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Synthesis of *C*-5a-substituted derivatives of 4-epi-isofagomine: notable β-galactosidase inhibitors and activity promotors of G_{M1}-gangliosidosis related human lysosomal β-galactosidase mutant R201C

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ABSTRACT

From an easily available partially protected analog of 1-deoxy-L-*gulo*-nojirimycin, by chain-branching at C-4 and suitable modification, lipophilic analogs of the powerful β-D-galactosidase inhibitor 4-epi-isofagomine have been prepared. New compounds exhibit considerably improved inhibitory activities when compared with the unsubstituted parent compound and may serve as leads toward new pharmacological chaperones for G_{M1}-gangliosidosis and Morquio B disease.

1. Introduction

Imino sugars in general and, in particular, iminoalditols, such as 1-deoxynojirimycin (**1**) and its epimer at C-4 (**2**) as well as bicyclic analog castanospermine (**3**) are typical examples of powerful inhibitors of glycoside hydrolases, and as such have proved to be valuable tools for enzymatic investigation and characterization.[1–5] Due to the vital roles of these enzymes, specific inhibitors may exert notable biological activities against various pathological processes, including certain forms of hereditary lysosomal disorders.[6–9] This latter group of about 50 metabolic diseases arises from mutations in specific genes that lead to deficiencies in enzymes involved in the lysosomal degradation of glycolipids and glycans. Considerable efforts have been made to provide novel therapeutic compounds that may relieve the various symptoms arising from the inability of lysosomal glycosidases to degrade their respective substrates. These glycosides consequently accumulate in the cells and lead to irreversible damage of nerve tissue, bones as well as various organs resulting in painful long-term suffering of patients and their families without the hope for a cure.

Efforts of many groups have shown that imino sugar- as well as carba sugar-based glycomimetics may provide suitable therapeutic agents that function either by inhibiting upstream enzymes and, thus, by reducing the production of metabolites that cannot be degraded rapidly enough by the respective mutant enzyme (substrate reduction therapy, SRT) or by assisting the folding and transport of mutant enzymes to the lysosome (chaperone mediated therapy, CMT), for which it was suggested that sub-inhibitory concentrations of active site specific molecules (pharmacological chaperones) could be exploited.[6] Whereas mutant proteins that cannot obtain/retain their functional conformation are recognized as misfolded by the quality control machinery in the endoplasmic reticulum and are eventually targeted for degradation; these carba or imino sugars coordinate and stabilize such mutant enzymes including lysosomal β-glucosidase, β-galactosidase or β-*N*-acetylhexosaminidase in their functional folded conformations thus supporting their exit from the endoplasmic reticulum and their subsequent transport to the lysosome.

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Guiding contributions by various leaders in the field and recent reviews[10,11] have shown that N-substituted iminoalditols and similarly functionalized structures bind better than their more polar parent compounds through stronger interactions with the aglycon binding site or with lipophilic pockets around the enzymes' active sites. In this context, N-butyl-1-deoxynojirimycin is one of the best studied iminosugars, thus far.[9] Other researchers such as Wong and his group[12] as well as Aerts and Overkleeft and their co-workers[13] have observed that large, lipophilic N-alkyl substituents, in particular adamantyl capped spacer arms, greatly improve the interaction between iminoalditol derivatives and the lysosomal β-glucosidase (Fig. 1).

Whereas compounds **1**–**3** have been found in nature, the so-called isoimino sugars invented by Lundt[14] and Bols[15] and their groups in 1994 are not natural products. They were designed on the hypothesis that sugar related compounds that can stabilize positive charge at the (formal) anomeric position may be interesting glycosidase inhibitors. Indeed, the first such isoimino sugar, isofagomine (**4**), was found to be a potent β-glucosidase inhibitor. *C*-5a-Chain extended derivatives such as *C*-nonyl analog **5**, introduced by Fan and co-workers,[16] turned out even more powerful. Other chain-extended derivatives **6** and **7** were synthesized and investigated by Withers and collaborators.[17]

For lysosomal β-galactosidase associated with G_{M1}-gangliosidosis and Morquio B disease, only two distinct types of β-D-galactosidase inhibitors have been investigated as potential pharmacological chaperones thus far. The first compound, carba sugar NOEV (N-octyl-*epi*-valienamine, **8**), turned out a powerful inhibitor[18] and even at sub-micromolar concentrations a highly potent pharmacological chaperone for a wide range of mutants of lysosomal β-galactosidase.[19] More recently, selected unbranched analogs lacking the hydroxymethyl group have been presented and their activities with patients' skin fibroblasts carrying the R201C mutation were reported.[20] Other potential chaperones were structurally based on compound **2** which by various types of structural modifications could be transformed into a relatively small number of typical derivatives (**10**–**16**, in case of several N-substituent-variations in the same publication, only the most active is depicted) with interesting properties as experimental pharmacological chaperones.[21–28]

Potentially interesting derivatives of the isoiminosugar 4-*epi*-isofagomine[29] (**17**), whose chaperoning activity was only recently reported,[28] for the same purpose have been lacking. To date, only 5a-*C*-methyl analog **18** has been prepared (in different context) by a *de-novo* approach.[30]

Motivated by the early encouraging results with our 1-deoxy-D-*galacto*-nojirimycin derivatives[25,26,31] we became interested in extending our collection of experimental chaperone candidates for G_{M1}-gangliosidosis and have recently targeted *C*-5a-modifications of parent compound **17**.[32]

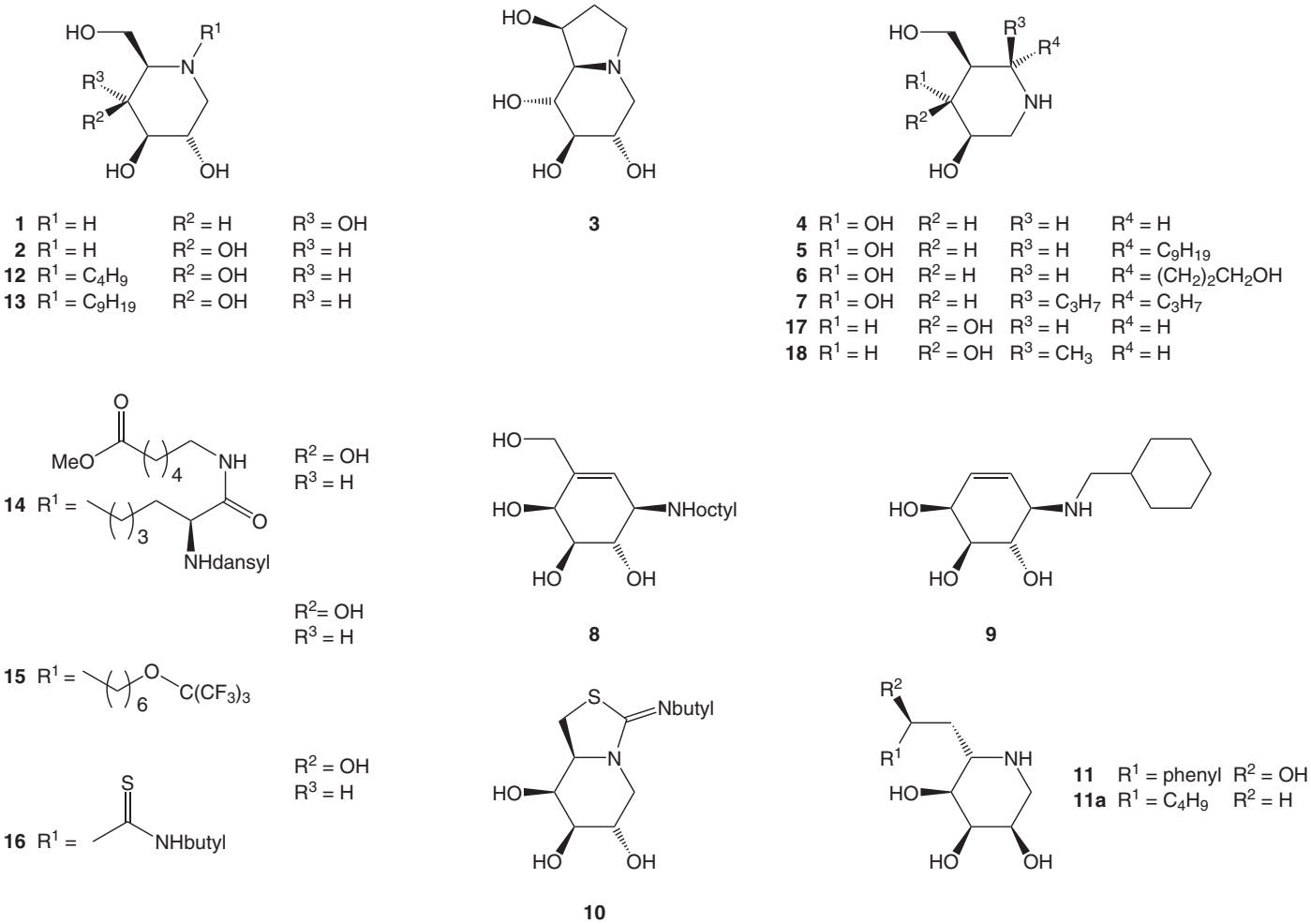

Fig. 1. Glucosidase and galactosidase inhibitors as potential therapeutic principles for lysosomal deficiencies of D-glucosidase and D-galactosidase, respectively.

