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Synthesis and glycosidase inhibition potency of all-*trans* substituted 1-*C*-perfluoroalkyl iminosugars

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ABSTRACT

Synthetic analogues of the naturally occurring iminosugar homoDMDP, which feature a perfluoroalkyl group at the pseudo-anomeric position, have been synthesized from the corresponding sugar-derived cyclic aldonitrone. The new fluorinated iminosugars as well as homoDMDP and its 6-deoxy counterpart were evaluated for their inhibitory activity against a panel of glycosidases. While the replacement of the (1′,2′)-dihydroxyethyl substituent of homoDMDP with $-C_4F_9$ proved detrimental for enzyme binding, introduction of a $-C_3F_7$ moiety tuned the inhibitory activity spectrum selectively towards α-fucosidase and α-glucosidase from yeast.

1. Introduction

Iminosugars have been reported for fifty years as potent inhibitors of carbohydrate processing enzymes such as glycoside hydrolases, glycosyltransferases or glycogen phosphorylase [1]. A number of these nitrogen-in-the-ring carbohydrate mimics are naturally occurring but [2], in parallel, many synthetic approaches were developed to fill the library of analogues [3]. Among the five families of natural iminosugars (polyhydroxy-piperidines, -pyrrolidines, -pyrrolizidines, -nortropanes and -indolizidines), pyrrolidines such as DMDP **1**, DAB **1′** and their homologues (compounds **2** and **3** in Fig. 1 but also broussonetines [4], which contain a C_{13} lipophilic side-chain) are particularly widespread [5]. Interestingly, the inhibitory potency of homologues of DAB is clearly dependent on the nature of the side-chain. For instance, while DAB is a modest inhibitor of trehalase from porcine kidney (IC_{50} = 61 μM) [6], homoDMDP **2** displays strong affinity (IC_{50} = 5.0 μM) for the same isoform and **3** shows no significant inhibition [7]. Mimicry of the natural substrate (for instance, α-D-glucopyranosyl-1,1-α-D-glucopyranoside in the case of trehalase) by iminosugar *C*-glycosides might place the extra substituent into the aglycon subsite, giving rise to favorable (increasing inhibition potency) or unfavorable (decreasing potency) interactions [8]. Thus, the synthesis of new iminosugar *C*-glycosides with unprecedented side-chains would help identifying more potent and more specific inhibitors of therapeutically relevant glycosidases. In this regard, the introduction of fluorine atoms or fluoroalkyl groups into the structures of iminosugars has been initiated to tune both their inhibition profile and their physico-chemical properties such as solubility, basicity or bioavailability [9]. The role of fluorine substitution in structure-activity relationships has been largely reviewed and exemplified with a series of biologically relevant pharmacophores [10]. With iminosugars, some studies focused on the replacement of one OH group by F, with limited success though [11]. Recently, first series of iminosugar *C*-glycosides with a polyfluoroalkyl side chain such as **4**–**7** (Fig. 1) have been designed and synthesized to inhibit glycosidases or glycosyltransferases. While 6,6-difluoro-homoDMDP **4** decreased slightly the activity of α-glucosidase from yeast and rice (no inhibition on other glycosidases), trifluoro-homoDMDP **5** was mostly active against β-glucosidase from almond [7], whereas difluoromethylphosphono-iminosugar **6** was inactive to inhibit chitin synthase [12] (a glycosyl transferase) or glycosidases [13]. The *fuco*-configured perfluoropropyl *C*-glycoside **7** was a moderate inhibitor of fucosidase (74% inhibition at 250 μM) [9b]. Nevertheless, the prediction of the activity of homoDMDP analogues bearing a fluorinated chain remains elusive, so that the extension of the library of fluorinated homoDMDP analogues may be of interest to identify new active and/or specific targets.

Herein, we report the synthesis of new analogues of homoDMDP **8a,b**, which feature a perfluoroalkyl chain at the C-1 position (carbohydrate numbering) and their inhibitory activities against a range of commercial glycosidases in comparison with iminosugars **2** and **3**, which were assayed under the same conditions.

