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Leiden
The Netherlands

Exploring chemical space in covalent and competitive glycosidase inhibitor design

Chen, Y.

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Novel Uronic Acid-Type 1-*N*-Iminosugars Derived from Siastatin B as Competitive Heparanase Inhibitors

5.1 Introduction

Heparan sulfate (HS) is a long linear polysaccharide consisting of repeating disaccharide units formed by a uronic acid (either D-glucuronic acid, GlcA or L-iduronic acid, IdoA) and a D-glucosamine (either N-acetylated, GlcNAc or N-sulfated, GlcNS) that is modified at various positions by sulfation, yielding domains with high sulfation along the HS chain separated by low/minimal sulfation segments.¹ The structure of heparan sulfate proteoglycans (HSPGs) consists of a core protein to which variable HS chains are covalently attached.^{2, 3} HSPGs are mainly localized in the extracellular matrix (ECM), on the cell surface and also in the basement membrane (BM), and contribute significantly to the structural integrity, self-assembly and insolubility of the ECM and BM.^{4, 5} Moreover, HSPGs provide a reservoir for the binding of numerous bioactive molecules such as growth factors, cytokines, chemokines and enzymes, with specific binding events mediated by local features (e.g. sulfation patterns or anionic charges)^{6, 7} of HS chains.

Heparanase (HPSE) is the only known mammalian *endo*- β -D-glucuronidase capable of cleaving within HS chains. It catalyses the hydrolysis of internal β -1,4-linked glycosidic bonds between GlcA and GlcNS (Figure 5.1) at a limited number of sites. HPSE processes its substrates through a Koshland double displacement mechanism, generating smaller HS fragments with net retention of the anomeric configuration.^{8, 9} Under normal physiological conditions, HPSE is expressed at high levels in placenta and a few cell types such as platelets, neutrophils and mast cells, and is poorly expressed in other human tissues. However, upregulated expression and increased enzymatic activity of HPSE have been reported in multiple pathological conditions,¹⁰⁻¹² particularly in cancer progression and inflammation disorders where HPSE-mediated degradation of HS and the consequent release of HS-sequestered molecules enables remodelling of the ECM network and facilitates cell migration, invasion and proliferation.

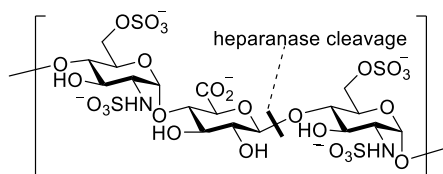


Figure 5.1. Heparanase cleavage site within the structure of heparan sulfate.

The pivotal role of HPSE in the establishment and development of numerous diseases has made it an attractive pharmacological target and several different classes of inhibitors have been developed over the past two decades, ranging from polysulfated oligo- and polysaccharides,

nucleic acids and monoclonal antibodies to small-molecule inhibitors.¹³⁻¹⁶ Amongst these, heparin/HS mimetics are the most intensively studied ones and four compounds (PI-88, SST0001, M402, and PG545)¹⁷⁻²⁰ of this class have been tested in clinical trials for various types of cancer. However, these compounds share some limitations related to their high-molecular weight nature, heterogeneous structure and parenteral administration, which may limit their potential therapeutic application. The discovery of small molecules endowed with anti-HPSE activity would provide another alternative to the development of novel therapeutic drugs because of their more conducive pharmacokinetic properties and ease of optimization for oral administration.^{15, 21}

Iminosugars are monosaccharide analogues which contain a nitrogen atom instead of an oxygen in the ring.²² Many compounds of this type, such as 1-deoxynojirimycin, are potent and selective inhibitors of enzymes associated with carbohydrate metabolism,^{23, 24} and have been investigated as potential therapeutics for tumor metastasis, diabetes, lysosomal storage disorders and other diseases.²⁵⁻²⁷ Siastatin B, a natural product originally isolated from a *Streptomyces* culture by Umezawa *et al.*,²⁸ is a *gem*-diamine 1-*N*-iminosugar in which the anomeric carbon is replaced by nitrogen²⁹ (Figure 5.2A). It effectively inhibits neuraminidases as well as β -D-glucuronidases and *N*-acetyl- β -D-glucosaminidases, presumably because it structurally resembles *N*-acetylneuraminic acid, β -D-glucuronic acid and *N*-acetyl- β -D-glucosamine. Upon achievement of the total synthesis³⁰ of siastatin B, Nishimura and co-workers further reported the synthesis of a series of siastatin B derivatives characterized by a trifluoroacetamido substituent at the 2 position of the piperidine scaffold (the ring numbering is depicted in siastatin B).^{31, 32} These 2-trifluoroacetamido derivatives **2-4** (Figure 5.2A) were all shown to be very potent inhibitors of bovine liver β -D-glucuronidase and also showed moderate inhibition of recombinant human HPSE. NMR analyses of compound **2** in the media of enzyme assays have suggested that it can undergo pH-dependent Amadori rearrangement in solution, yielding a hemiaminal/hydrated ketone that may act as the true inhibitor (Figure 5.2B).³³ This rearrangement starts by elimination of the 2-trifluoroacetamido group that can be followed by hydration of the resulting iminium to form hemiaminal **5**. Enolization of **6** can give enol **7**, which can further tautomerize to ketone **8**, followed by addition of a H₂O molecule to give hydrate **9**. This solvent-mediated rearrangement has not been demonstrated for siastatin B, which appears to be relatively stable in aqueous solutions.

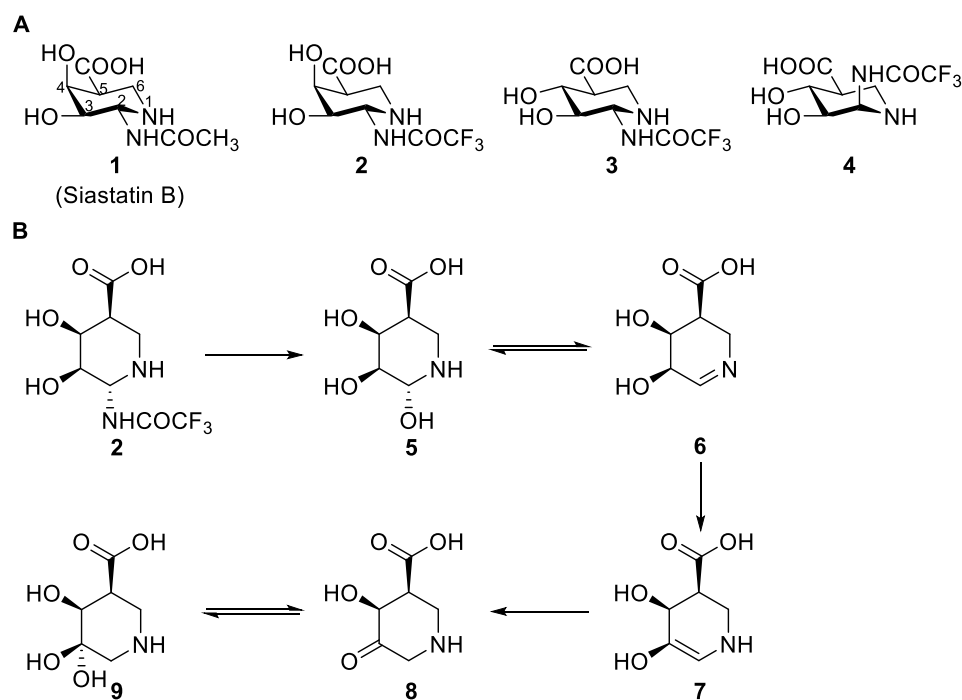


Figure 5.2. A) Chemical structures of siastatin B (**1**) and its 2-trifluoroacetamido derivatives **2-4**; B) Transformation of D-galacturonic acid-type gem-diamine 1-N-iminosugar **2** into hydrate **9** in aqueous acetate buffer (pH 5.0) as proposed by Nishimura and co-workers.

The crystal structural of human HPSE has recently been resolved by Wu *et al.*,³⁴ enabling structure-based design of HPSE inhibitors. Surprisingly, preliminary X-ray structures of human HPSE treated with siastatin B, as conducted by Liang Wu (University of York), has indicated that it is hydrate **9**, and not siastatin B, that occupies the enzyme active site. To establish the mode of action by which siastatin B inhibits heparanases and β -glucuronidases, X-ray crystallographic analysis of co-complexes between siastatin B and different enzymes are presented in this chapter. Furthermore, in order to understand the action of the breakdown products and their HPSE inhibitory properties, a panel of uronic acid-type 1-N-iminosugars **8-14** was synthesized (Figure 5.3). Besides the *galacto*-configured ketone **8** that may be formed by degradation of siastatin B, the *gluco*-configured analogue **12** was considered as well since it more closely mimics the glucose configuration of the natural HPSE substrates: glucuronic acid. Previous work on endo-glycosidase inhibitors as described in this thesis (Chapters 2 and 3) and elsewhere³⁵⁻³⁷ have demonstrated that endo-glycosidases have larger active sites to accommodate elongation at the non-reducing end sugars where the polysaccharide chain continues – here the O4 position. In line with this observation, O4-methylated compounds **10** and **13** were included in the panel as well, with the aim to achieve selectivity for HPSE over *exo*- β -D-glucuronidases. Additionally, uronic acid-type isofagomine **14** has previously been

reported as a potent inhibitor of β -D-glucuronidase,³⁸⁻⁴⁰ and comprises the reduced (keto/hydrate to alcohol) derivative of compound **2**. Since its inhibitory activity towards HPSE has yet to be explored, compound **14** as well as its *galacto*-configured isomer **11** were included in the panel.

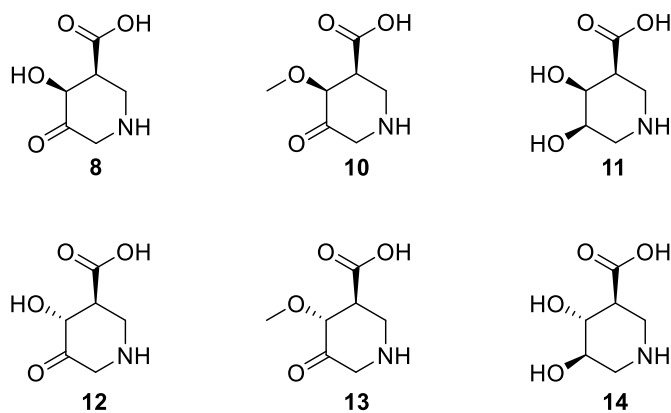


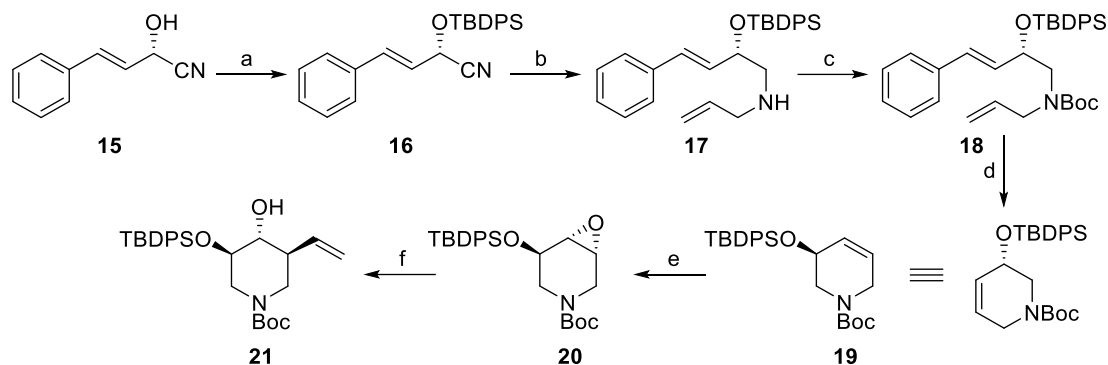
Figure 5.3. Structures of D-*galacto*- and D-*gluco*-uronic acid-type 1-*N*-iminosugars which are subject of the studies described in this chapter.

5.2 Results and discussion

5.2.1 Synthesis of D-glucuronic acid-type 1-*N*-iminosugars

The synthesis of glucuronic acid-type 1-*N*-iminosugars commenced with the preparation of key intermediate **21** (Scheme 5.1). Transformation of **15** into **19** was achieved using adaptations of the procedures described by Jiang *et al.* for the construction of 1-deoxy-L-fuconojirimycin.⁴¹ Starting from enantiomerically pure cyanohydrin **15**, prepared employing (*S*)-hydroxynitrile lyase from the *Hevea brasiliensis* rubber tree,⁴² the secondary alcohol was silylated to give **16** in excellent yield. Conversion of **16** via a one-pot DIBAL-H reduction-transimination-sodium borohydride reduction cascade sequence, using commercially available allylamine, followed by subsequent *N*-Boc protection, afforded compound **18** in 81% yield over two steps. Ring-closing metathesis of **18** using Grubbs' catalyst provided heterocyclic alkene **19**. The yield (45%) is however relatively low compared to those (88%–97%) of previously reported analogues,⁴¹ possibly due to the higher reaction concentration (0.2 M instead of 0.1 M) applied here, which led to the formation of undesired intermolecular metathesis side products. The last two steps in the synthesis route followed strategies as reported by Takahata *et al.*⁴³ Epoxidation of **19** was performed using methyl(trifluoromethyl) dioxirane (generated *in situ* from 1,1,1-trifluoroacetone and oxone⁴⁴), and epoxide **20** was formed preferably due to a favored attack of the dioxirane on the less hindered *anti*-side of the large TBDPS group. Nucleophilic opening of epoxide **20**

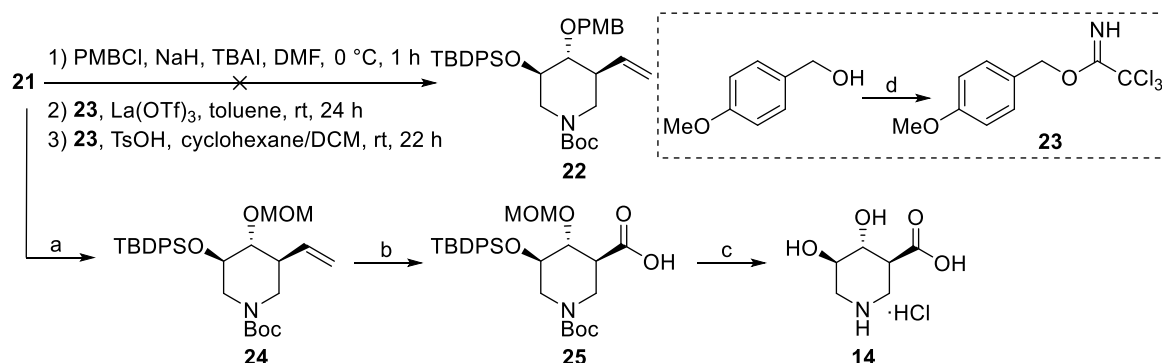
was carried out with *in situ* generated cuprate⁴⁵ $(\text{CH}_2=\text{CH})_2\text{CuCNLi}_2$ in the presence of Lewis acid at low temperature for 2 h, affording product **21** in 64% yield.



Scheme 5.1. Synthesis of key intermediate **21**. Reagents and conditions: a) TBDPSCl, imidazole, DMF, 0 °C to rt, 98%; b) i) DIBAL-H, Et_2O , -78 °C to 10 °C; ii) MeOH, -90 °C; iii) allylamine, NaOMe, rt; iv) NaBH_4 , 0 °C to rt; c) Boc_2O , Et_3N , DCM, rt, 81% over two steps; d) Grubbs 1st generation, DCM, reflux, 45%; e) oxone, CH_3COCF_3 , NaHCO_3 , EDTA, MeCN, H_2O , 0 °C, 60%; f) *n*-butyllithium, tetravinyltin, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, Et_2O , -78 °C, 2 h, 64%.

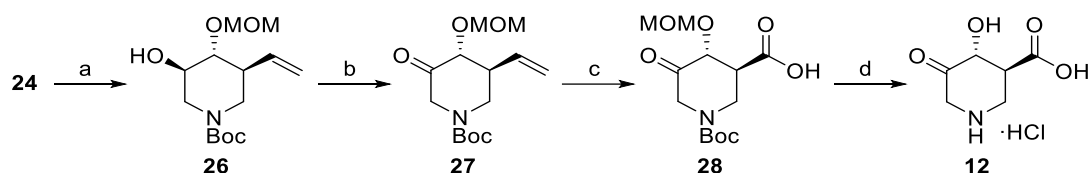
Glucuronic acid-type isofagomine **14** was synthesized by Ichikawa *et al.* starting from commercially available D-arabinose in 13 steps.³⁸ Here, an alternative synthesis route starting from the vinyl intermediate **21** is described (Scheme 5.2). It was envisioned that protecting the secondary alcohol in **21** with an acid-labile group would facilitate its removal together with the Boc and TBDPS groups in the final stage of the synthesis, therefore a 4-methoxybenzyl (PMB) group was first chosen for this purpose. However, treatment of **21** with PMBCl in the presence of sodium hydride (NaH) at 0 °C for 1 h only led to the formation of a desilylated side product. Other attempts by reacting **21** with freshly prepared PMB-imidate **23** using catalytic amounts of acid^{46–48} proved unproductive as well. As an alternative, protection of the hydroxyl group as the methoxymethyl (MOM) ether was investigated. While treatment of **21** with an excess of MOMCl (7.0 eq.) in the presence of DIPEA (8.0 eq.) proved unproductive when conducted at room temperature, heating the reaction mixture to reflux (40 °C) overnight afforded product **24** in good yield. It was found that performing the reaction in a sealed microwave tube, which could prevent the evaporation of solvent (CH_2Cl_2) and the low-boiling MOMCl during the refluxing process, allowed to achieve full conversion of the starting material. Oxidative cleavage of the terminal alkene to the corresponding carboxylic acid was best achieved in one-pot by using ruthenium tetroxide (generated *in situ* from ruthenium chloride and sodium periodate) in a solvent system of $\text{CCl}_4/\text{CH}_3\text{CN}/\text{H}_2\text{O}$ as developed by Sharpless and co-workers,⁴⁹ resulting in the isolation of **25** in 64% yield. Attempts to replace (toxic) CCl_4 by

EtOAc or CH₂Cl₂ led to incomplete conversion even after prolonged reaction times (15 h), and the corresponding vicinal diol and aldehyde intermediates (from **24**) were observed as the main products by TLC analysis. Ultimately, all of the protecting groups in **25** were removed by treatment with 3 M HCl under reflux for 4 h, yielding product **14** in excellent yield, and all spectroscopic data obtained for **14** proved to be in agreement with those reported in the literature.³⁸⁻⁴⁰



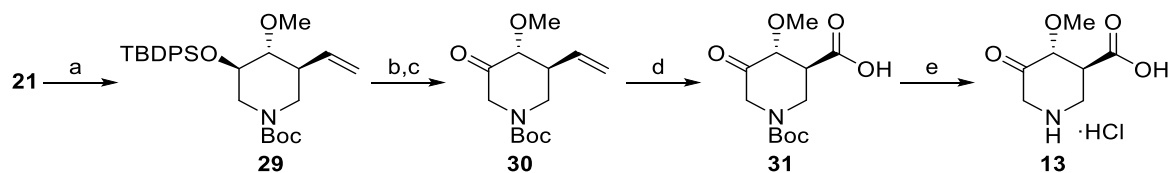
Scheme 5.2. Synthesis of glucuronic acid-type isofagomine **14**. Reagents and conditions: a) MOMCl, DIPEA, DCM, reflux, 87%; b) RuCl₃·3H₂O (10 mol%), NaIO₄ (5.0 eq.), CCl₄/CH₃CN/H₂O, rt, 3 h, 64%; c) 3 M HCl in H₂O, dioxane, 100 °C, 4 h, 95%; d) CCl₃CN, NaH, Et₂O, 0 °C to rt, 2 h, 98%.

Next, the synthesis of **12** from intermediate **24** was investigated (Scheme 5.3). The TBDPS group in **24** could be readily removed by tetrabutylammonium fluoride (TBAF) in THF, resulting in the formation of alcohol **26**, which was then oxidized to ketone **27** with Dess-Martin periodinane (DMP). Oxidative cleavage of the terminal alkene proceeded smoothly using the same conditions as described for the preparation of **25**. Complete conversion of the starting material was achieved after 2 h and carboxylic acid **28** was observed as the single product by TLC and LC-MS analyses. However, extraction of the product from the reaction mixture proved to be troublesome after quenching the reaction with saturated aqueous sodium thiosulfate (Na₂S₂O₃). It was found that the reaction mixture had an original pH of around 2 to 3, which after treatment with Na₂S₂O₃ solution increased to ~8, pH at which the product became water-soluble. This problem was circumvented by quenching the reaction with isopropanol³² and the product could be easily extracted into the organic phase. After purification by standard silica gel column chromatography, carboxylic acid **28** was isolated in good yield. Removal of the protecting groups could be accomplished by treatment with 4 M HCl in dioxane/H₂O for 5 h, providing compound **12** as the single product after precipitation from MeOH/Et₂O. Of note, deprotection of **28** under acidic conditions for prolonged reaction times (18 h) resulted in the formation of a complex mixture of several compounds.



Scheme 5.3. Synthesis of compound **12**. Reagents and conditions: a) TBAF, THF, rt, 94%; b) Dess-Martin periodinane, DCM, 0 °C to rt, 93%; c) $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (10 mol%), NaIO_4 (5 eq.), $\text{CCl}_4/\text{CH}_3\text{CN}/\text{H}_2\text{O}$, rt, 2 h, 83%; d) 4 M HCl in dioxane/ H_2O , rt, 70%.

