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Stereocontrolled synthesis of new iminosugar lipophilic derivatives and evaluation of biological activities

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ABSTRACT

Iminosugars' similarity to carbohydrates determines the exceptional potential for this class of polyhydroxylated alkaloids to serve as potential drug candidates for a wide variety of diseases such as diabetes, lysosomal storage diseases, cancer, bacterial and viral infections. The presence of lipophilic substituents has a significant impact on their biological activities. This work reports the synthesis of three new pyrrolidine lipophilic derivatives O-alkylated in C-6 position. The biological activities of our iminosugars' collection were tested in two cancer cell lines and, due to the pharmaceutical potential, in the model yeast system *Saccharomyces cerevisiae* to assess their toxicity.

1. Introduction

Iminosugars are polyhydroxylated compounds which differ from carbohydrates in the presence of the endocyclic nitrogen atom in place of oxygen. Based on their cyclic structures they are subdivided into five classes: pyrrolidines, piperidines, pyrrolizidines, indolizidines and nortropanes (Fig. 1) [1–3].

Iminosugars have ancient origins, dating to their therapeutic employment in traditional Chinese medicine. Thanks to their similarity to sugars, iminosugars are able to inhibit or modulate the activity of fundamental enzymes such as glucosidase, glycosyltransferase, glycogen phosphorylases, nucleoside-processing enzymes, a sugar nucleotide mutase and metalloproteinases [4–13]. The wide variety of enzymes inhibited by iminosugars holds promises for the creation of new generation of medicines to treat several disorders such as diabetes, lysosomal storage disorders, viral infections (including HIV and hepatitis), and tumor metastases [14–16].

Several studies of the numerous types of derivatives have found that derivatization of iminosugars with aliphatic side chains can improve their biological activity and bioavailability [17]. In fact, it has been proved that, in many cases, the presence of a lipophilic portion makes these derivatives more powerful and selective inhibitors compared to their un-alkylated analogues [18–22].

The correlation between the presence of alkyl chains and the improvement of the biological activities, due either to an increase of the permeability into the cell membrane or to a better/more specific interaction with the targeted enzyme, has been demonstrated for various diseases [23,24]. A collection of iminosugar lipophilic derivatives [25–31] have been tested for the treatment of Gaucher and sporadic Parkinson's disease, highlighting the relation between alkyl chain length, its position and pharmacological chaperoning activity (**I–VI**, Fig. 2). A recent work by Ballerau group has shown that a supplementary O-alkyl chain on N-alkylated-DNJ derivatives (**VII–IX,** Fig. 2) reinforces the lipophilic interactions and strengthens the binding of the ceramide transport protein CERT, which is associated with diseases such as cancer [24b].

Several iminosugar alkyl derivatives were evaluated for their inhibition activity toward different cancer cell lines. These studies showed that the inhibitory effect on the growth [23d,32] and on the migration [33] of the examined cancer cells depends on the length of the alkyl

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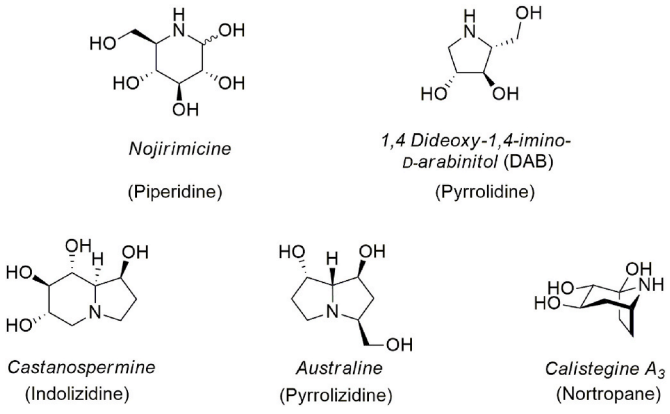


Fig. 1. Examples of natural iminosugars.

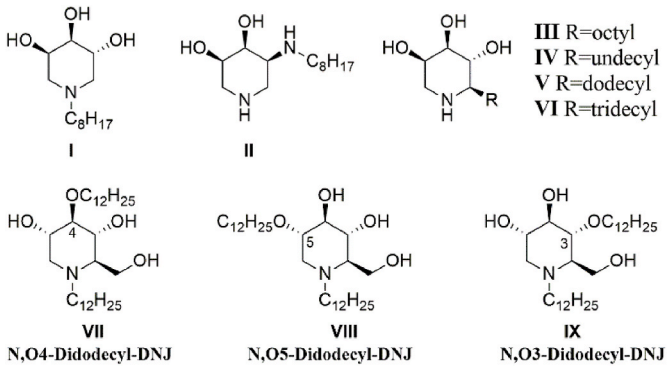


Fig. 2. Iminosugar lipophilic derivatives and O, N-alkylated-DNJ derivatives.

chain linked to the iminosugars.

2. Results and discussion

Many of the cited examples reported in literature take in exam N- and C-alkylated iminosugars derivatives, while the examples of O-alkylated derivatives are less described. In this context, we propose a synthetic strategy for the preparation of C-6 O-alkylated derivatives of pyrrolidine iminosugars (**2a**, **2b** and **2c**) and the evaluation of the biological activities of our iminosugars' collection (Fig. 3). The biological activity

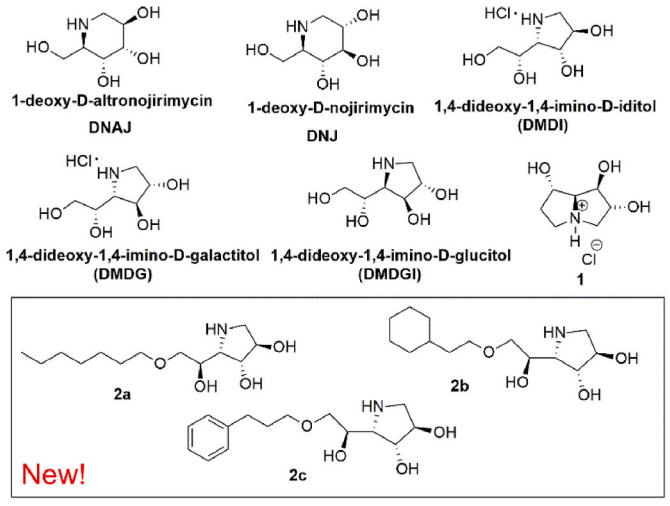


Fig. 3. Our iminosugars' collection synthesized and tested for biological activities.

tests were conducted on two cancer cell lines, the human histiocytic lymphoma U937 and the squamous cell carcinoma CAL-27. Further tests were performed on the eukaryotic model system *S. cerevisiae* to verify that the tested compounds have no toxic effect on these cells.

The synthesis of 1-deoxy- D-altronojirimycin (**DNAJ**), 1-deoxy-D-nojirimycin (**DNJ**), 1,4-dideoxy-1,4-imino-D-iditol (**DMDI**), 1,4-dideoxy-1,4-imino-D-galactitol (**DMDG**), 1,4-dideoxy-1,4-imino-D-glucitol (**DMDGl**) and of the pyrrolizidine iminosugar **1** was described in our previous works [34–37]. Our strategy is based on a stereocontrolled total synthetic approach that provides access to several classes of iminosugars from a common precursor, while controlling the four contiguous stereocenters. Control of the stereocenters is achieved through an opportune sequence of stereocontrolled key steps: the asymmetric Sharpless epoxidation (AE) [38], the asymmetric dihydroxylation (AD) [39], and the epoxide ring opening [40]. Through the opportune sequence of the key steps listed above, the suitable choice of the chiral ligands and the nucleophiles in the epoxide ring opening, is possible to obtain different classes of iminosugars and their derivatives, starting from the α,β-unsaturated epoxy ester **X** (Scheme 1).

2.1. Synthetic approach

At first, we considered various ways for introducing substituents on the four hydroxyl functions of the pyrrolidine ring (Fig. 4) by exploiting the synthetic strategy described above.

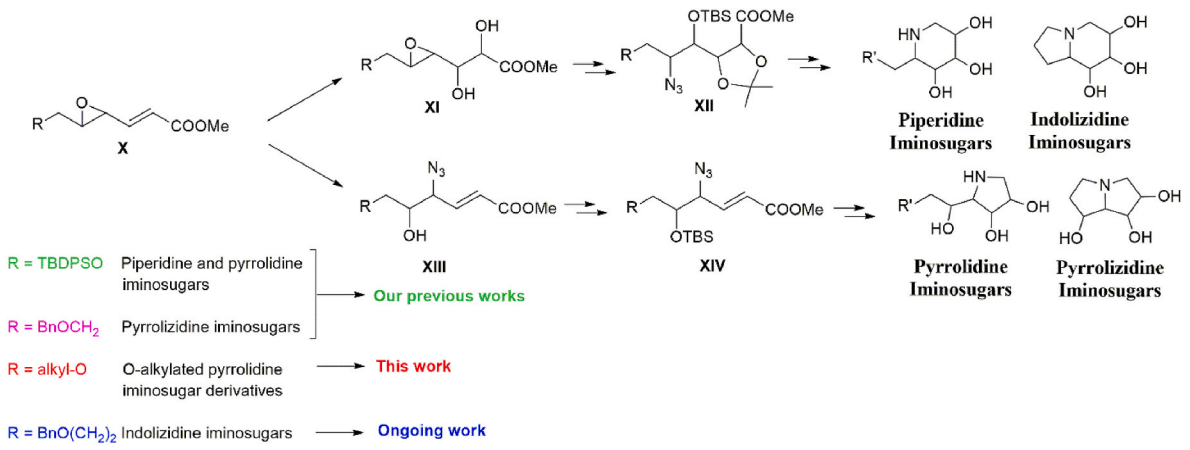
The numerous attempts carried out for the etherification of secondary hydroxyl groups in C-2, C-3 and C-5 position, led us only to unsatisfactory results and studies to this end are still ongoing.

Considering the O-functionalization in C-6 position, the best results among those we tested were achieved by introducing the desired substituent in the first step of the synthesis and performing the monoetherification reaction on *cis* 2-butene-1,4-diol **3**. In order to test how the ramification and unsaturation degree of the alkyl chain could intervene on the enzymatic inhibition properties or cytotoxicity of these compounds, three different substituents were chosen: a seven-carbon atoms straight chain (**4a**), a branched-chain (**4b**), and a substituent containing an aromatic function (**4c**). First, the monoetherification was performed using NaH as base, but this condition led to unsatisfactory yields (30–42% depending on the alkylating agent). Instead, by employing crushed KOH and the alkyl halide in dry DMF at 65 °C, the reaction carried out the formation of the expected product in just 2 h with good yields (Scheme 2).

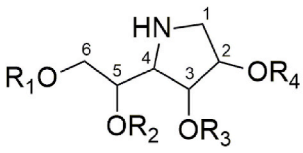
Since the subsequent Sharpless epoxidation on the *cis* allylic alcohols led to the corresponding epoxy alcohols with low yield and poor enantiomeric excess, we decided to perform the reaction on the corresponding *trans* allylic alcohols (**5a-c**, Scheme 3), achievable from the *cis* isomer through a simple isomerization of the double bond [41]. On the *trans* isomers the same reaction gave very good yield and enantiomeric excess (**6a–c**).

We then followed the synthetic pathway standard in our approach toward pyrrolidine iminosugars in order to achieve the suitable unsaturated azido alcohols **8a–c**. The subsequent asymmetric dihydroxylation was then performed using $(DHQ)_2AQN$, the chiral ligand of choice for obtaining excellent diastereomeric ratios as we have already observed for this type of substrates (Scheme 3) [35]. As shown in Table 1, $(DHQ)_2AQN$ worked as expected also on **9a-c**, giving a significant diastereofacial preference. The diastereomeric ratio was calculated by integrating the signals of the CHOH in α of the ester moiety on the ^1H NMR spectra of the crude mixture.

At this point, synthesis of the pyrrolidine derivatives **2a**, **2b** and **2c** was successfully achieved, by first closing the pyrrolidine rings through the *one-pot* reduction of azide moiety and subsequent lactamization (**11a–c**). Finally, the iminosugars' derivatives were obtained as hydrochlorides after the borane–dimethyl sulfide reduction in refluxing THF, followed by the acidic work-up to remove the silyl protecting groups (HCl 37% in MeOH) (Scheme 4).



