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Chapter 5

Synthesis of mannose configured cyclophellitol and its aziridine derivative[1]

Introduction

Carbohydrates are structurally the most diverse class of biopolymers. They not only occur as oligosaccharides and polysaccharides but are also constituents of glycoproteins and glycolipids.[2] These glycoconjugates are involved in numerous fundamental biological processes, such as communication processes[3] and cell-cell recognition.[4,5] Gaining insight into the role of carbohydrates and glycoconjugates in these biological processes is complicated by their complex structure, their transient occurrence and their biosynthesis that is not directly controlled by the genetic code.[6] The metabolism of glycoconjugates is guided by glycosyltransferases, enzymes that specifically introduce glycosidic bonds, and glycosidases, enzymes that specifically cleave glycosidic bonds.[7] Establishment of the activity of these enzymes in a defined biological context is an important approach to elucidate their role and the function of the corresponding glycans. In this respect activity-based protein profiling (ABPP) has became an important and rapidly advancing field of research in the past decade.[8] Central and vital for this type of research is the development of activity based probes (ABPs). These probes are characterized by the presence of an irreversible activity based inhibitor for a specific (class of) enzyme(s) and a reporter group or ligation handle.[9]

Recently, major advances in the development of APBs for glycosidases have been made.[10] Key to the success of these ABPs is the classical Koshland double-replacement mechanism of retaining glycosidases.[11] As shown in Figure 1a two carboxylic acid residues (Asp or Glu) in the active site of the enzyme are involved in the two-step process.[12] In the first step protonation by one carboxylic acid residue of the glycan substrate and nucleophilic displacement by the second carboxylate leads to a covalent enzyme-glycosyl intermediate with inversion of configuration. In the next step the formed carboxylate anion assists in the hydrolysis of the enzyme-glycosyl intermediate to give the stripped glycan with retention of configuration with respect to the substrate. Fluorinated glycosides and cyclitol epoxides are two classes of covalent inhibitors, qualified for the development of ABPs for retaining glycosidases.

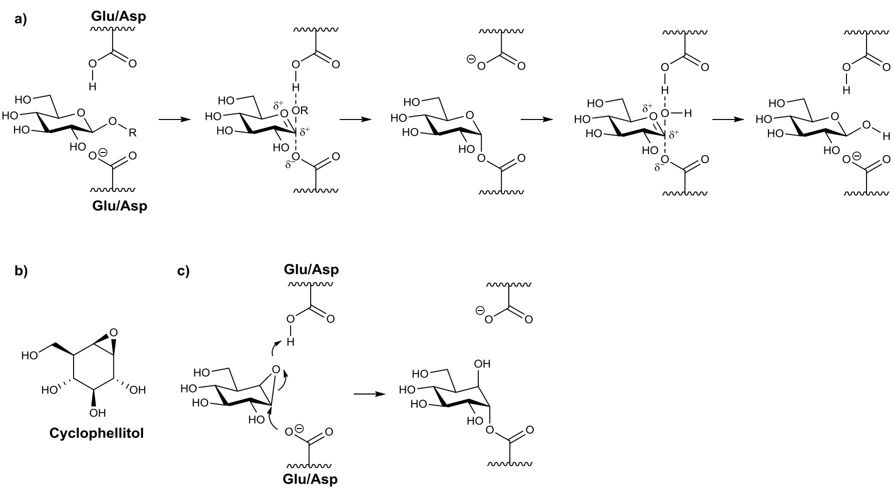


Figure 1: a) Classical Koshland double replacement mechanism of retaining beta-glucosidases. b) Structure of cyclophellitol. c) Proposed mechanism of cyclophellitol binding to retaining beta-glucosidases.

Cyclophellitol (Figure 1b), the cyclitol analogue of D-glucopyranose with an β-configured epoxide, is an irreversible and naturally occurring β-glucosidase inhibitor.[13] The inhibition mechanism comprises protonation of the epoxide in the active site by the general acid/base catalyst, followed by nucleophilic attack of the carboxylate to give a covalent cyclophellitol-enzyme adduct (Figure 1c).[14]

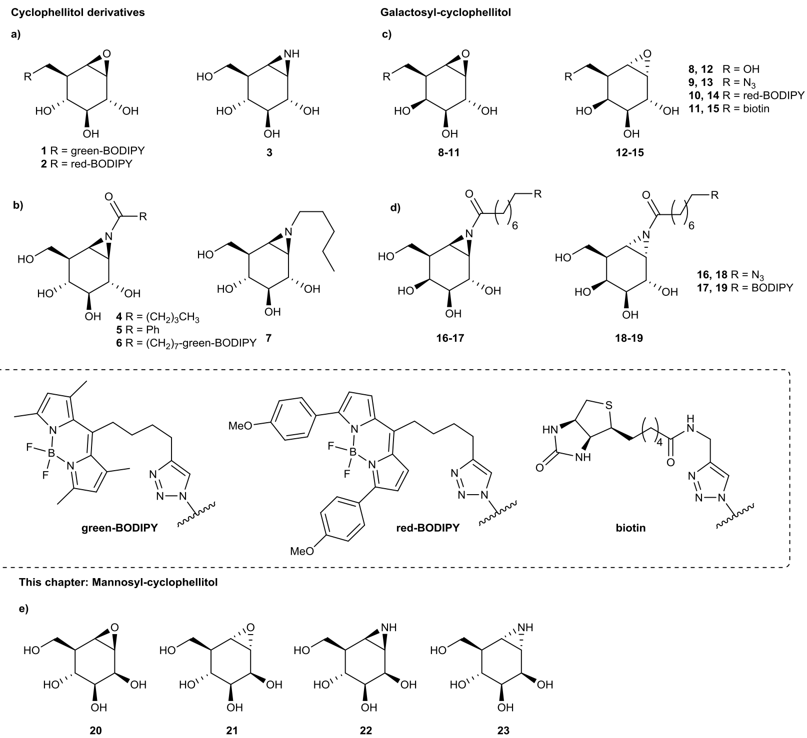


Figure 2: Structures of cyclophellitol based ABPs. a) Structures of BODIPY conjugated cyclophellitol **1** and **2** and its aziridine derivative **3**. b) Structures of functionalized aziridine cyclophellitol **4**-**7**. c) Structures of α/β-galactose configured cyclophellitol **8**-**15**. d) Structures of α/β-galactose configured functionalized aziridine cyclophellitol **16**-**19**. e) Structures of mannose configured α/β-cyclophellitol **20**, **21** and α/β-mannose configured aziridine cyclophellitol derivatives **22** and **23**.

Based on the mode of action of cyclophellitol, Witte *et al.*[15] developed two ABPs (**1** and **2**, Figure 2a) allowing highly specific and efficient labelling of active glucocerebrosidase, key enzyme in Gaucher disease.[16] Chapter 3 describes the synthesis of Man_1- and Man_3-BODIPY-cyclophellitol probes designed to selectively target the probes to Gaucher macrophages. Next to the epoxide based probes, broad spectrum ABPs for β-glucosidases were developed[17] by replacement of the epoxide electrophilic trap in cyclophellitol by functionalized aziridines[18,19] (as in **4-7**, Figure 2b**)**. In this manner the reporter group or ligation handle could be varied and installed at a position in the ABP pointing towards the aglycon site, where most glycosyl hydrolases (GHs) are more relaxed in their substrate specificity.

The synthesis of cyclophellitol analogs derived from the common monosaccharides found in mammalian and bacterial glycans will provide an ABP toolbox that can be used to interrogate different GHs that use the Koshland double-replacement mechanism. Willems *et al*. synthesized both α- and β-galactopyranose configured cyclophellitol analogues **8-15** and the α-aziridine derivatives **18** and **19** (Figure 2c-d).[20,21] Synthesis of β-Aziridine derivative **16** and **17** is currently in progress. With these probes GH27 human retaining α-galactosidases could be detected.[22]

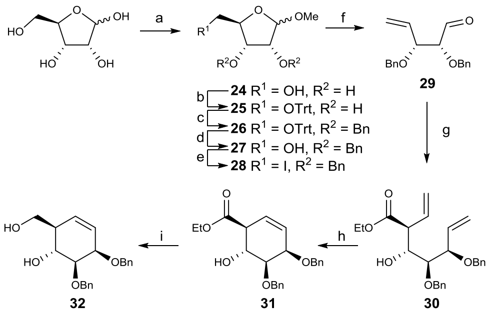
This chapter describes the synthesis of four cyclophellitol analogs having the D-mannose configuration (2-*epi*-cyclophellitol), bearing either an α- or β-configured epoxide (**20**, **21**, Figure 2e) or a α- or β- aziridine function (**22**, **23**), to use these both as covalent inhibitors (the epoxides and non-functionalized aziridines) and as a starting point for the generation of ABPs.

