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

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6 Synthesis of Cellobiosyl Cyclophellitol and Analogues as Potential Cellulase Inhibitors and Activity-Based Probes

6.1 Introduction

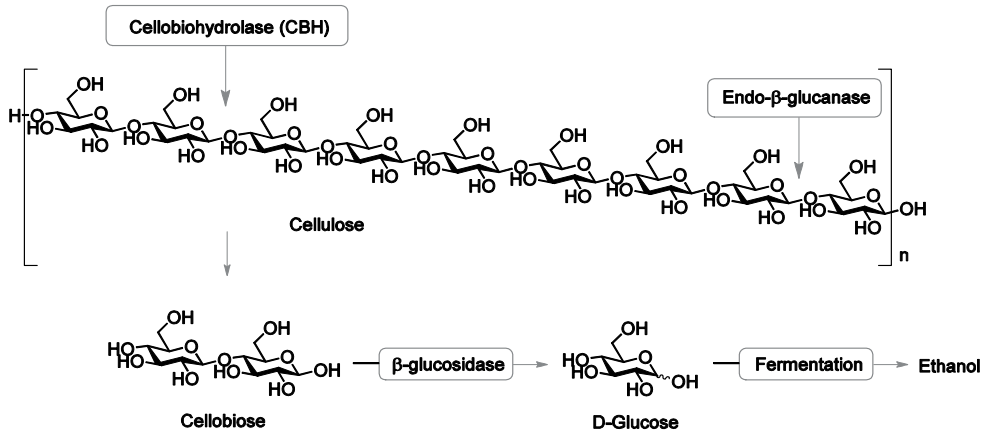
The demand for energy is steadily growing as the world population increases and more countries become industrialized. To date, fossil fuels are the major source to meet this increased energy demand. However, in view of its inevitable depletion, alternative energy sources are needed to meet the growing energy demand. The use of biomass (for instance, agriculture residues and forestry residues) has gained considerable interest as renewable energy sources.[1-5]

Today, ethanol is the most used renewable bio-fuel, which can be produced by fermentation of any carbohydrate-containing material. These materials are grouped into three types of carbohydrates: sugars, starch and lignocellulosic materials. Sugars (for instance, sugar cane and fruit) and starches (for instance, corn and cassava)

serve as the major source for the production of bio-ethanol today, since they can be easily converted into ethanol. However, utilizing these sources results in an undesirable conflict between land use for food and energy feedstock production.[2,4] This can be minimized when using lignocellulosic materials. Lignocellulosic materials are the most abundant global source of biomass and are waste products from agriculture and forestry, but it remains largely unutilized owing to their complex compositions consisting of cellulose, hemi-cellulose and lignin. Cellulose, the major component of lignocellulosic biomass, is a homopolysaccharide composed of β-1,4-linked glucosides with cellobiose as the smallest repeating unit that can be converted in fermentable glucose. Depending on the organization of various cellulose chains, cellulose can appear in its crystalline or amorphous form. The former is tightly packed together by hydrogen bonds and van der Waals interactions and therefore less susceptible to enzymatic degradation compared to their amorphous counterparts. Hemi-cellulose acts as a crosslinking material between cellulose and lignin, which in turn serves as a defensive matrix. The composition and percentage of these three polymers vary between biomass sources, thereby complicating the industrial production of ethanol from lignocellulosic materials.[2,6]

Industrial conversion of lignocellulosic biomass into bio-ethanol requires the liberation of cellulose from lignin and hemi-cellulose by pre-treating lignocellulosic biomass in a chemical and a physical manner. This is followed by the enzymatic hydrolysis of cellulose in fermentable carbohydrates and fermentation of the free sugars into ethanol.[7-11] Efficient enzymatic hydrolysis of cellulose into free sugars requires the

Figure 1. Overview of enzymes that are involved in the conversion of cellulose into bio-ethanol.

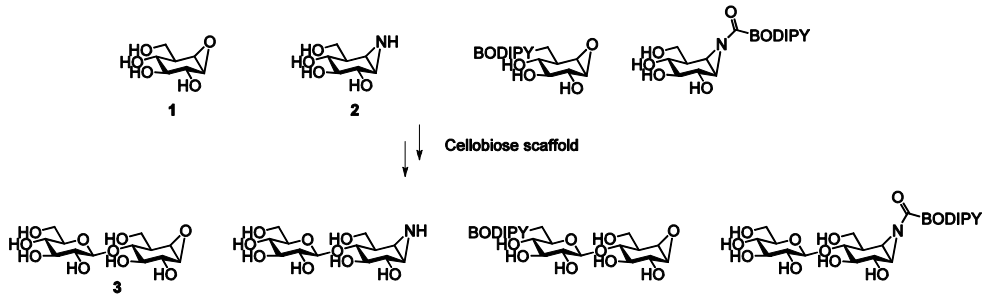


action of three different groups of cellulolytic enzymes to act synergistically: cellobiohydrolases (CBH, exo-1,4-β-D-glucanase), endoglucanases (EG, endo-1,4-β-D-glucanases) and β-glucosidases (Figure 1). EGs randomly catalyze the cleavage of internal glucosidic linkages positioned in the amorphous regions of cellulose and thereby creating new cleavage sites for CBHs. CBHs act on the termini of cellulose, releasing cellobiose, which in turn is hydrolyzed into glucose by β-glucosidase. Hydrolysis by these three cellulolytic enzymes proceeds via the classical glycosidase mechanisms as initially proposed by Koshland.[12]

To date, the cellulolytic system secreted by the filamentous fungus *Hyprocrea jecorina* (*H. jecorina*), previously reported as *Trichoderma reseei* (*T. reseei*) is one of the most efficient and this system is incorporated in the industrial production of bio-ethanol.[13-15] However, a commonly encountered problem during the industrial process is the "dying off" of one or more enzymes over time resulting in a lower ethanol production.[16] The origin of the observed loss of enzyme activity arises from inhibition by by-products that are generated during the pre-treatment process or depolymerisation of cellulose.[16] Currently, no methodologies are available to determine whether one or more enzymes of the cellulolytic mixture have become deficient or to point out which enzyme has become inactivated. Methodologies that allow the assessment and quantification of the actual live enzymes and their relative concentrations in the cellulolytic mixture are therefore needed. Additionally, such work enables the in real time monitoring of the *H. jecorina* enzyme mixture on its native substrates (cellulose) during the hydrolysis process with the ultimate goal to optimize the current protocols for industrial biomass conversion.

One strategy to accomplish these objectives entails the construction of ABPs that allow identification and quantification of the various cellulases in a complex mixture. Previous studies have demonstrated the merits of cyclophellitol **1** and cyclophellitol aziridine **2** as potent, irreversible mechanism-based inhibitors of retaining β-glucosidases from various biological origins.[17-20] Appending a reporter group to the inhibitors resulted in a new class of ABPs that proved to be useful in profiling of active glucocerebrosidase (GBA) in Gaucher disease.[21,22] Capitalizing on the remarkable ability of **1**, **2** and the corresponding ABP derivatives to selective inactivate human GBA, it was envisioned that incorporation of these mechanism-based inhibitors and ABPs on a cellobiose scaffold (Figure 2) generates useful tools to profile cellulases in a complex mixture.

Figure 2. Design of cyclophellitol and cyclophellitol aziridines-based compounds for cellulases.



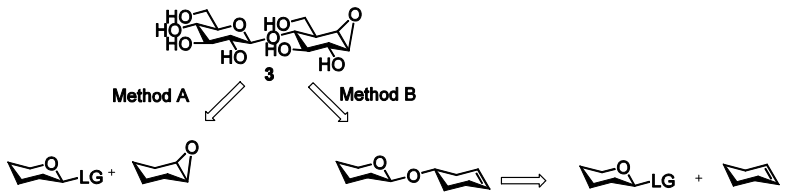
This Chapter describes a study towards the synthesis of mechanism-based inhibitors and ABPs for cellulases based on cyclophellitol **1** using cellobiose as a scaffold. The inhibitory potential of these cyclophellitol-based compounds will be discussed in Chapter 7 along with the synthesis and biological activity of cyclophellitol aziridine-based inhibitors and ABPs for *H. jecorina* cellulases.

6.2 Results and Discussion

6.2.1 Synthesis of cellobiosyl cyclophellitol as mechanism-based inhibitor

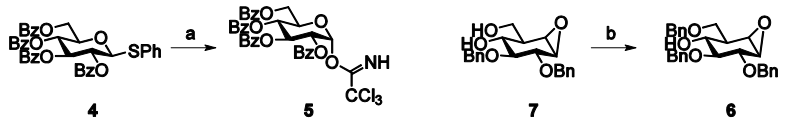
Two strategies are amenable for the synthesis of cellobiosyl cyclophellitol **3** as presented in Scheme 1. The first strategy involves the consecutive glycosylation of an appropriate donor with a cyclitol epoxide acceptor and removal of protective groups to yield **3** (Method A). For the second approach, the epoxide functionality is introduced after the glycosylation step (Method B). To this end, a suitable donor is condensed with a hydroxylated cyclic alkene and then subjected to epoxidation conditions. Glycosylation in the presence of the epoxide function (Method A) with a suitable donor appears to be, at the first glance the favorable route, since less functionalization steps are needed.

Scheme 1. Retrosynthetic analysis of cellobiosyl cyclophellitol **3**.



Glycosylation in the presence of an oxirane ring. In order to explore this route, two relevant donors **4** and **5**, and partially protected cyclcophellitol **6** were synthesized as depicted in Scheme 2.

Scheme 2. Synthesis of donors and acceptor **4**-**6**.

