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Chemoenzymatic Synthesis and α-Glucosidase Inhibitory Activity of Dimeric Neolignans Inspired by Magnolol

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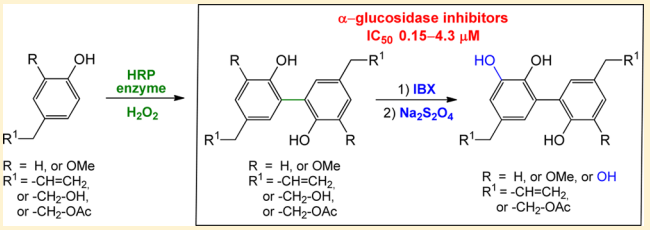
Supporting Information

ABSTRACT: A chemoenzymatic synthesis of a small library of dimeric neolignans inspired by magnolol (**1**) is reported. The 2-iodoxybenzoic acid (IBX)-mediated regioselective *ortho*-hydroxylation of magnolol is described, affording the bisphenols **6** and **7**. Further magnolol analogues (**12**, **13**, **15**−**17**, **19**−**23**) were obtained from eugenol (**3**), tyrosol (**4**), and homovanillic alcohol (**5**), through horseradish peroxidase (HRP)-mediated oxidative coupling and regioselective *ortho*-hydroxylation or *ortho*-demethylation in the presence of IBX, followed by reductive treatment with $Na_2S_2O_4$. A chemoselective protection/deprotection of the alcoholic group of **4** and **5** was carried out by lipase-mediated acetylation/deacetylation. The dimeric neolignans, together with **1** and honokiol (**2**), were evaluated as inhibitors of yeast α-glucosidase, in view of their possible utilization and optimization as antidiabetic drugs. The synthetic analogues of magnolol showed a strong inhibitory activity with IC_{50} values in the range 0.15−4.1 μM, much lower than those of honokiol and the reference compounds quercetin and acarbose. In particular, a very potent inhibitory activity, with an IC_{50} of 0.15 μM, was observed for 1,1′-dityrosol-8,8′-diacetate (**15**), and comparable inhibitory activities were also shown by bisphenols **6** (0.49 μM), **13** (0.50 μM), and **22** (0.86 μM). A kinetic study showed that **15** acts as a competitive inhibitor, with a K_i value of 0.86 μM.



Lignans and related compounds (neolignans, oxyneolignans, and mixed lignans) are a large family of natural products, showing an interesting variety of structures and biological activities.[1,2] They are normally biosynthesized through oxidative radical coupling of phenylpropanoid (C_6C_3) precursors, in turn originated by shikimic acid. In recent years, two simple dimeric neolignans, magnolol (**1**) and honokiol (**2**), have gained growing attention by researchers, and a recent literature search on these compounds afforded approximately 2000 results. Both are natural products originally isolated from the bark of *Magnolia* spp.,[3,4] used in Japanese and Chinese traditional medicine for various diseases such as gastrointestinal disorders, anxiety, and allergic diseases. *Magnolia officinalis* bark is reported for a number of biological activities including anticancer, anti-inflammatory, antidepressant, and antiplatelet activity.[5] Magnolol is probably the most cited among *M. officinalis* constituents, and a nonexhaustive list of its biological properties includes antitumor,[6,7] antiangiogenic,[8,9] anti-inflammatory,[9] antimicrobial,[10] antiviral,[11] and antioxidant[12] effects, as well as prevention of inflammation-induced tumorigenesis,[13] inhibition of osteoclast differentiation,[14] reduction of multidrug resistance through P-glycoprotein modulation,[15] and protection against cerebral ischemia injury.[16] A comparable range of biological properties has also been reported for honokiol.[12,17−19]

These properties have prompted a number of researchers to synthesize magnolol and honokiol analogues and evaluate their biological properties: this afforded new bisphenol neolignans and derivatives with antimicrobial/antiproliferative,[20] neuroprotective,[21] and anti-inflammatory activities,[22] cytotoxicity against cancer cell lines,[23] and modulation of GABA receptors.[24]

In recent years, growing attention has been devoted to phenolic substances as a new class of potential α-glucosidase inhibitors.[25] The search for new and effective α-glucosidase inhibitors has grown rapidly during the past decade, in view of the epidemic diffusion of diabetes and consequently of the efforts devoted to the discovery of potent glucosidase inhibitors able to retard glucose absorption and reduce blood glucose levels.[26] In this frame, we have recently investigated resveratrol-related synthetic glycosides[27] and natural phenols[28] as inhibitors of yeast α-glucosidase, the enzyme most frequently employed in the preliminary steps of the search for new antidiabetic drugs. Although some drugs based on carbohydrate-related glucosidase inhibitors, such as acarbose, miglitol, or voglibose, are commercially available, their effectiveness is hindered by certain side effects,[29] and there is a search for new, optimized inhibitors. Interestingly, a potent α-glucosidase inhibitory activity has been reported for honokiol and especially magnolol,[30] but this property was not further investigated, and only one report deals with inhibition of α-glucosidase by honokiol derivatives (namely, dimers and trimers).[31] Thus, as a continuation of our studies on bioactive phenols, reported herein are the chemoenzymatic synthesis of a series of

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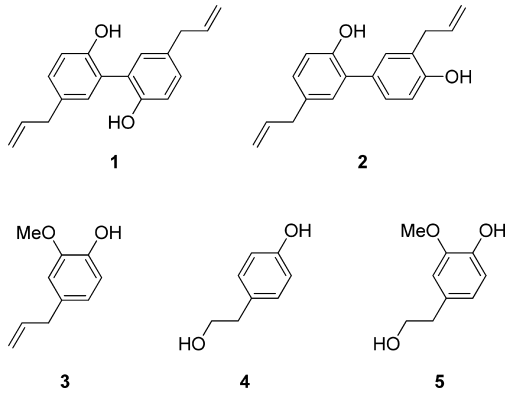
bisphenols inspired by magnolol (**1**) and their evaluation as yeast α-glucosidase inhibitors. To this aim, we employed both enzymatic dimerization of simple phenolic compounds and chemical conversion of monohydroxylated to *ortho*-dihydroxylated aromatic rings (catechols). The enzymatic dimerization of phenolic compounds through oxidative coupling is currently employed in biomimetic, eco-friendly synthesis of lignans and neolignans, and we have previously used this methodology to obtain bioactive dimeric compounds related to resveratrol,[32,33] as well as *p*-coumaric, ferulic, and caffeic acid derivatives.[34,35] Natural or natural-derived catechols are often reported for a variety of biological activities; in particular, their antioxidant activity is higher than that of their monohydroxylated or *meta*-dihydroxylated analogues.[36] Recently, a number of *ortho*-dihydroxylated phenols has been reviewed for promising glucosidase inhibition.[25] Hence, it was planned to obtain dimeric neolignans inspired by magnolol with both monohydroxylated and *ortho*-dihydroxylated substructures. For the latter purpose, IBX (1-hydroxy-1-oxo-1*H*-1λ^5-benz[*d*][1,2]-iodoxol-3-one, or 2-iodoxybenzoic acid) was used, a versatile and environmentally benign reagent of hypervalent iodine,[37] allowing *ortho*-regioselective oxygenation[38] of phenols with free *ortho* positions and phenolic methyl aryl ethers.[39] When combined with an in situ reduction of the products, IBX allows a facile conversion of phenolic compounds to their *ortho*-dihydroxylated derivatives,[40] with a selectivity similar to that mediated by enzymes in Nature.[41] This procedure is generally more convenient than alternative methods requiring the use of environmentally unsafe metal oxidants and hard reaction conditions.[42] Reported herein is the first IBX-mediated *ortho*-selective hydroxylation of magnolol (**1**). Subsequently, this method was applied to other bisphenols obtained through enzymatic dimerization of simple natural phenols, namely, eugenol (**3**), an allylphenol isolated from *Eugenia aromatica* and other plants, tyrosol (**4**), a phenol found in a variety of plants and in olive oil, and the dopamine metabolite homovanillic alcohol (**5**). The choice of eugenol, as well as of strictly related monomeric phenols, was reinforced by the reported anti-glycation activity of **3**, able to lower blood glucose level by inhibition of both α-glucosidase and advanced glycation end products.[43]



RESULTS AND DISCUSSION

Considering the above-cited biological properties of magnolol, a small library was generated of bisphenols inspired by **1**. First, **1** was employed as a substrate to test the IBX-mediated regioselective hydroxylation (Table 1). In this reaction, magnolol (**1**) was treated with IBX, and, after in situ reductive treatment with $Na_2S_2O_4$, both a mono- and a dihydroxylated product, namely, **6** and **7**, may be formed. Some preliminary experiments were performed varying solvent, temperature, reaction time, and equivalents of IBX. After the reductive treatment, the mixtures were analyzed by HPLC-UV on a C_{18} reversed-phase silica gel column, and the results obtained are summarized in Table 1.

