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# Galacto configured *N*-aminoaziridines: a new type of irreversible inhibitors of β-galactosidases

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A new type of galactose mimetics has been synthesized following a straightforward synthetic approach based on cyclohexene olefin aziridination reactions directed by hydroxyl substituents. These enantiomerically pure galacto-configured *N*-aminoaziridines are potent irreversible inhibitors of *Aspergillus oryzae* and *Escherichia coli* β-galactosidases.

## Introduction

Glycosidases[1] are a class of enzymes that assists the hydrolysis of glycosidic bonds in complex sugars and have interesting roles in nature.[2,3] Different classification[4] of these enzymes are known depending on the substrate specificity, mode of action[5] and, their amino acid sequence and predicted structural relationship. A variety of natural products[6] have been described as inhibitors of glycosidases[7] (Figure 1). However, other synthetic analogs[7-10] of these natural products (Figure 1) have been obtained in order to improve its inhibitory activity,[10] use them as therapeutics[3,11] or find applications as pharmacological tools.[12] For instance, the synthesis of cyclophellitol analogs and galactopyranose-configured isomers of cyclophellitol[13] have found recent applications in activity-based probes for glycosidases.[14,15] Although a variety of *N*-functionalized cyclophellitol aziridine[13,14] derivatives are reported, no precedents of *N*-aminoaziridine functionality in sugar mimetics have been yet described. As mechanism-based irreversible inhibitors of glycosidases are of interest,[16] we thought that novel types of functional groups could be capable of reacting with the enzyme machinery as mechanism based inhibitors. Therefore, we were attracted by the introduction of *N*-aminoaziridines and their corresponding hydrazone derivatives, that could be easily obtained with reactive carbonyl compounds. In addition, it should be noted that the introduction of *N*-aminoaziridines opens a new area of further development in the preparation of sugar analogs by

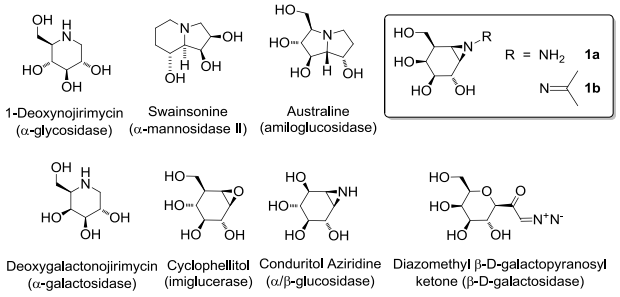


**Figure 1.** Known inhibitors of glycosidases (enzyme inhibited is given in brackets), and *N*-aminoaziridines **1a** and **1b**.

hydrazone formation, *N*-*N*-acylation or *N*-*N*-alkylation, that can be explored as irreversible inhibitors. Because of the reasons outlined above, the aim of this work is the synthesis of the galacto-configured *N*-aminoaziridines **1a** and its hydrazone derivative **1b** (Figure 1) as sugar mimetics to study the effect of these analogs on the activity of different glycosidases.

## Results and discussion

In order to synthesize the compounds of interest **1a** and **1b**, it was considered a report[17] on the synthesis of galacto-configured aminocyclitol phytoceramides. In this work[17] is described the preparation of olefin **2** (Scheme 1), which could be a key intermediate for the exploration of olefin aziridination reactions (Scheme 1). Although different literature approaches[18] to aziridination of olefins exist, the method described by Atkinson[19] was considered suitable for our purposes. Interestingly, it is reported[19] that the presence of hydroxyl groups in the vicinal positions of the double-bond could influence the stereoselectivity of the aziridination reaction by playing a directing role in the transition state. The application of this methodology to olefin **2**[17] would lead enantiomerically pure galacto-configured sugar analogs **1a** and **1b** (Scheme 1).

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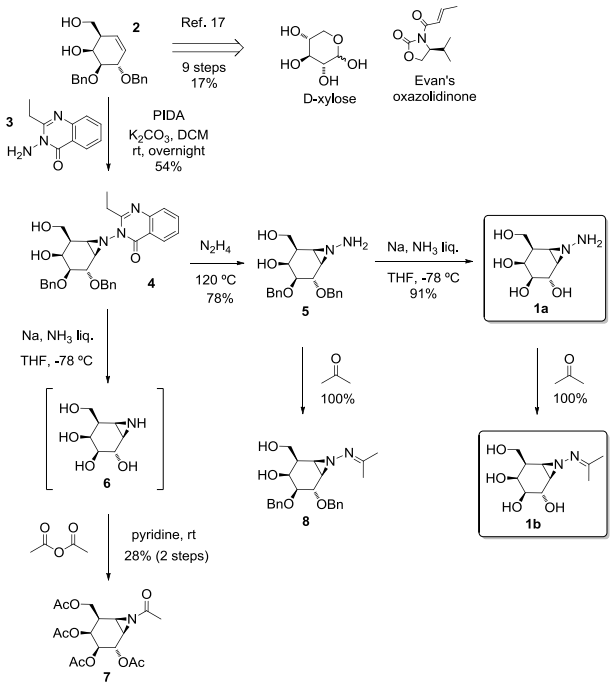
† Electronic Supplementary Information (ESI) available: Scheme S1, Figure S1, copies of NMR spectra, and HPLC methods and chromatograms. See DOI: 10.1039/x0xx00000x




**Scheme 1.** Aziridination reaction and reactivity of the subsequent intermediates to afford *N*-aminoaziridines **1a** and **1b**.

