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

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5 Cyclophellitol Analogues as Inhibitors for Human Retaining β-Glucosidases

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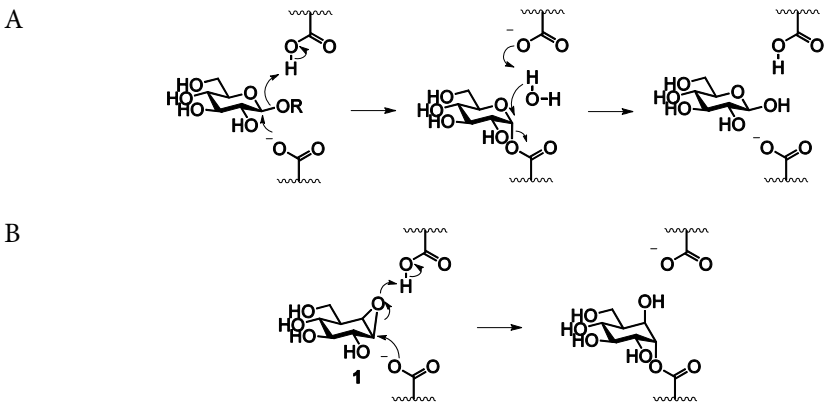
5.1 Introduction

Cyclophellitol **1**, first isolated in 1990 from *Phellinus sp*., is a potent and specific mechanism-based inhibitor of retaining β-glucosidases.[1-4] Cyclophellitol **1** is a close structural mimic of β-glucopyranose that fits well in the active pocket of retaining β-glucosidases. These enzymes, and retaining β-glycosidases in general, employ a catalytic mechanism that is commonly referred to as the Koshland double displacement mechanism.[5-6] This process (see Figure 1A) involves two acidic (Glu or Asp) amino acid residues residing in the enzyme active site. In retaining β-glucosidases, these acidic residues are positioned about 5.5 Å apart such that one can act as the nucleophile and the other as a general acid/base catalyst. When a retaining β-exoglucosidase binds to cyclophellitol **1**, the inhibitor is positioned in such a way that the epoxide can undergo

trans-diaxial opening by consecutive protonation and nucleophilic attack of the two catalytic carboxylic acid residues (Figure 1B). An ester linkage is formed that is considerably more stable than the corresponding acylal linkage that would result from the initial step in the enzymatic cleavage of β-glucosidic linkages. As a result hydrolysis of the covalently bound enzyme-cyclophellitol adduct is slowed down to a point that the enzyme is effectively and irreversibly inhibited.[7]

Figure 1. (A) General double-displacement mechanism of retaining β-glucosidases and (B) Mechanism of inactivation by cyclophellitol **1**.

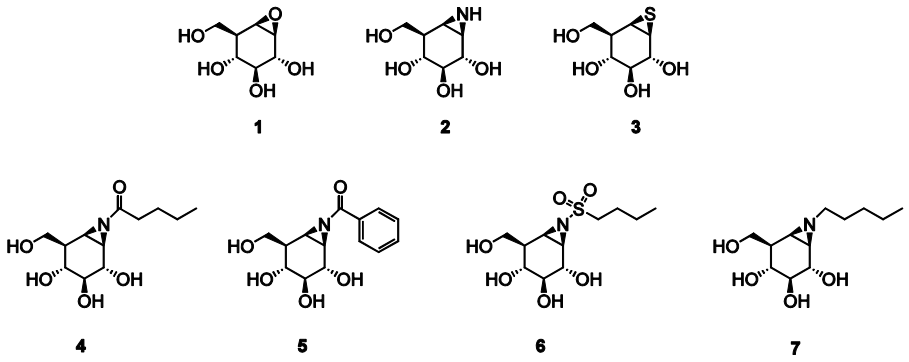


Following the discovery of cyclophellitol **1**, Tatsuta and co-workers reported the synthesis of cyclophellitol aziridine **2** and cyclophellitol thiirane **3** as well as a number of *N*-alkyl-and *N*-acyl cyclophellitol aziridine derivatives (Figure 2).[8-10] Inhibition studies performed on almond β-glucosidase revealed that the nature of the *N*-substituent on cyclophellitol aziridine influences inhibitory potency. Following these initial studies, research on cyclophellitol derivatives as glycosidase inhibitors has been relatively limited until recently, especially when compared to the multitude of reports describing the synthesis and evaluation of deoxynojirimycin-type competitive glycosidase inhibitors.[11-13] Arguably, this lack of interest stems from the covalent and irreversible mode of action exerted by this compound class – a feature not normally considered being ideal for drug development. Indeed, research on deoxynojirimycin derivatives is often conducted with drug discovery aims in mind, with a number of *N*-alkyl deoxynojirimycin derivatives currently in development or in clinical use.[14-16] Cyclophellitol derivatives and more in general mechanism-based glycosidase inactivators are, however, receiving increasing attention as discovery tools in chemical