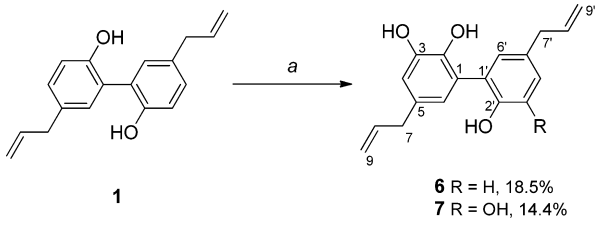
Table 1. Optimization of *ortho*-Selective Hydroxylation of 1 with IBX

entry	IBX (equiv)	solvent	*T* (°C)	time	**6** (%)[a]	**7** (%)[a]
1	1.2	DMSO[b]	rt[c]	2 h	<1	<1
2	1.5	DMSO[b]	rt[c]	4 h	<1	0[d]
3	2.1	DMSO[b]	rt[c]	2 h	<1	0[d]
4	1.5	DMF[b]	rt[c]	4 h	1.1	2.1
5	2.1	DMF[b]	rt[c]	2 h	<1	<1
6	1.2	MeOH[e]	rt[c]	40 min	7.1	6.9
7	1.2	MeOH[e]	0	30 min	14.3	11.3
8	2.1	MeOH[e]	0	30 min	2.5	8.0

[a]The yield was determined by HPLC-UV. [b]0.1 M solution of **1**. [c]Reaction was carried out at rt (27−30 °C). [d]Not obtained. [e]0.2 M solution of **1**.

The reactions carried out in dimethyl sulfoxide (DMSO; entries 1−3) and dimethylformamide (DMF; entries 4 and 5), irrespective of the reaction conditions, gave a high conversion of substrate **1**, but no significant amounts of the expected compounds were detected. The reaction was then carried out in methanol (MeOH) at room temperature, employing 1.2 equiv of IBX (entry 6): after 40 min two main products, more polar than **1**, and subsequently characterized as **6** and **7**, were formed. Further reactions were carried out, in the same solvent, at 0 °C with 1.2 (entry 7) or 2.1 (entry 8) equiv of IBX. The former conditions allowed a high conversion rate (>95%), and the two main products, **6** and **7**, were obtained with 14.3% and 11.3% yield, respectively, whereas a greater amount of IBX significantly lowered their yields. Furthermore, on prolonging the reaction time up to 1 h, no improvement of the yields was observed; thus 30 min was confirmed as the best reaction time. The reaction was then repeated in a preparative scale (Scheme 1), and the main products **6** and **7** were recovered, after purification, respectively with 18.5% and 14.4% yield.

Scheme 1. Synthesis of Magnolol Derivatives 6 and 7[a]



[a]Conditions: (a) MeOH, IBX (1.2 equiv), 0 °C, 30 min; $Na_2S_2O_4$ solution (H_2O), rt, 10 min.

The molecular formula of the least polar product **6**, $C_{18}H_{18}O_3$, was determined by elemental analysis and ESIMS, affording a $[M - H]^-$ peak at *m*/*z* 281.2, 15 amu higher than the molecular weight of magnolol, suggesting the monohydroxylation of **1**. The ^1H and ^{13}C NMR spectra of **6**, compared to those of magnolol, showed doubled signals, clearly due to

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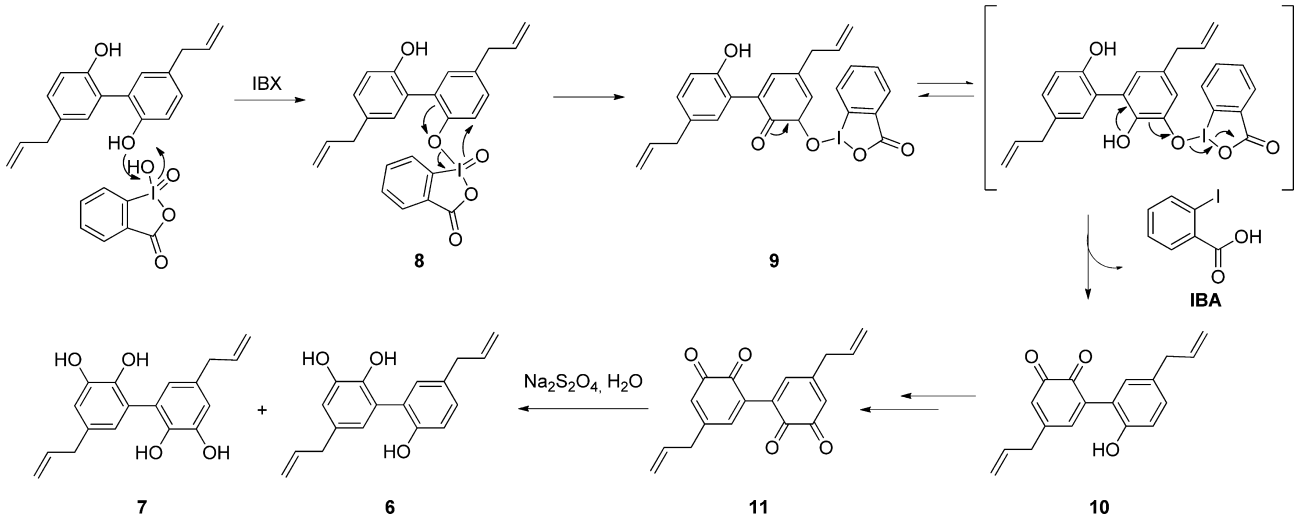
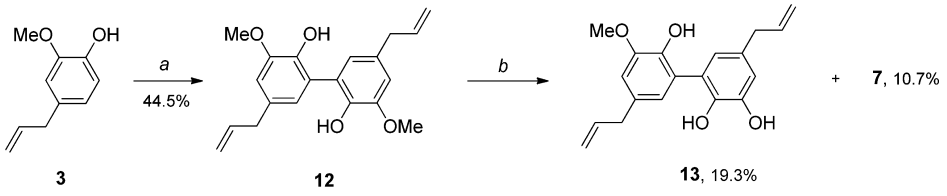
Scheme 2. Mechanism of Formation of 6 and 7



Scheme 3. Chemoenzymatic Synthesis of 13 and 7[a]



[a]Conditions: (a) MeOH, HRP (acetate buffer, 0.1 M, pH 5.0), H_2O_2, rt, 2 h; (b) THF, IBX (1.5 equiv), rt, 3 h; $Na_2S_2O_4$ solution (H_2O), rt, 10 min.

the loss of symmetry. In detail, the ^{13}C NMR spectrum exhibited two sp^3 methylene signals (39.8, 39.5 ppm), two sp^2 methylene signals (116.0, 115.9 ppm), seven sp^2 methine signals (137.7, 137.5, 131.4, 129.8, 122.3, 116.6, 115.1 ppm), and seven signals attributable to sp^2 quaternary carbons (150.4, 145.3, 138.8, 134.0, 133.6, 125.2, 124.5 ppm). Assignments of the ^1H and ^{13}C NMR signals (Experimental Section) were aided by analysis of two-dimensional NMR spectra (COSY, HSQC, and HMBC). In the ^1H NMR spectrum, two *meta*-coupled signals at 6.80 (d, J = 2.0 Hz) and 6.66 (d, J = 2.0 Hz) ppm were assigned, respectively, to H-4 and H-6 of the dihydroxylated ring A. The remaining aromatic signals clearly resembled those of magnolol, with an AMX system (ring B) observed at 7.13 (d, J = 2.0 Hz, H-6′), 7.10 (dd, J = 8.5, 2.0 Hz, H-4′), and 6.91 (d, J = 8.5 Hz, H-3′) ppm. The signals of the allyl chains were observed at 5.96 (m, H-8 and H-8′), 5.06 (m, H_2-9 and H_2-9′), 3.37 (d, J = 6.5 Hz, H_2-7′), and 3.32 (d, J = 6.5 Hz, H_2-7) ppm. The HBMC correlation, from H-6 and H-7 to C-5 (133.6 ppm) as well as from H-7′ to C-5′ (134.0 ppm), allowed the discrimination of the two allyl chains. Hence, the structure **6** was established as 3-hydroxymagnolol. The ESIMS of the most polar compound **7** gave an $[M - H]^-$ peak at m/z 297.2, suggesting the dihydroxylation of **1**. The ^1H and ^{13}C NMR spectra of **7** indicated that the symmetry of magnolol was maintained and suggested an *ortho*-dihydroxylation of both aromatic rings. A literature search showed a close agreement of NMR data with those previously reported for 3,3′-dihydroxymagnolol.[20]

In Scheme 2 we report the mechanism for the IBX-mediated regioselective hydroxylation of magnolol. According to the literature,[44] **1** adds to the iodine(V) center of IBX to form a λ^5-iodanil intermediate (**8**), subsequently leading to a more stable λ^3-iodanil intermediate (**9**), for which the tautomeric form is hydrolyzed to give the *ortho*-quinone (**10**) and 2-iodobenzoic acid (IBA). If these steps are repeated for the second phenolic ring, the bis-*ortho*-quinone **11** is formed, and the final reductive step affords both catechol **6** and **7**.