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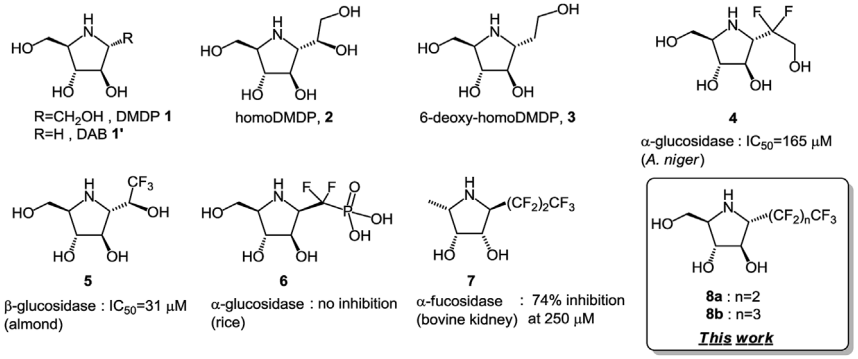
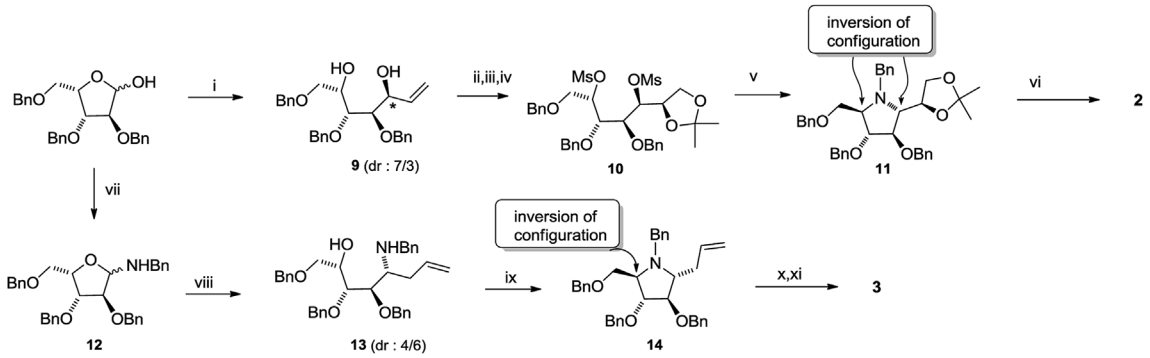


Fig. 1. Structures of iminosugars structurally related to homoDMDP.



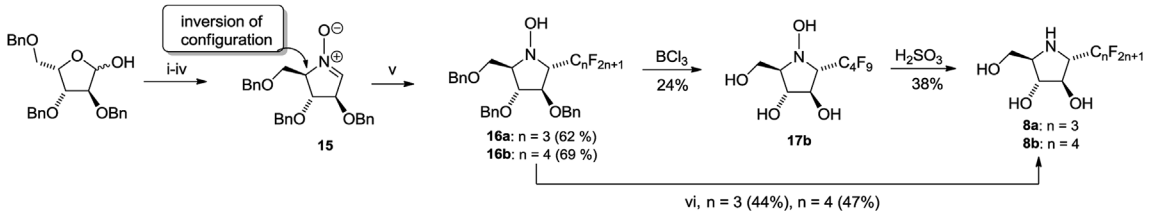
Scheme 1. Synthesis of iminosugars **2** and **3** [15,16]. Reagents and conditions. i: vinylMgBr, THF, 0 °C, 91%; ii: MsCl, NEt_3, CH_2Cl_2, 91%; iii: OsO_4, NMO, acetone/water, 80% (dr: 4/6); iv: acetone, TsOH, 89%; v: $BnNH_2$, 120 °C, 69%; vi: 80% HCOOH then H_2, $Pd(OH)_2/C$, 70%; vii: $BnNH_2$, CH_2Cl_2, 4 Å MS, quant.; viii: allylMgCl, THF, 0 °C, 91%; ix: MsCl, pyr., 79%; x: H_2SO_4, O_3, then $NaBH_4$, MeOH, 74%; xi: $HCOONH_4$, 10% Pd/C, MeOH, reflux, 40%.

2. Synthesis of homoDMDP and its analogues

Iminosugar *C*-glycosides **2** and **3** were obtained either from 2,3,5-tri-*O*-benzyl-L-xylofuranose or from glycosylamine **12** following reported procedures as depicted in Scheme 1 [14–16].

