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Synthesis and L-fucosidase inhibitory potency of a cyclic sugar imine and its pyrrolidine analogue

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ABSTRACT

The synthesis of a fully deprotected ketimine-type iminosugar is reported, starting from commercial D-mannose diacetonide. An appropriate solvent system was critical for the success of the key tandem addition/cyclization reaction. Reduction of the imine was also achieved to yield the corresponding pyrrolidine in a stereoselective manner. The cyclic sugar imine displayed modest fucosidase inhibitory activity when compared to the saturated analogue.

1. Introduction

Glycosidases are involved in a wide range of biochemical transformations, such as digestion, lysosomal catabolism of polysaccharides and glycoconjugates or biosynthesis of glycoproteins and glycolipids.[1] Hence, the control of these processes by specific inhibitors in vivo is of great therapeutic interest. Iminosugars are an important class of glycosidase inhibitors that have attracted considerable attention as antidiabetics, as well as anticancer or anti-infective agents.[2] The strong affinity of iminosugars for glycosidases is generally attributed to a structural analogy with the oxocarbenium-like transition state involved in the enzymatic reaction.[3] As a part of our search for potent α-L-fucosidase inhibitors, we have recently introduced the spirocyclopropyl moiety in the structures of fucose-derived iminosugars to flatten the heterocyclic ring in the proximity of the nitrogen atom to mimic the distorted half-chair conformation of the fucosyl cation (Fig. 1).[4] Promising results were obtained with pyrrolidine **1**, which displayed potent inhibition of α-L-fucosidase (K_i = 1.6 μM).[5] Another chemical motif might force the structures of iminosugars into the required conformation, that is, the C=N unsaturation as found in cyclic sugar imines.[6] Continuing our interest in more varied α-L-fucosidase inhibitors, we herein report the rapid synthesis of such an unsaturated iminosugar (compound **2**) and its hydrogenated analogue **3**, structurally related to the potent inhibitor **1**. The comparison of their inhibitory potencies will provide a first set of SAR studies.

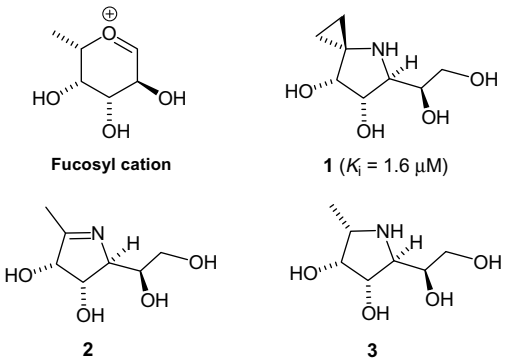

Figure 1. Structures of compounds **1**–**3**.

2. Results and discussion

Despite their resemblance to the putative transition state of glycosidases, only a few, fully deprotected sugar ketimines have been reported in the literature and assayed against glycosidases.[6f] We have recently described a general approach to polyhydroxypyrrolines, which was based on the tandem addition/cyclization reaction of Grignard reagents to the easily available ω-methanesulfonyl-glyconononitriles.[7] We wished to apply this methodology to the synthesis of the fucose-configured pyrroline **2**. In this regard, the addition of MeMgBr to the mannononitrile **4** might afford an intermediate imide salt **5a** that could undergo an intramolecular nucleophilic displacement to give the target ketimine **6** (Scheme 1).

The starting mannononitrile mesylester **4** was obtained from commercially available D-mannose diacetonide by reaction with hydroxylamine and subsequent treatment with an excess of methanesulfonyl chloride, as reported earlier.[5] Our initial attempts were focused on bringing about the addition/cyclization reaction in the optimized conditions from the literature.[7] Nevertheless, when glyconononitrile **4** was treated with 1.5 equiv of MeMgBr in toluene

