



A remarkable change in inhibition potency and selectivity of isofagomine by simple N-modification

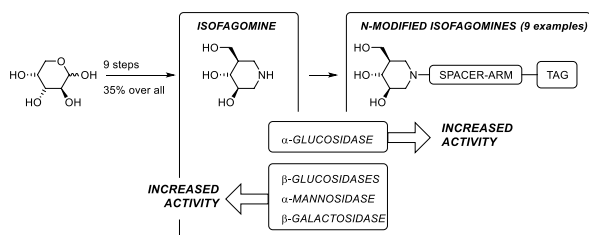
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Abstract

Herein, we present an alternative and elegant synthetic approach toward powerful β -glucosidase inhibitor isofagomine. Derivatizations of the ring nitrogen provided a selected set of N-modified isofagomine analogues. Biological evaluation of these compounds showed a remarkable change in potency as well as α/β -preference for various glycosidases from different sources when compared to the parent compound isofagomine. Overall, the conducted N-modification improved the potency against α -glucosidase from *Saccharomyces cerevisiae* (GH13). Coming along, significant diminished activities toward GH1 family β -glucosidases from three different sources have been observed for all tested derivatives. Moreover, and contrary to isofagomine, deactivations of β -galactosidase from *Escherichia coli* (GH2) as well as α -mannosidase from *Canavalia ensiformis* (GH38) have not been verified for this series of compounds.

Graphical abstract



Keywords Carbohydrates · Isofagomine · Glycoside hydrolases · N-modification · Glycosidase inhibitor · Henry reaction

Introduction

Isofagomine (**1**; IFG, 1,2,5-trideoxy-2-*C*-hydroxymethyl-1,5-imino-D-xylitol, or (3*R*,4*R*,5*R*)-5-(hydroxymethyl)piperidine-3,4-diol) [**1**], a nitrogen containing chimeric structure to D-glucose (**2**), has been proven as highly potent inhibitor for respective β -glucosidases (glycoside hydrolase family

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GH1 and GH30) [**2**, **3**]. Therefore, this prominent parent compound of isoiminosugars (**A**, Fig. **1**) gained profound interest in the broad field of glycosciences. Hence, developing synthetic approaches for access to suitable benchmark amounts and structural modifications for tailored biological elaborations is aimed for. Accordingly, dedicated derivatizations (Fig. **1**) of either the ring nitrogen (i.e., compound **4–9**) [**4–14**], selected alcohols (i.e., compound **10** and **11**) [**15–18**], or the carbon backbone (i.e., compound **12** and **13**) [**14**, **19**], as well as advanced combinations thereof (i.e., **14**) [**20**], provided a broad set of innovative inhibitors of respective glycosidases. In consequence, appropriate structures of this compound class have been elaborated upon their biological activity against selected human glycosidases related to lysosomal storage diseases [**3**, **21**]. In particular, Morbus

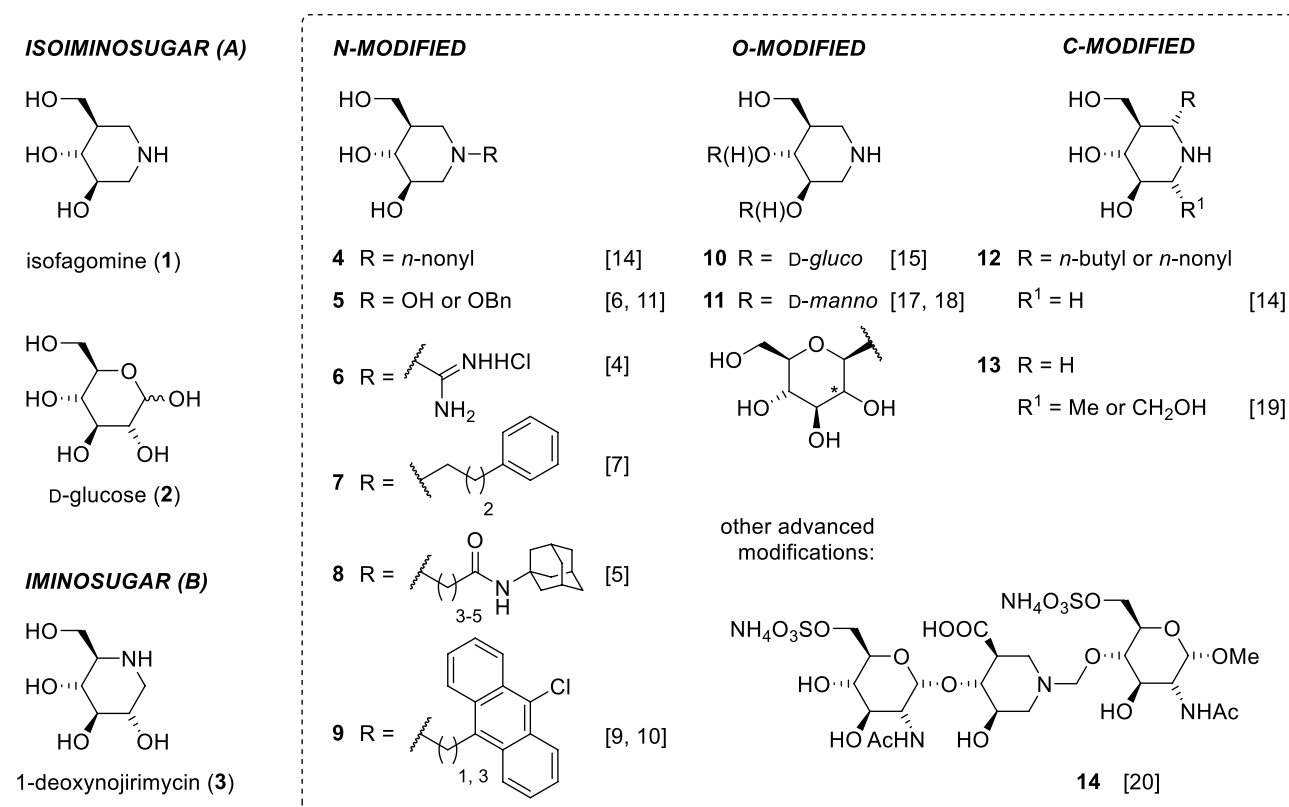


Fig. 1 Structures of isofagomine (1) and selected modified derivatives (3–14; dashed box) as representatives of isoiminosugars (A) and iminosugar analogue (B) 1-deoxynojirimycin (3) and D-glucose (2)

Gaucher (GD, OMIM #230,800, ORPHA35) [22], the disorder caused by impaired human β -glucosidase (glucocerebrosidase, GBA, EC. 3.2.1.45, GH30) is currently the focus of various dedicated investigations [5, 57].

Connected to this, guiding leaders in this field emphasized isofagomine (1) and analogues for their frequently strong complexation by general acid/base and catalytic nucleophilic amino acid residues (aspartic and/or glutamic acids) found in the active site of certain glycosidases [15, 16, 23–25]. Thereby, and contrasting the general behavior of related iminosugars (B), such isoiminosugars (A) interact with these two acid residues working as a “clamp” or “pincer” complexing the ring nitrogen and consequently exerting very powerful coordination (see Fig. 2).

This interesting feature of double coordination of the protonated inhibitor species with both active site residues can be seen as consequence of the inhibitors' structural characteristics. Accordingly, isofagomine (1) is not only unique in terms of active site binding but also poses synthetic challenges when compared to related iminosugars such as 1-deoxynojirimycin (3; 1,5-dideoxy-1,5-imino-D-glucitol, or (2*R*,3*R*,4*R*,5*S*)-2-(hydroxymethyl)piperidine-3,4,5-triol) [26]. Thus, respective C–C bond formation combined with the replacement of the anomeric carbon by a nitrogen atom

is required en route toward this carbon chain branched 2,5-dideoxygenated derivative of D-glucose (2).

The first synthesis of parent compound isofagomine (1) was reported by Lundt, Bols [1] and their co-workers taking advantage of the “Černý epoxide” [27] as their crucial intermediate. Valuable alternatives based on monosaccharidic starting materials were added to the repertoire by the groups of Bols [28], as well as by Stick [4, 29, 30]. Another sugar-based approach was published by Chmielewski who provided the (–) enantiomer [31]. In addition, a range of de novo syntheses were published starting, for example, from dihydrouracil [32], from butadiene monoxide [33–35], from enantiomerically enriched tris(hydroxymethyl)methane (2-hydroxymethyl-1,3-dihydroxypropane) [36, 37], and from chiral (lipase-resolved) *N*-BOC-5-hydroxy-3-piperidine [38]. Parent compound 1 was also accessed by intramolecular delivery of a D-tartaric acid-derived α -trimethylsilylmethylamine radical cation to a tethered acetylene moiety [39, 40], or starting from arecoline [41] and related intermediates [42, 43], as well as from methyl nicotinate [44].

Based on the indicated importance and exceptional biological properties of isofagomine (1) and related derivatives, complementing investigations connected to respective

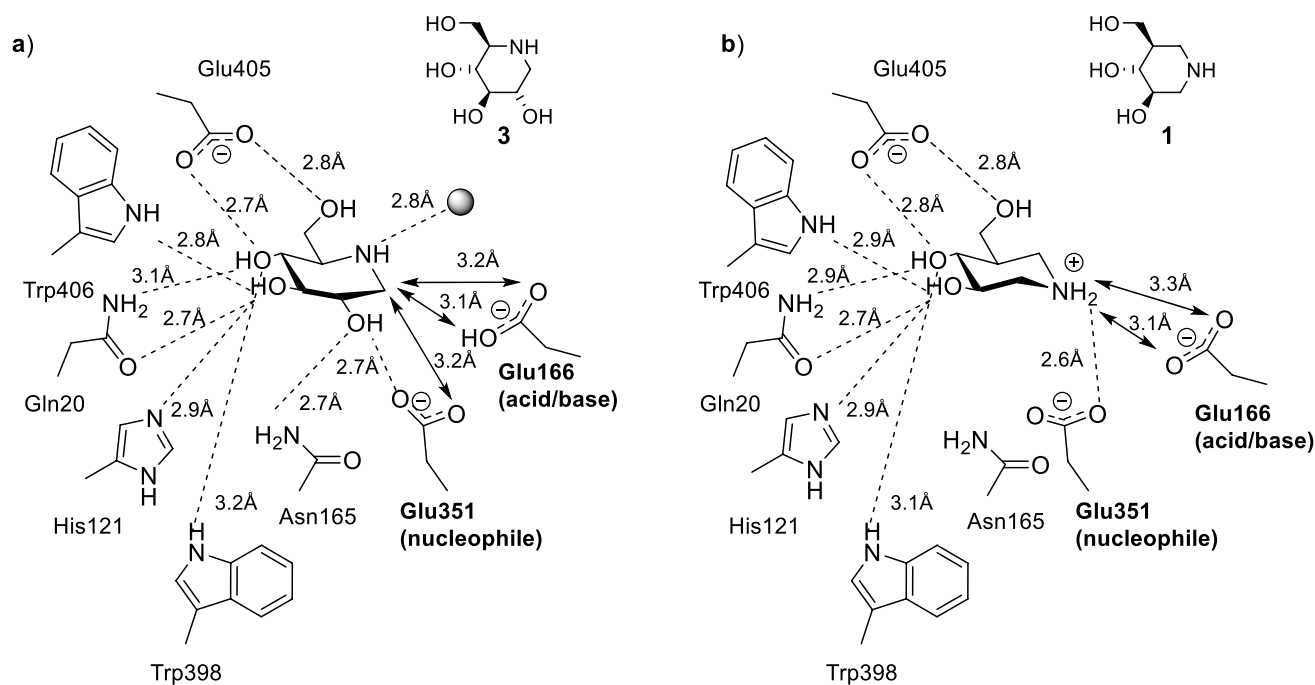


Fig. 2 Coordination studies based on co-crystals of **a** inhibitor **3** and **b** IFG (**1**), respectively, with β -glucosidase from *Thermotoga maritima* (*T. mar.*, GH1). Taken and rearranged from [25]