To carry out the synthesis of the desired aziridines, the key synthetic precursor **2**[17] was synthesized and it was used as a test substrate to explore the reactivity in olefin aziridination reactions by using *N*-aminophthalimide, 2-ethyl-3-aminoquinazolinone[20] (**3**) or 3-acetoxyamino-2-trifluoromethyl-3-aminoquinazolin-4-one[20,21] as nitrogen donors and phenyliodine diacetate ($PhI(OAc)_2$, PIDA)[22] or lead tetraacetate ($Pb(OAc)_4$) as oxidating agents. In our case, quinazolinone **3** and PIDA were the most efficient reagents and the aziridination of cyclohexene **2** was carried out in analogy to a reported procedure to control the diastereoselectivity of the aziridination reactions of chiral allylic alcohols.[23] Thus, the reactivity of olefin **2** with 2-ethyl-3-aminoquinazolinone (**3**)[20] was studied and, after a few attempts to optimize the reaction, a single diastereomeric adduct **4** with *syn* stereochemistry (see structural characterization of this compound in the Experimental section) was obtained in a moderate 54% yield (Scheme 1). At this point, we decided to explore its conversion to the desired final galacto-configured compounds of interest **1a** and **1b**. A described procedure[24] consisting in the treatment of the *N*-substituted aziridine **4** with Na or $Li/NH_3$ liq. in THF at – 78 ºC was first considered, because this would also remove the benzyl groups in one reaction step. However, the treatment of **4** under these reaction conditions (Scheme 1) gave a complex mixture and attempts to characterize the products formed were only possible after acetylation. We could conclude that aziridine **6** was mainly formed instead of the expected *N*-aminoaziridine **1a** due to the isolation of the peracetylated compound **7**. The extensive reduction of the *N-N* bond under these conditions prompted us to consider the debenzylation of **5** as an alternative route towards **1a**.

A literature procedure by Atkinson et al.[25] reporting the hydrazinolysis of an *N*-aminoaziridine attracted our interest. Thus, heating aziridine **4** with hydrazine at 120 ºC (Scheme 1) for 20 minutes showed total consumption of the starting material and compound **5** was consistently isolated in 65-75% yields. However, when running $^1$H-NMR, we observed a progressive transformation of compound **5** with time. We could deduce that hydrazone **8** (Scheme 1) was formed, presumably coming from the reaction of *N*-aminoaziridine **5** with adventitious acetone, present in solvents, laboratory ambient or glassware.

This was confirmed by spectroscopic and analytical data on **8**, which was quantitatively formed after simply mixing **5** with excess acetone. This result is in accordance with the reported[26] formation of hydrazones from 1-aminoaziridines and reactive carbonyl compounds or imines at temperatures below 40 ºC, to give a special family of *N*-aziridinylimines known as Eschenmoser hydrazones.[27] Gratifyingly, when more strict experimental conditions were employed, using acetone free glassware and solvents, *N*-aziridinylimine **5** could be isolated in 78% yield and was uneventfully characterized (Scheme 1). Interestingly, the desired *N*-amino-aziridine **1a** was obtained in 91% yield after debenzylation of intermediate **5** with $Na/NH_3$ liq. in THF at -78 ºC without reductive cleavage of the *N-N* bond (Scheme 1). Again, **1a** exhibited high reactivity in the presence of acetone and hydrazone **1b** was formed in quantitative yield.

With these compounds in hand, we next tested the activity of *N*-aminoaziridines **1a** and **1b** as inhibitors of different glycosidases (Figure 2). Whereas the compounds did not display significant

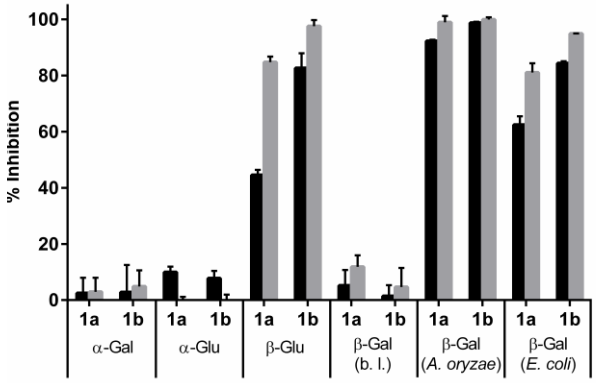



**Figure 2.** Inhibition of green coffee beans α-galactosidase (α-Gal), *Saccharomyces cerevisiae* α-glucosidase (α-Glu), almond β-glucosidase (β-Glu), bovine liver β-galactosidase (β-Gal b. l.), *Aspergillus oryzae* β-galactosidase (β-Gal *A. oryzae*) and *Escherichia coli* β-galactosidase (β-Gal *E. coli*) by compouds **1a** and **1b** after pre-incubation of enzyme and inhibitor for 5 min (black bar) and 120 min (gray bar). Compounds were assayed at 46 μM for β-Gal (b. l.) and α-Gal, 0.5 μM for β-Gal (*A. oryzae*) and 50 μM for the other enzymes.

**Table 1.** Apparent $IC_{50}$ and kinetic data of compounds **1a** and **1b** for *Aspergillus oryzae* and *Escherichia coli* β-galactosidases.

| | β-Galactosidase (*Aspergillus oryzae*) | | | | β-Galactosidase (*Escherichia coli*) | |
|---|---|---|---|---|---|---|
| | $IC_{50}$ (μM) | | | | | |
| compd | pH 5.2 (5 min)[a] | pH 5.2 (60 min)[a] | pH 7 (60 min)[a] | $k_i/K_i$ ($min^{-1}$ $\mu M^{-1}$) | $IC_{50}$ (μM) pH 7 (5 min)[a] | $k_i/K_i$ ($min^{-1}$ $\mu M^{-1}$) |
| **1a** | 0.108 | 0.027 | 0.042 | 0.298 ± 0.007 | 14.8 | $(0.228 \pm 0.018) \times 10^{-3}$ |
| **1b** | 0.025 | 0.007 | 0.014 | 0.881 ± 0.035 | 7.8 | $(0.465 \pm 0.040) \times 10^{-3}$ |

[a]Incubation time of enzyme and inhibitor

inhibition of bovine liver β-galactosidase, the *N*-aminoaziridines were found to be potent inhibitors of *Aspergillus oryzae* and *Escherichia coli* β-galactosidases. The structure of the *N*-aminoaziridines **1a** and **1b** was expected to have a preference for β-galactosidase inhibition according to the reported selectivity of similar aziridines and epoxides.[16] This was confirmed after testing on α-galactosidase (green coffee beans) and α-glucosidase (*Saccharomyces cerevisiae*), where the aminoaziridines showed no effect. However, compounds **1a** and **1b** inhibited almond β-glucosidase (see Figure 2), a fact that can be explained considering the reported[28] β-galactosidase activity of this enzyme.

