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Synthesis of isofagomine derivatives as new fluorescence pH indicators/glycosidase inhibitors.

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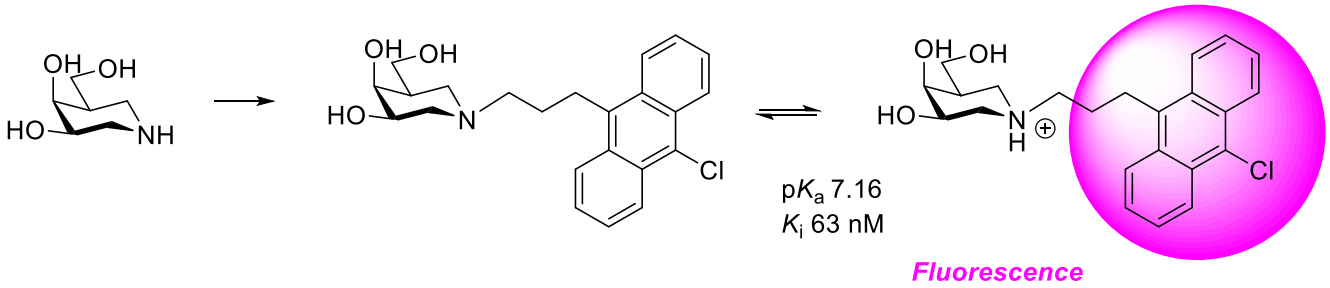
Key topic: Inhibitor protonation

Table of Contents (Graphical Abstract): To check pH conditions in the active site of an enzyme an inhibitor with on/off fluorescence upon protonation is required. Here the potent glycosidase inhibitor isofagomine is converted into 'glycosidase fluorescence indicators.'



Abstract: Inhibitor protonation of azasugars of the isofagomine type when bound to enzyme can be investigated using photon induced electron transfer (PET) quenching of an attached fluorophore. For this purpose Isofagomine and iso-D-*galacto*-fagomine and iso-L-*gulo*-fagomine were converted to *N*-(10-chloroantracenenyl-9-alkyl) derivatives where the alkyl group contained one, two or three methylene groups. The new derivatives displayed pH dependent fluorescence, as the ammonium forms were fluorescent while 90-99% of the fluorescence was quenched in the amine forms. The 3 isofagomine derivatives were competitive inhibitors of *T. Maritima* β-glucosidase with K_i values from 0.37-4.6μM. Similarly the iso-D-*galacto*-fagomines inhibited *A. Niger* β-galactosidase with K_i values from 63-2000 nM. When bound to the enzymes the inhibitors displayed between 1-15% fluorescence.

Introduction

Glycosidases or glycoside hydrolases are an extremely common type of enzyme that participates in many different biological events such as degradation of polysaccharides or processing of oligosaccharides.[1] Due to the very widespread occurrence of glycosidases, glycosidase inhibitors have a large drug potential. Some of the most widely applicable inhibitors are imino- and azasugars that has the sugar shaped amine general structure of 1-deoxynojirimycin (DNJ, **2**)[2] or isofagomine (**1**, Figure 1).[3] The basic nitrogen atom (pK_a(**1**)= 8.4; pK_a(**2**)= 6.7) which these compounds have in common with many other inhibitors and drugs,[4] means that these compounds are substantially protonated at neutral pH.[5]

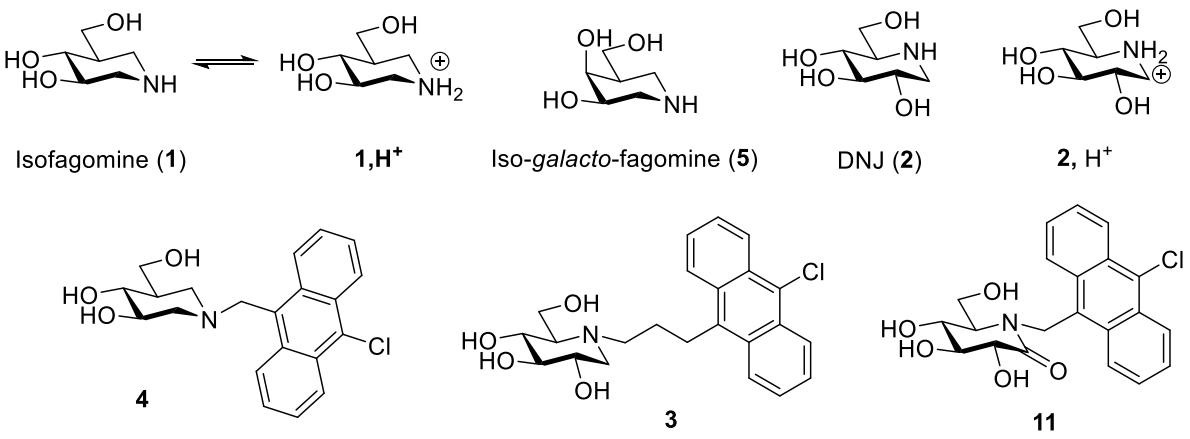
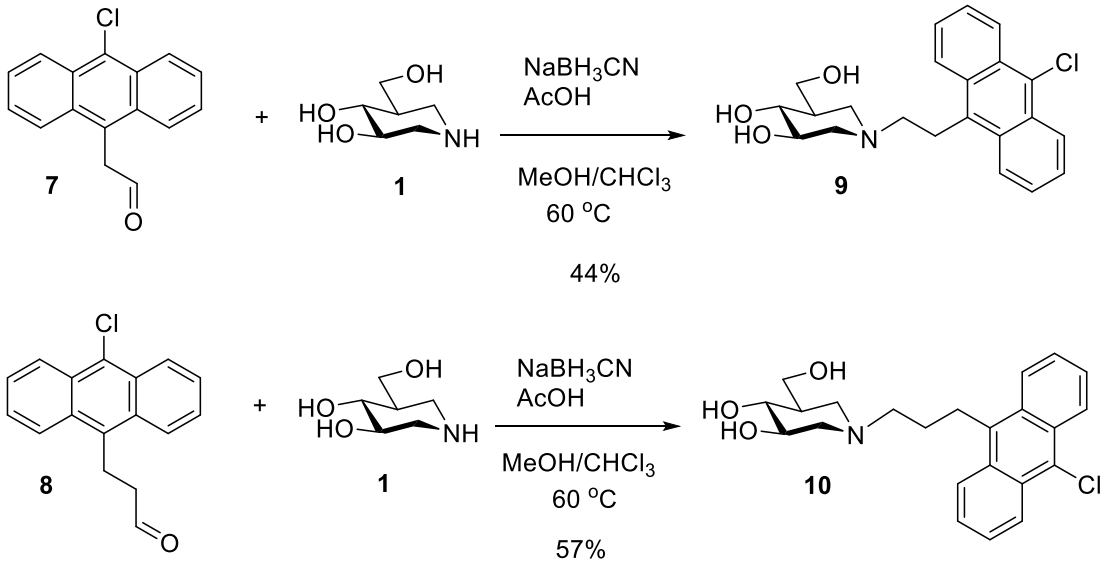


Figure 1: Aza- and iminosugars studied.

