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Chiral 1,4-Morpholine-2,5-diones. Synthesis and Evaluation as Glucosidase Inhibitors

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Summary. A series of chiral 1,4-morpholine-2,5-dione derivatives were synthesized. These substrates behave as non competitive inhibitors against α-glucosidase showing a moderate inhibition ($Ki <$ 0.8 mM) and were found inactive towards β-glucosidase.

Keywords. 1,4-Morpholine-2,5-diones; α-Glucosidase; Glycosidase inhibitors.

Introduction

Glycosidases are an important class of carbohydrate and glycoconjugate modifying enzymes and are involved in a wide range of important biological processes [1–3]. Synthetic and naturally occurring glycosidase inhibitors play an important role not only in elucidating the glycoside hydrolysis mechanism, but also in promising therapeutic applications (antiviral [4], anticancer [5], anti-HIV [4], *etc.*). In fact, many efforts to design and synthesize competitive inhibitors of glycosidases have been made over the last years. Continuing our studies on the asymmetric synthesis of biologically active compounds, we have focused our attention on a new family of 1,4-morpholine-2,5-dione derivatives as potential glucosidase inhibitors. Our interest in these derivatives is due to the fact that the geometry of morpholine-2,5-dione (with the heterocyclic ring in the preferred sofà conformation [6]) could be considered analogous to the transition state of *D*-gluconolactone [2b], a good competitive inhibitor of glycosidases, which unfortunately has limited application owing to its instability [2b, 7].

Results and Discussion

In this paper we wish to report the results of enzymatic kinetic studies performed on several chiral derivatives of 1,4-morpholine-2,5-dione prepared enantiomeri-

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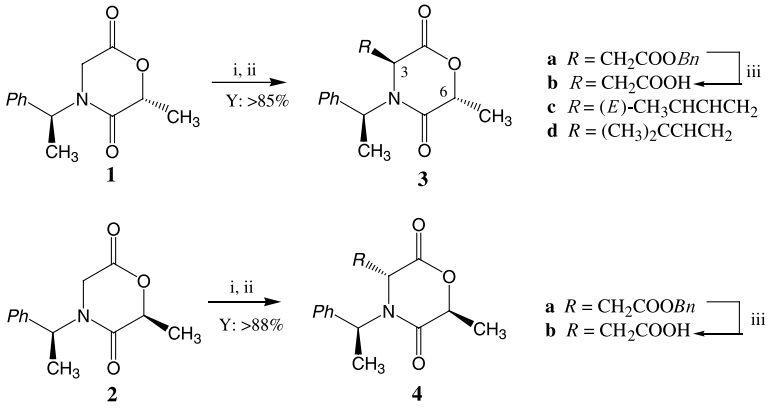
Table 1. Inhibition constants (*Ki*) towards α-glucosidase[a]

Substrates	1	3b	3c	3d	2	4b	7b	7c	10a	10b
Ki values/m*M*	4.80	0.70	0.80	2.38	0.30	0.70	23.70	n.i.[b]	3.35	0.86[c]

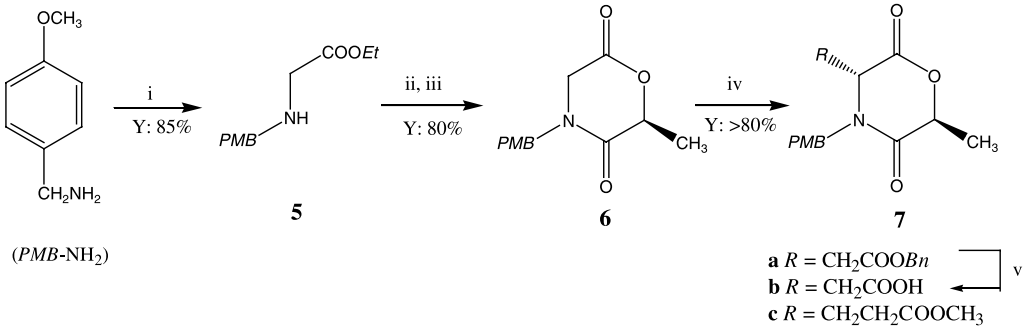
[a] In *HEPES* buffer ($pH = 6.85$) at 37°C; [b] no inhibition; [c] $Ki = 0.3$ towards β-glucosidase