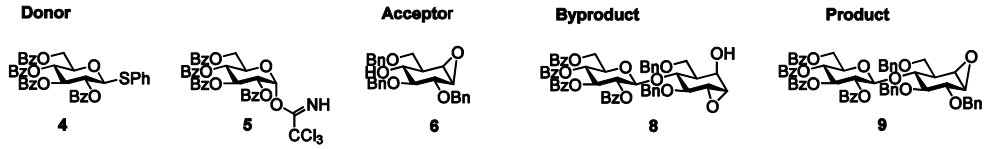


Reagents and conditions: (a) i. *N*-bromosuccinimide, acetone, H_2O, 0 °C, ii. CCl_3CN, DBU, DCM, 0 °C, 87 %; (b) BnBr, NaH, DMF, 0 °C, 58%.

Donor **4** was synthesized according to literature procedures as described by Sinay and coworkers[23] and **4** served as the starting material for donor **5**. NBS-mediated hydrolysis of the anomeric thio functionality in **4** was followed by the introduction of an anomeric trichloroimidate function under thermodynamic conditions (CCl_3CN, DBU, DCM) to yield imidate donor **5** (87%). Acceptor **6** was synthesized by treating **7** (see Chapter 3) with one equivalent of benzyl bromide to give a mixture of **6** (58%), 2,3,4-*O*-tribenzyl cyclophellitol (7.5%) and diol **7**, which was easy separated by silica column chromatography. Attempts to synthesize **6** by installment of the 4,8-O-benzylidene (cyclophellitol numbering) protective group ($PhC(OMe)_2$, *p*TsOH, DMF) and ensuing reductive regioselective opening (TESH, TFA, DCM or $NaCNBH_3$, TFA, DCM) were abortive as opening of the epoxide functionality was observed.

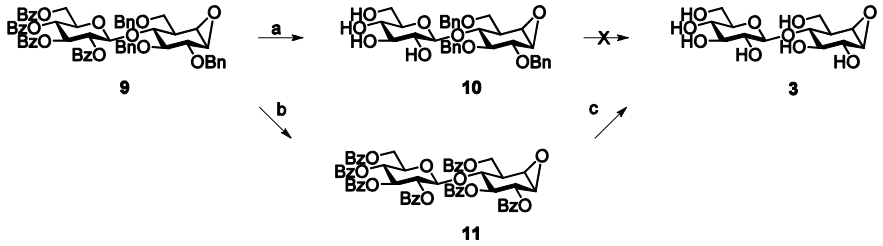
With donors **4**-**5** and acceptor **6** in hand, the attention was focused to find optimal conditions for glycosylation. The results of this study are summarized in Table 1. Condensation of imidate donor **5** with acceptor **6** under the agency of $BF_3.OEt_2$ yielded **8** as the single product (Entry 1). Disaccharide **8** was formed upon activation of the epoxide function by the Lewis acid and ensuing Sn^2 nucleophilic attack of the C2 benzyl group. Attempts to perform the glycosylations with triflic acid proved abortive in that only an unidentified ring opened product was isolated (Entry 2) or no full consumption of **6** occurred (Entry 3). Entry 4 showed that glycosylation (Ph_2SO, TTBP, Tf_2O, DCM) with thiodonor **4** was ineffective. Changing the activation system to NIS/TfOH yielded **9** in a moderate yield (43%, Entry 5), which was enhanced to 90% by increasing the amount of donor **4** (Entry 6).

Table 1. Glycosylation study with donor **4**-**5** and acceptor **6**.



Entry	Donor	Acceptor	Glycosylation conditions	Results/Yield
1	**5** (1.1 eq)	**6** (1.0 eq)	$BF_3.OEt_2$ (0.6 eq), DCM, 0 °C	**8** (46%)
2	**5** (1.1 eq)	**6** (1.0 eq)	TfOH (0.6 eq), DCM, 0 °C	Opening epoxide
3	**5** (1.1 eq)	**6** (1.0 eq)	TfOH (0.2 eq), DCM, 0 °C	**9** (28%) and **6** (37%)
4	**4** (1.2 eq)	**6** (1.0 eq)	Ph_2SO (1.4 eq), TTBP (1.15 eq), Tf_2O, DCM, -60 °C → 0 °C	0%
5	**4** (1.2 eq)	**6** (1.0 eq)	NIS (1.1 eq), TfOH (0.3 eq), DCM -40 °C → 0 °C	**9** (43%)
6	**4** (1.5 eq)	**6** (1.0 eq)	NIS (1.3 eq), TfOH (0.25 eq), DCM -40 °C → 0 °C	**9** (90%)

Scheme 3. Deprotection strategies towards cellobiosyl cyclophellitol **9**.



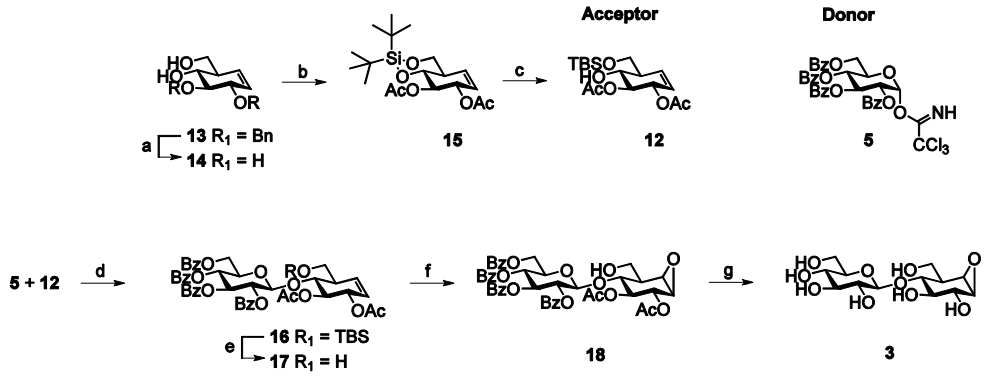
Reagent and conditions: (a) NaOMe, MeOH, 82%; (b) $RuCl_3$, $NaIO_4$, CCl_4, ACN, H_2O, 9%; (c) NaOMe, MeOH, DCM, 59%.

With an effective NIS/TfOH activating system for the synthesis of protected cellobiosyl cyclophellitol **9** in hand, the attention was directed to the last steps. Removal of the benzoyl and benzyl protecting group (Scheme 3) was much more complicated than it was initially foreseen. Removal of the benzoyl groups in **9** under Zemplén conditions proceeded uneventfully (82%), while ensuing palladium-catalyzed hydrogenation ($Pd(OH)_2$, H_2, MeOH/H_2O; 9/1; v/v) resulted in a mixture of 1-deoxy and 6-deoxy disaccharides. Varying the amount of $Pd(OH)_2$ catalyst, changing the Pd catalyst into 10%Pd/C, 5% Pd/C, $Pd(CaCO_3)$ or $Pd(BaSO_4)$ or the solvent system into THF/H_2O

(9/1; v/v) also resulted in a mixture of 1-and 6-deoxy disaccharides. When utilizing $Pd(CaCO_3)$ or $Pd(BaSO_4)$ as palladium source, the starting material **10** was not fully consumed. Treatment of **10** with lithium and liquid ammonia proved abortive as well and therefore the attention was turned to oxidize the benzyl ether in **9** to benzoyl ester that enables removal under Zemplén conditions. Oxidation of the benzyl ether groups in **9** by O_3 in dichloromethane and methanol at 0 °C group was ineffective in that only hydrolyzed benzoylated disacharride could be isolated. Ruthenium-mediated oxidation ($RuCl_3$, $NaIO_4$, CCl_4, ACN, H_2O) afforded a complex mixture of the desired product **11** and multiple by-products resulting from either a Cl-Sn^2 nucleophilic attack, glycosidic bond cleavage of **9** and incomplete consumption of **9**. Attempts to decrease the number of by-products by varying the amount of reagents, reducing the reaction time or lowering the reaction temperature to 0 °C were not successful. The desired **11** could nonetheless be isolated after HPLC purification under neutral conditions (acetonitrile/water) in a low yield (9%). Ensuing debenzoylation with sodium methoxide in methanol and dichloromethane afforded the target compound **3** in a yield of 59%.

Glycosylation in the presence of a double bond. Although cellobiosyl cyclophellitol **3** was synthesized by coupling of thiodonor **4** and epoxide acceptor **6** (Method A), an alternative strategy was needed, since the overall yield of **3** starting from **6** was only 4%. The most complicated step in this strategy was the removal or substitution of the benzyl ethers in **9** in the presence of an epoxide functionality. It was therefore envisioned that it would be beneficial if both the donor and acceptor are protected with a benzoyl or an acetyl group. Preliminary NIS/TfOH glycosylation studies with 2,3,8-tri-*O*-acetyl cyclophellitol and thiodonor **4** resulted solely in a ring opened product by sequential protonation of the oxirane ring and nucleophilic attack by the participating C2-acetyl group. Hence, glycosylation in the presence of a double bond (Method B) was selected as synthetic route towards cellobiosyl cyclophellitol **3**. This strategy commenced with the synthesis of acceptor **12** as described in Scheme 4.
Starting from cyclitol **13**[24] (See Chapter 3) acceptor **12** was readily prepared in five steps by first removal of the benzyl ether groups in **13** under Birch conditions (lithium, NH_3). Installation of the 4,8-di-tertbutylsilylene protective group ($(tBu)_2SiOTf_2$, pyridine, DMF) was followed by acetylation of the remaining secondary alcohols yielding **15** in a yield of 92%. Next, **15** was transformed in **12** by HF-mediated removal of the silylene group and selective protection of the primary alcohol with a TBS group

Scheme 4. Synthesis of accepter **12** and cellobiosyl cyclophellitol **3**.