The resemblance of the protonated amines (**1**,H^+ and **2**,H^+) with the transition state of glycoside hydrolysis have led to discussion of the possible transition state resemblance of these inhibitors.[6,7] It is clear from the pH dependent inhibition of **1** and **2** that they bind in one distinct protonation state[8] but the actual protonation state of these inhibitors cannot be elucidated from those data. This can however potentially be done by converting the inhibitor into a fluorescence indicator where photoinduced electron transfer (PET) quench the fluorescence when the lonepair of the amine is unoccupied by protonation.[9] We have recently shown with DNJ derivatives (i.e. **2**) with fluorescent anthracene groups attached (e.g. **3**) that they were unprotonated when bound to *Phanerochaete chrysosporium* β-glucosidase – a type 3 glycosidase.[10] Another smaller study with the isofagomine analogue **4** showed that this compound was unprotonated when bound to almond β-glucosidase – a type 1 glycosidase.[11] In the present work we wished to do a larger study with isofagomine derivatives and therefore required more compounds. We have here expanded the previous study with **4** with longer chain-length analogues and also prepared analogues of iso-D-*galacto*-fagomine (**5**) and iso-L-*gulo*-fagomine (**6**).

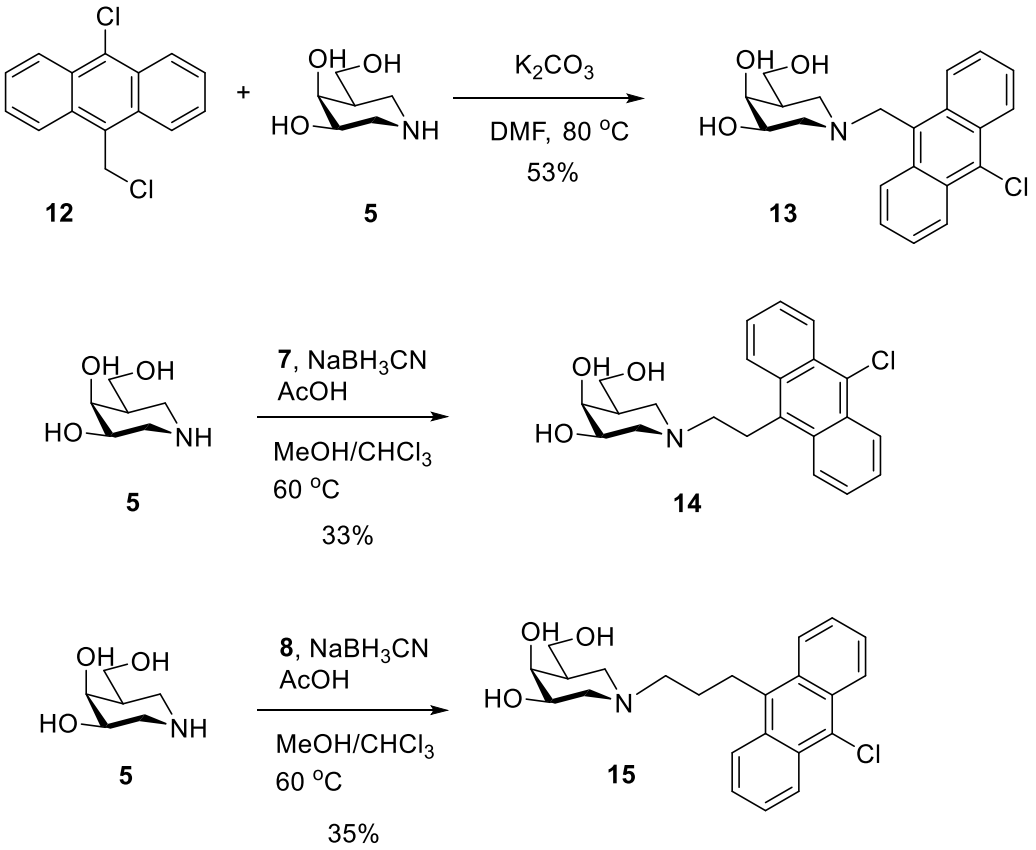


Scheme 1: Synthesis of 10-chloroanthracenyl substituted isofagomines **9** and **10**

Results and discussion

Synthesis. The isofagomine-chloroanthracene derivatives were prepared from **1**[12] and the aldehydes **7** and **8**[10] as outlined in scheme 1: Reductive amination using sodium cyanoborohydride in methanol-chloroform and acetic acid at 60 °C gave the products **9** and **10** in yields of 44 and 57%, respectively (Scheme 1). The reaction is sluggish requiring long time even at elevated temperature which is the reason for the generally modest yields in the reductive amination reactions with the chloroanthracene aldehydes. The shorter chain analogue **4** and the lactame reference compound **11** (Figure 1) were prepared as previously described.[11]

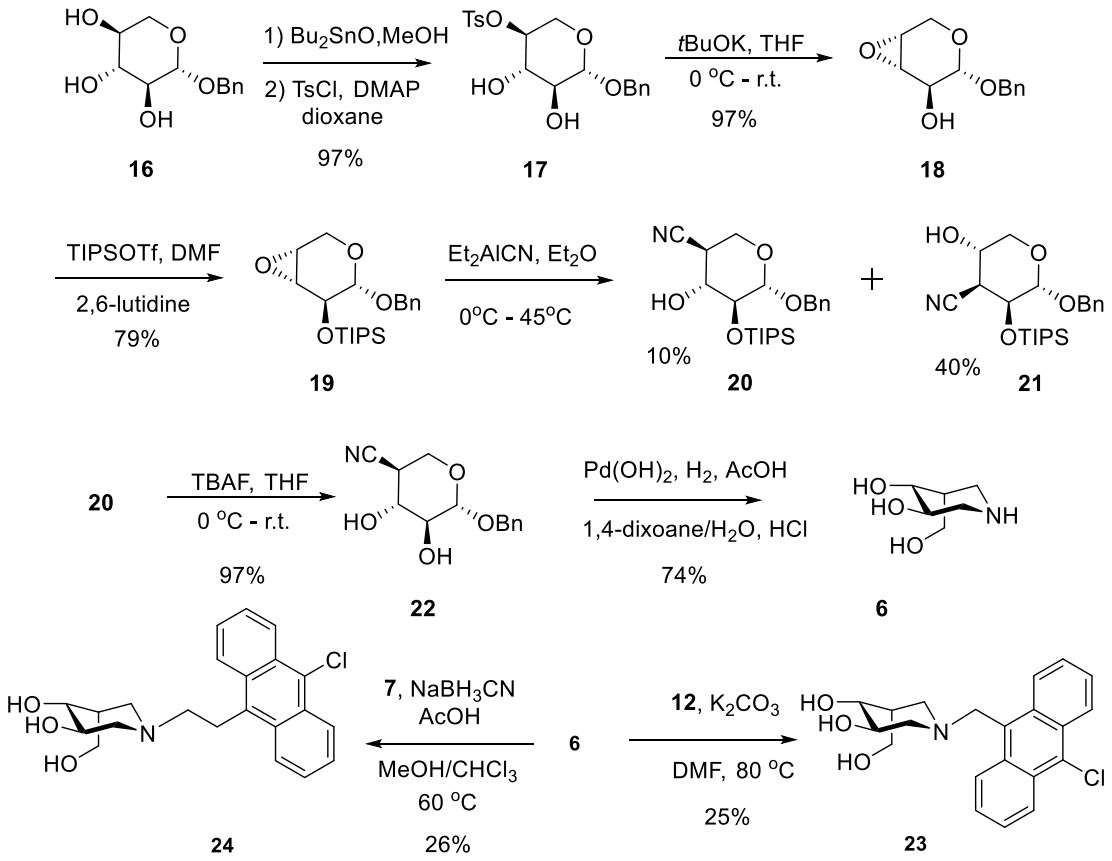
The iso-*galacto*-fagomine derivatives were made in a similar fashion (Scheme 2). Alkylation of **5**[13] with chloride **12** and potassium carbonate in DMF gave **13** in 53% yield (Scheme 2). Reductive amination of **7** and **8** with **5** using sodium cyanoborohydride in methanol-chloroform and acetic acid at 60 °C gave the products **14** and **15** in 33 and 35% yield, respectively (Scheme 2).