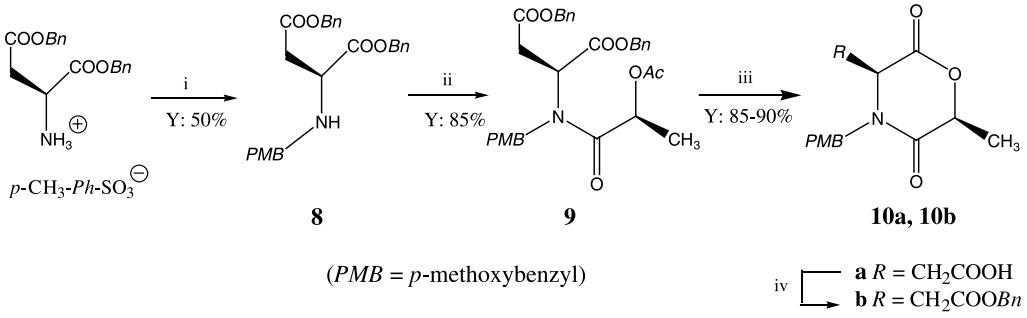
Scheme 1. i) 1 *M* Li*HMDS*/*THF*; ii) *R*-Br; iii) H_2 (3 atm), 10% Pd/C in CH_3OH

cally pure in good yields. The products **3b**–**3d** and **4b** reported in Table 1 were obtained by following a procedure already employed starting from (*S*)-phenylethylamine, ethyl bromoacetate and (*R*,*S*)-2-chloropropionyl chloride [6]. After separation by silica gel chromatography, the diastereomers (1′*S*,6*R*) **1** [6] and (1′*S*,6*S*)-6-methyl-4-*N*-(1′-phenethyl)-1,4-morpholin-2,5-dione (**2**) [6] were converted into the derivatives **3b**–**3d** and **4b**, respectively (Scheme 1). As previously observed [6], the alkylation occurred with a practically total 1,4-*trans* induction with respect to (C-6)–CH_3. However, the configuration of the C-3 stereocenter was confirmed by means of the shielding effect induced by the phenyl ring of the (*S*)-phenylethylamine group, as described in a previous paper [6], and by the N*O*E observed between (C-6)–CH_3 and (C-3)–H, the configuration of the C-6 stereocenter being note. The intermediates **3a** and **4a** were then submitted to hydrogenolysis and the substrates **3b** and **4b** were obtained in very good yields.

The substrates **7b** and **7c** were synthesized following the procedure reported in Scheme 2. The intermediate **5**, obtained by alkylating *p*-methoxybenzylamine with ethyl bromoacetate, was acylated with (*S*)-2-acetoxy-propanoyl chloride, and the crude reaction product was submitted to alkaline hydrolysis. The 1,4-morpholine-2,5-dione derivative **6** was then recovered after induced acid cyclization. In analogy to that observed for **1** and **2**, the alkylation of intermediate **6** with benzyl bromoacetate or with methyl 3-bromopropionate occurred with a practically total 1,4-*trans* induction giving **7a** and **7c**. The configuration of the new C-3 stereocentre was assigned by means of a N*O*E between (C-6)–CH_3 and (C-3)–H, the (*S*) configuration of the C-6 stereocentre being note. By submitting the intermediate **7a** to hydrogenolysis the substrate **7b** was easily obtained in very good yield.



Scheme 2. i) $BrCH_2COOEt/Et_3N$, CH_2Cl_2, 0°C; ii) (*S*)-$CH_3CH(OAc)COCl/Et_3N$, CH_2Cl_2, 0°C; iii) $NaOH/H_2O$, *Et*OH, then HCl; iv) 1 *M* Li*HMDS*/*THF*, *R*-Br; v) H_2 (3 atm), 10% Pd/C in CH_3OH



Scheme 3. i) *p*-methoxybenzylchloride, pyridine/acetone, 60°C; ii) (*S*)-$CH_3CH(OAc)COCl/Et_3N$, CH_2Cl_2; iii) NaOH in $H_2O/EtOH$, then HCl; iv) $PhCH_2Br$, Et_3N, acetone, rt

The substrates **10a** and **10b** were synthesized starting from (*L*)-aspartic acid dibenzyl ester *p*-toluenesulfonate and following the procedure reported in Scheme 3. The derivative **8**, obtained by alkylating (*L*)-dibenzyl aspartate with *p*-methoxybenzyl chloride, was acylated with (*S*)-2-acetoxypropanoyl chloride to afford the intermediate **9**. After alkaline hydrolysis, followed by acid induced cyclization, the *cis*-1,4-morpholine-2,5-dione derivative **10a** was obtained, which was then converted into the corresponding benzyl ester **10b**.

The various substrates, synthesized in optically pure form, were submitted to the inhibitory activities screening against both α- and β-glucosidases from bakers yeast and almonds, respectively, and the kinetic results are summarized in Table 1. The inhibition kinetic curves showed that the compounds behave as non-competitive inhibitors.