On the basis of the above results, initially a general route was tested for the synthesis of a small library of dimeric neolignans inspired by magnolol. Eugenol (**3**) was used as substrate for a preparative dimerization reaction mediated by the environmentally friendly oxidative enzyme horseradish peroxidase (HRP), employed with H_2O_2 in MeOH at room temperature (Scheme 3), according to a previous literature report.[45] This afforded, after purification, one major product (44.5% yield), for which the ESIMS and ^1H and ^{13}C NMR data were closely comparable with those reported in the literature for 1,1′-dieugenol (**12**).

Also for the IBX-mediated oxidative demethylation of **12**, a preliminary screening procedure was conducted varying solvent, temperature, reaction time, and equivalents of IBX. After in situ reduction with $Na_2S_2O_4$, the reactions were monitored by HPLC-UV on a C_{18} reversed-phase silica gel column, and the results are summarized in Table 2. The reactions carried out in MeOH (entry 1), DMSO (entry 2), and DMF (entry 3) gave a high conversion of substrate **12**, but no significant amounts of the expected compounds were detected. When the reaction was carried out in tetrahydrofuran (THF) for 3 h (entry 4), two main products, more polar than the substrate, were formed and subsequently identified as **13** (18.7%) and **7** (10.0%). The reaction in THF was monitored at regular time intervals up to 16 h, and quantitative analysis indicated 3 h as the best reaction time. Further experiments carried out at 0 °C with 1.5 equiv of IBX (entry 5) and at rt

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Table 2. Optimization of *ortho*-Selective Demethylation of 12 with IBX[a]

entry	IBX (equiv)	solvent	T (°C)	time	7 (%)[b]	13 (%)[b]
1	1.5	MeOH	rt[c]	3 h	<1	<1
2	1.5	DMSO	rt[c]	3 h	1.0	<1
3	1.5	DMF	rt[c]	3 h	1.8	2.1
4	1.5	THF	rt[c]	3 h	10.0	18.7
5	1.5	THF	0	3 h	4.5	9.8
6	1.2	THF	rt[c]	3 h	5.9	12.2

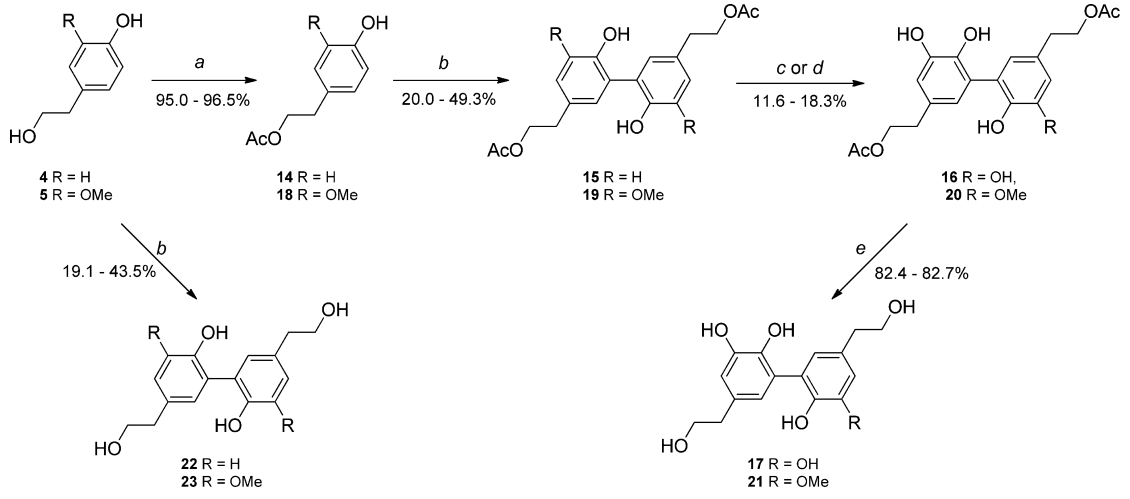
[a]All reactions were carried out using a 0.06 M solution of **12**. [b]The yield was determined by HPLC-UV. [c]Reaction was carried out at room temperature (27−30 °C).

with 1.2 equiv of IBX (entry 6) and afforded **13** and **7** with lower yields. Thus, a preparative reaction of **12** with IBX was carried out in THF at rt for 3 h, followed by in situ reduction with $Na_2S_2O_4$ (Scheme 3).

After purification, two main products were recovered, with the least polar **13** (19.3% yield) showing an ESIMS $[M - H]^-$ peak at *m/z* 311.1, suggesting that only one methyl group has been removed from **12**. Both the ^1H and ^{13}C NMR spectra indicated an unsymmetrical structure and were in close agreement with those previously reported for **13**,[20] which was thus established as 3-*O*-desmethyl-1,1′-dieugenol. The other main product (10.7% yield), markedly more polar than **13**, showed ESIMS and NMR data identical to those of 3,3′-dihydroxymagnolol (**7**), as expected for an IBX-mediated double demethylation reaction. On the basis of these results, the synthesis was conducted on a preparative scale of further magnolol-related bisphenols based on tyrosol (**4**) and homovanillic alcohol (**5**), employing HRP in the dimerization step, followed by treatment with IBX/$Na_2S_2O_4$. These reactions are summarized in Scheme 4.

Based on the literature, it was anticipated that the oxidative treatment of unprotected tyrosol/homovanillic alcohol dimers with IBX would provide unsatisfactory results.[45] Hence, tyrosol (**4**) was converted into tyrosol 8-acetate (**14**), using an eco-friendly, chemoselective acetylation procedure mediated by *Candida antarctica* lipase (CaL), with vinyl acetate as acyl donor. After purification, **14** was recovered with 95.0% yield. Its MS and NMR spectra were in close accord with previous data.[46] Tyrosol 8-acetate (**14**) was subjected to HRP-mediated dimerization in acetone at rt. Although after 4 h only a partial conversion of the substrate was observed, attempts to improve the conversion were unsuccessful. Hence, the preparative dimerization of **14** was carried out, thus obtaining the main product **15** with 20.0% yield, after purification. The molecular formula of **15** was found to be $C_{20}H_{22}O_6$, based on elemental analysis and the ESIMS ($[M - H]^-$ peak at *m/z* 357.0), indicating the formation of a dimeric product. Its ^{13}C NMR spectrum showed only 10 signals and suggested a symmetrical bisphenol. In the aromatic region, a quaternary carbon signal at 127.0 ppm (C-1/C-1′) was observed instead of the CH signal in the ^{13}C NMR of **14**. The low-field ^1H NMR signals were assigned readily to an aromatic AMX system analogous to that of magnolol (**1**), namely, 7.21 (d, *J* = 2.5 Hz, H-6), 7.15 (dd, *J* = 2.5, 8.5 Hz, H-4), and 6.95 (d, *J* = 8.5 Hz, H-3) ppm. At higher fields, the signals due to the $-CH_2CH_2OAc$ pendants were observed at 4.24 (t, *J* = 7.0 Hz, H_2-8), 2.91 (t, *J* = 7.0 Hz, H_2-7), and 1.99 (s, $-OCOCH_3$) ppm. The proton/carbon NMR assignments (Experimental Section) were corroborated by two-dimensional NMR experiments (COSY and HSQC), also confirming the structure of 1,1′-dityrosol-8,8′-diacetate (**15**). The reaction of **15** with IBX was carried out in MeOH at 0 °C, followed by in situ reduction, as reported for magnolol (**1**). However, in this case, the formation of only one major product was observed, even on increasing the reaction time. After purification, the main product **16** (11.6% yield) was subjected to spectroscopic characterization. Its elemental analysis and ESIMS ($[M - H]^-$ peak at *m/z* 389.0) gave the molecular formula $C_{20}H_{22}O_8$, indicating the addition of two oxygen atoms to **15** and suggesting the formation of a tetrahydroxylated product. The ^{13}C NMR spectrum of **16**, with 10 carbon resonances, indicated a symmetrical structure, and a deshielded quaternary carbon at 146.8 ppm replaced the CH signal observed in the ^{13}C NMR of **15**. The ^1H NMR spectrum of **16** showed two *meta*-coupled signals at 6.81 (d, *J* = 2.0 Hz, H-6) and 6.73 ppm (d, *J* = 2.0 Hz, H-4) and those due to the

Scheme 4. Chemoenzymatic Synthesis of 15−17 and 19−23[a]



[a]Conditions: (a) CaL, vinyl acetate, MTBE, 40 °C, 1 h; (b) acetone, HRP solution (aceate buffer, 0.1 M, pH 5.0), H_2O_2, rt, 4 h; (c) MeOH, IBX (1.2 equiv), 0 °C, 30 min; $Na_2S_2O_4$ solution (H_2O), rt, 10 min; (d) THF, IBX (1.5 equiv), rt, 3 h; $Na_2S_2O_4$ solution (H_2O), rt, 10 min; (e) CaL, *n*-butanol, MTBE, 40 °C, 92 h.