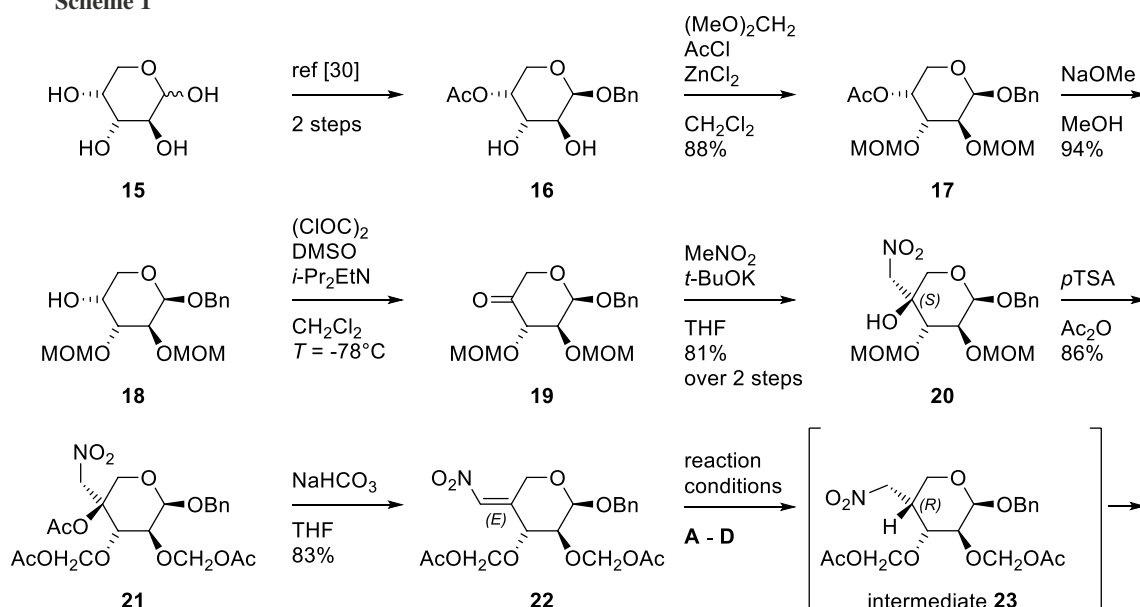
inhibition properties toward additional glycoside hydrolases, and in consequence a convenient access to suitable bench-amounts of the parent compound, can be deemed crucial. In this context, we herein present an adapted synthetic pathway starting from D-arabinose (**15**; see Scheme 1) inspired by “An Efficient” [28] as well as “Expeditious” [30] synthesis of isofagomine (**1**) provided by the teams of Bols and Stick, respectively. Starting from cheap and commercially available carbohydrate **15**, compound **18** was easily available by similar [30] and concise protecting group manipulations. Thus, initially selective access to position C-4 was gained (Scheme 1). In contrast to the following nucleophilic addition of a cyanide ion, we herein took advantage of a well-established [45–47] Henry reaction-based approach to simultaneously introduce a nitrogen atom combined with structurally required carbon chain branching at this position [28]. Accordingly, convenient Swern oxidation of alcohol **18** provided the respective ulose **19**, as suitable and analogous intermediate for the subsequent addition of nitromethane. As consequence of the sterically more demanding character of the herein globally O-protected compound **19**, this reaction turned out as highly stereoselective. Subsequently, similar and much to our delight also stereo controlled conversions of the resulted crystalline Henry-product **20** have been tested for an optimized access toward IFG (**1**). Thus, simple and straight forward, in this approach, virtually stereo selective as well as high yielding reactions enabled a convenient access to IFG (**1**) in nine steps and 35% overall yield. In the

following, we prepared a selected set of differently N-modified isofagomine derivatives (**27–35**) for in-depth biological evaluations with a series of different glycoside hydrolases. Two types of spacer arms providing a terminal azide functionality were introduced by simple N-alkylation reactions. To increase the water solubility of the final compounds, the first series of inhibitors provides an “ethylene glycol” based spacer arm (Scheme 2, series A). Their corresponding counterparts exhibit a simple alkyl chain (Scheme 2, series B), thereby targeting lipophilic interactions with respective areas adjacent to the active site of enzymes investigated herein. Subsequent reductive liberation of the primary amines **29** and **30** enabled concise conversions to selected sulfonamides, inhibitors **31–35** (see Scheme 3).

Results and discussion

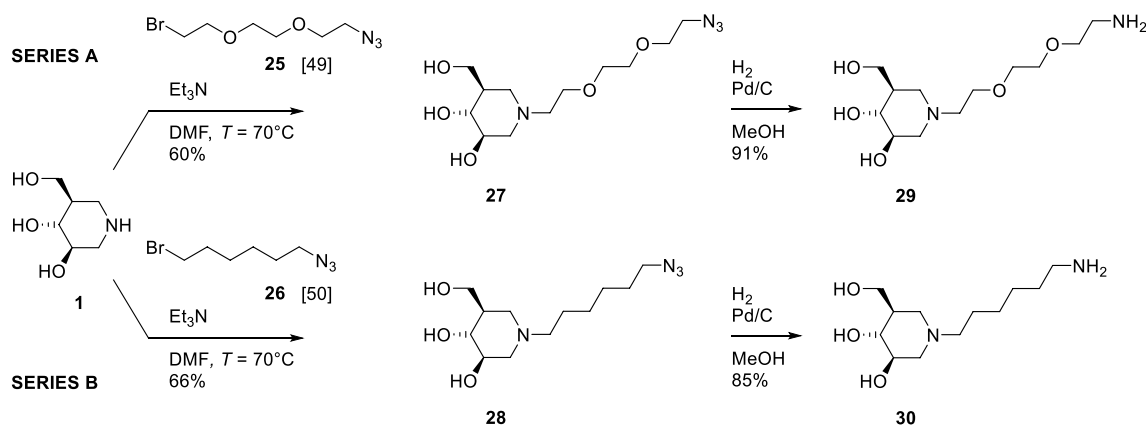
In detail, starting from crystalline (see Fig. 3) benzyl 4-O-acetyl- β -D-arabinopyranoside (**16**) [30], treatment with dimethoxymethane, acetyl chloride (AcCl), and catalytic amounts of zinc chloride (ZnCl_2) in dichloromethane (CH_2Cl_2) [48] provided global protected compound **17** in 88% (see Scheme 1). Subsequent liberation of OH-4 under Zemplén's conditions yielded alcohol **18**, which in turn was oxidized to ulose **19** employing Swern's methodology (93%, over 2 steps).

Scheme 1



	entry / ratio of	5- <i>epi</i> -IFG (24)	IFG (1)
24 R¹ = CH₂OH R² = H 1 R¹ = H R² = CH₂OH	A) methanolic HCl (3 <i>N</i>) then H ₂ , Pd(OH) ₂ /C in MeOH,	-	-
	B) methanolic HCl (3 <i>N</i>), and H ₂ , Pd(OH) ₂ /C, MeOH	1	2
	C) H ₂ , Pd(OH) ₂ /C in MeOH, then methanolic HCl (3 <i>N</i>)	1	3
	D) NaBH ₄ in MeOH, T = -10°C (yielding 23), then H ₂ , Pd(OH) ₂ /C, MeOH/HCl	traces	1

Scheme 2

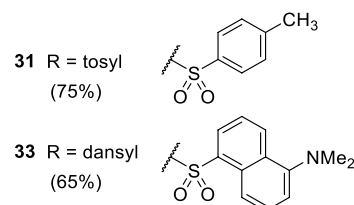
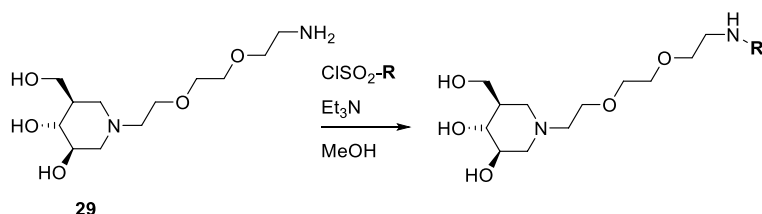


In a well-established synthetic procedure [45–47], including a common Henry reaction, followed by sequential activation and elimination sequence, the corresponding condensation product **22** was formed. In detail, compound **19** was treated with nitromethane (MeNO₂) and potassium *tert*-butoxide (*t*-BuOK) in tetrahydrofuran (THF) yielding the

Henry product **20** (81% starting from **18**). It is noteworthy to mention, that completed conversion of the starting material was already detected after stirring for 5 min at ambient temperature. To our delight, compound **20** was formed as virtually single and crystalline diastereomer confirmed by NMR analysis (4*S*/4*R* approx. 6/1 determined by NMR). Thereby,

Scheme 3

SERIES A



SERIES B

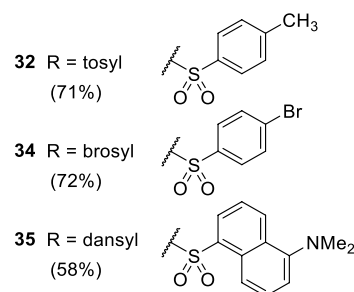
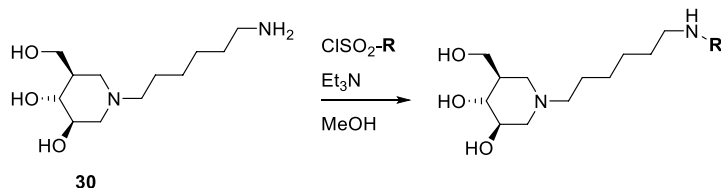
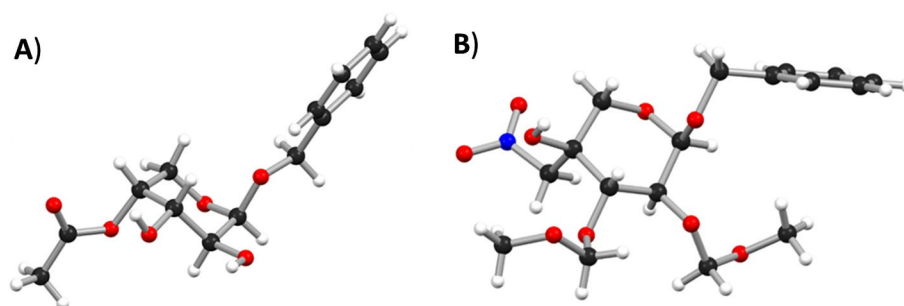


Fig. 3 Crystal structures of: **A** compound **16** (CCDC 2330318) and **B** compound **20** (CCDC 2330319), respectively



the nucleophilic attack predominantly (compare reiterated NMR-studies of corresponding crude reaction mixtures; Fig. SI-16a) occurred from *Ré-face*, which was structurally unambiguously confirmed by XRD-studies (see Fig. 3).

