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Summary and future prospects

8.1 Summary
This thesis describes the development of functional and configurational analogues of cyclophellitol aziridine as activity-based probes (ABPs) for various retaining glycoside hydrolases (GHs), namely α-L-fucosidases, β-glucosidases, α-glucosidases and β-glucuronidases (Figure 1). Attention is focused on the design and synthesis of the cyclophellitol aziridine derivatives and their application in chemical biology studies of various retaining GHs.

Chapter 1 introduces the research subject described in this thesis. Subjects introduced in this chapter include β-glucosidases classification, a description of the molecular mechanisms employed by these enzymes in the hydrolysis of their substrates, as well as the cyclophellitol aziridine-based ABPs targeting retaining β-glucosidases. The retaining glycosidases employ Koshland double displacement mechanism, and on the basis of which the research of this thesis was formulated. Chapter 2 reviews the literature on the synthesis of cyclitol aziridines as the scaffold of choice for the development of retaining GHs ABPs.
Chapter 8

Figure 1. Activity-based glycosidase probes (ABPs) discussed in this thesis. I-IV are configurational and functional cyclophellitol aziridine isomers emulating the structure of the parent monosaccharides as indicated; A-I are acyl and alkyl functional tags.

Retaining α-L-fucosidase (FUCA) is a member of the glycoside hydrolases family 29 (GH29). FUCA deficiency is at the basis of the rare lysosomal storage disorder, fucosidosis. Chapter 3 describes an activity-based protein profiling (ABPP) study on GH29 α-L-fucosidases. L-Fucopyranose-configured cyclophellitol aziridines (JJB237, JJB243, JJB244 and JJB256), are applied as ABPs for selective in vitro and in vivo labeling of GH29 α-L-fucosidases from bacteria, mice and man. The chapter also reports on the synthesis of eight configurational L-fucoojirimycin isomers, which were screened as potential α-L-fucosidase inhibitors in a competitive ABPP setting. The covalent aziridine-enzyme adduct between the carbon...
corresponding to the anomeric center in the substrate fucoside and the FUCA active site nucleophile was trapped in a co-crystal of *Bacteroides thetaiotaomicron* α-L-fucosidase treated with *N*-acetyl-L-fuco-cyclophellitol aziridine. The *trans*-diaxial skew boat conformation adopted by the covalently attached inhibitor also provides insight in the conformational changes substrate α-L-fucosides undergo during FUCA-mediated hydrolysis.

In order to improve the stability and availability of retaining GHs ABPs, a set of next generation probes, namely *N*-alkyl cyclophellitol aziridine ABPs, were investigated. The synthesis and evaluation of fluorescent *N*-alkyl probes directed at GH30 β-glucosidases (JJB339, JJB343) and GH29 α-L-fucosidases (JJB349, JJB380) are described in **Chapter 4**. In comparison with the corresponding acyl aziridine ABPs reported previously, the alkyl aziridine ABPs proved relatively easy to synthesize and are more stable in mildly acidic and basic media. The β-*gluco*-configured alkyl aziridine ABPs proved equally effective in inhibiting and labeling the lysosomal β-glucosidase (GBA) as its *N*-acyl counterparts. In contrast, the *N*-acyl aziridines targeting α-L-fucosidase outperform their *N*-alkyl counterparts. Therefore, *N*-alkyl cyclophellitol aziridines can be an attractive alternative in retaining GHs ABP design, but in targeting a new retaining glycosidase both *N*-alkyl and *N*-acyl aziridines are best considered at the onset of a new study.

**Chapter 5** describes the development of ABPP technology to study GH31 α-glucosidases *in vitro* and *in situ*. To this end a comprehensive set of α-*gluco*-cyclophellitol aziridines bearing either a fluorescent group (JJB347, JJB382, JJB383) or a biotin (JJB384) was synthesized. α-Glucosidases are involved in diverse physiological processes in the human body, including carbohydrate assimilation in the gastrointestinal tract, glycoprotein processing in the endoplasmic reticulum (ER), and intra-lysosomal glycogen catabolism. Inherited deficiency of the lysosomal acid α-glucosidase (GAA) causes Pompe disease, a relatively common lysosomal glycogen storage disorder. The developed ABPs proved to be highly potent and irreversible inhibitors towards recombinant α-glucosidase as established by enzyme inhibition assays and X-ray crystallography analysis. Moreover the ABPs can specifically label distinct retaining GH31 α-glucosidases, notably, the lysosomal GAA and the ER α-glucosidase II and this labelling can be tuned by pH. The chapter further describes a direct diagnostic application in Pompe disease patient fibroblast cells, and reports on the analysis of intestinal dietary α-glucosidases, such as sucrase-isomaltase (Sis) and maltase-glycoamylase (MGAM).

**Chapter 6** describes a synthesis strategy towards β-glucuronide-configured cyclophellitol, cyclophellitol aziridine and its derivatives (JJB133, JJB144, JJB249, JJB355, JJB391, JJB392, JJB395 and JJB397) as ABPs for GH2 and GH79 β-glucuronidases. The former enzyme is related to the glycosaminoglycan (GAG) storage disorder, Sly disease, and the latter enzyme is related to inflammation, tumour angiogenesis and cell migration. Uronic *N*-alkyl cyclophellitol
aziridines are easier to be prepared than uronic N-acyl cyclophellitol aziridines, and both alkyl and acyl inhibitors and probes proved equally effective in inhibition and labelling of β-glucuronidases, which is in line with the findings in Chapter 4. Crystallographical analysis on bacterial β-glucuronidase (AcaGH79) complexed with alkyl and acyl aziridines further confirmed the covalent modification of the enzyme nucleophile active site and showed a \( ^4C_1 \) chair conformation for both enzyme-inhibitor adducts. Applications of the β-glucuronide-configured N-alkyl cyclophellitol aziridine ABPs are described in Chapter 7 and include GH2 human lysosomal β-glucuronidase (GUSB) and GH79 human heparanase (HPSE) identification by activity-based proteomics. An interesting finding is that the endo-β-glucuronidase HPSE is effectively labeled and thus inhibited by cyclophellitol aziridine derivatives, which in essence are monosaccharide mimetics (thus expected to bind efficiently to exo-glycosidases, but not necessarily endo-glycosidases). Crystallographical analysis of the structures of recombinant exo-GUSB, exo-AcaGH79 and endo-HPSE as well as their corresponding nucleophile mutant (Glu to Ala) complexed with cyclophellitol aziridine JJB355, reveals the Koshland double displacement mechanism employed by these enzymes and their respective glycosidase activities.

8.2 Future prospects

The research described in this thesis entails the design, synthesis and application of functional configurational cyclophellitol aziridine derivatives as ABPs selective for in-class GH family retaining glycosidases. Besides red BODIPY probes of β-glucosidases and α-L-fucosidases shown in Chapter 3, also green BODIPY probes 3 JJB376, 6 JJB350, blue Cy5 probes 4 JJB367, 7 JJB381 and biotin probes 5 JJB377, 8 JJB385 ABPs (Figure 1) can be prepared following a similar strategy (Scheme 1).

By altering either configuration or substitution pattern, or both, selectivity of the resulting probes changes in a highly predictable manner. This bodes well for the development of ABPs directed at other retaining glycosidases. For instance, ABPs 12-14 (Figure 2) may target with good efficiency their respective underlying glycosidases: β-glucosidases (already a proven fact), β-galactosidases and β-mannosidases, which are of interest in the context of specific lysosomal storage disorders: Gaucher disease, GM1 gangliosidosis and mannosidosis respectively. In comparison with ABPP technology developed for serine hydrolases and cysteine proteases, however, the in-class GH family selectivity displayed by the ABPs described in this thesis may also be considered a disadvantage. Cysteine protease probes and especially serine hydrolase probes have been reported that efficiently label a large number – sometimes hundreds of – related enzymes, which may be of advantage in for instance competitive ABPP experiments aimed at the discovery of selective inhibitors. Arguably, the nature (substitution pattern, configuration) of the highly functionalized cyclitol aziridines (Figure 1) determines their glycosidase specificity, and deleting some substituents may yield probes that, though less
potent, are also less selective, in other words, more broad-spectrum. For instance, diol cyclohexane aziridine 15 (Figure 2) may be developed as broad spectrum ABP for retaining β-glucosidases, β-galactosidases and β-mannosidases based on the idea that the hydroxyl at C2 (distinguishing β-glucose from β-mannose) and C4 (distinguishing β-glucose from β-galactose) are removed.

**Scheme 1.** Synthesis of alkyl aziridine ABPs for β-glucosidases and α-L-fucosidases.

**Reagent and conditions:** (a) CuSO$_4$(1.0 M in H$_2$O), sodium ascorbate (1.0 M in H$_2$O), DMF, 3 JJB376: 30%, 4 JJB367: 13%, 5 JJB377: 34%, 6 JJB350: 8%, 7 JJB381: 13%, 8 JJB385: 20%.