Scheme 1. Our stereocontrolled synthetic approach toward several classes of iminosugars.

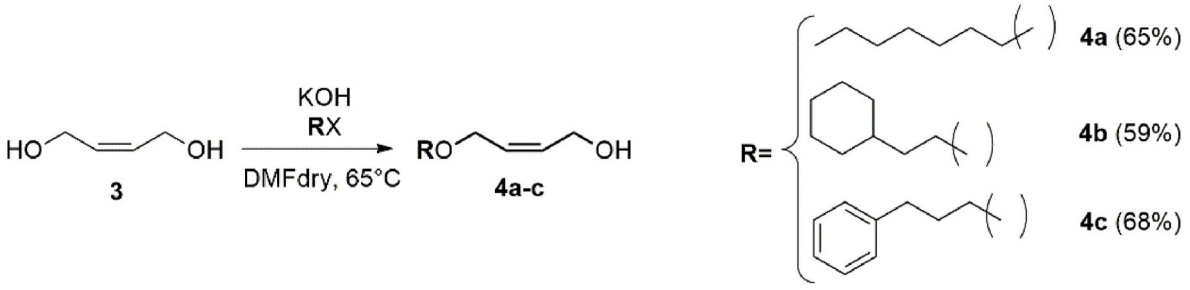


a) R_1=alkyl, R_2=R_3=R_4=H
b) R_2= alkyl, R_1=R_3=R_4=H
c) R_3= alkyl, R_1=R_2=R_4=H
d) R_4= alkyl, R_1=R_2=R_3=H

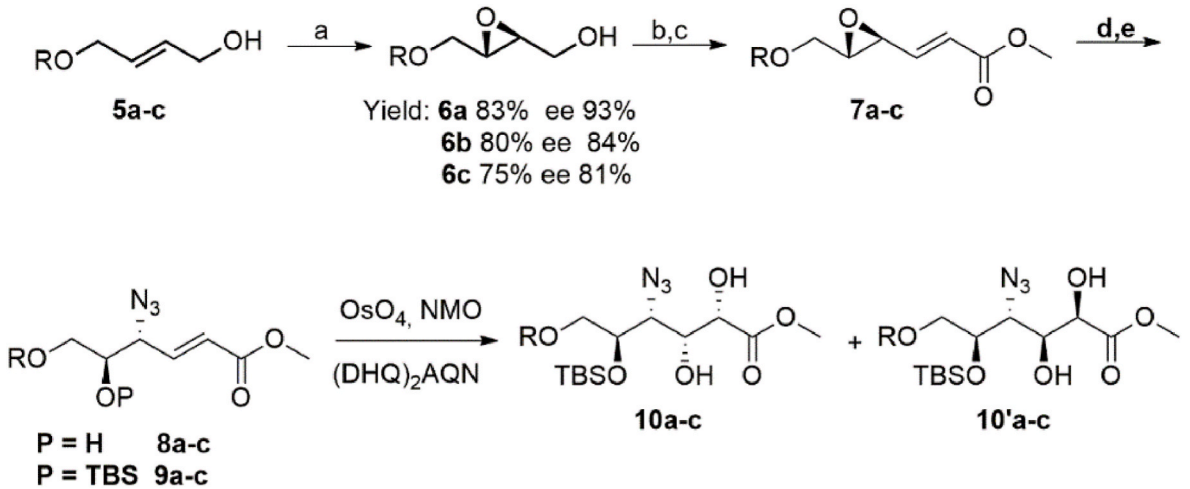
Fig. 4. Possible O-alkylated pyrrolidine iminosugar derivatives.

Table 1
Dihydroxylation of vinyl azido alcohols **9a–c**.

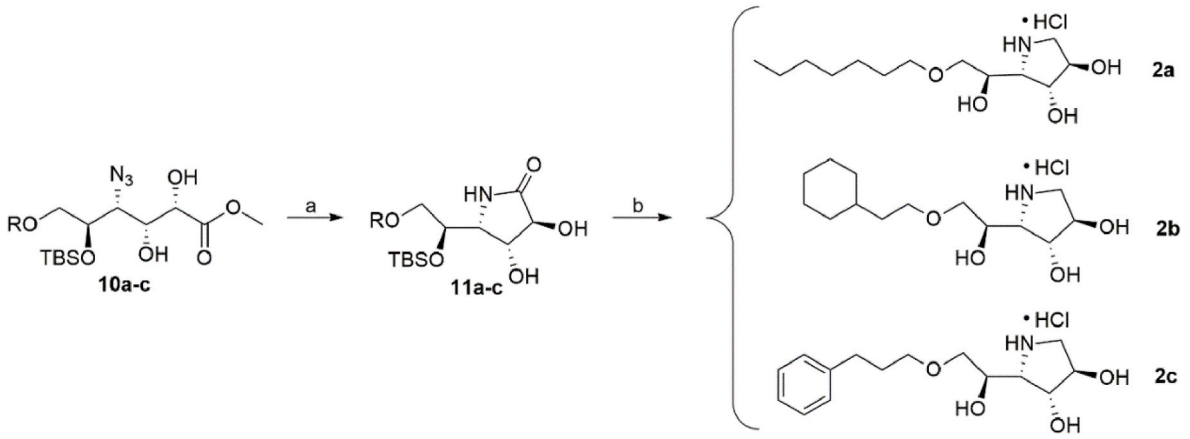
Substrate	Yield (%)	10a–c/10'a–c
9a	81	90:10
9b	83	90:10
9c	88	90:10



Scheme 2. General preparation of monoalkylated of *cis* 2-butene-1,4-diol.



Scheme 3. a) Ti(O-i-Pr)$_4$, (+)-DET, *t*-BuOOH, CH_2Cl_2, −20 °C, **6a:** 83%, *ee* 93%, **6b**: 80%, *ee* 84%, **6c** 75%, *ee* 81%; b) TEMPO, IBDA, CH_2Cl_2, rt; c) LiOH, TMPA, THF, reflux, **7a**: 57% from **6a**, **7b**: 59% from **6b**, **7c**: 55% from **6c**; d) $TMSN_3$, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt; e) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt, **9a**: 74% from **7a, 9b**: 70% from **7b, 9c**: 75% from **7c**.



Scheme 4. a) PPh_3, THF dry, rt for 48 h, then water; **11a**: 69%, **11b**:73% and **11c**: 63%; b) BH_3–SMe_2, THF dry, rt; then HCl (37%), MeOH, 70 °C, **2a**: 71%, **2b**: 68%, **2c**: 75%.

2.2. Biological tests

Biological tests of our iminosugars' collection (Fig. 3) were then performed.

2.2.1. Inhibition of metabolic activity in U937 and CAL-27 cancer cell lines

The potential biological activities of the compounds under study were investigated through an assay to assess their metabolic inhibition on two cancer cell lines *in vitro*. The compounds were tested within the 1000–10 μM range versus a monocytoid cell line (U937) growing in suspension, and within the 1000 and 1 μM versus an adherent tumor cell line (CAL-27). Table 2 shows that the concentration of most compounds at which cells are inhibited by 50% is higher than 1000 μM, apart from compound **2b** and compound **DNJ** which are able to inhibit U937 cells by 50% at 697 ± 113.1 and 427.4 ± 278.3 μM respectively. Notably, both **2b** and **DNJ** also inhibited metabolic activity of the adherent cell line CAL-27 (Table 2), although at higher maCC50. The inhibition of CAL-27 cells is a promising result because this adherent cell line was found to be resistant to several chemotherapeutic drugs. However, since U937 cells were more susceptible to **2b** and **DNJ** treatment, we decided to perform a dose effect assay on this cell line to determine the cytotoxic activity of **2b** and **DNJ** through a trypan blue exclusion assay using a 250, 500 and 1000 μM concentration.

The data in Fig. 5 show that both compound **2b** and compound **DNJ** have a cytotoxic effect, expressed as inhibition of percentage of viable cells, at the concentrations tested. In particular, **2b** showed a significantly higher inhibition compared to **DNJ** when used at 500 μM.These results indicate that the compound **DNJ** inhibits the metabolic activity of the cancer cell lines tested more evidently than compound **2b**, on the other hand the cytotoxic effect, investigated on U937 cells, is greater when compound **2b** is used (Fig. 5). Finally, **2b** and **DNJ** are endowed with good solubility in an aqueous environment and showed a notable inhibition activity toward lymphoid cell line metabolism and growth.

Table 2
Biological assays of metabolic inhibition in U937 and in CAL-27 cells.

Compound	maCC50 (μM)[a] U937	R square[b]	maCC50 (μM)[a] CAL-27	R square[c]
DMDG	>1000	0,9803		
DMDGl	>1000	0,9688		
1	>1000	0,9803		
2b	697 ± 113,1	0,8988	778,13 ± 35,03	0,8974
2c	>1000	0,9688		
2a	>1000	0,9688		
DMDI	>1000	0,9803		
DNJ	427,4 ± 278,3	0,9088	990,63 ± 16,22	0,8798
DANJ	>1000	0,9688		

[a] maCC50 is the concentration at which metabolic activity in the U937 and CAL-27 cell line is inhibited by 50% by means of the MTS assay.
[b] The coefficient of determination in U937 is the proportion of the variation in the dependent variable that is predictable from the independent variable(s).
[c] The coefficient of determination in CAL-27 is the proportion of the variation in the dependent variable that is predictable from the independent variable(s).

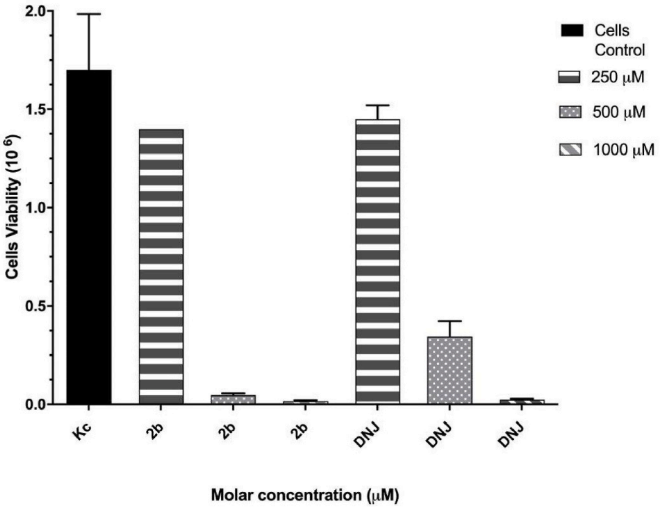


Fig. 5. Effect of compounds **2b** and **DNJ** at 250, 500 and 1000 μM on the viability of U937 cells.

2.2.2. Biological assays in yeast cells

We tested the toxicity of the compounds in *S. cerevisiae* W303 cells with a range of the concentrations. In Fig. 6, the effect of the tested compounds at the highest concentration (400 μM) for this model system is shown [42,43]. After treatment, the cultures' serial dilutions were spotted on YPD medium and grown at 28 °C. After 16 h, the yeast cells treated with **1** and **2c** compounds showed a reduced growth rate. Nevertheless, this effect was transient because after 48 h, no difference in growth was observed in comparison with the growth of the untreated cells.

As a control cells of *S. cerevisiae* were stained with DAPI (Sigma; D9542) in order to visualize the nuclear DNA. The results confirmed that the assayed compounds have no toxic effect on this model system (Fig. 7).

