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Synthesis of epoxide- and aziridine-based probes to target retaining galactosidases

5.1 Introduction

Glycosidases are hydrolytic enzymes responsible for the hydrolysis of glycosidic bonds in (oligo)saccharides and glycoconjugates and are crucial for the breakdown of glyco(sphingo)lipids in lysosomes. They can be classified according to the nature of their substrate and the mechanism of substrate hydrolysis, which generally involves cleavage with either retention or inversion of anomeric stereochemistry. For example, retaining β-glucosidases recognize β-linked glucoside substrates and hydrolyze the glycosidic bond with retention of stereochemistry at the anomeric position. The catalytic mechanism of retaining glycosidases is a two-step process that generally involves a double displacement mechanism as depicted schematically in Figure 5.1A.¹ In the first step the catalytic nucleophile, usually an aspartic or glutamic acid residue, attacks the anomeric center to cleave the glycosidic bond with inversion of configuration and thereby creates a covalent enzyme-substrate intermediate. Cleavage of the substrate aglycon is assisted by another glutamic or aspartic acid residue that acts as a general acid and protonates the leaving group. In the next step this residue, now acting as a general base, deprotonates a water molecule that hydrolyzes the glycosyl-enzyme intermediate, thereby releasing the glycoside from the active site. The configuration is again inverted, resulting in a net retention of anomeric stereochemistry. An
alternative mechanism for hydrolysis with retention of configuration can occur when the substrate carries an acetamide substituent at C2 that assists in the cleavage of the glycosidic bond by acting as a nucleophile. This mechanism, for example used by N-acetylhexosaminidases, does not involve a covalent substrate-enzyme intermediate (Figure 5.1B). The same holds true for the class of inverting glycosidases. These enzymes employ a one-step mechanism involving two carboxylic acid residues in the active site that act as a general base and a general acid. The first carboxylic acid residue deprotonates a water molecule during its attack on the anomeric center, while the second residue protonates the leaving aglycon (Figure 5.1C).

Many covalent and irreversible mechanism-based glycosidase inhibitors have been described in literature. These can generally be divided into two types: compounds that release a reactive aglycon after hydrolytic cleavage which then reacts with another residue in the active site, and inhibitors that contain a reactive electrophile that is attacked by the active site nucleophile to create a stable covalent bond. The latter class includes epoxide- and aziridine-based inhibitors as well as glycosides that are functionalized with one or more fluorine substituents at C2 and/or C5. Of these, especially the fluorosugars have received considerable attention. Drawbacks of these inhibitors are their modest potency and the reversibility of binding, although the covalent enzyme-probe intermediate is usually sufficiently stable to enable analysis of the adducts. In contrast, the epoxide based inhibitors

Figure 5.1. Mechanism of substrate hydrolysis by retaining β-glucosidases (A), N-acetylhexosaminidases (B) and inverting β-glucosidases (C). D) Proposed mechanism of cyclophellitol binding to retaining β-glucosidases.
have proven to enable very potent and selective irreversible inhibition of specific glycosidases. Cyclophellitol (1) is a naturally occurring β-glucosidase inhibitor that was first isolated in 1990 from the mushroom Phellinus sp. (Figure 5.2A). ⁵,⁶ In a follow-up study it was demonstrated that cyclophellitol is a selective and potent mechanism-based irreversible inhibitor of several retaining β-glucosidases.⁷ The β-glucopyranose-configured inhibitor is thought to bind in the active site by mimicking a β-linked glucoside substrate and to enable covalent bond formation through reaction of the epoxide with the catalytic nucleophile (Figure 5.1D). In later studies, the α-epoxide stereoisomers (1R,6S)- and (1R,2S,6S)-cyclophellitol (2 and 3, Figure 5.2A) were shown to be irreversible inhibitors of retaining α-glucosidases and α-mannosidases, respectively,⁸,⁹ while the β-epoxide isomer of inhibitor 3 was synthesized as a putative β-mannosidase inhibitor.¹⁰ Conduritol B epoxide (5), although structurally related to cyclophellitol, is less potent and also less selective as a result of its structural symmetry, resulting in the inhibition of both retaining β- and α-glucosidases.¹¹,¹² Two galactopyranose-configured isomers, conduritol C epoxides 6 and 7, have been identified as irreversible inhibitors of several retaining α- and β-galactosidases, respectively.¹³ In addition to the epoxide-based inhibitors, a few aziridine derivatives have been synthesized. For example, the aziridine analogue of cyclophellitol (4) is an inhibitor of

![Figure 5.2. A) Irreversible glycosidase inhibitors (1-8). B) ABPs for retaining β-exoglucosidases (9-10). C) Target compounds: inhibitors and ABPs for retaining α-galactosidases (11-16) and β-galactosidases (17-22).]
Chapter 5

retaining β-glucosidases and conduritol B aziridine (8) inhibits a number of retaining α- and β-glucosidases. The remarkable selectivity and potency of cyclophellitol, in combination with the highly conserved catalytic mechanism of this class of enzymes, opens up the potential to develop selective inhibitors for different retaining glycosidases by altering the configuration of the inhibitor to that of the natural substrates of the target enzyme. For example, galactopyranose-configured epoxides may enable specific targeting of catalytically active retaining galactosidases. In the same line, the structure of these inhibitors can be exploited for the design of ABPs that target a specific class of glycosidases. Cyclophellitol has already served as a basis to develop a number of epoxide- and aziridine-based probes for retaining β-exoglucosidases (e.g. 9 and 10, Figure 5.2B). Surprisingly, other than the glucosidase and mannosidase inhibitors described above, no differently configured isomers of cyclophellitol or ABPs 9 and 10 have been reported. It would therefore be desirable to develop new synthetic strategies that enable the synthesis of such compounds. This chapter describes the synthesis of two series of galactopyranose-configured ABPs (12-16 and 18-22, Figure 5.2C) for retaining α- and β-galactosidases, respectively, with the electrophilic aziridine and epoxide moieties in either an α- or a β-configuration to allow attack by the respective catalytic nucleophiles. The ABPs are functionalized with a fluorescent Bodipy tag, a biotin tag or an azide that can be used for two-step labeling. In addition, the epoxide-based inhibitors 11 and 17 are synthesized as α- and β-galactopyranose-configured cyclophellitol isomers.

5.2 Results and discussion

All target compounds were synthesized from a single precursor, dibenzylated compound 27, that was prepared in 3 steps from aldehyde 24 following conditions similar to those described in literature (Scheme 5.1). Dibutylboryl triflate mediated reaction of 24 with oxazolidinone 23 resulted in the stereoselective formation of syn-aldol adduct 25, which after removal of the Evans template (26) was subjected to ring-closing metathesis to provide cyclohexene 27. The synthetic route towards α- and β-epoxides 11 and 17 and their azide-modified derivatives 12 and 18 is depicted in Scheme 5.2. The azide moiety was installed at C6 (carbohydrate numbering) by selective tosylation of the primary alcohol in 27 followed by substitution with sodium azide to give 28. Next, the benzyl groups were removed with BCl3 and the free hydroxyl groups benzyolated (29) in order to enable facile deprotection after formation of the epoxide. Epoxidation was performed with mCPBA to give the α- and β-epoxides (30) in a ratio of approximately 4:1. After final deprotection with sodium methoxide the isomers could be separated by column chromatography to give
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**Scheme 5.1** Synthesis of dibenzylated cyclohexene 27

![Scheme 5.1](image)

**Reagents and conditions:**
- a) Bu₂BSO₃CF₃, Et₃N, DCM, -78 °C → -15 °C, 3.5 hrs, 63%;
- b) LiBH₄, THF/H₂O, 0 °C → rt, 2 hrs, 85%;
- c) Grubbs II, DCM, 40 °C, overnight, 78%.

Enantiomerically pure azide-functionalized α- and β-epoxides 12 and 18. The same strategy of deprotection, benzylation and epoxidation was used to synthesize the non-tagged epoxide inhibitors 11 and 17. In this case it proved impossible to separate the mixture of α- and β-epoxides (ratio 1:1) in the protected (32) or deprotected (33) stage. However, acetylation of 33 was found to give a separable mixture of mainly tri-acetylated β-epoxide 34β₁, a small amount of fully acetylated β-epoxide 34β₂, and fully acetylated α-epoxide 34α. After separation of the isomers by column chromatography, deprotection yielded the enantiomerically pure α- and β-epoxides 11 and 17. In order to synthesize the Bodipy- and

**Scheme 5.2** Synthesis of α- and β-galactopyranose-configured epoxides (11, 17) and azide-modified derivatives (12, 18)

![Scheme 5.2](image)

**Reagents and conditions:**
- a) i) pTsCl, Et₃N, DCM, rt, 4 days, ii) NaN₃, DMF, 60 °C, overnight, 63%;
- b) i) BCl₃, DCM, -78 °C, 4 hrs, ii) BzCl, pyridine, rt, overnight, 29 85%, 31 76%;
- c) mCPBA, DCM, rt, 4-8 days, 30 53%, 32 62%;
- d) NaOMe, MeOH, rt, 1.5 hrs, 18 8% + 12 42%, 33 76%;
- e) Ac₂O, pyridine, rt, 4 hrs, 34α 38% + 34β₁ 20% + 34β₂ 2%;
- f) NaOMe, MeOH, rt, 2 hrs, 11 94%, 17 84%.
biotin-tagged epoxide ABPs, their azide-functionalized precursors 12 and 18 were subjected to copper(I)-catalyzed azide-alkyne cycloadditions (‘click’ reactions) with Bodipy-alkyne 35 or biotin-alkyne 36 (Scheme 5.3). The click reactions proceeded smoothly to provide the fluorescently labeled α-epoxide 13 and biotinylated probe 14 as well as their β-epoxide isomers 19 and 20.