**Figure 2.** Chemical structures of β-glucose, β-galactose, β-mannose, ABPs 12-14 for β-glucosidases, β-galactosidase, β-mannosidase and broad spectrum ABP 15.
Activity-based protein profiling is a powerful technique both to discover new enzyme activities (comparative ABPP) and to study their expression, activity and sensitivity in a tissue- and condition-dependent setting (competitive ABPP). The research described in this thesis focused predominantly on the latter, with probes designed with a specific GH glycosidase family in mind. Research in Chapter 5, however also demonstrates the potential of biotin-modified cyclophellitol aziridines to identify, by means of chemical proteomics, enzyme activities in a tissue-dependent fashion. In line with this, and underscored in a literature study on the application of β-glucopyranose-configured cyclophellitol aziridines to annotate Arabidopsis retaining β-glucosidases, a comparative ABPP investigation can be envisaged in which all biotinylated cyclophellitol aziridine probes (Figure 1) are employed to screen tissue from various kingdoms and map their respective retaining glycosidase activities.

The research described in this thesis reveals that cyclophellitol aziridines are highly potent irreversible retaining glycosidase inhibitors, and that the inhibitory potency can be tuned by varying the nature of the aziridine N-substituent. For instance (see Chapter 3), N-acetyl-cyclophellitol aziridine (IC\textsubscript{50} = 46.8 nM) inhibits recombinant FUCA1 about 8 times more potently than the corresponding N-benzoyl-cyclophellitol aziridine (IC\textsubscript{50} = 371.6 nM). However, previously described in the literature, that N-alkylated derivatives of the natural product deoxynojirimycin (DNM) and in which the nitrogen substituent is large and hydrophobic, are considerably more potent GBA inhibitors than analogous derivatives that bear a small nitrogen substituent. For instance, N-(adamantanemethoxypentyl)-deoxynojirimycin (AMP-DNM) is a considerably more potent GBA inhibitor than DNM as well as N-butyl-DNM. In this light, it is of interest to explore cyclophellitol aziridine derivatives bearing as nitrogen substituent a variety of alkyl and acyl substituents. Scheme 2 describes the synthesis of several of these compounds, namely, N-adamantanemethoxypentanoyl aziridine 18 and N-carboxybenzyl aziridine 22 as potential GBA inhibitors and two N-substituted L-fucose cyclophellitol aziridine inhibitors (Cbz-aziridine 24 and p-nitrophenyloxycarbonyl aziridine 25) as potential FUCA inhibitors. Compound 18 was prepared with the appropriate acid under the agency of EEDQ as the condensation agent following a procedure as presented in Chapter 3 to yield JJB237. Unprotected aziridines 16 and 23 were transformed in the corresponding carboxybenzyl derivatives via activated anhydride 21 yielding 22 and 24. Interestingly, during the synthesis of fucose analogue 24, compound 25 was also isolated after HPLC purification using a neutral eluent in a yield of 7%.
The potency and selectivity of cyclophellitol and cyclophellitol aziridine as retaining β-glucosidase inhibitors can be attributed to their tight initial binding to the enzyme active site. Upon binding, the electrophilic carbon corresponding to C1 of the substrate glucoside is ideally positioned for reaction with the enzyme active site nucleophile, leading to covalent and irreversible modification of the enzyme active site. Glycosidase specificity is guided by the configuration and substitution pattern of the cyclophellitol derivative, as is amply demonstrated in this thesis. Looking at the conformation of cyclophellitol in comparison with that of a substrate β-glucoside, one could argue that initial binding happens, not so much as a substrate analogue, but as a transition analogue. Whereas β-glucopyranosides adopt a preferential 4C1 chair conformation of Michaelis complex (Figure 3A), cyclophellitol prefers to adopt 4H3 half-chair conformation (Figure 3B).

Figure 3. A) β-Glucopyranosides complex and B) cyclophellitol Michaelis complex conformations in enzyme catalysis.
Arguably, this conformation emulates that of an emerging oxocarbenium ion that may be formed as a transient intermediate in the enzyme active site and onto which the active site nucleophile will add. In a similar way, the configurational and substitutional isomers of cyclophellitol aziridine may resemble in conformation transition state oxocarbenium ions more than substrate glycosides of their underlying corresponding carbohydrates. Following this reason, cyclophellitol derivatives featuring the same substitution pattern and configuration, that are able to adopt a similar conformation but without an appropriate leaving group may turn out to be effective competitive inhibitors. With this reasoning in mind, carba-cyclophellitol analogues, with the epoxide oxygen substituted for methylene, can be proposed as a new class of glycosidase inhibitors. Scheme 3 represents the synthesis of compounds 28, 31 and 32, being carba-cyclophellitol analogues featuring α-L-fucopyranose (28, 31) and β-L-fucopyranose (32) configurations. Cyclopropanation of alkene 26 with diethylzinc/diiodomethane yielded cyclopropane 27 as the major stereoisomer, which was converted to α-cyclitol-cyclopropane 28 by palladium hydroxide catalyzed hydrogenolysis of the benzyl protective groups. Substituted cyclopropane derivatives 31 and 32 were prepared as follows. Benzylation of C4-OH in 26 yielded 29, which was treated with copper (II) acetylacetonate (Cu(AcAc)₂) and diazoacetate giving 30 as a stereomeric mixture of cyclopropane products in a 5:2 ratio. Following debenzylation using Pearlman’s catalyst and dihydrogen, cyclitol-cyclopropane 31 and 32 were isolated following silica gel column chromatography. Future studies will reveal whether these carba-cyclophellitol derivatives are able to inhibit FUCA1/2, and if so whether they do so by keeping a half-chair conformation within the enzyme active site.

**Scheme 3.** Synthesis of L-fuco-carba-cyclophellitol compounds 28, 31 and 32

**Reagent and conditions:** (a) Et₂Zn, BF₃·OEt₂, CH₂I₂, Et₂O, DCM, 83%; (b) Pd(OH)₂/C, H₂, MeOH, 28: 99%, 31: 26%, 32: 11% over two steps; (c) BnBr, NaH, TBAI, DMF, 0 °C→20 °C, 95%; (d) Cu(AcAc)₂, Ethyl diazoacetate, EtOAc.
Recently, Speciale et al. have identified a new sulfoquinovosidase in *Escherichia coli*, YihQ. This enzyme is a GH31 α-glycosidase that cleaves the modified glucose derivative named sulfoquinovose (SQ) from sulfoquiovosyl diacylglyceride (SQDG) sulfolipids. It would be of interest to develop YihQ activity-based probes to identify its activity in SQDG sulfolipids metabolism in bacteria. In Chapter 5, *epi*-cyclophellitol aziridine CF022, JJB347, JJB382-384 have already been successfully synthesized and applied in ABPP of GH31 α-glucosidasases.

Introduction of a sulfite moiety on to this cyclitol aziridine scaffold would yield potential YihQ ABPs. A proposed synthesis approach for YihQ ABPs is depicted in Scheme 4. O-sulfonation of primary alcohol with sulfur trioxide trimethylamine complex (SO$_3$·Et$_3$N) would lead to the corresponding sulfate derivative, following a sulfonaton protocol as previously reported.

Scheme 4. Synthesis of sulfo-aziridine ABPs.

![Scheme 4](image)

Reagent and conditions: (a) SO$_3$·Et$_3$N, DMF, 60 ºC; (b) CuSO$_4$ (1.0 M in H$_2$O), sodium ascorbate (1.0 M in H$_2$O), DMF, or 10 or 11.

Lysosomal β-glucuronidase (GUSB) is an *exo*-glucuronidase that removes D-glucuronic acid residues from the reducing end of glycosaminoglycans (GAGs), whereas heparanase (HPSE) is an *endo*-glucuronidase able to hydrolyse glycosidic linkages within heparin sulfate (HS) chains. Surprisingly, both of GUSB and HPSE are labeled by glucuronic acid emulating cyclophellitol aziridines JJB355, JJB392, JJB395 or JJB397 (compounds in Chapter 6, 7). In order to selectively modify HPSE in vitro and in vivo, it would be useful to have specific HPSE ABPs. Based on the chemical structure of HS and also the HPSE catalytic cleft spatial structure analysis recently performed by Wu et al., HPSE would prefer to recognize oligosaccharide substrate mimetics. Moreover, *exo*-glucuronidasases would not cleave the internal linkage of oligosaccharide. Based on these considerations disaccharide derivatives 36-43 may turn out to be selective HPSE inhibitors and probes (Figure 4), also considering that the *exo*-glycosidase, GUSB, would not be able to fit these disaccharide-like compounds.
Figure 4. Proposed disaccharide cyclophellitol epoxide and aziridine inhibitors and probes for HPSE.

Scheme 5. Synthesis of HPSE inhibitors and probes.

