Inhibitors and probes targeting endo-glycosidases

Boer, C. de

4 Synthesis of an activity-based probe based on the Psl motif

4.1 Introduction

PslG, a retaining endo-mannosidase expressed by the pathogen *Pseudomonas aeruginosa*, is capable of digesting biofilms, a potential treatment for chronic infections. In this chapter the chemical synthesis and biological evaluation of a putative activity-based probe for PslG is described.

Pseudomonas aeruginosa biofilms

Pseudomonas aeruginosa is an opportunistic Gram negative bacterium that may be present in healthy individuals and that forms a major health concern for hospitalized patients.[1] Following surgery as well as for individuals using a medical device like a catheter or ventilator, *Pseudomonas aeruginosa* can form biofilms on compromised skin area and cause infections.[2]

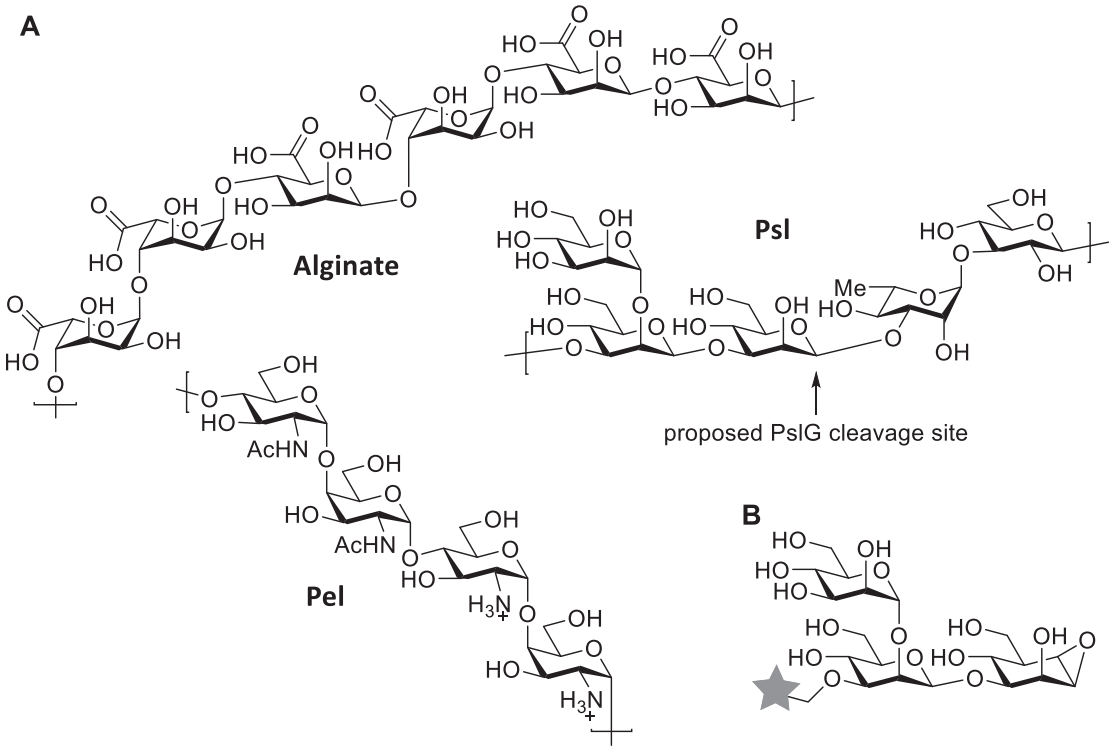


Figure 4.1| A) Simplified structures of Alginate[6] and Pel[8] and the repeating pentamer of the Psl polysaccharides. Based on molecular docking PslG was proposed to hydrolytically cleave Psl at the indicated position. **B**) Envisioned activity-based probe based on the structure of Psl and the proposed catalytic activity of PslG.

Treatment of *Pseudomonas aeruginosa* is difficult when it is embedded in a biofilm because it creates a micro environment shielding the bacterium from antibiotics and protecting it from the host immune system. Even upon eradicating the infective bacteria, the remaining biofilm offers an attractive environment for recolonization by the same or other pathogens leading to chronic infections.[3]

Pseudomonas aeruginosa generates a variety of biofilms containing proteins, DNA and at least three different polysaccharides allowing it to adapt to different environments.[4] Anionic alginate[5,6] is produced by *Pseudomonas aeruginosa* strains with a mucoid phenotype mostly found in the lungs of cystic fibrosis patients and is the most studied polysaccharide excreted by *Pseudomonas aeruginosa*. The structure is composed of β-linked and partially acetylated D-mannuronic and L-guluronic acid residues (Figure 4.1A). Strains isolated from other environments do not incorporate alginate in their biofilm, but incorporate the polysaccharides Pel or Psl instead.[4] Pel consists of partially acetylated D-glucosamine and D-galactosamine and is cationic which allows it to interact with negatively charged DNA in the biofilm.[7,8] Mutant

strains unable to produce Pel do not form pellicles, biofilms formed at the air water interface, a feature after which this particular sugar was named.[9]

Psl, the third polysaccharide produced by *Pseudomonas aeruginosa*, is a neutral polymer of pentameric repeating units consisting of D-mannose, D-glucose and L-rhamnose (Figure 4.1A).[10] The Psl polymer is produced by the combined activities of the enzymes encoded on the *polysaccharide locus*. On the locus are 15 co-transcribed proteins named PslA through to PslO of which 10, PslACDEFHIJKL, are essential for Psl biosynthesis, as determined by mutagenisis. Psl plays an important role in cell surface attachment and biofilm maintenance.[11] Mutant strains unable to synthesize Psl are unable to attach to human cells. Psl or fragments thereof also increase bis-(3',5')-cyclic dimeric GMP (c-di-GMP) levels in *Pseudomonas aeruginosa* leading to increased Psl production.[12] Psl and c-di-GMP both increase tolerance against antibiotics.[13]

PslG

PslG (47 kDa), postulated to be an endo-acting glycosyl hydrolase (GH) belonging to GH family 39, was originally believed to be essential for Psl production but this was later revoked by the same group.[10,14,15] The structure of PslG was reported[14,15] and it was postulated, based on molecular modeling, that PslG is an endo-mannosidase cleaving between the D-mannose and L-rhamnose residue of Psl.[15]

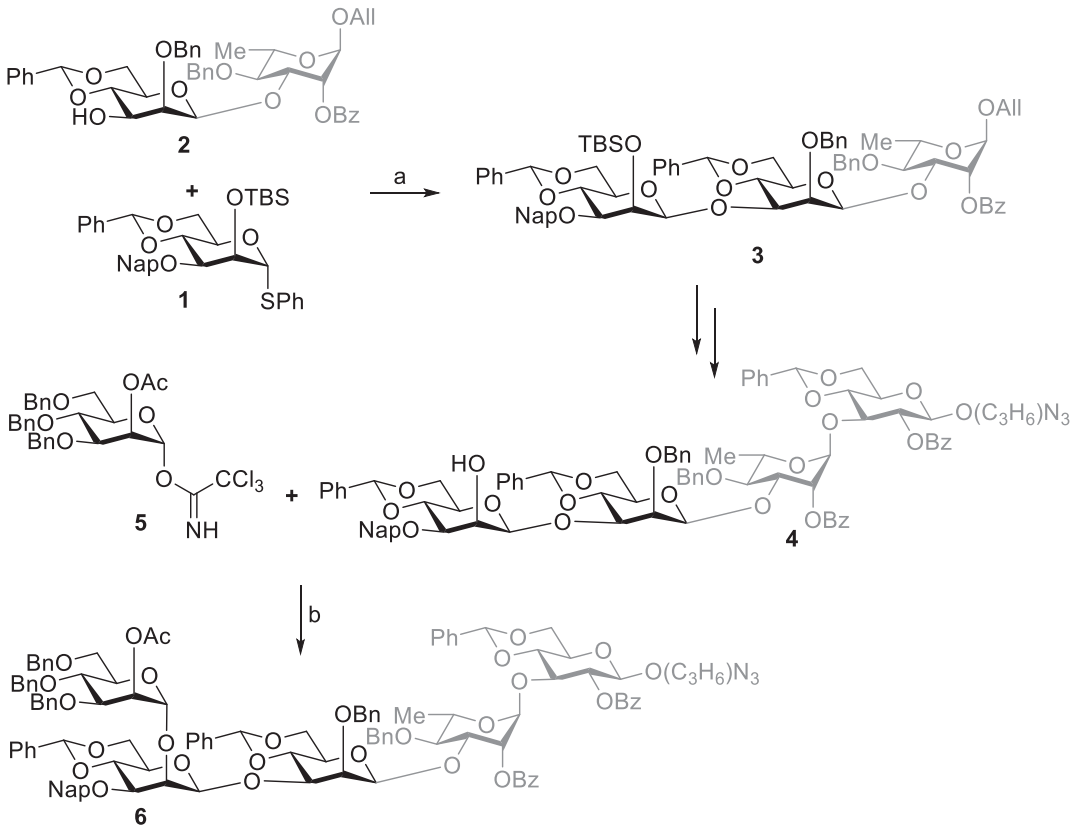
The hydrolase activity of PslG can be exploited to remove existing Psl containing biofilms and prevent biofilm formation by exogenous addition of the enzyme.[16] Exogenous addition of PslG to *Pseudomonas aeruginosa* cultures also leads to higher cell mobility, more random movement of the bacterium and slower microcolony formation.[17] PslG covalently attached to glass slides prevents the attachment of *Pseudomonas aeruginosa* and reduces biofilm formation.[18] These results show that PslG may be used to treat existing biofilms in patients with chronic infections and as a tool to design medical devices that are less susceptible to biofilm attachment. Despite the clinical potential of PslG, molecular understanding of the role endogenous PslG plays in *Pseudomonas aeruginosa* infection and biofilm formation requires more detailed studies.

