Versatile Diamondoids
Applications in Bioorganic Chemistry

PROEFSCHRIFT

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‘If we knew what we were doing it wouldn’t be research.’

Einstein, Albert

‘Probeer geen troep te maken want dat krijg je er gratis bij.’

OKL
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## Abbreviations

- **Δ** heated to reflux
- **δ** chemical shift
- **Å** ångström
- **Ac₂O** acetic anhydride
- **iBu** isobutyl
- **nBuLi** n-butyllithium
- **tBu** tert-butyl
- **J** coupling constant
- **p-TsCl** 4-toluenesulfonyl chloride
- **p-TsOH** 4-toluenesulfonic acid
- **R<sub>F</sub>** retardation factor
- **A** adenosine
- **Ac** acetyl
- **AcOH** acetic acid
- **Ada** adamantane
- **AIDS** acquired immune deficiency syndrome
- **aq** aqueous
- **BMT** bone marrow transplantation
- **Bn** benzyl
- **BOB** benzo[d]1,2,3-triazol-1-yl)tris(dimethylamino)phosphonium hexafluorophosphate
- **Bz** benzoyle
- **C** cytidine
- **calc** calculated
- **cat** catalytic
- **CE** cyanoethyl
- **cer** ceramide
- **CoA** coenzyme A
- **COSY** correlation spectroscopy
- **CSA** camphorsulfonic acid
- **CV** column volume
- **CVD** chemical vapor deposition
- **d** doublet
- **DCA** dichloroacetic acid
- **DCI** 4,5-dicyanoimidazole
- **dd** doublet of doublet
- **Dia** diamantane
- **DIBALH** diisobutyl aluminium hydride
- **DiPEA** diisopropylethylamine
- **DMAP** 4-dimethylaminopyridine
- **DMF** N,N-dimethylformamide
- **DMP** Dess-Martin periodinane
- **DMSO** dimethylsulfoxide
- **DMTr** 4,4’-dime-thoxy trityl
- **DNA** deoxyribonucleic acid
- **DNJ** 1-deoxynojirimycin
- **dT** deoxythymidine
- **e.g.** *exempli gratia*, for example
- **EDC** 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide
- **eq** (molar) equivalents
- **ER** endoplasmic reticulum
- **Et** ethyl
- **EtOAc** ethyl acetate
- **EtOH** ethanol
- **FCC** flash column chromatography
- **FDA** food and drug administration
- **G** guanosine
Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<td>g</td>
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<td>ON</td>
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</tr>
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<td>SPEM</td>
<td>solution-phase extraction method</td>
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<td>substrate reduction therapy</td>
</tr>
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<td>tetrabutylammonium iodide</td>
</tr>
<tr>
<td>TBG</td>
<td>2,3,4,6-tetra-O-benzyl-D-glucose</td>
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<tr>
<td>TBS</td>
<td>tert butylsilyl</td>
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<tr>
<td>TCEP</td>
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</tr>
<tr>
<td>TEAA</td>
<td>triethylammonium acetate</td>
</tr>
<tr>
<td>TEDT</td>
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<tr>
<td>TES</td>
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<td>TFA</td>
<td>trifluoroacetic acid</td>
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<tr>
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<td>tetrahydrofuran</td>
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<tr>
<td>TLC</td>
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<tr>
<td>TMS</td>
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<tr>
<td>Tof</td>
<td>time of flight</td>
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<td>Tol</td>
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<td>Trt</td>
<td>trityl</td>
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<tr>
<td>U</td>
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General introduction

Diamonds are forever. This is certainly not true for the Jupiter-size diamond found orbiting a pulsar.\(^1\) This gigantic astronomical diamond is the remainder of a white dwarf, and consists mostly of crystallized carbon. Diamonds can be found across the universe, from natural oil deposits on earth to asteroids afloat in distant star systems. The largest diamond ever found on earth is known as the 530-carat Star of Africa which resides in the Crown Jewels of the United Kingdom. The large colour variation found in natural diamonds are caused by metallic contaminations embedded in their structure. A beautiful example is the 46-carat Hope diamond which was found in India and contains traces of boron, resulting in a brilliant blue colour.\(^2,3\) These large diamonds are crystals of carbon arranged in a diamond lattice. The architecture of the diamond lattice was proposed as early as 1905\(^4\) and was confirmed in 1913 by Bragg and Bragg using X-ray diffraction analysis.\(^5\)

Figure 1.1: Diamond lattice.\(^4,5\)

Selection of diamondoids superimposed on diamond architecture.
1. General introduction

1.1 Introduction

Diamonds are the hardest natural substance currently known to men and consist of the most abundant interstellar dust forming element, carbon (C). Terrestrial diamonds usually are highly reflective and massive hydrocarbon crystals. These diamonds are formed under extremely high pressures and temperatures, such as found near the centre of the Earth. Besides that diamonds make precious gems, their remarkable properties such as thermal conductivity, dopability and optical transparency over a wide spectral range have led to their exhaustive research and applications. Puzzling are the black diamonds which originate from the cold emptiness of outer space. Such diamonds, better known as carbonado type diamonds, consists of a collection of aggregated crystals rather than a massive one. The unusual high hydrogen content and the presence of unusual elemental isotopes are conformations of the non-terrestrial origin of carbonado type diamonds. These porous hydrocarbon crystals have poor light reflecting properties but otherwise equal or surpass the hardness of natural diamonds (Figure 1.2).

**Figure 1.2:** (Non) terrestrial diamonds.

![Figure 1.2: (Non) terrestrial diamonds.](image)

Left: Selection of raw earth diamonds. Right: Carbonado diamond.

Mankind has been fascinated by diamonds and has speculated over their formation for a long time. Driven by synthetic curiosity coupled to high industrial demands, the quest for methods to synthesize or grow natural diamonds was undertaken. The natural method of terrestrial diamond formation is, to some extend, duplicated in specialized facilities for the production of synthetic diamonds. An account by J.B. Hannay that dates back to 1880 describes the first synthetic attempts to produce diamonds. This first attempt to man made diamonds involved heating of a mixture of hydrocarbons, bone, oil and lithium in sealed wrought iron tubes. This heroic effort was not without danger because of exploding tubes and only three out of the eight tubes

---

*a* Wrought iron is an iron alloy with a very low carbon content.
were intact after the experiment. The identification of the obtained substance seemed very conclusive, since it included a density of 3.5 g/cm$^3$ and a carbon content of 97.85%.$^b$ The quest for man made diamonds was continued by Sir Charles Parson, who tried for nearly thirty years to duplicate the work of Hannay and co-workers.$^{16}$ After several unsuccessful attempts, Parson re-examined all his work on the subject and finally concluded that neither he or anyone else had ever prepared diamonds in the laboratory.$^7$ The first successful and reproducible synthesis of diamonds is reported by R. H. Wentorf $et$ $al.$$^{17}$ who developed a pressure vessel which could operate at pressures up to 100,000 kg/cm$^2$ and temperatures around 2,300 K. With this apparatus in hand, processes were discovered which yielded diamonds, ranging in edge-size from 100 micron to more than 1 mm. The man made diamonds were subjected to several analytical methods including X-ray diffraction, chemical analysis and hardness tests. The developed technique was named a high pressure high temperature technique and proved to be highly reproducible and was repeated independently for more than a hundred times.

Direct transformation of graphite to diamond was first announced by the Stanford Research Institute of Menlo Park, California in 1960, and is accomplished by subjecting graphite to a very high, instantaneous stress developed by explosive shock.$^{18}$ Applying pressures up to 500 kilobars and estimated temperatures of 1000-1500$^\circ$C, black diamond crystals, resembling carbonado-type diamonds were obtained in yields <5%. Another process by which man made diamonds are grown, is known as chemical vapour deposition (CVD). This process can be used to generate single crystals diamonds or poly crystalline diamonds (carbonado type). The process of CVD diamond synthesis is conducted at reduced pressure (1 - 200 Torr)$^{19}$ in the presence of a diamond seedling (for single crystal diamonds) and the starting materials for diamond growth, which are dihydrogen (H$_2$) and methane.$^{20}$ This method is generally applied in the synthesis of diamond films, coatings or to produce diamonds destined for industrial purpose. Today, the need of a diamond expert to distinguish synthetic- from natural diamonds is a testimony of the quality of synthetic diamonds.

**Diamondoids**

The existence of a strain free caged hydrocarbon with the C$_{10}$H$_{16}$ formula and a diamond-like structure has been the subject of discussions for a long time. At a conference in 1924, a synthetic route was suggested by H. Decker and he named the target a decaterpene. Landa and co-workers where the first to isolate this illusive cage compound in 1933 from a petroleum sample originating from Czechoslovakia.$^{21}$ In fact, natural oil deposits can contain up to 200 ppm of diverse diamondoid derivatives and even higher concentration are present in refined oil fractions, which often leads to clogging of the oil pipes. After structural elucidation of the obtained crystalline hydrocarbon, the name adamantane from $\alpha\delta\alpha\mu\zeta$, the Greek word for diamond, was adopted. X-ray and electron diffraction studies revealed that adamantane crystallizes in a face-centered cubic lattice, which is highly unusual for organic compounds. Three fused cyclohexane rings, all in a stable chair conformation, form the interlocking cage structure of adamantane, which can be

$^b$The density of natural diamond is $\sim 3.5$-3.53 g/cm$^3$. 

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1. General introduction

Figure 1.3: A few diamondoids.

Diamondoids from left to right: adamantane, diamantane, triamantane, iso-tetramantane, skew-tetramantane and anti-tetramantane.

superimposed on the diamond lattice (Figure 1.1). Furthermore, all C-C bond lengths are 1.54 ± 0.01 Å with bond angles of 109.5 ± 1.5°.11

Adamantane is a highly lipophilic compound that is readily dissolved in organic solvents, sublimes at 209-212°C and has a melting point of 268°C, in a sealed tube. Diamondoids represents a class of caged hydrocarbons in which adamantane (tricyclo[3.3.1.1]decane, 1) is the first member and can be viewed as a nanometer-sized diamond of ~ 1 ± 10^{-21} karat.22 Soon after the isolation of adamantane, several hydrocarbons with the general formula C_{4n+6}H_{4n+12} were identified. Their structure can also be superimposed on the diamond lattice and a few examples are depicted in Figure 1.3.23 These polymantane homologues are categorized in lower- and higher diamondoids based on their spatial and structural arrangement. Adamantane (C_{10}H_{16}, 1), diamantane (C_{14}H_{20}, 2) and triamantane (C_{18}H_{24}, 3) are referred to as lower diamondoids because only one structural isomer is possible. The higher diamondoids, those with more than three fused adamantane units, can form multiple structural isomers. The three isomeric tetramantanes 4, 5 and 6 are respectively referred to as iso-, skew-, and anti-tetramantane. The number of possible structural- and non-structural isomers increases dramatically for the higher diamondoids. The isomeric complexity of higher diamondoids is evident starting from pentamantane of which there are nine isomers with the formula C_{26}C_{32} and one with the formula C_{25}H_{30}. For hexamantane the situation is even more complex with 39 possible isomers divided over three classes: 28 are C_{30}H_{36} isomers, 10 are C_{29}H_{34} analogues and one is a peri-condensed isomer namely cyclohexamantane C_{26}H_{30}. With several hundreds of possible isomeric octamantane also the additional complexity of chiral and non-chiral isomers exist.2,13,22

1.2 Synthesis of diamondoids

The beautiful architecture of diamondoids (Figure 1.3) has sparked the interest of chemists for many years, since the isolation of minute quantities of adamantane (1) from crude oil sources.10,21 The occurrence of diamondoids in crude oil has led to speculation of their natural formation deep in the earth crust. The natural formation of diamondoids is not understood in depth. However, it is believed that the lower diamondoids are formed through carbocation rearrangements of functionalized petroleum degrades, e.g. polycyclic terpenes, on the surface of natural minerals such as montmorillonite ((NaCa)_{1/3}(AlMg)_{2}(Si_{4}O_{10})(OH)_{2}·H_{2}O). The higher
1.2 Synthesis of diamondoids

**Scheme 1.1:** Adamantane synthesis.\(^{11}\)

\[
\begin{align*}
    \text{CH}_3\text{O}_2\text{C} & \quad \text{CO}_2\text{CH}_3 \quad \text{i}\quad \text{CH}_3\text{O}_2\text{C} \quad \text{CO}_2\text{CH}_3 \quad \text{ii}\quad \text{CH}_3\text{O}_2\text{C} \quad \text{CO}_2\text{CH}_3
\end{align*}
\]

Reagents and conditions: i) various conditions, 0.16% - 6.5%; ii) H\(_2\), PtO\(_2\), Et\(_2\)O, quant.; iii) 10 wt% AlCl\(_3\), 20-50%.

Diamondoids can be formed via homologation of the lower adamantologues at high pressure and temperature in the natural underground oil and gas reservoirs. Most crude oil components start to decompose at temperatures above 200°C to produce ultimately methane gas and graphite.\(^6,22,24\) Up to the 1950’s, the total synthesis of adamantane, starting from Meerwein’s ester\(^{25}\) (7), has been reported several times (Scheme 1.1), albeit the overall yields did not exceed a few per cents.\(^{11}\) During studies directed to the AlCl\(_3\) mediated \textit{endo} \rightleftharpoons \textit{exo} isomerisation of trimethyle-nenorbornane (8), Schleyer and co-workers observed the formation of a white crystalline solid.\(^8\) This crystalline compound proved to be adamantane (1) and after optimisation of the reaction condition the overall yield of this serendipitous discovery was increased to \(\sim 50\%\). The mechanism of AlCl\(_3\) catalyzed isomerisation of 8 \(\rightarrow\) 1 has been the subject of much speculation.\(^{26-28}\) The process is believed to be a thermodynamically controlled carbocation rearrangement, proceeding via multiple 1,2-carbon bond- and hydride shifts. Whitlock and Siefken used a general mathematical model and calculated that there are 2897 possible routes towards the product. The most direct route for the 8 \(\rightarrow\) 1 isomerisation involves five intermediates interconversions via 1,2-shifts only.\(^{29-32}\) Currently, adamantane can be obtained from several suppliers for as low as 1 €/gram.

**Scheme 1.2:** Diamantane synthesis.\(^{33,34}\)

Reagents and conditions: i) \(h\nu\); ii) 33 wt% AlCl\(_3\), \(\sim 1\%\); iii) CoBr\(_2\) \(\cdot\) 2 PPh\(_3\), BF\(_3\) \(\cdot\) Et\(_3\)O, toluene, 82-85%; iv) H\(_2\), PtO\(_2\), HCl, AcOH, 70°C, 90-94%; v) AlBr\(_3\), C\(_6\)H\(_{12}\), 60-62%.
1. General introduction

The Lewis acid promoted carbocation rearrangement pathway for adamantane (1) synthesis proved to be an effective method for the synthesis of the remaining lower diamondoids 2 and 3. The structure of diamantane (2) represented the emblem of the XIXth International Congress of Pure and Applied Chemistry in London in 1963, and was synthesized two years later by the group of Schleyer. The AlCl₃ catalysed rearrangement of norbornene (10) photodimerized product 11 produced a tar like mixture containing 1% of diamantane (2) (Scheme 1.2). A higher yielding route for the preparation of diamantane was reported by the same group, starting with a [4+4] cycloaddition of norbornadiene (12). The resulting polycyclic product, known as Binor-S (13) was hydrogenated over Adams catalyst and subsequently subjected to AlBr₃ isomerisation to furnish diamantane (2) in ~50% overall yield. The mechanism concerning the AlBr₃ rearrangement of 14 → 2 is not clear, but it has been estimated that at least 40,000 pentacyclotetradecanes are involved.

The last member of the lower diamondoids, triamantane (3), was synthesized via a similar approach as applied for diamantane (2). Initially, Schleyer et al. reported on the use of cyclooctatetraene (15) dimerized product 16 as a precursor in the synthesis of 3 (Scheme 1.3). The structural basis of triamantane was constructed via a Simmons-Smith cyclopropanation of the double bond of 16, followed by catalytic hydrogenation of 17. Subsequent AlCl₃ catalysed carbocation rearrangement of 17 → 3 was sluggish and gave only trace amounts (~1%) of triamantane (3).

**Scheme 1.3: Triamantane synthesis.**

\[\text{Reagents and conditions: } i) \text{ several methods; } ii) \text{ CH}_2\text{I}_2, \text{Zn(Cu); } iii) \text{ H}_2, \text{Pd; b. AlBr}_3, \text{tBuBr, CS}_2; \text{ vi) AgClO}_4, \text{benzene, 85°C, 64%; } v) \text{ butadiene, 160°C, 70%; vi) PtO}_2, \text{H}_2, \text{CH}_2\text{Cl}_2, \text{quant.; vii) AlCl}_3, \text{C}_8\text{H}_{12}, \text{60%}.\]
1.3 Functionalisation of diamondoids

The ease in which Binor-S (13) is converted into diamantane 2 led to renewed interest in its use as a precursor to build the triamantane framework. Indeed, silver perchlorate induced rearrangement of Binor-S provided intermediate olefins 18 and 19 in a near 1:1 mixture, in good yields. Although the obtained mixture of 18 and 19 are separable by distillation, this is not necessary due to the fact that they can both be converted to triamantane. Subsequent [4+2] cycloaddition with butadiene gave polycyclic intermediate 20 and 21, which upon hydrogenation over Adams catalyst yielded dihydro derivatives 22 and 23. Carbocation rearrangement of intermediates 22 and 23 in cyclohexane under the agency of AlCl₃ provided triamantane (3) in an overall yield of 27% from Binor-S (13).³⁷

The carbocation rearrangement method for the synthesis of the lower diamondoids has proven very effective and resulted in the preparation of the lower diamondoids in good yields. Unfortunately, because of the multiple structural isomers of the higher diamondoids, the carbocation rearrangement approach is not effective for their synthesis. Several synthetic efforts have been made towards the higher diamondoids.³⁹,⁴⁰ Their synthesis is often complex and entails numerous steps. Only minute amounts of e.g. anti-tetramantane was isolated by double homologation of functionalized diamantane derivatives.⁴¹

1.3 Functionalisation of diamondoids

The availability of large amounts of the lower diamondoids ada-, dia- and triamantane (1, 2 and 3) by a Lewis acid promoted carbocation rearrangement of a hydrocarbon framework, paved the way for their derivatization. Aliphatic substituted diamondoid derivatives can be obtained by carbocation rearrangement of a proper hydrocarbon precursor⁴² or by transformation of Meerwein’s ester (7).⁴³ The highly symmetrical architecture of the diamond lattice by which diamondoids are constructed, complicates their functionalization. There are several methods known to functionalize the diamondoid scaffold, such as radical, ionic, and oxidative transformations.¹¹,³⁹,⁴⁴–⁴⁹ It is beyond the scope of this Thesis to provide a comprehensive overview on the derivatization of the diamondoid skeleton. Instead, a selection of derivatization strategies regarding the lower diamondoids is discussed, involving ionic bridgehead functionalizations and oxidation of the bridging methylene group.

Ionic bridgehead bromination

Adamantane (1) is a highly symmetrical compound possessing tetrahedral rotational symmetry with reflection symmetry (Td symmetry).⁴⁵,⁵¹ As a result, adamantane (1) contains four equivalent bridgehead carbons (C-1,3,5,7) with a 1,3-relationship to each other and six equivalent methylene groups (Scheme 1.4). One of the earliest reported modifications of the adamantane framework is the ionic bromination in neat Br₂ (Scheme 1.4). It was observed that adamantane reacts with bromine at ambient temperatures to produce exclusively 1-bromoadamantane (24). Bromination of the remaining bridgeheads of 1-bromoadamantane becomes increasingly more difficult. These observations support an ionic halogenation mechanism for adamantane, involving a negatively charged halogen and an 1-adamantyl cation. The involvement of an
1-adamantyl cation is further supported by the catalytic action of strong Lewis acids on the halogenation reactions.\textsuperscript{50} For instance, monobromination of adamantane proceeds efficient at ambient temperature in neat bromine, whereas elevated temperatures are required to prepare 1,3-dibromoadamantane (25). The addition of a Lewis acid (BBr\(_3\) or AlBr\(_3\)) and elevated temperatures are required to obtain 1,3,5-tribromoadamantane (26) and 1,3,5,7-tetrabromoadamantane (27).

**Diamantane (2)** has a lower (D\(_3d\)) symmetry compared to adamantane (1) and contains two types of bridgehead carbons, namely, two apical- and six medial bridgehead carbons and six equivalent methylene groups. The different bridgehead types in diamantane renders the ionic bromination a more complicated process with respect to adamantane (Scheme 1.5). The medial bridgehead carbons of diamantane are more susceptible to ionic bromination compared to adamantane.\textsuperscript{51–53} In general, polybromination of diamantane is, as in the case of adamantane, controlled by inductive effect of the installed bromide. Subsequent bromination of 28 occurs at a position most removed from the first bromide, with respect to the inherent reactivity difference between the medial- and apical bridgeheads. In addition, there is a 3:1 statistical advantage for medial over apical attack in the ionic bromination of diamantane. As a consequence, selectivity in the ionic bromination of diamantane is difficult to achieve except in the formation of 1-bromodiamantane.

1-bromodiamantane (28) can be obtained by reaction of diamantane (2) in neat bromine for two hours. Prolonged exposure to liquid Br\(_2\) at elevated temperatures gives bromide 28 and the mixture of dibromides 1,6-dibromodiamantane (29) and 1,4-dibromodiamantane (30). Surprisingly, the 1,6- and 1,4-dibromodiamantane isomers are separable by crystallisation from hexane or by alumina column chromatography, where 1,6-dibromodiamantane (29) eludes first with hexane.\textsuperscript{54} Bromination of 2 with Br\(_2\) in the presence of excess tert-butyl bromide and catalytic AlBr\(_3\) results in the formation of a near 1:1 mixture of medial and apical bromides 28

**Scheme 1.4:** Adamantane bridgehead bromination.\textsuperscript{45,50}

\[
\begin{align*}
\text{24} & \xrightarrow{i} \text{1} & \xrightarrow{ii} \text{25} & \xrightarrow{iii} \text{26} & \xrightarrow{iv} \text{27}
\end{align*}
\]

**Reagents and conditions:** i) Br\(_2\), RT, 95%; ii) Br\(_2\), \(\Delta\), 79%; iii) AlBr\(_3\) – Br\(_2\) or BBr\(_3\) – Br\(_2\), \(\Delta\), 80%; iv) AlBr\(_3\) – Br\(_2\), 140°C, sealed tube, 75%.
1.3 Functionalisation of diamondoids

**Scheme 1.5:** Diamantane bridgehead bromination.\(^{54}\)

Reagents and conditions: i) Br\(_2\), RT, 2 h, 80%; ii) Br\(_2\), Δ, 28, 19%; 29, 48%; 30, 8%; iii) 100 wt% tBuBr, 5wt% AlBr\(_3\), Br\(_2\), 0°C, 28, 40%; 31, 58%; iv) 10 wt% AlBr\(_3\), Br\(_2\), 0°C, 29, 6%; 30, 38%; 32, 48%; v) excess tributyltin hydride.

and 31, respectively. When diamantane (2) is reacted at 0°C, in the presence of 10 wt% AlBr\(_3\), a mixture of bis-medial dibromide 29, 1,4-dibromodiamantane (30) and bis-apical dibromide 33 are obtained.\(^{54}\) A selective bis-apical bromination of 2 was reported in 2006,\(^{55}\) applying Br\(_2\) in Freon 113 in the presence of Fe powder. In this way, 4,9-dibromodiamantane (32) was isolated in 62% yield. Surprisingly, 4-bromodiamantane (31) can be obtained by the selective reduction of 4,9-dibromodiamantane (32) with tributyltin hydride.\(^{52,54}\) The preparation of polybromodiamantane derivatives from 2 with Br\(_2\) requires the addition of (excess) AlBr\(_3\) and elevated temperatures.

**Scheme 1.6:** Triamantane bridgehead bromination.\(^{48,56}\)

Reagents and conditions: i) Br\(_2\), 5-10 min, 0°C, 34, 37%; 35, 23%; 36, 1%; 37, 3%.

Triamantane (3) has a lower symmetry (C\(_{2v}\)) compared to ada- and diamantane, and is the first member of the diamondoid family that has a quaternary carbon.\(^{51}\) Furthermore, 3 has two equivalent apical bridgeheads at carbon atoms 9 and 15 (Scheme 1.6) and three sets of non equivalent medial bridgeheads at carbon atoms 2,12 and 3,7,11,12 and 4,6. In addition, 3
contains also three sets of non-equivalent methylene groups at carbon atoms 5 and 8,10,14,18 and 16,17. Brief exposure of triamantane to Br$_2$ at 0°C leads to a mixture of all possible bridgehead brominated triamantanes (34, 35, 36 and 37). As a result of the lower symmetry, some mono-functionalized triamantane derivatives such as 35 are chiral. The ionic bromination of 3 is hampered by the lack in selectivity and reproducibility, as noticed by Fokin et al. who could only isolate 34 in 37% yield after two crystallisations of a mixture of bromides from $n$-hexane. The generation of polybromo triamantane derivatives is cumbersome via direct ionic bromination of 3.

**Bridgehead hydroxylation**

Direct oxidation of the bridgehead carbons of adamantane (1) is reported using a number of conditions including the use of fuming sulfuric acid, chromium trioxide in glacial acetic acid and lead (IV) salts. However, the direct oxidation of adamantane generally results in the formation of a mixture of 1-and 2-hydroxyadamantanes. The hydrolysis of adamantyl bromides represents a straightforward method to obtain the corresponding hydroxy-adamantanes. 1-Bromoadamantane (24) is highly reactive in nucleophilic substitution reactions. Given the involvement of a (3°) bridgehead carbon, there is no possibility of a back-side nucleophillic attack, which should exclude a S$_N$2 mechanism. An S$_N$1 mechanism implies the involvement of a planar, 1-adamantyl cation, which is only possible by considerable distortion of the rigid ring system.

**Scheme 1.7: Solvolysis constants of 3°-bromides.**

It was observed that 1-bromoadamantane (24) reacts a thousand times faster than 1-bromobicyclo-[2,2,2]octane (38) and $10^{11}$ times faster than 39 (Scheme 1.7). These solvolysis constants were derived by comparison with tert-butyl bromide (40), which reacts a thousand times faster than 1-bromoadamantane (24). These findings led to a theoretical explanation for the involvement of the 1-adamantyl cation by Schleyer and Nicholas. It was hypothesized that the conformational strain (Pitzer strain) offers more resistance to the formation of bridgehead cations compared to bond-angle strain (Baeyer strain). Whereas 1-bromoadamantane (24) is free from both Baeyer- and Pitzer strain, 1-bromobicyclo-[2,2,2]octane (38) is not free of Pitzer strain, due to its unfavourable conformation. Bicyclo derivative 39 possess both Baeyer and Pitzer strain. The decreasing reactivity of mono, di, tri and tetra brominated adamantanes 24, 25, 26 and 27 provides additional evidence of the involvement of an adamantyl bridgehead cation.

The preparation of diamantane and triamantane bridgehead hydroxyls is possible by hydrolysis of the corresponding bromides. However, the selectivity in the halogenation of dia- and triamantane is often poor (except for 1-bromodiamantane, 28), giving a mixture of the corresponding
1.3 Functionalisation of diamondoids

Scheme 1.8: Two-step bridgehead hydroxylation of diamantane.\textsuperscript{58}

![Reaction Scheme](image)

Reagents and conditions: i) Br\textsubscript{2}, reflux, 95%; ii) H\textsubscript{2}O\textsuperscript{+}, \(\Delta\), near quant.; iii) HNO\textsubscript{3}, RT, 16 h, 46, \(\sim\)50%; iv) a. HNO\textsubscript{3}, RT, 40 min; b. evaporation of HNO\textsubscript{3}; v) conc. H\textsubscript{2}SO\textsubscript{4}, 4 min, 41, 24%; 44, 44%; 45, 22%; vi) conc. H\textsubscript{2}SO\textsubscript{4}, 90 min, 41, 7%; 44, 8%; 45, 78%.

Bridgehead bromides. Recently, Fokin \textit{et al.}\textsuperscript{47,58} showed that selective bridgehead oxidation of diamantane (2) and triamantane (3) is achievable \textit{via} a two-step procedure. First, depending on the degree of exposure to nitric acid, mono and dinitroxy diamantane esters can be obtained in high yield (Scheme 1.8). For instance, 16 h of exposure of 2 to concentrated nitric acid yields, after chromatographic purification, 1,4-dinitroxydiamantane (42) in \(\sim\)50%. When the reaction time is shortened to 40 minutes, a mixture of mono- and bis-nitroxy derivatives (43) is obtained, which are separable by column chromatography and can also be isomerized with concentrated sulphuric acid to produce the corresponding hydroxyl analogues. If the isomerisation of 43 is executed under kinetically controlled conditions (5 min concentrated H\textsubscript{2}SO\textsubscript{4}), 1-hydroxy-, 4-hydroxy-, and 4,9-bishydroxy diamantane derivatives 41, 44 and 45 are obtained in 24, 44 and 22\% yield, respectively. Thermodynamic isomerisation of 43 (90 minutes concentrated H\textsubscript{2}SO\textsubscript{4}) gives predominantly 4,9-bishydroxylated diamantane 45 in good yield.

Scheme 1.9: Triamantane bridgehead hydroxylation.\textsuperscript{58}

![Reaction Scheme](image)

Reagents and conditions: i) HNO\textsubscript{3} then H\textsubscript{2}O then separation, 46, 18%; 47, 40%; 48, 24%; 49, 7%.

Treatment of triamantane (3) with concentrated nitric acid and subsequent hydrolysis of the intermediate nitrate esters furnished a mixture of mono bridgehead hydroxylated triamantane
derivatives 46, 47, 48 and 49 (Scheme 1.9). Bishydroxylation of triamantane is achieved upon prolonged exposure to concentrated nitric acid followed by the hydrolysis of the thus formed bis-nitroxy esters.58

**Bridgehead carbonylation.**

The carbonylation of adamantane (1) is reported under Koch-Haaf conditions.44 This carbonylation method utilizes a hydride transfer between the initially formed tert-butyl cation (generated from tert-butanol) and adamantane, to produce the 1-adamantyl cation (50). Cation 50 is trapped by *in situ* generated carbon monoxide, to give after aqueous work-up the corresponding acid 51 (Scheme 1.10).

**Scheme 1.10:** Koch-Haaf carbonylation of ada- and diamantane (bromides).44,59

![Scheme 1.10](image)

**Reagents and conditions:** i) tBuOH, HCO$_2$H, H$_2$SO$_4$, CCl$_4$, 51, 90%; 53, 28%; ii) conc. H$_2$SO$_4$/fuming H$_2$SO$_4$ (1:1, v/v), tBuOH, HCO$_2$H, CCl$_4$, 53:54, 88:12, 9% total yield; iii) HCO$_2$H, H$_2$SO$_4$, CCl$_4$, 53, minor; 54, major, 52% total yield.

It was shown that 1-bromoadamantane (24) and also 1-adamantanol are susceptible to Koch-Haaf type carbonylation.44 The Koch-Haaf carbonylation of diamantane (2) is reported to produce solely 1-diamantanecarboxylic acid (53) in 28% yield (Scheme 1.10).59 However, the use of a mixture of concentrated (H$_2$SO$_4$)/fuming H$_2$SO$_4$ (1:1, v/v) in the Koch-Haaf
1.3 Functionalisation of diamondoids

**Scheme 1.11:** Koch-Haaf carbonylation of 9-bromotriamantane.\(^6^0\)

![Scheme 1.11: Koch-Haaf carbonylation of 9-bromotriamantane.](image)

**Reagents and conditions:** i) SOBr\(_2\), Pyr, CH\(_2\)Cl\(_2\), 3.5 h, RT, 95%; ii) 97% H\(_2\)SO\(_4\), HCOOH, CCl\(_4\), 5 h, -5°C → RT, 85%.

carbonylation gives a 88:12 mixture of 53 and 4-diamantancarboxylic acid (54) in 9% total yield. Application of 4-bromodiamantane (31) under highly diluted Koch-Haaf conditions, gives predominately 4-diamantancarboxylic acid (54) in 52% yield.\(^5^4,\(^5^9\) Recently, Fokina et al. reported the Koch-Haaf carbonylation of several diamondoid bromides, including 9-bromotriamantane (37).\(^6^0\) To this end, 37 was prepared from 9-triamantanol (48) and used under highly diluted conditions in the Koch-Haaf reaction (Scheme 1.11) to give acid 56.

**Oxidation of bridging methylene group.**

The preparations of ketones of cage compounds is well studied. 2-adamantanone is available by direct oxidation of adamantane (1) CrO\(_2\) in acetic anhydride. However, the major product is 1-adamantanol (57, 71%) in addition to 9% of the desired 58.\(^5^7\) Geluk et al. reported an convenient synthesis of 2-adamantanone (58) from 1 (Scheme 1.12).

**Scheme 1.12:** H\(_2\)SO\(_4\) oxidation of adamantane.\(^6^1\)

![Scheme 1.12: H\(_2\)SO\(_4\) oxidation of adamantane.](image)

**Reagents and conditions:** i) conc. H\(_2\)SO\(_4\), 54%.

It was found that 1-adamantanol (57) equilibrates in concentrated H\(_2\)SO\(_4\) to 2-adamantanol (59), via hydride transfer reactions. 2-Adamantanol (59) is subsequently further oxidized by H\(_2\)SO\(_4\) to 58 or undergoes a disproportionation with the 1-adamantyl cation, leading to 2-adamantanone (58) and adamantane (1), respectively.

Oxidation of diamantane (2), via the Geluk’s procedure, gives 3-diamantane (60) in 60% yield (Scheme 1.13).\(^5^9\) The oxidation of triamantane (3) with concentrated H\(_2\)SO\(_4\) at elevated
1. General introduction

Scheme 1.13: \( \text{H}_2\text{SO}_4 \) oxidation of dia- and triamantane.\(^{59,62}\)

\[
\begin{align*}
&\text{2} \quad \text{O} \\
\downarrow & \text{i} \\
\text{60}
\end{align*}
\]

Reagents and conditions: i) conc. \( \text{H}_2\text{SO}_4 \), 60%, 60%.

temperatures results in a mixture of oxygenated products.\(^{63}\) Kafka and co-workers\(^{62}\) analysed the mixture and found two isomeric triamantanone derivatives (61), in a 3:1 ration. The major ketone (62) could be isolated by crystallisation from \( n \)-hexane and the structural assignment was based on IR- and NMR analysis.

1.4 Application of diamondoids

Diamondoids are very stable, natural occurring compounds which make them suitable for numerous applications. The unique properties of (natural) diamondoids are for instance exploited in the investigation of oil spills.\(^{22}\) Oil spills and contaminations can be traced back by analysing the natural diamondoid composition, which act as a fingerprint signature of the oil source. The hydrophobic nature of especially 1 have resulted in its wide scale application. The solubility of adamantane (1) and diamantane (2) in several relative apolar solvent is listed in Table 1.1.\(^{64}\) The availability of sizeable quantities of the lower diamondoids 1, 2 and 3 paved the way for the exploration of their properties in different field of research. For instance, 1 is used in peptide nucleic acid (PNA) conjugates e.g. 63 (Figure 1.4) to increase cellular uptake.\(^{65,66}\) Numerous examples deal with the polymerisation of adamantane and/or diamantane monomers to obtain polymers, e.g. 64 with unique properties.\(^{24}\)

In drug design: The early observation of the prophylactic effects of hydrochloric salts of the adamantane amine, adamantine (65, Figure 1.5) against influenza A virus,\(^{68}\) started a revolution in the preparation of adamantane derivatives. Detailed analysis of the mode of action of 65 showed that early stages of viral replication is inhibited. Amine 65 was found to block the ion-channels which are formed by the transmembrane M\(_2\) protein of the influenza A virus.\(^{69}\) Closer evaluation of 65 showed that the drug is absorbed rapidly in man and is excreted in non-metabolized form in urine. Shortly thereafter, structure activity relationship (SAR) studies identified both isomers of (±)-ritamantine (66a and 66b) as a potent agent against influenza A
**Table 1.1:** Solubility (wt\%) of adamantane (1) and diamantane (2) in organic solvents.\textsuperscript{64}

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Adamantane</th>
<th>Diamantane</th>
<th>Solvent</th>
<th>Adamantane</th>
<th>Diamantane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentane</td>
<td>11.6</td>
<td>4.0</td>
<td>CCl\textsubscript{4}</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Hexane</td>
<td>10.8</td>
<td>3.9</td>
<td>m-Xylene</td>
<td>9.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>11.1</td>
<td>6.3</td>
<td>p-Xylene</td>
<td>9.6</td>
<td>4.5</td>
</tr>
<tr>
<td>Heptane</td>
<td>10.4</td>
<td>3.7</td>
<td>o-Xylene</td>
<td>9.6</td>
<td>4.1</td>
</tr>
<tr>
<td>Octane</td>
<td>10</td>
<td>3.9</td>
<td>Toluene</td>
<td>9.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Decane</td>
<td>8.9</td>
<td>3.5</td>
<td>THF</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Undecane</td>
<td>7.9</td>
<td>3.2</td>
<td>Benzene</td>
<td>10.9</td>
<td>4.3</td>
</tr>
<tr>
<td>Tridecane</td>
<td>7.3</td>
<td>2.7</td>
<td>Diesel fuel</td>
<td>7.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Tetradecane</td>
<td>7.5</td>
<td>2.3</td>
<td>1,3-dimethyl-adamantane</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Pentadecane</td>
<td>7.1</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.4:** Utilisation of the diamond scaffold.\textsuperscript{65,67}

S-15 (swine) in mice. However, 66 is metabolized in the human body, giving rise to three oxygenated derivatives, which also possess anti-viral activity.\textsuperscript{70} The amines were prepared either by N-alkylation of 1-bromoadamantane (24) or by a (modified) Ritter reaction of 24. Homoadamantane (67) was found to be slightly more active than 65 and di-adamantyl derivative 68 was one of the least active members of a vast library of N-alkylated adamantane derivatives.\textsuperscript{68,71}

In 1968, a 58-year-old woman with moderate Parkinson’s disease, treated twice daily with 100 mg of 65 against the flue, experienced a remarkable remission in her symptoms related to Parkinson’s disease.\textsuperscript{73} Neurodegenerative diseases, such as Parkinson’s disease and Alzheimer’s disease, are a group of pathologies characterized by a progressive and specific loss of certain brain cell populations. Several adamantane amine derivatives including amantadine (65), rimantadine (66) and memantine (69, Figure 1.6) exhibit antiparkinsonian activity.\textsuperscript{74} The ability of adamantane derivatives to pass the blood-brain barrier is contributed to the highly lipophilic nature of adamantane (1).\textsuperscript{75} The utilisation of the adamantane moiety in drug discovery pro-
Figure 1.5: Antiviral dose$_{50}$ of amantane amine derivatives against influenza A S-15 (swine) in mice.$^{71}$

![Chemical structures of amantane amine derivatives](image1.png)

Figure 1.6: A selection of adamantane based agents (in development) for the treatment of several conditions.$^{72}$

![Chemical structures of adamantane based agents](image2.png)

grams have resulted in a number of compound which are currently in clinical trails. These derivatives include adamantine (65), rimantadine (66) and memantine (69) for the treatment of Parkinson’s- and Alzheimer’s disease.$^{76}$ There is a growing number of examples reporting promising results with adamantane based agents in the treatment of several conditions such as iron overload disease, neurological conditions, malaria, type 2 diabetes, tuberculosis and cancer. The many faces of the adamantyl group in drug design was recognized in a recent review covering most of the adamantyl based agents,$^{72}$ of which a selection is shown in Figure 1.6. Contrary to adamantane, the application of diamantane (2) in drug discovery is still in its infancy. In the last decades, there have been only a few reports of the incorporation of diamantane derivatives in pharmacophores.$^{77-81}$
1.5 Outline thesis

The research described in this thesis aims at the exploitation of the unique properties of diamondoid derivatives and comprises of two parts. The first part involves the utilisation of adamantane derivatives in the solution-phase preparation of oligonucleotides. Chapter 2 gives an introduction into the development of a 3′→5′ directed solution-phase approach for the synthesis of native and phosphorothioate modified oligonucleotides, initiated by de Koning and co-workers in 2006. The reverse, 5′→3′ directed solution-phase oligonucleotide chain elongation, is described in chapter 3. In both these solution-phase approaches, the isolation of protected, oligonucleotide intermediates are isolated by extractive work-up procedures. Here, 1-adamantaneacetic acid served as either 3'- or 5'-O-nucleoside protective group and aided in the solubility of protected oligonucleotide fragment in organic solvents. Chapter 4 revolves around the development of an orthogonally cleavable adamantane - levulinoyl hybrid protective group for 3'-O-nucleoside protection.

The second part of the research focusses on the role of adamantane derivatives as part of iminosugar based modulators of glucosylceramide (GlcCer) metabolism and originated from two lead compounds identified by Aerts and co-workers in the period of 1999 to 2007. N-pentyloxymethyl-1-adamantane-1-deoxynojirimycin (MZ-21) and its L-ido congener, MZ-31, were found to be potent inhibitors of all the enzymes involved in the GlcCer metabolism. An introduction into the topic is presented in Chapter 5 and entails the synthesis and biological evaluation of N-alkylated 1-deoxynojirimycin (DNJ) and L-ido-1-deoxynojirimycin (L-ido-DNJ) derivatives. Chapter 6 describes the synthesis of several N-alkylated DNJ and L-ido-DNJ derivatives bearing an adamantane unit. Additionally, this chapter describes the synthesis of a DNJ-based photo-affinity probe, decorated with a bodipy fluorophore. To provide a better understanding on the mechanism by which iminosugars act on β-glucosidase, N-methyl quaternary ammonium salts of leads MZ-21 and MZ-31 are prepared as is described in chapter 7. In Chapter 8 the synthesis of two imidazol-D-glucopyranose bicyclic iminosugar derivatives are described. The synthesis of several derivatives of castanospermine, a natural glucosidase inhibitor, is explored in chapter 9. Finally, a general summary of the results described in the preceding chapters is given and also some future prospects are described.
Diamondoid assistance in the $3'\rightarrow 5'$ directed solution-phase synthesis of (PS) oligonucleotide fragments

2.1 Introduction

The blueprint of life is written in a four letter code in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules. These biomolecules are composed of adenosine (A), cytidine (C), guanosine (G), deoxythymidine (dT, only DNA) and uridine (U, only RNA). Oligonucleotides (ONs) are short sequences of nucleotides, containing typically up to 20 nucleosides. A nucleoside consists of (deoxy)ribose linked via a $\beta$-N-glycosidic bond to a nucleobase ($B$). The corresponding phosphate derivative represents a (deoxy)nucleotide (Figure 2.1).

ONs are applied as tools in diagnostics, molecular biology and in templated organic synthesis. Furthermore, (synthetic) ON analogs are finding widespread application as the active ingredients in therapeutic agents with mechanism of action including antisense, aptamer, ribozyme, microRNA, RNAi. The application of ONs in physiological conditions requires a high resistance against nuclease degradation. Native ONs are rapidly degraded by nucleases whereas modified ON analogs possess enhanced nuclease stability. For instance, the phosphorothioate (PS) oligonucleotides (Figure 2.1, b), in which one of the non-bridging oxygen atoms of the internucleotidic phosphodiester is replaced by sulfur, have a half-live in human serum of 9-10 hours compared to ~ 1 hour for native ONs. Various PS oligonucleotides are currently in different stages of human clinical trials as therapeutics for a range of diseases including cancer, cardiovascular diseases, autoimmune diseases, diabetes and infectious diseases.
2.1 Introduction

Figure 2.1: Nucleotides and analogues thereof.

(a) DNA \((R_1 = H)\), RNA \((R_1 = OH)\); (b) phosphorothioate, \(R_1 = H\) (phosphorothioate-DNA), \(R_2 = OH\) (phosphorothioate-RNA); (c) \(R_2 = H\) (phosphoramidate-DNA), \(R_2 = OH\) (phosphoramidate-RNA), \(R_2 = F\) (2'-fluorophosphoramidate); (d) Locked Nucleic Acid (LNA, \(X = O, S, NH, NMe\)).

Vitravene\(^\text{®}\), a 21-mer PS oligonucleotide, is currently on the market for the treatment of cytomegalovirus induced retinitis in AIDS patients via an antisense mechanism.\(^9^4\) The therapeutic and diagnostic potential of ONs has stimulated research on improved methods for their synthesis. The successful commercialisation and further development of ONs based therapeutics is dependent on the development of safe and economical methods to produce large quantities of ONs.\(^9^5\)

**ONs Synthesis**

The field of ONs synthesis has a rich history.\(^9^6\) The pioneering work of the groups of Todd and Khorana towards sequence defined ON, led to the development of a plethora of methods to construct the ON backbone.\(^9^7,9^8\) Utilizing acyl protective groups on the nucleobases (A, G and C) in combination with the acid labile trityl (Trt) group for nucleoside-5'-O protection, the basis was provided for chain elongation. Several approaches that led to the evolution of ON synthesis are the phosphodiester-, H-phosphonate-, phosphotriester and phosphoramidite method.\(^9^9-1^0^1\) The emergence of the solid-phase technology, pioneered by Merrifield for the synthesis of polypeptides,\(^1^0^2\) combined with phosphoramidite chemistry for ONs synthesis came to dominate the field of ON research.\(^1^0^3,1^0^4\)

**Solid-phase methodology toward ONs assembly.**

Currently, all ONs involved in clinical trials are produced via automated solid-phase DNA synthesisers, based on phosphoramidite chemistry.\(^1^0^5\) In the solid-phase approach (Scheme 2.1), reactions are conducted on the surface of an insoluble support.\(^1^0^2\) ON synthesis is usually carried out in the 3'→5' direction due to the higher reactivity of the 5'-OH with respect to the 3'-OH.\(^1^0^4\) The first monomer is attached (via a linker e.g. the succinyl linkage) to a solid support. Usually
Reagent and conditions: i) tetrazole, CH$_3$CN; ii) X = O: 0.02 M I$_2$, base, X = S: CH$_3$CN, Beaucage reagent; iii) Ac$_2$O, pyridine; iv) 3 vol% dichloroacetic acid in CH$_2$Cl$_2$. B$_{Pg}$ = A$^{Rg}$, C$^{Rg}$, G$^{Bu}$ and T$^H$.

polystyrene resins or controlled pore glass beads are used. After each coupling reaction, the resin is rinsed with an organic solvent to remove the excess reagent and possible by-products. In the first step of the elongation cycle acid mediated cleavage of the 5’-O-DMTr protective group of the immobilized nucleoside I (n = 0) yields II (n = 0) that is suitable for elongation. Next, a suitably protected phosphoramidite donor (III) is condensed with the free 5’-OH group under mild acidic conditions (a weak acid such as tetrazole or dicyanoimidazole (DCI) that does not affect the DMTr-group is often used). The excess phosphoramidite donor III is removed from the mixture by means of an additional washing step and the labile phosphite intermediate IV (n = 0) is subsequently oxidized with iodine to produce the phosphotriester I (n = 1, X = O). Sulfurisation of phosphite intermediate IV (n = 0), with for instance the Beaucage reagent, results in the formation of phosphorothioate triester I, (n = 0, X = S). To prevent accumulation of n-1 deletion fragments, unreacted 5’-OH of II (n = 0) is usually capped by the addition of Ac$_2$O in pyridine. The aforementioned steps are repeated until the desired ON-length is reached. The target ON is cleaved from the solid support, the protective groups are removed (ammonolysis) and the crude ON is subsequently purified by ion-exchange- or preparative HPLC.$^{96,106}$

Solid- vs solution-phase chemistry in the preparation of ONs.

The solid-phase methodology is well established, versatile, very efficient and produces ONs of high quality and purity.$^{105}$ These characteristics makes the solid-phase method an excellent tool for the laboratory scale (up to several milligrams) preparation of ONs. When one tries to synthe-
size larger quantities of ONs fragments several problems are encountered. The major limitations during scale-up of a specific ON sequence via the solid-phase methodology are the unpredictable results in terms of yield and purity. During large scale preparation, also the limitations of the solid-support is a bottleneck. Given the nature of the support, reactions conducted on resins or glass beads are usually shaken and not stirred. Considering the numbers of step involved in the synthesis of for instance a 21-mer ON, the final deprotection and isolation procedures, a drop in coupling efficiency below the 95% would be catastrophic for the overall yield. Furthermore, this approach requires the use of relatively large excesses of expensive reagents and costly solid supports. Additionally, as a consequence of the bi-phasic reaction systems, the reaction progress is difficult to monitor by conventional techniques (Table 2.1).

### Table 2.1: (Dis)advantages of solid-phase vs. solution-phase chemistry.

<table>
<thead>
<tr>
<th>Solid-phase</th>
<th>Solution-phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expensive resins</td>
<td>No solid support</td>
</tr>
<tr>
<td>Difficult to scale up</td>
<td>Easy to scale up</td>
</tr>
<tr>
<td>Lots of organic waste</td>
<td>Less organic waste</td>
</tr>
<tr>
<td>Multiple equivalents of reagents</td>
<td>Less excess of reagents</td>
</tr>
<tr>
<td>Reactions are difficult to monitor</td>
<td>Easy monitoring of reactions</td>
</tr>
<tr>
<td>Automation (synthesizer)</td>
<td>No automation</td>
</tr>
<tr>
<td>Short synthesis time</td>
<td>Long synthesis time</td>
</tr>
<tr>
<td>Easy isolation</td>
<td>Cumbersome isolation</td>
</tr>
</tbody>
</table>

A solution-phase approach may overcome the limitations encountered during the large scale solid-phase preparation of ONs, provided that time-consuming and expensive chromatographic purifications of ON intermediates can be avoided. In this vein, several approaches have been explored that combine the advantages of solution- and solid-phase syntheses. For instance, the HELP protocol exploits the solubility of PEG-based polymers during the reactions, while the isolation of intermediates is accomplished by precipitation and filtration. The PASS-process utilizes a resin that temporarily immobilizes the growing ON chain for removal of excess monomers. Also the use of immobilized reagents in the solution-phase synthesis of ONs (up to hexamers) was reported employing phosphoramidite- as well as H-phosphonate chemistry. The mentioned approaches all have in common that polymers (resins) are involved in the process and that they are often combined with recurrent purification methods such as precipitation and/or chromatography.

A new strategy for the solution-phase preparation of ONs was devised which is based on well established phosphoramidite chemistry and can be divided into the following three stages (Scheme 2.2):
2. Diamondoid assistance in the 3’→5’ directed solution-phase synthesis of ONs

- Elongation of the 5’-OH of properly protected nucleoside V (n = 0) with a slight excess of phosphoramidite monomer III.
- Hydrolysis of the excess phosphoramidite donor III, oxidation or sulfurisation of the reactive ON intermediate VI (n = 0) as well as the H-phosphonate monomer VII, followed by extractive work-up.
- Removal of the DMTr-group combined with the extractive removal of its by-products and the excess phosphodiester monomer.

Scheme 2.2: Strategy for the solution-phase synthesis of (PS) oligonucleotides.

Reagent and conditions: i) 4,5-dicyanoimidazole, CH₃CN; ii) X = O, 0.2M I₂ in H₂O/pyridine or X = S, Beaucage reagent, extractive work-up 1; iii) 80% aq. AcOH, extractive work-up 2.

2.2 Results and Discussion

A crucial factor to the success of this approach is the solubility of the ON in the organic phase. It is known that the solubility of protected ONs decreases with the increase length, especially when unprotected thymidines are used. The solubility of growing ON chain in the organic phase can be favourably influenced by the use of lipophilic protective groups. 1-Adamantane acyl serves not only as a base labile 3’-O-protective group but also acts as a hydrophobic anchor. The CE phosphate protective group and 5’-O-DMTr protections were selected because they enable the use of commercially available 3’-phosphoramidite donors. Commercially available N⁶-benzoyl adenosine-, N⁶-benzoyl cytidine- and N²-isobutyryl guanosine phosphoramidite monomers were used with 5’-O-DMTr protection. An exception is made for the thymidine phosphoramidite. Bz-protection of N³-thymidine was selected, based on an earlier observation.
that the use of $N^3$-benzoylated thymidines enhances the solubility of protected ON fragments.\textsuperscript{108}

**Scheme 2.3:** Synthesis of 3'-O-adamantane acetyl-$N^3$ protected thymidine monomers.

\[
\begin{align*}
& \text{Reagents and conditions: i) 1-adamantaneacetic acid (1.5 eq.), EDC (1.5 eq.), DMAP (cat.), CH}_2\text{Cl}_2, \text{RT, 12 h, 97%}; \text{ii) } R = \text{Bz, Bz-Cl (1.5 eq.), Pyr (0.17 M), 5.5 eq. DiPEA, RT, overnight, 79, 97%; } R = \text{Piv, Piv-Cl (1.5 eq.), Pyr (0.1 M), cat. DMAP, RT, 24 h, 80, 80%}; \text{iii) } R = \text{Pom, Pom-Cl (3 eq.), DMF (0.2 M), K}_2\text{CO}_3 (4 eq.), \text{RT, 12 h, 81, 89%}; \text{iv) 5 vol\% DCA in CH}_2\text{Cl}_2, \text{TES; 0°C, 30 min, 82, 92%; 84, 94%}. 
\end{align*}
\]

**Monomer synthesis**

The synthesis of 3'-adamantylacetyl-$N^3$-benzoylthymidine (82), the starting compound in the synthesis of ONs, is depicted in Scheme 2.3. Esterification of commercially available 3'-O-DMTr thymidine (77) and 1-adamantaneacetic acid under the agency of EDC/DMAP provided orthogonally protected \textit{78} in good yield. Subsequent protection of the nucleobase with BzCl gave compound \textit{79}. Acid mediated cleavage of the DMTr group in \textit{79} with cation scavenger triethylsilane (TES) afforded, after chromatographic purification, pure acceptor \textit{82}.

The requisite $N^3$-benzoylated thymidine phosphoramidite monomer 90 was prepared from known $N^3$-benzoylthymidine (87, Scheme 2.4).\textsuperscript{111} Treatment of 87 with DMTrCl in pyridine followed by phosphitylation of the 3'-OH in 88 using 2-cyanoethyl phosphoramidite 92 gave building block 90 in good overall yield. The bisfunctional phosphochloridite reagent 92 was prepared on large scale from PCl$_3$ according to known procedures.\textsuperscript{112,113} Special care must be taken during the \textit{in vacuo} distillation of the highly reactive and combustible reagent. P(III) species are highly combustible compounds at high temperatures ($\geq 150°C$). Direct cooling of the collection flask with liquid nitrogen proved to be essential to maintain high vacuum during the distillation of the P(III) species.

**Native ON synthesis**

**Stage 1:** To test the feasibility of the solution-phase approach, the synthesis of a pentameric ON sequence was undertaken on a 0.5 mmol scale. In the first stage, both the acceptor 82 and donor 90 were pre-mixed and co-evaporated several times with CH$_3$CN. Commercially available 4,5-dicyanoimidazole (DCI) in CH$_3$CN was used for the activation of phosphoramidite donor 90,
2. Diamondoid assistance in the 3'→5' directed solution-phase synthesis of ONs

Scheme 2.4: Synthesis of \( N^3 \)-protected thymidine phosphoramidite donors.

Reagents and conditions: i) LevOH (1.5 eq.), EDC (1.5 eq.), DMAP (cat.), dioxane (0.14M), RT, 2.5 h; ii) Pom-Cl (1.5 eq.), \( K_2CO_3 \) (3 eq.), DMF, RT, overnight, 96%, 2 steps; iii) \( N_2H_4 \cdot H_2O \) (0.5M), pyridine/\( AcOH \) (4:1, v/v), RT, 5 min, then acetylacetone (40 eq.), 97%; iv) DMTr-Cl (1.2 eq.), Pyr (0.20M), 87%; v) \( 92 \) (1.5 eq.), DiPEA (1.5 eq), \( CH_2Cl_2 \) (0.1M), 0°C, 30 min, 90, 79%; 91, 86%.

due to its higher stability with respect to traditional tetrazole activators. The reaction progress was monitored by means of HPLC and TLC, and showed completion within 30 minutes.

Stage 2: The excess phosphoramidite donor \( 90 \) was hydrolysed followed by oxidation of the thus obtained H-phosphonate \( VII \) (\( B^{pg} = T^{Bz} \)) and phosphite triester \( VI \) (\( n = 0, B^{pg} = T^{Bz} \), Scheme 2.2) by the addition of a 0.2M \( I_2 \) solution in THF/pyridine. The addition of THF was a necessity for extractive work-up procedures due to the fact that protected ON fragments dissolve better in a mixture of EtOAc/THF compared to EtOAc. Dilution of the mixture with EtOAc and extraction with 1M \( Na_2S_2O_3 \) (quench \( I_2 \), remove \( I^- \), 10% \( KHSO_4 \) (remove pyridine, diisopropylamine) and 10% \( NaHCO_3 \) (remove DCI) gave, after evaporation of the solvents, a mixture of fully protected dimer \( DMTrOdT^{Bz}d^{Bz}OAda \) and monomeric phosphodiester \( DMTrdT^{Bz}_{OP(=O)(OCE)OH} \).

Stage 3: A key step in the approach is the acid mediated detritylation followed by the extractive removal of the by-products there off. In this stage, the extractive removal of the phosphodiester, originating from excess phosphoramidite monomers, is explored. Detritylation by aqueous acetic acid did not lead to effective removal of the thus formed DMTr-OH. More importantly,
2.2 Results and Discussion

Figure 2.2: Pilot study utilizing $N^3$-benzoyl thymidine and 3'-O-adamantane acetyl protection.

Left: Thymidine dimer obtained after one elongation cycle. Right: Pentameric ON fragment.

... in several cases also retritylation of the 5'-OH was observed upon *in vacuo* concentration. To this end, novel conditions were developed that facilitated extractive removal of the lipophilic DMTr-cleavage product. Detritylation was carried out in a mixture of MeOH and CH$_3$CN (6:1, v/v) containing 0.1m HCl (1.9 eq. relative to $^{DMTrO}dT^{Bz}dT^{BzOAda}$ dimer. Reaction times varied between 5 min for short oligomers to 25 minutes for longer sequences. It appears that the size and composition of the oligonucleotide influences the acidity of the reaction mixture. The variation in reaction time can be contributed to the buffering capacity of nucleobases. Although this buffering effect leads to prolonged reaction times during detritylation reactions, no depurination products were observed by LCMS analysis. Upon completion of the reaction, as judge by TLC analysis, the acid was quenched with an excess of aqueous Et$_3$NHOAc (TEAA) and CH$_3$CN was added to give a final mixture of CH$_3$CN/MeOH/H$_2$O 2:2:1 (v/v/v). Repetitive extraction of this mixture with heptane/Et$_2$O (2:1, v/v) removed up to 90-95% of the DMTrOMe by-product. After concentration of the CH$_3$CN/MeOH layer, the foamy residue was redissolved in a mixture of THF/EtOAc and washed with 10% NaHCO$_3$ to remove excess phosphodiester $^{HO}dT^{Bz}_{OP(-O)(OCE)}OH$. Evaporation of the solvents afforded dimer $^{HO}dT^{Bz}_{OP(-O)(OCE)}OH$ in good yield and purity (Figure 2.2, left).$^a$

**Pentameric ON sequence:** Next, elongation of the obtained thymidine dimer via the above described procedure was undertaken with the commercially available phosphoramidite monomers. After four coupling cycles, the crude, partially protected pentameric ON fragment $^{HO}A^{Bz}G^{iBu}C^{Bz}T^{Pom}T^{PomOAda}$ (Figure 2.2, right) was obtained 78% yield (based on 82). However, LCMS-analysis showed major cleavage of the $N^3$-benzoyl group and to a lesser extend CE elimination. To circumvent this side reaction, the use of the pivaloyl group was investigated for $N^3$-protection of thymidine. $^{DMTrO}dT^{Piv}_{OAda}$ (80) was prepared in a similar manner as described for the benzoylated derivative (Scheme 2.3). Unfortunately, acidic treatment of 80 resulted in the complete cleavage of the 5'-O-DMTr and partial deblocking of the $N^3$-pivaloyl group. Interestingly, isolated 83 decomposed rapidly at room temperature while dissolved in methanol, as revealed by HPLC analysis. Next, our attention was focused on the use of the pivaloyloxymethyl (Pom) group, which was already known as $N^3$-protection for thymidine.$^{114}$ Installation of

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$^a$Yields were estimated by dividing the amount of obtained material (mg) with the expected amount (0.5 mmol x MW oligonucleotide).
2. Diamondoid assistance in the 3’ → 5’ directed solution-phase synthesis of ONs

**Figure 2.3:** Hexameric native ON fragment.

**Top left:** HPLC trace of the crude fully protected hexamer \( \text{HO} \text{dA}^\text{Bz} \text{dT}^\text{Pom} \text{dG}^\text{iBu} \text{dT}^\text{Pom} \text{dC}^\text{Bz} \text{dT}^\text{Pom} \text{OAda} \). **Top right:** HPLC trace of the crude, fully deprotected DNA hexamer \( \text{HO} \text{ATGCTT} \text{OH} \). **Bottom left:** HPLC trace of the purified ON fragment \( \text{HO} \text{ATGCTT} \text{OH} \). **Bottom right:** HPLC trace of the crude, fully deprotected hexameric fragment \( \text{HO} \text{ATGCTT} \text{OH} \), generated via automated solid-phase synthesis.

the Pom protective group was performed according to the described procedures and provided acceptor 84 (Scheme 2.3) and phosphoramidite donor 91 (Scheme 2.4) in good yields. For the large scale preparation of the monomeric building blocks, \( \text{DMTr} \text{dT}^\text{OH} \) (77) was prepared from cheap thymidine according to known procedures in excellent yields.\(^{111,114}\)

**Hexameric ON sequence:** The influence of \( \text{N}^3 \)-Pom thymidine protection was evaluated by the synthesis of hexameric ON fragment \( \text{HO} \text{ATGCTT} \text{OH} \) on 0.5 mmol scale. The synthesis commenced with the condensation of acceptor 84 with a slight excess of phosphoramidite donor 91. Successive execution of the three stages, also termed one full coupling cycle, yielded \( \text{HO} \text{dT}^\text{Pom} \text{dT}^\text{Pom} \text{OAda} \) in quantitative yield and good purity. Successive elongation of the \( \text{N}^3 \)-Pom protected dimer to the hexamer proceeded without complications. LCMS-analysis of the intermediate ON after each coupling cycle revealed the presence minor ON related products. These derivatives originate from the parent ON with the loss of one or more CE phosphate protective group. Gratifyingly, the presence of n-1 deletion fragments, resulting from incomplete coupling reactions, was not observed. The thus obtained hexameric ON fragment \( \text{HO} \text{dA}^\text{Bz} \text{dT}^\text{Pom} \text{dG}^\text{iBu} \text{dT}^\text{Pom} \text{dC}^\text{Bz} \text{dT}^\text{Pom} \text{OAda} \) (67% yield based on 84) was deprotected by ammonolysis and subsequently purified by ion-exchange chromatography and finally desalted (Sephadex G25). Pure hexameric \( \text{HO} \text{ATGCTT} \text{OH} \) was obtained in 39% yield\(^b\) and the identity and homogeneity confirmed with HPLC, \(^{31}\text{P}\) NMR and Maldi-Tof analysis. In addition, pure hexamer \( \text{HO} \text{ATGCTT} \text{OH} \) was compared to the same fragment independently prepared via automated solid-phase synthesis (Figure 2.3).\(^{115}\)

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\(^b\)Yields are based on 84 and was determined by \( A_{260} \) units, \( \varepsilon = 55100 \text{ L mol}^{-1} \text{cm}^{-1} \).
Decameric ON sequence: The low but unavoidable loss of the CE group during the basic washes is a crucial factor leading to cumbersome extractions due to poor separation of the organic- and aqueous layer. The premature $\beta$-elimination of the CE group results in a charged phosphodiester bond, which is detrimental for the solubility of protected ON fragments in organic solvents such as EtoAc. By reducing the concentration of the bicarbonate wash solution to 5%, the cleavage of the CE group could be suppressed.

**Scheme 2.5:** Evaluation of readily available phosphoramidites as capping reagent.

Reagents and conditions: i) DCI, CH$_3$CN, RT, 30 min; ii) X=O, I$_2$, THF/Pyr; X=S, phenylacetyl disulfide then 3-$H$-benzo[1,2]dithiol-3-one.

It is suspected that the synthesis of higher length ON fragments could suffer from a drop in coupling efficiency. To prevent tedious purification at the end of the synthesis, the implementation of a capping step of the residual free 5'-OH, resulting from incomplete coupling reactions was investigated. Ideally, this reagent and its capped products should be removed by extractions. It was envisaged that the introduction of a terminal phosphate by the addition of an excess of a readily available, inexpensive phosphoramidite reagent could serve as an effective capping reagent. Providing deletion fragments with a 5’-phosphate functionality can facilitate the final ON purification by means of ion-exchange chromatography.

Six easily accessible phosphoramidite reagents were selected and evaluated as capping reagent (Scheme 2.5). In a model experiment, a coupling efficiency of 95% was simulated by replicating the coupling condition with only 5% acceptor 84. The pre-set conditions include the use of a 20 fold excess of phosphoramidite capping reagent in combination with 5 minutes of reaction time. After the hydrolysis of excess reagent, the reaction mixture was subjected to the
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developed oxidation and work-up sequence. The formation of the intermediate phosphite triester proved to be efficient and proceeded rapidly for all the tested phosphoramidite reagents. However, hydrolysis of the excess capping reagent by the addition of water, was accompanied by hydrolysis of the 5’-phosphate triester of the deletion sequence. $^{31}P$-NMR and HPLC analysis of the capping reaction revealed that the phosphoramidite reagents containing one or more CE groups were less prone to hydrolysis.

**Figure 2.4:** Synthesis of a native decameric ON fragment on 0.5 mmol scale.

![HPLC traces](image)

**Top left:** HPLC trace of the crude fully protected decamer. **Top right:** HPLC trace of the crude, fully deprotected DNA decamer, $^{5}O_{H}A_{G}C_{T}A_{T}T_{T}G_{C}T_{O}H$. **Bottom left:** Purified ON fragment $^{5}O_{H}A_{G}C_{T}A_{T}T_{T}G_{C}T_{O}H$. **Bottom right:** HPLC-trace of crude, fully deprotected decameric fragment $^{5}O_{H}A_{G}C_{T}A_{T}T_{T}G_{C}T_{O}H$, generated via automated solid-phase synthesis.

Bis-cyanoethylphosphoramidite (100) was selected for implementation in the solution-phase ON synthesis method as the capping reagent. In order to explore the full potential of this method the synthesis of decameric $^{5}O_{H}A_{G}C_{T}A_{T}T_{T}G_{C}T_{O}H$ ON fragment was undertaken on 0.5 mmol scale. The synthesis started with the formation of dimer $^{5}O_{H}dC_{B};dT_{Pom}OAda$. The capping-step was introduced after completion of the coupling-reaction by the addition of 1 eq. of capping-reagent 100. After the execution of 6 coupling cycles, a slight decrease was observed in yield of the crude fragment, based on expected and recovered mass. The crude, fully protected decameric ON fragment was obtained as an off white foam in an overall yield of 70%. The crude material was subjected to ammonolysis followed by preparative HPLC-purification. Pure decameric fragment $^{5}O_{H}A_{G}C_{T}A_{T}T_{T}G_{C}T_{O}H$ was obtained in 33% yield. The identity and homogeneity of the thus obtained decameric ON fragment was confirmed by $^{31}P$ NMR, LCMS (Figure 2.4) and Maldi-Tof analysis and also by comparison with the same fragment prepared via automated solid-phase chemistry.

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*Yield is based on $^{5}O_{H}dT_{Pom}OAda$ and was determined by $A_{260}$ units, $ε=55100\text{ L mol}^{-1}\text{cm}^{-1}$. 

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2.2 Results and Discussion

**PS-oligonucleotide synthesis**

The most successful DNA analogue used in antisense therapeutics, \(^8^7\) PS-oligonucleotides, can be prepared by the same synthetic route as for DNA, except that the oxidation step is replaced with a sulfurization step. Expanding the scope of the newly developed solution-phase protocol towards the synthesis of PS-oligonucleotides requires a suitable sulfurization protocol. The sulfurization procedure should sulfurize both the phosphite triester intermediates obtained after each coupling step and also convert the excess of monomeric building block derivatives, predominantly present as H-phosphonates, into negatively charged phosphodiester to facilitate their extractive removal. Moreover, excess of the sulfur transfer reagent and the by-products should be removable by a simple extraction procedure. Numerous reagents that effectuate the sulfurization of phosphites to give the corresponding phosphorothioates have been reported over the years of which a selection is depicted in Figure 2.5. \(^1^1^6^-^1^2^7\)

![Figure 2.5: Commercially available sulfur transfer reagents.](image)

The most widely used reagent in laboratory scale solid-phase synthesis is the Beaucage reagent. \(^1^1^7\) The ability of Beaucage reagent \((101)\) in sulfurizing both phosphite triesters and H-phosphonate diesters, was an incentive for its implementation in the developed solution-phase protocol. To this end, a mixture of \(d^O d^T^P^o^m_{O^a^d^a} \) (84) and phosphoramidite monomer 91 was treated with DCI (4.5 eq; Scheme 2.6). Upon complete consumption of acceptor 84, as judged by HPLC, excess phosphoramidite donor 91 was hydrolysed to give the corresponding H-phosphonate 106. After evaporation of the volatiles, the thus obtained mixture of phosphite 107 and H-phosphonate 106 was treated with Beaucage reagent \((101, 2.25 \text{ eq.})\) in 2% \(H_2O/pyridine.\) \(^1^1^7\) Analysis of the reaction mixture by \(^3^1^P\) NMR showed that phosphite 107 as well as H-phosphonate 106 were converted to a large extend into the corresponding phosphorothioate triester 108 (67 ppm) and diester 109 (57 ppm), respectively. However, the presence of signals at -2 ppm indicated the formation of significant amounts (up to 11%) of phosphate esters. These results are in agreement with the finding that 3H-2,1-benzoxathiolan-3-one-1-oxide, which is generated during the sulfurization, is an potent oxidizing agent. \(^1^2^3\) The use of the mono-oxidized Beaucage derivative 102 gave similar results as the Beaucage reagent, albeit prolonged reaction time were required for complete conversions. Stawinski and co-workers reported that compound 103 is a suitable reagent for the conversion of H-phosphonates into H-phosphonothioates. \(^1^1^8\) Pilot experiments towards the sulfurization of trimethyl phosphite (PMe\(_3\)) suggested that this reagent may also be suitable for the sulfurization of phosphites. However,
when 103 was used in our protocol only H-phosphonate 106 was completely sulfurized to give 109 as shown by $^{31}P$ NMR analysis, whereas the phosphite triester 107 remained intact.

**Scheme 2.6:** Phosphorothioate oligonucleotide synthesis strategy.

Reagents and conditions: i) DCI (4.5 eq.), CH$_3$CN (0.15M), RT, 30 min; ii) phenylacetyl disulfide (105, 1.5 eq.), 30 min, RT then 3-H-benzo[1,2]dithiol-3-one (103, 1.5 eq.).

Next, the commercially available and relative inexpensive sulfur reagents tetraethylthiramidisulfide (TEDT, 104)$^{119}$ and phenylacetyl disulfide (PADS, 105)$^{122}$ were explored. First TEDT (104, 2.25 eq) was added to the mixture of phosphite 107 and H-phosphonate 106 in dry CH$_3$CN to reveal, after 30 minutes, that 107 was completely converted into the corresponding phosphorothioate 108. In contrast, H-phosphonate 106 remained stable towards 104 under the applied reaction conditions. The use of 104 also led to low, but persistent amounts of oxidation products. Analogous evaluation of 105 showed effective sulfurization of the phosphite triester 107 into phosphorothioate 108 whereas the H-phosphonate 106 remained untouched. Contrary to TEDT (104) the use of PADS (105) did not result in the formation of oxidation by-products. Attempts to completely remove H-phosphonate 106 by extractive work-up failed, thus demonstrating the necessity of their conversion into charged phosphodiesters.

The outcome of these experiments guided us to a sulfurization scheme in which both PADS (105) and the Stawinski reagent (103) were employed to sulfurize phosphite 107 and H-phosphonate 106, respectively. To this end, a mixture of phosphite triester 107 and H-phosphonate 106 (Figure 2.6, A) was treated with an excess (1.5 eq.) of 105 in dry CH$_3$CN. $^{31}P$ NMR analysis after 30 minutes showed the complete conversion of 107 into phosphorothioate 108 as well as the presence of unreacted H-phosphonate 106 (Figure 2.6, B). Addition of reagent 103 (1.5 eq) afforded, after 30 minutes, a mixture of the desired phosphorothioate triester 108 and the phosphorothioate diester 109 (Figure 2.6, C).
2.2 Results and Discussion

Scheme 2.7: Quenching of excess sulfur transfer reagents with TCEP.

Reagents and conditions: i) H$_2$O, Pyr (4:1, v/v).

The excess of sulfur transfer reagents was depleted by the addition of tris-(2-carboxyethyl)phosphine (TCEP) in pyridine and water (Scheme 2.7). The initially formed thioanhydride 111 and thiolactone 112 were hydrolysed in situ giving the water-soluble acids 114, 115 and 116. PS dimer 108 and PS diester 109 were isolated after extractive work-up involv-

Figure 2.6: $^{31}$P NMR spectra of the two-step sulfurisation protocol.

(A) Reaction mixture after DCI mediated coupling of $^{31}$P$_{Pom}$ dT$_{Oda}$ (84) and subsequent hydrolysis of phosphoramidite donor 91. (B) Reaction mixture after the addition of PADS (105). (C) Reaction mixture after the sequential addition of PADS (105) and 103. (D) Purified PS-hexamer $^{31}$PGACGTT$_{OH}$.
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Figure 2.7: Hexameric PS-oligonucleotide fragment.

Top left: HPLC trace of the crude fully protected PS-hexamer. Top right: HPLC trace of the crude, fully deprotected PS-hexamer. \( \text{HO}^{\text{GACGTT}} \text{OH} \). Bottom left: HPLC trace of the purified PS-fragment \( \text{HO}^{\text{GACGTT}} \text{OH} \). Bottom right: HPLC trace of crude, fully deprotected PS-hexameric fragment \( \text{HO}^{\text{GACGTT}} \text{OH} \), generated via automated solid-phase synthesis.

ing dilution of the reaction mixture with EtOAc/THF (5:2, v/v) and extraction with \( \text{H}_2\text{O}\), 10% KHSO\(_4\) (to remove pyridine) and 10% NaHCO\(_3\) (to remove DCI, reagent side products originating from the sulfurizing agents, TCEP (110) and tris-(2-carboxyethyl)thiophosphine (113)). The mixture of dimer 108 and diester 109 was detritylated with 0.1M HCl in CH\(_3\)CN/MeOH, followed by extractive work-up and further processing as described the preparation of regular phosphodiester oligonucleotides. The crude detritylated dinucleotide was isolated in \( \geq 95\% \) yield. Analysis by mass spectrometry, HPLC and \( ^{31}\text{P} \) NMR indicated the presence of trace amounts of H-phosphonate 106 (\( < 5\% \)).

Hexameric phosphorothioate ONs sequence: The applicability of the two-step sulfurization protocol is illustrated by the assembly of the hexameric oligothioate sequence \( \text{HO}^{\text{GACGTT}} \text{OH} \), representing the minimal sequence able to activate Toll-like receptor 9 in mice. The synthesis started from monomer 84 at 0.5 mmol scale and after five elongation cycles, crude PS-hexamer \( \text{HO}^{\text{G}^\text{Bu}^\text{A}^\text{Bu}^\text{C}^\text{Bu}^\text{G}^\text{Bu}^\text{T}^\text{Pom}^\text{T}^\text{Pom}^\text{OH}} \) was obtained in 67% overall yield (based on 84). After ammonolysis, the crude hexamer was subjected to preparative HPLC purification using triethylammonium acetate buffer at 60°C.\(^d\) The purified PS-hexanucleotide \( \text{HO}^{\text{GACGTT}} \text{OH} \) was obtained in 31% overall yield.\(^e\) The identity and homogeneity of purified the hexamer was confirmed by LC-MS, analytical HPLC analysis, \( ^{31}\text{P} \) NMR (Figure 2.6 D) and MALDI-TOF mass spectrometry (found: 1886.67, expected: 1886.23). Moreover, \( \text{HO}^{\text{GACGTT}} \text{OH} \) PS-oligonucleotide fragment was identical (HPLC, MS) with the same hexamer prepared via

\(^d\) Analytical HPLC-analysis of unprotected PS oligonucleotides were carried out at 60°C with hexafluoroisopropanol (HFIP) to suppress peak broadening due to PS-diastereoisomers.

\(^e\) Yield is based on \( \text{HO}^{\text{dT}} \text{Pom}^\text{OAda} \) and was determined by \( \text{A}^{260} \) units, \( \varepsilon = 58200 \text{ L mol}^{-1} \text{ cm}^{-1} \).
standard solid-phase synthesis (Figure 2.7). 129

2.3 Conclusion and Outlook

This chapter describes an efficient and high yielding protocol for the solution-phase synthesis of native and PS-oligonucleotide fragments up to 10 and 6 nucleotides respectively, via 3′ → 5′ elongation (Scheme 2.8).

Scheme 2.8: 3′→5′ Directed SPEM approach towards (PS) oligonucleotide fragments.

The presented method enables efficient large-scale preparation of (PS) oligonucleotide fragments by omitting costly chromatographic purification of ONs intermediates. The use of 1-adamantaneacetic acid for 3′-O-nucleoside protection, in combination with protective groups on the nucleobases and the internucleotide phosphate linkage, enabled the step-wise synthesis of ON fragments up to 10 nucleobases. It is envisaged that the utilization of higher diamondoid derivatives, eq. diamantane or triamantane, as 3′-O-nucleoside protection is a attainable strategy for the step-wise preparation of higher length ONs fragments.

2.4 Experimental Section

General. All reagents were used as received. CH₃CN (Biosolve, DNA synthesis grade) was used for the coupling reactions. HPLC monitoring of the coupling reactions as well as analysis of protected ONs were run on a JASCO system with simultaneous detection at 214, 254, and 280 nm. Solvent system: A: 75% H₂O in MeOH, B: 100% CH₃CN, C: 0.2M TEAA in MeOH. Usually a gradient of 20→90% B was applied for protected ON and solution C was fixed at 10%. Preparative HPLCs of completely deblocked ONs were conducted at 60°C, applying a gradient of 5→25% B and solution C was replaced by a 0.5M TEAA buffer and fixed at 10%. Analytical LC-MS of protected ONs was conducted on a JASCO system using an Alltima C₁₈ analytical column (5µm particle size, flow: 1.0 ml/min) or a Phenomenex® Gemini C₁₈ analytical column (3µm particle size, flow: 1.0 ml/min). Absorbance was measured at 214 nm and 254 nm. Solvent system: A: 100% H₂O, B: 100% CH₃CN, C: 1% TFA. Gradients of B in 10% C were applied over 15 minutes (Alltima column) or over 10 minutes (Phenomenex®)
2. Diamondoid assistance in the 3’→5’ directed solution-phase synthesis of ONs

Usually, for protected ONs a gradient of 50→90% B was used. Analytical LC-MS of completely deblocked ONs were conducted on a Surveyor HPLC system using an Altima C₁₈ column (5 µm particle size, flow: 1.0 ml/min). Solvent system: A: 0.4M HFiP, 16.3 mM TEA in a mixture of 5% MeOH/H₂O, B: 0.4M HFiP, 16.3 mM TEA in a mixture of 80% MeOH/H₂O. Gradients of 0→30% B were applied over 30 minutes. Mass spectra were recorded on a Perkin Elmer Sciex API 165 equipped with an electrospray interface (ESI) or on a LCQ Advantage Max equipped with an ESI interface. MALDI TOF spectra were recorded on a Voyager-DE PRO mass spectrometer (PerSeptive Biosystems). 

31P NMR spectra were measured on Bruker AC200 spectrometer. Chemical shifts (δ) are given in ppm relative to external standard H₃PO₄.

\[
\text{O} \quad \text{N} \quad \text{DMTrO} \quad \text{O} \quad \text{NH} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{3’-}\text{O-Adamantaneacetyl-5’-O-dimethoxytrityl-thymidine (78).}
\]

5’-O-Dimethoxytrityl-thymidine (10.9 g, 20 mmol) was coevaporated with CH₃CN and dissolved in CH₂Cl₂ (0.1 M). Molecular sieves (~10 g, 4Å), 1-adamantaneacetic acid (5.8 g, 30 mmol), EDC (6.14 g, 32 mmol) and DMAP (367 mg, 3 mmol) were added and the mixture was stirred overnight at RT. After TLC analysis (EtOAc/PE 1:1), which indicated completion of the reaction, the mixture was concentrated and the residue was dissolved in EtOAc (700 ml). The organic phase was washed with H₂O (250 ml), 10% KHSO₄ (250 ml), H₂O (250 ml), 10% NaHCO₃ (250 ml) and finally with brine (250 ml). The organic layer was dried (MgSO₄), filtrated and evaporated. The residue was subjected to flash column chromatography (FCC) (prepared in the presence of 3 vol% Et₃N, PE/EtOAc 1:3 → 3:1) to provide the title compound in 97% yield as a white foam. MS found (calc.) for [M+H]⁺: 721.5 (721.3). M.p. 104-108 °C.

\[
\text{1H NMR (300 MHz, CDCl₃) δ: 8.6 (bs, 1H, H-3), 7.6 (s, 1H, H-6), 7.4-7.2 (m, 9H, H-ar), 6.8 (d, J= 8.8, 4H, H-ar), 6.4 (t, J= 7.0, 1H, H-1’), 5.5 (bs, 1H, H-3’), 4.1 (s, 1H, H-4’), 3.8 (s, 6H, 2x OMe), 3.5 (s, 2H, H-5’), 2.4 (m, 2H, H-2’), 2.1 (s, 2H, CH₂COAda), 2.0 (bs, 3H, 3x CHAda), 1.7-1.6 (m, 12H, 6x CH₂Ada), 1.4 (s, 3H, CH₃ thymidine). 13C NMR (75 MHz; CDCl₃) δ: 171.0, 163.9, 158.5, 150.6, 144.1, 135.2, 135.0, 129.9, 127.9, 127.8, 127.0, 113.1, 111.4, 86.9, 84.2, 84.0, 74.5, 63.5, 55.0, 48., 42.2, 37.8, 36.4, 32.8, 28.3, 11.5. [α]D²⁰ = +4.6° (c = 1.0, CHCl₃).
\]

\[
\text{3’-O-Adamantaneacetyl-5’-O-dimethoxytrityl-N³-benzoylethymidine (79).}
\]

3’-O-Adamantaneacetyl-5’-O-dimethoxytrityl-thymidine (78, 23.8 g, 33 mmol) was dissolved in pyridine (0.17M) and DiPEA (30.9 ml, 181 mmol) and BzCl (5.9 ml, 51 mmol) were added. When TLC analysis (PE/EtOAc 2:1) indicated complete conversion of starting material the solvents were removed by evaporation and the crude mixture was taken up in EtOAc (300 ml), washed with 10% KHSO₄ (200 ml), water (200 ml) and 10% NaHCO₃ (200 ml). After drying (MgSO₄), filtration and evaporation the crude material was purified by FCC (column prepared in the presence of 3 vol% Et₃N, PE/EtOAc, 1:3 → 1:1) to give the title compound in 97% yield. HRMS found (calc) for [M+Na]⁺: 847.3573 (847.3565). M.p. 97-101 °C. 1H NMR (300 MHz; CDCl₃) δ: 8.0 (d,
2.4 Experimental Section

\[ J = 7.8, 2H, H-ar \), 7.8 (s, 1H, H-6), 7.7-7.2 (m, 12H, H-ar), 6.8 (d, \( J = 8.5, 4H, H-ar \)), 6.4 (t, \( J = 6.9, 1H, H-1' \)), 5.5 (s, 1H, H-3'), 4.2 (s, 1H, H-4'), 3.8 (s, 6H, 2x OMe), 3.5 (s, 2H, H-5'), 2.5 (bs, 2H, H-2'), 2.1 (s, 2H, CH\(_2\)COAda), 1.9 (bs, 3H, 3x CH Ada), 1.7-1.6 (m, 12H, 6x CH\(_2\)Ada), 1.4 (s, 3H, CH\(_3\) thymidine); \(^{13}\)C NMR (75 MHz; CDCl\(_3\)) \( \delta \): 171.1, 168.9, 162.7, 158.7, 149.3, 144.1, 135.1, 135.0, 134.9, 131.5, 130.4, 130.0, 129.0, 128.0, 127.9, 127.2, 113.2, 111.5, 87.2, 84.5, 84.3, 74.5, 63.5, 55.1, 48.5, 42.5, 38.1, 36.5, 32.9, 28.4, 11.5. \( [\alpha]_{D}^{20} = +5.6^\circ \) (c = 1.0, CHCl\(_3\)).