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aliphatic chain at 4.22 (t, J = 7.0 Hz, H_2-8) and 2.83 ppm (t, J = 7.0 Hz, H_2-7). COSY, HSQC, and HMBC experiments were used to confirm the structure of 3,3′-dihydroxy-1,1′-dityrosol-8,8′-diacetate (**16**), also allowing for the complete assignment of NMR signals (Experimental Section). Subsequently, a lipase-mediated alcoholysis procedure was employed for the deprotection of **16**, in which the substrate was treated with CaL, using *n*-butyl alcohol as acyl acceptor. The product **17** (3,3′-dihydroxy-1,1′-dityrosol) was obtained with 82.7% yield. Its elemental analysis and ESIMS data, with a main $[M - H]^-$ peak at m/z 305.1, gave the expected molecular formula, $C_{16}H_{18}O_6$, confirming the removal of two acetyl groups. The main differences in the ^1H and ^{13}C NMR spectra of **17** with respect to those of **16** were the lack of the acetate group.

On the basis of the above, also **5** was treated with vinyl acetate in the presence of CaL (Scheme 4) to obtain the acetate **18** (96.5% yield), showing spectroscopic data identical to those reported in the literature for homovanillic alcohol 8-acetate.[47] This was subjected to HRP-mediated oxidative coupling and afforded **19** with 49.3% yield. The purified product was subjected to spectroscopic analysis: HRMS gave an $[M - H]^-$ peak at m/z 417.2201, confirming the formation of a dimer. The ^1H NMR and ^{13}C NMR spectra of **19** indicated a symmetrical structure. The main differences with respect to **15** were the signals of the methoxy group (^1H NMR: 2.05 ppm; ^{13}C NMR: 20.0 ppm) and the lack of H-3 signal in the proton spectrum. A deshielded quaternary carbon at 147.8 ppm (C-3, bearing the methoxy group) replaced a CH signal in the ^{13}C NMR spectrum of **15**. The analysis of 2D NMR spectra (COSY and HSQC) was used to corroborate the structure of 1,1′-dihomovanillic alcohol 8,8′-diacetate (**19**) and allowed the complete assignment of all NMR resonances. This compound was subjected to a preparative IBX-mediated reaction in THF at rt for 3 h, with very good conversion of the substrate, and afforded two main products. The major product **20**, obtained with 20.0% yield, was the least polar. Its molecular formula, $C_{21}H_{24}O_8$, and the ESIMS, with a $[M - H]^-$ peak at m/z 403.0, suggested a monodemethylation of **19**. The ^1H and ^{13}C NMR spectra of **20** indicated the formation of an unsymmetrical bisphenol and resembled those of the dimeric bisphenol **13**. Eighteen carbon resonances were observed in its ^{13}C NMR spectrum and were assigned through the analysis of HSQC and HMBC spectra. In particular, the signal at 141.4 ppm was assigned to C-3′ on the basis of the HMBC correlation of the $-OCH_3$ signal (3.91 ppm in the proton spectrum) with the carbon resonance at 141.4 ppm. The HMBC correlation from H-4′ (6.78 ppm, d, J = 1.5 Hz) to C-3′ and the COSY correlation of H-4′ with H-6′ (6.70 ppm, d, J = 1.5 Hz) allowed an unambiguous assignment of the *meta*-coupled signals in ring B. Consequently, the *meta*-coupled aromatic proton signals at 6.94 (d, J = 1.5 Hz, H-4) and 6.83 ppm (d, J = 1.5 Hz, H-6) were assigned to ring A. Pertinent HMBC correlations allowed the assignments of the alkyl chain signals at 4.25 and 4.22 ppm (t, J = 7.0 Hz, respectively, H_2-8 and H_2-8′), 2.91 and 2.84 ppm (t, J = 7.0 Hz, respectively, H_2-7 and H_2-7′), and 1.99 and 1.98 ppm (s, $-OCOCH_3$). Thus, **20** was established as 3-hydroxy-3′-methoxy-1,1′-dityrosol 8,8′-diacetate. The other main product was obtained with 13.7% yield, and its MS and NMR spectra were identical to those of compound **16**, as expected for a double demethylation of **19**. Finally, **20** was submitted to a deprotection reaction through a CaL-mediated butanolysis. The product **21** (3-hydroxy-3′-methoxy-1,1′-dityrosol) was obtained with 82.4% yield. The molecular formula of **21**, $C_{17}H_{20}O_6$, its ESIMS spectrum, showing a $[M - H]^-$ peak at m/z 319.1, and its ^1H and ^{13}C NMR spectra confirmed the removal of two acetyl groups.

In order to increase the structural variety of this library, both tyrosol (**4**) and homovanillic alcohol (**5**) were submitted to an HRP-mediated dimerization reaction (Scheme 4), obtaining respectively the products **22** and **23**. The former was obtained, after purification, in 19.1% yield. Its HRMS gave a $[M - H]^-$ peak at m/z 273.1705, corroborating the formation of a dimer. Both NMR spectra indicated the presence of a symmetrical bisphenol, showing five proton signals and eight carbon signals (Experimental Section). The spectra strongly resembled those of **15**, with the main difference being the lack of acetate signals. Analysis of the NMR spectra was aided by the COSY and HSQC spectra and confirmed the structure of 1,1′-dityrosol (**22**). Analogously, the HRMS of **23**, obtained with 43.5% yield, showed a main $[M - H]^-$ peak at m/z 333.2061 as expected for a dimeric product. Both ^1H and ^{13}C NMR spectra were very similar to those of **19** apart from the lack of any acetate signals. The analysis, aided by 2D NMR spectra, confirmed **23** as 1,1′-dihomovanillic alcohol.

The synthesized bisphenols **6**, **7**, **12**, **13**, **15**−**17**, and **19**−**23** were evaluated as yeast α-glucosidase inhibitors using *p*-nitrophenyl-α-glucopyranoside (*p*NP-α-G) as the substrate. Also, the natural occurring neolignans magnolol (**1**) and honokiol (**2**) were tested for comparison and also to confirm previous reports on their inhibitory activity. Quercetin and the antidiabetic drug acarbose were employed as reference standards. The percent of inhibition at 1.5 μM of each compound and the IC_{50} values are reported in Table 3. The

Table 3. α-Glucosidase Inhibitory Activity of Magnolol-Related Bisphenols

compound	inhibition (%)[a]	IC_{50} ± SD (μM)[b]
magnolol (**1**)	29.8	2.0 ± 0.4
honokiol (**2**)	3.9	23.0 ± 2.4
6	97.6	0.49 ± 0.19
7	36.7	2.1 ± 0.6
12	46.7	2.0 ± 0.6
13	98.0	0.50 ± 0.13
15	98.9	0.15 ± 0.09
16	31.4	2.9 ± 0.3
17	18.2	4.1 ± 0.8
19	64.5	1.1 ± 0.4
20	21.3	3.3 ± 0.9
21		n. d.[c]
22	76.9	0.86 ± 0.25
23		n.d.[c]
quercetin	5.3	14.2 ± 2.1
acarbose	0.7	269.9 ± 34.6

[a]Inhibition determined at 1.5 μM. [b]Results are reported as mean ± SD (n = 3). [c]Not determined: no reliable IC_{50} value was obtained up to 100 μM.

synthetic analogues of magnolol showed a strong inhibitory activity, with IC_{50} values in the range 0.15−4.1 μM, much lower than those of quercetin (14.2 μM), acarbose (269.9 μM), and honokiol (23.0 μM). Magnolol was confirmed to be very active, with an IC_{50} value of 2.0 μM, close to that reported in the literature.[30]

Most interestingly, very potent inhibitory activity, with an IC_{50} of 0.15 μM and 98.9% inhibition at 1.5 μM, was observed

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for 1,1′-dityrosol-8,8′-diacetate (**15**). Comparable inhibitory activities were also shown by the bisphenols **6** (0.49 μM), **13** (0.50 μM), and **22** (0.86 μM), with percentage inhibitions in the approximate range 77−98%. These compounds are far more potent than the known carbohydrate-related glucosidase inhibitor acarbose.