PslG has been postulated to be responsible for the degradation of improperly processed or transported polymers in the periplasm.[4] However, PslG is not crucial for cell viability.[19] Recent results also suggest that PslG is important for dispersion of the bacterium by partially breaking

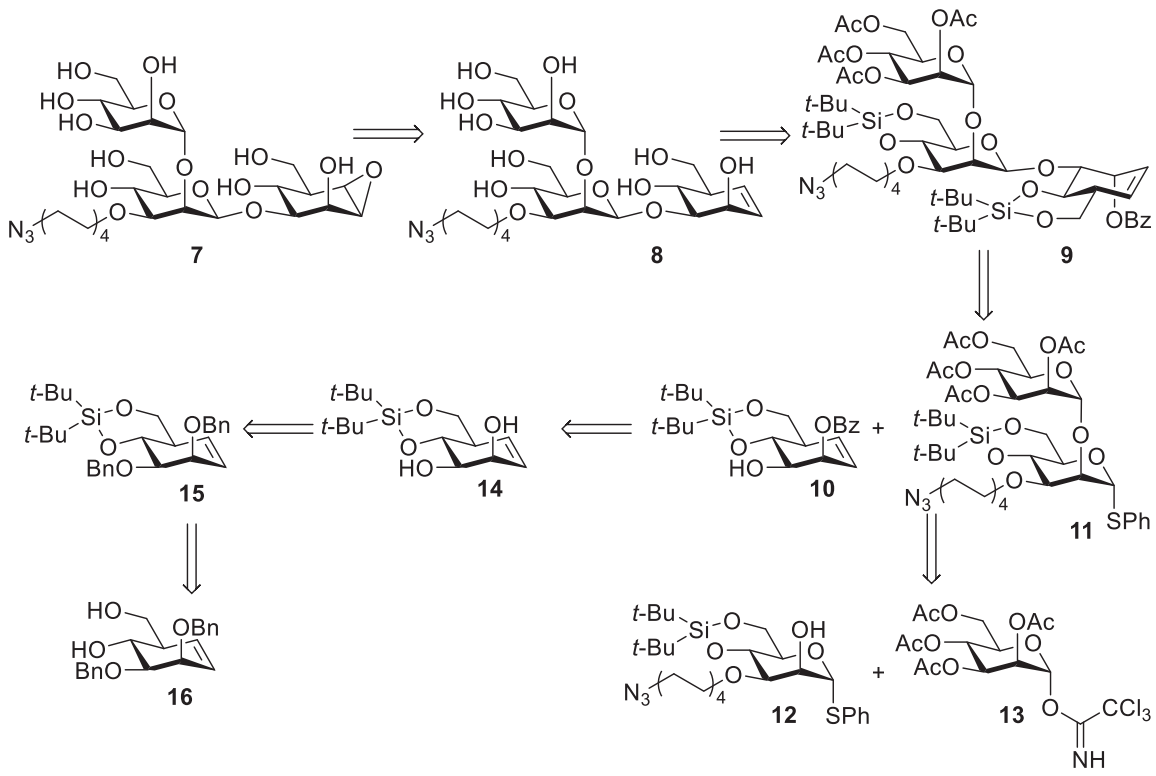
down the Psl polymers in the biofilm in a tightly regulated manner.[20] It could also be hypothesized that PslG is involved in intercellular signaling due to its ability to release complex Psl (fragments) which have been shown to have an inter cellular signaling function.[12]

PslG activity-based probes

A possible way to assess the role of PslG in *Pseudomonas aeruginosa* biology is by monitoring PslG activity *in situ* and *in vivo* by tailored activity-based probes (ABPs). A potent and selective ABP would show the activity of PslG in different cell environments and at different time points during infection and dispersion. It would also facilitate the observation of the direct effects of the inhibition of this activity compared to genetic knockouts. Because of the rare specificity of PslG the activity-based probe may serve as a tool to diagnose *Pseudomonas aeruginosa* infections. The probe may also aid in the improvement of the selectivity and stability of PslG as a therapeutic, by enabling high throughput assays for example by labeling active phage displayed PslG variants. Finally, a PslG ABP could be used for the unbiased screening of various enzyme sources looking for unknown enzymes with similar activities pointing towards undiscovered interactions in microbiology.



Scheme 4.1| Part of the synthesis of Psl fragments by the Boons group. Reagents and conditions: **a**) BSP, DTBMP, Tf_2O, DCM, -60°C, 72%, α/β 1/10. **b**) TMSOTf, DCM, -30°C, 82%.



Scheme 4.2| Retrosynthetic analysis of the trisaccharide activity-based probe.

Cyclophellitol and cyclophellitol aziridine equipped with reporter tags at various positions have proven to be effective scaffolds for exo-glycosidase ABPs.[21–23] More recently it was reported that the elongation of cyclophellitols with the appropriate carbohydrate can yield inhibitors and probes targeting various endo-glycosidases.[24,25] In the context of the work described in this chapter it was hypothesized that suitably configurated and substituted cyclophellitol derivatives equipped with a reporter tag may be effective probes to detect and monitor PslG activities as well. Based on the reported repeating unit and the proposed cleavage site a trisaccharide ABP featuring an electrophilic epoxide warhead as well as a reporter functionality was designed. The reporter tag was positioned at the non-reducing end to prevent possible degradation by exo-mannosidases (Figure 4.1B).

The synthesis strategy was inspired by the only reported chemical synthesis of Psl fragments to date, which was conducted by the Boons lab (Scheme 4.1).[26] For one of the two critical β-mannosylations donor **1** and acceptor **2** are used. This results in trisaccharide **3** in good yield and β-selectivity. **3** was elaborated into tetrasaccharide acceptor **4** which was reacted with trichloroacetimidate donor **5**. This resulted in pentasaccharide **6** containing the complete motif of the designed activity-based probe **7** (Scheme 4.2).

It was envisioned that epoxide **7** might be directly obtained from completely deprotected alkene **8** by hydrogen bond directed epoxidation. By making use of solely silyl ether- and ester-based protective groups it would be possible to conserve the azide during the deprotection sequence. Instead of using a benzylidene acetal as used by Boons to obtain β-selectivity in the mannosylation reaction, a 4,6-*O*-silylene was selected since the Bols group showed that these are also able to induce excellent β-selectivity in mannosylations.[27]

An alkyl spacer bearing an azide was attached on the central mannose to introduce the tag later in the synthesis or allow for two step activity-based protein profiling. Use of the azidosugar without the spacer on the carbohydrate moiety was avoided because of the poor availability of the required D-altrose configured starting material and the reported poor β-selectivity of 3-deoxy-3-azido mannosyl donors.[28] Based on these considerations protected trisaccharide **9** was proposed as intermediate towards **7** (Scheme 4.2).

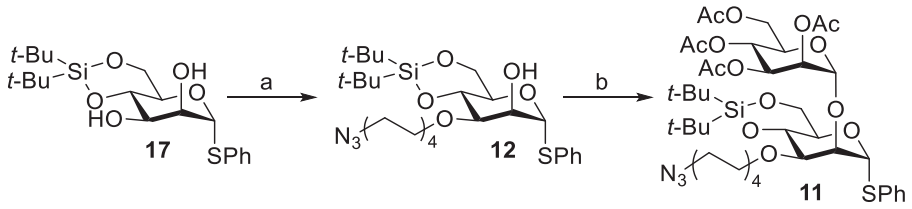
Trisaccharide **9** could be synthesized from cyclohexene acceptor **10** and disaccharide donor **11** to minimize the amount of steps after introduction of the valuable cyclohexene building block. The glycosylation in the Boons synthesis with donor **1** carrying a bulky TBS group on the 2-position is an indication that the glycosylation with the disaccharide could also yield predominantly a β-configured product. The donor could be obtained from acceptor **12** and donor **13** by neighboring group directed 1,2-trans glycosylation.

