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Tuning of β-glucosidase and α-galactosidase inhibition by generation and *in situ* screening of a library of pyrrolidine-triazole hybrid molecules

Macarena Martínez-Bailén,[a] Ana T. Carmona,[a] Elena Moreno-Clavijo,[a] Inmaculada Robina,[a,*] Daisuke Ide,[b] Atsushi Kato[b] and Antonio J. Moreno-Vargas[a,*]

[a]*Department of Organic Chemistry, Faculty of Chemistry, University of Seville, C/Prof. García González, 1, 41012-Seville, Spain.* [b]*Department of Hospital Pharmacy, University of Toyama, Toyama 930-0194, Japan. e-Mail: robina@us.es, ajmoreno@us.es*

Abstract

The preliminary screening of two libraries of epimeric (pyrrolidin-2-yl)triazoles (**14a-s** and **22a-s**), generated *via* click chemistry, allowed the rapid identification of four α-galactosidase (coffee beans) inhibitors (**22b**,**k**,**p**,**r**) and two β-glucosidase (almond) inhibitors (**14b**,**f**) in the low μM range. The additional biological analysis of **14b**,**f** towards β-glucocerebrosidase (human lysosomal β-glucosidase), as target enzyme for Gaucher disease, showed a good correlation with the inhibition results obtained for the plant (almond) enzyme. Surprisingly, although these compounds showed inhibition towards β-glucocerebrosidase as acid hydrolase, they did not inhibit bovine liver β-glucosidase as neutral hydrolase. In contrast to what was observed for β-glucosidase inhibition, the coffee beans α-galactosidase inhibitors of the epimeric library (**22b,k,p,r**) only showed weak inhibition towards human lysosomal α-galactosidase.

Keywords: iminosugars; glycosidase inhibitors; pyrrolidines; triazole; click reaction.

Introduction

Iminosugars, carbohydrate mimics containing a nitrogen atom instead of an oxygen in the ring system, constitute the most important class of glycosidase inhibitors.[1] Among them, the search for β-glucosidase and α-galactosidase inhibitors is attracting the attention of many researchers of the area in the last fifteen years due to the potential use of these compounds in the therapy of two lysosomal storage diseases (LSDs), Gaucher and Fabry disease.[2] In the case of Gaucher disease, surprisingly, some competitive lysosomal β-glucosidase (β-glucocerebrosidase) inhibitors at sub-inhibitory concentrations can act as pharmacological chaperones enhancing the activity of deficient β-glucocerebrosidase in cultured fibroblasts from patients with this disease, thus hydrolyzing the excess of β-glucosides stored in the lysosome.[3] A similar effect was observed for some competitive lysosomal α-galactosidase (α-galactosidase A) inhibitors in the Fabry disease.[4]

Unfortunately, after the enormous effort made in the last fifteen years in the search for pharmacological chaperones in LSDs, only 1-deoxygalactonojirimycin (DGJ, Galafold®) has reached all the clinical trials and has recently been approved by the European Commission as a drug for Fabry disease.[5] The most common problems found in the search for drug candidates for these diseases are related with the cell and ER permeability,[2] and with the lack of selectivity that could result in considerable side-effects in the clinic.[6] Thus, the search of low-molecular-

weight iminosugars with lipophilic substituents able to cross the cell membranes and selectively inhibit glycosidases remains as an interesting goal.

The generation and *in situ* biological screening of a library of glycosidase inhibitors is an interesting strategy for the rapid discovery of potent inhibitors. The choice of the key reaction for the generation of the library is crucial for the success of the strategy. Reactions that afford products in high (or quantitative) yields and do not generate by-products that could interfere in the further *in situ* biological analysis, are the most appropriate. In this regard, click reactions largely fulfill these requirements. This strategy has been previously explored by several authors using different reactions to generate libraries, such as reactions between an aminomethyl pyrrolidine and a set of aldehydes or acids leading to imines[7] or amides,[8] respectively. The combination of the well-known copper catalyzed azide alkyne cycloaddition (CuAAC), the most typical click reaction, and the *in situ* biological screening has been broadly used in the discovery of a wide range of bioactive molecules.[9] However this strategy has been scarcely explored in the glycosidase inhibition field. As far as we are aware, only two examples have been reported in the last years. The first one used this methodology for the search of aminocyclitol derived compounds as β-glucocerebrosidase inhibitors.[10] The second one was reported by our research group for the search of α-L-fucosidase inhibitors.[11] With this methodology we have been able to find one of the most potent α-L-fucosidase inhibitors containing a pyrrolidine iminosugar core. The results of this work, and other previous findings in our research,[12] demonstrate that the insertion of aromatic or heteroaromatic moieties into the pyrrolidine iminosugar core increases the selectivity and potency of the resulting inhibitor that, simultaneously, becomes more lipophilic. This structural feature is relevant for the therapeutic potential of pyrrolidine iminosugars. It circumvents the lack of selectivity in pyrrolidines as glycosidase inhibitors which can be attributed to their high conformational flexibility.

Based on our successful previous results, and following our interest in the search of new glycosidase inhibitors, we report herein the extension of this methodology for the rapid discovery of α-galactosidase and β-glucosidase inhibitors. We have used the CuAAC between epimeric azidomethyl pyrrolidines and a set of alkynes in combination with the *in situ* screening to choose the best triazole-derived aglycone for a five-membered iminosugar inhibitor. The new compounds are low-molecular-weight inhibitors containing a 3,4-dihydroxypyrrolidine core. This five-membered iminosugar core was chosen instead the six-membered one with the aim to preserve the inhibition activity but using a less hydrophilic iminosugar core that could facilitate the traffic of the inhibitor through the cell membranes. Additionally, the correlation of the inhibitory activity of the new compounds towards β-glucosidases and α-galactosidases of different sources (non-mammalian *vs* human lysosomal) will be also studied.

Results and discussion

Synthesis and selection of the appropriate pyrrolidine scaffolds.

The first step to successfully achieve our goal was the choice of an adequate pyrrolidine scaffold for the generation of the library. We planned to use 3,4-dihydroxypyrrolidines carrying a triazole moiety at C-2 as a model of non-complex small molecules that could act as potent

and selective inhibitor based on our previous results.[11] For this reason, we first synthesized and explored the biological activity of seven pyrrolidine-triazole hybrids (**1**-**4**, ***ent*-1**, ***ent*-2, 7**) in order to establish the adequate configuration of the pyrrolidine and the adequate distance between the pyrrolidine and the triazole moiety that generates the best inhibitors. The effect on the inhibitory activity provoked by the change of the triazole moiety for an amide or thiourea group (compounds **5** and **6**) was also analyzed.

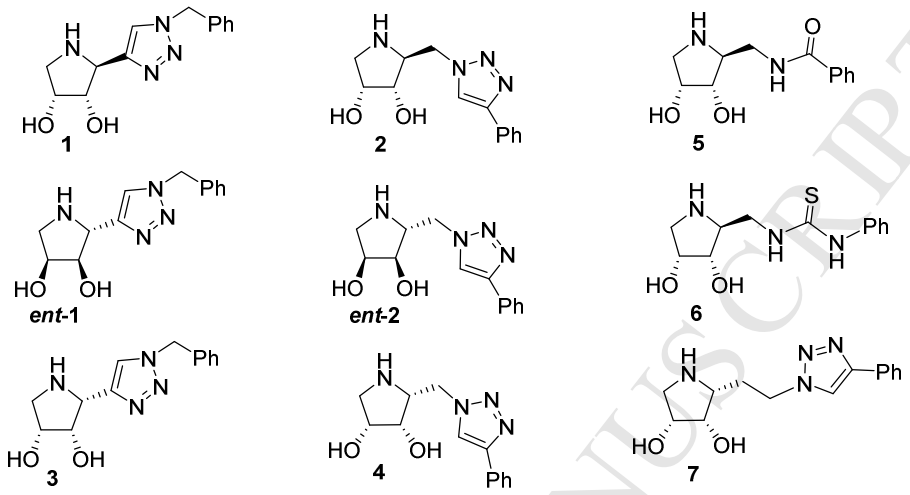
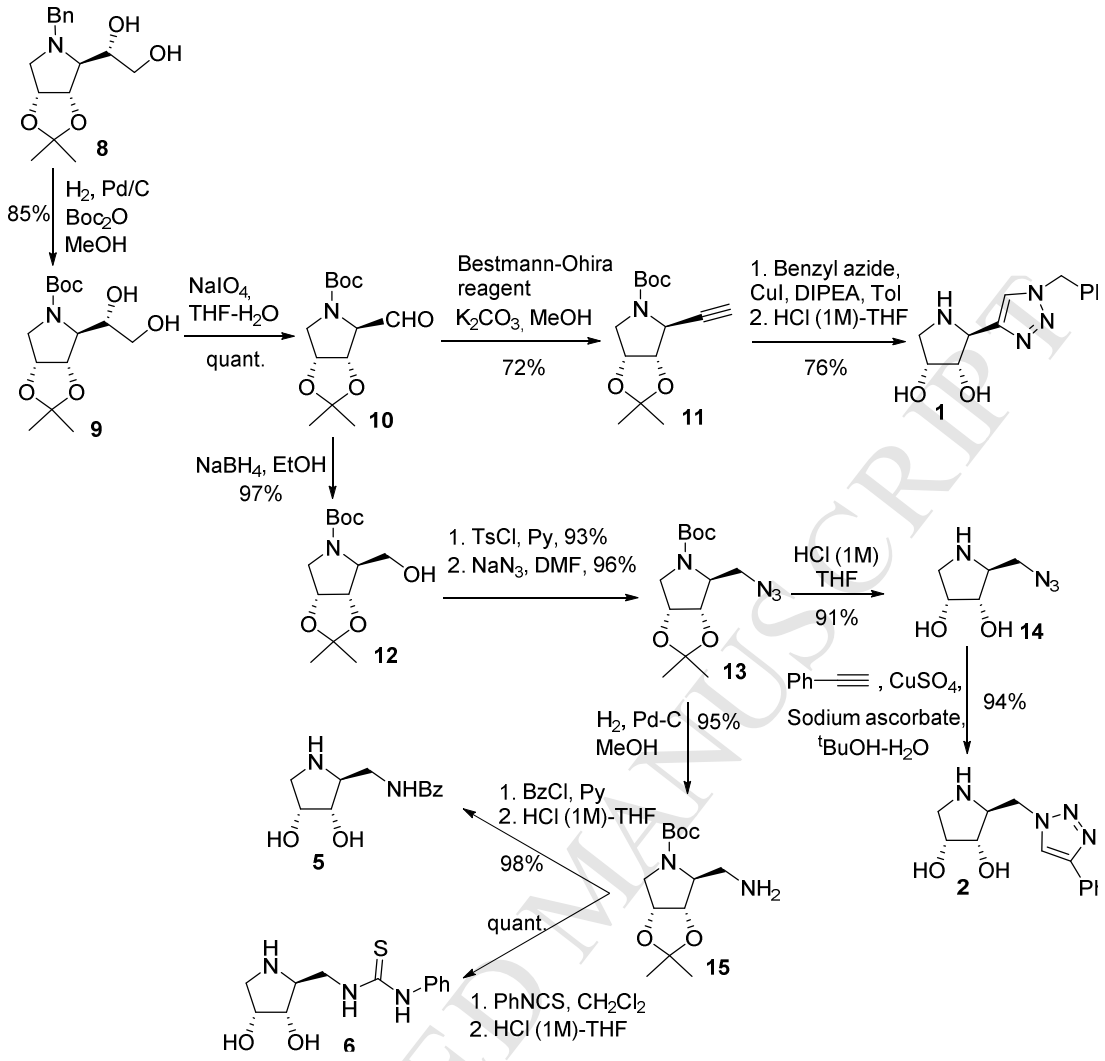


Figure 1. Pyrrolidine-triazole hybrid molecules and the amide/thiourea analogues synthesized for biological evaluation and scaffold selection.