The apparent $IC_{50}$ values of both *N*-aminoaziridines were measured by determination of the remaining activity after a 5 min pre-incubation of *Aspergillus oryzae* and *Escherichia coli* β-galactosidases with various concentrations of inhibitors (Table 1). *N*-aminoaziridines **1a** and **1b** were found to be far more potent inhibitors of *Aspergillus oryzae* β-galactosidase than *Escherichia coli* β-galactosidase. For the inhibition of *Aspergillus oryzae* β-galactosidase, the apparent $IC_{50}$ value of **1b** was 25 nM, which was 4-fold lower than that of **1a**. *N*-aminoaziridines **1a** and **1b** were also evaluated as inhibitors of *Aspergillus oryzae* β-galactosidase at pH 7.0 and 5.2 after pre-incubation of enzyme and inhibitor for 60 min. Interestingly, both compounds were more potent at neutral rather than acidic pH.

To study the activity of the *N*-aminoaziridines in greater detail, kinetic studies were performed to determine $K_i$ (the equilibrium constant for the initial binding), $k_i$ (the inactivation rate constant), and $k_i/K_i$ (the second-order rate constant) using a continuous substrate release assay.

Compounds **1a** and **1b** were tested as covalent inactivators of *Aspergillus oryzae* and *Escherichia coli* β-galactosidases by measuring residual enzyme activity as a function of time upon incubation with a range of concentrations of inhibitor. Pseudo-first-order inactivation rate constants ($k_{obs}$) were obtained by fitting the resulting progress curves (Figure S1, Supporting Information) to one-phase association equation, and a second replot of the individual $k_{obs}$ values as a function of irreversible inhibitor concentration is shown in Figure 3. However, the time-dependent loss of enzyme activity measured after treating the enzyme with either **1a** or **1b** was too fast to permit sampling at concentrations approaching saturation, so only the second-order rate constants $k_i/K_i$ could be measured. These values for both enzymes are shown in Table 1. The kinetic analysis for the inhibition of β-galactosidase from *Aspergillus oryzae* by the inhibitors showed that *N*-aminoaziridines **1a** and **1b** are effective irreversible inhibitors of this enzyme with $k_i/K_i$ of 0.298 ± 0.007 $min^{-1}$ $\mu M^{-1}$ and 0.881 ± 0.035 $min^{-1}$ $\mu M^{-1}$, respectively. Thus, **1b** is approximately 3-fold more effective than **1a**.

Compounds **1a** and **1b** were also found to function as irreversible inhibitors of β-galactosidase from *Escherichia coli*, but with approximately 1000-fold lower inactivation efficiency ($k_i/K_i$) than that of the other β-galactosidase.

Diazomethyl β-D-galactopyranosyl ketone (Figure 1) was shown to be an irreversible inactivator of the β-galactosidase from *A. oryzae* with $k_i/K_i$ of $0.018 \times 10^{-3}$ $min^{-1}$ $\mu M^{-1}$, and the inactivation was shown to be active site directed.[9] Thus, compounds **1a** and **1b** are approximately $1.65 \times 10^4$–fold and $4.89 \times 10^4$–fold, respectively, more effective than diazomethyl β-D-galactopyranosyl ketone. Moreover, examination of the inhibitory properties revealed that the *N*-aminoaziridines **1a** and **1b**, and the epoxide cyclophellitol (Figure 1) are comparably potent as irreversible inhibitors of glycosidases (cyclophellitol $k_i/K_i$ of 0.514 $min^{-1}$ $\mu M^{-1}$ for recombinant glucocerebrosidase).[29]

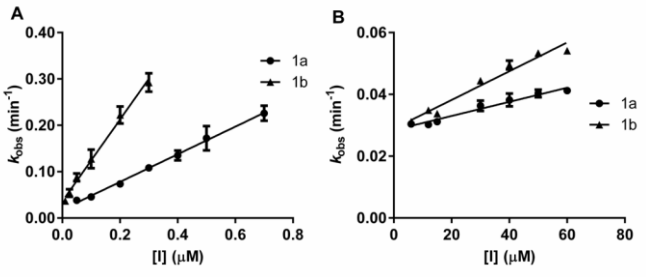



**Figure 3.** Inactivation of *Aspergillus oryzae* (A) and *Escherichia coli* (B) β-galactosidases with compounds **1a** and **1b**. Plot of the pseudo-first-order rate constants ($k_{obs}$) *versus* concentration of inhibitor.

The results obtained with the *N*-aminoaziridines **1a** and **1b** complement the literature on the inhibitory potency of aziridine and epoxide analogues as irreversible inhibitors of glycosidases.

