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Synthesis of α- and β-Galactopyranose-Configured Isomers of Cyclophellitol and Cyclophellitol Aziridine

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Cyclophellitol and cyclophellitol aziridine are potent and irreversible mechanism-based inhibitors of retaining β-glucosidases. Alterations in the configuration of these compounds can lead to irreversible inhibition of different classes of retaining glycosidases. We have recently reported on the design of a set of α-galactopyranose-configured cyclophellitol and cyclophellitol aziridine derivatives that inhibit human retaining α-galactosidases. Moreover, we have shown that fluorescently labeled derivatives enable the activity-based profiling of these enzymes in vitro. In this report we describe in detail the synthetic strategies that were used to obtain these epoxide- and aziridine-based probes. In addition, we describe the parallel synthesis of a set of β-galactopyranose-configured cyclophellitol isomers as putative inhibitors of retaining β-galactosidases.

Introduction

Cyclophellitol (**1**) is a naturally occurring β-glucosidase inhibitor that was first isolated from the mushroom *Phellinus* sp. in 1990 (Figure 1).[1,2] Follow-up studies demonstrated that this compound is a selective and potent mechanism-based irreversible inhibitor of several retaining β-exoglucosidases.[3] As first postulated by Koshland,[4] most retaining glycosidases, including retaining β-glucosidases, hydrolyze the glycosidic bond in their substrates by employing a double displacement mechanism that results in a net retention of anomeric stereochemistry.[4] This two-step process involves two acidic amino acid residues that act as a catalytic nucleophile and a general acid/base. During hydrolysis, a covalent enzyme–substrate intermediate is formed, and this is the cause of the irreversible inhibition by cyclophellitol. This β-glucopyranose-configured inhibitor binds with high affinity in the active site of a target enzyme by mimicking a terminal β-linked glucoside substrate. The epoxide is positioned in such a way that it can be protonated by the general acid/base residue and at the same time attacked by the catalytically active nucleophile, resulting in the formation of a covalent and stable linkage between the inhibitor and the enzyme.

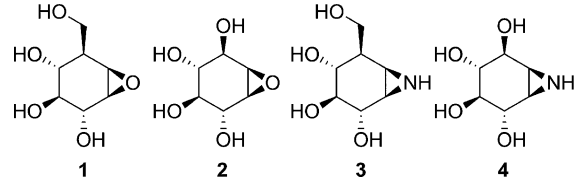

Figure 1. Structures of cyclophellitol (**1**), conduritol B epoxide (**2**), cyclophellitol aziridine (**3**), and conduritol B aziridine (**4**).

Conduritol B epoxide (**2**, Figure 1), which is structurally similar to cyclophellitol except for the substituent at C5 (carbohydrate numbering), is a less potent and also less selective retaining glucosidase inhibitor.[5,6] As a consequence of the symmetry in this molecule, it inhibits not only retaining β-glucosidases but also various retaining α-glucosidases, though generally with lower efficiency. The aziridine derivatives of these epoxide-based inhibitors – cyclophellitol aziridine (**3**)[7] and conduritol B aziridine (**4**)[8] – are irreversible inhibitors of the same enzyme classes.

An interesting application of potent and selective irreversible mechanism-based inhibitors such as cyclophellitol is the design of labeled derivatives, also known as activity-based probes (ABPs), that enable the selective monitoring of enzymatic activity in a biological sample. Using the structure of cyclophellitol and cyclophellitol aziridine as a scaffold, we have previously reported on the design and synthesis of several fluorescently labeled retaining β-glucosidase ABPs.[9,10] In these studies, reporter groups

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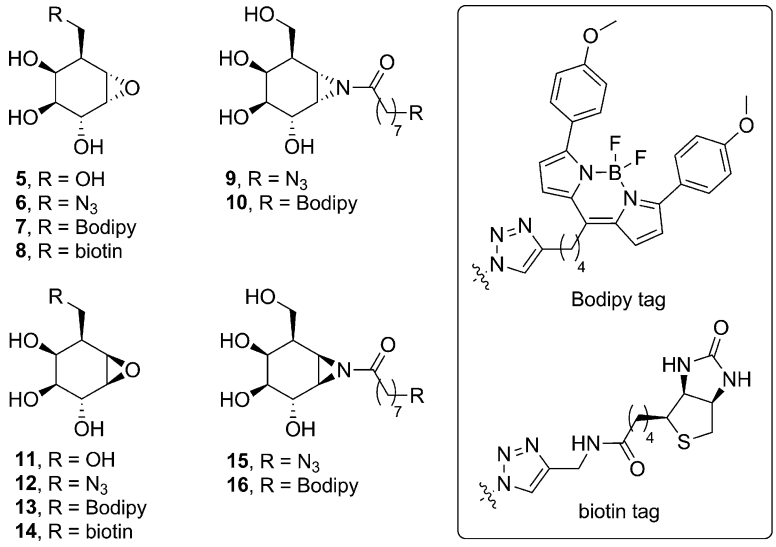


Figure 2. Structures of α-galactopyranose-configured cyclophellitol and cyclophellitol aziridine derivatives **5**–**10** and β-galactopyranose-configured isomers **11**–**16**.

were incorporated by means of an azide moiety installed at the C6 position of the epoxide inhibitor or through acylation of the aziridine nitrogen with various tagged substituents. The resulting probes enabled the sensitive detection of retaining β-exoglucosidase activities in situ and in vivo.

The remarkable specificities and potencies of these compounds, in combination with the highly conserved catalytic mechanism of retaining glycosidases, opens up the potential to develop selective inhibitors and ABPs for different classes of retaining glycosidases by modifying their configurations to that of the natural substrate of an enzyme of interest. Several cyclophellitol isomers have been described in the literature. The α-glucopyranose-configured isomer of inhibitor **1**, 1,6-*epi*-cyclophellitol, is an irreversible inhibitor of retaining α-glucosidases.[11] An α-mannopyranose-configured cyclophellitol isomer was demonstrated to be a retaining α-mannosidase inhibitor,[7] whereas its β-isomer has been synthesized as a putative retaining β-mannosidase inhibitor.[12] Recently we have reported on the synthesis of α-galactopyranose-configured epoxide **5** (Figure 2), which inhibits both retaining α- and β-galactosidases.[13] Moreover, we have also synthesized the various derivatives **6**–**8**, functionalized at C6 with reporter entities, as well as the acylated aziridine analogue **9** and its fluorescently labeled derivative **10** (Figure 2). We have demonstrated that the aziridine-based ABPs are very potent and selective inhibitors of the human retaining α-galactosidases, α-galactosidase A, and α-*N*-acetylgalactosaminidase, and that Bodipy-tagged probe **10** enables the labeling of the endogenous activity of these enzymes in cell extracts. In this report we give a detailed overview of the synthetic strategies that we have used to generate the α-galactopyranose-configured probes, including potential pitfalls and difficulties that we encountered during the synthesis and purification of these compounds. In addition, we describe the parallel synthesis of compounds **11**–**16**, a new series of epoxide- and aziridine-based probes with β-galactopyranose configuration.

Results and Discussion

The synthetic strategies previously developed in our laboratory for the synthesis of cyclophellitol, cyclophellitol aziridine, and their derivatives are based on key intermediate **17**, described by Madsen and co-workers (Figure 3).[14] With all hydroxy substituents in the correct configuration, this cyclohexene gave access to both epoxide- and aziridine-based β-glucosidase probes.[9,10] We reasoned that the similar dibenzylated compound **18**, recently reported by Llebaria et al.,[15] with the only difference being the configuration of the hydroxy substituent at C4 (carbohydrate numbering), would provide an ideal starting point for both α- and β-galactopyranose-configured epoxides and aziridines (Figure 3). The aziridine-based probes can be modified with reporter entities by acylation of the aziridine nitrogen. In the case of the epoxide inhibitors, an azide installed at C6 enables modification with alkyne-modified tags through copper(I)-catalyzed "click" reactions.[16]

Cyclohexene derivative **18** was synthesized in three steps from aldehyde **20**[14] and oxazolidinone **19**[17] essentially as described by Llebaria and co-workers (Scheme 1).[15] The key step in this procedure is the dibutylboryl-triflate-catalyzed stereoselective aldol condensation of **19** and **20**. When this reaction was performed as prescribed, by consecutive addition of all reagents at a temperature of –78 °C, followed by reaction at –20 °C to –15 °C for 3.5 h, compound **21** was obtained stereoselectively but with a considerably lower yield (44%) than reported (83%). Because a considerable amount of unreacted aldehyde could repeatedly be isolated after the reaction, we attempted to improve the yield by using freshly distilled Et_3N and by drying solutions of **19** and **20** in dichloromethane over activated molecular sieves (4 Å) for at least 1 h prior to use. Furthermore, we performed the reaction at –20 °C overnight. With this procedure, aldehyde **20** was completely consumed, and the desired product **21** could be isolated in 80% yield. Next, the

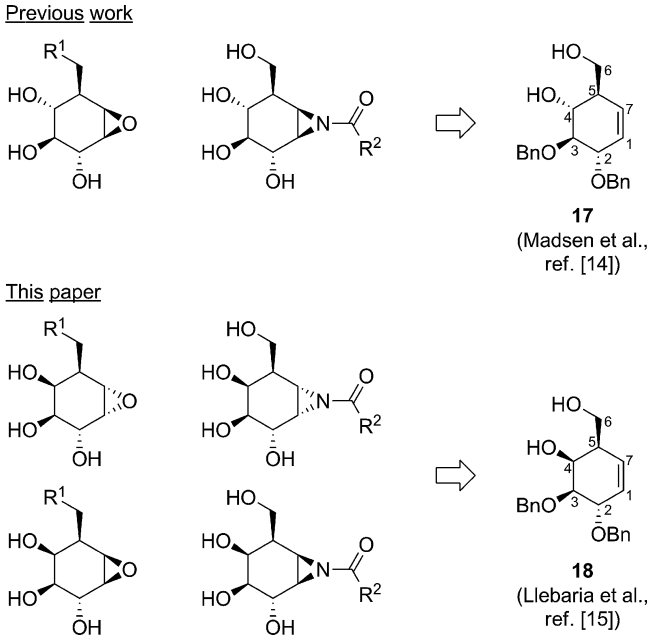
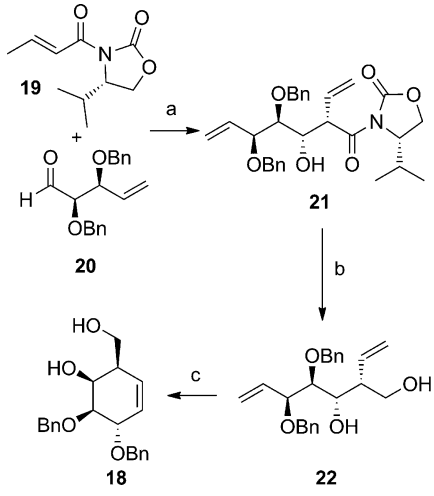


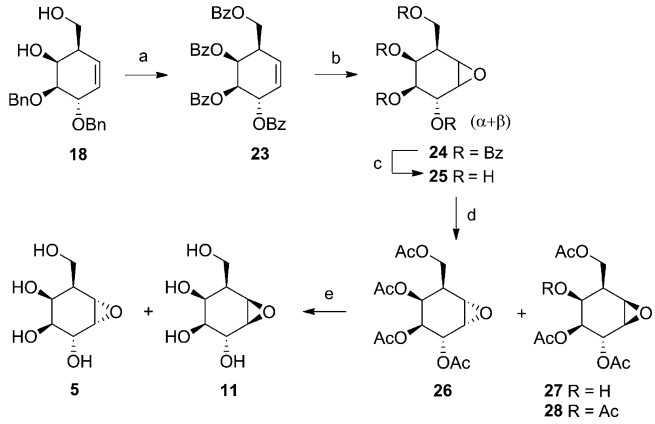
Figure 3. Key intermediate **17**, previously used for the synthesis of β-glucopyranose-configured probes, and key intermediate **18** used in this work for the synthesis of galactopyranose-configured inhibitors and probes. R^1 = OH, N_3, or tag; R^2 = N_3- or reporter-group-functionalized spacer.

Evans template was removed by reduction with $LiBH_4$, and the resulting alcohol **22** was subjected to ring-closing metathesis in the presence of the second-generation Grubbs catalyst to provide cyclohexene **18**.



Scheme 1. Reagents and conditions: a) $Bu_2BSO_3CF_3$, Et_3N, CH_2Cl_2, –78 °C to –20 °C, overnight, 80%; b) $LiBH_4$, THF/H_2O, 0 °C→room temp., 2 h, 85%; c) second-generation Grubbs catalyst, CH_2Cl_2, 40 °C, overnight, 78%.