The substrates differ for the substituents at C-3, at N-4, and at C-6 positions of the heterocyclic ring, and for the absolute configuration at C-3 and C-6 stereocenters. Some substrates were found to be weak inhibitors of α-glucosidase, whereas all are inactive towards β-glucosidase, except **10b**, which does, however, show a weak inhibitory activity. Better inhibition potency ($Ki \leq 0.8$ m*M*) towards α-glucosidase was displayed by **2**, **3b**, **3c**, **4b**, and **10b** (Table 1). By comparing *Ki* values of **1** and **2**, it appears that the absolute configuration at C-6 plays an important role. An analogous behaviour can be observed for the C-3 stereocenter when *R* is the *p*-methoxybenzyl group (compare **7b** and **10a**), whereas the *Ki* value is not affected by the C-3

configuration when $R = (S)$–C_6H_5–$CH(CH_3)$– (compare **3b** and **4b**). Thus, the chiral (*S*)-phenethyl group appears more effective towards the inhibitory activity in comparison to the *p*-methoxybenzyl substituent (compare **3b** with **10a** and **4b** with **7b**).

The moderate inhibition activity found for these substrates can probably be attributed to their inability to interact with the active site of the enzyme by hydrogen bonds and/or electrostatic interactions. In fact, competitive inhibitors, such as nojirimycin and 1-deoxynojirimycin, which is the reference compound for the biological activity against glycosidases, have hydroxyl groups, which may interact with the active site of enzyme by hydrogen bond [8].

In conclusion, from these preliminary results the heterocyclic structure of the 1,4-morpholine-2,5-dione appears to be a promising skeleton to design other substrates with a better inhibition ability towards glycosidases. Finally, it is interesting to underline that our synthetic pathway to the enantiomerically pure 1,4-morpholine-2,5-dione derivatives is rather simple and suitable for preparing other similar substrates.

Experimental

^1H and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 300 MHz using $CDCl_3$ as the solvent. The chemical shifts are reported in ppm relative to $CDCl_3$. The coupling constants (*J*) are in Hz. UV-Vis: Cary 100 spectrophotometer. Cary software for enzyme kinetics. Optical rotation values were measured at 25°C on a Perkin-Elmer 343 polarimeter. Dry *THF* was distilled from sodium benzophenone ketyl. Elemental analyses for **3b**, **3c**, **3d**, **4b**, **7b**, **10a**, and **10b** agreed favourably with the calculated values.

*General Procedure for the Alkylation of **1** and **2***

Li*HMDS* in *THF* (1 *M*, 22 cm^3) was added to a stirred solution of 5 g of **1** or **2** (21.7 mmol) in 100 cm^3 of dry *THF* cooled at −78°C. After about 1 h 25 mmol of alkylating reagent were added and the reaction was monitored by TLC. When the reaction was complete, the mixture was allowed to warm to room temperature with stirring. Diluted HCl and ethyl acetate were added and after separation the organic solution was evaporated *in vacuo*. The residue was purified by silica gel chromatography eluting with cyclohexane/ethyl acetate.

Benzyl (3S,6R)-[6-methyl-2,5-dioxo-N-((S)-1-phenethyl)-1,4-morpholin-3-yl]acetate (**3a**, $C_{22}H_{23}NO_5$)

Obtained in 87% yield by alkylating **1** with benzyl bromoacetate. The benzyl ester was separated by silica gel chromatography. ^1H NMR: $\delta = 1.58$ (d, 3H, $J = 7.2$ Hz), 1.69 (d, 3H, $J = 6.9$ Hz), 2.9 (dd, 1H, $J = 5.7$, 17.1 Hz), 3.02 (dd, 1H, $J = 3.6$, 17.1 Hz), 4.15 (m, 1H), 5.16 (m, 2H), 5.22 (q, 1H, $J = 6.9$ Hz), 5.71 (q, 1H, $J = 7.2$ Hz), 7.27–7.45 (m, 10H) ppm; ^{13}C NMR: $\delta = 17.1$, 17.4, 37.7, 52.9, 53.1, 67.4, 74.4, 127.4, 128.3, 128.4, 128.5, 128.9, 134.6, 137.1, 166.5, 167, 169.1 ppm; the product was not obtained in a sufficiently pure form to measure its specific rotation.