The remarkable specificities and potencies of these *N*-aminoaziridines open up the potential to develop selective inhibitors for different classes of glycosidases by modifying their configurations or the *N*-*N*-functionalization to that of the natural substrate of an enzyme of interest. Furthermore, an interesting application of potent and selective irreversible inhibitors such as the *N*-aminoaziridine **1a** is the design of labeled derivatives, also known as activity-based probes, which is the field of research that aims to monitor the enzymatic activity within the context of a native biological system. Therefore, the substitution of the *N*-aminoaziridine scaffold may lead to fluorescent activity-based probes for subsequent biological studies.[30]

## Conclusions

In conclusion, enantiomerically pure galacto-configured *N*-aminoaziridines **1a** and **1b** obtained by aziridination reactions of cyclic olefin **2** were studied as inhibitors of glycosidases and resulted to be potent irreversible inhibitors of *Aspergillus oryzae* and *Escherichia coli* β-galactosidases. The enzyme selectivity of the *N*-aminoaziridines is likely due to active site recognition of the similar configuration of the hydroxyl substituents to the galactose substrates. In addition, the reactivity of the aziridine with an enzyme catalytic nucleophile, would provide a possible explanation of the irreversibility of the inhibitor as it is found in other mechanism based covalent aziridines and epoxide inhibitors.[16] The proof of the potent inhibition of galactosidases by *N*-aminoaziridines describes a new class of reactive groups to design covalent inhibitors for glycoside hydrolases that can be useful in glycoscience.

## Experimental section

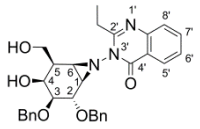
### Chemistry: material and methods

All moisture-sensitive reactions were carried out under nitrogen. All the materials were obtained commercially and used without further purification. Solvents were dried prior to use with Pure Solv-EN™ system or distilled and dried by standard methods. Thin-layer chromatography (TLC) was performed on silica gel (Alugram Sil G/UV) and *flash* chromatography was done using silica gel 60 (40-63 microns, Panreac) or Biotage® SNAP Cartridges when using Biotage® Isolera Prime™ *flash* purification system. Analytical samples were homogeneous as confirmed by TLC and afforded spectroscopic results consistent with the assigned structures. Chemical shifts are reported in δ (ppm) relative to the singlet at δ = 7.26 ppm of $CDCl_3$, the multiplet at δ = 3.31 ppm of Methanol-$d_4$ and the singlet at 4.79 ppm of $D_2O$ for $^1$H NMR, and to the centre line of the triplet at δ = 77.16 ppm of $CDCl_3$, the multiplet at δ = 49.0 ppm of Methanol-$d_4$ and a TMS external reference in $D_2O$ for $^{13}$C-NMR. $^1$H and $^{13}$C NMR spectra of compound **4** were measured on a Varian INOVA-500 ($^1$H at 499.81 MHz and $^{13}$C at 125.69 MHz) instrument in $CDCl_3$ (ref.: TMS for $^1$H and the $CHCl_3$ signal at δ 77.0 ppm for $^{13}$C) at 25 °C. The $^1$H DPFGSE-NOE, $^1$H–$^1$H COSY, $^1$H–$^1$H NOESY, $^1$H–$^{13}$C HSQC and $^1$H–$^{13}$C HMBC techniques from Chempack/VnmrJ32A (Agilent Technologies) were used for structural assignments. HSQC and HMBC NMR experiments for compound **1b** were recorded on a BRUKER DRX-500 spectrometer equipped with a 3-channel 5-mm cryoprobe incorporating a z-gradient coil. The basic frequencies are 500.13 MHz for $^1$H and 125.00 for $^{13}$C. All other NMR spectra were recorded on an Agilent VNMRS-400 ($^1$H at 400.10 MHz and $^{13}$C at 100.62 MHz). IR spectra were registered as film and were recorded with a Thermo Nicolet Avatar 360 FT-IR Spectometer. $[\alpha]_D$ values are given in $10^{-1}$ deg $cm^2$ $g^{-1}$ and were measured with a Perkin–Elmer 341 polarimeter. ESI/HRMS were recorded with a Waters LCT Premier mass spectrometer. Melting points were measured with a digital Stuart Scientific SMP10 melting point apparatus.

**(1*S*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-(hydroxymethyl)cyclohex-3-enol (2)** was synthesized according to literature procedures (see Scheme S1, Supporting Information).[17,31]

**3-amino-2-ethylquinazolin-4(3*H*)-one (3)** was synthesized according to a literature methodology.[20]

**3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (4)**



A suspension of (1*S*,2*R*,5*S*,6*S*)-5,6-bis(benzyloxy)-2-(hydroxymethyl)cyclohex-3-enol (**2**)[2] (422.0 mg, 1.24 mmol), potassium carbonate (1.71 g, 12.4 mmol) and 3-amino-2-ethylquinazolin-4(3*H*)-one (**3**,[3] 1.17 g, 6.20 mmol) in dry DCM (19 mL), was cooled to 0 ºC. To the previous suspension was added diacetoxy(phenyl) iodane (PIDA, 2.20 g, 6.82 mmol) and the resulting mixture was allowed to reach room temperature and stirred overnight. Then, the reaction mixture was diluted with brine (60 mL) and extractions were done with DCM (3 x 60 mL). The collected organic layers were dried over $Na_2SO_4$, filtered and the solvent was removed under reduced pressure to get a brown oil, which was purified by *flash* chromatography (silica gel, hexane-EtOAc 7:3 to 0:1, gradient) to afford aziridine **4** as a pale yellow solid (356.0 mg, 54%). mp 57-59 ºC; $[\alpha]_D^{20}$ +146.4 (*c* 3.94 in $CHCl_3$); $\nu_{max}$ /$cm^{-1}$ 3447, 3061, 3026, 2935, 2881, 1668, 1598, 1464, 1106, 1069; $\delta_H$ (500 MHz; $CDCl_3$) 8.10 (1H, dd, *J* = 8.0, 1.2 Hz, $CH_{ar}$ (8')), 7.65 (1H, td, *J* = 7.8, 1.4 Hz, $CH_{ar}$ (6')), 7.58 (1H, d, *J* = 8.0 Hz, $CH_{ar}$ (5')), 7.37 (1H, td, *J* = 7.5, 1.0 Hz, $CH_{ar}$ (7')), 7.35 - 7.21 (10H, m, 2Ph), 4.88 (1H, d, $J_{AB}$ = 11.4 Hz, A part of a C*H*-Ph (2) AB system), 4.80 (1H, d, $J_{AB}$ = 11.4 Hz, B part of a CH*H*-Ph (2) AB system), 4.74 (1H, d, $J_{AB}$ = 11.6 Hz, A part of a C*H*H-Ph (3) AB system), 4.58 (1H, d, $J_{AB}$ = 11.6 Hz, B part of a CH*H*-Ph (3) AB system), 4.51 (1H, br s, $CH_2$-

