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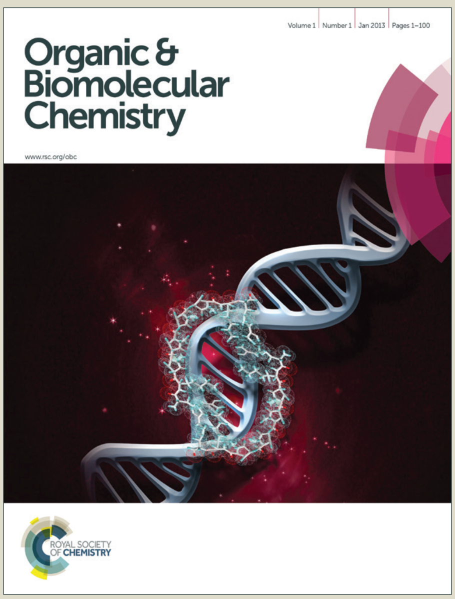

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ARTICLE

Rapid discovery of potent α-fucosidase inhibitors by *in situ* screening of a library of (pyrrolidin-2-yl)triazoles

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The synthesis of a small library of (pyrrolidin-2-yl)triazoles *via* copper catalysed cycloaddition of an alkynyl iminocyclopentitol and a set of commercial and synthetic azides has been achieved. The *in situ* screening for the activity towards α-fucosidase of the resulting triazoles has allowed the identification of one of the most potent and selective pyrrolidine derived inhibitors of this enzyme (K_i = 4 nM)

Introduction

α-Fucosidases are glycosidases involved in the biosynthesis of cell surface *O*-fucosylated oligosaccharides which play an important role in cell recognition, bacterial adhesion and viral invasion. Thus, α-fucosidases are associated to certain disorders as inflammation,[1] metastasis of certain cancer cells[2] and cystic fibrosis.[3] Recently, it has been reported that the activity of human α-fucosidase 2 is critical for the pathogenesis of *Helicobacter pylori* including gastric cancer[4] among other diseases. Moreover, α-fucosidases have been found in human seminal plasma and in the membrane of human sperm cells and facilitate sperm transport and sperm-egg interactions.[5] For these reasons, α-fucosidases are clinically important targets and the design of efficient routes for the synthesis of potent and selective inhibitors remains an attractive goal.

Over recent years, we have been actively working on the development of new iminocyclitols with inhibitory activity towards α-fucosidases.[6-9] We have shown that the presence of an additional aromatic or heteroaromatic binding component close to the five membered iminocyclitol increases notably their inhibitory activity; (**1**[10] *vs* **2**,[8] Figure 1). This fact has been also shown by Behr in the case of pyrrolidine **3**[11] and by Wong in the case of six-membered iminocyclitols (**4** *vs* **5**).[12] Nevertheless, attempts to explore chemical diversity on five-membered iminocyclitols by the systematic variation of the aromatic group remains a cumbersome task.

The Cu(I)-catalyzed alkyne-azide cycloaddition[13] (CuAAC) has become a widely used strategy for chemical space exploration in drug design[14] due to its quickness at room

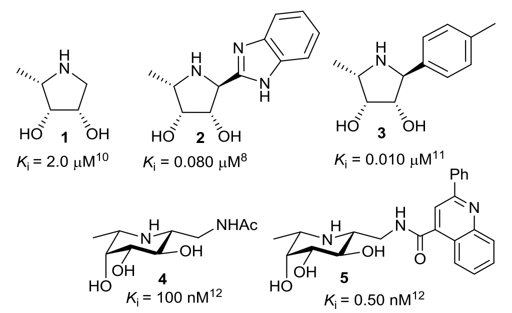
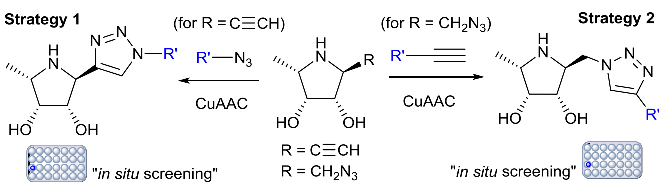


Fig 1. Inhibitory activity of representative iminocyclitols towards α-fucosidase from bovine kidney.

temperature and compatibility with water-rich solvent systems, which makes it very suitable for *in situ* screening. In the search for potent glycosidase inhibitors, the strategy of *in situ* screening of a library of compounds generated by a combinatorial procedure has been very scarcely explored to date.[12,15] Thus, the search for α-mannosidase inhibitors has been performed through imine condensation of the appropriate configured pyrrolidine carbaldehyde and amines.[15a] In the case of α-fucosidase inhibitors, L-*fuco*piperidine derivatives were sought through amide condensations from 1-aminofuconojirimycin as starting material.[12,15c] For the synthesis of pyrrolidine derivatives as hexosaminidase inhibitors, amide condensation and non-catalyzed Huisgen cycloaddition were used starting from convenient 1-amino- and 1-azido-iminocyclitols, respectively.[15b] Particularly, CuAAC has been only used in the *in situ* screening of aminocyclitols as glucocerebrosidase (a type of β-glucosidases) inhibitors,[15d] in spite that CuAAC has been broadly used in the last years as click reaction in the preparation of multivalent glycosidase inhibitors.[16]

We report herein the application of the CuAAC followed by *in situ* screening towards α-fucosidase for the preparation of analogues of compound **2** with improved inhibitory properties. As far as we are aware, no other optimization of the glycosidase inhibitory properties of iminocyclitols using this strategy has been reported. To achieve this goal and choose the appropriate lead compound, we planned two possible strategies (Scheme 1): 1) CuAAC between an unprotected alkynyl iminocyclitol with synthetic or commercial azides, and 2) CuAAC between an azidomethyl iminocyclitol and terminal alkynes. In both cases the biological analysis of the combinatorial library should be carried out by *in situ* screening, allowing determining whether the resulting substituted pyrrolidine-triazole derivatives are α-fucosidase inhibitors and if they could improve the activity of the parent pyrrolidines.

