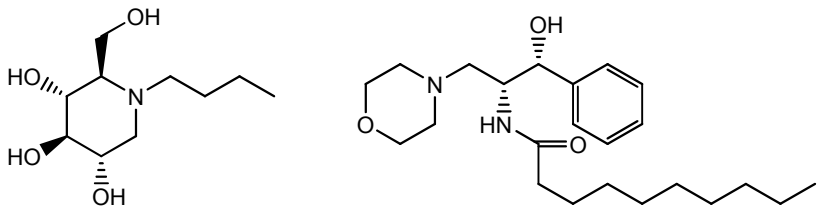
1.5.2.3 Substrate Reduction Therapy

Treatment of glycosphingolipidoses can also take place on the metabolite level. One of the methods under investigation is the so-called substrate reduction therapy (SRT). This method bases on the idea of controlling the catabolism of GSLs by the inhibition of their biosynthesis, which was promoted several years ago [115]. This means, however, that a minimum residual lysosomal glycosidase activity has to be existent.

Substrate reduction therapy is clinically available for the treatment of type I Gaucher disease in patients unable to receive ERT [116,117]. The concept of SRT is to inhibit an early step in glycosphingolipid biosynthesis employing well-tolerated compounds. To date, compounds inhibiting the first step in GSL biosynthesis, the conversion of ceramide to glucosylceramide, are available. This reaction is catalysed by glucosyl ceramide (GlcCer) synthase employing uridine diphosphate (UDP) glucose as the saccharide donor [116-118]. One of the targets of substrate reduction

therapy is the design of drugs that are able to cross the blood-brain barrier to reduce substrate burden in the central nervous system.

Currently, two distinct classes of molecules are applied in SRT: the ones based on D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) and the group based on iminosugar compounds, the prototype of which is *N*-butyl-1-deoxynojirimycin (NB-DNJ, miglustat, Zavesca® ; Actelion Pharmaceuticals Ltd.) [117].



NB-DNJ **PDMP**

Figure 1-26: Drugs for substrate reduction therapy.

Derivatives of PDMP mimic the alkyl chain and the charged transition state of GlcCer synthase, respectively, due to structural resemblance [119]. Concerning ceramide, PDMP is a mixed type 1 inhibitor (K_I, 0.7 µM), but shows uncompetitive inhibition for the sugar donor [116]. N-Alkylated derivatives of deoxynojirimycin show competitive inhibition for ceramide (NB-DNJ: K_I, 7.4 µM) and non-competitive inhibition for the UDP sugar [120]. The structural homology between NB-DNJ and ceramide may partly explain its inhibitory activity [121]. The therapeutic efficacy of NB-DNJ was validated in a tissue-culture model of Gaucher disease [122] and in the phase of preclinical development, no adverse effects could be observed in healthy mice that were administered high doses of NB-DNJ [123]. The clinical evaluation of miglustat began in the 1990s after the drug has been evaluated as anti-HIV agent due to the inhibitory action against glycoprotein trimming ER glycosidases [124]. Based on the data of these studies, the clinical evaluation of miglustat for the SRT of type I Gaucher disease was started [125]. It could be shown, that rapid clinical improvement can be achieved by the administration of 100 mg NB-DNJ three times daily [126,127].

Substrate reduction is a suited therapy for juvenile and adult-onset LSDs, where clinical benefit can be observed if SRT is initiated early [128]. However, in rapidly progressing infantile-onset disease types, SRT will provide limited therapeutic benefit due to the very low level of residual enzyme activity [129].

Combination of SRT with bone marrow or neuronal stem cell transplantation therapy showed synergies in the treatment of a mouse model of Sandhoff disease [128,130]. The combination of SRT with the treatment of secondary consequences of LSDs (*e.g.*, anti-inflammatory drugs) also exhibits synergistic effects in mouse models [131], suggesting a key role for SRT in combination therapy for lysosomal storage disorders.

1.5.2.4 Pharmaceutical Chaperone Therapy

Recently, pharmaceutical chaperone therapy (PCT) has been introduced for the treatment of lysosomal storage disorders. This therapy, also referred to as active-site-specific chaperone (ASSC) therapy is an emerging therapeutic strategy for the treatment of LSDs.

In many LSDs, residual enzyme activity can be observed, which presumably results from a small amount of mutated protein that is properly folded and transported to the lysosomes. This provides a basis for the therapeutic employment of pharmaceutical chaperones (PCs).

Certain missense mutations in the disease-causing enzymes result in the biosynthesis of misfolded proteins that are retarded in the endoplasmatic reticulum (ER) and are degraded by the so-called endoplasmatic-reticulum-associated-degradation (ERAD) pathway, one of the cells "quality-control" mechanisms, even though, they might be catalytically active, when properly folded [87,132]. Endoplasmatic reticulum associated degradation relies on the control of the protein maturation process in the ER, which bases molecular chaperones, such as calnexin and calreticulin, respectively, to bind to improperly folded proteins, facilitate correct folding and to prevent the aggregation of non-native forms [132,133]. Misfolded proteins are secreted to the cytosol, where they are subjected to ubiquitin-dependent proteasomal degradation.

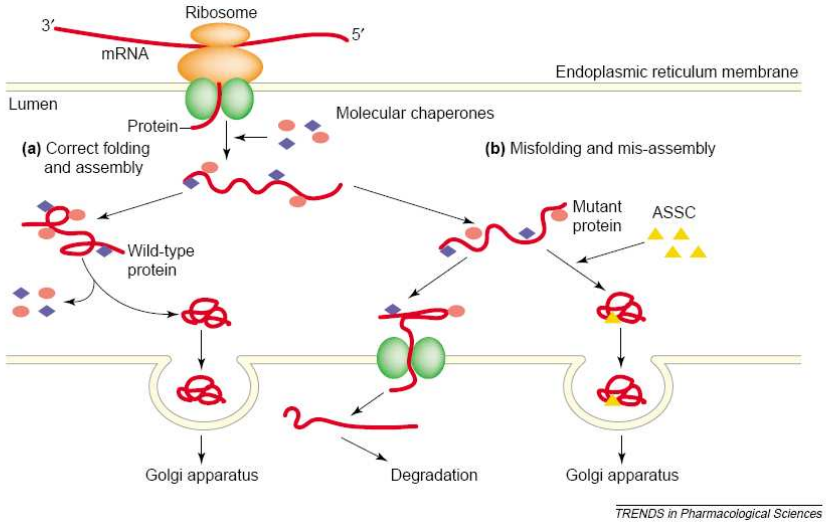


Figure 1-27: Mechanism of pharmaceutical chaperones. Adapted from Ref [96].

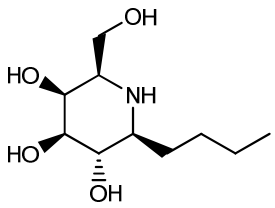
The principle of PCT is that enzyme inhibitors may stabilize or induce the proper conformation of the mutated enzyme, when present in subinhibitory concentrations and prevent their recognition by the quality control [134]. Once the mutant enzyme reaches its native conformation it may maintain conformational stability after post-translational modification and maturation, even after dissociation of the chemical chaperone off its active site. In the lysosomes, the highly concentrated substrate may be able to displace the inhibitor. This can promote normal trafficking of the enzyme and finally restores enzyme activity in the lysosomes [97].

The effect of PCs could be first demonstrated with Fabry disease. In the study, mutant α-galactosidase enzymes could be rescued by various α-Gal A inhibitors, among them 1-deoxygalactonojirimycin [37,135]. Consequently, pharmaceutical chaperones for Gaucher disease [136], GM1-gangliosidosis [137], GM2-gangliosidosis [138,139] and Pompe disease [140,141] could be identified [137,138,142,143].

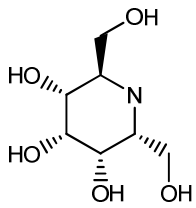
Compared to enzyme replacement therapy, pharmaceutical chaperones may have some advantages, such as the ability to cross the blood-brain-barrier, the ability to diffuse in connecting tissue and to reach target sites, which exogenously added enzymes cannot reach.

Lysosomal storage diseases can be considered ideal candidates for pharmaceutical chaperone therapy: It is assumed that an activity threshold of approximately 10 % is sufficient to prevent storage in LSDs [144]. Even a minor increase in residual enzyme activity thus might be beneficial and might have an impact on disease pathology.

Certain requirements to pharmaceutical chaperones exist. First, having a high affinity to the enzymes active-site is necessary. Higher affinities to the active-site imply a higher chaperone activity, as the comparison of several galactosidase inhibitors shows [145]. The second requirement to be an ASSC is the cellular permeability and the subcellular distribution. This effect was demonstrated by the comparison of two enzyme inhibitors, β-1-*C*-butyl-DGJ and α-*allo*-HNJ (Figure 1-28), regarding their chaperone power. Although α-*allo*-HNJ is the more active inhibitor, the chaperone potential of the C-alkylated derivative is higher. It is assumed that the higher lipophilicity of the latter improves cellular permeability [145].



β-1-*C*-butyl-1-DGJ

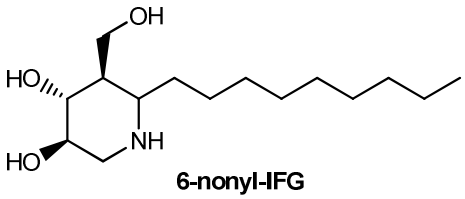


α-*allo*-HNJ

Figure 1-28

Third, a smooth dissociation off the enzymes active site is an important feature a PC should have. A properly rescued enzyme can only return to its function after the active-site has been liberated. 6-Nonyl-IFG is a very powerful inhibitor for glucosylceramidase (IC_{50}= 0.6 nM), combining the two elements of strong inhibition and lipophilicity to facilitate permeation and interaction with the aglycon binding site.

However, both parts of the molecule act as competitive inhibitors when tested sole, but the alkylated IFG shows a mixed type inhibition, thus it leaves the active site of the enzyme slowly, which is not favourable for a therapeutic molecule [87].

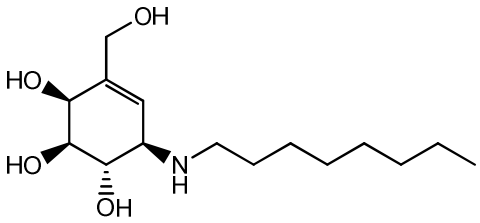


6-nonyl-IFG

Figure 1-29

One of the advantages of chemical chaperones is the better cellular distribution, as could be shown for NB-DNJ in rats, where the chaperone was widely distributed in inner organs [146].

The ability of crossing the blood-brain barrier could be demonstrated by Suzuki and co-workers [147] in a mouse model of a severe neuronopathic GM1-gangliosidosis.



NOEV

Figure 1-30

The synthetic chaperone *N*-octyl-4-*epi*-valienamine (NOEV), a carbasugar, was rapidly transported to the brain and was able to increase the lysosomal β-galactosidase activity levels in almost all regions of the brain. Ganglioside storage could be prevented and neurological deterioration could be delayed.

Insights to the mechanism chemical chaperones could recently be provided by the crystal structures of lysosomal enzymes and the co-crystal structures with chemical chaperones. [148-151]. It could be demonstrated that the pharmaceutical chaperones fit into the active site pocket like the natural substrate und undergo hydrogen bonding with the specific amino acid residues. The strength of binding and the interactions with the active-site residues have been elucidated and the stabilising effect has been shown [152].

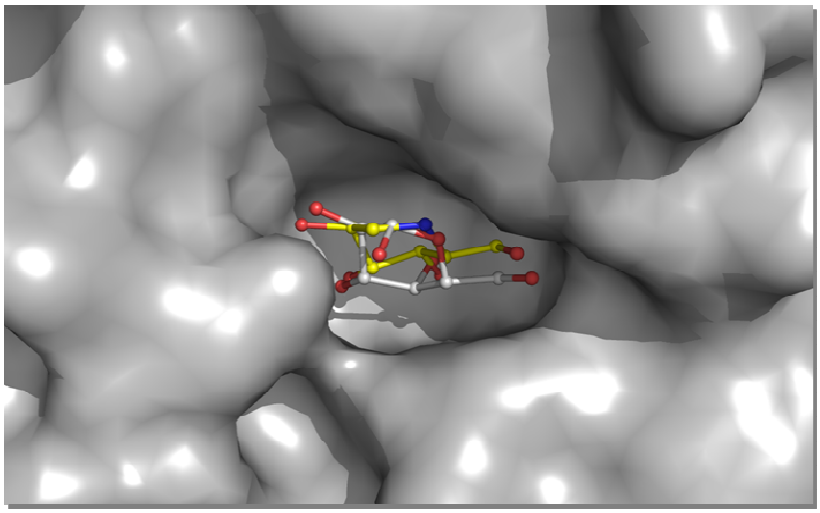


Figure 1-31: Superposition of α–D-galactose (grey) and 1-deoxygalactonojirimycin (yellow) in the active site of human lysosomal α-galactosidase A. (PDB codes 3GXT, 3GXP)

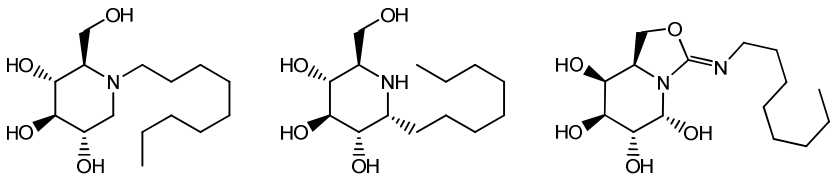
Pharmaceutical chaperone therapy still has drawbacks, since not all genotypes of lysosomal storage diseases are responsive to the chemical chaperone effect. In Gaucher disease, chaperone-sensitive mutations are relatively frequent, such as the N370S mutation that accounts for 34 % of Gaucher alleles in non-Ashkenazi patients and 71 % in Ashkenazi patients [153], whereas, *e.g.*, in Fabry disease almost all mutations are rare mutations and the rate of chaperone responsive mutations is difficult to estimate.

Besides substances such as NOEV or D-galactose, iminosugars were found to be effective chaperones for the treatment of lysosomal storage disorders.

Iminosugars can be dramatically altered in biological activity by the introduction of substituents to the ring nitrogen. In the field of pharmaceutical chaperones, *N*-substitution seems to be beneficial for activity. The modification of the ring nitrogen not only changes the basicity, but, depending on the chemical nature of the substituent, alters the polarity and hydrophobicity of the entire system, allows for the incorporation of diagnostic tags such as fluorophors or biotin and may enhance the molecules ability to cross the blood brain barrier.

It could be demonstrated that the length of the alkyl chain is of crucial importance for the chaperone activity of iminosugars. In the case of Gaucher disease, *N*-butyl-1-deoxynojirimycin is nearly inactive, whereas derivatives with *N*-nonyl- or *N*-decyl-chains show chaperone activity. However, *N*-dodecyl-DNJ is predominantly inhibitory [154].

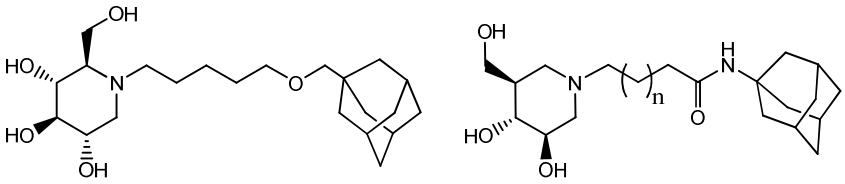
It could be shown, that the C-1-substituted iminoalditiol α-1-*C*-octyl-1-deoxynojirimycin is an active chemical chaperone for Gaucher disease, minimising side effects, such as the inhibition of ER glucosidases [155]. Bicyclic nojirimycin and galactonojirimycin derivatives with sp^2-hybridisation and an n-octyl-substitution could also be shown to be active chaperones for some Gaucher and GM1-gangliosidosis mutations, respectively [156].



N-nonyl-DNJ | α-1-C-octyl-DNJ | sp^2-hybridised bicyclic galactostatin derivative

Figure 1-32: Pharmaceutical chaperones for lysosomal storage disorders.

Overkleeft [157,158] could demonstrate that the introduction of a relatively large, hydrophobic 5-(1-adamant-1-yl-methoxy)pentyl residue linked to 1-deoxynojirimycin as N-substituent improves the inhibitory power of the molecules against human lysosomal glucocerebrosidase as well as glucosylceramid synthase up to 1,250 fold.



Overkleeft, 2007 | Wong, 2007, n = 1-3

Figure 1-33: Adamantyl substituted iminoalditols

Accordingly, Wong [159] hypothesises the interaction of a terminal adamantyl-amide in iminoalditol derivatives with a hydrophobic groove, close, but not next to the active site of human glucocerebrosidase and could show that the incorporation of the terminal adamantyl moiety to *N*-alkyl-deoxynojirimycin derivatives enhances the chaperone-like behaviour of the iminoalditols up to 9-fold.

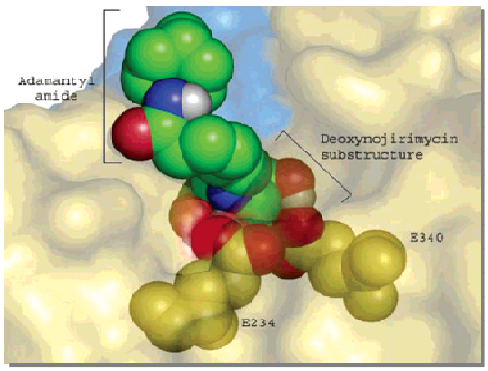


Figure 1-34: Molecular model of adamantylamidohexyl-substituted 1-deoxynojirimycin modelled into the active site of human glucocerebrosidase. The proposed hydrophobic groove is coloured blue, the active site glutamic acid residues are depicted in CPK format. Adapted from Ref. [159].

Another example of the substituents' role in iminosugars was shown by Wrodnigg, Stütz and co-workers [160] in the comparison of 1-deoxy-1-amino-DMDP with the corresponding napthylsulfonyl- and dansyl-modified derivatives (Figure 28).

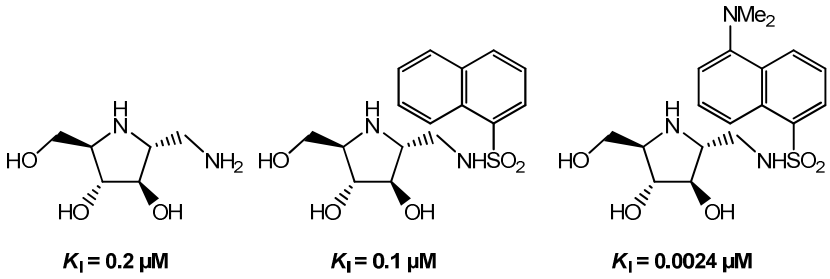


Figure 1-35 1-Amino-DMDP and two sulfonyl amide derivatives. K_I values determined against *Agrobacterium sp*. glucosidase. [160]

While the incorporation of the bicyclic aromatic system raised the inhibitor activity of the iminosugar against *Agrobacterium sp*. β-glycosidase by a factor of 250, the addition of an aromatic amine in the case of the dansylated derivative enhanced the inhibitor activity more than 10^4 fold with a K_I-value of 2.4 nM.

1.6 Fluorine in Medicinal Chemistry

As shown, the lipophilic substitution of pharmaceutical chaperones and glycosidase inhibitors generally improves their biological activity. To further increase the hydrophobic properties and possibly enhance the biological activity of iminosugars, fluorine substitution of the side chain may have advantageous effects.

The role of fluorine in organic and medicinal chemistry is well recognized. An increasing number of drugs on the market contain fluorine, which often is of major importance to activity. In the design of analogues of biologically active substances, the replacement of hydrogen or oxygen atoms with fluorine atoms has special advantages. Very early examples of fluorinated drugs were given by Peters [161], demonstrating that fluoroacetic acid leads to "suicide synthesis" of fluorocitric acid, requiring recognition of fluorous substrates by biosynthetic enzymes. On the other hand, fluorination of cortisone increases activity and selectivity [162]. Fluorinated nucleotides, such as 5-fluorouracil have been proven to be effective anti-cancer agents [163].

Fluorine, as expected from its position in the periodic table of elements possesses a small electronic radius and a very high electronegativity. Fluorine itself is highly reactive, so initial progress in organofluorine chemistry was impeded by the requirement of special expertise and technology. The development of electrophilic fluorination reagents and technology to control the

reactivity of elemental fluorine led to an impressive increase in the area. Recent studies suggest that the C-F bond (van der Waals radius 1.47 Å) is more similar to a C-O bond (van der Waals radius 1.52 Å) than to the C-H bond (van der Waals radius 1.2 Å). Fluorine is the smallest atom that can be used for the substitution of a hydrogen atom. Many examples can be found where the replacement of either oxygen or hydrogen by fluorine led to compounds with comparable activities but altered physicochemical properties.

The orthogonal reactivity of F^+ (not existing under normal conditions) and H^+, as well as of F^- and H^- have been exploited in enzyme inhibitor design [164,165].

The trifluoromethyl group has been shown to be sterically twice as demanding as a methyl group, to being up the size of a phenyl or tert-butyl group, respectively, depending on the methods employed to estimate the size [166,167]. Rotational energy studies suggest the trifluoromethyl group to be slightly larger in volume than an isopropyl group. X-ray crystallographic data put its volume closer to that of the isobutyl group [168-171].

Fluoro- or trifluoromethyl substitution will have an effect on the physico-chemical and/or the biological properties of the targeted molecule.

The strong electron withdrawing behaviour of fluorine substituents becomes evident in its effect on the acidity of neighbouring functional groups. Amines generally become less basic with fluorine substitution, and alcohols, carboxylic acids and phenols become more acidic by fluorine substitution. This lowering of pK_a values has an effect on various parameters, such as bioavailability, solubility, binding affinities and enhanced membrane penetration ability.

In central nervous system related drugs, fluorine substituents not only enhance the ability to cross the blood brain barrier, but also can be used as labelling agents (^{18}F) in positron emission tomography [172].

Beyond the inductive effects, altering reactivity and physical behaviour, there is now a greater appreciation for the part that fluorine substitution might play in direct binding interactions, which may occur between fluorine and the enzyme directly, bridged by a solvation sphere or may be caused by the change in conformation of the ligand molecule [173].

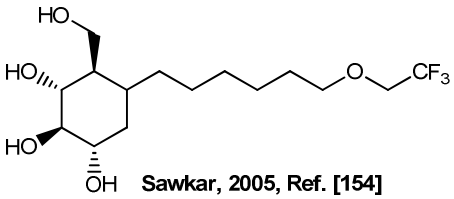
Fluorine substitution of lead molecules would be expected to have a profound effect on dipolar interactions, but the change in biological activity of fluorinated compounds compared to the respective hydrocarbon analogues sometimes might be reduced [174].

Fluorination of alkyl chains generally will decrease lipophilicity whereas the incorporation of fluorine substituents to an aryl ring or adjacent to a π-system increases lipophilicity and can have strong effects on the polarisation of the parent molecule [175].

Oligofluorinated or perfluorinated alkanes were developed as respiratory gas carriers and blood substitutes, impressively demonstrated by the “submerged mouse”, breathing in a perfluoroalkyl solution of oxygen [176]. In recent years, the potential of highly fluorinated molecules has been rediscovered and the field of so-called “fluorous chemistry” has been established. The term

"fluorous chemistry" was introduced in 1994 by Hórvath and Rabai [177] in context with biphasic separation techniques employing a third phase – the fluorous phase. Nowadays, various techniques exist for the separation of intermediates, excess reagents and side-products, respectively, exploiting the unique behaviour of fluorous phase techniques [178]. Additionally, perfluoroalkyl chains can be used for the non-covalent immobilisation of molecules to fluorinated glass slides, as recently reported by Schreiber's group [179].

Recently, an iminosugar featuring a trifluoromethyl substituent has been reported [154].



Sawkar, 2005, Ref. [154]

Figure 1-36

2 Aims and Synthetic Targets

N-Substituted iminoalditols have been shown to be powerful glycosidase inhibitors. Not only the interactions of the iminosugar with the active site of glycosidases, but also interactions of the N-substituents with hydrophobic pockets contribute to the biological activity.

Moreover, N-alkylated iminosugars gained importance as potential chemical chaperones for the treatment of lysosomal storage disorders.

The aim of this work is the synthesis of N-substituted 1-deoxynojirimycin derivatives for the evaluation of their chaperone and glycosidase inhibitor potential employing readily available D-galactose as starting material.

One set of target structures are derivatives of *N*-(6-aminohexyl)-1-deoxygalactonojirymicin, the primary amine of which can be derivatized by acylation or carbamate formation.

The synthetic strategy to obtain these products is the synthesis of the common intermediate by either the N-substitution of 1-deoxygalactonojirimycin with suitable alkylating agents or the cyclisation of L-*arabino*-hexos-5-ulose with aminated precursors of the spacer molecule (Figure 2-1).

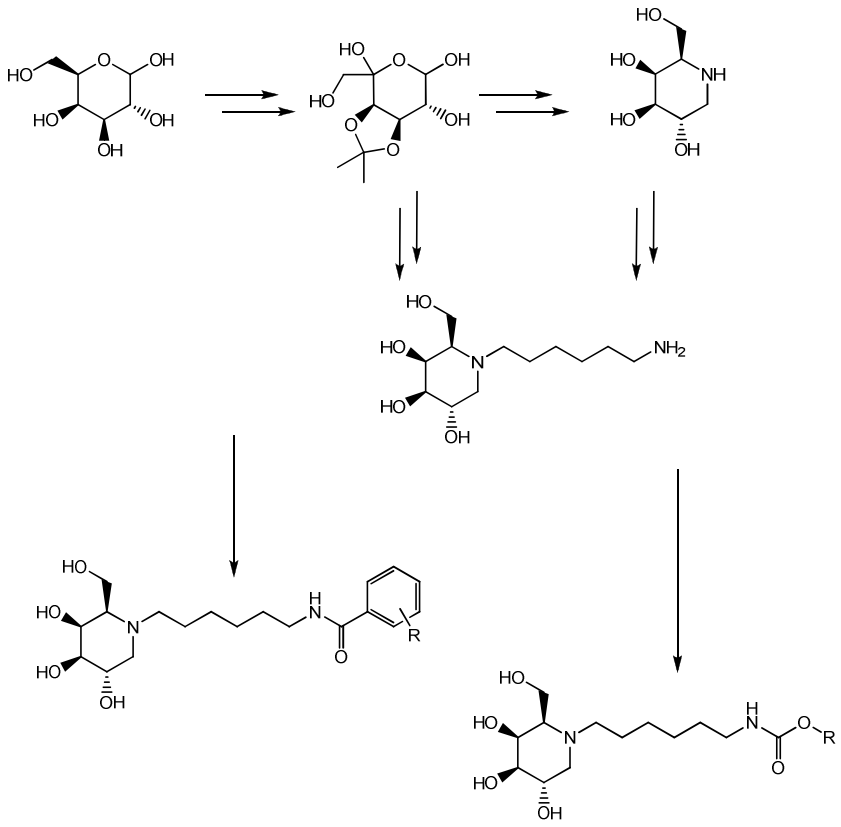


Figure 2-1:.Synthetic strategies towards lipophilic DGJ-derivatives.

Moreover, the incorporation of diagnostically relevant tags is an aim of this work. As model compound, a pyrenylated derivative for possible application as fluorescent probe, as well as a biotinyltated DGJ-derivative, in order to take advantage of the biotin-(strept)avidin interaction have been designed (Fig. 2-2)

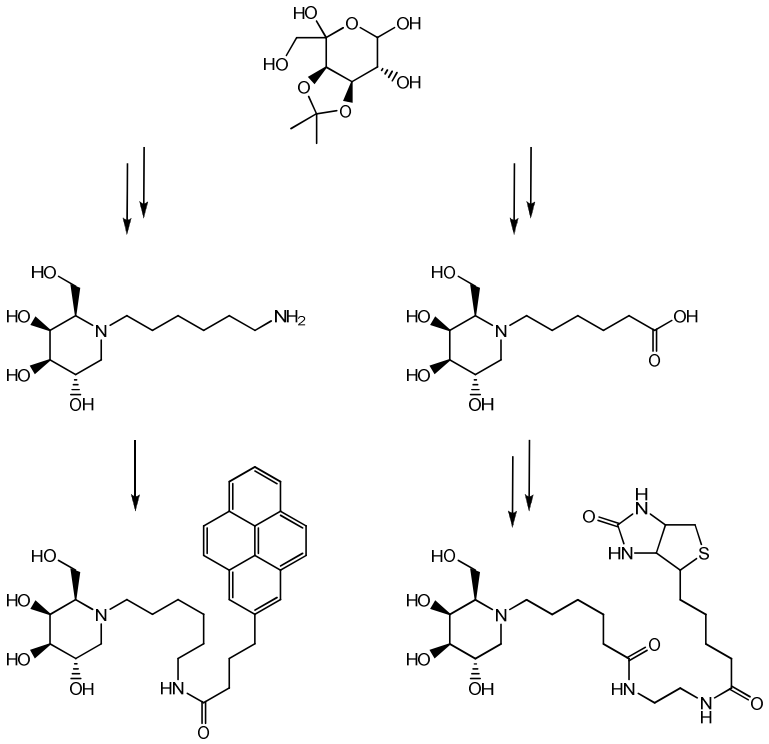


Figure 2-2: Target compounds of diagnostical relevance.

To further extend the hydrophobic properties, the synthesis of oligofluoroalkyl substituted 1-deoxygalactonojirimycin is a target of this work. Additionally, fluorous iminosugars might have additional advantages, such as the applicability to fluorous separation or immobilisation techniques. The synthesis of fluorously tagged 1-deoxygalactonojirimycin derivatives is carried out by cyclisation of L-*arabino*-hexos-5-ulose with suitable amine functionalised fluorous spacer molecules (Figure 2-3).

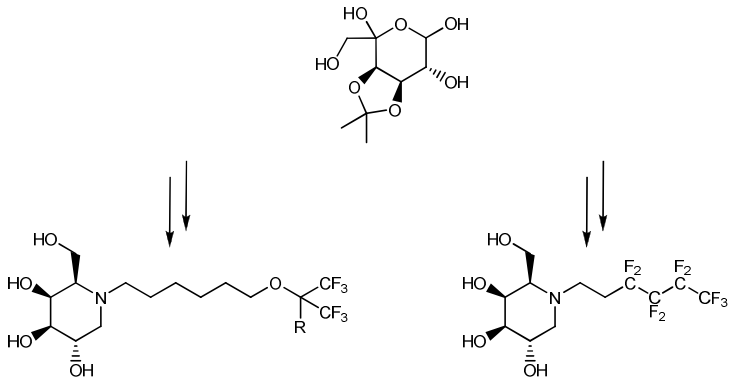


Figure 2-3: Synthetic strategies for the preparation of fluorous DGJ-derivatives.

3 Results and Discussion

In order to synthesise the target compounds by a double reductive amination procedure as the synthetic key step, a partially protected 5-ketoaldose derivative as sugar component had to be prepared. In general, the used synthetic approach relies on the formation of a hex-5-enopyranoside, combined with subsequent epoxidation and base catalyzed methanolysis to furnish the desired ketoaldose. In the next chapter the synthesis of 3,4-*O*-isopropylidene-L-*arabino*-hexos-5-ulose will be described in detail.

3.1 Synthesis of 3,4-O-Isopropylidene-L-arabino-hexos-5-ulose

Complementary to other routes leading to L-*arabino*-hexos-5-ulose derivatives as described by Catelani and co-workers [69] the carbonyl group at C-5 is introduced via the epoxidation of the corresponding 5,6-enogalactopyranose derivative in contrast to oxidation of a 4,5-unsaturated galactose derivative (see section 1.4.1).

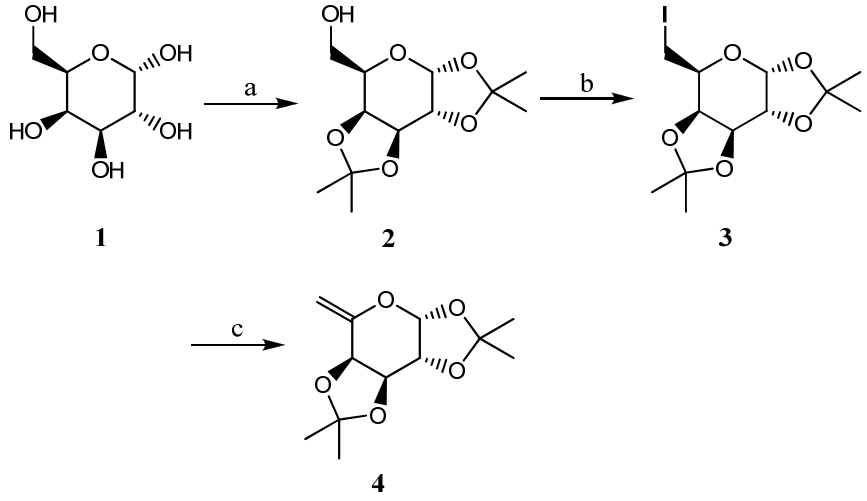


Figure 3-1: Synthesis of the protected enopyranose 5, a) Me_2CO, H_2SO_4; b) I_2, Ph_3P, imidazol, PhMe; c) KO^tBu, THF.

Nonetheless, OH-protection is necessary, as could be shown by previous studies in our group [70,71], since the common route to the 5,6-enopyranoside by the reaction of methyl galactopyranoside under conditions described by Garegg [180] yields the 4,6-anhydro derivative as sole product.

Due to these reasons, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose **2** was chosen as starting material for the iodination procedure. The diacetonide **2** was prepared by the sulphuric acid catalyzed reaction of D-galactose in acetone, furnishing derivative **2** in 51 % isolated yield.

Subsequent treatment with iodine, triphenylphosphine and 1-H imidazol in refluxing toluene, 6-deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**3**) can be isolated in nearly 80 % yield. Although reaction times are shorter when THF is used as solvent for this reaction, workup is more complicated due to the moderate miscibility of THF and water and product loss has to be accepted, so the original method using toluene as solvent is kept.

In contrast to earlier procedures for the synthesis of the 5,6-enopyranose **4**, where silver (I) fluoride was used as base during the elimination reaction, the complete protection of the enopyranoside allows for harsher conditions. Moreover, the use of silver (I) fluoride furnishes the 6-deoxy-6-fluoro derivative to some extent as side reaction [70].

Instead, potassium-tert-butoxide in THF is used for the elimination reaction to yield the 5,6-enopyranoside derivative **4** in nearly quantitative yield after recrystallisation from isopropanol. Heating the reaction mixture to shorten reaction times (typically 6 hours) is not recommendable since a secondary elimination product is formed in a side reaction that can be avoided by carrying out the reaction at room temperature.

The epoxidation of the double bond is accomplished with *m*CPBA in CH_2Cl_2 without any adjustment of the pH leading to a mixture of 5- and 6-chlorobenzoic acid esters **5** instead of the epoxide. This product mixture is directly taken into the next step after removal of excess 3-chloroperbenzoic acid by precipitation.

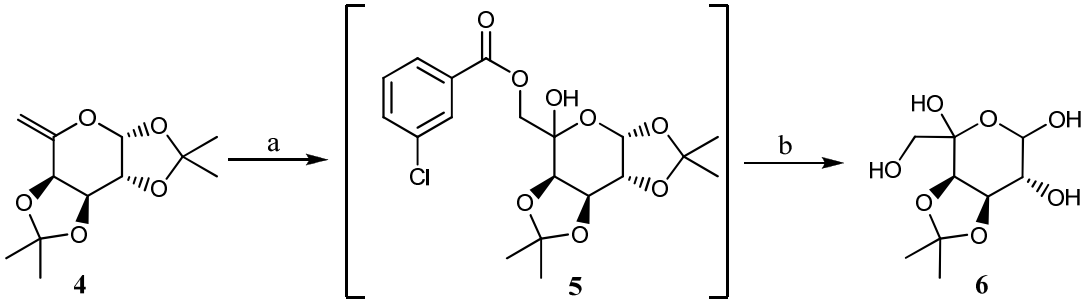


Figure 3-2: Oxidation of **5**, a) *m*CPBA, CH_2Cl_2; b) NaOMe, MeOH**;**

Zemplén saponification of this product mixture using NaOMe in MeOH yields the desired 3,4-*O*-isopropylidene-L-*arabino*-hexos-5-ulose (**6**) in 67 % yield after silica gel chromatography.

According to NMR spectroscopy (Figure 33), the compound is obtained as a mixture of at least 3 diastereomers due to the anomeric carbons C-5 and C-1, also TLC indicates several products. Still, flash chromatography has proven a suitable method for the isolation of the main product.

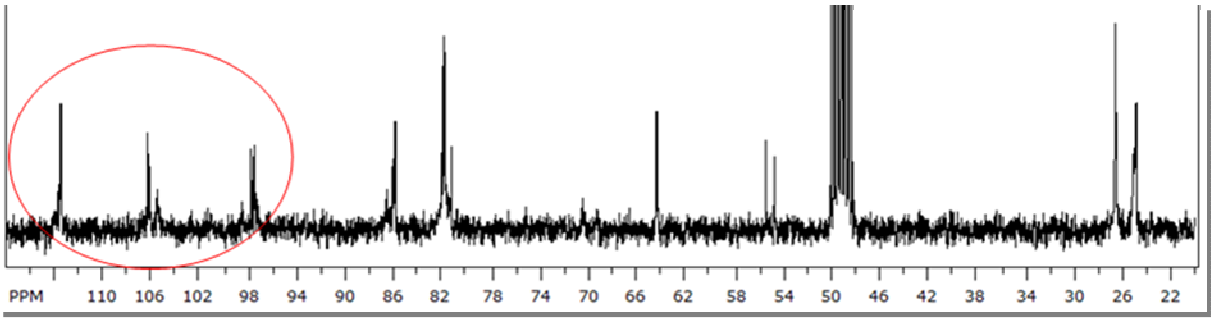


Figure 3-3: ^{13}C-NMR of L-*arabino*-hexos-5-ulose. The anomeric region is marked in red, showing signals from the isopropylidene protecting group and various anomeric signals.