\( 3'O\)-adamantaneacetyl-5'-O-dimethoxytrityl-\( N^3 \)-benzoyl-thymidine (80).

\( 3'O\)-Adamantaneacetyl-5'-O-dimethoxytrityl-thymidine (78, 0.72 g, 1.0 mmol) was coevaporated trice with toluene and dissolved in pyridine (0.1 M). Piv-Cl (1.5 mmol, 185 µl) and cat. DMAP were subsequently added and the reaction mixture was stirred for 24 h at RT. The reaction mixture was concentrated and the residue was dissolved in EtOAc and subsequently washed with 10% aq. KHSO\(_4\), sat. aq. NaHCO\(_3\), brine, dried (MgSO\(_4\)), filtered and concentrated. The residue was purified by silica gel column chromatography (PE/Et\(_2\)O, 1:0 \( \rightarrow \) 0:1), to provide the title compound in 80% yield. \(^1\)H NMR (200 MHz; CDCl\(_3\)) \( \delta \): 1.35 (s, 9H, tBu - Piv), 1.34 (s, 3H, CH\(_3\) - thymidine), 1.46-1.81 (m, 12H, 6x CH\(_2\) - Ada), 1.96 (br. s, 3H, 3x CH - Ada), 2.06 (s, 2H, CH\(_2\) -Ada), 2.37-2.60 (m, 1H, H2'), 3.40-3.60 (m, 2H, H5'), 3.80 (s, 6H, 2x OMe), 4.13 (br. s, 1H, H4'), 5.39-5.52 (m, 1H, H3'), 6.39 (t, 1H, \( J = 7.1, H1' \)), 6.75-6.95 (m, 4H, Har), 7.13-7.47 (m, 9H, Har), 7.63 (s, 1H, H6). \(^{13}\)C NMR (50 MHz; CDCl\(_3\)) \( \delta \): 27.2, 28.4, 29.5, 32.8, 36.5, 38.0, 40.0, 42.3, 43.6, 48.4, 55.0, 63.4, 74.4, 84.2, 87.1, 111.1, 113.2, 127.1, 127.9, 128.0, 129.9, 130.6, 134.9, 135.0, 135.1, 144.1, 149.0, 158.7, 162.6, 170.9, 174.2, 183.6.

\( 3'O\)-adamantaneacetyl-5'-O-dimethoxytrityl-\( N^3 \)-pivaloy-thymidine (81).

\( 3'O\)-Adamantaneacetyl-5'-O-dimethoxytritylthymidine (78, 21.7 g, 30 mmol) was dissolved in DMF (0.2 M). K\(_2\)CO\(_3\) (16.6 g, 120 mmol) and chloromethylpivalate (13.1 ml, 90 mmol) were added and the mixture was stirred overnight. Et\(_2\)O (550 ml) and H\(_2\)O (350 ml) were added and the layers were separated. The aqueous layer was extracted with Et\(_2\)O (200 ml). The combined organic layers were washed with H\(_2\)O (200 ml) and brine (200 ml), dried (MgSO\(_4\)), filtered and evaporated. FCC (prepared in the presence of 3 vol% Et\(_3\)N, PE/Et\(_2\)O 1:1 \( \rightarrow \) 1:0) gave the title compound in 89% yield. HRMS found (calc) for [M+Na]^+: 857.3976 (857.3984). M.p. 89-92 °C. \(^1\)H NMR (300 MHz; CDCl\(_3\)) \( \delta \): 7.6 (s, 1H, H-6), 7.4-7.2 (m, 9H, H-ar), 6.8 (d, \( J = 8.5, 4H, H-ar \)), 6.5 (t, \( J = 6.2, 1H, H-1' \)), 6.0 (s, 2H, CH\(_2\)Pom), 5.5 (s, 1H, H-3'), 4.1 (s, 1H, H-4'), 3.8 (s, 6H, 2x OMe), 3.5 (s, 2H, H-5'), 2.5 (bs, 2H, H-2'), 2.1 (s, 2H, CH\(_2\)COAda), 2.0 (bs, 3H, 3x CH Ada), 1.7-1.6 (m, 12H, 6x CH\(_2\)Ada), 14 (s, 3H, CH\(_3\) thymidine), 1.2 (s, 9H, tBuPom); \(^{13}\)C NMR (75 MHz; CDCl\(_3\)) \( \delta \): 177.4, 177.1, 162.4, 158.7, 150.3, 144.1, 135.2, 135.0, 130.0, 128.0, 127.1, 113.1, 110.7, 87.1, 85.0, 84.1, 74.5, 65.1, 63.5, 55.1, 48.5, 42.3, 38.7, 38.0.
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36.5, 32.9, 28.4, 26.9, 12.1. \([\alpha]_{D}^{20} = +6.6^\circ\) (c = 1.0, CHCl₃).

3'-O-adamantaneacetyl-N³-benzoyl-thymidine (82).

3'-O-Adamantaneacetyl-5'-O-dimethoxytrityl-N³-benzoyl-thymidine (79, 26.4 g, 32 mmol) was dissolved in CH₂Cl₂ (0.08 M). Triethylsilane (11 ml, 68 mmol) and dichloroacetic acid (21 ml, 5% vol) were added. After completion of the reaction (45 min, TLC: PE/EtOAc 3:1) CH₂Cl₂ (200 ml) was added and the organic phase was extracted with 2% Na₂CO₃ (300 ml). The aqueous layer was back-extracted with CH₂Cl₂ (200 ml) and the combined organic layers were dried (MgSO₄), filtrated and concentrated. The crude material was applied onto FCC (PE/EtOAc 1:2 → 2:1) to afford the title compound in 92% yield. HRMS found (calc) for [M+H]⁺: 523.2479 (523.2439). M.p. 94-96 °C.

1H NMR (300 MHz; CDCl₃) δ: 7.9 (d, \(J = 7.2\), 1H, H-6), 7.7-7.6 (m, 5H, CH Bz), 6.3 (t, \(J = 7.2\), 1H, H-1'), 5.3 (m, 1H, H-3'), 4.1 (m, 1H, H-4'), 3.9 (bs, 2H, H-5'), 2.4 (m, 2H, H-2'), 2.3 (bs, 1H, OH), 2.1 (s, 2H, CH₂COAda), 1.969, 1.966 (3x CHAda, CH₃ thymidine), 1.7-1.6 (m, 12H, CH₂Ada).

13C NMR (75 MHz; CDCl₃) δ: 171.3, 168.8, 162.8, 149.2, 136.2, 135.0, 131.3, 130.3, 139.0, 110.9, 85.4, 85.3, 74.2, 62.1, 48.4, 42.2, 37.5, 36.4, 32.8, 28.3, 12.4. \([\alpha]_{D}^{20} = -6.2^\circ\) (c = 1.0, CHCl₃).

3'-O-adamantaneacetyl-N³-pivaloyloxymethyl-thymidine (84).

3'-O-Adamantaneacetyl-5'-O-dimethoxytrityl-N³-pivaloyloxymethyl-thymidine (81, 19.4 g, 25.6 mmol) was dissolved in CH₂Cl₂ (0.1 M). Triethylsilane (8.2 ml, 51.2 mmol) and dichloroacetic acid (13 ml, 5 vol%) were added. After 30 minutes the initial colouring disappeared and TLC analysis (PE/Et₂O 1:2) indicated complete detritylation. CH₂Cl₂ (250 ml) was added and the resulting mixture was extracted with 2% Na₂CO₃ (400 ml). The aqueous layer was back-extracted with CH₂Cl₂ (150 ml) and the combined organics were dried (MgSO₄), filtrated and concentrated. FCC (PE/Et₂O 2:1 → 1:0) gave the title compound as a white foam in 94% yield. HRMS found (calc) for [M+H]⁺: 533.2860 (533.2857). M.p. 63-68 °C. ¹H NMR (300 MHz; CDCl₃) δ: 7.7 (s, 1H, H-6), 6.3 (t, \(J = 6.8\), H-1'), 6.0 (s, 2H, CH₂Pom), 5.3 (bs, 1H, H-3'), 4.1 (s, 1H, H-4'), 3.9 (m, 2H, H-5'), 2.9 (bs, 1H, OH), 2.4 (bs, 2H, H-2'), 2.1 (s, 2H, CH₂COAda), 1.9 (m, 3H, 3x CHAda), 1.7 (m, 12H, 6x CH₂Ada), 1.2 (s, 9H, tBuPom); ¹³C NMR (75 MHz; CDCl₃) δ: 171.3, 168.7, 162.8, 149.2, 136.2, 135.0, 131.3, 130.3, 139.0, 110.9, 85.4, 85.3, 74.2, 62.1, 48.4, 42.2, 37.5, 36.4, 32.8, 28.3, 12.4. \([\alpha]_{D}^{20} = -9.0^\circ\) (c = 1.0, CHCl₃).

5'-O-dimethoxytrityl-3'-O-levulinoyl-thymidine (85).

5'-O-Dimethoxytritylthymidine (77, 7.59 g, 14.0 mmol) was coevaporated with and dissolved in dioxane (0.14 M). To this solution were added levulinic acid (2.87 ml, 28.0 mmol), EDC (5.4 g, 28 mmol) and DMAP (171 mg, 0.1 mmol). After stirring for 2.5 h at RT, TLC analysis (CH₂Cl₂/Methanol 95:5) indicated complete conversion of the SM. The volatiles were removed and the
residue was dissolved in CH$_2$Cl$_2$ (100 ml) and washed with H$_2$O, 10% KHSO$_4$, and 3x 10% NaHCO$_3$, filtered (MgSO$_4$), filtered and concentrated to give a foam (85), which was directly used in the next reaction. MS found (calc) for [M+H]$^+$: 643.4 (643.3). M.p. 88-92 °C. $^1$H NMR (300 MHz; CDCl$_3$) δ 8.7 (s, 1H, H-3), 7.6 (s, 1H, H-6), 7.4-7.2 (m, 9H, H-ar), 6.9 (d, J= 8.9, 4H, H-ar), 6.4 (t, J= 7.0, 1H, H-1’), 5.5 (bs, 1H, H-3’), 4.1 (s, 1H, H-4’), 3.8 (s, 6H, 2x OMe), 3.5 (bs, 2H, H-5’), 2.8 (t, J= 6.8, 2H, CH$_2$ Lev), 2.6 (t, J= 6.3, 2H, CH$_2$Lev), 2.5 (m, 2H, H-2’), 2.2 (s, 3H, CH$_3$Lev), 1.4 (CH$_3$ thymidine). $[^\alpha]_{D}^{20} = +8.0$° (c = 1.0, CHCl$_3$).

5’-$O$-dimethoxytrityl-3’-$O$-levulinoyl-$N^3$-pivaloyloxymethyl-thymidine (86).
The foam obtained in the previous reaction (85, 12.1 g, ~14 mmol) was dissolved in DMF (0.2 M) and chloromethylpivalate (6.1 ml, 42 mmol) and K$_2$CO$_3$ (7.73 g, 56 mmol) were added and the mixture was stirred overnight at RT. TLC analysis (100% Et$_2$O) indicated the formation of a more lipophilic product. The reaction mixture was diluted with Et$_2$O (250 ml) and washed with H$_2$O (150 ml). The aqueous layer was back-extracted with Et$_2$O (100 ml) and the combined organics were concentrated to give an oil. The crude material was subjected to FCC (prepared using 1% Et$_3$N, 100% Et$_2$O, isocratic) to provide the title compound in 96% yield. HRMS found (calc) for [M+Na]$^+$: 779.3150 (779.3150). M.p. 59-64 °C. $^1$H NMR (300 MHz; CDCl$_3$) δ: 7.7 (s, 1H, H-6), 7.4-7.2 (m, 9H, H-ar), 6.8 (d, J= 8.7, 4H, H-ar), 6.3 (t, J= 6.6, 1H, H-1’), 5.8 (bs, 2H, CH$_2$Pom), 5.4 (bs, 1H, H-3’), 4.1 (s, 1H, H-4’), 3.7 (s, 6H, 2x OMe), 3.4 (bs, 2H, H-5’), 2.7 (t, J= 6.2, 2H, CH$_2$Lev), 2.5 (t, J= 6.2, 2H, CH$_2$Lev), 2.4 (m, 2H, H-2’), 2.1 (s, 3H, CH$_3$Lev), 1.3 (s, 3H, CH$_3$ - thymidine), 1.1 (9H, tBu Pom). $^{13}$C NMR (75 MHz; CDCl$_3$) δ: 206.6, 177.8, 172.5, 162.8, 159.0, 150.6, 144.5, 135.5, 134.7, 130.4, 128.4, 127.5, 113.6, 111.1, 87.5, 85.3, 84.3, 75.8, 65.4, 64.0, 55.5, 39.1, 38.2, 38.0, 30.0, 28.2, 27.3, 12.5. $[^\alpha]_{D}^{20} = +7.0$° (c = 1.0, CHCl$_3$).

5’-$O$-dimethoxytrityl-$N^3$-benzoyl-thymidine (88).
N3-benzoyl-thymidine (87, 9.7 g, 28 mmol) was coevaporated thrice with pyridine and dissolved in pyridine (0.2M). Dimethoxytritylchloride (11.2 g, 33 mmol) was added and the mixture was stirred for 45 min TLC analysis (PE/EtOAc 1:1) indicated complete conversion of the starting compound into a higher running product ($R_F$ = 0.4) and the mixture was concentrated. The residue was taken up in EtOAc (200 ml) and washed with H$_2$O (100 ml), 5% KHSO$_4$ (2x 100 ml), H$_2$O (100 ml) and finally brine (100 ml). After drying (MgSO$_4$), filtration and evaporation the crude product was applied subjected to FCC (prepared in the presence of 3 vol% Et$_3$N, PE/EtOAc 2:1 → 1:2) to provide the title compound in 87% yield. HRMS found (calc) for [M+H]$^+$: 649.2558 (649.2544). M.p. 100-105 °C. $^1$H NMR (300 MHz; CDCl$_3$) δ: 7.9 (d, J= 7.3, 2H, H-ar), 7.7 (s, 1H, H-6), 7.6-7.2 (m, 12H, H-ar), 6.8 (d, J= 8.5, 4H, H-ar), 6.4 (t, J= 7.1, 1H, H-1’), 4.5 (bs, 1H, H-3’), 4.0 (bs, 1H, H-4’), 3.8 (s, 6H, 2x OMe), 3.40 (dd, J= 10.6, 2.5,
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2.7, 1H, H-5’a), 3.3 (dd, J = 10.6, 2.6, 2.7, 1H, H-5’b), 2.5 (bs, 1H, OH), 2.3 (m, 2H, H-2âĂŽZ), 1.4 (s, 3H, CH$_3$ thymidine). $^{13}$C NMR (75 MHz; CDCl$_3$) δ: 168.9, 162.8, 158.4, 149.1, 144.0, 135.8, 135.1, 131.1, 130.2, 129.8, 128.9, 127.8, 126.9, 113.0, 110.8, 86.6, 86.8, 84.8, 71.8, 63.3, 55.0, 40.8, 11.4. [$\alpha$]$_D^{20}$ +5.4 (c 1.0, CHCl$_3$).

$^{5'}$-O-dimethoxytrityl-$N^3$-pivaloyloxymethyl-thymidine (89).

$^{5'}$-O-Dimethoxytrityl-$3'$-O-levulinoyl-$N^3$-pivaloyloxymethyl-thymidine (86, 30.2 g, 40 mmol) was dissolved in a mixture of pyridine and acetic acid (0.1 M, 4:1, v/v) and hydrazine monohydrate (1.9 ml, 60 mmol) was added. TLC analysis (100% EtOAc) after 30 min showed the formation of a more hydrophilic compound. Excess hydrazine was quenched by the addition of acetylacetone (8.3 ml, 80 mmol) and the mixture was stirred for 5 min. The solvents were removed by evaporation, the residue was taken up in Et$_2$O (350 ml) and washed with H$_2$O (350 ml), 10% KHSO$_4$ (2x 350 ml), 10% NaHCO$_3$ (2x 350 ml) and finally brine (350 ml). After drying (MgSO$_4$) and filtration the solvents were removed and the crude product was subjected to FCC (prepared with 3 vol% Et$_3$N, Et$_2$O, isocratic) to give the title compound in 97% yield. HRMS found (calc) for [M+Na]$^+$: 681.2792 (681.2783). M.p. 91-95 °C.

$^{5'}$-O-dimethoxytrityl-$N^3$-benzoylthymidine-3'-(2-cyanoethyl-$N,N$-diisopropyl)-phosphoramidite (90).

$^{5'}$-O-Dimethoxytrityl-$N^3$-benzoyl-thymidine (88, 13 g, 20 mmol) was coevaporated with CH$_3$CN, dissolved in CH$_2$Cl$_2$ (70 ml) and put under an argon atmosphere. DiPEA (13.6 ml, 80 mmol) was added followed by dropwise addition (dropping funnel) of a solution of chloro-2-cyanoethyl-$N,N$-diisopropylphosphoramidite (92, 5.9 g, 25 mmol) in CH$_2$Cl$_2$ (40 ml). When TLC analysis (EtOAc/PE, 1:2) indicated complete conversion of the starting compound, the mixture was concentrated and the residue applied onto a silica gel column (prepared in the presence of 3 vol% Et$_3$N) and eluted with a gradient of EtOAc in PE (1:2 → 1:1). Evaporation of the correct fractions yielded the title compound (13.4 g, 15.8 mmol) as a white foam (79% yield). M.p. 81-85°C. HRMS found (calc) for [M+H]$^+$: 849.3651 (849.3623). $^1$H NMR (300 MHz, CDCl$_3$, two diastereoisomers): δ 8.0 (d, J = 7.9, 2H, H arom Bz), 7.8, 7.7 (2x s, 1H, H-6), 7.7-7.2 (m, 14H, Har Bz, DMTr), 6.9 (m, 4H, Har, DMTr), 6.4 (m, 1H, H1’), 4.7 (br. s, 1H, H3’), 4.20, 4.14 (2x s, 1H, H-4’), 3.8 (s, 6H, 2x OMe), 3.7-3.4 (m, 3m, H-2’, H-5’), 3.3 (m, 1H, CH - iPr), 2.6-2.3 (m, 5H, 2x CH$_2$ - CE, CH - iPr), 1.4 (s, 3H, CH$_3$ - thymidine), 1.2, 1.0 (2x m,
2.4 Experimental Section

$^{13}$C NMR (50 MHz, CDCl$_3$, two diastereoisomers): $\delta$ 168.8, 162.4, 158.3, 148.9, 143.9, 135.3, 134.9, 134.6, 131.2, 129.7, 128.7, 127.8, 127.6, 126.8, 117.4, 117.2, 112.9, 110.7, 86.5, 85.4, 84.6, 73.1, 62.6, 57.9, 57.6, 54.8, 42.9, 42.6, 39.7, 24.1, 19.8, 11.3; $^{31}$P NMR (80.7 MHz, CDCl$_3$, two diastereoisomers): $\delta$ 149.3, 148.9. $[\alpha]_D^{20} = +20.6^\circ$ (c = 1.0, CHCl$_3$).

**5'-O-dimethoxytrityl-N$^3$-pivaloyloxymethylthymidine-3'-O-(2-cyanoethyl-N,N-diisopropyl)-phosphoramidite (91).**

5’-O-Dimethoxytrityl-N$^3$-pivaloyloxymethyl-thymidine (89, 25.2 g, 38.3 mmol) was coevaporated with CH$_3$CN, dissolved in CH$_2$Cl$_2$ (200 ml) and put under an argon atmosphere. DiPEA (23.2 ml, 153.2 mmol) was added, followed by slow addition (approx. 15 min) of a solution of chloro-2-cyanoethyl-N,N-diisopropylphosphoramidite (47.9 mmol, 10.7 ml) in CH$_2$Cl$_2$ (200 ml) using a dropping funnel. After complete addition, the mixture was stirred for another 30 min TLC analysis (100% Et$_2$O) indicated complete conversion of the starting compound into a higher running spot. After evaporation of the solvent, the residue was applied to silica gel column (prepared with 3 vol% Et$_3$N in diethylether) and eluted with Et$_2$O. Concentration of the right fractions afforded the title phosphoramidite as a white foam in 86% yield. Upon storage, the compound turns into a glass-like substance. HRMS found (calc) for [M+Na]$^+$: 881.3871 (881.3861). M.p. 70-75°C. $^1$H NMR (300 MHz, CDCl$_3$, two diastereoisomers): $\delta$ 7.7 (2x s, 1H, H6), 7.4-7.2 (m, 9H, Har DMTr), 6.8 (m, 4H, Har - DMTr), 6.4 (m, 1H, H1’), 6.0 (s, 2H, CH$_2$ - Pom), 4.7 (br. s, 1H, H3’), 4.2 (2x s, 1H, H4’), 3.8 (s, 6H, 2x O Me), 3.6-3.5 (m, 4H, H2’, H5’), 3.3 (m, 1H, CH - iPr), 2.6-2.4 (m, 4H, 2x CH$_2$ - CE), 1.6, 1.5 (2x s, 3H, CH$_3$ - thymidine), 1.197 (s, 9H, tBu - Pom), 1.95-1.0 (m, 12H, 4x CH$_3$ - iPr). $^{13}$C NMR (75 MHz, CDCl$_3$ two diastereoisomers): $\delta$ 177.0, 162.1, 158.3, 149.9, 143.9, 134.9, 134.3, 129.8, 127.8, 127.7, 127.6, 126.7, 177.3, 177.1, 122.9, 109.9, 86.5, 85.3, 85.0, 73.5, 73.3, 73.0, 72.8, 64.7, 62.7, 62.6, 58.0, 57.9, 57.8, 57.6, 54.8, 42.93, 42.87, 42.77, 42.70, 39.7, 38.4, 26.6, 24.2, 20.0, 19.9, 19.8, 19.7, 12.0; $^{31}$P NMR (80.7 MHz, CDCl$_3$, two diastereoisomers): $\delta$ 149.4, 148.9. $[\alpha]_D^{20} = +17.0^\circ$ (c = 1.0, CHCl$_3$).

Representable procedure for one elongation cycle en route to native ONs:

**Coupling and oxidation:** Compound 84 (when n=0, 0.5 mmol) and 1.5 eq. of phosphoramidite monomer (91) were mixed and coevaporated with CH$_3$CN (3x). The mixture was dissolved in CH$_3$CN (3.5 ml) under an argon atmosphere and DCI (266 mg, 2.25 mmol) was added. The resulting reaction mixture was stirred for 30 min at RT followed by the addition of capping reagent 100 (136 mg, 0.5 mmol). Samples for HPLC analysis were prepared by diluting an aliquot (10 µL) of the reaction mixture with a mixture of CH$_3$CN/MeOH 1/3 (1 ml). H$_2$O (175 µL) was added and the reaction mixture was stirred for 2 min A solution of I$_2$ (0.2M in THF/pyridine 4/1, 7.5 ml) was added and the mixture was stirred for 5 min The reaction mixture was transferred into a separatory funnel and diluted with EtOAc (15 ml). The mixture was
2. Diamondoid assistance in the 3'→5' directed solution-phase synthesis of ONs

subsequently extracted with a solution of 1M Na$_2$S$_2$O$_3$ (15 ml), 10% KHSO4 (2x 15 ml), 5% ceNaHCO3 (1x 15 ml) and finally with a mixture of brine/H$_2$O (1:1, 15 ml). The organic phase was dried (MgSO$_4$), filtrated and concentrated to give a white foam.

**Detritylation:** A stock-solution of 0.1M HCl was prepared by the (careful) addition of AcCl to a mixture of CH$_3$CN/ MeOH (1:6, v/v). The foam containing the ON and the monomer was dissolved in the acidic stock-solution (14 ml) and the progress of the detritylation was monitored by TLC (CH$_2$Cl$_2$/MeOH, 9:1). After complete disappearance of the orange ON-spot (spray with 20% aq. H$_2$SO$_4$ in EtOH) 0.23M aqueous TEAA solution (prepared by pre-mixing 0.7 ml of the commercial 2M TEAA solution and 5.3 ml of H$_2$O) was added. The resulting mixture was transferred into a separatory funnel. The flask was rinsed with CH$_3$CN (total amount 10 ml). The mixture was extracted with heptane/Et$_2$O (2:1, v/v, 4x 30 ml). Next, EtOAc (20 ml) and H$_2$O (20 ml) were added and the resulting layers were separated. The organic phase was washed with H$_2$O (20 ml), 5% NaHCO$_3$ (2x 20 ml) and finally with a mixture of brine/H$_2$O (1:1, v/v, 20 ml). After drying (MgSO$_4$), filtration and evaporation of the solvents the oligonucleotide was isolated as a white foam. Yields were estimated by dividing the amount of material obtained (in mg) with the expected amount (0.5 mmol x MW oligonucleotide).

**Representable procedure for one Elongation cycle and route to PS-oligonucleotides:**

Compound 84 (0.5 mmol) and 1.5 equiv of phosphoramidite monomer (91) were mixed and coevaporated with CH$_3$CN (3x). The mixture was dissolved in CH$_3$CN (3.5 ml) under an argon atmosphere and DCI (266 mg, 2.25 mmol) was added. After 30 min, samples for HPLC analysis were prepared by diluting an aliquot (10 µl) of the reaction mixture with a mixture of CH$_3$CN/MeOH (1:3, v/v, 1 ml). Upon completion of the reaction, water (175 µl was added, and after 2 min the reaction mixture was concentrated and coevaporated with CH$_3$CN (3x). The resulting foam was dissolved in CH$_3$CN (6.0 ml) under an argon atmosphere. PADS (105) (227 mg, 0.75 mmol) was added to the reaction mixture followed, after 30 min, by the addition of 103 (126 mg, 0.75 mmol). The reaction mixture was stirred for an additional 30 min and a solution of TCEP·HCl (645 mg, 2.25 mmol) in pyridine/H$_2$O (1:1, v/v, 4 ml) was added and the stirring was continued for another 45 min. The reaction mixture was transferred to a separatory funnel and diluted with a mixture of EtOAc /THF (5:2, v/v, 35 ml). The mixture was subsequently extracted with H$_2$O (15 ml), a solution of 10% KH$_2$O$_4$ (3 x 15 ml), 10% NaHCO$_3$ (3 x 15 ml) and finally with a mixture of brine/H$_2$O (1:1, v/v, 15 ml). The organic layer was dried (MgSO$_4$), filtrated and concentrated to give a light yellow foam. The foam containing the ON and the excess monomer were subjected to the detritylation step as described earlier. The protected ONs were analysed by LC-MS, HPLC and $^{31}$P-NMR. Yields were estimated by dividing the amount of obtained material (mg) with the expected amount (0.5 mmol x MW oligonucleotide).

**General procedure for complete deblocking of ON fragments:**

5'-OH ON (50 mg) was treated with concentrated NH$_3$ solution (25 ml) at 55°C for 48 h. After cooling of the reaction mixture to room temperature, the mixture was concentrated. The
residue was dissolved (cloudy mixture) in H₂O and centrifuged. The supernatant (appr. 6 ml) was analysed by LC/MS as well as mono-Q ion-exchange chromatography using a gradient of 1M NaCl in 10 mM NaCl (0-50%).

**Purification and desalting of**[^1]**HOAGCTATTGCT_{OH}**:
After deblocking and centrifugation as described above, the aqueous solution containing the crude, fully deprotected ON was purified using Q-Sepharose ion-exchange chromatography utilizing a gradient of 1M NaOH in 10 mM NaCl (0→35% over 1 CV, then 35→65% over 10 CV). After concentration of the appropriate fractions, the residue was dissolved in H₂O and desalted over a Sephadex G-25 column using 0.15M NH₄HCO₃ as the eluent. After pooling, evaporation and lyophilisation, the yield was determined by measuring the absorption at 260 nm (ε= 55100 L mol⁻¹ cm⁻¹).

**Purification of**[^2]**HOAGCGTT_{OH}**:
The supernatant containing the crude fully deprotected ON, obtained as described above, was purified over preparative HPLC at 60°C. The fractions containing the pure hexamer (determined by analytical HPLC at 60°C) were collected, concentrated and lyophilized. The yield was determined by measuring the absorption at 260 nm (ε = 58200 L mol⁻¹ cm⁻¹).
3

Diamondoid assistance in the 5’→3’ directed solution-phase synthesis of (PS) oligonucleotide fragments

3.1 Introduction

The solution-phase preparation of oligonucleotide (ON) fragments via step-wise chain assembly is hampered by the increase of hydrophilicity that accompanies the growth of chain length of protected ON fragments. The repeated cumbersome isolation procedures of protected, polar intermediate ON fragments is detrimental for the overall yield of the pure ON target. This drawback associated with linear approaches to synthetic ONs can be circumvented by a block-coupling approach. The block-coupling approach has been explored by numerous chemists which resulted in a plethora of methods for the formation of the internucleotide phosphate or phosphorothioate linkage between two suitably protected ON fragments.\textsuperscript{95,99,100,130,131} For instance, the H-phosphonate method was recently adapted in the synthesis of Vitravene®, a 21-mer phosphorothioate ON, by repeated block-coupling of a trimeric ON fragment.\textsuperscript{131}

**Block coupling approach**

The Solution-Phase Extraction Method (SPEM) for synthetic ONs (described in chapter 2) is efficient up to the synthesis of decameric native- and hexameric phosphorothioate ONs. A convergent synthetic strategy can be applied to produce larger ON fragments. An illustration of the envisaged block coupling approach is depicted in Scheme 3.1 and requires two partially protected ON fragments IX, X and bis-functional phosphorylation reagent XI. The linear,
3.1 Introduction

Scheme 3.1: Illustration of the envisaged block coupling approach towards ON chain assembly.

3’→5’ directed SPEM approach can be used to generate partially protected ON fragments X, with a free 5’-OH, in good overall yields (phosphate decamer ~70% yield, phosphorothioate hexamer ~67% yield, chapter 2). However, this method only allows the generation of partially protected ON fragments bearing a free 5’-OH. Liberation of the base labile 3’-protective group would also result in cleavage of the cyanoethyl- and the base-labile nucleobase protective groups.

It was envisaged that the reverse chain elongation can be used to generate partially protected ON fragment IX, with a free 3’-OH, via a SPEM approach. Hereto, a synthetic strategy was devised utilizing 5’-O-acyl-1-adamantane protection (OAda, 117, Figure 3.1). The DMTr (118) protective group for temporary masking of the 3’-OH would be ideal. However, the installment of the DMTr on the 3’-OH is cumbersome due to increase steric hindrance. Therefore, the levulinoyl (Lev) protective group was selected for temporary 3’-O-nucleotide protection. The Lev (119) group is frequently employed for hydroxyl protection and can be cleaved under basic- or buffered conditions.132

Figure 3.1: Selection of protective groups for 5’- and 3’-O-nucleoside protection.
3. Diamondoid assistance in the 5’→3’ directed solution-phase synthesis of ONs

3.2 Results and Discussion

The envisaged strategy for the preparation of ONs by 5’ → 3’ chain elongation via the SPEM approach, is depicted in Scheme 3.2. This strategy entails three similar stages as described in chapter 2 for the 3’→5’ directed ON chain assembly via the SPEM approach, namely:

- Elongation of the 3’-OH of properly protected nucleoside XIII (n = 0) with an excess of phosphoramidite donor XIV, followed by capping of unreacted acceptor XIII (n = 0) with phosphoramidite capping reagent 100.
- Hydrolysis of excess phosphoramidite donor XIV and capping reagent 100, oxidation or sulphurisation of the reactive ON intermediate XV (n = 0) as well as the H-phosphonate monomer XVI and 120, followed by extractive work-up.
- Removal of the 3’O-Lev protection in XVII (n = 1) combined with the extractive removal of its by-products and excess monomer.

Scheme 3.2: Strategy for the 5’→3’ directed synthesis of ON fragments via a SPEM approach.

Reagent and conditions: i) Elongation: 4,5-dicyanoimidazole, dry CH₃CN; ii) Capping: 1 eq. 100, iii) Oxidation: X = O, 0.2M I₂ in H₂O/pyridine (5:1, v/v). Sulfurisation: X = S, phenylacetyl disulphide (105) then 3H-1,2-benzodithiol-3-one (103) then TCEP·HCl in Pyr. Extractive work-up 1; iv) 3’-O-Lev cleavage: 0.5M N₂H₄·H₂O in Pyr/AcOH (4:1, v/v). Extractive work-up 2. Bpg = ABz, CBz, G³Bu or T⁵Pom.

Monomer synthesis: The synthesis of thymidine acceptor 121, bearing the 5’-O-Ada protective group was undertaken. To this end, the N³ position of thymidine (122, Scheme 3.3) was protected
3.2 Results and Discussion

with the pivaloyloxyxymethyl (Pom) group. Following reported procedures, $^{114}$ TBSO$d^{Pom}_{OTBS}$ (123) was obtained in 97% yield over two steps. TBAF promoted cleavage of the TMS-ethers in 123 was not productive and therefore Et$_3$N·3 HF was applied to give 124 in 75% yield. Next, selective Mitsunobu type esterification of the 5'-OH of 124 with 1-adamantaneacetic acid was executed to give pure acceptor $^{AdaO}d^{Pom}_{OTOH}$ (121) in 84% yield.

Scheme 3.3: 5'-adamantyl acyl-thymidine building block synthesis.

Reagent and conditions: i) TBSCI (2.2 eq.), imidazole (4.4 eq.), DMF (0.2 M), RT, 1 h; ii) pivaloyloxyxymethyl chloride (3.0 eq), K$_2$CO$_3$ (4.0 eq.), DMF (0.2 M), RT, 12 h, 97% over two steps; iii) Et$_3$N·3 HF (2.0 eq.), Pyr (0.5 M), RT, 12 h, 75%; iv) 1-adamantaneacetic acid (1.0 eq.), DIAD (1.0 eq.), PPh$_3$ (1.0 eq), DMF (0.25 M), RT, 12 h, 84%.

Scheme 3.4: 5'-phosphoramidite monomer synthesis.

Reagent and conditions: i) LevOH (2.0 eq.), EDC·HCl (2.0 eq.), DMAP (0.05 eq.), 1,4-dioxane (0.2 M), 129 = 97%, 130 = 96% and 131 = near quant.; ii) PomCl (3.0 eq.), K$_2$CO$_3$ (4.0 eq.), DMF (0.25 M), RT, 12 h, 92% (two steps); iii) 5 vol% DCA in CH$_2$Cl$_2$ (0.1 M), Et$_3$SiH (4.0 eq.), RT, 15 min, 132 = 95%, 133 = 75%, 134 = 87% and 135 = 76%; iv) (N-diisopropyl)-O-cyanoethyl-phosphorylchloride (1.25 eq.), DiPEA (4.0 eq.), CH$_2$Cl$_2$ (0.1 M), 0°C, 60 min, 136 = 90%, 137 = 52%, 138 = 57% and 139 = 53%.

The synthesis of the 5'-O-phosphoramidite nucleotides, with 3'-O-Lev protection was executed according to Scheme 3.4, from the commercially available 5'-O-DMTr protected nucle-
3. Diamondoid assistance in the 5’→3’ directed solution-phase synthesis of ONs

osides. First, the 3’-OH of nucleosides 125, 126, 127 and 128 were esterified with Lev-OH under the agency of EDC and catalytic DMAP. The resulting 3’-O-Lev protected nucleosides 85, 129, 130 and 131 were obtained in excellent yields. The N3 position of crude thymidine derivative 85 was protected by reaction with Pom-Cl to give 86 in 92% yield over two steps. Dichloroacetic acid (DCA) mediated detritylation of the 5’-O-DMTr group of thymidine derivative 86 proceeded without complications to give 132 in 95% yield. However, DCA mediated detritylation of purine derivatives 129 and 130 were accompanied by (substantial) depurination. It was found that depurination of adenosine and guanosine derivatives 129 and 130 can be suppressed by the immediate precipitation of products 133 and 134 from the reaction mixture and 133 and 134 were obtained in 75% and 87%, respectively. Detritylation of cytidine derivative 131 produced 135 in 76% yield.

Next, the 5’-OH of nucleosides 132, 133, 134 and 135 were phosphitylated with 2-cyanoethyl N,N-diisopropylchlorophosphoramidite. The phosphitylation reactions were usually complete within 60 min, however the isolation of the products was cumbersome and required multiple purification steps. Thymidine building block 136, which did not suffer from purification difficulties, was isolated in 86% yield over two steps. The corresponding 5’-O-phosphoramidite monomers 137, 138 and 139 were obtained in about 40% overall yield over three steps.

ON synthesis

Stage 1: With the 5’-O-phosphoramidite monomers 136, 137, 138 and 139 in hand, the feasibility of the envisaged 5’→3’ SPEM was evaluated. Hereto, the synthesis of dimer AdaOdT\text{Pom}dT\text{Pom}OH was undertaken. Applying the coupling conditions as reported for the SPEM in the 3’→5’ direction (chapter 2), an incomplete coupling was observed after 30 min between monomer AdaOdT\text{Pom}OH (121, 0.5 mmol) and 136. Therefore the condensation of 121 was executed with two equivalents of phosphoramidite 136. After 30 min, the reaction progress was analysed by TLC and HPLC, which revealed complete consumption of monomer 121. Traces of 121 were capped by the addition of equimolar amounts of bis-(2-cyanoethyl)-N,N-diisopropyl phosphoramidite (100) to the reaction mixture.

Stage 2: The excess of phosphoramidite donor and capping reagent were hydrolyzed by the addition of 5 vol% H2O. Subsequently, the thus obtained H-phosphonate intermediate XVI (and also the H-phosphonate 120 derived from 100, Scheme 3.2) and phosphite XV (n = 0) were oxidized with 0.2M I2 solution in Pyr/THF. After 5 min, the reaction mixture was diluted with EtOAc and subsequently extracted with 1M Na2S2O3, 10% KHSO4, 10% NaHCO3 and finally brine/H2O (1:1, v/v). After drying of the combined organics over MgSO4, filtration and evaporation of the solvents, crude protected dimer AdaOdT\text{Pom}dT\text{Pom}OHLev and phosphodiester HO(OCE)(O=)POdT\text{Pom}dT\text{Pom}OHLev were obtained.

Stage 3: The completion of one coupling cycle involves the selective cleavage of the temporary 3’-O-Lev group. Delevulinoylation can be accomplished under saponification conditions or by reaction with N2H4 · H2O under buffered conditions (Pyr/AcOH (4:1, v/v)). To avoid cleavage of the base labile nucleobase protective groups, the latter conditions were adopted for Lev
3.2 Results and Discussion

deprotection studies. Employing a 0.5M hydrazine solutions, TLC analysis showed complete consumption of the ON material after 5 min at RT. The reaction mixture was diluted with a mixture of EtOAc/THF (2:5, v/v) and subsequently washed with H₂O, 10% KHSO₄, 10% NaHCO₃ and finally with a mixture of brine/H₂O (1:1, v/v). Residual H₂O was depleted by drying of the reaction mixture over MgSO₄, and after filtration and evaporation of the solvent, crude dimer \( \text{AdaO}d\text{T}^{\text{Pom}}dT_{\text{OH}}^{\text{Pom}} \) was obtained in quantitative yield (based on 121) and high purity (Figure 3.2A).

Figure 3.2: HPLC trace of (protected) ON fragments and target hexamer \( \text{H}^0\text{TACTG}_{\text{OH}} \).

A - D: Crude intermediate ON fragments obtained after a full coupling cycle. E: Crude, fully protected target hexamer. F: Crude, fully deprotected hexamer \( \text{H}^0\text{TACTG}_{\text{OH}} \). G: Hexamer \( \text{H}^0\text{TACTG}_{\text{OH}} \) after ion-exchange chromatography and desalting (Sephadex G25).

Hexamer ON sequence: Next, the obtained dimer \( \text{AdaO}d\text{T}^{\text{Pom}}dT_{\text{OH}}^{\text{Pom}} \) was elongated via the above described procedure. The second elongation cycle was executed with amidite 137, to give expected trimer \( \text{AdaO}d\text{T}^{\text{Pom}}dT_{\text{OH}}^{\text{Pom}}d\text{A}^{\text{Bz}}_{\text{OH}} \) in quantitative yield and high purity. The subsequent coupling with amidite 139 proceeded less efficient and required prolonged reaction times (1 h) to reach completion. Additional, extractive work-up of the reaction mixture suffered from difficult separation of the organic- and aqueous layer. These difficulties resulted in a drop in yield of the obtained tetramer \( \text{AdaO}d\text{T}^{\text{Pom}}dT_{\text{OH}}^{\text{Pom}}d\text{A}^{\text{Bz}}d\text{C}^{\text{Bz}}_{\text{OH}} \) (≈96% yield based on isolated ON material, Figure 3.2C). Furthermore, LCMS analysis of the crude tetramer showed the presence of small amounts of by-products which are derived from the parent ON, in which one or more cyanoethyl- and/or benzoyl protective groups are cleaved. After two additional coupling cycles, crude \( \text{AdaO}d\text{T}^{\text{Pom}}dT_{\text{OH}}^{\text{Pom}}d\text{A}^{\text{Bz}}d\text{C}^{\text{Bz}}dT_{\text{OH}}^{\text{Pom}}d\text{G}^{\text{iBu}}_{\text{OLev}} \) was isolated in an overall yield of 86% based on isolated ON material. Yields were estimated by dividing the amount of obtained material (mg) with the expected amount (0.5 mmol x MW oligonucleotide).
on $^{3}Ado\,dT^{Pom}_{OH}$ (121). A small fraction (50 mg) of the obtained ON material was subjected to ammonolysis at 55°C. After ion-exchange chromatography and desalting (Sephadex G25), pure unprotected hexamer $^{HO}$TTACTG$_{OH}$ was isolated in 35% yield based on 121. The identity and homogeneity of pure hexamer $^{HO}$TTACTG$_{OH}$ were confirmed by analytical ion-exchange chromatography, LCMS- and by Maldi-Tof analysis.

**Hexameric phosphothioate sequence:** The outcome of the preparation of native hexameric ON $^{HO}$TTACTG$_{OH}$ was an incentive to explore the synthesis of PS oligonucleotides via a similar SPEM approach. To this end, two equivalents of phosphoramidite 136 were reacted with $^{3}Ado\,dT^{Pom}_{OH}$ (121) on 0.5 mmol scale, under the agency of DCI. Upon completion of the reaction (30 min), as revealed by HPLC-analysis, traces of 121 was capped by the addition of equimolar bis-(2-cyanoethyl)-N,N-diisopropyl phosphoramidite (100).

![Figure 3.3: HPLC trace of (protected) phosphorothioate ON and target hexamer $^{HO}$TTGTAC$_{OH}$.]

A - D: Consecutive crude PS oligonucleotide intermediates obtained after a full coupling cycle. 
E: Crude, fully protected target hexamer. 
F: Crude, fully deprotected hexamer $^{HO}$TTGTAC$_{OH}$. 
G: Hexamer $^{HO}$TTGTAC$_{OH}$ after preparative HPLC purification.

Excess phosphoramidite 136 and also excess capping reagent were hydrolysed by the addition of 5 mol% $H_{2}O$. The reaction mixture was concentrated and co-evaporated with $CH_{2}CN$. Reactive phosphite intermediate XV ($n = 0$) was sulfurized by the addition of phenylacetyl disulfide (105) and $^{31}$P NMR analysis of the reaction mixture confirmed total consumption of XV ($n = 0$). Next, 3H-1,2-benzodithiol-3-one (103) was added to the reaction mixture to sulfurize H-phosphonate XVI. It is of interest to note that bis-(2-cyanoethyl)-H-phosphonate (120),

\[^{b}\text{Yield was determined by } A_{260} \text{ units, } \varepsilon = 574300 \text{ L mol}^{-1} \text{cm}^{-1}.\]
derived from hydrolysis of the capping reagent 100 is sulfurized by both PADS and 3H-1,2-benzodithiol-3-one. Subsequently, the excess of sulfurizing reagents were converted into water soluble derivatives by the addition of tris(2-carboxyethyl)phosphine (TCEP) in pyridine and H2O. The reaction mixture was diluted with a mixture of EtOAc/THF (5:2, v/v) and subsequently washed with 10% KH2SO4 (2x), 10% NaHCO3 and finally with a mixture of brine/H2O (1:1, v/v). The organic layer was dried over MgSO4, filtered and after evaporation of the solvent, crude PS-dimer \( AdaO dT^{Pom}dT^{Pom}_{OH} \) was isolated in near quantitative yield (based on 121) and high purity (Figure 3.3A).

Next, the obtained PS-dimer \( AdaO dT^{Pom}dT^{Pom}_{OH} \) was elongated via repetition of the above described procedure. After four additional coupling cycles, crude hexameric PS oligonucleotide \( AdaO dT^{Pom}dT^{Pom}G^{iBu}dT^{Pom}dA^{Bz}dC^{Bz}_{OH} \) was isolated in 72% overall yield. A small sample (50 mg) was subjected to ammonolysis at 55°C and purified by preparative HPLC at 60°C.\(^c\) Pure \( HO^{TTGTAC}_{OH} \) was obtained in 33% yield (based on 121).\(^d\) The identity and homogeneity of pure phosphorothioate \( HO^{TTGTAC}_{OH} \) was confirmed by analytical HPLC- and Maldi-Tof analysis.

### 3.3 Conclusion and Outlook

**Scheme 3.5:** Solution-Phase Extraction Method (SPEM) towards the preparation of (PS) oligonucleotide fragments in the 5’→3’-direction.

![Scheme 3.5](image)

Yield is based on 121 for the 3’→5’- and 5’→3’- and was determined by \( A_{260} \) units; after preparative ion-exchange purification and desalting over a Sephadex G-25 column for native ON fragments and after preparative HPLC purification at 60°C for phosphorothioate ON fragments. \( B^{PS} = A^{Bz}, C^{Bz}, G^{iBu} \) or \( T^{Pom} \).

The solution phase extraction method (SPEM) for the 3’→5’ directed synthesis of (PS) oligonucleotide fragments (see chapter 2) was adapted for the synthesis in the reverse (5’→3’) direction. In this approach, 1-adamantaneacetic acid was installed at the 5’-OH of the first nucleoside as a permanent protective group (Scheme 3.5). The Lev group was used as temporary protection of the 3’-OH of the 5’-phosphoramidite monomers. The isolation of protected ON

\(^c\)Analytical HPLC-analysis of unprotected PS oligonucleotides were carried out at 60°C with hexafluoroisopropanol (HFIP) to suppress peak broadening due to PS-diastereoisomers.

\(^d\)Yield was determined by \( A_{260} \) units, \( \epsilon = 57800 \) L mol\(^{-1}\) cm\(^{-1}\).
intermediates was accomplished by extractive procedures only. Application of 2 equivalents of 5’-phosphoramidite monomers was necessary to achieve high coupling efficiencies and resulted in the preparation of a pure hexameric ON fragment in 35% overall yield (after ammonolysis, ion-exchange chromatography and desalting over Sephadex G25). Application of the earlier developed two-step sulfurization protocol for the preparation of phosphorothioates, resulted in the generation of a hexameric PS fragment in 33% yield based on 121.

The reverse directed SPEM for the synthesis of ON fragments can be used to generate partially protected ON with a free 3’-OH. This partially protected ON fragment, in combination with a ON fragment with a free 5’-OH (obtainable from the 3’→5’ directed SPEM) can be used in a block-coupling approach towards large ON fragments as illustrated in Scheme 3.1.

### 3.4 Experimental Procedures

**General.** All reagents were used as received. CH₃CN (Biosolve, DNA synthesis grade) was used for the coupling reactions. HPLC monitoring of the coupling reactions as well as analysis of protected ONs were run on a JASCO system with simultaneous detection at 214, 254, and 280 nm. Solvent system: A: 75% H₂O in MeOH, B: 100% CH₃CN, C: 0.2M TEAA in MeOH. Usually a gradient of 20→90% B was applied for protected ON and solution C was fixed at 10%. Preparative HPLCs of completely deblocked ONs were conducted at 60°C, applying a gradient of 5→25% B and solution C was replaced by a 0.5M TEAA buffer and fixed at 10%. Analytical LC-MS of protected ONs was conducted on a JASCO system using an Alltima C₁₈ analytical column (5µm particle size, flow: 1.0 ml/min) or a Phenomenex® Gemini C₁₈ analytical column (3µm particle size, flow: 1.0 ml/min). Absorbance was measured at 214 nm and 254 nm. Solvent system: A: 100% H₂O, B: 100% CH₃CN, C: 1% aq. TFA. Gradients of B in 10% C were applied over 15 minutes (Alltima column) or over 10 minutes (Phenomenex® column). Usually, for protected ONs a gradient of 50 → 90% B was used. Analytical LC-MS of completely deblocked ONs were conducted on a Surveyor HPLC system using a Altima C₁₈ column (5 µm particle size, flow: 1.0 ml/min). Solvent system: A: 0.4M HFIP, 16.3 mM Et₃N in a mixture of 5% MeOH/H₂O, B: 0.4M HFIP, 16.3 mM Et₃N in a mixture of 80% MeOH/H₂O. Gradients of 0 → 30% B were applied over 30 minutes. Mass spectra were recorded on a Perkin Elmer Sciex API 165 equipped with an electrospray interface (ESI) or on a LCQ Advantage Max equipped with an ESI interface. MALDI TOF spectra were recorded on a Voyager-DE PRO mass spectrometer (PerSeptive Biosystems). ³¹P NMR spectra were measured on Bruker AC200 spectrometer. Chemical shifts (δ) are given in ppm relative to external standard H₃PO₄.

**General Procedure A: Nucleoside 3’-OH esterification.** To a dry solution of the appropriate 5’-O-DMTr protected nucleoside in 1,4-dioxane (0.1M) were subsequently added 2.0 eq. Lev-OH, 2.0 eq. EDC·HCl, and 0.05 eq. DMAP. The resulting reaction mixture was stirred for 12 h at RT, after which the reaction progress was analysed by TLC (5 vol% MeOH in CH₂Cl₂). Upon complete consumption of the SM., the reaction mixture was concentrated. The residue was dissolved in EtOAc and subsequently washed with H₂O, 10% aq. KHSO₄ (2x), sat. aq.
3.4 Experimental Procedures

NaHCO$_3$ (3x), brine, dried (MgSO$_4$), filtered and concentrated. The residue was purified by silica gel flash column chromatography (silica gel FCC).

**General Procedure B: Nucleoside 5'-O-DMTr cleavage.** To a solution of the corresponding nucleoside in CH$_2$Cl$_2$ (0.1M) were subsequently added 5.0 eq. TES and 5 vol% DCA. The reaction progress was monitored by TLC analysis (100% Et$_2$O) and was usually complete in 30 min. Upon completion of the reaction, the reaction mixture was diluted with CH$_2$Cl$_2$ and subsequently washed with 2% aq. Na$_2$CO$_3$ (2x). The aqueous layer was back-extracted with CH$_2$Cl$_2$ and the combined organics was washed with brine, dried (Na$_2$SO$_4$), filtered and concentrated. The residue was purified by silica gel FCC.

**General Procedure C: Nucleoside 5'-O-H phosphitylation.** To a solution of the corresponding nucleoside in CH$_2$Cl$_2$ (0.1M) were subsequently added 4.0 eq. DiPEA and 1.25 eq. 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite at 0°C. The reaction progress was monitored by TLC analysis (100% Et$_2$O) and was usually complete in 1 h. The excess of phosphitylating reagent was quenched by the addition of 1 vol% H$_2$O and the reaction mixture was concentrated. The residue was purified by silica gel FCC.

3',5'-Di-O-butyldimethylsilyl-N$^3$-pivaloyloxymethyl-thymidine (123).

To a dry solution of thymidine (122, 7.7 g, 31.9 mmol) in DMF (0.2M) were subsequently added TBSCl (10.6 g, 2.2 eq.) and imidazole (9.4 g, 4.4 eq.) and the resulting reaction mixture was stirred for 1 h at RT. TLC analysis (100% Et$_2$O) showed total consumption of the SM and the reaction mixture was diluted with H$_2$O and extracted with Et$_2$O (5x). The combined ethereal layers were subsequently washed with H$_2$O, brine, dried (MgSO$_4$), filtered and concentrated. The residue coevaporated thrice with toluene and redissolved in DMF (0.2M) and Pom-Cl (13.9 ml, 3 eq.) and K$_2$CO$_3$ (17.6 g, 4 eq.) were subsequently added and the reaction was stirred for 12 h at RT. TLC-analysis (100% Et$_2$O) indicated complete conversion of the SM and the reaction mixture was diluted with H$_2$O and extracted with Et$_2$O. The combined ethereal layer was subsequently washed with H$_2$O, brine, dried (MgSO$_4$), filtered and concentrated. The residue was subjected to silica gel FCC (PE/Et$_2$O, 1:1, isocratic) to give the title compound in 97% yield. $^1$H NMR (300 MHz; CDCl$_3$): δ 0.01-0.10 (m, 12H, 4x CH$_3$), 0.89-1.18 (m, 18H, 2x tBu-TBS), 1.20-1.23 (m, 12H, tBu-Pom, CH$_3$-thymidine), 1.93-2.07 (m, 2H, H2'), 3.72-4.41 (m, 3H, H4', H5'), 5.32-5.35 (m, 1H, H3'), 5.96 (s, 2H, CH$_2$-Pom), 6.32-6.40 (m, 1H, H1'), 7.50 (s, 1H, H6). $^1$H NMR (75 MHz; CDCl$_3$): δ 12.7, 17.6, 18.0, 25.4, 25.6, 26.5, 26.7, 38.4, 41.0, 62.7, 64.7, 72.0, 85.2, 87.6, 109.5, 134.1, 149.9, 162.1, 176.9.
3. Diamondoid assistance in the 5’→3’ directed solution-phase synthesis of ONs

\[
\text{N}^3\text{-pivaloyloxymethyl-thymidine (124).}
\]
To a dry solution of 123 (18.1 g, 31 mmol) in pyridine (0.5 M) was added Et₃N·3 HF (10.4 ml, 2.0 eq) and the resulting mixture was stirred for 12 h at RT. TLC analysis (10 vol% MeOH in CH₂Cl₂) revealed complete conversion of the SM. The reaction mixture was concentrated and the residue was dissolved in a mixture of CH₃CN/MeOH/H₂O (3:3:1, v/v/v) and washed twice with a mixture of heptane/Et₂O (1:1, v/v). The CH₃CN/MeOH/H₂O layer was concentrated and coevaporated thrice with toluene to give the title compound in 75% yield.

\[
\begin{align*}
\text{1H NMR (300 MHz; CDCl₃): } & \delta 1.18 (s, 9H, tBu - Pom), 1.99 (s, 3H, CH₃ - thymidine), 2.12-2.20 (m, 3H, CH₂Ada, H₂’α), 2.43-2.51 (m, 1H, H₂’β), 3.72, 3.77 (2x s, 6H, 2x OMe), 4.32-4.32 (m, 1H, H4’), 5.54-5.56 (m, 1H, H3’), 6.50-6.80 (m, 1H, H1’), 6.78 (d, 4H, J = 8.7, Har), 7.14-7.61 (m, 12H, Har), 8.00-8.03 (m, 2H, Har), 8.18, 8.72 (2x s, 2H, H2, H8), 9.19 (br. s, 1H, NH). \\
\text{13C NMR (75 MHz; CDCl₃): } & \delta: 27.9, 29.7, 37.8, 37.9, 43.4, 55.1, 63.5, 75.4, 84.0, 84.3, 84.4, 86.6, 113.1, 123.3, 126.9, 127.8, 128.0.
\end{align*}
\]

\[
5’-O-Adamantaneacetyl-N³-pivaloyloxymethyl-thymidine (121).
\]
To a dry solution of HO dTPom (124, 6.3 g, 18.1 mmol) in DMF (0.25 M) were subsequently added 1.0 eq. 1-adamantaneacetic acid and 1.0 eq. PPh₃. Next, a solution of DIAD (1.0 eq.) in DMF (1 M) was added drop wise to the reaction mixture over a period of 30 min and stirring continued for 12 h at RT. The reaction mixture was concentrated and the residue was purified by silica gel FCC (PE/Et₂O, 1:1, isocratic) to provide the title compound in 84% yield as a white solid. HRMS found (calc) for [M+H]+: 533.28574 (533.28574). M.p. 64-67 °C.

\[
\begin{align*}
\text{1H NMR (300 MHz; CDCl₃): } & \delta: 1.18 (s, 9H, tBu), 1.43-1.81 (m, 12H, 6x CH₂-Ada), 1.99 (br. s, 6H, 3x CHAda, CH₃ - thymidine), 2.12-2.20 (m, 3H, CH₂Ada, H₂’α), 2.43-2.51 (m, 1H, H₂’β), 3.74 (s, 1H, OH), 4.18-4.20 (m, 1H, H4’), 4.23-4.28 (m, 1H, H’), 4.37-4.43 (m 2H, H5’), 5.94 (d, 2H, J = 3.1, CH3Pom), 6.33 (t, 1H, J = 6.9, H1’). \quad 7.36 (s, 1H, H6). \\
\text{13C NMR (75 MHz; CDCl₃): } & \delta: 12.8, 26.5, 27.8, 32.4, 36.2, 38.4, 39.9, 41.9, 43.3, 48.2, 63.1, 84.0, 84.3, 85.4, 109.7, 134.2, 149.7, 160.3, 162.2, 170.9, 177.2, \{\alpha\}_D^{20} = +7.2 ° (c= 1.0, CHCl₃).
\end{align*}
\]

\[
5’-O-dimethoxytrityl-N⁴-benzoyl-3’-O-levulinoyl-deoxyadenosine (129).
\]
Commercially available nucleoside DMTr dAdBz (126, 23.4 g, 35.6 mmol), was used in general procedure A. After silica gel FCC (MeOH/EtOAc (0:1 → 9:1) the title compound was isolated in 97% yield as a white solid. HRMS found (calc) for [M+H]+: 756.30919 (756.30279). M.p. 78-80 °C.

\[
\begin{align*}
\text{1H NMR (300 MHz; CDCl₃): } & \delta: 2.20 (s, 3H, CH₃ - Lev), 2.59-2.81 (m, 5H, 2x CH₂ - Lev, H₂’α), 3.00-3.08 (m, 1H, H₂’β), 3.44 (d, 2H, J= 3.9, H5’), 3.72, 3.77 (2x s, 6H, 2x OMe), 4.32-4.32 (m, 1H, H4’), 5.54-5.56 (m, 1H, H3’), 6.50-6.80 (m, 1H, H1’), 6.78 (d, 4H, J = 8.7, Har), 7.14-7.61 (m, 12H, Har), 8.00-8.03 (m, 2H, Har), 8.18, 8.72 (2x s, 2H, H2, H8), 9.19 (br. s, 1H, NH). \\
\text{13C NMR (75 MHz; CDCl₃): } & \delta: 27.9, 29.7, 37.8, 37.9, 43.4, 55.1, 63.5, 75.4, 84.0, 84.3, 84.4, 86.6, 113.1, 123.3, 126.9, 127.8, 128.0,
\end{align*}
\]
3.4 Experimental Procedures

128.7, 129.1, 129.9, 132.7, 133.6, 135.4, 141.2, 144.3, 149.5, 151.5, 152.5, 158.5, 172.1, 206.3. 

$[\alpha]^{20}_D = 0^\circ$ (c= 1.0, CHCl$_3$).

5'-O-dimethoxytrityl-N$^2$-2-isobutryl-3'-O-levulinoyl-deoxyguanosine (130).

Commerically available nucleoside $\text{DMTr}^\text{dG}_{Bz}^\text{OH}$ (127, 25.0 g, 39.1 mmol), was used in general procedure A. After silica gel FCC (MeOH/EtOAc (0:1 → 9:1) the title compound was isolated in 96% yield as a white solid. $^1$H NMR (300 MHz; CDCl$_3$) $\delta$: 0.94 (d, 3H, $J = 6.9$, CH$_3$-iBu), 1.04 (d, 3H, $J = 6.9$, CH$_3$-iBu), 2.0-2.16 (m, 1H, CH - iBu), 2.20 (s, 3H, CH$_3$-Lev), 2.39-2.67 (m, 3H, CH$_2$-Lev, H$_2'$α), 2.76 (t, 2H, $J = 6.6$, CH$_2$-Lev), 2.94-3.16 (m, 1H, H$_2'$β), 3.33 (ddd, 2H, $J = 3.3, 10.4, 24.0$, H$_5'$). $^{13}$C NMR (75 MHz; CDCl$_3$): $\delta$: 18.5, 27.6, 29.5, 35.6, 37.5, 54.9, 63.5, 75.0, 83.8, 84.0, 86.3, 112.9, 121.1, 126.7, 127.6, 127.8, 129.7, 135.2, 135.3, 137.1, 144.2, 147.7, 148.2, 155.5, 158.3, 172.0, 179.4, 206.9.

$[\alpha]^{20}_D = + 68.8^\circ$ (c= 1.0, CHCl$_3$).

5'-O-dimethoxytrityl-N$^3$-benzoyl-3'-O-levulinoyl-deoxycytidine (131).

Commerically available nucleoside $\text{DMTr}^\text{dT}_{Pom}^\text{OLev}$ (128, 25 g, 39.5 mmol), was used in general procedure A. After silica gel FCC (MeOH/EtOAc (0:1 → 9:1) the title compound was isolated in near quantitative yield as a white solid. HRMS found (calc) for [M+H]$^+$: 732.29103 (732.29156). M.p. 80-82°C. $^1$H NMR (300 MHz; CDCl$_3$) $\delta$: 2.2 (s, 3H, CH$_3$-Lev), 2.32-2.39 (m, 1H, H$_2'$α), 2.56-2.84 (m, 5H, 2x CH$_2$-Lev, H$_2'$β), 3.47-3.54 (m, 2H, H$_5'$), 3.78, 3.79 (2x s, 6H, 2x OMe). $^{13}$C NMR (75 MHz; CDCl$_3$) $\delta$: 27.8, 29.6, 37.6, 39.2, 55.0, 62.9, 74.5, 84.5, 86.9, 87.0, 96.5, 113.2, 127.0, 127.6, 127.9, 128.7, 129.8, 129.9, 132.9, 133.0, 134.9, 135.1, 143.9, 144.2, 154.5, 158.5, 162.3, 166.8, 172.0, 206.2. $[\alpha]^{20}_D = + 68.8^\circ$ (c= 1.0, CHCl$_3$).

3'-O-levulinoyl-N$^3$-pivaloyloxymethyl-thymidine (132).

Nucleoside $\text{DMTr}^\text{dT}_{Pom}^\text{OLev}$ (86, 30.2 g, 40 mmol, prepared as described in chapter 2) was detritylated according to general procedure B. After silica gel FCC (PE/EtOAc 1:0 → 0:1), the title compound was isolated in 95% yield as a white solid. HRMS found (calc) for [M+Na]$^+$: 477.18499 (477.18435). M.p. 86-89°C. $^1$H NMR (300 MHz; MeOD) $\delta$: 1.13 (s, 9H, tBu), 1.88 (s, 3H, CH$_3$-thymidine), 2.14 (s, 3H, CH$_3$-Lev), 2.31-2.34 (m, 2H, H$_2'$), 2.54 (t, 2H, $J = 6.5$, CH$_2$-Lev), 2.79 (t, 2H, $J = 6.0$, CH$_2$-Lev),
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3.76 (d, 2H, J= 2.8, H5’), 4.04-4.05 (br. s, 1H, H4’), 5.27 (t, 1H, J= 3.1, H3’), 5.87 (s, 2H, CH2-Pom), 6.26 (t, 1H, J= 6.6, H1’), 7.87 (s, 1H, H6). 13C NMR (75 MHz; CDCl3) δ: 12.7, 26.6, 27.6, 29.3, 37.1, 37.4, 38.4, 62.0, 64.6, 74.8, 84.8, 85.6, 109.8, 135.2, 150.0, 162.3, 172.0, 177.3, 206.6. [α]D20 = -4.0° (c= 1.0, CHCl3).

3’-O-levulinoyl-N4-benzoyl-deoxyadenosine (133).
Nucleoside 129 (26.0 g, 34.4 mmol) was used in general procedure B. However, only 4 vol% DCA was applied with 4.0 eq. TES. The progress of the reaction was monitored by TLC analysis and upon complete consumption of the SM, the reaction mixture was diluted with a mixture of PE/Et2O, (1:9). The reaction mixture was cooled to -20°C and the formed precipitated was collected and rinsed with an ice cold mixture of PE/Et2O, (1:9), to provide the title compound in 75% yield. HRMS found (calc) for [M+H]+: 454.17196 (454.17211). M.p. 45-50°C. 1H NMR (300 MHz; CDCl3) δ: 2.22 (s, 3H, CH3-Lev), 2.42-2.70 (m, 3H, CH2-Lev, H2’α), 2.81 (dd, 2H, J= 3.7, 10.02, CH2-Lev), 3.09-3.29 (m, 1H, H2’β), 3.83-4.08 (m, 2H, H5’), 4.28-4.36 (m, 1H, H4’), 5.57 (d, 1H, J= 5.6, H3’), 6.38 (dd, 1H, J= 5.4, 9.72, H1’), 7.45-7.71 (m, 3H, Har), 7.96-8.10 (m, 2H, Har), 8.16, 8.82 (2x s, H2, H8). 13C NMR (75 MHz; CDCl3) δ: 27.3, 29.1, 37.2, 62.1, 64.9, 75.4, 85.9, 86.1, 123.5, 127.5, 128.0, 132.1, 132.8, 149.5, 150.3, 151.2, 165.0, 171.7, 206.2. [α]D20 = - 23.0° (c= 1.0, CHCl3).

3’-O-levulinoyl-N2-isobutyryl-deoxyguanosine (134).
Nucleoside 130 (26.0 g, 34.4 mmol) was used in general procedure B. However, only 4 vol% DCA was applied with 4.0 eq. TES. The progress of the reaction was monitored by TLC analysis and upon complete consumption of the SM, the reaction mixture was diluted with a mixture of PE/Et2O, (1:9). The reaction mixture was cooled to -20°C and the formed precipitate was collected and rinsed with an ice cold mixture of PE/Et2O, (1:9), to provide the title compound in 87% yield. 1H NMR (200 MHz; CDCl3) δ: 1.12-1.28 (m, 6H, 4x CH3 - iBu), 2.21 (s, 3H, CH3 - Lev), 2.59-2.80 (m, 7H, 2x CH2 - Lev, K2’, CH -iBu), 3.87-3.92 (m, 2H, H5’), 4.17-4.21 (m, 1H, H4’), 5.12 (br. s, 1H, OH), 5.40-5.55 (m, 1H, H3’), 6.10-6.25 (m, 1H, H1’), 8.0 (s, 1H, H8), 9.51 (br. s, 1H, NH).

3’-O-levulinoyl-N4-benzoyl-deoxycytidine (135).
Nucleoside 131 (29.1 g, 39.4 mmol) was used in general procedure B. However, only 4 vol% DCA was applied with 4.0 eq. TES. The crude product was purified by crystallisation from PE/Et2O, (1:9). The title compound was obtained in 76% yield. 1H NMR (300 MHz; CDCl3) δ: 2.18 (s, 3H, CH3 - Lev), 2.21-3.36 (m, 6H, 2x CH2 - Lev, H2’), 3.70-3.97 (m, 2H, H5’), 4.07-4.25 (m, 1H, H4’), 5.15-5.43 (m, 1H,
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H3’), 6.23 (t, 1H, J = 6.0, H1’), 7.34-7.67 (m, 4H, Har), 7.92 (d, 2H, J = 7.0, H5, H6), 8.36 (d, 1H, J = 7.6, Har). $^{13}$C NMR (75 MHz; CDCl$_3$) $\delta$: 27.7, 29.4, 37.5, 38.7, 61.5, 74.9, 85.9, 87.4, 97.3, 127.7, 128.5, 132.8, 144.9, 155.9, 162.9, 167.3, 172.3, 207.1.

$^{5’-O-}$$(2$-cyanoethyl-$N,N$-diisopropyl)-phosphoramidite-$N^3$-pivaloyloxymethyl-$3’$-O-levulinoyl-thymidine (136).

Nucleoside $^{5’-O-dT}$$^{O_{Lev}}$ (132, 14.2 g, 31.3 mmol) was used in general procedure C. The crude product was purified by silica gel FCC (prepared in the presence of 3 vol% Et$_3$N, 100% Et$_2$O, isocratic) to give the title compound in 90% yield as a colourless oil. HRMS found (calc) for [M+H]$^+$: 655.31227 (655.31026).

$^1$H NMR (300 MHz; CDCl$_3$, two diastereoisomers) $\delta$: 1.17-1.23 (m, 9H, tBu - Pom, 4x CH$_3$-N,N-diisopropyl), 2.20 (s, 3H, CH$_3$-thymidine), 2.21-2.40 (m, 2H, 2x CH -N,N-diisopropyl), 2.59 (t, 2H, $J$ = 6.2, CH$_2$-Lev), 2.68 (t, 2H, $J = 6.1$, CH$_2$ - Lev), 2.77-2.81 (m, 2H, H2’), 3.60-3.64 (m, 2H, CH$_2$-OCE), 3.81-3.97 (m, 4H, H3’, H4’, CH$_2$ - Lev), 4.20-4.23 (m, 1H, H5’$^\alpha$), 5.29 (2x d, 1H, $J = 5.9$, H5’$^\beta$), 5.91-5.98 (2x s, 2H, CH$_2$-Pom), 6.33-6.45 (m, 1H, H1’), 7.59, 7.72 (2x s, 1H, H6). $^{13}$C NMR (75 MHz; CDCl$_3$, two diastereoisomers) $\delta$: 12.7, 12.9, 20.1, 20.2, 24.5, 27.6, 29.5, 37.2, 37.5, 38.5, 42.7, 42.8, 57.9, 58.2, 58.5, 62.9, 63.1, 63.7, 63.9, 64.7, 64.8, 75.0, 75.2, 84.0, 84.1, 85.0, 85.4, 109.8, 110.1, 117.2, 117.3, 134.1, 134.3, 150.0, 150.1, 162.2, 172.0, 172.1, 177.2, 206.1. $[\alpha]_{D}^{20}$ = -21$^\circ$ (c= 1.0, CHCl$_3$).

$^{5’-O-}$$(2$-cyanoethyl-$N,N$-diisopropyl)-phosphoramidite-$N^4$-levulinoyl-$N^4$-benzoyl-deoxyadenosine (137).

Nucleoside 133 (10.9 g, 24.1 mmol) was used in general procedure C. The crude product was first purified by silica gel FCC (prepared in the presence of 3 vol% Et$_3$N in EtOAc) and eluted with 100% EtOAc. The appropriate fractions were collected, the volatiles were removed and the oily residue was washed with Et$_2$O to give the title compound in 52% yield as a pale oil which solidified at -20$^\circ$C. HRMS found (calc) for [M+H]$^+$: 654.27947 (654.27996). M.P. 23$^\circ$C. $^1$H NMR (300 MHz; CDCl$_3$, two diastereoisomers) $\delta$: 1.08-1.31 (m, 12H, 4x CH$_3$-iPr, 2.24 (s, 3H, CH$_3$ - Lev), 2.57-2.93 (m, 8H, 2x CH$_2$ - Lev, H2’, 2xCH - iPr), 3.64-3.72 (m, 2H, CH$_2$ - CE), 3.72-4.06 (m, 4H, CH$_2$ - Lev, H5’), 4.29-4.44 (m, 1H, H4’), 5.41-5.62 (m, 1H, H3’), 6.51-6.72 (m, 1H, H1’), 7.42-7.67 (m, 3H, Har), 8.04 (d, 2H, J = 8.0, Har), 8.50, 8.82 (2x s, H2, H8), 9.08 (br. s, 1H, NH). $^{13}$C NMR (75 MHz; CDCl$_3$): $\delta$: 19.8, 20.0, 22.0, 24.1, 24.2, 27.5, 29.3, 37.4, 37.7, 42.5, 42.7, 57.8, 58.3, 63.0, 63.2, 75.1, 83.9, 84.1, 117.3, 123.1, 123.2, 127.6, 128.2, 132.2, 133.3, 149.3, 151.3, 151.9, 164.8, 171.8, 206.0. $[\alpha]_{D}^{20}$ = -24.4$^\circ$ (c= 1.0, CHCl$_3$).
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Nucleoside 134 (13.3 g, 30.6 mmol) was used in general procedure C. The crude product was first purified by silica gel FCC (prepared in the presence of 3 vol% Et₃N in EtOAc) and eluted with 100% EtOAc. The appropriate fractions were collected, the volatiles were removed and the oily residue was washed with Et₂O to give the title compound in 57% yield as a pale oil which solidified at -20°C. HRMS found (calc) for [M+H]⁺: 636.29394 (636.29052). M.P. 52-56°C. ¹H NMR (300 MHz; CDCl₃, two diastereoisomers): δ 0.94-1.24 (m, 16H, 8x CH₃, -iBu, N,N-diisopropyl), 2.07 (s, 3H, CH₃-Lev), 2.24-2.97 (m, 7H, 2x CH₂-Lev, H2’, CH-iBu), 3.26-3.63 (m, 2H, CH₂-OCE), 3.63-3.98 (m, 4H, CH₂-Lev, H5’), 4.03-4.28 (m, 1H, H4’), 5.17-5.40 (m, 1H, H3’), 5.59-6.25 (m, 1H, H1’), 8.03 (s, 1H, H8). ¹³C NMR (75 MHz; CDCl₃): δ 18.6, 18.8, 20.0, 20.1, 24.3, 27.5, 29.5, 35.6, 37.6, 38.0, 42.7, 42.9, 57.9, 58.3, 63.1, 75.5, 83.5, 83.7, 84.3, 91.6, 92.6, 117.5, 120.6, 147.9, 148.3, 155.7, 172.1, 179.6, 207.1. [α]₂⁰ = -12.2° (c = 1.0, CHCl₃).

Nucleoside 135, (12.8 g, 30 mmol) was used in general procedure C. The crude product was first purified by silica gel FCC (prepared in the presence of 3 vol% Et₃N in EtOAc) and eluted with 100% EtOAc. The appropriate fractions were collected, the volatiles were removed and the oily residue was washed with Et₂O to give the title compound in 53% yield as a pale oil which solidified at -20°C. HRMS found (calc) for [M+H]⁺: 630.27029 (630.26873). ¹H NMR (300 MHz; CDCl₃, two diastereoisomers): δ 1.11-1.37 (m, 12H, 4x CH₃-iPr), 2.22 (s, 3H, CH₃-Lev), 2.53-2.87 (m, 8H, 2x CH₂-Lev, 2x CH-iPr, H2’), 3.46-4.08 (m, 6H, 2x CH₂-CE, H5’), 4.18-4.39 (m, 1H, H4’), 5.26-5.50 (m, 1H, H3’), 6.22-6.48 (m, 1H, H1’), 7.41-7.69 (m, 4H, Har), 7.85-7.98 (m, 2H, H5,6), 8.33 (dd, 1H, J = 2.2, 7.52, Har). ¹³C NMR (50 MHz; CDCl₃): δ: 18.1, 19.3, 23.6, 27.0, 28.7, 34.7, 36.8, 38.0, 42.2, 57.3, 57.8, 62.6, 74.2, 84.0, 86.0, 86.5, 86.6, 95.9, 117.1, 127.2, 127.6, 131.9, 132.3, 143.4, 146.7, 147.8, 153.8, 155.6, 162.0, 166.4, 171.3, 177.6, 205.5. [α]₂⁰ = + 15.8° (c = 1.0, CHCl₃).

Representable procedure for one elongation cycle en route to native and PS oligonucleotides:

**Coupling and capping:** Compound 121 (when n = 0, 0.5 mmol) and 2.0 eq. of phosphoramidite monomer (136) were mixed and coevaporated with CH₃CN (3x). The mixture was dissolved in CH₃CN (3.5 ml) under an argon atmosphere and DCI (266 mg, 2.25 mmol) was added. The resulting reaction mixture was stirred for 30 min at RT followed by the addition of capping reagent 100 (136 mg, 0.5 mmol). Samples for HPLC analysis were prepared by diluting an aliquot (10 µL) of the reaction mixture with a mixture of CH₃CN/MeOH (1:3, v/v) (1 mL).
3.4 Experimental Procedures

**Oxidation (X=O):** H$_2$O (175 µL) was added and the reaction mixture was stirred for 2 min. A solution of I$_2$ (0.2M in THF/pyridine (4:1, v/v, 7.5 mL) was added and the mixture was stirred for 5 min. The reaction mixture was transferred into a separatory funnel and diluted with EtOAc (15 ml). The mixture was subsequently extracted with a solution of 1M aq. Na$_2$S$_2$O$_3$ (15 ml), 10% aq. KHSO$_4$ (2x 15 ml), 5% aq. NaHCO$_3$ (1x 15 ml) and finally with a mixture of brine/H$_2$O (1:1, v/v, 15 mL). The organic phase was dried (MgSO$_4$), filtrated and concentrated to give a white foam.

**Sulfurisation (X= S):** After the coupling and capping step, water (175 µl was added to the reaction mixture and after 2 min the reaction mixture was concentrated and coevaporated with CH$_3$CN (3x). The resulting foam was dissolved in CH$_3$CN (6.0 ml) under an argon atmosphere. PADS (105) (227 mg, 0.75 mmol) was added to the reaction mixture followed, after 30 minutes, by the addition of 103 (126 mg, 0.75 mmol). The reaction mixture was stirred for an additional 30 min and a solution of TCEP·HCl (645 mg, 2.25 mmol) in pyridine/H$_2$O (1:1, v/v, 4 ml) was added and the stirring was continued for another 45 minutes. The reaction mixture was transferred to a separatory funnel and diluted with a mixture of EtOAc /THF (5:2, v/v, 35 ml). The mixture was subsequently washed with H$_2$O (15 ml), 10% aq. KHSO$_4$ (2 x 15 ml), 10% NaHCO$_3$ (2 x 15 ml) and finally with a mixture of brine/H$_2$O (1:1, v/v, 15 ml). The organic layer was dried (MgSO$_4$), filtrated and concentrated to give a light yellow foam.

**Delevulinoylation:** A stock-solution of 0.5M N$_2$H$_4$·H$_2$O in Pyr/AcOH (4:1, v/v) was prepared. The foam containing the target ON XVII (n = 1, X = O or S) and the excess monomer was dissolved in the stock-solution (4.5 ml) and the progress of the delevulinoylation was monitored by TLC (CH$_2$Cl$_2$/MeOH, 9:1). After complete consumption of the starting materials, the reaction mixture was diluted with a mixture of EtOAc/THF (35 ml, 2:5, v/v) and subsequently washed with H$_2$O (20 ml), 10% aq. KHSO$_4$ (2x 15 ml), 10% aq. NaHCO$_3$ (2x 15 ml) and finally with a mixture of brine/H$_2$O (1:1, v/v, 20 ml). After drying (MgSO$_4$), filtration and evaporation of the solvents the elongated oligonucleotide XIII, (n = 1, X = O or S) was isolated as a white foam. Yields were estimated by dividing the amount of material obtained (in mg) with the expected amount (0.5 mmol x MW oligonucleotide).

**General procedure for complete deblocking of ON fragments:** 3’-OH ON (50 mg) was treated with conc. NH$_3$ solution (25 ml) at 55°C for 48 h. After cooling of the reaction mixture to room temperature, the mixture was concentrated. The residue was dissolved (cloudy mixture) in H$_2$O and centrifuged. The supernatant (appr. 6 ml) was analysed by LC/MS as well as mono-Q ion-exchange chromatography using a gradient of 1M NaOH in 10 mM NaCl (0-50%).

**Purification and desalting of native ON $^{HO}$TTACTG$_{OH}$:** After deblocking and centrifugation as described above, the aqueous solution containing the crude, fully deprotected ON was purified using Q-Sepharose ion-exchange chromatography utilizing a gradient of 1M NaOH in 10 mM NaCl (0→35% over 1 CV, then 35→65% over 10 CV). After concentration of the appropriate fractions, the residue was dissolved in H$_2$O and desalted over a Sephadex G-25
3. Diamondoid assistance in the $5'\rightarrow 3'$ directed solution-phase synthesis of ONs

column using 0.15M $\text{NH}_4\text{HCO}_3$ as the eluent. After pooling, evaporation and lyophilisation, the yield was determined by measuring the absorption at 260 nm ($\varepsilon = 574300 \text{ L mol}^{-1}\text{ cm}^{-1}$). The title compound was obtained in 35% overall yield from monomer 121. Maldi -Tof analysis (calc.) for [M+H]$^+$ 1782.10 (1782.34)

**Purification of PS oligonucleotide $\text{HO-TTGTA}C_{OH}$**: The supernatant containing the crude fully deprotected ON, obtained as described above, was purified using preparative HPLC at 60°C. The fractions containing the pure hexamer (determined by analytical HPLC at 60°C) were collected, concentrated and lyophilized. The yield was determined by measuring the absorption at 260 nm ($\varepsilon = 57800 \text{ L mol}^{-1}\text{ cm}^{-1}$). The title compound was obtained in 33% overall yield from monomer 121. Maldi -Tof analysis (calc.) for [M+H]$^+$ 1861.24 (1861.22).
Development of levulinoyl - adamantane hybrid protective groups for 3’-O-nucleoside protection

4.1 Introduction

The ability to mask functional groups, to prevent their participation in subsequent reactions is at the basis of multi-step synthesis in organic chemistry. An efficient protective group strategy is a prerequisite for the construction of biopolymers. In case of ON synthesis, the use of protective groups is a necessity to achieve regioselectivity and high coupling efficiencies. A block-coupling approach is a viable strategy for preparation of large ON fragments in solution. This approach requires two proper protected ON fragments, as illustrated in chapter 3 Scheme 3.1. The developed Solution-Phase Extraction Method (SPEM), described in chapters 2 and 3, is applicable in the generation of two partially protected ON fragments, suitable for a block coupling. However, the synthesis of partially protected fragments via a 5’→3’ directed SPEM approach suffers from a few drawbacks. The formation of the internucleotide phosphite linkage is affected by the decreased reactivity of the 3’-OH as coupling partner in comparison with the 5’-OH. As a consequence two equivalents of phosphoroamidite monomers XIV (Scheme 3.5), the preparation of which requires multistep synthesis protocols, is necessary to achieve high coupling efficiencies.