Reagent and conditions: (a) Levulinic acid, DIC, DMAP, DCM, 78%; (b) i) imidazole-1-sulfonyl azide, H$_2$O, CuSO$_4$·5H$_2$O; ii) BnBr, TBAI, NaH, DMF, 0 °C-20 °C, 43%; (c) N-iodosuccinimide (NIS), TFA, DCM/H$_2$O (v/v, 10/1), 94%; (d) CF$_3$C(NPh)Cl, Cs$_2$CO$_3$, acetone/H$_2$O (20/1), 68%; (e) N-formylmorpholine (NFM), NIS, TMSOTf, DCM, molecular sieves 3Å, 45, -20 °C, 28%; (f) NH$_2$NH$_2$, AcOH, pyridine/AcOH (4/1), 64%; (g) AcSH, pyridine, CHCl$_3$, 70%; (h) i) Trichloroacetonitrile, DCM; ii) I$_2$, H$_2$O, NaHCO$_3$, 62%; (i) i) 1.0 M aq. HCl, MeOH; ii) NaHCO$_3$, MeOH, 58%; (j) 1-azido-8-iodooctane, K$_2$CO$_3$, DMF, 50 °C; (k) i) TEMPO/BAIB, DCM/H$_2$O (v/v, 2/1), 0 °C; ii) Li, NH$_3$, THF, -60 °C; (l) BODIPY/Cy5/Biotin-OSu; (m) mCPBA, DCM, 40 °C; (n) TEMPO/BAIB, DCM/H$_2$O (v/v, 2/1), 0 °C; (o) Pd/C, H$_2$, MeOH.
Compounds 36, 40 can be synthesized via the strategy proposed in Scheme 5. Firstly, building blocks 45 (‘acceptor’) and 47, 49 (‘donor’) are prepared from diol 44 and thiophenyl 46, respectively. The primary alcohol in 44 is selectively protected as the levulinoyl ester yielding acceptor 45. The free amine in 46 is converted into the azide by treatment with Stick’s reagent, followed by benzylation of the remaining hydroxyl groups. The glycosylation step proved a major limiting step during these initial synthesis studies, because it involves selective α-1,4-glycosylation linkage formation. Literature procedures towards the installation of related 1,2-cis-glycosidic linkages make use of activation strategies including nucleophile modulators, such as dimethylacetamide (DMA), N-formylmorpholine (NFM) and diphenyl sulfoxide (DPSO). Herein, NFM was used as the modulator for α-selectivity of glycosylation from 47 to 50, and after the reaction, 28% α-glycosyl product 50 was isolated. The use of N-phenyl-trifluoroacetimidate 49 as donor could be an alternative option to increase the efficiency and α-selectivity in the desired glycosylation event. Disaccharide aziridine 54 was successfully synthesized using the intramolecular aziridination protocol described in Chapter 6. N-alkylation of the aziridine nitrogen in 54 followed by global deprotection and oxidation of the cyclophellitol aziridine primary hydroxyl would require some carefully designed synthesis strategies, but seems feasible based on methodology described in this thesis. The disaccharide cyclophellitol epoxide 36 could be prepared from intermediate 52 by mCPBA epoxidation, TEMPO/BAIB oxidation and debenzylation depicted in Scheme 5D.

Scheme 6. Synthesis of proposed new type aziridine ABPs 60 and 61 for inverting GHs.

Finally, carbasugar-derived spiro-aziridines compounds 58 and 59 (Scheme 6) were reported by Vasella and co-workers group in 2003. These aziridines, structurally related to the cyclophellitol aziridines described in this thesis, proved to be moderately weak inhibitors of Caldodcellum saccharololiticum β-glucosidase and yeast α-glucosidase. One might consider investigating whether spiro-aziridines 58 and 59 would be useful scaffolds for the development of a new generation of glycosidase activity-based probes. Possibly, the exocyclic aziridine would be able to expel water from the active site of inverting glycosidases to next react with the catalytic base. In case valid, this would lead to the discovery of inverting glycosidase ABPs, although it can not be excluded that compounds 58 and 59 also irreversibly
inhibit retaining glycosidases. The proposed ABPs 60 and 61 could be developed from spiro-aziridines 58 and 59 respectively via alkylation or acylation of the nitrogen on the aziridine ring and conjugation of reporter groups.

8.3 Experimental section

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 absorption (254 nm) and by spraying with a solution of (NH₄)₆Mo(VI)O₇(β-D2O) (25 g/L) and (NH₄)⁴Ce(SO₄)⁴(β-D2O) (10 g/L) in 10% sulfuric acid followed by charring at ~150 °C or by spraying with an aqueous solution of KMnO₄ (7%) and K₂CO₃ (2%) followed by charring at ~150 °C. Column chromatography was performed using either Baker or Screening Device silica gel 60 (0.04 - 0.063 mm) in the indicated solvents.

1H-NMR and 13C-NMR spectra were recorded on Bruker AV-850 (850/214 MHz), Bruker DMX-600 (600/150 MHz) and Bruker AV-400 (400/100 MHz) spectrometers 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 13C 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 an LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a C₁₈ column (Gemini, 4.6 mm x 50 mm, 3 μm particle size, Phenomenex) equipped with buffers A: H₂O, B: acetonitrile (MeCN) and C: 1% aqueous TFA or 50 mM NH₄HCO₃ in H₂O. For reversed-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: 25mM NH₄OAc or 50 mM NH₄HCO₃ in H₂O, B: MeCN.

(1R,2S,3S,4R,5R,6R)-7-(8-(4-(4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ,5λ-dipyrrolo[1,2-c:2',1'-f][1,2]-diazaborinin-10-yl)butyl)-1H-1,2,3-triazol-1-yl)octyl)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (3): Azide compound 1 (13 mg, 0.039 mmol, 1.0 eq.) was dissolved in DMF (0.80 mL). Green BODIPY 9 (15 mg, 0.045 mmol, 1.2 eq.), CuSO₄ (1.0 M in H₂O, 15 μL, 0.015 mmol, 0.38 eq.) and sodium ascorbate (1.0 M in H₂O, 16 μL, 0.016 mmol, 0.40 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 40%-65% B in A, 3 CV, solutions used A: 50 mM NH₄HCO₃ in H₂O, B: MeCN) and lyophilized resulting as orange product 3 JJB376 (7.7 mg, 11.7 μmol, 30%). 1H-NMR (400 MHz, CD₃OD): δ ppm 7.73 (s, 1H), 6.11 (s, 2H), 4.35 (t, J = 7.0 Hz, 2H), 3.99 (dd, J = 10.1, 4.4 Hz, 1H), 3.63 – 3.58 (m, 2H), 3.13 – 3.08 (m, 1H), 3.05 – 2.98 (m, 3H), 2.78 (t, J = 7.2 Hz, 2H), 2.43 (s, 6H), 2.37 (s, 6H), 2.36 – 2.29 (m, 1H), 2.16 – 2.07 (m, 1H), 2.01 – 1.95 (m, 1H), 1.94 – 1.82 (m, 5H), 1.68 – 1.59 (m, 3H), 1.58 – 1.50 (m, 2H), 1.37 – 1.19 (m, 8H); 13C-NMR (100 MHz, CD₃OD): δ ppm 154.91, 148.50, 147.87, 142.19, 132.57, 123.38, 122.61, 79.01, 73.88, 70.08, 70.08.
6-[3,3-dimethyl-2-((E)-1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl]-3\H-1H-indol-1-yl\)-N-[(1\R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-aza bicyclo[4.1.0]heptan-7-yl]octyl\)-1H-1,2,3-triazol-4-yl]methyl\)hexanamide (4): Azide compound 1 (20 mg, 0.061 mmol, 1 eq.) was dissolved in DMF (1.0 mL). Blue Cy5 10 (41 mg, 0.074 mmol, 1.2 eq.), CuSO4 (1.0 M in H2O, 25 μL, 0.025 mmol, 0.40 eq.) and sodium ascorbate (1.0 M in H2O, 28 μL, 0.028 mmol, 0.46 eq.) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 41%→49% B in A, 3 CV, solutions used A: 50 mM NH4HCO3 in H2O, B: MeCN) and lyophilized resulting as deep blue product 4 JJB367 (6.9 mg, 7.8 μmol, 13%). 1H-NMR (600 MHz, CD3OD): δ ppm 8.28 – 8.22 (m, 2H), 7.84 (s, 1H), 7.50 (d, J = 7.5 Hz, 2H), 7.44 – 7.39 (m, 2H), 7.32 – 7.25 (m, 4H), 6.62 (t, J = 12.4 Hz, 1H), 6.28 (dd, J = 13.7, 2.6 Hz, 2H), 4.45 – 4.32 (m, 4H), 4.09 (t, J = 7.5 Hz, 2H), 3.99 (dd, J = 10.1, 4.4 Hz, 1H), 3.69 – 3.54 (m, 5H), 3.12 – 3.09 (m, 1H), 3.00 (t, J = 9.8 Hz, 1H), 2.35 – 2.29 (m, 1H), 2.25 (t, J = 7.3 Hz, 2H), 2.14 – 2.09 (m, 1H), 1.99 – 1.97 (m, 1H), 1.93 (s, 6H), 1.90 – 1.79 (m, 4H), 1.73 – 1.68 (m, 10H), 1.63 (d, J = 6.3 Hz, 1H), 1.59 – 1.42 (m, 4H), 1.39 – 1.23 (m, 8H). 13C-NMR (150 MHz, CD3OD): δ ppm 175.74, 175.39, 174.59, 155.55, 155.47, 144.24, 143.54, 142.62, 142.51, 129.78, 129.74, 126.61, 126.28, 126.21, 124.17, 123.42, 123.28, 112.02, 111.85, 104.42, 104.23, 79.03, 73.91, 70.05, 63.72, 62.09, 51.33, 50.54, 45.54, 45.47, 44.75, 43.04, 36.46, 35.57, 31.51, 31.29, 30.37, 30.25, 29.94, 28.21, 28.13, 27.94, 27.79, 27.37, 27.31, 26.39; LC-MS: Rr 5.97 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: m/z = 848.60 (M+); HRMS: calculated for C60H73BF7N7O4 [M+H]+: 848.54329, found: 848.54304.