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2. Results and discussion

2.1. Synthesis

Starting from known 5-aminodeoxy septanuronic ester **19** (easily available from D-mannose in six steps), by reduction of the ester moiety (**20**) and subsequent catalytic hydrogenation/concomitant intramolecular reductive amination, partially protected 6-homo derivative **21** of 1-deoxy-L-*gulo*-nojirimycin was prepared and N-protected (**22**). Regioselective O-silylation at C-7 (**23**) followed by oxidation of C-4 provided ulose **24**. Its reaction with methylene triphenyl phosphorane or other one-carbon Wittig reagents such as methoxymethylene triphenyl phosphorane invariantly was met with failure to provide the desired branched chain compound due to pronounced enolization tendency of the carbonyl group. The same was true for attempted Peterson olefination approaches with (trimethylsilyl)methyl magnesium chloride under all conditions probed. Cerium chloride modification of the (trimethylsilyl)methyl Grignard reagent following a particular protocol by Li and Yang[33] and subsequent spontaneous elimination of trimethyl silanol during attempted chromatography of the reaction mixture finally gave desired *exo*-methylene compound **25**, but only in inacceptable yields (23%) (Scheme 1).

Despite all other efforts made, in our hands, only the Tebbe reaction allowed convenient access to the desired crucial intermediate **25** in fair yield. Stereoselective hydroboration led to the 4-*epi* isofagomine derivative **26** in 51% yield. Due to steric interference of the neighboring silyloxyethyl moiety with the approach of the borane, diastereomeric side product **27** was also formed in this reaction (21%). For unambiguous structural assessment, compound **27** was deprotected to free iminoalditol **28** which could be crystallized for X-ray crystallography (CCDC 1465280). Deprotection of **26** gave free iminosugar **29**.

By catalytic hydrogenation (Pd/C 5%), 6-deoxy derivative **30** and the corresponding free compound **31** were also prepared from compound **25** (Scheme 2).

To gain regioselective access to C-5a-modifications, the primary hydroxyl group **26** was protected as methoxymethylene acetal **32** followed by fluoride catalyzed cleavage of the silyl ether to provide alcohol **33**. X-Ray crystallography unambiguously revealed the identity of this key intermediate (CCDC 1465279) (Picture 1).

By oxidation to aldehyde **34** and subsequent chain extension, C_4 as well as C_6 chains featuring terminal nitrile groups (compounds **35** and **36**, respectively) were formed. From these, the corresponding primary amines **37** and **38**, respectively, were liberated by catalytic hydrogenation over Raney-Ni *en route* to the desired N-dansyl derivatives **39** and **40** which upon acidic deprotection provided "glycogroup-typical"[25,34] free inhibitors **41** and **42**.

2.2. D-Galactosidase inhibition

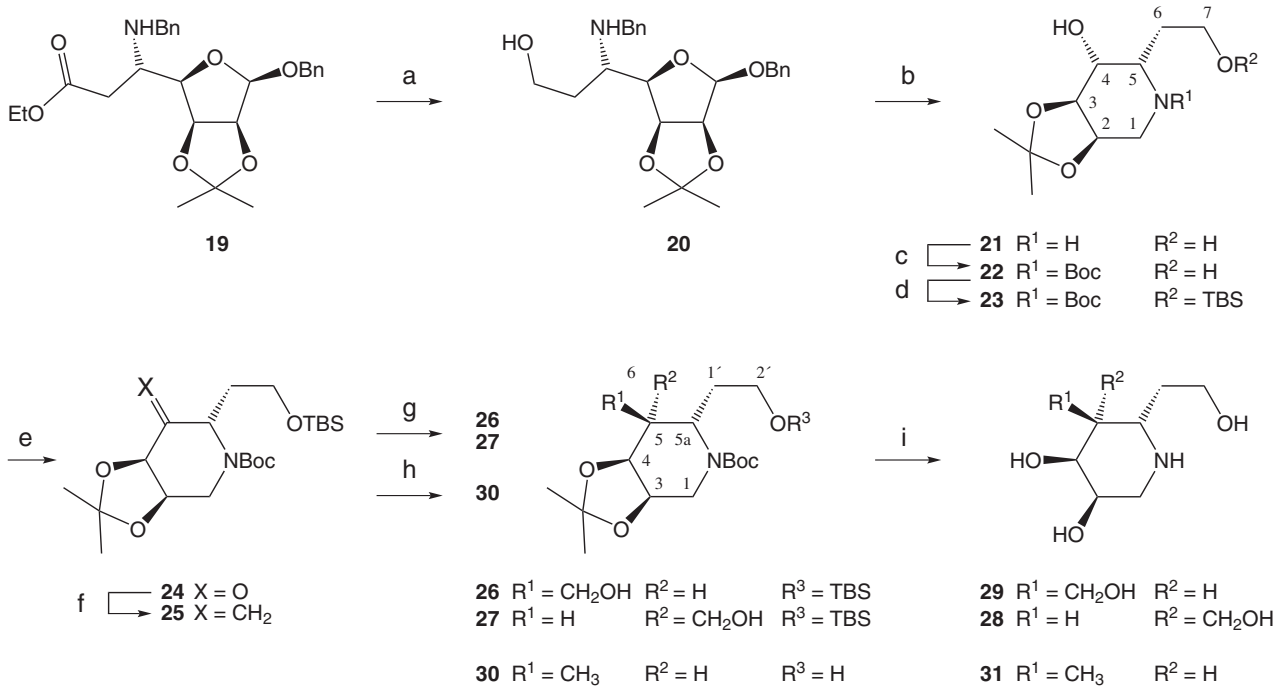
Inhibitory activities of free iminosugars **28**, **29**, **31**, **41** as well as **42** in comparison with unsubstituted parent compound **17** as well as bench mark molecule **8** are summarized in Table 1. In all cases of confirmed activity, the inhibition was competitive.

Interestingly, branched iminotetraol **29** was found to be a good inhibitor of β-D-galactosidases. Epimer **28** was practically devoid of inhibitory activity against the enzymes probed. 6-Deoxygenation (derivative **31**) mainly reduced the activity against *E. coli* β-galactosidase, whereas inhibitory power with the other two β-galactosidases probed was practically retained when compared to parent **29**. α-Galactosidase Fabrazyme was not inhibited by any of these new compounds.

Gratifyingly, dansylaminoalkyl substituted 4-*epi*-isofagomines **41** and **42** are powerful β-galactosidase inhibitors with practically no affinity to the α-galactosidases employed in this study.

With human lysosomal β-galactosidase, polyol **29** was an, albeit mediocre, inhibitor (IC_{50} = 40 μM). Compound **41** had IC_{50} = 1.5 μM, homolog **42** showed IC_{50} = 0.38 μM.

Evaluation of inhibitor **42** as a potential chaperone for the R201C mutant enzyme employing patients' skin fibroblasts revealed an effective concentration range of three orders of magnitude with an



Scheme 1. (a) LAH, THF, 94%; (b) H_2, $Pd(OH)_2/C$ (20%), MeOH, 86%; (c) $(Boc)_2O$, Et_3N, MeOH, 90%; (d) TBSCl, imidazole, DMF, 83%; (e) Dess–Martin oxidation, CH_2Cl_2, 97%; (f) Tebbe's reagent, THF, 94%; (g) BH_3·THF, THF, 72%; (h) H_2, $Pd(OH)_2/C$ (20%), MeOH, 88%; (i) HCl, MeOH, 85–92%.

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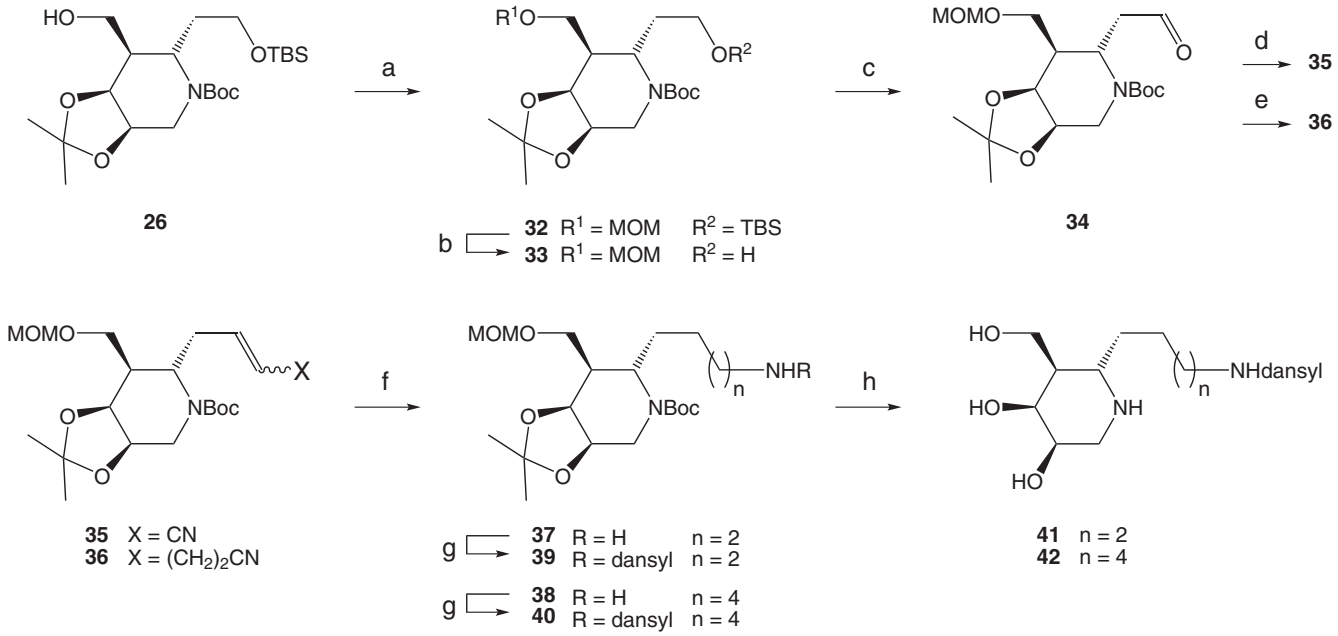