For the preparation of pyrrolidine-triazoles **1** and **2**, we started from diol **8** that was previously prepared by Fleet from D-mannose.[13]

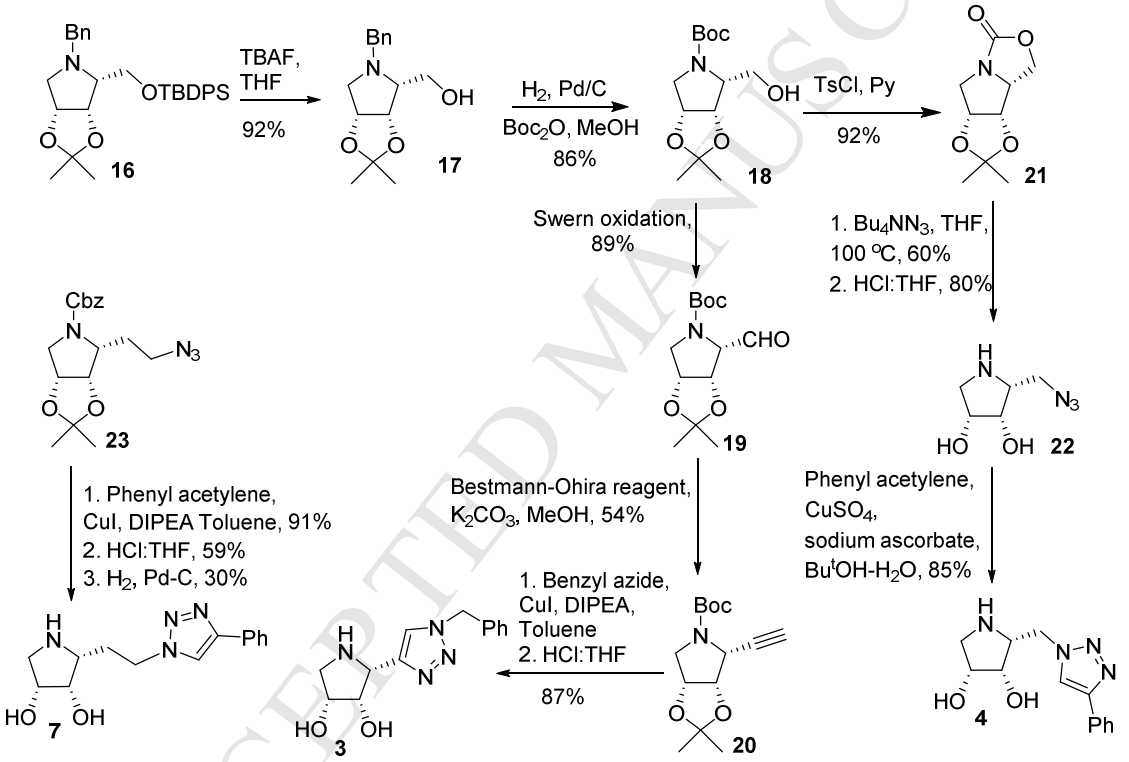


Scheme 1. Synthesis of pyrrolidine-triazoles **1** and **2**, pyrrolidine-amide **5** and pyrrolidine-thiourea **6**.

Protecting group manipulation of *N*-benzyl pyrrolidine **8** afforded *N*-Boc pyrrolidine **9** that was transformed into the aldehyde **10** by oxidative degradation of the diol chain. Reaction of **10** with Bestmann-Ohira reagent afforded alkyne **11** in moderate-to-good yield. Aldehyde **10** was also transformed into azidomethyl-pyrrolidine **13** through a conventional sequence of reactions.[14] Alkyne **11** reacted with benzyl azide under CuAAC conditions to afford triazole **1** after acidic deprotection. Azide derivative **13** was deprotected to give **14** which was transformed into triazole **2** by click reaction. Additionally, hydrogenation of azide **13** gave aminomethyl pyrrolidine **15** that was benzoylated to give **5** in excellent yield after acidic deprotection. Reaction of **15** with phenyl isothiocyanate followed by deprotection afforded **6** in good yield. Enantiomers *ent*-**1** and *ent*-**2** were prepared following the same methodology but starting from aldehyde *ent*-**10** (see Supplementary data, Scheme S1). The synthesis of this aldehyde was carried out, in this case, from commercial D-gulono-γ-lactone as was previously reported.[15]

The synthesis of **3** and **4** started from *N*,*O*-protected pyrrolidine **16** (Scheme 2) that was synthesized from L-ribose as described by Kim and coworkers.[16] Manipulation of protecting

groups in **16** gave alcohol **18**[17] which, after oxidation followed by reaction with Bestmann-Ohira reagent, afforded alkynyl pyrrolidine **20** in good yield. This compound was easily transformed into pyrrolidine-triazole **3** by CuAAC with benzyl azide followed by acidic deprotection. Attempts to synthesize the azidomethyl pyrrolidine **22** from **18** by standard tosylation followed by nucleophilic substitution with azide anion were unsuccessful. Instead, the carbamate **21**[17] was obtained after treatment with tosyl chloride. This carbamate showed high stability and the opening reaction leading to the corresponding azidomethyl derivative was difficult to achieve. Finally, carbamate **21** could be opened by reaction with tetrabutylammonium azide in a sealed tube at high temperature giving the azidomethyl pyrrolidine **22** with moderate-to-good yield, after acidic deprotection. Reaction of azide **22** with phenyl acetylene under CuAAC conditions gave pyrrolidine-triazole **4** in good yield. Besides, azide **23**, previously prepared by us,[18] was transformed into triazole **7** through CuAAC reaction and deprotection.



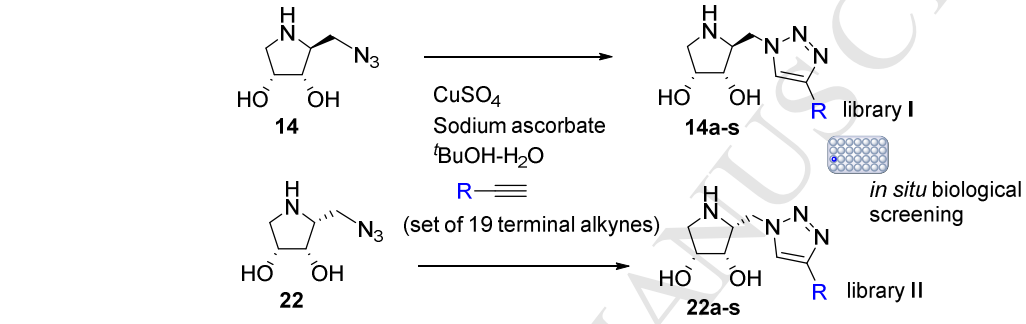
Scheme 2. Synthesis of pyrrolidine-triazoles **3**, **4** and **7**.

The analysis of the inhibition properties of compounds **1**-**7**, *ent*-**1** and *ent*-**2** against a panel of eleven commercial glycosidases was carried out (see Table S1 in Supplementary data, for details). This initial screening showed that compound **2** was a selective inhibitor of β-glucosidase from almond (IC_{50} = 8 μM) and compound **4** was a selective inhibitor of α-galactosidase from coffee beans (IC_{50} = 14 μM). Triazole **3** was a moderate and selective inhibitor of α-L-fucosidase from bovine kidney (IC_{50} = 168 μM). Amide **5** and thiourea **6** were moderate-to-weak inhibitors of β-glucosidase from almond and α-mannosidase from Jack beans. Triazoles **1**, *ent*-**1**, *ent*-**2** and **7** were weak and/or non-selective inhibitors of different glycosidases. Thus, we envisaged triazoles **2** and **4** as interesting leads for our study and we

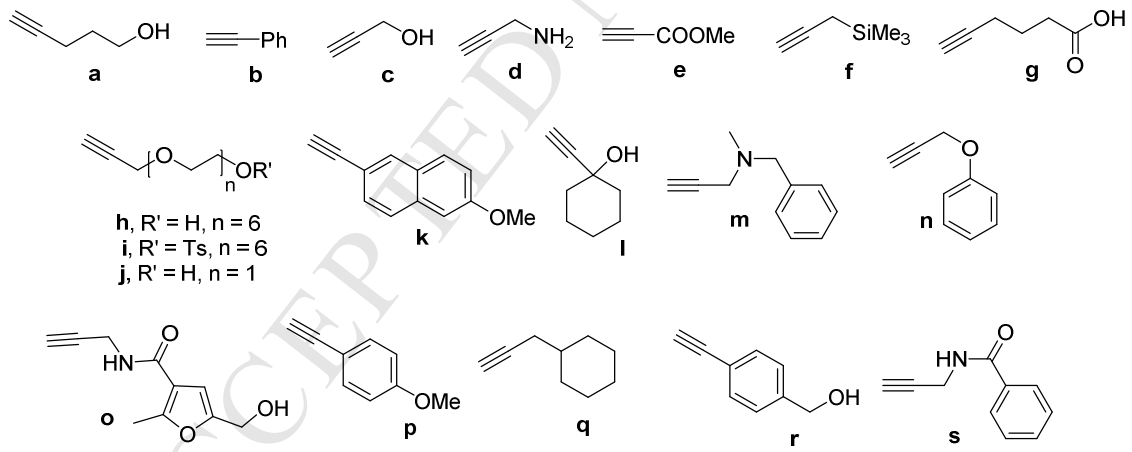
chose unprotected azides **14** (Scheme 1) and **22** (Scheme 2) as the appropriate scaffolds for the generation of the desired libraries.

Generation of two libraries of pyrrolidine-triazole hybrid molecules by parallel synthesis and *in situ* biological screening.

Epimeric azidomethyl pyrrolidines **14** and **22**, were reacted with 1.2 equiv.[19] of a set of commercial alkynes (**a**-**g**,**j**-**n**,**p**-**r**) and synthetic alkynes (**h**,**i**,**o**,**s**, see Supplementary data for synthetic details) under CuAAC conditions (0.068 equiv. $CuSO_4$/0.22 equiv. sodium ascorbate as catalyst in Bu^tOH-H_2O as solvent) in order to generate libraries **I** and **II**, as depicted in Scheme 3. After 24 h at room temperature, TLC and ESI-MS of the reaction mixtures showed complete conversion and the presence of the desired pyrrolidine-triazole derivatives (see Supplementary data for mass spectra analysis).



Terminal alkynes:



Scheme 3. Parallel synthesis of pyrrolidine-triazole libraries **I** and **II**.

The *in situ* screening of the resulting crude pyrrolidine-triazoles was carried out in a 96-well microtiter plate. In case of library **I**, the % inhibition of β-glucosidase from almond in a well containing 10 µM of each compound was measured (Figure 2). For library **II**, the inhibition assay was performed against α-galactosidase from coffee beans in a well containing 50 µM of each potential inhibitor (Figure 3). Blank experiments with $CuSO_4$, sodium ascorbate and each alkyne (**a**-**s**) were also carried out; none of these reagents showed any inhibition of the corresponding enzyme at the concentration of the experiments.