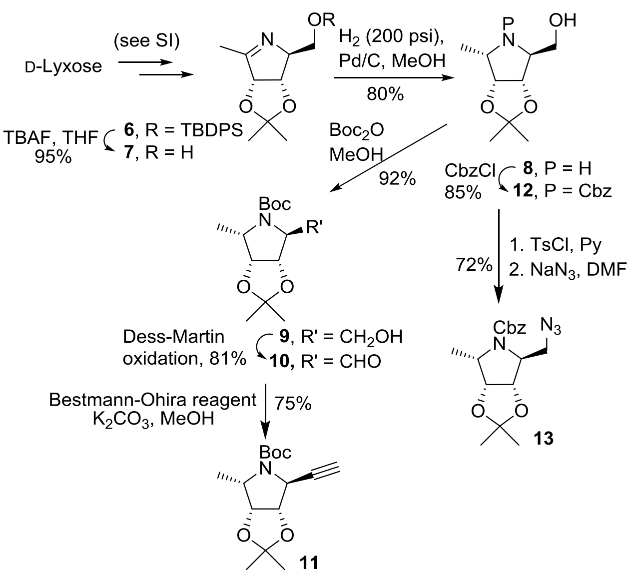


Scheme 1. Proposed strategies for the preparation of libraries of inhibitors.

Results and discussion

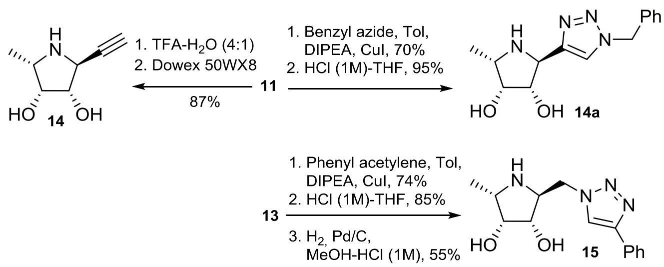
Synthesis of the lead compound for the generation of the library.

Protected precursors of the above iminocyclitols were prepared as described in Scheme 2. The synthesis of pyrroline **6** was carried out from D-lyxose following the procedure reported by Behr[17] but using the *tert*-butyldiphenylsilyl ether as protecting group.[18] Treatment of **6** with TBAF followed by stereoselective hydrogenation of **7** over Pd/C in MeOH at 200 psi gave **8**. *N*-Boc protection and oxidation furnished carbaldehyde **10**[19] which was transformed into alkyne derivative **11** after reaction with the Bestmann-Ohira reagent. On its side, *N*-Cbz protection of **8** and introduction of the azido moiety through tosylation and displacement, provided azido derivative **13**.



Scheme 2. Synthesis of protected alkynyl/azidomethyl pyrrolidines.

In order to choose the best strategy (strategy 1 or 2, Scheme 1) for the combinatorial preparation of a library of potential inhibitors, alkyne **11** and azide **13** were transformed into isomeric triazoles **14a** and **15**, respectively. Thus, CuAAC of **11** with benzyl azide using CuI/DIPEA followed by acid deprotection afforded triazole **14a** (Scheme 3). Azide **13** was transformed into triazole **15** by CuAAC with phenyl acetylene followed by deprotection. The inhibitory activity of both triazoles **14a** and **15** was studied towards eleven commercial glycosidases (Table 1). Triazole **14a** was twenty times better fucosidase inhibitor than the isomeric triazole **15**. The presence of a methylene group between the pyrrolidine skeleton and the triazole moiety proved to be detrimental for the inhibition of fucosidase. Thus, strategy 1 was chosen for the generation of the combinatorial library. For this reason, unprotected alkyne **14** was also synthesized from **11** and biologically analyzed.



Scheme 3.

Table 1. Inhibitory activities of compounds **14**, **14a** and **15** towards glycosidases. Percentage of inhibition at 1 mM of inhibitor, IC_{50} and K_i. Optimal pH for each enzyme, 37 ºC.[a,b]

Compounds/ Enzyme	14	14a	15
α-L-fucosidase (from bovine kidney)	99% IC_{50} = 0.5μM **K_i = 44 nM**	99% IC_{50} = 0.3μM **K_i = 24 nM**	99% IC_{50} = 2.1 μM **K_i = 500 nM**

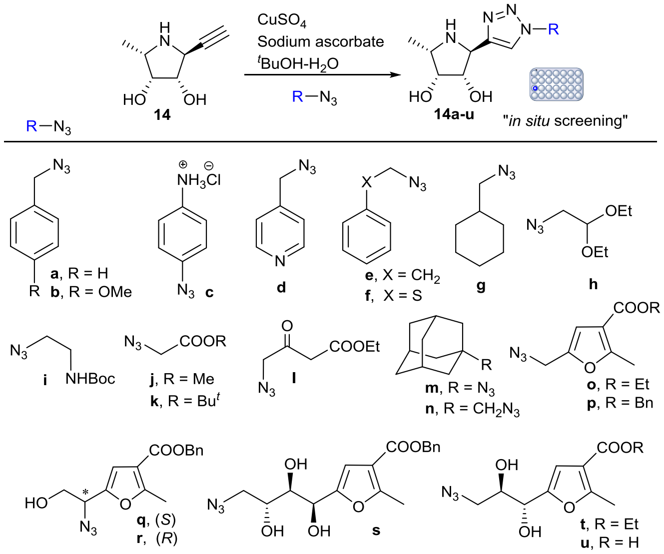
[a] No inhibition was detected towards β-galactosidases from *Aspergillus orizae* and from *Escherichia coli*, α-glucosidase from rice, α-mannosidase from Jack beans, β-*N*-acetylglucosaminidase from Jack beans, α-galactosidase from coffee beans, amyloglucosidase from *Aspergillus niger*, α-glucosidase from *Saccharomyces cerevisiae*, β-glucosidase from almonds and β-mannosidase from snail.
[b] Competitive inhibition was observed in all the cases, according to the Lineweaver-Burk plots (See Supporting Information).