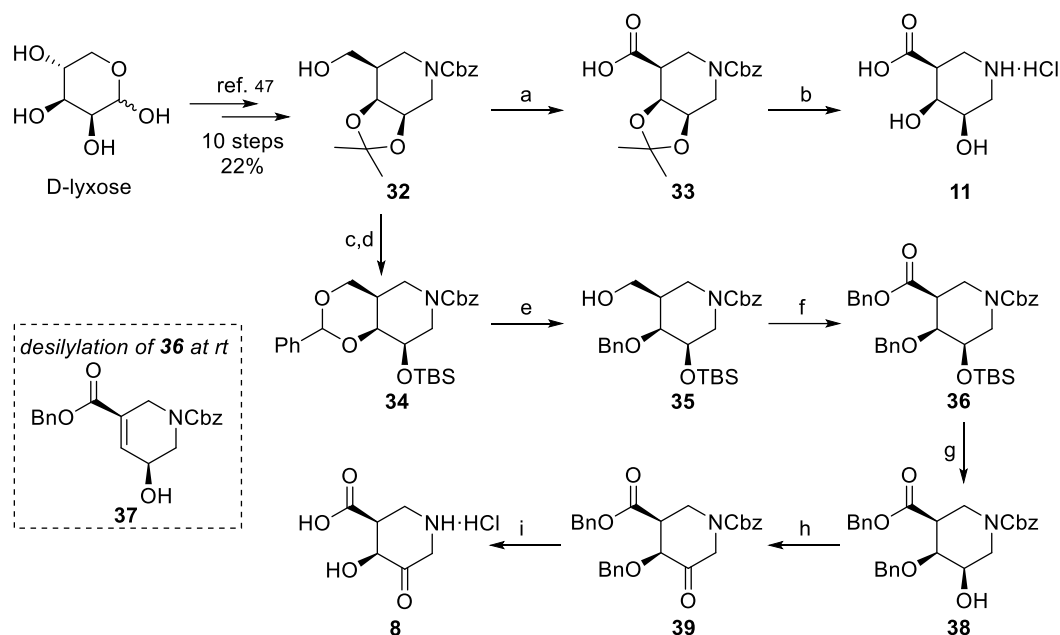
As the next objective, the versatility of alkene **21** as starting material for the synthesis of **13** was investigated (Scheme 5.4). The secondary alcohol in **21** was methylated by reaction with an excess (8.0 eq.) of trimethyloxonium tetrafluoroborate, affording compound **29** in good yield. Desilylation of **29** followed by oxidation resulted in the formation of ketone **30**. Oxidative cleavage of the terminal double bond in **30** had to be performed carefully. Treatment of **30** with 10 mol% of RuCl_3 and 5.0 equivalent of NaIO_4 at room temperature for 2 h (the same condition as used for the preparation of **28**) resulted in the isolation of carboxylic acid **31** in a low yield (28%). NMR analysis of the crude product revealed the presence of several impurities which were presumably intermediates resulting from incomplete oxidation. However, after prolonging the reaction time to 17 h, compound **31** could not be detected in the crude while the impurities were observed by NMR, which indicates that these impurities might be a result of over-oxidation since RuO_4 is known as a strong oxidant. To confirm this speculation, the reaction was performed with decreased amounts of RuCl_3 (5 mol%) and NaIO_4 (3.5 eq.) at 0 °C, and the oxidation process was monitored every 15 minutes by TLC analysis and terminated as soon as no intermediates (diol and aldehyde) remained. In this way carboxylic acid **31** was obtained in 75% yield and no over-oxidation side products were detected. The Boc group in **31** was then removed under acidic conditions to give the 4-methylated iminosugar **13** in around 80% purity.



Scheme 5.4. Synthesis of compound **13**. Reagents and conditions: a) trimethyloxonium tetrafluoroborate, proton sponge, 3 Å MS powder, DCM, 0 °C to rt, 75%; b) TBAF, THF, rt, 90%; c) Dess-Martin periodinane, DCM, 0 °C to rt, 83%; d) $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (5 mol%), NaIO_4 (3.6 eq.), $\text{CCl}_4/\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 0 °C for 1 h then rt for 30 min, 75%; e) 0.2 M HCl/HFIP, H_2O , rt, 80% in purity.

5.2.2 Synthesis of D-galacturonic acid-type 1-*N*-iminosugars

The synthesis towards galacturonic acid-type 1-*N*-iminosugars commenced with commercially available D-lyxose that was smoothly converted into key intermediate **32** (Scheme 5.5) in ten steps following procedures described by Ichikawa *et al.* for the preparation of the *N*-Boc protected version of compound **32**.⁵⁰ Oxidation of the primary alcohol in **32** with Dess-Martin periodinane gave an aldehyde intermediate that was further oxidized by means of Pinnick oxidation,⁵¹ providing carboxylic acid **33** in 65% yield over two steps. Deprotection of the isopropylidene acetal and the carboxybenzyl (Cbz) group was achieved by palladium catalyzed hydrogenolysis in the presence of acid, affording galacturonic acid-type isofagomine **11** in quantitative yield. Next, the preparation of **8** from common intermediate **32** was investigated. The 3,4-isopropylidene acetal in **32** was removed smoothly under acidic conditions followed by a thermodynamically controlled installation of the benzylidene acetal and subsequent silylation of the remaining 3-hydroxyl group, affording fully protected **34** in 68% yield over three steps. Regioselective cleavage of the benzylidene acetal using $\text{BH}_3 \cdot \text{THF}/\text{TMSOTf}$ ⁵² resulted in the isolation of compound **35** in excellent yield. Jones oxidation of the primary alcohol followed by benzyl protection of the resulting carboxylic acid gave benzyl ester **36** in moderate yield. Removal of the silyl group with TBAF needed to be performed carefully. Treatment of **36** with TBAF at room temperature for prolonged reaction times resulted in the isolation of unsaturated ester **37** which was formed by tetrabutylammonium hydroxide catalyzed β -elimination. This problem could be circumvented by lowering the temperature and shortening the reaction time, providing compound **38** in 94% yield. Oxidation of the secondary alcohol to the ketone followed by global deprotection using palladium catalyst under hydrogen atmosphere finally afforded target 1-*N*-iminosugar **8**.

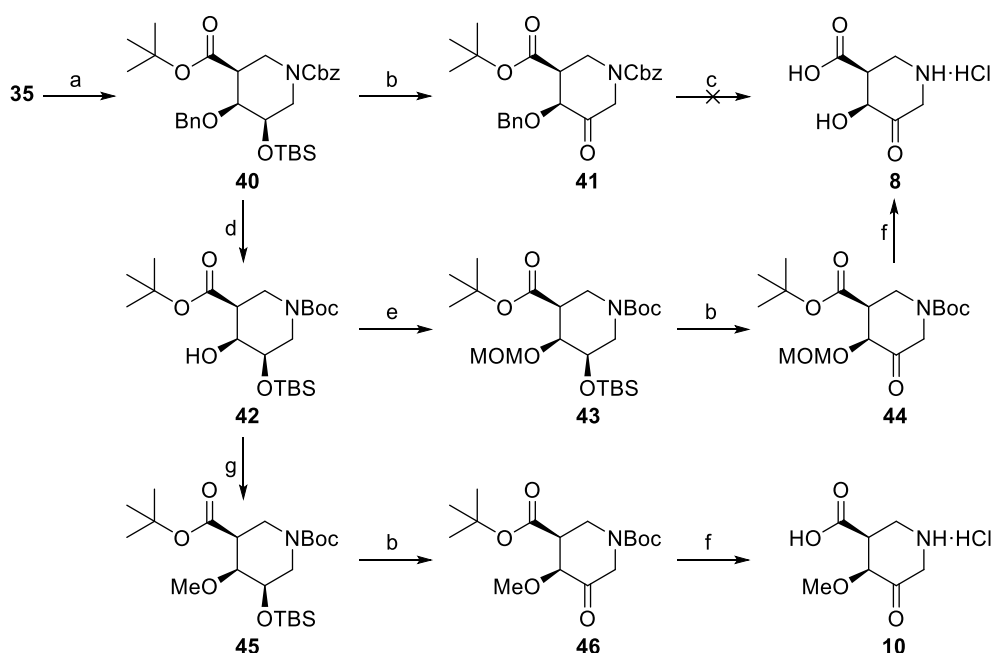


Scheme 5.5. Synthesis of compounds **8** and **11**. Reagents and conditions: a) i) Dess-Martin periodinane, DCM, rt; ii) NaClO_2 , NaH_2PO_4 , 30% H_2O_2 , CH_3CN , H_2O , 0 °C to rt, 65% over two steps; b) H_2 , 10% Pd/C, H_3O^+ , THF, rt, quant; c) i) 8 M HCl, MeOH, rt; ii) $\text{PhCH}(\text{OMe})_2$, CSA, DMF, 60 °C; d) TBSCl, imidazole, DCM, rt, 68% over three steps; e) $\text{THF} \cdot \text{BH}_3$, TMSOTf, DCM, 0 °C to rt, 97%; f) i) Jones reagent, acetone, rt; ii) benzyl alcohol, DIC, DMAP, DCM, rt, 46% over two steps; g) TBAF (75 wt% in H_2O), THF, **38**: 0 °C, 5 h, 94%, **37**: rt, overnight, 98%; h) Dess-Martin periodinane, DCM, rt, 71%; i) H_2 , 10% Pd/C, THF, H_3O^+ , rt, quant.

It was anticipated that installation of a *tert*-butyl ester instead of the benzyl ester in **36** would provide more possibilities for the preparation of the targeted keto-iminosugars. For this purpose, the primary alcohol in **35** was oxidized to a carboxylic acid via a two-step oxidation sequence (Scheme 5.6). Direct esterification of the carboxylic acid with *tert*-butanol under the activation of *N,N'*-diisopropylcarbodiimide (DIC) only afforded *tert*-butyl ester **40** in poor yield (30%). LC-MS analysis indicated the presence of a product with a mass corresponding to the acylurea rearrangement product as the major product. Alternatively, esterification of the carboxylic acid with the commercially available *tert*-butyl *N,N'*-diisopropylcarbamimidate was investigated and this transformation proceeded smoothly in toluene at 60 °C, affording ester **40** in 71% yield over three steps. Desilylation and subsequent oxidation gave protected ketone **41** in good yield. Unexpectedly, reductive deprotection of **41** by palladium catalyzed hydrogenation under acidic conditions resulted in a complex mixture of compounds.

Inspired by the preparation of the *gluco*-configured keto-iminosugars, a precursor that is fully protected with acid-labile groups was investigated (Scheme 5.6). Treatment of compound

40 with catalytic palladium under hydrogen atmosphere in methanol for a short reaction time (2 h) resulted in selective removal of the Cbz group to afford a free amine, which was subsequently protected with a Boc group. After work-up, the resulting Boc-protected intermediate was directly subjected to a second palladium catalyzed hydrogenolysis for prolonged reaction times (48 h) to remove the benzyl group at O4, affording alcohol **42** in 69% yield over three steps. Protection of the secondary alcohol in **42** with MOMCl was performed in a sealed microwave tube at 100 °C, providing compound **43** in good yield. Subsequent desilylation and oxidation provided ketone **44** which was deprotected under acidic conditions to afford compound **8**, of which the spectroscopic data are in accordance with those prepared by hydrogenolysis of **39** (Scheme 5.5). On the other hand, methylation of **42** with trimethyloxonium tetrafluoroborate as used for the preparation of **29** proved to be troublesome, and compound **45** could only be obtained in very low yield (13%). As an alternative, compound **42** was reacted with iodomethane in the presence of silver oxide and dimethyl sulfide,⁵³ giving **45** in satisfying yield (69%), which after desilylation, oxidation and final deprotection provided iminosugar **10**.



Scheme 5.6. Alternative synthetic routes toward the preparation of compound **8** and synthesis of compound **10**. Reagents and conditions: a) i) Dess-Martin periodinane, DCM, rt; ii) NaClO₂, NaH₂PO₄, 30% H₂O₂, CH₃CN, H₂O, 0 °C to rt; iii) *tert*-butyl *N,N*-diisopropylcarbamimidate, toluene, 60 °C, 5 h, 71% over three steps; b) i) TBAF (75 wt% in H₂O), THF, 0 °C, 5 h; ii) Dess-Martin periodinane, DCM, rt, **41** 85%, **44** 76%, **46** 73% over two steps; c) H₂, 10% Pd/C, H₃O⁺, rt; d) i) H₂, 10% Pd/C, MeOH, rt, 2 h; ii) Boc₂O, DIPEA, DCM, rt; iii) H₂, 10% Pd/C, MeOH, 48 h, 69% over three steps; e) MOMCl,

DIPEA, DCM, 100 °C in microwave tube, 88%; f) concentrated HCl (36%-38%), HFIP, H₂O, rt, **8** quant, **10** quant; g) CH₃I, Ag₂O, (CH₃)₂S, THF, rt, 69%.

5.2.3 Structural analysis of siastatin B-enzyme complexes

To determine how siastatin B inhibits both heparanases and β -glucuronidases, the structure of co-complexes between siastatin B and each of human heparanase (HPSE), *A. capsulatum* β -glucuronidase (AcGH79), *Burkholderia pseudomallei* heparanase (BpHep) and *E. coli* β -glucuronidase (EcGusB) were determined (see Table S5.1 for data collection and refinement statistics). Each of the structures contains a ligand present in the active site with full occupancy (Figure 5.4). Three of the four enzymes contain the same compound bound to the enzyme active site: AcGH79, BpHep and EcGusB all have the hemiaminal breakdown product **5** bound in the active site, as opposed to siastatin B (Figure 5.4A-C). Although all three enzymes normally accommodate glucuronic acid at the position where the inhibitor is located, compound **5** appears to bind productively to the active site. The 4-hydroxyl – with an epimeric configuration with respect to glucuronic acid – forms hydrogen bond interactions with the enzyme active site, for both AcGH79 and EcGusB. The 4-hydroxyl of **5** complexed to BpHep does not form a hydrogen bond, however it is exposed to the bulk solvent, and forms a hydrogen bond with an active site water molecule.

The co-complex structure of HPSE and siastatin B, unlike the three other co-complex structures, contains the 3-geminal diol derivative **9** bound to the enzyme active site which is a further breakdown product of **5** (Figure 5.4D). Despite the additional hydroxyl present at the 3-position of **9** this compound appears to be well accommodated in the active site of HPSE. Both hydroxyls present at the 3-position form hydrogen bond interactions with the active site: the hydroxyl on the β -face forms a hydrogen bond with Asp27 and the backbone nitrogen of Thr62 while the hydroxyl on the α -face is positioned to form hydrogen bonds with the catalytic nucleophile Glu343, Gln383 and Asp27. The hydroxyl at the 4-position of inhibitor **9** lacks the hydrogen bond with Trp391 which is observed for *gluco*-configured inhibitors,⁵⁴ but it does form hydrogen bonds with water molecules present in the crystal structure.

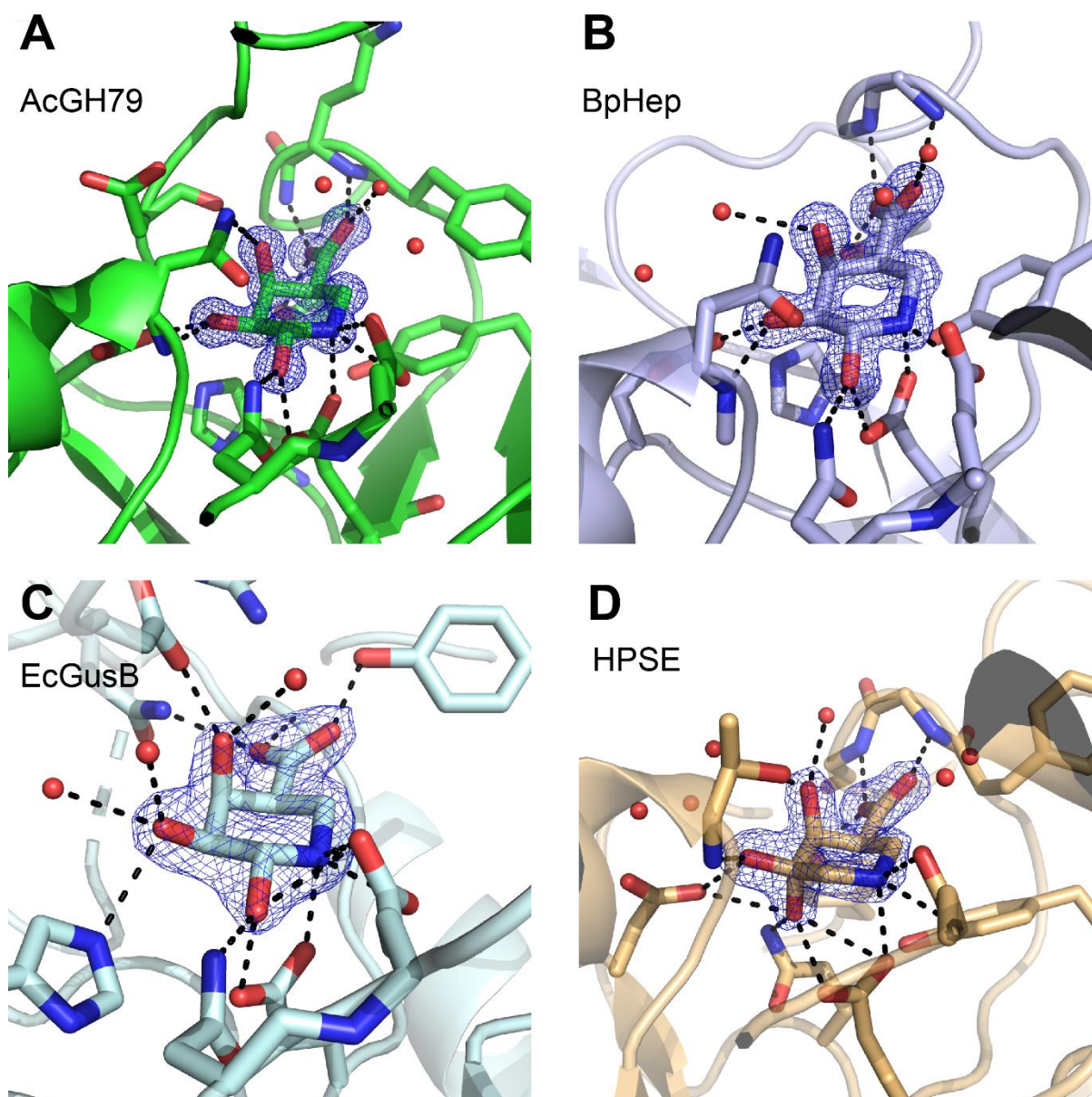


Figure 5.4. Structures of heparanases and β -glucuronidases in complex with siastatin B breakdown products. Siastatin B breakdown product **5** is shown bound to AcGH79 (A), BpHep (B), EcGusB (C) and further breakdown product **9** is shown in HPSE (D). Electron density ($2F_o - F_c$) is shown for the ligand as a blue mesh contoured at 2σ (AcGH79 = $0.92\text{ e}^-/\text{\AA}^3$, BpHep = $0.77\text{ e}^-/\text{\AA}^3$, EcGusB = $0.41\text{ e}^-/\text{\AA}^3$, HPSE = $0.61\text{ e}^-/\text{\AA}^3$). The polypeptide is shown in cartoon form with active site residues shown as sticks. Apparent hydrogen bonding interactions are shown as dotted black lines.

5.2.4 Structural basis for enzyme inhibition by synthetic iminosugars

To examine the structural basis for inhibition by the panel of synthetic *gluco*- and *galacto*-configured iminosugars, the co-crystal structures between two of these inhibitors and both HPSE and AcGH79 were determined (Figure 5.5). The uronic acid-type isofagomine **14** was well accommodated in the active sites of both AcGH79 and HPSE. For the co-complex between

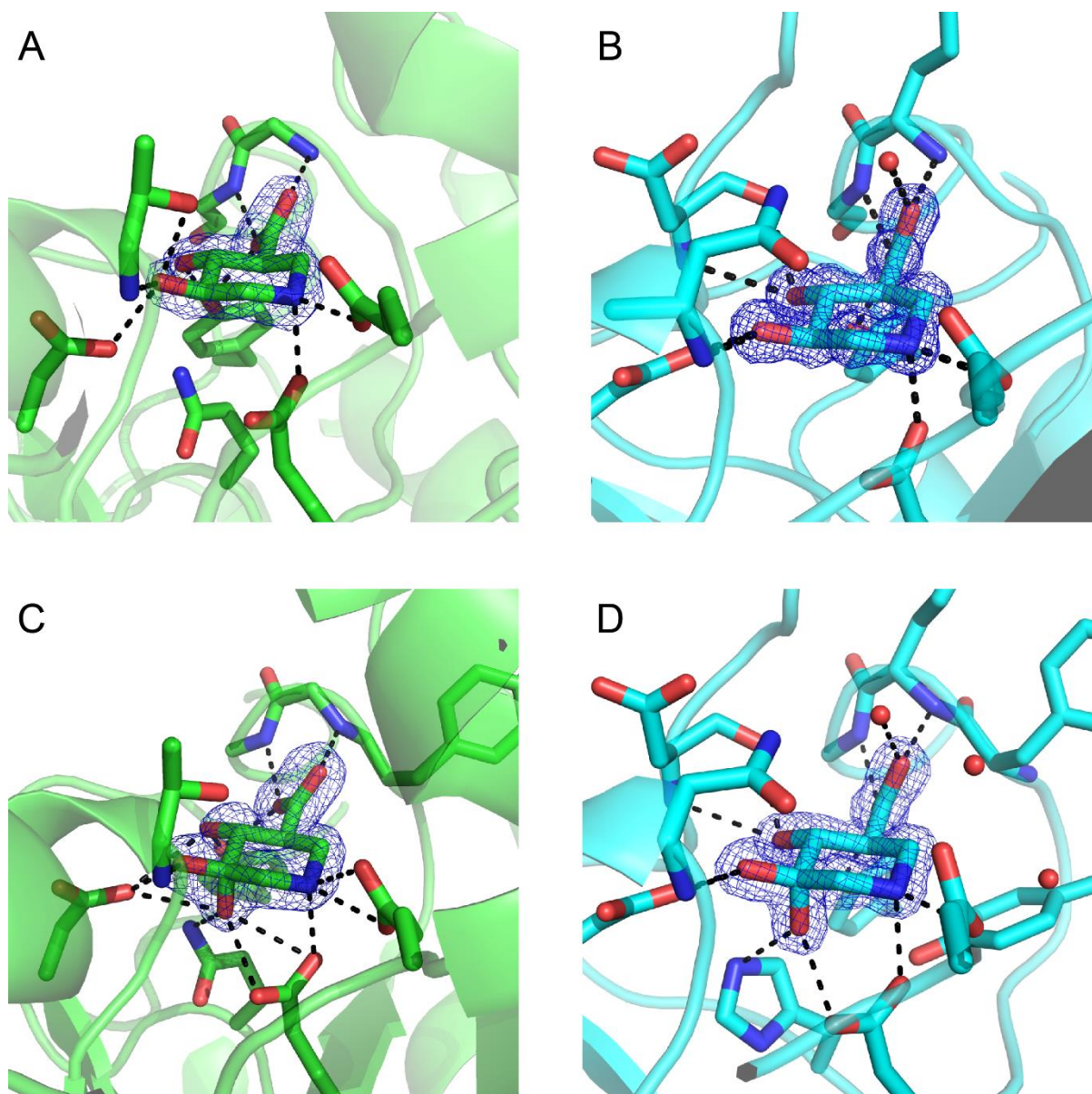


Figure 5.5. Structures of synthetic iminosugars **12** and **14** bound to HPSE and AcGH79. A) Complex between **14** and HPSE; B) Complex between **14** and AcGH79; C) Complex between **12** and HPSE; D) Complex between **12** and AcGH79. Electron density ($2F_o - F_c$) is shown for the ligand as a blue mesh contoured at 2σ (A = $0.53\text{ e}^-/\text{\AA}^3$, B = $0.85\text{ e}^-/\text{\AA}^3$, C = $0.60\text{ e}^-/\text{\AA}^3$, D = $0.69\text{ e}^-/\text{\AA}^3$). The polypeptide is shown in cartoon form with active site residues shown as sticks. Apparent hydrogen bonding interactions are shown as dotted black lines.

