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Synthesis of new chiral 1,4-morpholin-2,5-dione derivatives and evaluation as α-glucosidase inhibitors. Part 3☆

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Dedicated to Professor Sergio Sandri on occasion of his retirement

Abstract—A series of chiral 1,4-morpholin-2,5-dione derivatives were synthesized starting from chiral synthons **1** and **2**, monolactim ethers derived from L-valine, and the absolute configurations of the new stereocentres were assigned. The substrates investigated behave as noncompetitive inhibitors against α-glucosidases and are inactive towards β-glucosidase, α-mannosidase and α-galactosidase. Three of these substrates show very good and specific inhibition abilities towards α-glucosidase.

1. Introduction

In continuation of our studies directed towards producing biologically active compounds,[1,2] we have focused our attention on the asymmetric synthesis of new, optically active derivatives of 1,4-morpholin-2,5-dione. We believe that the heterocyclic 1,4-morpholin-2,5-dione is an effective skeleton to help further investigation and design substrates with specific inhibition abilities towards the α-glucosidase. In a previous paper[2] we observed that the inhibitory effect towards this enzyme is totally lost if the lactone function is not present in these substrates. Although it is not a mimetic of natural products, such a heterocyclic structure reveals interesting perspectives; in fact, some nonglycosidic inhibitors represent a new class of compounds with promising therapeutic potential.[3] It is possible that the skeleton of the morpholin-2,5-dione mimics the transition state of D-glucolactone, a good competitive inhibitor of glycosidases.[4] From energy calculations the heterocyclic ring of morpholin-2,5-dione in the preferred geometry adopts a sofà conformation[5] that is similar to the flattened half chair conformation which has been shown to be important for good glycosidase inhibitors.[6,7] Thus, we thought it would be interesting to synthesize new 1,4-morpholin-2,5-dione derivatives (making use of the experience previously acquired for analogous compounds) in order to test their inhibitory activity towards the α-glucosidase in connection with the derivatives previously investigated.[1,2]

It is important to underline that glucosidase inhibitors are still under careful consideration owing to the potential therapeutic application in the treatment of a variety of diseases.[3,7]

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 **7**–**10** and **19**–**22**, prepared, by following the procedures outlined in Schemes 1 and 2, respectively.

The synthetic path employed is based on the use of (6*S*)-**1** and (6*R*)-**2**, 4-*N*-[(*S*)-1-phenethyl]-6-methyl-1,4-morpholin-2,5-dione, a chiral synthon we have already used.[5,8] The alkylation of **1** and **2** occurred with practically total 1,4-*trans* induction with respect to the methyl group at C-6, as previously observed.[2,5,8] (*Z*)-Derivatives **3** and **15** were

