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Inhibitors and probes targeting endo-glycosidases

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Summary and future prospects

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In this dissertation covalent glycosidase inhibitors and activity-based probes (ABPs) for retaining endo-glycosidases are described. The design of the molecules is based on the natural product cyclophellitol. *Chapter 1* highlights previous applications of cyclophellitol derivatives, discusses mechanistic and conformational aspects of the irreversible reaction of cyclophellitol with retaining glycosidases and provides an overview of activity-based protein profiling (ABPP) protocols.

The subsequent chapters describe the chemical synthesis and biological evaluation of inhibitors and probes for xyloglucan active retaining glycosidases, human heparanase and PslG from *Pseudomonas aeruginosa*. This final chapter briefly summarizes the results and suggests directions for future research projects.

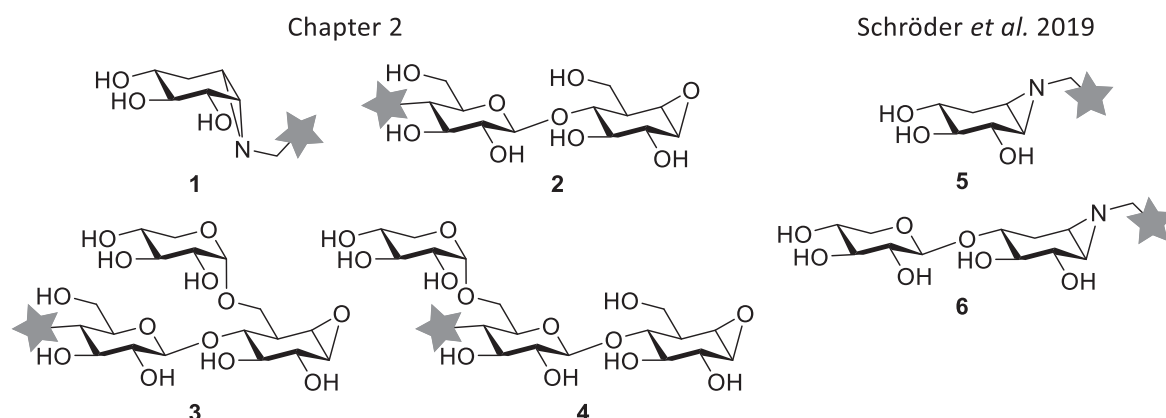


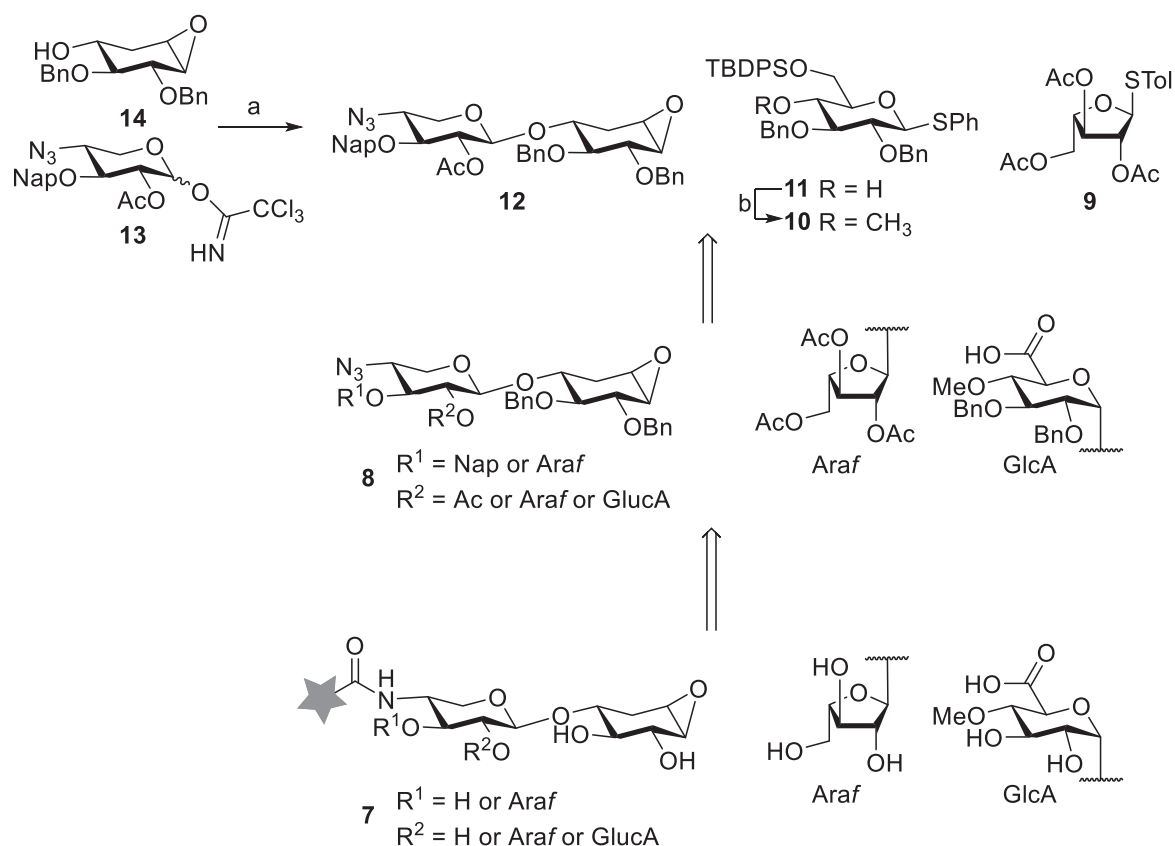
Figure 5.1 | Activity-based probes for enzymes acting on xyloglucan as described in chapter 2 and xylan as described in the literature⁵.

Glycosidases targeting plant glycans

In *chapter 2* activity-based probes for retaining glycosidases acting on various glycosidic linkages in xyloglucan are presented (**1-4**, Figure 5.1). The oligomeric probes, **2**, **3** and **4** were synthesized by chemical glycosylation under pre-activation conditions, using cyclophellitol epoxide acceptors.

ABPP in *Aspergillus niger* secretomes showed no cross reactivity between monosaccharide and oligosaccharide probes suggesting selective labeling of endo-glycosidases. Profiling with **2**, **3** and **4** also showed distinct labeling for the three probes. This indicates that glucanases and xyloglucanases with distinct substrate specificities can be distinguished with these probes. To assess the specificity of the probes in more depth, the probes can be used for ABPP of a variety of fungal secretomes and the labeled proteins can be analyzed by SDS-PAGE and by LC-MS proteomics. Together with further characterization of the synthesized probes these future studies may also reveal uncharacterized enzymes with desired characteristics, and provide new insights in fungal physiology.

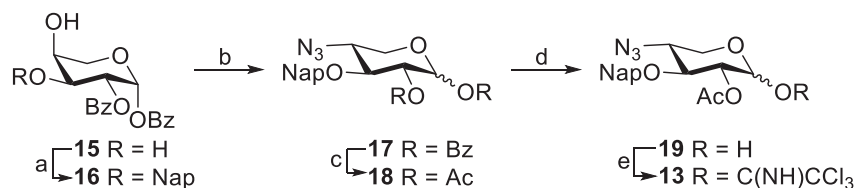
ABPP of xylanases with specificity for xylan with specific branching such as arabino-¹ and glucurono-xylanases²⁻⁴ could be envisaged via a similar approach as an extension of previously developed xylosidase (**5**) and xylanase (**6**) ABPs.⁵ The synthesis of probes **7** via protected **8** has been partially conducted (Scheme 5.1) with the aim to test this hypothesis. Orthogonal protection of the non-reducing end xylose allows selective introduction of arabinofuranose and 4-*O*-methyl glucuronic acid on the 2' and 3' position. L-Arabinofuranose donor **9**⁶ and 4-*O*-methyl glucose donor **10** may be suitable to introduce the respective side chains. Orthogonal



Scheme 5.1 | Reagents and conditions: **a**) TMSOTf, DCM, -30°C, 61%. **b**) MeI, NaH, THF, quant.

silyl ether protection of **10** allows selective oxidation to glucuronic acid post-glycosylation. **10** was synthesized from **11**⁷ by reaction with iodomethane. Pseudodisaccharide **12** was obtained by glycosylation of donor **13** with acceptor **14**⁵.

Donor **13** was obtained starting from partially benzoylated L-arabinopyranose **15**⁸ (Scheme 5.2). A naphthyl (Nap) ether was selectively introduced employing tin ketal catalysis affording **16**. The axial 4-OH was activated as a triflate and substituted with NaN₃ affording D-xylose configured **17**. Selective removal of the anomeric benzoyl group was unsuccessful but removal of both benzoyl esters followed by acetylation afforded **18** on which selective anomeric deprotection was achieved, affording lactol **19**. The lactol was transformed into trichloroacetimidate donor **13**.

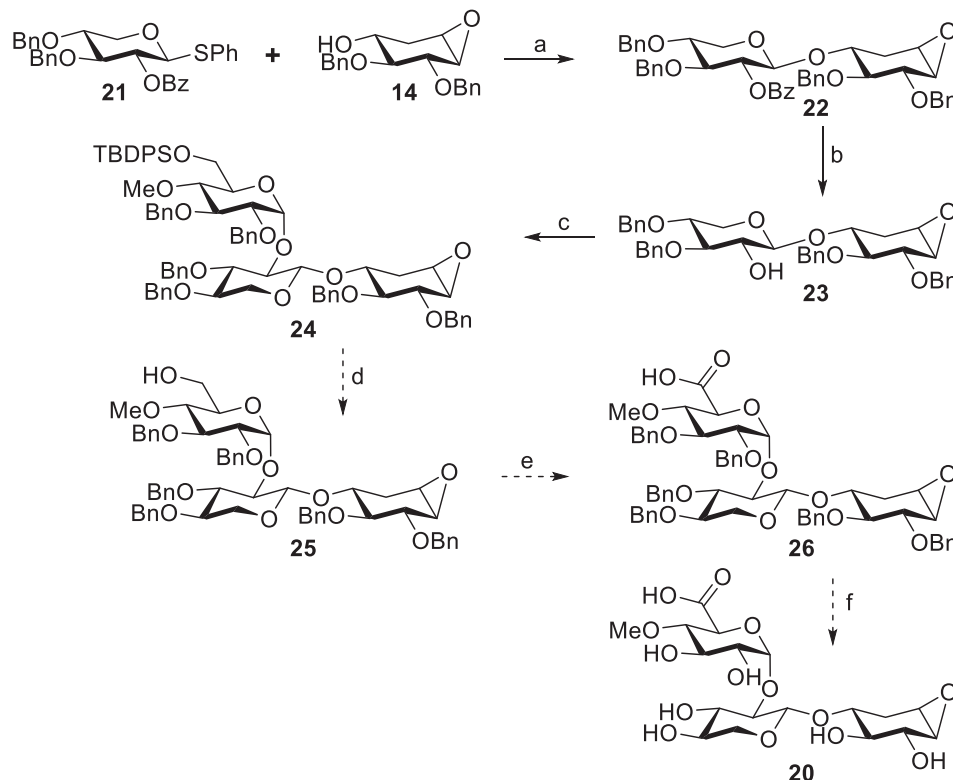


Scheme 5.2 | Reagents and conditions: **a**) i. Bu₂SnO, toluene; ii. NapBr, CsF, DMF, 56%. **b**) i. Tf₂O, pyr, DCM -20°C; ii. NaN₃, DMF, 90%. **c**) i. NaOMe, MeOH, DCM; ii. Ac₂O, pyr, 88%. **d**) piperidine, THF, 68%. **e**) CCl₃CN, K₂CO₃, DCM, 90%.

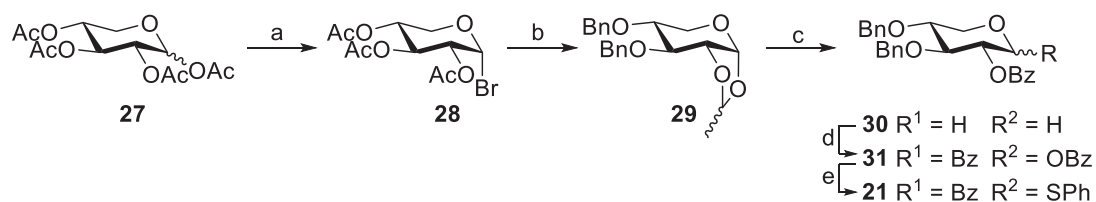
The synthesis of glucuronoxylanase inhibitor **20** was started as well (Scheme 5.3). Glycosylation of donor **21** with acceptor **14** provided pseudodisaccharide **22**. Removal of the benzoyl ester under basic conditions afforded alcohol **23**. Glycosylation of pseudodisaccharide acceptor **23** with donor **10** under pre-activation conditions provided product **24** in 30% yield. N-iodosuccinimide (NIS)/TMSOTf mediated glycosylation in DCM/Et₂O afforded the same product in 16% yield. In both cases only the α -configured product was observed.