glycobiology research. 2-Deoxy-2-fluoroglycosides have proven indispensable tools to unravel the mode of action of retaining glycosidases and to confirm the double displacement mechanism originally proposed by Koshland.[17-20] Owing to their general superior inhibitory potency, cyclophellitol derivatives are the most promising starting points for the development of reagents for activity-based profiling of retaining glycosidases. Previous research demonstrated that substitution of cyclophellitol **1** at the primary alcohol with a BODIPY reporter group yielded a highly potent and specific activity-based probe to monitor the human lysosomal retaining β-glucosidase, GBA, in cells extracts, *in situ* and *in vivo*.[21] *N*-acyl cyclophellitol aziridine in turn with (BODIPY or biotin) reporter groups occupying the space normally reserved for the aglycon within an exoglucosidase active site proved to be broad-spectrum retaining β-glucosidase probes.[22] These activity-based retaining β-glucosidase probes efficiently modified all four known human retaining β-glucosidases, GBA, GBA2, GBA3 and LPH. These latter results invite a more in-depth study of modified cyclophellitol derivatives as mechanism-based inhibitors, and possible starting points for activity-based probe development of mammalian retaining β-glucosidases.

This Chapter describes the synthesis of a comprehensive series of cyclophellitol derivatives sharing the absolute stereochemistry of the natural product, cyclophellitol **1** and varying in the nature of the electrophilic centre (O, S, NH, *N*-alkyl, *N*-acyl or *N*-sulfonyl) (Figure 2). The inhibitory potency of this compound series towards the three human retaining glucosidases GBA, GBA2 and GBA3 is reported as well.

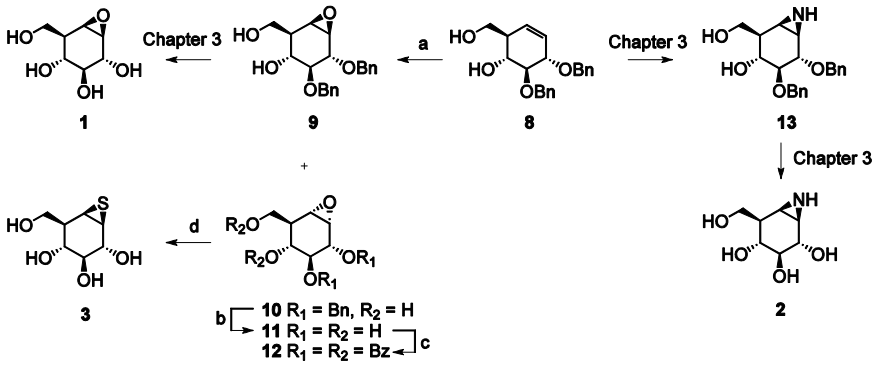
Figure 2. Structures of cyclophellitol **1** and cyclophellitol analogues **2**-**7** targeted in this study.



5.2 Results and Discussion

The synthetic scheme towards all target compounds is based on advanced cyclohexene intermediate **8**, first reported by Madsen and co-workers[23] (Scheme 1).

Scheme 1. Synthesis of cyclophellitol **1**, cyclophellitol aziridine **2** and cyclophellitol thiirane **3**.

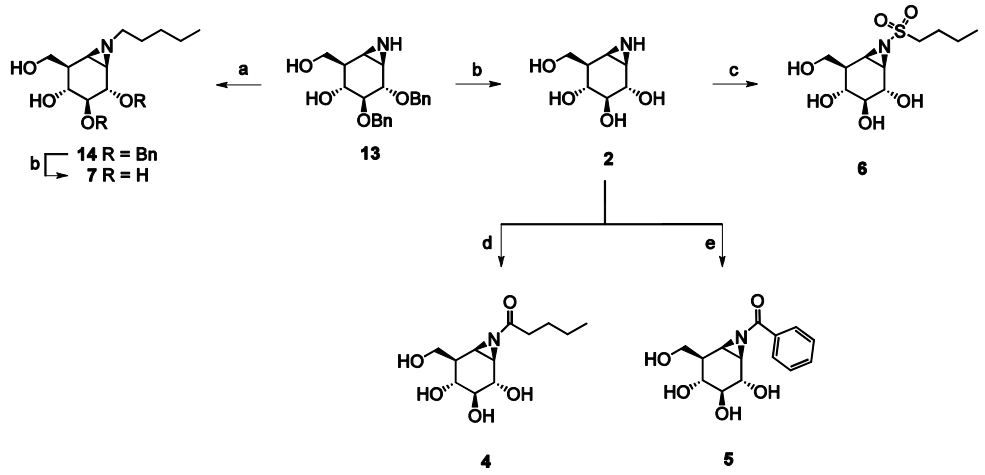


Reagents and conditions: (a) oxone, CF_3OCH_3, $NaHCO_3$, acetonitrile, 4 mM Na_2EDTA (aq.), 0 °C, **9**: 31%, **10**: 46%; (b) $Pd(OH)_2$, H_2, MeOH, 97%; (c) Bz_2O, DMAP, pyridine, 26%; (d) i. *N*,*N*-dimethylthioformamide, TFA, DCM, 40 °C, ii. NaOMe, MeOH, 7% over two steps.