Reagents and conditions: (a) Li, NH_3, THF, -60 °C, 78%; (b) i. $(tBu)_2SiOTf_2$, pyridine, DMF, -40 °C to 0 °C, ii. Ac_2O, pyridine, 92%; (c) i. HF/pyridine, pyridine, THF, 0 °C, ii. TBSCl, imidazole, DMF, 77%; (d) TfOH, DCM, 0 °C, 60%; (e) HF/pyridine, pyridine, THF, 0 °C, 78%; (f) *m*CPBA, 1 M Na_2HPO_4 (aq.), 1 M NaH_2PO_4 (aq.), dichloroethane, H_2O, 50 °C; (g) Na(s), MeOH, 8% (over two steps).

(TBSCl, imidazole, DMF) in 77% yield. Condensation of imidate donor **5** and acceptor **12** using triflic acid as promoter afforded **16** in a moderate yield (60%). Treatment of **16** with HF/pyridine resulted in the cleavage of the primary TBS protective group and provided **17**. Stereoselective epoxidation of alkene **17**, guided by the homo-allylic alcohol, with *m*CPBA in a buffered biphasic solvent system (1 M aqueous Na_2HPO_4, 1 M aqueous NaH_2PO_4, DCE) at 50 °C gave **18** in a low yield and the hydrolyzed 1,6-diol derivative was isolated as a major byproduct. In addition, alkene **17** was recovered as well. No significant improvement of the yield was observed when the reaction conditions were changed to *m*CPBA in the presence of a large excess of K_2HPO_4 (8 eq) in either an aqueous (DCE/H_2O) or a non-aqueous (DCE) solvent system at elevated temperature (50 °C). Full consumption of **17** occurred when using methyl(trifluoromethyl)-dioxirane (formed *in situ* from trifluoroacetone and oxone), but the 1,6-*epi*-isomer and 1,6-hydroxychloro derivatives were isolated as major products. Treatment of protected cellobiosyl cyclophellitol **18** with sodium in methanol afforded **3** (8%). Overall, cellobiosyl cyclophellitol **3** was prepared in 4% overall yield from **12**.

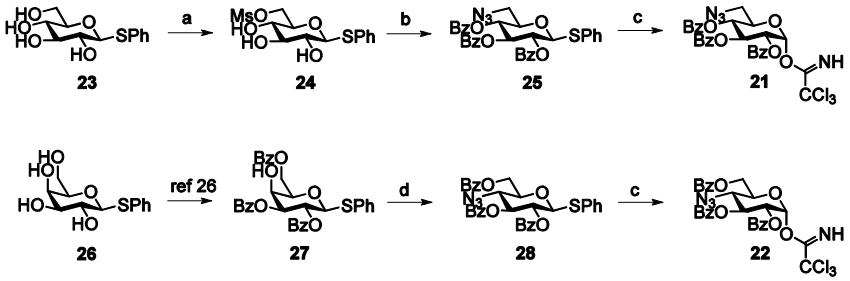
6.2.2. Synthesis of 6'-and 4'-BODIPY cellobiosyl cyclophellitol as ABP

The three-dimensional structures of various cellulases indicated that modifications should be tolerated on the 4'-and 6' position of cellobiosyl cyclophellitol **3**, hence it was

projected that a fluorescent BODIPY reporter group can be appended at these positions. The synthesis of 6'-and 4'-BODIPY cellobiosyl cyclophellitol **19** and **20** (Scheme 6) started with the synthesis of 6-and 4 azido imidate glucosyl donors **21** and **22** (Scheme 5).

Selective mesylation (MsCl, pyridine, 0 °C) of thioglucopyranosyl **23**[25] yielded mesylate **24** in 73%. Nucleophilic substitution of **24** with sodium azide in DMF and ensuing perbenzoylation of the remaining secondary hydroxyls groups in **24** with benzoyl chloride in pyridine afforded **25** (92%). 6-Azido imidate donor **21** was synthesized in two steps by first hydrolysis of the anomeric thiofunctionality with NBS in acetone and water and reaction with trichloroacetonitrile and DBU in dichloromethane, giving **21** in 75%. Thiogalactosyl **27** was synthesized as previously described from **26**.[26] The free hydroxyl in **27** was transformed in a triflate (Tf_2O, pyridine, DCM, 0 °C), which was treated with sodium azide to give the desired azide **28** in 81%. Consecutive NBS-mediated hydrolysis of the anomeric thio-functionality in **28** and treatment with trichloroacetronitrile under basic conditions afforded 4-azido imidate donor **22** (76%).

Scheme 5. Synthesis of 6-and 4- azido imidate donors **21** and **22**.

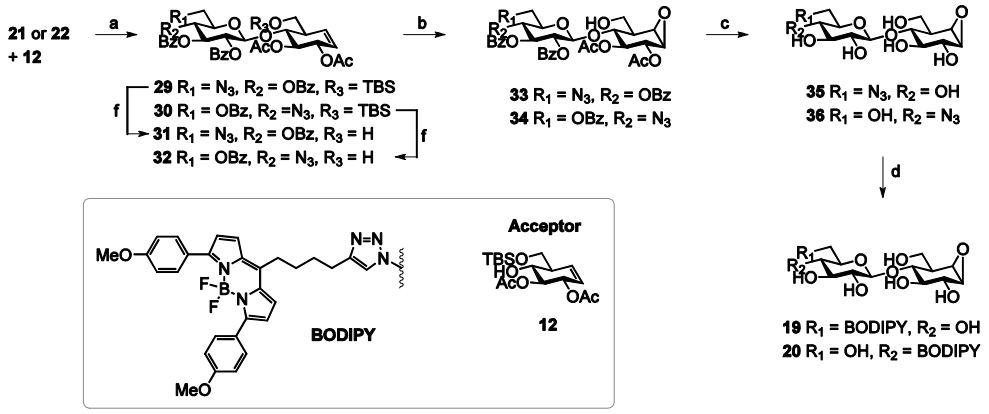


Reagents and conditions: (a) MsCl, pyridine, 0 °C, 73%; (b) i. NaN_3, DMF, 80 °C, ii. BzCl, pyridine, 92%; (c) i. *N*-bromosuccinimide, acetone, H_2O, 0 °C, ii. CCl_3CN, DBU, DCM, 0 °C, **21**: 75%, **22**: 76%; (d) i.Tf_2O, pyridine, DCM, 0 °C, ii. NaN_3, DMF, 81%.

Condensations of acceptor **12** with either 4-azido donor **22** or 6-azido donor **21** with triflic acid as promoter yielded **29** and **30** in a moderate yield (Scheme 6). Removal of the primary TBS protective group under the agency of HF/pyridine afforded respectively **31** and **32**. Stereoselective transformation of alkene **31** and **32** to epoxide **33** and **34** with the earlier mentioned condition (*m*CPBA, 1 M aqueous Na_2HPO_4, 1 M aqueous NaH_2PO_4, DCE, 50 °C) proved abortive. The starting material was recovered and the formation of hydrolyzed disaccharides was observed. Therefore, the suboptimal epoxidation conditions with *in situ* formed methyl(trifluoromethyl)-dioxirane were

used to construct epoxides **33** and **34**. This method gave complex mixtures of the desired products along with their 1,6-epimers and 1,6-hydroxylchloro derivatives. Attempts to separate the side products from the desired **33** and **34** were ineffective and hence the mixtures were deprotected with sodium in methanol and directly subjected to copper (I)-catalyzed click reaction with BODIPY-alkyne[27]. Unfortunately, attempts to purify and separate **19** and **20** from the unwanted by-products by HPLC were unsuccessful and therefore the synthesis of the potential ABPs **19** and **20** was not achieved. Explorations of other synthetic strategies are needed to attain the target compounds **19** and **20**.

Scheme 6. Synthesis of 6'-and 4'-BODIPY cellobiosyl cyclophellitol **19** and **20**.



Reagents and conditions: (a) TfOH, DCM, 0 °C, **29**: 52%, **30**: 58%; (b) HF/pyridine, pyridine, THF, 0 °C, **31**: 82%, **32**: 85%; (c) oxone, CF_3OCH_3, $NaHCO_3$, acetonitrile, 4 mM Na_2EDTA (aq.); (d) Na(s), MeOH; (e) BODIPY-alkyne[27], $CuSO_4$, sodium ascorbate, TBTA, MeOH.

6.3 Conclusion

This Chapter describes the synthesis of cellobiosyl cyclophellitol **3** as a potential cellulase inhibitor via two different strategies. The first strategy relies on chemoselective glycosylation in presence of an epoxide functionality, while the second strategy depends on condensation in the presence of a double bond, which is epoxidized in a later stage. Both strategies proved suboptimal, each with their own specific limitations. Next to cellobiosyl cyclophellitol **3**, efforts were undertaken to synthesize BOPDIPY-cellobiosyl cyclophellitol as potential cellulase ABPs, but this proved to be unsuccessful. The inhibitory activity of cellobiosyl cyclophellitol **3** will be discussed in Chapter 7.

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) or High resolution mass spectra were recorded in the mass spectrometry laboratory of the Chemistry Department at U.B.C. 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).

General procedure A: NBS-mediated hydrolysis of thioglucosides and formation of anomeric imidateglucosides 5, **21** and **22**

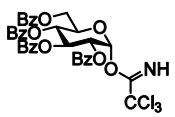
A solution of thioglucoside (1 eq) and NBS (4 eq) in acetone/water (9/1; v/v, 0.2 M) was stirred at ambient temperature until TLC showed full consumption of the starting material (2-4 h). The reaction mixture was diluted with EtOAc and subsequently washed with aqueous saturated $NaHCO_3$, 10% sodium thiosulphate, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified over a short pad of silica using EtOAc and petroleum ether before being dissolved in DCM (0.1 M) and cooled to 0 °C. The solution was treated with trichloroacetonitrile (1.5 eq) and DBU (0.05 eq) at stirred at 0 °C for 1 h before being concentrated *in vacuo*. Purification by silica column chromatography afforded the desired imidates as colorless oil.