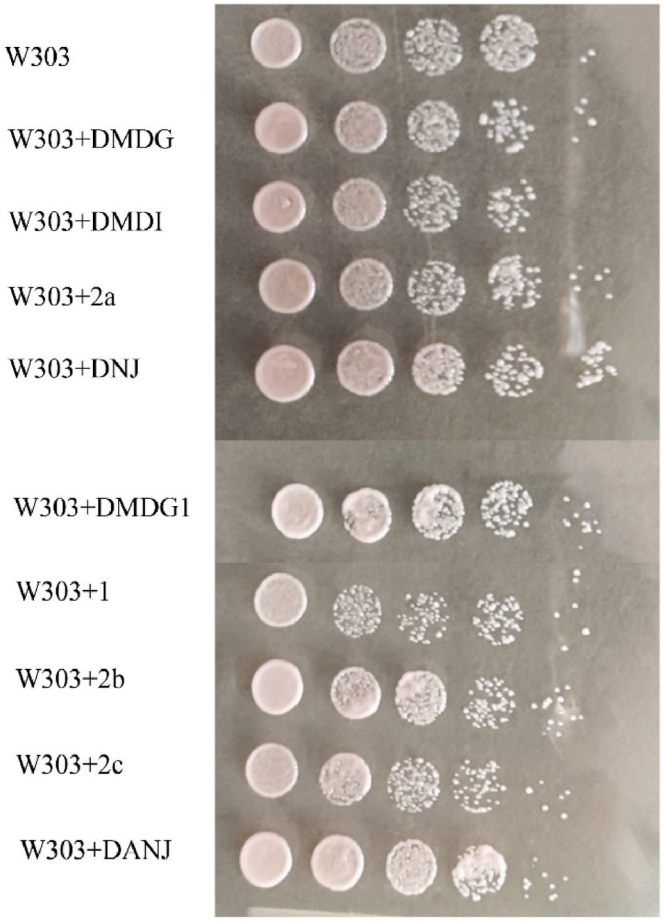


Fig. 6. Effect of the tested iminosugars on the growth of *S. cerevisiae* after 16 h of growth at 28 °C. Yeast cells were treated with the compounds at 400 μM overnight and serial dilutions were spotted on YPD complete medium.

3. Conclusion

In summary, we have reported the stereocontrolled synthesis of three new lipophilic pyrrolidine iminosugars O-alkylated in C-6 position. The new iminosugar derivatives were synthesized by exploiting a versatile synthetic strategy we developed, which allows to obtain different classes of iminosugars with control of the four contiguous stereocenters. The new iminosugars' lipophilic derivatives and those previously reported by us were tested for their potential biological activities in two cancer cell lines *in vitro* and for their toxicity on *S. cerevisiae*.

Regarding the metabolic inhibition of cancer cell lines, the results show that, among the tested iminosugars, **2b** and **DNJ** were endowed with notable metabolic inhibitory activity. Compound **2b** turned out to have a higher cytotoxic activity versus U937 cell line compared to **DNJ** at 500 μM concentration. These results may relate to the higher lipophilicity of **2b** that can have an influence on its tropism for cell membrane. Promising results were obtained also from the tests on CAL-27 cell line which is resistant to several chemotherapeutic drugs. All the tested compounds are not toxic in eukaryotic model system (*S. cerevisiae*) and the toxicity tests in yeast identified **1** and **2c** compounds as temporarily inhibiting the growth of yeast cells, however, after 48 h, no effect on yeast cells growth was observed. These preliminary results suggest the lack of toxicity in model system for healthy human cells. Suitable structure modifications aimed at the synthesis of new iminosugars derivatives are under study and further investigations on other cell lines will be evaluated.

4. Experimental section

4.1. General methods

Organic solvents and reagents were purchased and used without further purification unless otherwise stated. Reactions were monitored by thin-layer chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light (254 nm) and visualization was achieved by inspection under short-wave UV light (Mineralight UVG 11 254 nm) followed by staining with phosphomolybdic acid dip [polyphosphomolybdic acid (5g), ethanol (100 mL)] or ninhydrin dip [ninhydrin (5g), sulfuric acid (5 mL), n-butanol (100 mL)] and heating. Low temperature reactions were performed in a Haake EK 101cryostat using an acetone bath. Unless otherwise stated, reactions were carried out under standard atmosphere. ^{1}H and ^{13}C NMR spectra were recorded using Bruker 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) instrument. Residual solvent peaks were used as internal references: chloroform (^{1}H, d 7.26 ppm; ^{13}C, δ 77.00 ppm), methanol-d_4 (^{1}H, δ 4.87, 3.31 ppm; ^{13}C, δ 49.00 ppm) and deuterium oxide (^{1}H, δ 4.79 ppm). Chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard and coupling constant (J) in Hz. Splitting patterns are designated as s, singlet; br s, broad singlet; d, doublet; br d, broad doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; t, triplet; q, quartet; m, multiplet. Unless otherwise stated, all spectra are registered in deuterated chloroform. Optical rotations were measured with a Jasco Mod. DIP-370 polarimeter with a cell pathway length of 10 cm; solution concentrations are reported in grams per 100 mL. All chromatographic purifications were performed using forced flow on flash silica gel (Kieselgel 200–400 mesh from E. Merck, Germany). All procedures are referred to 1 mmol and the yields to isolated and spectroscopically homogeneous compounds.

Elemental analyses for C, H and N were performed on an EA 1110 CHNS–O instrument.

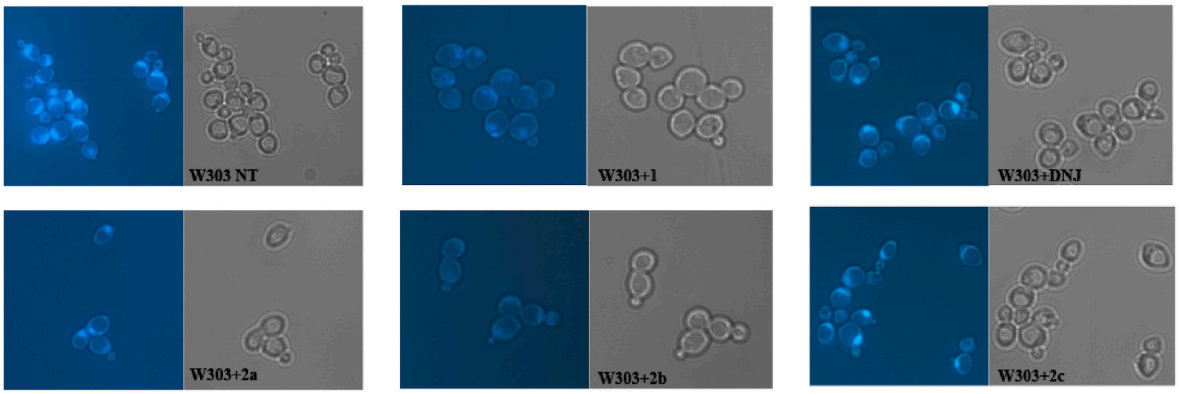


Fig. 7. The cells nuclei were counterstained with DAPI (blue) and visualized with fluorescence microscopy. The magnification is the same in each picture.

The syntheses of 1-deoxy- D-nojirimycin, 1-deoxy- D-altronojirimycin, 1,4-dideoxy-1,4-imino-D-galactitol, 1,4-dideoxy-1,4-imino-D-iditol, 1,4-dideoxy-1,4-imino-D-glucitol and pyrrolizidine iminosugars skeleton have been already described in detail in our previous articles [32–35].

4.2. (Z)-4-(heptyloxy)but-2-en-1-ol **4a**, (Z)-4-(2-cyclohexylethoxy)but-2-en-1-ol **4b** and (Z)-4-(3-phenylpropoxy)but-2-en-1-ol **4c**

73 mg (1,55 mmol) of crushed potassium hydroxide were dissolved in 4.3 mL of dry N,N-dimethylformamide under argon atmosphere and the reaction mixture was warmed to 65 °C. 88 mg (1 mmol) of *cis* 2-buten-1,4-diol **3** and 0,8 mmol of alkyl halide were added. The mixture was left stirring for 2 h (TLC monitoring), then hydrochloric acid 1 N was added until neutral pH. Water was added and the layers were separated; the aqueous layers was extracted three times with ethyl acetate and the combined organic layers were washed with brine and dried over sodium sulfate. After filtration the solvent evaporated in vacuum. The crude was purified by flash chromatography on silica gel (hexane/ethyl acetate 90:10–80:20) affording the desired product.

4a: Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.70 (dt, 1H, $J_1 = 12.6$, $J_2 = 6.3$ Hz, C<u>H</u>═CHCH$_2$OH); 5.59 (dt, 1H, $J_1 = 12.6$, $J_2 = 6.3$ Hz, CH═C<u>H</u>CH$_2$OH); 4.09 (d, 2H, $J = 6.3$ Hz, C<u>H</u>$_2$OH); 3.96 (d, 2H, $J = 6.3$ Hz, C<u>H</u>$_2$OCH$_2$R); 3.36 (t, 2H, $J = 6.7$ Hz, RCH$_2$C<u>H</u>$_2$O); 2.05 (bs, 1H, O<u>H</u>); 1.57 (quintet, 2H, $J = 6.7$ Hz, RC<u>H</u>$_2$CH$_2$O); 1.33–1.13 (m, 8H, CH$_3$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$); 0.81 (t, 3H, $J = 6.8$ Hz, C<u>H</u>$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.4, 128.9, 71.2, 66.8, 59.1, 32.1, 30.0, 29.4, 26.4, 22.9, 14.4. $C_{11}H_{22}O_2$ (186.16): C 70.92, H 11.90; found C 70.99, H 11.95.

4b: Yield 59% ^{1}H NMR (400 MHz, CDCl3) δ 5.74 (dt, 1H, C<u>H</u>═CH, $J_1 = 11.6$, $J_2 = 6.3$ Hz), 5.67–5.60 (m, 1H, CH═C<u>H</u>), 4.13 (d, 2H, C<u>H</u>$_2$CH═CH, $J = 6.3$ Hz), 3.98 (d, 2H, C<u>H</u>$_2$OH, $J = 6.1$ Hz), 3.42 (t, 2H, C<u>H</u>$_2$OCH$_2$, $J = 6.9$ Hz), 2.35–2.25 (m, 1H, OH) 1.72–1.54 (m, 5H, cyclohexyl+O<u>H</u>), 1.43 (q, 2H, RCH$_2$C<u>H</u>$_2$O, $J = 6.8$ Hz), 1.38–1.27 (m, 1H, cyclohexyl), 1.26–1.04 (m, 3H, cyclohexyl), 0.86 (m, 2H, cyclohexyl). 13C NMR (101 MHz, $CDCl_3$) δ 132.2, 128.6, 68.8, 66.6, 58.8 37.2, 34.7, 33.4, 26.7, 26.4. $C_{12}H_{22}O_2$ (198.16): C 72.68, H 11.18; found C 72.91, H 11.45.

4c: Yield 68% ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.26 (m, 2H, phenyl), 7.25–7.17 (m, 3H, phenyl), 5.86–5.76 (m, 1H, CH═CH), 5.75–5.67 (m, 1H, CH═CH), 4.20 (d, 2H, CH$_2$OH, $J = 6.3$ Hz), 4.05 (d, 2H, OCH$_2$, $J = 6.1$ Hz), 3.46 (t, 2H, CH$_2$C<u>H</u>$_2$O, $J = 6.5$ Hz), 3.02 (bs, 1H, O<u>H</u>), 2.70 (t, 2H, PhCH$_2$, $J = 7.3$ Hz), 2.63 (bs, 1H, O<u>H</u>), 1.96–1.88 (m, 2H, CH$_2$C<u>H</u>$_2$CH$_2$O). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 142.1, 132.5, 128.8, 128.7, 128.6, 126.2, 70.2, 66.8, 58.9, 32.6, 31.6. $C_{13}H_{18}O_2$ (206.13): C 75.69, H 8.80; found C 75.91, H 9.21.

4.3. (E)-4-(heptyloxy)but-2-en-1-ol **5a**, (E)-4-(2-cyclohexylethoxy)but-2-en-1-ol **5b** and (E)-4-(3-phenylpropoxy)but-2-en-1-ol **5c**

1.5 mmol (0.321g) of pyridinium chlorochromate was dissolved in 10 mL of dichloromethane and 0.160 mg of Celite were added. A solution of 1 mmol of **4a-c** in 10 mL of dichloromethane was added and the mixture left stirring at room temperature until completion (TLC monitoring). After evaporation of the solvent in vacuo, diethyl ether was added and the mixture left stirring for 1 h; it was then filtered through a pad of Celite and finally the solvent evaporated in vacuo to give the crude, that was used without purification. The crude was diluted in 2 mL of methanol and cooled to 0 °C. Sodium borohydride (2 mmol, 0.076 g) was added and the mixture left stirring at room temperature until complete consumption of the substrate (TLC monitoring). The reaction mixture was concentrated in vacuo, diluted with diethyl ether and washed with aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent removed in vacuo to leave the crude, which was then purified by chromatography on silica gel (hexane/ethyl acetate 90:10–80:20) to give the desired product.

