Activity-based protein profiling of glucosidases, fucosidases and glucuronidases

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In vitro and *in vivo* comparative and competitiveactivity-based protein profiling of GH29 α-L-Fucosidases

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3.1 Introduction

GH29 α-L-fucosidases catalyze the hydrolysis of terminal α-L-fucosidic linkages.[1] The GH29[2] glycoside hydrolase family of retaining α-L-fucosidases contains members from various kingdoms of life including eukaryota[3] and bacteria (for example, *Bacteroides thetaiotaomicron*,[4] *Sulfolobus solfataricus*,[5] and *Thermotoga maritima*[6]). GH29 α-L-fucosidases process their substrate with overall retention of configuration at the anomeric center of the cleaved fucopyranose and do so through a double-displacement mechanism. In this mechanism, first proposed by Koshland,[7] (Figure 1A), S_N2 displacement of the aglycon (activated through protonation by the general acid/base residue) by nucleophilic attack of the catalytic nucleophile yields a fucosyl-enzyme intermediate, which is subsequently hydrolyzed to yield α-L-fucopyranose together with the released aglycon.

Two GH29 α-L-fucosidases are expressed in man. Of these, FUCA1 is found in lysosomes whereas FUCA2 is secreted into the plasma.[8] Deficiency of FUCA1 α-L-fucosidase activity causes fucosidosis,[9] a rare autosomal recessive lysosomal storage disorder. Next to its role in lysosomal turnover of fucosylated substrates, FUCA1 is also involved in sperm transport and sperm-egg interactions.[10] FUCA1 activity levels are considered to be a biomarker for cellular senescence[11] as well as for the diagnosis of hepatocellular cancers.[12] Deficiency of human FUCA2 has been shown to protect against *Helicobacter pylori* adhesion to gastric cancer cells.[8]

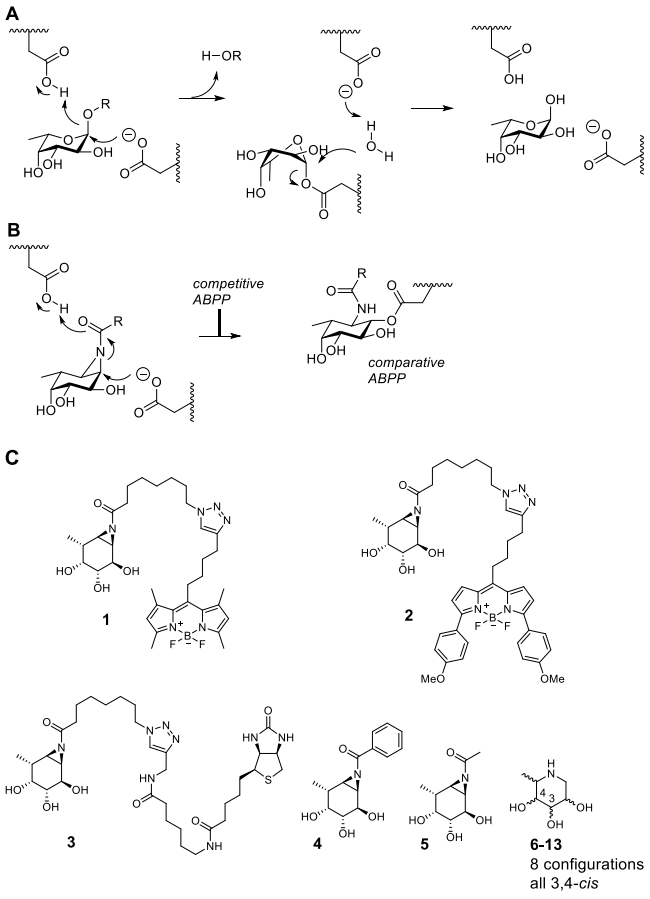


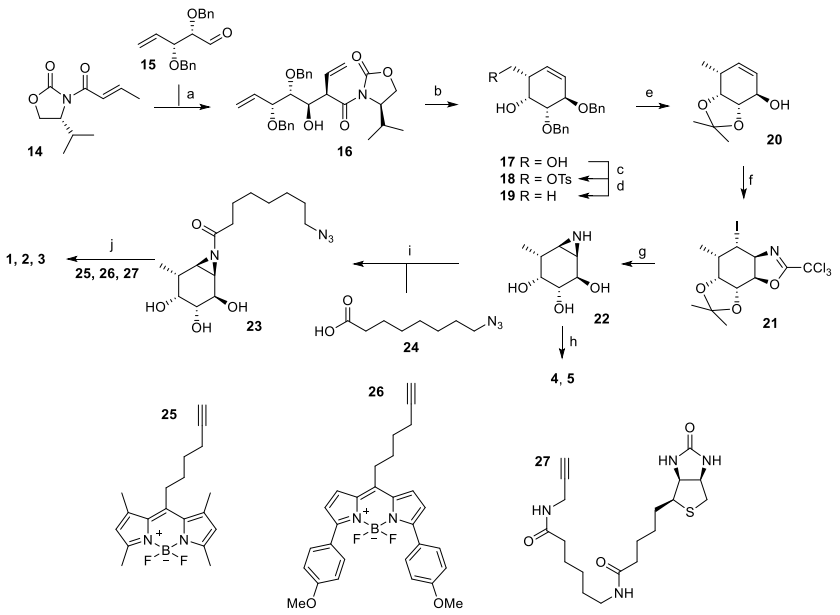
Figure 1. A) Double-displacement mechanism of retaining α-L-fucosidases. B) Comparative and competitive activity-based profiling of GH29 α-L-fucosidases presented here. C) Inhibitors and probes subject of this chapter.

The biological and biomedical relevance of GH29 retaining α-L-fucosidases warrant the development of efficient methods to monitor their functional state and activity *in vitro*, *in situ* and *in vivo*. In this respect, activity-based probes (ABPs) have shown their merit as tools to detect active enzyme molecules in their native environment (Figure1B).[13] It was previously

shown that cyclophellitol aziridines are useful scaffolds for the design of *in situ* and *in vivo* active ABPs directed at retaining β-glucosidases[14] and retaining α-galactosidases.[15] The specificity of these probes appeared due to their configuration, with the β-glucopyranose configured cyclitol aziridine being highly selective towards retaining β-glucosidases and their α-galacto-configured counterparts selective towards α-galactosidases.

In this chapter the development of retaining GH29 α-L-fucosidase ABPs is described. The ABPs are based on the cyclophellitol aziridine structure having the α-L-fucoside configuration and are equipped with a green (**1**, JJB256) or red (**2**, JJB244) BODIPY fluorophore or a biotin tag (**3**, JJB243) (Figure 1C). The probes turned out to be highly sensitive and selective and can be used for *in situ* and *in vivo* monitoring of mammalian and bacterial GH29 retaining α-L-fucosidases. ABPs **1** and **2** can also be used in a competitive activity-based protein profiling (ABPP) assay[16] to rapidly identify retaining α-L-fucosidase inhibitors from a library of eight configurational isomers of deoxy-L-fuconojirimycin (**6**-**13**); a library prepared specifically for this purpose. Finally the validity of the cyclophellitol aziridine design platform, for ABP development of retaining glycosidases, is established unambiguously by solving the crystal structure of a retaining α-L-fucosidase from *Bacteroides thetaiotaomicron* 2970, covalently bound to *N*-acyl cyclophellitol aziridines **4** and **5**.

Scheme 1. Synthesis of aziridine ABPs **1**, **2**, **3** and inhibitors **4**, **5**.



Reagents and conditions: (a) Bu_2BOTf (1.0 M in DCM), Et_3N, DCM, -78 °C, 71%; (b) i) $LiBH_4$, THF, 83%; ii) Grubbs 2nd generation, DCM, 95%; (c) *p*-TsCl, Et_3N, DCM, 87%; (d) $LiAlH_4$, THF, 0 °C to rt, 87%; (e) i) Li, NH_3 (liq.), THF, -60 °C, 73%, ii) 2,2-dimethoxypropane, CSA, 60%; (f) i) CCl_3CN, DBU, DCM; ii) $NaHCO_3$, I_2, H_2O, 46%; (g) i) 37% HCl (aq.), MeOH,

60 °C; ii) $NaHCO_3$, MeOH, 65%; (h) EEDQ, benzoic acid or acetic acid, DMF, 0 °C, **4**: 7% , **5**: 28%; (i) EEDQ, **24**, DMF, 0 °C 25%; (j) $CuSO_4$ (1.0 M in H_2O), sodium ascorbate (1.0 M in H_2O), DMF, **25** , **26** or **27**, **1**: 19% , **2**: 12%**, 3**: 13% .

3.2 Results and discussion

The synthesis of α-L-fucopyranose-configured cyclophellitol aziridine-based target compounds started with aldol condensation of aldehyde **15** and chiral acrylamide **14**, following the procedure reported[17] by Llebaria and co-workers for the enantiomer of **16** (Scheme 1). Reductive removal of the Evans template in **16** followed by ring-closing metathesis yielded, according to the Llebaria procedure,[17] partially protected L-galactopyranose-configured cyclohexene **17** in good yield. Tosylation of the primary alcohol in **17** was followed by hydride displacement of the tosylate to afford L-fucopyranose-configured cyclohexene **19**. The benzyl groups in **19** were reduced under Birch conditions, after which the *cis*-diol was protected as the isopropylidene acetal to give **20**. The secondary alcohol in **20** was transformed into the corresponding trichloroacetimidate, after which iodocyclisation yielded in a stereospecific fashion intermediate **21** analogous to the procedure reported for the synthesis of retaining β-glucosidase ABPs.[18] Acidic hydrolysis of both acetal and imino protections in **21** was followed by base-induced intramolecular nucleophilic substitution of the iodine to yield aziridine **22**. Acetylation or benzoylation of the aziridine in **22** under the agency of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ), following conditions developed previously,[18] yielded compounds **4** and **5**, respectively. EEDQ-induced acylation of **22** with 8-azidooctanoic acid[19] **24** provided azide **23**, which was transformed into target ABPs **1**, **2** and **3** by conjugation to BODIPY-alkynes **25**, **26** and biotin-alkyne **27**, respectively, via copper(I)-catalyzed Huisgen [2+3] cycloaddition. The final compounds were purified by reverse phase HPLC.

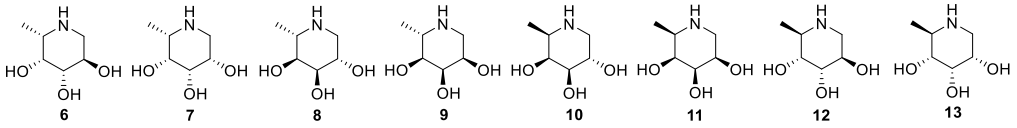
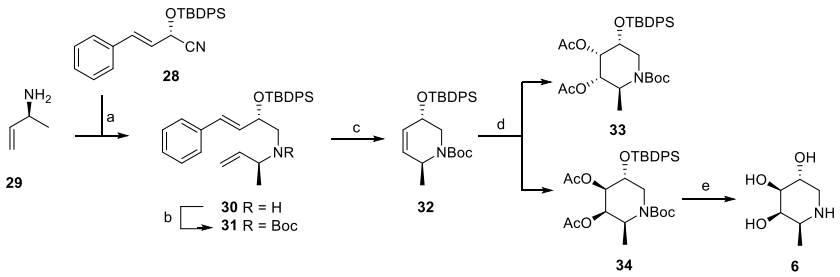


Figure 2. Structures of 1-deoxy-L-fuconojirimycin **6** and configurational isomers **7**-**13**.

The configurational fuconojirimycin isomers **6**-**13** (Figure 2) were synthesized following the strategy exemplified for 1-deoxy-L-fuconojirimycin **6** (Scheme 2). Key steps in the synthetic scheme include a DIBAL-H reduction-transimination-sodium borohydride reduction cascade of reactions involving enantiomerically pure cyanohydrin **28**, prepared employing (S)-hydroxynitrile lyase (*S*-*Hb*HNL) from *Hevea brasiliensis* rubber tree,[20] and the allylic amine **29** prepared by reported strategy,[21] to give secondary amine **30**. *N*-Boc protection (**30** to **31**), ring-closing metathesis (**31** to **32**) and Upjohn dihydroxylation afforded a mixture of *syn*-diols,

which were acetylated and separated by silica gel purification to yield diastereomers **33** and **34** in a 1:3 ratio.

Scheme 2. Synthesis of 1-deoxy-L-fuconojirimycin **6**.



Reagents and conditions: (a) i) Et_2O, DIBAL-H, -80 °C, ii) MeOH, -90 °C, iii) amine **29**, NaOMe, iv) $NaBH_4$,-15 °C to rt, 88%; (b) Boc_2O, 50 °C, 100%; (c) Grubbs 1st generation, DCM, 88%; (d) i) $K_2OSO_4{\cdot}2H_2O$, 4-methylmorpholine-N-oxide (NMO), acetone/H_2O (1:1, v/v), -10 °C, ii) Ac_2O, pyridine, DMAP, 0 °C, 88% (**33**:**34** = 1:3); (e) i) K_2CO_3, MeOH, ii) TBAF, THF, iii) HCl (6 M in H_2O), MeOH, 71%.

Global deprotection of **34** afforded 1-deoxy-L-fuconoijirimycin **6**, of which the analytical and spectroscopical data were in full agreement with those reported in the literature.[22] The seven configurational isomers **7**-**13** were prepared by alteration of the building blocks and/or the chemical transformations. It should be noted that four out of the eight isomers of fuconojirimycin were derived from **28**. To enable the synthesis of the four enantiomers, the enantiomer of cyanohydrin **28** was required, and this was prepared using almond (*R*)-hydroxynitrile lyase (*R*-*Pa*HNL).[23]

***In vitro* GH29 α-L-fucosidase activity assays**

Having the cyclophellitol aziridine inhibitors and probes in hand, their inhibitory potency towards the human lysosomal α-L-fucosidase, FUCA1, was determined. Inhibition potency was determined by measuring the residual enzyme activity using the fluorogenic substrate 4-methylumbelliferyl-α-L-fucopyranoside after pre-incubation of lysates of COS-7 cells over-expressing recombinant human FUCA1, with varying concentrations of the non-fluorescent, irreversible cyclophellitol aziridine inhibitors **4**, **5** and **23**; the ABPs **1** (JJB256), **2** (JJB244), **3** (JJB243), 1-deoxy-L-fuconojirimycin 6, and the seven fuconojirimycin isomers **7**-**13**. All *N*-acyl aziridines inhibited human FUCA1 activity with nanomolar IC_{50} activities (Table 1).

Table 1. *In vitro* and *in situ* inhibition of recombinant human GH29 α-L-fucosidase, given as half-maximal inhibitory concentration (IC_{50}). Data were averaged values of two separate experiments measured in duplicate, error ranges depict stand deviation.

Compounds	*In vitro* IC_{50} (nM)	*In situ* IC_{50} (nM)
1 (JJB256)	6.9 ± 0.8	28.9 ± 4.4
2 (JJB244)	8.7 ± 1.2	157.8 ± 22
3 (JJB243)	7.2 ± 1.1	25,512 ± 2,278
4	371.6 ± 21.2	515.4 ± 72.9
5	46.8 ± 3.4	77.9 ± 9.1
23	8.7 ± 1.1	65.4 ± 7.5
6	3,979 ± 257	ND
7 - 13	> 100,000	ND

ND: not determined. It should be noted that IC_{50} values on competitive inhibitors do not compare well to those obtained for mechanism-based inhibitors. The values given above allow for a comparison of inhibitory potency within the two classes of compounds studied.

Perusal of the inhibitory data does reveal some trends that allow some tentative conclusions. *N*-benzoyl aziridine **4**, bearing a bulky aromatic *N*-benzoate group, is about eight-fold less active compared to its *N*-acetylated counterpart, **5**. In contrast, comparing **5** with **23** reveals that a bulky aliphatic *N*-acyl substituent is in fact favored for enzyme inhibition (5-fold increase, Table 1). ABPs **1** and **2**, bearing a BODIPY fluorophore attached to the alkyl chain, inhibit FUCA1 in the same order as their azide precursor **23**. *In situ* inhibition of FUCA1 in living fibroblasts by ABP **1** and **2** occurs with similar efficacy as **23** and ABP **3**, the latter carrying a biotin attached to the alkyl chain. Exposure of cells to ABP **3** revealed in a dramatically decreased inhibitory potency, suggesting a reduced ability to penetrate into cells to reach the lysosomal FUCA1. The known competitive fucosidase inhibitor, 1-deoxy-L-fuconojirimycin **6** inhibits FUCA1 with an IC_{50} of 3.9 µM, in accordance with the literature values.[24] The seven configurational isomers **7**-**13** do not significantly inhibit FUCA1 activity up to 100 µM, a result that corroborates previous findings on some of the configurational analogues, which were reported as poor fucosidase inhibitors.[25]

As the next research objective, activity-based profiling of GH29 α-L-fucosidases from varying sources with green-fluorescent aziridine ABP **1** was examined, in the presence or absence of excess concentrations of, either the mechanism-based inhibitor **4** or the competitive inhibitor **6**. As is shown in Figure 3A, ABP **1** efficiently labels purified α-L-fucosidase from *Bacteroides thetaiotaomicron*. In lysates from an *E. coli* culture overexpressing recombinant α-L-fucosidase from *Bacteroides thetaiotaomicron* gene 2970, several fluorescent protein bands are visible upon labeling with **1** (Figure 3B), with the most prominent band at around 50 kDa,

corresponding to the predicted molecular weight of the enzyme. Labeling of the major band at 50 kD could moreover be blocked following pre-incubation with either 100 µM **4** or with 5 mM **6** (Figure 4B). Red fluorescent ABP **2** labels α-(1-2, 3, 4) and α-(1-6)-fucosidases from various bacterial sources in a similar fashion (Figure 3C).

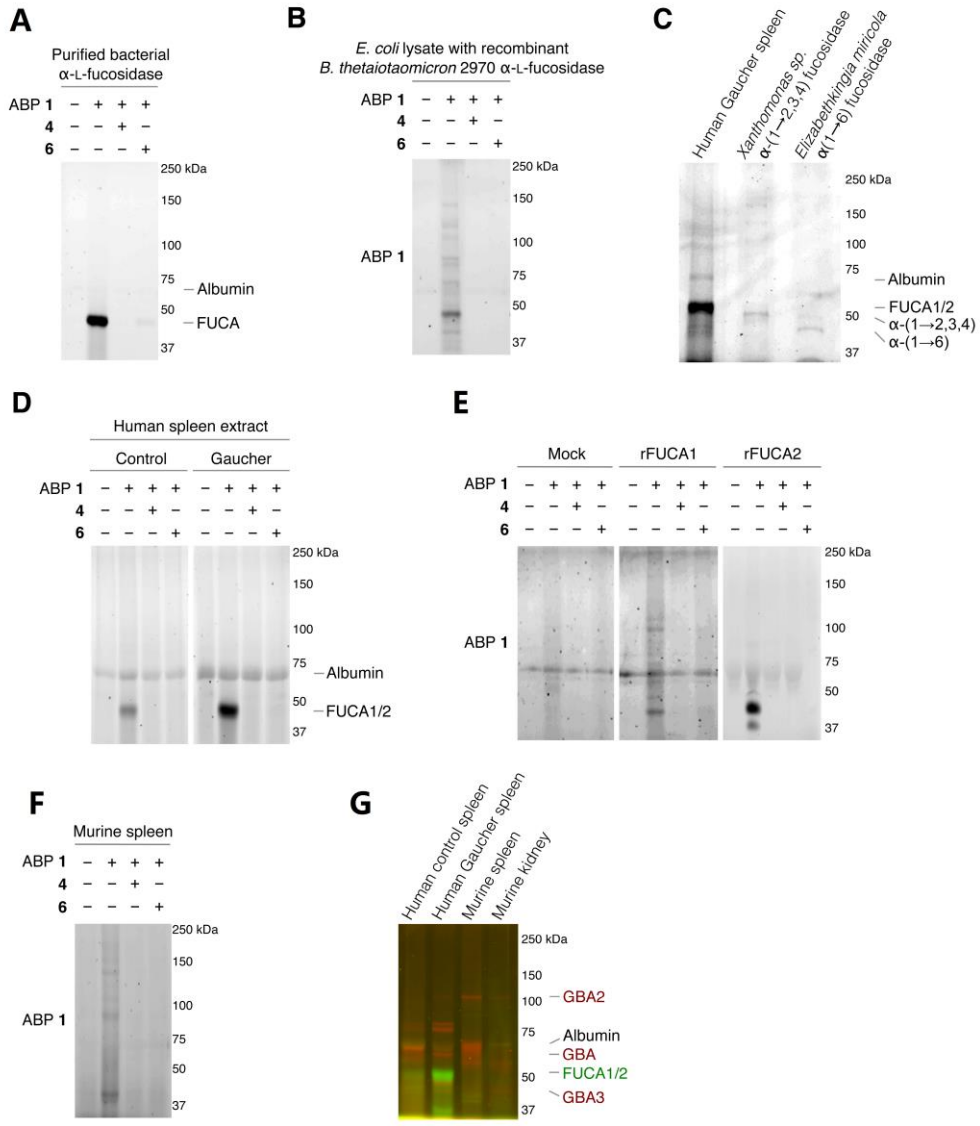


Figure 3. *In vitro* activity-based protein profiling of GH29 α-L-fucosidases. A), B) Labeling with ABP **1** of recombinant α-L-fucosidase and lysate of *E. coli* expressing recombinant α-L-fucosidase from *Bacteroides thetaiotaomicron* 2970. C) *In vitro* labeling of lysate of spleen from a Gaucher disease patient, α-(1-2,3,4)-fucosidase from *Xanthomonas sp.* and α-(1-6)-fucosidase from *Elizabethkingia miricola* with ABP **2**. D) *In vitro* labeling of human healthy and Gaucher disease spleen. E) ABP **1** labeling of α-L-fucosidases present in lysate of COS-7 cells transfected with empty plasmid (Mock) or

plasmid encoding human FUCA1 or FUCA2. F) Labeling of tissue homogenate of wild-type murine spleen with ABP **1**. G) Direct labeling of GH29 α-L-fucosidases with green-fluorescent ABP **1** and retaining β-glucosidases GBA, GBA2 and GBA3 with red-fluorescent JJB75.[18] The location of albumin autofluorescence is designated on each gel.

Subsequently, lysates of spleens from a healthy human individual and a patient suffering from Gaucher disease were exposed to ABP **1**. As can be seen in Figure 3D, a single fluorescent protein migrating slightly below 50 kDa was fluorescently labeled. The labeled band from human Gaucher spleen lysate is considerably more intense than the corresponding band in healthy human spleen, reflecting elevated α-L-fucosidase activity in the former. This result corroborates earlier observations that mRNA encoding lysosomal glycosidases are upregulated in Gaucher tissue.[26] As before, labeling with ABP **1** was suppressed after pre-treatment with either *N*-benzoyl aziridine **4** or L-fuconojirimycin **6**. To further ascertain that ABP **2** labels mammalian GH29 retaining α-L-fucosidases a number of control experiments were performed (Figure 3E). Compound **1** labeled both FUCA1 and FUCA2 that were overexpressed in COS cells. Treatment of murine spleen lysates with **1** yielded a result essentially as observed for healthy human tissue (Figure 3F).