Results and discussion

Up to now only one low yielding synthesis has been described for mannose configured cyclophellitol,[23] whereas the corresponding aziridine has not been reported. To develop a straightforward route of synthesis towards these 2-*epi*-cyclophellitol targets, it was reasoned that the Madsen route towards cyclophellitol[24] (see Chapter 3) could be adapted, starting from the appropriate epimeric starting material, that is, D-ribose instead of D-xylose. Scheme 1 shows the synthesis of cyclohexene **32**, the common precursor for the 2-*epi*-cyclophellitol target epoxides and aziridines. Treatment of D-ribose with acetyl chloride in methanol under kinetic conditions gave methyl-D-ribofuranose (**24**) as an α/β anomeric mixture. Selective tritylation of the primary alcohol in **24** and ensuing benzylation of the *cis*-diol to give fully protected ribose **26** followed by acid mediated detritylation furnishing ribose **27**[25] in 72% yield over 4 steps. Substitution of the primary alcohol in **27** by iodine and subsequent reductive ring opening of **28** with activated zinc yielded aldehyde **29**[26], the required starting compound for the ensuing indium catalyzed Barbier reaction.

In this key reaction the indium reagent derived from ethyl-4-bromocrotonate was formed *in situ* and added to aldehyde **29**. Following this procedure diene **30** was formed with excellent stereoselectivity (95:5 with respect to the undesired C-4 epimer).

Scheme 1: Synthesis of cyclohexene precursor **32**.



Reagents and conditions: (a) acetyl chloride, MeOH, 0 °C to rt.; (b) trityl chloride, pyridine, rt.; (c) BnBr, NaH, DMF, 0 °C to rt.; (d) *p*TsOH, DCM/MeOH (1:1), rt, 72% over four steps; (e) *i*. $(Ph)_3P$, imidazole, THF, reflux; *ii*. I_2, THF, reflux, 93%; (f) act. Zn, THF/H_2O (9:1), 60 °C, sonicate, 40%; (g) ethyl 4-bromocrotonate, indium (powder), $La(OTf)_3$, 61%; (h) Grubbs II, DCM, reflux 93%; (i) *i*. DIBAL-H, THF, 0 °C to rt. *ii*. $NaBH_4$, EtOAc/H_2O (2:1), 95%.

This stereoselectivity can be explained by a similar transition state as proposed by Madsen and co-workers in the Barbier reaction leading to the cyclophellitol octadiene precursor. As depicted in Figure 3, a 6-membered ring transition state, in which the aldehyde and benzyl ether moieties in **29** coördinate to indium, explain the observed stereochemistry at the two new stereocenters (C-4 and C-5) in **30**. Purification by column chromatography resulted in the isolation of homogenous **30** in 61% yield. RCM of diene **30** using Grubbs 2nd generation catalyst gave cyclohexene **31** in 93% yield. Reduction of the ethyl ester in **31** was accomplished by treatment with DIBAL-H and sodium borohydride to furnish primary alcohol **32**, the common precursor to both the target α/β-epoxides and α/β-aziridines.

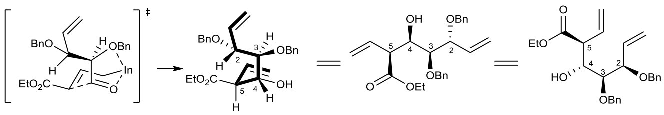


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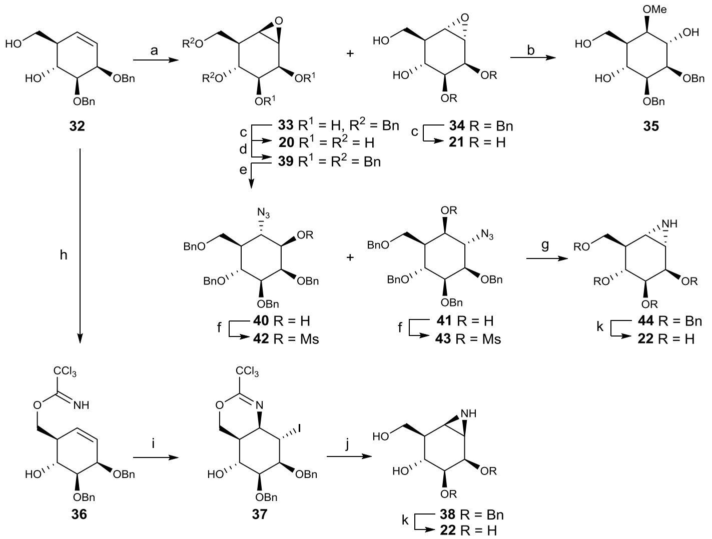
Figure 3: Possible transition state of the indium catalyzed Barbier reaction of aldehyde **29** with ethyl bromocrotonate leading to diene **30**.

The transformation of cyclohexene **32** into the four target compounds is shown in Scheme 2. Epoxidation of cyclohexene **32** using *m*CPBA resulted in a mixture of the α- and β-epoxides **33** and **34** in a 3:2 α/β-ratio and a combined 64% yield. Column chromatography gave the individual epoxides, the identity of which was ascertained by NMR experiments in combination with DFT calculations. The latter calculations were performed because the stereochemistry of the newly formed epoxides cannot be simply derived from the coupling constants of the ring protons.

Scheme 2: Scheme overview synthesis of target compounds **20**, **21**, **22** and **23**.



Reagents and conditions: (a) *m*CPBA, DCE, reflux (**33**: 38%, **34**: 26%); (b) Pd/C, H_2 (g), MeOH; (c) Pd/C, H_2 (g), 1,4-dioxane/*t*BuOH (9:1), rt. (**20**: 40%, **21**: 36%); (d) BnBr, NaH, DMF, 0 °C to rt (52%); (e) NaN_3, $LiClO_4$; (f) MsCl, pyridine (**40**: 88%, **41**: 72%); (g) $LiAlH_4$, THF, 0 °C (31%); (h) Cl_3CCN, DBU, DCM, 0 °C to rt, 94%; (i) I_2, $NaHCO_3$, THF/H_2O (4:1), 60 °C, 80%; (j) *i*. 1,4-dioxane/H_2O/AcOH (1:1:8), rt.; *ii*. $NaHCO_3$, MeOH, 93%; (k) Li, NH_3 (l), -60 °C, quantitative.

The spectroscopic data and calculated coupling constants for both the $^4H_3$ and the opposite $^3H_4$ half chair α- and β- epoxides are summarized in Table 1. Comparison of the experimental ^3J coupling constants of the two epoxide isomers with the calculated values show that the coupling constants for the protons of epoxide **34** match best with the series of coupling constants calculated for the α-epoxide in a $^4H_3$ conformation, where there is also good agreement between the recorded coupling constants of epoxide **35** and the calculated β-epoxide in a $^4H_3$ half chair. The measured and calculated coupling constants are summarized in Figure 4.

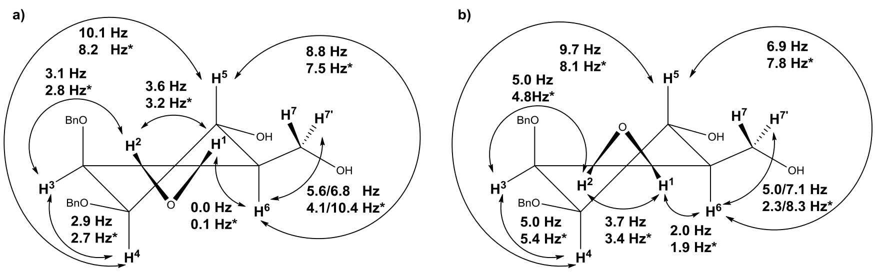


Figure 4: Absolute configuration of a) α-epoxide **34** b) and β-epoxide **35**. *Calculated coupling constants.