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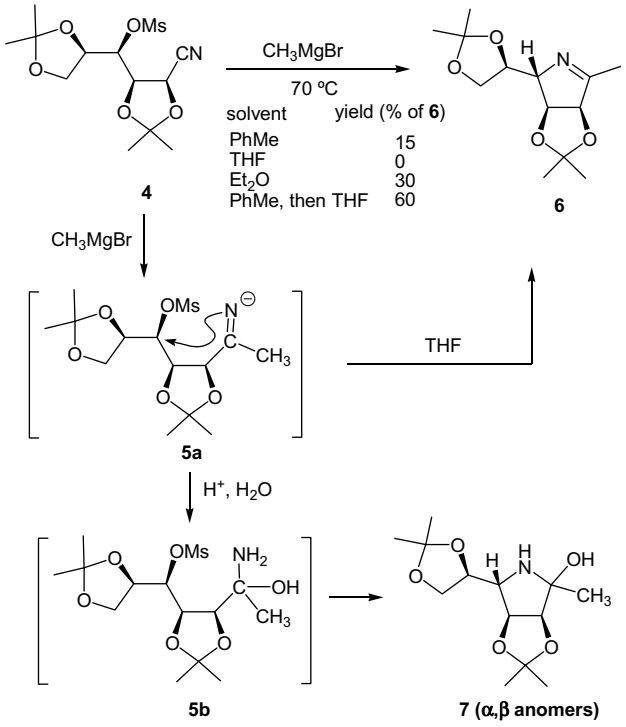
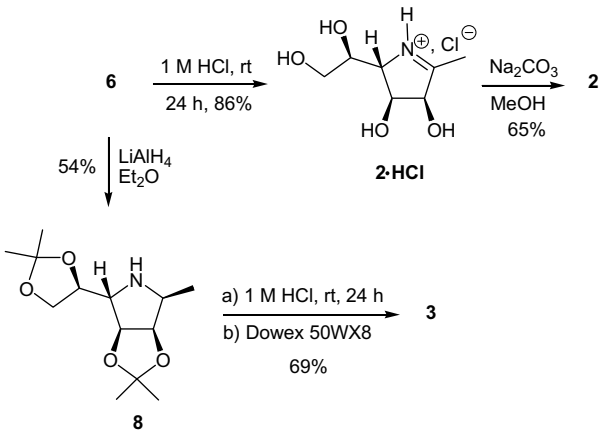


Scheme 1. Synthesis of cyclic sugar imine **6**.



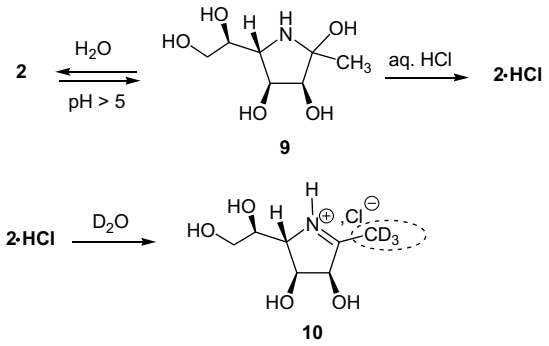
Scheme 2. Synthesis of compounds **2**·**HCl**, **2** and **3**.

at 70 °C for 1.5 h, pyrroline **6** was isolated in only 15% yield. The major product of the reaction was hemiaminal **7** (two anomers), the hydrate derivative of imine **6**. No equilibrium was observed between compounds **6** and **7** either in the presence of water or on silica gel. Both products were independently isolated in pure and stable form. Attempts to dehydrate **7** into the target compound **6** according to known procedures failed.[8] Furthermore, when the standard protocol was applied to ketimine **6** (addition of MeMgBr and treatment of the reaction mixture with an aqueous solution of NH_4Cl), no reaction occurred and imine **6** was recovered quantitatively without any trace of **7**. These observations provide evidence that the major product **7** does not derive from its possible precursor **6** by simple addition of water during the work-up. Alternatively, a less obvious reaction pathway must account for its formation. As depicted in Scheme 1, hemiaminal **7** could be obtained by cyclization of the amino intermediate **5b**, which might form during the work-up after hydrolysis of ketimine salt **5a**. Thus, the poor yields observed for pyrroline **6** could be attributed to the low efficiency of the cyclization step. Since it is known that such a ring closure is accelerated in more polar solvents,[9] we examined Et_2O and THF as the reaction solvents (Scheme 1). In Et_2O, the complete disappearance of the starting material occurred after 2 h at 35 °C to give a complex mixture of several products amongst which ketimine sugar **6** was isolated in 30% yield. When THF was used as the solvent, no reaction occurred, even at 70 °C, and the starting material was entirely recovered. Fortunately, the addition of THF once the initial nucleophilic addition in toluene at 70 °C is completed (1.5 h) is sufficient to promote the expected ring formation. This procedure afforded imine **6** in greatly improved yield (60%) after silica gel chromatography.