Scheme 2. (a) MOMCl, DIPEA, CH_2Cl_2, 87%; (b) TBAF·$3H_2O$, THF/H_2O, 92%; (c) Dess–Martin oxidation, 95%; (d) $(EtO)_2POCH_2CN$, *t*-BuOK, THF, 73%; (e) $Ph_3P(CH_2)_3CNBr$, LDA, THF, 60%; (f) H_2, Raney-Ni, MeOH, 75–84%; (g) dansyl chloride, Na_2CO_3, MeOH; (h) HCl, MeOH, 49–59% (over 2 steps).

enhancement of 3.5-fold β-galactosidase activity at a concentration as low as 0.02 μM, 6-fold activity at 0.1 μM and a maximum chaperone effect of 10-fold activity at 2.5 μM (Compound **8**: 5.2-fold at 2 μM; parent compound **17**: 2.7-fold at 10 μM). At higher concentrations, dose-dependent inhibition gradually reduced the desired enhancement effect to a level of 9-fold at 12.5 μM. Compound **41** was considerably less powerful but showing good activity enhancement of around 9-fold between 20 and 100 μM (Fig. 2).

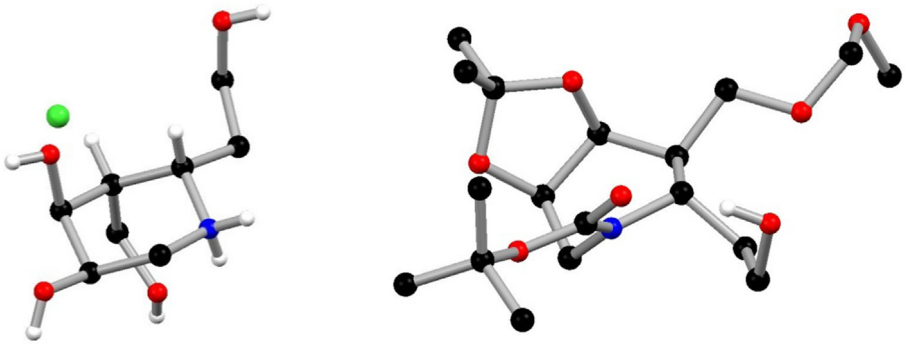
3. Conclusions

Following a simple protocol, a series of novel derivatives of 4-*epi*-isofagomine, bearing chain extensions at C-5a, have been made available and were screened with a range of D-galactosidases including human lysosomal β-galactosidase. Two new inhibitors, compounds **41** and **42**, turned out highly potent and exhibited interesting dose dependent chaperoning profiles at low concentrations with enzyme mutant R201H. Results obviously merit screening for potential chaperone activity with other frequent mutants of this enzyme. Screening with selected Morquio B cell lines is currently in progress.

4. Experimental

4.1. General methods

Optical rotations were measured at 20° C on a Perkin Elmer 341 polarimeter at a wavelength of 589 nm and a path length of 10 cm. NMR spectra were recorded on a Varian INOVA 500 operating at 599.82 MHz (^{1}H), and at 125.894 MHz (^{13}C) or on a Bruker Ultrashield spectrometer at 300.36 and 75.53 MHz, respectively. $CDCl_3$ was employed for protected compounds and methanol-d_4 or D_2O for unprotected inhibitors. Chemical shifts are listed in delta employing residual, non-deuterated solvent as the internal standard. Signals were assigned unambiguously by COSY, HSQC as well as APT analysis. The signals of the protecting groups as well as of the N-substituents were found in the expected regions and are only listed explicitly when overlapping with important spectral features of the iminoalditol. For crucial intermediates, structures were confirmed by XRD structural analysis. MALDI-TOF Mass Spectrometry was performed on a Micromass TofSpec 2E Time-of-Flight Mass Spectrometer. Analytical TLC was performed on precoated aluminum plates silica gel 60 F254 (E. Merck 5554) and detected with UV light



Picture 1. Crystal structures of compounds **28**·HCl (CCDC 1465280) and **33** (CCDC 1465279).

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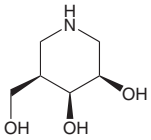
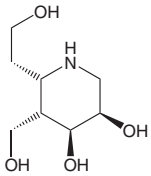
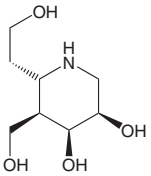
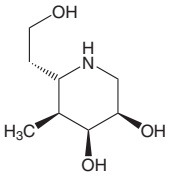
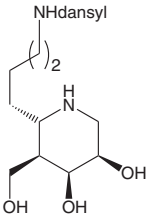
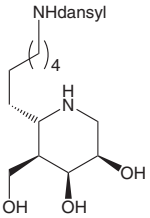
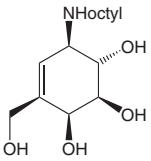
Table 1
K_i-values [μM] of compounds with ABG = β-glucosidase/β-galactosidase from *Agrobacterium* sp.; *E. coli* = *lac Z* β-galactosidase from *E. coli*; Bovine Liver = β-galactosidase from bovine liver; GCB = α-galactosidase from green coffee beans

	Compounds						
Enzyme							
	17	**28**	**29**	**31**	**41**	**42**	**8**
ABG	0.29	927	2.9	3.6	0.049	0.0175	–
E. coli	0.031	147.5	0.106	305	0.0037	0.0021	–
Bovine Liver	10	N.I.	4.89	6.57	0.151	0.0014	0.87 (ref. 35)
GCB	140	n.d.	n.d.	n.d.	848	n.d.	3.1 (ref. 35)
Fabrazyme	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	–
β-Gal human lys (IC_{50})	99.7 (ref. 36)	n.d.	30	n.d.	2.67	0.38	0.125 (ref. 37)

Fabrazyme = commercial recombinant lysosomal α-galactosidase; IC_{50} [μM] with β-Gal (human lysosomal); N.I. = no inhibition, with Ki > 2 mM; n.d., not determined.

(254 nm). For staining, a solution of vanillin (9 g) in a mixture of H_2O (950 mL)/EtOH (750 mL)/H_2SO_4 (120 mL) or ceric ammonium molybdate (100 g ammonium molybdate/8 g ceric sulfate in 1 L 10% H_2SO_4) was employed followed by heating on a hotplate. For column chromatography, silica gel 60 (230–400 mesh, E. Merck 9385) or silica gel 60 (Acros Organics, AC 24036) was used.

4.2. Kinetic studies

Kinetic studies were performed at 37 °C in an appropriate buffer using a known concentration of enzyme (specific conditions depicted below). K_i determinations were performed using the corresponding 4-nitrophenyl α- or β-D-galactopyranoside as substrate, except for Fabrazyme, where 2,4-dinitrophenyl α-galactopyranoside[38] was employed. In a typical assay, the enzyme was incubated with different inhibitor concentrations for up to 5 minutes before initiating the reaction by the addition of substrate. The initial reaction rate was measured by monitoring the increase in absorbance at 400 nm for up to ten minutes. K_i determinations were performed using at least two different substrate concentrations. For each inhibitor, a range of four to six inhibitor concentrations bracketing the K_i value ultimately determined was used for each substrate concentration. Dixon plots (1/v vs [I]) were constructed to validate the use of competitive inhibition model. The data were then fit using non-linear regression analysis with Grafit 7.0.

N.I. stands for no inhibition or weak inhibition with an estimated K_i value higher than 2 mM.

Specific assay conditions for each enzyme:

***Agrobacterium* sp. β-glucosidase:**[39,40] 50 mM sodium phosphate buffer (pH 7) using 1.85×10^{-4} mg/mL of enzyme (K_m = 4.1 mM).

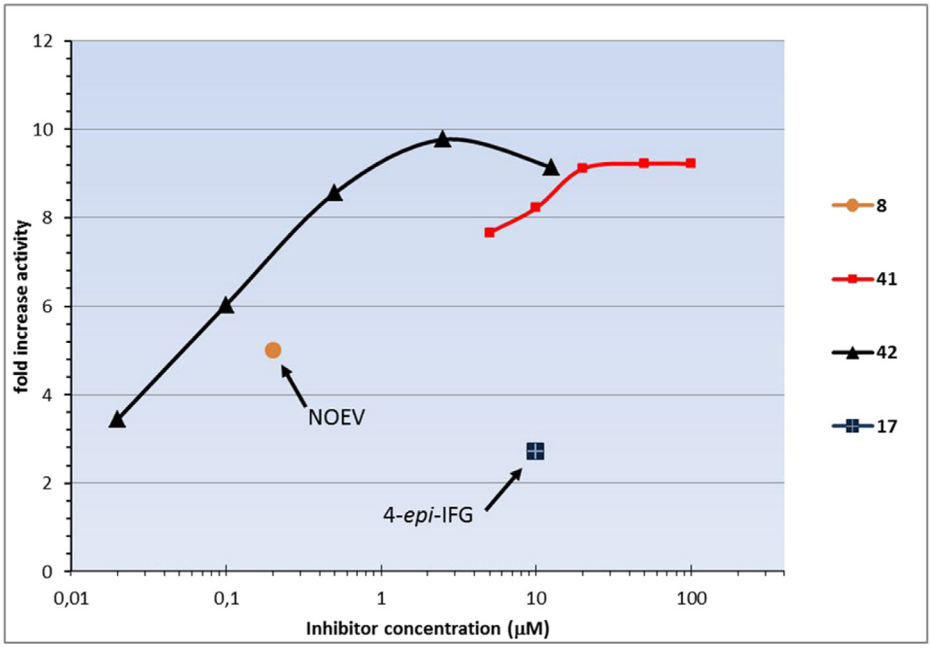


Fig. 2. Chaperoning activities of inhibitors **41** (black) and **42** (red) in comparison with values of NOEV (**8**, ref. 18) and parent 4-*epi*-isofagomine (**17**, ref. 36) as activity enhancement at given concentrations.