(3S,6R)-[6-Methyl-2,5-dioxo-N-((S)-1-phenethyl)-1,4-morpholin-3-yl]acetic acid (**3b**, $C_{15}H_{17}NO_5$)

The benzyl ester **3a** (0.76 g, 2 mmol) in 20 cm^3 of methanol was cleaved by hydrogenolysis in the presence of 10% Pd/C. The reaction performed at room temperature in a *Parr* hydrogenator under 36 psi (3 atm) of H_2, was complete after 1 h. The catalyst was filtered off on Celite and the alcoholic

solution was evaporated under vacuum to dryness. The oily product was recovered pure in 95% yield after purification by silica gel chromatography. ^{1}H NMR: $\delta = 1.64$ (d, 3H, $J = 6.8$ Hz), 1.69 (d, 3H, $J = 6.8$ Hz), 2.87 (dd, 1H, $J = 5.4$, 17.6 Hz), 3.05 (dd, 1H, $J = 3.2$, 17.6 Hz), 4.05 (dd, 1H, $J = 3.2$, 5.4 Hz), 5.48 (q, 1H, $J = 6.8$ Hz), 5.76 (q, 1H, $J = 6.8$ Hz), 7.38 (m, 5ArH) ppm; ^{13}C NMR: $\delta = 17.2$, 18, 37.3, 52.8, 53.5, 75.1, 127.9, 128.9, 129.4, 136.4, 167.5, 168.5, 172.2 ppm; $[\alpha]_D = 86.2^\circ$ cm^3 g^{-1} dm^{-1} ($c = 1$, CH_3OH).

(3S,6R)-3-(E)-But-2-enyl-6-methyl-N-[(S)-1-phenethyl]-1,4-morpholine-2,5-dione
(**3c**, $C_{17}H_{21}NO_3$)

Obtained in 85% yield by alkylating **1** with (*E*)-1-bromobut-2-ene. The reaction was worked up after 8 h and the product was isolated pure as an oil after silica gel chromatography. ^{1}H NMR: $\delta = 1.65$ (d, 3H, $J = 6.8$ Hz), 1.66 (d, 3H, $J = 7.1$ Hz), 1.68 (d, 3H, $J = 7.1$ Hz), 2.6 (m, 2H), 3.85 (t, 1H, $J = 6.5$ Hz), 5 (q, 1H, $J = 6.8$ Hz), 5.4 (m, 1H), 5.6 (m, 1H), 5.85 (q, 1H, $J = 7.1$ Hz), 7.3 (m, 5H) ppm; ^{13}C NMR: $\delta = 16.6$, 17.2, 17.4, 17.7, 52.2, 56.4, 73.4, 123.3, 126.9, 128, 128.8, 131.1, 138.8, 166.4, 166.9 ppm; $[\alpha]_D = 94.1^\circ$ cm^3 g^{-1} dm^{-1} ($c = 1.8$, $CHCl_3$).

(3S,6R)-N-[(S)-1-Phenethyl]-3-[3-methylbutyl]-6-methyl-1,4-morpholine-2,5-dione
(**3d**, $C_{18}H_{23}NO_3$)

Obtained in 86% yield by alkylating **1** with 1-bromo-3-methylbut-2-ene. The reaction was worked up after 4 h and the product was isolated pure as an oil after silica gel chromatography. ^{1}H NMR: $\delta = 1.62$ (d, 3H, $J = 7.2$ Hz), 1.64 (d, 3H, $J = 6.8$ Hz), 1.67 (s, 3H), 1.7 (s, 3H), 2.6 (m, 2H), 3.83 (dd, 1H, $J = 4.9$, 8.3 Hz), 5.05 (q, 1H, $J = 6.8$ Hz), 5.15 (m, 1H), 5.85 (q, 1H, $J = 7.2$ Hz), 7.3 (m, 5H) ppm; ^{13}C NMR: $\delta = 16.9$, 17.5, 18, 25.9, 32, 52.5, 56.4, 73.8, 116.7, 127.2, 128.1, 128.4, 137.5, 138.3, 166.8, 168 ppm; $[\alpha]_D = 108.7^\circ$ cm^3 g^{-1} dm^{-1} ($c = 1.4$, $CHCl_3$).

Benzyl (3R,6S)-[6-methyl-2,5-dioxo-N-((S)-1-phenethyl)-1,4-morpholin-3-yl]acetate
(**4a**, $C_{22}H_{23}NO_5$)

Obtained in 88% yield by alkylating **2** with benzyl bromoacetate. The benzyl ester was submitted to silica gel chromatography. ^{1}H NMR: $\delta = 1.67$ (m, 6H), 2.33 (dd, 1H, $J = 6.2$, 17.2 Hz), 2.5 (dd, 1H, $J = 4$, 17.2 Hz), 4.62 (dd, 1H, $J = 4$, 6.2 Hz), 4.95 (m, 2H), 5.24 (q, 1H, $J = 6.6$ Hz), 5.98 (q, 1H, $J = 7.2$ Hz), 7.31 (m, 10H) ppm; ^{13}C NMR: $\delta = 15.4$, 17.2, 36.6, 50.8, 52.2, 67.1, 74.4, 127.2, 128.2, 128.5, 128.6, 128.9, 134.8, 139.5, 166.7, 167.2, 168.9 ppm; the product was not obtained in a sufficiently pure form to measure the specific rotation.