3.2 Synthesis of 1-Deoxygalactonojirimycin and N-(6-Aminohexyl)-1-Deoxygalactonojirimycin

1-Deoxynojirimycin was synthesised from ulosose **6** in a reductive amination procedure. The amine functionality has been introduced via benzylamine, which has advantages concerning handling and ease of dosing as well as reactivity compared to aqueous ammonia. In this one pot reaction, the supposedly formed aldimine is first hydrogenated to the corresponding amine before a second imine formation with the keto-group and subsequent hydrogenation leads to 3,4-*O*-isopropylidene-1-deoxygalactonojirimycin (**7**). Although reaction times are long (typically 2-3 days) the yield of 76 % is acceptable.

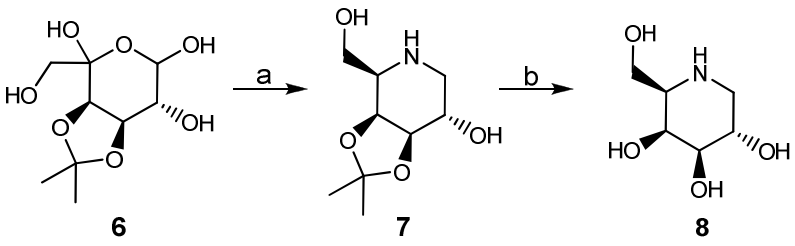


Figure 3-4: Synthesis of 1-DGJ, a) $BnNH_2$, H_2 (1 bar), Pd/C, MeOH, d.e. > 95 %; b) HCl, H_2O/MeOH;

In the case of ulosose **6**, the reaction product showing D-*galacto* configuration is formed exclusively, suggesting that the axial functional group at C-4 additionally influences the approach of the hydrogenation catalyst towards the formation of the D-*galacto*-configurated product (see section 1.4.1).

In order to obtain unprotected 1-deoxygalactonojirimycin (**8**), the remaining 3,4-*O*-isopropylidene protection has to be cleaved under acidic conditions, which can be accomplished by stirring compound **7** in a solution of hydrochloric acid in a mixture of methanol and water with typical yields

around 50 % after column chromatography. The remaining protecting group may be advantageous, when follow up chemistry is wished to be made on the iminoalditol ring system since the amine function and the remaining hydroxyl groups OH-2 and OH-6 can be conveniently orthogonally protected.

A key intermediate in this work is *N*-(6-aminohexyl)-1-deoxygalactonojirimycin (**15**). This compound has been prepared using various strategies, either employing suitable hexylamine derivatives in a reductive amination procedure or by *N*-alkylation of 1-deoxynojirimycin with alkylating agents. This substance is not only an important synthetic intermediate but may also be immobilised to suited resins and be used as an alternative to *p*-aminobenzyl-1-thio-β-D-galactopyranoside as affinity ligand for the purification of galactosidases [181,182].

In a first attempt, ulosose **6** was reacted in 39 % yield based on ulosose **6** with benzyl 6-azidohexylcarbamate **13** which had been previously prepared from 6-aminohexanol (**9**) by N-protection with di-tert-butyl-dicarbonate under standard conditions to yield alcohol **10**, followed by O-tosylation with toluene-4-sulfonyl chloride in CH_2Cl_2 using pyridine as base to yield compound **12**. Tosylate **12** then underwent azide exchange under S_N2-conditions using sodium azide in DMF to yield azide **13**.

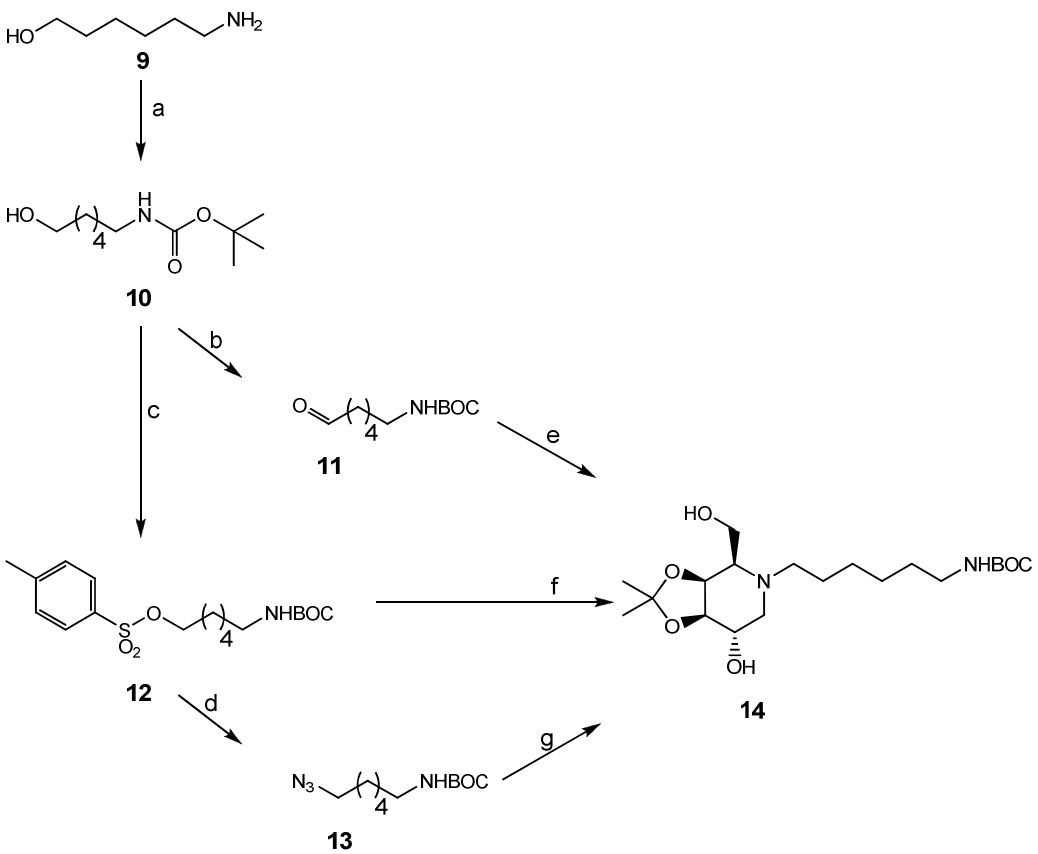
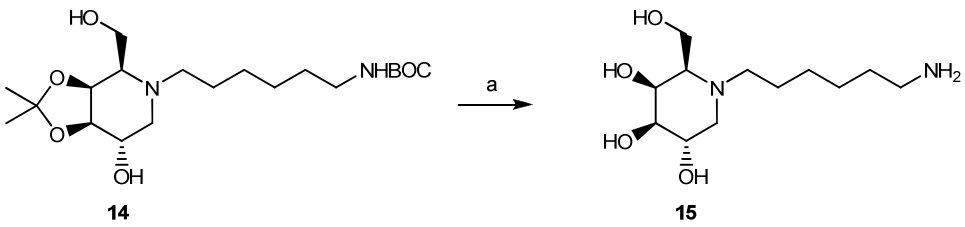


Figure 3-5: Syntheses of **14**. Reagents and conditions: a) BOC_2O, Na_2CO_3; b) Dess-Martin periodinane, CH_2Cl_2; c) TosCl, pyridine, CH_2Cl_2; d) NaN_3, DMF, 60 °C; e) **7**, H_2 (1 bar), Pd/C, MeOH; f) **7**, DMF, Na_2CO_3, 60 °C; g) **6**, H_2 (1 bar), Pd/C, MeOH.

In an attempt to improve yields, a reductive amination of the partially protected DGJ derivative **7** with aldehyde **11** was carried out. Aldehyde **11** was obtained by the Dess-Martin oxidation of alcohol **10** in nearly quantitative yields. The reductive amination of **7** with the aldehyde yielded **14** in 42 % based on ulosose **6**.

Another strategy to obtain the partially protected derivative **14** was the N-alkylation of **7** under S_N2-conditions using tosylate **12** as the alkylating agent. This strategy yielded the partially protected derivative **14** in 71 % referred to ulosose **6**.

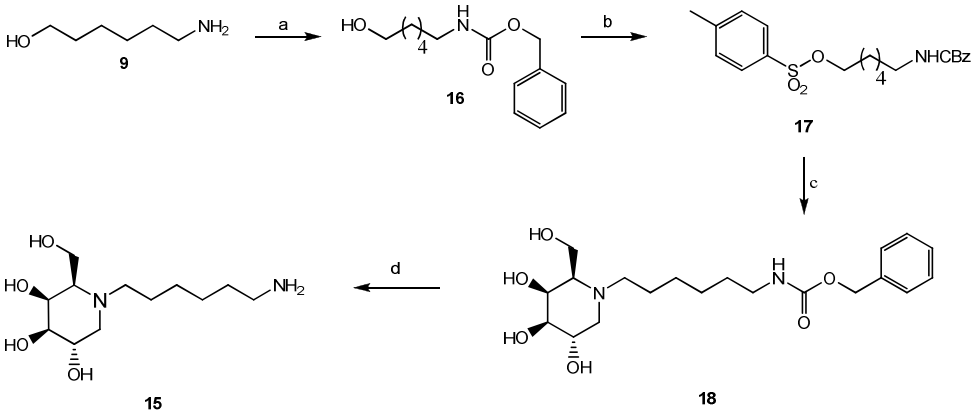
Compound **14** then was converted to the target compound *N*-(6-aminohexyl)-1-deoxygalactonojirimycin (**15**) by acidic cleavage of the protecting groups. Thus, compound **14** was dissolved in a mixture of water and methanol before concentrated hydrochloric acid was added and the reaction was stirred at room temperature. After completed conversion, the crude reaction product was subjected to silica gel chromatography to yield **15** as free base in 68 %.



14 **15**

Figure 3-6: Synthesis of **15** I. Reagents and conditions: a) HCl conc., H_2O/MeOH

In analogy to alcohol **10**, *N*-CBz-6-aminohexanol (**16**) was prepared in 89 % after recrystallisation by the reaction of **9** with benzyl chloroformate in methanol, again using sodium carbonate as base. Tosylate formation under the conditions described above yielded tosylate **17** in 64 % after column chromatography. Subsequently, DGJ (**8**) was reacted with tosylate **18** under S_N2-conditions described above to yield 77 % of *N*-(*N*-CBz-6-aminohexyl)-1-deoxygalactonojirimycin (**18**) after silica gel chromatography.



9 **16** **17** **18** **15**

Figure 3-7: Synthesis of **15** II. Reagents and conditions: a) $BnOC(O)Cl$, Na_2CO_3, MeOH; b) TosCl, pyridine, CH_2Cl_2; c) **8**, Na_2CO_3, 60 °C; d) H_2 (1 bar), Pd/C, MeOH.

For the hydrogenolytic cleavage of the benzyloxycarbonyl group, compound **18** was dissolved in methanol and Pd/C is added as hydrogenation catalyst. After completed conversion and removal of the catalyst, no further purification was necessary and amine **15** was obtained as free base in 86 %.

Although compound **15** was obtained as nearly colourless oil, the primary amine function renders the substance sensitive towards oxidation, thus a darkening of the material was observed upon exposure to air.

The overall yields of the three described routes to the target compound **15** range between 25 to 27 % for the direct cyclisation, the *N*-substitution of 1-DGJ (**8**) with compound **17** as well as the reductive amination of **7** with aldehyde **11**. In the case of the *N*-substitution of intermediate **7** with tosylate **12** the overall yield of 37 % suggests this route being the strategy of choice. Still purification of the target compound is complex, since due to the high polarity of the compound and the presence of a primary amine, yield losses during silica gel column chromatography cannot be avoided.

3.3 Synthesis of Lipophilic DGJ derivatives

3.3.1 Syntheses of *N*-(6-Aminohexyl)-1-Deoxygalactonojirimycin Derivatives

The primary amine of compound **15** is an attractive site for modifications of the spacer moiety in 1-DGJ derivatives. Acylation can be carried out with suitable acid chlorides or anhydrides as well as carbamate forming agents. Also, peptide coupling chemistry can be used to form derivates with unactivated carboxylic acids. In the following sections, the synthesis of N-modified derivatives will be described in detail.

The chemoselective acylation of the primary amine of **15** with various aromatic carboxylic acids was used to obtain a set of derivatives with different substituents, two of them, namely compounds **19** and **20** being isosteric to each other.

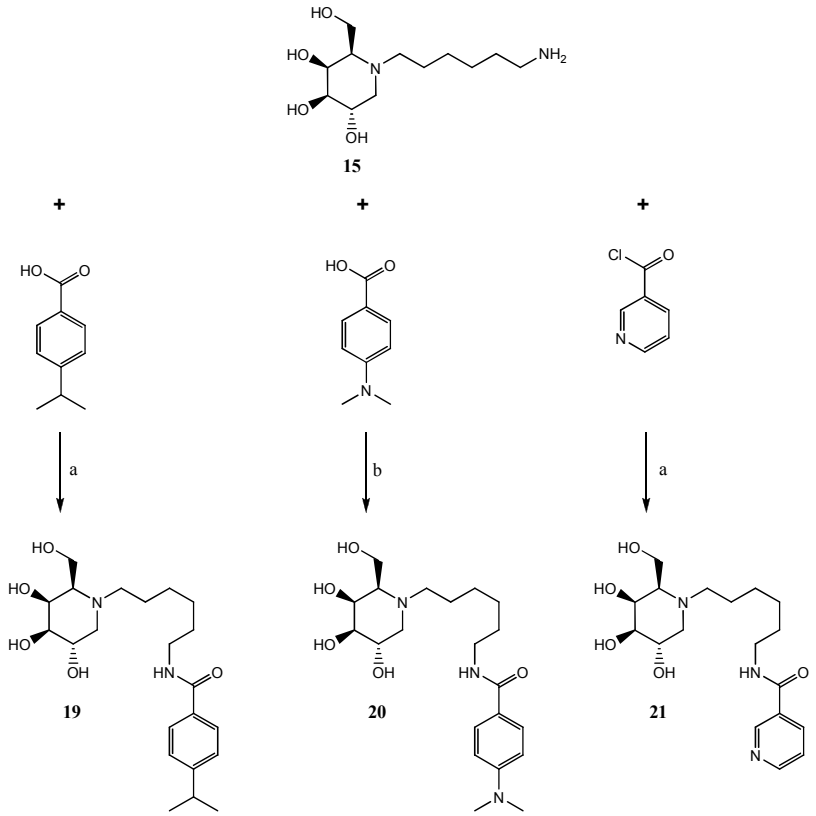


Figure 3-8: Synthesis of aromatic amide functionalised DGJ derivatives. Reagents and conditions: a) TBTU, Et_3N, DMF; b) DMF, Et_3N, 0 °C;

The reaction of **15** with 4-isopropylbenzoic acid under peptide coupling conditions yielded derivative **19** in 51 %. TBTU was used as coupling reagent in DMF employing Et_3N as base. Under the same conditions, nicotinic acid (3-pyridine carboxylic acid) was coupled to the primary amine to yield compound **21** in 24 %. Compound **20** was obtained by the N-acylation of the primary amine **15** using 4-dimethylamino benzoyl chloride as acylating agent in DMF, yielding compound **20** in 24 %. Compared to the synthesis of **19**, showing acceptable yields in the coupling step, the poor yields when ionisable acids were introduced might be explained by the presence of additional hydroxyl functions in **15**, lowering the yields of the coupling reactions and especially leading to side products in the N-acylation reaction with the acid chloride. More likely, the influence of the additional basic nitrogen atoms influenced the coupling reactions themselves due to the zwitterionic behaviour of the amino acids. A possible strategy to improve yields in the peptide coupling reaction is to change the coupling reagent from TBTU to other, possibly more suited reagents of the benzotriazole family. Additionally, purification of the obtained DGJ derivatives **19** - **21** proved to be non-trivial and a major loss of substance was observed. The substances were obtained in amounts allowing for the investigation of the biological properties of this set of substances which will be discussed later in this work.

To investigate the influence of an aromatic ring system as unpolar head group in N-alkylated DGJ derivatives, compound **23** has been prepared by the carbamate formation of **15** with di-tert.-butyl carbonate in methanol, yielding the target compound in 86 %. Its aromatic counterpart, namely the

benzyl carbamate **18** the synthesis of which has been discussed above was tested for its biological activity in the identical manner as compounds **22** and **23** which will be discussed later in this work.

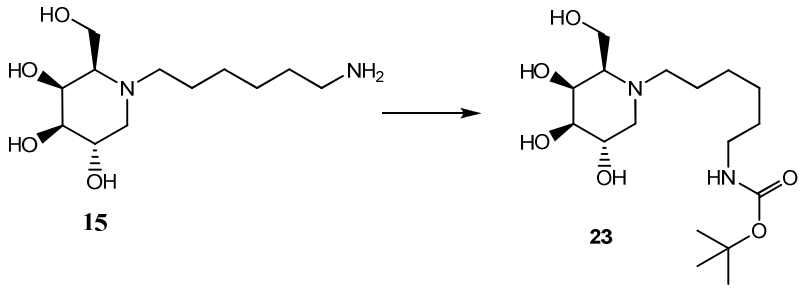


Figure 3-9: Synthesis of amide and carbamate functionalised DGJ derivatives. Reagents and conditions: a) TBTU, Et_3N, DMF; b) MeOH, Na_2CO_3;

All newly synthesised 1-DGJ derivatives described in this chapter were tested for their inhibitory activity against three different glycosidases. Selected compounds were also tested for their chaperone activity *in vitro*. The results of these tests will be discussed in section 3.6.

3.3.2 Synthesis of Diagnostically Relevant 1-Deoxygalacto-nojirimycin Derivatives

The incorporation of diagnostic tags to biological molecules produces powerful tools for the investigation of biological activity. Fluorescence is an important tool for the determination of concentrations or the localisation of compounds within a cell. Fluorescent DGJ derivatives have been previously prepared employing the dansyl moiety as fluorophor [70,71]. To alter the wavelength range, in this work, a pyrenylated DGJ derivative has been synthesised.

Another diagnostic tag of great importance is the biotinyl residue. The biotin-(strept)avidin biorecognition has proven to be an indispensable tool for diagnostics, biotechnology and nanotechnology [183,184]. Biotinylation was for example used in context with activity-based protein profiling [185] of exo- as well as endo-glycosidases and could be demonstrated to be a versatile tool for the investigation of the enzymes structure and function. Therefore, a mechanism-based, active-site specific inactivator was covalently bound to biotin via an either cleavable or non-cleavable linker the properties of which can be exploited for affinity chromatography or fluorescence spectroscopy [186,187]. In the course of the synthesis of biotinylated iminosugars with different stereochemical configurations, a biotinylated 1-DGJ derivative was targeted.

The acylation of amine **15** with 1-pyrene butyric acid using peptide coupling methodology with TBTU and Et_3N in DMF yielded derivative **22** in 60 %, a yield comparable to the synthesis of

compound **19**. The pyrene moiety is not only a bulky aromatic system, but also its physicochemical properties can be exploited in fluorescence spectroscopy, making this compound a putative tool for microbiological investigations.

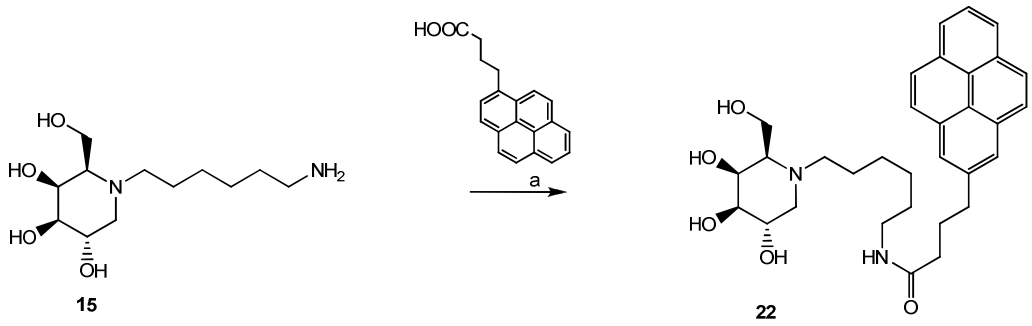


Figure 3-10: Synthesis of a pyrenylated DGJ-derivative.

In order to prepare target compound **27**, DGJ-derivative **24** was synthesised in the first step by the reductive amination cyclisation of ulosose **6** and methyl 6-aminohexanoate hydrochloride. The intermediate isopropylideneated compound had not been isolated but the isopropylidene group was cleaved by acidic hydrolysis using anhydrous HCl in methanol which gave methyl ester hydrochloride **24** in 92 % with respect to the staring material **6**. NMR as well as TLC analysis confirmed the required purity of the compound, thus, no additional purification was necessary.

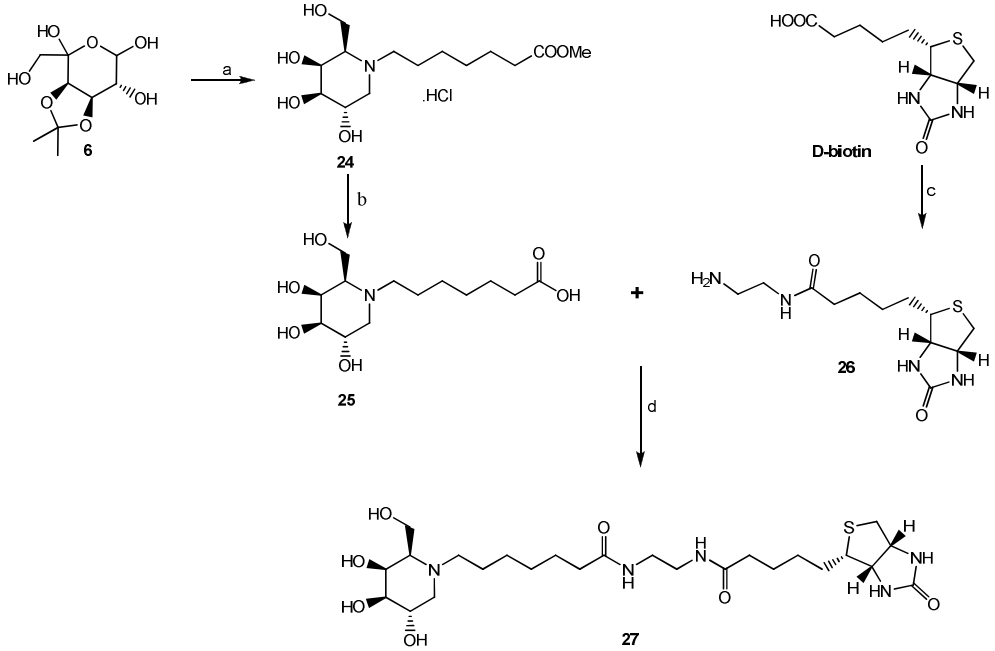


Figure 3-11: Synthesis of a biotinylated DGJ derivative. Reagents and conditions: a) 1: methyl 6-aminocaproate.HCl, H_2, Et_3N, Pd/C, MeOH; 2: AcCl, MeOH; b) 1: NaOH, 1,4-dioxane/water 1:1, 2: Amberlite IR-120 H^+ c) ethylene diamine, HBTU, Et_3N, DMF; d) TBTU, iPr_2NEt, DMF.

The saponification of the methyl ester was carried out under standard conditions and showed quantitative conversion of the starting material. After careful neutralization with acidic ion exchange resin and removal of the solvents, **25** was obtained in 69 % and was used as starting material for the biotinylation reaction without additional purification.

To introduce the biotinyl residue, aminoethyl biotinamide (**26**), which was prepared by the coupling of D-biotin with ethylene diamine under peptide bond forming conditions using HBTU as the coupling reagent, has been employed. Compound **26** was reacted with carboxylic acid **25** in the presence of TBTU and Hünig's base in DMF. The isolated yield of **27** after column chromatography was 50 %. Results of preliminary biological experiments will be discussed later in this work.

3.4 Synthesis of Fluorous 1-DGJ Derivatives

Based on recent observations that lipophilic derivatives of various iminoalditols, among them *N*-butyl- and *N*-nonyl-1-deoxynojirimycin, as well as other derivatives bearing sterically demanding groups, not only are powerful glycosidase inhibitors, but also are promising candidates as chemical chaperones for the treatment of lysosomal storage disorders, the possibility to modulate the chemical nature and the physical properties of the N-substituents by the incorporation of fluorine substituents led us to the design of model compounds containing fluorous moieties.

In order to synthesise fluorinated 1-deoxygalactonojirimycin derivatives, two major strategies have been employed. For the synthesis of the fluorous hexyl ether derivatives and the N-oligofluoro-substituted derivative, the spacer arms have been prepared as N-CBz protected precursors that can be employed as reagents in a catalytic reductive amination cyclisation procedure. The oligofluoroalkyl tagged benzyl carbamate containing compound **37** has been prepared by the derivatisation of *N*-(6-aminohexyl)-1-deoxynojirimycin (**15**).

Starting from CBz protected amino alcohol **16**, the reaction with suitable 1,1-bis(trifluoromethyl)methanol derivatives under Mitsunobu conditions, using triphenylphosphine and diethyl azodicarboxylate (DEAD), the ether-type molecules were prepared in good yields (> 80 %) and in very short reaction times. The used method was reported by Falck [188] and Sebesta [189], exploiting the relatively high acidity (pK_a ~ 9-10) of bis(trifluoromethyl)methanol derivatives, which enables them to formally act as acids in the reaction. However, in contrast to the classical Mitsunobu methodology, the fluorous alcohol has to be used in large excess to achieve acceptably high yields.

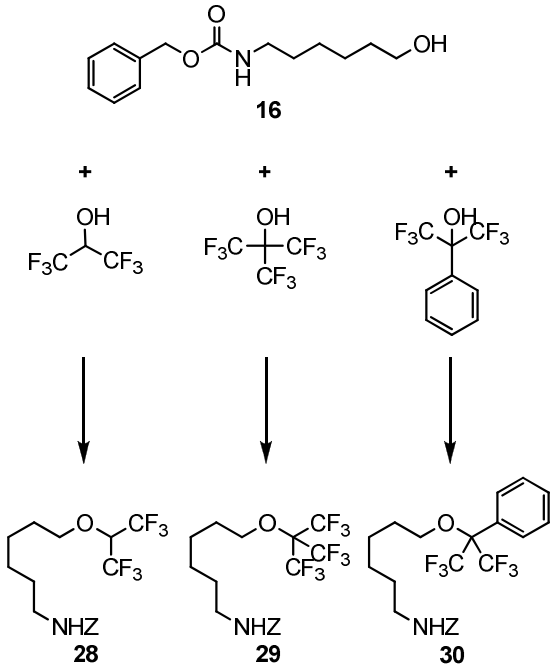


Figure 3-12: Synthesis of fluorous ether-type spacer molecules.
Reagents and conditions: Ph_3P, DEAD, 0 ℃ → r.t.

The purification of the substances was achieved by a two step method with fluorous solid phase extraction [190] (F-SPE) being the first step. F-SPE in fact turned out to be a suitable method for the removal of triphenylphosphine oxide, which usually is difficult to separate from the reaction mixture. Subsequent silica gel chromatography furnished the spacer molecules as pure compounds.

Azide **31** was prepared from the commercially available nonafluorohexyl iodide by the phase transfer catalysed azide substitution employing trioctylmethylammonium chloride as catalyst.

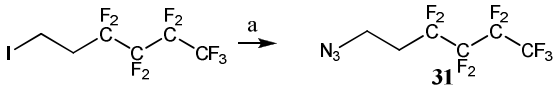


Figure 3-13: Synthesis of a fluorous hexyl azide. Reagents and conditions: a) NaN_3, Aliquat 336, H_2O.

Compounds **28** - **31** could be used directly as reagents in the reductive amination procedure employing ulosose **6** as dicarbonyl component. The reaction is carried out at room temperature in methanol using palladium on active charcoal as hydrogenation catalyst under a hydrogen atmosphere at atmospheric pressure for 30 – 40 hours.

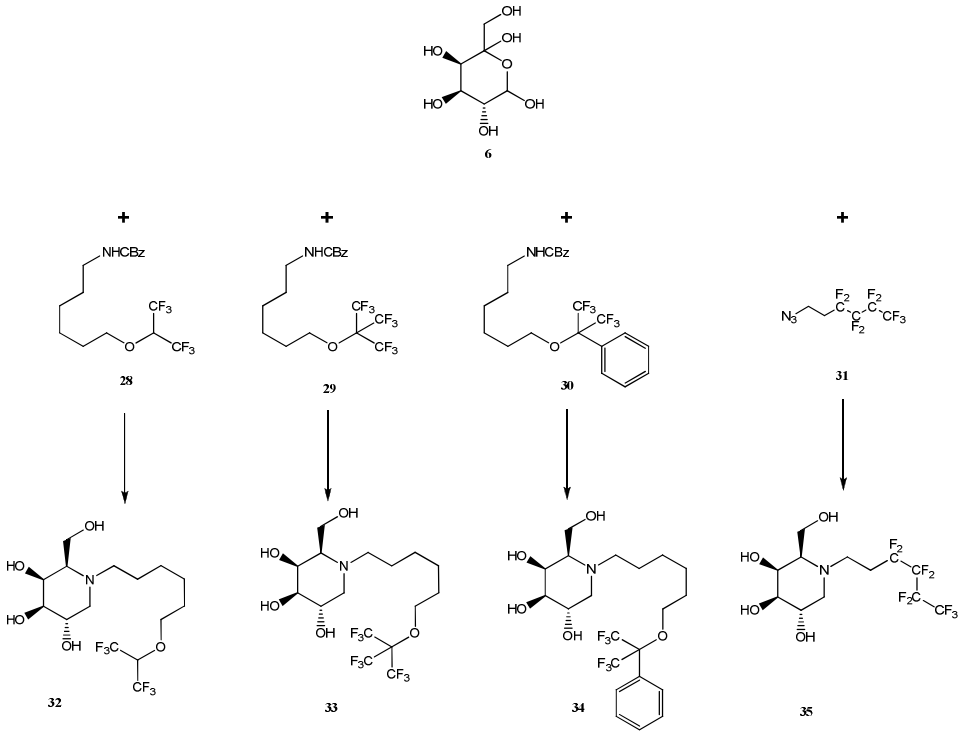


Figure 3-14: Synthesis of fluorously tagged 1-DGJ derivatives. Reagents and conditions: 1) H_2, Pd/C, MeOH; 2) HCl, MeOH/H_2O.

The isopropylidene protecting group was subsequently removed by acidic cleavage using concentrated hydrochloric acid in a mixture of methanol and water.

In the case of the fluoroalkoxy derivatives **32** - **34**, the abovementioned two-step purification strategy was applied and after F-SPE and silica gel chromatography, the compounds were isolated as free bases in yields between 26 and 52 %, where the major loss of substance apparently occurs at the step of silica gel chromatography.

The cyclisation/hydrogenation reaction of **6** with fluorous azide **31** under the hydrogenation conditions described above followed by hydrolysis of the isopropylidene moiety yielded 40 % of compound **35** as free base after silica gel chromatography. In this case, the moderate yield of the cyclisation reaction might also be explained by the low solubility of the fluorous azide in methanol.

Another type of fluorous iminosugar was prepared by the reaction of amine **15** with an activated fluorous benzyl carbamate. The activated carbamate incorporates a fluorous tag containing nine fluorine atoms and is a commercially available compound used for the fluorous tagging of amines.

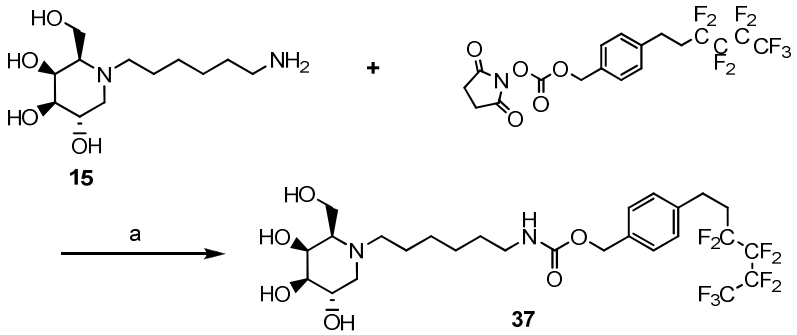


Figure 3-15: Synthesis of a fluorously tagged DGJ derivative. Reagents and conditions: a) Et_3N, DMF.

The reaction of the hydroxysuccinimide ester with amine **15** was carried out in DMF with triethylamine as base and yielded compound **37** in 46 % after silica gel chromatography.

The biological activities of the fluorous compounds described above will be discussed in section 3.6.

3.5 Scaling-Up of Iminosugar Syntheses

Multi-gram amounts of biologically active iminosugars are occasionally needed to conduct biological experiments beyond enzyme kinetics, such as confocal microscopy and chaperoning experiments. Additionally, there is mentionable interest to use iminosugars-amino acid hybrids for the modification of biological molecules and for the use as peptide building blocks.

Based on previous work in the GlycoGroup [70,71], the syntheses for DGJ-lysine hybrids, which have been usually carried out on a scale of typically 50 to 100 milligrams was a necessary task.

Fig. 16 shows the target compounds needed to be synthesised in a scale up to 5 grams.

Compounds **38** and **40** are recognised to be potent galactosidase inhibitors. Moreover, they show a good chaperoning profile to mutated lysosomal galactosidases. Compound **39** is a new compound in which the α-amine and the carboxylic acid function of lysine are protected orthogonally.

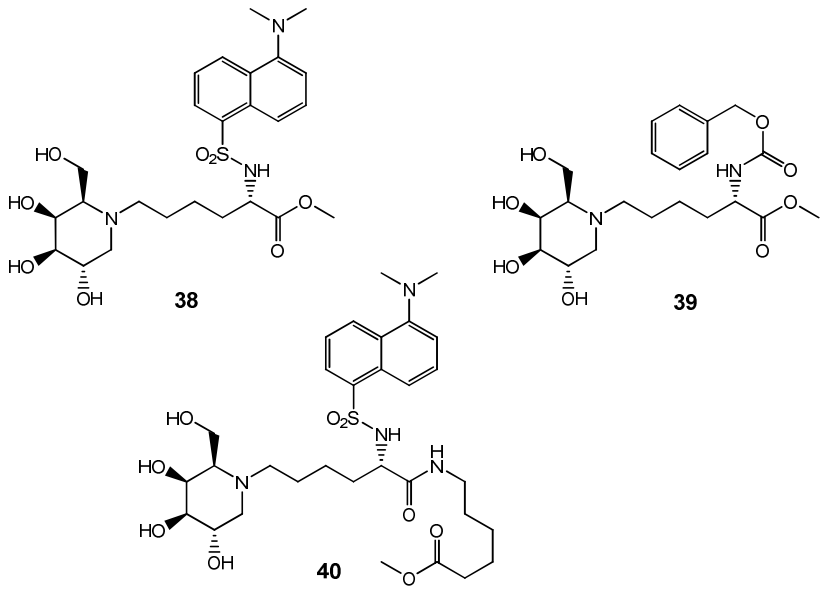


Figure 3-16: Primary target structures for the up-scaling of DGJ-lysine hybrids.

Starting from ulosose **6**, suitably protected lysine derivatives as the amine compounds for the reductive amination cyclisation method have been employed.

Lysine derivatives **41** and **42** were synthesised as reported [70,71], from commercially available BOC-L-Lys(Z)-OH and the synthesis of multigram quantities of the spacer molecules through direct scaling-up was possible.

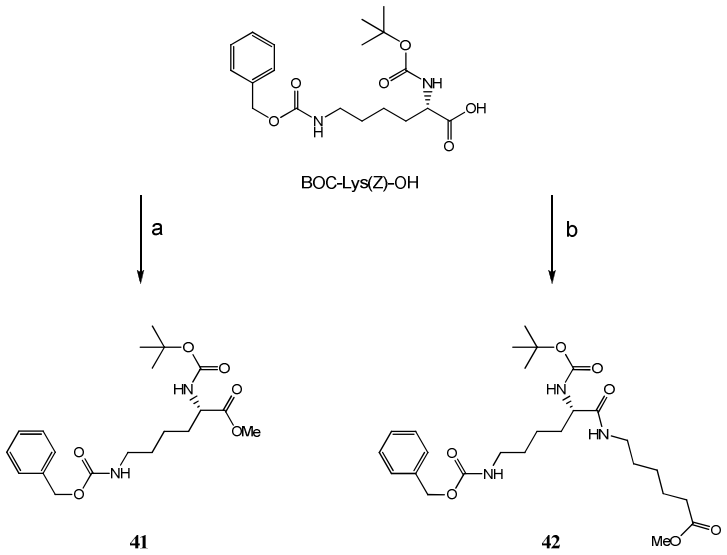


Figure 3-17: Synthesis of lysine spacers.

In contrast to the reported synthesis [70,71] the synthetic route to the target compounds was changed towards the use of ulosose **6** instead of an unprotected dicarbonyl derivative, yielding partially protected derivatives in the first step, which was supposed to facilitate the purification process on a larger scale.