O*H*), 4.38 - 4.33 (1H, m, A part of a C*H*H-OH AB system), 4.30 (1H, d, *J* = 8.2 Hz, CH-O (2)), 4.11 - 4.06 (1H, m, CH-O (4)), 4.02 -3.95 (1H, m, B part of a CH*H*-OH AB system), 3.43 (1H, dd, J = 7.9, 2.6 Hz, CH-N (6)), 3.40 (1H, dd, *J* = 8.3, 2.3 Hz, CH-O (3)), 3.02 (1H, br d, *J* = 8.2 Hz, CH-O*H* (4)), 2.98 - 2.87 (1H, m, A part of a C*H*H-$CH_3$ AB system), 2.77 - 2.68 (1H, m, B part of a CH*H*-$CH_3$ AB system), 2.66 (1H, d, *J* = 7.9 Hz, CH-N (1)), 2.19 - 2.14 (1H, m, C*H*-$CH_2OH$), 1.24 (3H, t, *J* = 7.3 Hz, $CH_3$); $\delta_C$ (126 MHz; $CDCl_3$) 159.9 (C=O), 156.0 (C=N), 145.8 ($C_{ar}$ (4a')), 137.8 ($C_{ar}$ (phenyl)), 137.7 ($C_{ar}$ (phenyl)), 134.3 ($CH_{ar}$ (6')), 128.7 ($CH_{ar}$), 128.6 ($CH_{ar}$), 128.5 ($2CH_{ar}$), 128.2 ($CH_{ar}$), 128.1 ($2CH_{ar}$), 128.0 ($CH_{ar}$), 127.9 ($CH_{ar}$), 127.8 ($CH_{ar}$), 127.0 ($CH_{ar}$ (5')), 126.8 ($CH_{ar}$ (7')), 126.4 ($CH_{ar}$ (8')), 120.8 ($C_{ar}$ (8a')), 83.8 (CH-O (3)), 74.9 (CH-O (2)), 74.3 ((C$H_2$-Ph (2)), 71.5 ((C$H_2$-Ph (3)) , 67.5 (CH-O (4)), 62.3 ($CH_2$-OH), 52.1 (CH-N (1)), 47.5 (CH-N (6)), 40.9 (*C*H-$CH_2OH$), 27.7 ($\underline{C}H_2$-$CH_3$), 10.6 ($CH_3$); HRMS calculated for $C_{31}H_{33}N_3O_5$: 550.2318 $[M+Na]^+$; found: 550.2316.

**(1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-7-amino-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-3-ol (5)**

A mixture of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**) (172.4 mg, 0.327 mmol) and hydrazine (6.5 mL, 132 mmol) was heated to 120 ºC for 20 min. Then, the reaction mixture was concentrated under reduced pressure to give a residue, which was purified by *flash* chromatography (silica gel, MeOH-EtOAc 0.5:9.5 + 1% aq $NH_3$) to afford pure product **5** as a pale yellow oil (111.9 mg, 78%). $[\alpha]_D^{20}$ +74.8 (*c* 2.1 in MeOH); $\nu_{max}$ /$cm^{-1}$ 3311, 3085, 3062, 3029, 2919, 2879, 1682-1600, 1454, 1101, 1062; $\delta_H$ (400 MHz; $CDCl_3$) 7.43 - 7.22 (10H, m, 2Ph), 4.77 (2H, s, $CH_2$ benz), 4.73 (1H, d, $J_{AB}$ = 12.0 Hz, A part of a C*H*H-Ph AB system), 4.63 (1H, d, $J_{AB}$ = 12.0 Hz, B part of a CH*H*-Ph AB system), 4.02 (1H, d, *J* = 8.1 Hz, CH-O), 4.01 - 3.98 (1H, m, CH-O), 3.98 - 3.89 (2H, m, C$H_2$-OH), 3.42 (4H, br s, 2OH and $NH_2$), 3.26 (1H, dd, *J* = 8.0, 2.3 Hz, CH-O), 2.15 (1H, dd, *J* = 7.3 Hz, 1.0 Hz, CH-N (1)), 2.10 (1H, d, *J* = 7.3 Hz, CH-N (6)), 1.99 - 1.96 (1H, m, CH (2)). ); $\delta_C$ (101 MHz; $CDCl_3$) 138.4 ($C_{ar}$), 138.1 ($C_{ar}$), 128.6 ($CH_{ar}$), 128.4 ($CH_{ar}$), 128.2 ($CH_{ar}$), 128.0 ($CH_{ar}$), 128.0 ($CH_{ar}$), 127.7 ($CH_{ar}$), 82.8 (CH-O), 77.6 (CH-O), 73.3 (C$H_2$-Ph), 71.9 (C$H_2$-Ph), 67.6 (CH-O), 62.5 ($CH_2$-OH), 45.6 (CH-N (1)), 45.3 (CH-N (6)), 39.7 (CH (2)); HRMS calculated for $C_{21}H_{27}N_2O_4$: 371.1971 $[M+H]^+$; found: 371.1968. The following hydrazone **8** was isolated as trace by-product in the same reaction. It could also be obtained quantitatively by treatment of compound **5** with an excess of acetone at room temperature overnight and after removal of the solvent under reduced pressure. **(1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-aza bicyclo[4.1.0]heptan-3-ol (8)**