It is worth noting that **6**, the catechol analogue of magnolol (**1**), was found to be significantly more potent than **1**, and a similar difference was observed for **13** with respect to **12**. Nevertheless, both **15** and **22**, lacking a catechol moiety, showed potent inhibition and suggest that the dityrosol scaffold is especially promising for future optimization of antidiabetic drugs based on natural lead compounds. The markedly lower potency of honokiol (**2**) suggests that an OH group *ortho* to the allyl chain in ring B may be detrimental for the resultant activity. However, other compounds, closely related to those showing a potent inhibitory activity, were less potent (IC_{50} values in the range 1.1−4.1 μM). Hence, further studies are required to establish clear structural determinants for the α-glucosidase inhibitory activity of magnolol-related bisphenols.

To gain some insight about the mode of action of these neolignans, a kinetic study of the inhibitory effect of the most potent compound **15** on yeast α-glucosidase was carried out. In Figure 1, the Lineweaver−Burk plots of α-glucosidase

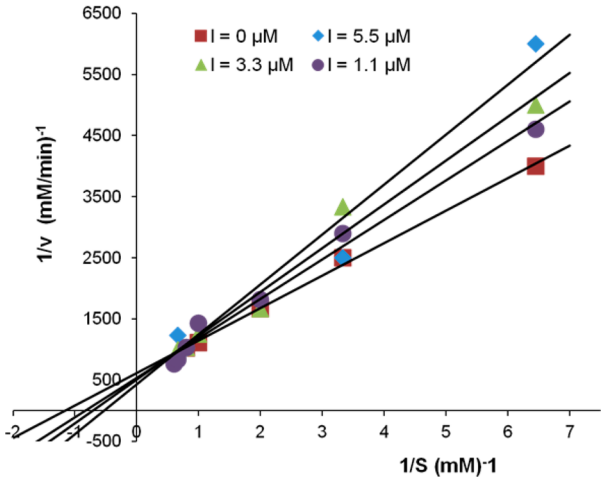

Figure 1. Lineweaver−Burk plots of α-glucosidase inhibition at different concentrations of substrate and compound **15** (I; 0, 1.1., 3.3, and 5.5 μM). The data points present the average of two experiments.

inhibition by **15** is shown, with reference to different concentrations of substrate (*p*NP-α-G) and inhibitor. The results indicated that **15** acts as a competitive inhibitor, with a K_i value of 0.86 μM.

In conclusion, this study has highlighted a new class of magnolol-related neolignans with potent yeast α-glucosidase inhibitory activity, which is a promising property in view of their possible optimization as new antidiabetic drugs.

EXPERIMENTAL SECTION

General Experimental Procedures. α-Glucosidase inhibition assay measurements were carried out by employing a JASCO V630 spectrometer (Milan, Italy), and the kinetic measurements were performed on an Eppendorf PlateReader AF 2200 (Hamburg, Germany). NMR spectra were run on a Varian Unity Inova spectrometer (Milan, Italy) operating at 499.86 (^{1}H) and 125.70 MHz (^{13}C) and equipped with a gradient-enhanced, reverse-detection probe. Chemical shifts (δ) were recorded in parts per million (ppm) and were referenced to tetramethylsilane using the residual solvent signals, chloroform-d_1 (7.26 ppm) and acetone-d_6 (2.05 ppm). ^{1}H NMR splitting patterns are designated as s (singlet), bs (broad singlet), d (doublet), dd (double doublet), t (triplet), m (multiplet); J values are given in Hz. The 2D g-HSQCAD experiments were performed with matched adiabatic sweeps for coherence transfer, corresponding to a central ^{13}C−^{1}H J-value of 146 Hz. g-HMBCAD experiments were optimized for a long-range ^{13}C−^{1}H coupling constant of 8.0 Hz. All NMR experiments, including 2D spectra, i.e., g-COSY, g-HSQCAD, and g-HMBCAD, were performed using software supplied by the manufacturer and acquired at constant temperature (300 K). Mass spectra were acquired with a Thermo Scientific LCQ-DECA ion trap mass spectrometer (Waltham, MA, USA) equipped with ESI ion source, operating in the negative-ion mode. Samples were directly infused, and electrospray mass spectra were acquired from m/z 150 to 2000 using the following electrospray ion source parameters: capillary temperature 220 °C; capillary voltage −18 V; spray voltage 3.5 kV; gas flow rate 30 au. Other mass spectra were performed on a Waters QTof Premier mass spectrometer (Milford, MA, USA) equipped with ESI ion source operating in negative-ion mode. Elemental analyses were performed on a PerkinElmer 240B microanalyzer (Milan, Italy). High-performance liquid chromatography (HPLC) was carried out using an Agilent Series (Milan, Italy) G1354A pump and an Agilent UV G1315D as diode array detector. An Agilent Series 1100 G1313A autosampler was used for sample injection; an analytical reversed-phase column (Luna C_{18}, 5 μm; 4.6 × 250 mm; Phenomenex, Castel Maggiore, BO, Italy) was employed to monitor the course of the reactions, eluting at 1 mL/min with the following gradient of CH_3CN−HCOOH (99:1 v/v; A) in H_2O−HCOOH (99:1 v/v; B): $t_{0\ min}$ A = 60%, $t_{7\ min}$ A = 100%, $t_{10\ min}$ A = 100%, $t_{15\ min}$ A = 60%. PLC was performed on LiChroprep Diol silica gel (40−63 μm; Merck Millipore, Milan, Italy) using different solvent systems. TLC was carried out using precoated silica gel F254 plates (Merck Millipore, Milan, Italy), with visualization of reaction components achieved under UV light at a wavelength of 254 and 366 nm or by staining with a solution of cerium sulfate or phosphomolybdic acid, followed by heating.

All chemicals were of reagent grade and were used without further purification. Horseradish peroxidase (type I), α-glucosidase from *Saccharomyces cerevisiae* (type I, lyophilized powder, 10 units/mg protein), acarbose, quercetin, and *p*-nitrophenyl-α-D-glucopyranoside, tyrosol, and homovanillyl alcohol were purchased from Sigma-Aldrich (Milan, Italy). *Candida antarctica* lipase (Chirazyme, L-2, c.-f. C2, lyo) was purchased from Roche (Monza, MB, Italy). Magnolol, honokiol, and eugenol were purchased from TCI Europe N.V. (Zwijndrecht, Belgium). IBX was prepared in our laboratory according to the literature.[48] The stabilized IBX (SIBX), kindly offered by Prof. S. Quideau, was employed in the early stages of this study, in preliminary experiments (details not reported).

Preliminary Screening for the *ortho*-Selective Hydroxylation. Magnolol (**1**) was dissolved in three different solvents, namely, DMSO, DMF, and MeOH. The solutions were treated with different amounts of IBX and in different reaction conditions as reported below. Finally, the reaction mixtures were treated with $Na_2S_2O_4$. All reactions were monitored at selected time intervals by HPLC-UV on a reversed-phase C_{18} column as reported in the General Experimental Procedures.

Experiments in DMSO. Different amounts of **1** (5.0 mg, 0.018 mmol) were solubilized in DMSO (0.18 mL, 0.1 M) and stirred in capped vials with (a) IBX (6.0 mg, 1.2 equiv) at rt for 2 h; (b) IBX (7.5 mg, 1.5 equiv) at rt for 4 h; and (c) IBX (10.5 mg, 2.1 equiv) at rt for 2 h. The reactions were treated with a solution of $Na_2S_2O_4$ (3.1 mg, 0.017 mmol in 0.18 mL of H_2O) and stirred for 10 min.

Experiments in DMF. Two samples of **1** (5.0 mg, 0.018 mmol) were solubilized in DMF (0.18 mL, 0.1 M), and the solutions were stirred in two capped vials with (a) IBX (7.5 mg, 1.5 equiv) at rt for 4 h and (b) IBX (10.5 mg, 2.1 equiv) at rt for 2 h. The mixtures were treated with a solution of $Na_2S_2O_4$ (3.1 mg, 0.017 mmol in 0.18 mL of H_2O) for 10 min.

Experiments in MeOH. Different amounts of **1** (10.0 mg, 0.037 mmol) were solubilized in MeOH (0.18 mL, 0.2 M) and stirred in

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capped vials with (a) IBX (12.6 mg, 1.2 equiv) at rt for 1 h and (b) IBX (12.6 mg, 1.2 equiv) at 0 °C for 1 h. The reactions were treated with a solution of $Na_2S_2O_4$ (3.1 mg, 0.017 mmol in 0.18 mL of H_2O) and stirred for 10 min, and (c) a 0.2 M solution of **1** in MeOH (10.0 mg, 0.037 mmol, 0.18 mL) was stirred in a capped vial with IBX (21.1 mg, 2.1 equiv) at 0 °C for 1 h; after this time the reaction was treated with a solution of $Na_2S_2O_4$ (6.4 mg, 0.037 mmol in 0.18 mL of H_2O) for 10 min.

