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Cyclophellitol analogues for profiling of exo- and endo-glycosidases

Schröder, S.P.

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Author: Schröder, Sybrin P.

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Chapter 4

D-*arabino*- and D-*lyxofuranosyl* cyclitol aziridines for unbiased glycosidase profiling

4.1 Introduction

Activity-based protein profiling (ABPP) has proven to be a powerful method for the identification of several classes of hydrolytic enzymes, including glycosidases.¹ Recent research has shown that carbasugars mimicking the natural substrate configuration are potent irreversible glycosidase inhibitors. For example, the configurational β -glucopyranose mimic ABP **1** (Figure 1) effectively labels the endogenous β -glucosidases, GBA1, GBA2, GBA3 and LPH in various mouse tissue lysates without off-target labeling of other proteins present in the complex biological sample.² Similarly, other configurational pyranoside probes such as **2** and **3** have been employed to selectively identify α -D-galactosidases and α -L-fucosidases, respectively (Figure 1).^{3,4} With these specific ABPs in hand, the glycosidase composition of various biological samples could be studied. Moreover, this technique allows the study on the effect of small-molecule inhibitors on the activity of the target enzymes by competitive ABPP experiments.⁵ However, the scope of glycosidases studied in such experiments is limited by the high specificity of these ABPs. Therefore it would be of interest to develop non-specific ABPs which display activity towards a wide variety of

glycosidases. With such probes, the overall glycosidase composition in biologically relevant settings as well as its response to external factors could be studied.

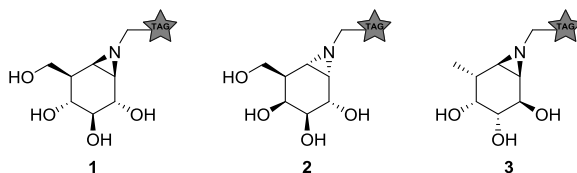


Figure 1 Activity-based probes **1-3** used for the selective identification of β -glucosidases, α -galactosidases and α -L-fucosidases, respectively.

The selective glycosidase ABPs discussed above were pyranoside carbasugar mimics. Probes emulating furanosides have not been investigated, while glycosidases acting on furanoside substrates are present in GH families 32, 43, 62, 68, 117 and 130.⁶ Bols and co-workers have published the synthesis of *D-arabinofuranosyl* aziridine **4** (Figure 2),⁷ and shown that it adopts a $^{1,4}B$ conformation (carbohydrate numbering) in solution. While the five-membered ring resembles a furanoside, one could argue that this conformation also mimics pyranoside carbohydrates in a $^{1,4}B$ conformation, which is the Michaelis complex conformation found during the catalytic itinerary of several (GH 1, 5, 7, 12, 18, 20 and 26) retaining glycosidases acting on pyranosides.⁸ Therefore, *D-arabinofuranosyl* aziridines could potentially serve as covalent inhibitors of retaining glycofuranosidases, as well as glycopyranosidases. While aziridine **4** displayed zero activity towards α -glucosidase (yeast) or β -glucosidase (almonds), alkylation of the aziridine with a methyl (**5**) or benzyl group (**6**) positively induced competitive inhibition towards these enzymes. Although inhibition was moderate, it was anticipated that the incorporation of a large aliphatic reporter tag could further increase its potency (a phenomenon also observed for **1**).⁹

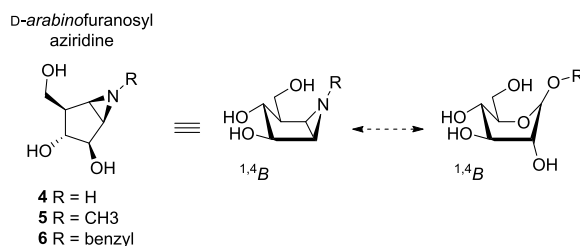
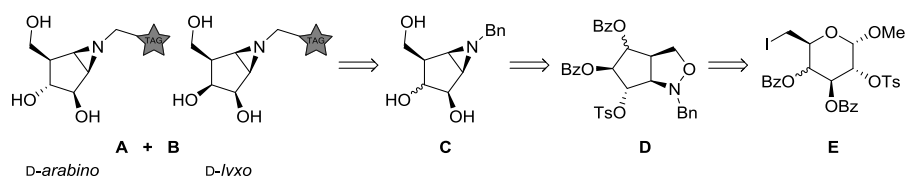


Figure 2 *D-arabinofuranosyl* aziridines **4-6** synthesized by Bols and co-workers adopt a $^{1,4}B$ conformation in solution.

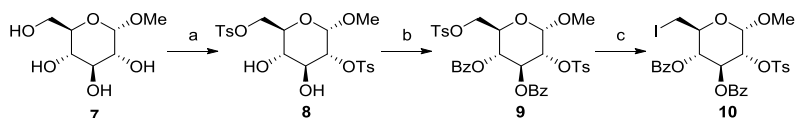
In this Chapter, the synthesis of furanosyl aziridine inhibitors equipped with a reporter tag is described. The synthesis route of Bols and co-workers is adapted and further optimized to afford D-arabinofuranosyl aziridine derivatives, and the synthesis strategy is then translated to a similar starting material to afford novel D-lyxofuranosyl aziridines. It was envisioned that D-arabinofuranosyl aziridines **A** as well as D-lyxofuranosyl aziridines **B** would be available from configurationally matching **C**, which would be accessible by N-O reduction of cyclopentaisoxazolidine **D**. This cis-fused bicyclic system would be available *via* fragmentation of **E** into the corresponding aldehyde, followed by nitron formation and subsequent [3+2] cycloaddition. The biochemical evaluation of these compounds focused on labeling of glycosidases in an unbiased fashion, as analyzed by SDS-PAGE and proteomics.



Scheme 1 Retrosynthetic analysis of the target D-arabino- and D-lyxofuranosyl aziridines.

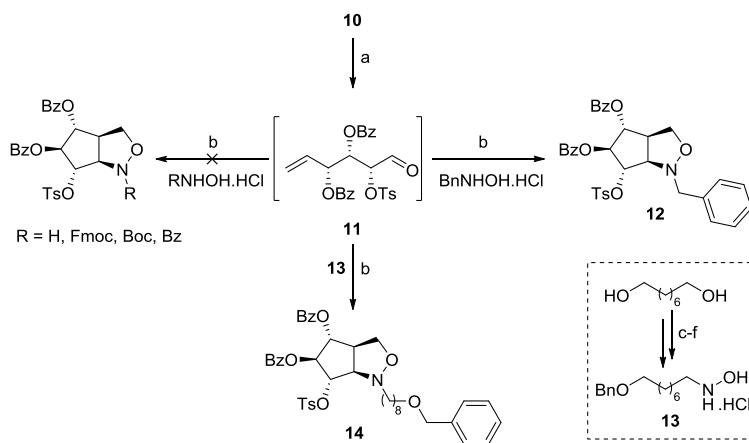
4.2 Results and Discussion

The synthesis of D-arabinofuranosyl aziridine **4** commenced with the preparation of synthon **10** (Scheme 2). A previously described procedure by Ferrier¹⁰ to prepare intermediate **8** from **7** proved to be low yielding, therefore an alternative route was employed. Methyl α -glucopyranoside **7** was selectively tosylated at position O2 and O6 using a catalytic amount of dibutyltin dichloride, affording bis-tosylate **8**.^{11,12} Subsequent benzoylation of the remaining hydroxyls furnished compound **9** which was then regioselectively iodinated at C-6 to afford key-intermediate **10**.



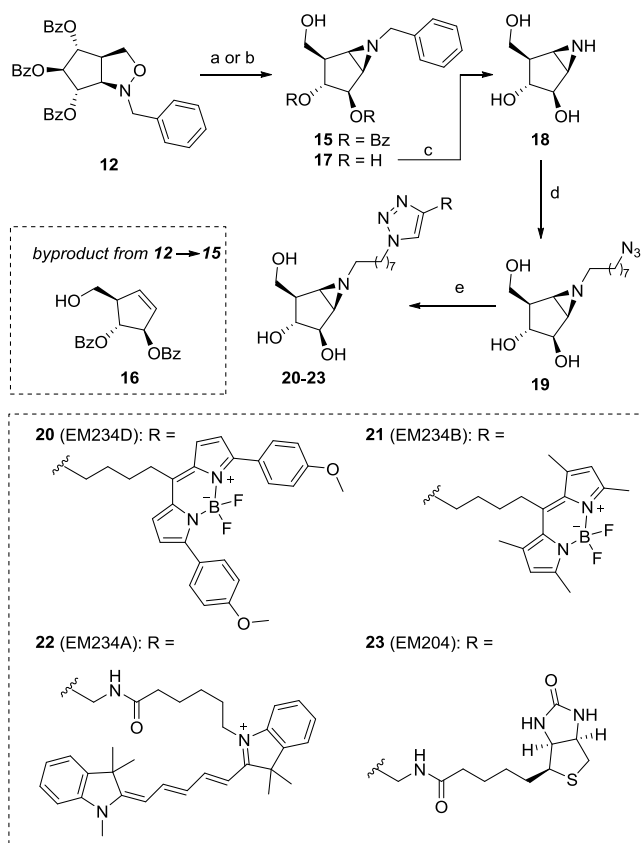
Scheme 2 Synthesis of iodopyranoside **10**. Reagents and conditions: a) TsCl, Bu₂SnCl₂ (40 mol%), Et₃N, MeCN, rt, 24h, 97%; b) Bz₂O, DMAP, pyridine, rt, 16h, 90%; c) NaI, Ac₂O, reflux, 4h, quant.

Iodide **10** was then subjected to a Vasella fragmentation¹³ using zinc dust in refluxing ethanol to afford intermediate aldehyde **11**, which was found to be unstable during isolation and was therefore directly used in the next step (Scheme 3). The aldehyde was reacted with *N*-benzyl hydroxylamine, affording the corresponding nitrone intermediate which underwent smooth intramolecular [3+2] cycloaddition with the terminal alkene to afford *N*-benzyl isoxazolidine **12** as a single isomer. Additionally, it would be of interest to investigate the incorporation of other functionalities/protecting groups on the isoxazolidine nitrogen. Therefore, condensation of aldehyde **11** with other hydroxylamines was studied. While the commercially available unprotected hydroxylamine as well as Fmoc-, Boc- and Bz-protected hydroxylamines were found to give complex mixtures upon reaction with aldehyde **11**, *N*-(8-(benzyloxy)octyl)hydroxylamine **13** (obtained by mono-benylation of 1,8-octanediol followed by oxidation, reductive amination and reduction, respectively) reacted with the aldehyde and underwent subsequent [3+2] cycloaddition to give bicyclic **14**. Although this method could ensure preliminary installation of the alkyl linker in the synthesis route, the [3+2] cycloaddition with hydroxylamine **13** was low yielding and therefore not further pursued. Instead, the synthesis was continued with bicyclic *N*-benzylated **12**.



