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## **Cyclophellitol and its derivatives: synthesis and application as beta-glycosidase inhibitors**

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# 9

## Summary and Future Prospects

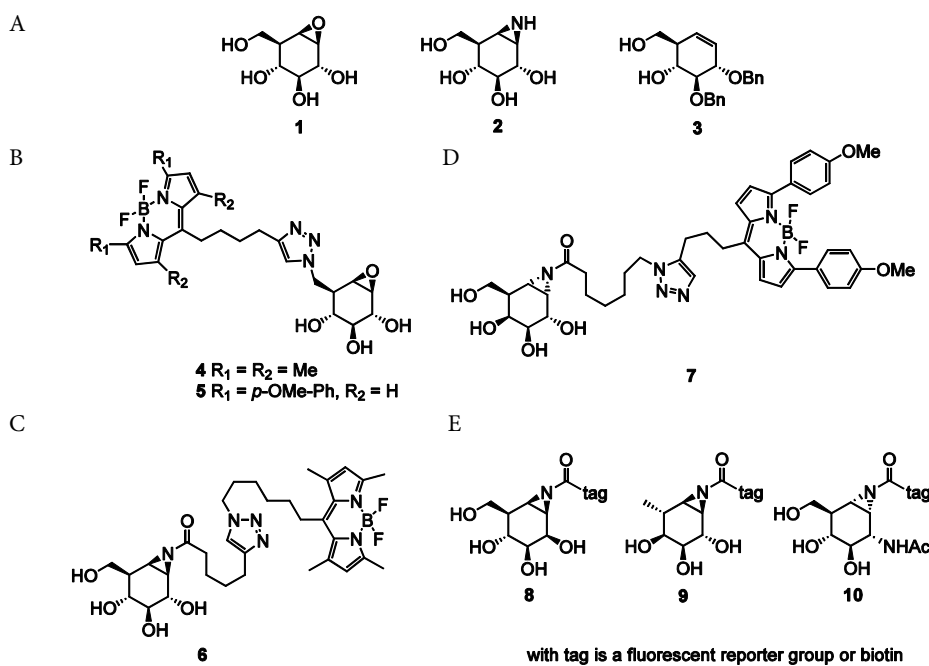
### Introduction

The research described in this Thesis aims to develop inhibitors (mechanism-based and competitive) and activity-based probes (ABPs) for  $\beta$ -glycosidases. Glycosidases are a large, evolutionary conserved enzyme family that catalyze the hydrolysis of acetal linkages in glycopolymers to form hemiacetal linkages. Both the role of glycosidases and their hydrolytic features have been studied extensively as a result of their involvement in (patho)physiological processes and their (potential) applications in the glycopolymer assembly and biotechnological industry. **Chapter 1** provides an overview of the miscellaneous aspects of glycosidases along with their inhibitors and activity-based probes (ABPs).

Proteomic profiling of glycosidases has evolved progressively in the past decades. This is illustrated by the various classes of glycosidase ABPs that have been developed by careful examination of the active site and the catalytic hydrolytic mechanism. Two classes of ABPs based on 2-deoxy-2-fluoroglycosides and cyclitol epoxides are highlighted in **Chapter 2**. In this Chapter their design principles and biological applications for retaining  $\beta$ -glucosidases, in particular for glucocerebrosidase (GBA) are discussed. GBA is involved in the penultimate step of glycosphingolipid metabolism, which if defective results in the lysosomal storage disorder Gaucher disease.

The study conducted for this Thesis started with the development and optimization of a route adapted from Madsen and co-workers, for the large scale synthesis of  $\beta$ -glucosidase inhibitors cyclophellitol **1** and cyclophellitol aziridine **2** as presented in **Chapter 3**. Together with the key intermediate **3**, these two compounds are the

**Figure 1.** Chemical structures of (A) cyclophellitol **1**, cyclophellitol aziridine **2** and key intermediate **3** from **Chapter 3**, (B) Cyclophellitol-derived ABPs **4** and **5**, (C) Broad-spectrum retaining  $\beta$ -glucosidase cyclophellitol aziridine ABP **6**, (D) ABP **7** for  $\alpha$ -galactosidase, (E) Putative ABPs for  $\beta$ -mannosidase **8**,  $\alpha$ -fucosidase **9** and  $\alpha$ -*N*-acetyl-glucosaminidase **10**.



starting points for the synthesis of cyclophellitol-type inhibitors and ABPs presented in this Thesis (Figure 1A).

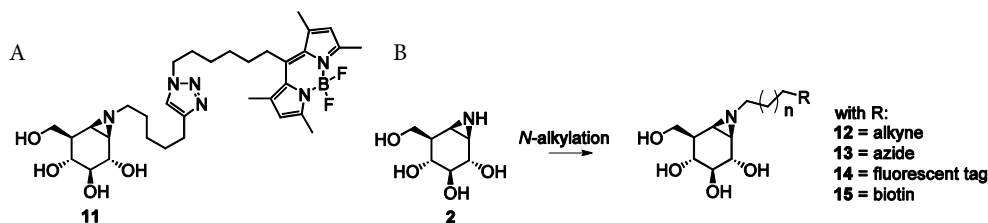
The development of a novel broad-spectrum ABP for retaining  $\beta$ -glucosidases is outlined in **Chapter 4**. In contrast to the previously reported cyclophellitol-derived ABPs **4** and **5** (Figure 1B) a fluorescent BODIPY reporter group was appended near the less restrictive aglycon binding site of retaining  $\beta$ -glucosidases. Acylation of the nitrogen function of cyclophellitol aziridine generated the highly potent retaining  $\beta$ -glucosidase ABP **6** (Figure 1C), which enabled labeling of numerous mammalian (GBA, GBA2, GBA3 and LPH) and bacterial retaining  $\beta$ -glucosidases. Interestingly, labeling of GBA by ABP **6** occurs even in the absence of the catalytic general acid/base residue, presumably caused by the high reactivity of the acylated aziridine moiety. Consequently, ABP **6** cannot be considered as a “true” ABP as it is able to label enzymes that lack catalytic activity on their natural substrate.

Arguably, the design of ABP **6** with a reporter group grafted on a position near the aglycon binding site is amenable to design ABPs that target retaining glycosidases with a different substrate specificity and thereby providing valuable tools to study the related (patho)physiological processes. In this line, a highly potent and specific cyclophellitol aziridine-based ABP **7** for  $\alpha$ -galactosidases has been developed recently to study their role in Fabry disease (Figure 1D).<sup>1,2</sup> This example implicates that the described approach is extendable to other retaining  $\beta$ -glycosidases by the synthesis of configurational isomers of **6**, for instance **8-10** to target  $\beta$ -mannosidases,  $\alpha$ -fucosidases and  $\alpha$ -*N*-acetyl-glucosaminidases respectively (Figure 1E).

The effect of altering the electrophilic centre in cyclophellitol **1** and cyclophellitol aziridine **2** on the inhibitory potency of GBA, GBA2 and GBA3 is described in **Chapter 5**. Herein, it was demonstrated that *N*-acylation, *N*-sulfonylation and *N*-alkylation of the aziridine moiety in **2** yielded viable GBA, GBA2 and GBA3 inhibitors with some subtle differences in the inhibitory activity. The *N*-alkylated analogue was the most potent inhibitor with a considerable enhanced stability during its synthesis and purification in comparison to its *N*-acylated counterpart. This opens up the possibility to develop ABP **11** (Figure 2A), the *N*-alkylated counterpart of ABP **6** (**Chapter 4**). *N*-alkylated cyclophellitol aziridine ABP **11** and analogues **12-15** may be synthesized by alkylating the aziridine function with a brominated azide- or alkyne linker, followed by a copper (I)-catalyzed click reaction with a suitable reporter group like BODIPY or biotin (Figure 2B). *N*-alkylated cyclophellitol aziridine ABPs hold great potential, perhaps even more than their *N*-acylated congeners, to serve as a blueprint for the

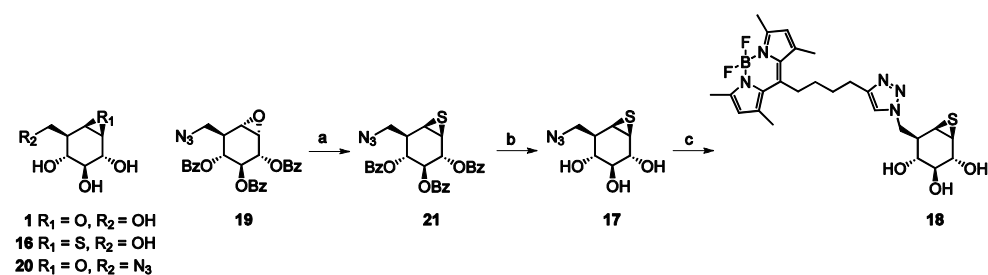
development of ABPs aimed at other glycosides families by emulating the configuration and substitution pattern of the corresponding glycoside substrate.