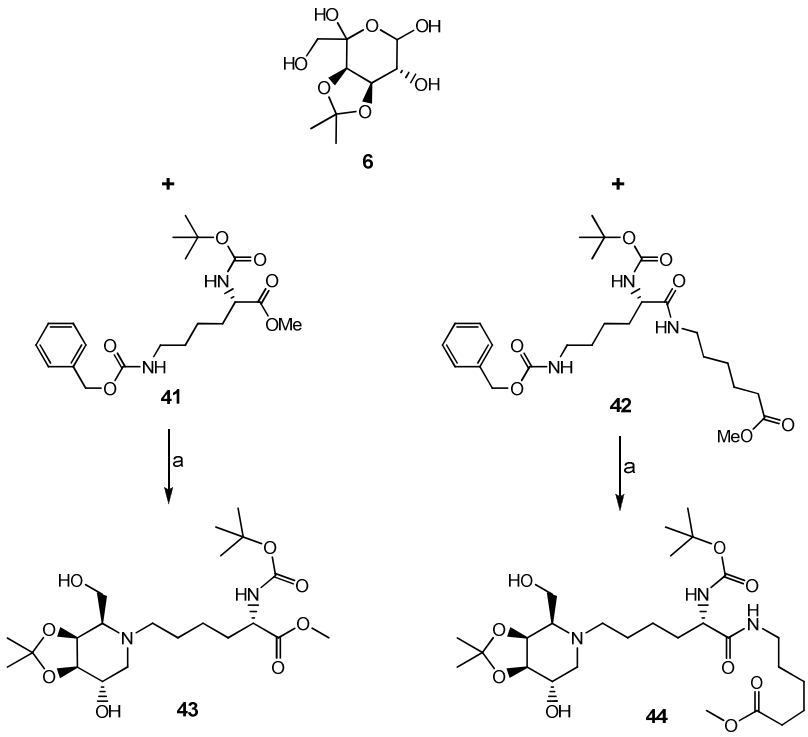


Figure 3-18: Synthesis of partially protected DGJ-L-lysine hybrids. Reagents and conditions: a) H_2, Pd/C, MeOH.

The partially protected compounds **43** and **44** were synthesised by the reductive amination of ulosose **6** using Pd/C as catalyst and H_2 at atmospheric pressure. In the linear scaling-up of the synthesis of **43**, yields were not satisfying but could be improved by carrying out the cyclisation procedure in higher dilution. Table 3-1 gives an overview on optimised and non-optimised conditions. The cyclisation of **6** with lysine derivative **42** yielded compound **44** in yields of 83 %.

Table 3-1: Comparison of reaction conditions in the scaling-up of the synthesis of **43.**

Batch	6	41	catalyst	MeOH	Yield
A	8.4 g (35.4 mmol)	16.8 g (42.5 mmol)	3.4 %	300 ml (~ 3%)	13 %
B	3.4 g (14.2 mmol)	5.05 g (12.8 mmol)	3.0 %	750 ml (~ 0.5 %)	48 %

The deprotection of **43** and **44**, respectively, was accomplished using anhydrous HCl in methanol to avoid hydrolysis of the methyl ester and the obtained amine hydrochlorides **45** and **48** were not further purified, since NMR and TLC analysis showed satisfactory purity.

The introduction of the dansyl moiety was then carried out by the reaction of **45** and **48,** respectively**,** with dansyl chloride in DMF. Silica gel chromatography finally yielded dansylated compound **40** in 47 % with respect to ulosose **6.** Overall, more than 1.3 g of the dansylated compound **40** could be prepared to be investigated towards biological activity.

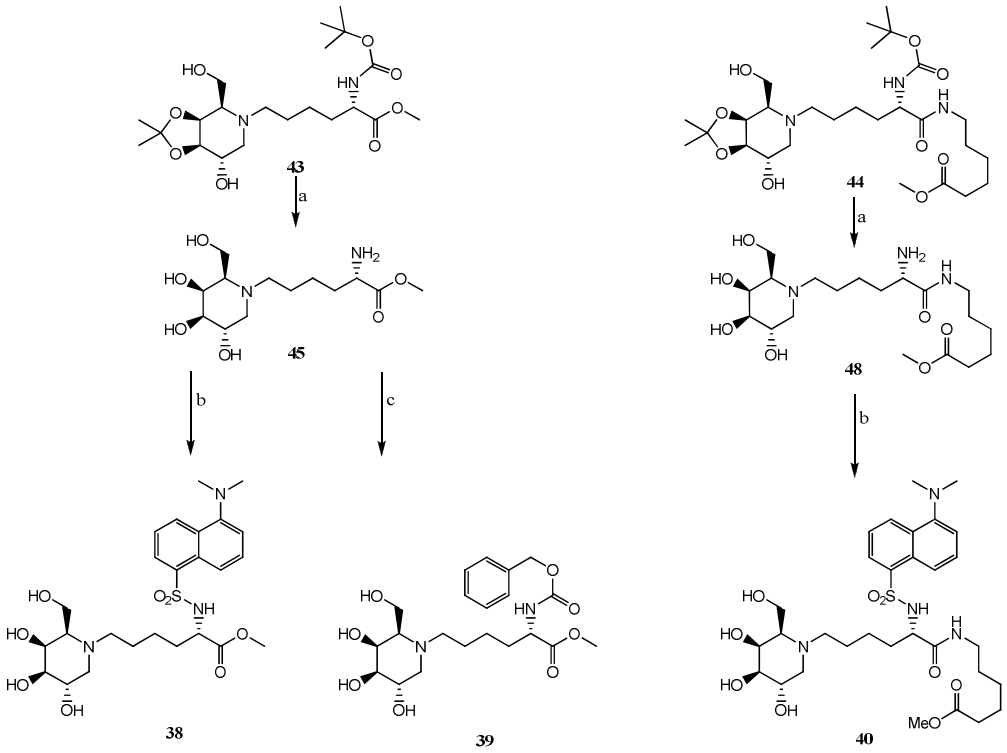


Figure 3-19: Routes for the gram-scale syntheses of target compounds **38** – **40**. Reagents and conditions: a) AcCl, MeOH; b) dansyl chloride, Et_3N, DMF; c) BnOC(O)Cl, Na_2CO_3, MeOH.

Although the purification of **40** by silica gel chromatography proved to be successful, in the case of **38** the difference in retention of the side product dansylic acid and the target compound was minimal and separation attempts by silica gel column chromatography failed. In the small scale purification (< 50 mg) of dansylated compound **38**, preparative thin layer chromatography (pTLC) was used to isolate the product, but on a scale of 1 g, pTLC is not very efficient in terms of economics and handling.

To overcome this limitation, contaminated compound **38** was acetylated under standard acetylation conditions using acetic acid anhydride in pyridine. The reaction was quantitative and peracetate **49** could be isolated in nearly quantitative yield. The subsequent Zemplén saponification with sodium methoxide in methanol was unproblematic and finally target compound **38** could be isolated in 44 % yield with respect to the starting material **6**.

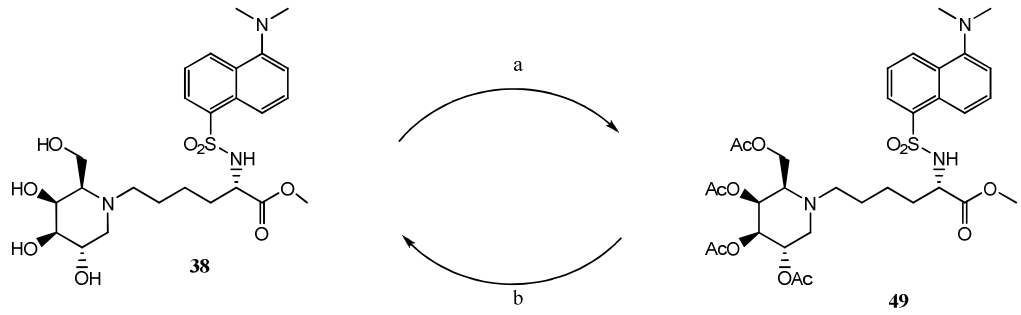


Figure 3-20: Acetylation/deacetylation procedure for efficient purification of **38**. Reagents and conditions: a) Ac_2O, DMAP, pyridine; b) NaOMe, MeOH, -30 °C.

The N-CBz protected target compound **39** in a first attempt was tried to be prepared by the reaction of benzyl chloroformate with amine **45** in methanol using triethylamine as base, which turned out to be fatal, since the resulting triethylammonium species could not be separated from the final product by any means, even using adsorbents such as Amberlite XAD-4. The change to sodium carbonate as base finally solved this problem and overall 3.8 grams of the target compound could be prepared in parallel batches in a yield of 27.8 % with respect to ulosose **6**.

The critical phase in the large scale production of the DGJ-L-lysine hybrids has turned out to be the reductive amination procedure itself, since isolated yields went worse by increase of the reaction volumes. Furthermore, the amine compound which is usually added in a slight excess has to be used in a stoichiometrically deficient proportion to minimise side products due to possible aminolysis of the methyl ester by the terminal primary amine function of the spacer which is set free by hydrogenolysis of the CBz protecting group. The formation of this side product can also be minimised by mixing ulosose **6** and the CBz-protected spacer molecule directly together and carrying out the cyclisation procedure without previous deprotection of the amine compound.

Due to the high dilution required, reaction volumes become very large on scaling-up, so it turned out to be more efficient, to run parallel batches for the cyclisation reactions instead of attempting a linear scaling-up. Finally, amounts in the gram-scale could be synthesised by this approach.

Moreover, two novel compounds could be prepared, namely CBz-containing compound **39** and the free amine **48**, and have been and tested for their biological activity. Together with the biological activities of all newly synthesised compounds described in this work, the promising results of the biological investigations will be discussed in the next section.

3.6 Biological Activities of the New Compounds

All newly synthesised 1-deoxygalactonojirimycin derivatives presented in this work were evaluated towards their activity as inhibitors against three galactosidases from various sources. The latter are *Agrobacterium sp*. glycosidase (ABG), which shows glucosidase as well as galactosidase activity, α-galactosidase from green coffee beans (α-Gal GCB) and *E. coli* β-galactosidase (β-Gal EC).

3.6.1 Inhibition Profile of Lipophilic Derivatives

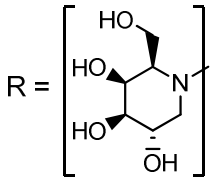
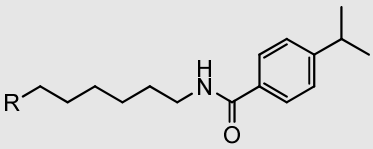
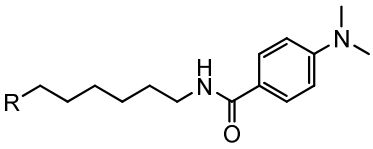
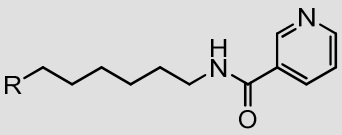
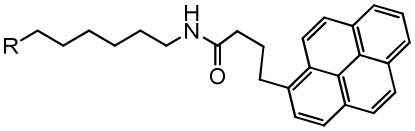
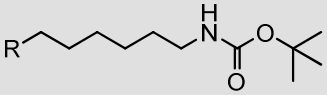
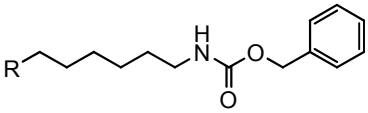
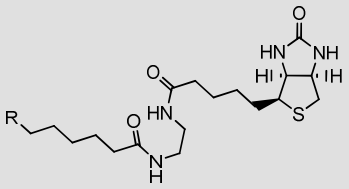
In Table 3-2, the K_I-values of the presented lipophilic derivatives of 1-DGJ are collected. The inhibition constants against ABG are in the low micromolar range with compound **22** even showing a K_I value of 60 nM. In comparison to the parent compound 1-deoxygalactonojirimycin (**8**), this means an increase in activity by up to three orders of magnitude.

In comparison to the strongly inhibiting parent compound, DGJ, K_I-values are generally larger against α-Gal GCB, but still they are in the low micromolar and submicromolar (compound **20**) range, respectively, which makes them attractive for applications where moderate inhibitors are needed.

The prepared substances show generally better inhibition against β-Gal EC by at least one order of magnitude in comparison with DGJ (**8**). K_I values are in the low micromolar, and submicromolar range which makes them interesting candidates for further studies.

No general trend can be derived from the available data, although compounds featuring aromatic substituents generally show better inhibition than their aliphatic counterparts. The direct comparison of compounds **18** and **23** shows an increase in inhibitory power against α-Gal GCB and β-Gal EC.

Table 3-2: Collected K_I-values of the prepared lipophilic 1-DGJ derivatives. ABG: Glycosidase from *Agrobacterium sp.*; α-Gal: α-galactosidase from green coffee beans; β-Gal: b-galactosidase from *E. coli.*

R = 	**K_I-values / µM**			
	#	**ABG**	**α-Gal GCB**	**β-Gal EC**
R−H	8	100	0.013	13
	19	13	2.6	1.3
	20	3.8	0.49	1.1
	21	1.5	2.2	1.3
	22	0.06	7	0.25
	23	3.0	4.3	2.4
	18	6.0	2.2	0.4
	27	11	1.1	2.3

In comparison of compounds **19** and **20**, it is obvious, that the introduction of the amine functionality to the aromatic ring systems has an impact on the inhibitory power of the compounds,

increasing inhibitory activity against α-Gal GCB 5-fold, which is in accordance with previously measured inhibition data [160], comparing dansyl- and naphtylsulfonyl-substituents. Pyrenylated compound **22** shows promising results for the inhibition of ABG and β-Gal EC. Preliminary investigations in cell cultures showed a relatively high toxicity of the substance [191,192].

Amine **48** could not yet be tested for its inhibitory power, but preliminary results suggest this compound being significantly more active compared to the N-dansylated analogue **40**. [191]

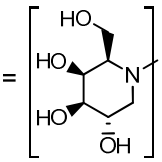
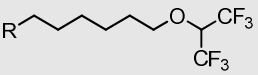
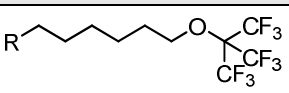
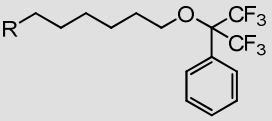
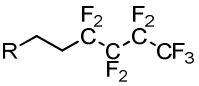
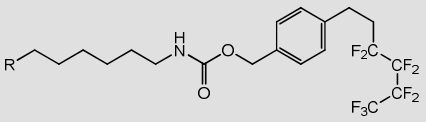
This finding is in contrast to previous studies, showing that an unprotected primary or secondary alkyl amine in relatively close distance to the iminoalditol ring system decreases inhibitory activity in comparison to derivatized analogues [160]. On the other hand, several examples, such as the observations made concerning the 1-amino-DMDP system [160] and the herein described compounds **19** and **20** respectively, where the introduction of an aromatic amine in a distance of 9 to 12 atoms from the ring nitrogen led to a significant increase of biological activity.

3.6.2 Inhibition Profile of Fluorous Derivatives

In Table 3-3, the inhibition constants of the presented fluorous derivatives are collected. The substances were tested for their inhibition ability of the abovementioned enzymes ABG, α-Gal GCB, and β-Gal EC, respectively. The fluorinated derivatives of 1-DGJ are excellent galactosidase inhibitors with inhibition constants in the low micromolar and submicromolar range. The comparison of aromatic and aliphatic oligofluorinated derivatives shows the same trend as already mentioned, meaning the aromatically substituted substances generally being more powerful inhibitors than their aliphatic analogues. The comparison of the fluorous ether-type iminosugars **32**, **33**, and **34**, respectively, clearly supports this hypothesis. Aromatic compound **34** shows considerably lower K_I values than aliphatic analogues **32** and **33**.

Interestingly, compound **37**, possessing a fluorous tagged benzyl carbamate moiety shows no significant difference in inhibitor strength for ABG and ß-Gal EC compared to the non-fluorous benzyl carbamate **18**, however, the inhibition constant against α-Gal GCB is larger in comparison to non-fluorous compound **18**.

Table 3-3: Collected K_I-values of the described fluorous 1-DGJ derivatives. ABG: Glycosidase from *Agrobacterium sp.;* α-Gal: α-galactosidase from green coffee beans; β-Gal: β-galactosidase from *E. coli.*

R = 	K_i-values / µM			
	#	ABG	α-Gal	β-Gal
R−H	8	100	0.013	13
	32	17	4.0	13
	33	2.1	1.4	0.55
	34	0.7	0.36	1.1
	35	450	3.2	0.37
	37	5.0	11	3.5

3.6.3 Chaperone Potential

Selected substances prepared in this work were chosen to be used in experiments in cell cultures, feline fibroblasts as well as human patient fibroblasts, carrying galactosidase mutations leading to lysosomal storage disorders such as GM1-gangliosidosis.

At the Medical University of Graz, a set of selected substances has been tested towards chaperone activity for mutated β-galactosidases in patient cell-lines.

Prior to the chaperoning experiments, IC_{50} and K_I values for human lysosomal β-galactosidase were measured in confluent fibroblasts from healthy patients. The measured data are summarized in Table 3-4.

Table 3-4: IC_{50} and K_I-values of the tested compounds. Data by courtesy of K. Fantur

Compound	*IC_{50} [µM]*	*K_I [µM]*
8	25	10.5 ± 1.03
18	3.1 ± 0.1	0.9 ± 0.09
23	10.9 ± 0.3	2.0 ± 0.2
32	11.0 ± 0.8	4.9 ± 0.3
33	4.4 ± 0.4	0.8 ± 0.05
34	8.1 ± 0.7	0.8 ± 0.04

Figure 3-21 shows the relative increase of residual β-galactosidase activity in a cell line, carrying the R201C/R201C mutation leading to β-galactosidase activity deficiency. The growing cells were treated with fluorous and non-fluorous galactosidase inhibitors.

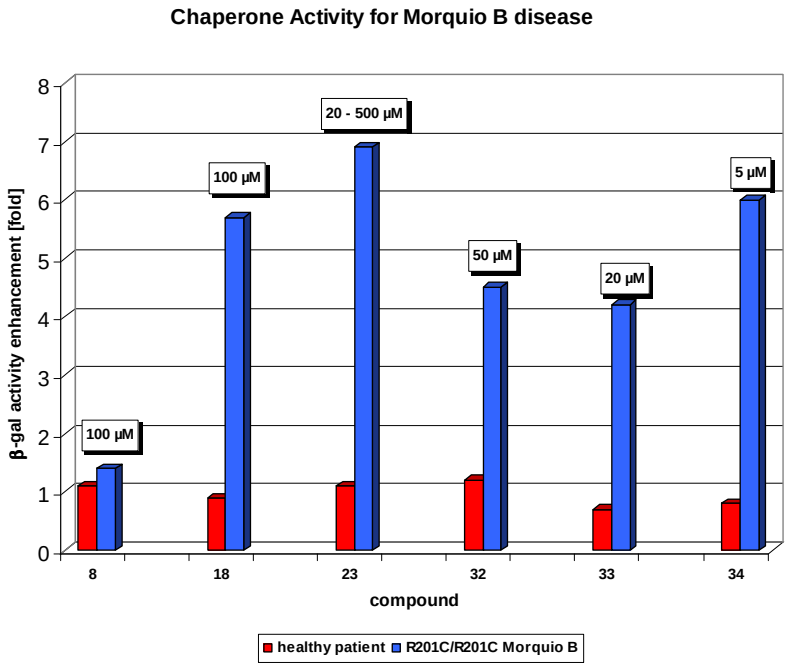


Figure 3-21: Relative increase of residual β-galactosidase activity by the treatment of cells with galactosidase inhibitors. Optimum inhibitor concentration is noted on top of the bars. Data by courtesy of K. Fantur [191].

In comparison to 1-deoxygalactonojirimycin (**8**), all tested compounds were able to increase the lysosomal β-galactosidase activity up to 6-fold. Remarkably, the most effective concentration of the oligofluorinated compound **34** was 5 µM, which is a very promising result for further studies. No

general trend concerning fluorination can be observed, since also non-fluorinated compound **18** shows a significant result in terms of chaperone behaviour.

Dansylated compound **40** and de-dansylated amine **48** were tested for their chaperone potential. In previous experiments it could be shown that compound **40** is a powerful chemical chaperone for GM1-β-galactosidase. Due to concerns about the unknown toxicological potential of the dansyl moiety and the possible mutageneity of aromatic compounds, amine **48** was prepared. The chaperone potential of the de-dansylated compound even outreaches the performance of its parent compound and nearly doubled the rate of GM1-degradation in certain patient cell lines. Moreover, **48** shows these results at comparatively low concentration in a cell-line exhibiting infantile GM1-gangliosidosis phenotype (Figure 3-22).

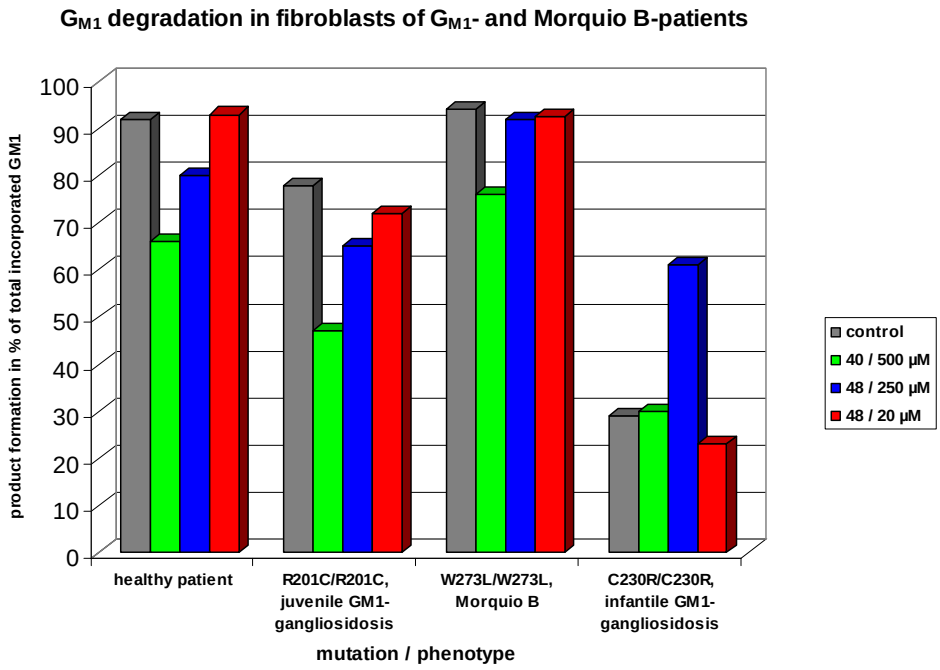


Figure 3-22: GM1-degradation in fibroblasts of GM1-gangliosidosis and Morqiuo B patients. The bars represent the degradation product formation of GM1-gaglioside in treated cells in comparison to the untreated cells. Data by courtesy by K. Fantur.

At the Hospital for Sick Children in Toronto, selected substances were tested for their chaperone potential towards β-galactosidases in GM1-gangliosidosis affected cell-lines. The tested cells either were feline fibroblasts or patient cell lines.

Figure 3-23 shows the first results obtained by the treatment of GM1-gangliosidosis cat fibroblasts upon treatment with substances **32** and **35,** both of them fluorous compounds, compared to the parent compound 1-deoxygalactonojrimycin (**8**).

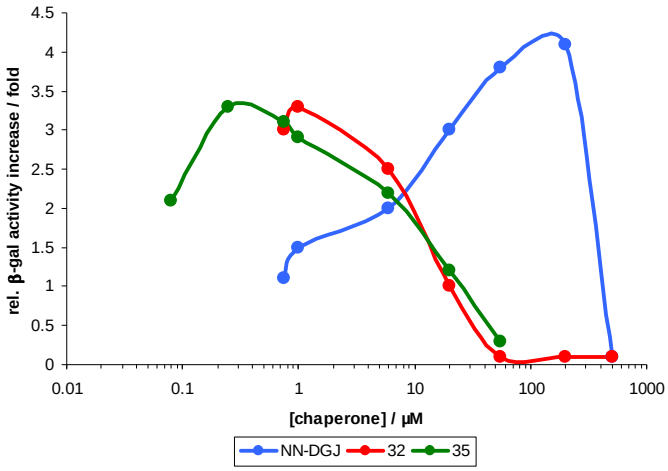


Figure 3-23: Compounds tested in β-galactosidase deficient feline fibroblasts in comparison with *N*-nonyl-1-DGJ (NN-DGJ). By courtesy of M.B. Tropak [192].

What can be seen is that in comparison to *N*-nonyl-1-deoxygalactonojirimycin (NN-DGJ), one of the lead substances in the area, both substances, containing nonafluorohexyl residues show a remarkable chaperoning potential, meaning that the residual activity of the mutated β-galactosidase is elevated upon the treatment with the substances more than three-fold. Although, in comparison with NN-DGJ, the level of residual activity could not be reached, the concentration range in which the compounds show their activity maxima are about 2 orders of magnitude lower. Especially compound **35** shows promising results compared to NN-DGJ which is structurally very similar but lacks the fluorine substitution in the N-substituent.

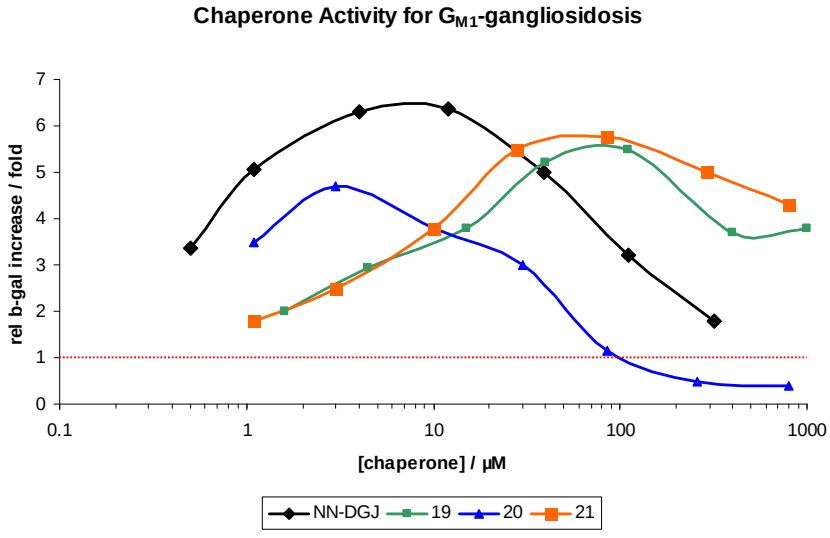


Figure 3-24: Compounds tested in β-galactosidase deficient patient fibroblasts in comparison to *N*-nonyl-1-deoxygalactonojirimycin (NN-DGJ). By courtesy of M.B. Tropak [192]

Figure 3-24 shows the results obtained by the treatment of GM1-patient cell-lines in comparison to NN-DGJ. In this set of various benzamido-type substituted 1-DGJ derivatives, the chaperone activity is not as good as of NN-DGJ in terms of activity enhancement and also in terms of the optimum concentration range. Only compound **20**, featuring an aromatic dimethylamino substituent has a concentration profile comparable to NN-DGJ. Those investigations have shown that the toxicity of the compounds might be lower compared to NN-DGJ [193].

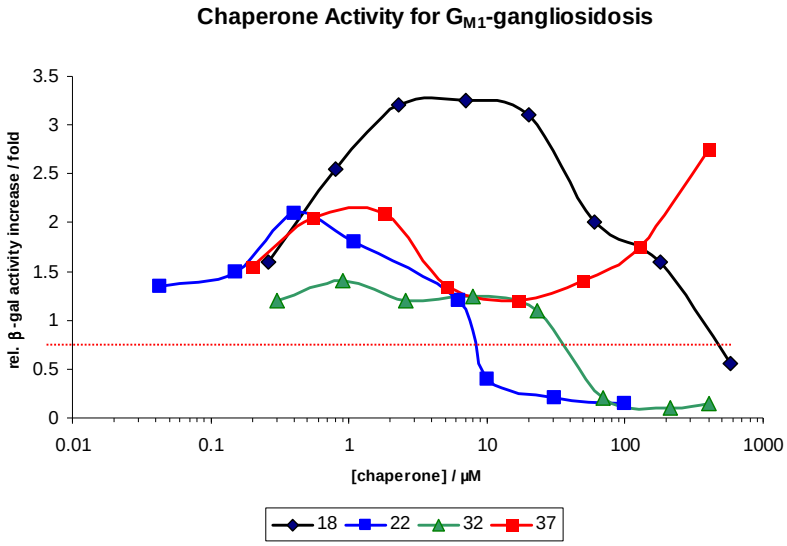


Figure 3-25: Compounds tested in β-galactosidase deficient patient fibroblasts. By courtesy of M.B. Tropak [192].

Figure 3-25 shows the results of chaperone experiments with fluorous as well as non-fluorous galactosidase inhibitors in a GM1-gangliosidosis patient cell-line. Substance **18** is the best pharmacological chaperone tested in this set with an enhancement of residual β-galactosidase activity more than three-fold. The pyrenylated compound **22**, which showed to be a powerful inhibitor for *E. coli* β-galactosidase also proves to be an active pharmaceutical chaperone for mutated β-galactosidases with a two-fold activity increase and a relatively low optimum concentration. The fluorous compounds **32** and **37** show activity increase to a minor extent. Especially compound **32** proved to be more active in the chaperone experiments with feline fibroblasts. Remarkably, both fluorous substances show a very broad concentration range before inhibition gets predominant. Compound **37** even raises residual enzyme activity further in a higher concentration range.

4 Conclusion

The synthetic approaches presented in this thesis allow for the synthesis of new, N-modified derivatives of 1-deoxygalactonojirimycin. The key step of the synthesis, the double reductive amination, catalysed by a supported palladium hydrogenation catalyst is a well-suited method for the laboratory scale preparation of 1-DGJ derivatives.

The synthesis of the dicarbonyl sugar is straightforward and can be conducted on a large scale, even though work-up procedures start to become more complicated upon scaling-up. The presented synthetic strategies, using 3,4-*O*-isopropylidene-L-*arabino*-hexos-5-ulose as starting material are suited for the direct synthesis of partially protected 1-DGJ derivatives as well as for the preparation of synthetic key intermediates allowing for a broad range of follow-up chemistry.

Following this approach, various 1-DGJ-derivatives have been prepared, carrying either lipophilic or amphiphilic N-substituents. A critical step in the synthesis of these molecules turned out to be the purification, since yield losses had to be accepted due to the high polarity of the compounds. A change to reverse-phase chromatography, thus, seems to be reasonable.

Also, fluorine containing compounds were prepared using the double reductive amination method. Those molecules were either synthesised by a direct cyclisation approach with suitable fluorous precursor molecules or by the modification of an 6-aminohexyl substituted 1-DGJ derivative.

Especially in the case of the fluorous ether type spacer, the modified Mitsunobu method proved to be versatile and might be expanded to other fluorous systems.

Additionally, fluorous tagging of the iminosugars allows for facile purification by fluorous solid phase extraction. Another possible application of these molecules might be fluorous immobilisation to fluoroalkyl-coated glass slides.

Scaling-up the synthesis of 1-DGJ-L-lysine hybrid compounds turned out to be a challenging task but also clearly shows the frontiers of the double reductive amination procedure. For the large-scale preparation of those compounds a more robust method will have to be developed.

All of the presented compounds have turned out to be potent glycosidase inhibitors. Especially iminoalditols with aromatic rings incorporated to the N-substituent show promising results, some of them exceeding the activity of the parent compound up to three orders of magnitude.

Pleasantly, chaperone ability of selected compounds that were tested by treating cat fibroblasts as well as patient cell-lines could be observed. Compared to the one of the benchmark molecules, *N*-nonyl-1-deoxygalactonojirimycin, some of the compounds are able to restore residual galactosidase activity in the same range or even better. Preliminary results confirm the relatively low toxicity of selected substances, thus making them interesting candidates for further studies.

The most recent biological tests showed unexpected results concerning the biological activity of a DGJ-derivative carrying an unprotected amine on the N-substituent, being twice as active as the (already very powerful) dansylated analogue. Possibly these results help to create a new generation of glycosidase inhibitors and pharmaceutical chaperones.

5 Experimental Section

5.1 General Methods

Analytic thin-layer chromatography was performed on silica gel 60 F_{254} precoated plates (MERCK 5554). Compounds were detected by quenching of UV fluorescence and by spraying with one of the following reagents and subsequent heating.

- vanillin/sulfuric acid: Vanillin (1 g) dissolved in sulfuric acid (100 mL).
- ammoniummolybdate/ceric sulfate: Ammoniummolybdate (100 g) dissolved in 10% sulfuric acid (1000 mL), ceric sulfate (8 g) in 10% sulfuric acid (80 mL). Subsequently the solutions were mixed.

Flash chromatography was performed with the indicated solvent system on silica gel 60, 230-400 mesh (MERCK 9385).

Melting points were determined with a Tottoli apparatus (BÜCHI 530) and are uncorrected. Specific rotations were measured at room temperature on a Perkin Elmer 341 polarimeter with a path length of 10 cm at a wavelength of 589 nm.

Mass spectra were recorded on an Agilent Systems 6120 quadrupole LC-MS employing APCI in positive mode.

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Inova 500 MHz spectrometer at 499.82 MHz and 125.69 MHz, respectively, as well as on a Bruker Utrashield 300 MHz spectrometer at 300.36 MHz and 75.53MHz, respectively. Chemical shifts are listed in ppm employing residual, non-deuterated solvent as the internal standard. Structures of crucial intermediates have been unambiguously assigned by APT, COSY, and HSQC spectroscopy.

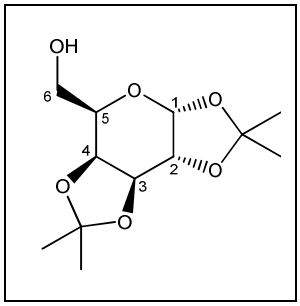
NMR-Abbreviations:

s	singlet	dt	duplet of triplets
d	dublet	q	quadruplet
dd	duplet of duplets	p	pentet
ddd	double duplet of duplets	h	heptet
t	triplet		

5.2 Procedures

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (2)

D-Galactose (25 g, 139.1mmol) is suspended in 300 ml dry acetone and anhydrous $CuSO_4$ (5g) were added. Concentrated H_2SO_4 (10 ml, ~ 188 mmol) are added at once. After 72 hours of stirring, the reaction becomes almost clear. The reaction is cooled with an ice-bath, neutralised with solid $NaHCO_3$, filtered off and concentrated under reduced pressure to yield 25.4 g (70 %) of a yellow oil, which is subjected to silica gel chromatography yielding 18.64 g (51.0 %) of the desired product as colourless sirup.



$C_{12}H_{20}O_6$

MW = 260.3 g/mol

TLC: Cy/EA 1/1(v/v)

Purification: SGC Cy →Cy/EtOAc 1/1 (v/v)

$[\alpha]_D^{20} = 55.0°$ (c 1.3, $CHCl_3$)

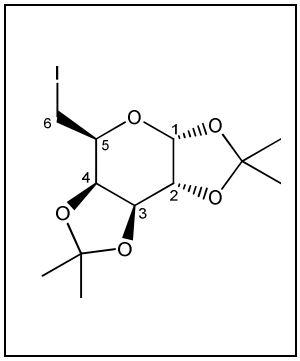
^{1}H-NMR, (300 MHz, $CDCl_3$) δ in ppm: 5.57 (d, 1H, $J_{1,2}$ = 5.0 Hz, H-1), 4.62 (dd, 1H, $J_{2,3}$ = 2.4 Hz, $J_{3,4}$ = 8.0 Hz, H-3), 4.34 (dd, 1H, H-2), 4.28 (dd, 1H, $J_{4,5}$ = 1.0 Hz, H-4), 3.92 – 3.80 (m, 2H, H-5, H-6a), 3.75 (dd, 1H, $J_{5,6b}$ = 6.8 Hz, $J_{6a,6b}$ = 8.9 Hz, H-6b), 1.54 (s, 3H), 1.46 (s, 3H), 1.34 (s, 6H).

^{13}C-NMR, (75 MHz, $CDCl_3$) δ in ppm: 109.4, 108.7, 93.3 (C-1), 71.5 (C-4), 70.7 (C-3), 70.6 (C-2), 68.1 (C-5), 62.2 (C-6), 26.0, 25.9, 24.9, 24.3.

6-Deoxy-6-iodo-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (3)

Compound **2** (38.7 g, 0.149 mol), imidazol (30.2 g, 0.443 mol) and triphenylphosphine (116.0 g, 0.442 mol) are dissolved in dry toluene, before iodine (75.1 g, 0.296 mol) is added to the stirred solution. After 3 hours of stirring, complete conversion is indicated by TLC control and the brown solution is brought to room temperature and poured into 1.5 L of saturated $NaHCO_3$ solution. After 20 minutes of stirring, the organic phase is decoloured. Iodine (~ 3 g) is added until the organic phase stays brown. After 15 minutes of stirring, saturated $Na_2S_2O_3$ solution is added and the biphasic system is stirred, until the organic phase stays decoloured. The phases are separated and the aqueous phase is washed with toluene. The pooled organic phases are washed with water, dried with Na_2SO_4 and evaporated to dryness. The residue (~ 250 g) is taken up in a little amount of EtOAc and petrol ether is added to precipitate triphenyl phosphine oxide. The solid is filtered off and the filtrate is concentrated under reduced pressure.

The residue is subjected to column chromatography to yield 42.66 g (0.115 mol, 78 %) of **3** as colourless oil.