(3R,6S)-[6-Methyl-2,5-dioxo-N-((S)-1-phenethyl)-1,4-morpholin-3-yl]acetic acid
(**4b**, $C_{15}H_{17}NO_5$)

The benzyl ester **4a** was cleaved by hydrogenolysis, following the procedure described for **3b**. After purification by silica gel chromatography, the product was recovered as an oil in 93% yield. ^{1}H NMR: $\delta = 1.69$ (m, 6H), 2.38 (dd, 1H, $J = 6.3$, 17.1 Hz), 2.47 (dd, 1H, $J = 4.2$, 17.1 Hz), 4.63 (dd, 1H, $J = 4.2$, 6.3 Hz), 5.2 (q, 1H, $J = 6.9$ Hz), 5.98 (q, 1H, $J = 6.9$ Hz), 7.4 (m, 5ArH) ppm; ^{13}C-NMR: $\delta = 15.5$, 17, 36.2, 51.7, 52.3, 74.3, 127.1, 128.3, 128.9, 138.8, 167.1, 167.3, 172.5 ppm; $[\alpha]_D = -144.7^\circ$ cm^3 g^{-1} dm^{-1} ($c = 1$, CH_3OH).

3-N-(p-Methoxybenzyl)glycine ethylester (**5**, $C_{12}H_{17}NO_3$)

Ethyl bromoacetate (12 cm^3, 110 mmol) was dropped to a solution of 15.5 cm^3 of *p*-methoxybenzylamine (110 mmol) and 15.3 cm^3 of triethylamine (110 mmol) in 50 cm^3 of CH_2Cl_2 cooled at 0°C. When

the addition was complete, the cooling bath was removed allowing the reaction mixture to warm up to rt. The reaction was then monitored by TLC and after 3 h H_2O was added. The organic phase was separated, washed with H_2O, and dried ($CaCl_2$). The organic solvent was evaporated *in vacuo* and the crude reaction product was submitted to purification by silica gel chromatography. The product was recovered in 85% yield. ^{1}H NMR: $\delta = 1.28$ (t, 3H, $J = 7$ Hz), 2 (bs, NH), 3.4 (s, 2H), 3.82 (m, 5H) 4.2 (q, 2H, $J = 7$ Hz), 6.9 (m, 2ArH), 7.3 (m, 2ArH) ppm; ^{13}C-NMR: $\delta = 13.8$, 49.5, 52.2, 54.7, 60.2, 113.4, 129, 131.3, 158.4, 172 ppm.

(6S)-N-(p-Methoxybenzyl)-6-methyl-1,4-morpholine-2,5-dione (**6**, $C_{13}H_{15}NO_4$)

To a solution of 11.5 g of **5** (50 mmol) in 50 cm^3 of CH_2Cl_2 and 8.3 cm^3 of triethylamine (60 mmol), cooled at 0°C, 8.25 g of (*S*)-2-acetoxy propanoylchloride (55 mmol) was dropped under stirring. After 30 min the cooling bath was removed allowing the reaction mixture to warm up to rt and the reaction was monitored by TLC. After 1 h, diluted HCl was added, the organic phase separated and dried ($CaCl_2$). The organic solvent was evaporated under vacuum to dryness and the residue was dissolved in 50 cm^3 of ethanol. To the solution it was added under stirring 8 g of NaOH (200 mmol) dissolved in 80 cm^3 of 50% *Et*OH/H_2O and the reaction was monitored by TLC. After the starting material had disappeared, the ethanol was evaporated and the aqueous solution acidified by adding concentrated HCl and stirred at rt. The reaction product was extracted with ethyl acetate and the organic solution evaporated *in vacuo*. The residue was purified by silica gel chromatography. The pure product was recovered as an oil in 80% yield. ^{1}H NMR: $\delta = 1.67$ (d, 3H, $J = 7$ Hz), 3.82 (s, 3H), 4 (q_{AB}, 2H, $J = 18$ Hz), 4.56 (q_{AB}, 2H, $J = 14.7$ Hz), 4.94 (q, 1H, $J = 7$ Hz), 6.89 (m, 2H), 7.2 (m, 2H) ppm; ^{13}C NMR: $\delta = 17.3$, 42, 48.8, 55.2, 74.9, 114.3, 126.5, 129.6, 159.5, 165.2, 166 ppm; $[\alpha]_D = -35°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.2$, $CHCl_3$).