The strategy towards β-aziridine ABPs 21 and 22 is based on the previously reported procedure to synthesize β-glucosidase probe 10 and relies on the stereocontrolled formation of a β-aziridine with the aid of the primary alcohol at C6 (Scheme 5.4). Briefly, the primary hydroxyl in 27 was selectively transformed into the trichloroacetimidate which was then subjected to iodocyclization. Acidic hydrolysis of the intermediate imidate was followed by the addition of base to effect nucleophilic displacement of the iodine by the amine, providing aziridine 39. In case of the α-aziridine ABPs 15 and 16 it might be possible to apply a similar procedure by creating an imidate with the hydroxyl group at C2 in order to achieve iodocyclization below the plane of the cyclohexane ring. However, such a strategy would require a number of additional protection and deprotection steps in order to selectively unmask this hydroxyl group. Therefore another route was evaluated to synthesize the α-aziridine probes, commencing with the formation of fully benzylated β-epoxide 42. Although an α/β mixture was formed by mCPBA-mediated epoxidation of 41, the most prevalent product was β-epoxide 42 with only a small amount of the corresponding α-epoxide (ratio 6:1). Moreover, the two isomers could easily be separated by column chromatography. Next, the β-epoxide was opened with sodium azide and lithium perchlorate as a Lewis acid to give a mixture of azido-alcohols (43). Treatment of 43 with

Scheme 5.3 Synthesis of biotin- and Bodipy-functionalized epoxide ABPs (13, 14, 19, 20) and Bodipy-spacer 38

Reagents and conditions: a) Bodipy-alkyne 35 or biotin-alkyne 36, CuSO₄·5H₂O, sodium ascorbate, DMF, rt, overnight, 13 88%, 14 68%, 19 90%, 20 68%; b) Bodipy-alkyne 35, CuSO₄·5H₂O, sodium ascorbate, DMF, 80 °C, 4.5 hrs, 87%.
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Scheme 5.4 Synthesis of α- and β-galactopyranose-configured aziridine ABPs (15, 16, 21)

Reagents and conditions: a) i) CCl₃CN, DBU, DCM, 0 °C, 1.5 hrs, ii) I₂, NaHCO₃, H₂O, rt, overnight, iii) HCl, MeOH, rt, 3.5 hrs, iv) HCl, dioxane, 60 °C, 1 hr, v) NaHCO₃, MeOH, rt, 4 days, 52%; b) Li, NH₃ (l), THF, -60 °C, 45 min; c) 37 or 38, EEDQ, DMF, 0 °C, 90 min, 15 14%, 16 15%; d) NaH, BnBr, TBAI, DMF, rt, overnight, 85%; e) mCPBA, DCM, rt, overnight, 63%; f) NaN₃, LiClO₄, ACN, 80 °C, overnight, 73%; g) Ph₃P, ACN, 80 °C, 2 hrs, 26%.

triphenylphosphine led to the formation of the α-aziridine moiety (44) via an intramolecular Staudinger-like ring closure. The yield of this reaction was rather low, partly due to the fact that it proved difficult to remove the formed triphenylphosphine oxide. In addition, it might be possible that the intramolecular rearrangement of the iminophosphorane intermediate is hindered by steric factors or ring strain, resulting in reduction of the azide to an amine during workup. Nonetheless, the α-aziridine 44 could be obtained in enantiomerically pure form in four steps starting from 27. It proved impossible to synthesize β-aziridine 39 using the same strategy since no product was formed by opening of the benzylated epoxide (the α-isomer of 42) under the same conditions as described above, nor under acidic ‘nonchelating’ conditions (NaN₃, MeOH/1.2 M NH₄Cl (4:1), 80 °C) or with the use of phase transfer conditions (NaN₃, Bu₄NCl, ACN/H₂O, 80 °C).

The final stage in the synthetic route towards the aziridine ABPs involved deprotection of the benzyl groups in 39 and 44 by Birch reduction and acylation of the aziridine nitrogen with an azide- or Bodipy-functionalized spacer (37, 38) using EEDQ as the coupling reagent (Scheme 5.4). In case of the α-aziridine 44, deprotection and subsequent acylation followed by HPLC purification provided the azide- and Bodipy-tagged products 15 and 16. After deprotection of the β-aziridine 39, however, some byproduct formation was observed by ¹H NMR analysis. Furthermore, attempts to acylate the crude product 40 with azido-
spacer 37 were unsuccessful since $^1$H NMR analysis indicated significant degradation and almost complete disappearance of the aziridine proton peaks after HPLC purification and ensuing lyophilization. Further analysis by LC/MS revealed that the aziridine was opened with HCl, despite the fact that no acids were used during purification. Instability of the acylated aziridine moiety is a recurring problem during the synthesis of such ABPs and opening of the aziridine was also repeatedly observed with the $\alpha$-galactosidase ABPs and the $\beta$-glucosidase ABPs described previously.\textsuperscript{16} Hence, care has to be taken that the acylation reaction as well as ensuing HPLC purification and lyophilization are performed in the absolute absence of any traces of acid. It appears that the $\beta$-aziridine moiety is very unstable even under these conditions and consequently the $\beta$-aziridine ABP 21 could not be isolated.

The diastereomeric configuration of the $\alpha$- and $\beta$-epoxides 11 and 17 was determined by $^1$H NMR analysis and was supported by a comparison of the experimental coupling constants with the corresponding values obtained from DFT calculations (Table 5.1). The optimized structures obtained by these calculations are shown in Figure 5.3. The assignment of the diastereomeric configuration of epoxides 12, 18 and 42 could be deduced from epoxides 11 and 17. In all cases a characteristic configuration-dependent pattern of epoxide proton peaks was observed in which the H1 and H7 peaks (carbohydrate numbering) of the $\beta$-epoxides are consistently closer together than those of the $\alpha$-epoxides. Moreover, the H1-H2 coupling is smaller and the H5-H7 coupling larger for the $\beta$-isomers than the $\alpha$-isomers. The correct configuration of $\beta$-epoxide 42 was further supported by the fact that it leads to $\alpha$-aziridine 44, which can in turn be confirmed by comparison with $\beta$-aziridine 39 of which the configuration is known.

**Table 5.1. Comparison of experimental and calculated coupling constants for epoxides 11 and 17**

<table>
<thead>
<tr>
<th>$\alpha$-epoxide 11</th>
<th>$J$ exp. (Hz)</th>
<th>$J$ calc. (Hz)</th>
<th>$\beta$-epoxide 17</th>
<th>$J$ exp. (Hz)</th>
<th>$J$ calc. (Hz)</th>
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</thead>
<tbody>
<tr>
<td>H1-H7</td>
<td>4.0</td>
<td>3.7</td>
<td>H1-H7</td>
<td>3.7</td>
<td>3.4</td>
</tr>
<tr>
<td>H1-H2</td>
<td>2.5</td>
<td>2.2</td>
<td>H1-H2</td>
<td>n.d. (a)</td>
<td>0.04</td>
</tr>
<tr>
<td>H2-H3</td>
<td>8.6</td>
<td>6.9</td>
<td>H2-H3</td>
<td>8.6</td>
<td>6.6</td>
</tr>
<tr>
<td>H3-H4</td>
<td>1.9</td>
<td>1.8</td>
<td>H3-H4</td>
<td>n.d. (b)</td>
<td>2.7</td>
</tr>
<tr>
<td>H4-H5</td>
<td>3.6</td>
<td>4.0</td>
<td>H4-H5</td>
<td>4.0</td>
<td>3.5</td>
</tr>
<tr>
<td>H5-H7</td>
<td>n.d. (a)</td>
<td>0.04</td>
<td>H5-H7</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>H4-H7</td>
<td>1.8</td>
<td>1.1</td>
<td>H4-H7</td>
<td>n.d. (a)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Coupling constants obtained from DFT calculations (‘calc.’) as compared to experimental data from $^1$H NMR analysis (‘exp.’). n.d.: values were not determined because of a) very small coupling constants ($J < 1$ Hz) or b) peak overlap.
In the synthetic strategies described here, the formation of both α- and β-epoxides by mCPBA-mediated epoxidation is favorable, as long as the two isomers can be separated at some point in the route of synthesis. In other cases, however, it may be desirable to obtain only one isomer and for that purpose other oxidation methods should be evaluated. It is worth noting that the fully benzoylated compound 31 gives a 1:1 ratio of α- and β-epoxides whereas an α:β ratio of 4:1 was obtained with the C6-azide-functionalized analogue 29. Full benzylation (41) on the other hand leads to more β-selectivity during epoxidation (α:β 1:6). These results indicate that formation of the β-epoxide is favored in the absence of chelating effects and that the presence of a benzoyl group at either the allylic (C2) or homoallylic (C6) position can enhance α- or β-selectivity, respectively. As expected, deprotection of either 29 or 31 prior to epoxidation led to the formation of mainly the α-epoxide (α:β > 15:1) due to strong coordination of the allylic OH to mCPBA.