Scheme 2: Synthesis of 10-chloroanthracenyl substituted iso-*galacto*-fagomines **13**, **14** and **15**

Iso-L-*gulo*-fagomine **6** was obtained from L-xylose as outlined in Scheme 3. Starting from the 1-*O*-benzylated L-xylopyranoside **16**[14] dibutyltin oxide assisted tosylation of **16** gave the 4-*O*-tosylate **17** in high yield (Scheme 3). Treatment of this compound with potassium *tert*-butoxide gave the 3,4-epoxide **18** also in high yield. Reaction with triisopropylsilyl triflate and lutidine gave the 2-*O*-triisopropyl (TIPS) protected derivative **19**. Epoxide opening with cyanide afforded some difficulties but was achieved using cyanodiethyl aluminum which afforded two regioisomers **20** and **21** in 10% and 40% yield, respectively. The minor regioisomer **20** was desilylated with tetrabutylammonium fluoride to give **22** and then subjected to hydrogenolysis and hydrogenation to give **6** in good yield.

With **6** in hand two chloroanthracene derivatives were prepared (Scheme 3). Reaction of **6** with **12** and potassium carbonate gave *N*-alkylation to **23**. Reductive amination of **7** with **6** and sodium cyanoborohydride gave **24**.



Scheme 3: Synthesis of 10-chloroanthracenyl substituted iso-L-*gulo*-fagomines **23** and **24**

Fluorescence measurements. The fluorescence as a function of pH of compound **4** and the new chloroanthracenyl substituted isofagomine derivatives **9-10, 13-15, 23** and **24** were determined by measuring their fluorescence spectra at different pH. Due to the poor solubility of these derivatives in pure water these experiments had to be carried out with 10% DMSO being added as cosolvent. The influence on pK_a and K_i values was expected to be minor with this low content of DMSO. The excitation wave-length was 358 nm and the fluorescence spectra were measured from 380-680 nm. From plots of the fluorescence intensity at maximum versus pH (Figure 2) pK_a values for the compounds were determined as well as relative intensity of fluorescence remaining in the free amine form (Table 1). All 8 compounds worked as fluorescence indicators with 76-95% of the fluorescence being quenched in the amine. The pK_a values were from 1.6-2.4 units lower than those of **1** and **5** (pK_a(**1**)= 8.4; pK_a(**5**)= 8.8) which in part is due to the greater sterical hindrance associated with protonation in these tertiary amines and in part is due to an inductive electron withdrawing-effect from the anthracene. The basicity increases with increasing chain length in each series consistent with the decreasing influence of the anthracene on the amine. There is also a consistent pK_a difference between stereoisomers, which is due to the higher electron-withdrawing effect of the equatorial OH.[5] In otherwise similar compounds the *gulo* isomer is more basic than the *galacto* isomer, which is more basic than the *gluco* isomer i.e. pK_a decreases in the methylene series pK_a(**23**)> pK_a(**13**)> pK_a(**4**) and in the ethylene series pK_a(**24**)> pK_a(**14**)> pK_a(**9**).

The effectiveness of photo induced electron transfer (PET) is decreased with chain-length: In the short chain analogues **4**, **13** and **23** quenching is almost complete as the fluorescence is reduced to 5-6 %, while the residual fluorescence increases to 7-10% in the C2-chain analogues **9**, **14** and **24** and to 20-24% in the C3-chain analogues **10** and **15**.

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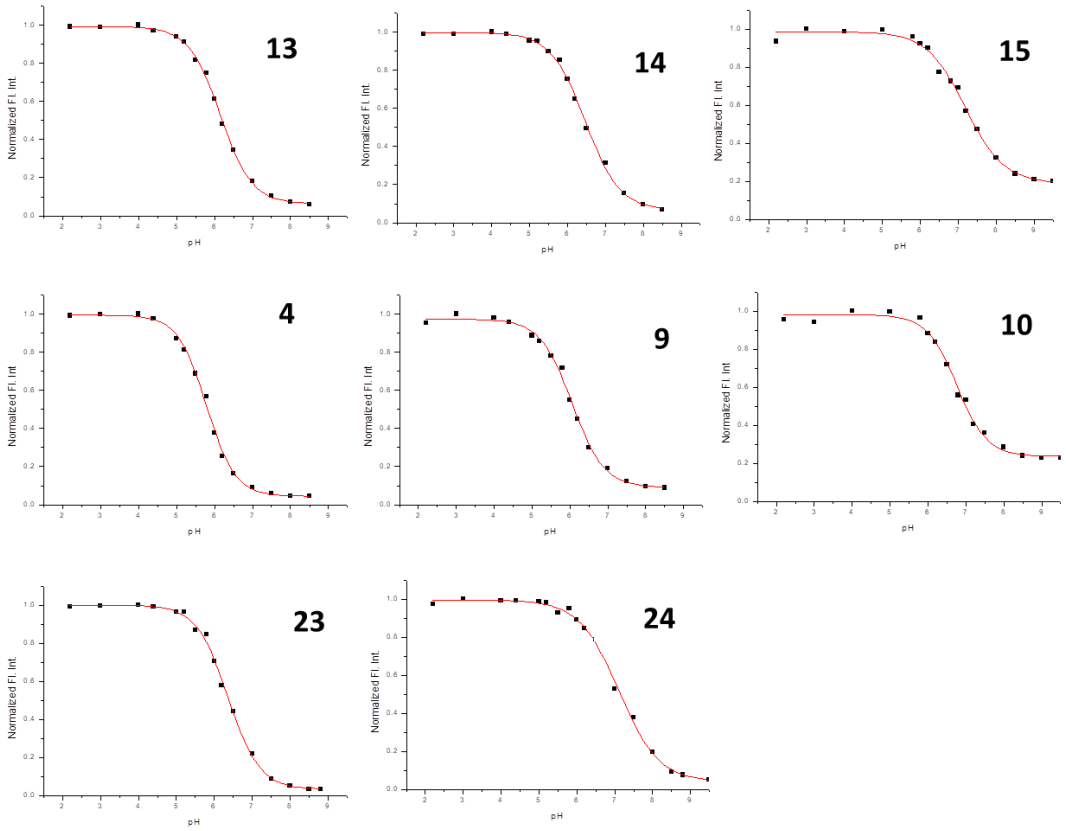
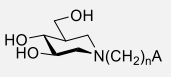
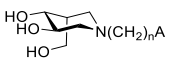
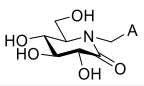
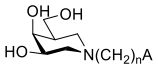


Figure 2: Fluorescence titration curves for inhibitors **4**, **9**-**10**, **13**-**15** and **23**-**24**.