Finally, simultaneous labeling of retaining β-glucosidases and retaining α-L-fucosidase was evaluated. To this end tissue lysates were incubated with **1** and the previously reported broad-spectrum activity-based retaining β-glucosidase probe, JJB75.[18] As can be seen (Figure 3G) the applied ABPs label a distinct set of proteins. Since they bear complementary fluorophores they can be used jointly to profile both retaining glycosidase families in a single experiment.

The FUCA1 enzymatic activity is maximal at around pH 5.0 (Figure 4A, B), consistent with the acidic pH of its natural lysosomal environment. Labeling efficiency with **1** largely reflects the pH dependence of FUCA1 at pH below 7. Of note, the pH dependence of labeling of FUCA1 does not follow that of its enzymatic activity. At alkaline conditions, where enzymatic activity is low, labeling still proceeds. Similar observations were made in the past for ABPP of retaining *exo*-β-glucosidases using cyclophellitol β-aziridine ABPs.[14] This result reveals the high reactivity of the aziridine probes in the initial displacement step employed by retaining glycosidases, a feature which, in conjunction with their selectivity in binding and relative stability in physiological environment, explains their efficacy as activity-based glycosidase probes.[27] Assessment of fluorescent labeling kinetics by employing 10 nM **1** labeling at stoichiometric concentrations of rhFUCA1 at 4 °C and 37 °C revealed that, during time-course experiments, examined on SDS-PAGE gels, labeling is near complete within the first minute at 4 °C (Figure 4C), impairing accurate determination of kinetic constants.

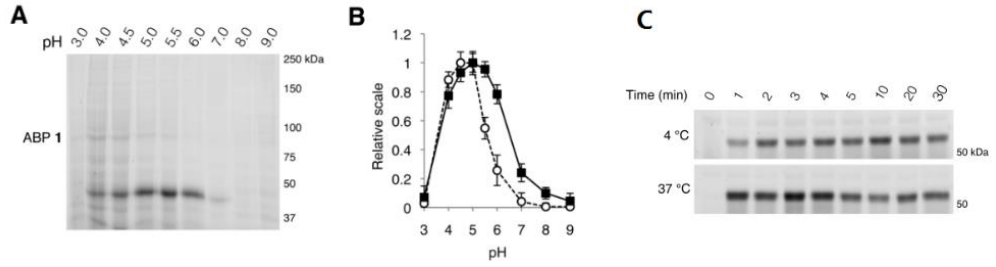


Figure 4. *In vitro* pH profile and labeling kinetics of recombinant FUCA1. A) *In vitro* labeling of COS-7 cell lysate containing over-expressed human FUCA1 at various pH with ABP **1**. B) Relative *in vitro* labeling of FUCA1 with ABP **1** (closed squares) compared to relative enzymatic activity towards artificial 4-methylumbelliferyl-α-L-fucopyranoside substrate (open circles) at various pH values. Data average of *n*=3 experiments, ± standard deviation. C) Stoichiometric *in situ* labeling of COS-7 lysate containing over-expressed human FUCA1 with 10 nM ABP **1** at 4 °C and 37 °C.

In vivo GH29 α-L-fucosidase assays

The ability of ABP **1** to label α-L-fucosidase in living mice was investigated next. Four wild-type C57Bl/6J male mice were injected with 100 µL phosphate-buffered saline (PBS) or PBS containing 10, 100 or 1000 pmol ABP **1**. After two hours, the mice were anesthetized, perfused with PBS and then brain, spleen, liver and kidney tissues were isolated. Tissue homogenates were prepared and each lysate was labeled prior to gel electrophoresis with red-fluorescent cyclophellitol β-aziridine JJB75, which labels *exo*-β-glucosidases as loading control (Figure 5). Furthermore, tissue homogenates of vehicle-treated animals were labeled with excess ABP **1** to visualize the maximal α-L-fucosidase labeling achievable in each tissue.

After treatment of mice with ABP **1**, a dose-dependent labeling of retaining α-L-fucosidases is observed in spleen, liver and kidney (Figure 5). Injection of 1000 pmol ABP **1** results in substantial labeling of α-fucosidase in spleen, liver and kidney. Detected fluorescence levels are comparable to that in matching samples from vehicle-treated mice incubated *in vitro* with excess **1**. In contrast, no *in vivo* brain FUCA1 labeling was observed after any of the administered doses of ABP **1**, and it can be concluded that ABP **1**, just as its β-glucose and α-galactose congeners,[14, 15] does not penetrate the brain.

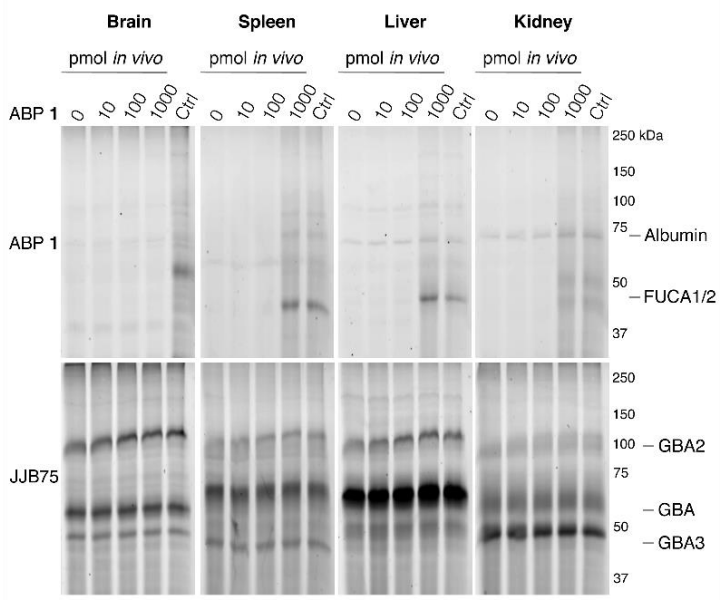


Figure 5. *In vivo* labeling of α-L-fucosidases in mice with various concentrations of ABP **1** during 2 hours. Top: *in vivo* labeling compared to maximal *in situ* labeling with excess ABP **1** of matched homogenates of untreated animals (Ctrl). Bottom: *in vitro* labeling of retaining β-glucosidases GBA, GBA2 and GBA3 with JJB75 in all homogenates as loading control.

Competitive activity-based GH29 α-L-fucosidase profiling

Having established the efficacy of ABP **2** to selectively label GH29 retaining α-L-fucosidases from various sources in an activity-based manner, the inhibition potential of deoxyfuconojirimycin **6** and its **7** stereoisomers **7**-**13** was determined in a competitive ABPP format (Figure 6). In contrast to **6**, none of the seven configurational isomers **7**-**13** were capable of blocking ABP **1** labeling of α-L-fucosidase, a result that matches with the data on inhibition of recombinant FUCA1 in the fluorogenic activity assay (Table 1).

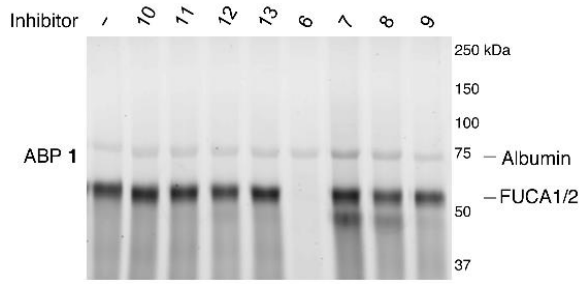


Figure 6. Competitive ABPP on recombinant FUCA1 with deoxyfuconojirimycin **6** and configurational analogues **7**-**13** towards α-L-fucosidases, with ABP **1** labeling as readout.

Identification of biotin labeled GH29 α-L-fucosidases

To further determine the specificity of the developed ABPs, ABP labeling of proteins was analyzed in complex tissue homogenates. For this purpose, Gaucher spleen lysate was incubated with biotinylated ABP **3**; with DMSO (control) or competitive ABP **3**-labeling by first incubating with fluorescent ABP **1**. Glycosylated proteins were then enriched via concanavilin A (ConA), followed by affinity purification with Streptavidin-coated paramagnetic beads. The identity of biotinylated and ABP **3**-labeled proteins was determined by on-bead digestion with trypsin, peptide analysis by LC-MS/MS and matching against the human UniProt database, using the Mascot search engine as previously reported.[14] FUCA1 was identified after ABP **3** pull-down as one of the top identified proteins (SI Table S1A) in the supplementary data), but was not found in the competitive (SI Table S1B) or untreated control (SI Table S1C). Proteins with higher scores were background proteins such as abundant endogenously biotinylated propionyl-CoA carboxylase alpha chain (PCCA), pyruvate carboxylase (PC), and keratin contaminations. FUCA1 was selectively found in the ABP **3**-pull down experiment only. Table 2 shows the analysis parameters of the identified peptides from FUCA1, with accuracy below 5 ppm, and Mascot ion scores above 40 (indicating reliable MS/MS fragment annotation and match) and manually curated fragmentation patterns. These results show FUCA1 can be undisputedly affinity purified and identified via biotinylated ABP **3**. Moreover the binding of ABP **3** can be completely blocked by pre-incubation with ABP **1**, which may indicate that both ABPs bind at the same site of the enzyme.

Table 2. Analysis parameters of peptides derived from the P04066 Tissue alpha-L-fucosidase, FUCA1 protein after affinity purification, on bead digest and LC-MS/MS analysis.

start-end	*m/z* obs.	z	ppm	Ion score	Sequence
114-130	982.4723	2	2	75	FFHPEEWADLFQAAGAK
163-173	572.3213	2	-1	58	DLVGELGTALR
392-420	1049.211	3	0	84	GSAVYAIFLHWPENGVLNLESPITTSTTK
439-461	828.1304	3	1	49	GLFISLPQLPPSAVPAEFAWTIK

Start-end gives the position of the identified peptide in the protein sequence, *m/z* observed is the measured *m/z* of the peptide, z is the charge, ppm is the measurement accuracy between the calculated and the observed *m/z*, ion score is the Mascot search engine score calculated for the match of the MS/MS fragmentation to the human protein database, sequence is the identified peptide sequence.

3-D crystal structure analysis of bacterial FUCA1 complexed to 4 and 5

In order to obtain experimental evidence for the formation of a covalent adduct between α-L-fucosidase and ABPs **1**, **2** and **3**, a crystal structure of *Bt*Fuc2970 (often used as a surrogate for the mammalian enzyme) with mechanism-based inhibitor **4** was obtained (PDB code: 4WSK). While the resulting crystal structure clearly demonstrates the formation of an

enzyme-**4** complex, electron density around the aryl group of the inhibitor "aglycon" moiety was close to the side-chain of the catalytic acid/base of the enzyme, residue E288 (Figure 7A), resulting in disorder. This likely reflects steric clashing and considerable conformational flexibility in the **4** aglycon when bound to *Bt*Fuc2970. “Aglycon” here reflects the aziridine *N*-acyl moiety to which the BODIPY tags are grafted in ABPs **1** and **2** and which is designed to occupy the space normally occupied by the substrate fucoside.

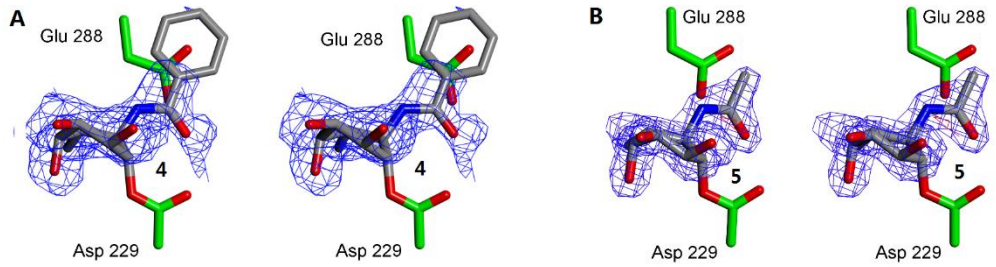


Figure 7. Crystal structures of α-L-fucosidase from *Bacteroides thetaiotaomicron* in complex with A) **4** and B) **5**. Catalytic residues are annotated: Asp 229 (nucleophile) and Glu 288 (acid/base). Electron density displayed is F_o-F_c density from phases calculated prior to the inclusion of **4** and **5** in refinement, contoured at 2σ and 3σ, respectively. Figure was prepared using CCP4MG[29]. Note that there is no electron density, at this level, for the aryl group of **4** presumably reflecting considerable disorder and/or steric clashes.

In the here-presented design of aziridine-based retaining *exo*-glycosidase ABPs it was assumed that such aglycon-like moieties would not interfere with enzyme binding (this also based on the numerous fluorogenic substrates that are in use to study *exo*-glycosidases and in which the aglycon moiety can take on made shapes and sizes). The 3-D fold of *Bt*Fuc2970, however, appears not to provide sufficient space to accommodate an extended aryl aglycon pendant to the atom, which would equate to the ring oxygen in fucose. In order to minimize steric clashes, *Bt*Fuc2970 was subsequently incubated with **5**. The crystal structure of the resulting complex (PDB code: 4WSJ) revealed unambiguous electron density for the presence of a covalent enzyme-**5** complex (Figure 7B). The complex has C-O bond lengths between the *Bt*Fuc2970 catalytic nucleophile and **5** of *ca*. 1.43 Å as would be expected for a C-O ester bond. As expected from the reduction in aglycon size, **5** is better ordered than **4** when bound to the bacterial enzyme and provides a clearer definition of the resulting conformation and interactions. Upon trans-diaxial opening of the acylaziridine the covalently bound and substituted cyclohexane adopts a slightly distorted $^3H_4$ conformation (between $^3H_4$ and $^3S_1$; consistent with the expected catalytic itinerary.[28]

3.3 Conclusions

This chapter reports on the development of potent and selective aziridine-based ABPs **1**, **2** and **3** for selective profiling of active GH29 α-L-fucosidases in cell extracts from bacteria, mice and man as well as *in vivo* in mice. Labeling of GH29 retaining α-L-fucosidases with the L-fucopyranose-configured, cyclophellitol aziridine-based ABPs proceeds with good potency and high selectively both *in vitro* and *in vivo* with the single caveat that the probes do not penetrate brain tissue in mice. The covalent irreversible aziridine inhibitors proved much more potent than their iminosugar counterparts, of which L-fuconojirimycin **6** appeared to be the single compound from this set of configurational isomers that is able to inhibit FUCA1 in a competitive ABPP setting. The crystal structures of α-L-fucosidase from *Bacteroides thetaiotaomicron* in complex with compound **4** and **5** provide strong evidence for the covalent binding of cyclophellitol aziridine to active α-L-fucosidases and by this virtue the validity of the cyclophellitol aziridine design for activity-based profiling of retaining glycosidases that employ the Koshland double displacement mechanism. Whereas aziridines and epoxides that are annulated to cyclohexane rings preferably open in a trans-diaxial fashion through a chair-like transition state, reaction with the α-L-fucosidase nucleophile takes place at the aziridine carbon corresponding to the anomeric center of a substrate α-L-fucoside. This corresponds to ring opening to yield a skew boat, as is observed in the trapped enzyme active site in the co-crystal.

In conclusion, ABPs **1**, **2** and **3** are useful reagents for the discovery and annotation of new members of the GH29 family of α-L-fucosidase in comparative ABPP experiments, for monitoring retaining α-L-fucosidase activities in health and disease, and for the discovery of inhibitors able to interfere with specific α-L-fucosidases in competitive ABPP experiments. ABPs **1**, **2** and **3** add to the growing series of *in situ* and *in vivo* active retaining glycosidase ABPs and moreover their design hold promise for the design of ABPs targeting retaining glycosidases recognizing and processing differently configured and substituted carbohydrates.

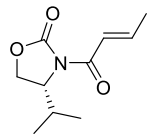
3.4 Experimental section

Synthesis:

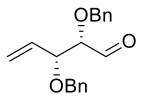
General synthetic methods. All reagents were of a commercial grade and were used as received unless stated otherwise. Dichloromethane (DCM), tetrahydrofuran (THF) and *N,N*-dimethylformamide (DMF) were stored over 4 Å molecular sieves, which were dried *in vacuo* before use. All reactions were performed under an argon atmosphere unless stated otherwise. Solvents used for flash column chromatography were of pro analysis quality. Reactions were monitored by TLC analysis using Merck aluminium sheets pre-coated with silica gel 60 with detection by UV absorbtion (254 nm) and by spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot H_2O$ (10 g/L) in 10% sulfuric acid followed by charring at ~150 °C or by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C. Column chromatography was performed using either Baker - or Screening Device silica gel 60 (0.04-0.063 mm) in the indicated solvents. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DMX-600 (600/150 MHz) and

a Bruker AV-400 (400/100 MHz) spectrometer in the given solvent. Chemical shifts are given in ppm relative to the chloroform residual solvent peak or tetramethylsilane (TMS) as internal standard. Coupling constants are given in Hz. All given ^{13}C-NMR spectra are proton decoupled. High-resolution mass spectra were recorded with a LTQ Orbitrap (Thermo Finnigan). Optical rotations were measured on Propol automatic polarimeter (Sodium D-line, λ = 589 nm). IR spectra were recorded on a Shimadzu FT-IR 83000 spectrometer. LC-MS analysis was performed on a Jasco HPLC-system (detection simultaneously at 214 nm and 254 nm) equipped with buffers A: H_2O, B: acetonitrile (MeCN) and C: 10% 0.5 M NH_4OAc, and coupled to a Perkin Elmer Sciex API 165 mass instrument. For reverse phase HPLC purifications an Agilent Technologies 1200 series instrument equipped with a semi preparative Gemini C18 column (10 x 250 mm) was used. The applied buffers were A: H_2O, and B: MeCN.

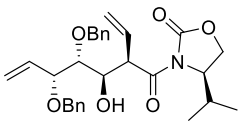
Synthesis of compounds 1-5:



(*R,E*)-3-(But-2-enoyl)-4-isopropyloxazolidin-2-one (14): Compound **14** was prepared from Boc-D-Valine via the strategy reported by Evans[30] *et al.* for its enantiomer, giving compound **14** (3.6 g, 18 mmol, 38% over three steps) as a yellow oil. TLC: R_f 0.59 (pentane/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$ -104 (*c* = 1, $CHCl_3$); lit.[1] for enantiomer: $[\alpha]_D^{20}$ -105 (*c* = 1.97, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.28 (d, *J* = 15.8 Hz, 1H), 7.20-7.11 (m, 1H), 4.51-4.47 (m, 1H), 4.34 – 4.18 (m, 2H), 2.48 – 2.27 (m, 1H), 1.96 (d, *J* = 6.7 Hz, 4H), 0.93 (d, *J* = 7.2 Hz, 3H); 0.89 (d, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 165.01, 154.13, 146.71, 121.92, 63.39, 58.55, 28.51, 18.56, 18.06, 14.72; IR (neat, cm^{-1}): 2965, 1773, 1684, 1638, 1389, 1364, 1233, 1202, 1119, 1061, 1036, 970, 926, 754, 714; HRMS: calculated for $C_{10}H_{15}NO_3$ [M+H$^+$] 198.11247, found: 198.11224;

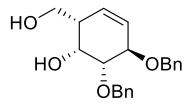


(2*R*,3*S*)-2,3-Bis(benzyloxy)pent-4-enal (14): Building block **15** was prepared from L-(-)-xylose by the reported strategy[31] of Hansen *et. al.* for its enantiomer. Compound **15** was obtained as a clear oil (6.1 g, 21 mmol, overall yield 47%). TLC: R_f 0.45 (pentane/EtOAc, 5/1, v/v); $[\alpha]_D^{20}$ -88 (*c* = 1, $CHCl_3$); lit.[31] for enantiomer: $[\alpha]_D^{20}$ +68.7 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 9.66 (d, *J* = 7.6 Hz, 1H), 7.41 – 7.20 (m, 10H), 5.97 – 5.87 (m, 1H), 5.44 – 5.22 (m, 2H), 4.73 (d, *J* = 12.1 Hz, 1H), 4.60 (t, *J* = 12.0 Hz, 2H), 4.33 (d, *J* = 12.1 Hz, 1H), 4.17 – 4.13 (dd, *J* = 7.7, 4.1 Hz, 1H), 3.81 (d, *J* = 4.1 Hz, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 202.38, 137.53, 137.06, 133.79, 128.46, 128.35, 128.12, 128.08, 127.90, 127.75, 119.84, 85.11, 79.86, 73.39, 70.61; IR (neat, cm^{-1}): 2862, 1732, 1454, 1207, 1069, 1028, 934, 737, 696; HRMS: calculated for $C_{19}H_{20}O_3$ [M+H$^+$] 297.14852, found 297.14922.

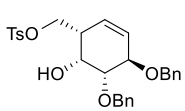


(*R*)-3-((2*R*,3*R*,4*R*,5*R*)-4,5-Bis(benzyloxy)-3-hydroxy-2-vinylhept-6-enoyl)-4-isopropyloxazolidin-2-one (16): The oxazolidinone **14** (2.5 g, 13 mmol, 1.2 eq.) was dissolved in anhydrous DCM (20 mL). After addition of a solution of 1.0 M dibutylboryl trifluoromethanesulfonate (Bu_2BOTf) in anhydrous DCM (13 mL, 13 mmol, 1.2 eq.) at -78 °C, the resulting dark green mixture was removed from the cold bath to dissolve any frozen triflate and cooled again to -78 °C. Triethylamine (2.1 mL, 15 mmol, 1.3 eq.) was added subsequently, causing the dark green color to fade. The solution was stirred for 50 minutes at -78 °C and then at 0 °C for 15 minutes (the solution turned yellow). While the reaction mixture was being cooled back down to -78 °C, a solution of aldehyde **15** (3.4 g, 11 mmol, 1.0 eq.) in anhydrous DCM (20 mL) was added to the reaction mixture via a syringe. The temperature was slowly raised to

-20 °C for one hour and then maintained at this temperature for an additional hour. The resulting yellow solution was then stirred at -15 °C for another hour and then warmed to -5 °C and quenched with a phosphate buffer (pH 7) solution (25 mL). A 30% H_2O_2 solution was then added dropwise while maintaining the internal temperature below 5 °C. Addition of the peroxide was continued until the internal temperature remained constant. The mixture was stirred for an additional 45 minutes while slowly warming to room temperature. The reaction was then poured into aq. sat. $NaHCO_3$ (100 mL) and the aq. layer extracted with DCM (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (2%→20% EtOAc in pentane) giving product **16** as colorless oil (4.4 g, 8.9mmol, 71%). TLC: R_f 0.47 (pentane/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ +24 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.37 – 7.20 (m, 10H), 6.08-6.00 (m, 1H), 5.96 – 5.82 (m, 1H), 5.42 (m, 2H), 5.37 (m, 1H), 5.28 (d, *J* = 9.9 Hz, 1H), 5.02 – 4.97 (m, 1H), 4.68 (d, *J* = 11.5 Hz, 2H), 4.55 – 4.33 (m, 3H), 4.30 – 4.26 (m, 1H), 4.07 – 3.95 (m, 1H), 3.83 (dd, *J* = 8.9, 3.1 Hz, 1H), 3.57 (dd, *J* = 8.3, 3.9 Hz, 1H), 3.35 (d, *J* = 2.1 Hz, 1H), 3.24 (t, *J* = 8.8 Hz, 1H), 2.21 (m, 1H), 0.77 (d, *J* = 7.0 Hz, 3H), 0.72 (d, *J* = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 172.58, 153.62, 138.06, 137.76, 134.48, 133.61, 128.53, 128.34, 128.00, 127.91, 127.66, 127.42, 120.58, 119.37, 81.75, 79.88, 73.04, 71.23, 70.73, 62.45, 58.04, 50.21, 28.02, 17.88, 14.49; IR (neat, cm^{-1}): 3503, 2963, 1776, 1697, 1385, 1371, 1300, 1202, 1099, 1061, 928, 739, 698; HRMS: calculated for $C_{29}H_{35}NO_6$ [M+H$^+$] 494.25371, found: 494.25344.