AcGH79 and **14** interactions between the endocyclic nitrogen, 3-hydroxyl and the carboxylic acid moiety are the same as for the structure with compound **5** (Figure 5.5B and 5.4A). However, as the 4-hydroxyl is in the ‘gluco’ configuration it is now able to form hydrogen bonds with both the backbone carbonyl and the nitrogen of Asp105, instead of Asn80. The co-complex of **14** with HPSE also shows the anticipated switch in hydrogen bonding interactions for a *gluco*-

configured compound, with the 4-hydroxyl now hydrogen bonding to Tyr391 instead of an active site water (Figure 5.5A).

Ketone **12** was also soaked into pre-formed crystals of HPSE and AcGH79. In both structures the inhibitor is present as the geminal diol (Figure 5.5C and D). This mirrors compound **9** in the active site of HPSE when it was soaked with siastatin B. The positioning of the **12**-derived geminal diol is nearly identical to the structure with **9**, with the only exception being the hydrogen bonding of the 4-hydroxyl to Tyr391 (Figure 5.5C). The **12**-derived geminal diol in the active site of AcGH79 has the same hydrogen bonding network as the structure containing **14** and additional hydrogen bonds between the hydroxyl on the ' α -face' of 3-position with the nucleophile (Glu287) and His327 (Figure 5.5D).

5.3 Conclusion

This chapter describes the in-depth structural analysis of co-complexes between siastatin B and four kinds of β -D-glucuronidases including HPSE, AcGH79, BpHep and EcGusB, showing that siastatin B, in contrast to previous reports describing this to be a stable inhibitor, can provide hemiaminal **5** and geminal diol **9**, that are responsible for enzyme inhibition. In particular, binding of geminal diol **9** in the active site of HPSE suggests that this new 1-*N*-iminosugar may serve as a potent inhibitor of HPSE. In line with this consideration, the synthesis of a panel of *galacto*- and *gluco*-configured 1-*N*-iminosugar derivatives of compound **9** is presented in this chapter. The synthesis of the *gluco*-configured iminosugars comprises the preparation of a common intermediate that is protected with acid-labile groups, followed by oxidation and global deprotection under acidic conditions. The key acid-labile protecting group strategy can also be applied to the synthesis of the *galacto*-configured iminosugars **8** and **10**. In addition, an alternative benzyl-protection route is also described for the preparation of compound **8**, demonstrating that these ketone iminosugars are compatible with hydrogenolysis conditions as well.

Structural analysis for enzyme inhibition by the synthetic *gluco*-configured iminosugars showed that both compounds **12** and **14** are well accommodated in the active sites of HPSE and AcGH79. Compound **12** bound in the active sites of both enzymes in a hydrated form similar to compound **9** that was derived from siastatin B when it was soaked with HPSE. In the future, the inhibition potency of this panel of synthetic iminosugars toward HPSE can be evaluated, which will provide useful information for the development of potent HPSE inhibitors as potential therapeutics.

5.4 Acknowledgements

Zachary Armstrong, Liang Wu and Gideon Davies from University of York, UK are kindly acknowledged for crystallographic studies and valuable discussion. Rian van den Nieuwendijk is acknowledged for the synthesis of D-galacturonic acid-type 1-*N*-iminosugars and valuable discussion.

5.5 Experimental methods

5.5.1 Biochemical experiments

Recombinant protein production and purification

HPSE – Human HPSE was expressed and purified according to previously reported procedures.³⁴

AcGH79 – AcGH79 was expressed and purified according to previously reported procedures.⁵⁴

BpHep – The coding sequence of BpHep was cloned into the pET28a vector (Novagen), behind an N-terminal 6xHis tag and thrombin cleavage site and used to transform *E. coli* strain BL21 Gold (DE3) (Agilent). Transformants were grown in TB media supplemented with 50 mg/mL kanamycin at 37 °C until cultures reached an OD600 of 0.8-1.0, whereupon expression was induced by the addition of 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG; Sigma). Induced cultures were grown at 16 °C overnight, then harvested by 4,000 *g* centrifugation at 4 °C for 15 min.

Harvested cells were resuspended in ~50 mL HisTrap buffer (20 mM Tris pH 8.0, 500 mM NaCl, 20 mM imidazole, 1 mM DTT), supplemented with DNase I (Sigma; bovine pancrease), and cOmplete™ EDTA protease inhibitors (Roche). Cells were lysed using a cell disruptor (Constant systems) at 40 kPSI operating pressure, and lysate clarified by centrifugation at 40,000 *g* at 4 °C for 30 min. Clarified supernatant was loaded onto a 5 mL HisTrap FF crude column (Cytiva) pre-equilibrated with HisTrap buffer A, washed with 10 column volumes (CV) of HisTrap buffer A, before eluting with HisTrap buffer B (20 mM Tris pH 8.0, 500 mM NaCl, 1000 mM imidazole, 1 mM DTT) over a 20 CV linear gradient. BpHep containing fractions were pooled, and buffer exchanged into 20 mM HEPES pH 7.4, 100 mM NaCl by at least 3 rounds of sequential concentration/dilution using a 30 kDa molecular weight cut-off (MWCO) Vivaspin centrifugal concentrator (Cytiva). Buffer exchanged BpHep was digested overnight at ambient temperature with thrombin (Sigma; bovine plasma) at 1:100 mass ratio thrombin:BpHep.

Digested BpHep was rerun over a 5 mL HisTrap FF crude column pre-equilibrated with HisTrap buffer A, which was further washed with 3 CV of HisTrap buffer A. Combined flowthrough and wash fractions were concentrated to ~2 mL volume using a 30 kDa MWCO Vivaspin centrifugal concentrator, then loaded onto a Superdex S75 16/600 pg size exclusion chromatography (SEC) column (Cytiva) pre-equilibrated in SEC buffer (20 mM HEPES pH 7.4, 200 mM NaCl, 1 mM DTT). BpHep containing fractions were pooled and concentrated using a 30 kDa MWCO Vivaspin centrifugal concentrator to a

final concentration of ~20 mg/ml. Purified protein was flash frozen in liquid nitrogen and stored at – 80 °C for use in further experiments.

EcGUSB – The coding sequence of EcGUSB was cloned into the pET28a vector, behind an N-terminal 6xHis tag, and used to transform *E. coli* strain BL21 Gold (DE3). Transformants were grown in TB media supplemented with 50 µg/mL kanamycin at 37 °C until cultures reached an OD600 of 0.8-1.0, whereupon gene expression was induced by the addition of 0.5 mM IPTG. Induced cultures were grown at 16 °C overnight, then harvested by 4,000 *g* centrifugation at 4 °C for 15 min.

Harvested cells were resuspended in ~50 mL HisTrap buffer (20 mM Tris pH 8.0, 500 mM NaCl, 20 mM imidazole, 1 mM DTT), supplemented with DNase I, and cOmplete™ EDTA protease inhibitors. Cells were lysed using a cell disruptor at 40 kPSI operating pressure, then lysate clarified by centrifugation at 40,000 *g* at 4 °C for 30 min. Clarified supernatant was loaded onto a 5 mL HisTrap FF crude column pre-equilibrated with HisTrap buffer A, washed with 10 CV of HisTrap buffer A, before eluting with HisTrap buffer B (20 mM Tris pH 8.0, 500 mM NaCl, 1000 mM imidazole, 1 mM DTT) over a 20 CV linear gradient. BpHep containing fractions were pooled, and concentrated using a 30 kDa MWCO Vivaspin centrifugal concentrator to a volume of ~ 2 mL. Concentrated protein was loaded onto a Superdex S200 16/600 pg SEC column pre-equilibrated in SEC buffer (20 mM HEPES pH 7.4, 200 mM NaCl, 1 mM DTT). EcGUSB containing fractions were pooled and concentrated using a 30 kDa MWCO Vivaspin centrifugal concentrator to a final concentration of ~24.5 mg/ml. Purified protein was flash frozen in liquid nitrogen and stored at -80 °C for use in further experiments.

Crystallization

AcGH79 – Well diffracting crystals of AcGH79 were obtained by the sitting-drop vapor-diffusion method at 20 °C using a well solution containing 0.5 M ammonium sulfate, 1 M lithium sulfate, 0.1 M trisodium citrate, and a protein to well solution ratio of 500 nl: 500 nl. Crystals typically appeared after 1 week.

Inhibitor ligand complexes were obtained by transferring AcGH79 crystals to drops containing 2 M lithium sulfate and 1 mM inhibitor. Crystals were incubated with ligand for ~0.5 – 1 h, then directly harvested and flash-cooled in liquid nitrogen for data collection.

BpHep – Well diffracting crystals of BpHep were obtained by the sitting-drop vapor-diffusion method at 20 °C using a well solution containing 0.1 M sodium citrate pH 5.0, 14% (w/v) PEG 6000, and a protein to well solution ratio of 300 nl: 500nl. Crystals typically appeared after 3 days.

Inhibitor ligand complexes were obtained by transferring BpHep crystals to drops of well solution supplemented with 25 % (v/v) ethylene glycol and 1–5 mM inhibitor. Crystals were incubated with ligand for ~24 h, then directly harvested and flash-cooled in liquid nitrogen for data collection.

EcGusB – Initial crystals of EcGUSB were obtained by the sitting-drop vapor-diffusion method at 20 °C using a well solution containing 0.1 M Bis-Tris propane pH 7.5, 20% (w/v) PEG 3350, 0.2 M NaNO₃. These initial crystals were used to prepare a microseed stock using Seed Beads (Hampton), then used to seed well diffracting crystals of EcGUSB in the same well conditions, at a protein to seed to well solution ratio of 500 nL: 200 nL: 1000 nL.

Inhibitor ligand complexes were obtained by transferring EcGUSB crystals to drops of well solution supplemented with 25 % (v/v) ethylene glycol and 1–5 mM inhibitor. Crystals were incubated with ligand for ~2 – 4 h, then directly harvested and flash-cooled in liquid nitrogen for data collection.

HPSE – Well diffracting crystals of HPSE were obtained by the sitting-drop vapor-diffusion method at 20 °C using a well solution containing 0.1 M MES pH 5.5, 0.1 M MgCl₂, 17 % (w/v) PEG 3350, and a protein to well solution ratio of 200 nL : 500 nL. Crystals typically appeared after 1 week.

Inhibitor ligand complexes were obtained by transferring HPSE crystals to drops of well solution supplemented with 25 % (v/v) ethylene glycol and 1 mM inhibitor. Crystals were incubated with ligand for ~0.5-1h, then directly harvested and flash-cooled in liquid nitrogen for data collection.

X-ray data collection and structure solution

X-ray diffraction data were collected at 100 K at beamlines i03, i04 and i04-1 of the Diamond Light Source UK. Reflections were autoprocessed using the XIA2 pipeline.⁵⁵ Complexes were solved by directly refining against their unliganded structures where solved using molecular replacement with PHASER4 (search model PDB accessions 5E98 (HPSE), 3VNY (AcGH79), 3K46 (EcGUSB)). Solved structures were iteratively improved by rounds of manual model building and maximum-likelihood refinement using COOT⁵⁶ and REFMAC5⁵⁷ respectively. Ligand coordinates were built using jLigand.⁵⁸ Diagrams were generated using PyMOL.

5.5.2 Chemical synthesis

General experimental details

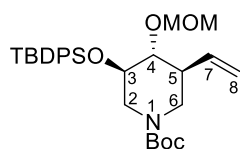
All reagents were of experimental grade and were used without further purification unless stated otherwise. Dichloromethane (DCM) and tetrahydrofuran (THF) were stored over 3 Å molecular sieves and *N,N*-dimethylformamide (DMF) was stored over 4 Å molecular sieves, which were dried *in vacuo* before use. All reactions were performed under an Argon or N₂ atmosphere unless stated otherwise. Reactions were monitored by analytical thin layer chromatography (TLC) using Merck aluminum sheets pre-coated with silica gel 60 with detection by UV-absorption (254 nm) and by spraying with a solution of (NH₄)₆Mo₇O₂₄·H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·H₂O (10 g/mL) in 10% sulfuric acid followed by charring at ~150 °C or by spraying with an aqueous solution of KMnO₄ (7%) and K₂CO₃ (2%) followed by charring at ~150 °C. Column chromatography was performed manually using Screening Device b.v. silica gel 60 (0.04-0.063 mm) in the indicated solvents. LC-MS analysis was performed on a LCQ

Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI⁺) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a C18 column (Gemini, 4.6 mm x 50 mm, 5 μ M particle size, Phenomenex). The applied buffers were H₂O, acetonitrile (MeCN) and 1% aqueous trifluoroacetic acid (TFA). ¹H-NMR and ¹³C-NMR spectra were recorded on Bruker AV-400 (400/101 MHz), Bruker AV-500 (500/126 MHz), and Bruker AV-850 (850/214 MHz) spectrometers in the given solvent. Chemical shifts (δ) are given in ppm relative to tetramethylsilane (TMS) as internal standard (¹H NMR in CDCl₃) or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in Hz. All given ¹³C-NMR spectra are proton decoupled. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), Ar (aromatic), C_q (quarternary carbon). 2D NMR experiments (COSY, HSQC) were carried out to assign protons and carbons of the new structures and assignment follows the general numbering shown in compounds **24**. High-resolution mass spectrometry (HRMS) analysis was performed with a LTQ Orbitrap mass spectrometer (Thermo Finnigan), equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution *R* = 60000 at *m/z* 400 (mass range *m/z* = 150 – 2000) and dioctyl phthalate (*m/z* = 391.28428) as a “lock mass”. The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

Experimental Procedures and Characterization Data of Products

The spectroscopic data of known compounds **15-16**⁴¹, **19-21**⁴³ and **S3**⁵⁰ are in agreement with those previously reported.

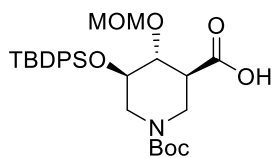
Compound 24



Compound **21** (218 mg, 0.454 mmol) was co-evaporated with toluene (3 x) and dissolved in dry DCM (3.0 mL) in a microwave tube. After cooling to 0 °C, DIPEA (632 μ L, 3.63 mmol) and MOMCl (241 μ L, 3.18 mmol) were added successively. The tube was then sealed and the mixture was heated to reflux at 40 °C overnight. TLC-analysis indicated full conversion of the starting material and the reaction was quenched with sat. aq. NaHCO₃ at 0 °C and diluted with DCM. The layers were separated and the aqueous layer was extracted with DCM (3 x). The combined organic layers were washed with H₂O (2 x), brine, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (pentane/EtOAc 50:1→20:1) affording compound **24** (208 mg, 0.396 mmol, 87%) as a clean oil. ¹H NMR (400 MHz, CDCl₃ at 293K) δ 7.71 (ddt, *J* = 13.7, 6.7, 1.6 Hz, 4H, CH Ar), 7.50 – 7.31 (m, 6H, CH Ar), 5.88 (s, 1H, H7), 5.25 – 5.05 (m, 2H, H8), 4.88 (s, 1H, CHH MOM), 4.58 – 4.40 (m, 1H, CHH MOM), 3.75 (brs, 2H, H3 and H6a), 3.63 (d, *J* = 13.2 Hz, 1H, H2a), 3.44 (dd, *J* = 8.3, 6.9 Hz, 1H, H4), 3.28 (s, 3H, CH₃ MOM), 2.85 (brs, 2H, H2b and H6b), 2.26 (qd, *J* = 8.6, 4.4 Hz, 1H, H5), 1.28 (s, 9H, (CH₃)₃Si), 1.06 (s, 9H, (CH₃)₃CO). ¹³C NMR (101 MHz, CDCl₃ at 293K) δ 154.6 (C=O), 137.5 (C7), 135.6 (4CH Ar), 134.1 (C_q Ar), 133.3 (C_q Ar), 129.9 (2CH Ar), 127.7 (4CH Ar), 117.1 (C8), 98.0 (CH₂

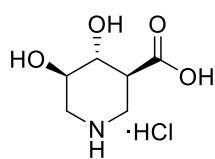
MOM), 82.5 (C4), 79.8 (C_q Boc), 73.0 (C3), 56.0 (CH₃ MOM), 48.4 (C2), 46.1 (C6), 45.2 (C5), 28.4 ((CH₃)₃CSi), 27.1 ((CH₃)₃CO), 19.3 ((CH₃)₃CSi) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₃₀H₄₃NO₅SiNa 548.2803, found 548.2800.

Compound 25



To a solution of compound **24** (26 mg, 50 μmol) in a mixture of CCl₄ (0.3 mL) and MeCN (0.3 mL) was added a solution of NaIO₄ (53 mg, 0.25 mmol) and RuCl₃·3H₂O (0.1 M in H₂O, 50 μL, 5.0 μmol) in water (0.45 mL) at 0 °C. The reaction mixture was then stirred vigorously at rt for 3 h. After which, sat. aq. Na₂S₂O₃ was added to quench the reaction and the resulting mixture was stirred for another 15 min at rt. The layers were separated and the aqueous layer was extracted with EtOAc (3 x). The combined organic layers were washed with H₂O, brine, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (pentane/EtOAc 15:1→5:1, with 0.1% HOAc) affording compound **25** (17.5 mg, 32.0 μmol, 64%) as a clean oil. ¹H NMR (500 MHz, CDCl₃ at 293K) δ 7.74 – 7.66 (m, 4H, CH Ar), 7.48 – 7.34 (m, 6H, CH Ar), 4.67 (s, 1H, CHH MOM), 4.49 (s, 1H, CHH MOM), 3.99 (t, *J* = 6.3 Hz, 1H, H4), 3.84 – 3.64 (brs, 1H, H6a), 3.73 (q, *J* = 5.7 Hz, 1H, H3), 3.60 – 3.29 (brs, 2H, H6b and H2a), 3.22 (s, 3H, CH₃ MOM), 2.59 (brs, 1H, H2b), 1.33 (s, 9H, (CH₃)₃CSi), 1.05 (s, 9H, (CH₃)₃CO). ¹³C NMR (126 MHz, CDCl₃ at 293K) δ 136.0 (4CH Ar), 130.0 (2CH Ar), 127.8 (4CH Ar), 97.4 (CH₂ MOM, assigned by HSQC), 78.8 (C4, assigned by HSQC), 70.9 (C3, assigned by HSQC), 56.0 (CH₃ MOM), 47.4 (C2, assigned by HSQC), 46.0 (C5, assigned by HSQC), 41.9 (C6), 28.4 ((CH₃)₃CSi), 27.1 ((CH₃)₃CO), 19.3 ((CH₃)₃CSi) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₉H₄₁NO₇SiNa 566.2545, found 566.2544.

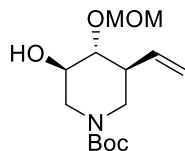
Compound 14



A solution of compound **25** (16 mg, 29 μmol) in dioxane (0.25 mL) in a microwave tube was added aqueous 3 M HCl (1.5 mL). The tube was then sealed and the mixture was stirred at 100 °C for 4 h until LC-MS indicated full deprotection. After cooling to rt, Et₂O (5 mL) was added and the mixture was stirred vigorously at rt for 5 min. The phases were then separated and the organic layer was taken out carefully. The remaining H₂O layer was washed two more times with Et₂O (2 x 5 mL) and concentrated. The residue was purified by silica gel column chromatography (isopropanol/H₂O/28-30% NH₄OH 12:1:1→10:2:1) to give chromatographically pure **14**. Milli-Q water (1 mL) and aq. 1 M HCl (2 mL) were added to the residue, and the solution was evaporated to form a hydrochloride salt of **14**, which was further purified by a column of Sephadex G-25 with Milli-Q as eluent, affording the title compound **14** (5.5 mg, 28 μmol, 95%) as a white solid after lyophilization. ¹H NMR (500 MHz, D₂O at 293K) δ 4.04 – 3.96 (m, 1H, H4), 3.90 – 3.82 (m, 1H, H3), 3.49 (dp, *J* = 12.9, 3.6, 2.7 Hz, 2H, H6a and H2a), 3.30 (tt, *J* = 10.8, 2.6 Hz, 1H, H6b), 3.08 – 2.99 (m, 1H, H2b), 2.81 (td, *J* = 7.9, 3.6 Hz, 1H, H5). ¹³C NMR (126 MHz, D₂O at

293K) δ 174.3 (C=O), 70.2 (C4), 66.9 (C3), 45.7 (C2), 45.0 (C5), 42.5 (C6). HRMS (ESI) m/z : $[M+H]^+$ calc for $C_6H_{12}NO_4$ 162.0761, found 162.0763.