Sugar-derived cyclic nitrones represent appropriate building-blocks for the introduction of various substituents on an iminosugar scaffold through 1,3-dipolar cycloaddition or nucleophilic addition of organometallic reagents [17]. The stereochemical outcome of these reactions is typically controlled by the stereocenter next to the electrophilic carbon and substantial diastereoisomeric excesses are usually obtained. To access homoDMDP analogues, the D-arabino configured nitrone **15** was required (Scheme 2), which was prepared in four steps from 2,3,5-tri-*O*-benzyl-L-xylose, according to the reported procedure [18]. With nitrone **15** in hands, we investigated the addition step of a perfluoroalkyl organometallic nucleophile. Perfluoroalkyl Grignard reagents such as C_3F_7MgBr or C_4F_9MgBr are unstable species which decompose above −30 °C and thus need to be prepared *in situ* [19]. Here, a pre-formed solution of perfluoroalkyl Grignard was obtained by addition of EtMgBr to C_3F_7I or C_4F_9I in Et_2O at −45 °C. Nitrone **15**, solubilized in a minimal volume of THF, was added and the mixture was left to react for 1 h. An excess of the organometallic was needed (2.5 equivalents) to secure complete conversion, affording the expected hydroxylamines **16a** and **16b** in 62% and 69% respectively after purification on silica gel (ethyl acetate/petroleum ether, 1/9, v/v). Surprisingly, no conversion was observed when Et_2O was replaced by THF as the solvent during the process. Nucleophilic additions of organometallics to nitrone **15** usually occur with good stereoselectivities affording the (1*S*) configured hydroxylamine as the major diastereoisomer [20]. The same observation accounted here for the addition of the perfluoroalkyl species. Only one isomer was isolated, arising from exclusive nucleophilic attack at the less hindered *Re* side, as controlled by the bulky substituents at C-2 and C-4. The configuration of the newly formed stereogenic center was confirmed by HOESY experiments. The relative *cis* position of the perfluoroalkyl chain with regard to H-2 and H-4 in **16b** was deduced from the corresponding correlations (Fig. 2). The configuration of **16a** was attributed by analogy.

Deprotection and N-O reduction of pyrrolidines **16** was finalized next. A two-step procedure was experienced starting with **16b**. Treatment of **16b** with BCl_3 afforded the expected debenzylated hydroxylamine in only 24% yield [21]. Reduction of **17b** was effected next with sulfurous acid, giving the targeted homoDMDP analogue **8b** in 38% yield. Alternatively, concomitant debenzylation and hydrogenolysis were carried out successfully after prolonged reaction time



Scheme 2. Synthesis of fluorinated iminosugars **8a,b**. i: H_2N-OTBDPS, $MgSO_4$, PPTS, PhMe, 100 °C, 30 min; ii: MsCl, NEt_3, CH_2Cl_2, 0 °C to rt, 30 min; iii: TBAF, THF, 0 °C, 5 min; iv: NH_2OH-HCl, $NaHCO_3$, MeOH-H_2O, rfx 16 h; v: I-C_nF_{2n+1}, EtMgBr, Et_2O, − 45 °C, 30 min; vi: H_2, Pd/C, HCl 6 M, rt, 48 h.

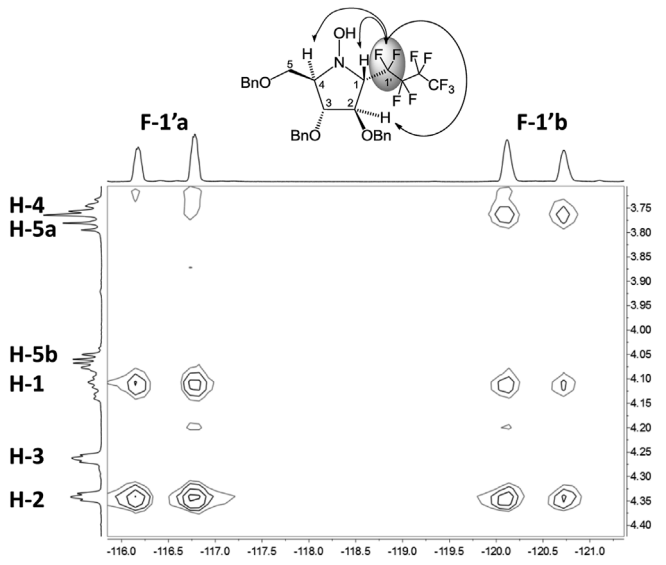


Fig. 2. Two-dimensional heteronuclear (HOESY) correlations for **16b**.

(48 h) using Pd/C in the presence of 6 M HCl, affording **8a** and **8b** in 44% and 47% yields respectively. The low polarity and poor protonation ability of perfluorinated iminosugars **7** allowed easy purification by silica gel chromatography with ethyl acetate as the eluent. The all-*trans* configuration of mannitol-derived **8a,b** was confirmed here by the characteristic coupling constants of such systems ($J_{1,2}$, $J_{2,3}$, $J_{3,4}$ above 6 Hz) [22].