Cyclophellitol **1** and cyclophellitol aziridine **2** were synthesized as previously described in Chapter 3. As demonstrated in Chapter 3, epoxidation of the homo-allylic alcohol with *m*CBPA furnished the required β-epoxide in the natural product, cyclophellitol **1** with good stereoselectivity. The synthesis of β-thiirane **3** however required a route that proceeded through 1,6-*epi*-cyclophellitol **10**. In order to obtain sufficient quantities of this epimeric cyclophellitol derivative, epoxidation of **8** was carried out with *in situ* formed methyl(trifluoromethyl)dioxirane. This process yielded a diastereoisomeric mixture of **9** and **10**, with the desired protected 1,6-*epi*-cyclophellitol **10** as the major isomer (46%). Removal of the benzyl ethers in **10** by $Pd(OH)_2$-catalyzed hydrogenolysis gave 1,6-*epi*-cyclophellitol **11**. Following the procedure previously reported by Tatsuta and co-workers in their synthesis of thiirane **3**, 1,6-*epi*-cyclophellitol derivative **11** was treated with 4-methoxybenzyl chloride (PMBCl) and sodium hydride.[8] However the expected PMB protected 1,6-*epi*-cyclophellitol could not be isolated following this procedure. Altering the protective group pattern in benzoyl groups by treating **11** with benzoic anhydride in pyridine yielded perbenzoylated **12** in a moderate yield and di- and tribenzoylated 1,6-*epi*-cyclophellitol analogues were isolated as major side

products. Attempts to improve the yield of **12** by heating the reaction mixture to 80 °C, prolonging the reaction time or by treating **11** with benzoyl chloride in pyridine were abortive as opening of the epoxide ring was observed under these conditions. Transformation of epoxide **12** into its thiirane analogue was performed by refluxing **12** with *N*,*N*-dimethylthioformamide (DMTF) and trifluoroacetic acid.[23] Ensuing deacetylation under Zemplén conditions furnished cyclophellitol thiirane **3** in 7% yield over two steps.

Scheme 2. Synthesis of *N*-substituted cyclophellitol aziridine **4**-**7**.



Reagents and conditions: (a) 1-iodopentane, K_2CO_3, DMF, 90 °C, 61%; (b) Li, NH_3, THF, -60 °C, **2**: 70%, **7**: 26%; (c) 1-butanesulfonyl chloride, $NaHCO_3$, DMF, 36%; (d) i. valeric acid, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), DMF, 0 °C, ii. HPLC (acetonitrile/H_2O), 23%; (e) i. benzoic acid, EEDQ, DMF, 0 °C, ii. HPLC (acetonitrile/H_2O), 23% .

The synthesis of substituted cyclophellitol aziridines **4**-**7**, starting from either partially protected cyclophellitol aziridine **13** or cyclophellitol aziridine **2** is depicted in Scheme 2. Selective *N*-alkylation of **13** with 1-iodopentane and potassium carbonate, followed by the removal of benzyl ethers via a Birch reduction afforded **7** in an overall yield of 16%. Installment of a sulfonyl functionality in **2** (1-butanesulfonyl chloride, $NaHCO_3$, DMF) furnished sulfonylated cyclophellitol aziridine **6**. Acylation of **2** with a pre-activated solution of valeric acid or benzoic acid with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ)[24] furnished **4** an **5** respectively. Acylated cyclophellitol aziridines are prone to nucleophilic ring opening as stated in Chapter 4 and therefore -**4** and **5** were purified by HPLC using a neutral (acetonitrile/water) gradient. In contrast

to the acylated cyclophellitol aziridines **4** and **5**, the intrinsically more stable sulfonylated - and alkylated cyclophellitol aziridine **6** and **7** could be isolated in high purity by silica gel column chromatography.

The inhibitory potential of cyclophellitol **1** and derivatives **2**-**7** towards the three human retaining β-glucosidases GBA, GBA2 and GBA3 was evaluated by determining their apparent IC_{50} values (Table 1). To this end, the enzymes were incubated with a concentration series of the inhibitor for 30 min followed by measuring the residual enzymatic activity using the fluorogenic substrate 4-methylumbelliferyl β-D-glucopyranoside.