**Figure 2.** (A) Putative *N*-alkylated cyclophellitol ABP **11** and (B) General alkylation strategy towards **11-15**.



Moreover, it was demonstrated that substitution of the epoxide function in **1** for a thiirane group was detrimental for the inhibitory activity. However, it is known that incorporation of a bulky hydrophobic fluorophore in cyclophellitol **1** strongly enhanced its inhibitory activity towards GBA,<sup>3</sup> which could be true for cyclophellitol thiirane **16** as well. To this end, 8-deoxy-8-azidocyclophellitol thiirane **17** and the BODIPY derivative **18** were constructed starting from the previously reported **19**<sup>3</sup> (Scheme 1), a predominant byproduct in the synthesis of cyclophellitol ABPs **4,5** and 8-deoxy-8-azidocyclophellitol **20**. Treatment of **19** with *N,N*-dimethylthioformamide and trifluoroacetic acid gave the benzoylated protected thiirane derivative **21** (59%). Ensuing Zemplén debenzoylation yielded **17** (72%), which was subjected to copper (I)-catalyzed click reaction with **22**<sup>4</sup> to form the BODIPY thiirane analogue **18** (38%). The inhibitory potency of **17** and **18** were tested on GBA as previously reported and these results are summarized together with their cyclophellitol-derived congeners in Table 1.

**Scheme 1.** Synthesis of 8-deoxy-8-azidocyclophellitol thiirane **17** and BODIPY-thiirane derivative **18**.

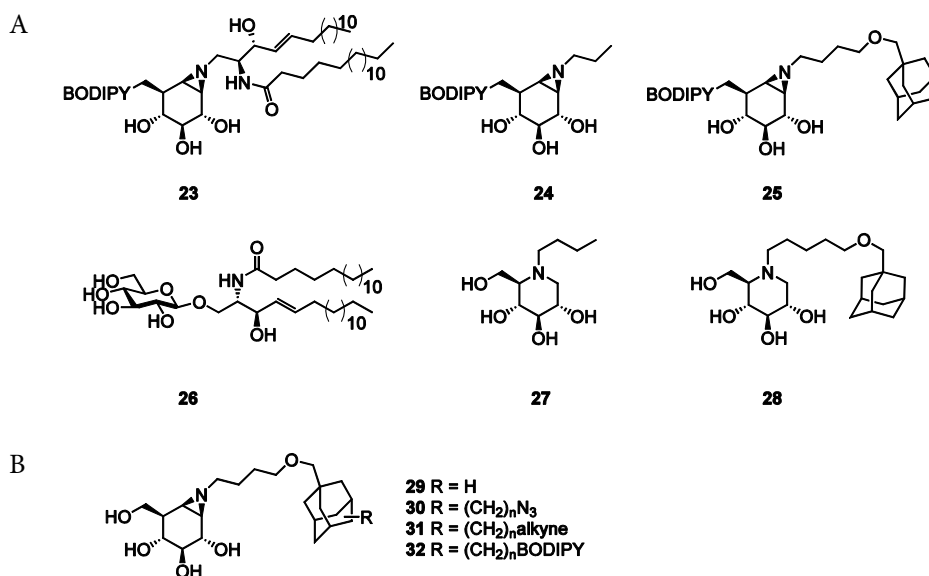


Reagents and conditions: (a) *N,N*-dimethylthioformamide, TFA, DCM, 40 °C, 59%; (b) NaOMe, MeOH, 72%; (c) BODIPY-alkyne **22**<sup>4</sup>, CuSO<sub>4</sub>, sodium ascorbate, DMF, 38%.

**Table 1.** Apparent IC<sub>50</sub> values in  $\mu\text{M}$  of cyclophellitol **1**, cyclophellitol thiirane **16** and derivatives **4**, **17**, **18**, **20**.

Enzyme	Compounds					
	<b>1</b>	<b>20</b>	<b>4</b>	<b>16</b>	<b>17</b>	<b>18</b>
GBA	0.36	0.12	0.0012	>100	>100	10

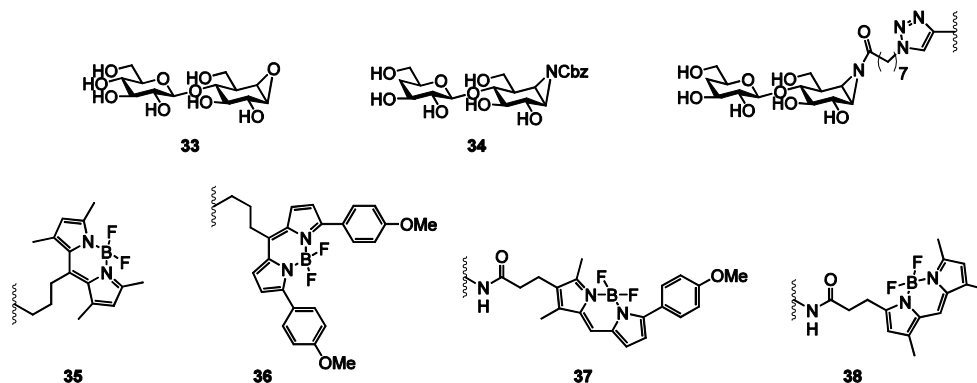
The inhibitory potency of **16** was not affected when the primary hydroxyl group was substituted for the bioorthogonal azide handle (**17**), while indeed a more potent inhibitor **18** was formed after the introduction of a BODIPY. This result clearly confirms that attaching a bulky hydrophobic group near the primary hydroxyl group of cyclophellitol **1** or derivatives thereof strongly enhances their inhibitory capacity towards GBA. In combination with the improved potency of *N*-alkylated cyclophellitol aziridine, it is of interest to synthesize *N*-alkylated cyclophellitol aziridine analogues bearing a BODIPY-reporter group near the glycan binding site as GBA inhibitor. *N*-alkylated analogues such as **23-25** that show similarities with the natural substrate of GBA, glucosylceramide **26** or potent inhibitors such as Zavesca **27** and AMP-DNM **28** (MZ21) hold great potential to serve as potent and selective GBA inhibitors.

**Figure 3.** (A) Putative *N*-alkylated cyclophellitol aziridine analogues **23-25** with their parent compounds **26-28** and (B) putative selective GBA2 inhibitors **29-32**.

One interesting observation made in the past on studies involving *N*-alkylated deoxynojirimycin derivatives is that AMP-DNM **28** bearing a *N*-5-(adamantan-1-yl-methoxy)-pentyl group is a more potent inhibitor of GBA2 ( $IC_{50} = 1$  nM) than for other mammalian retaining  $\beta$ -glucosidases: GBA ( $IC_{50} = 200$  nM), GBA3 ( $IC_{50} = 25$   $\mu$ M) and LPH ( $IC_{50} = 35$   $\mu$ M).<sup>5</sup> If this enzyme specificity can be transferred to *N*-alkylated cyclophellitol aziridine **29**, specific ABPs for GBA2 may lie ahead by the introduction of a bio-orthogonal handle (azide, alkyne) or reporter group at the adamantane core via a short alkyl spacer (ABP **30-32**).

The research described in **Chapter 6** and **7** entails the development of cyclophellitol- and cyclophellitol aziridine-based inhibitors and ABPs for *H. jecorina* cellulases. The cellulolytic system secreted by this fungus is incorporated in the industrial production of bio-ethanol with the substantial problem that either one or more cellulases are “dying off”. Cellobiosyl cyclophellitol **33** appears to be a broad-spectrum inhibitor of the four studied cellulases (CBHI, CBHII, EGI and EGII), while 4'-deoxycellobiosyl cyclophellitol Cbz-aziridine **34** was a selective inhibitor of EGII. Transformation of **34** into its fluorescent congeners **35** and **36** yielded only **35** as a viable EGII ABP, indicating that the aglycon binding site of EGII is restrictive to relatively less bulky reporter groups. It will therefore be of interest to synthesize ABP **37** and **38** with a differently orientated BODIPY group.