In the next step, removal of the isopropylidene groups was achieved efficiently by treatment of **6** with a 1 M HCl solution overnight (Scheme 2). The crude hydrochloride salt **2**·**HCl** was isolated as a yellow solid after evaporation of the solvent. Pure material was obtained as a colourless foam in 86% yield, after purification on a RP support (elution with 10 mM HCl). Compounds **6** and **2**·**HCl** proved to be stable at −20 °C for weeks and at room temperature for at least 7 days, since no decomposition was noticed in the ^1H NMR spectrum after this time. The free ketimine **2** was prepared by neutralization of a methanolic solution of the corresponding hydrochloride salt with Na_2CO_3, evaporation of the solvent and solubilization of the ketimine in a CH_2Cl_2/MeOH solution. Cyclic sugar imine **2** was isolated as a yellow oil in 65% yield following this procedure.

Preparation of the saturated analogue **3** required the reduction of the C=N bond (Scheme 2). The reaction of **6** with $LiAlH_4$ proved to be completely stereoselective,[10] affording pyrrolidine **8** in 54% yield (not optimized) after purification by silica gel chromatography. The (2*S*)-configuration for pyrrolidine **8** was unambiguously deduced from NMR analysis of the deprotected derivative **3** (see below). The selective formation of isomer **8** might be explained by an exclusive hydride delivery from the preferred *Re* face; the upper *Si* face of the C=N bond being impeded by the 3,4-*O*-isopropylidene moiety. No trace of the epimer was detected in the crude mixture. The modest yield for this transformation is mainly due to a loss of product during the work-up. Acidic treatment of **8** (1 M HCl) allowed the removal of the isopropylidene groups and purification by ion-exchange chromatography (elution, 0.8 M NH_4OH) afforded pyrrolidine **3** in 69% yield. As observed for analogues, the $^3J_{H\text{-}2,H\text{-}3}$ coupling constant (3.4 Hz) in **3** indicates a *cis*-orientation of both hydrogen atoms, which ascertains the (2*S*)-configuration. A *trans*-orientation between H-2 and H-3 in 2-methylpolyhydroxypyrrolidines is usually characterized by a 7 Hz coupling constant.[11]

Before performing the biological assays, we analyzed the chemical behaviour and stability of the cyclic imines by NMR analysis (Scheme 3). In CD_3OD, compounds **2** and **2**·**HCl** appeared as a single spin system, which remained completely stable, even after 7 days at room temperature. The imine salt **2**·**HCl** showed δ = 198.1 ppm for C-2, which is consistent with protonation at nitrogen,[7,12] whereas the free imine showed δ = 182.2 ppm for C-2. Compound **2**·**HCl** was stable in water at pH 4 (natural pH). However, after 24 h, a minor species arose, which was identified as the hydrate **9** by analytical and spectral data (Scheme 3).[13] Formation of **9** was much faster at higher pH or in an aqueous solution of the free imine **2**. At pH 7, equilibrium between the imine and its hydrate took place rapidly leading to a stable mixture of both compounds in a 6:4 ratio that did not decompose with time. Reversibility of the reaction was observed by lowering the pH (<2) with aqueous HCl. Under these conditions, only the imine form existed in solution and lyophilization of the reaction mixture gave back compound **2**·**HCl**. The chemical behaviour of ketimines **2** or **2**·**HCl** might be correlated to that of cyclic aldimines, which are known to follow such reversible transformations in water.[14] Furthermore,

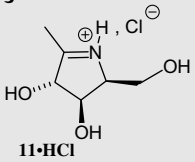


Scheme 3. Chemical behaviour of cyclic sugar imines.

in D_2O, a rapid deuteriation of the methyl substituent was observed ($t_{1/2}$ = 2 h) as deduced from the disappearance of the signal at δ = 2.48 ppm in the ^{1}H NMR spectrum of ketimine **2·HCl**. No deuteriation occurred at other positions, in contrast with that observed with 2-methylpyrroline.[12]

Imines **2·HCl** and **2**, as well as pyrrolidine **3** were assayed against α-L-fucosidase (Table 1) and a panel of 12 other glycosidases. Glycosidase activities were determined at 37 °C at the optimal pH of each enzyme (typically, pH < 7), at which the ketimine existed mainly as a mixture of the unsaturated and the hydrated form (see above).