Table 1. Apparent IC_{50} in μM of inhibitors **1**-**7** for GBA, GBA2 and GBA3.

	Inhibitor						
Enzyme	**1**	**2**	**3**	**4**	**5**	**6**	**7**
GBA	0.35	0.5	>100	0.07	1.4	2.5	0.017
GBA2	2	0.4	>100	0.2	0.055	0.3	0.003
GBA3	>100	>100	>100	0.5	20	0.3	0.7

In agreement with the literature values on almond β-glucosidase, cyclophellitol thiirane **3** proved inactive towards all three human retaining β-glucosidases.[8] The corresponding aziridine **2** inhibits all enzymes with equal or slightly higher potency than **1** and subtle, though persistent, differences are observed in the series **4**-**7** when compared to **2**. GBA3 is inhibited 50-200 fold more effectively by **4**-**7** than unsubstituted aziridine **2**. Whereas *N*-sulfonylation, as in **6**, results in a small drop in inhibitory activity for GBA and GBA2, *N*-acylation (**4** and **5**) yields GBA and GBA2 inhibitors with potency about equal to that of unsubstituted **2**. The most potent inhibitor of this series turned out to be *N*-alkyl derivative **7**. This compound inhibits GBA, GBA2 and GBA3 10-100 fold more effectively when compared to **2** as a possible consequence of the high affinity of protonated *N*-alkyl derivative **7** towards the rather negatively charged (acidic) active site of the three studied enzymes. This result is of importance since compound **7** appeared considerably more stable during its synthesis and purification, and is presumably also more stable under physiological conditions. *N*-alkylation of cyclophellitol aziridine derivatives is therefore a promising strategy to obtain mechanism-based retaining β-glucosidase inhibitors and the same holds possibly true for the design of inhibitors targeting retaining glycosidases that hydrolyze differently configured glycosides. Moreover, installation of a bioorthogonal handle via

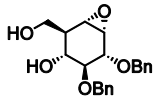
N-alkylation opens the way to design activity-based retaining β-glucosidase probes related to the in Chapter 4 described *N*-acyl analogues, but with improved intrinsic stability and therefore easier-to-handle probes for chemical glycobiology studies.

5.3 Conclusion

This Chapter reveals that modifications of the epoxide in cyclophellitol **1** can have a profound effect on their inhibitory potency. As evident, substitution of the epoxide oxygen for sulfur is detrimental for inhibitory activity. The corresponding aziridine derivative is, however, a viable retaining β-glucosidase inhibitor and *N*-alkyl, *N*-acyl and *N*-sulfonyl substituents are all tolerated. A subtle though important improvement is found in changing an *N*-acyl substituent to an *N*-alkyl substituent. This modification leads to an improved inhibitor with respect to potency, but also to an intrinsically more stable compound. This result may have a significant impact on the future design of activity-based probes directed to retaining β-glucosidases, and to retaining glycosidases in general.

Experimental section

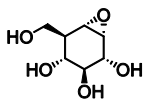
General methods: All reagents and solvents were of a commercial grade and used as received unless stated otherwise. THF and dichloromethane were stored over flamed-dried 3Å molecular sieves. All reactions were performed under an inert atmosphere unless stated otherwise. Solvents used for flash chromatography 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 $(NH_4)_6Mo_7O_{24}.H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4.H_2O$ (10 g/L) in 10% sulfuric acid followed by charring at ~150 °C or by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C. Column chromatography was performed using Screening Device silica gel in the indicated solvents. ^{1}H NMR, ^{13}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 δ-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}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 Quadropole MS 6130, equipped with a semi-preparative Gemini C18 column (Phenomenex, 250 x 10, 5 µ particle size).



2,3- Di-*O*-benzyl-1,6-*epi*-cyclophellitol 10

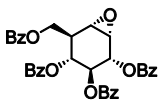
A solution of 0.4 mM aqueous Na_2EDTA (21 mL) and trifluoroacetone (9.32 mL, 104 mmol) were added to **8** (see Chapter 3, 2.36 g, 6.94 mmol) in acetonitrile (6.7