6-(5-\{(3,5,S,6R)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl\}pentanamido)-N-[(1\R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl]octyl\)-1H-1,2,3-triazol-4-yl]methyl\)hexanamide (5): Azide compound 1 (13 mg, 0.039 mmol, 1.0 eq.) was dissolved in DMF (0.8 mL). Biotin compound 11 (18.6 mg, 0.047 mmol, 1.2 eq.), CuSO4 (1.0 M in H2O, 15 μL, 0.015 mmol, 0.38 eq.) and sodium ascorbate (1.0 M in H2O, 16 μL, 0.016 mmol, 0.40 eq.) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 15%→35% B in A, 3 CV, solutions used A: 50 mM NH4HCO3 in H2O, B: MeCN) and lyophilized resulting in white powder product 5 JJB377 (9.6 mg, 13.4 μmol, 34%). 1H-NMR (600 MHz, CD3OD): δ ppm 7.84 (s, 1H), 4.49 (dd, J = 7.9, 4.9 Hz, 1H), 4.42 (s, 2H), 4.38 (t, J = 7.1 Hz, 2H), 4.30 (dd, J = 7.9, 4.5 Hz, 1H), 3.99 (dd, J = 10.1, 4.4 Hz, 1H), 3.67 – 3.55 (m, 2H), 3.23 – 3.19 (m, 1H), 3.18 – 3.14 (m, 2H), 3.13 – 3.09 (m, 1H), 3.02 (t, J = 9.8 Hz, 1H), 2.93 (dd, J = 12.7, 5.0 Hz, 1H), 2.70 (d, J = 12.7 Hz, 1H), 2.36 – 2.31 (m, 1H), 2.27 – 2.10 (m, 5H), 1.99 (dd, J = 6.3, 3.5 Hz, 1H), 1.94 (s, 1H), 1.92 – 1.86 (m, 3H), 1.79 – 1.40 (m, 13H), 1.38 – 1.28 (m, 10H). 13C-NMR (150 MHz, CD3OD): δ ppm 176.01, 175.98, 166.11, 146.26, 124.17, 79.05, 73.93, 70.11, 63.77, 63.39, 62.10, 61.63, 57.01, 51.35, 45.54, 45.46, 43.04, 41.05, 40.19, 36.83, 36.76, 35.59, 31.27, 30.37,
(1R,2R,3R,4R,5R,6R)-7-(8-[4-(4-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4,5,6,7-tetrahydro-3H-pyrrolo[1,2-c:2,1'-f][1,3,2]diazaborinin-10-yl)butyl]-1H-1,2,3-triazol-1-yl)octyl)-5-methyl-7-azabicyclo[4.1.0]heptane-2,3,4-triol (6): Azide compound 2 (14 mg, 0.045 mmol, 1.0 eq.) was dissolved in DMF (1.0 mL). Green BODIPY 9 (20 mg, 0.054 mmol, 1.2 eq.), CuSO₄ (1.0 M in H₂O, 18 μL, 0.018 mmol, 0.4 eq.) and sodium ascorbate (1.0 M in H₂O, 19 μL, 0.019 mmol, 0.42 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 47%→53% B in A, 3 CV, solutions used A: 50 mM NH₄HCO₃ in H₂O, B: MeCN) and lyophilized resulting as orange product 6 JJB350 (2.3 mg, 3.5 μmol, 8%). ¹H-NMR (850 MHz, CD₂OD): δ ppm 7.73 (s, 1H), 6.12 (s, 2H), 4.35 (t, J = 7.0 Hz, 2H), 3.99 (dd, J = 8.7, 4.3 Hz, 1H), 3.54 – 3.52 (m, 1H), 3.34 – 3.32 (m, 1H), 3.06 – 3.00 (m, 2H), 2.79 (t, J = 7.3 Hz, 2H), 2.44 (s, 6H), 2.39 (s, 6H), 2.31 – 2.27 (m, 1H), 2.13 – 2.10 (m, 1H), 1.96 – 1.80 (m, 6H), 1.69 – 1.62 (m, 2H), 1.56 – 1.52 (m, 2H), 1.36 – 1.21 (m, 8H), 1.14 (d, J = 7.5 Hz, 3H); ¹³C-NMR (214 MHz, CD₂OD): δ ppm 154.93, 148.50, 147.89, 123.37, 123.37, 122.60, 122.60, 76.04, 75.14, 75.14, 74.62, 51.21, 46.47, 45.73, 36.95, 32.27, 31.22, 30.82, 30.56, 30.42, 29.89, 29.09, 28.21, 27.32, 25.90, 16.77, 16.50, 14.43; LC-MS: Rᵣ 6.26 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: m/z = 641.33 (M+H⁺); HRMS: calculated for C₃₆H₄₃F₂N₂O₃ [M+H⁺] 641.41265, found: 641.41541.

Chapter 8

3,3-dimethyl-1-(6-oxo-6-(((1-(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)amino)hexyl)-2-((1E,3E)-5-((E)-1,3,3-trimethylindolin-2-ylidene)penta-1,3-dien-1-yl)-3H-indol-1-ium (7): Azide compound 2 (8.3 mg, 0.027 mmol, 1.0 eq.) was dissolved in DMF (0.80 mL). Blue Cy5 10 (16 mg, 0.030 mmol, 1.1 eq.), CuSO₄ (1.0 M in H₂O, 12 μL, 0.012 mmol, 0.44 eq.) and sodium ascorbate (1.0 M in H₂O, 13 μL, 0.013 mmol, 0.48 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 45%→55% B in A, 3 CV, solutions used A: 50 mM NH₄HCO₃ in H₂O, B: MeCN) and lyophilized resulting as a purple powder product 7 JJB381 (6.9 mg, 7.8 μmol, 13%). ¹H-NMR (850 MHz, CD₂OD): δ ppm 8.28 – 8.22 (m, 2H), 7.84 (s, 1H), 7.50 (d, J = 7.4 Hz, 2H), 7.44 – 7.38 (m, 2H), 7.34 – 7.22 (m, 4H), 6.62 (t, J = 12.4 Hz, 1H), 6.28 (dd, J = 13.7, 3.3 Hz, 2H), 4.41 (s, 2H), 4.36 (t, J = 7.1 Hz, 2H), 4.09 (t, J = 7.3 Hz, 2H), 3.99 (dd, J = 8.7, 4.3 Hz, 1H), 3.63 (s, 2H), 3.53 – 3.52 (m, 1H), 2.66 (s, 1H), 2.31 – 2.27 (m, 1H), 2.25 (t, J = 7.3 Hz, 2H), 2.15 – 2.10 (m, 1H), 1.91 – 1.79 (m, 5H), 1.73 – 1.58 (m, 16H), 1.56 – 1.50 (m, 2H), 1.50 – 1.44 (m, 2H), 1.35 – 1.27 (m, 8H), 1.14 (d, J = 7.5 Hz, 3H); ¹³C-NMR (214 MHz, CD₂OD): δ ppm 175.72, 175.41, 174.61, 174.61, 155.56, 155.48, 146.13, 144.24, 143.54, 142.54, 142.52, 129.78, 129.74, 126.61, 126.29, 126.22, 124.12, 123.42, 112.02, 111.85, 104.41, 104.23, 76.04, 75.11, 70.13, 62.29, 51.34, 50.54, 50.52, 46.47, 45.71, 44.71, 36.94, 36.46, 35.57, 31.50, 31.30, 30.56, 30.48, 29.99, 28.26, 28.12, 27.94, 27.79, 27.40, 27.31, 26.38; LC-MS: Rᵣ 4.83 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: m/z = 833.60 (M⁺); HRMS: calculated for C₃₆H₄₅N₂O₅ [M⁺] 833.55621, found: 833.55124.
6-(5-(3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)-N-{1-(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 hexanamide (8): Azide compound 2 (8.3 mg, 0.027 mmol, 1.0 eq.) was dissolved in DMF (0.80 mL). Biotin compound 11 (12 mg, 0.030 mmol, 1.1 eq.), CuSO$_4$ (1.0 M in H$_2$O, 12 μL, 0.012 mmol, 0.44 eq.) and sodium ascorbate (1.0 M in H$_2$O, 13 μL, 0.013 mmol, 0.48 eq) were added to the solution under argon atmosphere. After stirring at room temperature for 12 h, the reaction volatiles were removed under reduced pressure. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 5%→40% B in A, 3 CV, solutions used A: 50 mM NH$_4$HCO$_3$ in H$_2$O, B: MeCN) and lyophilized resulting as white product 8 JJB385 (3.8 mg, 5.3 μmol, 20%).