$C_{12}H_{19}O_5I$

MW = 370.2 g/mol

TLC: Cy/EA 4/1(v/v)

Purification: SGC: Cy/EtOAc 4/1 (v/v)

m.p.: 62-64°C

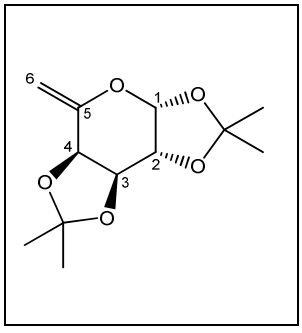
$[\alpha]_D^{20}$ = -50.7° (c = 1.2, $CHCl_3$)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 5.47 (d, 1H, $J_{1,2}$ = 4.9 Hz, H-1), 4.55 (dd, 1H, $J_{2,3}$ = 2.4 Hz, $J_{3,4}$ = 7.9 Hz, H-3), 4.33 (dd, 1H, $J_{4,5}$ = 1.8 Hz, H-4), 4.24 (dd, 1H, H-2), 3.88 (ddd, $J_{5,6a}$ = $J_{5,6b}$ = 6.9 Hz, H-5), 3.25 (dd, 1H, $J_{6a,6b}$ = 9.9 Hz, H-6_a), 3.14 (dd, 1H, H-6_b) 1.48 (s, 3H), 1.38 (s, 3H), 1.29 (s, 3H), 1.17 (s, 3H).

^{13}C-NMR, (75 MHz, ,MeOD) δ in ppm: 109.5, 108.8, 96.6 (C-1), 71.5 (C-3), 71.0 (C-4), 70.5 (C-2), 68.9 (C-5), 26.0, 25.9, 24.8, 24.4, 2.3 (C-6).

3,4-*O*-Isopropylidene-6-deoxy-α-L-*arabino*-hex-5-enopyranose (4)

22.87 g (0.062 mol) of **3** are dissolved in 250 ml of dry THF. KO^{t}Bu (13.9 g, 0.124 mol) is added at once and the reaction is stirred at room temperature for 16 hours. TLC control of the brown suspension indicated complete conversion. The reaction mixture is diluted with 200 ml of diethyl ether and the KO^{t}Bu is hydrolysed by slow addition of H_2O. The phases are separated and the aqueous phase is re-extracted with Et_2O two times. The combined organic phases are dried over Na_2SO_4, filtered off and concentrated in vacuuo to yield 14.61 g (97.6 %) of the crude title compound as white solid. Re-crystallisation from 2-propanol yields 12.28 g (82 %) of the title compound as white crystals.



$C_{12}H_{18}O_5$

MW = 242.3 g/mol

TLC: Cy/EA 4/1(v/v)

Purification: recrystallisation from 2-propanol

m.p.: 84 - 86 °C (2-propanol)

$[\alpha]_D^{20}$ = -153.9° (c = 2.1, $CHCl_3$)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 5.61 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.79 (s, 1H, H-6_a), 4.68 (s, 1H, H-6_b), 4.60 (m, 2H, H-3, H-4), 4.28 (d, 1H, H-2), 1.50 (s, 3H), 1.49 (s, 3H), 1.39 (s, 3H), 1.37 (s, 3H).

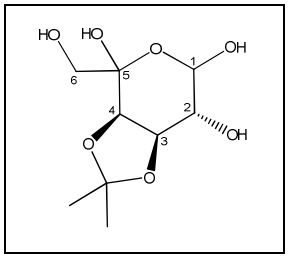
^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 152.2 (C-5), 110.3, 109.7, 100.5 (C-6), 97.2 (C-1), 73.0 (C-2), 77.1, 76.6 (C-3, C-4), 26.8, 26.5, 25.6, 24.4)

3,4-*O*-Isopropylidene-L-*arabino*-hexos-5-ulose (6)

13.8 g (61.9 mmol) *m*CPBA (77 %) are dissolved in 150 ml dry CH_2Cl_2 and dried with Na_2SO_4. The clear solution is filtered directly onto a solution of 10 g (41.3 mmol) **4** in 50 ml of dry CH_2Cl_2. The reaction turns slightly warm and is stirred for 4 h at room temperature. After complete conversion of the starting material (TLC), the reaction is cooled to -18 °C to furnish a white precipitate that is filtered off. The filtrate is washed twice with saturated $NaHCO_3$ solution and is consecutively washed with water until the pH of the aqueous phase is neutral. The organic phase is dried with Na_2SO_4, filtered and concentrated to dryness to yield 14.3 g of brownish foam.

The crude product is taken up in absolute MeOH and cooled to -30 °C. To this solution NaOMe (1M in MeOH) is added dropwise to adjust a pH ~10. Upon addition of NaOMe, the solution turns intensely yellow.

After complete conversion of the starting material (TLC), Amberlite IR-120 is added in portions to adjust a neutral pH. The resin is filtered off and the reaction is evaporated to dryness. Silica gel chromatography yields 9.8 g (67 %) of the title compound as off-white foam.



$C_9H_{16}O_7$

MW = 236.2 g/mol

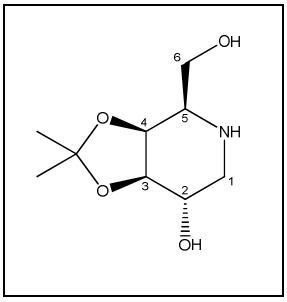
TLC: Cy/EA 7/1(v/v)

Purification: SGC: CH_2Cl_2/MeOH 16/1, **(**v/v)

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 113.8, 113.7 (isopropylidene), 106.5, 106.4, 97.8, 97.6, multiple anomeric carbons.

3,4-*O*-Isopropylidene-1,5-dideoxy-1,5-imino-D-galactitol (7)

1.6 g (6.77 mmol) **6** and 875 mg (8.11 mmol) benzyl amine are dissolved in 250 ml of MeOH, before 200 mg (~ 0.19 mmol Pd) Pd/C are added and a hydrogen atmosphere (1 bar) is applied. The reaction is stirred at room temperature for 3 days until no further reaction progress is monitored by TLC analysis. The catalyst is filtered off and the reaction is evaporated to dryness. Silica gel chromatography yields 1.04 g (5.11 mmol, 76 %) of the title compound as colourless syrup.



$C_9H_{17}NO_4$

MW = 203.2 g/mol

TLC: $CHCl_3$/MeOH/NH_3 3:1, 1% (v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 700/100/8 (v/v/v)

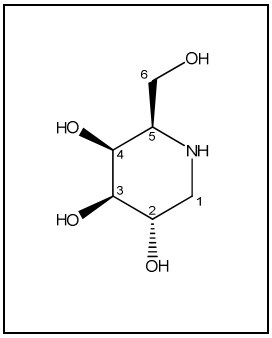
$[\alpha]_D^{20}$ = 46.73 °(c = 1.0, MeOH)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 4.13 (dd, 1H, $J_{4,5}$ = 2.7 Hz, $J_{3,4}$ = 5.3 Hz, H-4), 3.18 (dd, 1H, $J_{2,3}$ = 7.3 Hz, H-3), 3.62 (dd, 1H, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6_a), 3.60-3.50 (ddd, 1H, $J_{1eq,2}$ = 5.0 Hz, $J_{1ax,2}$ = 10.6 Hz, H-2), 3.54 (dd, 1H, $J_{5,6b}$ = 5.1 Hz, H-6_b), 2.91 (dd, 1H, $J_{1ax,1eq}$ = 12.5 Hz, H-1_{eq}), 2.89 (ddd, 1H, H-5), 2.25 (dd, 1H, H-1_{ax}), 1.39 (s, 3H), 1.23 (s, 3H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 110.5, 81.5 (C-3), 75.2 (C-4), 71.7 (C-2), 63.5 (C-6), 58.9 (C-5), 49.9 (C-1), 28.5, 26.7.

1,5-Dideoxy-1,5-imino-D-galactitol (8)

7 (0.62 g, 3.05 mmol) is dissolved in 12 ml of a mixture of H_2O and MeOH (10/2, v/v). Then 2 ml of concentrated HCl are added (pH ~1) and the reaction is stirred at room temperature for 20 hours. The reaction is evaporated to dryness, taken up in MeOH and evaporated again three times to remove excess HCl. The residue is taken up in 40 ml MeOH containing 4 ml of NH_3, evaporated and subjected to silica gel chromatography to yield 196.5 mg (1.20 mmol. 53 %) of the title compound as colourless syrup.



$C_6H_{13}NO_4$

MW = 163.17 g/mol

TLC: $CHCl_3$/MeOH/NH_3 4/4/1 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 300:100, 4 (v/v/v)

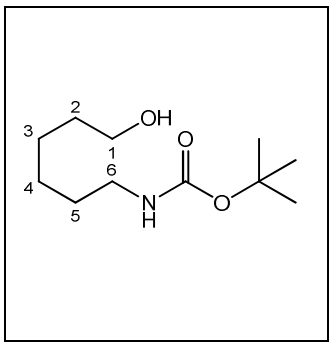
$[\alpha]_D^{20}$ = 53.9 °, (c = 0.9, H_2O)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 4.01 (dd, 1H, $J_{4,5}$ = 1.0 Hz, $J_{3,4}$ = 2.6 Hz, H-4), 3.91 (ddd, 1H, $J_{1eq,2}$ = 5.4 Hz, $J_{2,3}$ = 9.8 Hz, $J_{1ax,2}$ = 11.5 Hz, H-2), 3.72 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 12.2 Hz, H-6_a), 3.64 (dd, 1H, $J_{5,6b}$ = 9.3 Hz, H-6_b), 3.48 (dd, 1H, H-3), 3.35 (dd, 1H, $J_{1ax,1eq}$ = 12.7 Hz, H-1_{eq}), 3.26 (ddd, 1H, H-5), 2.72 (dd, H-1_{ax})

^{13}C-NMR, (125 MHz, MeOD) δ in ppm: 73.0 (C-3), 67.0 (C-4), 64.8 (C-2), 60.2 (C-6), 59.2 (C-5), 46.2 (C-1)

***N*-BOC-6-aminohexanol (10)**

6-Amino-1-hexanol (**9**) (2.0 g, 17 mmol) is dissolved in dry methanol, before. di-tert-butyl dicarbonate (7.5 g, 34 mmol) and Na_2CO_3 (3.6 g, 34 mmol) are added, and the reaction mixture is stirred for 12 h at room temperature. The solids are filtered off and the filtrate is evaporated under reduced pressure. The residue is taken up in CH_2Cl_2 and the solution is consecutively washed with saturated $NaHCO_3$ solution and water, dried over Na_2SO_4, filtered and concentrated in vacuuo. Subsequent silica gel chromatography yields 3.2 g of alcohol **10** (15 mmol, 88%).



$C_{11}H_{23}NO_3$

MW = 217.3 g/mol

TLC: Cy/EtOAc 2/1 (v/v)

Purification: SGC; Cy/EtOAc 4/1, (v/v)

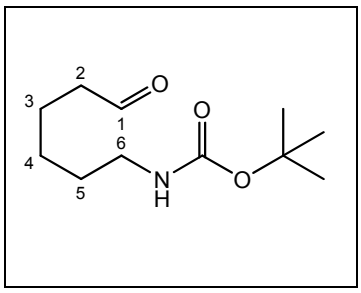
m.p.: 36 -38 ℃

^{1}H-NMR, (300 MHz, $CDCl_3$) δ in ppm: 4.65 (bs, 1H), 3.61 (t, 1H $J_{1,2}$ = 6.5 Hz, H-1), 3.10 (quad., $J_{5,6}$ = $J_{6,NH}$ = 6.2 Hz, H-6), 2.19 (bs, 1H), 1.64 – 1.28 (m, 8H), 1.43 (s, 9H).

^{13}C-NMR, (75 MHz, $CDCl_3$) δ in ppm: 156.1, 79.0, 62.5, 40.4, 32.6, 30.0, 28.4 (3C), 26.4, 25.3.

***N*-BOC-6-aminohexanal (11)**

1g (4.6 mmol) of **10** are dissolved in 50 ml of CH_2Cl_2 and 2.14 g (5.06 mmol) of Dess-Martin-periodinan are added at once. After few seconds, the reaction mixture gets cloudy and forms a white precipitate. TLC indicates quantitiative conversion after 10 minutes. Saturated $NaHCO_3$ solution and saturated NaS_2O_3 solution (15 ml each) are added to the reaction mixture and the reaction is stirred until both phases are clear. The phases are separated and the organic phase is washed with brine and water, dried over Na_2SO_4, filtered off and concentrated under reduced pressure to yield 0.95 g (96 %) of **11** as a colourless syrup.



$C_{11}H_{21}NO_3$

MW = 215.3 g/mol

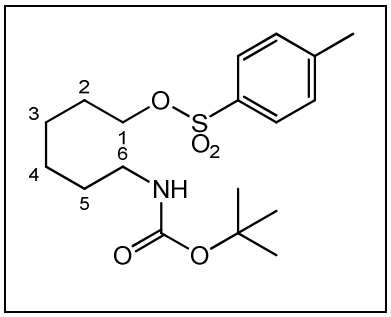
TLC: Cy/EtOAc 1/1 (v/v)

^{1}H-NMR, (300 MHz, $CDCl_3$) δ in ppm: 9.7 (m, 1H, $J_{1,2}$ = 1.6 Hz H-1), 4.64 (bs, 1H, NH), 3.06 (m, 2H, H-6), 2.39 (dt, 2H, $J_{5,6}$ = 7.8 Hz, H-6), 1.62 (m, 2H), 1.48-1.32 (m, 2H), 1.41 (s, 9H), 1.32 – 1.20 (m, 2H).

^{13}C-NMR, (75 MHz, $CDCl_3$) δ in ppm: 202.5 (C-1), 156.0, 43.7 (C-2), 40.3 (C-6), 29.8, 28.4 (3C), 26.2, 21.6.

***N*-BOC-*O*-tosyl-6-aminohexanol (12)**

4 g (18.4 mmol) **10** are dissolved in 100 ml CH_2Cl_2 and 3.7 g (46.4 mmol) pyridine are added. TsCl (7.2 g, 37.8 mmol) are added in 4 portions. After 12 h of stirring at room temperature, quantitative conversion is indicated by TLC control. The solution is extracted twice with 1N HCl and subsequently washed with saturated $NaHCO_3$ solution. After drying over Na_2SO_4, the filtrate is concentrated under reduced pressure and subsequent column chromatography yields 5.06 g (74.0 %) of the title compound as colourless oil.



$C_{11}H_{21}NO_3$

MW = 215.3 g/mol

TLC: Cy/EA 2/1 (v/v)

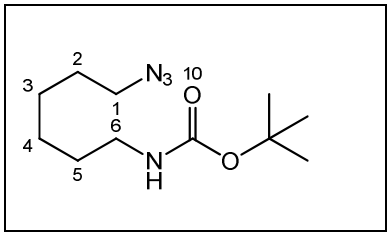
Purification: SGC: Cy/EtOAc 10/1 → 5/1 (v/v)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 7.74 (d, 2H), 7.29 (d, 2H), 4.0 (t, 2H, $J_{1,2}$ = 6.0 Hz, H-1), 3.06 (d, 2H, $J_{5,6}$ = 7.0 Hz, H-6), 2.44 (s, 3H) 1.64 (p, 2H, $J_{2,3}$ = 7.3 Hz, H-2), 1.45 – 1.37 (m, 2H, H-5) 1.42 (s, 9H), 1.36 – 1.20 (m, 4H, H-3, H-4).

^{13}C-NMR, (125 MHz, ,MeOD) δ in ppm: 156.2, 144.9, 133.3, 130.1, 128.1, 79.3, 70.7 (C-1), 40.6 (C-6), 30.1 (C-2), 28.7(3C), 27.1, 26.3, 25.3 (C-3, C-4, C-5), 21.9.

***N*-BOC-6-aminohexyl azide (13)**

0.87 g (3.34 mmol) **12** are dissolved in 50 ml dry DMF and 0.60 g (9.36 mmol) NaN_3 and 0.99 g (9.36 mmol) Na_2CO_3 are added at once at room temperature. The reaction is stirred at room temperature for 20 hours, before TLC analysis showed complete conversion of the starting material. The solvent is removed in vacuuo and the syrupy residue is taken up in 50 ml of CH_2Cl_2 and washed four times with 50 ml of H_2O. The organic phases are combined, dried over Na_2SO_4, filtered and concentrated to furnish 800 mg (140 %) of a brownish sirup. Silica gel column chromatography yields 770 mg (135 %) of the title compound as pale yellow oil.



$C_{11}H_{22}N_4O_2$

MW = 215.3 g/mol

TLC: Cy/EtOAc 2:1 (v/v)

Purification: SGC: Cy/EtOAc 5/1 (v/v)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 3.26 (t, 2H, $J_{1,2}$ = 6.8 Hz, H-1), 3.11 (d, 2H, $J_{5,6}$ = 6.8 Hz, H-6), 1.65 (m, 2H), 1.50-1.30 (m, 4H), 1.43 (s, 9H).

^{13}C-NMR, (125 MHz, ,MeOD) δ in ppm: 162.4, 79.4, 51.6 (C-1), 40.7 (C-6), 30.22, 28.9, 28.6 (3C), 26.7, 26.6.

***N*-[6-(tert-butoxycarbonylamino)hexyl]-3,4-*O*-isopropylidene-1,5-dideoxy-1,5-imino-D-galactitol (14)**

a)

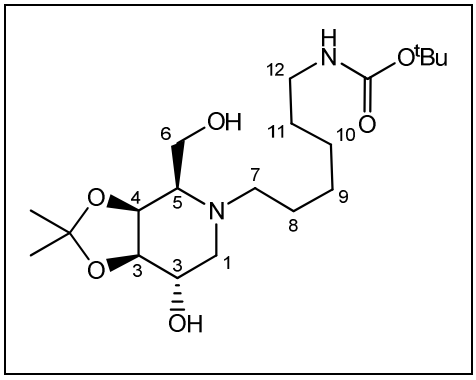
220 mg (1.08 mmol) of **7** and **12** (2.16 mmol) are dissolved in15 ml of dry DMF and 424 mg (4 mmol) of Na_2CO_3 are added. The suspension is heated to 60 °C. After 30 hours, complete conversion is indicated by TLC analysis. The reaction is allowed to cool to room temperature and subsequently the solids are filtered off. The solvent is removed under reduced pressure and the resulting slurry is subjected to silica gel chromatography to yield 278 mg (0.77 mmol, 71 %) of the title compound as colourless syrup.

b)

308 mg (1.27 mmol) **13** are dissolved in 50 ml of MeOH, before 120 mg (~ 0.12 mmol Pd) Pd/C are added and a hydrogen atmosphere (1 bar) is applied. After complete reduction of the azide, monitored by TLC analyis, 300 mg (1.27 mmol) 3,4-*O*-isopropylidene-L-arabino-hexos-5-ulose in 5 ml MeOH are added and the reaction is stirred under a hydrogen atmosphere (1 bar) at room temperature for 2 days until no further reaction progress is monitored by TLC analysis. The catalyst is filtered off and the reaction is evaporated to dryness. Silica gel chromatography yields 199.4 mg (0.49 mmol, 39 %) of the title compound as colourless syrup.

c)

220 mg (1.08 mmol) of **7** are dissolved in 20 ml of dry methanol, before 350 mg (1.62 mmol) of **11** and 50 mg of Pd/C are added. A hydrogen atmosphere (1 bar) is applied and the heterogeneous reaction mixture is stirred for 18 hours at room temperature. After complete conversion of the starting material, the catalyst is filtered off and the solvent is evaporated. Silica gel chromatography yields 239.2 mg (0.594 mmol, 55 %) of the title compound as colourless syrup.



$C_{20}H_{38}N_2O_6$

MW = 402.5 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 700/100/8 (v/v/v)

$[\alpha]_D^{20}$ = 5.32 °(c = 0.9, MeOH)

^{1}H-NMR, (300 MHz, $CDCl_3$) δ in ppm: 4.24 (m, 1H, $J_{3,4}$ = 4.6 Hz, $J_{4,5}$ = 3.6 Hz, H-4), 3.89 (dd, 1H, J = 5.3 Hz, J = 11.0 Hz, H-6a), 3.80 (m, 3H, H-2, H-3, H-6_b), 3.03 (dd, 2H, $J_{11,12}$ = 5.4 Hz, $J_{12,12'}$ = 11.8 Hz, H-12, H-S12'), 2.92 (dd, 1H, $J_{1eq,2}$ = 3.8 Hz, $J_{1ax,1eq}$ = 11.8 Hz, H-1_{eq}), 2.68 (dd $J_{7,8}$ = 6.8 Hz, $J_{7,7'}$ = 13.2 Hz, H-7), 2.59 (m, 1H, $J_{5,6a}$ = 5.8 Hz, H-5), 2.50 (dd, 1H, H-7'), 2.05 (m, 1H, H-1_{ax}), 1.46 (s, 3H), 1.37 (s, 9H), 1.31 (s, 3H), 1.45 – 1.33 (m, 4H), 1.30 – 1.14 (m, 1H).

^{13}C-NMR, (75 MHz, $CDCl_3$) δ in ppm: 156.0, 109.8, 80.6, 77.3 (C-4), 70.4 (C-3), 61.5 (C-2), 60.6 (C-5), 54.2 (C-6), 53.4, 52.5 (C-1, C-7), 40.4 (C-12), 29.9, 29.7, 28.4, 28.3, 26.5, 26.3, 24.4.

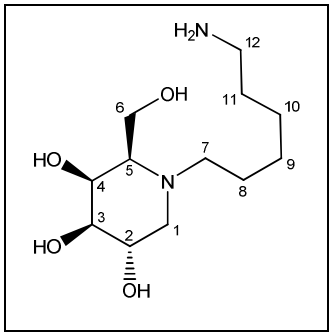
***N*-(6-Aminohexyl)-1,5-dideoxy-1,5-imino-D-galactitol (15)**

a)

18 (285.6 mg, 0.72 mmol) is dissolved in 30 ml of MeOH and 100 mg (~ 0.095 mmol Pd) Pd/C is added. A hydrogen atmosphere (1 bar) is applied and the reaction is stirred for 2 hours at room temperature. After complete conversion of the starting material (TLC), the catalyst is filtered off and the solvent is removed under reduced pressure to yield 163 mg (0.62 mmol, 86 %) of the title compound as pale-yellow syrup, which darkens upon exposure to air. The product is taken into the next step without further purification.

b)

14 (3.24 mg, 0.78 mmol) is dissolved in 30 ml of a 1/1 (v/v) – mixture of MeOH and H_2O and 1 ml of concentrated hydrochloric acid is added (pH ~1). TLC control after 8 hours does not show complete conversion, so further 1 ml of HCl is added (pH ~ 1) and the reaction is warmed to 50 °C. After 14 hours, TLC control indicates complete conversion of the starting material. The reaction is evaporated to dryness, taken up in MeOH, co-evaporated three times to remove excess HCl and subjected to silica gel chromatography to yield 139.2 mg (0.53 mmol, 68 %) of the title compound as pale-brown, strongly hygroscopic foam after lyophilisation from H_2O, which darkens upon exposure to air.



$C_{12}H_{26}N_2O_4$

MW = 262.2 g/mol

TLC:$CHCl_3$/MeOH/NH_3 4/4/1 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 4/4/1 (v/v/v)

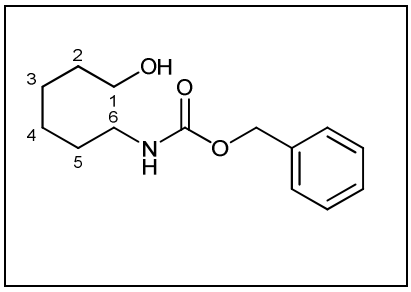
$[\alpha]_D^{20}$ = 6.54 °(c = 1.5, MeOH)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 4.19 (dd, 1H, $J_{4,5}$ = 1.1 Hz, $J_{3,4}$ = 2.6 Hz, H-4), 4.07 (ddd, 1H, $J_{1eq,2}$ = 5.0 Hz, $J_{2,3}$ = 9.8 Hz, $J_{1ax,2}$ = 11.2 Hz, H-2), 4.00 (m, $J_{5,6a}$ = 4.4 Hz, H-6_a, H-6_b), 3.56 (dd, 1H, H-3), 3.44 (dd, 1H, $J_{1ax,1eq}$ =12.1 Hz, H-1_{eq}), 3.37 – 3.22 (m, 2H, H-7, H-7'), 3.05 – 2.95 (m, 3H, H-1_{ax}, H-12), 1.95 - 1.68 (m, 2H), 1.75 (m, 2H), 1.58 – 1.40 (m, 4H, (H-8, H-9. H-10, H-11)

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 74.6 (C-3), 71.7 (C-4), 66.4 (C-2), 66.2 (C-5), 61.4 (C-6), 55.2 (C-1), 54.7 (C-7), 40.7 (C-12), 27.0, 26.9, 23.13 (C-8, C-9, C-10, C-11)

_N_-CBz-6-aminohexanol (16)

2.0 g (12.6 mmol) of 6-aminohexanol (9) are suspended in 40 ml of dry methanol, before 1.99 g (18.8 mmol) Na_2CO_3 are added. To this stirred suspension, benzyl chloroformate (2.57 g, 15.1 mmol) is added dropwise. After complete conversion of the starting material, the suspension is filtered and the filtrate is concentrated under reduced pressure. The residue is taken up in CH_2Cl_2 and the solution is consecutively washed with 1N HCl and saturated $NaHCO_3$ solution. The combined organic phases are dried over Na_2SO_4, filtered off and evaporated to dryness. The syrupy residue is recristallysed from EtOAc and cyclohexane to yield 3.1 g (12.3 mmol, 98 %) of the title compound as white crystals.



$C_{14}H_{21}NO_3$

MW = 251.2 g/mol

TLC: Cy/EtOAc 1:1 (v/v)

Purification: Recrystallisation, Cy/EtOAc

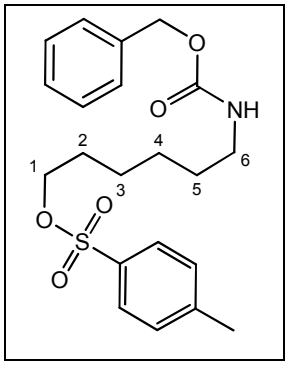
m.p.: 79 – 81 °C

^{1}H-NMR, (300 MHz, $CDCl_3$) δ in ppm: 7.40 – 7.34 (m, 5H, phenyl), 5.10 (s, 2H, Ph-CH_2), 4.95 (bs, 1H), 3.62 (t, 2H, $J_{1,2}$ = 6.4 Hz, H-1), 3.20 (quad., 2H, $J_{5,6}$ = $J_{6,NH}$ = 6.7 Hz, H-6), 2.01 (bs, 1H), 1.64 – 1.46 (m, 4H), 1.44 – 1.30 (m, 4H)

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 156.5, 136.7, 128.5 (3C), 128.1 (2C), 66.6, 62.6 (C-1, Ph-CH_2), 40.9 (C-6), 32.5, 29.9, 26.4, 25.3.

***N*-CBz-*O*-tosyl-6-aminohexanol (17)**

16 (2.93g, 11.7 mmol) is dissolved in 120 ml of absolute DCM, before 2.12 g (35.1 mmol) pyridine followed by 3.34 g (17.5 mmol) of TsCl are added. After 16 hours of reaction time, another 2.24 g (11.7 mmol) TsCl and 1.6 g (23.4 mmol) of pyridine are added. After complete conversion of the starting material (additional 4 hours, indicated by TLC), the reaction mixture is diluted with 100 ml of dichloromethane, washed with 1N HCl, saturated $NaHCO_3$ solution and water. The combined organic phases are dried over Na_2SO_4, filtered off and the filtrate is evaporated to dryness. Silica gel chromatography yields 3.05 g (7.52 mmol, 64.3 %) of the title compound as colourless oil.



$C_{21}H_{27}NO_5S$

MW = 405.2 g/mol

TLC:Cy/EtOAc 1/1 (v/v)

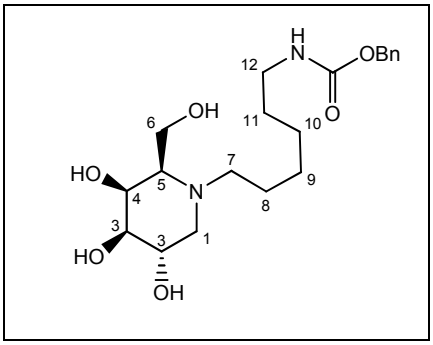
Purification: SGC: Cy/EtOAc 2/1 (v/v)

^{1}H-NMR, (500 MHz, $CDCl_3$) δ in ppm: 7.78 (d, 2H), 7.37 – 7.29 (m, 7H), 5.08 (s, 2H, CH_2-Ph), 4.00 (t, 2H, $J_{1,2}$ = 6.4 Hz, H-1), 3.20 – 3.05 (m, 2H, H-6), 2.44 (s, 3H), 1.68 – 1.56 (m, 2H), 1.49 – 1.39 (m, 2H), 1.36 – 1.20 (m, 4H).

^{13}C-NMR, (125 MHz, $CDCl_3$) δ in ppm: 156.6, 145.0, 136.8, 133.3, 130.1, 128.8, 128.3, 128.1, 70.6 (CH_2-Ph), 66.8 (C-1), 41.1 (C-6), 30.0, 28.9, 26.2, 25.3, 21.9.

***N*-[6-(benzyloxycarbonylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (18)**

370.5 mg (2.27 mmol) of 1-deoxygalactonojirimycin and 3.05 g (7.52 mmol) **17** are dissolved in 16 ml of dry DMF before 1.2 g (11.32 mmol) of Na_2CO_3 are added. The suspension is stirred at 50 °C for 18 hours before complete conversion of the starting material is observed by TLC analysis. The solids are filtered off and the filtrate is evaporated to dryness. Subsequent column chromatography followed by treatment with Merck-III basic ion exchange resin yields 688.9 mg (1.74 mmol, 77 %) of **18** as colourless syrup.



$C_{20}H_{32}N_2O_6$

MW = 396.5 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 700/100/8 (v/v/v)

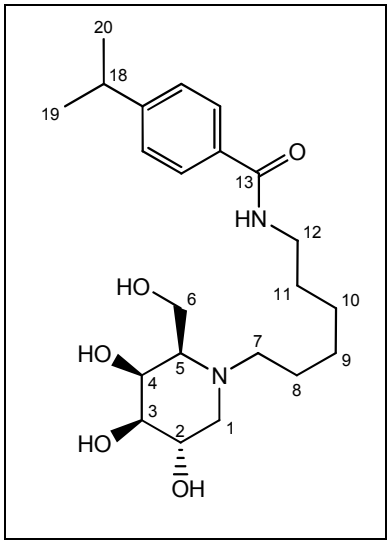
$[\alpha]_D^{20}$ = -14 °(c = 1.2, MeOH)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 7.39 – 7.23 (m, 5H), 5.06 (s, 2H, CH_2-Ph), 4.02-3.95 (m, 1H, H-4), 3.87 – 3.75 (m, 3H, H-2, H-6_a, H-6_b), 3.25 – 3.17 (m, 1H, $J_{2,3}$ = 8.8 Hz, H-3), 3.10 (m, 2H, $J_{11,12}$ = 5.8, H-12), 3.00 – 2.93 (m, 1H, $J_{1eq,2}$ = 7.3 Hz, H-1_{eq}), 2.74 – 2.64 (m, 1H, $J_{7,7'}$ = 13.0 Hz, H-7), 2.54 – 2.44 (m, 1H, H-7'), 2.39 – 2.32 (m, 1H, H-5), 2.10 (dd, 1H, $J_{1ax,1eq}$ = $J_{1ax,2}$ = 10.5 Hz, H-1_{ax}), 1.60 – 1.43 (m, 4H), 1.43 – 1.19 (m, 4H).

^{13}C-NMR, (125 MHz, MeOD) δ in ppm: 157.8, 137.1, 128.3, 127.8, 127.6, 76.1(C-3), 71.0 (C-4), 67.9 (C-2), 66.1 (CH_2-Ph), 64.0 (C-5), 61.1 (C-6), 56.9 (C-1), 52.8 (C-7), 40.6 (C-12), 29.6, 27.9, 26.5, 23.8.

N-[6-(4-Isopropylbenzoylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (19)

93.9 mg (0.57 mmol) of 4-isopropylbenzoic acid are dissolved in 5 ml of dry DMF before 115.6 mg of Et_3N (1.14 mmol) are added. Then, 201.9 mg TBTU (0.63 mmol) are added to the stirred solution. After 10 minutes of stirring, 150 mg of **15** are added and the solution is stirred at room temperature for 1 hour before TLC analysis shows complete conversion of the amine. The solvent is removed under reduced pressure and the partly solid residue is taken up in MeOH and subjected to silica gel chromatography twice to yield 62.6 mg of a syrupy substance. After lyophilisation from H_2O, 47.1 mg (20.2 %) of a hygroscopic white foam are obtained.



$C_{22}H_{36}N_2O_5$

MW = 408.5 g/mol

TLC:$CHCl_3$/MeOH/NH_3 700/100/8 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

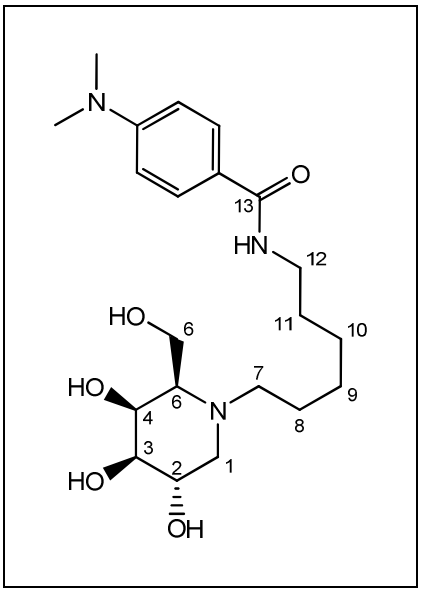
$[\alpha]_D^{20}$ = 5.20 °(c = 2.4, MeOH)

^{1}H-NMR, (300 MHz, MeOD/$CDCl_3$ 2/1 v/v) δ in ppm: 7.76 (d, 2H), 7.31 (d, 2H), 4.20 (dd, 1H, $J_{4,5}$ = 1.8 Hz, $J_{3,4}$ = 2.3 Hz, H-4), 4.07 (m, 1H, $J_{2,3}$ = 8.9 Hz, $J_{1ax,2}$ = 3.9 Hz, H-2), 3.97 (m, 2H H-6_a, H-6_b), 3.52, (dd, 1H, H-3), 3.52 (m, 2H, H-12), 3.16 (dd, $J_{1eq,\,2}$ = 11.9 Hz, H-1_{eq}), 3.05 – 2.81 (m, 2H, H-7, H-7') 2.55 (dd, 1H, $J_{1ax,2}$ = 11.2 Hz, H-1_{eq}), 2.94 (m, 1H, H-18), 1.95 – 1.65 (m, 2H), 1.65 – 1.55 (m, 2H), 1.55 – 1.35 (m, 4H), 1.26 (d, 6H, $J_{18,19}$ = 6.9 Hz, H-19, H-19')

^{13}C-NMR, (75 MHz, MeOD/$CDCl_3$ 2/1 v/v) δ in ppm: 170.4 (C-13), 154.2, 133.2, 127.7, 127.6, 72.6 (C-3), 69.8 (C-4), 64.4 (C-2), 64.2 (C-5), 59.5 (C-6), 53.2 (C-1), 52.9 (C-7), 38.8 (C-12), 33.4 (C-18), 28.3, 25.5, 25.4 (C-8, C-10, C-11), 22.6 (C-19, C-20), 21.4 (C-9)

N-[6-(4-Dimethylaminobenzoylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (20)

57.6 mg (0.22 mmol) of **15** are dissolved in 4 ml of dry DMF and 44.7 mg (0.44 mmol) of Et_3N are added and cooled to 0 °C. To this stirred solution, a solution of 4-dimethylaminobenzoic acid chloride hydrochloride in 1 ml of dry DMF is added dropwise with a syringe. After complete addition of the reagent, the reaction is allowed to come to room temperature and stirring is continued for 2 hours before TLC analysis showed complete conversion of the starting material. 1ml of MeOH is added and the reaction is stirred for 20 minutes before the solvents are removed under reduced pressure. The resulting slurry is subjected to silica gel chromatography twice to yield: 20.8 mg (0.51 mmol, 23.1 %) as off-white, strongly hygroscopic material after lyophilisation from H_2O.



$C_{21}H_{35}N_3O_5$

MW = 409.5 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

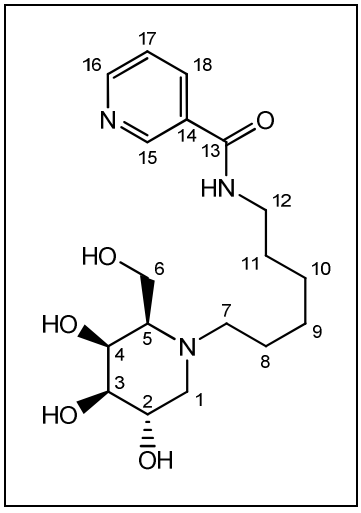
$[\alpha]_D^{20}$ = 5.61 °(c = 1.6, MeOH)

^{1}H-NMR, (300 MHz, MeOD/$CDCl_3$ 2/1 v/v) δ in ppm: 7.55 (d, 2H), 6.72 (d, 2H), 3.89 (m, 1H, H-4), 3.69 – 3.56 (m, 3H, H-2, H-6_a, H-6_b), 3.32 – 3.22 (m, 3H, H-3, H-12, H-12'), 2.94 – 2.84 (m, 1H, H-1_{eq}), 2.82 (s, 6H, NMe_2), 2.62 - 2.48 (m, 1H, H-7), 2.46 - 2.31 (m, 2H, *J,* H-7', H-5), 2.03 (dd, $J_{1ax,1eq}$ = $J_{1ax,2}$ = 11.1 Hz, H-1_{ax}), 1.45 (m, 2H), 1.31 (m, 2H), 1.23 (m, 2H), 1.15 (m, 2H)

^{13}C-NMR, (75 MHz, MeOD/$CDCl_3$ 2/1 v/v) δ in ppm: 170.0 (C-13), 153.4, 128.5, 121.0, 112.3), 75.1 (C-3), 70.1 (C-4), 67.0 (C-2), 62.5 (C-5), 60.4 (C-6), 55.9 (C-1), 52.2 (C-7), 39.7 NMe_2), 39.7 (C-12), 28.5, 26.4, 26.0, 22.6 (C-8, C-9, C-10, C-11)

_N_-[6-Nicotinoylaminohexyl]-1,5-dideoxy-1,5-imino-D-galactitol (21)

59.1 mg 3-pyridinecarboxylic acid (0.48 mmol) are dissolved in 5 ml of dry DMF and 132.4 mg (1.31 mmol) of Et_3N are added. TBTU (154.1 mg, 0.48 mmol) is added and the solution is stirred for 10 minutes at room temperature before 114.5 mg (0.44 mmol) of **15** are added in one portion. After 90 minutes, complete conversion of the starting material is observed by TLC analysis. The solvent is removed under reduced pressure and the title compound is purified by silica gel chromatography to yield 34.3 mg (0.09 mmol, 21.2 %). After lyophilisation, the product is obtained as strongly hygroscopic, white foam.