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***E. coli* lac *z* β-galactosidase:** 50 mM sodium phosphate, 1.0 mM $MgCl_2$ (pH 7) using 6.4×10^{-4} mg/mL of enzyme (K_m = 60 μM).
***Bovine liver* β-galactosidase:** 50 mM sodium phosphate buffer (pH 7) using 4.9×10^{-2} mg/mL of enzyme (K_m = 0.65 mM).
***Green Coffee Bean* α-galactosidase:** 50 mM sodium citrate buffer (pH 6.5) using 4.6×10^{-3} mg/mL of enzyme (K_m = 0.56 mM).
Fabrazyme (Acid α-galactosidase): 20 mM sodium citrate, 50 mM sodium phosphate, 1.0 mM tetrasodium EDTA, 0.25% v/v Triton X-100® and 0.25% w/v taurocholic acid buffer (pH 5.5) using 5×10^{-5} mg/mL of enzyme (K_m = 0.65 mM).
Human β-galactosidase and **β-hexosaminidase** activity measurements were performed in duplicate assays, unless otherwise stated. Fibroblast cells were harvested by trypsinization in 0.9% NaCl containing 0.01% Triton, homogenized by sonication (2 × 10 sec, Branson Sonifier II, W-250) and centrifuged at 13,000 rpm for 2 min in a table top centrifuge (Biofuge Pico, Heraeus). Protein amounts were determined according to the method of Lowry.[41]

For assessment of β-Gal activity, 20 μL of cell homogenate were mixed with 100 μL of 0.5 mM 4-methylumbelliferyl-β-D-galactopyranoside (Sigma-Aldrich), in 100 mM citrate buffer (pH 4.0) containing 100 mM NaCl and 0.02% NaN_3. After incubation at 37 °C for 30 min, the reaction was stopped by adding 2.5 ml 400 mM glycine/NaOH (pH 10.4).

β-Hexosaminidase activity was measured by adding 10 μL homogenate to 90 μL 0.9% NaCl and 100 μL of 1 mM 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (Sigma-Aldrich) in 100 mM citrate buffer (pH 4.6) containing 0.2% BSA and 0.04% NaN_3. The reaction was stopped after 10 min at 37 °C by addition of 2.5 ml of 400 mM glycine/NaOH (pH 10.4). The amount of hydrolyzed 4-methylumbelliferone was determined with a fluorescence spectrometer (Hitachi F7000).

Modified β-Gal assays were used to estimate the half maximal inhibitory concentration (IC_{50}) of the particular chaperone. For IC_{50} determination, 0.001 to 100 μM of chaperone was added to the assay mixture. Activity was measured in normal fibroblasts. Data analysis was performed with Microcal™ Origin® v6.0 using a non-linear curve fitting module based on sigmoid curve fitting.

4.2.1. Patients and cell lines

Cell lines from one G_{M1}-Gangliosidosis-patient (R201C) and one WT were exposed to compounds **29**, **41** as well as **42** for evaluation of their chaperone effects.

Human skin fibroblasts were grown in minimal essential medium (MEM) with Earle's Salts (SIGMA) containing 10% fetal bovine serum, 400 μM L-glutamine, and 50 μg/mL gentamycin at 37 °C and 5% CO_2. All cells used in this study were between the third and nineteenth passages.

Potential chaperones were dissolved in DMSO and diluted in 10 mM phosphate buffer (pH 7.0) containing 100 mM NaCl, 0.01% NaN_3, and 0.01% Triton for the IC_{50}-measurements and in MEM for the *in-vivo* tests. The total DMSO concentration in the media was less than 1% and had no effect on β-Gal activity or cell viability.

4.2.2. In-vivo treatment of cultured fibroblasts

Fibroblasts were grown to semi-confluency in 6-well plates. Particular chaperone was added to the culture medium at following concentrations: **41**: 100 μmol to 5 μmol; **42**: 12.5 μmol to 0.02 μmol. Cells were incubated for four additional days at 37 °C. Cells were harvested by scraping and prepared for β-galactosidase and β-hexosaminidase assays as described above.

4.3. General procedures

4.3.1. General procedure A

The respective starting material was dissolved in MeOH and HCl concd was added dropwise to adjust to pH 1. After completed removal of all protecting groups, the solvents were removed under reduced pressure. Subsequent purification on silica gel provided the respective desired title compound.

4.3.2. General procedure B

To a 10% solution of the respective alcohol in CH_2Cl_2, Dess–Martin periodinane (1.1–1.2 equiv) was added. After completed conversion of the starting material, the reaction was carefully quenched with saturated aq $NaHCO_3$. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification of the remaining residue by column chromatography on silica gel afforded the respective oxidation product.

4.3.3. General procedure C

A 10% solution of the respective nitrile in MeOH was stirred with Raney-Ni (moist in H_2O) under an atmosphere of H_2 at ambient pressure for 30–60 min. After complete conversion of the starting material, the catalyst was filtered off. Removal of the solvent under reduced pressure and subsequent chromatography on silica gel afforded the respective desired amine.

4.3.4. General procedure D

To a suspension of the respective amine and Na_2CO_3 (2.5–3.0 equiv) in MeOH, dansyl chloride (1.2 equiv) was added. After completed conversion of the starting material, the reaction mixture was evaporated to dryness. The remaining residue was quickly passed through silica gel to provide the crude product which was immediately taken to the next step.

4.4. Benzyl N-benzyl-5-amino-5,6-dideoxy-2,3-O-isopropylidene-α-L-gulo-heptofuranoside (*20*)

To a 10% suspension of $LiAlH_4$ (0.68 g, 18.00 mmol) in dry THF, a 3% solution of ester **19** (5.3 g, 12.00 mmol) in dry THF was added dropwise at –0 °C. After completed conversion of the starting material (5–15 min), the reaction was quenched with H_2O (3 mL). By addition of 10 mL NaOH (3 N) and stirring for further 60 min at ambient temperature aluminum salts were precipitated and could be removed by filtration. The filtrate was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The resulting syrup was purified by flash column chromatography (C/EE 1:2 v/v) to give alcohol **20** (4.5 g, 11.26 mmol, 93.8%). $[\alpha]_D^{20} = -20.3$ (c 2.02, MeOH); ^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.72 (d, 1H, $J_{1,2}$ 4.2 Hz, H-1), 4.60 (dd, 1H, $J_{2,3}$ 6.0 Hz, $J_{3,4}$ 3.6 Hz, H-3), 4.49 (dd, 1H, H-2), 3.63 (m, 2H, 2× H-7), 3.53 (dd, 1H, $J_{4,5}$ 8.8 Hz, H-4), 3.11 (ddd, 1H, $J_{5,6a}$ 4 Hz, $J_{5,6b}$ 7.5 Hz, H-5), 1.80–1.53 (bm, 2H, H-6a, H-6b); ^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 140.6, 139.1 (*ipso* NBn, *ipso* OBn), 129.7–128.3 (aromatic OBn, NBn), 114.4 [$C(CH_3)_2$], 103.4 (C-1), 81.1, 81.0, 80.1 (C-2, C-3, C-4), 73.0 (OCH_2Ph), 61.2 (C-7), 56.6 (C-5), 52.1 (NCH_2Ph), 32.8 (C-6), 26.2, 25.6 [($CH_3)_2C$]. MS: Calcd for [$C_{20}H_{31}NO_5H$]: *m/z* 414.2281[M + H]$^+$; Found [M + H]$^+$ 414.2273; MS: Calcd for [$C_{20}H_{31}NO_5Na$]: *m/z* 436.2100 [M + Na]$^+$; Found [M + Na]$^+$ 436.2107.