Synthesis of Compounds 6 and 7. A 0.2 M solution of magnolol (**1**, 100 mg, 0.37 mmol) in MeOH (1.8 mL) was stirred with IBX (123.1 mg, 1.2 equiv) at 0 °C until complete disappearance of the substrate (30 min). Subsequently, a solution of $Na_2S_2O_4$ (76.6 mg, 0.43 mmol in 1.8 mL of H_2O) was added, and the solution was stirred at rt for 10 min. The solvent was removed under vacuum, and the residue was solubilized with ethyl acetate (20 mL) and partitioned with a saturated $NaHCO_3$ solution (3 × 20 mL). The recovered aqueous phase was partitioned with ethyl acetate (1 × 50 mL). The combined organic layers were washed with a saturated NaCl solution (1 × 50 mL) and dried over Na_2SO_4. After filtration, the solvent was evaporated under vacuum. The residue was subjected to flash chromatography on DIOL silica gel, eluting with *n*-hexane−$CHCl_3$ (30:70 → 0:100) and $CHCl_3$−EtOAc (99:1 → 80:20).

*5,5′-Diallyl-(1,1′-biphenyl)-2,2′,3-triol (**6**):* yellow oil (19.3 mg; 18.5%): R_f (TLC) 0.46 (*n*-hexane−acetone, 60:40); ^{1}H NMR (chloroform-d_1, 500 MHz) δ 7.13 (1H, d, J = 2.0 Hz, H-6′), 7.10 (1H, dd, J = 8.5, 2.0 Hz, H-4′), 6.91 (1H, d, J = 8.5 Hz, H-3′), 6.80 (1H, d, J = 2.0 Hz, H-4), 6.66 (1H, d, J = 2.0 Hz, H-6), 6.17 (1H, bs, OH), 5.97 (2H, m, 5.99−5.92, H-8/8′), 5.85 (2H, bs, OH), 5.06 (4H, m, 5.11−5.05, H_2-9/9′), 3.37 (2H, d, J = 6.5 Hz, H_2-7′), 3.32 (2H, d, J = 7.0 Hz, H_2-7); ^{13}C NMR (chloroform-d_1, 125 MHz) δ 150.4 (C, C-2′), 145.3 (C, C-3), 138.8 (C, C-2), 137.7 (CH, C-8), 137.5 (CH, C-8′), 134.0 (C, C-5′), 133.6 (C, C-5), 131.4 (CH, C-6′), 129.8 (CH, C-4′), 125.2 (C, C-1), 124.5 (C, C-1′), 122.3 (CH, C-6), 116.6 (CH, C-3′), 116.0 (CH_2, C-9), 115.9 (CH_2, C-9′), 115.1 (CH, C-4), 39.8 (CH_2, C-7), 39.5 (CH_2, C-7′); ESIMS m/z 281.2 $[M - H]^-$ (calcd for $C_{18}H_{17}O_3$: 281.1); anal. C 76.54; H, 6.42%, calcd for $C_{18}H_{18}O_3$, C 76.57, H, 6.43%.

*5,5′-Diallyl-(1,1′-biphenyl)-2,2′,3,3′-tetraol (**7**):* yellow oil (15.9 mg, 14.4%). The spectroscopic data were in agreement with those reported in the literature.[20]

Synthesis of Compound 12. Eugenol (**3**, 300.0 mg, 1.82 mmol) was solubilized in MeOH (12.0 mL), and the solution was stirred at rt with an HRP solution (3.1 mg in 12 mL of acetate buffer 0.1 M, pH 5.0) and H_2O_2 (30% v/v, 0.1 mL); two further additions of H_2O_2 were done within 2 h. The reaction was stopped after 4 h, the mixture was concentrated in vacuo, and the residue was extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with water (60 mL), dried over Na_2SO_4, and evaporated under a vacuum. Flash chromatography with Diol silica gel, eluted with *n*-hexane−acetone (100:0 → 50:50), gave the expected compound **12** (131.6 mg, 44.5%): R_f (TLC) 0.36 (*n*-hexane−acetone, 60:40); spectroscopic data were in agreement with those reported in the literature.[49]

Preliminary Experiments for the *ortho*-Selective Demethylation. Dieugenol (**12**) was dissolved in four different solvents, namely, DMSO, DMF, MeOH, and THF. The solutions were treated with different amounts of IBX and in different reaction conditions as reported below. Then, the mixtures were treated with $Na_2S_2O_4$. All reactions were monitored at selected time intervals by HPLC-UV on a reversed-phase C_{18} column, as reported in the General Experimental Procedures.

Experiment in DMSO. Compound **12** (4.0 mg, 0.012 mmol) was solubilized in DMSO (0.15 mL) to a final concentration of 0.06 M and stirred with IBX (5.0 mg, 1.5 equiv) at rt up to 16 h. The mixture was treated with a solution of $Na_2S_2O_4$ (3.1 mg, 0.017 mmol in 0.15 mL of H_2O) and stirred for 10 min.

Experiments in DMF. Compound **12** (4.0 mg, 0.012 mmol) was solubilized in DMF (0.20 mL) to a final concentration of 0.06 M and stirred with IBX (5.0 mg, 1.5 equiv) at rt up to 16 h. The mixture was treated with a solution of $Na_2S_2O_4$ (3.1 mg, 0.017 mmol in 0.20 mL of H_2O) and stirred for 10 min.

Experiment in MeOH. Compound **12** (4.0 mg, 0.012 mmol) was solubilized in MeOH (0.20 mL) to a final concentration of 0.06 M and stirred with IBX (5.0 mg, 1.5 equiv) at rt up to 16 h. The mixture was treated with a solution of $Na_2S_2O_4$ (3.1 mg, 0.017 mmol in 0.20 mL of H_2O) and stirred for 10 min.

Experiments in THF. Different amounts of **12** (4.0 mg, 0.012 mmol) were solubilized in THF (0.20 mL) to a final concentration of 0.06 M, and the solutions were stirred with (a) IBX (5.0 mg, 1.5 equiv) at rt up to 16 h; (b) IBX (5.0 mg, 1.5 equiv) at 0 °C up to 16 h; and (c) IBX (4.1 mg, 1.2 equiv) at rt up to 16 h. The reactions were treated with a solution of $Na_2S_2O_4$ (3.1 mg, 0.017 mmol in 0.20 mL of H_2O) and stirred for 10 min.

Synthesis of Compounds 7 and 13. A 0.06 M THF solution of **12** (180 mg, 0.55 mmol in 9.2 mL) was stirred with IBX (229.5 mg, 1.5 equiv) at rt for 3 h. After this time, the mixture was treated with an aqueous solution of $Na_2S_2O_4$ (142.7 mg, 0.81 mmol in 9.2 mL) and stirred at rt for 10 min. After the evaporation of the solvent under vacuum, the residue was diluted with EtOAc (30 mL) and partitioned with a saturated $NaHCO_3$ solution (3 × 20 mL). The combined aqueous phase was partitioned with ethyl acetate (1 × 50 mL). The organic phases were washed with a saturated NaCl solution (1 × 50 mL) and dried over Na_2SO_4. After filtration, the solvent was evaporated under vacuum. Flash chromatography on Diol silica gel, eluted with a gradient of *n*-hexane−$CHCl_3$ (30:70 → 0:100) and $CHCl_3$−EtOAc (99:1 → 70:30), gave the neolignan **13** (33.2 mg, 19.3%), for which the spectroscopic data were in agreement with those reported in the literature,[20] and also a more polar product that showed spectroscopic data identical to those of 3,3′-dihydroxymagnolol (**7**, 17.6 mg, 10.7%).

Enzymatic Acetylation of Compounds 14 and 18. *Candida antarctica* lipase (CaL, 300 mg) and vinyl acetate (3.8 mL, 42.0 mmol) were added to a solution of the substrate (**4** or **5**, 2.17 mmol) in *tert*-butyl methyl ether (MTBE) (75.0 mL). The resulting mixture was stirred at 400 rpm at 40 °C, and the progress of the reactions was monitored by TLC ($CHCl_3$−MeOH, 98:2). After the completion of the reaction, the enzyme was filtered off and the filtrate was evaporated in vacuo. The crude mixture was purified by flash chromatography on Diol silica gel (*n*-hexane−CH_2Cl_2, 30:70 → 0:100).