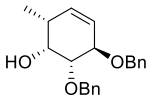


(1*R*,2*S*,5*R*,6*R*)-5,6-Bis(benzyloxy)-2-(hydroxymethyl)cyclohex-3-en-1-ol (17): The product **16** (4.41 g, 8.90 mmol, 1.0 eq.) was dissolved in a mixture of THF (65 mL) and H_2O (3.3 mL). Next, $LiBH_4$ (2 M solution in THF, 26 mL, 52 mmol) was added at 0 °C. After stirring at 0 °C for one hour, the reaction mixture was warmed to room temperature and stirring was continued for one hour. The reaction was quenched with aq. 2 M NaOH (50 mL) and diluted with Et_2O (50 mL). After stirring for five minutes the reaction mixture was extracted with Et_2O (100 mL), and the separated organic phase was washed with aq. sat. $NaHCO_3$ (20 mL) and brine (100 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude alcohol that was purified by silica gel column chromatography (10-50%, EtOAc in pentane) giving the intermediate primary alcohol as a white solid (2.72 g, 7.38 mmol, 0.83 eq.) that was dissolved in DCM (260 mL). After addition of the second generation Grubbs catalyst (313 mg, 0.37 mmol, 0.040 eq.), the mixture was stirred at 40 °C in the dark for 24 h. DMSO (0.50 mL) was next added, and the solution was stirred at room temperature for another 3 h. The solvent was evaporated under reduced pressure to give a crude mixture, which was purified by silica gel column chromatography (20-50% EtOAc in pentane) giving product **17** as a black solid (2.4 g, 6.9 mmol, 78%). TLC: R_f 0.41 (pentane/EtOAc, 3/2, v/v); $[\alpha]_D^{20}$ -147 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.41 – 7.25 (m, 10H), 5.89 – 5.84 (m, 1H), 5.60 – 5.56 (m, 1H), 4.80 – 4.66 (m, 4H), 4.38 (br, 1H), 4.35 – 4.27 (m, 1H), 3.92 – 3.76 (m, 2H), 3.68 (dd, *J* = 7.8, 2.2 Hz, 1H), 2.65 (m, 1H), 2,60 (s, 1H), 2.50 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 138.64, 138.14, 128.66, 128.53, 128.07, 128.01, 127.94, 127.78, 127.64, 126.97, 81.88, 76.69, 72.40, 72.36, 70.47, 63.88, 41.97; IR (neat, cm^{-1}) 3422, 2872, 1454, 1206, 1090, 1051, 1026, 860, 801, 733, 696; HRMS: calculated for $C_{21}H_{24}O_4$ [M+H$^+$] 341.17474, found: 341.17486.

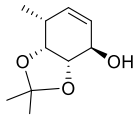


((1*S*,4*R*,5*R*,6*R*)-4,5-Bis(benzyloxy)-6-hydroxycyclohex-2-en-1-yl)methyl 4-methylbenzenesulfonate (18): A solution of **17** (1.73 g, 5.09 mmol, 1.0 eq.) in anhydrous DCM (40 mL), containing Et_3N (1.8 mL, 13 mmol, 2.5 eq.) was cooled to 0 °C and treated with

p-TsCl (2.2 g, 11 mmol, 2.2 eq.). The reaction mixture was stirred at room temperature for 3 h, followed by extra addition of Et_3N (0.80 mL, 5.7 mmol, 1.1 eq.) and *p*-TsCl (1.0 g, 5.0 mmol, 1.0 eq.). After TLC confirmed full conversion of the starting material, the reaction was diluted with water and extracted with DCM. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (5%→35% EtOAc in pentane) giving product **18** as a pale yellow solid (2.18 g, 4.42 mmol, 87%). TLC: R_f 0.30 (pentane/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ -133 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.77 (d, *J* = 8.0 Hz, 2H), 7.39 - 7.23 (m, 12H), 5.80 – 5.73 (m, 1H), 5.34 (d, *J* = 9.5 Hz, 1H), 4.69 - 4.62 (m, 4H), 4.24 (m, 1H), 4.16 (m, 2H), 4.04 – 3.98 (m, 1H), 3.57 (dd, *J* = 7.6, 2.0 Hz, 1H), 2.73 – 2.63 (m, 1H), 2.51 (br, 1H), 2.39 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 144.89, 138.43, 137.93, 132.65, 129.88, 128.47, 128.45, 128.36, 127.92, 127.86, 127.80, 127.62, 123.64, 81.72, 76.57, 72.31, 72.17, 69.84, 66.83, 40.25, 21.64; IR (neat, cm^{-1}): 3032, 2872, 1597, 1497, 1454, 1358, 1175, 1096, 964, 787, 698, 664; HRMS: calculated for $C_{28}H_{30}O_6S$ [M+H$^+$] 495.18359, found: 495.18300;

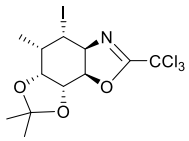


(1*R*,2*R*,5*R*,6*R*)-5,6-Bis(benzyloxy)-2-methylcyclohex-3-en-1-ol (19): Compound **18** (2.2 g, 4.4 mmol, 1.0 eq.) was dissolved in dry THF (18 mL) at 0 °C. A solution of $LiAlH_4$ (2 M in THF) (3.3 mL, 6.6 mmol, 1.5 eq.) was added dropwise. The reaction mixture was stirred at room temperature for 3h, diluted with Et_2O and quenched with dropwise addition of sat. aq. NaCl. The solid material was removed by filtration and the residue washed thoroughly 3 times with hot EtOAc. The filtrate was dried over $MgSO_4$, filtered again and the solvents removed under reduced pressure. The crude product was purified by silica gel column chromatography (10-20% EtOAc in pentane) giving product **19** as yellow oil (1.2 g, 3.8 mmol, 87%). TLC: R_f 0.53 (pentane/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$ -121 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.41 – 7.20 (m, 10H), 5.73 – 5.68 (m, 1H), 5.47 – 5.42 (m, 1H), 4.76 – 4.62 (m, 4H), 4.33 – 4.28 (m, 1H), 4.06 (br, 1H), 3.67 (dd, *J* = 7.7, 2.2 Hz, 1H), 2.45-2.39 (m, 1H), 2.25 (br, 1H), 1.12 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 138.77, 138.37, 131.27, 128.54, 128.44, 127.91, 127.90, 127.86, 127.64, 125.26, 82.73, 76.99, 72.28, 72.09, 70.90, 35.00, 16.36; IR (neat, cm^{-1}) : 3456, 2872, 1497, 1454, 1207, 1090,1057, 980, 785, 735, 696; HRMS: calculated for $C_{21}H_{24}O_3$ [M+H$^+$] 325.17982, found: 325.17995.

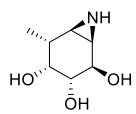


(3*S*,4*R*,7*R*,7*R*)-2,2,7-trimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (20): Ammonia (50 mL) was condensed at -60 °C. Lithium (525 mg, 76 mmol, 10 eq.) was added and the mixture was stirred until the lithium was completely dissolved. To this solution was added a solution of cyclohexene **19** (2.5 g, 7.6 mmol, 1.0 eq.) in dry THF (60 mL). The reaction mixture was stirred for 30 minutes at -60 °C and subsequently quenched with water (10 mL). The resulting solution was allowed to come to room temperature and stirred until all ammonia had evolved. Then the solution was concentrated under reduced pressure, re-dissolved in water and neutralized with Amberlite H$^+$. The resin was removed by filtration, and the filtrate was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (5%→20% MeOH in DCM) giving a white crystaline product (1.0 g, 7.0 mmol, 0.92 eq.) which was dissolved in 2,2-dimethoxypropane (70 mL) and cooled to 0 °C. A catalytic amount of D-(+)-10-camphorsulfonic acid (CSA) (162 mg, 0.70 mmol, 0.10 eq.) was added and the mixture was stirred at 0 °C for 2 h. TLC analysis showed complete conversion and the mixture was diluted by MeOH/H_2O (50 mL, 9/1, v/v) and stirred at room temperature for 30 minutes. The reaction mixture was neutralized with Et_3N, concentrated under reduced pressure, extracted with DCM, washed with

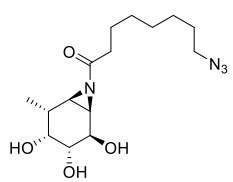
brine, dried over $MgSO_4$, filtered and concentrated. After purification by silica gel column chromatography (0%→8% MeOH in DCM) product **20** was obtained as a pale yellow oil (870 mg, 4.73 mmol, 63%). TLC: R_f 0.40 (DCM/MeOH, 9/1, v/v); $[\alpha]_D^{20}$ -126 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 5.93-5.89 (m, 1H), 5.76 (dd, *J* = 10.0, 3.6 Hz, 1H), 4.42 - 4.39 (m, 1H), 4.28-4.24 (m, 2H), 2.69-2.66 (m, 1H), 1.79 (br, 1H), 1.40 (s, 3H), 1.35 (s, 3H), 1.15 (d, *J* = 7.4 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 135.50, 128.09, 108.20, 80.17, 76.46, 67.80, 31.11, 26.85, 24.91, 16.12; IR(neat, cm^{-1}): 3345, 2913, 2699, 1161, 1084, 1053, 986, 858, 783; HRMS: calculated for $C_{10}H_{16}O_3$ [M+H$^+$] 185.11722, found: 185.11714.



(3*R*,4*S*,5*S*,5*S*,8*R*,8*R*)-5-iodo-2,2,4-trimethyl-7-(trichloromethyl)-3,4,5,5a,8a,8b-hexahydro-[1, 3]dioxolo[4',5':3,4]benzo[1,2-d]oxazole (21): Compound **20** (870 mg, 4.7 mmol, 1.0 eq.) was dissolved in anhydrous DCM (70 mL). The solution was cooled to 0 °C and treated with trichloroacetonitrile (946 µL, 9.5 mmol, 2.0 eq.) and 1,8-diazobicyclo[5.4.0]undec-7-ene (68 µL, 0.47 mmol, 0.10 eq.). After 2h stirring at 0 °C, TLC analysis revealed complete conversion to a higher running product. To the resulting solution was added water (18 mL), $NaHCO_3$ (3.9 g, 47 mmol, 10 eq.) and iodine (4.3 g, 17 mmol, 3.5 eq.). The reaction mixture was stirred overnight at room temperature before being quenched with aq. 10% $Na_2S_2O_3$ solution and extracted three times with EtOAc. The organic layer was dried over $MgSO_4$, filtered, concentrated under reduced pressure and the residue purified by silica gel column chromatography (0%→8% EtOAc in pentane) giving product **21** as brown oil (980 mg, 2.16 mmol, 46%). TLC: R_f 0.49 (pentane/EtOAc, 9/1, v/v); $[\alpha]_D^{20}$ +34 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 5.12 (dd, *J* = 10.2, 4.1 Hz, 1H), 4.89 (dd, *J* = 10.2, 7.0 Hz, 1H), 4.47 – 4.43 (m, 1H), 4.35 (dd, *J* = 8.1, 4.0 Hz, 1H), 4.12 (dd, *J* = 7.0, 3.0 Hz, 1H), 2.11-2.07 (m, 1H), 1.58 (s, 3H), 1.32 (s, 3H), 1.17 (d, *J* = 7.0 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 162.43, 109.70, 84.10, 74.88, 73.75, 73.30, 35.47, 26.11, 25.09, 23.93, 14.98; IR (neat, cm^{-1}): 2982, 1661, 1381, 1207, 1067, 1045, 988, 953, 837, 791, 665, 652; HRMS: calculated for $C_{12}H_{15}Cl_3INO_3$ [M+H$^+$] 453.92350, found: 453.92347.

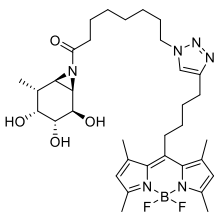


(1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-5-methyl-7-azabicyclo[4.1.0]heptane-2, 3, 4-triol (22): Compound **21** (980 mg, 2.2 mmol, 1.0 eq.) was dissolved in MeOH (32 mL). The solution was treated with concentrated HCl (8.0 mL) at 60 °C overnight. LC-MS analysis showed complete conversion. The solution was concentrated under reduced pressure and re-dissolved in MeOH (30 mL), $NaHCO_3$ (3.9 g, 47 mmol, 22 eq.) was added. After stirring at room temperature for 4 days, the reaction mixture was filtered and concentrated under reduced pressure. After purification by silica gel column chromatography (5%→20% MeOH in DCM) product **22** was obtained as a colorless oil (225 mg, 1.4 mmol, 65%). TLC: R_f 0.26 (DCM/MeOH, 5/1, v/v); $[\alpha]_D^{20}$ -97 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): *δ* ppm 4.07 (dd, J = 8.8, 4.2 Hz, 1H), 3.57-3.56 (m, 1H), 3.36 – 3.32 (m, 2H), 2.51 (dd, *J* = 6.3, 4.2 Hz, 1H), 1.95 (d, *J* = 6.3 Hz, 1H), 1.92 – 1.84 (m, 1H), 1.17 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): *δ* ppm 75.90, 74.39, 70.23, 37.32, 36.92, 36.38, 16.82; IR (neat, cm^{-1}): 3283, 1456, 1090, 1065, 995, 914, 874, 752; HRMS: calculated for $C_7H_{13}NO_3$ [M+H$^+$] 160.09682, found: 160.09711.

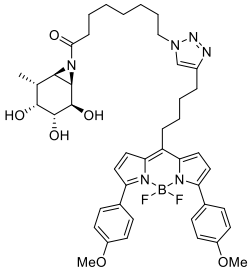


8-Azido-1-((1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptan-7-yl)-octan-1-one (23): 8-azido-octanoic acid **24**[19] (207 mg, 1.1 mmol, 1.3 eq.) and EEDQ (277 mg, 1.1 mmol, 1.3 eq.) were dissolved in anhydrous DMF (1.1 mL) and stirred at

room temperature for 2h. This pre-activated mixed anhydride solution (600 µL, 0.61 eq.) was added to a solution of aziridine **22** (137 mg, 0.86 mmol, 1.0 eq.) in DMF (5.0 mL) at 0 °C and stirred for 30 minutes after which the remaining portion of the pre-activated mixed anhydride solution (500 µL, 0.51 eq.) was added. The resulting mixture was stirred at 0 °C for 3h. The reaction was quenched by 2 mL MeOH and the mixture was concentrated *in vacuo*. Then the crude product was purified by silica gel column chromatography (1-10% MeOH in DCM) giving **23** as a colorless oil (162 mg, 0.50 mmol, 58% yield)（or by semi-preparative reversed HPLC (linear gradient: 27-33% B in A, 12 min, solutions used A: H_2O, B: MeCN) followed by lyophilization yielding white powder (2.14mg, 6.6 µmol, 2%). TLC: R_f 0.31 (DCM/MeOH, 10/1, v/v); $[\alpha]_D^{20}$ -29 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 4.06 (dd, *J* = 8.7, 3.9 Hz, 1H), 3.67 – 3.55 (m, 1H), 3.38 (dd, *J* = 8.0, 1.6 Hz, 1H), 3.28 (t, *J* = 6.9 Hz, 2H), 2.96 (dd, *J* = 6.0, 3.6 Hz, 1H), 2.59 – 2.49 (m, 1H), 2.45 (t, *J* = 7.6 Hz, 1H), 2.42 – 2.36 (m, 1H), 2.03 – 1.95 (m, 1H), 1.64 – 1.55 (m, 4H), 1.41-1.33 (m, 6H), 1.20 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 188.56, 75.71, 74.42, 69.20, 52.36, 43.48, 42.60, 36.94, 36.57, 30.10, 29.90, 29.79, 27.60, 25.83, 16.17; IR (neat, cm^{-1}): 3402, 2932, 2959, 2093, 1674, 1425, 1258, 1167, 1063, 997, 816; LC-MS: R_t 5.35 min, linear gradient 10-90% B in 15 min; ESI-MS: *m/z* = 327.4 $(M+H)^+$; HRMS: calculated for $C_{15}H_{26}N_4O_4$ $[M+H^+]$ 327.20268, found: 327.20266.

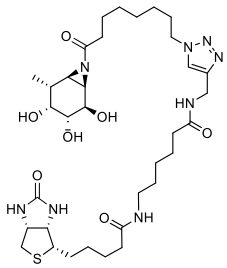


8-(4-(4-(5,5-Difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diaza-borinin-10-yl)butyl)-1H-1,2,3-triazol-1-yl)-1-((1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptan-7-yl)octan-1-one (1): Azide **23** (40 mg, 0.12 mmol) was dissolved in DMF (3 mL), Bodipy compound **25**[32] (44 mg, 0.13 mmol, 1.1 eq.) and aq. solutions of $CuSO_4$ (1.0 M 24 µL, 0.024 mmol, 0.2 eq.) and aq. solutions of sodium ascorbate (1.0 M, 25 µL, 0.025 mmol, 0.20 eq) were added to the solution under argon atmosphere. The mixture was stirred at room temperature for 2h. The volatiles were removed under reduced pressure and the crude product was purified by semi-preparative reversed HPLC (linear gradient: 44%→46% B in A, 12 min, solutions used A: H_2O, B: MeCN) and the pure product **1** was obtained as orange powder after lyophilization (9.5 mg, 0.0145 mmol, 12% yield). ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 7.73 (s, 1H), 6.11 (s, 2H), 4.34 (t, *J* = 6.9 Hz, 2H), 4.05 (dd, *J* = 8.7, 3.9 Hz, 1H), 3.65 – 3.55 (m, 1H), 3.37 (dd, *J* = 8.7, 1.8 Hz, 1H), 3.02 – 2.94 (m, 2H), 2.92 (dd, *J* = 6.0, 3.9 Hz, 1H), 2.77 (t, *J* = 7.3 Hz, 2H), 2.54 – 2.47 (m, 1H), 2.43 (s, 6H), 2.39 – 2.33 (m, 1H), 2.36 (s, 6H), 2.22 (t, *J* = 7.5 Hz, 1H), 2.00-1.93 (m, 1H), 1.92-1.82 (m, 4H), 1.69 – 1.50 (m, 4H), 1.33-1.26 (m, 6H), 1.18 (d, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 188.59, 154.91, 148.51, 147.89, 142.20, 132.58, 123.38, 122.61, 75.83, 74.49, 69.27, 51.17, 43.53, 42.66, 36.89, 36.76, 36.67, 32.22, 32.18, 31.16, 30.83, 29.98, 29.70, 29.65, 29.04, 27.28, 27.18, 26.84, 25.86, 25.75, 16.48, 16.18, 14.45; LC-MS: R_t 8.58 min, linear gradient 10%→90% B in 15 min; ESI-MS: *m/z* = 655.5 $(M+H)^+$; HRMS: calculated for $C_{34}H_{49}BF_2N_6O_4$ $[M+H^+]$ 655.39552, found: 655.39549.

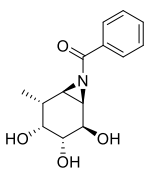


8-(4-(4-(5, 5-Difluoro-3, 7-bis(4-methoxyphenyl)-5H-4l4, 5l4-dipyrrolo[1, 2-c:2',1'-f]-[1, 3,2]diazaborinin-10-yl)butyl)-1H-1,2,3-triazol-1-yl)-1-((1R,2R,3R,4R,5R,6R)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptan-7-yl)octan-1-one (2): Azide **23** (31 mg, 0.099 mmol, 1.0 eq) was dissolved in DMF (3 mL), Bodipy compound **26**[32] (53 mg, 0.11 mmol, 1.1 eq), and aq. solutions of $CuSO_4$ (1.0 M, 20 µL, 0.019 mmol, 0.20 eq.) and aq. solutions of sodium ascorbate (1.0 M, 21 µL, 0.021 mmol, 0.20 eq) were added to the

solution under argon atmosphere and the mixture was stirred at room temperature for 2 h. The reaction was checked with LC-MS within the elution system of 10% NH_4OAc. The volatiles were removed under reduced pressure and the crude product was purified by semi-preparative reversed HPLC (linear gradient: 52%→58% B in A, 12 min, solutions used A: H_2O, B: MeCN) resulting a dark blue powder as the product **2** after lyophilization (15.32 mg, 0.019 mmol, 19%). ^{1}H-NMR (400 MHz, CD_3CN): δ ppm 7.83 – 7.74 (m, 4H), 7.51 – 7.49 (s, 1H), 7.46 (d, *J* = 4.4 Hz, 2H), 7.03 – 6.94 (m, 4H), 6.69 (d, *J* = 4.4 Hz, 2H), 4.27 (t, *J* = 7.0 Hz, 2H), 3.91 (dd, *J* = 8.5, 3.9 Hz, 1H), 3.84 (s, 6H), 3.56 – 3.54 (m, 1H), 3.25 (dd, *J* = 8.6, 1.8 Hz, 1H), 3.09 – 3.00 (m, 2H), 2.82 (dd, *J* = 6.1, 3.9 Hz, 1H), 2.78 – 2.69 (m, 2H), 2.44 – 2.23 (m, 3H), 1.88 – 1.74 (m, 6H), 1.55 – 1.45 (m, 2H), 1.31-1.20 (m, 7H), 1.11 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3CN): δ ppm 161.85, 158.30, 148.12, 147.71, 137.18, 132.13, 132.09, 132.05, 128.79, 126.09, 122.37, 121.27, 118.40, 114.62, 101.03, 74.97, 74.36, 69.23, 56.15, 50.66, 42.70, 41.99, 36.77, 36.05, 34.07, 31.01, 30.89, 30.34, 29.64, 29.28, 26.88, 25.72, 25.52, 16.24; LC-MS: R_t 9.15 min, linear gradient 0-90% B in 15 min; ESI-MS: *m/z* = 811.8 $(M+H)^+$; HRMS: calculated for $C_{44}H_{53}BF_2N_6O_6$ [$M+H^+$] 811.41681, found: 811.41690.