Design of hybrid levulinoyl - adamantane protective groups: The promising results achieved via the 3’→5’ directed SPEM for ON synthesis, led to the design of a levulinoyl - adamantane
4. Development of levulinoyl - adamantane hybrid protective groups

This hybrid protective group combines the favourable hydrophobic properties of the adamantane moiety with the orthogonal cleavage procedures inherent to the Lev group. Installation of this hybrid protective group at the 3’-OH of the first nucleoside and elongation with 5’-O-DMTr phosphoramidite building blocks III (Scheme 2.8) in a SPEM approach would result in an orthogonally protected ON fragment. Selective liberation of either the 3’-OH or the 5’OH results in two properly protected ON fragments suitable for block-coupling strategies.

Scheme 4.1: Design and retrosynthetic analysis of hybrid adamantane - levulinoyl protective group.

It was envisaged that the synthesis of hybrid 140 (Scheme 4.1) can be initiated from commercial available 1-adamantaneacetic acid (141). Retrosynthetic analysis shows that the Lev moiety can be introduced to 141 via the addition of homoallylmagnesium bromide to the Weinreb amide 143, followed by oxidative olefin cleavage of 142.

4.2 Results and Discussion

Three novel hybrid protective groups were designed, varying in the number of methylene groups between the 1-adamantane core and the adjacent carbonyl functionality. These protective groups were named Klev(n), where (n) indicates the number of methylene groups, ranging from zero in Klev0 (144) to two in Klev2 (145). The optimized route towards the Klev(n) protective groups is depicted in Scheme 4.2.

Klev1 (140) synthesis was initiated with the conversion of 1-adamantaneacetic acid (141) into the corresponding Weinreb amide 143. Discouragingly, the addition of commercially available homoallylmagnesiumbromide to 143 was not productive and therefore, alkylation of adamantane-acetaldehyde (147) was explored. Although 147 is available through LiAlH₄ reduction of Weinreb amide 143, Swern oxidation of 1-adamantanecarboxylic acid (51) proved to be a superior method. The addition of homoallylmagnesiumbromide to 147 proceeded smoothly and yielded alcohol 148 in 80%. Direct conversion of alcohol 148 to Klev1 was not successful using NaIO₄ and cat. RuO₂. Fortunately, oxidative olefin cleavage of ketone 142, obtained by quantitative Swern oxidation of 148, gave Klev1 (140) in an overall yield of 57%.

Klev0 (144) was prepared according to the strategy developed for the synthesis of Klev1 and was initiated from commercially available 1-adamantanecarboxylic acid (51). LiAlH₄ reduction of 51 gave, after crystallisation from ethanol, 1-adamantanemethanol (149) in near quantitative yield. Swern oxidation of alcohol 149 gave aldehyde 150 in excellent yield, after chromatographic
4.2 Results and Discussion

Scheme 4.2: Synthesis of novel Klev(n) protective groups.

Reagents and conditions: i) LiAlH₄ (1.25 eq.), THF (3.5 M), 0°C → RT, overnight, 146 and 149, near quant.; ii) CH₂Cl₂ (1M), oxalyl chloride (1.32 eq.), DMSO (2.5 eq.), -78°C → -5°C, 147 and 150, near quant.; iii a) (methoxymethyl)triphenylphosphonium bromide (1.5 eq.), nBuLi (1.5 eq.), Et₂O (0.1M), -15°C → RT, 12h; b) Et₅O (0.5M), 35% aq. HClO₄; iv) 154 (1.5 eq.), nBuLi (1.5 eq.), Et₂O (0.1M), -15°C → RT, RT, 12h; quant. v) EtOAc (0.1M), 5 mol% Pd/C (10 wt%), H₂, near quant.; vi) DIBALH (1.2 eq.), Et₂O (0.1M), -78°C, 71%; vii) homoallylmagnesium bromide (1.4 eq.), ET₂O (1.0M), RT, overnight, 148, 80%; 151, 83%; 157, 82%; viii) CH₂Cl₂ (1M), oxalyl chloride (1.32 eq.), DMSO (2.5 eq.), -78°C, 2 h then add Et₃N (5 eq.), -78°C → -5°C, 142, 97%; 152, 91%; 158, 99%; ix) NaIO₄ (8 eq.), RuO₂ (cat.); H₂O/ acetone (2:1, v/v), 140, 73%; 144, 71% and 145, 75%.

purification. The subsequent Grignard alkylation of 150 with homoallylmagnesium bromide, freshly prepared from 4-bromo-1-butene and magnesium turnings, gave secondary alcohol 148. In the penultimate step, Swern oxidation of secondary alcohol 148 furnished intermediate ketone 152, which underwent oxidative olefin cleavage efficiently, producing Klev0 (144) in an overall yield of 54% yield.

Klev2 (145) synthesis via the developed strategy, implements the homoallylation of adamantanepropanal (153). Due to the low availability of 1-adamantanepropionic acid, homologation of adamantaneethanal (147) was addressed to obtain 153. To this end, Wittig olefination of 147 was executed with (methoxymethyl)triphenylphosphonium bromide. A slight excess of nBuli was used at -15°C to liberate the corresponding phosphonium ylide, followed by the addition of aldehyde 147. Multiple products could be detected by TLC-analysis and upon acidic hydrolysis of the crude material obtained after extractive work-up of the reaction mixture, only traces of adamantane propanal (153) could be detected by LCMS analysis. It is hypothesized that the
α-protons of aldehyde 147 are at the basis of numerous side reactions. Therefore, the olefination of 150, lacking α-protons, was explored with Wittig salt 154 to give α,β-unsaturated ester 155 in near quantitative yield. Subsequent Pd/C mediated hydrogenation gave crude ester 156 which was reduced at -78°C with DIBALH to give pure aldehyde 153 in 71% yield. Homoallylation of 153 as described for the preparation of 151 gave alcohol 157 in 82% yield. Swern oxidation of 157 proceeded quantitatively and the subsequent oxidative olefin cleavage gave acid 145 in 40% overall yield.

Scheme 4.3: Synthesis of 3’-Klev(n) monomers.

Reagents and conditions: i) corresponding Klev(n) (0.8 eq.), EDC (1.8 eq.), DMAP (cat), DiPEA (3.0 eq.) CH₂Cl₂ (0.1M), RT, overnight, 159, 95%; 160, 88%; 161, 66%; ii) TES (2.0 eq.), CH₂Cl₂ (0.1M), 5 vol% DCA in CH₂Cl₂ (M), RT, 2 h, 162, 87%; 163, 90%; 164, 78%.

Evaluation of the Klev(n) protective groups: Next, the evaluation of the three Klev(n) derivatives for 3’-O-nucleoside protection was undertaken. To this end, DMTrOₙdTₙPomOAda (81, chapter 2, Scheme 2.3) and DMTrOₙdTₙPomOLev (86, chapter 3, Scheme 3.4) were analysed by HPLC, and their retention times were compared to those of DMTrOₙdTₙPomOH (89) and DMTrOₙdTₙPomOKlev₁ (160, Figure 4.1). It was found that installation of the Klev₁ group at the 3’-OH of thymidine derivative 89 results in an increase in hydrophilicity, comparable to 1-adamantaneacyl protection.

3’-O-Klev(n) cleavage: After dichoroacetic acid (DCA) mediated detritylation, HOₙdTₙPomOKlev₁ (160, Figure 4.1) Initial studies revealed that the cleavage of the Klev(n) protective groups is at least 20 times slower compared to the parent Lev group, which is cleaved in ~5 min. Interestingly, it appeared that the rate of Klev(n) cleavage depends on the number of carbon atoms between the ketone
**Figure 4.1:** HPLC trace of 5’-O-DMTr protected thymidine derivatives.

A: \( \text{DMTr}^O dT_{OH} \) (89); B: \( \text{DMTr}^O dT_{OLev} \) (86); C: \( \text{DMTr}^O dT_{OAda} \) (81); D: \( \text{DMTr}^O dT_{OKlev} \) (160).

and the adamantane group, embedded in the Klev(n) derivative. Whereas both Klev1 (163) and Klev2 (164) derivatives are cleaved within 180 minutes, Klev0 required more than 12 hours to reach completion.

(Re)design of Klev protective group: It is likely that prolonged exposure of protected ON fragments to the hydrazine cleavage cocktail, would result in unwanted side reactions such as premature cleavage of the base labile protective groups on the nucleobase. Guided by the mechanism of hydrazine mediated Klev cleavage (Scheme 4.4), it was hypothesized that formation of hydrazone 165 is the rate determining step and that the rate of its formation is negatively influenced by the adjacent, sterically congested and electron donating adamantane moiety.

**Scheme 4.4:** Mechanism of hydrazine mediated Klev cleavage.
As reported in chapter 6, oxime formation of 2-adamantanone (58) with hydroxylamine was complete within 30 minutes. These findings led to the design of the adamantane - levulinoyl hybrid protective group in which the ketone moiety is incorporated in the adamantane skeleton (iKlev, 167). It is expected that the distorted geometry (ring strain) of the adamantane core due to the presence of a carbonyl functionality would positively affect hydrazone (168) formation, thus increasing the overall reaction speed (Scheme 4.4).

**Scheme 4.5: Synthesis of novel iKlev protective group.**

Reagents and conditions: i) BF$_3$·OEt$_2$ (1.5 eq.), TMSCHN$_2$ (1.5 eq.), CH$_2$Cl$_2$ (0.17 M), -15°C → RT, 12 h, 64%; ii) SeO$_2$ (1.1 eq.), 1,4-dioxane (1M), 4 vol% H$_2$O, quant.; iii) HIO$_4$·2H$_2$O (1.75 eq.), 1.4-dioxane/H$_2$O (3:1, v/v), 70°C, 24 h, 94%; iv) SOCl$_2$ (0.2M), reflux, 2 h; v) H$_2$O, 77%; vi) BF$_3$·OEt$_2$ (1.05 eq.), TMSCN (1.05 eq.), CH$_2$Cl$_2$ (2.0M), 90 min, RT, 90%; vii) LiAlH$_4$ (6 eq.), Et$_2$O (2.5M), RT, 2 h, 86%; viii) NaNO$_2$ (3 eq.), Ac$_2$O, H$_2$O (0.16M), 57%.

iKlev (167) was synthesized in the 1970’s in four steps from commercially available 2-adamantanone (58). The method of Black and Gill was adapted to obtain 167 (Scheme 4.5). First, the ring expansion of 2-adamantanone (58) by an Arndt Eistert reaction was executed. Homologation of 58 was performed with TMS-diazomethane since the procedure is safer and more simple in comparison to the use of Diazald®, as reported.12 TLC monitoring of the reaction progress was not possible because during analysis both product and starting material co-eluted on TLC-plates. The progress of the reaction could be monitored by means of TLC/MS and after sublimation, pure 170 was isolated in 64% yield. Subsequent selenium dioxide-mediated oxidation of 170 in refluxing aqueous 1,4-dioxane gave crude dione 171 in near quantitative yield and high purity. The periodic acid cleavage of crude 171 was sluggish and required overnight reaction times to reach completion. Crude di-acid 172 was isolated in excellent yield after repetitious extraction of the aqueous phase with EtOAc/THF (5:2, v/v) and was used without purification in the next step. Finally, 172 was converted to acid chloride 173 in refluxing thionyl chloride, which upon hydrolysis provided iKlev 167 in good yield. For large scale preparations of iKlev, the use of expensive TMS-diazomethane for the preparation of 170.
was circumvented. To this end, 2-adamantanone (58) was converted in three step, involving cyanohydrin 174 formation, reduction to the amine 175 and finally rearrangement to ketone 170, in a overall yield of 46%.

**Scheme 4.6:** Synthesis of 3’-iKlev monomers.

Reagents and conditions: i) 173 (1.5 eq), Et$_3$N (1.5 eq.), CH$_2$Cl$_2$ (0.1 M), RT, 12 h, 12%; ii) TES (2.0 eq.), 5 vol% DCA in CH$_2$Cl$_2$ (0.1 M), RT, 2 h, 90%.

**Evaluation of the iKlev as a protective group:** Similar to the evaluation of the Klev($n$) protective groups, iKlev (167) was installed on the 3’-OH of $^{DMTrO}dT^{Pom}_{OH}$ (89, Scheme 4.6). The EDC mediated esterification of 167 proved to be troublesome. Total consumption of 167 was observed at 0°C in CH$_2$Cl$_2$, while only traces of product 176 could be isolated and thymidine monomer 89 remained intact. Possibly, iKlev (167) decomposed under the reaction conditions, e.g. by decarboxylation, yielding the stable tertiary 1-adamantyl cation. Application of acid chloride 173 in the esterification reaction was contributive, giving $^{DMTrO}dT^{Pom}_{iKlev}$ in a yield of 12%. After detritylation, $^{HO}dT^{Pom}_{iKlev}$ was subjected to the same cleavage conditions as described for the Klev($n$) derivatives. Pilot experiments revealed that complete hydrazine mediated cleavage of the iKlev protection was achieved within 10 minutes.

### 4.3 Conclusion and Outlook

Three levulinoyl - adamantane hybrid protective groups 140, 144 and 145 were developed and evaluated for usage as 3’-O-nucleoside protection. These novel protective group combines the lipophilic properties of the adamantane moiety with the orthogonal cleavage method of the levulinoyl group. The Klev($n$) derivatives (140, 144 and 145) were prepared in good overall yields and installed at the 3’-OH of $^{DMTrO}dT^{Pom}_{OH}$ (89). Unfortunately, the Klev($n$) esters were cleaved ~ at least 20 times slower compared to the original Lev.

Additionally, a levulinoyl - adamantane hybrid protective group, iKlev 167, in which the ketone moiety is incorporated in the adamantane skeleton was prepared in good overall yield. Installation of the iKlev at the 3’-OH of protected thymidine derivative 89 was troublesome, giving only trace amounts of $^{DMTrO}dT^{Pom}_{iKlev}$. However, preliminary studies indicate that the iKlev protective group rivals the parent Lev in terms of cleavage conditions and reaction times.
4. Development of levulinoyl-adamantane hybrid protective groups

Improvement of the introduction of the iKlev can result in a new protective group, usable as a lipophilic alternative to the Lev in the solution phase synthesis of partially protected ON fragments. Alternatively, the incorporation of a methylene group between the adamantane unit and the acid functionality in iKlev can prevent the degradation of the iKlev acid during esterification reactions.

4.4 Experimental Procedures

General methods: Solvents and reagents were obtained commercially and used as received unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere and residual water was removed from starting compounds by coevaporation with toluene (3x), unless stated otherwise. Reaction grade solvents were stored on 4Å molecular sieves or 3Å for MeOH, CH₃CN, and DMSO. All solvents were removed by in vacuo evaporation at ~45°C. Reactions were monitored by TLC analysis using silica gel coated aluminium plates (0.2 mm thickness) an detection by UV absorption (254 nm) and/or by spraying with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·4H₂O in 10% H₂SO₄, followed by charring at ~150°C. Visualisations of olefins was achieved by spraying with a solution of KMnO₄ (5 g/L) and K₂CO₃ (25 g/L) in H₂O, followed by charring at ~150°C. Nucleosides were visualized by spraying with a solution of 20% H₂SO₄ in EtOH and charring at ~150°C and for adamantane containing compounds a solution of H₃PMo₁₂O₄₀ (100 g/L) was used. Flash column chromatography was performed on silica gel (40-63 μm). ¹H- and ¹³C NMR spectra were recorded on a 400/100, 500/125 or 600/150 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard for all ¹H NMR measurements in CDCl₃ and the deuterated solvent signal for all other NMR experiments. ¹H NMR peak assignments were made using COSY and HSQC experiments, where the structure is numbered arbitrarily (as shown in the accompanied figure) and coupling constants (J) are given in Hz. All ¹³C NMR spectra are proton decoupled. For LC/MS analysis a HPLC-system (detection simultaneously at 213 nm, 254 nm and evaporative light detection) equipped with an analytical C18 column (4.6 mmD x 250 mmL, 5μm particle size) in combination with buffers A: H₂O, B: CH₃CN, C: 1.0% aqueous trifluoroacetic acid and coupled with an electrospray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a semi-preparative C18 column (5 μm C18, 10, 150 x 21.2 mm) was used. The applied buffers were A: H₂O + trifluoroacetic acid (1% mM) and B: CH₃CN. High resolution mass spectra were recorded by direct injection (2 μL of a 2 μM solution in H₂O/CH₃CN; 50:50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Optical rotations were measured on an automatic polarimeter (Sodium D-line, λ = 589 nm).
4.4 Experimental Procedures

**General procedure A: LiAlH₄ reduction.** To a dry solution of the corresponding carboxylic acid (1.0 eq.) in THF (3.5 M) was added LiAlH₄ (1.25 eq.) as a 4 M solution in THF, over a period of 15 min at 0°C under an argon atmosphere. The resulting reaction mixture was allowed to warm up to room temperature over a period of 90 min. After TLC analysis showed complete consumption of the starting material, the reaction mixture was cooled to 0°C and excess LiAlH₄ was quenched with EtOAc followed by H₂O. The formed metallic salts were dissolved with 1 M aq. HCl and the reaction mixture was extracted three times with EtOAc. The combined organic layers were subsequently washed with 1 M aq. HCl (3x), 10% aq. NaHCO₃, brine, dried (MgSO₄), filtered and concentrated. The crude material was subjected to silica gel flash column chromatography (FCC) purification.

**General Procedure B: Swern oxidation.** To a cooled (-78°C) solution of oxalyl chloride (1.3 eq.) in CH₂Cl₂ (1 M) was added DMSO (2.5 eq.) as a 2 M solution in CH₂Cl₂, over a period of 30 min under an argon atmosphere. During the addition of DMSO, the internal reaction temperature was maintained at ±70 °C. Next, a dry solution of the adamantane derivative (1 eq.) in CH₂Cl₂ (1.3 M) was added dropwise over a period of 30 min., while keeping the internal reaction temperature ≤ -78°C. TLC monitoring of the reaction was possible by treating a few drops of the reaction mixture with Et₃N. The reaction mixture was stirred for 2 h at -70°C, and after complete consumption of the starting material, Et₃N (5 eq.) was slowly added to the reaction mixture at -70°C, after which the mixture was allowed to warm up to -5°C. The reaction mixture was diluted with 1 M aq. HCl and transferred to a separatory funnel and extracted three times with CH₂Cl₂. The combined organic layers were subsequently washed with 1 M aq. HCl, 10% aq. NaHCO₃, brine, dried (Na₂SO₄), filtered and concentrated. The crude material was purified by silica gel FCC.

**General procedure C: Grignard addition.** To a dry solution of the corresponding aldehyde in Et₂O (1 M) was added homoallyl magnesiumbromide (1.4 eq.) as a ~ 1 M solution in Et₂O dropwise at 0 °C. The reaction mixture was stirred overnight at RT and after TLC analysis indicated complete consumption of the starting material, the excess Grignard reagent was quenched by the addition of H₂O. The resulting metallic salts were dissolved with 1 M aq. HCl and the reaction mixture was extracted three times with Et₂O. The combined organic layer was subsequently washed with 10% aq. NaHCO₃, brine, dried (MgSO₄), filtrated and concentrated. The crude material was purified by silica gel FCC.

**General Procedure D: oxidative olefin cleavage.** To a solution of the corresponding olefin in acetone (0.025 M) was added NaIO₄ (8 eq.) as a 0.35 M solution in H₂O. To this vigorously stirred mixture was added RuO₂ (0.07 eq.) and stirring was continued for 3 h at room temperature. After TLC analysis indicated complete consumption of the starting material, excess NaIO₄ was depleted by the addition of isopropanol. Celite® was added and the reaction mixture was filtered over Celite®. The volatiles were removed via a gentle air stream and the pH of the
residue was adjusted to $\sim$ 7 with NaHCO$_3$(s). The aqueous layer was washed once with CHCl$_3$ and subsequently acidified by the careful addition of 4M aq. HCl and extracted three times with CHCl$_3$. The combined organic layer was subsequently washed with sat. aq. Na$_2$S$_2$O$_3$, brine, dried (MgSO$_4$), filtered and concentrated. The crude material was purified by silica gel FCC.

**General Procedure E: Esterification of** $^{DMTrO}dT^{Pom}_{\text{OH}}$. To a dry solution of $^{DMTrO}dT^{Pom}_{\text{OH}}$ (89, prepared in chapter 2 and chapter 3) in CH$_2$Cl$_2$ (0.1M) was added the corresponding carboxylic acid (0.8 eq.) under an argon atmosphere. Subsequently, EDC·HCl (1.8 eq.), DiPEA (3.0 eq.) and catalytic DMAP were added and the reaction mixture was allowed to stir overnight at RT. Upon completion of the reaction, as judged by TLC analysis (PE/Et$_2$O, 4:1), the reaction mixture was diluted with H$_2$O and extracted three times with CH$_2$Cl$_2$. The combined organic layer was subsequently washed with 10% aq. KH$_2$SO$_4$, 10% aq. NaHCO$_3$ (3x), brine/water (1:1, v/v). The organic layer was dried over MgSO$_4$, filtered, concentrated and the residue was subjected to silica gel FCC purification (prepared in the presence of 3 vol% Et$_3$N).

**General Procedure F: Acid mediated DMTr-cleavage.** To a dry solution of the corresponding 5'-O-DMTr derivative in CH$_2$Cl$_2$ (0.1M) was added TES (2 eq.) followed by the addition of DCA (5 vol%). The reaction mixture was stirred for 2h at RT, at which point TLC analysis usually show full conversion of the starting material. The reaction mixture was diluted with CH$_2$Cl$_2$ and subsequently washed with H$_2$O, 10% aq. NaHCO$_3$ (2x). The combined aqueous layer were back-extracted with CH$_2$Cl$_2$ and the combined organic layers were subsequently washed with brine, dried (MgSO$_4$), filtered and concentrated. The obtained residue was subjected to silica gel FCC purification.

**General Procedure G: Hydrazine mediated cleavage of the 3'-O-nucleoside protection.** The corresponding Klev($n$) or iKlev derivative was coevaporated thrice with toluene and dissolved in a 0.5M N$_2$H$_4$·H$_2$O solution in a mixture of Pyr/AcOH (4:1, v/v). After complete consumption of the SM., as shown by TLC analysis, excess N$_2$H$_4$·H$_2$O was quenched by the addition of 20 eq. acetylacetone. The reaction mixture was diluted with EtOAc and subsequently washed with 1M HCl, sat. aq. NaHCO$_3$, brine and dried (MgSO$_4$), filtered and concentrated. The residue was purified by silica gel FCC purification.

**but-3-enyl magnesiumbromide.**

Magnesium turnings (1.0 g, 41.14 mmol, 2.1 eq.) were heated under an argon atmosphere in the presence of an iodine crystal, followed by the addition of freshly distilled Et$_2$O ($\sim$ 5 ml). Next, a solution of 4-bromobutene (2.0 ml, 19.6 mmol, 1 eq.) in freshly distilled Et$_2$O (3 ml) was added to the reaction mixture at such a rate that the heat of the exothermic reaction allowed for a gentle reflux. The reaction mixture was diluted with Et$_2$O (15 ml) and refluxed for 90 min. The concentration of resulting Grignard reagent was determined by the reaction with benzaldehyde and was found to be $\sim$1M.
N-methoxy-N-methyl-2-(1-adamant-1-yl)-ethanamide (143).

To a dry solution of 1-adamantylacetic acid (141, 4.9 g, 25.4 mmol) in CH₂Cl₂ (0.2M) were subsequently added N,O-dimethylhydroxylamine (5.0 g, 50.8 mmol), DIPEA (30.0 ml, 102 mmol) and BOP (22.5 g, 50.8 mmol). The resulting reaction mixture was stirred for 3 h at RT and diluted with CH₂Cl₂ and subsequently washed with 5% aq. KHSO₄, sat. aq. NaHCO₃, brine, dried (MgSO₄), filtered and concentrated. The residue was subjected to silica gel FCC (PE/EtOAc, 8:2, isocratic) to give the title compound in 88% yield. Rf= 0.3 (8:2 PE/EtOAc). 300 MHz, CDCl₃: δ 1.67-1.71 (br. s, 12H, 6x CH₂- Ada), 1.96 (br. s, 6H, 3x CH Ada), 2.19 (s, 2H, CH₂, H11), 3.18 (s, 3H, N-Me), 3.66 (s, 3H, O-Me).

1-adamantane ethanol (146).

1-Adamantaneacetic acid (141, 8.14 g, 42 mmol) was subjected to LiAlH₄ reduction according to general method A. The crude material was purified by silica gel FCC (PE/Et₂O, 4:1 → 2:1) and gave the title compound in a quantitative yield as a white solid. Analytical data of the obtained compound was identical with that of commercially available 1-adamantane ethanol. ¹H NMR (300 MHz, CDCl₃): δ 1.38 (t, 2H, J= 7.3, H11), 1.51-1.66 (m, 12H, H2,4,6,8,9,10), 1.93 (br. s, 3H, H3,5,7), 3.71 (t, 2H, J= 7.3, H12). ¹³C NMR (75 MHz, CDCl₃): δ 28.5, 33.3, 36.6, 42.5, 44.7, 60.6, 172.3

1-adamantane ethanal (147).

1-Adamantane ethanol (146, 8.4 g, 47 mmol) was oxidized according to general procedure B. The crude material was purified by silica gel FCC (Tol/Et₂O, 1:0 → 1:1) and gave the title compound in quantitative yield as an colourless oil which solidified upon cooling to -20°C. ¹H NMR (200 MHz, CDCl₃): δ 1.63-1.73 (br. s, 12H, H2,4,6,8,9,10), 1.78 (br. s, 3H, H3,5,7), 2.13 (d, 2H, J= 3.6, H11), 9.87 (t, 1H, J= 3.7, H12). ¹³C NMR (50 MHz, CDCl₃): δ 28.0, 31.2, 36.1, 36.3, 36.6, 42.1, 42.2, 42.4, 56.6, 202.2.

1-adamantylhex-5-en-2-ol (148).

1-Adamantane acetaldehyde (147, 7.5 g, 42 mmol, 1.0 eq.) was subjected to general protocol C. The crude compound was purified by silica gel FCC (Tol/Et₂O, 1:0 → 2:3) and gave the title compound in 80% yield as a white crystalline solid. ¹H NMR (400 MHz, CDCl₃): δ 1.25 (m, 2H, H11), 1.48-1.54 (m, 2H, H13), 1.55-1.60 (m, 6H, H2,8,9), 1.66-1.78 (m, 6H, H4,6,10), 1.95 - 2.0 (br. s, 3H, H3,5,7), 2.10-2.25 (m, 2H, H14), 3.74-3.80 (m, 1H, H12), 4.89-5.00 (m, 2H, H16), 5.73-5.83 (m, 1H, H15). ¹³C NMR (100 MHz, CDCl₃): δ 28.7, 28.9, 30.1, 32.3, 37.1, 38.6, 43.1, 52.4, 67.5, 114.7, 138.7.
1-adamantylhex-5-en-2-one (142).
1-Adamantylhex-5-en-2-ol (148, 6.5 g, 28 mmol, 1.0 eq.) was subjected to Swern oxidation according to general procedure B. The crude material was purified by silica gel FCC (PE/Et₂O, 1:0 → 2:3) and gave the title compound in 97% yield as a yellow oil. ¹H NMR (400 MHz, CDCl₃): δ 1.63-1.70 (m, 12H, H₂,4,6,8,9,10), 1.95 (br. s, 3H, H₃,5,7), 2.15 (s, 2H, H₁₁), 2.26-2.31.1 (m, 2H, H₁₃), 2.43-2.49 (m, 2H, 14), 4.95-5.04 (m, 2H, H₁₆), 5.75-5.85 (m, 1H, H₁₅). ¹³C NMR (100 MHz, CDCl₃): δ 27.8, 28.8, 33.7, 36.9, 42.7, 45.0, 56.3, 155.2, 137.5.

1-adamantyl-2-oxopentanoicacid (140).
1-Adamantylhex-5-en-2-one (142, 4.7 g, 18.7 mmol, 1.0 eq.) was subjected to oxidative olefin cleavage according to general protocol D. The crude compound was purified by silica gel FCC (PE/Et₂O, 1:0 → 9:1) which gave the title compound in 73% yield as a white solid. Mp = 54-58 °C. ¹H NMR (400 MHz, CDCl₃): δ 1.42-1.64 (m, 12H, H₂,4,6,8,9,10), 1.95-2.07 (m, 3H, H₃,5,7), 2.10 (s, 2H, H₁₁), 2.49-2.58 (m, 2H, H₁₃), 2.68-2.89 (m, 2H, H₁₄), 9.80 (br. s, 1H, OH). ¹³C NMR (100 MHz, CDCl₃): δ 28.0, 28.8, 33.7, 36.9, 40.0, 42.6, 56.1, 179.1.

1-adamantane methanol (149).
1-Adamantanecarboxilic acid (51, 25.0 g, 138.9 mmol) was subjected to LiAlH₄ reduction according to general method A. The crude material was purified via crystallisation from ethanol to provide the title compound in near quantitative yield as white crystals. Analytical data of the obtained compound was identical with that of commercially available 1-adamantane methanol. ¹H NMR (400 MHz, CDCl₃): δ 1.40 (br. s, 1H, OH), 1.53 (m, 6H, H₄,6,10), 1.66 - 1.77 (m, 6H, H₂,8,9), 2.01 (br. s, 3H, H₃,5,7), 3.22 (s, 2H, H₁₁). ¹³C NMR (100 MHz, CDCl₃): δ 28.2, 34.2, 37.2, 39.0, 73.8.

1-adamantane methanal (150).
1-Adamantane methanol (149, 10.1 g, 60.1 mmol) was oxidized according to general procedure B. The crude material was purified by silica gel FCC (Tol/Et₂O, 1:0 → 1:1) and gave the title compound in quantitative yield as an colourless oil. ¹H NMR (400 MHz, CDCl₃): δ 1.69-1.78 (m, 12H, H₂,4,6,8,9,10), 2.08 (br. s, 3H, H₃,5,7), 9.32 (s, 1H, H₁₁). ¹³C NMR (100 MHz, CDCl₃): δ 27.3, 29.0, 35.8, 36.5, 205.8.

1-adamantylpent-4-en-1-ol (151).
1-Adamantanemethanal (150, 3.0 g, 18 mmol, 1.0 eq.) was subjected to Grignard addition according to general protocol C. The crude compound was purified by silica gel FCC (Tol/Et₂O, 1:0 → 4:1) and gave the title compound in 83% yield as a yellow oil. ¹H NMR (400 MHz, CDCl₃): δ 1.34-1.42 (m, 1H, H₁₂a), 1.50-1.80 (m, 13H, H₂,4,6,8,9,10, 12b), 2.0 (br. s, 3H, H₃,5,7), 2.07-2.16 (m, 1H, H₁₃a), 2.29-2.40 (m,
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1H, H13b), 3.01-3.07 (dd, 1H, \( J = 10.6, 1.8, \text{H11} \)), 3.65 (s, 1H, OH), 4.96-5.12 (m, 2H, H15), 5.82-5.93 (m, 1H, H14). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 27.9, 28.3, 29.2, 31.1, 36.6, 37.2, 37.9, 79.3, 114.5, 138.8.

1-adamantylpent-4-en-1-one (152).

1-Adamantylpent-5-en-2-ol (151, 1.7 g, 7.7 mmol, 1.0 eq.) was subjected to Swern oxidation according to general procedure B. The crude material was purified by silica gel FCC (Tol/Et\(_2\)O, 1:0 \( \rightarrow \) 9:1) and gave the title compound in 91% yield as a yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.69-1.78 (m, 6H, H2,8,9), 1.82-1.83 (m, 6H, H4,6,10), 2.06 (br. s, 3H, H3,5,7), 2.27-2.33 (m, 2H, H13), 2.54-2.57 (m, 2H, H12), 4.95-5.04 (m, 2H, H15), 5.77-5.87 (m, 1H, H14). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 27.7, 28.0, 35.3, 36.6, 38.2, 46.3, 114.9, 137.7, 214.6.

1-adamantyl-1-oxobutanoic acid (144).

1-Adamantylpent-4-en-1-one (152, 1.5 g, 7.0 mmol, 1.0 eq.) was subjected to oxidative olefin cleavage according to general protocol D. The crude compound was purified by silica gel FCC (PE/Et\(_2\)O, 1:0 \( \rightarrow \) 9:1) which gave the title compound in 71% yield as a white solid. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.63-1.72 (m, 6H, H2,8,9), 1.78-1.79 (m, 6H, H4,6,10), 2.00 (br. s, 3H, H3,5,7), 2.54 (t, 2H, \( J = 2.5 \), H12), 2.73 (t, 2H, \( J = 2.7 \), H13), 11.3 (br. s, 1H, OH). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 27.8, 27.9, 30.9, 36.5, 38.2, 46.1, 179.1, 213.7.

(Z)-ethyl 3-(adamantan-1-yl) acrylate (155).

To a dry solution of ethyl 2-(bromotriphenylphosphoranyl)acetate (154, 11 mmol, 1.1 eq.) in Et\(_2\)O (0.1M) was dropwise added n-BuLi (1.1 eq., 1M in THF) over a period of 5 min at -15°C. To the resulting yellow suspension was added 1-adamantane methanal (150, 10 mmol as a 5M solution in Et\(_2\)O) and the reaction mixture was allowed to stir overnight at RT. After TLC analysis (Tol/PE, 3:2) showed complete consumption of the SM, excess n-Buli was quenched by the addition of H\(_2\)O. The reaction mixture was extracted three times with Et\(_2\)O and the combined ethereal layer was washed with 1M aq. HCl, 10% aq. NaHCO\(_3\), brine, dried (NaSO\(_4\)) filtered and concentrated. The crude material was purified by silica gel FCC (PE/Tol, 1:0 \( \rightarrow \) 1:1) to give the intermediate \( \alpha\beta \)-unsaturated ester in near quantitative yield as a colourless oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.29 (t, 3H, \( J = 16.0 \), H15), 1.62-1.76 (m, 12H, H2,4,6,8,9,10), 2.01 (br. s, 3H, H3,5,7), 4.18 (q, 2H, \( J = 7.1 \), H14), 5.67 (d, 1H, \( J = 16.0 \), H11), 6.81 (d, 1H, \( J = 16.0 \), H12). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 14.3, 28.0, 35.6, 36.6, 41.1, 60.1, 116.5, 158.8, 167.5.
4. Development of levulinoyl - adamantane hybrid protective groups

**ethyl 3-(adamantan-1-yl)-propanoate (156).**

\[ \text{OEt} \]

\[ \alpha, \beta \text{-Unsaturated ester 155 was dissolved in EtOAc (1M), purged of oxygen and stirred overnight in the presence of a catalytic amount of Pd/C (5 mol%) under an H}_2 \text{ atmosphere (balloon). After TLC analysis (PE/Tol, 3:2) indicated complete conversion of the starting material in to a more polar compound, the reaction mixture was filtrated over Celite® and the volatiles were removed. The crude material was used without further purification in the next step.} \]

\[ ^1\text{H NMR (400 MHz, CDCl}_3\text{): } \delta 1.26 \text{ (t, 3H, } J= 7.1, \text{ H15), 1.40-1.47 \text{ (m, 8H, H4,6,10, 11), 1.61-1.72 \text{ (m, 6H, H2,8,9), 1.96 \text{ (br. s, 3H, H3,5,7), 2.24-2.28 \text{ (m,2H, H12), 4.12 (q, 2H, } J= 7.1, \text{ H14).} \text{ } ^{13}\text{C NMR (100 MHz, CDCl}_3\text{): } \delta 14.2, 28.2, 28.6, 31.9, 36.0, 39.0, 42.00, 60.2, 174.7.} \]

**1-adamantane propanal (153).**

\[ \text{O} \]

To a dry solution of 156 (0.26 g, 1.0 mmol) in Et\(_2\)O (0.1 M) was added DIBALH (1.2 eq., 1.0 M in hexane) at -78 °C. The reaction mixture was stirred for 90 min at -78 °C after which TLC analysis (PE/Et\(_2\)O, 3:2) revealed complete conversion of the starting material into a more polar compound. Excess DIBALH was quenched by the addition of 1 ml MeOH at -78 °C and the reaction mixture was allowed to warm up to RT. The resulting metallic salts were dissolved by the addition of 10% aq. NH\(_4\)Cl and the reaction mixture was extracted three times with Et\(_2\)O. The combined etheral layer was washed with 10% aq. NaHCO\(_3\), brine, dried (Na\(_2\)SO\(_4\)), filtered and concentrated. The crude material was purified by silica gel FCC to furnish the title compound in 71% yield as a colourless oil. \[ ^1\text{H NMR (400 MHz, CDCl}_3\text{): } \delta 1.39 \text{ (t, 2H, } J= 8.0, \text{ H11), 1.45 \text{ (br. s, 6H, H4,6,10), 1.60-1.72 \text{ (m, 6H, H2,8,9), 1.96 \text{ (br. s, 3H, H3,5,7), 2.35-2.40 \text{ (m,2H, H12), 9.76 (t, 1H, } J= 1.9, \text{ H13).} \text{ } ^{13}\text{C NMR (100 MHz, CDCl}_3\text{): } \delta 28.5, 31.7, 35.8, 37.8, 42.0, 203.3.} \]

**1-adamantylhep-6-en-3-ol (157).**

\[ \text{OH} \]

1-Adamantanepropanal (153, 3.84 g, 20 mmol) was subjected to the Grignard addition according to general protocol C. The crude compound was purified by silica gel FCC (PE/Et\(_2\)O, 1:0 → 7:3) and gave the title compound in 82% yield as a colourless crystalline solid. \[ ^1\text{H NMR (400 MHz, CDCl}_3\text{): } \delta 0.98-1.09 \text{ (m, 1H, H11a), 1.19-1.28 \text{ (m, 1H, H11b), 1.33-1.58 \text{ (m, 10H, H4,6,10,14,15), 1.58-1.75 \text{ (m, 6H, 2,8,9), 1.96 \text{ (br. s, 3H, H3,5,7), 2.09-2.30 \text{ (m, 2H, H12), 4.95-5.10 \text{ (m, 2H, H17).} \text{ } ^{13}\text{C NMR (100 MHz, CDCl}_3\text{): } \delta 28.7, 30.1, 30.3, 32.0, 36.4, 37.2, 40.3, 42.5, 72.4, 114.7, 138.7.} \]

**1-adamantylhep-6-en-3-one (158).**

\[ \text{OH} \]

1-Adamantylhep-6-en-3-ol (157, 4.1 g, 16.5, 1.0 eq.) was subjected to a Swern oxidation according to general procedure B. The crude material was purified by silica gel FCC (PE/Et\(_2\)O, 1:0 → 1:1) and gave the title compound
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in 99% yield as a yellow oil. 

\[ ^1H \text{NMR (400 MHz, CDCl}_3\]: 1.42-1.46 (m, 2H, H11), 1.53-1.54 (m, 6H, H4,6,10), 1.69-1.81 (m, 6H, H2,8,9), 2.04 (br. s, 3H, H3,5,7), 2.38-2.45 (m, 4H, H14,15), 2.59 (t, 2H, J = 7.4, H12), 5.05-5.16 (m, 2H, H17), 5.84-5.95 (m, 1H, H16). 

\[ ^13C \text{NMR (100 MHz, CDCl}_3\]: } \delta 28.0, 28.7, 31.9, 36.6, 37.2, 37.8, 41.8, 42.3, 115.2, 125.4, 211.1.

**1-adamantyl-3-oxohexanoic acid (145).**

1-Adamantylhep-6-en-3-one (158, 4.4 g, 16.4 mmol, 1.0 eq.) was subjected to oxidative olefin cleavage according to general protocol D. The crude compound was purified by silica gel FCC (PE/Et\(_2\)O, 1:1 → 0:1) which gave the title compound in 75% yield as a white solid.

\[ ^1H \text{NMR (400 MHz, CDCl}_3\]: } \delta 1.35-1.42 (m, 2H, H11), 1.45-1.48 (m, 6H, H4,6,10), 1.62-1.74 (m, 6H, H2,8,9), 1.97 (s, H3,5,7), 2.40-2.44 (m, 2H, H12), 2.65 (t, 2H, J = 6.4, H14), 2.76 (t, 2H, J = 6.4, H15). 

\[ ^13C \text{NMR (100 MHz, CDCl}_3\]: } \delta 27.9, 28.6, 31.8, 36.4, 36.8, 37.1, 37.7, 42.2, 178.8, 209.7.

**5'-O-dimethoxytrityl-3'-O-Klev0-N\(^3\)-pivaloyloxymethyl-thymidine (159).**

5'-O-Dimethoxytrityl-N\(^3\)-pivaloyloxymethyl-thymidine (89, 1.35 g, 2.0 mmol) was acetylated with 144 as described in General procedure E. The title compound was obtained as a colourless solid in 95% yield after silica gel FCC (prepared in the presence of 3 vol% Et\(_3\)N and eluted with PE/Et\(_2\)O, 1:1 → 0:1). 

\[ ^1H \text{NMR (400 MHz, CDCl}_3\]: } \delta 1.18 (s, 9H, t−Bu - Pom), 1.38 (s, 3H, CH\(_3\)- thymine), 1.64-1.76 (m, 6H, 3x CH\(_2\)-Ada), 1.82 (br. s, 6H, 3x CH\(_2\)-Ada), 2.03 (br. s, 3H, 3x CH - Ada), 2.44-2.51 (m, 2H, CH-2'), 2.55 (t, 2H, J = 6.3, CH\(_2\)2), 2.78 (t, 2H, J = 6.3, CH\(_2\)2), 3.47 (d, 2H, J = 5.3, H5'), 3.75 (s, 6H, 2x OMe), 4.15 (d, 1H, J = 1.5, H4'), 5.49 (d, 1H, J = 5.3, H3'), 5.88-6.00 (m, 2H, CH\(_2\)-Pom), 6.47 (dd, 1H, J = 8.5, H3'), 6.83 (d, 4H, J = 8.9, Har, DMTr), 7.19 -7.42 (m, 9H, Har, DMTr), 7.66 (s, 1H, H6). 

\[ ^13C \text{NMR (100 MHz, CDCl}_3\]: } \delta 12.2, 27.0, 27.9, 28.0, 31.0, 37.9, 38.3, 38.8, 63.7, 66.2, 75.4, 84.0, 86.0, 87.2, 101.7, 113.3, 127.2, 128.0, 128.1, 130.1, 130.1, 134.5, 135.1, 135.2, 144.2, 150.4, 158.7, 162.5, 172.4, 177.5, 213.5.

5'-O-dimethoxytrityl-3'-O-Klev1-N\(^3\)-pivaloy laxymethyl-thymidine (160). 5'-O-Dimethoxytrityl-N\(^3\)-pivaloyloxymethyl-thymidine (89, 3.29 g, 5 mmol) was acetylated with 140 as described in General procedure E. The crude material was purified by silica gel FCC (prepared in the presence of 3 vol% Et\(_3\)N and eluted with PE/Et\(_2\)O, 1:4 → 1:9), to give the title compound in 88% yield. 

\[ ^1H \text{NMR (400 MHz, CDCl}_3\]: } \delta 1.18 (s, 9H, t−Bu - Pom), 1.62-1.69 (m, 12H, 6 x CH\(_2\)-Ada), 1.93 (br. s, 3H, 3 x CH - Ada), 2.12 (s, 2H, CH\(_2\)2), 2.18 (s, 2H, CH\(_2\)2), 2.46-2.53 (m, 4H, CH\(_2\)2, H2'), 2.70 (t, 2H, J = 12.4, CH\(_2\)2), 3.45-3.48 (m, 2H, H5'), 3.74 (s, 6H, 2x OMe), 4.16 (s, 1H, 1 x H4'), 5.51 (d, J = 5.2, 1H, H3'), 5.94 (s, 2H, CH\(_2\)2 - Pom), 6.47 (dd, 1H, J = 14.0, H1'), 6.82 (m, 4H, Har, DMTr), 7.20-7.41 (m,
4. Development of levulinoyl - adamantane hybrid protective groups

9H, Har, DMTr), 7.67 (s, 1H, H6); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 26.8, 27.7, 33.3, 36.5, 37.7, 38.6, 39.8, 42.2, 54.9, 63.5, 75.2, 83.9, 84.9, 87.0, 110.5, 113.1, 127.0, 127.8, 127.9, 129.9, 134.3, 134.9, 135.1, 144.1, 150.2, 158.6, 162.2, 177.1.

$\textit{5'-O-dimethoxytrytilyl-3'-O-Klev2-N}^3\text{-pivaloyloxy-methyl-thymidine (161).}$  $\textit{5'-O-Dimethoxytrytilyl-N}^3\text{-pivaloyloxyethyl-thymidine (89, 2.17 g, 8.2 mmol) was acetylated with 145 as described in General procedure E. The title compound was obtained as a white foam in 66% yield after silica gel FCC (prepared in the presence of 3 vol% Et$_3$N and eluted with PE/Et$_2$O, 4:1).}$

$^{1}$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.19 (9H, s, tBu - Pom), 1.25-1.35 (2H, m, CH$_2$), 1.47 (s, 3H, CH$_3$ - thymine), 1.57-1.62 (12H, m, 6x CH$_2$ - Ada), 1.97 (br. s, 3H, 3x CH - Ada), 2.37-2.55 (m, 2H, CH$_2$), 2.57-2.65 (m, 2H, CH$_2$), 2.75-2.81 (m, 2H, CH$_2$), 3.46 (2H, m, H5'), 3.79 (6H, s, 2x OMe), 4.12-4.22 (m, 1H, H4'), 5.47-5.53 (m, 1H, H3'), 5.95 (m, 2H, CH$_2$ - Pom), 6.46-6.53 (m, 1H, H1'), 6.86 (m, 4H, Har, DMTr), 7.26-7.38 (9H, m, Har, DMTr), 7.63 (1H, s, H6).

$\textit{3'-O-Klev0-N}^3\text{-pivaloyloxy-methyl-thymidine (162).}$  $\textit{5'-O-dimethoxytrytilyl-3'-O-Klev1-N}^3\text{-pivaloyloxymethyl-thymidine (159, 1.7 g, 1.9 mmol) was detritylated according to General procedure F. The crude material was purified by silica gel FCC (PE/EtOAc, 2:1 → 0:1) to furnish the title compound in 87% yield.}$

$^{1}$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.5 (s, 9H, t- Bu - Pom), 1.60-1.76 (m, 6x CH$_2$ - Ada), 1.80 (br. s, 6H, 3x CH$_2$ - Ada), 1.90 (s, 3H, CH$_3$ - thymine), 2.02 (br. s, 3H, 3x CH - Ada), 2.29-2.43 (m, 2H, H2'), 2.53 (t, 2H, $J$ = 6.3, CH$_2$), 2.77 (t, 2H, $J$ = 6.3, 2H, CH$_2$), 3.21 (br. s, OH), 3.82-3.95 (m, 2H, H5'), 4.04-4.14 (m, 1H, H4'), 5.33 (dt, 1H, $J$ = 2.4, 5.0, H3'), 5.87-5.97 (m, 2H, CH$_2$ - Pom), 6.29 (dd, 1H, $J$ = 6.3, 7.8, H1'), 7.69 (s, 1H, H6). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 13.0, 26.8, 27.7, 27.8, 30.8, 36.3, 37.2, 38.1, 38.6, 45.9, 62.3, 64.9, 74.7, 84.9, 86.0, 110.2, 135.2, 150.2, 162.4, 172.6, 177.5, 213.8

$\textit{3'-O-Klev1-N}^3\text{-pivaloyloxy-methyl-thymidine (163).}$  $\textit{5'-O-dimethoxytrytilyl-3'-O-Klev1-N}^3\text{-pivaloyloxymethyl-thymidine (160, 3.65 g, 4.41 mmol) was detritylated according to General procedure E. The title compound was obtained in 90% yield as a white foam after silica gel FCC.}$

$^{1}$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.17 (s, 9H, t-Bu - Pom), 1.60-1.70 (m, 12H, 6 x CH$_2$ - Ada), 1.92-1.93 (s, 3H, CH$_3$ - thymine), 1.94 (br. s, 3H, 3x CH - Ada), 2.20 (s, 2H, CH$_2$), 2.36-2.40 (m, 2H, H2'), 2.48 (t, 2H, $J$ = 6.3, CH$_2$) 2.69 (dd, 2H, $J$ = 12.3, 6.2, CH$_2$), 3.90 (d, 2H, $J$ = 2.4, H5'), 4.06 (d, 1H, $J$ = 2.2, H4'), 5.31 (dd, 1H, $J$ = 5.1, 2.8, H3'), 5.89 (m, 2H, CH$_2$ - Pom), 6.28 (dd, 1H, $J$ = 8.1, 6.1, H1'), 7.75 (s, 1H, H6). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 26.6, 27.6, 32.3, 36.3, 37.1, 33.2, 36.3, 37.1, 38.5, 39.7, 42.1, 55.5, 64.7, 74.7, 84.2, 85.8, 110.0, 135.1, 150.0, 162.3, 177.3.
3'-O-Klev2-N3'-pivaloyloxy-methyl-deoxythymidine (164).

5'-O-dimethoxytrityl-3'-O-Klev2-N3'-pivaloyloxy-methyl-thymidine (161, 1.81 g, 2.0 mmol) was detritylated according to General procedure E. The title compound was obtained as a white foam in 78% yield after silica gel FCC (PE/Et2O, 4:1 → 0:1). 1H NMR (400 MHz, CDCl3): δ 1.18 (s, 9H, t-Bu - Pom), 1.34 (m, 2H, CH2), 1.44 (m, 6H, CH2-Ada, CH3-thymine), 1.59-1.71 (m, 6H, CH2-Ada), 1.95 (m, 6H, CH2-Ada), 2.34 (2H, m, H2'), 2.41 (m, 2H, CH2), 2.61 (t, 2H, J= 6.4, CH2), 2.77-2.82 (m, 2H, CH2), 3.90-4.01 (m, 2H, CH2-Pom), 6.27 (t, 1H, J= 7.0, H1'), 7.55 (1H, s, H6). 13C NMR (400 MHz, CDCl3): δ 13.0, 27.0, 28.0, 28.6, 36.4, 37.2, 37.0, 42.15, 62.3, 65.0, 74.6, 84.9, 86.6, 110.9, 135.2.

2-cyanoadamantan-2-ol (174).

To a dry solution of commercially available 2-adamantanone (21.0 g, 140 mmol) in CH2Cl2 (2m) was dropwise added BF3·OEt2 (18.7 ml, 147 mmol) at -10°C, followed by the addition of TMS-CN (18.5 ml, 147 mmol) under a argon atmosphere. The reaction mixture was allowed to stir for 90 min at RT after which TLC analysis (PE/Et2O, 1:1) showed complete consumption of the SM. The reaction mixture was subsequently washed with 10% aq. NaHCO3 (3x), H2O, dried MgSO4, filtered and concentrated. The residue was dissolved in 1M aq. HCl/dioxane (200 ml, 1:1, v/v) and stirred overnight at room temperature, to cleave the intermediate TMS-ether. The reaction mixture was diluted with toluene and concentrated in vacuo and redissolved in CH2Cl2. The reaction mixture was subsequently washed with 10% aq. NaHCO3 (2x), H2O, brine, dried (MgSO4), filtered and concentrated. The crude material was obtained as a crystalline compound in 90% yield and was engaged in the next step without further purifications. 1H NMR (400 MHz, CDCl3): δ 1.57-1.58 (br. s, 1H, H5), 1.59-1.61 (br. s, 1H, H7), 1.71 (br. s, 2H, H6), 1.78 (br. s, 1H, H3), 1.85-1.92 (m, 3H, H3,4), 2.03-2.16 (m, 6H, H8,9,10), 4.05 (1H, s, OH). 13C NMR (100 MHz, CDCl3): δ 26.2, 26.4, 30.7, 34.8, 36.8, 37.1, 73.7, 122.5.

2-aminomethyladamantan-2-ol (175).

To a cooled (-15°C) and well stirred suspension of LiAlH4 (22.2 g, 0.6 mol) in dry Et2O (2.5m) was carefully added a dry solution of cyanohydrin 174 (21.24 g, 120 mmol) in Et2O (3.0m). The reaction mixture was allowed to warm up to RT in 2 h, after which excess LiAlH4 was quenched by the careful addition of aq. KOH (48 ml, 4m) at -15°C. The reaction mixture was stirred overnight at RT and the formed precipitate was filtered off and rinsed with Et2O. The combined filtrates was dried (MgSO4), filtered and concentrated to give the title compound as a colourless crystalline compound in 86% yield. 1H NMR (400 MHz, CDCl3): δ 1.55-1.58 (br. s, 1H, H5), 1.59-1.61 (br. s, 1H, H7), 1.71 (br. s, 2H, H6), 1.78 (br. s, 1H, H3), 1.85-1.92 (m, 3H, H3,4), 2.03-2.16 (m, 6H, H8,9,10), 4.05 (1H, s, OH). 13C NMR (100 MHz, CDCl3): δ 26.2, 26.4, 30.7, 34.8, 36.8, 37.1, 73.7, 122.5.
2-homoadamantanone (170).

**Method 1:** To a dry solution of 2-adamantanone (58, 3.0 g, 20 mmol) in CH$_2$Cl$_2$ (0.17M) were subsequently added BF$_3$·Et$_2$O (3.8 ml, 30 mmol) and TMSCH$_2$N$_2$ (0.25M solution in CH$_2$Cl$_2$, 30 mmol) at -15 °C under an argon atmosphere. The reaction was stirred overnight at RT and excess BF$_3$·OEt$_2$ was quenched with ice. The reaction mixture was washed with 10% aq. NaHCO$_3$, H$_2$O, dried (MgSO$_4$), filtered and concentrated. The title compound was obtained as a white crystalline compound in 64% yield after sublimation. **Method 2:** A well stirred suspension of 175 (18.1 g, 100 mmol) in water (0.16M) was acidified with AcOH (22.8 ml). Next, a solution of NaNO$_2$ (20.7 g, 300 mmol) in water (1.66M) was added dropwise to reaction mixture at room temperature. The reaction mixture was stirred 4 h at 90°C the resulting white precipitate was filtered off. The white precipitate was purified by silica gel FCC (PE/Et$_2$O, 8:2) to furnish the title compound as a white crystalline solid in 57% yield. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.65-2.10 (m, 13H), 2.58 (d, 2H, $J$ = 3.6, H$_3$), 2.72 (t, 1H, $J$ = 6.5, H$_1$). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 26.3, 27.0, 30.9, 35.0, 37.3, 49.0, 49.9, 203.2.

2,3-homoadamantandione (171).

To a well stirred suspension of 170 (8.2 g, 50 mmol) in dioxane (1M) was added SeO$_2$ (6.13 g, 56.3 mmol) followed by the addition of 2 ml H$_2$O. The reaction mixture was refluxed for 2 h after which TLC analysis showed complete conversion of the starting material (PE/Et$_2$O, 1:1). The insolubles were filtered off and the filtrate was concentrated to give the crude title compound in quantitative yield as a colourless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.78 (br. s, 2H, H$_7$), 1.86-2.20 (10H, m, H$_5$,9,10,11,6,8), 2.93 (t, 2H, $J$ = 5.9, H$_1$,4). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 26.3, 27.0, 30.9, 35.0, 37.3, 49.0, 49.9, 203.2.

bicyclo[3.3.1]nonane-3α,7α-dicarboxylic acid (172).

To a well stirred suspension of 171 (8.72 g, 49 mmol) in dioxane/H$_2$O (0.33M, 3:1, v/v) was added HIO$_4$ (18.9 g, 85.8 mmol). The reaction mixture was stirred for 24 h at 70°C and excess HIO$_4$ was quenched with solid sodium thiosulfate. The resulting precipitate was filtered off and the filter cake was rinsed with EtOAc. The filtrate was transferred to a separatory funnel and extracted three times with EtOAc. The combined organic layers was dried over Na$_2$SO$_4$, filtered and concentrated. The residue was purified over silica gel FCC (PE/Et$_2$O, 1:1 → Et$_2$O/AcOH, 95:5) to provide the title compound in 94% yield as a crystalline solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.46 (d, 2H, $J$ = 2.9, H$_{10}$), 1.93-2.2 (m, 10H, H$_3$,4,5,8,9,11), 2.65-2.70 (m, 2H, H$_2$,H$_{2,6}$), 10.0 (br. s, 2H, 2x COOH). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 24.0, 28.3, 29.9, 34.9, 183.3.
**1-carboxylic acid-adamantan-2-one (167).**

A dry solution of diacid 172 (1.1 g, 5.0 mmol) in SOCl₂ (0.2 M) was refluxed for two hours under argon atmosphere. Excess SOCl₂ was removed under reduced pressure and the residue was redissolved in dioxane/water (0.2 M, 1:1, v/v) and stirring was continued for 30 min at RT. The volatiles were evaporated and the residue was dissolved in EtOAc and subsequently washed with 10% aq. NaHCO₃, H₂O, brine, dried MgSO₄, filtered and concentrated. The crude title compound was obtained in 77% yield as a colourless solid. ¹H NMR (400 MHz, CDCl₃): δ 1.79-1.90 (m, 2H, H₆), 1.97-2.06 (m, 4H, H₄,9), 2.10-2.16 (m, 4H, H₈,10), 2.27-2.36 (m, 2H, H₅,7), 2.56 - 2.62 (m, 1H, H₃), 10.7(br. s, 1H, COOH). ¹³C NMR (100 MHz, CDCl₃): δ 27.3, 35.0, 38.8, 41.1, 46.4, 57.2, 175.8, 213.2.

**5’-O-dimethoxytrityl-3’-O-iKlev-N³-pivaloyloxy-methyl-thymidine (176).** To a dry solution of 5’-O-dimethoxytrityl-3’-O-iKlev-N³-pivaloyloxy-methyl-thymidine (89, 1.0 g, 1.5 mmol) in CH₂Cl₂ (0.1 M) were subsequently added 167 (1.5 eq.) and Et₃N (1.5 eq.). The resulting reaction mixture was stirred for 12 h at RT and subsequently diluted with CH₂Cl₂, washed with 1M aq. HCl, sat. aq. NaHCO₃, dried (MgSO₄), filtered and concentrated. The title compound was obtained as white foam in 12% yield after silica gel FCC (prepared in the presence of 3 vol% Et₃N and eluted with PE/Et₂O, 7:3 → 3:7). ¹H NMR (400 MHz, CDCl₃): δ 1.23 (s, 9H, t- Bu - Pom), 1.41-2.21 (m, 13H, 5x CH₂, 3x CH), 1.59 (s, 3H, CH₃ - thymine), 2.64 (m, 2H, H₂'), 3.54 (m, 2H, H₅', H₄'), 3.80 (s, 6H, 2x OMe, DMTr), 4.25 (m, 1H, H₃'), 5.61 (m, 1H, H₃'), 5.98 (m, 2H, CH₂ - Pom), 6.50 (dd, 1H, J= 5.3, 9.14, H₁'), 6.87-7.00 (m, 4H, Har, DMTr), 7.27-7.33 (m, 9H, Har, DMTr), 7.63 (1H, s, H₆). ¹³C NMR (100 MHz, CDCl₃): δ 12.2, 27.1, 27.4, 35.2, 37.8, 38.7, 40.7, 46.5, 55.3, 57.81, 63.8, 65.2, 75.9, 84.3, 85.1, 110.9, 113.35, 127.2, 128.1, 128.2, 130.1, 130.2, 130.1, 135.2, 135.3, 144.3, 150.1, 150.1, 155.6, 171.5, 171.8, 179.8, 211.5.

**3’-O-iKlev-N³-pivaloyloxy-methyl-thymidine (177).**

5’-O-Dimethoxytrityl-3’-O-iKlev-N³-pivaloyloxy-methyl-thymidine (176, 0.92 g, 1.2 mmol) was detritylated according to General procedure F. The title compound was obtained as a white foam in 90% yield after silica gel FCC (PE/Et₂O, 1:1 → 3:7). ¹H NMR (400 MHz, CDCl₃): δ 1.20 (s, 9H, t–Bu - Pom), 1.84-2.00 (m, 5H, CH₃ - thymine, CH₂ - Ada), 2.04-2.15 (m, 4H, 2x CH₂ - Ada), 2.19-2.24 (br. s, 2H, 2x CH - Ada), 2.38-2.67 (m, 7H, 2x CH₂ - Ada, CH - Ada, H₂'), 3.97-4.02 (m, 2H, H₅'), 4.17-4.23 (m, 1H, H₄'), 5.43-5.50 (m, 1H, H₃'), 5.95-6.01 (m, 2H, CH₂ -Pom), 6.26 -6.30 (m, 1H, H₁'), 7.63 (1H, s, H₆). ¹³C NMR (100 MHz, CDCl₃): δ 15.2, 27.0, 27.4, 35.2, 37.2, 38.8, 40.7, 46.5, 57.9, 62.6, 65.1, 75.0, 85.0, 86.5, 110.6, 135.3, 150.5, 162.5, 171.8, 179.8, 211.8.
5


5.1 Introduction

Nature has gifted us with many plants and herbs having mystical healing properties and their rudimentary application as medicines is a universal phenomenon. In this respect, Asian and Occident phytomedicines prepared from leaves or other parts of the Morus Alba (mulberry, genus Salix) tree are respected for their many benefits. Haarlem oil was the first medication produced on industrial scale for the treatment of a range of medical conditions including diabetes. The major constituent of Haarlem oil, which is still produced to date in the Netherlands, is an extract from leaves of the white mulberry. This plant extract contains a great diversity of (natural) compounds which can effect the human body in numerous ways. One family of such compounds are polyhydroxylated alkaloids, of which nojirimycin, a polyhydroxylated iminosugar, is the first isolated natural glycosidase inhibitor.

Iminosugars

The early 1960’s sparked the academic exercise to synthesize analogues of natural sugars in which the ring oxygen is replaced by another heteroatom. Iminosugars are carbohydrate analogues in which the endocyclic oxygen atom is replaced by a nitrogen atom.

Early reports of the group of Paulsen described the synthesis of 1,5-dideoxy-5-amino-D-glucose (178) from L-sorbose (Scheme 5.1). Bis(tosylate) 179 was obtained from L-sorbose and was converted to hydrochloric salt 180 in 21% yield over four steps. The free base of furanose 180 was immediately hydrogenated, which prevented aromatisation leading to pyridine derivative 181, and gave 178. Shortly after the iminosugar synthesis, the groups of Ishida
5.1 Introduction

Scheme 5.1: Paulsen synthesis of 1-deoxynojirimycin (178).\textsuperscript{137}

![Scheme 5.1](image)

**Reagents and conditions:** i) NaN\textsubscript{3}, DMF, 100°C; ii) Pt, H\textsubscript{2}, MeOH; iii) Na/Hg, aq. MeOH; iv) aq. HCl, RT, 21% over four steps; v) Amberlite OH\textsuperscript{−}; vi) Pt, H\textsubscript{2}, H\textsubscript{2}O.

and Inouye described remarkable antimicrobial properties of a culture broth of *Streptomyces roseochromogenes* R-468.\textsuperscript{138,139} Analysis of the culture broth revealed that the active ingredient was the relatively unstable iminosugar 182 (Figure 5.1). Upon structural elucidation, the isolated iminosugar was given the trivial name nojirimycin (182), after its isolation from *Streptomyces nojiriensis*. Besides antimicrobial activity, 182 possesses moderate inhibitory activity against α- and β-glucosidases. The assessment of the biological activity of DNJ (178) as a glycosidase inhibitor has triggered a renaissance in iminosugar analogues as inhibitors of glycoside processing enzymes.

**Taxonomy of iminosugars**

Following the isolation of poly-hydroxylated piperidines from micro-organisms, chemists started to explore plants as a source of iminosugars. The abundance of iminosugars across different plants, fungi and bacteria has led to the isolation and characterisation of different classes of polyhydroxylated alkaloids (Figure 5.1). Iminosugars are organized in five different classes based on their structural characteristics. The first class are the piperidines which include nojirimycin (182), galactonojirimycin and mannojirimycin derivatives. The second class, the pyrrolidines resembles furanoses, in which the ring oxygen is substituted by an amine. The pyrrolizidines and indolizidines are bicyclic derivatives possessing a bridging nitrogen atom. The nortropanes are the most recent addition to the field of iminosugars. These structurally different classes of iminosugars are unified in their ability to inhibit glycosidases and to a lesser extent glycosyl transferases.\textsuperscript{140,141}

**Glycoside-processing enzymes**

The formation and cleavage of glycosidic bonds is of utmost importance for the propagation of life. To further illustrate their biological importance, 1-3% of the average genome is dedicated to carbohydrate-active enzymes.\textsuperscript{142} The formation of either α- or β-glycosidic bonds is dictated
by specific glycosyl transferases. Glycosidases are abundant in almost all terrestrial life forms and are responsible for the hydrolytic cleavage of glycosidic bonds. Glycosidases are organized in two types namely inverting and retaining glycosidases, depending on the mode of action. The enzymatic cleavage of glycosidic bonds is crucial in various biological pathways including cellular recognition, eukaryotic glycoprotein processing, polysaccharide- and glycoconjugate metabolism. The importance of glycoside processing enzymes is illustrated by numerous diseases originating from defective glycosidases. For instance, glycosidases are involved in various metabolic disorders and diseases such as diabetes, viral or bacterial infection and cancer formation. Lysosomal storage disorders are one of the major disorders originating from defective glycosidases. These disorders are a result of a disruption in the homeostasis of glycosphingolipids (GSLs), a class of highly complexed biomolecules which are essential for life.

Glycosphingolipids
GSLs were discovered around the year 1870 as constituents of the human brain by J. Thudichum. These cerebrosides (a name derived from the cerebellum) are constructed of a sphingolipid linked via a glycosidic bond to an (oligo)sacharide. A great number of different GSLs decorate the cell membrane forming regions or microdomains known as lipid rafts. These enriched microdomains are able to cluster different receptor proteins, thereby forming the major functional part of the cell membrane. Anabolism: Many different GSLs are derived from a common biological pathway and consist of one or more carbohydrate residues attached to a sphingolipid (SL) that resides in the lipid bilayer. The biosynthesis of these SL commences in the cytosolic leaflet of the ER membranes. Here, a cascade of enzymatic transformation produce sphinganine, a common SL precursor, from L-serine and palmitoyl-CoA. Dihydroceramide (Scheme 5.2) is synthesised from sphinganine by N-acylation with palmitoyl-CoA, catalyzed by dihydroceramide synthase. Depending on the function and structure of the (G)SLs, various CoA-activated esters are used in the N-acylation. Finally, dihydroceramide desaturase converts dihydroceramide into ceramide,
5.1 Introduction

Scheme 5.2: Biosynthesis of glucosyl- and galactosyl ceramide.

Ceramide is a key precursor in the biosynthetic pathway towards complex GSLs. After equilibration to the luminal side of the ER, some cell types transform ceramide into galactosylceramide (GalCer) in the lumen of the ER, by ceramide galactosyl transferase. GalCer is further decorated by either sulfation or glycosylation at the 3- or 4-O-position. For the synthesis of glucosylceramide, ceramide is transported to the cytosolic side of the cis-Golgi apparatus. Here condensation of ceramide and UDP-glucose, by the action of the membrane bound glucosylceramide synthase (GCS), takes place. Given the nature of GCS, being a membrane bound enzyme, very little structural data is available concerning this β-glucosyl transferase. Further synthesis of complex glycosphingolipids with GlcCer as precursor occurs exclusively at the luminal inside the Golgi-apparatus.152

Catabolism: GSL destined for degradation can reach the lysosomes by either receptor-mediated endocytosis, phagocytosis or by endocytosis of the plasma membrane.152,153 A glycocalix, composed of lysosomal resistant glycoproteins, protects the lysosomal membrane against deterioration. GSL degradation takes place at the membrane surface of the internal lysosomal membrane vesicles. Here, glycan specific exoglycosidases are involved in the sequential hydrolysis of carbohydrate residues from the non reducing terminus of the GSLs. Additionally, non-enzymatic proteins are essential for the hydrolysis of GSLs containing only a few
carbohydrate residues. The penultimate hydrolysis is carried out by specialized glycosceramidases. GlcCer is hydrolysed to ceramide and α/β-glucose by the enzyme glucocerebrosidase (GBA1, glucosylceramide-β-glucosidase). Finally, ceramidases are involved in the hydrolysis of ceramide to sphingosine and a fatty acid. The resulting glycon and aglycon degradation products can be scavenged for re-utilisation in the cytosol. The aforementioned GBA1 is a lysosomal membrane protein involved in the cleavage of glucocerebroside. GBA2 is a microsomal β-glucosidase involved in the hydrolysis of bile acid 3-O-glucosides. GBA3 is a cytosolic β-glucosidase and is predominantly expressed in the liver. GBA3 efficiently hydrolyzes β-D-glucosides and β-D-galactoside. In addition, a significant neutral glycosylceramidase activity of GBA3 was described, suggesting its involvement in nonlysosomal glucosylceramide catabolism pathways.

**Lysosomal Storage Disorders (LSD)**

In healthy mammalian cells the influx and efflux of GSLs is in homeostasis. Distortion in the catalytic activity of lysosomal hydrolases results in the accumulation of (G)SL fragments in the lysosomes. Progressive accumulation of partially degraded substrates in several tissues ultimately leads to multiple organ dysfunction and clinical complications. Considering the many steps involved in the synthesis and processing of lysosomal hydrolases, it is not surprising that there are many ways to render them dysfunctional. Almost all LSDs arise from single gene mutations encoding lysosomal hydrolases and are autosomal recessive disorders. The exceptions are Hunter-, Fabry- and Danon’s disease. Over 40 different disorders have been described since the identification of Pompe’s disease nearly half a century ago. Interestingly, the first description of a LSD was that of Tay-Sachs disease in 1881, however the lysosome was not discovered until 1955, by Christian De Duve. A ‘critical threshold’ of enzyme activity was proposed in 1983 by Conzelmann and Sandhoff. Residual enzyme activity above this threshold level can cope with substrate influx whereby enzyme activity below this level leads to substrate accumulation. The residual enzyme activity has a profound influence on the age of onset and the severity of the disease. LSDs are categorized according to the substances which are stored, in glycoprotein- and glyco- gen storage diseases, mucopolysaccharidoses, mucolipidoses and sphingolipidoses. A selection of (glyco)sphingolipids and their associated lysosomal disorders are depicted in Figure 5.2.

**Gaucher disease (GD):** A group of inherit disorders caused by impaired lysosomal degradation of GSLs is known as sphingolipidoses. Gaucher disease, an auto recessive disorder which is characterized by massive splenomegaly, is the most prevalent sphingolipidosis. Phillipe Gaucher described the disease, that bears his name, already in 1882. The fact that it represent a metabolic abnormality was made 20 years later, after the discovery of the lipid GlcCer engorged macrophages, the pathological hallmark of GD. Deficiencies in the dissimilation of GlcCer by GBA1, with the aid of activator protein saposin C, lies at the basis of GD. Over 200 different mutations have been identified thus far in the GBA1 encoding gene, located on chromosome 1,
5.1 Introduction

**Figure 5.2:** Selection of (glyco)sphingolipids and their associated lysosomal storage disorders.

![Figure 5.2: Selection of (glyco)sphingolipids and their associated lysosomal storage disorders.](image)


resulting in impaired glucosylcerebroside degradation. GlCer accumulates intracellularly, primarily in cells of mononuclear phagocyte origin which are coined Gaucher cells. Depending on the severity, GD is categorized in three types:  

- **Type 1:** adult or non-neuronopathic and is commonly observed during adulthood and is the most common type. Symptoms include hepatospleomegaly, anaemia, trombocytopenia, leukopenia, undermineralization and fracture of the long bones.
- **Type 2:** neuronopathic and is initiated after birth (also known as infantile or acute neuronopathic type). Symptoms include hepatosplenomegaly, brain impairment and infants die before the age of two.
- **Type 3:** neuronopathic and occurs during childhood or adolescence (also known as juvenile or chronic neuronopathic type). This type can be further categorized in three sub classes (3a-c) with symptoms ranging from mild to massive hepatosplenomegaly, myoclonic seizures, kyphoscoliosis, diminished speech, mitral- and aortic valve calcification.

**Therapeutic treatment**

There has been a rapid understanding of Gaucher disease in the past few decades, culminating in the development of several successful therapeutic treatments. The recognition that a single gene mutation resulted in defective GBA1 enzymes triggered the investigation of enzyme replacement therapy and gene therapy.  

**Enzyme Replacement Therapy (ERT):** Starting with the isolation of GBA1 from human placenta in 1966, ERT led to the successfully treatment of Type 1 GD patients in 1990. Currently,
this therapy employs an analogue of the human enzyme β-glucocerebrosidase, produced by recombinant DNA technology using mammalian cell culture (Chinese hamster ovary). The recombinant enzyme, imiglucerase is a monomeric glycoprotein of 497 amino acids, containing 4 N-linked glycosylation sites and is commercialized under the trade name Cerezyme®. The glycosylation sites in imiglucerase have been engineered to terminate in mannose residues, which are specifically recognized by endocytic mannose receptors on typical Gaucher cells. More than five thousand patients worldwide with the symptoms and risks of Type 1 Gaucher disease, have been augmented with Cerezyme® since its FDA approval in 1994. Unfortunately, with an annual cost of U.S. $200,000 per patients, Cerezyme® is one of the most expensive drugs.

**Gene therapy:** Patients suffering from severe GD, primarily those with chronic neurologic involvement (type 3), can benefit from bone marrow transplantation (BMT). The morbidity and mortality associated with BMT limit the broad application of this therapy in the treatment of GD. However, when successfully engrafted, BMT can correct the metabolic abnormality, improve blood counts and reduce hepatosplenomegaly.

**Chaperone therapy:** The folding and maturation of lysosomal enzymes, like many other proteins, are constantly monitored by the ER quality control system. Aberrant proteins do not pass the quality control system of the ER. In general, the mechanisms that distinguish native from non-native conformations and assist in folding of lysosomal enzymes have only begun to be elucidated. Only those proteins that are correctly folded and stable leave this cellular compartment efficiently and progress through the secretory pathway to their final destination. Although there is still some residual enzyme activity, mutant GBA1 proteins do not pass the ER quality control system. As a consequence, compromised GBA1 proteins are targeted to the proteasome for degradation. Recently, it was demonstrated that orally administered small molecules, called pharmacological chaperones, can ameliorate endogenous GBA1 activity. Pharmacological chaperones thereby selectively bind to newly synthesized target proteins, increasing protein stability, modulate protein processing and trafficking. The postulated mechanism of action involve the occupation of the active site of the target enzymes by reversible inhibitors such as iminosugar derivatives. Clinical efficacy dictated that the increase in lysosomal enzyme activity must be sustained over an extended period to restore GSL homeostasis. The implementation of pharmacological chaperones may be limited to patients with diseases that are associated with residual enzyme activity and that are amenable to enhancement.

**Substrate Reduction Therapy (SRT):** Glycosphingolipidoses originate from a disruptive metabolism of GSL. Despite residual enzyme activity, malfunctioning lysosomal enzymes can not cope with the substrate influx. Small molecules can inhibit substrate precursor synthesis to match the rate of degradation in the lysosomes, thus relieving the symptoms associated with LSD. This therapeutic strategy is known as substrate reduction therapy and was already proposed in the 1970’s by Radin et al. The archetypal GCS inhibitor 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP, Figure 5.3) was designed to simulate the structures of ceramide and GlcCer. This substrate based inhibitor is active at micromolar level and apparently acts at the enzyme’s active site, as well as on a similar binding site. Noteworthy is the fact that...
the optical enantiomer of d-threo-PDMP (187), the L-threo configuration, is not active in GCS inhibition.

Figure 5.3: Selection of glucosylceramide synthase inhibitors and their apparent IC\textsubscript{50} values.

The implementation of SRT was retarded by the general idea that the inhibition of an essential enzyme in the biosynthesis of complex GSL would have prohibitively toxic side effects. In the 1990’s, the evaluation of the iminosugar miglustat (N-butyl-1-deoxynojirimycin) in HIV infections, revealed that its toxicity was much lower than had been anticipated and triggered exhaustive research towards potent and selective GCS inhibitors. Since its FDA approval in 2003, miglustat is marketed under the name Zavesca\textsuperscript{R} for the treatment of Type 1 Gaucher disease when ERT is not a viable option. Unfortunately, patients suffer from side effects such as diarrhoea, flatulence, abdominal pain, nausea, dizziness and headache, partly due to the poor selectivity of the marketed drug.

Glucosylceramide modulators
There is compelling evidence that partial inhibition of GCS improve insulin sensitivity in type 2 diabetes.\textsuperscript{171,172} Furthermore, GCS is implicated in several other therapeutic areas which include hepatosteatosis, atherosclerosis, inflammatory- and polycystic diseases. Potent and selective inhibitors of GCS hold considerable therapeutic value and their development is pursued by a growing number of researchers worldwide. In the search for selective GCS inhibitors, particular attention is given to the \( \beta \)-glucocerebrosidase (GBA1) and \( \beta \)-glucosidase GBA2. These two hydrolytic enzymes are capable of hydrolysing GlcCer and as indicated before, genetic impaired GBA1 lays at the basis of Gaucher disease. Further impairment of GBA1 by small molecule inhibitors can offset the beneficiary effect of its substrate reduction. Furthermore, selective GBA1 inhibitors hold potential as pharmacophoric chaperones, increasing the concentration of lysosomal GBA1. With respect to GBA2, the physiological relevance to Gaucher disease or other (patho)-physiological processes is unclear. Selective GBA2 inhibitors may well serve to aid in unravelling its role in a chemical knock-out strategy. Thus, whereas the main objective of the research described here is to identify selective GCS inhibitors, selective GBA1 and GBA2 inhibitors may have value in gaining insight in the development of LSD. Therefore, the prepared iminosugar libraries are also evaluated against GBA1 and GBA2.

Over the past decade, several 1-deoxynojirimycin derivatives were developed as inhibitors for the enzymes involved in GlcCer metabolism (Figure 5.3).\textsuperscript{171 153 151 173} In the course of
these studies, it was found that increasing the size and hydrophobic character of the \( N \)-alkyl substituent results in a drastic increase in GCS inhibitory potency. The installment of an \( N \)-pentyloxymethyl-1-adamantane moiety on DNJ (190, MZ-21) results in a ∼200 times increase in potency compared to Zavesca (189), possessing an \( N \)-butyl tail (Table 5.1). This increased activity is accompanied by an increased inhibitory potency against GBA1 and GBA2 as well as several intestinal digestive glycosidases (sucrase, isomaltase).\(^{174}\) The C-5-epimer of 190, the \( L \)-ido derivative (191) was identified as a much-improved inhibitor in this respect.\(^{175}\) Its GCS inhibitory activity is equal to, or even surpasses MZ-21 (190), whereas its activity toward GBA1 and GBA2 is about a 10-fold lower and its activity toward intestinal glycosidases next to non-existent. Apparently the alteration of the iminosugar configuration from \( D \)-gluco to \( L \)-ido holds no consequence for GCS recognition, whereas the hydrolases are more sensitive to the nature of the carbohydrate or carbohydrate mimetic. One strategy toward iminosugars with exclusive selectivity for GCS would be to explore the nature of the \( N \)-alkyl substituent. This chapter describes the systematically introduction of (non)linear alkyl- and alkyloxyalkyl groups onto both \( D \)-gluco and \( L \)-ido-configured deoxynojirimycins.

5.2 Results and Discussions

The preparation of the iminosugar library follows well established routes of synthesis.\(^{175}\) Large quantities of 1-deoxynojirimycin (178) and its \( L \)-ido congener 192 were prepared according to

![Scheme 5.3: Synthesis of \( L \)-ido-1-deoxynojirimycin and 1-deoxynojirimycin.](image)

Reagents and conditions: i) \( \text{LiAlH}_4 \) (3.5 eq.), THF (0.17M), \( 0^\circ\text{C} \rightarrow \text{RT} \), 24 h; ii) \( \text{(COCl)}_2 \) (4.1 eq), DMSO (5 eq.), \( \text{Et}_3\text{N} \) (10 eq.), \( \text{CH}_2\text{Cl}_2 \) (2m); \( -78^\circ\text{C} \); iii) \( \text{NH}_4\text{HCO}_2 \) (20 eq.), \( \text{NaCNBH}_3 \) (4 eq.), \( \text{CH}_2\text{Cl}_2 /\text{MeOH} (1:1, \text{v/v}, 0.1\text{M}), 0^\circ\text{C} \rightarrow \text{RT} \), 24 h, 67% over two steps; iv) 4 bar \( \text{H}_2 \) (g), 10 mol% \( \text{Pd/C} \) (10 wt%), \( \text{EtOH} \) (2m) pH ∼1 (adjusted with 37% aq. HCl), 24 h, RT, 178, 90%; 192, 89%; v) \( \text{MsCl} \) (2.5 eq.), \( \text{Pyr} \) (0.1M), \( 0^\circ\text{C} \rightarrow \text{RT} \), 24 h; vi) allyl amine (0.2M), \( \Delta \), 72 h; vii) \( \text{KOTBu} \) (0.5 eq.), DMSO (0.2M), 100°C, 2h; viii) 1M HCl (0.2M), 71% over four steps.
5.2 Results and Discussions

Scheme 5.4: General strategies: \(N\)-alkylation of 178 and 192 and synthesis of pentyloxyalkyl bromides.

![Scheme 5.4](image_url)

Reagents and conditions: i) Requisite (pentyloxyalkyl) bromide, \(K_2\)CO\(_3\) (3 eq.), DMF (0.2M), 80°C, 24 h, \(~50-80\%\); ii) Trt-Cl (1.0 eq.), Pyr (0.1M), 80°C, 24 h; iii) pTsCl (1.5 eq.), Et\(_3\)N (1.5 eq.), CH\(_2\)Cl\(_2\) (0.1M), 83%; iv) NaH (1.5 eq.), DMF (0.1M), 2 h, 80°C, R\(_2\)CH\(_2\)OH (0.9 eq.), \(~60-80\%\); v) cat. \(p\)-TsOH, CH\(_2\)Cl\(_2\)/MeOH (1:1, v/v, 0.1M), \(~90-95\%\); vi) PPh\(_3\) (2 eq.), CBr\(_4\) (2 eq), CH\(_2\)Cl\(_2\) (0.1M), 0°C, 2 h, 80-90%; vii) R\(_2\)CH\(_2\)OH (0.25 eq.), NaH (0.3 eq.), DMF (0.1M), 80°C, 24 h, \(~50\%\).

in house developed procedures\(^{176}\) based on the work of Matos et al.\(^{177}\) Briefly, commercially available 2,3,4,6-tetra-O-benzylglucose (193) was reduced with LiAlH\(_4\) to yield crude glucitol 194 in high purity (Scheme 5.3). Crude 194 was subjected to a Swern oxydation and a subsequent double reductive amination protocol, to produce 195 in good yield. After Pd/C mediated hydrogenation and subsequent purification, 178 was isolated as its HCl salt in high overall yield (60%). The 5-epimer, 192 was prepared from glucitol 194 via \(S_N\)2 substitution of bismesylate 196 with allylamine. After \(N\)-dealallylation of 197 and subsequent hydrogenation of amine 198, \(L\)-ido-DNJ (192) was obtained in a overall yield of 71%, after purification over neutral AlO\(_2\).