General procedure B: TfOH-mediated imidate glycosylation 16, **29** and **30**

Imidate donor (1.5 eq) and cyclitol acceptor **12** (1 eq) were co-evaporated with toluene thrice and DCM (0.2 M) was added. The solution was stirred over 3Å activated molecular sieves for 30 min and cooled down to 0 °C. A catalytic amount of triflic acid (0.2 eq) was added to the solution and stirred at 0 °C for 1 h. The reaction was quenched with Et_3N, diluted with EtOAc, washed with aqueous saturated $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by size-exclusion chromatography and silica column chromatography afforded the desired disaccharides.

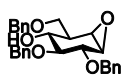
General procedure C: HF/pyridine-mediated removal of TBS-protected disaccharides 17, **31** and **32**

A solution of TBS-protected disacharride (1 eq) was dissolved in HF.pyridine/pyridine/THF (4/4/9, v/v/v, 0.17 M) at 0 °C and the reaction mixture was stirred at 0 °C for 18 h before being quenched with solid $NaHCO_3$, extracted with EtOAc, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica column chromatography yielded the deprotected target compounds.



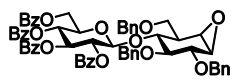
2,3,4,6-tetra-*O*-benzoyl-1-*O*-trichloroacetimidoyl-α-D-glucopyranoside 5

Procedure A with thioglucoside 4.[23] Silica column chromatography (petroleum ether/EtOAc, 80:20→76:24). Yield (2.60 g, 4.36 mmol, 87%).[1]H NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H, NH imidate), 8.05 (dd, *J* = 1.2, 6.8 Hz, 2H, H_{Ar}Bz), 7.98-7.95 (m, 4H, H_{Ar}Bz), 7.88 (dd, *J* = 1.2, 6.8 Hz, 2H, H_{Ar}Bz), 7.55-7.51 (m, 1H, H_{Ar}Bz), 7.49-7.46 (m, 2H, H_{Ar}Bz), 7.42-7.38 (m, 3H, H_{Ar}Bz), 7.34 (dt, *J* = 1.2, 6.4 Hz, 4H, H_{Ar}Bz), 7.28-7.24 (m, 2H, H_{Ar}Bz), 6.88 (d, *J* = 2.8 Hz 1H, H-1α), 6.31 (t, *J* = 8.0 Hz, 1H, H-3), 5.86 (t, *J* = 8.0 Hz, 1H, H-4), 5.66 (dd, *J* = 3.2, 8.0 Hz, 1H, H-2), 4.70-4.63 (m, 2H, H-5, H-6), 4.53 (dd, *J* = 4.0, 10.0 Hz, 1H, H-6). [13]C NMR (100 MHz, $CDCl_3$): δ 165.9, 165.5, 165.3, 165.1, 160.4, 133.4, 133.2, 133.0, 129.9, 129.8, 129.7, 129.6, 129.5, 128.7, 128.5, 128.4, 128.3, 128.3, 128.2, 93.0, 90.6, 70.6, 79.6, 70.1, 68.6, 62.4. HRMS: found 740.0858 $[M+H]^+$, calculated for $[C_{36}H_{29}Cl_3NO_{10}]$ 740.0852.



2,3,8-tri-*O*-benzyl-cyclophellitol 6

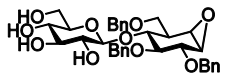
A solution of 2,3-di-*O*-benzyl-cyclophellitol **7** (see Chapter 3, 2.32 g, 6.52 mmol) and benzyl bromide (0.19 mmol, 1.63 mmol) in DMF (35 mL) was cooled to 0 °C before sodium hydride (60% dispersion in mineral oil, 143 mg, 3.57 mmol) was added. The reaction mixture was stirred at ambient temperature for 18 h. The mixture was cooled to 0 °C and subsequently quenched with MeOH, diluted with H_2O, extracted with Et_2O, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/EtOAc, 90:10→85:15) afforded the title compound **6** (422 mg, 0.95 mmol, 58%), 2,3,4-tri-*O*-benzyl-cyclophellitol (petroleum ether/EtOAc, 75:25→70:30, 54 mg, 0.12 mmol, 7.5%) and the starting cyclitol **7** (petroleum ether/EtOAc, 60:40→20:80, 1.61 g, 4.52 mmol). [1]H NMR (400 MHz, $CDCl_3$): δ 7.36-7.21 (m, 15H, H_{Ar}Bn), 4.89 (d, *J* = 11.6 Hz, 1H, CH_2Bn), 4.75 (d, *J* = 11.6 Hz, 1H, CH_2Bn), 4.68 (d, *J* = 11.6 Hz, 1H, CH_2Bn), 4.62 (d, *J* = 11.2 Hz, 1H, CH_2Bn), 4.56-4.50 (m, 2H, CH_2Bn), 3.87 (d, *J* = 4.8, 9.2 Hz, 1H, H-8), 3.78 (d, *J* = 7.2 Hz, 1H, H-2), 3.39-3.38 (m, 1H, H-6), 3.35-3.28 (m, 2H, H-3, H-4), 3.15 (d, *J* = 3.6 Hz, 1H, H-1), 2.83 (br s, 1H, OH), 2.26-2.21 (m, 1H, H-5). [13]C NMR (100 MHz, $CDCl_3$): δ 138.2, 138.0, 137.4, 128.4, 128.3, 128.2, 128.2, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 126.7, 83.6, 79.1, 74.7, 73.2, 72.4, 69.7, 67.1, 54.5, 53.7, 54.6, 53.7, 41.9. HRMS: found 447.2168 $[M+H]^+$, calculated for $[C_{28}H_{31}O_5]$ 447.2166.



2,3,8-tri-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-cellobiosyl cyclophellitol 9

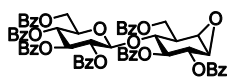
Perbenzoylated thioglucopyranoside **4**[23] (207 mg, 0.3 mmol) and cyclophellitol derivative **6** (89 mg, 0.2 mmol) were coevaporated thrice with anhydrous toluene and was then dissolved in DCM (1.0 mL). After the addition of activated 3Å molecular sieves, the solution

was stirred for 30 min at ambient temperature before *N*-iodosuccinimide (59 mg, 0.26 mmol) was added. The mixture was cooled to -40 °C and a catalytic amount of triflic acid (4.4 µL, 0.05 mmol) was added. The reaction mixture was stirred from -40 °C to 0 °C over 2 h until TLC analysis showed full consumption of **6**. Anhydrous triethylamine (0.2 mL) was added and the solution was further diluted with saturated aqueous $NaHCO_3$, followed by extraction with EtOAc, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica column chromatography (petroleum ether/EtOAc, 70:30→50:50) and size-exclusion chromatography (MeOH/dichloromethane) yielded **9** (187 mg, 0.18 mmol, 91%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (dd, *J* = 1.6, 7.2 Hz, 4H, H_{Ar}Bz), 7.71 (d, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 7.80 (d, *J* = 7.6 Hz, 2H, H_{Ar}Bz), 7.52 (m, 3H, H_{Ar}Bz), 7.42-7.29 (m, 12H, H_{Ar}Bz, H_{Ar}Bn), 7.27-7.17 (m, 12H, H_{Ar}Bz, H_{Ar}Bn), 5.75 (t, *J* = 9.6 Hz, 1H, H-3'), 5.57 (t, *J* = 9.6 Hz, H-4'), 5.50 (dd, *J* = 8.0, 9.6 Hz, 1H, H-2'), 5.03 (d, *J* = 11.6 Hz, 1H, CH_2Bn), 4.94 (d, *J* = 8.0 Hz, 1H, H-1'β), 4.67 (d, *J* = 11.6 Hz, 1H, CH_2Bn), 4.63 (d, *J* = 11.6 Hz, 1H, CH_2Bn), 4.38 (d, *J* = 12.0 Hz, 1H, CH_2Bn), 4.33 (dd, *J* = 3.6, 12.4 Hz, 1H, H-6'), 4.24 (d, *J* = 12.0 Hz, 1H, CH_2Bn), 4.13 (dd, *J* = 4.8, 12.0 Hz, 1H, H-6'), 3.83 (d, *J* = 7.6 Hz, 1H, H-2), 3.77-3.71 (m, 2H, H-4, H-5'), 3.63 (dd, *J* = 3.2, 8.8 Hz, 1H, H-8), 3.57-3.47 (m, 2H, H-8, H-3), 3.32 (d, *J* = 3.6 Hz, 1H, H-6), 3.12 (d, *J* = 3.6 Hz, 1H, H-1), 2.23-2.19 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 165.8, 165.0, 165.0, 139.2, 138.1, 137.6, 133.4, 133.3, 133.1, 132.9, 129.7, 129.7, 128.8, 128.8, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 127.8, 127.8, 127.7, 127.6, 127.1, 126.9, 101.4, 82.9, 79.3, 76.3, 74.4, 73.2, 73.0, 72.7, 72.0, 69.7, 68.2, 62.9, 55.6, 53.1, 41.9. HRMS: found 1047.3565 $[M+Na]^+$, calculated for $[C_{62}H_{56}O_{14}Na]$ 1047.3562.