$\delta_H$ (400 MHz; $CDCl_3$) δ 7.41 - 7.24 (10H, m, 2Ph), 4.82 - 4.62 (4H, m, 2C$H_2$-Ph), 4.12 (1H, d, *J* = 8.1 Hz, CH-O), 4.12 - 4.07 (1H, m, CH-O (3)), 3.96 (2H, ddd, *J* = 17.3, 11.0, 7.3 Hz, C$H_2$-OH), 3.89 - 3.80 (1H, br s, OH (3)), 3.37 (1H, dd, *J* = 8.0, 2.3 Hz, CH-O), 2.60 - 2.55 (1H, m, CH-N (1)), 2.37 (1H, d, *J* = 7.2 Hz, CH-N (6)), 2.13 - 2.06 (1H, m, C*H*-$CH_2OH$), 1.94 (3H, s, $CH_3$), 1.91 (3H, s, $CH_3$), 1.61 (1H, br s, OH); $\delta_C$ (101 MHz; $CDCl_3$) 168.1 (C=N), 138.4 ($C_{ar}$), 138.1 ($C_{ar}$), 128.6 ($CH_{ar}$), 128.5 ($CH_{ar}$), 128.2 ($CH_{ar}$), 128.1 ($CH_{ar}$), 128.0 ($CH_{ar}$), 127.8 ($CH_{ar}$), 83.2 (CH-O), 77.7 (CH-O), 73.3 (C$H_2$-Ph), 71.9 (C$H_2$-Ph), 67.1 (CH-O (3)), 62.3 ($CH_2$-OH), 44.6 (2CH-N), 39.8 (*C*H-$CH_2OH$), 25.2 ($CH_3$), 19.0 ($CH_3$); HRMS calculated for $C_{24}H_{31}N_2O_4$: 411.2284 $[M+H]^+$; found: 411.2288.

**(1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (1a)**

A suspension of sodium (50.0 mg, 2.18 mmol) in dry THF (6 mL) was cooled to -78 ºC and it was treated with liquid ammonia for 5 minutes until the starting suspension became a dark blue solution. Then, a solution of (1*R*,2*R*,3*S*,4*S*,5*S*,6*R*)-7-amino-4,5-bis(benzyloxy)-2-(hydroxymethyl)-7-azabicyclo[4.1.0]heptan-3-ol (**5**) (19.2 mg, 0.052 mmol) in THF (4 mL) was added. The resulting mixture was stirred at -78 ºC for 1 hour. After that, MeOH was added dropwise until the blue color disappeared and then, the reaction mixture was allowed to reach room temperature and concentrated *in vacuo* to give a residue that was purified by ion-exchange chromatography with Dowex 50W X8 (729.0 mg) after adsorption with water (10 mL) and desorption by 30% aq ammonia (100 mL) to afford *N*-amino aziridine **1a** as a white waxy solid (9.0 mg, 91%). $\delta_H$ (400 MHz; $CD_3OD$) 3.90 (1H, d, *J* = 8.2 Hz, CH-O (2)), 3.82 (2H, d, *J* = 7.3 Hz, $CH_2$-O), 3.71 - 3.67 (1H, m, CH-O (4)), 3.15 (1H, dd, *J* = 8.2, 2.1 Hz, CH-O (3)), 2.25 - 2.20 (1H, br d, *J* = 7.0 Hz, CH-N (6)), 2.03 (1H, d, *J* = 7.1 Hz, CH-N (1)), 2.03 - 1.97 (1H, m, C*H*-$CH_2OH$); $\delta_C$ (101 MHz; $CD_3OD$) 77.9 (CH-O (3)), 71.9 (CH-O (4)), 71.5 (CH-O (2)), 62.2 ($CH_2$-O), 48.3 (CH-N (1)), 46.0 (CH-N (6)), 41.9 (*C*H-$CH_2OH$); HRMS calculated for $C_7H_{14}N_2NaO_4$: 213.0851 $[M+Na]^+$; found: 213.0838. The following hydrazone **1b** was isolated as reaction by-product in the same reaction (0.9 mg, 8%). In addition, the treatment of compound **1a** (1.4 mg, 7.36 μmol) with an excess of acetone (2 mL) at room temperature overnight afforded pure compound **1b** (1.7 mg, 100%) after removal of the solvent under reduced pressure. The purity of the compound **1a** determined by HPLC-ELS (see Supporting Information) is 100%. The HPLC-MS confirms the identity of the compound.

**(1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(hydroxymethyl)-7-(propan-2-ylideneamino)-7-azabicyclo[4.1.0]heptane-2,3,4-triol (1b)**

$\delta_H$ (400 MHz; $D_2O$) 4.10 (1H, d, *J* = 8.7 Hz, CH-O), 3.92 - 3.90 (1H, m, CH-O), 3.89 (2H, d, *J* = 7.4 Hz, $CH_2O$), 3.45 (1H, dd, *J* = 8.7, 2.4 Hz, CH-O), 2.69 (1H, ddd, *J* = 7.2, 3.0, 1.1 Hz, CH-N), 2.31 (1H, d, *J* = 7.2 Hz, CH-N), 2.30 - 2.25 (1H, m, C*H*-$CH_2O$), 2.08 and 2.07 (3H, s, $CH_3$ invertomer), 1.98 and 1.94 (3H, s, $CH_3$ invertomer); $\delta_C$ (101 MHz; $D_2O$) 174.7 (C=N), 77.7 (CH-O), 71.9 (CH-O), 71.4 (CH-O), 62.7 ($CH_2O$), 48.4 (CH-N), 45.8 (CH-N), 41.6 (*C*H-$CH_2OH$), 26.1 ($CH_3$), 21.0 ($CH_3$); HRMS calculated for $C_{10}H_{19}N_2O_4$: 231.1345 $[M+H]^+$; found: 231.1334. The purity of the compound **1b** determined by HPLC-ELS (see Supporting Information) is 96%. The HPLC-MS confirms the identity of the compound.