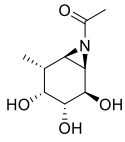


N-((1-(8-oxo-8-((1R,2R,3R,4R,5R,6R)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptan-7-yl)octyl)-1H-1,2,3-triazol-4-yl)methyl)-6-(5-((3S,4S,6R)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)hexanamide (3): Azide compound **23** (31 mg, 0.099 mmol, 1.0 eq) was dissolved in DMF(3.0 mL), biotin-ahx-alkyne **27**[33] (38 mg,0.099 mmol, 1.0 eq), aq. solutions of $CuSO_4$ (1.0 M, 20 µL, 0.019 mmol, 0.20 eq) and aq. solutions of sodium ascorbate (1.0 M, 21 µL,0.021 mmol, 0.22 eq) was added to the solution under argon atmosphere, and the mixture was stirred at 80 °C overnight, the reaction was followed by LC-MS. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 18%→24% B in A, 12min, solutions used A: H_2O, B: actonitrile) and the fraction were freeze-dried without concentration resulting white powder product **3** (9.631 mg, 0.013 mmol, 13%). ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 7.85 (s, 1H), 4.51 (dd, *J* = 7.6, 4.8 Hz, 1H), 4.42 (s, 2H), 4.38 (t, *J* = 3.6 Hz, 2H), 4.32 (dd, *J* = 8.0, 4.4 Hz, 1H), 4.08 (dd, *J* = 8.8, 4.0 Hz, 1H), 3.61 (t, *J* = 1.6 Hz, 1H), 3.39 (dd, *J* = 8.8, 1.6 Hz, 1H), 3.22 - 3.14 (m, 3H), 2.95 - 2.90 (m, 2H), 2.72 (d, *J* = 12.8 Hz, 1H), 2.58 - 2.50 (m, 1H), 2.47 - 2.38 (m, 2H), 2.25 - 2.17 (m, 4H), 2.01 - 1.96 (m, 1H), 1.95 - 1.87 (m, 2H), 1.79 - 1.58 (m, 8H), 1.53 - 1.41 (m, 4H), 1.37 - 1.30 (m, 8H), 1.21 (d, *J* =2.4Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 188.7, 177.0, 176.0, 166.1, 146.3, 124.2, 75.8, 74.5, 69.3, 63.55, 63.4, 61.6, 57.0, 51.3, 43.6, 42.7, 41.1, 40.2, 36.9, 36.8, 36.7, 36.7, 35.6, 31.2, 30.1, 30.0, 29.9, 29.8, 29.7, 29.5, 27.5, 27.3, 27.2, 26.9, 26.8, 26.5, 25.8, 16.2; LC-MS: R_t 4.31min, linear gradient 10→90% B in 15 min; ESI-MS: *m/z* = 721.7 $(M+H)^+$; HRMS: calculated for $C_{34}H_{56}N_8O_7S$ [$M+H^+$] 721.40655, found: 721.40661.



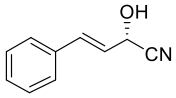
Phenyl((1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptan-7-yl)methanone (4): Benzoic acid (49 mg, 0.40 mmol, 2.0 eq.) and EEDQ (99 mg, 0.40 mmol, 2.0 eq.) were dissolved in anhydrous DMF (0.40 mL) and stirred at room temperature for 2 h. This pre-activated mixed anhydride solution (200 µL) was added to aziridine **22** (32 mg, 0.20 mmol, 1.0 eq.) in dry DMF (1.0 mL) at 0 °C and stirred for 30 minutes. The remaining half of the pre-activated mixed anhydride solution (200 µL) was added and the resulting mixture was stirred at 0 °C for 2 h. The reaction was quenched with MeOH (1.0 mL) and the mixture was concentrated *in vacuo*. The crude product was purified by HPLC

(linear gradient: 15%→21% B in A, 12 min, solutions used A: H_2O, B: actonitrile) giving compound **4** as white powder (3.8 mg, 15 µmol, 7% yield). TLC: R_f 0.52 (DCM/MeOH, 5/1, v/v); ^{1}H-NMR (400 MHz, CD_3OD): δ ppm 8.21 – 8.11 (m, 2H), 7.60 (m, 1H), 7.48 (dd, *J* = 7.6 Hz, 2H), 4.18 (dd, *J* = 8.8, 4.0 Hz, 1H), 3.73-3.71(m, 1H) , 3.55 (dd, *J* = 8.8, 1.8 Hz, 1H), 3.14 (dd, *J* = 6.2, 4.0 Hz, 1H), 2.43 (d, *J* = 6.2 Hz, 1H), 2.31 – 2.23 (m, 1H), 1.90 (s, 1H), 1.21 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): δ ppm 181.40, 134.11, 134.06, 130.50, 129.50, 75.78, 74.65, 69.40, 44.43, 44.34, 36.94, 16.30; LC-MS: R_t 5.35min; linear gradient 0%→90% B in 15 min; ESI-MS: *m/z* = 264.3 $(M+H)^+$; HRMS: calculated for $C_{14}H_{17}NO_4$ $[M+H^+]$ 264.12304, found: 264.12308.



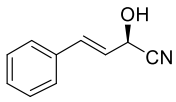
1-((1*R*,2*R*,3*R*,4*R*,5*R*,6*R*)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptan-7-yl)ethan-1-one (5): Acetic acid (16 µL, 0.28 mmol, 2.0 eq.) and EEDQ (68 mg, 0.28 mmol, 2.0 eq.) were dissolved in anhydrous DMF (0.30 mL) and stirred at room temperature for 2h. This pre-activated mixed anhydride solution (150 µL) was added to aziridine **22** (22 mg, 0.14 mmol, 1.0 eq.) in dry DMF (0.70 mL) at 0 °C and stirred for 30 min. The remaining half of the pre-activated mixed anhydride solution (150 µL) was added and the resulting mixture was stirred at 0 °C for 2 h. The reaction was quenched by adding MeOH (0.50 mL) and the mixture was concentrated *in vacuo*. The crude product was purified by semi-preparative reversed HPLC (linear gradient: 0%→10% B in A, 12 min, solutions used A: H_2O, B: MeCN) giving compound **5** as white powder after lyophilization (6.9 mg, 34 µmol, 25% yield). TLC: R_f = 0.38 (DCM/MeOH, 10/3, v/v); ^{1}H-NMR (400 MHz, D_2O): δ ppm 4.06 (dd, *J* = 8.9, 4.0 Hz, 1H), 3.76 – 3.63 (m, 1H), 3.46 (dd, *J* = 8.9, 1.8 Hz, 1H), 3.12 (dd, *J* = 6.0, 4.0 Hz, 1H), 2.56 (d, *J* = 6.1 Hz, 1H), 2.16 (s, 3H), 2.13-2.07 (m, 1H) 1.16 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 187.12, 74.33, 72.46, 67.67, 42.08, 42.03, 34.32, 22.58, 14.83; LC-MS: R_t 2.13 min, linear gradient 0%→90% B in 15 min; ESI-MS: *m/z* = 202.2 $(M+H)^+$; HRMS: calculated for $C_8H_{15}NO_4$ $[M+H^+]$ 202.10738, found: 202.10740.

Synthesis of compounds 28 and 29:

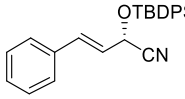


(*S,E*)-2-Hydroxy-4-phenylbut-3-enenitrile:[20] (**Caution!!!** Toxic gas (HCN) may evolve! Work in a well ventilated hood!) In an erlenmeyer flask KCN (26 g, 406 mmol, 3.6 eq.) was dissolved in H_2O (90 mL). On top a layer of methyl *tert*-butyl ether (MTBE, 80 mL) was placed and the mixture was magnetically stirred at such a rate that the two layer system remains. Under slight ice-cooling an aq. 20% (w/w) citric acid solution was added in portions until a pH of 5.45 was reached (pH meter control). At that time the mixture was transferred into a separation funnel, shaken firmly and separated. The water layer was extracted once more with MTBE (80 mL) and the combined MTBE layers were combined and kept on ice. In the meantime a 500 mL three necked flask, equipped with a magnetic stirrer and a thermometer, was charged with a citrate buffer (75 mL, 0.10 M, pH = 5.45), MTBE (20 mL) and cinnamon aldehyde (14.7 g, 111 mmol, 1.0 eq.). The mixture was cooled on an ice bath and *Hb*HNL extract (4.5 g) was added. Under argon and vigorous stirring the ice cold HCN buffer was added drop wise in 15 minutes at 8 °C. The reaction was stirred at this temperature for one hour and for 24 hours at room temperature. At this time TLC showed almost complete conversion and the reaction was stopped. The layers were separated, the water layer extracted once more with MTBE (50 mL). The combined MTBE layers were dried ($MgSO_4$), filtered and evaporated to afford the crude product as a yellow oil (20.6 g, 93% *e.e.* as determined by chiral HPLC). After two crystallizations from DCM/pentane the target cyanohydrin was obtained (9.71 g, 55 %, *e.e.* = 99%) as colorless crystals. $[\alpha]_D^{20}$ -30 (*c* = 1, $CHCl_3$), lit.[34a] $[\alpha]_D^{20}$ -21.8 (*c* = 0.97, $CHCl_3$), lit.[34b] $[\alpha]_D^{20}$ -24.1 (*c* =

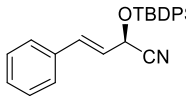
0.8, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.41 - 7.37 (m, 2H), 7.37 - 7.27 (m, 3H), 6.88 (d, *J* = 16.1 Hz, 1H), 6.23 (dd, *J* = 16.1, 6.2 Hz, 1H), 5.14 (dd, *J* = 6.2, 6.2, Hz, 1H), 3.28 (d, *J* = 7.2 Hz, 1H); Chiral HPLC: R_t 14.8 min (Daicel Chiralcel OD, UV 254 nm, Hexane/2-propanol/acetic acid = 85/15/0.1, v/v/v; 1.0 mL/min).



(*R,E*)-2-Hydroxy-4-phenylbut-3-enenitrile:[23] (**Caution!!!** Toxic gas (HCN) may evolve! Work in a well ventilated hood!) In an erlenmeyer flask KCN (28.3 g, 435 mmol, 2.6 eq.) was dissolved in water (100 mL). On top a layer of MTBE (100 mL) was placed and mixture was magnetically stirred at such a rate that the two layer system remains. Under slight ice-cooling an aq. 20% (w/w) citric acid solution was added in portions until a pH of 5.45 was reached (pH meter control). At that time the mixture was transferred into a separation funnel, shaken firmly and separated. The water layer is extracted once more with MTBE (100 mL) and the combined MTBE layers were combined and kept on ice. In the meantime, a 500 mL three necked flask, equipped with a magnetic stirrer and a thermometer, was charged with a citrate buffer (50 mL, 0.1 M, pH = 5.45), MTBE (60 mL) and cinnamon aldehyde (21.5 g, 163 mmol, 1.0 eq.). The mixture was cooled on an ice bath and *pa*HNL (142 mg) was added. Under vigorous stirring the ice cold HCN buffer was added drop wise in 15 minutes. After 64 hours the reaction was stopped, the layers separated and the water layer extracted once more with MTBE (50 mL). The combined MTBE layers were dried ($MgSO_4$), filtered and evaporated to afford a yellow oil (28.8 g) as the crude product. The oil was dissolved in DCM (150 mL) and pentane (200 mL) was added. After standing at room temperature for 2 hours and 2 hours at 4 ºC the formed crystals were collected by filtration and washed with cold pentane twice. Drying afforded the title compound as colorless crystals (11.8 g, 74 mmol, 46 %, *e.e.* = 99%). $[\alpha]_D^{20}$ +30 (*c* = 1, $CHCl_3$), lit.[35] $[\alpha]_D^{20}$ +28.8 (*e.e*. = 92%; *c* = 1.02, $CHCl_3$), lit.[23] $[\alpha]_D^{20}$ +30.5 (*c* = 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.45 - 7.40 (m, 2H), 7.40 - 7.30 (m, 3H), 6.92 (d, *J* = 15.8 Hz, 1H), 6.26 (dd, *J* = 15.8, 6.0 Hz, 1H), 5.17 (dd, *J* = 6.2, 6.2 Hz, 1H), 2.86 (d, *J* = 7.1 Hz).

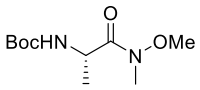


(*S,E*)-2-((*tert*-Butyldiphenylsilyl)oxy)-4-phenylbut-3-enenitrile((*S*)-28): *tert*-Butylchlorodiphenyl- silane (7.2 g, 26 mmol, 1.3 eq.) was dissolved in DMF (80 mL) and imidazole (2.7 g, 40.0 mmol) was added. The mixture was stirred at room temperature for 15 min. Then it was cooled on ice and (*S,E*)-2-hydroxy-4-phenylbut-3-enenitrile (3.2 g, 20 mmol, 1.0 eq.) was added and the reaction stirred for 24 h. TLC analysis revealed complete conversion and the reaction was quenched with H_2O (250 ml), extracted with Et_2O (3 x 100 mL). The combined organic layers were washed with H_2O (50 mL) and brine (50 mL), dried with $MgSO_4$ and evaporated. The mixture was purified by silicagel column chromatography (1%→2%, EtOAc in pentane) to afford the title compound as a colorless oil (7.8 g, 19 mmol, 98 %). $[\alpha]_D^{20}$ +75 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.75 (d, *J* = 7.6, 2H), 7.67 (d, *J* = 7.6, 2H), 7.52 - 7.23 (m, 11H), 6.56 (d, *J* = 15.8 Hz, 1H), 6.13 (d, *J* = 15.8, 6.4 Hz, 1H), 4.97 (d, *J* = 6.4 Hz, 1H), 1.12 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 135.92, 135.87, 135.13, 131.97, 131.59, 130.57, 130.47, 128.85, 128.78, 128.13, 128.05, 127.05, 134.47, 123.38, 118.28, 63.61, 26.77, 19.40; IR (neat, cm^{-1}): 3024, 2933, 2860, 1472, 1428, 1116, 1112, 1075, 1060, 965, 753, 741, 700, 613, 504.

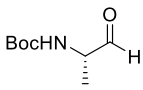


(*R,E*)-2-((*tert*-Butyldiphenylsilyl)oxy)-4-phenylbut-3-enenitrile((*R*)-28): Prepared as described for compound **28**, obtained as pale yellow oil (54 mmol scale, yield 21 g, 99%). $[\alpha]_D^{20}$ -75 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.75 (d, *J* = 7.7, 2H), 7.67 (d, *J* = 7.7,

2H), 7.51 - 7.22 (m, 11H), 6.56 (d, *J* = 15.8 Hz, 1H), 6.13 (dd, *J* = 15.8, 6.4 Hz, 1H), 4.97 (d, *J* = 6.4 Hz, 1H), 1.18 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 135.93, 135.88, 135.75, 135.14, 134.89, 131.99, 131.60, 130.67, 130.58, 130.47, 129.68, 128.86, 128.79, 128.39, 128.14, 128.05, 127.77, 127.06, 126.66, 134.49, 123.40, 118.28, 63.62, 26.78, 19.41.

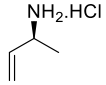


***tert*-Butyl(*S*)-(1-(methoxy(methyl)amino)-1-oxopropan-2-yl)carbamate:**[21] A solution of Boc-L-Alanine (19.2 g, 102 mmol, 1.0 eq.) in DCM (400 mL) was cooled to -15 °C followed by addition of *N,O*-dimethylhydroxylamine hydrochloride (10.1 g, 103 mmol, 1.0 eq.) and then NMM (11.3 ml, 103 mmol). *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (19.8 g, 103 mmol, 1.0 eq.) was added portion wise as a solid over 30 minutes. The reaction was stirred at room temperature for 24 hours. After cooling on an ice-bath, 1M HCl was added (30 mL).The aq. layer was extracted twice with DCM (150 mL) and the combined organic layers were washed with a sat. aq. $NaHCO_3$ solution (60 mL), dried ($MgSO_4$), filtered and the solvent was evaporated under vacuum to give the Weinreb amide as a white solid (22.1 g, 96 mmol, 94%) that was used without purification. ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 5.28 (d, *J* = 7.0 Hz), 4.75 - 4.60 (m, 1H), 3.77 (s, 3H), 3.21 (s, 3H), 1.44 (s, 9H), 1.31 (d, *J* = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.36, 79.58, 61.71, 46.62, 32.25, 28.47, 18.78.

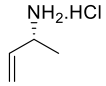


***tert*-Butyl(*S*)-(1-oxopropan-2-yl)carbamate:**[21] Weinreb amide from above (22 g, 95 mmol, 1.0 eq.) was dissolved in anhydrous THF (300 mL) and cooled to 0 °C. A solution of 2.0 M $LiAlH_4$ in THF (47 ml, 95 mmol, 1.0 eq.) was added dropwise and the mixture was stirred for another 30 minutes. The reaction was cooled to -15 °C and a sat. aq. $KHSO_4$ solution (250 mL) was added carefully. The solution was diluted with Et_2O (500 mL) and stirred vigorously for 30 min. The organic layer was separated, dried with $MgSO_4$, filtered and the solvent was evaporated under vacuum to give the aldehyde as a white solid (16 g, 95 mmol, quant.) that was used crude. ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 9.58 (s, 1H), 5.36 – 4.91 (m, 1H), 4.30 - 4.07 (m, 1H), 1.46 (s, 9H), 1.33 (d, *J* = 7.3 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 199.93, 155.41, 80.06, 55.61, 28.39, 14.92.

***tert*-Butyl(*S*)-but-3-en-2-ylcarbamate:**[21] Methyltriphenylphosphonium bromide (57 g, 159 mmol, 17 eq.) was suspended in THF (750 mL) at room temperature and KHMDS (30 g, 152 mmol, 16 eq.) was added. The resultant yellow suspension was stirred at room temperature for 1 hour and then cooled to -78 °C and a solution of the aldehyde from above (16 g, 95 mmol, 1.0 eq.) dissolved in THF (150 mL) was added dropwise. The cooling bath was removed and the mixture was stirred for another 2 hours. The reaction was quenched with MeOH (100 mL) and the resulting mixture was poured into sat. ammonium chloride solution (500 mL). Extraction with Et_2O (3 x 200 mL), dry $MgSO_4$ and evaporation of the solvent *in vacuo* afforded an orange semi-solid that was treated several times with pentane. The combined pentane fractions were filtered and the solvent was evaporated to give the target compound as a white solid that was purified by silica gel column chromatography (2%→5%, EtOAc in pentane) to afford the title compound as a white solid (15 g, 86 mmol, 91 %). $[\alpha]_D^{20}$ -4.2 (*c* = 1, $CHCl_3$), lit.[36] $[\alpha]_D^{20}$ -6.33 (*c* = 1.2, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 5.82 (ddd, *J* = 17.2, 10.4, 5.0 Hz), 5.24 (d, *J* = 7.7 Hz, 1H), 5.12 (d, *J* = 17.2 Hz, 1H), 5.01 (d, *J* = 10.4 Hz, 1H), 4.31 - 4.14 (m, 1H), 1.43 (s, 1H), 1.20 (d, *J* = 7.0 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 154.70, 139.88, 112.84, 78.14, 47.57, 27.93, 20.04. IR (neat, cm^{-1}): 3459, 3020, 1703, 1498, 1215, 1170, 748; HRMS: calculated for $C_9H_{17}NO_2$ [M+H$^+$] 173.13321, found: 173.13307.



(*S*)-But-3-en-2-amine hydrochloride ((*S*)-29): The Boc-protected amine (14 g, 83 mmol, 1.0 eq.) from above was dissolved in MeOH (110 mL), aq. 6.0 M HCl (100 mL) was added and the mixture stirred overnight at room temperature. The solvents were evaporated using a water aspirator affording the title salt as an off white solid (10 g, 76 mmol, quant., 92% overall, *e.e.* > 99%). $[\alpha]_D^{20}$ +2.4 (*c* = 1, MeOH), lit.[37] $[\alpha]_D^{20}$ -3.5 (*c* = 1, EtOH); ^{1}H-NMR (400 MHz, D_2O): δ ppm 5.90 (ddd, *J* = 17.2, 10.6, 6.7 Hz, 1H), 5.37 (d, *J* = 17.2 Hz, 1H), 5.33 (d, *J* = 10.6 Hz, 1H), 3.92 (c, *J* = 6.7 Hz, 1H), 2.20 (s, 2H), 1.37 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 134.76, 118.62, 49.34, 17.90. IR (neat, cm^{-1}): 3400 - 3200, 1649, 1610, 1483, 1425, 1385, 1019, 995, 927, 659.

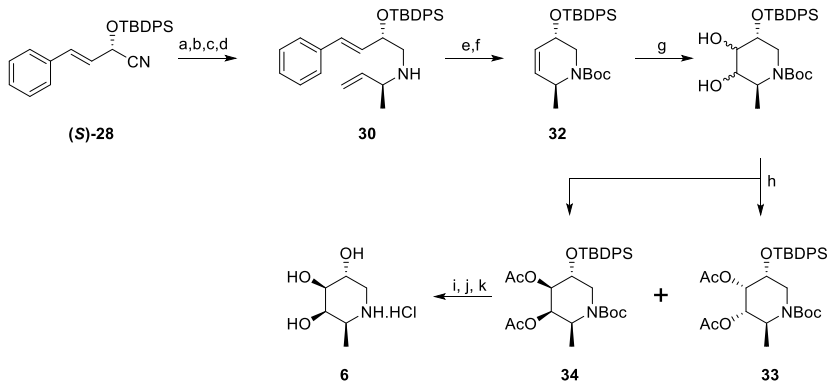


(*R*)-But-3-en-2-amine hydrochloride ((*R*)-29): Prepared from Boc-D-Alanine in the same manner as described above for the (*S*)-enantiomer, *e.e.* = 95%. $[\alpha]_D^{20}$ -3.6 (*c* = 1, MeOH). All spectral data were identical.

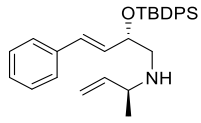
Determination of enantiomeric excess: Analytical samples of both obtained amine hydrochlorides were treated with benzoyl chloride in DCM in the presence of triethyl amine. After work up and purification these samples were subjected to Chiral HPLC analysis on a Daicel Chiralcel OD column (250 x 4.5 mm) using Hexane/2-propanol = 90/10 (v/v), 1.0 mL/min, UV detection (254 nm). (*R*)-isomer, *e.e.* = 95% (left chromatogram); (*S*)-isomer, *e.e.* > 99% (left chromatogram); See chromatograms below.

Preparation of fuconojirimycin (6) and the configurational isomers 7 – 13:

Scheme 3: Preparation of fuconojirimycin **(6)** from cyanohydrin **(S)-28**.