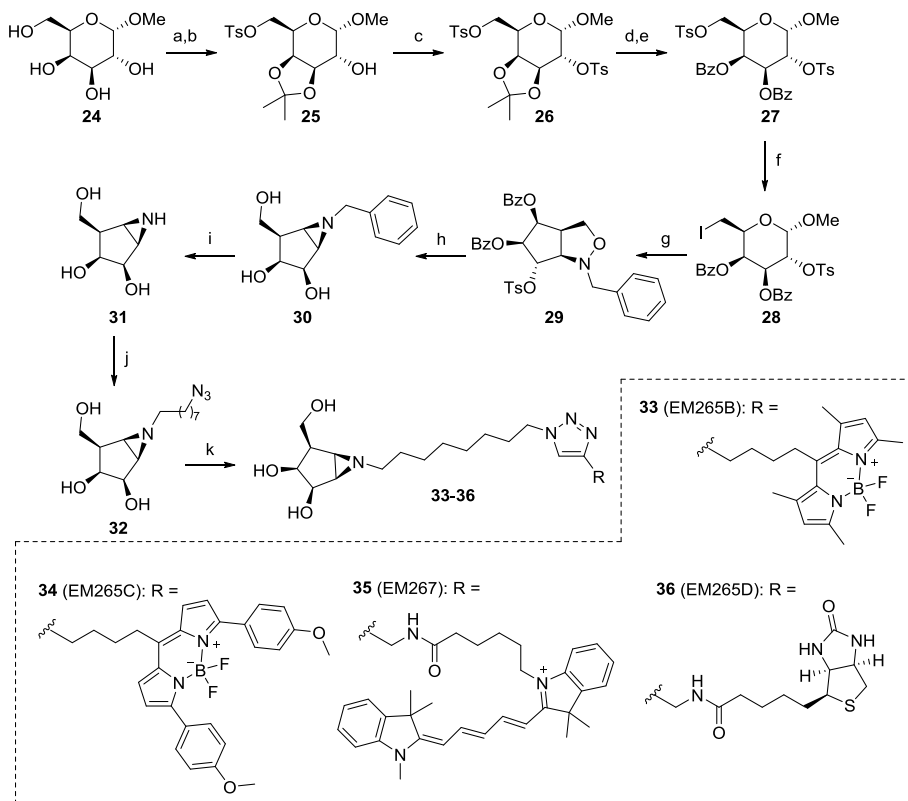
Scheme 3 Synthesis of the isoxazolidines. Reagents and conditions: a) Zn, EtOH, reflux, 2h, work-up; b) RNHOH.HCl, Na₂CO₃, toluene, 50 °C, 2h, for **12**: 61% over 2 steps; for **14**: 24% over 2 steps; c) BnBr, NaH, TBAI, THF, 16h; d) (COCl)₂, DMSO, DCM, -78 °C, 1h; e) NH₂OH.HCl, NaOH, EtOH, reflux, 1h; f) NaBH₃CN, HCl, MeOH, rt, 5h, 17% overall yield over 4 steps.

As reported by Bols et al.,⁷ reductive N-O bond cleavage can be achieved using Raney nickel under hydrogen pressure. However the reported yield was moderate (48%), possibly due to concomitant *N*-debenzylation. The use of Raney nickel under hydrogen atmosphere indeed resulted in a moderate yield (52%) after prolonged reaction times (72 h). Alternatively, zinc in acetic acid is reported to reductively cleave N-O bonds.¹⁴ While the reaction of isoxazolidine **12** with zinc in acetic acid gave no reaction at room temperature, heating the reaction to 55 °C resulted in the isolation of product **15** (50%) as well as byproduct **16** (25%, Scheme 4). Lowering the temperature to 35 °C and subsequent work-up followed by basification with Et₃N resulted in the isolation of



Scheme 4 Synthesis of *N*-substituted D-arabinofuranosyl aziridines. Reagents and conditions: a) Zn, AcOH, 35 °C, 2h, work-up, then Et₃N, MeOH, 97% **15**; b) Zn, AcOH, 35 °C, 2h, work-up, then NaOMe, MeOH, quant. **17**; c) Pd(OH)₂/C, H₂, H₂O, 1h, 45%; d) 1-azido-8-iodooctane, K₂CO₃, DMF, 80 °C, 16h, 40%; e) tag-alkyne, CuSO₄, sodium ascorbate, DMF/H₂O, rt, 16h, **20** 37%; **21** 57%; **22** 22%; **23** 40%.

15 in 96% yield. Furthermore, basification of the crude amine with sodium methoxide led to the cyclization to the aziridine followed by global debenzoylation, leading to *N*-benzyl cyclitol **17** in quantitative yield. The aziridine was debenzoylated using Pearlman's catalyst in water under hydrogen atmosphere. To avoid reductive opening of the aziridine ring the reaction times were minimized and aziridine **18** could be isolated in 45% yield. Finally, the aziridine was alkylated with 1-azido-8-iodooctane to afford *N*-octylazido aziridine **19** which was equipped with various reporter tags via a Huisgen azide/alkyne 1,3-dipolar cycloaddition¹⁵ to afford *N*-substituted D-arabinofuranosyl aziridines **20-23** after HPLC purification.



Scheme 5 Synthesis of D-lyxofuranosyl activity based probes. Reagents and conditions: a) TsCl, Et₃N, MeCN, rt, 16h; b) *p*-TsOH, 2,2-dimethoxypropane, DMF, 63% over 2 steps; c) TsCl, Et₃N, MeCN, 40 °C, 16h, 50%; d) HCl, MeOH, 16h, quant.; e) Bz₂O, DMAP, pyridine, rt, 16h, 70%; f) NaI, Ac₂O, reflux, 4h, 88%; g) Zn, EtOH, reflux, 6h, work-up, then BnNH₂·HCl, Na₂CO₃, toluene, 50 °C, 16h, 95%; h) Zn, AcOH, 40 °C, work-up, then NaOMe, MeOH, rt, 3h, quant.; i) Pd(OH)₂/C, H₂, H₂O, 30 min, 46%; j) 1-azido-8-iodooctane, K₂CO₃, DMF, 80 °C, 16h, 46%; k) tag-alkyne, CuSO₄, sodium ascorbate, DMF/H₂O, rt, 16h, **33** 35%; **34** 29%; **35** 24%; **36** 29%.

The synthesis of D-*lyxofuranosyl* aziridine follows a similar strategy (Scheme 5). Starting from commercially available methyl α -D-galactopyranoside **24**, the primary alcohol was tosylated and the resulting product was protected with a 3,4-isopropylidene acetal resulting in compound **25** in 63% over two steps. Tosylation of the remaining secondary alcohol required gentle heating and product **26** could be isolated in moderate yield. Quantitative deprotection of the isopropylidene acetal was achieved using hydrochloric acid in methanol, which after benzylation yielded product **27**. S_N2 displacement of the tosyl group by iodine afforded iodopyranoside **28**. Vasella fragmentation followed by work-up, nitron formation and *in situ* [3+2] dipolar cycloaddition resulted in isoxazolidine **29**. Next, reductive cleavage of the N-O bond and subsequent ring-closure proceeded smoothly, and the crude product was directly deprotected under Zemplén conditions affording *N*-benzyl aziridine **30** in excellent yield over two steps. Debenzylation using Pearlman's catalyst under hydrogen atmosphere afforded aziridine cyclitol **31**, which was alkylated yielding *N*-azidoalkyl aziridine **32**. Several reporter tags were attached to the linker moiety *via* a Huisgen azide/alkyne 1,3-dipolar cycloaddition to afford *N*-alkylated D-*lyxofuranosyl* aziridines **33-36**.

With fluorescent D-*arabino*- (**22**) and D-*lyxofuranosyl* (**35**) aziridines in hand, their ability to label various glycosidases in human c920 fibroblast lysates was evaluated at lysosomal (4.0) and cytosolic (7.0) pH (Figure 3A and 3B, respectively). Both aziridines **22** and **35** showed significant labeling of bands at 10 μ M; at higher concentrations background fluorescence was observed. At this concentration, fibroblast lysate was incubated with the aziridines at different pH's (Figure 3C and 3D). Several bands appeared following labeling at pH ranging from 4-9 indicating the high reactivity of the aziridine warhead over a wide pH-range, consistent with labeling patterns of analogous cyclitol aziridines.¹⁶ These initial results suggested that for both **22** and **35**, the optimal pH for labeling in fibroblast lysate was ~5.0. Both fluorescent aziridines displayed a similar labeling pattern in fibroblast lysate, with a strong band at ~42 kDa. Moreover, for both aziridines a band at the molecular weight of GBA1 (MW ~56 kDa, annotated with an asterisk) was visualized indicating a possible covalent interaction with this enzyme. Additionally, the unbiased labeling of glycosidases in WT mouse liver lysate (C57bl/6j, Jackson's laboratories) was probed with **21** and **33** (Figure 3E). Again, a band at ~42 kDa was labeled by **21**. However, while the presence of GBA1 could clearly be indicated by the GBA1 selective probe MDW933,¹⁷ the enzyme did not appear to be labeled with 10 μ M **21**. In contrast, while

labeling with **33** also showed a band at ~42 kDa, a strong band at ~56 kDa appeared as well, possibly caused by labeling of GBA1.

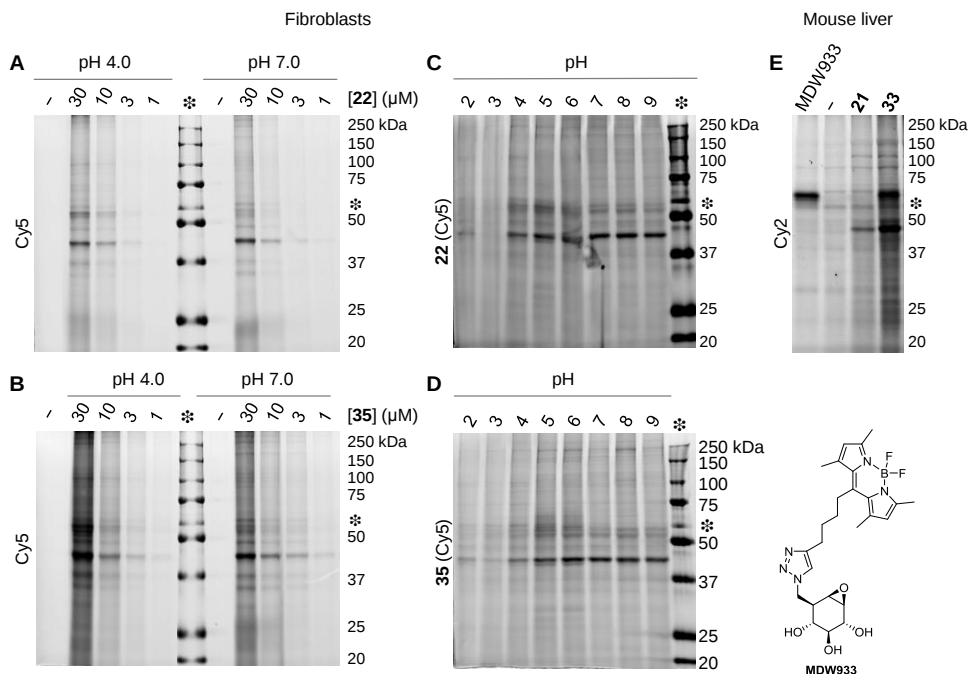


Figure 3 Biochemical evaluation of fluorescent D-arabino- (**21** and **22**) and D-lyxofuranosyl aziridines (**33** and **35**) in c920 fibroblast lysates; A) concentration range for D-arabino-aziridine **22** at cytosolic and lysosomal pH; B) concentration range for D-lyxofuranosyl aziridine **35** at cytosolic and lysosomal pH; C) pH range for D-arabino-aziridine **22**; D) pH range for D-lyxofuranosyl aziridine **35**; E) labeling of **21** and **33** in C57bl/6j mouse liver lysates; MDW933 is a GBA1 selective probe; (-) represents a control experiment without addition of probe; the band for GBA1 is annotated with an asterisk (*).

Next, competitive ABPP experiments were performed (Figure 4). Fibroblast lysates were pre-incubated with different concentrations (1, 10 or 100 μM) of fluorescent aziridines **21** or **33** (Figure 4A and 4B, respectively) and subsequently labeled with matching concentrations of biotin-aziridines (**23** or **36**, respectively). The biotin-labeled proteins were then visualized by a streptavidin-Cy5 blot and the bands were compared to the matching non-competed lanes. Unfortunately, no significant competition of the bands at ~42 kDa, ~56 kDa or other bands was observed for either **23** or **36** at any of the concentrations used. A similar competition experiment was executed in mouse liver lysates at pH 5.0 (Figure 4C). The samples were treated with

10 μ M biotin-aziridine (**23** or **36**, respectively) with or without competition of 10 μ M fluorescent aziridine (**21** or **33**, respectively). Again, no significant competition of biotin-labeled proteins could be observed, although detection might have been hampered by the high amount of endogenous biotinylated proteins present. Ultimately, streptavidin pull-down experiments were performed with **23** and **36** on various lysates (human fibroblast, mouse kidney, liver, duodenum and brain) and the labeled proteins were analyzed after tryptic digestion followed by LC-MS/MS proteomics. No glycosidase hits could be identified in any of the lysates, therefore further optimization of labeling conditions was abandoned.