The synthetic route to α- and β-epoxides[18] **5** and **11** is depicted in Scheme 2. We decided to change the protecting group pattern prior to epoxidation in order to enable facile deprotection of the hydroxy groups in the presence of the epoxide. Hence, the benzyl groups in **18** were removed with anhydrous BCl_3 in dichloromethane, after which the crude product was treated with benzoyl chloride in pyridine to afford fully benzoylated product **23**. Epoxidation was then performed with *m*-chloroperoxybenzoic acid, which gave a mixture of α- and β-epoxides **24** in a ratio of approximately 1:1. Separation of the two stereoisomers proved impossible at this stage, and also after removal of the benzoyl esters by methanolysis under basic conditions to give **25**. However, acetylation of **25** with acetic anhydride in pyridine gave a separable mixture of fully acetylated α-epoxide **26**, triacetylated β-epoxide **27**, and a small amount of fully acetylated β-epoxide **28**. These results indicate that acetylation at the C4 hydroxy group is sterically hindered by the β-epoxide. After separation of the isomers by column chromatography, deprotection by basic methanolysis yielded the enantiomerically pure α- and β-epoxides **5** and **11**, respectively.



Scheme 2. Reagents and conditions. (a) (*i*) BCl_3, CH_2Cl_2, –78 °C, 4 h, (*ii*) BzCl, pyridine, room temp., overnight, 76%; (b) *m*CPBA, CH_2Cl_2, room temp., 4–8 d, 62%; (c) NaOMe, MeOH, room temp., 1.5 h, 76%; (d) Ac_2O, pyridine, room temp., 4 h, **26** (38%) + **27** (20%) + **28** (2%); (e) NaOMe, MeOH, room temp., 2 h, **5** (94%), **11** (84%).

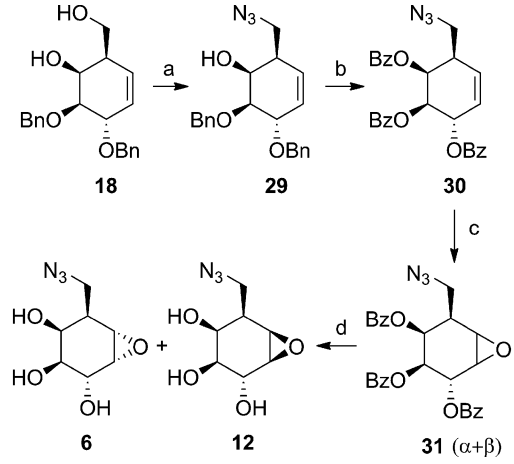
The diastereomeric configuration of epoxides **5** and **11** was determined by ^1H NMR analysis and further confirmed by DFT calculations. The optimized structures obtained by these calculations reveal that both epoxides adopt half-chair conformations (see the Supporting Information). The calculated coupling constants agree well with the corresponding experimentally measured values (Table 1), supporting the experimentally assigned configurations. Evidence is provided by the absence of an observable coupling constant between H1 and H2 of the β-epoxide (calculated value $J_{1,2}$ = 0.04 Hz), in comparison with $J_{1,2}$ = 2.5 Hz for the α-epoxide, and the absence of an observable coupling constant between H5 and H7 of the α-epoxide (calculated value $J_{5,7}$ = 0.04 Hz) in comparison with $J_{5,7}$ = 1.7 Hz for the β-epoxide. Characteristic proton peaks for α-epoxide **5** are double doublets at δ = 3.3 ppm (J_{H1} = 4.0, 2.5 Hz) and δ = 3.1 ppm (J_{H7} = 4.0, 1.8 Hz). For β-epoxide **11**, the corresponding peaks are a multiplet at δ = 3.3 ppm (H7) and a doublet at δ = 3.2 ppm (J_{H1} = 3.7 Hz). Very similar patterns of epoxide proton peaks were observed for the acetyl-

ated epoxides **26**–**28** (although, as might be expected, the exact chemical shift values depend on the substitution of the hydroxy groups), as well as for the C6-modified analogues **6**–**8** and **12**–**14** and the benzylated epoxide **36** described below.

Table 1. Comparison of experimentally measured [^{1}H NMR analysis ("*J* exp.")] and calculated [from DFT calculations ("*J* calcd.")] coupling constants for epoxides **5** and **11**.

α-Epoxide **5**	*J* exp. [Hz]	*J* calcd. [Hz]	β-Epoxide **11**	*J* exp. [Hz]	*J* calcd. [Hz]
H1,H7	4.0	3.7	H1,H7	3.7	3.4
H1,H2	2.5	2.2	H1,H2	n.d. [a]	0.04
H2,H3	8.6	6.9	H2,H3	8.6	6.6
H3,H4	1.9	1.8	H3,H4	n.d. [b]	2.7
H4,H5	3.6	4.0	H4,H5	4.0	3.5
H5,H7	n.d. [a]	0.04	H5,H7	1.7	1.5
H4,H7	1.8	1.1	H4,H7	n.d. [a]	0.8

[a] Values not determined (n.d.) because of very small coupling constants ($J < 1$ Hz). [b] Values not determined because of peak overlap.

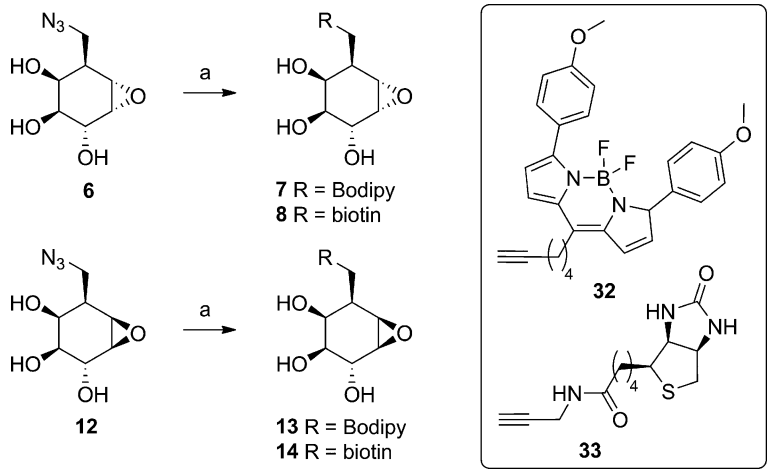


Scheme 3. Reagents and conditions: (a) (*i*) *p*TsCl, Et_3N, CH_2Cl_2, room temp., 4 d, (*ii*) NaN_3, DMF, 60 °C, overnight, 63%; (b) (*i*) BCl_3, CH_2Cl_2, –78 °C, 4 h, (*ii*) BzCl, pyridine, room temp., overnight, 85%; (c) *m*CPBA, CH_2Cl_2, room temp., 4–8 d, 53%; (d) NaOMe, MeOH, room temp., 1.5 h, **6** (42%) + **12** (8%).

The synthesis of azide-functionalized epoxides **6** and **12** from **18** commenced with the installment of the azide moiety at C6 by selective tosylation of the primary alcohol, followed by substitution with sodium azide to give **29** (Scheme 3). Next, the same strategy of deprotection, benzoylation, and epoxidation as used for the non-tagged epoxide inhibitors was employed to synthesize **31**, which was obtained as a 4:1 mixture of α- and β-epoxides. The α- and β-epoxides were not separable at this stage. However, after a final deprotection step the two isomers could be separated by column chromatography to give the enantiomerically pure azide-functionalized α- and β-epoxides **6** and **12**, respectively.

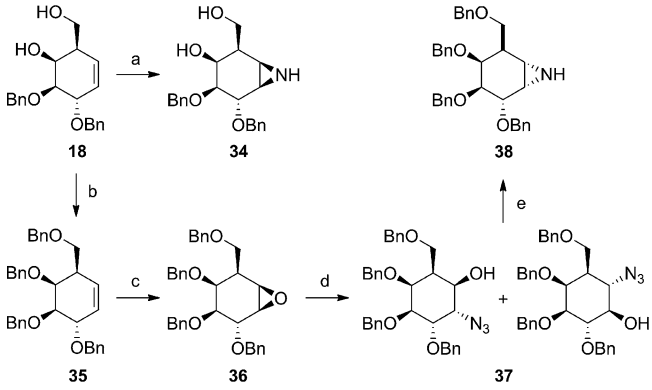
The azide groups in compounds **6** and **12** provide a means to install reporter groups through copper(I)-catalyzed azide–alkyne Huisgen [2+3] cycloaddition ("click" reactions)[16] with alkyne-derivatized tags. The Bodipy- and biotin-tagged α-epoxide ABPs **7** and **8** were prepared from azide-functionalized epoxide **6** by treatment either with Bodipy-alkyne **32**[19] or with biotin-alkyne **33**[20] in the presence of copper(II) sulfate and sodium ascorbate overnight (Scheme 4). Similarly, the Bodipy- and biotin-tagged β-epoxides **13** and **14** were synthesized from azido-epoxide **12**. These reactions proceeded smoothly to afford the fluorescently labeled and biotinylated epoxide probes in good yield.

The synthetic strategy directed towards the β-aziridine ABPs **15** and **16** is based on a procedure previously developed in our laboratory for the corresponding β-glucopyranose-configured probes.[10] The approach relies on the stereocontrolled formation of a β-aziridine with the aid of the primary alcohol at C6 in cyclohexene **18** (Scheme 5). By the reported tandem one-pot procedure, the primary hydroxy group in **18** was first selectively transformed into a



Scheme 4. Reagents and conditions: Bodipy-alkyne **32** or biotin-alkyne **33**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF, room temp., overnight, **7** (88%), **8** (68%), **13** (90%), **14** (68%).

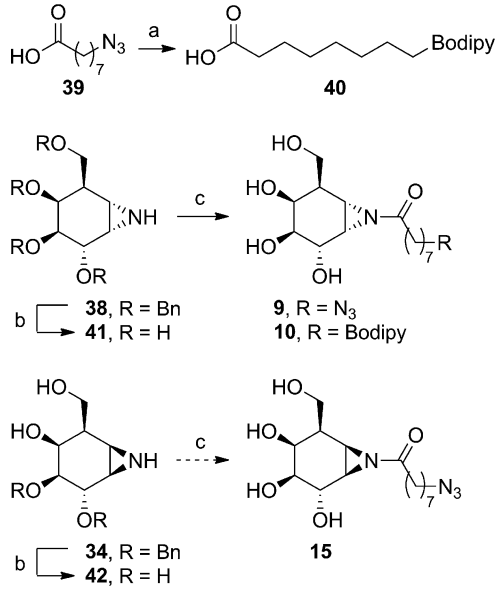
trichloroacetimidate, and this was then subjected to iodocyclization by treatment with iodine under basic conditions. Acidic hydrolysis of the intermediate imidate was followed by the addition of base to effect intramolecular nucleophilic displacement of the iodine by the amine to provide partially protected aziridine **34**, which can be used for subsequent functionalization with a reporter group.



Scheme 5. Reagents and conditions: (a) (*i*) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 1.5 h, (*ii*) I_2, $NaHCO_3$, H_2O, room temp., overnight, (*iii*) HCl, MeOH, room temp., 3.5 h, (*iv*) HCl, dioxane, 60 °C, 1 h, (*v*) $NaHCO_3$, MeOH, room temp., 4 d, 52%; (b) NaH, BnBr, TBAI, DMF, room temp., overnight, 85%; (c) *m*CPBA, CH_2Cl_2, room temp., overnight, 63%; (d) NaN_3, $LiClO_4$, MeCN, 80 °C, overnight, 73%; (e) Ph_3P, MeCN, 80 °C, 2 h, 26%.

In the cases of the α-aziridine ABPs **9** and **10**, the application of a similar procedure would entail the formation of a trichloroacetimidate with the hydroxy group at C2 to achieve iodocyclization below the plane of the cyclohexane ring. Such a strategy would also require several additional protective group manipulations in order to obtain this hydroxy group selectively unmasked. With the aim of finding a shorter synthetic route to the α-aziridine probes, we decided to use an alternative approach in which the α-aziridine moiety is generated from a β-epoxide. As depicted in Scheme 5, this strategy commenced with the formation of fully benzylated compound **35**. Epoxidation under the same conditions as described above yielded a mixture of α- and β-epoxides. Fortunately, the more prevalent product was the desired β-epoxide **36**, together with only a minor 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 treated according to literature procedures with sodium azide and lithium perchlorate as a Lewis acid to give a mixture of *trans*-azidoalcohols **37**.[21] Subsequent treatment with triphenylphosphine led to the formation of α-aziridine **38** through an intramolecular Staudinger-like ring closure.[22] The yield of this reaction was rather low (26%), partly due to the fact that it proved difficult to remove the triphenylphosphine oxide byproduct. In addition, the intramolecular rearrangement of the imino-phosphorane intermediate is likely hindered either by steric factors or by ring strain. Nonetheless, the α-aziridine **38** could be obtained in enantiomerically pure form in sufficient quantities. Notably, it proved impossible to synthesize β-aziridine **34** by the same strategy, with no product being formed by attempted opening of the benzylated α-epoxide isomer of **36** with sodium azide and lithium perchlorate. Attempts to open the α-epoxide with sodium azide under acidic "nonchelating" conditions [MeOH/1.2 M NH_4Cl (4:1), 80 °C][21] or with use of phase-transfer conditions (Bu_4NCl, MeCN/H_2O, 80 °C) were also unsuccessful.