4.5. 1,5,6-Trideoxy-1,5-imino-2,3-O-isopropylidene-L-gulo-heptitol (*21*)

A solution of Compound **20** (4.5 g, 11.26 mmol) in MeOH (15 mL) was stirred with $Pd(OH)_2$/C under an atmosphere of H_2 at ambient pressure overnight. After completed conversion of the starting material (indicated by TLC), the catalyst was filtered off and the solvent was removed under reduced pressure. Chromatography on silica gel

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($CHCl_3$/MeOH/concd NH_4OH 8:4:0.01 v/v/v) afforded compound **21** (2.1 g, 9.66 mmol, 85.8%) as a colorless syrup. $[\alpha]_D^{20} = -28.4$ (c 1.12, MeOH); ^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.30 (ddd, 1H, $J_{1,2ax}$ 6.3 Hz, $J_{1,2eq}$ 4.8 Hz, $J_{2,3}$ 6 Hz, H-2), 4.18 (dd, 1H, $J_{3,4}$ 3 Hz, H-3), 3.80 (dd, 1H, $J_{4,5}$ 3 Hz, H-4), 3.68 (m, 2H, 2× H-7), 3.25–3.13 (m, 2H, H-1eq, H-5), 2.88 (dd, 1H, $J_{1ax,1eq}$ 13.5 Hz, H-1ax), 1.77 (m, 2H, 2× H-6); ^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 110.3 [*C*(CH_3)$_2$], 76.9 (C-3), 71.4 (C-2), 68.5 (C-4), 59.98 (C-7), 52.6 (C-5), 45.4 (C-1), 33.8 (C-6), 27.9, 25.7 [(*C*H_3)$_2$C]; MS: Calcd for [$C_{10}H_{19}NO_4Na$]: *m/z* 240.1212 [M + Na]$^+$; Found [M + Na]$^+$ 240.1225.

4.6. *N-tert-Butyloxycarbonyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-L-gulo-heptitol (**22**)*

To a suspension of compound **21** (2.1 g, 9.66 mmol) in MeOH (8 mL) and Et_3N (4.0 mL, 28.99 mmol), $(Boc)_2O$ (2.5 g, 11.59 mmol) was added and the mixture was stirred for 8 h. When TLC ($CHCl_3$/MeOH/concd NH_4OH 3:1:0.01 v/v/v) indicated full conversion of the starting material, the suspension was concentrated and the remaining residue was diluted with CH_2Cl_2 and consecutively washed with HCl (6%) and saturated aq $NaHCO_3$. The combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness. Chromatographic purification on silica gel (C/EE 1:1 v/v) gave compound **22** (2.75 g, 8.66 mmol, 89.7%) as a pale yellow syrup. $[\alpha]_D^{20} = +25.3$ (c 4.75, $CHCl_3$); ^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.18 (m, 2H, H-2, H-3), 4.00 (dd, 1H, $J_{1ax/1eq}$ 14.Hz, $J_{1eq,2}$ 2.8 Hz, H-1eq), 3.95 (m, 2H, H-5), 3.88-3.83 (m, 1H, H-4), 3.58-3.40 (m, 2H, 2× H-7), 3.28–3.08 (m, 1H, H-1ax), 2.00–1.60 (m, 2H, 2× H-6); ^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 158.0, (C = O, Boc), 110.3 [*C*(CH_3)$_2$], 81.2, 81.1 [O*C*(CH_3)$_3$], 76.6, 76.2, 77.5, 77.4 (C-2, C-3), 68.2 (C-4), 59.8 (C-7), 53.0, 51.9 (C-5), 52.6, 41.5 (C-1), 31.8, 30.7 (C-6), 28.7 [OC(*C*H_3)$_3$], 27.3, 24.9 [(*C*H_3)$_2$C]; MS: Calcd for [$C_{15}H_{27}NO_6Na$]: *m/z* 340.1736 [M + Na]$^+$; Found [M + Na]$^+$ 340.1731.

4.7. *N-tert-Butyloxycarbonyl-7-O-tert.butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-L-gulo-heptitol (**23**)*

To a 20% solution of compound **22** (2.75 g, 8.66 mmol) in dry DMF, imidazole (1.77 g, 25.99 mmol) and TBSCl (1.40g, 9.53 mmol) were added at ambient temperature. After full conversion of the starting material (indicated by TLC) the reaction was quenched with MeOH (15 mL) and the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and consecutively washed with HCl (6%) and saturated $NaHCO_3$. The combined organic layers were dried (Na_2SO_4), filtered and concentrated. Chromatography on silica gel (C/EE 10:1 v/v) provided compound **23** (3.1 g, 7.18 mmol, 82.9%) as a pale colorless syrup. $[\alpha]_D^{20} = +53.5$ (c 0.94, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.48–4.35 (m, 2H, H-2, H-3), 4.35-3.89 (m, 2H, 2× H-7), 3.40–3.19 (m, 1H, H-1ax), 2.08–1.88 (m, 2H, 2× H-6); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.7 (C = O, Boc), 109.3 [*C*(CH_3)$_2$], 79.5 [O*C*(CH_3)$_3$], 73.8 (C-3), 73.1, 72.9 (C-4), 66.7, 66.6 (C-2), 60.6, 60.4 (C-7), 53.3, 52.8 (C-5), 41.9, 40.9 (C-1), 32.4, 31.4 (C-6), 28.6 [OC(*C*H_3)$_3$], 26.5, 24.4 [(*C*H_3)$_2$C], 25.94, 18.25, –5.6 (TBS); MS: Calcd for [$C_{21}H_{41}NO_6SiNa$]: *m/z* 454.2601 [M + Na]$^+$; Found [M + Na]$^+$ 454.2617.

4.8. *N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-L-ribo-hept-4-ulose (**24**)*

Following general procedure B, alcohol **23** (1.39 g, 3.22 mmol) was treated with Dess–Martin periodinane (1.64 g, 3.86 mmol). Purification on silica gel (C/EE 7:1 v/v) afforded **24** (1.34g, 3.12 mmol, 96.9%) as a colorless syrup. $[\alpha]_D^{20} = +24.5$ (c 2.17, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.80 (bs, 1H, H-5), 4.60–4.19 (m, 3H, H-1eq, H-2, H-3), 3.66 (m, 2H, 2× H-7), 3.08 (m, 1H, H-1ax), 1.99 (m, 2H, 2× H-6); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 204.8 (C-4), 155.2 (C = O, Boc), 111.7 [*C*(CH_3)$_2$], 80.7 [O*C*(CH_3)$_3$], 76.7, 76.6 (C-3, C-2), 59.7 (C-7), 59.1 (C-2), 42.2 (C-1), 32.8 (C-6), 28.5 [OC(*C*H_3)$_3$], 26.6, 25.0 [(*C*H_3)$_2$C], 26.1, 18.43, –5.2, –5.3 (TBS); MS: Calcd for [$C_{21}H_{39}NO_6SiNa$]: *m/z* 452.2444 [M + Na]$^+$; Found [M + Na]$^+$ 452.2544.

4.9. *N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-4-C-methylene-L-ribo-heptitol (**25**)*

To a solution of compound **24** (964 mg, 2.24 mmol) in dry THF (45 mL), Tebbe's reagent (0.5 M in toluene, 4.9 mL, 2.45 mmol) was added under an atmosphere of nitrogen at –25 °C. After 120 min at –20 °C, the reaction was stirred at ambient temp for another 12 h. The reaction mixture was diluted with Et_2O (80 mL), and NaOH (1.5 N, 1.94 mL, 2.91 mmol) was added dropwise. After vigorous stirring for further 12 h, the mixture was filtered. The filtrate was washed with water and the organic layer was dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel, C/EE 8:1 v/v) to give **25** (897 mg, 2.09 mmol, 93.5%). $[\alpha]_D^{20} = +9.0$ (c 4.33, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.30, 5.19 (2s, 2H, 2× exo-methylene), 4.72 (bs, 1H, H-5), 4.64 (d, 1H, $J_{2,3}$ 7.1 Hz, H-3), 4.25 (bs, 1H, H-2), 4.05 (m, 1H, H-1eq), 3.75-3.57 (2H, 2× H-7), 2.82 (m, 1H, H-1ax), 2.09–1.76 (m, 2H, 2× H-6); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.6 (C = O, Boc), 143.0 (C-4), 116.0 (exo-methylene), 109.5 [*C*(CH_3)$_2$], 79.9, 79.6 [O*C*(CH_3)$_3$], 76.9, 75.3 (C-2, C-3), 60.7, 60.4 (C-7), 52.5, 51.7 (C-5), 43.4, 42.4 (C-1), 35.4, 35.2 (C-6), 28.5 [OC(*C*H_3)$_3$], 26.1 (TBS), 26.7, 24.9 [(*C*H_3)$_2$C], 18.4, –5.2 (TBS); MS: Calcd for [$C_{22}H_{41}NO_5SiNa$]: *m/z* 450.2652 [M + Na]$^+$; Found [M + Na]$^+$ 450.2767.