Table 1: Calculated and experimental *J* coupling α-epoxide **34** and β-epoxide **35**.

entry	correlation	$^3H_4$ *J* calc. (Hz)		$^4H_3$ *J* calc. (Hz)		*J* exp. (Hz)	
		α-**34**	β-**35**	α-**34**	β-**35**	α-**34**	β-**35**
1	H1-H2	3.6	3.6	3.2	3.4	3.6	3.7
2	H2-H3	0.01	3.8	2.8	4.8	3.1	5.0
3	H3-H4	4.8	8.5	2.7	5.4	2.9	5.0
4	H4-H5	4.4	8.2	8.2	8.1	10.1	9.7
5	H5-H6	1.6	7.9	7.5	7.8	8.8	6.9
6	H6-H7	0.5	2.3	4.1	2.3	5.6	5.0
7	H6-H7’	6.4	8.3	10.4	8.3	6.8	7.1
8	H6-H1	1.3	1.1	0.1	1.9	0.0	2.0

To conclude the synthesis of the epoxides, the removal of the benzyl groups in epoxides **33** and **34** was undertaken (Scheme 2). Hydrogenolysis of the benzyl groups in **34** with Pd/C and H_2 in MeOH gave a mixture of products. With the aid of NMR spectroscopy and mass spectrometry it was revealed that the crude reaction mixture contained the desired compound **21** next to side product **35**, originating from ring opening of the epoxide by methanol. Changing the solvent mixture of the hydrogenolysis to 1,4-dioxane/*t*BuOH (9:1) prevented the undesired epoxide ring opening and led to the isolation of target epoxides **20** and **21** in 40% and 36% yield, respectively, after crystallization.

β-Configured aziridine (**22)** was obtained by adaptation of the procedure that was used to prepare β-cyclophellitol aziridine[18] (Scheme 2). First, the trichloroacetimidate function was regioselectively introduced at the primary hydroxyl group by treatment of cyclohexene **32** with trichloroacetonitrile and DBU to give **36**. Next, stereospecific iodo-cyclisation gave oxazine **37** in 80% yield. Hydrolysis of oxazine could be effected by treatment with 80% AcOH (1,4-dioxanes/H_2O/AcOH, 1:1:8) at ambient temperature. It is of interest to note that for the same opening of cyclophellitol oxazine heating at 60 °C in an HCl solution is prescribed. Base treatment of the crude 1,2-*trans* amino iodide provided β-aziridine **38** in 93% yield. The removal of the benzyl protective groups required some optimization. Using a reported procedure, in which Birch reduction is followed by treatment with Amberlite IR-H^+ to remove the lithium salts, gave an inseparable mixture of compounds. Treating the crude product with Amberlite IR-NH_4^+ was successful and β-manno-aziridine **22** was isolated in 70% overall yield starting from cyclohexene **38**.

α-Mannose configured aziridine cyclophellitol **23** was obtained from β-manno-epoxide **33** as depicted in Scheme 2. Perbenzylation of β-epoxide **33** was followed by epoxide opening by the azide anion in presence of $LiClO_4$ to afford a 1:1 mixture of azido alcohol regioisomers **40** and **41**. Separation by column chromatography gave the individual isomers **40** and **41** in 48% and 52% yield, respectively. Mesylation of the free hydroxyl in **40** and **41** yielded compound **42** and **43**, suitable for α-aziridine formation. To this end cyclitol **43** was subjected to $LiAlH_4$ treatment. Monitoring of the reaction by TLC-MS showed formation of the amine product, the desired aziridine and hydrolyzed aziridine. Unfortunately, a prolonged reaction time, to convert more of the amine into the desired aziridine was accompanied by an increase of hydrolyzed side product and decrease of yield. After 4h the mixture was quenched and purification by column chromatography provided the benzylated α-aziridine **44** in a yield of 31%. Finally, removal of the benzyl groups by Birch reduction and treatment of resulting mixture with Amberlite NH_4^+ IR-120 as described for the β-aziridine yielded α-manno-aziridine **23** in 14% overall yield starting from β-epoxide **32**.

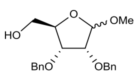
Conclusion

This chapter describes the synthesis of the α- and β-mannose configured cyclophellitol derivatives **20** and **21** and the corresponding aziridine analogues **22** and **23**. The key indium catalyzed Barbier reaction, in which two new stereocenters were introduced, proceeded with excellent stereoselectively. Central intermediate cyclohexene **32** was used for the synthesis of both the α- and β-epoxides **20** and **21** and the β-aziridine **22**, while the α-aziridine **23** was constructed from the β-epoxide **20**.

The obtained epoxides and aziridines can be explored as mechanism based covalent inhibitors for α- and β-mannosidases, that hydrolyze mannosidic linkages with retention of configuration, such as the glycosyl hydrolases from CAZy GH-family 38[27,28], 47[29], 92[30,31] and 99[32,33]. Aziridines **22** and **23** can also be further processed by installation of *N*-alkyl and *N*-acyl groups to deliver ABPs that can report on α- and β-mannosidase activity.

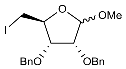
Experimental

General: Traces of water in the starting materials were removed by co-evaporation with toluene for all moisture and oxygen sensitive reactions and the reactions were performed under an argon atmosphere. Dichloromethane was distilled over P_2O_5 and stored over activated 3 Å molecular sieves under an argon atmosphere. All other solvents and chemicals (Acros, Fluca, Merck) were of analytical grade and used as received. Column chromatography was performed on Screening Device silica gel 60 (0.040-0.063 mm). Size exclusion was performed on Sepadex LH20 (eluent DCM/MeOH, 1:1). TLC analysis was conducted on HPTLC aluminium sheet (Merck, TLC silica gel 60, F_{254}). Compounds were visualized by UV absorption (λ = 254 nm), staining with *p*-anisaldehyde (3.7 mL in 135 mL EtOH, 1.5 mL AcOH and 5 mL H_2SO_4), 20% H_2SO_4 in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25g/L) in 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^{1}H and ^{13}C NMR were recorded on a Bruker DPX 300 (300 and 75 MHz respectively), Bruker AV 400 (400 and 100 MHz respectively), Bruker DMX 400 (400 and 100 MHz respectively) or Bruker DMX 600 (600 and 125 MHz respectively). Chemical shifts are given in ppm (δ) relative to the residual solvent peak or TMS (0 ppm) as internal standard. *J* couplings are given in Hz. Optical rotations were measured on a Propol automatic polarimeter. IR spectra (thin film) were conducted on a Perkin Elmer FTIR Spectrum Two UATR (Single reflection diamond). LC-MS measurements were conducted on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI+) coupled to a Thermo Finnigan Surveyor HPLC system equipped with a standard C_{18} (Gemini, 4.6 mm x 50 mm, 5µm particle size, Phenomenex) analytical column and buffers A: H_2O, B: MeCN, C: 0.1% TFA (aq.). High-resolution mass spectra were recorded on a LTQ Orbitrap (Thermo Finnigan) mass spectrometer.