To complete the synthesis three more steps could be executed. The silyl protecting group in pseudotrisaccharide **24** may be deprotected with a fluorine source to provide primary alcohol **25**. TEMPO/BAIB oxidation may provide carboxylic acid **26** which could be deprotected to provide inhibitor **20** by either dissolving metal or Pd catalyzed hydrogenation conditions.

Donor **21** was synthesized from peracetylated D-xylose (**27**) (Scheme 5.4). Treatment with HBr provided bromide **28** which was reduced to a diastereomeric mixture of anomeric ethylidenes. Removal of the acetyl esters under basic conditions followed by benzyl ether formation afforded **29**. The acetal masking group was hydrolyzed under acidic conditions and the resulting diol was benzoylated providing **31**. Lewis acid catalyzed introduction of an anomeric thiophenol provided thioglycoside donor **21** as an anomeric mixture.



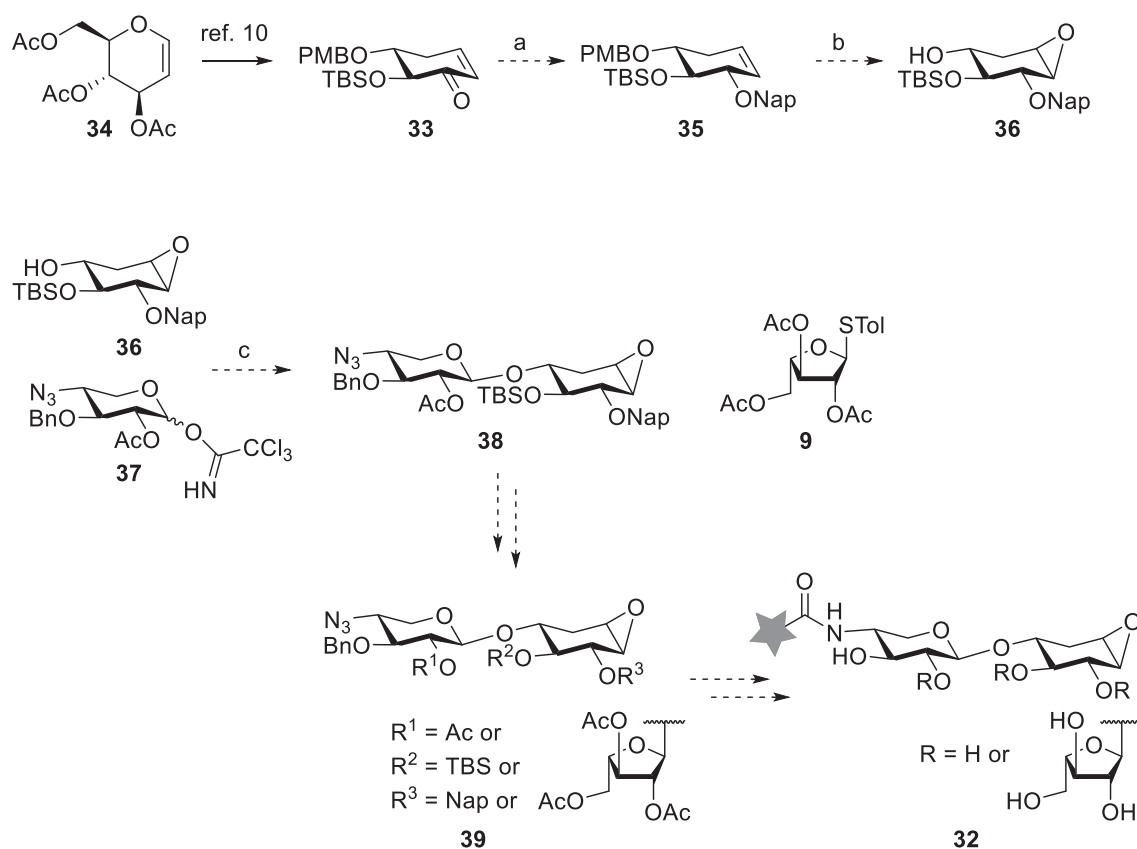
Scheme 5.3 | Reagents and conditions: **a)** TMSOTf, NIS, DCM, -70°C to -50°C, 73%. **b)** NaOMe, MeOH, DCM, 78%. **c)** **10**, Tf₂O, Ph₂SO, TTBP, DCM, Et₂O, -70 to -50°C, 30%. Proposed reagents and conditions: **d)** 3HF·Et₃N, THF. **e)** TEMPO, BAIB, DCM, *t*-BuOH, H₂O. **f)** Na(s), *t*-BuOH, NH₃, THF.



Scheme 5.4 | Reagents and conditions: **a**) HBr, AcOH, DCM. **b**) i. NaBH₄, TBAI, MeCN; ii. NaOMe, MeOH; iii. BnBr, NaH, TBAI, DMF, 44% over 4 steps. **c**) H₂SO₄, H₂O, dioxane; **d**) BzCl, pyr, DCM 67% over 2 steps. **e**) HSPH, BF₃·Et₂O, DCM, 79%.

A set of activity-based probes with L-arabinofuranose substitution on the xylose configured cyclophellitol (**32**, Scheme 5.5) could also be of interest. The structures could be useful to discover and study arabinoxylanases with specificity for substituted xylosides in the active site such as CTXyl5A⁹ from *Clostridium thermocellum*.

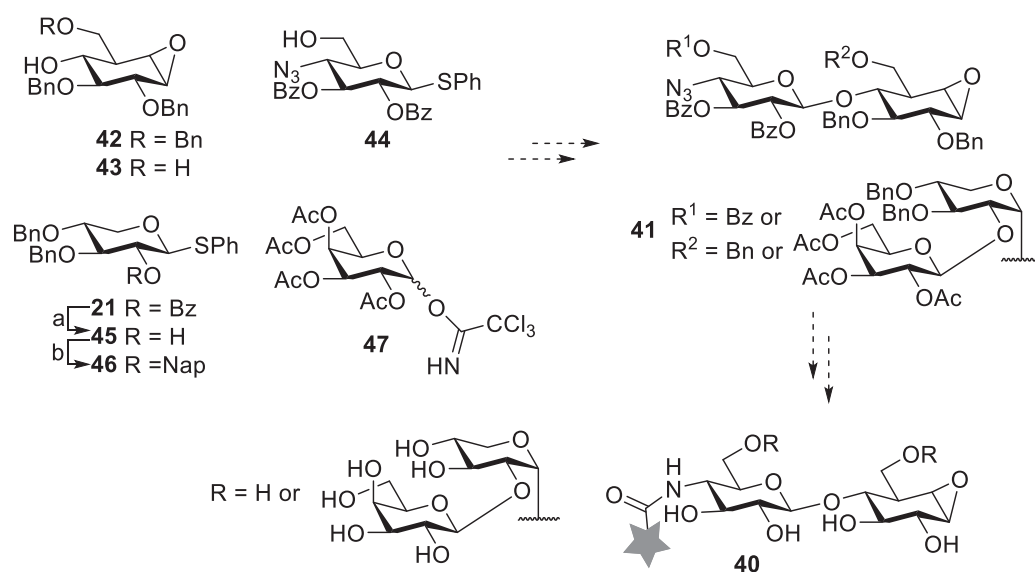
Pivotal to the successful synthesis of these structures is the choice of suitable orthogonal protecting groups on the xylose configured cyclophellitol building block. *p*-Methoxybenzyl (PMB) and silyl ether protected enone **33**¹⁰, of which a synthesis has been reported starting from readily accessible peracetylated glucal (**34**), may be a suitable starting material. Luche reduction¹¹ could provide the allyl alcohol which can be protected with a naphthyl ether



Scheme 5.5 | Proposed reagents and conditions: **a**) i. NaBH₄, CeCl₃, MeOH; ii. NapBr, NaH, DMF. **b**) i. DDQ, H₂O, DCM; ii. 1,1,1-trifluoroacetone, Oxone, NaHCO₃. **c**) TMSOTf, DCM.

providing **35**. Selective removal of the PMB protecting group by either HCl/HFIP¹², DDQ¹³ or ceric ammonium nitrate (CAN)¹⁴ followed by epoxide formation with *in situ* generated methyl(trifluoromethyl)dioxirane could provide acceptor **36**. Donor **37**, which could be prepared analogous to **13** (Scheme 5.2), could be reacted with acceptor **36** to provide orthogonally protected pseudodisaccharide **38**. Treatment with a nucleophilic base, fluorine source or DDQ, would allow selective removal of the acetyl, silyl ether or naphthyl ether protecting groups respectively. Glycosylation of the liberated alcohol(s) with arabinofuranose donor **9**⁶ could provide a set of protected inhibitors with diverse arabinofuranosylation patterns on the 2O, 3O and 2'O position (**39**). Removal of the remaining protecting groups and reduction of the azide would allow introduction of fluorophores or affinity tags by amide bond formation using the functionalized triethylene glycol spacers and reaction conditions as described in chapter 2.

A final suggestion for future synthesis of ABPs in the biomass utilization field would be the extension of the XG and GX configured probes described in chapter 2 with a β -D-galactose residue because it has been reported that some xyloglucanases have increased reactivity towards more extensively branched substrates (Scheme 5.6).¹⁵ A set of LG, GL and LL configured probes (**40**) may be constructed via **41** using cyclophellitol building blocks **42** and **43**, and 4-deoxy-4-azido-glucoside **44** described in chapter 2 together with orthogonally protected D-xylose configured building blocks **21**, **45** and **46** and D-galactose donor **47**¹⁶.



Scheme 5.6 | Reagents and conditions: **a**) NaOMe, MeOH, DCM quant. **b**) NapBr, NaH, DMF, 55%.

Heparanase

Heparanase (HPSE) is the only known human endo-glycosidase that modifies heparan sulfate in the extracellular matrix. Overexpression of HPSE is implicated in a wide range of pathologies inciting the development of HPSE inhibitors and activity assays. A set of mechanism-based covalent and irreversible inhibitors of HPSE and HPSE activity-based probes are presented in *chapter 3*. The HPSE inhibitors are synthesized via common intermediate **48** (Figure 5.2). By selective oxidation and deprotection the set of inhibitors **49–53** was synthesized. The relative potency of the inhibitors was assessed by competitive ABPP. 6'O sulfated inhibitor **49** was found to be the most potent inhibitor followed by **50** and **51**. Inhibitors **52** and **53** were found to be significantly less potent.

Inhibitors **54** and **55** may also be synthesized from **48**. With these inhibitors the effect of *N*-sulfation on inhibitor potency could be determined. 6'O and 2'N sulfated inhibitor **54** might be the most potent inhibitor in this series in line with known polysulfated inhibitors.^{17–19} Synthesis of aziridine derivatives **56** and **57** with substitution on the aziridine nitrogen might be another possibility towards potent HPSE inhibitors.

Although inhibitor **50** has shown effect *in vivo*, the stability of the glycosidic linkage in the structure might be of concern. Enzymatic hydrolysis would release glucuronic acid configured cyclophellitol which is a covalent inhibitor of retaining exo- β -glucuronidases such as the human enzyme GUSB.²⁰ This enzymatic hydrolysis of the inhibitor might lead to lower efficacy

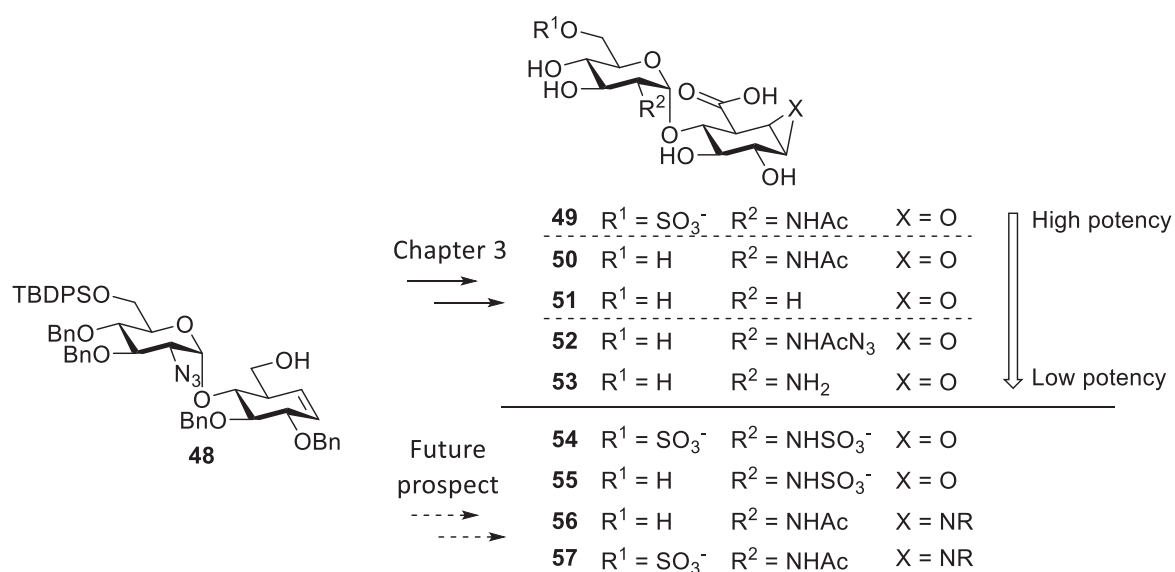
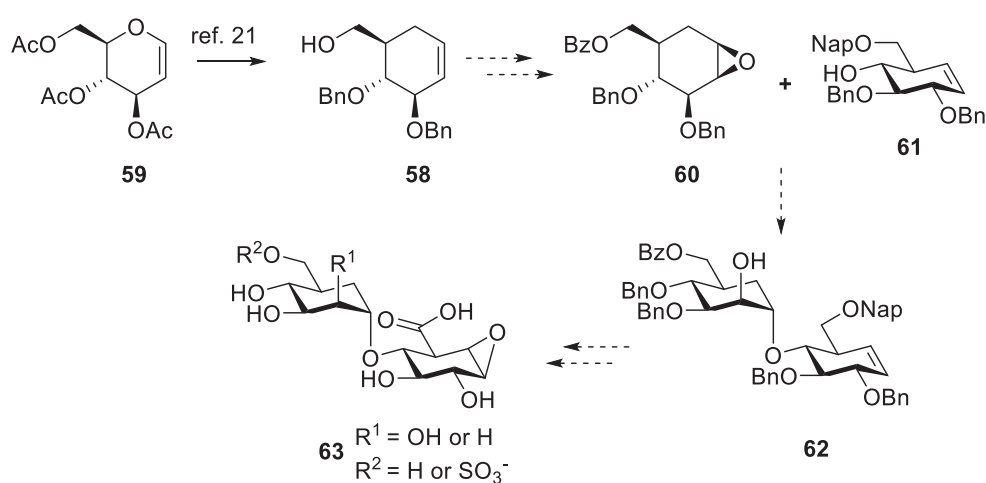