Table 1

Entry		% Inhibition at 1 mM (K_i)
1	**1**[a]	97 (1.6 μM, competitive)
2	**2·HCl**	0
3	**2**	60
4	**3**	99 (0.2 μM, competitive)
5	 **11·HCl**	86 (9.8 μM, mixed)

[a] See Ref. 5.

Pyrrolidine **3** was the most potent inhibitor of α-L-fucosidase amongst the compounds tested (99% inhibition at 1 mM) and displayed sub-μM K_i value (0.2 μM). Surprisingly, unsaturated iminosugars **2·HCl** and **2** exhibit different inhibition profiles: imine **2** was a weak fucosidase inhibitor (60% inhibition at 1 mM), whereas **2·HCl** was completely inactive at this concentration. We also assayed compound **11·HCl**, an analogue of **2·HCl**, which has recently been synthesized.[7] Surprisingly, imine **11·HCl** induced 86% inhibition of α-L-fucosidase at 1 mM, with K_i = 9.8 μM (mixed inhibition). These results deserve comment. Firstly, comparison of the inhibition potencies of pyrrolidines **1** and **3** clearly demonstrates that the presence of the constraining and sterically demanding spirocyclopropyl substituent is detrimental for the insertion in the fucosidase active site, despite the presence of a large surrounding hydrophobic pocket. Next, the presence of the double bond in the structure of **2** diminishes dramatically the affinity for the enzyme. Either steric or electronic structural differences with the putative carbocation might explain this unexpected result. According to the pK_a of the conjugated acid, both imine **2** (pK_a ca. 7) and pyrrolidine **3** (pK_a ca. 9) must be protonated in the enzymatic assay mixture (pH = 6.0 for α-L-fucosidase). A difference in the protonation state of these ligands cannot account for the large differences in binding. On the other hand, pyrroline **11·HCl** displays a strong affinity for the enzyme, even though it seems not adapted to mimic the hydroxyl topography of the fucosyl cation. These results suggest that the unsaturated iminosugars bind in a different manner than their saturated counterparts, which has to be investigated further with other isomers.

Concerning the other glycosidases, compounds **2·HCl**, **2**, **3** and **11·HCl** were inactive at the tested concentration of 1 mM towards coffee bean α-galactosidase, *Escherichia coli* and *Aspergillus orizae* β-galactosidase, yeast and rice α-glucosidase, *Aspergillus niger* amyloglucosidase, almonds β-glucosidase, Jack bean α-mannosidase, snail β-mannosidase, *A. niger* β-xylosidase, Jack bean and bovine kidney β-*N*-acetyl-glucosaminidase.

3. Conclusion

The straightforward synthesis of a ketimine-type iminosugar is reported in a three-step sequence from commercially available diacetone-D-mannose. The experimental conditions of the key tandem addition/cyclization reaction with MeMgBr, as well as the stability and chemical behaviour of the unsaturated iminosugar were carefully studied. Reduction of the imine with $LiAlH_4$ gave rise to the corresponding pyrrolidine in a stereoselective manner. The deprotected pyrroline **2** and pyrrolidine **3** were evaluated for their inhibitory activity against a panel of glycosidases. The ketimine-type iminosugar **2** showed a modest, but very specific, inhibition of α-L-fucosidase (60% at 1 mM). Interestingly, pyrrolidine **3** displayed a sub-μM K_i value (0.2 μM) and was also completely selective towards α-L-fucosidase. The synthesis and biological evaluation of other unsaturated iminosugars are under investigation to establish the biological significance of these compounds.

4. Experimental

4.1. General

All non-aqueous reactions were carried out under an argon atmosphere for the exclusion of moisture and air. THF and toluene were distilled over potassium/benzophenone. Analytical thin layer chromatography was performed on Merck silica gel (0.2 mm) TLC plates with F-254 indicator. Flash column chromatography was performed using Merck 9385 (40–63 μm) Kieselgel 60 Silica Gel. IR spectra were obtained on a IR^{TM} plus MIDAC spectrophotometer. Optical rotations were measured on a Perkin–Elmer Model 241 polarimeter. The HRMS data were performed by D. Harakat on Q-TOF Micro micromass positive ESI (CV = 30 V). ^{1}H and ^{13}C NMR spectra were recorded on a Brucker AC 250 spectrometer. All chemical shifts are referenced to internal TMS or residual undeuterated solvent.