4.10. *(5aS)-N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-5a-C-(tert-butyldimethylsilyloxy)ethyl-4-epi-isofagomine (**26**) and (5aS)-N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-5a-C-(tert-butyldimethylsilyloxy)ethyl-4,5-di-epi-isofagomine (**27**)*

To a stirred solution of compound **25** (453 mg, 1.06 mmol) in dry THF (4 mL), BH_3·THF (1 M, 5.3 mL) was added dropwise at 0 °C under an atmosphere of nitrogen. After stirring for 12 h, the reaction was allowed to reach ambient temperature. To the ice cooled reaction mixture, H_2O (5 mL), NaOH (3 M, 8 mL) and H_2O_2 (33%, 8 mL) were added and the mixture was stirred for additional 8 h. The organic layer was separated and dried (Na_2SO_4) and was filtered, and the solvent was evaporated to dryness. Purification of the residue on silica gel (C/EE 15:1 v/v) afforded alcohols **26** (241 mg, 0.54 mmol, 51.0%) and **27** (101 mg, 0.23 mmol, 21.4%). Compound **26**: $[\alpha]_D^{20} = +52.6$ (c 5.32, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.49 (m, 1H, H-4), 4.32–3.94 (m, 3H, H-2eq, H-3, H-5a), 3.82 (bs, 2H, 2× H-6), 3.69 (bs, 2H, 2× H-2′), 2.94–2.48 (m, 2H, H-2ax, 6-OH), 2.05–1.60 (m, 3H, H-5, 2× H1′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.8 (C = O, Boc), 108.9 [*C*(CH_3)$_2$], 79.8, 79.4 [O*C*(CH_3)$_3$], 74.6, 74.5 (C-4), 74.0, 73.8 (C-3), 63.2, 63.0 (C-6), 60.7, 60.6 (C-2′), 47.5, 47.0 (C-5a), 42.9, 41.9 (C-2), 41.1, 40.8 (C-5), 36.0, 35.7 (C-1′), 28.6 [OC(*C*H_3)$_3$], 26.2, 24.3 [(*C*H_3)$_2$C], 26.0, 18.3, –5.3 (TBS). MS: Calcd for [$C_{22}H_{43}NO_6SiNa$]: *m/z* 468.2757 [M + Na]$^+$; Found [M + Na]$^+$ 468.3375. Compound **27**: $[\alpha]_D^{20} = -24.1$ (c 1.75, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.45-4.17 (bs, 2H, H-2eq, H-5a), 4.16–4.02 (m, 2H, H-3, H-4), 3.89–3.49 (m, 4H, 2× H-6, 2× H-2′), 3.24 (bs, 1H, H-2ax), 2.09 (m, 1H, H-5), 1.76–1.51 (m, 2H, 2× H-1′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.5 (C = O, Boc), 108.9 [*C*(CH_3)$_2$], 80.1 [O*C*(CH_3)$_3$], 74.4, 72.0 (C-3, C-4), 63.3 (C-6), 61.0 (C-2′), 49.1 (C-5a), 43.9 (C-5), 40.4, 39.4 (C-2), 29.7 (C-1′), 28.4 [OC(*C*H_3)$_3$], 28.3, 26.4 [(*C*H_3)$_2$C], 26.0, 18.4, –5.3, –5.4 (TBS); MS: Calcd for [$C_{22}H_{43}NO_6SiNa$]: *m/z* 468.2757 [M + Na]$^+$;Found [M + Na]$^+$ 468.2781.

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*4.11. (5aS)-5a-C-(Hydroxy)ethyl-4,5-di-epi-isofagomine (**28**)*

Following general procedure A, compound **27** (79 mg, 0.18 mmol) was converted into title compound **28**, which was an amorphous solid. Recrystallization in MeOH and Et_2O in the presence of a few drops of aq HCl afforded the corresponding hydrochloride **28**·HCl (36 mg, 0.16 mmol, 89.2%) as colorless crystals. $[\alpha]_D^{20} = -13.6$ (c 0.91, H_2O) (hydrochloride); ^{1}H NMR (300 MHz, D_2O, DCl): δ = 4.22 (ddd, 1H, $J_{2ax,3}$ 7.9 Hz, $J_{2eq,3}$ 3.8 Hz, $J_{3,4}$ 6.8 Hz, H-3), 4.04 (dd, 1H, $J_{4,5}$ 3.1 Hz, H-4), 3.96 (dd, 1H, $J_{5,6}$ 5.4 Hz, $J_{6,6}$ 11.7 Hz, 1× H-6), 3.88–3.68 (m, 4H, H-5a, 1× H-6, 2× H-2′), 3.35 (dd, 1H, $J_{2ax,2eq}$ 12.9 Hz, H-2eq), 3.23 (dd, 1H, H-2ax), 2.35 (m, 1H, H-5), 2.05–1.93 (m, 2H, 2× H-1′); ^{13}C NMR (75.5 MHz, D_2O, DCl): δ = 67.9 (C-4), 64.1 (C-3), 59.0 (C-2′), 58.9 (C-6), 52.5 (C-5a), 43.0 (C-2), 40.8 (C-5), 28.0 (C-1′); MS: Calcd for $[C_8H_{17}NO_4H]$: *m/z* 192.1236 $[M+H]^+$; Found $[M+H]^+$ 192.1229.

*4.12. (5aS)-5a-C-(2-Hydroxy)ethyl-4-epi-isofagomine (**29**)*

Following general procedure A, compound **26** (61 mg, 0.14 mmol) was converted into **29**. Purification on silica gel ($CHCl_3$/MeOH/concd NH_4OH 8:4:1 v/v/v) afforded **29** (24 mg, 0.13 mmol, 91.7%) as amorphous solid. $[\alpha]_D^{20} = -18.1$ (c 0.73, H_2O) (hydrochloride); ^{1}H NMR (300 MHz, D_2O, DCl): δ = 4.22 (dd, 1H, $J_{3,4}$ 2.7 Hz, $J_{4,5}$ 2.5 Hz, H-4), 3.94 (ddd, 1H, $J_{2ax,3}$ 11.7 Hz, $J_{2eq,3}$ 5 Hz, H-3), 3.87–3.73 (m, 3H, $J_{5,6}$ 5 Hz, 1× H-6, 2× H-2′), 3.70 (dd, 1H, $J_{5,6}$ 7.3 Hz, $J_{6,6}$ 11.5 Hz, 1× H-6), 3.45 (ddd, 1H, $J_{5,5a}$ 11.5 Hz, $J_{5,1'}$ 3.2 Hz, $J_{5a,1'}$ 8.4 Hz, H-5a), 3.25 (dd, 1H, $J_{2ax,2eq}$ 12 Hz, H-2eq), 3.12 (dd, 1H, H-2ax), 2.15–2.05 (m, 1H, 1× H-1′), 2.03–1.92 (m, 1H, H-5), 1.86–1.71 (m, 1H, 1× H-1′); ^{13}C NMR (75.5 MHz, D_2O, DCl): δ = 66.9 (C-4), 65.6 (C-3), 59.6 (C-6), 58.2 (C-2′), 51.7 (C-5a), 42.5 (C-5), 42.2 (C-2), 31.0 (C-1′); MS: Calcd for $[C_8H_{17}NO_4H]$: *m/z* 192.1236 $[M+H]^+$; Found $[M+H]^+$ 192.1240.

*4.13. (5aS)-N-tert-Butyloxycarbonyl-6-deoxy-3,4-O-isopropylidene-5a-C-(tert-butyldimethylsilyloxy)ethyl-4-epi-isofagomine (**30**)*

A solution of compound **25** (165 mg, 0.39 mmol) in MeOH (10 mL) was stirred with $Pd(OH)_2$/C (20%, ca. 50 mg), under an atmosphere of H_2 at ambient pressure for 16 h. After completed conversion of the starting material (indicated by TLC), the catalyst was filtered off and the solvent was removed under reduced pressure. Chromatography on silica gel (C/EE 3:1 v/v) afforded compound **30** (107 mg, 0.34 mmol, 87.9%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.39–4.09 (m, 2H, H-3, H-4), 3.94 (dd, 1H, $J_{2ax,2eq}$ 14.8 Hz, $J_{2eq,3} < 1$ Hz, H-2eq), 3.87 (m, 1H, H-5a), 3.60–3.38 (m, 2H, 2× H-2′), 2.71 (dd, 1H, $J_{2ax,3} < 1$ Hz, H-2ax), 1.92 (m, 1H, 1× H-1′), 1.46 (m, 1H, H-5a), 1.22 (m, 1H, 1× H-1′), 1.16 (d, 3H, 3× H-6); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 157.4 (C = O, Boc), 108.4 [$C(CH_3)_2$], 80.3 [$OC(CH_3)_3$], 76.0, 73.9 (C-3, C-4), 58.2 (C-2′), 50.2 (C-5a), 42.0 (C-2), 36.4 (C-1′), 35.5 (C-5), 28.5 [$OC(CH_3)_3$], 26.33, 24.2 [$(CH_3)_2C$], 16.7 (C-6); MS: Calcd for $[C_{16}H_{29}NO_5Na]$: *m/z* 338.1943 $[M+Na]^+$; Found $[M+Na]^+$ 338.1945.

*4.14. (5aS)-6-Deoxy-5a-C-hydroxyethyl-4-epi-isofagomine (**31**)*

Following general procedure A, compound **30** (37 mg, 0.12 mmol) was converted into title compound **31**, which was an amorphous solid. Recrystallization in MeOH and Et_2O in the presence of a few drops of aq HCl afforded the corresponding hydrochloride **31**·HCl (21 mg, 0.10 mmol, 84.6%) as white crystals. $[\alpha]_D^{20} = -10.7$ (c 0.48, H_2O) (hydrochloride); ^{1}H NMR (300 MHz, D_2O, DCl): δ = 3.97 (m, 1H, H-3), 3.92 (m, 1H, H-4), 3.78 (m, 2H, 2× H-2′), 3.30–3.17 (m, 2H, H-2eq, H-5a), 3.08 (dd, 1H, $J_{2ax,2eq} = J_{2ax,3}$ 11.5 Hz, H-2ax), 2.13–2.00 (m, 1H, 1× H-1′), 1.92 (ddq, 1H, $J_{4,5}$ 2 Hz, J5,5a 13.7 Hz, $J_{5,6}$ 6.9 Hz, H-5), 1.82–1.67 (m, 1H, 1× H-1′), 1.06 (d, 3H, 3× H-6); ^{13}C NMR (75.5 MHz, D_2O, DCl): δ = 70.4 (C-4), 65.6 (C-3), 58.2 (C-2′), 54.2 (C-5a), 42.35 (C-2), 35.83 (C-5), 30.9 (C-1′), 13.9 (C-6); MS: Calcd for $[C_8H_{17}NO_3H]$: *m/z* 176.1287 $[M+H]^+$; Found $[M+H]^+$ 176.178.