Inhibition. The isofagomine analogues **9-10** and **23-24** were investigated for inhibition of *Thermotoga maritima* β-glucosidase, while the *galacto*-analogues **13-15** were tested for inhibition of *Aspergillus Niger* β-galactosidase. Due to the solubility issue discussed above all these assays were done in aqueous buffers containing 10% DMSO. The added DMSO resulted in homogeneous solutions of the inhibitors and did not disrupt these two enzymes. In all cases competitive inhibition was found with the listed K_i values, which mostly are submicromolar (Table 1). The gluconolactame **11**[10] was also tested against the β-glucosidase and was a reasonably good inhibitor (K_i 12.1 μM). Inhibition was measured at pH 6.5 unless otherwise noted, which is close to the optimum pH of all these inhibitors.

Table 1. pK_a values, fluorescence quenching, optimum pH for inhibition and inhibition constants of **4**, **9**-**11** **13**-**15** and **23**-**24**. A means 10-Chloro-anthracene, - means not relevant or not tested. Optimum pH was calculated as previously described.[8]

Compound	Structure	n	pK_a	*Fluorescence in amino-form*	K_i (μM, pH 6.5) β-glucosidase *T. Maritima*
4		1	5.78	5%	0.37
9		2	6.00	10%	4.3
10		3	6.75	24%	2.3
23		1	6.36	5%	1.1
24		2	7.09	7%	4.6

11		-	-	100%	12
					K_i (μM, pH 6.5) β-galactosidase *A. Niger*
13		1	6.15	6%	0.29
14		2	6.47	8%	2.1
15		3	7.16	20%	0.063

Fluorescence during inhibition. Investigation of inhibitor fluorescence upon binding to the enzyme was performed. This was done by recording the fluorescence spectrum of the inhibitor at pH 6.5 in buffer containing 10% DMSO and adding increasing amounts of excess enzyme. With β-glucosidase *T. Maritima* the glucosidase inhibitors **4**, **9** and **11** had a decrease in intensity of the fluorescence proportional to the added glycosidase (Figure 3 and 4). This means that the fluorescence is quenched when the inhibitor binds to this enzyme. A change in pH to more acidic pH makes the quenching more apparent (Figure 3 left) as more inhibitor is protonated and hence fluorescent outside the enzyme. The decrease in fluorescence of one of these inhibitors increase with enzyme concentration so that the data fit the equation F = Fo[I] + Fx[EI] where [I] is the concentration of free inhibitor, [EI] is the concentration of inhibitor-enzyme complex, F is the total fluorescence, Fo is the fluorescence from inhibitor with no enzyme and Fx is fluorescence of the inhibitor when totally bound (Figure 5).

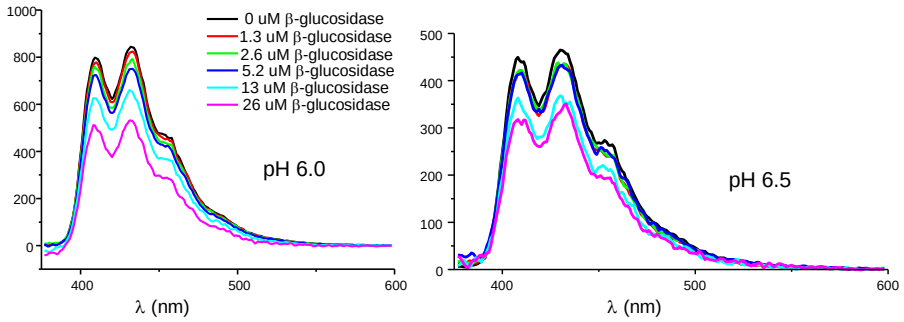


Figure 3. Difference fluorescence spectra of inhibitor **4** (0.8 μM) with increasing concentration of β-glucosidase (the corresponding spectra without inhibitor have been subtracted). It is seen that fluorescence decrease with increasing enzyme concentration.

Unfortunately, the fluorescence of lactam **11**, a control compound, is also quenched by the enzyme in an identical manner. This means that this enzyme is inherently quenching the fluorescence of the anthracene, and the decrease in fluorescence is probably unrelated to the protonation state of the inhibitors. This quenching is presumably caused by close proximity of tryptophans in the enzyme and the anthracene groups upon binding of the inhibitor as tryptophans are known to be able to quench the fluorescence.[15]

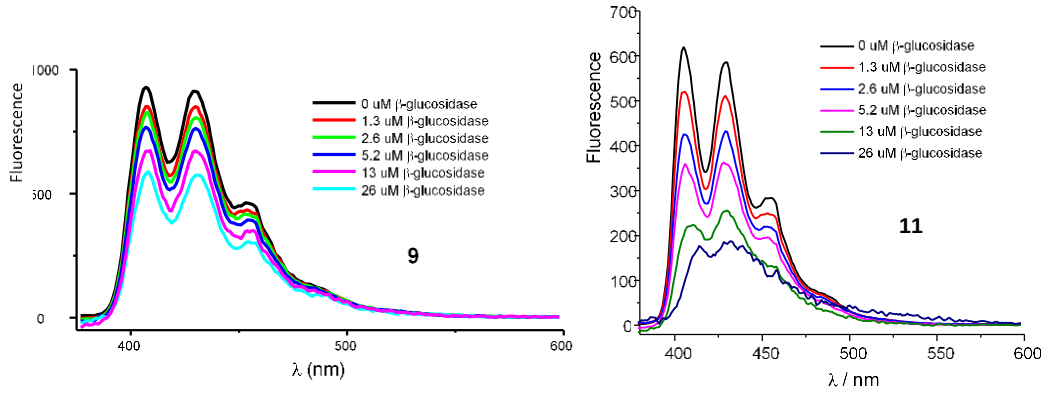


Figure 4. Difference fluorescence spectra of inhibitors **9** and **11** (0.8 μM) with increasing concentration of β-glucosidase (*T. maritima*) at pH 6.5 (the corresponding spectra without inhibitor have been subtracted). It is seen that fluorescence decrease with increasing enzyme concentration.

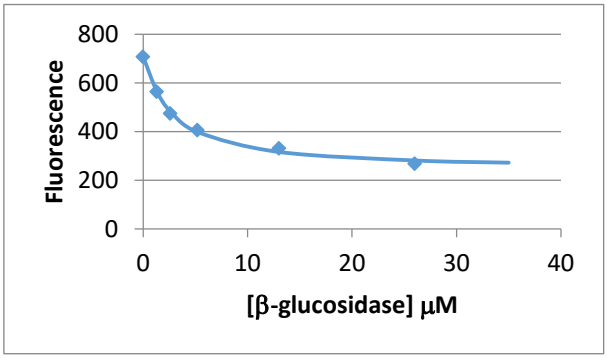


Figure 5. Decrease in fluorescence at 410 nm from inhibitor **9** as of result of increasing concentration of β-glucosidase (*T. maritima*) at pH 6.0. The data fit to the equation F = Fo[I] + Fx[EI] where Fo is the fluorescence from inhibitor with no enzyme and Fx is fluorescence when totally bound. This shows that the fluorescence change is a result of the simple binding event E + I → EI.