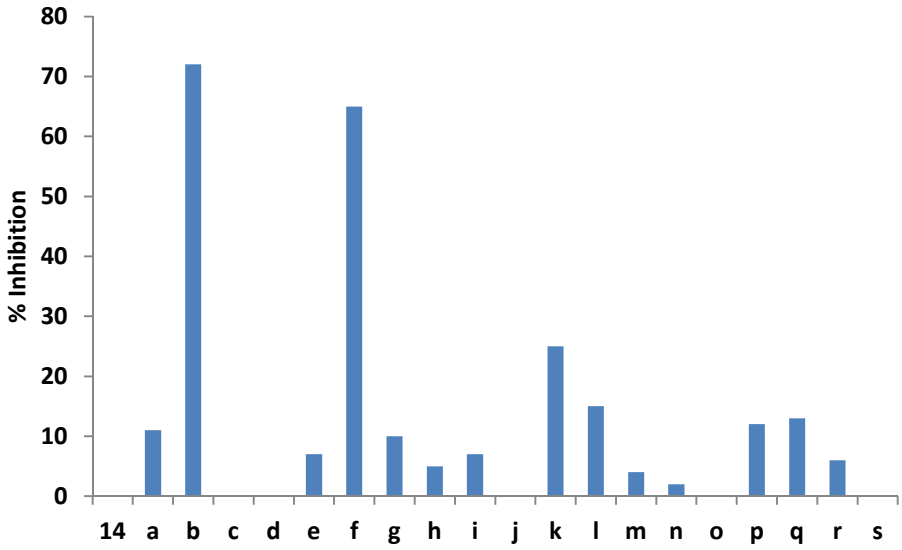


Figure 2. Biological screening of library **I**: % inhibition of β-glucosidase from almond at 10 μM of pyrrolidine-triazoles (pH 5, 37 ºC).

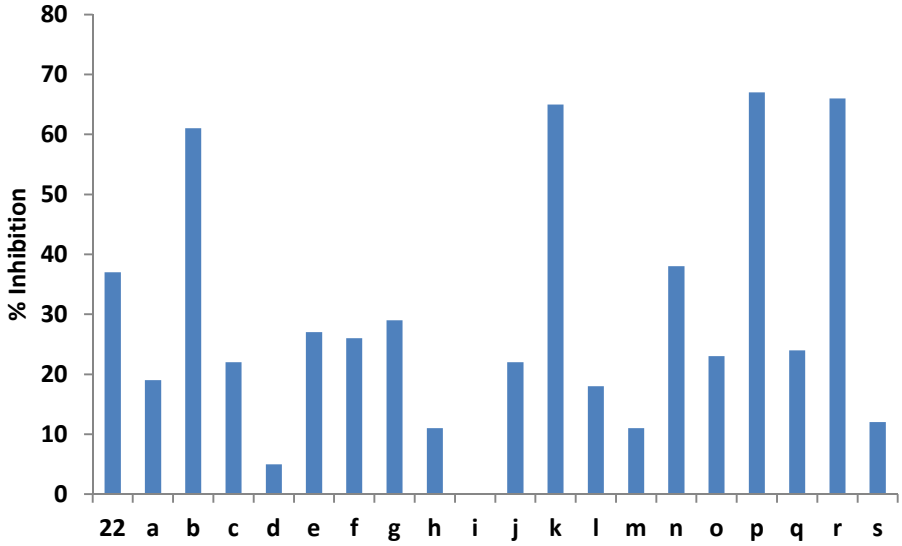


Figure 3. Biological screening of library **II**: % inhibition of α-galactosidase from coffee beans at 50 μM of pyrrolidine-triazoles (pH 6, 37 ºC).

The preliminary biological screening of library **I** showed two compounds with outstanding inhibitory activity, **14b** and **14f** (72 and 65% inhibition, respectively). It must be said that, by chance, compound **14b** had been already prepared and evaluated in the scaffold selection process (numbered as **2**, Figure 1) but **14f** was a new bioactive triazole derivative. The initial azidomethyl-pyrrolidine scaffold **14** was not inhibitor at 10 μM and the other seventeen triazoles of the library showed weak or no inhibition at this concentration, displaying the high influence of the C-4 substituent on the triazole ring. In the case of library **II**, the corresponding screening towards α-galactosidases showed four compounds with remarkable inhibition, compounds **22b**,**k**,**p**,**r** (62, 64, 67 and 66% inhibition, respectively). Compounds **22b**,**k**,**p**,**r** have in common an aromatic ring directly linked to the triazole moiety, being the only compounds in the library with this unique structural characteristic. In this case, the azidomethyl-pyrrolidine

scaffold **22** showed moderated-to-weak inhibition (38%) under the same conditions, being more active than the majority of the triazoles.

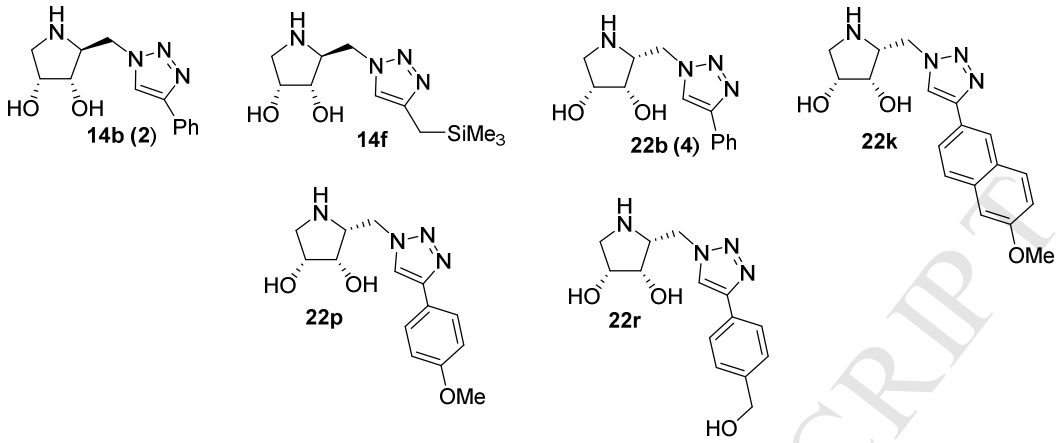


Figure 4. Structure of the most potent inhibitors detected by *in situ* screening.

The new bioactive triazoles selected from the libraries (Figure 4) were synthesized in a higher scale and purified by column chromatography in order to perform a more accurate inhibition study. Their inhibitory properties were studied towards twenty commercial glycosidases and the results are summarized in Table 1. As compounds **14b**,**f** and **22b**,**k,p,r** showed promising inhibition properties towards β-glucosidase from almond and α-galactosidase from coffee beans, respectively, human lysosomal enzymes and other mammalian enzymes were also included in this study.

Compound **14b**, that had shown good inhibition towards β-glucosidase from almonds, showed now an unusual specificity between two different mammalian β-glucosidases; this compound was a good inhibitor of β-glucocerebrosidase from human lysosome (IC_{50} = 11 μM) but did not inhibit β-glucosidase from bovine liver. Usually, a good inhibition of bovine liver β-glucosidase has been used as a preliminary parameter to select compounds for human lysosome β-glucocerebrosidase inhibition.[20] Significantly, this is one of the very few cases reported to date where the β-glucosidase inhibition pattern is unusual. The result for the inhibition of the non-mammalian enzyme (β-glucosidase from almond) correlates perfectly with those obtained in the human version. A similar behavior was observed for compound **14f**. The similarity on the inhibition data between **14b** and **14f** allows us to assume that the trimethylsilyl substituent in **14f** may interact with the enzyme in a similar way than the phenyl group in **14b**, in spite of their structural difference. These two compounds were specific β-glucosidase inhibitors, presenting only weak inhibition of β-galactosidase (bovine liver) and not inhibiting any other of the enzymes tested. Almond β-glucosidase is not fully sequenced but is deduced to belong to CAZy[21] family GH1 by limited sequence analysis.[22] The X-ray structure of human lysosomal beta-glucosidase has been reported and, on the basis of sequence similarity, this enzyme was classified as a member of the family GH30.[23] Both enzymes belong to the same clan fold, named GH-A. Bovine liver beta-glucosidase belongs to the same CAZy family and clan fold than almond beta-glucosidase. Thus, the three enzymes share three-dimensional structural similarities (similar clan fold) but the human one would have only limited sequence similarity with the others (different CAZy family). The level of selectivity detected in the inhibition of these enzymes by our compounds could be explained by decisive non-glycone allosteric

interactions close to the active site that should be more important than the glycone interactions.

Table 1. Concentration of pyrrolidine-triazole inhibitors giving 50% inhibition (IC_{50}) of a set of glycosidases.

Compound	**14b**	**14f**	**22b**	**22k**	**22p**	**22r**
Enzymes[1]						
β-glucosidase (almond)	8 μM	7 μM	NI	NI	NI	NI
β-glucosidase (bovine liver)	NI	NI	240 μM	NI	887 μM	888 μM
β-glucocerebrosidase (human lysosome)	11 μM	19 μM	28 μM	19 μM	31 μM	187 μM
α-galactosidase (coffee beans)	NI	NI	14 μM	17 μM	13 μM	14 μM
α-galactosidase A (human lysosome)	NI	NI	171 μM	NI	185 μM	159 μM
β-galactosidase (bovine liver)	458 μM	151 μM	173 μM	42 μM	338 μM	268 μM

NI: non inhibition (or less than 50% inhibition) at 1 mM of inhibitor.

[1]Non inhibition was detected towards these enzymes: α-L-fucosidase from bovine kidney, α-glucosidase from yeast and rice, β-galactosidases from *Escherichia coli* and *Aspergillus oryzae*, amyloglucosidases from *Aspergillus niger* and *Rhizopus sp*, α-mannosidase from Jack beans, β-mannosidase from snail, α,α-trehalase from porcine kidney, α-rhamnosidase from *Penicillium decumbens*, β-glucuronidases from *Escherichia coli* and bovine liver, and β-*N*-acetylglucosaminidase from Jack beans.

Compounds **22b**,**k**,**p**,**r** were less specific than the corresponding epimers in the inhibition assays. In general, they showed good inhibition towards α-galactosidase from coffee beans (IC_{50} = 14-17 μM) but the inhibition potency decreased 10-fold towards human lysosomal α-galactosidase A (IC_{50} = 159-185 μM), although both enzymes belong to the same CAZy family (GH27).[21] Surprisingly, human lysosomal β-glucocerebrosidase did not discriminate between epimers **14b** and **22b**, showing both compounds the same range of activity towards this enzyme. Moreover, **22b** showed weak inhibition towards other mammalian glycosidases, such as β-galactosidase and β-glucosidase from bovine liver. The presence of a bulkier group in the triazole substituent of compound **22k** led to an increase in the inhibition selectivity, reaching good inhibitory activity in α-galactosidase from coffee beans (IC_{50} = 17 μM) and human lysosomal β-glucocerebrosidase (IC_{50} = 19 μM). The inhibition pattern of compounds **22p** and **22r** was similar to that of analogue **22b**.