5.3 Conclusion

Two sets of galactopyranose-configured glycosidase probes were synthesized that are equipped with an electrophilic α- or β-configured epoxide moiety in order to enable targeting of retaining α- and β-galactosidases, respectively. Each set consists of a non-tagged inhibitor (11, 17), an ABP for two-step labeling with an azide as a ligation handle (12, 18), and two ABPs that are functionalized with a fluorescent Bodipy tag (13, 19) or a biotin tag (14, 20). Next to the epoxide-based ABPs, efforts were undertaken to synthesize analogous probes with an aziridine as the electrophile in which an azide or Bodipy tag is installed via acylation of the aziridine moiety. Whereas the α-aziridine ABPs 15 and 16 were successfully obtained, it proved impossible to isolate β-aziridine probe 21 since even the use of a completely acid-free purification procedure resulted in opening of the labile acylated aziridine. Hence, other methods or a different probe design will be necessary to realize the synthesis of aziridine-based β-galactosidase ABPs. Evaluation of the inhibitory potency of the synthesized α- and β-configured probes as well as their application for the labeling of human retaining α- and β-galactosidases is described in Chapters 6 and 7, respectively.
Experimental procedures

General

All reagents were commercial grade and were used as received unless stated otherwise. DCM, DCE, DMF and THF (Biosolve) were of analytical grade and were used under anhydrous conditions stored over flame-dried 3 Å molecular sieves. EtOAc (Riedel-de Haën) used for column chromatography was of technical grade and distilled before use. Reactions were monitored by TLC-analysis using DC-alufolien (Merck, Kieselgel60, F254) with detection by UV-absorption (254/366 nm), spraying with 20% H2SO4 in ethanol or a solution of (NH4)6Mo7O24.4H2O (25 g/L) and (NH4)4Ce(SO4)4.2H2O (10 g/L) in 10% aqueous sulfuric acid followed by charring at ~150 °C or by spraying with an aqueous solution of KMnO4 (7%) and K2CO3 (2%). Column chromatography was performed on silica gel (Screening Devices BV, 0.040 - 0.063 mm, 60 Å). LC/MS analysis was performed on an LCQ Adventage 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, 5μm particle size, Phenomenex). The applied buffers were A: H2O, B: ACN and C: 1 % aqueous TFA. Reported gradients represent the percentage of buffer B in buffer A with 10% buffer C. Alternatively, where indicated LC/MS analysis was performed using an API 3000 ESI (Q1) mass spectrometer (Applied Biosystems) coupled to a Jasco (900 series) HPLC system. The applied buffers were A: H2O, B: ACN and C: 100 mM NH4OAc in H2O. Reported gradients represent the percentage of buffer B in buffer A with 10% buffer C. HRMS analysis was performed on an LTQ Orbitrap (Thermo Finnigan) mass spectrometer equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL min−1, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 - 2000) and dioctylphthalate (m/z = 391.28428) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). For reversed-phase HPLC purification an automated HPLC system equipped with a C18 semiprep column (Gemini C18, 250 mm x 10 mm, 5μm particle size, 110 Å pore size, Phenomenex) was used. 1H- and 13C-APT-NMR spectra were recorded on a Brüker AV-400 (400/100MHz) or a Bruker DMX600 (600/150 MHz) instrument with a cryoprobe. Chemical shifts are given in ppm (δ) relative to the solvent peak or to tetramethylsilane as internal standard. Coupling constants (J) are given in Hz. All presented 13C-APT spectra are proton decoupled. Peak assignments are based on 2D 1H-COSY and 13C-HSQC NMR experiments.

(S)-3-((2S,3S,4S,5S)-4,5-bis(benzyloxy)-3-hydroxy-2-vinylhept-6-enoyl)-4-isopropyloxazolidin-2-one (25)

Note: Solutions of aldehyde 24 and oxazolidinone 23 in DCM were put under argon atmosphere and dried over activated 4Å molsieves for at least 1 hr prior to use. Et3N was distilled over CaH2 and stored on activated 4Å molsieves under argon atmosphere.

A solution of oxazolidinone 23 (32 mmol, 6.3 g, 2.0 eq.) in DCM under argon atmosphere was cooled to -78 °C with the use of a cryostate before addition of Bu3BSO2CF3 (32 mmol, 32 mL 1 M in DCM, 2.0 eq.) and Et3N (35 mmol, 4.9 mL, 2.2 eq.). The reaction mixture was stirred at -78 °C for 1 hr, then at 0 °C (ice bath) for 15 min and subsequently cooled back to -78 °C. A solution of aldehyde 24 (16 mmol, 4.7 g, 1.0 eq.) in DCM under argon atmosphere was added via canula after which the temperature was allowed to rise to -20 °C over 2 hrs and maintained at -15 °C for 1.5 hrs. Next, the mixture was removed from the cold bath and put in an ice bath of -5 °C. After quenching with PBS (20 mL), H2O2 (30% aqueous solution) was added dropwise while keeping the temperature of the mixture below 5 °C, until no more rise in temperature was observed. At this point the mixture was stirred for another 45 min while slowly warming to room temperature after which aqueous saturated NaHCO3 was added and the aqueous layer was extracted with DCM (3x). The combined organic layers were dried over MgSO4, filtered and concentrated in vacuo. The crude product was purified by column chromatography (pentane → 10% EtOAc in pentane) to afford the aldol adduct 25 (4.9 g, 10 mmol, 63%). 1H NMR (400 MHz, CDCl3): δ (ppm) 7.39-7.25 (m, 10H), 6.06 (ddd, J = 18.5, 9.7, 7.1
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(2R,3S,4S,5S)-4,5-bis(benzyloxy)-2-vinylhept-6-ene-1,3-diol (26)

Oxazolidinone 25 (13.8 mmol, 6.8 g) was dissolved in THF (100 mL) at 0 °C after which H2O (8 mL) and LiBH4 (35 mmol, 17.5 mL 2 M in THF, 2.5 eq.) were added. The reaction mixture was stirred at 0 °C for 1 hr and then at room temperature for 1 hr, before being quenched with 2 M NaOH. The resulting mixture was extracted with Et2O (2x) and the combined organic layers were washed with aqueous saturated NaHCO3 and Brine, dried over MgSO4 and concentrated in vacuo. Purification by column chromatography (pentane → 40% EtOAc in pentane) yielded alcohol 26 as a white solid (4.3 g, 12 mmol, 85%).1H NMR (400 MHz, CDCl3): δ (ppm) 7.45-7.21 (m, 10H), 6.14-5.94 (m, 2H), 5.49-5.33 (m, 2H), 5.26 (dd, J = 11.8 Hz, 1H), 4.67 (d, J = 11.8 Hz, 1H), 4.57 (d, J = 11.1 Hz, 1H), 4.48 (d, J = 11.1 Hz, 1H), 4.39 (d, J = 11.8 Hz, 1H), 4.21 (dd, J = 7.9, 5.7, 2.9 Hz, 1H), 2.20 (bs, 1H). 13C NMR (100 MHz, CDCl3): δ (ppm) 137.75, 137.26, 135.28, 133.85, 128.55, 128.36, 127.93, 127.86, 127.80, 119.51, 119.05, 79.58, 78.97, 73.08, 72.53, 70.97, 65.58, 47.33.

(3S,4S,5S,6R)-6-(hydroxymethyl)-3,4-dibenzyloxy-5-hydroxy-cyclohex-1-ene (27)

To a solution of 26 (7.8 mmol, 2.9 g) in DCM under argon atmosphere was added second generation Grubbs catalyst (0.4 mmol, 0.34 g, 5 mol%). The reaction mixture was stirred at 40 °C in the dark for 20 hrs, DMSO (40 mmol, 2.8 mL, 100 eq. with respect to catalyst) was added and the mixture was stirred overnight at room temperature before being concentrated in vacuo. Purification by column chromatography (pentane → pentane/EtOAc 1/1 (v/v)) gave cyclohexene 27 (7.2 mmol, 2.5 g, 92%). 1H NMR (400 MHz, CDCl3): δ (ppm) 7.38-7.28 (m, 10 H), 5.84 (d, J = 10.4 Hz, 1H), 5.55 (d, J = 10.1 Hz, 1H), 4.71 (d, J = 8.5 Hz, 4H), 4.33-4.31 (m, 2H), 3.82-3.80 (m, 2H), 3.65 (d, J = 7.4 Hz, 1H), 2.95 (bs, 2H), 2.45 (d, J = 6.1 Hz, 1H). 13C NMR (100 MHz, CDCl3): δ (ppm) 138.45, 137.93, 128.45, 128.32, 133.85, 128.55, 128.36, 127.93, 127.86, 127.80, 119.51, 119.05, 79.58, 78.97, 73.08, 72.53, 70.97, 65.58, 47.33.

(3S,4S,5S,6R)-6-(azidomethyl)-3,4-dibenzyloxy-5-hydroxy-cyclohex-1-ene (28)

A solution of (3S,4S,5S,6R)-6-(hydroxymethyl)-3,4-dibenzyloxy-5-hydroxy-cyclohex-1-ene (27) (0.76 mmol, 0.26 g) in DCM under argon atmosphere was cooled to 0 °C before pTsCl (0.84 mmol, 0.16 g, 1.1 eq.) and Et3N (1.4 mmol, 0.19 mL, 1.8 eq.) were added. The reaction mixture was stirred overnight at room temperature, after which TLC analysis indicated incomplete reaction. Therefore another 0.5 eq. of pTsCl (75 mg) and 1.8 eq. of Et3N (0.19 mL) were added after 24 hrs and again after 48 hrs. The mixture was stirred for a total of 4 days, after which 1 M aqueous HCl was added and the aqueous layer was extracted with Et2O (3x). The combined organic layers were dried over MgSO4, filtered and concentrated in vacuo. The crude tosylated product was then redissolved in DMF under argon atmosphere and NaN3 (7.6 mmol, 0.50 g, 10 eq.) was added. The reaction mixture was heated to 60 °C, stirred overnight and concentrated in vacuo. The residue was then redissolved in EtOAc and washed with 1 M aqueous HCl (1x), aqueous saturated NaHCO3 (1x) and Brine (1x), dried over MgSO4, filtered and concentrated in vacuo. Purification by column chromatography (pentane → 12% EtOAc in pentane in steps of 2%) gave the dibenzylated azide 28 (0.17 g, 0.48 mmol, 63% over two steps). 1H NMR (400 MHz, CDCl3): δ (ppm) 7.46-7.31 (m, 10H), 5.86 (dt, J = 10.2, 2.6 Hz, 1H), 5.48 (dd, J = 9.6, 1.6 Hz, 1H), 4.81-4.73 (m, 4H), 4.40-4.27 (m, 2H), 3.70 (dd, J = 7.8, 2.2 Hz, 1H), 3.60 (dd, J = 11.9, 9.3 Hz, 1H), 3.40 (dd, J = 11.9, 6.5 Hz, 1H), 2.56 (dt, J = 6.3, 3.1 Hz, 1H), 2.50-2.47
Chapter 5