Subsequently, acid-catalyzed esterification of alcohol **20** in acetic anhydride (Ac_2O) provided compound **21** in 86% yield. Concomitantly, the methoxymethyl (MOM) protecting groups at positions O-2 and O-3 were converted into acetyloxymethylene groups. Assumed issues regarding chemical stability of these adapted protecting groups (coincident lability against acids as well as bases) could not have been verified in our hands, compound **21** turned out as highly bench stable. Moreover, stirring at $T=80^\circ\text{C}$ in toluene for 3 days only provided insignificant amounts of nitroalkene **22** and did not lead to any other decomposition of the starting material **21**. Even treatment with excess amounts of sodium borohydride (NaBH_4) in MeOH showed no influence on the protecting groups. Accordingly, the formal elimination of AcOH was induced by treatment of intermediate **21** with NaHCO_3 in THF at $T=40^\circ\text{C}$ (see Scheme 1). Following this, complete conversion of the starting material was

already obtained after 3 h in 83%. Thereby, predominantly the (*E*) configured stereoisomer of product **22** (*E/Z* approx. 8/1, in extended NMR-experiments (APT, NOE); see Fig. SI-16b and SI-16c) was formed. To optimize the final cyclization-deprotection sequence leading to IFG (**1**), different synthetic strategies (see Scheme 1) starting from nitroalkene **22** were tested. Initial cleavage of the protecting groups under strong acidic conditions, followed by catalytic hydrogenolysis (entry A) was not successful in our hands. Next, treatment with hydrogen in methanolic HCl over Pd/C (entry B) provided a mixture of isofagomine (**1**) and its C-5 epimer **24** in a ratio about 2:1, as well as several unidentified side products. In consequence, unsatisfying isolated yields (max. 20% of **1**) verified this entry B as not convenient for us. In contrast, initial stirring of compound **22** under an atmosphere of hydrogen over $\text{Pd}(\text{OH})_2/\text{C}$ in MeOH until complete reductive cyclization, followed by subsequent treatment with methanolic HCl (3 N), afforded free inhibitors **1** (71%) and **24** in a ratio of approximately 3:1 (entry C). Following entry D, this desired steric outcome was significantly increased by precedent reduction of the double bond with

sodium borohydride (NaBH_4) in MeOH, thereby obtaining the corresponding intermediate **23**. Triggered by lowered reaction temperatures, this stereoselective 1,4-addition of the hydride ion, predominantly formed the (4*R*) configured diastereomer (compare summarized NMRs of the respective crude reaction mixtures in Fig. SI-16d). Subsequent catalytic hydrogenolysis of intermediate **23** under strong acidic conditions in MeOH resulted in global O-deprotection and reduction of the nitro moiety. Concomitant cyclization in an intramolecular reductive amination reaction yielded the desired free IFG (**1**) in 88% starting from **22** (see Scheme 1).

For N-modification (Scheme 2), IFG (**1**) was treated with 1-azido-2-[2-(2-bromoethoxy)ethoxy]ethane (**25**) [49] in the presence of trimethylamine (Et_3N) in dimethylformamide (DMF) at $T = 70^\circ\text{C}$ to provide compound **27** in 60% yield. Accordingly, N-alkylated analogue **28** (66%) was obtained by conversion with 1-azido-6-bromohexane (**26**) [50]. Catalytic reduction of the respective azides in MeOH provided the corresponding amines **29** (91% yield from **27**) and **30** (85% yield from **28**), respectively.

In the following, common conversions of amines **29** and **30** with different sulfonyl chlorides and Et_3N in MeOH provided the corresponding sulfonamides **31–35** (see Scheme 3). In detail, conversions with 4-methylbenzene-1-sulfonyl chloride (“tosyl” chloride; TsCl) yielded the electron-rich aromatic sulfonamides **31** and **32** in 75% and

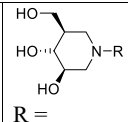
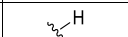
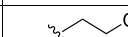
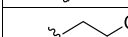
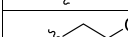
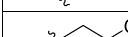
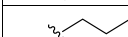
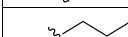
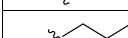
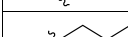
71%, respectively. The electron-poor aromatic system was provided by the formal exchange of the involved methyl group (+I effect) by a bromo substituent (–I effect). Thus, amine **30** was treated with 4-bromobenzene-1-sulfonyl chloride (“brosyl” chloride; BsCl) to give the corresponding NHbrosyl derivative **34** in 72%. As consequence of long-standing experiences with inhibitors providing 5-(dimethylamino)naphthalene-1-sulfonamides (“dansyl”), known to significantly increase biological activities with respective enzymes [51–55], we herein wanted to take advantage of equal functional modification. Accordingly, fluorescently tagged compounds **33** and **35** were synthesized via conversions of amines **29** and **30** with 5-(dimethylamino)naphthalene-1-sulfonyl chloride (“dansyl” chloride; dansylCl) in 65% and 58% yield, respectively.

Biological evaluation

All title compounds **27–35** were evaluated concerning their inhibitory activities against several glycoside hydrolases from various sources. In Table 1, the respective inhibition constants are collected and compared with parent compound isofagomine (**1**).

Series A inhibitors **27**, **31**, and **33**, N-derivatives with ethylene glycol spacer arms, showed a significant loss (up to 5700-fold) of activity toward tested GH1 family

Table 1 K_i -values [μM] of inhibitors **1** and **27–35**

	 R = Nr	β -Glc/Gal	β -Glc	β -Glc	α -Glc	β -Gal	α -Man
		Abg (GH1)	<i>T.mar.</i> (GH1)	<i>P.dul.</i> (GH1)	<i>S.cer.</i> (GH13)	<i>E.coli</i> (GH2)	<i>C.ens.</i> (GH38)
SERIES A	 1	0.015 ± 0.004	0.021 ± 0.002	0.41 ± 0.12	35.4 ± 3.1	86 ± 23	167 ± 14
	 27	31 ± 2^b	45.8 ± 6.1	65 ± 10	61.1 ± 4.4	N.I.	N.I.
	 29	6.3 ± 1.2^a	35.3 ± 4.9^c	31.4 ± 6.4	1154 ± 142	N.I.	N.I.
	 31	85 ± 14^a	97 ± 31	170 ± 17	184 ± 27^a	N.I.	N.I.
SERIES B	 33	27.8 ± 5.1^a	69 ± 7	78 ± 10	221 ± 24	N.I.	N.I.
	 28	4.5 ± 0.7	11.5 ± 2.1	25.2 ± 7.1	3.1 ± 0.2	N.I.	N.I.
	 30	0.14 ± 0.02	0.66 ± 0.13	1.24 ± 0.16	56.3 ± 3.6	N.I.	N.I.
	 32	18.7 ± 3.4^a	45.8 ± 6.1	70 ± 13	2.8 ± 0.3	1277 ± 183^b	N.I.
	 34	36.8 ± 6.8^a	89 ± 15	69 ± 17	1.8 ± 0.1	452 ± 38^b	N.I.
	 35	8.2 ± 1.2	35.4 ± 4.5	41.5 ± 5.9	9.7 ± 2.3^a	626 ± 33^b	N.I.

Abg β -glucosidase/ β -galactosidase from *Agrobacterium sp.* (GH1), *T. mar.* β -glucosidases from *Thermotoga maritima* (GH1), *P. dul.* β -glucosidases from *Prunus dulcis*, sweet almonds (GH1), *S. cer.* α -glucosidase from *Saccharomyces cerevisiae* (GH13), *E. coli* β -galactosidase from *Escherichia coli* (GH2), *C. ens.* α -mannosidase from *Canavalia ensiformis* (GH38), N.I. no inhibition, with $K_i > 1 \text{ mM}$

^aMixed-type inhibition

^bUncompetitive/non-competitive inhibition

^cNon-competitive inhibition

β -glucosidases when compared to parent compound IFG (**1**). In relation to this, according deactivation potencies toward α -glucosidase from *Saccharomyces cerevisiae* (*S. cer.*, GH13) were only slightly reduced (by factors of 2–6). However, amino derivative **29** provided a relatively stronger affinity toward tested β -glucosidases when compared to its corresponding “terminal nonpolar” analogues **27**, **31**, and **33**. Compared to all inhibitors presented herein, a considerably decreased inhibition of α -glucosidase from *S. cer.* (GH13) was observed for **29** ($K_i = 1154 \mu\text{M}$ vs. corresponding K_i -values ranging from 1.8 to 221 μM). Except relatively high potencies toward GH1 family β -glucosidases, related influences on the respective interactions with the remaining probed enzymes were not observed for corresponding amino derivative **30** from Series B. In contrast, investigations with the more lipophilic alkyl chain-based derivatives **28**, **32**, **34**, and **35** from Series B showed a remarkable change in the potency and α/β -preference. These compounds showed significantly improved (by factors of 4–20) activities against α -glucosidase from *S. cer.* (GH13). In this context, our best candidate (NHbrosyl derivative **34**, $K_i = 1.8 \mu\text{M}$) effectively provided a 20-fold increased potency compared to IFG (**1**, $K_i = 35.4 \mu\text{M}$). Simultaneously, an impressively diminished inhibition of β -glucosidases from *Thermotoga maritima* (*T. mar.*), *Prunus dulcis* (*P. dul.*) and β -glucosidase/ β -galactosidase from *Agrobacterium* sp. (Abg), members of the GH1 family, was observed (up to 4200-fold decreased activity) with the mentioned inhibitors **28**, **32**, **34**, and **35**. In this context, supporting depictions of the presented K_i -values are provided in Fig. SI-17a and Fig. SI-17b This observed trend regarding inhibition potency of the presented compounds is additionally indicated in Fig. 4, in which respective activities of each compound with probed enzymes are normalized and depicted as relatives ($f = K_{i \text{ isofagomine}}/K_{i \text{ compound}}$) to their parent compound **1**.