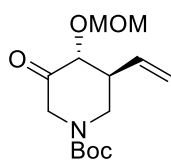
Compound 26



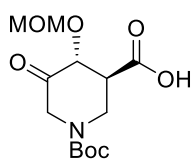
Compound **24** (187 mg, 0.35 mmol) was dissolved in dry THF (3.5 mL). TBAF (1 M in THF, 2.1 mL, 2.1 mmol) was added and the mixture was stirred at rt for 1.5 h. The reaction was quenched with sat. aq. NH_4Cl and concentrated *in vacuo* to remove THF.

The resulting residue was diluted with DCM, washed with H_2O (2 x), brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (pentane/EtOAc 9:1→3:1) affording compound **26** (96 mg, 0.33 mmol, 94%) as a clean oil. 1H NMR (500 MHz, $CDCl_3$ at 293K) δ 5.74 – 5.63 (m, 1H, H7), 5.19 (dd, J = 12.1, 1.3 Hz, 1H, H8a), 5.19 – 5.14 (m, 1H, H8b), 4.77 (dd, J = 6.9, 0.9 Hz, 1H, CHH MOM), 4.65 (dd, J = 7.0, 1.1 Hz, 1H, CHH MOM), 4.53 – 4.17 (m, 2H, H6a and H2a), 3.50 – 3.40 (H3), 3.46 (s, 3H, CH_3 MOM), 3.08 (dd, J = 10.2, 8.3 Hz, 1H, H4), 2.64 – 2.47 (m, 2H, H6b and H2b), 2.35 – 2.23 (m, 1H, H5), 1.46 (d, J = 1.3 Hz, 9H, $(CH_3)_3C$). ^{13}C NMR (126 MHz, $CDCl_3$ at 293K) δ 154.6 (C=O), 135.9 (C7), 117.9 (C8), 98.5 (CH_2 MOM), 89.2 (C4), 80.2 ($(CH_3)_3C$), 70.0 (C3), 56.0 (CH_3 MOM), 48.5 (C2, assigned by HSQC), 46.3 (C6, assigned by HSQC), 44.9 (C5, assigned by HSQC), 28.5 ($(CH_3)_3C$). HRMS (ESI) m/z : $[M+Na]^+$ calc for $C_{14}H_{25}NO_5Na$ 310.1625, found 310.1623.

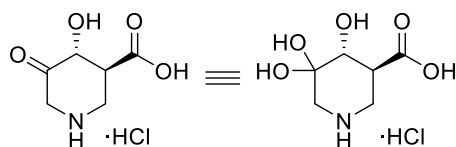
Compound 27



To a solution of compound **26** (96 mg, 0.33 mmol) in dry DCM (5 mL) at 0 °C was added Dess-Martin periodinane (283 mg, 0.66 mmol). The mixture was stirred at rt for 1 h until TLC-analysis indicated total consumption of the starting material. A mixture of sat. aq. $Na_2S_2O_3$ (3 mL) and sat. aq. $NaHCO_3$ (3 mL) was added to quench the reaction and the mixture was stirred at rt for another 15 min until the white emulsion became clear solution. The layers were separated and the aqueous layer was extracted with DCM (2 x). The combined organic layers were washed with H_2O , brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (pentane/EtOAc 9:1→3:1) affording compound **27** (88 mg, 0.31 mmol, 93%) as a clean oil. 1H NMR (500 MHz, $CDCl_3$ at 293K) δ 5.77 (ddd, J = 17.6, 10.4, 7.6 Hz, 1H, H7), 5.30 – 5.20 (m, 2H, H8), 4.79 (dd, J = 7.1, 1.0 Hz, 1H, CHH MOM), 4.67 (dd, J = 7.0, 1.0 Hz, 1H, CHH MOM), 4.28 (dd, J = 16.7, 1.5 Hz, 1H, H2a), 4.10 (d, J = 10.2 Hz, 1H, H4), 4.07 – 3.99 (brs, 1H, H6a), 3.84 (brs, 1H, H2b), 3.39 (d, J = 1.0 Hz, 3H, CH_3 MOM), 3.37 – 3.17 (m, 1H, H6b), 2.71 (qd, J = 9.7, 5.0 Hz, 1H, H5), 1.46 (d, J = 1.0 Hz, 9H, $(CH_3)_3C$). ^{13}C NMR (126 MHz, $CDCl_3$ at 293K) δ 202.5 (C3), 154.3 (C=O Boc), 135.3 (C7), 118.5 (C8), 96.5 (CH_2 MOM), 81.1 ($(CH_3)_3C$), 80.0 (C4), 56.3 (CH_3 MOM), 54.2 (C2, assigned by HSQC), 46.6 (C5), 46.0 (C6, assigned by HSQC), 28.4 ($(CH_3)_3C$) ppm. HRMS (ESI) m/z : $[M+Na]^+$ calc for $C_{14}H_{23}NO_5Na$ 308.1468, found 308.1465.

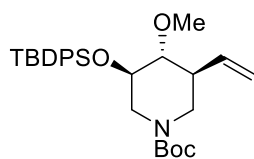
Compound 28

To a solution of compound **27** (35 mg, 0.12 mmol) in a mixture of CCl_4 (1.4 mL) and MeCN (1.4 mL) was added a solution of NaIO_4 (130 mg, 0.60 mmol, 5.0 eq.) and $\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$ (2.5 mg, 0.012 mmol, 0.1 eq.) in water (2.1 mL) at 0 °C. The reaction mixture was then stirred vigorously at rt for 2 h until TLC and LC-MS analysis indicated complete conversion. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 15 mL). To the combined organic extracts was added isopropanol (0.2 mL) and the mixture was stirred at rt for an additional 1 hour. The mixture was then washed with H_2O (15 mL), brine (15 mL), dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (DCM/MeOH 100:0→100:4) affording compound **28** (31 mg, 0.10 mmol, 83%) as a clean oil. ^1H NMR (500 MHz, CDCl_3 at 333K) δ 4.79 (d, J = 6.7 Hz, 1H, CHH MOM), 4.71 (d, J = 6.7 Hz, 1H, CHH MOM), 4.49 (d, J = 8.5 Hz, 1H, H4), 4.09 (d, J = 16.8 Hz, 1H, H2a), 3.99 (d, J = 16.7 Hz, 2H, H2b and H6a), 3.79 (dd, J = 13.7, 7.4 Hz, 1H, H6b), 3.39 (s, 3H, CH_3 MOM), 3.05 – 2.97 (m, 1H, H5), 1.46 (s, 9H, $(\text{CH}_3)_3\text{C}$). ^{13}C NMR (126 MHz, CDCl_3 at 333K) δ 174.0 (COOH), 154.4 (C=O Boc), 96.9 (CH_2 MOM), 81.8 ($(\text{CH}_3)_3\text{C}$), 77.2 (C4), 56.4 (CH_3 MOM), 53.4 (C2, assigned by HSQC), 48.1 (C5), 43.4 (C6), 28.4 ($(\text{CH}_3)_3\text{C}$) ppm. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calc for $\text{C}_{13}\text{H}_{21}\text{NO}_7\text{Na}$ 326.1210, found 326.1210; $[\text{M}+\text{H}_2\text{O}+\text{Na}]^+$ calc for $\text{C}_{13}\text{H}_{23}\text{NO}_8\text{Na}$ 344.1316, found 344.1316.

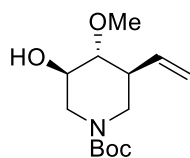
Compound 12

Compound **28** (24 mg, 79 μmol) was dissolved in 4 M dioxane/ H_2O (1.5 mL) and stirred at rt for 5 h. After which, water (1.0 mL) and Et_2O (~8.0 mL) were added. The mixture was stirred vigorously for a while and stood for stratification.

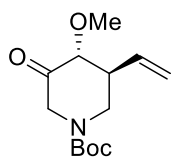
The Et_2O layer was taken out carefully and the remaining water layer was washed two more times with Et_2O (2 x 8.0 mL) and concentrated *in vacuo*. The resulting dry residue was re-dissolved in MeOH (1.0 mL) and Et_2O (~8.0 mL) was added slowly under vigorous stirring. Upon addition of Et_2O , a lot of light yellow solid appeared. After stirring vigorously for a while, the mixture was stood for solid precipitation and the Et_2O supernatant was taken out carefully. The solid was washed repeatedly with Et_2O (3 x 6.0 mL), re-dissolved in Milli-Q water (~1.5 mL) and filtered over a Whatman filter paper. After lyophilization, the target product (10.8 mg, 55 μmol , 70%) was obtained as a light yellow solid (HCl salt). ^1H NMR after lyophilization (500 MHz, D_2O at 293K) [hydrate form] δ 4.07 (d, J = 7.5 Hz, 1H, H4), 3.48 (ddd, J = 13.2, 4.5, 1.1 Hz, 1H, H6a equatorial), 3.38 (dd, J = 13.2, 8.0 Hz, 1H, H6b axial), 3.35 (dd, J = 12.9, 1.0 Hz, 1H, H2a), 3.14 (d, J = 12.9 Hz, 1H, H2b), 3.04 (td, J = 8.0, 7.5, 4.5 Hz, 1H, H5). ^{13}C NMR after lyophilization (126 MHz, D_2O at 293K) [hydrate form] δ 173.6 (COOH), 90.7 (C3 hydrate), 71.1 (C4), 49.0 (C2), 44.4 (C5), 41.9 (C6) ppm. HRMS (ESI) m/z : $[\text{M}_{\text{ketone}}+\text{H}]^+$ calc for $\text{C}_6\text{H}_{10}\text{NO}_4$ 160.06043, found 160.06059; $[\text{M}_{\text{hydrate}}+\text{H}]^+$ calc for $\text{C}_6\text{H}_{12}\text{NO}_5$ 178.07100, found 178.07119.

Compound 29

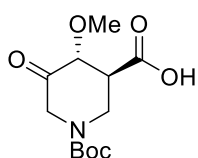
Compound **21** (0.15 g, 0.31 mmol) was co-evaporated with toluene (3 x) and dissolved in dry DCM (3 mL) under nitrogen in a microwave tube and stirred over fresh flame-dried 3 Å molecular sieve powder. After cooling to 0 °C, proton sponge (669 mg, 3.1 mmol) and trimethyloxonium tetrafluoroborate (0.37 mg, 2.5 mmol) were added successively. The tube was then sealed and protected from light. After stirring at rt for 22 h, the reaction slurry was diluted with EtOAc and filtered over a small pad of celite. The filter pad was washed with EtOAc (2 x) and the combined filtrates were washed with sat. aq. NH₄Cl (2 x), sat. aq. NaHCO₃, H₂O and brine, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (pentane/EtOAc 100:1→30:1) affording compound **29** (116 mg, 0.23 mmol, 75%) as a clean oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.78 – 7.65 (m, 4H, CH Ar), 7.45 – 7.28(m, 6H, CH Ar), 5.92 – 5.81 (m, 1H, H7), 5.20 – 5.05 (m, 2H, H8), 3.85 – 3.61 (m, 3H, H3, H6a and H2a), 3.30 (s, 3H, CH₃O), 3.01 (ddt, *J* = 8.2, 6.6, 1.8 Hz, 1H, H4), 2.96 – 2.65 (m, 2H, H6b and H2b), 2.31 – 2.10 (m, 1H, H5), 1.32 (s, 9H, (CH₃)₃CSi), 1.09 (q, *J* = 2.5, 1.4 Hz, 9H, (CH₃)₃CO). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 154.7 (C=O), 137.4 (C7), 136.2 (2CH Ar), 136.1 (2CH Ar), 134.5 (C_q Ar), 134.0 (C_q Ar), 129.9 (CH Ar), 129.8 (CH Ar), 127.8 (2CH Ar), 127.7 (2CH Ar), 116.6 (C8), 86.6 (C4), 79.8 ((CH₃)₃CO), 71.8 (C3), 59.5 (CH₃O), 48.6 (C2, assign by HSQC), 46.2 (C6), 45.2 (C5), 28.5 ((CH₃)₃CSi), 27.3 ((CH₃)₃CO), 19.5 ((CH₃)₃CSi) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₉H₄₁NO₄SiNa 518.2697, found 518.2693.

Compound S1

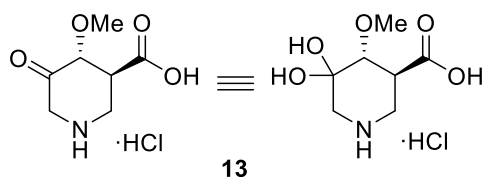
Compound **29** (153 mg, 0.31 mmol) was dissolved in dry THF (3.1 mL). TBAF (1 M in THF, 1.85 mL, 1.85 mmol) was added and the mixture was stirred at rt for 2 h. The reaction was quenched with sat. aq. NH₄Cl and concentrated *in vacuo* to remove THF. The resulting residue was diluted with DCM, washed with H₂O, brine, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (pentane/EtOAc 11:1→3:1) affording compound **S1** (72 mg, 0.28 mmol, 90%) as a clean oil. ¹H NMR (500 MHz, CDCl₃ at 293K) δ 5.76 (ddd, *J* = 17.8, 10.4, 7.9 Hz, 1H, H7), 5.28 – 5.12 (m, 2H, H8), 4.20 (brs, 1H, H2a), 4.11 – 3.77 (m, 1H, H6a), 3.54 (brs, 1H, H3), 3.51 (s, 3H, CH₃O), 2.95 (dd, *J* = 9.9, 8.4 Hz, 1H, H4), 2.81 (brs, 1H, OH), 2.74 – 2.50 (m, 2H, H2b and H6b), 2.28 (brs, 1H, H5), 1.45 (s, 9H, (CH₃)₃C). ¹³C NMR (126 MHz, CDCl₃ at 293K) δ 154.7 (C=O), 136.3 (C7), 117.7 (C8), 87.3 (C4), 80.3 (C_q Boc), 70.3 (C3), 59.8 (CH₃O), 48.1 (C2, assign by HSQC), 47.1 (C6, assign by HSQC), 45.5 (C5), 28.5 ((CH₃)₃C) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₁₃H₂₃NO₄Na 280.1519, found 280.1517.

Compound 30

To a solution of compound **S1** (79 mg, 0.30 mmol) in dry DCM (4.5 mL) at 0 °C was added Dess-Martin periodinane (260 mg, 0.60 mmol). The mixture was stirred at rt for 3.5 h until TLC-analysis indicated total consumption of the starting material. A mixture of sat. aq. Na₂S₂O₃ (3 mL) and sat. aq. NaHCO₃ (3 mL) was added to quench the reaction and the mixture was stirred at rt for another 15 min until the white emulsion became clear solution. The layers were separated and the aqueous layer was extracted with DCM (2 x). The combined organic layers were washed with H₂O, brine, dried over Na₂SO₄, filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (pentane/EtOAc 9:1→4:1) affording compound **30** (65 mg, 0.25 mmol, 83%) as a colourless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 5.77 (ddd, *J* = 17.4, 10.7, 6.8 Hz, 1H, H7), 5.24 – 5.16 (m, 2H, H8), 4.13 (dd, *J* = 16.8, 1.2 Hz, 1H, H2a), 3.90 (dd, *J* = 17.6, 13.2 Hz, 2H, H6a and H2b), 3.57 (d, *J* = 8.6 Hz, 1H, H4), 3.45 (s, 3H, CH₃O), 3.44 – 3.37 (m, 1H, H6b), 2.72 (dddt, *J* = 12.8, 8.0, 4.7, 1.3 Hz, 1H, H5), 1.46 (s, 9H, (CH₃)₃C). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 202.8 (C3), 154.4 (C=O Boc), 135.3 (C7), 117.8 (C8), 85.1 (C4), 80.9 (C_q Boc), 58.9 (CH₃O), 53.6 (C2), 45.6 (C5), 45.4 (C6), 28.4 ((CH₃)₃C). HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₁₃H₂₁NO₄Na 278.1363, found 278.1361.

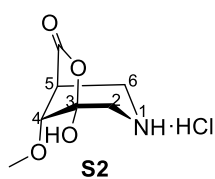
Compound 31

To a stirred solution of compound **30** (20 mg, 0.078 mmol) in CCl₄ (0.45 mL) and MeCN (0.45 mL) was added a solution of NaIO₄ (60 mg, 0.28 mmol, 3.6 eq.) and RuCl₃·3H₂O (0.1 M in H₂O, 40 μL, 4.0 μmol, 0.05 eq.) in water (0.67 mL) at 0 °C. The reaction mixture was stirred vigorously at 0 °C for 1 h. TLC-analysis indicated the presence of the aldehyde intermediate, so the mixture was warmed to rt and stirred for another 30 min. The phases were separated and the aqueous phase was extracted with EtOAc (3 x 10 mL). To the combined organic extracts was added isopropanol (0.2 mL) and the mixture was stirred at rt for an additional 1 h. The mixture was then washed with brine (10 mL), dried over Na₂SO₄, filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (DCM/MeOH 80:1→20:1) affording compound **31** (16 mg, 59 μmol, 75%) as a pale yellow oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 4.08 – 3.97 (m, 3H, H4 and H2ab), 3.97 – 3.85 (m, 1H, H6a), 3.84 – 3.76 (m, 1H, H6b), 3.49 (s, 3H, CH₃O), 3.03 – 2.93 (m, 1H, H5), 1.45 (s, 9H, (CH₃)₃C). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 154.5 (C=O Boc), 81.7 (C_q Boc), 81.5 (C4), 59.3 (CH₃O), 53.1 (C2, assigned by HSQC), 47.8 (C5), 43.0 (C6), 28.4 ((CH₃)₃C) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₁₂H₁₉NO₆Na 296.1105, found 296.1102.

Major product 13 and minor product S2

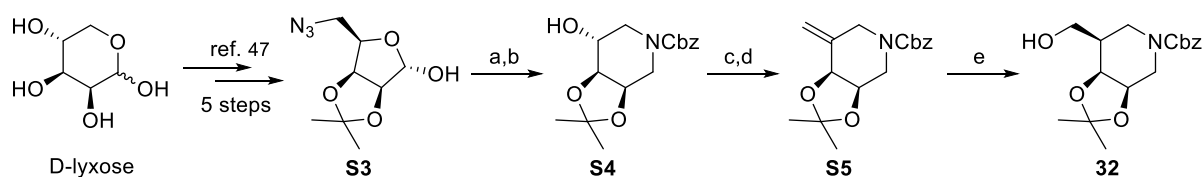
To a stirred white suspension of compound **31** (17 mg, 62 μ mol) in the mixture of HFIP (600 μ L) and H₂O (300 μ L) was added 0.2 M HCl/HFIP (340 μ L, 68 μ mol, 1.1 eq.) and the resulting clear solution was stirred at rt for 1 h. TLC-

analysis indicated the presence of the starting material, so more 0.2 M HCl/HFIP (340 μ L, 68 μ mol) was added and the mixture was stirred for another 1 h. After which, 37% HCl (12 M, 5.7 μ L, 68 μ mol) was added and the mixture was stirred for additional 1.5 h until TLC-analysis indicated full deprotection. The solvent was concentrated under reduced pressure and the resulting residue was re-dissolved in Milli-Q water (~1.5 mL), washed with Et₂O (3 x 8.0 mL) and filtered over a Whatman filter paper. After lyophilization, the product was obtained as a mixture of two compounds (10.9 mg, 52 μ mol, 84%, ratio $\approx$ 4:1) as a hygroscopic light yellow solid. Major product **13**: ¹H NMR (500 MHz, D₂O at 328K) [hydrate form] δ 4.13 (d, J = 6.0 Hz, 1H, H4), 3.88 (s, 3H, CH₃O), 3.81 (dd, J = 13.3, 6.1 Hz, 1H, H6a), 3.70 (dd, J = 13.3, 4.3 Hz, 1H, H6b), 3.62 (d, J = 12.9 Hz, 1H, H2a), 3.58 – 3.52 (m, 1H, H5), 3.49 (d, J = 12.9 Hz, 1H, H2b). ¹³C NMR (126 MHz, D₂O at 328K) [hydrate form] δ 173.9 (COOH), 91.2 (C3 hydrate), 80.6 (C4), 59.6 (CH₃O), 49.1 (C2), 41.8 (C5), 41.4 (C6) ppm. HRMS (ESI) m/z : [$M_{\text{ketone}} + H$]⁺ calc for C₇H₁₂NO₄ 174.07608, found 174.07607; [$M_{\text{hydrate}} + H$]⁺ calc for C₇H₁₄NO₅ 192.08665, found 192.08655.



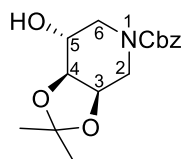
Proposed structure of minor product **S2** which may be formed by dehydration of **13**.

The protons of **S2** could not be assigned confidently due to the peaks partially overlap with those of major product **13**. The carbon peaks were observed at δ 91.9 (C3), 79.9 (C4), 61.4 (CH₃O), 47.2 (C2), 42.0 (C5), 39.4 (C6) ppm. Of note the carbonyl carbon was not found on the NMR spectrum.

Synthesis of key intermediate 32

Scheme 5.6. Synthesis of key intermediate **32** following reported procedures for the preparation of its *N*-Boc protected analogue. Reagents and conditions: a) H₂, 10% Pd/C, MeOH, rt; b) CbzCl, sat. aq. NaHCO₃, THF, 0 °C to rt, 74% over two steps; c) Dess-Martin periodinane, DCM, 0 °C to rt; d) Ph₃PCH₃Br, ^tBuOK, THF, -20 °C to rt, 84% over two steps; (e) i) 9-BBN, THF, 0 °C to rt; ii) 30% H₂O₂, 1 M NaOH in H₂O, rt, 73%.