Acceptor **10** was anticipated to be accessible from diol **14** by selective acylation on the allylic alcohol. The diol could be obtained by debenzylation of fully protected **15,** which in turn could be derived from **16** of which the synthesis has been reported.[29]

4.2 Results and Discussion

Synthesis of a putative PslG activity-based probe

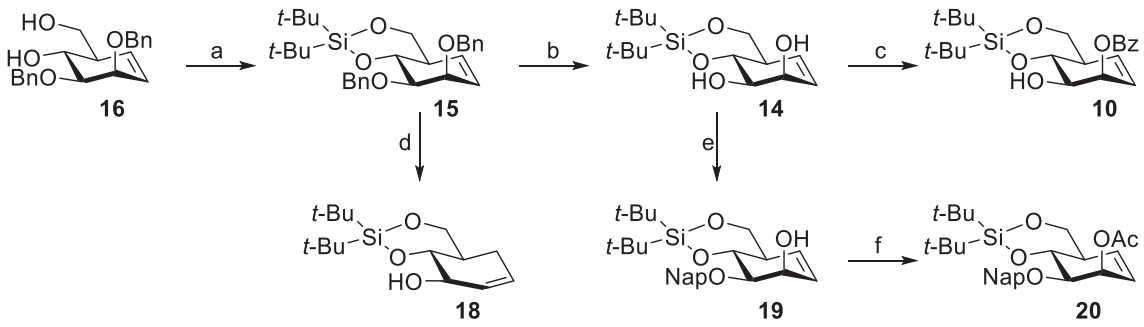
The first aim was to obtain azide tagged disaccharide donor **11** (Scheme 4.3). To this end diol **17**[27] was regioselectively alkylated in a borinate catalyzed reaction with a freshly prepared alkyl triflate under conditions similar to the alkylations described by the Taylor group.[30] Attempts to perform this reaction with the alkyl iodide were sluggish. Glycosylation of **12** with donor **13**[31] afforded disaccharide donor **11** in good yield on a gram scale.



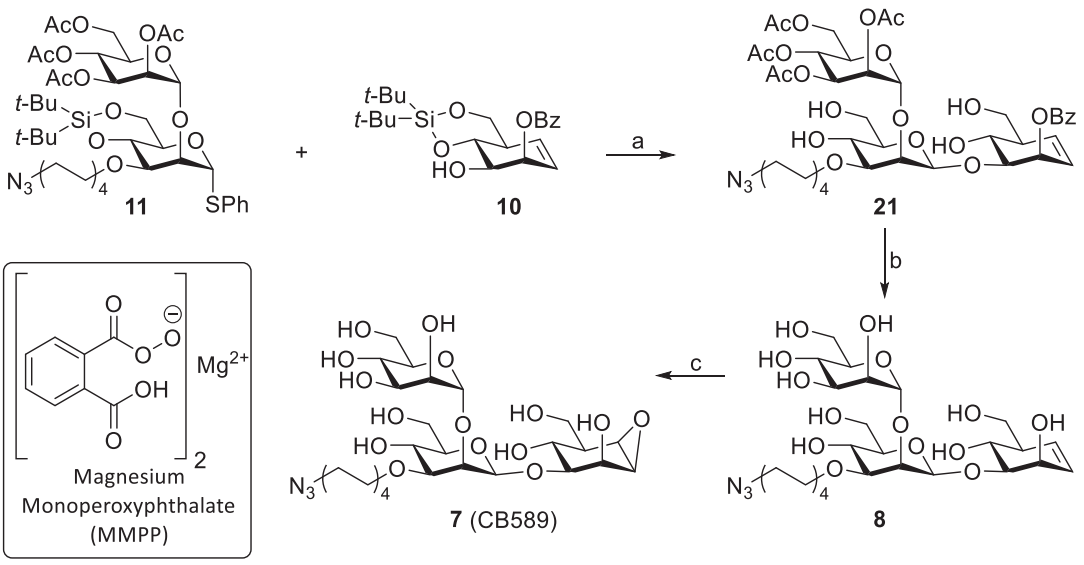
Scheme 4.3| Reagents and conditions: **a**) 8-azidooctyl trifluoromethanesulfonate, 2-aminoethyl diphenylborinate, K_2CO_3, MeCN, 0°C, 80%. **b**) **13**[31], TMSOTf, DCM, -20°C to 5°C, 78%.

The next target was the generation of a suitably protected mannose configured cyclohexene acceptor (Scheme 4.4). To this end diol **16**[29], synthesized based on the chemistry developed by Madsen *et al.*[32] was silylated to obtain **15**. Attempts to remove the benzyl ethers by dissolving metal hydrogenolysis gave low and irreproducible yield. From the conditions studied, reactions with sodium and without a proton source provided the highest yield of the desired product. The mayor observed side product, especially when using lithium and adding a proton source, was alkene **18**, which is consistent with observations made by Birch on his studies on allylic alcohols.[33] The limited solubility of the starting material in ammonia combined with the difficulty in monitoring the progress of these type of reactions by TLC analysis often led to the recovery of a large amount of starting material. Attempts to obtain the product by using lithium naphthalinide[34] or Lewis acidic BCl_3[35] failed as well. Eventually diol **14** was obtained reproducibly in high yield by debenzylation under Lewis acidic conditions using $TiCl_4$.[36]

Selective acylation of the pseudo axial, allylic alcohol in **14** was achieved in moderate yield by borinate catalysis providing **10**.[30] Attempts to orthogonally protect this alcohol with a naphthyl group provided the other regioisomer **19**.



Scheme 4.4| Reagents and conditions: **a**) Di-tert-butylsilyl ditriflate, imidazole, DMF, 73%. **b**) $TiCl_4$, DCM, toluene, 0°C, 82%. **c**) BzCl, 2-aminoethyl diphenylborinate, DIPEA, MeCN, rt, 65%. **d**) Li(s), *t*-BuOH, THF, NH_3. **e**) NapBr, 2-aminoethyl diphenylborinate, KI, K_2CO_3, MeCN. **f**) Ac_2O, pyr, DCM.



Scheme 4.5| Reagents and conditions: **a**) i. Ph_2SO, Tf_2O, TTBP, DCM, cyclohexene, -80°C -> -40°C; ii. $3HF{\cdot}Et_3N$, THF, 35% over 2 steps, 65% based on recovered **10**. **b**) NaOMe, MeOH. **c**) MMPP, NaOH, H_2O, 15% over 2 steps.

The regioselectivity was confirmed by acetylation of the remaining hydroxyl to afford **20** with a chemical shift of the allylic alcohol of 5.65 ppm compared to 3.51 ppm for the starting material. A possible mechanistic explanation for this difference could be that both reactions, the acylation and alkylation, afford mono alkylated and acylated products at the pseudo equatorial 3 OH. In the case of acylation, the acyl group can migrate to the allylic position leading to **10** whereas for alkylation migration is not possible affording **19**. Glycosylation of diol **14** with donor **11** under pre-activation conditions gave mostly the undesired 2*O*-glycosylated product.

Attempts to stereoselectively manipulate the alkene via Boc protection of **14** followed by iodine[37] ,IBr[38], or N-iodosuccinimide[39] induced iodocarbonylation were not productive presumably because of the inflexibility of the locked ring system.

Donor **11** and acceptor **10** were glycosylated under pre-activation conditions (Scheme 4.5).[40] The acceptor was partially recovered but the obtained product was difficult to separate from the hydrolyzed donor side product. This mixture was desilylated and this afforded the pure pseudo trisaccharide **21** after column chromatography. Only the β-configured product was obtained.

Deacylation with NaOMe in MeOH afforded crude **8** which was epoxidized in water with magnesium monoperoxyphthalate and NaOH to force the epoxidation to go via a hydrogen

bond directed mechanism leading to high diastereoselectivity.[41,42] This afforded epoxide **7** after size exclusion purification.

The H2 in the product shows a triplet with a coupling constant of 5.0 Hz in ^{1}H NMR which is exactly the same as the monomeric β-configured epoxide of mannose configured cyclophellitol.[29] The undesired α-configured epoxide shows a double doublet with coupling constant 2.9 and 3.1 Hz in the monomer confirming the formation of the expected β-configured epoxide.

Affinity for- and reactivity with PslG

X-ray crystallography of crystals of recombinant PslG soaked in a solution of epoxide **7** showed noncovalent binding of the alkyl spacer of the probe to a hydrophobic pocket of the enzyme (Figure 4.2). Treatment of recombinant PslG in solution at pH 5 and pH 7 did not lead to covalent attachment of the probe to the enzyme as monitored by ESI-MS of the intact protein.