Selective \(N\)-alkylation of iminosugars 178 and 192 was accomplished by the use of the appropriate alkyl bromide in DMF and potassium carbonate as the base (Scheme 5.4). The required alkyl bromides were obtained commercially. The yields of the \(N\)-alkylations varied between 50 and 80% and in general, \(L\)-ido-1-deoxynojirimycin gives lower yields. In this fashion, linear aliphatic alkyl substitutions ranging from butyl to nonyl were introduced onto both piperidine cores, leading to \(D\)-\(\text{gluco}\) iminosugars 189, 199, 200, 201, 202 and 203 (Table 5.1) and \(L\)-ido derivatives 204, 205, 206, 207, 208 and 209.

Previous studies have indicated that iminosugars equipped with large alkyl chains are cellularly toxic and that introducing an ether functionality at a strategic position may prevent this undesired effect. With this rationale in mind, and considering that the two leads 190 and 191...
5. Evaluation of N-alkylated iminosugars for the treatment of Gaucher’s disease

encompass a five-carbon spacer, a series of N-(alkyloxypentyl)-deoxynojirimycin derivatives was prepared. N-alkylation of DNJ (178) and 1-ido-DNJ (192) with the pentyloxyalkyl moiety ranging from butyl to nonyl gave iminosugar derivatives 216, 217, 218, 219, 220 and 221, along with the related L-ido series 222, 223, 224, 225, 226 and 227 (Scheme 5.4, Table 5.1). Additionally, a selection of branched alkyl groups, natural compounds such as cholesterol and the farnesyl groups were decorated with an pentyloxy spacer and used in the N-alkylation of the two iminosugar cores (Table 5.2). The requisite pentyloxyalkyl spacer were prepared from 1,5-pentanediol (210) in four steps. First, mono tritylation of 210 followed by the extractive removal of the excess diol 210 and subsequent tolsylation gave pure 211 in high yield. Alkylation of tosylate 211 was accomplished in DMF with sodium hydride and the appropriate alcohol in yields ranging from 60-80%. After acid mediated detritylation of 212, alcohol (213) was transformed into the corresponding bromide via an Appel reaction in about 80-90% yield. Alternatively, the preparation of the pentyloxy alkyl bromide spacers was accomplished in one step. Here a reaction with 1,5-dibromopentane (215) was applied to produce the pentyloxyalkyl bromides in ~50% yield.

Biological evaluation

Next, the inhibitory potency of the newly synthesized compounds against GCS, GBA1, GBA2, sucrase, lactase, and maltase was evaluated.

**Linear alkyl substituents:** The results of the linear (pentyloxy)alkyl chains are given in Table 5.1. The first three entries depict the results obtained from Zavesca (189) and leads MZ-21 (190) and MZ-31 (191), data which corroborates previous results. In general, extension of the N-alkyl chain results in more potent GCS inhibitors. Within the D-gluco series, the N-alkyl derivatives are moderate GCS inhibitors, with octyl and nonyl deoxynojirimycin derivatives 202 and 203 being the most potent. Interestingly, the N-pentyloxyalkyl-substituted deoxynojirimycin derivatives are in general more potent, with the compounds having the larger substituents being the most active. A similar trend is seen in the L-ido-configured series. The GCS inhibitory potency increases with increasing N-alkyl substituent, and also here, the N-pentyloxyalkyl derivatives outperform the N-alkyl species. Head-to-head comparison of two stereoisomers from the two series reveals that in general the L-ido congener is the most potent GCS inhibitor. This trend is most obvious when considering that the N-alkyloxyalkyl series, wherein 227 (IC\(_{50}\) ≤50 nM), outperforms its D-gluco isomer (221, IC\(_{50}\) = 100 nM) by at least two-fold.

GBA1 inhibitory data reveal a related trend, wherein larger substituents give more potent inhibitors, with the important difference that the increase in inhibitory activity within the L-ido series is less pronounced than that observed in the D-gluco series. Without exception, the D-gluco compound is the more potent GBA inhibitor when directly compared with the respective L-ido diastereomer. The inhibitory data on the other enzymes reveal a trend that was already observed for leads 190 and 191. GBA2 appears sensitive to most compounds. Indeed, this enzyme was found to be sensitive to almost all iminosugar type inhibitors that were screened over the years.
### Table 5.1: Enzyme inhibition assay results: Apparent IC$_{50}$ values in micromolar (µM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GCS</td>
</tr>
<tr>
<td>Zavesca</td>
<td></td>
</tr>
<tr>
<td>189</td>
<td>50</td>
</tr>
<tr>
<td>MZ-21</td>
<td>0.2</td>
</tr>
<tr>
<td>MZ-31</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**R = (in situ)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>189</td>
<td>Butyl 50 400 0.23 0.6 750 9</td>
</tr>
<tr>
<td>199</td>
<td>Pentyl &gt;20 500 0.4 2 &gt;1000 10</td>
</tr>
<tr>
<td>200</td>
<td>Hexyl &gt;20 80 0.11 1 700 10</td>
</tr>
<tr>
<td>201</td>
<td>Heptyl 40 18.5 0.045 0.5 500 10</td>
</tr>
<tr>
<td>202</td>
<td>Octyl 4 4 0.020 0.75 300 8</td>
</tr>
<tr>
<td>203</td>
<td>Nonyl ~ 4 1.5 0.007 0.4 200 8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>204</td>
<td>Butyl 20 &gt;1000 1 &gt;1000 &gt;1000 10</td>
</tr>
<tr>
<td>205</td>
<td>Pentyl 15 &gt;1000 0.25 &gt;1000 &gt;1000 10</td>
</tr>
<tr>
<td>206</td>
<td>Hexyl 40 &gt;1000 0.14 600 500 10</td>
</tr>
<tr>
<td>207</td>
<td>Heptyl 4 700 0.04 &gt;1000 200 10</td>
</tr>
<tr>
<td>208</td>
<td>Octyl 4 25 0.02 60 100 600</td>
</tr>
<tr>
<td>209</td>
<td>Nonyl 2 50 0.01 &gt;1000 &gt;1000 &gt;1000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
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</thead>
<tbody>
<tr>
<td>216</td>
<td>Butyl 4 30 0.06 2 300 8</td>
</tr>
<tr>
<td>217</td>
<td>Pentyl 2 705 0.04 1.2 300 4</td>
</tr>
<tr>
<td>218</td>
<td>Hexyl 1 205 0.008 0.8 200 5</td>
</tr>
<tr>
<td>219</td>
<td>Heptyl 0.3 1.75 0.015 1 250 7</td>
</tr>
<tr>
<td>220</td>
<td>Octyl 0.2 0.5 0.010 1 200 10</td>
</tr>
<tr>
<td>221</td>
<td>Nonyl 0.1 0.5 0.040 2.5 350 25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>222</td>
<td>Butyl 2 &gt;1000 0.09 &gt;1000 400 &gt;1000</td>
</tr>
<tr>
<td>223</td>
<td>Pentyl 0.15 100 0.015 &gt;1000 200 &gt;1000</td>
</tr>
<tr>
<td>224</td>
<td>Hexyl 0.1 95 0.025 &gt;1000 200 &gt;1000</td>
</tr>
<tr>
<td>225</td>
<td>Heptyl 0.05 40 0.015 &gt;1000 250 &gt;1000</td>
</tr>
<tr>
<td>226</td>
<td>Octyl 0.05 15 0.015 &gt;1000 300 &gt;1000</td>
</tr>
<tr>
<td>227</td>
<td>Nonyl &lt;0.05 12 0.045 &gt;1000 350 &gt;1000</td>
</tr>
</tbody>
</table>

With respect to the intestinal enzymes, these are inhibited to various extents by the D-gluco compounds but are hardly targeted by the L-ido compounds.

**Branched pentyloxyalkyl substituents:** The results of the branched pentyloxyalkyl chains are given in Table 5.2. In general, DNJ derivatives 228, 229, 230 and L-ido derivatives 234, 235, 236...
Table 5.2: Enzyme inhibition assay results: Apparent IC$_{50}$ values in micromolar ($\mu$M).

<table>
<thead>
<tr>
<th>Compound</th>
<th>GCS $^\text{Facs}$</th>
<th>GCS $^\text{NBD}$</th>
<th>GBA 1</th>
<th>GBA 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>228: Isobutyl</td>
<td>± 10</td>
<td>8</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>229: Prenyl</td>
<td>1-10</td>
<td>12.5</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>230: Neopentyl</td>
<td>&gt;10</td>
<td>4</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>231: Methylcyclohexane</td>
<td>± 0.1</td>
<td>0.5</td>
<td>1.5</td>
<td>0.004</td>
</tr>
<tr>
<td>232: Farnesyl</td>
<td>0.1-1</td>
<td>2.5</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>233: Cholesterol</td>
<td>± 0.1</td>
<td>1</td>
<td>0.5</td>
<td>0.005</td>
</tr>
<tr>
<td>234: Isobutyl</td>
<td>± 0.1</td>
<td>1.5</td>
<td>800</td>
<td>0.02</td>
</tr>
<tr>
<td>235: Prenyl</td>
<td>0.1-1</td>
<td>500</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>236: Neopentyl</td>
<td>± 0.1</td>
<td>100</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>237: Methylcyclohexane</td>
<td>± 0.01</td>
<td>0.07</td>
<td>60</td>
<td>0.006</td>
</tr>
<tr>
<td>238: Farnesyl</td>
<td>0.1-1</td>
<td>2.5</td>
<td>50</td>
<td>0.04</td>
</tr>
<tr>
<td>239: Cholesteryl</td>
<td>0.1-1</td>
<td>1.0</td>
<td>15</td>
<td>0.2</td>
</tr>
</tbody>
</table>

are relatively weak GCS inhibitors, but still about four times more potent compared to Zavesca (189). The inhibitory potency of N-alkylated DNJ derivatives 231, 232 and 233 rivals that of lead MZ-31 (190) and the same holds true for analogues 237, 238, 239 with respect to lead MZ-31 (191). The GBA1 inhibitory data reveal that the D-gluco compound is the more potent GBA1 inhibitor when directly compared with the respective L-ido diastereomer. Interestingly, these branched DNJ derivatives are potent and selective inhibitors of GBA2. In this respect, the L-ido analogues are relative weak inhibitors of GBA1. The most promising candidates are DNJ derivatives 228, 229, 230 in which the neopentyl derivative 230 is identified as the most potent (IC$_{50} = 0.1$ nM) and selective GBA2 inhibitor.

5.3 Conclusion and Outlook

This chapter described the design, synthesis and biological profiling of iminosugar libraries as modulators of glucosylceramide metabolism. A general trend is observed regarding the D-gluco derivatives compared to the L-ido analogues. The D-gluco based inhibitors are in general less potent and selective than the L-ido congeners towards GCS inhibition. Additionally, the incorporation of a N-pentoyloxy spacer proved to have a beneficiary effect on the inhibition profile of the iminosugar-based inhibitors. In this study, L-ido derivative 227 was identified as a potent GCS inhibitor (IC$_{50}$ ≤ 50 nM). The installment of a N-pentoxynopentyl group onto L-ido-1-deoxynojirimycin, resulted in selective and subnanomolar inhibition of GBA2.
5.4 Experimental Procedures

**General Methods:** Solvents and reagents were obtained commercially and used as received unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere and residual water was removed from starting compounds by coevaporation with toluene (3x), unless stated otherwise. Reaction grade solvents were stored on 4Å molecular sieves or 3Å for MeOH, CH₃CN, and DMSO. All solvents were removed by *in vacuo* evaporation at ~ 45°C. Reactions were monitored by TLC analysis using silica gel coated aluminium plates (0.2 mm thickness) an detection by UV absorption (254 nm) and/or by spraying with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·4H₂O in 10% H₂SO₄, followed by charring at ~ 150°C. Visualisations of olefins and N-alkylated iminosugars was achieved by spraying with a solution of KMnO₄ (5 g/L) and K₂CO₃ (25 g/L) in H₂O, followed by charring at ~ 150°C. Glycosides and hemiacetals were visualized by spraying with a solution of 20% H₂SO₄ in EeOH and charring at ~ 150°C and for adamantane containing compounds a solution of H₃PMo₁₂O₄₀ (100 g/L) in EtOH was used. Flash column chromatography was performed on silica gel (40-63 µm). NMR spectra were recorded on a 400/100), 500/125 or 600/150 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard for all ¹H NMR measurements in CDCl₃ and the deuterated solvent signal for all other NMR experiments. All ¹H NMR peak assignments were made using COSY and HSQC experiments, the α/β protons were assigned on the basis of their occurrence at high and low field, respectively and coupling constants (J) are given in Hz. All ¹³C NMR spectra are proton decoupled. For LC/MS analysis a HPLC-system (detection simultaneously at 213 nm, 254 nm and evaporative light detection) equipped with an analytical C18 column (4.6 mmD x 250 mmL, 5µm particle size) in combination with buffers A: H₂O, B: CH₃CN, C: 1.0% aqueous trifluoroacetic acid and coupled with an electrospray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a semi-preperative C18 column (5 µm C18, 10, 150 x 21.2 mm) was used. The applied buffers were A: H₂O + trifluoroacetic acid (1% mM) and B: CH₃CN. High resolution mass spectra were recorded by direct injection (2 µL of a 2µM solution in H₂O/CH₃CN; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylpthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Optical rotations were measured on an automatic polarimeter (Sodium D-line, λ = 589 nm).

**Measurement of enzyme activities:** IC₅₀ values of the iminosugars for the various enzyme activities were determined by exposing cells or enzyme preparations to an appropriate range of iminosugar concentrations (DMSO stock solutions diluted with RPMI medium).

**In vivo glucosylceramide synthase assay:** The mouse macrophage cell line RAW-267 was grown to 90-100% confluence in growth medium at 37°C in a 5% CO₂ incubator. The growth
medium consisted of RPMI -1640 + 10% FCS + penicillin (150 μg/ml) and streptomycin (250 μg/ml) + 50 μM) Hepes buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). The incubation flask was washed with RPMI medium without serum (3.5 ml) to remove serum. Cells were taken up in 3 ml RPMI + 50 mM) Hepes + 300 μM conduritol B epoxide (10 mM) stock in RPMI + the inhibitor (0.1, 1, 10 μM) (20 mM) stock in DMSO diluted in RPMI) and 5 nmol C6-NBD-ceramide/ BSA complex (N-[7-(4-nitrobenzo-2-oxa-1,3-diazole]-6-aminocaproyl-D-erythrospingosine) were added successively to the cell culture. The cells were incubated for 1 hour at 37°C in a 5% CO₂ incubator. At the end of incubation, the flask was inspected for potential cytotoxicity of the inhibitor and washed with medium without serum (5.5 ml). A 50 mM potassium phosphate buffer (KPi buffer, pH 5.8, 0.75 ml) was added and the flask was placed in ice. Cells were removed from the flask by scraping and the harvested cells were collected in a capped plastic vial and immediately immersed in liquid nitrogen. The frozen cell lysate (~0.75 ml) was suspended in methanol (3 ml) and extracted with chloroform (3 ml). After extraction a 0.73% NaCl solution (2.0 ml) was added to biphasic system. The aqueous phase was extracted once more with chloroform (1 ml). The combined chloroform layers were isolated and concentrated at 30-40°C under a nitrogen flow. Lipids were separated by thin-layer chromatography (HP-TLC plates 20-10 silicagel 60 of Merck) using chloroform/methanol/15 mM) aq. CaCl₂ (60:35:8, v/v/v) as the developing solvent. The C6-NBD-labeled (glyco)sphingolipids were identified using standards, visualized with a Typhoon Trio Variable Mode Imager (λex 488 nM, λem 520 nM and quantified with ImageQuant TL software. Glucocerebrosidase activity was measured using recombinant enzyme and 4-methylumbelliferyl-β-glucose as substrate. GBA2 activity was measured using enzyme-containing membrane preparations from Gaucher spleen and 4-methylumbelliferyl-β-glucoside as substrate. Lysosomal α-glucosidase activity was measured using purified enzyme from human urine and 4-methylumbelliferyl-α-glucoside as substrate. Lactase, maltase and sucrase activities were determined with homogenates of freshly isolated rat intestine by measuring liberated glucose from the corresponding disaccharides. Debranching enzyme activity (α-1,6-glucosidase activity) was measured by determining liberated glucose from dextrin with an erythrocyte preparation as enzyme source.

**General procedure for the synthesis of N-alkyl-DNJs, N-alkyl-L-ido-DNJs, N-pentyloxyalkyl-DNJs and N-pentyloxyalkyl-L-ido-DNJs.**

To a dry solution of DNJ (178) or L-ido-DNJ (192, 0.20 mmol) in DMF (1 ml) were added the requisite alkyl bromide or alkylxypentyl bromide (0.30 mmol) in DMF (1 ml) and K₂CO₃ (0.60 mmol). The reaction mixture was stirred for 16h at 80°C. Monitoring of the reaction progress was accomplished by TLC analysis or analytical LC/MS analysis. Upon complete consumption of the starting material, the solids were removed by filtration over a 50 μm filter and the filter-cake was rinsed with DMF. The volatiles were removed in vacuo and the residue was purified by silica gel column chromatography. Elution was performed with MeOH/EtOAc/NH₄OH (0:100:0 → 30:70:0.03) affording a colorless oil. The obtained compounds were further purified by RP-HPLC (via an automated HPLC system).
5.4 Experimental Procedures

2,3,4,6-tetra-O-benzyl-D-glucitol (194).
Commercially available 2,3,4,6-tetra-O-benzyl-D-glucopyranose (193, 135.0 g, 250 mmol) was co-evaporated trice with dioxane and dissolved in THF (1.5 l). A solution of LiAlH₄ (360 ml, 2.4 M in THF, 875 mmol) was added dropwise to the cooled reaction mixture (0°C) over a period of 2h, after which the reaction mixture was allowed to warm up to RT and stirring continued for 12h. The excess of LiAlH₄ was quenched by the careful addition of EtOAc (∼250 ml) at 0°C followed by sat. NH₄Cl (1.5 l). The reaction mixture was transferred to a separatory funnel with EtOAc (2.5 l) and a 2M HCl solution was used to dissolve the metallic salts before the layers were separated. The aqueous layer was back-extracted with EtOAc (1 l) and the combined organics were washed with brine (800 ml), dried (MgSO₄), filtered and concentrated. The crude material (160 g, quant.) was used in the next reaction without further purifications. 

1H NMR (400 MHz, CDCl₃): δ 7.59-7.22 (m, 2H, Har, Bn), 4.72-4.55 (m, 8H, 4x CH₂-Bn), 4.12 (ddd, 1H, J = 11.1, 7.8, 3.4, H-5), 3.98 (dd, 1H, J = 9.6, 6.7, H3), 3.90-3.82 (m, 2H, H2,4), 3.80 (dd, 1H, J = 9.6, 6.4, H1α), 3.75-3.68 (m, 2H, H6), 3.64 dd, 1H, J = 11.9, 4.7, H1β), 2.67 (s, 2H, OH). 13C NMR (100 MHz, CDCl₃): δ 138.2-137.9, 128.4-127.8, 79.6, 79.1, 77.5, 74.5-73.1, 71.2, 70.7, 61.8.

2,3,4,6-tetra-O-benzyl-1-deoxynojirimycin (195).
To a cooled (-78 °C) solution of oxalylchloride (17.5 ml, 205 mmol) in CH₂Cl₂ (200 ml) was added a 2M solution of DMSO (17.7 ml, 250 mmol) in CH₂Cl₂ over a period of 30 min. During the addition of DMSO, the internal reaction temperature was maintained at ± -70°C. Next, a dry solution of glucitol 194 (28.1 g, 50 mmol) in CH₂Cl₂ (2M) was added to the reaction mixture at such a rate that the internal temperature remains ≤ -70°C. The reaction mixture was stirred for 2h at -70°C. TLC monitoring of the reaction was possible by treating a few drops of the reaction mixture with Et₃N. Upon complete consumption of the starting material, Et₃N (84 ml, 600 mmol) was slowly added to the reaction mixture, after which the reaction was allowed to warm up to -5°C. The cold reaction mixture (∼-5°C) was added to a well stirred solution of ammoniumformiate (63.1 g, 1.0 mol) in MeOH (2 l) at 0°C. Subsequently, NaCNBH₃ (12.6 g, 200 mmol) and Na₂SO₄ (30 g, 210 mmol) were added to the reaction cocktail and stirring continued for 48 h at RT. After TLC analysis indicated complete disappearance of the starting material, the acidity of the reaction mixture was adjusted to pH 8/9 with a 1M NaOH solution and the volatiles were removed. The residue was suspended in H₂O and extracted with CH₂Cl₂ (3x 1.2 l) and the combined organics were dried (MgSO₄), filtered and concentrated. After FCC (PE/EtOAc 1:1 → 0:1), the title compound was obtained as an off white solid in 67% yield. Rf = 0.2 (1:1 PE / EtOAc). 1H NMR (400 MHz, CDCl₃): δ 1.90 (br. s, 1H, NH), 2.68-2.81 (m, 1H, H1α), 3.06-3.21 (m, 1H, H5), 3.53-2.68 (m, 1H, H1β), 3.73-4.04 (m, 4H, H2,3,4,6α), 4.10-4.29 (m, 1H, H6β), 4.29-5.04 (m, 8H, 4x CH₂Ph), 7.06-7.40 (m, 20H, Har). 13C NMR (100 MHz, CDCl₃): δ 45.0, 58.5, 65.1, 73.3, 73.6, 75.4, 75.5, 75.6, 75.8, 85.0, 127.6, 127.64, 127.9, 128.0, 128.4.
1-deoxynojirimycin (178).

A solution of 195 (5.2 g, 10 mmol) in aldehyde free EtOH (250 ml) was acidified with 37% aq. HCl to pH ∼1 and purged of oxygen by sonification under an argon atmosphere. Pd/C (10 wt%, ∼0.1 g) was added and the reaction mixture was exposed to 4 bar of H₂ for 12 h, after which TLC analysis indicated the formation of a highly polar compound. The reaction mixture was filtered over celite and the residue was rinsed with MeOH and the filtrate was concentrated. The residue was applied to neutral Al₂O₃ column chromatography (first 1:1 EtOAc/MeOH then 10% H₂O/MeOH) to furnish the title compound in 90% yield as an off white solid. 

\[ \text{RF} = 0.2 \text{ (10:12:3 MeOH/EtOAc/NH₄OH).} \]

\[ \text{1H NMR (400 MHz, D₂O): } \delta \text{ 2.42 (dd, } J = 10.8, 12.3, 1H, H₁α), \text{ 2.50 (ddd, } J = 3.0, 6.2, 9.5, 1H, H₅), \text{ 3.08 (dd, } J = 5.2, 12.3, 1H, H₁β), \text{ 3.16-3.22 (dd, } J = 9.1, 9.4, 1H, H₃), \text{ 3.28 (dd, } J = 9.1, 1H, H₃), \text{ 3.45 (ddd, 1H, } J = 9.1, 9.4, 10.8, 1H, H₃), \text{ 3.59 (dd, 1H, } J = 6.3, 11.7, H₆alpha), \text{ 3.79 (dd, } J = 3.0, 11.7, 1H, H₆β). \]

\[ \text{13C NMR (100 MHz, D₂O): } \delta \text{ 48.6, 60.4, 61.3, 70.8, 71.4, 78.3.} \]

1,5-bismesyl-2,3,4,6-tetra-O-benzyl-D-glucitol (196).

To a dry and cooled solution (0°C) of 194 (160 g, 250 mmol) in dry pyridine (2.5 l) was added methanesulfonyl chloride (48 ml, 625 mmol) dropwise over a period of 1 h. The reaction mixture was stirred for 12h at RT, after which TLC indicated the complete conversion of the starting material into a more apolar product. Excess methanesulfonyl chloride was quenched by the addition of H₂O (0.5 l) and the reaction mixture was concentrated to a volume of ~ 600 ml. The resulting slurry was transferred to a separatory funnel with EtOAc (2 l) and subsequently washed with 1M HCl (2x 1 l), sat. NaHCO₃ (1 l), brine (0.5 l), dried (MgSO₄), filtered and concentrated. The resulting yellow oil (200 g, quant.) was used as such in the next reaction. 

\[ \text{RF} = 0.4 \text{ (2:1 PE/EtOAc).} \]

\[ \text{1H NMR (400 MHz, CDCl₃): } \delta \text{ 2.83 (s, 3H, CH₃), 2.95 (s, 3H, CH₃), 3.71-4.25 (m, 3H, H₂,3,4), 4.44-4.45 (m, 10H, H₁,6, 3x CH₂Ph), 4.90-5.10 (m, 1H, H₅), 7.22-7.41 (m, 20H, Har).} \]

\[ \text{13C NMR (100 MHz, CDCl₃): } \delta \text{ 53.3, 54.0, 63.5, 64.8, 72.0, 72.8, 74.5, 74.7, 77.9, 78.0, 86.8, 117.8, 126.9, 127.0, 127.2, 127.8, 128.3, 133.1, 137.3, 138.2, 138.5.} \]
5.4 Experimental Procedures

2,3,4,6-tetra-O-benzyl-1-ido-1-deoxynojirimycin (198).
To a dry solution of 197 (145 g, 250 mmol) in DMSO (1.35 l) was added KOtBu (14.0 g, 125 mmol) and the resulting brown mixture was stirred for 1 h at 100°C.

A small sample (a few drops) was treated with 1M HCl, to reveal complete consumption of the starting material. The reaction mixture was charged with 1M HCl (1.25 l) at 0°C and stirred vigorously for 2h at RT. The reaction mixture was diluted with H2O (1.25 l), neutralized by the controlled addition of Na2CO3(s) and extracted with Et2O (3x 1.5 l). The combined organics were washed with brine (1 l), dried (MgSO4), filtered and concentrated.

The resulting brownish oil was subjected to FCC (PE/EtOAc 1: 1 → 0:1) to provide the title compound in 71% yield (92.3 g) as a brown oil. RF = 0.2 (2:1 PE/EtOAc).

1H NMR (400 MHz, CDCl3): δ 1.7 (s, 1H, NH), 2.87 (dd, 1H, J = 6.7, 12.9, H1α), 3.01 (dd, 1H, J = 6.9, 14.1, H1β), 3.38-3.73 (m, 6H, H2-6), 4.50-4.65 (m, 8H, 4x CH2Ph), 7.26-7.33 (m, 20H, Har).

1H NMR (400 MHz; MeOD): δ 0.95 (t, J = 7.4, 3H, H10), 1.38 (m, 2H, H9), 1.68 (m, 2H, H8), 2.88 (d, J = 12.0, 11.6, 1H, H1α), 2.93 (m, 1H, H5), 3.12 (ddd, J = 6.8, 1H, H7α), 3.29 (t, J = 9.2, 1H, H3), 3.36 (dd, J = 12.0, 4.8, 1H, H1β), 3.53 (dd, J = 9.6, 10.0, 1H, H4), 3.61 (m, 1H, H2), 3.84 (d, J = 11.2, 1H, H6α), 4.03 (d, J = 12.4, 1H, H6β). 13C NMR (100 MHz; MeOD): δ 44.8, 56.3, 58.5, 66.1, 67.0, 67.1.

LC/MS analysis: Rt 2.46 min (linear gradient 0-50% B), ES (ESI): m/z = 220.2 [M + H]+, 439.0 [2M + H]+. HR-MS [QTOF, MH]+ m/z calculated for C10H22NO4 220.15433, found 220.15449. [α]20D = -5.5° (c = 0.07, MeOH).

1-ido-1-deoxynojirimycin (192).
Prior to the reaction, 198 was dissolved in CH2Cl2 and washed with a 0.7% aqueous hypochloride solution, to oxidise all sulfur containing residues to prevent deactivation of the Pd catalyst. A solution of 198 (15.5 g, 30 mmol) in aldehyde free EtOH (250 ml) was acidified with 37% aq. HCl to pH ∼ 1 and purged of oxygen by sonification under an argon atmosphere. Pd/C (10 wt %, ∼ 0.1 g) was added and the reaction mixture was exposed to 4 bar of H2 for 12 h, after which TLC analysis indicated the formation of a highly polar compound. The reaction mixture was filtered over celite and the residue was rinsed with MeOH and the filtrate was concentrated. The residue was applied to neutral AlO2 column chromatography (first 1:1 EtOAc/MeOH then 10% H2O/MeOH) to furnish the title compound in 89% yield as a brownish semi-solid, with an unpleasant odour. RF = 0.2 (10:12:3 MeOH / EtOAc / NH4OH).

1H NMR (400 MHz, D2O): δ 3.17 (dd, 1H, J = 3.2, 13.5, H1α), 3.26 (dd, 1H, J = 2.5, 13.5, H1β), 3.42 (ddd, 1H, J = 1.8, 5.4, 7.5, H5), 3.65-3.78 (m, 2H, H6), 3.80-3.85 (m, 2H, H3,4), 3.85-3.90 (m, 1H, H2).

13C NMR (100 MHz, D2O): δ 44.8, 56.3, 58.5, 66.1, 67.0, 67.1.
5. Evaluation of N-alkylated iminosugars for the treatment of Gaucher’s disease

\[ \text{N-pentyl-1-deoxynojirimycin (199).} \]
\[
^1H \text{ NMR (400 MHz; MeOD): } \delta 0.96 (t, J = 6.8, 3H, H11), 1.39 (m, 4H, H9,10), 1.75 (m, 2H, H8), 2.97 (dd, J = 12.0, 11.6, 1H, H1\alpha), 3.03 (br. d, J = 9.2, 1H, H5), 3.17 (d, J = 9.2, 1H, H7\alpha), 3.37 (m, 2H, H3,7β), 3.46 (dd, J = 12.0, 4.4, 1H, H1β), 3.60 (t, J = 9.6, 1H, H4), 3.69 (m, 1H, H2), 3.90 (d, J = 11.2, 1H, H6α), 4.11 (d, J = 12.4, 1H, H6β). \]
\[
^13C \text{ NMR (100 MHz; MeOD): } \delta 14.2, 23.1, 23.8, 29.7, 54.4, 54.8, 54.9, 67.5, 67.8, 68.8, 78.1. \text{LC/MS analysis: Rt 4.03 min (linear gradient 0-50% B), ES (ESI): m/z = 234.2 [M + H]+, 467.4 [2M + H]+. HR-MS [QTOF, MH\textsuperscript+] m/z calculated for C\textsubscript{11}H\textsubscript{24}NO\textsubscript{4} 234.16998, found 234.17009. [\alpha\textsubscript{D}\textsuperscript{20}] = -2.0\degree (c = 1.08, MeOH). \]

\[ \text{N-hexyl-1-deoxynojirimycin (200).} \]
\[
^1H \text{ NMR (400 MHz; MeOD): } \delta 0.94 (t, J = 7.2, 3H, H-12), 1.38 (m, 6H, H9,10,11), 1.75 (m, 2H, H8), 2.97 (dd, J = 12.0, 11.2, 1H, H1\alpha), 3.03 (br. d, J = 10.4, 1H, H5), 3.18 (m, 1H, H7\alpha), 3.36 (m, 2H, H3,7β), 3.44 (dd, J = 12.0, 4.4, 1H, H1β), 3.59 (t, J = 9.6, 1H, H4), 3.69 (dd, J = 11.2, 5.2, 10.8, 1H, H2), 3.89 (d, J = 11.4, 1H, H6β). \]
\[
^13C \text{ NMR (100 MHz; MeOD): } \delta 14.2, 23.4, 27.3, 32.3, 24.1, 54.4, 54.8, 54.8, 67.4, 67.8, 68.8, 78.1. \text{LC/MS analysis: Rt 5.18 min (linear gradient 0-50% B), ES (ESI): m/z = 248.1 [M + H]+, 495.8 [2M + H]+. HR-MS [QTOF, MH\textsuperscript+] m/z calculated for C\textsubscript{12}H\textsubscript{26}NO\textsubscript{4} 248.18563, found 248.18574. [\alpha\textsubscript{D}\textsuperscript{20}] = -1.2\degree (c = 0.17, MeOH). \]

\[ \text{N-heptyl-1-deoxynojirimycin (201).} \]
\[
^1H \text{ NMR (400 MHz; MeOD): } \delta 0.92 (t, J = 6.8, 3H, H13), 1.37 (m, 8H, H9-12), 1.75 (m, 2H, H7), 2.96 (dd, J = 12.0, 11.6, 1H, H1\alpha), 3.01 (br. d, 1H, H5), 3.17 (m, 1H, H7\alpha), 3.36 (m, 2H, H3,7β), 3.44 (dd, J = 12.0, 4.4, 1H, H1β), 3.59 (t, J = 9.6, 1H, H4), 3.67 (m, 1H, H2), 3.90 (d, J = 11.6, 1H, H6α), 4.11 (d, J = 12.4, 1H, H6β). \]
\[
^13C \text{ NMR (100 MHz; MeOD): } \delta 14.3, 23.5, 27.6, 29.8, 32.7, 24.2, 54.4, 54.8, 54.9, 67.4, 67.8, 68.8, 78.1. \text{LC/MS analysis: Rt 6.04 min (linear gradient 0-50% B), ES (ESI): m/z = 262.1 [M + H]+, 523.2 [2M + H]+. HR-MS [QTOF, MH\textsuperscript+] m/z calculated for C\textsubscript{13}H\textsubscript{28}NO\textsubscript{4} 262.20128, found 262.20143. [\alpha\textsubscript{D}\textsuperscript{20}] = -3.5\degree (c = 0.17, MeOH). \]

\[ \text{N-octyl-1-deoxynojirimycin (202).} \]
\[
^1H \text{ NMR (400 MHz; MeOD): } \delta 0.91 (t, J = 7.2, 3H, H14), 1.35 (m, 10H, H9-13), 1.75 (m, 2H, H8), 2.97 (dd, J = 12.0, 11.2, 1H, H1\alpha), 3.03 (br. d, J = 9.6, 1H, H5), 3.17 (m, 1H, H7\alpha), 3.36 (m, 2H, H3,7β), 3.45 (dd, J = 12.0, 4.4, 1H, H1β), 3.59 (t, J = 9.6, 1H, H4), 3.67 (m, 1H, H2), 3.89 (d, J = 12.4, 1H, H6α), 4.11 (d, J = 12.4, 1H, H6β). \]
\[
^13C \text{ NMR (100 MHz; MeOD): } \delta 14.3, 23.6, 27.6, 30.1, 30.2, 32.8, 24.1, 54.4, 54.8, 54.9, 67.5, 67.8, 68.8, 78.1. \text{LC/MS analysis: Rt 4.27 min (linear gradient 10-90% B), ES (ESI): m/z = 275.9 [M + H]+, 551.9 [2M + H]+. HR-MS [QTOF, MH\textsuperscript+] m/z calculated for C\textsubscript{14}H\textsubscript{30}NO\textsubscript{4} 276.21693, found 276.21702. [\alpha\textsubscript{D}\textsuperscript{20}] = -1.6\degree (c =...
5.4 Experimental Procedures

0.36, MeOH).

**N-nonyl-1-deoxynojirimycin (203).**

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.84 (t, $J = 6.4$, H-15), 1.28 (m, 12H, H-9-14), 1.67 (m, 2H, H8), 2.90 (dd, $J = 12.0$, 11.6, 1H, H1$\alpha$), 3.03 (br. d, $J = 9.6$, 1H, H5), 3.10 (m, 1H, H7$\alpha$), 3.30 (m, 2H, H3,7$\beta$), 3.38 (dd, $J = 12.0$, 4.8, 1H, H1$\beta$), 3.53 (dd, $J = 10.0$, 9.6, 1H, H4), 3.61 (m, 1H, H-2), 3.83 (d, $J = 11.6$, 1H, H6$\alpha$), 4.04 (d, $J = 12.4$, 1H, H6$\beta$). $^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.4, 23.6, 27.6, 30.1, 30.2, 30.4, 32.9, 24.1, 54.4, 54.8, 54.9, 67.4, 67.8, 68.8, 78.1. LC/MS analysis: Rt 7.68 min (linear gradient 0-50% B), ES (ESI): m/z = 290.1 [M + H]$^+$, 579.8 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{15}$H$_{32}$NO$_4$ 290.23258, found 290.23268. $[\alpha]_D^{20}$ = -8.5° (c = 0.23 MeOH).

**N-butyl-L-ido-1-deoxynojirimycin (204).**

$^1$H NMR (500 MHz; D$_2$O): $\delta$ 0.96 (t, $J = 7.5$, 3H, H10), 1.42 (m, 2H, H9), 1.74 (m, 2H, H8), 3.58 (br. t, $J = 7.5$, 2H, H7), 3.43 (m, 1H, H1$\alpha$), 3.52 (dd, $J = 13.5$, 3.5, 1H, H1$\beta$), 3.67 (br. s, 1H, H5), 3.93 (br. s, 1H, H3), 4.06 (br. s, 3H, H2,6), 4.16 (br. s, 1H, H4). $^{13}$C-NMR (125 MHz; D$_2$O): $\delta$ 12.8, 19.2, 24.5, 52.1, 53.7, 58.1, 61.5, 66.3, 68.0, 69.1. LC/MS analysis: Rt 2.93 min (linear gradient 0-50% B), ES (ESI): m/z = 220.2 [M + H]$^+$, 439.4 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{10}$H$_{22}$NO$_4$ 220.15433, found 220.15440. $[\alpha]_D^{20}$ = +9.6° (c = 0.17, MeOH).

**N-pentyl-L-ido-1-deoxynojirimycin (205).**

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.95 (t, $J = 6.8$, 3H, H11), 1.38 (m, 6H, H9-11), 1.78 (br. d, 2H, H8), 3.31 (m, 2H, H7), 3.35 (br. d, $J = 16.0$, 1H, H1$\alpha$), 3.49 (br. d, 2H, H5,1$\beta$), 3.86 (br. s, 1H, H3), 3.97 (br. s, 3H, H2,6), 4.02 (br. s, 1H, H4). $^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.2, 23.4, 27.3, 32.3, 54.4, 55.1, 61.3, 63.8, 68.0, 68.9, 72.3. LC/MS analysis: Rt 4.29 min (linear gradient 0-50% B), ES (ESI): m/z = 234.1 [M + H]$^+$, 439.4 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{11}$H$_{24}$NO$_4$ 234.16998, found 234.17010. $[\alpha]_D^{20}$ = +12.8° (c = 0.47, MeOH).

**N-hexyl-L-ido-1-deoxynojirimycin (206).**

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.91 (t, $J = 6.8$, 3H, H12), 1.38 (m, 6H, H9-11), 1.77 (br. d, 2H, H8), 3.30 (m, 2H, H7), 3.34 (br. d, $J = 14.0$, 1H, H1$\alpha$), 3.49 (br. d, 2H, H5,1$\beta$), 3.86 (br. s, 1H, H3), 3.97 (br. s, 3H, H6,2), 4.02 (br. s, 1H, H4). $^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.2, 23.4, 27.3, 32.3, 54.4, 55.2, 61.3, 63.8, 68.1, 68.9, 72.3. LC/MS analysis: Rt 5.28 min (linear gradient 0-50% B), ES (ESI): m/z = 248.1 [M + H]$^+$, 495.5 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{12}$H$_{26}$NO$_4$ 248.18563, found 248.18571. $[\alpha]_D^{20}$ = +4.8° (c = 0.46, MeOH).
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\(N\)-heptyl-\(L\)-ido-1-deoxynojirimycin (207).

\(^1\)H NMR (400 MHz; CDCl\(_3/\)MeOD): \(\delta\) 0.88 (t, \(J = 6.8\), 3H, H13), 1.32 (m, 8H, H9-12), 1.69 (br. s, 2H, H8), 3.12 (m, 3H, H7,1\(\alpha\)), 3.26 (br. d, \(J = 12.4\), 1H, H1\(\beta\)), 3.39 (br. d, 1H, H5), 3.75 (br. t, 1H, H3), 3.85 (br. s, 1H, H2), 4.94 (d, \(J = 4.8\), 3H, H6,4). \(^{13}\)C NMR (100 MHz; CDCl\(_3/\)MeOD): \(\delta\) 14.2, 23.2, 24.9, 27.4, 29.6, 53.2, 54.9, 59.3, 63.1, 69.0, 70.4, 71.7. LC/MS analysis: Rt 6.21 min (linear gradient 0-50% B), ES (ESI): \(m/z = 262.1\) [M + H]\(^+\), 523.3 [2M + H]\(^+\) HR-MS [QTOF, MH\(^+\)] \(m/z\) calculated for \(C_{13}H_{28}NO_4\) 262.20128, found 262.20140. \(\left[\alpha\right]_{D}^{20} = +12.0^\circ\) (c = 0.48, MeOH).

\(N\)-octyl-\(L\)-ido-1-deoxynojirimycin (208).

\(^1\)H NMR (400 MHz; MeOD): \(\delta\) 0.91 (t, \(J = 6.8\), 3H, H14), 1.35 (m, 10H, H9-13), 1.77 (br. d, 2H, H8), 3.30-3.37 (m, 3H, H1,7\(\alpha\)), 3.47-3.51 (br. m, 2H, H5,7\(\beta\)), 3.86 (br. s, 1H, H3), 3.97 (br. s, 3H, H-2, H6), 4.02 (br. s, 1H, H4). \(^{13}\)C NMR (100 MHz; MeOD): \(\delta\) 14.3, 23.6, 27.6, 30.1, 32.8, 54.4, 55.2, 61.3, 63.8, 68.1, 68.9, 72.3. LC/MS analysis: Rt 4.39 min (linear gradient 10-90% B), ES (ESI): \(m/z = 275.8\) [M + H]\(^+\), 551.8 [2M + H]\(^+\) HR-MS [QTOF, MH\(^+\)] \(m/z\) calculated for \(C_{14}H_{30}NO_4\) 276.21693, found 276.21700. \(\left[\alpha\right]_{D}^{20} = +11.7^\circ\) (c = 0.48, MeOH).

\(N\)-nonyl-\(L\)-ido-1-deoxynojirimycin (209).

\(^1\)H NMR (400 MHz; MeOD): \(\delta\) 0.91 (t, \(J = 6.4\), 3H, H15), 1.22-1.39 (m, 12H, H9-14), 1.71-1.81 (br. d, 2H, H8), 3.30-3.35 (m, 3H, H1,7\(\alpha\)), 3.44-3.51 (br. m, 2H, H5,7\(\beta\)), 3.83 (br. s, 1H, H3), 3.97 (br. s, 1H, H-2), 3.94-3.99 (m, 3H, H4,6). \(^{13}\)C NMR (100 MHz; MeOD): \(\delta\) 14.4, 23.7, 27.7, 30.1, 30.2, 30.3, 30.5, 32.9, 54.1, 55.1, 60.2, 63.8, 68.1, 69.0, 72.3. LC/MS analysis: Rt 7.89 min (linear gradient 0-50% B), ES (ESI): \(m/z = 290.0\) [M + H]\(^+\), 579.5 [2M + H]\(^+\) HR-MS [QTOF, MH\(^+\)] \(m/z\) calculated for \(C_{15}H_{32}NO_4\) 290.23258, found 290.23263. \(\left[\alpha\right]_{D}^{20} = +9.3^\circ\) (c = 0.13, MeOH).

\(N\)-butoxypentyl-1-deoxynojirimycin (216).

\(^1\)H NMR (400 MHz; MeOD): \(\delta\) 0.93 (t, \(J = 6.6\), 3H, H15), 1.38 (m, 2H, H14), 1.47 (m, 2H, H9), 1.55 (m, 2H, H13), 1.65 (m, 2H, H-10), 1.78 (br. s, 2H, H8), 2.90 (t, \(J = 11.6\), 1H, H1\(\alpha\)), 2.96 (br. d, \(J = 9.6\), 1H, H5), 3.13 (m, 1H, H7\(\alpha\)), 3.29 (m, 2H, H3,7\(\beta\)), 3.38 (m, 5H, H1\(\beta\), H11,12), 3.53 (dd, \(J = 9.6\), 1H, H4), 3.61 (m, 1H, H2), 3.84 (d, \(J = 11.6\), 1H, H6\(\alpha\)), 4.04 (d, \(J = 12.0\), 1H, H6\(\beta\)). \(^{13}\)C NMR (100 MHz; MeOD): \(\delta\) 14.2, 20.4, 23.9, 24.5, 30.1, 32.9, 54.2, 54.8, 54.9, 67.4, 67.8, 68.8, 71.3, 71.7, 78.1. LC/MS analysis: Rt 5.93 min (linear gradient 0-50% B), ES (ESI): \(m/z = 306.1\) [M + H]\(^+\), 611.4 [2M + H]\(^+\) HR-MS [QTOF, MH\(^+\)] \(m/z\) calculated for \(C_{15}H_{32}NO_5\) 306.22750, found 306.22762. \(\left[\alpha\right]_{D}^{20} = -2.7^\circ\) (c = 0.44, MeOH).
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**N-pentoxypentyl-1-deoxynojirimycin (217).**

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.91 (t, $J$ = 6.3, 3H, H16), 1.33 (m, 4H, H14,15), 1.47 (m, 2H, H9), 1.56 (m, 2H, H13), 1.64 (m, 2H, H10), 1.78 (br. s, 2H, H8), 2.98 (dd, $J$ = 12.0, 11.6, 1H, H1α), 3.03 (br. d, $J$ = 10.0, 1H, H5), 3.20 (m, 1H, H7α), 3.36 (m, 2H, H3,7β), 3.44 (m, 5H, H1β, H11,12), 3.59 (dd, $J$ = 10.0, 9.6, 1H, H4), 3.67 (m, 1H, H2), 3.90 (d, $J$ = 12.4, 1H, H6α), 4.11 (d, $J$ = 12.8, 1H, H6β). $^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.3, 23.5, 23.9, 24.5, 29.5, 30.1, 30.4, 54.3, 54.8, 54.9, 67.4, 67.8, 68.8, 71.3, 72.0, 78.1. LC/MS analysis: Rt 4.27 min (linear gradient 10-90% B), ES (ESI): m/z = 320.5 [M + H]$^+$, 639.6 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{16}$H$_{34}$NO$_5$ 320.24315, found 320.24323. [$\alpha$]$^D_{20}$ = -3.7° (c = 0.27, MeOH).

**N-hexoxypentyl-1-deoxynojirimycin (218).**

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.91 (t, $J$ = 6.3, 3H, H17), 1.31 (m, 6H, H14-16), 1.47 (m, 2H, H9), 1.56 (m, 2H, H13), 1.64 (m, 2H, H10), 1.78 (br. s, 2H, H8), 2.98 (dd, $J$ = 12.0, 11.6, 1H, H1α), 3.03 (br. d, $J$ = 10.0, 1H, H5), 3.19 (m, 1H, H7α), 3.36 (m, 2H, H3,7β), 3.44 (m, 5H, H1β, H11,12), 3.60 (dd, $J$ = 10.0, 9.6, 1H, H4), 3.68 (m, 1H, H2), 3.90 (d, $J$ = 12.0, 1H, H6α), 4.11 (d, $J$ = 12.4, 1H, H6β). $^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.3, 23.6, 23.9, 24.4, 26.9, 30.1, 30.7, 32.8, 54.3, 54.8, 54.9, 67.5, 67.8, 68.8, 71.3, 72.0, 78.1. LC/MS analysis: Rt 4.77 min (linear gradient 10-90% B), ES (ESI): m/z = 334.0 [M + H]$^+$, 667.5 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{17}$H$_{36}$NO$_5$ 334.25880, found 334.25888. [$\alpha$]$^D_{20}$ = -1.0° (c = 0.78, MeOH).

**N-heptoxyoctyl-1-deoxynojirimycin (219).**

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.91 (t, $J$ = 6.4, 3H, H18), 1.32 (m, 8H, H14-17), 1.47 (m, 2H, H9), 1.56 (m, 2H, H13), 1.64 (m, 2H, H10), 1.76 (br. s, 2H, H8), 2.86 (dd, $J$ = 12.0, 11.6, 1H, H1α), 2.92 (br. d, $J$ = 10.0, 1H, H5), 3.10 (m, 1H, H7α), 3.28 (m, 2H, H3,7β), 3.38 (m, 5H, H1β, H11,12), 3.52 (t, $J$ = 9.6, 1H, H4), 3.61 (m, 1H, H2), 3.84 (d, $J$ = 10.8, 1H, H6α), 4.02 (d, $J$ = 12.0, 1H, H6β). $^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.3, 23.6, 24.2, 24.5, 27.3, 30.1, 30.2, 30.7, 33.0, 54.1, 54.9, 55.1, 67.4, 67.9, 68.8, 71.4, 72.0, 78.3. LC/MS analysis: Rt 8.42 min (linear gradient 0-50% B), ES (ESI): m/z = 348.0 [M + H]$^+$, 695.4 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{18}$H$_{38}$NO$_5$ 348.27445, found 348.27452. [$\alpha$]$^D_{20}$ = -4.4° (c = 0.09, MeOH).

**N-octoxypentyl-1-deoxynojirimycin (220).**

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.90 (t, 3H, $J$ = 6.2, H19), 1.29 (m, 10H, H14-18), 1.46 (m, 2H, H9), 1.55 (m, 2H, H13), 1.64 (m, 2H, H10), 1.78 (br. s, 2H, H8), 2.98 (dd, $J$ = 12.0, 11.2, 1H, H1α), 3.04 (br. d, $J$ = 10.0, 1H, H5), 3.19 (m, 1H, H7α), 3.37 (m, 2H, H3,7β), 3.43 (m, 5H, H1β, H11,12), 3.60 (t, $J$ = 9.6, 1H, H4), 3.70 (m, 1H, H2), 3.91 (d, $J$ = 12.0, 1H, H6α), 4.11 (d, $J$ = 12.0, 1H, H6β). $^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.3, 23.6, 23.9, 24.4, 27.2, 30.0, 30.3, 30.5, 30.7, 32.9,
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54.3, 54.8, 54.9, 67.4, 67.7, 68.8, 71.3, 72.0, 78.0. LC/MS analysis: Rt 5.67 min (linear gradient 10-90% B), ES (ESI): m/z = 362.1 [M + H]$^+$, 723.9 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{19}$H$_{40}$NO$_5$ 362.29010, found 362.29016. $[\alpha]_D^{20} = -2.4^\circ$ (c = 1.65, MeOH).

$N$-nonoxypentyl-1-deoxynojirimycin (221).

$^1$H NMR (400 MHz; MeOD): δ 0.90 (t, $J$ = 6.2, 3H, H20), 1.30 (m, 12H, H14-19), 1.46 (m, 2H, H9), 1.56 (m, 2H, H13), 1.64 (m, 2H, H10), 1.76 (br. s, 2H, H8), 2.85 (dd, $J$ = 9.6, 9.6, 1H, H1α), 2.91 (br. d, $J$ = 10.0, 1H, H5), 3.09 (m, 1H, H7α), 3.28 (m, 2H, H3,7β), 3.37 (m, 5H, H1β, H11,12), 3.51 (dd, $J$ = 10.0, 9.6, 1H, H4), 3.70 (ddd, $J$ = 9.6, 4.4, 10.4, 1H, H2), 3.83 (dd, $J$ = 12.0, 2.8, 1H, H6α), 4.02 (d, $J$ = 12.0, 1H, H6β). $^{13}$C NMR (100 MHz; MeOD): δ 14.3, 23.7, 24.2, 24.5, 27.3, 30.1, 30.4, 30.6, 30.7, 30.8, 33.0, 54.1, 54.9, 55.2, 67.4, 67.8, 68.9, 71.4, 72.0, 78.3. LC/MS analysis: Rt 9.84 min (linear gradient 0-50% B), ES (ESI): m/z = 376.1 [M + H]$^+$, 751.6 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{20}$H$_{42}$NO$_5$ 376.30575, found 376.30581. $[\alpha]_D^{20} = -9.5^\circ$ (c = 0.08, MeOH).

$N$-butoxypentyl-1-ido-1-deoxynojirimycin (222).

$^1$H NMR (400 MHz; MeOD): δ 0.93 (t, $J$ = 7.2, 3H, H15), 1.38 (m, 2H, H14), 1.48 (m, 2H, H9), 1.55 (m, 2H, H13), 1.80 (br. s, 2H, H8), 3.30-3.35 (m, 3H, H1,7α), 3.37-3.51 (m, 6H, H7β, H11,12), 3.87 (br. s, 1H, H3), 3.97 (br. s, 3H, H2,6), 4.02 (br. s, 1H, H4). $^{13}$C NMR (100 MHz; MeOD): δ 14.2, 23.7, 24.2, 24.5, 27.3, 30.1, 32.9, 54.4, 55.0, 61.3, 63.8, 68.0, 68.9, 71.4, 71.7, 72.3. LC/MS analysis: Rt 0.50 min (linear gradient 10-90% B), ES (ESI): m/z = 306.2 [M + H]$^+$, 611.4 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{15}$H$_{32}$NO$_5$ 306.22750, found 306.22762. $[\alpha]_D^{20} = +24.1^\circ$ (c = 0.17, MeOH).

$N$-pentoxypentyl-1-ido-1-deoxynojirimycin (223).

$^1$H NMR (400 MHz; MeOD): δ 0.92 (t, $J$ = 7.0, 3H, H16), 1.33 (m, 4H, H14,15), 1.48 (m, 2H, H9), 1.57 (m, 2H, H13), 1.81 (br. d, 2H, H8), 3.30-3.36 (m, 3H, H1,7α), 3.41-3.48 (m, 5H, H7β H11,12), 3.51 (br. s, 1H, H5), 3.87 (br. s, 1H, H3), 3.97 (br. s, 3H, H2,6). $^{13}$C NMR (100 MHz; MeOD): δ 14.3, 23.3, 24.5, 29.5, 30.1 30.4, 54.4, 55.1, 61.3, 63.8, 68.1, 68.9, 71.4, 72.0, 72.4. LC/MS analysis: Rt 4.11 min (linear gradient 10-90% B), ES (ESI): m/z = 320.4 [M + H]$^+$, 639.7 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{16}$H$_{34}$NO$_5$ 320.24315, found 320.24323. $[\alpha]_D^{20} = +8.6^\circ$ (c = 0.30, MeOH).
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*N*-hexoxypentyl-1-*ido*-1-deoxynojirimycin (224).

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.91 (t, $J = 7.0$ Hz, 3H, H17), 1.32 (m, 6H, H14-16), 1.46 (m, 2H, H9), 1.57 (m, 2H, H13), 1.64 (m, 2H, H10), 1.82 (br. d, 2H, H8), 3.30-3.36 (m, 3H, H2, H7$\alpha$), 3.41-3.47 (m, 5H, H7$\beta$, H11,12), 3.51 (br. s, 1H, H5), 3.87 (br. s, 1H, H3), 3.97 (br. s, 3H, H2,6 ), 4.02 (br. s, 1H, H4).

$^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.3, 23.3, 23.9, 24.5, 26.9, 30.1 30.7, 32.8, 54.1, 55.1, 61.3, 63.8, 68.1, 68.9, 71.4, 72.0, 72.4. LC/MS analysis: Rt 4.88 min (linear gradient 10-90% B), ES (ESI): m/z = 334.6 [M + H$^+$], 667.5 [2M + H$^+$] HR-MS [QTOF, MH$^+$] m/z calculated for C$_{17}$H$_{36}$NO$_5$ 334.25880, found 334.25890. $[\alpha]_{D}^{20}$ = +10.0° (c = 0.30, MeOH).

*N*-heptoxypentyl-1-*ido*-1-deoxynojirimycin (225).

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.91 (t, $J = 6.8$ Hz, H18), 1.32 (m, 8H, H14-17), 1.48 (m, 2H, H9), 1.58 (m, 2H, H13), 1.63 (m, 2H, H10), 1.78 (br. d, 2H, H8), 3.28-3.35 (m, 3H, H1,7$\alpha$), 3.41-3.47 (m, 5H, H7$\beta$, H11,12), 3.81 (br. s, 1H, H5), 3.89 (br. s, 1H, H3), 3.93-4.01 (m, 4H, H2,4,6).

$^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.4, 23.6, 24.5, 27.2, 30.2 30.3, 30.8, 33.0, 54.2, 54.9, 61.1, 63.8, 68.5, 69.0, 71.4, 72.0, 72.2. LC/MS analysis: Rt 8.57 min (linear gradient 0-50% B), ES (ESI): m/z = 348.0 [M + H$^+$], 695.4 [2M + H$^+$] HR-MS [QTOF, MH$^+$] m/z calculated for C$_{18}$H$_{38}$NO$_5$ 348.27445, found 348.27450. $[\alpha]_{D}^{20}$ = +6.5° (c = 0.12, MeOH).

*N*-octoxypentyl-1-*ido*-1-deoxynojirimycin (226).

$^1$H NMR (400 MHz; MeOD): $\delta$ 0.90 (t, $J = 7.2$ Hz, H19), 1.32 (m, 10H, H14-18), 1.48 (m, 2H, H9), 1.58 (m, 2H, H13), 1.63 (m, 2H, H10), 1.78 (br. d, 2H, H8), 3.30-3.37 (m, 3H, H1,7$\alpha$), 3.41-3.47 (m, 5H, H7$\beta$, H11,12), 3.52 (br. s, 1H, H5), 3.85 (br. s, 1H, H3), 3.91-4.02 (m, 4H, H2,4,6).

$^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.4, 23.6, 24.5, 27.2, 30.1 30.3, 30.5, 30.7, 32.9, 54.3, 54.5, 61.2, 63.8, 68.0, 68.9, 71.4, 72.0, 72.2. LC/MS analysis: Rt 5.86 min (linear gradient 10-90% B), ES (ESI): m/z = 362.5 [M + H$^+$], 723.6 [2M + H$^+$] HR-MS [QTOF, MH$^+$] m/z calculated for C$_{19}$H$_{40}$NO$_5$ 362.29019, found 362.29019. $[\alpha]_{D}^{20}$ = +11.4° (c = 0.51, MeOH).

*N*-nonoxypentyl-1-*ido*-1-deoxynojirimycin (227).

$^1$H NMR (400 MHz; MeOD): $\sigma$: 0.91 (t, $J = 6.4$ Hz, H20), 1.32 (m, 12H, H14-19), 1.48 (m, 2H, H9), 1.58 (m, 2H, H13), 1.63 (m, 2H, H10), 1.78 (br. d, 2H, H7), 3.30-3.36 (m, 3H, H1-1$\alpha$), 3.41-3.47 (m, 5H, H7$\beta$, H11,12), 3.52 (br. s, 1H, H5), 3.84 (br. s, 1H, H3), 3.91-4.02 (m, 4H, H2,4,6).

$^{13}$C NMR (100 MHz; MeOD): $\delta$ 14.4, 23.7, 24.5, 27.2, 30.1 30.4, 30.6, 30.7, 30.8, 33.0, 54.3, 55.0, 61.3, 63.8, 68.2, 69.0, 71.4, 72.0, 72.2. LC/MS analysis: Rt 9.88 min (linear gradient 0-50% B), ES (ESI): m/z = 376.1 [M + H$^+$], 751.9 [2M + H$^+$] HR-MS [QTOF, MH$^+$] m/z calculated for C$_{20}$H$_{42}$NO$_5$ 376.30575, found 376.30585. $[\alpha]_{D}^{20}$ = +5.1° (c = 0.27, MeOH).
5. Evaluation of N-alkylated iminosugars for the treatment of Gaucher’s disease

**N-isobutoxypentyl-1-deoxynojirimycin (228).**

\[\text{H NMR (400 MHz; CDCl}_3\text{): } \delta \text{ 0.93 (d, } J = 6.7, 6H, H14,15), 1.31-1.44 (m, 2H, H9), 1.50-1.67 (m, 4H, H9,10), 1.85-1.90 (m, 1H, H13), 2.14 (dt, } J = 2.7, 9.5, 1H, H5), 2.19 (dd, } J = 7.7, 14.0, 1H, H1\alpha), 2.55-2.66 (m, 1H, H7\alpha), 2.772.87 (m, 1H, H7\beta), 3.05 (dd, } J = 4.9, 11.2, 1H, H1\beta), 3.15 (t, } J = 9.06 Hz, 1H, H3), 3.21 (d, } J = 6.66, 2H, H12), 3.38 (dd, } J = 6.80, 11.8, 1H, H4), 3.45 (t, } J = 6.4, 2H, H11), 3.48-3.53 (m, 1H, H2), 3.83-3.93 (m, 2H, H6). 13C NMR (100 MHz; CDCl3): \(\delta\) 18.3, 23.7, 23.9, 28.2, 29.2, 52.4, 56.3, 58.1, 66.0, 69.4, 70.5, 70.7, 77.5, 79.2. LC/MS analysis: Rt 4.75 min (linear gradient 0-90% B), ES (ESI): m/z = 306.4 [M + H]+, 611.8 [2M + H]+ \([\alpha]_{D}^{20} = -4.5^\circ (c = 0.44, \text{ CHCl}_3)\).
N-(5-farnesoxy)pentyl-1-deoxynojirimycin (232).

\(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 1.59 (s, 6H, H20,21), 1.66 (s, 3H, H10), 1.67 (s, 3H, H15), 1.93-2.13 (m, 8H, H11,12,16,17), 2.21-2.29 (m, 2H, H5, H11\(\alpha\)), 3.07 (dd, \(J=\) 4.4, 11.2, 1H, H11\(\beta\)), 3.18-3.30 (m, 2H, H3, H7\(\alpha\)), 3.42-3.58 (m, 3H, H2,3,7\(\beta\)), 3.88-3.90 (m, 2H, H6). 5.05-5.12 (m, 2H, H13,18), 5.22-5.28 (m, 1H, H8). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 15.4, 15.9, 17.0, 25.1, 26.1, 26.3, 39.3, 39.5, 55.4, 57.12, 65.1, 68.5, 69.7, 78.4, 116.7, 123.1, 123.8, 130.8, 135.0, 141.1. LC/MS analysis: Rt 6.61 min (linear gradient 0-90% B), ES (ESI): m/z = 368.1 [M + H]\(^+\), 735.7 [2M + H]\(^+\). HR-MS [QTOF, MH\(^+\)] m/z calculated for C\(_{21}\)H\(_{38}\)NO\(_4\) 368.27954, found 368.27952. \([\alpha]^{20}_D\) = -12.6\(^\circ\) (c = 1.1, CHCl\(_3\)).

N-(5-cholesteroxy)pentyl-1-deoxynojirimycin (233).

\(^1\)H NMR (600 MHz; CDCl\(_3\)): \(\delta\) 0.88 (s, 3H, CH\(_3\)-cholesterol), 1.04-1.07 (m, 6H, 2x CH\(_3\)-cholesterol), 1.20 (s, 3H, CH\(_3\)-cholesterol), 1.23-2.57 (m, 35H, 11x CH-cholesterol, 7x CH-cholesterol, H8-10), 2.81-2.98 (m, 2H, H-1\(\alpha\), H5), 3.11-3.22 (m, 1H, H7\(\alpha\)), 3.26-3.40 (m, 2H, H7\(\beta\), H1\(\beta\)), 3.43-3.56 (m, 1H, H3), 3.67-3.76 (m, 3H, H11,4), 3.80-3.90 (m, 1H, H2), 4.04-4.08 (m, 1H, H6\(\alpha\)), 4.19-4.23 (m, 1H, H6\(\beta\)), 5.52-5.56 (m, 1H, olefin-cholesterol). \(^{13}\)C NMR (150 MHz; CDCl\(_3\)): \(\delta\) 11.5, 17.4,18.4, 19.0, 21.0, 22.1, 22.4, 23.2, 23.6, 23.7, 24.1, 27.9, 28.1, 28.3, 29.5, 31.8, 31.8, 35.8, 36.1, 36.7, 37.1, 39.0, 39.4, 39.7, 42.2, 50.2, 52.5, 54.6, 56.1, 56.7, 58.4, 65.8, 67.6, 79.2, 121.7, 140.5. LC/MS analysis: Rt 9.9 min (linear gradient 10-90% B), ES (ESI): m/z = 618.5 [M + H]\(^+\), 1236.2 [2M + H]\(^+\). HR-MS [QTOF, MH\(^+\)] m/z calculated for C\(_{38}\)H\(_{68}\)NO\(_5\) 618.50920, found 618.50906. \([\alpha]^{20}_D\) = -16.9\(^\circ\) (c = 1.54, CHCl\(_3\)).

N-isobutoxypentyl-1-ido-1-deoxynojirimycin (234).

\(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 0.93 (d, \(J=\) 6.7, 6H, H14,15), 1.37-1.46 (m, 2H, H9), 1.49-1.66 (m, 4H, H8, 10), 1.79-1.89 (m, 1H, H13), 2.57-2.84 (m, 4H, H1,7), 3.05 (dd, \(J=\) 5.3, 11.0, 1H, H5), 3.21 (d, \(J=\) 6.7, 2H, H12), 3.37-3.42 (m, 1H, H3), 3.45 (t, \(J=\) 6.5, 2H, H11), 3.51-3.58 (m, 1H, H2), 3.71 (dd, \(J=\) 5.2, 8.9, 1H, H4), 3.80-3.90 (m, 2H, H6). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 19.7, 25.0, 28.5, 29.6, 30.6, 52.8, 55.5, 57.6, 64.3, 71.3, 72.0, 72.8, 76.0, 78.9. LC/MS analysis: Rt 4.85 min (linear gradient 0-90% B), ES (ESI): m/z = 306.4 [M + H]\(^+\), 611.8 [2M + H]\(^+\). \([\alpha]^{20}_D\) = +11.76\(^\circ\) (c = 0.20, CHCl\(_3\)).

N-(5-prenoxy)pentyl-1-ido-1-deoxynojirimycin (235).

\(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 1.63 (s, 3H, H10), 1.7 (s, 6H, H15,16), 2.03-2.19 (m, 4H, H11,12), 2.58 (dd, \(J=\) 9.3, 12.4, 1H, H1\(\alpha\)), 2.83 (dd, \(J=\) 4.8, 12.4, 1H, H1\(\beta\)), 3.03 (dd, \(J=\) 5.2, 10.6, 1H, H5), 3.29-3.40 (m, 2H, H7), 3.45 (t, \(J=\) 7.1, 1H, H3), 3.52-3.60 (m, 1H, H2), 3.87 (dd, \(J=\) 5.1, 8.6, 1H, H4), 3.83-3.93 (m, 2H, H6), 5.10-5.17 (m, 1H, H13), 5.25-5.31 (m, 1H, H8). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 16.5, 17.8, 25.9, 27.5, 40.9, 52.3, 53.0, 57.9, 64.1, 71.2, 72.7, 75.8, 122.7,
5. Evaluation of N-alkylated iminosugars for the treatment of Gaucher’s disease

125.2, 132.5, 40.1. LC/MS analysis: Rt 5.48 min (linear gradient 0-90% B), ES (ESI): m/z = 300.3 [M + H]^+, 599.8 [2M + H]^+. HR-MS [QTOF, MH^+] m/z calculated for C_{16}H_{30}NO_{4} 300.21693, found 300.21690. \([\alpha]^D_{20} = + 4.71^\circ (c = 0.42, \text{CHCl}_3)\).

**N-neopentoxypentyl-L-ido-1-deoxynojirimycin (236).**

1H NMR (600 MHz; CDCl₃): \(\delta\) 0.78 (s, 9H, H14-16), 1.23-1.30 (m, 2H, H9), 1.40-1.54 (m, 4H, H8, 10), 2.48-2.88 (m, 4H, H1, H7), 3.08-3.12 (m, 3H, H12,5), 3.34-3.41 (m, 1H, H3), 3.43 (t, J = 6.6, 2H, H11), 3.51-3.58 (m, 1H, H2), 3.65-3.70 (m, 1H, H4), 3.75-3.81 (m, 2H, H6).

**N-(5-cyclohexylmethoxy)pentyl-L-ido-1-deoxynojirimycin (237).**

1H NMR (400 MHz; CDCl₃): \(\delta\) 0.92-1.01 (m, 2H, H16), 1.15-1.81 (m, 15H, H8-10, 13-18), 2.54-2.58 (m, 4H, H1, H7), 3.05 (dd, J = 5.3, 11.0, 1H, H5), 3.24 (d, J = 6.5, 2H, H12), 3.37-3.47 (m, 3H, H12,3), 3.55 (dd, J = 5.0, 8.2, 9.6, 1H, H2), 3.72 (dd, J = 5.2, 8.9, 1H, H4), 3.81-3.89 (m, 2H, H6).

**N-(5-farnesoxy)pentyl-L-ido-1-deoxynojirimycin (238).**

1H NMR (400 MHz; CDCl₃): \(\delta\) 1.63 (m, 6H, H20,21), 1.69 (s, 3H, H15), 1.70 (s, 3H, H10), 1.97-2.22 (m, 8H, H11,12,16,17), 2.59 (dd, J = 9.4, 12.4, 1H, H1\(\alpha\)), 2.83 (dd, J = 4.2, 12.4, 1H, H1\(\beta\)), 3.04 (dd, J = 5.1, 10.7, 1H, H5), 3.28-3.40 (m, 2H, H7), 3.45 (t, J = 7.3, 1H, H3), 3.53-3.61 (m, 1H, H2), 3.75 (dd, J = 5.1, 10.7, 1H, H4), 3.82-3.91 (m, 2H, H6), 5.07-5.19 (m, 2H, H13,18), 5.24-5.32 (m, 1H, H8). 13C NMR (100 MHz; CDCl₃): \(\delta\) 15.9, 16.4, 17.6, 25.7, 27.3, 27.6, 40.6, 40.7, 52.1, 52.8, 57.7, 63.7, 71.0, 72.5, 75.5, 122.5, 125.0, 125.1, 131.8, 136.0, 139.9. LC/MS analysis: Rt 6.82 min (linear gradient 0-90% B), ES (ESI): m/z = 368.1 [M + H]^+, 736.6 [2M + H]^+. HR-MS [QTOF, MH^+] m/z calculated for C_{21}H_{38}NO_{4} 368.27954, found 368.27951. \([\alpha]^D_{20} = + 4.96^\circ (c = 0.52, \text{CHCl}_3)\).

**N-(5-cholesteroxy)pentyl-L-ido-1-deoxynojirimycin (239).**

1H NMR (600 MHz; CDCl₃): \(\delta\) 1.17 (s, 3H, CH₃-cholesterol), 1.34-1.36 (m, 6H, 2x CH₃-cholesterol), 1.49 (s, 3H, CH₃-cholesterol), 1.51-2.87 (m, 35H, 11x CH₂-cholesterol, 7x CH-cholesterol, H8-10), 3.2-3.8 (m, 6H, H1,3,5,7), 4.2-4.4 (m, 4H, H2,4,6), 5.81-5.86 (m, 1H,
olefin. $^{13}$C NMR (150MHz; CDCl$_3$): $\delta$ 11.7, 18.5, 19.2, 21.0, 22.3, 22.6, 23.7, 23.7, 24.2, 27.9, 28.1, 28.3, 29.6, 31.8, 31.9, 35.7, 36.1, 36.8, 37.1, 39.0, 39.4, 39.7, 42.2, 50.2, 53.8, 56.1, 56.7, 64.0, 67.4, 67.7, 79.2, 121.7, 140.6. LC/MS analysis: Rt 10.18 min (linear gradient 10-90% B), ES (ESI): m/z = 618.4 [M + H]$^+$, 1236.1 [2M + H]$^+$ HR-MS [QTOF, MH$^+$] m/z calculated for C$_{38}$H$_{68}$NO$_5$ 618.50920, found 618.50908. $[\alpha]_{D}^{20} = -11.87^\circ$ (c = 0.44, CHCl$_3$).
6

Diamondoids in iminosugar based glucosylceramide metabolism modulators

6.1 Introduction

The role of the adamantane moiety in drug design is multidimensional. For instance, several adamantane based drugs are able to cross the blood brain barrier and this capability is attributed to the adamantane scaffold. Additionally, the biological availability of pharmacophores can be affected by the incorporation of an adamantane unit. For instance, the decoration of a biologically active compound with a diamondoid results in an increased in hydrophobicity of the corresponding pharmacophore. As a result, the cellular-uptake of the diamondoid derivatized pharmacophore by passive diffusion through the lipid by-layer is promoted. Furthermore, the increase in steric bulk, associated with these fused polycyclic cyclohexanes, can restrict excess to (hydrolytic) enzymes thereby increasing drug stability and prolonging plasma half life.

In what has become known as substrate reduction therapy (SRT), small molecule glucosylceramide synthase (GCS) inhibitors are employed to manipulate cellular glycosphingolipid (GSL) levels. In the development of potent and selective iminosugar based GCS inhibitors, Aerts and co-workers reported the functionalization of 1-deoxynojirimycin (DNJ) with a N-pentyloxymethyl-1-adamantane unit. This N-alkylated DNJ derivative, MZ-21 (190), was found to possess enhanced inhibitory potency against GCS (Figure 6.1), compared to the marketed DNJ derivative Zavesca (189) equipped an N-butyl chain. Structure activity relationship studies of MZ-21 revealed that the L-ido-congener MZ-31 (191) is a more
selective inhibitor of GCS with respect to β-glucocerebrosidase (GBA1) inhibition. An-Other example of an adamantane derivatized iminosugar is the clinically under investigation isofagomine derivative 240. In a new therapeutic strategy, iminosugar derivatives such as 240 serve as pharmacological chaperones for the treatment of several LSD. In contrast to SRT, the small molecule inhibitors target the newly synthesized GBA1 and assist in their correct folding and lysosomal trafficking leading to increased lysosomal GBA1 concentrations.

In the search for potent and selective inhibitors for the enzymes involved in GlcCer metabolism, it was decided to further explore adamantane derivatized iminosugars based on leads MZ-21 (190) and MZ-31 (191). To this end, two libraries of iminosugar derivatives were designed and the bromides depicted in Figure 6.2 were selected for N-alkylation with DNJ and L-ido-DNJ. First, 6-bromohexylamide-1-adamantane (241), was selected based on isofagomine derivative 240 (Figure 6.1). Next, 6-bromohexyl-1-adamantane (242) and

Figure 6.1: Iminosugar based modulators of glucosylceramide levels and their IC50 values.175,179

<table>
<thead>
<tr>
<th></th>
<th>GCS IC50 = 50 uM</th>
<th>GBA1 IC50 = 400 uM</th>
<th>GBA2 IC50 = 0.23 uM</th>
</tr>
</thead>
<tbody>
<tr>
<td>189</td>
<td>IC50 = 0.2 uM</td>
<td>IC50 = 0.2 uM</td>
<td>IC50 = 0.001 uM</td>
</tr>
<tr>
<td>190</td>
<td>IC50 = 0.1 uM</td>
<td>IC50 = 2 uM</td>
<td>IC50 = 0.03 uM</td>
</tr>
<tr>
<td>191</td>
<td>IC50 = 94 uM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>IC50 = 0.2 uM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 6.2: Selected bromides for the synthesis of two libraries of adamantane decorated iminosugars.
6. Diamondoids in iminosugar based glucosylceramide metabolism modulators

6-bromohexyloxymethyl-1-adamantane (243) were selected to study the influence of an ether linkage. Furthermore, derivatives of leads 190 and 191 were designed by alteration of the spacer attachment point on the adamantane core, hence 2-adamantyl bromide (244) was selected. For the second library, adamantane bromides with an oxime linkage (245) and an ‘internal amide’ linkage (246) were selected for N-alkylation with DNJ and L-ido-DNJ. To further increase the hydrophobicity of the diamond functionalized iminosugar derivatives, the interesting bis-adamantyl derivative 247 was also selected. Finally, adamantane-diazirine photo crosslinker probe (248) was designed based on MZ-21, to gain more insight in the cellular behaviour of adamantane decorated iminosugar derivatives.

6.2 Results and Discussions

The synthesis of the iminosugar libraries follows a well established route of synthesis (Scheme 6.1). DNJ (178) and L-ido-DNJ (192) were prepared on large scale, as discussed in chapter 5, following literature procedures. Selective N-alkylation of these iminosugars with the appropriate bromide was accomplished under Schotten-Baumann conditions at 80°C in DMF with K₂CO₃ as the base. After removal of the insoluble base and concentration of the filtrate, the crude N-alkylated iminosugar derivatives were obtained. Pre-purification by a flash silica column chromatography followed by preparative HPLC purification, provided the pure N-alkylated iminosugars as their corresponding TFA salts.

Scheme 6.1: N-alkylated (L-ido) 1-deoxynojirimycin derivatives.

Reagents and conditions: i) Appropriate bromide (1.5 eq.), K₂CO₃ (3 eq.), DMF (0.1 M), 80°C, 12 h, ∼50-80% yield; then preparative HPLC purification.

1-adamantanoids: The preparation of bromide 241 was executed in three steps from commercially available 1-adamantanamine (65, Scheme 6.2). To this end, readily available 6-(benzyloxy)hexanoic acid (249) was condensed with amine 65, under the agency of EDC and catalytic DMAP, to give amide 250 in good yield. After Pd/C mediated debenzylation of 250 the resulting alcohol 251 was brominated via an Appel reaction. Bromide 241 was obtained in 79% yield over three steps. Alkylation of both D-gluco and L-ido iminosugar building blocks with bromide 241 provided derivatives 252 and 253 in reasonable yields after preparative HPLC-purification.

Next, analogues of lead 190 and 191 were prepared by omitting the ether functionality in the spacer. To this end, a Wittig olefination of readily available 1-adamantane ethanal (147, chapter 4,
Scheme 6.2: Synthesis of 1-adamantane derivatives.

Reagents and conditions: i) 249 (1.25 eq.), EDC·HCl (2 eq.), DiPEA (2 eq.), cat. DMAP, CH₂Cl₂ (0.2 M), 2 h, RT, 87%; ii) 5 mol% Pd/C (10 wt%), H₂, EtOAc (0.2 M), 12 h, RT; iii) PPh₃ (2 eq.), CBr₄ (2 eq.), CH₂Cl₂ (0.1 M), 0°C, 91% over two steps; iv) CH₂Cl₂ (1 M), oxalyl chloride (1.32 eq.), DMSO (2.5 eq.), -78°C, 2 h then add Et₃N (5 eq.), -78°C → -5°C, near quant. (see chapter 4, Scheme 4.2); v) 254 (1.5 eq.), NaOH (2 eq.), THF (0.5 M), 24 h, Δ, 32%; vi) NaH (3 eq.), 1,4-dioxane (0.25 M), 1 h, Δ, 1,6-dibromohexane (5 eq., 10 M) in DMF), 12 h, 80°C, 30%.

Scheme 4.2) and phosphonium salt 254 was applied for the preparation of the necessary bromide 255 (Scheme 6.2). The required Wittig salt 254 was prepared in one step from PPh₃ and 1,4-dibromobutane according to reported procedures, in good yields. The subsequent olefination was carried out in dry THF with NaOH as the base, to provide Z-olefin 255 in 32% yield. Because TLC analysis indicated a clean conversion, it is believed that prolonged exposure of this relative volatile bromide to high vacuum resulted in diminished yield. Bromide 255 was used in the selective N-alkylation of DNJ (178) and L-ido-DNJ (192) to provide derivatives 256 and 257. Subsequent Pd/C mediated hydrogenation of the double bond in 256 and 257 furnished derivatives 258 and 259.

Additionally, analogues of lead 190 and 191 were prepared by the addition of a methylene group in the spacer. To this end, 1-adamantanemethanol (149) was reacted with a five fold excess of 1,6-dibromohexane to provide 243 in one step in 30% yield (Scheme 6.2). N-alkylation of both DNJ and L-ido-DNJ with 243 provided derivatives 260 and 261.

2-adamantanoids: Both leads MZ-21 and MZ-31 bear a bridgehead functionalized 1-adamantane-methoxy group. To effect the overall spatial orientation of the adamantane moiety, iminosugar derivatives were prepared with a 2-adamantanemethoxy linkage. To this

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aThe Z-conformation was assigned on the basis of ¹H NMR.
Scheme 6.3: Synthesis of 2-amantantane derivatives.

Reagents and conditions: i) (methoxymethyl)triphenylphosphonium bromide (1.2 eq.), nBuli (1.2 eq.), Et₂O (0.2M), 1 h 10°C then add ketone 58, 12 h, RT; ii) 35 vol% HClO₄, 1 h, Δ; iii) NaBH₄ (1 eq.), EtOH, 12 h, RT, 54% over three steps; iv) 5-(benzyloxy)pentyl p-toluenesulfonate (1.5 eq.), NaH (1.5 eq.), cat. TBAI, DMF (0.1M), 57%; v) 5 mol% Pd/C (10 wt%), H₂, EtOAc (0.2M), 12 h, RT; vi) PPh₃ (2 eq.), CBr₄ (2 eq.), CH₂Cl₂ (0.1M), 0°C, 244, 75% over two steps; 245, 56%; 246, 85%; vii) NH₂OH·HCl (2 eq.), Pyr (0.3M), 4 h, RT, 85%; viii) THF (0.3M), 5-(trityloxy)pentyl p-toluenesulfonate (1.1 eq., 10M in DMF), NaH (1.5 eq.) cat. TBAI, THF (0.1M), 20 h, 67%; ix) BF₃·OEt₂ (0.15 eq.), MeOH/Tol (1:1, v/v), 1.5 h, RT, 246, 89%; 271, near quant.; x) AlCl₃ (2.5 eq.), cyclohexane (0.2M), 30 min, 85%; xi) 5-(trityloxy)pentyl p-toluenesulfonate (1.5 eq.), NaH (1.5 eq.) cat. TBAI, 20 h, RT, 64%.

end, 2-adamananemethanol (264) was prepared from 2-adamantanone (58) in three steps (Scheme 6.3), according to procedures described by Strating et al. First, Wittig olefination of 58 with (methoxymethyl)triphenylphosphonium bromide provided enol ether 262 after work-up procedures. Subsequent acidic hydrolysis of enol ether 262 produced aldehyde 263. The crude aldehyde was subjected to sodium borohydride reduction to furnish alcohol 264 in 54% yield over the three steps. Direct alkylation of alcohol 264 with 1,5-dibromopentane was not effective
and thus etherification of 264 was carried out with 5-(benzyloxy)pentyl p-toluenesulfonate in 57% yield. After Pd/C mediated cleavage of the benzyl ether in 265, the resulting alcohol 266 was transformed into bromide 244 via the Appel reaction in 75% yield over two steps. The resulting iminosugar derivatives 273 and 274 were obtained after selective N-alkylation of DNJ (178) and L-ido-DNJ (192) with bromide 244.

**Preliminary biological results:** The inhibitory potency of the newly synthesized compounds against GCS, GBA1 and GBA2 was evaluated. The inhibitory profile of the already tested compounds are depicted in Table 6.1.