Conclusions

We have used two epimeric and structurally simple azidomethyl-pyrrolidines as scaffolds for the generation of two libraries of pyrrolidine-triazole glycosidase inhibitors. We have demonstrated that the biological analysis of the library can be performed *in situ* without purification and isolation of the individual compounds. This rapid preliminary analysis allowed us to select several β-glucosidase and α-galactosidase inhibitors as models for the assessment towards human glycosidases. The derivatives that showed the best inhibition towards β-glucosidase from almonds were also found to be inhibitors of the corresponding human enzyme. However this correspondence was not observed between the latter and the bovine liver enzyme. Although the new pyrrolidines described in this work do not contain a hydroxymethyl recognition motif on the skeleton, which is present in the major part of synthetic and natural pyrrolidine-derived glycosidase inhibitors,[24,25] they showed selective inhibition towards β-glucosidases or α-galactosidases. In this sense, compounds **14b**,**f** are the first examples of β-glucocerebrosidase inhibitors on the low micromolar range derived from a 3,4-dihydroxypyrrolidine. The absence of the hydroxymethyl group and the incorporation of an important hydrophobic component in these compounds may improve their cell and ER permeability for potential pharmacological applications. As far as we are aware, the interaction of a hydroxymethyl group, installed on other differently configured pyrrolidine iminosugars, with the Asp127 of the active site of human β-glucocerebrosidase has been recently reported.[26] We are currently working on the application of our method on other scaffolds, once we have already established the appropriate tool for the rapid assessment. In this way, we would be able to evaluate the convenience of the presence of that group in our systems.

Experimental part.

General methods

Optical rotations were measured in a 1.0 cm or 1.0 dm tube with a Jasco P-2000 spectropolarimeter. Infrared spectra were recorded with a Jasco FTIR-410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX300, AV300, AV500 and AVIII500 for solutions in $CDCl_3$, CD_3OD and DMSO-d_6 at room temperature except when indicated. δ are given in ppm and *J* in Hz. All the assignments were confirmed by COSY and HSQC experiments. Mass spectra (CI and ESI) were recorded on Micromass AutoSpeQ and QTRAP spectrometers. NMR and mass spectra were registered in CITIUS (University of Seville). TLC was performed on silica gel HF_{254} (Merck), with detection by UV light charring with H_2SO_4, *p*-anisaldehyde, vanillin, ninhydrin, $KMnO_4$ or with Pancaldi reagent [$(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O]. Silica gel 60 (Merck, 63–200 μm) was used for preparative chromatography.

Inhibition studies with commercial enzymes

The % of inhibition towards the corresponding glycosidase was determined in the presence of 1 mM of the inhibitor on the well. Each enzymatic assay (final volume 0.12 mL) contains 0.01 to 0.5 units/mL of the enzyme and 10 mM aqueous solution of the appropriate *p*-nitrophenyl glycopyranoside (substrate) buffered to the optimal pH of the enzyme. Enzyme and inhibitor were preincubated for 5 min at rt, and the reaction started by addition of the substrate. After

20 min of incubation at 37 °C, the reaction was stopped by addition of 0.1 mL of sodium borate solution (pH 9.8). The *p*-nitrophenolate formed was measured by visible absorption spectroscopy at 405 nm. Under these conditions, the *p*-nitrophenolate released led to optical densities linear with both reaction time and concentration of the enzyme. For the best inhibitors (% inhibition ≥ 80), the IC_{50} value (concentration of inhibitor required for 50% inhibition of enzyme activity) was determined from plots of % inhibition *versus* inhibitor concentration. Each experiment (%, IC_{50}) is performed by duplicate and the average value is given (see Table S1 in Supplementary data).

Inhibition studies with human lysosomal enzymes

The inhibitory activity toward human lysosomal β-glucocerebrosidase and α-galactosidase A was measured with 4-methylumbelliferyl-β-D-glucopyranoside and 4-methylumbelliferyl-α-D-galactopyranoside as substrate. The reaction mixture consisted on 100 mM McIlvaine buffer (pH 5.2), 0.25% sodium taurocholate and 0.1% Triton X-100, and the appropriate amount of enzyme. The reaction mixture was pre-incubated at 0˚C for 45 min, and the reaction was started by adding 3 mM substrate solution, followed by incubation at 37 ˚C for 30 min. The reaction was stopped by the addition of 1.6 mL of the solution of 400 mM glycine-NaOH solution (pH 10.6). The released 4-methylumbelliferone was measured (excitation 362 nm, emission 450 nm) with a F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

*Generation of the libraries **I** and **II** followed by in situ biological screening.*

Library **I**: To a 0.30 mL of a solution of azide **14** (23 mM in ^{t}BuOH–H_2O (2 : 1)) in an eppendorf, 0.10 mL of a solution of the corresponding alkyne (**a**-**s**) (83 mM in ^{t}BuOH) was added followed by 25 µL of an aqueous solution of sodium ascorbate (30 mM) and 25 µL of an aqueous solution of $CuSO_4$ (9.4 mM). The final concentration of the azide **14** in each eppendorf was 15 mM. The resulting mixtures were left at room temperature for 24 h and monitored for completion by TLC and ESI-MS (see Supplementary data for mass spectra analysis). Then, the reactions were diluted with water to the desired concentration and placed in a 96-well microtiter plate in order to perform the enzymatic assays. In the preliminary screening of the resulting crude (pyrrolidin-2-yl)triazoles **14a**-**s**, % of inhibition towards β-glucosidase from almonds was determined at 10 µM of the pyrrolidine-triazole on each well (supposing quantitative conversion in the click reaction).

Library **II**: This library was generated and screened following the same procedure that for Library **I**, except that azide **22**, instead of **14**, was used as starting material and the biological analysis was performed towards α-galactosidases from coffee beans. In this case, the % inhibition of crude triazoles **22a**-**s** was determined at 50 µM on each well.

Synthesis of compounds

N-tert-Butoxycarbonyl-1,4-dideoxy-1,4-imino-2,3-O-isopropylidene-D-talitol (**9**).[14] To a solution of **8**[13] (1.05 g, 3.58 mmol) in MeOH (25 mL), Boc_2O (1.59 g, 7.15 mmol) and Pd/C (10%, cat.) were added and the reaction mixture was stirred under an atmosphere of hydrogen for 2 h. The crude was filtered through celite and the solvent removed in vacuo. The resulting residue was purified by chromatography column on silica gel (ether:cyclohexane 4:1) to give **9** (0.92 g,

3.04 mmol, 85%) as a white solid. $[\alpha]_D^{26}$ + 44.8 (*c* 1.17, CH_2Cl_2) (Lit: . $[\alpha]_D^{25}$ + 54 (*c* 1.0, $CHCl_3$)). IR (ν cm^{-1}) 3606-3243 (OH), 2989, 2934, 1655 (C=O). ^{1}H-NMR (300 MHz, DMSO-d_6, 363 K, δ ppm, *J* Hz) δ 4.73-4.66 (m, 2H, H-2, H-3), 4.52 (d, 1H, $J_{OH,5}$=4.8, O*H*-5), 4.11 (t, 1H, $J_{OH,6a} = J_{OH,6b}$ = 5.9, O*H*-6), 4.03 (ap. bs, 1H, H-4), 3.73-3.63 (m, 2H, H-5, H-6a), 3.44-3.25 (m, 3H, H-6b, H-1a, H-1b), 1.42 (s, 9H, -C(CH_3)$_3$), 1.33 (s, 3H, -C(CH_3)$_2$), 1.26 (s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, DMSO-d_6, 363 K, δ ppm) δ 154.0 (*C*=O, Boc), 109.8 (-*C*(CH$_3$)$_2$), 82.4, 79.0 (C-2, C-3), 78.4 (-*C*(CH$_3$)$_3$), 72.4 (C-5), 64.8 (C-4), 62.6 (C-1), 53.2 (C-6), 27.7 (-C(*C*H$_3$)$_3$), 26.5 (-C(*C*H$_3$)$_2$), 24.6 (-C(*C*H$_3$)$_2$). HRCIMS *m/z* found 304.1760, calc. for $C_{14}H_{25}NO_6$ $[M+H]^+$: 304.1760.

(2R,3S,4R)-N-tert-Butoxycarbonyl-2-formyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (**10**).[14] To a solution of **9** (353 mg, 1.17 mmol) in THF (5 mL) at 0 °C, a solution of $NaIO_4$ (502 mg, 2.34 mmol) in water (8 mL) was added. The reaction mixture was stirred at r.t. for 2 h and then THF was removed in vacuo and the aqueous crude was extracted twice with CH_2Cl_2. The organic layers were washed with sat. aq. sol. of $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and evaporated to give **10** (317 mg, 1.17 mmol, quant.) as a colorless oil. $[\alpha]_D^{26}$ + 55.9 (*c* 0.84, CH_2Cl_2) (Lit: $[\alpha]_D^{25}$ + 35.5 (*c* 1.0, $CHCl_3$)). IR (ν cm^{-1}) 2979, 2936, 1735 (C=O), 1692 (C=O), 1300, 1158, 1075, 1051. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 9.65, 9.61[1] (s, 1H, C*H*O), 4.83 (ap. t, 1H, H-3), 4.70-4.65 (m, 2H, H-2, H-4), 3.96-3.83 (m, 1H, H-5a), 3.42-3.30 (m, 1H, H-5b), 1.48 (s, 9H, -C(CH_3)$_3$), 1.47 (s, 3H, -C(CH_3)$_2$), 1.30 (s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 198.2, 198.0[1] (*C*HO), 154.2 (*C*=O, Boc), 112.7 (-*C*(CH$_3$)$_2$), 81.1, 81.0[1] (-*C*(CH$_3$)$_3$), 80.0, 79.0[1] (C-3), 79.6, 78.6, 71.5[1] (C-2, C-4), 52.4, 51.9[1] (C-5), 28.5, 28.4, 28.4, 25.7[1] (-C(*C*H$_3$)$_3$), 27.1, 27.0[1] (-C(*C*H$_3$)$_2$), 25.1 (-C(*C*H$_3$)$_2$). HRCIMS *m/z* found 272.1491, calc. for $C_{13}H_{21}NO_5$ $[M+H]$: 272.1498.

(2S,3S,4R)-N-tert-Butoxycarbonyl-2-ethynyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (**11**). To a solution of **10** (317 mg, 1.17 mmol) in anhydrous MeOH (16 mL) at 0 °C, K_2CO_3 (327 mg, 2.34 mmol) and Bestmann-Ohira reagent (200 µL, 1.29 mmol), were slowly added. The reaction mixture was stirred at r.t. for 4 h and then Et_2O and sat. aq. sol. of $NaHCO_3$ were added. The aqueous phase was extracted twice with CH_2Cl_2. The organic layers were washed with brine, dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:5) to give **11** (223 mg, 0.83 mmol, 71%) as a colorless oil. $[\alpha]_D^{27}$ + 105.1 (*c* 0.67, CH_2Cl_2). IR (ν cm^{-1}) 3262 (≡CH), 2978, 2936, 1698 (C=O), 1391, 1158, 880. ^{1}H-NMR (300 MHz, DMSO-d_6, 363 K, δ ppm, *J* Hz) δ 4.81 (ap. t, 1H, H-4), 4.68 (d, 1H, $J_{3,4}$ = 5.7, H-3), 4.54 (ap. bs, 1H, H-2), 3.68 (d, 1H, $^2J_{5a,5b}$ = 12.9, H-5a), 3.31 (dd, 1H, $J_{5b,4}$ = 4.5, H-5b), 3.20 (d, 1H, $J_{2',2}$ = 2.4, H-2'), 1.43 (s, 9H, -C(CH_3)$_3$), 1.35 (s, 3H, -C(CH_3)$_2$), 1.27 (s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, DMSO-d_6, 363 K, δ ppm) δ 153.0 (*C*=O, Boc), 110.9 (-*C*(CH$_3$)$_2$), 84.4 (C-3), 79.6 (-*C*(CH$_3$)$_3$), 79.1 (C-1'), 78.2 (C-4), 74.7 (C-2'), 54.1 (C-2), 50.3 (C-5), 27.6 (-C(*C*H$_3$)$_3$), 26.0 (-C(*C*H$_3$)$_2$), 24.3 (-C(*C*H$_3$)$_2$).HRESIMS *m/z* found 290.1364, calc. for $C_{14}H_{21}NO_4$ $[M+Na]^+$: 290.1363.