4.2. (3*R*,4*S*,5*S*)-3,4-Dihydroxy-5-[(1′*S*)-1′,2′-dihydroxyethyl]-3,4:1′,2′-di-*O*-isopropylidene-2-methyl-1-pyrroline 6

A solution of glyconitrile **4** (110 mg, 0.33 mmol) in toluene (5 mL) under argon was heated at 70 °C and the Grignard reagent (235 μL of a 1.4 M solution, 1.0 equiv) was added dropwise. The solution was stirred at 70 °C for 1.5 h, and THF (0.5 mL) was then added. The suspension was allowed to cool down slowly to room temperature while stirring for 2 h. Then Et_2O (10 mL) and a saturated solution of NH_4Cl (20 mL) were successively added at 0 °C and the resulting organic phase was separated. The aqueous layer was extracted with Et_2O (2 × 10 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography with petroleum ether/EtOAc (50/50) to yield pure **6** (50 mg, 60%) as a yellow oil; R_f = 0.4 [petroleum ether/EtOAc (50/50)]; $[\alpha]_D^{20} = -59.7$ (*c* 1.26, $CHCl_3$); IR (film) 2987, 2936, 1651, 1372, 1255,

1213, 1083 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 4.80 (d, 1H, J = 5.3 Hz), 4.49 (d, 1H, J = 5.3 Hz), 4.32 (dt, 1H, J = 6.8, 3.2 Hz), 4.15 (br s, 1H), 4.00 (m, 2H), 2.26 (s, 3H), 1.38 (s, 6H), 1.35 (s, 3H), 1.22 (s, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 176.7, 112.1, 109.8, 87.4, 81.2, 76.9, 76.8, 66.3, 27.3, 26.3, 26.2, 25.9, 17.4; ESI-HRMS: calcd for $C_{13}H_{22}NO_4$ $[M+H]^+$ 256.1549; found 256.1550.

4.3. (2*S*,3*R*,4*S*,5*S*)-3,4-Dihydroxy-5-[(1′*S*)-1′,2′-dihydroxyethyl]-3,4:1′,2′-di-*O*-isopropylidene-2-methylpyrrolidine 8

To a solution of imine **6** (50 mg, 0.196 mmol) in Et_2O at 0 °C under argon was added dropwise a solution of $LiAlH_4$ in Et_2O (98 μL of a 4 M solution, 0.392 mmol). The suspension was stirred for 1 h and left to warm up to room temperature. THF (1 mL) was then added, and the resulting clear solution was treated with water (32 μL), 3 M NaOH (32 μL) and water again (100 μL). After 1 h the solution was passed through a pad of Celite, dried over $MgSO_4$ and concentrated. Analysis of the crude residue revealed the presence of only one product. Purification by silica gel chromatography with petroleum ether/EtOAc (50/50) afforded pure **8** (27 mg, 54%) as a colourless oil; R_f = 0.5 [petroleum ether/EtOAc (50/50)]; $[\alpha]_D^{20} = -15.0$ (c 0.3, $CHCl_3$); IR (film) 2985, 2934, 1380, 1371, 1209 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 4.80 (dd, 1H, J = 5.7, 4.0 Hz), 4.44 (d, 1H, J = 5.7 Hz), 4.03 (m, 2H), 3.80 (m, 1H), 3.22 (dq, 1H, J = 6.5, 4.0 Hz), 3.12 (d, 1H, J = 6.3 Hz), 1.47, 1.42, 1.34 and 1.31 (4 × s, 4 × 3H), 1.20 (d, 3H, J = 6.5 Hz); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ 111.5, 109.6, 84.9, 83.6, 75.8, 67.1, 66.9, 57.1, 26.6, 26.3, 25.5, 24.3, 14.1; ESI-HRMS: calcd for $C_{13}H_{24}NO_4$ $[M+H]^+$ 258.1705; found 258.1707.