Benzyl (3R,6S)-[N-(p-methoxybenzyl)-6-methyl-2,5-dioxo-1,4-morpholin-3-yl]acetate
(**7a**, $C_{22}H_{23}NO_6$)

The intermediate **6** was alkylated with benzyl bromoacetate following the procedure employed to synthesize **3a**. The benzyl ester was obtained as an oil in 85% yield. ^{1}H NMR: $\delta = 1.64$ (d, 3H, $J = 7$ Hz), 2.93 (dd, 1H, $J = 4.4$, 17.4 Hz), 3.19 (dd, 1H, $J = 4.4$, 17.4 Hz), 3.79 (s, 3H), 4.15 (d, 1H, $J = 14.8$ Hz), 4.32 (t, 1H, $J = 4.4$ Hz), 5.03 (d, 1H, $J = 14.8$ Hz), 5.10 (q_{AB}, 2H, $J = 12.2$ Hz), 5.26 (q, 1H, $J = 7$ Hz), 6.86 (m, 2H), 7.16 (m, 2H), 7.36 (m, 5H) ppm; ^{13}C NMR: $\delta = 17.8$, 35.5, 46.7, 54.5, 55.2, 67.5, 74.7, 114.4, 126.8, 128.4, 128.6, 129.3, 134.5, 159.4, 166.5, 166.8, 169.4 ppm; the product was not obtained in a sufficiently pure form to measure the specific rotation.

(3R,6S)-[4-(p-Methoxybenzyl)-6-methyl-2,5-dioxo-1,4-morpholin-3-yl]acetic acid
(**7b**, $C_{15}H_{17}NO_6$)

The benzyl ester **7a** was cleaved by hydrogenolysis, as described for **3b**. The product was recovered as an oil in 94% yield after purification by silica gel chromatography. ^{1}H-NMR: $\delta = 1.68$ (d, 3H, $J = 7$ Hz), 2.95 (dd, 1H, $J = 4.6$, 17.6 Hz), 3.12 (dd, 1H, $J = 4$, 17.6 Hz), 3.8 (s, 3H), 4 (d, 1H, $J = 15$ Hz), 4.26 (dd, 1H, $J = 4$, 4.6 Hz), 5.24 (d, 1H, $J = 15$ Hz), 5.35 (q, 1H, $J = 7$ Hz), 6.9 (m, 2H), 7.15 (m, 2H), 7.66 (bs, 1H) ppm; ^{13}C NMR: $\delta = 18$, 36.6, 45.6, 54.4, 55.2, 74.6, 114.2, 126.6, 129.4, 159.2, 166.8, 168.1, 174.1 ppm; $[\alpha]_D = -87.1°\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.6$, CH_3OH).

Methyl (3R,6S)-3-[N-(p-methoxybenzyl)-6-methyl-2,5-dioxo-1,4-morpholin-3-yl]propionate
(**7c**, $C_{17}H_{21}NO_6$)

It was obtained in 80% yield by alkylating the intermediate **6** with methyl 3-bromopropionate. The product was recovered as an oil after silica gel chromatography. ^{1}H NMR: $\delta = 1.65$ (d, 3H, $J = 6.9$ Hz),

2.1 (m, 2H), 2.46 (m, 2H), 3.68 (s, 3H), 3.8 (s, 3H), 4.04 (d, 1H, $J = 14.7$ Hz), 4.05 (m, 1H), 5.02 (q, 1H, $J = 6.9$ Hz), 5.1 (d, 1H, $J = 14.7$ Hz), 6.86 (m, 2H), 7.2 (m, 2H) ppm; ^{13}C NMR: $\delta = 16.6$, 25.3, 28.9, 47, 51.8, 55.1, 57.3, 73.4, 114.2, 127, 129.5, 159.3, 165.8, 166.5, 172.1 ppm; the product was not obtained in a sufficiently pure form to measure the specific rotation.

Dibenzyl (L)-N-(p-methoxybenzyl)aspartate (**8**, $C_{26}H_{27}NO_5$)

