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8

Synthesis of potential glycosyltransferase inhibitors based on carba-cyclophellitol\textsuperscript{1}

8.1 Introduction

The enzymatic formation of glycosidic linkages is achieved by glycosyltransferases (GTs). At the end of their lifetime, glycosidic linkages within oligosaccharides and glycoconjugates (combined termed glycans) are hydrolysed by glycosidases (GHs), and thus GTs and GHs combined control glycan levels and diversity within cells and organisms. Just as is the case with GH inhibitors, GT inhibitors are widely sought after with the aim to exert control over glycan metabolism. Indeed, numerous potent and selective GH inhibitors have been discovered in the past decades, and some are in clinical use.\textsuperscript{2,3} However, and despite the therapeutic potential of GT inhibitors, potent GT inhibitors are scarce.\textsuperscript{4,5}
Retaining galactosyltransferases (GalT) catalyse the transfer of galactose from UDP-α-D-galactose (donor) to an acceptor molecule such as proteins, (oligo)saccharides and lipids (Figure 8.1). A double displacement mechanism is suggested for galactosyltransferases of the GT6 family, including α3GalT (E.C. 2.4.1.87), α-1,3-N-acetylgalactosaminyltransferase (GTA, E.C. 2.4.1.40) and α-1,3-galactosyltransferase (GTB, E.C. 2.4.1.37). In this (putative) mechanism (Figure 8.1A), a covalent enzyme-donor intermediate is formed via an oxocarbenium ion-like transition state. An incoming nucleophile (the acceptor-OH to be glycosylated) would then release the enzyme-bound galactoside to yield the galactosylated product with concomitant reforming of the enzyme active site.

An alternative mechanism (Figure 8.1B), is suggested for lipopolysaccharyl-α-1,4-galactosyltransferase C (LgtC, GT8 family). In this $S_{N}i$-like mechanism, a front-side

![Figure 8.1](image_url)
attack of the acceptor on the anomeric position of the donor takes place, while the leaving group departs simultaneously from the same face resulting in the formation of the product with retention of configuration. This mechanism, implies an oxocarbenium ion-like transition state or a potentially short-lived oxocarbenium ion intermediate.

Finally, inverting galactosyltransferases, such as β-1,4-galactosyltransferase T1 (β4Gal-T1), employ a SN2 nucleophilic attack of the acceptor at the anomeric center (Figure 8.1). Indeed, the oxocarbenium-like transition state is formed as well in this mechanism, followed by expulsion of the UDP-moiety, resulting in product formation with inverted configuration at the anomeric center.8

The promising results of carba-cyclophellitol derivatives as described in Chapter 6 and 7 wherein the oxocarbenium ion-like transition state was mimicked by carbacyclophellitols, which employs a 4H3 conformation, served as a source of inspiration for the here-described research. Known α-galactosidase inhibitors, such as 1-deoxy-galactosnojirimycin (DGJ, with an IC50 value of 4.7 nM for lysosomal α-galactosidase A9), do not inhibit galactosyltransferases, such as α3GalT.10 On the other hand, uridine diphosphate (UDP, 1) is a relatively potent α3GalT inhibitor with an IC50 of 13 μM (Figure 8.2A).10 Based on these considerations, compound 3 (AB209) was designed as a potential inhibitor of galactosyltransferases (Figure 8.2B). Furthermore, compound 4 (AB209) was synthesized, based on 2 which is a moderately potent α3GalT inhibitor (IC50 value of 320 μM).11 In compound 2, an O-sulfamoyl amide bond was introduced to mimic the pyrophosphate moiety of the natural donor, UDP-galactose.

Figure 8.2 A) Galactosyltransferase inhibitors described in the literature,10,11 B) Potential galactosyltransferase inhibitors that are the subject of the research described in this chapter.
In the second part of this chapter the synthesis of glucose-configured isomer 5 is described (Figure 8.3). Glucopyranose-configured carba-cyclophellitol derivatives are described in Chapter 6 and were used as a starting point to obtain this potential inhibitor towards glucosyltransferases including glucosylceramide synthase.

![Figure 8.3 Structure of compound 5.](image)

**8.2 Results and discussion**

The synthesis of compound 3 was inspired by the phosphoramidite-based sugar nucleotide synthesis as described by Gold and co-workers.12 α-Galactopyranose-configurated carba-cyclophellitol 6 was synthesized as described in Chapter 6. Once a phosphodiester bond is formed however, all protective groups in the penultimate intermediate are ideally removed in a single (basic) step. Protection group manipulations were therefore executed to transform 6 into a suitable substrate (10) in which all alcohols barring the one intended for phosphorylation are masked as the acetate (Scheme 8.1). Briefly, the primary alcohol of compound 6 was reacted with 3,4-dihydro-2H-pyran (DHP) and catalytic para-toluenesulfonic acid followed by removal of the benzyl ethers in the resulting THP ether 7 under Birch reduction conditions to give compound 8. Global acetylation and acid catalyzed THP deprotection afforded key intermediate 10.

![Scheme 8.1 Synthesis of alcohol 10.](image)

**Reagents and conditions:** a) 3,4-dihydro-2H-pyran, pTsOH, CH₂Cl₂, rt, 30 min, 79%; b) Li, NH₃ (l), -60 °C, 1 h; c) Ac₂O, 4-dimethylaminopyridine, pyridine, rt, overnight, 88% over 2 steps; d) pTsOH, MeOH, rt, 1 h, 85%.
The obtained alcohol 10 was then coupled with uridine phosphoramidite 113 (Scheme 8.2). After 30 min, the phosphite moiety was oxidized with tert-butylhydroperoxide giving phosphate diester 13. In the following three-step-procedure all protecting groups were removed. Treatment with DBU resulted in the removal of the 2-cyanoethyl group and partial deacetylation was observed by LC/MS analysis. Next, aqueous ammonia was added to the reaction mixture. However, after stirring overnight the deacetylation process was not completed as determined by LC/MS analysis. Therefore, the reaction mixture was concentrated in vacuo and stirred overnight in Et$_3$N/H$_2$O/MeOH, providing the target compound. The crude product mixture was purified by size-exclusion chromatography followed by HPLC purification. Finally, treatment with Dionex sodium exchange resin provided compound 3 as the sodium salt in 22% yield.

**Scheme 8.2 Synthesis of compound 3.**

Reagents and conditions: a) DCI, acetonitrile, rt, 30 min; b) tBuOOH, rt, 30 min; c) DBU, rt, 1h; d) aqueous NH$_3$, rt, 24 h; e) i) Et$_3$N/H$_2$O/MeOH (1:1:3), rt, overnight; ii) size-exclusion, HPLC purification, Dionex sodium exchange, 22% over 6 steps.

In order to prepare compound 4, the UDP mimic had to be synthesized first (Scheme 8.3). The synthesis of O-sulfamoylamide 16 was accomplished according to the literature procedure. Iodine-catalyzed acetalization of uridine 14 and acetone gave 2',3'-isopropylidene-uridine 15. Installment of the sulfamoyl functionality to give 16 was accomplished by first generation of sulfamoyl chloride from chlorosulfamoyl isocyanate and formic acid followed by addition to the reaction mixture of compound
Chapter 8

15. Purification of $O$-sulfamoylamide 16 however turned out to be troublesome. An alternative strategy comprises the protection of the uracil-nitrogen as the Boc derivative (15 to 17). $O$-sulfamoylation and ensuing purification now proceeded uneventfully yielding compound 18, which was used in the following steps instead of 16.

Scheme 8.3 Synthesis of $O$-sulfamoyluridines.