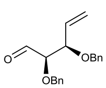


methyl 2,3-di-*O*-benzyl-D-ribofuranoside (27): To a 0 °C cooled solution of D-(-)-ribose (37.5 g, 250 mmol) in MeOH (500 mL) was added dropwise AcCl (3.5 mL, 50 mmol) and the reaction mixture was allowed to warm to rt. After complete conversion of the starting material the reaction was quenched with Et_3N till pH ≥7 and the mixture was concentrated *in vacuo* giving OMe-ribose **24** as an α/β mixture (1:0.3). The crude OMe-ribose **24** was co-evaporated with toluene and dissolved in pyridine (500 mL). To the solution was added trityl chloride (76.7 g, 275 mmol) and the mixture was stirred overnight at rt. The reaction was quenched with MeOH and the mixture was concentrated *in vacuo*. The product was dissolved in EtOAc and the organic phase was washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude tritylated OMe-ribose **25** was taken up in DMF (1 L) and to the solution was added BnBr (90 mL, 750 mmol) and the mixture was cooled to 0 °C. To the cooled mixture was added (60%) NaH (25 g, 625 mmol) in small portions over a period of 6h. The reaction was gradually allowed to warm to rt and stirred overnight. The reaction mixture was cooled to 0 °C and quenched with MeOH after which the solvents were removed *in vacuo*. The product was dissolved in Et_2O and the organic phase was washed with H_2O (3x), brine (2x), and dried over $MgSO_4$. The crude was filtered over a bed of silica to remove the bulk of impurities, concentrated and dissolved in DCM/MeOH (1:1) (1 L). To the solution was added *p*TsOH (4.8 g, 25 mmol) and the reaction mixture was stirred overnight at rt. The mixture was neutralized with Et_3N and concentrated *in vacuo*. Purification by column chromatography yielded benzylated OMe-ribose **27** as a colourless oil (57.4 g, 179 mmol, 72%). Spectroscopic data were in accordance with known literature data.[25] (α-product) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 10H), 4.88 (s, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.60 (d, *J* = 12.0 Hz, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.27 (dt, *J* = 6.9, 3.4 Hz, 1H), 4.11 (dd, *J* = 7.0, 4.7 Hz, 1H), 3.85 (d, *J* = 4.7 Hz, 1H), 3.78 (d, *J* = 12.2 Hz, 1H), 3.55 (ddd, *J* = 10.9, 7.3, 3.5 Hz, 1H), 3.34 (s, 3H), 2.15 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.7, 137.7, 128.5, 128.0, 127.9, 127.9, 106.8, 82.3, 80.1, 77.3, 72.7, 72.5, 62.8, 55.6.

(β-product) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.27 (m, 10H), 4.85 (d, *J* = 4.1 Hz, 1H), 4.74 (d, *J* = 12.7 Hz, 1H), 4.66 (d, *J* = 12.3 Hz, 1H), 4.65 – 4.54 (m, 2H), 4.17 (q, *J* = 3.5 Hz, 1H), 3.84 (dd, *J* = 6.9, 3.5 Hz, 1H), 3.73 (dd, *J* = 6.9, 4.2 Hz, 1H), 3.63 (dd, *J* = 12.0, 3.3 Hz, 1H), 3.45 (s, 3H), 3.39 (dd, *J* = 12.7, 3.5 Hz, 1H), 1.97 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 137.8, 128.4, 128.4, 128.2, 128.0, 127.9, 127.8, 102.7, 83.2, 78.1, 74.7, 72.7, 72.6, 62.7, 55.6.

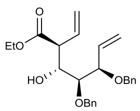


methyl 6-deoxy-6-iodo-2,3-di-*O*-benzyl-D-ribofuranoside (28): To a solution of benzylated α-OMe-ribose **27** (51.7 g, 113.8 mmol) in THF (455 mL) was added imidazole (15.5 g, 227.6 mmol), Ph_3P (44.8 g, 170.7 mmol) and the mixture was heated till reflux. After complete consumption of the starting material a 1M I_2 solution (170.7 mL, 170 mmol) in THF was added dropwise to the boiling reaction mixture and refluxed overnight. The mixture was cooled to rt and Et_2O was added, upon addition of Et_2O crystalline precipitate was formed. The mixture was cooled to -20 °C and the solids were filtered. The filtrate was washed with 10% $Na_2S_2O_3$ (aq.) (2x), H_2O (3x), brine (2x) dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude was immobilized on silica and purification by column chromatography yielded iodo ribose **28** as a colourless oil (47.8 g, 105.4 mmol, 93%). Spectroscopic data were in accordance with known literature data.[34] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 – 7.28 (m, 10H), 4.92 (s, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.14 (t, *J* = 6.6 Hz, 1H), 3.94 (dd, *J* = 6.6, 4.5 Hz, 1H), 3.88 (d, *J* = 4.5 Hz, 1H), 3.38 – 3.33 (m, 4H), 3.26 (dd, *J* = 10.5, 6.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.7, 128.6, 128.1, 128.0, 106.3, 81.8, 20.4, 80.2, 72.7, 72.5, 55.4, 8.8.

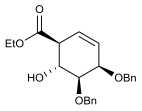


(2R,3R)-2,3-dibenzyloxypent-4-enal (29): Iodo ribose **28** (47.8 g, 105.4 mmol) was dissolved in THF/H_2O (9:1) (1 L) and the solution was purged with argon under sonication. To the solution was added activated zinc (65.4 g, 1.0 mol) and the mixture was further sonicated at 60 °C under argon atmosphere. After complete conversion of the starting material the excess of zinc was filtered and rinsed with DCM. The crude mixture was diluted with brine and the product was extracted with DCM (5x). The combined organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded aldehyde **29** as a colourless oil (12.47 g, 42 mmol, 40%). Spectroscopic data were in accordance with known literature data.[35] ^{1}H NMR (400 MHz,

$CDCl_3$) δ 9.63 (d, *J* = 2.1 Hz, 1H), 7.39 – 7.24 (m, 10H), 5.87 (dt, *J* = 17.6, 10.5, 7.6 Hz, 1H), 5.38 (d, *J* = 9.1 Hz, 1H), 5.34 (d, *J* = 16.9 Hz, 1H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 12.2 Hz, 2H), 4.40 (d, *J* = 12.0 Hz, 1H), 4.16 (dd, *J* = 7.6, 4.6 Hz, 1H), 3.89 (dd, *J* = 4.6, 2.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7, 137.8, 137.3, 134.1, 128.6, 128.5, 128.1, 127.8, 120.2, 85.0, 80.3, 73.1, 70.6.

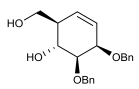


ethyl (2*S*,3*R*,4*S*,5*R*)-4,5-bis(benzyloxy)-3-hydroxy-2-vinylhept-6-enoate (30) To a solution of aldehyde **29** (1.01 g, 3.41 mmol) in H_2O (15.4 mL) was added 75% ethyl 4-bromocrotonate (2.04 mL, 11.1 mmol), $La(OTf)_3$ (4.00 g, 6.83 mmol), indium powder (0.90 g, 7.85 mmol) and the mixture was vigorously stirred overnight at rt. The reaction mixture turned into a white slurry in which sand was added till small balls were formed. The mixture was filtered over a pad of celite and rinsed with Et_2O. The layers were separated and the aqueous layer was extracted with Et_2O (3x). The combined organic phase was washed with H_2O (3x), brine (3x), dried over $MgSO_3$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded manno diene **30** as a colourless oil (0.853 g, 2.078 mmol, 61%). ^1H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 10H), 5.89 (ddd, *J* = 17.5, 10.4, 7.2 Hz, 1H), 5.72 (ddd, *J* = 17.1, 10.3, 9.4 Hz, 1H), 5.41 (dt, *J* = 9.2, 1.3 Hz, 1H), 5.37 (t, *J* = 1.2 Hz, 1H), 5.14 (dd, *J* = 10.3, 1.4 Hz, 1H), 5.08 (d, *J* = 17.1 Hz, 1H), 4.68 (d, *J* = 11.2 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.44 (d, *J* = 11.3 Hz, 1H), 4.40 (d, *J* = 11.7 Hz, 1H), 4.24 (ddd, *J* = 9.5, 6.8, 1.3 Hz, 1H), 4.17 (t, *J* = 6.6 Hz, 1H), 4.13 (q, *J* = 7.3 Hz, 2H), 3.44 (dd, *J* = 5.8, 1.3 Hz, 1H), 3.34 (t, *J* = 9.5 Hz, 1H), 3.14 (d, *J* = 6.9 Hz, 1H), 1.24 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 137.9, 137.9, 135.5, 133.1, 128.5, 128.5, 128.1, 128.0, 128.0, 127.8, 120.0, 119.6, 80.2, 79.0, 73.2, 72.0, 71.0, 60.9, 55.0, 14.2. HRMS: $[M+H]^+$ calculated for $C_{25}H_{31}O_5$ 411.21660, found 411.21653.