(*L*)-Aspartic acid dibenzyl ester *p*-toluenesulfonate (purchased from Aldrich) (4.85 g, 10 mmol) dissolved 20 cm^3 of acetone was stirred at about 60°C with 1.7 g of *p*-methoxybenzyl chloride (11 mmol) in the presence of 1.6 cm^3 of pyridine. When the TLC showed the reaction practically complete (after 2–3 days) the mixture was evaporated *in vacuo* and the residue dissolved in ethyl acetate. The organic solution was washed with H_2O, dried, and after removal of the organic solvent *in vacuo* the residue was submitted to silica gel chromatography. The product was recovered as an oil in 50% yield. ^{1}H NMR: $\delta = 1.65$ (bs, 1H), 2.7 (dd, 1H, $J = 6.9$, 15.6 Hz), 2.78 (dd, 1H, $J = 6$, 15.6 Hz), 3.62 (d, 1H, $J = 12.6$ Hz), 3.71 (dd, 1H, $J = 6$, 6.9 Hz), 3.76 (d, 1H, $J = 12.6$ Hz), 3.77 (s, 3H), 5.1 (m, 4H), 6.8 (m, 2H), 7.15 (m, 2H), 7.3 (m, 10H) ppm; ^{13}C NMR: $\delta = 36$, 50.8, 54.9, 55.2, 66.9, 67.8, 114, 124.8, 128.1, 128.2, 128.3, 130.7, 134.4, 134.9, 159.6, 169, 169.5 ppm; the product was not obtained in a sufficiently pure form to measure the specific rotation.

Dibenzyl (L)-N-(p-methoxybenzyl)-N-[(2S)-acetoxypropionyl]aspartate (**9**, $C_{31}H_{33}NO_8$)

(*S*)-2-Acetoxypropanoyl chloride (1.5 cm^3, 10 mmol) was added to a stirred solution of 4.33 g of **8** (10 mmol) in 22 cm^3 of CH_2Cl_2 and 15 cm^3 of triethylamine, cooled at 0°C. The reaction, monitored by TLC, after 1 h was slowly warmed to rt and acidified with diluted HCl. The organic phase was separated, washed, dried ($CaCl_2$), and the solvent was evaporated *in vacuo*. The residue was submitted to silica gel chromatography and the product was recovered in 85% yield. ^{1}H NMR: $\delta = 1.35$ (d, 3H, $J = 6.6$ Hz), 2 (s, 3H), 2.81 (dd, 1H, $J = 7.2$, 17.1 Hz), 3.25 (dd, 1H, $J = 6.6$, 17.1 Hz), 3.71 (s, 3H), 4.33 (d, 1H, $J = 16.2$ Hz), 4.48 (dd, 1H, $J = 6.6$, 7.2 Hz), 4.57 (d, 1H, $J = 16.2$ Hz), 5.05 (m, 4H), 5.38 (q, 1H, $J = 6.6$ Hz), 6.69 (m, 2H), 7.3 (m, 12H) ppm; ^{13}C NMR: $\delta = 16.6$, 20.4, 34.1, 51.3, 54.9, 56, 66.4, 67, 67.1, 113.6, 127.9, 128.1, 128.2, 128.3, 129.2, 135.1, 135.3, 159, 168.8, 169.9, 170.3, 170.8 ppm; the product was not obtained in a sufficiently pure form to measure the specific rotation.

(3S,6S)-[4-(p-Methoxybenzyl)-6-methyl-2,5-dioxo-1,4-morpholin-3-yl]acetic acid
(**10a**, $C_{15}H_{17}NO_6$)

NaOH (0.4 g, 10 mmol) dissolved in 10 cm^3 of 50% *Et*OH/H_2O was added to 5.5 g of **9** (10 mmol) in 10 cm^3 of ethanol under stirring at rt and the reaction was monitored by TLC. After about 2 h the reaction mixture was concentrated *in vacuo*, acidified with 5 *N* HCl, and then extracted with ethyl acetate. The organic solution was dried and evaporated *in vacuo*. The pure product was recovered in 90% yield after silica gel chromatography. ^{1}H NMR: $\delta = 1.54$ (d, 3H, $J = 7.2$ Hz), 3 (dd, 1H, $J = 5.4$, 17.4 Hz), 3.13 (dd, 1H, $J = 3.9$, 17.4 Hz), 3.82 (s, 3H), 4.12 (d, 1H, $J = 15$ Hz), 4.37 (m, 1H), 5.11 (q, 1H, $J = 7.2$ Hz), 5.2 (d, 1H, $J = 15$ Hz), 6.8 (m, 2H), 7.2 (m, 2H) ppm; ^{13}C NMR: $\delta = 19.1$, 35.1, 46.2, 53.2, 55.3, 75.6, 114.6, 126.4, 129.4, 159.6, 165.9, 166.3, 172.8 ppm; $[\alpha]_D = -17.9°$ $cm^3\,g^{-1}\,dm^{-1}$ ($c = 0.2$, CH_3OH).