The final stage in the synthetic route to the aziridine-based ABPs involves removal of the benzyl groups in **34** and **38** and acylation of the aziridine nitrogen with azide- or Bodipy-functionalized spacers **39** and **40**, respectively (Scheme 6). Removal of the benzyl groups was achieved by Birch reduction, after which acylation in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as the coupling reagent at 0 °C yields the *N*-acylated product.[10] As we have reported previously, these steps are complicated by the lability of the acylated aziridine under either basic or acidic conditions, with opening of the aziridine during reversed-phase HPLC purification and/or ensuing lyophilization being a major problem. Therefore, it is essential that the acylation step and the purification of the final product are carried out under neutral conditions, including acetonitrile/water gradients for HPLC purification and the use of MilliQ for workup. In the case of α-aziridine **38**, this procedure uneventfully provided the azide- and Bodipy-tagged products **9** and **10**.



Scheme 6. Reagents and conditions: (a) Bodipy-alkyne **32**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF, 80 °C, 4.5 h, 87%; (b) Li, NH_3 (l), THF, –60 °C, 45 min; (c) **39** or **40**, EEDQ, DMF, 0 °C, 90 min, **9** (14%), **10** (15%).

The synthesis of the β-aziridine ABPs, however, proved more problematic. After debenzylation of β-aziridine **34** (Scheme 6), ^1H NMR analysis of the crude product **42** revealed a small amount of byproduct formation. Attempts to acylate **42** with azido-spacer **39** followed by reversed-

phase HPLC purification and lyophilization resulted in significant degradation of the aziridine. LC/MS analysis revealed the major product to be an H_2O adduct, presumably resulting from opening of the aziridine. In some cases an HCl adduct was also observed, despite the fact that no acids and no chloride salts were used during purification. Further analysis by ^1H NMR indicated almost complete disappearance of the aziridine proton peaks. Hence, it appears that the *N*-acylated β-aziridine moiety is rather labile and prone to decomposition, even under our optimized conditions. As a result, ABPs **15** and **16** could not be synthesized.

In the synthetic strategies described here, the formation of both α- and β-epoxides by *m*-chloroperoxybenzoic acid-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 might be desirable to obtain only one isomer, and for that purpose other oxidation methods should be evaluated. It is worth noting that epoxidation of the perbenzoylated compound **23** 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 **30**. Full benzylation, as in compound **35**, on the other hand, leads to more β-selectivity during epoxidation (α/β ratio of 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 the homoallylic (C6) position can enhance α- or β-selectivity, respectively. As expected, epoxidation of unprotected cyclohexene derivatives, obtained by debenzylation either of **18** or of **29**, led mainly to the formation of α-epoxides (α/β > 15:1), due to stereoselective control by the allylic hydroxy group, which leads to *syn* epoxidation.

Conclusions

We describe the parallel synthesis of α- and β-galactopyranose-configured cyclophellitol and cyclophellitol aziridine isomers as (putative) inhibitors and ABPs for retaining α- and β-galactosidases, respectively. We present the synthesis of non-tagged epoxide inhibitors **5** and **11**, as well as derivatives of these functionalized at C6 with an azide, a Bodipy fluorophore, or a biotin tag (compounds **6**–**8** and **12**–**14**). As well as the epoxide-based ABPs, α-galactopyranose-configured aziridine-based probes in which an azide or Bodipy tag is installed through *N*-acylation of the aziridine were also synthesized (compounds **9** and **10**). Unfortunately, it proved impossible to isolate the acylated β-aziridine isomers, due to severe lability of the aziridine during purification procedures. Efforts to synthesize differently substituted aziridine-based β-galactosidase ABPs are currently being undertaken in our laboratory.

The synthetic strategies directed towards both α- and β-galactopyranose-configured probes, epoxides as well as aziridines, are based on key cyclohexene intermediate **18**, from which all compounds were synthesized in a maximum of six steps. Although our goal was to obtain the epoxides as mixtures of α- and β-isomers, stereoselective synthesis is also possible through alteration of the protective group patterns and/or by application of different epoxidation procedures, as was reported previously for the synthesis of β-glucopyranose-configured epoxide ABPs.[9] In the case of the aziridine-based probes, two separate routes were required to obtain the α- and β-configured isomers. The β-configured aziridine **34** was synthesized stereoselectively by an intramolecular iodocyclization procedure, whereas α-aziridine **38** was obtained by azidolysis of the corresponding β-epoxide. The ability of the α-galactosidase probes presented here to inhibit recombinant human retaining α-galactosidases and to label the endogenous activities of these enzymes in vitro has already been established.[13] Evaluation of the inhibitory potencies of the β-galactopyranose-configured epoxides will be the subject of future research.

Experimental Section

General Methods: All reagents were commercial grade and were used as received unless stated otherwise. Dichloromethane (CH_2Cl_2), dichloroethane (DCE), dimethylformamide (DMF), and tetrahydrofuran (THF) (Biosolve) were of analytical grade and, when used under anhydrous conditions, stored over flame-dried molecular sieves (3 Å). Ethyl acetate (EtOAc, Riedel-de Haën) used for column chromatography was of technical grade and distilled before use. Reactions were monitored by TLC analysis (DC-alufolien, Merck, Kieselgel 60, F_{254}) with detection by UV absorption (254/366 nm), by spraying with H_2SO_4 in ethanol (20%) or a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g L^{-1}) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g L^{-1}) in 10% aqueous sulfuric acid, followed by charring at ca. 150 °C, or by spraying with an aqueous solution of $KMnO_4$ (7%) and K_2CO_3 (2%). Column chromatography was performed on silica gel (Screening Devices BV, 0.040–0.063 mm, 60 Å). LC/MS analysis was performed with 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 × 50 mm, 5 μm particle size, Phenomenex). The applied buffers were A: H_2O, B: MeCN, and C: aqueous TFA (1%). Reported gradients represent the percentage of buffer B in buffer A with 10% buffer C. Alternatively, where indicated, LC/MS analysis was performed with an API 3000 ESI (Q1) mass spectrometer (Applied Biosystems) coupled to a Jasco (900 series) HPLC system. The applied buffers were A: H_2O, B: MeCN, and C: NH_4OAc in H_2O (100 mM). Reported gradients represent the percentage of buffer B in buffer A with 10% buffer C. HRMS analysis was performed with an LTQ Orbitrap (Thermo Finnigan) mass spectrometer equipped with an electronspray 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 dioctyl phthalate (*m*/*z* = 391.28428) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). For reversed-phase HPLC purification an automated HPLC system equipped with a C18 semiprep column (Gemini C18, 250 mm × 10 mm, 5 μm particle size, 110 Å pore size, Phenomenex) was used. ^1H- and ^{13}C-APT-NMR spectra were recorded with a Bruker AV 400 (400/100 MHz) or a Bruker DMX 600 (600/150 MHz) instrument and a cryoprobe. All NMR spectra were recorded at room temperature (18–22 °C). 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 ^{13}C-APT spectra are proton-decoupled. Peak assignments are based on 2D ^{1}H-COSY and ^{13}C-HSQC NMR experiments.

Note: Numbering of proton peaks in cyclohexene and cyclohexane epoxide derivatives is according to the numbering in Figure 3.

(*S*)-3-[(2*S*,3*S*,4*S*,5*S*)-4,5-Bis(benzyloxy)-3-hydroxy-2-vinylhept-6-enoyl]-4-isopropyloxazolidin-2-one (21): *Note:* Solutions of aldehyde **20**[14] and oxazolidinone **19**[17] in CH_2Cl_2 were put under argon and dried with activated molecular sieves (4 Å) for at least 1 h prior to use. Et_3N was freshly distilled from CaH_2 and stored on activated molecular sieves (4 Å) under argon prior to use.

A solution of oxazolidinone **19** (12 mmol, 2.4 g, 1.15 equiv.) in CH_2Cl_2 under argon was cooled to –78 °C with the aid of a cryostate before addition of $Bu_2BSO_3CF_3$ (12 mmol, 12 mL 1 M in CH_2Cl_2, 1.15 equiv.) and Et_3N (14 mmol, 1.9 mL, 1.3 equiv.). The reaction mixture was stirred at –78 °C for 1 h, then at 0 °C (ice bath) for 15 min, and subsequently cooled back to –78 °C. A solution of aldehyde **20** (11 mmol, 3.2 g, 1.0 equiv.) in CH_2Cl_2 under argon was added by cannula, after which the reaction mixture was stirred at –20 °C overnight. Next, the mixture was removed from the cold bath and put in an ice bath (–5 °C). After quenching with PBS (20 mL), H_2O_2 (30% aqueous solution) was added dropwise while the temperature of the mixture was kept below 5 °C, until no more rise in temperature was observed. At this point the mixture was stirred for another 45 min while being allowed to warm slowly to room temperature, after which aqueous saturated $NaHCO_3$ was added and the aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by column chromatography (pentane→10% EtOAc in pentane) to afford the aldol adduct **21** (4.2 g, 8.6 mmol, 80%). $[\alpha]_D^{20}$ = –48 (c = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.25 (m, 10 H, C*H* arom.), 6.06 (ddd, J = 18.5, 9.7, 7.1 Hz, 1 H, C*H*=CH$_2$), 5.92 (ddd, J = 17.3, 10.1, 8.8 Hz, 1 H, C*H*=CH$_2$), 5.45–5.37 (m, 3 H, CH=C*H*$_2$, CH=CH*H*), 5.29 (dd, J = 10.2, 1.6 Hz, 1 H, CH=CH*H*), 5.02 (dd, J = 9.0, 7.2 Hz, 1 H, C*H*-CH=CH$_2$), 4.69 (dd, J = 11.5, 4.0 Hz, 2 H, C*H*$_2$ Bn), 4.55–4.40 (m, 3 H, C*H*$_2$ Bn, C*H*-OH), 4.29 (dd, J = 7.3, 3.9 Hz, 1 H, C*H*-OBn), 4.03 (dt, J = 8.6, 3.5 Hz, 1 H, C*H* oxazolidinone), 3.84 (dd, J = 9.0, 3.0 Hz, 1 H, CH*H* oxazolidinone), 3.58 (dd, J = 8.3, 3.9 Hz, 1 H, C*H*-OBn), 3.39 (br. s, 1 H, O*H*), 3.26 (t, J = 8.8 Hz, 1 H, CH*H* oxazolidinone), 2.26–2.14 [m, 1 H, C*H*-(CH$_3$)$_2$], 0.75 (dd, J = 18.8, 7.0 Hz, 6 H, 2×C*H*$_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.53 (*C*=O), 153.53 (*C*=O), 138.01, 137.76 (C_q arom.), 134.55, 133.56 (2×*C*H=CH$_2$), 128.17, 128.00, 127.64, 127.53, 127.32, 127.09 (*C*H arom.), 120.43, 119.14 (2×CH=*C*H$_2$), 81.78, 79.90 (2×*C*H-OBn), 73.04, 71.12 (2×*C*H$_2$ Bn), 70.66 (*C*H-OH), 62.38 (*C*H$_2$ oxazolidinone), 57.94 (*C*H oxazolidinone), 50.06 (*C*H-CH=CH$_2$), 27.96 [*C*H-(CH$_3$)$_2$], 17.75, 14.41 (2×*C*H$_3$) ppm. HRMS: calcd. for $[C_{29}H_{36}NO_6]^+$ 494.25371; found 494.25349. HRMS: calcd. for $[C_{29}H_{35}NO_6Na]^+$ 516.23566; found 516.23543.

(2*R*,3*S*,4*S*,5*S*)-4,5-Bis(benzyloxy)-2-vinylhept-6-ene-1,3-diol (22): Oxazolidinone **21** (13.8 mmol, 6.8 g) was dissolved in THF (100 mL) at 0 °C, after which H_2O (8 mL) and $LiBH_4$ (35 mmol, 17.5 mL 2 M in THF, 2.5 equiv.) were added. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h, before being quenched with NaOH (2 M). The resulting mixture was extracted with Et_2O (2×), and the combined organic layers were washed with aqueous saturated $NaHCO_3$ and brine, dried with $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (pentane→40% EtOAc in pentane) yielded alcohol **22** as a white solid (4.3 g, 12 mmol, 85%). $[\alpha]_D^{20}$ = +8 (c = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45–7.21 (m, 10 H, C*H* arom.), 6.14–5.94 (m, 2 H, 2×C*H*=CH$_2$), 5.49–5.33 (m, 2 H, 2×CH=CH*H*), 5.26 (dd, J = 10.4, 2.1 Hz, 1 H, CH=CH*H*), 5.13 (dd, J = 17.5, 2.2 Hz, 1 H, CH=CH*H*), 4.67 (d, J = 11.8 Hz, 1 H, CH*H* Bn), 4.57 (d, J = 11.1 Hz, 1 H, CH*H* Bn), 4.48 (d, J = 11.1 Hz, 1 H, CH*H* Bn), 4.39 (d, J = 11.8 Hz, 1 H, CH*H* Bn), 4.21 (dd, J = 7.0, 3.8 Hz, 1 H, C*H*-OH), 4.05 (d, J = 9.1 Hz, 1 H, C*H*-OBn), 3.77–3.64 (m, 2 H, C*H*$_2$-OH), 3.51 (dd, J = 9.1, 3.7 Hz, 1 H, C*H*-OBn), 3.39 (br. s, 1 H, O*H*), 2.58 (ddt, J = 7.9, 5.7, 2.9 Hz, 1 H, C*H*-CH=CH$_2$), 2.20 (br. s, 1 H, O*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 137.75, 137.26 (C_q arom.), 135.28, 133.85 (2×*C*H=CH$_2$), 128.55, 128.36, 127.93, 127.86, 127.80 (*C*H arom.), 119.51, 119.05 (2×CH=*C*H$_2$), 79.58, 78.97 (2×*C*H-OBn), 73.08 (*C*H$_2$ Bn), 72.53 (*C*H-OH), 70.97 (*C*H$_2$ Bn), 65.58 (*C*H$_2$-OH), 47.33 (*C*H-CH=CH$_2$) ppm. HRMS: calcd. for $[C_{23}H_{28}O_4Na]^+$ 391.18798; found 391.18897.