The fluorescence of the galactosidase inhibitors **13**, **14** and **15**, when bound to β-galactosidase from *A. Niger* (pH 6.5 in buffer containing 10% DMSO) was also investigated (Figure 6). In all cases the fluorescence was quenched when these inhibitors bound to the galactosidase very similar to what was seen above. As the quenching could be inherent quenching by tryptophanes near the active site no conclusion with regard to protonation of the inhibitors can be made.

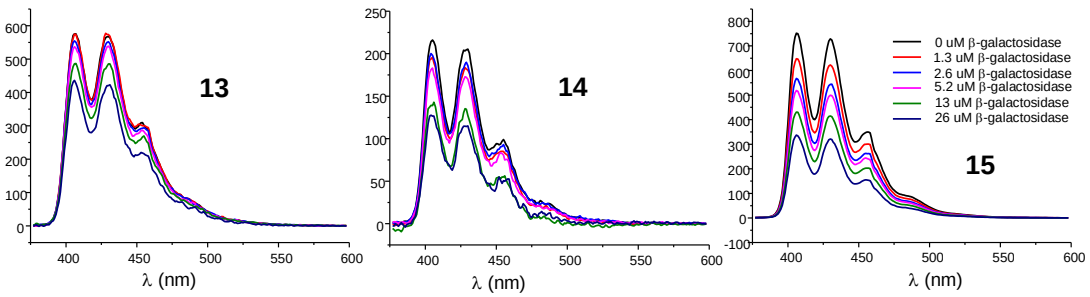


Figure 6. Difference fluorescence spectra of inhibitors **13-15** with increasing concentration of β- galactosidase at

pH 6.5 (the corresponding spectra without inhibitor have been subtracted). It is seen that the fluorescence decrease with increasing enzyme concentration.

Conclusions In summary in this work we have synthesized eight new isofagomine anthracene conjugates and shown that they function as pH indicators and are good to excellent inhibitors of two glycosidases. In all cases the enzymes quenched the fluorescence from the protonated inhibitors which could be due to removal of a proton or quenching of the fluorescence by nearby tryptophan residues in the enzymes. For β-glucosidase from *T. maritima* it was seen that the enzyme was capable of quenching fluorescence from the pH independent and permanently fluorescent and related inhibitor **11**.

Experimental part

General information

Dry solvents were trapped from a solvent purification system. Reactants were purchased from commercial sources and used without further purification. HRMS were recorded on a Bruker Solarix XR mass spectrometer analyzing TOF. NMR spectra were recorded on a Brüker 500 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to the residue solvent signals ($CDCl_3$: δ = 7.26 for ^{1}H-NMR and 77.16 for ^{13}C-NMR. D_2O: δ = 4.79 for ^{1}H-NMR. DMSO-d_6: δ = 2.50 for ^{1}H-NMR and 39.52 for ^{13}C-NMR. CD_3OD: δ = 3.31 for ^{1}H-NMR and 49.00 for ^{13}C-NMR). Assignments were aided by COSY and HSQC experiments.

Determination of p*K*a value of iminosugars by fluorescence titration.
Iminosugar was dissolved in DMSO to give 8 μmol/L stock solution. 0.1 mL was added to 0.9 mL of citrate-phosphate buffers with different pH values (2.2, 3.0, 4.0, 4.4, 5.0, 5.2, 5.5, 5.8, 6.0, 6.2, 6.5, 6.8, 7.0, 7.2, 7.5, 8.0, 8.5, 9.0, 9.5). The fluorescence spectra were recorded from 380-680 nm with excitation at 358 nm.

Inhibition constants (K_i) of isofagomine derivatives to different glycosidases were measured in phosphate buffer (pH 6) containing 10% DMSO

Procedure for fluorescence measurements. The inhibitor was dissolved in DMSO to create a stock solution of approximately 8.0 μM. Normally, fluorescence was measured on four types of solutions:

A. Blank (0.45 mL phosphate buffer and 0.05 mL DMSO)
B. Inhibitor (0.45 mL phosphate buffer, and 0.05 mL inhibitor stock solution, [I] = 0.8 μM)
C. Enzyme (0.1-0.2 mL enzyme stock solution in phosphate buffer, and 0.25-0.35 mL phosphate buffer and 0.05 mL DMSO. [E] = 5.8-72 μM)
D. Enzyme and inhibitor (0.1-0.2 mL enzyme stock solution in phosphate buffer, and 0.25-0.35 mL phosphate buffer and 0.05 mL inhibitor stock solution. [I] = 0.8 μM, [E] = 5.8-62 μM

Fluorescence spectra was taken of samples A-D exciting at 358 nm on a Perkin Elmer LS50 instrument. The subtracted spectra (B-A), (D-C) gave the net effect of inhibitor fluorescence when free and bound.

Benzyl 3,4-anhydro-α-D-arabinopyranoside (18)

To a solution of compound **16**[14] (50 mg, 0.208 mmmol, 1 equiv.) in dry methanol (5 mL), Bu_2SnO (53 mg, 0.208 mmol, 1 equiv.) was added. The reaction mixture was refluxed at 66°C overnight. The reaction was then cooled down to room temperature and concentrated under reduced pressure. After dried under vacuum, the residue of stannyl acetal was dissolved in 1,4-dioxane (5 mL). TsCl (45 mg, 0.229 mmol, 1.1 equiv.) and DMAP (5 mg, 0.416 mmol, 0.2 equiv.) were added. After being stirred at r.t. for 2 hrs, the reaction mixture was concentrated under reduced pressure. The flash chromatographical column (PE/EtOAc: 2/1, then 1/2) gave the tosylated xylose **17** (80 mg, 97%) as a white solid (R_f= 0.25 in DCM/MeOH: 7/1). To a solution of **17** (0.88 g, 2.23 mmol, 1 equiv.) in THF (20 mL), *t*BuOK (300 mg, 2.68 mmol, 1.2 equiv.) was added at 0 °C. The reaction mixture was then stirred at r.t. for 1 hr and sat. NH_4Cl (15 ml) was added. After being stirred for another 30 min, the mixture was extracted with EtOAc (3×20 mL). The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuum* to give **18** (0.48 g, 97%) as a colorless oil, which was used in the next step without further purification.

R_f= 0.20 (PE/EtOAc: 2/1). $[\alpha]_D^{20}$=+31.3(c 0.3, $CHCl_3$). **^{1}H NMR** ($CDCl_3$, 500 MHz): δ 7.23-7.20 (m, 5H, H-Ar^{tert}), 4.78 (d, 1H, ^2J = 11.0 Hz, CHPh), 4.44 (d, 1H, ^2J = 11.2 Hz, CHPh), 4.17 (d, 1H, $^2J_{5,5'}$ = 13.7 Hz, H-5a), 4.08 (d, 1H, $^3J_{1,2}$ = 6.7 Hz, H-1), 3.82 (d, 1H, $^2J_{5,5'}$ = 13.7 Hz, H-5a), 3.68 (d, 1H, $^3J_{1,2}$ = 6.7 Hz, H-2), 3.14 (d, 1H, $^3J_{3,4}$ = 4.1 Hz, H-3), 3.05 (d, 1H, $^3J_{3,4}$ = 4.1 Hz, H-4), 2.31 (b, 1H, OH-2). **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 137.05 (C-Ar^{quat}), 128.68(2C), 128.34(2C), 128.21 (5×C-Ar^{tert}), 101.51 (C-1), 70.44 (CH_2Ph), 66.97 (C-2), 62.98 (C-5), 54.05 (C-3), 49.84 (C-4). **HRMS (ESP):** Calcd. For $C_{12}H_{14}O_4Na$ $[M+Na]^{1+}$: 245.2297; Found: 245.2298