Benzyl (3S,6S)-[4-(p-Methoxybenzyl)-6-methyl-2,5-dioxo-1,4-morpholin-3-yl]acetate
(**10b**, $C_{22}H_{23}NO_6$)

To a solution of 1.6 g of **10a** (5 mmol) in 15 cm^3 of acetone and 1.5 cm^3 of triethylamine was added 0.63 g of benzyl chloride (5 mmol) and the reaction was stirred at rt. After 12 h acetone was evaporated

in vacuo and first ethyl acetate and then diluted HCl were added to the residue. The organic phase was separated, washed, dried, and the organic solvent was evaporated *in vacuo*. The residue was purified by silica gel chromatography and the product was recovered in 85% yield. ^{1}H NMR: $\delta = 1.84$ (d, 3H, $J = 7$ Hz), 2.99 (dd, 1H, $J = 5.2$, 17.2 Hz), 3.12 (dd, 1H, $J = 4$, 17.2 Hz), 3.79 (s, 3H), 4.21 (d, 1H, $J = 15.4$ Hz), 4.41 (dd, 1H, $J = 4$, 5.2 Hz), 4.99 (d, 1H, $J = 15.4$ Hz), 5.08 (q, 1H, $J = 7$ Hz), 5.09 (q_{AB}, 2H, $J = 12$ Hz), 6.87 (m, 2H), 7.17 (m, 2H), 7.35 (m, 5H) ppm; ^{13}C NMR: $\delta = 19.2$, 35.9, 46.4, 53.8, 55.2, 67.2, 75.5, 114.3, 126.9, 128.2, 128.4, 128.5, 129.1, 134.7, 159.3, 165.8, 166, 169 ppm; $[\alpha]_D = 11.3^\circ\ cm^3\ g^{-1}\ dm^{-1}$ ($c = 0.4$, $CHCl_3$).

Enzyme Kinetics

Materials: α-Glucosidase (EC 3.2.1.20) from baker yeast, β-glucosidase (EC 3.2.1.2) from almonds, *p*-nitrophenyl glucosides and *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid potassium salt (*HEPES*) were purchased from Sigma.

The kinetic hydrolyses of glucosides were carried out at $pH = 6.85$ in presence of 0.1 *M HEPES* buffer solution and 0.05–0.2 units of enzyme. The stock solutions of the inhibitors were prepared by dissolving **1**, **2**, **3c**, **3d**, **7c**, and **10b** in *Et*OH and **3b**, **4b**, **7b**, and **10a** in *HEPES*. Stock solution (20–60 mm^3) was added to buffered enzyme solutions contained in 10–12 cells placed in the multicell holder accessory of a Cary 100 spectrophotometer and thermostated at 37 ± 0.02°C. The solutions were incubated for 15 min. Then, appropriate aliquots of the *p*-nitrophenyl glucoside (thermostated at 37 ± 1°C in Hamilton syringes) were added to the enzyme solutions and the initial rates were followed at $\lambda = 400$ nm by monitoring the formation of *p*-nitrophenol. Non-competitive inhibition constants (*K*i) were calculated from the equation $K_i = v'_{max}[I_o]/(v_{max} - v'_{max})$, where v'_{max} and v_{max} are the maximum rates measured in presence and in absence of inhibitor and $[I_o]$ is the inhibitor concentration. The *K*i values were obtained by means of the Cary software (Enzyme Kinetics) and are reported in Table 1. The reproducibility was in the range 5–12%.

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References

[1] Lillelund VH, Jensen HH, Liang X, Bols M (2002) Chem Rev **102**: 515
[2] Stutz AE (ed) Iminosugars as Glycosidase Inhibitors. Wiley-VCH, Weinheim, 1999, a) p 8; b) p 188
[3] a) Ganem B (1996) Acc Chem Res **29**: 340; b) Heightman TD, Vasella A (1999) Ang Chem Int Ed **38**: 750
[4] a) Papandreou MJ, Barbouche R, Guieu R, Kieny MP, Fenouillet E (2002) Mol Pharmacol **61**: 186; b) Cai J, Davison BE, Ganellin CR, Thaisrivongs S, Wibley KS (1997) Carbohydr Res **300**: 109
[5] a) Johnson HA, Thomas NR (2002) Biorg Med Chem Let **12**: 2037; b) Goss PE, Reid CL, Bailey D, Dennis JW (1997) Clin Cancer Res **3**: 1077; c) Pili R, Chang J, Partis RA, Mueller RA, Chrest FJ, Passaniti A (1995) Cancer Res **55**: 2920
[6] Porzi G, Sandri S (1996) Tetrahedron Asymm **7**: 189
[7] Reese ET, Parrish FW, Ettlinger M (1971) Carbohydr Res **18**: 381
[8] Liu TKKK-C, Pederson RL, Ichikawa ZZY, Porco JA, Wong CH (1991) J Am Chem Soc **113**: 6187