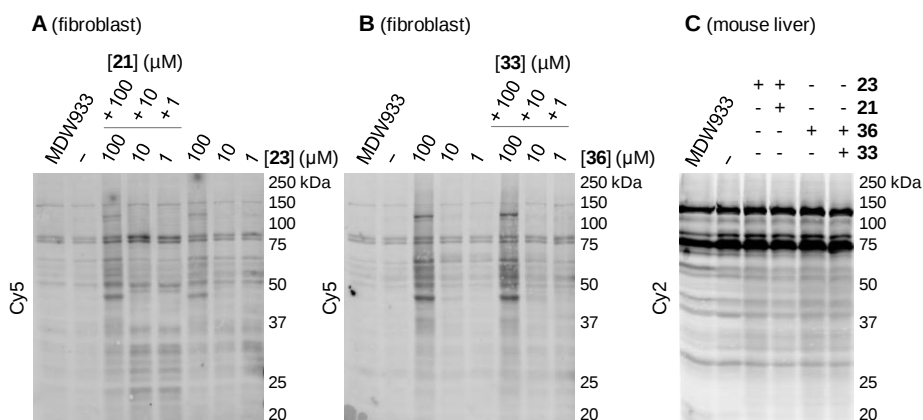


Figure 4 Competition experiments matching biotin and Cy2 tagged aziridines; A) competition of **23** with **21** in human c920 fibroblast lysates; B) competition of **36** with **33** in c920 fibroblast lysates; C) competition of **23** with **21** and **36** with **33** in mouse liver lysate.

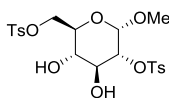
4.3 Conclusion

In the past, activity-based glycosidase profiling studies employed covalent pyranose-configured inhibitors to mimic the substrate of the specific enzyme of interest. In this Chapter, this method is expanded to furanosyl configured aziridines, with the aim to label glycosidases in an unbiased fashion. Fluorescent cyclitol aziridines with *D-arabino* (**20-23**) and *D-lyxo* (**33-36**) configurations were synthesized and their labeling efficiency at different concentrations and pH values was evaluated in human fibroblast- and mouse kidney lysates. While for both configurations different fluorescent bands could be observed in complex biological samples, competitive ABPP with the matching biotinylated aziridines did not demonstrate significant competition of these signals. Additionally, pull-down experiments were performed on various biological samples but no glycosidase hits could be identified. Ultimately, it was concluded that there were no enzymes present in human fibroblast and mouse kidney lysates which were capable of recognizing *D-arabino*- or *D-lyxo*furanose configured cyclitols as their substrates, although it would be of interest to study the viability of other furanose-based cyclitols (see future prospects, Chapter 8).

Experimental procedures

General: Chemicals were purchased from Acros, Sigma Aldrich, Biosolve, VWR, Fluka, Merck and Fisher Scientific and used as received unless stated otherwise. Tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF) and toluene were stored over molecular sieves before use. Traces of water from reagents were removed by co-evaporation with toluene in reactions that required anhydrous conditions. All reactions were performed under an argon atmosphere unless stated otherwise. TLC analysis was conducted using Merck aluminum sheets (Silica gel 60 F₂₅₄) with detection by UV absorption (254 nm), by spraying with a solution of (NH₄)₆Mo₇O₂₄·4H₂O (25 g/L) and (NH₄)₄Ce(SO₄)₄·2H₂O (10 g/L) in 10% sulfuric acid or a solution of KMnO₄ (20 g/L) and K₂CO₃ (10 g/L) in water, followed by charring at ~150 °C. Column chromatography was performed using Screening Device b.v. silica gel (particle size of 40 – 63 µm, pore diameter of 60 Å) with the indicated eluents. For reversed-phase HPLC purifications an Agilent Technologies 1200 series instrument equipped with a semi-preparative column (Gemini C18, 250 x 10 mm, 5 µm particle size, Phenomenex) was used. LC/MS analysis was performed on a Surveyor HPLC system (Thermo Finnigan) equipped with a C₁₈ column (Gemini, 4.6 mm x 50 mm, 5 µm particle size, Phenomenex), coupled to a LCQ Advantage Max (Thermo Finnigan) ion-trap spectrometer (ESI⁺). The applied buffers were H₂O, MeCN and 1% aqueous TFA. ¹H NMR and ¹³C NMR spectra were recorded on a Brüker AV-400 (400 and 101 MHz respectively) or a Brüker DMX-600 (600 and 151 MHz respectively) spectrometer in the given solvent. Chemical shifts are given in ppm (δ) relative to the residual solvent peak or tetramethylsilane (0 ppm) as internal standard. Coupling constants are given in Hz. High-resolution mass spectrometry (HRMS) analysis was performed with a LTQ Orbitrap mass spectrometer (Thermo Finnigan), equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL/min, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150 – 2000) and dioctyl phthalate (m/z = 391.28428) as a “lock mass”. The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

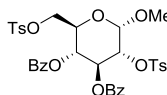
Compound 8



To a stirred solution of dry Et₃N (0.97 mL, 7 mmol) in dry MeCN (30 mL) was added methyl-α-D-glucopyranoside (485 mg, 2.5 mmol), Bu₂SnCl₂ (304 mg, 1.0 mmol) and tosyl chloride (1.14 g, 6.0 mmol) at rt under argon atmosphere and stirred for 24 hours. The mixture was quenched with water and extracted with EtOAc (300 mL). The extract was washed successively with water, aqueous sodium hydrogen carbonate and again with water, followed by drying over MgSO₄ and evaporation. Purification of the crude product by column chromatography (pentane/EtOAc, 1:1) yielded product **8** (1.22 g, 97%). ¹H-NMR (400 MHz, CDCl₃) δ 7.80 (m, 4H), 7.36 (dd, J = 7.8, 6.6 Hz, 4H), 4.64 (d, J = 3.7 Hz, 1H), 4.29 (dd, J = 11.1, 4.6 Hz, 1H), 4.25 – 4.17 (m, 2H), 3.91 (t, J = 9.3 Hz, 1H), 3.72 (ddd, J = 10.0, 4.5, 2.0 Hz, 1H), 3.47 (t, J = 9.4 Hz, 1H), 3.25 (s, 3H), 2.78 (s, 10H), 2.63 (s, OH), 2.46 (s, 6H). ¹³C-NMR (101 MHz, CDCl₃) δ 145.4, 130.0, 129.9, 128.1, 127.9, 97.3, 78.8, 77.3, 77.0, 76.7, 71.0, 69.7, 68.7, 68.3, 55.6, 29.7, 21.7. IR (neat, cm⁻¹): ν 3510, 2929,

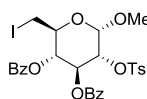
1597, 1355, 1172, 1033, 970, 929, 810, 665. $[\alpha]_D^{20}$ (c 1.0, CH_2Cl_2): +47. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{21}\text{H}_{26}\text{O}_{10}\text{S}_2$ 503.10538 found 503.10437.

Compound 9



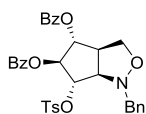
Compound **8** (5.7 g, 11.4 mmol) was dissolved in dry pyridine (300 mL). Benzoyl anhydride (10.3 g, 45 mmol) and DMAP (0.28 g, 3.3 mmol) were added and stirred 16 h at rt. The mixture was quenched with sat. aq. NaHCO_3 and extracted with EtOAc. The extract was washed successively with water, sat. aq. NaHCO_3 and water, dried over MgSO_4 and evaporated. Purification of the crude product by column chromatography (pentane/EtOAc, 2:1) yielded product **9** (7.3 g, 10.3 mmol, 90%). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.79 – 7.67 (m, 2H), 7.64 – 7.54 (m, 2H), 7.48 (td, J = 7.4, 1.3 Hz, 1H), 7.36 – 7.24 (m, 2H), 7.20 (d, J = 8.0 Hz, 1H), 6.93 (d, J = 8.0 Hz, 1H), 5.82 (t, J = 9.7 Hz, 0H), 5.02 (d, J = 3.6 Hz, 0H), 4.53 (dd, J = 10.0, 3.6 Hz, 1H), 4.23 – 4.13 (m, 1H), 4.06 (dd, J = 11.5, 5.9 Hz, 1H), 3.46 (s, 1H), 2.33 (s, 1H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.0, 149.0, 145.1, 145.0, 137.0, 133.7, 133.2, 133.1, 132.7, 132.3, 130.1, 130.0, 129.9, 129.9, 129.8, 129.8, 128.8, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.7, 97.8, 76.4, 69.5, 68.8, 67.8, 67.4, 56.3, 21.7. IR (neat, cm^{-1}): ν 2943, 1720, 1357, 1273, 1174, 1093, 1066, 1020, 975, 812, 657. $[\alpha]_D^{20}$ (c 0.6, CH_2Cl_2): +15. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{35}\text{H}_{34}\text{O}_{12}\text{S}_2$ 711.15765, found 711.15680.

Compound 10



Sodium iodide (5.0 g, 34 mmol) was added to a mixture of **9** (19.9 g, 28 mmol) in acetic anhydride (50 mL) and refluxed for 4 hours. After cooling to rt, the mixture was filtered over celite, diluted with dichloromethane (200 mL), washed with aqueous sodium thiosulphate, water, dried over MgSO_4 , filtered, and concentrated. The solid residue was recrystallized from methanol to yield compound **10** (18.6 g, 28 mmol, quant.) as a white solid. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.86 (d, J = 7.9 Hz, 2H), 7.62 (dd, J = 17.9, 8.1 Hz, 4H), 7.49 (q, J = 7.2 Hz, 2H), 7.34 (t, J = 7.7 Hz, 2H), 7.29 (d, J = 7.7 Hz, 2H), 6.96 (d, J = 8.2 Hz, 2H), 5.89 (t, J = 9.7 Hz, 1H), 5.20 (t, J = 9.6 Hz, 1H), 5.09 (d, J = 3.6 Hz, 1H), 4.60 (dd, J = 10.0, 3.6 Hz, 1H), 4.05 – 3.95 (m, 1H), 3.57 (s, 3H), 3.34 (dd, J = 11.0, 2.5 Hz, 1H), 3.19 (dd, J = 11.0, 8.5 Hz, 1H), 2.21 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.4, 165.0, 145.0, 133.8, 133.2, 130.3, 130.0, 129.9, 129.8, 128.6, 128.5, 128.2, 127.8, 97.9, 76.8, 72.7, 69.2, 69.1, 56.4, 3.6. IR (neat, cm^{-1}): ν 1681, 1452, 1323, 1178, 931, 802, 667, 634, 617. $[\alpha]_D^{20}$ (c 0.7, CH_2Cl_2): +33. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{28}\text{H}_{27}\text{IO}_9\text{S}$ 689.03183, found 689.03107.