mL). A mixture of oxone (21.3 g, 34.7 mmol) and $NaHCO_3$ (4.08 g, 48.6 mmol) was added to the solution over a period of 15 min at 0 °C. After stirring at 0 °C for 3 h, the reaction mixture was diluted with H_2O, extracted with EtOAc, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/Et_2O, 12:88→10:90 and 8:92→0:100) furnished β-epoxide **9** (767 mg, 2.15 mmol, 31%) and α-epoxide **10** (1.14 g, 3.19 mmol, 46%) respectively as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.17 (m, 10H, H_{Ar}Bn), 4.97 (d, *J* = 11.2 Hz, 1H, CH_2Bn), 4.79 (dd, *J* = 12.0, 21.2 Hz, 2H, CH_2Bn), 4.64 (d, *J* = 11.2 Hz, 1H, CH_2Bn), 3.86-3.77 (m, 3H, H-2, H-8), 3.56 (t, *J* = 10.0 Hz, 1H, H-3), 3.41 (t, *J* = 10.0 Hz, 1H, H-4), 3.34-3.33 (m, 1H, H-6), 3.10 (d, *J* = 3.6 Hz, 1H, H-1), 2.88 (br s, 1H, OH), 2.54 (br s, 1H, OH), 2.15-2.10 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.2, 137.9, 128.5, 128.4, 128.0, 127.9, 127.8, 80.8, 79.4, 75.4, 72.5, 70.2, 62.5, 54.4, 54.0, 43.5. HRMS: found 357.1699 $[M+H]^+$, calculated for $[C_{21}H_{25}O_5]$ 357.1697.



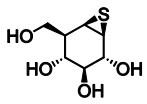
1,6-*epi*-cyclophellitol 11

A catalytic amount of $Pd(OH)_2$ was added to a solution of **10** (170 mg, 0.48 mmol) in MeOH (2.5 mL). The solution was stirred under H_2 atmosphere for 6 h before being filtered over a small pad of celite and concentrated under reduced pressure. The crude product was resuspended in chloroform and filtered to yield **11** as white crystals (82.0 mg, 0.47 mmol, 97%). ^{1}H NMR (400 MHz, MeOD): δ 3.93–3.89 (m, 2H, H-2, H-8), 3.78 (dd, *J* = 5.6, 11.2 Hz, 1H, H-8), 3.46 (dd, *J* = 2.0, 4.4 Hz, 1H, H-6), 3.41 (dd, *J* = 8.4, 10.0 Hz, 1H, H-3), 3.36–3.31 (m, 2H, H-1, H-4), 2.05–2.02 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 73.0, 71.2, 69.3, 60.3, 57.5, 55.1, 44.1. HRMS: found 177.0758 $[M+H]^+$, calculated for $[C_7H_{12}O_5]$ 177.0758.



2,3,4,8-tetra-*O*-benzoyl-1,6-*epi*-cyclophellitol 12

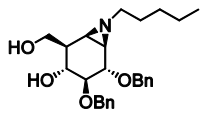
1,6-*epi*-cyclophellitol **11** was taken up in pyridine (2.5 mL). Benzoic anhydride (1.09 g, 4.8 mmol) and a catalytic amount of DMAP were added to the solution and the reaction mixture was stirred at 50 °C for 18 h. The mixture was quenched with 1 M HCl, extracted with EtOAc, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/EtOAc, 84:16→80:20) afforded **12** (73 mg, 0.12 mmol, 26%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14-8.02 (m, 5H, H_{Ar}Bz), 7.86 (d, *J* = 7.2 Hz, 1H, H_{Ar}Bz), 7.78 (d, *J* = 7.2 Hz, 1H, H_{Ar}Bz), 7.65-7.59 (m, 1H, H_{Ar}Bz), 7.57-7.33 (m, 8H, H_{Ar}Bz), 7.31-7.27 (m, 2H, H_{Ar}Bz), 7.21 (t, *J* = 7.6 Hz, 2H, H_{Ar}Bz), 5.99 (dd, *J* = 9.2, 10.4 Hz, 1H, H-3), 5.82 (dd, *J* = 2.4, 9.2 Hz, 1H, H-2), 5.67 (t, *J* = 10.4 Hz, 1H, H-4), 4.67 (dd, *J* = 3.6, 11.6 Hz, H-8), 4.49 (dd, *J* = 5.6, 11.6 Hz, 1H, H-8), 3.79 (dd, *J* = 1.6, 3.6 Hz, 1H, H-6), 3.48 (d, *J* = 4.0 Hz, 1H, H-1), 3.00-2.96 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 166.1, 165.8, 165.6, 133.6, 133.4, 133.4, 133.3, 133.1, 133.0, 130.1, 129.9, 129.8, 129.7, 129.7, 129.5, 129.4, 129.0, 128.9, 128.8, 128.7, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 127.8, 72.4, 70.2, 69.3, 62.9, 54.4, 53.6, 40.7. HRMS: found 593.1807 $[M+H]^+$, calculated for $[C_{35}H_{29}O_9]$ 593.1806.