3. Glycosidase inhibition assays

The strong interaction of iminosugars with glycoenzymes has attracted great attention in medicinal chemistry. Indeed, the depletion of glycoenzyme's activity with iminosugar inhibitors or the control of the native conformation of glycosidases with iminosugar chaperones were experienced in the study or the therapy of several disorders [23]. Nevertheless, the lack of selectivity of iminosugars usually hampers their medical use, the inhibition of the targeted enzyme being accompanied by undesirable inhibitions of isoforms that display essential biological roles. Selectivity might be induced by an additional substituent at the pseudo-anomeric position [24]. In the case of DAB **1′** and its analogues, the insertion of CF_2 or CF_3 on the side chain affords analogues such as **4** or **5**, which display retained or even increased biological potential towards certain glycosidases [7]. With highly fluorinated compounds **8a,b** it is possible to go further in the analysis of structure-activity relationships. To this aim, iminosugars **8a,b** were tested against a panel of glycosidases in parallel with the non-fluorinated analogues **2** and **3**. Inhibition of rice α-glucosidase, yeast α-glucosidase, almond β-glucosidase, β-glucosidase from *Aspergillus niger*, β-galactosidase from *Aspergillus oryzae*, Jack bean α-mannosidase, bovine liver α-fucosidase and α-rhamnosidase from *Aspergillus niger* was first evaluated at 1 mM concentration of inhibitor (Table 1). When almost complete inhibition (> 95%) occurred at this high concentration, IC_{50} (the concentration of inhibitor affording half initial rate of the considered glycosidase) was determined further. In the case where inhibition rate was between 80% and 95%, additional assay was conducted at a concentration of 100 µM. According to the literature, heptafluoropropylpyrrolidines (p*K*a = 4.5–4.6) are significantly less basic than DMDP (p*K*a = 7.2), which impacts the protonation state of the iminosugar in biological medium [9b]. Thus, all the assays were performed at buffered pH = 5.7, to ensure proper enzyme activity and, at least partial, protonation at nitrogen. Glycosidases are particularly sensitive to pH, as examplified on Fig. 3 with α-glucosidase from yeast: changing the pH from 6.9 to 6.2 or 5.7 results in significant loss in initial rate as reflected by the decrease in absorbance due to released *p*-nitrophenolate [25]. However, at pH 5.7, α-glucosidase from yeast is still sufficiently active to perform the biological assays. Another border line case was α-glucosidase from rice, which was significantly more active at pH 5.2 than pH 5.6. Thus, contrarily to all the other tested glycosidases, the isoform from rice was assayed at pH 5.2.

Table 1
Inhibitory activity against glycosidases.[a,b]

Enzyme	2	3	8a	8b	DAB (1′)
α-glucosidase rice[c]	79%[d]	12%	33%	6%	IC_{50} = 0.15 µM[h]
α-glucosidase *S. cerevisiae*	84%[e]	63%	99% **IC_{50} = 6.2 µM**	9%	IC_{50} = 250 µM[h]
β-glucosidase almond	96% **IC_{50} = 0.41 µM**	67%	90%[g]	60%	IC_{50} = 250 µM[h]
β -glucosidase *A. niger*	36%	NI	11%	31%	
β -galactosidase *A. orizae*	91%[f]	15%	31%	NI	
α-mannosidase Jack bean	25%	19%	40%	NI	IC_{50} = 320 µM[h]
α-rhamnosidase *A. niger*	26%	20%	20%	23%	
α-fucosidase bovine kidney	13%	NI	99% **IC_{50} = 56 µM**	9%	NI[h]

[a] Expressed as % inhibition at 1 mM concentration of inhibitor. [b] NI means no inhibition at 1 mM. [c] Assayed at pH 5.2. [d] 34% at 100 µM. [e] 36% at 100 µM. [f] 47% at 100 µM. [g] 33% at 100 µM.[h] taken from Ref. [7].

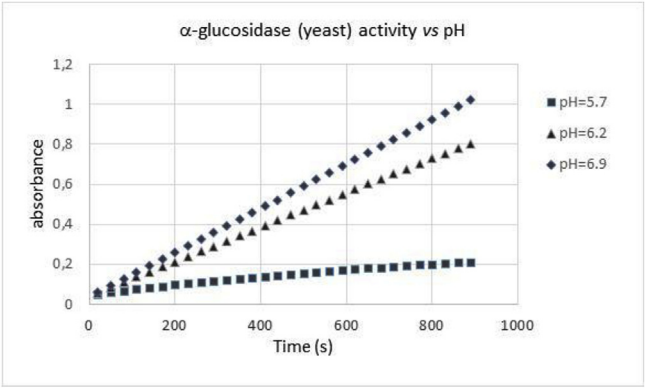


Fig. 3. α-glucosidase catalysed release of *p*NP from α-*p*NP-Glc at different pH.