$C_{18}H_{29}N_3O_5$

MW = 367.4 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

$[\alpha]_D^{20}$ = -10.1 °(c = 1.0, MeOH)

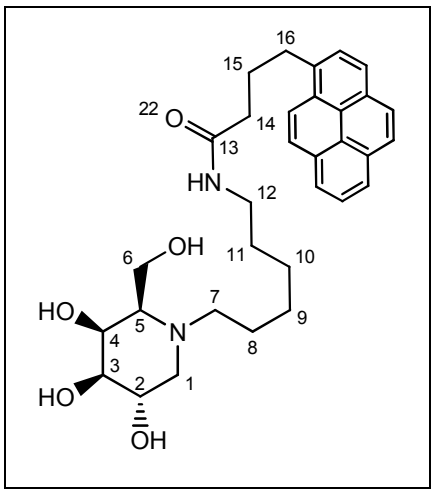
^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 8.99 (dd, 1H, *J* = 0.7 Hz, *J* = 2.2 Hz, H-15), 8.71 (dd, 1H, *J* =1.62 Hz, $J_{16,17}$ = 4.9 Hz, H-16), 8.26 (ddd, 1H, *J* = 2.0 Hz, *J* = 1.79 Hz, $J_{18,17}$ = 4.9 Hz, H-18), 7.57 (dd, 1H, H-17), 4.01 (dd, 1H, $J_{4,5}$ = 1.8 Hz, $J_{3,4}$ = 3.29 Hz, H-4), 3.83 (m, 1H, $J_{1eq,2}$ = 4.9 Hz, $J_{1ax,2}$ = 10.0 Hz, H-2), 3.82 (m, 2H, H-6_a, H-6_b), 3.42 (t, 2H, $J_{11,12}$ = 7.1 Hz, H-12), 3.24 (dd, 1H, $J_{2,3}$ = 9.25 Hz, H-3), 3.01 (dd, 1H, $J_{1ax,1eq}$ = 11.2 Hz, H-1_{eq}), 2.85 – 2.68 (m, 1H, H-7), 2.58 – 2.47 (m, 1H, H-7'), 2.39 (ddd, 1H, $J_{5,6a}$ = 5.2 Hz, H-5), 2.13 (dd, 1H, H-1_{ax}), 1.73 – 1.61 (m, 2H), 1.61 – 1.47 (m, 2H), 1.47 - 1.30 (m, 4H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 167.8 (C-13), 152.6 (C-16), 149.1 (C-15), 137.0 (C-18), 132.2 (C-14), 125.2 C-17), 77.3 (C-3) 72.6 (C-4), 69.3 (C-2), 65.5 (C-5), 62.7 (C-6), 58.4 (C-1), 54.2 (C-7), 41.3 (C-12), 30.6, 28.6, 28.2, 25.3 (C-8, C-9, C-10, C-11)

***N*-[(4-Pyrenebutanoylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (22)**

Pyrenebutyric acid (123.4 mg, 0.42 mmol) and 61.9 mg (0.61 mmol) of Et_3N are dissolved in 15 ml of dry DMF before 144 mg (0.44 mmol) of TBTU are added to the stirred solution. Then, 111.9 mg (0.42 mmol) of **15** are added in one portion and the reaction is stirred for 45 minutes at room temperature before complete conversion of the starting material is observed by TLC analysis.

10 ml of MeOH are added and after 10 minutes of stirring, the solvents are removed under reduced pressure. The crude mixture is subjected to column chromatography and is subsequently treated with Merck III basic ion exchange resin to yield 134 mg (0.25 mmol, 59 %) of the title compound as white crystals.



$C_{32}H_{40}N_2O_5$

MW = 532.67 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 900/100/10 →600/100/10 (v/v/v)

$[\alpha]_D^{20}$ = 3.60 °(c = 1.0, MeOH/$CHCl_3$ 1/1 (v/v))

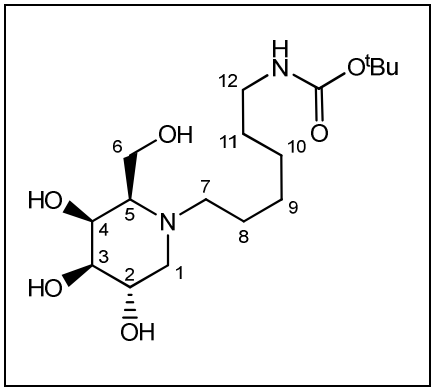
m.p.: 189 – 191 ℃

^{1}H-NMR, (500 MHz, D_2O) δ in ppm: 7.50 – 6.40 (m, 9H, pyrene), 3.94 – 3.87 (bs, 1H, H-4), 3.87 – 3.78 (m, 1H, $J_{1ax,2}$ = 10.4 Hz, H-2), 3.62 – 3.46 (m, 2H, $J_{6a,6b}$ = 10.3 Hz, H-6_a, H-6_b), 3.26 – 3.28 (m, 1H, $J_{2,3}$ = 9.3 Hz, H-3), 3.14 – 3.06 (m, 1H, $J_{1eq,2}$ = 6.9 Hz, H-1_{eq}), 2.82 – 2.68 (m, 2H), 2.66 – 2.44 (m, 3H), 2.44 – 2.24 (m, 3H) (H-1_{ax}, H-5, H-7, H-7', H-12, H-12', H-16, H-16'), 1.80 – 1.60 (m, 2H), 1.49 – 1.28 (m, 2H), 1.14 – 0.96 (m, 2H), 0.84 – 0.70 (m, 2H).

^{13}C-NMR, (75 MHz, D_2O) δ in ppm: 174.5 (C-13), 135.5, 130.5, 130.2, 129.0, 127.8 126.7, 125.8, 125.2, 124.0, 122.7 (pyrene), 72.7 (C-3), 69.9 (C-4), 64.5 (C-2), 64.0 (C-5), 59.3 (C-6), 53.4 (C-1), 53.1(C-7), 39.2 (C-12), 35.6 (C-14), 32.1 (C-16), 28.5, 27.3, 25.6, 25.4, 21.7.

***N*-[6-(tert-butyloxycarbonylamino)hexyl]-1,5-dideoxy-1,5-imino-D-galactitol (23)**

80 mg (0.30 mmol) of **15** are dissolved in 15 ml of absolute DMF and 121 mg (1.20 mmol) of Et_3N are added. To the stirred solution, 80 mg (0.37 mmol) Boc_2O are added and the reaction is stirred at room temperature for 30 minutes. 1 ml MeOH is added and the reaction is evaporated to dryness to yield 94.9 mg (0.26 mmol, 86 %) of the title compound after silica gel chromatography.



$C_{14}H_{34}N_2O_6$

MW = 362.5 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 700/100/8 (v/v/v)

$[\alpha]_D^{20}$ = -14.3 °(c = 1.1, MeOH)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 3.98 (bs, 1H, H-4), 3.85 – 3.74 (m, 3H, H-2, H-6a, H-6b), 3.20 (dd, 1H, $J_{3,4}$ = 1.9 Hz, $J_{2,3}$ = 9.1 Hz, H-3), 3.02 (t, $J_{11,12}$ = 6.9 Hz, H-12), 2.96 (dd, 1H, $J_{1eq,2}$ = 4.5 Hz, $J_{1ax,1eq}$ = 11.7 Hz, H-1_{eq}), 2.74 – 2.64 (m, 1H, H-7), 2.54 (m, 1H, H-7'), 2.38 – 2.31 (m, 1H, H-5), 2.10 (dd, 1H, $J_{1ax,2}$ = 10.7 Hz, H-1_{ax}), 1.56 – 1.40 (m, 4H), 1.43 (s, 9H), 1.37 – 1.24 (m, 4H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 158.9, 80.0, 77.6 (C-3), 72.44 (C-4), 69.3 (C-2), 65.4 (C-5), 62.6 (C-6), 58.4 (C-1), 54.3 (C-7), 41.5 (C-12), 31.2, 29.1 (3C), 28.6, 28.0, 25.2.

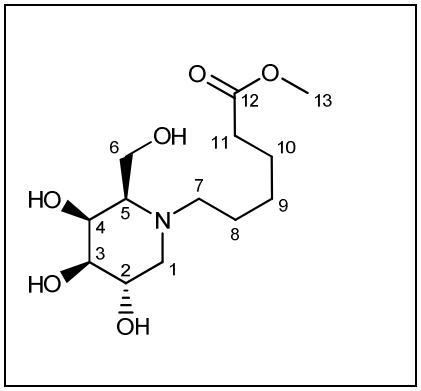
N-Methoxycarbonylpentyl-1,5-dideoxy-1,5-imino-D-galactitol hydrochloride (24)

N-Carboxypentyl-1,5-dideoxy-1,5-imino-D-galactitol (25)

0.45 g (1.89 mmol) of 6 are dissolved in 50 ml of dry methanol before 0.34 g (1.89 mmol) of methyl 6-aminohexanoate hydrochloride and 80 mg $Pd(OH)_2$/C are added. Et_3N (0.28 g, 2.84 mol) is added and a hydrogen atmosphere (1 bar) is applied. The reaction is stirred for 18 hours, before no further reaction progress can be monitored by TLC analysis. The catalyst is filtered off and the solvent is removed under reduced pressure to yield 0.57 g (1.71 mmol, 91 %) of a pale-brown oil.

The residue is taken up in 15 ml of dry methanol containing 1.5 ml of acetyl chloride. The reaction is stirred at room temperature for 14 hours before quantitative conversion of the starting material is observed by TLC.

The solvent is removed under reduced pressure, taken up in methanol and evaporated three times to remove excess HCl yielding 0.57 g of a pale-brown foam. After NMR analysis of the crude material, showing satisfactory purity, it is directly taken to the next step without further purification.



$C_{13}H_{25}NO_6$

MW = 291.2 g/mol

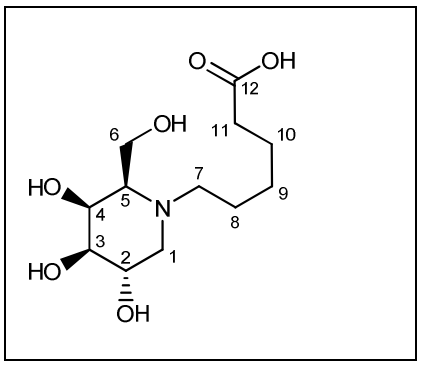
TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

$[\alpha]_D^{20}$ = 8.8° (c = 4.3, MeOH)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 4.35 – 4.16 (m, 1H, H-4), 4.15 – 4.02 (m, 1H, H-2), 4.02 – 3.92 (m, 2H, H-6a, H-6b), 3.68 (s, 3H, H-13), 3.56 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-3), 3.52 – 3.29 (m, 4H, H-5, H-1_{eq}, H-7, H-7'), 2.99 (dd, 1H, $J_{1ax,1eq}$ = 11.5 Hz, $J_{1ax,2}$ = 11.0 Hz, H-1_{ax}), 2.39 (t, 2H, $J_{11,12}$ = 7.3 Hz), 1.94 – 1.63 (m, 2H), 1.70 (m, 2H), 1.52 – 1.34 (m, 2H)

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 175.7 (C-12), 74.6 (C-3), 71.8 (C-4), 66.4 (C-2), 66.3 (C-5), 55.2 (C-1), 54.7 (C-7), 52.1 (C-13), 34.5 (C-11), 27.13 (C-9), 25.5 (C-10), 23.2 (C-8)

Crude **24** is taken up in a 1/1 (v/v) mixture of 1,4-dioxane and H_2O (15 ml) and 0.5 M NaOH is used to adjust pH 10. The reaction is stirred at room temperature for 5 hours, before complete conversion of the starting material is observed by TLC analysis. Amberlite IR-120 acidic ion exchange resin is carefully added in portions to adjust neutral pH. The resin is filtered off and the solvents are removed under reduced pressure to yield 0.36 g (1.30 mmol, max. 69 %) of the desired crude compound, as yellow slurry, which is taken into the next step without further purification.



$C_{12}H_{23}NO_6$

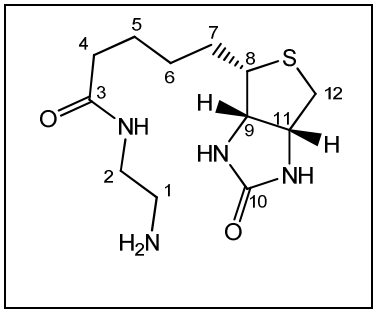
MW = 277.2 g/mol

TLC:$CHCl_3$/MeOH/NH_3 2/2/2 (v/v/v) (stays at baseline)

***N'-Biotinoyl-1,2-diaminoethan* (26)**

1 g (4.09 mmol) of D-biotin, 1.55 g (4.10 mmol) of HBTU and 435 mg (4.3 mmol) of Et_3N are dissolved in 25 ml of dry DMF. This solution is added dropwise to a solution of ethylene 1,2-diaminoethane (609 mg, 10.1 mmol) in 15 ml of DMF. The solution is stirred at room temperature for 6 hours before complete conversion of the starting material, the reaction is quenched by the dropwise addition of 3 ml of dry methanol. The solvents are removed under reduced pressure and

the resulting slurry is subjected to silica gel chromatography to yield 678 mg (2.37 mmol, 58 %) of the title compound **26** as white chalk.



$C_{12}H_{22}N_4O_2S$

MW = 286.15 g/mol

TLC:$CHCl_3$/MeOH/NH_3 100/100/2 (v/v/v)

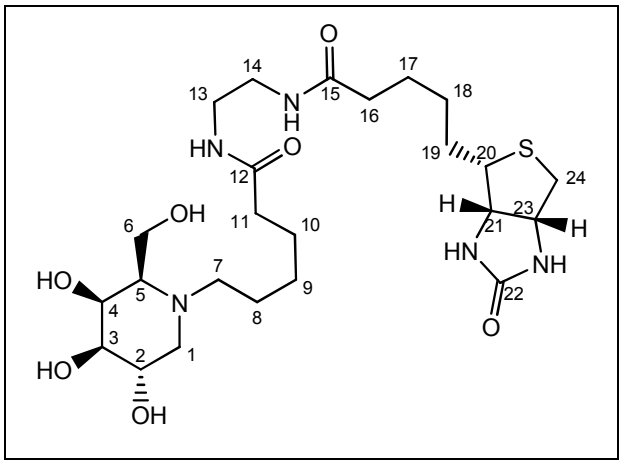
Purification: SGC: $CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 4.52 (dd, 1H, $J_{11',12}$ = 4.7 Hz, $J_{9,11}$ = 7.6 Hz, H-11), 4.33 (dd, $J_{8,9}$ = 4.4 Hz, H-9), 3.38 (t, 2H, $J_{1,2}$ = 6.0 Hz, H-1), 3.30 – 3.17 (m, H-8), 2.95 (dd, 1H, $J_{9,9'}$ = 12.8 Hz, H-9), 2.93 (t, 2H, H-2), 2.73 (d, 1H, H-9'), 2.27 (t, 2H, $J_{4,5}$ = 7.4 Hz, H-4), 1.85 – 1.55 (m, 4H), 1.48 (p, 2H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 176.9 (C-3), 166.2 (C-10), 63.4, 61.7 (C-9, C-11), 41.4, 41.0 (C-1, C-2), 40.3 (C-12), 36.7 (C-4), 29.8, 29.5, 26.7.

***N*-(*N*'-Biotinoylaminoethyl)aminocarbonylpentyl-1,5-dideoxy-1,5-imino-D-galactitol (27).**

To a solution of **25** (180 mg, 650 µmol) in DMF (15 ml), iPr_2NEt (127.3 µL, 840 µmol), TBTU (230 mg, 715 µmol) and **26** (204.3 mg, 715 µmol) are added and the reaction is stirred for 7 hours at room temperature. The solvent is evaporated, and the residue is purified by silica gel chromatography to give the title compound as off white solid after lyophilisation (185.7 mg, 50%).



$C_{24}H_{43}N_5O_7S$

MW = 545.7 g/mol

TLC:$CHCl_3$/MeOH/NH_3 4/4/1 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

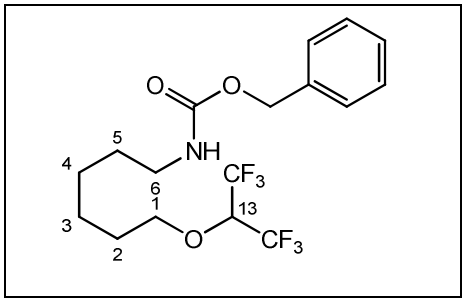
$[\alpha]_D^{20}$ = 14.3 °(c = 0.6, DMSO)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 4.77 (dd, 1H, $J_{21,23}$ = 8.2 Hz, $J_{23,24}$ = 4.5 Hz, H-23), 4.58 (dd, 1H, $J_{20,21}$ = 4.4 Hz,), 4.23 (dd, 1H, $J_{4,5}$ = 1.5 Hz, $J_{3,4}$ = 2.7 Hz, H-4), 4.11 (ddd, 1H, $J_{1eq,2}$ = 4.8 Hz, $J_{2,3}$ = 9.7 Hz, $J_{1ax,2}$ = 10.9 Hz, H-2), 4.01 (m, 2H, $J_{6b,5}$ = 4.3 Hz, H-6_a, H-6_b), 3.59 (dd, 1H, H-3), 3.32 (m, 1H, H-5), 3.48 (dd, 1H, $J_{1ax,1eq}$ = 12.0 Hz, H-1_{eq}), 3.44 – 3.29 (m, 8H, H-20, H-7, H-13, H-14), 23.06 (dd, 1H, $J_{24,24'}$ = 13.0, H-24), 3.03 (dd, 1H, H-1_{ax}), 2.87 (d, 1H, H-24'), 2.39 (m, 4H, *J* = 2.3 Hz, H-11, H-16), 2.0 – 1.35 (m, 12H)

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 177.6, 177.4 (C-12, C-15), 165.5 (C-22), 74.6 (C-3), 71.6 (C-4), 66.4 (C-2), 66.3 (C-5), 64.8 (C-21), 63.32 (C-23), 61.3 (C-6), 56.7 (C-20), 55.2 (C-7), 54.6 (C-1), 40.6 (C-24), 40.4 (C-13), 40.3 (C-14), 36.2, 36.1, (C-11, C-16), 29.7, 29.3, 27.1, 26.8, 26.4, 23.1

Benzyl 6-(1,1,1,3,3,3-hexafluoropropane-2-yloxy)hexylcarbamate (28)

1 g (3.98 mmol) of **16** are dissolved in 50 ml of absolute THF before 3.15 g (11.95 mmol) of triphenylphosphine are added at once. The mixture is cooled to 0 °C and 1.84 ml DEAD (11.94 mmol) are added dropwise. The reaction is allowed to warm to room temperature before 2.17 g (11.94 mmol) of 1,1,1,3,3,3-hexafluoro-2-methylpropan-2-ol are added rapidly. After 20 minutes, complete conversion is indicated by TLC control. The solvent is removed under reduced pressure and the crude mixture is pre-purified by fluorous SPE using MeOH/H_2O (80:20) as loading and washing solvent and THF as elution solvent. Subsequent silica gel column chromatography yields 1.31 g (82.1 %) of the title compound as colourless sirup.



$C_{17}H_{21}F_6NO_3$

MW = 401.3 g/mol

TLC: : Cy/EtOAc 2/1 (v/v)

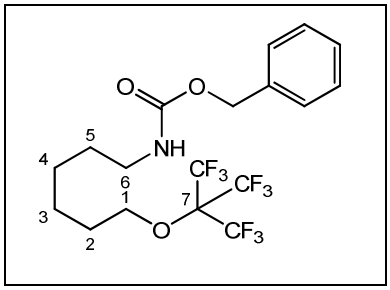
Purification: F-SPE, SGC: Cy/EtOAc 8/1 (v/v)

^{1}H-NMR, (500 MHz, $CDCl_3$) δ in ppm: 7.4 – 7.2 (m, 5H), 5.12 (s, 2H, CH_2-Ph), 4.92 (h, 1H, $J_{13,F}$ = 5.3 Hz, H-7, 4.47 (bs, 1H, N<u>H</u>), 4.02 (t, 2H, $J_{1,2}$ = 6.2 Hz, H-1), 3.19 (q., 2H, $J_{5,6} = J_{6,NH}$ = 6.7 Hz, H-6), 1.68 (m, 2H), 1.52 (m, 2H,), 1.44 – 1.30 (m, 4H)

^{13}C-NMR, (125 MHz, $CDCl_3$) δ in ppm: 156.2, 136.8, 128.6, 128.4 128.4, 121.2 (q., $J_{C,F}$ = 292 Hz, 2x CF_3), 69.7 (CH_2-Ph), 69.6 (p, $J_{7,F}$ = 34 Hz, C-7), 66.8 (C-1), 41.0 (C-6), 30.0, 29.5, 26.3, 25.1.

Benzyl-6-(1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-yloxy)hexylcarbamate (29)

1 g (3.98 mmol) of **16** are dissolved in 50 ml of absolute THF before 3.15 g (11.95 mmol) of triphenylphosphine are added at once. The mixture is cooled to 0 °C and 1.84 ml DEAD (11.94 mmol) are added dropwise. The reaction is allowed to warm to room temperature, before 2.81 g (11.94 mmol) of 1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-ol are added rapidly. After 15 minutes, complete conversion is indicated by TLC control. The solvent is removed under reduced pressure, and the mixture is pre-purified by fluorous SPE using MeOH/H_2O (80:20) as loading and washing solvent and THF as elution solvent. Subsequent silica gel column chromatography yields 1.49 g (79.8 %) of the title compound as colourless sirup.



$C_{18}H_{20}F_9NO_3$

MW = 469.3 g/mol

TLC: : Cy/EtOAc 2/1 (v/v)

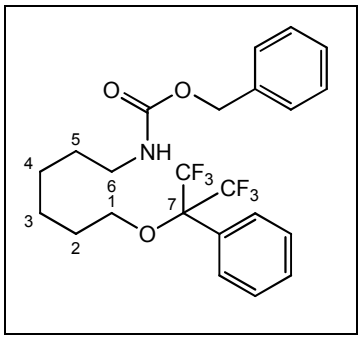
Purification: F-SPE, SGC: Cy/EtOAc 8/1 (v/v)

^{1}H-NMR, (500 MHz, $CDCl_3$) δ in ppm: 7.4 – 7.2 (m, 5H), 5.19 (s, 2H, CH_2-Ph), 4.76 (bs, NH), 3.55 (t, 2H, $J_{1,2}$ = 6.8 Hz, H-1), 3.18 (dd, 2H, $J_{6,6'}$ = 13.2 Hz, $J_{5,6}$= 6.8 Hz, H-6), 1.7 (m, 2H), 1.53 (m, 2H), 1.43 (m, 2H), 1.35 (m, 2H).

^{13}C-NMR, (125 MHz, $CDCl_3$) δ in ppm: 156.7, 136.7, 128.8, 128.7, 128, 4, 122.1 (q, $J_{C,F}$ = 291 Hz, 3x CF_2), 82.9 (t, $J_{13,F}$ = 28 Hz, C-7), 66.9, 66.48 (C-1, CH_2-Ph), 41.2 (C-6), 30.1, 29.9, 26.7, 25.6.

Benzyl 6-(1,1,1,3,3,3-hexafluoro-2-phenylpropan-2-yloxy)hexylcarbamate (30)

1 g (3.98 mmol) of **16** are dissolved in 50 ml of absolute THF before 3.15 g (11.95 mmol) of triphenylphosphine are added at once. The mixture is cooled to 0 ℃ and 1.84 ml DEAD (11.94 mmol) are added dropwise. The reaction is allowed to warm to room temperature, before 3.00 g (11.94 mmol) of 1,1,1,3,3,3-hexafluoro-2-phenylpropan-2-ol are added rapidly. After 15 minutes, complete conversion is indicated by TLC control. The solvent is removed under reduced pressure, and the mixture is prepurified by fluorous SPE using MeOH/H_2O (80:20) as loading and washing solvent and THF as elution solvent. Subsequent silica gel column chromatography yields 1.68 g (90.5 %) of the title compound as slightly yellow sirup.



$C_{23}H_{25}F_6NO_3$

MW = 477.4 g/mol

TLC: Cy/EtOAc 2/1 (v/v)

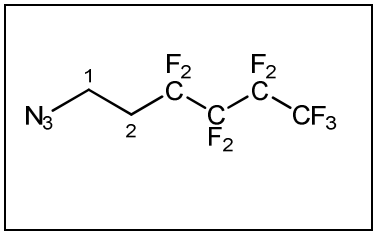
Purification: F-SPE, SGC: Cy/EtOAc 8/1 (v/v)

^{1}H-NMR, (500 MHz, $CDCl_3$) δ in ppm: 7.4 – 7.2 (m, 10H), 5.19 (s, 2H, CH_2-Ph), 4.74 (bs, NH), 3.98 (t, 2H, $J_{1,2}$ = 5.9 Hz, H-1), 3.18 (dd, 2H, $J_{6,6'}$ = 13.2 Hz, $J_{5,6}$ = 6.9 Hz, H-6), 1.67 (m, 2H), 1.51 (m, 2H), 1.45 – 1.30 (m, 4 H).

^{13}C-NMR, (125 MHz, $CDCl_3$) δ in ppm: 156.6, 136.8 134.0, 133.9, 129.0, 128.8, 128.7, 128.4, 121.8 (q, $J_{C,F}$ = 293 Hz, 2x CF_3) 69.9, 66.9 (C-1, CH_2-Ph), 41.1 (C-6), 30.0, 29.8, 26.5, 25.2.

3,3,4,4,5,5,6,6,6-Nonafluorohexylazide (31)

728 mg (11.2 mmol) NaN_3 are dissolved in 1.7 ml deionised H_2O. 112 mg (0.27 mmol) methyl trioctylammoniumchloride (Aliquat 336) and 1.57 g (5.44 mmol) 1,1,1,2,2,3,3,4,4-nonafluoro-6-iodohexane are added to the stirred solution to yield an opaque suspension. The reaction is stirred at 90 °C for 24 hours until complete conversion is indicated by TLC control. The reaction is transferred to a separatory funnel and diluted with 50 ml H_2O and is washed twice with 50 ml CH_2Cl_2. The combined organic fractions are dried over Na_2SO_4 and evaporated to dryness to yield 1.51 g (96 %) of the crude title compound, which is taken into the next step without further purification.



$C_6H_4F_9N_3$

MW = 289.1 g/mol

TLC: Cy/EtOAc 3/1 (v/v)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 3.59 (t, 2H, $J_{1,2}$ = 7.3 Hz, H-1), 2.37 (tt, 2H, $J_{2,F}$ = 18.3 Hz, H-2)

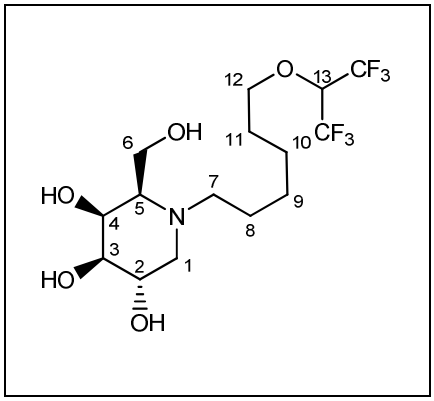
^{13}C-NMR, (125 MHz, ,MeOD) δ in ppm: 118.7 – 110.4 (m, $J_{C,F}$ = 285 Hz, $J_{C,F}$ = 30 Hz. 3x CF_2, CF_3), 43.5 (t, $J_{1,F}$ = 4.1 Hz, C-1), 30.9 (t, $J_{2,F}$ = 22 Hz, C-2).

***N*-{6-[2,2,2-Trifluoro-1-(trifluoromethyl)ethoxy]hexyl}-1,5-dideoxy-1,5-imino-D-galactitol (32)**

415 mg (1.75 mmol) of **6** are dissolved in 50 ml of dry methanol and 670 mg (1.67 mmol) of **28** are added. To the stirred solution 100 mg Pd/C (0.094 mmol) are added. The flask is evacuated three times and refilled with hydrogen at atmospheric pressure and room temperature until complete conversion is indicated by TLC (30 hours). The catalyst is filtered off and the reaction is concentrated to dryness.

The residue is taken up in 10 ml of a mixture of MeOH and H_2O (1/1, v/v) and concentrated HCl is added to adjust a pH < 1.

After complete conversion, the solution is concentrated under reduced pressure and subsequently co-evaporated with methanol 3 times. Purification is achieved by fluorous SPE using H_2O as loading solvent and methanol as elution solvent. Subsequent silica gel chromatography (CH_2Cl_2/MeOH/NH_3 800/100/9 (v/v/v/)) yields 222.3 mg (26 %) as a white foam.



$C_{15}H_{25}F_6NO_5$

MW = 413.4 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: F-SPE SGC: $CHCl_3$/MeOH/NH_3 100/100/2 (v/v/v)

$[\alpha]_D^{20}$ = -11.5 °(c = 0.8, MeOH)

m/z: 414.2 (MH^+), 426.1 (MNa^+)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 5.76 (m, 1H, $J_{13,14F}$ = 5.3 Hz, H-13), 4.09 (m, 2H), 3.98 (m, 1H, H-4), 3.79 (m, 3H, $J_{5,6b}$ = 2.9 Hz, H-6_a, H-6_b, H-2), 3.20 (m, 1H, $J_{2,3}$ = 9.3 Hz, H-3), 2.98 (dd, 1H, $J_{1eq,2}$ = 2.8 Hz, $J_{1ax,1eq}$ = 10.3 Hz, H-1_{eq}), 2.77 – 2.67 (m, 1H, $J_{7,7'}$ = 13.4 Hz, H-7), 2.55 – 2.45 (m, 1H, H-7'), 2.37 (m, 1H, H-5), 2.11 (dd, 1H, $J_{1ax,2}$ = 10.9 Hz, H-1_{ax}), 1.80 – 1. 69 (m, 2H), 1.60 – 1.48 (m, 2H), 1.48 – 1.40 (m, 2H), 1.37 – 1.25 (m, 2H)

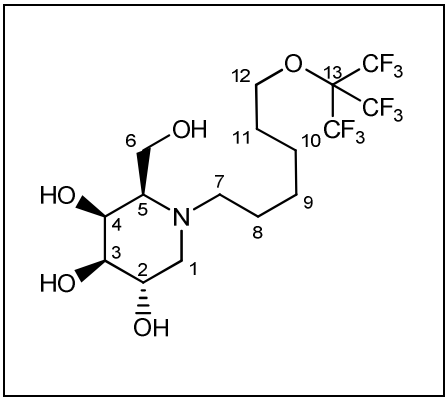
^{13}C-NMR, (125 MHz, MeOD) δ in ppm: 120.2 (q., $J_{C,F}$ = 293 Hz, 2x CF_3) 76.1 (C-3), 71.0 (C-4), 70.0 (C-2), 69.0 (h, $J_{13,F}$ = 34 Hz, C-13), 67.8 (C-5), 64.0 (C-12), 61.2 (C-6), 56.8 (C-1), 52.8 (C-7), 29.2, 26.9, 25.1, 23.8 (C-8, C-9, C-10, C-11)

_N_-{6-[2,2,2-Trifluoro-1,1-bis(trifluoromethyl)ethoxy]hexyl}-1,5-dideoxy-1,5-imino-D-galactitol (33)

100 mg (0.63 mmol) **6** are dissolved in 30ml of dry methanol and 281 mg (0.60 mmol) of **29** are added. To the stirred solution 50mg Pd/C (0.047 mmol) are added. The flask is evacuated three times and refilled with hydrogen at atmospheric pressure and room temperature until complete conversion is indicated by TLC (30 hours). The catalyst is filtered off and the reaction is concentrated to dryness.

The residue is taken up in 10 ml of a mixture of MeOH and H_2O (1/1, v/v) and concentrated HCl is added to adjust a pH < 1.

After complete conversion, the solution is concentrated under reduced pressure and subsequently co-evaporated with methanol 3 times. Purification is achieved by fluorous SPE using H_2O as loading solvent and methanol as elution solvent. Subsequent silica gel chromatography (CH_2Cl_2/MeOH/NH_3 800/100/9 (v/v/v/)) yields 157.7 mg (52 %) as a white foam.



$C_{16}H_{24}F_9NO_5$

MW = 481.4 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: F-SPE SGC: $CHCl_3$/MeOH/NH_3 100/100/2 (v/v/v)

$[\alpha]_D^{20}$ = -14.7 °(c = 1.9, MeOH)

m/z: 482.1 (MH^+)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 4.08 (dd, 2H, $J_{11,12}$ = 5.3 Hz, $J_{12.11'}$ = 5.9 Hz, H-12), 3.98 (m, 1H, H-4), 3.80 (m, 3H, $J_{1eq,2}$ = 4.8 Hz, $J_{1ax,2}$ = 10.1Hz, H-2, H-6_a, H-6_b), 3.21 (dd, 1H, $J_{3,4}$ = 2.8 Hz, $J_{2,3}$ = 9.3 Hz, H-3), 2.98 (dd, 1H, $J_{1eq,2}$ = 4.4 Hz, $J_{1ax,1eq}$ = 11.2 Hz, H-1_{eq}), 2.72 (m, 1H, $J_{7,7'}$ = 13.5 Hz, H-7), 2.51 (m, 1H, H-7'), 2.42 – 2.37 (m, 1H, H-5), 2.11 (dd, 1H, H-1_{ax}), 1.70 m, 2H), 1.51 (m, 2H), 1.43 (m, 2H), 1.32 (m, 2H).

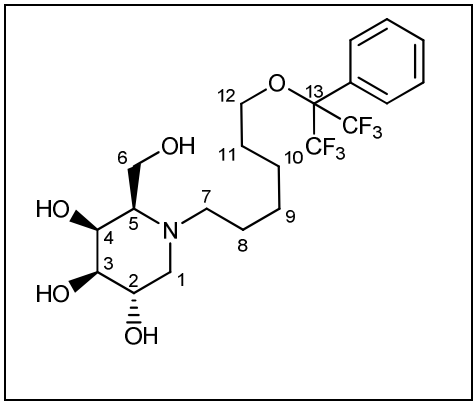
^{13}C-NMR, (125 MHz, ,MeOD) δ in ppm: 120.6 (q, $J_{C,F}$ = 294 Hz, 3x CF_3), 79.8 (t, $J_{13,F}$ = 28 Hz, C-13), 76.0 (C-3), 70.9 (C-4), 70.3 (C-2), 67,8 (C-12), 64.0 (C-5), 61.2 (C-6), 56.8 (C-1), 52.8 (C-7), 29.6, 26.9, 25.3, 23.7

N-{6-[2,2,2-Trifluoro-1-(trifluoromethyl)-1-phenylethoxy]hexyl}-1,5-dideoxy-1,5-imino-D-galactitol (34)

100 mg (0.63 mmol) of **6** is dissolved in 30ml of dry methanol and 286 mg (0.60 mmol) of **30** are added. To the stirred solution 50mg Pd/C (0.047 mmol) are added. The flask is evacuated three times and refilled with hydrogen at atmospheric pressure and room temperature until complete conversion is indicated by TLC (30 hours). The catalyst is filtered off and the reaction is concentrated to dryness.

The residue is taken up in 10 ml of a mixture of MeOH and H_2O (1/1, v/v) and concentrated HCl is added to adjust a pH < 1.

After complete conversion, the solution is concentrated under reduced pressure and subsequently co-evaporated with methanol 3 times. Purification is achieved by fluorous SPE using H_2O as loading solvent and methanol as elution solvent. Subsequent silica gel chromatography (CH_2Cl_2/MeOH/NH_3 800/100/9 (v/v/v/)) yields 147.3 mg (47.9 %) as a white foaṃ.