2,3,8-tri-*O*-benzyl-4-O-(β-D-glucopyranosyl)-cellobiosyl cyclophellitol 10

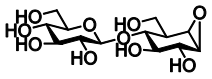
A catalytic amount of NaOMe was added to a solution of **9** (187 mg, 0.18 mmol) in MeOH (1.0 mL) and stirred at ambient temperature for 18 h. After TLC analysis showed full consumption of the starting material, the reaction mixture was neutralized with Amberlite IR-120 H^+, filtered and concentrated under reduced pressure. Purification by silica column chromatography (dichloromethane/MeOH, 85:15→80:20) yielded **10** (79 mg, 0.13 mmol, 72%) as colorless oil. ^{1}H NMR (400 MHz, MeOD/$CDCl_3$): δ 7.44-7.28 (m, 15H, H_{Ar}Bn), 5.01 (d, *J* = 10.4 Hz, 1H, CH_2Bn), 4.84 (d, *J* = 12.0 Hz, 1H, CH_2Bn), 4.68 (t, *J* = 11.6 Hz, 2H, CH_2Bn), 4.64 (d, *J* = 10.0 Hz, 1H, CH_2Bn), 4.58 (d, *J* = 12.0 Hz, 1H, CH_2Bn), 4.35 (d, *J* = 7.6 Hz, 1H, H-1), 4.00-3.92 (m, 2H, H-8), 3.83-3.74 (m, 3H, H-2, H-4, H-6'), 3.56 (dd, *J* = 8.0, 10.0 Hz 1H, H-3), 3.50 (dd, *J* = 6.0, 12.8 Hz, 1H, H-6'), 3.46-3.44 (m, 1H, H-6), 3.30-3.20 (m, 4H, H-1, H-2', H-3', H-5'), 3.15-3.12 (m, 1H, H-4'), 2.44-2.39 (m, 1H, H-5). ^{13}C NMR (100 MHz, MeOD/$CDCl_3$): δ 139.8, 139.6, 139.3, 129.8, 129.5, 129.4, 129.2, 129.1, 128.9, 128.9, 128.7, 128.6, 104.4, 84.7, 80.2, 78.5, 78.0, 77.1, 76.0, 75.1, 72.1, 69.6, 57.3, 53.8, 44.2. HRMS: found 631.2511 $[M+Na]^+$, calculated for $[C_{34}H_{40}O_{10}Na]$ 631.2513.



2,3,8-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl) cellobiosyl cyclophellitol 11

A solution of **9** (174 mg, 0.17 mmol), sodium periodate (373 mg, 1.53 mmol) and ruthenium trichloride (19 mg, 0.09 mmol) in CCl_4 (7.8 mL), ACN (7.8 mL) and H_2O (11 mL) was stirred at ambient temperature for 18 h. The reaction was quenched with 10% sodium thiosulphate, subsequently extracted with EtOAc, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was again subjected to the same reaction condition and after

stirring for 18 h at ambient temperature, 10% aqueous sodium thiosulphate was added. The aqueous solution was extracted with EtOAc, dried over $MgSO_4$, filtered and reduced *in vacuo*. Purification by HPLC (water/acetonitrile) afforded **11** (16 mg, 0.015 mmol, 9.0%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 7.92-7.85 (m, 7H, H_{Ar}Bz), 7.72 (d, *J* = 7.6 Hz, 2H, H_{Ar}Bz), 7.66 (d, *J* = 7.6 Hz, 2H, H_{Ar}Bz), 7.84-7.46 (m, 3H, H_{Ar}Bz), 7.40-7.32 (m, 8H, H_{Ar}Bz), 7.24-7.14 (m, 11H, H_{Ar}Bz), 5.72 (t, *J* = 9.6 Hz, 1H, H-3'), 5.61 (t, *J* = 8.4 Hz, 1H, H-3), 5.41-5.38 (m, 2H, H-2, H-2'), 5.21 (t, *J* = 9.6 Hz, 1H, H-4'), 4.87 (d, *J* = 8.0 Hz, H-1'), 4.60-4.52 (m, 2H, H-7), 4.07-4.02 (m, 2H, H-4, H-6'), 3.85-3.79 (m, 2H, H-5', H-6'), 3.37 (s, 1H, H-6), 3.20 (d, *J* = 3.6 Hz, 1H, H-1), 2.62-2.54 (m, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1, 165.8, 165.6, 165.5, 165.4, 165.1, 164.8, 133.5, 133.4, 133.2, 133.2, 130.9, 129.9, 129.8, 129.7, 129.6, 129.6, 129.5, 129.0, 128.7, 128.6, 128.6, 128.3, 128.3, 128.2, 127.9, 101.7, 74.2, 72.9, 72.4, 72.3, 72.1, 72.1, 69.7, 63.1, 62.0, 55.3, 52.8, 38.7. HRMS: found 1089.2948 $[M+Na]^+$, calculated for $[C_{62}H_{56}O_{17}Na]$ 1089.2946.

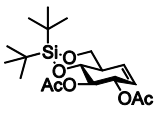


Cellobiosyl cyclophellitol 3

A solution of **11** (16 mg, 0.015 mmol) in MeOH (2 mL) was treated with 50 μL of 0.5 M NaOMe solution. The reaction mixture was stirred at ambient temperature for 18 h, before being neutralized with Amberlite IR-120 H^+, filtered and concentrated under reduced pressure. Purification by silica column chromatography (EtOAc/MeOH/H_2O, 70:20:10) afforded the title compound **3** (0.30 mg, 0.89 μmol, 59%) as a solid. ^{1}H NMR (600 MHz, MeOD): δ 4.44 (d, *J* = 7.8 Hz, 1H, H-1'), 4.05 (dd, *J* = 3.6, 7.2 Hz, 1H, H-8), 3.90 (dd, *J* = 6.6, 11.4 Hz, 1H, H-8), 3.86 (dd, *J* = 1.8, 12.0 Hz, 1H, H-6'), 3.83 (d, *J* = 7.2 Hz, 1H, H-2), 3.69 (dd, *J* = 7.2, 12.6 Hz, 1H, H-6'), 3.53 (d, *J* = 3.6 Hz, 1H, H-6), 3.52-3.45 (m, 3H, H-4, H-3', H-5'), 3.44-3.89 (m, 2H, H-3, H-4'), 3.30 (dd, *J* = 8.4, 9.6 Hz, 1H, H-2'), 3.21 (d, *J* = 4.2 Hz, 1H, H-1), 2.30-2.27 (m, 1H, H-5). ^{13}C NMR (150 MHz, MeOD): δ 104.0, 78.6, 76.9, 76.4, 75.7, 74.3, 71.8, 70.2, 61.3, 60.8, 57.5, 56.1, 43.7. HRMS: found 361.1103 $[M+Na]^+$, calculated for $[C_{13}H_{22}O_{10}Na]$ 361.1105.

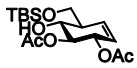
D-gluco-cyclohexene derivative 14

Ammonia (20 mL) was condensed at -60 °C. Lithium (200 mg) was added and the mixture was stirred until lithium was completely dissolved. To this solution was added a solution of cyclitol **13** (see Chapter 3, 1.36 g, 4 mmol) in THF (30 mL). The reaction mixture was stirred for 30 min at -60 °C and subsequently quenched with MilliQ-H_2O. The solution was allowed to warm to room temperature and stirred until all ammonia had evolved. Next, the solution was concentrated *in vacuo*, re-dissolved in MilliQ-H_2O and neutralized with Amberlite IR-120 H^+, filtered and concentrated under reduced pressure. Purification by silica column chromatography (EtOAc/MeOH, 84:16→80:20) afforded tetrol **14** (497 mg, 3.11 mmol, 78%) as an amorphous solid. ^{1}H NMR (400 MHz, MeOD): δ 5.63-5.48 (m, 2H, H-1, H-6), 4.02 (br s, 1H, H-3), 3.77 (dd, *J* = 5.6, 14.4 Hz, 1H, H-7), 3.59 (dd, *J* = 12.0, 14.0 Hz, 1H, H-7), 3.47-3.38 (m, 2H, H-2, H-4), 2.26 (br s, 1H, H-5). ^{13}C NMR (100 MHz, MeOD): δ 131.0, 128.6, 78.8, 73.6, 71.9, 63.4, 47.7. HRMS: found 183.0628 $[M+Na]^+$, calculated for $[C_7H_{12}O_4Na]$ 183.0629.



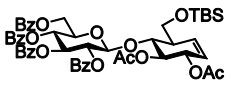
4,7-*O*-di-tertbutylsilanediyl, 2,3-di-*O*-acyl-D-glucocyclohexene 15

Di-tertbutylsilyl bis(trifluoromethanesulfonate) (1.28 mL, 3.9 mmol) and pyridine (2.52 mL, 31.2 mmol) was added to a solution of tetrol **14** (500 mg, 3.12 mmol) in DMF (28 mL) at -40 °C. After stirring at -40 °C for 1 h, the reaction mixture was diluted with EtOAc, washed with aqueous saturated $NaHCO_3$, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was taken up in a solution of pyridine (9 mL) and acetic anhydride (6 mL). The reaction was stirred at ambient temperature for 2 h before being subsequently diluted with Et_2O, washed with H_2O, dried over $MgSO_4$, concentrated *in vacuo* and co-evaporated thrice with toluene. Purification by silica column chromatography (petroleum ether/Et_2O, 92:8→88:12) afforded **15** (1.11 g, 2.87 mmol, 92%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.60 (t, *J* = 2.8, 10.0 Hz, 1H, H-6), 5.55-5.53 (m, 1H, H-2), 5.38 (dt, *J* = 1.6 Hz, 10.0 Hz, 1H, H-1), 5.28 (dd, *J* = 8.0, 10.4 Hz, 1H, H-3), 4.14 (dd, *J* = 4.4, 10.4 Hz, 1H, H-7), 3.96 (t, *J* = 10.0 Hz, 1H, H-4), 3.78 (dd, *J* = 10.8, 12.0 Hz, 1H, H-7), 2.75-2.71 (m, 1H, H-5), 2.08 (s, 3H, CH_3OAc), 2.07 (s, 3H, CH_3OAc), 1.02 (s, 9H, CH_3tBuSi), 0.98 (s, 9H, CH_3tBuSi). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.3, 126.8, 126.4, 75.0, 74.8, 72.7, 67.8, 43.1, 27.4, 27.2, 26.8, 22.7, 21.0, 20.7, 19.8. HRMS: found 385.2043 $[M+H]^+$, calculated for $[C_{19}H_{33}O_6Si]$ 385.2041.