ethyl (1*S*,4*R*,5*S*,6*R*)-4,5-bis(benzyloxy)-6-hydroxycyclohex-2-enecarboxylate (31) To a solution of manno diene **30** (0.575 g, 1.4 mmol) in DCM (56 mL) was added Grubbs 2nd catalyst (95 mg, 0.11 mmol, 8 mol%) and the reaction mixture was refluxed in the dark for 2 h. The mixture was concentrated *in vacuo* and directly purified by column chromatography without further workup yielding cyclohexene ethyl ester **31** as a slightly brown oil (0.498 g, 1.302 mmol, 93%). $[\alpha]_D^{22}$ + 42.8° (c = 1.0, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 10H), 5.89 (ddd, *J* = 9.9, 5.2, 2.8 Hz, 1H), 5.82 (dd, *J* = 9.9, 2.4 Hz, 1H), 4.74 (d, *J* = 11.9 Hz, 1H), 4.69 (s, 2H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.55 (ddd, *J* = 10.4, 8.8, 1.8 Hz, 1H), 4.21 (qd, *J* = 7.1, 0.8 Hz, 2H), 4.09 (t, *J* = 4.4 Hz, 1H), 3.46 (dd, *J* = 10.2, 4.0 Hz, 1H), 3.13 (ddt, *J* = 8.9, 2.5, 0.9 Hz, 1H), 2.91 (d, *J* = 1.9 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 138.6, 138.1, 128.6, 128.5, 128.1, 128.0, 128.0, 127.8, 127.5, 126.7, 80.3, 72.2, 71.9, 69.6, 67.3, 61.4, 51.2, 14.3. HRMS: $[M+H]^+$ calculated for $C_{23}H_{27}O_5$ 383.18530, found 383.18548.

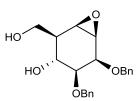
(1*S*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-(hydroxymethyl)cyclohex-3-enol



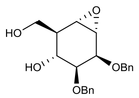
(32): To a 0 °C cooled solution of cyclohexene ethyl ester **31** (0.463 g, 1.2 mmol) in THF (40 mL) was added a 1M DIBAL-H sol. (6 mL, 6 mmol) in THF dropwise and the mixture was warmed to rt. After 30 min the mixture was cooled to 0 °C and to the mixture was added EtOAc (2.4 mL, 24.4 mmol), H_2O (1.2 mL) and $NaBH_4$ (0.295 g, 7.8 mmol) in small portions. After stirring for 20 min at 0 °C TLC showed full conversion of the starting material and the mixture was diluted with EtOAc. The mixture was transferred to a separation funnel and H_2O was added giving a white slurry. 1M HCl (aq.) was added till a clear two phase system was formed. The layers were separated and the aqueous layer was extracted with EtOAc (3x). The combined organic phase was washed with sat. $NaHCO_3$ (aq.), H_2O (3x), brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded manno cyclohexene **32** as a white amorphous solid $[\alpha]_D^{22}$ + 48.5° (c = 1.0, DCM). (0.387 g, 1.137 mmol, 95%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.27 (m, 10H), 5.88 (ddd, *J* = 9.9, 5.3, 2.8 Hz, 1H), 5.64 (dd, *J* = 9.9, 2.3 Hz, 1H), 4.72 (d, *J* = 11.6 Hz, 1H), 4.68 (d, *J* = 12.3 Hz, 1H), 4.67 (d, *J* = 12.1 Hz, 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.13 – 4.08 (m, 2H), 3.81 – 3.73 (m, 2H), 3.46 (dd, *J* = 10.2, 3.9 Hz, 1H), 2.97 (s, 1H), 2.64 (s, 1H), 2.44 – 2.37 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.6, 137.9, 130.7, 128.7, 128.5, 128.1, 128.1, 128.1, 127.9, 81.1, 71.8, 71.7, 70.1, 69.4, 65.9, 46.6. HRMS: $[M+H]^+$ calculated for $C_{21}H_{25}O_4$ 341.17474, found 341.17501.

Epoxidation

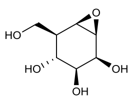
To a solution of manno cyclohexene **32** (0.953 g, 2.8 mmol) in DCE (48 mL) was added *m*CPBA (55%) (1.32 g, 4.2 mmol) and the mixture was heated to reflux. After complete conversion of the starting material the mixture was cooled to rt and silica was added to the mixture after which the solvents were removed *in vacuo*. The immobilized product was directly purified by column chromatography yielding benzylated β-manno cyclophellitol **33** (0.283 g, 0.794 mmol, 29%) and benzylated α-manno cyclophellitol **34** (0.180 g, 0.505 mmol, 18%) both as a white amorphous solid.



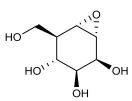
β-2,3-*O*-dibenzyl-2-*epi*-cyclophellitol (33): $[\alpha]_D^{22}$ + 64.7° (c = 1.0 DCM). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 – 7.41 (m, 2H), 7.39 – 7.27 (m, 8H), 4.85 (d, *J* = 12.1 Hz, 1H), 4.65 (d, *J* = 11.6 Hz, 1H), 4.63 (d, *J* = 12.1 Hz, 1H), 4.42 (d, *J* = 11.6 Hz, 1H), 4.10 – 4.05 (m, 2H), 3.93 (dd, *J* = 10.7, 5.1 Hz, 2H), 3.91 (t, *J* = 9.7 Hz, 1H), 3.28 (dd, *J* = 3.7, 2.0 Hz, 1H), 3.24 (dd, *J* = 4.9, 3.7 Hz, 1H), 3.20 (dd, *J* = 10.1, 5.0 Hz, 1H), 2.76 (s, 1H), 2.61 (s, 1H), 2.10 (dddd, *J* = 9.1, 7.1, 5.2, 2.0 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 137.8, 137.5, 128.7, 128.6, 128.5, 128.2, 128.1, 79.7, 71.5, 71.4, 68.5, 67.1, 64.5, 54.6, 50.4, 44.8. HRMS: $[M+H]^+$ calculated for $C_{21}H_{25}O_5$ 357.16965, found 357.16965.



α-2,3-*O*-dibenzyl-2-*epi*-cyclophellitol (34): $[\alpha]_D^{22}$ -32.3° (c = 1.0 DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 10H), 4.83 (d, J = 12.0 Hz, 1H), 4.65 (d, J = 11.9 Hz, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.51 (d, J = 11.6 Hz, 1H), 4.28 (t, J = 2.9 Hz, 1H), 3.93 (dd, J = 9.8, 3.4 Hz, 1H), 3.91 (d, J = 10.2 Hz, 1H), 3.83 (dd, J = 10.7, 5.8 Hz, 1H), 3.53 (dd, J = 10.1, 3.1 Hz, 1H), 3.26 (t, J = 3.1 Hz, 1H), 3.07 (d, J = 3.6 Hz, 1H), 2.69 (s, 2H), 2.18 (dt, J = 8.8, 5.9 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.2, 137.8, 128.8, 128.6, 128.2, 128.1, 128.1, 128.0, 79.6, 74.0, 72.6, 71.6, 67.5, 63.7, 54.5, 53.7, 44.1. HRMS: $[M+H]^+$ calculated for $C_{21}H_{25}O_5$ 357.16965, found 357.16967



