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Chiral 1,4-morpholin-2,5-dione derivatives as α-glucosidase inhibitors: Part 2

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Abstract—A series of chiral 1,4-morpholin-2,5-dione derivatives were synthesized starting from chiral synthons **1** and **2**, diastereomeric monolactim ethers derived from L-valine. The compounds investigated, were inactive toward β-glucosidase, α-mannosidase and α-galactosidase but behave as noncompetitive inhibitors against the α-glucosidase (from *Saccharomices cervisiae*) with some showing a good inhibition ability ($0.05 < K_i < 0.18$ mM).

1. Introduction

In continuation of our program aimed at the asymmetric synthesis of biologically active compounds, we have focused our attention on a new series of optically active 1,4-morpholin-2,5-diones. Previously[1] we observed that such a heterocyclic framework, which is not mimetic of natural products, is a promising skeleton for designing substrates with an inhibition ability toward glycosidases. Thus, as continuation of our studies we have directed our interest toward the synthesis new chiral 1,4-morpholin-2,5-dione derivatives containing polar groups in order to increase the water solubility and ability to interact with the enzyme through hydrogen bonds and hydrophobic and electrostatic interactions.

This work was also stimulated because glucosidase inhibitors have recently received considerable attention as chemotherapeutic agents[2] toward not only diabetes,[3a] but also several viral diseases such as influenza,[3b] hepatitis,[3c] HIV,[3d] and cancer.[3e]

2. Results and discussion

Herein we report the results of enzymatic kinetic studies performed on several enantiomerically pure 1,4-morpholin-2,5-dione derivatives **3**–**12**, which have been prepared in good yields following the procedure outlined in Scheme 1. The synthesis is based on the use of (6*S*)-**1**, (6*R*)-**2** and 4-*N*-[(*S*)-1-phenethyl]-6-methyl-1,4-morpholin-2,5-dione, a useful chiral synthon we have already employed.[4a] The alkylation of **1** and **2** with allyl bromide occurred with a practically total 1,4-*trans* induction with respect to the methyl group at C-6, as previously observed.[4]

Diastereomers **3** and **4** were then converted into epoxides **5**, **6** and **7**, **8**, respectively. While the epoxidation of **3** occurred with a diastereomeric ratio **5**/**6** ≈ 1, in the case of **4**, prevalence (≈3:1) of diastereomer **7** with respect to **8** was observed. It is noteworthy that unfortunately both the diastereomeric mixtures **5**, **6** and **7**, **8** were difficult to separate by silica gel chromatography. In fact, while the less polar (greater R_f) epoxides **5** and **7** were obtained pure, the more polar (smaller R_f) ones, **6** and **8**, were always recovered as diastereomeric mixtures (92% and 67%, respectively).

By acid catalyzed ring opening of pure epoxides **5** and **7**, diols **9** and **11** were obtained, respectively. In addition, when performing the reaction on the diastereomeric mixtures of epoxides **5**, **6** and **7**, **8** it was possible to establish that from the less polar epoxide, the less polar diol was obtained while the more polar epoxide gave the more polar diol. Fortunately, it was now possible to separate, by silica gel chromatography, the diastereomeric mixture of diols **9**, **10** and **11**, **12**.