7-*O*-tertbutylsilyl-2,3-di-*O*-acyl-D-glucocyclohexene 12

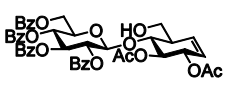
A solution of **15** (1.11 g, 2.87 mmol) in HF.pyridine/pyridine/THF (13 mL; 1/2/10; v/v/v) was stirred at 0 °C for 2 h. The reaction mixture was then quenched with solid $NaHCO_3$, diluted with brine and extracted several times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure before the crude product was dissolved in DMF (14.5 mL). After the addition of TBSCl (476 mg, 3.16 mmol) and imidazole (489 mg, 7.18 mmol), the reaction mixture was stirred at ambient temperature for 2 h before being consecutively diluted with Et_2O, washed with H_2O, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/Et_2O, 70:30) yielded **12** (797 mg, 2.22 mol, 77%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.53-5.48 (m, 2H, H-1, H-6), 5.44 (dd, *J* = 3.2, 8.0 Hz, 1H, H-4), 5.16 (dd, *J* = 8.4, 10.4 Hz, 1H, H-3), 3.81-3.75 (m, 2H, H-2, H-7), 3.66 (t, *J* = 7.6 Hz, 1H, H-7), 2.52-2.48 (m, 1H, H-5), 2.07 (s, 3H, CH_3OAc), 2.00 (s, 3H, CH_3OAc), 0.85 (s, 9H, CH_3tBuSi), 0.03 (s, 6H, CH_3OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.0, 170.6, 128.6, 125.7, 75.2, 72.3, 71.1, 65.1, 45.6, 25.7, 20.9, 18.1, -5.67. HRMS: found 359.1882 $[M+H]^+$, calculated for $[C_{17}H_{31}O_6Si]$ 359.1884.



7-*O*-tertbutylsilyl-2,3-di-*O*-acyl-4-O-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-D-glucocyclohexene 16

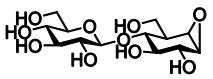
Procedure B with donor **5** (333 mg, 0.45 mmol) and acceptor **12** (108 mg, 0.3 mmol). Silica column chromatography (petroleum ether/Et_2O, 72:28→68:32). Yield (169 mg, 0.18 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, *J* = 6.8 Hz, $H_{Ar}Bz$), 7.95-7.89 (m, 4H, $H_{Ar}Bz$), 7.80 (d, *J* = 7.2 Hz, 2H, $H_{Ar}Bz$), 7.57-7.44 (m, 4H, $H_{Ar}Bz$), 7.43-7.33 (m, 6H, $H_{Ar}Bz$), 7.28-7.25 (m, 2H, $H_{Ar}Bz$), 5.84 (t, *J* = 9.6 Hz, 1H, H-3'), 5.64 (t, *J* = 9.6 Hz,1H, H-4'), 5.55-5.49 (m, 2H, H-6, H-2'), 5.46-5.43 (m, 2H, H-1, H-2), 5.29 (dd, *J* = 8.0, 10.8 Hz, 1H, H-3), 4.95 (d, *J* = 8.0 Hz, 1H, H-1'), 4.68 (dd, *J* = 2.8, 12.0 Hz, 1H, H-6'), 4.46 (dd, *J* = 6.0, 12.4 Hz, 1H, H-6'), 4.08-4.03 (m, 2H, H-4, H-5'), 3.82 (dd, *J* =

3.6, 10.4 Hz, 1H, H-7), 3.43 (dd, *J* = 2.0, 10.4 Hz, 1H, H-7), 2.36-2.34 (m, 1H, H-5), 2.02 (s, 6H, CH_3OAc), 0.90 (s, 9H, CH_3tBuSi), 0.15 (s, 3H, CH_3Si), 0.02 (s, 3H, CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 166.0, 165.8, 165.2, 164.5, 133.4, 133.3, 133.2, 133.2 131.0, 130.9, 129.8, 129.7, 129.5, 129.1, 129.0, 128.8, 128.7, 128.7, 128.5, 128.4, 128.3, 125.6, 101.7, 76.3, 73.2, 72.8, 72.5, 72.2, 72.2, 69.5, 63.0, 60.6, 45.3, 25.8, 21.0, 18.1, -5.14, -5.42. HRMS: found 959.3271 $[M+Na]^+$, calculated for $[C_{51}H_{56}O_{15}SiNa]$ 959.3281.



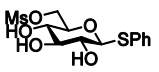
2,3-di-*O*-acyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-D-glucocyclohexene 17

Procedure C with **16** (169 mg, 0.18 mmol). Silica column chromatography (petroleum ether/acetone, 70:30→60:40). Yield (113 mg, 0.14 mmol, 78%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.07 (d, *J* = 7.2 Hz, 2H, $H_{Ar}Bz$), 7.96 (d, *J* = 7.2 Hz, 2H, $H_{Ar}Bz$), 7.88 (d, *J* = 7.6 Hz, 2H, $H_{Ar}Bz$), 7.80 (d, *J* = 7.6 Hz, 2H, $H_{Ar}Bz$), 7.59-7.56 (m, 1H, $H_{Ar}Bz$), 7.53-7.44 (m, 4H, $H_{Ar}Bz$), 7.43-7.32 (m, 4H, $H_{Ar}Bz$), 7.28-7.24 (m, 3H, $H_{Ar}Bz$), 5.94 (t, *J* = 9.6 Hz, 1H, H-3'), 5.69 (t, *J* = 9.6 Hz, 1H, H-4'), 5.60-5.50 (m, 4H, H-1, H-6, H-2, H-2'), 5.31 (dd, *J* = 8.0, 10.4 Hz, 1H, H-3), 5.01 (d, *J* = 8.0 Hz, 1H, H-1'), 4.69 (dd, J = 2.4, 12.0 Hz, 1H, H-6'), 4.45 (dd, *J* = 5.2, 12.0 Hz, 1H, H-6'), 4.20-4.15 (m, 1H, H-5'), 4.08 (t, *J* = 9.6 Hz, 1H, H-4), 3.79 (dd, *J* = 4.0, 10.8 Hz, 1H, H-7), 3.58 (dd, *J* = 2.0, 10.8 Hz, 1H, H-7), 2.43-2.42 (m, 1H, H-5), 2.02 (s, 3H, CH_3OAc), 1.98 (s, 3H, CH_3OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 166.1, 165.7, 165.1, 164.6, 133.4, 133.3, 133.3, 133.2, 129.7, 129.6, 129.3, 128.9, 128.5, 128.4, 128.3, 128.2, 126.8, 101.8, 76.7, 73.0, 72.6, 72.3, 72.1, 72.1, 69.4, 62.9, 60.7, 45.5, 20.9, 20.9. HRMS: found 845.2416 $[M+H]^+$, calculated for $[C_{45}H_{43}O_{15}]$ 845.2416.



Cellobiosyl cyclophellitol 3

A solution of **17** (82 mg, 0.1 mmol) and *m*CPBA (70-77%, 74 mg, 0.3 mmol) in DCE (0.5 mL), 1 M aqueous Na_2HPO_4 (0.25 mL) and 1 M aqueous NaH_2PO_4 (0.25 mL) was heated to 50 °C. The reaction mixture was stirred at 50 °C for 18 h, before additional *m*CPBA (70-77%, 74 mg, 0.3 mmol) was added. The reaction was again stirred at 50 °C for 18 h before being diluted with H_2O, extracted with EtOAc, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/EtOAc, 56:44→54:46) afforded the semi-pure protected cellobiosyl cyclophellitol **18** (24 mg, 0.028 mmol, 28%), which was directly subjected to NaOMe deprotection conditions. A solution of **18** (24 mg, 0.028 mmol) in MeOH (2 mL) was treated with 60 µL of 0.5 M NaOMe solution. The reaction mixture was stirred at ambient temperature for 18 h before being neutralized with Amberlite IR-120 H^+, filtered and concentrated under reduced pressure. Purification by silica column chromatography (EtOAc/MeOH/H_2O, 70:18:2→70:15:5) afforded the title compound **3** (2.8 mg, 8.3 µmol, 30%) as a solid. ^1H NMR (600 MHz, MeOD): δ 4.44 (d, *J* = 7.8 Hz, 1H, H-1'), 4.05 (dd, *J* = 3.6, 7.2 Hz, 1H, H-8), 3.90 (dd, *J* = 6.6, 11.4 Hz, 1H, H-8), 3.86 (dd, *J* = 1.8, 12.0 Hz, 1H, H-6'), 3.83 (d, *J* = 7.2 Hz, 1H, H-2), 3.69 (dd, *J* = 7.2, 12.6 Hz, 1H, H-6'), 3.53 (d, *J* = 3.6 Hz, 1H, H-6), 3.52-3.45 (m, 3H, H-4, H-3', H-5'), 3.44-3.89 (m, 2H, H-3, H-4'), 3.30 (dd, *J* = 8.4, 9.6 Hz, 1H, H-2'), 3.21 (d, *J* = 4.2 Hz, 1H, H-1), 2.30-2.27 (m, 1H, H-5). ^{13}C NMR (150 MHz, MeOD): δ 104.0, 78.6, 76.9, 76.4, 75.7, 74.3, 71.8, 70.2, 61.3, 60.8, 57.5, 56.1, 43.7. HRMS: found 361.1103 $[M+Na]^+$, calculated for $[C_{13}H_{22}O_{10}Na]$ 361.1105.