(2S,3S,4R)-2-[(1-benzyl)-1H-1,2,3-triazol-4-yl]-pyrrolidine-3,4-diol hydrochloride (**1**). To a solution of **11** (61 mg, 0.23 mmol) in toluene (2.1 mL), benzylazide (47 mg, 0.35 mmol), DIPEA (153 µL, 0.87 mmol) and CuI (13 mg, 0.07 mmol) were added. After stirring at r.t. for 1 d., aq. sat. sol. of $NaHCO_3$ (5 mL) was added and the aqueous phase was extracted with EtOAc. The organic layers were dried, filtered and evaporated. The resulting residue was purified by

[1] The signals are unfolded by the presence of two rotamers.

chromatography column on silica gel (EtOAc:cyclohexane 1:2) to give the protected pyrrolidine-triazole (70 mg, 0.17 mmol, 76%) as a white solid. A solution of this compound (37 mg, 0.10 mmol) in HCl (1M):THF (1:1, 2.4 mL) was stirred at r.t. for 2 d., and then evaporated to give **1** (32 mg, 0.11 mmol, quant) as a solid. $[\alpha]_D^{26}$ - 47.0 (*c* 0.60, MeOH). IR (ν cm^{-1}) 3285 (OH, NH), 1445, 1121, 814, 719. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.22 (s, 1H, H-5'), 7.39-7.35 (m, 5H, H-aromat.), 5.65 (s, 2H, -CH_2Ph), 4.72 (d, 1H, $J_{2,3}$ = 8.1, H-2), 4.51 (dd, 1H, $J_{3,4}$ = 3.9, H-3), 4.44-4.42 (m, 1H, H-4), 3.63 (dd, 1H, $^2J_{5a,5b}$ = 12.6, $J_{5a,4}$ = 4.2, H-5a), 3.35 (dd, 1H, $J_{5b,4}$=1.5, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 142.5 (C-4'), 136.5 (Cq aromat.), 130.1 (C aromat.), 129.7 (C aromat.), 129.4 (C aromat.), 125.7 (C-5'), 76.8 (C-3), 70.8 (C-4), 57.1 (C-2), 55.1 (-CH$_2$Ph), 51.2 (C-5). HRCIMS *m/z* found 261.1345, calc. for $C_{13}H_{17}N_4O_2$ [M]: 261.1352.

(2S,3S,4R)-N-tert-Butoxycarbonyl-2-hydroxymethyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (**12**).[14] A solution of crude aldehyde **10** (1.26 g, 4.66 mmol) in ethanol was added to a suspension of $NaBH_4$ (184 mg, 4.66 mmol) in ethanol (10 mL). After stirring at r.t. for 50 min, aq. sol. of. NH_4Cl was added and the aqueous phase was extracted with EtOAc, dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 2:1) to give **12** (1.24 g, 4.54 mmol, 97%) as a colorless oil. $[\alpha]_D^{25}$ + 40.7 (*c* 0.64, CH_2Cl_2) (Lit: $[\alpha]_D^{24}$ + 56 (*c* 1.0, $CHCl_3$)). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.72-4.54 (m, 2H), 4.14-4.02 (m, 1H), 3.88-3.63 (m, 3H), 3.49 (dd, 1H, *J* = 12.6, *J* = 5.4), 2.82 (bs, 1H, OH), 1.46 (s, 12H, -C(CH_3)$_2$, -C(CH_3)$_3$), 1.32 (s, 3H, -C(CH_3)$_2$).

(2S,3S,4R)-N-tert-Butoxycarbonyl-2-azidomethyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (**13**).[14] To a solution of **12** (1.25 g, 4.57 mmol) in anhydrous pyridine (20 mL) at 0 °C, TsCl (4.40 g, 22.8 mmol) was added. After stirring overnight, water was added and the solvent was removed in vacuo. The crude was dissolved in CH_2Cl_2, washed with HCl 1M, aq. sol. of. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (ether:cyclohexane 1:1) to give the corresponding tosylate derivative (1.81 g, 4.23 mmol, 93%) as a colorless oil. To a solution of this compound (301 mg, 0.70 mmol) in DMF (6 mL), NaN_3 (114 mg, 1.75 mmol) was added. After stirring at 70 °C for 3 h the solvent was removed in vacuo and the crude was dissolved in CH_2Cl_2, washed with water and brine, dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (ether:cyclohexane 1:2) to give **13** (200.1 mg, 0.67 mmol, 96%) as a white solid. $[\alpha]_D^{26}$ + 38.0 (*c* 0.57, CH_2Cl_2) (Lit: $[\alpha]_D^{26}$ + 39.0 (*c* 1.0, $CHCl_3$)). IR (ν cm^{-1}) 2970, 2101 (N_3), 1687 (C=O), 1408, 1121. ^{1}H-NMR (300 MHz, DMSO-d_6, 363 K, δ ppm, *J* Hz) δ 4.74 (ap. td, 1H, H-4), 4.54 (dd, 1H, $J_{3,4}$ = 5.7, $J_{3,2}$ = 0.3, H-3), 4.00 (ap. t, 1H, H-2), 3.67 (dd, 1H, $^2J_{5a,5b}$ = 12.8, $J_{5a,4}$ = 0.8, H-5a), 3.50 (dd, 1H, $J_{1'a,1'b}$ = 12.6, $J_{1'a,2}$ = 6.0, H-1'a), 3.44 (dd, 1H, $J_{1'b,2}$ = 6.0, H-1'b), 3.39 (dd, 1H, $J_{5b,4}$ = 5.0, H-5b), 1.43 (s, 9H, -C(CH_3)$_3$), 1.37 (ap. s, 3H, -C(CH_3)$_2$), 1.27 (ap. s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, DMSO-d_6, 363 K, δ ppm) δ 153.1 (*C*=O, Boc), 110.5 (-*C*(CH$_3$)$_2$), 81.9 (C-3), 78.8 (-*C*(CH$_3$)$_3$), 78.2 (C-4), 62.4 (C-2), 51.2 (C-5), 50.4 (C-1'), 27.6 (-C(*C*H$_3$)$_3$), 26.4 (-C(*C*H$_3$)$_2$), 24.5 (-C(*C*H$_3$)$_2$). HRESIMS *m/z* found 321.1531, calc. for $C_{13}H_{22}N_4O_4$ $[M+Na]^+$: 321.1533.

(2S,3S,4R)-2-Azidomethyl-pyrrolidine-3,4-diol (**14**). A solution of **13** (205 mg, 0.69 mmol) in HCl (1M):THF 1:1 (17 mL), was stirred at r.t. overnight. Evaporation of the solvent and chromatographic purification on Dowex 50WX8 eluting with MeOH, H_2O and NH_4OH 25%,

afforded **14** (99 mg, 0.63 mmol, 91%) as a crystalline white solid. $[\alpha]_D^{25}$ - 44.1 (*c* 1.09, MeOH). IR (ν cm^{-1}) 3495, 3302 (OH, NH), 2101 (N_3), 1263, 945. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 4.04 (td, 1H, $J_{4,3} = J_{4,5a}$ = 5.1, $J_{4,5b}$ = 3.3, H-4), 3.72 (dd, 1H, $J_{3,2}$ = 7.5, H-3), 3.58 (dd, 1H, $^2J_{1'a,1'b}$ = 12.5, $J_{1'a,2}$ = 4.1, H-1'a), 3.40 (dd, 1H, $J_{1'b,2}$ = 6.8, H-1'b), 3.14 (dd, 1H, $^2J_{5a,5b}$ = 12.0, H-5a), 3.11-3.08 (m, 1H, H-2), 2.83 (dd, 1H, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 75.6 (C-3), 72.5 (C-4), 62.4 (C-2), 54.4 (C-1'), 52.4 (C-5). HRESIMS *m/z* found 159.0874, calc. for $C_5H_{11}N_4O_2$ $[M+H]^+$: 159.0877.

(2S,3S,4R)-2-[(4-Phenyl-1H-1,2,3-triazol-1-yl)methyl)]-pyrrolidine-3,4-diol (**2**). To a solution of **14** (66 mg, 0.42 mmol) in ^{t}BuOH:H_2O 2:1 (14 mL), phenylacetylene (57 µL, 0.50 mmol), sodium ascorbate (9 mg, 0.05 mmol) and $CuSO_4$ (2 mg, 0.01 mmol) were added. After stirring overnight at r.t. the solvent was evaporated and the resulting residue was purified by chromatography column on silica gel (CH_2Cl_2:MeOH 10:1 → 5:1, 0.5% Et_3N) to give **2** (102 mg, 0.39 mmol, 94%) as a white solid. $[\alpha]_D^{26}$ - 33.9 (*c* 1.37, MeOH). IR (ν cm^{-1}) 3283, 3128 (OH, NH), 2923, 1424, 1109, 765, 692. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.35 (s, 1H, H-5''), 7.83-7.80 (m, 2H, H-aromat.), 7.46-7.40 (m, 2H, H-aromat), 7.37-7.32 (m, 1H, H-aromat), 4.69 (dd, 1H, $^2J_{1'a,1'b}$ = 14.1, $J_{1'a,2}$ = 4.2, H-1'a), 4.47 (dd, 1H, $J_{1'b,2}$ = 8.1, H-1'b), 4.05 (td, 1H, $J_{4,3} = J_{4,5a}$ = 4.8, $J_{4,5b}$ = 2.9, H-4), 3.79 (dd, 1H, $J_{3,2}$ = 7.8, H-3), 3.53 (ap. td, 1H, H-2), 3.15 (dd, 1H, $^2J_{5a,5b}$ = 12.0, H-5a), 2.88 (dd, 1H, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 148.8 (C4''), 131.7 (Cq aromat), 130.0 (C aromat.), 129.4 (C aromat.), 126.7 (C aromat.), 123.0 (C-5''), 76.1 (C-3), 72.1 (C-4), 62.5 (C-2), 53.7 (C-1'), 52.3 (C-5). HRESIMS *m/z* found 261.1342, calc. for $C_{13}H_{17}N_4O_2$ $[M+H]^+$: 261.1346.

(2S, 3S, 4R)-N-tert-Butoxycarbonyl-2-aminomethyl-3,4-O-isopropylidene-pirrolidine-3,4-diol (**15**). To a solution of **13** (88 mg, 0.30 mmol) in MeOH (2.1 mL), Pd/C (10%, cat.) was added and the reaction mixture was stirred under an atmosphere of hydrogen for 1.5 h. The crude was filtered through celite and the solvent removed in vacuo. The resulting residue was purified by chromatography column on silica gel (CH_2Cl_2:MeOH 40:1, 1% Et_3N) to give **15** (77 mg, 0.28 mmol, 95%) as a colorless oil. $[\alpha]_D^{26}$ + 36.8 (*c* 0.72, CH_2Cl_2). IR (ν cm^{-1}) 3352, 2977 (OH, NH), 1696, 1671, 1402, 1158, 1036, 852. ^{1}H-NMR (300 MHz, DMSO-d_6, 363 K, δ ppm, *J* Hz) δ 4.70 (ap. td, 1H, H-4), 4.61 (d, 1H, $J_{3,4}$ = 6.0, H-3), 3.80 (ap. t, 1H, H-2), 3.60 (dd, 1H, $^2J_{5a,5b}$ = 12.8, $J_{5a,4}$ = 0.8, H-5a), 3.36 (dd, 1H, $J_{5b,4}$ = 5.1, H-5b), 2.66 (dd, 1H, $J_{1'a,1'b}$ = 12.8, $J_{1'a,2}$ = 5.3, H-1'a), 2.60 (dd, 1H, $J_{1'b,2}$ = 6.8, H-1'b), 1.42 (s, 9H, -C(CH_3)$_3$), 1.35 (s, 3H, -C(CH_3)$_2$), 1.26 (s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, DMSO-d_6, 363 K, δ ppm) δ 153.4 (*C*=O, Boc), 110.0 (-*C*(CH$_3$)$_2$), 82.0 (C-3), 78.3 (C-4), 78.1 (-*C*(CH$_3$)$_3$), 65.3 (C-2), 51.5 (C-5), 41.6 (C-1'), 27.7 (-C(*C*H$_3$)$_3$), 26.4 (-C(*C*H$_3$)$_2$), 24.6 (-C(*C*H$_3$)$_2$). HRESIMS *m/z* found 273.1811, calc. for $C_{15}H_{25}N_2O_4$ $[M+H]^+$: 273.1809.