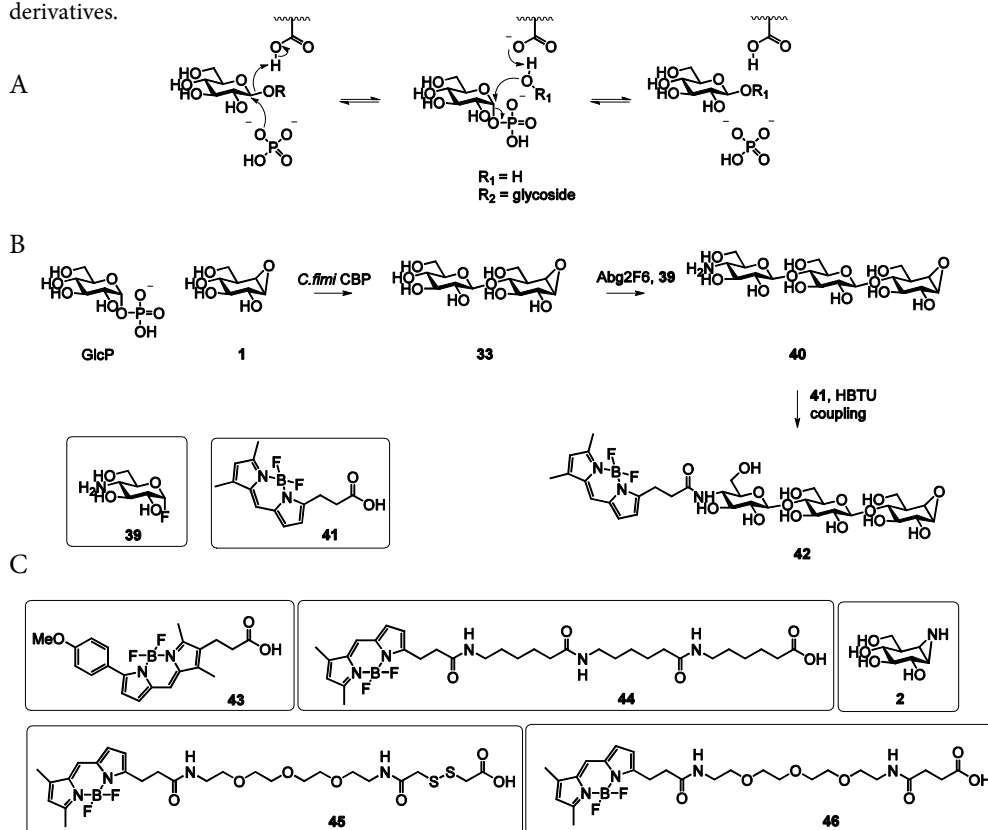
**Figure 4.** Cellobiosyl cyclophellitol **33**, 4'-deoxycellobiosyl cyclophellitol Cbz-aziridine **34** and (putative) derivatives **35-38**.



Furthermore, it was demonstrated that **34-36** could be synthesized by a glycosynthase (Abg2F6)-mediated approach. Glycosynthases facilitate, in general, the synthesis of oligosaccharides as illustrated by numerous studies.<sup>6,7</sup> Another class that holds the same potential is cellobiose phosphorylase (CBP), an enzyme that catalyzes the reversible

phosphorolysis of cellobiose in  $\alpha$ -D-glucose-1-phosphate (Glc1P) and D-glucose (Figure 5A).<sup>8</sup> Due to the reversibility, CBP enables the synthesis of 1,4- $\beta$ -disaccharides starting from diverse monosaccharides as acceptor and Glc1P or GlcF as donor.<sup>9</sup> Cyclophellitol **1** was used in a preliminary study as acceptor to generate cellobiosyl cyclophellitol **33** in the presence of donor Glc1P and *Cellulomonas fimi* CBP (Figure 5B).<sup>10</sup> Cellobiosyl cyclophellitol **33** was then transferred to 4-amino-4-deoxyglucosyl- $\alpha$ -fluoride (4-aminoGlcF) **39** by Abg2F6 to yield 4''-aminocellobiosyl cyclophellitol **40**. Ensuing HBTU-mediated amide coupling with BODIPY **41** gave the fluorescent cellobiosyl cyclophellitol derivative **42**. Initial inhibition data of **42** on EGII reveals that the enzyme could still be inactivated using  $\sim 0.25 \mu\text{M}$  of **42**, indicating that cellobiosyl cyclophellitol **40** is a more potent scaffold than cellobiosyl cyclophellitol **33**.<sup>10</sup> Perusal of

**Figure 5.** (A) Phosphorolysis and reverse phosphorolysis mechanism of cellobiophosphorylase (CBP), (B) Chemo-enzymatic synthesis of cellobiosyl cyclophellitol **33** and BODIPY-cellobiosyl cyclophellitol **42**, (C) Reporter group **43**, reporter groups with a linker **44-46** and cyclophellitol aziridine **2** for the synthesis of cellobiosyl- and cellobiosyl cyclophellitol and cyclophellitol aziridine derivatives.

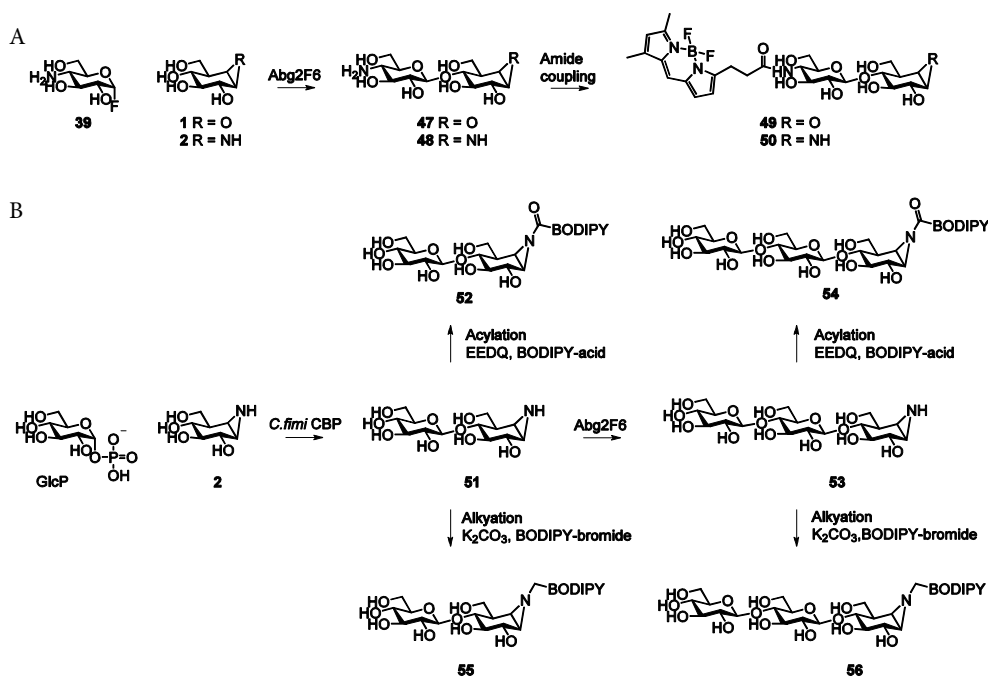




literature confirmed the enhanced inhibitory activity of cellotriosylated iminosugars towards various cellulases with the possible occurrence of internal cleavage.<sup>11</sup> It is therefore of importance to have access to both the cellobiosylated and cellotriosylated analogues of cyclophellitol as inhibitors and ABPs for effective profiling of *H. Jecorina* cellulases. Figure 5C presents the putative chemo-enzymatic routes with the combined action of Abg2F6 and CBP towards cellotriosyl cyclophellitol derivatives linked with different reporter groups as in **43** or in the presence of linkers as in **44-46**. Contingently, this strategy can be extended to their non-*N*-substituted cyclophellitol aziridine analogues with **2** as acceptor.

Initial transglycosylations studies<sup>11</sup> with 4-aminoGlcF **39**, D-glucose and Abg2F6 proceeded uneventfully and with this, an alternative route is provided for the synthesis of a 4'-BODIPY-cellobiosyl cyclophellitol derivative, which could not be synthesized (see **Chapter 6**). The synthesis of 4'-BODIPY cellobiosyl cyclophellitol derivative such as **49** may be accomplished by first reacting AbgF6 with donor **39** and acceptor **1** or **2** to furnish 4'-aminocellobiosyl cyclophellitol **47** and its aziridine analogue **48**.

**Scheme 2.** Putative synthetic routes towards (A) 4'-BODIPY cellobiosyl cyclophellitol and cyclophellitol aziridine derivative **49-50** and (B) cellobiosyl cyclophellitol aziridine **51**, cellotriosyl cyclophellitol aziridine **53** and their *N*-alkylated or *N*-acylated derivatives **52, 54, 55** and **56**.

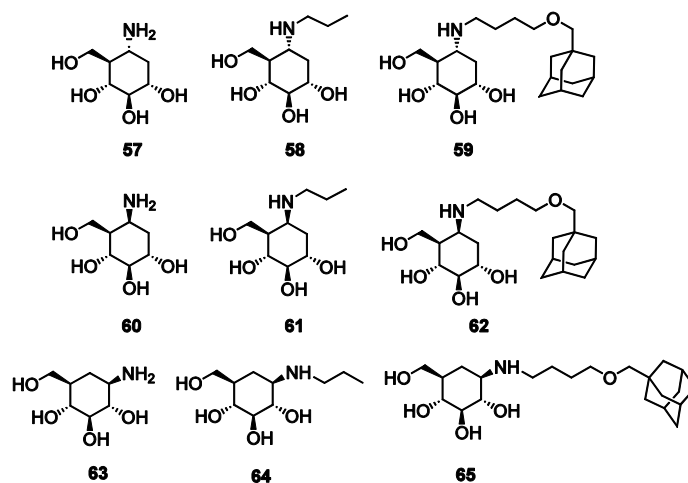


Ensuing amide ligation with a suitable reporter group (with or without a linker, see Figure 5C) may give, for example **49** and **50** (Scheme 2A).