(3S,4R,5S,6R)-6-(azidomethyl)-3,4,5-tribenzoyloxy-cyclohex-1-ene (29)

A solution of dibenzylated azide 28 (0.47 mmol, 0.17 g) in DCM was cooled to -78 °C under argon atmosphere before addition of BCl₃ (4.8 mmol, 4.8 mL 1 M in DCM, 10 eq.). The reaction mixture was stirred at -78 °C for 4 hrs, quenched with MeOH, concentrated in vacuo and coevaporated with toluene (3x). The deprotected product was then dissolved in pyridine, BzCl was added (4.7 mmol, 0.55 mL, 10 eq.) and the mixture was stirred overnight at room temperature. After quenching with aqueous saturated NaHCO₃ the mixture was extracted with DCM (3x) and the combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo. The product was purified by column chromatography (pentane → 7.5% EtOAc in pentane) to yield the tribenzylated product 29 (0.20 g, 0.40 mmol, 85% over two steps). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.19-7.86 (m, 6H), 7.66-7.28 (m, 9H), 6.21-6.12 (m, 1H), 6.12-6.07 (m, 1H), 6.03 (dt, J = 10.3, 2.7 Hz, 1H), 5.89-5.82 (m, 1H), 5.71 (dd, J = 8.6, 2.3 Hz, 1H), 3.52 (dd, J = 12.1, 7.7 Hz, 1H), 3.42 (dd, J = 12.1, 7.8 Hz, 1H), 3.10-3.08 (m, 1H). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 166.11, 165.84, 165.51, 133.75, 133.55, 133.28, 133.21, 130.17, 129.78, 129.76, 129.46, 129.20, 129.17, 128.66, 128.46, 128.40, 128.31, 127.48, 126.55, 73.14, 70.49, 69.85, 51.46, 39.87. HRMS: calcd. for [C₂₁H₂₃O₇N₃Na]⁺ 520.14791, found 520.14724. [α]D²⁰ (c = 0.2, CHCl₃): +265°.

(2S,3S,4S,5S)-5-(azidomethyl)-2,3,4-trihydroxy-7-oxabicyclo[4.1.0]heptane (30)

Tribenzylated azide 29 (0.40 mmol, 0.20 g) was dissolved in DCM under argon atmosphere and 3-chloroperoxybenzoic acid (1.2 mmol, 0.27 g, 3.0 eq.) was added. The reaction mixture was stirred at room temperature for a total of 4 days, concentrated in vacuo and purified by column chromatography (pentane → 10% EtOAc in pentane) giving the azido-epoxide 30 as a mixture of α- and β-isomers (ratio 4:1) (0.11 g, 0.21 mmol, 53%). ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.12-7.81 (m, 6H α + 6H β), 7.68-7.25 (m, 9H α + 9H β), 6.04 (dd, J = 9.3, 2.3 Hz, 1H α), 5.95-5.86 (m, 1H α + 1H β), 5.75 (d, J = 9.4 Hz, 1H β), 5.60 (dd, J = 9.3, 1.9 Hz, 1H α), 5.53 (dd, J = 9.3, 2.6 Hz, 1H β), 3.88 (dd, J = 3.9, 2.3 Hz, 1H α), 3.68-3.42 (m, 2H α + 4H β), 3.35 (dd, J = 3.9, 1.7 Hz, 1H α), 2.80-2.74 (m, 1H α + 1H β). ¹³C NMR (100 MHz, CDCl₃): δ (ppm) 166.25, 165.68, 165.65, 165.54, 165.47, 165.26, 154.59, 133.66, 133.62, 133.53, 133.48, 133.36, 133.30, 133.88, 129.80, 129.79, 129.76, 129.73, 129.71, 129.64, 129.01, 128.96, 128.92, 128.89, 128.83, 128.67, 128.64, 128.58, 128.56, 128.49, 128.43, 128.33, 128.32, 72.17, 70.85, 70.32, 70.18, 68.47, 66.77, 54.44, 54.14, 53.67, 52.04, 49.77, 49.75, 39.01, 38.27. LC/MS analysis: R₂₈ ṕ₂₃ ṕ₃₇. The product was purified by multiple rounds of column chromatography (pentane to 3% MeOH in DCM) giving the azido-epoxide 30 (0.19 mmol, 96 mg) was dissolved in MeOH (2.5 mL) and deprotected with NaOMe (38 µmol, 6.8 µL 5.6 M in MeOH, 20 mol%) for 1.5 hrs at room temperature. The reaction mixture was then quenched by addition of Amberlite-H⁺ IR-200, filtered and concentrated in vacuo. The product was purified by multiple rounds of column chromatography (DCM → 3% MeOH in DCM in steps of 0.5%) to separate the α-epoxide 12 (16 mg, 80 µmol, 42%) and β-epoxide 18 (3.0 mg, 15 µmol, 8%) (yield of mixed α/β product 7.0 mg, 35 µmol, 18%). ¹H NMR (600 MHz, MeOD) α-epoxide 12: δ (ppm) 4.07 (dd, J = 8.6, 2.5 Hz, 1H), 3.78 (dd, J = 3.4, 1.8 Hz, 1H), 3.59 (dd, J = 12.2, 8.0 Hz, 1H), 3.48 (dd, J = 12.3, 8.3 Hz, 1H), 3.39 (dd, J = 8.5, 1.9 Hz, 1H), 3.32 (dd, J = 3.9, 2.4 Hz, 1H), 2.97 (dd, J = 3.9, 1.8 Hz, 1H), 2.09 (td, J = 8.2, 3.5 Hz, 1H). ¹³C NMR (150 MHz, MeOD) α-epoxide 12: δ (ppm) 73.56, 72.15, 70.53, 58.08, 55.29, 51.73, 41.83. ¹H NMR (600 MHz, MeOD) β-epoxide 18: δ (ppm) 3.92 (dd, J = 8.7, 0.7 Hz, 1H), 3.82 (dd, J = 4.1, 2.5, 1.3 Hz, 1H), 3.70 (dd, J = 12.1, 8.2 Hz, 1H), 3.63 (dd, J = 12.1, 7.2 Hz, 1H), 3.33 (dd, J = 8.6, 2.6 Hz, 1H), 3.26-
chloroperoxybenzoic acid was added and the reaction mixture was stirred again for 4 days at room temperature, and the reaction mixture was stirred for 4 days at room temperature. Then, an additional 1 eq. of 3-
(0.42 mmol, 0.24 g) in DCM was added. 3-chloroperoxybenzoic acid (1.1 mmol, 0.24 g, 2.5 eq.)
to a solution of yielded a mixture of the α-
α) 4.52 δ (ppm) 76.39, 70.36, 69.50, 58.03, 55.21, 51.58, 40.43. [α]D20 (c = 0.1 (β)/0.3 (α), MeOH): 12° +81°, 18° +64°.

(35,4R,5S,6R)-6-(benzoyloxy)methyl)-3,4,5-tribenzoyloxy-cyclohex-1-ene (31)

A solution of (35,4S,5S,6R)-6-(hydroxymethyl)-3,4-dibenzyloxy-5-hydroxy-cyclohex-1-ene (27) (0.5 mmol, 0.17 g) in DCM under argon atmosphere was cooled to -78 °C before BCl3 was added (5 mmol, 5 mL 1M in DCM, 10 eq.). The reaction mixture was stirred under argon at -78 °C for 4 hrs and then quenched with MeOH, concentrated in vacuo and coevaporated with toluene (3x). Purification by column chromatography (10% MeOH in EtOAc → 20% MeOH in EtOAc) afforded the fully deprotected compound, which was redissolved in pyridine and cooled to 0 °C. Next, benzoyl chloride was added (5 mmol, 0.6 mL, 10 eq.) and the mixture was stirred overnight at room temperature before being quenched with a saturated aqueous NaHCO3 solution. The aqueous layer was extracted with DCM (3x) and the combined organic layers were dried over MgSO4, filtered and concentrated in vacuo. Purification by column chromatography (pentane → 10% EtOAc in pentane) yielded the fully benzoylated product 31 (0.22 g, 0.38 mmol, 76% over two steps). 1H NMR (400 MHz, CDCl3): δ (ppm) 8.00-7.88 (m, 8H), 7.62-7.30 (m, 12H), 6.23-6.18 (m, 2H), 6.09-6.06 (m, 1H), 5.88 (dd, J = 1.2, 10.4 Hz, 1H), 5.78 (dd, J = 2.2, 8.6 Hz, 1H), 4.59 (dd, J = 6.4, 10.8 Hz, 1H), 4.32 (dd, J = 8.8, 10.8 Hz, 1H), 3.44-3.40 (m, 1H). 13C NMR (100 MHz, CDCl3): δ (ppm) 166.28, 165.69, 165.58, 133.59, 133.50, 133.44, 133.25, 133.20, 133.13, 129.80, 129.77, 129.74, 129.68, 129.51, 129.35, 129.24, 128.60, 128.47, 128.39, 128.31, 128.66, 126.41, 73.10, 70.60, 69.30, 63.05, 39.47. LC/MS analysis: Rt 10.1 min (linear gradient 10 → 90% B in 15 min), m/z 599.1 [M+Na]+, 1194.9 [2M+Na]+. HRMS: calcd. for [C35H29O9Na]+ 615.16255, found 615.16189.