$^1$H-NMR (850 MHz, CD$_3$OD): δ ppm 7.84 (s, 2H), 4.50–4.48 (m, 1H), 4.42 (s, 2H), 4.38 (t, $J$ = 7.1 Hz, 2H), 4.30 (dd, $J$ = 7.9, 4.5 Hz, 1H), 4.00 (dd, $J$ = 8.7, 4.3 Hz, 1H), 3.54–3.52 (m, 1H), 3.36–3.32 (m, 1H), 3.23–3.18 (m, 1H), 3.17–3.13 (m, 2H), 2.92 (dd, $J$ = 12.8, 5.0 Hz, 1H), 2.70 (d, $J$ = 12.7 Hz, 1H), 2.66 (s, 1H), 2.34–2.30 (m, 1H), 2.23 (t, $J$ = 7.5 Hz, 2H), 2.21–2.18 (m, 2H), 2.17–2.12 (m, 2H), 1.92–1.83 (m, 3H), 1.78–1.71 (m, 1H), 1.71–1.53 (m, 7H), 1.53–1.47 (m, 2H), 1.45–1.40 (m, 2H), 1.40–1.26 (m, 10H), 1.15 (d, $J$ = 7.5 Hz, 3H); $^{13}$C-NMR (214 MHz, CD$_3$OD): δ ppm 176.00, 175.96, 166.11, 146.22, 124.14, 76.06, 75.10, 70.14, 63.38, 62.30, 61.62, 57.02, 51.35, 46.48, 45.73, 41.05, 40.18, 36.94, 36.81, 36.75, 35.59, 31.29, 30.57, 30.12, 29.79, 29.50, 27.55, 27.39, 26.93, 26.52, 16.79; LC-MS: $R_t$ 4.48 min, linear gradient 10%→90% B in 12.5 min; ESI-MS: $m/z$ = 707.47 (M+H)$^+$; HRMS: calculated for C$_{34}$H$_{58}$N$_8$O$_6$S [M+H]$^+$ 707.42728, found: 707.42759.

5-(((3R,5R,7R)-adamantan-1-yl)methoxy)-1-((1R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl)pentan-1-one (18):

N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (79 mg, 0.32 mmol, 1.6 eq.) and adamantan-acid 17 (85 mg, 0.32 mmol, 1.6 eq.) were dissolved in anhydrous DMF (0.32 mL) and stirred at room temperature for 2 h. Pre-activated mixed anhydride solution (160 µL, 0.80 eq) was added to deprotected aziridine 16 (35 mg, 0.20 mmol, 1.0 eq.) in dry DMF (1.2 mL) at 0 °C and stirred for 30 min. Additional pre-activated solution (160 µL, 0.80 eq) was added. The resulting mixture was stirred at 0 °C for 2 h. The reaction was quenched by MeOH (1.0 mL) and the mixture was concentrated in vacuo. Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 38%→47% B in A, 12min, solutions used A: H$_2$O, B: MeCN) and the fractions were lyophilized directly yielding 18 as white powder (21 mg, 0.05 mmol, 25%).$^1$H-NMR (400 MHz, CD$_3$OD): δ ppm 4.08 (d, $J$ = 10.4, 2H), 3.72–3.66 (m, 2H), 3.41 (t, $J$ = 6.4 Hz, 2H), 3.23 (dd, $J$ = 10.0, 8.0 Hz, 1H), 3.10–3.02 (m, 2H), 2.74 (d, $J$ = 5.6 Hz, 1H), 2.55–2.51 (m, 2H), 2.01–1.95 (m, 4H), 1.77–1.66 (m, 8H); 1$^3$C-NMR (100 MHz, CD$_3$OD): δ ppm 188.5, 83.0, 79.1, 73.4, 72.2, 69.4, 63.6, 45.3, 42.4, 41.1, 40.8, 38.3, 36.6, 35.2, 30.1, 29.9, 29.8, 22.9; LC-MS: $R_t$ 7.87 min; linear gradient 10%→90% B in 15 min, ESI-MS: $m/z = 424.4$ (M+H)$^+$; HRMS: calculated for C$_{14}$H$_{17}$NO$_4$ [M+H]$^+$ 424.26936, found: 424.26921.

Benzyl (1R,2S,3S,4R,5R,6R)-2,3,4-trihydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-1-one (22): Benzyl alcohol (28 μL, 0.28 mmol, 1.1 eq) and p-nitrophenyl chloroformate (105 mg, 0.32 mmol, 2.0 eq.) were dissolved in DCM (6.0 mL). Pyridine (104 μL, 1.3 mmol, 5.0 eq) was added into the solution, the resulting mixture was stirred...
at room temperature for 4 h. After quenching the reaction with brine (1.5 mL), the mixture was transferred into a separatory funnel, washed the organic layer with brine, dried over \( \text{MgSO}_4 \), concentrated \textit{in vacuo}, redissolved in dry DMF 1mL, followed by addition of Et_3N (0.2 mL). Aziridine compound (45.6 mg, 0.26 mmol, 1.0 eq.) in DMF (1.0 mL) was added dropwise into active ester solution under argon atmosphere at room temperature overnight. The resulting solution was concentrated \textit{in vacuo} and Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 17%→23% B in A, 3 CV, solutions used A: \textit{H}_2\text{O}, B: \textit{MeCN}) and the fractions were lyophilized directly yielding 22 as white powder product (30 mg, 0.11 mmol, 42%). TLC: \( R_f \) 0.31 (DCM/MeOH, 5/1, v/v); \( ^1\text{H}-\text{NMR} \) (600 MHz, CD_3OD): \( \delta \) ppm 7.39 – 7.29 (m, 5H), 5.15 – 5.09 (m, 2H), 4.04 (dd, \( J = 10.4, 4.4 \text{ Hz}, 1\text{H} \)), 3.78 (dd, \( J = 10.4, 8.4 \text{ Hz}, 1\text{H} \)), 3.12 – 3.02 (m, 2H), 2.75 (d, \( J = 6.0 \text{ Hz}, 1\text{H} \)), 1.98 – 1.90 (m, 1H); \( ^{13}\text{C}-\text{NMR} \) (150 MHz, CD_3OD): \( \delta \) ppm 164.6, 137.4, 129.6, 129.3, 129.1, 129.0, 79.0, 73.3, 69.3, 69.3, 63.6, 45.1, 13.42; LC-MS: \( R_t \) 5.16 min, linear gradient 00→90% B in 15 min; ESI-MS: \( m/z \) = 310.3 (M+H)^+; HRMS: calculated for \textit{C}_{15}\textit{H}_{19}\textit{NO}_5\textit{[M+H]+} 310.12851, found: 310.12876.

**Benzyl (1R,2R,3R,4R,5R,6R)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptane-7-carboxylate (24) and 4-nitrophenyl(1R,2R,3R,4R,5R,6R)-2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptane-7-carboxylate (25):** Benzyl alcohol (23 μL, 0.23 mmol, 1.1 eq) and \( p \)-nitrophenol chloroformate (80.6 mg, 0.40 mmol, 2.0 eq.) were dissolved in DCM (5 mL). Pyridine (80 μL, 1 mmol, 5.0 eq.) was added into the solution, the resulting mixture was stirred at room temperature for 4 h. After quenching the reaction with brine (1.2 mL), the mixture was transferred into a separatory funnel, washed the organic layer with brine, dried over \( \text{MgSO}_4 \), concentrated \textit{in vacuo}, redissolved in dry DMF (1.0 mL), followed by addition of Et_3N (0.20 mL). Aziridine compound (32 mg, 0.20 mmol, 1.0 eq.) in DMF (1.5mL) was added dropwise under argon atmosphere at room temperature overnight. The resulting solution was concentrated \textit{in vacuo} and Then the crude product was purified by semi-preparative reversed HPLC (linear gradient: 19%→23% B in A, 3 CV, solutions used A: \textit{H}_2\text{O}, B: \textit{MeCN}) giving product 24 (10 mg, 34 μmol, 17% yield) and 25 (4.3 mg, 13 μmol, 7% yield) as white powder. 24 TLC: \( R_f \) 0.51 (DCM/MeOH, 8/1, v/v); \( ^1\text{H}-\text{NMR} \) (600 MHz, CD_3OD): \( \delta \) ppm 7.39 – 7.32 (m, 4H), 5.15 (dd, \( J = 21.6, 12 \text{ Hz}, 2\text{H} \)), 4.03 (dd, \( J = 9.0, 4.2 \text{ Hz}, 1\text{H} \)), 3.60 – 3.59 (m, 1H), 3.37 (dd, \( J = 9.0, 1.8 \text{ Hz}, 1\text{H} \)), 2.98 (dd, \( J = 6.0, 3.6 \text{ Hz}, 1\text{H} \)), 2.41 (d, \( J = 6.6 \text{ Hz}, 1\text{H} \)), 2.04 – 2.02 (m, 1H); 1.89 (s, 1H), 1.19 (d, \( J = 7.8 \text{ Hz}, 3\text{H} \)); \( ^{13}\text{C}-\text{NMR} \) (150 MHz, CD_3OD): \( \delta \) ppm 165.0, 137.5, 129.6, 129.3, 129.2, 129.0, 75.7, 74.4, 69.2, 69.2, 44.6, 44.3, 36.7, 16.2; LC-MS: \( R_t \) 4.28 min, linear gradient 10→90% B in 15 min; ESI-MS: \( m/z \) = 294.3 (M+H)^+; HRMS: Calculated for \textit{C}_{15}\textit{H}_{19}\textit{NO}_5\textit{[M+H]+} 294.13360. Found: 264.13364. 25 \( ^1\text{H}-\text{NMR} \) (400 MHz, CD_3OD): \( \delta \) ppm 8.24 – 8.20 (m, 2H), 7.18 – 7.15 (m, 2H), 4.78 (t, \( J = 7.8 \text{ Hz}, 1\text{H} \)), 4.66 (t, \( J = 6.4 \text{ Hz}, 1\text{H} \)), 4.26 (t, \( J = 6.4 \text{ Hz}, 1\text{H} \)), 3.98 – 3.93 (m, 2H), 2.56 – 2.52 (m, 1H), 1.04 (d, \( J = 7.6 \text{ Hz}, 3\text{H} \)); \( ^{13}\text{C}-\text{NMR} \) (100 MHz, CD_3OD): \( \delta \) ppm 164.7, 161.6, 143.2, 126.9, 116.6, 81.3, 78.9, 73.4, 73.0, 55.6, 35.4, 11.3; 25 LC-MS: \( R_t \) 4.15min, linear gradient 10→90% B in 15 min; ESI-MS: \( m/z \) = 342.1 (M+NH_4)^+.