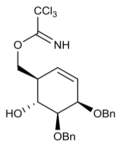
β-5-*epi*-cycloophellitol (20): Benzylated β-manno cyclophellitol **33** (41 mg, 0.115 mmol) was dissolved in dioxanes/*t*BuOH (9:1) (2.5 mL) and purged with argon gas. To the solution was added Pd/C (10%) and the mixture was stirred under a H_2 atmosphere. After complete conversion of the starting material to the fully debenzylated product the mixture was filtered over a pad of celite and rinsed with H_2O. The filtrate was concentrated *in vacuo*. The product was crystallized in MeOH yielding β-manno cyclophellitol **20** as a colourless crystalline solid (8.1 mg, 46 µmol, 40%). mp 164 °C. ^{1}H NMR (500 MHz, D_2O) δ 4.37 (t, J = 5.1 Hz, 1H), 3.99 (dd, J = 11.2, 4.2 Hz, 1H), 3.83 (dd, J = 11.2, 8.0 Hz, 1H), 3.56 (dd, J = 4.0, 1.9 Hz, 1H), 3.52 (dt, J = 8.3, 3.7 Hz, 2H), 3.46 (dd, J = 10.1, 8.9 Hz, 1H). ^{13}C NMR (126 MHz, D_2O) δ 72.3, 65.9, 65.3, 60.8, 56.1, 53.6, 44.1. HRMS: $[M+H]^+$ calculated for $C_7H_{13}O_5$ 177.07575, found 177.07576



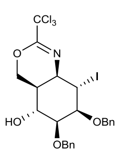
α-5-*epi*-cyclophellitol (21): Benzylated β-manno cyclophellitol **34** (71.2 mg, 0.2 mmol) was dissolved in dioxanes/*t*BuOH (9:1) (5 mL) and purged with argon gas. To the solution was added Pd/C (10%) and the mixture was stirred under a H_2 atmosphere. After complete conversion of the starting material to the fully debenzylated product, the mixture was filtered over a pad of celite and rinsed with H_2O. The filtrate was concentrated *in vacuo*. The product was crystallized in MeOH yielding α-manno cyclophellitol **21** as a white solid

(12.6 mg, 72 µmol, 36%). mp 135-137 °C. ^{1}H NMR (400 MHz, MeOD) δ 4.30 – 4.26 (m, 1H), 3.91 (dd, *J* = 10.8, 4.0 Hz, 1H), 3.67 (dd, *J* = 10.8, 7.9 Hz, 1H), 3.46 (dd, *J* = 8.2, 2.0 Hz, 1H), 3.41 (dd, *J* = 10.2, 3.3 Hz, 1H), 3.24 (t, *J* = 3.1 Hz, 1H), 3.19 (dd, *J* = 3.6, 0.8 Hz, 1H), 1.94 (td, *J* = 8.2, 3.9 Hz, 1H). ^{13}C NMR (100 MHz, MeOD) δ 72.2, 69.1, 67.5, 62.4, 56.9, 55.1, 46.6. HRMS: $[M+H]^+$ calculated for $C_7H_{13}O_5$ 177.07575, found 177.07575

(1*S*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-

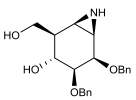


(methylthrichloroacetimidate)cyclohex-3-enol (36) To a solution of manno cyclohexene **32** (68.1 mg, 0.2 mmol) in DCM (4.25 mL) was added a 0.6 M Cl_3CCN (0.5 mL, 0.3 mmol) in DCM and the mixture was cooled to 0 °C. To the cooled solution was added dropwise a 0.4 M DBU (0.25 mL, 0.1 mmol) solution in DCM to the reaction mixture. The mixture was warmed to rt and stirred for 30 min. The mixture was diluted with DCM and silica was added. The solvents were removed *in vacuo* and the immobilized product was directly purified by column chromatography yielding cyclohexene imidate **36** as a colourless oil (75.4 mg, 0.16 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.39 – 7.26 (m, 10H), 5.95 – 5.84 (m, 2H), 4.74 (d, *J* = 11.7 Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.61 (dd, *J* = 6.5, 4.1 Hz, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.16 – 4.06 (m, 2H), 3.48 (dd, *J* = 10.1, 3.8 Hz, 1H), 2.87 (s, 1H), 2.62 (ddd, *J* = 8.3, 7.8, 3.5 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 138.6, 138.0, 131.0, 128.6, 128.5, 128.1, 128.1, 128.0, 127.8, 125.8, 81.2, 71.8, 71.3, 69.5, 69.5, 66.8, 44.3. TLC-MS: $[M + Na]^+$ 508.0

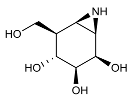


iodo oxazine (37) To a solution of cyclohexene imidate **36** (0.484 g, 1 mmol) in THF/H_2O (4:1) (25 mL) was added $NaHCO_3$ (0.294 g, 10 mmol), I_2 (0.888 g, 3.5 mmol) and the mixture was heated till reflux. After complete conversion of the starting material the mixture was cooled to rt and diluted with EtOAc. To the solution was added 10% $Na_2S_2O_3$ (aq.) till the organic phase was colourless.

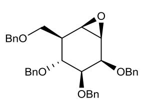
The two phases were separated and the aqueous phase was extracted with EtOAc (3x). The combined organic layers were washed with H_2O (3x), brine (3x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded iodo oxazine **37** as a yellow oil (0.459 g, 0.75 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (ddq, *J* = 9.6, 6.9, 2.1 Hz, 10H), 5.05 (t, *J* = 2.4 Hz, 1H), 4.88 (dd, *J* = 11.2, 1.6 Hz, 1H), 4.79 (d, *J* = 12.3 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.45 (d, *J* = 12.3 Hz, 1H), 4.41 (d, *J* = 11.6 Hz, 1H), 4.19 (dd, *J* = 7.0, 2.6 Hz, 1H), 4.16 (dd, *J* = 5.6, 2.7 Hz, 1H), 4.13 – 4.11 (m, 1H), 4.10 (d, *J* = 2.7 Hz, 1H), 4.07 (q, *J* = 2.6 Hz, 1H), 2.63 (s, 1H), 2.50 (ddt, *J* = 9.9, 4.7, 2.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.6, 137.5, 128.7, 128.5, 128.4, 128.2, 128.1, 127.9, 79.3, 77.1, 71.8, 71.1, 67.2, 64.7, 59.3, 33.8, 26.9. TLC-MS: $[M + Na]^+$ 633.8. HRMS: $[M+H]^+$ calculated for $C_9H_{14}INO_4$ 328.00403, found 328.00409.



β-2,3-*O*-dibenzyl-2-*epi*-cyclophellitol azirine (38) Iodo oxazine **37** (0.459 g, 0.75 mmol) was dissolved in a 1,4-dioxane/H_2O/AcOH (1:1:8) mixture (30 mL) and stirred overnight at rt. The mixture was concentrated *in vacuo*, co-evaporated with toluene (3x) and the residue was dissolved in MeOH (30 mL). To the solution was added $NaHCO_3$ (1.26 g, 15 mmol) and stirred overnight at rt. The solids were filtered over a pad of celite and the filtrate was concentrated *in vacuo*. The crude was dissolved in DCM and washed with H_2O (1x). The aqueous layer was extracted with DCM (5x) and the combined organic layers was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography using neutral and activated silica followed by precipitation in cold Et_2O yielded benzylated aziridine **38** as a white powder (0.155 g, 0.437 mmol, 58%). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 7.46 – 7.25 (m, 10H), 4.77 (d, *J* = 11.5 Hz, 1H), 4.62 (d, *J* = 11.5 Hz, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.18 (t, *J* = 5.2 Hz, 1H), 3.92 (dd, *J* = 10.7, 5.7 Hz, 1H), 3.84 (dd, *J* = 10.8, 4.8 Hz, 1H), 3.77 (t, *J* = 9.8 Hz, 1H), 3.23 (dd, *J* = 10.1, 4.7 Hz, 1H), 2.82 (s, 2H), 2.42 – 2.29 (m, 2H), 2.05 (s, 1H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 138.6, 128.9, 128.9, 128.7, 128.5, 128.3, 81.2, 71.6, 71.5, 69.7, 66.1, 64.9, 44.7. HRMS: $[M+H]^+$ calculated for $C_{21}H_{26}NO_4$ 356.18563, found 356.18570.