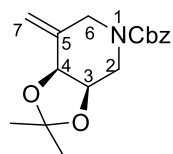
Compound S4



Azide **S3** (10.7 g, 49.8 mmol) was dissolved in MeOH (1000 mL) and nitrogen was bubbled through the solution before 20% Pd(OH)₂/C (2.0 g) was added. While stirring vigorously, the mixture was flushed with two H₂ balloons. After stirring for 20 h under H₂ atmosphere, NMR analysis of a small amount of the reaction mixture revealed almost no conversion. Therefor 10% Pd/C (2.06 g) was added and the reaction was stirred for another 20 h until NMR analysis indicated full conversion of the starting material. The reaction mixture was filtered and concentrated to give the iminosugar intermediate (8.2 g, 47 mmol) as a white solid. The crude amine was directly dissolved in a mixture of THF (350 mL) and sat. aq. NaHCO₃ (250 mL). After cooling to 0 °C, CbzCl (95%, 10.5 mL, 69.8 mmol) was added dropwise and the reaction was stirred at rt for 40 h until LC-MS analysis indicated full conversion. The layers were separated and the water layer was extracted with EtOAc (2 x 200 mL). The combined organic layers were washed with brine (100 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The product was purified by silica gel column chromatography (pentane/EtOAc 9:1→1:1) affording compound **S4** (11.4 g, 37.1 mmol, 74% over two steps) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.43 – 7.14 (m, 5H, CH Ar), 5.12 (s, 2H, CH₂ Cbz), 4.33 – 4.27 (m, 1H, H5), 4.07 (dd, *J* = 6.5, 4.1 Hz, 1H, H4), 3.97 – 3.83 (m, 2H, H3, H6b), 3.63 (dd, *J* = 13.5, 3.2 Hz, 1H, H2b), 3.51 (dd, *J* = 14.2, 3.3 Hz, 1H, H6a), 3.36 (dd, *J* = 13.5, 5.2 Hz, 1H, H2a), 3.11 (brs, 1H, OH), 1.41 (s, 3H, CH₃), 1.31 (s, 3H, CH₃). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 156.5 (C=O), 136.9 (C_q Ar), 128.5, 128.0, 127.8 (CH Ar), 109.2 (OCO), 75.6 (C4), 72.0 (C5), 67.5 (C3), 67.3 (CH₂ Cbz), 44.9 (C2), 42.8 (C6), 27.3 (CH₃), 25.0 (CH₃) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) [mixture of rotamers 2:1] δ 7.37 – 7.23 (m, 5H, CH Ar), 5.15 – 5.06 (m, 2H, CH₂ Cbz), 4.38 – 4.27 (m, 1H, H5), 4.12 – 4.04 (m, 1H, H4), 4.03 – 3.85 (m, 2H, H3, H6b), 3.83 – 3.56 (m, 2H, OH and H2b), 3.55 – 3.43 (m, 1H, H6a), 3.43 – 3.31 (m, 1H, H2a), 1.43 (s, 2H, CH₃), 1.41 (s, 1H, CH₃), 1.32 (s, 3H, CH₃). ¹³C NMR (101 MHz, CDCl₃ at 293K) [mixture of rotamers 2:1] δ 156.5 (C=O), 136.5 (C_q Ar), 128.5, 128.0, 127.807, 127.7 (Ar), 109.1 (OCO), 75.1 (C4), 71.9 & 71.8 (C5), 67.2 (CH₂ Cbz), 67.2 (C3), 44.6 & 44.4 (C2), 42.5 & 42.2 (C6), 27.2 & 27.1 (CH₃), 24.9 & 24.8 (CH₃) ppm. HRMS (ESI) *m/z*: [M+H]⁺ calc for C₁₆H₂₂NO₅ 308.14925, found 308.14907.

Compound S5

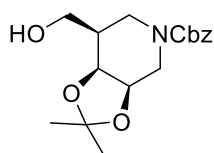


Alcohol **S4** (11.3 g, 36.8 mmol) was dissolved in dry DCM (400 mL) under argon. After cooling to 0 °C, Dess-Martin periodinane (27.3 g, 64.4 mmol) was added in several portions. After stirring for 30 minutes at 0 °C and for 2 h at room temperature, the reaction was diluted with EtOAc (600 mL) and washed successively with 2 M Na₂S₂O₃ solution (2 x 250 mL), sat. aq. NaHCO₃ (2 x 200 mL) and brine (150 mL). The organic layer was dried over MgSO₄, filtrated and concentrated *in vacuo* to afford the crude ketone. In the meantime methyltriphenylphosphonium bromide (55.3 g, 155 mmol) was suspended in dry THF (250 mL) and

cooled to 0 °C, ^tBuOK (15.4 g, 138 mmol) was added. The resulting yellow suspension was stirred for 1 h at 0 °C and for 2 h at rt. After which, the suspension was cooled to -20 °C and a solution of the crude ketone in THF (150 mL) was added dropwise over 20 minutes. The reaction was then stirred overnight at room temperature and quenched by addition of sat. aq. NH₄Cl (250 mL). The layers were separated and the water layer extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine (100 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 95:5→3:1) affording compound **S5** (9.4 g, 31 mmol, 84% over two steps) as a slightly orange oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.53 – 7.08 (m, 5H, CH Ar), 5.28 – 5.14 (m, 4H, H7ab and CH₂ Cbz), 4.61 (d, *J* = 7.4 Hz, 1H, H4), 4.32 (m, 2H, H3 and H6b), 3.97 – 3.76 (m, 2H, H6a and H2b), 3.11 (dd, *J* = 14.3, 2.8 Hz, 1H, H2a), 1.38 (s, 3H, CH₃), 1.33 (s, 3H, CH₃). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 155.9 (C=O), 139.7 (C5), 137.0 (C_q Ar), 128.4, 127.9, 127.8 (CH Ar), 116.4 (C7), 109.7 (OCO), 76.3 (C4), 74.6 (C3), 67.1 (CH₂ Cbz), 46.6 (C6), 44.2 (C2), 26.6 (CH₃), 24.8 (CH₃) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 7.40 – 7.24 (m, 5H, CH Ar), 5.29 – 5.07 (m, 4H, H7ab and CH₂ Cbz), 4.65 (dd, *J* = 7.4, 3.8 Hz, 1H, H4), 4.44 – 4.30 (m, 2H, H3 and H6b), 3.97 (dd, *J* = 14.4, 2.7 Hz, 1H, H2b), 3.87 (dd, *J* = 14.1, 8.5 Hz, 1H, H6a), 3.07 (ddd, *J* = 14.1, 7.7, 2.3 Hz, 1H, H2a), 1.40 (s, 1.5H, CH₃), 1.37 (s, 1.5H, CH₃), 1.35 (s, 3H, CH₃). ¹³C NMR (101 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 156.0 & 155.8 (C=O), 139.3 (C5), 136.8 (C_q Ar), 128.4, 128.4, 128.0, 127.9, 127.9, 127.8 (CH Ar), 117.1 & 116.8 (C7), 109.6 (OCO), 76.2 & 76.1 (C4), 74.5 (C3), 67.1 & 67.0 (CH₂ Cbz), 46.5 & 46.3 (C6), 44.1 & 43.8 (C2), 26.5 (CH₃), 24.6 (CH₃). HRMS (ESI) *m/z*: [M+H]⁺ calc for C₁₇H₂₂NO₄ 304.15433, found 304.15419.

Compound 32

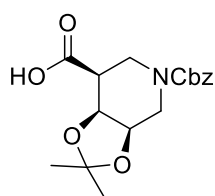


Alkene **S5** (4.70 g, 15.5 mmol) was dissolved in dry THF (150 mL) under argon. After cooling to 0 °C, 9-BBN (0.5 M in THF, 140 mL, 70 mmol) was added dropwise over 30 minutes and the mixture was stirred for 20 h at rt until TLC-analysis indicated full conversion of the starting material. Then the mixture was cooled to 0 °C again, and water (10 mL), 1 M NaOH in H₂O (10 mL) and 30% H₂O₂ (10 mL) were added successively. After stirring overnight at rt, the mixture was diluted with EtOAc (250 mL) and washed with brine (2 x 200 mL). The combined water layers were extracted with EtOAc (100 mL) and the combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 1:1→3:7) to afford compound **32** (3.66 g, 11.4 mmol, 73%) as a thick oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.37 – 7.22 (m, 5H, Ar), 5.13 (s, 2H, CH₂ Cbz), 4.42 (dd, *J* = 7.1, 2.7 Hz, 1H, H4), 4.29 (s, 1H, H3), 3.80 (d, *J* = 10.9 Hz, 1H, H2b), 3.68 (m, 2H, H7ab), 3.56 (dd, *J* = 12.3, 5.0 Hz, 1H, H6b), 3.29 (d, *J* = 13.9 Hz, 1H, H2a), 3.22 (app t, *J* = 12.3 Hz, 1H, H6a), 2.50 (brs, 1H, OH), 1.99 (m, 1H, H5), 1.39 (s, 3H, CH₃), 1.31 (s, 3H, CH₃). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 156.1 (C=O), 137.0 (C_q Ar), 128.5, 127.9, 127.8 (CH Ar), 108.9

(OCO), 72.6 (C3), 72.4 (C4), 67.1 (CH₂ Cbz), 62.5 (C7), 43.2 (C2), 40.3 (C6), 38.3 (C5), 26.7 (CH₃), 24.7 (CH₃) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 7.38 – 7.24 (m, 5H, Ar), 5.13 (s, 2H, CH₂ Cbz), 4.44 (dd, J = 7.3, 2.2 Hz, 1H, H4), 4.39 – 4.26 (m, 1H, H3), 3.93 – 3.75 (m, 1H, H2b), 3.69 (s, 2H, H7ab), 3.55 (m, 1H, H6b), 3.24 (m, 2H, H6a and H2a), 2.99 (s, 0.5H, OH), 2.89 (s, 0.5H, OH), 2.00 (s, 1H, H5), 1.40 (s, 3H, CH₃), 1.32 (s, 3H, CH₃). ¹³C NMR (101 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 156.3 & 155.9 (C=O), 136.7 (C_q Ar), 128.4, 127.9, 127.8, 127.7 (CH Ar), 108.7 (OCO), 72.4 (C4), 72.1 & 72.0 (C3), 67.0 (CH₂ Cbz), 62.3 & 62.2 (C7), 43.0 & 42.6 (C2), 40.2 & 39.9 (C6), 38.0 (C5), 26.6 (CH₃), 24.6 (CH₃) ppm. HRMS (ESI) m/z : [M+Na]⁺ calc for C₁₇H₂₃NO₅Na 344.1468, found 344.1477.

Compound 33

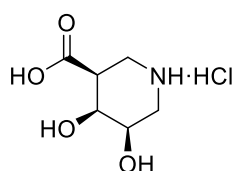


Alcohol **32** (347 mg, 1.08 mmol) was dissolved in dry DCM (14 mL) under argon and Dess-Martin periodinane (720 mg, 1.70 mmol) was added. After stirring at rt for 3 h, TLC-analysis confirmed complete conversion of the starting material. The mixture was diluted with EtOAc (60 mL) and washed successively with aqueous 2 M Na₂S₂O₃ (2 x 15 mL), sat. aq. NaHCO₃ (2 x 15 mL) and brine (20 mL), dried over MgSO₄, filtrated and concentrated to afford the crude aldehyde as a colorless oil (350 mg). The aldehyde was directly dissolved in a mixture of MeCN (18 mL) and water (3.5 mL) and NaH₂PO₄·2H₂O (1.31 g, 8.40 mmol) was added. The mixture was cooled to 0 °C and 30% H₂O₂ (142 μ L, 1.39 mmol) and a solution of NaClO₂ (80% purity, 157 mg, 1.14 mmol) in water (8.5 mL) were added subsequently. The slightly yellow solution was stirred at 0 °C for 15 minutes and at rt for 1 h until TLC-analysis confirmed full conversion of the aldehyde. The reaction was quenched by addition of Na₂SO₃ (0.5 g) and stirred for another 15 minutes. After which, the mixture was diluted with EtOAc (60 mL) and the layers were separated. The aqueous layer was extracted with EtOAc (2 x 10 mL) and the combined organic layers were washed with brine (20 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification by silica gel column chromatography (pentane/EtOAc 1:1→0:1) afforded the target compound **33** (236 mg, 0.703 mmol, 65%) as a glassy solid. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 8.14 (s, 1H, OH), 7.37 – 7.23 (m, 5H, CH Ar), 5.15 (s, 2H, CH₂ Cbz), 4.72 (dd, J = 6.9, 2.9 Hz, 1H, H4), 4.35 (s, 1H, H3), 3.94 – 3.72 (m, 2H, H6b and H2b), 3.50 (app t, J = 12.7 Hz, 1H, H6a), 3.34 (d, J = 13.6 Hz, 1H, H2a), 2.80 (ddd, J = 12.6, 4.8, 3.2 Hz, 1H, H5), 1.41 (s, 3H, CH₃), 1.33 (s, 3H, CH₃). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 173.8 (C=O acid), 156.1 (C=O Cbz), 136.8 (C_q Ar), 128.6, 128.1, 128.0 (CH Ar), 109.5 (OCO), 72.3 (C3), 71.9 (C4), 67.5 (CH₂ Cbz), 43.3 (C2), 42.0 (C5), 38.6 (C6), 26.7 (CH₃), 24.8 (CH₃) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 9.41 (brs, 1H, OH), 7.82 – 7.12 (m, 5H, CH Ar), 5.15 (s, 2H, CH₂ Cbz), 4.74 (dd, J = 7.0, 2.7 Hz, 1H, H4), 4.56 – 4.25 (m, 1H, H3), 4.09 –

3.67 (m, 2H, H6b and H2b), 3.49 (m, 1H, H6a), 3.32 (m, 1H, H2a), 2.87 – 2.75 (m, 1H, H5), 1.45 – 1.39 (m, 3H, CH₃), 1.33 (s, 3H, CH₃). ¹³C NMR (101 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 174.3 (C=O acid), 156.3 & 155.9 (C=O Cbz), 136.5 (C_q Ar), 128.5, 128.1, 128.0 (CH Ar), 109.2 (OCO), 72.1 (C3), 71.6 (C4), 67.4 (CH₂ Cbz), 43.1 & 42.7 (C2), 41.6 (C5), 38.3 & 38.1 (C6), 26.6 & 26.5 (CH₃), 24.7 & 24.6 (CH₃) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₁₇H₂₁NO₆Na 358.12611, found 358.12580.

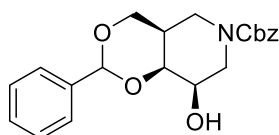
Compound 11



Method A (deprotection of compound **33**): Carboxylic acid **33** (135 mg, 0.403 mmol) was dissolved in a mixture of dioxane (9 mL) and aq. 4 M HCl (1 mL), 10% Pd/C (100 mg) was added subsequently. The mixture was stirred vigorously under a balloon of hydrogen for 16 h, filtered over a Whatman filter and concentrated to give a white residue (130 mg). NMR analysis revealed removal of the isopropylidene acetal while the Cbz-group was still present. Therefore the residue was re-dissolved in a mixture of THF (10 mL) and water (500 μ L), aq. 8 M HCl (100 μ L) and 10% Pd/C (139 mg) were added. After stirring vigorously overnight under hydrogen atmosphere, the mixture was filtered over a Whatman filter and concentrated to give a crude product, of which NMR analysis proved removal of the Cbz-group. The residue was then dissolved in absolute ethanol (1.2 mL) and added dropwise to a stirred solution of dry diethyl ether (20 mL) affording a white precipitate. The precipitate was collected on a filter, washed with dry diethyl ether (2 x 5 mL) and dried *in vacuo* to give the target compound **11** (85 mg, quant) as an off-white powder.

Method B (deprotection of compound **S8**): To a stirred solution of diol **S8** (40 mg, 0.126 mmol) in dry diethyl ether (1 mL) at 0 °C was added 4 M HCl/dioxane (4 mL). The mixture was stirred at 0 °C for 10 min and at rt for 4 h. At which LC-MS analysis revealed complete removal of the Boc group while the *tert*-butyl ester was still present in large amount. Water (200 μ L) was added and the mixture was left stirring overnight until LC-MS confirmed full conversion. The solvents were removed *in vacuo* and the residue was re-dissolved in anhydrous 2-propanol (1 mL). Upon addition of dry diethyl ether a white precipitate appeared. The supernatant was taken out carefully and the residue washed with dry diethyl ether (3 x 2 mL). Evaporation of the volatiles afforded the target compound (19 mg, 0.096 mmol, 76%) as a white foam. ¹H NMR (400 MHz, D₂O at 293K) δ 4.42 (s, 1H, H4), 3.99 (dt, *J* = 11.2, 3.6 Hz, 1H, H3), 3.40 (dd, *J* = 12.9, 4.4 Hz, 1H, H6b), 3.29 – 3.18 (m, 2H, H2b and H6a), 3.09 – 2.99 (m, 2H, H2a and H5). ¹³C NMR (101 MHz, D₂O at 293K) δ 173.3 (C=O), 66.8 (C4), 65.3 (C3), 42.7 (C5), 41.9 (C2), 38.5 (C6). HRMS (ESI) *m/z*: [M+H]⁺ calc for C₆H₁₂NO₄ 162.07608, found 162.07572.

Compound S6

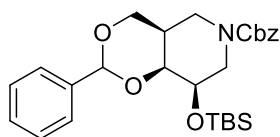


To a stirred solution of alcohol **32** (3.65 g, 11.4 mmol) in MeOH (100 mL) was added 8 M HCl solution (2.24 mL) and the mixture stirred at room temperature overnight until TLC-analysis confirmed full conversion of the

starting material. The reaction was quenched by addition of Et₃N (5 mL). The solvent was evaporated and the residue treated with EtOAc (100 mL) and filtered. The filter cake was washed with EtOAc (3 x 10 mL) and the combined filtrates were concentrated to afford the crude triol that was directly dissolved in DMF (80 mL). After addition of benzaldehyde dimethyl acetal (3.40 mL, 22.6 mmol) and (+)-camphorsulfonic acid (1.57 g, 6.76 mmol), the mixture was stirred at 60 °C for 20 h until LC-MS analysis showed full conversion. After cooling to rt, sat. aq. NaHCO₃ (300 mL) was added and the mixture was extracted with EtOAc (3 x 130 mL). The combined organic layers were washed with brine (2 x 50 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 4:1→1:1) affording compound **S6** (3.03 g, 8.20 mmol, 72% over two steps) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.49 – 7.39 (m, 2H, CH Ar), 7.40 – 7.26 (m, 8H, CH Ar), 5.53 (s, 1H, CHPh), 5.13 (dd, *J* = 12.0 Hz, 2H, CH₂ Cbz), 4.24 (s, 1H, H₄), 4.17 (d, *J* = 9.1 Hz, 1H, H_{2b}), 4.09 – 3.96 (m, 3H, H_{6b} and H_{7ab}), 3.63 (ddd, *J* = 10.9, 5.2, 3.3 Hz, 1H, H₃), 3.41 (t, *J* = 12.7 Hz, 1H, H_{6a}), 2.89 (t, *J* = 11.9 Hz, 1H, H_{2a}), 2.35 (s, 1H, OH), 1.68 (d, *J* = 9.3 Hz, 1H, H₅). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 155.6 (C=O), 138.2 (C_q Ar), 136.9 (C_q Ar), 129.3, 128.7, 128.5, 128.2, 128.1, 126.3 (CH Ar), 102.0 (OCHO), 76.1 (C₄), 68.7 (C₇), 67.8 (C₃), 67.5 (CH₂ Cbz), 45.2 (C₂), 41.5 (C₆), 34.3 (C₅) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) δ 7.45 (dd, *J* = 6.6, 3.1 Hz, 2H, CH Ar), 7.42 – 7.26 (m, 8H, CH Ar), 5.53 (s, 1H, CHPh), 5.12 (s, 2H, CH₂ Cbz), 4.29 – 3.89 (m, 5H, H₄, H_{2b}, H_{6b} and H_{7ab}), 3.62 (s, 1H, H₃), 3.38 (m, 1H, H_{6a}), 2.95 – 2.82 (m, 1H, H_{2a}), 2.60 (d, *J* = 10.5 Hz, 1H, OH), 1.79 – 1.59 (m, 1H, H₅). ¹³C NMR (101 MHz, CDCl₃ at 293K) δ 155.4 (C=O), 137.9 (C_q Ar), 137.2 (C_q Ar), 129.4, 128.6, 128.4, 128.2, 128.0, 126.3 (CH Ar), 101.8 (OCHO), 75.9 (C₄), 68.6 (C₇), 67.7 (C₃), 67.4 (CH₂ Cbz), 44.9 (C₂), 41.2 (C₆), 33.8 (C₅) ppm. HRMS (ESI) *m/z*: [M+H]⁺ calc for C₂₁H₂₄NO₅ 370.1649, found 344.1644.

Compound 34



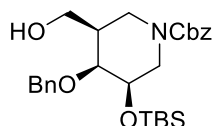
Alcohol **S6** (2.35 g, 6.36 mmol) was dissolved in DCM (35 mL) and imidazole (1.96 g, 28.8 mmol) and TBSCl (2.3 g, 15 mmol) were added subsequently.