(2R,3S,4S,5R)-5-(hydroxymethyl)-7-oxabicyclo[4.1.0]heptane-2,3,4-triol: mix of α- and β-epoxides (33)

A solution of tetrabenzoylated epoxides 32 (α- and β-epoxides in 1:1 ratio) (0.42 mmol, 0.24 g) in DCM was added 3-chloroproxybenzoic acid (1.1 mmol, 0.24 g, 2.5 eq.) and the reaction mixture was stirred for 4 days at room temperature. Then, an additional 1 eq. of 3-chloroproxybenzoic acid was added and the reaction mixture was stirred again for 4 days at room temperature, before being concentrated in vacuo. Purification by column chromatography (pentane → 15% EtOAc in pentane) yielded a mixture of the α- and β-epoxides 32 in a 1:1 ratio (0.15 g, 0.26 mmol, 62%). 1H NMR (400 MHz, CDCl3): δ (ppm) 8.19-7.76 (m, 8H α + 8H β), 7.68-7.20 (m, 12H α + 12H β), 6.14-5.98 (m, 2H α + 1H β), 5.78 (dd, J = 9.4 Hz, 1H β), 5.68 (dd, J = 1.8, 9.2 Hz, 1H α), 5.61 (dd, J = 2.6, 9.4 Hz, 1H β), 4.78 (dd, J = 6.7, 11.1 Hz, 1H β), 4.70-4.62 (m, 1H α), 4.52-4.46 (m, 1H α + 1H β), 3.92 (dd, J = 2.3, 3.7 Hz, 1H α), 3.50 (s, 2H β), 3.43 (dd, J = 1.5, 3.8 Hz, 1H α), 3.21-3.01 (m, 1H α + 1H β). 13C NMR (100 MHz, CDCl3): δ (ppm) 166.23, 166.14, 165.87, 165.51, 133.71, 133.42, 133.26, 133.20, 133.13, 129.80, 129.77, 129.74, 129.68, 129.51, 129.35, 129.24, 128.60, 128.47, 128.39, 128.31, 128.66, 126.41, 73.10, 70.60, 69.30, 63.05, 39.47. LC/MS analysis: Rt 10.1 min (linear gradient 10 → 90% B in 15 min), m/z 599.1 [M+Na]+, 1194.9 [2M+Na]+. HRMS: calcd. for [C35H29O9Na]+ 615.16255, found 615.16189; calcd. for [C35H32O9Na]+ 599.16764, found 599.16727. [α]D20 (c = 0.1, CHCl3): +250°.
Separation of α- and β-epoxides by acetylation-deacetylation (11 + 17)

An α/β mixture of deprotected epoxides 33 (0.26 mmol, 45 mg) was coevaporated with toluene before being dissolved in pyridine under argon atmosphere. After addition of acetic anhydride (10 mmol, 0.9 mL, 38 eq.) the mixture was stirred at room temperature for 4 hrs, then concentrated in vacuo and coevaporated with toluene (3x). After purification by column chromatography (DCM → 0.6% MeOH in DCM in steps of 0.1%) three different products were isolated: the tetra-acetylated form of the α-isomer 34α (0.10 mmol, 35 mg, 38%), a small amount of tetra-acetylated β-isomer 34β2 (4.4 µmol, 1.5 mg, 2%) and tri-acetylated β-isomer 34β1 (53 µmol, 16 mg, 20%). 1H NMR (400 MHz, CDCl3) 34α: δ (ppm) 5.52-5.41 (m, 2H), 5.00 (dd, J = 9.6, 1.8 Hz, 1H), 4.24-4.07 (m, 2H), 3.59 (dd, J = 4.0, 2.4 Hz, 1H), 3.10 (dd, J = 4.0, 1.6 Hz, 1H). 2.68 (td, J = 8.1, 3.7 Hz, 1H), 2.13 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H). 1H NMR (400 MHz, CDCl3) 34β2: δ (ppm) 5.25-5.45 (m, 1H), 5.26 (dd, J = 9.4 Hz, 1H). 4.95 (dd, J = 9.6, 2.0 Hz, 1H), 4.35-4.20 (m, 2H), 3.26-3.17 (m, 2H), 2.12 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.96 (s, 3H). 1H NMR (400 MHz, CDCl3) 34β1: δ (ppm) 5.35 (d, J = 9.1 Hz, 1H), 4.90 (dd, J = 9.1, 2.6 Hz, 1H), 4.46-4.38 (m, 2H), 3.99 (d, J = 9.3 Hz, 1H), 3.44-3.37 (m, 1H), 3.30 (d, J = 3.6 Hz, 1H), 2.54 (td, J = 7.6, 3.5, 1.3 Hz, 1H), 2.46 (d, J = 11.0 Hz, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H). The β-isomers 34β1 and 34β2 were then combined and deprotected separately from 34α by treatment with sodium methoxide (30 µmol, 5 µL 5.6 M in MeOH, 0.3 eq. for 34α and 17 µmol, 3 µL 5.6 M in MeOH, 0.3 eq. for 34β) in MeOH at room temperature for 2 hrs. The mixtures were neutralized with Amberlite-H+ IR-200, filtered and concentrated in vacuo. The crude products were finally purified by column chromatography (10% MeOH in DCM → 15% MeOH in DCM) to give α-epoxide 11 (17 mg, 94 µmol, 94%) and β-epoxide 17 (8.5 mg, 48 µmol, 84%). 1H NMR (600 MHz, MeOD) α-isomer 11: δ (ppm) 4.09 (dd, J = 8.5, 2.5 Hz, 1H), 3.85 (ddd, J = 3.6, 1.8, 1.8 Hz, 1H), 3.78 (dd, J = 11.0, 6.6 Hz, 1H), 3.74 (dd, J = 11.0, 8.0 Hz, 1H), 3.41 (dd, J = 8.6, 1.9 Hz, 1H), 3.33 (dd, J = 4.0, 2.5 Hz, 1H), 3.10 (dd, J = 4.0, 1.7 Hz, 1H), 2.04 (ddd, J = 8.0, 6.6, 3.5 Hz, 1H). 13C NMR (150 MHz, MeOD) α-epoxide 11: δ (ppm) 73.76, 72.77, 70.79, 62.28, 58.10, 55.46, 44.34. 1H NMR (600 MHz, MeOD) β-isomer 17: δ (ppm) 3.93 (d, J = 8.6 Hz, 1H), 3.88-3.82 (m, 3H), 3.33-3.31 (m, 2H), 3.17 (d, J = 3.7 Hz, 1H), 2.18 (tdd, J = 7.3, 4.0, 1.7 Hz, 1H). 13C NMR (150 MHz, MeOD) β-epoxide 17: δ (ppm) 76.72, 70.76, 69.96, 61.87, 57.76, 55.36, 42.82. [α]D20 (c = 0.2, MeOH): 11 +85°, 17 +67°.

(1R,2R,3S,4S,5R,6S)-5-((Bodipy-triazolyl)methyl)-2,3,4-trihydroxy-7-oxabicyclo[4.1.0]heptane (13)

Azido-epoxide 12 (25 µmol, 5.0 mg) and Bodipy-alkyne 35 (25 µmol, 12 mg, 1.0 eq.) were dissolved in DMF (0.5 mL) under argon atmosphere. After addition of copper(I)Iisulfate pentahydrate (5 µmol, 5 µL 1 M in H2O, 0.2 eq.) and sodium ascorbate (10 µmol, 10 µL 1 M in H2O, 0.4 eq.) the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated in vacuo and purified by column chromatography (DCM → 3% MeOH in DCM) to yield Bodipy-epoxide 13 as a purple solid (15 mg, 22 µmol, 88%). 1H NMR (400 MHz, MeOD): δ (ppm) 7.89-7.82 (m, 4H), 7.78 (s, 1H), 7.43 (d, J = 4.4 Hz, 2H), 7.01-6.94 (m, 4H), 6.70 (d, J = 4.3 Hz, 2H), 4.60-4.53 (m, 2H), 4.13 (dd, J = 8.5, 2.4 Hz, 1H), 3.85 (s, 6H), 3.66-3.61 (m, 1H), 3.42 (dd, J = 8.5, 1.8 Hz, 1H), 3.37-3.34 (m, 1H), 3.07-3.02 (m, 2H), 2.97 (dd, J = 3.9, 1.8 Hz, 1H), 2.79-2.75 (m, 2H), 2.55 (td, J = 8.2, 3.5 Hz, 1H), 1.88-1.83 (m, 4H). 13C NMR (100 MHz, MeOD): δ (ppm) 160.77, 157.39, 145.31, 136.08, 130.71, 127.03, 125.10, 119.66, 115.98, 113.22, 72.03, 70.53, 69.06, 56.67, 54.41, 53.30, 49.13, 41.27, 32.86, 29.58, 29.00, 24.48. LC/MS analysis: R; 10.4 min (linear gradient 10 → 50% B in 15 min), m/z 666.3 [M-F]+, 685.9 [M+H]+, 1371.0 [2M+H]+. HRMS: calcd. for [C36H39O6N5BF2]+ 686.29560, found 686.29599; calcd. for [C34H43O6N6BF2Na]+ 708.27754, found 708.27719.