Figure 5.2 | Covalent HPSE inhibitors synthesized in this thesis in order of potency as assessed by a competitive ABPP assay with recombinant HPSE and suggestions for possible inhibitors that may be accessible from **48**.

in vivo due to the lower effective concentration and to side effects by inhibition of off target enzymes by the degradation product. An attractive way to stabilize the glycosidic linkage is the replacement of the acetal functionality with an ether linkage by substitution of one of the two oxygen atoms with a methylene group (Scheme 5.7).

Carbaglucal **58**²¹ prepared from tri-*O*-acetyl-D-glucal (**59**) could be transformed into properly protected epoxide **60**. Lewis acid catalyzed trans-diaxial opening of the epoxide with cyclophellitol alkene **61** could provide alcohol **62**. The inversion of the axial alcohol in **62** might be difficult due to elimination side reactions. However, since there are mannose configured HPSE inhibitors (mupafostat) it could be argued that this mannose configured compound might also be a sufficiently potent inhibitor. Another possibility is deoxygenation of the 2' alcohol since deoxygenated inhibitor **51** has shown no significant drop in potency compared to 2'NHAc substituted **50**. Naphthyl deprotection followed by oxidation and debenzoylation would allow selective 6'-*O*-sulfation and hydrogenation may provide the set of carba-pseudodisaccharides (**63**).

The conformation of the glycosidic linkage is largely dictated by the *exo*-anomeric effect.²² Overlap of one of the exocyclic oxygens lone pairs with the anti-bonding orbital of the polarized O5-C1 bond stabilizes the 'exo-conformation' and reduces the flexibility around the glycosidic bond. This conformational preference is not present in analogues where the acetal group is replaced by an ether such as carbasugars or C-glycosides.²³ Inhibitors bind in one specific conformation leading to an entropy penalty and consequently reduced potency for more flexible inhibitors. This may explain why some stabilized carbohydrate mimics have a lower inhibitor potency compared to the parent structures.^{24,25}

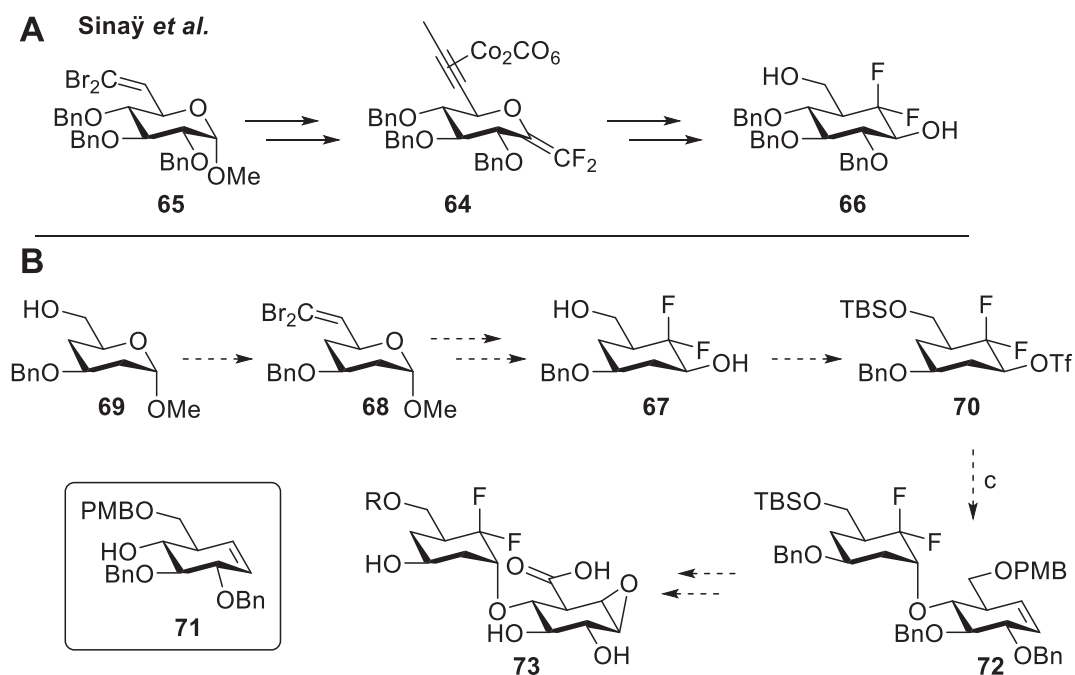


Scheme 5.7 | Synthesis of carbasugar analogues of the HPSE inhibitors presented in this thesis.

A way to (partially) restore the exo-anomeric effect is the introduction of a gem-difluorocarbasugar.^{26,27} A synthesis of gem-difluorocarba- β -D-glucose has been reported with the triisobutylaluminium (TIBAL) catalyzed sugar-to-carbocycle rearrangement of **64** as key step (Scheme 5.8A).²⁸ The alkyne-cobalt complex, obtained from dibromoalkene **65**, is electron donating and is needed to stabilize the positive charge during the rearrangement. Ozonolysis followed by stereoselective reduction provides diol **66**.

In a similar way diol **67** may be obtained from dibromide **68** which in turn could be accessible from alcohol **69**²⁹ (Scheme 5.8B). A silyl ether may be selectively introduced on the primary alcohol in diol **67** followed by generation of the secondary triflate (**70**). The triflate might be substituted with alcohol **71** to obtained **72**. Putative HPSE inhibitor **73** might be obtained via the reaction sequence describes for the inhibitors in chapter 3. Reduced hydrophilicity by removal of the 2' and 4' alcohol groups and hydrolytic stability with preservation of the optimal anomeric conformation might make **73** a potent HPSE inhibitor.

The rest of chapter 3 is dedicated to the development of selective ABPs for HPSE based on the developed covalent inhibitors. Epoxide **74** was synthesized as the first design of a potentially selective HPSE ABP (Figure 5.3). The molecule displayed diminished potency for HPSE (compared to **75**²⁰) while maintaining potency for proHPSE. The reaction of proHPSE



Scheme 5.8 | **A**) Sugar to carbocycle rearrangement approach to gem-difluorocarba- β -D-glucose. **B**) Proposed synthesis route towards deoxygenated gem-difluorocarbasugar analogues of HPSE inhibitors synthesized in this thesis.

with inhibitors might be insignificant since proHPSE has no reported enzymatic activity. However, it has been postulated that the beneficial effects of HPSE inhibitors may be the result of the reduction of intracellular HPSE activity by limiting cellular uptake of HPSE.³⁰ Inhibition of HPSE inside the cell is not directly possible since the most used HPSE inhibitors are not cell permeable. The irreversible ‘inhibition’ of proHPSE might be a unique opportunity to achieve intracellular HPSE inhibition. Because proHPSE is transported outside the cell during HPSE biosynthesis, the extracellular proHPSE could be irreversibly acylated with cyclophellitol derivatives. Internalization followed by activation by cathepsin L will yield intracellular inhibited HPSE.

Although **74** is not a potent ABP for HPSE the design of covalent HPSE and proHPSE inhibitors based on this monosaccharide mimetic approach remains attractive because the compounds based on this scaffold might have a more suitable physicochemical profile compared to the disaccharide mimics. Scaffolds **76** and **77** would allow the late stage

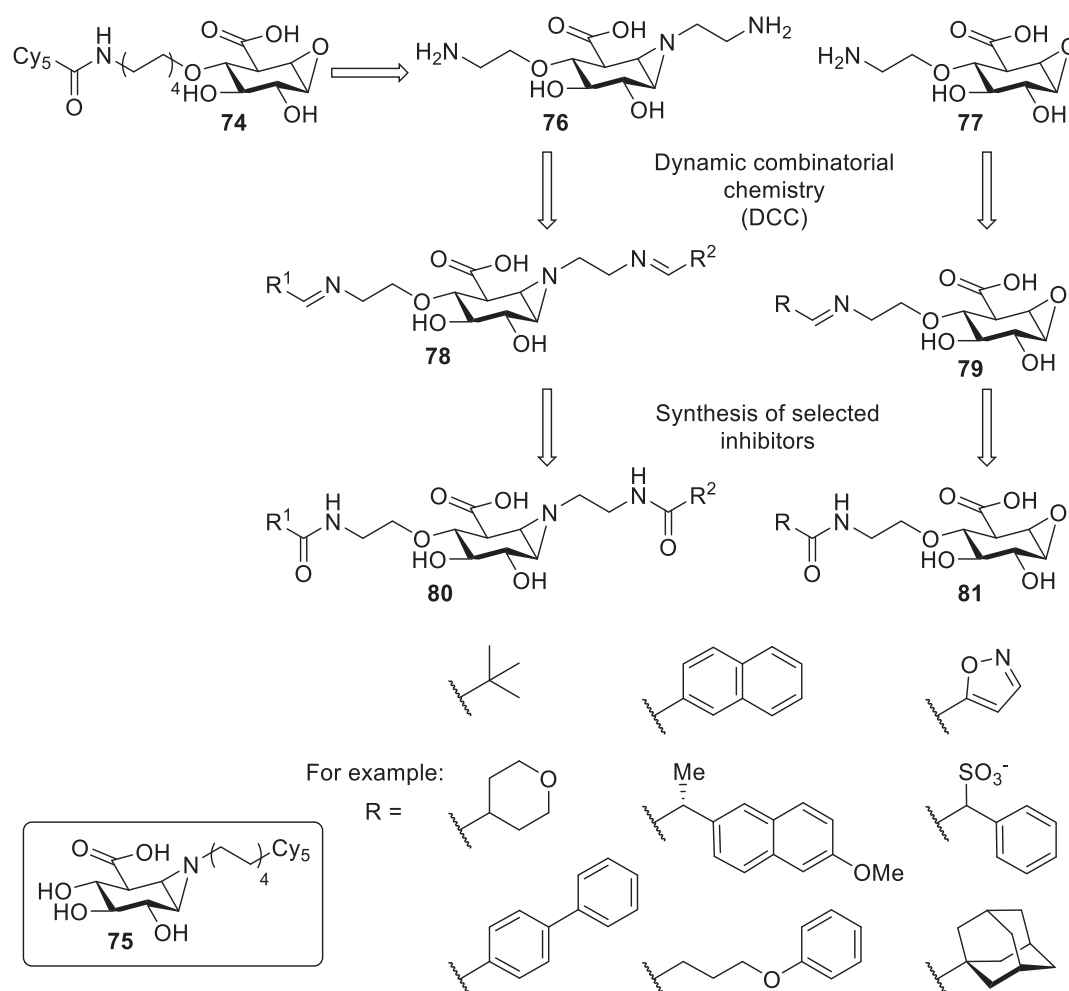


Figure 5.3 | Potential irreversible proHPSE and HPSE inhibitor screening with Dynamic combinatorial chemistry followed by chemical synthesis of selected inhibitors.