Generation of a library of (pyrrolidin-2-yl)triazoles and *in situ* screening as α-fucosidase inhibitors.

In order to obtain a library of triazoles from alkyne **14**, alkyl and aryl azide reactants (Scheme 4) were selected from commercial sources or prepared from commercially available materials following standard protocols (see ESI). As we had previously reported that appropriate configured furyl iminocyclitols showed good α-fucosidase inhibition,[6,7] a batch of synthetic azides containing the furan moiety (compounds **o-u**)[20] was also used.

Parallel CuAAC between (2-ethynyl)iminocyclitol **14** and azides **a-u** were carried out under the same reaction conditions using H_2O-^{t}BuOH as solvent system (Scheme 4).[21] The *in situ* screening of the resulting crude (pyrrolidin-2-yl)triazoles towards α-fucosidase was carried out in a 96-well microtiter plate. Each inhibition assay was performed in a well containing 0.5 μM of the potential inhibitor (see Experimental for details). Blank experiments with the CuAAC reagents ($CuSO_4$ and sodium ascorbate) and with each of the azides **a-u** were also carried out: no inhibition was observed. Interestingly, several potent inhibitors were found among the 21 library members studied, their % inhibition values at 0.5 μM are shown in Figure 2. All the triazole derivatives resulted to be better inhibitors than the alkyne precursor. Compounds containing the triazole linked to other aromatic moieties showed better inhibition than when linked to non-aromatic ones (% inhibition of **14b**,**d**,**e**,**o-t** >85% *vs* % inhibition of **14h-l** < 68%). It is worth noting the high inhibition presented by the derivatives bearing the furan moiety (**14o-14u**), being **14p** the best inhibitor of the library (93% inhibition at 0.5 μM). These results were used as criteria for preliminary screening and compound selection.

In order to perform a more accurate inhibition study, selected triazole **14p** was synthesized in higher scale and purified by column chromatography. Its inhibition properties were studied towards eleven commercial glycosidases (footnote in Table 1). At 1 mM concentration, **14p** only showed inhibition towards α-fucosidase from bovine kidney, being the IC_{50} = 17 nM and K_i = 4 nM (competitive inhibition), which confirms the high potency of this inhibitor.



Scheme 4. Reaction of (2-ethynyl)iminocyclitol **14** with azides **a-u** for *in situ* screening in a microtiter plate.

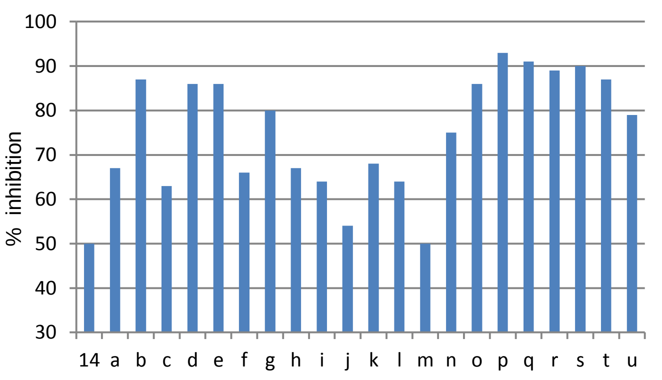


Fig 2. Inhibitory activities towards bovine kidney α-fucosidase (pH 6, 37 ºC) measured for triazole derivatives **14a-u** at 0.5 μM on the well.

Conclusions

In conclusion, we have demonstrated that the fucosidase inhibitory activity of a library of (pyrrolidin-2-yl)triazoles generated by CuAAC can be *in situ* analysed after the click reaction, avoiding the tedious isolation/purification steps. By this strategy, one of the best α-fucosidase inhibitor reported so far belonging to the pyrrolidine-iminosugar family has been identified. This is the first combinatorial method for the rapid discovery of α-fucosidase inhibitors that employs the combination of a CuAAC click reaction and *in situ* screening.

Experimental

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. ^1H 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 LSI) were recorded on Micromass AutoSpeQ and QTRAP spectrometers. The LSI was performed using thioglycerol as the matrix. 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 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.

(3*R*,4*S*,5*S*)-5-Hydroxymethyl -3,4-*O*- isopropylidene-2-methyl-1-pyrroline-3,4-diol (7).