Benzyl 3,4-anhydro-2-*O*-triisopropylsilyl-α-D-arabinopyranoside (19)

To a solution of **18** (480 mg, 2.16 mmol, 1 equiv.) in DMF (10 mL), 2,6-lutidine (0.6 mL, 5.18 mmol, 2.4 equiv.) was added. The reaction mixture was then cooled down to 0°C. TIPSOTf (0.79 mL, 2.59 mmol, 1.2 equiv.) was added. The reaction mixture was then stirred at r.t. overnight. The reaction was diluted with EtOAc (10 mL) and washed with HCl (1 M), (2×10mL), water (2×10mL) and brine. The combined organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuum*. The residue was purified by flash chromatography (PE/EtOAc: 95/5, then 4/1) to give **19** (0.65 g, 79%) as a colorless oil. R_f= 0.8 (PE/EtOAc: 2/1). $[\alpha]_D^{25}$=+49.3(c 0.3, $CHCl_3$). **^{1}H NMR** ($CDCl_3$, 500 MHz): δ 7.35-7.27 (m, 5H, H-Ar^{tert}), 4.84 (d, 1H, ^2J = 10.6 Hz, CHPh), 4.52 (d, 1H, ^2J = 10.6 Hz, CHPh), 4.22 (d, 1H, $^2J_{5,5'}$ = 12.8 Hz, H-5a), 4.18 (d, 1H, $^3J_{1,2}$ = 6.4 Hz, H-1), 3.92-3.89 (m, 2H, H-5a, H-2), 3.19 (d, 1H, $^3J_{3,4}$ = 4.0 Hz, H-3), 3.16 (d, 1H, $^3J_{3,4}$ = 4.1 Hz, H-4), 1.06-1.02 (m, 21H, TIPS). **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 137.33 (C-Ar^{quat}), 128.41(2C), 128.34(2C), 127.80 (5×C-Ar^{tert}), 101.83 (C-1), 70.25 (CH_2Ph), 67.84 (C-2), 62.64 (C-5), 55.39 (C-3), 50.11 (C-4), 18.10, 18.04, 12.39 (TIPS). **HRMS (ESP):** Calcd. For $C_{12}H_{34}O_4SiNa$ $[M+Na]^{1+}$: 401.2119; Found: 401.2105

Benzyl 4-cyano-4-deoxy-2-*O*-triisopropylsilyl-β-L-xylopyranoside (20) and benzyl 3-deoxy-cyano-2-O-triisopropylsilyl-α-D-lyxopyranoside (21)

To a solution of **19** (0.635 g, 1.68 mmol, 1 equiv.) in dry Et_2O (15 mL) at 0 °C, Et_2AlCN (1.85 mL, 1.85 mmol, 1.1 equiv., 1 M in Et_2O) was added. The reaction mixture was then refluxed at 45 °C for 3h30, and stirred at r.t. for another 1 hr. The reaction mixture was cooled down to -40°C, and sat. NH_4Cl was added dropwise. After being stirred for 1 hr., the solid was filter and washed with EtOAc. The filtrate was evaporated and the residue was purified by flash chromatography (PE/EtOAc: 6/1, then 5/1) to give **20** (67 mg, 10%), **21** (270 mg, 40%), and starting material **19** (120 mg, 19%) as colorless oil.

20: R_f= 0.55 (PE/EtOAc: 3/1), **^{1}H NMR** ($CDCl_3$, 500 MHz): δ 7.38-7.29 (m, 5H, H-Ar^{tert}), 4.81 (d, 1H, ^2J = 11.5 Hz, CHPh), 4.64 (d, 1H, $^3J_{1,2}$ = 3.7 Hz, H-1), 4.54 (d, 1H, ^2J = 11.5 Hz, CHPh), 4.18 (dd, 1H, *J* = 12.1, 3.0 Hz, H-5a), 3.97 (dd, 1H, *J*= 12.7, 5.6 Hz, H-3), 3.76 (dd, 1H, *J* = 12.1, 5.1 Hz, H-5a), 3.71–3.68 (m, 1H, H-2), 3.33 (d, 1H, *J* = 7.5 Hz, OH-3), 2.81 (m, 1H, H-4), 1.06–0.99 (m, 21H, TIPS). **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 136.26 (C-Ar^{quat}), 128.70(2C), 128.53(2C), 128.46 (5×C-Ar^{tert}), 128.26 (CN), 100.36 (C-1), 70.95 (C-2), 70.60 (CH_2Ph), 70.43 (C-3), 57.81 (C-5),

32.03 (C-4), 18.10, 18.08, 12.44 (TIPS) **HRMS (ESP):** Calcd. For $C_{22}H_{35}NO_4SiNa$ $[M+Na]^{1+}$: 428.5998; Found: 428.6100

21: R_f = 0.50 (PE/EtOAc: 3/1), $[\alpha]_D^{20}$=+61.4(c 1.0, $CHCl_3$), **^{1}H NMR** ($CDCl_3$, 500 MHz): δ 7.38-7.31 (m, 5H, H-Ar^{tert}), 4.74 (d, 1H, ^2J = 11.9 Hz, CHPh), 4.64 (d, 1H, $^3J_{1,2}$ = 2.3 Hz, H-1), 4.50 (d, 1H, ^2J = 11.9 Hz, CHPh), 4.26 (ddd, 1H, *J* = 15.2, 10.1, 5.2 Hz, H-4), 4.18 (t, 1H, $^3J_{1,2}$ = 2.5 Hz, H-2), 3.81 (dd, 1H, *J* = 11.2, 5.0 Hz, H-5a), 3.56 (t, 1H, *J* = 10.8 Hz, H-5b), 3.08 (dd, 1H, *J* = 2.5, 10.0 Hz, H-3), 2.15 (d, 1H, *J* = 5.9 Hz, OH-4), 1.06–1.03 (m, 21H, TIPS). **^{13}C NMR** ($CDCl_3$, 125 MHz): δ 136.77 (C-Ar^{quat}), 128.71(2C), 128.38(2C), 128.33 (5×C-Ar^{tert}), 118.88 (CN), 96.40 (C-1), 69.37 (CH_2Ph), 69.36 (C-2), 63.75 (C-4), 63.21 (C-5), 39.60 (C-3), 18.13, 18.05, 12.65 (TIPS). **HRMS (ESP):** Calcd. For $C_{22}H_{35}NO_4SiNa$ $[M+Na]^{1+}$: 428.2155; Found: 428.2223

Benzyl 4-deoxy-cyano-β-L-xylopyranoside (22)