Reagents and conditions: a) I$_2$, acetone, rt, overnight, 83%; b) formic acid, chlorosulfamoyl isocyanate, NaH, THF, overnight, 0 °C to rt, 79%; c) i) TBDMS-Cl, $N,N$-dimethylpyridin-4-amine, pyridine, overnight, 88%; ii) Et$_3$N, $N,N$-dimethylpyridin-4-amine, di-tert-butyl dicarbonate, CH$_2$Cl$_2$, rt, overnight, quantitative; iii) tetra-$n$-butylammoniumfluoride (TBAF), THF, 0 °C to rt, 40 min, 81%; d) formic acid, chlorosulfamoyl isocyanate, pyridine, 0 °C to reflux, overnight, 30%;

α-Galactopyranose-configured carba-cyclophellitol 19 was synthesized in sufficient quantities following the procedure described in Chapter 6. Treatment with lithium hydroxide in a mixture of THF, MeOH and H$_2$O resulted in acid 20 (Scheme 8.4). Acylation of 20 using oxalyl chloride in toluene gave acid chloride 21. After having acid chloride 21 and $O$-sulfamoylamide 18 available, the key step in the sequence was investigated. Coupling of acid chloride 21 with $O$-sulfamoylamide 18 resulted in compound 22, which was purified by size-exclusion chromatography followed by silica gel column chromatography. In contrast to cyclophellitol and cyclophellitol aziridine$^{15}$-$^{17}$, carba-cyclophellitol derivatives are stable towards acidic conditions. Therefore, the isopropylidene and tert-butyloxycarbonyl protecting groups of 22 could be successfully removed by treatment with TFA and H$_2$O in CH$_2$Cl$_2$. The remaining benzyl groups
were removed under the agency of BCl$_3$ as the Lewis acid. Purification by HPLC provided compound 4 in 69% yield.

**Scheme 8.4 Synthesis of compound 4.**

![Scheme 8.4](image_url)

**Reagents and conditions:** a) LiOH, THF/MeOH/H$_2$O (8:2:1), rt, overnight, 86%; b) oxalyl chloride, toluene, 40 °C, overnight c) 18, N,N-diisopropylethylamine, CH$_2$Cl$_2$, rt, 3 h, 60% over 2 steps; d) TFA/H$_2$O (9:1), CH$_2$Cl$_2$, 0 °C to rt, 4.5 h, 81%; e) BCl$_3$, CH$_2$Cl$_2$, -78 °C to 0 °C, 4.5 h, 69%.

The synthesis of α-glucopyranose-configured carba-cyclophellitol 5 (Scheme 8.5), was based on the strategy as described above towards α-galactopyranose-configured analogue 4. The synthesis of compound 5 requires access to sufficient quantities of acid chloride 29. Cyclopropanation of cyclohexene 24 yielded ethyl ester 25 as an α/β-mixture (see also Chapter 6). Crystallization in ethanol resulted in obtaining a pure fraction of α-ethyl ester 25. Hydrolysis by LiOH resulted in carboxylic acid 27. In order to obtain larger quantities of carboxylic acid 26, ethyl ester 25 as an α/β-mixture was first reduced with di-isobutyl aluminum hydride after which alcohol 27 was isolated by column chromatography (Chapter 6). Alcohol 27 was oxidized to carboxylic acid 26 using Jones oxidation conditions. Having large quantities of carboxylic acid 26 available, the synthesis was continued as described for compound 4. Briefly, acylation of acid 26 using oxalyl chloride afforded compound 28, which was coupled with previously synthesized uridine sulfamoyl amide 16 to obtain compound 29 as the DIPEA salt. Finally, global deprotection by treatment with BCl$_3$, followed by quenching with MeOH gave compound 5, which was purified by precipitation in chloroform, followed by HPLC purification.
Scheme 8.5 Synthesis of compound 5.

Reagents and conditions: a) LiOH, THF/MeOH/H₂O (6.5:1.5:1), rt, 5 h, 91%; b) Jones reagent, acetone, 0 °C, 70 min, 75%; c) oxalyl chloride, toluene, 40 °C, 4 h; d) 16, N,N-diisopropylethylamine, CH₂Cl₂, rt, overnight, 70% over 2 steps; e) i) BCl₃, CH₂Cl₂, 0 °C, 3 h; ii) MeOH, 30 min, 54% over 2 steps.

The potency of the synthesised UDP-glucose and UDP-galactose mimetics to inhibit GH activities was established next (Table 8.1). Compound 4 turned out to be a potent human lysosomal α-galactosidase (α-GalA) inhibitor, with considerable less potency towards human lysosomal β-galactosidase (GALC). As can be seen from Table 8.1, no inhibition was observed for any of the α-glucosidases (GAA, GANAB) or β-glucosidases (GBA1, GBA2) tested for compound 5 (ML128). Furthermore, 5 failed to inhibit glucosylceramide synthase (GCS), a glucosyltransferase. Inhibition assays to assess the potential of the compounds to target other transferases, including the α-galactosyl transferases for which the galactose-configured compounds were designed, could not be performed in the timeframe of the PhD research described here, though compounds have been shared with interested groups who may shed light on their GT inhibitory properties, or lack thereof, in the (near) future.
Table 8.1 Apparent IC₅₀ values of compounds 3 – 5 towards glycosidases and glucosyltranferase (μM).

<table>
<thead>
<tr>
<th>Compound</th>
<th>α-galactosidase</th>
<th>β-galactosidase</th>
<th>GlucT</th>
<th>α-glucosidase</th>
<th>β-glucosidase</th>
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<tbody>
<tr>
<td></td>
<td>α-GalA</td>
<td>GALC</td>
<td>GCS</td>
<td>GAA</td>
<td>GANAB</td>
</tr>
<tr>
<td>3 (AB209)</td>
<td>&gt;500</td>
<td>&gt;500</td>
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<tr>
<td>4 (AB180)</td>
<td>18.84 ± 1.33</td>
<td>180.27 ± 4.52</td>
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</tr>
<tr>
<td>5 (ML128)</td>
<td>--</td>
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<td>&gt;50</td>
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8.3 Conclusion

In this chapter the versatility of carba-cyclophellitol as analogues of the oxocarbenium ion-like transition state is demonstrated by synthesizing potential inhibitors (3 and 4) towards galactosyltransferases. In the (near) future, their GT specific inhibition potency will be examined. Furthermore, compound 4 turned out to be a potent carba-cyclophellitol-based α-galactosidase inhibitor. In a similar fashion compound 5 (ML128) was successfully synthesized. However, inhibition towards GCS or various glucosidases (GAA, GANAB, GBA1 or GBA2) was not observed.

Experimental

General methods: All chemicals were purchased from Acros, Sigma Aldrich, Biosolve, VWR, Fluka, Merck and Fisher Scientific and used as received unless stated otherwise. Dichloromethane (DCM), tetrahydrofuran (THF) and toluene were stored over flame-dried 4 Å molecular sieves before use. Pyridine and triethylamine were stored on KOH pellets before use. Traces of water from reagents were removed by co-evaporation with toluene in reactions that require anhydrous conditions. All reactions were performed under an argon atmosphere unless stated otherwise. TLC analysis was conducted using Merck aluminium sheets (Silica gel 60 F 254) with detection by UV absorption (254 nm), by spraying with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) in 10% sulfuric acid, a solution of KMnO₄ (20 g/L) and K₂CO₃ (10 g/L) in water or ninhydrin (0.75 g/L), followed by charring at ~150 °C. Size exclusion was performed on Sephadex LH-20 (eluent DCM/MeOH, 1:1). Column chromatography was performed using Screening Device b.v. Silica Gel (particle size of 40 – 63 μm, pore diameter of 60 Å) in the indicated solvents. For reversed-phase HPLC purifications a HPLC system equipped with a C18 semiprep column (Gemini C18, 250x10 mm, 5 μm particle size, Phenomenex) was used. LC/MS analysis was performed on a Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 5 μm particle size, Phenomenex), coupled to a LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI+). The applied buffers were H2O, MeCN and 1% aqueous TFA. ³¹P NMR, ¹H NMR and ¹³C NMR spectra were recorded on a Bruker AV-400 (161.7, 400 and 101 MHz respectively) spectrometer in the given solvent. Chemical shifts are given in ppm (δ) relative to the residual solvent peak or
tetramethylsilane (0 ppm) as internal standard. Coupling constants are given in Hz. High-resolution mass spectrometry (HRMS) analysis was performed with a LTQ Orbitrap mass spectrometer (Thermo Finnigan), equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150–2000) and dioctyl phthalate (m/z = 391.28428) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