To a solution of **6** (3.6 g, 8.5 mmol) in THF (20 mL), TBAF (1 M in THF, 8.5 mL, 8.5 mmol) was added. After stirring at r.t. for 3 h, the solvent was evaporated and the residue purified by chromatography column on silica gel (CH_2Cl_2/MeOH, 15:1) to give **7** (1.5 g, 8.1 mmol, 95%) as a colourless oil. NMR and IR data are in accordance with those of its enantiomer.[22] $[\alpha]_D^{27}$ -78.2 (*c* 0.96, CH_2Cl_2). IR (ν cm^{-1}) 3181 (OH), 2983, 2826, 1648, 1207, 1069, 868. ^1H-NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.88 (d, 1H, $J_{4,3}$= 5.6, H-3), 4.58 (d, 1H, H-4), 4.16 (br.s, 1H, H-5), 3.87 (dd, 1H, $^2J_{1'a,1'b}$= 11.6, $J_{1'a,5}$= 3.3, H-1'a), 3.76 (dd, 1H, $J_{1'b,5}$= 3.5, H-1'b), 2.76 (br.s, 1H, OH), 2.09 (d, 3H, $J_{H,H}$= 1.1, Me), 1.35 (s, 3H, -C(CH_3)$_2$), 1.34 (s, 3H, -C(CH_3)$_2$). ^{13}C-NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 176.1 (C-2), 111.9 (-*C*(CH_3)$_2$), 87.4 (C-3), 80.8 (C-4), 78.1 (C-5), 62.6 (C-1'), 27.0 (-C(CH_3)$_2$), 25.8 (-C(CH_3)$_2$), 17.1 (Me). LSIMS *m/z* 186 [33%, $(M+H)^+$]. HRLSIMS *m/z* found 186.1134, calc. for $C_9H_{16}NO_3$: 186.1130.

(2*S*,3*S*,4*R*,5*S*)-2-Hydroxymethyl-3,4-*O*-isopropylidene-5-methyl-pyrrolidine-3,4-diol (8).

A solution of pyrroline **7** (2.0 g, 10.8 mmol) in MeOH (40 mL) was stirred under H_2 (200 psi) in the presence of 10% Pd/C. After 24 h, the catalyst was filtered through celite and washed with MeOH. The solvent was evaporated and the residue purified by chromatography column on silica gel (CH_2Cl_2/MeOH, 15:1→10:1, 1% Et_3N) to give **8** (1.6 g, 8.6 mmol, 80%) as a white solid. NMR and IR data are in accordance with those of its enantiomer.[22] $[\alpha]_D^{27}$ -13.3 (*c* 0.89, CH_2Cl_2). IR (ν cm^{-1}) 3239 (OH, NH), 2977, 2874, 1369, 841. ^1H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.48 (dd, 1H, $J_{4,3}$= 5.4, $J_{4,5}$= 3.9, H-4), 4.40 (d, 1H, H-3), 3.50 (dd, 1 H, $^2J_{1'a,1'b}$= 10.2, $J_{1'a,2}$= 5.3, H-1'a), 3.32-3.24 (m, 2H, H-1'b, H-2), 3.09 (qd, 1H, $J_{5,Me}$ = 6.6, H-5), 2.93 (s, 2H, NH, OH), 1.47, 1.29 (2s, 3H each, -C(CH_3)$_2$), 1.21 (d, 3 H, Me). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 111.2 (-*C*(CH_3)$_2$), 84.1 (C-3), 83.3 (C-4), 66.0 (C-2), 60.1 (C-1'), 56.0 (C-5), 26.3, 24.1 (-C(*C*H_3)$_2$), 13.4 (Me). LSIMS *m/z* 188 [13%, $(M+H)^+$]. HRLSIMS *m/z* found 188.1285, calc. for $C_9H_{18}NO_3$: 188.1287.

(2*S*,3*S*,4*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-2-hydroxymethyl-3,4-*O*-isopropylidene-5-methylpyrrolidine-3,4-diol (9).

To a solution of **8** (198 mg, 1.06 mmol) in MeOH (8 mL), $(Boc)_2O$ (302 mg, 1.4 mmol) was added. The mixture was left at r.t. for 6 h. The solvent was evaporated and the obtained residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH, 40:1→5:1) to give **9** (281.2 mg, 0.98 mmol, 92%) as a colourless oil. $[\alpha]_D^{23}$ +59.1 (*c* 1.01, CH_2Cl_2); IR (ν cm^{-1}) 3433 (OH), 2986, 2938, 1665 (C=O), 1367, 1165, 1024, 857, 774. ^{1}H NMR (500 MHz, DMSO-d_6, 363 K, δ ppm, *J* Hz) δ 4.64 (dd, 1H, $J_{3,4}$= 6.2, $J_{3,2}$= 0.75, H-3), 4.59 (t, 1H, $J_{4,5}$= 6.2, H-4), 3.82-3.77 (m, 2H, H-2, H-5), 3.57-3.51 (m, 2H, H-1'a, H-1'b), 1.42 (s, 9H, -C(CH_3)$_3$), 1.41 (s, 3H, -C(CH_3)$_2$), 1.30 (s, 3H, -C(CH_3)$_2$), 1.25 (d, 3H, $J_{5,Me}$= 6.5, Me). ^{13}C NMR (125.7 MHz, DMSO-d_6, 363 K, δ ppm) δ 153.4 (C=O), 109.7 (-*C*(CH_3)$_2$), 80.3 (C-3), 79.7 (C-4), 78.1 (-*C*(CH_3)$_3$), 63.9, 56.4 (C-2, C-5), 59.9 (C-1'), 27.7 (-C(*C*H_3)$_3$), 25.4, 24.5 (-C(*C*H_3)$_2$), 14.9 (Me). CIMS *m/z* 288 [63%, $(M+H)^+$], 256 [56%, $(M-CH_2OH)^+$], 188 [100%, $(M+2H-Boc)^+$]. HRCIMS *m/z* found 288.1806, calc. for $C_{14}H_{26}O_5N$: 288.1811.

(2*R*,3*S*,4*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-2-formyl-3,4-*O*-isopropylidene-5-methylpyrrolidine-3,4-diol (10).