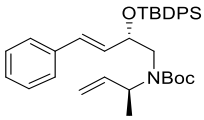


Reagents and conditions: (a) DIBAL-H, -78→5 °C; (b) MeOH, -90 °C; (c) amine **(*S*)-29**, NaOMe, RT, 18 h; (d) $NaBH_4$, 5 °C→RT, 4 h; (e) Boc_2O, 50 °C; (f) Grubbs 1st generation, DCM, reflux; (g) $K_2OsO_4 \cdot 2H_2O$, NMO, acetone/H_2O; (h) Ac_2O, pyridine, DMAP, 0 °C→RT; (i) K_2CO_3, MeOH; (j) TBAF, THF; (k) HCl (6 M in H_2O), MeOH.

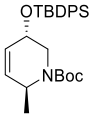


(*S,E*)-*N*-((*S*)-But-3-en-2-yl)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-amine (30): In a flame dried flask and under argon atmosphere, a solution of ((*S,E*)-2-((tert-butyldiphenylsilyl)oxy)-4-phenylbut-3-enenitrile (3.6 g, 9.0 mmol, 1.0 eq.) in

dry Et_2O (80 mL) was cooled to -78 °C. A 1 M solution of DIBAL-H in toluene (14 mL, 14 mmol, 1.5 eq.) was added dropwise and the reaction was allowed to warm up slowly to 10 °C. After cooling to -90 °C, absolute MeOH (14 mL) was added at once, followed by a solution (*S*)-but-3-en-2-amine hydrochloride (3.2 g, 29 mmol, 3.3 eq.) in MeOH (20 mL). Subsequently dry sodium methoxide (2.4 g, 45 mmol, 5.0 eq.) was added to deprotonate the (*S*)-but-3-en-2-amine hydrochloride *in situ*. The cooling bath was removed and the mixture stirred overnight at room temperature under a light flow of argon to reduce the volume of the reaction by half. The mixture was cooled on an ice bath and $NaBH_4$ (1.2 g, 33 mmol, 3.6 eq.) was added in three portions. After stirring for 30 min on the ice bath and two hours at room temperature, the reaction was poured in to an aq. 0.50 M NaOH (90 mL) solution and extracted with diethyl ether (3 x 80 mL). The combined organic layers were washed with a cold aq. 1.0 M HCl solution (100 mL). Evaporation of this acidic aq. layer afforded recovered (*S*)-but-3-en-2-amine hydrochloride (2.07 g, 19.2 mmol, 2.1 eq.). The organic layer was washed subsequently with aq. 0.50 M NaOH (60 mL) solution and brine (30 mL). Drying on $MgSO_4$, filtering and evaporation of the solvent afforded the crude product that was purified by silica gel column chromatography (3%→10%, EtOAc in pentane) to afford the target compound as a yellow oil (3.6 g, 7.9 mmol, 88%). $[\alpha]_D^{20}$ +128 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400MHz, $CDCl_3$): δ ppm 7.72 - 7.62 (m, 4H), 7.46 - 7.13 (m, 11H), 6.19 (d, *J* = 16.0 Hz, 1H), 6.10 (dd, *J* = 16.0, 7 Hz, 1H), 5.60 (ddd, *J* = 17.4, 10.0, 7.7 Hz, 1H), 5.01 (d, *J* = 17.4 Hz, 1H), 4.98 (d, *J* = 10.0 Hz, 1H), 4.45 (dt, *J* = 12.5, 6.3 Hz, 1H), 3.11 (m, 1H), 2.72 (dd, *J* = 11.8, 6.3 Hz, 1H), 2.68 (dd, *J* = 11.8, 5.8 Hz, 1H), 1.08 (s, 9H), 1.05 (d, *J* = 3.4 Hz, 3H); ^{13}C-NMR (101MHz, $CDCl_3$): δ ppm 142.24, 136.80, 136.12, 136.03, 135.68, 134.97, 134.15, 134.06, 131.46, 130.68, 129.80, 129.69, 128.50, 127.71, 127.58, 126.60, 114.88, 74.34, 56.73, 53.64, 27.22, 21.50, 19.50; IR (neat, cm^{-1}): 3071, 2958, 2930, 2856, 1471, 1427, 1109, 740; HRMS: calculated for $C_{30}H_{37}NOSi$ [M+H$^+$] 456.27172, found: 456.27122.

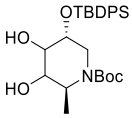


***tert*-Butyl((*S*)-but-3-en-2-yl)((*S,E*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-yl)carba-mate (31):** To compound **30** (8.3 g, 15 mmol, 1.0 eq.) was added Boc_2O (5.1 g, 23 mmol, 1.5 eq.) and the mixture was stirred overnight at 50 °C. TLC analysis showed complete conversion and after evaporation of the solvent the mixture was purified by silica gel column chromatography (2%→5%, EtOAc in pentane) to afford the title compound **31** as a colorless oil (8.7 g, 15 mmol, 100%). $[\alpha]_D^{20}$ +27 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.78 – 7.62 (m, 4H), 7.47 – 7.12 (m, 11H), 6.15 – 6.00 (m, 2H), 5.77 (ddd, *J* = 16.3, 10.6, 5.2 Hz, 1H), 5.00 – 4.86 (m, 2H), 4.53 – 4.37 (m, 1H), 3.60 – 3.37 (m, 1H), 3.37 – 3.19 (m, 1H), 3.19 - 3.01 (m, 1H), 1.47 - 1.18 (m, 9H), 1.07 (s, 9H), 1.06 (d, *J* = 3.4 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 139.54, 136.15, 136.05, 135.52, 131.12, 130.82, 129.78, 129.69, 128.43, 127.93, 129.69, 128.43, 127.93, 127.69, 127.56, 126.56, 115.13, 73.55, 50.13, 45.54, 28.45, 27.20, 19.44, 17.69; IR (neat, cm^{-1}): 3072, 2933, 2858, 1690, 1428, 1391, 1365, 1164, 1111, 736; HRMS: calculated for $C_{35}H_{45}NO_3Si$ [M+H$^+$] 556.32415, found: 556.32387.

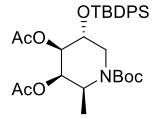


***tert*-Butyl (3*S*,6*S*)-3-((*tert*-butyldiphenylsilyl)oxy)-6-methyl-3,6-dihydropyridine-1(2*H*)-carboxylate (32):** Boc-protected diene **31** (8.7 g, 15 mmol, 1.0 eq.) was dissolved in DCM and argon was bubbled through the solution for five minutes. Grubbs 1st generation (260 mg, 0.32 mmol, 0.020 eq.) was added and the reaction refluxed under argon for 48 hours after which TLC analysis revealed complete conversion. Evaporation of the solvent and silica gel column chromatography (3%→5%, EtOAc in pentane) afforded the compound **32** as a colorless oil (6.85 g, 14 mmol, 97%). $[\alpha]_D^{20}$ +158 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.72 (d, *J* = 6.8

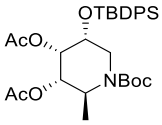
Hz, 2H), 7.66 (d, *J* = 6.8 Hz, 2H), 7.46 – 7.32 (m, 6H), 5.74 – 5.43 (m, 1H), 5.60 - 5.43 (m, 1H), 4.67 – 4.46 (m, 1H), 4.27 – 4.08 (m, 1H), 4.08 – 3.97 (m, 1H), 2.96 – 2.75 (m, 1H), 1.50 (s, 9H), 1.08 (d, *J* = 7.0 Hz, 3H), 1.05 (s, 9H);. ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 154.80, 135.93, 134.93, 134.12, 133.58, 129.80, 127.78, 125.39, 79.46, 64.20, 49.91, 41.94, 28.63, 27.02, 19.34, 17.18. IR (neat, cm^{-1}): 2965, 2931, 2858, 1695, 1416, 1384, 1175, 1131, 1106, 1073, 702; HRMS: calculated for $C_{27}H_{37}NO_3Si$ [M+H$^+$] 452.2615, found: 452.26159.



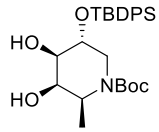
Upjohn dihydroxylation of compound 32: Compound **32** (8.3 g, 18 mmol, 1.0 eq.) was dissolved in a mixture of acetone (70 mL) and water (70 mL) and cooled to -10 °C. *N*-Methylmorpholine-*N*-oxide monohydrate (6.7 g, 50 mmol, 16 eq.) and $K_2OsO_4{\cdot}2H_2O$ (72 mg, 0.19 mmol, 1.0 mol %) were added subsequently. After 24 - 48 hours TLC analysis showed complete conversion of the starting material **32**. The reaction was quenched with an aq. sat. Na_2SO_3 solution (100 mL) and stirred for 30 min. The mixture was diluted with water (100 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with aq. 0.6 M HCl, sat. aq. $NaHCO_3$ and brine. After drying (Na_2SO_4), filtering and evaporation of the solvent, afforded a 3:1 mixture of diastereoisomers (5.4 g, 11 mmol, 61%) that could not be separated by silica gel column chromatography.



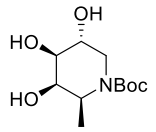
(2*S*,3*R*,4*R*,5*R*)-1-(*tert*-butoxycarbonyl)-5-((*tert*-butyldiphenylsilyl)oxy)-2-methylpiperidine-3,4-diyl diacetate (34): Mixture from above (5.4 g, 11 mmol, 1.0 eq.) was dissolved in pyridine (25 mL) and cooled to 0 °C. Acetic anhydride (6.0 mL, 63 mmol, 5.7 eq.) and a few crystals of DMAP were added and the reaction was stirred for 24 – 48 hours at room temperature. TLC analysis showed complete conversion of the starting material. The reaction was diluted with toluene (100 mL) and the solvents evaporated. The resulting mixture was diluted with EtOAc (100 mL) and washed with H_2O (50 mL), 1 M HCl (50 mL), sat. aq. $NaHCO_3$ solution (50 mL) and brine (50 mL). After drying (Na_2SO_4), filtering and evaporation of the solvent, the silica gel column chromatography (3%→7%, EtOAc in pentane) afforded compound **32** as the first eluting isomer, pale yellow oil (4.0 g, 6.9 mmol, 63%). $[\alpha]_D^{20}$ +12 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.72 – 7.66 (m, 4H), 7.47 – 7.34 (m, 6H), 5.44 (dd, *J* = 6.9, 3.1 Hz, 1H), 5.12 (dd, *J* = 3.9, 3.9 Hz, 1H), 4.54 (qd, *J* = 6.9, 6.9 Hz, 1H), 3.94 (d, *J* = 14.2 Hz, 1H), 3.76 (ddd, *J* = 3.9, 1.5, 1.5 Hz, 1H), 3.07 (dd, *J* = 14.2, 1.5 Hz, 1H) 2.03 (s, 3H), 1.93 (s, 3H), 1.46 (s, 9H), 1.20 (d, *J* = 6.9 Hz, 3H), 1.10 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 169.79, 169.37, 154.89, 135.97, 135.94, 133.13, 133.05, 130.03, 129.94, 127.85, 127.80, 80.03, 70.99, 68.68, 67.41, 48.08, 40.83, 28.48, 26.94, 21.00, 20.96, 19.27, 12.50; IR (neat, cm^{-1}): 2933, 2859, 1752, 1697, 1418, 1366, 1284, 1162, 703; HRMS: calculated for $C_{31}H_{43}NO_7Si$ [M+H$^+$] 570.28816, found: 570.28780.



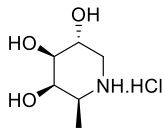
(2*S*,3*S*,4*S*,5*R*)-1-(*tert*-butoxycarbonyl)-5-((*tert*-butyldiphenylsilyl)oxy)-2-methylpiperidine-3,4-diyl di- acetate (33): Obtained as the later eluting isomer, pale yellow oil (1.2 g, 2.0 mmol, 19%). ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.78 – 7.69 (m, 4H), 7.45 – 7.32 (m, 6H), 5.03 (m, 1H), 4.93 (t, *J* = 3.2 Hz, 1H), 4.60 (q, *J* = 7.2 Hz, 1H), 4.06 – 3.97 (m, 2H), 2.89 (d, *J* = 11.2 Hz, 1H), 2.15 (s, 3H), 1.83 (s, 3H), 1.41 (s, 9H), 1.19 (d, *J* = 7.4 Hz, 3H), 1.11 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 170.89, 170.16, 154.99, 136.10, 136.01, 133.69, 133.23, 129.75, 129.60, 127.63, 127.29, 79.90, 70.90, 68.12, 67.33, 51.01, 44.19, 28.32, 26.76, 21.35, 20.77, 19.52, 14.61.



***tert*-Butyl(2*S*,3*R*,4*R*,5*R*)-5-((*tert*-butyldiphenylsilyl)oxy)-3,4-dihydroxy-2-methylpiperidine-1-carboxy- late:** Compound **34** (4.0 g, 7.0 mmol, 1.0 eq.) was dissolved in MeOH (100 mL) and K_2CO_3 (1.3 g, 9.4 mmol, 1.3 eq,) was added. The reaction was stirred for 24 hours after which TLC analysis showed complete conversion of the material **34**. The reaction was acidified with AcOH until pH 5, subsequently diluted with EtOAc (80 mL) and washed with brine (80 mL). Drying (Na_2SO_4), filtering, evaporation of the solvent and silica gel column chromatography (10%→50%, EtOAc in pentane) afforded the title compound as a pale yellow oil (3.4 g, quant.). $[\alpha]_D^{20}$ -4.2 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.71 (dd, *J* = 7.8, 1.2 Hz, 2H), 7.68 (dd, *J* = 7.8, 1.2 Hz, 2H), 7.50 - 7.37 (m, 6H), 4.51 (qd, *J* = 6.9, 6.9 Hz, 1H), 4.14 (dd, *J* = 6.4, 3.1 Hz, 1H), 3.93 - 3.84 (m, 2H), 3.77 (dd, *J* = 3.1, 3.1 Hz, 1H), 3.16 (dd, *J* = 14.5, 1.8 Hz, 1H), 2.80 - 2.17 (m, 2H), 1.48 (s, 9H), 1.23 (d, *J* = 7.1 Hz, 3H), 1.10 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.44, 135.98, 135.82, 133.86, 133.38, 130.00, 128.22, 128.22, 128.01, 127.86, 127.85, 127.09, 127.09, 79.83, 72.46, 70.70, 66.59, 49.83, 39.67, 28.59, 27.09, 19.34, 12.04. IR (neat, cm^{-1}): 3412, 2832, 2858, 1662, 1426, 1365, 1158, 1092, 1017, 755, 740, 700; HRMS: calculated for $C_{27}H_{39}NO_5Si$ [M+H$^+$] 486.26703, found: 486.26669.

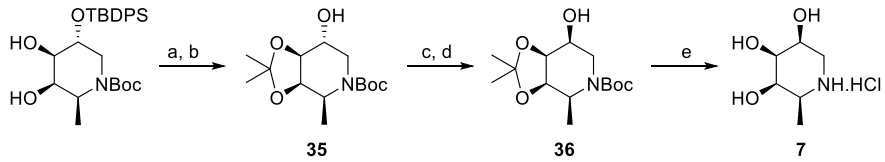


***tert*-Butyl (2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxy-2-methylpiperidine-1-carboxylate:** The TBDPS-ether from above (1.5 g, 3.1 mmol, 1.0 eq.) was dissolved in THF (30 mL) and TBAF·$3H_2O$ (2.8 g, 8.8 mmol, 2.7 eq.) was added at room temperature. The reaction was stirred at ambient temperature overnight. TLC indicated complete conversion and the mixture was concentrated. The crude compound was purified by silica gel column chromatography (50%→100%, EtOAc in pentane) to afford the title compound as a colorless oil (727 mg, 2.9 mmol, 94%). $[\alpha]_D^{20}$ +19 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, CD_3OD): *δ* ppm 4.28 (dq, *J* = 6.9, 6.9 Hz, 1H), 3.86 (dd, *J* = 6.9, 2.9 Hz, 1H), 3.85 – 3.75 (m, 3H), 3.27 (dd, *J* = 14.2, 1.7 Hz, 1H), 1.46 (s, 9H), 1.25 (d, *J* = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): *δ* ppm 157.62, 81.17, 73.43, 71.01, 67.57, 52.28, 40.98, 28.90, 12.71; IR (neat, cm^{-1}): 3400 - 3200, 2977, 2931, 1659, 1420, 1365, 1315, 1252, 1158, 1069, 1044, 1015, 732; HRMS: calculated for $C_{11}H_{21}NO_5$ [M+H$^+$] 248.14925, found: 248.14920.



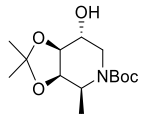
(2*S*,3*R*,4*S*,5*R*)-2-methylpiperidine-3,4,5-triol hydrochloride (6): The Boc-protected-imino sugar from above (645 mg, 2.6 mmol, 1.0 eq.) was dissolved in a mixture of MeOH (20 mL) and aq. 6.0 M HCl (3.0 mL) and stirred overnight at room temperature. The mixture was concentrated to afford the title compound **6** as a white foam (366 mg, 2.0 mmol, 76%). $[\alpha]_D^{20}$ -36 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, D_2O): *δ* ppm 4.04 – 3.92 (m, 2H), 3.60 (dd, *J* = 9.9, 2.8 Hz, 1H), 3.45 – 3.37 (m, 2H), 2.81 (t, *J* = 12.0 Hz, 1H), 1.30 (d, *J* = 6.7 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): *δ* ppm 73.04, 69.75, 64.29, 54.92, 46.01, 14.01. IR (neat, cm^{-1}): 3400 - 3200, 2942, 2816, 2464, 1457, 1388, 1076, 1003; HRMS: calculated for $C_6H_{13}NO_3$ [M+H$^+$] 148.09682, found: 148.09658.

Scheme 4: Preparation of iminosugar **7** from intermediate **34**.

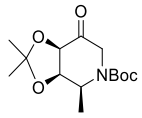


35 **36** **7**

Reagents and conditions: (a) Acetone/2,2-dimethoxypropane, $BF_3{\cdot}EtO_2$, 5 °C; (b) TBAF, THF; (c) Dess-Martin periodinane, DCM; (d) $NaBH_4$, EtOH, -78 °C→RT; (e) HCl (6.0 M in H_2O), MeOH.

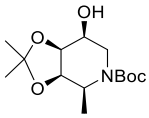


***tert*-Butyl(3*R*,4*S*,7*R*,7*S*)-7-hydroxy-2,2,4-trimethyltetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-carb-oxylate (35):** The diol derived from diacetate **32** (2.2 g, 4.5 mmol, 1.3 eq.) was dissolved in a mixture of acetone (40 mL) and 2,2-dimethoxypropane (10 ml) and cooled to 5 °C. Boron trifluoride diethyl etherate (200 µL) was added and the reaction stirred on an ice bath during 30 minutes and at room temperature for 18 hours. The reaction was quenched with TEA (2.0 mL) and diluted with EtOAc (125 mL). The mixture was washed with brine (60 mL), dried with $MgSO_4$, filtered and evaporated. The crude product was purified by silica gel column chromatography (1%→5%, EtOAc in pentane) to afford the title compound as a yellow oil (2.0 g, 3.8 mmol, 85%). The TBDPS-protected compound (1.8 g, 3.5 mmol, 1.0 eq.) was dissolved in THF (40 mL) and TBAF·3H_2O (3.4 g, 10 mmol, 3.0 eq.) was added and the reaction stirred at room temperature for 24 hours. TLC analysis confirmed complete conversion. The mixture was diluted with EtOAc (150 mL) and washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered and concentrated. The crude mixture was purified by silica gel column chromatography (5%→25%, EtOAc in pentane) to afford compound **35** (0.78 g, 2.7 mmol, 77%). $[\alpha]_D^{20}$ +2.0 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.33 (dd, *J* = 6.8, 5.8 Hz, 1H), 4.16 – 4.10 (m, 1H), 4.08 (dd, *J* = 6.8, 3.1 Hz, 1H), 3.90 (dd, *J* = 6.6, 3.1 Hz, 1H), 3.64 – 3.54 (m, 1H), 3.44 (dd, *J* = 13.5, 3.1 Hz,1H), 2.82 – 2.45 (m 1H), 1.48 (s, 3H), 1.46 (s, 9H), 1.34 (s , 3H), 1.28 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 156.58, 108.79, 80.26, 77.33, 74.00, 68.39, 47.71, 42.98, 28.52, 26.65, 24.63, 17.56; IR (neat, cm^{-1}): 3500 – 3200, 2929, 1672, 1405, 1381, 1367, 1253, 1215, 1166, 1049, 751; HRMS: calculated for $C_{14}H_{25}NO_5$ [M+H$^+$] 288.18055, found: 288.18061.

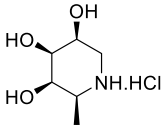


***tert*-Butyl(3*R*,4*S*,7*R*)-2,2,4-trimethyl-7-oxotetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-carboxylate:** The alcohol **35** (690 mg, 2.4 mmol, 1.0 eq.) was dissolved in DCM (30 mL) and at 0 °C Dess-Martin periodinane (1.8 g, 4.3 mmol, 1.8 eq.) was added. The reaction mixture was allowed to warm up to room temperature and stirred overnight. TLC indicated complete conversion and the reaction was quenched with a mixture of sat. aq. $NaHCO_3$ (30 mL) and sat. aq. $Na_2S_2O_3$ (30 mL) and stirred for 5 minutes. The mixture was extracted with EtOAc (2 x 50 mL), dried with $MgSO_4$, filtered and concentrated. The crude product was purified by silica gel column chromatography (5%→10%, EtOAc in pentane) to afford the title compound (613 mg, 2.1 mmol, 89%). $[\alpha]_D^{20}$ -13 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.92 – 4.75 (m, 2H), 4.55 (d, *J* = 9.0 Hz, 1H), 4.51 – 4.29 (m, 1H), 3.74 (d, *J* = 19.2 Hz, 1H), 1.53 (s, 3H), 1.48 (s, 9H), 1.39 (s, 3H), 1.16 (d, *J* = 7.0 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 204.12, 154.05, 110.92, 81.12, 77.39, 74.47, 50.20, 28.20, 26.05, 24.33, 12.70. IR (neat, cm^{-1}):

2979, 2932, 1699, 1369, 1247, 1219, 1159, 1016, 773, 745; HRMS: calculated for $C_{14}H_{23}NO_5$ [M+H$^+$] 286.16490, found: 286.16488.