In the same line, it is of interest to synthesize the cellobiosylated and cellotriosylated analogues of **6** and **11** by following the strategy as described in Scheme 2B. Transglycosylation of GlcP with cyclophellitol aziridine **2** may yield cellobiosyl cyclophellitol aziridine **51**, which can be subjected to EEDQ-mediated acylation conditions as described in Chapter 4 to yield **52**. The addition of a glucose moiety to **51** to yield **53** may be performed with Abg2F6, after which **53** can be acylated again with the desired reporter group to furnish **54**. Additionally, their intrinsically more stable *N*-alkylated congeners **55** and **56** may be synthesized by alkylation of **51** and **53** (see Chapter 5) with an appropriate BODIPY and potassium carbonate.

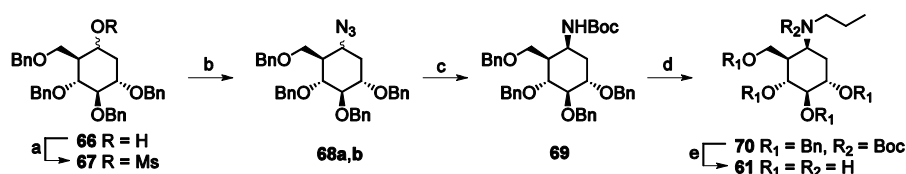
Chapter 8 describes the synthesis of *exo-N*-cyclic-deoxynojirimycin **57** and analogues **58** and **59** as potential inhibitors for enzymes that are involved in the glucosylceramide metabolism (GBA, GBA2, GBA3 and GCS). Inhibitory analysis of the compounds proved they were inactive, which is quite remarkable as numerous related 1-deoxynojirimycin and aminocyclitol analogues are potent inhibitors of the carbohydrate processing enzymes.<sup>13-17</sup> Additionally, **57** can be considered as an analogue of cyclophellitol aziridine **2** with a removed C1 carbon-nitrogen bond. To elucidate the impotency of *exo-N*-cyclic-deoxynojirimycin **57** and its analogues **58-59**, it is of interest to synthesize their corresponding isomers **60-62**, 1-epivalidamine **63**<sup>18</sup> and analogues **64-65** to study the structure-activity relationships (Figure 6).

**Figure 6.** Structures *exo-N*-deoxynojirimycin **57**, epimer *exo-N*-deoxynojirimycin **60**, 1-epivalidamine **63** and derivatives **58**, **59**, **61**, **62**, **64** and **65**.



In an initial attempt **61** was synthesized using the epimeric alcohol mixture **66** as starting material (Scheme 3). Mesylation (MsCl, DMAP, pyridine) of **66** afforded **67** as an inseparable mixture (68%). Ensuing nucleophilic substitution of the mesylate in **67** with sodium azide provided azide **68** as a separable mixture. Azide **68a** then was converted into **61** by following a strategy as described in **Chapter 8**. Staudinger reduction of **68a** was followed by protection of the corresponding amine with a BOC group (Boc<sub>2</sub>O, Et<sub>3</sub>N, DCM) to yield **69** (65% over two steps). Alkylation of **69** with propyl bromide (25%) and Pd-catalyzed hydrogenation (Pd/C, MeOH, HCl) afforded **61**. This strategy should be applicable for the construction of adamantane-analogue **62** with adamantane **71**.

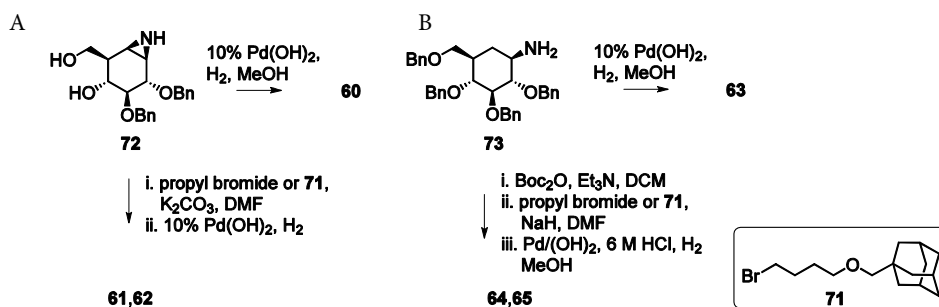
**Scheme 3.** Synthesis of *epi-exo-N*-cyclic deoxynojimiycin **61**.



Reagents and conditions: (a) MsCl, pyridine, 68%; (b) NaN<sub>3</sub>, DMF, 120 °C; (c) i. PMe<sub>3</sub>, THF, H<sub>2</sub>O (9/1; v/v), ii. Boc<sub>2</sub>O, Et<sub>3</sub>N, DCM, 65%; (d) propyl bromide, NaH, DMF, 80 °C, 25%; (e) H<sub>2</sub>, Pd/C, 37% HCl, MeOH, 2 days, 63% .

Alternatively, **60-62** may be stereoselectively synthesized from the partial protected cyclophellitol aziridine **73** (see Chapter 3). Pd-catalyzed hydrogenation of **60** or *N*-alkylated aziridine derivatives (alkylation with propyl bromide or **71**) should furnish the reduced aziridine compounds **60-62** (Scheme 4A). After the synthesis of protected 1-epivalidamine **73**<sup>18</sup>, 1-epivalidamine **63** can be obtained by Pd-catalyzed hydrogenation of **73**. A similar sequence as described in **Chapter 8** can be followed for

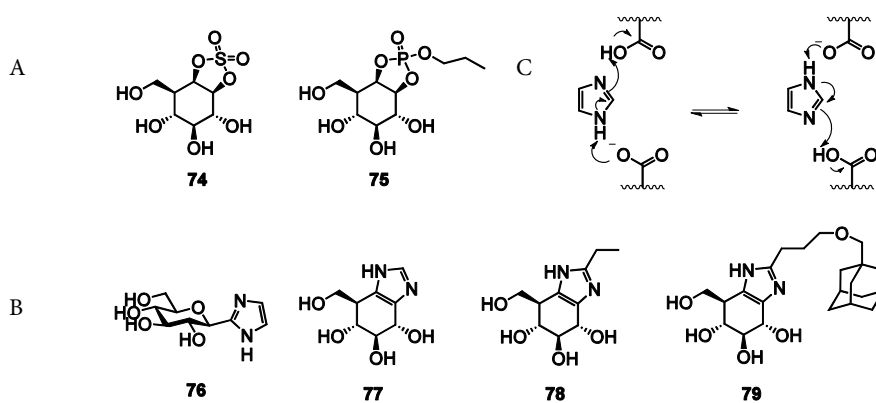
**Scheme 4.** Putative synthetic routes towards (A) **60-61** and (B) **63-65**.



the construction of **64** and **65**. Subsequent introduction of a Boc group in **73**, nucleophilic substitution with propyl bromide or **71** and Pd-catalyzed hydrogenation should afford **64** and **65** (Scheme 4B).

Cyclophellitol **1** and cyclophellitol aziridine **2** and derivatives are highly potent inhibitors of retaining  $\beta$ -glucosidases as demonstrated in this Thesis and previously reported studies. The cyclitol core, which resembles the absolute stereochemistry of the natural substrate  $\beta$ -glucoside, is possibly a contributing factor to the high inhibitory potency. Substitution of the epoxide or aziridine moiety for another electrophilic trap such as sulphate or phosphate may result in the formation of cyclophellitol sulphate **74** and cyclophellitol phosphate **75** as putative  $\beta$ -glucosidase inhibitors (Figure 7A).

**Figure 7.** Putative  $\beta$ -glucosidase inhibitors (A) cyclophellitol analogues **74**, **75**, (B) imidazole derivatives **76-79** and (C) Proton-transfer/coordination mechanism of **77-79**.