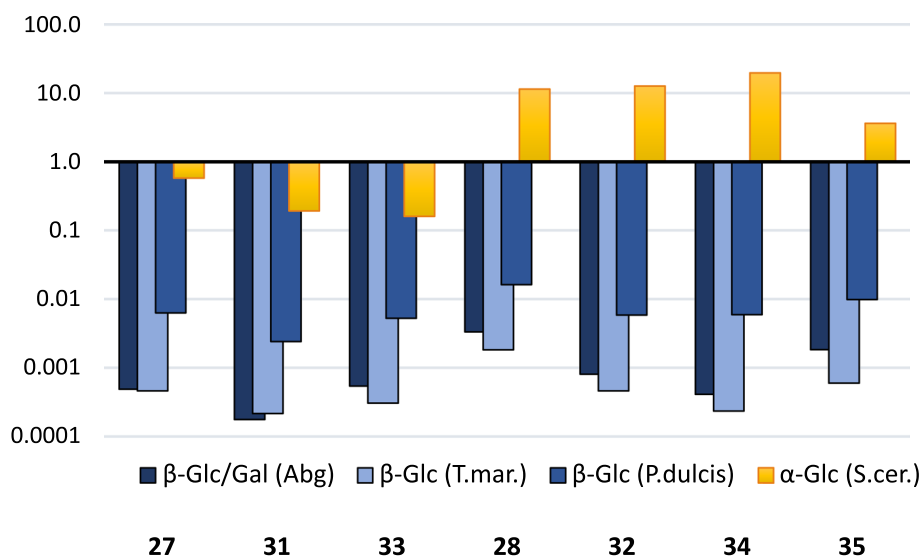
This trend in α/β -preference is additionally indicated in Fig. SI-17c with $F_{\text{sel}} = K_{i \beta\text{-glucosidases}}/K_{i \alpha\text{-glucosidase}}$ for respective inhibitors. In addition, and contrary to isofagomine (**1**), all presented inhibitors **27–35** showed no mentionable deactivation of β -galactosidase from *Escherichia coli* (*E. coli*, GH2) as well as α -mannosidase from *Canavalia ensiformis* (*C. ens.*, GH38).

Conclusion

We herein present an adapted, alternative synthesis of isofagomine (**1**) starting from cheap D-arabinose (**15**). Following a versatile protecting group strategy, selective access toward position C-4 was gained. Subsequent oxidation of the accessible alcohol, followed by consecutive treatment with nitromethane, provided predominantly one crystalline diastereomer of the corresponding “Henry-products”, compound **20**. Conversion to nitroalkene **22**, followed by distinct reduction and cyclization methods, provided isofagomine **1** in nine steps and 35% overall yield. Conventional *N*-alkylation of inhibitor **1**, followed by derivatizations of the introduced spacer arms, yielded a set of *N*-modified isofagomine derivatives **27–35**.

Compared to parent compound **1**, all tested compounds provided significantly reduced inhibition activities of GH1 family β -glucosidases from *Thermotoga maritima* (*T. mar.*), *Prunus dulcis* (*P. dul.*) and β -glucosidase/ β -galactosidase from *Agrobacterium* sp. (Abg). Simultaneously, an improved inhibitory potency against α -glucosidase from *Saccharomyces cerevisiae* (*S. cer.*, GH 13) was observed for *N*-alkyl modified inhibitors **28**, **32**, **34**, and **35** when compared to IFG (**1**). In addition, and contrary to IFG (**1**), all presented *N*-derivatives provided herein showed no mentionable deactivation of β -galactosidase from *E. coli* (GH2) as

Fig. 4 Relative inhibition activities of inhibitors **27**, **28**, and **31–35** compared to parent compound IFG (**1**) ($f = K_{i \text{ IFG}}/K_{i \text{ compound}}$)



well as α -mannosidase from *Canavalia ensiformis* (*C. ens.* (GH38). Possibly, these findings help in the development of novel compounds selectively interacting with (impaired) α -glucosidases, clinically relevant for a potential treatment of Pompe disease.

Experimental

Optical rotations were measured at 20 °C using a Perkin Elmer 341, or Schmidt&Haensch VariPol C101R-NMPVS polarimeter at 589 nm with a path length of 10 cm. $[\alpha]_D^{20}$ values are given in $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. NMR spectra were recorded on a Bruker Ultrashield spectrometer at 75.53 MHz (^{13}C) and 300.36 MHz (^1H), respectively. CDCl_3 was employed for protected compounds and CD_3OD as well as D_2O for unprotected inhibitors. Chemical shifts are listed in δ employing residual, non-deuterated solvent or residual H_2O (CD_3OD) as the internal standard. CDCl_3 : 7.26 ppm (^1H), 77.16 ppm (^{13}C); CD_3OD : 4.87 ppm (^1H), 49.0 ppm (^{13}C); D_2O : 4.79 ppm (^1H). The signals were unambiguously assigned by COSY (correlation spectroscopy) and HSQC (heteronuclear single-quantum correlation spectroscopy) analysis. For all structures, the respective numbering of each atom is indicated in the supporting information. MALDI-TOF was performed on a Micromass ToFSpec 2E Time-of-Flight mass spectrometer. High-resolution MS data were recorded using LC-TOF instrument (Agilent Technologies 6230 TOF LC/MS) equipped with an electrospray ionization source (ESI). Accurate mass determination was corrected by calibration using ESI-L Low Concentration Tuning Mix by Agilent Technologies. All reactions were monitored by thin-layer chromatography (TLC) performed on pre-coated aluminum plates with silica gel 60 F254 and detected with UV light (254 nm). For staining, a solution of vanillin (9 g) in a mixture of $\text{H}_2\text{O}/\text{EtOH}/\text{H}_2\text{SO}_4$ (950 cm^3 – 750 cm^3 – 120 cm^3) or ceric ammonium molybdate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ (100 g)/ $\text{Ce}(\text{SO}_4)_2\cdot 4\text{H}_2\text{O}$ (8 g) in 10% H_2SO_4 (1000 cm^3)) were employed, followed by heating on a hotplate.

General procedure A (hydrogenolysis over Pearlman's catalyst)

A 10% solution of the respective starting material in MeOH was stirred with $\text{Pd}(\text{OH})_2/\text{C}$ under an atmosphere of H_2 at ambient pressure. After completed conversion of the starting material, the catalyst was filtered off and the solvent was removed under reduced pressure.

General procedure B (N-alkylation)

A 5% solution of the respective amine in dry DMF was stirred with Et_3N (2.5–3.0 equiv.) and the selected

halocarbon (1.1 equiv.) at $T=70^\circ\text{C}$. After completed conversion of the starting material, the solvent was removed under reduced pressure to give the crude product.

General procedure C (sulfonamide formation)

To a 10% solution of the respective amine and Et_3N (2.5–3.0 equiv.) in MeOH, the corresponding sulfonyl chloride (1.2 equiv.) was added. After completed conversion of the starting material, the reaction mixture was evaporated to dryness.

Benzyl 4-O-acetyl-2,3-di-O-methoxymethylene- β -D-arabinopyranoside (17, $\text{C}_{18}\text{H}_{26}\text{O}_8$) To a stirred dispersion of dimethoxymethane (15.7 cm^3 , 214.6 mmol) and catalytic amounts of zinc chloride (48 mg, 0.35 mmol) in CH_2Cl_2 (250 cm^3), acetyl chloride (15.3 cm^3 , 214.6 mmol) was added dropwise. After stirring for 4 h, a solution of compound **16** (10.1 g, 35.7 mmol) and *i*-Pr₂EtN (74.9 cm^3 , 129.3 mmol) in CH_2Cl_2 (10 cm^3) was added dropwise. After stirring for additional 16 h, the reaction mixture was consecutively washed with HCl (2 N) and saturated aqueous NaHCO_3 . Removal of the dried (Na_2SO_4) solvent under reduced pressure followed by silica gel chromatography (cyclohexane/EtOAc, 8:1 v/v) provided compound **17** (11.7 g, 31.6 mmol , 88.3%) as a pale yellow oil. $R_f=0.4$ (CH/EA, 2:1 v/v); $[\alpha]_D^{20}=-123.6$ (MeOH, $c=1.10$); ^1H NMR (300 MHz, CDCl_3): $\delta=7.42$ – 7.22 (m, 5H, aromatic), 5.26 (m, 1H, H-4), 5.02 (d, 1H, $J_{1,2}=3.6 \text{ Hz}$, H-1), 4.79–4.51 (m, 6H, 4x $-\text{OCH}_2\text{OCH}_3$, 2x $-\text{OCH}_2\text{Ph}$), 4.10 (dd, 1H, $J_{2,3}=10.2 \text{ Hz}$, $J_{3,4}=3.6 \text{ Hz}$, H-3), 3.92 (dd, 1H, H-2), 3.88–3.80 (m, 1H, H-5a), 3.62 (dd, 1H, $J_{5a,5b}=12.8 \text{ Hz}$, $J_{4,5b}=2.0 \text{ Hz}$, H-5b), 3.33 (s, 3H, $-\text{OCH}_2\text{OCH}_3$), 3.24 (s, 3H, $-\text{OCH}_2\text{OCH}_3$), 2.10 (s, 3H, $\text{CH}_3\text{CO}-$) ppm; ^{13}C NMR (75.5 MHz, CDCl_3): $\delta=169.7$ (carbonyl), 136.5 (ipso aromatic), 127.7, 127.6, 127.2 (aromatic), 96.8 (C-1), 96.7, 95.5 (2x $-\text{OCH}_2\text{OCH}_3$), 73.9 (C-2), 71.3 (C-3), 69.7 (C-4), 68.7 ($-\text{OCH}_2\text{Ph}$), 60.0 (C-5), 54.8, 54.6 (2x $-\text{OCH}_2\text{OCH}_3$), 20.4 ($\text{CH}_3\text{CO}-$) ppm; MS (MALDI-TOF): m/z calculated for $[\text{C}_{18}\text{H}_{26}\text{O}_8\text{Na}]$ ($[\text{M}+\text{Na}]^+$) 393.1525, found 393.1524.