As reported in Table 1, homoDMDP **2** was a potent inhibitor of β-glucosidase from almond (IC_{50} = 0.41 µM) and had some effect on the other tested glucosidases, though to a lesser extent [26]. This was accompanied by a strong inhibition of β-galactosidase at 1 mM, which decreased rapidly (only 47% remaining inhibition at 100 µM). For its part, 6-deoxy-homoDMDP elicited only weak inhibition at 1 mM concentration towards yeast α-glucosidase and β-glucosidase from almond. Interestingly, the biological behaviour of fluorinated homoDMDP analogues **8a,b** differed significantly from their model **2**. Whereas (C_4F_9) substituted **8b** had almost no effect on the tested glycosidases (only 60% inhibition of β-glucosidase from almond), iminosugar **8a** proved strongly active against α-glucosidase from yeast (IC_{50} = 6.2 µM) and α-fucosidase (IC_{50} = 56 µM). It is commonly admitted that favorable IC_{50} (*ie*, below 100 µM) might be obtained only for iminosugars showing almost complete inhibition (> 95%) at 1 mM concentration. This is ensured here with control assays at 100 µM, conducted when % inhibition is in the borderline range 80–95%, actually providing inhibition rates < 50% in each case at this concentration.

As observed earlier with *gem*-difluoromethylene or trifluoromethyl substituted iminosugars **4**,**5**, the electronic impact of the perfluorinated

substituent as well as its hydrophobic character might influence the conformation of the molecule and spatial distribution of hydroxyl groups [27], which would lead to unexpected inhibition profile. This is clearly evidenced here with **8a**, which displays strong inhibition of yeast α-glucosidase, an enzyme that is usually sparsely impacted by homoDMDP analogues. Iminosugar **8a** is also a strong inhibitor of α-fucosidase, which is uncommon for DMDP-scaffolded pyrrolidines. Moreover, a C_4F_9 substituent seems to be the upper limit of fluorine introduction since an almost complete loss in inhibition potency was observed with analogue **8b**.

4. Conclusions

In conclusion, homoDMDP analogues with a perfluoroalkyl chain at the pseudo anomeric position were prepared using a stereoselective nucleophilic addition of fluorinated Grignard reagents onto a cyclic nitrone in the key step of the synthetic sequence. These two fluorinated iminosugars with a perfluoropropyl or a perfluorobutyl chain were tested against a panel of glycosidases. While the replacement of the dihydroxyethyl side-chain of homoDMDP by $–C_4F_9$ proved deleterious for enzyme binding, introduction of a $–C_3F_7$ moiety afforded potent and selective inhibition of α-fucosidase and α-glucosidase from yeast. Nevertheless, concerning iminosugars, the impact of fluorine atoms on their glycosidase inhibition potencies is so far difficult to predict. Indeed, introduction of fluoroalkyl chains might deeply influence hydrophobicity or electron density partitioning of the molecule, as well as p*K*a of the amine, as previously described [9b]. As a consequence, intramolecular interactions (conformation) and intermolecular glycosidase-inhibitor interactions (binding) might be deeply affected to afford new and rather unexpected inhibition profiles.

5. Acknowledgements

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6. Experimental section

6.1. General methods

Reactants and reagents were purchased from Aldrich and Sigma and were used without further purification. Silica gel F254 (0.2 mm) was used for TLC plates, detection being carried out by spraying with an alcoholic solution of phosphomolybdic acid or an aqueous solution of $KMnO_4$ (2%)/Na_2CO_3 (4%), followed by heating. Flash column chromatography was performed over silica gel M 9385 (40–63 μm) Kieselgel 60. NMR spectra were recorded on Bruker AC 250 (250 MHz for ^1H, 62.5 MHz for ^{13}C and 235 MHz for ^{19}F), 500 (500 MHz for ^1H, 125 MHz for ^{13}C and 470 MHz for ^{19}F) or 600 (600 MHz for ^1H, 150 MHz for ^{13}C) spectrometers. Chemical shifts are expressed in parts per million (ppm) and were calibrated to the residual solvent peak for ^1H and ^{13}C spectra and to CCl_3F peak for ^{19}F spectra. Coupling constants are in Hz and splitting pattern abbreviations are: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet. Optical rotations were determined at 20 °C with a Perkin-Elmer Model 241 polarimeter in the specified solvents. High Resolution Mass Spectra (HRMS) were performed on Q-TOF Micro micromass positive ESI (CV = 30 V).