**Table 6.1:** Enzyme inhibition assay preliminary results: Apparent IC_{50} values in micromolar (µM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R =</th>
<th>GCS Facs</th>
<th>GCS NBD</th>
<th>GBA1</th>
<th>GBA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>252: Hexylamide-N-1-adamantane</td>
<td>± 0.1</td>
<td>1</td>
<td>0.5</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>256: (Z)-hex-5-enyl-1-adamantane</td>
<td>2-20</td>
<td>0.5</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>258: Hexyl-1-adamantane</td>
<td>&gt;20</td>
<td>0.6</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>260: Hexyloxymethyl-1-adamantane</td>
<td>0.01-0.1</td>
<td>0.3</td>
<td>0.1</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>273: Pentyloxymethyl-2-adamantane</td>
<td>&lt;0.1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>253: Hexylamide-N-1-adamantane</td>
<td>± 0.01</td>
<td>0.4</td>
<td>40</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>257: (Z)-hex-5-enyl-1-adamantane</td>
<td>± 10</td>
<td>2-20</td>
<td>12</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>259: Hexyl-1-adamantane</td>
<td>1-10</td>
<td>2-20</td>
<td>25</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>261: Hexyloxy-1-adamantane</td>
<td>0.01-0.1</td>
<td>0.25</td>
<td>5</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td>274: Pentyloxymethyl-2-adamantane</td>
<td>&lt;0.1</td>
<td>0.08</td>
<td>10</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

The general trend that is observed in chapter 5,\textsuperscript{173} in that DNJ derivatives are more potent in the inhibition of the hydrolytic enzymes GBA1 and GBA2 compared to the L-ido-DNJ analogues is also observed here. Furthermore, the L-ido-DNJ derivatives are the more potent and selective GCS inhibitors compared to the DNJ analogues. In general, the presence of an amide-linked adamantane in compounds 252 and 253 or elongation of the spacer in 260 and 261 have little effect on the activity or selectivity compared to leads 190 and 191. Interestingly, the omission of the ether linkage in combination with a Z double bond in 256 and 257 have a detrimental effect on the inhibitory potency towards GCS. However, derivatives 256 and 257 are highly active against GBA2. The absence of the double bond in 258 and 259 results in partial loss of inhibition of the tested enzymes. It appears that the alteration of the attachment point of the spacer on the adamantane core in 273 and 274 has no influence on the degree or selectivity of inhibition on the tested enzymes compared to leads 190 and 191.
Second library of adamantane derivatives: It is hypothesized that the introduction of an oxime linkage will result in a decreased overall lipophilicity, compared to a 2-ether linkage. Therefore, 2-oxime linked adamantane derivatives 275 and 276 were prepared (Scheme 6.3). Furthermore, the oxime linkage can influence both the orientation and interaction of the adamantane unit. Oxime 267 was easily obtained from 2-adamananone and hydroxylamine and was subsequently alkylated with 5-(trityloxy)pentyl p-toluene sulfonate in moderate yield. Acidic mediated detritylation of 268 with a catalytic amount of p-TsOH resulted in complete degradation of the starting material. Instead, BF$_3$·OEt$_2$ mediated detritylation of 268 provided alcohol 269 in good yield. Subsequently, alcohol 269 was transformed into bromide 245 via the Appel reaction.

Next, the incorporation of a ‘internal’ amide functionality in the adamantane core was investigated. To this end, oxime 267 was subjected to an AlCl$_3$ promoted Beckmann rearrangement, according to a slightly modified literature procedure, to give 270 in 85% yield. Lactam 270 was alkylated with 5-(trityloxy)pentyl p-toluene sulfonate in good yield. Unexpectedly, detritylation of 271 using cat. p-TsOH·H$_2$O in MeOH/CH$_2$Cl$_2$ (1:1, v/v) was not productive and led to deterioration of the starting amide. Fortunately, BF$_3$·OEt$_2$ mediated detritylation went smoothly and provided alcohol 272 in near quantitative yield. Bromide 246 was obtained in good yields from alcohol 272 via an Appel reaction.

Scheme 6.4: Synthesis of bis-adamantyl derivatives.

Reagents and conditions: i) Br$_2$, 1 drop H$_2$O, 30 min RT, quant.; ii) Mg, Et$_2$O, cat. I$_2$, 40°C; iii) 1-adamanatanecarbonyl chloride, CuBr (0.25 eq.), 0°C → RT, 12 h, 25% over two steps; iv) (methoxymethyl)triphenylphosphonium bromide, nBuLi (1.2 eq.), Et$_2$O, ~10°C; v) LiAlH$_4$ (2 eq.), Et$_2$O, 0°C → RT, 12 h, quant.; vi) 5-(trityloxy)pentyl p-toluene sulfonate (1.5 eq.), DMF, NaH (3 eq.), 30 min, 40°C, 45%; vii) CH$_2$Cl$_2$/MeOH (1:1, v/v, 0.1 M), cat. p-TsOH, RT.

Bis-adamantanoid synthesis: N-alkylated DNJ (178) and L-ido-DNJ (192) with an bis-adamantyl moiety are interesting derivatives, in terms of the increased hydrophobicity and steric bulk. The synthesis of bis-adamantyl derivative 247 (Scheme 6.4) was undertaken, following procedures described by Dubois et al.$^{182,183}$ First, mild ionic bromination of adamantane (1) with
Br₂ at 0°C provided exclusively 1-bromoadamantane in excellent yield (24, Scheme 6.4). Formation of organomagnesium compound 277 was carried out in the absence of mechanical stirring, as deemed crucial according to the group of Dubois. Addition of 1-adamantanecarbonyl chloride to 277 resulted in the formation of 247 in reasonable yields. Unfortunately, homologation via a Wittig olefination of 247 was unsuccessful. Next, ketone 247 was reduced with LiAlH₄ to provide alcohol 279 in near quantitative yield. Alkylation of 279 with 5-(trityloxy)pentyl p-toluenesulfonate gave 280 in 45% yield. However, upon detritylation of 280 using p-TsOH in a mixture of CH₂Cl₂ and MeOH resulted in the cleavage of the 5-(trityloxy)pentyl spacer giving alcohol 279 in near quantitative yield.

Scheme 6.5: Synthesis of 2-amantantane derivatives.

Reagents and conditions: i) a. 4M NH₃, MeOH (0.1M); b. H₂NOSO₃H, MeOH (0.1M, 0°C); c. CrO₃H₂SO; conc. HNO₃, 70 h then aq. H₂SO₄, 52%; iii) conc. formic acid, oleum (30%), 56%; vi) p-TsOH (cat), ethyleneglycol (20 eq.), Tol, δ; v) LiAlH₄ (2.0 eq), THF (0.1M), 2 h, RT, 72% over two steps; vi) NaH (1.1 eq.), DMF (0.1M), 5-(trityloxy)pentyl p-toluenesulfonate (1.1 eq.), 65%; vii) THF/H₂O (5:1, v/v, 0.1M), cat. CSA, 60°C, 12 h, 96%; viii) TBDPS-Cl (1.5 eq.), imidazole 1.5 eq.), DMF (0.1M), 67%; ix) hydroxylamine (1.5 eq.), Pyr (0.1M), 50°C, 89%; x) a. p-TsCl, Et₃N, CH₂Cl₂ (0.1M); b. NH₃ in MeOH (4M); xi) a. p-TsCl, Et₃N, CH₂Cl₂; b. NaBr (5 eq.), acetone (0.1M), Δ.
Adamantane-diazirine photo crosslinker: The design of crosslinker probe 248 was tailored after the reported conversion of 2-adamantanone (58) to diazirine 282 (Scheme 6.5).\textsuperscript{185,186} To this end, 5-hydroxyadamantane2-one (283) was prepared according to reported procedures,\textsuperscript{61} and used in the Koch-Haaf carbonylation reaction. The crude acid 284 was immediately subjected to acetal formation with 1,2-ethane diol and subsequently reduced with LiAlH\textsubscript{4} to give 285 in 72\% yield from 2-adamantanone (291) over the two steps. The installment of a 5-trityloxypentyl spacer to give 286 proceeded in good yield. Subsequently, acid mediated detritylation of 286 with simultaneously acetal deprotection was accomplished with catalytic camphorsulfonic acid in excellent yield. The resulting alcohol 287 was silylated with TBDMS-Cl in 67\% yield. Unfortunately, ketone 288 remained in-reactive towards imine formation with concentrated NH\textsubscript{3} as described in the formation of 282.\textsuperscript{185,186} Alternatively, ketone 288 was subjected to oxime formation with hydroxyl amine, which provide pure 290 in good yield. However, exposure of oxime 290 to p-TsCl resulted in complete degradation of the starting material. These setbacks let to the abortion of the adamantane-diazirine probe and the design of a phenyl-diazirine probe.

6.3 Conclusions and Outlook

In this study, two libraries of adamantane derivatized iminosugars were designed based on leads MZ-21 (190) and MZ-31 (191). The members of the library were designed to incorporate different linkages between the adamantane moiety and the iminosugar. Preliminary results indicate that the introduction of an unsaturated spacer between the iminosugar and diamondoid core, as present in derivatives 256 and 257 results in remarkable inhibitor activity towards GBA2. Furthermore, the importance of an ether linkage in the design of potent GCS inhibitors is emphasized in 258 and 259, which lack this functionality.

Scheme 6.6: Design of phenyl-diazirine photo crosslinker probe.
In the second library, the synthesis of adamantane photo crosslinker probe 248 presented several difficulties. The promising results obtained in chapter 5 with \(N\)-pentyloxy aromatic iminosugar derivatives inspired the design of crosslinker probe 292 (Scheme 6.6). The new probe was designed to incorporate a bodipy fluorophore at the C-6 atom of the iminosugar. The C-6 position was chosen as a ligation point for the bodipy fluorophore based on the recent findings that the enzymes involved in GlcCer metabolism tolerate the presence of a sterically congested unit.\(^{144}\) Retro-synthetic analysis revealed that probe 292 can be constructed from alkyne 293, iminosugar 294 and aziridine 295.

Scheme 6.7: Synthesis of 6-azido DNJ precursor.

Reagents and conditions: i) \(\text{Ac}_2\text{O}/\text{TFA} (4:1, v/v, 0.15\text{M})\), 3 h, \(0^\circ\text{C}\); ii) \(\text{MeOH} (0.5\text{M})\), cat. NaOMe, 2 h, RT; iii) \(\text{NaBH}_4 (2.0\text{eq.})\), EtOH (0.1M); iv) \(\text{CH}_3\text{CN}, 2,2\)-dimethoxypropane (2.0 eq.), cat. CSA; v) \(\text{Ac}_2\text{O} (3.0\text{eq.})\), Et\(_3\text{N} (6.0\text{eq.})\), cat. DMAP, \(\text{CH}_2\text{Cl}_2 (0.1\text{M})\); vi) \(\text{CH}_3\text{CN}, 5\text{vol}\% \text{H}_2\text{O}\), cat. CSA; 60\(^\circ\text{C}\), 30 min, 67\% yield over six steps; vii) Trt-Cl (1.5 eq.), Pyr (0.1M), 50\(^\circ\text{C}\), 12 h, 92\%; MeOH (0.5M), cat. NaOMe, 30 min, RT, near quant.; ix) \(a\). oxalyl chloride (2.2 eq.), DMSO (4.4 eq.), \(\text{CH}_2\text{Cl}_2 (0.2\text{M})\), -78\(^\circ\text{C}\), 2h, Et\(_3\text{N} (10\text{eq.})\); b. add. reaction mixture to \(\text{MeOH} (0.2\text{M})\), \(\text{Na}_2\text{SO}_4 (\sim 1\text{g/mmol})\), \(\text{NH}_4\text{HCO}_3 (20\text{eq.})\), \(\text{NaCNBH}_3 (4\text{eq.})\), 0\(^\circ\text{C}\) \(\rightarrow\) RT, 24 h, 60\% over two steps; x) benzaldehyde (2.0 eq.), \(\text{Na}_2\text{SO}_4 (\sim 1\text{g/mmol})\), \(\text{NaBH(OAc)}_3 (2.0\text{eq.})\), 85\%; xi) AcOH (0.2M), TES (2.0 eq.), 50\(^\circ\text{C}\), 12 h, 81\%; xii) Ms-Cl (1.5 eq.), \(\text{Et}_3\text{N} (1.5\text{eq.})\), \(\text{CH}_2\text{Cl}_2 (0.1\text{M})\), 0\(^\circ\text{C}\), 1 h; xiii) \(\text{NaNO}_3 (5.0\text{eq.})\), DMF (0.1M), 71\% over two steps; xiv) BCl\(_3 (20\text{eq.})\), \(\text{CH}_2\text{Cl}_2 (0.1\text{M})\), -78\(^\circ\text{C}\), 6 h, 51\%.

First, the synthesis of 6-azido DNJ (294) was explored similar to the preparation of DNJ (178).\(^{175}\) To this end, diol 296 was prepared according to literature procedures\(^{187}\) in 67\% yield from 2,3,4,6-tetra-O-benzyl glucose (193). After selective tritylation, acetate 297 was saponified to give diol 298 in excellent yield over the two steps. Next, diol 298 was subjected to the Swern oxidation - reductive amination protocol, as reported for the synthesis of DNJ (178). This multi-step protocol furnished DNJ derivative 299 in about 60\% yield. Amine 299 was \(N\)-benzylated with benzaldehyde, under reductive amination conditions, and subsequently treated with acetic acid at 80\(^\circ\text{C}\) to give alcohol 300 in good yield. Alcohol 300 was mesylated and immediately
reacted with NaN₃ in DMF, to give azide 301 in good yield over two steps. Finally, debenzylation with BCl₃ at -78°C followed by ion-exchange purifications provided sufficient amounts of azide 294.

**Scheme 6.8: Synthesis of phenyl-diazirine precursor.**

Reagents and conditions:  

- i) NH₂OH·HCl (1.5 eq.), Pyr (0.1 M), 50°C, 87%; ii) p-TsCl (1.5 eq.), Et₃N (1.5 eq.), CH₂Cl₂ (0.1 M), 90%; iii) Et₂O (0.1 M), NH₃ (~1 vol%), -78°C → RT (caution: pressure exceeds 8 bar); iv) Et₃N (2.0 eq.), I₂ (1.0 eq.), 30 min, RT, 85%; v) MeOH (0.1 M), conc. HCl (pH = 1), 2 h, RT, 92%; vi) DMP (1.5 eq.), CH₂Cl₂ (0.1 M), 2 h, 0°C, 79%.

The synthesis of phenyl diazirines 295 was executed based on reported procedures for the preparation of a similar compound (295 with a C₅H₁₀ alkyl chain instead of a C₅H₁₀). Briefly, silyl ether 302 was converted via intermediate oxime 303 and tosylate 304 to diaziridine 305. After iodine mediated oxidation of 305, diazirine 306 was obtained in good yield. Acidic removal of the silyl protective group and subsequent Dess-Martin periodinane oxidation of resulting alcohol 295, provide diazirine precursor 295 in good yield.

The bodipy precursor 293 was prepared in house and was kindly provided by Mark Ruben. With all three precursors in hand, the synthesis of photo crosslinker probe 292 was investigated. The N-alkylation of azide 294 was accomplished via reductive amination with aldehyde 295. To minimize the exposure of the diazirine unit to reductive agents, a two step protocol was applied. First, imine formation between azide 294 and a large excess of aldehyde 295 was executed in MeOH at pH = 5.5 (adjusted with AcOH). Complete consumption of azide 294 was observed after 12 h at room temperature, and equimolar NaCNBH₃ was added to the reaction cocktail. After preparative HPLC purification, the N-alkylated product 308 was obtained in sufficient quantity and was used in a azide-alkyn-Huisgen-cycloaddition with alkyne 293. The cycloaddition was executed in tBuOH/H₂O/Tol (1:1:1, v/v/v) with CuSO₄ and sodium ascorbate. Preparative HPLC-purification provided benzyl-diazirine photo cross linker probe 116.
6.4 Experimental Procedures

Scheme 6.9: Synthesis of phenyl-diazirine photo crosslinker.

Reagents and conditions: i) a. MeOH (0.1 M), pH = 5.5 (adjusted with AcOH), Na$_2$SO$_4$ (~1 g/mmol), 295 (3 eq.), 12 h, RT; b. add NaN$_3$(1.0 eq.), 12 h, 10%; ii) 293 (3.0 eq.), CuSO$_4$ (21 µl, 100 mM in H$_2$O), sodium ascorbate (41 µl, 100 mM in H$_2$O), tBuOH/H$_2$O/Tol (1:1:1, v/v/v, 1.5 ml).

292 in high purity.

6.4 Experimental Procedures

General Methods: Solvents and reagents were obtained commercially and used as received unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere and residual water was removed from starting compounds by coevaporation with toluene (3x), unless stated otherwise. Reaction grade solvents were stored on 4 molecular sieves or 3 for MeOH, CH$_3$CN, and DMSO. All solvents were removed by *in vacuo* evaporation at ~45°C. Reactions were monitored by TLC analysis using silica gel coated aluminium plates (0.2 mm thickness) an detection by UV absorption (254 nm) and/or by spraying with a solution of (NH$_4$)$_6$Mo$_7$O$_{24}$.4H$_2$O (25 g/L) and (NH$_4$)$_4$Ce(SO$_4$)$_4$.4H$_2$O in 10% H$_2$SO$_4$, followed by charring at ~150°C. Visualisations of olefins and N-alkylated iminosugars was achieved by spraying with a solution of KMnO$_4$ (5 g/L) and K$_2$CO$_3$ (25 g/L) in H$_2$O, followed by charring at ~150°C. Glycosides and hemiacetals were visualized by spaying with a solution of 20% H$_2$SO$_4$ in MeOH and charring at ~150°C and for adamantane containing compounds a solution of H$_3$PMo$_{12}$O$_{40}$ (100 g/L) was used. Flash column chromatography was performed on silica gel (40-63 µm). NMR spectra were recorded on a 400/100 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethyl silane as internal standard for all $^1$H NMR measurements in CDCl$_3$ and the deuterated solvent signal for all other NMR experiments. $^1$H NMR peak assignment were made using COSY and HSQC experiments and coupling constants (J) are given in Hz. All $^{13}$C NMR spectra are proton decoupled. For LC/MS analysis a HPLC-system (detection simultaneously at 213 nm, 254 nm and evaporative light detection) equipped with an analytical C18 column (4.6 mmD x 250 mmL, 5µm particle size) in combination with buffers
A: H$_2$O, B: CH$_3$CN, C: 1.0% aqueous trifluoroacetic acid and coupled with an electrospray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a semi-preparative C18 column (5 µm C18, 10, 150 x 21.2 mm) was used. The applied buffers were A: H$_2$O + trifluoroacetic acid (1% mM) and B: CH$_3$CN. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in H$_2$O/CH$_3$CN; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250°C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Optical rotations were measured on an automatic polarimeter (Sodium D-line, λ = 589 nm).

**General procedure A:** synthesis of N-alkyl-DNJ$s$, N-alkyl-Lido-DNJ$s$, N-pentyloxyalkyl-DNJ$s$ and N-pentyloxyalkyl-Lido-DNJ$s$: To a dry solution of DNJ (178) or Lido-DNJ (192)(0.20 mmol) in DMF (1 ml) were added the requisite alkylbromide or alkylxypentylbromide (0.30 mmol) in DMF (1 ml) and K$_2$CO$_3$ (0.60 mmol). The reaction mixture was stirred for 16 h at 80°C. Monitoring of the reaction progress was accomplished by TLC analysis or analytical LC/MS analysis. Upon complete consumption of the SM, the solids were removed by filtration over a 50 µm filter and the filter-cake was rinsed with DMF. The volatiles were removed in vacuo and the residue was purified by silica gel column chromatography. Elution was performed with MeOH/EtOAc/NH$_4$OH (0:100:0 → 30:70:0.03) affording a colourless oil. The obtained compounds were further purified by RP-HPLC (via an automated HPLC system).

**General procedure B:** O-alkylation of ada- and diamantane methanol: To a dry and cooled (0°C) solution of the correct alcohol (1 eq.) in DMF (0.1M) were subsequently added 5-(benzyloxy)pentyl p-toluenesulfonate (1.5 eq.) or 5-(trityloxy)pentyl p-toluenesulfonate (1.5 eq.), NaH (1.5 eq.) and a catalytic amount of TBAI. The resulting reaction mixture was stirred for 12 h at RT. After TLC analysis revealed complete disappearance of the starting alcohol, excess NaH was quenched at 0°C by the addition of EtOH. The reaction mixture was diluted with H$_2$O and extracted five times with Et$_2$O. The combined ethereal layer was subsequently washed with H$_2$O, brine, dried (MgSO$_4$), filtered, concentrated and the residue was purified by silica gel FCC.

**General procedure C:** Pd/C mediated hydrogenations: The appropriate substrate was dissolved in either aldehyde free EtOH (for polar substrates) or EtOAc (0.1M) and was depleted from oxygen by sonification under an argon atmosphere. 5 mol% Pd/C (10 wt%) was added and the resulting mixture was stirred under an H$_2$ atmosphere for 12 h. The reaction mixture was filtered over a pad of Celite and the residue was rinsed with the solvent of choice. The volatiles were removed to provide the crude product.
6.4 Experimental Procedures

**General procedure D: Bromination of primary alcohols via the Appel reaction.** To a dry solution of the 1°-alcohol in CH₂Cl₂ (0.1M) was added PPh₃ (2 eq.) and the reaction mixture was cooled down to 0°C. Next, CBr₄ (2 eq.) was added and the resulting reaction mixture was allowed to stir for 2 h at RT, after which TLC analysis usually indicates complete conversion of the starting alcohol. Celite (∼1 g/mmol) was added and the volatiles were removed and the residue was subjected to silica gel FCC.

**General procedure E: Homologation of 2-adamantanone and 3-diamantanone:** To a dry and cooled (∼10°C) solution of (methoxymethyl)triphenylphosphonium bromide (1.3 eq.) in Et₂O (0.2M) was dropwise added nBuLi (1.3 eq., 1.6M solution in hexane) and the reaction mixture was stirred for 1 h at ~10°C. Next, a dry solution of the proper ketone (1 eq.) in Et₂O (0.2M) was added to the resulting yellow suspension and the reaction mixture was stirred for 12 h at RT. TLC analysis indicated the formation of a more apolar olefin (intense colouration with aq. KmNO₄). The resulting triphenylphosphine oxide (O−PPh₃) was removed from the reaction mixture by complexation with ZnCl₂ (0.5 g/mmol) and subsequent filtration of the reaction mixture. The residue was rinsed with Et₂O and the combined filtrates were concentrated to provide the crude, intermediate enol ether.

To a dry solution of the crude enol ether in Et₂O (0.3M) was added aq. perchloric acid (35 vol%) and the resulting reaction mixture was stirred under reflux for 1 h. The reaction mixture was allowed to cool down to RT and diluted with H₂O and the organic layer was collected. The aqueous layer was extracted twice with Et₂O and the combined ethereal layer was subsequently washed with H₂O, brine, dried (Na₂SO₄) and filtered. The volatiles were removed in vacuo to provide the crude aldehyde, which was used in the next step.

To a dry and cooled (0°C) solution of the aldehyde in EtOH (0.3M) was added NaBH₄ (1.5 eq.) and the reaction mixture was stirred for 12 h at RT. After TLC analysis indicated completed consumption of the starting aldehyde, excess sodiumborohydride was quenched by with AcOH at 0°C. The reaction mixture was concentrated and the residue was dissolved in Et₂O and subsequently washed with H₂O, sat. aq. NaHCO₃ (2x) and finally brine. The organic layer was dried over MgSO₄, filtered, concentrated and the residue was subjected to silica gel FCC.

\[
\text{HN} \quad \text{O} \\
\text{OBn} \quad \text{N} \quad \text{(adamantan-1-yl)-6-(benzyloxy)hexanamide (250).}
\]

To a dry solution of 1-adamantanamine (65, 0.30 g, 2.0 mmol) in CH₂Cl₂ (0.2M) were subsequently added 6-(benzyloxy)hexanoic acid (249, 0.56 g, 2.5 mmol), EDC·HCl (0.77 g, 4 mmol), DiPEA (0.7 ml, 4 mmol) and a catalytic amount of DMAP. The resulting reaction mixture was stirred for 2 h at RT after which TLC analysis indicated the formation of a more apolar compound. The reaction mixture was diluted with CH₂Cl₂ and subsequently washed with 0.2M aq. HCl (2x), sat. aq. NaHCO₃, a mixture of brine/H₂O (1:1, v/v). The organic layer was dried (MgSO₄), filtered and concentrated. The residue was subjected to silica gel FCC (PE/Et₂O, 5:1 → 1:1) to give the title compound in 87% yield. \( R_F = 0.9 \) (100% EtOAc). \(^1\)H NMR (400 MHz; CDCl₃): \( \delta \)
6. Diamondoids in iminosugar based glucosylceramide metabolism modulators

1.38-1.47 (m, 2H, H16), 1.66-2.20 (m, 21H, 6x CH\(_2\), 3x CH - Ada, H12,13,15), 3.47 (t, 2H, J=5.8, H16), 4.48 (s, 2H, CH\(_2\)-Bn), 5.17 (br. s, 1H, NH), 7.30-7.3 (m, 5H, Har). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 25.3, 25.5, 29.1, 36.1, 37.2, 41.3, 51.3, 69.9, 72.5, 127.2, 127.2, 128.0, 138.3, 171.9.

\(\text{N-(adamantan-1-yl)-6-hydroxyhexanamide (251).}\)

A solution of benzyl ether 250 (0.83 g, 2.4 mmol) in EtOAc (0.2M) was debenzylated according to general procedure C. After removal of the solvents, the title compound was obtained and used in the next step. RF = 0.4 (100% EtOAc). \(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 1.21-1.66 (m, 12H, 3x CH\(_2\)-Ada, H13-15), 1.99-2.14 (m, 11H, 3x CH\(_2\), 3x CH - Ada, H12), 3.03 (br. s, 1H, OH), 3.61 (t, 2H, J=5.8, H16), 5.46 (br. s, 1H, NH). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 25.3, 39.3, 32.1, 36.3, 37.4, 41.5, 51.6, 62.0, 172.3.

\(\text{N-(adamantan-1-yl)-6-bromohexanamide (241).}\)

A dry solution of crude alcohol 251 (0.53 g, \(\sim 2\) mmol) in CH\(_2\)Cl\(_2\) (0.1M) was brominated according to general procedure D. The residue was subjected to silica gel FCC (PE/Et\(_2\)O, 4:1 \(\rightarrow\) 0:1) to provide the title compound in 91% yield. RF = 0.3 (100% Et\(_2\)O). \(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 1.38-1.74 (m, 8H, 3x CH\(_2\)-Ada, H14), 1.782-2.19 (m, 15H, 3x CH\(_2\), 3x CH - Ada, H12, 13, 15), 3.40 (t, 2H, J=5.8, H16), 5.46 (br. s, 1H, NH). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 24.5, 27.2, 29.0, 32.1, 33.3, 36.0, 36.8, 41.2, 43.2, 51.2, 171.5.

\(\text{N-heptylamide-N-1-adamantane-1-deoxy-nojirimycin (252).}\)

\(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 1.30-1.45 (m, 2H, H-9), 1.59-1.78 (m, 10H, 3x CH\(_2\)Ada, H-8,10), 2.02-2.10 (9H, 3x CH\(_2\)Ada, 3x CHAda), 2.17 (t, J= 7.32 Hz, 2H, H-11), 2.65-2.77 (m, 2H, H-5,1b), 2.94-3.06 (m, 1H, H-7a), 3.11-3.22 (m, 1H, H-7b), 3.27-3.38 (m, 2H, H-1b,3), 3.49-3.59 (m, 1H, H-4), 3.62-3.69 (m, 1H, H-2), 3.91-4.06 (m, 2H, H-6). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 24.36, 26.62, 27.42, 30.86, 37.48, 37.56, 41.06, 42.35, 52.75, 53.78, 55.94, 56.96, 67.39, 68.97, 70.14, 79.04. LC/MS analysis: Rt 5.6 min (linear gradient 0-90% B), ES (ESI): m/z = 411.2 [M + H]\(^+\). \([\alpha]^{20}_{D}\) = - 2.39 (c = 0.75, CHCl\(_3\)).

\(\text{N-heptylamide-N-1-adamantane-\textit{Lido}-1-deoxy-nojirimycin (253).}\)

\(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 1.61-1.81 (m, 2H, H-9), 1.96-2.27 (m, 10H, 3x CH\(_2\)Ada, H-8,10), 2.34-2.46 (m, 9H, 3x CH\(_2\)Ada, 3x CHAda), 2.52 (t, J=7.27, 2H, H-11), 3.63-3.98 (m, 6H, H-1,3,5,7), 4.22-4.50 (m, 4H, H-2,4,6). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 24.27,24.3, 24.90, 28.66, 35.44, 35.57, 40.35, 50.94, 52.85, 58.0, 62.5, 66.56, 67.0, 75.0. LC/MS analysis: Rt 5.6
min (linear gradient 0-90% B), ES (ESI): m/z = 411.2 [M + H]+. \([\alpha]^2_D = + 1.15\) (c = 1.1, CHCl₃).

**1-((Z)-6-bromohex-2-en-1-yl)adamantane (255)** To a dry solution of readily available adamantane ethanal (147, 0.80 g, 4.5 mmol, see chapter 4) in THF (0.5M) were subsequently added 1-(4-bromobutyl)triphenylphosphonium bromide (254, 3.2 g, 6.8 mmol) and NaOH (0.27 g, 6.8 mmol). The resulting reaction mixture was heated to reflux and stirred for 24 h after which the reaction mixture was slowly cooled to 0°C. The insolubles were filtered off and the filtrate was concentrated and the residue was subjected to silica gel FCC (PE/Et₂O, 1:0 → 95:5) to provide the title compound in 32% yield. \(R_F = 0.9\) (PE/Et₂O, 9:1).

\(^1H\) NMR (600 MHz; CDCl₃): \(\delta 1.50-1.59\) (br. s, 6H, 3x CH₂-Ada), \(1.62-1.74\) (m, 6H, 3x CH₂-Ada), \(1.85\) (d, \(J = 7.8\), H11), \(1.90-1.99\) (m, 5H, 3x CH-Ada, H15), \(2.21\) (q, 2H, \(J = 7.1, 6.9\), H14), \(3.44\) (t, 2H, \(J = 7.1, 6.9\), H14), \(5.40-5.47\) (m, 1H, H13), \(5.50-5.57\) (m, 1H, H12).

\(^13C\) NMR (100 MHz; CDCl₃): \(\delta 25.7, 28.6, 32.7, 33.2, 37.0, 41.6, 42.2, 126.9, 128.9\).

\(N\)-hex-(Z)-10-ene-1-adamantane-1-deoxy-nojirimycin (256).

\(^1H\) NMR (600 MHz; CDCl₃): \(\delta 1.77-1.83\) (m, 6H, 3x CH₂Ada), \(1.84-2.05\) (m, 8H, 3x CH₂Ada, H-8), \(2.11\) (d, \(J = 6.45\) Hz, 2H, H-12), \(2.25\) (br. s, 3H, 3x CHAda), \(2.32-2.42\) (m, 2H, H-9), \(2.54-2.69\) (m, 2H, H-1a,5), \(2.94-3.05\) (m, 1H, H-7a), \(3.11-3.19\) (m, 1H, H-7b), \(3.38\) (dd, \(J = 5.08, 11.63\) Hz, 1H, H-6b), \(5.73-5.83\) (m, 2H, H-10,11). \(^13C\) NMR (150MHz; CDCl₃): \(\delta 23.78, 24.79, 28.69, 32.20, 36.99, 41.62, 42.28, 52.13, 55.81, 57.60, 65.25, 68.92, 70.26, 78.67, 126.21, 129.97. LC/MS analysis: Rt 6.74 min (linear gradient 0-90% B), ES (ESI): m/z = 380.1 [M + H]+, 759.7 [2M + H]+. HR-MS [QTOF, MH+] m/z calculated for C_{22}H_{38}NO_{3} 380.27954, found 380.27945. \([\alpha]^2_D = - 22.2\) (c = 0.80, CHCl₃).

\(N\)-hex-(Z)-10-ene-1-adamantane-1-deoxy-nojirimycin (257).

\(^1H\) NMR (600 MHz; CDCl₃): \(\delta 1.67-1.70\) (m, 6H, 3x CH₂Ada), \(1.76-1.94\) (m, 8H, 3x CH₂Ada, H-8), \(1.99\) (d, \(J = 6.51\) Hz, 2H, H-12), \(2.13\) (br. s, 3H, 3x CHAda), \(2.23-2.29\) (m, 2H, H-9), \(2.85-3.20\) (m, 4H, H-1,7), \(3.28-3.35\) (m, 1H, H-5), \(3.723.80\) (m, 1H, H-3), \(3.82-3.89\) (m, 1H, H-2), \(3.97-4.03\) (m, 2H, H-4,6a), \(4.09\) (dd, \(J = 5.08, 11.63\) Hz, 1H, H-6b), \(5.61-5.68\) (m, 2H, H-10,11). \(^13C\) NMR (150 MHz; CDCl₃): \(\delta 24.56, 28.67, 32.36, 41.57, 42.33, 51.68, 53.69, 61.37, 63.69, 69.16, 71.51, 126.04, 130.04. LC/MS analysis: Rt 6.97 min (linear gradient 0-90% B), ES (ESI): m/z = 380.1 [M + H]+, 759.6 [2M + H]+. HR-MS [QTOF, MH+] m/z calculated
for C_{22}H_{38}NO_{4} 380.27954, found 380.27945. \([\alpha]_{D}^{20} = + 1.2 (c = 0.48, \text{CHCl}_3).\)

**N-hexyl-1-adamantane-1-deoxy-nojirimycin (258).**

\(1^H\ NMR\ (400\ MHz; \text{CDCl}_3): \delta\ 1.79-1.86\ (m, 2H, H-9), 2.00-2.21\ (m, 6H, H-8,10,11), 2.25-2.28\ (m, 6H, 3x CH_2Ada), 2.39-2.56\ (m, 8H, 3x CH_2Ada, H-12), 2.71\ (br. s, 3H, 3x CHAda), 3.68\ (t, J = 11.64 Hz, 1H, H-1a), 3.72-3.79\ (m, 3x CHAda), 3.85-3.97\ (m, 1H, H-7a), 3.98-4.06\ (m, 1H, H-7), 4.12-4.23\ (m, 2H, H-1b,3), 4.37\ (t, J = 2.80, 12.85 Hz, 1H, H-6a), 4.44-4.25\ (m, 1H, H-2), 4.64\ (dd, J = 9.67 Hz, 1H, H-4), 4.44-4.25\ (m, 1H, H-2), 4.64\ (dd, J = 2.80, 12.85 Hz, 1H, H-6a), 4.80-4.95\ (m, 1H, H-6b).\n
**13C\ NMR\ (100\ MHz; \text{CDCl}_3): \delta\ 21.1, 22.03, 25.57, 27.93, 29.08, 31.18, 36.24, 41.52, 43.64, 51.0, 52.63, 53.37, 65.02, 65.55, 66.62, 75.72.**

**LC/MS analysis:** Rt 7.077 min (linear gradient 0-90% B), ES (ESI): m/z = 382.1 [M + H]^+; 763.6 [2M + H]^+. HR-MS [QTOF, MH]^+ m/z calculated for C_{22}H_{40}NO_{4} 382.29519, found 382.29519. \([\alpha]_{D}^{20} = - 3.52 (c = 0.91, \text{CHCl}_3).\)

**N-hexyl-1-adamantane-lido-1-deoxy-nojirimycin (259).**

\(1^H\ NMR\ (400\ MHz; \text{CDCl}_3): \delta\ 1.00\ (m, 2H, H-12), 1.22-1.44\ (m, 8H, H-8-11), 1.44-1.49\ (br. s, 6H, 3x CH_2Ada), 1.92\ (br. s, 3H, 3x CHAda), 2.3-3.54\ (m, 6H, H-1,7,3,5), 3.86-4.07\ (m, 4H, H-6,2,4).\n
**13C\ NMR\ (100\ MHz; \text{CDCl}_3): \delta\ 27.98, 30.14, 31.38, 33.44, 38.50, 43.80, 45.92, 54.63, 55.39, 61.78, 63.47, 67.51, 68.97, 72.80.**

**LC/MS analysis:** Rt 7.27 min (linear gradient 0-90% B), ES (ESI): m/z = 382.2 [M + H]^+; 763.6 [2M + H]^+. HR-MS [QTOF, MH]^+ m/z calculated for C_{22}H_{40}NO_{4} 382.29519, found 382.29516. \([\alpha]_{D}^{20} = - 10.45 (c = 0.57, \text{CHCl}_3).\)

**1-((6-bromohexyloxy)methyl)adamantane (243)**

To a dry solution of 1-adamantanemethanol (149, 4.16 g, 25 mmol) in 1,4-dioxane (0.25M) was carefully added NaH (3.0 g, 60 wt% dispersion in mineral oil, 75 mmol) at RT. The resulting reaction mixture was heated to reflux and stirred for 1 h after which the formed suspension was cooled to RT. Next, a solution of 1,6-dibromohexane (19.2 ml, 125 mmol) in DMF (10M) was added and the reaction mixture was heated up to 80°C and stirred for 12 h. TLC analysis indicated complete disappearance of the starting alcohol and the reaction mixture was cooled to ~50°C and diluted with ice-cold H_2O. The reaction mixture was transferred to a separatory funnel and extracted five times with EtOAc. The combined organic layer was subsequently washed with H_2O, brine, dried (MgSO_4), filtered and concentrated. The residue was subjected silica gel FCC (PE/Tol, 1:0 → 9:1) to give the title compound in 30% yield as a pale yellow oil. **1H NMR (400 MHz; CDCl_3): \delta\ 1.37-1.96\ (m, 23H, 6x CH_2, 3x CH - Ada, H13-16), 2.95\ (s, 2H, H11), 3.34-3.45\ (m, 4H, H12,17).**

**13C\ NMR\ (100\ MHz; \text{CDCl}_3): \delta\ 25.3, 28.0, 28.3, 29.3, 32.8, 33.8, 34.0, 37.2, 39.7, 71.3, 81.8**
**6.4 Experimental Procedures**

**N-hexyloxymethyl-1-adamantane-1-deoxy-nojirimycin (260).**

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 1.25-1.34 (m, 2H, H-9), 1.34-1.44 (m, 2H, H-10), 1.46-1.60 (m, 10H, 3x CH$_2$Ada, H-8,11), 1.64-1.79 (m, 6H, 3x CH$_2$Ada). 1.96 (br. s, 3H, 3x CHAda). 2.20 (d, $J$ = 9.33, 1H, H-5), 2.27 (t, $J$ = 10.87, 1H, H-1a), 2.59-2.67 (m, 1H, H-1b). 2.77-2.84 (m, 1H, H-1b), 2.97 (s, 2H, H-13). 2.97 (s, 2H, H-13). 3.03 (dd, $J$ = 5.23, 8.90 Hz, 1H, H-4). 3.72 (d, $J$ = 6.4 Hz, H-4). 3.72 (dd, $J$ = 4.47, 11.17 Hz, 1H, H-1). 3.81-3.92 (m, 2H, H-6). 13C NMR (100 MHz; CDCl$_3$): $\delta$ 23.81, 25.96, 27.18, 28.18, 33.96, 37.06, 39.57, 52.48, 56.06, 57.81, 65.39, 69.13, 70.44, 71.51, 78.89, 81.86. HR-MS [QTOF, MH$^+$] m/z calculated for C$_{23}$H$_{42}$NO$_5$ 412.30575, found 412.30561. [$\alpha$]$_D^{20}$ = - 9.17 (c = 1.22, CHCl$_3$).

**N-hexyloxymethyl-1-adamantane-lido-1-deoxy-nojirimycin (261).**

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 1.31-1.48 (m, 4H, H-9,10), 1.46-1.64 (m, 10H, 3x CH$_2$Ada, H-8,11), 1.67-1.83 (m, 6H, 3x CH$_2$Ada), 1.98 (br. s, 3H, 3x CHAda). 2.54-2.71 (m, 2H, H-1a,7a), 2.73-2.84 (m, 2H, H-7b,1b), 2.99 (s, 2H, H-13). 3.05 (dd, $J$ = 5.23, 8.90 Hz, 1H, H-4). 3.80-3.89 (m, 2H, H-6). 13C NMR (100 MHz; CDCl$_3$): $\delta$ 27.23, 28.19, 28.67, 29.78, 30.64, 33.96, 37.06, 39.57, 52.48, 56.06, 57.81, 65.39, 69.13, 70.44, 71.51, 78.89, 81.86. HR-MS [QTOF, MH$^+$] m/z calculated for C$_{23}$H$_{42}$NO$_5$ 412.30575, found 412.30562. [$\alpha$]$_D^{20}$ = - 5.44 (c = 0.59, CHCl$_3$).

**2-adamantanemethanol (264).**

A dry solution of 2-adamantanone (58, 7.5 g, 50 mmol) in Et$_2$O (0.2M) was used in general procedure E. After work-up procedures, the residue was subjected to silica gel FCC (PE/Et$_2$O, 1:0 $\rightarrow$ 0:1) to provide the title compound in 54% yield over three steps. $R_F$ = 0.2 (PE/Et$_2$O, 9:1, colours purple with molybdenum visualizing reagent). M.p = 99-100$^\circ$C. $^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 1.53-1.87 (m, 16H, 5x CH$_2$-Ada, 5x CH - Ada, OH), 3.72 (dd, 2H, $J$ = 11.0, 5.3 Hz, 1H, H-5). 3.80-3.89 (m, 2H, H-6). 13C NMR (100 MHz; CDCl$_3$): $\delta$ 27.23, 28.19, 28.67, 29.78, 30.64, 33.96, 37.06, 39.57, 52.48, 56.06, 57.81, 65.39, 69.13, 70.44, 71.51, 78.89, 81.86. GC analysis: Rt 4.80 min, 130$^\circ$C $\rightarrow$ 200$^\circ$C, gradient 10$^\circ$C/min.

**2-(((5-(benzyloxy)pentyl)oxy)methyl)adamantane (265).**

A dry and cooled (0$^\circ$C) solution of alcohol 264 (0.83 g, 5.0 mmol) in DMF (0.1M) was used in general procedure B. After extractive work-up the reaction mixture was subjected to silica gel FCC (PE/Et$_2$O, 1:0 $\rightarrow$ 0:1) to provide the title compound in 57% yield. $^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 0.85-0.97 (m, 2H, H-14), 1.12-2.05 (m, 20H, 5x CH$_2$ - Ada, 5x CH - Ada, OH). 3.72 (d, 2H, $J$ = 11.4, H11). 13H NMR (100 MHz; CDCl$_3$): $\delta$ 27.29, 28.4, 29.2, 31.9 38.1, 38.8, 47.1, 65.0. GC analysis: Rt 4.80 min, 130$^\circ$C $\rightarrow$ 200$^\circ$C, gradient 10$^\circ$C/min.
5-(adamantan-2-ylmethoxy)pentan-1-ol (266).
A solution of benzyl ether 265 (2.8 mmol, 0.96 g) in EtOAc (0.1 M) was depleted from oxygen by sonification under an argon atmosphere. 5 mol% Pd/C (10 wt%) was added and the resulting mixture was stirred under an H₂ atmosphere for 12 h. The reaction mixture was filtered over a pad of Celite and the residue was rinsed with EtOAc. The volatiles were removed to provide the crude title compound which was used in the next step.

\[ \text{1}^1\text{H NMR (400 MHz; CDCl}_3\text{): } \delta 1.33-1.43 \text{ (m, 2H, H}_{14}\text{), 1.46-1.98 (m, 20H, 5x CH}_2\text{- Ada, 5x CH- Ada, H}_{13,15}\text{), 2.80 (br. s, 1H, OH), 3.40 (t, 2H, J = 6.5, H}_{12}\text{), 3.46 (d, 2H, J = 7.3, H}_{11}\text{), 3.57 (t, 2H, J = 6.6, H}_{16}.} \]

2-(((5-bromopentyl)oxy)methyl) adamantane (244).
To a dry and cooled (0°C) solution of crude alcohol 266 (0.70 g, ~ 2.8 mmol) in CH₂Cl₂ (0.1 M) were subsequently added PPh₃ (1,5 g, 5.6 mmol) and CBr₄ (1.85 g, 5.6 mmol). The resulting reaction mixture was stirred for 2 h at 0°C after which TLC analysis revealed complete consumption of the starting material. Celite (~ 5 g) was added to the reaction mixture and the volatiles were removed. The residue was subjected to silica gel FCC (PE/Et₂O 1:0 → 1:1) to provide the title compound in 75% yield over two steps.

\[ \text{1}^3\text{C NMR (100 MHz; CDCl}_3\text{): } \delta 24.8, 27.9, 28.3, 28.8, 29.5, 32.0, 32.5, 33.6, 38.2, 38.6, 38.8, 44.1, 70.6, 73.1 \]

N-pentyloxymethyl-2-adamantane-1-deoxy-nojirimycin (309).
\[ \text{1}^1\text{H NMR (400 MHz; CDCl}_3\text{): } \delta 1.30-1.42 \text{ (m, 2H, H}_{9}\text{), 1.48-1.68 (m, 6H, H-8,10, CH}_2\text{Ada), 1.76-1.98 (m, 12H, 4x CH}_2\text{Ada, 4x CHAda), 2.00 (t, J = 6.93, 1H, H-13 (Ada)), 2.13 (dt, J = 2.67, 9.54 Hz, 1H, H-5), 2.20 (t, J = 10.85, 1H, H-1a), 2.55-2.64 (m, 1H, H-7a), 2.77-2.86 (m, 1H, H-7b), 3.01 (dd, J = 4.88, 11.14 Hz, 1H, H-1b), 3.16 (t, J = 9.06, 1H, H-3), 3.35-3.41 (m, 1H, H-4), 3.44-3.53 (3H, H-11,2), 3.55 (d, J = 7.13 Hz, 2H, H-12), 3.83-3.91 (m, 2H, H-6).} \]

N-pentyloxymethyl-2-adamantane-Lido-1-deoxy-nojirimycin (310).
\[ \text{1}^1\text{H NMR (400 MHz; CDCl}_3\text{): } \delta 1.35-1.46 \text{ (m, 2H, H-9), 1.49-1.66 (m, 6H, H-8,10, CH}_2\text{Ada), 1.78-1.95 (m, 12H, 4x CH}_2\text{Ada, 4x CHAda), 2.00 (t, J = 7.23, 1H, H-13 (Ada)), 2.56-2.71 (m, 2H, H-7a,1a), 2.74-2.84 (m,2H, H-7b,1b), 3.05 (dd, J = 5.27, 10.87 Hz, 1H, H-5), 3.40 (t, J = 8.48, 1H, H-3), 3.48 (t, J = 5.56 Hz, 2H, H-11), 3.51-3.59 (3H, H-11,2), 3.72 (dd, J = 5.21,
8.85 Hz, H-4), 3.80-3.90 (m, 2H, H-6). $^{13}$C NMR (100 MHz; CDCl$_3$): δ 24.98, 28.4, 29.44, 29.85, 30.59, 30.93, 33.01, 39.35, 40.06, 45.52, 52.82, 55.54, 57.62, 64.30, 71.26, 71.93, 72.07, 72.78, 74.22, 75.97. LC/MS analysis: Rt 6.53 min (linear gradient 0-90% B), ES (ESI): m/z = 398.3 [M + H]$^+$, 795.6 [2M + H]$^+$. $[\alpha]_{D}^{20} = -8.64$ (c = 0.65, CHCl$_3$).

adamantan-2-one oxime (267). To a dry solution of 2-adamantanone (311, 1.5 g, 10 mmol) in pyridine (0.3 M) was added NH$_2$OH·HCl (1.4 g, 20 mmol) and the resulting reaction mixture was stirred for 4 hours at RT. Upon completion of the reaction, as judged by TLC analysis, the reaction mixture was concentrated and the residue was dissolved in EtOAc and transferred to a separatory funnel. The reaction mixture was washed with 1 M aq. HCl and the aqueous layer was back extracted with EtOAc. The combined organic layer was subsequently washed with H$_2$O, sat. aq. NaHCO$_3$, brine, dried (MgSO$_4$), filtered and concentrated. The residue was subjected to silica gel FCC (PE/EtOAc, 1:0 → 3:1) to provide the title compound in 85% yield as white crystals. $R_F = 0.5$ (PE/EtOAc, 3:1). $^1$H NMR (400 MHz, CDCl$_3$): δ 1.82-1.99 (m, 12H, 5x CH$_2$ - Ada, 2x CH - Ada), 2.58 (s, 1H, H3), 3.58 (s, 1H, H1), 9.23 (br. s, 1H, OH). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 28.0, 28.9, 36.3, 36.6, 37.6, 39.0, 167.0.

adamantan-2-one O-(5-(trityloxy)pentyl) oxime (268). To a dry and cooled (0°C) solution of oxime 267 (0.50 g, 3.0 mmol) in THF (0.1 M) was added NaH (0.18 g, 4.5 mmol) and the resulting reaction mixture was stirred for 30 min at RT. Subsequently, a catalytic amount of TBAI and a solution of 5-(trityloxy)pentyl p-toluenesulfonate (1.65 g, 3.3 mmol) in DMF (1 M) were added to the reaction mixture and stirring was continued for 20 h at RT. Upon completion of the reaction, as judged by TLC analysis, excess NaH was quenched by the addition of sat aq. NH$_4$Cl at 0°C. The the reaction mixture was diluted with H$_2$O and extracted with Et$_2$O (5x). The combined ethereal layer was subsequently washed with H$_2$O, brine, dried (MgSO$_4$), filtered and concentrate. The residue was subjected to silica gel FCC (PE/Tol, 1:0 → 0:1) to give the title compound in 67% yield as a white solid. $R_F = 0.8$ (PE/EtOAc, 19:1). $^1$H NMR (400 MHz, CDCl$_3$): δ 1.43-1.49 (m, 2H, H13), 1.58-1.68 (m, 4H, H12, H14), 1.75-1.92 (m, 10H, H4, H6, H8-10), 1.95 (s, 2H, H5, H7), 2.53 (s, 1H, H3), 3.06 (t, J = 6.4, 2H, H15), 3.49 (s, 1H, H1), 3.99 (t, J = 6.4, 2H, H11), 7.17-7.28 (m, 9H, Har), 7.43-7.45 (m, 6H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 22.9, 27.9, 29.0, 29.6, 30.0, 36.3, 36.6, 37.6, 39.0, 63.6, 73.0, 126.9, 127.8, 144.5, 166.4. HRMS: found 516.2871 [M+Na]$^+$, calculated for [C$_{34}$H$_{39}$NO$_2$Na]$^+$ 516.2873.

adamantan-2-one O-(5-hydroxypentyl) oxime (269). To a dry solution of trityl ether 268 (1.0 g, 2.0 mmol) in a mixture of MeOH/toluene (0.15 M, 1:1, v/v) was added BF$_3$·OEt$_2$ (0.88 ml, 50 wt% solution in Et$_2$O). The resulting reaction mixture was stirred for 1.5 h at RT. Upon completion of the reaction, as judged by TLC analysis, the reaction mixture was diluted with EtOAc
and subsequently washed with sat. aq. NaHCO₃, dried (MgSO₄), filtered and concentrated. The residue was subjected to silica gel FCC (PE/EtOAc, 1:0 → 1:1) to provide the title compound in 89% yield as a colourless oil. \( R_F = 0.1 \) (PE/EtOAc, 9:1). \(^1\)H NMR (400 MHz, CDCl₃): δ 1.42-1.48 (m, 2H, H13), 1.58-1.69 (m, 4H, H12, H14), 1.78-1.98 (m, 12H, H4-10), 2.54 (s, 1H, H3), 3.49 (s, 1H, H1), 3.65 (t, \( J = 6.4 \), 2H, H15), 4.01 (t, \( J = 6.6 \), 2H, H11). \(^1\)3C NMR (100 MHz, CDCl₃): δ 22.2, 27.8, 28.8, 29.6, 32.5, 36.2, 36.5, 37.6, 39.0, 62.8, 72.8, 166.7. HRMS: found 252.1958 [M+H]⁺, calculated for [C₁₅H₂₅NO⁺H]⁺ 252.1958.

**adamantan-2-one O-(5-bromopentyl) oxime (245).** A dry and cooled (0°C) solution of alcohol 269 (0.4 g, 1.6 mmol) in CH₂Cl₂ (0.1M) was brominated according to general procedure D. The residue was subjected to silica gel FCC (PE/Tol 1:0 → 0:1) to provide the title compound in 56% yield as a colourless oil. \( R_F = 0.7 \) (PE/EtOAc, 9:1). \(^1\)H NMR (400 MHz, CDCl₃): δ 1.51 (s, 2H, H13). 1.65-1.66 (m, 2H, H12), 1.78-2.03 (m, 14H, H4-10, H14), 2.53 (s, 1H, H3), 3.41 (t, \( J = 1.6 \), 2H, H15), 3.49 (s, 1H, H1), 4.00 (t, \( J = 1.2 \), 2H, H11). \(^1\)3C NMR (100 MHz, CDCl₃): δ 24.5, 27.6, 28.0, 29.3, 32.3, 33.4, 35.9, 36.2, 37.3, 38.7, 72.3, 166.1. HRMS: found [M+H]⁺ 314.1116, calculated for [C₁₅H₂₅BrNO⁺H]⁺ 314.1114.

**4-azatricyclo[4.3.1.1³,₈]undecan-5-one (270).** A dry solution of oxime (0.33 g, 2.0 mmol) in cyclohexane (0.2M) was heated to reflux (90°C). Next, AlCl₃ (0.67 g, 5.0 mmol) was added portion wise and the reaction mixture was allowed to stir under reflux for 30 min. The reaction mixture was poured in to ice-cold H₂O and subsequently extracted with CHCl₃ (5x). The combined organic layer was dried (MgSO₄), filtered and concentrated. The residue was subjected to silica gel FCC (EtOAc: MeOH, 1:0 → 9:1 with 5 vol% NH₄OH) to provide the title compound in 85% yield as off-white crystals. \( R_F = 0.1 \) (PE/EtOAc, 1:9). \(^1\)H NMR (400 MHz; CDCl₃): δ 1.73 (br. s, 2H, H7), 1.80-1.98 (m, 8H, H5,6,8, 10, 11), 2.10 ( br. s, 2H, H9), 2.66 (dd, \( J = 1.2 \), 4.8, 1H, H1), 3.33 (d, \( J = 2.4 \), 1H, H4), 7.82 (s, 1H, NH). \(^1\)3C NMR (100 MHz; CDCl₃): δ 26.4, 30.0, 34.0, 35.6, 41.0, 44.5, 182.0.

**4-(5-(trityloxy)pentyl)-4-azatricyclo[4.3.1.1³,₈]undecan-5-one (271).** To a dry and cooled (0°C) solution of lactam 270 (2.0 g, 12.1 mmol) in THF (0.4M) was added NaH (0.728g, 60 wt% dispersion in mineral oil, 18.2 mmol) and the resulting suspension was allowed to stir for 30 minutes at RT. Next, a solution of 5-(trityloxy)pentyl p-toluensulfonate (9.1 g, 18.2 mmol) in DMF (10M) and a catalytic amount of TBAI were added to the reaction mixture and stirring was continued for 20 h at RT. Upon completion of the reaction, as judged by TLC analysis, the reaction mixture was poured into ice-cold sat. aq. NH₄Cl and extracted thrice with CH₂Cl₂. The combined organic layer was subsequently washed with H₂O, brine, dried (MgSO₄), filtered and concentrated. The residue was subjected to silica gel FCC (PE/EtOAc, 1:1 → 1:2 + 5vol%
Et$_3$N) to provide the title compound in 64% yield as a white solid. 

$R_F = 0.7$ (PE/EtOAc, 1:4). 

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 1.38-1.49 (m, 4H, H13,14), 1.62-1.66 (m, 2H, H15), 1.69 (br. s, 2H, H7), 1.75-1.92 (m, 8H, H5, H9-11), 2.02 (s, 2H, H6,8), 2.84 (br. s, 1H, H1), 3.04 (t, $J$= 6.6, 2H, H16), 3.32-3.36 (m, 3H, H4,12), 7.20-7.30 (m, 9H, Har), 7.42-7.44 (m, 6H, Har). 

$^{13}$C NMR (100 MHz; CDCl$_3$): $\delta$ 23.9, 26.5, 28.1, 30.1, 31.3, 34.7, 36.0, 42.7, 49.0, 53.0, 63.6, 126.9, 127.8, 128.8, 144.6, 178.6. HRMS: found 516.2871 [M+Na]$^+$, calculated for [C$_{34}$H$_{39}$NO$^+$2Na]$^+$ 516.2873.

**4-(5-hydroxypentyl)-4-azatricyclo[4.3.1.13,8]undecan-5-one (272).**

To a dry solution of trityl ether 271 (0.49 g, 1.0 mmol) in a mixture of MeOH/toluene (0.15 M, 1:1, v/v) was added BF$_3$·OEt$_2$ (0.44 ml, 50 wt% solution in Et$_2$O). The resulting reaction mixture was stirred for 1.5 h at RT. Upon completion of the reaction, as judged by TLC analysis, the reaction mixture was diluted with EtOAc and subsequently washed with sat. aq. NaHCO$_3$, dried (MgSO$_4$), filtered and concentrated. The residue was subjected to silica gel FCC (EtOAc/MeOH, 1:0 → 9:1 + 5 vol% NH$_4$OH) to provide the title compound in near quantitative yield as a colourless oil. $R_F = 0.1$ (PE/EtOAc, 1:19). 

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 1.38-1.36 (m, 2H, H13), 1.52-1.60 (m, 4H, H14,15), 1.69 (s, 2H, H7), 1.77-1.95 (m, 8H, H5, H9-11, H-15), 2.04 (br. s, 2H, H6,8), 2.84 (s, 1H, H1), 3.36-3.38 (m, 3H, H4,16), 3.63 (s , 2H, H12). 

$^{13}$C NMR (100 MHz; CDCl$_3$): $\delta$ 22.8, 26.2, 27.6, 31.0, 32.3, 34.4, 35.7, 42.4, 48.7, 52.9, 62.4, 178.6. HRMS: found 252.1958 [M+H]$^+$, calculated for [C$_{15}$H$_{25}$NO$^+$H]$^+$ 252,1958.

**4-(5-bromopentyl)-4-azatricyclo[4.3.1.13,8]undecan-5-one (246).**

A dry solution of 272 (0.25 g, 1.0 mmol) in dry CH$_2$Cl$_2$ (01M) was brominated according to general procedure D. The residue was subjected to silica gel FCC (PE/EtOAc/MeOH, 1:0 → 9:1 + 5 vol% NH$_4$OH) to provide the title compound in 85% yield as a colourless oil. $R_F = 0.7$ (PE/EtOAc, 1:4). 

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 1.38-1.36 (m, 2H, H13), 1.52-1.60 (m, 4H, H14,15), 1.69 (s, 2H, H7), 1.77-1.95 (m, 8H, H5, H9-11, H-15), 2.04 (br. s, 2H, H6,8), 2.84 (s, 1H, H1), 3.36-3.38 (m, 3H, H4,16), 3.63 (s , 2H, H12). 

$^{13}$C NMR (100 MHz; CDCl$_3$): $\delta$ 25.2, 26.9, 26.2, 30.9, 32.3, 33.6, 34.2, 35.6, 42.2, 48.5, 52.7, 178.3. HRMS: found 314.1117 [M+H]$^+$, calculated for [C$_{15}$H$_{23}$BrNO$^+$H]$^+$ 314,1114.

**1-bromoadamantane (24).**

Adamantane (1, 20 g, 0.15 mol) was coevaporated trice with toluene and dissolved in neat Br$_2$ (3M) at 0°C. Next, H$_2$O (1.3 ml) was added and the reaction mixture was stirred for 1 h at 0°C. The reaction mixture was diluted with CHCl$_3$ and poured in to crushed ice (~0.5 kg). Excess bromine was depleted by the addition of NaHSO$_3$ (s) to the well stirred dark red/brown suspension. The resulting yellowish suspension was extracted transferred to a separatory funnel and the organic layer was collected. The aqueous layer was extracted twice with CHCl$_3$ and the combined organic layer was dried
6. Diamondoids in iminosugar based glucosylceramide metabolism modulators

(DMSO4), filtered and concentrated. The residue was purified by crystallization from MeOH, to provide the title compound in 26% yield as colourless needles. Mp. 118.0-119.9°C. 1H NMR (400 MHz; CDCl3): δ 1.70 (br. s, 6H, 3x CH2- Ada), 2.11 (br. s, 3H, 3x CH -Ada), 2.33 (br. s, 6H, 3x CH2 - Ada). 13C NMR (100 MHz, CDCl3): δ 32.6, 35.5, 49.3, 66.7. GC-analysis: RT = 4.43 min (150°C → 300°C, gradient 10°C/min).

**di-(adamant-1-yl)methanone (247).**

Magnesium turnings (4.5 g) were heated in the presence of a iodine crystal under an argon atmosphere. Next, a dry solution of bromide 24 (2.2 g, 10 mmol) in Et2O (0.5M) was added (NO mechanical stirring) and the reaction mixture was heated to reflux for 12 h. The reaction mixture containing the organomagnesium derivative was transferred dropwise to a dry and cooled (0°C) solution of commercially available 1-adamantanecarboxylic acid chloride (1.0 g, 5 mmol) in Et2O (0.5M), prepared in the presence of CuBr (0.25 g, 1.7 mmol). The resulting reaction mixture was stirred for 12 h at RT and excess Grignard reagent was quenched by the addition of H2O. The reaction mixture was diluted with 1M aq. HCl and extracted twice with Et2O. The combined ethereal layer was subsequently washed with sat. aq. NaHCO3, brine, dried (MgSO4), filtered and concentrated. The residue was subjected to silica gel FCC (100% Tol) to provide the title compound in 25% yield as an off white solid. Rf = 0.4 (100% Tol). M.p. 183-186°C (lit. 185°C). 1H NMR (400 MHz; CDCl3): δ 1.61-1.77 (m, 12H, 6x CH2- Ada), 1.83-1.94 (m, 12H, 6x CH2-Ada), 2.01 (br. s, 6H, 6x CH -Ada). 13C NMR (100 MHz, CDCl3): δ 28.0, 36.5, 38.8, 40.5, 217.0. GC-analysis: RT = 10.35 min (150°C → 300°C, gradient 10°C/min).

**di-adamantan-1-yl)methanol (279)**

To a dry and cooled (0/degreeC) solution of 312 (100 mg, 0.33 mmol) in Et2O (0.1M) was added LiAlH4 (0.2 ml, 4M in Et2O). the resulting reaction mixture was stirred for 12 h at RT. Excess LiAlH4 was quenched by the careful addition of EtOAc at 0°C. The reaction mixture was diluted with 1M aq. HCl and extracted twice with Et2O. The combined ethereal solution was subsequently washed with sat. aq. NaHCO3, brine, dried (MgSO4), filtered and concentrated. The residue was purified by silica gel FCC (100% Tol) to provide the title compound in near quantitative yield. Rf = 0.3 (100% Tol). 1H NMR (400 MHz; CDCl3): δ 1.42 (d, 1H, J = 5.8, OH), 1.69-1.74 (m, 12H, 6x CH2 - Ada), 1.76-1.88 (m, 12H, 6x CH2-Ada), 1.95-2.03 (br. s, 6H, 6x CH -Ada), 2.59 9d, 1H, J = 4.7, CH). 13C NMR (100 MHz, CDCl3): δ 28.8, 29.7, 37.2, 40.1, 86.5.

**1,1’-((5-(trityloxy)pentyl)oxy)methylene)bis(adamantane) (280).**

A dry solution of 279 (67 mg, 0.22 mmol) in DMF (0.1M) was used in general procedure B with 1.5 eq. 5-(trityloxy)pentyl p-toluenesulfonate. The residue was purified by silica gel FCC (100% Tol, isocratic) to provide the title compound in 45% yield. 1H NMR (400 MHz; CDCl3): δ 1.46-1.57 (m, 2H, H3), 1.58-1.67
5-hydroxyadamantan-2-one (283).
Commercially available 2-adamantanone (58, 12.0 g, 80.0 mmol) was dissolved in concentrated HNO₃ (0.8M) at 10-15°C. The resulting reaction mixture was carefully heated up to 60°C and stirring continued for 2 h. The reaction mixture was allowed to cool down to RT and was poured in to crushed ice (~ 0.25 kg) and stirred vigorously for 30 min at 0°C. The resulting white precipitate was collected by filtration and subsequently suspended in H₂O (4M). The suspension was acidified with concentrated H₂SO₄ (7.5 ml) and heated up 60°C. Next, the resulting clear solution was allowed to cool down to RT and was subsequently poured in to crushed ice (~ 0.25 kg) and neutralized with a 30% aq. NaOH solution. The reaction mixture was extracted with CHCl₃ (3x). The combined organic layer was subsequently washed with brine, dried (MgSO₄), filtered and concentrated to provide the crude product. After crystallization from toluene, the title compound was isolated in 52% yield. Spectral data correspond to that reported in literature.⁶¹

methyl 4-oxoadamantane-1-carboxylate (284).
A solution of 283 (0.5 g, 3.0 mmol) in dry formic acid (2M) was dropwise added to 10 ml oleum (30%a). The resulting reaction mixture was stirred for 1 h at 60°C. The reaction mixture was cooled to 0°C and subsequently diluted with MeOH (0.1M) and stirring was continued for 12 h at RT. The reaction mixture was poured in to crushed ice (~ 0.25 kg) and subsequently extracted three times with CHCl₃. The combined organic layer was subsequently washed with brine, dried (MgSO₄), filtered and concentrated. The residue was purified by silica gel FCC (PE/Et₂O, 0:1 → 1:0) to give the title compound in 56% yield as a white solid.⁶¹ Rf=0.5 (PE/Et₂O, 2:3). Spectral data correspond to that reported in literature.⁶¹

spiro[adamantane-2,2’-[1,3]dioxolan]-5-ylmethanol (285).
To a dry solution of ester (6.5 g, 31.3 mmol) in toluene (0.1M) were subsequently added 1,2-ethanediol (15 ml, 0.63 mol) and a catalytic amount of p-TsOH. The resulting reaction mixture was heated to reflux and stirred for 12 h. The reaction mixture was allowed to cool down to RT and was neutralize by the addition of Et₃N. The reaction mixture was concentrated and coevaporated trice with toluene and the residue was dissolved in THF (0.1M). Next, LiAlH₄ (15.6 ml, 4M in THF) was added and the resulting reaction mixture was stirred for 2 h at RT. Excess LiAlH₄ was quenched at 0°C.

⁶¹Oleum is excess sulfur trioxide dissolved in sulfuric acid
⁶¹Oleum was replaced by concentrated H₂SO₄ for large scale preparations to give the product in 33% yield.
by the addition of EtOH and the reaction mixture was diluted with 1M aq. HCl and extracted five times with Et₂O. The combined ethereal layer was subsequently washed with sat. aq. NaHCO₃, brine, dried (Na₂SO₄), filtered and concentrated. The residue was purified by silica gel FCC (PE/Et₂O, 2:1 → 1:2) to give the title compound in 72% yield as a white solid. R_F =0.6 (PE/Et₂O, 2:3). ¹H NMR (400 MHz; CDCl₃): δ 1.41-1.97 (m, 14H, 3x CH, 5x CH₂ - Ada, OH), 3.20 (s, 2H, H13), 3.94 (s, 4H, H11,12). ¹³C NMR (100 MHz; CDCl₃): δ 26.8, 33.7, 34.4, 36.2, 36.3, 38.5, 64.2, 72.7, 111.3. [M+H]⁺ 225.3 (calc. for [C_{13}H_{20}O₃]+H⁺ 225.14)

5-(((5-(trityloxy)pentyl)oxy)methyl)spiro[adamantane-2,2’-[1,3]dioxolane] (286).
A dry solution of 285 (3.0 g, 12.5 mmol) in DMF (0.1M) was alkylated according to general procedure B. After work-up procedures, the residue was purified over silica gel FCC (Tol/Et₂O, 1:0 → 1:1) to provide the title compound in 65% yield as a pale yellow oil. R_F =0.9 (PE/Et₂O, 2:3). ¹H NMR (400 MHz; CDCl₃): δ 1.58-2.16 (m, 19H, 3x CH, 5x CH₂-Ada, H15-17), 3.09 (s, 2H, H13), 3.18 (t, 2H, J= 6.5, H14), 3.47 (t, 2H, J= 5.4, H18), 4.03 (s, 4H, H11,12), 7.19-7.62 (m, 15H, Har). ¹³C NMR (100 MHz; CDCl₃): δ 23.1, 27.1, 29.6, 30.0, 33.4, 34.5, 36.4, 37.1, 39.3, 63.6, 64.3, 71.6, 81.0, 86.4, 111.5, 127.2, 128.8, 129.7, 144.6.

5-(((5-hydroxypentyl)oxy)methyl)adamantan-2-one (287).
To a dry solution of 286 (0.5 g, 0.9 mmol) in a mixture of THF/H₂O (5:1, v/v) was acidified to pH =1 by the addition of CSA. The resulting reaction mixture was stirred for 12 h at 60°C after which the mixture was allowed cool down to RT. Next, the reaction mixture was diluted with sat. aq. NaHCO₃ and extracted trice with EtOAc. The combined organic layer was subsequently washed with brine, dried (MgSO₄), filtered and concentrated. The residue was purified by silica gel FCC (PE/EtOAc, 1:0 → 0:1) to provide the title compound in 96% yield. R_F =0.2 (100% Et₂O). ¹H NMR (400 MHz; CDCl₃): δ 1.28-1.40 (m, 2H, H14), 1.45-1.59 (m, 4H, H13,15), 1.62-1.83 (m, 6H, H6,8,10), 1.84-1.98 (m, 4H, H4,9), 2.02-2.09 (m, 1H, H7), 2.42-2.51 (br. s, 2H, H1,3), 2.65 (br. s, OH), 2.99 (s, 2H, H11), 3.32 (t, 2H, J= 6.4, H12), 3.55 (t, 2H, J= 6.6, H16). ¹³C NMR (100 MHz; CDCl₃): δ 22.2, 27.2, 29.1, 32.2, 33.9, 38.2, 38.7, 40.6, 45.9, 62.3, 71.3, 79.4.

5-(((5-((tert-butyldiphenylsilyl)oxy)pentyl)oxy)methyl)adamantan-2-one (288).
To a dry solution of 287 (0.23 g, 0.86 mmol) in DMF (0.1M) were subsequently added imidazole (0.11 g, 1.29 mmol) and TBDPS-Cl (0.35 g, 1.29 mmol). The resulting reaction mixture was stirred for 12 h at RT after which TLC analysis indicated complete consumption of the starting material. The reaction mixture was diluted with CH₂Cl₂ and subsequently washed with 1M aq. HCl (2x), sat. aq. NaHCO₃, brine, dried (MgSO₄), filtered and concentrated. The residue was purified by silica gel FCC (PE/Et₂O, 1:0 → 1:1)
to give the title compound in 67% yield. \(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 1.10 (s, 9H, tBu), 1.41-1.54 (m, 2H, H14), 1.54-1.69 (m, 4H, H13,15), 1.75-1.91 (m, 6H, H6,8,10), 1.95-2.06 (m, 4H H4,9), 2.10-2.18 (br. s, 1H, H7), 2.55-2.61 (br. s, 2H, H11), 3.07 (s, 2H, H11), 3.41 (t, 2H, \(J = 6.3\), H12), 3.72 (t, 2H, \(J = 6.4\), H16), 7.37-7.50 (m, 6H, Har), 7.67-7.75 (m, 4H, Har).

\(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 19.3, 22.5, 26.9, 27.6, 29.4, 32.4, 34.2, 38.6, 39.0, 40.9, 46.2, 63.9, 71.6, 79.7, 127.7, 129.6, 134.1, 135.6.

**5-(((5-((tert-butyldiphenylsilyl)oxy)pentyl)oxy)methyl)adamantan-2-one oxime (290).**

To a dry solution of ketone 288 (0.15 g, 0.3 mmol) in pyridine (0.1 M) was added NH\(_2\)OH·HCl (0.10 g, 1.5 mmol) and the resulting reaction mixture was stirred for 4 hours at RT. Upon completion of the reaction, as judged by TLC analysis, the reaction mixture was concentrated and the residue was dissolved in EtOAc. The organic layer was collected and washed with 1M aq. HCl and the aqueous layer was back extracted with EtOAc. The combined organic layer was subsequently washed with H\(_2\)O, sat. aq. NaHCO\(_3\), brine, dried (MgSO\(_4\)), filtered and concentrated. The residue was subjected to silica gel FCC (PE/EtOAc 1:1 → 0:1) to give the title compound in 89% yield. \(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 1.10 (s, 9H, tBu), 1.42-1.48 (m, 2H, H14), 1.53-1.94 (m, 14H, 5x CH\(_2\)-Ada, H13,15), 2.11 (br. s, 1H, H7), 2.65 (br. s, 1H, H1), 3.02 (s, 2H, H11), 3.39 (t, 2H, \(J = 6.4\), H12), 3.66 (br. s, 1H, H3), 3.70 (t, 2H, \(J = 6.4\), H16), 7.32-7.47 (m, 6H, Har), 7.70-7.77 (m, 4H, Har), 8.65-8.67 (m, 1H, OH). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 19.3, 22.5, 26.9, 27.9, 28.5, 29.4, 32.4, 34.4, 36.0, 37.1, 38.5, 38.7, 39.4, 40.7, 63.9, 71.6, 80.4, 127.6, 129.6, 134.1, 135.6, 166.6. [M+H]\(^+\) 520.33 (calc. for [C\(_{32}\)H\(_{45}\)NO\(_3\)Si+H]\(^+\) 520.32).

**OH
\[\text{BnO} \quad \text{BnO} \quad \text{OBn} \quad \text{OAc} \]**

**OH
\[\text{BnO} \quad \text{BnO} \quad \text{OBn} \quad \text{OAc} \]**

(2S,3R,4R,5R)-2,3,4-tris(benzyloxy)-5,6-dihydroxyhexyl acetate (296).

Commercially available 2,3,4,6-tetra-O-benzylglucose (TBG) was converted in to the title compound according to literature procedures. \(^{187}\) \(R_F = 0.5\) (100% Et\(_2\)O) \(^1\)H NMR (400 MHz; CDCl\(_3\)): \(\delta\) 2.01 (s, 3H, CH\(_3\)), 2.27 (t, 1H, \(J = 6.3\), C6-OH), 3.34 (d, 1H, \(J = 4.1\), C5-OH), 3.66-3.73 (m, 1H, H3), 3.73-3.83 (m, 3H, H1, H2), 3.83-3.92 (m, 1H, H4), 3.95-4.04 (m, 1H, H5), 4.25 (dd, 1H, \(J = 6.0, 11.7\), H6\(\alpha\)), 4.36 (dd, 1H, \(J = 4.9, 11.7\), H6\(\beta\)), 4.60-4.77 (m, 6H, 3x CH\(_2\)-Bn), 7.24-7.45 (m, 15H, Har). \(^{13}\)C NMR (100 MHz; CDCl\(_3\)): \(\delta\) 20.9, 63.5, 63.6, 71.8, 73.3, 73.7, 73.9, 76.4, 76.8, 78.1, 128.0, 128.2, 128.5, 128.5, 137.5, 137.6, 17.8, 170.7.

**OH
\[\text{BnO} \quad \text{OH} \quad \text{OAc} \quad \text{BnO} \quad \text{Trt} \quad \text{OAc} \]**

**OH
\[\text{BnO} \quad \text{OH} \quad \text{OAc} \quad \text{BnO} \quad \text{Trt} \quad \text{OAc} \]**

(2S,3R,4R,5R)-2,3,4-tris(benzyloxy)-5-hydroxy-6-(trityloxy)hexyl acetate (297).

To a dry solution of diol 296 (3.0 g, 6.0 mmol) in pyridine (0.1M) was added Trt-Cl (2.5 g, 9.0 mmol). The resulting reaction mixture was stirred for 12 h at 50°C. The reaction mixture was concentrated to a volume of \(\sim 10\) ml and diluted with EtOAc. The reaction mixture was subsequently washed with 1M aq. HCl (2x), sat.
aq. NaHCO₃, brine, dried (MgSO₄), filtered and concentrated. The residue was purified by silica gel FCC (Tol/Et₂O, 1:0 → 9:1) to provide the title compound in 92% yield. $R_F = 0.9$ (Tol/Et₂O, 4:1). $^1$H NMR (400 MHz; CDCl₃): δ 2.02 (s, 3H, CH₃), 3.11 (br. s, 1H, OH), 3.43 (d, 2H, $J = 4.9$, H1), 3.85-3.89 (m, 2H, H3,4), 4.12-4.19 (m, 1H, H5), 4.23 (dd, 1H, $J = 6.0$, 11.9, H1α), 4.43 (dd, 1H, $J = 3.5$, 12.0, H1β), 4.49-4.77 (m, 6H, 3x CH₂-Bn), 7.04-7.80 (m, 30H, Har).

$^{13}$C NMR (100 MHz; CDCl₃): δ 20.9, 64.1, 64.8, 70.7, 72.9, 73.2, 74.3, 76.3, 77.1, 78.4, 86.7, 125.4, 127.2, 127.3, 127.7, 127.8, 128.0, 128.1, 12.2, 12.3, 128.4, 128.5, 128.8, 129.1, 137.9, 137.9, 138.1, 143.8, 170.8.

(2S,3R,4R,5R)-2,3,4-tris(benzyloxy)-6-(trityloxy)hexane-1,5-diol (298).

To a dry and cooled (0°C) solution of 297 (3.7 g, 5.0 mmol) in MeOH (0.5M) was added a catalytic amount of NaOMe (pH ~12) and the resulting reaction mixture was stirred for 30 min at 0°C. At this point, TLC analysis revealed complete consumption of the starting material and the reaction mixture was neutralized by the addition of Amberlite™ H⁺ resin. The reaction mixture was filtered and the volatiles were removed. The residue was purified by silica gel FCC (Tol/Et₂O, 1:0 → 8:2) to give the title compound in near quantitative yield. $R_F = 0.5$ (Tol/Et₂O, 4:1). $^1$H NMR (400 MHz; CDCl₃): δ 2.28 (br. s, 1H, C1-OH), 3.19 (br. s, 1H, C5-OH), 3.38 (dd, 1H, $J = 9.6$, 5.3, H1α), 3.44 (dd, 1H, $J = 9.5$, 4.3, H1β), 3.63 (dd, 1H, $J = 11.9$, 4.2, H6α), 3.78 (dd, 1H, $J = 12.0$, 3.9, H6β), 3.83-3.97 (m, 3H, H2-4), 4.10-4.18 (m, 1H, H5), 4.44-4.79 (m, 6H, 3x CH₂-Bn), 7.12-7.58 (m, 30H, Har). $^{13}$C NMR (100 MHz; CDCl₃): δ 61.8, 64.5, 70.6, 72.8, 73.0, 74.3, 76.7, 79.2, 79.4, 86.6, 127.0, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 128.7, 137.5, 137.7, 138.1, 143.7.

(2S,3R,4R,5R)-2,3,4-tris(benzyloxy)-6-(trityloxy)hexane-1,5-diol (298).

Diol 298 (3.5 g, 5.0 mmol) was subjected to the procedure as described in chapter 5 for the preparation of 2,3,4,6-tetra-O-benzyl-1-deoxynojirimycin (195, chapter 5). The title compound was isolated in 60% yield after silica gel FCC (PE/Et₂O, 1:0 → 1:1). $R_F = 0.4$ (PE/Et₂O, 2:1). $^1$H NMR (400 MHz; CDCl₃): δ 2.16 (br. s, 1H, NH), 2.65-2.76 (m, 1H, H1α), 2.82-2.94 (m, 1H, H5), 3.34 (dd, 1H, $J = 5.9$, 8.8, H6α), 3.44-3.54 (m, 2H, H1β, H4), 3.63-3.76 (m, 3H, H2,3,6β), 4.36 (d, 1H, $J = 10.6$, CH - Bn), 4.80-4.91 (m, 3H, CH, CH₂-Bn), 4.95 (d, 1H, $J = 10.8$, CH - Bn), 5.10 (d, 1H, $J = 9.5$, CH - Bn), 7.01-7.62 (m, 30H, Har). $^{13}$C NMR (100 MHz; CDCl₃): δ 48.5, 60.5, 63.6, 72.9, 75.2, 76.0, 80.4, 81.0, 86.7, 87.6, 127.2, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.6, 128.9, 138.3, 138.7, 139.0, 143.8. [M+H]+ 676.4 (calc for [C₄₆H₄₅NO₄+H]+ 676.33)

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6.4 Experimental Procedures

*N-benzyl-2,3,4-tri-O-benzyl-6-O-trityl-1-deoxynojirimycin (313)*

To a dry solution of amine 299 (6.75 g, 10.0 mmol) in DCE (0.1 M) were subsequently added 2.0 eq benzaldehyde, Na$_2$SO$_4$ (1.0 g) and 2.0 eq. NaBH(OAc)$_3$. The resulting reaction mixture was stirred under reflux for 2 h and diluted with H$_2$O. The reaction mixture was extracted thrice with CH$_2$Cl$_2$ and the combined organic layer was subsequently washed with brine, dried (Na$_2$SO$_4$), filtered and concentrated. The residue was subjected to silica gel FCC (PE/Et$_2$O, 1:0 → 4:1) to provide the title compound in 85% yield. $R_F$ = 0.9 (PE/Et$_2$O, 2:1). $^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 2.02-2.16 (m, 1H, H$_1$$\alpha$), 2.70-2.81 (m, 1H, H$_5$), 3.11-3.29 (m, 2H, H$_1$$\beta$, CH$\alpha$-N-Bn), 3.36 (dd, 1H, $J = 4.5, 10.1$, H$_6$$\alpha$), 3.66-3.90 (m, 4H, H$_2$-4, H$_6$$\beta$), 4.28 (d, 1H, $J = 13.8$, CH$\beta$-N-Bn), 4.47-5.14 (m, 6H, 3x CH$_2$-Bn), 7.08-7.72 (m, 35H, Har). $^{13}$C NMR (100 MHz; CDCl$_3$): $\delta$ 54.1, 57.5, 62.3, 66.5, 72.5, 74.8, 75.5, 78.5, 78.6, 86.8, 87.7, 126.8, 127.1, 127.4, 127.6, 127.7, 127.8, 127.9, 128.1, 128.3, 128.4, 128.5, 128.6, 128.7, 129.0, 139.4, 144.0.