5a: Yield 53% from **4a.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90–5.74 (m, 2H, C<u>H</u>═C<u>H</u>); 4.12 (dd, 2H, $J_1 = 4.9$ $J_2 = 1.1$ Hz, C<u>H</u>$_2$OH); 3.94 (dd, 2H, $J_1 = 5.4$ $J_2 = 1.1$ Hz, OC<u>H</u>$_2$); 3.40 (t, 2H, $J = 6.7$ Hz RC<u>H</u>$_2$O); 2.06 (bs, 1H, O<u>H</u>); 1.56 (m, 2H $J = 7.1$ Hz, RC<u>H</u>$_2$CH$_2$O); 1.28 (m, 8H, CH$_3$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$); 0.86 (t, 3H, $J = 7.1$ Hz). ^{13}C NMR (101 MHz, $CDCl_3$) δ 132.2, 128.4, 71.0, 71.0, 63.2, 32.1, 30.0, 29.5, 26.4, 22.9, 14.4. $C_{11}H_{22}O_2$ (186.16): C 70.92, H 11.90; found C 70.97, H 11.94.

5b: Yield 55% from **4b.** ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.88–5.67 (m, 2H, ROCH═CH), 4.06 (d, 2H, CH$_2$OH, $J = 4.6$ Hz), 3.89 (d, 2H, OCH$_2$CH═CH, $J = 4.8$ Hz), 3.39 (t, 2H, RCH$_2$CH$_2$O, $J = 6.8$ Hz), 2.89 (s, 1H, OH), 1.71–1.52 (m, 5H, cyclohexyl),1.49–1.35 (m, 2H, RCH$_2$CH$_2$O), 1.35–1.24 (m, 1H, cyclohexyl), 1.23–1.03 (m, 3H, cyclohexyl), 0.92–0.75 (m, 2H, cyclohexyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 132.2, 127.7, 70.8, 68.5, 62.7, 37.1, 34.6, 33.4, 26.6, 26.3. $C_{12}H_{22}O_2$ (198.16): C 72.68, H 11.18; found C 72.94, H 11.35.

5c: Yield 61% from **4c** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.24 (m, 2H, phenyl), 7.23–7.16 (m, 3H, phenyl), 5.93–5.77 (m, 2H, CH═CH), 4.14 (dd, 2H, CH$_2$OH, $J_1 = 4.8$, $J_2 = 0.9$ Hz), 3.98 (dd, 2H, OCH$_2$, $J_1 = 5.3$, $J_2 = 0.8$ Hz), 3.45 (t, 2H, CH$_2$OCH$_2$, $J = 6.5$ Hz), 2.74–2.68 (m, 2H, PhCH$_2$), 2.43 (s, 1H, OH), 1.97–1.87 (m, 4H, PhCH$_2$C<u>H</u>$_2$C<u>H</u>$_2$). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 141.9, 132.2, 128.5, 128.3, 127.8, 125.8, 70.8, 69.6, 62.8, 32.3, 31.3. $C_{13}H_{18}O_2$ (206.13): C 75.69, H 8.80; found C 75.97, H 9.25.

4.4. [(2S,3S)-3-(heptyloxymethyl)oxiran-2-yl]methanol **6a**, [(2S,3S)-3-(2-cyclohexylethoxymethyl)oxiran-2-yl]methanol **6b** and [(2S,3S)-3-(3-phenylpropoxymethyl)oxiran-2-yl]methanol **6c**

In a three-neck flask equipped with a dropping funnel under argon atmosphere, 98 mg of activated powdered 4 Å molecular sieves were dissolved in 5 mL of dry dichloromethane and cooled to −20 °C. Then 0,03 mL (0,18 mmol) of (+)-L-diethyltartrate and 0.04 mL (0.15 mmol) of titanium(IV) isopropoxide were added. The reaction mixture was stirred at −20 °C as 0.4 mL of a *tert*-butyl hydroperoxide solution 5.5 M in decane (2 mmol of *t*-BuOOH) were added through the addition funnel at a moderate rate (over ca. 5 min.). The resulting mixture was stirred at −20 °C for 30 min **5a-c** (1 mmol), dissolved in 0.5 mL of dichloromethane, was then added dropwise through the same addition funnel over a period of 5 min, being careful to maintain the reaction temperature between −20 and −15 °C. The reaction mixture was left stirring for 8 h (TLC monitoring). A freshly prepared solution of 698 mg of ferrous sulfate heptahydrate and 300 mg of tartaric acid in a total volume of 2 mL of deionized water is cooled to ca. 0 °C, by means of an ice water bath. The epoxidation reaction mixture was allowed to warm to ca. 0 °C and then was slowly poured into a beaker containing the pre-cooled stirring ferrous sulfate solution (external cooling is not essential during or after this addition). The two-phase mixture was stirred for 5–10 min and then transferred into a separatory funnel. The phases were separated and the aqueous phase was extracted with diethyl ether. The combined organic layers were treated with a precooled (0 °C) solution of 30% sodium hydroxide (w/v) in saturated brine. The two-phase mixture was stirred vigorously for 1 h at 0 °C. Following transfer to a separatory funnel and dilution with water, the phases were separated and the aqueous layer was extracted with diethyl ether. The combined organic layers were dried over sodium sulfate, filtered, and concentrated. The crude was purified by flash chromatography on silica gel (hexane/ethyl acetate 80:20).

6a: Yield 83%, $[\alpha]_D$**:** −23,16 ($c = 3.2$ g/100 mL, $CHCl_3$); the enantiomeric excess (93%) was determined on naphthoyl derivative by HPLC with a Chiralpak IB column (250 × 4.6 mm, 5 μm): *n*-hexane/2-propanol 96:4; flow rate: 1 mL/min; UV detection at 220 nm, T = 30 °C. Rt$_1$ = 9.45 min, rt$_2$ = 12.15 min.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (bd, 1H, $J = 12.5$ Hz, C<u>H</u>aHbOH), 3.61 (dd, 1H CHa<u>H</u>bOH, $J_1 = 11.7$ $J_2 = 3.0$ Hz), 3.55–3.43 (m, 1H OC<u>H</u>cCHd), 3.41–3.29 (m, 3H, OCHcC<u>H</u>d, C<u>H</u>$_2$O), 3.22 (bs, 1H, O<u>H</u>), 3.06 (dt, 1H, $J_1 = 5.4$ $J_2 = 2.7$ Hz, C<u>H</u>OCH), 2.95 (dt, 1H, $J_1 = 4.7$ $J_2 = 2.7$ Hz, CHOC<u>H</u>), 1.47 (m, 2H, $J = 7.1$ Hz, RC<u>H</u>$_2$CH$_2$O), 1.27–1.09 (m,

8H, $CH_3CH_2CH_2CH_2CH_2$), 0.78 (t, 3H, $J = 7.1$ Hz, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 72.1, 69.0, 61.1, 56.0, 55.0, 32.1, 29.9, 29.4, 26.3, 22.9, 14.4. $C_{11}H_{22}O_3$ (202.16): C 65.31, H 10.96; found C 65.69, H 11.27.

6b: Yield 80% $[\alpha]_D$: −19,47 ($c = 3.2$ g/100 mL, $CHCl_3$); the enantiomeric excess (84%) was determined on naphthoyl derivative by HPLC with a Chiralpak IB column (250 × 4.6 mm, 5 μm): mobile phase *n*-hexane/2-propanol 98:2; flow rate: 1 mL/min; UV detection at 220 nm, T = 30 °C. $Rt_1 = 13.85$ min, $rt_2 = 18.46$ min.

^1H NMR (400 MHz, $CDCl_3$) δ 3.93 (dd, 1H, OCHaHb, $J_1 = 12.7$, $J_2 = 2.2$ Hz), 3.70 (dd, 1H, OCHcHdOH, $J_1 = 11.6$, $J_2 = 3.0$ Hz), 3.65 (dd, 1H, OCHaHb, $J_1 = 12.7$, $J_2 = 4.0$ Hz), 3.55–3.42 (m, 3H, OCHcHdOH, CH_2O), 3.20–3.16 (m, 1H, $CHOCHCH_2OH$), 3.09–3.03 (m, 1H, $CHOCHCH_2OH$), 2.48 (bs, 1H, OH), 1.73–1.57 (m, 5H, cyclohexyl, 1.46 (q, 2H, RCH_2CH_2O, $J = 6.8$ Hz), 1.42–1.28 (m, 1H, cyclohexyl), 1.28–1.09 (m, 3H, cyclohexyl), 0.95–0.85 (m, 2H, cyclohexyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ 70.4, 69.8, 61.3, 55.9, 54.5, 37.2, 34.6, 33.5, 33.4, 26.7, 26.4. $C_{12}H_{22}O_3$ (214.16): C 67.26, H 10.35; found C 67.51, H 10.61.

6c: Yield 75% $[\alpha]_D$: −12,86 ($c = 3.2$ g/100 mL, $CHCl_3$); The enantiomeric excess (81%) was determined by HPLC with a Chiralpak IB column (250 × 4.6 mm, 5 μm): mobile phase *n*-hexane/2-propanol 90:10; flow rate: 0.8 mL/min; UV detection at 258 nm, T = 30 °C. $Rt_1 = 14.22$ min, $rt_2 = 14.68$ min.

^1H NMR (400 MHz, CDCl3) δ 7.32–7.24 (m, 2H, phenyl), 7.23–7.16 (m, 3H, phenyl), 3.93 (bd, 1H, OCHaHbOH, $J = 12.7$ Hz), 3.72 (dd, 1H, OCHcHd, $J_1 = 11.6$, $J_2 = 2.9$ Hz), 3.64 (bd, 1H, OCHaHbOH, $J = 12.4$ Hz), 3.54–3.42 (m, 3H, OCHcHd, RCH_2O), 3.22–3.16 (m, 1H, $CHOCH_2CH_2OH$), 3.10–3.05 (m, 1H, $CHOCHCH_2OH$), 2.69 (t, 2H, $PhCH_2$, $J = 7.4$ Hz), 2.47 (s, 1H, OH), 1.96–1.86 (m, 2H, $PhCH_2CH_2CH_2O$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 141.8, 128.5, 128.4, 125.9, 70.7, 70.4, 61.3, 55.9, 54.4, 32.2, 31.2, 26.4. $C_{13}H_{18}O_3$ (222.13): C 70.24, H 8.16; found C 70.53, H 8.48.

*4.5. (E)-methyl 3-[(2S,3S)-3-(heptyloxymethyl)oxiran-2-yl]acrylate **7a**, (E)-methyl 3-[(2S,3S)-3-(2-cyclohexylethoxymethyl)oxiran-2-yl]acrylate **7b** and (E)-methyl 3-[(2S,3S)-3-(3-phenylpropoxymethyl)oxiran-2-yl] acrylate **7c***

In a round bottom flask 1 mmol of alcohol was dissolved in 1 mL of dichloromethane and then 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, 0.1 mmol, 0.016 g) and iodosobenzene diacetate (IBDA, 1.1 mmol, 0.354 g) were added; the mixture was stirred at room temperature for 3 h or until consumption of the substrate (TLC monitoring). The reaction mixture was then diluted with dichloromethane and washed with a saturated solution of sodium thiosulfate then the organic layer was washed with saturated solutions of sodium bicarbonate and sodium chloride until pH = 7. The organic layer was dried on anhydrous sodium sulfate and evaporated in vacuo to leave the crude product that was used without any purification.