Alcohol 6 (see Chapter 6, 708 mg, 1.25 mmol) was dissolved in DCM (15 mL) and 3,4-dihydropyran (170 μL, 1.9 mmol, 1.5 eq.) and a catalytic amount of pTsOH were added. After stirring for 30 min at room temperature, the reaction mixture was quenched with saturated aqueous NaHCO₃, extracted with DCM, dried with Na₂SO₄, filtered and concentrated in vacuo. Purification by column chromatography (5% EtOAc in pentane → 20% EtOAc in pentane) gave a diastereomeric mixture of compound 7 (0.64 g, 0.99 mmol, 79%) as a clear oil. 1H NMR (400 MHz, CDCl₃): δ (ppm) 7.51 – 7.19 (m, 20H, CH arom-Bn), 5.01 – 4.81 (m, 2H, CH₂-Bn), 4.79 – 4.54 (m, 2H, CH₂O), 4.38 (t, J = 7.4 Hz, 1H, H-2), 3.96 – 3.87 (m, 1H, H-4), 3.70 – 3.53 (m, 1H, H-8), 3.50 – 3.35 (m, 1H, H-1, 3 x CC₂H₂C-THP), 3.28 (dd, J = 10.6, 6.6 Hz, 1H, OCH₃C-THP) 3.20 (d, J = 8.2 Hz, 1H, H-3), 1.94 (m, 1H, H-5), 1.85 – 1.40 (m, 7H, H-1, 3 x CC₂H₂C-THP), 1.08 (m, 1H, H-7), 0.78 – 0.63 (m, 1H, H-6). 13C NMR (101 MHz, CDCl₃): δ (ppm) 190.3, 190.3, 190.3, 190.3, 190.1, 190.1, 188.3, 188.3 (C q-Bn), 128.4, 128.4, 128.2, 128.2, 127.9, 127.8, 127.8, 127.7, 127.6, 127.4, 127.3, 127.2 (CH₃-Bn), 98.2, 97.3 (OCHO-THP), 83.5 (C-3), 76.7, 76.7 (C-2), 76.0, 76.0 (C-4), 73.9, 73.9, 73.2, 73.1, 72.8, 72.7, 71.0, 70.9 (CH₂-Bn), 70.8, 70.8 (C-8), 70.5 (OCH₃C-THP), 62.0, 61.9 (CH₂O), 41.6 (C-5), 30.7, 30.6, 25.5 (CCH₂C-THP), 24.3, 24.1 (C-7), 21.8, 21.5 (C-1), 19.8 (C-6), 19.4, 19.3 (CCH₂C-THP), 19.2 (C-6). HRMS: calculated for [C₄₂H₄₈O₆Na]+ 671.33431, found 671.33379.

NH₃ (50 mL) was condensed in a three necked flask at -60 °C after which solid lithium (0.25 g) was added resulting in a blue solution. After addition of a solution of 7 (0.34 g, 0.52 mmol) in THF (4 mL), the reaction mixture was stirred for 1 h before cautiously quenching with MilliQ (5 mL). The reaction mixture was gradually allowed to warm to room temperature and concentrated in vacuo to afford debenzylated product 8. The crude product 44 was redissolved in pyridine (15 mL) and acetic anhydride (10 mL) and a catalytic amount of DMAP were added. After stirring overnight at room temperature, the reaction was quenched with H₂O. The solution was extracted with EtOAc, washed with H₂O (3x), dried with MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (10% EtOAc in pentane → 50% EtOAc in pentane) gave a diastereomeric mixture of peracetylated 9 (210 mg, 0.46 mmol, 88% over two steps) as a pale oil. 1H NMR (400 MHz, CDCl₃): δ (ppm) 5.41 (dd, J = 9.2, 6.8 Hz, 1H, H-2), 5.32 (s, 1H, H-4), 4.65 – 4.57 (m, 2H, H-3, OCHO-THP), 4.14 – 4.05 (m, 2H, H-8), 3.85 – 3.75 (m, 1H, H-8).
Synthesis of potential glycosyltransferase inhibitors based on carba-cyclophellitol

OCH₂C-THP), 3.56 (dd, J = 10.7, 6.6 Hz, 0.5H, H-9), 3.50 – 3.47 (m, 1H, OCH₂C-THP), 3.40 (dd, J = 6.3, 2.7 Hz, 1H, H-9), 3.26 (dd, J = 10.7, 6.6 Hz, 0.5H, H-9), 2.35 – 2.31 (m, 1H, H-5), 2.09 (d, J = 15.6 Hz, 6H, CH₃-Ac), 2.02 (d, J = 7.6 Hz, 6H, CH₃-Ac), 1.80 – 1.54 (m, 3x CCH₂C-THP), 1.19 – 1.15 (m, 1H, H-7), 0.79 (dd, J = 13.3, 5.5 Hz, 1H, H-6). ¹³C NMR (101 MHz, CDCl₃): δ (ppm) 170.7, 170.7, 170.7, 170.4, 170.2 (C=O-Ac), 98.3, 97.4 (OCHHO-THP), 72.2 (C-3), 69.7 (C-2), 69.6, 69.1 (C-9), 69.1 (C-4), 62.9, 62.9 (C-8), 62.0, 61.5 (OCH₂C-THP), 38.2, 38.1 (C-5), 30.5, 30.4, 25.4, 25.3 (CCH₂C-THP), 24.4, 24.2 (C-7), 21.1, 21.0 (C-1), 20.8, 20.7, 20.7, 20.7 (CH₃-Ac), 19.3, 19.0 (CCH₂C-THP), 18.2, 17.9 (C-6). HRMS: calculated for [C₂₂H₃₂O₁₀Na]+ 479.18877, found 479.18854.

(1R,2S,3R,4S,5R,6R,7R)-5-(acetoxymethyl)-7-(hydroxymethyl)bicyclo[4.1.0]heptane-2,3,4-triyl triacetate (10)

A solution of compound 9 (150 mg, 0.33 mmol) in MeOH (10 mL) was acidified to pH 2 with p-TsOH. After stirring for 1 h at room temperature, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with DCM (3x). The combined organic layers were washed with brine, dried with MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (50% DCM in pentane → 10% MeOH in DCM) gave alcohol 10 (104 mg, 0.28 mmol, 85%) as a white solid. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 5.39 (dd, J = 9.2, 6.8 Hz, 1H, H-2), 5.31 (s, 1H, H-4), 4.64 (dd, J = 9.3, 1.6 Hz, 1H, H-3), 4.11 (d, J = 8.1 Hz, 2H, H-8), 3.49 (dd, J = 11.4, 6.4 Hz, 1H, CH₂), 3.42 (dd, J = 11.4, 6.4 Hz, 1H, CH₂), 2.36 – 2.25 (m, 1H, H-5), 2.09 (d, J = 15.6 Hz, 6H, CH₃-Ac), 2.02 (d, J = 7.6 Hz, 6H, CH₃-Ac), 1.67 (dt, J = 8.8, 6.5 Hz, 1H, H-1), 1.16 (t, J = 5.5 Hz, 1H, H-7), 0.78 (ddd, J = 8.2, 4.7, 2.6 Hz, 1H, H-6). ¹³C NMR (101 MHz, CDCl₃): δ (ppm) 171.2, 170.9, 170.6, 170.3 (C=O-Ac), 72.3 (C-3), 69.9 (C-2), 69.1 (C-4), 65.1 (CH₂O), 62.9 (C-8), 38.2 (C-5), 27.0 (C-7), 21.2 (C-1), 20.8, 20.8, 20.8, 20.7 (CH₃-Ac), 17.9 (C-6). HRMS: calculated for [C₁₇H₂₄O₉Na]+ 395.13125, found 395.13103.