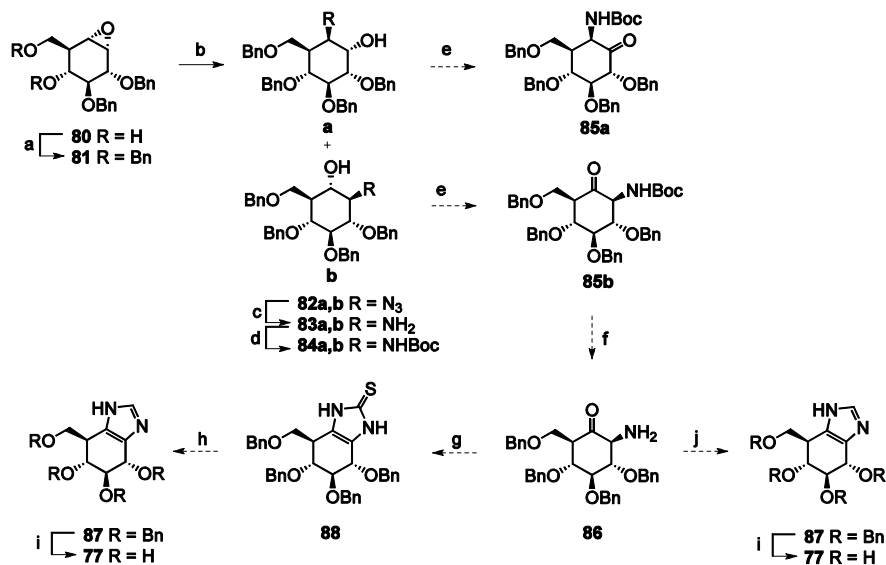


Another putative class of  $\beta$ -glucosidase cyclitol inhibitors is cyclophellitol imidazole **77**, which theoretically enables the inhibition of glycosidases in a proton transfer/coordination fashion as previously demonstrated for **76** (Figure 7B and 7C).<sup>19</sup> The synthesis of cyclophellitol imidazoles **77** and analogues (for example **78** and **79**) may be synthesized according Scheme 5.

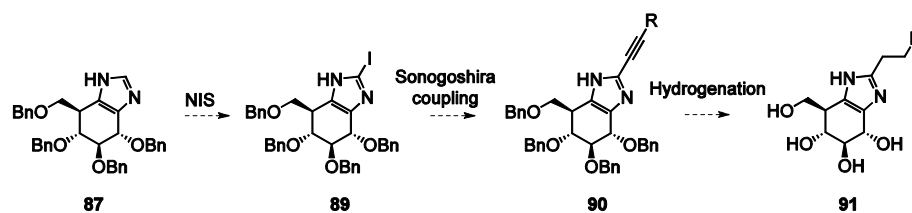
The two hydroxyl functions in 1,6-*epi*-cyclophellitol **80** (see **Chapter 5**) were benzylated with benzyl bromide and sodium hydride to afford **81** in a moderate yield (66%). Next, the epoxide moiety was opened with sodium azide in the presence of lithium perchlorate as Lewis acid to give a mixture of azido-alcohols **82a,b** as an inseparable mixture (89%). Ensuing Staudinger reduction ( $\text{PMe}_3$ , pyridine,  $\text{H}_2\text{O}$ ) of **82a,b** and Boc-protection ( $\text{Boc}_2\text{O}$ ,  $\text{Et}_3\text{N}$ , DCM) of the resulting amines **83a,b** gave

**84a,b** as a separable mixture (**84a**: 31%, **84b**: 59%). Cyclophellitol imidazole **77** can presumably be synthesized according to the synthetic route described in Scheme 5 starting from **84b**. A similar sequence should be applicable for **84a** as well. Oxidation of the secondary hydroxyl group in **84a,b** with Dess-Martin periodane may give ketone **85a,b**. Subsequent removal of the Boc protecting group should yield amine **86**, which can be treated with formamidine acetate to form the protected cyclophellitol imidazole **87** directly. Alternatively, **87** may be constructed in two steps. First, treatment of **86** with potassium thiocyanate and triethylamine is then followed by desulfurization of the resulting thiourea analogue **88** with either benzoic peroxide or hydrogen peroxide under acidic conditions. Removal of the benzyl ether protective groups should yield the desired compound **77**. The introduction of alkyl substituents in **77** may be possible as depicted as Scheme 6. Treatment of **87** with NIS should the iodine derivative **89**, which can be subjected to Sonogoshira conditions with a given terminal alkyne. Finally, Pd-catalyzed hydrogenation of the triple bond and benzyl ether group in **90** may furnish the desired target compound **91**.

**Scheme 5.** Putative synthetic route towards cyclophellitol imidazole **77**.



Reagents and conditions: (a) BnBr, NaH, DMF, 0 °C, 66%; (b) LiClO<sub>4</sub>, NaN<sub>3</sub>, toluene, 120 °C, 89%; (c) PMe<sub>3</sub>, pyridine, H<sub>2</sub>O (9/1; v/v); (d) Boc<sub>2</sub>O, Et<sub>3</sub>N, DCM, **84a**: 31%, **84b**: 59%; (e) Dess-Martin periodane, DCM; (f) 4 M HCl, dioxane; (g) KSCN, Et<sub>3</sub>N, toluene; (h) i. Bz<sub>2</sub>O<sub>2</sub> or ii. H<sub>2</sub>O<sub>2</sub> (i) Pd(OH)<sub>2</sub>, H<sub>2</sub>, MeOH; (j) formamidine acetate, toluene

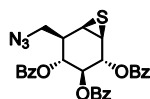
**Scheme 6.** Putative route towards substituted cyclophellitol imidazole derivative **91**.

## Conclusion

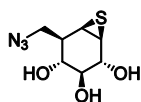
The work described in this Thesis reveals that cyclophellitol, a naturally occurring product, is a useful template for the development of mechanism-based and competitive inhibitors of retaining  $\beta$ -glycosidases. At the same time, cyclophellitol and its derivatives serve as a scaffold for the development of retaining  $\beta$ -glycosidase activity-based probes. This opens up the possibility to construct inhibitors and ABPs for glycosidases that follow the general Koshland mechanisms by emulating the configuration and substitution pattern of the corresponding substrate glycosides.

## Experimental section

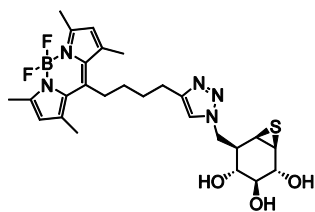
General methods: All reagents and solvents were of a commercial grade and used as received unless stated otherwise. THF and dichloromethane were stored over flamed-dried 3 Å molecular sieves. All reactions were performed under an inert atmosphere unless stated otherwise. Solvents used for flash chromatography were of pro analysi quality. Reactions were monitored by TLC analysis using aluminum sheets pre-coated with silica gel 60 with detection by UV-absorption (254 nm) and by spraying with a solution of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot\text{H}_2\text{O}$  (25 g/L) and  $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4\cdot\text{H}_2\text{O}$  (10 g/L) in 10% sulfuric acid followed by charring at  $\sim 150^\circ\text{C}$  or by spraying with 20% sulfuric acid in ethanol followed by charring at  $\sim 150^\circ\text{C}$ . Column chromatography was performed using Screening Device silica gel in the indicated solvents.  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, COSY and HSQC spectra were recorded on a Bruker DMX-400 (400/100 MHz), Bruker AV-400 (400/100 MHz) and Bruker AV-600 (600/150 MHz) spectrometer in the given solvent. Chemical shifts are reported as  $\delta$ -values in ppm relative to the chloroform residual solvent peak or tetramethylsilane (TMS) as internal standard or the signal of the deuterated solvent. Coupling constants are given in Hz. All given  $^{13}\text{C}$  spectra are proton decoupled. High resolution mass spectra were recorded with a LTQ Orbitrap (Thermo Finnigan). HPLC-MS purifications were performed on an Agilent Technologies 1200 series automated HPLC system with a Quadrupole MS 6130, equipped with a semi-preparative Gemini C18 column (Phenomenex, 250 x 10, 5  $\mu$ , particle size).