6.2. Synthesis of perfluoroalkylhydroxylamines *16a,b*

A solution of EtMgBr (3 M, 0.73 mL, 2.2 mmol) was added to perfluoroalkyl iodide (2.2 mmol) in dry Et_2O (6 mL) at −45 °C. The mixture was stirred for 40 min. A solution of nitrone **15** (150 mg, 0.877 mmol) in THF/Et_2O (0.6 mL/1.5 mL) was added dropwise during 15 min and the mixture was stirred for 1 h after which the temperature was raised slowly to −15 °C. A solution of sat. NH_4Cl was then added and the mixture was extracted with Et_2O (3 × 15 mL). The organic layers were dried ($MgSO_4$), concentrated and purified by column chromatography (EtOAc/Petroleum ether: 1/9) to yield pure hydroxylamine **16a,b**.

6.2.1. (1S)-N-hydroxy-2,3,5-tri-O-benzyl-1-C-heptafluoropropyl-1,4-dideoxy-1,4-imino-D-arabinitol *16a*

(62%, colourless oil): R_f = 0.25 (EtOAc/Petroleum ether: 1/9); $[\alpha]_D^{20}$ = −14.3 (*c* 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 3.66–3.73 (m, 2H, H-4 and H-5a), 3.96–4.02 (m, 2H, H-1 and H-5b), 4.17 (dd, 1H, $J_{4,5}$ 4.5 Hz, $J_{4,3}$ 3.5 Hz, H-3), 4.24 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{3,2}$ 3.5 Hz, H-2), 4.39 (d, 1H, $J_{Ha,Hb}$ 11.5 Hz, CH_aH_b-Ph), 4.48 (d, 1H, $J_{Hb,Ha}$ 11.5 Hz, CH_aH_b-Ph), 4.51 (d, 1H, $J_{Ha',Hb'}$ 12 Hz, $CH_{a'}H_{b'}$-Ph), 4.52 (s, 2H, CH_2-Ph), 4.56 (d, 1H, $J_{Hb',Ha'}$ 12 Hz, $CH_{a'}H_{b'}$-Ph), 5.25 (s, 1H, OH), 7.28–7.34 (m, 15H, 3 Ph); ^{13}C NMR (150 MHz, $CDCl_3$) δ 65.81 (C-5), 69.17 (C-4), 72.19 (CH_2), 72.29 (CH_2), 72.64 (dd, $J_{C,F}$ 18.7 Hz and 25.7 Hz, C-1), 73.33 (CH_2), 82.37 (C-3), 83.11 (C-2), 107.18–111.45 (m, CF_3 and 2 x CF_2), 127.81–128.58 (m, Ar), 137.29 (C_{quat}), 137.64 (C_{quat}), 138.11 (C_{quat}); ^{19}F NMR (470 MHz, $CDCl_3$): δ −80.49 (t, 3F, $J_{F,F}$ 10.4 Hz, CF_3), −116.83 (dm, 1F, $J_{F,F}$ 284 Hz), −121.48 (dm, 1F, $J_{F,F}$ 284 Hz), −125.35 (ddd, 1F, $J_{F,F}$ 290 Hz, 11.6 Hz and 4.6 Hz), −126.4 (ddd, 1F, $J_{F,F}$ 290 Hz, 11.5 Hz and 2.7 Hz); HRMS- ESI$^+$ (*m/z*): $[M+Na]^+$ calcd for $C_{29}H_{28}NO_4F_7Na$: 610.1804; found 610.1807.