$C_{21}H_{29}F_6NO_5$

MW = 489.5 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: F-SPE SGC: $CHCl_3$/MeOH/NH_3 100/100/2 (v/v/v)

$[\alpha]_D^{20}$ = -15.2 °(c = 0.5, MeOH)

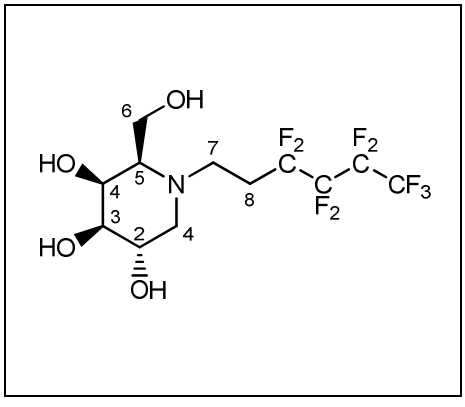
m/z: 490.1 (MH^+)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 7.61 – 7.56 (m, 2H), 7.55 – 7.50 (m, 3H), 4.00 – 3.96 (m, 1H, H-4), 3.84 – 3.76 (m, 3H, $J_{5,6b}$ = 4.9 Hz, H-2, H-6a, H-6b), 3.57 (dd, 2H, $J_{11,12}$ = 5.8 Hz, $J_{11',12}$ = 6.4 Hz, H-12), 3.24 – 3.18 (dd, 1H, $J_{3,4}$ = 3.3 Hz. $J_{2,3}$ = 9.7 Hz, H-3), 2.98 (dd, 1H, $J_{1eq,2}$ = 4.7 Hz, $J_{1ax,1eq}$ = 11.2 Hz, H-1_{eq}), 2.7 – 2.66 (m, 1H, H-7), 2.56 – 2.46 (m, 1H, H-7'), 2.41 – 2.34 (m, 1H, H-5), 2.12 (dd, 1H, $J_{1ax,2}$ = 10.8 Hz, H-1_{ax}), 1.73 (m, 2H), 1.58 – 1.49 (m, 2H), 1.49 – 1.42 (m, 2H), 1.36 – 1.27 (m, 2H).

^{13}C-NMR, (125 MHz, ,MeOD) δ in ppm: 131.9, 130.2, 129.6, 129.5, 125.5 – 123.0 (m, $J_{C,F}$ = 292 Hz, 2x CF_3), 84.4 (m, $J_{13,F}$ = 29 Hz, C-13), 77.6 (C-3), 72.5 (C-4), 69.3 (C-2), 65.4, 62.7, 58.4, 54.3, 31.0, 28.6, 27.0, 25.2.

***N*-3,3,4,4,5,5,6,6,6-Nonafluorohexyl-1,5-dideoxy-1,5-imino-D-galactitol (35)**

406 mg (1.72 mmol) of **6** are dissolved in 60 ml of dry methanol and 290 mg (1.00 mmol) of **31** are added. To the stirred solution 50 mg Pd/C (0.047 mmol) are added. The flask is evacuated three times and refilled with hydrogen at atmospheric pressure and room temperature until complete conversion is indicated by TLC (40 hours). The catalyst is filtered off and the reaction is concentrated to dryness. The crude mixture is taken up in 15 ml of a mixture of MeOH/H_2O 1/1 (v/v) and chloroform is added until a clear solution is obtained (ca. 6 ml). Concentrated HCl is added to maintain a pH < 1. After complete conversion of the starting material indicated by TLC, the reaction is evaporated to dryness and subjected to silica gel chromatography ($CHCl_3$/MeOH/NH_3 600/100/7 (v/v/v/)) to yield 163 mg (40 %) as a white chalk.



$C_{12}H_{16}F_9NO_4$

MW = 409.2 g/mol

TLC:$CHCl_3$/MeOH 10/1 (v/v)

Purification: F-SPE SGC: $CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

$[\alpha]_D^{20}$ = -6.9 °(c = 1.9, MeOH)

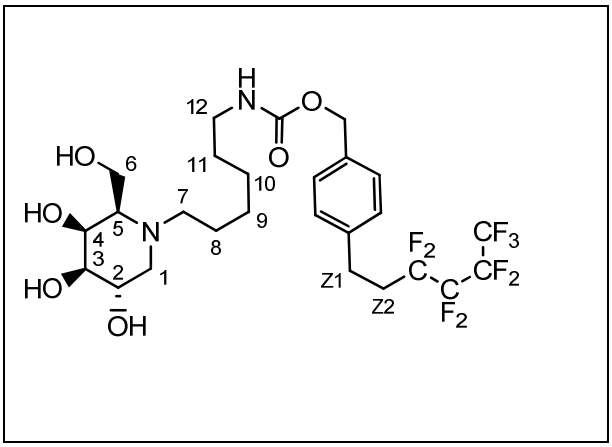
m/z: 410.0 (MH^+), 432.0 (MNa^+)

^{1}H-NMR, (500 MHz, MeOD) δ in ppm: 3.93 (m, 1H, 1H, H-4), 3.81 (m, 3H, H-2, H-6_a, H-6_b), 3.24 (dd, $J_{3,4}$ = 2.5 Hz, $J_{2,3}$ = 8.8 Hz, H-3), 3.15 – 3.06 (m, 1H, $J_{7,7'}$ = 10.4 Hz, H-7), 3.06 – 2.97 (m, 1H, H-7'), 2.92 (dd, 1H, $J_{1ax,1eq}$ = 11.3 Hz, $J_{1eq,2}$ = 2.7 Hz, H-1_{eq}), 2.60 – 2.28 (m, 2H, $J_{8,8'}$ = 11.5 Hz, Hz, $J_{8,9F}$ = 17.3 Hz, H-8, H-8), 2.18 (dd, 1H, $J_{1ax,2}$ = 10.1 Hz, H-1_{ax})

^{13}C-NMR, (125 MHz, ,MeOD) δ in ppm: 119.0 – 110.8 (m, $J_{C,F}$ = 249 Hz, $J_{C,F}$ = 18 Hz, $J_{C,F}$ = 49 Hz, 3x CF_2, CF_3), 75.8 (C-3), 71.4 (C-4), 67.7 (C-2), 63.6 (C-5), 62.0 (C-6), 56.6 (C-1), 44.0 (t, $J_{7,F}$ = 3.9 Hz, C-7), 25.8 (t, $J_{8,F}$ = 22 Hz, C-8).

N-{{6-[4-(3,3,4,4,5,5,6,6,6-Nonafluorohexyl)benzyl]oxycarbonylamino}hexyl}-1,5-dideoxy-1,5-imino-D-galactitol (37)

108.3 mg (0.41 mmol) **15** are dissolved in 15 ml of dry DMF before 85.65 mg (0.82 mmol) of Et_3N are added. To this stirred solution (**36**) (204 mg, 0.42 mmol) is added and the reaction is stirred for 90 minutes at room temperature. After complete conversion, the reaction is quenched by the addition of 5 ml of methanol. The solvents are removed under reduced pressure and subsequent column chromatography yields 128 mg (0.19 mmol, 46 %) of the pure product.



$C_{26}H_{35}F_9N_2O_6$

MW = 642.6 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification: SGC: $CHCl_3$/MeOH/ NH_3 600/100/7 (v/v/v)

$[\alpha]_D^{20}$ = +4.5 °(c = 0.7, MeOH)

m/z: 457.2

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 7.40 – 7.26 (m, 4H), 5.08 (s, 3H, CH_2-Ph), 4.03 (dd, 1H, $J_{4,5}$ = 1.9 Hz, $J_{3,4}$ = 3.0 Hz, H-4), 3.91 – 3.81 (m, 1H, $J_{2,3}$ = 9.2 Hz, $J_{1eq,2}$ = 4.7 Hz, H-2), 3.86 – 3.81 (m, 2H, H-6a, H-6-b), 3.27 (dd, 1H, H-3), 3.14 (t, 2H, $J_{11,12}$ = 6.9 Hz, H-12), 3.05 (dd, 1H, $J_{1ax,1eq}$ = 11.2 Hz, H-1_{eq}), 3.0 – 2.92 (m, 2H, H-Z2), 2.86 – 2.70 (m, 1H, H-7), 2.66 – 2.52 (m, 1H, H-7'), 2.54 – 2.38 (m, 3H, H-5, H-Z1), 2.21 (dd, 1H, $J_{1ax,2}$ = 10.5 Hz, H-1_{ax}), 1.65 – 1.47 (m, 4H), 1.46 – 1.26 (m, 4H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 158.9, 140.3, 136.9, 129.5, 129.3 122.1 – 108.0 (m, 3x CF_2, CF_3), 77.1 (C-3), 72.13 (C-4), 68.8 (C-2), 67.0 (O-CH_2-Ph), 65.4 (C-5), 62.2 (C-6), 57.9 (C-1), 54.0 (C-7), 41.7 (C-12), 33.6 (t, $J_{C,F}$ = 22 Hz, C-Z2), 30.9, 28.2, 27.7, 27.0 (t, $J_{C,F}$ = 5 Hz, C-Z1), 25.0.

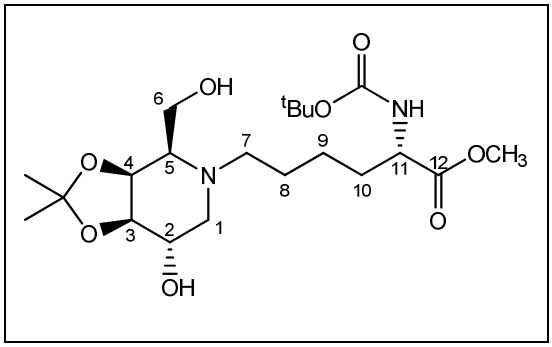
Methyl N^2-(tert-butoxycarbonyl)-N^6-(3,4-*O*-isopropylidene-1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinate (43)

a) direct scaling-up

8.36 g (35.4 mmol) of **6** and 16.77 g (42.5 mmol) of **41** are dissolved in 300 ml dry methanol. 1.2 g (~3.4 %mol) Pd/C, 10 %, are added and the reaction is stirred under a hydrogen atmosphere (1 bar). After 6 days of stirring, TLC control still does not indicate complete conversion of the starting material. The catalyst is filtered off and the reaction is evaporated to dryness. Subsequent column chromatography yields 2.04 g (4.56 mmol, 13 %) as colourless oil

b) improved conditions

3.36 g (14.2 mmol) of **6** and 5.05 g (12.8 mmol) of **41** are dissolved in 750 ml dry methanol. 0.45 g (~3.0 %mol) Pd/C, 10 %, are added and the reaction is stirred under a hydrogen atmosphere (1 bar). After 2 days of stirring, TLC control still indicates no further conversion of the starting material. The catalyst is filtered off and the reaction is evaporated to dryness. Subsequent column chromatography yields 3.06 g (6.85 mmol, 48.26 %) as pale-brown oil



$C_{21}H_{38}N_2O_8$.

MW = 446.3 g/mol

TLC:$CHCl_3$/MeOH/NH_3 700/100/8 (v/v/v)

Purification:SGC: $CHCl_3$/MeOH/NH_3 200/10/2 → 100/10/2 (v/v/v)

$[\alpha]_D^{20}$ = -11.0 °(c = 0.7, MeOH)

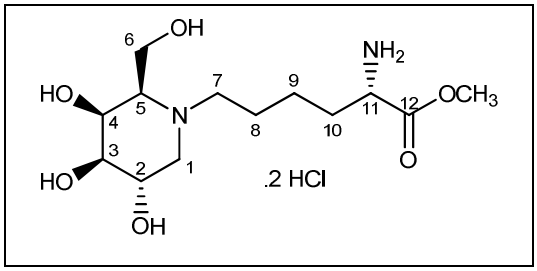
^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 4.37 (dd, 1H, $J_{4,5}$ = 3.0 Hz, $J_{3,4}$ = 4.9 Hz, H-4), 4.12 (dd, 1H, $J_{10,11}$ = 8.7 Hz, $J_{10',11}$ = 5.0 Hz, H-10), 3.84 (m, 2H, $J_{5,6a}$ = 5.6 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6a, H-6b), 3.82 – 3.70 (m, H-2, H-3), 3.74 (s, 3H, H-13), 2.90 (dd, 1H, $J_{1eq,2}$ = 3.9 Hz, $J_{1ax,1eq}$ = 11.9 Hz, H-1_{eq}), 2.86 – 2.74 (m, 1H, $J_{7,7'}$ = 13.4 Hz, H-7), 2.71 (dd, 1H, H-5), 2.64 – 2.52 (m, 1H, H-7'), 2.12 (dd, 1H, $J_{1ax,2}$ = 10.4 Hz, H-1_{ax}), 1.94 – 1.60 (m, 2H, n.r), 1.60 – 1.30 (m, 4H), 1.52 (s, 3H, H-18), 1.47 (s, 9H, H-16, H-16', H-16''), 1.36 (s, 3H, H-19)

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 175.0 (C-12), 158.2 (C-14), 110.2 (C-17), 81.3 (C-15), 76.6 (C-3), 70.8 (C-4), 63.2 (C-2), 62.5 (C-5), 56.3 (C-6), 55.0 (C-7), 54.8 (C-13), 54.0 (C-1), 52.6 (C-11), 32.5, 28.7 (C-16, C-16', C-16''), 28.6 (C-18), 26.5 (C-19), 25.2, 24.8

Methyl N^6-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinate (45)

3.06 g (6.85 mmol) of **43** are dissolved in 200 ml of absolute methanol containing 3.5 ml of acetyl chloride. The reaction is stirred at 40 °C for 12 hours before TLC control indicates complete cleavage of both protecting groups. The solvent is removed under reduced pressure and the

residue is co-evaporated with methanol three times to remove excess HCl to yield 2.77 g (6.94 mmol, 101 %) of a clear syrup, which is taken to the next step without further purification.



$C_{13}H_{26}N_2O_6$ .2HCl

MW = 399.3 g/mol

TLC:$CHCl_3$/MeOH/NH_3 4/4/1 (v/v/v)

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 171.32 (C-12), 75.2 (C-3), 72.3 (C-4), 67.02, 66.9 (C-2, C-5), 62.1 (C-6), 55.8, 54.8, 54.3, 54.2 (C-1, C-7, C-11, C-13), 31.5, 23.8, 23.4 (C-8, C-9, C-10)

Methyl *N*^{2}-(benzyloxycarbonyl)-*N*^{6}-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinate (39)

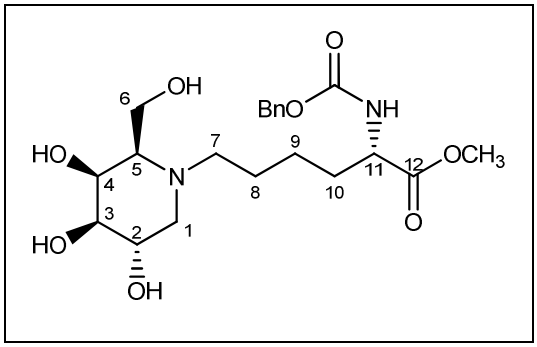
a)

2.77 g (max. 6.85 mmol) of crude **45** are dissolved in 100 ml of absolute methanol before 3.3 g (31.7 mmol) of Et_3N are added. To this stirred solution, 2.2 g (12.8 mmol) CBz-Cl are added dropwise. The reaction is stirred for 90 minutes at room temperature, before complete conversion is indicated by TLC analysis.

The reaction is brought to a neutral pH by the dropwise addition of anhydrous HCl (AcCl in methanol) and the reaction mixture is concentrated under reduced pressure to yield 16.1 g of the crude product contaminated triethyl ammonium chloride. Subsequent column chromatography yields 2.52 g (5.72 mmol, 83.5 %). NMR analysis of the product shows still unelectable amounts of triethylamine. Repeated column chromatography still doesn't separate the compounds. The product mixture is taken up in H_2O and loaded to a column of Amberlite XAD-4 (~10 ml). Elution of triethylamine is achieved by washing the column with 10 bed volumes of H_2O, before the desired product is eluted with 5 bed volumes of methanol, to yield 1.81 g (4.1 mmol, 60 %) of the title compound still containing traces of triethylamine/triethylammonium salts.

b)

3.0 g (7.5 mmol) of crude **45** are dissolved in 50 ml of dry methanol before 3.6 g (34 mmol) of Na_2CO_3 are added. To this stirred suspension, 1.5 g (8.7 mmol) of benzyl chloroformate are added dropwise. After 2 h of stirring at room temperature, the solids are filtered off and the filtrate is evaporated to dryness. Consecutive silica gel chromatography yields 1.92 g (4.35 mmol, 58 %) of the title compound as colourless syrup. After lyophilisation from H_2O, the title compound is obtained as hygroscopic white foam.



$C_{21}H_{32}N_2O_8$.

MW = 440.5 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification:SGC: $CHCl_3$/MeOH/NH_3 200/10/2 (v/v/v), Amberlite XAD-4.

$[\alpha]_D^{20}$ = -7.9 °(c = 1.1, MeOH)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 7.30 (m, 5H), 5.11 (s, 2H, O-CH_2-Ph), 4.21 (dd, 1H, $J_{11,NH}$ = 5.0 Hz, $J_{10,11}$ = 8.9 Hz, H-11), 4.03 (dd, 1H, $J_{4,5}$ = 1.8, $J_{3,4}$ = 3.0, H-4), 3.86 (m, 1H, $J_{1eq,2}$ = 4.8 Hz, $J_{2,3}$ = 9.9 Hz, H-2), 3.74 (s, 3H, H-13), 3.3 (dd, 1H, H-3), 3.28 (m, 2H, H-6_a, H-6_b), 3.05 (dd, 1H, $J_{1ax,1eq}$ = 11.3, H-1_{eq}), 2.79 (m, 1H, $J_{7,8}$ = 6.6 Hz, $J_{7,7'}$ = 10.0 Hz, H-7), 2.62 (m, 1H, $J_{7',8}$ = 6.2 Hz, H-7'), 2.23 (dd, 1H, H-1_{ax}), 1.78 (m, 2H), 1.57 (m, 2H), 1.40 (m, 2H)

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 174.8 (C-12), 158.8, 133.6, 129.5, 129.1, 128.9, 77.0 (C-3), 72.1 (C-4), 68.7 (C-2), 67.7 (C-22), 65.4 (C-5), 62.2 (C-6), 57.5 (C-1), 55.5 (C-11), 53.6 (C-7), 52.8 (C-13), 32.4, 24.7, 24.6 (C-8, C-9, C-10)

Methyl *N*^{2}-(dansyl)-*N*^{6}-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysinate (38)

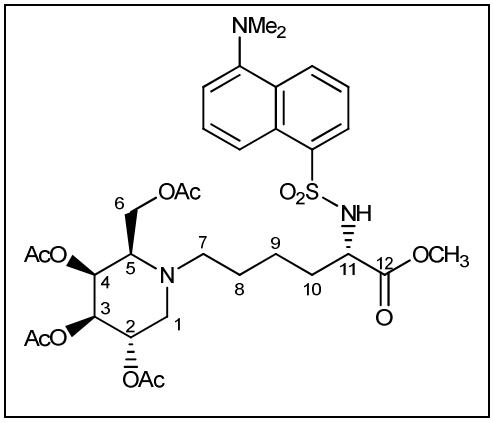
Methyl *N*^{2}-(dansyl)-*N*^{6}-(1,5-dideoxy-2,3-4-6-tetra-*O*-acetyl-D-galactitol-1,5-diyl)-L-lysinate (49)

1.00 g (3.26 mmol) of **39** are taken up in 100 ml of dry methanol before 100 mg (~ 3 %mol Pd) of Pd/C 10 % are added and the heterogeneous reaction mixture is stirred under hydrogen atmosphere (1 bar) at room temperature for 90 minutes. The catalyst is filtered off and Et_3N (365 mg, 3.59 mmol) and dansyl chloride (0.97 g, 3.59 mmol) is added. The reaction is stirred overnight before complete conversion of the starting material is observed by TLC analysis.

The solvent is removed under reduced pressure and the resulting brown syrup is subjected to column chromatography to yield 1.12 g (2.08 mmol, 64 %) of the title compound as a green-brown syrup which is contaminated by DNS-OH. Repeated column chromatography doesn't separate the compounds.

The residue is taken up in absolute pyridine, cooled to 0 ℃ and a spatula tip of DMAP is added. Acetic acid anhydride (432 mg, 4.2 mmol) is added dropwise.

After 12 hours, complete conversion is indicated by TLC analysis. The reaction is quenched by the slow addition of 10 ml of methanol. The solvents are removed in vaccuo and the resulting brown residue is subjected to column chromatography to yield the *O*-peracetylated target compound as bright green oil.



$C_{33}H_{45}N_3O_{12}S$.

MW = 707.3 g/mol

TLC:$CHCl_3$/MeOH/NH_3 20/1 (v/v)

Purification:SGC: $CHCl_3$/MeOH/NH_3 100/0 → 100/10 (v/v)

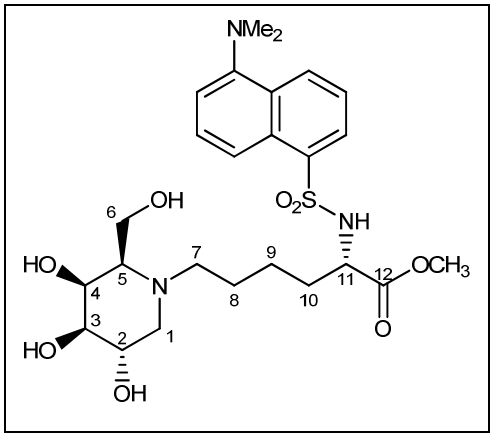
$[\alpha]_D^{20}$ = 5.20 °(c = 1.6, MeOH)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 8.60 (d, 1H), 8.42 (d, 1H), 8.23 (dd, 1H), 7.63 (dd), 7.61 (dd, 1H), 7.33 (d), 5.45 (dd, 1H, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 2.5 Hz, H-4), 5.11 (dd, 1H, $J_{1eq,2}$ = 4.4 Hz, $J_{2,3}$ = $J_{1ax,2}$ = 9.5 Hz, H-2), 4.5.01 – 4.78 (m, 1H, H-11)4.74 – 4.58 (m, 1H, H-5), 4.14 (dd, 1H, $J_{5,6a}$ = 5.7 Hz, $J_{6a,6b}$ = 11.2 Hz, H-6a), 4.06 (dd, 1H, $J_{5,6b}$ = 7.4 Hz, H-6b), 3.76 (dd, 1H, H-3), 3.39 (s, 3H, H-13), 3.02 (dd, 1H, $J_{1ax,1eq}$ =11.5 Hz, H-1_{eq}), 2.92 (s, 6H, H-26, H-25), 2.32 (dd, 1H, H-1_{ax}), 2.42 – 2.24 (m, 2H, H-7, H-7'), 2.14 (s, 3H, acetyl), 2.09 (s, 3H, acetyl), 2.04 (s, 3H, acetyl), 2.01 (s, 3H, acetyl), 1.45 – 1.53 (m, 2H), 1.25 – 1.00 (m, 4H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 173.7, 172.2 (2C), 171.9, 171.7 (C-12, 4x acetyl), 153.2, 137.0, 131.5, 131.1, 131.0, 130.6, 129.1, 124.3, 120.9, 116.6 (C-14, C-15, C-16, C-17, C-18, C-19, C-20, C-21, C-22, C-23), 73.5 (C-3), 69.5 (C-4), 68.8 (C-2), 62.6 (C-5), 59.9 (C-6), 56.9 (C-13), 53.5, 53.4 (C-1, C-7), 52.4 (C-11), 45.9 (C-25, C-26), 33.0, 28.0, 24.0 (C-8, C-9, C-10), 20.9, 20.8, 20.7 (4x acetyl)

Peracetate **49** is taken up in absolute methanol and the solution is cooled to -30 °C. MeONa (1 M in methanol) is added dropwise to adjust pH 10. The reaction is stirred for 1 hour before the cooling is removed and stirring is continued for 4 hours. After complete cleavage of the acetyl groups, the reaction is neutralised by the careful addition of Amberlite IR-120 to adjust pH 7-8. The resin is filtered off, washed thoroughly with methanol and the combined solutions are evaporated to dryness.

Lyophilisation of the product from H_2O yields 0.77 g (1.43 mmol, 44 % over all steps) of the desired compound as green, hydrophobic glass.



$C_{25}H_{37}N_3O_8S$.

MW = 539.23 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification:SGC: $CHCl_3$/MeOH/NH_3 800/100/9 (v/v/v), Amberlite XAD-4.

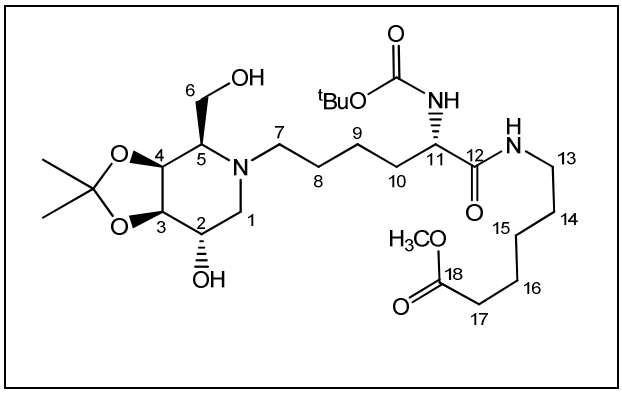
$[\alpha]_D^{20}$ = - 3.8 °(c = 1.0, MeOH)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 8.55 (d, 1H, dansyl), 8.41 (d, 1H, dansyl), 8.18 (d, 1H, dansyl), 7.59 (t, 1H, dansyl), 7.55 (t, 1H, dansyl), 7.28 (d, 1H, dansyl), 3.95 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 3.76 (ddd, 1H, $J_{1eq,2}$ = 4.9 Hz, $J_{1ax,2}$ = 10.3 Hz, $J_{2,3}$ = 9.3 Hz, H-2), 3.73 (m, 1H, H-11), 3.70 (m, 2H, H-6a, H-6b), 3.20 (s, 3H, OCH_3), 3.18 (dd, 1H, H-3), 2.88 (s, 6H, dansyl), 2.82 (dd, 1H, $J_{1ax,1eq}$ = 11.2 Hz, H-1_{eq}), 2.40 (m, 1H, H-7), 2.27 (m, 2H, H-5, H-7'), 1.99 (dd, 1H, H-1_{ax}), 1.58-1.52 (m, 2H), 1.24-0.96 (m, 4H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 172.9 (C-12), 151.8, 136.2, 130.0, 129.9, 129.9, 129.2, 127.8, 123.1, 119.9, 115.2 (dansyl), 76.1 (C-3), 71.0 (C-4), 67.8 (C-2), 63.7 (C-5), 61.1 (C-6), 56.8, 56.1, 52.2 (C-1, C-11, C-7), 51.0 (OCH_3), 44.7, 44.7 (dansyl), 32.2, 23.1, 22.7.

Methyl 6-{[*N*^{2}-(tert-butoxycarbonyl)-*N*^{6}-(3,4-*O*-isopropylidene-1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysyl]amino}hexanoate (44)

1.40 g (5.91 mmol) of **6** and 3.0 g (5.91 mmol) of **42** are dissolved in 100 ml dry methanol. 630 mg (~10 %mol) Pd/C, 10 %, are added and the reaction is stirred under a hydrogen atmosphere (1 bar). After 18 hours of stirring, TLC control indicates complete conversion of the starting material. The catalyst is filtered off and the reaction is evaporated to dryness. Subsequent column chromatography yields 2.76 g (4.93 mmol, 83 %) as pale-brown oil



$C_{27}H_{49}N_3O_9$

MW = 559.35 g/mol

TLC:$CHCl_3$/MeOH/NH_3 700/100/8 (v/v/v)

Purification:SGC: $CHCl_3$/MeOH/NH_3 180/10/2 (v/v/v)

$[\alpha]_D^{20}$ = -7.4 °(c = 2.0, MeOH)

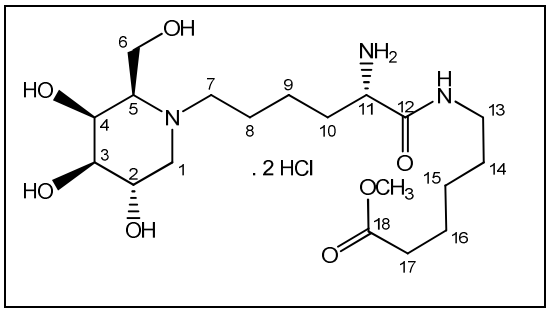
^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 4.39 (dd, 1H, $J_{4,5}$ = 3.0 Hz, $J_{3,4}$ = 4.9 Hz, H-4), 4.01 (dd, 1H, $J_{10,11}$ = 5.5 Hz, $J_{10',11}$ = 7.7 Hz, H-11), 3.90 – 3.84 (m, 2H, $J_{6a,5}$ = 5.6 Hz, H-6a, H-6b), 3.84 – 3.80

(m, 1H, H-3), 3.81 – 3.73 (m, 1H, H-2), 3.69 (s, 3H, OCH_3), 3.25 (dd, 1H, $J_{13,14}$ = 6.8 Hz, $J_{13,13'}$ = 13.4 Hz, H-13), 3.20 (dd, 1H, $J_{13',14}$ = 6.9 Hz, H-13'), 2.92 (dd, 1H, $J_{1eq,\ 2}$ = 3.9 Hz, $J_{1eq.\ 1ax}$ = 11.5 Hz, H-1eq), 2.88 – 2.74 (m, 1H, $J_{7,7'}$ = 13.3 Hz, H-7), 2.79 (m, 1Hm H-5), 2.68 – 2.54 (m, 1H, H-7'), 2.67 (dd, 2H, $J_{16,17}$ = 7.4 Hz, H-17), 2.17 (dd, 1H, $J_{1ax,2}$ = 10.9 Hz, H-1_{ax}), 1.85 – 1.30 (m, 14 H), 1.53 (s, 3H), 1.48 (s, 9H), 1.37 (s, 3H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 175.6 (C-18), 174.9 (C-12), 157.6 (C-20), 110.1, 81.2 (C-3), 80.4, 76.4 (C-4), 70.5 (C-2), 63.0 (C-5), 62.3 (C-6), 56.1 (C-1, C-11), 54.1 (C-7), 52.0 (OCH_3), 40.0 (C-13), 34.6 (C-17), 33.3, 29.9, 28.7, 27.2, 26.4, 25.5, 25.2, 24.8.

Methyl 6-{[*N*^{6}-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysyl]amino}hexanoate dihydrochloride (48)

1 ml of acetyl chloride are added dropwise to methanol (50 ml) at 0 ℃ and the solution is stirred for 20 minutes after the cooling bath has been removed. Then, 1.52 g of compound **44** (2.7 mmol) are added as a methanol solution (10 ml) and the reaction is stirred at room temperature for 12 hours. After complete conversion of the starting material, the solvent is removed under removed pressure at ambient temperature. The residue is taken up in methanol and co-evaporated again, to remove excess HCl and yield 2.2 g (4.47 mmol, 165 %) of a pale-brown slurry, which is taken crude into the next reaction.



$C_{19}H_{37}N_3O_7$.2 HCl

MW = 492.4 g/mol

TLC:$CHCl_3$/MeOH/NH_3 4/4/1 (v/v/v)

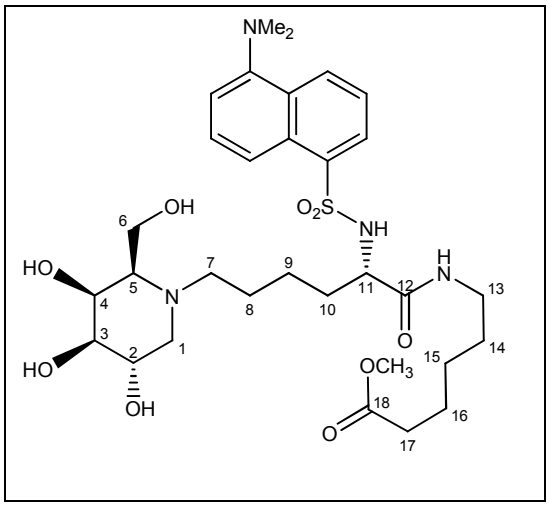
^{1}H-NMR, (300 MHz, D_2O) δ in ppm: 4.32 – 4.26 (m, 1H, H-4), 4.10 (ddd, 1H, $J_{1eq,2}$ = 4.8 Hz, $J_{1ax,2}$ = 10.8 Hz, H-2), 4.04 – 3.98 (m, 2H, $J_{5,6a}$ = 4.3 Hz, H-6a, H-6b), 3.96 (t, 1H, $J_{11,\ 10}$ = 6.7 Hz, H-11), 3.69 (s, 3H, H-19), 3.63 (dd, 1H, $J_{3,4}$ = 3.0 Hz, $J_{2,3}$ = 9.8 Hz, H-3), 3.55, (dd, 1H, $J_{1eq,\ 1ax}$ = 11.7 Hz, H-1_{eq}), 3.53 – 3.47 (m, 1H, H-5), 3.44 -3.12 (m, 4H, H-7, H-7', H-13, H-13'), 3.02 (dd, 1H, H-1_{ax}), 2.40 (t, 2H, $J_{16,17}$ = 7.4 Hz, H-17), 2.02 – 1.72 (m, 4.5 H), 1.68 – 1.24 (m, 9.5 H).

^{13}C-NMR, (75 MHz, D_2O) δ in ppm: 177.5 (C-18), 169.2 (C-12), 72.6 (C-3), 70.0 (C-4), 64.5 (C-2), 64.2 (C-5), 59.4 (C-6), 53.4 (C-1), 53.1 (C-11), 52.8 (C-7), 52.1 (OCH_3), 39.3 (C-13), 33.7 (C-17), 33.6, 30.4, 27.8, 25.6, 23.9, 21.6, 21.5

Methyl 6-{[*N*^{2}-(dansyl)-*N*^{6}-(1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysyl]amino}hexanoate (40)

670.3 mg of crude **48** (max 0.82 mmol) are dissolved in 5 ml of dry DMF, before 246 mg (2.46 mmol) of Et_3N are added. 265 mg (0.98 mmol) of dansyl chloride are added in portions and the

reaction is stirred at room temperature for 3 hours. After complete conversion of the starting material, the reaction is quenched by the dropwise addition of 1 ml of H_2O. The solvents are removed under reduced pressure and the resulting slurry is subjected twice to silica gel chromatography to yield the product as green oil. After lyophilisation from H_2O, 305.1 mg (0.47 mmol, 57 %) of the title compound are obtained as green hygroscopic foam.



$C_{31}H_{48}N_4O_9S$

MW = 652.3 g/mol

TLC:$CHCl_3$/MeOH/NH_3 300/100/4 (v/v/v)

Purification:SGC: $CHCl_3$/MeOH/NH_3 800/100/9 (v/v/v)

$[\alpha]_D^{20}$ = -6.4°(c = 0.9, MeOH)

^{1}H-NMR, (300 MHz, MeOD) δ in ppm: 8.54 (d, 1H, dansyl), 8.43 (d, 1H, dansyl), 8.19 (d, 1H, dansyl), 7.61 (t, 1H, dansyl), 7.55 (t, 1H, dansyl), 7.27 (d, 1H, dansyl), 3.95 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, H-4), 3.76 (ddd, 1H, $J_{1eq,2}$ = 4.9 Hz, $J_{1ax,2}$ = 10.7 Hz, $J_{2,3}$ = 9.3 Hz, H-2), 3.70 (m, 2H, H-6a, H-6b), 3.65 (s, 3H, OCH_3), 3.56 (m, 1H, H-11), 3.17 (dd, 1H, H-3), 2.88 (s, 6H, dansyl), 2.82 (dd, 1H, $J_{1ax,1eq}$ = 11.2 Hz, H-1_{eq}), 2.82 (m, 1H, H-7), 2.77 (m, 1H, 7'), 2.37 (m, 1H, H-13), 2.28 (m, 3H, H-5, H-17), 2.23 (m, 1H, H-13'), 2.00 (dd, 1H, H-1_{ax}), 1.58-0.88 (m, 12H).

^{13}C-NMR, (75 MHz, MeOD) δ in ppm: 174.6, 173.4 (C-12, C-18), 152.0, 136.4, 129.9, 129.9, 129.9, 129.1, 127.9, 123.2, 119.7, 115.2 (dansyl), 76.1 (C-3), 70.9 (C-4), 67.8 (C-2), 63.8 (C-5), 61.0 (C-6), 57.3, 56.9, 52.3 (C-1, C-11, C-7), 50.9 (OCH_3), 44.7 (dansyl), 38.7 (C-13), 33.5 (C-17), 32.9, 28.5, 26.1, 24.4, 23.3, 22.9.