To a solution of **9** (50.7 mg, 0.176 mmol) in CH_2Cl_2 (3 mL), Dess-Martin reagent (112 mg, 0.26 mmol) was added. The mixture was stirred at r.t. for 2 h. Then CH_2Cl_2 (18 mL), sat. aq. sol. of $NaHCO_3$ (10 mL) and $Na_2S_2O_3 \cdot 5H_2O$ (250 mg, 1.0 mmol) were successively added and the resulting solution was stirred for 5 min. The organic phase was washed with sat. aq. sol. of $NaHCO_3$ and brine, dried with Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc/cyclohexane, 1:1) to give **10** (40.5 mg, 0.142 mmol, 81%) as a white solid. $[\alpha]_D^{25}$ +68.9 (*c* 0.95, CH_2Cl_2); IR (ν cm^{-1}) 2986, 1737 (C=O), 1684 (C=O), 1367, 1211, 1024, 865, 736. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 9.58 (br.s, 1H, CHO), 4.64-4.59 (m, 2H, H-4, H-3), 4.46-4.38 (m, 1H, H-2), 3.99-3.91 (m, 1H, H-5), 1.52 (s, 3H, -C(CH_3)$_2$), 1.47-1.38 (m, 12H, -C(CH_3)$_3$, Me), 1.33 (s, 3H, -C(CH_3)$_2$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 198.0 (CHO), 112.7 (-*C*(CH_3)$_2$), 81.1 (-*C*(CH_3)$_3$), 80.6, 78.2 (C-3, C-4), 71.6 (C-2), 57.6 (C-5), 28.3 (-C(*C*H_3)$_3$), 26.3, 25.2 (-C(*C*H_3)$_2$), 14.6 (Me). CIMS *m/z* 286 [10%, $(M+H)^+$], 256 [49%, $(M-CHO)^+$], 186 [100%, $(M+2H-Boc)^+$]. HRCIMS *m/z* found 286.1652, calc. for $C_{14}H_{24}O_5N$: 286.1654.

(2*S*,3*S*,4*R*,5*S*)-*N*-*tert*-Butoxycarbonyl-2-ethynyl-3,4-*O*-isopropylidene-5-methylpyrrolidine-3,4-diol (11).

To a solution of **10** (31.7 mg, 0.111 mmol) in anhydrous MeOH (1.5 mL) at 0 ºC, K_2CO_3 (31 mg, 0.22 mmol) and Bestmann-Ohira reagent (25 μL, 0.166 mmol) were successively and slowly added. The mixture was stirred at r.t. for 7 h. Diethyl ether (5 mL) and sat. aq. sol. of $NaHCO_3$ were successively added and the aqueous phase was extracted twice with CH_2Cl_2. The organic layers were washed with brine, dried with Na_2SO_4, filtered and evaporated. The resulting residue was purified by chromatography column on silica gel (EtOAc/cyclohexane,1:5) to give **11** (23.4 mg, 0.083 mmol, 75%) as a yellow oil. $[\alpha]_D^{25}$ +145.6 (*c* 0.41, CH_2Cl_2); IR (ν cm^{-1}) 2986, 2933, 1706 (C=O), 1366, 1163, 1024, 859. ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm, *J* Hz) δ 4.66-4.64 (m, 2H, H-4, H-3), 4.58 (br.s, 1H, H-2), 3.79-3.71 (m, 1H, H-5), 2.28 (d, 1H, $J_{2',2}$ = 2.4, H-2'), 1.47 (s, 12H, -C(CH_3)$_2$, -C(CH_3)$_3$), 1.42 (d, 3H, $J_{Me,5}$= 6.3, Me), 1.31 (s, 3H, -C(CH_3)$_2$). ^{13}C NMR (75.4 MHz, $CDCl_3$, δ ppm) δ 155.7 (C=O), 111.8 (-*C*(CH_3)$_2$), 82.9, 81.6 (C-4, C-3), 80.8 (-*C*(CH_3)$_3$), 80.6 (C-1'), 72.5 (C-2'), 56.5 (C-2), 55.6 (C-5), 28.5 (-C(*C*H_3)$_3$), 26.2, 25.2 (-C(*C*H_3)$_2$), 14.8 (Me). CIMS *m/z* 282 [8%, $(M+H)^+$], 226 [100%, $(M-C(CH_3)_3+2H)^+$]. HRCIMS *m/z* found 282.1707, calc. for $C_{15}H_{24}O_4N$: 282.1705.

(2*S*,3*S*,4*R*,5*S*)-*N*-Benzyloxycarbonyl-2-hydroxymethyl-3,4-*O*-isopropyliden-5-methylpyrrolidine-3,4-diol (12).

To a solution of compound **8** (265.2 mg, 1.42 mmol) in EtOH:H_2O (1:1, 20 mL), $NaHCO_3$ (120 mg, 1.43 mmol) and CbzCl (224 μL, 1.56 mmol) were added. After stirring 2 h at r.t., sat. aq. sol. of $NaHCO_3$ was added and the mixture was extracted with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered and concentrated. The resulting residue was purified by chromatography column on silica gel (EtOAc/cyclohexane, 1:2) to give **12** (385.7 mg, 1.20 mmol, 85%) as a colourless oil. $[\alpha]_D^{24}$ + 93.1 (*c* 0.53, CH_2Cl_2). IR (ν cm^{-1}) 3447 (OH), 2986, 2938, 1681 (C=O), 1410, 1210, 1026, 697. ^{1}H NMR (300 MHz, DMSO-d_6, 363 K, δ ppm, *J* Hz) δ 7.37-7.31 (m, 5H, H-aromat.), 5.10 (d, 1H, $^2J_{H,H}$ = 12.6, CH_2 of Cbz), 5.05 (d, 1H, CH_2 of Cbz), 4.68-4.60 (m, 3H, H-3, H-4, OH), 3.92-3.84 (m, 2H, H-2, H-5), 3.56-3.53 (m, 2H, H-1'a, H-1'b), 1.39, 1.29 (2s, 3H each, -C(CH_3)$_2$), 1.27 (d, 3H, $J_{Me,5}$= 6.6, Me). ^{13}C NMR (75.4 MHz, DMSO-d_6 363 K, δ ppm) δ 154.1 (C=O of Cbz), 136.6, 127.9, 127.4, 127.2 (C-aromat.), 109.9 (-*C*(*C*H_3)$_2$), 80.5, 79.8 (C-3, C-4), 65.5 (*C*H_2 of Cbz), 64.3, 56.9 (C-2, C-5), 60.0 (C-1'), 25.5, 24.6 (-C(*C*H_3)$_2$), 14.9 (Me). CIMS *m/z* 322 [2%, $(M+H)^+$], 290 [22%, $(M-CH_2OH)^+$]. HRCIMS *m/z* obsd. 322.1647, calc. for $C_{17}H_{24}NO_5$: 322.1654.