The crude of the epoxy aldehyde was dissolved in 10 mL of tetrahydrofuran and lithium hydroxide (1.1 mmol, 0.027 g), then trimethyl phosphonoacetate (1.1 mmol, 0.200 g, 0.16 mL) were added. The mixture was stirred at 30 °C for 15 h or until consumption of the substrate (TLC monitoring). A saturated solution of ammonium chloride was added, and the reaction mixture concentrated in vacuo to evaporate the tetrahydrofuran. The aqueous residue was then extracted with ethyl acetate and the organic layer washed with ammonium chloride saturated solution and brine until pH = 7. The combined organic extracts were dried over sodium sulfate and evaporated in vacuo to leave the crude product that was then purified by flash chromatography on silica gel (hexane/ethyl acetate 90:10).

7a: Yield 57% from **6a** ^1H NMR (400 MHz, $CDCl_3$) δ 6.66 (dd, 1H, $J_1 = 15.7$, $J_2 = 7.1$ Hz, CH═CHCO), 6.13 (d, 1H, $J = 15.7$ Hz, CH═CHCO), 3.71 (s, 3H, OCH_3), 3.68 (dd, 1H, $J_1 = 11.8$ $J_2 = 3.7$ Hz, OCHaHb), 3.52–3.40 (m, 3H, OCHaHb, RCH_2O), 3.38 (dd, 1H, $J_1 = 7.1$ $J_2 = 1.6$ Hz, CHOCH), 3.08 (ddd, 1H, $J_1 = 5.1$ $J_2 = 3.1$ $J_3 = 2.1$ Hz, CHOCH), 1.54 (m, 2H, $J = 7.0$ Hz RCH_2CH_2O), 1.34–1.18 (m, 8H, $CH_3CH_2CH_2CH_2CH_2$), 0.84 (t, 3H, $J = 7.3$ Hz, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 144.5, 124.1, 72.2, 70.1, 59.9, 53.9, 52.0, 32.1, 29.9, 29.4, 26.3, 22.9, 14.4. $C_{14}H_{24}O_4$ (256.17): C 65.60, H 9.44; found C 65.81, H 9.79.

7b: Yield 59% from **6b** ^1H NMR (400 MHz, $CDCl_3$) δ 6.67 (dd, 1H, CH═CH, $J_1 = 15.7$, $J_2 = 7.1$ Hz), 6.14 (dd, 1H, CH═CH, $J_1 = 15.7$, $J_2 = 0.5$ Hz), 3.73 (s, 3H, OCH_3), 3.68 (dd, 1H, CHaHbO, $J_1 = 11.8$, $J_2 = 3.1$ Hz), 3.52–3.45 (m, 3H, CHaHbO, RCH_2O), 3.38 (dd, 1H, CHOCH, $J_1 = 7.1$, $J_2 = 1.6$ Hz), 3.09 (ddd, 1H, CHOCH, $J_1 = 5.0$, $J_2 = 3.1$, $J_3 = 2.1$ Hz), 1.71–1.60 (m, 5H, cyclohexyl), 1.45 (q, 2H, CH_2CH_2O, $J = 6.8$ Hz), 1.39–1.28 (m, 1H, cyclohexyl), 1.27–1.04 (m, 3H, cyclohexyl), 0.95–0.81 (m, 2H, cyclohexyl). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 144.3, 123.9, 69.9, 69.9, 59.7, 53.7, 51.8, 37.1, 34.6, 33.4, 33.4, 26.6, 26.3. $C_{15}H_{24}O_4$ (268.17): C 67.14, H 9.01; found C 67.48, H 9.39.

7c: Yield 55% from **6c** ^1H NMR (400 MHz, $CDCl_3$) δ 7.32–7.25 (m, 2H, phenyl), 7.22–7.15 (m, 3H, phenyl), 6.69 (dd, 1H, CH═CH, $J_1 = 15.7$, $J_2 = 7.1$ Hz), 6.17 (dd, 1H, CH═CH, $J_1 = 15.7$, $J_2 = 0.7$ Hz), 3.81 (s, 3H, OCH_3), 3.78 (dd, 1H, OCHaHb, $J_1 = 11.7$, $J_2 = 3.1$ Hz), 3.55–3.46 (m, 3H, OCHaHb, RCH_2O), 3.18 (ddd, 1H, CHOCH, $J_1 = 5.1$, $J_2 = 3.1$, $J_3 = 1.9$ Hz), 3.40 (dd, 1H, CHOCH, $J_1 = 7.2$, $J_2 = 1.9$ Hz), 2.69 (t, 2H, $PhCH_2$, $J = 7.6$ Hz), 1.95–1.87 (m, 2H, $CH_2CH_2CH_2O$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 144.3, 141.8, 128.5, 128.5, 125.9, 123.9, 70.9, 70.0, 59.6, 53.8, 51.9, 32.3, 31.3. $C_{16}H_{20}O_4$ (276.14): C 69.54, H 7.30; found C 69.85, H 7.68.

*4.6. (4R,5R,E)-methyl 4-azido-5-(tert-butyldimethylsilyloxy)-6-(heptyloxy)hex-2-enoate **9a**, (4R,5R,E)-methyl 4-azido-5-(tert-butyldimethylsilyloxy)-6-(2-cyclohexylethoxy)hex-2-enoate **9b** and (4R,5R,E)-methyl 4-azido-5-(tert-butyldimethylsilyloxy)-6-(3-phenylpropoxy)hex-2-enoate **9c***

To a stirred solution of **7a–c** (1 mmol) in dry dichloromethane (3 mL) were added trimethylsilyl azide (1 mmol, 115 mg, 0.13 mL) and boron trifluoride diethyl etherate (2 mmol, 283 mg, 0.25 mL) dropwise and the mixture left stirring at room temperature. After complete consumption of the substrate (12 h, TLC monitoring), the mixture was diluted with dichloromethane and washed with aq. sodium bicarbonate and brine until pH 7. The organic layer was dried over sodium sulfate and after filtration the solvent evaporated under vacuum to give **8a–c**, used without purification. The crude of **8a–c**, put in a two-neck flask under argon atmosphere, was dissolved in 6 mL of dry dichloromethane; then 2 mmol of 2,6-lutidine and 3 mmol of tert-butyldimethylsilyl trifluoromethanesulfonate were added and the mixture stirred at room temperature until completion (12 h, TLC monitoring). The reaction was quenched with water, the two phases were separated, and the aqueous layer was extracted twice with dichloromethane. The combined organic layers were washed with brine and sodium bicarbonate saturated solution until pH 7. The combined organic layers were dried over sodium sulfate and after filtration the solvent evaporated in vacuo. The crude was purified by flash chromatography on silica gel (hexane/ethyl acetate 98:2).

9a: Yield 74% from **7a** ^1H NMR (400 MHz, $CDCl_3$) δ 6.89 (dd, 1H, $J_1 = 15.7$ $J_2 = 6.9$ Hz, CH═CHCO), 6.06 (d, 1H, $J = 15.7$ Hz, CH═CHCO), 4.21 (t, 1H, $J = 6.9$ Hz, CHN_3), 3.90 (q, 1H, $J = 5.5$ Hz, CHOTBS), 3.75 (s, 3H, OCH_3), 3.46–3.27 (m, 4H, CH_2OCH_2), 1.60–1.47 (m, 2H, RCH_2CH_2O), 1.39–1.21 (m, 8H, $CH_3CH_2CH_2CH_2CH_2$), 0.88 (s, 12H, CH_3, $(CH_3)_3C$), 0.10 (s, 3H, CH_3Si), 0.08 (s, 3H, CH_3Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 142.3, 124.8, 74.1, 72.3, 71.4, 65.4, 52.4, 32.5, 30.3, 29.8, 26.7, 26.4, 23.3, 18.7, 14.7, −3.9, −4.3. $C_{20}H_{39}N_3O_4Si$ (413.27): C 58.08, H 9.50; found C 58.42, H 9.88.

9b Yield 70% from **7b** ^1H NMR (400 MHz, $CDCl_3$) δ 6.89 (dd, 1H, CH═CH, $J_1 = 15.7$, $J_2 = 7.0$ Hz), 6.05 (dd, 1H, CH═CH, $J_1 = 15.7$, $J_2 = 1.3$ Hz), 4.23–4.18 (m, 1H, CHN_3), 3.93–3.87 (m, 1H, CHOTBS), 3.76 (s, 3H, OCH_3), 3.40 (dt, 3H, RCH_2, OCHaHb, $J_1 = 7.7$, $J_2 = 6.0$ Hz), 3.30 (dd, 1H, OCHaHb, $J_1 = 9.8$, $J_2 = 6.0$ Hz), 1.72–1.62 (m, 5H, cyclohexyl), 1.47–1.37 (m, 2H, RCH_2CH_2O), 1.38–1.29 (m, 1H, cyclohexyl),

1.29–1.11 (m, 3H, cyclohexyl), 1.01–0.79 (m, 11H, $(CH_3)_3$), cyclohexyl), 0.10 (s, 3H, CH_3Si), 0.08 (s, 3H, CH_3Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 166.2, 141.8, 124.4, 73.6, 71.8, 69.7, 64.9, 51.9, 37.2, 34.7, 33.6, 33.4, 26.7, 26.4, 25.9, 18.2, −4.4, −4.8. $C_{21}H_{39}N_3O_4Si$ (425.27): C 59.26, H 9.24, N 9.87; found C 59.61, H 9.60, N 9.98.

9c: Yield 75% from **7c** ^1H NMR (400 MHz, $CDCl_3$) δ 7.38–7.30 (m, 2H, phenyl), 7.27–7.20 (m, 3H, phenyl), 6.96 (dd, 1H, CH═CH, J_1 = 15.7, J_2 = 6.9 Hz), 6.13 (dd, 1H, CH═CH, J_1 = 15.7, J_2 = 1.3 Hz), 4.27 (ddd, 1H, CHN_3, J_1 = 6.9, J_2 = 4.1, J_3 = 1.3 Hz), 3.98 (td, 1H, CHOTBS, J_1 = 4.2, J_2 = 5.6 Hz), 3.81 (s, 3H, OCH_3), 3.52–3.37 (m, 4H, CH_2O, OCH_2), 2.76–2.70 (m, 2H, $PhCH_2$), 1.98–1.89 (m, 2H, $PhCH_2CH_2CH_2O$), 0.96 (s, 9H, $(CH_3)_3$), 0.18 (s, 3H, CH_3Si), 0.16 (s, 3H, CH_3Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 141.9, 141.8, 128.5, 128.4, 126.0, 124.2, 73.6, 71.9, 70.9, 64.9, 51.9, 32.4, 31.3, 25.8, 18.2, −4.4, −4.8. $C_{22}H_{35}N_3O_4Si$ (433.24): C 60.94, H 8.14, N 9.69; found C 61.26, H 8.48, N 9.88.

*4.7. (2S,3R,4R,5R)-methyl 4-azido-5-(tert-butyldimethylsilyloxy)-6-(heptyloxy)-2,3-dihydroxyhexanoate **10a**, (2S,3R,4R,5R)-methyl 4-azido-5-(tert-butyldimethylsilyloxy)-6-(2-cyclohexylethoxy)-2,3-dihydroxyhexanoate **10b** and (2S,3R,4R,5R)-methyl 4-azido-5-(tert-butyldimethylsilyloxy)-2,3-dihydroxy-6-(3-phenylpropoxy)hexanoate **10c***

To a solution of 1 mmol of **9a–c** in 9 mL of acetone/water (8:1) were added N-methylmorpholine N-oxide (2 mmol, 270 mg), $(DHQ)_2AQN$ (0.15 mmol, 128.5 mg) and osmium tetroxide (0.05 mmol, 0.63 mL of a 2.5% solution of OsO_4 in tert-butanol). The mixture was left stirring overnight at room temperature. The reaction was then quenched with 5 mL of a saturated solution of sodium-thiosulfate and the mixture left stirring for 1 h before transfer into a separating funnel. The aqueous layer was extracted with 10 mL of ethyl acetate, the combined organic layers dried over sodium sulfate and the solvent removed under reduced pressure. The crude was purified by flash chromatography on silica gel (hexane/ethyl acetate 8:2).