β-5-*epi*-cycloophellitol aziridine (22) NH_3 gas was condensated at -60 °C and liquid NH_3 was collected (± 2.5 mL). To the liquid NH_3 was added lithium (16.5 mg, 2.5 mmol), upon addition of the lithium the solution turned dark blue. The mixture was stirred till all the lithium was completely dissolved and a solution of benzylated aziridine **38** (35.5 mg, 0.1 mmol) in THF (2 mL) was added drop wise. The reaction was stirred for 30 min at -60 °C and H_2O (1.5 mL) was added dropwise to the reaction mixture. The mixture was gradually warmed to rt and co-evaporated with H_2O (3x). The crude product was dissolved in H_2O and treated with Amberlite IR-120 NH_4^+ for 2 h. The resin was filtered, the filtrate was concentrated *in vacuo* and retreated with Amberlite IR-120 NH_4^+ (3x). The product was dissolved in MeOH and precipitated in 0 °C ether under vigorous stirring. The precipitate was filtered and dried over a stream of air yielding β-aziridine **22** as a white powder (17.4 mg, 0.1 mmol, quantitative). ^1H NMR (400 MHz, Deuterium Oxide) δ 4.27 (t, *J* = 5.3 Hz, 1H), 3.89 (dd, *J* = 10.9, 4.5 Hz, 1H), 3.70 (dd, *J* = 10.9, 8.7 Hz, 1H), 3.44 (dd, *J* = 9.3, 4.9 Hz, 1H), 3.35 (t, *J* = 8.8 Hz, 1H), 2.66 – 2.52 (m, 3H), 2.01 (ddd, *J* = 8.3, 4.6, 3.6 Hz, 1H). ^{13}C NMR (100 MHz, D_2O) δ 73.4, 66.4, 65.5, 62.3, 43.6, 33.1, 32.6. HRMS: $[M+H]^+$ calculated for $C_7H_{13}NO_4$ 176.09173, found 176.09170.

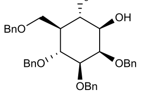


β-3,4,5,7-*O*-tetrabenzyl-5-*epi*-cyclophellitol (39): To a 0 °C cooled solution of β-manno cyclophellitol **33** (0.104 g, 0.29 mmol) in DMF (2.9 mL) was added BnBr (86 µl, 0.725 mmol) and NaH (60%) (29 mg, 0.725 mmol) in small portions and the reaction mixture was allowed to warm to rt. After complete conversion of the starting material the mixture was cooled to 0 °C and quenched with MeOH. The solvent was removed *in vacuo* and the crude was dissolved in Et_2O. The product was washed with H_2O (3x), brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded per-benzylated β-manno cyclophellitol **39** as a white amorphous solid (80.6 mg, 0.15 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.41 (m, 2H), 7.40 – 7.26 (m, 16H), 7.24 – 7.18 (m, 2H), 4.83 (d, *J* = 12.5 Hz, 1H), 4.79 (d, *J* = 11.2 Hz, 1H), 4.72 (d, *J* = 12.5 Hz, 1H), 4.60 (s, 2H), 4.56 (d, *J* = 12.1 Hz, 1H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.42 (d, *J* = 11.2 Hz, 1H), 4.04 (t, *J* = 4.6 Hz, 1H), 3.77 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.69 (t, *J* = 8.8 Hz, 1H), 3.60 (dd, *J* = 8.7, 7.6 Hz, 1H), 3.47 (dd, *J* = 4.8, 2.1 Hz, 1H), 3.46 (t, *J* = 4.9 Hz, 1H), 3.27 (t, *J* = 4.0 Hz, 1H), 2.30 (dddd, *J* = 8.8, 7.6, 4.9, 2.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.5, 138.4, 138.3, 128.6, 128.5, 128.5, 128.5, 128.3, 128.2, 128.0, 128.0, 127.8, 127.8, 127.8, 127.7, 79.7, 74.4, 73.6, 73.4, 72.7, 71.5, 70.9, 69.4, 54.7, 52.0, 42.6. HRMS: $[M+H]^+$ calculated for $C_{35}H_{36}O_5$ 537.26355, found 537.26347.

Azido ringopening

To a solution of per-benzylated β-manno cyclophellitol **39** (53.7 mg, 0.1 mmol) in MeCN (2 mL) was added $LiClO_4$ (16 mg, 0.15 mmol), NaN_3 (65 mg, 1.0 mmol) and the reaction mixture was stirred overnight at 80 °C under an argon atmosphere. The reaction mixture was cooled to rt and quenched with H_2O. The product was extracted with DCM (5x) from the water and the combined organic layers were dried over $MgSO_4$, filtered and concentred *in vacuo* giving a 1:1 mixture of two products. Purification by column chromatography yielded 0-azido-1-hydroxy **40** (28.0 mg, 0.048 mmol, 48%) and 0-hydroxy-1-azido **41** (30.9 mg, 0.052 mmol, 52%) (cyclophellitol numbering).

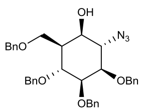
(1*R*,2*S*,3*R*,4*R*,5*S*,6*R*)-2-azido-4,5,6-tris(benzyloxy)-3-((benzyloxy)methyl) cyclohexan-1-ol (40)



^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15 (d, *J* = 11.6 Hz, 1H), 4.90 (d, *J* = 10.7 Hz, 1H), 4.75 (d, *J* = 11.8 Hz, 1H), 4.71 (d, *J* = 11.9 Hz, 1H), 4.67 (d, *J* = 11.7 Hz, 1H), 4.54 (d, *J* = 10.6 Hz, 1H), 4.51 (d, *J* = 12.1 Hz, 1H), 4.45 (d, *J* = 12.1 Hz, 1H), 4.10 (t, *J* = 10.2 Hz, 1H), 4.05 (t, *J* = 2.7 Hz, 1H), 3.87 – 3.77 (m, 2H), 3.63

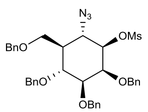
(dd, *J* = 9.2, 2.4 Hz, 1H), 3.44 (dd, *J* = 9.8, 2.1 Hz, 1H), 3.40 (dd, *J* = 10.2, 2.8 Hz, 1H), 1.34 (tt, *J* = 11.2, 2.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.6, 138.6, 138.5, 138.3, 128.7, 128.6, 128.5, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7, 127.7, 84.2, 77.8, 75.8, 75.7, 74.8, 74.2, 73.2, 73.2, 65.1, 62.4, 45.0. TLC-MS $[M+Na]^+$ 602.5. HRMS: $[M+H]^+$ calculated for $C_{35}H_{37}N_3O_5$ 580.28060, found 580.28062.

(1*R*,2*S*,3*R*,4*R*,5*S*,6*R*)-2-azido-4,5,6-tris(benzyloxy)-3-((benzyloxy)methyl) cyclohexan-1-ol (41)



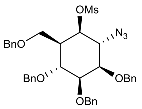
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.41 – 7.14 (m, 20H), 4.72 – 4.37 (m, 8H), 3.98 – 3.84 (m, 3H), 3.75 (t, *J* = 4.7 Hz, 1H), 3.72 (t, *J* = 3.9 Hz, 1H), 3.66 (dd, *J* = 8.1, 2.9 Hz, 1H), 3.62 (dd, *J* = 9.5, 5.5 Hz, 1H), 3.45 (s, 1H), 2.57 (dq, *J* = 9.8, 4.6 Hz, 1H). TLC-MS $[M+Na]^+$ 602.5. HRMS: $[M+H]^+$ calculated for $C_{35}H_{37}N_3O_5$ 580.28060, found 580.28056.