Cyclohexene 18: The second-generation Grubbs catalyst (0.4 mmol, 0.34 g, 5 mol-%) was added under argon to a solution of **22** (7.8 mmol, 2.9 g) in CH_2Cl_2. The reaction mixture was stirred at 40 °C in the dark for 20 h, DMSO (40 mmol, 2.8 mL, 100 equiv. 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 **18** (7.2 mmol, 2.5 g, 92%). $[\alpha]_D^{20}$ = +104 (c = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 10 H, C*H* arom.), 5.84 (d, J = 10.4 Hz, 1 H, C*H*=CH), 5.55 (d, J = 10.1 Hz, 1 H, C*H*=CH), 4.71 (d, J = 8.5 Hz, 4 H, 2×C*H*$_2$ Bn), 4.33–4.31 (m, 2 H, C*H*-2, C*H*-4), 3.82–3.80 (m, 2 H, C*H*$_2$-OH), 3.65 (d, J = 7.4 Hz, 1 H, C*H*-3), 2.95 (br. s, 2 H, 2×O*H*), 2.45 (d, J = 6.1 Hz, 1 H, C*H*-5) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.45, 137.93 (C_q arom.), 128.45, 128.32, 127.85, 127.75, 127.57, 127.43, 126.75 (*C*H arom., 2×*C*H=CH), 81.80 (*C*H-4), 77.31, 76.58 (*C*H-2, *C*H-3), 72.15 (*C*H$_2$-OH), 41.87 (*C*H-5) ppm. HRMS: calcd. for $[C_{21}H_{25}O_4]^+$ 341.17474; found 341.17519. HRMS: calcd. for $[C_{21}H_{24}O_4Na]^+$ 363.15668; found 363.15714.

Benzoyl-cyclohexene 23: A solution of cyclohexene **18** (0.5 mmol, 0.17 g) in CH_2Cl_2 under argon was cooled to –78 °C, after which BCl_3 was added (5 mmol, 5 mL 1 M in CH_2Cl_2, 10 equiv.). The reaction mixture was stirred under argon at –78 °C for 4 h and then quenched with MeOH, concentrated in vacuo, and coevaporated with toluene (3×). 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 equiv.) and the mixture was stirred overnight at room temperature before being quenched with a saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2 (3×), and the combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification by column chromatography (pentane→10% EtOAc in pentane) yielded the fully benzoylated product **23** (0.22 g, 0.38 mmol, 76% over two steps). $[\alpha]_D^{20}$ = +250 (c = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00–7.88 (m, 8 H, C*H* arom.), 7.62–7.30 (m, 12 H, C*H* arom.), 6.23–6.18 (m, 2 H, C*H*-3, C*H*-4), 6.09–6.06 (m, 1 H, C*H*=CH), 5.88 (dd, J = 1.2, 10.4 Hz, 1 H, C*H*=CH), 5.78 (dd, J = 2.2, 8.6 Hz, 1 H, C*H*-2), 4.59 (dd, J = 6.4, 10.8 Hz, 1 H, CH*H*), 4.32 (dd, J = 8.8, 10.8 Hz, 1 H, CH*H*), 3.44–3.40 (m, 1 H, C*H*-5) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.23, 166.14, 165.87, 165.51 (4×*C*=O), 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, 126.86, 126.81 (*C*H and C_q arom., 2×*C*H=CH), 73.10, 70.60, 69.30 (*C*H-

2, *C*H-3, *C*H-4), 63.05 (*C*H$_2$), 39.47 (*C*H-5) ppm. LC/MS analysis: R$_t$ 10.1 min (linear gradient 10→90% B in 15 min), *m*/*z* 599.1 [M + Na]$^+$, 1174.9 [2M + Na]$^+$. HRMS: calcd. for [$C_{35}H_{29}O_8$]$^+$ 577.18569; found 577.18602. HRMS: calcd. for [$C_{35}H_{28}O_8Na$]$^+$ 599.16764; found 599.16727.

Benzoyl-epoxide 24: 3-Chloroperoxybenzoic acid (1.1 mmol, 0.24 g, 2.5 equiv.) was added to a solution of **23** (0.42 mmol, 0.24 g) in CH_2Cl_2 and the reaction mixture was stirred for 4 d at room temperature. Then, an additional 1 equiv. of 3-chloroperoxybenzoic acid was added and the reaction mixture was stirred again for 4 d 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 **24** in a 1:1 ratio (0.15 g, 0.26 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19–7.76 (m, 8 H, 8×C*H* arom. α, 8×C*H* arom. β), 7.68–7.20 (m, 12 H, 12×C*H* arom. α, 12×C*H* arom. β), 6.14–5.98 (m, 1.5 H, C*H*-2 α, C*H*-4 α, C*H*-4 β), 5.78 (d, *J* = 9.4 Hz, 0.5 H, C*H*-2 β), 5.68 (dd, *J* = 1.8, 9.2 Hz, 0.5 H, C*H*-3 α), 5.61 (dd, *J* = 2.6, 9.4 Hz, 0.5 H, C*H*-3 β), 4.78 (dd, *J* = 6.7, 11.1 Hz, 0.5 H, CH*H* β), 4.70–4.62 (m, 0.5 H, CH*H* α), 4.52–4.46 (m, 1 H, CH*H* α, CH*H* β), 3.92 (dd, *J* = 2.3, 3.7 Hz, 0.5 H, C*H*-1 α), 3.50 (s, 1 H, C*H*-1 β, C*H*-7 β), 3.43 (dd, *J* = 1.5, 3.8 Hz, 0.5 H, C*H*-7 α), 3.21–3.01 (m, 1 H, C*H*-5 α, C*H*-5 β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.28, 165.69, 165.58 (*C*=O of α- and β-isomers), 133.59, 133.50, 133.44, 133.25, 133.20, 133.16, 130.00, 129.86, 129.77, 129.68, 129.12, 129.08, 129.01, 128.68, 128.58, 128.47, 128.40, 128.28 (*C*H and C_q arom. of α- and β-isomers), 72.13, 70.85, 70.30, 69.87, 68.71, 66.25 (*C*H-2, *C*H-3 and *C*H-4 of α- and β-isomers), 61.92, 61.79 (*C*H$_2$ of α-and-β isomers), 54.17, 53.98, 53.42, 51.73 (*C*H-1 and *C*H-7 of α- and β-isomers), 38.59, 37.75 (*C*H-5 of α- and β-isomers) ppm. LC/MS analysis: R$_t$ 9.7 min (linear gradient 10→90% B in 15 min), *m*/*z* 593.1 [M + H]$^+$, 1206.9 [2M + Na]$^+$. HRMS: calcd. for [$C_{35}H_{29}O_9$]$^+$ 593.18061; found 593.18073. HRMS: calcd. for [$C_{35}H_{28}O_9Na$]$^+$ 615.16255; found 615.16184.

Mixture of α- and β-Epoxides 25: A solution of tetrabenzoylated epoxides **24** (α- and β-epoxides in 1:1 ratio, 0.30 mmol, 180 mg) in MeOH was treated with sodium methoxide (0.12 mmol, 21 μL 5.6 M in MeOH, 0.4 equiv.) at room temperature for 1.5 h. After neutralization with Amberlite-H$^+$ IR-200 the reaction mixture was filtered and concentrated in vacuo. Purification by column chromatography (10% MeOH in CH_2Cl_2→15% MeOH in CH_2Cl_2) yielded the fully deprotected product **25** (α- and β-epoxides in 1.5:1 ratio) (40 mg, 0.23 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.07 (dd, *J* = 2.4, 8.4 Hz, 0.6 H, C*H*-2 α), 3.91 (d, *J* = 8.4 Hz, 0.4 H, C*H*-2 β), 3.87–3.82 (m, 3 H, C*H*-4 α, C*H*-4 β, C*H*$_2$ α, C*H*$_2$ β), 3.38 (dd, *J* = 1.6, 8.4 Hz, 0.6 H, C*H*-3 α), 3.32–3.29 (m, 1.4 H, C*H*-1 α, C*H*-7 β, C*H*-3 β), 3.15 (d, *J* = 4.0 Hz, 0.4 H, C*H*-1 β), 3.08 (dd, *J* = 1.6, 4.0 Hz, 0.6 H, C*H*-7 α), 2.18–2.16 (m, 0.4 H, C*H*-5 β), 2.05–2.00 (m, 0.6 H, C*H*-5 α) ppm.

Separation of α- and β-Epoxides by Acetylation/Deacetylation (5, 11): An α/β mixture of deprotected epoxides **25** (0.26 mmol, 45 mg) was coevaporated with toluene before being dissolved in pyridine under argon. After addition of acetic anhydride (10 mmol, 0.9 mL, 38 equiv.) the mixture was stirred at room temperature for 4 h and was then concentrated in vacuo and coevaporated with toluene (3×). After purification by column chromatography (CH_2Cl_2→0.6% MeOH in CH_2Cl_2 in steps of 0.1%) three different products were isolated: the tetraacetylated form of the α-isomer **26** (0.10 mmol, 35 mg, 38%), a small amount of tetraacetylated β-isomer **28** (4.4 μmol, 1.5 mg, 2%), and triacetylated β-isomer **27** (53 μmol, 16 mg, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) **26**: δ = 5.52–5.41 (m, 2 H, C*H*-2, C*H*-4), 5.00 (dd, *J* = 9.6, 1.8 Hz, 1 H, C*H*-3), 4.24–4.07 (m, 2 H, C*H*$_2$), 3.59 (dd, *J* = 4.0, 2.4 Hz, 1 H, C*H*-1), 3.10 (dd, *J* = 4.0, 1.6 Hz, 1 H, C*H*-7), 2.68 (td, *J* = 8.1, 3.7 Hz, 1 H, C*H*-5), 2.13 (s, 3 H, C*H*$_3$), 2.10 (s, 3 H, C*H*$_3$), 2.06 (s, 3 H, C*H*$_3$), 2.00 (s, 3 H, C*H*$_3$) ppm. ^{1}H NMR (400 MHz, $CDCl_3$) **28**: δ = 5.52–5.45 (m, 1 H, C*H*-4), 5.26 (d, *J* = 9.4 Hz, 1 H, C*H*-2), 4.95 (dd, *J* = 9.6, 2.0 Hz, 1 H, C*H*-3), 4.35–4.20 (m, 2 H, C*H*$_2$), 3.26–3.17 (m, 2 H, C*H*-1, C*H*-7), 2.78–2.74 (m, 1 H, C*H*-5), 2.12 (s, 3 H, C*H*$_3$), 2.09 (s, 3 H, C*H*$_3$), 2.05 (s, 3 H, C*H*$_3$), 1.96 (s, 3 H, C*H*$_3$) ppm. ^{1}H NMR (400 MHz, $CDCl_3$) **27**: δ = 5.35 (d, *J* = 9.1 Hz, 1 H, C*H*-2), 4.90 (dd, *J* = 9.1, 2.6 Hz, 1 H, C*H*-3), 4.46–4.38 (m, 2 H, C*H*$_2$), 3.99 (d, *J* = 9.3 Hz, 1 H C*H*-4), 3.44–3.37 (m, 1 H, C*H*-7), 3.30 (d, *J* = 3.6 Hz, 1 H, C*H*-1), 2.54 (tdd, *J* = 7.6, 3.5, 1.3 Hz, 1 H, C*H*-5), 2.46 (d, *J* = 11.0 Hz, 1 H, O*H*), 2.13 (s, 3 H, C*H*$_3$), 2.12 (s, 3 H, C*H*$_3$), 2.10 (s, 3 H, C*H*$_3$) ppm. The β-isomers **27** and **28** were then combined and deprotected separately from **26** by treatment with sodium methoxide (30 μmol, 5 μL 5.6 M in MeOH, 0.3 equiv. for **26** and 17 μmol, 3 μL 5.6 M in MeOH, 0.3 equiv. for **27**/**28**) in MeOH at room temperature for 2 h. 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 CH_2Cl_2→15% MeOH in CH_2Cl_2) to give α-epoxide **5** (17 mg, 94 μmol, 94%) and β-epoxide **11** (8.5 mg, 48 μmol, 84%).