*N-benzyl-2,3,4-tri-O-benzyl-1-deoxynojirimycin (300)*

To dry a solution of 313 (3.82 g, 5.0 mmol) in AcOH (0.2 M) was added 2.0 eq. triethylsilane. The resulting reaction mixture was stirred for 12 h at 50°C and diluted with toluene. The volatiles were removed and the residue was coevaporated twice with toluene. The residue was purified by silica gel FCC (PE/Et$_2$O, 1:0 → 0:1) to give the title compound in 81% yield. $^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 2.14-2.22 (m, 1H, H$_1$$\alpha$), 2.40-2.42 (m, 1H, H$_5$), 3.10-3.18 (m, 1H, H$_1$$\beta$), 3.44 (d, 1H, $J = 13.6$, H$_6$$\alpha$), 3.55-3.70 (m, 2H, H$_2$,$\beta$), 3.75-3.84 (m, 1H, H$_4$), 3.89-4.20 (m, 3H, H$_6$$\beta$, CH$_2$N-Bn), 4.53-5.16 (m, 6H, 3x CH$_2$-Bn), 7.11-7.77 (m, 20H, Har). $^{13}$C NMR (100 MHz; CDCl$_3$): $\delta$ 54.1, 56.6, 58.1, 66.0, 72.7, 75.3, 75.6, 78.0, 78.1, 86.8, 86.9, 127.5, 127.6, 127.7, 127.8, 127.9, 128.0, 128.4, 128.5, 128.6, 128.9, 129.0, 129.3, 137.3, 138.4, 138.5, 138.9.

*N-benzyl-2,3,4-tri-O-benzyl-6-deoxy-6-azido-1-deoxynojirimycin (300)*

To a dry and cooled (0°C) solution of alcohol 300 (2.61 g, 5.0 mmol) in CH$_2$Cl$_2$ (0.1 M) were subsequently added 1.5 eq. Ms-Cl and 1.5 eq. Et$_3$N. The resulting reaction mixture was stirred for 2 h at 0°C and subsequently diluted with CH$_2$Cl$_2$. The reaction mixture was subsequently washed with H$_2$O, 1M aq. HCl, sat. NaHCO$_3$, dried (MgSO$_4$), filtered and concentrated. The residue was coevaporated twice with toluene and dissolved in DMF (0.1M). Next, NaN$_3$ (1.65 g, 25 mmol) was added and the reaction mixture was stirred for 12 h at 50°C. The reaction mixture was diluted with H$_2$O and extracted five times with Et$_2$O. The combined ethereal layer was subsequently washed with H$_2$O, brine, dried (MgSO$_4$), filtered and concentrated. The residue was purified by silica gel FCC (PE/Et$_2$O, 1:0 → 0:1) to give the title compound in 71% yield. $^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 2.06-2.12 (m, 1H, H$_1$$\alpha$), 2.52 (dt, 1H, $J = 2.6, 9.2$, H$_5$), 3.16 (dd, 1H, $J = 4.28, 11.5$, H$_6$$\alpha$), 3.38 (d, 1H, $J = 13.4$, CH$\beta$-N-Bn), 3.60-3.77 (m, 4H, H$_2$,$\beta$, H$_1$$\beta$), 3.95 (dd, 1H, $J = 2.6, 13.5$, H$_6$$\beta$), 4.21 (d, 1H, CH$\alpha$-N-Bn), 4.60-5.21 (m, 6H, 3x CH$_2$-Bn), 7.15-7.77 (m, 20H, Har). $^{13}$C NMR
6. Diamondoids in iminosugar based glucosylceramide metabolism modulators

(100 MHz; CDCl₃): δ 48.5, 54.0, 57.0, 64.8, 72.6, 75.4, 75.6, 78.2, 78.8, 87.0, 127.4, 17.5, 127.7, 127.8, 127., 128.1, 128.4, 128.5, 128.5, 128.6, 128.6, 128.7, 129.0, 129.1, 129.2, 137.6, 138.4, 138.9.

6-deoxy-6-azido-1-deoxynojirimycin (294)

To a dry and cooled (-78°C) solution of azide 300 (0.54 g, 1.0 mmol) in CH₂Cl₂ (M) was added 20 eq. of BCl₃ and stirring was continued for 6 h at -78°C. Excess BCl₃ was quenched at -78°C by the addition of MeOH. The reaction mixture was allowed to warm-up to room temperature and was extracted trice with H₂O. The combined aqueous layer was concentrated and the crude material was suspended in H₂O and a few drops of MeOH were added until a clear solution was obtained. The resulting solution was applied to a column loaded with DOWEX-resin (H⁺ form). The column was rinsed with H₂O and the product was eluted with a 5% NH₄OH solution. The title compound was obtained in 51% yield. 

¹H NMR (400 MHz; CDCl₃): δ 0.09 (s, 6H, 2x CH₃), 1H NMR (400 MHz; CDCl₃): δ 2.72-2.82 (m, 1H, H₁α), 2.98-3.05 (m, 1H, H₅), 3.23-3.44 (m, 3H, H₂,3, 1β), 3.56-3.69 (m, 1H, H₄), 3.77 (dd, 1H, J = 5.8, 13.2, H₆α), 3.87 (dd, 1H, J = 2.8, 13.2, H₆β).

¹³C NMR (100 MHz; CDCl₃): δ 48.0, 49.9, 58.7, 68.5, 69.8, 77.5. [M+H]+ 189.00 (calc. for [C₆H₁₂N₄O₃]+H+ 189.09)

(4-((5-((tert-butyldimethylsilyl)oxy)pentyl)oxy)methyl)phenyl)-2,2,2-trifluoroethanone oxime (303)

Prepared according to adapted literature procedures. 

¹H NMR (400 MHz; CDCl₃): δ 0.11 (s, 6H, 2x CH₃), 0.94 (s, 9H, tBu), 1.44-1.49 (m, 2H, H₃), 1.49-1.74 (m, 4H, H₂,4), 3.52-3.57 (m, 2H, H₁), 3.65-3.7 (m, 2H, H₅), 4.58 (2x s, 2H, CH₂ - Bn), 7.39-7.53 (m, 4H, Har), 10.38 and 10.72 (s, 1H, OH).

¹³C NMR (100 MHz; CDCl₃): δ -5.3, 18.4, 22.4, 24.1, 25.4, 26.0, 26.3, 29.4, 32.4, 44.8, 47.0, 63.4, 70.7, 70.8, 72.3, 72.4, 115.2, 117.2, 118.1, 119.5, 120.0, 122.3, 125.8, 127.4, 127.5, 128.4, 128.8, 129.9, 140.4, 140.7, 146.5, 146.8. [M+Na]+ 442.2 (calc. for C₂₀H₃₂F₃NO₃SiNa+ 442.2)

(4-((5-((tert-butyldimethylsilyl)oxy)pentyl)oxy)methyl)phenyl)-2,2,2-trifluoroethanone O-tosyl oxime (304)

Prepared according to adapted literature procedures. 

¹H NMR (400 MHz; CDCl₃): δ 0.86 (s, 6H, 2x CH₃), 0.93 (s, 9H, tBu), 1.43-1.71 (m, 6H, H₂-4), 2.47-2.49 (m, 3H, CH₃), 3.50-3.56 (m, 2H, H₁), 3.64-3.67 (m, 2H, H₅), 4.55 (2x s, 2H, CH₂ - Bn), 7.29-7.48 (m, 6H, Har), 7.89-7.93 (m, 2H, Har). 

¹³C NMR (100 MHz; CDCl₃): δ -5.3, 18.4, 21.7, 22.5, 26.0, 29.5, 32.7, 63.1, 70.9, 71.0, 72.0, 72.0, 116.0, 118.8, 121.1, 123.5, 126.7, 127.0, 127.5, 127.5, 128.5, 128.9, 129.1, 129.2, 129.9, 130.3, 131.2, 131.4, 143.0, 143.2, 146.1, 146.2, 153.7, 154.0
3-(4-(((5-((tert-butyldimethylsilyl)oxy)pentyl)oxy)methyl)phenyl)-3-(trifluoromethyl)diaziridine (305)
Prepared according to adapted literature procedures.\(^{188}\) ¹H NMR (400 MHz; CDCl\(_3\)): δ 0.09 (s, 6H, 2x CH\(_3\)), 0.94 (s, 9H, tBu), 1.50-1.54 (m, 2H, H3), 1.56-1.60 (m, 2H, H4), 1.64-1.70 (m, 2H, H2), 2.23 (br. s, 1H, NH), 2.85 (br. s, 1H, NH), 3.52 (t, 2H, J = 6.4, H5), 3.66 (t, 2H, J = 6.4, H1), 4.53 (s, 2H, CH\(_2\)-Bn), 7.41 (d, 2H, J = 8.0, Har), 7.60 (d, 2H, J = 8.0, Har).\(^{13}\)C NMR (100 MHz; CDCl\(_3\)): δ -5.3, 18.4, 22.5, 26.0, 29.5, 32.7, 63.1, 65.8, 70.7, 72.2, 119.5, 122.2, 125.0, 127.7, 128.2, 130.8, 141.0.

3-(4-(((5-((tert-butyldimethylsilyl)oxy)pentyl)oxy)methyl)phenyl)-3-(trifluoromethyl)-3H-diazirine (306)
Prepared according to adapted literature procedures.\(^{188}\) ¹H NMR (400 MHz; CDCl\(_3\)): δ 0.08 (s, 6H, 2x CH\(_3\)), 0.93 (s, 9H, tBu), 1.53-1.59 (m, 2H, H3), 1.60-1.64 (m, 2H, H4), 1.66-1.70 (m, 2H, H2), 3.49 (t, 2H, J = 6.4, H5), 3.63 (t, 2H, J = 6.4, H1), 7.18 (d, 2H, J = 8.4, Har), 7.34 (d, 2H, J = 8.4, Har).\(^{13}\)C NMR (100 MHz; CDCl\(_3\)): δ -5.4, 18.3, 22.5, 25.9, 29.5, 32.6, 62.5, 63., 70.6, 72.0, 120.8, 123.5, 126.3, 127.7, 128.1, 129.2, 140.5.

5-((3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)pentan-1-ol (307)
Prepared according to adapted literature procedures.\(^{188}\) ¹H NMR (400 MHz; CDCl\(_3\)): δ 1.52-1.58 (m, 2H, H3), 1.60-1.66 (m, 4H, H2,4), 3.09 (br. s, 1H, OH), 3.46 (t, 2H, J = 6.4, H5), 3.56 (t, 2H, J = 6.4, H1), 4.48 (s, 2H, CH\(_2\)-Bn), 7.15 (d, 2H, J = 8.4, Har), 7.34 (d, 2H, J = 8.4, Har).\(^{13}\)C NMR (100 MHz; CDCl\(_3\)): δ 21.3, 28.5, 32.3, 62.3, 65.8, 70.6, 72.0, 119.3, 120.8, 123.5, 126.2, 127.7, 128.2, 140.4.

5-((4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)oxy)pentanal (295)
Prepared according to adapted literature procedures.\(^{188}\) ¹H NMR (400 MHz; CDCl\(_3\)): δ 1.42-1.94 (m, 5H, H2,3,4a), 2.32-2.79 (m, 1H, H4b), 3.46-3.54 (m, 2H, H1), 4.50 (s, 2H, CH\(_2\)-Bn), 7.22 (d, 2H, J = 8.1, Har), 7.43 (d, 2H, J = 8.3, Har), 9.69 (t, 1H, J = 1.5, CHO).\(^{13}\)C NMR (100 MHz; CDCl\(_3\)): δ 21.3, 28.8, 34.2, 70.0, 70.3, 71.5, 120.9, 123.6, 125.8, 126.4, 127.6, 141.1, 203.0.
**N-(5-((4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)oxy)pentyl-6-deoxy-6-azido-1-deoxynojirimycin (308).**

A dry solution of azide 294 (40 mg, 0.21 mmol) and aldehyde 295 (3 eq.) in MeOH (0.1 M) was acidified with AcOH to pH = 5.0. Next, Na₂SO₄ (∼1 g/mmol) was added and the resulting reaction mixture was stirred for 12 h at room temperature. Next, NaCNBH₃ (1.0 eq.) was added to the reaction mixture and stirring was continued for 12 h. The reaction mixture was filtrated and subsequently purified by preparative HPLC to give the title compound (10 mg). LC/MS analysis: Rt 6.71 min (linear gradient 0-50% B), ES (ESI): m/z = 473.07 [M + H]⁺ (calc. for [C₂₀H₂₇F₃N₆O₄+H]⁺ 473.20).

**1H NMR (400 MHz, MeOD):**

δ 1.26-1.59 (m, 6H), 1.61-1.72 (m, 2H), 2.12 (dd, J = 22.8, 11.9, 1H), 2.16-2.30 (m, 1H), 2.34-2.54 (m, 1H), 2.78 (dt, J = 13.4, 7.9, 1H), 3.02 (dd, J = 11.3, 4.9, 1H), 3.13 (t, J = 9.0, 1H), 3.40-3.61 (m, 3H), 3.67 (dd, J = 13.6, 2.9, 1H), 3.79 (dd, J = 13.5, 2.6, 1H), 4.54 (d, J = 14.3, 2H), 7.26 (d, J = 8.1, 2H), 7.47 (d, J = 8.4, 2H).

**13C NMR (100 MHz; MeOD):** δ 22.8, 23.8, 23.8, 29.17, 52.2, 56.2, 61.5, 64.4, 69.3, 70.2, 70.9, 71.5, 78.8, 126.2, 127.8, 141.1.

**N-(5-((4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)oxy)pentyl-6-[1H-1,2,3-triazol-4-yl]butyl)-5,5-difluoro-1,3,7,9-tetramethyl-5H-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-4-ium-5-uide]-1-deoxynojirimycin (292).**

To a solution of 308 (10 mg, 0.02 mmol) in tBuOH/H₂O/Tol (1:1:1, v/v/v, 1.5 ml) and 293 (3.0 eq.) were subsequently added CuSO₄ (50 µl, 100 mM in H₂O) and sodium ascorbate (50 µl, 100 mM in H₂O). The resulting reaction mixture was stirred for 12 h at room temperature. LCMS analysis indicated an incomplete reaction, however the insolubles were filtered off and the filtrate was concentrated. The residue was purified by preparative HPLC/ms to give 1 mg of the title compound and 2.5 mg of the starting material. LC/MS analysis: Rt 6.71 min (linear gradient 0-50% B), ES (ESI): m/z = 801.20 [M + H]⁺, 823.27 [M + Na]⁺, 1601.07 [2M + H]⁺ (calc. for [C₃₉H₅₀BF₃N₈O₄+H]⁺ 801.40).
7

Inhibition of β-glucocerebrosidase by \( N \)-pentyloxy-1-adamantane-1-deoxy-nojirimycinium ions

7.1 Introduction

Glycosidases are widespread in nature and selectively hydrolyze glycosidic bonds. These crucial enzymes are involved in various biological pathways including polysaccharide and glycoconjugate catabolism. The hydrolysis of glycosidic bonds proceeds with either retention or inversion of the anomic configuration. The mechanisms for glycoside hydrolysis was first proposed by Koshland in 1953\(^ {190}\) and corroborated by Withers and co-workers in 2001.\(^ {191}\) Lysosomal β-glucocerebrosidase (GBA1) hydrolyzes β-O-glucosidic bonds with retention of the anomic configuration (Scheme 7.1). This is achieved via a double displacement mechanism, and the formation of a covalent enzyme - α-glucoside via a short lived oxocarbenium-ion TS. Subsequent hydrolysis of this covalent enzyme-glucoside intermediate, via a oxocarbenium-ion TS, completes the catalytic cycle.\(^ {190–192}\) Since the discovery of the glucosidase inhibitory properties of 1-deoxynojirimycin (178, Scheme 7.2), the first stable iminosugar isolated from natural sources,\(^ {193}\) chemists and biochemists are involved in the preparation and derivatization thereof.\(^ {140}\) In the past decades \( N \)-alkylated iminosugars have emerged as a new class of highly potent inhibitors, in the treatment of several LSD.\(^ {194,195}\)

There is some controversy about the action of iminosugars with respect to their promiscuous inhibition of several glucosidases. It is widely believed that the powerful inhibition of glycosyl
7. Synthesis and evaluation of N-pentyloxymethyl-1-adamantane-1-deoxy-nojirimycinum ions

**Scheme 7.1:** Mechanism of glucosylceramide hydrolysis by β-glucocerebrosidase (GBA1).\(^{191}\)

Hydrolases by \(N\)-alkylated iminosugars, is based on their resemblance of the transition state of glycosidic bond cleavage.\(^{196-198}\) Modulators of the glucosylceramide metabolism (Scheme 7.2), \(N\)-alkylated iminosugars, can be roughly characterized as glucose analogue which are (partially) protonated at physiological pH, in the enzyme active site. These \(N\)-quaternized iminosugars therefore can act as mimics of the oxocarbenium-ion TS, where the positive charge is located at the endocyclic oxygen atom.

Another theory proposed by Butters et al.\(^{194}\) concerning the polyhydroxy piperidine \(189\), dictates that \(189\) act as a ceramide mimic (Scheme 7.2). As a consequence of this hypothesis, \(N\)-alkylated DNJ analogs bearing an additional lipophilic tail at the 2-\(O\)-position would possess an enhanced inhibitory profile with respect to \(189\). Recent findings by several groups\(^{175,199}\) disproved this theory by the synthesis and evaluation of di- or trialkylated DNJ derivatives, which resulted in weaker inhibition compared to \(189\).

In the development of potent iminosugar based inhibitors of the enzymes involved in the GlcCer metabolism, two highly potent lead compounds were identified being MZ-21 (\(190\)) and its C5 epimer MZ-31 (\(N\)-pentyloxymethyl-1-adamantane-\(L\)-ido DNJ, \(191\)). To gain insight in the mode of action of these inhibitors, it was decided to prepare \(N\)-quaternized MZ-21 and MZ-31 derivatives 314 and 315, respectively. In addition, two interesting \(N\)-spirocyclic derivative 316 and 317 were also targeted (Figure 7.1).

**Figure 7.1:** Interesting \(N\)-alkylated 1-deoxynojirimycinum ions.
Scheme 7.2: Modulation of glucosylceramide levels by N-alkylated 1-deoxynojirimycin derivatives and their apparent IC\textsubscript{50} values in micromolar (\(\mu\)M).\textsuperscript{175}

<table>
<thead>
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<th>Compound</th>
<th>R =</th>
<th>GCS</th>
<th>GBA 1</th>
<th>GBA 2</th>
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<td>DNJ (178)</td>
<td>H</td>
<td>(2 \cdot 10^3)</td>
<td>(2.4 \cdot 10^5)</td>
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</tr>
<tr>
<td>Zavesca (189)</td>
<td>Butyl</td>
<td>50</td>
<td>400</td>
<td>0.23</td>
</tr>
<tr>
<td>MZ-21 (190)</td>
<td>Pentyloxymethyl-1-adamantane</td>
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<td>0.2</td>
<td>0.001</td>
</tr>
<tr>
<td>MZ-31 (191)</td>
<td>(N)-pentyloxymethyl-1-adamantane-L-ido-1-deoxynojirimycin</td>
<td>0.1</td>
<td>2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

7.2 Results and Discussion

The preparation of quaternary ammonium salts 314 and 315 (Scheme 7.3) commenced with the synthesis of leads MZ-21 (190) and MZ-31 (191), according to earlier described procedures.\textsuperscript{175} MZ-21 (190) and MZ-31 (191) were obtained in respectively 40 and 36% overall yield, from commercially available 2,3,4,6-tetra-O-benzyl glucose (193). Next, 190 and 191 were treated with an excess of MeI, in the presence of 18-Crown-6 in a screw-cap flask in DMF. After \textit{in vacuo} evaporation of the solvent and subsequent HPLC purification, pure 314 and 315 were obtained in 47% and 34% yield, respectively.

Next, the synthesis of \(N\)-spiro derivative 316 via the sequential inter/intra molecular bis-alkylation of DNJ (178) with 1,5-dibromopentane (215) was investigated (Scheme 7.4). To this end, DNJ (178), was treated with a large excess of 215, in the presence of 18-Crown-6 and \(K_2CO_3\) in DMF. However, prolonged exposure of the reaction mixture to elevated temperatures only resulted in the formation of mono \(N\)-alkylated DNJ. The addition of TBAI or NaI to the reaction was not productive in promoting the formation of 316. Also the use of bis-mesylate 318, as the alkylating agent in the formation of 316 proved to be abortive. These setbacks led to a two-step cyclisation strategy employing reductive amination of tetra-O-benzylated DNJ (195) with aldehyde 319 followed by \(N\)-alkylation procedure to give after the final step \(N\)-spirocyclic compound 316 (Scheme 7.4). Thus, reductive amination of DNJ (178) with aldehyde 319 in EtOH at pH 5.5 (set with AcOH) and NaCNBH\textsubscript{3} gave derivative 320 in 15% yield after flash column chromatography. Optimization of the reductive amination conditions resulted in a protocol which uses DCE as the solvent, omission of acetic acid, and the employment of NaBH(OAc)\textsubscript{3} as the reductive agent. Application of the optimized reductive amination protocol yielded pure
7. Synthesis and evaluation of $N$-pentyloxymethyl-1-adamantane-1-deoxy-nojirimycinium ions

**Scheme 7.3:** One step methylation of leads MZ-21 and MZ-31.

Reagent and conditions: i) 5-bromopentyloxymethyl-1-adamantane (1.5 eq.), $K_2CO_3$ (3 eq), DMF (0.1M), 190, 76%; 191, 5%. ii) MeI (6 eq.), cat. 18-crown-6, DMF (0.1M), screw-cap vial, 80°C, 12 h, HPLC purification, 314, 47%; 315, 34%.

320 in 87%. Acid mediated detritylation of 320 provided alcohol 321, which upon an Appel reaction with PPh$_3$ and CBr$_4$ at 0°C in CH$_2$Cl$_2$, revealed the formation of intermediate 322 by HPLC analysis. The reaction mixture was diluted with DMF and stirred at 80°C for 12 h and after work-up and chromatographic purifications, ammonium salt 323 was obtained in 36% yield. Unfortunately, HPLC-analysis showed the presence of minor and inseparable contaminants.

A less elaborate, one-pot strategy was devised to obtain spiro derivative 316 by the use of bi-functional tosylate 324 (obtained in two steps from 1,5-pentanediol). Reaction of iminosugar 195 with tosylate 324, under the conditions as described for the formation of 320 using pentanal 319, showed the formation of spiro-cyclic compound 323. Surprisingly, the major side products which were identified by HPLC analysis, presumably resulted from reductive-opening of the $N$-quaternized product 323. Tweaking of the reaction conditions gave ammonium salt 323 in 84% yield, after chromatographic purification. BCl$_3$ promoted debenzylation of 323 at 0°C went to completion within one hour, as revealed by HPLC analysis of the reaction mixture. However, several purification methods, e.g. size exclusion-, alumina/silica column chromatography, ion-exchange chromatography and crystallization studies, proved to be ineffective in the isolation of 316, due to its highly polar nature. The synthesis of the L-ido derivative 317 from 2,3,4,6-tetra-$O$-benzyl-L-ido-DNJ (198) was executed analogously to the optimized synthesis of 316. $N$-spirocyclisation of 198 with aldehyde 324 proceeded efficiently and gave 325 in excellent yield. Also here, after debenzylation of 325, the isolation of $N$-spirocyclic derivative 317 was ineffective. The isolation the highly polar bis-$N$-alkylated ammonium salts 316 and 317 was abandoned. Next, the inhibitory profile of $N$-methylated MZ-21 and MZ-31 derivatives 314

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$^a$The yield of 323 is increased to 39% when the bromination is executed in refluxing CH$_3$CN.
7.2 Results and Discussion

Scheme 7.4: \(N\)-spirocyclisation of 1-deoxynojirimycin (178) and \(L\)-ido-1-deoxynojirimycin (192).

Reagent and conditions: i) 215 (10 eq.) or 318 (10 eq.), \(K_2\)CO₃ (5 eq.), cat. 18-crown-6, cat. TBAI, DMF (0.1M), 80°C; ii) 319 (3 eq.), NaBH(OAc)₃ (3 eq.), DCE (0.1M), \(Na_2\)SO₄ (∼1g/mmol), 12 h, \(\Delta\), 94%; iii) cat. pTsOH, MeOH/CH₂Cl₂ (v/v, 1:1), 12h, RT, 94%; iv) a. PPh₃ (2 eq.), CBr₄ (2 eq.), CH₂Cl₂ (0.1M), 0°C, 2 h; b. diluted with DMF (0.05M), 80°, 12h, 36%; v) 324 (3 eq.), NaBH(OAc)₃ (3 eq.), DCE (0.1M), \(Na_2\)SO₄ (∼1g/mmol), \(\Delta\), 12h, 323, 84%; 325, 97%; vi) 10 eq. BCl₃, CH₂Cl₂ (0.06M), 0°C, compounds were not isolated.

and 315 against GCS, GBA1 and GBA2 was assessed.

**Biological evaluation**

In Table 7.1 the inhibitory result of ammonium salts 314 and 315 are depicted. With respect to the \(N\)-quaternized iminosugars, in general, the \(N\)-methylation of leads MZ-21 (190) and
MZ-31 (191) is detrimental for its inhibitory profile. The N-methylated MZ-21 and MZ-31 derivatives are roughly 10 times weaker inhibitors of GCS and GBA1 compared to MZ-21 and MZ-32. The weaker inhibitory profile of N-quaternized iminosugars might result from a lower bio-availability. The ammonium salts 314 and 315 might complex to the negatively charged lipid bilayer in the GCS bio-assay. These findings suggest that ammonium salts of N-alkylated iminosugar derivatives are able to enter the enzymes active site. However, it still remains unclear whether MZ-21 and MZ-31 are protonated in the enzymes active site or that they are protonated at an earlier stage.

Table 7.1: Enzyme inhibition assay results: Apparent IC\textsubscript{50} values in micromolar (µM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>GCS Facs</th>
<th>GCS NBD</th>
<th>GBA1</th>
<th>GBA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>314</td>
<td>∼1</td>
<td>&gt;2</td>
<td>2</td>
<td>∼0.1</td>
</tr>
<tr>
<td>315</td>
<td>∼1</td>
<td>2</td>
<td>6</td>
<td>∼0.5</td>
</tr>
</tbody>
</table>

7.3 Conclusions and Outlook

N-methylated derivatives of leads MZ-21 (190) and MZ-31 (191) were prepared and tested against GCS, GBA1 and GBA2. The inhibitory profile of 314 and 315 notably resembles their non-methylated counterpart 190 and 191, in terms of selectivity. However, the prepared ammonium salts are roughly 10 times less powerful in inhibiting the aforementioned enzymes. These results suggest that the active site of these enzymes allow the inclusion of N-quaternized iminosugars and corroborates the generally accepted hypothesis that N-alkylated iminosugars act as an oxocarbenium-ion TS mimic.

To overcome the difficulties encountered during the isolation of the polar ammonium salts 316 and 317, the application of silyl-protective groups on the DNJ-core was investigated. It was envisaged that the final cleavage of for instance the O-TBS protective groups, can be accomplished under acidic conditions coupled with the extractive removal of the relative apolar silyl-reaction products. Therefore, DNJ (178) was treated with a large excess of TBS-Cl in the
Scheme 7.5: Envisaged strategy for the synthesis and isolation of extremely polar $N,N$-alkylated 1-deoxynojirimycinium ions.

Reagent and conditions: i) TBS-Tf (12 eq.), 2,6-lutidine (12 eq.), DMF (0.1 M), 65°C, 12h, 75%; ii) MeI (20 eq.), K$_2$CO$_3$ (3 eq.), cat. 18-crown-6, DMF (0.1 M), 80°C, 12 h, 35%; iii) MeOH, HCl (20 eq., 4M solution in dioxane), 12 h, RT, extractive work-up, quant.

presence of imidazole, which gave a mixture of tri- and tetra-$O$-silylated products (Scheme 7.5). Employment of the more reactive TBS-triflate in conjunction with 2,6-lutidine gave pure 2,3,4,6-tetra-$O$-TBS-DNJ (326) in 75% yield. The viability of the purification strategy was demonstrated with the synthesis of the extremely polar $N,N$-dimethyl DNJ (328). To this end, 326 was bis-$N$-methylated under the conditions as described for the methylation of MZ-21 (Scheme 7.3), with a twenty fold excess of methyl iodide. Chromatographic purification of the concentrated reaction mixture provided $O$-TBS protected ammonium salt 327 in moderate yields. The deblocking of the $O$-TBS groups was readily accomplished in dry MeOH with 20 equivalents HCl and after extractive work-up procedures, 328 was obtained in excellent yields and high purity. It was envisaged that pure $N$-spirocyclic derivatives 316 and 317 might be accessible via the developed procedure for the synthesis of 328.

7.4 Experimental Procedures

General: Solvents and reagents were obtained commercially and used as received unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere and residual water was removed from starting compounds by coevaporation with toluene (3x), unless stated otherwise. Reaction grade solvents were stored on 4Å molecular sieves or 3Å for MeOH, CH$_3$CN, and DMSO. All solvents were removed by in vacuo evaporation at $\sim$ 45°C. Reactions were monitored by TLC analysis using silica gel coated aluminium plates (0.2 mm thickness) and detection by UV absorption (254 nm) and/or by spraying with a solution of (NH$_4$)$_6$Mo$_7$O$_{24}.4$H$_2$O (25 g/L) and (NH$_4$)$_4$Ce(SO$_4$)$_4.4$H$_2$O in 10% H$_2$SO$_4$, followed by charring at $\sim$ 150°C. Visualisations of olefins and $N$-alkylated iminosugars was achieved by spraying with a solution of KMnO$_4$ (5 g/L) and K$_2$CO$_3$ (25 g/L) in H$_2$O, followed by charring at $\sim$ 150°C. Glycosides and hemiacetals were visualized by spraying with a solution of 20% H$_2$SO$_4$ in EtOH and charring at $\sim$ 150°C and for adamantane containing compounds a solution of H$_3$PMo$_{12}$O$_{40}$ (100 g/L) was used. Flash column chromatography was performed on silica gel (40-63 $\mu$m). NMR spectra were recorded on a 400/100 MHz spectrometer. Chemical shifts are given in ppm ($\delta$) relative to tetramethylsilane as internal standard for all $^1$H NMR measurements.
7. Synthesis and evaluation of \(N\)-pentyloxymethyl-1-adamantane-1-deoxy-nojirimycin ions in CDCl\(_3\) and the deuterated solvent signal for all other NMR experiments. \(^1\)H NMR peak assignments were made using COSY and HSQC experiments and coupling constants (\(J\)) are given in Hz. All \(^1\)C NMR spectra are proton decoupled. For LC/MS analysis a HPLC-system (detection simultaneously at 213 nm, 254 nm and evaporative light detection) equipped with an analytical C18 column (4.6 mm x 250 mmL, 5 \(\mu\)m particle size) in combination with buffers A: \(\text{H}_2\text{O}\), B: \(\text{CH}_3\text{CN}\), C: 1.0\% aqueous trifluoroacetic acid and coupled with an electrospray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a semi-preparative C18 column (5 \(\mu\)m C18, 10, 150 x 21.2 mm) was used. The applied buffers were A: \(\text{H}_2\text{O} + \text{trifluoroacetic acid (1% m/M)}\) and B: \(\text{CH}_3\text{CN}\). High resolution mass spectra were recorded by direct injection (2 \(\mu\)L of a 2 \(\mu\)M solution in \(\text{H}_2\text{O/CH}_3\text{CN; 50/50; v/v and 0.1% formic acid}\)) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250\(^{\circ}\)) with resolution R = 60000 at m/z = 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Optical rotations were measured on an automatic polarimeter (Sodium D-line, \(\lambda = 589\) nm).

\(N\)-pentyloxymethyl-1-adamantane-1-deoxy-nojirimycin (190).

To a dry solution of 1-deoxynojirimycin (178, 33 mg, 0.2 mmol) in DMF (0.1m) were subsequently added 5-bromopentyloxymethyl-1-adamantane (100 mg, 0.3 mmol) and \(\text{K}_2\text{CO}_3\) (82 mg, 0.6 mmol). The resulting reaction mixture was stirred overnight at 80\(^{\circ}\)C at which point TLC and LC/MS analysis indicated the formation of a more hydrophilic compound. The reaction mixture was filtered over a 100 \(\mu\)m filter and the residue was rinsed with DMF. The combined filtrates were subjected to silica gel flash chromatography (FCC, EtOAc/MeOH/N\(_4\text{OH} 100:0:0 \rightarrow 78:20:2\)) to give the title compound in 76\% yield. \(^1\)H NMR (400 MHz; \(\text{d}_4\)-methanol): \(\delta\) 3.86 (dd, 1H, \(J = 2.7, 12.1, \text{H}_6\alpha\)), 3.82 (dd, 1H, \(J = 2.7, 12.1, \text{H}_6\beta\)), 3.46 (dt, 1H, \(J = 4.9, 9.1, 10.6, \text{H}_2\)), 3.38 (t, 2H, \(J = 6.3, \text{H}_1\text{I}\)), 3.35 (dd, 1H, \(J = 9.4, \text{H}_4\)), 3.12 (dd, 1H, \(J = 9.1, \text{H}_3\)), 2.97 (dd, 1H, \(J = 4.8, 10.1, \text{H}_1\alpha\)), 2.96 (s, 2H, \(\text{H}_1\beta\)), 2.79 (m, 1H, \(\text{H}_7\alpha\)), 2.58 (m, 1H, \(\text{H}_7\beta\)), 2.17 (dd, 1H, \(J = 10.2, 10.6, \text{H}_1\beta\)), 2.10 (dt, 1H, \(J = 9.4, 2.7, \text{H}-5\)), 1.94 (s, 3H, 3x CH - Ada), 1.77-1.66 (m, 6H, 3x CH\(_2\) - Ada), 1.58 (m, 2H, \(\text{H}_1\alpha\)), 1.51-1.58 (m, 6H, 3x CH\(_2\) - Ada), 1.51 (m, 2H, \(\text{H}_8\)), 1.33 (m, 2H, \(\text{H}_9\)). \(^{13}\)C NMR (100 MHz, \(\text{d}_4\)-methanol): \(\delta\) 83.1, 80.1, 72.4, 71.4, 70.2, 67.3, 58.6, 57.1, 52.3, 40.9, 38.3, 36.0, 30.5, 29.8, 25.2, 24.8. LC/MS analysis: Rt 7.16 min (linear gradient 10-90\% B), ES (ESI): m/z = 398.27 [M+H]\(^+\).

\(N\)-pentyloxymethyl-1-adamantane-1-ido-1-deoxynojirimycin (191).

To a dry solution of L-ido-1-deoxynojirimycin (192, 57 mg, 0.35 mmol) in DMF (0.1m) were subsequently added 5-bromopentyloxymethyl-1-adamantane (0.17 g, 0.5 mmol) and \(\text{K}_2\text{CO}_3\) (0.15 g, 1.1 mmol). The resulting reaction mixture was stirred overnight at 80\(^{\circ}\)C at which point TLC and LC/MS analysis indicated the formation of a more hydrophilic compound. The reaction mixture was filtered over a 100 \(\mu\)m filter and the residue was rinsed with DMF. The combined filtrates were subjected to silica gel flash chromatography (FCC, EtOAc/MeOH/N\(_4\text{OH} 100:0:0 \rightarrow 78:20:2\)) to give the title compound in 76\% yield. \(^1\)H NMR (400 MHz; \(\text{d}_4\)-methanol): \(\delta\) 3.86 (dd, 1H, \(J = 2.7, 12.1, \text{H}_6\alpha\)), 3.82 (dd, 1H, \(J = 2.7, 12.1, \text{H}_6\beta\)), 3.46 (dt, 1H, \(J = 4.9, 9.1, 10.6, \text{H}_2\)), 3.38 (t, 2H, \(J = 6.3, \text{H}_1\text{I}\)), 3.35 (dd, 1H, \(J = 9.4, \text{H}_4\)), 3.12 (dd, 1H, \(J = 9.1, \text{H}_3\)), 2.97 (dd, 1H, \(J = 4.8, 10.1, \text{H}_1\alpha\)), 2.96 (s, 2H, \(\text{H}_1\beta\)), 2.79 (m, 1H, \(\text{H}_7\alpha\)), 2.58 (m, 1H, \(\text{H}_7\beta\)), 2.17 (dd, 1H, \(J = 10.2, 10.6, \text{H}_1\beta\)), 2.10 (dt, 1H, \(J = 9.4, 2.7, \text{H}-5\)), 1.94 (s, 3H, 3x CH - Ada), 1.77-1.66 (m, 6H, 3x CH\(_2\) - Ada), 1.58 (m, 2H, \(\text{H}_1\alpha\)), 1.51-1.58 (m, 6H, 3x CH\(_2\) - Ada), 1.51 (m, 2H, \(\text{H}_8\)), 1.33 (m, 2H, \(\text{H}_9\)). \(^{13}\)C NMR (100 MHz, \(\text{d}_4\)-methanol): \(\delta\) 83.1, 80.1, 72.4, 71.4, 70.2, 67.3, 58.6, 57.1, 52.3, 40.9, 38.3, 36.0, 30.5, 29.8, 25.2, 24.8. LC/MS analysis: Rt 7.16 min (linear gradient 10-90\% B), ES (ESI): m/z = 398.27 [M+H]\(^+\).
TLC and LC/MS analysis indicated the formation of a more hydrophobic compound. The reaction mixture was filtered over a 100 µm filter and the residue was rinsed with DMF. The combined filtrates were concentrated and subjected to silica gel FCC (EtOAc/MeOH/NH$_4$OH 100:0:0 → 78:20:2) to give the title compound in 51% yield.

$^1$H NMR (400 MHz; d$_4$-methanol): δ 3.91 (d, 2H, J = 5.1, H6), 3.86 (br. s, 1H, H3), 3.74 (br. s, 1H, H2), 3.63 (br. s, 1H, H4), 3.42 (t, 2H, J = 6.2, H11), 3.33-3.29 (m, 1H, H5), 3.25-2.94 (m, 4H, H1,7), 2.97 (s, 2H, H12), 1.95 (s, 3H, 3x CH-Ada), 1.82-1.61 (m, 10H, H8,10, 3x CH$_2$-Ada), 1.54-1.39 (m, 2H, H9).

$^{13}$C NMR (100 MHz; d$_4$-methanol): δ 83.1, 76.0, 72.4, 72.7, 71.2, 64.2, 58.6, 55.5, 52.3, 40.9, 38.3, 36.0, 30.5, 29.8, 25.2, 24.8.

LC/MS analysis: Rt 7.18 min (linear gradient 10-90% B), ES (ESI): m/z = 398.27 [M+H]$^+$. 

**N-methyl,N-pentyloxymethyl-1-adamantane-1-deoxy-nojirimycinium iodide (314).** To a dry solution of 190 (60 mg, 0.15 mmol) in DMF (0.1M) were subsequently added MeI (56 µl, 0.9 mmol), K$_2$CO$_3$ (46 mg, 0.3 mmol) and a catalytic 18-crown-6-ether (10 µl). The resulting reaction mixture was stirred overnight at 80°C at which point TLC analysis indicated the formation of a highly polar compound. The reaction mixture was filtered over a 100 µm filter and the residue was rinsed with DMF and the combined filtrates were concentrated. The residue was subjected to HPLC purifications utilizing a gradient of 32% → 36% CH$_3$CN in 0.1%aq.TFA, to furnish the title compound in 47% yield. $^1$H NMR (400 MHz; d$_4$-methanol): δ 4.18 (dd, 1H, J = 2.7, 12.1, H6α), 4.10 (dd, 1H, J = 2.7, 12.1, H6β), 3.88 (ddd, 1H, J = 9.4, H4), 3.62 (dd, 1H, J = 9.1, H3), 3.43 (dd, 1H, J = 10.6, H2), 3.85 (t, 2H, J = 6.3, H11), 3.83 (dd, 1H, J = 9.4, H4), 3.62 (dd, 1H, J = 9.1, H3), 3.43 (dd, 1H, J = 10.6, H11), 3.41 (s, 2H, H12), 3.39 (m, 1H, H7α), 3.32 (m, 1H, H7β), 3.19 (dd, 1H, J = 10.2, 10.6, H1β), 2.98 (dt, 1H, J = 9.4, 2.7, H5), 2.55 (s, 3H, N-Me), 1.94 (s, 3H, 3x CH - Ada), 1.85-1.77 (m, 6H, 3x CH$_2$ - Ada), 1.69 (m, 2H, H10), 1.54-1.58 (m, 6H, 3x CH$_2$ - Ada), 1.51 (m, 2H, H8), 1.33 (m, 2H, H9). $^{13}$C NMR (100 MHz, d$_4$-methanol): δ 83.1, 79.0, 73.6, 72.1, 68.5, 67.8, 66.2, 65.8, 47.8, 40.9, 38.3, 35.1, 30.1, 29.7, 24.4, 22.9. LC/MS analysis: Rt 6.41 min (linear gradient 10-90% B), ES (ESI): m/z = 412.27 [M+H]$^+$. 

**N-methyl,N-pentyloxymethyl-1-adamantane-1-ido-1-deoxy-nojirimycinium iodide (315).** To a dry solution of 191 (70 mg, 0.18 mmol) in DMF (0.1M) were subsequently added MeI (66 µl, 1.1 mmol), K$_2$CO$_3$ (54 mg, 0.4 mmol) and catalytic 18-crown-6-ether (10 µl). The resulting reaction mixture was stirred overnight at 80°C, at which point TLC analysis revealed the formation of a highly polar compound. The reaction mixture was filtered over a 100 µm filter and the filter cake was rinsed with DMF. The combined filtrates were concentrated and the residue was subjected to HPLC purification utilizing a gradient of 34% → 38% CH$_3$CN in aqTFA (0.1%) to furnish the title compound in 34% yield. $^1$H NMR (400 MHz; d$_4$-methanol): δ 4.18 (dd, 1H, J = 2.7, 12.1, H6α), 4.10 (dd, 1H, J = 2.7, 12.1, H6β),
7. Synthesis and evaluation of N-pentyloxymethyl-1-adamantane-1-deoxy-nojirimycinium ions

3.88 (ddd, 1H, J = 4.9, 9.1, 10.6, H2), 3.85 (t, J = 6.3 Hz, 2H, H11), 3.83 (dd, 1H, J = 9.4, 1H, H4), 3.62 (dd, J = 9.1, 1H, H3), 3.43 (dd, J = 4.8, 10.12, 1H, H1α), 3.41 (s, 2H, H-12), 3.39 (s, 2H, H-12), 3.32 (m, 1H, H-7α), 3.19 (dd, J = 10.2, 10.6, 1H, H1β), 2.98 (dt, 1H, J = 9.4, 2.7, H5), 2.55 (s, 3H, N-Me), 1.94 (s, 3H, 3x CH - Ada), 1.85-1.77 (m, 6H, 3x CH2 - Ada), 1.69 (m, 2H, CH10), 1.54-1.58 (m, 6H, 3x CH2 - Ada), 1.51 (m, 2H, H8), 1.33 (m, 2H, H9).

13C NMR (100 MHz; d4-methanol): δ 83.1, 79.0, 73.3, 72.2, 68.5, 67.8, 66.2, 65.8, 56.8, 40.9, 38.3, 35.1, 30.2, 29.7, 24.4, 23.2. LC/MS analysis: Rt 6.58 min (linear gradient 10-90% B), ES (ESI): m/z = 412.27 [M]+.

5-(trityloxy)pentanal (319).
To a dry solution of 1,5-pentanediol (0.11 l, 1.0 mol) in pyridine (0.1 M) was added triphenylmethyl chloride (70 g, 2.5 mol) and the resulting reaction mixture was stirred overnight at RT. The reaction mixture was diluted with H2O and the volatiles were removed in vacuo. The resulting slurry was dissolved in EtOAc (1 l) and washed with 0.1 M HCl (2 x 0.25 l), sat. NaHCO3 (0.25 l), brine (0.25 l), dried MgSO4, filtered and concentrated. The residue was subjected to Swern oxydation. Intermediate 5-trityloxypentan-1-ol: 1H NMR (400 MHz; CDCl3): δ 7.57-7.17 (m, 15H, Har), 3.62 (t, 2H, J = 6.5, 2H, H5), 3.11 (t, 2H, J = 6.6, H1), 1.75-1.63 (m, 2H, H3), 1.57 (dt, 2H, J = 12.9, 6.4, H2), 1.52-1.42 (m, 2H, H4). 13C NMR (100 MHz; CDCl3): δ 144.4, 128.6, 127.6, 126.7, 86.2, 63.4, 62.2, 32.6, 29.8, 22.5.

To a dry solution of oxalyl chloride (4.2 ml) in dry CH2Cl2 (75 ml) was dropwise added a solution of DMSO (5.4 ml) in CH2Cl2 (25 ml), over a period of 30 min at -70°C. The resulting reaction mixture was allowed to stir for 30 min at -70°C, followed by the dropwise addition of 5-(trityloxy)pentan-1-ol (13 g, 37 mmol) in CH2Cl2 (50 ml) over a period of 1 h at -70°C. After the addition of the alcohol, stirring continued for 2 h at -70°C, followed by the slow addition of Et3N (25 ml, 0.19 mol) over a period of 10 minutes at -70°C. The reaction mixture was allowed to warm up to RT over a period of 2 h and subsequently washed with 1M HCl (2 x 50 ml), sat. NaHCO3 (2 x 50 ml), brine (50 ml). The organic layer was dried (Na2SO4), filtered and concentrated. The residue was subjected to silica gel FCC (EtOAc/PE 9:1 → 8:2) to give the title compound in 70% yield. 1H NMR (400 MHz; CDCl3): δ 9.73 (t, 1H, J = 1.6, CHO), 7.44-7.13 (m, 15H, Har), 3.22 (t, 2H, J = 6.0, H5), 2.41 (dd, 2H, J = 7.1, 1.5, H2), 1.87-1.69 (m, 4H, H3,4). 13C NMR (100 MHz; CDCl3): δ 202.5, 144.3, 128.6, 127.6, 126.7, 86.4, 62.8, 43.4, 29.3, 18.9.

5-oxopentyl p-toluenesulfonate (324).
To a dry solution of 1,5-pentandiol (0.26 ml, 0.25 mol) in EtOAc (2M) were subsequently added TsCl (9.5 g, 50 mmol) and Et3N (11 ml). The resulting reaction mixture was stirred overnight at RT after which TLC analysis showed complete consumption of pTsCl. The reaction mixture was washed with H2O (0.5 l), 1M HCl (0.5 l), brine (0.5 l), dried (MgSO4), filtered and concentrated. The residue was subjected to silica gel FCC (EtOAc/PE 1:1 → 1:0) to provide the intermediate tosylate in 70% yield as a brownish semi-solid. Intermediate 5-hydroxypentyl p-toluenesulfonate: Rf = 0.7 (EtOAc/MeOH 9:1).
7.4 Experimental Procedures

1H NMR (400 MHz; CDCl₃): δ 7.75-7.77 (m, 2H, Har), 7.32-7.30 (m, 2H, Har), 4.01 (t, 2H, J = 6.4, H5), 3.55 (t, 2H, J = 6.4, H1), 2.43 (s, 3H, CH₃), 1.65 (dt, 2H, J = 14.3, 6.5, H3), 1.54-1.44 (m, 2H, H2), 1.37 (dt, 2H, J = 8.7, 7.2, H4). 13C NMR (100 MHz; CDCl3): δ 144.7, 132.7, 129.7, 127.6, 70.5, 61.8, 31.6, 28.3, 21.5, 21.3.

To a dry solution of 5-hydroxypentyl p-toluenesulfonate (0.51 g, 2.0 mmol) in dry CH₂Cl₂ (20 ml) was added Dess-Martin periodinane (1.3 g, 3.0 mmol) at 0°C. The resulting reaction mixture was stirred for 2 h at 0°C after which TLC analysis showed complete consumption of the starting material. The reaction mixture was diluted with a mixture of sat. Na₂S₂O₃ / sat. NaHCO₃ (1:1, v/v, 12.5 ml) and stirring was continued for 15 min. The organic layer was collected and the aqueous layer was back-extracted with CH₂Cl₂ (2x). The combined organic layers were washed with a mixture of H₂O/brine (1:1, v/v), dried (Na₂SO₄), filtered and concentrated. The residue was applied to silica gel FCC (EtOAc/PE 9:1 → 1:1) to provide the title compound in 24% yield. RF = 0.75 (PE/EtOAc 5:1). 1H NMR (400 MHz; CDCl₃): δ 9.70 (t, 1H, J = 1.3, CHO), 7.78-7.76 (m, 2H, Har), 7.36-7.34 (m, 2H, Har), 4.01 (dd, 2H, J = 6.7, 5.0, H5), 2.43-2.33 (m, 5H, CH₃, H2), 1.69-1.60 (m, 4H, H3,4). 13C NMR (100 MHz; CDCl₃): δ 201.5, 144.7, 132.7, 129.7, 127.5, 69.9, 42.6, 27.8, 21.3, 17.7. (Note: the title compound was also obtained via Swern oxidation of 5-hydroxypentyl p-toluenesulfonate in 84% yield).

N-5′-trityloxypentyl-2,3,4,6-tetra-O-benzyl-1-deoxyojirimycin (320).

To a cooled (0°C) solution of 2,3,4,6-tetra-O-benzyl-1-deoxyojirimycin (195, 0.52 g, 1 mmol) in dry DCE (1 M) were subsequently added 319 (1.5 g, 3 mmol), NaBH(OAc)₃ (0.63 g, 3 mmol) and Na₂SO₄ (~3 g). The resulting reaction mixture was stirred overnight under reflux, diluted with H₂O and extracted with CHCl₃ (3 x). The combined organic layer was subsequently washed with brine (2x), dried (MgSO₄), filtered and concentrated. The residue was subjected to silica gel FCC (PE/Et₂O, 1:0 → 1:1) to provide the title compound in 87%. 1H NMR (400 MHz, CDCl₃): δ 7.57-7.22 (m, 35H, Har), 5.07-4.48 (m, 8H, CH₂ -Bn), 3.79-3.65 (m, 2H, H3, H6α), 3.63-3.51 (m, 3H, H11, H6β), 3.20-3.14 (m, 1H, H2), 3.11 (t, J = 6.5, 1H, H4), 2.80-2.61 (m, 1H, H5), 2.41-2.35 (m, 2H, H1), 2.31-2.30 (m, 2H, H7), 1.85-1.20 (m, 6H, H8-10). 13C NMR (100 MHz, CDCl₃): δ 144.5, 139.1-137.8, 128.8-127.0, 87.4, 86.4, 78.6, 78.5, 75.4-72.8, 65.4, 63.7, 63.5, 54.4, 52.3, 29.9, 24.3, 23.3.
7. Synthesis and evaluation of N-pentyloxyethyl-1-adamantane-1-deoxy-nojirimycinium ions

\[ \text{N, N-cyclohexyl-2,3,4,6-tetra-O-benzyl-1-deoxynojirimycinium 4-toluene-sulfonate (323).} \]

To a dry solution of 195 (51 mg, 0.2 mmol) in dry DCE (0.1 M) were subsequently added 324 (0.15 g, 0.6 mmol), flame dried \( \text{Na}_2\text{SO}_4 \) (~ 0.2 g) and \( \text{NaBH(OAc)}_3 \) (0.13 g, 3 mmol) and the resulting mixture was stirred under reflux for 12 h. The reaction progress was monitored by LC/MS analysis which indicated the formation of the target compound. The reaction mixture was filtrated over celite and the volatiles were removed in vacuo. The residue was subjected to silica gel FCC (EtOAc/MeOH/NH\(_4\)OH 1:0:0 → 12:10:3) to provide the title compound in 84% yield. \( R_F = 0.2 \) (EtOAc/MeOH 8:2). \(^{1}H\) NMR (400 MHz; d\(_4\)-methanol): \( \delta \) 7.76-7.14 (m, 24H, Har), 4.82-4.48 (m, 8H, 4 x CH\(_2\)-Bn), 4.17 (t, 1H, \( J = 7.7 \), H4), 4.14-4.03 (m, 2H, H6), 4.02-3.89 (m, 2H, H11), 3.89-3.77 (m, 3H, H1\( \beta \), H2,3), 3.58-3.40 (m, 3H, H5,7), 3.25 (dd, 1H, \( J = 8.5, 13.6 \), H1\( \alpha \)), 2.35 (s, 3H, CH\(_3\)), 1.90-1.48 (m, 6H, H8,9,10). \(^{13}C\) NMR (100 MHz, d\(_4\)-methanol): \( \delta \) 142.5, 139.4, 139.1, 138.9, 138.4, 129.7, 129.6, 129.5, 129.3, 129.2, 129.1, 129.0, 128.9, 128.7, 127.0, 80.6, 75.6, 75.5, 75.3, 74.3, 74.3, 74.2, 65.1, 64.6, 59.2, 56.0, 21.9, 21.2, 21.1, 20.7. LC/MS analysis: Rt 9.12 min (linear gradient 10-90% B), ES (ESI): \( m/z = 592.47 \) [M]\(^{+}\).

\[ \text{N,N-cyclohexyl-2,3,4,6-tetra-O-benzyl-L-ido-1-deoxynojirimycinium 4-toluene-sulfonate (325).} \]

To a dry solution of 2,3,4,6-tetra-O-benzyl-L-ido-1-deoxynojirimycin (198, 0.52 g, 1.0 mmol) in dry DCE (0.1 M) were subsequently added 324 (0.77 g, 3 mmol), flame dried \( \text{Na}_2\text{SO}_4 \) (~ 3 g) and \( \text{NaBH(OAc)}_3 \) (0.63 g, 3 mmol) and the resulting mixture was stirred under reflux for 12 h. The reaction progress was monitored by LC/MS analysis which indicated the formation of the target compound. The reaction mixture was filtrated over celite and the volatiles were removed in vacuo. The residue was subjected to silica gel FCC (EtOAc/MeOH/NH\(_4\)OH 1:0:0 → 12:10:3) to provide the title compound in 97% yield. \(^{1}H\) NMR (400 MHz, d\(_4\)-methanol): \( \delta \) 7.70-7.20 (m, 24H, Har), 4.74-4.53 (m, 8H, 4 x CH\(_2\)-Bn), 4.26 (dd, \( J = 4.2, 12.0 \), 1H, H6\( \beta \)), 4.12-3.87 (m, 7H, H1\( \beta \),2,5,6\( \alpha \),4,11), 3.79 (t, \( J = 10.0 \), 1H, H3\( \beta \)), 3.58-3.49 (m, 1H, H1\( \beta \)), 3.49-3.40 (m, 2H, H7), 2.32 (s, 3H, CH\(_3\)), 1.94-1.48 (m, 6H, H8,9,10). \(^{13}C\) NMR (100 MHz; d\(_4\)-methanol): \( \sigma \): 143.8, 141.5, 139.2, 139.0, 138.6, 138.4, 129.8, 129.6, 129.6, 129.5, 129.3, 129.2, 129.1, 129.0, 128.9, 128.7, 127.0, 80.6, 75.6, 75.5, 75.3, 74.3, 74.3, 74.2, 65.1, 64.6, 59.2, 56.0, 21.9, 21.2, 21.1, 20.7. LC/MS analysis: Rt 9.19 min (linear gradient 10-90% B), ES (ESI): \( m/z = 592.47 \) [M]\(^{+}\).

\[ 2,3,4,6-tetra-O-trimethylsilyl-1-deoxynojirimycin (326). \]

To a solution of 1-deoxynojirimycin (178, 0.16 g, 1 mmol) in DMF (0.1 M) were subsequently added TBS-Tf (12 mmol) and 2,6-lutidine (12 mmol). The reaction mixture was heated for 12 h at 65°C and diluted with H\(_2\)O and extracted with a mixture of THF / EtOAc (2:5) and the combined organic layers were washed with brine, dried (MgSO\(_4\)), filtered and concentrated in vacuo. The crude material was subjected to silica gel FCC to provide the title compound in 75% yield. LC/MS analysis: Rt 10.64 (linear...
7.4 Experimental Procedures

gradient 10-90% B), ES (ESI): m/z = 620.4 [M+H]+.

\[ \text{N,N-dimethyl-2,3,4,6-tetra-O-trimethylsilyl-1-deoxynojirimycinium iodide (327).} \]

To a dry solution of 326 (0.3 g, 0.5 mmol) in DMF (0.1 M) was subsequently added an excess of MeI (0.6 ml, 10 mmol), K₂CO₃ (0.15 g, 1.1 mmol) and catalytic amount of 18-crown-6-ether. The reaction mixture was stirred overnight at 80°C at which point TLC analysis indicated complete consumption of the starting material and the formation of a highly polar compound. Excess K₂CO₃ was removed from the reaction mixture by filtration over a 100 µm filter. The filtrate was concentrated in vacuo and subjected to silica gel FCC (EtOAc/MeOH/NH₄OH 100:0:0 → 20:3:2) to furnish the title compound in 35% yield. ¹H NMR (400 MHz; CDCl₃): δ 4.27 (m, 1H, H₆α), 4.17 (m, 1H, H₆β), 4.02 (m, 1H, H₂), 3.97 (m, 1H, H₄), 3.78 (s, 3H, N-Me), 3.76 (m, 2H, H₁), 3.69 (m, 1H, H₅), 3.60 (s, 3H, N-Me), 3.32 (m, 1H, H₃), 0.98-0.85 (m, 36H, 4x tBu), 0.33-0.03 (m, 12H, 4x CH₃). ¹³C NMR (100 MHz; CDCl₃): δ 78.7, 72.8, 70.3, 69.6, 61.6, 59.2, 58.0, 56.2, 26.0-25.7, 18.2-17.9, -4.1, -5.4. LC/MS analysis: Rt 12.03 min (linear gradient 0-90% B), ES (ESI): m/z = 648.5 [M]+.

\[ \text{N,N-dimethyl-1-deoxynojirimycinium chloride (328).} \]

A dry solution of 327 (30 mg, 46 µmol) in dry MeOH (1 ml) was acidified with HCl (1 ml, 4M in dioxane). The resulting reaction mixture was stirred overnight at RT after which TLC analysis showed complete disappearance of the starting material. The reaction mixture was diluted with H₂O and washed with Et₂O (3x) and the aqueous layer was concentrated to give the title compound in near quantitative yield. ¹H NMR (400 MHz; D₂O): δ 4.22 (m, 1H, H₆α), 4.13 (m, 1H, H₆β), 3.93 (dt, 1H, J = 11.1, 7.8, 3.4, H₂), 3.87 (dd, 1H, J = 9.6, 6.7, H₃), 3.58 (dd, 1H, J = 5.0, 4.5, H₄), 3.52 (m, 1H, H₁α), 3.47 (dd, 1H, J = 9.6, 6.4, H₁β), 3.34 (m, 1H, H₅), 3.30 (s, 3H, N-Me), 3.16 (s, 3H, N-Me). ¹³C NMR (100 MHz, D₂O): δ 76.8, 74.0, 67.7, 66.1, 64.5, 59.9, 55.7, 55.4. LC/MS analysis: Rt 0.92 min (linear gradient 0-50% B), ES (ESI): m/z = 192.1 [M]+.
8

Synthesis of C-2 derivatized imidazo-D-gluco-pyranoses

8.1 Introduction

It is widely accepted that the effect of (N-alkylated) iminosugars on β-glycosidases principally arise by their ability to imitate the TS involved in enzymatic hydrolysis. The TS for enzymatic glycoside hydrolysis involves an putative oxocarbenium-ion (Figure 8.1, A) which displays a sp² anomic hybridization. A partial positive charge is predominantly located across the bond between the anomic carbon and endocyclic oxygen of the putative oxocarbenium-ion TS. As a consequence of this, the pyranoside conformation is distorted from a chair to a half-chair (e.g. ⁴H₃ and ³H₄) or boat (e.g. ²⁵B or B₂,₅) conformations (Figure 8.1, B).

In 1940 Ezaki et al. observed that δ-D-gluconolactone (329) is a strong inhibitor of β-glycosidases. These findings led Leaback and Reese to suggested that δ-D-gluconolactone (329a, Figure 8.1, C) is a true mimic of the transition state involved in glycosidic bond cleavage. The observation that δ-D-gluconolactone (329a) rivals the positively charged piperidine, DNJ (178a, Figure 8.1, D), in terms of β-glucosidase inhibition, resulted in the synthesis of several piperidine derivatives bearing a sp² hybridized C-1 atom (Figure 8.2A). Systematic analysis of the inhibitory profile of these ‘transition state’ inhibitors against several β-glycosidases, including almond β-glucosidase, revealed that both the shape and charge are important factors in designing potent and selective β-glycosidases inhibitors. X-ray analysis of the prepared derivatives revealed that the presence of a nonhydrolyzable C=N or C=C bond at the C-1 atom accurately mimics the assumed flattened half-chair/boat conformation.
8.1 Introduction

Figure 8.1: (A) Putative oxocarbenium-ion TS involved in glycosidic bond cleavage and (B) a selection of possible conformers.\textsuperscript{202} (C) Inhibition of sweet almond $\beta$-glucosidase by $\delta$-D-gluconolactone and (D) 1-deoxynojirimycin.\textsuperscript{203}

Figure 8.2: (A) $K_i$ values of a selection of ‘transition state’ inhibitors against almond $\beta$-glucosidase. (B-E) Illustration of the relative position of the catalytic carboxylate-ion and carboxylic acid active site residue with respect to the substrate or inhibitor.\textsuperscript{203}
of the putative oxacarbenium-ion TS. It was hypothesized that the proton transfer from the enzyme to the inhibitor and the stabilization of the protonated inhibitor are crucial factors, determining the effectiveness of TS-based β-glucosidase inhibitors. Protonation of the inhibitor is governed by the relative position of the catalytic carboxylic acid residue in the active site of β-glucosides, relative to the inhibitor. Based on X-ray analysis of lysozyme (N-acetylmuramide glycanhydrolase) it was generally assumed that the two key catalytic active site residues are on the opposite sites of the mean plane of the carbohydrate substrate (Figure 8.2B), and this assumption was extrapolated to other β-glycosidases. In an attempt to rationalized the results obtained from several ‘transition state’ inhibitors Vasella and co-workers hypothesized that the catalytic carboxylic acid residue is much closer to the mean plane of the carbohydrate substrate (Figure 8.2C). The newly developed hypothesis was corroborated by the striking activity difference between the two annulated triazoles 332 (Figure 8.2D) and 333 (Figure 8.2E).211

**D-gluco-imidazopyranose derivatives:** The most active β-glucosidase inhibitors known to date are tailored after D-gluco-configured tetrahydroimidazopyridine (331). Synthetic approaches towards 331 derivatives are well documented, starting from either D-glucose or L-xylose.212,213,215,216 The imidazole functionality in 335 can be introduced in two steps via a Hg(OAc)$_2$-promoted reaction of D-thiogluconolactam (336) with aminoacetaldehyde dimethyl acetal followed by acid mediated annulation (Scheme 8.1).213 A high yielding synthesis of D-thiogluconolactam (336) is reported from tetra-benzylated glucose (TBG).217,218 Another approach towards 335 involves the alkylation of protected furanoses, e.g. 337, with lithiated N-tritylimidazole.208,210,219 This one-step introduction of the imidazole unit results in the formation of 338 as a mixture of two diastereoisomers, ultimately leading to the D-gluco and D-manno imidazopyranose derivatives.

**Scheme 8.1:** Known synthesis of D-gluco-imidazopyranose derivatives.208,210,212

![Scheme 8.1](image)

Reagent and conditions: i) 74% over six steps; ii) aminoacetaldehyde dimethyl acetal, Hg(OAc)$_2$, THF, ~ 90%; iii) toluene, p-TsOH·H$_2$O, 85%; iv) N-iodosuccinimide, DMF, 80°C, 83-92%; v) EtMgBr, THF or CH$_2$Cl$_2$, 0°C, 82-94%; vi) N-tritylimidazole, nBuLi, 72% (2:3 mixture of the L-ido/L-gulo epimer); vii) 68% over four steps.
Panday et al.\textsuperscript{213} reported that C-2 substitution of 331 resulted in highly potent β-glucosidase inhibitors. The C-2 derivatisation of 331 was accomplished \textit{via} known key intermediate 339.\textsuperscript{212} The C-2 substituent was introduced \textit{via} a Sonogashira coupling of 339 with an alkyne, \textit{via} a Heck reaction with an alkene or by the alkylation of the organomagnesium derivative of 331. Systematic analysis of different C-2 substituents revealed that the installment of an aliphatic moiety at the C-2 atom strongly influence the binding affinities against several β-glucosidases (Table 8.1). Recently, Li \textit{et al.}\textsuperscript{220} reported the synthesis of a library of C-2 analogues of 331 \textit{via} a similar strategy as reported by Panday \textit{et al.}\textsuperscript{213} A selection from both reported libraries of C-2 substituted 331 with their $K_i$ values are listed in Table 8.1. From the results obtained by Panday \textit{et al.}\textsuperscript{213} it became apparent that the installment of a C$_2$H$_4$phenyl unit at the C-2 atom of 331 results in highly potent ($K_i = 1.2$ nM) inhibition of sweet almond β-glucosidase. Li \textit{et al.}\textsuperscript{220} confirmed the results obtained by Panday \textit{et al.}\textsuperscript{213} with regard to 340 and identified 341, having a C$_2$H$_4$tBu side chain at the C-2 position as a highly active β-glucosidase inhibitor with subnanomolar binding affinity ($K_i = 0.64$ nM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R =</th>
<th>$K_i$ in nM$^{213}$</th>
<th>R =</th>
<th>$K_i$ in nM$^{220}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>331: H</td>
<td></td>
<td>100</td>
<td>331: H</td>
<td>100</td>
</tr>
<tr>
<td>342: CH$_2$OH</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>340: C$_2$H$_4$Ph</td>
<td>1.2</td>
<td>341: C$_2$H$_4$tBu</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>343: Phenyl</td>
<td>100</td>
<td>340: C$_2$H$_4$Ph</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>345: CONHPh</td>
<td>3000</td>
<td>344: C$<em>8$H$</em>{17}$</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td>346: C$_2$H$_4$Biphenyl</td>
<td>24.5</td>
<td></td>
<td></td>
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</tbody>
</table>

The powerful inhibition of β-glucocerebrosidase (GBA1) by $N$-pentyloxymethyl-1-adamantane-1-deoxynojirimycin (MZ-21, 190) and its \textit{l-ido} congeners, MZ-31 (191 (see chapter 5) is partly attributed to the adamantane moiety. However, MZ-21 and to a lesser extent MZ-31, are not selective towards the other enzymes involved in the metabolism of glucosylceramide, such as the non-lysosomal β-glucosidase GBA2 and glucosylceramide
synthase (GCS). Therefore it was decided to prepare D-gluco-imidazopyranose derivatives based on lead compounds MZ-21 (190) and 340 (Figure 8.3) and evaluate their inhibitory profile against the hydrolases GBA1, GBA2 and the synthase GCS. The results described in chapter 6, revealed that omission of an ether linkage between the adamantane unit and the pentyl spacer in MZ-21 derivatives, resulted in weaker inhibition of GCS, compared to parent MZ-21. Therefore, derivatives 347 and 348 were designed without an ether linkage between the aliphatic moiety and the imidazopyranose core. The synthetic strategy devised by the group of Vasella,203,212,213 involving a Sonogashira coupling of iodine 339 with the appropriate alkyne, is applicable for the preparation of imidazopyranoses 347 and 348.

8.2 Results and Discussions

The synthesis of Sonogashira coupling partner 339 commenced with the preparation of lactam 349 from commercially available 2,3,4,6-tetra-O-benzyl-D-glucose, according to described procedures.217,218 Next, lactam 349 was exposed to Lawesson’s reagent (Scheme 8.2), which gave pure thiolactam 336 in near quantitative yield after chromatographic purification. Subsequently, thiolactam 336 was treated with aminoacetaldehyde dimethyl acetal, under the agency of Hg(OAc)₂. LCMS analysis of the crude reaction mixture revealed the presence of two diastereoisomers, which is in agreement with the findings of Gragnier et al.,212 who obtained a 2:1 mixture of the desired D-gluco imidazopyranose 350 and the corresponding D-manno epimer 351, respectively. After extractive work-up and evaporation of the volatiles, the residue was subjected to acid mediated annulation and the resulting diastereoisomers were separated by column chromatography. D-Gluco-imidazopyranose 335 and the D-manno-epimer 352 were isolated in 46% and 28% yield, respectively. D-Gluco-imidazopyranose 335 was treated with N-iodosuccinimide (NIS), which gave the bis-halogenated derivative 353 in excellent yields. Subsequent regioselective deiodination with EtMgBr furnished the desired Sonogashira coupling partner 339 in 53% yield in addition to 8% of the fully dehalogenated product 335.

Next, the preparation of the required alkynes 354 and 355 was undertaken via the Corey-Fuchs reaction with the proper aldehyde precursor. (Scheme 8.3). To this end, readily available 1-adamantane ethanal (147) was allowed to react with a mixture of PPh₃ and CBr₄ to give olefin 356 in 72% yield. After treatment with nBuLi, the resulting alkyne 354 was obtained in 72% yield. Analogously, commercially available phenylacetaldehyde (357) was transformed via intermediate olefin 358 to 3-phenylpropyne (355) in 68% yield over two steps. The penultimate step in generating imidazopyranose derivatives 347 and 348 involves the Sonogashira coupling of iodine 339 with alkynes 354 and 355, respectively. Employing catalytic Pd(PPh₃)₄ with the addition of copper iodine furnished Sonogashira coupling products 359 and 360 in 12% and 50% respectively. Reduction of the triple bond and simultaneous deblocking of the O-benzyl protective groups was accomplished with Pd/C under an hydrogen atmosphere. D-Gluco-imidazopyranose derivatives 347 and 348 were isolated in reasonable yields and high purity after preparative HPLC purification and their inhibitory profile was evaluated.
8.2 Results and Discussions

Scheme 8.2: Synthesis of Sonogashira coupling partner 339.

Reagent and conditions: i) Lawesson’s reagent (0.75 eq.), toluene (0.1 M), RT, 12 h; 99%; ii) H$_2$NCH$_2$CH(OMe)$_2$ (5 eq.), Hg(OAc)$_2$ (1.4 eq.), THF (0.1 M), 5°C, 2 h; iii) pTsOH - H$_2$O (2.6 eq), toluene/H$_2$O (10:1, v/v, 0.1 M), 65°C, 17 h, 335, 46% over two steps; 352, 28% over two steps; iv) NIS (10 eq.), DMF (0.16 M), 80°C, 1.5 h, 90%; vii) EtMgBr (1.4 eq.), THF (0.1 M), 0°C, 20 min, 52%.

Scheme 8.3: Synthesis of C-2 substituted d-glucopyranose derivatives 347 and 348.

Reagent and conditions: i) CBr$_4$ (2 eq.), PPh$_3$ (2 eq.), CH$_2$Cl$_2$ (0.1 M), 0°C, 1 h, 356, 77%; 358, 99%; ii) nBuLi (2 eq.), THF (0.1 M), -78°C, 1 h, 354, 72%; 355, 69%; iii) 339 (0.2 eq.), Pd(PPh$_3$)$_4$ (0.2 eq.), Cul (0.2 eq.), Et$_3$N (4.5 eq.), DMF (0.1 M), 80°C, 3 h, 359, 12%; 360, 50%; iv) 10 mol% Pd/C (10 wt%), cat. Pd black, H$_2$, EtOH (∼0.1 M), pH ∼ 5 (AcOH), RT, 12 h, 347, 21%; 348, 30%.
8. Synthesis of C-2 derivatized imidazo-d-gluco-pyranoses

Biological evaluation
The prepared imidazopyranose derivatives 347 and 348 were tested against GBA1, GB2 and GCS and the results are depicted in Table 8.2. Both inhibitors proved to be highly potent against GBA1 and the most potent, 348, has an IC_{50} of 2 nM. Unexpectedly, the C-2 adamantane derivative 347 was 10 times less potent compared to the C-2 phenyl analogue 348. Remarkably, the non-lysosomal β-glucosidase GBA2, which has the similar capability as GBA1, and glycosyltransferase (GCS) are apparently not affected by the prepared inhibitors.

Table 8.2: Enzyme inhibition assay results: Apparent IC_{50} values in micromolar (µM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R=</th>
<th>GCS</th>
<th>GCS</th>
<th>GBA1</th>
<th>GBA 2</th>
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<tr>
<td></td>
<td>Facs NBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>347:</td>
<td>Adamantane</td>
<td>&gt;10</td>
<td>&gt;20</td>
<td>0.02</td>
<td>8</td>
</tr>
<tr>
<td>348:</td>
<td>Phenyl</td>
<td>&gt;10</td>
<td>&gt;20</td>
<td>0.002</td>
<td>40</td>
</tr>
</tbody>
</table>

8.3 Conclusions and Outlook
In the search of potent and selective inhibitors for the enzymes involved in the glucosylceramide metabolism, two C-2 substituted d-Gluco- imidazopyranose derivatives 347 and 348 were prepared. Evaluation of their inhibitory profile against GBA1, GBA2 and GCS revealed that both derivatives 347 and 348 are potent inhibitors of GBA1. Strikingly, the C-2 adamantane derivative 347 was outperformed by the C-2 phenyl derivative 348. In particular, the installment of a C_{3}H_{6}Ph group at the C-2 atom of the d-glucoimidazopyranose ring resulted in a nanomolar range inhibitor of GBA1 (348, IC_{50} = 2 nM) with exceptional selectivity against GCS and GBA2.

8.4 Experimental section
General: Solvents and reagents were obtained commercially and used as received unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere and residual water was removed from starting compounds by coevaporation with toluene (3x), unless stated otherwise. Reaction grade solvents were stored on 4Å molecular sieves or 3Å for MeOH, CH_{3}CN, and DMSO. All solvents were removed by in vacuo evaporation at ∼45°C. Reactions were monitored by TLC analysis using silica gel coated aluminium plates (0.2 mm thickness) an detection by UV absorption (254 nm) and/or by spraying with a solution of (NH_{4})_{6}Mo_{7}O_{24}.4H_{2}O (25 g/L) and (NH_{4})_{4}Ce(SO_{4})_{4}.4H_{2}O in 10% H_{2}SO_{4}, followed by charring at ∼150°C. Visualisations of olefins and N-alkylated iminosugars was achieved by spraying with a solution of KMnO_{4} (5 g/L) and K_{2}CO_{3} (25 g/L) in H_{2}O, followed by charring.
8.4 Experimental section

at ∼ 150°C. Glycosides and hemiacetals were visualized by spraying with a solution of 20% H₂SO₄ in EtOH and charring at ∼ 150°C and for adamantane containing compounds a solution of H₃PMo₁₂O₄₀ (100 g/L) in EtOH was used. Flash column chromatography was performed on silica gel (40-63 μm). NMR spectra were recorded on a 400/100 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard for all ¹H NMR measurements in CDCl₃ and the deuterated solvent signal for all other NMR experiments. ¹H NMR peak assignments were made using COSY and HSQC experiments and coupling constants (J) are given in Hz. All ¹³C NMR spectra are proton decoupled. For LC/MS analysis a HPLC-system (detection simultaneously at 213 nm, 254 nm and evaporative light detection) equipped with an analytical C18 column (4.6 mmD x 250 mmL, 5 μm particle size) in combination with buffers A: H₂O, B: CH₃CN, C: 1.0% aqueous trifluoroacetic acid and coupled with an electrospray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a semi-preparative C18 column (5 μm C18, 10, 150 x 21.2 mm) was used. The applied buffers were A: H₂O + trifluoroacetic acid (1% mM) and B: CH₃CN.

5-amino-2,3,4,6-tetra-O-benzyl-5-deoxy-D-gluconothio-1,5-lactam (336).

To a dry solution of δ-D-gluconolactam (349, 7.15 g, 13.3 mmol) in toluene was added Lawesson’s reagent (4.09 g, 10.1 mmol) and the resulting mixture was stirred overnight at RT. After TLC analysis indicated complete consumption of the SM, the volatiles were removed. The residue was subjected to silica gel FCC (PE/EtOAc 95:5 → 8:2) to provide the title compound in 99% yield as a smelly yellow powder. Rf = 0.7 (PE/EtOAc 8:2). ¹H NMR (400 MHz; CDCl₃): δ 3.36-3.40 (m, 1H, H5), 3.56-3.59 (m, 2H, H6), 3.61-3.65 (m, 1H, H4), 3.8-3.91 (m, 2H, H2,3), 4.34- 5.03 (m, 8H, 4x CH₂ - Bn), 7.13-7.41 (m, 20H, Har). ¹³C NMR (100 MHz; CDCl₃) δ 55.9, 68.2, 72.4, 72.5, 72.7, 73.4, 78.3, 81.2, 82.4, 127.8, 127.9, 127.95, 127.97, 128.00, 128.04, 128.1, 128.16, 128.18, 128.20, 128.26, 128.34, 128.37, 128.41, 128.51, 128.54, 129.0, 137.0, 137.3, 137.4, 200.3. LC/MS analysis: Rt 11.6 min (linear gradient 10-90% B, CN-column), ES (ESI): m/z = 554.4 [M+H]+ and 576.6 [M+Na]+.

2,3,4,6-tetra-O-benzyl-1,5-dideoxy-1-[(2′,2′dimethoxyethyl) imino]-1-5-imino-D-glicitol (350) and 2,3,4,6-tetra-O-benzyl-1,5-dideoxy-1-[(2′,2′dimethoxyethyl) imino]-1-5-imino-D-mannitol (351).

To a dry solution of thiolactam 336 (8.1 g, 15 mmol) in distilled THF (0.1M) were subsequently added aminoacetaldehyde dimethyl acetal (8.2 ml, 76 mmol) and Hg(OAc)₂ (6.5 g, 21 mmol) at 5°C. The resulting dark reaction mixture was stirred for 2 h at 5°C followed by the addition of celite. The insolubles were removed by filtration over a Büchner funnel containing a layer of celite, sand and a Whatman filter. The filtrate was diluted with Et₂O (35 ml) and washed with H₂O (100 ml) and the aqueous layer was back-extracted with Et₂O. The combined organic layers were subsequently washed with H₂O, sat. NaHCO₃/brine (1:1, v/v), dried Na₂SO₄, filtered and concentrated. The crude product was
8. Synthesis of C-2 derivatized imidazo-D-glucopyranoses

used in the next reaction. $R_F = 0.1$ (100% EtOAc). LC-MS: m/z 624.9 [M+H]$^+$. 

(5R,6R,7S,8S)-6,7,8-tris(benzyloxy)-5-((benzyloxy)methyl)-5,6,7,8-tetrahydro imidazo[1,2-a]pyridine (335).

To a solution of crude 350 (5.1 g, 15 mmol) in toluene/water (0.1 M, 10:1, v/v) was added pTsOH·H$_2$O (7.2 g, 38 mmol) and the resulting reaction mixture was stirred for 17 h at 65°C. After TLC analysis revealed complete disappearance of the SM, the reaction mixture was diluted with Et$_2$O and washed with H$_2$O. The aqueous layer was back-extracted with Et$_2$O and the combined organic layers were subsequently washed with H$_2$O, sat. NaHCO$_3$ (3x), brine, dried (MgSO$_4$), filtered and concentrated. The residue was subjected to silica gel FCC (PE/EtOAc 8:2 → 1:1) to provide the title compound in 46% yield over two steps. $R_F = 0.5$ (PE/EtOAc 1:1).

$^1$H NMR (400MHz, CDCl$_3$): $\delta$ 3.69-3.73 (m, 1H, H5), 3.79-3.86 (m, 2H, H9), 4.06-4.09 (m, 1H, H7), 4.15 (m, 1H, H6), 4.38-5.19 (m, 8H, 4x CH$_2$-Bn), 4.74-4.75 (m, 1H, H8), 7.00-7.02 (m, 1H, H2), 7.11-7.14 (m, 1H, H3), 7.17-7.42 (m, 20H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 57.8, 68.1, 72.4, 73.0, 73.8, 74.0, 75.7, 81.8, 117.1, 127.3 - 128.3, 129.0, 137.1 - 138.0, 143.7. LC-MS: m/z 561.27 [M+H]$^+$ and 1120.80 [2M+H]$^+$. 

The mannose derivative 352 was isolated in 28% yield over two steps. $R_F = 0.2$ (PE/EtOAc 1:1).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.58-3.63 (m, 1H, H9$^\alpha$), 3.72-3.76 (m, 1H, H9$^\beta$), 3.83-3.86 (m, 1H, H7), 4.08-4.14 (m, 1H, H5), 4.27-4.31 (m, 1H, H6), 4.39-5.00 (m, 8H, 4x CH$_2$-Bn), 4.80-4.81 (m, 1H, H8), 7.04 (s, 1H, H2), 7.14-7.16 (m, 1H, H3), 7.17-7.42 (m, 20H, Har). $^{13}$C NMR (100 MHz; CDCl$_3$): $\delta$ 59.6, 67.9, 70.2, 70.7-74.7, 74.0, 80.0, 119.1, 127.4 - 128.4, 129.1, 137.7 - 137.9, 143.0. LC-MS: m/z 561.27 [M+H]$^+$ and 1120.87 [2M+H]$^+$. 

(5R,6R,7S,8S)-6,7,8-tris(benzyloxy)-5-((benzyloxy)methyl)-2,3-diiodo-5,6,7,8-tetrahydro imidazo[1,2-a]pyridine (353).

To a dry solution of 335 (2.6 g; 4.7 mmol) in DMF (0.16 M) was added NIS (11 g, 47 mmol) and the resulting reaction mixture was stirred for 90 min at 80°C. TLC analysis at this point revealed an incomplete reaction thus the reaction mixture was allowed to stir over night at RT. The mixture was diluted with Et$_2$O and washed with sat. Na$_2$S$_2$O$_3$ (4x) and the combined aqueous layers were back-extracted with Et$_2$O. The combined organic layers were subsequently washed with H$_2$O, brine, dried (MgSO$_4$), filtered and concentrated in vacuo. The crude material was subjected to silica gel FCC (PE/EtOAc 9:1 → 7:3) to afford the title compound in 90% yield. $R_F = 0.7$ (PE/EtOAc 8:2).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.58-3.61 (m, 1H, H9$^\alpha$), 3.65-3.71 (m, 1H, H9$^\beta$), 4.04-4.05 (m, 1H, H8), 4.30-4.31 (m, 1H, H7), 4.36-4.37 (m, 2H, CH$_2$ - Bn), 4.40-4.45 (m, 2H, CH$_2$ - Bn), 4.49-4.50 (m, 1H, H6), 4.53-4.64 (m, 2H, CH$_2$ - Bn), 4.66-4.67 (m, 1H, H5), 4.78-5.09 (m, 2H, CH$_2$ - Bn), 7.14-7.39 (m, 20H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 60.6, 69.2, 72.0, 72.0-72.9, 73.0, 77.7, 81.2, 96.8, 127.4, 128.3, 137.1 - 137.9, 148.8. LC-MS: m/z 813.0 [M+H]$^+$. 

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(5R,6R,7S,8S)-6,7,8-tris(benzyloxy)-5-((benzyloxy)methyl)-2-iodo-5,6,7,8-tetrahydro imidazo[1,2-alpyridine (339)).