((2R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydronuran-2-yl)methyl
(((1R,2S,3R,4S,5R,6R,7R)-2,3,4-trihydroxy-5-(hydroxymethyl)bicyclo[4.1.0]heptan-7-yl)methyl) phosphate sodium salt (3)

To a solution of phosphoramidite 11 (52 mg, 0.09 mmol) in ACN (2 mL) was added a solution of alcohol 10 (43 mg, 0.11 mmol, 1.2 eq.) in ACN (3 mL). After stirring for 30 min at room temperature tBuOOH (5.5 M in decane, 70 μL, 0.40 mmol, 4.4 eq.) was added. After stirring for additional 30 min, DBU (75 μL, 0.50 mmol, 5.6 eq.) was added and the reaction mixture was stirred for 1 h before addition of aqueous NH₃ (30-35%, 0.50 mL). After stirring overnight additional aqueous NH₃ (30-35%, 0.50 mL) was added and stirred for 6 h. The reaction mixture was concentrated in vacuo and redissolved in MeOH (3 mL), H₂O (1 mL) and Et₃N (1 mL) in order to complete the deacetylation. After stirring overnight, the mixture was concentrated in vacuo and purified by size-exclusion chromatography (DCM/MeOH, 1:1) followed by HPLC purification (C18 column, linear gradient: A → 10% A in B, A = 100 mM NH₄OAc in H₂O, B = ACN, 15 min). Next, the purified product was dissolved in water and flushed through a tube of Dionex sodium exchange resin. Lyophilization afforded compound 3 (10 mg, 0.02 mmol, 22%) as a white solid. ³¹P
NMR (161.7 MHz, D₂O): δ (ppm) 0.64. ¹H NMR (400 MHz, D₂O): δ (ppm) 7.91 (d, J = 8.1 Hz, H-6), 5.97 – 5.92 (m, 1H, H-1’ and H-5), 4.38 – 4.31 (m, 2H, H-2’ and H-3’), 4.25 (s, 1H, H-4’), 4.22 – 4.06 (m, 3H, H-5’, H-2’’), 3.83 (s, 1H, H-4’’), 3.80 – 3.66 (m, 4H, H-8’’, H-9’’), 3.20 (d, J = 9.1 Hz, 1H, H-3’’), 1.90 – 1.85 (m, 1H, H-5’’), 1.43 – 1.39 (m, 1H, H-1’’), 1.16 – 1.12 (m, 1H, H-7’’), 0.81 – 0.77 (m, 1H, H-6’’). ¹³C NMR (101 MHz, D₂O): δ (ppm) 166.6 (C-4), 152.1 (C-2), 141.5 (C-6), 102.5 (C-5), 88.7 (C-1’), 83.1 (C-4’), 74.0 (C-3’), 73.7 (C-2’), 71.5 (C-4’’), 69.6 (C-3’), 69.4, 69.3 (C-9’’), 68.5 (C-2’’), 64.4, 64.3 (C-5’’), 62.5 (C-8’’), 42.5 (C-5’’), 24.2, 24.1 (C-7’’), 23.5 (C-1’’), 18.2 (C-6’’). HRMS: calculated for [C₁₈H₂₈N₂O₁₃P]+ 511.13235, found 511.13220.

2’,3’-O-Isopropylideneuridine (15)

To a solution of uridine 14 (4.88 g, 20.0 mmol) in acetone (200 mL) was added iodine (0.50 g, 2.0 mmol, 0.1 eq.). After stirring overnight at room temperature, the reaction mixture was quenched with saturated aqueous Na₂S₂O₃ (30 mL) and concentrated in vacuo. The crude product was redissolved in DCM, dried with MgSO₄, filtered and concentrated in vacuo. Purification by column chromatography (5% MeOH in DCM → 10% MeOH in DCM) gave compound 15 (4.72 g, 16.6 mmol, 83%) as a white solid. ¹H NMR (400 MHz, MeOD): δ (ppm) 7.83 (d, J = 8.0 Hz, 1H, H-6), 5.87 (d, J = 2.8 Hz, 1H, H-1’), 5.68 (d, J = 8.0 Hz, 1H, H-5), 4.91 - 4.82 (m, 2H, H-2’ and H-3’), 4.21 (dd, J = 8.0, 3.6 Hz, 1H, H-4’), 3.78 (dd, J = 11.9, 3.6 Hz, 1H, H-5’’), 3.71 (dd, J = 11.9, 4.5 Hz, 1H, H-5’’), 1.54 (s, 3H, CH₃), 1.35 (s, 3H, CH₃). ¹³C NMR (101 MHz, MeOD): δ (ppm) 166.2 (C=O), 152.1 (C=O), 143.9 (C-6), 115.1 (C₆), 102.6 (C-5), 94.1 (C-1’), 88.4 (C-4’), 85.8 (C-2’), 82.2 (C-3’), 63.1 (C-5’), 27.5, 25.5 (CH₃). HRMS: calculated for [C₁₂H₁₇N₂O₆]+ 285.10811, found 285.10818.

Sulfamoyl chloride

Formic acid (1 eq.) was added to chlorosulfamoyl isocyanate (1 eq.) and stirred at 0 °C. When the evolution of gas stopped, the reaction mixture was concentrated under reduced pressure at room temperature to afford sulfamoyl chloride as a clear oil, which was directly used without further purification.

2’,3’-O-Isopropylidene-5’-O-sulfamoyluridine (16)

2’,3’-O-isopropylideneuridine 15 (1.00 g, 3.51 mmol) was dissolved in THF (20 mL) and cooled to 0 °C, before sodium hydride (60% dispersion in oil, 0.700 g, 17.6 mmol, 5 eq.) was added. After stirring for 15 minutes, sulfamoyl chloride (7.0 mmol, 2 eq.) in THF (4 mL) was added. After stirring for 4 hours at 0 °C, the reaction mixture was allowed to come to room temperature and stirred for an additional 12 h. The reaction mixture was quenched with MeOH (5 mL) and saturated aqueous NH₄Cl (5 mL), filtrated and concentrated under reduced pressure. Purification by silica column chromatography (30% acetone in chloroform → 40% acetone in chloroform) resulted in O-sulfamoylamide 16 (1.0 g, 2.8 mmol, 79%) as a white foam. ¹H NMR (400 MHz, MeOD): δ (ppm) 7.64 (d, J = 8.1 Hz, 1H, H-2’’), 5.82 (s, 1H, H-1’’), 5.72 (d, J = 8.0 Hz, 1H, H-1’’), 5.03 (d, J = 8.6 Hz, 1H, H-2’’), 4.88 (dd, J = 6.3, 3.6 Hz, 1H, H-3’’), 4.41 – 4.36 (m, 1H, H-4’’), 4.36 – 4.27 (m, 2H, H-5’’), 1.54 (s, 3H, CH₃), 1.35 (s, 3H, CH₃). ¹³C NMR (101 MHz, MeOD): δ (ppm) 166.2 (C=O), 152.2 (C=O), 144.3
Synthesis of potential glycosyltransferase inhibitors based on carba-cyclophellitol

135

(C-1’’), 115.4 (Cq), 102.8 (C-2’’), 95.1 (C-1’), 86.1 (C-4’), 85.4 (C-3’), 82.4 (C-2’), 70.0 (C-5’), 27.6, 25.5 (CH3). HRMS: calculated for [C12H18N3O8S]+ 364.08091, found 364.08074.