**(1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-5-(acetoxymethyl)-7-acetyl-7-azabicyclo[4.1.0]heptane-2,3,4-triyl triacetate (7)**

A suspension of sodium (148.0 mg, 6.45 mmol) in anhydrous THF (6 mL) was cooled to -78 ºC and it was treated with liquid ammonia until the starting suspension became a dark blue solution. Then, a solution of 3-((1*R*,2*S*,3*S*,4*S*,5*R*,6*R*)-2,3-bis(benzyloxy)-4-hydroxy-5-(hydroxymethyl)-7-azabicyclo[4.1.0] heptan-7-yl)-2-ethylquinazolin-4(3*H*)-one (**4**, 126.0 mg, 0.24 mmol) in anhydrous THF (3 mL) was added. The resulting solution was stirred at -78 ºC for 1 hour. After that, ammonium chloride (345.0 mg, 6.45 mmol) was added and the reaction mixture was stirred for 10 minutes while ammonia was allowed to remove. Then, the resulting solution was allowed to reach 0 ºC and methanol (10 mL) was added dropwise. The solvent of the reaction mixture was removed under reduced pressure to give a residue that was redissolved in pyridine (10 mL) and acetic anhydride (2 mL, 20.7 mmol) was added. This mixture was stirred at room temperature for 18 hours. Then, the solvent of the reaction mixture was removed under reduced pressure, and it was redissolved in EtOAc (10 mL). The organic layer was washed with 1N aq HCl (2 x 10 mL) and 0.5 M aq KOH (2 x 10 mL), it was dried over $MgSO_4$, filtered and the solvent of the filtrate was removed in vacuo to give a yellow oil that was purified by *flash* chromatography (silica gel, DCM-EtOAc 9:1 to 0:1, gradient) to afford peracetate (**7**) (25.8 mg, 28%). $\delta_H$ (400 MHz; $CDCl_3$) 5.48 - 5.45 (1H, m, CH-O), 5.11 (1H, d, *J* = 9.7 Hz, CH-O), 4.93 (1H, dd, *J* = 9.7, 2.6 Hz, CH-O), 4.27 - 4.16 (2H, m, $CH_2$-O), 2.77 - 2.73 (1H, m, CH-N), 2.73 - 2.67 (1H, m, C*H*-$CH_2$OAc), 2.65 (1H, d, *J* = 5.8 Hz, CH-N), 2.23 (3H, s, $CH_3$), 2.14 (3H, s, $CH_3$), 2.07 (3H, s, $CH_3$), 2.04 (3H, s, $CH_3$), 2.01 (3H, s, $CH_3$); $\delta_C$ (101 MHz; $CDCl_3$) 182.2 (N-C=O), 170.8 (*C*OAc), 170.6 (*C*OAc ), 170.4 (2 *C*OAc), 71.7 (CH-O), 68.7 (CH-O), 65.5 (CH-O) , 61.8 ($CH_2$-O), 38.9 (CH-N), 36.3 (CH), 35.5 (CH-N), 23.6 ($CH_3$), 23.3 ($CH_3$), 21.1 ($CH_3$), 20.9 ($CH_3$), 20.9 ($CH_3$); HRMS calculated for $C_{17}H_{23}NO_9Na$: 408.1271 $[M+Na]^+$; found: 408.1257.

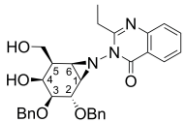
**Structural Characterization of Compound 4**

Structural characterization of aziridine-fused sugar unit (**4**) was done using homonuclear ($^1$H-$^1$H 2D COSY and NOESY) and heteronuclear ($^1$H-$^{13}$C 2D HSQC and HMBC) experiments to assign proton and carbon resonances and to determine the stereochemistry.

The presence of an aziridine ring in compound **4** was supported by characteristic upfield chemical shift signals for the *CH*NH protons (3.43 (H6) and 2.66 (H1) ppm) and carbons (47.54 (H6) and 52.08 (H1)). The vicinal coupling constant of aziridine-ring protons *J*(1,6) was 7.9 Hz and for aziridine H6 proton we observed an additional coupling constant of 2.6 Hz for the coupling with C*H*(5), supporting *trans* orientation between H(6)/H(1) and H(2).

The 2D NOESY spectrum of **4** allowed us to identify OH protons (in exchange between them and with the water residual signal in $CDCl_3$ solvent). Also, in the NOESY spectra was observed a strong NOE correlation between the two aziridine protons H(1) and H(6), a strong correlation between NC*H*(6) and C*H*(5), and a weak correlation between NC*H*(1) and C*H*(2), supporting that both aziridine protons are on the same side of the aziridine-fused sugar ring and at the same side than C*H*(5) protons.

Due to the low sample concentration, in 2D NOESY experiments some peaks were difficult to detect. Therefore, to confirm the structure assignment, 1D selective irradiation experiments, using 1D DPFGSE-NOE pulse sequence, were acquired. The stereochemistry of **4** in the figure below was supported by 1D NOE/2D NOESY spectra. Summarizing NOE results:




- Irradiation of C*H*3CH2 resulted in the enhancement of C*H*(2) and the attached group benzyl signals, supporting its presence in the same side of the molecule.
- Irradiation of C*H*(2) resulted in the enhancement of O*H*(4), but not C*H*(4), signaling that C*H*(2) is on the same side of the sugar ring that O*H*(4).
- Irradiation of C*H*(2) resulted in the enhancement of NC*H*(1) but not NC*H*(6), supporting that NC*H*(6) is on the opposite side of the sugar ring.
- Irradiation of C*H*(5) resulted in the enhancement of C*H*(3), C*H*(4), NC*H*(1), NC*H*(6) but not C*H*(2), confirming that the latter is on the opposite side of the molecule respect to all of the other sugar ring protons. We arrived at similar conclusions by irradiating C*H*(3) or C*H*(4).
- We confirmed the previous 1D NOE results for protons which overlap in monodimensional spectra (for example, C*H*(3) and C*H*(6)) using 2D NOESY.