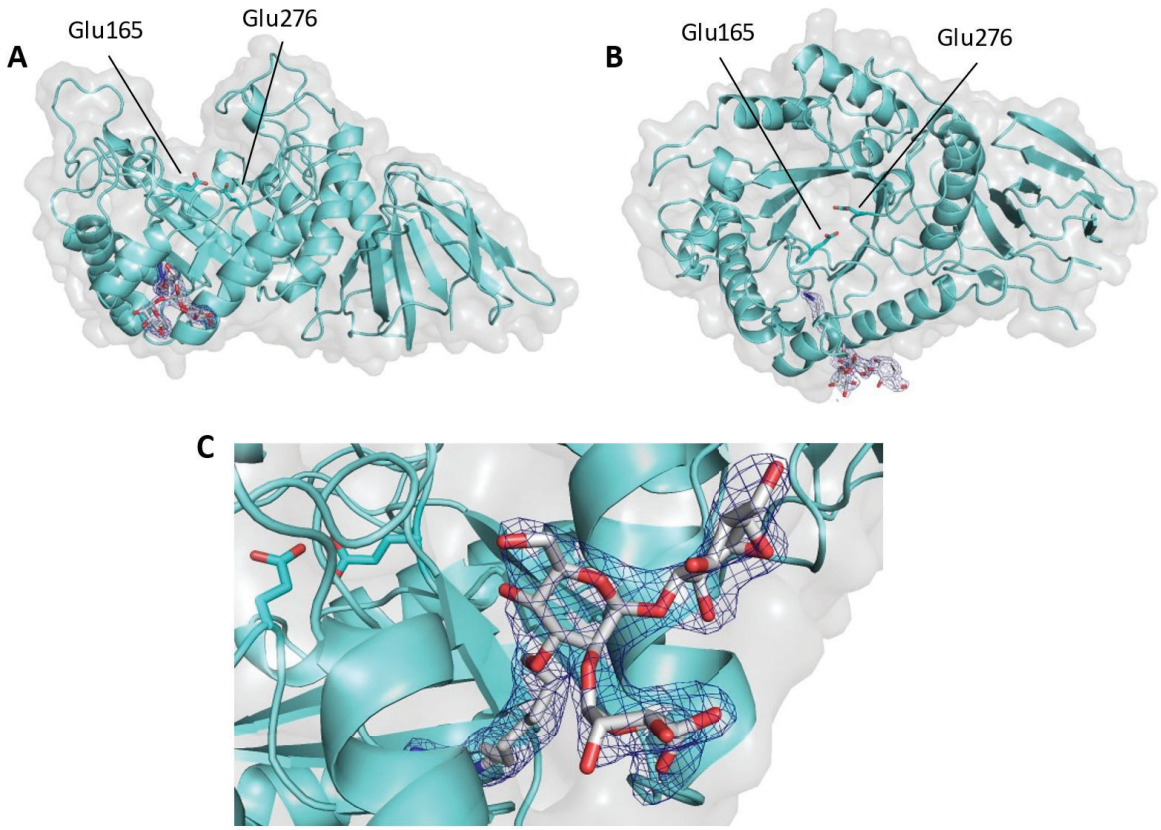


Figure 4.2| Ribbon and surface representation of PslG with **7** noncovalently bound away from the active site. Glu165 (putative acid/base) and Glu276 (putative nucleophile) are shown as stick representation. Electron density of **7** is REFMAC5 maximum-likelihood/σ_A weighted $2F_o–F_c$ map contoured to 0.8 σ (0.19 $e^-/Å^3$. **A**) Side view showing the active side cleft typical of endo-glycosidases. **B**) Top view. **C**) Closeup of **7** bound to PslG.

Attempts to compete binding to the hydrophobic pocket with 0.1 mM or 1 mM *n*-octyl-β-D-glucoside, decyl-β-D-maltoside or dodecyl-β-D-maltoside and the use of relatively high concentrations (5 μM) of probe did not lead to covalent probe binding to recombinant PslG.

4.3 Conclusion

Trisaccharide epoxide **7** was synthesized by a pre-activation protocol from a thioglycoside donor and an epi-cyclophellitol alkene acceptor further expanding the scope of glycosylation chemistry compatible with cyclophellitols and cyclophellitol precursors. The epoxide was generated late stage on a fully deprotected trisaccharide in water with good stereocontrol. The final stereochemistry of the product was confirmed by NMR spectroscopy and X-ray crystallography. The probe did not interact covalently with PslG (GH39).

CAZY[43] (www.cazy.org) GH family 39 contains mainly β-xylosidases (12) and α-L-iduronidases (4). It also contains a multifunctional enzyme (Bgxg1)[44] having β-xylosidase, β-glucosidase and β-galactosidase activities and two arabinosidases releasing disaccharides: α-L-(β-1,2)-arabinobiosidase NF2152 and D-galacto-(α-1,2)-L-arabinosidase NF2523.[45] It also contains one recently reported endo-α-L-rhamnosidase (BN863_22200)[46]. All these enzymes act on carbohydrate substrates with a 1,2-trans glycosidic linkage.

The postulation that PslG is an endo-mannosidase is based on intrinsic tryptophan fluorescence quenching with mannose, but the authors suggest this could also indicate mannose binding away from the active site.[14] In the crystal structure obtained after soaking with 3 M mannose the electron density of the mannose bound in the active side is poor.[14] Based on the results presented in this chapter showing the inability of PslG to interact with **7**, the minimal experimental evidence for the hypothesized cleavage position as well as the activities of the other GH39 family members, the postulated classification of PslG as an endo-mannosidase should perhaps be reconsidered.

4.4 Acknowledgements

Liang Wu, Nicholas McGregor and Gideon Davies from York University, UK are kindly acknowledged for the X-ray and LC-MS experiments and valuable discussion. Thijs Voskuilen and Michaela Ferrari are acknowledged for their synthesis work in the context of their Bachelor and Erasmus internships.

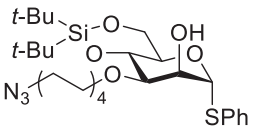
4.5 Experimental

General experimental procedures are shown in the experimental section of chapter 2.

8-azidooctyl trifluoromethanesulfonate

Tf_2O (0.54 ml, 3.22 mmol) was dissolved in DCM (7 ml) and cooled to -20°C. A solution of 8-azido-1-octanol (0.46 g, 2.68 mmol) and pyridine (0.25 ml, 3.22 mmol) in DCM (7 ml) was added and the reaction was stirred for 1 hour at the same temperature. The reaction mixture was diluted with DCM and subsequently washed with cold water and cold brine, dried with $MgSO_4$ and concentrated *in vacuo*. The reagent was used immediately without further purification.

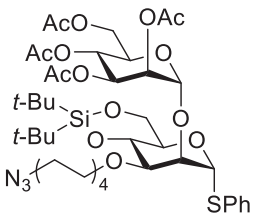
Phenyl 3-(8-azidooctyl)-4,6-*O*-ditertbutylsilyl-1-thio-α-D-mannopyranose (12)



Diol **17**[27] (1.12 g, 2.69 mmol) and K_2CO_3 (0.42 g, 3.0 mmol) were coevaporated with toluene. Freshly prepared 8-azidooctyl trifluoromethanesulfonate (1.22 g, 4.03 mmol) was dissolved in MeCN (7 ml) and added at 0°C. 2-aminoethyl diphenylborinate (0.06 g, 0.27 mmol) was dissolved in MeCN (7 ml) and added to the reaction mixture. DCM (1 ml) was added and the reaction was slowly warmed to rt. After 2 hours the reaction was quenched with $NaHCO_3$ (aq. sat.) and diluted with H_2O. The water layer was extracted with EtOAc (2x) and the combined organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The product was purified by column chromatography. (pentane/EtOAc, 10/1, v/v) (1.21 g, 2.15 mmol, 80%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.42 (m, 2H), 7.34 – 7.23 (m, 3H), 5.55 (d, *J* = 1.3 Hz, 1H, H1), 4.28 – 4.13 (m, 3H, H4/H2/H5), 4.05 – 3.92 (m, 2H, H6ab), 3.89 – 3.81 (m, 1H, CH_2O), 3.76 – 3.69 (m, 1H, CH_2O), 3.50 (dd, *J* = 8.4, 3.4 Hz, 1H, H3), 3.26 (t, *J* = 6.9 Hz, 2H, CH_2N_3), 2.83 (s, 1H, OH), 1.65 – 1.55 (m, 4H, CH_2 (2x)), 1.44 – 1.29 (m, 8H, CH_2 (4x)), 1.06 (s, 9H, (*t*-Bu), 1.04 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 133.9, 131.5, 129.3, 127.6, 87.6 (C1), 79.0 (C3), 75.1, 72.0 (CH_2O), 71.7 , 68.3, 66.5 (C6), 51.6 (CH_2N_3), 30.2 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.0 (CH_2), 27.5 (SiC(**C**H_3)$_3$), 27.2 (SiC(**C**H_3)$_3$), 26.8 (CH_2), 26.1 (CH_2), 22.7 (Si**C**(CH_3)$_3$), 20.1 (Si**C**(CH_3)$_3$). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{28}H_{47}N_3O_5SSiNa$, 588.2903 found 588.2902.

Phenyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-3-*O*-(8-azidooctyl)-4,6-*O*-ditertbutylsilyl-1-thio-α-D-mannopyranose (11)

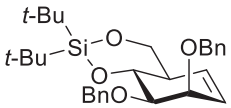


Acceptor **12** (1.21 gram, 2.14 mmol) and donor **13**[31] (1.22 gram, 2.57 mmol) were coevaporated with toluene (3x) and dissolved in DCM (11 ml, 0.2 M). 4Å molecular sieves were added and the mixture was stirred for 30 minutes. The reaction was cooled to -20°C and TMSOTf (0.08 ml, 0.43 mmol) was added. The reaction was allowed to warm to 5°C in 3 hours. The reaction was quenched with Et_3N and diluted with DCM. The organic layer was washed with $NaHCO_3$ (aq sat.). The water layer was extracted with DCM and the combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was removed *in vacuo* and the product was isolated by column chromatography (PE/EtOAc, 4/1, v/v) to afford a white sticky solid. (1.38 g, 1.67 mmol, 78%)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.42 (m, 2H), 7.37 – 7.25 (m, 3H), 5.46 (d, *J* = 1.4 Hz, 1H, H1), 5.38 (dd, *J* = 3.4, 1.8 Hz, 1H, H2'), 5.33 (dd, *J* = 9.9, 3.4 Hz, 1H, H3'), 5.21 (t, *J* = 9.8 Hz, 1H, H4'), 5.16 (d, *J* = 1.8 Hz, 1H, H1), 4.26 – 4.15 (m, 4H, H5/H4/H2/H6a), 4.07 – 3.97 (m, 4H, H6b/H6ab'/H5'), 3.85 (dt, *J* = 9.3, 6.4 Hz, 1H, C**H**$_2$O), 3.62 (dt, *J* = 9.3, 6.3 Hz, 1H, C**H**$_2$O), 3.53 (dd, *J* = 8.8, 3.1 Hz, 1H, H3), 3.26 (t, *J* = 7.0 Hz, 2H, C**H**$_2$N$_3$), 2.15 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.90 (s, 3H, OAc), 1.64 – 1.49 (m, 4H, spacer CH_2 (2x)), 1.40 – 1.28 (m, 8H, spacer CH_2 (4x)), 1.09 (s, 9H, *t*-Bu), 1.03 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8 (OAc), 170.0 (OAc), 169.8 (OAc), 169.7 (OAc), 133.9 (SPh), 131.4 (SPh), 129.4 (SPh), 127.8 (SPh), 99.6 (C1'), 87.8 (C1), 79.2 (C3), 78.4, 75.4, 72.3 (CH_2O), 69.4 (C2'), 69.2, 69.0, 68.9 (C3'), 66.5 (C4'), 66.4 (C6), 62.7 (C6), 51.6 (CH_2N_3), 30.2 (spacer), 29.4 (spacer), 29.2 (spacer), 28.9 (spacer), 27.6 (*t*-Bu), 27.2 (*t*-Bu), 26.8 (spacer), 26.1 (spacer), 22.8 (*t*-Bu), 21.0 (OAc), 20.8 (OAc), 20.8

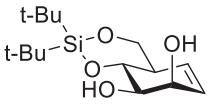
(OAc), 20.6 (OAc), 20.1 (*t*-Bu). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{42}H_{65}N_3O_{14}SSiNa$ 918.3862, found 918.3854.

2,3-*O*-benzyl-4,6-*O*-ditertbutylsilyl-mannosecyclophellitolalkene (15)



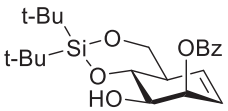
Alkene **16**[29] (1.1 g, 3.23 mmol) was coevaporated with toluene (2x), imidazole (0.9 g, 13.2 mmol) was added and the mixture was dissolved in DMF (32 ml, 0.1 M). The solution was cooled to 0°C, di-tert-butyl-silyltriflate (2.8 ml, 8.7 mmol) was added dropwise and the reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched with MeOH and the product was extracted with Et_2O (2x), the organic phase was washed with HCl (1 M), $NaHCO_3$ (aq. sat.) and brine, dried over $MgSO_4$ and volatiles were removed under reduced pressure. The product was obtained after column chromatography (PE/EtOAc, 20/1, v/v) as a colorless oil. (1.14 g, 2.36 mmol, 73%)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.26 (m, 10H), 5.69 (ddd, *J* = 9.8, 5.0, 3.0 Hz, 1H, alkene), 5.30 (dd, *J* = 9.8, 1.8 Hz, 1H, alkene), 5.06 (d, *J* = 12.4 Hz, 1H, CH_2Bn), 4.92 (d, *J* = 12.4 Hz, 1H, CH_2Bn), 4.82 (d, *J* = 12.4 Hz, 1H, CH_2Bn), 4.74 (d, *J* = 12.4 Hz, 1H, CH_2Bn), 4.43 (dd, *J* = 10.3, 9.1 Hz, 1H, H4), 4.08 (dd, *J* = 10.4, 4.6 Hz, 1H, H6a), 4.03 (t, *J* = 4.4 Hz, 1H, H2), 3.87 (dd, *J* = 12.0, 10.3 Hz, 1H, H6b), 3.52 (dd, *J* = 10.2, 4.3 Hz, 1H, H3), 2.53 – 2.44 (m, 1H, H5), 1.09 (s, 9H, *t*-Bu), 1.05 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 139.7, 139.2, 128.4, 128.4, 128.2, 127.7, 127.7, 127.6, 127.5, 127.3, 81.3 (C3), 75.6 (C4), 74.0 (CH_2Bn), 73.4 (CH_2Bn), 73.2 (C2), 68.5 (C6), 45.7 (C5), 27.6 (*t*-Bu), 27.4 (*t*-Bu), 22.9 (*t*-Bu), 20.0 (*t*-Bu). HRMS (ESI) m/z: $[M+NH_4]^+$ calc for $C_{29}H_{44}O_4SiN$ 498.3034, found 498.3033.

4,6-*O*-ditertbutylsilyl-mannocyclophellitolalkene (14)



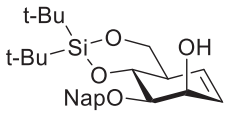
Alkene **15** (0.99 g, 2.06 mmol) was dissolved in DCM (20 ml, 0.1 M) and cooled to 0°C. A solution of $TiCl_4$ (1 M in toluene, 8.24 ml, 8.24 mmol) was added slowly. After 20 minutes, the reaction was quenched by careful addition of $NaHCO_3$ (aq. sat.). The obtained suspension was filtered over celite. The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine and dried over $MgSO_4$. The solvent was evaporated *in vacuo* and the product was isolated by column chromatography (Pentane/EtOAc, 9/1 to 7.5/2, v/v) as a colorless oil. (0.51 g, 1.7 mmol, 82%)
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.89 (ddd, *J* = 9.8, 4.8, 2.9 Hz, 1H, alkene), 5.42 (dd, *J* = 9.9, 1.8 Hz, 1H, alkene), 4.39 (t, *J* = 4.5 Hz, 1H, H2), 4.13 (dd, *J* = 10.4, 4.8 Hz, 1H, H6a), 4.02 (t, *J* = 9.7 Hz, 1H, H4), 3.83 (dd, *J* = 12.0, 10.4 Hz, 1H, H6b), 3.65 (dd, *J* = 10.1, 4.5 Hz, 1H, H3), 3.11 (s, 1H, OH), 2.87 (s, 1H, OH), 2.56 – 2.45 (m, 1H, H5), 1.05 (s, 9H, *t*-Bu), 1.01 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 128.0 (alkene), 127.7 (alkene), 74.1 (C4), 73.7 (C3), 68.1 (C6), 66.1 (C2), 43.8 (C5), 27.6 (*t*-Bu), 27.2 (*t*-Bu), 22.8 (*t*-Bu), 20.0 (*t*-Bu). HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{15}H_{28}O_4SiNa$, 323.1649 found 323.1647.

2-*O*-benzoyl-4,6-*O*-ditertbutylsilyl-mannocyclophellitolalkene (10)



Diol **14** (0.28 g, 0.94 mmol) was dissolved in MeCN (4.8 ml, 0.2 M). DIPEA (0.82 ml, 4.72 mmol), BzCl (0.33 ml, 2.83 mmol) and 2-aminoethyl diphenylborinate (21 mg, 0.094 mmol) were added and the mixture was stirred for 17 hours at rt. The reaction was diluted with Et_2O and washed with HCl (1 M), $NaHCO_3$ (aq. sat.) and brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under educed pressure. The product was obtained by column chromatography (Pentane/Et_2O, 95/5 to 85/15, v/v) as an orange oil. (250 mg, 0.611 mmol, 65%)
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 8.02 (m, 2H), 7.60 – 7.52 (m, 1H), 7.44 (m, 2H), 5.93 (ddd, *J* = 9.7, 5.0, 2.9 Hz, 1H, alkene), 5.83 (td, *J* = 4.8, 1.1 Hz, 1H, H2), 5.55 (dd, *J* = 9.7, 1.9 Hz, 1H, alkene), 4.24 – 4.15 (m, 2H, H6a/H4), 3.94 – 3.84 (m, 2H, H6b/H3), 2.63 – 2.53 (m, 1H, H5), 1.09 (s, 9H, *t*-Bu), 1.03 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 166.2 (PhCOO), 133.1 (Bz), 130.4 (Bz), 129.9 (Bz), 129.7 (alkene), 128.5 (Bz), 125.5 (alkene), 74.7 (C4), 72.5 (C3), 68.4 (C2), 68.1 (C6), 44.2 (C5), 27.6 (*t*-Bu), 27.2 (*t*-Bu), 23.0 (*t*-Bu), 20.0 (*t*-Bu). HRMS (ESI) m/z: $[M+H]^+$ calc for $C_{22}H_{33}O_5Si$ 405.2092, found 405.2089.