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7 Appendix

2D-Correlation NMR Spectra of Final Products

List of Figures

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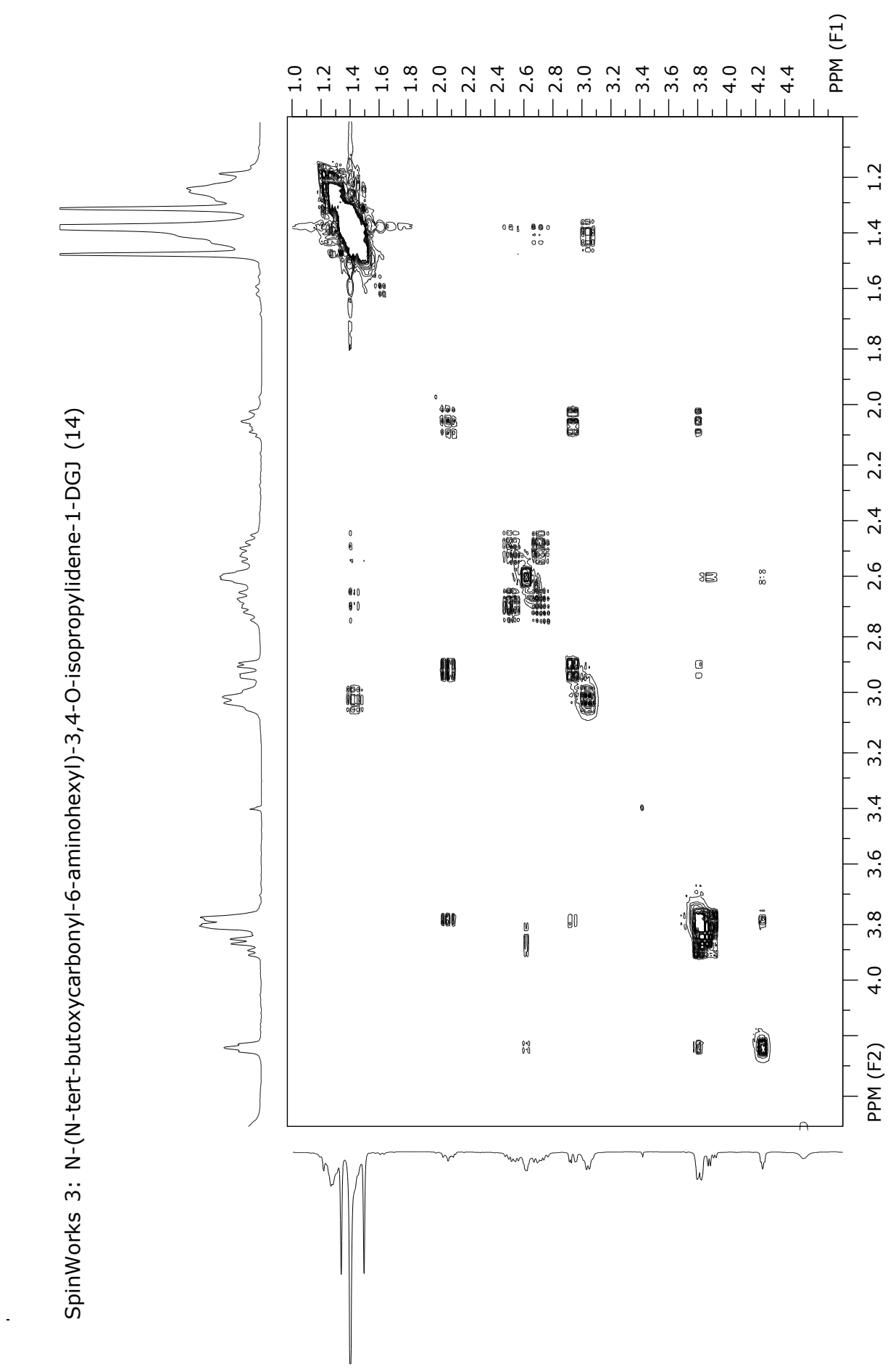
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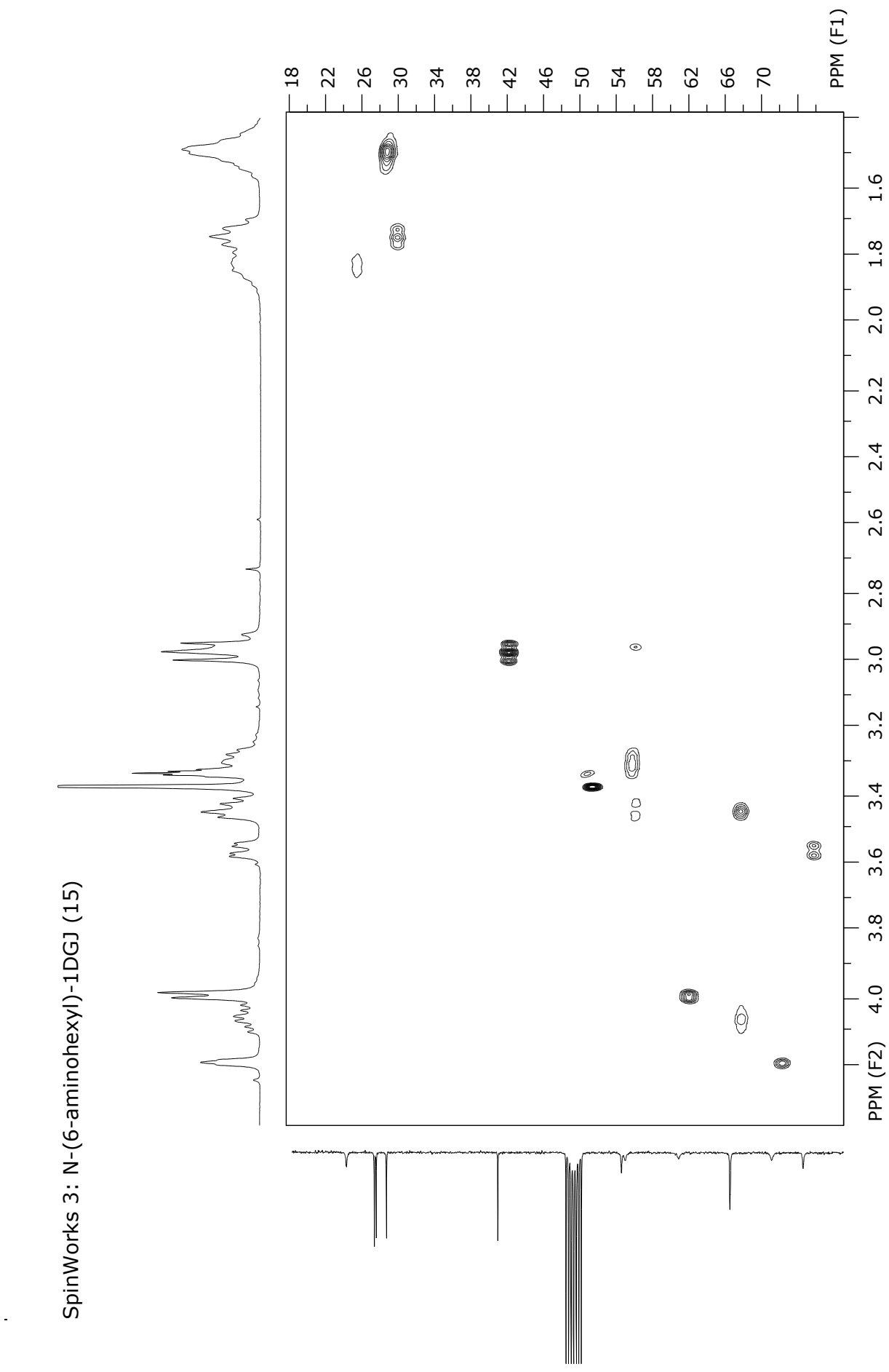
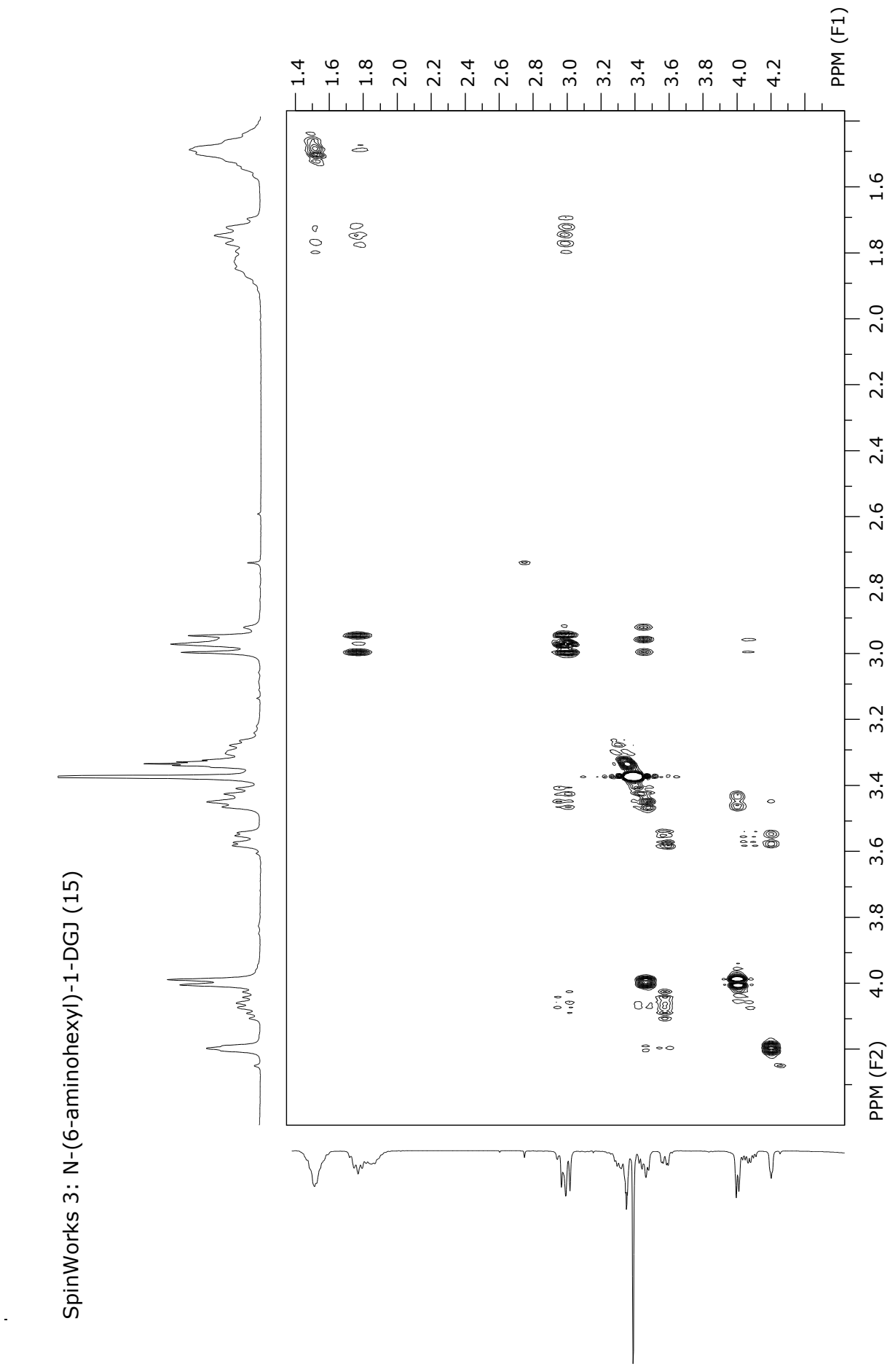
PPM (F2)


SpinWorks 3: N-(6-aminohexyl)-1DGJ (15)
PPM (F1)
18
22
26
30
34
38
42
46
50
54
58
62
66
70
PPM (F2)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6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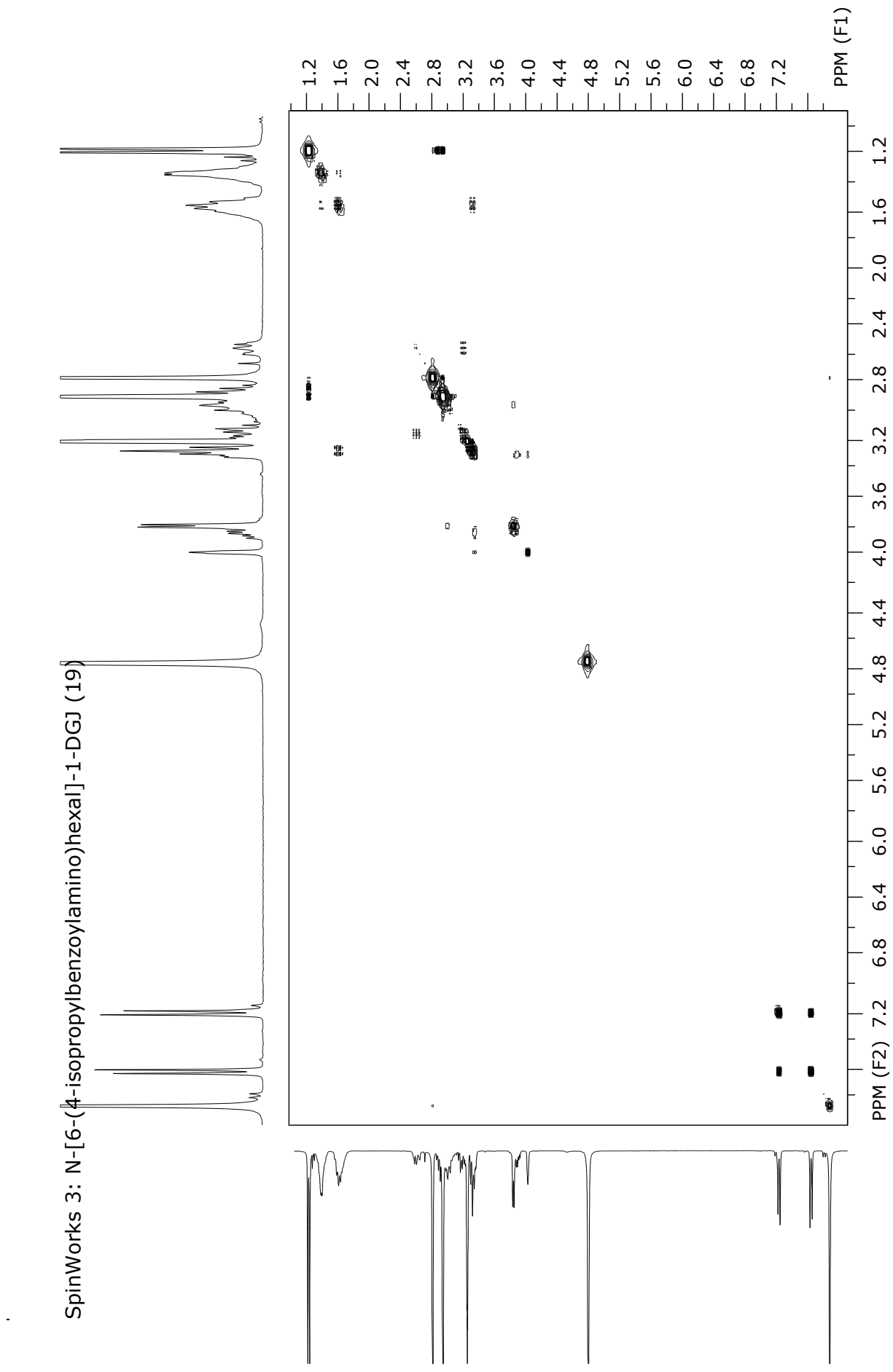
PPM (F1)
PPM (F2)
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2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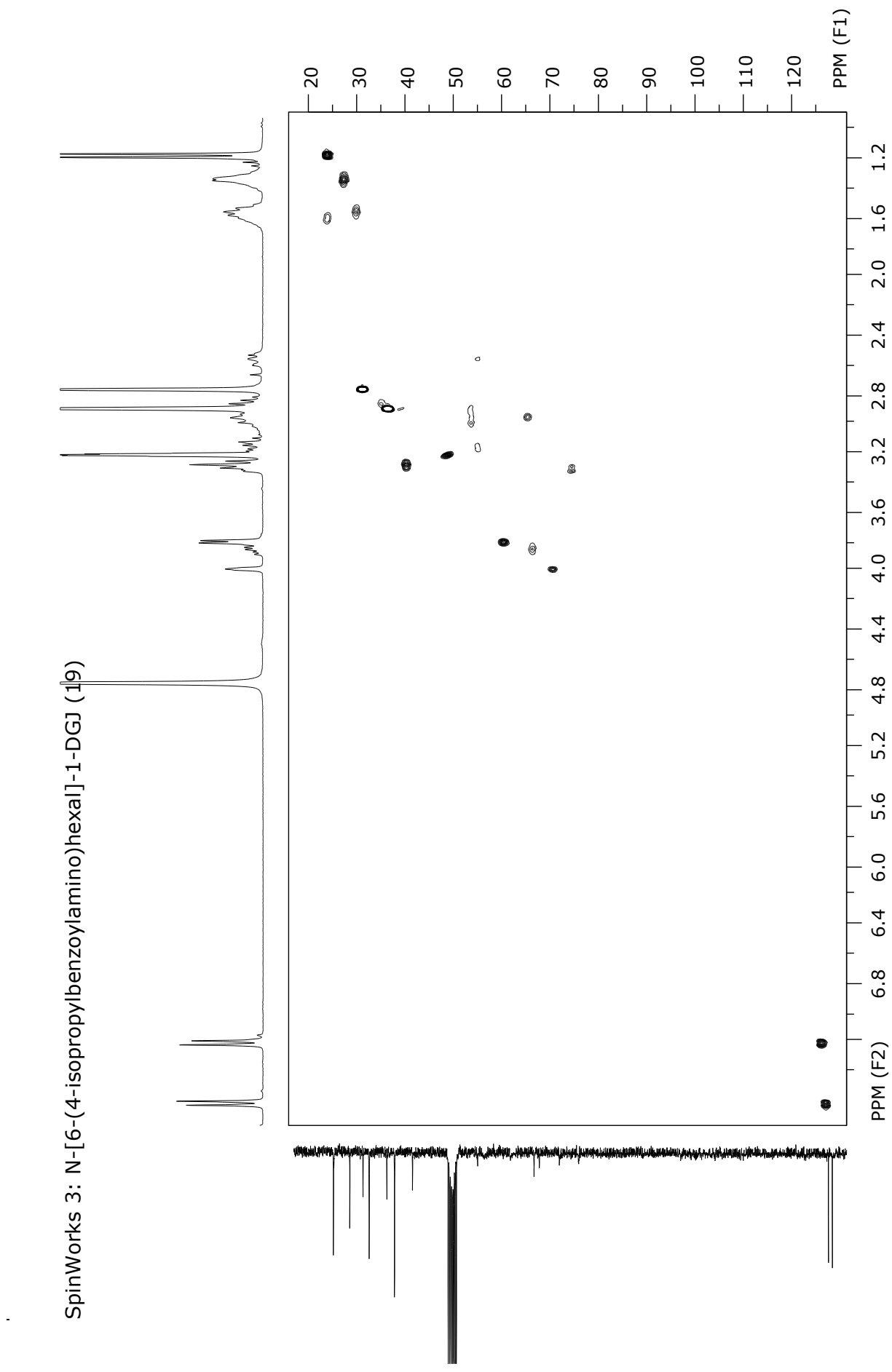
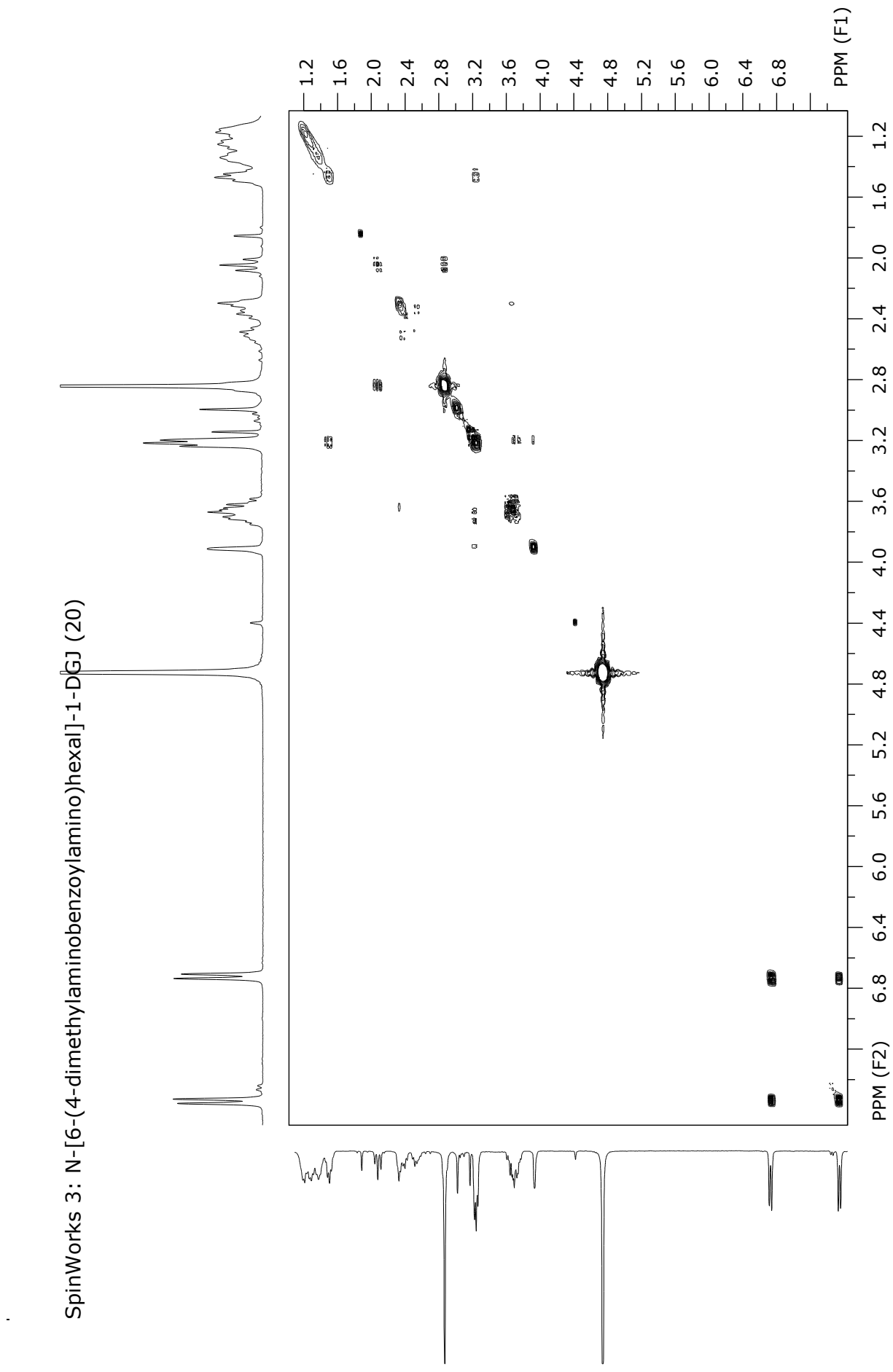
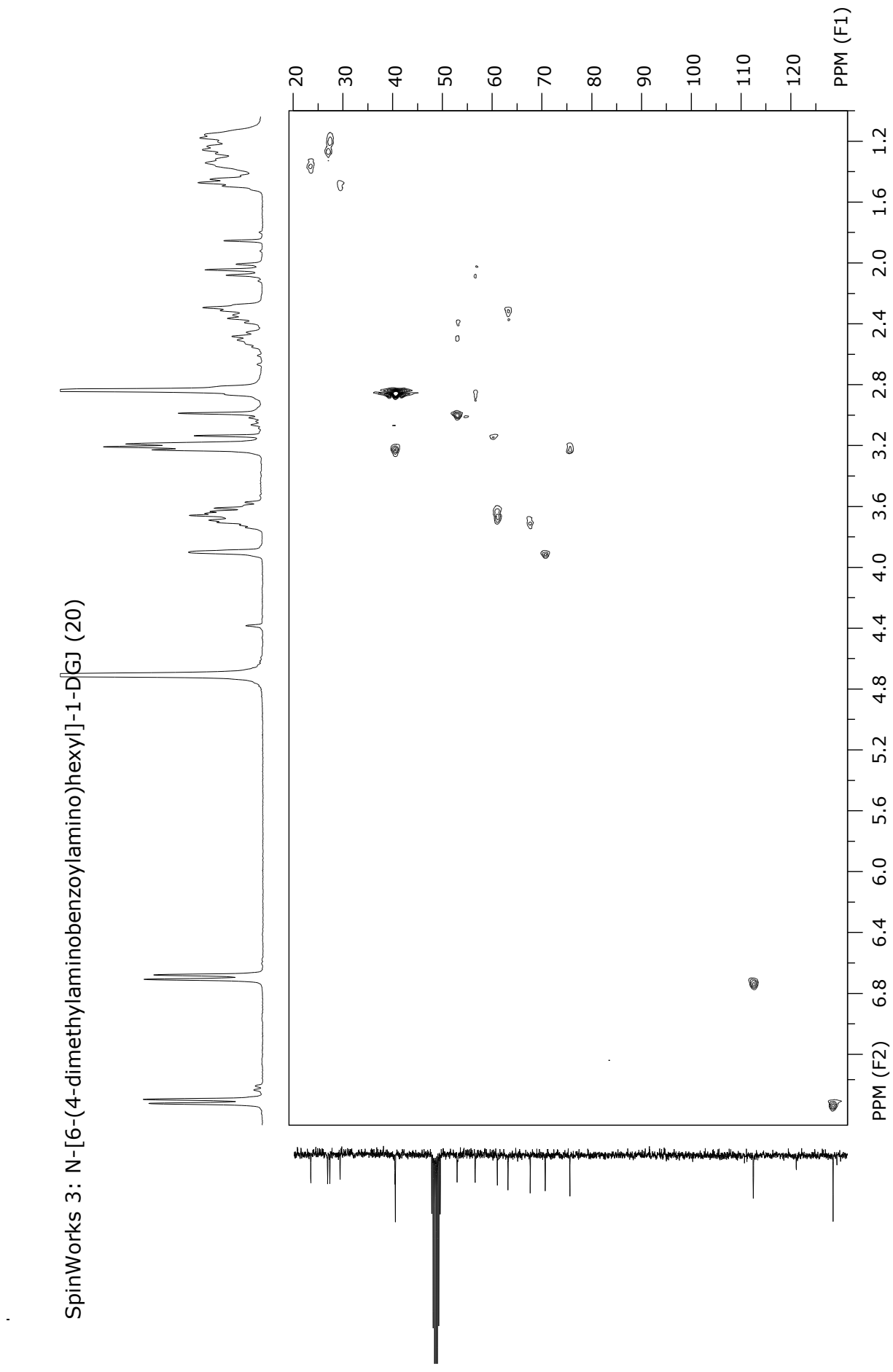
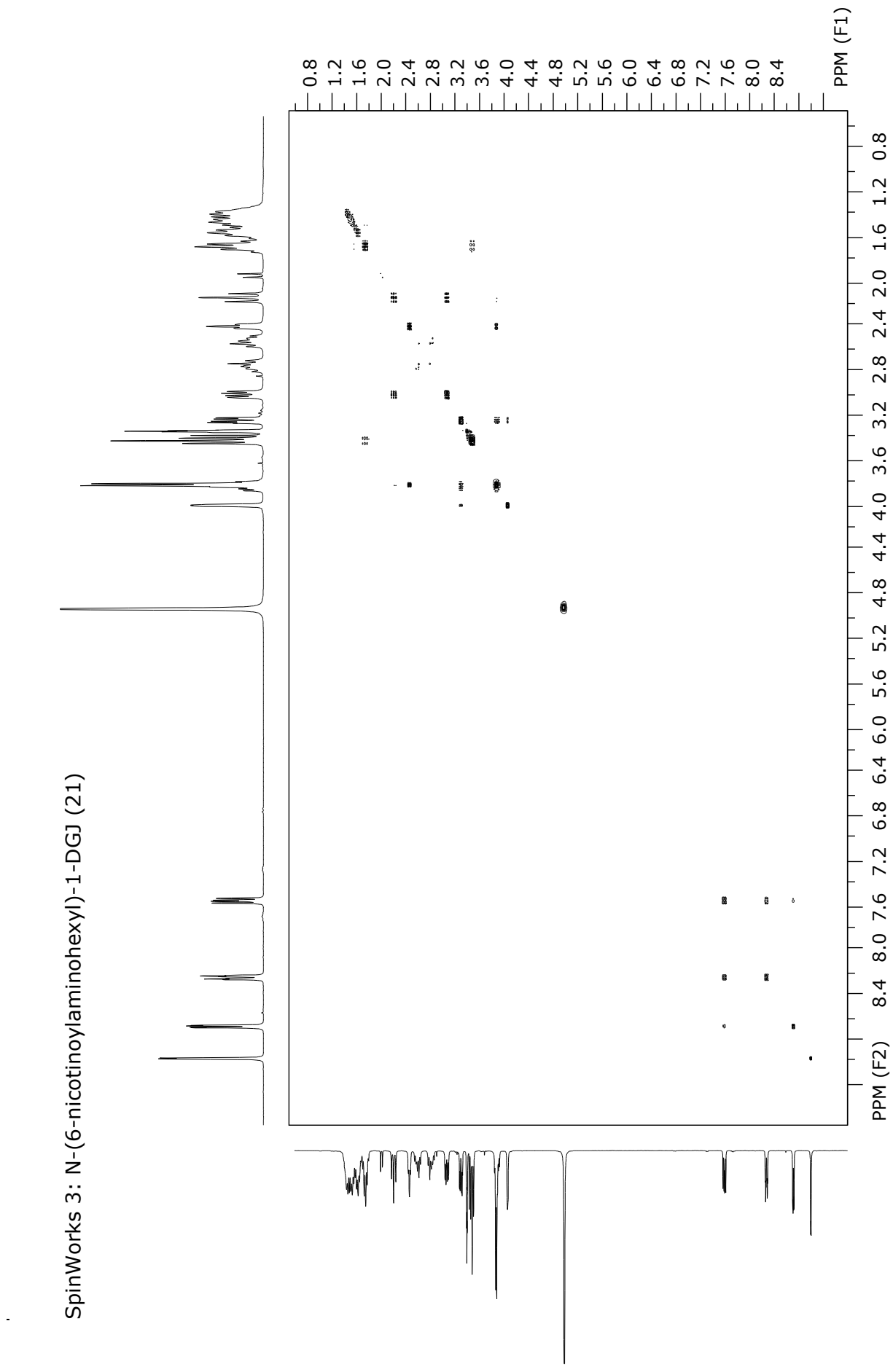
PPM (F1)
PPM (F2)
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4
4.8
5.2
5.6
6.0
6.4
6.8
7.2


SpinWorks 3: N-[6-(4-isopropylbenzoylamino)hexal]-1-DGJ (19)
PPM (F1)
20
30
40
50
60
70
80
90
100
110
120
PPM (F2)
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2


SpinWorks 3: N-[6-(4-dimethylaminobenzoylamino)hexal]-1-DGJ (20)
PPM (F1)
PPM (F2)
1.2
1.6
2.0
2.4
2.8
3.2
3.6
4.0
4.4
4.8
5.2
5.6
6.0
6.4
6.8