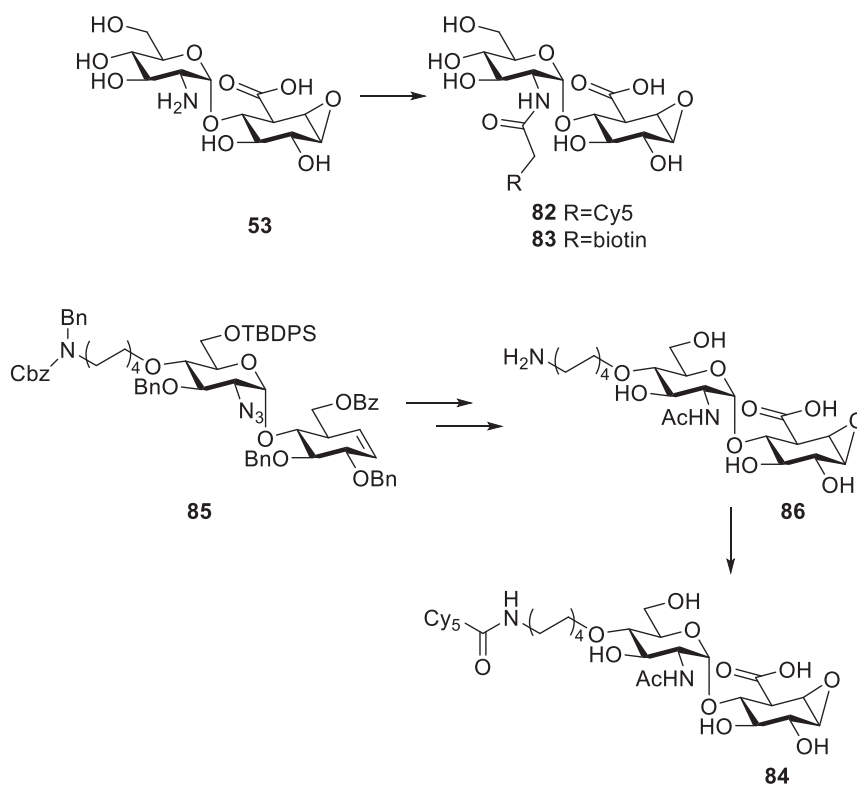
introduction of diverse substituents allowing the synthesis of a library to determine a structure activity relationship. The chemical synthesis and purification of a large enough library of derivatives would be challenging due to the limited availability of the cyclophellitol building block and the difficult purification of the products.

Dynamic combinatorial chemistry (DCC) might be a suitable approach to screen a large chemical space without the need to synthesize many individual derivatives.^{31,32} For this approach two sets of fragments with compatible functional groups are mixed. The functional groups, in this case amines **76** and **77** and a set of aldehydes, will reversibly react and form a thermodynamic equilibrium of reaction products, in this case imines **78** and **79**. Addition of the target protein will increase the stability of binders to the protein and shift the equilibrium towards these products. Quenching of the reaction, in this case by reduction of the imine, and analysis of the product distribution may reveal inhibitors for the enzyme.

DCC methodology is normally applied to find reversible inhibitors. However, in this case the amine derivatives (**80** and **81**) are covalent inhibitors of the enzyme. The product will in this case be covalently attached to the enzyme so LC-MS analysis of the resulting products will not be possible. A suitable approach might be the direct detection of binding ligands by X-ray crystallography.³³ DCC could be performed on crystals of HPSE and proHPSE and the resulting electron density maps could directly reveal the relevant binding modes. Stable analogues of the discovered inhibitors could be synthesized and the potency for both HPSE and proHPSE could be determined by ABPP with broad spectrum β -glucuronidase ABP **75**.²⁰

Putative HPSE ABPs based on the disaccharide inhibitors are also reported in chapter 3 (Scheme 5.9). Fluorescent and biotin conjugated inhibitors **82** and **83** with substitution on the 2'*N* position were synthesized by selective acylation of inhibitor **53**. **82** was however unable to label HPSE. The 4'*O* alkylated derivative **84** was prepared via **85** and **86** by a similar reaction sequence as used for the inhibitors. The second amine in the scaffold required orthogonal protection to the azide. The deprotection of the chosen NCbzBn protecting groups was however only partially compatible with the epoxide functionality in the molecule. In the future instead of the NCbzBn other protecting groups such as PMB could be considered to improve the yield of the deprotection step.

84 did show labeling of HPSE. The labeling was evaluated in different lysates and was found to be more selective than labeling with broad spectrum β -glucuronidase probe **75**. The ability



Scheme 5.9 | Synthesis of HPSE inhibitor derivatives carrying fluorescent reporter moieties on different positions.

to selectively visualize HPSE activity may provide insight in HPSE related pathology and allow for efficient screening of HPSE inhibitors.

PslG

The chemical synthesis of a putative two step ABP for PslG (**87**, Figure 5.4) is described in *chapter 4*. PslG is reported in the literature as an endo-mannosidase expressed by *Pseudomonas aeruginosa* to hydrolyze the polysaccharide Psl, a constituent of *Pseudomonas Aeruginosa* derived biofilms. Covalent attachment of **87** with recombinant PslG could not be observed by X-ray crystallography and mass spectrometry.

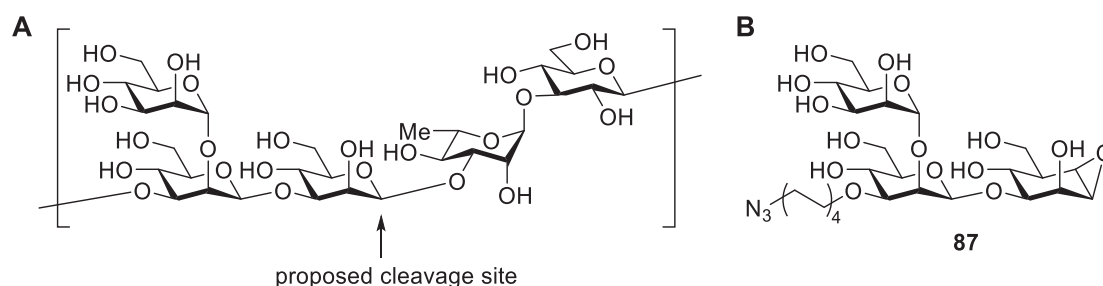


Figure 5.4 | **A)** Repeating pentamer of the Psl polysaccharide and the proposed cleavage site of PslG. **B)** Synthesized activity-based probe based on the proposed catalytic activity of PslG.

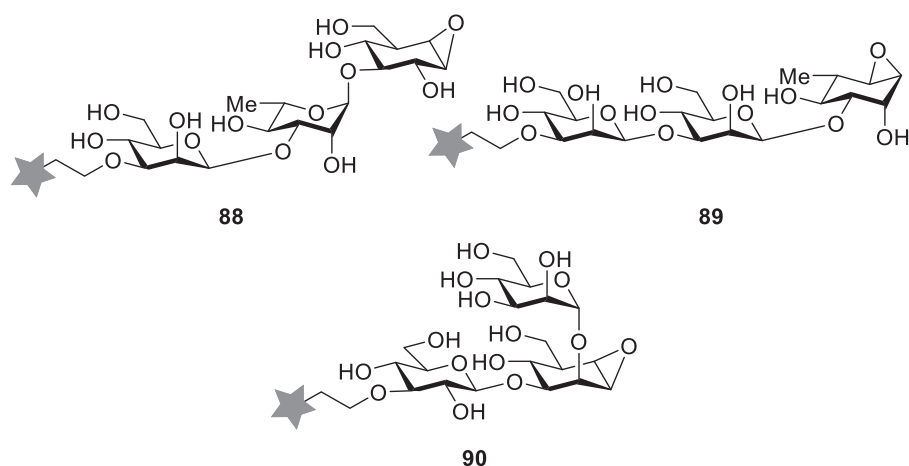
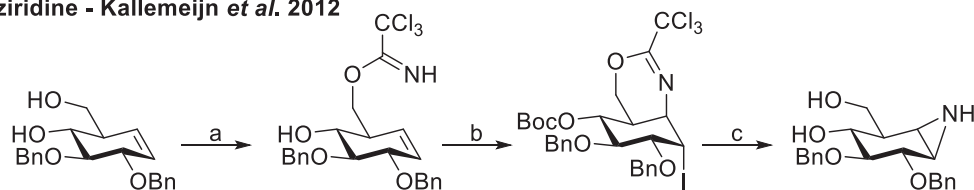
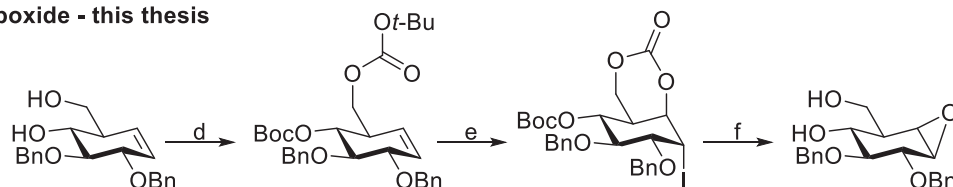
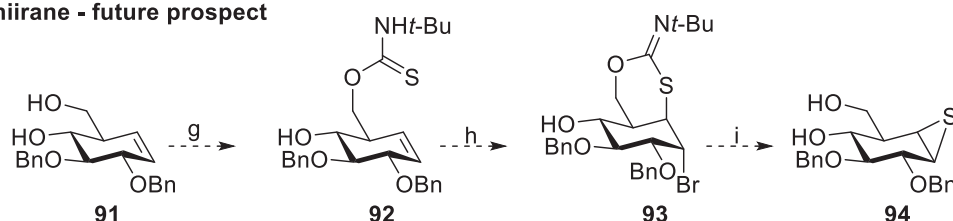


Figure 5.5| Three possible activity-based probes for PslG depending on its classification as a β -glucosidase **88**, an α -L-rhamnosidase **89** or a β -mannosidase **90**. Star denotes various reporter groups.

To confirm the proposed specificity of PslG synthetic or isolated Psl fragments should be reacted with PslG and the reaction products should be analyzed by NMR or MS methods. ABPs could be generated based on the newly found cleavage point. Several examples of potential PslG probes are depicted in figure 5.5.

Efficient (late stage) transformation of an alkene into a reactive warhead is important for the successful synthesis of increasingly complex cyclophellitol-based ABPs. Aziridines³⁴ and epoxides have been stereospecifically introduced on the cyclophellitol scaffold by intramolecular iodocyclization reactions (Scheme 5.10). The main advantage of this approach is the complete stereo control which is not always achieved with other methods such as epoxide formation using peracid reagents (chapter 3). The methodology may be extended to the stereoselective synthesis of thiiranes to stimulate the inclusion of these warheads in covalent inhibitor and activity-based probe development projects.

Primary alcohol **91** may be reacted with an isothiocyanate³⁵ or thiocarbamoyl chloride to produce thiocarbamate **92**. Bromocyclization might produce bromide **93** which might be transformed into thiirane **94** by reaction with ammonium hydroxide.³⁶

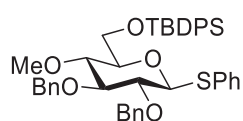
Aziridine - Kallemeijn *et al.* 2012**Epoxide - this thesis****Thiirane - future prospect**

Scheme 5.10 | Reagents and conditions used in iodocyclization approaches for stereoselective aziridine and epoxide formation: **a)** CCl_3CN , DBU, DCM, 0°C . **b)** I_2 , NaHCO_3 , H_2O . **c)** i. HCl, MeOH; ii. HCl, dioxane 60°C ; iii. NaHCO_3 , MeOH, 60% over 5 steps. **d)** Boc_2O , DMAP, THF. **e)** NIS, AcOH. **f)** K_2CO_3 , MeOH, 75% over 3 steps. Proposed conditions for stereoselective thiirane synthesis: **g)** $t\text{-BuNCS}$, pyr.³⁵ **h)** Br_2 , DMF.³⁶ **i)** NH_3 , H_2O .³⁶

5.1 Experimental

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

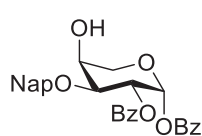
Phenyl 2,3-di-*O*-benzyl-4-*O*-methyl-6-*O*-*t*-butyldiphenylsilyl-1-thio- β -D-glucopyranoside (**10**)



Alcohol **11**⁷ (0.800 g, 1.16 mmol) was dissolved in THF (2.32 ml). MeI (0.43 ml, 6.95 mmol) and NaH (60% in mineral oil, 0.278 g, 6.95 mmol) were added and the reaction was stirred for 3 hours. The reaction was quenched with MeOH.

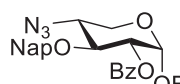
Water was added and the product was extracted with Et_2O (2x). The combined organic layers were washed with brine, dried over MgSO_4 and concentrated under reduced pressure. Column chromatography (Et_2O /pentane, 0/1 \rightarrow 1/9, v/v) afforded the product (0.820 g, 1.16 mmol, quant.).

^1H NMR (400 MHz, CDCl_3) δ 7.80 – 7.72 (m, 4H), 7.60 – 7.56 (m, 2H), 7.44 – 7.27 (m, 16H), 7.22 – 7.17 (m, 3H), 4.89 – 4.81 (m, 3H), 4.72 (d, J = 10.3 Hz, 1H), 4.65 (d, J = 9.6 Hz, 1H), 3.93 (qd, J = 11.4, 2.7 Hz, 2H), 3.65 – 3.56 (m, 4H), 3.55 – 3.45 (m, 2H), 3.29 (ddd, J = 9.5, 3.5, 1.9 Hz, 1H), 1.09 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 138.5, 138.4, 136.0, 135.8, 134.3, 133.7, 133.2, 131.8, 129.8, 129.7, 129.0, 128.6, 128.6, 128.3, 128.3, 128.0, 127.9, 127.8, 127.4, 87.5, 87.0, 80.7, 80.1, 79.2, 76.1, 75.5, 62.8, 60.9, 27.0, 19.5. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calc for $\text{C}_{43}\text{H}_{48}\text{O}_5\text{SSiNa}$ 727.2884, found 727.2878.