*4.15. (5aS)-N-tert-Butyloxycarbonyl-5a-C-(tert-butyldimethylsilyloxy)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (**32**)*

To a solution of compound **17** (162 mg, 0.36 mmol) and diisopropyl ethyl amine (172 µL, 1.02 mmol) in CH_2Cl_2 (5 mL), chloromethyl methyl ether (MOMCl, 36 µL, 0.47 mmol) was added dropwise. After completed conversion, the reaction mixture was consecutively washed with HCl (6%) and saturated $NaHCO_3$. The dried (Na_2SO_4) combined organic layers were evaporated to dryness. Chromatographic purification of the remaining residue on silica gel (C/EE 8:1 v/v) gave **32** (155 mg, 0.32 mmol, 87.1%). $[\alpha]_D^{20} = +86.5$ (c 1.10, $CDCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.48 (m, 1H, H-4), 4.20–3.87 (m, 2H, H-2eq, H-3), 3.82–3.54 (m, 5H, H-5a, 2× H-6, 2× H-2′), 2.88–2.67 (m, 1H, H-2ax), 1.94–1.54 (m, 3H, H-5, 2× H-1′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.4 (C = O, Boc), 108.2 [$C(CH_3)_2$], 96.5 (MOM), 79.6, 79.2 [$OC(CH_3)_3$], 73.3, 73.2 (C-3), 71.3, 71.2 (C-4), 66.9 (C-6), 60.6, 60.4 (C-2′), 55.2 (MOM), 48.3, 47.5 (C-5a), 52.9, 41.9 (C-2), 39.9, 39.4 (C-5), 36.7, 36.3 (C-1′), 28.4 [$OC(CH_3)_3$], 26.2, 24.2 [$(CH_3)_2C$], 26.0, 18.3, –5.3 (TBS); MS: Calcd for $[C_{24}H_{47}NO_7SiNa]$: *m/z* 512.3019 $[M+Na]^+$; Found $[M+Na]^+$ 512.3071.

*4.16. (5aS)-N-tert-Butyloxycarbonyl-5a-C-(2-hydroxyl)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (**33**)*

Silyl ether **23** (98 mg, 0.20 mmol) and TBAF·$3H_2O$ (200 mg) were stirred in THF/H_2O (100:1 v/v) at 50 °C. When TLC indicated completed cleavage of the protecting group, the solvents were removed under reduced pressure. Purification of the remaining slurry on silica gel (C/EE 1:1 v/v) afforded the free alcohol **33** (69 mg, 0.18 mmol, 91.7%). $[\alpha]_D^{20} = +37.1$ (c 1.93, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.52 (dd, 1H, $J_{3,4}$ 7.7 Hz, $J_{4,5}$ 2.4 Hz, H-4), 4.22–4.05 (m, 1H, H-3), 3.99–3.86 (m, 2H, H-2eq, H-5a), 3.75–3.60 (m, 2H, 2× H-6), 3.58–3.40 (m, 2H, 2× H-2′), 2.73 (dd, 1H, $J_{2ax,2eq}$ 14.7 Hz, $J_{2ax,3}$ 2 Hz, H-2ax), 1.99–1.87 (m, 1H, 1× H-1′), 1.73–1.62 (m, 1H, H-5), 1.40–1.29 (m, 1H, 1× H1′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 157.2 (C = O, Boc), 108.5 [$C(CH_3)_2$], 96.6 (MOM), 80.5 [$OC(CH_3)_3$], 73.15 (C-3), 71.4 (C-4), 67.3 (C-6), 58.1 (C-2′), 55.4 (MOM), 46.5 (C-5a), 42.4 (C-2), 41.1 (C-5), 36.8 (C-1′), 28.4 [$OC(CH_3)_3$], 26.3, 24.2 [$(CH_3)_2C$]; MS: Calcd for $[C_{18}H_{33}NO_7Na]$: *m/z* 398.2155 $[M+Na]^+$; Found $[M+Na]^+$ 398.2517.

*4.17. (5aS)-N-tert-Butyloxycarbonyl-5a-C-(1-oxo)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (**34**)*

Following general procedure B, alcohol **24** (110 mg, 0.29 mmol) was treated with Dess–Martin periodinane (149 mg, 0.35 mmol). Purification on silica gel (C/EE 1:1 v/v) afforded **34** (104 mg, 0.27 mmol, 94.8%) as a colorless syrup. $[\alpha]_D^{20} = +56.2$ (c 3.43, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.96 (s, 1H, H-2′), 4.43 (m, 1H, H-4), 4.28–3.87 (m, 3H, H-2eq, H-3, H-5a), 3.74–3.48 (m, 2H, 2× H-6), 2.99–2.78 (m, 1H, H-2ax), 2.77–2.37 (m, 2H, 2× H-1′), 1.99–1.81 (m, 1H, H-5); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 199.9, 199.4 (C-2′), 154.5, 153.9 (C = O, Boc), 107.7 [$C(CH_3)_2$], 95.6 (MOM), 79.7, 79.0 [$OC(CH_3)_3$], 72.7 (C-4), 70.9 (C-3), 66.7 (C-6), 54.43 (MOM), 47.0, 46.3 (C-5a), 46.6, 46.3 (C-1′), 41.7, 40.7 (C-2), 39.3, 38.3 (C-5), 27.4 [$OC(CH_3)_3$], 25.2, 23.3 [$(CH_3)_2C$]; **34 hydrate**: MS: Calcd for $[C_{18}H_{33}NO_8Na]$: *m/z* 414.2104 $[M+Na]^+$; Found $[M+Na]^+$ 414.0195.

*4.18. 4-[(5aS)-(N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomin-5a-yl)]-but-2-enoic nitrile (**35**)*

To a suspension of *t*-BuOK (45 mg, 0.40 mmol) in dry THF (3 mL), diethyl cyanomethyl phosphonate (48 µL, 0.45 mmol) was added

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dropwise at 0° C. The mixture was stirred for 10 min before allowing the reaction to warm up to ambient temperature. After additional 40 min, a 5% solution of compound **34** (83 mg, 0.22 mmol) in dry THF was added dropwise. The reaction mixture was stirred until full conversion of the starting material was detected (TLC). The mixture was consecutively extracted with HCl (6%) and with saturated aq $NaHCO_3$, dried (Na_2SO_4) and filtered. The filtrate was concentrated under reduced pressure and the remaining crude product was purified on silica gel (C/EE 4:1 v/v) to provide **35** (mixture of the *E*/*Z*-isomers, 64 mg, 0.16 mmol, 72.6%). ^{1}H NMR (300 MHz, $CHCl_3$): δ = 6.83–6.45 (m, 1H, H-2′), 5.34 (m, 1H, H-3′), 4.49 (m, 1H, H-4), 4.30–3.78 (m, 3H, H-2eq, H-3, H-5a), 3.77–3.53 (m, 2H, 2× H-6), 2.91–2.53 (m, 3H, H-2ax, 2× H-1′), 1.76 (bs, 1H, H-5); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.4, 155.1 (C = O, Boc), 152.2, 151.78 (C-2′), 117.2, 116.0 (C-4′), 108.6, 108.5 [*C*$(CH_3)_2$], 102.0, 100.9 (C-3′), 96.6 (MOM), 80.1, 79.8 [O*C*$(CH_3)_3$], 73.1-71.5 (C-3, C-4), 67.4, 67.3 (C-6), 55.4 (MOM), 50.6, 50.3, 49.6, 49.5 (C-5a), 43.2, 42.7, 42.1, 41.9 (C-2), 39.9, 39.8, 39.5, 38.8 (C-5), 37.9, 37.0, 36.6, 36.1 (C-1′), 28.4, 28.3 [OC(*C*$H_3)_3$], 26.2, 24.2 [(*C*$H_3)_2$C].

4.19. 6-[(5aS)-(N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomin-5a-yl)]-hex-4-enoic nitrile (**36**)

A solution of freshly prepared LDA [by dropwise addition of 2.5 M *n*-BuLi (0.38 mL) in hexane to diisopropyl amine (0.14 mL, 0.99 mmol, 20% solution in dry THF)] in dry THF was added dropwise at –78 °C under an atmosphere of nitrogen to a suspension of triphenyl-(3-cyano)propyl phosphonium bromide (421 mg, 1.03 mmol). After stirring at –40 °C for 60 min, a solution of compound **34** (110 mg, 0.29 mmol, 3% in dry THF) was added dropwise. After having been stirred for 12 h, the reaction mixture was diluted with CH_2Cl_2 and consecutively washed with HCl (6%) and saturated $NaHCO_3$. The combined organic layers were dried (Na_2SO_4) and solvents were removed under reduced pressure. Chromatographic purification (silica gel, C/EE 4:1 v/v) of the crude product gave **36** (75 mg, 0.18 mmol, 60.1%) as a mixture of the *E*/*Z*-isomers. ^{1}H NMR (300 MHz, $CHCl_3$): δ = 5.71–5.39 (m, 2H, H-2′, H-3′), 4.51 (m, 1H, H-4), 4.26–3.94 (m, 2H, H-2eq, H-3), 3.93–3.69 (m, 1H, H-5a), 3.63 (bs, 2H, 2× H-6), 2.86–2.66 (m, 1H, H-2ax), 2.65–2.08 (m, 6H, 2× H-1′, 2× H-4′, 2× H-5′), 1.86 (bs, 1H, H-5); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.4, 155.2 (C = O, Boc), 128.4, 128.3, 128.2 (C-2′, C-3′), 119.4 (C-6′), 108.5, 108.4 [*C*$(CH_3)_2$], 96.5, 96.4 (MOM), 79.8, 79.5 [O*C*$(CH_3)_3$], 73.3, 73.1, 71.7, 71.5 (C-3, C-4), 67.1, 66.7 (C-6), 55.3, 55.2 (MOM), 50.4, 49.6 (C-5a), 43.7, 42.8 (C-2), 38.5, 37.9 (C-5), 30.5, 29.4 (C-1′), 28.4 [OC(*C*$H_3)_3$], 26.2, 24.2 [(*C*$H_3)_2$C], 23.3 (C-4′), 17.4 (C-5′).