**8-deoxy-2,3,4-tri-O-benzoyl-8-azidocyclophellitol thiirane 21**

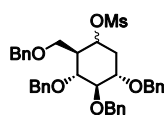
Trifluoroacetic acid (0.04 mL, 0.50 mmol) and *N,N*-dimethylthioformamide (0.09 mL, 1.0 mmol) were added dropwise to a solution of epoxide<sup>3</sup> (220 mg, 0.42 mmol) in DCM (19 mL) at 40 °C. The reaction mixture was stirred at 40 °C for 18 h, before being concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/ EtOAc, 94:6→92:8) yielded **21** (130 mg, 0.25 mmol, 59%) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.04 (d, *J* = 7.2 Hz, 2H, H<sub>Ar</sub>Bz), 7.87 (d, *J* = 7.2 Hz, 2H, H<sub>Ar</sub>Bz), 7.79 (d, *J* = 7.2 Hz, 2H, H<sub>Ar</sub>Bz), 7.58-7.55 (m, 1H, H<sub>Ar</sub>Bz), 7.52-7.38 (m, 4H, H<sub>Ar</sub>Bz), 7.32 (t, *J* = 8.0 Hz, 2H, H<sub>Ar</sub>Bz), 7.26-7.22 (m, 2H, H<sub>Ar</sub>Bz), 5.82-5.77 (m, 2H, H-2, H-3), 5.56-5.48 (m, 1H, H-4), 3.71 (dd, *J* = 4.0, 12.0 Hz, 1H, H-8), 3.66 (dd, *J* = 3.6, 6.0 Hz, 1H, H-6), 3.55 (dd, *J* = 9.6, 12.0 Hz, 1H, H-8), 3.37 (d, *J* = 6.0 Hz, 1H, H-1), 3.03-2.96 (m, 1H, H-5). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 165.8, 165.6, 165.6, 133.5, 133.4, 133.2, 129.9, 129.7, 129.6, 129.1, 128.8, 128.7, 128.5, 128.3, 128.2, 74.2, 73.5, 68.2, 53.8, 41.0, 39.5, 37.2. HRMS: found 552.1198 [M+Na]<sup>+</sup>, calculated for [C<sub>28</sub>H<sub>23</sub>N<sub>3</sub>O<sub>6</sub>SNa] 552.1199.

**8-deoxy-8-azidocyclophellitol thiirane 17**

A catalytic amount of NaOMe was added to a solution of **21** (102 mg, 0.19 mmol) in MeOH (2 mL) and the reaction was stirred at ambient temperature for 1 h. Next, the mixture was neutralized with Amberlite IR-120 H<sup>+</sup>, filtered and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/EtOAc, 0:100) yielded **17** (30 mg, 0.14 mmol, 72%). <sup>1</sup>H NMR (400 MHz, MeOD): δ 4.05 (d, *J* = 4.8 Hz, 1H, H-2), 3.92 (dd, *J* = 3.6, 12.0 Hz, 1H, H-8), 3.48 (dd, *J* = 4.0, 6.8 Hz, 1H, H-6), 3.35 (dd, *J* = 9.2, 11.6 Hz, 1H, H-8), 3.26-3.17 (m, 2H, H-3, H-4), 3.14 (d, *J* = 6.4 Hz, 1H, H-1), 2.40-2.33 (m, 1H, H-5). <sup>13</sup>C NMR (100 MHz, MeOD): δ 79.8, 75.5, 68.5, 55.7, 44.1, 41.7, 41.5. HRMS: found 240.0414 [M+Na]<sup>+</sup>, calculated for [C<sub>7</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>SNa] 240.0413.

**BODIPY-cyclophellitol thiirane derivative 18**

CuSO<sub>4</sub> (20 μL of 1M in H<sub>2</sub>O) and sodium ascorbate (20 μL of 1M in H<sub>2</sub>O) were added to a solution of **17** (33 mg, 0.15 mmol) and BODIPY-alkyne<sup>4</sup> (49 mg, 0.15 mmol) in DMF (5 mL). The reaction was stirred at ambient temperature for 2 h before being diluted with Et<sub>2</sub>O, washed with H<sub>2</sub>O and concentrated under reduced pressure. Purification by HPLC (acetonitrile/H<sub>2</sub>O) yielded **18** (32 mg, 57 μmol, 38%) as an orange powder. <sup>1</sup>H NMR (400 MHz, MeOD): δ 7.76 (s, 1H, CH<sub>trz</sub>), 6.09 (s, 2H, H<sub>Ar</sub>), 4.87 (below D<sub>2</sub>O signal is H-8), 4.43 (dd, *J* = 9.2, 14.0 Hz, 1H, H-8), 3.94 (d, *J* = 7.2 Hz, 1H, H-2), 3.02-2.97 (m, 2H, H-4, H-5), 2.96 (d, *J* = 6.4 Hz, 1H, H-6), 2.82 (dd, *J* = 4.0, 6.4 Hz, 1H, H-1), 2.77 (t, *J* = 7.2 Hz, 2H, CH<sub>2</sub>alkyl), 2.72-2.63 (m, 1H, H-5), 2.40 (s, 1H, 6H, OMe), 2.35 (s, 6H, OMe), 1.92-1.91 (m, 2H, CH<sub>2</sub>alkyl), 1.65-1.16 (m, 3H, CH<sub>2</sub>alkyl), 1.33-1.26 (m, 3H, CH<sub>2</sub>alkyl). <sup>13</sup>C NMR (100 MHz, MeOD): δ 154.9, 148.1, 147.9, 142.2, 132.6, 124.5, 122.6, 79.6, 75.4, 68.4, 53.6, 44.7, 41.3, 40.3, 32.3, 31.1, 30.9, 29.1, 25.9, 16.5, 14.4. HRMS: found 546.2520 [M+H]<sup>+</sup>, calculated for [C<sub>26</sub>H<sub>25</sub>BF<sub>2</sub>N<sub>5</sub>O<sub>3</sub>S] 546.2524.



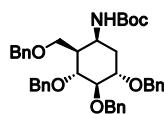
**(2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)cyclohexyl methanesulfonate 67**

Alcohol **66** (140 mg, 0.26 mmol) was dissolved in pyridine (3 mL). A catalytic amount of DMAP (10 mg, 0.08 mmol) and MsCl (201  $\mu$ L, 2.60 mmol) was added to the solution and the mixture was stirred for overnight at room temperature. After cooling down to 0 °C, the reaction mixture was carefully quenched with 1 M HCl before being poured into EtOAc. The organic layer was washed with 1 M HCl, dried over MgSO<sub>4</sub> and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/EtOAc, 90:10→80:20) yielded **67** (108 mg, 0.18 mmol, 68%). The mixture of epimers could not be purified at this stage. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.62-7.04 (m, 36H), 5.16-5.15 (m, 1H), 5.02-4.88 (m, 6H), 4.73 (d,  $J$  = 11.0 Hz, 1H), 4.72-4.67 (m, 1H), 4.53 (d,  $J$  = 10.8 Hz, 1H), 4.49-4.36 (m, 5H), 3.89 (ddd,  $J$  = 4.7, 9.1, 12.0 Hz, 1H), 3.75 (dd,  $J$  = 4.1, 9.2 Hz, 1H), 3.69 (dd,  $J$  = 4.1, 9.2 Hz, 1H), 3.54-3.51 (m, 4H), 3.50-3.47 (m, 1H), 3.43 (dd,  $J$  = 2.4, 9.0 Hz, 1H), 3.36 (dd,  $J$  = 8.0, 8.0 Hz, 1H), 2.82 (s, 5H), 2.63 (dt,  $J$  = 4.3, 14.6 Hz, 1H), 2.25 (ddd,  $J$  = 3.4, 5.1, 12.9 Hz, 1H), 2.09 (tq,  $J$  = 2.6, 3.2, 10.4 Hz, 1H), 1.95-1.80 (m, 1H), 1.75-1.71 (m, 1H), 1.55 (ddd,  $J$  = 2.1, 11.7, 14.0 Hz, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  140.3, 140.0, 139.8, 139.8, 139.7, 139.6, 139.5, 139.2, 130.10, 130.1, 130.0, 130.0, 129.7, 129.6, 129.6, 129.6, 129.5, 129.5, 129.5, 129.5, 129.4, 129.4, 129.4, 129.3, 129.3, 129.3, 129.2, 129.2, 129.2, 89.1, 87.8, 84.8, 83.5, 79.5, 79.0, 78.8, 78.7, 78.4, 77.5, 77.4, 77.3, 77.0, 74.9, 74.8, 74.4, 70.5, 68.3, 47.7, 40.3, 39.7, 39.4, 36.6, 33.2.



**(((1S,2R,3R,4R)-5-azido-4-((benzyloxy)methyl)cyclohexane-1,2,3-triyl)-tris(oxy))tris(methylene)tribenzene 68a**

Mesylate **67** (108 mg, 0.18 mmol) was co-evaporated thrice with toluene before DMF (3 mL) was added. After addition of sodium azide (114 mg, 1.75 mmol) the reaction mixture was stirred overnight at 120 °C. The reaction mixture was then diluted with water and extracted twice with Et<sub>2</sub>O. The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (petroleum ether/EtOAc, 96:4→90:10) allowed the separation of the diastereoisomers and afforded **68a** as a single epimer (84 mg, 0.15 mmol, 83%). <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>):  $\delta$  7.74-6.34 (m, 20H), 4.94 (d,  $J$  = 10.6 Hz, 1H), 4.88 (d,  $J$  = 11.0 Hz, 1H), 4.82 (d,  $J$  = 10.7 Hz, 1H), 4.73 (s, 2H), 4.99 (d,  $J$  = 10.9 Hz, 1H), 4.41 (s, 2H), 4.00-3.99 (m, 1H), 3.87 (t,  $J$  = 9.3 Hz, 1H), 3.67 (dd,  $J$  = 4.3, 9.0 Hz, 1H), 3.54 (dd,  $J$  = 3.4, 9.5 Hz, 1H), 3.46 (dd,  $J$  = 9.1, 10.8 Hz, 1H), 3.39 (dd,  $J$  = 2.6, 9.1 Hz, 1H), 2.05-1.93 (m, 1H), 1.82 (dt,  $J$  = 3.7, 14.6 Hz, 1H), 1.61-1.53 (m, 1H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  139.0, 138.8, 138.5, 138.1, 128.6, 128.5, 128.1, 128.0, 128.0, 127.7, 127.7, 83.8, 83.1, 80.6, 76.0, 75.4, 73.1, 69.6, 59.6, 37.6, 29.3. HRMS: found 564.2856 [M+H]<sup>+</sup>, calculated for [C<sub>35</sub>H<sub>38</sub>N<sub>3</sub>O<sub>3</sub>] 564.2857.