☆ Refs. 1 and 2 are considered to be Part 1 and 2, respectively.
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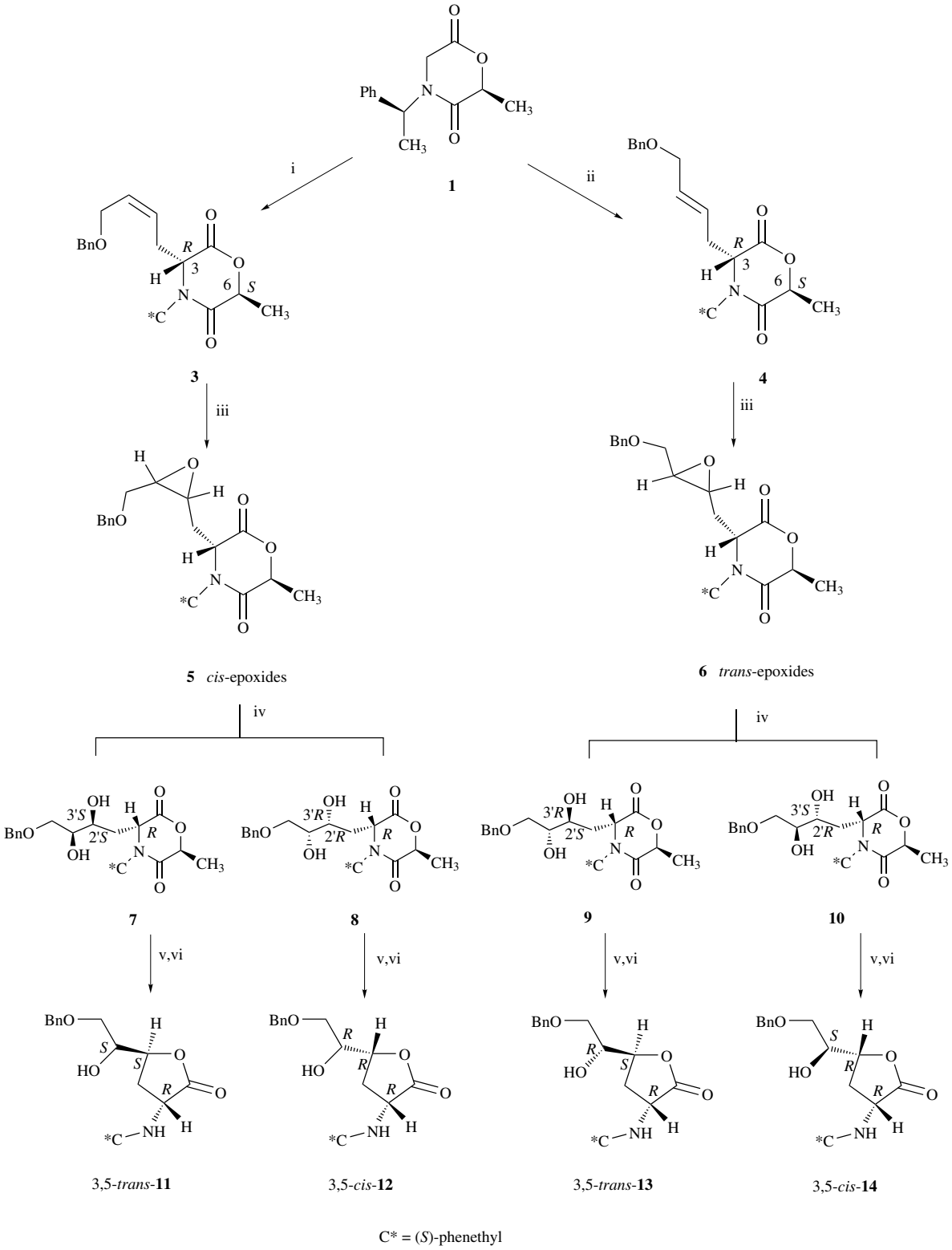
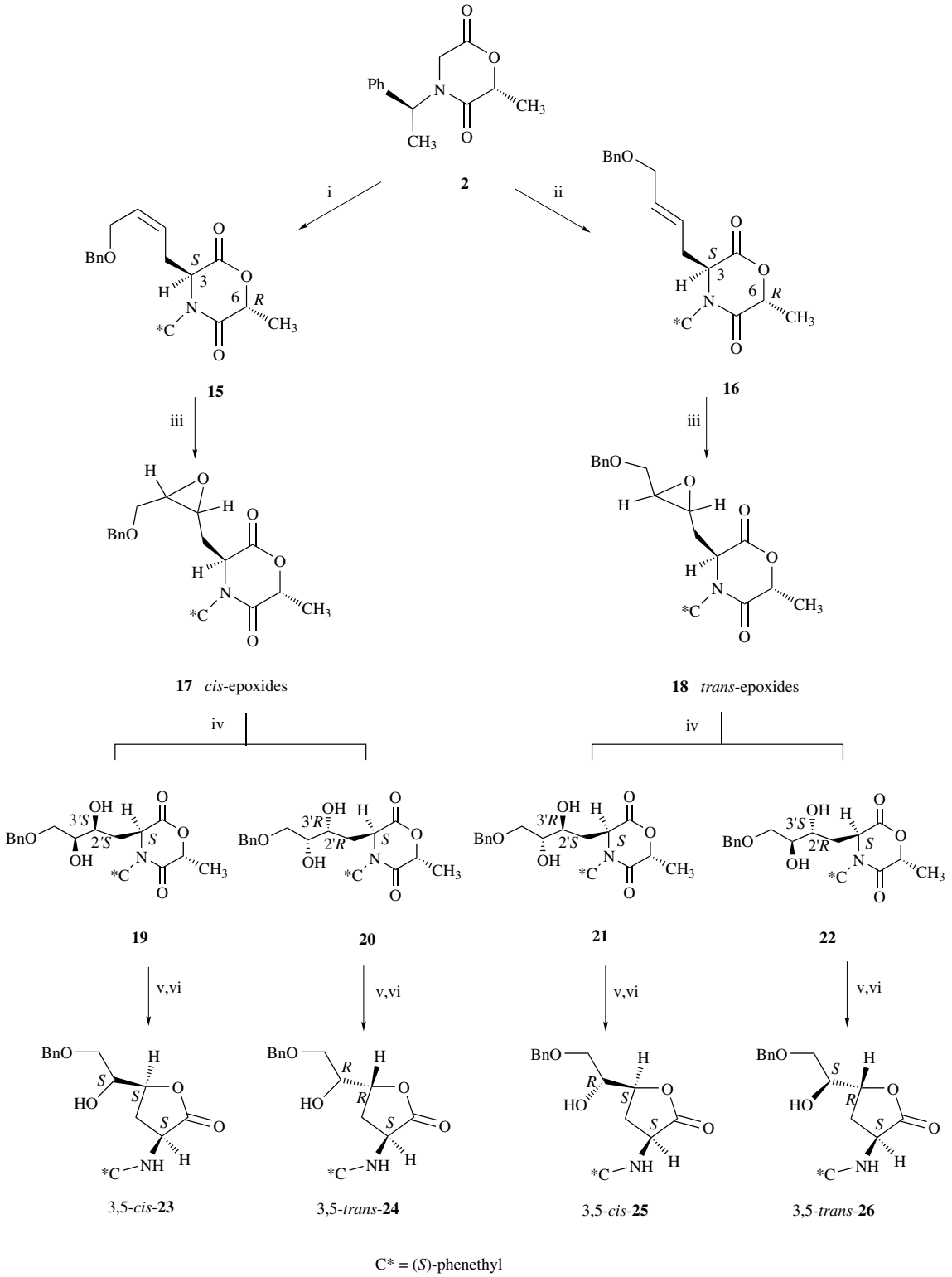


Scheme 1. Reagents and conditions: (i) 1 M LHMDS/THF, then (*Z*)-4-(benzyloxy)buten-2-ylmethansulfonate; (ii) 1 M LHMDS/THF, then (*E*)-iodo-4-benzyloxy-2-butene; (iii) MCPBA/CH_2Cl_2; (iv) 1 M H_2SO_4/THF; (v) NH_3/isopropanol at rt; (vi) reflux in toluene/ethanol 9:1.

obtained from chiral synthon **1** or **2**, respectively, by using (*Z*)-4-(benzyloxy)buten-2-ylmethansulfonate, while (*E*)-derivatives **4** and **16** were synthesized by employing (*E*)-iodo-4-benzyloxy-2-butene, as an electrophile. The treatment of (*Z*)-4-(benzyloxy)buten-2-ylmethansulfonate with NaI does not give the corresponding (*Z*)-iodo-derivative, but instead provided the isomer (*E*)-iodo-4-benzyloxy-2-butene.