**Enzyme activity studies of the analogs as inhibitors of glycosidases**

**Materials**: The glycosidases α-glucosidase (from *Saccharomyces cerevisiae*), β-glucosidase (from almond), β-galactosidase (from bovine liver and *Aspergillus oryzae*), and α-galactosidase (from green coffee beans) that were used in the inhibition studies, as well as *o*- and *p*-nitrophenyl glycoside substrates, were purchased from Sigma-Aldrich.

**Inhibition assay against glycosidases**

Commercial enzyme solutions were prepared with the appropriate buffer and incubated in 96-well plates at 37 ºC without (control) or with inhibitor for 5 min, 60 min or 120 min. After addition of the corresponding substrate solution, incubations were prolonged for different time periods: 3 min for β-glucosidase (from almond) and α-glucosidase (from *Saccharomyces cerevisiae*), 5 min for β-galactosidase (from bolive liver), 10 min for α-glucosidase (from rice), 13 min for α-galactosidase and β-Galactosidase (from *Aspergillus oryzae*) and 15 min for β-galactosidase (from *Escherichia coli*) and stopped by addition of Tris solution (50 μL, 1M) or $Na_2CO_3$ (180 μL, 1M), depending on the enzymatic inhibition assay. The amount of *o*- or *p*-nitrophenol formed was determined at 405 nm

with a SpectraMax M5 (Molecular Devices Corporation) spectrophotometer. β-Galactosidase (from bolive liver) activity was determined with *p*-nitrophenyl-β-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, 0.1 mM $MgCl_2$, pH 7.2). β-Galactosidase (from *Aspergillus oryzae*) activity was determined with *o*-nitrophenyl-β-D-galactopyranoside (1 mM) in McIlvaine buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2 or pH 7.0). β-galactosidase (from *Escherichia coli*) activity was determined with *o*-nitrophenyl-β-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, 1 mM $MgCl_2$, 50 mM mercaptoethanol, pH 7.0). α-Galactosidase activity was determined with *p*-nitrophenyl-α-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 6.8). β-Glucosidase activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (1 mM) in sodium acetate buffer (100 mM, pH 5.0). α-glucosidase activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 6.8). The commercial glycosidase solutions were prepared as follows: α-glucosidase from *Saccharomyces cerevisiae*: 0.1 mg $mL^{-1}$ buffer; β-glucosidase from almond: 0.1 mg $mL^{-1}$ buffer; α-galactosidase from green coffee beans: 3.7 μL $mL^{-1}$ buffer; β-galactosidase from bovine liver: 0.5 mg $mL^{-1}$ buffer; β-galactosidase from *Aspergillus oryzae*: 0.1 mg $mL^{-1}$ buffer; β-galactosidase from *Escherichia coli*: 1 μg $mL^{-1}$ buffer.

**Determination of inhibition constants**

Previously discontinuous method has been used to determine the inhibition constants of β-galactosidase inhibitors. However, this method proved to be unsuitable for the determination of the inhibition constants of **1a** and **1b** due to their high affinity/fast binding. Therefore, the inhibition constants have been determined in a continuous substrate assay as reported for other glycosidases.[29] All enzyme reactions were performed at 37 ºC.

**β-Galactosidase from *Aspergillus oryzae*:** To 189 μL McIlvaine Buffer (100 mM sodium citrate and 200 mM sodium phosphate buffer, pH 5.2) containing 50 μL of the substrate *o*-nitrophenyl β-D-galactopyranoside (4 mM) in 96-well plate was added 2 μL of several concentrations of inhibitor (stock in milliQ water). The resulting mixture was preincubated at 37 ºC for 5 min. β-Galactosidase from *Aspergillus oryzae* (0.56 mg $mL^{-1}$ McIlvaine buffer pH 5.2) was also preheated to 37 ºC for 5 min and 9 μL of this enzyme solution was added to the substrate-inhibitor mixture. The amount of *o*-nitrophenol formed was determined at 405 nm with a SpectraMax M5 (Molecular Devices Corporation) spectrophotometer for 30 min.

**β-Galactosidase from *Escherichia coli*:** To 189 μL of sodium phosphate buffer (100 mM, 1 mM $MgCl_2$, 50 mM mercaptoethanol, pH 7) containing 50 μL of the substrate *o*-nitrophenyl β-D-galactopyranoside (4 mM) in 96-well plate was added 2 μL of several concentrations of inhibitor (stock in milliQ water). The resulting mixture was preincubated at 37 ºC for 5 min. β-Galactosidase from *Escherichia coli* (5 μg $mL^{-1}$ sodium phosphate buffer) was also preheated to 37 ºC for 5 min and 9 μL of this enzyme solution was added to the substrate-inhibitor mixture. The amount of *o*-nitrophenol formed was determined at 405 nm with a SpectraMax M5 (Molecular Devices Corporation) spectrophotometer for 30 min.

All readings were corrected by subtracting blanks (200 μL buffer and 50 μL of the substrate solution). Apparent rate constants $k_{obs}$ were obtained by fitting the resulting progress curves (Figure S1, Supporting Information) to one-phase association equation $y=(y_{max}-y_o)(1-\exp^{-kt}) + y_o$ in GraphPad Prism version 6. It was not possible to calculate the individual $k_i$ and $K_i$ values for **1a** and **1b**, since inactivation was too fast at concentrations approaching saturation to allow sampling. However, a second-order rate constants of inactivation ($k_i/K_i$) were determined by plotting the individual $k_{obs}$ values versus the concentration of inhibitor (Figure 3) and fit to the equation: $k_{obs}= (k_i[I]/K_i)$.

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