**Diethyl 2,3,4-trihydroxy-5-methyl-7-azabicyclo[4.1.0]heptane-7-carboxylate (26):** Diethyl zinc solution (1.0 M in Et_2O, 930 μL, 930 μmol, 10.0 eq.), BF_3-OEt_2 liquid (56.7 μL, 463 μmol, 5.0 eq.) and Et_2O (97 μL, 930 μmol, 10.0 eq.) were dissolved in 0.5 mL dry DC. Diiodomethane (149 μL, 1.8...
mmol, 20 eq.) was added to the solution and stirred at room temperature for 5 min. A solution of compound 26 (30 mg, 0.093 mmol 1.0 eq.) in dry DCM (0.50 mL) was added to the mixture dropwise. After stirring overnight, the reaction was quenched by saturated aqueous NH₄Cl, the mixture was poured into H₂O and extracted by EtOAc. The organic layer was washed with brine and dried over MgSO₄. The solvent was removed under reduced pressure and residue was purified by silica gel column chromatography (5%-20% EtOAc in pentane) yielding cyclopropane 27 (26 mg, 0.077 mmol, 83%). TLC: Rf 0.45 (EtOAc/pentane, 1/5, v/v); [α]D₂₀ -101 (c = 0.5, CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ ppm 7.46 – 7.21 (m, 10H), 4.82 (d, J = 11.8 Hz, 1H), 4.77 – 4.59 (m, 3H), 4.25 (t, J = 7.3 Hz, 1H), 3.75 (s, 1H), 3.17 – 3.13 (m, 1H), 2.08 (s, 1H), 1.65 – 1.59 (m, 1H), 1.51 – 1.41 (m, 1H), 1.22 (d, J = 12.0 Hz, 3H), 0.89 – 1.74 (m, 2H), 0.27 – 0.20 (m, 1H); ¹³C-NMR (100 MHz, CDCl₃): δ ppm 139.24, 138.69, 128.51, 128.41, 127.91, 127.86, 127.76, 127.52, 82.61, 77.48, 77.16, 76.84, 76.47, 73.01, 72.59, 71.00, 36.12, 18.42, 16.94, 16.18, 11.63; HRMS: calculated for C₂₂H₂₆O₃ [M+H⁺] 339.19547, found: 339.19599.

(1R,2R,3R,4R,5R,6R)-5-methylbicyclo[4.1.0]heptane-2,3,4-triol (28): A mixture of product 27 (26 mg, 0.077 mmol, 1.0 eq) and Pd(OH)₂/C (20 wt.% loading (dry basis), 5.0 mg) in MeOH (5.0 mL) was stirred at room temperature under hydrogen atmosphere for 24 h. The catalyst was then filtered off and washed with MeOH. The filtrate was combined and concentrated under reduced pressure. Crude product was purified by silica gel column chromatography (5%-10%, MeOH in DCM) giving the title compound 28 (12 mg, 0.076 mmol, 99%). TLC: Rf 0.60 (1/5, MeOH/DCM, v/v); [α]D₂₀ -102 (c = 0.2, MeOH); ¹H-NMR (850 MHz, CD₃OD): δ ppm 4.19 (dd, J = 8.9, 6.5 Hz, 1H), 3.53 – 3.48 (m, 1H), 3.05 (dd, J = 8.9, 1.8 Hz, 1H), 1.70 – 1.66 (m, 1H), 1.35 – 1.31 (m, 1H), 1.17 (d, J = 7.3 Hz, 3H), 0.74 – 0.70 (m, 1H), 0.69 – 0.65 (m, 1H), 0.22 – 0.17 (m, 1H); ¹³C-NMR (214 MHz, CD₃OD): δ ppm 76.96, 75.85, 70.40, 38.05, 19.89, 18.77, 17.94, 11.82; IR (neat, cm⁻¹): 3321, 2965, 2905, 1366, 1250, 1045, 1022, 995, 929, 812, 682; HRMS: calculated for C₈H₁₄O₃ [M+H⁺] 159.10157, found: 159.10149.

((((1R,2S,3R,6R)-6-methylcyclohex-4-ene-1,2,3-triyl)tris(oxy))tris(methylene)) tribenzene (29): A solution of 26 (65 mg, 0.2 mmol, 1.0 eq.) in dry DMF (1 mL), benzyl bromide (47.6 μL, 0.4 mmol, 2.0 eq.) and tetrabutylammonium iodide (1.5 mg, 2.0 μmol, 0.02 eq.) were added. The mixture was cooled down to 0 °C, and NaH (60% (w/w) in mineral oil, 16 mg, 0.40 mmol, 2.0 eq.) was added slowly and keep the low temperature for 2 h. After stirring at room temperature overnight, the reaction was quenched with H₂O (0.5 mL), and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over MgSO₄. The solvent was removed under reduced pressure and residue was purified by silica gel column chromatography (0%-20% EtOAc in pentane) yielding cyclohexane 29 (80 mg, 0.19 mmol, 95%). TLC: Rf 0.62 (EtOAc/pentane, 1/8, v/v); [α]D₀⁻ -103 (c = 1, CHCl₃); ¹H-NMR (400 MHz, CDCl₃): δ ppm 7.54 – 7.33 (m, 15H), 5.85 – 5.80 (m, 1H), 5.58 (d, J = 10.0 Hz, 1H), 5.03 (d, J = 11.8 Hz, 1H), 4.95 – 4.76 (m, 5H), 4.58 – 4.53 (m, 1H), 4.02 – 3.98 (m, 1H), 3.90 – 3.87 (m, 1H), 2.63 – 2.50 (m, 1H), 1.19 (d, J = 7.3 Hz, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ ppm 139.93, 132.05, 128.41, 128.37, 128.34, 128.10, 127.85, 127.58, 127.52, 127.48, 127.45, 125.52, 83.30, 77.95, 77.58, 73.82, 72.39, 70.17, 35.84, 16.69; IR (neat, cm⁻¹): 3028, 2872, 1497, 1454, 1089, 1068, 734, 696; HRMS: calculated for C₅₂H₃₂O₃ [M+H⁺] 415.22677, found: 415.22683.
methylbicyclo[4.1.0]heptane-7-carboxylate (32): A 2-necked pear flask was charged with 29 (80 mg, 0.19 mmol, 1.0 eq.), Copper(II) acetylacetonate (Cu(AcAc)$_2$) (5.0 mg, 0.019 mmol, 0.10 eq.) and dry EtOAc (0.2 mL). The mixture was refluxed at 90 °C and ethyl diazoacetate (13% wt. in DCB, 46 μL, 0.38 mmol, 2.0 eq.) was added in dry EtOAc (0.80 mL) by syringe pump over 6 h. After full conversion of starting material, the mixture was concentrated under reduced pressure, and silica gel column chromatography (1%-→10%, EtOAc in pentane) produced the mixture of α and β cyclopropane ester mixture 30, which was treated by 20 mg Pb(OH)$_2$/C (20% wt. loading (dry basis)) in MeOH (2.0 mL) at room temperature under hydrogen atmosphere overnight. The reaction mixture was filtered with celite and washed with MeOH. The solvent was removed under reduced pressure and residue was purified by silica gel column chromatography (0%-→10% MeOH in DCM) giving target α-cyclopropane 31 (11 mg, 0.049 mmol, 26%) and byproduct β-cyclopropane 32 (4.6 mg, 0.020 mmol, 11%). 31 TLC: R$_f$ 0.34 (MeOH/DCM, 1/9, v/v); 31 H-NMR (400 MHz, CD$_3$OD): δ ppm 4.20 (dd, $J$ = 8.9, 6.2 Hz, 1H), 4.10 (q, $J$ = 7.3 Hz, 2H), 3.55 – 3.51 (m, 1H), 3.08 – 3.03 (m, 1H), 1.98 – 1.92 (m, 1H), 1.84 – 1.76 (m, 1H), 1.58 (t, $J$ = 4.6 Hz, 1H), 1.29 – 1.22 (m, 4H), 1.19 (d, $J$ = 7.3 Hz, 3H); 13C-NMR (100 MHz, CD$_3$OD): δ ppm 175.52, 76.17, 75.59, 68.90, 61.70, 36.92, 30.95, 29.08, 26.80, 18.47, 14.53; HRMS: calculated for C$_{18}$H$_{18}$O$_5$ [M+H$^+$] 231.11261, found: 231.11058. 