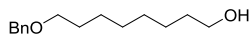
Compound 12



To a suspension of **10** (1.0 g, 1.50 mmol) in EtOH (20 mL) was added Zn dust (1.0 g, 15.30 mmol). The mixture was refluxed for 2 h and then filtered through Celite and concentrated to give a yellow oil that was dissolved in CH_2Cl_2 (20 mL), washed with water (2 $\times$ 10 mL), dried over MgSO_4 , filtered and concentrated to give unstable aldehyde **11** as an oil. To a solution of the aldehyde in toluene (8 mL) was added *N*-benzylhydroxylamine hydrochloride (359 mg, 2.25 mmol) and Na_2CO_3 (225 mg, 2.25 mmol), and the

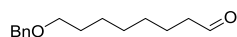
reaction was stirred at 50 °C for 2 h. The mixture was then concentrated and the residue was dissolved in CH₂Cl₂ (15 mL), washed with water (2 × 10 mL), dried over MgSO₄, filtered and concentrated. After crystallization from methanol, product **12** was obtained (0.56 g, 0.92 mmol, 61%) as a white solid. ¹H-NMR (400 MHz, CDCl₃) δ 8.00 – 7.93 (m, 2H), 7.88 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.55 (t, *J* = 7.4 Hz, 2H), 7.41 (td, *J* = 7.6, 5.5 Hz, 4H), 7.36 – 7.22 (m, 6H), 7.07 (d, *J* = 8.1 Hz, 2H), 5.91 (t, *J* = 8.2 Hz, 1H), 5.18 (dd, *J* = 8.1, 6.3 Hz, 1H), 5.09 (dd, *J* = 8.2, 5.6 Hz, 1H), 4.28 – 4.17 (m, 2H), 3.95 (d, *J* = 13.5 Hz, 1H), 3.91 – 3.80 (m, 2H), 3.25 (dtd, *J* = 10.0, 6.5, 3.7 Hz, 1H), 2.20 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 166.2, 165.0, 145.0, 136.7, 133.7, 133.5, 133.4, 130.1, 130.0, 129.9, 129.1, 128.9, 128.7, 128.5, 128.4, 128.0, 127.7, 84.1, 79.1, 77.4, 70.8, 70.1, 59.4, 50.2, 21.7. IR (neat, cm⁻¹): ν 3061, 2879, 1714, 1598, 1355, 1261, 1180, 1109, 1093, 1068, 962, 844, 688. [α]_D²⁰ (c 0.8, CH₂Cl₂): +15. HRMS (ESI) *m/z*: [M+H]⁺ calc for C₃₄H₃₁NO₈S 614.20931, found 614.18433.

8-(benzyloxy)octan-1-ol



To a stirred suspension of NaH (3.2 g, 60 wt%, 141 mmol) in dry THF (150 mL) was added a solution of octane-1,8-diol (8.9 g, 61 mmol) in dry THF (100 mL) at 0 °C, and stirring was continued for 30 min. Then, BnBr (6.9 mL, 58 mmol) in dry THF (20 mL) was added slowly. The mixture was warmed to rt, then slowly heated with a heatgun to 50 °C and then cooled to rt. TBAI (11.1 g, 30 mmol) was added and the stirring was continued for 16 h. The reaction was cooled to 0 °C and slowly quenched with sat. NH₄Cl followed by extraction with EtOAc (3×150 mL). The organic extracts were combined and washed with H₂O and brine, and then dried over MgSO₄. The solvent was evaporated, followed by purification of the crude product by column chromatography (pentane/EtOAc, 5:1), to afford the product (7.51 g, 32 mmol, 52%). ¹H-NMR (400 MHz, CDCl₃) δ 7.39 – 7.12 (m, 5H), 4.50 (s, 2H), 3.63 (t, *J* = 6.6 Hz, 2H), 3.46 (t, *J* = 6.6 Hz, 2H), 1.65 – 1.50 (m, 4H), 1.42 – 1.20 (m, 8H). ¹³C-NMR (101 MHz, CDCl₃) δ 138.8, 128.5, 127.8, 127.6, 73.0, 70.6, 63.2, 32.9, 29.9, 29.6, 29.5, 26.3, 25.8. IR (neat, cm⁻¹): ν 3360, 2927, 2854, 1454, 1361, 1097, 731, 694. HRMS (ESI) *m/z*: [M+H]⁺ calc for C₁₅H₂₄O₂ 237.18645, found 237.18486.

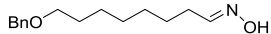
8-(benzyloxy)octanal



To a stirred solution of (COCl)₂ (0.51 mL, 6 mmol) in dry CH₂Cl₂ (3.6 mL) was added a pre-mixed solution of dry DMSO (0.85 mL, 12 mmol) and dry CH₂Cl₂ (5.4 mL) at -78 °C. After 30 min, a solution of 8-(benzyloxy)octan-1-ol (0.717 g, 3 mmol) in CH₂Cl₂ (3.6 mL) was added slowly at the same temperature. The resulting mixture was stirred for 1 h at -78 °C, and then Et₃N (2.11 mL, 24 mmol) was added. The mixture was warmed to rt, and stirring was continued for 1 h. A saturated solution of NH₄Cl (2 mL) was added slowly to quench the reaction followed by addition of brine, and then the mixture was extracted with CH₂Cl₂. The combined organic extracts were washed with brine, dried over MgSO₄, filtered and concentrated. The resulting aldehyde was purified by column chromatography (pentane/EtOAc 4:1), to afford the product (0.589 g, 2.5 mmol, 84%). ¹H-NMR (400 MHz, CDCl₃) δ 9.75 (t, *J* = 1.8 Hz, 1H), 7.31 (dd, *J* = 19.3, 15.5 Hz, 8H), 4.49 (s, 3H), 3.45 (t, *J* = 6.6 Hz, 3H), 2.41 (td, *J* = 7.4, 1.8 Hz, 3H), 1.66 – 1.52 (m, *J* = 12.8, 6.9 Hz, 6H), 1.40 –

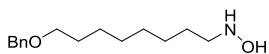
1.27 (m, $J = 6.0, 3.4$ Hz, 9H). ^{13}C -NMR (101 MHz, CDCl_3) δ 203.0, 138.8, 128.5, 127.8, 127.6, 73.0, 70.5, 44.0, 29.8, 29.3, 29.2, 26.1, 22.2. IR (neat, cm^{-1}): ν 2929, 2854, 1722, 1454, 1361, 1097, 1028, 731, 696. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{15}\text{H}_{22}\text{O}_2$ 235.17080 found 235.16914.

8-(benzyloxy)octanal oxime



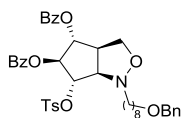
To a solution of the aldehyde (0.354 g, 1.5 mmol) and hydroxylamine hydrochloride (0.37 g, 5.3 mmol) in EtOH (6 mL) were added NaOH pellets (0.54 g, 13.5 mmol) in small portions. The mixture was allowed to stir at rt for 1 h and then refluxed for another 1 h. The reaction mixture was then cooled to rt and 1M HCl was added until pH $\approx$ 7 and the solution became clear. The mixture was extracted with CH_2Cl_2 (4x10 mL), washed with brine, dried over MgSO_4 and concentrated to afford the product (0.373 g, 1.5 mmol, quant.). ^1H -NMR (400 MHz, CDCl_3) δ 7.39 – 7.26 (m, 10H), 4.50 (s, 4H), 3.46 (dd, $J = 7.4, 5.8$ Hz, 4H), 2.18 (dt, 2H), 1.66 – 1.55 (m, $J = 10.4, 4.0$ Hz, 5H), 1.51 – 1.39 (m, $J = 7.4$ Hz, 4H), 1.40 – 1.26 (m, $J = 9.4, 8.9, 4.4$ Hz, 13H). ^{13}C -NMR (101 MHz, CDCl_3) δ 128.5, 127.8, 127.6, 73.0, 70.5, 29.8, 29.6, 29.4, 29.3, 29.2, 26.6, 26.2, 25.0. IR (neat, cm^{-1}): ν 3250, 2927, 2854, 1454, 1361, 1095, 1028, 923, 904, 732, 696. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{15}\text{H}_{25}\text{NO}_2$ 250.18176, found 250.18009.

Compound 13



The oxime (0.317 g, 1.27 mmol) was dissolved in MeOH (4 mL) and cooled to 0 $^\circ\text{C}$. NaBH_3CN (0.168 g, 2.67 mmol) was added and 12 M HCl (2.1 mL, 2.54 mmol) was added drop wise. After addition the reaction mixture was allowed to stir at rt for 5 h before adding 1M NaOH until pH $\approx$ 9. The reaction mixture was concentrated and the product was extracted with CH_2Cl_2 (4 x 10ml), washed with brine, dried over MgSO_4 , filtered, and concentrated. The crude product was recrystallized from pentane/EtOAc (4:1) to afford product **13** (0.12 g, 0.5 mmol, 40%). ^1H -NMR (400 MHz, CDCl_3) δ 7.44 – 7.27 (m, 5H), 4.50 (s, 2H), 3.46 (t, $J = 6.6$ Hz, 2H), 2.96 – 2.89 (m, 2H), 1.68 – 1.56 (m, 2H), 1.56 – 1.42 (m, 2H), 1.42 – 1.25 (m, 8H). ^{13}C -NMR (101 MHz, CDCl_3) δ 128.5, 127.8, 127.6, 73.0, 70.6, 54.2, 29.9, 29.6, 29.5, 27.2, 26.3. IR (neat, cm^{-1}): ν 3255, 2922, 2484, 1359, 1105, 1060, 740, 694. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{15}\text{H}_{25}\text{NO}_2$ 252.19741 found 252.19576.

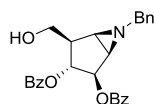
Compound 14



To a suspension of compound **10** (0.5 g, 0.75 mmol) in aqueous EtOH (10 mL) was added Zn dust (0.5 g, 8.05 mmol). The mixture was refluxed for 2 h and then filtered through Celite and concentrated to give a yellow oil that was dissolved in CH_2Cl_2 (20 mL), washed with water (2 $\times$ 10 mL), dried over MgSO_4 , filtered and concentrated to give aldehyde **11** as an oil. To a solution of the aldehyde in toluene (8 mL) was added **13** (420 mg, 1.5 mmol) and Na_2CO_3 (150 mg, 1.5 mmol), and the reaction was heated at 50 $^\circ\text{C}$ for 2 h. The mixture was then concentrated, and the residue was dissolved in CH_2Cl_2 (15 mL), washed with water (2 $\times$ 10 mL), dried over MgSO_4 , filtered and concentrated. The product was

crystallized from methanol to yield compound **14** (220 mg, 0.18 mmol, 24%). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.01 – 7.94 (m, 2H), 7.92 – 7.86 (m, 2H), 7.74 (d, J = 8.3 Hz, 2H), 7.58 – 7.50 (m, 2H), 7.44 – 7.36 (m, 4H), 7.34 (d, J = 4.4 Hz, 5H), 7.10 (d, J = 8.0 Hz, 2H), 5.88 (t, J = 8.2 Hz, 1H), 5.15 (dd, J = 8.0, 6.3 Hz, 1H), 5.01 (dd, J = 8.1, 5.7 Hz, 1H), 4.51 (s, 2H), 4.20 (dd, J = 9.4, 2.8 Hz, 1H), 4.07 (dd, J = 9.4, 7.3 Hz, 1H), 3.71 (dd, J = 9.6, 5.5 Hz, 1H), 3.47 (t, J = 6.6 Hz, 2H), 3.22 – 3.11 (m, 1H), 2.75 – 2.65 (m, 1H), 2.55 – 2.44 (m, 1H), 1.69 – 1.54 (m, 2H), 1.54 – 1.42 (m, 2H), 1.42 – 1.25 (m, 8H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 144.9, 133.6, 133.5, 130.1, 130.0, 129.8, 129.2, 128.7, 128.5, 128.4, 128.1, 127.8, 127.6, 79.2, 77.4, 73.0, 70.7, 50.3, 29.9, 29.6, 27.9, 27.2, 26.3. IR (neat, cm^{-1}): ν 2916, 2852, 1726, 1705, 1597, 1450, 1267, 1178, 1111, 970, 846, 813, 705, 665. $[\alpha]_{\text{D}}^{20}$ (c 0.2, CH_2Cl_2): -21. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{42}\text{H}_{47}\text{NO}_9$ 742.30617, found 742.30479.