6.2.2. (1S)-N-hydroxy-2,3,5-tri-O-benzyl-1-C-nonafluorobutyl-1,4-dideoxy-1,4-imino-D-arabinitol *16b*

(69%, colourless oil): R_f = 0.25 (EtOAc/Petroleum ether: 1/9); $[\alpha]_D^{20}$ = −11.8 (*c* 1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 3.73–3.79 (m, 2H, H-4 and H-5a), 4.05–4.14 (m, 2H, H-1 and H-5b), 4.26 (dd, 1H, $J_{4,5}$ 4.0 Hz, $J_{4,3}$ 3.0 Hz, H-3), 4.34 (dd, 1H, $J_{3,4}$ 3.3 Hz, $J_{3,2}$ 3.3 Hz, H-2), 4.47 (d, 1H, $J_{Ha,Hb}$ 11.5 Hz, CH_aH_b-Ph), 4.56 (d, 1H, $J_{Hb,Ha}$ 11.5 Hz, CH_aH_b-Ph), 4.58 (d, 1H, $J_{Ha',Hb'}$ 11.7 Hz, $CH_{a'}H_{b'}$-Ph), 4.59 (s, 2H, CH_2-Ph), 4.63 (d, 1H, $J_{Hb',Ha'}$ 11.7 Hz, $CH_{a'}H_{b'}$-Ph), 5.92 (s, 1H, OH), 7.33–7.42 (m, 15H, 3 Ph); ^{13}C NMR (125 MHz, $CDCl_3$) δ 65.80 (C-5), 69.07 (C-4), 72.10 (CH_2), 72.25 (CH_2), 72.82 (dd, $J_{C,F}$ 18.7 Hz and 25.0 Hz, C-1), 73.22 (CH_2), 82.42 (C-3), 83.19 (C-2), 106.67–121.29 (m, CF_3 and 3 x CF_2), 127.79–128.50 (m, Ar), 137.30 (C_{quat}), 137.66 (C_{quat}), 138.04 (C_{quat}); ^{19}F NMR (470 MHz, $CDCl_3$): δ −80.85 (t, 3F, $J_{F,F}$ 9.5 Hz, CF_3), −116.48 (dm, 1F, $J_{F,F}$ 284 Hz), −120.41 (dm, 1F, $J_{F,F}$ 284 Hz), −121.96 (dm, 1F, $J_{F,F}$ 298 Hz), −122.76 (dm, 1F, $J_{F,F}$ 298 Hz), −125.29 (dm, 1F, $J_{F,F}$ 294 Hz), −126.37 (dm, 1F, $J_{F,F}$ 294 Hz); HRMS- ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_{30}H_{29}NO_4F_9$: 638.1953; found 638.1948.

6.3. Synthesis of perfluoroalkyliminosugars *8a,b*

A solution of **16a** (80 mg, 0.136 mmol) in MeOH (4 mL) and 6 M HCl (0.8 mL) was hydrogenated in the presence of Pd/C (10%) (40 mg) under H_2 (1 bar). After 24 h, more Pd-C (40 mg) was added and the solution was stirred for additional 24 h. The catalyst was then removed by filtration over Celite® and the solution was concentrated. The resulting crude product was diluted in MeOH, neutralized with Amberlyst A-26 (HO^-) resin and purified by column chromatography (ethyl acetate) to yield **8a** as colorless oil (18 mg, 44%).

6.3.1. (1S)-1-C-heptafluoropropyl-1,4-dideoxy-1,4-imino-D-arabinitol *8a*

R_f = 0.27 (EtOAc); $[\alpha]_D^{20}$ = +15.2 (*c* 0.8, MeOH); ^1H NMR (250 MHz, CD_3OD): δ 2.90 (ddd, 1H, $J_{5,4}$ 8.5 Hz, $J_{5,6a}$ 4.2 Hz, $J_{5,6b}$ 3.2 Hz, H-4), 3.65 (dd, 1H, $J_{Ha',Hb'}$ 11.5 Hz $J_{5,6}$ 4.2 Hz, H-5a), 3.65–3.80 (m, 1H, H-1), 3.70 (dd, 1H, $J_{Ha',Hb'}$ 11.6 Hz $J_{5,6}$ 3.2 Hz, H-5b), 3.84 (dd, 1H, $J_{4,5}$ 8.5 $J_{4,3}$ 6.7 Hz, H-3), 4.27 (t, 1H, $J_{3,4}$ $J_{3,2}$ 6.7 Hz, H-2); ^{13}C NMR

(62.5 MHz, CD_3OD) δ 61.1 (C-5), 62.8 (dd, $J_{C,F}$ 20.3 Hz and 24.3 Hz, C-1), 64.3 (C-4), 78.5 (C-2), 78.5 (C-3), 108.0–129.0 (m, CF_3, 2 × CF_2); ^{19}F NMR (235 MHz, $CDCl_3$): δ −82.4 (t, 3F, $J_{F,F}$ 9.9 Hz, CF_3), −122.4 (dm, 1F, $J_{F,F}$ 276 Hz), −125.2 (dm, 1F, $J_{F,F}$ 276 Hz), −127.1 (d, 1F, $J_{F,F}$ 7.5 Hz), −127.3 (d, 1F, $J_{F,F}$ 7.5 Hz); HRMS- ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_9H_{11}NO_3F_9$: 352.0595; found 352.0597.