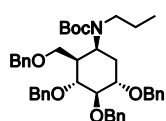


**tert-butyl ((1S,2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)-cyclohexyl)-carbamate 69a**

PMe<sub>3</sub> (1 M in THF, 0.58 mL, 0.58 mmol) was added to a solution of **68a** (54 mg, 0.01 mmol) in pyridine/H<sub>2</sub>O (9/1; v/v; 0.9 mL/ 0.1 mL). The reaction was stirred for 6 h at ambient temperature, concentrated under reduced pressure and coevaporated thrice with toluene. The crude amine was redissolved in DCM (1 mL). After the addition of Boc<sub>2</sub>O (31 mg, 0.14

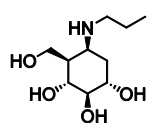


mmol) and Et<sub>3</sub>N (40 μL, 0.29 mmol), the reaction mixture was stirred overnight at ambient temperature and concentrated under reduced pressure. Purification by silica gel column chromatography (petroleum ether/EtOAc, 95:5→90:10) afforded **69a** (40 mg, 0.063 mmol, 65%) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.44-6.93 (m, 20H), 4.93 (d, *J* = 10.8 Hz, 1H), 4.87 (d, *J* = 10.8 Hz, 1H), 4.81-4.73 (m, 2H), 4.70 (d, *J* = 11.3 Hz, 1H), 4.52 (d, *J* = 11.2 Hz, 2H), 4.43 (s, 2H), 4.20-4.18 (m, 1H), 3.71 (dd, *J* = 4.2, 9.0 Hz, 1H), 3.63-3.47 (m, 3H), 3.44 (dd, *J* = 2.6, 9.0 Hz, 1H), 2.19 (dt, *J* = 3.6, 14.5 Hz, 1H), 1.88 (ddd, *J* = 3.6, 9.7, 13.2 Hz, 1H), 1.61-1.46 (m, 1H), 1.44 (s, 9H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 156.0, 139.0, 138.8, 138.6, 138.1, 128.5, 128.2, 128.0, 127.9, 127.7, 83.9, 81.2, 80.4, 79.6, 75.9, 75.4, 73.2, 71.6, 69.9, 47.4, 37.5, 28.9, 28.5. HRMS: found 638.3476 [M+H]<sup>+</sup>, calculated for [C<sub>40</sub>H<sub>48</sub>NO<sub>6</sub>] 638.3476.



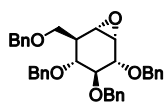
**tert-butyl propyl((1S,2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)cyclohexyl)carbamate **70****

To a suspension of NaH (60% dispersion in mineral oil) (16.5 mg, 0.41 mmol) in DMF (2.5 mL) was added compound **69** (40 mg, 0.063 mmol) at 0 °C. After stirring for 30 min at room temperature, propyl bromide (40 μL, 0.44 mmol) was added and the resulting mixture was stirred at 80 °C for 2 days. After cooling down to room temperature, the reaction mixture was then diluted with water and extracted twice with Et<sub>2</sub>O. The combined organic layers were dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. Purification by silica gel column chromatography (petroleum ether/EtOAc, 90:10) afforded the desired secondary amine **70** (10 mg, 0.015 mmol, 25%) as a colorless oil. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ 7.54-6.79 (m, 20H), 4.61 (d, *J* = 11.7 Hz, 1H), 4.57-4.37 (m, 8H), 3.76-3.74 (m, 1H), 3.73-3.72 (m, 1H), 3.62-3.59 (m, 3H), 3.30-3.24 (m, 1H), 3.20-3.17 (m, 1H), 2.44-2.32 (m, 1H), 2.27-2.19 (m, 1H), 1.72-1.37 (m, 13H), 0.77 (t, *J* = 7.4 Hz, 3H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ 155.9, 138.9, 138.8, 138.5, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 79.8, 79.3, 73.2, 73.0, 72.6, 72.2, 71.1, 49.3, 46.8, 38.3, 29.9, 28.7, 23.9, 11.6. HRMS: found 680.3944 [M+H]<sup>+</sup>, calculated for [C<sub>43</sub>H<sub>54</sub>NO<sub>6</sub>] 680.3946.



**(1S,2R,3R,4R,5S)-4-(hydroxymethyl)-5-(propylamino)cyclohexane-1,2,3-triol **61****

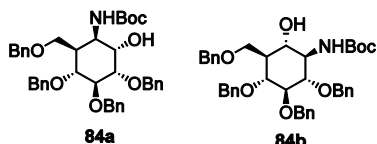
The secondary amine **70** (10 mg, 0.015 mmol) was dissolved in aldehyde-free MeOH (1 mL) and HCl (37%, 60 μL, 0.36 mmol) was added. Oxygen was depleted from the reaction mixture by sonication under an argon atmosphere. 5 mol% Pd/C (10 wt%) was added and hydrogen was bubbled through the solution for 15 min. The reaction mixture was then stirred 2 d under an H<sub>2</sub> atmosphere. The crude was filtered through a pad of celite. Solvent was removed *in vacuo* and the desired **61** was obtained (2 mg, 9.45 μmol, 63%). <sup>1</sup>H NMR (600 MHz, MeOD) δ 3.75 (dd, *J* = 3.7, 10.7 Hz, 1H), 3.69 (dd, *J* = 4.5, 9.1 Hz, 1H), 3.66-3.61 (m, 1H), 3.54 (d, *J* = 4.5 Hz, 1H), 3.48 (t, *J* = 8.5 Hz, 1H), 3.25-3.17 (m, 1H), 3.07-2.98 (m, 2H), 2.15-2.08 (m, 1H), 1.84-1.60 (m, 3H), 1.02 (t, *J* = 7.4 Hz, 3H). <sup>13</sup>C NMR (150 MHz, MeOD): δ 75.4, 73.4, 71.8, 63.2, 58.5, 49.6, 40.47, 24.7, 20.0, 11.2. HRMS: found 22.1543 [M+H]<sup>+</sup>, calculated for [C<sub>10</sub>H<sub>22</sub>NO<sub>4</sub>] 220.1543.



**2,3,4,8-tetra-O-benzyl-1,6-*epi*-cyclophellitol **81****

Sodium hydride (60% dispersion in mineral oil, 453 mg, 11.3 mmol) was added to a cooled (0 °C) solution of benzyl bromide (0.54 mL, 4.44 mmol) and 2,3-di-O-

benzyl-1,6-*epi*-cyclophellitol **80** (721 mg, 2.0 mmol, see Chapter 5) in DMF (21 mL). After stirring for 4 h at 0 °C, the reaction was quenched with MeOH at 0 °C, diluted with H<sub>2</sub>O, extracted with Et<sub>2</sub>O, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/EtOAc, 90:10→80:20) yielded **81** (714 mg, 1.32 mmol, 66%) as a colorless oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.41-7.24 (m, 20H, H<sub>A</sub>,Bn), 4.88-4.80 (m, 5H, CH<sub>2</sub>Bn), 4.41 (d, *J* = 12.4 Hz, 1H, CH<sub>2</sub>Bn), 4.38 (d, *J* = 11.2 Hz, 1H, CH<sub>2</sub>Bn), 4.33 (d, *J* = 12.0 Hz, 1H, CH<sub>2</sub>Bn), 3.88 (dd, *J* = 2.0, 8.8 Hz, 1H, H-2), 3.73 (d, *J* = 10.0 Hz, 1H, H-8), 3.55 (d, *J* = 3.6 Hz, 2H, H-8, H-3), 3.46 (t, *J* = 10.0 Hz, 1H, H-6), 3.33 (dd, *J* = 1.6, 4.6 Hz, 1H, H-4), 3.16 (d, *J* = 4.0 Hz, 1H, H-1), 2.22 (dt, *J* = 3.6, 10.0 Hz, 1H, H-5). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 138.6, 138.3, 138.3, 137.8, 128.4, 128.3, 128.2, 127.9, 127.9, 127.7, 127.7, 127.7, 127.6, 127.5, 127.4, 82.2, 79.7, 77.5, 75.6, 75.2, 73.0, 72.7, 68.1, 54.9, 54.7, 42.7.