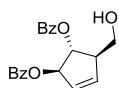
Compound 15



Compound **12** (2.0 g, 0.50 mmol) was dissolved in acetic acid (40 mL). Zinc powder (5 g, 20 mmol) was added and the mixture was stirred in a preheated oil bath at 35°C . After 2 hours the mixture was filtered over Celite and rinsed with methanol.

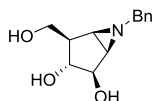
The solvents were evaporated and the solid residue was redissolved in methanol followed by addition of Et_3N until $\text{pH} \approx 7$, and the reaction was stirred overnight. The mixture was then concentrated, and the residue was dissolved in CH_2Cl_2 (15 mL), washed with water (2×10 mL), dried over MgSO_4 , filtered and concentrated. After recrystallization from methanol compound **15** (0.21 g, 0.49 mmol, 97%) was obtained. $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.10 – 8.03 (m, 3H), 7.57 – 7.52 (m, 2H), 7.47 – 7.36 (m, 7H), 7.16 – 7.06 (m, 3H), 5.59 (dd, J = 6.1, 3.1 Hz, 1H), 5.45 (dd, J = 7.2, 6.2 Hz, 1H), 3.98 (dd, J = 11.1, 5.4 Hz, 1H), 3.93 – 3.84 (m, 1H), 3.60 (d, J = 13.7 Hz, 1H), 3.25 (d, J = 13.7 Hz, 1H), 2.66 (dd, J = 4.9, 3.1 Hz, 1H), 2.50 (dd, J = 4.9, 3.0 Hz, 1H), 2.48 – 2.39 (m, 1H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 166.7, 166.5, 138.6, 136.0, 133.5, 133.4, 133.3, 133.2, 130.00, 129.9, 129.8, 129.7, 129.6, 129.5, 128.6, 128.5, 128.4, 128.4, 128.3, 127.6, 127.2, 84.1, 81.8, 80.0, 76.8, 64.3, 62.5, 61.3, 55.1, 46.4, 43.1, 42.1. IR (neat, cm^{-1}): ν 3514, 2922, 1724, 1701, 1450, 1261, 1099, 1070, 1026, 705, 686. $[\alpha]_{\text{D}}^{20}$ (c 0.5, CH_2Cl_2): -87. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{27}\text{H}_{25}\text{NO}_5$ 444.18098, found 444.18027.

Compound 16



Byproduct obtained when the reaction of **12** with zinc in acetic acid was performed at 55°C , yield 25%. $^1\text{H NMR}$ (400 MHz, CDCl_3) δ 8.13 – 7.95 (m, 5H), 7.65 – 7.51 (m, 2H), 7.50 – 7.42 (m, 3H), 6.17 (s, 1H), 6.08 – 5.97 (m, 2H), 5.49 (t, J = 2.8 Hz, 1H), 3.90 (dd, J = 11.1, 4.4 Hz, 1H), 3.78 (dd, J = 11.1, 7.4 Hz, 1H), 3.06 – 2.96 (m, 1H). TLC-MS m/z : $[\text{M}+\text{Na}]^+$ calc for $\text{C}_{20}\text{H}_{18}\text{O}_5\text{Na}$ 361.1, found 361.1. These data are in accordance with literature reports.¹⁸

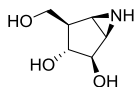
Compound 17



Compound **12** (80 mg, 0.13 mmol) was dissolved in acetic acid (10 mL). Zinc powder (1.2 g, 5 mmol) was added and the mixture was transferred to a preheated oil bath at 35°C . After 2 hours the mixture was filtered over Celite and

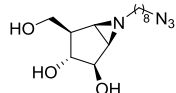
rinsed with methanol. The solvents were evaporated and co-evaporated with toluene. The solid residue was dissolved in methanol (10 mL) and methanolic sodium methoxide was added until pH $\approx$ 10 and stirred for 3 h. Subsequently, acetic acid was added until pH $\approx$ 7 and the solvent was evaporated followed by purification of the crude product by column chromatography (DCM/MeOH, 9:1) to afford compound **17** (30 mg, 0.13 mmol, quant.) as a white solid. $^1\text{H-NMR}$ (400 MHz, D_2O) δ 7.43 – 7.26 (m, 5H), 4.09 (dd, J = 6.5, 2.8 Hz, 1H), 3.71 (dd, J = 10.7, 4.9 Hz, 1H), 3.66 – 3.52 (m, 1H), 3.47 (d, J = 13.8 Hz, 1H), 3.40 – 3.30 (m, 1H), 3.25 (dd, J = 8.2, 6.5 Hz, 1H), 2.60 – 2.48 (m, 2H), 2.08 (dddt, J = 9.5, 7.7, 4.9, 2.5 Hz, 1H). $^{13}\text{C-NMR}$ (101 MHz, D_2O) δ 138.3, 128.6, 128.0, 127.5, 78.2, 75.6, 61.0, 60.5, 46.7, 45.0, 42.4. IR (neat, cm^{-1}): ν 2887, 1724, 1450, 1365, 1274, 1259, 1174, 1093, 995, 844, 812, 659. $[\alpha]_{\text{D}}^{20}$ (c 0.8, H_2O): +39. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{13}\text{H}_{17}\text{NO}_3$ 236.12940, found 236.12816.

Compound 18

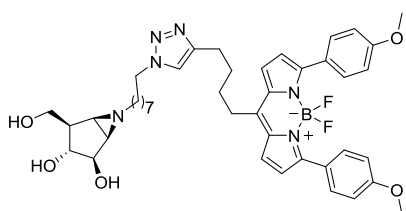


To a solution of **17** (290 mg, 1.23 mmol) in water (24 mL) under argon was added $\text{Pd}(\text{OH})_2/\text{C}$ (10%wt, 280 mg, 2 mmol) and the mixture was purged with H_2 gas with a balloon. After vigorously stirring for 1 h, the mixture was filtered through Celite and concentrated followed by purification of the crude product by column chromatography (DCM/MeOH, 9:1), to afford product **18** (80 mg, 0.55 mmol, 45%) as a white solid. $^1\text{H-NMR}$ (400 MHz, D_2O) δ 4.04 (d, J = 6.1 Hz, 1H), 3.73 (dd, J = 10.9, 4.8 Hz, 1H), 3.67 – 3.56 (m, 1H), 3.16 (dd, J = 7.2 Hz, 1H), 2.66 (s, 2H), 2.08 – 1.96 (m, J = 4.6 Hz, 1H). $^{13}\text{C-NMR}$ (101 MHz, D_2O) δ 78.0, 74.7, 60.8, 48.8, 46.2, 44.1, 35.6, 32.6. IR (neat, cm^{-1}): ν 3387, 3197, 2947, 2509, 2385, 1382, 1091, 1020, 877, 678. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_6\text{H}_{11}\text{NO}_3$ 146.08199, found 146.08104.

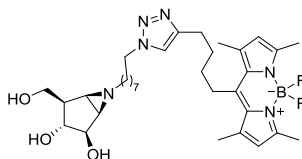
Compound 19



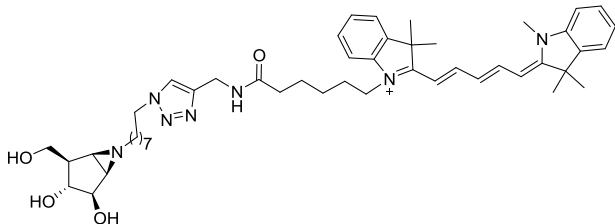
Aziridine **18** (82 mg, 0.57 mmol) was dissolved in dry DMF (2.5 mL). 1-azido-8-iodooctane (0.32 g, 1.13 mmol) and potassium carbonate (0.23 g, 1.7 mmol) were added and the mixture was heated to 80°C and stirred overnight. The mixture was then concentrated and the product was purified by column chromatography on neutralized silica gel (DCM/MeOH, 10:1). After freeze drying product **19** was obtained (69 mg, 0.23 mmol, 40%) as a fluffy powder. $^1\text{H-NMR}$ (400 MHz, MeOD) δ 3.92 (dd, J = 6.3, 3.0 Hz, 1H), 3.79 (dd, J = 10.2, 4.4 Hz, 1H), 3.68 (t, J = 10.0 Hz, 1H), 3.30 (t, J = 6.8 Hz, 2H), 3.20 (dd, J = 8.0, 6.3 Hz, 1H), 2.32 (dt, J = 11.6, 7.5 Hz, 1H), 2.18 (dd, J = 5.2, 2.7 Hz, 1H), 2.14 (dd, J = 5.1, 3.0 Hz, 1H), 2.02 (dt, J = 11.7, 7.3 Hz, 1H), 1.98 – 1.90 (m, 1H), 1.65 – 1.51 (m, 4H), 1.42 – 1.33 (m, 8H). $^{13}\text{C-NMR}$ (101 MHz, MeOD) δ 80.2, 77.3, 62.6, 59.6, 52.4, 49.1, 46.2, 43.0, 30.5, 30.5, 30.2, 29.9, 28.2, 27.7. IR (neat, cm^{-1}): ν 3344, 2929, 2856, 2090, 1728, 1359, 1271, 1174, 1091, 1020, 812, 705, 659. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{14}\text{H}_{26}\text{N}_4\text{O}_3$ 299.20940, found 299.20779.

Compound 20 (EM234D)

Compound **19** (5.1 mg, 17 μ mol) was dissolved in DMF (0.6 mL). BODIPY-red-alkyne (16.2 mg, 34 mmol), CuSO₄ (33 μ L of 100 mM solution in H₂O) and sodium ascorbate (50 μ L of 100 mM solution in H₂O) were added and the solution was stirred overnight at room temperature. The solvent was evaporated and the product was purified by semi-preparative reversed phase HPLC (linear gradient: 49%→55%, solutions used: A: 50 mM NH₄HCO₃ in H₂O, B: acetonitrile). Freeze drying yielded product **20** as a dark blue powder (4.9 mg, 6.3 μ mol, 37%). ¹H-NMR (600 MHz, MeOD) δ 7.84 (d, J = 8.9 Hz, 3H), 7.69 (s, 1H), 7.43 (d, J = 4.3 Hz, 2H), 6.97 (d, J = 8.9 Hz, 3H), 6.69 (d, J = 4.3 Hz, 2H), 4.32 (t, J = 7.0 Hz, 2H), 3.87 (dd, J = 6.2, 3.0 Hz, 1H), 3.85 (s, 5H), 3.75 (dd, J = 10.2, 4.4 Hz, 1H), 3.64 (t, J = 10.0 Hz, 1H), 3.16 (dd, J = 7.9, 6.3 Hz, 1H), 3.06 (t, J = 7.2 Hz, 2H), 2.78 (t, J = 6.8 Hz, 2H), 2.22 (dt, J = 11.6, 7.4 Hz, 1H), 2.10 (dd, J = 5.1, 2.8 Hz, 1H), 2.06 (dd, J = 5.1, 3.1 Hz, 1H), 1.96 – 1.77 (m, 9H), 1.54 – 1.41 (m, 2H), 1.33 – 1.15 (m, 9H). ¹³C-NMR (151 MHz, MeOD) δ 162.2, 158.8, 148.6, 146.8, 137.5, 132.2, 132.1, 132.1, 128.4, 126.5, 123.3, 121.0, 114.6, 80.2, 77.4, 62.6, 59.6, 55.8, 51.2, 49.8, 49.6, 49.4, 49.3, 49.1, 49.0, 48.9, 48.7, 48.6, 46.2, 43.0, 34.1, 31.2, 31.1, 31.0, 30.5, 30.4, 30.3, 29.9, 28.1, 27.3, 25.8. HRMS (ESI) m/z : [M+H]⁺ calc for C₃₃H₄₉BF₂N₆O₃ 783.42093, found 783.42211.