2’,3’-O-Isopropylidene-5’-O-tert-butyldimethylsilyluridine (30)
To a solution of alcohol 15 (2.0 g, 7.0 mmol) in pyridine (50 mL) was added TBDMS-Cl (1.26 g, 8.4 mmol, 1.2 eq.) and a catalytic amount of 4-dimethylaminopyridine. After stirring overnight at room temperature, additional TBDMS-Cl (0.63 g, 3.5 mmol, 0.5 eq.) was added followed by stirring for 1.5 h before the reaction mixture was quenched with MeOH (5 mL) and concentrated in vacuo. The crude product was redissolved in EtOAc and washed with saturated aqueous NaHCO3 and brine. The organic layer was dried with MgSO4, filtered, and concentrated in vacuo. Purification by column chromatography (20% EtOAc in pentane → 50% EtOAc in pentane) gave title compound 30 (2.46 g, 6.2 mmol, 88%) as a white foam. 1H NMR (400 MHz, CDCl3): δ (ppm) 9.48 (s, 1H, NH) 7.71 (d, J = 8.1 Hz, 1H, H-6), 6.00 (d, J = 2.7 Hz, 1H, H-1’), 5.71 (d, J = 8.1 Hz, 1H, H-5’), 4.78 - 4.71 (m, 2H, H-2’ and H-3’), 4.35 – 4.30 (m, 1H, H-4’), 3.94 (dd, J = 11.5, 2.7 Hz, 1H, H-5’a’), 3.82 (dd, J = 11.5, 3.0 Hz, 1H, H-5’a’), 1.60 (s, 3H, CH3), 1.37 (s, 3H, CH3), 0.92 (s, 9H, CH3), 0.10 (d, J = 2.6 Hz, 6H, CH3). 13C NMR (101 MHz, CDCl3): δ (ppm) 163.6 (C-4), 150.3 (C-2), 140.7 (C-6), 114.2 (Cq), 102.3 (C-5), 92.0 (C-1’), 86.8 (C-4’), 85.5 (C-2’), 80.4 (C-3’), 63.5 (C-5’), 27.4 (CH3), 25.9 (Cq), 25.5 (CH3), 18.4 (Cq), -5.3, -5.4 (CH3). HRMS: calculated for [C18H31N2O6Si]+ 399.19459, found 399.19400.

2’,3’-O-Isopropylidene-5’-O-tert-butyldimethylsilyl-3-N-tetrt-butyloxy carbonyluridine (31)
Silyl-protected alcohol 30 (2.04 g, 5.1 mmol) was taken up in DCM (30 mL). Et3N (2.2 mL, 15.3 mmol, 3 eq.), 4-dimethylaminopyridine (300 mg, 2.5 mmol, 0.5 eq.) and di-tert-butyldicarbonate (2.22 g, 10.2 mmol, 2 eq.) were added to the solution. After stirring overnight at room temperature, the reaction mixture was concentrated in vacuo, and purified by column chromatography (5% EtOAc in pentane → 10% EtOAc in pentane) to yield title compound 31 (3.14 g, 5.1 mmol, quantitative) as a yellow oil. 1H NMR (400 MHz, CDCl3): δ (ppm) 7.71 (d, J = 8.2 Hz, 1H, H-6), 5.93 (d, J = 2.1 Hz, 1H, H-1’), 5.70 (d, J = 8.2 Hz, 1H, H-5’), 4.75 (m, 2H, H-2’ and H-3’), 4.37 – 4.34 (m, 1H, H-4’), 3.92 (dd, J = 11.6, 2.3 Hz, 1H, H-5’a’), 3.79 (dd, J = 11.6, 3.0 Hz, 1H, H-5’a’), 1.60 (s, 9H, CH3), 1.58 (s, 3H, CH3), 1.36 (s, 3H, CH3), 0.90 (s, 9H, CH3), 0.09 (d, J = 3.2 Hz, 6H, CH3). 13C NMR (101 MHz, CDCl3): δ (ppm) 160.5 (C-4), 148.4 (C-2), 147.6 (C=O-Boc) 139.8 (C-6), 114.0 (Cq), 101.7 (C-5), 92.8 (C-1’), 87.1 (C-4’), 86.8 (Cq), 85.6 (C-2’), 80.4 (C-3’), 63.4 (C-5’), 27.5 (CH3), 27.3 (CH3), 25.9 (Cq), 25.4 (CH3), 18.4 (Cq), -5.4, -5.5 (CH3).
To a solution of Boc-protected uridine  (2.29 g, 4.6 mmol) in THF (50 mL) was added TBAF (6.9 mL, 6.9 mmol, 1.5 eq., 1 M in THF) at 0 °C. After stirring for 10 min, the reaction mixture was allowed to warm to room temperature and stirred for additional 30 min before the reaction mixture was partitioned between H2O (50 mL) and EtOAc (150 mL). The aqueous layer was separated and extracted with EtOAc (3x). The combined organic layers were washed with brine, dried with MgSO4, filtered and concentrated in vacuo. Purification by column chromatography (20% EtOAc in pentane → 50% EtOAc in pentane) afforded compound  (1.43 g, 3.7 mmol, 81%) as a white foam. 

1H NMR (400 MHz, CDCl3): δ (ppm) 7.72 (d, J = 8.2 Hz, 1H, H-6), 5.82 (d, J = 1.8 Hz, 1H, H-1'), 5.73 (d, J = 8.2 Hz, 1H, H-5), 4.87 (m, 2H, H-2' and H-3'), 3.34 – 3.41 (m, 1H, H-4'), 3.88 (dd, J = 11.9, 2.4 Hz, 1H, H-5 a'), 3.77 (dd, J = 11.9, 2.4 Hz, 1H, H-5 b'), 1.59 (s, 9H, CH3), 1.56 (s, 3H, CH3), 1.35 (s, 3H, CH3). 13C NMR (101 MHz, CDCl3): δ (ppm) 160.6 (C-4), 148.4 (C-2), 147.5 (C=O-Boc) 113.9 (Cq), 102.3 (C-5), 94.8 (C-1'), 87.6 (C-4'), 84.5 (C-2'), 80.4 (C-3'), 27.5, 27.1, 25.3 (CH3). HRMS: calculated for [C17H25N2O8]+ 385.16054, found 385.16056.

To a solution of alcohol  (552 mg, 1.4 mmol) in DCM (15 mL) was added pyridine (0.3 mL) and sulfamoyl chloride (345 mg, 3.0 mmol, 2.1 eq.). After the reaction mixture was refluxed overnight, the reaction mixture was concentrated in vacuo and purified by column chromatography (20% EtOAc in pentane → 50% EtOAc in pentane) to yield O-sulfamoylamide  (194 mg, 0.42 mmol, 30%) as a white solid. 

1H NMR (400 MHz, CDCl3): δ (ppm) 7.40 (d, J = 8.2 Hz, 1H, H-6), 5.75 (d, J = 1.8 Hz, 1H, H-1'), 5.00 (dd, 1H, H-2'), 4.87 (dd, 1H, H-3'), 4.38 – 4.40 (m, 1H, H-4'), 4.38 – 4.35 (m, 1H, H-5'), 1.59 (s, 9H, CH3), 1.56 (s, 3H, CH3), 1.35 (s, 3H, CH3). 13C NMR (101 MHz, CDCl3): δ (ppm) 160.7 (C-4), 148.5 (C-2), 147.8 (C=O-Boc) 114.8 (Cq), 102.3 (C-5), 94.8 (C-1'), 87.6 (C-4'), 84.5 (C-2'), 80.6 (C-3'), 27.5, 27.1, 25.3 (CH3). HRMS: calculated for [C12H18N3O8S]+ 364.08091, found 364.08088.

Ester  (see Chapter 6, 0.30 g, 0.49 mmol) was dissolved in THF/MeOH/H2O (8:2:1, 25 mL) and LiOH (95 mg, 4 mmol, 8.0 eq.) was added. After stirring overnight at room temperature, the reaction mixture was acidified to pH 2 with aqueous HCl (1 M). The reaction mixture was diluted with H2O and extracted with EtOAc. The organic layer was dried with MgSO4, filtered and concentrated in vacuo. Purification by column chromatography (20% EtOAc in pentane → 30% EtOAc in pentane) afforded carboxylic acid  (0.244 g, 0.42 mmol, 86%) as a clear oil. 