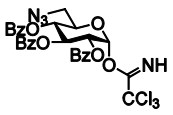


Phenyl-6-mesyl-6-deoxy-β-D-thioglucopyranoside 24

Mesylchloride (0.32 mL, 11 mmol) was added to a solution of thioglucoside **23**[25] (2.72 g, 10 mmol) in pyridine (25 mL) at 0 °C. After stirring at 0 °C for 15 min, the reaction was carefully quenched with 1 M HCl and diluted with EtOAc. The mixture was washed with brine and the organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by silica column chromatography (EtOAc/MeOH, 98:2→96:4) yielded **24** (2.55 g, 7.29 mmol, 73%) as an oil. ^{1}H NMR (400 MHz, MeOD): δ 7.56-7.53 (m, 2H, H_{Ar}SPh), 7.33-7.24 (m, 3H, H_{Ar}SPh), 4.66 (d, *J* = 9.6 Hz, 1H, H-1), 4.52 (dd, *J* = 2.0, 11.6 Hz, 1H, H-6), 4.35 (dd, *J* = 6.4, 11.6 Hz, 1H, H-6), 3.59-3.54 (m, 1H, H-5), 4.40 (t, *J* = 9.2 Hz, 1H, H-3), 3.31-3.29 (m, 1H, H-4, below MeOD solvent), 3.23 (dd, *J* = 7.6, 9.6 Hz, 1H, H-2), 3.04 (s, 3H, CH_3mesyl). ^{13}C NMR (100 MHz, $CDCl_3$): δ 134.6, 133.0, 130.0, 128.5, 89.0, 79.4, 78.9, 73.6, 70.8, 70.8, 37.5. HRMS: found 351.0569 $[M+H]^+$, calculated for $[C_{13}H_{19}O_7S_2]$ 351.0567.

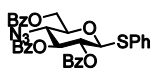
Phenyl-2,3,4-tri-*O*-benzoyl-6-azido-6-deoxy-β-D-thioglucopyranoside 25

Mesylate **24** (2.55 g, 7.29 mmol) was taken up in DMF (50 mL). Sodium azide (2.60 g, 40 mmol) was added to the solution and stirred at 120 °C for 19 h before being diluted with brine, extracted with Et_2O, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was redissolved in pyridine (40 mL) and benzoyl chloride (3.39 mL, 29 mmol) was added. The reaction mixture was stirred at ambient temperature for 2 h and subsequently diluted with water, extracted with EtOAc, dried over $MgSO_4$, filtered and concentrated under reduced pressure. Purification by silica column chromatography (petroleum ether/EtOAc, 92:8→88:12) yielded **25** (3.55 g, 5.83 mmol, 80%) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 7.90 (d, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 7.79 (d, *J* = 7.6 Hz, 2H, H_{Ar}Bz), 7.56-7.47 (m, 5H, H_{Ar}Bz, H_{Ar}SPh), 7.42-7.31 (m, 9H, H_{Ar}Bz, H_{Ar}SPh), 7.27-7.23 (m, 4H, H_{Ar}Bz), 5.89 (t, *J* = 9.6 Hz, 1H, H-3), 5.46 (dt, *J* = 4.0, 9.6 Hz, 2H, H-2, H-4), 5.05 (d, *J* = 10.0 Hz, 1H, H-1), 3.99-3.95 (m, 1H, H-5), 3.52 (dd, *J* = 6.8, 13.6 Hz, 1H, H-6), 3.43 (dd, *J* = 2.4, 13.2 Hz, 1H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7, 165.2, 165.0, 133.8, 133.6, 133.3, 133.2, 131.0, 130.1, 129.8, 129.7, 129.1, 129.0, 128.7, 128.6, 128.4, 128.3, 86.2, 77.6, 74.0, 70.3, 69.9, 51.4. HRMS: found 610.1644 $[M+H]^+$, calculated for $[C_{33}H_{28}N_3O_7S]$ 610.1643.



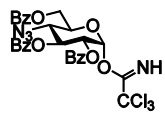
2,3,4-tri-*O*-benzoyl-6-azido-6-deoxy-1-*O*-trichloroacetimidoyl-α-D-gluco-pyranoside 21

Procedure A. Silica column chromatography (petroleum ether/EtOAc, 88:12→84:16). Yield (2.30 g, 3.48 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92 (s, 1H, NH imidate), 7.98 (d, *J* = 6.4 Hz, 4H, H_{Ar}Bz), 7.88 (d, *J* = 6.0 Hz, 2H, H_{Ar}Bz), 7.45-7.40 (m, 2H, H_{Ar}Bz), 7.33-7.28 (m, 5H, H_{Ar}Bz), 7.11 (t, *J* = 2.4 Hz, 2H, H_{Ar}Bz), 6.95 (d, *J* = 2.8 Hz, 1H, H-1), 6.34 (t, *J* = 8.0 Hz, 1H, H-3), 5.78 (t, *J* = 8.0 Hz, 1H, H-4), 5.72 (dd, *J* = 2.8, 8.4 Hz, 1H, H-2), 4.57-4.53 (m, 1H, H-5), 3.56-3.50 (m, 2H, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.3, 165.2, 164.9, 164.9, 133.4, 133.2, 133.0, 130.2, 129.7, 129.5, 129.4, 129.3, 128.9, 128.8, 128.4, 128.2, 128.2, 128.1, 128.0, 92.7, 90.4, 71.6, 70.3, 69.7, 69.0, 50.4. HRMS: found 661.0661 $[M+H]^+$, calculated for $[C_{29}H_{24}Cl_3N_4O_8]$ 661.0654.



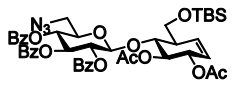
Phenyl-2,3,6-tri-*O*-benzoyl-4-azido-4-deoxy-β-D-thioglucopyranoside 28

Thiogalactopyranoside[26] (2.94 g, 4,82 mmol) was dissolved in a mixture of DCM (45 mL) and pyridine (5 mL) and the solution was cooled to 0 °C. After dropswise addition of Tf_2O (0.89 mL, 5.29 mmol), the reaction mixture was stirred at 0 °C until TLC analysis showed full consumption of the starting material (2 h). The mixture was then diluted with cold DCM and the organic layer was washed with water, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The resulting crude triflate was taken up in DMF (40 mL) and sodium azide (1.54 g, 24 mmol) was added. The reaction mixture was stirred at ambient temperature for 18 h before being diluted with Et_2O, washed with water, dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by silica column chromatography (petroleum ether/EtOAc, 92:8→90:10) afforded **28** (2.38 g, 3.90 mmol, 81%) as a white amorphous solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13-8.08 (m, 2H, H_{Ar}Bz), 7.97-7.93 (m, 4H, H_{Ar}Bz), 7.65-7.58 (m, 1H, H_{Ar}Bz), 7.54-7.45 (m, 7H, H_{Ar}Bz), 7.40-7.34 (m, 4H, H_{Ar}Bz, H_{Ar}SPh), 7.26-7.23 (m, 1H, H_{Ar}Bz), 7.16-7.13 (m, 2H, H_{Ar}SPh), 5.72 (t, *J* = 9.2 Hz, 1H, H-3), 5.38 (t, *J* = 9.6 Hz, 1H, H-2), 4.97 (d, *J* = 10.0 Hz, H-1), 4.82 (dd, *J* = 2.0, 12.0 Hz, 1H, H-6), 4.60 (dd, *J* = 4.8, 12.0 Hz, 1H, H-6), 3.90-3.81 (m, 2H, H-4, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 165.6, 165.1, 133.7, 133.5, 133.4, 133.3, 131.4, 130.1, 129.8, 129.8, 129.5, 129.0, 128.8, 128.6, 128.5, 128.4, 128.4, 128.3, 86.0, 76.4, 75.0, 70.2, 63.4, 60.7. HRMS: found 632.1461 $[M+Na]^+$, calculated for $[C_{33}H_{27}O_7S]$ 632.1462.



2,3,6-tri-*O*-benzoyl-4-azido-4-deoxy-1-*O*-trichloroacetimidoyl-α-D-gluco-pyranoside 22

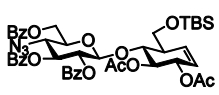
Procedure A. Silica column purification (petroleum ether/EtOAc, 88:12→84:16). Yield (898 mg, 1.36 mmol, 76%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.64 (s, 1H, NH imidate), 8.10 (dd, *J* = 1.2, 8.0 Hz, 2H, H_{Ar}Bz), 8.00 (dd, *J* = 1.2, 8.0 Hz, 2H, H_{Ar}Bz), 7.96 (dd, *J* = 1.2, 8.0 Hz, 2H, H_{Ar}Bz), 7.60-7.57 (m, 1H, H_{Ar}Bz), 7.51-7.44 (m, 4H, H_{Ar}Bz), 7.38-7.32 (m, 4H, H_{Ar}Bz), 6.14 (t, *J* = 10.0 Hz, 1H, H-3), 5.54 (dd, *J* = 3.5, 10.0 Hz, H-2), 4.75-4.67 (m, 2H, H6), 4.32 (dt, *J* = 3.5, 10.5 Hz, 1H, H-5), 4.07 (t, *J* = 10.0 Hz, 1H, H-4). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.9, 165.3, 165.3, 160.2, 133.5, 133.5, 133.2, 129.9, 129.8, 129.7, 129.6, 129.4, 128.6, 128.4, 128.4, 128.3, 128.3, 93.0, 70.9, 70.7, 70.4, 62.7, 60.2. HRMS: found 661.0661 $[M+H]^+$, calculated for $[C_{29}H_{24}Cl_3N_4O_8]$ 661.0654.