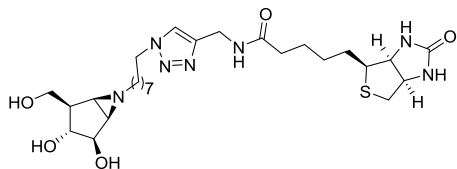
Compound 21(EM234B)

Compound **19** (5.1 mg, 17 μ mol) was dissolved in DMF (0.6 mL). BODIPY-green-alkyne (11 mg, 34 mmol), CuSO₄ (33 μ L of 100 mM solution in H₂O) and sodium ascorbate (50 μ L of 100 mM solution in H₂O) were added and the solution was stirred overnight at room temperature. The solvent was evaporated and the product was purified by semi-preparative reversed phase HPLC (linear gradient: 42%→48%, solutions used: A: 50 mM NH₄HCO₃ in H₂O, B: acetonitrile). Freeze drying yielded compound **21** as a yellow powder (6.0 mg, 9.6 μ mol, 57%). ¹H-NMR (600 MHz, MeOD) δ 7.74 (s, 1H), 6.12 (s, 2H), 4.35 (t, J = 7.0 Hz, 2H), 3.88 (dd, J = 6.3, 3.0 Hz, 1H), 3.76 (dd, J = 10.2, 4.4 Hz, 1H), 3.65 (t, J = 10.0 Hz, 1H), 3.17 (dd, J = 8.0, 6.3 Hz, 1H), 3.06 – 2.97 (m, 2H), 2.79 (t, J = 7.2 Hz, 2H), 2.44 (s, 7H), 2.39 (s, 7H), 2.25 (dt, J = 11.6, 7.4 Hz, 1H), 2.13 (dd, J = 5.1, 2.8 Hz, 1H), 2.08 (dd, J = 5.1, 3.1 Hz, 1H), 1.98 – 1.83 (m, 7H), 1.69 – 1.61 (m, 2H), 1.56 – 1.47 (m, 2H), 1.36 – 1.22 (m, 11H). ¹³C-NMR (151 MHz, MeOD) δ 154.9, 148.5, 147.9, 142.2, 132.6, 131.0, 123.4, 122.6, 80.2, 77.4, 62.7, 59.7, 51.2, 49.6, 46.2, 43.0, 32.3, 31.2, 31.1, 30.8, 30.5, 30.4, 29.9, 29.1, 28.1, 27.3, 25.9, 16.5 14.4. HRMS (ESI) m/z : [M+H]⁺ calc for C₃₃H₄₉BF₂N₆O₃ 627.40062, found 627.40088.

Compound 22 (EM234A)

Compound **19** (5.1 mg, 17 μ mol) was dissolved in DMF (0.6 mL). Cy5-alkyne (18.6 mg, 34 mmol), CuSO₄ (33 μ L of 100 mM solution in H₂O) and sodium ascorbate (50 μ L of 100 mM solution in H₂O) were added and the solution was

stirred overnight at room temperature. The solvent was evaporated and the product was purified by semi-preparative reversed phase HPLC (linear gradient: 45%→51%, solutions used: A: 50 mM NH₄HCO₃ in H₂O, B: acetonitrile). Freezedrying yielded product **22** as a blue powder (3.2 mg, 3.7 μ mol, 22%). ¹H-NMR (600 MHz, MeOD) δ 8.30 – 8.17 (m, 3H), 7.84 (s, 1H), 7.50 (d, J = 7.3 Hz, 2H), 7.46 – 7.37 (m, 2H), 7.33 – 7.21 (m, 5H), 6.62 (t, J = 12.4 Hz, 1H), 6.28 (dd, J = 13.7, 2.2 Hz, 3H), 4.40 (s, 2H), 4.36 (t, J = 7.1 Hz, 2H), 4.09 (t, J = 7.5 Hz, 2H), 3.88 (dd, J = 6.3, 3.0 Hz, 1H), 3.76 (dd, J = 10.2, 4.4 Hz, 1H), 3.65 (d, J = 9.9 Hz, 1H), 3.63 (s, 3H), 3.16 (dd, J = 8.0, 6.3 Hz, 1H), 2.29 – 2.21 (m, 3H), 2.13 (dd, J = 5.1, 2.8 Hz, 1H), 2.09 (dd, J = 5.1, 3.1 Hz, 1H), 1.96 (dt, J = 11.7, 7.1 Hz, 1H), 1.93 – 1.85 (m, 8H), 1.82 (dt, J = 15.3, 7.9 Hz, 2H), 1.73 (s, 12H), 1.56 – 1.43 (m, 4H), 1.38 – 1.25 (m, 8H). ¹³C-NMR (151 MHz, MeOD) δ 175.8, 175.4, 174.6, 155.6, 155.5, 146.1, 144.2, 143.5, 142.6, 142.5, 129.8, 129.8, 126.6, 126.3, 126.2, 124.2, 123.4, 123.3, 112.0, 111.9, 104.4, 104.2, 80.2, 77.4, 62.7, 59.7, 56.1, 51.3, 50.5, 50.5, 49.9, 49.6, 46.2, 44.7, 43.0, 36.5, 35.6, 31.5, 31.3, 31.1, 30.5, 30.4, 29.9, 28.2, 28.1, 27.9, 27.8, 27.4, 27.3, 26.4. HRMS (ESI) m/z : [M]⁺ calc for C₄₉H₆₈N₇O₄ 818.5327, found 818.55349.

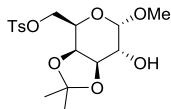
Compound 23 (EM204)

Compound **19** (6.2 mg, 21 μ mol) was dissolved in DMF (0.9 mL). Biotin-alkyne (6.5 mg, 23 mmol), CuSO₄ (17 μ L of 100 mM solution in H₂O) and sodium ascorbate (18 μ L of 100 mM solution in H₂O) were added and the solution was stirred overnight at room temperature. The solvent was

evaporated and the product was purified by semi-preparative reversed phase HPLC (linear gradient: 19%→25%, solutions used: A: 50 mM NH₄HCO₃ in H₂O, B: acetonitrile). Freeze drying yielded compound **23** as a white powder (4.8 mg, 3.7 μ mol, 40%). ¹H-NMR (600 MHz, MeOD) δ 7.84 (s, 1H), 4.50 (dd, J = 7.8, 4.6 Hz, 1H), 4.42 (s, 2H), 4.37 (t, J = 7.1 Hz, 2H), 4.29 (dd, J = 7.9, 4.5 Hz, 1H), 3.89 (dd, J = 6.3, 3.0 Hz, 1H), 3.76 (dd, J = 10.2, 4.4 Hz, 1H), 3.65 (t, J = 10.0 Hz, 1H), 3.24 – 3.13 (m, J = 10.3, 8.9, 6.0 Hz, 2H), 2.93 (dd, J = 12.8, 5.0 Hz, 1H), 2.71 (d, J = 12.7 Hz, 1H), 2.28 (dd, J = 7.9, 4.3 Hz, 1H), 2.24 (t, J = 7.6 Hz, 2H), 2.15 (dd, J = 5.1, 2.8 Hz, 1H), 2.11 (dd, J = 5.1, 3.1 Hz, 1H), 2.02 – 1.94 (m, J = 7.5, 4.6 Hz, 1H), 1.94 – 1.83 (m, 4H), 1.79 – 1.48 (m, 6H), 1.43 (dd, J = 15.4, 7.6 Hz, 2H), 1.39 – 1.24 (m, 8H). ¹³C-NMR (151 MHz, MeOD) δ 176.0, 166.1, 146.2, 124.2, 80.2, 77.4, 63.3, 62.6, 61.6, 59.6, 57.0, 51.4,

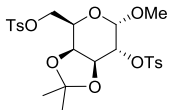
49.8, 49.6, 46.2, 43.0, 41.1, 36.5, 35.6, 31.3, 30.5, 30.4, 29.9, 29.7, 29.4, 28.2, 27.4, 26.7. HRMS (ESI) m/z : $[M+H]^+$ calc for $C_{27}H_{45}N_7O_5S$ 580.32826, found 580.32788.

Compound 25



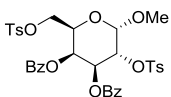
To a stirred solution of dry acetonitrile (200 mL) was added methyl- α -D-galactopyranoside (10.1 g, 51 mmol), triethylamine (12.5 mL, 65 mmol) and tosylchloride (13.7 g, 65 mmol) and stirred overnight. The white precipitate was filtered and washed with pentane. The solid was dissolved in toluene, evaporated and dissolved in dry DMF (200 mL). *p*-Toluenesulfonic acid (0.78 g, 0.15 mmol) and 2,2-dimethoxypropane (102 mmol) were added and the mixture was stirred over night at rt. Then it was quenched with triethylamine until pH $\approx$ 7. The solvent was evaporated followed by purification of the crude product by column chromatography (DCM/MeOH, 20:1) to afford product **25** (12.4 g, 32 mmol, 63% over 2 steps). 1H -NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.70 (d, J = 3.9 Hz, 1H), 4.29 – 4.17 (m, 4H), 4.14 (d, J = 6.2 Hz, 1H), 3.79 (td, J = 6.0, 3.9 Hz, 1H), 3.42 (s, 3H), 2.48 (d, J = 5.8 Hz, 1H), 2.46 (s, 3H), 1.42 (s, 3H), 1.28 (s, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 145.0, 129.9, 128.1, 110.1, 98.0, 75.7, 72.6, 69.3, 68.8, 66.6, 55.6, 27.5, 25.8, 21.8. IR (neat, cm^{-1}): ν 3221, 2941, 1720, 1336, 1253, 1172, 1095, 806, 702. $[\alpha]_D^{20}$ (c 0.8, H_2O): +56. HRMS (ESI) m/z : $[M+H]^+$ calc $C_{17}H_{30}O_8S$ 389.12989, found 389.12976.

Compound 26



Compound **25** (12 g, 32 mmol) was dissolved in dry acetonitrile (150 mL), then triethylamine (15 mL, 80 mmol) and tosylchloride (18 g, 80 mmol) were added and the mixture was stirred overnight at 40 °C. The reaction was quenched with water and extracted with EtOAc (3x). The combined extracts were washed successively with water (3x), aqueous $NaHCO_3$ (3x), brine and dried over $MgSO_4$. The solution was concentrated followed by purification of the crude product by column chromatography (pentane/EtOAc, 3:1) affording product **26** (8.0 g, 16 mmol, 50%). 1H -NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, J = 8.3, 5.4 Hz, 4H), 7.34 (t, J = 8.6 Hz, 4H), 4.81 (d, J = 3.1 Hz, 1H), 4.27 – 4.14 (m, 5H), 4.11 (dd, J = 4.9, 2.1 Hz, 1H), 3.32 (s, 3H), 2.46 (s, 3H), 2.43 (s, 3H), 1.18 (s, 3H), 1.05 (s, 3H). ^{13}C -NMR (101 MHz, $CDCl_3$) δ 145.1, 145.1, 133.3, 133.0, 130.4, 130.0, 129.8, 128.4, 128.1, 110.1, 97.7, 78.4, 73.4, 73.2, 68.7, 65.5, 56.1, 27.5, 26.3, 21.8, 21.8. IR (neat, cm^{-1}): ν 1724, 1452, 1352, 1172, 1091, 974, 810, 704, 677. $[\alpha]_D^{20}$ (c 0.2, CH_2Cl_2): +60. HRMS (ESI) m/z : $[M+H]^+$ calc for $C_{21}H_{26}O_{10}S_2$ 543.13703, found 543.13521.