Scheme 2. Reagents and conditions: (i) 1 M LHMDS/THF, then (*Z*)-4-(benzyloxy)buten-2-ylmethansulfonate; (ii) 1 M LHMDS/THF, then (*E*)-iodo-4-benzyloxy-2-butene; (iii) MCPBA/CH_2Cl_2; (iv) 1 M H_2SO_4/THF; (v) NH_3/isopropanol at rt; (vi) reflux in toluene/ethanol 9:1.

Intermediates **3** and **15** were then converted into diastereomeric mixture of *cis*-epoxides **5** and **17**, respectively. Similarly, intermediates **4** and **16** when submitted to the epoxidation gave the diastereomeric mixture of *trans*-epoxides **6** and **18**, respectively.

The diastereomeric mixtures of both *cis*- and *trans*-epoxides were not separable by silica gel chromatography and were submitted to acid cleavage. Hence, from the diastereomeric mixture of *cis*-epoxides **5** or **17** we obtained the diastereomeric mixture of diols **7** and **8** or **19** and **20**,

respectively, while from the diastereomeric mixture of *trans*-epoxides **6** or **18** the diastereomeric mixture of diols **9** and **10** or **21** and **22** were recovered, respectively (see Schemes 1 and 2). Despite the R_f being rather similar, it was possible to separate the diastereomeric diols by silica gel chromatography although with some difficulty. However, while diols **7** and **8** could be isolated in a pure form, diols **10** and **19** were recovered in 80% and 90% diastereomeric excess, respectively. The purities of diols **9**, **20**, **21** and **22**, as determined by HPLC–MS analysis, were greater than 95%.

The C-3 configuration of diastereomers **3**, **4**, **15** and **16** was established through the shielding effect on (C-3)–H induced by the phenyl ring of the phenethyl group at N-4,[2,5] the configuration of the C-6 stereocentre being known from the starting material.

The absolute configurations of the new stereocentres C-2′ and C3′ in **7**–**10** and **19**–**22** was assigned by following the same procedure previously used[2] and summarized in Schemes 1 and 2. γ-Lactones **11**–**14** and **23**–**26** were obtained by treating diols **7**–**10** and **19**–**22**, respectively, with ammonia in isopropanol (through the assisted opening of the morpholinone ring as described in our previous papers[2,8,9]) and then by refluxing in toluene/ethanol 9:1. As previously observed,[2] the formation of γ-lactones was ascertained by the IR spectra which showed a characteristic carbonyl absorption at shorter wavelengths (i.e., at $\nu = 1795$–$1760\ cm^{-1}$) than δ-lactones.[10] Thus, it was possible to establish the absolute configuration of the C-5 stereocentre of the γ-lactones by NOE experiments (the configuration of C-3 stereocentre being known) and consequently, the absolute configuration of the C-2′ stereocentre of diols could be deduced.[2] Finally, because the acid catalyzed opening of the epoxides occurs in a regio- and stereocontrolled fashion, the absolute configuration at the C-3′ stereocentre was established.

The diols synthesized were then submitted to the kinetic tests of inhibitory activities against α-glucosidases (from both baker's yeast and *Bacillus stearothermophilus*) and the results are summarized in Table 1. The inhibition kinetic curves showed that all compounds investigated behaved as noncompetitive inhibitors. It is interesting to note that all substrates showed inhibition activity exclusively towards α-glucosidase. In fact, at 500 μM concentration, β-glucosidase (from almonds), α-mannosidase (from jack beans) and α-galactosidase (from green coffee beans) did not suffer inhibition.

Table 1. Inhibition constants against α-glucosidases

Substrates	C-3	C-6	C-2′	C-3′	K_i (μM)	
					[a]	[b]
7	(*R*)	(*S*)	(*S*)	(*S*)	n.i.[c]	804
8	(*R*)	(*S*)	(*R*)	(*R*)	n.i.[c]	831
9	(*R*)	(*S*)	(*S*)	(*R*)	40	400
10[d]	(*R*)	(*S*)	(*R*)	(*S*)	300	33
19[e]	(*S*)	(*R*)	(*S*)	(*S*)	370	194
20	(*S*)	(*R*)	(*R*)	(*R*)	520	696
21	(*S*)	(*R*)	(*S*)	(*R*)	12	4
22	(*S*)	(*R*)	(*R*)	(*S*)	1030	250

[a] From baker's yeast.
[b] From *Bacillus stearothermophilus*.
[c] No inhibition at 500 μM.
[d] de = 80% (see text and Section 4).
[e] de = 90% (see text and Section 4).