To a solution of **20** (0.067 g, 0.165 mmol, 1 equiv.) in dry THF (5 mL) at 0 °C, TBAF (0.314 mL, 0.314 mmol, 1.9 equiv., 1 M in THF) was added. The reaction mixture was stirred at r.t. for 3 hrs. The solvent was evaporated and the residue was purified by flash chromatography (DCM, then DCM/Acetone: 9/1) to give **22** (40 mg, 97%) as a white solid. R_f = 0.33 (DCM/acetone: 9/1), **^{1}H NMR** (CD_3OD, 500 MHz): δ 7.42–7.27 (m, 5H, H-Ar^{tert}), 4.87–4.84 (d, 1H, *J* = 11.8 Hz, CHPh), 4.64 (d, 1H, *J* = 11.8 Hz, CHPh), 4.36 (d, 1H, *J* = 7.6 Hz, H-1), 4.18–4.13 (dd, 1H, *J* = 4.6, 11.8 Hz, H-5a), 3.67 (dd, 1H, *J* = 9.0, 10.6 Hz, H-3), 3.60 (t, 1H, *J* = 11.4, H-5b), 3.21 (dd, 1H, *J* = 8.7, 7.6 Hz, H-2), 2.87 (td, 1H, *J* = 10.8, 4.8 Hz, H-4)). **^{13}C NMR** (CD_3OD, 125 MHz): δ 138.90 (C-Ar^{quat}), 129.36(2C), 129.21(2C), 128.81 (5×C-Ar^{tert}), 118.80 (CN), 104.10 (C-1), 75.58 (C-2), 73.51 (C-3), 71.95 (CH_2Ph), 63.23 (C-5), 36.88 (C-4), **HRMS (ESP):** Calcd. For $C_{13}H_{16}NO_4$ $[M+H]^{1+}$: 250.1079; Found: 250.1080

Iso-L-*gulo*-fagomine HCl salt (6)

Compound **22** (40 mg, 0.190 mmol) was dissolved in a 5 mL mixture of dioxane/water (4/1). To this mixture, 40 mg $Pd(OH)_2/C$ (20% w/w) was added, together with acetic acid (0.016 mL, 1.5 equiv.). The reaction mixture was stirred under hydrogen atmosphere for 48 hrs. The mixture was then filtrated through a Celite pad and the solvent was evaporated. After co-evaporation with HCl (1 M), iso-L-*gulo*-fagomine hydrochloride salt **6** (22 mg, 74%) was afforded as a white solid. NMR data was identical to previously published data.[16]

General procedures for *N*-alkylation of iminosugars

To a solution of iminosugar (0.060 mmol, 1 equiv.) in DMF (5 mL), anhydrous K_2CO_3 (0.018 mmol, 3 equiv) was added, followed by addition of 10-chloro-9-anthracenemethyl chloride (0.066 mmol, 1.1 equiv.). The reaction mixture was stirred at 80°C for 4 hrs. The reaction mixture was filtered and the solvent was evaporated. The residue was purified by flash chromatography ($CHCl_3$, then $CHCl_3$/MeOH 8/1) to give the corresponding 10-chloroanthracenyl iminosugars as yellow solid.

General procedures for reductive amination of iminosugars

A mixture of iminosugar (0.060 mmol, 1 equiv.) and aldehyde (0.075 mmol, 1.25 equiv.) in methanol (10 mL) and chloroform (1 mL) was heated to reflux until a homogenous solution was formed. Then $NaBH_3CN$ (0.15 mmol, 2.5 equiv.) and acetic acid (0.075 mmol, 1.25 equiv.) were added. The reaction mixture was refluxed overnight. After that, the solvent was evaporated and the residue was purified by flash chromatography ($CHCl_3$, then $CHCl_3$/MeOH 8/1) to give the corresponding 10-chloroanthracenyl iminosugars as yellow solid.

N-(2-(10-Chloroanthracen-9-yl)ethyl) isofagomine (9)

Yield (44%). R_f = 0.16 (DCM/MeOH: 7/1). **^{1}H NMR** (CD_3OD, 500 MHz): δ 8.50 (d, 2H, $^3J_{Ha,Hb}$ = 8.6 Hz, 2×H-a), 8.47 (d,

2H, $^{3}J_{Ha',Hc}$ = 8.7 Hz, 2×H-a'), 7.65-7.61 (m, 4H, 2×H-b, 2×H-c), 3.90 (dd, 1H, $^{3}J_{5',5}$ = 4.5 Hz, ^{2}J = 9.8 Hz, H-5'), 3.89-3.85 (m, 2H, 2×H-d), 3.69-3.62 (m, 2H, H-3, H-5'), 3.37-3.32 (m, 2H, H-2, H-6), 3.22 (t, 1H, ^{3}J = 8.4 Hz, H-4), 2.83 (t, 2H, *J* = 8.5 Hz, 2×H-e), 2.24-2.18 (m, 2H, H-2, H-6), 1.91 (m, 1H, H-5). **^{13}C NMR** (CD_3OD, 125 MHz): δ 131.12, 130.14(2C), 128.47(2C), 127.43 (6×C^{quat}), 126.31(2C) (2×C-b), 126.00(2C) (2×C-c), 124.95(2C) (2×C-a), 124.12(2C) (2×C-a'), 74.27 (C-4), 71.59 (C-3), 61.10 (C-5'), 58.07 (C-6), 57.80 (C-e), 54.81 (C-2), 43.68 (C-5), 24.72 (C-d). **HRMS (ESP):** Calcd. For $C_{22}H_{25}ClNO_3$ $[M+H]^{1+}$: 386.1447; Found: 386.1515

N-(3-(10-Chloroanthracen-9-yl)propyl) isofagomine (10)

Yield (57%). **R_f** = 0.23 (DCM/MeOH: 7/1). **^{1}H NMR** (CD_3OD, 500 MHz): δ 8.51 (d, 2H, $^{3}J_{Ha,Hc}$ = 8.7 Hz, 2×H-a), 8.38 (d, 2H, $^{3}J_{Ha',Hc}$ = 8.3 Hz, 2×H-a'), 7.63-7.58 (m, 4H, 2×H-b, 2×H-c), 3.80 (dd, 1H, $^{3}J_{5',5}$ = 3.5 Hz, ^{2}J = 11.0 Hz, H-5'), 3.67 (t, 2H, *J* = 7.9 Hz, 2×H-d), 3.60-3.53 (m, 2H, H-3, H-5'), 3.19-3.13 (m, 3H, H-2, H-4, H-6), 2.78 (t, 2H, *J* = 7.1 Hz, 2×H-f), 2.14-2.00 (m, 4H, H-2, H-6, 2×H-e), 1.81 (m, 1H, H-5) 7.65 (m, 2H,). **^{13}C NMR** (CD_3OD, 125 MHz): δ 135.38, 131.39(2C), 129.87(2C), 128.35 (6×C^{quat}), 127.65(2C) (2×C-b), 127.12(2C) (2×C-c), 126.28(2C) (2×C-a), 125.87(2C) (2×C-a'), 75.24 (C-4), 72.50 (C-3), 62.18 (C-5'), 59.02 (C-6), 58.41 (C-f), 55.99 (C-2), 44.64 (C-5), 29.00 (C-e), 26.08 (C-d). **HRMS (ESP):** Calcd. For $C_{23}H_{27}ClNO_3$ $[M+H]^{1+}$: 400.1680; Found: 400.1689