10a: Yield 81%, d.r. 90:10. ^1H NMR (400 MHz, $CDCl_3$) δ: 4.37 (d, 1H, J = 1.4 Hz, CHOHCO), 4.24 (dt, 1H, J_1 = 7.3 J_2 = 3.5 Hz, CHOTBS), 3.90 (dd, 1H, J_1 = 10.0 J_2 = 2.9 Hz, CHN_3), 3.80–3.75 (m, 4H, CHN_3CHOH, OCH_3), 3.69 (dd, 1H, J_1 = 8.9 J_2 = 8.2 Hz, OCHaHb), 3.48–3.34 (m, 3H, OCHaHb, RCH_2O), 3.21 (bs, 2H, OH), 1.55 (m, 2H, J = 6.3 Hz, RCH_2CH_2O), 1.39–1.21 (m, 8H, $CH_3CH_2CH_2CH_2CH_2$), 0.90–0.82 (m, 12H, CH_3, $(CH_3)_3C$), 0.10 (s, 3H, CH_3Si), 0.10 (s, 3H, CH_3Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6, 71.9, 71.8, 71.5, 71.2, 71.1, 65.4, 52.6, 31.7, 29.5, 29.1, 26.0, 25.7, 22.6, 18.0, 14.1, −4.8, −4.9. $C_{20}H_{41}N_3O_6Si$ (447.28): C 53.66, H 9.23, N 9.39; found C 53.98, H 9.52, N 9.78.

10b: Yield 83% d.r. 90:10 ^1H NMR (400 MHz, $CDCl_3$) δ: 4.38 (bs, 1H, CHOHCO), 4.24 (dt, 1H, CHOTBS, J_1 = 8.0, J_2 = 3.6 Hz), 3.92 (dd, 1H, CHN_3, J_1 = 9.9, J_2 = 2.9 Hz), 3.85–3.74 (m, 4H, OCH_3, CHOH), 3.70 (dd, 1H, OCHaHb, J_1 = 9.4, J_2 = 8.1 Hz), 3.51–3.45 (m, 2H, CH_2O), 3.38 (dd, 1H, OCHaHb, J_1 = 9.4, J_2 = 3.2 Hz), 3.32 (s, 1H, OH), 3.01 (s, 1H, OH), 1.73.1.60 (m, 5H, cyclohexyl), 1.47–1.40 (m, 2H, RCH_2CH_2O), 1.35–1.07 (m, 4H, cyclohexyl), 0.98–0.83 (m, 11H, $(CH_3)_3$, cyclohexyl), 0.13 (s, 3H, CH_3Si, 0.13 (s, 3H, CH_3Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.9, 72.0, 71.6, 71.3, 71.2, 70.0, 65.5, 52.9, 37.0, 34.7, 33.5, 33.3, 26.6, 26.3, 25.8, 18.2, −4.7, −4.7. $C_{21}H_{41}N_3O_6Si$ (459.28): C 54.87, H 8.99, N 9.14; found C 55.19, H 9.34, N 9.25.

10c: Yield 88% d.r. 90:10 ^1H NMR (400 MHz, $CDCl_3$) δ: 7.40–7.34 (m, 2H, phenyl), 7.22–7.14 (m, 3H, phenyl), 4.43 (d, 1H, CHOHCO, J = 1.5 Hz), 4.33–4.28 (m, 1H, CHOTBS), 3.95 (dd, 1H, CHN_3, J_1 = 10.0, J_2 = 2.9 Hz), 3.87 (dd, 1H, CHOH, J_1 = 10.1, J_2 = 1.5 Hz), 3.75 (s, 3H, OCH_3), 3.55–3.40 (m, 4H, CH_2O, OCH_2), 3.12 (bs, 2H, OH), 2.71–2.59 (m, 2H, $PhCH_2$), 1.93–1.83 (m, 2H, $PhCH_2CH_2CH_2O$), 0.92 (s, 9H, $(CH_3)_3$, 0.15 (s, 3H, CH_3Si), 0.14 (s, 3H, CH_3Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 173.6, 141.6, 128.4, 128.4, 128.4, 126.0, 71.8, 71.5, 71.2, 71.1, 70.9, 65.5, 52.7, 32.3, 31.3, 25.7, 18.0, −4.7, −4.9. $C_{22}H_{37}N_3O_6Si$ (467.25): C 56.51, H 7.98, N 8.99; found C 56.89, H 8.27, N 9.34.

*4.8. (3S,4R,5R)-5-[(R)-1′-(tert-butyldimethylsilyl)oxy-2′-(heptyloxy) ethyl]-3,4-dihydroxypyrrolidin-2-one **11a**, (3S,4R,5R)-5-[(R)-1′-(tert-butyldimethylsilyl)oxy-2′-(2-cyclohexylethoxy)ethyl]-3,4-dihydroxypyrrolidin-2-one **11b** and (3S,4R,5R)-5-[(R)-1′(tert-butyldimethylsilyl)oxy-2′-(3-phenylpropoxy)ethyl]-3,4-dihydroxypyrrolidin-2-one **11c***

To a solution of **10a-c** (1 mmol) in tetrahydrofuran (3 mL) was added triphenylphosphine (1 mmol, 223 mg) in one portion at 0 °C. After stirring at 0 °C for 10 min, the reaction mixture was warmed to room temperature and stirred for 48 h. Water (20 mL) was added. After stirring at room temperature for an additional 12 h, the reaction mixture was concentrated to dryness. The crude was purified by flash chromatography on silica gel (hexane/ethyl acetate 8:2).

11a: Yield 69% ^1H NMR (400 MHz, $CDCl_3$) δ: 6.17 (s, 1H, NH), 4.32–4.28 (m, 2H, COCHOH, CHOH), 3.88 (dt, J_1 = 6.4 J_2 = 4.6 Hz, 1H CHOTBS), 3.54 (dd, 1H, J_1 = 9.8, J_2 = 4.0 Hz, $CH_2OCHaHb$) 3.51–3.38 (m, 6H, $CH_2OCHaHb$, RCH_2CH_2O, CHNH, OHa, OHb), 1.61–1.50 (m, 2H, RCH_2CH_2O), 1.39–1.21 (m, 8H, $CH_3CH_2CH_2CH_2CH_2$), 0.91–0.85 (m, 12H, CH_3, $(CH_3)_3C$), 0.10 (s, 6H, CH_3Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 76.3, 75.8, 74.0, 72.7, 71.9, 66.2, 61.3, 32.0, 29.8, 29.4, 26.3, 26.0, 22.9, 18.3, 15.6. 14.4, −4.1, −4.5. $C_{19}H_{39}NO_5Si$ (389.26): C 58.57, H 10.09, N 3.60; found C 58.75, H 10.35, N 3.71.

11b: Yield 73% ^1H NMR (400 MHz, $CDCl_3$) δ: 6.37 (s, 1H, NHCO), 4.31 (m, 2H, COCHOH, CHOTBS),3.92–3.30 (m, 9H, CHOH, CHOH, CHNH, CH_2OCH_2), 1.74–1.58 (m, 5H, cyclohexyl), 1.51–1.40 (m, 2H, CH_2CH_2O), 1.38–1.09 (m, 4H, cyclohexyl), 0.88 (m, 11H, $(CH_3)_3$, cyclohexyl), 0.10 (s, 6H, $(CH_3)_2Si$). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 174.5, 75.6, 75.5, 73.7, 71.4, 70.5, 61.2, 37.0, 34.7, 33.6, 33.4, 29.8, 26.6, 26.3, 26.3, 25.8, 18.1, −4.3, −4.6. $C_{20}H_{39}NO_5Si$ (401.26): C 59.81, H 9.79, N 3.49; found C 60.15, H 9.98, N 3.59.

11c: Yield 63% ^1H NMR (400 MHz, DMSO-d_6) δ: 7.52 (s, 1H, NHCO), 7.29–7.19 (m, 2H, phenyl), 7.21–7.10 (m, 3H, phenyl), 5.47 (d, 1H, OH, J = 5.3 Hz), 5.32 (d, 1H, OH, J = 6.4 Hz), 4.0–3.94 (m, 1H, COCHOH), 3.92–3.85 (m, 1H, CHOTBS), 3.82 (dd, 1H, CHOH, J_1 = 7.1, J_2 = 5.3 Hz), 3.45–3.35 (m, 4H, OCHaHb, CH_2O), 3.17 (dd, 1H, CHNH, J_1 = 6.9, J_2 = 2.0 Hz), 2.60 (t, 2H, $PhCH_2$, J = 7.07 Hz), 1.83–1.71 (m, 2H, $PhCH_2CH_2CH_2O$), 0.85 (s, 9H, $(CH_3)_3$), 0.04 (s, 3H, CH_3Si), 0.03 (s, 3H, CH_3Si). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 173.6, 141.8, 128.3, 128.3, 125.7, 76.2, 72.9, 72.3, 70.7, 69.8, 58.8, 31.8, 31.1, 25.8, 18.0, −4.4, −4.8. $C_{21}H_{35}NO_5Si$ (409.23): C 59.81, H 9.79, N 3.49; found C 60.15, H 9.98, N 3.59.

*4.9. (2S,3R,4R)-2-[(R)-2-(heptyloxy)-1-hydroxyethyl]-3,4-dihydroxypyrrolidin-1-ium chloride **2a**, (2S,3R,4R)-2-[(R)-2-(2-cyclohexylethoxy)-1-hydroxyethyl]-3,4-dihydroxypyrrolidin-1-ium chloride **2b** and (2S,3R,4R)-2-[(R)-1-hydroxy-2-(3-phenylpropoxy) ethyl]-3,4-dihydroxypyrrolidin-1-ium chloride **2c***

To a solution of **11a-c** (1 mmol) in dry tetrahydrofuran (4.34 mL) was added BH_3-DMS (5 mmol, 0.15 mL) at 0 °C. The reaction mixture was stirred under nitrogen at reflux temperature for 5 h and then at room temperature until complete consumption of the substrate (TLC monitoring); the reaction was quenched by cautiously adding 1,5 mL of hydrogen chloride 1 M. The mixture was refluxed for 2 h and after cooling, was concentrated to dryness. The crude was coevaporated 4 times with a solution of methanol/hydrogen chloride 37% (1:1). The product was washed 3 times with hexane removing the supernatant and the solid residue was crystallized from methanol/water 90:10 affording **2a-c** as HCl salt.

2a: Yield: 71%; $[\alpha]_D^{28}$ +24.1 (c 0.35, DMSO); ^1H NMR (400 MHz, MeOD) δ: 7.70–7.50 (m, 1H, NH), 4.35–4.25 (m, 1H, CHOH), 4.22–4.10 (m, 1H, CHOH), 4.05–3.95 (m, 1H, CHOH), 3.70–3.20 (m, 10H, CH_2OCH_2, CHN, 3 OH, CH_2N) 1.75–1.50 (m, 2H, alkyl chain), 1.30–1.15 (m, 8H, alkyl chain), 0.80 (t, 3H, J = 6.9 Hz, alkyl chain). ^{13}C NMR (101 MHz, MeOD) δ: 75.2, 74.9, 71.9, 71.5, 68.2, 67.1, 50.6, 31.5, 29.1, 28.8,

25.6, 22.2, 12.9. $C_{13}H_{28}ClNO_4$ (297.17): C 52.43, H 9.48, N 4.70; found C 52.75, H 9.85, N 4.87.

2b: Yield: 68%; $[\alpha]_D^{28}$ +18.2 (c 0.58, DMSO); ^{1}H NMR (400 MHz, MeOD) *δ*: 4.35–4.25 (m, 1H, CHOH), 4.23–4.14 (m, 1H, CHOH), 4.05–3.95 (m, 1H, CHOH), 3.75–3.40 (m, 8H, CH_2OCH_2, CHN, 3 OH), 3.32–3.25 (m, 2H, CH_2N), 1.80–1.55 (m, 5H, cyclohexyl), 1.42 (q, 2H, CH2CH2O, J = 7.0 Hz), 1.30–1.10 (m, 1H, cyclohexyl), 1.25–1.05 (m, 3H, cyclohexyl), 1.05–0.79 (m, 2H, cyclohexyl). ^{13}C NMR (101 MHz, MeOD) *δ*: 75.2, 74.8, 71.9, 69.4, 68.0, 67.1, 50.6, 36.6, 34.3, 33.0, 33.0, 26.2, 25.9. $C_{14}H_{28}ClNO_4$ (309.17): C 54.27, H 9.11, N 4.52; found C 54.61, H 9.48, N 4.68.