#### 1,2-hydroxyyl-tertbutylcarbamate-3,4,5,7-tetra-O-benzylcyclitol **84a** and **84b**

A mixture of **81** (714 mg, 1.32 mmol) and lithium perchlorate (702 mg, 6.6 mmol) was coevaporated thrice with anhydrous toluene before being dissolved in acetonitrile (20 mL) and DMF (20 mL). After stirring for 30 min at ambient temperature, sodium azide (858 mg, 13.2 mmol) was added and the reaction was stirred at 120 °C until TLC analysis showed full consumption of the starting material (18 h). The mixture was diluted with H<sub>2</sub>O, extracted with Et<sub>2</sub>O, dried over MgSO<sub>4</sub>, filtered and concentrated *in vacuo*. Purification by silica column chromatography (petroleum ether/EtOAc, 90:10→80:20) yielded **82a,b** (674 mg, 1.17 mmol, 87%) as an inseparable mixture. PMe<sub>3</sub> (1M in THF, 7.0 mL, 7.0 mmol) was added to a solution of **82a,b** (406 mg, 0.7 mmol) in pyridine (6.3 mL) and H<sub>2</sub>O (0.7 mL). After stirring for 5 h, the reaction was concentrated and co-evaporated thrice with toluene before being dissolved in DCM (7 mL). Boc<sub>2</sub>O (229 mg, 1.05 mmol) and triethylamine (0.29 mL, 2.1 mmol) were added and subsequently stirred for two days. The mixture was concentrated *in vacuo* and purified by silica column chromatography (petroleum ether/EtOAc, 80:20) and (petroleum ether/ EtOAc (78:22→70:30) to furnish **84a** (144 mg, 0.22 mmol, 31%) and **84b** (271 mg, 0.42 mmol, 59%) respectively. **84a**: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.28-7.18 (m, 20H, H<sub>A</sub>,Bn), 5.65 (br, 1H, NHBoc), 4.89 (d, *J* = 11.6 Hz, 1H, CH<sub>2</sub>Bn), 4.86 (d, *J* = 8.0 Hz, 1H, CH<sub>2</sub>Bn), 4.79 (d, *J* = 11.2 Hz, 1H, CH<sub>2</sub>Bn), 4.66 (s, 2H, CH<sub>2</sub>Bn), 4.58 (d, *J* = 10.8 Hz, 1H, CH<sub>2</sub>Bn), 4.44-4.30 (m, 1H, H-1), 4.40 (s, 2H, CH<sub>2</sub>Bn), 3.98 (dd, *J* = 4.8, 9.2 Hz, 1H, H-7), 3.94 (q, *J* = 3.6 Hz, 1H, H-6), 3.88 (t, *J* = 9.2 Hz, 1H, H-3), 3.74 (t, *J* = 11.6 Hz, 1H, H-4), 3.67 (dd, *J* = 2.8, 9.2 Hz, 1H, H-2), 3.58 (d, *J* = 9.6 Hz, 1H, H-7), 2.40 (dt, *J* = 3.6, 11.2 Hz, 1H, H-5), 1.42 (s, 9H, CH<sub>3</sub>Boc). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 156.1, 139.2, 139.1, 138.8, 138.2, 137.9, 129.9, 128.9, 128.5, 128.4, 128.3, 128.0, 127.7, 127.7, 127.6, 127.5, 127.4, 127.3, 83.5, 80.1, 77.6, 75.4, 75.1, 73.6, 72.5, 69.6, 66.2, 47.8, 38.6, 28.4. **84b**: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.29-7.18 (m, 20H, H<sub>A</sub>,Bn), 4.87-4.83 (m, 4H, CH<sub>2</sub>Bn), 4.66 (d, *J* = 11.2 Hz, 1H, CH<sub>2</sub>Bn), 4.55 (d, *J* = 11.2 Hz, 1H, CH<sub>2</sub>Bn), 4.51-4.41 (m, 3H, CH<sub>2</sub>Bn, NHBoc), 3.88 (dd, *J* = 2.0, 8.8 Hz, 1H, H-7), 3.73 (dd, *J* = 3.2, 8.8 Hz, 1H, H-7), 3.63-3.54 (m, 3H, H-3, H-4, H-6), 3.41-3.38 (m, 2H, H-1, H-2), 1.71-1.66 (m, 1H, H-5), 1.43 (s, 9H, CH<sub>3</sub>Boc). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 157.2, 138.8, 138.8, 138.5, 128.3, 128.5, 128.4, 128.3, 128.3, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 87.0, 80.1, 79.4, 77.6, 75.4, 75.3, 74.9, 73.4, 69.6, 66.2, 47.8, 28.4.

## References

- 1 L.I. Willems, T.J.M. Beenakker, B. Murray, S. Scheij, W.W. Kallemeijn, R.G. Boot, M. Verhoek, W.E. Donker-Koopman, M.J. Ferraz, E.R. van Rijssel, B.I. Florea, J.D.C. Codée, G.A. van der Marel, J.M.F.G. Aerts, H.S. Overkleeft, *J. Am. Chem. Soc.* **2014**, *136*, 11622-11625
- 2 L.I. Willems, T.J.M. Beenakker, B. Murray, B. Gagestein, H. van den Elst, E. R. van Rijssel, J.D.C. Codée, W.W. Kallemeijn, J.M.F.G. Aerts, G.A. van der Marel, H.S. Overkleeft, *Eur. J. Org. Chem.* **2014**, *27*, 6044-6056
- 3 M.D. Witte, W.W. Kallemeijn, J. Aten, K.Y. Li, A. Strijland, W.E. Donker-Koopman, A.M. van den Nieuwendijk, B. Bleijlevens, G. Kramer, B.I. Florea, B. Hooibrink, C.E. Hollak, R. Ottenhoff, R.G. Boot, G.A. van der Marel, H.S. Overkleeft, J.M. Aerts, *Nat. Chem. Biol.* **2010**, *6*, 907-913.
- 4 M. Verdoes, U. Hillaert, B.I. Florea, M. Sae-Heng, M.D.P Risseeuw, D.V. Filippov, G.A van der Marel, H.S. Overkleeft, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6169-6171
- 5 J.M. Aerts, R. Ottenhoff, A.S. Powlson, A. Grefhorst, M. van Eijk, P. F. Dubbelhuis, J. Aten, F. Kuipers, M.J. Serlie, T. Wennekes, J.K. Sethi, S. O’Rahilly, H.S. Overkleeft, *Diabetes*, **2007**, *56*, 1341-1349
- 6 F.A. Shaikh, S.G. Withers, *Biochem. Cell. Biol.* **2008**, *86*, 169-177
- 7 L.F. Mackenzie, Q. Wang, R.A.J. Warren, S.G. Withers, *J. Am. Chem. Soc.* **1998**, *120*, 5583-5584
- 8 K.L. Schimz, B. Broll, B. John, *Arch. Microbiol.* **1983**, *135*, 241-249
- 9 H. Nakai, M. A. Hachem, B. O. Petersen, Y. Westphal, K. Mannerstedt, M. J. Baumann, A. Dilokpimol, H.A. Schols, J. Duus, B. Svensson, *Biochimie* **2010**, *92*, 1818-1826
- 10 T. Rasmussen, S.G. Withers, *personal communication*
- 11 M. Arai, K. Sugimoto, T. Kawaguchi, *Chem. Express* **1992**, *7*, 565-586
- 12 M. Chambers, S.G. Withers, *personal communication*
- 13 A. Delgado, J. Casas, A. Llebaria, J.L. Abad, G. Fabrias, *Biochim. Biophys. Acta. Biomembr.* **2006**, *1758*, 1957-1977
- 14 A. Delgado, J. Casas, A. Llebaria, J.L. Abad, G. Fabrias, *ChemMedChem* **2007**, *2*, 580-606
- 15 E.B. de Melo, A.S. Gomes, I. Carvalho, *Tetrahedron* **2006**, *62*, 10277-10302
- 16 S. Ogawa, M. Ashiura, C. Uchida, S. Watanabe, C. Yamazaki, K. Yamgishi, J. Inokuchi, *Bioorg. Med. Chem.* **1998**, *6*, 1955-1962
- 17 M. Egido-Gabas, P. Serrano, J. Casa, A. Llebaria, A. Delgado, *Org. Biomol. Chem.* **2005**, *3*, 1195-1201
- 18 P. Kapferer, V. Birault, J-F. Poisson, A. Vasella, *Helv. Chim. Acta* **2003**, *86*, 2210-2227
- 19 T. Granier, A. Vasella, *Helv. Chim. Acta* **1995**, *78*, 1738-1746