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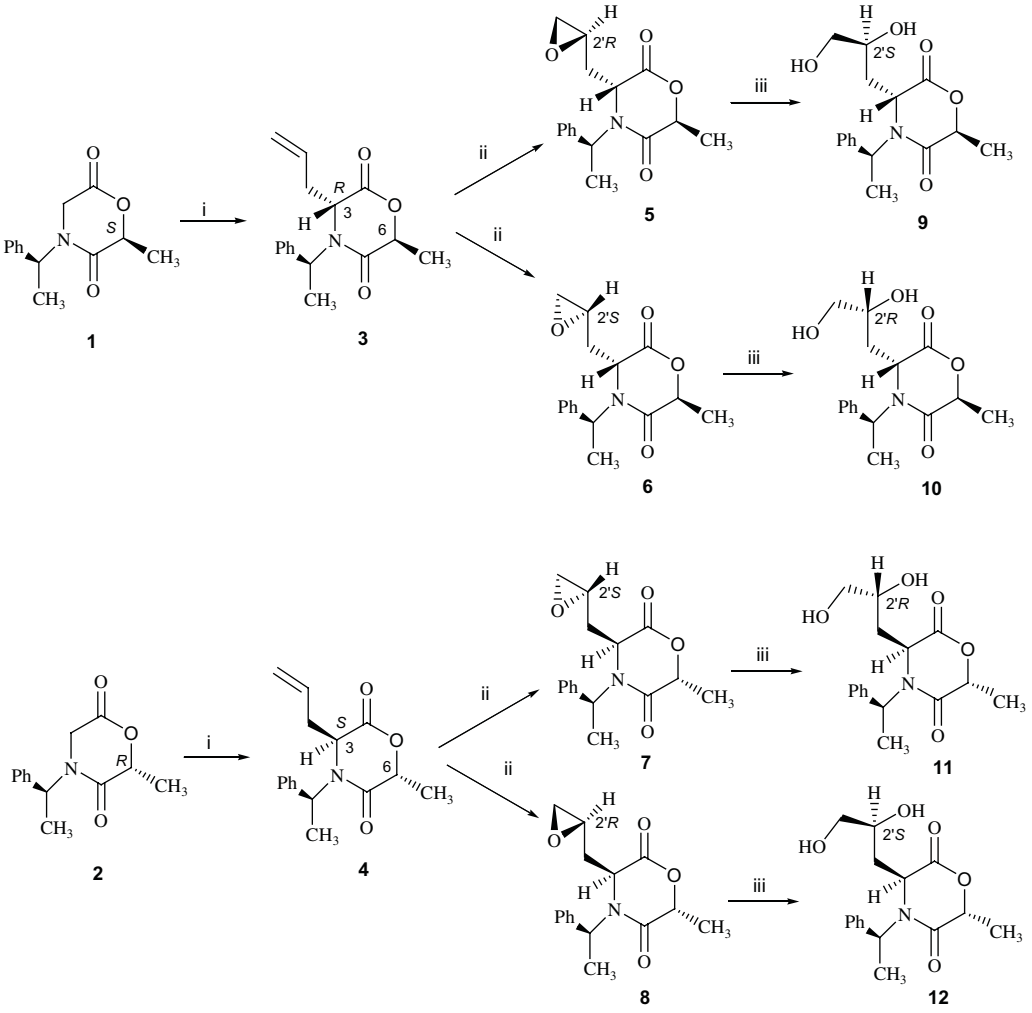


Scheme 1. Reagents and conditions: (i) 1 M LHMDS/THF, $CH_2{=}CHCH_2Br$; (ii) MCPBA/CH_2Cl_2; (iii) 1 M H_2SO_4/THF.

Substrates **3**–**12** were then submitted to the kinetic tests of inhibitory activities against both α- and β-glucosidases (from baker's yeast and almonds, respectively), α-glucosidases (from *Bacillus stearothermophilus*), α-mannosidase (from jack beans), and α-galactosidase (from green coffee beans). The results are summarized in Table 1. The inhibition kinetic curves showed that all the compounds investigated, behaved as noncompetitive inhibitors. Moreover, it is interesting to emphasize that all substrates showed inhibition activity exclusively toward the α-glucosidase from baker's yeast. At 1.3 mM concentration, β-glucosidase, α-mannosidase, and α-galactosidase did not suffer inhibition.

The inhibition constants reported in Table 1 allow for some observations. In all substrates, the C-3 configuration appears quite important for the inhibition efficacy. In fact, for substrates **3** and **4**, the (*S*)-configuration of C-3 improves the biological activity. Furthermore, for epoxides **5**–**8** and diols **9**–**12** it appears evident that the relative stereochemistry between the C-3 and C-2′ stereocenters plays a relevant role. In fact, while epoxides **5** and **7**, which displayed the best K_i values, possess the same configuration at C-3 and C-2′, the most effective diols **9** and **11** possess the opposite configurations. It is also interesting to observe that the most efficient epoxide **7** and diol **11** display the same configuration at the C-3 and C-6 stereocenters. Finally, it was observed that the inversion of the C-3, C-6 and C-2′ stereocenters did not considerably affect the inhibition constant values (in the range 0.05–0.18 mM) for both epoxides and diols (compare **5**, **7** and **9**, **11** in Table 1).

Among the substrates investigated, the diols show an interesting behavior. Since they are soluble both in water and in organic solvents, we performed kinetic experiments to evaluate the solvent effect in the inhibition ability. For substrate **11**, as the concentration of EtOH increases, reaching a maximum of 1% (v/v), in buffer solution, the activity toward α-glucosidase was greatly affected; the affinity binding significantly increases by more than an order of magnitude (Table 2). The same behavior was also observed in the presence of 1% (v/v) isopropanol and 2-methyl-2,4-pentandiol (an organic solvent valuable in the crystallization of proteins[5]). In fact in these solvents, the initial rate was reduced by 35% at $[S] = 2K_m$, while 1% of MeOH (v/v) reduces only 5% of the initial rate. Also, **9** and to a lesser extent **12**,

Table 1. Inhibition constants K_i/mM toward α-glucosidase from *Saccharomices cervisiae*[a]

Substrates	C-3	C-6	C-2′	K_i/mM[b]	K_i/mM[c]	K_i/mM[d]
3	*R*	*S*	—	3.35	4.52	
4	*S*	*S*	—	1.50		
5	*R*	*S*	*R*	0.11		
6[e]	*R*	*S*	*S*	1.02		
7	*S*	*R*	*S*	0.05	0.96	
8[f]	*S*	*R*	*R*	0.96		
9	*R*	*S*	*S*	0.18		1.26
10	*R*	*S*	*R*	1.00		1.20
11	*S*	*R*	*R*	0.11	1.61	4.60
12	*S*	*R*	*S*	0.52[g]		1.65

[a] In HEPES buffer (pH = 6.85) at 37 °C.
[b] In the presence of 1% EtOH.
[c] In the presence of 1% DMSO.
[d] In water.
[e] In de = 84% (see text and Experimental).
[f] In de = 34% (see text and Experimental).
[g] Toward α-glucosidase from *Bacillus stearothermophilus*: K_i = 1.35 (in the presence of 1% EtOH) and $K_i \geqslant 1200$ (in H_2O).