(1R,2R,3S,4S,5R,6S)-5-((biotin-triazolyl)methyl)-2,3,4-trihydroxy-7-oxabicyclo[4.1.0]heptane (14)

Azido-epoxide 12 (25 µmol, 5.0 mg) and biotin-alkyne 36 (25 µmol, 7.0 mg, 1.0 eq.) were dissolved in DMF (0.5 mL) under argon atmosphere. After addition of copper(I)Iisulfate pentahydrate (5 µmol, 5 µL 1 M in H2O, 0.2 eq.) and sodium ascorbate (10 µmol, 10 µL 1 M in H2O, 0.4 eq.) the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated in vacuo and purified by column chromatography (DCM → 30%
Synthesis of epoxide- and aziridine-based probes to target retaining galactosidases

MeOH in DCM) to yield biotin-epoxide 14 as a white solid (8.0 mg, 17 µmol, 68%). $^1$H NMR (400 MHz, MeOD): δ (ppm) 7.97 (s, 1H), 4.65-4.60 (m, 2H), 4.52 (ddd, J = 8.0, 5.0, 1.0 Hz, 1H), 4.47 (s, 2H), 4.32 (dd, J = 7.9, 4.5 Hz, 1H), 4.14 (dd, J = 8.6, 2.4 Hz, 1H), 3.66 (dt, J = 3.7, 1.8 Hz, 1H), 3.43 (dd, J = 8.6, 1.8 Hz, 1H), 3.37-3.36 (m, 1H), 3.22 (ddd, J = 8.3, 5.9, 4.4 Hz, 1H), 3.02 (dd, J = 4.0, 1.8 Hz, 1H), 2.96 (dd, J = 12.8, 5.0 Hz, 1H), 2.74 (d, J = 12.7, 1.8 Hz, 1H), 2.58 (td, J = 8.1, 3.5 Hz, 1H), 2.27 (t, J = 7.3 Hz, 2H), 1.78-1.57 (m, 4H), 1.48-1.42 (m, 2H). $^{13}$C NMR (100 MHz, MeOD): δ (ppm) 174.63, 145.05, 123.62, 72.00, 70.51, 69.03, 61.95, 59.03, 49.23, 43.45, 39.66, 35.14, 34.22, 28.30, 27.05, 25.30. LC/MS analysis: R$_t$ 174.63, 145.05, 123.89, 123.06, 119.63, 113.22, 74.73, 68.51, 67.89, 56.70, 55.59, 53.32, 49.23, 43.45, 39.66, 35.14, 34.20, 28.28, 28.05, 25.29. LC/MS analysis: R$_t$ 28.30, 28.05, 25.29. LC/MS analysis: R$_t$ 174.63, 145.05, 123.62, 72.00, 70.51, 69.03, 61.95, 59.03, 49.23, 43.45, 39.66, 35.14, 34.22, 28.30, 28.04, 25.30.

Azido-epoxide

Azido-epoxide as a purple solid (15 mg, 22 µmol, 90%). $^1$H NMR (400 MHz, MeOD): δ (ppm) 1.91-1.72 (m, 4H), 7.77 (s, 1H), 7.44 (d, J = 4.4 Hz, 2H), 7.03-6.93 (m, 4H), 6.70 (d, J = 4.3 Hz, 2H), 4.70 (d, J = 7.8 Hz, 2H), 3.97 (d, J = 8.5 Hz, 1H), 3.86 (s, 6H), 3.66-3.58 (m, 1H), 3.42-3.40 (m, 1H), 3.18 (d, J = 3.7 Hz, 1H), 3.11-2.97 (m, 3H), 2.86-2.76 (m, 2H), 2.73 (ddt, J = 7.6, 4.3, 2.2 Hz, 1H), 1.91-1.82 (m, 4H). $^{13}$C NMR (100 MHz, MeOD): δ (ppm) 160.78, 157.38, 136.09, 130.79, 127.03, 125.11, 123.06, 117.32, 74.73, 68.51, 67.89, 56.77, 55.41, 53.27, 48.74, 39.84, 32.86, 29.59, 29.02, 24.46. LC/MS analysis: R$_t$ 10.5 min (linear gradient 10 → 50% B in 15 min), m/z 666.3 [M-F]$,^+$, 686.0 [M+H]$^+$, 1371.0 [2M+H]$^+$, 505.18850. HRMS: calcd. for [C$_{36}$H$_{36}$O$_{5}$N$_{5}$BF$_{2}$]$^+$ 686.29560, found 686.29615; calcd. for [C$_{36}$H$_{36}$O$_{5}$N$_{5}$BF$_{2}$Na]$^+$ 708.27754, found 708.27725.

Azido-epoxide

Azido-epoxide as a purple solid (8.0 mg, 17 µmol, 68%). $^1$H NMR (400 MHz, MeOD): δ (ppm) 7.93 (s, 1H), 4.75 (d, J = 7.7 Hz, 2H), 4.52 (ddd, J = 8.0, 5.0, 0.9 Hz, 1H), 4.46 (s, 2H), 4.32 (ddd, J = 7.9, 4.5 Hz, 1H), 3.98 (d, J = 8.5 Hz, 1H), 3.63 (ddd, J = 4.1, 2.7, 1.2 Hz, 1H), 3.38-3.36 (m, 1H), 3.25-3.22 (m, 1H), 3.21 (d, J = 3.8 Hz, 1H), 3.14-3.08 (m, 1H), 2.96 (dd, J = 7.7, 5.0 Hz, 1H), 2.78 (ddt, J = 12.7, 5.4, 2.7 Hz, 1H), 2.73 (d, J = 12.8 Hz, 1H), 2.27 (t, J = 7.3 Hz, 2H), 1.81-1.55 (m, 4H), 1.50-1.38 (m, 2H). $^{13}$C NMR (100 MHz, MeOD): δ (ppm) 174.62, 144.82, 123.89, 74.73, 68.46, 67.88, 61.95, 60.23, 56.79, 55.59, 53.25, 48.86, 39.87, 39.65, 35.14, 34.22, 28.29, 28.04, 25.30. LC/MS analysis: R$_t$ 3.7 min (linear gradient 10 → 50% B in 15 min), m/z 483.3 [M+H]$^+$, 965.0 [2M+H]$^+$, 1447.1 [3M+H]$^+$. HRMS: calcd. for [C$_{36}$H$_{36}$O$_{5}$N$_{5}$BF$_{2}$]$^+$ 686.29560, found 686.29615; calcd. for [C$_{36}$H$_{36}$O$_{5}$N$_{5}$BF$_{2}$Na]$^+$ 708.27754, found 708.27725.

8-(Bodipy-triazole)-octanoic acid 38

8-Azidoocanoic acid (37$^{25}$) (0.50 mmol, 93 mg) and Bodipy-alkyne 35$^{21}$ (0.50 mmol, 0.24 g, 1.0 eq.) were dissolved in DMF under argon atmosphere. After addition of copper(I) sulfate pentahydrate (100 µmol, 10 µL 1 M in H$_{2}$O, 0.2 eq.) and sodium ascorbate (200 µmol, 200 µL 1 M in H$_{2}$O, 0.4 eq.) the reaction mixture was stirred for 4.5 hrs. The mixture was then concentrated in vacuo and purified by column chromatography (DCM → 2% MeOH in DCM) to yield Bodipy-acid 38 as a purple solid (0.29 g, 0.43 mmol, 87%). $^1$H NMR (400 MHz, CDCl$_{3}$): δ
(35,4R,5S,6R)-6-(benzyloxymethyl)-3,4,5-tribenzyloxy-cyclohex-1-ene (41)

A solution of (35,4S,5S,6R)-6-(hydroxymethyl)-3,4-dibenzyloxy-5-hydroxy-cyclohex-1-ene (27) (3.5 mmol, 1.2 g) in DMF under argon atmosphere was cooled to 0 °C before tetrabutylammonium iodide (35 μmol, 13 mg, 1 mol%), benzyl bromide (8.4 mmol, 1.0 mL, 2.4 eq.) and sodium hydride (8.4 mmol, 0.33 g (60% in mineral oil), 2.4 eq.) were added. The reaction mixture was stirred overnight at room temperature and subsequently quenched with MeOH. Next, H2O was added and the resulting mixture was extracted with EtOAc (3x). The combined organic layers were washed with H2O (1x) and Brine (1x), dried over MgSO4, filtered and concentrated in vacuo, yielding a mixture of products having 3 or 4 benzyl groups. These were separated by column chromatography (pentane → 5% EtOAc in pentane → 20% EtOAc in pentane) after which the tribenzylated products were again subjected to the benzylation reaction as before. After work-up and column chromatography purification (pentane → 5% EtOAc in pentane) the fully benzylated product 41 was obtained (total yield 1.5 g, 3.0 mmol, 85%). 1H NMR (400 MHz, CDCl3): δ (ppm) 7.39-7.23 (m, 20H), 5.80-5.76 (m, 1H), 5.48 (d, J = 10.4 Hz, 1H), 4.93 (d, J = 11.6 Hz, 1H), 4.78-4.68 (m, 4H), 4.64 (d, J = 11.6 Hz, 1H), 4.50-4.43 (m, 3H), 4.20 (dd, J = 1.6, 1.6 Hz, 1H), 3.75 (dd, J = 1.6, 8.0 Hz, 1H), 3.62-3.46 (m, 2H), 2.72-2.68 (m, 1H). 13C NMR (100 MHz, CDCl3): δ (ppm) 139.03, 138.77, 138.70, 138.15, 128.40, 128.32, 128.30, 128.17, 128.77, 128.71, 127.79, 127.67, 127.49, 127.47, 127.44, 127.38, 126.66, 83.20, 77.66, 75.12, 74.03, 73.28, 72.37, 72.16, 70.17, 41.92. LC/MS analysis: Rf 10.8 min (linear gradient 10 → 90% B in 15 min), m/z 521.1 [M+H]+, 543.3 [M+Na]+. HRMS: calcld. for [C35H36O5Na]+ 521.26864, found 521.26864; calcld. for [C36H38O5] 543.25058, found 543.24988. [α]D10 (c = 0.1, CHCl3): +105°.