1,2-di-*O*-benzoyl-3-*O*-(2-naphthylmethyl)-L-arabinopyranose (16)

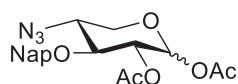
Diol **15**⁸ (0.90 g, 2.5 mmol) was dissolved in toluene (25 ml, 0.1 M). Bu₂SnO (0.75 g, 3.0 mmol) was added and the mixture was heated to 90 °C and stirred for 1 hour. The mixture was allowed to cool and was subsequently concentrated under reduced pressure. The crude product was dissolved in DMF (20 ml, 0.1 M) and 2-naphthylene bromide (0.66 g, 3.0 mmol), and cesium fluoride (0.61 g, 4.0 mmol) were added and the mixture was stirred overnight at rt. The reaction mixture was then poured into water (200 ml) and the product was extracted with Et₂O (3 x 70 ml). The combined organic layers were washed with brine, then dried over MgSO₄, filtered and concentrated. Column chromatography (pentane/EtOAc, 5/1-→ 2/1, v/v) afforded the product as an oil (0.69 g, 1.38 mmol, 55%).

¹H NMR (300 MHz, CDCl₃): δ 7.93 – 7.28 (m, 19H), 6.60 (d, *J* = 3.5 Hz, 1H), 5.79 (dd, *J* = 9.6, 3.5 Hz, 1H), 4.89 (q, *J* = 12.2 Hz, 2H), 4.30 – 4.18 (m, 2H), 4.03 (m, 2H), 2.77 (m, 1H). ¹³C NMR (101 MHz, CDCl₃): δ 165.3, 164.5, 134.3, 133.3, 133.1, 132.9, 132.9, 129.6, 129.5, 129.3, 129.2, 128.3, 128.2, 127.7, 127.6, 126.8, 126.1, 126.0, 125.6, 91.3, 74.1, 72.0, 68.9, 66.5, 64.1, 60.2, 14.0. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₃₀H₂₆O₇Na 521.1571, found 521.1567.

1,2-di-*O*-benzoyl-3-*O*-(2-naphthylmethyl)-4-deoxy-azido-α-D-xylopyranose (17)

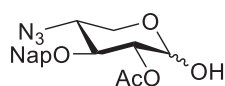
Alcohol **16** (6.2 g, 12.4 mmol) was co-evaporated with toluene and was subsequently dissolved in dry DCM (120 ml, 0.1 M), then pyridine (1.5 ml, 18.6 mmol) was added and the mixture was cooled to -20 °C. Tf₂O (2.5 ml, 14.9 mmol) was added dropwise and the reaction was stirred for 1 hour at -20 °C. The reaction was diluted with DCM (250 ml), then washed with H₂O (3 x 120 ml), then dried over MgSO₄, filtered and concentrated at rt. The crude was then dissolved in DMF (31 ml) and sodium azide (8.06 g, 124 mmol) was added, the mixture was stirred overnight at rt. The reaction was diluted with H₂O (300 ml), then extracted with Et₂O (3 x 100 ml), and the combined organic layers were washed with brine, then dried over MgSO₄, filtered and concentrated. Column chromatography (pentane/EtOAc, 8/1, v/v) afforded the product as an oil (5.8 g, 10.6 mmol, 90%).

¹H NMR (400 MHz, CDCl₃): δ 8.02 – 7.97 (m, 2H), 7.89 – 7.84 (m, 2H), 7.77 – 7.73 (m, 1H), 7.69 – 7.60 (m, 4H), 7.53 – 7.29 (m, 8H), 6.58 (d, *J* = 3.5 Hz, 1H), 5.43 (dd, *J* = 9.7, 3.6 Hz, 1H), 5.09 – 4.92 (m, 2H), 4.18 (t, *J* = 9.3 Hz, 1H), 3.99 – 3.87 (m, 2H), 3.76 – 3.69 (m, 1H). ¹³C NMR (101 MHz, CDCl₃): δ 165.4, 164.6, 134.7, 133.9, 133.6, 133.3, 133.2, 129.9, 129.8, 129.3, 129.1, 128.8, 128.6, 128.5, 128.0, 127.8, 127.3, 126.2, 126.2, 126.2, 90.9, 78.2, 75.6, 72.3, 62.4, 61.1. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₃₀H₂₅N₃O₆Na 546.1636, found 546.1633.

1,2-di-*O*-acetyl-3-*O*-(2-naphthylmethyl)-4-deoxy-azido-D-xylopyranose (18)

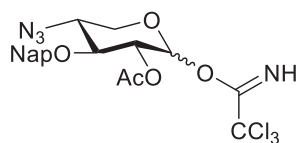
17 (3.3 g, 6.2 mmol) was dissolved in DCM/MeOH (1/1, v/v, 30 ml), then NaOMe solution in MeOH was added (0.28 ml, 1.2 mmol) and stirred overnight. The reaction was quenched with Et₃N·HCl (0.3 g, 1.9 mmol), after which the mixture was concentrated and co-evaporated with dioxane (3x). The crude was then dissolved in pyridine/Ac₂O (2:1, v/v, 30 ml) and stirred overnight at rt. The reaction was quenched with H₂O (4 ml) at 0 °C and the diluted with EtOAc (120 ml). The mixture was then washed with HCl (1M aq. 3x 40 ml), NaHCO₃ (sat. aq. 3x 40 ml) and brine, dried over MgSO₄, filtered and concentrated. Column chromatography (pentane/EtOAc, 9/1, v/v) afforded the product as an oil (2.2 g, 5.2 mmol, 88% over 2 steps).

For α isomer: ¹H NMR (400 MHz, CDCl₃): δ 7.87 – 7.75 (m, 4H), 7.52 – 7.41 (m, 3H), 6.23 (d, *J* = 3.6 Hz, 1H), 5.06 – 4.81 (m, 3H), 3.91 – 3.81 (m, 2H), 3.77 – 3.67 (m, 1H), 3.66 – 3.53 (m, 1H), 2.14 (s, 3H), 1.94 (s, 3H). ¹³C NMR (101 MHz, CDCl₃): δ 169.8, 169.5, 169.2, 135.1, 134.8, 133.3, 133.2, 128.5, 128.4, 128.1, 128.0, 127.8, 127.0, 126.7, 126.4, 126.4, 126.3, 126.2, 125.9, 125.7, 90.0, 78.2, 75.5, 71.8, 62.1, 60.9, 21.0, 20.7. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₀H₂₁N₃O₆Na 422.1323, found 422.1327.

2-O-acetyl-3-O-(2-naphthylmethyl)-4-deoxy-azido-D-xylopyranose (19)

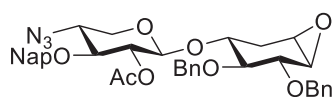
Ester **18** (2.2 g, 5.4 mmol) was dissolved in THF (11 ml, 0.5 M), then piperidine (0.6 ml, 5.9 mmol) was added and the mixture was stirred overnight at rt. The mixture was diluted with EtOAc (120 ml) and washed with HCl (1 M aq. 3x 40 ml) and brine, then dried over MgSO_4 , filtered and concentrated. Column chromatography (pentane/EtOAc, 3/1, v/v) over silica afforded the product as a white solid (1.3 g, 3.6 mmol, 68%, α/β 3/1).

Major isomer: ^1H NMR (500 MHz, CDCl_3): δ 7.85 – 7.76 (m, 4H), 7.50 – 7.40 (m, 3H), 5.36 (d, J = 3.5 Hz, 1H), 4.99 (t, J = 11.3 Hz, 1H), 4.93 – 4.87 (m, 1H), 4.85 (dd, J = 9.5, 3.5 Hz, 1H), 3.94 (t, J = 9.2 Hz, 1H), 3.78 – 3.70 (m, 2H), 3.67 – 3.59 (m, 1H), 2.95 (s, 1H), 2.01 (d, J = 8.7 Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 170.4, 135.3, 133.4, 133.2, 128.5, 128.4, 128.1, 127.9, 127.8, 127.0, 126.8, 126.4, 126.3, 126.3, 126.2, 125.9, 125.9, 90.9, 77.8, 75.4, 73.6, 61.2, 60.1, 21.0. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calc for $\text{C}_{18}\text{H}_{19}\text{N}_3\text{O}_5\text{Na}$ 380.1217, found 380.1215.

2-O-acetyl-3-O-(2-naphthylmethyl)-4-deoxy-azido-D-xylopyranosyl 2,2,2-trichloroacetimidate (13)

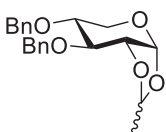
Lactol **19** (0.12 g, 0.32 mmol) was dissolved in DCM (1.6 ml, 0.2 M), then CCl_3CN (64 μL , 0.64 mmol) and K_2CO_3 (88 mg, 0.64 mmol) were added and the mixture was stirred overnight at rt. The mixture was filtered over celite and concentrated. Column chromatography (pentane/EtOAc, 5/1, v/v) afforded the product as an oil (0.15 g, 0.29 mmol, 90%, 1/2, α/β).

β : ^1H NMR (400 MHz, CDCl_3): δ 8.68 (s, 1H), 7.88 – 7.77 (m, 4H), 7.52 – 7.44 (m, 3H), 5.86 (d, J = 5.8 Hz, 1H), 5.31 – 5.25 (m, 1H), 4.96 – 4.89 (m, 2H), 4.28 – 4.23 (m, 1H), 3.75 – 3.71 (m, 2H), 3.58 – 3.51 (m, 1H), 1.98 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3): δ 161.2, 134.7, 128.5, 128.1, 127.9, 127.1, 127.0, 126.4, 126.3, 126.1, 125.9, 96.0, 78.0, 74.2, 69.5, 63.2, 59.1, 20.9.

4-O-(2-O-acetyl-3-O-(2-naphthylmethyl)-4-deoxy-azido-D-xylopyranosyl)-2,3-di-O-benzyl-xylocyclophellitol (12)

Donor **13** (0.13 g, 0.26 mmol) and acceptor **14** (61 mg, 0.19 mmol) were combined and co-evaporated with toluene. The mixture was dissolved in dry DCM (2 ml), 3 Å molecular sieves were added and the mixture was stirred for 1 hour before cooling to -30°C . TMSOTf (9 μL , 0.05 mmol) was added and the mixture was stirred for 1 hour at -30°C . The reaction was quenched with Et_3N (12 μL), diluted with DCM (50 ml), washed with brine, dried over MgSO_4 , filtered and concentrated under reduced pressure. Column chromatography (pentane/EtOAc, 6/1 \rightarrow 4/1, v/v) afforded the product as a white solid (77 mg, 0.12 mmol, 61%).

^1H NMR (400 MHz, CDCl_3): δ 7.87 – 7.73 (m, 4H), 7.50 – 7.39 (m, 3H), 7.39 – 7.22 (m, 12H), 5.00 – 4.88 (m, 3H), 4.81 (d, J = 11.6 Hz, 1H), 4.74 – 4.63 (m, 3H), 4.42 (d, J = 6.7 Hz, 1H), 3.99 (dd, J = 12.0, 4.9 Hz, 1H), 3.82 – 3.70 (m, 2H), 3.65 – 3.57 (m, 1H), 3.53 (t, J = 8.3 Hz, 1H), 3.38 (dd, J = 10.1, 7.7 Hz, 1H), 3.26 – 3.21 (m, 1H), 3.14 – 3.05 (m, 2H), 2.49 (m, 1H), 1.95 (s, 3H), 1.71 (m, 1H). ^{13}C NMR (101 MHz, CDCl_3): δ 169.5, 138.9, 137.9, 134.9, 133.3, 133.2, 128.7, 128.64, 128.57, 128.42, 128.39, 128.31, 128.25, 128.14, 128.09, 128.06, 127.97, 127.8, 127.5, 127.0, 126.3, 126.23, 126.19, 126.0, 99.4, 82.6, 80.0, 79.5, 75.3, 73.7, 73.5, 72.6, 63.0, 60.4, 54.0, 53.4, 30.0, 21.0. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calc for $\text{C}_{38}\text{H}_{39}\text{N}_3\text{O}_8\text{Na}$ 688.2629, found 688.2625.

3,4-di-O-benzyl-1,2-O-ethylidene- α -D-xylopyranose (29)

1,2,3,4-Tetra-O-acetyl-D-xylopyranose (**27**) (8.60 g, 27.0 mmol) was dissolved in DCM (180 ml, 0.15 M) and cooled to 0°C . HBr (33% in AcOH, 11 ml) was added and the mixture was stirred for 2 hours. TLC (2/1, pent/EtOAc) indicated complete consumption of the starting material and the mixture was diluted with DCM and washed subsequently with H_2O , NaHCO_3 (2x) and brine. The organic phase was dried with MgSO_4 and concentrated *in vacuo* at 30°C .