(2*S*,3*S*,4*R*,5*S*)-*N*-Benzyloxycarbonyl-2-azidomethyl-3,4-*O*-isopropyliden-5-methylpyrrolidine-3,4-diol (13).

To a 0 ºC solution of the alcohol **12** (602 mg, 1.87 mmol) in dry pyridine (16 mL), TsCl (717 mg, 3.76 mmol) was slowly added. After stirring at r.t overnight, the mixture was cooled to 0 ºC, water was slowly added, and the mixture was allowed to warm to r.t. Solvent was then removed, and the residue was diluted with EtOAc, washed with HCl (1N), sat. aq. sol. of $NaHCO_3$ and brine, dried, filtered, and concentrated. To a solution of this compound in DMF (16 mL), NaN_3 (305 mg, 4.69 mmol) was added. After heating at 70 ºC for 2 h, the solvent was evaporated and the residue diluted with CH_2Cl_2 and washed with water and brine. The organic phase was dried, filtered, and concentrated. Purification by chromatography column (EtOAc:cyclohexane 1:6) afforded **13** (464 mg, 1.34 mmol, 72%) as a colourless oil. $[\alpha]_D^{28}$ + 60.4 (*c* 0.55, CH_2Cl_2). IR (ν cm^{-1}) 2986, 2938, 2103 (N_3), 1693 (C=O), 1403, 1210, 1026, 697. ^{1}H NMR (300 MHz, DMSO-d_6, 363 K, δ ppm) δ 7.38-7.30 (m, 5H, H-aromat.), 5.14 (d, 1H, $^2J_{H,H}$ = 12.5, CH_2 of Cbz), 5.08 (d, 1H, CH_2 of Cbz), 4.68 (t, 1H, $J_{4,3}$= $J_{4,5}$= 6.2, H-4), 4.56 (dd, 1H, $J_{3,2}$ = 1.0, H-3), 4.01-3.98 (m, 1H, H-2), 3.90 (q, 1H, $J_{5,Me}$= 6.4, H-5), 3.66 (dd, 1H, $^2J_{1'a,1'b}$= 12.8, $J_{1'a,2}$= 6.0, H-1'a), 3.53 (dd, 1H, $J_{1'b,2}$= 3.5, H-1'b), 1.40, 1.30 (2s, 3H each, -C(CH_3)$_2$,) 1.28 (d, 3H, Me). ^{13}C NMR (75.4 MHz, DMSO-d_6, 363 K, δ ppm) δ 153.6 (C=O of Cbz), 136.2, 127.8, 127.4, 127.2 (C-aromat.), 110.4 (-*C*(*C*H_3)$_2$), 80.5 (C-3), 79.4

(C-4), 65.8 (CH_2 of Cbz), 61.8 (C-2), 56.6 (C-5), 50.5 (C-1'), 25.4 , 24.4 (-C(CH_3)$_2$), 14.6 (Me). CIMS *m/z* 347 [2%, $(M+H)^+$], 290 [26%, $(M-CH_2N_3)^+$]. HRCIMS *m/z* found 347.1727, calc. for $C_{17}H_{23}N_4O_4$: 347.1719.

(2*S*,3*S*,4*R*,5*S*)-2-ethynyl-5-methylpyrrolidine-3,4-diol (14).

Compound **11** (44.7 mg, 0.16 mmol) was dissolved in TFA:H_2O 4:1 (0.5 mL) and the mixture was stirred at r.t. for 2.5 h. Evaporation of the solvent and chromatographic purification on Dowex 50WX8 eluting with MeOH (50 mL), H_2O (50 mL) and NH_4OH 10%, afforded **14** (19.2 mg, 0.14 mmol, 87 %) as a yellow solid. $[\alpha]_D^{26}$ -10.6 (*c* 0.83, MeOH); IR (ν cm^{-1}) 3306 (OH, NH), 1662, 1454, 1190, 1134, 844, 799, 724. ^{1}H NMR (300 MHz, MeOD, δ ppm, *J* Hz) δ 4.17 (dd, 1H, $J_{3,2}$= 6.8, $J_{3,4}$= 4.4, H-3), 3.91 (t, 1H, $J_{4,5}$ = 4.4, H-4), 3.77 (dd, 1H, $J_{2,2'}$= 2.3, H-2), 3.37-3.28 (m, 1H, H-5), 2.73 (d, 1H, H-2'), 1.16 (d, 3H, $J_{Me,5}$= 6.7, Me). ^{13}C NMR (75.4 MHz, MeOD, δ ppm) δ 85.3 (C-1'), 81.0 (C-3), 74.5 (C-4), 73.2 (C-2'), 56.6 (C-5), 54.3 (C-2), 14.6 (Me). CIMS *m/z* 142 [86%, $(M+H)^+$]. HRCIMS *m/z* found 142.0866, calc. for $C_7H_{12}O_2N$: 142.0868.