SpinWorks 3: N-[6-(4-dimethylaminobenzoylamino)hexyl]-1-DGJ (20)
PPM (F1)
20
30
40
50
60
70
80
90
100
110
120
PPM (F2)
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2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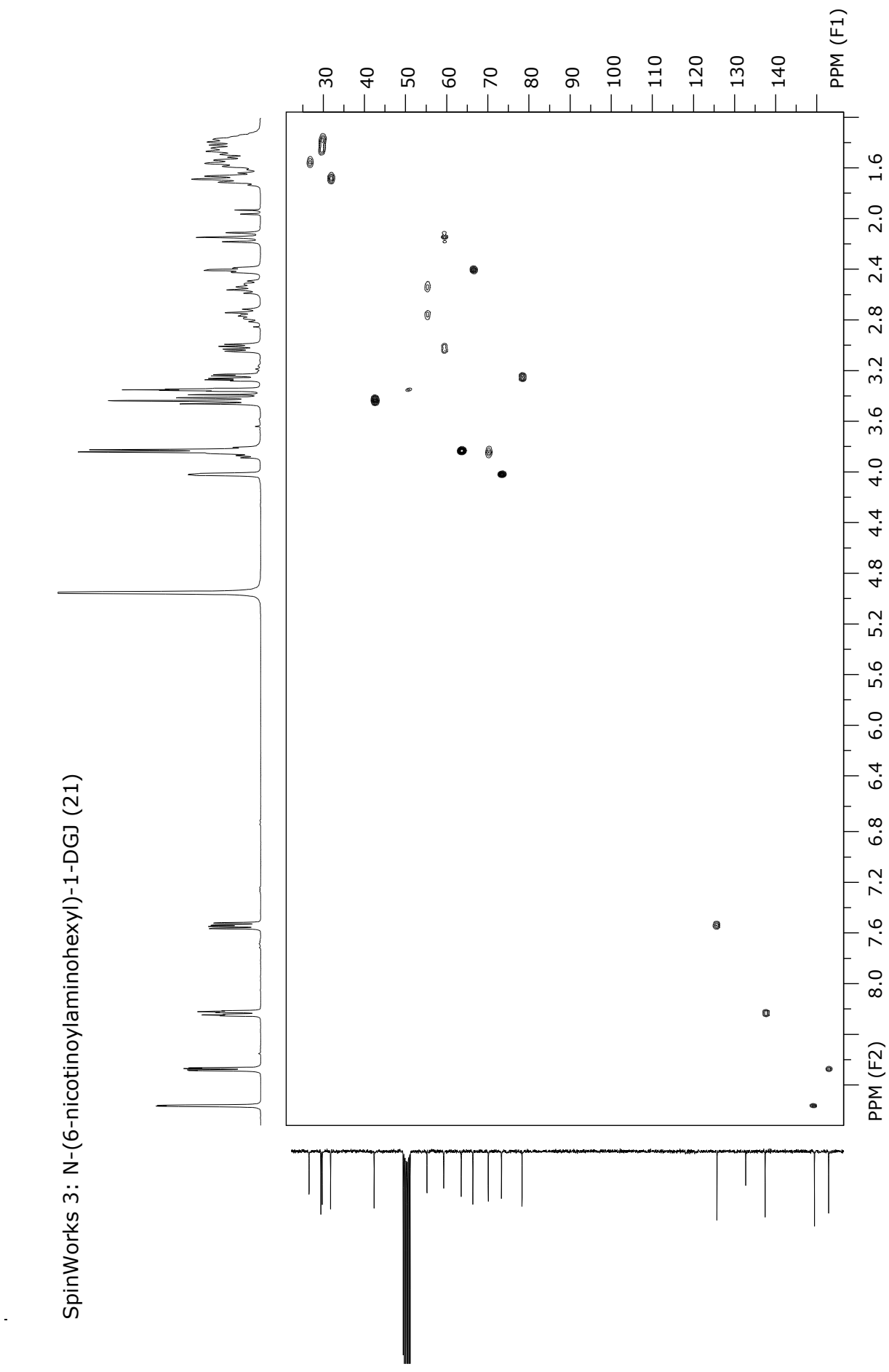
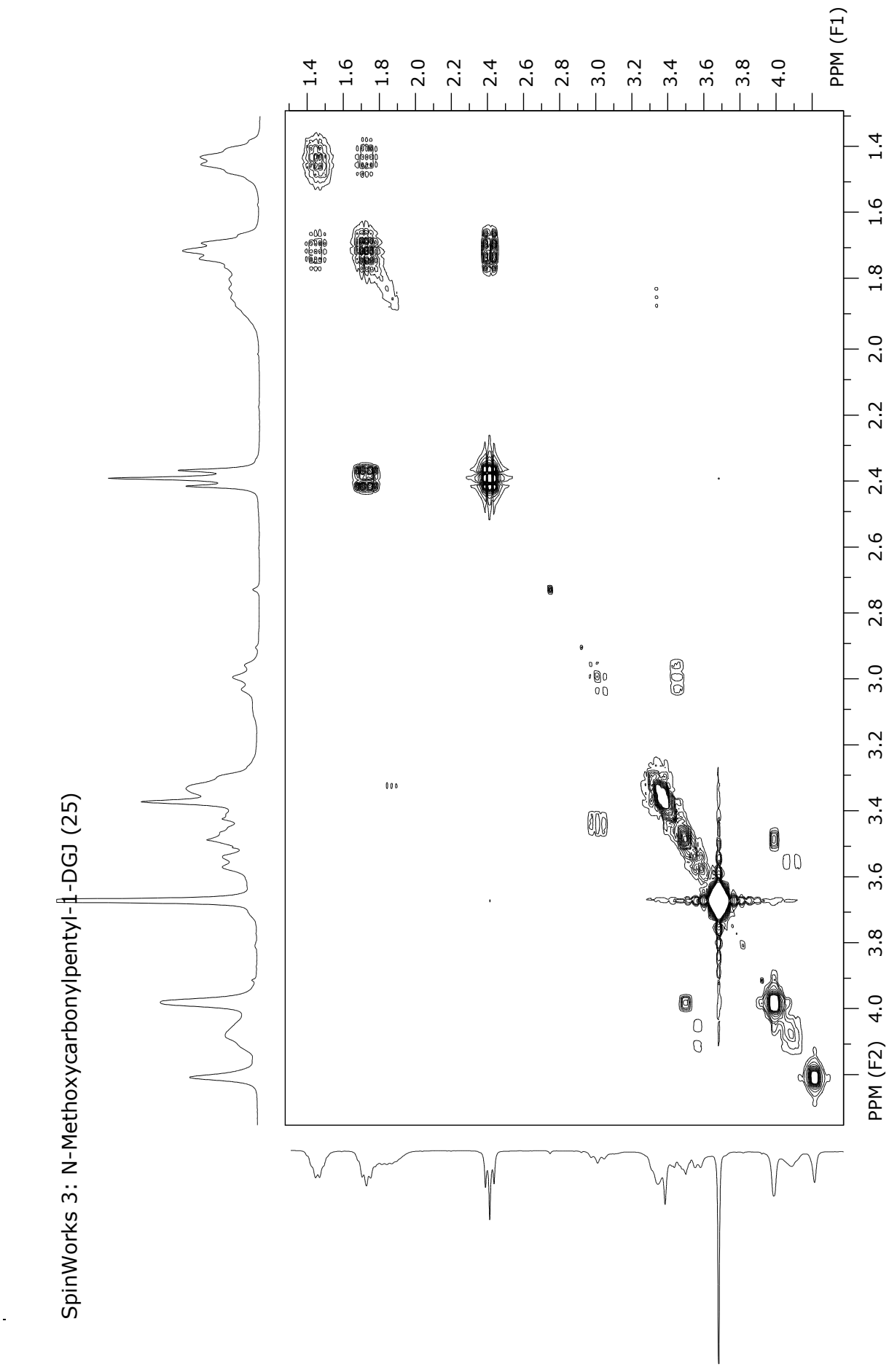
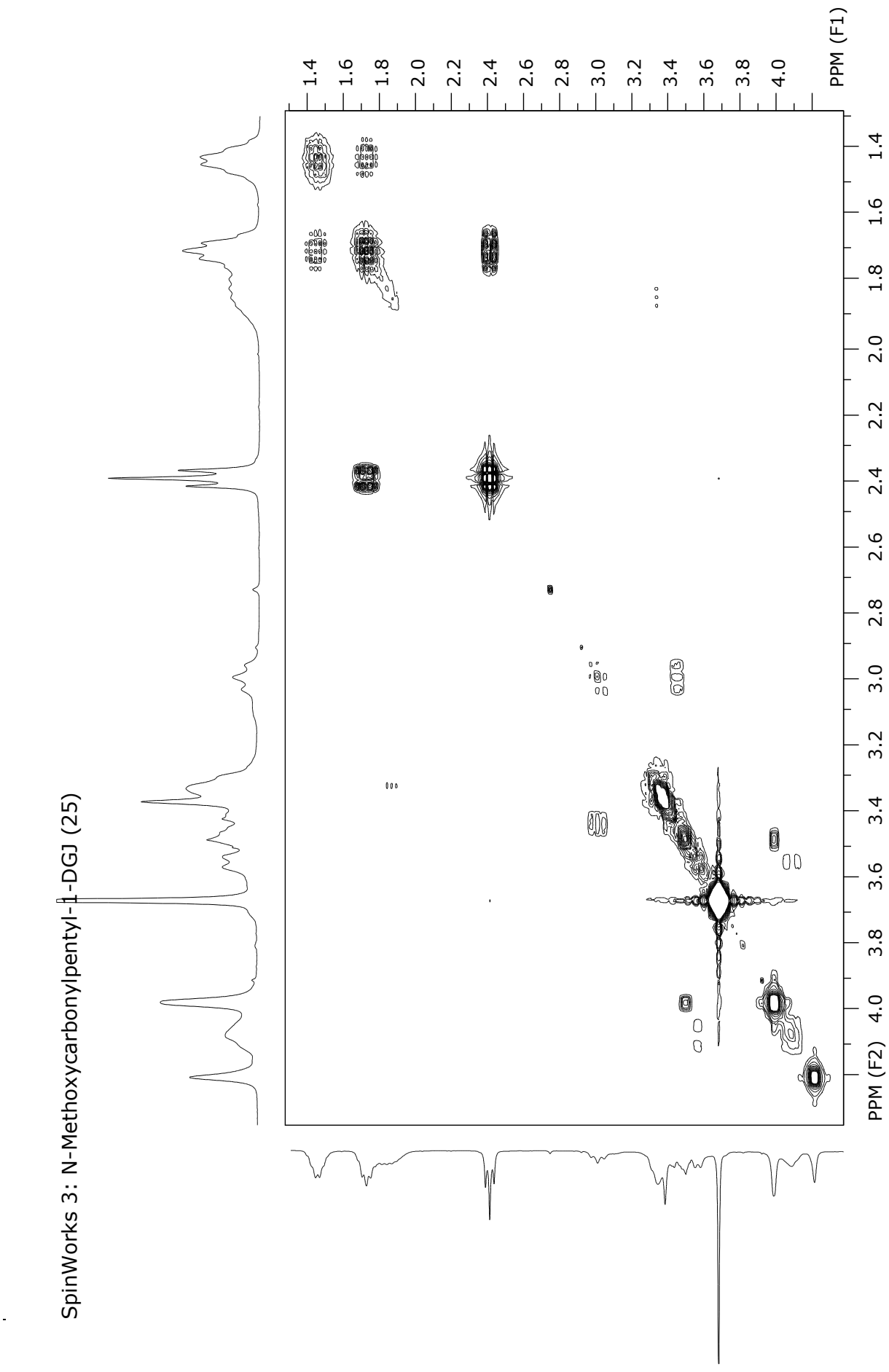
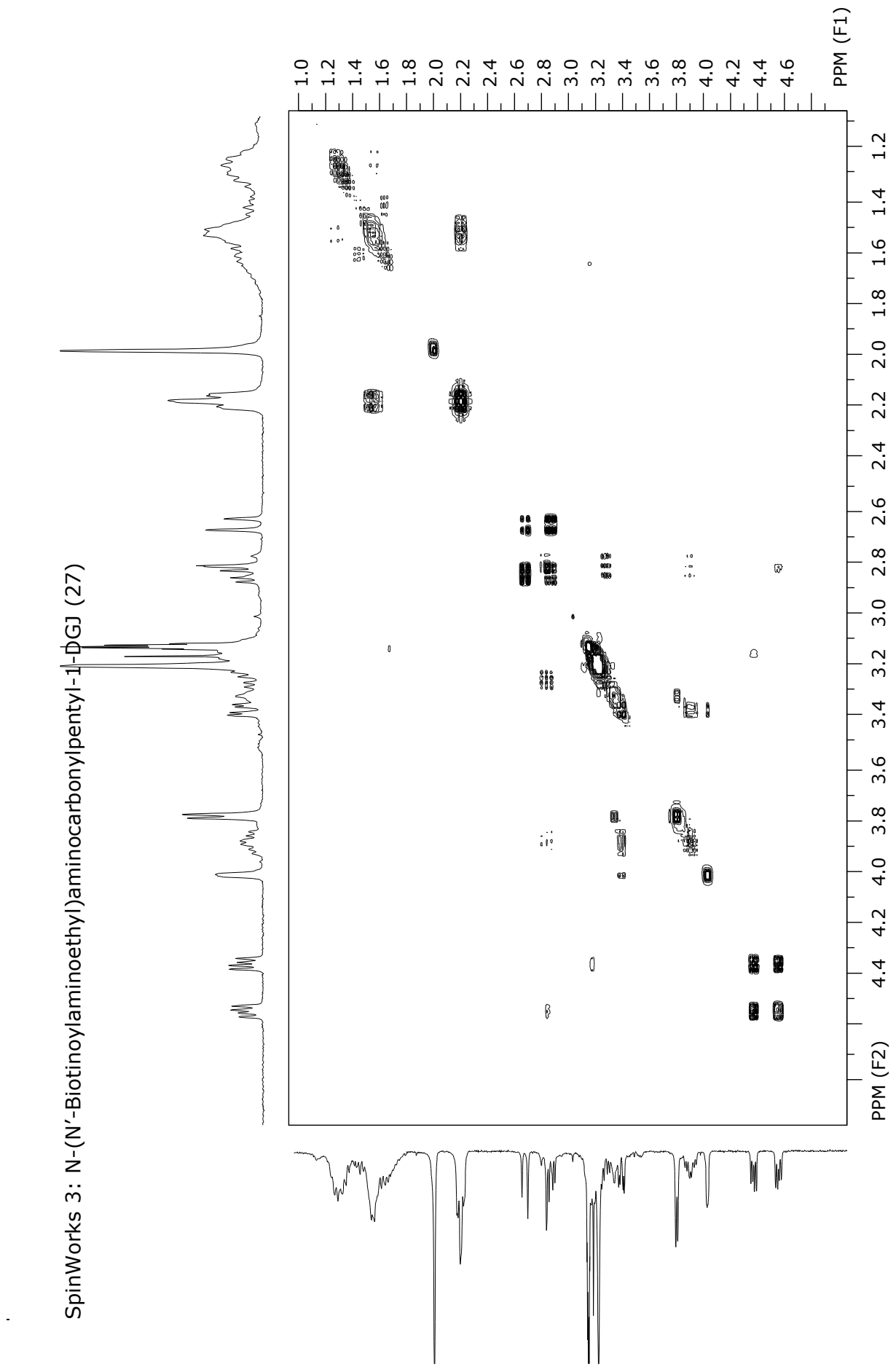
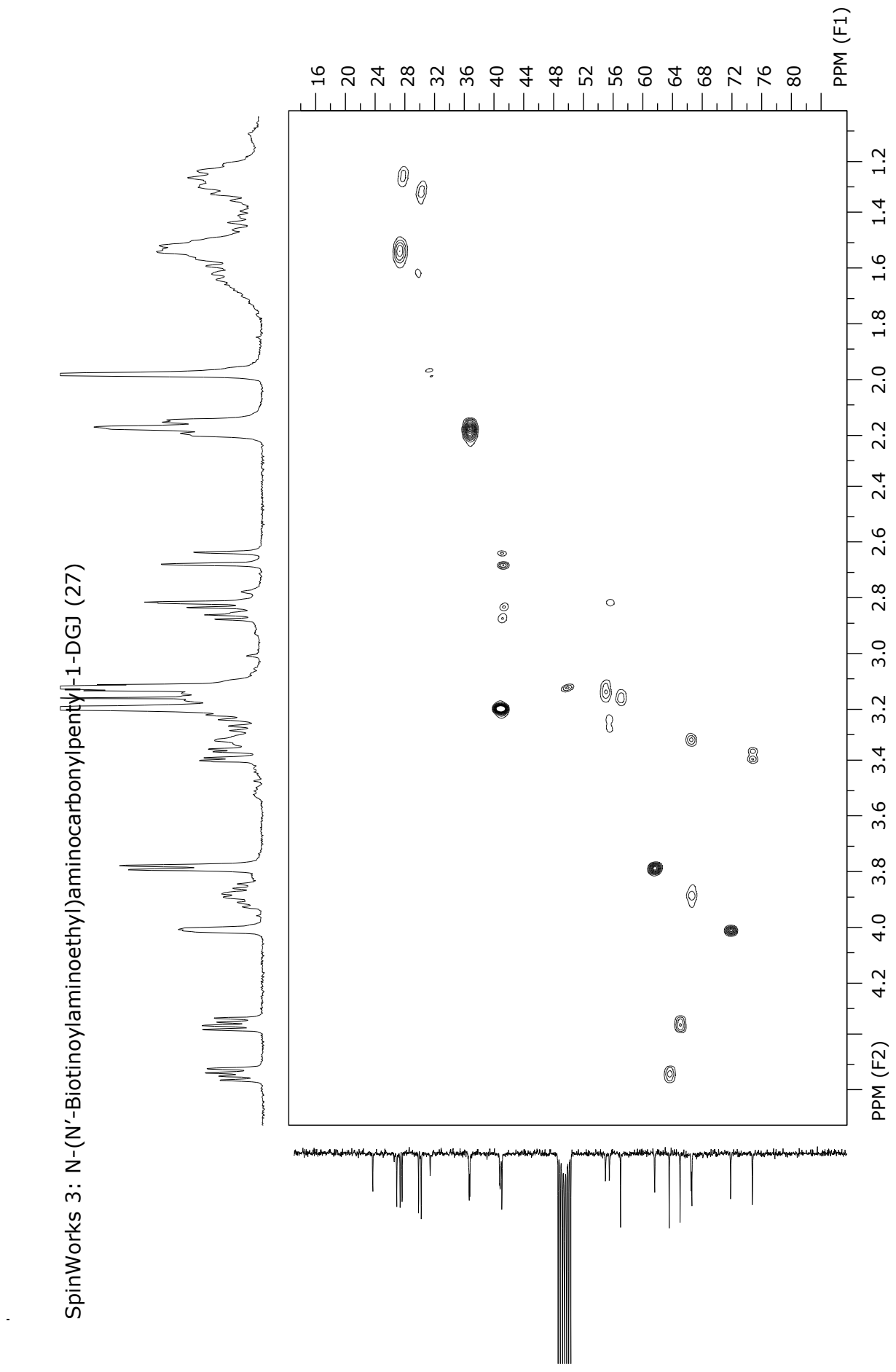
PPM (F1)
0.8 1.2 1.6 2.0 2.4 2.8 3.2 3.6 4.0 4.4 4.8 5.2 5.6 6.0 6.4 6.8 7.2 7.6 8.0 8.4
PPM (F2)
8.4 8.0 7.6 7.2 6.8 6.4 6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8

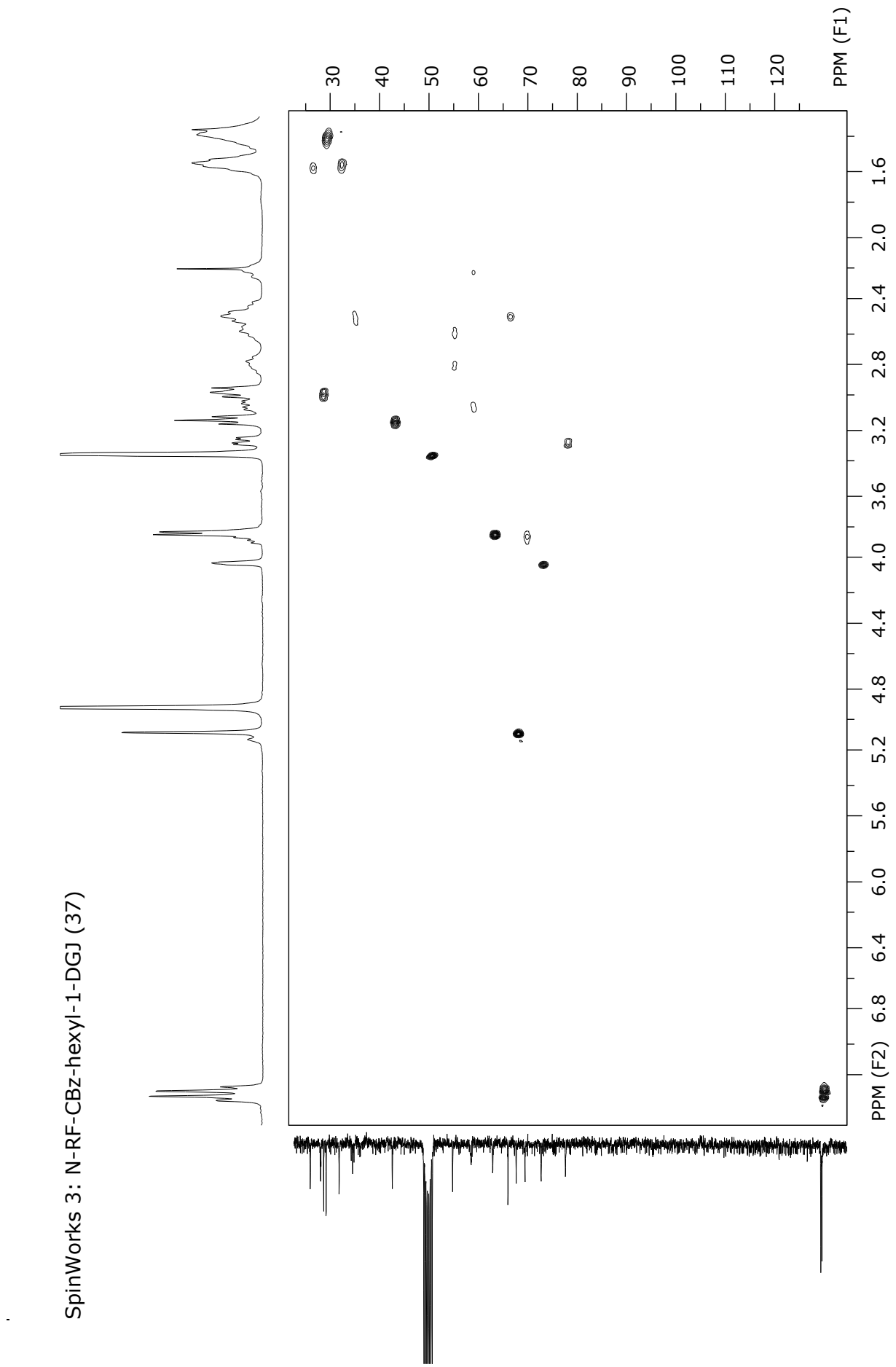

SpinWorks 3: N-(6-nicotinoylaminohexyl)-1-DGJ (21)
PPM (F1)
30
40
50
60
70
80
90
100
110
120
130
140
PPM (F2)
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6

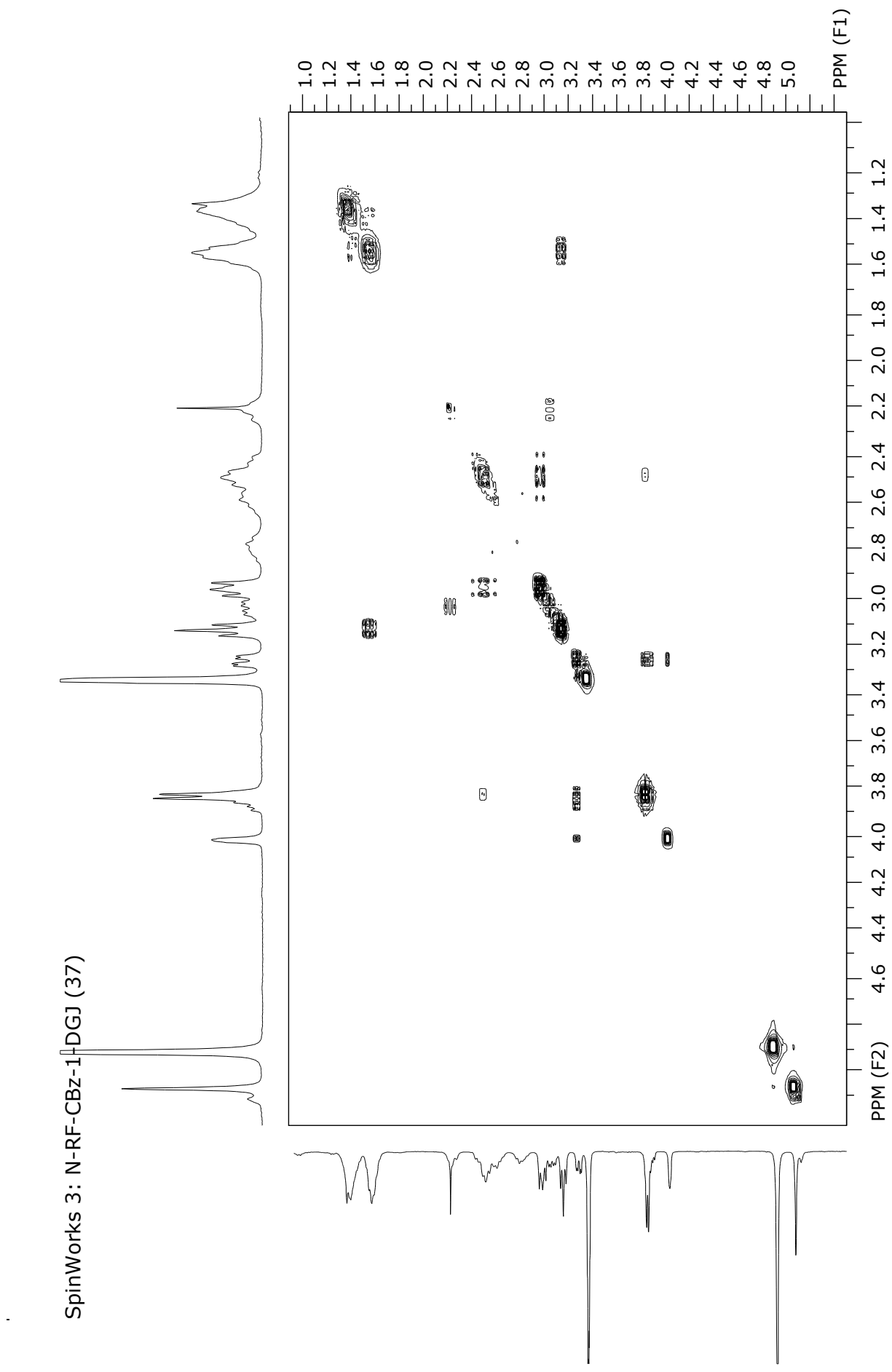

SpinWorks 3: N-Methoxycarbonylpentyl-1-DGJ (25)
PPM (F1)
PPM (F2)
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0


SpinWorks 3: N-Methoxycarbonylpentyl-1-DGJ (25)
PPM (F1)
PPM (F2)
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0


SpinWorks 3: N-(N'-Biotinoylaminoethyl)aminocarbonylpentyl-1-DGJ (27)
PPM (F1)
PPM (F2)
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6


SpinWorks 3: N-(N'-Biotinoylaminoethyl)aminocarbonylpentyl-1-DGJ (27)
PPM (F1)
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76
80
PPM (F2)
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2


SpinWorks 3: N-RF-CBz-hexyl-1-DGJ (37)
PPM (F1)
30
40
50
60
70
80
90
100
110
120
PPM (F2)
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6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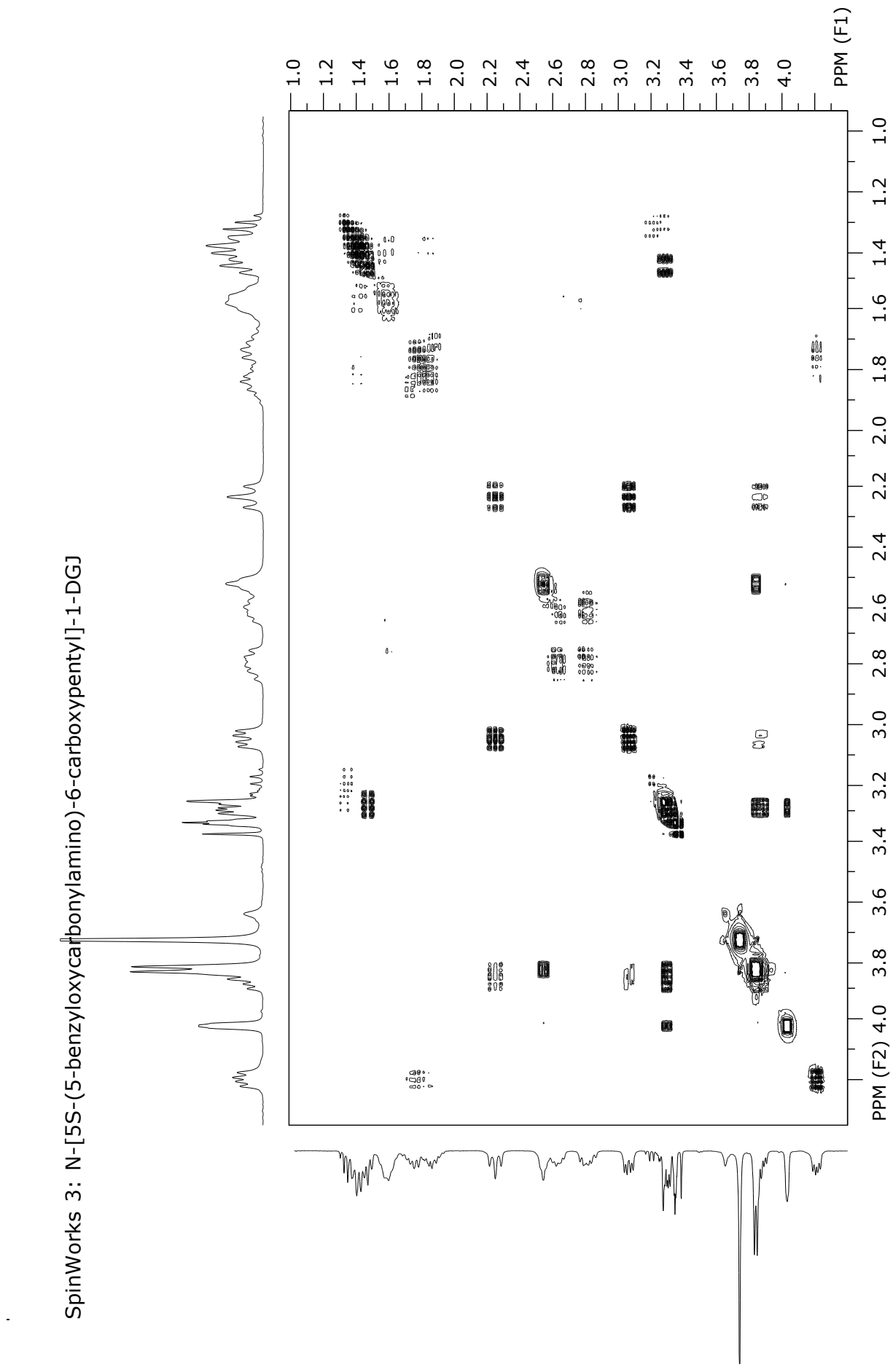
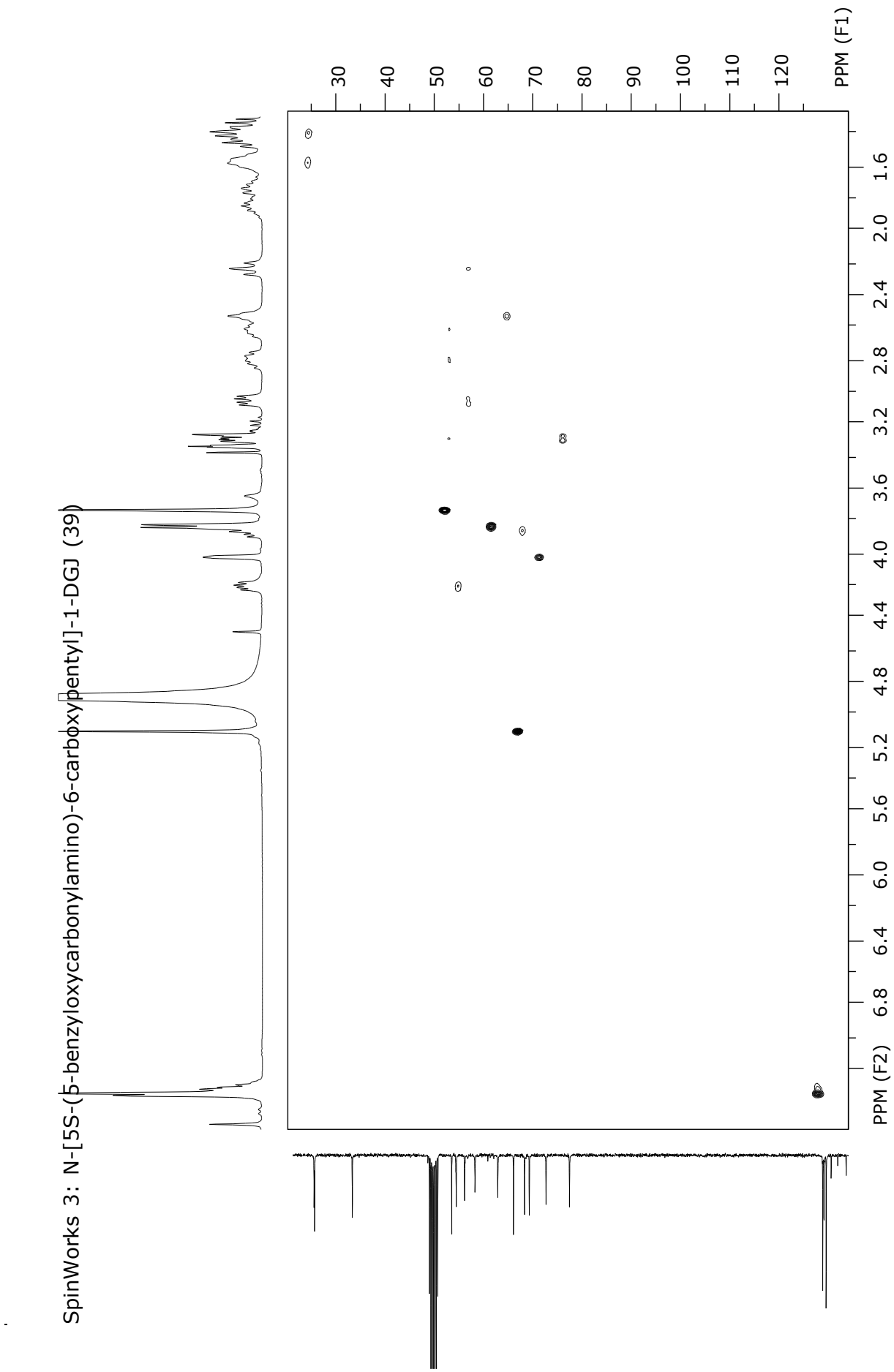
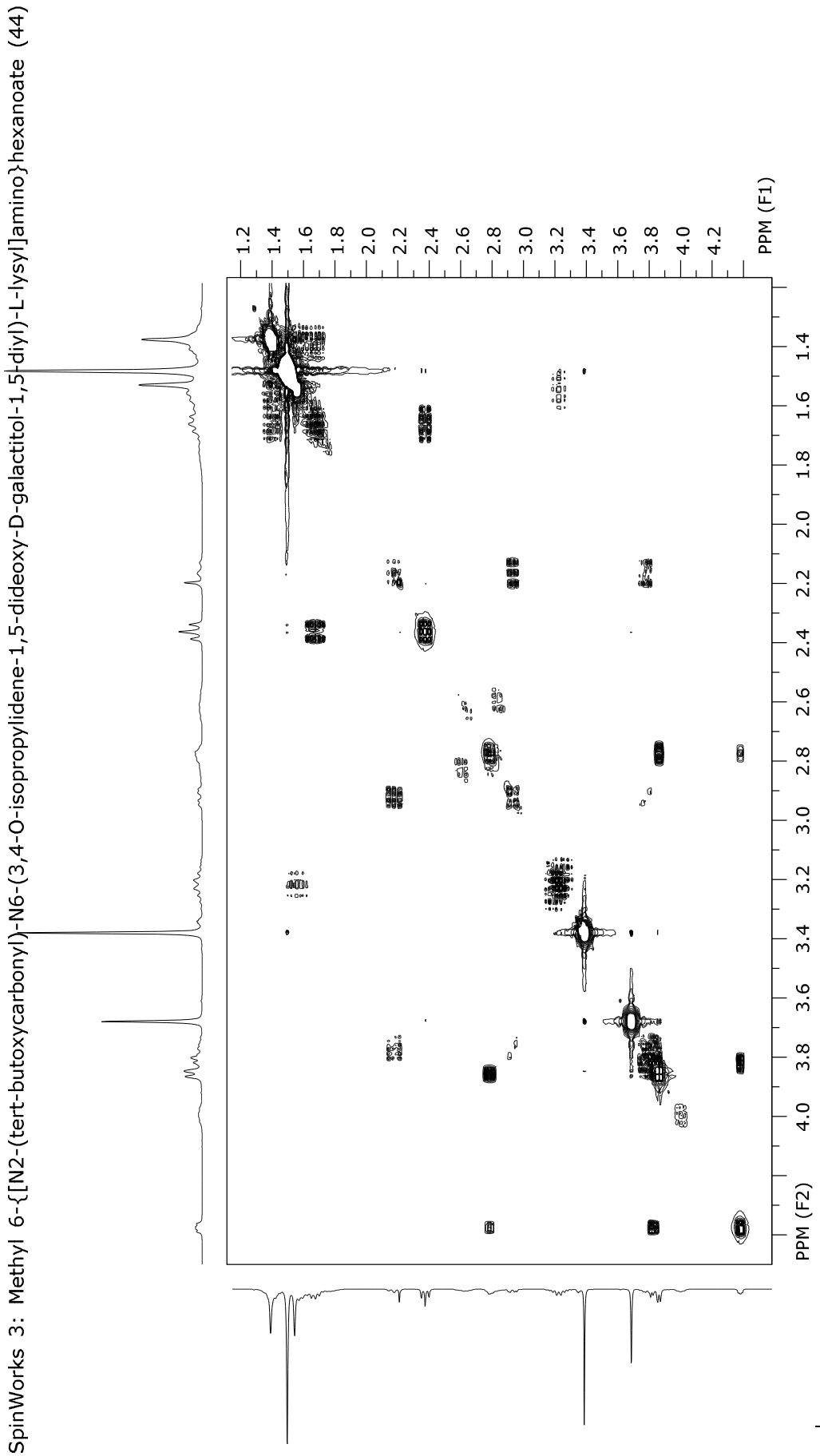
PPM (F2)


SpinWorks 3: N-[5S-(5-benzyloxycarbonylamino)-6-carboxypentyl]-1-DGJ
PPM (F1)
PPM (F2)
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0


SpinWorks 3: N-[5S-(5-benzyloxycarbonylamino)-6-carboxypentyl]-1-DGJ (39)
PPM (F1)
30
40
50
60
70
80
90
100
110
120
PPM (F2)
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6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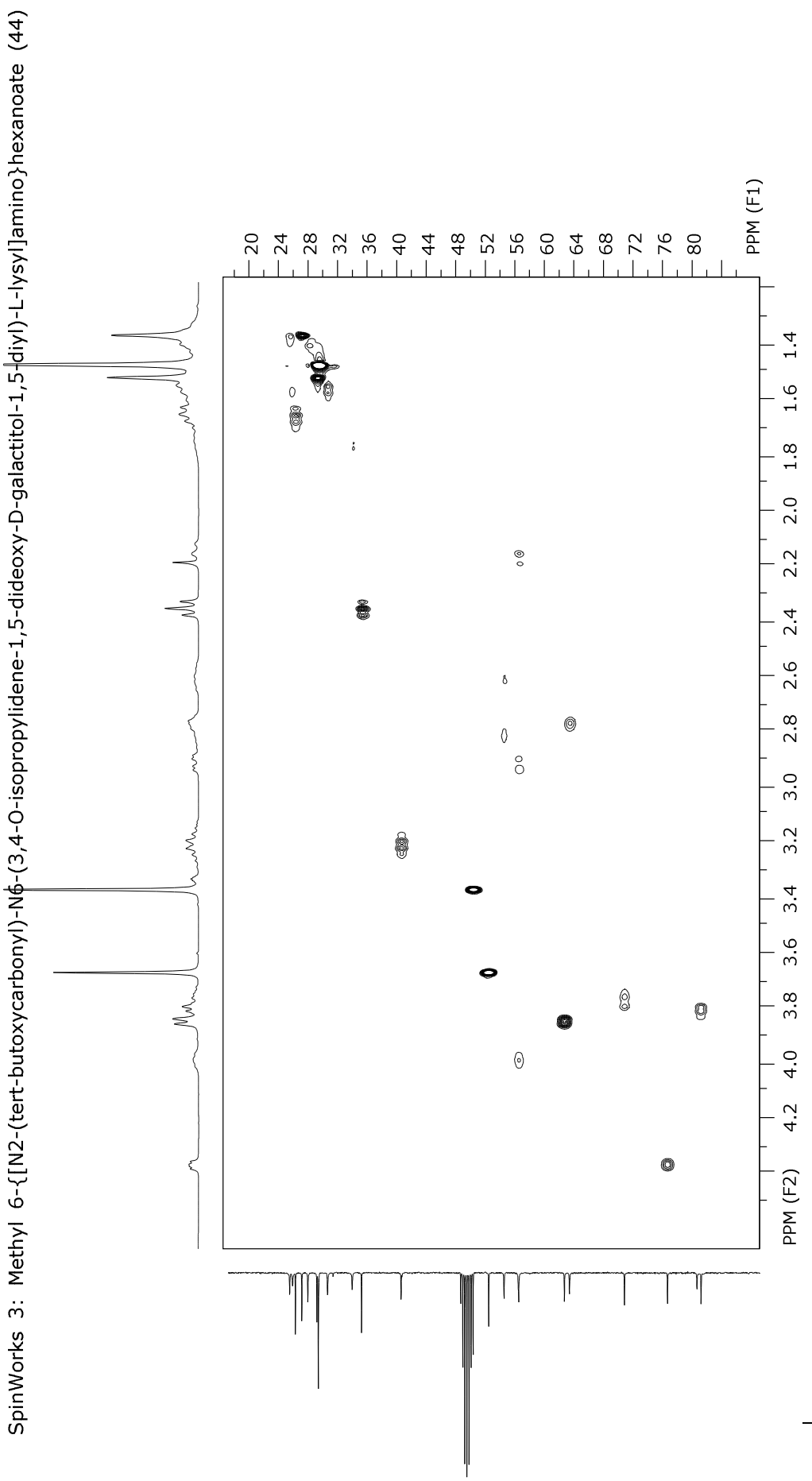
PPM (F1)
PPM (F2)
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2


SpinWorks 3: Methyl 6-{[N2-(tert-butoxycarbonyl)-N6-(3,4-O-isopropylidene-1,5-dideoxy-D-galactitol-1,5-diyl)-L-lysyl]amino}hexanoate (44)
PPM (F1)
PPM (F2)

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8 Curriculum vitae

Georg Schitter

Born March 25, 1981 in Graz, Austria

georg.schitter@telering.at

Education

Aug 2007 - present	PhD thesis: ***„Pharmaceutical Chaperones for GM1-Gangliosidosis and Morbus Morquio B: Synthesis of Lipophilic and Fluorophilic 1-Deoxygalactonojirimycin Derivatives”***, GlycoGroup, Institute of Organic Chemistry, Graz University of Technology, advisor: Prof. Dr. Arnold E. Stütz.
Dec 2005 - Jul. 2007	Scientific co-worker, “*Pharmaceutical Engineering”* with Prof. Dr. Johannes Khinast, Graz University of Technology, working on surface chemistry of silicon.
Oct 20, 2005	2nd Diploma exam - degree “Diplom-Ingenieur” received.
Jan 2005 - Oct 2005	Diploma thesis: ***"Enantiopure Alditols from meso-Sugars: Classical versus Enzymatical Methods"***, GlycoGroup, Institute of Organic Chemistry, Graz University of Technolgy, advisor: Prof. Dr. Arnold E. Stütz.
Oct 2000 - Oct 2005	Undergraduate studies, Technical Chemistry, Graz University of Technology.
Jan 2000 - Sept 2000	Military Service, AufklB 1, Gratkorn.
Jul 1999	University Entrance Certificate, BG Rein.

9 Publications

9.1 Articles in peer-reviewed journals

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