4.4. (3*R*,4*S*,5*S*)-3,4-Dihydroxy-5-[(1′*S*)-1′,2′-dihydroxyethyl]-2-methyl-1-pyrroline hydrochloride 2·HCl

Imine **6** (62 mg, 0.24 mmol) was dissolved in a small amount of EtOH (ca. 0.2 mL), and a solution of 1 M HCl (3 mL) was added. The resulting clear solution was stirred at room temperature overnight and concentrated. The residue (60 mg) was purified by chromatography on a RP-HP20 support by elution with 10 mM HCl to afford **2·HCl** (43 mg, 86%) as a colourless solid after lyophilization of the product-containing fractions. $[\alpha]_D^{20} = -122.0$ (c 0.3, MeOH); IR (film) 1588, 1413 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 5.04 (d, 1H, J = 5.4 Hz), 4.46 (d, 1H, J = 5.4 Hz), 4.33 (br s, 1H), 4.00 (m, 1H), 3.69 (dd, 1H, J = 11.3, 5.2 Hz), 3.57 (dd, 1H, J = 11.3, 6.3 Hz), 2.48 (s, 3H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 198.1, 78.8, 76.8, 73.7, 71.8, 64.4, 16.3; MS (ESI) m/z: 176.1 (100%, MH^+); ESI-HRMS: calcd for $C_7H_{14}NO_4$ $[M+H]^+$ 176.0923; found 176.0929.

4.5. (3*R*,4*S*,5*S*)-3,4-Dihydroxy-5-[(1′*S*)-1′,2′-dihydroxyethyl]-2-methyl-1-pyrroline 2

A solution of pyrroline **2·HCl** (24 mg, 0.11 mmol) in 2 mL of MeOH was stirred at rt for 1 h in the presence of Na_2CO_3 (50 mg). The solvent was evaporated and the residue was washed with 3 × 2 mL of CH_2Cl_2/MeOH (50/50). Filtration of the residue and evaporation of the solvent gave imine **2** as a yellow oil (13 mg, 65%). $[\alpha]_D^{20} = -21.0$ (c 0.16, MeOH); ^1H NMR (250 MHz, CD_3OD) δ 4.45 (d, 1H, J = 5.7 Hz), 4.02 (d, 1H, J = 5.7 Hz), 3.90 (br s, 1H), 3.84 (m, 1H), 3.64 (dd, 1H, J = 11.3, 4.8 Hz), 3.52 (dd, 1H, J = 11.3, 7.3 Hz), 2.04 (s, 3H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 182.2, 80.4, 79.8, 74.5, 73.8, 65.4, 17.1.

4.6. (2*S*,3*R*,4*S*,5*S*)-3,4-Dihydroxy-5-[(1′*S*)-1′,2′-dihydroxyethyl]-2-methylpyrrolidine 3

Pyrrolidine **8** (23 mg, 0.089 mmol) was dissolved in a small amount of EtOH (ca. 0.2 mL) and a solution of 1 M HCl (2 mL) was added. The resulting clear solution was stirred at room temperature overnight and concentrated. The residue (25 mg) was purified by ion-exchange chromatography on Dowex 50WX8 (H^+ form) by elution with 0.8 M NH_4OH to yield 11 mg (69%) of pyrrolidine **3** as a yellow oil. $[\alpha]_D^{20} = -46.0$ (c 0.22, MeOH); IR (film) 3353, 2925, 1114, 1032 cm^{-1}; ^1H NMR (250 MHz, CD_3OD) δ 4.42 (dd, 1H, J = 4.1, 8.2 Hz), 3.79 (dd, 1H, J = 4.1, 3.4 Hz), 3.63 (m, 1H), 3.54 (m, 2H), 3.15 (dq, 1H, J = 6.6, 3.4 Hz), 3.06 (dd, 1H, J = 8.2, 3.6 Hz), 1.11 (d, 3H, J = 6.6 Hz); ^{13}C NMR (62.5 MHz, CD_3OD) δ 76.1, 75.3, 72.7, 65.7, 64.2, 56.9, 14.8; ESI-HRMS: calcd for $C_7H_{16}NO_4$ $[M+H]^+$ 178.1079; found 178.1084.

4.7. Enzymatic assays[15]

The experiments were performed essentially as follows: 0.01–0.5 unit/mL of enzyme (1 unit = 1 mol of glycoside hydrolyzed/min), preincubated for 5 min at 20 °C with the inhibitor, and increasing concentration of aqueous solution of the appropriate *p*-nitrophenyl glycoside substrates (buffered to the optimum pH of the enzyme) were incubated for 20 min at 37 °C. The reaction was stopped by the addition of 100 μL of 0.3 M sodium borate buffer pH 9.8. The *p*-nitrophenolate formed was quantified at 405 nm, and IC_{50} values were calculated. Double-reciprocal (Lineweaver-Burk) plots were used to determine the inhibition characteristics of compounds **3** and **11·HCl**.

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