***N*-(10-Chloroanthracen-9-yl)methyl) iso-D-*galacto*-fagomine (13)**

Yield (53%). **R_f** = 0.33 (DCM/MeOH: 8/1). **^{1}H NMR** (CD_3OD, 500 MHz): δ 8.59 (d, 2H, $^{3}J_{Ha,Hb}$ = 8.4 Hz, 2×H-a), 8.54 (d, 2H, $^{3}J_{Ha',Hc}$ = 8.5 Hz, 2×H-a'), 7.65-7.58 (m, 4H, 2×H-b, 2×H-c), 4.53 (m, 2H, 2×H-d), 3.90 (br, 1H, H-4), 3.63 (dd, 1H, *J* = 10.0 Hz, *J* =6.6 Hz,H-5'a), 3.55-3.50 (m, 2H, H-5'b, H-3), 2.72-2.70 (m, 2H, H-6a, H-2), 2.58 (t, 1H, *J* = 10.4 Hz, H-2a), 2.47–2.38 (m, 1H, H-6a), 1.79 (m, 1H, H-5). **^{13}C NMR** (CD_3OD, 125 MHz): δ 133.12(3C), 129.76(3C) (6×C^{quat}), 127.68(2C) (2×C-b), 127.04(2C) (2×C-c), 126.78(2C) (2×C-a), 125.99(2C) (2×C-a'), 70.88 (C-3), 69.52 (C-4), 63.07 (C-5'), 54.90 (C-2), 54.65 (C-d), 51.10 (C-6), 43.88 (C-5). **HRMS (ESP):** Calcd. For $C_{21}H_{22}ClNaNO_3$ $[M+Na]^{1+}$: 394.1186; Found: 394.1184

***N*-(2-(10-Chloroanthracen-9-yl)ethyl) iso-D-*galacto*-fagomine (14)**

Yield (33%). **R_f** = 0.19 (DCM/MeOH: 7/1). **^{1}H NMR** (CD_3OD, 500 MHz): δ 8.54 (m, 2H, 2×H-a), 8.38 (m, 2H, 2×H-a'), 7.64-7.62 (m, 4H, 2×H-b, 2×H-c), 4.01 (t, 1H, ^{3}J = 2.2 Hz, H-4), 3.89 (m, 2H, 2×H-d), 3.78 (ddd, 1H, ^{3}J= 10.9, 4.7, 2.9 Hz, H-3), 3.73 (dd, 1H, *J* = 10.8 Hz, *J* = 6.4 Hz,H-5'), 3.63 (dd, 1H, *J* = 10.9 Hz, *J* = 7.2 Hz,H-5'), 3.00-2.94 (m, 2H, H-2, H-6), 2.81-2.78 (m, 2H, 2×H-e), 2.52 (t, 1H, ^{2}J = 10.6 Hz, H-2), 2.34 (t, 1H, ^{2}J = 11.6 Hz, H-6), 1.98 (m, 1H, H-5). **^{13}C NMR** (DMSO, 125 MHz): δ 133.05, 131.59(2C), 129.93(2C), 128.70 (6×C^{quat}), 127.74(2C) (2×C-b), 127.37(2C) (2×C-c), 126.37(2C) (2×C-a), 125.61(2C) (2×C-a'), 70.52 (C-3), 69.16 (C-4), 63.02 (C-5'), 59.62 (C-e), 54.69 (C-2), 50.64 (C-6), 43.66 (C-5), 26.12 (C-d). **HRMS (ESP):** Calcd. For $C_{22}H_{25}ClNO_3$ $[M+H]^{1+}$: 386.1523; Found: 386.1521

***N*-(3-(10-Chloroanthracen-9-yl)propyl) iso-D-*galacto*-fagomine (15)**

Yield (35%). **R_f** = 0.13 (DCM/MeOH: 7/1). **^{1}H NMR** (CD_3OD, 500 MHz): δ 8.52 (d, 2H, $^{3}J_{Ha,Hc}$ = 8.1 Hz, 2×H-a), 8.39 (d, 2H, $^{3}J_{Ha',Hc}$ = 7.8 Hz, 2×H-a'), 7.63-7.61 (m, 4H, 2×H-b, 2×H-c), 3.94 (t, 1H, ^{3}J = 2.6 Hz, H-4), 3.71-3.63 (m, 4H, H-3, H-5', 2×H-d), 3.53 (dd, 1H, $^{3}J_{5',5}$ = 7.9 Hz, ^{2}J = 10.9 Hz, H-5'), 2.83-2.74 (m, 4H, H-2, H-6, 2×H-f), 2.40 (t, 1H, 1H, ^{2}J = 11.4 Hz, H-2), 2.22 (t, 1H, 1H, ^{2}J = 11.5 Hz, H-6), 2.07-2.01 (m, 2H, 2×H-e), 1.91 (m, 1H, H-5). **^{13}C NMR** (CD_3OD, 125 MHz): δ 135.59, 131.39(2C), 129.87(2C), 128.29 (6×C^{quat}), 127.66(2C) (2×C-b), 127.10(2C) (2×C-c), 126.27(2C) (2×C-a), 125.91(2C) (2×C-a'), 70.02 (C-3), 68.87 (C-4), 62.75 (C-5'), 58.81 (C-f), 54.42 (C-2), 50.62 (C-6), 43.19 (C-5), 29.11 (C-e), 26.23 (C-d). **HRMS (ESP):** Calcd. For $C_{23}H_{27}ClNO_3$ $[M+H]^{1+}$: 400.1680; Found: 400.1679

***N*-(10-Chloroanthracen-9-yl)methyl) iso-D-*galacto*-fagomine (23)**

Yield (25%). $\mathbf{R_f}$ = 0.32 (DCM/MeOH: 8/1). **^{1}H NMR** (CD_3OD, 500 MHz): δ 8.55-8.52 (m, 4H, 2×H-a, 2×H-a'), 7.64-7.58 (m, 4H, 2×H-b, 2×H-c), 4.52 (m, 2H, 2×H-d), 3.69-3.66 (m, 1H, H-5'), 3.63-3.58 (m, 2H, H-4, H-5'a), 3.52-3.49 (m, 1H, H-3), 2.89-2.86 (m, 2H, H-6, H-2), 2.62 (dd, 1H, *J* = 11.6, 3.5 Hz, H-2), 2.42 (br, 1H, H-6), 2.09-2.04 (m, 1H, H-5). **HRMS (ESP):** Calcd. For $C_{21}H_{22}ClNaNO_3$ $[M+H]^{1+}$: 372.1366; Found: 372.1358

***N*-(2-(10-Chloroanthracen-9-yl)ethyl) iso-L-*gulo*-isofagomine (24)**

Yield (26%). $\mathbf{R_f}$ = 0.20 (DCM/MeOH: 7/1). **^{1}H NMR** (CD_3OD, 500 MHz): δ 8.54 (m, 2H, 2×H-a), 8.38 (m, 2H, 2×H-a'), 7.64-7.62 (m, 4H, 2×H-b, 2×H-c), 4.07-3.92 (m, 3H, 2×H-d, H-3), 3.87 (t, 1H, H-4), 3.77 (dd, 1H, *J* = 10.9, 5.8 Hz, H-5'), 3.66 (dd, 1H, *J* = 10.9, 7.5 Hz, H-5'), 3.29-3.14 (m, 5H, 2×H-e, 2×H-2, H-6), 3.00 (t, 1H, *J* = 11.5 Hz, H-6), 2.49 (br, 1H, H-5). **HRMS (ESP):** Calcd. For $C_{22}H_{25}ClNO_3$ $[M+H]^{1+}$: 386.1523; Found: 386.1583

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