(2*S*,3*S*,4*R*,5*S*)-2-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)-5-methyl-pyrrolidine-3,4-diol (14a).

DIPEA (115 μL, 0.658 mmol) and CuI (10 mg, 0.05 mmol) were added to a solution of **11** (47.1 mg, 0.167 mmol) and benzyl azide (29.7 mg, 0.223 mmol) in toluene (1.5 mL). The mixture was stirred at r.t. for 6 h and then, a sat. aq. sol. of $NaHCO_3$ was added and extracted with EtOAc. The organic phases were dried, filtered and concentrated under reduced pressure. The resulting residue was purified by chromatography column on silica gel (EtOAc/cyclohexane, 1:3) to give the corresponding protected triazole derivative (47.9 mg, 0.116 mmol, 70%) as a white solid. Deprotection of this compound (47.9 mg, 0.116 mmol) was carried out in HCl (4 M):THF 1:1 (3 mL) at r.t. for 20 h. Solvent was then evaporated and the residue was purified by chromatography column on silica gel (CH_2Cl_2/MeOH/NH_4OH, 9:1:0.1) to give **14a** (29.9 mg, 0.11 mmol, 95%) as a yellow solid. $[\alpha]_D^{26}$ -34.0 (*c* 0.85, MeOH); IR (ν cm^{-1}) 3259 (OH, NH), 2919, 2360, 1453, 1051, 727, 611. ^{1}H NMR (500 MHz, DMSO-d_6, 363K, δ ppm, *J* Hz) δ 8.00 (s, 1H, H-5'), 7.38-7.32 (m, 5H, H-aromat.), 5.57 (s, 2H, -CH_2Ph), 4.24-4.20 (m, 2H, H-2, H-3), 3.89 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 3.5, H-4), 3.47-3.42 (m, 1H, H-5), 1.13 (d, 3H, $J_{Me,5}$= 6.5, Me). ^{13}C NMR (125.7 MHz, DMSO-d_6, 363K, δ ppm) 147.2 (C-4'), 135.5, 128.2, 127.6, 127.5 (C-aromat.), 122.1 (C-5'), 77.5 (C-3), 72.3 (C-4), 57.1 (C-2), 55.1 (C-5), 52.5 (-CH_2Ph), 13.8 (Me). HRCIMS *m/z* found 275.1506, calc. for $C_{14}H_{19}O_2N_4$: 275.1508.

(2*S*,3*R*,4*S*,5*S*)-2-Methyl-5-[(4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl]pyrrolidine-3,4-diol (15).

To a solution of azide **13** (50.1 mg, 0.145 mmol) in toluene (3 mL), phenylacetylene (19 μL, 0.17 mmol), DIPEA (97 μL, 0.56 mmol) and CuI (7 mg, 0.037 mmol) were added. The mixture was stirred for 18 h at r.t. Then, the mixture was diluted with EtOAc and washed with sat. aq. sol. of $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered and evaporated. The resulting residue was purified by column chromatography on silica gel (Toluene/acetone, 10:1), affording the corresponding protected triazole derivative (47.8 mg, 0.107 mmol, 74%). A solution of this compound (40.9 mg, 0.091 mmol) in HCl (1M)-THF (1:1, 3.5 mL) was stirred for 24 h, then the mixture was evaporated. The resulting crude was purified by chromatography column (EtOAc/Hex, 1:1) to give pure diol (85%). The 3,4-*O*-unprotected pyrrolidine (28 mg, 0.069 mmol) was dissolved in MeOH-HCl (1M) (1:1, 3 mL), a catalytic amount of Pd/C (10%) was added and the resulting mixture was hydrogenated under atmospheric pressure for 2 h. The catalyst was filtered over celite and the filtered solution was evaporated. The resulting residue was purified by chromatography column on silica gel (DCM/MeOH/NH_4OH, 5:1:0.05) affording **15** (10.4 mg, 0.038 mmol, 55%). $[\alpha]_D^{28}$ - 24.2 (*c* 0.72, MeOH). IR (ν cm^{-1}) 3300 (OH), 1452, 1237, 1119, 764, 692. ^{1}H NMR (500 MHz, CD_3OD, δ ppm, *J* Hz) δ 8.37 (s, 1H, H-5''), 7.82-7.80 (m, 2H, H-aromat.), 7.45-7.41 (m, 2H, H-aromat.), 7.36-7.32 (m, 1H, H-aromat.), 4.63 (dd, 1H, $^2J_{1'a,1'b}$= 13.9, $J_{1'a,5}$= 4.3, H-1'a), 4.48 (dd, 1H, $J_{1'b,5}$= 8.1, H-1'b), 3.99 (dd, 1H, $J_{4,5}$= 8.1, $J_{4,3}$ = 4.1, H-4), 3.81 (br. dd, 1H, $J_{3,2}$= 3.0, H-3), 3.64 (td, 1H, H-5), 3.18 (qd, 1H, $J_{Me,2}$= 6.7, H-2), 1.16 (d, 1H, Me). ^{13}C NMR (125.7 MHz, CD_3OD, δ ppm) δ 148.8 (C-4''), 131.8, 130.0, 129.3, 126.7 (C-aromat.), 123.0 (C-5''), 77.6 (C-4), 75.1 (C-3), 62.8 (C-5), 56.7 (C-2), 54.6 (C-1'), 14.4 (Me). HRCIMS *m/z* found 275.1508, calc. for $C_{14}H_{19}O_2N_4$: 275.1508.