The crude bromide (**28**) was dissolved in MeCN (135 ml, 0.2 M). TBAI (6.0 g, 16.2 mmol) and NaBH₄ (1.23 g, 32.4 mmol) were added and the mixture was stirred overnight. The reaction was quenched with water and extracted with EtOAc (3x). The combined organic layers were washed with HCl (1M, 2x), NaHCO₃ and brine, dried with MgSO₄ and concentrated *in vacuo*.

The crude product was dissolved in MeOH (90 ml, 0.3 M), NaOMe (5.4 M in MeOH, 1.1 ml) was added and the mixture was stirred at rt until TLC (2/1, pent/EtOAc) indicated full conversion of starting material into a polar product. The mixture was neutralized with amberlite (H⁺), filtered and concentrated under reduced pressure.

DMF (10 ml) was added and the solution was coevaporated with toluene (3x). DMF (135 ml, 0.2 M), TBAI (0.87 g, 2.7 mmol) and BnBr (12.84 ml, 108.1 mmol) were added and the mixture was cooled to 0°C before adding NaH (60% in mineral oil, 3.78 g, 94.6 mmol). The mixture was stirred overnight before it was quenched with MeOH. Et₂O and H₂O were added. The layers were separated and the aqueous layer was extracted with Et₂O (3x). The combined organic layers were washed with H₂O (2x) and brine (2x), dried with MgSO₄, filtered and concentrated *in vacuo*. The products were purified by column chromatography (Et₂O/pentane, 1/9 → 1/4, v/v) yielding the products as a colorless oil as a 3/1 mixture of diastereomers (4.27 g, 11.9 mmol, 44%).

Mayor isomer: ¹H NMR (400 MHz, CDCl₃) δ 7.38 – 7.26 (m, 10H), 5.45 – 5.40 (m, 1H), 5.09 (q, *J* = 4.9 Hz, 1H), 4.67 – 4.52 (m, 4H), 4.06 – 4.02 (m, 1H), 3.88 (dd, *J* = 4.0, 3.0 Hz, 1H), 3.82 – 3.78 (m, 1H), 3.77 – 3.64 (m, 2H), 1.47 (d, *J* = 4.9 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ = 138.1, 137.8, 128.6, 128.6, 128.5, 128.5, 128.0, 128.0, 127.9, 127.9, 127.9, 100.8, 97.0, 78.3, 76.4, 74.8, 72.2, 71.9, 60.2, 20.3. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₁H₂₄O₅Na 379.1516, found 379.1513.

3,4-di-*O*-benzyl-D-xylopyranose (**30**)



29 (2.57 g, 7.21 mmol) was dissolved in dioxane (36 ml, 0.2 M). H₂SO₄ (0.5 M aq, 36 ml) was added and the mixture was stirred at 75°C overnight. TLC (9/1, v/v, pent/EtOAc) indicated consumption of starting material. H₂O was added and the mixture was extracted with EtOAc (2x). The combined organic layers were washed with NaHCO₃ (aq. sat.) and brine, dried with MgSO₄, filtered and concentrated under reduced pressure. This yielded the product which was used without further purification (2.24 g, 6.81 mmol).

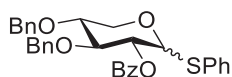
Mayor isomer: ¹H NMR (400 MHz, CDCl₃) δ 7.38 – 7.27 (m, 10H), 4.98 – 4.91 (m, 1H), 4.73 – 4.62 (m, 3H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.04 – 3.97 (m, 1H), 3.91 – 3.82 (m, 2H), 3.81 – 3.73 (m, 1H), 3.68 – 3.62 (m, 1H), 3.50 – 3.45 (m, 1H), 3.34 (d, *J* = 10.3 Hz, 1H). ¹³C NMR (101 MHz, CDCl₃) δ = 137.8, 137.2, 128.8, 128.7, 128.7, 128.3, 128.2, 128.2, 128.1, 127.9, 127.8, 92.5, 76.1, 74.1, 73.1, 71.7, 69.8, 62.5. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₁₉H₂₂O₅Na 353.1359, found 353.1354.

1,2-di-*O*-benzoyl-3,4-di-*O*-benzyl-D-xylopyranose (**31**)



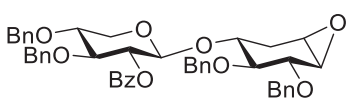
Crude diol **30** (2.25 g, 6.81 mmol) was dissolved in DCM (13.6 ml, 0.5 M). The solution was cooled to 0°C. Pyridine (2.74 ml, 34.1 mmol) and BzCl (2.77 ml, 23.8 mmol) were added and the reaction was stirred overnight. H₂O was added and the mixture was extracted with EtOAc (2x). The combined organic layers were washed with NaHCO₃ (aq. sat.) and brine, dried with MgSO₄, filtered and concentrated under reduced pressure. Column chromatography (Et₂O/pentane, 1/9 → 1/4, v/v) yielded the product (2.62 g, 4.86 mmol, 67% over 2 steps, α/β 5/4).

¹H NMR (400 MHz, CDCl₃) δ 8.13 – 8.02 (m, 6H), 8.00 – 7.96 (m, 2H), 7.70 – 7.64 (m, 1H), 7.62 – 7.50 (m, 5H), 7.46 – 7.36 (m, 15H), 7.33 – 7.23 (m, 10H), 6.64 (d, *J* = 3.6 Hz, 1H), 6.13 (d, *J* = 6.3 Hz, 1H), 5.56 (dd, *J* = 7.5, 6.3 Hz, 1H), 5.44 (dd, *J* = 9.8, 3.6 Hz, 1H), 4.99 (d, *J* = 11.2 Hz, 1H), 4.94 – 4.70 (m, 7H), 4.32 – 4.19 (m, 2H), 4.04 – 3.85 (m, 5H), 3.73 (dd, *J* = 11.9, 7.9 Hz, 1H). ¹³C NMR (101 MHz, CDCl₃) δ 165.5, 165.4, 165.0, 164.7, 138.1, 137.8, 137.8, 133.7, 133.7, 133.4, 130.2, 130.2, 129.9, 129.9, 129.8, 129.5, 129.5, 129.4, 129.0, 128.7, 128.7, 128.6, 128.5, 128.5, 128.4, 128.2, 128.1, 128.1, 128.0, 127.8, 127.8, 93.0, 90.8, 79.0, 78.7, 77.6, 76.3, 75.5, 74.3, 74.0, 72.9, 72.0, 70.6, 63.5, 62.6.

Phenyl 2-O-benzoyl-3,4-di-O-benzyl-1-thio-D-xylopyranoside (21)

Benzoyl ester **31** (1.07 g, 1.99 mmol) was dissolved in DCM (9.9 ml, 0.1 M). Thiophenol (0.22 ml, 2.19 mmol) was added and the mixture was cooled to 0°C. $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.05 ml, 0.40 mmol) was added and the mixture was slowly warmed to room temperature overnight. The next day the mixture was cooled to 0°C and more $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.05 ml, 0.40 mmol) was added and the mixture was stirred at 0°C for 5 hours. Thiophenol (0.10 ml and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.05 ml, 0.40 mmol) were added and the solution was warmed to room temperature overnight. The solution was diluted with DCM and quenched with Et_3N . The solution was washed with NaOH (1M, aq. 4x) and brine, dried over MgSO_4 filtered and the volatiles were removed under reduced pressure. Column chromatography (Et_2O /pentane, 1/19 \rightarrow 3/17, v/v) provided the product as an anomeric mixture (0.830 g, 1.57 mmol, 79%, 1/2 α/β).

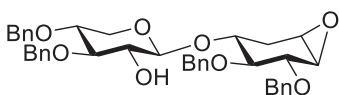
^1H NMR (500 MHz, CDCl_3) δ 8.12 – 8.07 (m, 1H), 8.06 – 8.03 (m, 2H), 7.64 – 7.57 (m, 2H), 7.51 – 7.41 (m, 7H), 7.36 – 7.18 (m, 23H), 5.85 (d, J = 5.2 Hz, 1H), 5.34 – 5.27 (m, 2H), 4.95 (d, J = 8.0 Hz, 1H), 4.90 – 4.84 (m, 1H), 4.80 – 4.62 (m, 6H), 4.26 (dd, J = 11.8, 4.5 Hz, 1H), 4.17 (dd, J = 11.6, 9.8 Hz, 1H), 4.04 (dd, J = 9.2, 8.1 Hz, 1H), 3.86 – 3.79 (m, 2H), 3.77 – 3.70 (m, 2H), 3.53 – 3.45 (m, 1H). ^{13}C NMR (126 MHz, CDCl_3) δ 165.8, 165.4, 138.0, 137.9, 133.8, 133.5, 133.3, 132.3, 131.9, 130.1, 130.0, 130.0, 129.7, 129.1, 129.0, 128.6, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.8, 127.5, 87.0, 86.2, 80.4, 79.0, 77.6, 76.9, 75.3, 74.6, 73.5, 73.1, 73.1, 71.6, 66.1, 61.7. HRMS (ESI) m/z : $[\text{M}+\text{NH}_4]^+$ calc for $\text{C}_{32}\text{H}_{34}\text{O}_5\text{SN}$ 544.2152, found 544.2151.

4-O-(2-O-benzoyl-3,4-di-O-benzyl- β -D-xylopyranosyl)-2,3-di-O-benzyl-xylocyclophellitol (22)

Thioglycoside donor **21** (87 mg, 0.17 mmol) and acceptor **14** (49 mg, 0.15 mmol) were co-evaporated with toluene. DCM (1.0 ml) and NIS (40 mg, 0.18 mmol) were added and the solution was cooled to -70°C. TMSOTf (0.015 mmol, 0.1 ml of 0.15 M solution in DCM) was added and the mixture was warmed to -50°C over 30 minutes. Et_3N (0.05 ml) was added and solution was diluted with EtOAc and washed with NaHCO_3 (aq. sat.), $\text{Na}_2\text{S}_2\text{O}_3$ (aq. 1 M) and brine, dried over MgSO_4 , filtered and the volatiles were removed under reduced pressure.

Column chromatography (Et_2O /pentane, 1/9 \rightarrow 3/7, v/v) afforded the product (81 mg, 0.11 mmol, 73%).

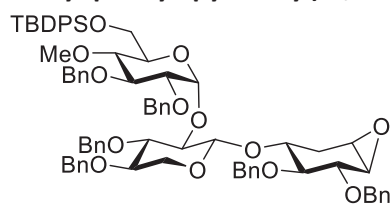
^1H NMR (400 MHz, CDCl_3) δ 8.03 – 7.98 (m, 2H), 7.60 – 7.53 (m, 1H), 7.43 (t, J = 7.8 Hz, 2H), 7.38 – 7.22 (m, 15H), 7.14 (tdd, J = 9.6, 4.5, 1.8 Hz, 5H), 5.22 – 5.17 (m, 1H), 4.95 (d, J = 10.9 Hz, 1H), 4.77 (d, J = 11.5 Hz, 1H), 4.71 – 4.54 (m, 7H), 3.97 (dd, J = 12.0, 3.9 Hz, 1H), 3.80 – 3.68 (m, 4H), 3.33 (dd, J = 10.1, 7.7 Hz, 1H), 3.26 – 3.18 (m, 1H), 3.09 (d, J = 3.4 Hz, 1H), 3.04 (d, J = 3.6 Hz, 1H), 2.41 (ddd, J = 14.6, 5.3, 2.4 Hz, 1H), 1.56 (ddd, J = 14.6, 10.5, 1.5 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 165.3, 138.9, 138.0, 138.0, 137.9, 133.2, 130.0, 129.8, 128.6, 128.5, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.9, 127.7, 127.4, 99.7, 82.5, 80.3, 79.4, 77.5, 75.3, 74.4, 73.8, 73.4, 73.2, 73.2, 63.3, 54.0, 53.4, 29.9. HRMS (ESI) m/z : $[\text{M}+\text{NH}_4]^+$ calc for $\text{C}_{46}\text{H}_{50}\text{O}_9\text{N}$ 760.3480, found 760.3477.

4-O-(3,4-di-O-benzyl- β -D-xylopyranosyl)-2,3-di-O-benzyl-xylocyclophellitol (23)

Benzoyl ester **22** (79 mg, 0.11 mmol) was dissolved in DCM/MeOH (1.0 ml, 7/3, v/v). NaOMe (5.4 M, 0.01 ml) was added and the solution was stirred overnight. The reaction was quenched with NH_4Cl and the volatiles were removed under reduced pressure. Column chromatography (EtOAc/pentane, 1/4 \rightarrow 3/7, v/v) provided the product (53 mg, 0.083 mmol, 78%).

^1H NMR (400 MHz, CDCl_3) δ 7.36 – 7.24 (m, 21H), 4.87 (d, J = 11.1 Hz, 1H), 4.79 (s, 2H), 4.77 (s, 0H), 4.73 – 4.64 (m, 3H), 4.60 (d, J = 11.7 Hz, 1H), 4.44 – 4.39 (m, 1H), 4.00 – 3.94 (m, 1H), 3.88 – 3.79 (m, 2H), 3.57 – 3.50 (m, 3H), 3.42 (dd, J = 10.2, 7.7 Hz, 1H), 3.29 – 3.18 (m, 2H), 3.13 (d, J = 3.6 Hz, 1H), 2.87 (s, 1H), 2.63 (ddd, J = 14.6, 5.3, 2.4 Hz, 1H), 1.82 (ddd, J = 14.6, 10.4, 1.5 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 138.6, 138.0, 137.8, 128.6, 128.6, 128.6, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.6, 100.7, 82.4, 81.6, 79.8, 76.9, 75.1, 74.5, 73.4, 73.0, 72.0, 70.9, 62.9, 53.9, 53.7, 30.6. HRMS (ESI) m/z : $[\text{M}+\text{NH}_4]^+$ calc for $\text{C}_{39}\text{H}_{46}\text{O}_8\text{N}$ 656.3218, found 656.3213.