To a dry solution of compound 353 (3.4 g, 4.2 mmol) in THF (0.1 M) was dropwise added EtMgBr (5.7 ml, 1 M in THF) over a period of 5 min at 0°C. The resulting reaction mixture was stirred for 15 min at 0°C and excess EtMgBr was quenched by the addition of sat. NH₄Cl. The reaction mixture was diluted with EtOAc and washed with H₂O. The aqueous layer was back-extracted with EtOAc and the combined organic layers were subsequently washed with H₂O, brine, dried (MgSO₄), filtered, and concentrated. The crude material was redissolved in EtOAc and celite (~ 10 g) was added, the volatiles were removed in vacuo and the residue was subjected to silica gel FCC (PE/EtOAc 8:2, isocratic) to provide the title compound in 52% yield. R_F = 0.3 (PE/EtOAc 8:2). ¹H NMR (400 MHz; CDCl₃): δ 3.65-3.69 (m, 1H, H₉α), 3.74-3.81 (m, 2H, H₉β, H₇), 4.03-4.17 (m, 2H, H₅, H₈), 4.38-4.82 (m, 8H, 4x CH₂-Bn), 5.10-5.13 (m, 1H, H₅), 7.07 (s, 1H, H₃), 7.15-7.40 (m, 20H, Har). ¹³C NMR (100 MHz, CDCl₃): δ 58.2, 68.0, 73.1-73.9, 73.4, 75.6, 81.2, 82.1, 123.1, 127.5-128.4, 137.0-137.9, 145.7. LC-MS: m/z 687.2 [M+H]⁺. [α]D²⁰ = + 34.2° (c= 0.14, CHCl₃).

1-(3,3-dibromoallyl)adamantane (356).

To a dry solution of 1-adamantane ethanal (147 (0.9 g, 5.0 mmol)) in CH₂Cl₂ (0.1 M) were subsequently added CBr₄ (2.6 g, 9.9 mmol) and PPh₃ (6.6 g, 20 mmol) at 0°C. The resulting reaction mixture was stirred for 1 h at RT, after which TLC analysis indicated complete conversion of the SM into a more hydrophobic compound. The reaction mixture was diluted with CH₂Cl₂ and washed with sat. NH₄Cl, and the aqueous layer was back-extracted with Et₂O. The combined organic layers were washed with H₂O, brine, dried (Na₂SO₄), filtered and concentrated. The crude material was subjected to silica gel FCC (PE/Et₂O 99:1 isocratic) to provide the title compound in 77% yield. R_F = 0.8 (100% hexane). ¹H NMR (400 MHz, CDCl₃): δ 1.51 (br. s, 6H, 3x CH₂-Ada), 1.61-1.72 (m, 6H, 3x CH₂-Ada), 1.86-1.88 (d, J = 8.0, 2H, H1), 1.96 (br. s, 3H, 3x CH-Ada), 6.41-6.45 (t, 1H, J = 8.0, H2). ¹³C NMR (100 MHz, CDCl₃): δ 28.6, 33.6, 36.8, 42.2, 47.3, 89.1, 135.4.

1-(prop-2-yn-1-yl)adamantane (354).

To a dry solution of 356 (1.3 g; 3.8 mmol) in THF (0.1 M) was dropwise added nBuLi (4.8 ml, 1.6 M in hexane) over a period 5 min at -78°C. The resulting reaction mixture was allowed to stir for 90 min at -78°C, after which excess nBuLi was depleted by the careful addition sat. NH₄Cl. The reaction mixture was diluted with Et₂O and subsequently washed with sat. NH₄Cl, and the aqueous layer was back-extracted with Et₂O. The combined organic layers were washed with H₂O, brine, dried (Na₂SO₄), filtered and concentrate. The crude material was subjected to silica gel FCC (100% PE, isocratic) to provide the title compound in 72% yield as a colourless oil. R_F = 0.7 (100% hexane). ¹H NMR (400 MHz; CDCl₃): δ 1.57-1.58 (br. s, 6H, 3x CH₂-Ada), 1.62-1.80 (m, 6H, 3x CH₂-Ada), 1.94-1.98 (m, 6H, H1, H3, 3x CH-Ada). ¹³C NMR (100 MHz, CDCl₃): δ 28.6, 32.3, 33.6, 36.8, 41.8, 70.0, 81.8.
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(3,3-dibromoallyl)benzene (358).

To a dry solution of phenacetaldehyde (357, 2.3 ml, 20 mmol) in CH₂Cl₂ (0.1M) were subsequently added CBr₄ (11 g, 40 mmol) and PPh₃ (27 g, 80 mmol) at 0°C. The resulting reaction mixture was stirred for 1h at RT after which TLC analysis revealed complete conversion of the SM. The reaction mixture was diluted with CH₂Cl₂ and with H₂O, brine, dried (MgSO₄), filtered and concentrated in vacuo. The crude material was purified by silica gel FCC (100% PE, isocratic) to provide the title compound in near quantitative yield. R_F = 0.8 (100% PE). ¹H NMR (400 MHz, CDCl₃): δ 3.39 (d, 2H, J = 7.2, 2H, H1), 6.53 (t, 1H, J = 7.2, H2), 7.12-7.40 (m, 5H, Har). ¹³C NMR (100 MHz, CDCl₃): δ 39.4, 90.3, 127.0, 128.7, 129.0, 137.5, 137.7. LC-MS: m/z 275.7 [M+H]⁺.

prop-2-yn-1-ylbenzene (355).

To a dry solution of 358 (2.8 g, 10 mmol) in THF (0.1M) was added nBuLi (13 ml, 1.6M in hexane) dropwise over a period of 5 min at -78°C. The resulting reaction mixture was stirred for 90 min at -78°C followed by the addition of sat. NH₄Cl to quench the excess nBuLi. The reaction mixture was diluted with Et₂O and washed with sat. NH₄Cl. The aqueous layer was back-extracted with Et₂O and the combined organic layers were subsequently washed with H₂O, brine, dried (MgSO₄), filtered and concentrated. The crude material was subjected to silica gel FCC (100%, isocratic) to provide the title compound in 69% yield as a colourless oil. R_F = 0.6 (100% PE). ¹H NMR (400 MHz, CDCl₃): δ 2.18 (s, 1H, H1), 3.60 (s, 2H, H3), 7.21-7.36 (m, 5H, Har). ¹³C NMR (100 MHz, CDCl₃): δ 24.8, 70.4, 81.9, 126.7, 127.3, 127.8, 128.4, 136.1.

General procedure for Sonogashira cross-couplings

To a dry solution of the corresponding iminosugar in DMF (0.1M) were subsequently added the required alkyne (5 eq.) and Et₃N (4.5 eq.) and the resulting mixture was degassed by ultrasonic sonification under an argon atmosphere for 5 min. Simultaneously, a dry solution of Pd(PPh₃)₄ (0.2 eq.) and CuI (0.2 eq.) in DMF (0.02M) was also depleted of oxygen by ultrasonic sonification under an argon atmosphere. Next, the two solutions were combined and the resulting reaction mixture was stirred for 3 h at 80°C and stirring continued overnight at RT. Upon completion of the reaction, the reaction mixture was filtrated over celite, and the filtrate was diluted with H₂O and extracted with Et₂O (3x). The combined organic layers were subsequently washed with H₂O, brine, dried (MgSO₄), filtered and concentrated. The crude material was subjected to Pd/C hydrogenations without further purifications.

2-[3-(1-adamantyl)-prop-1-ynyl]-5,6,7,8-tetra-O-benzyl-imidazo-[1,2] nojirimycin (359).

Compound 339 (0.14 g, 0.2 mmol) and alkyne 354 (0.2 g, 1.0 mmol) were used in the general procedure for Suzuki cross-couplings. After
work up the title compound was obtained in 12% yield and used in the next step. $R_F = 0.61$ (PE/EtOAc 4:1). LCMS: m/z 733.8 [M+H]$^+$. 

2-(3-phenylprop-1-ynyl)-5,6,7,8-tetra-O-benzyl-imidazo[1,2]-nojirimycin (360).

Compound 339 (0.14 g, 0.2 mmol) and alkyne 355 (0.12 g, 1.0 mmol) were used in the general procedure for Suzuki cross-couplings. After work up, the crude title compound was obtained in 50% yield and used in the next step. $R_F = 0.53$ (PE/EtOAc 8:2). LCMS: m/z 675.3 [M+H]$^+$ and 1349.2 [2M+H]$^+$. 

2-[3-(1-adamantyl)-prop-1-ynyl]-imidazo[1,2-a]-imino-nojirimycin (347).

To a dry solution of 359 (47 mg) in AcOH/EtOH (4.0 ml, 3:1, v/v) was added cat. Pd/C 10 wt% and the resulting mixture was depleted of oxygen by ultrasonic sonification under an argon atmosphere for 5 min. The resulting reaction mixture was exposed for 12 h to an H$_2$ atmosphere (H$_2$ balloon) and subsequently filtrated over celite. The filtrate was diluted with toluene, concentrated and subjected to preparative HPLC purification. $R_F = 0.61$ (EtOAc/MeOH/NH$_4$OH, 50:45:5). LCMS: 377.2 [M+H]$^+$. 

2-(3-phenylprop-1-ynyl)-imidazo[1,2]-nojirimycin (348).

To a dry solution of 360 (64 mg) in AcOH/EtOH (4.0 ml, 3:1, v/v) were added cat. Pd/C 10 wt% and cat. Pd-black and the resulting mixture was depleted of oxygen by ultrasonic sonification under an argon atmosphere for 5 min. The resulting reaction mixture was exposed for 12 h to an H$_2$ atmosphere (H$_2$ balloon) and subsequently filtrated over celite. The filtrate was diluted with toluene, concentrated and subjected to preparative HPLC-purification to provide the title compound in 30% yield. $R_F = 0.67$ (EtOAc/MeOH/NH$_4$OH, 50:45:5). LCMS: 319.2 [M+H]$^+$. 
Design and synthesis of C-3 substituted 1-deoxy castanospermine derivatives

9.1 Introduction

Numerous plants across the world produce polyhydroxylated alkaloids to gain protection from predators by the inhibition of several glycoside processing enzymes.\(^{221-223}\) This holds true for castanospermine (361), a polyhydroxylated indolizidine alkaloid, first isolated from seeds of the Australian legume *Castanospermum australe*.\(^{224}\) Castanospermine was later also isolated from dry pods of *Alexa leiopetala*, originating from the wetlands of South America.\(^{225}\) The inhibition of α- and β-glucosidases by this natural iminosugar and its analogs, render these suitable as the active pharmaceutical ingredient for the treatment of a range of diseases including HIV, malaria, cancer and diabetes.\(^{222,226-228}\)

The very diverse and important physiological properties of castanospermine and its derivatives are reflected by the sheer number of publications over the past 50 years, dealing with their synthesis and biological evaluation.\(^{229-235}\) For instance, castanospermine (361) was found to be a relatively potent inhibitor (IC\(_{50} = \sim 50 \mu M\)) of β-glucocerebrosidase (GBA1) in fibroblast extracts.\(^{221}\) Interestingly, 361 was found to be equally active against lysosomal α-glucosidases, however other lysosomal α- and β-glycosidases such as galactosidases and mannosidases remained untouched. Surprisingly, castanospermine was not effective against yeast α-glucosidase.
9.1 Introduction

Figure 9.1: Iminosugar based inhibitors of enzymes involved in glucosylceramide metabolism.

\[
\begin{align*}
\text{IC}_{50} &= 50 \, \mu\text{M} \\
\text{IC}_{50} &= 0.2 \, \mu\text{M} \\
\text{IC}_{50} &= 0.1 \, \mu\text{M}
\end{align*}
\]

\[\text{N-alkylated 1-deoxynojirimycin (178, Figure 9.1) derivatives are known to be potent inhibitors of the enzymes involved in glucosylceramide (GlcCer) metabolism.}^{140}\] DNJ derivative bearing a \(N\)-butyl chain (189) is marketed under the trade name Zavesca\textsuperscript{R}, and is a relatively potent inhibitor of glucosylceramide syntase (GCS). It was found that the installment of a \(N\)-pentyloxymethyl-1-adamantane moiety on the DNJ core resulted in a \(\sim 250\) times more potent inhibitor (MZ-21, 190) of GCS compared to 189.\textsuperscript{174} The corresponding \(L\)-ido congener, MZ-31 (191) was reported to be \(\sim 20\) times more selective compared to MZ-21 towards GCS with respect to the inhibition of \(\beta\)-glucocerebrosidase (GBA1) and \(\beta\)-glucosidase (GBA2).\textsuperscript{175} \(\beta\)-glucoserebroside (GBA1) and especially the non-lysosomal \(\beta\)-glucosidase (GBA2) are simultaneously targeted by most DNJ-based GCS inhibitors.

Scheme 9.1: Castanospermine derivatives.

In the treatment of several lysosomal storage disorders via substrate reduction therapy (SRT), there is a need for potent and selective GCS inhibitors. The promising inhibitory profile of castanospermine (361) against diverse glycoside processing enzymes invited a
9. Synthesis of C-3 substituted 1-deoxy castanospermine derivatives

study towards the design of analogues, aimed at influencing cellular GlcCer levels. The synthesis of castanospermine (361) is well documented in the literature and generally entails complex synthetic procedures.229–235 It was decided to prepare both castanospermine (361) and 1-deoxy-castanospermine (362) via synthetic procedures developed by the group of Pyne.232,236 Additionally, 1-deoxy-castanospermine derivatives were designed to incorporate an aliphatic side chain at the C-3 atom, based on the finding that the N-alkylation of DNJ enhances its inhibitory potency. To this end, derivatives (3R)-363 and (3S)-363 were designed with a C-3 butyl chain. Analogues (3R)-364 and (3S)-364 were tailored after lead compound MZ-21 (190) to display a C-3 butyloxymethyl-1-adamantane side chain.

9.2 Results and Discussion

The synthesis of castanospermine (361) commenced with the synthesis of known amino-tetraol 365 (Scheme 9.2).236,237 The Petasis borono-Mannich reaction of L-xylene, allylamine and (E)-styrene boronic acid proceeded in excellent diastereoselectivity, dictated by the fixed stereochemistry at the C-2 of L-xylene. Contrary to the procedures described by Pyne et al.232 the isolation of 365 by means of ion-exchange (Amberlite® H+ resin) proved to be cumbersome and yields deviate from 30 to 48%. Fortunately, the highly polar nature of 365 with respect to the impurities, allowed for its easy isolation by means of extractive work-up in both high yield and purity. The identity and homogeneity of 365 was proven by 1H- and 13C NMR and HPLC. The installment of the N-Boc-protective group was accomplished under Schotten-Baumann reaction conditions, providing pure 366 in near quantitative yield. Subsequent ring-closing metathesis (RCM) of 366, utilizing Grubb’s 1th generation catalyst in refluxing CH2Cl2, gave 367 in excellent yield. Next, selective protection of the primary alcohol with Trt-Cl gave 368 and subsequent benzylation of the remaining secondary alcohol functions gave fully protected 369 in good yield.

The formation of bi-cyclic intermediate 370 was undertaken, analogous to the synthesis of Uniflorine A, as reported by Davis et al.237 The one step de-blocking of the O-Trt and N-Boc protective group in 369, followed by the intramolecular ring closure to 370 was cumbersome. Following the described procedures, only traces of 370 could be isolated, in addition to many unidentified products as revealed by TLC-analysis. Therefore two other strategies were explored to obtain 370 in decent yields. First, acid mediated removal of the trityl protective group, followed by DMP mediated oxidation of resulting alcohol 371 gave aldehyde 372 in good yield. Subsequently, aldehyde 372 was dissolved in TFA/CHCl2 (v/v, 1:1) at -15°C for 15 min, diluted with toluene and concentrated. The residue was subjected to reductive amination, utilizing MeOH and NaCNBH3. This three step cyclisation procedure resulted in the isolation of 370 in a overall yield of ~ 46% yield. Secondly, the S2 cyclisation via mesylate 373 was explored. To this end, mesylate 373, obtained in good yield from 371, was treated with TFA/CH2Cl2 (v/v, 1:1) at -15° for 15 min, diluted with toluene and concentrated. The resulting residue was dissolved in DMF, K2CO3 was added and the reaction mixture was heated to 80°C and allowed to stir for 12 h. After extractive work-up and chromatographic purification, pure 370 was isolated in 63% yield over three steps.
Scheme 9.2: Synthesis of castanospermine precursor.

Reagent and conditions: i) EtOH (0.5 M), trans-2-phenyl vinylboronic acid (1.0 eq.), allylamine (1.0 eq.), RT, 12 h, quant.; ii) Boc₂O (5.0 eq), CH₂Cl₂/NaHCO₃ (1:1 v/v), quant.; iii) 5 mol% Grubb’s 1st generation cat. CH₂Cl₂, Δ, 12 h, 91%; iv) Trt-Cl (1.5 eq.), Pyr (0.1 M), 50°C, 12 h, 94%; v) BnBr (4.5 eq.), DMF (0.1 M), NaH (5.0 eq.), 50°C, 12 h, quant.; vi) TFA/CH₂Cl₂ (1:1, v/v), anisole (5.0 eq.), 0°C, 2 h, ≤5%; vii) cat. p-TsOH, CH₂Cl₂/MeOH (1:1 v/v, 1 M), 2 h, RT, 92%; viii) DMP (1.5 eq.), CH₂Cl₂ (0.1 M), 0°C, 2 h, 89%; ix) a. TFA/CH₂Cl₂ (1:1, v/v), 15 min, -15°C then add toluene and concentrate; b. MeOH (0.1 M), pH ∼ 1 (adjusted with AcOH), NaCNBH₃ (2.0 eq.), 12 h, RT, 53% over two steps; x) Ms-Cl (1.5 eq.), Et₃N (1.5 eq.), CH₂Cl₂, 12 h; xi) a. TFA/CH₂Cl₂ (1:1, v/v), 15 min, -15°C then add toluene and concentrate; b. DMF (0.1 M), K₂CO₃ (5 eq.), 80°C, 12 h, 63% over three steps.

With pure 370 in hand, the bis-hydroxylation of the double bond was explored, according to reported procedures.²³²,²³³ Employing cat. K₂OsO₄ · 2H₂O and NMO as the co-oxidant, a 2:1 diastereomeric mixture was obtained in 82% yield (Scheme 9.3). After column chromatography, the major diastereoisomer (374) was isolated in 57%. At this point, the absolute stereochemistry of the two isomers was not known and determined. The major diastereomer (374) was used in the formation of cyclic sulphate 375, in one step utilizing sulfuryl chloride. Reductive opening of sulphate 375 with NaBH₄ in dimethylacetamide and subsequent acidic hydrolysis of the resulting sulphate provided 376 in moderate yield. Upon complete debenzylation of 376, the identity of castanospermine (361) was undisputed confirmed by comparison of the optical rotation, NMR and LCMS-data with literature data.²³² 1-Deoxy castanospermine (362) was gained after Pd/C mediated hydrogenation of benzylated 370 and subsequent ion-exchange purification, in 68% yield.
9. Synthesis of C-3 substituted 1-deoxy castanospermine derivatives

Scheme 9.3: Synthesis of castanospermine and 1-deoxy castanospermine.

Reagent and conditions: i) cat. K₂OsO₄·2H₂O, NMO (2 eq.), 12h, RT, major diastereoisomer (374), 57%; minor diastereoisomer, 25%; ii) SO₂Cl₂ (2 eq.), Et₃N (20 eq.), CH₂Cl₂, -15°C, 30 min, 88%; iii) NaBH₄ (5 eq.), DMA (0.25M), 12 h, RT 50°C then concentrate and dissolve in THF/3 M aq. H₂SO₄ (3:2, v/v, 0.1 M), 12 h, 50°C, 29% over three steps; iv) 5 mol% Pd/C (10 wt%), EtOH, H₂, 12 h, RT, 89%; v) same as iv, 68%.

1-deoxy castanospermine derivatives: With castanospermine (361) in hand, the synthesis of castanospermine derivatives 363 and 364 was undertaken via adaptation of the strategy as described for castanospermine (361, Scheme 9.2). The secondary, optically pure amines, which are required for the Petasis borono-Mannich reactions were prepared utilizing the chiral tert-butane sulfinamide group, based on the pioneering work of Ellman.²³⁸–²⁴⁰ To this end, 1-adamantanemethanol was equipped with a 5-(triyloxy)penty1 spacer to provide 377 in good yield. Acid mediated detritylation of 377 (Scheme 9.4) followed by Swern oxidation of alcohol 378 gave aldehyde 379 in quantitative yield. Next, aldehyde 379 was condensed with both commercially available (Rₛ)- and (Sₛ)- tert-butylsulfinamide. Imine formation proceeded efficiently in the presence of excess Cu₂SO₄ in CH₂Cl₂.²⁴⁰ However, the isolation of the imines from the reaction mixture was cumbersome, resulting in decreased yields. Alternatively, imine formation in toluene, with azeotropic removal of H₂O in vacuo at moderate temperatures, gave the crude (Rₛ)-380 and (Sₛ)-381 in quantitative yield and high purity.

Initially, the Grignard addition of vinylmagnesium bromide to sulfinimides (Rₛ)-380 and (Sₛ)381 was executed in CH₂Cl₂ at -78°C (Table 9.1).²⁴⁰ After extractive work-up, chromatographic separation of the resulting diastereomers was ineffective. Besides the moderate isolated yield, ¹H NMR analysis revealed a poor ratio of diastereomers. It is proposed that the Grignard addition to N-sulfinyl aldimines in noncoordinating solvents such as CH₂Cl₂ and toluene can proceed through a cyclic six-membered transition state with Mg coordinated to the oxygen of the sulfinyl group (Scheme 9.5).²⁴¹ Based on this assumption, the major diastereo-isomer, formed through a Ireland-Claisen type transition state would posses the
9.2 Results and Discussion

Scheme 9.4: Synthesis of optically active allylic-amines.

Reagent and conditions: i) MeOH/CH₂Cl₂ (1:1, v/v, 0.5M), cat. p-TsOH, 90%; ii) oxalylchloride (1.2 eq.), DMSO (1.5 eq.), CH₂Cl₂ (0.1M), -78°C, 1 h, then Et₃N (5 eq.), 2h, -78°C → 0°C, quant.; iii) 0.95 eq. (R)- or (S)-tert-butane sulfinamide, toluene (0.5M), 12 h, 45°C, p= 100 mbar, 380, quant.; 381, quant.; 386, 80%; 387, 80%; iv) vinylmagnesium bromide (2 eq.), toluene (0.01M), (R,S)-382, 93%, (R,S)/(R,R), 20:1; (S,R)-383, 96%, (S,R)/(S,S), 13:1; (R,S)-388, 89% ((R,S)/(R,R), 11:1); (S,R)-389, 87% (S,R)/(S,S), 10:1; v) HCl (2 eq.), MeOH (0.1M), 384, 93%; 385, 96%; 390, 82%; 391, 88%.

Scheme 9.5: Proposed transition states for Grignard addition to N-sulfinyl aldmines.
Table 9.1: Alkylation of chiral tert-butanesulfinamides 380 and 381.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Conditions</th>
<th>Yield</th>
<th>Diastereomeric Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(RS)-380</td>
<td>CH₂Cl₂ 0.1 M -78</td>
<td>59</td>
<td>1:2</td>
</tr>
<tr>
<td></td>
<td>Toluene 0.1 M -78</td>
<td>41</td>
<td>2:1</td>
</tr>
<tr>
<td></td>
<td>Toluene 0.1 M RT</td>
<td>94</td>
<td>6:1</td>
</tr>
<tr>
<td></td>
<td>Toluene 0.01 M RT</td>
<td>93</td>
<td>20:1</td>
</tr>
<tr>
<td>(SS)-381</td>
<td>CH₂Cl₂ 0.1 M -78</td>
<td>69</td>
<td>1:5</td>
</tr>
<tr>
<td></td>
<td>Toluene 0.1 M -78</td>
<td>63</td>
<td>1:2</td>
</tr>
<tr>
<td></td>
<td>Toluene 0.1 M RT</td>
<td>75</td>
<td>5:1</td>
</tr>
<tr>
<td></td>
<td>Toluene 0.01 M RT</td>
<td>96</td>
<td>13:1</td>
</tr>
<tr>
<td>(RS)-386</td>
<td>Toluene 0.01 M RT</td>
<td>89</td>
<td>11:1</td>
</tr>
<tr>
<td>(SS)-387</td>
<td>Toluene 0.01 M RT</td>
<td>87</td>
<td>10:1</td>
</tr>
</tbody>
</table>

*All experiments were executed with vinylmagnesium bromide (2 eq., 1.0 M solution in THF)*. 
*Isolated yields after chromatographic purifications, diastereoisomers were not separated.*
*Diastereomeric ratio was determined on the basis of ¹H NMR analysis.*

(R₉R) or (S₉S) stereochemistry with respect to aldimines (RS)-380 and (SS)-381, respectively.*

Replacement of the reaction solvent for toluene resulted in a lower selectivity for 381 and with respect to 380, a complete reversal in selectivity was observed. Furthermore, elevated reaction temperatures resulted in both higher diastereoselectivity and yields. The alkylation of N-sulfinyl aldimines with Grignard reagents can also proceed via an open transition state. It is hypothesized that the reversal in diastereoselectivity, affected by the solvent and elevated temperatures, originates from a Felkin-Ahn type transition state, as shown in Scheme 9.5. Further optimisation of the reaction conditions, including a high dilution (0.01 M) gave 382 and its epimer 383 in high diastereoselectivity and excellent yields. After acidic cleavage of the tert-butyl sulfinyl group, both (R)- and (S)-allylic amines 385 and 384 were obtained as their hydrochloric salts in good yields. Next, the optimized reaction sequence was repeated with

*Based on the alkylation with vinylmagnesium bromide.*
butanal, with both \((R_s)-\) and \((S_s)-\) tert-butylsulfinamide. The corresponding imines, 386 and 387 were alkylated with vinylmagnesium bromide to give amines 388 and 389 in good yield. Acidic cleavage of the tert-butyl sulfinyl group gave \((S)-\)hex-1-en-3-amine (390) and \((R)-\)hex-1-en-3-amine (391) in comparable selectivity and slightly lower yields as for the adamantane derivatives.

1-deoxy castanospermine hybrids: With the hydrochloric salt of the substituted allylic amines 384, 385, 390 and 391 in hand, the synthesis of C-3 substituted castanospermine derivatives was initiated. First, the Petasis borono-Mannich reaction with L-xylose, \((E)-\)styrene boronic acid and \((S)-\)hex-1-en-3-amine (390) was explored (Scheme 9.6). Employing Et₃N to liberate the free

### Scheme 9.6: Synthesis of C-3 substituted castanospermine derivatives.

Reagent and conditions: i) trans-2-phenyl vinylboronic acid (1.0 eq.), 1.0 eq. Et₃N, 50°C, EtOH (0.5M), 12 h, either 1.0 eq. of amine 390, \((S)-\)392, 79%; or 1.0 eq. of amine 391, \((R)-\)392, 77%; ii) Trt-Cl (1.5 eq.), Pyr (0.1M), 50°C, 12 h, \((S)-\)393, 53%; \((R)-\)393, 86%; iii) 10 mol% Grubb’s 1th generation cat., toluene (0.05M), 2 h, 100°C, MW-irradiation, \((S)-\)394, 90%, \((R)-\)394, 64%; iv) Boc₂O (5.0 eq.), NaHCO₃ (1:1, v/v, 1.0M), 12 h, RT, \((S)-\)395, 86%; \((R)-\)395, 86%; v) BnBr (4.5 eq.), DMF (0.1M), NaH (5 eq.), 50°C, 12 h, \((S)-\)396, 92%; \((R)-\)396, 97%; vi) cat. p-TsOH, CH₂Cl₂/MeOH (1:1 v/v, 0.1M), 2 h, RT, \((S)-\)397, 81%; \((R)-\)397, 92%; vii) Ms-Cl (1.5 eq.), Et₃N (1.5 eq.), \(\text{CH}_2\text{Cl}_2\) (0.1M), 2 h, 0°C; viii) a. TFA /\(\text{CH}_2\text{Cl}_2\) (1:1, v/v, 0.5M), 15 min -15°C then add toluene and concentrate; b. DMF (0.1M), \(\text{K}_2\text{CO}_3\) (5 eq.), 80°C, 12 h, \((S)-\)399, 43% over three steps; \((R)-\)399, 52% over three steps; ix) 5 mol%Pd/C (10 wt%), EtOH (0.5M), H₂, 12 h, RT, (35)-363, 51%; (3R)-363, 93%.
base of \textit{(S)}-390, in addition to elevated reaction temperatures provided \textit{(S)}-392 in good yields and high purity after extractive work-up. The subsequent \textit{N}-Boc protection of \textit{(S)}-392, as described for the synthesis of castanospermine (361) was troublesome. Amine \textit{(S)}-392 did not react under mild conditions (e.g. \textit{Boc}_2\textit{O} or \textit{Boc}-ON), and multiple product formation was observed at elevated temperatures. At this point, \textit{N}-Boc protection still remained illusive, and therefore the RCM reaction was investigated.\textsuperscript{242} It is well known that RCM in the presence of free amines is cumbersome, requiring additives such as (Lewis) acids. The starting diene \textit{(S)}-392 remained inert with Grubb’ 1\textsuperscript{st} generation cat. in refluxing \textit{CH}_2\textit{Cl}_2. The addition of \textit{p}-TsOH, \textit{CSA} or utilizing \textit{AcOH}\textsuperscript{243} as the solvent at 50°C, in the RCM only resulted in detritylation of diene \textit{(S)}-392. Application of Grubb’ 2\textsuperscript{nd} generation cat. or the Hoveyda-Grubb’s 1\textsuperscript{st} and 2\textsuperscript{nd} generation cat. was ineffective in the RCM of \textit{(S)}-392.

It was decided to first explore the selective tritylation of the primary hydroxyl in \textit{(S)}-392 and then to re-examine the \textit{N}-Boc protection. To this end, \textit{(S)}-392 was reacted with Trt-\textit{Cl} in pyridine at 50°C, to give trityl ether \textit{(S)}-393 in reasonable yields. However, \textit{N}-Boc protection of \textit{(S)}-393 was sluggish and only trace amounts of the product could be isolated. Interestingly, RCM of \textit{(S)}-393 with 10 mol\% Grubb’s 1\textsuperscript{st} generation catalyst in refluxing \textit{CH}_2\textit{Cl}_2 showed the formation of a more polar olefin (indicated by rapid colouration with \textit{KMnO}_4) in addition to a highly apolar spot and the starting diene. Substitution of the reaction solvent for toluene, in addition to a high dilution (0.05 M) under microwave (MW) irradiation, resulted in the clean conversion of diene \textit{(S)}-393, providing \textit{(S)}-394 in 90% yield. Remarkably, \textit{N}-Boc protection of \textit{(S)}-394 was accomplished with \textit{Boc}_2\textit{O} under Schotten-Boumann’s conditions in high yields. Subsequent benzylation of the remaining hydroxides in \textit{(S)}-395 gave intermediate \textit{(S)}-396 in good yield. After acid mediated detritylation of \textit{(S)}-396, alcohol \textit{(S)}-397 was converted via mesylate \textit{(S)}-398 into bicyclic \textit{(S)}-399 in reasonable yields. Finally, \textit{(S)}-399 was subjected to Pd/C promoted hydrogenation, which furnished after ion-exchange purifications, pure \textit{(3S)}-363.

\textbf{Figure 9.2:} Observed correlation by 2D NOESY experiment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{noesy.png}
\end{figure}

Next, the synthesis of \textit{(3R)}-363 was executed analogously to the preparation of \textit{(3S)}-363. Diene \textit{(R)}-393 was obtained in comparable yields as for the \textit{(S)}-epimer. However, diene \textit{(R)}-393 was partially consumed in the RCM, under MW irradiation. The addition of more (20 mol\%) Grubb’s 1\textsuperscript{st} generation cat. was ineffective, and \textit{394} was obtained in 64% yield. Alkene \textit{(R)}-394 was subsequently converted to \textit{(R)}-399 via the procedures as described for \textit{(S)}-399 and was obtained in comparable yields. After Pd/C mediated hydrogenation and subsequent
ion-exchange purification, pure (3R)-363 was obtained in respectable yield. With both (3R)- and (3S)-1-deoxy castanospermine derivatives (363) in hand, the stereochemistry at the C-3 position was determined by 2D-correlation 1H NMR Figure 9.2. 2D NOESY experiments revealed a strong correlation of H-3 with H-8 in (3R)-363 and this correlation is not observed in (3S)-363.

**Scheme 9.7: Synthesis of C-3 substituted castanospermine derivatives.**

Reagent and conditions: i) trans-2-phenyl vinylboronic acid (1.0 eq.), Et3N (1.0 eq.), EtOH (0.5M), 50°C, 12 h, 1.0 eq. of amine 385, (S)-400, 76%; 1.0 eq. amine 384, (R)-400, 63%; ii) i) Trt-Cl (1.5 eq.), Pyr (0.1M), 50°C, 12 h, (S)-401, 61%; (R)-401, 64%; iii) 10 mol% Grubb’s 1st generation cat., toluene (0.05M), 2 h, 100°C, MW-irradiation, (S)-402, 68%; (R)-402, ≤ 5%; iv) Boc2O (5.0 eq.), CH2Cl2/NaHCO3 (1:1, v/v, 0.1M), 12 h, RT, 82%; v) BnBr (4.5 eq.), DMF (0.1M), NaH (5.0 eq.), 50°C, 12 h, near quant.; vi) cat. p-TsOH, CH2Cl2/MeOH (1:1 v/v, 0.1M), 2 h, RT, 92%; vii) Ms-Cl (1.5 eq.), 1.5 eq. Et3N, CH2Cl2 (0.1M), 2 h, 0°C; viii) a. TFA/CH2Cl2 (1:1, v/v, 0.5M), 15 min -15°C then add toluene and concentrate; b. DMF (0.1M), K2CO3 (5 eq.), 80°C, 12h, 58% over three steps; ix) 5 mol% Pd/C (10 wt%), EtOH (0.5M), H2, 73%.
9. Synthesis of C-3 substituted 1-deoxy castanospermine derivatives

**Castanospermine - MZ-21 hybrids:** The synthesis of 1-deoxy castanospermine derivatives (3S)-364 and (3R)-364 was undertaken next (Scheme 9.7). The Petasis borono-Mannich reaction with L-xylose, (E)-styrene boronic acid with 384 or 385 proceeded smoothly at 50°C. The extractive purification of tetrols (S)-400 and (R)-400 was not effective. Therefore, chromatographic purification thereof was executed to give (S)-400 and (R)-400 in good yields. The subsequent reaction with Trt-Cl proceeded without complications, giving both (S)-401 and (R)-401 in decent yields. RCM of (S)-401 went efficiently and furnished (S)-402 in 88%. However, diene (S)-401 showed sluggish conversion in the RCM, giving only trace amounts of (R)-402 in addition to a row of unidentified, highly apolar spots on TLC. The synthesis of C-3 castanospermine derivatives was only continued with olefin (S)-402. Next, (S)-402 was subjected to N-bocylation to give (S)-403 in high yield. Subsequent benzylation of the remaining secondary hydroxyl functions in (S)-403 followed by acid mediated detritylation of (S)-404 provided alcohol (S)-405 in excellent yield. Alcohol (S)-405 was transformed into pyrolidine (S)-364 via mesylate (S)-406. Finally, MZ-21 castanospermine hybrid (3S)-364 was obtained in respectable yield, after Pd/C mediated hydrogenation and subsequent ion-exchange purification.

**9.3 Conclusions and Outlook**

Castanospermine 361 was prepared from L-xylose in 13 steps via adapted literature procedures, and its identity was confirmed by comparison with literature data. In addition, 1-deoxy castanospermine (408) and C3-derivatives thereof with an C3H7 or a C4H8OCH2Ada side chain were prepared. The required substituted allylic amines were prepared enantio-selectively utilizing the tert-butane sulfinamide template. The Petasis borono-Mannich reaction with L-xylose, (E)-styrene boronic acid and substituted allylic amines was accomplished at 50°C. The key RCM of the resulting diene, bearing a free secondary amine was accomplished under MW irradiation conditions in good yields. The hampering RCM of diene R-401 resulted in its abandonment from the synthetic schemes. The inhibitory profile of the prepared 1-deoxy castanospermine hybrids (3R)-363, (3S)-363 and (3S)-364 are currently evaluated against GCS, GBA1 and GBA2.

Given the better selectivity of MZ-31 (191 over MZ-31 (190, the development of the C8a-epimer of castanospermine analogues is desirable. The presented synthetic scheme towards castanospermine (361) can be adopted for this purpose, provided that the stereochemistry of the Petasis borono mannich reaction can be controlled. The recent report of the Lewis acid promoted Petasis borono mannich with high diastereoselectivity, can serve as a good starting point for future endeavours.
9.4 Experimental section

**General:** Solvents and reagents were obtained commercially and used as received unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere and residual water was removed from starting compounds by coevaporation with toluene (3x), unless stated otherwise. Reaction grade solvents were stored on 4Å molecular sieves or 3Å for MeOH, CH₃CN, and DMSO. All solvents were removed by *in vacuo* evaporation at ∼ 45°C. Reactions were monitored by TLC analysis using silica gel coated aluminium plates (0.2 mm thickness) an detection by UV absorption (254 nm) and/or by spraying with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·4H₂O in 10% H₂SO₄, followed by charring at ∼ 150°C. Visualisations of olefins and iminosugars was achieved by spraying with a solution of KMnO₄ (5 g/L) and K₂CO₃ (25 g/L) in H₂O, followed by charring at ∼ 150°C. Glycosides and hemiacetals were visualized by spaying with a solution of 20% H₂SO₄ in EtOH and charring at ∼ 150°C and for adamantane containing compounds a solution of H₃PMo₁₂O₄₀ (100 g/L) in EtOH was used. Flash column chromatography was performed on silica gel (40-63 μm). NMR spectra were recorded on a 400/100 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard for all ¹H NMR measurements in CDCl₃ and the deuterated solvent signal for all other NMR experiments. ¹H NMR peak assignments were made using COSY and HSQC experiments and coupling constants (J) are given in Hz. Compounds were numbered arbitrary for NMR peak assignments and do not correspond to the UPAC-nomenclature. All ¹³C NMR spectra are proton decoupled. For LC/MS analysis a HPLC-system (detection simultaneously at 213 nm, 254 nm and evaporative light detection) equipped with an analytical C18 column (4.6 mmD x 250 mmL, 5μm particle size) in combination with buffers A: H₂O, B: CH₃CN, C: 1.0% aqueous trifluoroacetic acid and coupled with an electronspray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a semi-preperative C18 column (5 μm C18, 10, 150 x 21.2 mm) was used. The applied buffers were A: H₂O + trifluoroacetic acid (1% mM) and B: CH₃CN.

**General Procedure A:** *N*-Boc protection. To a well stirred suspension of the requisite amine in CH₂Cl₂/sat. aq.NaHCO₃ (1M, v/v, 1:1) was added 5.0 eq. Boc₂O. The reaction mixture was stirred overnight at RT and transferred to a separatory funnel. The organic layer was isolated and the aqueous layer was extracted once with CH₂Cl₂. The combined organics were washed with brine, dried (MgSO₄), filtered, concentrated and applied to FCC.

**General Procedure B:** MW assisted ring-closing metathesis (RCM). A solution of the requisite diene in toluene (0.05m) was depleted of oxygen by sonification under an argon atmosphere. 10 Mo% of Grubb’s 1th generation cat. was added and the resulting mixture was heated for 2 h at 100°C by MW irradiation. After TLC analysis, the volatiles were removed and the residue was subjected to FCC.
**General Procedure C: O-Trityl protection.** To a dry solution of the corresponding alcohol in pyridine (0.1M) was added 1.5 eq. Trt-Cl and the resulting mixture was stirred overnight at 50°C. After TLC analysis indicated complete conversion of the SM, the reaction mixture was diluted with H₂O and the volatiles were removed. The residue was dissolved in Et₂O and subsequently washed with 1M aq. HCl (2x), sat. aq. NaHCO₃, brine, dried (MgSO₄), filtered, concentrated and subjected to FCC.

**General Procedure D: O-Benzyl protection.** To a dry solution of the corresponding alcohol in DMF (0.1M) was added 1.5 eq. benzylbromide /hydroxide and the resulting mixture was cooled to 0°C. After the careful addition of NaH (1.5 eq. /hydroxide) the reaction mixture was stirred overnight at 50°C. When TLC-analysis indicated complete conversion of the SM, the reaction mixture was diluted with H₂O and extracted with Et₂O (5x). The combined ethereal solution was subsequently washed with 1M aq. HCl (2x), sat. aq. NaHCO₃, brine, dried (MgSO₄), filtered, concentrated and subjected to FCC.

**General Procedure E: Acid mediated de-tritylation.** To a dry solution of the corresponding O-Trt derivative in MeOH/CHCl₂ (0.1M, v/v, 1:1, 0.1M) was added cat. p-TsOH and the resulting solution was stirred at RT for 2 h. After TLC-analysis indicated complete conversion of the SM, the reaction mixture was neutralize with Et₃N, concentrated and subjected to FCC.

**General Procedure F/G: O-Mesylation followed by N-Boc deprotection and intramolecular cyclisation.** To a dry solution of the corresponding alcohol in CH₂Cl₂ (0.1M) was added 1.5 eq. Et₃N and 1.5 eq. of MsCl and the resulting mixture was stirred 2h at 0°C. After TLC-analysis indicated complete conversion of the SM, the reaction mixture was diluted with H₂O and subsequently washed with 1M aq. HCl (2x), sat. aq. NaHCO₃, brine, dried (MgSO₄), filtered and concentrated. The corresponding N-Boc protected mesylate was stirred in a mixture of TFA/CH₂Cl₂ (0.5M, 1:1, v/v) at -15°C for 15 min. Upon complete consumption of the SM, as dictated by TLC analysis, the reaction mixture was diluted with toluene en concentrated. The residue was coevaporated trice with toluene, dissolved in DMF (0.1M) and 5 eq. K₂CO₃ was added. The resulting mixture was stirred for 12 h at 80°C, after which the insoluble material was filtered off. After removal of the solvent, the residue was applied on to FCC.

**General Procedure H: Pd/C catalysed hydrogenations.** The corresponding substrate was dissolved in aldehyde free ethanol (0.5M) and acidified with 37% HCl to pH ~ 1. Oxygen was depleted from the reaction mixture by sonification under an argon atmosphere. 5 mol% Pd/C (10 wt%) was added and the resulting mixture was stirred under an H₂ atmosphere. Upon completion of the reaction, as dictated by TLC-analysis, the volatiles were removed and the residue was subjected to chromatographic purifications.
General Procedure I: Iminisation with either (R)- or (S)-tBu-sulfinamide. The corresponding aldehyde was dissolved in toluene (0.05 M) and the appropriate tBu-sulfinamide (0.95 eq) was added. The reaction mixture was stirred at 0.1 bar at 45°C for 5 h. When TLC-revealed complete consumption of the sulfinamide, the volatiles were removed and crude material was used directly in the next step or was subjected to FCC.

General Procedure J: Grignard addition to sulfinimides. To a dry solution of the corresponding sulfinimide in toluene (0.01 M) was added 2 eq. of vinylmagnesium bromide (1 M in THF) at RT. The resulting reaction mixture was stirred overnight at RT, and after TLC-analysis, the RM was concentrate. The residue was dissolved in Et₂O and washed subsequently with 1 M aq. HCl, sat. aq. NaHCO₃, dried (MgSO₄), filtered and concentrated. The residue was purified by FCC.

General Procedure K: Cleavage of sulfinamides. To a dry solution of the corresponding sulfinamide in MeOH (0.1 M) was added 2 eq. of HCl (4 M solution in 1,4-dioxane) at 0°C. The resulting reaction mixture was allow to warm-up to RT in 1h. After TLC-analysis indicated complete consumption of the SM, the reaction mixture was concentrated and purified by FCC.

General Procedure L: Petasis boro-Mannich reaction with substituted amines. To a dry solution of L-xylose (1 eq.) in EtOH (0.5 M) was added Et₃N (1.5 eq.), (E)-styrene boronic (1.0 eq.) and the appropriate substituted amine·HCl (1.0 eq.). The resulting reaction mixture was stirred for 12 h at 50°C. The reaction mixture was diluted with H₂O and washed with Et₂O (5x). The combined ethereal solution was back-extracted with H₂O. The combined aqueous layer was concentrated to give the crude tetrol.

(6E)-5-(allylamino)-5,6,7-trideoxy-7-phenyl-D-glucohept-6-enitol (365).

To a dry mixture of L-xylose (2.0 g, 13.3 mmol) and E-2-phenylvinyl boronic acid (2.0 g, 13.3 mmol) in absolute ethanol (0.5 M) was added allyl amine (1.0 ml, 13.3 mmol). The resulting reaction mixture was stirred at RT for 16 h, at that point TLC analysis indicated complete consumption of the starting saccharide. The reaction mixture was diluted with H₂O and washed with Et₂O (3X). The combined ethereal solution was extracted with H₂O and the combined aqueous layers were concentrated to furnish the crude title compound as a brownish foam in quantitative yield. Rₚ = 0.6 (1% ceNH4OH in MeOH/EtOAc, 9:1). ¹H NMR (400 MHz; MeOD): δ 3.24 (dd, 1H, J = 6.6, 13.8, H1’α), 3.39 (dd, 1H, J = 5.6, 13.8, H1’β), 3.50 (dd, 1H, J = 4.5, 9.1, H5), 3.63 (m, 2H, H1), 3.71 (dd, 1H, J = 5.6, 13.8, H3), 3.78 (dd, 1H, J = 4.5, 9.1, H2), 3.90 (t, 1H, J = 4.9, H4), 5.22 (dd, 2H, J = 13.7, 28.3, H3’), 5.95 (m, 1H, H2’), 6.20 (dd, 1H, H = 12.3, 24.6, H6), 6.61 (d, 1H, J = 16.0, H7), 7.25 (t, 1H, J = 7.2, Har.), 7.33 (t, 1H, J = 7.5, 2H, Har), 7.5 (t, 2H, J = 7.7, Har). ¹³C NMR (100 MHz, MeOD): δ 63.2, 64.3, 72.7, 72.9, 74.2, 118.4, 126.8, 127.5, 128.8, 129.1, 129.5, 135.8, 136.1, 137.7.
9. Synthesis of C-3 substituted 1-deoxy castanospermine derivatives

**NBoc**

(6E)-5-[allyl(tert-butylcarbonyl)amino]-5,6,7-trideoxy-7-phenyl-D-glucopyranose hept-6-enitol (366).

Crude 365 (3.5 g, 12.1 mmol) was used in general procedure A. After FCC (EtOAc/MeOH 1:0 → 9:1), the title compound was obtained as a white foam in quantitative yield. $R_F = 0.3$ (9:1, EtOAc/MeOH). $^1$H NMR (400 MHz; MeOD): δ 1.48 (s, 9H, tBu), 3.60-3.76 (m, 3H, H1', H3), 3.80-3.92 (m, 2 H, H1), 4.0-4.3 (m, 2H, OH), 4.46-4.58 (m, 1H, H5), 5.1 - 5.26 (m, 2H, H3'), 5.83-5.96 (m, 1H, H6), 6.50-6.60 (m, 2H, H7), 7.23 (t, 1H, J = 7.2, Har), 7.31 (t, 2H, J = 7.5, Har), 7.41 (t, 2H, J = 7.6, Har).

$^{13}$C NMR (100 MHz, MeOD): δ 27.7, 48.4, 62.1, 62.9, 71.0, 72.6, 72.9, 81.6, 116.9, 127.3, 128.5, 135.2, 136.0, 138.3, 156.0 (Multiple signals present due to rotamers). LC/MS analysis: Rt 7.22 min (linear gradient 10-90% B), ES (ESI): m/z = 394.00 [M+H]$^+$, 809.13 [2M+Na]$^+$. 

**NBoc**

tert-butyl (2R)-2-[(2S,3S)-1,2,3,4- tetrahydroxybutyl]-2,5-dihydro-1H-pyrrrole-1-carboxylate (367).

A solution of diene 366 in CH$_2$Cl$_2$ (0.1M) was purged of oxygen by sonification under an argon atmosphere and subsequently 5 Mol% Grubb’s 1$^{st}$ gen. cat. was added. The resulting mixture was stirred overnight under reflux after which the volatiles were removed. The residue was subjected to FCC ((EtOAc/MeOH 1:0 → 9:1), the title compound was obtained in 91% yield. $R_F = 0.5$ (9:1) EtOAc/MeOH). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.43 (s, 9H, tBu), 3.53-3.81 (m, 5H, H1,2,3,4), 3.99 (d, 1H, J = 15.5, H8$\alpha$), 4.16 (d, 1H, J = 15.4, H8$\beta$), 4.35-4.57 (br. s, 2H, OH), 4.57-4.66 (m, 1H, H5), 5.77-5.99 (m, 2H, H6,7).

$^{13}$C NMR (100 MHz, CDCl$_3$): δ 26.1, 54.3, 63.77, 67.0068.3, 70.7, 71.9, 72.8, 80.6, 126.64, 128.07, 156.1. (Multiple signals present due to rotamers).

**NBoc**

tert-butyl (2R)-2-[(2S,3S)-4-O-trityl-1,2,3- trihydroxybutyl]-2,5-dihydro-1H-pyrrrole-1-carboxylate (368).

Tetrol 367 (1.7 g, 6.0 mmol) was used in general procedure C. After FCC (PE/Et$_2$O 1:0 → 0:1), the title compound was isolated in 94% yield. $R_F = 0.9$ (EtOAc). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.56 (s, 9H, tBu), 3.22 (dd, 1H, J = 9.0, 16.7, H1$\alpha$), 3.37-3.57 (m, 1H, H1$\beta$), 3.57-3.65 (m, 1H, H3), 3.90-3.95 (m, 2H, H2,4), 4.01-4.09 (m, 1H, H8$\alpha$), 4.19-4.36 m, 3H, H8$\beta$, 2x OH), 4.71-4.78 (m, 1H, H5), 5.81-5.87 (m, 1H, H7), 5.92 (m, 1H, H6), 7.23-7.30 (m, 1H, Har), 7.34 (t, 2H, J = 7.5, Har), 7.49-7.54 (m, 2H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 27.8, 54.1, 64.0, 66.71, 69.4, 72.2, 75.2, 80.5, 86.3, 126.0, 126.7, 127.6, 128.4, 143.7, 156.3 (Multiple signals present due to rotamers).

**NBoc**

tert-butyl(2R)-2-[(2S,3S)-4-O-trityl-1,2,3-tri-O-benzyl]-2,5-dihydro-1H-pyrrrole-1-carboxylate (369).

Triol 368 (3.0 g, 5.7 mmol) was used in general procedure D. After FCC (PE/Et$_2$O 1:0 → 1:5), the title compound was isolated in near quantitative yield. $R_F = 0.8$ (Et$_2$O). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.5 and 1.69 (2x s, 9H, tBu), 3.58-3.72 (m, 2H,
9.4 Experimental section

H1), 3.89 (t, 1H, J = 5.5, H3), 3.99-4.12 (m, 2H, H2,4), 4.35-4.58 (m, 2H, H8), 4.65-4.70 (m, 1H, H5), 4.74-5.07 (m, 6H, 3x CH2-Bn), 5.90-6.06 (m, 2H, H6,7), 7.22-7.79 (30H, Har).

13C NMR (100 MHz, CDCl3): δ 28.7, 53.7, 63.5, 64.1, 66.4, 67.3, 64.1, 67.3, 69.3, 73.3, 73.7, 74.7, 74.9, 75.0, 75.1, 79.5, 79.7, 80.0, 80.5, 81.0, 81.2, 87.01, 87.13, 95.8, 126.5, 126.7, 127.1, 127.2, 127.5, 127.55, 127.6, 127.71, 127.9, 128.0, 128.2, 128.3, 128.33, 128.4, 128.5, 128.6, 128.7, 128.9, 129.1, 138.4, 138.5, 138.6, 138.8, 138.9, 139.0, 144.2, 144.22, 154.1, 154.7 (Multiple signals present due to rotamers).

NBoc OH

tert-butyl(2R)-2-[(2S,3S)-1,2,3-tri-O-benzyl-4-hydroxy]-2,5-dihydro-1H-pyrrole-1-carboxylate (371).

Compound 369 (1.7 g, 2.1 mmol) was used in general procedure E. After FCC (PE/Et2O 4:1 → 0:1), the title compound was isolated in 92% yield. Rf = 0.3 (4:1, PE/EtOAc).

1H NMR (400 MHz; CDCl3): δ 1.54 (s, 9H, tBu), 2.4 (br. s, 1H, OH), 3.58 (dd, 1H, J = 5.4, 11.6, H1α), 3.72-3.91 (m, 4H, H1β, H8, H3), 4.02-4.34 (m, 2H, H2,4), 4.40-4.97 (m, 7H, 3x CH2-Bn, H5), 5.82-5.95 (m, 2H, H6,7), 7.23-7.41 (m, 15H, Har).

13C NMR (100 MHz, CDCl3): δ 28.6, 28.7, 53.6, 53.7, 61.4, 61.5, 66.9, 67.2, 73.0, 73.2, 74.6, 74.9, 75.2, 78.9, 79.6, 79.7, 80.0, 80.3, 80.8, 126.54, 126.6, 127.3, 127.6, 127.7, 127.8, 128.0, 128.2, 128.27, 128.3, 128.37, 128.4, 128.49, 128.5, 128.6, 137.95, 138.0, 138.1, 138.3, 138.4, 138.7, 154.1, 154.3 (Multiple signals present due to rotamers).

N BnO

BnO

BnO

H

(6S,7R,8R,8aS)-6,7,8-tris(benzyloxy)-1,5,6,7,8,8a-hexahydroindolizine (370).

Compound 371 (1.1 g, 1.9 mmol) was used in general procedure F/G. After silica gel FCC (Et2O, isocratic), the title compound was isolated in 63% yield over three steps. Rf = 0.7 (5% NH4OH in 3:2 PE/EtOAc).

1H NMR (400 MHz; CDCl3): δ 2.66 (t, 1H, J = 10.4, H5α), 3.32-3.47 (m, 3H, H5β, H3α, H5,8a), 3.52 (t, 1H, J = 9.1, H8), 3.66-3.76 (m, 2H, H3β, H7), 3.83-3.92 (m, 1H, H6), 5.96-6.01 (m, 1H, H1), 6.06-6.11 (m, 1H, H2), 7.37-7.54 (m, 15H, Har). 13C NMR (100 MHz, CDCl3): δ 28.6, 28.7, 53.6, 53.7, 61.4, 61.5, 66.9, 67.2, 73.0, 73.2, 74.6, 74.9, 75.8, 79.6, 79.7, 80.0, 80.3, 80.8, 126.54, 126.6, 127.3, 127.6, 127.7, 127.8, 128.0, 128.2, 128.27, 128.3, 128.37, 128.4, 128.49, 128.5, 128.6, 137.95, 138.0, 138.1, 138.3, 138.4, 138.7, 154.1, 154.3 (Multiple signals present due to rotamers).

(6S,7R,8R,8aR)-octahydroindolizine-6,7,8-triol (362).

Indolizine 370 (0.1 g, 0.6 mmol) was subjected to General procedure H, and the residue was dissolved in H2O and purified over a DOWEX - resin column (H+ form). The column was rinsed with H2O and the product was eluted with a 5% NH4OH solution, to give the title compound in 68% yield. MS analysis: ES (ESI): m/z = 442.20 [M+H]+

1H NMR (400 MHz; D2O): δ 1.64-.185 (m, 1H, H1α), 1.94-2.15 (m, 2H, H2), 2.21(q, 1H, J = 9.22, H1β), 2.88 (t, 1H, J = 11.0, H8), 3.04-3.24 (m, 2H, H3α, H5α), 3.37-3.64 (m, 4H,
9. Synthesis of C-3 substituted 1-deoxy castanospermine derivatives

H3β, H5β, H8a, H6), 3.68-3.82 (m, 1H, H7). 13C NMR (100 MHz, D2O): δ 20.2, 25.7, 52.9, 66.0, 67.5, 70.7, 76.28, 76.28. [α]20D = +33.9° (c = 0.36, H2O).

(1R,2S,6S,7R,8R,8aR)-6,7,8-tris(benzyloxy)octahydroindolizine-1,2-diol (374).

To a mixture of 370 (1.2 g, 2.7 mmol) in acetone/H2O (1:1, v/v) was added 2 eq. NMO and cat. K2OsO4·2H2O. The reaction mixture was stirred overnight at RT, after which TLC analysis (3:1 PE/EtOAc) revealed complete disappearance of the SM. The reaction mixture was diluted with toluene and concentrated. TLC-analysis (5% NH4OH in MeOH/EtOAc 9:1) showed the presence of two diastereoisomers, RP = 0.4 (major), 0.6 minor. The two diastereoisomers were separated by FCC (PE/EtOAc 1:0 → 2:8) and the title compound (major) was obtained in 57% yield. MS analysis: ES (ESI): m/z = 476.3 [M + H]+.

1H NMR (400 MHz; CDCl3): δ 2.01 (t, 1H, J = 10.4, H5α), 2.09 (dd, 1H, J = 2.8, 9.4, H8a), 2.41-2.51 (m, 1H, H3α), 2.99 (d, 1H, J = 10.5, H3β), 3.23-3.31 (m, 1H, H5β), 3.63 (t, 1H, J = 8.9, H7), 3.77-3.86 (m, 1H, H6), 3.89 (t, 1H, J = 9.2, H8), 4.19-4.33 (m, 2H, H1,2), 4.75 (q, 2H, J = 11.6, CH2Ph), 4.93-5.10 (m, 4H, 2xCH2Ph), 7.24-7.60 (m, 15H, Har).

13C NMR (100 MHz, CDCl3): δ 54.3, 61.6, 70.2, 70.4, 71.3, 74.8, 75.8, 77, 77.2, 78.8, 87.2, 125.4, 127.6, 127.8, 127.9, 128.6, 128.1, 128.4, 128.44, 128.5, 128.54, 129.2, 138.5, 139.0.

(3aS,7S,8R,9R,9aS,9bR)-7,8,9-tris(benzyloxy)octahydro-[1,3,2]dioxathiolino-[4,5-a]indolizine 2,2-dioxide (375).

To a dry solution of 374 (300 mg, 0.6 mmol) in CH2Cl2 (0.1M) was added 20 eq. Et3N (1.7 ml, 12 mmol) and 2 eq. sulfuryl chloride (100 µL, 1.2 mmol) at -15°C. The reaction mixture was stirred for 30 min at -15°C, after which TLC analysis (100% Et2O) showed the disappearance of the SM and the formation of a more apolar compound. The reaction mixture was diluted with CH2Cl2 and subsequently washed with H2O, 1M aq. HCl (2x), sat. aq. NaHCO3 and finally with brine. The organic layer was dried over MgSO4, filtered, and concentrated. The residue was subjected to FCC (PE/Et2O 1:1 → 0:1) to give the title compound in 88% yield. RP = 0.7 (100% Et2O). 1H NMR (400 MHz; CDCl3): δ 2.08 (t, 1H, J = 10.3, H5α), 2.31 (d, 1H, J = 9.2, H8a), 2.48 (d, 1H, J = 11.8, H3α), 3.30-3.48 (m, 3H, H3β, H5β, H6), 3.60 (t, 1H, J = 8.7, H8), 3.79 (t, 1H, J = 8.4, H7), 4.64-5.07 (m, 6H, 3xCH2Ph), 5.30 (br. s, 2H, H1,2), 7.16-7.59 (m, 15H, Har). 13C NMR (100 MHz, CDCl3): δ 52.6, 61.1, 69.5, 73.1, 75.2, 75.7, 76.6, 78.8, 82.3, 82.4, 86.7, 127.7, 127.9, 127.9, 128.0, 128.4, 128.5, 128.5, 128.6, 138.2, 138.6. LC/MS analysis: Rt 11.07 min (linear gradient 10-90% B), ES (ESI): m/z = 538.20 [M+H]+.

(6,7,8)-tri-O-benzyl-castanospermine (376).

To a dry solution of 375 (0.26 g, 0.5 mmol) was dissolved in DMA (0.25M) and 5 eq. NaBH4 (0.1 g, 2.5 mmol) and the resulting reaction mixture was allowed to stir overnight at RT. The reaction mixture was diluted with toluene.
and concentrated in vacuo (40°C, ~ 1 mbar). The residue was dissolved in a mixture of THF/3M aq. HCl (0.1m, 3:2, v/v) and stirred overnight at 50°C. The reaction mixture was neutralized by the addition of Na₂CO₃(s) and diluted with H₂O and extracted with Et₂O (5x). The combined ethereal solution was subsequently washed with sat. aq. NaHCO₃ and brine, dried (MgSO₄), filtered and concentrated. The residue was subjected to FCC (PE/Et₂O 1:0 → 0:1) to give the title compound in 29% yield. LC/MS analysis: Rt 7.57 min (linear gradient 10-90% B), ES (ESI): m/z = 460.2 [M + H]⁺.

1H NMR (400 MHz; CDCl₃): δ 1.73-1.89 (m, 1H, H₁α), 1.98-2.13 (m, 2H, H₃α, H₈a), 2.16-2.29 (m, 2H, H₂β, H₅α), 3.17 (t, 1H, J = 7.8, H₅β), 3.34 (dd, 1H, J = 5.0, 10.5, H₃β), 3.64 (t, 1H, J = 9.0, H₇), 3.72-3.85 (m, 2H, H₆, H₈), 4.26-4.42 (m, 1H, H₁), 4.67-5.1 (m, 6H, 3x CH₂Ph), 7.18-7.52 (m, 15H, Har).

13C NMR (100 MHz, CDCl₃): δ 33.8, 51.8, 54.4, 70.7, 72.0, 73.0, 74.5, 75.7, 76.9, 79.2, 87.3, 127.6, 127.8, 127.9, 128.0, 128.2, 128.4, 128.5, 128.6, 138.5, 138.9, 139.0. LC/MS analysis: Rt 7.57 min (linear gradient 10-90% B), ES (ESI): m/z = 460.20 [M+H]⁺.

376 (100 mg, 0.22 mmol) was used in General Procedure H, and the residue was dissolved in H₂O and purified over a DOWEX - resin column (H⁺ form). The column was rinsed with H₂O and the product was eluted with a 5% NH₄OH solution, to give the title compound in 89% yield. 1H NMR (400 MHz; D₂O): δ 1.48-1.65 (m, 1H, H₂α), 1.83-1.97 (m, 2H, H₅α, H₈a), 2.08 (dd, 1H, J = 9.1, 18.1, H₃α), 2.14-2.25 (m, 1H, H₂β), 2.94 (t, 1H, J = 8.5, H₃β), 3.04 (dd, 1H, J = 5.0, 10.8, H₅β), 3.19 (dd, 1H, J = 6.4, 15.6, H₇), 3.41-3.55 (m, 2H, H₆, H₈), 4.28 (br. s, 1H, H₁).

13C NMR (100 MHz, D₂O): δ 32.6, 51.5, 55.3, 68.9, 69.5, 70.0, 71.3, 78.9. [α]D²⁰ = +61.7° (c = 0.46, MeOH, TFA-salt).

5-(trityloxy)pentyloxymethyl-1-adamantane (377).

To a dry solution of 1-adamantane methanol (16.6 g, 0.1 mol) in DMF (0.4m) was added 1.3 eq. NaH (0.15 mol, 6.0 g of 60 wt% dispersion in mineral oil) at 0°C. The reaction mixture was carefully heated to 75°C and stirred for 1 h and a 2M solution of 5-(trityloxy)pentyl-p-toluenesulfonate (55 g, 110 mmol) in DMF was added dropwise over a period of 30 min. The resulting mixture was stirred 2 h at 75°C after which TLC analysis indicated complete disappearance of the SM. The reaction mixture was cooled to 0°C and excess NaH was quenched with EtOH, diluted with H₂O and extracted with Et₂O (5x). The combined organics were subsequently washed with 1M aq. HCl, sat. aq. NaHCO₃, dried (MgSO₄), filtered and concentrated. The crude material was purified by FCC (PE/Et₂O 1:0 → 0:1) to provide the title compound in 97% yield. RF = 0.9 (4:1, Tol/Et₂O). 1H NMR (400 MHz; CDCl₃): δ 1.48-1.57 (m, 2H, H₃), 1.57-1.64 (m, 8H, H₁₀, H₁₂, H₁₆, H₄), 1.69-1.82 (m, 8H, 8H, 8H, 15.2), 2.04 (br. s, 3H, H₉, H₁₁, H₁₃), 3.03 (s, 2H, H₆), 3.15 (t, 2H, J = 6.6, H₅), 3.44 (t, 2H, J = 6.3, H₁), 7.23-7.57 (m, 15H, Har). 13C NMR (100 MHz, CDCl₃): δ 23.1, 28.4, 29.4, 30.0, 34.2, 37.3, 39.8, 63.3, 67.6, 82.0, 86.4, 126.9, 126.9, 128.0,
5-((adamantan-1-ylmethoxy)pentan-1-ol (378).

5-(adamantan-1-ylmethoxy)pentan-1-ol (378). 377 (22.2 g, 45 mmol) was used in general procedure E. After FCC (PE/Et₂O 1:0 → 1:1) the title compound was obtained in 90% yield. \( R_F = 0.2 \) (4:1 Tol/Et₂O). \(^1\)H NMR (400 MHz; CDCl₃): \( \delta 1.36-1.51 \) (m, 2H, \( H₃ \)), \( 1.52-1.77 \) (m, 18H, 6x CH₂ (Ada), H₃,4,2), \( 2.98 \) (s, 2H, H₆), \( 3.41 \) (t, \( J = 6.45 \), H₅), \( 3.67 \) (t, \( J = 6.6 \), H₁). \(^{13}\)C NMR (100 MHz, CDCl₃): \( \delta 22.4, 28.3, 29.3, 32.5, 34.1, 37.3, 39.8, 62.9, 71.6, 82 \).

5-((adamantan-1-ylmethoxy)pentanal (379).

To a dry solution of oxalyl chloride (8.5 ml, 99 mmol) in dry CH₂Cl₂ (0.2M) was added a solution of DMSO (14 ml, 198 mmol) in CH₂Cl₂ (15 ml), dropwise at -70°C over a period of 30 min. The resulting reaction mixture was allowed to stir for 30 min at -70°C, followed by the dropwise addition of 378 (11.3 g, 45 mmol) in CH₂Cl₂ (15 mL), dropwise at -70°C over a period of 30 min. The resulting reaction mixture was allowed to warm up to RT over a period of 2 h and subsequently washed with 1M HCl, sat. NaHCO₃, and brine. The organic layer was dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was subjected to silica gel chromatography (PE/Et₂O 1:0 → 0:1) to give the title compound in quantitative yield. \(^1\)H NMR (400 MHz; CDCl₃): \( \delta 1.52-1.79 \) (m, 16H, 6x CH₂-Ada, H₃,4), \( 1.98 \) (br. s, 3H, H₉,11,13), \( 2.45-2.50 \) (m, 2H, H₂), \( 2.97 \) (s, 2H, H₆), \( 3.41 \) (t, \( J = 6.1 \), H₅), \( 9.78 \) (t, \( J = 1.8 \), H₁). \(^{13}\)C NMR (100 MHz, CDCl₃): \( \delta 19.0, 28.3, 29.0, 34.1, 37.3, 39.8, 43.7, 70.9, 82.0, 202.7 \).

(R)-N-(5-((adamantan-7-ylmethoxy)pentylidene)-2-methylpropane-2-sulfinamide 380.

(R)-N-(5-((adamantan-7-ylmethoxy)pentylidene)-2-methylpropane-2-sulfinamide 380. Aldehyde 379 (4.0 g, 16 mmol) was used in general procedure I. After FCC (Tol/Et₂O 1:0 → 1:1), the title compound was obtained in quantitative yield. \( R_F = 0.5 \) (4:1 Tol/Et₂O). \(^1\)H NMR (400 MHz; CDCl₃): \( \delta 1.20 \) (s, 9H, tBu), \( 1.51-1.55 \) (m, 6H, H₈,14,15), \( 1.61-1.76 \) (m, 10H, H₃,4,10,12,16), \( 1.93-1.99 \) (br. s, 3H, H₉,11,13), \( 2.53-2.59 \) (m, 2H, H₂), \( 2.96 \) (s, 2H, H₆), \( 3.41 \) (t, \( J = 6.1 \)), \( 8.09 \) (t, \( J = 4.6 \), H₁). \(^{13}\)C NMR (100 MHz, CDCl₃): \( \delta 22.3, 28.3, 29.1, 34.1, 35.9, 37.2, 39.7, 56.5, 70.9, 81.9, 169.6 \).

(S)-N-(5-((adamantan-7-ylmethoxy)pentylidene)-2-methylpropane-2-sulfinamide (381).

Aldehyde 379 (3.8 g, 15 mmol) was used in general procedure I. After FCC (Tol/Et₂O 1:0 → 1:1), the title compound was obtained in quantitative yield. \( R_F = 0.5 \) (4:1 Tol/Et₂O). \(^1\)H NMR (400 MHz; CDCl₃): \( \delta 1.20 \) (s, 9H,
tBu), 1.50-1.55 (m, 6H, H8,14,15), 1.60-1.75 (m, 10H, H3,4, 10,12,16), 1.93-1.99 (br. s, 3H, H9,11,13), 2.56 (td, 2H, J= 4.7, 7.32, 7.57, H2), 2.95 (s, 2H, H6), 3.40 (t, 2H, J= 6.1), 8.09 (t, 1H, J= 4.6, H1). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 22.3, 28.2, 29.1, 34.0, 35.9, 37.2, 39.7, 56.5, 70.9, 81.9, 169.5.

**(R)-N-((S)-7-adamantan-9-ylmethoxy)hept-1-en-3-yl)-2-methylpropane-2-sulfonamide (382).**

Sulfonamide 380 (4.8 g, 13 mmol) was used in general procedure J. After FCC (Tol/Et$_2$O 1:0 → 0:1), the title compound was obtained in 93%. $R_F$ = 0.2 (1:1, PE/Et$_2$O). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.24 (s, 9H, tBu), 1.35-1.61 (m, 12H, H4,5,6, 10,16, 17), 1.61-1.77 (m, 6H, H12, 14, 18), 1.97 (br. s, 3H, H11,13,15), 2.96 (s, 2H, H8), 3.14 (d, 1H, J= 4.9, NH), 3.38 (t, 2H, J= 6.1, H7), 3.69-3.81 (m, 1H, H3), 5.14-5.32 (m, 2H, H2), 5.78-5.93 (m, 1H, H1). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 22.3, 28.2, 29.1, 34.0, 35.9, 37.2, 39.7, 56.5, 70.9, 81.9, 169.5.

**(S)-N-((R)-7-adamantan-9-ylmethoxy)hept-1-en-3-yl)-2-methylpropane-2-sulfonamide (383).**

Sulfonamide 381 (2.2 g, 6.3 mmol) was used in general procedure J. After FCC (Tol/Et$_2$O 1:0 → 0:1), the title compound was obtained in 93%. $R_F$ = 0.2 (1:1, PE/Et$_2$O). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.24 (s, 9H, tBu), 1.35-1.61 (m, 12H, H4,5,6, 10,16, 17), 1.61-1.77 (m, 6H, H12, 14, 18), 1.97 (br. s, 3H, H11,13,15), 2.96 (s, 2H, H8), 3.14 (d, 1H, J= 4.9, NH), 3.38 (t, 2H, J= 6.1, H7), 3.69-3.81 (m, 1H, H3), 5.14-5.32 (m, 2H, H2), 5.78-5.93 (m, 1H, H1). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 22.3, 28.2, 29.1, 34.0, 35.9, 37.2, 39.7, 56.5, 70.9, 81.9, 169.5.

**(S)-7-adamantan-9-ylmethoxy)hept-1-en-3-amine (384).**

Sulfonamide 382 (4.6 g, 12 mmol) was used in general K. After FCC (5% NH$_4$OH in EtOAc/MeOH (1:0 → 8:2)), the title compound was isolated in quantitative yield. $R_F$ = 0.8 (5% NH$_4$OH in EtOAc/MeOH, 9:1). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.40-1.93 (18H, 6x CH$_2$(Ada), H4,5,6), 1.93-1.99 (br. s, 3H, H11,13,15), 2.99 (s, 2H, H8), 3.33 (m, 2H, H7), 3.69-3.80 (m, 1H, H3), 5.01-5.27 (br. s, 2H, H2), 5.39-5.54 (m, 2H, H2), 5.81-6.01 (m, 1H, H1). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 21.90, 28.4, 29.0, 32.4, 33.8, 37.0, 39.5, 54.1, 70.7, 81.7, 120.0, 134.0.

**(R)-7-adamantan-9-ylmethoxy)hept-1-en-3-amine (385).**

Sulfonamide 383 (4.4 g, 12 mmol) was used in general K. After FCC (5% NH$_4$OH in EtOAc/MeOH (1:0 → 8:2)), the title compound was isolated in quantitative yield. $R_F$ = 0.8 (5% NH$_4$OH in EtOAc/MeOH, 9:1). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.29-1.83 (18H, 6x CH$_2$(Ada), H4,5,6), 1.83-2.02 (br. s, 3H, H11,13,15), 2.99 (s, 2H, H8), 3.33 (t, 2H, J= 6.0, H7), 3.62-3.75 (m, 1H,
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H3), 5.39 (dd, 2H, J = 13.7, 34.0, H1), 5.76-5.95 (m, 1H, H2), 8.46 (br. s, 2H, NH2). 13C NMR (100 MHz, CDCl3): δ 22.0, 28.1, 28.8, 32.6, 33.9, 37.1, 39.6, 54.5, 70.8, 81.7, 120.8, 133.3.

(R)-N-butyldiene-tBu-sulfinamide (386).
Butanal (25 mmol) was used in general procedure I, with (R)-tBu-sulfinamide. The crude material was purified by FCC (PE/Et2O 0:1 → 1:0) to give the title compound in 80% yield. 1H NMR (400 MHz; CDCl3): δ 0.93 (t, 3H, J = 6.94, H3), 1.13 (s, 9H, tBu), 1.55-1.68 (m, 2H, H2), 2.39-2.52 (m, 2H, H3), 8.01 (t, 1H, J = 4.7, H1). 13C NMR (100 MHz, CDCl3): δ 13.7, 18.9, 22.3, 38.0, 56.4, 169.5.

(S)-N-butyldiene-tBu-sulfinamide (387).
Butanal (25 mmol) was used in general procedure I, with (S)-tBu-sulfinamide. The crude material was purified by FCC (PE/Et2O 0:1 → 1:0) to give the title compound in 80% yield. 1H NMR (400 MHz; CDCl3): δ 0.90 (t, 3H, J = 7.4, H1), 1.10 (s, 9H, tBu), 1.53-1.61 (m, 2H, H2), 2.39-2.43 (m, 2H, H3), 7.98 (t, 1H, J = 4.7, H1). 13C NMR (100 MHz, CDCl3): δ 13.7, 18.8, 22.2, 37.9, 56.3, 169.5.

(R)- N-(S)-hex-1-en-3-yl)-tBu-sulfinamide (388).
Sulfinimide 386 (3.5 g, 20 mmol) was alkylated according to general procedure J. After FCC (PE/Et2O 2:1 → 1:2) the title compound was obtained in 89% yield. 1H NMR (400 MHz; CDCl3): δ 0.90 (t, 3H, J = 5.6, H6), 1.21 (s, 9H, tBu), 1.29-1.39 (m, 2H, H5), 1.42-1.68 (m, 2H, H4), 3.10 (d, 1H, J = 6.1, NH), 3.65-3.76 (m, 1H, H3), 5.09-5.30 (m, 2H, H1), 5.74-5.87 (m, 1H, H2). 13C NMR (100 MHz, CDCl3): δ 13.8, 18.7, 22.6, 37.5, 55.7, 58.8, 116.3, 140.

(S)- N-(R)-hex-1-en-3-yl)-tBu-sulfinamide (389).
Sulfinimide 387 (3.5 g, 20 mmol) was alkylated according to general procedure H. After FCC (PE/Et2O 2:1 → 1:2) the title compound was obtained in 87% yield. 1H NMR (400 MHz; CDCl3): δ 0.92 (t, 3H, J = 7.3, H6), 1.23 (s, 9H, tBu), 1.31-1.42 (m, 2H, H5), 1.42-1.69 (m, 2H, H4), 3.1 (d, 1H, J = 6.3, NH), 3.72-3.76 (m, 1H, H3), 5.11-5.32 (m, 2H, H1), 5.77-5.90 (m, 1H, H2). 13C NMR (100 MHz, CDCl3): δ 13.9, 18.7, 22.6, 37.5, 55.7, 58.6, 116.3, 140.

(2S,3S,4R,5R,ɛ)-5-((R)-hex-1-en-3-ylamino)-7-phenylhept-6-ene-1,2,3,4-tetraol ((R)-392).
The HCl salt of substituted amine 391 (13.6 mmol, 1.84 g) was used in general procedure L. After extractive work-up, the title compound was obtained in 77% yield. RF = 0.2 (MeOH/EtOAc/NH4OH, 10:12:3). 1H NMR (400 MHz; CDCl3): δ 0.84 (t, 3H, J = 7.3, H1'), 1.11-1.40 (m, 2H, H2'), 1.56-1.75 (m, 1H, H3'α), 1.75-1.91 (m, 1H, H3'β), 3.34-3.49 (m, 1H, H4'), 3.65-3.85 (m, 2H, 182
H2.4), 3.87-4.02 (m, 1H, H3), 4.17-4.27 (m, 1H, H5), 5.08-5.42 (m, 2H, H6'), 5.69-5.84 (m, 1H, H5'), 6.34 (dd, 1H, J = 8.9, 15.0, H6), 6.69 (d, 1H, J = 10.6, H7), 7.21-7.34 (m, 3H, Har), 7.43-7.51 (m, 2H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 13.7, 19.01, 35.4, 59.6, 61.6, 64.0, 71.7, 72.1, 72.7, 121.1, 126.9, 128.5, 128.7, 135.0, 135.7, 137.4.

N$_2$H$_3$TrtO OH OH OH

4-O-trityl-(2S,3S,4R,5R,$\varepsilon$)-5-((($R$)-hex-1-en-3-ylamino)-7-phenylhept-6-ene-2,3,4-triol (($R$)-393).

Tetrol ($R$)-392 (1.3 g, 3.9 mmol) was used in general procedure C. After FCC (Et$_2$O/EtOAc 1:0 → 0:1 then 5 % NH$_4$OH in EtOAc/MeOH, 9:1), the title compound was obtained in 86% yield. $R_F$ = 0.4 (5% NH$_4$OH in EtOAC/MeOH 9:1). $^1$H NMR (400 MHz; MeOD): δ 0.93 (t, 3H, J = 7.4, H1'), 1.25-1.50 (m, 2H, H2'), 1.60-1.73 (m, 1H, H3' $\alpha$), 1.76-1.89 (m, 1H, H3' $\beta$), 3.18 (dd, 1H, J = 5.0, 8.9, H1 $\alpha$), 3.29-3.35 (m, 1H, H1 $\beta$), 3.49-3.72 (m, 2H, H5, H4'), 3.90-4.10 (m, 3H, H2, H3, 4') 5.31-5.58 (m, 2H, H6'), 5.69-5.82 (m, 1H, H5'), 6.35-6.49 (m, 1H, H6), 6.81 (d, 1H, J = 16.0, H7), 7.07-7.59 (m, 20H, Har). $^{13}$C NMR (100 MHz, MeOD): δ 12.7, 18.6, 34.0, 59.4, 61.4, 64.17, 69.5, 72.2, 71.4, 86.5, 119.8, 122.4, 126.7, 127.3, 127.4, 127.8, 127.9, 128.4, 128.5, 128.6, 129.1, 133.0, 135.6, 138.7, 143.9.

NH$_2$OTrt OH OH OH

(1R,2S,3S)-1-((2R,5R)-5-propyl-2,5-dihydro-1H-pyrrol-2-yl)-4-(trityloxy)butane-1,2,3-triol (($R$)-394).

Diene ($R$)-393 (0.83 g, 1.4 mmol) was used in general procedure B. After FCC (PE/Et$_2$O 1:0 → 0:1), the title compound was obtained in 64% yield. $^1$H NMR (400 MHz; MeOD) δ 0.93 (t, 3H, J = 7.3, H8''), 1.39-1.56 (m, 2H, H7''), 1.62-1.81 (m, 2H, H6''), 3.23 (dd, 1H, J = 5.1, 9.4, H4$\alpha$), 3.35-3.41 (m, 1H, H4$\beta$), 3.76-3.85 (m, 1H, H1), 3.93-4.01 (m, 2H, H2, 3), 4.23-4.35 (m, 1H, H5$'$), 4.52-4.65 (m, 1H, H2''), 5.91 (d, 1H, J = 6.4, H3''), 6.03 (d, 1H, J = 6.3, H4''), 7.10-7.58 (m, 20H, Har). $^{13}$C NMR (100 MHz, MeOD): δ 12.8, 19.3, 37.0, 64.6, 65.1, 69.2, 71.0, 71.4, 71.6, 86.6, 126.8, 127.1, 127.4, 128.5, 131.9, 144.0.

OTrt OH OH OH

(2R,5R)$\varepsilon$-tert-buty1 2-propyl-5-((1R,2S,3S)-1,2,3-trihydroxy-4-(trityloxy)butyl)-2,5-dihydro-1H-pyrrole-1-carboxylate (($R$)-395).

Amine ($R$)-394 (1.5 mmol, 0.7 g) was used in general procedure A. After FCC (PE/Et$_2$O 1:0 → 1:2), the title compound was isolated in 86% yield. $R_F$ = 0.7 (4:1 Et$_2$O/PE). $^1$H NMR (400 MHz; CDCl$_3$) δ 0.91 (t, 3H, J = 5.9, H8''), 1.29-1.41 (m, 2H, H7''), 1.47 (s, 9H, tBu), 1.55-1.82 (m, 2H, H6''), 2.88-3.00 (br. s, 1H, OH), 3.14-3.27 (m, 1H, H4$\alpha$), 3.30-3.39 (m, 1H, H4$\beta$), 3.84-3.97 (m, 2H, H2,3), 4.45-4.54 (m, 1H, H1), 4.56 (d, 1H, J = 7.1, H5''), 4.70-4.81 (m, 1H, H2''), 5.80-5.90 (m, 1H, H3''), 5.97-6.01 (m, 1H, H4''), 7.19-7.59 (m, 15H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): δ 14.2, 17.0, 28.5, 35.1, 64.8, 65.1, 69.3, 69.33, 72.8, 73.8, 86.8, 126.4, 127.0, 127.8, 127.9, 128.7, 131.7, 143.9, 156.2.

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9. Synthesis of C-3 substituted 1-deoxy castanospermine derivatives

\[
(\text{2R,5R}-\text{tert-butyl}\quad 2\text{-propyl-5-}\text{-(1R,2S,3S)-1,2,3-tris(benzyloxy)-4-(trityloxy)butyl)-2,5-dihydro-1H-pyrrole-1-carboxylate (}(\text{R})\text{-396).}
\]

Triol \text{(R)}\text{-395} (1.0 g, 1.8 mmol) was benzylated according to general procedure D. After FCC (PE/Et\textsubscript{2}O 1:0 → 1:1), the title compound was obtained in 97% yield. \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}): \(\delta\) 0.83-1.06 (m, 3H, H8'), 1.1-1.33 (m, 2H, H7'), 1.33 and 1.55 (s, 9H, tBu), 1.68-1.98 (m, 2H, H6'), 3.48-3.59 (m, 2H, H4), 3.85-3.96 (m, 1H, H2), 4.06-4.21 (m, 1H, H3), 4.30-5.11 (m, 9H, 3x CH\textsubscript{2}Ph, H2', H5'), 5.73-5.82 (m, 1H, 4'), 5.85-5.96 (m, 1H, H3'), 7.11-7.61 (m, 30H, Har).