Cyclophellitol thiirane 3

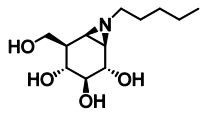
N,*N*-dimethylthioformamide (24.5 µL, 0.29 mmol) and trifluoroacetic acid (10.7 µL, 0.14 mmol) were added to a solution of **12** (73 mg, 0.12 mmol) in anhydrous DCM (7.2 mL) at 40 °C. After stirring for 18 h at 40 °C, the reaction mixture was

concentrated under reduced pressure and the resulting crude product was dissolved in MeOH (1 mL). A catalytic amount of NaOMe was added to the solution and stirred for 5 h at ambient temperature. The reaction mixture was neutralized with Amberlite IR-120 H^+ resin, filtered and concentrated *in vacuo*. Purification by silica column chromatography (dichloromethane/MeOH, 98:2→96:4) afforded **3** (3.7 mg, 19 µmol, 7.0%) as a white solid. ^{1}H NMR (400 MHz, MeOD): δ 4.09 (dd, *J* = 4.4, 10.4 Hz, 1H, H-8), 3.97 (d, *J* = 7.2 Hz, 1H, H-2), 3.53 (dd, *J* = 8.4, 9.2 Hz, 1H, H-8), 3.50 (dd, *J* = 2.4, 4.8 Hz, 1H, H-6), 3.21-3.16 (m, 2H, H-3, H-4), 3.07 (d, *J* = 6.4 Hz, 1H, H-1), 2.33-2.27 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 79.9, 75.6, 68.7, 66.0, 45.9, 41.6, 41.3. HRMS: found 193.0530 $[M+H]^+$, calculated for $[C_7H_{13}O_4S]$ 193.0529.



2,3-Di-*O*-benzyl-7-*N*-pentylcyclophellitol aziridine 14

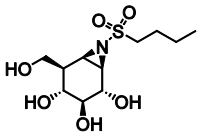
A solution of **13** (see Chapter 3, 91 mg, 0.21 mmol), 1-iodopentane (60 µL, 0.46 mmol) and potassium carbonate (87 mg, 0.63 mmol) in DMF (1.0 mL) was stirred at 90 °C for 18 h. The reaction mixture was diluted with water and subsequently extracted with Et_2O, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica column chromatography (dichloromethane/MeOH, 97:3→96:4) gave **14** (56 mg, 0.13 mmol, 61%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.26 (m, 10H, H_{Ar}Bn), 4.97 (d, *J* = 11.2 Hz, 1H, CH_2Bn), 4.77 (d, *J* = 11.6 Hz, 1H, CH_2Bn), 4.65 (dd, *J* = 9.2, 11.6 Hz, 2H, CH_2Bn), 3.95 (dd, *J* = 6.4, 10.8 Hz, 1H, H-8), 3.87 (dd, *J* = 4.4, 10.8 Hz, 1H, H-8), 3.71 (d, *J* = 8.4 Hz, 1H, H-2), 3.53-3.45 (m, 2H, H-4, OH), 3.31 (dd, *J* = 8.4, 10.0 Hz, 1H, H-3), 2.78 (br s, 1H, OH), 2.27 (dt, *J* = 7.2, 14.8 Hz, 1H, CH_2alkyl), 2.12-2.05 (m, 1H, CH_2alkyl), 2.02-1.97 (m, 1H, H-5), 1.72 (dd, *J* = 3.6, 6.0 Hz, 1H, H-6), 1.55 (d, *J* = 6.0 Hz, 1H, H-1), 1.47 (q, *J* = 7.2 Hz, 2H, CH_2alkyl), 1.31-1.19 (m, 4H, CH_2alkyl), 0.90 (t, *J* = 6.4 Hz, 3H, CH_3alkyl). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.4, 137.8, 128.8, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7, 84.1, 81.0, 74.7, 72.1, 69.6, 68.1, 65.0, 61.0, 42.5, 41.5, 40.3, 29.5, 29.0, 22.5, 14.0. HRMS: found 427.2662 $[M+H]^+$, calculated for $[C_{26}H_{36}NO_4]$ 427.2672.