α-Isomer 5: $[\alpha]_D^{20}$ = +85 (*c* = 0.2, MeOH). ^{1}H NMR (600 MHz, MeOD): δ = 4.09 (dd, *J* = 8.5, 2.5 Hz, 1 H, C*H*-2), 3.85 (ddd, *J* = 3.6, 1.8, 1.8 Hz, 1 H, C*H*-4), 3.78 (dd, *J* = 11.0, 6.6 Hz, 1 H, CH*H*), 3.74 (dd, *J* = 11.0, 8.0 Hz, 1 H, CH*H*), 3.41 (dd, *J* = 8.6, 1.9 Hz, 1 H, C*H*-3), 3.33 (dd, *J* = 4.0, 2.5 Hz, 1 H, C*H*-1), 3.10 (dd, *J* = 4.0, 1.7 Hz, 1 H, C*H*-7), 2.04 (ddd, *J* = 8.0, 6.6, 3.5 Hz, 1 H, C*H*-5) ppm. ^{13}C NMR (150 MHz, MeOD): δ = 73.76 (*C*H-3), 72.77 (*C*H-4), 70.79 (*C*H-2), 62.28 (*C*H$_2$), 58.10 (*C*H-1), 55.46 (*C*H-7), 44.34 (*C*H-5) ppm.

β-Isomer 11: $[\alpha]_D^{20}$ = +67 (*c* = 0.2, MeOH). ^{1}H NMR (600 MHz, MeOD): δ = 3.93 (d, *J* = 8.6 Hz, 1 H, C*H*-2), 3.88–3.82 (m, 3 H, C*H*-4, C*H*$_2$), 3.33–3.31 (m, 2 H, C*H*-3, C*H*-7), 3.17 (d, *J* = 3.7 Hz, 1 H, C*H*-1), 2.18 (tdd, *J* = 7.3, 4.0, 1.7 Hz, 1 H, C*H*-5) ppm. ^{13}C NMR (150 MHz, MeOD): δ = 76.72 (*C*H-3), 70.76 (*C*H-4), 69.96 (*C*H-2), 61.87 (*C*H$_2$), 57.76 (*C*H-1), 55.36 (*C*H-7), 42.82 (*C*H-5) ppm.

Azido-cyclohexene 29: A solution of cyclohexene **18** (0.76 mmol, 0.26 g) in CH_2Cl_2 under argon was cooled to 0 °C, after which *p*TsCl (0.84 mmol, 0.16 g, 1.1 equiv.) and Et_3N (1.4 mmol, 0.19 mL, 1.8 equiv.) were added. The reaction mixture was stirred overnight at room temperature, after which TLC analysis indicated that the reaction was not complete. Therefore, another 0.5 equiv. of *p*TsCl (75 mg) and 1.8 equiv. of Et_3N (0.19 mL) were added after 24 h and again after 48 h. The mixture was stirred for a total of 4 d, after which aqueous HCl (1 M) was added and the aqueous layer was extracted with Et_2O (3×). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude tosylated product was then redissolved in DMF under argon, and NaN_3 (7.6 mmol, 0.50 g, 10 equiv.) 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 aqueous HCl (1 M, 1×), saturated aqueous $NaHCO_3$ (1×), and brine (1×), dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification by column chromatography (pentane→12% EtOAc in pentane in steps of 2%) gave the dibenzylated azide **29** (0.17 g, 0.48 mmol, 63% over two steps). $[\alpha]_D^{20}$ = +118 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46–7.31 (m, 10 H, C*H* arom.), 5.86 (dt, *J* = 10.2, 2.6 Hz, 1 H, C*H*=CH), 5.48 (dd, *J* = 9.6, 1.6 Hz, 1 H, C*H*=CH), 4.81–4.73 (m, 4 H, 2×C*H*$_2$ Bn), 4.40–

4.27 (m, 2 H, C*H*-2, C*H*-4), 3.70 (dd, *J* = 7.8, 2.2 Hz, 1 H, C*H*-3), 3.60 (dd, *J* = 11.9, 9.3 Hz, 1 H, CH*H*-N_3), 3.40 (dd, *J* = 11.9, 6.5 Hz, 1 H, CH*H*-N_3), 2.56 (dt, *J* = 6.3, 3.1 Hz, 1 H, C*H*-5), 2.50–2.47 (br. s, 1 H, O*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.51, 138.03 (C_q arom.), 128.56, 128.44, 127.96, 127.92, 127.86, 127.70 (*C*H arom., *C*H=CH), 125.50 (*C*H=CH), 82.12 (*C*H-3), 76.80 (*C*H-2), 72.42 (CH_2 Bn), 72.26 (CH_2 Bn), 67.58 (*C*H-4), 51.77 (CH_2-N_3), 40.63 (*C*H-5) ppm. HRMS: calcd. for $[C_{21}H_{24}O_3N_3]^+$ 366.18122; found 366.18143. HRMS: calcd. for $[C_{21}H_{23}O_3N_3Na]^+$ 388.16316; found 388.16322.

Azido-cyclohexene 30: A solution of dibenzylated azide **29** (0.47 mmol, 0.17 g) in CH_2Cl_2 was cooled to –78 °C under argon before addition of BCl_3 (4.8 mmol, 4.8 mL 1 M in CH_2Cl_2, 10 equiv.). The reaction mixture was stirred at –78 °C for 4 h, quenched with MeOH, concentrated in vacuo, and coevaporated with toluene (3×). The deprotected product was then dissolved in pyridine, BzCl (4.7 mmol, 0.55 mL, 10 equiv.) was added, and the mixture was stirred overnight at room temperature. After quenching with aqueous saturated $NaHCO_3$, the mixture was extracted with CH_2Cl_2 (3×), and the combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo. The product was purified by column chromatography (pentane→7.5% EtOAc in pentane) to yield the tribenzoylated product **30** (0.20 g, 0.40 mmol, 85% over two steps). $[\alpha]_D^{20}$ = +265 (*c* = 0.2, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.19–7.86 (m, 6 H, C*H* arom.), 7.66–7.28 (m, 9 H, C*H* arom.), 6.21–6.12 (m, 1 H, C*H*-2), 6.12–6.07 (m, 1 H, C*H*-4), 6.03 (dt, *J* = 10.3, 2.7 Hz, 1 H, C*H*=CH), 5.89–5.82 (m, 1 H, C*H*=CH), 5.71 (dd, *J* = 8.6, 2.3 Hz, 1 H, C*H*-3), 3.52 (dd, *J* = 12.1, 7.7 Hz, 1 H, CH*H*), 3.42 (dd, *J* = 12.1, 7.8 Hz, 1 H, CH*H*), 3.10–3.08 (m, 1 H, C*H*-5) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.11, 165.84, 165.51 (3× *C*=O), 133.75, 133.55, 133.28, 133.21, 130.17, 129.78, 129.76, 129.72, 129.46, 129.20, 129.17, 128.66, 128.46, 128.40, 128.31 (*C*H and C_q arom.), 127.48, 126.55 (2× *C*H=CH), 73.14 (*C*H-3), 70.49 (*C*H-2), 69.85 (*C*H-4), 51.46 (CH_2), 39.87 (*C*H-5) ppm. HRMS: calcd. for $[C_{28}H_{23}O_6N_3Na]^+$ 520.14791; found 520.14724.

Azido-epoxide 31: Tribenzoylated azide **30** (0.40 mmol, 0.20 g) was dissolved in CH_2Cl_2 under argon, and 3-chloroperoxybenzoic acid (1.2 mmol, 0.27 g, 3.0 equiv.) was added. The reaction mixture was stirred at room temperature for a total of 4 d, concentrated in vacuo, and purified by column chromatography (pentane→10% EtOAc in pentane), giving the azido-epoxide **31** as a mixture of α- and β-isomers (ratio 4:1, 0.11 g, 0.21 mmol, 53%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.12–7.81 (m, 6 H, 6× C*H* arom. α, 6× C*H* arom. β), 7.68–7.25 (m, 9 H, 9× C*H* arom. α, 9× C*H* arom. β), 6.04 (dd, *J* = 9.3, 2.3 Hz, 0.8 H, C*H*-2 α), 5.93–5.86 (m, 1 H, C*H*-4 α, C*H*-4 β), 5.75 (d, *J* = 9.4 Hz, 0.2 H, C*H*-2 β), 5.60 (dd, *J* = 9.3, 1.9 Hz, 0.8 H, C*H*-3 α), 5.53 (dd, *J* = 9.3, 2.6 Hz, 0.2 H, C*H*-3 β), 3.88 (dd, *J* = 3.9, 2.3 Hz, 0.8 H, C*H*-1 α), 3.68–3.42 (m, 2.4 H, CH_2 α, CH_2 β, C*H*-1 β, C*H*-7 β), 3.35 (dd, *J* = 3.9, 1.7 Hz, 0.8 H, C*H*-7 α), 2.80–2.74 (m, 1 H, C*H*-5 α, C*H*-5 β) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.25, 165.68, 165.65, 165.54, 165.47, 165.26 (*C*=O of α- and β-isomers), 134.59, 133.66, 133.62, 133.53, 133.48, 133.36, 133.30, 130.88, 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 (*C*H and C_q arom. of α- and β-isomers), 72.17, 70.85 (*C*H-3 of α- and β-isomers), 70.32, 70.18 (*C*H-2 α, *C*H-4 α), 68.47 (*C*H-2 β), 66.77 (*C*H-4 β), 54.44 (*C*H-1 or *C*H-7 β), 54.14 (*C*H-1 α), 53.67 (*C*H-7 α), 52.04 (*C*H-1 or *C*H-7 β), 49.77, 49.75 (CH_2 of α- and β-isomers), 39.01 (*C*H-5 α), 38.27 (*C*H-5 β) ppm. LC/MS analysis: R_t 9.3 min (linear gradient 10→90% B in 15 min), *m*/*z* 514.1 $[M + H]^+$, 1026.7 $[2M + H]^+$. HRMS: calcd. for $[C_{28}H_{24}O_7N_3]^+$ 514.16088; found 514.16066. HRMS: calcd. for $[C_{28}H_{23}O_7N_3Na]^+$ 536.14282; found 536.14242.

α-Epoxide 6 and β-Epoxide 12: Epoxide **31** (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-%) over 1.5 h 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 (CH_2Cl_2→3% MeOH in CH_2Cl_2 in steps of 0.5%) to separate the α-epoxide **6** (16 mg, 80 μmol, 42%) and β-epoxide **12** (3.0 mg, 15 μmol, 8%) (additional yield of mixed α/β product 7.0 mg, 35 μmol, 18%).

α-Epoxide 6: $[\alpha]_D^{20}$ = +81 (*c* = 0.3, MeOH). ^{1}H NMR (600 MHz, MeOD): δ = 4.07 (dd, *J* = 8.6, 2.5 Hz, 1 H, C*H*-2), 3.78 (dd, *J* = 3.4, 1.8 Hz, 1 H, C*H*-4), 3.59 (dd, *J* = 12.2, 8.0 Hz, 1 H, CH*H*), 3.48 (dd, *J* = 12.3, 8.3 Hz, 1 H, CH*H*), 3.39 (dd, *J* = 8.5, 1.9 Hz, 1 H, C*H*-3), 3.32 (dd, *J* = 3.9, 2.4 Hz, 1 H, C*H*-1), 2.97 (dd, *J* = 3.9, 1.8 Hz, 1 H, C*H*-7), 2.09 (td, *J* = 8.2, 3.5 Hz, 1 H, C*H*-5) ppm. ^{13}C NMR (150 MHz, MeOD): δ = 73.56 (*C*H-3), 72.15 (*C*H-4), 70.53 (*C*H-2), 58.08 (*C*H-1), 55.29 (*C*H-7), 51.73 (CH_2), 41.83 (*C*H-5) ppm.

β-Epoxide 12: $[\alpha]_D^{20}$ = +64 (*c* = 0.1, MeOH). ^{1}H NMR (600 MHz, MeOD): δ = 3.92 (dd, *J* = 8.7, 0.7 Hz, 1 H, C*H*-2), 3.82 (ddd, *J* = 4.1, 2.5, 1.3 Hz, 1 H, C*H*-4), 3.70 (dd, *J* = 12.1, 8.2 Hz, 1 H, CH*H*), 3.63 (dd, *J* = 12.1, 7.2 Hz, 1 H, CH*H*), 3.33 (dd, *J* = 8.6, 2.6 Hz, 1 H, C*H*-3), 3.26–3.22 (m, 1 H, C*H*-7), 3.17 (d, *J* = 3.7 Hz, 1 H, C*H*-1), 2.28 (dddd, *J* = 8.2, 7.2, 4.1, 1.8 Hz, 1 H, C*H*-5) ppm. ^{13}C NMR (150 MHz, MeOD): δ = 76.39 (*C*H-3), 70.36 (*C*H-4), 69.50 (*C*H-2), 58.03 (*C*H-1), 55.21 (*C*H-7), 51.58 (CH_2), 40.43 (*C*H-5) ppm.