Table 2. Effect of the ethanol concentration on the inhibition constant (K_i) of **11**

% (v/v) EtOH in water[a]	K_i/mM	% (v/v) EtOH in DMSO[b]	K_i/mM
0	4.58	0	1.61
0.5	0.7	0.17	0.7
0.7	0.26	0.23	0.26
1.0	0.13	1.0	0.1

[a] In the aqueous stock solution.
[b] In the DMSO stock solution.

behave similarly in the presence of 1% (v/v) EtOH (Table 1).

The far and near UV CD spectra obtained in the presence and absence of 1% (v/v) EtOH showed that there was not any solvent-induced conformational change in the enzyme. In all probability, the diol hydrogen bonded to the EtOH (present in solution in very large molar concentration in comparison to both inhibitor and enzyme) could gain an easy access in the closeness of the active site inducing change in the solvation, which destabilizes the transition state. Alternatively, it can be hypothesized that the EtOH could modify the pK_a of the carboxyl group responsible for the catalysis, as observed for thermolysin in the presence of alcohols.[6] The importance of the interactions amongst the diol/enzyme/solvent (water and DMSO) is strengthened by the behavior of substrate **3** for which the K_i value in the presence of EtOH (K_i = 3.35 mM) is close to that found in DMSO (K_i = 4.52 mM).

To the best of our knowledge, examples in which the EtOH increases the binding inhibition ability toward the α-glucosidase have not yet been reported in the literature. Conversely, one example where the EtOH works in the opposite direction, that is, decreasing the affinity by up to one order of magnitude, is reported for the inhibition of *Zincpeptidase thermolysin* by acyclic phosphinate.[7]