1H NMR (400 MHz, CDCl3): δ (ppm) 7.41 – 7.20 (m, 20H, H arom), 4.87 (d, J = 11.6 Hz, 1H, CH2Bn), 4.77 – 4.61 (m, 4H, CH2Bn), 4.55 (d, J = 11.6 Hz, 1H, CH2Bn), 4.45 (s, 2H, CH2Bn), 4.11 – 4.29 (m, 1H, H-2), 3.89 (s, 1H, H-4), 3.63 (t, J = 8.9, 1H, H-8), 3.55 (t, J = 7.5 Hz, 1H, H-8), 3.15 (d, J = 8.2 Hz, 1H, H-3), 2.14 – 2.07 (m, H-1), 1.99 – 1.93 (m, 1H, H-5), 1.61 (t, J = 4.3 Hz, 1H, H-7), 1.40 –
1.36 (m, 1H, H-6). 13C NMR (100 MHz, CDCl3): δ (ppm) 179.4 (C=O), 139.1, 138.8, 138.1 (Cq), 128.6, 128.4, 128.0, 127.9, 127.6, 127.5 (CH arom), 82.7 (C-3), 76.0 (C-2), 75.6 (C-4), 74.1, 73.4, 72.9, 71.8 (CH2Bn), 70.4 (C-8), 41.2 (C-5), 28.0 (C-1), 25.8 (C-7), 25.5 (C-6). HRMS: calculated for [C37H38O6Na]+ 593.30066, found 596.29994.

tert-butyl 3-((3aR,4R,6R,6aR)-2,2-dimethyl-6-(((N-((1R,2S,3R, 4S,5R,6R,7R)-2,3,4-tris(benzyloxy)-5-((benzyloxy)methyl)bicyclo[4.1.0]heptane-7-carbonyl)sulfamoyl)oxy)methyl)tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-2,6-dioxo-3,6-dihydropyrimidine-1(2H)-carboxylate (22)
To a solution of carboxylic acid 20 (98 mg, 0.17 mmol) in toluene (5 mL) was added oxalyl chloride (0.15 mL, 1.7 mmol, 10 eq.). After stirring overnight at 40 °C, the reaction mixture was concentrated in vacuo followed by coevaporation with toluene (3x). The residu was redissolved in DCM (5 mL) and a solution of O-sulfamoylamide 18 (100 mg, 0.22 mmol, 1.3 eq.) in DCM (2 mL) and DIPEA (60 μL, 0.34 mmol, 2 eq.) were added. After stirring for 3 h at room temperature, the reaction mixture was washed with an aqueous HCl solution (1 M), saturated aqueous NaHCO3 and brine. The organic layer was dried with Na2SO4, filtered and concentrated in vacuo. Purification by size exclusion chromatography (DCM/MeOH, 1:1) followed by silica column chromatography (20% EtOAc in pentane → 50% EtOAc in pentane) yielded compound 22 (100 mg, 98 μmol, 60%) as a white solid. 1H NMR (400 MHz, CDCl3): δ (ppm) 7.36 – 7.24 (m, 21H, H-6 and H arom), 5.79 (d, J = 1.8 Hz, 1H, H-1’), 5.76 (d, J = 8.2 Hz, 1H, H-5), 4.88 – 4.43 (m, 12H, CH2Bn, , H-2’, H-3’ and H-5’), 4.39 – 4.33 (m, 2H, H-2” and H-4’), 3.93 (s, 1H, H-4’’), 3.64 – 3.54 (m, 2H, H-8’’), 3.13 (d, J = 8.5 Hz, 1H, H-3’’), 2.13 – 2.09 (m, 1H, H-1’’), 2.02 – 1.97 (m, 1H, H-5’’), 1.66 – 1.61 (m, 1H, H-7’’), 1.58 (s, 9H, CH3), 1.55 (s, 3H, CH3), 1.51 – 1.45 (m, 1H, H-6’’). 13C NMR (101 MHz, CDCl3): δ (ppm) 170.9 (C-9’’), 160.5 (C-4), 148.6 (C-2), 147.7 (C=O-Boc) 140.8 (C-6), 138.9, 138.5, 138.1, 138.0, (Cq), 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.6, 127.6, 127.5 (CH arom), 114.9 (Cq), 102.6 (C-5), 93.4 (C-1’), 87.3 (Cq), 84.3 (C-2’), 83.9 (C-4’), 82.6 (C-3’’), 80.3 (C-3’), 76.2 (C-2’’), 75.1 (C-4’’), 74.2 (CH2Bn), 73.4 (CH2Bn), 72.6 (C-5’), 72.5, 71.5 (CH2Bn), 70.4 (C-8’), 45.9 (Cq) 41.0 (C-5’’), 28.6 (C-1’’), 27.5 (Cq), 27.4 (C-7’’), 27.2, 25.3 (CH3), 24.6 (C-6’’). HRMS: calculated for [C49H54N3O13S]+ 924.33719, found 924.33760.

((2R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl
((1R,2S,3R,4S,5R,6R,7R)-2,3,4-tris(benzyloxy)-5-((benzyloxy)methyl)bicyclo[4.1.0]heptane-7-carbonyl)sulfamate (23)
To a cooled (0 °C) solution of compound 22 (100 mg, 98 μmol) in DCM (2 mL) was dropwise added a solution of TFA/H2O (9:1, 500 μL). After stirring for 4.5 h the reaction mixture was concentrated in vacuo followed by coevaporation with toluene (3x). Purification by column chromatography (50% EtOAc in pentane → EtOAc) afforded title compound 23 (70 mg, 79 μmol, 81%) as a white solid. 1H NMR (400 MHz, MeOD): δ (ppm) 7.69 (d, J = 8.1 Hz, 1H, H-6), 7.39 –
Chapter 8

7.17 (m, 20H, H$_{arom}$), 5.89 (d, $J = 3.9$ Hz, 1H, H-1’), 5.72 (d, $J = 8.1$ Hz, 1H, H-5’), 4.78 – 4.37 (m, 10H, CH$_2$Bn and H-5’), 4.32 (dd, $J = 8.3$, 6.3 Hz, 1H, H-2’), 4.21 – 4.10 (m, 3H, H-2’, H-3’ and H-4’), 3.90 (s, 1H, H-4’), 3.63 – 3.51 (m, 2H, H-8’), 3.19 (d, $J = 9.0$ Hz, 1H, H-3’), 2.17 – 2.09 (m, 1H, H-1’), 2.01 – 1.97 (m, 1H, H-5’), 1.65 (t, $J = 4.5$ Hz, 1H, H-7’), 1.50 – 1.42 (m, 1H, H-6’). 13C NMR (101 MHz, MeOD): δ (ppm) 173.5 (C-9’), 166.0 (C-4), 152.3 (C-2), 142.3 (C-6), 140.2, 140.0, 139.9, 139.5 (C$_{aq}$), 129.5, 129.4, 129.3, 129.1, 129.1, 128.8, 128.7, 128.6, 128.5 (CH$_{arom}$), 103.1 (C-5), 90.5 (C-1’), 83.6 (C-3’), 82.7 (C-4’), 76.9 (C-2’), 76.7 (C-4’’), 75.2 (C-2’), 75.0, 74.2, 73.4, 72.7 (CH$_2$Bn), 71.9 (C-5’), 71.8 (C-8’’), 71.0 (C-3’), 42.2 (C-5’’), 29.3 (C-1’’), 29.1, 28.4 (C-7’’), 26.7 (C-6’’). HRMS: calculated for [C$_{46}$H$_{50}$N$_3$O$_{13}$S]+ 884.30589, found 884.30684.