7-*N*-Pentylcyclophellitol aziridine 7

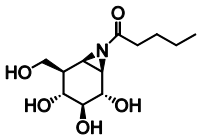
Ammonia (5 mL) was condensed at -60 °C. Lithium (22 mg) was added and the mixture was stirred for 30 min at -60 °C until lithium was completely dissolved. To this solution was added a solution of **14** (56 mg, 0.13 mmol) in THF (3.0 mL). The reaction mixture was stirred for 30 min at -60 °C and subsequently quenched with MilliQ-water (2 mL). The solution was allowed to come to ambient temperature and stirred until all ammonia had evolved. Next, the solution was concentrated *in vacuo*, redissolved in MilliQ-water and neutralized with Amberlite IR-120 H^+ resin. Product bound to the resin was washed with water and subsequently eluted with 1 M NH_4OH solution and evaporated under reduced pressure. The resulting solid was again purified on Amberlite IR-120 NH_4^+ resin using MilliQ-water as eluens until the eluate was neutral. Evaporation of the combined eluate under reduced pressure gave crude **7**, which was purified by silica column chromatography (dichloromethane/MeOH, 92:8) yielding **7** (8.2 mg, 34 µmol, 26%) as a white solid. ^{1}H NMR (400 MHz, MeOD): δ 3.97 (dd, *J* = 4.4, 10.0 Hz, 1H, H-8), 3.62-3.54 (m, 2H, H-2, H-8), 3.08 (t, *J* = 9.6 Hz, 1H, H-3), 3.00 (t, *J* = 9.6 Hz, 1H, H-4), 2.33 (dt, *J* = 8.0, 11.6 Hz, 1H, CH_2alkyl), 2.16-2.10 (m, 1H, CH_2alkyl), 1.98 (dd, *J* = 3.6, 6.0 Hz, 1H, H-6), 1.89-1.83 (m, 1H, H-5), 1.63 (d, *J* = 6.4 Hz, H-1), 1.56 (q, *J* = 7.6 Hz, 2H, CH_2alkyl), 1.32-1.27 (m, 4H, CH_2alkyl), 0.90 (t,

J = 1.6 Hz, 3H, CH_3alkyl). ^{13}C NMR (100 MHz, MeOD): δ 79.0, 73.9, 70.1, 63.7, 62.2, 45.5, 43.0, 30.7, 30.1, 23.7, 14.4. HRMS: found 246.1699 $[M+H]^+$, calculated for $[C_{12}H_{24}NO_4]$ 246.1699.



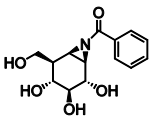
7-(*N*-butylsulfonyl)-cyclophellitol aziridine 6

A solution of cyclophellitol aziridine **2** (see Chapter 3, 18 mg, 0.1 mmol), sodium hydrogencarbonate (56 mg, 0.67 mmol) and 1-butanesulfonyl chloride (13 µmol, 0.1 mmol) in DMF (0.5 mL) was stirred at ambient temperature for 18 h. The reaction mixture was filtered over a small pad of celite and the filtrate was concentrated under reduced pressure. Purification by silica column chromatography (dichloromethane/MeOH, 92:8) afforded **6** (11 mg, 36 µmol, 36%) as a colorless oil. ^{1}H NMR (400 MHz, MeOD): δ 4.02 (dd, *J* = 4.4, 10.4 Hz, 1H, H-8), 3.68 (d, *J* = 8.0 Hz, 1H, H-2), 3.54 (t, *J* = 9.6 Hz, 1H, H-8), 3.32-3.23 (m, 2H, CH_2alkyl and MeOD solvent peak), 3.21-3.15 (m, 2H, H-3, H-6), 3.03 (t, *J* = 10.0 Hz, 1H, H-4), 2.87 (d, *J* = 6.8 Hz, 1H, H-1), 2.03-1.96 (m, 1H, H-5), 1.87-1.79 (m, 2H, CH_2 alkyl), 1.53-1.44 (m, 2H, CH_2alkyl), 0.95 (t, 3H, CH_3alkyl). ^{13}C NMR (100 MHz, MeOD): δ 78.6, 73.0, 69.2, 63.0, 52.3, 44.5, 44.4, 43.7, 26.3, 22.6, 13.9. HRMS: found 308.1128 $[M+H]^+$, calculated for $[C_{11}H_{22}NO_6S]$ 308.1162.



7-(*N*-pentoyl)-cyclophellitol aziridine 4

A pre-activated mixed anhydride solution (1 M) was prepared by dissolving EEDQ (209 mg, 0.85 mmol) and valeric acid (0.09 mL, 0.85 mmol) in DMF (0.85 mL) and the reaction mixture was stirred at ambient temperature for 2 h before use. Cyclophellitol aziridine **2** (see Chapter 3, 21 mg, 0.12 mmol) was dissolved in DMF (0.6 mL) and the solution was cooled to 0 °C before 1 M pre-activated mixed anhydride solution (0.06 mL, 0.06 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min and additional pre-activated solution (0.06 mL, 0.06 mmol) was added. After stirring for 30 min at 0 °C, the reaction mixture was quenched with MeOH and concentrated under reduced pressure. Purification by semi preparative reversed phase HPLC (linear gradient: 13%→16%, 3 CV, solutions used: A: H_2O, B: acetonitrile) and lyophilization gave the title compound **4** (7.1 mg, 27 µmol, 23%) as a white solid. ^{1}H NMR (400 MHz, MeOD): δ 4.05 (dd, *J* = 4.4, 10.4 Hz, 1H, H-8), 3.70-3.65 (m, 2H, H-2, H-8), 3.19 (dd, *J* = 8.0, 10.0 Hz, 1H, H-3), 3.06 (t, *J* = 9.6 Hz, 1H, H-4), 3.01 (dd, *J* = 3.2, 6.0 Hz, 1H, H-6), 2.71 (d, *J* = 6.0, Hz, 1H, H-1), 2.49 (dt, *J* = 1.2, 7.2 Hz, 2H, CH_2alkyl), 1.99-1.93 (m, 1H, H-5), 1.62-1.55 (m, 2H, CH_2alkyl), 1.40-1.30 (m, 2H, CH_2alkyl), 0.93 (t, *J* = 7.2 Hz, CH_3alkyl). ^{13}C NMR (100 MHz, MeOD): δ 188.6, 79.1, 73.4, 69.3, 63.5, 62.2, 45.3, 42.4, 41.0, 36.7, 28.3, 23.4, 14.2. HRMS: found 260.1493 $[M+H]^+$, calculated for $[C_{12}H_{22}NO_5]$ 260.1493.