4-O-(2-O-(2,3-di-O-benzyl-4-O-methyl-6-O-*t*-butyl-diphenylsilyl- α -D-glucopyranosyl) 3,4-di-O-benzyl- β -D-xylopyranosyl)-2,3-di-O-benzyl-xylocyclophellitol (24)



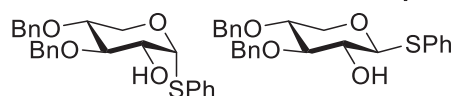
Donor **10** (67 mg, 0.095 mmol) TTBP (78 mg, 0.32 mmol) and Ph₂SO (19 mg, 0.094 mmol) were co-evaporated with toluene and dissolved in DCM (0.5 ml). Et₂O (0.5 ml) and 3 Å molecular sieves were added and the mixture was stirred for 1 hour. The solution was cooled to -65°C and Tf₂O (0.07 ml of a 1.2 M solution in DCM, 0.081 mmol) was added. The reaction was allowed to warm to -

50°C over 25 minutes and was cooled to -70°C. Acceptor **23** (40 mg, 0.063 mmol) was added as a solution in DCM (0.4 ml). The mixture was allowed to warm to -30°C and was quenched with Et₃N.

The solution was filtered, dilute with DCM and washed with NaHCO₃ (aq. sat.), dried over MgSO₄, filtered and concentrated under reduced pressure. Column chromatography (EtOAc/pentane, 1/19 → 3/17, v/v) afforded the product (24 mg, 0.019 mmol, 30%).

¹H NMR (500 MHz, CDCl₃) δ 7.68 – 7.62 (m, 4H), 7.45 – 7.14 (m, 36H), 5.51 (d, *J* = 3.6 Hz, 1H), 4.98 – 4.86 (m, 5H), 4.77 – 4.68 (m, 5H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.55 (d, *J* = 11.6 Hz, 1H), 4.50 (d, *J* = 7.0 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.88 (ddd, *J* = 14.1, 11.1, 5.1 Hz, 2H), 3.79 (d, *J* = 7.6 Hz, 1H), 3.69 – 3.52 (m, 10H), 3.40 (dd, *J* = 11.6, 2.9 Hz, 1H), 3.35 (dd, *J* = 10.2, 7.6 Hz, 1H), 3.12 (dd, *J* = 11.7, 9.3 Hz, 1H), 3.04 (d, *J* = 3.6 Hz, 1H), 2.78 (dd, *J* = 3.5, 2.1 Hz, 1H), 2.45 (ddd, *J* = 14.5, 5.3, 2.4 Hz, 1H), 1.37 – 1.35 (m, 1H), 1.02 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 138.9, 138.7, 138.1, 138.1, 138.1, 135.9, 135.7, 133.9, 133.6, 129.6, 129.5, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 100.3, 96.3, 82.5, 82.2, 81.4, 80.5, 79.8, 79.6, 79.2, 75.8, 75.7, 75.4, 74.7, 74.3, 73.4, 73.3, 71.2, 71.2, 63.8, 62.4, 60.6, 53.9, 53.4, 29.8, 28.9, 27.0, 19.5. HRMS (ESI) *m/z*: [M+NH₄]⁺ calc for C₇₆H₈₈O₁₃SiN 1250.6019, found 1250.6016.

Phenyl 3,4-di-O-benzyl-1-thio- α / β -D-xylopyranoside (45)



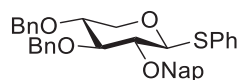
Thioglycoside **21** (354 mg, 0.672 mmol, α / β 1/2) was dissolved in MeOH/DCM (7 ml, 6/1, v/v). A catalytic amount of NaOMe was added and the mixture was stirred for 2 days. The

reaction was quenched with AcOH and the volatiles were removed under reduced pressure. The crude was dissolved in a minimal amount of toluene and loaded on a silica column. Elution (Et₂O/pentane, 1/9 → 1/4, v/v) provided the product as two separate isomers: α (200 mg, 0.473 mmol 70%) and β (90 mg, 0.213 mmol 32%).

α : ¹H NMR (500 MHz, CDCl₃) δ 7.52 – 7.47 (m, 2H), 7.36 – 7.20 (m, 13H), 5.29 (d, *J* = 2.6 Hz, 1H), 4.65 (d, *J* = 12.1 Hz, 3H), 4.57 (d, *J* = 11.8 Hz, 1H), 4.10 (ddd, *J* = 12.3, 4.7, 1.0 Hz, 1H), 3.94 (dddd, *J* = 9.9, 5.4, 2.7, 0.9 Hz, 1H), 3.85 (dd, *J* = 12.4, 2.7 Hz, 1H), 3.78 (td, *J* = 5.2, 1.0 Hz, 1H), 3.52 (tdd, *J* = 4.8, 2.7, 0.9 Hz, 1H), 3.44 (d, *J* = 9.8 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 137.8, 137.3, 135.1, 131.0, 129.0, 129.0, 128.6, 128.1, 128.1, 127.9, 127.8, 127.1, 88.0, 76.8, 74.5, 73.4, 71.9, 70.8, 64.2.

β : ¹H NMR (500 MHz, CDCl₃) δ 7.53 – 7.47 (m, 2H), 7.38 – 7.21 (m, 13H), 4.91 (d, *J* = 6.0 Hz, 1H), 4.82 (d, *J* = 11.6 Hz, 1H), 4.73 (d, *J* = 11.6 Hz, 1H), 4.61 (s, 2H), 4.27 (dd, *J* = 11.9, 3.3 Hz, 1H), 3.71 (q, *J* = 6.1 Hz, 1H), 3.62 (t, *J* = 6.2 Hz, 1H), 3.55 (td, *J* = 6.4, 3.3 Hz, 1H), 3.49 (dd, *J* = 11.9, 6.6 Hz, 1H), 3.29 (d, *J* = 6.3 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 138.1, 137.6, 134.2, 131.9, 129.0, 128.6, 128.5, 128.1, 127.9, 127.9, 127.8, 127.6, 89.0, 79.4, 75.9, 73.9, 72.4, 70.9, 63.6. HRMS (ESI) *m/z*: [M+NH₄]⁺ calculated for C₂₅H₃₀O₄S 440.1890, found 440.1888.

Phenyl 2-O-(2-naphthylmethyl)-3,4-di-O-benzyl-1-thio- α / β -D-xylopyranoside (46)



Alcohol **45** (192 mg, 0.454 mmol, only β) was dissolved in DMF (2.3 ml, 0.2 M). NapBr (151 mg, 0.682 mmol), TBAI (17 mg, 0.045 mmol) and NaH (27 mg, 0.682 mmol) were added and the solution was stirred overnight. The mixture was

quenched and diluted with water and extracted with Et₂O (3x). The combined organic layers were washed with water (3x) and brine, dried over MgSO₄, filtered and concentrated under reduced

pressure. Column chromatography (Et₂O/pentane, 1/19 → 1/9, v/v) provided the product (138 mg, 0.245 mmol, 55%).

¹H NMR (500 MHz, CDCl₃) δ 7.85 – 7.75 (m, 4H), 7.55 – 7.51 (m, 3H), 7.50 – 7.44 (m, 2H), 7.35 – 7.26 (m, 13H), 5.01 (d, *J* = 10.6 Hz, 1H), 4.94 – 4.89 (m, 2H), 4.85 (d, *J* = 11.0 Hz, 1H), 4.75 – 4.69 (m, 2H), 4.64 (d, *J* = 11.6 Hz, 1H), 4.10 – 4.05 (m, 1H), 3.71 – 3.62 (m, 2H), 3.53 – 3.47 (m, 1H), 3.27 (dd, *J* = 11.5, 9.3 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 138.6, 138.2, 135.7, 133.9, 133.4, 133.2, 132.1, 129.1, 128.6, 128.6, 128.3, 128.1, 128.1, 128.0, 127.9, 127.8, 127.7, 127.0, 126.4, 126.2, 126.0, 88.6, 85.5, 80.6, 77.9, 75.8, 75.7, 73.4, 67.6. HRMS (ESI) *m/z*: [M+Na]⁺ calculated for C₃₆H₃₄O₄Na 585.2070, found 585.2069.

5.2 References

1. Labourel, A.; Crouch, L. I.; Brás, J. L. A.; Jackson, A.; Rogowski, A.; Gray, J.; Yadav, M. P.; Henrissat, B.; Fontes, C. M. G. A.; Gilbert, H. J.; et al. The Mechanism by Which Arabinoxylanases Can Recognize Highly Decorated Xylans. *J. Biol. Chem.* **2016**, *291* (42), 22149-22159.
2. Vršanská, M.; Kolenová, K.; Puchart, V.; Biely, P. Mode of Action of Glycoside Hydrolase Family 5 Glucuronoxylan Xylanohydrolase from *Erwinia Chrysanthemi*. *FEBS J.* **2007**, *274* (7), 1666-1677.
3. Nakamichi, Y.; Fouquet, T.; Ito, S.; Watanabe, M.; Matsushika, A.; Inoue, H. Structural and Functional Characterization of a Bifunctional GH30-7 Xylanase B from the Filamentous Fungus *Talaromyces Cellulolyticus*. *J. Biol. Chem.* **2019**, *294* (11), 4065-4078.
4. Urbániková, L.; Vršanská, M.; Mørkeberg Krogh, K. B. R.; Hoff, T.; Biely, P. Structural Basis for Substrate Recognition by *Erwinia Chrysanthemi* GH30 Glucuronoxylanase. *FEBS J.* **2011**, *278* (12), 2105-2116.
5. 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.
6. Crich, D.; Pedersen, C. M.; Bowers, A. A.; Wink, D. J. On the Use of 3,5-O-Benzylidene and 3,5-O-(Di-Tert-Butylsilylene)-2-O-Benzylarabinothiofuranosides and Their Sulfoxides as Glycosyl Donors for the Synthesis of β-Arabinofuranosides: Importance of the Activation Method. *J. Org. Chem.* **2007**, *72* (5), 1553-1565.
7. Wang, L.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. Reagent Controlled Stereoselective Synthesis of α-Glucans. *J. Am. Chem. Soc.* **2018**, *140* (13), 4632-4638.
8. Ibatullin, F. M.; Shabalin, K. A.; Jänis, J. V.; Selivanov, S. I. Stereoselective Synthesis of Thioxyloligosaccharides from S-Glycosyl Isothiourea Precursors. *Tetrahedron Lett.* **2001**, *42* (27), 4565-4567.
9. Correia, M. A. S.; Mazumder, K.; Brás, J. L. A.; Firbank, S. J.; Zhu, Y.; Lewis, R. J.; York, W. S.; Fontes, C. M. G. A.; Gilbert, H. J. Structure and Function of an Arabinoxylan-Specific Xylanase. *J. Biol. Chem.* **2011**, *286* (25), 22510-22520.
10. Tanimoto, H.; Saito, R.; Chida, N. Formal Synthesis of (–)-Morphine from D-Glucal Based on the Cascade Claisen Rearrangement. *Tetrahedron Lett.* **2008**, *49* (2), 358-362.
11. Luche, J.-L.; Rodriguez-Hahn, L.; Crabbé, P. Reduction of Natural Enones in the Presence of Cerium Trichloride. *J. Chem. Soc. Chem. Commun.* **1978**, *14*, 601-602.
12. Volbeda, A. G.; Kistemaker, H. A. V.; Overkleeft, H. S. Chemoselective Cleavage of P-Methoxybenzyl and 2-Naphthylmethyl Ethers Using a Catalytic Amount of HCl in Hexafluoro-2-Propanol. *J. Org. Chem.* **2015**, *80* (17), 8796-8806.
13. Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. Specific Removal of O-Methoxybenzyl Protection by DDQ Oxidation. *Tetrahedron Lett.* **1982**, *23* (8), 885-888.
14. Wright, J. A.; Yu, J.; Spencer, J. B. Sequential Removal of the Benzyl-Type Protecting Groups PMB and NAP by Oxidative Cleavage Using CAN and DDQ. *Tetrahedron Lett.* **2001**, *42* (24), 4033-4036.
15. Attia, M. A.; Nelson, C. E.; Offen, W. A.; Jain, N.; Davies, G. J.; Gardner, J. G.; Brumer, H. In Vitro and in Vivo Characterization of Three *Cellvibrio Japonicus* Glycoside Hydrolase Family 5 Members Reveals Potent Xyloglucan Backbone-Cleaving Functions. *Biotechnol. Biofuels* **2018**, *11* (1), 45.
16. Vudhigiri, S.; Koude, D.; Veeragoni, D. K.; Misra, S.; Prasad, R. B. N.; Jala, R. C. R. Synthesis and Biological Evaluation of 5-Fatty-Acylamido-1, 3, 4-Thiadiazole-2-Thioglycosides. *Bioorg. Med. Chem. Lett.* **2017**, *27* (15), 3370-3373.
17. Loka, R. S.; Sletten, E. T.; Barash, U.; Vlodavsky, I.; Nguyen, H. M. Specific Inhibition of Heparanase by a Glycopolymer with Well-Defined Sulfation Pattern Prevents Breast Cancer Metastasis in Mice. *ACS Appl. Mater. Interfaces* **2019**, *11* (1), 244-254.