Bodipy-α-epoxide 7: Azido-epoxide **16** (25 μmol, 5.0 mg) and Bodipy-alkyne **32**[19] (25 μmol, 12 mg, 1.0 equiv.) were dissolved in DMF (0.5 mL) under argon. After addition of copper(II)-sulfate pentahydrate (5 μmol, 5 μL 1 M in H_2O, 0.2 equiv.) and sodium ascorbate (10 μmol, 10 μL 1 M in H_2O, 0.4 equiv.) the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated in vacuo and purified by column chromatography (CH_2Cl_2→3% MeOH in CH_2Cl_2) to yield Bodipy-epoxide **7** as a purple solid (15 mg, 22 μmol, 88%). ^{1}H NMR (400 MHz, MeOD): δ = 7.89–7.82 (m, 4 H, C*H* arom.), 7.78 (s, 1 H, C*H* triazole), 7.43 (d, *J* = 4.4 Hz, 2 H, 2× C*H* pyrrole), 7.01–6.94 (m, 4 H, C*H* arom.), 6.70 (d, *J* = 4.3 Hz, 2 H, 2× C*H* pyrrole), 4.60–4.53 (m, 2 H, CH_2-6), 4.13 (dd, *J* = 8.5, 2.4 Hz, 1 H, C*H*-2), 3.85 (s, 6 H, 2× OCH_3), 3.66–3.61 (m, 1 H, C*H*-4), 3.42 (dd, *J* = 8.5, 1.8 Hz, 1 H, C*H*-3), 3.37–3.34 (m, 1 H, C*H*-1), 3.07–3.02 (m, 2 H, CH_2), 2.97 (dd, *J* = 3.9, 1.8 Hz, 1 H, C*H*-7), 2.79–2.75 (m, 2 H, CH_2), 2.55 (td, *J* = 8.2, 3.5 Hz, 1 H, C*H*-5), 1.88–1.83 (m, 4 H, 2× CH_2) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 160.77 (C_q arom.), 157.39 (C_q arom.), 145.31 (C_q arom.), 136.08 (C_q arom.), 130.71 (*C*H arom.), 127.03 (*C*H pyrrole), 125.10 (C_q arom.), 123.38 (*C*H triazole), 119.66 (*C*H pyrrole), 113.22 (*C*H arom.), 72.03 (*C*H-3), 70.53 (*C*H-4), 69.06 (*C*H-2), 56.67 (*C*H-1), 54.41 (2× OCH_3), 53.30 (*C*H-7), 49.13 (CH_2-6), 41.27 (*C*H-5), 32.86, 29.58, 29.00, 24.48 (4× CH_2) ppm. LC/MS analysis: R_t 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 $[C_{36}H_{39}O_6N_5BF_2]^+$ 686.29560; found 686.29599. HRMS: calcd. for $[C_{36}H_{38}O_6N_5BF_2Na]^+$ 708.27754; found 708.27719.

Biotin-α-epoxide 8: Azido-epoxide **6** (25 μmol, 5.0 mg) and biotin-alkyne **33**[20] (25 μmol, 7.0 mg, 1.0 equiv.) were dissolved in DMF (0.5 mL) under argon. After addition of copper(II)sulfate pentahydrate (5 μmol, 5 μL 1 M in H_2O, 0.2 equiv.) and sodium ascorbate

(10 μmol, 10 μL 1 M in H_2O, 0.4 equiv.) the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated in vacuo and purified by column chromatography ($CH_2Cl_2 \rightarrow 30\%$ MeOH in CH_2Cl_2) to yield biotin-epoxide **8** as a white solid (8.0 mg, 17 μmol, 68%). $[\alpha]_D^{20}$ = +48 (*c* = 0.1, MeOH). ^{1}H NMR (400 MHz, MeOD): δ = 7.97 (s, 1 H, C*H* triazole), 4.65–4.60 (m, 2 H, CH_2-6), 4.52 (ddd, *J* = 8.0, 5.0, 1.0 Hz, 1 H, C*H*-NH), 4.47 (s, 2 H, CH_2-NH), 4.32 (dd, *J* = 7.9, 4.5 Hz, 1 H, C*H*-NH), 4.14 (dd, *J* = 8.6, 2.4 Hz, 1 H, C*H*-2), 3.66 (dt, *J* = 3.7, 1.8 Hz, 1 H, C*H*-4), 3.43 (dd, *J* = 8.6, 1.8 Hz, 1 H, C*H*-3), 3.37–3.36 (m, 1 H, C*H*-1), 3.22 (ddd, *J* = 8.9, 5.9, 4.4 Hz, 1 H, C*H*-S), 3.02 (dd, *J* = 4.0, 1.8 Hz, 1 H, C*H*-7), 2.96 (dd, *J* = 12.8, 5.0 Hz, 1 H, CH*H*-S), 2.74 (d, *J* = 12.7 Hz, 1 H, CH*H*-S), 2.58 (td, *J* = 8.1, 3.5 Hz, 1 H, C*H*-5), 2.27 (t, *J* = 7.3 Hz, 2 H, CH_2), 1.78–1.57 (m, 4 H, 2 × CH_2), 1.48–1.42 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 174.63 (*C*=O), 145.05 (*C*=O), 123.62 (*C*H triazole), 72.00 (*C*H-3), 70.51 (*C*H-4), 69.03 (*C*H-2), 61.95, 60.23 (2 × *C*H-NH), 56.70 (*C*H-1), 55.59 (*C*H-S), 53.32 (*C*H-7), 49.23 (*C*H_2-6), 41.34 (*C*H-5), 39.66 (*C*H_2-S), 35.14, 34.20, 28.28, 28.05, 25.29 (5 × *C*H_2) ppm. LC/MS analysis: *Rt* 3.6 min (linear gradient 10 → 50% B in 15 min), *m*/*z* 483.3 $[M + H]^+$, 965.1 $[2M + H]^+$, 1446.9 $[3M + H]^+$. HRMS: calcd. for $[C_{20}H_{31}O_6N_6S]^+$ 483.20203; found 483.20176. HRMS: calcd. for $[C_{20}H_{30}O_6N_6SNa]^+$ 505.18397; found 505.18347.

Bodipy-β-epoxide 13: Azido-epoxide **12** (25 μmol, 5.0 mg) and Bodipy-alkyne **32**[19] (25 μmol, 12 mg, 1.0 equiv.) were dissolved in DMF (0.5 mL) under argon. After addition of copper(II)sulfate pentahydrate (5 μmol, 5 μL 1 M in H_2O, 0.2 equiv.) and sodium ascorbate (10 μmol, 10 μL 1 M in H_2O, 0.4 equiv.) the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated in vacuo and purified by column chromatography ($CH_2Cl_2 \rightarrow 3\%$ MeOH in CH_2Cl_2) to yield Bodipy-epoxide **13** as a purple solid (15 mg, 22 μmol, 90%). ^{1}H NMR (400 MHz, MeOD): δ = 1.91–7.82 (m, 4 H, C*H* arom.), 7.77 (s, 1 H, C*H* triazole), 7.44 (d, *J* = 4.4 Hz, 2 H, 2 × C*H* pyrrole), 7.03–6.93 (m, 4 H, C*H* arom.), 6.70 (d, *J* = 4.3 Hz, 2 H, 2 × C*H* pyrrole), 4.70 (d, *J* = 7.8 Hz, 2 H, CH_2-6), 3.97 (d, *J* = 8.5 Hz, 1 H, C*H*-2), 3.86 (s, 6 H, 2 × OCH_3), 3.66–3.58 (m, 1 H, C*H*-4), 3.42–3.40 (m, 1 H, C*H*-3), 3.18 (d, *J* = 3.7 Hz, 1 H, C*H*-1), 3.11–2.97 (m, 3 H, C*H*-7, CH_2), 2.86–2.76 (m, 2 H, CH_2), 2.73 (ddt, *J* = 7.6, 4.3, 2.2 Hz, 1 H, C*H*-5), 1.91–1.82 (m, 4 H, 2 × CH_2) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 160.78 (C_q arom.), 157.38 (C_q arom.), 144.42 (C_q arom.), 136.09 (C_q arom.), 130.79 (C*H* arom.), 127.03 (C*H* pyrrole), 125.11 (C_q arom.), 123.06 (C*H* triazole), 119.63 (C*H* pyrrole), 113.22 (C*H* arom.), 74.73 (*C*H-3), 68.51 (*C*H-4), 67.89 (*C*H-2), 56.77 (*C*H-1), 54.41 (2 × OCH_3), 53.27 (*C*H-7), 48.74 (CH_2-6), 39.84 (*C*H-5), 32.86, 29.59, 29.02, 24.46 (4 × *C*H_2) ppm. 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 $[2M + H]^+$. HRMS: calcd. for $[C_{36}H_{39}O_6N_5BF_2]^+$ 686.29560; found 686.29615. HRMS: calcd. for $[C_{36}H_{38}O_6N_5BF_2Na]^+$ 708.27754; found 708.27725.

Biotin-β-epoxide 14: Azido-epoxide **12** (25 μmol, 5.0 mg) and biotin-alkyne **33**[20] (25 μmol, 7.0 mg, 1.0 equiv.) were dissolved in DMF (0.5 mL) under argon. After addition of copper(II)sulfate pentahydrate (5 μmol, 5 μL 1 M in H_2O, 0.2 equiv.) and sodium ascorbate (10 μmol, 10 μL 1 M in H_2O, 0.4 equiv.) the reaction mixture was stirred at room temperature overnight. The mixture was then concentrated in vacuo and purified by column chromatography ($CH_2Cl_2 \rightarrow 30\%$ MeOH in CH_2Cl_2) to yield biotin-epoxide **14** as a white solid (8.0 mg, 17 μmol, 68%). $[\alpha]_D^{20}$ = +36 (*c* = 0.1, MeOH). ^{1}H NMR (400 MHz, MeOD): δ = 7.93 (s, 1 H, C*H* triazole), 4.75 (d, *J* = 7.7 Hz, 2 H, CH_2-6), 4.52 (ddd, *J* = 8.0, 5.0, 0.9 Hz, 1 H, C*H*-NH), 4.46 (s, 2 H, CH_2-NH), 4.32 (dd, *J* = 7.9, 4.5 Hz, 1 H, C*H*-NH), 3.98 (d, *J* = 8.5 Hz, 1 H, C*H*-2), 3.63 (ddd, *J* = 4.1, 2.7, 1.2 Hz, 1 H, C*H*-4), 3.38–3.36 (m, 1 H, C*H*-3), 3.25–3.22 (m, 1 H, C*H*-S), 3.21 (d, *J* = 3.8 Hz, 1 H, C*H*-1), 3.14–3.08 (m, 1 H, C*H*-7), 2.96 (dd, *J* = 12.7, 5.0 Hz, 1 H, CH*H*-S), 2.78 (ddt, *J* = 7.7, 5.4, 2.7 Hz, 1 H, C*H*-5), 2.73 (d, *J* = 12.8 Hz, 1 H, CH*H*-S), 2.27 (t, *J* = 7.3 Hz, 2 H, CH_2), 1.81–1.55 (m, 4 H, 2 × CH_2), 1.50–1.38 (m, 2 H, CH_2) ppm. ^{13}C NMR (100 MHz, MeOD): δ = 174.62 (*C*=O), 144.82 (*C*=O), 123.89 (*C*H triazole), 74.73 (*C*H-3), 68.46 (*C*H-4), 67.88 (*C*H-2), 61.95, 60.23 (2 × *C*H-NH), 56.79 (*C*H-1), 55.59 (*C*H-S), 53.25 (*C*H-7), 48.86 (*C*H_2-6), 39.87 (*C*H-5), 39.65 (*C*H_2-S), 35.14, 34.22, 28.29, 28.04, 25.30 (5 × *C*H_2) ppm. 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 $[C_{20}H_{31}O_6N_6S]^+$ 483.20203; found 483.20179. HRMS: calcd. for $[C_{20}H_{30}O_6N_6SNa]^+$ 505.18397; found 505.18357.

Aziridine 34: Cyclohexene **18** (0.50 mmol, 0.17 g) was coevaporated with toluene before being dissolved in CH_2Cl_2 (10 mL) at 0 °C under argon. Next, CCl_3CCN (0.50 mmol, 51 μL, 1.0 equiv.) and DBU (25 μmol, 2.0 μL, 0.05 equiv.) were added and the reaction mixture was stirred at 0 °C for 1.5 h. The mixture was removed from the ice bath, after which H_2O (1.5 mL), $NaHCO_3$ (5.0 mmol, 0.40 g, 10 equiv.), and I_2 (1.5 mmol, 0.38 g, 3.0 equiv.) were added. After stirring at room temperature overnight, the reaction mixture was quenched with aqueous $Na_2S_2O_3$ (10%), and the aqueous layer was extracted with EtOAc (2 ×). The combined organics were dried with $MgSO_4$, filtered, and concentrated in vacuo. The residue was redissolved in MeOH and cooled to 0 °C, and concentrated HCl (6.3 mmol, 0.6 mL, 13 equiv.) was added, after which the reaction mixture was stirred at room temperature for 3.5 h. Next, the mixture was concentrated in vacuo, the residue was redissolved in dioxane, and concentrated HCl (16 mmol, 1.5 mL, 32 equiv.) was again added. The mixture was stirred at 60 °C for 1 h, concentrated in vacuo, and coevaporated with toluene (3 ×). The residue was redissolved in MeOH, $NaHCO_3$ (10 mmol, 0.8 g, 20 equiv.) was added, and the reaction mixture was stirred at room temperature for 4 d. After addition of H_2O, the aqueous layer was extracted with EtOAc, and the organic layer was dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by column chromatography ($CH_2Cl_2 \rightarrow 6\%$ MeOH in CH_2Cl_2) to afford dibenzylated β-aziridine **34** (92 mg, 0.26 mmol, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.46–7.22 (m, 10 H, C*H* arom.), 4.83–4.61 (m, 4 H, 2 × CH_2 Bn), 4.13–4.04 (m, 2 H, C*H*-2, C*H*-4), 3.99 (dd, *J* = 10.9, 7.5 Hz, 1 H, CH*H*-6), 3.90 (dd, *J* = 10.9, 6.2 Hz, 1 H, CH*H*-6), 3.39 (dd, *J* = 7.9, 2.3 Hz, 1 H, C*H*-3), 2.50–2.32 (m, 2 H, C*H*-1, C*H*-7), 2.06–1.96 (m, 1 H, C*H*-5) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.31, 138.17 (C_q arom.), 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, 127.80, 127.78, 127.63 (*C*H arom.), 83.29 (*C*H-3), 78.57 (*C*H-2), 73.10, 71.69 (2 × *C*H_2 Bn), 67.42 (*C*H-4), 62.40 (*C*H_2-6), 39.47 (*C*H-5), 32.63, 32.53 (*C*H-1, *C*H-7) ppm. LC/MS analysis: R_t 5.4 min (linear gradient 10 → 90% B in 15 min), *m*/*z* 356.1 $[M + H]^+$, 711.1 $[2M + H]^+$.