*4-Hydroxyphenethyl acetate (**14**):* yellow oil (371.1 mg, 95.0%). The spectroscopic data were in agreement with those reported in the literature.[47]

*4-Hydroxy-3-methoxyphenetyl acetate (**18**):* yellow oil (439.7 mg, 96.5%). The spectroscopic data were in agreement with those reported in the literature.[47]

General Procedure for the Synthesis of Magnolol-Related Bisphenols 15, 19, 22, and 23. A solution of a monomer (**4**, **5**, **14**, **18**; 0.50 mmol in 32.0 mL of acetone) was stirred with a solution of HRP (4.2 mg in 16.0 mL of acetate buffer, 0.1 M, pH 5.0) and in the presence of H_2O_2 (0.1 mL of 0.3% solution) at rt for 4 h. Two further aliquots of H_2O_2 (2 × 0.1 mL) were added within 2 h. The reaction mixture was concentrated in vacuo, and the crude residue was partitioned with EtOAc (3 × 25 mL). The combined organic layer was dried over Na_2SO_4 and filtered, and the solvent was evaporated under vacuum. The crude residue was purified by flash chromatography on Diol silica gel (*n*-hexane−acetone 100:0 → 50:50 for **15** and **19** and *n*-hexane−CH_2Cl_2 40:60 → 0:100 and CH_2Cl_2−MeOH 99:1 → 94:6 for **22** and **23**) to give the pure products.

*[6,6′-Dihydroxy-(1,1′-biphenyl)-3,3′]diylbis(ethane-2,1-diyl) diacetate (**15**):* yellow oil (20.1 mg, 20.0%); R_f (TLC) 0.50 (*n*-hexane−acetone, 50:50); ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.29 (2H, bs, OH), 7.21 (2H, d, J = 2.5 Hz, H-6), 7.15 (2H, dd, J = 2.5, 8.5 Hz, H-4), 6.95 (2H, d, J = 8.5 Hz, H-3), 4.24 (4H, t, J = 7.0 Hz, H_2-8), 2.91 (4H, t, J = 7.0 Hz, H_2-7), 1.99 (6H, s, $OCOCH_3$); ^{13}C NMR (acetone-d_6, 125 MHz) δ 170.9 (C, C-9), 153.4 (C, C-2), 132.9 (CH, C-6), 130.8 (C, C-5), 130.0 (CH, C-4), 127.0 (C, C-1), 117.4 (CH, C-3), 65.6 (CH_2, C-8), 34.9 (CH_2, C-7), 20.8 (CH_3, $OCOCH_3$); ESIMS m/z 357.0 $[M - H]^-$ (calcd for $C_{20}H_{21}O_6$, 357.1); anal. 67.06, H, 6.21%, calcd for $C_{20}H_{22}O_6$, C 67.03, H, 6.19%.

*[6,6′-Dihydroxy-5,5′-dimethoxy-(1,1′-biphenyl)-3,3′-diyl]bis-(ethane-2,1-diyl) diacetate (**19**):* yellow oil (146.5 mg, 49.3%); R_f

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(TLC) 0.36 (*n*-hexane−-acetone, 70:30); ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.45 (2H, bs, OH), 7.10 (2H, d, J = 2.0 Hz, H-4), 6.83 (2H, d, J = 2.0 Hz, H-6), 4.29 (4H, t, J = 7.0 Hz, H_2-8), 3.93 (6H, s, 3-OCH_3), 2.93 (2H, t, J = 7.0 Hz, H_2-7), 2.05 (6H, s, OCOCH_3); ^{13}C NMR (acetone-d_6, 125 MHz) δ 170.0 (C, C-9), 147.8 (C, C-3), 142.3 (C, C-2), 128.8 (C, C-5), 125.4 (C, C-1), 123.7 (CH, C-6), 111.2 (CH, C-4), 64.8 (CH_2, C-8), 55.5 (CH_3, OCH_3-3), 34.6 (CH_2, C-7),20.0 (CH_3, OCOCH_3); HRESIMS m/z 417.2201 $[M - H]^-$ (calcd for $C_{22}H_{25}O_8$, 417.4306).

*5,5′-Bis(2-hydroxyethyl)-[1,1′-biphenyl]-2,2′-diol (**22**):* yellow oil (20.1 mg, 19.1%); R_f (TLC) 0.28 (CH_2Cl_2−MeOH, 94:6); ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.13 (2H, d, J = 1.5 Hz, H-6), 7.06 (2H, dd, J = 1.5, 7.5 Hz, H-4), 6.86 (2H, d, J = 8.0 Hz, H-3), 3.70 (4H, t, J = 7.0 Hz, H_2-8), 2.74 (4H, t, J = 7.0 Hz, H_2-7); ^{13}C NMR (acetone-d_6, 125 MHz) δ 153.0 (C, C-2),132.9 (CH, C-6), 132.3 (C, C-5), 130.0 (CH, C-4), 127.0 (C, C-1), 117.3 (CH, C-3), 64.14 (CH_2, C-8), 39.54 (CH_2, C-7); HRESIMS m/z 273.1705 $[M - H]^-$ (calcd for $C_{16}H_{17}O_4$, 273.2968).

*5,5′-Bis(2-hydroxyethyl)-3,3′-dimethoxy-[1,1′-biphenyl]-2,2′-diol (**23**):* yellow oil (34.2 mg, 43.5%); R_f (TLC) 0.40 (CH_2Cl_2−MeOH, 94:6); ^{1}H NMR (acetone-d_6, 500 MHz) δ 8.15 (2H, bs, OH), 6.86 (2H, d, J = 2.0 Hz, H-4), 6.74 (2H, d, J = 2.0 Hz, H-6), 3.89 (6H, s, OCH_3-3), 3.77 (4H, t, J = 7.0 Hz, H_2-8), 2.78 (4H, t, J = 7.0 Hz, H_2-7); ^{13}C NMR (acetone-d_6, 125 MHz) δ 150.4 (C, C-3), 144.5 (C, C-2), 132.7 (C, C-5), 128.2 (C, C-1), 126.2 (CH, C-6), 113.9 (CH, C-4), 65.8 (CH_2, C-8), 58.0 (CH_3, OCH_3-3), 41.5 (CH_2, C-7); HRESIMS m/z 333.2061 $[M - H]^-$ (calcd for $C_{18}H_{21}O_6$, 333.3570).

Synthesis of [5,5′,6,6′-Tetrahydroxy-(1,1′-biphenyl)-3,3′-diyl]bis(ethane-2,1-diyl) Diacetate (16). A 0.2 M solution of **15** (175 mg, 0.48 mmol) in MeOH (2.4 mL) was stirred with IBX (161.23 mg, 1.2 equiv) at 0 °C for 30 min. Finally, a solution of $Na_2S_2O_4$ (83.5 mg, 0.47 mmol in 2.4 mL of H_2O) was added, and the resulting mixture was stirred at rt for 10 min. After the evaporation of the solvent under vacuum, the residue was solubilized with ethyl acetate (20 mL) and partitioned with a saturated $NaHCO_3$ solution (3 × 20 mL). The combined aqueous phase was extracted with ethyl acetate (1 × 50 mL). The organic phases were washed with saturated NaCl and dried over Na_2SO_4. After filtration, the solvent was evaporated in vacuo. The flash chromatography on Diol silica gel, eluted with *n*-hexane−$CHCl_3$ (30:70 → 0:100) and $CHCl_3$−MeOH (99:1 → 97:3) gave the expected product **16** (22.6 mg, 11.6%) as a brown oil: R_f (TLC) 0.51 (CH_2Cl_2−MeOH, 93:7); ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.81 (2H, d, J = 2.0 Hz, H-6), 6.73 (2H, d, J = 2.0 Hz, H-4), 4.22 (4H, t, J = 7.0 Hz, H_2-8), 2.83 (4H, t, J = 7.0 Hz, H_2-7), 1.98 (6H, s, OCOCH_3); ^{13}C NMR (acetone-d_6, 125 MHz) δ 171.0 (C, C-9), 146.8 (C, C-3), 141.1 (C, C-2), 131.5 (C, C-5), 127.4 (C, C-1), 122.5 (CH, C-4), 115.2 (CH, C-6), 65.7 (CH_2, C-8), 35.3 (CH_2, C-7), 20.9 (CH_3, OCOCH_3); ESIMS m/z 389.0 $[M - H]^-$ (calcd for $C_{20}H_{21}O_8$, 389.1); anal. C 61.49, H 5.65%, calcd for $C_{20}H_{22}O_8$, C 61.53, H 5.68%.