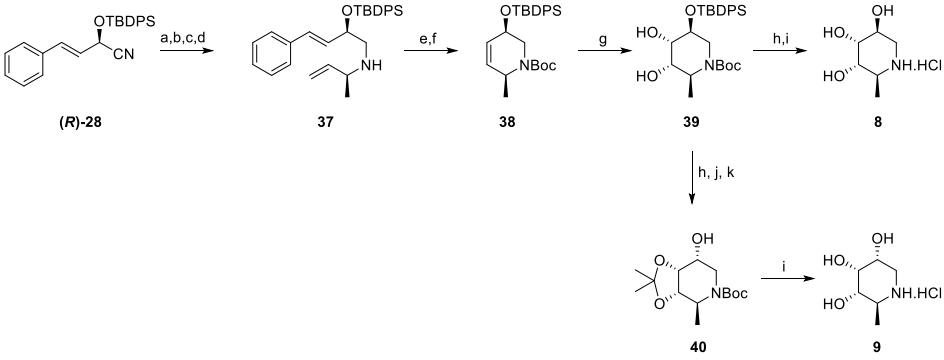


***tert*-Butyl(3*R*,4*S*,7*S*,7*S*)-7-hydroxy-2,2,4-trimethyltetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-carboxylate (36):** The ketone from above (456 mg, 1.6 mmol, 1.0 eq.) was dissolved in EtOH (20 mL) and at -78 °C $NaBH_4$ (48 mg, 1.3 mmol, 0.80 eq.) was added and the reaction was allowed to warm up slowly over night. TLC indicated complete conversion and the mixture was diluted with EtOAc (50 mL), washed subsequently with water (30 mL) and brine (20 mL), dried with $MgSO_4$, filtered and evaporated to give a crude product that was purified by silica gel column chromatography (25%→100%, EtOAc in pentane) to afford compound **36** (184 mg, 0.64 mmol, 40%). $[\alpha]_D^{20}$ +2.0 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 4.43 – 4.33 (m, 2H), 4.01 (dq, *J* = 6.6, 6.6 Hz, 1H), 3.88 (dd, *J* = 12.0, 3.8 Hz, 1H), 3.61 (ddd, *J* = 10.2, 4.3, 4.3 Hz, 1H), 2.99 (t, *J* = 12.0 Hz, 1H), 2.92 – 2.60 (m, 1H), 1.53 (s, 3H), 1.46 (s, 9H), 1.39 (s, 3H), 1.34 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 155.13, 108.78, 80.12, 76.03, 72.85, 66.35, 47.44, 41.89, 28.48, 26.42, 24.80, 16.77; IR (neat, cm^{-1}): 3500 – 3200, 2980, 2934, 1688, 1393, 1366, 1251, 1212, 1159, 1023, 867, 773, 734; HRMS: calculated for $C_{14}H_{25}NO_5$ [M+H$^+$] 288.18056, found: 288.18057.

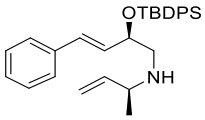


(2*S*,3*R*,4*S*,5*S*)-2-methylpiperidine-3,4,5-triol hydrochloride (7): The Boc-protected-iminosugar **36** (144 mg, 0.50 mmol, 1.0 eq.) was dissolved in a mixture of MeOH (20 mL) and aq. 6.0 M HCl (3.0 mL) and stirred over night at room temperature. The mixture was concentrated to afford the title compound **7** as a white foam (92 mg, quant.). $[\alpha]_D^{20}$ +13 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, D_2O): δ ppm 4.20 – 4.16 (m, 1H), 4.00 – 3.97 (m, 1H), 3.83 (t, *J* = 3.3 Hz, 1H), 3.40 (dd, *J* = 13.8, 2.8 Hz, 1H), 3.41 – 3.36 (m, 1H), 3.22 (dd, *J* = 13.8, 1.2 Hz, 1H), 1.36 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): δ ppm 70.46, 67.34, 66.21, 55.03, 48.21, 14.12; IR (neat, cm^{-1}): 3676, 3400 – 3200, 2971, 2925, 1724, 1568, 1148, 1407, 1394, 1250, 1118, 1075, 1066; HRMS: calculated for $C_6H_{13}NO_3$ [M+H$^+$] 148.09682, found: 148.09675.

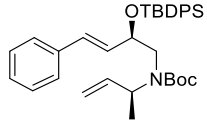
Scheme 5: Preparation of iminosugars **8** and **9**.



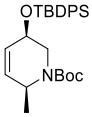
Reagents and conditions: (a) DIBAL-H, -78→5 °C; (b) MeOH, -90 °C; (c) amine **(*S*)-29**, NaOMe, 18 h; (d) $NaBH_4$, 5 °C→RT, 4 h; (e) Boc_2O, 50 °C; (f) Grubbs 1st generation, DCM, reflux; (h) i) $K_2OsO_4{\cdot}2H_2O$, NMO, acetone/H_2O; ii) TBAF, THF; (i) HCl (6.0 M in H_2O), MeOH; (j) Dess-Martin periodinane, DCM; (k) $NaBH_4$, EtOH, -78 °C→RT.



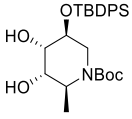
(*R, E*)-*N*-((*S*)-But-3-en-2-yl)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-amine (37): Prepared as described for compound **30** (18 mmol scale, yield 6.8 g, 83%). $[\alpha]_D^{20}$ -92 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.71 (d, *J* = 7.1 Hz, 2H), 7.68 (d, *J* = 7.1 Hz, 2H), 7.48 - 7.09 (m, 11H), 6.21 (d, *J* = 16.0 Hz, 1H), 6.10 (dd, *J* = 16.0, 6.9 Hz, 1H), 5.57 (ddd, *J* = 17.4, 10.3, 7.8 Hz, 1H), 4.98 (d, *J* = 10.3 Hz, 1H), 4.95 (d, *J* = 7.8 Hz, 1H), 4.46 (dd, *J* = 12.0, 6.3 Hz, 1H), 3.04 (c, *J* = 6.7 Hz, 1H), 2.78 (dd, *J* = 12.0, 6.7 Hz, 1H), 2.59 (dd, *J* = 12.0, 5.2 Hz, 1H), 1.08 (s, 9H), 1.02 (d, *J* = 6.4Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 142.45, 136.76, 136.03, 135.92, 135.36, 134.10, 134.07, 133.96, 131.20, 130.79, 129.72, 129.16, 128.40, 127.65, 127.50, 126.48, 114.51, 74.07, 56.25, 53.64, 27.16, 21.77, 19.43; IR (neat, cm^{-1}): 2931, 2858, 1219, 1112, 772, 702; HRMS: calculated for $C_{30}H_{37}NOSi$ [$M+H^+$] 456.27172, found: 456.27144.



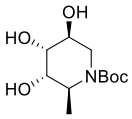
***tert*-Butyl((*S*)-but-3-en-2-yl)((*R,E*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-yl) carba- mate:** Prepared as described for **31** (3.9 mmol scale, yield 2.25 g, quant.). $[\alpha]_D^{20}$ -68 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.69 (d, *J* = 6.8 Hz, 2H), 7.65 (d, *J* = 6.8 Hz, 2H), 7.48 – 7.09 (m, 11H), 6.17 – 6.00 (m, 2H), 5.71 (ddd, *J* = 16.1, 10.3, 4.8 Hz, 1H), 5.08 – 4.78 (m, 2H), 4.47 – 4.34 (m, 1H), 3.59 – 3.40 (m, 1H), 3.37 – 3.18 (m, 1H), 3.15 – 3.00 (m, 1H), 1.42 – 1.22 (m, 9H), 1.07 (s, 9H), 1.06 - 1.04 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.61, 143.50, 136.15, 136.05, 135.68, 131.05, 130.89, 129.81, 129.71, 128.02, 127.94, 127.73, 127.58, 126.58, 114.89, 79.64, 73.93, 50.39, 41.47, 28.44, 27.21, 19.44, 17.40; IR (neat, cm^{-1}): 3072, 2932, 2858, 1693, 1266, 1167, 1113, 1070, 741, 702; HRMS: calculated for [$C_{35}H_{45}NO_3Si$ [$M+H^+$] 556.32415, found: 556.32436.



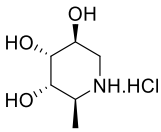
***tert*-Butyl (3*R*, 6*S*)-3-((*tert*-butyldiphenylsilyl)oxy)-6-methyl-3, 6-dihydropyridine-1(2*H*)-carboxy-late (38):** Prepared as described for **32**. Compound **38** was obtained as a clear oil (3.8 mmol scale, yield 1.6 g, 96%). $[\alpha]_D^{20}$ +22 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) *δ* ppm 7.75 - 7.60 (m, 4H), 7.47 – 7.32 (m, 6H), 5.76 – 5.37 (m, 2H), 4.44 – 4.28 (m, 1H), 4.28 – 4.15 (m, 1H), 4.09 – 3.88 (m, 1H), 2.80 – 2.67 (m, 1H), 1.34 (s, 9H), 1.16 (d, *J* = 6.6 Hz, 3H), 1.08 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$) *δ* ppm 153.93, 135.91, 135.80, 135.46, 130.86, 130.57, 129.88, 129.78, 128.31, 127.79, 127.57, 126.52, 79.61, 65.63, 47.42, 43.99, 28.48, 27.06, 19.31, 17.85. IR (neat, cm^{-1}): 3072, 2932, 2858, 1697, 1453, 1366, 1162, 1112, 763, 741, 702; HRMS: calculated for $C_{27}H_{37}NO_3Si$ [$M+H^+$] 452.26155, found: 452.26185.



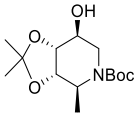
***tert*-Butyl(2*S*,3*S*,4*S*,5*S*)-5-((*tert*-butyldiphenylsilyl)oxy)-3,4-dihydroxy-2-methylpiperidine-1-carbox ylate (39):** Prepared as described in the Upjohn procedure concerning compound **32**. Compound **37** was obtained as a clear oil (1.1 mmol scale, yield 430 mg, 79%). $[\alpha]_D^{20}$ +21 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.70 (dd, *J* = 7.9, 1.5 Hz, 2H), 7.68 (dd, *J* = 7.9, 1.5 Hz, 2H), 7.48 - 7.36 (m, 6H), 4.55 - 4.29 (m, 1H), 4.29 - 3.96 (m, 1H), 3.90 (td, J = 10.3, 5.4 Hz, 1H), 3.84 - 3.74 (m, 1H), 3.71 (dd, *J* = 8.8, 2.9 Hz, 1H), 2.77 (dd, *J* = 13.2, 10.7 Hz, 1H), 2.38 - 2.00 (m, 2H), 1.34 (s, 9H), 1.13 (d, *J* = 7.3 Hz, 3H), 1.09 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.32, 135.92, 135.81, 133.75, 130.16, 130.13, 128.03, 127.97, 80.03, 73.46, 72.52, 70.24, 48.03, 41.78, 28.42, 27.14, 19.46, 14.18; IR (neat, cm^{-1}): 3020, 1215, 1111, 770, 748, 668; HRMS: calculated for $C_{27}H_{39}NO_5Si$ [$M+H^+$] 486.26703, found: 486.26723.



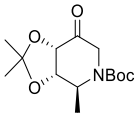
***tert*-Butyl (2*S*, 3*S*, 4*R*, 5*S*)-3,4,5-trihydroxy-2-methylpiperidine-1-carboxylate:** The TBDPS-ether **39** (1.9 g, 3.9 mmol, 1.0 eq.) was dissolved in THF (40 mL) and TBAF·$3H_2O$ (3.5 g, 11 mmol, 2.8 eq.) was added at room temperature. The reaction was stirred at ambient temperature overnight. TLC indicated complete conversion and the mixture was concentrated. The crude compound was purified by silica gel column chromatography (50%→100%, EtOAc in pentane) to afford the title compound as a colorless oil (957 mg, 3.8 mmol, 98%). $[\alpha]_D^{20}$ +38 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, CD_3OD): *δ* ppm 4.35 (m, 1H), 4.08 (dd, *J* = 13.0, 5.4 Hz, 1H), 3.83 – 3.69 (m, 2H), 3.55 (dd, *J* = 9.5, 3.1 Hz, 1H), 2.74 – 2.61 (m, 1H), 1.46 (s, 9H), 1.15 (d, *J* = 7.3 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): *δ* ppm 157.27, 81.38, 73.99, 73.38, 68.19, 56.32, 45.39, 28.84, 14.45; IR (neat, cm^{-1}): 3020, 1215, 770, 747; HRMS: calculated for $C_{11}H_{21}NO_5$ [M+H$^+$] 248.14925, found: 248.14924.



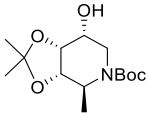
(2*S*,3*S*,4*R*,5*S*)-2-methylpiperidine-3,4,5-triol hydrochloride (8): Prepared as described for iminosugar **6**. Boc-iminosugar from above was used and **8** was obtained as a white foam (2.7 mmol scale, yield 445 mg, 90%). $[\alpha]_D^{20}$ -10 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, D_2O): *δ* ppm 4.14 (ddd, *J* = 4.9, 3.3, 3.3 Hz, 1H), 4.00 (dd, *J* = 4.1, 3.1 Hz, 1H), 3.85 (dd, *J* = 9.9, 3.1 Hz, 1H), 3.39 (dq, *J* = 9.9, 6.6 Hz, 1H), 3.33 (dd, *J* = 13.6, 1.9 Hz, 1H), 3.17 (dd, *J* = 13.6, 3.1 Hz, 1H), 1.39 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): 68.45, 68.09, 66.32, 51.07, 43.88, 12.72; IR (neat, cm^{-1}): 3369, 3271, 3022, 2952, 1727, 1583, 1437, 1260, 1086, 1069, 1051, 965, 701; HRMS: calculated for $C_6H_{13}NO_3$ [M+H$^+$] 148.09682, found: 148.09672.



***tert*-Butyl(3*S*,4*S*,7*S*,7*R*)-7-hydroxy-2,2,4-trimethyltetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-carb-oxylate:** Prepared as described en route towards **35** (scheme 2, steps b, c). The title compound was obtained as a clear oil (4.8 mmol scale, yield 1.25 g, 91%). $[\alpha]_D^{20}$ +48 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.68 – 4.52 (m, 1H), 4.05 (d, *J* = 5.4 Hz, 1H), 4.00 (t, *J* = 6.3 Hz, 1H), 3.93 (dd, *J* = 13.5, 4.7 Hz, 1H), 3.76 (m, 1H), 2.92 (br, 1H), 2.82 (dd, *J* = 13.5, 10.2 Hz, 1H), 1.48 (s, 3H), 1.47 (s, 9H), 1.35 (s, 3H), 1.20 (d, *J* = 7.3Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.43, 108.88, 80.31, 78.10, 77.85, 69.50, 48.18, 41.46, 28.48, 28.22, 26.20, 16.86; IR (neat, cm^{-1}): 3020, 1215, 748, 668; HRMS: calculated for $C_{14}H_{25}NO_5$ [M+H$^+$] 288.18055, found: 288.18053.

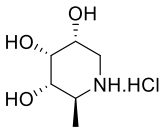


***tert*-Butyl(3*S*,4*S*,7*S*)-2,2,4-trimethyl-7-oxotetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-carboxylat-e:** Prepared as described en route towards **36** (scheme 2, step d). The title ketone was obtained as a white solid (0.50 mmol scale, yield 108 mg, 76%). $[\alpha]_D^{20}$ -2.4 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.76 – 4.57 (m, 2H), 4.36 (d, *J* = 6.6 Hz, 1H), 4.30 (d, *J* = 6.6 Hz, 1H), 3.66 (d, *J* = 18.5 Hz, 1H), 1.48 (s, 9H), 1.46 (s, 3H), 1.35 (s, 3H), 1.16 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 203.20, 157.70, 111.69, 80.86, 80.25, 75.54, 50.94, 49.23, 26.67, 26.18, 24.98, 15.47; IR (neat, cm^{-1}): 2980, 2935, 1737, 1693, 1408, 1367, 1221, 1157, 1049, 867; HRMS: calculated for $C_{14}H_{23}NO_5$ [M+H$^+$] 286.16490, found: 286.16478.



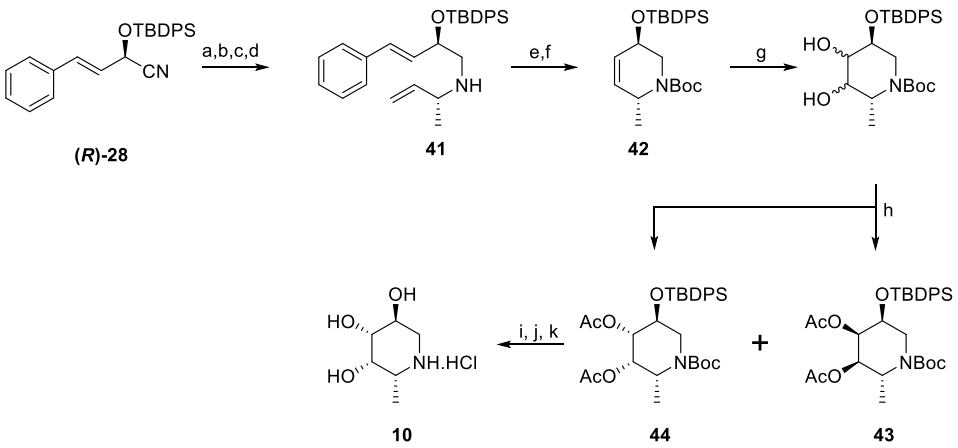
***tert*-Butyl(3*S*,4*S*,7*R*,7*R*)-7-hydroxy-2,2,4-trimethyltetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-c-arboxylate (40):** Prepared as described for alcohol **36** from the ketone described above. Alcohol **40** was obtained as a mixture of two diastereoisomers (ratio 93:7, 0.42 mmol scale, yield 104 mg, 86%). $[\alpha]_D^{20}$ +42 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.43 (dd, *J* = 6.8, 4.3 Hz, 1H), 4.33

(dd, *J* = 7.4, 2.0 Hz, 1H), 4.27 – 4.19 (m, 1H), 3.96 (ddd, *J* = 11.3, 4.5, 4.5 Hz, 1H), 3.61 (dd, *J* = 11.7, 4.4 Hz, 1H), 3.08 (t, *J* = 11.7 Hz, 1H), 2.68 – 2.48 (m, 1H), 1.50 (s, 3H), 1.47 (s, 9H), 1.38 (s, 3H), 1.17 (d, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.14, 108.85, 79.82, 77.35, 71.79, 65.03, 47.68, 42.46, 28.49, 26.23, 24.37, 19.10; IR (neat, cm^{-1}): 3437, 2978, 2935, 1683, 1401, 1369, 1255, 1212, 1169, 1049, 877, 731; HRMS: calculated for $C_{14}H_{25}NO_5$ [M+H$^+$] 288.18056, found: 288.18063.

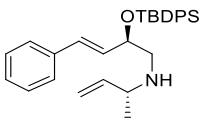


(2*S*,3*S*,4*R*,5*R*)-2-methylpiperidine-3,4,5-triol hydrochloride (9): Prepared as described for iminosugar **6** from Boc-protected-iminosugar **38**. Iminosugar **8** was obtained as a colorless foam (1.7 mmol scale, yield 311 mg, quant.) in a *d.r.* of 93:7. *N*-Boc-protection, column chromatography and subsequent deprotection (HCl/MeOH) afforded the diastereomerically pure compound **9**. $[\alpha]_D^{20}$ -20 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, D_2O): *δ* ppm 4.19 – 4.12 (m, 1H), 4.00 (ddd, *J* = 11.5, 4.9, 2.6 Hz, 1H) 3.62 (dd, *J* =10.4, 2.5 Hz, 1H), 3.33 (dq, *J* = 4.9, 4.9 Hz, 1H), 3.25 (dd, *J* = 12.0, 4.9 Hz, 1H), 3.09 (t, *J* = 12.0 Hz, 1H), 1.37 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): *δ* ppm 69.95, 69.61, 64.72, 49.92, 41.65, 14.16; IR (neat, cm^{-1}): 3400 - 3200, 2939, 2804, 1456, 1158, 1106, 1072, 1043, 1018, 996, 962, 816, 707; HRMS: calculated for $C_6H_{13}NO_3$ [M+Na$^+$] 170.07876, found: 170.07865.

Scheme 6: Preparation of iminosugar **10**.

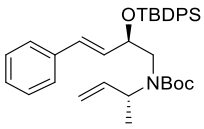


Reagents and conditions: (a) DIBAL-H, -78→5 °C; (b) MeOH, -90 °C; (c) amine **(*R*)-29**, NaOMe, 18 h; (d) $NaBH_4$, 5 °C→RT, 4 h; (e) Boc_2O, 50 °C; (f) Grubbs 1st generation, DCM, reflux; (g) $K_2OsO_4{\cdot}2H_2O$, NMO, acetone/H_2O; (h) Ac_2O, pyridine, DMAP, 0 °C→RT; (i) K_2CO_3, MeOH; (j) TBAF, THF; (k) HCl (6.0 M in H_2O), MeOH.

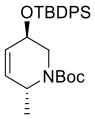


(*R,E*)-*N*-((*R*)-But-3-en-2-yl)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-amine (41): Prepared as described for compound **30**. (35 mmol scale, yield 13 g, 83%). $[\alpha]_D^{20}$ -109 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.72 – 7.62 (m, 4H), 7.43 – 7.15 (m, 11H), 6.20 (d, *J* = 16.0 Hz, 1H), 6.10 (dd, *J* = 16.0, 7.0 Hz, 1H), 5.60 (ddd, *J* = 16.8, 10.2, 7.0 Hz, 1H), 5.01 (d, *J*

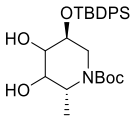
= 16.8 Hz, 1H), 4.97 (d, *J* = 10.2 Hz, 1H), 4.44 (dt, *J* = 12.6, 6.2 Hz, 1H), 3.10 (m, 1H), 2.72 (dd, *J* = 11.8, 6.3 Hz, 1H), 2.69 (dd, *J* = 11.8, 5.6 Hz, 1H), 1.08 (s, 9H), 1.05 (t, *J* = 6.8 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 142.46, 136.88, 136.14, 136.06, 135.49, 134.24, 134.14, 131.40, 130.83, 129.79, 129.69, 128.51, 127.71, 127.59, 126.61, 114.69, 74.45, 56.72, 53.77, 27.25, 21.58, 19.52; IR (neat, cm^{-1}): 3500 - 3200, 2967, 1653, 1111, 1055, 1033, 1015, 741, 700; HRMS: calculated for $C_{30}H_{37}NOSi$ [M+H$^+$] 456.27172, found: 456.27139.



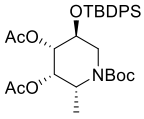
***tert*-Butyl((*R*)-but-3-en-2-yl)((*R,E*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-yl)carba-mate:** Prepared as described for **29** (29 mmol scale, yield 16 g, 99%). $[\alpha]_D^{20}$ -25 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.78 – 7.62 (m, 4H), 7.47 – 7.10 (m, 11H), 6.15 - 6.05 (m, 2H), 5.78 (ddd, *J* = 16.6, 10.6, 5.2 Hz, 1H), 5.00 – 4.86 (m, 2H), 4.55 – 4.38 (m, 1H), 3.59 – 3.37 (m, 1H), 3.37 – 3.20 (m, 1H), 3.20 – 3.04 (m, 1H), 1.40 - 1.20 (m, 9H), 1.08 (s, 9H), 1.08 – 1.04 (m, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 155.57, 146.87, 136.14, 135.12, 134.92, 131.13, 130.84, 129.78, 128.42, 127.68, 126.56, 79.64, 73.60, 50.16, 43.52, 28.45, 27.54, 19.43, 17.62; IR (neat, cm^{-1}): 2977, 2933, 2858, 1808, 1757, 1691, 1396, 1370, 1212, 1166, 1113, 1065, 739, 701; HRMS: calculated for $C_{35}H_{45}NO_3Si$ [M+H$^+$] 556.32415, found: 556.32416.