18. Chhabra, M.; Ferro, V. PI-88 and Related Heparan Sulfate Mimetics In *Heparanase. Advances in Experimental Medicine and Biology*. Vlodavsky, I.; Sanderson, R. D.; Ilan, N.; Springer, Cham, **2020**, 473-491.
19. Hammond, E.; Dredge, K. Heparanase Inhibition by Pixatimod (PG545): Basic Aspects and Future Perspectives. In *Heparanase. Advances in Experimental Medicine and Biology*. Vlodavsky, I.; Sanderson, R. D.; Ilan, N.; Springer, Cham, **2020**, 539-565.
20. Wu, L.; Jiang, J.; Jin, Y.; Kallemijn, 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.
21. Gao, Q.; Zaccaria, C.; Tontini, M.; Poletti, L.; Costantino, P.; Lay, L. Synthesis and Preliminary Biological Evaluation of Carba Analogues from Neisseria Meningitidis A Capsular Polysaccharide. *Org. Biomol. Chem.* **2012**, *10* (33), 6673-6681.
22. Wolfe, S.; Myung-Hwan, W.; Mitchell, D. J. On the Magnitudes and Origins of the "Anomeric Effects", "Exo-Anomeric Effects", "Reverse Anomeric Effects", and C-X and C-Y Bond Lengths in XCH₂YH Molecules. *Carbohydr. Res.* **1979**, *69* (1), 1-26.
23. García-Herrero, A.; Montero, E.; Muñoz, J. L.; Espinosa, J. F.; Vián, A.; García, J. L.; Asensio, J. L.; Cañada, F. J.; Jiménez-Barbero, J. Conformational Selection of Glycomimetics at Enzyme Catalytic Sites: Experimental Demonstration of the Binding of Distinct High-Energy Distorted Conformations of C-, S-, and O-Glycosides by E. C Oli β -Galactosidases. *J. Am. Chem. Soc.* **2002**, *124* (17), 4804-4810.
24. Link, J. J.; Sorensen, B. K. A Method for Preparing C-Glycosides Related to Phlorizin. *Tetrahedron Lett.* **2000**, *41* (48), 9213-9217.
25. Ellsworth, B. A.; Meng, W.; Patel, M.; Girotra, R. N.; Wu, G.; Sher, P. M.; Hagan, D. L.; Obermeier, M. T.; Humphreys, W. G.; Robertson, J. G.; et al. Aglycone Exploration of C-Arylglucoside Inhibitors of Renal Sodium-Dependent Glucose Transporter SGLT2. *Bioorg. Med. Chem. Lett.* **2008**, *18* (17), 4770-4773.
26. Xu, B.; Unione, L.; Sardinha, J.; Wu, S.; Ethève-Quekquejeu, M.; Pilar Rauter, A.; Blériot, Y.; Zhang, Y.; Martín-Santamaría, S.; Díaz, D.; et al. Gem-Difluorocarbadisaccharides: Restoring the Exo-Anomeric Effect. *Angew. Chem. Int. Ed.* **2014**, *53* (36), 9597-9602.
27. Linclau, B.; Ardá, A.; Reichardt, N.-C.; Sollogoub, M.; Unione, L.; Vincent, S. P.; Jiménez-Barbero, J. Fluorinated Carbohydrates as Chemical Probes for Molecular Recognition Studies. Current Status and Perspectives. *Chem. Soc. Rev.* **2020**, *49*, 3863-3888.
28. Deleuze, A.; Menozzi, C.; Sollogoub, M.; Sinaÿ, P. Synthesis of Gem-Difluorocarba-D-Glucose: A Step Further in Sugar Mimesis. *Angew. Chem. Int. Ed.* **2004**, *43* (48), 6680-6683.
29. Aurrecochea, J. M.; Gil, J. H.; López, B. Substituent Effects on the Sml₂/Pd(0)-Promoted Carbohydrate Ring-Contraction of 5-Alkynylpyranosides. *Tetrahedron* **2003**, *59* (36), 7111-7121.
30. Ilan, N.; Bhattacharya, U.; Barash, U.; Boyango, I.; Yanku, Y.; Gross-Cohen, M.; Vlodavsky, I. Heparanase-The Message Comes in Different Flavors. In *Heparanase. Advances in Experimental Medicine and Biology*. Vlodavsky, I.; Sanderson, R. D.; Ilan, N.; Springer, Cham, **2020**, 253-283.
31. Frei, P.; Hevey, R.; Ernst, B. Dynamic Combinatorial Chemistry: A New Methodology Comes of Age. *Chem. Eur. J.* **2019**, *25* (1), 60-73.
32. Mondal, M.; Hirsch, A. K. H. Dynamic Combinatorial Chemistry: A Tool to Facilitate the Identification of Inhibitors for Protein Targets. *Chem. Soc. Rev.* **2015**, *44* (8), 2455-2488.
33. Congreve, M. S.; Davis, D. J.; Devine, L.; Granata, C.; O'Reilly, M.; Wyatt, P. G.; Jhoti, H. Detection of Ligands from a Dynamic Combinatorial Library by X-Ray Crystallography. *Angew. Chem. Int. Ed.* **2003**, *42* (37), 4479-4482.
34. Kallemijn, 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. Chem. Int. Ed.* **2012**, *51* (50), 12529-12533.
35. Len, C.; Postel, D.; Ronco, G.; Villa, P. Synthesis of Bis(Carbamoyl Ester) Derivatives of D-Glucose as Antifungal Products. *J. Carbohydr. Chem.* **1997**, *16* (7), 1029-1049.
36. Aksinenko, A. Y.; Pushin, A. N.; Sokolov, V. B. Synthesis of Thiiranylmethylamides from N(S)-Allylthiooxamides. *Phosphorus Sulfur Silicon Relat. Elem.* **2019**, *194* (8), 775-779.

Nederlandse samenvatting

Glycosidases zijn enzymen die de hydrolyse van een glycosidische binding katalyseren. De enzymen kunnen onder andere geclassificeerd worden als endo- en exo-glycosidases. De exo-varianten hydrolyseren de eindstandige koolhydraat van een langere keten, terwijl de endo-varianten activiteit op de gehele keten kunnen hebben. Glycosidases spelen een belangrijke rol in uiteenlopende processen in levende systemen en zijn dus overal in de natuur te vinden.

Natuurlijk voorkomende glycosidases kunnen toegepast worden om op een milde en selectieve wijze glycosiden af te breken, bijvoorbeeld om producten te maken uit biomassa of om schadelijke biofilms op te lossen. Ook kunnen in sommige gevallen mensen met bepaalde aandoeningen gebaat zijn bij de remming van een glycosidase. Vanwege al deze toepassingen wordt er veel onderzoek gedaan naar het vinden, karakteriseren en remmen van glycosidases met uiteenlopende specificiteit.

In dit proefschrift staat de chemische synthese en de biologische toepassing van irreversibel covalente remmers van endo-glycosidases beschreven. De structuur van de ontworpen en gesynthetiseerde moleculen is opgebouwd rond de in de natuur gevonden enzymremmer cyclophellitol. De structuur van cyclophellitol wordt door de glycosidase herkend als een koolhydraat, maar in tegenstelling tot een koolhydraat bevat cyclophellitol een epoxide die fungeert als electrofiele val. Hierdoor kan het enzym wel een covalente interactie met de remmer aangaan maar deze niet meer hydrolyseren en wordt de activiteit van het enzym blijvend geremd. De covalente remmers kunnen uitgerust worden met een label waardoor de enzymen die covalent aan de remmers gebonden zijn gedetecteerd kunnen worden. Dit soort moleculen, die selectief enzymen met een bepaalde activiteit detecteerbaar kunnen maken, staan in de Engelstalige vakliteratuur bekend als ‘activity-based probes’ (ABPs). Tot op heden zijn de meeste ABPs ontwikkeld voor exo-glycosidases. In dit proefschrift ligt de nadruk op de ontwikkeling van ABPs en covalente remmers van verschillende endo-glycosidases.

In hoofdstuk 1 wordt de voorkennis, opgedaan in voorgaande studies met cyclophellitol ABPs, samengevat aan de hand van voorbeelden van ontwerpstrategieën en toepassingen van op cyclophellitol gebaseerde ABPs. Het hoofdstuk beschrijft ook de huidige kennis over de mechanistische en conformationele aspecten van de reactie tussen cyclophellitol derivaten en verschillende klassen van — voornamelijk exo — glycosidases. Aan het eind van het

hoofdstuk wordt een overzicht gegeven van de meest gangbare experimenten die met een ABP uitgevoerd kunnen worden.

Hoofdstuk 2 richt zich op glycosidases die gebruikt kunnen worden om stugge biomassa om te zetten in makkelijk te verwerken bouwstenen. Om voldoende binding met de endo-glycosidase enzymen te bewerkstelligen moeten de ABPs een uitgebreider herkenningselement bevatten dan de bekende ABPs voor exo-glycosidases. In het hoofdstuk wordt de chemische synthese van ABPs voor cellulases, xyloglucanases en α -xylosidases beschreven. Deze ABPs kunnen de opsporing, karakterisatie en activiteitscontrole van de enzymen vergemakkelijken. De cellulase en xyloglucanase ABPs worden gesynthetiseerd via chemische glycosyleringen, onder pre-activatie condities, op cyclophellitol nucleofielen. De activiteit van de gesynthetiseerde endo-glycosidase ABPs bleek, in de enzymmengsels uitgescheiden door de schimmel *Aspergillus niger*, geen overlap te vertonen met exo-glycosidase ABPs. Dit nieuwe ABP ontwerp blijkt dus ABPs selectief voor endo-glycosidases op te kunnen leveren. Ook lieten de ABPs ontwikkeld voor cellulases en xyloglucanases verschil in specificiteit zien, wat erop kan wijzen dat met verschillend gesubstitueerde ABPs aparte subklassen van enzymen onderscheiden kunnen worden.

In hoofdstuk 3 worden drie verschillende ontwerpen voor ABPs en een set covalente remmers voor heparanase (HPSE) gesynthetiseerd. HPSE is de enige bekende endo-glycosidase in de extracellulaire matrix met activiteit voor heparansulfaat. Hoge HPSE activiteit wordt in verband gebracht met een verscheidenheid aan aandoeningen. De detectie en remming van HPSE activiteit zou dus kunnen bijdragen aan de diagnose en behandeling van verschillende ziekten. De remmers werden gesynthetiseerd uit één gezamenlijk tussenproduct en verschillen in substitutie op de -2 positie waardoor een structuur-activiteitsrelatie voor deze positie gemaakt kan worden. Het is gebleken dat sulfatering op de 6'O en acetylering of verwijdering van de 2'N positief effect hebben op de HPSE remming. Het installeren van een 2'amine of een 2' azido acetyl bleek geen potente remmers op te leveren. Twee van de remmers zijn effectief bevonden in verschillende HPSE inhibitie testen. Deze remmers kunnen gebruikt worden om het effect van HPSE inhibitie in detail te bestuderen en als beginpunt voor de ontwikkeling van een nieuwe klasse HPSE remmers voor medicinale toepassingen. ABPs met een label op de 2'N bleken, in lijn met de activiteit van de remmers, ook niet actief te zijn en cyclophellitol met een label op de 4O was niet selectief voor HPSE. Uiteindelijk bleek de disaccharide ABP met het label op de 4'O selectief voor HPSE.

In hoofdstuk 4 wordt de synthese beschreven van een azide gelabeld cyclophellitol derivaat gelijkend op de chemische structuur van de polysaccharide, genaamd Psl. Deze polysaccharide wordt gevonden in de biofilms van de pathogene bacterie *Pseudomonas aeruginosa*. Het molecuul werd gesynthetiseerd door middel van een selectieve β -mannosylering op een mannose geconfigureerd cyclophellitol alkeen derivaat. Vervolgens werd het volledige molecuul ontschermd waarna een stereoselectieve epoxidering met een peroxycarbonzuur in loog het gewenste product opleverde. Het door dezelfde bacterie tot expressie gebrachte enzym PslG is in staat deze biofilms af te breken. De verwachting was dat de gesynthetiseerde ABP zou kunnen reageren met dit enzym. Dit was echter niet het geval, wat aanleiding zou kunnen geven om de karakterisatie van PslG te herzien. De ABP zou gebruikt kunnen worden om andere enzymen te vinden die met deze specificiteit Psl zouden kunnen afbreken.

Afsluitend staat in hoofdstuk 5 een samenvatting van de verkregen resultaten met aanbevelingen voor vervolgonderzoek.