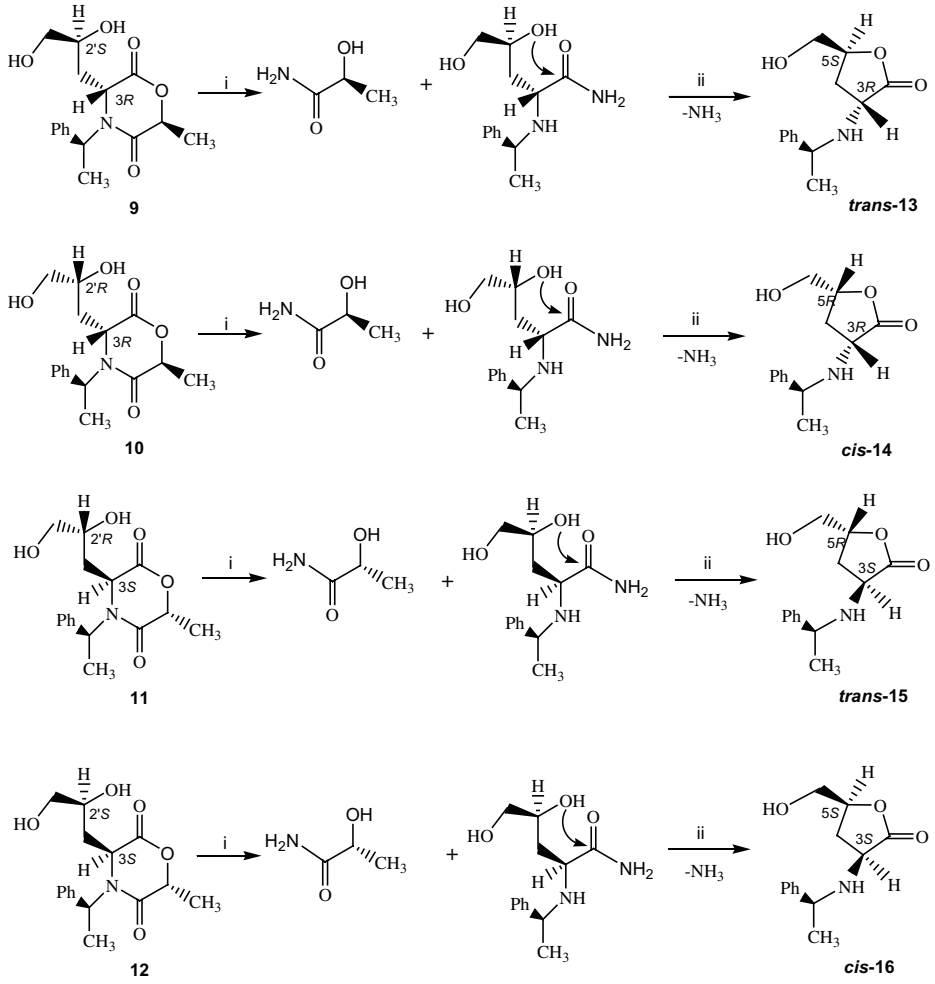
3. Stereochemical assignments

The C-3 configuration of diastereomers **3** and **4** was established through the shielding effect on (C-3)–H induced by the phenyl ring of the phenethyl group at N-4,[4] the configuration of the C-6 stereocenter being already known.

The absolute configuration of the stereocenter C-2′ in diols **9**–**12**, and in epoxides **5**–**8**, was established by converting the diols into γ-lactones **13**–**16**, respectively (Scheme 2). The reaction was accomplished by treating the diols with ammonia in isopropanol, that is, through the assisted opening of the morpholinone ring, which occurs as described in our previous papers.[4,8]

By refluxing in toluene, the intermediate 4,5-dihydroxy-2-[(*S*)-phenyl-ethylamino]-pentanamides were then cyclized into γ-lactones **13**–**16**. The formation of a γ-lactone, instead of a δ-lactone, was established by converting the cyclic product into the corresponding acetate, the ^{1}H NMR spectrum of which showed a significant shift in the CH_2O protons, due to the proximal acetate group. In fact, these protons in the γ-lactone **16** resonate as double doublets at 3.49 and 3.83 ppm and in the corresponding acetate are deshielded at 4.33 ppm. In contrast, in the case of a δ-lactone the downfield shift in the corresponding acetate would involve the multiplet of the C*H*O proton. In addition, the IR spectra of lactones **13**–**16** showed a characteristic carbonyl absorption in the region at shorter wavelengths than δ-lactones or esters, that is, at $\nu > 1760\ cm^{-1}$ (see Section 5).

The absolute configuration of the C-5 stereocenter of γ-lactones was then established by means of NOE experiments, the configuration of the C-3 stereocenter being known. In fact, the *cis*-configuration of γ-lactones **14** and **16** was established on the basis of the NOE registered on (C-3)–H by irradiating the (C-5)–H at 4.4 or 4.37 ppm, respectively, while the absence of the NOE



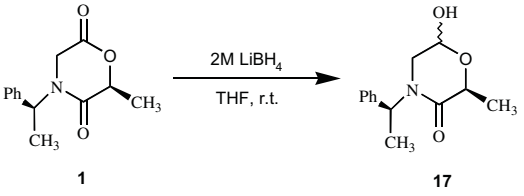
Scheme 2. Reagents and conditions: (i) NH_3/isopropanol at rt; (ii) refluxing in toluene/ethanol 9:1.

in the case of **13** and **15** allowed us to assign the *trans*-configuration to these diastereomers.

Consequently, the absolute configuration of C-5 was assigned. On this basis, it was possible to also deduce the absolute configuration of the C-2′ stereocenter of diols **9**–**12**. Finally, because the acid catalyzed opening of the epoxides occurs in a regio- and stereocontrolled fashion, the absolute configuration of epoxides **5**–**8** was consequently deduced.

4. Conclusions

In conclusion, the results reported above suggest that the heterocyclic 1,4-morpholine-2,5-dione might be a suitable skeleton for further investigation and to design substrates with specific inhibition ability toward the α-glucosidase. It is noteworthy that the inhibitory effect is totally lost if the lactone function is not present in the substrate. In fact, while compound **1** is a weak inhibitor toward α-glucosidase ($K_i = 0.31$ mM), the diastereomeric mixture of derivative **17** (very difficult to separate by silica gel chromatography), obtained by reducing the carbonyl function of **1** (Scheme 3), is totally inactive at 0.3 mM.



Scheme 3. Reagents and conditions: (i) 2 M $LiBH_4$ in THF at rt overnight.

Lastly, all the substrates investigated, although are not oxonium ion transition state analogous, behave as noncompetitive inhibitors against the α-glucosidase ($0.05 < K_i < 0.18$ mM), were inactive toward β-glucosidase, α-mannosidase, and α-galactosidase.

5. Experimental

5.1. General

^1H and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 300 MHz using $CDCl_3$ as the solvent, unless otherwise stated. Chemical shifts are reported in ppm relative to $CDCl_3$ and the coupling constants (*J*)

are in hertz. IR spectra were recorded on a Nicolet 210 spectrometer. Optical rotation values were measured at 25 °C on a Perkin–Elmer 343 polarimeter. Melting points are uncorrected. The products isolated in not sufficiently pure form for elemental analysis were submitted to HPLC–MS analysis on a Hewlett–Packard Model 1100 liquid chromatograph-single-quadrupole mass-selective detector system, with an Atmospheric Pressure Chemical Ionization-ElectroSpray interface, using a Zorbax Eclipse XDB-C 8 column. Cary100 UV spectrophotometer and Cary software was employed for enzyme kinetics. Dry THF was distilled from sodium benzophenone ketyl. Synthesis and spectroscopic data of **1**, **2** and **3**, **4** are reported in Refs. 4a and 4b, respectively.