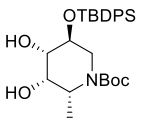
***tert*-Butyl (3*R*, 6*R*)-3-((*tert*-butyldiphenylsilyl)oxy)-6-methyl-3,6-dihydropyridine-1(2*H*)-carboxylate(42):** Prepared as described for compound **32** from the Boc-protected diene mentioned above. Compound **42** was obtained as a colorless oil (8.4 mmol scale, yield 3.6 g, 95%). $[\alpha]_D^{20}$ -153 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.72 (d, *J* = 7.7 Hz), 7.66 (d, *J* = 7.7 Hz, 2H), 7.46 – 7.32 (m, 6H), 5.71 – 5.62 (m, 1H), 5.58 – 5.47 (m, 1H), 4.64 – 4.48 (m, 1H), 4.28 – 4.09 (m, 1H), 4.07 – 4.00 (m, 1H), 2.96 – 2.76 (m, 1H), 1.50 (s, 9H), 1.08 (d, *J* = 6.9 Hz, 3H**),** 1.05 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 154.80, 135.94, 134.12, 129.80, 129.65, 127.78, 127.58, 79.45, 64.21, 46.44, 28.63, 27.03, 19.35, 17.27; IR (neat, cm^{-1}): 2978, 2933, 2858, 1808, 1757, 1691, 1470, 1212, 1166, 1113, 1065, 739, 701; HRMS: calculated for $C_{27}H_{37}NO_3Si$ [M+H$^+$] 452.26155, found: 452.26155.



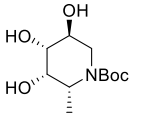
***tert*-Butyl(2*R*, 5*S*)-5-((tert-butyldiphenylsilyl)oxy)-3, 4-dihydroxy-2-methylpiperidine-1-carboxylate:** The procedure described for the Upjohn dihydroxylation of compound **32** afforded a 3 : 1 mixture of inseparable diastereoisomers (27 mmol scale, yield 10 g, 78%). To separate these diastereoisomers the mixture was directly converted into the diacetates.



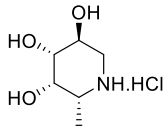
(2*R*,3*S*,4*S*,5*S*)-1-(*tert*-butoxycarbonyl)-5-((*tert*-butyldiphenylsilyl)oxy)-2-methylpiperidine-3,4-diyl di-acetate (44): Prepared as described for **34**. Compound **44** was obtained as a pale yellow oil (21 mmol scale, yield 7.2 g, 61%). $[\alpha]_D^{20}$ -10 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.73 – 7.67 (m, 4H), 7.46 – 7.35 (m, 6H), 5.44 (dd, *J* = 6.9, 3.2 Hz, 1H), 5.12 (dd, *J* = 3.2, 3.6 Hz, 1H), 4.54 (qd, *J* = 6.9, 6.9 Hz, 1H), 3.94 (d, *J* = 14.3 Hz, 1H), 3.77 (dd, *J* = 3.6, 1.5 Hz, 1H), 3.08 (dd, *J* = 14.3, 1.5 Hz, 1H) 2.03 (s, 3H), 1.92 (s, 3H), 1.47 (s, 9H), 1.20 (d, *J* = 6.9 Hz, 3H**),** 1.09 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 169.75, 169.33, 154.87, 135.95, 135.91, 133.11, 133.04, 130.01, 129.92, 127.83, 127.78, 80.00, 70.98, 68.67, 67.39, 48.07, 40.83, 28.46, 26.92, 20.97, 20.92, 19.25, 12.48; IR (neat, cm^{-1}): 3073, 2933, 2859, 1750, 1694, 1417, 1365, 1237, 1218, 1160, 1026, 753, 740, 701; HRMS: calculated for $C_{31}H_{43}NO_7Si$ [M+H$^+$] 570.28816, found: 570.28791.



***t*ert-Butyl(2*R*,3*S*,4*S*,5*S*)-5-((*tert*-butyldiphenylsilyl)oxy)-3,4-dihydroxy-2-methylpiperidine-1-carbo xy-late:** Prepared as described en route towards fucononojirimycin (**6**) and was obtained as a colorless oil (12 mmol scale, yield 5.5 g, 85%). $[\alpha]_D^{20}$ +5.4 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.68 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.64 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.45 – 7.32 (m, 6H), 4.48 (qd, *J* = 6.9, 6.9 Hz, 1H), 4.11 (dd, *J* = 6.4, 3.2 Hz, 1H), 3.89 – 3.80 (m, 2H), 3.74 (t, *J* = 3.1 Hz, 1H), 3.12 (dd, *J* = 14.5, 1.7 Hz, 1H), 2.81 (br, 1H), 2.44 (br, 1H), 1.44 (s, 9H), 1.19 (d, *J* = 7.1 Hz, 3H), 1.07 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.45, 135.95, 135.80, 133.81, 133.36, 129.97, 127.83, 79.84, 72.36, 70.61, 66.48, 49.81, 39.62, 28.57, 27.06, 19.32, 12.03; IR (neat, cm^{-1}): 3500 – 3200, 2932, 2859, 1663, 1426, 1365, 1156, 1093, 1017, 753, 701; HRMS: calculated for $C_{27}H_{39}NO_5Si$ [M+H$^+$] 486.26703, found: 486.26708.

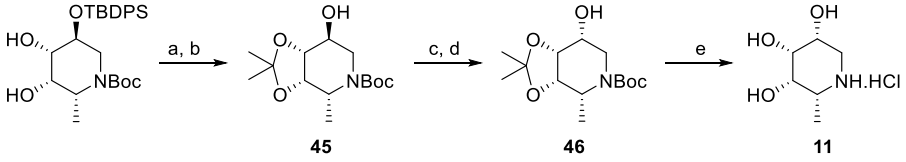


***tert*-Butyl(2*S*,3*R*,4*S*,5*R*)-3,4,5-trihydroxy-2-methylpiperidine-1-carboxylate:** Prepared as described for alcohol **35** and was obtained as a colorless oil (3.8 mmol scale, yield 860 mg, 92%). $[\alpha]_D^{20}$ -20 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, CD_3OD): *δ* ppm 4.28 (dq, *J* = 6.9, 6.9 Hz, 1H), 3.86 (dd, *J* = 6.9, 2.3 Hz, 1H), 3.85 – 3.75 (m, 3H), 3.27 (dd, *J* = 14.2, 1.5 Hz, 1H), 1.46 (s, 9H), 1.24 (d, *J* = 6.9 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): *δ* ppm 157.57, 81.14, 73.37, 70.95, 67.53, 52.23, 40.96, 28.90, 12.72; IR (neat, cm^{-1}): 3400 – 3200, 2978, 2931, 1660, 1421, 1366, 1157, 1068, 907, 729; HRMS: calculated for $C_{11}H_{21}NO_5$ [M+H$^+$] 248.14925, found: 248.14933.

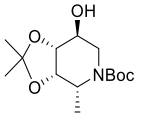


(2*R*,3*S*,4*R*,5*S*)-2-methylpiperidine-3,4,5-triol hydrochloride (10): Prepared as described for **5** and was obtained as a white foam (3.0 mmol scale, yield 549 mg, quant.). $[\alpha]_D^{20}$ +31 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, D_2O): *δ* ppm 4.07 – 3.98 (m, 2H), 3.64 (dd, *J* = 9.9, 3.0 Hz, 1H), 3.50 – 3.40 (m, 2H), 2.85 (t, *J* = 12.0 Hz, 1H), 1.35 (d, *J* = 6.7 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): *δ* ppm 73.07, 69.79, 64.33, 54.96, 46.05, 14.05; IR (neat, cm^{-1}): 3400 – 3200, 2939, 2805, 1583, 1444, 1386, 1159, 1074, 1022, 999, 708; HRMS: calculated for $C_6H_{13}NO_3$ [M+H$^+$] 148.09682, found: 148.09658.

Scheme 7: Preparation of iminosugar **11**.

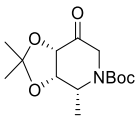


Reagents and conditions: (a) Acetone/2,2-dimethoxypropane, $BF_3{\cdot}EtO_2$, 5 °C; (b) TBAF, THF; (c) Dess-Martin periodinane, DCM; (d) $NaBH_4$, EtOH, -78 °C→RT; (e) HCl (6.0 M in H_2O), MeOH.

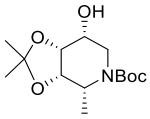


***tert*-Butyl(3*S*,4*R*,7*S*,7*R*)-7-hydroxy-2,2,4-trimethyltetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-car b- oxylate (45):** Prepared as described for alcohol **33** from the silyl ether mentioned above (4.6 mmol scale, yield 1.2 g, 85% for two steps). $[\alpha]_D^{20}$ -3.4 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.34 (dd, *J* = 6.9, 5.7 Hz, 1H), 4.13 (qd, *J* = 6.6, 6.6 Hz, 1H), 4.08 (dd, *J* = 6.5, 3.3 Hz, 1H), 3.96

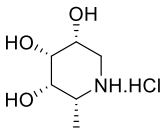
(ddd, *J* = 6.5, 6.5, 3.3 Hz, 1H), 3.60 (dd, *J* = 13.2, 6.5 Hz, 1H), 3.44 (dd, *J* = 13.2, 3.2 Hz, 1H), 2.55 – 2.36 (m 1H), 1.48 (s, 3H), 1.47 (s, 9H), 1.35 (s, 3H), 1.28 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 158.48, 108.84, 80.26, 77.38, 73.99, 68.53, 47.74, 42.97, 28.54, 26.67, 24.66, 17.56; IR (neat, cm^{-1}): 3500 – 3200, 2980, 2934, 1670, 1403, 1367, 1253, 1212, 1166, 1056, 868, 773; HRMS: calculated for $C_{14}H_{25}NO_5$ [M+H$^+$] 288.18055, found: 288.18059.



***tert*-Butyl(3*S*,4*R*,7*S*)-2,2,4-trimethyl-7-oxotetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-carboxylate:** Prepared as described en route towards compound **36** (scheme 2, step d). The title compound was obtained as a white solid (3.9 mmol scale, yield 919 mg, 82 %). $[\alpha]_D^{20}$ +13 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.89 – 4.71 (m, 2H), 4.52 (d, *J* = 9.0 Hz, 1H), 4.49 – 4.27 (m, 1H), 3.75 (d, *J* = 19.2 Hz, 1H), 1.51 (s, 3H), 1.49 (s, 9H), 1.39 (s , 3H), 1.16 (d, *J* = 7.0 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 204.28, 154.21, 111.07, 81.28, 80.25, 75.55, 74.62, 50.35, 48.12, 28.35, 26.20, 24.49, 12.86; IR (neat, cm^{-1}): 2979, 2935, 1741, 1696, 1409, 1368, 1381, 1162, 1081, 1031, 875; HRMS: calculated for [$C_{14}H_{23}NO_5$ + H]$^+$ 286.16490, found: 286.16488.

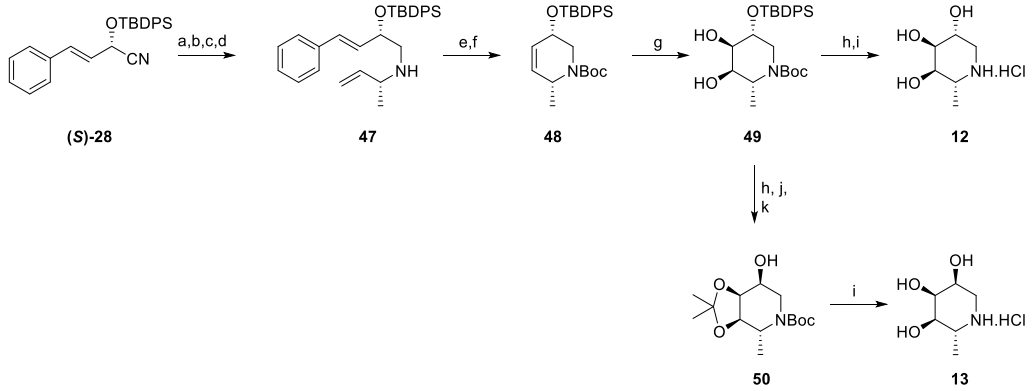


***tert*-Butyl(3*S*,4*R*,7*R*,7*R*)-7-hydroxy-2,2,4-trimethyltetrahydro-[1,3]dioxolo[4,5-c]pyridi-ne-5(4*H*)-carboxylate (46):** Prepared as described for **36** from the ketone mentioned above. Alcohol **46** was obtained (3.8 mmol scale, yield 610 mg, 56%). $[\alpha]_D^{20}$ -4.0 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.44 – 4.34 (m, 2H), 4.06 – 3.96 (m, 1H), 3.88 (dd, *J* = 12.0, 3.8 Hz, 1H), 3.68 – 3.56 (m, 1H), 2.99 (t, *J* = 12.0 Hz, 1H), 2.79 – 2.61 (m 1H), 1.53 (s, 3H), 1.46 (s, 9H), 1.39 (s, 3H), 1.34 (d, *J* = 6.6 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.14, 108.79, 80.13, 76.06, 72.83, 66.36, 47.44, 41.92, 28.49, 26.43, 24.81, 16.78; IR (neat, cm^{-1}): 3500 – 3200, 2979, 2934, 1688, 1406, 1393, 1367, 1250, 1221, 1156, 1061, 1046, 1033, 867; HRMS: calculated for $C_{14}H_{25}NO_5$ [M+ H$^+$] 288.18056, found: 288.18058.

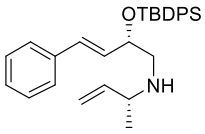


(2*R*,3*S*,4*R*,5*R*)-2-methylpiperidine-3,4,5-triol hydrochloride (11): Prepared as described for **6** from the protected iminosugar **46**. The title compound **10** was obtained as a white foam (1.5 mmol scale, yield 275 mg, quant.). $[\alpha]_D^{20}$ -16 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, D_2O): *δ* ppm 4.2 (ddd, *J* = 4.5, 2.8, 1.4 Hz, 1H), 4.00 – 3.97 (m, 1H), 3.84 (t, *J* = 3.5 Hz, 1H), 3.44 (dd, *J* = 13.7, 2.8 Hz, 1H), 3.42 – 3.37 (m, 1H), 3.23 (dd, *J* = 13.7, 1.4 Hz, 1H), 1.36 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): *δ* ppm 70.48, 67.33, 66.22, 55.05, 48.26, 14.20; IR (neat, cm^{-1}): 3676, 3348, 3130, 2971, 1559, 1443, 1312, 1277, 1249, 1121, 1056, 1007, 988; HRMS: calculated for [$C_6H_{13}NO_3$ + Na]$^+$ 170.07876, found: 170.07864.

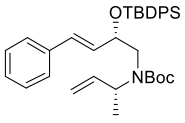
Scheme 8: Preparation of iminosugars **12** and **13**



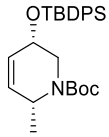
Reagents and conditions: (a) DIBAL-H, -78→5 °C; (b) MeOH, -90 °C; (c) amine **(*R*)-29**, NaOMe, 18 h; (d) $NaBH_4$, 5 °C→RT, 4 h; (e) Boc_2O, 50 °C; (f) Grubbs 1st generation, DCM, reflux; (h) $K_2OsO_4 \cdot 2H_2O$, NMO, acetone/H_2O; (h) TBAF, THF; (i) HCl (6.0 M in H_2O), MeOH; (j) Dess-Martin periodinane, DCM; (k) $NaBH_4$, EtOH, -78 °C→RT.



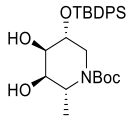
(*S,E*)-*N*-((*R*)-But-3-en-2-yl)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-amine (47): Preparation as described for compound **30** from **(*S*)-27** (3.6 g, 9.1 mmol) and **(*R*)-29·HCl** (3.2 g, 29 mmol, 3.3 eq.) afforded the target compound (3.6 g, 87 %). $[\alpha]_D^{23}$= +95 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.74 – 7.62 (m, 4H) 7.44 – 7.13 (m, 11H), 6.20 (d, *J* = 16.0 Hz, 1H), 6.11 (dd, *J* = 16.0, 6.9 Hz, 1H), 5.57 (ddd, *J* = 17.4, 10.5, 7.5 Hz, 1H), 4.98 (d, *J* = 17.4 Hz, 1H), 4.96 (d, *J* = 10.5 Hz, 1H), 4.43 (td, *J* = 6.8, 5.1 Hz, 1H), 3.04 (m, 1H), 2.78 (dd, *J* = 11.9, 6.7 Hz, 1H), 2.59 (dd, *J* = 11.9, 5.2 Hz, 1H), 1.65, (br, 1H), 1.08 (s, 9H), 1.02 (d, *J* = 6.4Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 142.57, 136.86, 136.10, 136.00, 134.99, 134.19, 134.06, 131.24, 130.91, 129.77, 129.66, 128.47, 127.70, 127.55, 126.56, 114.55, 74.13, 56.32, 53.72, 27.21, 21.84, 19.49. IR (neat, cm^{-1}): 3053, 2957, 2930, 2857, 1471, 1428, 1111, 772, 701; HRMS: calculated for $C_{30}H_{37}NOSi$ $[M+H^+]$ 456.27172, found: 456.27147.



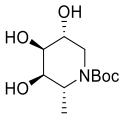
***tert*-Butyl((*R*)-but-3-en-2-yl)((*S,E*)-2-((*tert*-butyldiphenylsilyl)oxy)-4-phenylbut-3-en-1-yl)carbamate:** Prepared as described for compound **31** (3.7 mmol scale, 2.0 g in 98% yield). $[\alpha]_D^{20}$ +76 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.71 (d, *J* = 6.8 Hz, 2H), 7.65 (d, *J* = 6.8 Hz, 2H), 7.48 - 7.16 (m, 11H), 6.19 - 6.00 (m, 2H), 5.78 - 5.64 (m, 1H), 5.06 - 4.80 (m, 2H), 4.47 - 4.32 (m, 1H), 3.59 - 3.39 (m, 1H), 3.39 - 3.20 (m, 1H), 3.14 - 3.00 (m, 1H), 1.43 - 1.19 (m, 9H), 1.07 (s, 9H), 1.05 (d, *J* = 2.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 139.08, 136.15, 136.05, 135.51, 131.02, 130.87, 129.81, 129.71, 128.44, 128.36 127.73, 127.58, 126.56, 114.92, 79.63, 73.92, 50.33, 47.00, 28.43, 27.55, 19.43, 17.40; IR (neat, cm^{-1}): 3057, 2931, 2858, 1691, 1391, 1365, 1165, 1110, 1069, 740, 700; HRMS: calculated for $C_{35}H_{45}NO_3Si$ $[M+H^+]$ 556.32415, found: 556.32427.



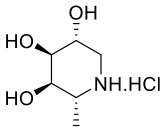
***tert*-Butyl(3*S*,6*R*)-3-((*tert*-butyldiphenylsilyl)oxy)-6-methyl-3,6-dihydropyridine-1(2*H*)-carboxylate (48):** Prepared as described for compound **32** (3.6 mmol scale, 1.6 g, 98%). $[\alpha]_D^{20}$ -12 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.74 - 7.63 (m, 4H), 7.46 - 7.32 (m, 6H), 5.72 - 5.42 (m, 2H), 4.44 - 4.28 (m, 1H), 4.28 - 4.16 (m, 1H), 3.98 (dd, *J* = 12.5, 5.6 Hz, 1H), 2.79 - 2.68 (m, 1H), 1.35 (s, 9H), 1.16 (d, *J* = 6.7 Hz, 3H**),** 1.08 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 153.92, 135.79, 134.91, 130.84, 130.55, 129.87, 128.77, 127.78, 126.59, 79.65, 65.60, 47.40, 43.96, 28.44, 27.21, 19.44, 17.82; IR (neat, cm^{-1}): 2963, 2930, 2858, 1697, 1427, 1410, 1365, 1157, 1109, 1059, 740; HRMS: calculated for $C_{27}H_{37}NO_3Si$ [M+H$^+$] 452.26155, found: 452.26158.



***tert*-Butyl(2*R*,3*R*,4*R*,5*R*)-5-((*tert*-butyldiphenylsilyl)oxy)-3,4-dihydroxy-2-methylpiperidine-1-carbo xy-late (49):** Compound **48** (6.9 g, 15 mmol, 1.0 eq.) was dissolved in a mixture of acetone (70 mL) and water (70 mL) and cooled to -10 °C. *N*-Methylmorpholine-*N*-oxide monohydrate (6.7 g, 50 mmol, 3.3 eq.) and $K_2OsO_4.2H_2O$ (60 mg, 0.16 mmol, 1.1 mol %) were added subsequently. After 24 - 48 hours TLC analysis showed complete conversion of the starting material **48**. The reaction was quenched with an aq. sat. Na_2SO_3 solution (100 mL) and stirred for 30 min. The mixture was diluted with water (100 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with aq. 0.60 M HCl, sat. $NaHCO_3$ and brine. After drying (Na_2SO_4), filtering and evaporation of the solvent, the silicagel column chromatography (10%→50%, EtOAc in pentane) afforded the compound **49** as a colorless oil (6.40 g, 1.3 mmol, 86%). $[\alpha]_D^{20}$ -34 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.71 (dd, *J* = 7.9, 1.5 Hz, 2H), 7.66 (dd, *J* = 7.9, 1.5 Hz, 2H), 7.48 - 7.34 (m, 6H), 4.56 - 4.26 (m, 1H), 4.26 - 3.95 (m, 1H), 3.90 (td, *J* = 10.3, 5.2 Hz, 1H), 3.83 - 3.73 (m, 1H), 3.71 (dd, *J* = 8.8, 3.0 Hz, 1H), 2.77 (dd, *J* = 13.3, 10.3 Hz, 1H), 2.40 - 2.09 (m, 2H), 1.49 - 1.19 (s, 9H), 1.13 (d, *J* = 7.3 Hz, 3H), 1.09 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.32, 135.90, 135.81, 133.73, 130.13, 130.10, 128.00, 127.95, 80.03, 73.41, 72.50, 70.21, 48.65, 43.18, 28.40, 27.13, 19.45 , 14.15; IR (neat, cm^{-1}): 3300, 3020, 2254, 1683, 1112, 904, 725; HRMS: calculated for $C_{27}H_{39}NO_5Si$ [M+H$^+$] 486.26703, found: 486.26736.