Benzyl-cyclohexene 35: A solution of cyclohexene **18** (3.5 mmol, 1.2 g) in DMF under argon was cooled to 0 °C, after which tetrabutylammonium iodide (35 μmol, 13 mg, 1 mol-%), benzyl bromide (8.4 mmol, 1.0 mL, 2.4 equiv.), and sodium hydride [8.4 mmol, 0.33 g (60% in mineral oil), 2.4 equiv.] were added. The reaction mixture was stirred overnight at room temperature and subsequently quenched with MeOH. Next, H_2O was added and the resulting mixture was extracted with EtOAc (3 ×). The combined organic layers were washed with H_2O (1 ×) and brine (1 ×), dried

with $MgSO_4$, filtered, and concentrated in vacuo, yielding a mixture of products containing three or four 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 workup and column chromatography purification (pentane→5% EtOAc in pentane) the fully benzylated product **35** was obtained (total yield 1.5 g, 3.0 mmol, 85%). $[\alpha]_D^{20}$ = +105 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.23 (m, 20 H, C*H* arom.), 5.80–5.76 (m, 1 H, C*H*=CH), 5.48 (d, *J* = 10.4 Hz, 1 H, C*H*=CH), 4.93 (d, *J* = 11.6 Hz, 1 H, CH*H* Bn), 4.78–4.68 (m, 4 H, 2 × CH_2 Bn), 4.64 (d, *J* = 11.6 Hz, 1 H, CH*H* Bn), 4.50–4.43 (m, 3 H, C*H*-2, CH_2 Bn), 4.20 (dd, *J* = 1.6, 1.6 Hz, 1 H, C*H*-4), 3.75 (dd, *J* = 1.6, 8.0 Hz, 1 H, C*H*-3), 3.62–3.46 (m, 2 H, CH_2-6), 2.72–2.68 (m, 1 H, C*H*-5) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.03, 138.77, 138.70, 138.15 (C_q arom.), 128.40, 128.32, 128.30, 128.17, 127.87, 127.81, 127.79, 127.67, 127.49, 127.47, 127.44, 127.38, 126.66 (*C*H arom., 2 × *C*H=CH), 83.20 (*C*H-3), 77.66 (*C*H-2), 75.12 (*C*H-4), 74.03, 73.28, 72.37, 72.16 (4 × *C*H_2 Bn), 70.17 (*C*H_2-6), 41.92 (*C*H-5) ppm. LC/MS analysis: R_t 10.8 min (linear gradient 10→90% B in 15 min), *m*/*z* 521.1 [M + H]$^+$, 543.3 [M + Na]$^+$. HRMS: calcd. for $[C_{35}H_{37}O_4]^+$ 521.26864; found 521.26864. HRMS: calcd. for $[C_{35}H_{36}O_4Na]^+$ 543.25058; found 543.24988.

β-Epoxide 36: 3-Chloroperoxybenzoic acid (1.7 mmol, 0.30 g, 2.5 equiv.) was added under argon to a solution of **35** (0.70 mmol, 0.36 g) in CH_2Cl_2. After stirring overnight, the reaction mixture was quenched with saturated aqueous Na_2SO_3 and extracted with CH_2Cl_2 (2×). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (1×), dried with $MgSO_4$, filtered, and concentrated in vacuo. The crude products were purified and separated by column chromatography (pentane→10% EtOAc in pentane) to afford β-epoxide **36** (0.24 g, 0.43 mmol, 63%) and its α-epoxide isomer (37 mg, 70 μmol, 10%).

α-Epoxide: $[\alpha]_D^{20}$ = +30 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41–7.22 (m, 20 H, C*H* arom.), 4.90–4.79 (m, 3 H, CH*H* Bn, CH_2 Bn), 4.70 (dd, *J* = 11.8, 17.8 Hz, 2 H, CH_2 Bn), 4.53–4.45 (m, 3 H, CH*H* Bn, CH_2 Bn), 4.25 (dd, *J* = 2.4, 8.4 Hz, 1 H, C*H*-2), 3.93–3.92 (m, 1 H, C*H*-4), 3.62 (dd, *J* = 8.6, 1.0 Hz, 1 H, C*H*-3), 3.58 (d, *J* = 8.0 Hz, 2 H, CH_2-6), 3.37 (dd, *J* = 2.4, 4.0 Hz, 1 H, C*H*-1), 2.96 (dd, *J* = 1.4, 4.0 Hz, 1 H, C*H*-7), 2.32–2.28 (m, 1 H, C*H*-5) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) α-epoxide: δ = 138.68, 138.60, 138.58, 137.89 (C_q arom.), 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 (*C*H arom.), 81.10 (*C*H-3), 76.73 (*C*H-2), 75.68 (*C*H-4), 74.23, 73.68, 73.35, 73.04 (4 × *C*H_2 Bn), 68.53 (*C*H_2-6), 55.20 (*C*H-1), 54.35 (*C*H-7), 40.89 (*C*H-5) ppm. LC/MS analysis: R_t 10.3 min (linear gradient 10→90% B in 15 min), *m*/*z* 537.0 [M + H]$^+$, 559.3 [M + Na]$^+$. HRMS: calcd. for $[C_{35}H_{37}O_5]^+$ 537.26355; found 537.26385. HRMS: calcd. for $[C_{35}H_{36}O_5Na]^+$ 559.24550; found 559.24515.

β-Epoxide 36: $[\alpha]_D^{20}$ = +66 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.20 (m, 20 H, C*H* arom.), 4.86 (d, *J* = 12.0 Hz, 1 H, CH*H* Bn), 4.81–4.66 (m, 4 H, 2 × CH_2 Bn), 4.56 (d, *J* = 12.0 Hz, 1 H, CH*H* Bn), 4.49–4.42 (m, 2 H, CH_2 Bn), 4.14 (d, *J* = 8.8 Hz, 1 H, C*H*-2), 3.94 (dd, *J* = 4.0, 2.0 Hz, 1 H, C*H*-4), 3.73–3.62 (m, 2 H, CH_2-6), 3.45 (dd, *J* = 2.0, 8.8 Hz, 1 H, C*H*-3), 3.23 (d, *J* = 4.0 Hz, 1 H, C*H*-1), 3.16–3.15 (m, 1 H, C*H*-7), 2.32–2.30 (m, 1 H, C*H*-5) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.80, 138.44, 137.96 (C_q arom.), 128.36, 128.33, 128.25, 128.05, 127.81, 127.77, 127.71, 127.68, 127.63, 127.44, 127.42, 127.21 (*C*H arom.), 82.47 (*C*H-3), 76.11 (*C*H-2), 74.27, 73.38, 73.15 (3 × *C*H_2 Bn), 72.79 (*C*H-4), 72.62 (CH_2 Bn), 68.79 (CH_2-6), 54.37 (*C*H-1), 52.38 (*C*H-7), 40.19 (*C*H-5) ppm. LC/MS analysis: R_t 10.3 min (linear gradient 10→90% B in 15 min), *m*/*z* 537.1 [M + H]$^+$, 559.3 [M + Na]$^+$. HRMS: calcd. for $[C_{35}H_{37}O_5]^+$ 537.26355; found 537.26391. HRMS: calcd. for $[C_{35}H_{36}O_5Na]^+$ 559.24550; found 559.24491.

***trans*-Azido Alcohols 37:** A solution of β-epoxide **36** (0.48 mmol, 0.26 g) in MeCN was put under argon, sodium azide (24 mmol, 1.6 g, 50 equiv.) and lithium perchlorate (4.8 mmol, 0.51 g, 10 equiv.) were added, and the mixture was heated to 80 °C. After stirring under reflux overnight, the reaction mixture was allowed to cool to room temperature, quenched with H_2O, and extracted with CH_2Cl_2 (3×). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated in vacuo. Purification by column chromatography (pentane→10% EtOAc in pentane) yielded a mixture of the two *trans*-azido alcohols **37** in a ratio of 1:1.3 (0.20 g, 0.35 mmol, 73%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.18 (m, 20 H, 20 × C*H* arom. isomer 1, 20 × C*H* arom. isomer 2), 5.02–4.67 (m, 5 H, 2 × CH_2 Bn isomer 1, CH*H* Bn isomer 1, 2 × CH_2 Bn isomer 2, CH*H* Bn isomer 2), 4.51–4.40 (m, 3 H, CH_2 Bn isomer 1, CH*H* Bn isomer 1, CH_2 Bn isomer 2, CH*H* Bn isomer 2), 4.30–4.26 (m, 1.57 H, C*H*-2 isomer 1, C*H*-4 isomer 1, C*H*-4 isomer 2), 4.11–4.09 (m, 0.57 H, C*H*-1 isomer 1), 3.91–3.87 (m, 1 H, O*H* isomer 1, C*H*-2 isomer 2), 3.79–3.77 (m, 1.57 H, C*H*-3 isomer 1, C*H*-7 isomer 1, O*H* isomer 2), 3.69–3.41 (m, 3.29 H, CH_2-6 isomer 1, CH_2-6 isomer 2, C*H*-3 isomer 2, C*H*-1 isomer 2, C*H*-7 isomer 2), 2.05–2.02 (m, 0.57 H, C*H*-5 isomer 1), 1.68–1.62 (m, 0.43 H, C*H*-5 isomer 2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.14, 138.04, 137.99, 137.85, 137.80 (C_q arom.), 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 (*C*H arom.), 83.58 (*C*H-3 isomer 2), 81.21 (*C*H-2 isomer 2), 80.68 (*C*H-3 isomer 1), 78.25 (*C*H-2 isomer 1), 76.93 (*C*H-1 isomer 2), 76.60 (*C*H-4 isomer 1), 75.86, 75.59, 75.02, 73.54 (4 × CH_2 Bn), 73.43 (*C*H-4 isomer 2), 73.39, 73.32, 73.28, 72.50 (4 × CH_2 Bn), 71.55 (*C*H-7 isomer 1), 67.71 (CH_2-6 isomer 1, CH_2-6 isomer 2), 64.75 (*C*H-1 isomer 1), 61.58 (*C*H-7 isomer 2), 42.31 (*C*H-5 isomer 2), 38.59 (*C*H-5 isomer 1) ppm. LC/MS analysis: R_t 10.4 min (linear gradient 10→90% B in 15 min), *m*/*z* 579.9 [M + H]$^+$; and R_t 10.6 min, *m*/*z* 580.0 [M + H]$^+$. HRMS: calcd. for $[C_{35}H_{38}N_3O_5]^+$ 580.28060; found 580.28078. HRMS: calcd. for $[C_{35}H_{37}N_3O_5Na]^+$ 602.26254; found 602.26211.

Benzyl-aziridine 38: A mixture of *trans*-azido alcohols **37** (0.50 mmol, 0.29 g) was dissolved in MeCN under argon. After addition of triphenylphosphine (0.65 mmol, 0.17 g, 1.3 equiv.), the reaction mixture was heated to 80 °C and stirred under reflux for 2 h. The mixture was then allowed to cool to room temperature and concentrated in vacuo. The crude product was purified by column chromatography (CH_2Cl_2→0.8% MeOH in CH_2Cl_2) to give α-aziridine **38** (pure yield 70 mg, 0.13 mmol, 26%, together with an additional 77 mg product containing triphenylphosphine oxide). $[\alpha]_D^{20}$ = +56 (*c* = 0.1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47–7.22 (m, 20 H, C*H* arom.), 4.89–4.79 (m, 3 H, CH_2 Bn, CH*H* Bn), 4.70 (dd, *J* = 11.6, 22.8 Hz, 2 H, CH_2 Bn), 4.55–4.43 (m, 3 H, CH_2 Bn, CH*H* Bn), 4.23 (dd, *J* = 4.0, 8.4 Hz, 1 H, C*H*-2), 3.88–3.87 (m, 1 H, C*H*-4), 3.61–3.55 (m, 3 H, C*H*-3, CH_2-6), 2.55–2.52 (m, 1 H, C*H*-1), 2.19–2.15 (m, 1 H, C*H*-5), 2.02 (d, *J* = 6.0 Hz, 1 H, C*H*-7) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 139.09, 138.92, 138.84, 138.06 (C_q arom.), 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 (*C*H arom.), 81.88 (*C*H-3), 76.87 (*C*H-2), 76.25 (*C*H-4), 73.99, 73.10, 72.91, 72.35 (4 × CH_2 Bn), 69.81 (CH_2-6), 41.89 (*C*H-5), 34.15 (*C*H-1), 32.08 (*C*H-7) ppm. LC/MS analysis: *Rt* 7.7 min (linear gradient 10→90% B in

15 min), *m/z* 536.1 $[M + H]^+$, 1071.2 $[2M + H]^+$. HRMS: calcd. for $[C_{35}H_{38}NO_4]^+$ 536.27954; found 536.27943.