(1*R*,2*S*,3*R*,4*R*,5*S*,6*S*)-2-azido-4,5,6-tris(benzyloxy)-3-((benzyloxy)methyl) cyclohexyl methanesulfonate (42)

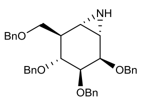


To a 0 °C cooled solution of **40** (22.9 mg, 39.5 µmol) in pyridine (1.2 mL) was added dropwise a 0.5M mesyl chloride solution (0.237 mL, 119 µmol) in toluene and the mixture was allowed to warm to rt. After complete conversion of the starting material the reaction was quenched with H_2O and the product was extracted with EtOAc (5x). The combined organic phase was washed with brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mesylated compound **42** (18.7 mg, 28.4 µmol, 72%). FT-IR: v_{max} (neat)/cm^{-1} 962.12, 991.55, 1075.14, 1091.00, 1179.40, 1360.29, 1454.53, 1496.68, 2112.75, 2918.66, 3030.95. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.22 (m, 16H), 7.21 – 7.15 (m, 4H), 4.69 (dd, *J* = 10.3, 6.2 Hz, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 4.47 (q, *J* = 6.3, 5.8 Hz, 5H), 4.38 (d, *J* = 11.7 Hz, 1H), 4.31 (d, *J* = 11.8 Hz, 1H), 4.08 (t, *J* = 3.3 Hz, 1H), 4.03 (t, *J* = 10.0 Hz, 1H), 3.87 (t, *J* = 9.5 Hz, 1H), 3.77 (dd, *J* = 9.4, 5.9 Hz, 1H), 3.73 (t, *J* = 3.2 Hz, 1H), 3.67 (dd, *J* = 9.7, 3.2 Hz, 1H), 3.06 (s, 3H), 2.93 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.3, 137.7, 137.6, 128.6, 128.6, 128.5, 128.5, 128.2, 128.1, 128.1, 128.0, 127.7, 79.7, 78.5, 74.8, 73.3, 73.2, 72.8, 72.8, 66.3, 61.5, 42.8, 38.4. TLC-MS $[M+Na]^+$ 580.9. HRMS: $[M+H]^+$ calculated for $C_{36}H_{39}N_3O_7S$ 658.25815, found 658.25808.

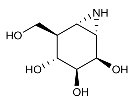
(1*R*,2*R*,3*R*,4*S*,5*R*,6*R*)-2-azido-3,4,5-tris(benzyloxy)-6-(benzyloxy)methyl)



cyclohexyl methanesulfonate (43) To a 0 °C cooled solution of **41** (21.0 mg, 36.2 µmol) in pyridine (1.0 mL) was added dropwise a 0.5M mesyl chloride solution (0.217 mL, 108 µmol) in toluene and the mixture was allowed to warm to rt. After complete conversion of the starting material the reaction was quenched with H_2O and the product was extracted with EtOAc (5x). The combined organic phase was washed with brine (2x), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded mesylated compound **43** (21.0 mg, 32 µmol, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.39 (m, 2H), 7.37 – 7.25 (m, 16H), 7.17 (dd, *J* = 7.4, 2.2 Hz, 2H), 4.94 (d, *J* = 11.8 Hz, 1H), 4.89 (d, *J* = 10.8 Hz, 1H), 4.86 (d, *J* = 11.9 Hz, 1H), 4.60 (d, *J* = 11.7 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 4.54 – 4.44 (m, 2H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.37 (dd, *J* = 10.5, 2.5 Hz, 1H), 4.30 (t, *J* = 2.4 Hz, 1H), 4.24 (t, *J* = 10.8 Hz, 1H), 4.10 (dd, *J* = 10.8, 9.5 Hz, 1H), 3.79 (dd, *J* = 9.4, 1.9 Hz, 1H), 3.61 (dd, *J* = 9.4, 2.5 Hz, 1H), 3.43 (dd, *J* = 9.6, 2.3 Hz, 1H), 3.07 (s, 3H), 1.38 (dt, *J* = 11.4, 2.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 138.4, 138.2, 138.0, 128.6, 128.5, 128.5, 128.4, 128.3, 128.0, 127.8, 127.8, 127.7, 127.6, 83.1, 82.3, 76.3, 75.8, 75.1, 75.0, 73.1, 72.6, 64.7, 59.1, 45.1, 38.5. TLC-MS $[M+Na]^+$ 581.0. HRMS: $[M+H]^+$ calculated for $C_{36}H_{39}N_3O_7S$ 658.25815, found 658.25822.



α-3,4,5,7-*O*-tetrabenzyl-5-*epi*-cyclophellitol aziridine (44) A solution of mesyl **43** (21 mg, 32 µmol) in THF (0.3 mL) was added dropwise to a 0 °C 0.1 M $LiAlH_4$ solution (0.5 mL, 50 µmol) in THF. After 4 h the reaction was diluted with THF and quenched with 3M NaOH (aq.) (0.167 mL, 0.5 mmol). The mixture was dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography yielded perbezylated α-manno aziridine **44** (5.5 mg, 10 μmol, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.37 (m, 2H), 7.37 – 7.24 (m, 16H), 7.23 – 7.19 (m, 2H), 4.93 (d, *J* = 12.2 Hz, 1H), 4.84 (d, *J* = 11.3 Hz, 1H), 4.73 (d, *J* = 12.4 Hz, 1H), 4.70 (d, *J* = 11.9 Hz, 1H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.52 (d, *J* = 12.1 Hz, 1H), 4.46 (t, *J* = 11.2 Hz, 2H), 4.22 (t, *J* = 2.5 Hz, 1H), 3.76 (dd, *J* = 9.7, 2.6 Hz, 1H), 3.71 (dd, *J* = 9.8, 7.7 Hz, 1H), 3.61 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.51 (t, *J* = 8.7 Hz, 1H), 2.46 (dd, *J* = 6.0, 2.4 Hz, 1H), 2.36 (d, *J* = 5.8 Hz, 1H), 2.26 (td, *J* = 8.0, 4.1 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.3, 139.1, 139.0, 138.6, 128.5, 128.5, 128.4, 128.4, 128.2, 127.8, 127.8, 127.7, 127.6, 127.5, 80.8, 75.7, 75.2, 74.7, 73.3, 73.1, 73.1, 70.9, 43.4, 34.6, 31.9. TLC-MS $[M+H]^+$ 536.3 HRMS: $[M+H]^+$ calculated for $C_{35}H_{37}NO_4$ 536.27954, found 536.27957.



α-5-*epi*-cyclophellitol aziridine (23) NH_3 gas was condensated at -60 °C and liquid NH_3 was collected (± 2.5 mL). To the liquid NH_3 was added lithium (3 mg, 0.43 mmol), upon addition of the lithium the solution turned dark blue. The mixture was stirred till all the lithium was completely dissolved and a solution of perbenzylated α-manno-aziridine **44** (5.5 mg, 10 μmol) in THF (1.0 mL) was added drop wise. The reaction was stirred for 30 min at -60 °C and H_2O (1.0 mL) was added dropwise to the reaction mixture. The mixture was gradually warmed to rt and co-evaporated with H_2O (3x). The crude product was dissolved in H_2O and treated with Amberlite IR-120 NH_4^+ for 2 h. The resin was filtered, the filtrate was concentrated *in vacuo* and retreated with Amberlite IR-120 NH_4^+ (3x) yielding α-aziridine **23** (1.8 mg, 10 μmol, quantitative). ^{1}H NMR (600 MHz, D_2O) δ 3.89 (s, 1H), 3.47 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.27 (dd, *J* = 11.0, 7.7 Hz, 1H), 3.04 (t, *J* = 9.7 Hz, 1H), 3.01 (dd, *J* = 10.3, 3.3 Hz, 1H), 2.08 (d, *J* = 6.0 Hz, 1H), 1.90 (d, *J* = 5.8 Hz, 1H), 1.43 (td, *J* = 8.3, 3.8 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 72.1, 69.1, 68.3, 63.2, 46.2, 36.8, 32.3. HRMS: $[M+H]^+$ calculated for $C_7H_{13}NO_4$ 176.09173, found 176.09169.

DFT calculations

The calculated ^{1}H NMR coupling constants were obtained by first finding the lowest energy conformation of both epoxide isomers, for which a library of gas phase conformations was generated using conformer distribution option included in Spartan 04 program employing DFT/B3LYP 6-31G(d). All conformers were further optimized by Gaussion 03 at DFT/B3LYP 6-311G(d,p), their zero-point energies were calculated and the energies corrected for solvent by another optimization step employing a Polarizable Continuum Model set for water. The energies of these conformers, corrected for their zero-point energies, were compared and of the lowest energy conformer an NMR calculation was performed using Gauche-Independent Atomic Orbital (GIAO) method with added spin-spin coupling calculation.

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