5.2. Epoxidation of 3 and 4

MCPBA (7 g, 21.9 mmol, 77%) was added to a solution of **3** or **4** (5 g, 18.3 mmol) in 50 mL of CH_2Cl_2. The reaction mixture was stirred at room temperature and monitored by TLC. When the starting material **3** or **4** was completely reacted, *m*-chlorobenzoic acid was filtered off and the organic phase extracted with 10% aqueous solution of Na_2CO_3 and then dried on $CaCl_2$. After evaporation of the organic solvent under vacuum, the residue was submitted to silica gel chromatography, eluting with hexane/ethyl acetate, to separate the diastereomeric epoxides obtained in practically quantitative yield. While the less polar (greater R_f) diastereomers **5** and **7** were isolated pure, the more polar (smaller R_f) isomers **6** and **8** were always recovered as diastereomeric mixtures.

5.2.1. (3*R*,6*S*,2′*R*)-6-Methyl-3-oxiranylmethyl-4-[(*S*)-1-phenethyl]-morpholine-2,5-dione, 5. The epoxidation of **3** gave a 1:1 diastereomeric mixture of **5** and **6**. The pure product was isolated as a wax. ^{1}H NMR: δ 1.05 (m, 1H), 1.57 (m, 1H), 1.61 (d, 3H, $J = 7.2$), 1.67 (d, 3H, $J = 7$), 2.2 (dd, 1H, $J = 2.8, 4.8$), 2.63 (m, 1H), 2.7 (m, 1H), 4.36 (dd, 1H, $J = 3.4, 10$), 5.06 (q, 1H, $J = 7$), 6.02 (q, 1H, $J = 7.2$), 7.4 (m, 5ArH). ^{13}C NMR: δ 15.9, 16.6, 34.7, 47.5, 48.1, 51.5, 53.3, 73.7, 127.9, 128.6, 138.5, 166.1, 167.2. $[\alpha]_D = -235$ (*c* 2.7, $CHCl_3$). Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.7; H, 6.61; N, 4.85.

5.2.2. (3*R*,6*S*,2′S)-6-Methyl-3-oxiranylmethyl-4-[(*S*)-1-phenethyl]-morpholine-2,5-dione, 6. A waxy product was recovered in 92% diastereomeric mixture with **5**. ^{1}H NMR: δ 1.1 (m, 1H), 1.55 (m, 1H), 1.62 (d, 3H, $J = 7$), 1.68 (d, 3H, $J = 6.6$), 2.05 (dd, 1H, $J = 2.8, 4.8$), 2.61 (m, 1H), 2.73 (m, 1H), 4.24 (dd, 1H, $J = 5, 10.2$), 5.05 (q, 1H, $J = 6.6$), 5.97 (q, 1H, $J = 7$), 7.4 (m, 5ArH). ^{13}C NMR: δ 16, 16.3, 35.1, 46.5, 47.8, 51.6, 53.8, 73.8, 128.2, 128.7, 129, 138.4, 166.1, 167.4. HPLC–MS: 290.1 $[M+1]^+$, 312.1 $[M+Na]^+$. The product was not isolated in sufficiently pure form to measure the specific rotation.

5.2.3. (3*S*,6*R*,2′*S*)-6-Methyl-3-oxiranylmethyl-4-[(*S*)-1-phenethyl]-morpholine-2,5-dione, 7. The epoxidation of **4** gave a 3:1 diastereomeric mixture of **7** and **8**, respectively. The prevalent isomer **7** was isolated as a pure solid (mp 78–79 °C).^{1}H NMR: δ 1.68 (d, 3H, $J = 7$), 1.72 (d, 3H, $J = 7.2$), 1.87 (m, 1H), 2.32 (m, 1H), 2.58 (dd, 1H, $J = 2.6, 4.8$), 2.88 (t, 1H, $J = 4.8$), 3.03 (m, 1H), 4.1 (dd, 1H, $J = 3.2, 9.8$), 5.12 (q, 1H, $J = 7.2$), 5.9 (q, 1H, $J = 7$), 7.3 (m, 5ArH). ^{13}C NMR: δ 16.6, 17.3, 36.4, 48, 48.1, 52.6, 53.9, 73.6, 127, 128.3, 129, 138, 166.4, 167. $[\alpha]_D = +87.6$ (*c* 0.9, $CHCl_3$). Anal. Calcd for $C_{16}H_{19}NO_4$: C, 66.42; H, 6.62; N, 4.84. Found: C, 66.2; H, 6.59; N, 4.8.