(2S, 3S, 4R)-2-Benzamidomethyl-pyrrolidine-3,4-diol (**5**). To a solution of **15** (251 mg, 0.92 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C, benzoyl chloride (220 mL, 1.85 mmol) and Et_3N (270 mL, 1.85 mmol) were added. After stirring at r.t. for 2 h, the solution was washed with aq. sol. of. $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered and evaported. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:2 → EtOAc:cyclohexane 1:1) to give the corresponding protected pyrrolidine-amide (356 mg, 0.94 mmol, quant.) as a white solid. A solution of this compound (278 mg, 0.74 mmol) in HCl (4M):THF (1:1, 18 mL) was stirred at r.t. for 3 h. Evaporation of the solvent and chromatographic purification on Dowex 50WX8 eluting with MeOH, H_2O and

NH_4OH 25%, afforded **5** (171 mg, 0.72 mmol, 98%) as a whitish solid. $[\alpha]_D^{25}$ - 37.8 (*c* 0.65, MeOH). IR (ν cm^{-1}) 3290, 3061 (OH, NH), 1635 (C=O), 1541, 1400, 1099, 807, 690. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 7.86-7.82 (m, 2H, H-aromat.), 7.54 (ap. tt, 1H, H-aromat), 7.49-7.43 (m, 2H, H-aromat), 4.08 (ap. td, 1H, H-4), 3.77 (dd, 1H, $J_{3,2}$ = 6.9, $J_{3,4}$ = 5.1, H-3), 3.61 (dd, 1H, $^2J_{1'a,1'b}$ = 13.7, $J_{1'a,2}$ = 5.3, H-1'a), 3.49 (dd, 1H, $J_{1'b,2}$ = 7.2, H-1'b), 3.24-3.20 (m, 1H, H-2), 3.17 (dd, 1H, $^2J_{5a,5b}$ = 11.9, $J_{5a,4}$ = 5.3, H-5a), 2.84 (dd, 1H, $J_{5b,4}$ = 3.6, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 170.8 (*C*=O), 135.4 (Cq aromat), 132.8 (C aromat.), 129.6 (C aromat.), 128.3 (C aromat.), 76.2 (C-3), 72.3 (C-4), 63.1 (C-2), 52.0 (C-5), 43.2 (C-1'). HRESIMS *m/z* obsd. 237.1231, calc. for $C_{12}H_{17}N_2O_3$ $[M+H]^+$: 237.1234.

(2S, 3S, 4R)-2-Phenylthioureidomethyl-pyrrolidine-3,4-diol hydrochloride (**6**). To a solution of **15** (76 mg, 0.28 mmol) in anhydrous CH_2Cl_2 (5 mL), phenyl isothiocyanate (44 mL, 0.36 mmol) was added. After stirring at r.t. for 6 h, the solvent was removed under vacuo and the resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:2) to give the corresponding protected pyrrolidine-thiourea (120 mg, 0.29 mmol, quant.) as a colorless oil. A solution of this compound (63 mg, 0.15 mmol) in HCl (4M):THF (1:1, 3.8 mL) was stirred at r.t. for 3 h, and evaporated to give **6** (45 mg, 0.15 mmol, quant.) as a colorless oil. $[\alpha]_D^{25}$ + 2.1 (*c* 0.61, MeOH). IR (ν cm^{-1}) 3240, 3045 (OH, NH), 2359, 1539, 1313, 1136, 1040, 696. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 7.50-7.39 (m, 2H, H-aromat), 7.35-7.33 (m, 2H, H-aromat), 7.29-7.23 (m, 1H, H-aromat.), 4.31-4.25 (m, 1H, H-4), 4.14-4.06 (m, 2H, H-3, H-1'a), 3.98 (dd, 1H, $^2J_{1'b,1'a}$ = 15.0, $J_{1'b,2}$ = 3.9, H-1'b), 3.73 (ap. td, 1H, H-2), 3.50 (dd, 1H, $^2J_{5a,5b}$ = 12.5, $J_{5a,4}$ = 3.8, H-5a), 3.25 (dd, 1H, $J_{5b,4}$ = 1.5, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 183.6 (*C*=S), 139.0 (Cq aromat), 130.4 (C aromat.), 127.4 (C aromat.), 125.9 (C aromat.), 74.4 (C-3), 70.8 (C-4), 63.7 (C-2), 50.7 (C-5), 44.5 (C-1'). HRESIMS *m/z* found 268.1117, calc. for $C_{12}H_{18}N_3O_2S$ $[M]^+$: 268.1114.

(2R,3S,4R)-N-Benzyl-2-hydroxymethyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (**17**).[27] To a solution of **16**[28] (13.44 g, 26.8 mmol) in THF (185 mL), TBAF (30 mL, 29.5 mmol) was added. After stirring at r.t. for 2.5 d, the solvent was removed and the resulting residue was purified by chromatography column on silica gel (ether:cyclohexane 1:1→2:1) to give **17** (6.46 g, 24.5 mmol, 92%) as a colorless oil. $[\alpha]_D^{25}$ - 81.8 (*c* 0.57, CH_2Cl_2) (Lit: $[\alpha]_D^{20}$ – 80.3 (*c* 1.3, $CHCl_3$)). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 7.29-7.18 (m, 5H, H-aromat.), 4.67 (dd, 1H, *J* = 6.3, *J* = 4.8), 4.55 (dd, 1H, *J* = 4.7, *J* = 6.5), 4.01 (d, 1H, *J* = 13.2), 3.91 (d, 1H, *J* = 1.8), 3.90 (s, 1H), 3.20 (d, 1H, *J* = 13.2), 3.04 (d, 1H, *J* = 11.1), 2.56 (bs, 1H, O*H*), 2.34 (ap. q, 1H), 2.09 (dd, 1H, *J* = 11.1, *J* = 4.5), 1.51 (s, 3H, -C(CH_3)$_2$), 1.28 (s, 3H, -C(CH_3)$_2$).

(2R,3S,4R)-N-tert-Butoxycarbonyl-2-hydroxymethyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (**18**).[17] To a solution of **17** (6.43 g, 24.4 mmol) in MeOH (180 mL), Boc_2O (10.88 g, 48.8 mmol) and Pd/C (10%, cat.) were added and the reaction mixture was stirred under hydrogen for 4 h. The crude was filtered through celite and the solvent removed in vacuo. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:2 → 1:1) to give **18** (5.73 g, 21.0 mmol, 86%) as a colorless oil. $[\alpha]_D^{25}$ - 40.1 (*c* 0.58, CH_2Cl_2) (Lit: $[\alpha]_D^{25}$ – 33.8 (*c* 2.1, $CHCl_3$)). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.74 (ap. d, 3H), 3.85-3.79 (m, 3H), 3.52 (bs, 2H), 1.48 (s, 3H, -C(CH_3)$_2$), 1.44 (s, 9H, -C(CH_3)$_3$), 1.31 (s, 3H, -C(CH_3)$_2$).

(2S,3S,4R)-N-tert-Butoxycarbonyl-2-formyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (**19**).[17] To a solution of oxalyl chloride (510 µL, 5.86 mmol) in anhydrous CH_2Cl_2 (6 mL) at -78 °C, DMSO (840 µL, 11.7 mmol) in CH_2Cl_2 (3 mL) was added and the solution was stirred at -78 °C for 45 min. A solution of **18** (1.07 g, 3.91 mmol) in CH_2Cl_2 (6 mL) was then added and the reaction mixture was stirred at -78 °C for 2.5 h. Finally, Et_3N (3.0 mL, 21 mmol) was added and the mixture stirred at -78 °C for 30 min. and allowed to reach r.t. The reaction was diluted with CH_2Cl_2 and washed with HCl 1M, sat. aq. sol. of $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:2) to give **19** (948 mg, 3.49 mmol, 89%) as a white solid. $[\alpha]_D^{25}$ - 94.6 (*c* 0.68, CH_2Cl_2) (Lit: $[\alpha]_D^{27}$ - 51.6 (*c* 3.06, $CHCl_3$)). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 9.42 (bs, 1H, C*H*O), 4.97 (t, 1H, *J* = 6.3), 4.81 (td, 1H, *J* = 5.7, *J* = 1.8), 4.08-4.04 (m, 1H), 3.80-3.76 (m, 1H), 3.63-3.60 (m, 1H), 1.46 (s, 3H, -C(CH_3)$_2$), 1.41 (s, 9H, -C(CH_3)$_3$), 1.29 (s, 3H, -C(CH_3)$_2$).

(2R,3S,4R)-N-tert-Butoxycarbonyl-2-ethynyl-3,4-O-isopropylidene-pyrrolidine-3,4-diol (**20**). To a suspension of 4-acetamidobenzenosulfonyl azide (587 mg, 2.37 mmol) and K_2CO_3 (1.16 g, 8.29 mmol) in anhydrous CH_3CN (18 mL) at 0 °C, dimethyl 2-oxopropylphosphonate (324 µL, 2.22 mmol) was added. After stirring at r.t. for 6 h, a solution of **19** (402 mg, 1.48 mmol) in anhydrous MeOH (18 mL) was added and the mixture was stirred at r.t. overnight. The mixture was filtered through celite and the solvent removed in vacuo. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:5) to give **20** (214 mg, 0.80 mmol, 54%) as a colored liquid. $[\alpha]_D^{25}$ + 104.3 (*c* 0.68, CH_2Cl_2). IR (ν cm^{-1}) 3220 (≡C−H), 2978, 2938, 2115 (≡C−C), 1696 (C=O), 1392, 1157, 881. ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.78-4.60 (m, 3H, H-4, H-3, H-2), 3.86-3.79 (m, 1H, H-5a), 3.39 (dd, 1H, $^2J_{5b,5a}$ = 12.8, $J_{5b,4}$ = 4.7, H-5b), 2.29 (d, 1H, $J_{2',2}$ = 2.4, H-2'), 1.47 (s, 9H, -C(CH_3)$_3$), 1. 42 (s, 3H, -C(CH_3)$_2$), 1.29 (s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 154.4 (*C*=O, Boc), 112.2 (-*C*(CH$_3$)$_2$), 85.6, 85.1[1] (C-3), 80.6 (-*C*(CH$_3$)$_3$), 80.1 (C-1'), 79.7, 78.8[1] (C-4), 72.8 (C-2'), 55.0, 54.5[1] (C-2), 51.4, 50.9[1] (C-5), 28.5 (-C(*C*H$_3$)$_3$), 26.7 (-C(*C*H$_3$)$_2$), 25.0 (-C(*C*H$_3$)$_2$). HRESIMS *m/z* found 290.1364, calc. for $C_{14}H_{21}NO_4$ [M+Na]$^+$: 290.1363.