The resulting suspension was stirred for 20 h at room temperature. The mixture was diluted with EtOAc (150 mL), washed with water and brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 95:5→9:1) to afford the target compound **34** (2.88 g, 5.95 mmol, 94%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.48 (d, *J* = 7.4 Hz, 2H, CH Ar), 7.38 – 7.23 (m, 8H, CH Ar), 5.51 (s, 1H, CHPh), 5.18 (d, *J* = 12.4 Hz, 1H, CHH Cbz), 5.10 (d, *J* = 12.4 Hz, 1H, CHH Cbz), 4.13 (s, 1H, H₄), 4.08 – 3.89 (m, 4H, H_{7ab}, H_{6b} and H_{2b}), 3.68 (d, *J* = 9.9 Hz, 1H, H₃), 3.45 (t, *J* = 12.5 Hz, 1H, H_{6a}), 3.08 (t, *J* = 11.5 Hz, 1H, H_{2a}), 1.64 (d, *J* = 10.1 Hz, 1H, H₅), 0.88 (s, 9H, (CH₃)₃C), 0.06 (s, 6H, 2CH₃). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 155.7 (C=O), 138.8, 137.1 (2C_q Ar), 128.9, 128.7, 128.3, 128.2, 128.1, 126.2 (CH Ar), 101.5 (CHPh), 77.2 (C₄), 69.9 (C₃), 69.0

(C7), 67.4 (CH₂ Cbz), 45.1 (C2), 41.8 (C6), 34.7 (C5), 25.9 ((CH₃)₃C), 18.3 ((CH₃)₃C), -4.4 (CH₃), -4.3 (CH₃) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 7.49 (m, 2H, CH Ar), 7.37 – 7.26 (m, 8H, CH Ar), 5.51 (s, 1H, CHPh), 5.22 – 5.14 (m, 1H, CHH Cbz), 5.09 (d, J = 12.4 Hz, 1H, CHH Cbz), 4.11 (s, 1H, H4), 4.10 – 3.84 (m, 4H, H7ab, H6b and H2b), 3.68 (m, 1H, H3), 3.50 – 3.36 (m, 1H, H6a), 3.09 (m, 1H, H2a), 1.62 (s, 1H, H5), 0.89 (s, 9H, (CH₃)₃C), 0.09 (s, 3H, CH₃), 0.06 (s, 3H, CH₃). ¹³C NMR (101 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 155.4 (C=O), 138.4, 136.7 (2C_q Ar), 128.7, 128.5, 128.1, 128.0, 127.9, 126.0 (CH Ar), 101.2 (OCHO), 76.8 (C4), 69.6 & 69.2 (C3), 68.7 (C7), 67.2 (CH₂ Cbz), 44.7 & 44.5 (C2), 41.3 (C6), 34.4 & 34.1 (C5), 25.8 ((CH₃)₃C), 18.2 ((CH₃)₃C), -4.6 (2CH₃) ppm. HRMS (ESI) m/z : [M+Na]⁺ calc for C₂₇H₃₇NO₅SiNa 506.2333, found 506.2338.

Compound 35

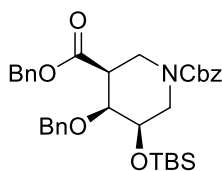


Compound **34** (2.59 g, 5.36 mmol) was dissolved in dry DCM (60 mL) and cooled on an ice-bath. BH₃·THF (1.0 M in THF, 22 mL, 22 mmol) was added dropwise in five minutes and followed by addition of TMSOTf (0.16 mL, 0.88 mmol). The resulting solution was stirred for 10 minutes on the ice-bath and for 3 h at room temperature. TLC-analysis confirmed full conversion and the reaction was quenched with TEA (2.5 mL) at 0 °C followed by careful addition of MeOH (25 mL). After stirring for another 1 h, the reaction was concentrated, co-evaporated twice with MeOH to give the crude product. Purification by silica gel column chromatography (pentane/EtOAc 4:1→1:1) afforded the target compound **35** (2.53 g, 5.21 mmol, 97%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.35 – 7.22 (m, 10H, CH Ar), 5.18 (d, J = 12.4 Hz, 1H, CHH Cbz), 5.05 (d, J = 12.4 Hz, 1H, CHH Cbz), 4.94 (d, J = 11.5 Hz, 1H, CHH Bn), 4.59 (d, J = 11.5 Hz, 1H, CHH Bn), 3.85 – 3.75 (m, 2H, H4 and H2b), 3.74 – 3.65 (m, 2H, H3 and H6b), 3.62 (m, 2H, H7ab), 3.38 – 3.30 (m, 1H, H2a), 3.13 (dd, J = 12.9, 10.7 Hz, 1H, H6a), 2.05 (s, 1H, OH), 1.84 (s, 1H, H5), 0.91 (s, 9H, (CH₃)₃C), 0.10 (s, 6H, 2CH₃). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 155.7 (C=O), 139.1, 137.0 (2C_q Ar), 128.5, 128.5, 128.0, 128.0, 127.9, 127.7 (CH Ar), 77.7 (C4), 74.1 (CH₂ Bn), 71.7 (C3), 67.3 (CH₂ Cbz), 61.9 (C7), 46.1 (C2), 42.4 (C5), 41.7 (C6), 25.9 ((CH₃)₃C), 18.1 ((CH₃)₃C), -4.7, -4.7 (2CH₃) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 7.45 – 7.16 (m, 10H, CH Ar), 5.19 (d, J = 12.4 Hz, 1H, CHH Cbz), 5.08 – 4.89 (m, 2H, CHH Cbz and CHH Bn), 4.60 (d, J = 11.5 Hz, 1H, CHH Bn), 3.97 – 3.51 (m, 6H, H4, H2b, H3, H6b and H7ab), 3.46 – 3.22 (m, 1H, H2a), 3.20 – 3.06 (m, 1H, H6a), 2.36 (s, 0.5H, OH), 2.18 (s, 0.5H, OH), 1.94 – 1.76 (m, 1H, H5), 0.92 (s, 4.5H, (CH₃)₃C), 0.89 (s, 4.5H, (CH₃)₃C), 0.22 – 0.01 (m, 6H, 2CH₃). ¹³C NMR (101 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 155.6 (C=O), 138.9, 136.7 (2C_q Ar), 128.5, 128.5, 128.1, 127.9, 127.9, 127.8 (CH Ar), 77.4 (C4), 74.1 & 73.9 (CH₂ Bn), 71.4 (C3), 67.3 (CH₂ Cbz), 62.1 & 61.6 (C7), 46.1 & 45.6 (C2), 42.1

& 41.9 (C5), 41.6 & 41.3 (C6), 25.8 ((CH₃)₃C), 18.1 ((CH₃)₃C), -4.8 (2CH₃) ppm. HRMS (ESI) m/z: [M+H]⁺ calc for C₂₇H₄₀NO₅Si 486.26703, found 486.26696.

Compound 36

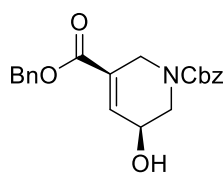


To a stirred solution of alcohol **35** (930 mg, 1.92 mmol) in acetone (40 mL) was added 2.4 M Jones reagent dropwise until an orange/brown color persisted. The reaction was stirred for an additional 10 minutes and then quenched by the addition of 2-propanol (3.0 mL). Subsequently sat. aq. NaHCO₃ (50 mL) and water (50 mL) were added and the acetone was carefully (foaming) evaporated from the mixture. Next acetic acid was added drop by drop until pH = 5. EtOAc (50 mL) was added and the mixture stirred vigorously for a few minutes after which the mixture was filtered over a pad of celite. The layers were separated and the water layer extracted with EtOAc (50 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated to give the crude carboxylic acid (0.95 g) as a colorless oil. The crude acid was dissolved in DCM (20 mL) and benzyl alcohol (540 μ L, 5.1 mmol) and DMAP (33 mg, 0.27 mmol) were added. After cooling on an ice-bath DIC (320 μ L, 2.05 mmol) was added dropwise and the mixture stirred for one more hour on the ice-bath and for 6 h at rt. At that time TLC indicated circa 50% conversion, so the reaction was left overnight. However, the situation did not change. Extra portions of benzyl alcohol (540 μ L) and DIC (320 μ L) were added and the reaction stirred for an extra 6 h upon which TLC did not show any further progress of the reaction. The mixture was concentrated and purified by column chromatography (pentane/EtOAc 95:5 $\rightarrow$ 4:1) to afford the benzyl ester **36** as a colorless oil (520 mg, 0.882 mmol) in 46% yield. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.34 – 7.16 (m, 15H, CH Ar), 5.17 (d, *J* = 12.4 Hz, 1H, CHH Cbz), 5.11 (d, *J* = 12.3 Hz, 1H, CHH Bn_{ester}), 5.07 (d, *J* = 12.4 Hz, 1H, CHH Cbz), 4.99 (d, *J* = 12.3 Hz, 1H, CHH Bn_{ester}), 4.95 (d, *J* = 11.3 Hz, 1H, CHH Bn), 4.47 (d, *J* = 11.3 Hz, 1H, CHH Bn), 4.26 (s, 1H, H6b), 4.23 (s, 1H, H4), 3.98 (s, 1H, H2b), 3.68 (d, *J* = 7.6 Hz, 1H, H3), 3.29 (app t, *J* = 12.7 Hz, 1H, H6a), 3.13 (app t, *J* = 11.6 Hz, 1H, H2a), 2.61 (d, *J* = 9.7 Hz, 1H, H5), 0.91 (s, 9H, (CH₃)₃C), 0.10 (s, 6H, 2CH₃) ppm. ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 170.3 (OC=O), 155.4 (NC=O), 139.3, 136.9, 135.9 (3C_q Ar), 128.6, 128.6, 128.4, 128.2, 128.1, 128.0, 127.4, 127.4 (CH Ar), 77.9 (C4), 75.2 (CH₂ Bn), 71.5 (C3), 67.4 (CH₂ Cbz), 66.6 (CH₂ Bn_{ester}), 46.3 (C5), 45.0 (C2), 39.9 (C6), 25.9 ((CH₃)₃C), 18.1 ((CH₃)₃C), -4.6, -4.7 (2CH₃) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 7.41 – 7.14 (m, 15H, CH Ar), 5.18 (d, *J* = 12.3 Hz, 1H, CHH Cbz), 5.12 (d, *J* = 12.2 Hz, 1H, CHH Bn_{ester}), 5.06 (d, *J* = 12.3 Hz, 1H, CHH Cbz), 4.98 (m, 2H, CHH Bn_{ester} and CHH Bn), 4.46 (d, *J* = 11.3 Hz, 1H, CHH Bn), 4.37 – 4.14 (m, 3H, H6b and H4), 4.12 – 3.84 (m, 1H, H2b), 3.77 – 3.58 (m, 1H, H3), 3.39 – 3.21 (m, 1H, H6a), 3.21 – 3.00 (m, 1H, H2a), 2.63 (s, 1H, H5), 0.90 (s, 9H, (CH₃)₃C), 0.24 – 0.02 (m, 6H, 2CH₃). ¹³C NMR (101 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 170.4 (OC=O), 155.3 (NC=O), 139.1, 136.6, 135.6 (3C_q Ar), 128.6, 128.6, 128.4, 128.2, 128.1, 128.0, 127.4 (CH Ar), 77.6 (C4), 75.1 (CH₂ Bn), 71.4 & 71.0 (C3), 67.4 (CH₂ Cbz), 66.6 (CH₂ Bn_{ester}), 46.1 & 45.9 (C5), 44.8 (C2), 39.6 (C6), 25.8 ((CH₃)₃C), 18.1

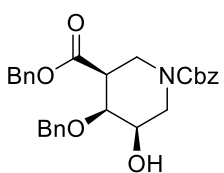
((CH₃)₃C), -4.8 (2CH₃) ppm. HRMS (ESI) *m/z*: [M+H]⁺ calc for C₃₄H₄₄NO₆Si 590.29324, found 590.29347.

Compound 37



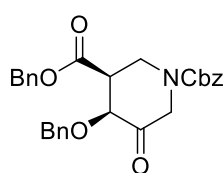
To a stirred solution of TBS-ether **36** (0.52 g, 0.88 mmol) in THF (20 mL) was added TBAF (75 wt% in H₂O, 680 mg, 1.95 mmol) and the mixture was left stirring overnight. The mixture was concentrated under reduced pressure to remove most of the solvent, then diluted with EtOAc, washed with brine, dried over MgSO₄, filtered and concentrated to give the crude product. Purification by silica gel column chromatography (pentane/EtOAc 9:1→1:1) afforded a colorless oil which after NMR and LC-MS analyses turned out to be the title unsaturated ester **37** (0.32 mg, 0.87 mmol, 98%). ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.36 – 7.22 (m, 10H, CH Ar), 6.98 (d, *J* = 2.6 Hz, 1H, H₄), 5.17 (s, 2H, CH₂ Bn_{ester}), 5.12 (s, 2H, CH₂ Cbz), 4.28 (s, 1H, H₃), 4.23 (d, *J* = 18.6 Hz, 1H, H_{6b}), 4.13 (d, *J* = 18.6 Hz, 1H, H_{6a}), 3.84 (dd, *J* = 12.8, 3.8 Hz, 1H, H_{2b}), 3.25 (dd, *J* = 12.8, 6.2 Hz, 1H, H_{2a}), 2.84 (br. s, 1H, OH). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 164.8 (OC=O), 155.6 (NC=O), 139.4 (C₄), 136.5 (C_q Ar), 135.8 (C_q Ar), 128.7, 128.6, 128.4, 128.2, 128.2, 128.0 (CH Ar), 67.6 (CH₂ Cbz), 66.7 (CH₂ Bn_{ester}), 63.4 (C₃), 47.0 (C₂), 42.8 (C₆).

Compound 38



To an ice cold solution of TBS-ether **36** (325 mg, 0.551 mmol) in THF (8 mL) was added TBAF (75 wt% in H₂O, 420 mg, 1.20 mmol) and the mixture was left stirring on the ice bath. After 5 h TLC-analysis showed complete conversion of the TBS-ether. The mixture was diluted with EtOAc (40 mL), washed with brine (2 x 15 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by column chromatography (pentane/EtOAc 95:5→7:3) to afford the title alcohol **38** (247 mg, 0.519 mmol, 94%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃ at 333K) δ 7.36 – 7.12 (m, 15H, CH Ar), 5.07 (m, 4H, CH₂ Cbz and CH₂ Bn_{ester}), 4.60 (m, 1H, CHH Bn), 4.48 (d, *J* = 11.6 Hz, 1H, CHH Bn), 4.26 – 3.83 (m, 3H, H₄, H_{6b} and H_{2b}), 3.61 (brs, 1H, H₃), 3.35 (app. t, *J* = 12.3 Hz, 1H, H_{6a}), 3.12 – 2.78 (m, 2H, H_{2a} and OH), 2.61 (brs, 1H, H₅). ¹³C NMR (101 MHz, CDCl₃ at 333K) δ 170.7 (OC=O), 155.2 (NC=O), 138.3, 136.4, 135.2 (3C_q Ar), 128.6, 128.5, 128.5, 128.5, 128.4, 128.1, 127.9, 127.7, 127.4 (CH Ar), 76.9 (C₄), 74.3 (CH₂ Bn), 68.5 (C₃), 67.4 (CH₂ Cbz), 66.8 (CH₂ Bn_{ester}), 45.6 & 45.3 (C₅), 45.2 & 44.9 (C₂), 39.7 (C₆). HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₈H₂₉NO₆Na 498.18871, found 498.18891.

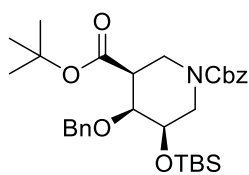
Compound 39



To a stirred solution of compound **38** (225 mg, 0.474 mmol) in DCM (9 mL) was added Dess-Martin periodinane (60%, 682 mg, 0.965 mmol) and the mixture was left stirring at rt for 3 h. The reaction mixture was then diluted with EtOAc (50 mL), washed with aq. 2 M Na₂S₂O₃ (2 x 15 mL), sat. aq. NaHCO₃ (2 x 15 mL) and brine (15 mL), dried over MgSO₄, filtered and concentration *in vacuo*. The crude was purified by silica

gel column chromatography (pentane/EtOAc 4:1→7:3) to afford the ketone **39** (160 mg, 0.338 mmol, 71%) as a colorless oil. ^1H NMR (400 MHz, CDCl_3 at 293K) δ 7.37 – 7.14 (m, 15H, CH Ar), 5.19 – 4.94 (m, 4H, CH_2 Cbz and CH_2 Bn_{ester}), 4.69 (d, J = 12.0 Hz, 1H, CHH Bn), 4.52 – 4.41 (m, 1H, CHH Bn), 4.35 – 3.77 (m, 5H, H2ab, H6ab and H4), 3.16 (brs, 1H, H5). ^{13}C NMR (101 MHz, CDCl_3 at 293K) δ 200.3 (C=O), 169.5 (OC=O), 154.9 (NC=O), 136.8, 136.0, 135.2 (3C_q Ar), 128.5, 128.5, 128.4, 128.3, 128.3, 128.1, 127.8, 127.4 (CH Ar), 78.31 (C4), 72.4 (CH_2 Bn), 67.8 (CH_2 Cbz), 67.1 (CH_2 Bn_{ester}), 52.7 (C2), 46.4 (C5), 41.8 (C6) ppm. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calc for $\text{C}_{28}\text{H}_{29}\text{NO}_6\text{Na}$ 496.17306, found 496.17304.

Compound 40

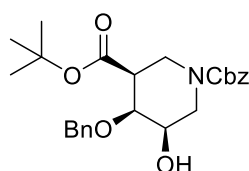


Alcohol **35** (1.25 g, 2.58 mmol) was dissolved in dry DCM (25 mL). Dess-Martin periodinane (60% purity, 2.65 g, 3.75 mmol) was added and the reaction was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc (100 mL) and washed successively with sat. aq. $\text{Na}_2\text{S}_2\text{O}_3$ (2 x 50 mL), sat. aq. NaHCO_3 (2 x 50 mL) and brine (50 mL). The organic layer was dried over MgSO_4 , filtered and concentrated to afford the crude aldehyde (1.47 g). To the stirred solution of the crude aldehyde in MeCN (45 mL) and water (8.5 mL), $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (3.12 g, 20.0 mmol) was added and the mixture was stirred at rt until it turned homogeneous. After which, the reaction was cooled to 0 °C and 30% H_2O_2 (340 μL , 4.35 mmol) and a solution of NaClO_2 (80% purity, 375 mg, 3.31 mmol) in water (8.5 mL) were added. The mixture was stirred at 0 °C for 15 minutes and 1 h at rt until TLC-analysis confirmed full conversion. The reaction was quenched by addition of Na_2SO_3 (0.95 g) and stirred for 10 more minutes. The mixture was diluted with EtOAc (60 mL) and the layers were separated. The water layer was extracted with EtOAc (2 x 30 mL) and the combined organic layers were washed with brine (30 mL), dried over MgSO_4 , filtered and concentrated to afford the crude carboxylic acid (1.47 g). The crude acid was co-evaporated twice with dry toluene and then dissolved in dry toluene (10 mL). *tert*-Butyl *N,N'*-diisopropylcarbaimidate (1.94 g, 9.70 mmol) was added and the reaction stirred at 60 °C for 5 h. LC-MS analysis confirmed complete conversion of the acid and the mixture was concentrated and filtered over a plug of silica gel eluting with pentane/Et₂O (9:1) to afford the crude ester. Purification by silica gel column chromatography (pentane/Et₂O 98:2→9:1) afforded target compound **40** (1.01g, 1.82 mmol, 71% over three steps) as a colorless oil. ^1H NMR (500 MHz, CDCl_3 at 333K) δ 7.33 – 7.24 (m, 9H, CH Ar), 7.22 – 7.16 (m, 1H, CH Ar), 5.18 (d, J = 12.4 Hz, 1H, CHH Cbz), 5.10 – 5.01 (m, 2H, CHH Cbz and CHH Bn), 4.59 (d, J = 11.1 Hz, 1H, CHH Bn), 4.26 – 4.16 (m, 2H, H4 and H6b), 3.98 (s, 1H, H3), 3.67 (d, J = 7.3 Hz, 1H, H2b), 3.20 (t, J = 12.7 Hz, 1H, H6a), 3.13 (t, J = 11.6 Hz, 1H, H2a), 2.52 – 2.44 (m, 1H, H5), 1.40 (s, 9H, $(\text{CH}_3)_3\text{CO}$), 0.91 (s, 9H, $(\text{CH}_3)_3\text{CSi}$), 0.11 (s, 3H, CH_3Si), 0.10 (s, 3H, CH_3Si). ^{13}C NMR (126 MHz, CDCl_3 at 333K) δ 169.5 (OC=O), 155.3 (NC=O), 139.3, 136.9 (2C_q Ar), 128.5, 128.1, 128.0, 127.9, 127.2 (CH Ar), 81.2 ($(\text{CH}_3)_3\text{CO}$), 78.1 (C4), 75.2 (CH_2 Bn), 71.6 (C3),

67.2 (CH₂ Cbz), 46.9 (C5), 44.9 (C2), 40.0 (C6), 28.1 ((CH₃)₃CO), 25.8 ((CH₃)₃CSi), 18.0 ((CH₃)₃CSi), -4.7, -4.8 (2CH₃Si) ppm.

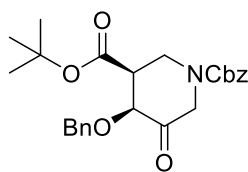
¹H NMR (400 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 7.37 – 7.18 (m, 10H, CH Ar), 5.23 – 5.15 (d, *J* = 12.4 Hz, 1H, CHH Cbz), 5.06 (m, 2H, CHH Cbz and CHH Bn), 4.58 (d, *J* = 11.1 Hz, 1H, CHH Bn), 4.31 – 3.85 (m, 3H, H4, H6b and H2b), 3.78 – 3.57 (m, 1H, H3), 3.30 – 3.01 (m, 2H, H2a and H6a), 2.51 (s, 1H, H5), 1.41 (s, 9H, (CH₃)₃CO), 0.91 (s, 9H, (CH₃)₃CSi), 0.16 (s, 3H, CH₃Si), 0.09 & 0.06 (s, 3H, CH₃Si). ¹³C NMR (101 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 169.6 (OC=O), 155.1 (NC=O), 139.1, 136.6 (2C_q Ar), 128.4, 128.0, 128.0, 127.8, 127.2 (CH Ar), 81.2 ((CH₃)₃CO), 77.9 (C4), 75.2 (CH₂ Bn), 71.4 & 71.1 (C3), 67.2 (CH₂ Cbz), 46.5 (C5), 44.7 (C2), 39.8 (C6), 28.0 ((CH₃)₃CO), 25.7 ((CH₃)₃CSi), 18.0 ((CH₃)₃CSi), -4.9 (2CH₃Si) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₃₁H₄₅NO₆SiNa 578.29084, found 578.29100.