2c: Yield 75%; $[\alpha]_D^{28}$ +16.3 (c 0.31, DMSO); ^{1}H NMR (400 MHz, MeOD) *δ*: 7.68–7.47 (m, 1H, NH) 7.40–7.30 (m, 2H, phenyl), 7.29–7.10 (m, 3H, phenyl), 4.37–4.28 (m, 1H, CHOH), 4.22–4.15 (m, 1H, CHOH) 4.08–3.96 (m, 1H, CHOH) 3.72–3.40 (m, 8H, CH_2OCH2, CHN, 3OH), 3.32–3.29 (m, 2H, CH_2NH) 2.82 (t, 2H, J = 6.5 Hz, CH_2Ph) 1.98–1.80 (m, 2H, CH_2CH_2O). ^{13}C NMR (101 MHz, MeOD) *δ*: 130.4, 129.7, 128.9, 127.9, 127.2, 126.6, 75.2, 74.9, 71.9, 70.5, 68.0, 67.1, 50.7, 40.7, 31.0. $C_{15}H_{24}ClNO_4$ (317.14): C 52.43, H 9.48, N 4.70; found C 52.75, H 9.85, N 4.87.

4.10. Cell lines

The human monocytoid cell line U937 and the human oral squamous cell carcinoma of the head and neck (OSCC) cell line CAL-27 were grown in RPMI Medium (Corning, Turin, Italy) supplemented with 10% heat-inactivated fetal bovine serum (FBS; SIGMA), 50 U/ml penicillin, and 50 U/mL streptomycin (Hyclone), at 37 °C, in humidified atmosphere with 5% CO_2. The compounds were solubilized in DMSO, aliquoted at a final concentration of 0.5 M and stored at −20 °C until use.

4.10.1. MTS and trypan blue exclusion assays

Metabolic inhibition was evaluated by MTS assay. Inhibition of cell metabolic activity was detected through formazan product formation, using a commercial colorimetric kit MTS (Cell Titer 96 Aqueous One Solution; Promega). The assay was performed by seeding 5×10^4 U937 cells and 5×10^3 CAL-27 in 100 mL/well of culture medium in 96 wells plate in triplicate in absence or in presence of different concentrations of the different compounds, for 48 h. At the end of incubation 20 μL of MTS reagent was added directly to culture wells, samples were incubated for 1 h, and the absorbance subsequently was read at 490 nm.

4.10.2. Cytotoxic assay

The effect of compounds on cell viability of the U-937 cell line was assessed by evaluating dead and living cells, respectively, using the trypan blue dye exclusion test, after 48 h of culture. The U-937 cells were seeded in duplicate at 5×10^4/well in absence or presence of 1000, 100 and 10 μM of the different compounds. After the incubation time, cells were stained with a colorant selective for dead cells, trypan blue diluted 1:5 in phosphate buffered saline. The viable cells were counted and expressed as millions of viable cells per milliliter.

4.10.3. Statistical analysis

The values of CC_{50} and R square were obtained using the GraphPad Prism v.6 software (GraphPad Software, Inc., La Jolla, CA, USA).

4.11. Yeast cell culture

The yeast strain used in this study is *Saccharomyces cerevisiae* strain W303 (MATa leu2–3,112, trp1–1, can1–100, ura3–1, ade2–1, his3–11,15). The growth test was described in [55] briefly, a pre-culture of yeast strain was grown in YPD, a culture-rich medium (1% bacto-peptone, 1% yeast extract and 2% glucose), for 24 h, then tubes with 2 mL of YPD liquid medium were inoculated and the compounds were added at the final concentration of 400 μM. After an overnight growth, the cells were counted, and serial dilutions (1×10^7, 1×10^6, 1×10^5, 1×10^4, 1×10^3/mL) were spotted on YPD plates and grown at 28 °C for 24 h.

4.11.1. Microscopy yeast cells

Cells were grown in YPD supplemented with adenine at 28 °C for 24 h. Aliquots of cells were immediately fixed and 4′,6-diamidino-2-phenylindole (DAPI) was added until a final concentration of 2.5 μg/mL was reached. Cells were visualized with fluorescence microscopy. Cells were observed with a Zeiss Axio Imager Z1 Fluorescence Microscope with AxioVision 4.8 Digital Image Processing System, the objective lens used was 63 × Oil. Filter sets: 38HE (GFP), 43HE (DsRed). Filters for DAPI (365-nm excitation and 445/450-nm emission), were used Metamorph software (Universal Imaging, West Chester, PA) was used to deconvolute Z-series and treat the images [44,45].

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

[1] A.E. Stütz, Iminosugars as Glycosidase Inhibitors. Nojirimycin and beyond, Wiley-VCH Verlag GmbH, Weinheim-Germany, 1999, pp. 8–14.
[2] a) R.J. Nash, A. Kato, C.Y. Yu, G.W.J. Fleet, Iminosugars as therapeutic agents: recent advances and promising trends, Future Med. Chem. 3 (2011) 1513–1521, https://doi.org/10.4155/fmc.11.117;
b) G. Horne, F.X. Wilson, J. Tinsley, D.H. Williams, R. Storer, Iminosugars past, present and future: medicines for tomorrow, Drug Discov. Today 16 (2011) 107–118, https://doi.org/10.1016/j.drudis.2010.08.017.
[3] a) V.K. Harit, N.G. Ramesh, Amino-functionalized iminocyclitols: synthetic glycomimetics of medicinal interest, RSC Adv. 6 (2016) 109528–109607, https://doi.org/10.1039/C6RA23513A;
b) N.G. Ramesh, Iminosugars, in: V.K. Tiwari (Ed.), Carbohydrates in Drug Discovery and Development, Elsevier Inc., The Netherland, 2020, pp. 331–381, https://doi.org/10.1016/B978-0-12-816675-8.00008-7.
[4] P. Compain, O.R. Martin, Iminosugars: from Synthesis to Therapeutic Applications, John Wiley and Sons, Ltd, England, 2007, p. 1.
[5] H. Paulsen, Preparation of 5-Acetamido-5-deoxy-D-xylopiperidinose, 597-597, Angew. Chem. 1 (1962), https://doi.org/10.1002/anie.196205971.
[6] J.K. Jones, J.C. Turner, 5-Acetamido-5-deoxy-L-arabinose: a sugar derivative containing nitrogen as the hetero-atom in the ring, J. Chem. Soc. (1962) 4699–4703, https://doi.org/10.1039/JR9620004699.
[7] H. Stephen, T.H. Haskel, Synthesis of 5-Acetamido-5-deoxypentoses. sugar derivatives containing nitrogen in the ring, J. Org. Chem. 28 (1963) 2604–2610. https://pubs.acs.org/doi/abs/10.1021/jo01045a029.
[8] H. Paulsen, K. Todt, Cyclic monosaccharides having nitrogen or sulfur in the ring, Adv. Carbohydr. Chem. 23 (1968) 115–232, https://doi.org/10.1016/S0096-5332(08)60169-1.
[9] S. Inouye, T. Tsuruoka, T. Niida, The structure of nojirimycin, a piperidinose sugar antibiotic, J. Antibiot. (1966) 288–292.
[10] S. Inouye, T. Tsuruoka, Y. Koaze, T. Niida, Structure and synthesis of nojirimycin, Tetrahedron 24 (1968) 2125–2144, https://doi.org/10.1016/0040-4020(68)88115-3.
[11] N. Asano, R.J. Nash, R.J. Molyneux, G.W.J. Fleet, Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and prospects for therapeutic application, Tetrahedron Asymmetry 11 (2000) 1645–1680, https://doi.org/10.1016/S0957-4166(00)00113-0.
[12] P. Compain, O.R. Martin, Design, synthesis and biological evaluation of iminosugar-based glycosyltransferase inhibitors, Curr. Top. Med. Chem. 3 (2003) 541–560, https://doi.org/10.2174/1568026033452474.
[13] H. Moriyama, T. Tsukida, Y. Inoue, K. Yokota, K. Yoshino, H. Kondo, N. Miura, S. I. Nishimura, Azasugar-based MMP/ADAM inhibitors as antipsoriatic agents, J. Med. Chem. 47 (2004) 1930–1938, https://doi.org/10.1021/jm0304313.

[14] Y. Suzuki, S. Ichinomiya, M. Kurosawa, M. Ohkubo, H. Watanabe, H. Iwasaki, J. Matsuda, Y. Noguchi, K. Takimoto, M. Itoh, M. Tabe, M. Iida, T. Kubo, S. Ogawa, E. Nanba, K. Higaki, K. Ohno, R.O. Brady, Chemical chaperone therapy: clinical effect in murine GM1-gangliosidosis. NOEV - 5, Ann. Neurol. 62 (2007) 671–675, https://doi.org/10.1002/ana.21284.

[15] M.-J.R. Papandréeou, R. Barbouche, M.P.K. Guieu, T. Fenouille, The α-glucosidase inhibitor 1-deoxynojirimycin blocks human immunodeficiency virus envelope Glycoprotein-mediated membrane fusion at the CXCR4 binding step, Mol. Pharmacol. 61 (2002) 186–193, https://doi.org/10.1124/mol.61.1.186.

[16] T.M. Gloster, G.J. Davies, Glycosidase inhibition: assessing mimicry of the transition state, Org. Biomol. Chem. 8 (2010) 305–320, https://doi.org/10.1039/B915870G.

[17] M.P. Elías-Rodríguez, E. Moreno-Clavijo, S. Carrión-Jiménez, A.T. Carmona Asenjo, A.J. Moreno-Vargas, I. Caffa, F. Montecucco, M. Cea, A. Nencioni, I. Robina Ramirez, Synthesis and cancer growth inhibitory activities of 2-fatty-alkylated pyrrolidine-3,4-diol derivatives, Arkivoc 3 (2014) 197–214, https://doi.org/10.3998/ark.5550190.p008.492.

[18] T.D. Butters, R.A. Dwek, F.M. Platt, Inhibition of glycosphingolipid biosynthesis: application to lysosomal storage disorders, Chem. Rev. 100 (2000) 4683–4696, https://doi.org/10.1021/cr990292q.

[19] H.S. Overkleeft, G.H. Renkema, J. Neele, P. Vianello, I.O. Hung, A. Strijland, A. M. Van Der Burg, G.-J. Koomen, U.K. Pandit, J.M. Aerts, Generation of specific deoxynojirimycin-type inhibitors of the non-lysosomal glucosylceramidase, J. Biol. Chem. 273 (1999), 26522, https://doi.org/10.1074/jbc.273.41.26522.

[20] H.R. Mellor, F.M. Platt, R.A. Dwek, T.D. Butters, Membrane disruption and cytotoxicity of hydrophobic N-alkylated imino sugars is independent of the inhibition of protein and lipid glycosylation, Biochem. J. 374 (2003) 307–314, https://doi.org/10.1042/bj20030348.

[21] A. Mehta, S. Ouzounov, R. Jordan, E. Simsek, X. Lu, R.M. Moriarty, G. Jacob, R. A. Dwek, T.M. Block, Imino sugars that are less toxic but more potent as antivirals, in vitro, compared with N-n-nonyl DNJ, Antivir. Chem. Chemother. 13 (2002) 299–304, https://doi.org/10.1177/095632020201300505.

[22] N. Asano, H. Kizu, K. Oseki, E. Tomioka, K. Matsui, M. Okamoto, M. Baba, N-Alkylated nitrogen in the ring sugars: conformational basis of inhibition of glycosidases and HIV-1 replication, J. Med. Chem. 38 (1995) 23–49, https://doi.org/10.1021/jm00013a012.