A solution of **16b** (209 mg, 0.328 mmol) in MeOH (5 mL) and 6 M HCl (1.0 mL) was hydrogenated in the presence of Pd/C (10%) (50 mg) under H_2 (1 bar). After 24 h, more Pd-C (50 mg) was added and the solution was stirred for additional 24 h. The catalyst was then removed by filtration over Celite® and the solution was concentrated. The resulting crude product was diluted in MeOH, neutralized with Amberlyst A-26 (HO^-) resin and purified by column chromatography (ethyl acetate) to yield **8b** as colorless oil (54 mg, 47%).

*6.3.2. (1S)-1-C-nonafluorobutyl-1,4-dideoxy-1,4-imino-D-arabinitol **8b***

R_f = 0.32 (EtOAc); $[\alpha]_D^{20}$ = +19.6 (*c* 0.56, MeOH); ^1H NMR (250 MHz, CD_3OD): δ 2.87 (ddd, 1H, $J_{5,4}$ 9.0 Hz, $J_{5,6a}$ 4.2 Hz, $J_{5,6b}$ 3.2 Hz, H-4), 3.63 (dd, 1H, $J_{Ha',Hb'}$ 11.6 Hz $J_{5,6}$ 4.2 Hz, H-5a), 3.65–3.80 (m, 1H, H-1), 3.70 (dd, 1H, $J_{Ha',Hb'}$ 11.6 Hz $J_{5,6}$ 3.2 Hz, H-5b), 3.81 (dd, 1H, $J_{4,5}$ 9.0 $J_{4,3}$ 6.8 Hz, H-3), 4.24 (t, 1H, $J_{3,4}$ $J_{3,2}$ 6.8 Hz, H-2); ^{13}C NMR (62.5 MHz, CD_3OD) δ 61.3 (C-5), 62.9 (dd, $J_{C,F}$ 20.0 Hz and 24.5 Hz, C-1), 64.4 (C-4), 78.6 (C-2), 78.6 (C-3), 105.0–127.0 (m, CF_3, 3 × CF_2); ^{19}F NMR (235 MHz, $CDCl_3$): δ −82.6 (t, 3F, $J_{F,F}$ 9.9 Hz, CF_3), −122.0 (dm, 1F, $J_{F,F}$ 280 Hz), −123.4 to −123.6 (m, 2F), −124.6 (dm, 1F, $J_{F,F}$ 280 Hz), −127.4 (tm, 2F, $J_{F,F}$ 13.9 Hz); HRMS- ESI$^+$ (*m/z*): $[M+H]^+$ calcd for $C_8H_{11}NO_3F_7$: 302.0627; found 302.0634.

6.4. Glycosidase assay

α-Glucosidases (from rice and yeast), β-glucosidases (from almond and *A. niger*), β-galactosidase (from *A. oryzae*), α-mannosidase (from Jack bean), α-rhamnosidase (from *A. niger*), α-fucosidase (from bovine liver) and *p*-nitrophenyl glycosides were purchased from Sigma Chemical Co. In a typical experiment, the glycosidase (0.013 U/mL) was pre-incubated at 33 °C for 5 min in the presence of 1 mM of the inhibitor in 50 mM acetate buffer (pH 5.7 for all enzymes except α-glucosidase from rice, which was assayed at pH 5.2). The reaction was started by addition of the appropriate *p*-nitrophenyl glycoside substrate (final concentration 1 mM) to a final volume of 250 µL. The reaction was stopped after 15 min by addition of 350 µL of 0.4 M Na_2CO_3. The released *p*-nitrophenolate was quantified spectrometrically at 415 nm with a microplate reader (96-well plates filled with 300 µL assay solution in each well). The control experiment contained no inhibitor. Percentage of inhibition was calculated as follows:

$$\%I = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100$$

In cases where inhibition was above 95%, IC_{50} values were determined after assaying decreasing concentrations of inhibitor. When inhibition rate at 1 mM was between 80% and 95%, a control assay was performed at an inhibitor concentration of 100 µM, which always demonstrated % inhibitions below 50% (ie, IC_{50} > 100 µM). All the assays were done in duplicate (less than 10% variability in each case).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.carres.2018.05.004.

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