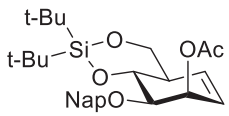
3-*O*-2-methylnaphtalene-4,6-*O*-ditertbutylsilyl-mannocyclophellitolalkene (19)



Diol **14** (0.129 g, 0.430 mmol) was coevaporated with toluene and dissolved in MeCN (2.0 ml). 2-aminoethyl diphenylborinate (0.01 g, 0.04 mmol), K_2CO_3 (0.071 g, 0.52 mmol), KI (0.071 g, 0.43 mmol) and NapBr (0.11 g, 0.53 mmol) were added and the solution was stirred at rt overnight. The reaction was quenched with water and extracted with Et_2O (2x). The combined organic phase was washed with $NaHCO_3$ (aq. sat.) and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was analyzed without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 – 7.76 (m, 4H), 7.60 – 7.52 (m, 1H), 7.51 – 7.42 (m, 2H), 5.81 (ddd, *J* = 9.8, 4.8, 2.9 Hz, 1H, alkene), 5.33 (dd, *J* = 9.8, 1.8 Hz, 1H, alkene), 5.21 (dd, *J* = 11.9, 0.8 Hz, 1H, CH_2Nap), 4.98 (d, *J* = 11.9 Hz, 1H, CH_2Nap), 4.37 – 4.25 (m, 2H, H3/H4), 4.10 (dd, *J* = 10.4, 4.7 Hz, 1H, H6a), 3.85 (dd, *J* = 12.0, 10.4 Hz, 1H, H6b), 3.51 (dd, *J* = 10.1, 4.6 Hz, 1H, H2), 3.05 (s, 1H, OH), 2.57 – 2.47 (m, 1H, H5), 1.10 (s, 9H, *t*-Bu), 1.06 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 136.3, 133.3, 133.1, 128.3, 128.0, 127.9 (alkene), 127.8, 127.6 (alkene), 126.7, 126.2, 126.1, 126.0, 126.0, 79.8 (C2), 75.2 (C3), 74.2 (CH_2Nap), 68.4 (C6), 66.8 (C4), 44.8 (C5), 27.6 (*t*-Bu), 27.3 (*t*-Bu), 22.8 (Cq *t*-Bu), 20.0 (Cq *t*-Bu).

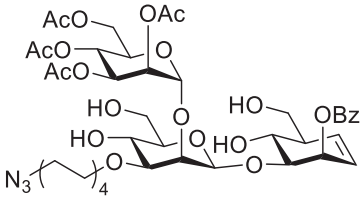
2-*O*-acetyl-3-*O*-(2-methylnaphtalene)-4,6-*O*-ditertbutylsilyl-mannocyclophellitolalkene (20)



Crude alcohol **19** was dissolved in pyridine (1 ml) and Ac_2O (1 ml) and stirred overnight. The was concentrated under reduced pressure, coevaporated with toluene and analyzed without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 – 7.78 (m, 4H), 7.57 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.49 – 7.42 (m, 2H), 5.70 (ddd, *J* = 9.5, 5.0, 2.9 Hz, 1H, alkene), 5.65 (t, *J* = 4.8 Hz, 1H, H2), 5.42 (dd, *J* = 9.6, 1.9 Hz, 1H, alkene), 5.03 – 4.93 (m, 2H, CH_2Nap), 4.27 (dd, *J* = 10.3, 9.1 Hz, 1H, H4), 4.11 (dd, *J* = 10.4, 4.6 Hz, 1H, H6a), 3.87 (dd, *J* = 12.0, 10.4 Hz, 1H, H6b), 3.58 (dd, *J* = 10.3, 4.6 Hz, 1H, H3), 2.11 (s, 3H, OAc), 1.11 (s, 9H, *t*-Bu), 1.05 (s, 9H, *t*-Bu). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.8 (OAc), 136.5 (alkene), 133.3, 133.1, 129.5, 129.1, 128.3, 128.2, 128.0, 127.8, 126.4, 126.1, 126.0, 125.8, 125.4 (alkene), 78.1 (C3), 75.2 (C4), 73.5 (CH_2Nap), 68.3 (C6), 67.6 (C2), 45.1 (C5), 27.6 (*t*-Bu), 27.4 (*t*-Bu), 22.9 (Cq *t*-Bu), 21.4 (OAc), 20.0 (Cq *t*-Bu).

2-*O*-benzoyl-3-*O*-(2-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-3-*O*-(8-azidooctyl)-β-D-mannopyranosyl)-mannocyclophellitolalkene (21)



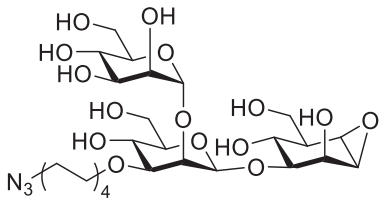
Disaccharide donor **11** (0.20 g, 0.22 mmol), diphenyl sulfoxide (0.045 g, 0.22 mmol) and TTBP (0.15 g, 0.59 mmol) were coevaporated with toluene (2x). The dry starting materials were dissolved in DCM (2 ml), 4Å molecular sieves were added and the mixture was stirred at rt for 30 minutes. The reaction was cooled to -72°C and Tf_2O (0.3 M in DCM, 0.7 ml, 0.21 mmol) was added. The reaction was warmed to -60°C over 30 minutes and was subsequently cooled to -80°C. Acceptor **10** (0.060 g, 0.15 mmol) and cyclohexene (0.08 ml, 0.74 mmol) were dissolved in DCM (1 ml) and added slowly to the reaction mixture. The reaction was allowed to warm to -40°C and was quenched with Et_3N at that temperature. The mixture was diluted with EtOAc. The molecular sieves were removed and the solution was washed with $NaHCO_3$ (aq. sat.) and brine. The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. Column chromatography (Pentane/EtOAc, 90/10 to 84/16, v/v) yielded a mixture of product (**9**) and donor (**11**) (85 mg) and unreacted acceptor **10** (26 mg, 43%).

The product mixture was taken up in THF (5 ml) and $3HF{\cdot}Et_3N$ (0.15 ml, 6,59 mmol) was added. The reaction was stirred overnight. More $3HF{\cdot}Et_3N$ (0.15 ml, 6.59 mmol) was added and the reaction was stirred for 5 hours. The mixture was diluted with THF and $CaCO_3$ (1.0 g, 10 mmol) was added. The suspension was stirred for 30 minutes before it was filtered over celite. The solvent was removed *in vacuo* and the pure product was obtained by column chromatography (DCM/Acetone, 1/0 to 2/8, v/v) as a colorless oil. (47 mg, 0.053 mmol, 35%, 65% based on recovered acceptor)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 7.98 (m, 2H, Bz), 7.60 – 7.54 (m, 1H, Bz), 7.45 (m, 2H, Bz), 5.92 – 5.82 (m, 3H, alkene (2x)/H2), 5.34 (dd, *J* = 10.1, 3.4 Hz, 1H, H3”), 5.22 – 5.14 (m, 2H, H4”/H2”), 4.90 (d,

J = 2.0 Hz, 1H, H1”), 4.80 (s, 1H, H1’), 4.25 (dt, *J* = 10.0, 3.3 Hz, 1H, H5”), 4.12 (dd, *J* = 10.0, 3.5 Hz, 1H, H3), 4.09 – 3.99 (m, 2H, H6a”/H4), 3.99 – 3.79 (m, 6H, H2’/H6ab/H6ab’/H4’), 3.60 (dt, *J* = 9.1, 6.5 Hz, 1H, spacer), 3.55 – 3.41 (m, 2H, H6b”/spacer), 3.38 (ddd, *J* = 9.7, 5.0, 3.0 Hz, 1H, H5’), 3.30 (dd, *J* = 9.4, 2.5 Hz, 1H, H3’), 3.26 (t, *J* = 7.0 Hz, 2H, CH_2N3), 2.53 (m, 1H, H5), 2.08 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.59 (m, 4H, spacer), 1.39 – 1.27 (m, 8H, spacer). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7, 170.2, 169.8, 166.5, 133.6, 133.4, 129.8, 129.7, 128.8, 123.6 (alkene), 97.7 (C1”), 97.3 (C1’), 82.2 (C3’), 77.3 (C3), 76.6 (H5’), 72.8 (C2’), 70.2 (spacer), 69.5 (C2”), 69.2 (3”), 68.6 (C5”/C4), 66.7 (C4’), 66.1 (C4”), 65.7 (C2), 64.9 (C6’), 62.3 (C6”), 62.1 (C6), 51.6 (CH_2N_3), 46.7 (C5), 29.8, 29.4, 29.1, 28.9, 26.8, 26.0 (spacer 6x), 21.0, 20.9, 20.8 (OAc 4x). $^1J_{H,C}$ (H1”) 4.90 ppm, 97.7 ppm = 171 Hz, $^1J_{H,C}$ (H1’) 4.80 ppm, 97.3 ppm = 154 Hz. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{42}H_{59}N_3O_{19}Na$ 932.3640, found 932.3654.