Compound S7

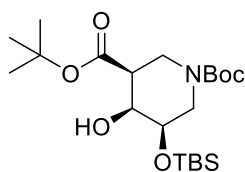


To an ice cold solution of TBS-ether **40** (0.35 g, 0.63 mmol) in THF (8 mL) was added TBAF (75 wt% in H₂O, 430 mg, 1.24 mmol) and the mixture was left stirring on the ice bath. After 5 h TLC-analysis showed complete conversion of the TBS-ether. The mixture was diluted with EtOAc (40 mL), washed with brine (2 x 10 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 9:1→4:1) to afford the title alcohol **S7** (267 mg, 0.605 mmol, 96%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 7.34 – 7.19 (m, 10H, CH Ar), 5.11 (s, 2H, CH₂ Cbz), 4.73 (dd, *J* = 11.4 Hz, 2H, CH₂ Bn), 4.20 (s, 1H, H4), 4.06 (dd, *J* = 13.5, 4.4 Hz, 1H, H6b), 3.92 (d, *J* = 8.9 Hz, 1H, H2b), 3.61 (s, 1H, H3), 3.32 (dd, *J* = 13.5, 11.0 Hz, 1H, H6a), 3.11 – 3.03 (m, 1H, H2a), 2.78 (d, *J* = 7.6 Hz, 1H, OH), 2.54 – 2.46 (m, 1H, H5), 1.43 (s, 9H, (CH₃)₃C). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 170.1 (OC=O), 155.4 (NC=O), 138.6, 136.8 (2C_q Ar), 128.5, 128.4, 128.0, 127.8, 127.7, 127.4 (CH Ar), 81.7 ((CH₃)₃C), 77.3 (C4), 74.4 (CH₂ Bn), 68.8 (C3), 67.3 (CH₂ Cbz), 46.5 (C5), 45.5 (C2), 40.2 (C6), 28.1 ((CH₃)₃C) ppm.

¹H NMR (500 MHz, CDCl₃ at 293K) [mixture of rotamers 1:1] δ 7.48 – 6.99 (m, 10H, CH Ar), 5.09 (s, 2H, CH₂ Cbz), 4.88 – 4.62 (m, 2H, CH₂ Bn), 4.20 (s, 1H, H4), 4.13 – 3.80 (m, 2H, H6b and H2b), 3.71 – 3.54 (m, 1H, H3), 3.35 – 3.27 (m, 1H, H6a), 3.22 – 2.98 (m, 2H, H2a and OH), 2.50 (s, 1H, H5), 1.43 (s, 9H, (CH₃)₃C). ¹³C NMR (126 MHz, CDCl₃) [mixture of rotamers 1:1] δ 170.1 (OC=O), 155.3 (NC=O), 138.3, 136.5 (2C_q Ar), 128.4, 128.3, 128.0, 127.8, 127.6, 127.3 (CH Ar), 81.7 ((CH₃)₃C), 77.0 (C4), 74.3 (CH₂ Bn), 68.6 & 68.5 (C3), 67.3 (CH₂ Cbz), 46.4 & 46.2 (C5), 45.3 & 45.0 (C2), 40.0 (C6), 27.9 ((CH₃)₃C) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₅H₃₁NO₆Na 464.20436, found 464.20419.

Compound 41

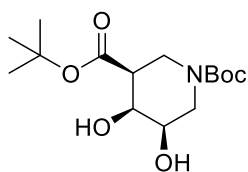
Compound **S7** (135 mg, 0.306 mmol) was dissolved in dry DCM (6 mL) and Dess-Martin periodinane (252 mg, 0.594 mmol) was added. The reaction was stirred at rt for 2 h until TLC-analysis indicated complete conversion of the alcohol. The mixture was diluted with EtOAc (25 mL) and washed subsequently with aq. 2 M Na₂S₂O₃ (2 x 10 mL), sat. aq. NaHCO₃ (2 x 10 mL) and brine (10 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 4:1→7:3) to afford the target ketone **41** (119 mg, 0.271 mmol, 89%) as a colorless oil. ¹H NMR (400 MHz, CDCl₃ at 333K) δ 7.39 – 7.21 (m, 10H, CH Ar), 5.10 (s, 2H, CH₂ Cbz), 4.76 (d, *J* = 12.1 Hz, 1H, CHH Bn), 4.57 (d, *J* = 12.1 Hz, 1H, CHH Bn), 4.30 (d, *J* = 17.0 Hz, 1H, H2b), 4.08 (br. s, 1H, H6b), 4.00 (d, *J* = 4.5 Hz, 1H, H4), 3.96 (br. s, 1H, H2a), 3.79 (d, *J* = 11.4 Hz, 1H, H6a), 3.05 (s, 1H, H5), 1.38 (s, 9H, (CH₃)₃C). ¹³C NMR (101 MHz, CDCl₃ at 333K) δ 200.6 (C=O), 168.8 (OC=O), 154.8 (NC=O), 137.0, 136.0 (2C_q Ar), 128.6, 128.5, 128.3, 128.1, 128.0, 127.8 (CH Ar), 82.4 ((CH₃)₃C), 78.8 (C4), 72.4 (CH₂ Bn), 67.8 (CH₂ Cbz), 52.9 (C2), 47.4 (C5), 42.3 (C6), 27.8 ((CH₃)₃C) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₅H₃₁NO₆Na 462.18871, found 462.18832.

Compound 42 and S8

Compound **40** (700 mg, 1.26 mmol) was dissolved in methanol (25 mL) under argon and 10% Pd/C (140 mg) was added. While stirring vigorously, the mixture was flushed with a H₂ balloon. After stirring for 2 h under H₂ atmosphere TLC and LC-MS analyses confirmed complete removal of the Cbz-group. The mixture was filtered over a Whatman filter and concentrated. The crude residue was directly taken up in DCM (10 mL) and DIPEA (250 μL, 1.44 mmol) and Boc₂O (700 mg, 3.21 mmol) were added. The mixture was stirred at rt overnight until LC-MS analysis confirmed complete *N*-protection. The mixture was concentrated, and eluted over a small plug of silica gel (pentane / EtOAc = 7 / 3) and the eluate was concentrated again. The material was redissolved in methanol (20 mL) under argon and 10% Pd/C (140 mg) was added. After stirring for 16 h under H₂ atmosphere, LC-MS indicated only circa 20% conversion of the starting material, so more 10% Pd/C (280 mg) and 20% Pd(OH)₂/C (300 mg) were added and the mixture was stirred over the weekend until LC-MS analysis indicated full conversion. The mixture was filtered over a Whatman filter and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/EtOAc 9:1→4:1) to afford compound **42** (372 mg, 0.862 mmol, 69% over three steps) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 4.20 (s, 1H, H4), 4.02 (d, *J* = 11.3 Hz, 1H, H6b), 3.85 (d, *J* = 7.4 Hz, 1H, H2b), 3.60 (ddd, *J* = 10.6, 5.2, 2.8 Hz, 1H, H3), 3.11 (t, *J* = 12.7 Hz, 1H, H6a), 2.88 (t, *J* = 10.9 Hz, 1H, H2a), 2.44 (m, 2H, H5 and OH), 1.47 (s, 9H, (CH₃)₃CO), 1.46 (s, 9H, (CH₃)₃CO), 0.91 (s, 9H, (CH₃)₃CSi), 0.13 (s, 6H, 2CH₃Si). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 169.9 (OC=O), 154.7 (NC=O), 81.2, 79.9 ((CH₃)₃CO), 69.5 (C4), 69.4 (C3), 46.1

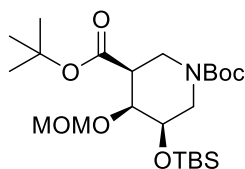
(C5), 44.1 (C2), 39.1 (C6), 28.4, 28.1 ((CH₃)₃CO), 25.8 ((CH₃)₃CSi), 18.1 ((CH₃)₃CSi), -4.6 (CH₃Si), -4.8 (CH₃Si) ppm.

¹H NMR (400 MHz, CDCl₃ at 293K) δ 4.21 (s, 1H, H4), 4.16 – 3.71 (m, 2H, H6b and H2b), 3.60 (s, 1H, H3), 3.10 (s, 1H, H6a), 3.01 – 2.67 (m, 1H, H2a), 2.58 – 2.37 (m, 2H, H5 and OH), 1.48 (s, 9H, (CH₃)₃CO), 1.46 (s, 9H, (CH₃)₃CO), 0.91 (s, 9H, (CH₃)₃CSi), 0.13 (s, 3H, CH₃Si), 0.12 (s, 3H, CH₃Si). ¹³C NMR (101 MHz, CDCl₃ at 293K) δ 170.2 (OC=O), 154.7 (NC=O), 81.4, 80.1 ((CH₃)₃CO), 69.4 (C4), 69.2 (C3), 45.9 (C5), 44.5 (C2), 38.7 (C6), 28.5, 28.1 ((CH₃)₃CO), 25.8 ((CH₃)₃CSi), 18.1 ((CH₃)₃CSi), -4.6 (2CH₃Si) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₁H₄₁NO₆SiNa 454.25954, found 454.25934.

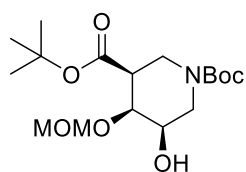


Further elution from the column with pentane/EtOAc = 1:1 afforded a byproduct (90 mg, 0.28 mmol, 22%) that was identified as diol **S8**. ¹H NMR (400 MHz, MeOD at 293K) δ 4.26 (app t, *J* = 2.3 Hz, 1H, H4), 4.03 – 3.91 (m, 1H, H6b), 3.90 – 3.78 (m, 1H, H2b), 3.50 (ddd, *J* = 10.9, 5.1, 2.7 Hz, 1H, H3), 3.19 – 3.00 (m, 1H, H6a), 2.99 – 2.75 (m, 1H, H2a), 2.49 (ddd, *J* = 11.8, 4.7, 2.5 Hz, 1H, H5), 1.48 (s, 9H, (CH₃)₃CO), 1.46 (s, 9H, (CH₃)₃CO). ¹³C NMR (101 MHz, MeOD at 293K) δ 172.0 (OC=O), 156.4 (NC=O), 82.2 ((CH₃)₃CO), 81.3 ((CH₃)₃CO), 69.9 (C4), 69.3 (C3), 47.4 (C5), 44.6 (C2, assign by HSQC), 39.8 (C6, assign by HSQC), 28.6 ((CH₃)₃CO), 28.1 ((CH₃)₃CO) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₁₅H₂₇NO₆Na 340.17306, found 340.17280.

Compound 43

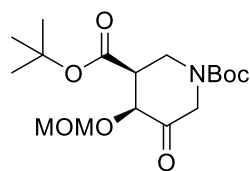


In a microwave tube compound **42** (0.18 g, 0.42 mmol) was dissolved in a mixture of dry DCM (4 mL), DIPEA (1.5 mL) and MOMCl (0.5 mL). The tube was sealed and heated for 1 h at 100 °C in a microwave (Biotage initiator+). After which the mixture was diluted with EtOAc (25 mL), washed with water (10 mL), sat. aq. NaHCO₃ (10 mL) and brine (10 mL), dried over MgSO₄, filtered and concentrated. The crude was purified by silica gel column chromatography (pentane/EtOAc 98:2→95:5) to give the target compound **43** (176 mg, 0.370 mmol, 88%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 4.81 (d, *J* = 5.8 Hz, 1H, CHHO MOM), 4.77 (d, *J* = 5.8 Hz, 1H, CHHO MOM), 4.19 (s, 1H, H4), 4.10 (d, *J* = 9.3 Hz, 1H, H6b), 3.86 (d, *J* = 9.1 Hz, 1H, H2b), 3.56 (ddd, *J* = 10.8, 4.9, 2.2 Hz, 1H, H3), 3.35 (s, 3H, OCH₃), 3.09 (t, *J* = 12.8 Hz, 1H, H6a), 2.99 (t, *J* = 11.3 Hz, 1H, H2a), 2.43 (ddd, *J* = 12.0, 4.5, 2.0 Hz, 1H, H5), 1.47 (s, 9H, (CH₃)₃CO), 1.46 (s, 9H, (CH₃)₃CO), 0.92 (s, 9H, (CH₃)₃CSi), 0.12 (s, 6H, 2CH₃Si). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 169.6 (OC=O), 154.8 (NC=O), 98.2 (CH₂ MOM), 81.3 ((CH₃)₃CO), 79.9 ((CH₃)₃CO), 76.0 (C4), 71.3 (C3), 56.2 (CH₃ MOM), 46.9 (C5), 44.8 (C2), 39.5 (C6), 28.5 ((CH₃)₃CO), 28.2 ((CH₃)₃CO), 25.9 ((CH₃)₃CSi), 18.2 ((CH₃)₃CSi), -4.6 (CH₃Si), -4.8 (CH₃Si) ppm. HRMS (ESI) *m/z*: [M+H]⁺ calc for C₂₃H₄₆NO₇Si 476.30381, found 476.30378.

Compound S9

Compound **43** (175 mg, 0.368 mmol) was dissolved in THF (5 mL) and cooled on an ice-bath. TBAF (75 wt% in H₂O, 230 mg, 0.66 mmol) was added and the mixture was stirred on the ice bath for 5 h. The mixture was then diluted with EtOAc (30 mL), washed with brine (2 x 10 mL), dried over Na₂SO₄, filtered and concentrated. The crude was purified by silica gel column chromatography (pentane/EtOAc 95:5 → 7:3) to afford compound **S9** (127 mg, 0.351 mmol, 95%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 4.72 (d, *J* = 6.5 Hz, 1H, CHH MOM), 4.68 (d, *J* = 6.5 Hz, 1H, CHH MOM), 4.19 (t, *J* = 2.5 Hz, 1H, H4), 4.10 (d, *J* = 10.6 Hz, 1H, H6b), 4.00 – 3.86 (m, 2H, OH and H2b), 3.52 – 3.45 (m, 1H, H3), 3.44 (s, 3H, CH₃ MOM), 3.08 (dd, *J* = 13.5, 11.6 Hz, 1H, H6a), 2.83 (app t, *J* = 11.7 Hz, 1H, H2a), 2.54 (ddd, *J* = 11.4, 4.8, 2.5 Hz, 1H, H5), 1.46 (s, 18H, 2(CH₃)₃CO). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 169.6 (OC=O), 154.9 (NC=O), 98.9 (CH₂ MOM), 81.4 ((CH₃)₃CO), 80.8 (C4), 80.1 ((CH₃)₃CO), 67.8 (CH₃ MOM), 56.2 (C3), 46.7 (C5), 45.5 (C2), 39.7 (C6), 28.5, 28.2 ((CH₃)₃CO) ppm.

¹H NMR (500 MHz, CDCl₃ at 293K) δ 4.73 (d, *J* = 6.7 Hz, 1H, CHH MOM), 4.69 (d, *J* = 6.7 Hz, 1H, CHH MOM), 4.27 – 3.88 (m, 4H, H4, OH, H2b and H6b), 3.54 – 3.47 (m, 1H, H3), 3.45 (s, 3H, CH₃ MOM), 3.12 – 2.99 (m, 1H, H6a), 2.89 – 2.70 (m, 1H, H2a), 2.57 (d, *J* = 9.3 Hz, 1H, H5), 1.46 (s, 18H, 2(CH₃)₃CO). ¹³C NMR (126 MHz, CDCl₃ at 293K) δ 169.5 (OC=O), 154.8 (NC=O), 98.7 (CH₂ MOM), 81.4 ((CH₃)₃CO), 81.1 (C4), 80.1 ((CH₃)₃CO), 67.6 (CH₃ MOM), 56.2 (C3), 46.4 (C5), 28.4, 28.1 ((CH₃)₃CO) ppm. HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₁₇H₃₁NO₇Na 384.19927, found 384.19904.

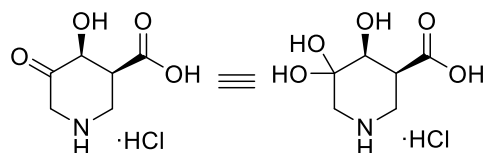
Compound 44

To a stirred solution of alcohol **S9** (126 mg, 0.349 mmol) in dry DCM (5 mL) was added Dess-Martin periodinane (248 mg, 0.585 mmol) and the mixture was stirred at rt for 3 h until complete conversion was confirmed by TLC and LC-MS analyses. The mixture was diluted with EtOAc (30 mL) and washed subsequently with aq. 2 M Na₂S₂O₃ (2 x 10 mL), sat. aq. NaHCO₃ (2 x 10 mL) and brine (10 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/acetone 98:2 → 85:15) to afford ketone **44** (100 mg, 0.278 mmol, 80%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 4.76 (d, *J* = 6.8 Hz, 1H, CHH MOM), 4.73 (d, *J* = 6.8 Hz, 1H, CHH MOM), 4.31 (dd, *J* = 17.4, 1.2 Hz, 1H, H2b), 4.22 – 4.13 (m, 2H, H4 and H6b), 3.87 (d, *J* = 17.4 Hz, 1H, H2a), 3.68 (dd, *J* = 14.1, 4.2 Hz, 1H, H6a), 3.38 (s, 3H, CH₃ MOM), 3.13 (dd, *J* = 9.6, 4.8 Hz, 1H, H5), 1.45 (s, 9H, (CH₃)₃CO), 1.43 (s, 9H, (CH₃)₃CO). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 200.3 (C=O), 169.2 (OC=O), 154.1 (NC=O), 96.6 (CH₂ MOM), 82.4, 81.0 ((CH₃)₃CO), 77.5 (C4), 56.1 (CH₃ MOM), 53.6 (C2), 47.7 (C5), 43.1 (C6), 28.4, 28.0 ((CH₃)₃CO) ppm.

¹H NMR (500 MHz, CDCl₃ at 293K) δ 4.80 (d, *J* = 6.9 Hz, 1H, CHH MOM), 4.75 (d, *J* = 6.9 Hz, 1H, CHH MOM), 4.43 – 4.11 (m, 3H, H2b, H4 and H6b), 3.96 – 3.75 (m, 1H, H2a), 3.75 – 3.52 (m, 1H,

H6a), 3.40 (s, 3H, CH₃ MOM), 3.19 (br. s, 1H, H5), 1.45 (s, 9H, (CH₃)₃CO), 1.44 (s, 9H, (CH₃)₃CO). ¹³C NMR (126 MHz, CDCl₃ at 293K) δ 200.5 (C=O), 169.3 (OC=O), 154.0 (NC=O), 96.3 (CH₂ MOM), 82.4, 80.9 ((CH₃)₃CO), 77.3 (C4), 56.0 (CH₃ MOM), 54.0 (C2), 47.7 (C5), 43.0 (C6), 28.3, 27.9 ((CH₃)₃CO). HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₁₇H₃₁NO₇Na 382.18362, found 382.18337.

Compound 8

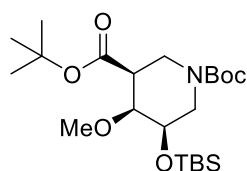


Method A (deprotection of ketone **39**): Ketone **39** (68 mg, 0.14 mmol) was dissolved in a mixture of THF (10 mL) and aqueous 3 M HCl (300 μ L). Subsequently, 10% Pd/C (72 mg) was added and the mixture was stirred under hydrogen

atmosphere for 18 h. The mixture was filtered over a Whatman® filter and concentrated *in vacuo* to afford the target compound (31 mg) as a white foam in quantitative yield.

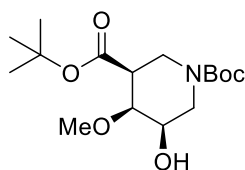
Method B (deprotection of ketone **44**): Ketone **44** (23 mg, 64 μ mol) was dissolved in a mixture of hexafluoro-2-propanol (960 μ L) and water (200 μ L). Concentrated HCl (40 μ L) was added and the mixture stirred at rt for 5 h after which the mixture was concentrated *in vacuo* and co-evaporated with water at 35 °C (3 x) to afford the target compound (14 mg) in quantitative yield. ¹H NMR (500 MHz, D₂O at 293K) [hydrate form] δ 4.20 – 4.15 (m, 1H, H4), 3.44 (dd, *J* = 12.1, 3.8 Hz, 1H, H6b), 3.32 (ddd, *J* = 12.7, 3.8, 2.5 Hz, 1H, H5), 3.26 (d, *J* = 12.4 Hz, 1H, H6a), 3.21 (d, *J* = 12.7 Hz, 1H, H2b), 3.16 (d, *J* = 12.7 Hz, 1H, H2a). ¹³C NMR (126 MHz, D₂O) δ 173.3 (C=O), 91.1 (C3), 69.4 (C4), 46.1 (C2), 41.9 (C5), 38.4 (C6). HRMS (ESI) *m/z*: [M_{hydrate}+H]⁺ calc for C₆H₁₂NO₅ 178.07100, found 178.07068.