5.2.4. (3*S*,6*R*,2′*R*)-6-Methyl-3-oxiranylmethyl-4-[(*S*)-1-phenethyl]-morpholine-2,5-dione, 8. The product was obtained as a wax in 67% diastereomeric mixture with **7**. ^{1}H NMR: δ 1.66 (d, 3H, $J = 7$), 1.74 (d, 3H, $J = 7.2$), 2.05 (m, 1H), 2.28 (m, 1H), 2.58 (dd, 1H, $J = 2.6, 4.8$), 2.82 (t, 1H, $J = 4.8$), 3.05 (m, 1H), 3.99 (dd, 1H, $J = 5.6, 9.2$), 5.08 (q, 1H, $J = 7.2$), 5.9 (q, 1H, $J = 7$), 7.3 (m, 5ArH). ^{13}C NMR: δ 16.3, 17.3, 36.2, 46.4, 47.9, 52.3, 53.6, 73.5, 126.9, 127, 128.2, 128.9, 166.3, 167. HPLC–MS: 290.1 $[M+1]^+$, 312.1 $[M+Na]^+$. The product was not isolated in sufficiently pure form to measure the specific rotation.

5.3. Opening of epoxides 5–8 to diols

To epoxide **5**–**8** (0.7 g, 2.4 mmol), dissolved in 15 mL of THF, 1 M H_2SO_4 (0.5 mL) was added. The reaction was monitored by TLC and stirred at room temperature for 24 h. After neutralization with 1 M NaOH, the reaction mixture was concentrated in vacuo at about 40 °C. The crude reaction product was dissolved in ethyl acetate and the Na_2SO_4 filtered off. The organic solution was evaporated under vacuum and the residue submitted to silica gel chromatographic separation eluting with ethyl acetate. The reaction products were isolated in at least 90% yield.

5.3.1. (3*R*,6*S*,2′*S*)-3-(2,3-Dihydroxy-propyl)-6-methyl-4-[(*S*)-1-phenethyl]-morpholine-2,5-dione, 9. The product, obtained starting from **5**, was isolated as a pure solid (mp 115–116 °C). ^{1}H NMR: δ 1.51 (d, 3H, $J = 6.8$), 1.65 (m, 1H), 1.78 (d, 3H, $J = 7$), 2.26 (m, 1H), 3.43 (dd, 1H, $J = 3, 12.6$), 3.83 (dd, 1H, $J = 5.4, 12.6$), 3.9 (dd, 1H, $J = 8.1, 10.8$), 4.7 (m, 1H), 4.76 (q, 1H, $J = 7$), 5.15 (q, 1H, $J = 6.8$), 7.4 (m, 5ArH). ^{13}C NMR: δ 17, 21.6, 28.4, 52.8, 54.3, 64.1, 64.7, 78.1, 127.1, 128.5, 129.1, 138.7, 173.1, 174.2. $[\alpha]_D = -23.3$ (*c* 0.8, $CHCl_3$). Anal. Calcd for $C_{16}H_{21}NO_5$: C, 62.53; H, 6.89; N, 4.56. Found: C, 62.83; H, 6.91; N, 4.55.

5.3.2. (3*R*,6*S*,2′*R*)-3-(2,3-Dihydroxy-propyl)-6-methyl-4-[(*S*)-1-Phenethyl]-morpholine-2,5-dione, 10. The product, obtained starting from **6**, was isolated as a pure solid (mp 157–158 °C). ^{1}H NMR: δ 1.52 (d, 3H, $J = 7$), 1.6 (m, 1H), 1.8 (d, 3H, $J = 7$), 2.35 (m, 1H), 3.72 (dd, 1H, $J = 5.1, 12.6$), 3.79 (t, 1H, $J = 9.8$), 3.9 (dd, 1H, $J = 2.6, 12.6$), 4.42 (m, 1H), 4.8 (q, 1H, $J = 7$), 5.19 (q, 1H, $J = 7$), 7.28 (m, 5ArH). ^{13}C NMR: δ 16.5, 21.1, 26.5, 53.2, 53.8, 63.5, 64.3, 77.5, 126.7, 128.1, 128.6, 138, 172.5, 173.7. $[\alpha]_D = +35.5$ (*c* 0.8, $CHCl_3$). Anal. Calcd for $C_{16}H_{21}NO_5$: C, 62.53; H, 6.89; N, 4.56. Found: C, 62.6; H, 6.9; N, 4.54.