[23] a) A. Mehta, N. Zitzmann, P.M. Rudd, T.M. Block, R.A. Dwek, α-Glucosidase inhibitors as potential broad based anti-viral agents, FEBS Lett. 430 (1998) 17–22, https://doi.org/10.1016/S0014-5793(98)00525-0;
b) G. Godin, P. Compain, O.R. Martin, K. Ikeda, L. Yu, N. Asano, α-1-C-Alkyl-1-deoxynojirimycin derivatives as potent and selective inhibitors of intestinal isomaltase: remarkable effect of the alkyl chain length on glycosidase inhibitory profile, Bioorg. Med. Chem. Lett 14 (2004) 5991–5995, https://doi.org/10.1016/j.bmcl.2004.09.086;
c) P.H. Liang, W.C. Cheng, Y.L. Lee, H.P. Yu, Y.T. Wu, Y.L. Lin, C.H. Wong, Novel five-membered iminocyclitol derivativesas selective and potent glycosidase inhibitors: new structures for antivirals and Osteoarthritis, Chembiochem 7 (2006) 165–173, https://doi.org/10.1002/cbic.200500321;
d) M. Padró, J.A. Castillo, L. Gómez, J. Joglar, P. Clapés, C. de Bolós, Cytotoxicity and enzymatic activity inhibition in cell lines treated with novel iminosugar derivatives, Glycoconj. J. 27 (2010) 277–285, https://doi.org/10.1007/s10719-009-9276-3.

[24] a) G.L. Zhang, C. Chen, Y. Xiong, L.H. Zhang, J. Ye, X.S. Ye, Synthesis of N-substituted iminosugar derivatives and their immunosuppressive activities, Carbohydr. Res. 345 (2010) 780–786, https://doi.org/10.1016/j.carres.2010.01.021;
b) T. Castellan, C. Santos, F. Rodriguez, M.L. Lepage, Y. Liang, A. Bodlenner, P. Compain, Y. Génisson, C. Dehoux, S. Ballereau, N,O-Dialkyl deoxynojirimycin derivatives as CERT START domain ligands, Bioorg. Med. Chem. Lett 30 (2020), 126796, https://doi.org/10.1016/j.bmcl.2019.126796;
c) J. Désiré, Z. Debbah, D. Gueyrard, J. Marrot, Y. Blériot, A. Kato, Evaluation of nonnatural L-iminosugar C,C-glycosides, a new class of C-branched iminosugars, as glycosidase inhibitors, Carbohydr. Res. 532 (2023), 108903, https://doi.org/10.1016/j.carres.2023.108903.

[25] T. Cox, R. Lachmann, C. Hollak, J. Aerts, S. van Weely, M. Hrebícek, F. Platt, T. Butters, R. Dwek, C. Moyses, I. Gow, D. Elstein, A. Zimran, Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis, Lancet 355 (2000) 1481–1485, https://doi.org/10.1016/S0140-6736(00)02161-9.

[26] C. Parmeggiani, S. Catarzi, C. Matassini, G. D'Adamio, A. Morrone, A. Goti, P. Paoli, F. Cardona, Human acid β-glucosidase inhibition by carbohydrate derived iminosugars: towards new pharmacological chaperones for Gaucher disease, Chembiochem 16 (2015) 2054–2064, https://doi.org/10.1002/cbic.201500292.

[27] C. Matassini, J. Warren, B. Wang, A. Goti, F. Cardona, A. Morrone, M. Bols, Imino and azasugar protonation inside human acid β-glucosidase, the enzyme that is defective in Gaucher disease, Angew. Chem. Int. Ed. 59 (2020) 10466–10469, https://doi.org/10.1002/anie.202002850.

[28] F. Clemente, C. Matassini, C. Faggi, S. Giachetti, C. Cresti, A. Morrone, P. Paoli, A. Goti, M. Martínez-Bailén, F. Cardona, Glucocerebrosidase (GCase) activity modulation by 2-alkyl trihydroxypiperidines: inhibition and pharmacological chaperoning, Bioorg. Chem. 98 (2020), 103740, https://doi.org/10.1016/j.bioorg.2020.103740.

[29] M. Martínez-Bailén, A.T. Carmona, F. Cardona, C. Matassini, A. Goti, M. Kubo, A. Kato, I. Robina, A.J. Moreno-Vargas, Synthesis of multimeric pyrrolidine iminosugar inhibitors of human β-glucocerebrosidase and α-galactosidase A: first example of a multivalent enzyme activity enhancer for Fabry disease, Eur. J. Med. Chem. 192 (2020), 112173, https://doi.org/10.1016/j.ejmech.2020.112173.

[30] M.G. Davighi, F. Clemente, C. Matassini, A. Morrone, A. Goti, M. Martínez-Bailén, F. Cardona, Synthesis of "All-cis" trihydroxypiperidines from a carbohydrate-derived Ketone: hints for the design of new β-gal and GCase inhibitors, Molecules 25 (2020) 4526, https://doi.org/10.3390/molecules25194526.

[31] F. Clemente, C. Matassini, A. Goti, A. Morrone, P. Paoli, F. Cardona, Stereoselective synthesis of C-2 alkylated trihydroxypiperidines: novel pharmacological chaperones for Gaucher disease, ACS Med. Chem. Lett. 10 (2019) 621–626, https://doi.org/10.1021/acsmedchemlett.8b00602.

[32] M.D.P. Elías Rodríguez, E. Moreno Clavijo, S. Carrión Jiménez, A.T. Carmona Asenjo, A.J. Moreno Vargas, I. Caffa, I. Robina Ramírez, Synthesis and cancer growth inhibitory activities of 2-fatty-alkylated pyrrolidine-3,4-diol derivatives, Arkivoc 3 (2014) 197–214, https://doi.org/10.3998/ark.5550190.p008.492.

[33] N. Gueder, G. Allan, M.S. Telliez, F. Hague, J.M. Fernandez, E.M. Sanchez-Fernandez, C. Ortiz-Mellet, A. Ahidouch, H. Ouadid-Ahidouch, sp2-Iminosugar α-glucosidase inhibitor 1-C-octyl-2-oxa-3-oxocastanospermine specifically affected breast cancer cell migration through Stim1, β1-integrin, and FAK signaling pathways, J. Cell. Physiol. 232 (2017) 3631–3640, https://doi.org/10.1002/jcp.25832.

[34] M. De Angelis, C. Sappino, E. Mandic, M. D'Alessio, M.G. De Dominicis, S. Sannino, L. Primitivo, P. Mencarelli, A. Ricelli, G. Righi, Stereodivergent synthesis of piperidine iminosugars 1-deoxy-D-nojirimycin and 1-deoxy-D-altronojirimycin, Tetrahedron 79 (2021), 131837, https://doi.org/10.1016/j.tet.2020.131837.

[35] M. De Angelis, L. Primitivo, C. Lucarini, S. Agostinelli, C. Sappino, A. Ricelli, G. Righi, Stereocontrolled total synthesis of iminosugar 1,4-dideoxy-1,4-imino-D-iditol, Carbohydr. Res. 492 (2020) 108082–108088, https://doi.org/10.1016/j.carres.2020.108028.

[36] G. Righi, E. Mandic, C. Sappino, E. Dema, P. Bovicelli, Asymmetric routes toward polyhydroxylated pyrrolidines: synthesis of 1,4-dideoxy-1,4-imino-D-galactitol and 1,4-dideoxy-1,4-imino-D-glucitol, Carbohydr. Res. 435 (2016) 100–105, https://doi.org/10.1016/j.carres.2016.09.018.

[37] M. De Angelis, L. Primitivo, F. Lizzio, S. Agostinelli, C. Sappino, I. Ben Rondam, L. Bonanni, A. D'Annibale, R. Antonioletti, A. Ricelli, G. Righi, Total stereocontrolled synthesis of a novel pyrrolizidine iminosugar, Carbohydr. Res. 511 (2022) 108484–108492, https://doi.org/10.1016/j.carres.2021.108484.

[38] a) T. Katsuki, K.B. Sharpless, The first practical method for asymmetric epoxidation, J. Am. Chem. Soc. 102 (1980) 5974–5976, https://doi.org/10.1021/ja00538a077;
b) B.E. Rossiter, T. Katsuki, K.B. Sharpless, Asymmetric epoxidation provides shortest routes to four chiral epoxy alcohols which are key intermediates in syntheses of methymycin, erythromycin, leukotriene C-1, and disparlure, J. Am. Chem. Soc. 103 (1981) 464–465, https://doi.org/10.1021/ja00392a038.

[39] a) H.C. Kolb, M.S. Van Nieuwenhze, K.B. Sharpless, Catalytic asymmetric dihydroxylation, Chem. Rev. 94 (1994) 2483–2547, https://doi.org/10.1021/cr00032a009;
b) G. Righi, P. Bovicelli, I. Tirotta, C. Sappino, E. Mandic', Double stereodifferentiation in the asymmetric dihydroxylation of optically active olefins, Chirality 28 (2016) 387–393, https://doi.org/10.1002/chir.22587;
c) G. Righi, P. Bovicelli, E. Mandic', G.C.M. Naponiello, I. Tirotta, Study on the stereochemical control of dihydroxylation of vinyl epoxides and their derivatives, Tetrahedron 68 (2012) 2984–2992, https://doi.org/10.1016/j.tet.2012.02.029.

[40] a) G. Righi, L. Salvati Manni, P. Bovicelli, R. Pelagalli, A new, simple and mild azidolysis of vinylepoxides, Tetrahedron Lett. 52 (2011) 3895–3896, https://doi.org/10.1016/j.tetlet.2011.05.085;
b) C.H. Beherens, K.B. Sharpless, Selective transformations of 2,3-epoxy alcohols and related derivatives. Strategies for nucleophilic attack at carbon-3 or carbon-2, J. Org. Chem. 50 (1985) 5696–5704, https://doi.org/10.1021/JO00350A051;
c) N. Di Blasio, M.T. Lopardo, P. Lupattelli, 1,2-diarylethanols by alternative regioselective reductive ring-opening of 2,3-diaryloxyranes, Eur. J. Org. Chem. 6 (2009) 938–944, https://doi.org/10.1002/ejoc.200800992.

[41] W.R. Roush, J.A. Straub, M.S. van Nieuwenhze, A stereochemically general synthesis of 2-deoxyhexoses via the asymmetrically allylboration of 2,3-epoxy aldehydes, J. Org. Chem. 56 (1991) 1636–1648, https://doi.org/10.1021/jo00004A053.

[42] A. Cirigliano, A. Amelina, B. Biferali, A. Macone, C. Mozzetta, M.M. Bianchi, M. Mori, B. Botta, E. Pick, R. Negri, T. Rinaldi, Statins interfere with the attachment of S. cerevisiae mtDNA to the inner mitochondrial membrane, J. Enzym. Inhib. Med. Chem. 35 (2019) 129–137, https://doi.org/10.1080/14756366.2019.1687461.

[43] L. Botta, S. Filippi, C. Zippilli, S. Cesarini, B.M. Bizzarri, A. Cirigliano, T. Rinaldi, A. Paiardini, D. Fiorucci, R. Saladino, R. Negri, P. Benedetti, Artemisinin derivatives with antimelanoma activity show inhibitory effect against human DNA topoisomerase 1, ACS Med. Chem. Lett. 11 (2020) 1035–1040, https://doi.org/10.1021/acsmedchemlett.0c00131.

[44] L. Bramasole, A. Sinha, D. Harshuk, A. Cirigliano, G. Sylvia, Z. Yu, R.L. Carmeli, M. H. Glickman, T. Rinaldi, E. Pick, The proteasome lid triggers COP9 signalosome activity during the transition of Saccharomyces cerevisiae cells into quiescence, Biomolecules 9 (2019) 449, https://doi.org/10.3390/biom9090449.

[45] D. Ottaviano, A. Montanari, L. De Angelis, R. Santomartino, A. Visca, L. Brambilla, T. Rinaldi, C. Bello, M. Reverberi, M.M. Bianchi, Unsaturated fatty acids-dependent linkage between respiration and fermentation revealed by deletion of hypoxic regulatory KlMGA2 gene in the facultative anaeroberespiratory yeast Kluyveromyces lactis, FEMS Yeast Res. 15 (2015) fov028, https://doi.org/10.1093/femsyr/fov028.