7-(*N*-benzoyl)-cyclophellitol aziridine 5

EEDQ (84 mg, 0.34 mmol) and benzoic acid (41 mg, 0.34 mmol) were dissolved in DMF (0.34 mL) and the solution was stirred at room temperature for 2 h. Pre-activated mixed anhydride solution (0.17 µL, 0.17 mmol) was added to cyclophellitol aziridine **2** (see Chapter 3, 46 mg, 0.26 mmol) in DMF (1.0 mL) at 0 °C. After stirring for 30 min at 0 °C, an additional amount of pre-activated mixed anhydride solution (0.17 µL, 0.17 mmol) was added. The reaction mixture was stirred for 2 h at 0 °C before being quenched with MeOH

(1 mL) and concentrated *in vacuo*. Purification by semi-preparative reversed HPLC (linear gradient: 10%→16%, 3 CV, solutions used A: H_2O, B: acetonitrile) and lyophilization yielding **5** (17 mg, 0.06 mmol, 23%) as a white powder. ^{1}H NMR (400 MHz, MeOD): δ 7.97 (d, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 7.59 (t, *J* = 7.2 Hz, 1H, H_{Ar}Bz), 7.48 (t, *J* = 7.6 Hz, 2H, H_{Ar}Bz), 4.04-4.00 (m, 2H, H-2, H-8), 3.73 (dd, *J* = 8.0, 10.4 Hz, 1H, H-8), 3.31-3.22 (m, 2H, H-3, H-4), 3.19 (dd, *J* = 3.2, 6.0 Hz, 1H, H-6), 2.72 (d, *J* = 6.0 Hz, 1H, H-1), 2.08-2.00 (m, 1H, H-5). ^{13}C NMR (100 MHz, MeOD): δ 181.5, 134.3, 134.1, 134.0, 130.1, 129.7, 129.5, 128.5, 79.3, 73.5, 69.7, 63.7, 45.3, 45.1, 40.8. HRMS: found 280.1177 [M+H$^+$], calculated for [$C_{14}H_{17}NO_4$] 280.1179.

Enzyme activity assays - Determination of the IC_{50}

The activity of GBA was assayed at 37 °C by incubating with 3.75 mM 4-methylumbelliferyl-β-D-glucopyranoside (4mu-β-D-Glc) as substrate in 150 mM McIlvain buffer, pH 5.2, containing 0.1% (w/v) BSA, 0.2% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100. Purified recombinant GBA (Cerezyme, Genzyme, U.S.A) was employed as enzyme source. Activity of GBA3 was measured at 37 °C in 150 mM McIlvain buffer, pH 6.0, containing 0.1% (w/v) BSA and 3.75 mM substrate. A human splenectomized N370S/RecNcI Gaucher spleen was homogenized by sonication in cold Nanopure water, centrifuged for 30 min at 12000 rpm (Sorvall RC-5b, Dupont Instruments, rotor SS-34), and supernatant was used as source for GBA3. The pellet was homogenized by sonication in cold Nanopure water, washed thrice by centrifugation, *vide supra*, resuspended in 50 mM potassium phosphate buffer, pH 5.8, and incubated with 5 mM conduritol β epoxide for 30 min at 4 °C before being used as GBA2 source. Activity of GBA2 was measured at 37 °C in 150 mM McIlvaine buffer, pH 5.8, containing 0.1% (w/v) BSA and 3.75 mM substrate. To determine the apparent IC_{50} value, enzyme was pre-incubated with a range of inhibitor dilutions for 30 min at 37 °C. After stopping the substrate reaction with excess NaOH-glycine (pH 10.3), fluorescence was measured with a fluorimeter LS30 (Perkin Elmer) using λ_{EX} 366 nm and λ_{EM} 445 nm.

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