7-*O*-tertbutylsilyl-2,3-di-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-benzoyl-6-azido-6-deoxy-β-glucopyranosyl)-D-glucocyclohexene 29

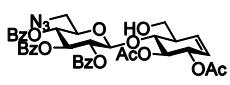
Procedure B. Silica column chromatography (petroleum ether/Et_2O, 60:40→56:44). Yield (221 mg, 0.26 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (dd, *J* = 3.6, 6.8 Hz, 4H, H_{Ar}Bz), 7.79 (d, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 7.54-7.49 (m, 2H, H_{Ar}Bz), 7.42-7.36 (m, 5H, H_{Ar}Bz), 7.26 (t, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 5.81 (t, *J* = 9.6 Hz, 1H, H-3'), 5.55-5.44 (m, 4H, H-1, H-2, H-6, H-4'), 5.40 (t, *J* = 10.0 Hz, 1H, H-2'), 5.30 (dd, *J* = 8.0, 10.4 Hz, 1H, H-3), 4.99 (d, *J* = 8.0 Hz, 1H, H-1'), 4.14 (t, *J* = 9.6 Hz, 1H, H-4), 3.90-3.85 (m, 1H, H-5'), 3.81 (dd, *J* = 4.0, 10.4 Hz, 1H, H-7), 3.57 (dd, *J* = 7.6, 13.6 Hz, 1H, H-6'), 3.50 (dd, *J* = 2.0, 10.4 Hz, 1H, H-7), 3.34 (dd, *J* = 2.0, 13.6 Hz, 1H, H-6'), 2.37-2.36 (m, 1H, H-5), 2.16 (s, 3H, CH_3OAc), 2.06 (s, 3H, CH_3OAc), 0.96 (s, 9H, CH_3tBuSi), 0.16 (s, 3H, CH_3Si), 0.06 (s, 3H, CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 165.6, 165.3, 164.4, 133.6, 133.2, 131.0, 129.9, 129.8, 129.6, 129.6, 129.5, 129.0, 128.5, 128.4, 128.4, 128.3, 128.2, 101.0, 75.1, 73.7,

72.9, 72.8, 72.5, 72.1, 70.2, 60.8, 51.0, 45.2, 25.8, 21.2, 21.0, 18.1, -5.20, -5.41. HRMS: found 880.3083 $[M+Na]^+$, calculated for $[C_{44}H_{51}N_3O_7SiNa]$ 880.3083.



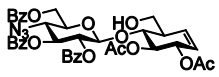
7-*O*-tertbutylsilylsilyl-2,3-di-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-benzoyl-4-azido-4-deoxy-β-D-glucopyranosyl)-D-glucocyclohexene 30

Procedure B. Silica column chromatography (petroleum ether/Et_2O, 60:40→56:44). Yield (435 mg, 0.51 mmol, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 7.92 (t, *J* = 8.8 Hz, 4H, H_{Ar}Bz), 7.64-7.60 (m, 1H, H_{Ar}Bz), 7.54-7.48 (m, 4H, H_{Ar}Bz), 7.37 (dt, *J* = 2.8, 8.0 Hz, 4H, H_{Ar}Bz), 5.61 (t, *J* = 10.0 Hz, 1H, H-3'), 5.50-5.47 (m, 1H, H-6), 5.43-5.39 (m, 3H, H-1, H-2, H-2'), 5.24 (dd, *J* = 8.0, 10.4 Hz, 1H, H-3), 4.82 (d, *J* = 8.0 Hz, 1H, H-1'), 4.77 (dd, *J* = 2.0, 12.4 Hz, 1H, H-6'), 4.59 (dd, *J* = 4.8, 12.0 Hz, 1H, H-6'), 4.00 (t, *J* = 9.6 Hz, 1H, H-4), 3.92 (t, *J* = 10.0 Hz, 1H, H-4'), 3.79 (dd, *J* = 3.6, 10.0 Hz, 1H, H-7), 3.68 (ddd, *J* = 2.0, 4.8, 7.2 Hz, 1H, H-5'), 3.39 (dd, *J* = 2.0, 10.4 Hz, 1H, H-7), 2.31-2.28 (m, 1H, H-5), 2.00 (s, 3H, CH_3OAc), 1.94 (s, 3H, CH_3OAc), 0.89 (s, 9H, CH_3tBuSi), 0.12 (s, 3H, CH_3Si), -0.01 (s, 3H, CH_3Si). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 165.9, 165.5, 164.6, 133.5, 133.3, 131.0, 129.0, 129.7, 129.6, 129.6, 129.3, 128.9, 128.6, 128.5, 128.4, 128.3, 128.2, 125.6, 101.6, 76.3, 73.9, 72.7, 72.5, 71.9, 63.3, 62.8, 60.8, 60.7, 60.5, 45.2, 26.9, 25.7, 20.9, 20.9, 18.1, -5.20, -5.42. HRMS: found 880.3073 $[M+Na]^+$, calculated for $[C_{44}H_{51}N_3O_{13}Si]$ 880.3083.



2,3-di-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-benzoyl-6-azido-6-deoxy-β-D-glucopyranosyl)-D-glucocyclohexene 31

Procedure C. Silica column chromatography (petroleum ether/acetone, 70:30→60:40). Yield (159 mg, 0.21 mmol, 82%).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96-7.90 (m, 4H, H_{Ar}Bz), 7.82-7.78 (m, 2H, H_{Ar}Bz), 7.54-7.49 (m, 2H, H_{Ar}Bz), 7.43-7.36 (m, 5H, H_{Ar}Bz), 7.28-7.24 (m, 3H, H_{Ar}Bz), 5.88 (t, *J* = 9.6 Hz, 1H, H-3'), 5.61 (dt, *J* = 2.4, 10.0 Hz, 1H, H1/6), 5.56-5.45 (m, 3H, H-2, H-4', H1/6), 5.44 (t, *J* = 9.6 Hz, 1H, H-2'), 5.32 (dd, *J* = 8.0, 10.4 Hz, 1H, H-3), 5.04 (d, *J* = 7.6 Hz, 1H, H-1'), 4.14 (t, *J* = 9.6 Hz, 1H, H-4), 4.01-3.96 (m, 1H, H-5'), 3.80 (dd, *J* = 3.6, 10.8 Hz, 1H, H-7), 3.63 (dd, *J* = 2.4, 10.8 Hz, 1H, H-7), 3.58 (dd, *J* = 7.2, 13.6, 1H, H-6'), 3.43 (dd, *J* = 2.4, 13.2 Hz, 1H, H-6'), 2.44-2.42 (m, 1H, H-5), 2.17 (s, 3H, CH_3OAc), 2.04 (s, 3H, CH_3OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.3, 165.7, 165.3, 164.6, 133.6, 133.3, 133.2, 130.0, 129.8, 129.6, 129.6, 128.9, 128.6, 128.6, 128.4, 128.4, 128.2, 126.9, 125.4, 101.1, 75.6, 73.4, 72.8, 72.7,72.5, 72.4, 72.2, 70.2, 60.8, 51.2, 40.4, 21.8, 21.0. HRMS: found 766.2216 $[M+Na]^+$, calculated for $[C_{38}H_{37}N_3O_{13}]$ 766.2219.



2,3-di-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-benzoyl-4-azido-4-deoxy-β-D-glucopyranosyl)-D-glucocyclohexene 32

Procedure B. Silica column chromatography (petroleum ether/acetone, 70:30→60:40). Yield (332 mg, 0.43 mmol, 85%).^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (d, *J* = 7.2 Hz, 2H, H_{Ar}Bz), 7.95 (dd, *J* = 1.6, 7.2 Hz, 4H, H_{Ar}Bz), 7.62 (t, *J* = 7.6 Hz, 1H, H_{Ar}Bz), 7.54-7.48 (m, 4H, H_{Ar}Bz), 7.38-7.33 (m, 4H, H_{Ar}Bz), 5.71 (t, *J* = 10.0 Hz, 1H, H-3'), 5.57-5.43 (m, 4H, H-1, H-6, H-2, H-2'), 5.28 (dd, *J* = 8.0, 10.4 Hz, 1H, H-3), 4.93 (d, *J* = 8.0 Hz, 1H, H-1'), 4.78 (dd, *J* = 1.6, 12.0 Hz, 1H, H-6'), 4.60 (dd, *J* = 4.8, 12.4 Hz, 1H, H-6'), 4.08 (t, *J* = 9.6 Hz, 1H, H-4), 3.98 (t, *J* = 10.0 Hz, 1H, H-4'), 3.85-3.78 (m, 2H, H-5', H-7), 3.56 (d, *J* = 9.2 Hz, 1H, H-7), 2.39-2.37 (m, 1H, H-5), 2.00 (s, 3H, CH_3OAc), 1.93 (s, 3H, CH_3OAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.3, 165.9, 165.5, 164.7, 133.4, 133.3, 129.9,

129.6, 129.5, 129.1, 128.7, 128.5, 128.4, 128.3, 126.4, 101.6, 76.6, 73.7, 72.5, 72.3, 72.2, 72.0, 63.3, 60.6, 60.3, 45.4, 20.8, 20.7. HRMS: found 766.2209 $[M+Na]^+$, calculated for $[C_{38}H_{37}N_3O_{13}]$ 766.2219.

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