3. Conclusion

From the values of the inhibition constants reported in Table 1 some considerations can be inferred. First of all, the compounds investigated are generally more effective and selective than those previously reported.[2] As a result, we believe that the extension of the side chain at C-3, by the introduction of a CH_2OBn group, increases the biological activity. The data collected in Table 1 show that compounds **9**, **10** and **21** are the most active inhibitors towards both the α-glucosidases tested with substrate **21** in particular exhibiting very good inhibition ability. Since both **9** and **21** have the same configurations at C-2′ and C-3′, their stereochemistry appears more important than that of the C-3 and C-6 stereocentres. In order to determine the influence of the benzyl group on the biological activity, we performed the debenzylation of **21**, through hydrogenolysis in the presence of Pd/C, to obtain the corresponding triol, which showed an inhibitory activity more than 100-fold smaller than substrate **21**, the K_i being 1500 μM towards α-glucosidase from baker's yeast. Such a result suggests that the benzyl group at the C-3′ position of the side chain probably favours the binding to an enzyme hydrophobic zone, more than the hydroxyl group, which is only able to form hydrogen bonds.

4. Experimental

4.1. General

^1H and ^{13}C NMR spectra were recorded on a Gemini spectrometer at 300 MHz using $CDCl_3$ as the solvent. Chemical shifts are reported in ppm relative to $CDCl_3$ and the coupling constants (*J*) are in Hz. IR spectra were recorded on a Nicolet 210 spectrometer. The products isolated, which were not sufficiently pure for elemental analysis and to measure the specific rotation, 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. Optical rotation values were measured at 25 °C on a Perkin–Elmer 343 polarimeter. The enzyme kinetics were followed by Cary100 UV spectrophotometer and Cary software was employed for calculating the inhibition constants. Dry THF was distilled from sodium benzophenone ketyl. For the synthesis and spectroscopic data of **1** and **2** (see Refs. 5 and 8).

4.2. Alkylation of 1 and 2

LHMDS (1 M, 10 mL) in dry THF (10 mmol) was dropped into a solution of 10 mm of **1** or **2** in dry THF (100 mL), cooled at −40 °C under an inert atmosphere. After about

1 h, the bath was cooled at −78 °C and the alkylating reagent (10 mmol) added. After about 5 h, the cooling bath was removed allowing the reaction mixture to warm up to rt, then 10 mL of 1 M HCl were added and the mixture extracted with ethyl acetate. The organic extract was dried, evaporated in vacuo and the residue purified by silica gel chromatography eluting with hexane/ethyl acetate.

4.2.1. (3*R*,6*S*)-3-(4′-Benzyloxy-(2′*Z*)-butenyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 3. The compound was obtained in 90% yield by alkylating **1** with (*Z*)-4-(benzyloxy)buten-2-ylmethansulfonate prepared from the (*Z*)-4-(benzyloxy)-2-buten-1-ol. ^{1}H NMR: δ 1.55 (d, 3H, $J = 6.6$); 1.6 (d, 3H, $J = 6.9$); 1.75 (m, 1H); 2.15 (m, 1H); 3.72 (m, 2H); 4.1 (dd, 1H, $J = 4.8$, 10.5); 4.25 (q_{AB}, 2H, $J = 10$); 4.94 (q, 1H, $J = 6.6$); 5.29 (m, 1H); 5.67 (m, 1H); 5.96 (q, 1H, $J = 6.9$), 7.4 (m, 10ArH). ^{13}C NMR: δ 15.4, 15.9, 29.3, 51, 55.1, 64.8, 71.6, 73.1, 124.8, 127.1, 127.5, 127.8, 127.9, 128, 128.4, 129.9, 137.6, 138.3, 165.6, 166.2. HPLC–MS: 394.2 $[M+1]^+$, 416.2 $[M+Na]^+$. The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.2.2. (3*R*,6*S*)-3-(4′-Benzyloxy-(2′*E*)-butenyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 4. The compound was obtained in 90% yield by alkylating **1** with (*E*)-iodo-4-benzyloxy-2-butene prepared from (*Z*)-4-(benzyloxy)buten-2-ylmethansulfonate. ^{1}H NMR: δ 1.59 (d, 3H, $J = 7.2$); 1.65 (d, 3H, $J = 6.9$); 1.76 (m, 1H); 2.01 (m, 1H); 3.89 (m, 2H); 4.15 (dd, 1H, $J = 4.5$, 9.6); 4.49 (s, 2H); 5.02 (q, 1H, $J = 6.9$); 5.39 (m, 2H); 6.1 (q, 1H, $J = 7.2$); 7.4 (m, 10ArH). ^{13}C NMR: δ 15.8, 16.4, 34.7, 51.4, 55.9, 69.5, 71.9, 73.5, 125.4, 127.4, 127.5, 127.8, 128.2, 128.4, 128.7, 131.7, 137.9, 138.6, 165.9, 166.8. $[\alpha]_D = -207$ (*c* 0.4, $CHCl_3$). Anal. Calcd for $C_{23}H_{27}NO_4$: C, 72; H, 7.13; N, 3.67. Found: C, 72.1; H, 7.15; N, 3.68.

4.2.3. (3*S*,6*R*)-3-(4′-Benzyloxy-(2′*Z*)-butenyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 15. The compound was obtained in 90% yield by alkylating **1** with (*Z*)-4-(benzyloxy)buten-2-ylmethansulfonate prepared from the (*Z*)-4-(benzyloxy)-2-buten-1-ol. ^{1}H NMR: δ 1.62 (d, 3H, $J = 6.6$); 1.65 (d, 3H, $J = 7.2$); 2.70 (m, 2H); 3.87 (dd, 1H, $J = 5.4$, 8.7); 4.04 (m, 2H); 4.53 (m, 2H); 4.97 (q, 1H, $J = 6.6$); 5.62 (m, 1H); 5.88 (m, 2H); 7.4 (m, 10ArH). ^{13}C NMR: δ 16.3, 17.2, 31.1, 52.1, 55.8, 65.2, 72.2, 73.4, 125.4, 126.8, 127.6, 128.1, 128.2, 128.8, 130.5, 137.6, 138.0, 166.3, 166.5. $[\alpha]_D = +84.8$ (*c* 0.9, $CHCl_3$). Anal. Calcd for $C_{23}H_{27}NO_4$: C, 72; H, 7.13; N, 3.67. Found: C, 71.84; H, 7.12; N, 3.65.