8-(Bodipy-triazole)-octanoic Acid 40: 8-Azidooctanoic acid **39**[23] (0.50 mmol, 93 mg) and Bodipy-alkyne **32**[19] (0.50 mmol, 0.24 g, 1.0 equiv.) were dissolved in DMF under argon. After addition of copper(II)sulfate pentahydrate (100 μmol, 100 μL 1 M in H_2O, 0.2 equiv.) and sodium ascorbate (200 μmol, 200 μL 1 M in H_2O, 0.4 equiv.) the reaction mixture was heated to 80 °C and stirred for 4.5 h. The mixture was then concentrated in vacuo and purified by column chromatography ($CH_2Cl_2 \rightarrow 2\%$ MeOH in CH_2Cl_2) to yield Bodipy-acid **40** as a purple solid (0.29 g, 0.43 mmol, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (d, *J* = 8.8 Hz, 4 H, C*H* arom.), 7.27–7.26 (m, 2 H, 2 × C*H* pyrrole), 7.18 (s, 1 H, C*H* triazole), 6.92 (d, *J* = 8.8 Hz, 4 H, C*H* arom.), 6.58 (d, *J* = 3.2 Hz, 2 H, 2 × C*H* pyrrole), 4.30–4.28 (m, 2 H, CH_2-N), 3.83 (s, 6 H, 2 × OCH_3), 2.96–2.95 (m, 2 H, CH_2-C=CH), 2.79–2.77 [m, 2 H, CH_2-$(CH_2)_3$-C=CH], 2.32 (t, *J* = 7.2 Hz, 2 H, CH_2-CO_2H), 1.90–1.83 (m, 6 H, 3 × CH_2), 1.62–1.58 (m, 2 H, CH_2), 1.32–1.25 (m, 6 H, 3 × CH_2) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 178.12 (*C*=O), 160.29, 157.15 (C_q arom.), 144.67 (C_q triazole), 135.88 (C_q arom.), 130.71 (*C*H triazole), 126.73 (*C*H pyrrole), 124.89 (C_q arom.), 119.73 (*C*H pyrrole), 113.47 (2 × *C*H arom.), 55.00 (2 × O*C*H_3), 53.33 (*C*H_2-CO_2H), 33.77, 32.72, 30.02, 29.78, 29.05, 28.49, 28.30, 25.95, 24.71, 24.31 (10 × *C*H_2) ppm.

α-Aziridine 41: Ammonia was condensed at –60 °C (ca. 5 mL) under argon flow with use of Birch equipment. Lithium (50 mg) was added and the solution was stirred for 30 min at –60 °C, after which a solution of α-aziridine **38** (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 with aqueous NH_4OH solution (1 M, 10 × 1 mL). After evaporation in vacuo the product was redissolved in MilliQ, and Amberlite-NH_4^+ 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 **41**, which was used without further purification. ^{1}H NMR (400 MHz, D_2O): δ = 4.01 (dd, *J* = 4.2, 9.0 Hz, 1 H, C*H*-2), 3.78 (s, 1 H, C*H*-4), 3.68–3.64 (m, 2 H, CH_2), 3.28 (d, *J* = 8.8 Hz, 1 H, C*H*-3), 2.54–2.51 (m, 1 H, C*H*-1 or C*H*-7), 2.08 (d, *J* = 6.4 Hz, 1 H, C*H*-1 or C*H*-7), 1.90–1.86 (m, 1 H, C*H*-6) ppm.

Azido-α-aziridine 9: 8-Azidooctanoic acid (**39**[23], 0.40 mmol, 78 mg) was preactivated with EEDQ (0.40 mmol, 99 mg, 1.0 equiv.) in DMF (0.4 mL) under argon for 2 h at room temperature. Deprotected aziridine **41** (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 equiv.) and the mixture was stirred at 0 °C for 30 min. Then another 0.5 equiv. 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 with use of a gradient of $22 \rightarrow 28\%$ MeCN in H_2O in 12 min, and lyophilized to yield aziridine **9** as a white powder (3.0 mg, 9.0 μmol, 14%). ^{1}H NMR (600 MHz, MeOD): δ = 4.08 (dd, *J* = 4.2, 9.0 Hz, 1 H, C*H*-2), 3.87–3.86 (m, 1 H, C*H*-4), 3.82–3.75 (m, 2 H, CH_2-6), 3.37 (dd, *J* = 1.8, 8.4 Hz, 1 H, C*H*-3), 3.30–3.27 (m, 2 H, CH_2-N_3), 2.97 (dd, *J* = 4.2, 6.0 Hz, 1 H, C*H*-1), 2.61 (d, *J* = 6.0 Hz, 1 H, C*H*-7), 2.56–2.44 (m, 2 H, CH_2-C=O), 2.02 (td, *J* = 7.8, 7.2, 3.6 Hz, 1 H, C*H*-5), 1.66–1.57 (m, 4 H, 2 × CH_2), 1.39–1.34 (m, 6 H, 3 × CH_2) ppm. ^{13}C NMR (150 MHz, MeOD): δ = 188.72 (*C*=O), 74.36 (*C*H-3), 73.13 (*C*H-4), 69.15 (*C*H-2), 62.84 (*C*H_2-6), 52.44 (*C*H_2-N_3), 44.54 (*C*H-5), 43.27 (*C*H-1), 39.44 (*C*H-7), 37.12 (*C*H_2-C=O), 30.17, 29.94, 27.68, 27.66, 25.90 (5 × *C*H_2) ppm. LC/MS analysis (ammonium acetate): R_t 7.5 min (linear gradient $0 \rightarrow 50\%$ B in 15 min), *m/z* 343.4 $[M + H]^+$, 360.5 $[M + NH_4]^+$. HRMS: calcd. for $[C_{15}H_{27}N_4O_5]^+$ 343.19760; found 343.19757. HRMS: calcd. for $[C_{15}H_{27}N_4O_5Na]^+$ 365.17954; found 365.17939.

Bodipy-α-aziridine 10: Bodipy acid **40** (0.10 mmol, 67 mg) was preactivated with EEDQ (0.10 mmol, 25 mg, 1.0 equiv.) in DMF (1 mL) under argon over 2 h at room temperature. Deprotected aziridine **41** (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 equiv.) and the mixture was stirred at 0 °C for 30 min. Then another 0.5 equiv. 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 with use of a gradient of $48 \rightarrow 51\%$ MeCN in H_2O in 12 min, and lyophilized to yield aziridine **10** as a purple powder (7.4 mg, 9.0 μmol, 15%). ^{1}H NMR (400 MHz, MeOD): δ = 7.87 (d, *J* = 8.8 Hz, 4 H, C*H* arom.), 7.72 (s, 1 H, C*H* triazole), 7.46 (d, *J* = 4.3 Hz, 2 H, 2 × C*H* pyrrole), 7.00 (d, *J* = 9.2 Hz, 4 H, C*H* arom.), 6.72 (d, *J* = 4.3 Hz, 2 H, 2 × C*H* pyrrole), 4.36 (t, *J* = 7.0 Hz, 2 H, CH_2-N), 4.10 (dd, *J* = 8.6, 4.0 Hz, 1 H, C*H*-2), 3.88 (s, 6 H, 2 × OCH_3), 3.87–3.80 (m, 1 H, C*H*-4), 3.79 (dd, *J* = 7.4, 4.3 Hz, 2 H, CH_2-6), 3.38 (dd, *J* = 8.6, 1.8 Hz, 1 H, C*H*-3), 3.12–3.06 (m, 2 H, CH_2-C=CH), 2.96 (dd, *J* = 6.0, 4.0 Hz, 1 H, C*H*-1), 2.82–2.78 [m, 2 H, CH_2-$(CH_2)_3$-C=CH], 2.62–2.58 (m, 1 H, C*H*-7), 2.55–2.35 (m, 2 H, CH_2-C=O), 2.03 (td, *J* = 7.8, 7.3, 3.6 Hz, 1 H, C*H*-5), 1.94–1.82 (m, 6 H, 3 × CH_2), 1.63–1.52 (m, 2 H, CH_2), 1.32–1.21 (m, 6 H, 3 × CH_2) ppm. LC/MS analysis: R_t 7.4 min (linear gradient $10 \rightarrow 90\%$ B in 15 min), *m/z* 827.07 $[M + H]^+$, 807.47 $[M - F]^+$, 1653.40 $[2M + H]^+$. Notably, under these acidic LC/MS conditions the aziridine is partially opened to give a second peak at R_t 7.2 min (linear gradient $10 \rightarrow 90\%$ B in 15 min), *m/z* 845.07 $[(M + H_2O) + H]^+$, 825.40 $[(M + H_2O) - F]^+$, 1689.13 $[2(M + H_2O) + H]^+$. HRMS: calcd. for $[C_{44}H_{54}BF_2N_6O_7]^+$ 827.41096; found 827.41180.

β-Aziridine 42: The β-aziridine **34** (0.10 mmol, 36 mg) was deprotected by the same procedure as described for α-aziridine **41** to give β-aziridine **42**. ^{1}H NMR (400 MHz, D_2O): δ = 4.10 (d, *J* = 8.0 Hz, 1 H, C*H*-2), 3.87–3.78 (m, 3 H, CH_2, C*H*-4), 3.43 (d, *J* = 8.0 Hz, 1 H, C*H*-3), 2.44 (br. s, 1 H, C*H*-1 or C*H*-7), 2.34–2.33 (m, 1 H, C*H*-1 or C*H*-7), 2.04 (br. s, 1 H, C*H*-5) ppm.

Azido-β-aziridine 15: Deprotected β-aziridine **42** (0.10 mmol) was acylated with azido-spacer **39**[23] by the same procedure as described for α-aziridine **9** to give β-aziridine **15**. However, LC/MS analysis (under basic conditions under which the aziridine should be stable) revealed the major product to be an H_2O adduct, presumably formed by opening of the aziridine. LC/MS analysis (ammonium acetate): minor peak R_t 7.6 min (linear gradient $0 \rightarrow 50\%$ B in 15 min), *m/z* 343.4 $[M + H]^+$, 360.4 $[M + NH_4]^+$; major peak R_t 7.0 min (linear gradient $0 \rightarrow 50\%$ B in 15 min), *m/z* 361.4 $[(M + H_2O) + H]^+$, 378.7 $[(M + H_2O) + NH_4]^+$. After HPLC purification and lyophilization, NMR analysis revealed that no aziridine proton peaks were present, indicating that the aziridine had completely decomposed.

DFT Calculations: The calculated ^{1}H NMR coupling constants were obtained by first finding the lowest-energy conformations of both epoxide isomers (**5** and **11**), for which a library of gas-phase conformations was generated by use of the conformer distribution option included in the Spartan 04[24] program with employment of

DFT/B3LYP 6–31G(d). All conformers were further optimized by use of Gaussian 03[25] at DFT/B3LYP 6–311G(d,p), their zero-point energies were calculated, and the energies were corrected for solvent by another optimization step with employment of a Polarizable Continuum Model set for water. The energies of these conformers, corrected for their zero-point energies, were compared, and for the lowest energy-conformer an NMR calculation was performed with the aid of the Gauge-Independent Atomic Orbital (GIAO) method with added spin–spin coupling calculation.

Supporting Information (see footnote on the first page of this article): ^{1}H NMR and ^{13}C-APT NMR spectra for all compounds and optimized structures of epoxides **5** and **11** obtained by DFT calculations.

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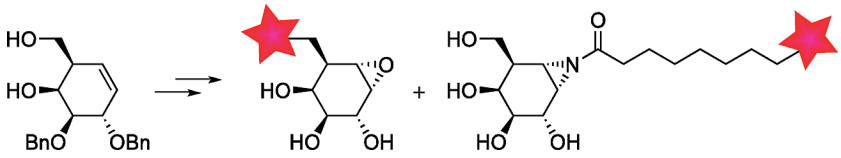

The synthesis of α- and β-galactopyranose-configured derivatives of cyclophellitol and of cyclophellitol aziridine, either non-tagged or functionalized with various reporter entities, is described. All epoxide- and aziridine-based compounds are synthesized from a single cyclohexene precursor – (3*S*,4*S*,5*S*,6*R*)-3,4-dibenzyloxy-5-hydroxy-6-(hydroxymethyl)cyclohex-1-ene.

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Synthesis of α- and β-Galactopyranose-Configured Isomers of Cyclophellitol and Cyclophellitol Aziridine

Keywords: Cyclitols / Enzyme inhibitors / Irreversible inhibitors / Aziridines / Epoxides / Fluorescent probes