(2R,3S,5R)-5-(benzyloxymethyl)-2,3,4-tribenzyloxy-7-oxabicyclo[4.1.0]heptane: β-epoxide (42)

To a solution of 41 (0.70 mmol, 0.36 g) in DCM under argon atmosphere was added 3-chloroperoxybenzoic acid (1.7 mmol, 0.30 g, 2.5 eq.). After stirring overnight, the reaction mixture was quenched with saturated aqueous Na2SO3 and extracted with DCM (2x). The combined organic layers were washed with saturated aqueous NaHCO3 (1x), dried over MgSO4, filtered and concentrated in vacuo. The crude products were purified and separated by column chromatography (pentane → 10% EtOAc in pentane) to afford α-epoxide isomer of 42 (37 mg, 70 μmol, 10%) and β-epoxide 42 (0.24 g, 0.43 mmol, 63%). 1H NMR (400 MHz, CDCl3) α-epoxide: δ (ppm) 7.41-7.22 (m, 20H), 4.90-4.79 (m, 3H), 4.70 (dd, J = 11.8, 17.8 Hz, 2H), 4.53-4.45 (m, 3H), 4.25 (dd, J = 2.4, 8.4 Hz, 1H), 3.93-3.92 (m, 1H), 3.62 (dd, J = 8.6, 1.0 Hz, 1H), 3.58 (d, J = 8.0 Hz, 2H), 3.37 (dd, J = 2.4, 4.0 Hz, 1H), 2.96 (dd, J = 1.4, 4.0 Hz, 1H), 2.32-2.28 (m, 1H). 13C NMR (100 MHz, CDCl3) α-epoxide: δ (ppm) 138.68, 138.60, 138.58, 137.89, 128.40, 128.31, 128.26, 128.21, 128.10, 127.97, 127.88, 127.85, 127.75, 127.73, 127.67, 127.52, 127.45, 81.10, 76.73, 75.68, 74.23, 73.68, 73.35, 73.04, 68.53, 55.20, 54.35, 40.89. 1H NMR (400 MHz, CDCl3) β-epoxide 42: δ (ppm) 7.38-7.20 (m, 20H), 4.86 (d, J = 12.0 Hz, 1H), 4.81-4.66 (m, 4H), 4.56 (d, J = 12.0 Hz, 1H), 4.49-4.42 (m, 2H), 4.14 (d, J = 8.8 Hz, 1H), 3.94 (dd, J = 4.0, 2.0 Hz, 1H), 3.73-3.62 (m, 2H), 3.45 (dd, J = 2.0, 8.8 Hz, 1H), 3.23 (d, J = 4.0 Hz, 1H), 3.16-3.15 (m, 1H), 2.32-2.30 (m, 1H). 13C NMR (100 MHz, CDCl3) β-epoxide 42: δ (ppm) 138.80, 138.44, 137.96, 128.36, 128.33, 128.25, 128.05, 128.03, 127.77, 127.11, 127.68, 127.63, 127.44, 127.42, 127.21, 82.47, 76.11, 74.27, 73.38, 73.15, 72.79, 72.62, 68.79, 54.37, 52.38, 40.19. LC/MS analysis: α-epoxide: Rf 10.3 min (linear gradient 10 → 90% B in 15 min), m/z 537.0 [M+H]+, 559.3 [M+Na]+; 42: Rf 10.3 min (linear gradient 10 → 90% B in 15 min), m/z 537.1 [M+H]+, 559.3 [M+Na]+. HRMS: calcld. for [C35H36O5Na]+ 537.26355, found for α-epoxide 537.26385, for 42 537.26391; calcld. for [C36H38O5Na]+ 559.24550, found for α-epoxide 559.24515, for 42 559.24491. [α]D10 (c = 0.1, CHCl3): α-epoxide +30°, 42 +66°.
Synthesis of epoxide- and aziridine-based probes to target retaining galactosidases

(1R,2S,3R,4S,5S,6S)-2-azido-3-(benzyloxymethyl)-4,5,6-tribenzyloxy-cyclohexanol and (1R,2S,3S,4S,5S,6S)-2-azido-6-(benzyloxymethyl)-3,4,5-tribenzyloxy-cyclohexanol (43)

A solution of β-epoxide (0.48 mmol, 0.26 g) in ACN under argon atmosphere was heated to 80 °C before addition of sodium azide (24 mmol, 1.6 g, 50 eq.) and lithium perchlorate (4.8 mmol, 0.51 g, 10 eq.). After stirring under reflux overnight, the reaction mixture was allowed to cool to room temperature, quenched with H₂O and extracted with DCM (3x). The combined organic layers were dried over MgSO₄, filtered and concentrated in vacuo.

Purification by column chromatography (pentane → 10% EtOAc in pentane) yielded a mixture of two azido alcohols in a ratio of 1:1.3 (0.20 g, 0.35 mmol, 73%). 1H NMR (400 MHz, CDCl₃): δ (ppm) 7.39-7.18 (m, 20H isomer 1 + 20H isomer 2), 5.02-4.67 (m, 5H isomer 1 + 5H isomer 2), 4.51-4.40 (m, 3H isomer 1 + 3H isomer 2), 4.30-4.26 (m, 2H isomer 1 + 1H isomer 2), 4.11-4.09 (m, 1H isomer 1), 3.91-3.87 (m, 1H isomer 1 + 1H isomer 2), 3.79-3.77 (m, 2H isomer 1 + 5H isomer 2), 2.05-2.02 (m, 1H isomer 1), 1.68-1.62 (m, 1H isomer 2). 13C NMR (100 MHz, CDCl₃): δ (ppm) 138.14, 138.04, 137.99, 137.85, 137.80, 128.59, 128.45, 128.43, 128.41, 128.35, 128.23, 128.05, 127.94, 127.90, 127.81, 127.78, 127.75, 127.70, 127.66, 127.59, 127.51, 127.44, 127.42, 83.58, 81.21, 80.68, 78.25, 76.93, 76.56, 75.59, 75.02, 73.62, 72.35, 71.55, 67.71, 64.75, 61.58, 42.31, 38.59. LC/MS analysis: Rₜ 10.4 min (linear gradient 10 → 90% B in 15 min), m/z 579.9 [M+H]+; and Rₜ 10.6 min, m/z 580.0 [M+H]+. HRMS: calcd. for [C₃₅H₃₈N₃O₅]+ 580.28060, found 580.28078; calcd. for [C₃₅H₃₇N₃O₅Na]+ 602.26254, found 602.26211. [α]D²₀ (c = 0.1, CHCl₃): -2°.

(1S,2S,3S,4S,5R,6S)-5-(benzyloxymethyl)-2,3,4-tribenzyloxy-7-azabicyclo[4.1.0]heptane (44)

A mixture of azido alcohols (0.50 mmol, 0.29 g) was dissolved in ACN under argon atmosphere. After addition of triphenylphosphine (0.65 mmol, 0.17 g, 1.3 eq.), the reaction mixture was heated to 80 °C and stirred under reflux for 2 hrs. The mixture was then allowed to cool to room temperature and concentrated in vacuo. The crude product was purified by column chromatography (DCM → 0.8% MeOH in DCM) to give α-aziridine (pure yield 70 mg, 0.13 mmol, 26%; and an additional 77 mg product containing triphenylphosphine oxide). 1H NMR (400 MHz, CDCl₃): δ (ppm) 7.47-7.22 (m, 20H), 4.89-4.79 (m, 3H), 4.70 (dd, J = 11.6, 22.8 Hz, 2H), 4.55-4.43 (m, 3H), 4.23 (dd, J = 4.0, 8.4 Hz, 1H), 3.88-3.87 (m, 1H), 3.61-3.55 (m, 3H), 2.55-2.52 (m, 1H), 2.19-2.15 (m, 1H), 2.02 (d, J = 6.0 Hz, 1H). 13C NMR (100 MHz, CDCl₃): δ (ppm) 139.09, 138.92, 138.84, 138.06, 132.03, 131.93, 128.47, 128.35, 128.32, 128.25, 128.20, 128.13, 128.11, 127.85, 127.79, 127.72, 127.65, 127.60, 127.36, 127.31, 127.27, 81.88, 76.87, 76.25, 73.99, 73.10, 72.91, 72.35, 69.81, 41.89, 34.15, 32.08. LC/MS analysis: Rₜ 7.7 min (linear gradient 10 → 90% B in 15 min), m/z 536.1 [M+H]+; and Rₜ 10.6 min, m/z 580.0 [M+H]+. HRMS: calcd. for [C₃₅H₃₈NO₅]+ 536.27954, found 536.27943. [α]D²₀ (c = 0.1, MeOH): +56°.

(1S,2S,3S,4S,5R,6S)-5-(hydroxymethyl)-2,3,4-trihydroxy-7-azabicyclo[4.1.0]heptane (45)

Ammonia was condensed at -60 °C (~5 mL) under argon flow using Birch equipment. Lithium (50 mg) was added and the solution was stirred for 30 min at -60 °C, before a solution of α-aziridine (0.10 mmol, 36 mg) in THF (5 mL) was added. The reaction mixture was stirred at -60 °C under argon for 45 min, quenched with MilliQ (2 mL) and allowed to warm to room temperature. After all ammonia had evaporated, the mixture was further concentrated in vacuo and redissolved in MilliQ. Neutralization with Amberlite-H⁺ resulted in resin-bound product, which was filtered, washed with MilliQ and then eluted using 1M aqueous NH₄OH solution (10x 1 mL). After evaporation in vacuo the product was redissolved in MilliQ and Amberlite-NH₄⁺ was added. Product was eluted from the resin by filtration and washing with MilliQ until the pH of the eluted solution was neutral (pH 7). The combined eluates were concentrated in vacuo to yield the crude deprotected α-aziridine, which was used without further purification. 1H NMR (400 MHz, D₂O): δ (ppm) 4.01 (dd, J = 4.2, 9.0 Hz, 1H), 3.78 (s, 1H), 3.68-3.64 (m, 2H), 3.28 (d, J = 8.8 Hz, 1H), 2.54-2.51 (m, 1H), 2.08 (d, J = 6.4 Hz, 1H), 1.90-1.86 (m, 1H).
Chapter 5

(15,2S,3S,4S,5R,6S)-5-(hydroxymethyl)-2,3,4-trihydroxy-N-(8-azidoctanoyl)-7-azabicyclo[4.1.0]heptane (15)