Benzyl 2,3-di-O-methoxymethylene- β -D-arabinopyranoside (18, $\text{C}_{16}\text{H}_{24}\text{O}_7$) To a 10% solution of compound **17** (6.2 g, 16.7 mmol) in MeOH, catalytic amounts of NaOMe were added to adjust pH 12. After completed conversion of the starting material, the reaction mixture was neutralized by addition of strongly acidic ion-exchange resin (Amberlite IR-120H⁺). After stirring for 5 min, filtration and evaporation of the solvent, the crude product was purified by silica gel chromatography (cyclohexane/EtOAc, 10:1 v/v) to give compound **18** (5.2 g, 15.8 mmol , 94.6%) as a pale yellow syrup. $R_f=0.3$ (CH/EA, 1:1 v/v); $[\alpha]_D^{20}=-110.0$ (MeOH, $c=1.30$); ^1H NMR (300 MHz, CDCl_3): $\delta=7.44$ – 7.23 (m, 5H, aromatic), 5.01 (d, 1H, $J_{1,2}=3.2 \text{ Hz}$, H-1), 4.85–4.49

(m, 6H, 4x $-\text{OCH}_2\text{OCH}_3$, 2x $-\text{OCH}_2\text{Ph}$), 4.05–3.90 (m, 3H, H-2, H-3, H-4), 3.82 (d, 1H, $J_{5a,5b} = 12.5$ Hz, $J_{4,5a} < 1$ Hz, H-5a), 3.69 (d, 1H, H-5b), 3.38 (s, 3H, $-\text{OCH}_2\text{OCH}_3$), 3.24 (s, 3H, $-\text{OCH}_2\text{OCH}_3$), 2.95 (bs, 1H, $-\text{OH}$) ppm; ^{13}C NMR (75.5 MHz, CDCl_3): $\delta = 137.1$ (ipso aromatic), 128.2, 128.0, 127.8 (aromatic), 97.3 (C-1), 97.1, 96.6 (2x $-\text{OCH}_2\text{OCH}_3$), 74.9 (C-4), 74.2 (C-2), 69.1 ($-\text{OCH}_2\text{Ph}$), 68.5 (C-3), 61.9 (C-5), 55.5, 55.1 (2x $-\text{OCH}_2\text{OCH}_3$) ppm; MS (MALDI-TOF): m/z calculated for $[\text{C}_{16}\text{H}_{24}\text{O}_7\text{Na}]$ ($[\text{M} + \text{Na}]^+$) 351.1420, found 351.1833.

Benzyl 4-C-nitromethyl-2,3-di-O-methoxymethylene- α -L-xylopyranoside (20, $\text{C}_{17}\text{H}_{25}\text{NO}_9$) To a solution of DMSO (3.0 cm^3 , 42.8 mmol) in 25 cm^3 CH_2Cl_2 , oxalyl chloride (2.7 cm^3 , 32.1 mmol) was added dropwise at $T = -78$ °C. After stirring for 30 min, alcohol **18** (5.0 g, 15.3 mmol, dissolved in 25 cm^3 CH_2Cl_2) was added dropwise and the reaction mixture was stirred for 20 min. Addition of Et_3N (11.6 cm^3 , 84.0 mmol) and stirring for additionally 20 min completed the conversion of the reactants. The reaction mixture was consecutively washed with HCl (2 N) and saturated aqueous NaHCO_3 . Removal of the dried (Na_2SO_4) solvent under reduced pressure gave the crude ulose **19** (4.9 g) which was immediately employed in the next step without further purification. [Crude compound **19** is practically stable at $T = -18$ °C for ca. 1 week. However, purification on silica gel induces undesired enolization/epimerization reactions.] $R_f = 0.4$ (CH/EA, 1:1 v/v).

To a freshly prepared suspension of MeNO_2 (11.5 cm^3 , 214.6 mmol) and $t\text{-BuOK}$ (1.7 g, 15.3 mmol) in THF (30 cm^3), crude compound **19** (calculated for 15.3 mmol of alcohol **18**, dissolved in 20 cm^3 THF) was dropwise added. After TLC indicated completed conversion of the starting material (ca. 15 min), the reaction mixture was diluted with CH_2Cl_2 and consecutively washed with HCl (2 N) and aqueous saturated NaHCO_3 . [Longer reaction times induced the formation of additional products; most likely resulted from Retro-Henry and/or connected isomerization reactions.] Subsequently, the combined organic layers were dried over Na_2SO_4 , filtered off and evaporated to give crude compound **20** (please compare Supporting Information Fig. SI-16a) as a white solid. Purification on silica gel (cyclohexane/EtOAc, 5:1 v/v) provided product **20** (4.7 g, 12.13 mmol, 79.4%) as a colorless crystals. Re-crystallization from cyclohexane/EtOAc (approx. 7:1 v/v) enabled XRD studies for the unambiguous structural assignment of compound **20**. $R_f = 0.5$ (CH/EA, 1:1 v/v); $[\alpha]_D^{20} = +27.3$ (EA, $c = 1.28$); ^1H NMR (300 MHz, CDCl_3): $\delta = 7.35\text{--}7.21$ (m, 5H, aromatic), 4.89 (d, 1H, $J_{1,2} = 3.8$ Hz, H-1), 4.76–4.46 (m, 6H, 4x $-\text{OCH}_2\text{OCH}_3$, 2x $-\text{OCH}_2\text{Ph}$), 4.64 (s, 2H, H-4'), 3.87 (d, 1H, $J_{5a,5b} = 11.9$ Hz, H-5a), 3.81 (d, 1H, $J_{2,3} = 9.9$ Hz, H-3), 3.66 (d, 1H, H-5b), 3.46 (s, 3H, $-\text{OCH}_2\text{OCH}_3$), 3.33 (dd, 1H, H-2), 3.17 (s, 3H, $-\text{OCH}_2\text{OCH}_3$) ppm; ^{13}C NMR (75.5 MHz,

CDCl_3): $\delta = 136.6$ (ipso aromatic), 128.6, 128.6, 128.3 (aromatic), 99.2, 97.3 (2x $-\text{OCH}_2\text{OCH}_3$), 96.2 (C-1), 84.7 (C-3), 76.6 (C-4'), 74.6 (C-2), 71.7 (C-4), 69.5 ($-\text{OCH}_2\text{Ph}$), 61.3 (C-5), 56.7, 55.6 (2x $-\text{OCH}_2\text{OCH}_3$) ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{17}\text{H}_{25}\text{NO}_9\text{Na}]$ ($[\text{M} + \text{Na}]^+$) 410.1427, found 410.1411; m.p.: 114–116 °C (recrystallized from ethyl acetate and cyclohexane).

Benzyl 4-O-acetyl-4-C-nitromethyl-2,3-di-O-acetoxymethylene- α -L-xylopyranoside (21, $\text{C}_{21}\text{H}_{27}\text{NO}_{12}$) Compound **20** (3.50 g, 9.03 mmol) was dissolved in 35 cm^3 Ac_2O . After addition of catalytic amounts of *para*-toluenesulfonic acid (*pTSA*), the reaction mixture was stirred for 18 h. After consecutive addition of crushed ice cubes and (ice-cooled) water (ca. 100 cm^3) under vigorous stirring (for 30 min), the reaction mixture was diluted with CH_2Cl_2 . Two additional washing steps of the aqueous phase with CH_2Cl_2 and subsequent neutralization of the organic layer via extraction with saturated aqueous NaHCO_3 provided crude product **21**. Purification on silica gel (cyclohexane/EtOAc, 10:1 v/v) gave product **21** (3.8 g, 7.83 mmol, 86.6%) as a colorless syrup. $R_f = 0.8$ (CH/EA, 1:1 v/v); $[\alpha]_D^{20} = -83.1$ (EA, $c = 0.92$); ^1H NMR (300 MHz, CDCl_3): $\delta = 7.35\text{--}7.21$ (m, 5H, aromatic), 5.26 (m, 2H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 5.16 (m, 1H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 5.05 (m, 1H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 5.03 (d, 1H, $J_{4'a,4'b} = 12.1$ Hz, H-4'a), 4.84 (d, 1H, H-4'b), 4.82 (d, 1H, H-1), 4.73 (d, 1H, $J = 12.4$ Hz, $-\text{OCH}_2\text{Ph}$), 4.53 (m, 2H, H-3, $-\text{OCH}_2\text{Ph}$), 4.12 (d, 1H, $J_{5a,5b} = 12.0$ Hz, H-5a), 4.05 (d, 1H, H-5b), 3.66 (dd, 1H, $J_{1,2} = 3.6$, $J_{2,3} = 9.4$ Hz, H-2), 2.06 (s, 3H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 2.04 (s, 3H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 1.89 (s, 3H, $-\text{O}(\text{CO})\text{CH}_3$) ppm; ^{13}C NMR (75.5 MHz, CDCl_3): $\delta = 170.4$, 170.2, 170.1 (3x $-\text{O}(\text{CO})\text{CH}_3$), 136.6 (ipso aromatic), 128.7, 128.2, 128.0 (aromatic), 95.9 (C-1), 89.0 (2x $-\text{CH}_2\text{O}(\text{CO})\text{CH}_3$), 80.1 (C-4), 78.2 (C-3), 77.5 (C-2), 73.3 (C-4'), 69.7 ($-\text{OCH}_2\text{Ph}$), 60.2 (C-5), 21.9, 21.1, 21.0 (3x $-\text{O}(\text{CO})\text{CH}_3$) ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{21}\text{H}_{27}\text{NO}_{12}\text{NH}_4]$ ($[\text{M} + \text{NH}_4]^+$) 503.1912, found 503.1865.