(2R,3S,4R)-2-[(1-Benzyl)-1H-1,2,3-triazol-4-yl]-pyrrolidine-3,4-diol (**3**). To a solution of **20** (81 mg, 0.30 mmol) in toluene (2.8 mL), benzyl azide (80 mg, 0.60 mmol), DIPEA (200 µL, 1.15 mmol) and CuI (18 mg, 0.09 mmol) were added. After stirring at r.t. for 6 h, aq. sat. sol. of $NaHCO_3$ was added and the aqueous phase was extracted with EtOAc. The organic layers were dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:2) to give the corresponding protected pyrrolidine-triazole (116 mg, 0.29 mmol, 96%) as a white solid. A solution of this compound (72 mg, 0.18 mmol) in HCl (4M):THF (1:1, 4.4 mL) was stirred at r.t. for 3 h. Evaporation of the solvent and chromatographic purification on Dowex 50WX8 eluting with MeOH, H_2O and NH_4OH 25%, afforded **3** (43 mg, 0.16 mmol, 91%) as a crystalline white solid. $[\alpha]_D^{26}$ - 61.2 (*c* 0.62, MeOH). IR (ν cm^{-1}) 3280, 3133 (OH, NH), 1441, 1128, 917, 720. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 7.91 (s, 1H, H-5'), 7.40-7.30 (m, 5H, H-aromat.), 5.58 (s, 2H, H-1''), 4.19 (ap. td, 1H, H-4), 4.15 (d, 1H, $J_{2,3}$ = 7.8, H-2), 4.09 (dd, 1H, $J_{3,4}$ = 5.0, H-3), 3.37 (dd, 1H, $^2J_{5a,5b}$ = 12.2, $J_{5a,4}$ = 5.3, H-5a), 2.91 (dd, 1H, $J_{5b,4}$ = 3.2, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 149.0 (C-4'), 136.8 (Cq aromat), 130.0 (C aromat.), 129.6 (C aromat.), 129.2 (C aromat.),

123.9 (C-5'), 78.8 (C-3), 72.3 (C-4), 59.0 (C-2), 54.9 (C-1''), 52.7 (C-5). HRESIMS *m/z* found 261.1347, calc. for $C_{13}H_{17}N_4O_2$ $[M+H]^+$: 261.1346.

(5S,6R,7S,7aR)-6,7-O-isopropylidene-tetrahydropirrolo[1,2-c]-oxazol-3-one-6,7-diol (**21**).[17] To a solution of **18** (704 mg, 2.58 mmol) in anhydrous piridine (10 mL) at 0 °C, TsCl (1.24 g, 6.44 mmol) was added. After stirring at 0 °C to r.t. overnight, the solvent was removed in vacuo and the resulting residue was purified by chromatography column on silica gel (EtOAc:cyclohexane 1:1 → 2:1) to give **21** (473 mg, 2.37 mmol, 92%) as a white solid. $[\alpha]_D^{26}$ - 19.8 (*c* 0.79, CH_2Cl_2) (Lit: $[\alpha]_D^{27}$ – 4.92 (*c* 2.40, $CHCl_3$)). ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.81 (dd, 1H, *J* = 5.1, *J* = 4.5), 4.59 (ap. t, 1H), 4.54 (dd, 1H, *J* = 8.7, *J* = 2.4), 4.41 (ap. t, 1H), 3.98 (d, 1H *J* = 13.5), 3.86 (ap. ddd, 1H), 3.09 (dd, 1H, *J* = 13.5, *J* = 4.2), 1.44 (s, 3H, -C(CH_3)$_2$), 1.31 (s, 3H, -C(CH_3)$_2$).

(2R,3S,4R)-2-Azidomethyl-pyrrolidine-3,4-diol (**22**). A solution of **21** (71 mg, 0.36 mmol) and Bu_4NN_3 (708 mg, 2.49 mmol) in THF (7 mL) was heated at 100 °C in a sealed tube for 14 d. The solvent was evaporated and the residue was purified by chromatography column on silica gel (ether→ether:MeOH 30:1, 0.5% Et_3N) to give the (2*R*,3*S*,4*R*)-2-azidomethyl-3,4-*O*-isopropylidene-pyrrolidine-3,4-diol (42 mg, 0.21 mmol, 60%) as a yellowish oil. A solution of this compound (101 mg, 0.51 mmol) in HCl (1M):THF 1:1 (12 mL), was stirred at r.t. overnight. Evaporation of the solvent and chromatographic purification on Dowex 50WX8 eluting with MeOH, H_2O and NH_4OH 25%, afforded **22** (64 mg, 0.40 mmol, 80%) as a yellowish oil. $[\alpha]_D^{26}$ + 5.6 (*c* 1.33, MeOH). IR (ν cm^{-1}) 3323 (OH, NH), 2098 (N_3), 1267, 1121. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 4.18 (td, 1H, $J_{4,5a}$ = $J_{4,5b}$ = 6.8, $J_{4,3}$ = 4.6, H-4), 4.03 (t, 1H, $J_{3,2}$ = 4.6, H-3), 3.62 (dd, 1H, $^2J_{1'a,1'b}$ = 12.2, $J_{1'a,2}$ = 6.2, H-1'a), 3.36 (dd, 1H, $J_{1'b,2}$ = 8.1, H-1'b), 3.19 (ddd, 1H, H-2), 3.03 (dd, 1H, $^2J_{5a,5b}$ = 11.1, H-5a), 2.81 (dd, 1H, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 73.7 (C-4), 72.9 (C-3), 61.2 (C-2), 52.4 (C-1'), 51.2 (C-5). HRESIMS *m/z* found 159.0875, calc. for $C_5H_{11}N_4O_2$ $[M+H]^+$: 159.0877.

(2R,3S,4R)-2-[(4-Phenyl-1H-1,2,3-triazol-1-yl)methyl)]-pyrrolidine-3,4-diol (**4**). To a solution of **22** (32 mg, 0.20 mmol) in ^{t}BuOH:H_2O 2:1 (8 mL), phenylacetylenene (27 µL, 0.24 mmol), sodium ascorbate (9 mg, 0.04 mmol) and $CuSO_4$ (2 mg, 0.01 mmol) were added and the solution was stirred at r.t. for 1 d. The solvent was evaporated and the resulting residue was purified by chromatography column on silica gel (CH_2Cl_2:MeOH:NH_4OH 6:1:0.1) to give **4** (45 mg, 0.17 mmol, 85%) as a whitish solid. $[\alpha]_D^{26}$ + 15.5 (*c* 0.68, MeOH). IR (ν cm^{-1}) 3254, 3130 (OH, NH), 1467, 1318, 1224, 1091, 766, 689. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.33 (s, 1H, H-5''), 7.83-7.79 (m, 2H, H-aromat.), 7.46-7.40 (m, 2H, H-aromat), 7.33 (ap. tt, H-aromat.), 4.75 (dd, 1H, $^2J_{1'a,1'b}$ = 13.8, $J_{1'a,2}$ = 5.7, H-1'a), 4.50 (dd, 1H, $J_{1'b,2}$ = 8.1, H-1'b), 4.21 (td, 1H, $J_{4,5a}$ = $J_{4,5b}$ = 6.6, $J_{4,3}$ = 4.6, H-4), 4.08 (t, 1H, $J_{3,2}$ = 4.7, H-3), 3.68-3.62 (m, 1H, H-2), 3.08 (dd, 1H, $^2J_{5a,5b}$ = 11.3, H-5a), 2.90 (dd, 1H, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 148.7 (C-4''), 131.7 (Cq aromat), 130.0 (C aromat.), 129.4 (C aromat.), 126.7 (C aromat.), 123.1 (C-5''), 73.4 (C-4), 72.9 (C-3), 61.7 (C-2), 51.7 (C-1'), 51.0 (C-5). HRESIMS *m/z* found 261.1349, calc. for $C_{13}H_{17}N_4O_2$ $[M+H]^+$: 261.1346.

(2R,3S,4R)-2-[(4-Phenyl)-1H-1,2,3-triazol-1-yl)ethyl)]-pyrrolidine-3,4-diol (**7**). To a solution of **23**[18] (94 mg, 0.27 mmol) in toluene (2.5 mL), phenylacetylene (122 µL, 1.08 mmol), DIPEA (180 µL, 1.03 mmol) and CuI (17 mg, 0.08 mmol) were added. After stirring at 50 °C for 3.5 d., aq. sat. sol. of $NaHCO_3$ (15 mL) was added and the aqueous phase was extracted with EtOAc. The

organic layers were dried, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (AcOEt:cyclohexane 1:2) to give the corresponding protected pyrrolidine-triazol (111 mg, 0.25 mmol, 91%) as a white solid. A solution of this compound (75 mg, 0.17 mmol) in HCl (1M):THF (1:1, 6.8 mL) was stirred at r.t. for 2.5 h and then evaporated. Chromatographic purification on silica gel (CH_2Cl_2:MeOH, 50:1 → 20:1) afforded the 3,4-*O*-unprotected derivative (43 mg, 0.10 mmol). A solution of this compound in MeOH (2 mL) was hydrogenated in the presence of Pd/C (10%) and HCl (5M) (73 µL) for 2 h. Filtration through celite, evaporation and chromatographic purification on Dowex 50WX8 eluting with MeOH, H_2O and NH_4OH 25%, afforded **7** (7 mg, 0.03 mmol, 30%) as a white solid. $[\alpha]_D^{24}$ + 66.9 (*c* 0.65, MeOH). IR (ν cm^{-1}) 3268 (OH, NH), 1426, 1219, 1044, 762, 690. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.35 (s, 1H, H-5''), 7.81 (ap. d, 2H, H-aromat.), 7.43 (ap. t, 2H, H-aromat.), 7.38-7.30 (m, 1H, H-aromat.), 4.63-4.49 (m, 2H, H-2'), 4.22 (ap. td, 1H, H-4), 3.99 (t, 1H, $J_{3,4}$ = $J_{3,2}$ = 4.4, H-3), 3.05 (dd, 1H, $^2J_{5a,5b}$ = 11.4, $J_{5a,4}$ = 7.2, H-5a), 3.00-2.95 (m, 1H, H-2), 2.86 (dd, 1H, $J_{5b,4}$ = 6.5, H-5b), 2.41-2.29 (m, 1H, H-1'a), 2.21-2.10 (m, 1H, H-1'b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 148.9 (C-4''), 131.7 (Cq aromat.), 130.0 (C aromat.), 129.4 (C aromat.), 126.7 (C aromat.), 122.3 (C-5''), 73.8 (C-4), 73.1 (C-3), 59.6 (C-2), 51.4 (C-5), 49.2 (C-2'), 31.4 (C-1'). HRESIMS *m/z* found 275.1498, calc. for $C_{14}H_{19}N_4O_2$ $[M+H]^+$: 275.1503.