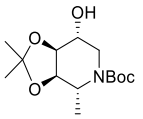


***tert*-Butyl(2*R*,3*R*,4*S*,5*R*)-3,4,5-trihydroxy-2-methylpiperidine-1-carboxylate:** The silyl ether **49** (1.7 g, 3.5 mmol, 1.0 eq.) was dissolved in THF (30 mL) and TBAF·$3H_2O$ (3.5 g, 11 mmol, 3.1 eq.) was added at room temperature. The reaction was stirred at ambient temperature overnight. TLC indicated complete conversion and the mixture was concentrated. The crude compound was purified by silica gel column chromatography (50%→100%, EtOAc in pentane) to afford the title compound as a clear oil (794 mg, 3.2 mmol, 91%). $[\alpha]_D^{20}$ -40 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, CD_3OD): *δ* ppm 4.36 (m, 1H), 4.09 (dd, *J* = 13.2, 5.6 Hz, 1H), 3.78 – 3.71 (m, 2H), 3.55 (dd, *J* = 9.6, 2.4 Hz, 1H), 2.67 (t, *J* = 12.5 Hz, 1H), 1.46 (s, 9H), 1.16 (d, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, CD_3OD): *δ* ppm 157.28, 81.41, 74.02, 73.41, 68.22, 54.77, 46.40, 28.85, 14.45; IR (neat, cm^{-1}): 3400 - 3200, 2977, 2932, 1665, 1419, 1366, 1167, 1074, 731; HRMS: calculated for $C_{11}H_{21}NO_5$ [M+H$^+$] 248.14925, found: 248.14920.

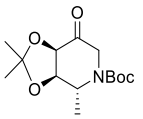


(2*R*,3*R*,4*S*,5*R*)-2-methylpiperidine-3,4,5-triol hydrochloride (12): *N*-Boc-protected-iminosugar from above (502 mg, 2.0 mmol, 1.0 eq.) was dissolved in a mixture of MeOH (20 mL) and aq. 6.0 M HCl (3.0 mL) and the reaction was stirred overnight at room temperature. TLC indicated complete conversion and the mixture was concentrated to afford the title iminosugar **12** as a white foam (317 mg, 1.7 mmol, 86%). $[\alpha]_D^{20}$ +13 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, D_2O): *δ* ppm 4.16 (dd, *J* = 6.4, 4.8

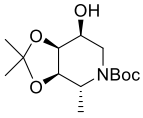
Hz, 1H), 4.02 (dd, *J* = 3.2, 3.2 Hz, 1H), 3.88 (dd, *J* = 9.8, 3.2 Hz, 1H), 3.42 (dq, *J* = 9.8, 6.7 Hz, 1H), 3.36 (m, 1H), 3.20 (dd, *J* = 13.4, 3.2 Hz, 1H), 1.47 (d, *J* = 6.7 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): *δ* ppm 68.50, 68.15, 66.36, 51.16, 43.95, 14.26; IR (neat, cm^{-1}): 3271, 3022, 2951, 2914, 1583, 1438, 1260, 1086, 1069, 1051, 965; HRMS: calculated for $C_6H_{13}NO_3$ [M+H$^+$] 148.09682, found: 148.09674.



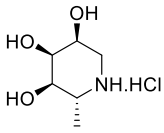
***tert*-Butyl(3*R*,4*R*,7*R*,7*S*)-7-hydroxy-2,2,4-trimethyltetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-carboxylate:** Preparation from TBDPS-ether **49** as described en route towards compound **40** (9.4 mmol scale), afforded the title compound (2.3 g, 7.9 mmol, 84% over two steps). $[\alpha]_D^{20}$ -38 (*c* = 0.3, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.64 – 4.54 (m, 1H), 4.05 (d, *J* = 5.8 Hz, 1H) 4.00 (t, *J* = 5.8 Hz, 1H), 3.93 (dd, *J* = 13.6, 4.9 Hz, 1H), 3.76 (ddd, *J* = 10.2, 6.4, 4.9 Hz, 1H), 2.53 – 2.19 (br, 1H), 2.82 (dd, J = 13.6, 10.2 Hz, 1H), 1.49 – 1.45 (m, 12H), 1.35 (s, 3H), 1.26 (d, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.42, 108.92, 80.31, 78.13, 77.90, 69.67, 48.21, 41.48, 28.52, 28.24, 26.22, 16.91; IR (neat, cm^{-1}): 3500 – 3200, 2979, 2929, 1694, 1671, 1413, 1367, 1166, 1059, 873.



***tert*-Butyl(3*R*,4*R*,7*R*)-2,2,4-trimethyl-7-oxotetrahydro-[1,3]dioxolo[4,5-c]pyridine-5(4*H*)-carboxylate:** Preparation from TBDPS-ether **49** as described en route towards compound **40** (6.9 mmol scale), afforded the title compound (1.49 g, 5.2 mmol, 76%). $[\alpha]_D^{20}$ +2.2 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.75 – 4.55 (m, 2H), 4.36 (dd, *J* = 6.8, 1.4 Hz, 1H), 4.30 (d, *J* = 6.8 Hz, 1H), 3.65 (d, *J* = 18.5 Hz, 1H), 1.48 (s, 9H), 1.46 (s, 3H), 1.35 (s, 3H), 1.16 (d, *J* = 7.5 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 203.12, 154.74, 111.62, 80.78, 80.22, 75.51, 54.68, 49.15, 28.49, 26.64, 24.95, 15.43; IR (neat, cm^{-1}): 3426, 2981, 2837, 1773, 1728, 1695, 1369, 1252, 1214, 1150, 1050, 870, 753.



***tert*-Butyl(3*R*,4*R*,7*S*,7*S*)-7-hydroxy-2,2,4-trimethyltetrahydro-[1,3]dioxolo[4,5-c]-pyridine-5(4*H*)-carboxylate (50):** Prepared as described for alcohol **40** from the ketone mentioned above (4.3 mmol scale), yielded title compound (1.0 g, 3.6 mmol, 83%, *d.r.* = 93:7). $[\alpha]_D^{20}$ -35 (*c* = 1, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 4.42 (dd, *J* = 7.3, 4.4 Hz, 1H), 4.33 (dd, *J* = 7.3, 1.8 Hz, 1H), 4.28 – 4.19 (m, 1H), 3.95 (ddd, *J* = 10.7, 4.4, 4.4 Hz, 1H), 3.61 (dd, *J* = 11.9, 4.4 Hz, 1H), 3.07 (t, *J* = 11.9 Hz, 1H), 2.55 – 2.36 (m 1H), 1.50 (s, 3H), 1.47 (s, 9H), 1.38 (s, 3H), 1.17 (d, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): *δ* ppm 155.21, 108.95, 79.91, 77.42, 71.80, 65.15, 47.70, 42.56, 28.56, 26.30, 24.45, 19.16; IR (neat, cm^{-1}): 3432, 2979, 2936, 1739, 1687, 1401, 1366, 1167, 1049, 877, 750; HRMS: calculated for $C_{14}H_{25}NO_5$ [M+H$^+$] 288.18056, found: 288.18056.



(2*R*,3*R*,4*S*,5*S*)-2-methylpiperidine-3,4,5-triol hydrochloride (13): Prepared as described for **6** from protected iminosugar **50**, to afford **13** as a colorless foam (3.4 mmol scale, yield 613 mg, 98%) in a *d.r.* of 93:7. $[\alpha]_D^{20}$ +21 (*c* = 1, MeOH); ^{1}H-NMR (400 MHz, D_2O): *δ* ppm 4.13 – 4.17 (m, 1H), 3.96 (ddd, *J* = 11.5, 4.9, 2.6 Hz, 1H) 3.57 (dd, *J* =10.4, 2.5 Hz, 1H), 3.28 (dq, *J* = 6.5, 2.5 Hz, 1H), 3.20 (dd, *J* = 12.0, 4.9 Hz, 1H), 3.05 (t, *J* = 12.0 Hz, 1H), 1.33 (d, *J* = 6.5 Hz, 3H); ^{13}C-NMR (100 MHz, D_2O): *δ* ppm 69.93, 69.61, 64.70, 49.08, 41.22, 14.16; IR (neat, cm^{-1}): 3400 - 3200, 2942, 2810, 2512, 1457, 1044, 1016, 962, 818, 707; HRMS: calculated for $C_6H_{13}NO_3$ [M+Na$^+$] 148.09682, found: 148.09658.

Biological assays:

Materials. Cyclophellitol β-aziridine ABP JJB75 was synthesized as described earlier.[18] Gaucher patients were diagnosed on the basis of reduced GBA activity and demonstration of an abnormal genotype.[38] Cell lines were cultured in HAMF12-DMEM medium (Invitrogen) supplied with 10% (v/v). Spleens from a normal subject and a patient suffering from type 1 Gaucher were collected after splenectomy and immediately stored frozen.

Molecular cloning and recombinant expression. The coding sequences of *H. sapiens* FUCA1 (NCBI reference sequence XM_005245821.1, using forward primer 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCACCATGCGGGCTCCGGGGATG-3' and reverse primer 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACTTCACTCCTGTCAGCTTTAT-3'), and of *H. sapiens* FUCA2 (NCBI reference sequence NM_032020.4, using forward primer 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCCACCACCATGCGGCCCCAGGAGCTC-3' and reverse primer 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGATCACATTAGTCAGGGCTA-3') were amplified via PCR and cloned into pDNOR-221 and thereafter sub-cloned in pcDNA™-DEST40 vector using the Gateway system (Invitrogen). Correctness of all constructs was verified by sequencing. Confluent COS-7 cells were transfected with pcDNA3.1 empty vector (Mock) or the vector with described insert in conjunction with FuGENE (Roche). After 72 hours, medium isolated and frozen at -80 °C and cells were harvested by scraping in 25 mM potassium phosphate buffer (pH 6.5, supplemented with 0.1% (v/v) Triton X-100 and protease inhibitor cocktail (Roche)). After determination of the protein concentration (BCA kit, Pierce), lysates were aliquoted and frozen at -80 °C.

Enzyme activity assays. The enzymatic activity of α-L-fucosidase was assayed at 37 °C by incubating with 1.5 mM 4-methylumbelliferyl-α-L-fucopyranoside as substrate in 150 mM McIlvaine buffer, pH 4.5, supplemented with 0.1% (w/v) BSA. To determine the apparent IC_{50} value, COS-7 cell lysate containing over-expressed human recombinant FUCA1 was pre-incubated with a range of inhibitor dilutions for 30 min at 37 °C. The reaction was quenched by adding excess NaOH-glycine (pH 10.6), after which fluorescence was measured with a fluorimeter LS55 (Perkin Elmer) using λ_{EX} 366 nm and λ_{EM} 445 nm.

***In vitro* labeling and SDS-PAGE analysis.** All pre-incubations and ABP labeling-reactions occurred for 30 min at 37 °C. Total lysates (50 µg), medium (500 µg) or purified protein preparations (5.0 µg) were labeled with 1.0 µM JJB256 **1** or JJB244 **2**, dissolved in 150 mM McIlvaine buffer, pH 4.5, incubating for 30 min at 37 °C. For ABPP, protein preparations were pre-incubated with compounds **4** (100 µM), **6** (100 µM), **7**-**13** (1 or 5 mM, specified in the main text) prior to the addition of 100 nM fluorescent ABPs. Influence of pH on ABP labeling involved pre-incubation at pH 3-10 prior to addition of 100 nM ABP **1**. Direct labeling of retaining β-glucosidases occurred at pH 5.0 in conjunction with 100 nM ABP JJB75. Samples were then denatured with 5× Laemmli buffer (50% (v/v) 1.0 M Tris-HCl, pH 6.8, 50% (v/v) 100% glycerol, 10% (w/v) DTT, 10% (w/v) SDS, 0.01% (w/v) bromophenol blue), boiled for 4 min at 100 °C, and separated by gel electrophoresis on 10% (w/v) SDS-PAGE gels running continuously at 90 V[13, 14]. Wet slab-gels were scanned on fluorescence using a Typhoon Variable Mode Imager (Amersham Biosciences) using λ_{EX} 488 nm and λ_{EM} 520 nm (band pass filter 40 nm) for green fluorescent JJB256 **1** and λ_{EX} 532 nm and λ_{EM} 610 nM (band pass filter 30 nm) for red fluorescent JJB244 **2** and JJB75.

***In vivo* labeling.** The appropriate ethics committee for animal experiments approved all experimental procedures. Wild-type C57Bl/6J male mice were obtained from Harlan and fed a commercially available lab diet (RMH-B; Hope Farms). Four C57BL/6J mice were injected intraperitoneally with 100 µL sterile PBS (vehicle) or PBS containing 10, 100, or 1000 pmol of ABP **1** (about 0.20 µg kg−1, 2 µg kg−1, and 20 µg kg−1, respectively). At 2 h post-administration, urine was collected and the mice were anesthetized with FFM mix (25/25/50 fentanylcitrate/ 67idazolam/H_2O), blood was collected and perfused with PBS at 3.0 mL min^{-1}. Then urine and organs were collected and directly frozen in liquid nitrogen. Homogenates were made in 25 mM potassium phosphate buffer, pH 6.5, supplemented with 0.10% (v/v) Triton X-100 and protease inhibitor cocktail (Roche). After determination of protein concentration (BCA kit, Pierce), 50 µg total protein was incubated with 100 nM red fluorescent JJB75 and analyzed by SDS-PAGE. As controls, matching tissue homogenates of vehicle-treated animals were concomitantly labeled with 1.0 µM ABP **1** and 100 nM JJB75 prior to SDS-PAGE.

ABP pulldown and LC-MS/MS analysis. Gaucher spleen lysate (1.0 g) was cut with a sterile scalpel, mixed with 4.0 mL 25 mM potassium phosphate buffer (pH 6.5, supplemented with 0.10% (v/v) Triton X-100 and protease inhibitor cocktail (Roche)) and sonicated three times for 10 seconds at 100% strength and kept on ice. After 2 min, procedure repeated twice. Lysate was then centrifuged for 10 min at 10,000 *g*, supernatant collected carefully, protein concentration determined and 200 mg total protein was then incubated with either 0.10% (v/v) DMSO, ABP **1** or ABP **3**, or firstly with 10 µM ABP **1** followed by 10 µM of ABP **3**, each step taking 30 min at 37 °C, in a total volume of 1.0 mL McIlvaine buffer, pH 4.5. Glycosylated biomolecules were enriched by using 1.0 mL ConA-Sepharose per sample, according to manufacturer's instructions (Amersham Pharmacia Biotech AB, Sweden) and subsequently denatured by the presence of 2.0% (w/v) SDS and boiling for 5 min at 100 °C. From here on, samples were prepared for MS as published earlier.[14] After desalting on StageTips, peptides were analyzed with a 2 h gradient of 5-25% CAN on nano-LC, hyphenated to an LTQ-Orbitrap and identified via the Mascot protein search engine.[14]

X-ray Crystallography. Recombinant *Bacteroides thetaiotaomicron* 2970 α-L-fucosidase (*Bt*Fuc2970) was prepared as described previously.[28] Protein crystals were obtained through hanging (Crystals soaked with compound **5**) or sitting (crystals soaked with compound **4**) drop vapor diffusion (for further details see PDB file headers). Compounds **4** and **5** were dissolved in crystallization mother liquor at a concentration of **5** or 20 mM respectively and added to crystallization drops containing crystals of *Bt*Fuc2970 in a 1:1 ratio. After *ca*. 1 h soaking with ligands, crystals were fished into cryo-protectant solutions (mother liquor supplemented with 20% v/v glycerol) and cryo-cooled in liquid nitrogen. Diffraction data were collected at Diamond Light Source. Diffraction images were indexed and integrated using MOSFLM[29] (**4**) or XDS[39] (**5**) and scaled and merged using AIMLESS.[40] Crystals grew in an almost isomorphous space group to PDB entry 4JFV, and coordinates from this entry were used directly to obtain a starting model for refinement. Iterative stages of model-building (COOT[41]) and maximum-likelihood refinement (REFMAC5[42]) were conducted to yield final models. Maximum-likelihood restraints for compounds **4** and **5** were created using the PRODRG online server[43] and link restraints generated using JLIGAND.[44] X-ray crystallographic data statistics are available in supplemental Table 3.

Table 3. X-ray crystallographic data table

	*Bt*Fuc2970-**5** PDB: 4WSJ	*Bt*Fuc2970-**4** PDB:4WSK
data collection		
Beamline/Date	Diamond i03 18/10/14	Diamond i03 02/02/14
Wavelength (Å)	0.97625	0.97625
Cell dimensions		
a, *b*, *c* (Å)	55.5, 187.0, 98.2	55.6, 186.5, 98.2
α, β, γ (°)	90, 94.2, 90	90, 94.2, 90
Resolution (Å)	93.5-1.64	62.17-1.92
R_{merge}	0.058(0.62)	0.095(0.86)*
$I / \sigma I$	11.2(2.0)	7.0(1.8)
Completeness (%)	98.1(98.5)	96.9(96.7)
Redundancy	4.1(4.3)	4.0(3.7)
Wilson B value	20.4	26.1
Refinement		
Resolution (Å)	97.9-1.64	97.9-1.71
No. reflections	328440	292076
R_{work} / R_{free}	0.16 / 0.19	0.18 / 0.23
R.m.s. deviations		
Bond lengths (Å)	0.019	0.019
Bond angles (°)	1.78	1.81
Ramachandran Statistics (%)		
Preferred	96.7	95.8
Allowed	2.4	3.1
Outliers	0.9	1.1

Values in parentheses are for highest-resolution shell.

3.5 References and notes

[1] J. Intra, M.-E. Perotti, G. Pavesi and D. Horner, *Gene* **2007**, 392, 34-46.

[2] V. Lombard, H. Golaconda Ramulu, E. Drula, P. M. Coutinho and B. Henrissat, *Nucleic Acids Res.* **2014**, 42, D490-D495.

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3.6 Supporting Information

Table S1 (A): Proteins identified by affinity purification with the biotinylated Probe **3**(JJB243, on bead digest, LC-MS/MS analysis and Mascot search engine match to the human Uniprot database (Jan. 2015).

nr	accession	protein description	score	cover	emPAI
1	P04264	Keratin, type II cytoskeletal 1, KRT1	677	18	0,62
2	P13645	Keratin, type I cytoskeletal 10, KRT10	580	22	0,92
3	P05165	Propionyl-CoA carboxylase, mitochondrial, PCCA	541	17	0,38
4	P35527	Keratin, type I cytoskeletal 9, KRT9	374	23	0,44
5	P35908	Keratin, type II cytoskeletal 2 epidermal, KRT2	366	10	0,34
6	P11498	Pyruvate carboxylase, mitochondrial, PC	262	8	0,19
7	P04066	Tissue alpha-L-fucosidase, FUCA1	210	17	0,35
8	H0YA55	Serum albumin, ALB	192	5	0,2
9	U3KQK0	Histone H2B, HIST1H2BN	191	9	0,39
10	H7C469	Uncharacterized protein	144	12	0,25
11	H0YFX9	Histone H2A, HISTH2A	142	21	0,82
12	P19013	Keratin, type II cytoskeletal 4, KRT4	132	4	0,12
13	Q14956	Transmembrane glycoprotein NMB, GPNMB	131	3	0,11
14	P81605	Dermcidin OS, DCD	96	13	0,70
15	F8W6P5	LVV-hemorphin-7, HBB	93	26	0,85
16	P31025	Lipocalin-1, LCN1	71	6	0,18
17	P69905	Hemoglobin subunit alpha, HBA1	71	37	0,83
18	A5A3E0	POTE ankyrin domain family member F, POTEF	70	2	0,03
19	F5GWP8	Keratin, type I cytoskeletal 17, KRT17	69	5	0,17

Table S1 (B): Proteins identified after competitive experiment between the fluorescent fucosidase probe **1** and the biotin probe **3**, followed by affinity purification, on bead digest, LC-MS/MS analysis and Mascot search engine match to the human UniProt database (Jan. 2015)

nr	accession	protein description	score	cover	emPAI
1	P05165	Propionyl-CoA carboxylase, mitochondrial, PCCA	426	22	0,69
2	P13645	Keratin, type I cytoskeletal 10, KRT10	418	21	1,03
3	E9PS68	Pyruvate carboxylase, mitochondrial, PC	276	16	0,47
4	Q14956	Transmembrane glycoprotein NMB, GPNMB	236	3	0,11
5	A0A087WY73	Proline-rich protein 4, PRR4	227	14	0,44
6	P35908	Keratin, type II cytoskeletal 2 epidermal, KRT2	218	12	0,48
7	P04264	Keratin, type II cytoskeletal 1, KRT1	218	12	0,4
8	P35527	Keratin, type I cytoskeletal 9, KRT9	213	11	0,29
9	U3KQK0	Histone H2B, HIST1H2BN	147	9	0,39
10	H0YI76	Keratin, type II cytoskeletal 5, KRT5	126	12	0,31
11	H7C469	Uncharacterized protein	97	5	0,12
12	H0YFX9	Histone H2A, H2A	84	21	0,35
13	P81605	Dermcidin, DCD	64	10	0,3
14	A8MUF7	Hemoglobin subunit epsilon, HBE1	48	12	0,37
15	P69905	Hemoglobin subunit alpha, HBA1	47	11	0,22
16	F8W0V3	Extracellular glycoprotein lacritin, LACRT	43	6	0,26

Table S1 (C): Proteins identified after no probe control experiment, aspecific background stickiness of proteins to the paramagnetic beads, followed by affinity purification, on bead digest, LC-MS/MS analysis and Mascot search engine match to the human UniProt database (Jan. 2015)

nr	accession	protein description	score	cover	emPAI
1	P04264	Keratin, type II cytoskeletal 1, KRT1	1166	35	1,91
2	P35527	Keratin, type I cytoskeletal 9, KRT9	972	39	1,67
3	P05165	Propionyl-CoA carboxylase, mitochondrial, PCCA	701	28	0,98
4	P35908	Keratin, type II cytoskeletal 2 epidermal, KRT2	427	20	0,71
5	P13645	Keratin, type I cytoskeletal 10, KRT10	342	12	0,46
6	P02533	Keratin, type I cytoskeletal 14, KRT14	341	18	0,54
7	P13647	Keratin, type II cytoskeletal 5, KRT5	283	13	0,43
8	P11498	Pyruvate carboxylase, mitochondrial, PC	282	7	0,16
9	P02538	Keratin, type II cytoskeletal 6A, KRT6A	270	12	0,38
10	P48668	Keratin, type II cytoskeletal 6C, KRT6C	257	12	0,38
11	P08779	Keratin, type I cytoskeletal 16, KRT16	252	15	0,55
12	P04259	Keratin, type II cytoskeletal 6B, KRT6B	222	9	0,31
13	Q7Z794	Keratin, type II cytoskeletal 1b, KRT77	179	4	0,11
14	P81605	Dermcidin, DCD	136	23	1,22
16	F8W6P5	LVV-hemorphin-7, HBB	82	36	1,52
17	Q14956	Transmembrane glycoprotein NMB, GPNMB	73	3	0,11
18	P31025	Lipocalin-1, LCN1	48	6	0,18
19	P69905	Hemoglobin subunit alpha, HBA1	47	11	0,22
20	A0A075B6N7	Ig alpha-2 chain C region, IGHA2	46	3	0,09
21	Q5T8M7	Actin, alpha skeletal muscle, ACTA1	43	3	0,09

Protein score is the Mascot score calculated for the peptide matches of the protein to the human protein database, protein mass is given in kDa, protein coverage is the percentage of the amino acid sequence that has been identified, emPAI value gives an indication of the abundancy or relative concentration of the protein in the LC-MS run.