(25,3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-[[benzylxoy)methyl]-2-(phenylthio)tetrahydro-2H-pyran (47): A mixture of compound 46 (5.7 g, 21 mmol, 1.0 eq.), ‘Stick’ reagent, imidazole-1-sulfonyl azide (5.5 g, 26 mmol, 1.2 eq.) and CuSO$_4$·5H$_2$O (52 mg, 0.21 mmol, 0.010 eq.) in 100 mL EtOH was stirred at room temperature overnight. The resulting solution was concentrated to remove most of EtOH in vacuo, diluted by 1.0 M aqueous HCl (100 mL), extracted by EtOAc. The organic layer was washed over NaHCO$_3$ and brine and dried over MgSO$_4$ and concentrated in vacuo. After three times co-evaporation with toluene, the residue was dissolved in dry DMF (60 mL), followed by addition of BnBr (6.5 mL, 55 mmol, 2.6 eq.), tetrabutylammonium iodide (225 mg, 1.0 mmol, 1.0 eq.), and TFA (0.74 mL, 1.0 mmol, 1.0 eq.) and byproduct 30, which was treated by 20 mg Pd(OH)$_2$/C (20% wt. loading (dry basis)) in MeOH (2.0 mL) at room temperature under hydrogen atmosphere overnight. The reaction mixture was filtered with celite and washed with MeOH. The solvent was removed under reduced pressure and residue was purified by silica gel column chromatography (0%-→15% MeOH in DCM) giving target α-cyclopropane 31 (11 mg, 0.049 mmol, 26%) and byproduct β-cyclopropane 32 (4.6 mg, 0.020 mmol, 11%). 

TLC: R$_f$ 0.29 (1/9, MeOH/DCM, v/v); H-NMR (400 MHz, CD$_3$OD): δ ppm 4.15 – 4.06 (m, 2H), 3.82 (d, $J$ = 8.7 Hz, 1H), 3.68 – 3.65 (m, 1H), 3.28 – 3.24 (m, 1H), 2.20 – 2.10 (m, 1H), 2.08 (t, $J$ = 4.6 Hz, 1H), 1.57 – 1.51 (m, 2H), 1.27 – 1.21 (m, 3H), 1.12 (d, $J$ = 6.9 Hz, 3H); 13C-NMR (100 MHz, CD$_3$OD): δ ppm 78.07, 74.08, 70.34, 61.58, 33.45, 30.32, 29.37, 23.83, 16.82, 14.55; HRMS: calculated for C$_{18}$H$_{18}$O$_5$ [M+H$^+$] 231.11261, found: 231.11058. 

(3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-[[benzylxoy)methyl]tetrahydro-2H-pyran-2-ol (48): Compound 47 (568 mg, 1.0 mmol, 1.0 eq.) was dissolved in DCM/H$_2$O (11 mL, 10/1, v/v) and cooled to 0°C. N-iodosuccinimide (225 mg, 1.0 mmol, 1.0 eq.) and TFA (0.74 mL, 1.0 mmol, 1.0 eq.) were added into the solution and stirred for 3 h, and quenched by sat. aq. Na$_2$S$_2$O$_5$ (25 mL). The resulting mixture
was diluted by EtOAc, washed by sat. aq. NaHCO₃ twice and dried over MgSO₄. After concentration in vacuo, the residue was purified by silica gel column chromatography (10%→40%, EtOAc in pentane) to afford mixture product 48 as white crystals (449 mg, 0.94 mmol, 94%). ¹H-NMR (400 MHz, CDCl₃): δ ppm 7.43 – 7.08 (m, 22H), 4.88 (s, 2H), 4.61 – 4.47 (m, 5H), 4.11 – 4.05 (m, 1H), 4.04 – 3.96 (m, 1H), 3.71 – 3.53 (m, 4H), 3.52 – 3.34 (m, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ ppm 137.94, 137.92, 128.62, 128.59, 128.27, 128.22, 128.14, 128.12, 128.05, 127.99, 127.96, 127.93, 96.30, 92.21, 83.19, 80.25, 78.58, 77.79, 75.73, 75.69, 75.18, 75.15, 75.00, 73.68, 73.63, 70.76, 68.70, 68.64, 67.56, 64.13.

(3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl (E)-2,2,2-trifluoro-N-phenylacetimidate (49): Compound 48 (316 mg, 0.66 mmol, 1.0 eq.) was dissolved in 7 mL actone/H₂O (20/1, v/v) and cooled to 0 °C. N-phenyl trifluoroacetimidoyl chloride (280 mg, 1.3 mmol, 2.0 eq.) and Cs₂CO₃ (261 mg, 0.80 mmol, 1.2 eq.) were added into the solution and stirred for 24 h, quenched by adding Et₃N dropwise. The resulting mixture was diluted by EtOAc, washed by H₂O and brine aqueous and dried over MgSO₄. After concentration in vacuo, the residue was purified by silica gel column chromatography (0%→10%, EtOAc in pentane) yielding product 49 as light yellow oil (293 mg, 0.45 mmol, 68%). ¹H-NMR (400 MHz, CDCl₃): δ ppm 7.42 – 7.23 (m, 18H), 7.17 (d, J = 6.2 Hz, 3H), 7.10 (t, J = 7.4 Hz, 1H), 6.82 (d, J = 7.7 Hz, 2H), 4.92 (s, 2H), 4.88 – 4.76 (m, 1H), 4.67 – 4.45 (m, 4H), 4.07 – 3.87 (m, 2H), 3.86 – 3.77 (m, 2H), 3.76 – 3.60 (m, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ ppm 177.75, 143.39, 137.85, 137.78, 137.76, 137.72, 128.87, 128.63, 128.56, 128.51, 128.17, 128.11, 128.02, 128.00, 127.95, 124.51, 93.89, 93.83, 80.37, 77.58, 75.79, 75.39, 75.18, 73.65, 73.51, 67.92, 63.03.

((1R,4S,5R,6R)-6-(((2S,3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)-4,5-bis(benzyloxy)cyclohex-2-en-1-yl)methyl 4-oxopentanoate (50): Mixture of thioglycoside donor 47 (108 mg, 0.19 mmol, 1.2 eq.) and flame-dried molecular sieve (3Å) was suspended in dry DCM (1.0 mL), the final concentration of the thioglycoside donor was 60 mM. Then, N-formylmorpholine (306 μL, 3.0 mmol, 16 eq.) was added to the mixture. The resulting mixture was stirred at room temperature for 10 min and at reaction temperature specified for particular reaction for additional 10 min. Subsequently, NIS (43 mg, 0.19 mmol, 1.2 eq.) and trimethylsilyltriflate (62 μL, 0.34 mmol, 1.8 eq.) were added, the reaction progress was monitored by TLC (pentane/EtOAc, 5/2, v/v). Upon completion activation of the glycosyl donor, acceptor 45 (70 mg, 0.16 mmol, 1.0 eq.) was added to the reaction mixture. The progress of glycosylation was monitored by TLC. Upon completion of reaction sat. aq. NaHCO₃ and Na₂SO₃ were added to the mixture, followed by vigorous stirring until the brown color of the reaction mixture faded away. The resulting mixture was filtered and extracted by DCM, dried over MgSO₄, filtered and concentrated before silica gel column chromatography (EtOAc in pentane, 10%→30%) yielding pure product 50 as light yellow oil (40 mg, 0.045 mmol, 28%). ¹H-NMR (400 MHz, CDCl₃): δ ppm 7.39 – 7.18 (m, 23H), 7.19 – 7.10 (m, 2H), 5.82 – 5.73 (m, 2H), 5.59 – 5.51 (m, 1H), 5.08 (d, J = 10.9 Hz, 1H), 4.95 – 4.80 (m, 3H), 4.76 (d, J = 10.8 Hz, 1H), 4.71 – 4.57 (m, 3H), 4.55 – 4.44 (m, 2H), 4.30 (dd, J = 11.0, 3.4 Hz, 1H), 4.24 – 4.18 (m, 1H), 4.06 (dd, J = 11.0, 5.3 Hz, 1H), 3.98 – 3.86 (m, 3H), 3.85 – 3.67 (m, 3H), 3.61 – 3.55 (m, 1H), 3.28 (dd, J = 10.4, 4.0 Hz, 1H), 2.69 – 2.60 (m, 3H), 2.52 – 2.47 (m, 2H), 2.15 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ ppm 206.35, 172.63, 138.99, 138.19, 138.03, 138.00, 137.98, 128.56, 128.56, 128.54.
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128.49, 128.40, 128.12, 128.04, 128.02, 127.99, 127.94, 127.93, 127.85, 127.80, 127.63, 127.40, 127.36, 98.35, 84.17, 80.90, 80.25, 78.19, 75.50, 75.14, 74.76, 74.52, 73.64, 71.77, 71.59, 68.05, 64.52, 63.30, 42.90, 37.91, 29.96, 27.89;

HRMS: calculated for C_{53}H_{57}N_{3}O_{10} [M+Na]^{+} 918.39362, found: 918.39371.

(1R,4S,5R,6R)-6-(((2S,3R,4R,5S,6R)-3-azido-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetracyclo[2.2.1.0^{2,4}]hepta-2,5-dien-1-yl)oxy)-4,5-bis(benzyloxy)cyclohex-2-en-1-yl)methanol (51):

Compound 50 (40 mg, 0.045 mmol, 1.0 eq.) was dissolved in pyridine/AcOH mixture (0.5 mL, 4/1, v/v) at room temperature, followed by addition of hydrazine acetate, \( \text{NH}_2\text{NH}_2\cdot\text{AcOH} \) (20.5 mg, 2.23 mmol, 5.0 eq.). The resulting mixture was stirred at room temperature for 1 h and quenched by acetone. The reaction mixture was diluted by EtOAc, washed by H\(_2\)O and brine aqueous and dried over MgSO\(_4\). After concentration in vacuo, the residue was purified by silica gel column chromatography (EtOAc in pentane, 20% \(\rightarrow\) 40%) yielding colorless oil product 51 (30 mg, 0.038 mmol, 84%).