(E)-Benzyl 4-deoxy-4-C-nitromethylene-2,3-di-O-ethanoylmethylene- β -D-arabinopyranoside (22, $\text{C}_{19}\text{H}_{23}\text{NO}_{10}$) Compound **21** (3.7 g, 7.62 mmol) was dissolved in 40 cm^3 THF and stirred for 10 h at $T = 45$ °C after addition of catalytic amounts of NaHCO_3 . Subsequently, the solvents were removed under reduced pressure to provide crude compound **22** (please compare Supporting Information Fig. SI-16b). Purification on silica gel (cyclohexane/EtOAc, 10:1 v/v) gave product **22** (2.70 g, 6.35 mmol, 83.3%) as a pale yellow syrup. $R_f = 0.7$ (CH/EA, 3:2 v/v); $[\alpha]_D^{20} = -251.6$ (EA, $c = 1.13$); ^1H NMR (300 MHz, CDCl_3): $\delta = 7.35\text{--}7.26$ (m, 5H, aromatic), 7.11 (s, 1H, H-4'), 5.39–5.22 (m, 3H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 5.14 (d, 1H, H-5a), 5.09 (d, 1H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 4.95 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 4.75

(d, 1H, $J = 12.3$ Hz, $-\text{OCH}_2\text{Ph}$), 4.66 (d, 1H, H-3), 4.56 (d, 1H, $-\text{OCH}_2\text{Ph}$), 4.25 (d, 1H, $J_{5a,5b} = 14.8$ Hz, H-5b), 3.70 (dd, 1H, $J_{2,3} = 9.8$ Hz, H-2), 2.03 (s, 3H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 1.90 (s, 3H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$) ppm; ^{13}C NMR (75.5 MHz, CDCl_3): $\delta = 170.5$, 170.2 (2x $-\text{O}(\text{CO})\text{CH}_3$), 145.3 (C-4), 136.6 (ipso aromatic), 134.9 (C-4'), 128.7, 128.3, 128.1 (aromatic), 96.2 (C-1), 89.2, 88.7 (2x $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 81.2 (C-2), 76.0 (C-3), 69.8 ($-\text{OCH}_2\text{Ph}$), 56.7 (C-5), 21.0 (2x $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$) ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{19}\text{H}_{23}\text{NO}_{10}\text{NH}_4]$ ($[\text{M} + \text{NH}_4]^+$) 443.1701, found 443.1646.

4-Deoxy-4-C-nitromethyl-2,3-di-O-ethanoylmethylene- β -D-arabinopyranoside (23, $\text{C}_{19}\text{H}_{25}\text{NO}_{10}$) To a solution of compound **22** (2.19 g, 5.15 mmol) in MeOH (30 cm^3), NaBH_4 (1.29 g, 33.97 mmol) was carefully added at $T = -10^\circ\text{C}$. After TLC indicated completed conversion of the starting material (ca. 5–10 min), the reaction mixture was neutralized by addition of acidic ion-exchange resin (amberlite IR-120H $^+$). Subsequently, filtration and evaporation of the solvent followed by purification on silica gel (cyclohexane/EtOAc, 15:1 v/v) provided a mixture of compound **23** (2.05 g, 4.80 mmol, 93.2%) and traces of an inseparable unidentified species (most likely an isomeric analogue of **23**) as a white wax. $R_f = 0.6$ (CH/EA, 3:2 v/v); $[\alpha]_D^{20} = -222.0$ (CHCl_3 , $c = 1.56$); ^1H NMR (300 MHz, CDCl_3): $\delta = 7.35$ – 7.18 (m, 5H, aromatic), 5.39 (d, 1H, $J = 6.5$ Hz, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 5.30 (d, 1H, $J = 6.5$ Hz, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 5.09 (m, 2H, 2x $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 4.82 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1), 4.70 (d, 1H, $J = 12.3$ Hz, $-\text{OCH}_2\text{Ph}$), 4.59 (dd, 1H, $J_{4,4'a} = 3.7$, $J_{4'a,4'b} = 13.1$ Hz, H-4'a), 4.48 (m, 2H, 1x $-\text{OCH}_2\text{Ph}$, H-4'b), 4.12 (dd, 1H, $J_{2,3} = 9.4$, $J_{3,4} = 5.5$ Hz, H-3), 3.87 (dd, 1H, $J_{4,5a} = 2.8$, $J_{5a,5b} = 12.3$ Hz, H-5a), 3.50 (dd, 1H, H-2), 3.45 (dd, 1H, $J_{4,5b} = 9.2$ Hz, H-5b), 2.92 (m, 1H, H-4), 2.03 (s, 3H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 1.89 (s, 3H, $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$) ppm; ^{13}C NMR (75.5 MHz, APT, CDCl_3): $\delta = 170.5$, 170.5 (2x $-\text{O}(\text{CO})\text{CH}_3$), 136.9 (ipso aromatic), 128.6, 128.1, 128.1 (aromatic), 89.1 (C-1), 89.2, 88.7 (2x $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$), 76.5 (C-2), 76.3 (C-3), 58.9 (C-4'), 69.6 ($-\text{OCH}_2\text{Ph}$), 58.9 (C-5), 39.5 (C-5), 21.0 (2x $-\text{OCH}_2\text{O}(\text{CO})\text{CH}_3$) ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{19}\text{H}_{25}\text{NO}_{10}\text{NH}_4]$ ($[\text{M} + \text{NH}_4]^+$) 445.1857, found 445.1823.

1,2,5-Trideoxy-2-C-hydroxymethyl-1,5-imino-D-xylitol; or (3R,4R,5R)-5-(hydroxymethyl)piperidine-3,4-diol; or isofagomine (1) **Entry C:** Compound **22** (0.81 g, 1.90 mmol) was dissolved in 15 cm^3 MeOH and stirred over catalytic amounts of $\text{Pd}(\text{OH})_2/\text{C}$ under an atmosphere of H_2 . After 18 h, 2 cm^3 methanolic HCl (3 N) were added and the reaction mixture was stirred for additional 12 h. Subsequently, the catalyst was filtered off and the solvent was removed under reduced pressure. Purification on silica ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 8:1:1 v/v/v) provided compound **24** (49 mg,

0.34 mmol, 17.9%) and isofagomine (**1**, 201 mg, 1.36 mmol, 71.4%) as a white solid, respectively.

Entry D: To a solution of compound **22** (0.85 g, 1.99 mmol) in MeOH (10 cm^3), NaBH_4 (0.45 g, 11.98 mmol) was carefully added at $T = -10^\circ\text{C}$. After TLC indicated completed conversion of the starting material (ca. 5–10 min), the reaction mixture was neutralized by addition of acidic ion-exchange resin (amberlite IR-120H $^+$). Subsequently, filtration and evaporation of the solvent provided crude intermediate **23** (please compare Supporting Information Fig. SI-16d) which was directly dissolved in a mixture of 10 cm^3 MeOH and 2 cm^3 methanolic HCl (3 N) and stirred over catalytic amounts of $\text{Pd}(\text{OH})_2/\text{C}$ under an atmosphere of H_2 for 16 h. Subsequently, the catalyst was filtered off and the solvent was removed under reduced pressure. Purification on silica provided title compound isofagomine (**1**, 260 mg, 1.77 mmol, 88.4%) as a white solid, respectively. NMR data fits to the literature [1].

(3R,4R,5R)-1-[2-[2-(2-Azidoethoxy)ethoxy]-ethyl]-5-(hydroxymethyl)piperidine-3,4-diol; or N-(8-azido-3,6-dioxaoctyl)isofagomine (27, $\text{C}_{12}\text{H}_{24}\text{N}_4\text{O}_5$) Following general procedure B, compound **1** (121 mg, 0.815 mmol) was treated with 1-azido-2-[2-(2-bromoethoxy)ethoxy]ethane (**25**, 214 mg, 0.897 mmol) [49]. Purification on silica gel ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 4:4:1 v/v/v) provided N-alkylated derivative **27** (160 mg, 0.532 mmol, 64.5%) as a colorless syrup. $R_f = 0.5$ ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 4:4:1 v/v/v); $[\alpha]_D^{20} = +10.0$ (MeOH, $c = 1.10$); ^1H NMR (300 MHz, D_2O): $\delta = 3.81$ (dd, 1H, $J_{5,6a} = 3.4$, $J_{6a,6b} = 11.4$ Hz, H-6a), 3.77–3.66 (m, 8H, 2x H-2', 2x H-3', 2x H-4', 2x H-5'), 3.65–3.57 (m, 2H, H-3, H-6b), 3.51 (t, 2H, $J = 4.9$ Hz, 2x H-6'), 3.24 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 3.12 (dd, 1H, H-2eq), 3.06 (dd, 1H, H-5aeq), 2.70 (t, 2H, $J = 5.6$ Hz, 2x H-1'), 2.08 (dd, 1H, $J_{5aax,5aeq} = J_{5,5aax} = 11.9$ Hz, H-5aax), 2.07 (dd, 1H, $J_{2ax,2eq} = J_{2ax,3} = 11.0$ Hz, H-2ax), 1.77 (m, 1H, H-5) ppm; ^{13}C NMR (75.5 MHz, D_2O): $\delta = 73.7$ (C-4), 71.1 (C-3), 69.6, 69.4, 69.2, 67.6 (C-2', C-3', C-4', C-5'), 60.7 (C-6), 57.5 (C-2), 56.0 (C-1'), 54.5 (C-5a), 50.2 (C-6'), 42.7 (C-5) ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{12}\text{H}_{24}\text{N}_4\text{O}_5\text{H}]$ ($[\text{M} + \text{H}]^+$) 305.1825, found 305.1835.

(3R,4R,5R)-1-[2-[2-(2-Aminoethoxy)ethoxy]-ethyl]-5-(hydroxymethyl)piperidine-3,4-diol; or N-(8-amino-3,6-dioxaoctyl)isofagomine (29, $\text{C}_{12}\text{H}_{26}\text{N}_2\text{O}_5$) According to general procedure A, compound **27** (122 mg, 0.394 mmol) was converted into its corresponding crude amine **29**. Subsequent purification on silica gel ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 4:4:1 v/v/v) provided compound **29** (103 mg, 0.361 mmol, 91.1%) as a colorless syrup. $R_f = 0.4$ ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 1:2:1 v/v/v); $[\alpha]_D^{20} = +12.4$ (MeOH, $c = 1.45$); ^1H NMR (300 MHz, D_2O): $\delta = 3.80$ (dd, 1H, $J_{5,6a} = 3.4$, $J_{6a,6b} = 11.4$ Hz, H-6a), 3.73–3.53 (m, 10H, H-3, H-6b, 2x H-2', 2x H-3', 2x H-4', 2x H-5'), 3.22

(dd, 1H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4), 3.10 (dd, 1H, H-2eq), 3.04 (dd, 1H, H-5aeq), 2.80 (t, 2H, $J=5.4$ Hz, $2\times$ H-6'), 2.68 (t, 2H, $J=5.6$ Hz, $2\times$ H-1'), 2.05 (dd, 1H, $J_{5ax,5aeq}=J_{5,5ax}=11.8$ Hz, H-5aax), 2.04 (dd, 1H, $J_{2ax,2eq}=J_{2ax,3}=10.7$ Hz, H-2ax), 1.77 (m, 1H, H-5) ppm; ^{13}C NMR (75.5 MHz, D_2O): $\delta=73.7$ (C-4), 71.1 (C-3), 71.9, 69.5, 69.5, 67.7 (C-2', C-3', C-4', C-5'), 60.7 (C-6), 57.6 (C-2), 54.6 (C-1'), 54.5 (C-5a), 42.8 (C-5), 39.8 (C-6') ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{12}\text{H}_{26}\text{N}_2\text{O}_5\text{H}]$ ($[\text{M}+\text{H}]^+$) 279.1920, found 279.1927.