4.2.4. (3*S*,6*R*)-3-(4′-Benzyloxy-(2′*E*)-butenyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 16. The compound was obtained in 90% yield by alkylating **1** with (*E*)-iodo-4-benzyloxy-2-butene prepared from the (*Z*)-4-(benzyloxy)buten-2-ylmethansulfonate. ^{1}H NMR: δ 1.64 (d, 3H, $J = 6.6$); 1.68 (d, 3H, $J = 6.6$); 2.68 (m, 2H); 3.91 (t, H, $J = 6.6$); 3.99 (m, 2H); 4.51 (s, 2H); 5.06 (q, 1H, $J = 6.6$); 5.74 (m, 2H); 5.86 (q, 1H, $J = 6.6$ Hz); 7.4 (m, 10ArH). ^{13}C NMR δ 16.6, 17.6, 36.3, 52.5, 56.3, 69.5, 72.2, 73.6, 125.2, 127.0, 127.6, 128.3, 129.0, 132.3, 137.9, 138.1, 166.5, 166.8. HPLC–MS: 394.2 $[M+1]^+$, 416.2 $[M+Na]^+$. The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.3. General procedure for the formation of diols 7, 8, 9, 10, 19, 20, 21 and 22

MCPBA (77%, 7 g, 21.9 mmol) was added to a solution of **3** or **4** or **15** or **16** (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 had completely reacted, *meta*-chlorobenzoic acid was filtered off, 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 diastereomeric mixture of epoxides was obtained in practically quantitative yield. To the epoxide (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 85% yield.

4.3.1. (3*R*,6*S*,2′*S*,3′*S*)-3-(4′-Benzyloxy-2′,3′-dihydroxybutyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 7. The product was obtained from **3** in 40% yield. ^{1}H NMR: δ 1.45 (m, 1H); 1.52 (d, 3H, $J = 6.9$); 1.78 (d, 3H, $J = 6.9$); 1.78 (d, 3H, $J = 6.9$); 2.35 (m, 1H); 3.57 (m, 2H); 3.66 (m, 1H); 3.98 (dd, 1H, $J = 8.1$, 10.2); 4.54 (s, 2H); 4.65 (m, 1H); 4.79, (q, 1H, $J = 6.9$); 5.12 (q, 1H, $J = 6.9$); 7.4 (m, 10ArH). ^{13}C NMR: δ 16.8, 21.2, 28.8, 51.2, 54.1, 64.6, 70.7, 71.9, 73.2, 77, 127, 127.6, 127.7, 128.2, 128.3, 128.8, 137.4, 138.6, 173.7, 174.3. $[\alpha]_D = +13.5$ (*c* 2, $CHCl_3$). Anal. Calcd for $C_{24}H_{29}NO_6$: C, 67.43; H, 6.84; N, 3.28. Found: C, 67.12; H, 6.86; N, 3.22.

4.3.2. (3*R*,6*S*,2′*R*,3′*R*)-3-(4′-Benzyloxy-2′,3′-dihydroxybutyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 8. The product was obtained from **3** in 40% yield. ^{1}H NMR: δ 1.52 (d, 3H, $J = 6.6$); 1.6 (m, 1H); 1.79 (d, 3H, $J = 6.9$); 2.4 (m, 1H); 3.61 (m, 2H); 3.78 (t, 1H, $J = 10.2$); 3.85 (m, 1H); 4.51 (q_{AB}, 2H, $J = 12$); 4.52 (m, 1H); 4.79 (q, 1H, $J = 6.6$); 5.17 (q, 1H, $J = 6.9$); 7.4 (m, 10ArH). ^{13}C NMR: δ 16.7, 21.2, 27.3, 53.4, 54.1, 64.7, 70.1, 71.3, 76.6, 127, 127.5, 128.2, 128.9, 137.7, 138.3, 172.9, 173.9. $[\alpha]_D = -10.6$ (*c* 0.5, $CHCl_3$). Anal. Calcd for $C_{24}H_{29}NO_6$: C, 67.43; H, 6.84; N, 3.28. Found: C, 67.66; H, 6.85; N, 3.25.