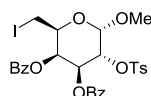
Compound 27



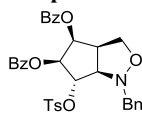
Compound **26** (2.8 g, 5.1 mmol) was dissolved in methanol (80 mL) and HCl (12 M, 0.2 mL) was added. The mixture was stirred overnight followed by addition of Na_2CO_3 until pH $\approx$ 7 and filtered. The solvent was evaporated and purification of the crude product by column chromatography (DCM/MeOH, 50:1) yielded the 3,4-diol (2.6 g, 5.1

mmol, quant.). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.80 (dd, $J = 14.0, 8.3$ Hz, 4H), 7.35 (dd, 4H), 4.61 (d, $J = 3.7$ Hz, 1H), 4.55 (dd, $J = 9.2, 3.7$ Hz, 1H), 4.22 (dd, $J = 10.6, 5.4$ Hz, 1H), 4.14 (dd, $J = 10.6, 7.0$ Hz, 1H), 4.07 – 3.92 (m, 3H), 3.26 (s, 3H), 2.76 (d, $J = 3.5$ Hz, 1H), 2.58 (s, 1H), 2.45 (d, $J = 3.1$ Hz, 6H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 145.6, 145.3, 132.7, 130.1, 130.1, 128.1, 128.1, 97.4, 77.8, 77.5, 77.2, 76.8, 69.1, 68.5, 67.6, 67.3, 55.8. IR (neat, cm^{-1}): ν 3537, 3464, 2920, 1597, 1352, 1190, 1170, 1002, 974, 837, 812, 769, 678. $[\alpha]_{\text{D}}^{20}$ (c 1.0, CH_2Cl_2): +80. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{21}\text{H}_{26}\text{O}_{10}\text{S}_2$ 503.10538, found 503.10428. Subsequently, the 3,4-diol (2.55 g, 5.1 mmol) was dissolved in dry pyridine (50 mL). Benzoyl anhydride (4.6 g, 20.4 mmol) and DMAP (30 mg, 0.3 mmol) were added and the mixture was stirred overnight. The reaction was quenched by addition of aqueous NaHCO_3 and extracted with EtOAc (3 x 100 mL). The combined extracts were washed with water, aqueous NaHCO_3 , and brine, dried over MgSO_4 , and concentrated. Purification of the crude product by column chromatography (pentane/EtOAc, 2:1) yielded compound **27** (2.56 g, 3.6 mmol, 70%). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.89 – 7.83 (m, 2H), 7.72 – 7.63 (m, 3H), 7.60 (d, $J = 8.3$ Hz, 2H), 7.52 – 7.44 (m, 5H), 7.25 – 7.18 (m, 4H), 6.96 (d, $J = 8.1$ Hz, 2H), 5.70 (d, $J = 2.6$ Hz, 1H), 5.63 (dd, $J = 10.5, 3.4$ Hz, 1H), 5.09 (d, $J = 3.5$ Hz, 1H), 4.93 (dd, $J = 10.4, 3.5$ Hz, 1H), 4.38 (t, $J = 6.3$ Hz, 1H), 4.17 (dd, $J = 10.3, 7.1$ Hz, 1H), 4.01 (dd, $J = 10.3, 5.6$ Hz, 1H), 3.44 (s, 3H), 2.32 (s, 3H), 2.22 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.1, 164.9, 145.2, 144.9, 133.8, 133.3, 130.0, 129.9, 129.9, 129.8, 129.0, 128.9, 128.7, 128.2, 128.0, 127.8, 98.2, 74.3, 69.1, 67.5, 67.1, 66.7, 56.3, 21.7. IR (neat, cm^{-1}): ν 1720, 1357, 1271, 1064, 1020, 977, 813, 705, 659. $[\alpha]_{\text{D}}^{20}$ (c 0.2, CH_2Cl_2): +59. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{35}\text{H}_{34}\text{O}_{12}\text{S}_2$ 711.15765, found 711.15689.

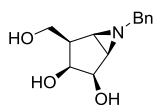
Compound 28



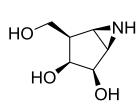
Sodium iodide (612 mg, 4.1 mmol) was added to a mixture of compound **27** (2.44 g, 3.4 mmol) in acetic anhydride (15 mL) and refluxed for 4 hours. After cooling to rt the mixture was filtered over Celite to remove the solids. The residue was dissolved in DCM, washed with aqueous sodium thiosulphate, water, dried over MgSO_4 and filtered. The solvent was evaporated followed by crystallization from methanol to yield product **28** (2.0 g, 88%). $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 8.06 – 7.88 (m, 2H), 7.66 (dd, $J = 14.8, 7.9$ Hz, 3H), 7.56 – 7.42 (m, 5H), 7.23 (dd, $J = 8.1, 7.6$ Hz, 2H), 6.99 (d, $J = 8.0$ Hz, 2H), 5.86 (dd, 1H), 5.69 (dd, $J = 10.5, 3.4$ Hz, 1H), 5.13 (d, $J = 3.6$ Hz, 1H), 5.00 (dd, $J = 10.5, 3.6$ Hz, 1H), 4.32 (dt, $J = 7.0$ Hz, 1H), 3.54 (s, 3H), 3.20 (d, $J = 7.1$ Hz, 2H), 2.24 (s, 3H). $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 165.4, 165.0, 144.9, 133.8, 133.3, 130.0, 129.9, 129.8, 129.0, 129.0, 128.8, 128.2, 127.8, 98.3, 74.4, 70.6, 69.9, 67.7, 56.4, 21.7, 0.5. IR (neat, cm^{-1}): ν 1720, 1371, 1273, 1247, 1093, 1020, 842, 817, 705, 655. $[\alpha]_{\text{D}}^{20}$ (c 0.3, CH_2Cl_2): +202. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{28}\text{H}_{27}\text{IO}_9\text{S}$ 689.03183, found 689.03131.

Compound 29

To a suspension of compound **28** (2.3 g, 6.8 mmol) in EtOH (60 mL) was added Zn dust (4 g, 60 mmol). The mixture was refluxed for 6 h and then filtered through Celite and concentrated to give a yellow oil that was dissolved in CH₂Cl₂ (200 mL), washed with water (2 × 100 mL), dried over MgSO₄, filtered and concentrated to give the aldehyde as an oil. To a solution of the aldehyde in toluene (30 mL) was added *N*-benzylhydroxylamine hydrochloride (2.4 g, 14 mmol) and Na₂CO₃ (1.6 g, 14 mmol), and the reaction was stirred at 50 °C for 16 h. The mixture was then concentrated, and the residue was dissolved in CH₂Cl₂ (150 mL), washed with water (2 × 100 mL), dried over MgSO₄, filtered and concentrated. Purification of the crude product by column chromatography (pentane/EtOAc, 3:1) yielded product **29** (4.0 g, 6.5 mmol, 95%). ¹H-NMR (400 MHz, CDCl₃) δ 7.92 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.80 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.59 – 7.44 (m, 3H), 7.43 – 7.27 (m, 10H), 7.11 (d, *J* = 8.0 Hz, 2H), 5.71 (dd, *J* = 6.7, 4.9 Hz, 1H), 5.50 (dd, *J* = 7.7, 4.8 Hz, 1H), 5.26 (dd, *J* = 7.7, 4.9 Hz, 1H), 4.07 – 3.95 (m, 3H), 3.89 – 3.79 (m, 2H), 3.68 – 3.58 (m, 1H), 2.24 (s, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 165.5, 165.1, 145.0, 136.8, 133.7, 133.6, 133.4, 130.0, 129.9, 129.1, 129.0, 128.6, 128.6, 128.4, 127.9, 127.7, 85.2, 75.4, 71.5, 70.3, 64.9, 59.9, 47.0, 21.7. IR (neat, cm⁻¹): ν 1722, 1598, 1450, 1363, 1274, 1174, 1024, 844, 812, 707, 696. [α]_D²⁰ (c 0.3, CH₂Cl₂): +17. HRMS (ESI) *m/z*: [M+H]⁺ calc for C₃₄H₃₁NO₈S 614.18431, found 614.18427.

Compound 30

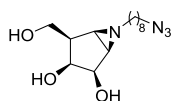
Compound **29** (480 mg, 0.8 mmol) was dissolved in acetic acid (15 mL). Zinc powder (1.0 g, 6 mmol) was added and the mixture was stirred in a preheated oil bath at 40 °C. After 7 h the mixture was filtered over Celite and rinsed with methanol. The solvents were evaporated and the crude was co-evaporated with toluene. The solid residue was dissolved in methanol (10 mL) and methanolic sodium methoxide was added until pH ≈ 10 and stirred for 3 h. Acetic acid was added until pH ≈ 7 and the solvent was evaporated, followed by purification of the crude product by column chromatography (DCM/MeOH, 9:1), to afford product **30** (188 mg, 0.8 mmol, quant.). ¹H-NMR (400 MHz, MeOD) δ 7.42 – 7.28 (m, 5H), 4.12 (dd, *J* = 5.2, 2.2 Hz, 1H), 3.84 – 3.72 (m, 2H), 3.65 – 3.55 (m, 2H), 3.17 (d, *J* = 13.5 Hz, 1H), 2.46 (s, 1H), 2.41 (s, 1H), 2.23 – 2.13 (m, 1H). ¹³C-NMR (400 MHz, MeOD) δ 140.3, 129.4, 128.9, 128.2, 75.0, 71.3, 61.9, 60.2, 47.5, 46.7, 44.0. IR (neat, cm⁻¹): ν 2885, 1724, 1598, 1450, 1274, 1259, 1174, 1093, 995, 908 844, 812, 659. [α]_D²⁰ (c 0.6, MeOH): +24. HRMS (ESI) *m/z*: [M+H]⁺ calc for C₁₃H₁₇NO₃ 236.12940, found 236.12809.

Compound 31

To a solution of **30** (500 mg, 2.13 mmol) in water (50 mL) under argon was added Pd(OH)₂/C (10%wt, 500 mg) and the mixture was purged with H₂ gas with a balloon. After vigorously stirring for 30 minutes, the mixture was filtered through Celite and concentrated followed by purification of the crude product by column chromatography (DCM/MeOH, 9:1), to afford product **31** (144 mg, 0.99 mmol, 46%). ¹H-NMR (400 MHz, MeOD) δ 4.24 (dd, *J* = 5.5, 2.2 Hz, 1H), 3.91 – 3.84 (m, 2H), 3.75 (dd, *J* = 10.8, 7.6 Hz, 1H), 2.59 – 2.55 (m, 1H), 2.54 (dd, *J* = 3.6, 2.0

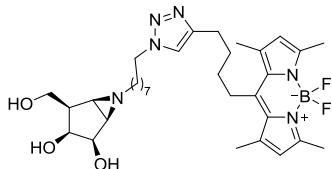
Hz, 1H), 2.33 (qd, $J = 7.4, 1.9$ Hz, 1H). ^{13}C -NMR (101 MHz, MeOD) δ 75.2, 69.7, 60.4, 46.8, 39.0, 35.8. IR (neat, cm^{-1}): ν 3259, 2922, 1730, 1408, 1375, 1101, 1085, 1051, 1031, 867, 682. $[\alpha]_{\text{D}}^{20}$ (c 0.8, MeOH): +8. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_6\text{H}_{11}\text{NO}_3$ 146.0812, found 146.0815.