((2R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl ((1R,2S,3R,4S,5R,6R,7R)-2,3,4-trihydroxy-5-(hydroxymethyl)bicyclo[4.1.0]heptane-7-carbonyl)sulfamate (4)

To a cooled (0 °C) solution of compound 23 (32 mg, 36 μmol) in DCM (2 mL) was dropwise added BCl$_3$ (1 M in DCM, 300 μL, 0.30 mmol, 8 eq.). After stirring for 30 min, the reaction mixture was allowed to warm to 0 °C. After stirring for additional 4 h, the reaction mixture was cooled to -78 °C and quenched with MeOH. The reaction mixture was concentrated in vacuo, redissolved in CHCl$_3$ and stirred vigorously for 30 min before the suspension was filtered. The residue was taken up in methanol and concentrated in vacuo. Purification by HPLC (C18, linear gradient: 5 → 10% B in A, solutions used A: H$_2$O, B: acetonitrile, 0.5% TFA, 15 min) afforded title compound 4 (13 mg, 25 μmol, 69%) as a white solid. 1H NMR (400 MHz, D$_2$O): δ (ppm) 7.66 (d, $J = 8.1$ Hz, 1H, H-6), 5.93 – 5.81 (m, 2H, H-1’ and H-5), 4.59 (dd, $J = 11.4$, 2.3 Hz, 1H, H-5’), 4.54 (dd, $J = 11.4$, 3.4 Hz, 1H, H-5’), 4.31 – 4.27 (m, 2H, H-2’ and H-4’), 4.25 – 4.21 (m, 2H, H-2” and H-3’), 3.83 (s, 1H, H-4’), 3.74 – 3.51 (m, 2H, H-8’), 3.20 (d, $J = 9.1$ Hz, 1H, H-3’), 2.08 (ddd, $J = 9.4$, 6.1, 4.9 Hz, 1H, H-1”), 1.96 – 1.90 (m, 1H, H-5”), 1.66 (t, $J = 4.5$ Hz, 1H, H-7”), 1.45 – 1.40 (m, 1H, H-6”). 13C NMR (101 MHz, D$_2$O): δ (ppm) 173.9 (C-9’), 166.1 (C-4), 151.6 (C-2), 141.6 (C-6), 102.4 (C-5), 89.3 (C-1’), 80.9 (C-4’), 73.2 (C-3’), 73.1 (C-2’), 70.9 (C-4’’), 70.7 (5”), 69.3 (C-3’’), 67.4 (C-2’’), 62.1 (C-8”), 42.1 (C-5”), 29.8 (C-1”), 27.1 (C-7”), 24.9 (C-6”). HRMS: calculated for [C$_{18}$H$_{26}$N$_3$O$_{13}$S]+ 524.11808, found 524.11793.

(1R,2S,3R,4R,5R,6R,7R)-2,3,4-tris(benzyloxy)-5-(benzyloxy)methyl)bicyclo[4.1.0]heptane-7-carboxylic acid (26)

Method A: To a solution of ester 25α (see Chapter 6, 0.202 g, 0.33 mmol) in THF/MeOH/H$_2$O (6.5:1.5:1, 18 mL) was added LiOH (32 mg, 1.3 mmol, 4 eq.). After stirring for 5 h at room temperature, the reaction mixture was quenched with HCl (1 M) until pH 2. The reaction mixture was extracted with EtOAc (2x) and the combined organic layers where dried over MgSO$_4$, filtered and concentrated in vacuo. Purification by column chromatography (10% EtOAc in pentane → 25% EtOAc in pentane) gave carboxylic acid 26 (0.173 g, 0.30 mmol, 91%) as a white oil.
Method B: Chromic acid stock solution (1.0 M) (Caution: Chromic acid is corrosive, toxic and carcinogenic): Concentrated H$_2$SO$_4$ (2.25 mL, 40.5 mmol) is taken up in H$_2$O (12.5 mL). To this solution was added CrO$_3$ (2.50 g, 25.0 mmol) and the resulting bright red solution was stirred until all solids were completely dissolved. The solution was then diluted with H$_2$O to a total volume of 25 mL. Alcohol 27 (see Chapter 6, 0.116 g, 0.20 mmol) was dissolved in acetone (40 mL) and cooled to 0 °C, after which chromic acid stock solution (1M, 0.4 mL, 0.40 mmol, 2 eq.) was added. After stirring for 70 min, the reaction mixture was diluted with EtOAc (150 mL) and washed with HCl (3 M, 2 x 100 mL) and brine (100 mL). The organic layer was dried over MgSO$_4$, filtered and concentrated in vacuo.

Purification by column chromatography (10% EtOAc in pentane → 25% EtOAc in pentane) resulted in carboxylic acid 26 (87 mg, 0.15 mmol, 75%) as a white solid. 1H NMR (400 MHz, CDCl$_3$): δ (ppm) 7.39 – 7.22 (m, 20H, H arom), 4.89 – 4.62 (m, 5H, CH$_2$Bn), 4.43 (m, 3H, CH$_2$Bn), 4.09 – 4.05 (m, 1H, H-2), 3.61 (dd, $J = 8.9, 4.5$ Hz, 1H, H-8), 3.55 (dd, $J = 8.9, 2.8$ Hz, 1H, H-8), 3.40 (t, $J = 10.2$ Hz, 1H, H-4), 3.29 – 3.22 (m, 1H, H-3), 2.06 – 2.05 (m, 1H, H-1), 1.99 – 1.90 (m, 1H, H-5), 1.84 – 1.80 (m, 1H, H-6), 1.69 (t, $J = 4.5$ Hz, 1H, H-7). 13C NMR (101 MHz, CDCl$_3$): δ (ppm) 178.7 (C=O), 138.9, 138.5, 138.2, 138.2 (Cq), 128.5, 128.5, 128.5, 128.5, 128.2, 128.0, 128.0, 127.9, 127.8, 127.7 (CH$_{arom}$), 84.0 (C-3), 79.0 (C-2), 78.4 (C-4), 75.7, 75.4, 73.3, 71.7 (CH$_2$:Bn), 70.2 (C-8), 43.0 (C-5), 28.3 (C-1), 26.2 (C-9), 24.7 (C-6). HRMS: calculated for [C$_{37}$H$_{38}$O$_6$Na]+ 601.25606, found 601.25564.

DIPEA salt of ((3a$R$,4$R$,6$R$,6a$R$)-6-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl ((1$R$,2$S$,3$R$,4$R$,5$R$,6$R$,7$R$)-2,3,4-tris(benzyloxy)-5-((benzyloxy)methyl)bicyclo[4.1.0]heptane-7-carbonyl)sulfamate (29)

Carboxylic acid 26 (74 mg, 0.12 mmol) was dissolved in toluene (6.0 mL) and oxalyl chloride (1 M in DCM, 0.24 mL, 0.24 mmol, 2.0 eq.) was added. The reaction mixture was stirred at 40 °C for 4 hours and concentrated in vacuo. The obtained acyl chloride was redissolved in DCM (7.0 mL) and O-sulfamoylamide 16 (0.100 g, 0.27 mmol, 2.3 eq.) and DIPEA (40 μL, 0.24 mmol, 2 eq.) were added. After stirring overnight at room temperature, the reaction mixture was concentrated under reduced pressure and purified by size-exclusion column chromatography (DCM/MeOH, 1:1), resulting in product 29 (89 mg, 84 μmol, 70%) as a white wax. 1H NMR (400 MHz, MeOD): δ (ppm) 7.75 (dd, $J = 13.8, 7.7$ Hz, 1H, H-6), 7.41 – 7.18 (m, 20H, H arom), 5.97 – 5.85 (m, 1H, H-1''), 5.71 (d, $J = 8.0$ Hz, 1H, H-5'), 4.89 – 4.86 (m, 1H, H-2''), 4.84 – 4.70 (m, 4H, H-3''), 4.59 (d, $J = 11.5$ Hz, 1H, H-4''), 4.50 – 4.33 (m, 5H, CH$_2$:Bn), 4.31 – 4.23 (m, 2H, H-5''), 4.06 (dd, $J = 7.9, 5.9$ Hz, 1H, H-2'''), 3.70 – 3.66 (m, 3H, Me-DIPEA), 3.58 – 3.57 (m, 2H, H-1'''), 3.35 – 3.27 (m, 2H, H-4'''' and H-3''''), 3.21 – 3.15 (m, 2H, CH$_2$:DIPEA) 2.09 – 2.02 (m, 1H, H-1'''''), 1.95 (dd, $J = 12.9, 8.3$ Hz, 1H, H-5'''''), 1.74 (dd, $J = 8.6, 5.3$ Hz, 2H, H-7'''' and H-6'''''), 1.56 – 1.47 (m, 3H, CH$_3$:), 1.39 – 1.30 (m, 15H, CH$_3$ and isopropyl DIPEA). 13C NMR (101 MHz, MeOD): δ (ppm) 166.1 (C=O), 162.7 (C=O), 152.1 (C=O), 143.6 (C-2''''), 140.3, 140.0, 139.9, 139.6 (Cq), 129.4, 129.3, 129.3, 129.2, 129.1, 129.0, 129.0, 128.7, 128.5, 128.5 (CH$_{arom}$), 115.2 (Cq), 103.2 (C-1’’’), 93.7 (C-1’’’’), 85.5 (C-2’’’), 85.4 (C-4’’’), 85.1 (C-5’’), 82.3 (C-3’’), 80.4 (C-2’’), 79.9 (C-4’’), 76.3, 76.0, 74.1, 71.9 (CH$_2$:Bn), 71.4 (C-8), 70.1 (C-5’’’), 55.8 (Me-DIPEA), 44.4 (C-5’’), 43.8 (CH$_2$:DIPEA), 29.6 (C-7’’), 27.6 (C-1’’), 26.2 (CH$_3$), 26.2 (C-6), 25.6 (CH$_3$).
Chapter 8