(2S,3S,4R)-2-[(4-((Trimethylsilyl)methyl)-1H-1,2,3-triazol-1-yl)methyl)]-pyrrolidine-3,4-diol (**14f**). To a solution of **14** (21 mg, 0.13 mmol) in ^{t}BuOH:H_2O 2:1 (4.5 mL), trimethyl(propargyl)silane (54 µL, 0.33 mmol), sodium ascorbate (6 mg, 0.03 mmol) and $CuSO_4$ (1.4 mg, 0.01 mmol) were added. After stirring 40 h. at r.t. the solvent was evaporated and the resulting residue was purified by chromatography column on silica gel (CH_2Cl_2:MeOH 5:1, 0.5% Et_3N) to give **14f** (24 mg, 0.09 mmol, 67%) as a yellowish oil. $[\alpha]_D^{25}$ - 35.9 (*c* 0.86, MeOH). IR (ν cm^{-1}) 3248 (OH, NH), 2954, 1542, 1428, 1248, 1047, 840. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 7.63 (s, 1H, H-5''), 4.57 (dd, 1H, $^2J_{1'a,1'b}$ = 14.0, $J_{1'a,2}$ = 4.1, H-1'a), 4.38 (dd, 1H, $J_{1'b,2}$ = 7.8, H-1'b), 4.01 (td, 1H, $J_{4,5a}$ = $J_{4,3}$ = 4.8, $J_{4,5b}$ = 3.0, H-4), 3.72 (dd, 1H, $J_{3,2}$ = 7.5, H-3), 3.45 (td, 1H, H-2), 3.11 (dd, 1H, $^2J_{5a,5b}$ = 12.0, H-5a), 2.86 (dd, 1H, H-5b), 2.11 (ap. s, 2H, -CH_2Si$(CH_3)_3$), 0.04 (ap. s, 9H, (-Si(CH_3)$_3$). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 146.6 (C-4''), 123.0 (C-5''), 76.1 (C-3), 72.3 (C-4), 62.7 (C-2), 53.7 (C-1'), 52.2 (C-5), 15.7 (-CH_2Si$(CH_3)_3$), -1.8 (-Si(CH_3)$_3$). HRESIMS *m/z* found 271.1579, calc. for $C_{11}H_{23}N_4O_2Si$ $[M+H]^+$: 271.1585.

(2R,3S,4R)-2-[(4-(6-Methoxynaphthalen-2-yl)-1H-1,2,3-triazol-1-yl)methyl)]-pyrrolidine-3,4-diol (**22k**). To a solution of **22** (28 mg, 0.18 mmol) in ^{t}BuOH:H_2O 2:1 (7.2 mL), 2-ethynyl-6-methoxynaphthalene (41 mg, 0.22 mmol), sodium ascorbate (8 mg, 0.04 mmol) and $CuSO_4$ (2 mg, 0.01 mmol) were added and the solution was stirred at r.t. for 1 d. The solvent was evaporated and the resulting residue was purified by chromatography column on silica gel (CH_2Cl_2:MeOH:NH_4OH 7:1:0.1 → 6:1:0.1) to give **22k** (46 mg, 0.14 mmol, 76%) as a whitish solid. $[\alpha]_D^{25}$ + 28.6 (*c* 0.60, MeOH). IR (ν cm^{-1}) 3253, 2919, (OH, NH), 1614, 1482, 1210, 1023, 853, 819. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm, *J* Hz) δ 8.56 (s, 1H, H-5''), 8.31 (bs, 1H, H-aromat.), 7.95 (dd, 1H, *J* = 8.6, *J* = 1.7, H-aromat.), 7.88 (ap. d, 2H, H-aromat.), 7.33 (d, 1H, *J* = 2.3, H-aromat.), 7.18 (dd, 1H, *J* = 9.0, *J* = 2.4, H-aromat), 4.81 (bs, 1H, O*H*), 4.75 (bs, 1H, O*H*), 4.56 (dd, 1H, $^2J_{1'a,1'b}$ = 13.8, $J_{1'a,2}$ = 4.8, H-1'a), 4.32 (dd, 1H, $J_{1'b,2}$ = 8.9, H-1'b), 4.03-3.93 (m, 2H, H-4, H-3), 3.88 (s, 3H, -OCH_3), 3.54-3.47 (m, 1H, H-2), 3.32 (bs, 1H, N*H*), 2.96 (dd, 1H, $^2J_{5a,5b}$ = 10.8, $J_{5a,4}$ = 6.6, H-5a), 2.70 (dd, 1H, $J_{5b,4}$ = 6.3, H-5b). ^{13}C-NMR (75.4 MHz, DMSO-d_6, δ ppm) δ

157.4 (Cq aromat.), 146.1 (C-4''), 133.8 (Cq aromat), 129.5 (C aromat.), 128.6 (Cq aromat.), 127.3 (C aromat.), 126.3 (Cq aromat.), 124.1 (C aromat.), 123.3 (C aromat.), 121.8 (C-5''), 119.1 (C aromat.), 106.0 (C aromat.), 72.2 (C-4), 71.8 (C-3), 59.9 (C-2), 55.2 (-O*C*H_3), 51.3 (C-1'), 50.4 (C-5). HRESIMS *m/z* found 341.1603, calc. for $C_{18}H_{21}N_4O_3$ $[M+H]^+$: 341.1608.

(*2R, 3S, 4R*)-*2-[(4-(4-Methoxyphenyl)-1H-1,2,3-triazol-1-yl)methyl)]-pyrrolidine-3,4*-diol (**22p**). The synthesis of this compound was carried out following the procedure of **22k**, except that 4-methoxyphenylacetylene was used as alkyne. The resulting residue was purified by chromatography column on silica gel (CH_2Cl_2:MeOH:NH_4OH 6:1:0.1) to give **22p** (50 mg, 0.17 mmol, 89%) as a whitish solid. $[\alpha]_D^{26}$ + 11.8 (*c* 0.82, MeOH). IR (ν cm^{-1}) 3254, 2924, (OH, NH), 1558, 1496, 1245, 1028, 835, 799. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.22 (s, 1H, H-5''), 7.75-7.70 (m, 2H, H-aromat.), 7.01-6.96 (m, 2H, H-aromat.), 4.73 (dd, 1H, $^2J_{1'a,1'b}$ = 13.8, $J_{1'a,2}$ = 5.7, H-1'a), 4.49 (dd, 1H, $J_{1'b,2}$ = 8.3, H-1'b), 4.21 (td, 1H, $J_{4,5a}$ = $J_{4,5b}$ = 6.7, $J_{4,3}$ = 4.5, H-4), 4.08 (t, 1H, $J_{4,3}$ = 4.6, H-3), 3.83 (s, 3H, -OCH_3), 3.69-3.62 (m, 1H, H-2), 3.08 (dd, 1H, $^2J_{5a,5b}$ = 11.3, H-5a), 2.90 (dd, 1H, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 161.3 (Cq aromat.), 148.7 (C-4''), 128.0 (C aromat.), 124.3 (Cq aromat.), 122.3 (C-5''), 115.4 (C aromat.), 73.6 (C-4), 73.0 (C-3), 61.7 (C-2), 55.8 (-O*C*H_3), 51.9 (C-1'), 51.2 (C-5). HRESIMS *m/z* found 291.1453, calc. for $C_{14}H_{19}N_4O_3$ $[M+H]^+$: 291.1452.

(2R, 3S, 4R)-2-[(4-(4-Hydroxymethylphenyl)-1H-1,2,3-triazol-1-yl)methyl)]-pyrrolidine-3,4-diol (**22r**). The synthesis of this compound was carried out following the procedure of **22k**, except that 4-hydroxymethylphenylacetylene was used as alkyne. The resulting residue was purified by chromatography column on silica gel (CH_2Cl_2:MeOH:NH_4OH 5:1:0.1 → 4:1:0.1) to give **22r** (42 mg, 0.14 mmol, 76%) as a whitish solid. $[\alpha]_D^{25}$ + 17.8 (*c* 0.82, MeOH). IR (ν cm^{-1}) 3408, 3245, 2923 (OH, NH), 1457, 1110, 1016, 972, 797. ^{1}H-NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 8.33 (s, 1H, H-5''), 7.80 (d, 2H, *J* = 8.4, H-aromat.), 7.43 (d, 2H, H-aromat.), 4.78 (dd, 1H, $^2J_{1'a,1'b}$ = 14.0, $J_{1'a,2}$ = 5.3, H-1'a), 4.63 (s, 2H, -CH_2OH), 4.55 (dd, 1H, $J_{1'b,2}$ = 8.6, H-1'b), 4.24 (td, 1H, $J_{4,5a}$ = $J_{4,5b}$ = 6.7, $J_{4,3}$ = 4.4, H-4), 4.11 (t, 1H, $J_{3,4}$ = $J_{3,2}$ = 4.5, H-3), 3.75-3.70 (m, 1H, H-2), 3.13 (dd, 1H, $^2J_{5a,5b}$ = 11.4, H-5a), 2.94 (dd, 1H, H-5b). ^{13}C-NMR (75.4 MHz, MeOD, δ ppm) δ 148.6 (C-4''), 143.0 (Cq aromat.), 130.7 (Cq aromat.), 128.5 (C aromat.), 126.7 (C aromat.), 123.0 (C-5''), 73.5 (C-4), 73.0 (C-3), 64.9 (-*C*H_2OH), 61.7 (C-2), 51.7 (C-1'), 51.1 (C-5). HRESIMS *m/z* found 291.1454, calc. for $C_{14}H_{19}N_4O_3$ $[M+H]^+$: 291.1452.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at…

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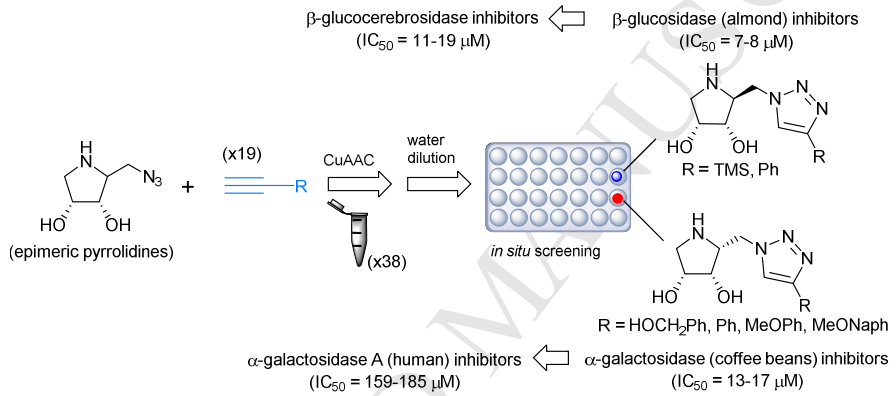
28. See supplementary data for details of preparation of **16**.

Tuning of β-glucosidase and α-galactosidase inhibition by generation and *in situ* screening of a library of pyrrolidine-triazole hybrid molecules

Macarena Martínez-Bailén,[a] Ana T. Carmona,[a] Elena Moreno-Clavijo,[a] Inmaculada Robina,[a,*] Daisuke Ide,[b] Atsushi Kato[b] and Antonio J. Moreno-Vargas[a,*]

[a]*Department of Organic Chemistry, Faculty of Chemistry, University of Seville, C/Prof. García González, 1, 41012-Seville, Spain.* [b]*Department of Hospital Pharmacy, University of Toyama, Toyama 930-0194, Japan. e-Mail: robina@us.es, ajmoreno@us.es*

Graphical Abstract



Highlights

Epimeric azidomethyl pyrrolidines were used as scaffolds for glycosidase inhibitors.

Click reaction/*in situ* screening were combined for the fast discovery of inhibitors.

β-Glucosidase (almond) inhibition correlates with β-glucocerebrosidase inhibition.

No correlation was observed between plant and human α-galactosidase inhibitors.

The new inhibitors incorporate a lipophilic group that could help to cross membranes.