(2*S*,3*S*,4*R*,5*S*)-2-[1-((4-Benzyloxycarbonyl-5-methylfuran-2-yl)methyl)-1*H*-1,2,3-triazol-4-yl]-5-methylpyrrolidine-3,4-diol (14p).

A solution of alkyne **14** (13.9 mg, 0.098 mmol) and azide **p** (32.1 mg, 0.118 mmol) in a 2:1 mixture of ^{t}BuOH/H_2O (3.3 mL) was treated with a catalytic amount of $CuSO_4$ (0.54 mg, $3.37\cdot10^{-3}$ mmol) followed by sodium ascorbate (2 mg, 0.011 mmol). After 24 h at r.t., the mixture was concentrated under reduced pressure. The resulting residue was purified by chromatography column on silica gel (CH_2Cl_2/MeOH, 8:1) to give **14p** (22 mg, 0.053 mmol, 54%) as a yellow solid. $[\alpha]_D^{22}$ -33.2 (*c* 0.52, MeOH); IR (ν cm^{-1}) 3346 (OH, NH), 2912, 1711, 1218, 1076, 770, 623. ^{1}H NMR (500 MHz, DMSO-d_6, 363K, δ ppm, *J* Hz) δ 7.90 (s, 1H, H-5'), 7.41-6.76 (m, 5H, H-aromat.), 6.76 (s, 1H, H-3'''), 5.56 (s, 2H, H-1''), 5.28 (s, 2H, -CH_2Ph), 4.13-4.12 (m, 2H, H-2, H-3), 3.83 (t, 1H, $J_{4,3}$ = $J_{4,5}$ = 4.0, H-4), 3.33-3.31 (m, 1H, H-5), 2.52 (s, 3H, Me), 1.07 (d, 3H, $J_{Me,5}$ = 6.5, Me). ^{13}C NMR (125.7 MHz, DMSO-d_6, 363K, δ ppm) 162.1 (C=O), 158.8, 149.3, 146.8, 135.8, 127.9, 127.5, 127.3, 113.4 (C-aromat.), 121.3 (C-5'), 109.8 (C-3'''), 78.1, 57.7 (C-2, C-3), 72.8 (C-4), 65.0 (-CH_2Ph), 54.7 (C-5), 45.0 (C-1''),14.7 (Me), 12.9 (Me). HRCIMS *m/z* found 413.1822, calc. for $C_{21}H_{25}O_5N_4$: 413.1825.

***In situ* screening towards α-fucosidase. Glycosidase inhibition assays.**

To a solution of alkyne **14** (0.3 mL, 30 mM in ^{t}BuOH-H_2O (2:1)) in an eppendorf, a solution of the corresponding azide (**a**-**u**) was added (0.1 mL, 108 mM in ^{t}BuOH) followed by 25 μL of an aqueous solution of sodium ascorbate (40 mM) and 25 μL of an aqueous solution of $CuSO_4$ (12 mM). The final concentration of the alkyne in each eppendorf was 20 mM. The resulting mixtures were left at room temperature for 24 h (the reactions were monitored for completion by TLC). Then, the reactions were diluted to the desired concentration in order to use it in the enzymatic assays. In the preliminary screening of the resulting crude (pyrrolidine-2-yl)triazoles, % of inhibition towards α-fucosidase from bovine kidney (EC 3.2.1.51) was determined in the presence of 0.5 μM of the inhibitor on the well (concentration corresponding to the IC_{50} of the alkyne **14**) with *p*-nitrophenyl α-L-fucopyranoside (Sigma-Aldrich) as substrate. 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 glycoside 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 ºC, the reaction was stopped by addition of 0.1 mL of sodium borate buffer (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. Blank experiments with sodium ascorbate/$CuSO_4$ and with each of the azides **a-u** were also carried out. An aqueous solution of sodium ascorbate (0.66 μM), $CuSO_4$ (1 μM), a solution of $CuSO_4$-sodium ascorbate (0.5 μM and 0.33 μM, respectively) and a solution of each azide (1.8 μM) were prepared in different wells. Each solution was assayed towards α-fucosidase as described above and no inhibition was observed for all cases. For the highest inhibition rate (triazole **14p**) and for compounds **14**, **14a** and **15**, the IC_{50} value (concentration of inhibitor required for 50% inhibition of enzyme activity) and K_i towards α-fucosidase were calculated. IC_{50} values were calculated from plots of percentage of inhibition *versus* inhibitor concentration and the K_i values were determined from the Lineweaver-Burk plots (See ESI for details). All the experiments were performed by duplicate. In the case of the other ten enzymes, % of inhibition was determined at 1 mM of inhibitor on the well with the appropriate *p*-nitrophenyl glycosides as substrates. Commercially available glycosidases from Sigma-Aldrich: β-galactosidase (EC 3.2.1.23) from *Aspergillus orizae* and from *Escherichia coli*, α-mannosidase (EC 3.2.1.24) from Jack beans, β-*N*-acetylglucosaminidase (EC 3.2.1.30) from Jack beans, α-galactosidase (EC 3.2.1.22) from coffee beans, amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger*, α-glucosidase (EC 3.2.1.20) from rice, α-glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae*, β-glucosidase (EC 3.2.1.21) from almonds and β-mannosidase (EC 3.2.1.25) from snail.

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Notes and references

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Electronic Supplementary Information (ESI) available: experimental details for the preparation of **6** and azides **a-u**, enzymatic test details, ^{1}H- and ^{13}C-NMR spectra. See DOI: 10.1039/b000000x/

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