3-*O*-(2-*O*-(α-D-mannopyranosyl)-3-*O*-(8-azidooctyl)-β-D-mannopyranosyl)-β-mannocyclophellitol (7)



Alkene **21** (17 mg, 19 µmol) was dissolved in MeOH (0.5 ml) a catalytic amount of NaOMe was added and the reaction was monitored by LC-MS. Upon completion the reaction was quenched with AcOH and the solvent was evaporated under reduced pressure. The crude product (**8**) was dissolved in H_2O (1 ml), NaOH (20 mg, 500 µmol) and magnesium monoperoxyphthalate (80%, 21 mg) were added. The mixture was stirred for 5 hours followed by purification on HW40 (150 mM NH_4HCO_3, H_2O). This yielded the product after elution of the salts. (1.83 mg, 2.9 µmol, 15%)

^{1}H NMR (850 MHz, D_2O) δ 5.04 (d, *J* = 1.7 Hz, 1H, H1”), 4.70 (H1’, obscured by HDO), 4.43 (t, *J* = 5.0 Hz, 1H, H2), 4.38 (d, *J* = 2.7 Hz, 1H, H2’), 4.08 (ddd, *J* = 10.2, 5.6, 2.3 Hz, 1H, H5”), 3.95 (dd, *J* = 3.5, 1.7 Hz, 1H, H2”), 3.89 (dd, *J* = 11.1, 4.4 Hz, 1H, H6a), 3.86 – 3.82 (m, 2H, H6a’/H3”), 3.79 (dd, *J* = 12.1, 2.3 Hz, 1H, H6a”), 3.75 (dd, *J* = 11.2, 8.0 Hz, 1H, H6b), 3.72 – 3.64 (m, 4H, H3/H6b’/H6b”/spacer), 3.58 (t, *J* = 8.6 Hz, 1H, H4”), 3.57 – 3.51 (m, 3H, H4’/H4/spacer), 3.47 (dd, *J* = 4.1, 2.1 Hz, 1H, epoxide), 3.45 – 3.42 (m, 2H, epoxide/H3’), 3.32 (ddd, *J* = 9.4, 6.7, 2.3 Hz, 1H, H5’), 3.24 (t, *J* = 6.9 Hz, 2H, CH_2N_3), 2.06 (tdd, *J* = 8.2, 4.4, 2.2 Hz, 1H, H5), 1.58 – 1.49 (m, 4H, spacer), 1.34 – 1.23 (m, 8H, spacer). ^{13}C NMR (214 MHz, D_2O) δ = 100.4 (C1”), 98.3 (C1’), 81.9 (C3’), 79.1 (C3), 76.8 (C5’), 72.4 (C5”), 71.5 (C2’), 70.6 (spacer), 70.3 (C3”), 70.0 (C2”), 66.7 (C4”), 66.0 (C4’), 64.2 (C4), 63.6 (C2), 61.0 (C6’/C6”), 60.7 (C6), 55.6 (epoxide), 53.4 (epoxide), 51.2 (CH_2N_3), 44.0 (C5), 28.8, 28.3, 28.1, 27.9, 25.8, 25.1 (spacer 6x). $^1J_{H,C}$ (H1”) 5.04 ppm, 100.4 ppm = 172 Hz, $^1J_{H,C}$ (H1’) 4.70 ppm, 98.3 ppm = 160 Hz. HRMS (ESI) m/z: $[M+Na]^+$ calc for $C_{27}H_{47}N_3O_{15}Na$ 676.2905, found 676.2914.

4.6 References

1. Driscoll, J. A.; Brody, S. L.; Kollef, M. H. The Epidemiology, Pathogenesis and Treatment of Pseudomonas Aeruginosa Infections. *Drugs* **2007**, *67* (3), 351-368.
2. Percival, S. L.; Suleman, L.; Vuotto, C.; Donelli, G. Healthcare-Associated Infections, Medical Devices and Biofilms: Risk, Tolerance and Control. *J. Med. Microbiol.* **2015**, *64* (4), 323-334.
3. Koo, H.; Allan, R. N.; Howlin, R. P.; Stoodley, P.; Hall-Stoodley, L. Targeting Microbial Biofilms: Current and Prospective Therapeutic Strategies. *Nat. Rev. Microbiol.* **2017**, *15* (12), 740-755.
4. Franklin, M. J.; Nivens, D. E.; Weadge, J. T.; Howell, P. L. Biosynthesis of the Pseudomonas Aeruginosa Extracellular Polysaccharides, Alginate, Pel, and Psl. *Front. Microbiol*. **2011**, *2*, 167
5. Linker, A.; Jones, R. S. A Polysaccharide Resembling Alginic Acid from a Pseudomonas Micro-Organism. *Nature* **1964**, *204* (4954), 187-188.
6. Zhang, Q.; van Rijssel, E. R.; Walvoort, M. T. C.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Acceptor Reactivity in the Total Synthesis of Alginate Fragments Containing α-L-Guluronic Acid and β-D-Mannuronic Acid. *Angew. Chemie Int. Ed.* **2015**, *54* (26), 7670-7673.
7. Jennings, L. K.; Storek, K. M.; Ledvina, H. E.; Coulon, C.; Marmont, L. S.; Sadovskaya, I.; Secor, P. R.; Tseng, B. S.; Scian, M.; Filloux, A.; et al. Pel Is a Cationic Exopolysaccharide That Cross-Links Extracellular DNA in the Pseudomonas Aeruginosa Biofilm Matrix. *Proc. Natl. Acad. Sci.* **2015**, *112* (36), 11353-11358.
8. Wang, L.; Zhang, Y.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Reagent Controlled

Glycosylations for the Assembly of Well-Defined Pel Oligosaccharides. *J. Org. Chem.* **2020**, doi:acs.joc.0c00703.

9. Friedman, L.; Kolter, R. Genes Involved in Matrix Formation in Pseudomonas Aeruginosa PA14 Biofilms. *Mol. Microbiol.* **2003**, *51* (3), 675-690.
10. Byrd, M. S.; Sadovskaya, I.; Vinogradov, E.; Lu, H.; Sprinkle, A. B.; Richardson, S. H.; Ma, L.; Ralston, B.; Parsek, M. R.; Anderson, E. M.; et al. Genetic and Biochemical Analyses of the Pseudomonas Aeruginosa Psl Exopolysaccharide Reveal Overlapping Roles for Polysaccharide Synthesis Enzymes in Psl and LPS Production. *Mol. Microbiol.* **2009**, *73* (4), 622-638.
11. Ma, L.; Jackson, K. D.; Landry, R. M.; Parsek, M. R.; Wozniak, D. J. Analysis of Pseudomonas Aeruginosa Conditional Psl Variants Reveals Roles for the Psl Polysaccharide in Adhesion and Maintaining Biofilm Structure Postattachment. *J. Bacteriol.* **2006**, *188* (23), 8213-8221.
12. Irie, Y.; Borlee, B. R.; O'Connor, J. R.; Hill, P. J.; Harwood, C. S.; Wozniak, D. J.; Parsek, M. R. Self-Produced Exopolysaccharide Is a Signal That Stimulates Biofilm Formation in Pseudomonas Aeruginosa. *Proc. Natl. Acad. Sci.* **2012**, *109* (50), 20632-20636.
13. Murakami, K.; Ono, T.; Viducic, D.; Somiya, Y.; Kariyama, R.; Hori, K.; Amoh, T.; Hirota, K.; Kumon, H.; Parsek, M. R.; et al. Role of Psl Genes in Antibiotic Tolerance of Adherent Pseudomonas Aeruginosa. *Antimicrob. Agents Chemother.* **2017**, *61* (7), e02587-16.
14. Baker, P.; Whitfield, G. B.; Hill, P. J.; Little, D. J.; Pestrak, M. J.; Robinson, H.; Wozniak, D. J.; Howell, P. L. Characterization of the Pseudomonas Aeruginosa Glycoside Hydrolase PslG Reveals That Its Levels Are Critical for Psl Polysaccharide Biosynthesis and Biofilm Formation. *J. Biol. Chem.* **2015**, *290* (47), 28374-28387.
15. Yu, S.; Su, T.; Wu, H.; Liu, S.; Wang, D.; Zhao, T.; Jin, Z.; Du, W.; Zhu, M. J.; Chua, S. L.; et al. PslG, a Self-Produced Glycosyl Hydrolase, Triggers Biofilm Disassembly by Disrupting Exopolysaccharide Matrix. *Cell Res.* **2015**, *25* (12), 1352-1367.
16. Baker, P.; Hill, P. J.; Snarr, B. D.; Alnabelseya, N.; Pestrak, M. J.; Lee, M. J.; Jennings, L. K.; Tam, J.; Melnyk, R. A.; Parsek, M. R.; et al. Exopolysaccharide Biosynthetic Glycoside Hydrolases Can Be Utilized to Disrupt and Prevent Pseudomonas Aeruginosa Biofilms. *Sci. Adv.* **2016**, *2* (5), e1501632.
17. Zhang, J.; He, J.; Zhai, C.; Ma, L. Z.; Gu, L.; Zhao, K. Effects of PslG on the Surface Movement of Pseudomonas Aeruginosa. *Appl. Environ. Microbiol.* **2018**, *84* (13), e00219-18.
18. Asker, D.; Awad, T. S.; Baker, P.; Howell, P. L.; Hatton, B. D. Non-Eluting, Surface-Bound Enzymes Disrupt Surface Attachment of Bacteria by Continuous Biofilm Polysaccharide Degradation. *Biomaterials* **2018**, *167*, 168-176.
19. Jain, S.; Ohman, D. E. Role of an Alginate Lyase for Alginate Transport in Mucoid Pseudomonas Aeruginosa. *Infect. Immun.* **2005**, *73* (10), 6429-6436.
20. Cherny, K. E.; Sauer, K. Untethering and Degradation of the Polysaccharide Matrix Are Essential Steps in the Dispersion Response of Pseudomonas Aeruginosa Biofilms. *J. Bacteriol.* **2019**, *202* (3), e00575-19.
21. Witte, M. D.; Kallemeijn, W. W.; Aten, J.; Li, K.-Y.; Strijland, A.; Donker-Koopman, W. E.; van den Nieuwendijk, A. M. C. H.; Bleijlevens, B.; Kramer, G.; Florea, B. I.; et al. Ultrasensitive in Situ Visualization of Active Glucocerebrosidase Molecules. *Nat. Chem. Biol.* **2010**, *6* (12), 907-913.
22. Kallemeijn, W. W.; Li, K.-Y.; Witte, M. D.; Marques, A. R. A.; Aten, J.; Scheij, S.; Jiang, J.; Willems, L. I.; Voorn-Brouwer, T. M.; van Roomen, C. P. A. A.; et al. Novel Activity-Based Probes for Broad-Spectrum Profiling of Retaining β-Exoglucosidases In Situ and In Vivo. *Angew. Chemie Int. Ed.* **2012**, *51* (50), 12529-12533.
23. Wu, L.; Jiang, J.; Jin, Y.; Kallemeijn, W. W.; Kuo, C.-L.; Artola, M.; Dai, W.; van Elk, C.; van Eijk, M.; van der Marel, G. A.; et al. Activity-Based Probes for Functional Interrogation of Retaining β-Glucuronidases. *Nat. Chem. Biol.* **2017**, *13* (8), 867-873.
24. Caner, S.; Zhang, X.; Jiang, J.; Chen, H. M.; Nguyen, N. T.; Overkleeft, H.; Brayer, G. D.; Withers, S. G. Glucosyl Epi-Cyclophellitol Allows Mechanism-Based Inactivation and Structural Analysis of Human Pancreatic α-Amylase. *FEBS Lett.* **2016**, *590* (8), 1143-1150.
25. Schröder, S. P.; de Boer, C.; McGregor, N. G. S.; Rowland, R. J.; Moroz, O.; Blagova, E.; Reijngoud, J.; Arentshorst, M.; Osborn, D.; Morant, M. D.; et al. Dynamic and Functional Profiling of Xylan-Degrading Enzymes in Aspergillus Secretomes Using Activity-Based Probes. *ACS Cent. Sci.* **2019**, *5* (6), 1067-1078.
26. Li, H.; Mo, K.-F. F.; Wang, Q.; Stover, C. K.; Digiandomenico, A.; Boons, G.-J. Epitope Mapping of Monoclonal Antibodies Using Synthetic Oligosaccharides Uncovers Novel Aspects of Immune Recognition of the Psl Exopolysaccharide of Pseudomonas Aeruginosa. *Chem. Eur. J.* **2013**, *19* (51), 17425-17431.
27. Heuckendorff, M.; Bendix, J.; Pedersen, C. M.; Bols, M. β-Selective Mannosylation with a 4,6-Silylene-Tethered Thiomannosyl Donor. *Org. Lett.* **2014**, *16* (4), 1116-1119.