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((2R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-3,4-
dihydroxytetrahydrofuran-2-yl)methyl
\]
\[
((1R,2S,3R,4R,5R,6R,7R)-2,3,4-trihydroxy-5-
(hydroxymethyl)bicyclo[4.1.0]heptane-7-carbonyl)sulfamate
(5)
\]

Compound 29 (86 mg, 81 μmol) was dissolved in DCM (10 mL) and cooled to 0 °C, before BCl3 (1 M in DCM, 1.11 mL, 1.11 mmol, 13.7 eq.) was added. After stirring for 3 h, the reaction mixture was quenched with MeOH. The reaction mixture was concentrated in vacuo, redissolved in CHCl3 and stirred vigorously for 30 min before the suspension was filtered. The residue was taken up in methanol and concentrated in vacuo. Purification by HPLC (C18, linear gradient: 5 → 10% B in A, solutions used A: H2O, B: acetonitrile, 0.5% TFA, 15 min) resulted into product 5 (23 mg, 43.5 μmol, 54%) as white solid. 1H NMR (400 MHz, MeOD): δ (ppm) 7.73 (t, J = 10.0 Hz, 1H, H-6), 5.90 (d, J = 4.4 Hz, 1H, H-1'), 5.73 (dd, J = 19.7, 8.3 Hz, 1H, H-5), 4.55 – 4.47 (m, 2H, H-5'), 4.20 (d, J = 4.1 Hz, 1H, H-4'), 4.18 – 4.11 (m, 2H, H-2', H-3'), 3.98 – 3.90 (m, 1H, H-2''), 3.84 (dd, J = 10.5, 3.6 Hz, 1H, H-8''), 3.70 – 3.59 (m, 1H, H-8''), 3.14 (t, J = 10.1 Hz, 1H, H-1''), 3.04 – 2.95 (m, 1H, H-3''), 2.03 – 1.97 (m, 1H, H-1''), 1.80 – 1.65 (m, 3H, H-5'', H-6'', H-7''). 13C NMR (101 MHz, MeOD): δ (ppm) 172.8 (C=O), 152.6 (C=O), 142.4 (C-2''), 103.1 (C-1''), 90.7 (C-1'), 82.8 (C-4'), 76.2 (C-3), 75.0 (C-2'), 72.3 (C-2'), 72.1 (C-4), 71.7 (C-2), 71.1 (C-3'), 64.2 (C-8), 46.2 (C-5), 31.3 (C-1), 27.0 (C-6), 26.2 (C-7). HRMS: calculated for \([C_{18}H_{26}N_3O_{13}S]^+\) 524.11808, found 524.11807.

Materials of biological assays
Recombinant human GBA1 (Cerezyme), recombinant human GAA (Myozyme) and recombinant human α-GalA (Fabrazyme) were obtained from Genzyme, USA. Bacterial GAA homologue CJAgd31B was kindly provided by professor Gideon Davies (University of York, UK). Cellular homogenates of a stable human embryonic kidney 293 (HEK293) over-expressing GBA2 cell line were pre-incubated for 30 min with an inhibitor of GBA1 (1 mM conduritol B epoxide (CBE)). Galactocerebrosidase (GALC) was obtained by expressing recombinant murine GALC in HEK293 cells after which the secreted recombinant protein in the culture medium was directly used. Fibroblasts of Pompe patients diagnosed on the basis of reduced GAA activity (obtained with consent from donors) were used for GANAB determinations.

Enzyme activity assays, determination of the apparent IC50 values
All activity assays were performed at 37 °C. To determine the apparent IC50 values, enzyme preparations were pre-incubated with a range of inhibitor concentrations for 30 min, where after the residual activity was measured by addition of the appropriated 4-methylumbelliferyl-based substrate mix. GBA1 residual activity was measured with 3.7 mM 4-methylumbelliférole(4-mu)-β-D-glucopyranoside in 150 mM McIlvaine buffer, pH 5.2, supplemented with 0.2% Taurocholate (w/v), 0.1% Triton X-100 (v/v), 0.1% Bovine Serum albumin (BSA) (w/v) for 30 min. GBA2 substrate mix consisted of 3.7 mM 4-mu-β-D-glucopyranoside in 150 mM McIlvaine, pH 5.8, 0.1% BSA (w/v) and was incubated for 1 h. α-GalA activity was determined with 2 mM 4-mu-α-D-galactopyranoside dissolved in 150 mM McIlvaine, pH 4.5, 0.1 % BSA (w/v) for 30 min. GAA substrate mix consisted of 3 mM 4-mu-α-D-glucopyranoside pH 5.0, 0.1% BSA (w/v) for 1 h, GALC was assayed with 1.25 mM
Synthesis of potential glycosyltransferase inhibitors based on carba-cyclophellitol

4-mu-β-D-galactopyranoside in 150 mM McIlvaine pH 4.3 containing 0.2 M NaCl and 0.1% BSA (w/v) for 1h and GANAB with 3 mM 4-mu-α-D-glucopyranoside pH 7.0, 0.1% BSA (w/v) for 2 h. The bacterial enzyme activity of CJAgd31B was determined with 3 mM 4-mu-α-D-glucopyranoside in 50 mM citrate buffer at the optimal pH 6.5, 0.1% BSA (w/v) for 1 h. After stopping the substrate reaction with an excess of 1 M NaOH-glycine (pH 10.3), liberated 4-mu fluorescence was measured with a fluorimeter LS55 (Perkin Elmer) using λEx 366 nm and λEm 445 nm. All IC50 values were determined in triplicate with Graphpad Prism 5 software.

IC50 values for GCS were determined in situ with NBD-ceramide as substrate as previously described. Briefly, RAW 264.7 cells were grown to confluence in 6-well plates and pre-incubated for 1 h with an inhibitor of GBA1 activity (300 µM CBE), followed by 1 h incubation at 37 °C with 1 nmol C6-NBD-ceramide (6-[N-methyl-N-(7-nitrobenz-2-oxa-1,3-diazol-4-y1) aminododecanoyl] sphingosine) and in the presence of a range of inhibitor concentrations. The cells were washed 3x with PBS and harvest by scraping. After lipid extraction, the C6-NBD lipids were separated and detected by HPLC (λEx 470 nm and λEm 530 nm). IC50 values were determined in duplicate from the titration curves of observed formed C6-NBD-glucosylceramide.

References

(1) Beenakker, T. J. M.; Bakker, A. T.; van de L’Isle, M. O. N.; Ferraz, M. J.; Codée, J. D. C.; van der Marel, G. A.; Aerts, J. M. F. G. and Overkleeft, H. S. contributed to the work described in this chapter.
(3) Asano, N. Glycobiology 2003, 13, 93R.
95.