5.3.3. (3*S*,6*R*,2′*R*)-3-(2,3-Dihydroxy-propyl)-6-methyl-4-[(*S*)-1-phenethyl]-morpholine-2,5-dione, 11. The product, obtained starting from **7**, was isolated as a pure solid (mp 147–148 °C). ^{1}H NMR: δ 1.42 (d, 3H, J = 6.6), 1.71 (d, 3H, J = 7.2), 2.42 (m, 1H), 2.55 (m, 1H), 3.58 (dd, 1H, J = 3.3, 12.3), 3.9 (dd, 1H, J = 8.1, 10.8), 3.93 (dd, 1H, J = 2.4, 12.3), 4.61 (q, 1H, J = 6.6), 4.84 (m, 1H), 5.14 (q, 1H, J = 7.2), 7.3 (m, 5ArH). ^{13}C NMR (CD_3OD): δ 20.1, 20.8, 31, 53.8, 56.1, 64.7, 66.4, 79.9, 128, 128.1, 129.5, 140.9, 175.1, 176.2. $[\alpha]_D = -39$ (*c* 1, CH_3OH). Anal. Calcd for $C_{16}H_{21}NO_5$: C, 62.53; H, 6.89; N, 4.56. Found: C, 62.72; H, 6.86; N, 4.57.

5.3.4. (3*S*,6*R*,2′*S*)-3-(2,3-Dihydroxy-propyl)-6-methyl-4-[(*S*)-1-phenethyl]-morpholine-2,5-dione, 12. The product, obtained starting from **8**, was isolated as a pure solid (mp 116–117 °C). ^{1}H NMR: δ 1.46 (d, 3H, J = 6.6), 1.7 (d, 2H, J = 7), 2.5 (m, 2H), 3.5 (m, 4H), 4.5 (m, 1H), 4.6 (q, 1H, J = 6.6), 5.19 (q, 1H, J = 7), 7.4 (m, 5ArH). ^{13}C NMR: δ 20, 21.6, 28.5, 53.3, 54.7, 63.8, 64.9, 76.4, 126.7, 128.1, 129, 138.1, 172.2, 174.7. $[\alpha]_D = -53$ (*c* 1, $CHCl_3$). Anal. Calcd for $C_{16}H_{21}NO_5$: C, 62.53; H, 6.89; N, 4.56. Found: C, 62.45; H, 6.87; N, 4.54.

5.4. Conversion of diols 9–12 into γ-lactones 13–16

A solution of diol **9**–**12** (1.5 g, 5 mmol) in 100 mL of isopropanol was cooled at 0 °C and then saturated with NH_3 by bubbling for about 30 min. The reaction flask was stopped and kept for 3 days at rt. After testing by TLC, the ammonia and the organic solvent were evaporated in vacuo. The residue was submitted to cyclization by refluxing in 50 mL of toluene/ethanol = 9:1 for 24 h and the reaction monitored by TLC. The organic solvents were evaporated to dryness under vacuum and the residue submitted to silica gel chromatography eluting with ethyl acetate.

5.4.1. (3*R*,5*S*)-5-Hydroxymethyl-3-[(*S*)-1-phenethyl-amino]-dihydrofuran-2-one, 13. The pure product was isolated as an oil in 45% overall yield. ^{1}H NMR: δ 1.43 (d, 3H, J = 6.6), 2.18–2.4 (m, 2H), 3.56 (t, 1H, J = 8.7), 3.6 (dd, 1H, J = 4.8, 12), 3.82 (dd, 1H, J = 3, 12), 3.84 (q, 1H, J = 6.6), 4.68 (m, 1H), 7.35 (m, 5ArH). ^{13}C NMR: δ 24, 31.6, 53.7, 56.3, 64.1, 78.4, 126.4, 127.4, 128.7, 143.8, 178. IR ($CHCl_3$) ν (cm^{-1}) = 3622 (OH), 1773 (C=O). $[\alpha]_D = -4.8$ (*c* 0.6, $CHCl_3$). Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.45; H, 7.3; N, 5.95.

5.4.2. (3*R*,5*R*)-5-Hydroxymethyl-3-[(*S*)-1-phenethyl-amino]-dihydrofuran-2-one, 14. The product was recovered as an oil in 47% overall yield. ^{1}H NMR: δ 1.41 (d, 3H, J = 6.6), 2 (m, 1H), 2.38 (m, 1H), 3.4 (dd, 1H, J = 9, 10.2), 3.62 (dd, 1H, J = 4.8, 12.6), 3.81 (q, 1H, J = 6.6), 3.88 (dd, 1H, J = 3, 12.6), 4.33 (m, 1H), 7.35 (5ArH). ^{13}C NMR: δ 24.1, 31.4, 54.7, 56.3, 63.5, 78.4, 126.4, 127.6, 128.9, 143.2, 176.6. IR ($CHCl_3$) ν (cm^{-1}) = 3610 (OH), 1770 (C=O). HPLC–MS: 236.1 $[M+1]^+$, 258.1 $[M+Na]^+$. The product was not isolated in sufficiently pure form to measure the specific rotation.