***N*-[2-[2-[2-[(3*R*,4*R*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)piperidin-1-yl]ethoxy]ethoxy]ethyl]-4-methylbenzenesulfonamide; or *N*-(8-(tosylamino)-3,6-dioxaoctyl)isofagomine (31, $\text{C}_{19}\text{H}_{32}\text{N}_2\text{O}_7\text{S}$)** Following general procedure C, compound **29** (52.3 mg, 0.19 mmol) was treated with 4-methylbenzene-1-sulfonyl chloride (42.9 mg, 0.23 mmol). Purification on silica gel ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 4:4:1 v/v/v) provided Nhtosyl derivative **31** (61.0 mg, 0.14 mmol, 75.1%) as a colorless syrup. $R_f=0.5$ ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 4:4:1 v/v/v); $[\alpha]_{\text{D}}^{20}=+10.0$ (MeOH, $c=0.90$); ^1H NMR (300 MHz, D_2O): $\delta=7.77$ (d, 2H, $J=8.3$ Hz, aromatic), 7.46 (d, 2H, $J=8.0$ Hz, aromatic), 3.80 (dd, 1H, $J_{5,6a}=3.4$, $J_{6a,6b}=11.4$ Hz, H-6a), 3.70–3.46 (m, 10H, H-3, H-6b, $2\times$ H-2', $2\times$ H-3', $2\times$ H-4', $2\times$ H-5'), 3.22 (dd, 1H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4), 3.22 (t, 2H, $J=5.2$ Hz, $2\times$ H-6'), 3.05 (m, 2H, H-2eq, H-5aeq), 2.66 (t, 2H, $J=5.6$ Hz, $2\times$ H-1'), 2.43 (s, 3H, Ar- CH_3 tosyl), 2.05 (dd, 1H, $J_{5ax,5aeq}=J_{5,5ax}=11.9$ Hz, H-5aax), 2.04 (dd, 1H, $J_{2ax,2eq}=J_{2ax,3}=10.5$ Hz, H-2ax), 1.76 (m, 1H, H-5) ppm; ^{13}C NMR (75.5 MHz, D_2O): $\delta=145.0$, 135.3 (ipso), 130.1, 126.7 (aromatic), 73.7 (C-4), 71.1 (C-3), 69.4, 69.3, 68.8, 67.7 (C-2', C-3', C-4', C-5'), 60.7 (C-6), 57.5 (C-2), 55.9 (C-1'), 54.5 (C-5a), 42.7 (C-5), 42.2 (C-6'), 20.7 (Ar- CH_3 tosyl) ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{19}\text{H}_{32}\text{N}_2\text{O}_7\text{SH}]$ ($[\text{M}+\text{H}]^+$) 433.2008, found 433.2025.

***N*-[2-[2-[2-[(3*R*,4*R*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)piperidin-1-yl]ethoxy]ethoxy]ethyl]-5-(dimethylamino)naphthalene-1-sulfonamide; or *N*-(8-(dansylamino)-3,6-dioxaoctyl)isofagomine (33, $\text{C}_{24}\text{H}_{37}\text{N}_3\text{O}_7\text{S}$)** Following general procedure C, compound **29** (43.1 mg, 0.15 mmol) was treated with 5-(dimethylamino)naphthalene-1-sulfonyl chloride (50.1 mg, 0.18 mmol). Subsequent purification on silica gel ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 4/4/1 v/v/v) provided NH-dansylated derivative **33** (52.0 mg, 0.10 mmol, 65.6%) as a fluorescent wax. $R_f=0.4$ ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 4:4:1 v/v/v); $[\alpha]_{\text{D}}^{20}=+8.0$ (MeOH, $c=0.88$); ^1H NMR (300 MHz, MeOD): $\delta=8.49$ (d, 1H, $J=8.5$ Hz, aromatic), 8.28 (d, 1H, $J=8.6$ Hz, aromatic), 8.14 (d, 1H, $J=7.3$ Hz, aromatic), 7.52 (m, 2H, aromatic), 7.21 (d, 1H, $J=7.5$ Hz, aromatic), 3.74 (dd, 1H, $J_{5,6a}=3.7$, $J_{6a,6b}=11.0$ Hz, H-6a), 3.54–3.21 (m, 10H, H-3, H-6b, $2\times$ H-2', $2\times$ H-3', $2\times$ H-4', $2\times$ H-5'), 3.07 (m, 3H, H-2eq,

H-4, H-5aeq), 2.99 (t, 2H, $J=6.8$ Hz, $2\times$ H-6'), 2.82 (s, 6H, Ar- $\text{N}(\text{CH}_3)_2$ dansyl), 2.64 (t, 2H, $J=5.4$ Hz, $2\times$ H-1'), 2.04 (dd, 1H, $J_{5ax,5aeq}=J_{5,5ax}=11.9$ Hz, H-5aax), 2.01 (dd, 1H, $J_{2ax,2eq}=J_{2ax,3}=11.0$ Hz, H-2ax), 1.76 (m, 1H, H-5) ppm; ^{13}C NMR (75.5 MHz, MeOD): $\delta=153.2$, 137.4, 131.2, 131.1 (ipso), 131.0, 130.0, 129.1, 124.3, 120.7, 116.4 (aromatic), 75.5 (C-4), 72.6 (C-3), 71.2, 71.1, 70.6, 69.0 (C-2', C-3', C-4', C-5'), 62.4 (C-6), 59.5 (C-2), 58.2 (C-1'), 56.5 (C-5a), 45.8 (Ar- $\text{N}(\text{CH}_3)_2$ dansyl), 44.6 (C-5), 43.8 (C-6') ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{24}\text{H}_{37}\text{N}_3\text{O}_7\text{SH}]$ ($[\text{M}+\text{H}]^+$) 512.2430, found 512.2454.

(3*R*,4*R*,5*R*)-1-(6-Azidoheptyl)-5-(hydroxymethyl)piperidine-3,4-diol; or *N*-(6-azidoheptyl)isofagomine (28, $\text{C}_{12}\text{H}_{24}\text{N}_4\text{O}_3$) Following general procedure B, compound **1** (153 mg, 1.04 mmol) was treated with 1-azido-6-bromohexane (**26**, 236 mg, 1.14 mmol) [50]. Purification on silica gel ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 8:4:1 v/v/v) provided N-alkylated derivative **28** (182 mg, 0.67 mmol, 64.3%) as a pale yellow syrup. $R_f=0.4$ ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 8:4:1 v/v/v); $[\alpha]_{\text{D}}^{20}=+14.6$ (MeOH, $c=0.96$); ^1H NMR (300 MHz, MeOD): $\delta=3.82$ (dd, 1H, $J_{5,6a}=3.6$, $J_{6a,6b}=10.9$ Hz, H-6a), 3.52 (m, 2H, H-3, H-6b), 3.30 (m, 2H, $2\times$ H-6'), 3.11 (dd, 1H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4), 3.07–2.99 (m, 2H, H-2eq, H-5aeq), 2.39 (m, 2H, $2\times$ H-1'), 1.86 (dd, 1H, $J_{2ax,2eq}=J_{2ax,3}=11.0$ Hz, H-2ax), 1.86 (dd, 1H, $J_{5ax,5aeq}=J_{5,5ax}=11.0$ Hz, H-5aax), 1.79–1.70 (m, 1H, H-5), 1.67–1.49 (m, 4H, $2\times$ H-2', $2\times$ H-5'), 1.48–1.30 (m, 4H, $2\times$ H-3', $2\times$ H-4') ppm; ^{13}C NMR (75.5 MHz, MeOD): $\delta=76.0$ (C-4), 73.0 (C-3), 62.7 (C-6), 59.8 (C-2), 59.3 (C-1'), 56.5 (C-5a), 52.4 (C-6'), 44.9 (C-5), 29.8, 28.1, 27.7, 27.6 (C-2', C-3', C-4', C-5') ppm; MS (ESI-TOF): m/z calculated for $[\text{C}_{12}\text{H}_{24}\text{N}_4\text{O}_3\text{H}]$ ($[\text{M}+\text{H}]^+$) 273.1926, found 273.1936.

(3*R*,4*R*,5*R*)-1-(6-Aminoheptyl)-5-(hydroxymethyl)piperidine-3,4-diol; or *N*-(6-aminoheptyl)isofagomine (30, $\text{C}_{12}\text{H}_{26}\text{N}_2\text{O}_3$) According to general procedure A, compound **28** (173 mg, 0.64 mmol) was converted into its corresponding crude amine **30**. Subsequent purification on silica gel ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 8:4:1 v/v/v) provided compound **30** (133 mg, 0.54 mmol, 85.0%) as a colorless syrup. $R_f=0.2$ ($\text{CHCl}_3/\text{MeOH}/\text{NH}_4\text{OH}$ (conc.), 8:4:1 v/v/v); $[\alpha]_{\text{D}}^{20}=+13.7$ (MeOH, $c=1.03$); ^1H NMR (300 MHz, MeOD): $\delta=3.81$ (dd, 1H, $J_{5,6a}=3.7$, $J_{6a,6b}=10.9$ Hz, H-6a), 3.52 (m, 2H, H-3, H-6b), 3.13–2.98 (m, 3H, H-2eq, H-4, H-5aeq), 2.91 (t, 2H, $J=6.8$ Hz, $2\times$ H-6'), 2.43 (m, 2H, $2\times$ H-1'), 1.90 (dd, 1H, $J_{5ax,5aeq}=J_{5,5ax}=11.1$ Hz, H-5aax), 1.90 (dd, 1H, $J_{2ax,2eq}=J_{2ax,3}=11.1$ Hz, H-2ax), 1.79–1.49 (m, 5H, $2\times$ H-2', $2\times$ H-5', H-5), 1.48–1.30 (m, 4H, $2\times$ H-3', $2\times$ H-4') ppm; ^{13}C NMR (75.5 MHz, MeOD): $\delta=75.8$ (C-4), 72.9 (C-3), 62.5 (C-6), 59.6 (C-2), 59.1 (C-1'), 56.4 (C-5a), 44.8 (C-5), 40.7 (C-6'), 28.5, 28.0, 27.4, 27.2 (C-2', C-3', C-4', C-5')

ppm; MS (ESI-TOF): m/z calculated for $[C_{12}H_{26}N_2O_3H]^+$ ($[M+H]^+$) 247.2021, found 247.2024.