\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta\) 14.4, 14.5, 17.1, 17.5, 28.7, 28.8, 33.8, 35.6, 63.4, 63.7, 64.1, 64.4, 64.7, 67.6, 67.9, 68.01, 73.2, 73.5, 73.8, 74.6, 74.8, 75.02, 77.1, 78.0, 79.2, 79.4, 80.8, 87.03, 87.2, 126.6, 126.8, 127.1, 127.2, 127.5, 127.6, 127.95, 128.0, 128.2, 128.3, 128.4, 128.40, 128.43, 128.5, 128.9, 129.1, 130.9, 138.4, 138.44, 138.77, 138.8, 138.9, 139.0, 144.2, 144.3, 153.1, 153.6 (multiple rotameric signals were observed).

\[
(\text{2R,5R}-\text{tert-butyl}\quad 2\text{-propyl-5-}\text{-(1R,2S,3S)-1,2,3-tris(benzyloxy)-4-hydroxybutyl)-2,5-dihydro-1H-pyrrole-1-carboxylate (}(\text{R})\text{-397).}
\]

Compound \text{(R)}\text{-396} (1.0 gr, 1.2 mmol) was used in general procedure E. After FCC (PE/Et\textsubscript{2}O 1:0 → 1:1), the title compound was obtained in 92% yield. \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}): \(\delta\) 1.01 (t, 3H, H8'), 1.18-1.48 (m, 2H, H7'), 1.61 (s, 9H, tBu), 1.71-2.02 (m, 2H, H6'), 3.47-3.59 (m, 1H, H2), 3.69-4.05 (m, 4H, H1,3,4), 4.17-5.03 (m, 8H, 3x CH\textsubscript{2}Ph, H2', H5'), 5.76-5.88 (m, 1H, H4'), 5.90-6.01 (m, 1H, H3'), 7.25-7.48 (m, 15H, Har).

\textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta\) 14.2, 14.3, 17.1, 17.3, 28.6, 33.6, 25.4, 61.3, 61.4, 61.8, 64.2, 64.6, 64.9, 65.9, 68.0, 72.9, 73.0, 73.2, 74.0, 74.3, 74.6, 74.9, 75.1, 78.7, 79.2, 79.5, 79.6, 80.0, 80.2, 80.7, 81.1, 126.9, 127.0, 127.6, 127.7, 127.8, 127.9, 128.0, 128.03, 128.3, 128.4, 128.5, 128.7, 130.6, 130.9, 131.0, 138.0, 138.02, 138.2, 138.3, 138.5, 138.7, 153.1, 153.9 (multiple rotameric signals were observed).

\[
(\text{3R,6S,7R,8R,8aR}-6,7,8\text{-tris(benzyloxy)-3-propyl-3,5,6,7,8,8a-hexahydroindolizine (}(\text{R})\text{-399).}
\]

Alcohol \text{(R)}\text{-397} (0.6 g, 1.0 mmol) was used in general procedure F/G. After FCC (PE/Et\textsubscript{2}O 1:0 → 1:1), the title compound was isolated in 52% yield over three steps. LC/MS analysis: Rt 8.55 min ES (ESI): m/z = 483.3 [M + H\textsuperscript{+}]. \textsuperscript{1}H NMR (400 MHz; CDCl\textsubscript{3}): \(\delta\) 1.0 (m, 3H, H1'), 1.20-1.47 (m, 2H, H2'), 1.47-1.62 (m, 2H, H3'), 2.76 (dd, 1H, J = 10.1, 12.7, H5\alpha), 3.24-3.37 (m, 2H, H5\beta, H6), 3.55-3.77 (m, 4H, H3, 7, 8, 8a), 4.45-5.16 (m, 6H, 3x CH\textsubscript{2}Ph), 5.92 (d, 1H, J = 6.2, H2), 6.07 (d, 1H, J = 6.2, H1), 7.26-7.48 (m, 15H, Har). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \(\delta\) 14.4, 19.4, 34.6, 47.2, 68.81, 71.5, 73.0, 75.1, 75.7, 78.2, 83.7, 87.1, 127.1, 127.4, 127.5, 127.7, 127.8, 127.83, 127.9, 128.0, 128.1, 128.14, 128.35, 128.5, 128.57, 128.59, 128.64, 130.5, 130.7, 133.3, 138.7, 138.9, 139.1.
\( (3R,6S,7R,8R,8aR)-3\)-propylcatalydrindolizine-6,7,8-tiol \( ((R)-363) \).

\( (R)-399 \) (100 mg, 0.21 mmol) was subjected to general procedure H. The crude material was suspended in \( \text{H}_2\text{O} \) and a few drops of \( \text{MeOH} \) was added until a clear solution was obtained. The resulting solution was applied to a column loaded with DOWEX-resin (\( \text{H}^+ \) form). The column was rinsed with \( \text{H}_2\text{O} \) and the product was eluted with a 5\% \( \text{NH}_4\text{OH} \) solution. The title compound was obtained in 93\% yield. MS analysis: ES (ESI): \( m/z = 216.13 \ [M + H]^+ \).

\[ \text{H NMR (400 MHz; CDCl}_3\text{):} \delta \ 0.76 \ (t, \ 3\text{H}, J = 7.2, H1'), 0.93-1.12 \ (m, 2\text{H}, H2'), 1.12-1.26 \ (m, 1\text{H}, H2α), 1.35-1.50 \ (m, 2\text{H}, H2β, H3'α), 1.50-1.62 \ (m, 1\text{H}, H1α), 1.75-2.00 \ (m, 2\text{H}, H1β, H3'β), 2.37 \ (dd, 1\text{H}, J = 11.0, 12.59, H5α), 2.58 \ (dt, 1\text{H}, J = 6.7, 9.1, H8a), 2.80-2.91 \ (m, 1\text{H}, H3), 2.98 \ (dd, 1\text{H}, J = 5.0, 12.7, H3', H5β), 3.04-3.16 \ (m, 2\text{H}, H7, 8), 3.5-3.61 \ (m, 1\text{H}, H6). \]

\[ \text{13C NMR (100 MHz, CDCl}_3\text{):} \delta \ 13.5, 19.3, 25.9, 27.8, 33.3, 48.9, 59.5, 63.2, 68.1, 73.0, 78.4. \]

\[ [\alpha]^{20}_{D} = -24.8° \ (c = 0.46, \text{H}_2\text{O}). \]

\( (2S,3S,4R,5R)\)-5-(\( (S\)-hex-1-en-3-ylamino)-7-phenylhept-6-ene-1,2,3,4-tetraol \( ((S)-392) \).

The HCl salt of substituted amine 390 (13.2 mmol, 1.80 g) was used in general procedure L. After extractive work-up, the title compound was obtained in 79\% yield. \( R_F = 0.2 \) (MeOH/EtOAc/\( \text{NH}_4\text{OH}, 10:12:3 \)). \[ \text{H NMR (400 MHz; CDCl}_3\text{):} \delta \ 0.84 \ (t, \ 3\text{H}, J = 7.3, H1'), 1.11-1.40 \ (m, 2\text{H}, H2'), 1.56-1.75 \ (m, 1\text{H}, H3'α), 1.75-1.91 \ (m, 1\text{H}, H3'β), 3.34-3.49 \ (m, 1\text{H}, H4'), 3.65-3.85 \ (m, 2\text{H}, H2,4), 3.87-4.02 \ (m, 1\text{H}, H5), 4.17-4.27 \ (m, 1\text{H}, H5), 5.08-5.42 \ (m, 2\text{H}, H6'), 5.69-5.84 \ (m, 1\text{H}, H5'), 6.34 \ (dd, 1\text{H}, J = 8.9, 15.0, H6), 6.69 \ (d, 1\text{H}, J = 10.6, H7), 7.21-7.34 \ (m, 3\text{H}, Har), 7.43-7.51 \ (m, 2\text{H}, Har). \]

\[ \text{13C NMR (100 MHz, CDCl}_3\text{):} \delta \ 13.7, 19.01, 35.4, 59.6, 61.6, 64.0, 71.7, 72.1, 72.7, 121.1, 126.9, 128.5, 128.7, 135.0, 135.7, 137.4. \]

\( \text{4-O-trityl-(2S,3S,4R,5R)\)-5-(\( (S\)-hex-1-en-3-ylamino)-7-phenylhept-6-ene-2,3,4-triol \( ((S)-393) \). Tetroil (S)-392 (1.7 g, 5.2 mmol) was used in general procedure C. After FCC (Et\(_2\)O/EtOAc 1:0 \rightarrow 0:1 then 5 \% \( \text{NH}_4\text{OH} \) in EtOAc/MeOH 9:1), the title compound was obtained in 53\% yield. \( R_F = 0.4 \) (5\% \( \text{NH}_4\text{OH} \) in EtOAc/MeOH 9:1). \[ \text{H NMR (400 MHz; MeOD):} \delta \ 0.93 \ (t, \ 3\text{H}, J = 7.2, H1'), 1.24-1.52 \ (m, 2\text{H}, H2'), 1.56-1.71 \ (m, 1\text{H}, H3'α), 1.72-1.80 \ (m, 1\text{H}, H3'β), 3.17 \ (dd, 1\text{H}, J = 5.2, 8.9, H1α), 3.29-3.35 \ (m, 2\text{H}, H1β, H4'), 3.62-3.72 \ (m, 1\text{H}, H5), 3.83-3.99 \ (m, 3\text{H}, H2,3,4), 5.11-5.28 \ (m, 2\text{H}, H6'), 5.61-5.67 \ (m, 1\text{H}, H5'), 6.25-6.38 \ (m, 1\text{H}, H6), 6.63 \ (d, 1\text{H}, J = 16.0, H7), 7.07-7.54 \ (m, 20\text{H}, Har). \]

\[ \text{13C NMR (100 MHz, MeOD):} \delta \ 13.1, 18.7, 38.8, 58.5, 59.6, 64.4, 70.0, 71.5, 71.9, 86.5, 116.7, 126.2, 126.6, 127.4, 128.4, 128.5, 133.8, 136.8, 139.5, 144.0. \]
9. Synthesis of C-3 substituted 1-deoxy castanospermine derivatives

\[
(1R,2S,3S)-1-((2R,5S)-5-propyl-2,5-dihydro-1H-pyrrole-2-yl)-4-trityloxy)butane-1,2,3-triol ((S)-394).
\]

Diene (S)-393 (0.92 g, 1.5 mmol) was used in general procedure B. After FCC (PE/Et₂O 1:0 → 0:1), the title compound was obtained in 90% yield. 

\[ ^1H \text{NMR (400 MHz; MeOD)} \delta 1.03 (t, 3H, J = 7.3, H8'), 1.42-1.56 (m, 2H, H7'), 1.69-1.80 (m, 2H, H6'), 3.23 (dd, 1H, J = 5.7, 9.6, H4 α), 3.32-3.41 (m, 1H, H4 β), 3.79-3.85 (m, 1H, H1), 5.91 (d, 1H, J = 6.4, H4'), 6.07 (d, 1H, J = 6.4, H3'), 7.16-7.56 (m, 15H, Har). \]

\[ ^13C \text{NMR (100 MHz, MeOD): } \delta 12.6, 18.9, 34.5, 64.7, 65.8, 68.9, 60.0, 71.0, 71.1, 125.4, 126.8, 127.5, 128.4, 128.5, 131.0, 144.0. \]

\[
(2R,5S)-\text{tert-butyl} \ 2\text{-propyl-5-}((1R,2S,3S)-1,2,3-trihydroxy-4-(trityloxy)-butyl)-2,5-dihydro-1H-pyrrole-1-carboxylate ((S)-395).
\]

Amine (S)-394 (2.5 mmol, 1.2 g) was used in general procedure A. After FCC (PE/Et₂O, 1:0 → 1:2), the title compound was isolated in 86% yield.

\[ R_F = 0.7 (4:1 \text{ Et}_2\text{O/PE}). \]

\[ ^1H \text{NMR (400 MHz; CDCl}_3\text{): } \delta 0.84-0.97 (m, 3H, H8'), 1.10-1.44 (m, 2H, H7'), 3.32-3.43 (m, 1H, H4 α), 3.47-3.61 (m, 1H, H4 β), 3.88-4.00 (m, 2H, H2, H3), 4.45-4.54 (m, 1H, H1), 5.63 (d, 1H, J = 7.1, H5'), 5.80-5.90 (m, 1H, H3'), 5.97-6.01 (m, 1H, H4'), 7.19-7.59 (m, 15H, Har). \]

\[ ^13C \text{NMR (100 MHz, CDCl}_3\text{): } \delta 12.6, 18.9, 34.5, 64.3, 65.8, 65.9, 66.7, 64.8, 72.8, 77.9, 86.7, 127.0, 127.8, 128.5, 130.3, 143.9, 157.1. \]

\[
(2R,5S)-\text{tert-butyl} \ 2\text{-propyl-5-}((1R,2S,3S)-1,2,3-tris(benzyloxy)-4-hydroxybutyl)-2,5-dihydro-1H-pyrrole-1-carboxylate ((S)-396).
\]

Triol (S)-395 (2.5 g, 4.3 mmol) was benzylated according to general procedure D. After FCC (PE/Et₂O 1:0 → 1:1), the title compound was obtained in 92% yield.

\[ ^1H \text{NMR (400 MHz; CDCl}_3\text{): } \delta 0.93-1.06 (m, 3H, H8'), 1.10-1.68 (m, 13H, H7', tBu), 3.52-3.69 (m, 1H, H4 α), 3.99-4.21 (m, 3H, H1, H4 β), 4.30-5.11 (m, 7H, 3x CH₂Ph, H2'), 5.73-5.82 (m, 1H, H3), 5.55-5.76 (m, 2H, H3', H4'), 7.18-7.61 (m, 30H, Har). \]

\[ ^13C \text{NMR (100 MHz, CDCl}_3\text{): } \delta 12.6, 18.9, 34.5, 64.3, 64.9, 69.5, 70.0, 70.7, 71.1, 73.4, 73.5, 73.7, 73.9, 74.2, 75.8, 77.34, 80.0, 80.3, 80.5, 80.9, 81.6, 93.0, 83.8, 84.6, 85.6, 127.7, 127.7, 127.9, 129.7, 129.1, 129.8, 129.9, 134.6, 137.3, 137.5, 137.7, 137.8, 138.1, 138.3, 138.4, 153.0, 153.2 (multiple rotameric signals were observed). \]

\[
(2R,5S)-\text{tert-butyl} \ 2\text{-propyl-5-}((1R,2S,3S)-1,2,3-tris(benzyloxy)-4-hydroxybutyl)-2,5-dihydro-1H-pyrrole-1-carboxylate ((S)-397).
\]

Compound (S)-396 (2.5 g, 2.9 mmol) was used in general procedure E. After FCC (PE/Et₂O, 1:0 → 1:1), the title compound was obtained in 81% yield.

\[ ^1H \text{NMR (400 MHz; CDCl}_3\text{): } \delta 1.01 (t, 3H, H8'), 1.18-1.48 (m, 2H, H7'). \]
9.4 Experimental section

1.61 (s, 9H, tBu), 1.71-2.02 (m, 2H, H6’), 3.47-3.59 (m, 1H, H2), 3.69-4.05 (m, 4H, H1,3,4), 4.17-5.03 (m, 8H, 3x CH2Ph, H2’, H5’), 5.76-5.88 (m, 1H, H4’), 5.90-6.01 (m, 1H, H3’), 7.25-7.48 (m, 15H, Har). 13C NMR (100 MHz, CDCl3): δ 14.2, 14.3, 17.1, 17.3, 25.4, 61.3, 61.4, 61.8, 64.2, 64.6, 64.9, 65.9, 68.0, 72.9, 73.0, 73.2, 74.0, 74.3, 74.6, 74.9, 75.1, 78.7, 79.2, 79.5, 79.6, 80.0, 80.2, 80.7, 81.1, 126.9, 127.0, 127.6, 127.7, 127.8, 127.9, 128.0, 128.03, 128.3, 128.4, 128.5, 128.7, 130.6, 130.9, 131.0, 138.0, 138.02, 138.2, 138.3, 138.5, 138.7, 153.1, 153.9 (multiple rotameric signals were observed).

Alcohol (S)-397 (0.6 g, 1.0 mmol) was used in general procedure F/G. After FCC (PE/Et2O, 1:0 → 1:1), the title compound was isolated in 43% yield over three steps. 1H NMR (400 MHz; CDCl3): δ 1.0 (t, 3H, J = 7.1, H1’), 1.27-1.70 (m, 4H, H2’, H3’), 2.41 (t, 1H, J = 6.1, H5α), 3.24-3.32 (m, 1H, H6), 3.37 (dd, 2H, J = 5.7, 10.5, H5β), 3.41-3.53 (m, 1H, 8a), 3.65 (t, 1H, J = 8.6, 1H, H7), 3.74-3.85 (m, 1H, H3), 4.45-5.11 (m, 6H, 3x CH2Ph), 5.87-5.92 (m, 1H, H2), 6.01 (d, 1H, J = 6.1, H1), 7.26-7.57 (m, 15H, Har). 13C NMR (100 MHz, CDCl3): δ 14.7, 19.0, 35.2, 49.7, 67.0, 72.0, 73.0, 74.8, 75.8, 80.9, 93.8, 87.9, 127.6, 127.8, 128.0, 128.00, 128.02, 128.4, 128.5, 128.6, 133.9, 138.7, 138.9, 139.2.

Alcohol (S)-397 (0.6 g, 1.0 mmol) was used in general procedure F/G. After FCC (PE/Et2O, 1:0 → 1:1), the title compound was isolated in 43% yield over three steps. 1H NMR (400 MHz; CDCl3): δ 1.0 (t, 3H, J = 7.1, H1’), 1.27-1.70 (m, 4H, H2’, H3’), 2.41 (t, 1H, J = 6.1, H5α), 3.24-3.32 (m, 1H, H6), 3.37 (dd, 2H, J = 5.7, 10.5, H5β), 3.41-3.53 (m, 1H, 8a), 3.65 (t, 1H, J = 8.6, 1H, H7), 3.74-3.85 (m, 1H, H3), 4.45-5.11 (m, 6H, 3x CH2Ph), 5.87-5.92 (m, 1H, H2), 6.01 (d, 1H, J = 6.1, H1), 7.26-7.57 (m, 15H, Har). 13C NMR (100 MHz, CDCl3): δ 14.7, 19.0, 35.2, 49.7, 67.0, 72.0, 73.0, 74.8, 75.8, 80.9, 93.8, 87.9, 127.6, 127.8, 128.0, 128.00, 128.02, 128.4, 128.5, 128.6, 133.9, 138.7, 138.9, 139.2.

(3S,6S,7R,8R,8aR)-6,7,8-tris(benzyloxy)-3-propyl-3,5,6,7,8,8a-hexahydroindolizine ((S)-399).

Alcohol (S)-397 (0.6 g, 1.0 mmol) was used in general procedure F/G. After FCC (PE/Et2O, 1:0 → 1:1), the title compound was isolated in 43% yield over three steps. 1H NMR (400 MHz; CDCl3): δ 1.0 (t, 3H, J = 7.1, H1’), 1.27-1.70 (m, 4H, H2’, H3’), 2.41 (t, 1H, J = 6.1, H5α), 3.24-3.32 (m, 1H, H6), 3.37 (dd, 2H, J = 5.7, 10.5, H5β), 3.41-3.53 (m, 1H, 8a), 3.65 (t, 1H, J = 8.6, 1H, H7), 3.74-3.85 (m, 1H, H3), 4.45-5.11 (m, 6H, 3x CH2Ph), 5.87-5.92 (m, 1H, H2), 6.01 (d, 1H, J = 6.1, H1), 7.26-7.57 (m, 15H, Har). 13C NMR (100 MHz, CDCl3): δ 14.7, 19.0, 35.2, 49.7, 67.0, 72.0, 73.0, 74.8, 75.8, 80.9, 93.8, 87.9, 127.6, 127.8, 128.0, 128.00, 128.02, 128.4, 128.5, 128.6, 133.9, 138.7, 138.9, 139.2.

(3S,6S,7R,8R,8aR)-3-propyloctahydroindolizine-6,7,8-triol ((S)-363).

(S)-399 (100 mg, 0.21 mmol) was subjected to general procedure H. The crude material was suspended in H2O and a few drops of MeOH were added until a clear solution was obtained. The resulting solution was applied to a column loaded with Dowex-resin (H+ form). The column was rinsed with H2O and the product was eluted with a 5% NH4OH solution. The title compound was obtained in 51% yield. 1H NMR (400 MHz; D2O): δ 0.77 (t, 3H, J = 7.3, H1’), 0.93-1.37 (m, 5H, H2’, H1α, H2α, H3’α), 1.53-1.63 (m, 1H, H2β), 1.75-2.00 (m, 3H, H1β, H3’β, H5α), 2.00-2.06 (m, 1H, H8a), 2.15-2.26 (m, 1H, H3), 3.08-3.21 (m, 3H, H5β, H7, H8), 3.49-3.55 (m, 1H, H3). 13C NMR (100 MHz, D2O): δ 13.6, 19.5, 25.4, 28.5, 34.4, 53.6, 64.5, 68.0, 70.5, 74.4, 78.7. [α]20 = +79.8° (c = 0.84, H2O).

(2S,3S,4R,5R,6E)-5-(((S)-7-adamantan-1-ylmethoxy)hept-1-en-3-yl)amino-7-phenylhept-6-ene-1,2,3,4-tetraol ((S)-400).

The HCl salt of substituted amine 384 (0.81 g, 2.9 mmol) was used in general procedure L with omission of the extractive work-up procedure. Upon complete consumption of the starting material, as judged by TLC analysis, the reaction mixture was concentrated. The residue was subjected to FCC (Et2O/EtOAc/MeOH 1:0:0 → 0:9:1 with 5 vol% NH4OH), to provide the title compound in 76% yield. MS analysis: ES (ESI): m/z = 514.3 [M + H]+. Rf =
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0.4 (5% NH₄OH in EtOAc/MeOH, 9:1). ¹H NMR (400 MHz; CDCl₃): δ 1.12-1.58 (m, 12H, 6x CH₂, H4’, 5’, 6’, 10’, 16’, 17’), 1.62-1.77 (m, 6H, H12’, 14’, 18’), 1.94 (br. s, 3H, H11’, 13’, 15’), 2.95 (s, 2H, H8’), 3.17-3.30 (m, 1H, H5), 3.35 (t, 2H, J = 6.3, H7’), 3.60-3.96 (m, 6H, H1-4, H3’), 4.50-4.95 (br. s, 4H, 4x OH), 5.09-5.22 (m, 2H, H1’), 3.61-5.79 (m, 1H, H2’), 6.20 (dd, 1H, J = 8.8, 15.8, H6), 6.56 (d, 1H, J = 15.8, H7), 7.22-7.45 (m, 5H, Har).

¹³C NMR (100 MHz, CDCl₃): δ 22.1, 28.3, 29.5, 29.7, 33.2, 34.0, 37.3, 39.7, 58.9, 61.6, 64.5, 71.3, 72.0, 72.9, 74.1, 81.9, 117.0, 125.3, 126.7, 134.2, 136.3, 139.6.

\[
\text{NH-TrtO} \quad \text{OH} \quad \text{OH} \quad \text{OH} \quad \text{O} \\
(2S,3S,4R,5R,6E)-5-(((S)-7-adamantan-1-ylmethoxy)hept-1-en-3-yl)amino-7-phenyl-1-(trityloxy)hept-6-ene-2,3,4-triol ((S)-401).
\]

Tetrol (S)-400 (5.2 g, 18.7 mmol) was used in general procedure C. After FCC (Et₂O/EtOAc/MeOH 1:0:0 → 0:9:1), the title compound was isolated in 61% yield. \( R_F = 0.7 \) (EtOAc/MeOH, 9:1). ¹H NMR (400 MHz; CDCl₃): δ 1.12-1.58 (m, 12H, 6x CH₂, H4’, 5’, 6’, 10’, 16’, 17’), 1.62-1.79 (m, 6H, H12’, 14’, 18’), 1.97 (br. s, 3H, H11’, 13’, 15’), 2.97 (s, 2H, H8’), 3.15 (dd, 1H, J = 7.1, 14.52, H5), 3.32 (d, 2H, J = 4.5, H1), 3.38 (t, 2H, J = 6.5, H7’), 3.73-3.98 (m, 7H, H2,3,4, H3’, 3x OH), 5.04-5.24 (m, 2H, H1’), 5.45-5.56 (m, 1H, H2’), 6.13 (dd, 1H, J = 7.9, 15.9, H6), 6.56 (d, 1H, J = 16.0, H7), 7.18-7.53 (m, 20H, Har).

¹³C NMR (100 MHz, CDCl₃): δ 22.6, 28.3, 29.5, 29.7, 33.8, 35.9, 37.3, 39.8, 58.2, 61.0, 65.0, 70.6, 71.4, 73.2, 75.6, 81.9, 86.9, 117.2, 126.5, 127.1, 127.3, 127.7, 127.8, 129.7, 128.7, 134.2, 136.5, 139.8, 143.7.

\[
\text{NH-TrtO} \quad \text{OH} \quad \text{OH} \quad \text{OH} \\
(1R,2S,3S)-1-((2R,5S)-5-(4-adamantan-1-ylmethoxy)butyl)-2,5-dihydro-1H-pyrrol-2-yl-4-(trityloxy)butane-1,2,3-triol ((S)-402).
\]

Diene (S)-401 (4.8 g, 6.4 mmol) was used in general procedure B. After FCC (Et₂O/PE , 1:1 then 100% EtOAc), the title compound was isolated in 68% yield. \( R_F = 0.4 \) (100% EtOAc). ¹H NMR (400 MHz; CDCl₃): δ 1.12-1.58 (m, 12H, 6x CH₂, H4’, 5’, 6’, 10’, 16’, 17’), 1.97 (br. s, 3H, H11’, 13’, 15’), 2.97 (s, 2H, H8’), 3.15 (dd, 1H, J = 7.1, 14.52, H5), 3.32 (d, 2H, J = 4.5, H1), 3.38 (t, 2H, J = 6.5, H7’), 3.73-3.98 (m, 7H, H2,3,4, H3’, 3x OH), 5.04-5.24 (m, 2H, H1’), 5.45-5.56 (m, 1H, H2’), 6.13 (dd, 1H, J = 7.9, 15.9, H6), 6.56 (d, 1H, J = 16.0, H7), 7.18-7.53 (m, 20H, Har).

¹³C NMR (100 MHz, CDCl₃): δ 23.3, 26.3, 26.8, 26.9, 28.3, 29.4, 34.1, 35.9, 37.3, 39.8, 58.2, 61.0, 65.0, 70.6, 71.4, 73.2, 75.6, 81.9, 86.9, 117.2, 126.5, 127.1, 127.3, 127.7, 127.8, 129.7, 128.7, 134.2, 136.5, 139.8, 143.7.
82% yield. $^1$H NMR (400 MHz; CDCl$_3$) $\delta$:1.33-1.62 (m, 21H, tBu, H6’,7’,8’, H12’, 19’, 18’). 1.62-1.81 (m, 1H, H5’), 3.13-3.26 (m, 1H, H1α), 3.31-3.46 (m, 4H, H9’, H1β), 3.55-3.61 (m, 1H, H3), 3.80 (s, 3H, 3x OH), 4.06-4.18 (m, 6H, 3x CH$_2$Ph), 7.04-7.70 (m, 15H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$: 22.7, 28.3, 32.4, 34.1, 37.3, 39.8, 64.0, 65.9, 66.8, 68.8, 71.3, 72.8, 77.8, 81.9, 86.7, 127.0, 127.8, 128.7, 130.2, 143.9, 157.0.

(2S,5R)-tert-butyl 2-(4-(adamantan-1-ylmethoxy)butyl)-5-((1R,2S,3S)-1,2,3-tris(benzyloxy)-4-(trityloxy)butyl)-2,5-dihydro-1H-pyrrole-1-carboxylate ((S)-404).

Compound (S)-403 (2.5 g, 3.3 mmol) was used in general procedure D. After FCC (PE/Et$_2$O 1:0 $\rightarrow$ 1:1), the title compound was isolated in near quantitative yield. $^1$H NMR (400 MHz; CDCl$_3$) $\delta$:1.25-1.62 (m, 21H, tBu, H6’,7’,8’, H12’, 19’, 18’). 1.66-1.82 (m, 6H, H14’, 16’, 20’), 2.01 (br. s, H13’,15’,17’), 2.96 (s, 2H, H10’), 3.25-3.35 (m, 2H, H9’), 3.46-3.60 (m, 2H, H1), 3.66-3.96 (m, 2H, H2, H5’), 4.06-4.18 (m, 6H, 3x CH$_2$Ph), 5.37-6.00 (m, 2H, H3’, 4’), 7.04-7.70 (m, 30H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$: 23.1, 28.4, 28.6, 29.6, 34.1, 37.3, 39.8, 63.6, 65.3, 66.0, 67.5, 71.5, 73.3, 74.6, 75.1, 75.6, 78.8, 79.1, 79.5, 80.6, 81.8, 87.0, 125.6, 126.9, 127.5, 127.6, 127.8, 128.1, 128.4, 128.8, 128.82, 130.9, 131.2, 138.4, 138.7, 144.1, 154.6. (Multiple rotameric signals were observed.)

(2S,5R)-tert-butyl 2-(4-(adamantan-1-ylmethoxy)butyl)-5-((1R,2S,3S)-1,2,3-tris(benzyloxy)-4-hydroxybutyl)-2,5-dihydro-1H-pyrrole-1-carboxylate ((S)-405).

Compound (S)-404 (2.6 g, 2.5 mmol) was used in general procedure E. After FCC (PE/Et$_2$O 1:0 $\rightarrow$ 1:1), the title compound was isolated in 92% yield. $^1$H NMR (400 MHz; CDCl$_3$) $\delta$: 1.23-1.63 (m, 21H, tBu, H6’,7’,8’, H12’, 19’, 18’). 1.63-1.78 (m, 6H, H14’, 16’, 20’), 2.01 (br. s, H13’,15’,17’), 2.21-2.40 (br. s, 1H, OH), 2.96 (s, 2H, H10’), 3.25-3.35 (m, 2H, H9’), 3.46-3.60 (m, 2H, H1), 3.62-3.78 (m, 1H, H1β), 4.65-5.02 (m, 6H, 3x CH$_2$Ph), 5.78-5.90 (m, 1H, H3’), 5.90-6.00 (m, 1H, H4’), 7.02-7.50 (m, 15H, Har). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$: 22.3, 28.3, 29.9, 32.4, 34.1, 37.3, 39.8, 71.5, 73.0, 74.8, 81.9, 127.3, 127.5, 127.7, 127.9, 128.4, 128.44. (Some CH-signals were not observed on $^{13}$C APT-spectrum.)

(3S,6S,7R,8R,8aR)-3-(4-(adamantan-1-ylmethoxy)butyl)-6,7,8-tris(benzyloxy)-3,5,6,7,8,8a-hexahydropindolizine ((S)-407).

Alcohol (S)-405 (1.8 g, 2.3 mmol) was used in general procedure F/G. After FCC (PE/Et$_2$O 1:1, isocratic), the title compound was obtained in 58% yield over 3 steps. $^1$H NMR (400 MHz; CDCl$_3$) $\delta$: 1.30-1.70 (12H,
9. Synthesis of C-3 substituted 1-deoxy castanospermine derivatives

H1’,H2’, H3’, H7’, H13’, H14’), 1.70-1.85 (m, 6H, H9’, H11’, H15’), 2.04 (br. s, 3H, H8’, H10’, H12’), 2.38 (t, 1H, J= 10.2, H5α), 3.05 (s, 2H, H5’), 3.20-3.28 (m, 1H, H3), 3.34 (dd, 1H, J= 5.6, 10.5, H5β), 3.38-3.50 (m, 3H, H4’, H8), 3.61 (t, 1H, J= 8.6, H7), 3.68-3.81 (m, 1H, H6), 4.69-5.12 (m, 6H, 3x CH2Ph), 5.86 (d, 1H, J= 5.3, H2), 6.01 (d, 1H, J= 6.0, H1), 7.16- 7.59 (m, 15H, Har). 13C NMR (100 MHz, CDCl3): δ 22.1, 28.2, 29.6, 29.8, 32.4, 34.0, 37.2, 39.7, 49.4, 66.9, 71.4, 71.8, 72.8, 74.6, 75.6, 76.7, 77.0, 77.3, 80.8, 81.8, 82.9, 87.7, 127.4, 127.6, 127.7, 127.8, 128.2, 128.3, 128.5, 128.7, 130.8, 133.6, 133.8, 138.5, 138.7, 139.0.

(NH)O

Ohio

(3S,6S,7R,8R,8aR)-3-(4-adamantan-1-ylmethoxy)butyl)octahydro - indolizine-6,7,8-triol ((S)-364). Derivative (S)-407 (100 mg, 0.15 mmol) was subjected to general procedure H. The crude material was suspended in H2O and a few drops of MeOH were added until a clear solution was obtained. The resulting solution was applied to a column loaded with DOWEX-resin (H+ form). The column was rinsed with H2O and the product was eluted with a 5% NH4OH solution. The title compound was obtained in 73% yield. 1H NMR (400 MHz; CDCl3/MeOD, 1:1): δ 1.09-1.78 (m, 18H, 6x CH2Ada, H1’, 2’, 3’), 1.87 (t, 1H, J= 10.5, H5α), 1.92-2.11 (m, 4H, 3x CHAda, H3), 2.17-2.28 (m, 1H, H8a), 2.98 (s, 2H, H5’), 3.14-3.27 (m, 3H, H5β, H7, H8), 3.41 (t, 2H, H4’), 3.55-3.64 (m, 1H, H6), 4.12 (br. s, 1H, OH), 4.29 (br. s, 2H, 2x OH). 13C NMR (100 MHz, CDCl3/MeOD, 1:1): δ 22.9, 25.6, 28.0, 28.8, 29.4, 32.7, 36.9, 39.4, 54.1, 64.6, 68.3, 70.5, 71.3, 74.5, 79.4, 81.7. [α]D20 = -37.6° (c = 0.50, H2O).
Summary and future prospects

Summary

The exploitation of the unusual hydrophobic properties of diamondoid derivatives has been the primary goal of the research described in this Thesis. A general introduction into the diamondoid family is given in Chapter 1 and the earliest attempts to man made diamonds, the ultimate synthetic diamondoid, are discussed first. Next, the natural occurrence and the synthesis of the lower diamondoids being ada-, dia- and triamantane (1, 2 and 3) are discussed. In particular, the Lewis acid promoted carbocation rearrangement pathway, discovered by Schleyer et al.\textsuperscript{8} for the synthesis of adamantane (1) proved to be invaluable in the preparation of dia- and triamantane (2 and 3). The functionalization of the diamondoid scaffold lies at the basis for their exploration in different fields of research. Selective functionalization of the diamondoid frame-work is hard to obtain due to its highly symmetrical nature. However, the ionic-bridgehead derivatization strategy represent a straightforward method and a selection of the ionic bromination, nitration and carbonylation of the lower diamondoids is presented.

\[1\text{-Adamantaneacetic acid (141)}\] was used as a protective group in the solution-phase synthesis of (phosphorothioate) oligonucleotides and this subject is introduced in Chapter 2. Pioneered by de Koning et al. a Solution-Phase Extraction Method (SPEM) was developed for the preparation of ON fragments (Scheme 10.1). The formation of the internucleosidic phosphodiester (PO) or phosphorothioate (PS) linkage was achieved by phosphoramidite mediated chain elongation. \(3'\text{-O-adamantane acyl protection was used in combination with commercially available (except for thymidine) 5'\text{-O-DMTr phosphoramidite building blocks for 3'} \rightarrow 5'\text{ directed chain elongation. Furthermore, only extractive work-up procedures were applied for the isolation of protected intermediate ON fragments. The developed SPEM approach was optimized by the implementation of a capping step to minimize n-1 deletion fragments, resulting from incomplete coupling reactions.}
**Scheme 10.1:** Solution-Phase Extraction Method (SPEM) for oligonucleotide synthesis.

Left: $5' \rightarrow 3'$ directed elongation. Right: $3' \rightarrow 5'$ directed elongation. **Reagent and conditions:** i) Elongation: 4,5-dicyanoimidazole, dry CH$_3$CN; ii) Capping: 1 eq. **100**; iii) Oxidation: X = O, 0.2M I$_2$ in H$_2$O/pyridine (5:1, v/v), Sulfurisation: X = S, phenylacetyl disulphide (105) then 3H-1,2-benzodithiol-3-one (103) then TCEP·HCl in Pyr, extractive work-up 1; iv) 5'-O-DMTr cleavage: 0.1M HCl in MeOH, extractive work-up 2; v) 3'-O-Lev cleavage: 0.5M N$_2$H$_4$·H$_2$O in Pyr/AcOH (4:1, v/v), extractive work-up 2.
The effectiveness of the SPEM approach was demonstrated with the synthesis of a decameric oligonucleotide fragment. After nine successive coupling cycles on 0.5 mmol scale via a SPEM approach, partially protected oligonucleotide fragment $\text{HO}A^BzG^iBuCBz^T\text{Pom}^T\text{Pom}^G^iBuCBz^T\text{Pom}^T\text{Pom}$ was isolated in 70% overall yield.\(^a\) For the synthesis of phosphorothioate (PS) ON via the SPEM approach, a two step sulfurization protocol was developed. The desired phosphorothioate triester was obtained by exposure of the reactive phosphite triester to phenylacetyl disulfide (PADS, 105). Extractive removal of the excess phosphoramidite donor was possible after its hydrolysis and conversion into a charged phosphorothioate diester by reaction with 3-$H$-benzo[1,2]dithiol-3-one (103). Partially protected phosphorothioate fragment $\text{HO}G^iBuA^BzCBz^G^iBu^T\text{Pom}^T\text{Pom}^T\text{Pom}^OAda$ was obtained in 67% overall yield.\(^b\)

The preparation of large ON fragments by stepwise chain assembly is cumbersome due to the highly polar nature of (partially protected) ON fragments and usually block-coupling strategies are adopted. The convergent approach requires two, suitably protected ON fragments of which the first fragment (with free 5'-OH) is excessable via the 3'→5' SPEM approach. To generate the second fragment (with free 3'-OH) needed for block-coupling strategies, the 5'→3' directed solution-phase ON synthesis via a SPEM approach has been explored in Chapter 3. In this case, 5’-O-adamantane acyl protection was used in combination with 3’-O-Lev phosphoramidite building blocks. After the execution of five coupling cycles, partially protected hexameric ON fragment $\text{AdaO}T\text{Pom}^T\text{Pom}^A^BzCBz^T\text{Pom}^G^iBu^T\text{Pom}^OAda$ was obtained in 86% overall yield\(^c\) and phosphorothioate fragment $\text{AdaO}T\text{Pom}^T\text{Pom}^G^iBu^T\text{Pom}^A^BzCBz^T\text{Pom}^OAda$ in 72% yield.\(^d\) The synthesis of levulinoyl - adamantane hybrid protective group for 3’-O-nucleoside protection is described in Chapter 4. Four hybrid protective groups (140, 144, 145 and 167, Figure 10.1) were synthesized and preliminary results indicated that the lipophilicity of the adamantan moiety with the orthogonal cleavage procedures inherent to the Lev protective group are combined in hybrid 167. However, the installment of hybrid 167 at the 3’OH of $\text{DMTr}dT\text{Pom}^T\text{Pom}^OAda$ was troublesome and requires further research to improve the esterification yields. It is envisaged that utilisation of 167 for 3’-O-nucleoside protection in a 3’→5’ SPEM approach allows the preparation of two partially protected ON fragments suitable for block-coupling strategies.

\[\text{Figure 10.1: Hybrid protective group for 3’-O-nucleoside protection.}\]

\[\text{Figure 10.1: Hybrid protective group for 3’-O-nucleoside protection.}\]

\(^a\)Complete deblocking and final chromatographic purification provided (PO) $\text{HO}AGCTATT\text{GCT}^T\text{OH}$ ON in 33% yield.

\(^b\)Complete deblocking and final chromatographic purification provided (PS) $\text{HO}GACGT^T\text{OH}$ ON in 31% yield.

\(^c\)Complete deblocking and final chromatographic purification provided (PO) $\text{HO}TTACTG^T\text{OH}$ ON in 35% yield.

\(^d\)Complete deblocking and final chromatographic purification provided (PS) $\text{HO}TTG\text{ACTG}^T\text{OH}$ ON in 33% yield.
The second part of this research is directed to explore the striking effect of the adamantane moiety as part of iminosugar-based inhibitors for the enzymes involved in glucosylceramide (GlcCer) metabolism namely glucosylceramide synthase (GCS), \( \beta \)-glucocerebrosidase (GBA1) and \( \beta \)-glucosidase (GBA2). Aerts and co-workers\textsuperscript{174,175} reported that the \( N \)-alkylation of DNJ (178, Table 10.1), a natural glycosidase inhibitor, with an \( N \)-pentyloxymethyl-1-adamantane (190) unit results in enhanced inhibitory profile compared to the marketed drug Zavesca\textsuperscript{R} possessing a \( N \)-butyl chain (189). Structure activity relationship (SAR) studies revealed that the C5-epimer of MZ-21 (190) namely \( N \)-pentyloxymethyl-1-adamantane-L-ido-DNJ (MZ-31, 191) is a more selective inhibitor compared to MZ-21 (190). The importance of potent and selective inhibitors to modulate glucosylceramide levels is briefly introduced in Chapter 5. The synthesis and evaluation of libraries of \( N \)-alkylated DNJ and L-ido-DNJ are described in Chapters 5 and 6. In these libraries the role of the adamantane unit was explored by the systematically replacement thereof with linear and branched hydrocarbons. The influence of a \( N \)-pentyloxy spacer between the iminosugar and the hydrophobic unit was demonstrated by the observation that the omission thereof (e.g. 221 vs. 203) results in decreased inhibitory potency (Table 5.1). Furthermore, the effect of an ether linkage between the hydrophobic unit and the pentyloxy spacer on the selectivity and potency towards GCS inhibition is pronounced (e.g. 260 vs. 258). Additionally, Chapter 6 describes the design and synthesis of a photo crosslinker based on MZ-21 (190).

Chapter 7 deals with the synthesis of quaternary ammonium salts of leads MZ-21 and MZ-31 by \( N \)-methylation. The ammonium salts, termed nojirimycininium ions, were found to be about 10 times less potent inhibitors compared to the parent iminosugar derivative. These findings suggest that the active site of the involved enzymes can bind nojirimycininium ions and support the general dogma that \( N \)-alkylated iminosugars act as oxocarbenium-ion transition state mimics. The synthesis of C-2 decorated imidazopyranose derivatives are described in Chapter 8. Two iminosugar derivatives decorated with either an \( \mathrm{C}_3 \mathrm{H}_6 \text{− adamantanate (347) or C}_3 \mathrm{H}_6 \text{− phenyl (348) were prepared based on the work of Vasella and co-workers. Evaluation of these derivatives against inhibition of GCS, GBA1 and GBA2 revealed that both derivatives are potent and selective GBA1 inhibitors. It was found that the adamantane derivative 347 was outperformed by the phenyl derivative 348 in terms of GBA1 inhibition. A different approach towards potent and selective inhibitors to influence GlcCer homeostasis is described in Chapter 9 by the design and synthesis of 1-deoxy castanospermine, a natural bi-cyclic iminosugar, decorated with an adamantane moiety. Castanospermine was synthesized, based on slightly modified procedures developed by the group of Pyne via a Petasis borono-Mannich reaction of \( \mathrm{L} \)-xylose, allylamine and (\( E \))-styrene boronic acid.\textsuperscript{237} C-3 modified 1-deoxy castanospermine synthesis commenced with the generation of chiral pure 2° allylic amines by stereoselective alkylation of chiral sulfinamides. A key step in the synthesis involved a ring closing metathesis reaction in the presence of a free secondary amine, under microwave irradiation conditions.
### Summary and future prospects

Table 10.1: Enzyme inhibition assay preliminary results: Apparent IC₅₀ values in micromolar (µM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R =</th>
<th>GCS</th>
<th>GBA 1</th>
<th>GBA 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>178: H</td>
<td></td>
<td>50ᵃ</td>
<td>400</td>
<td>0.23</td>
</tr>
<tr>
<td>189: C₄H₉</td>
<td></td>
<td>190: N-pentyloxymethyl-1-adamantane</td>
<td>0.2ᵃ</td>
<td>0.2</td>
</tr>
<tr>
<td>203: Nonyl</td>
<td>~ 4ᵃ</td>
<td>1.5</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>211: N-Nonoxypentyl</td>
<td>0.1ᵃ</td>
<td>0.5</td>
<td>0.040</td>
<td></td>
</tr>
<tr>
<td>258: Hexyl-1-adamantane</td>
<td>&gt;20ᵇ</td>
<td>0.6</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>191: N-pentyloxymethyl-1-adamantane</td>
<td>0.1ᵃ</td>
<td>2</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>209: Nonyl</td>
<td>2ᵃ</td>
<td>50</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>227: N-Nonoxypentyl</td>
<td>&lt;0.05ᵃ</td>
<td>12</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>261: Hexyloxy-1-adamantane</td>
<td>0.01-0.1ᵇ</td>
<td>5</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td>259: Hexyl-1-adamantane</td>
<td>1-10ᵇ</td>
<td>25</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>314: N-pentyloxymethyl-1-adamantane</td>
<td>~1ᵇ</td>
<td>2</td>
<td>~0.1</td>
<td></td>
</tr>
<tr>
<td>315: N-pentyloxymethyl-1-adamantane</td>
<td>~1ᵇ</td>
<td>6</td>
<td>~0.5</td>
<td></td>
</tr>
<tr>
<td>347: Adamantane</td>
<td>&gt;10ᵇ</td>
<td>0.02</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>348: Phenyl</td>
<td>&gt;10ᵇ</td>
<td>0.002</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

ᵃ *In situ* measurement. ᵇ *Facs* analysis.
Work in progress

The research described in this thesis aimed to exploit the highly lipophilic nature of adamantane (1) both in the solution-phase synthesis of oligonucleotide fragments and as part of iminosugar based inhibitors to influence cellular GlCer levels. However, the next member of the diamondoid family, diamantane (2) remains largely unexplored in both areas of research. Therefore, it was decided to prepare diamantane (2) and explore some derivatization strategies. The incorporation of a bromopentyloxymethyl (BrC₅H₁₀OCH₂) chain on the diamantane core is a necessity for its implementation in iminosugar based GCS inhibitors. The use of diamantane (2) as a protective group for 3'-O-nucleoside protection requires the installment of an acetic acid functionality.

The large scale preparation of diamantane (2) was executed according to reported procedures by the group of Schleyer and commenced with the [4+4] cycloaddition of norbornadiene (12, Scheme 10.2). The resulting polycyclic product, known as Binor-S (13) was obtained, after in vacuo distillation, in 80% yield. Hydrogenation of 13 was carried out in glacial acetic acid, over

Scheme 10.2: Preparation of 1-diamantane derivatives.

Reagents and conditions: i) cat. CoBr₂, 2 PPh₃, cat. BF₃·Et₂O, toluene (5.5M), 80%; ii) H₂, cat. PtO₂, AcOH (0.1M), RT, quant.; iii) cat. AlBr₃, C₆H₁₂ (0.1M), 64%.; iv) Br₂ (~1M), 2 h, RT, quant.; v) aq. HCl (0.8M)/DMF (1:1, 0.2M), 2 h, Δ, 96%; vi) CCl₄, conc. H₂SO₄, formic acid, 0°C, quant.; vii) LiAlH₄ (2.3 eq.), THF (0.1M), 77%; viii) 5-(benzyloxy)pentyl p-toluenesulfonate (1.5 eq.), NaH (1.5 eq.), cat. TBAI, DMF(0.1M), 22%; ix) 5 mol% Pd/C (10 wt%), H₂, THF (0.2M), 12 h, RT, 75%; x) PPh₃ (2 eq.), CBr₄ (2 eq.), CH₂Cl₂ (0.1M), 2 h, 0°C, 59%.
Adams catalyst at 4 bar \( \text{H}_2 \). It is noteworthy that the addition of concentrated hydrochloric acid and elevated temperatures, as dictated by Schleyer \textit{et al.}\textsuperscript{35} can be omitted during hydrogenation of 13 if the operating pressure is maintained at \( \sim 3.5 - 4 \text{ bar} \). Subsequent AlBr\(_3\) mediated rearrangement of hydrogenated product 14 provided diamantane (2) in 64\% yield as colourless crystals. The identity and homogeneity of diamantane was proven by NMR, MS and GC and by comparison (co-elution on GC) with an authentic sample which was obtained commercially.

\textbf{1-diamantanoids:} Diamantane (2) has two types of bridgehead positions namely 6 equivalent medial- and 2 equivalent apical bridgeheads. The medial or belt positions of diamantane (2) are the most reactive in ionic functionalization. Although carboxylation of diamantane (2) under Koch-Haaf conditions\textsuperscript{44} is reported to produce 1-diamantancarboxylic acid (53)\textsuperscript{59} in moderate yields, application of these conditions to diamantane (and also adamantane) was not productive. Therefore the mild ionic bridgehead bromination of diamantane in Br\(_2\) was explored, which resulted in the exclusive formation of 1-bromodiamantane (28) in excellent yield (Scheme 10.2). Acidic hydrolysis of 28 in aq. DMF at elevated temperatures gave 1-diamantanol (41) in quantitative yield. The Koch-Haaf reaction to introduce a carboxyl group was explored utilizing 41 as the starting material. First, the reaction conditions were optimized with 1-adamantanol which yielded crude 1-adamantanecarboxylic acid in near quantitative yield and high purity. Under the optimal conditions, the Koch-Haaf carboxylation of alcohol 41 gave 1-diamantancarboxylic acid (53) in near quantitative yield after precipitation in H\(_2\)O. Reduction of carboxylic acid 53 with LiAlH\(_4\) in THF proceeded slowly, and gave pure 1-diamantanemethanol (409) in 77\% yield. Alkylation of 409 with 5-(benzylxylo)pentyl p-toluenesulfonate was sluggish and benzyl ether 410 was isolated in 22\% yield (29\% based on recovered starting material). After Pd/C mediated cleavage of the benzyl ether in 410 and subsequent bromination \textit{via} of resulting alcohol 411 \textit{via} the Appel reaction, bromide 412 was isolated in 59\% yield.

\textbf{3-diamantanoids:} The synthesis of 3-diamantanone (60) was executed according to procedures described by Schlyer \textit{et al.}\textsuperscript{54,59} using diamantane (2) as the starting compound. Oxidation of 2 in concentrated H\(_2\)SO\(_4\) gave 60 in 74\% yield (Scheme 10.3). 3-Diamantanone (60) was transformed to 3-diamantanemethanol (415) in 61\% yield, analogously to the preparation of 2-adamantanemethanol (264). Subsequent alkylation of alcohol 415 with 5-(benzylxylo)pentyl p-toluenesulfonate went smoothly, giving trityl ether 416 in good yield. Acid mediated detritylation of 416 and subsequent conversion of the resulting alcohol 417 to bromide 418, proceeded without difficulties in good yields.

\textbf{4-diamantanoids:} Apical bridgehead functionalization of diamantane (2) is hampered by its inherent lower reactivity with respect to the medial bridgeheads. Recently, Schreiner and co-workers reported the development of an optimized protocol to obtain 4-diamantanol (44) from diamantane (2) via nitroxylation and subsequent isomerisation.\textsuperscript{58} To this end, diamantane (2) was triturated with CH\(_2\)Cl\(_2\) and treated with fuming HNO\(_3\) for 30 min at RT ( Scheme 10.4). The reaction mixture was concentrated and the residue was dissolved in concentrated H\(_2\)SO\(_4\) at 0\(^\circ\)C and stirring was continued for 90 min at 0\(^\circ\)C. Chromatographic purification of the crude
Scheme 10.3: Synthesis of 3-diamantane derivative 419.

Scheme 10.4: Synthesis of 4-diamantane derivatives.

Reagents and conditions: i) conc. H₂SO₄ (2M), 48 h, 70°C, 74%; ii) (methoxymethyl)triphenylphosphonium bromide (1.2 eq.), nBuLi (1.2 eq.), Et₂O, ∼10°C; iii) Et₂O, 35% perchloric acid, 2 h, Δ; iv) MeOH, NaBH₄, 61% over three steps; v) 5-(trityloxy)pentyl p-toluenesulfonate (1.5 eq.), NaH (1.5 eq.), DMF (0.1M), 12 h, RT, 86%; vi) MeOH/CH₂Cl₂ (1:1, v/v), cat. p-TsOH, 96%; vii) PPh₃ (1.5 eq.), CBr₄ (1.5 eq.), CH₂Cl₂, 0°C, 2 h, 79%.

Reagents and conditions: i) conc. HNO₃, 40 min RT; ii) conc. H₂SO₄, 90 min, O°C, 25%; iii) CH₂Cl₂, allyltrimethylsilane (4 eq.), BF₃·OEt₂ (1.2 eq.), -78°C → RT, 12 h, 45% (77 based on recovered SM); iv) KOtBu (1 eq.), DMSO, 100°C; v) OsO₄, NaIO₄, THF/H₂O (4:1, v/v); vi) CCl₄, conc. H₂SO₄, formic acid, 0°C; vii) TMSCN (4 eq.), BF₃·OEt₂ (1.2 eq.), -78°C → RT.
Summary and future prospects

The product mixture provided pure 4-diamantanol (44) in 25% yield and 1-diamantanol (41) in 48% yield. Discouragingly, Koch-Haaf type carbonylation of 44 gave 1-diamantanecarboxylic acid (53). Presumably the low reactivity of CO, which is generated insitu from formic acid in the Koch-Haaf carbonylation, in addition to the greater stability of the 1-diamantyl cation leads to equilibration of the intermediate 4-diamantyl cation.

Considering the harsh conditions required for the preparation of a diamantyl cation from the corresponding alcohol (conc. H₂SO₄), milder methods to generate these cations were explored. It is known that the adamantyl cation can be generated from 1-adamantane acetate using BF₃·OEt₂. Furthermore, Ohno and co-workers described the alkylation of the thus obtained 1-adamatyl cation using allyltrimethyl silane. It was envisaged that 4-diamantanecarboxylic acid (54) could be obtained from 4-allyldiamantane (420) after allyl - vinyl double bond isomerisation followed by oxidative olefin cleavage. As a model reaction, readily available 1-diamantanol (41) was acetylated in neat acetic anhydride with catalytic iodine in 96% yield. Allylation of 1-diamantane acetate was achieved in 88% under mild conditions (CH₂Cl₂, BF₃·OEt₂, allyltrimethyl silane at -78°C) and was an inspiration to investigate the allylation of 4-diamantane acetate (419). To this end, 4-diamantanol (44) was acetylated to give 419 in 88% yield. The allylation of 419 did not go to completion and provided 4-allyldiamantane (420) in 45% yield (77% based on RSM), however unreacted 419 was recovered from the reaction mixture. Unfortunately, double bond isomerisation of 420 was not effective using potassium tert-butoxide. Alternatively, the installment of a 4-cyanide group on the diamantane core might represent a straightforward method to give the 423.

Experimental procedures

General Methods: Solvents and reagents were obtained commercially and used as received unless stated otherwise. All moisture sensitive reactions were performed under an argon atmosphere and residual water was removed from starting compounds by coevaporation with toluene (3x), unless stated otherwise. Reaction grade solvents were stored on 4 molecular sieves or 3 for MeOH, CH₃CN, and DMSO. All solvents were removed by in vacuo evaporation at ∼ 45°C. Reactions were monitored by TLC analysis using silica gel coated aluminium plates (0.2 mm thickness) an detection by UV absorption (254 nm) and/or by spraying with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·4H₂O in 10% H₂SO₄, followed by charring at ∼ 150°C. Visualisations of olefins and N-alkylated iminosugars was achieved by spraying with a solution of KMnO₄ (5 g/L) and K₂CO₃ (25 g/L) in H₂O, followed by charring at ∼ 150°C. Glycosides and hemiacetals were visualized by spying with a solution of 20% H₂SO₄ in MeOH and charring at ∼ 150°C and for adamantane containing compounds a solution of H₃PMo₁₂O₄₀ (100 g/L) was used. Flash column chromatography was performed on silica gel (40-63 μm). NMR spectra were recorded on a 400/100 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethyl silane as internal standard for all ¹H NMR measurements in CDCl₃ and the deuterated solvent signal for all other NMR experiments. ¹H NMR peak assignment were made using COSY and HSQC experiments and coupling constants (J) are
given in Hz. All $^{13}$C NMR spectra are proton decoupled. For LC/MS analysis a HPLC-system (detection simultaneously at 213 nm, 254 nm and evaporative light detection) equipped with an analytical C18 column (4.6 mm ID x 250 mmL, 5µm particle size) in combination with buffers A: H$_2$O, B: CH$_3$CN, C: 1.0% aqueous trifluoroacetic acid and coupled with an electrospray interface (ESI) was used. For RP-HPLC purifications, an automated HPLC system equipped with a semi-preparative C18 column (5 µm C18, 10, 150 x 21.2 mm) was used. The applied buffers were A: H$_2$O + trifluoroacetic acid (1% mM) and B: CH$_3$CN.

**diamantane (2).**

Binor-S (13), a precursor in the synthesis of diamantane (2), was prepared on 2.5 mol scale according to the (organic synthesis) procedure described by the group of Schleyer. $^{34}$ 13 was isolated by in vacuo distillation (110-115°C, 0.8 mbar) and was obtained in 80% yield. GC-analysis: RT = 4.90 min (150°C → 300°C, gradient 10°C/min). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.07 (s, 6H), 1.29 (s, 4H, 2x CH$_2$), 1.61 (s, 2H), 1.89 (s, 4H). $^{13}$C NMR (100 MHz; CDCl$_3$): δ 15.5, 16.7, 31.8, 33.5, 39.9.

**4H Binor-S (14)** was prepared via a slightly modified procedure. $^{34}$ Binor-S (13, 25 g, 136 mmol) was dissolved in acetic acid (1M) at 45°C and PtO$_2$ (250 mg, 1.1 mmol) was added to the resulting solution. The reaction mixture was depleted of oxygen by sonification under an argon atmosphere and put under a H$_2$ atmosphere (4 bar). During the course of the reaction, the pressure was maintained around ~3.5-4 bar, until no further hydrogen consumption was detected. The reaction mixture was allowed to stir for 12 h at 4 bar H$_2$, after which the catalyst was removed by filtration of the reaction mixture over a pad of Celite. The filtrate was concentrated to provide the intermediate 4H Binor-S (14), which was used in the final step. GC-analysis: RT = 5.23 min (150°C → 300°C, gradient 10°C/min).

**Diamantane (2)** was prepared by AlBr$_3$ mediated isomerisation of 14, according to described procedures and was isolated by crystallisation from toluene in 64% yield. GC-analysis: RT = 2.55 min (150°C → 300°C, gradient 10°C/min). $^1$H NMR (400 MHz; CDCl$_3$): δ 1.70-1.77 (m, 20H). $^{13}$C NMR (100 MHz; CDCl$_3$): δ 25.9, 37.6, 38.4. MS: found [M+H]$^+$ 189.80, calculated for [C$_{14}$H$_{20}$+H]$^+$ 189.16.
1-bromodiamantane (28).

Diamantane (2) (6.0 g, 31.9 mmol) was co-evaporated three times with toluene, suspended in neat Br$_2$ (∼1M) and stirred for 2 h at RT. The reaction mixture was diluted with CHCl$_3$ (0.1M) and poured in to ∼ 500 g of crushed ice. Excess bromide was depleted by portion wise addition of NaHSO$_3$ (s), until the reaction mixture turns from dark brown to slightly yellow. Next, the organic layer was collected via a separatory funnel and the aqueous layer was extracted twice with CHCl$_3$. The combined organic layer was subsequently washed with 1 M aq. NaHSO$_3$, and a mixture of brine/H$_2$O (1:1, v/v). The organic layer was dried (MgSO$_4$), filtered and concentrated to provide the crude title compound in near quantitative yield, which was used in the next step. GC-analysis: >98% purity, RT = 7.65 min (150°C → 300°C, gradient 10°C/min). $^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 1.56 (br. s, 1H, H$_3$$^\alpha$), 1.60 (br. s, 1H, H$_3$$^\beta$), 1.71-1.78 (m, 8H, H$_4$-6, H$_8$,11), 1.92 (br. s, 1H, H$_9$), 2.05 (br. s, 2H, H$_2$,13), 2.11 (br. s, 2H, H$_7$,12), 2.41-2.46 (m, 4H, H$_{10}$,14). $^{13}$C NMR (100 MHz; CDCl$_3$): $\delta$ 25.1, 31.3, 34.0, 34.7, 36.4, 37.2, 38.5, 41.4, 45.9, 51.5, 78.7.

1-diamantanol (41).

A suspension of crude bromide 28 (8.5 g, ∼32 mmol) in DMF (0.2M) was diluted with 0.8M aq. HCl to a final concentration of 0.1M. The resulting suspension was stirred under reflux for 2 h and the resulting suspension was poured on to crushed ice (∼ 500 g). The insolubles were collected by filtration and rinsed with H$_2$O. The residue was dissolved in toluene and concentrated to provide the title compound in 96% yield. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.41 (s, 1H, OH), 1.47 and 1.50 (2x br. s, 2H, H$_3$), 1.60-1.67 (m, 10H, H$_5$-8,10,11), 1.72-1.74 (m, 1H, H$_4$), 2.0 (br. s, 2H, H$_2$,13), 2.07-2.09 (m, 1H, H$_9$), 2.18 and 2.21 (2x br. s, 2H, H$_{14}$). $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 25.2, 30.4, 32.4, 36.6, 37.4, 37.9, 39.9, 43.3, 46.3, 70.7.

1-diamantanecarboxylic acid (53).

A 100 ml three-neck round bottom flask was charged with 20 ml 97% H$_2$SO$_4$ under an argon atmosphere and cooled to 0°C. Next, formic acid (5 ml, 99% grade) was dropwise added via a dropping funnel (∼ 5 drops/min). CAUTION : CO (g) is generated! Simultaneously, a dry solution of 1-diamantanol (424, 2.0 g, 9.8 mmol) in CCl$_4$ (1M) was dropwise added to the reaction mixture (∼ 5 drops/min). Upon complete addition of the alcohol and formic acid, the reaction mixture was allowed to slowly warm-up to RT over a period of 4 h at 0°C after which stirring was continued for 1 h at RT. The reaction mixture was poured on to crushed ice (∼ 150 g) and stirred vigorously for 30 min and re-cooled to 0°C. The precipitate was collected by filtration and rinsed with H$_2$O to provide the crude title compound in near quantitative yield and high purity. $R_F = 0.2$ (5 vol% AcOH in Tol/Et$_2$O, 1:1). $^1$H NMR (400 MHz, MeOD) $\delta$ 1.55 and 1.59 (br. s, 2H, H$_3$), 1.69-1.89 (15H), 2.11 (br. s, 2H, H$_{10}$). $^{13}$C NMR (100 MHz, MeOD): $\delta$ 24.4, 25.5, 34.2, 35.8, 34.4, 36.5, 36.6,
36.9, 40.9, 56.6, 63.3, 179.2. MS: found [M+H]+ 233.1, calculated for [C_{15}H_{20}O_2 + H]^+ 233.15.

1-diamantanemethanol (409).
To a dry and cooled (0°) solution of crude acid 53 (2.0 g, 8.6 mmol) in THF (0.1 M) was carefully added LiAlH4 (5 ml, 4M solution in THF) and the resulting reaction mixture was stirred for 48 h at RT. TLC analysis of the reaction mixture revealed complete consumption of the starting material and excess LiAlH4 was quenched at 0°C by the addition of EtOH followed by H2O. The reaction mixture was diluted with 1M HCl and extracted trice with Et2O. The combined ethereal layer was subsequently washed with H2O and brine, dried (MgSO4) , filtered and concentrated. The resulting residue was subjected to silica gel FCC (Tol/Et2O, 1:0 → 8:2) to provide the title compound in 77% yield. Rf = 0.4 (Tol/Et2O, 3:2). 1H NMR (400 MHz; CDCl3): δ 1.41 and 1.45 (2x br. s, 2H, H3), 1.55 (d, 2H, J= 1.2, H10), 1.59 (br. s, 2H, H7,13), 1.67-1.80 (m, 9H, H2,4,5 ,8,11,12), 1.90-1.91 (m, 2H, H6,9), 1.99 and 2.01 (2x br. s, 2H, H14), 3.23 (s, 1H, OH ), 3.58 (s, 2H, H15). 13C NMR (100 MHz, CDCl3): δ 25.7, 27.2, 32.7, 36.8, 38.0, 38.03, 38.2, 38.9, 40.0, 68.2, 73.1. MS: found [M+NH4]^+ 236.1, calculated for [C_{15}H_{20}O_2 + NH4]^+ 236.20.

1-((5-benzyloxy)pentyloxymethyl)diamantane (410).
A dry solution of 409 (1.09 g, 5.0 mmol) in DMF (0.1 M) was used in general procedure B, as described in chapter 6. After extractive work-up procedures, the residue was purified by silica gel FCC (PE/Et2O, 1:0 → 1:1) to give the title compound in 22% yield as an off white solid. Rf = 0.9 (PE/Et2O, 3:1). 1H NMR (400 MHz; CDCl3): δ 1.50-1.1.94 (H3,5,8,11,10,17-19), 1.95-2.03 (m, 1H, H9), 2.08-2.17 (m, 2H, H14), 3.43 (s, 2H, H15), 3.49 (t, 2H, J= 6.4, H20), 3.58 (t, 2H, J= 6.6, H16), 4.46 (s, 2H, CH2 - Bn), 7.30-7.45 (m, 5H, Har). 13C NMR (100 MHz, CDCl3): δ 22.8,25.9, 27.4, 29.4, 29., 33.0, 37.2, 37.6, 37.8, 38.1, 38.2, 38.4, 39.1, 40.5, 41.0, 70.4, 71.5, 72.8, 76.4, 127.4, 127.5, 128.3, 138.7.

1-((5-hydroxy)pentyloxymethyl)diamantane (411).
A dry solution of benzyl ether 410 (0.44 g, 1.1 mmol) in THF (0.1 M) was used in general procedure C, as described in chapter 6. After work-up procedures, the residue was purified by silica gel FCC (Tol/Et2O, 1:0 → 0:1) to give the title compound in 75% yield. Rf = 0.5 Tol/Et2O, 1:1). 1H NMR (400 MHz; CDCl3): δ 1.38-1.92 (m, 20H, H2-8,10-13, 17-19), 1.98-2.06 (m, 2H, H14), 2.25 (br. s, OH), 3.33 (s, 2H, H15),3.40 (t, 2H, J= 6.4, H20), 3.62 (t, 2H, J= 6.6, H16). 13C NMR (100 MHz, CDCl3): δ 22.3, 25.8, 27.3, 29.2, 29.21, 32.4, 32.9, 37.1, 37.5, 37.5, 37.7, 37.8, 38.1, 38.3, 39.0, 40.4, 40.9, 62.6, 71.4, 76.4.
**1-((5-bromo)pentyloxymethyl)diamantane (412).**

A dry solution of alcohol 411 (0.25 g, 0.82 mmol) was brominated according to general procedure D, as described in chapter 6. The residue was purified by silica gel FCC (Tol/Et₂O, 1:0 → 0:1) to give the title compound in 59% yield. ¹H NMR (400 MHz; CDCl₃): δ 1.38-1.85 (H₃,5,8,11,10,17-19), 1.86-1.96 (1.92 (m, 1H, H9), 3.34 (s, 2H, H15), 3.39-3.48 (m, 4H, H16,20). ¹³C NMR (100 MHz, CDCl₃): δ 24.9, 25.0, 25.8, 25.9, 27.3, 28.7, 29.7, 32.1, 32.6, 33.0, 33.8, 37.2, 37.6, 37.8, 37.9, 38.1, 38.3, 39.0, 40.5, 41.0, 71.1, 76.5.

**3-diamantanone (60).**

Diamantane (2, 6.1 g, 32.4 mmol) was coevaporated trice and subsequently dissolved in 97% H₂SO₄ (2M). The resulting reaction mixture was stirred for 48 h at 70°C. The reaction mixture was allowed to cool down to RT and subsequently poured in to crushed ice (~0.25 kg) and stirred for 30 min. The reaction mixture was transferred to a separatory funnel and extracted five times with Et₂O. The combined ethereal layer was subsequently washed with sat. aq. NaHCO₃ (2x), brine, dried (MgSO₄), filtered and concentrated. The residue was subjected to silica gel FCC (Tol/Et₂O, 1:0 → 8:2) to provide the title compound in 74% yield. R_F = 0.2 (100% Tol). ¹H NMR (400 MHz; CDCl₃): δ 1.69-2.11 (m, 16H, 5x CH₂, 6x CH - Dia), 2.43-2.52 (m, 2H, H-3, OH). ¹³C NMR (100 MHz, CDCl₃): δ 25.3, 36.13, 36.3, 36.4, 37.0, 37.5, 38.3, 38.4, 39.3, 43.5, 55.6, 217.7.

**3-diamantanemethanol (425).**

A dry solution of 3-diamantanone (60, 2.0 g, 10 mmol) in Et₂O (0.2M) was used in general procedure E as described in chapter 6. After work-up procedures, the residue was subjected to silica gel FCC (Tol/Et₂O, 1:0 → 1:1) to provide the title compound in 61% yield. ¹H NMR (400 MHz; CDCl₃): δ 1.42-1.80 (m, 5x CH₂, 8x CH - Dia), 2.03-2.07 (m, 2H, H-3, OH), 3.71 (dd, 2H, J= 3.1, 8.9, H15). ¹³C NMR (100 MHz, CDCl₃): δ 26.3, 27.0, 32.1, 32.9, 37.3, 37.4, 37.7, 37.8, 38.0, 38.1, 38.5, 38.6, 39.3, 48.2, 64.1.

**4-diamantanol (44).**

Diamantane (2) was subjected to concentrated HNO₃ according to literature procedures. Chromatographic separation of the product mixture provided 1-diamantanol (41, 48%) and the title compound in 25% yield. R_F = 0.7 (Tol/Et₂O, 1:1). ¹H NMR (400 MHz; CDCl₃): δ 1.65 (br. s, 6H, 3x CH₂), 1.80 (br. s, 4H, 4x CH), 2.0 (br. s, 3H, 3x CH), 2.10 (br. s, 6H, 3x CH₂). ¹³C NMR (100 MHz, CDCl₃): δ 25.1, 36.0, 36.6, 39.6, 39.8, 89.5. LC/MS analysis: Rt 14.02 min (CN-column, 30 min, linear gradient 10-90% B), ES (ESI): m/z = 222.1 [M + NH₄]⁺.
Summary and future prospects

**diamantan-4-yl acetate (419).**

4-Diamantanol (44, 204 mg, 1.0 mmol) was coevaporated trice with toluene and dissolved in Ac₂O (0.1M). A catalytic amount of iodine was added and the resulting reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with Et₂O and subsequently washed with sat. aq. NaHCO₃ (5x), sat. aq. Na₂S₂O₃, brine, dried (MgSO₄), filtered and concentrated. The residue was purified by silica gel FCC (Tol/Et₂O, 1:0 → 0:1) to provide the title compound in 88% yield. RF = 0.4 (Tol).

1H NMR (400 MHz; CDCl₃): δ 1.69 (br. s, 6H, 3x CH₂), 1.72 (br. s, 4H, 4x CH), 1.95 (br. s, 6H, 3x CH, CH₃), 2.07 (br. s, 6H, 3x CH₂). 13C NMR (100 MHz, CDCl₃): δ 22.6, 25.4, 35.8, 36.3, 39.1, 41.5, 79.5, 170.2.

**4-allyldiamantane (420).**

To a dry and cooled (-78°C) solution of acetate 419 (130 mg, 0.5 mmol) in CH₂Cl₂ (0.1M) were subsequently added allyl trimethylsilane (320 µl, 2 mmol) and BF₃·OEt (75 µl, 0.6 mmol). The resulting reaction mixture was allowed to warm up to room temperature and stirring was continued for 12 h. The reaction mixture was diluted with CH₂Cl₂ and subsequently washed with H₂O, brine, dried (MgSO₄) and concentrated. The residue was purified by silica gel FCC (PE/Tol 1:0 → 0:1) to give the title compound in 45% yield. RF = 0.9 (Tol).

1H NMR (400 MHz; CDCl₃): δ 1.47 (br. s, 6H, 3x CH₂), 1.66 (br. s, 4H, 4x CH), 1.73 (br. s, 6H, 3x CH₂), 1.80 (br. s, 3H, 3x CH), 1.88 (d, 2H, J= 7.7, CH₂ - allyl), 4.92-5.09 (m, 2H, =CH₂), 5.75-5.95 (m, 1H, =CH). 13C NMR (100 MHz, CDCl₃): δ 25.8, 37.1, 37.9, 38.1, 43.1, 48.0, 116.4, 135.2.
References


Samenvatting

De exploitatie van de ongewone hydrofobe eigenschappen van diamantachtige verbindingen is het primaire doel van het in dit proefschrift beschreven onderzoek. De familie van diamantachtigen wordt ingeleid in hoofdstuk 1 en tevens worden de eerste pogingen om synthetische diamanten te verkrijgen beschreven. Vervolgens wordt de aanwezigheid van diamantachtige moleculen in natuurlijke bronnen gerapporteerd, gevolgd door de synthese van de eerste drie leden van de familie van diamantachtigen, namelijk ada-, dia- en triamantan. Met name de Lewis zuur gekatalysde carbocation omlegging, ontdekt door Schleyer et al. voor de synthese van adamantanaan, bleek van onschatbare waarde voor de bereiding van diamantaan en triamantaan. Het functionaliseren van diamantachtige verbindingen is cruciaal voor hun toepassing in verschillende gebieden van onderzoek. Selectieve functionalisering is lastig vanwege de symetrie in diamantachtige moleculen waarbij de ionische brug-hoofd derivatisering een mogelijke strategie is. Een selectie van ionische brug-hoofd brominering, nitrering en carbonyleringen van de diamantachtige verbindingen wordt gepresenteerd.

1-Adamantane azijnzuur werd in de synthese van (natuurlijke) oligonucleotide (ON) fragmenten in oplossing gebruikt als een beschermende groep. Geïnitieerd door de Koning et al. werd een Oplossing-fase Extractie Methode (SPEM) ontwikkeld die hoofdstuk 2 wordt geïntroduceerd. De internucleostidische fosfodiester (PO) of fosforothioaat (PS) binding werd gevormd met behulp van de fosforamidiet procedure. 3’-O-adamantanaan acyl bescherming werd gebruikt in combinatie met commercieel verkrijgbare (behalve thymidine) 5’-O-DMTr fosforamidiet bouwstenen voor ketenverlenging in de 3’→5’ richting. Er werden alleen extractieve opwerking protocollen toegepast voor de isolatie van beschermde oligonucleotide intermediairen. De ontwikkelde SPEM benadering werd geoptimaliseerd door de implementatie van een ‘capping’ stap, ter minimalisatie van n-1 deletie fragmenten als gevolg van incomplete koppelingreacties. De doeltreffendheid van de SPEM benadering werd aangetoond met de synthese van een decamere oligonucleotide fragment. Na negen opeenvolgende koppelingscycli op 0.5 mmol schaal werd het partiële beschermde (PO) oligonucleotide fragment geïsoleerd in een opbrengst van 70%. Voor de synthese van fosforothioaat (PS) oligonucleotide fragmenten via de SPEM benadering werd een tweestaps verzwavelings protocol ontwikkeld.
De gewenste fosforthioaat triester werd verkregen door blootstelling van de reactieve fosfortriest er intermediair aan fenylacetyl disulfide (PADS). Extractieve verwijdering van de overmaat fosforamid donor was mogelijk door het om te zetten in een geladen phophorothioate diester, door reactie met 3-\(H\)-benzo [1,2]dithiol-3-on. Gedeeltelijk beschermd fosforothioaat (PS) fragment \(\text{HO}\text{CijBu}A\text{BlzCijBu}\text{Tpom}T\text{Pom}\text{Oada}\) werd verkregen in een opbrenst van 67%.

De bereiding van lange oligonucleotide fragmenten door stapsgewijze ketenverlenging is lastig vanwege de toenemende polaire aard van (gedeeltelijk beschermd) fragmenten. Veelal wordt een blok-koppeling strategie toegepast tussen twee beschermd ON fragmenten. Het eerste benodigde partiëel beschermd ON fragment met een vrije 5’-OH is beschikbaar via de 3’→5’ SPEM benadering. Voor een convergente methode is een tweede, partieel beschermd ON fragment vereist, welke een vrije 3’-OH bevat. Voor de synthese van dergelijke fragmenten werd de 5’→3’ SPEM benadering onderzocht (hoofdstuk 3). In dit geval werd 5’-O-adamantaan acyl bescherming gebruikt in combinatie met 3’-O-Lev fosforamid donor bouwstenen. Na de voltooiing van vijf koppings cycli op 0.5 mmol schaal werd het gedeeltelijk beschermd hexamere (PS) fragment \(\text{Adao}T\text{Pom}T\text{Pom}A\text{BlzCijBu}G\text{iBu}\) verkregen in een opbrenst van 86%. Toepassing van het twee staps verzwavelings protocol in the 5’→3’ SPEM benadering resulteerde, na vijf koppeling cycli, in de isolatie van (PS) fragment \(\text{Adao}T\text{Pom}T\text{Pom}G\text{iBu}A\text{BlzCijBu}\text{OH}\) in een opbrenst van 72%.

In hoofdstuk 4 wordt de synthese van vier hybride levulinoyl-adamantaan beschermgroepen beschreven voor 3’-O-nucleoside protectie. Voorlopige resultaten laten zien dat deze hybride beschermgroepen de hoge lipofiliciteit van de adamantaan eenheid combineren met de orthogonal afspitsing procedures, inherent aan de Lev beschermgroep. Echter, de protectie van een 3’-O-nucleoside met de meest belovende hybride beschermgroep (iKlev) bleek lastig en vereist nader onderzoek. Gebruik van de hybride beschermgroep iKlev voor 3’-O-nucleoside protectie in de 3’→5’ gerichte SPEM benadering, zal resuleren in een orthogonaal beschermd nucleotide fragment. Selectieve ontscherming van of de 5’-O-DMTr of de 3’-O-iKlev bescherm groep, zal resuleren in twee gedeeltelijk beschermd ON fragmenten die geschikt zijn voor een block-koppeling strategie.

Het tweede deel van het onderzoek is gericht op het onderzoeken van het opvallende effect van de adamantaan op de remmende werking van iminosuikers, ter beïnvloeding van cellulair glucosylceramide (GlcCer) concentraties. De enzymen betrokken in het GlcCer metabolisme zijn respectievelijk glucosylceramide synthase (GCS), \(\beta\)-glucocerebrosidase (GBA1) en \(\beta\)-glucosidase (GBA2). Het belang van potente en selectieve inhibitory voor het moduleren van glucosylceramide concentraties wordt kort uitgelegd in hoofdstuk 5. Aerts en medewerkers rapporteerden dat de N-alkylering van deoxynojirimycin (DNJ), een natuurlijk voorkomende glycosidase remmer, met N-pentyloxy methyl-1-adamantaan (MZ-21) eenheid resulteert in een verbeterd profiel ten opzichte van het commercieel verkrijgbare geneesmiddel Zavesca®, met een N-butyl keten. Uit structuur-activiteit relaties (SAR) studies is gebleken dat de C5-epimeer van MZ-21 te weten N-pentyloxy methyl-1-adamantane-1-ido-DNJ (MZ-31) een selectievere remmer is in vergelijking met MZ-21. De synthese en evaluatie van N-gealkyleerde DNJ en
L-ido-DNJ bibliotheken wordt beschreven in hoofdstukken 5 en 6. De rol van de adamantaan eenheid op de biologische activiteit van MZ-21 en MZ-31 werd onderzocht door het systematisch introduceren van een reeks lineaire en vertakte koolwaterstoffen. De noodzaak van een N-pentyloxy keten tussen de iminosuiker en de hydrofobe eenheid voor potente GCS remming werd aangetoond door de observatie dat het weglaten daarvan resulteert in een verminderd remmend vermogen. Bovendien werd aangetoond dat een etherbinding tussen de hydrofobe eenheid en de pentyl keten een uitgesproken effect heeft op de potentie en selectiviteit van GCS remming. Verder wordt het ontwerp en synthese van een foto crosslinker op basis van MZ-21 besproken.

In hoofdstuk 7 wordt de synthese van quaternaire ammoniumzouten van MZ-21 en MZ-31 door N-methylering beschreven. Deze ammoniumzouten, nojirimycinium ionen genoemd, bleken ongeveer 10 maal minder potente remmers in vergelijking met de moeder verbinding. Deze bevindingen suggereren dat de actieve plaats van de betrokken enzymen nojirimycinium ionen kunnen binden. Hiermee wordt de algemene veronderstelling dat N-gealkyleerde iminosuikers de oxocarbenium-ion overgangstoestand nabootsen ondersteund.

De synthese van C-2 gemodificeerde imidazopyranose derivaten worden beschreven in hoofdstuk 8. Twee iminozuiker derivaten met ofwel een C\(_3\)H\(_6\)−adamantaan of een C\(_3\)H\(_6\)−fenyl werden bereid op basis van het werk van Vasella en collega’s. Evaluatie van deze derivaten tegen remming van GCS, GBA1 en GBA2 liet zien dat beide derivaten zeer potent en selectief GBA1 remmen. Het bleek dat de adamantaan imidazopyranose derivaat werd overtroffen door de fenyl imidazopyranose derivaat met betrekking tot GBA1 remming.

Een alternative benadering tot het verkrijgen van potente en selective inhibitoren ter begeïnvloeding van de GlcCer homeostase wordt beschreven in hoofdstuk 9. Hier wordt het ontwerp en synthese van 1-deoxycastanospermine, een natuurlijke bi-cyclische iminosuiker, gemodificeerd met een adamantaan groep onderzocht. Castanospermine werd gesynthetiseerd, op basis van enigszins gewijzigde procedures die zijn ontwikkeld door de groep van Pyne via een Petasis borono-Mannich reactie van L-xylose, allylamine en (E)-styreen boorzuur. De synthese C-3 gemodificeerde 1-deoxy castanospermine begon met de productie van chiraal zuivere 2°-allylische aminen, door stereoselectieve alkylering van chirale sulfinamides. Een belangrijke stap in de synthese betreft een ringsluiting metatheise, in aanwezigheid van een vrije secundair amine, onder invloed van microgolfbestraling.
List of publications

Conversion of chiral unsaturated cyanohydrins into chiral carba- and heterocycles via ring-closing metathesis.

Simple and efficient solution-phase synthesis of oligonucleotides using extractive work-up.

A two-step sulfurization for efficient solution-phase synthesis of phosphorothioate oligonucleotides.

Assessment of partially deoxygenated deoxynojirimycin derivatives as glucosylceramide synthase inhibitors.

Identification of potent and selective glucosylceramide synthase inhibitors from a library of *N*-alkylated iminosugars.