4.20. (5aS)-N-tert-Butyloxycarbonyl-5a-C-(4-amino)butyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (**37**)

Nitrile **35** (64 mg, 0.16 mmol) was converted into amine **37** (49 mg, 0.12 mmol, 75.4%) employing general procedure C. $[\alpha]_D^{20} = +33.8$ (c 2.36, MeOH); ^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.56 (dd, 1H, $J_{3,4}$ 8 Hz, $J_{4,5}$ 2 Hz, H-4), 4.24 (m, 1H, H-3), 3.99 (m, 1H, H-2eq), 3.79 (ddd, 1H, $J_{5,5a}$ 10.6 Hz, $J_{5a,1}$′ 7.3 Hz, $J_{5a,1}$′ 3.0 Hz, H-5a), 3.72–3.55 (m, 2H, 2× H-6), 2.98–2.78 (m, 3H, H-2ax, 2× H-4′), 1.89 (m, 1H, H-5), 1.79–1.30 (m, 6H, 2× H-1′, 2× H-2′, 2× H-3′); ^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 157.7, 157.4 (C = O, Boc), 109.4 [*C*$(CH_3)_2$], 97.5 (MOM), 81.2, 80.9 [O*C*$(CH_3)_3$], 81.2, 80.9 (C-3), 74.8, 74.6 (C-4), 68.4 (C-6), 55.6 (MOM), 52.0, 50.7 (C-5a), 43.8, 43.2 (C-2), 41.1 (C-4′), 40.4, 40.2 (C-5), 34.3, 34.1 (C-1′), 30.2, 29.4 (C-3′), 28.8 [OC(*C*$H_3)_3$], 26.8, 24.4, 24.2 [(*C*$H_3)_2$C], 23.5, 23.3 (C-2′); MS: Calcd for [$C_{20}H_{38}N_2O_6$Na]: *m/z* 425.2628 [M + Na]$^+$; Found [M + Na]$^+$ 425.2699.

4.21. (5aS)-N-tert-Butyloxycarbonyl-5a-C-(6-amino)hexyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (**38**)

Nitrile **36** (75 mg, 0.17 mmol) was converted into amine **38** by general procedure C. Purification on silica gel ($CHCl_3$/MeOH/concd NH_4OH 8:4:0.01 v/v/v) provided **38** (64 mg, 0.15 mmol, 84.1%). $[\alpha]_D^{20} = +27.4$ (c 1.05, MeOH); ^{1}H NMR (300 MHz, $CHCl_3$): δ = 4.57 (dd, 1H, $J_{3,4}$ 7.9 Hz, $J_{4,5}$ 2.4 Hz, H-4), 4.25 (m, 1H, H-3), 3.99 (m, 1H, H-2eq), 3.84–3.55 (m, 3H, H-5a, 2× H-6), 2.96–2.77 (m, 3H, H-2ax, 2× H-6′), 1.88 (m, 1H, H-5), 1.76–1.32 (m, 10H, 2× H-1′, 2× H-2′, 2× H-3′, 2× H-4′, 2× H-5′); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 157.7, 157.5 (C = O, Boc), 109.5 [*C*$(CH_3)_2$], 97.6, 97.5 (MOM), 80.8 [O*C*$(CH_3)_3$], 74.7, 74.5 (C-4), 73.4, 73.2 (C-3), 68.4 (C-6), 55.6 (MOM), 52.1, 50.8 (C-5a), 43.8, 43.1, 40.8, 40.7, 34.8, 34.6 (C-2, C-5, C-6′), 30.5, 30.4 (C-1′), 28.8 (OC(*C*$H_3)_3$), 28.6, 26.8 [(*C*$H_3)_2$C], 28.7-26.04 (C-2′, C-3′, C-4′, C-5′); MS: Calcd for [$C_{22}H_{42}N_2O_6$H]: *m/z* 431.3121 [M + H]$^+$; Found [M + H]$^+$ 431.3120; MS: Calcd for [$C_{22}H_{42}N_2O_6$Na]: *m/z* 453.2941 [M + Na]$^+$; Found [M + Na]$^+$ 453.2926.

4.22. (5aS)-N-tert-Butyloxycarbonyl-5a-C-(4-dansylamino)butyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (**39**)

Following general procedure D, compound **37** (28 mg, 0.07 mmol) was treated with Na_2CO_3 (19 mg, 0.18 mmol) and dansyl chloride (23 mg, 0.08 mmol) to give crude compound **39** which was immediately taken to the next step.

4.23. (5aS)-N-tert-Butyloxycarbonyl-5a-C-(6-dansylamino)hexyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (**40**)

Following general procedure D, compound **38** (64 mg, 0.15 mmol) was treated with Na_2CO_3 (44 mg, 0.42 mmol) and dansyl chloride (52 mg, 0.19 mmol) to give crude compound **40** which was immediately taken to the next step.

4.24. (5aS)-5a-C-(4-Dansylamino)butyl-4-epi-isofagomine (**41**)

Following general procedure A, crude compound **39** was converted into title compound **41**. Purification on silica gel ($CHCl_3$/MeOH/concd NH_4OH 8:4:0.01 v/v/v) and subsequent recrystallization in MeOH in the presence of HCl afforded **41**·HCl (21 mg, 0.41 mmol, 59.1% over 2 steps) as faintly green fluorescent solid. $[\alpha]_D^{20} = -15.2$ (c 1.1, MeOH); ^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.10 (dd, $J_{3,4} = J_{4,5}$ 2.5 Hz, Hz), 3.75 (ddd, 1H, $J_{2ax,3}$ 10.5 Hz, $J_{2eq,3}$ 6.1 Hz, H-3), 3.65 (bd, 2H, 2× H-6), 3.18 (ddd, 1H, $J_{5,5a}$ 10.9 Hz, $J_{5a,1}$′ 6.9 Hz, $J5_{a,1}$′ 3.1 Hz, H-5a), 3.14–3.00 (m, 2H, H-2eq, H-2ax) 2.85 (m, 2H, 2× H-4′), 1.69–1.155 (m, 8H, H-5, 2× H-1′, 2× H-2′, 2× H-3′); ^{13}C NMR (75.5 MHz, MeOD): δ = 153.3–116.5 (dansyl), 69.1 (C-4), 67.6 (C-3), 61.6 (C-6), 53.6 (C-5a), 45.8 (2xNMe$_2$), 45.1 (C-5), 44.4 (C-2), 43.4 (C-4′), 30.8, 30.5 (C-1′, C-3′), 22.5 (C-2′); MS: Calcd for [$C_{22}H_{33}N_3O_5$SH]: *m/z* 452.2219 [M + H]$^+$; Found [M + H]$^+$ 452.2336; MS: Calcd for [$C_{22}H_{33}N_3O_5$SNa]: *m/z* 474.2039 [M + Na]$^+$; Found [M + Na]$^+$ 474.2052.

4.25. (5aS)-5a-C-(6-Dansylamino)hexyl-4-epi-isofagomine (**42**)

Following general procedure A, crude compound **40** was converted into title compound **42**. Purification on silica gel ($CHCl_3$/MeOH/concd NH_4OH 8:4:0.01 v/v/v) provided **42** (35 mg, 0.07 mmol, 49.1% over 2 steps) as fluorescent wax. $[\alpha]_D^{20} = -10.3$ (c 2.17, MeOH); ^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.11 (dd, 1H, $J_{3,4} = J_{4,5}$ 2.5 Hz, H-4), 3.66 (bd, 2H, 2× H-6), 3.62 (ddd, $J_{2ax,3}$ 10.58 Hz, $J_{2eq,3}$ 6.12 Hz, H-3), 2.95–2.78 (m, 5H, H-2ax, H-2eq, H-5a, 2× H-6′), 1.64–1.03 (m, 11H, H-5, 2× H1′, 2× H-2′, 2× H-3′, 2× H-4′, 2× H-5′); ^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 153.2–116.4 (dansyl), 70.0 (C-4), 69.6 (C-3), 61.9

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(C-6), 52.6 (C-5a), 46.8 (C-5), 45.9 (C-2), 45.8 (2xNMe$_2$), 43.7 (C-6′)32.5 (C-1′), 30.4, 30.1, 27.2, 25.7 (C-2′, C-3′, C-4′, C-5′); MS: Calcd for [$C_{24}H_{37}N_3O_5SH$]: *m/z* 480.2532 [M + H]$^+$; Found [M + H]$^+$ 480.2480; MS: Calcd for [$C_{24}H_{37}N_3O_5SNa$]: *m/z* 502.2352 [M + Na]$^+$; Found [M + Na]$^+$ 502.2273.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.carres.2016.03.020.

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