Compound 32



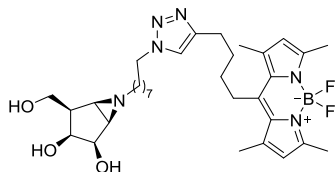
Aziridine **31** (39 mg, 0.27 mmol) was dissolved in dry DMF (1.3 mL). 1-azido-8-iodooctane (0.15 g, 0.53 mmol) and K_2CO_3 (0.11 g, 0.8 mmol) were added and the mixture was heated to 80°C and stirred overnight. The mixture was then concentrated and purified by column chromatography on neutralized silica gel (DCM/MeOH, 10:1) and by semi-preparative reversed phase HPLC (linear gradient: 36% $\rightarrow$ 42%, solutions used: A: 50 mM NH_4HCO_3 in H_2O , B: acetonitrile). After freeze drying compound **32** was obtained (37 mg, 0.12 mmol, 46%). ^1H -NMR (600 MHz, MeOD) δ 4.04 (dd, $J = 5.2, 2.3$ Hz, 1H), 3.87 (dd, $J = 10.7, 6.9$ Hz, 1H), 3.73 (dd, $J = 10.7, 7.8$ Hz, 1H), 3.69 (t, $J = 5.1$ Hz, 1H), 3.28 (t, $J = 6.9$ Hz, 2H), 2.37 (dt, $J = 11.8, 7.2$ Hz, 1H), 2.23 – 2.20 (m, 1H), 2.20 – 2.17 (m, 1H), 2.16 – 2.11 (m, 1H), 1.98 (dt, $J = 11.8, 6.8$ Hz, 1H), 1.63 – 1.56 (m, 2H), 1.56 – 1.49 (m, 2H), 1.44 – 1.32 (m, 9H). ^{13}C -NMR (151 MHz, MeOD) δ 74.9, 71.4, 60.4, 58.4, 52.5, 47.0, 46.8, 43.5, 30.8, 30.5, 30.2, 29.9, 28.3, 27.8. IR (neat, cm^{-1}): ν 3323, 2927, 2854, 2090, 1463, 1253, 1093, 1024, 906, 723, 623. $[\alpha]_{\text{D}}^{20}$ (c 1.0, MeOH): +12. HRMS (ESI) m/z : $[\text{M}+\text{Na}]^+$ calc for $\text{C}_{14}\text{H}_{26}\text{N}_4\text{O}_3$ 321.1897, found 321.1900.

Compound 33 (EM265B)

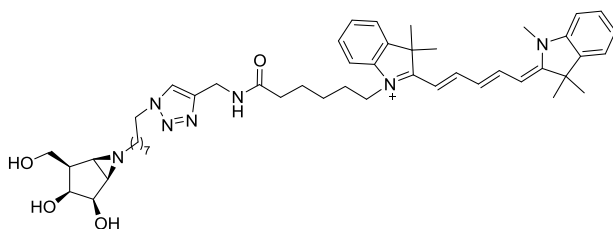


Compound **32** (5.0 mg, 17 μmol) was dissolved in DMF (0.6 mL). BODIPY-green-alkyne (11 mg, 34 μmol), CuSO_4 (33 μL of 100 mM solution in H_2O) and sodium ascorbate (50 μL of 100 mM solution in H_2O) were added and the solution was stirred overnight at room temperature. The solvent was evaporated and the product was purified by by semi-preparative reversed

phase HPLC (linear gradient: 50% $\rightarrow$ 53%, solutions used: A: 50 mM NH_4HCO_3 in H_2O , B: acetonitrile). After freezedrying product **33** was obtained as a yellow powder (3.8 mg, 6.0 μmol , 35%). ^1H -NMR (500 MHz, MeOD) δ 7.74 (s, 1H), 6.12 (s, 2H), 4.35 (t, $J = 6.9$ Hz, 2H), 4.03 (dd, $J = 5.1, 2.1$ Hz, 1H), 3.85 (dd, $J = 10.7, 6.9$ Hz, 1H), 3.74 – 3.66 (m, 2H), 3.06 – 2.98 (m, 2H), 2.78 (t, $J = 7.2$ Hz, 2H), 2.43 (s, 6H), 2.38 (s, 6H), 2.35 – 2.28 (m, 1H), 2.22 – 2.17 (m, 1H), 2.17 – 2.14 (m, 1H), 2.14 – 2.10 (m, 1H), 1.89 – 1.82 (m, 3H), 1.69 – 1.59 (m, 2H), 1.52 – 1.43 (m, 2H), 1.40 – 1.19 (m, 9H). ^{13}C -NMR (125 MHz, MeOD) δ 154.9, 142.2, 122.6, 74.9, 71.4, 60.4, 58.3, 51.2, 47.0, 46.8, 43.5, 43.5, 40.4, 32.2, 31.2, 30.8, 30.7, 30.3, 29.9, 29.1, 28.1, 27.3, 25.9, 16.5, 14.4. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{33}\text{H}_{49}\text{BF}_2\text{N}_6\text{O}_3$ 627.4000, found 627.4024.

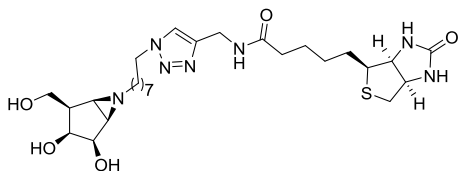
Compound 34 (EM265C)

Compound **32** (5.0 mg, 17 μ mol) was dissolved in DMF (0.6 mL). BODIPY-red-alkyne (16.2 mg, 34 mmol), CuSO₄ (33 μ L of 100 mM solution in H₂O) and sodium ascorbate (50 μ L of 100 mM solution in H₂O) were added and the solution was stirred overnight at room temperature. The solvent was evaporated and the product was purified by semi-preparative reversed phase HPLC (linear gradient: 55%→61%, solutions used: A: 50 mM NH₄HCO₃ in H₂O, B: acetonitrile). After freeze-drying product **34** was obtained as a dark blue powder (3.8 mg, 4.9 μ mol, 29%). ¹H-NMR (500 MHz, MeOD) δ 7.84 (d, J = 8.9 Hz, 4H), 7.69 (s, 1H), 7.43 (d, J = 4.3 Hz, 2H), 6.97 (d, J = 8.9 Hz, 4H), 6.69 (d, J = 4.3 Hz, 2H), 4.33 (t, J = 7.0 Hz, 2H), 4.01 (dd, J = 5.1, 2.2 Hz, 1H), 3.84 (s, 7H), 3.72 – 3.64 (m, 2H), 3.05 (t, J = 7.2 Hz, 2H), 2.78 (t, J = 6.7 Hz, 2H), 2.28 (dt, J = 11.8, 7.0 Hz, 1H), 2.15 (s, 1H), 2.14 – 2.07 (m, 2H), 1.91 – 1.78 (m, 8H), 1.49 – 1.37 (m, 2H), 1.37 – 1.17 (m, 9H). ¹³C-NMR (125 MHz, MeOD) δ 132.1, 128.4, 123.3, 121.0, 114.6, 74.9, 71.4, 60.3, 58.3, 55.8, 51.2, 46.9, 46.8, 43.5, 34.1, 30.3, 27.3. HRMS (ESI) m/z : [M+H]⁺ calc for C₄₃H₅₃BF₂N₆O₅ 783.4211, found 783.4241.

Compound 35 (EM267)

Compound **32** (5.0 mg, 17 μ mol) was dissolved in DMF (0.6 mL). Cy5-alkyne (18.6 mg, 34 mmol), CuSO₄ (33 μ L of 100 mM solution in H₂O) and sodium ascorbate (50 μ L of 100 mM solution in H₂O) were added and the solution was

stirred overnight at room temperature. The solvent was evaporated and the product was purified by semi-preparative reversed phase HPLC (linear gradient: 55%→61%, solutions used: A: 50 mM NH₄HCO₃ in H₂O, B: acetonitrile). After freeze-drying product **35** was obtained as a blue powder (3.5 mg, 4.1 μ mol, 24%). ¹H-NMR (500 MHz, MeOD) δ 8.27 (t, J = 13.0 Hz, 2H), 7.87 (s, 1H), 7.52 (d, J = 7.4 Hz, 3H), 7.48 – 7.39 (m, 3H), 7.37 – 7.24 (m, 5H), 6.64 (t, J = 12.4 Hz, 1H), 6.30 (d, J = 13.5 Hz, 2H), 4.43 (s, 2H), 4.39 (t, J = 7.0 Hz, 3H), 4.11 (t, J = 7.4 Hz, 2H), 4.07 – 4.03 (m, 1H), 3.87 (dd, J = 10.6, 6.9 Hz, 1H), 3.76 – 3.68 (m, 2H), 3.65 (s, 3H), 2.27 (t, J = 7.3 Hz, 2H), 2.23 (s, 1H), 2.21 – 2.18 (m, 1H), 2.18 – 2.12 (m, 1H), 1.93 – 1.80 (m, 7H), 1.55 – 1.44 (m, 5H), 1.44 – 1.27 (m, 13H). ¹³C-NMR (125 MHz, MeOD) δ 174.6, 155.5, 129.8, 126.6, 126.2, 124.1, 123.4, 123.3, 112.0, 111.8, 104.4, 104.2, 74.9, 71.4, 60.4, 58.3, 51.3, 50.5, 47.0, 46.8, 44.8, 43.5, 36.5, 35.6, 31.5, 31.3, 30.7, 30.3, 29.9, 28.1, 27.9, 27.8, 27.3, 26.4. HRMS (ESI) m/z : [M]⁺ calc for C₄₉H₆₈N₇O₄ 818.5327, found 818.5339.

Compound 36 (EM265D)

Compound **32** (5.0 mg, 17 μ mol) was dissolved in DMF (0.6 mL). Biotin-alkyne (9.3 mg, 34 mmol), CuSO_4 (33 μ L of 100 mM solution in H_2O) and sodium ascorbate (50 μ L of 100 mM solution in H_2O) were added and the solution was stirred overnight at room temperature. The solvent was

evaporated and the product was purified by semi-preparative reversed phase HPLC (linear gradient: 20%→26%, solutions used: A: 50 mM NH_4HCO_3 in H_2O , B: acetonitrile). After freeze-drying product **36** was obtained as a white powder (2.9 mg, 5.0 μ mol, 29%). ^1H -NMR (500 MHz, MeOD) δ 7.85 (s, 2H), 4.49 (dd, J = 7.8, 4.9 Hz, 2H), 4.42 (s, 4H), 4.38 (t, J = 7.0 Hz, 4H), 4.29 (dd, J = 7.9, 4.5 Hz, 2H), 4.04 (dd, J = 5.1, 2.2 Hz, 1H), 3.86 (dd, J = 10.7, 6.9 Hz, 1H), 3.74 – 3.67 (m, 2H), 3.53 (t, J = 6.6 Hz, 1H), 3.19 (dt, J = 10.0, 5.6 Hz, 2H), 2.93 (dd, J = 12.7, 5.0 Hz, 2H), 2.70 (d, J = 12.7 Hz, 2H), 2.36 (dt, J = 11.8, 7.1 Hz, 1H), 2.26 – 2.20 (m, 5H), 2.20 – 2.17 (m, 1H), 2.16 – 2.10 (m, 1H), 1.97 (dt, J = 12.1, 6.7 Hz, 1H), 1.78 – 1.55 (m, 8H), 1.55 – 1.46 (m, 4H), 1.46 – 1.27 (m, 19H). ^{13}C -NMR (125 MHz, MeOD) δ 74.9, 71.4, 63.3, 62.9, 61.6, 60.4, 58.3, 57.0, 51.4, 47.0, 46.8, 43.5, 41.1, 36.5, 35.6, 31.3, 30.8, 30.4, 30.0, 29.7, 29.5, 28.2, 27.4, 26.7. HRMS (ESI) m/z : $[\text{M}+\text{H}]^+$ calc for $\text{C}_{27}\text{H}_{45}\text{N}_7\text{O}_5\text{S}$ 580.3276, found 580.3289.

General procedure for SDS-PAGE experiments

Mouse liver lysate (Jackson's laboratories, C57Bl6/J, 40 μ g total protein per sample) or human fibroblast lysate (product code CC-2511, lot no. C92030, Lonza, 10 μ g total protein per sample) was diluted in 150 mM phosphate buffer with appropriate pH. A solution of the ABP with appropriate concentration was added and the mixture was incubated for 30 minutes at 37 $^\circ\text{C}$. In the case of competition experiments, the enzyme solution was pre-incubated with appropriate inhibitor concentrations for 30 minutes at 37 $^\circ\text{C}$. Then, laemmli (4X) was added and the mixture was heated at 100 $^\circ\text{C}$ for 5 minutes. The samples were loaded on 10% acrylamide SDS-PAGE gels and the gels were ran at a constant 90V. Wet slab gels were scanned on fluorescence using a Typhoon FLA9500 Imager (GE Healthcare) using λ_{EX} 635 nm; λ_{EM} > 665 nm. Images were acquired, processed and quantified with Image Quant (GE Healthcare).

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