Synthesis of [5,6,6′-Trihydroxy-5′-methoxy-(1,1′-biphenyl)-3,3′-diyl]bis(ethane-2,1-diyl) Diacetate (20). A 0.06 M solution of **19** (171.6 mg, 0.41 mmol) in THF (6.8 mL) was stirred with IBX (172.1 mg, 1.5 equiv) at rt for 3 h. Then, a solution of $Na_2S_2O_4$ (71.38 mg, 0.61 mmol in 6.8 mL of H_2O) was added, and the mixture was stirred at rt for 10 min. After the evaporation of the solvent under vacuum, the residue was solubilized with ethyl acetate (20 mL) and partitioned with a saturated $NaHCO_3$ solution (3 × 20 mL). The total aqueous phase was partitioned with ethyl acetate (1 × 50 mL), and finally the combined organic layer was washed with a saturated NaCl solution. The organic phase was dried over Na_2SO_4, filtered, and evaporated under vacuum. The crude mixture was purified by flash chromatography on Diol silica gel, eluting with *n*-hexane−$CHCl_3$ (15:85 → 0:100) and $CHCl_3$−MeOH (99:1 → 93:7), affording the product **20** (33.1 mg, 18.3%) as a brown oil: R_f (TLC) 0.46 (CH_2Cl_2−MeOH, 95:5); ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.94 (1H, d, J = 1.5 Hz, H-4), 6.83 (1H, d, J = 1.5 Hz, H-6), 6.78 (1H, d, J = 1.5 Hz, H-4′), 6.70 (1H, d, J = 1.5 Hz, H-6′), 4.25 (2H, t, J = 7.0 Hz, H_2-8), 4.22 (2H, t, J = 7.0 Hz, H_2-8′), 3.91 (3H, s, OCH_3-3′), 2.91 (2H, t, J = 7.0 Hz, H_2-7), 2.84 (2H, t, J = 7.0 Hz, H_2-7′), 1.99 (3H, s, CH_3-10), 1.98 (3H, s, CH_3-10′); ^{13}C NMR (acetone-d_6, 125 MHz) δ 171.0 (C, C-9/9′), 148.5 (C, C-3), 147.4 (C, C-2′), 142.1 (C, C-2), 141.4 (C, C-3′), 131.2 (C, C-5′), 131.1 (C, C-5), 127.5 (C, C-1′), 126.3 (C, C-1), 124.5 (CH, C-6), 122.9 (CH, C-6′), 115.6 (CH, C-4′), 112.2 (CH, C-4), 65.8 (CH_2, C-8/8′), 56.6 (CH_3, OCH_3-3), 35.56 (CH_2, C-7), 35.3 (CH_2, C-7′), 20.9 (CH_3, OCOCH_3); ESIMS m/z 403.0 $[M - H]^-$ (calcd for $C_{21}H_{23}O_8$, 403.1); anal. C 62.40, H 5.96%, calcd for $C_{21}H_{24}O_8$, C 62.37, H 5.98%.

Flash chromatography afforded also a more polar product with a 13.7% yield, which showed the same spectroscopic data as **16**.

Enzymatic Butanolysis of 16 and 20. *Candida antarctica* lipase (10.0 mg) and *n*-butyl alcohol (0.05 mL) were added to a solution of a substrate (**16** and **20**, 10.0 mg) in MTBE (1.20 mL). The resulting mixture was stirred (400 rpm) at 40 °C, and the progress of each reaction was monitored by TLC (CH_2Cl_2−MeOH, 90:10). After the completion of each reaction, the enzyme was filtered off and the filtrate was evaporated in vacuo. The crude mixtures were purified by flash chromatography on Diol silica gel (CH_2Cl_2−MeOH, 98:2 → 90:10).

*5,5′-Bis(2-hydroxyethyl)-[1,1′-biphenyl]-2,2′,3,3′-tetraol (**17**):* yellow oil (6.5 mg, 82.7%); R_f (TLC) 0.25 (CH_2Cl_2−MeOH, 90:10); ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.76 (2H, s, H-6), 6.70 (2H, s, H-4), 3.73 (4H, t, J = 7.5 Hz, H_2-8), 2.71 (4H, t, J = 7.5 Hz, H_2-7); ^{13}C NMR (acetone-d_6, 125 MHz) δ 146.7 (C, C-3), 140.8 (C, C-2), 132.7 (C, C-5), 127.4 (C, C-1), 123.1 (CH, C-4), 115.7 (CH, C-6), 64.2 (CH_2, C-8), 39.9 (CH_2, C-7); ESIMS m/z 305.2 $[M - H]^-$ (calcd for $C_{16}H_{17}O_6$, 305.1); anal. C 62.76, H 5.95%, calcd for $C_{16}H_{18}O_6$, C 62.74, H 5.92%.

*5,5′-Bis(2-hydroxyethyl)-3′-methoxy-[1,1′-biphenyl]-2,2′,3-triol (**21**):* yellow oil (6.6 mg, 82.4%); R_f (TLC) 0.30 (CH_2Cl_2−MeOH, 90:10); ^{1}H NMR (acetone-d_6, 500 MHz) δ 6.90 (1H, s, H-4), 6.79 (1H, s, H-6), 6.74 (1H, d, J = 1.5, H-4′), 6.66 (1H, d, J = 1.5 Hz, H-6′), 3.89 (3H, s, OCH_3-3), 3.75 (2H, t, J = 7.1 Hz, H_2-8), 3.71 (2H, t, J = 7.1 Hz, H_2-8′), 2.75 (2H, t, J = 7.1 Hz, H_2-7), 2.71 (2H, t, J = 7.1 Hz, H_2-7′); ^{13}C NMR (acetone-d_6, 125 MHz) δ 147.4 (C, C-3), 147.3 (C, C-2′), 141.5 (C, C-2), 140.9 (C, C-3′), 132.7 (C, C-5′), 132.5 (C, C-5), 127.4 (C, C-1′), 126.3 (C, C-1), 124.5 (CH, C-6), 122.2 (CH, C-6′), 115.7 (CH, C-4′), 112.3 (CH, C-4), 64.2 (CH_2, C-8), 64.1 (CH_2, C-8′), 56.5 (CH_3, OCH_3-3), 40.1 (CH_2, C-7), 39.9 (CH_2, C-7′); ESIMS m/z 319.1 $[M - H]^-$ (calcd for $C_{17}H_{19}O_6$, 319.1); anal. C 63.71, H 6.32%, calcd for $C_{17}H_{20}O_6$, C 63.74, H 6.29%.

α-Glucosidase Inhibition Activity Assay. The inhibitory activity on yeast α-glucosidase was assessed through a slight modification of a previously reported method employing *p*NP-α-G as substrate.[50] Stock solutions at different concentrations of the compounds (**1**, **2**, **6**, **7**, **12**, **13**, **15**−**17**, **19**−**23**) were prepared in MeOH (in the range 0.5−100 μM). For each assay, different amounts (10, 20, 30, 40, 60 μL) of each sample, the α-glucosidase from *Saccharomyces cerevisiae* solution (10 U/mL; 50 μL), and finally a 6.0×10^{-3} M *p*NP-α-G solution (0.05 M buffer Na_2HPO_4−KH_2PO_4, pH 7.2; 30 μL) were added in a 5 mL volumetric flask. The final concentration of MeOH did not exceed 1.5%. The solutions were incubated at 37 °C for 30 min and reactions stopped by adding 1 M Na_2CO_3 solution (200 μL). Enzymatic activity was quantified by measuring absorbance at 405 nm. The assay was performed in triplicate with five different concentrations; quercetin and acarbose were used as positive controls. The inhibition percentage was calculated by the equation

$$\text{Inhibition\%} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

The IC_{50} value is the concentration that inhibits 50% of α-glucosidase activity.

Kinetics of α-Glucosidase Inhibition. The inhibitory mode of compound **15** on α-glucosidase (0.05 U/mL) was measured with increasing concentrations of *p*NP-α-G (0, 0.15, 0.33, 0.50, 0.80, 1.00, 1.25, 1.50, and 2.00 mM solutions in 0.05 M buffer Na_2HPO_4−KH_2PO_4, pH 7.2) as a substrate in the absence and presence of **15**.[51] Optimal amounts of the test compound were determined on the basis of the α-glucosidase inhibition assay (1.1, 3.3, and 5.5 μM). The reactions were incubated at 37 °C for 20 min, and the optical density was read at regular time intervals of 1 min. The experiment was performed in duplicate. The mode of inhibition was resolved by

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Lineweaver–Burk plot analysis of the data that were calculated by Michaelis–Menten kinetics. The inhibition constant K_i for the competitive inhibitor **15** was calculated by the following equation:

$$1/v = K_m(1 + [I]/K_i)/(V_{max}[S]) + 1/V_{max}$$

where v is the initial velocity, [I] and [S] are the concentration of inhibitor and substrate, V_{max} is the maximum velocity, and K_m is the Michaelis–Menten constant.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00250.

ESIMS and ^{1}H and ^{13}C NMR spectra of compounds **6**, **15**–**17**, **19**–**23**; 2D NMR spectra of **6**, **15**, **16**, **19**, **20**, **22**, **23** (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This article is dedicated to the memory of our coauthor Dr. Carmela Spatafora, a friend, colleague, and talented scientist who prematurely passed away in August 2016.

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