4.3.3. (3*R*,6*S*,2′*S*,3′*R*)-3-(4′-Benzyloxy-2′,3′-dihydroxybutyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 9. The product was obtained from **4** in 30% yield. ^{1}H NMR: δ 1.51 (d, 3H, $J = 6.6$); 1.60 (m, 1H); 1.76 (d, 3H, $J = 7$); 2.15 (m, 1H); 3.40 (m, 2H); 3.86 (dd, 1H, $J = 7.8$, 10.6); 3.94 (m, 1H); 4.50 (m, 2H); 4.68 (m, 1H); 4.79 (q, 1H, $J = 6.6$); 5.12 (q, 1H, $J = 7$); 7.4 (m, 10ArH). ^{13}C NMR: δ 16.9, 21.3, 27.3, 53.4, 54.3, 64.8, 70.2, 71.2, 73.5, 77.1, 127.1, 127.7, 127.8, 128.4, 128.5, 129.0, 137.6, 138.3, 173.1, 174.1. HPLC–MS: 410.1 $[M+1-H_2O]^+$, 428.1

$[M+1]^+$, 450.1 $[M+Na]^+$, 877.2 [2M+Na]. The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.3.4. (3*R*,6*S*,2′*R*,3′*S*)-3-(4′-Benzyloxy-2′,3′-dihydroxybutyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 10. The product, obtained from **4**, was recovered in 40% yield as a wax in a 90:10 diastereomeric mixture with **9**, respectively. ^{1}H NMR: δ 1.50 (d, 3H, $J = 6.6$); 1.60 (m, 1H); 1.77 (d, 3H, $J = 7$); 2.42 (m, 1H); 3.58 (m, 2H); 3.74 (t, 1H, $J = 10$); 4.15 (m, 1H); 4.38 (m, 1H); 4.52 (s, 2H); 4.79 (q, 1H, $J = 7$); 5.18 (q, 1H, $J = 6.6$); 7.4 (m, 10ArH). ^{13}C NMR: δ 17.0, 21.4, 26.9, 52.5, 54.4, 64.7, 70.1, 71.0, 73.4, 78.3, 127.0, 127.7, 127.9, 128.4, 128.5, 128.9, 137.3, 138.5, 174.2. HPLC–MS: 410.1 $[M+1-H_2O]^+$, 428.1 $[M+1]^+$, 450.1 $[M+Na]^+$, 877.2 [2M+Na]. The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.3.5. (3*S*,6*R*,2′*S*,3′*S*)-3-(4′-Benzyloxy-2′,3′-dihydroxybutyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 19. The product, obtained from **15**, was recovered in 40% yield as a wax in 95:5 diastereomeric mixture with **20**, respectively. ^{1}H NMR: δ 1.42 (d, 3H, $J = 6.9$); 1.65 (d, 3H, $J = 6.6$); 2.34 (m, 1H); 2.63 (m, 1H); 3.63 (m, 2H); 3.80 (t, 1H, $J = 10.2$); 3.93 (m, 1H); 4.59 (m, 4H); 5.18 (q, 1H, $J = 6.6$); 7.4 (m, 10ArH). ^{13}C NMR: δ 19.7, 21.3, 28.8, 53.0, 54.5, 64.7, 70.1, 71.4, 73.3, 76.9, 126.6, 127.6, 127.9, 128.2, 128.8, 137.7, 138.1, 172.0, 174.6. HPLC–MS: 410.1 $[M+1-H_2O]^+$, 428.1 $[M+1]^+$, 450.1 $[M+Na]^+$, 877.2 [2M+Na]. The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.3.6. (3*S*,6*R*,2′*R*,3′*R*)-3-(4′-Benzyloxy-2′,3′-dihydroxybutyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 20. The product was obtained from **15** in 35% yield. ^{1}H NMR: δ 1.41 (d, 3H, $J = 6.6$); 1.68 (d, 3H, $J = 7$); 2.4–2.7 (m, 2H); 3.6 (m, 2H); 3.8 (m, 1H); 3.97 (dd, 1H, $J = 8.6, 10$); 4.56 (s, 2H); 4.59 (m, 1H); 4.74 (m, 1H); 5.12 (q, 1H, $J = 7$); 7.4 (m, 10ArH). ^{13}C NMR: δ 20.0, 21.6, 30.4, 52.0, 54.6, 64.7, 70.7, 72.2, 73.5, 76.6, 126.6, 127.8, 127.9, 128.4, 128.5, 128.9, 137.4, 138.3, 173.3, 174.8. HPLC–MS: 410.1 $[M+1-H_2O]^+$, 428.1 $[M+1]^+$, 450.1 $[M+Na]^+$, 877.2 $[2M+Na]^+$. The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.3.7. (3*S*,6*R*,2′*S*,3′*R*)-3-(4′-Benzyloxy-2′,3′-dihydroxybutyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 21. The product was obtained from **16** in 40% yield. ^{1}H NMR: δ 1.44 (d, 3H, $J = 6.6$); 1.68 (d, 3H, $J = 7.0$); 2.42 (m, 1H); 2.64 (m, 1H); 3.64 (m, 2H); 3.77 (t, 1H, $J = 9.8$); 4.20 (q, 1H, $J = 5.8$); 4.47 (m, 1H); 4.56 (s, 2H); 4.61 (m, 1H); 5.16 (q, 1H, $J = 7$); 7.4 (m, 10ArH). ^{13}C NMR: δ 19.9, 21.6, 28.9, 52.9, 54.6, 64.8, 70.2, 71.1, 73.5, 76.7, 126.7, 127.7, 127.8, 128.1, 128.4, 129.0, 137.6, 138.1, 172.1, 174.9. HPLC–MS: 410.1 $[M+1-H_2O]^+$, 428.1 $[M+1]^+$, 450.1 $[M+Na]^+$, 877.2 $[2M+Na]^+$. The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.3.8. (3*S*,6*R*,2′*R*,3′*S*)-3-(4′-Benzyloxy-2′,3′-dihydroxybutyl)-6-methyl-4-[(*S*)-phenethyl]-morpholine-2,5-dione 22. The product was obtained from **16** in 40% yield. ^{1}H NMR: δ 1.41 (d, 3H, $J = 6.6$); 1.66 (d, 3H, $J = 7$); 2.45 (m,2H); 3.52 (m,2H); 3.82 (dd, 1H, $J = 8.4, 9.8$); 3.92 (m, 1H); 4.53 (q_{AB}, 2H, $J = 11.6$); 4.52 (m, 1H); 4.76 (m, 1H); 5.13 (q, 1H, $J = 6.6$); 7.4 (m, 10ArH). ^{13}C NMR: δ 19.7; 21.2; 28.2; 52.0; 54.3; 64.7; 70.1; 70.6; 73.2; 77.8; 126.5; 127.5; 127.6; 128.2; 128.7; 137.3; 138.3; 173.3; 174.6. HPLC–MS: 410.1 $[M+1-H_2O]^+$, 428.1 $[M+1]^+$, 450.1 $[M+Na]^+$, 877.2 $[2M+Na]^+$. The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.4. Conversion of diols into γ-lactones