***N*-[6-[(3*R*,4*R*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)-piperidin-1-yl]hexyl]-4-methylbenzenesulfonamide; or *N*-((6-*to*-sylamino)hexyl)isofagomine (32, $C_{19}H_{32}N_2O_5S$)** Following general procedure C, compound **30** (45.0 mg, 0.18 mmol) was treated with 4-methylbenzene-1-sulfonyl chloride (41.8 mg, 0.22 mmol). Purification on silica gel ($CHCl_3/MeOH/NH_4OH$ (conc.), 8:4:1 v/v/v) provided Nhtosyl derivative **32** (52.0 mg, 0.1323 mmol, 71.1%) as a colorless syrup. R_f = 0.4 ($CHCl_3/MeOH/NH_4OH$ (conc.), 8:4:1 v/v/v); $[\alpha]_D^{20}$ = 14.5 (MeOH, c = 1.10); 1H NMR (300 MHz, MeOD): δ = 7.73 (d, J = 8.3 Hz, 2H, aromatic), 7.38 (d, J = 8.0 Hz, 2H, aromatic), 3.83 (dd, 1H, $J_{5,6a}$ = 3.7, $J_{6a,6b}$ = 11.0 Hz, H-6a), 3.52 (m, 2H, H-3, H-6b), 3.10 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.2 Hz, H-4), 3.07–2.99 (m, 2H, H-2eq, H-5aeq), 2.83 (t, 2H, J = 6.8 Hz, 2 × H-6'), 2.44 (s, 3H, Ar-CH₃ tosyl), 2.42–2.33 (m, 2H, 2 × H-1'), 1.90 (dd, 1H, $J_{5aax,5aeq}$ = $J_{5,5aax}$ = 11.0 Hz, H-5aax), 1.89 (dd, 1H, $J_{2ax,2eq}$ = $J_{2ax,3}$ = 11.0 Hz, H-2ax), 1.75 (m, 1H, H-5), 1.57–1.38 (m, 4H, 2 × H-2', 2 × H-5'), 1.37–1.19 (m, 4H, 2 × H-3', 2 × H-4') ppm; ^{13}C NMR (75.5 MHz, MeOD): δ = 144.5, 139.1 (ipso), 130.7, 128.0 (aromatic), 75.9 (C-4), 73.0 (C-3), 62.6 (C-6), 59.6 (C-2), 59.2 (C-1'), 56.4 (C-5a), 44.9 (C-5), 43.9 (C-6'), 30.5, 28.0, 27.5, 27.5 (C-2', C-3', C-4', C-5'), 21.4 (Ar-CH₃ tosyl) ppm; MS (ESI-TOF): m/z calculated for $[C_{19}H_{32}N_2O_5SH]^+$ ($[M+H]^+$) 401.2110, found 401.2124.

4-Bromo-*N*-[6-[(3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)-piperidin-1-yl]hexyl]benzenesulfonamide; or *N*-((6-brosylamino)hexyl)isofagomine (34, $C_{18}H_{29}BrN_2O_5S$) Following general procedure C, compound **30** (42.0 mg, 0.17 mmol) was treated with 4-bromobenzene-1-sulfonyl chloride (52.3 mg, 0.2046 mmol). Purification on silica gel ($CHCl_3/MeOH/NH_4OH$ (conc.), 8:4:1 v/v/v) provided Nhbrosyl derivative **34** (57.1 mg, 0.12 mmol, 71.8%) as a white solid. R_f = 0.4 ($CHCl_3/MeOH/NH_4OH$ (conc.), 8:4:1 v/v/v); $[\alpha]_D^{20}$ = + 8.0 (MeOH, c = 1.00); 1H NMR (300 MHz, MeOD): δ = 7.75 (s, 4H, aromatic), 3.83 (dd, 1H, $J_{5,6a}$ = 3.7, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.54 (m, 2H, H-3, H-6b), 3.26–3.03 (m, 3H, H-2 eq, H-4, H-5aeq), 2.86 (t, J = 6.8 Hz, 2H, 2 × H-6'), 2.44 (m, 2H, 2 × H-1'), 1.98 (dd, 1H, $J_{5aax,5aeq}$ = $J_{5,5aax}$ = 11.7 Hz, H-5aax), 1.96 (dd, 1H, $J_{2ax,2eq}$ = $J_{2ax,3}$ = 11.0 Hz, H-2ax), 1.75 (m, 1H, H-5), 1.56–1.38 (m, 4H, 2 × H-2', 2 × H-5'), 1.34–1.24 (m, 4H, 2 × H-3', 2 × H-4') ppm; ^{13}C NMR (75.5 MHz, MeOD): δ = 141.4, 128.0 (ipso), 133.4, 129.8 (aromatic), 75.6 (C-4), 72.7 (C-3), 62.4 (C-6), 59.3 (C-2), 59.1 (C-1'), 56.2 (C-5a), 44.7 (C-5), 43.9 (C-6'), 30.5, 27.9, 27.4, 27.3 (C-2', C-3', C-4', C-5') ppm; MS (ESI-TOF): m/z calculated for $[C_{18}H_{29}BrN_2O_5SH]^+$ ($[M+H]^+$) 467.1038, found 467.1043.

***N*-[6-[(3*R*,4*R*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)piperidin-1-yl]hexyl]-5-(dimethylamino)naphthalene-1-sulfonamide; or *N*-((6-dansylamino)hexyl)isofagomine (35, $C_{24}H_{37}N_3O_5S$)** Following general procedure C, compound **30** (43 mg, 0.17 mmol) was treated with 5-(dimethylamino)naphthalene-1-sulfonyl chloride (56.5 mg, 0.2095 mmol). Purification on silica gel ($CHCl_3/MeOH/NH_4OH$ (conc.), 4:4:1 v/v/v) provided NH-dansylated derivative **35** (49.2 mg, 0.10 mmol, 58.5%) as a fluorescent wax. R_f = 0.3 ($CHCl_3/MeOH/NH_4OH$ (conc.), 8:4:1 v/v/v); $[\alpha]_D^{20}$ = + 9.8 (MeOH, c = 1.22); 1H NMR (300 MHz, MeOD): δ = 8.56 (d, 1H, J = 8.5 Hz, aromatic), 8.36 (d, 1H, J = 8.5 Hz, aromatic), 8.19 (d, 1H, J = 7.5 Hz, aromatic), 7.62–7.53 (m, 2H, aromatic), 7.28 (d, 1H, J = 7.5 Hz, aromatic), 3.82 (dd, 1H, $J_{5,6a}$ = 3.5, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.51 (m, 2H, H-3, H-6b), 3.08 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.3 Hz, H-4), 2.97 (m, 2H, H-2eq, H-5aeq), 2.89 (s, 6H, Ar-N(CH₃)₂ dansyl), 2.84 (m, 2H, 2 × H-6'), 2.24 (m, 2H, 2 × H-1'), 1.82 (dd, 1H, $J_{5aax,5aeq}$ = $J_{5,5aax}$ = 11.2 Hz, H-5aax), 1.80 (dd, 1H, $J_{2ax,2eq}$ = $J_{2ax,3}$ = 11.2 Hz, H-2ax), 1.30 (m, 1H, H-5), 1.34–1.24 (m, 4H, 2 × H-2', 2 × H-5'), 1.19–0.98 (m, 4H, 2 × H-3', 2 × H-4') ppm; ^{13}C NMR (75.5 MHz, MeOD): δ = 153.2, 137.3, 131.2, 131.0 (ipso), 131.1, 130.2, 129.1, 124.3, 120.7, 116.4, (aromatic), 76.1 (C-4), 73.0 (C-3), 62.7 (C-6), 59.7 (C-2), 59.2 (C-1'), 56.5 (C-5a), 45.9 (Ar-N(CH₃)₂ dansyl), 44.9 (C-5), 43.8 (C-6'), 30.4, 28.0, 27.5, 27.3 (C-2', C-3', C-4', C-5') ppm; MS (MALDI-TOF): m/z calculated for $[C_{24}H_{37}N_3O_5SNa]^+$ ($[M+Na]^+$) 502.2352, found 502.2362.

Kinetic studies with compounds 1 and 27–35

Enzyme activities were assayed spectrophotometrically using 96-well plates from SARSTEDT on a Spark® Multimode Microplate Reader (TECAN Group AG, Switzerland). In the inhibition kinetic experiments, three different substrate concentrations ($[S] < K_m$, $[S] = K_m$, $[S] > K_m$) were used. For each substrate concentration, the before optimized concentration of enzyme was pre-incubated with the inhibitors at seven different concentrations and one control sample without inhibitor, referring to 100% of activity at the specific conditions. The initial rate was determined by measuring the release of *p*-nitrophenol at the expense of *p*-nitrophenyl glycoside specific for each enzyme at 405 nm for up to 5 min. Initial rates were calculated in Excel and Lineweaver–Burk plots ($1/v$ vs. $1/[S]$ for each inhibitor concentration) were constructed to validate the use of competitive, non-competitive or mixed-type inhibition models and to assess the fit of the data. The data were then fit to the appropriate inhibition model using non-linear regression analysis with GraFit 7.0.3. (Erithacus Software).

Specific assay conditions for each enzyme:

Agrobacterium sp. β -glucosidase GH1 (Abg): 50 mM sodium phosphate buffer (pH 7) at 25 °C. Substrate: *p*NP β -Glc, K_m =0.2 mM, [S]=0.1 mM, 0.2 mM, 0.4 mM.

Thermotoga maritima β -glucosidase GH1 (*T. mar.*): 50 mM sodium maleate buffer (pH 6.5) at 37 °C. Substrate: *p*NP β -Glc, K_m =0.7 mM, [S]=0.4 mM, 0.7 mM, 1.4 mM [56].

Prunus dulcis β -glucosidase GH1 (*P. dul.*, sweet almonds): 50 mM sodium phosphate buffer (pH 7) at 25 °C. Substrate: *p*NP β -Glc, K_m =4.8 mM, [S]=3.6 mM, 4.8 mM, 6.0 mM [56].

Saccharomyces cerevisiae α -glucosidase GH13 (*S. cer.*): 50 mM sodium phosphate buffer (pH 7) at 25 °C. Substrate: *p*NP α -Glc, K_m =3.2 mM, [S]=1.6 mM, 3.2 mM, 6.3 mM.

Escherichia coli β -galactosidase GH2 (*E. coli*): 50 mM sodium phosphate buffer, 1.0 mM $MgCl_2$ (pH 7) at 25 °C. Substrate: *p*NP β -Gal, K_m =53 μ M, [S]=35 μ M, 55 μ M, 100 μ M.

Canavalia ensiformis α -mannosidase GH38 (*C. ens.*): 50 mM sodium phosphate buffer (pH 7) at 25 °C. Substrate: *p*NP α -Manno, K_m =0.9 mM, [S]=0.5 mM, 0.9 mM, 2.0 mM.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00706-024-03210-7>.

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Data availability Not applicable.

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