Compound 45

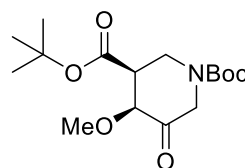


To a solution of alcohol **42** (0.35 g, 0.81 mmol) in THF (8 mL) were added Ag₂O (2.44 g, 10.5 mmol), CH₃I (3.0 mL, 48.2 mmol) and (CH₃)₂S (100 μ L, 1.36 mmol). The flask was protected from light by packing in alumina foil and the mixture was stirred vigorously at rt overnight. The mixture was filtered over

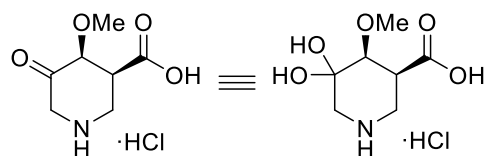
a pad of celite and the filter cake washed with EtOAc (3 x). The filtrate was concentrated *in vacuo* to give a thick oil (377 mg) that was purified by silica gel column chromatography (pentane/EtOAc 98:2→95:5) to afford the target product (0.25 g, 0.56 mmol) in 69% yield. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 4.01 (d, *J* = 10.3 Hz, 1H, H6b), 3.86 – 3.74 (m, 2H, H4, H2b), 3.56 – 3.47 (m, 4H, H3, OCH₃), 2.99 – 2.84 (m, 2H, H6a and H2a), 2.35 (ddd, *J* = 11.9, 4.6, 2.3 Hz, 1H, H5), 1.42 (s, 9H, (CH₃)₃CO), 1.40 (s, 9H, (CH₃)₃CO), 0.88 (s, 9H, (CH₃)₃CSi), 0.07 (s, 6H, 2CH₃Si). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 169.9 (OC=O), 154.8 (NC=O), 80.9 ((CH₃)₃CO), 79.8 (C4), 79.8 ((CH₃)₃CO), 71.6 (C3), 61.5 (OCH₃), 46.7 (C5), 44.8 (C2), 39.6 (C6), 28.5 ((CH₃)₃CO), 28.2 ((CH₃)₃CO), 25.9 ((CH₃)₃CSi), 18.1 ((CH₃)₃CSi), -4.6 (CH₃Si), -4.8 (CH₃Si). HRMS (ESI) *m/z*: [M+Na]⁺ calc for C₂₂H₄₃NO₆SiNa 468.27519, found 468.27500.

Compound S10

To an ice cold solution of TBS-ether **45** (0.25 g, 0.56 mmol) in THF (10 mL) was added TBAF (75 wt% in H₂O, 410 mg, 1.17 mmol). The mixture was allowed to warm up slowly to room temperature and stirred for 5 h until TLC analysis indicated complete conversion of the starting material. The mixture was diluted with EtOAc (30 mL) and washed with brine (2 x 10 mL), dried over Na₂SO₄, filtered and concentrated. The crude was purified by silica gel column chromatography (pentane/EtOAc 95:5→2:1) to give the target alcohol (178 mg, 0.537 mmol, 96%) as a colorless solid. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 3.95 – 3.87 (m, 2H, H6b and H4), 3.82 (d, J = 9.9 Hz, 1H, H2b), 3.61 (s, 1H, H3), 3.53 (s, 3H, OCH₃), 3.19 (dd, J = 13.7, 10.7 Hz, 1H, H6a), 3.03 – 2.95 (m, 1H, H2a), 2.71 (s, 1H, OH), 2.52 – 2.45 (m, 1H, H5), 1.48 (s, 9H, (CH₃)₃CO), 1.45 (s, 9H, (CH₃)₃CO). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 170.5 (OC=O), 154.9 (NC=O), 81.6 ((CH₃)₃CO), 80.1 ((CH₃)₃CO), 79.2 (C4), 68.7 (C3), 60.6 (OCH₃), 46.5 (C5), 45.7 (C2), 40.1 (C6), 28.5 ((CH₃)₃CO), 28.2 ((CH₃)₃CO). HRMS (ESI) m/z : [M+Na]⁺ calc for C₁₆H₂₉NO₆Na 354.18871, found 354.18850.

Compound 46

To a stirred solution of alcohol **S10** (176 mg, 0.53 mmol) in dry DCM (10 mL) was added Dess-Martin periodinane (488 mg, 1.15 mmol) and the mixture was stirred at rt for 4 h until complete conversion was confirmed by TLC analysis. The mixture was diluted with EtOAc (30 mL) and washed subsequently with aq. 2 M Na₂S₂O₃ (2 x 10 mL), sat. aq. NaHCO₃ (2 x 10 mL) and brine (10 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (pentane/acetone 9:1→7:3) to afford ketone **46** (135 mg, 0.41 mmol, 76%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃ at 333K) δ 4.10 (d, J = 17.1 Hz, 1H, H2b), 4.02 (d, J = 17.1 Hz, 1H, H2a), 3.87 (d, J = 5.9 Hz, 2H, H6), 3.80 (d, J = 4.6 Hz, 1H, H4), 3.45 (s, 3H, OCH₃), 3.04 (q, J = 5.9 Hz, 1H, H5), 1.45 (s, 9H, (CH₃)₃CO), 1.44 (s, 9H, (CH₃)₃CO). ¹³C NMR (126 MHz, CDCl₃ at 333K) δ 200.7 (C=O), 168.9 (OC=O), 154.3 (NC=O), 82.2 ((CH₃)₃CO), 81.8 (C4), 81.0 ((CH₃)₃CO), 58.7 (OCH₃), 52.9 (C2), 47.2 (C5), 41.9 (C6), 28.5 ((CH₃)₃CO), 28.1 ((CH₃)₃CO). HRMS (ESI) m/z : [M+Na]⁺ calc for C₁₆H₂₇NO₆Na 352.17306, found 352.17283.

Compound 10

Ketone **46** (47 mg, 0.14 mmol) was dissolved in a mixture of hexafluoro-2-propanol (1.5 mL) and water (0.4 mL). After cooling on an ice bath, concentrated hydrochloric acid (0.1 mL) was added. After five minutes the cooling bath was removed and the mixture stirred at room temperature for 4 h. The solvents were removed *in vacuo* and the residue co-evaporated with water (2 x) to afford the target product (33 mg) as a foam in quantitative

yield. ^1H NMR (400 MHz, D_2O at 293K) [hydrate form] δ 3.82 (d, $J = 2.4$ Hz, 1H, H4), 3.48 (s, 3H, OCH_3), 3.38 (dd, $J = 12.6, 4.3$ Hz, 1H, H6b), 3.27 (ddd, $J = 12.6, 4.3, 2.8$ Hz, 1H, H5), 3.19 – 3.05 (m, 3H, H6a and H2ab). ^{13}C NMR (101 MHz, D_2O at 293K) [hydrate form] δ 173.3 (COOH), 91.5 (C3), 79.3 (C4), 61.0 (OCH_3), 46.5 (C2), 41.4 (C5), 38.8 (C6). HRMS (ESI) m/z : [$\text{M}_{\text{ketone}} + \text{H}$] $^+$ calc for $\text{C}_7\text{H}_{12}\text{NO}_4$ 174.07608, found 174.07624; [$\text{M}_{\text{hydrate}} + \text{H}$] $^+$ calc for $\text{C}_7\text{H}_{14}\text{NO}_5$ 192.08665, found 192.08675; [$\text{M}_{\text{hydrate}} + \text{Na}$] $^+$ calc for $\text{C}_7\text{H}_{13}\text{NO}_5\text{Na}$ 214.06859, found 214.06866.

5.6 References

- [1] Li, J. P.; Kusche-Gullberg, M., *Academic Press* **2016**, 325, 215-273.
- [2] Sarrazin, S.; Lamanna, W. C.; Esko, J. D., *Cold Spring Harb Perspect Biol.* 2011, 3, 1-33.
- [3] Iozzo, R. V.; Schaefer, L., *Matrix Biol.* 2015, 42, 11-55.
- [4] Iozzo, R. V., *Nat. Rev. Mol. Cell Biol.* **2005**, 6, 646-656.
- [5] Masola, V.; Bellin, G.; Gambaro, G.; Onisto, M., *Cells* **2018**, 7, 236-246.
- [6] Billings, P. C.; Pacifici, M., *Connect Tissue Res.* **2015**, 56, 272-280.
- [7] Matsuo, I.; Kimura-Yoshida, C., *Phil. Trans. R. Soc. B* **2014**, 369, 1-9.
- [8] Koshland, D.E., *Biol. Rev. Camb. Philos. Soc.* **1953**, 28, 416-436.
- [9] Wilson, J. C.; Laloo, A. E.; Singh, S.; Ferro, V., *Biochem. Biophys. Res. Commun.* **2014**, 443, 185-188.
- [10] Goldberg, R.; Meirovitz, A.; Hirshoren, N.; Bulvik, R.; Binder, A.; Rubinstein, A. M.; Elkin, M., *Matrix Biol.* **2013**, 32, 234-240.
- [11] Sanderson, R. D.; Elkin, M.; Rapraeger, A. C.; Ilan, N.; Vlodavsky, I., *FEBS J.* **2017**, 284, 42-55.
- [12] Vlodavsky, I.; Singh, P.; Boyango, I.; Gutter-Kapon, L.; Elkin, M.; Sanderson, R. D.; Ilan, N., *Drug Resist. Updat.* **2016**, 29, 54-75.
- [13] Rivara, S.; Milazzo, F. M.; Giannini, G., *Future Med. Chem.* **2016**, 8, 647-680.
- [14] Jia, L.; Ma, S., *Eur. J. Med. Chem.* **2016**, 121, 209-220.
- [15] Mohan, C. D.; Hari, S.; Preetham, H. D.; Rangappa, S.; Barash, U.; Ilan, N.; Nayak, S. C.; Gupta, V. K.; Basappa; Vlodavsky, I.; Rangappa, K. S., *iScience* **2019**, 15, 360-390.
- [16] Vlodavsky, I.; Sanderson, R. D.; Ilan, N., *Springer Cham.* **2020**, 567-603.
- [17] Karoli, T.; Liu, L.; Fairweather, J. K.; Hammond, E.; Li, C. P.; Cochran, S.; Bergefall, K.; Trybala, E.; Addison, R. S.; Ferro, V., *J. Med. Chem.* 2005, 48, 8229-8236.
- [18] Ritchie, J. P.; Ramani, V. C.; Ren, Y.; Naggi, A.; Torri, G.; Casu, B.; Penco, S.; Pisano, C.; Carminati, P.; Tortoreto, M.; Zunino, F.; Vlodavsky, I.; Sanderson, R. D.; Yang, Y., *Clin. Cancer Res.* **2011**, 17, 1382-1393.
- [19] O'Reilly, E. M.; Roach, J.; Miller, P.; Yu, K. H.; Tjan, C.; Rosano, M.; Krause, S.; Avery, W.; Wolf, J.; Flaherty, K.; Nix, D.; Ryan, D. P., *Oncologist* **2017**, 22, 1429-e139.
- [20] Dredge, K.; Brennan, T. V.; Hammond, E.; Lickliter, J. D.; Lin, L.; Bampton, D.; Handley, P.; Lankesheer, F.; Morrish, G.; Yang, Y.; Brown, M. P.; Millward, M., *Br. J. Cancer* **2018**, 118, 1035-1041.
- [21] Madia, V. N.; Messori, A.; Pescatori, L.; Saccoliti, F.; Tudino, V.; De Leo, A.; Bortolami, M.; Scipione, L.; Costi, R.; Rivara, S.; Scalvini, L.; Mor, M.; Ferrara, F. F.; Pavoni, E.; Roscilli, G.; Cassinelli, G.; Milazzo, F. M.; Battistuzzi, G.; Di Santo, R.; Giannini, G., *J. Med. Chem.* **2018**, 61, 6918-6936.
- [22] Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J., *Phytochemistry* **2001**, 56, 265-295.
- [23] Gloster, T. M.; Davies, G. J., *Org. Biomol. Chem.* **2010**, 8, 305-320.
- [24] Conforti, I.; Marra, A., *Org. Biomol. Chem.* **2021**, 19, 5439-5475.
- [25] Platt, F. M.; Neises, G. R.; Dwek, R. A.; Butters, T. D., *J. Bio. Chem.* **1994**, 269, 8362-8365.
- [26] Gross, P. E.; Baker, M. A.; Carver, J. P.; Dennis, J. W., *Clin. Cancer Res.* **1995**, 1, 935-944.

- [27] Scott, L. J.; Spencer, C. M., *Drugs* **2000**, *59*, 521-549.
- [28] Umezawa, H.; Aoyagi, T.; Komiyama, H.; Morishima, H.; Hamada, M.; Takeuchi, T., *J. Antibiot.* **1974**, *27*, 963-969.
- [29] Nishimura, Y., *J. Antibiot.* **2009**, *62*, 407-423.
- [30] Nishimura, Y.; Wang, W.; Kondo, S.; Aoyagi, T.; Umezawa, H., *J. Am. Chem. Soc.* **1988**, *110*, 7249-7250.
- [31] Nishimura, Y.; Kudo, T.; Kondo, S.; Takeuchi, T., *J. Antibiot.* **1994**, *47*, 101-107.
- [32] Nishimura, Y.; Shitara, E.; Adachi, H.; Toyoshima, M.; Nakajima, M.; Okami, Y.; Takeuchi, T., *J. Org. Chem.* **2000**, *65*, 2-11.
- [33] Kondo, K.; Adachi, H.; Shitara, E.; Kojima, F.; Nishimura, Y., *Bioorg. Med. Chem.* **2001**, *9*, 1091-1095.
- [34] Wu, L.; Viola, C. M.; Brzozowski, A. M.; Davies, G. J., *Nat. Struct. Mol. Biol.* **2015**, *22*, 1016-1022.
- [35] Caner, S.; Zhang, X.; Jiang, J.; Chen, H. M.; Nguyen, N. T.; Overkleeft, H.; Brayer, G. D.; Withers, S. G., *FEBS Lett.* **2016**, *590*, 1143-1151.
- [36] Schroder, 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.; Abbate, E.; Stringer, M. A.; Krogh, K.; Raich, L.; Rovira, C.; Berrin, J. G.; van Wezel, G. P.; Ram, A. F. J.; Florea, B. I.; van der Marel, G. A.; Codee, J. D. C.; Wilson, K. S.; Wu, L.; Davies, G. J.; Overkleeft, H. S., *ACS Cent. Sci.* **2019**, *5*, 1067-1078.
- [37] de Boer, C.; McGregor, N. G. S.; Peterse, E.; Schroder, S. P.; Florea, B. I.; Jiang, J.; Reijngoud, J.; Ram, A. F. J.; van Wezel, G. P.; van der Marel, G. A.; Codee, J. D. C.; Overkleeft, H. S.; Davies, G. J., *RSC Chem. Biol.* **2020**, *1*, 148-155.
- [38] Igarashi, Y.; Ichikawa, M.; Ichikawa, Y., *Tetrahedron Lett.* **1996**, *37*, 2707-2708.
- [39] Ichikawa, Y.; Igarashi, Y.; Ichikawa, M.; Suhara, Y., *J. Am. Chem. Soc.* **1998**, *120*, 3007-3018.
- [40] Jip Kim, Y.; Ichikawa, M.; Ichikawa, Y., *J. Org. Chem.* **2000**, *65*, 2599-2602.
- [41] Jiang, J.; Kallemijn, W. W.; Wright, D. W.; van den Nieuwendijk, A.; Rohde, V. C.; Folch, E. C.; van den Elst, H.; Florea, B. I.; Scheij, S.; Donker-Koopman, W. E.; Verhoek, M.; Li, N.; Schurmann, M.; Mink, D.; Boot, R. G.; Codée, J. D. C.; van der Marel, G. A.; Davies, G. J.; Aerts, J.; Overkleeft, H. S., *Chem. Sci.* **2015**, *6*, 2782-2789.
- [42] Griengl, H.; Klempier, N.; Pöchlauer, P.; Schmidt, M.; Shi, N.; A. Zabelinskaja-Mackova, A., *Tetrahedron* **1998**, *54*, 14477-14486.
- [43] Ouchi, H.; Mihara, Y.; Takahata, H., *J. Org. Chem.* **2005**, *70*, 5207-5214.
- [44] Yang, D.; Wong, M.-K.; Yip, Y.-C., *J. Org. Chem.* **1995**, *60*, 3887-3889.
- [45] Lipshutz, B. H.; Wilhelm, R. S.; Kozlowski, J. A., *Tetrahedron* **1984**, *40*, 5005-5038.
- [46] Joly, G. D.; Jacobsen, E. N., *Org. Lett.*, **2002**, *4*, 1795-1798.
- [47] Custar, D. W.; Zabawa, T. P.; Hines, J.; Crews, C. M.; Scheidt, K. A., *J. Am. Chem. Soc.* **2009**, *131*, 12406-12414.
- [48] Kato, T.; Sato, T.; Kashiwagi, Y.; Hosokawa, S., *Org. Lett.* **2015**, *17*, 2274-2277.
- [49] Carlson, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B., *J. Org. Chem.* **1981**, *46*, 3936-3938.
- [50] Ichikawa, Y.; Igarashi, Y., *Tetrahedron Lett.* **1995**, *36*, 4585-4586.
- [51] Bal, B. S.; Childers, W. E.; Pinnick, H. W., *Tetrahedron* **1981**, *37*, 2091-2096.
- [52] Daragics, K.; Fügedi, P., *Tetrahedron Lett.* **2009**, *50*, 2914-2916.
- [53] Werz, D. B.; Seeberger, P. H., *Angew. Chem. Int. Ed. Engl.* **2005**, *44* (39), 6315-6318.
- [54] 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.; Codée, J. D. C.; Florea, B. I.; Aerts, J. M. F. G.; Overkleeft, H. S.; Davies, G. J., *Nat. Chem. Biol.* **2017**, *13*, 867-873.
- [55] Winter, G., *J. Appl. Crystallogr.* **2010**, *43*, 186-190.
- [56] Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K., *Acta. Crystallogr. D Biol. Crystallogr.* **2010**, *66*, 486-501.
- [57] Murshudov, G. N.; Skubak, P.; Lebedev, A. A.; Pannu, N. S.; Steiner, R. A.; Nicholls, R. A.; Winn, M. D.; Long, F.; Vagin, A. A., *Acta. Crystallogr. D Biol. Crystallogr.* **2011**, *67*, 355-367.
- [58] Lebedev, A. A.; Young, P.; Isupov, M. N.; Moroz, O. V.; Vagin, A. A.; Murshudov, G. N., *Acta. Crystallogr. D Biol. Crystallogr.* **2012**, *68*, 431-440.

APPENDIX

Table 5.S1. Data collection and refinement statistics

	HPSE + SiasB	HPSE + 14	HPSE + 12	AcGH79 + SiasB
Data collection				
Space group	P2 ₁	P2 ₁	P2 ₁	I2 ₁
Cell dimensions				
<i>a</i> , <i>b</i> , <i>c</i> (Å)	46.11, 70.92, 78.56	44.51, 71.57, 78.36	44.45, 71.16, 78.29	82.75 44.67 136.27
α , β , γ (°)	90.0, 95.5, 90.0	90.0 98.34 90.0	90.0, 98.48, 90.0	90.0, 97.46, 90.0
Resolution (Å)	45.90-1.70 (1.73-1.70)	52.64-1.90 (1.94-1.90)	44.01-1.80 (1.84-1.80)	67.56-1.05 (1.07-1.05)
<i>I</i> / σ <i>I</i>	10.8 (1.6)	10.6 (1.2)	9.2 (1.3)	15.1 (1.4)
Completeness (%)	99.6 (99.9)	100 (100)	99.2 (98.3)	96.5 (88.8)
Redundancy	1.9 (1.9)	2.0 (2.0)	6.9 (6.5)	6.3 (4.5)
Refinement				
Resolution (Å)	1.70	1.90	1.80	1.05
No. reflections	104,973	75,449	306,166	1,385,607
<i>R</i> _{work} / <i>R</i> _{free}	0.17/0.21	0.19/0.23	0.20/0.23	0.17/0.18
No. atoms				
Protein	3,674	3,654	3,643	3,613
Ligand/ion	104/3	51/1	34/1	17/0
Water	249	100	183	585
<i>B</i> -factors				
Protein	29.83	37.63	30.97	13.12
Ligand/ion	61.33/ 35.52	53.34/ 35.99	42.17/35.28	14.48
Water	35.04	36.17	35.92	24.68
R.m.s. deviations				
Bond lengths (Å)	0.01	0.01	0.01	0.02
Bond angles (°)	1.7	1.6	1.6	2.0

*Number of xtals for each structure should be noted in footnote. *Values in parentheses are for highest-resolution shell.

Table S1, continued

	AcGH79 + 14	AcGH79 + 12	BpHep + SiasB	EcGusB + SiasB
Data collection				
Space group	I2 ₁	I2 ₁	P2 ₁ 2 ₁ 2 ₁	C2
Cell dimensions				
<i>a</i> , <i>b</i> , <i>c</i> (Å)	83.05, 44.67, 137.17	82.04, 42.61, 140.14	76.37, 104.61, 113.71	126.28, 76.71, 141.02
α , β , γ (°)	90.0, 97.6, 90.0	90.0, 99.42, 90.0	90.0, 90.0, 90.0	90.0, 102.01, 90.0
Resolution (Å)	42.44-1.25 (1.27-1.25)	40.72-1.50 (1.53-1.50)	61.68-1.27 (1.29-1.27)	67.04-1.95 (1.98-1.95)
<i>I</i> / σI	10.2 (1.6)	11.0 (1.0)	12.6 (1.1)	8.1 (1.2)
Completeness (%)	99.0 (87.9)	100.0 (100.0)	99.9 (98.5)	98.4 (97.4)
Redundancy	6.2 (3.8)	6.4 (6.3)	8.2 (7.4)	4.1 (4.2)
Refinement				
Resolution (Å)	1.25	1.50	1.27	1.95
No. reflections	840,749	489,904	1,960,048	384,016
<i>R</i> _{work} / <i>R</i> _{free}	0.16/0.18	0.20/0.22	0.16/0.18	0.22/0.28
No. atoms				
Protein	3,487	3,435	6,441	9,574
Ligand/ion	11/0	17/0	56/0	24/0
Water	357	185	1037	316
<i>B</i> -factors				
Protein	15.07	24.02	18.44	54.8
Ligand/ion	10.47	22.25	25.01	42.05
Water	22.38	26.77	33.14	43.74
R.m.s. deviations				
Bond lengths (Å)	0.01	0.01	0.02	0.01
Bond angles (°)	1.9	1.8	1.9	1.6

*Number of xtals for each structure should be noted in footnote. *Values in parentheses are for highest-resolution shell.