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. The γ-lactones were recovered in 70–80% yield.

4.4.1. (3*R*,5*S*,1′*S*)-5-(2′-Benzyloxy-1′-hydroxyethyl)-3-[(*S*)-phenylethylamino]-dihydro-furan-2-one 11. The product was obtained from **7**. ^{1}H NMR: δ 1.42 (d, 3H, $J = 6.9$); 2.25 (m, 1H); 2.5 (m, 1H); 3.5 (m, 2H); 3.66 (t, 1H, $J = 8.7$); 3.79 (m, 1H); 3.85 (q, 1H, $J = 6.9$); 4.55 (s, 2H); 4.6 (m, 1H); 7.4 (m, 10ArH). ^{13}C NMR: δ 24, 32.6, 53.4, 56.2, 70.7, 71.9, 73.4, 77.3, 126.3, 127.3, 127.7, 127.9, 128.4, 128.6, 137.4, 143.9, 178. HPLC–MS: 356.3 $[M+1]^+$, 378.2 $[M+Na]^+$. IR ($CHCl_3$) ν (cm^{-1}) = 3620 (OH), 1772 (C=O). The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.4.2. (3*R*,5*R*,1′*R*)-5-(2′-Benzyloxy-1′-hydroxyethyl)-3-[(*S*)-phenylethylamino]-dihydro-furan-2-one 12. The product was obtained from **8**. ^{1}H NMR: δ 1.45 (d, 3H, $J = 6.9$); 2.1 (m, 1H); 2.4 (m, 1H); 3.4 (t, 1H, $J = 8.7$); 3.62 (m, 2H); 3.8 (m, 2H); 4.55 (m, 1H); 4.6 (s, 2H); 7.4 (m, 10ArH). ^{13}C NMR: δ 24.1, 31.9, 54.3, 56.2, 70.3, 71.4, 73.6, 77.8, 126.4, 127.5, 127.8, 127.9, 128.5, 128.8, 137.6, 143.3, 176.5. $[\alpha]_D = -65.6$ (c 0.7, $CHCl_3$). IR ($CHCl_3$) ν (cm^{-1}) = 3615 (OH), 1774 (C=O). Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.11; H, 7.1; N, 3.93.

4.4.3. (3*R*,5*S*,1′*R*)-5-(2′-Benzyloxy-1′-hydroxyethyl)-3-[(*S*)-phenylethylamino]-dihydro-furan-2-one 13. The product was obtained from **9**. ^{1}H NMR: δ 1.43 (d, 3H, $J = 6.9$); 2.15 (m, 1H); 2.57 (m, 1H); 3.46–3.62 (m, 3H); 3.77 (m, 1H); 3.83 (q, 1H, $J = 6.9$); 4.55 (m, 1H); 4.56 (s, 2H); 7.4 (m, 10ArH). ^{13}C NMR: δ 24.1, 30.7, 53.1, 56.3, 70.3, 70.6, 73.6, 77.5, 126.4, 127.4, 127.8, 128.0, 128.5, 128.7, 137.3, 143.8, 177.5. HPLC–MS: 356.3 $[M+1]^+$, 378.2 $[M+Na]^+$. IR ($CHCl_3$) ν (cm^{-1}) = 3625 (OH), 1770 (C=O). The

product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.4.4. (3*R*,5*R*,1′*S*)-5-(2′-Benzyloxy-1′-hydroxyethyl)-3-[(*S*)-phenylethylamino]-dihydro-furan-2-one 14. The product was obtained from **10**. ^{1}H NMR: δ 1.44 (d, 3H, $J = 6.6$); 2.09 (m, 1H); 2.48 (m, 1H); 3.37 (dd, 1H, $J = 8.4$, 10.2); 3.60 (m, 2H); 3.82 (q, 1H, $J = 6.6$); 3.97 (m, 1H); 4.39 (m, 1H); 4.56 (s, 2H); 7.4 (m, 10ArH). ^{13}C NMR: δ 24.2, 31.9, 54.5, 56.3, 70.3, 70.9, 73.6, 77.4, 126.4, 127.5, 127.8, 128.0, 128.5, 128.8, 137.5, 143.4, 176.7. HPLC–MS: 356.3 $[M+1]^+$, 378.2 $[M+Na]^+$. IR ($CHCl_3$) ν (cm^{-1}) = 3612 (OH), 1775 (C=O). The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.4.5. (3*S*,5*S*,1′*S*)-5-(2′-Benzyloxy-1′-hydroxyethyl)-3-[(*S*)-phenylethylamino]-dihydro-furan-2-one 23. The product was obtained from **19**. ^{1}H NMR: δ 1.43 (d, 3H, $J = 6.6$); 1.82 (m, 1H); 2.08 (m, 1H); 3.46 (t, 1H, $J = 8.4$); 3.57 (m, 2H); 3.77 (m, 1H); 4.19 (q, 1H, $J = 6.6$); 4.42 (m, 1H); 4.55 (s, 2H); 7.4 (m, 10ArH). ^{13}C NMR: δ 24.4, 33.0, 55.6, 57.5, 70.4, 71.2, 73.5, 77.4, 127.0, 127.4, 127.7, 127.9, 128.4, 128.5, 137.6, 144.5, 177.0. $[\alpha]_D = -18.7$ (*c* 0.6, $CHCl_3$). IR ($CHCl_3$) ν (cm^{-1}) = 3620 (OH), 1770 (C=O). Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.22; H, 7.07; N, 3.95.