5.4.3. (3*S*,5*R*)-5-Hydroxymethyl-3-[(*S*)-1-phenethyl-amino]-dihydrofuran-2-one, 15. The product was obtained as a pure wax in 50% overall yield. ^{1}H NMR: δ 1.42 (d, 3H, J = 6.6), 1.85–2.05 (m, 2H), 3.56 (dd, 1H, J = 4.5, 12), 3.62 (t, 1H, J = 8.7), 3.81 (dd, 1H, J = 3, 12), 4.13 (q, 1H, J = 6.6), 4.58 (m, 1H), 7.35 (5ArH). ^{13}C NMR: δ 24.4, 32.7, 55.1, 57.7, 64.1, 78.4, 127, 127.3, 128.4, 144.7, 178.2. IR ($CHCl_3$) ν (cm^{-1}) = 3614 (OH), 1772 (C=O). $[\alpha]_D = -94.4$ (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.65; H, 7.26; N, 5.98.

5.4.4. (3*S*,5*S*)-5-Hydroxymethyl-3-[(*S*)-1-phenethyl-amino]-dihydrofuran-2-one, 16. The product was isolated as an oil in 48% overall yield. ^{1}H NMR: δ 1.42 (d, 3H, J = 6.6), 1.76 (m, 1H), 2 (m, 1H), 3.49 (dd, 1H, J = 8.4, 10.2), 3.55 (dd, 1H, J = 4.2, 12.6), 3.83 (dd, 1H, J = 3, 12.6), 4.17 (q, 1H, J = 6.6), 4.35 (m, 1H), 7.35 (5ArH). ^{13}C NMR: δ 24.3, 32.4, 56.2, 58, 63.4, 78, 127.1, 127.5, 128.6, 144.1, 176.9. IR ($CHCl_3$) ν (cm^{-1}) = 3620 (OH), 1775 (C=O). HPLC–MS: 236.1 $[M+1]^+$, 258.1 $[M+Na]^+$. The product was not isolated in sufficiently pure form to measure the specific rotation.

5.5. (2*R*,*S*)(6*S*)-4N-[(*S*)-1-Phenethyl]-2-hydroxy-6-methyl-1,4-morpholin-5-one, 17

Compound **17** was obtained in about 80% yield by the reduction of **1** with 2 M $LiBH_4$ in THF at rt overnight.[8] The oily product was isolated as a diastereomeric mixture (≈1:1) nonseparable by silica gel chromatography. ^{1}H NMR: δ 1.53 (d, 3H, J = 6.9), 1.55 (d, 3H, J = 6.9), 1.56 (d, 3H, J = 6.9), 1.59 (d, 3H, J = 6.9), 2.88 (m, OH), 2.92 (dd, 1H, J = 2.4, 12), 3.03 (dd, 1H, J = 2.7, 12), 3.22 (dd, 1H, J = 7.5, 12), 3.37 (m, OH), 3.46 (dd, 1H, J = 3.9, 12), 4.37 (q, 1H, J = 6.9), 4.58 (q, 1H, J = 6.9), 5.04 (m, 1H), 5.34 (m, 1H), 7.35 (m, 5ArH). ^{13}C NMR: δ 15.1, 15.7, 17.8, 18.3, 44.8, 45.6, 49.7, 50, 67.3, 71.8, 80.2, 90.5, 127.1, 127.5, 127.7, 128.5, 128.6, 139.3, 139.4, 168.9, 169.6. HPLC–MS: 236.3 $[M+1]^+$, 258.3 $[M+Na]^+$.

5.6. Enzyme kinetics

5.6.1. Materials. α-Glucosidase (EC 3.2.1.20) from baker's yeast, β-glucosidase (EC 3.2.1.2) from almonds, α-mannosidase (EC 3.2.1.24) from jack bean, α-galactosidase (EC 3.2.1.22) from green coffee beans, *p*-nitrophenyl glucosides, and 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid and potassium salt (HEPES) were purchased from Sigma.

5.6.2. Kinetics. The kinetic hydrolyses of glucosides were carried out at pH = 6.85 in the presence of 0.1 M of HEPES buffer solution and 0.05–0.2 units of enzyme. Stock solutions of various inhibitors were prepared by dissolving the substrates at 0.01–0.04 M concentration in EtOH, H_2O or EtOH/H_2O, and EtOH/DMSO mixtures. Stock solution (10–100 μL) were added to enzyme buffered solutions contained in 10–12 cells placed in the multicell holder accessory of a Cary100 UV spectrophotometer and thermostated at 37 ± 0.01 °C for 15 min. Then, appropriate aliquots of a glucoside solution contained in 10–12 Hamilton syringes, thermostated at

37 ± 1 °C, were added to the solutions and the initial rates followed at $\lambda = 400$ nm by monitoring the formation of *p*-nitrophenol. Noncompetitive 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 the presence and absence of inhibitor, respectively, and $[I_o]$ is the inhibitor concentration. These values are obtained from the Cary software (Enzyme Kinetics) and are reported in Table 1. The reproducibility was in the range 5–12%.

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