28. Crich, D.; Xu, H. Direct Stereocontrolled Synthesis of 3-Amino-3-Deoxy-β-Mannopyranosides: Importance of the Nitrogen Protecting Group on Stereoselectivity. *J. Org. Chem.* **2007**, *72* (14), 5183-5192.
29. Armstrong, Z.; Kuo, C. L.; Lahav D.; Liu, B.; Johnson, R.; Beenakker T. J. M.; de Boer, C.; Wong C. S.; van Rijssel E. R., Debets M. F.; et al. Manno-epi-cyclophellitols Enable Activity-Based Protein Profiling of Human α-Mannosidases and Discovery of New Golgi Mannosidase II Inhibitors. *J. Am. Chem. Soc.* **2020**, DOI: 10.1021/jacs.0c03880
30. Lee, D.; Williamson, C. L.; Chan, L.; Taylor, M. S. Regioselective, Borinic Acid-Catalyzed Monoacylation, Sulfonylation and Alkylation of Diols and Carbohydrates: Expansion of Substrate Scope and Mechanistic Studies. *J. Am. Chem. Soc.* **2012**, *134* (19), 8260-8267.
31. Mori, M.; Ito, Y.; Ogawa, T. Total Synthesis of the Mollu-Series Glycosyl Ceramides α-D-Man*p*-(1→3)-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer and α-D-Man*p*-(1→3)-[β-D-Xyl*p*-(1→2)]-β-D-Man*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer. *Carbohydr. Res.* **1990**, *195* (2), 199-224.
32. Hansen, F. G.; Bundgaard, E.; Madsen, R. A Short Synthesis of (+)-Cyclophellitol. *J. Org. Chem.* **2005**, *70* (24), 10139-10142.
33. Birch, A. J. 212. Reduction by Dissolving Metals. Part II. *J. Chem. Soc.* **1945**, 809-813.
34. Liu, H.-J.; Yip, J.; Shia, K.-S. Reductive Cleavage of Benzyl Ethers with Lithium Naphthalenide. A Convenient Method for Debenzylation. *Tetrahedron Lett.* **1997**, *38* (13), 2253-2256.
35. Okano, K.; Okuyama, K.; Fukuyama, T.; Tokuyama, H. Mild Debenzylation of Aryl Benzyl Ether with BCl_3 in the Presence of Pentamethylbenzene as a Non-Lewis-Basic Cation Scavenger. *Synlett.* **2008**, *13*, 1977-1980.
36. Kamal, A.; Krishnaji, T.; Reddy, P. V. Enantioselective Total Synthesis of Both the Stereoisomers of Dihydrokawain-5-Ol. *Tetrahedron: Asymmetry* **2007**, *18* (15), 1775-1779.
37. Bartlett, P. A.; Meadows, J. D.; Brown, E. G.; Morimoto, A.; Jernstedt, K. K. Carbonate Extension. A Versatile Procedure for Functionalization of Acyclic Homoallylic Alcohols with Moderate Stereocontrol. *J. Org. Chem.* **1982**, *47* (21), 4013-4018.
38. Duan, J. J.-W.; Sprengeler, P. A.; Smith, A. B. Iodine Monobromide (IBr) at Low Temperature: A Superior Protocol for Diastereoselective Cyclizations of Homoallylic Carbonates. *Tetrahedron Lett.* **1992**, *33* (43), 6439-6442.
39. Shan, M.; Sharif, E. U.; O'Doherty, G. A. Total Synthesis of Jadomycin A and a Carbasugar Analogue of Jadomycin B. *Angew. Chemie Int. Ed.* **2010**, *49* (49), 9492-9495.
40. Codée, J. D. C.; Litjens, R. E. J. N.; den Heeten, R.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. Ph_2SO/Tf_2O: A Powerful Promotor System in Chemoselective Glycosylations Using Thioglycosides. *Org. Lett.* **2003**, *5* (9), 1519-1522.
41. Washington, I.; Houk, K. N. Epoxidations by Peracid Anions in Water: Ambiphilic Oxenoid Reactivity and Stereoselectivity. *Org. Lett.* **2002**, *4* (16), 2661-2664.
42. Ye, D.; Fringuelli, F.; Piermatti, O.; Pizzo, F. Highly Diastereoselective Epoxidation of Cycloalkenols with Monoperoxyphthalic Acid in Water. *J. Org. Chem.* **1997**, *62* (11), 3748-3750.
43. Lombard, V.; Golaconda Ramulu, H.; Drula, E.; Coutinho, P. M.; Henrissat, B. The Carbohydrate-Active Enzymes Database (CAZy) in 2013. *Nucleic Acids Res* **2014**, *42* , 490-495.
44. Morrison, J. M.; Elshahed, M. S.; Youssef, N. A Multifunctional GH39 Glycoside Hydrolase from the Anaerobic Gut Fungus Orpinomyces Sp. Strain C1A. *PeerJ* **2016**, *4*, e2289.
45. Jones, D. R.; Uddin, M. S.; Gruninger, R. J.; Pham, T. T. M.; Thomas, D.; Boraston, A. B.; Briggs, J.; Pluvinage, B.; McAllister, T. A.; Forster, R. J.; et al. Discovery and Characterization of Family 39 Glycoside Hydrolases from Rumen Anaerobic Fungi with Polyspecific Activity on Rare Arabinosyl Substrates. *J. Biol. Chem.* **2017**, *292* (30), 12606-12620.
46. Reisky, L.; Préchoux, A.; Zühlke, M.-K.; Bäumgen, M.; Robb, C. S.; Gerlach, N.; Roret, T.; Stanetty, C.; Larocque, R.; Michel, G.; et al. A Marine Bacterial Enzymatic Cascade Degrades the Algal Polysaccharide Ulvan. *Nat. Chem. Biol.* **2019**, *15* (8), 803-812.