4.4.6. (3*S*,5*R*,1′*R*)-5-(2′-Benzyloxy-1′-hydroxyethyl)-3-[(*S*)-phenylethylamino]-dihydro-furan-2-one 24. The product was obtained from **20**. ^{1}H NMR: δ 1.41 (d, 3H, $J = 6.6$); 1.98 (m, 2H); 2.20; 5.25 (m, 2H); 3.71 (m, 2H); 4.11 (q, 1H, $J = 6.6$); 4.42 (m, 1H); 4.53 (s, 2H); 7.4 (m, 10ArH). ^{13}C NMR: δ 24.5, 33.8, 54.8, 57.9, 70.7, 72.0, 73.5, 77.1, 127.0, 127.2, 127.8, 127.9, 128.4, 128.5, 137.4, 144.9, 178.3. $[\alpha]_D = -94.6$ (*c* 0.5, $CHCl_3$). IR ($CHCl_3$) ν (cm^{-1}) = 3624 (OH), 1772 (C=O). Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.1; H, 7.11; N, 3.94.

4.4.7. (3*S*,5*S*,1′*R*)-5-(2′-Benzyloxy-1′-hydroxyethyl)-3-[(*S*)-phenylethylamino]-dihydro-furan-2-one 25. The product was obtained from **21**. ^{1}H NMR: δ 1.41 (d, 3H, $J = 6.6$); 1.9 (m, 1H); 2.1 (m, 1H); 3.45 (dd, 1H, $J = 8.7$, 9.6); 3.53 (m, 2H); 3.97 (m, 1H); 4.22 (q, 1H, $J = 6.6$); 4.32 (m, 1H); 4.55 (q_{AB}, 2H, $J = 10.5$); 7.4 (m, 10ArH). ^{13}C NMR: δ 24.3, 32, 55.6, 57.3, 70.1, 73.4, 77.1, 126.9, 127.2, 127.6, 127.8, 128.4, 137.4, 144.4, 177.4. $[\alpha]_D = -63.7$ (*c* 0.4, $CHCl_3$). IR ($CHCl_3$) ν (cm^{-1}) = 3618 (OH), 1772 (C=O). Anal. Calcd for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 71.21; H, 7.1; N, 3.95.

4.4.8. (3*S*,5*R*,1′*S*)-5-(2′-Benzyloxy-1′-hydroxyethyl)-3-[(*S*)-phenylethylamino]-dihydro-furan-2-one 26. The product was obtained from **22**. ^{1}H NMR: δ 1.42 (d, 3H, $J = 6.6$); 1.9 (m, 1H); 2.2 (m, 1H); 3.54 (m, 3H); 3.82 (m, 1H); 4.16 (q, 1H, $J = 6.6$); 4.45 (m, 1H); 4.54 (q_{AB}, 2H, $J = 11.7$); 7.4 (m, 10ArH). ^{13}C NMR: δ 24.5, 31.7, 54.3, 57.4, 70.3, 70.7, 73.5, 77.4, 126.9, 127.3, 127.6, 128, 128.4, 128.5, 137.3, 144.7, 177.8. HPLC–MS: 356.3 $[M+1]^+$, 378.2 $[M+Na]^+$. IR ($CHCl_3$) ν (cm^{-1}) = 3623 (OH), 1773 (C=O). The product was not isolated in sufficiently pure form for elemental analysis or to measure the specific rotation.

4.5. Enzyme kinetics

4.5.1. Materials. α-Glucosidase (EC 3.2.1.20) from baker's yeast and from *B. stearothermophilus*, 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, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid and its potassium salt (HEPES) were purchased from Sigma.

4.5.2. Kinetics. The kinetic hydrolyses of glucosides were carried out at pH = 6.85 and were followed at $\lambda = 400$ nm by Carry 100 UV spectrophotometer at 37 ± 0.01 °C. Ten cells, filled with 0.1 M HEPES buffer solution containing 0.05–0.2 units of enzyme, were thermostated in the cell holder of UV spectrophotometer. Stock solutions (10–100 μL) of inhibitors, dissolved in ethanol, were added to enzyme buffer solution and thermostated for about 15 min. The glucosides solutions were added to the buffered enzyme solutions by ten Hamilton syringes previously thermostated. Noncompetitive inhibition constants (K_i) were calculated from the equation $K_i = V'_{max}[I_0]/(V_{max} - V'_{max})$ where V'_{max} and V_{max} are the maximum rates measured in the presence and in the absence of inhibitor, respectively, and $[I_0]$ is the inhibitor concentration. The kinetics were carried out in duplicate runs and the K_i reproducibility was in the range 10–15%.

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