\(^{1}H\)-NMR (400 MHz, CDCl\(_3\)): \( \delta \) ppm 7.38 – 7.24 (m, 23H), 7.11 – 7.06 (m, 2H), 5.84 – 5.75 (m, 2H), 5.66 – 5.59 (m, 1H), 5.10 (d, \( J = 10.9 \) Hz, 1H), 4.95 – 4.81 (m, 3H), 4.76 – 4.63 (m, 1H), 4.13 (t, \( J = 9.6 \) Hz, 1H), 3.99 – 3.89 (m, 4H), 3.77 – 3.61 (d, \( J = 10.3 \) Hz, 2H), 3.41 – 3.20 (m, 3H), 2.50 – 2.44 (m, 1H); \(^{13}C\)-NMR (100 MHz, CDCl\(_3\)): \( \delta \) ppm 139.16, 138.29, 137.84, 137.42, 137.10, 129.66, 128.66, 128.64, 128.54, 128.39, 128.27, 128.23, 128.19, 128.16, 128.05, 127.81, 127.76, 97.94, 84.79, 81.54, 80.64, 78.79, 75.62, 75.31, 74.64, 73.65, 73.01, 71.75, 71.70, 68.73, 63.27, 61.42, 45.60; HRMS: calculated for C_{48}H_{51}N_{3}O_{8} [M+Na]^{+} 820.35684, found: 820.35694.

N-((2S,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)tetracyclo[2.2.1.0^{2,4}]hepta-2,5-dien-1-yl)oxy)tetrahydro-2H-pyran-3-yl)acetamide (52):

Compound 51 (22 mg, 0.027 mmol, 1.0 eq.) was dissolved in CHCl\(_3\) (350 μL), followed by addition of pyridine (300 μL) and thioacetic acid (300 μL). The resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted by EtOAc, washed by H\(_2\)O and brine aqueous and dried over MgSO\(_4\). After concentration in vacuo, the residue was purified by silica gel column chromatography (EtOAc in pentane, 50% \(\rightarrow\) 90%) yielding product 52 as colorless oil (15.4 mg, 0.019 mmol, 70%).

\(^{1}H\)-NMR (400 MHz, CDCl\(_3\)): \( \delta \) ppm 7.37 – 7.24 (m, 23H), 7.16 – 7.11 (m, 2H), 6.33 (d, \( J = 9.7 \) Hz, 1H), 5.88 (dt, \( J = 10.3, 2.7 \) Hz, 1H), 5.72 – 5.67 (m, 1H), 5.05 (d, \( J = 3.7 \) Hz, 1H), 4.90 (d, \( J = 11.3 \) Hz, 1H), 4.79 – 4.62 (m, 4H), 4.60 – 4.50 (m, 4H), 4.44 (d, \( J = 10.8 \) Hz, 1H), 4.34 (td, \( J = 9.9, 3.6 \) Hz, 1H), 4.19 – 4.14 (m, 1H), 4.03 – 3.86 (m, 3H), 3.77 (dd, \( J = 8.3, 5.9 \) Hz, 1H), 3.73 – 3.65 (m, 3H), 3.64 – 3.51 (m, 3H), 2.47 – 2.39 (m, 1H), 1.50 (s, 3H); \(^{13}C\)-NMR (100 MHz, CDCl\(_3\)): \( \delta \) ppm 170.10, 138.46, 137.97, 137.88, 137.69, 129.62, 128.73, 128.67, 128.61, 128.49, 128.28, 128.16, 128.10, 128.06, 128.03, 127.98, 127.88, 127.85, 127.70, 126.83, 126.45, 99.55, 81.53, 81.41, 78.67, 78.12, 76.51, 75.11, 75.06, 74.36, 73.57, 72.39, 71.55, 69.03, 62.56, 52.70, 46.10, 23.01; HRMS: calculated for C_{50}H_{55}NO_{9} [M+Na]^{+} 837.37690, found: 837.37663.

N-((2S,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((4aR,5R,6S,7R,8S,8aR)-6,7-bis(benzyloxy)-8-iodo-2-(trichloromethyl)-4a,5,6,7,8,8a-hexahydro-4H-benzo[d][1,3]oxazin-5-yl)oxy)tetrahydro-2H-pyran-3-yl)acetamide (53):

Product 52 (11 mg,
0.013 mmol, 1.0 eq.) was thrice co-evaporated with toluene and was subsequently dissolved in dry DCM (100 mL). The solution was cooled down to 0 °C. DBU (2.7 µL, 0.027 mmol, 2.0 eq.) and trichloroacetonitrile (0.95 µL, 1.4 µmol, 0.10 eq.) were added to the solution. The reaction was stirred at 0°C for 2 h, TLC showed that the starting material was completely converted into a higher running product. Subsequently, H₂O (42 µL), NaHCO₃ (15 mg, 0.18 mmol, 13 eq.) and iodine (11 mg, 0.044 mmol, 3.1 eq.) were added to the solution. It was stirred overnight at room temperature. The reaction mixture was quenched with Na₂S₂O₃ (10% aqueous), concentrated in vacuo and extracted with EtOAc. The organic layer with the intermediate product was dried over MgSO₄ and concentrated for purification by silica gel column chromatography (EtOAc in pentane, 10%→40%) yielding product 53 as colorless oil (9.0 mg, 8.3 µmol, 62%).

1H-NMR (400 MHz, CDCl₃): δ ppm 7.42–7.04 (m, 25H), 6.46 (d, J = 9.8 Hz, 1H), 5.14 (d, J = 11.6 Hz, 1H), 5.04 (d, J = 10.3 Hz, 1H), 4.94 (d, J = 3.5 Hz, 1H), 4.85 (t, J = 3.5 Hz, 1H), 4.82–4.69 (m, 3H), 4.59 (d, J = 11.0 Hz, 3H), 4.56–4.47 (m, 3H), 4.43–4.34 (m, 1H), 4.21–4.13 (m, 1H), 4.09–4.04 (m, 1H), 4.03–3.99 (m, 1H), 3.90 (t, J = 9.3 Hz, 1H), 3.77–3.70 (m, 4H), 3.53 (t, J = 10.0 Hz, 1H), 2.75 (dd, J = 9.3, 3.8 Hz, 1H), 2.69–2.62 (m, 1H), 1.36 (s, 3H); HRMS: calculated for C₅₂H₅₄Cl₃N₂O₉ [M+H⁺] 1083.20123, found: 1083.20164.

N-((2S,3R,4R,5S,6R)-4,5-bis(benzyloxy)-6-((benzyloxy)methyl)-2-(((1R,2R,3R,4S,5S,6R)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-3-yl)oxy)tetrahydro-2H-pyran-3-yl)acetamide (54): The intermediate 53 (9.0 mg, 8.3 µmol, 1.0 eq.) was dissolved in dioxane (0.5 mL) and 2 drops of HCl 37% in water. The reaction mixture was refluxed at 60 °C for 4 h. The reaction progress was monitored by TLC with pentane/EtOAc mixture (2/1, v/v). After which it was concentrated in vacuo and redissolved in MeOH (0.50 mL), and NaHCO₃ (14 mg, 0.17 mmol, 20 eq.) was added making the pH > 7. The reaction mixture was stirred at room temperature for three days. The reaction mixture was concentrated in vacuo, redissolved in H₂O (5.0 mL) and extracted with EtOAc. The organic layer was dried with MgSO₄ and concentrated in vacuo. The crude product was purified via silica gel column chromatography (2%→6%, MeOH in DCM) yielding aziridine 54 as white solid (4.0 mg, 4.8 µmol, 58%). TLC: Rf 0.43 (MeOH/DCM, 1/9, v/v); 1H-NMR (400 MHz, CDCl₃): δ ppm 7.37–7.24 (m, 20H), 7.24–7.13 (m, 5H), 4.96 (d, J = 11.1 Hz, 1H), 4.90 (d, J = 3.6 Hz, 1H), 4.77 (d, J = 11.2 Hz, 2H), 4.66 (d, J = 10.2 Hz, 3H), 4.63–4.52 (m, 3H), 4.52–4.44 (m, 3H), 4.32 (td, J = 9.8, 3.6 Hz, 1H), 4.26–4.19 (m, 1H), 4.09–4.00 (m, 3H), 3.82 (d, J = 7.7 Hz, 1H), 3.73–3.59 (m, 6H), 3.58–3.50 (m, 1H), 2.59–2.55 (m, 1H), 2.35 (d, J = 6.2 Hz, 1H), 2.07–1.98 (m, 1H), 1.42 (s, 3H); 13C-NMR (100 MHz, CDCl₃): δ ppm 170.25, 138.56, 138.11, 138.05, 137.75, 127.96, 127.54, 127.17, 100.62, 81.63, 78.03, 75.25, 75.10, 74.85, 73.57, 72.47, 72.41, 69.18, 68.30, 62.80, 53.28, 44.01, 31.52, 31.50, 30.49, 29.85, 29.07, 23.87, 23.14, 22.82; HRMS: calculated for C₁₂₂H₁₄₅N₁₀O₉ [M+H⁺] 829.40586, found: 829.40593.

8.4 References