8-Azidoctanoic acid 37 (0.40 mmol, 78 mg) was preactivated with EEDQ (0.40 mmol, 99 mg, 1.0 eq.) in DMF (0.4 mL) under argon for 2 hours at room temperature. Deprotected aziridine 45 (65 µmol) was dissolved in DMF (1 mL) under argon and cooled to 0 °C, after which 33 µL of the activated ester solution was added (33 µmol, 0.5 eq.) and the mixture was stirred at 0 °C for 30 min. Then another 0.5 eq. of the activated ester was added and the reaction mixture was stirred at 0 °C for another 60 min. After quenching with MeOH, the mixture was concentrated in vacuo, purified by HPLC under neutral conditions using a gradient of 22 → 28% ACN in H2O in 12 min, and lyophilized to yield aziridine 15 as a white powder (3.0 mg, 9.0 µmol, 14%). 1H NMR (600 MHz, MeOD): δ (ppm) 4.08 (dd, J = 4.2, 9.0 Hz, 1H), 3.87-3.86 (m, 1H), 3.82-3.75 (m, 2H), 3.37 (dd, J = 1.8, 8.4 Hz, 1H), 3.30-3.27 (m, 2H), 2.97 (dd, J = 4.2, 6.0 Hz, 1H), 2.61 (d, J = 6.0 Hz, 1H), 2.56-2.44 (m, 2H), 2.02 (td, J = 7.8, 7.2, 3.6 Hz, 1H), 1.66-1.57 (m, 4H), 1.39-1.34 (m, 6H). 13C NMR (150 MHz, MeOD): δ (ppm) 188.72, 74.36, 73.13, 69.15, 62.84, 52.44, 44.54, 43.27, 39.44, 37.12, 30.17, 29.94, 27.68, 27.66, 25.90. LC/MS analysis (ammonium acetate): Rt 7.5 min (linear gradient 0 → 50% B in 15 min), m/z 343.4 [M+H]+, 360.5 [M+NH4]+. HRMS: calcd. for [C15H27N4O5]+ 343.19760, found 343.19757; calcd. for [C15H27N4O5Na]+ 365.17954, found 365.17939.

(15,2S,3S,4S,5R,6S)-5-(hydroxymethyl)-2,3,4-trihydroxy-N-(8-(Bodipy-triazole)-octanoyl)-7-azabicyclo[4.1.0]heptane (16)

Bodipy acid 38 (0.10 mmol, 67 mg) was preactivated with EEDQ (0.10 mmol, 25 mg, 1.0 eq.) in DMF (1 mL) under argon for 2 hours at room temperature. Deprotected aziridine 45 (60 µmol) was dissolved in DMF (0.5 mL) under argon and cooled to 0 °C, after which 0.3 mL of the activated ester solution was added (30 µmol, 0.5 eq.) and the mixture was stirred at 0 °C for 30 min. Then another 0.5 eq. of the activated ester was added and the reaction mixture was stirred at 0 °C for another 60 min. After quenching with MeOH, the mixture was concentrated in vacuo, purified by HPLC under neutral conditions using a gradient of 48 → 51% ACN in H2O in 12 min, and lyophilized to yield aziridine 16 as a purple powder (7.4 mg, 9.0 µmol, 15%). 1H NMR (400 MHz, MeOD): δ (ppm) 7.87 (d, J = 8.8 Hz, 4H), 7.72 (s, 1H), 7.46 (d, J = 4.3 Hz, 2H), 7.00 (d, J = 9.2 Hz, 4H), 6.72 (d, J = 4.3 Hz, 2H), 4.36 (t, J = 7.0 Hz, 2H), 4.10 (dd, J = 8.6, 4.0 Hz, 1H), 3.88 (s, 6H), 3.87-3.80 (m, 1H), 3.79 (dd, J = 7.4, 4.3 Hz, 2H), 3.38 (dd, J = 8.6, 1.8 Hz, 1H), 3.12-3.06 (m, 2H), 2.96 (dd, J = 6.0, 4.0 Hz, 1H), 2.82-2.78 (m, 2H), 2.62-2.58 (m, 1H), 2.55-2.35 (m, 2H), 2.03 (td, J = 7.8, 7.3, 6.3 Hz, 1H), 1.94-1.82 (m, 6H), 1.63-1.52 (m, 2H), 1.32-1.21 (m, 6H). LC/MS analysis: Rt 7.4 min (linear gradient 10 → 90% B in 15 min), m/z 827.07 [M+H]+, 807.47 [M-F]+, 1653.40 [2M+H]+. Of note, under these acidic LC/MS conditions the aziridine is partially opened to give a second peak at Rt 7.2 min (linear gradient 10 → 90% B in 15 min), m/z 845.07 [(M+H2O)+H]+, 825.40 [(M+H2O)-F]+, 1689.13 [2(M+H2O)+H]+. HRMS: calcd. for [C44H54BF2N6O7]+ 827.41096, found 827.41180.

(1R,2S,3S,4S,5R,6R)-5-(hydroxymethyl)-2,3-dibenzyloxy-4-hydroxy-7-azabicyclo[4.1.0]heptane (39)

Cyclohexene 27 (0.50 mmol, 0.17 g) was coevaporated with toluene before being dissolved in DCM (10 mL) at 0 °C under argon atmosphere. Next, CCl3CCN (0.50 mmol, 51 µL, 1.0 eq.) and DBU (25 µmol, 2.0 µL, 0.05 eq.) were added and the reaction mixture was stirred at 0 °C for 1.5 hrs. The mixture was removed from the ice bath before H2O (1.5 mL), NaHCO3 (5.0 mmol, 0.40 g, 10 eq.) and I2 (1.5 mmol, 0.38 g, 3.0 eq.) were added. After stirring at room temperature overnight, the reaction was quenched with 10% aqueous Na2S2O3 and the aqueous layer was extracted with EtOAc (2x). The combined organics were dried over MgSO4, filtered and concentrated in vacuo. The residue was redissolved in MeOH, cooled to 0 °C and concentrated HCl (6.3 mmol, 0.6 mL, 13 eq.) was added after which the reaction mixture was stirred at room temperature for 3.5 hrs. Next, the mixture was concentrated in vacuo, the residue was redissolved in dioxane and again concentrated HCl (16 mmol, 1.5 mL, 32 eq.) was added. The mixture was stirred at 60 °C for 1 hr, concentrated in vacuo and coevaporated with toluene (3x). The residue was redissolved in MeOH, NaHCO3 (10 mmol, 0.8 g, 20 eq.) was added and the reaction mixture was stirred at room
temperature for 4 days. After addition of H$_2$O the aqueous layer was extracted with EtOAc and the organic layer was dried over MgSO$_4$, filtered and concentrated in vacuo. The crude product was purified by column chromatography (DCM → 6% MeOH in DCM) to afford dibenzylated β-aziridine 39 (92 mg, 0.26 mmol, 52%). $^1$H NMR (400 MHz, CDCl$_3$): δ (ppm) 7.46-7.22 (m, 10H), 4.83-4.61 (m, 4H), 4.13-4.04 (m, 2H), 3.99 (dd, $J$ = 10.9, 7.5 Hz, 1H), 3.90 (dd, $J$ = 10.9, 6.2 Hz, 1H), 3.39 (dd, $J$ = 7.9, 2.3 Hz, 1H), 2.50-2.32 (m, 2H), 2.06-1.96 (m, 1H). $^{13}$C NMR (100 MHz, CDCl$_3$): δ (ppm) 138.31, 138.17, 128.62, 128.58, 128.54, 128.49, 128.46, 128.43, 128.35, 128.29, 128.17, 128.15, 128.02, 127.96, 127.93, 127.90, 127.87, 127.85, 127.78, 127.63, 83.29, 78.57, 73.10, 71.69, 67.42, 62.40, 39.47, 32.36, 32.53. LC/MS analysis: R$_t$ 5.4 min (linear gradient 10 → 90% B in 15 min), m/z 356.1 [M+H$^+$], 711.1 [2M+H$^+$].

$(1R,2S,3S,4S,5R,6R)$-5-(hydroxymethyl)-2,3,4-trihydroxy-7-azabicyclo[4.1.0]heptane (40)

The β-aziridine 39 (0.10 mmol, 36 mg) was deprotected using the same procedure as described for α-aziridine 45 to give β-aziridine 40. $^1$H NMR (400 MHz, D$_2$O): δ (ppm) 4.10 (d, $J$ = 8.0 Hz, 1H), 3.87-3.78 (m, 3H), 3.43 (d, $J$ = 8.0 Hz, 1H), 2.44 (bs, 1H), 2.34-2.33 (m, 1H), 2.04 (bs, 1H).

$(1R,2S,3S,4S,5R,6R)$-5-(hydroxymethyl)-2,3,4-trihydroxy-N-(8-azidoctanoyl)-7-azabicyclo[4.1.0]heptane (21)

Deprotected β-aziridine 40 (0.10 mmol) was acylated with azido-spacer 37 using the same procedure as described for α-aziridine 15 to give β-aziridine 21. After HPLC purification and lyophilization, however, NMR analysis revealed that no aziridine proton peaks were present, indicating that the product had degraded by opening of the aziridine.

DFT calculations

The calculated $^1$H NMR coupling constants were obtained by first finding the lowest energy conformation of both epoxide isomers, for which a library of gas phase conformations was generated using the conformer distribution option included in the Spartan 0426 program employing DFT/B3LYP 6-31G(d). All conformers were further optimized by Gaussian 03 at DFT/B3LYP 6-311G(d,p), their zero-point energies were calculated and the energies corrected for solvent by another optimization step employing a Polarizable Continuum Model set for water. The energies of these conformers, corrected for their zero-point energies, were compared and of the lowest energy conformer an NMR calculation was performed using the Gauge-Independent Atomic Orbital (GIAO) method with added spin-spin coupling calculation.

References

3. For a review, see: Rempel, B. P.; Withers, S. G. Glycobiology 2008, 18, 570.
17. The designation of the α-/β-stereochemistry of all galactopyranose-configured epoxides and aziridines is based on α-/β-galactopyranose nomenclature.