Chemical Aspects of Metoprolol Metabolism. Asymmetric Synthesis and Absolute Configuration of the 3-[4-(1-Hydroxy-2-methoxyethyl)phenoxy]-1-(isopropylamino)-2-propanols, the Diastereomeric Benzylic Hydroxylation Metabolites

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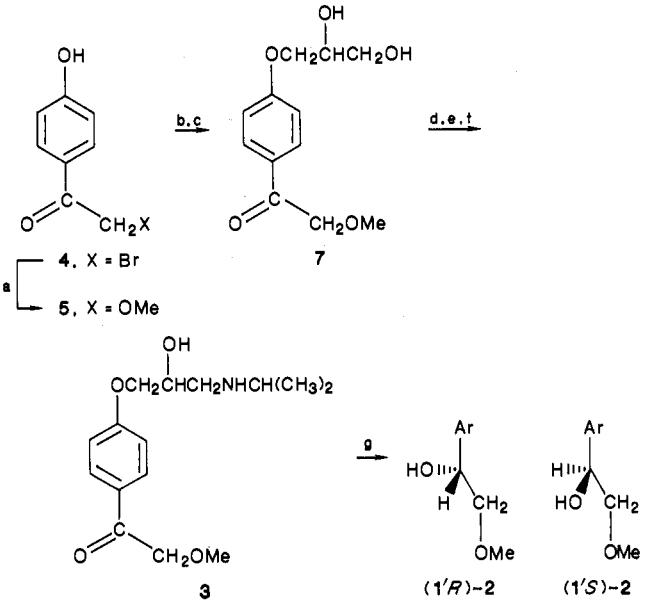
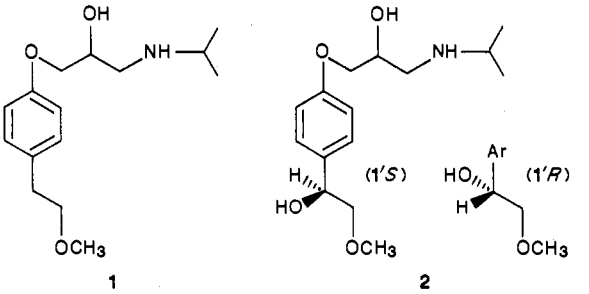
Asymmetric synthesis of 3-[4-(1-hydroxy-2-methoxyethyl)phenoxy]-1-(isopropylamino)-2-propanol (2), the benzylic hydroxylation metabolite of metoprolol (1), is described, and the absolute configurations of the diastereoisomers were assigned. Ketone 3, prepared in a multistep synthesis, was reduced with a complex of (2S)-(−)-2-amino-3-methyl-1,1-diphenylbutan-1-ol (9) and borane, yielding 2, with a ratio of 82:18 for the diastereomers. The absolute configurations 1′S,2S and 1′S,2R were assigned for the diastereomers formed in excess on the basis of reductions on closely related alkyl phenyl ketones and the circular dichroism spectrum. Derivatization of the 1′-hydroxyl group of oxazolidinone 10 with a chiral Mosher acid chloride and the use of an HPLC procedure to resolve the resulting esters enabled us to determine the metabolic product stereoselectivity for 2. In the presence of the rat liver microsomal fraction, the benzylic hydroxylation of 1 was highly product stereoselective favoring 1′R stereochemistry at the new asymmetric center in racemic 1 and in both enantiomers of 1. Determination of the stereochemistry of 2 will facilitate study of this polymorphically controlled metabolic process.

Metoprolol (1), a β_1-selective adrenergic antagonist, is extensively metabolized exclusively via oxidative pathways.[1-3] In humans and in rats, these oxidative pathways include O-demethylation and N-dealkylation processes, both followed by further oxidation and by benzylic hydroxylation, which produces 2, a pharmacologically active metabolite. In dogs, 2 is about 10 times less potent than 1 in reducing exercise heart rate,[4] and in humans, 2 has been suggested to be a major contributor to the effects of 1 in reduction of essential tremor.[5] The formation of 2 has the potential to yield diastereomeric products because a new chiral center is formed upon benzylic hydroxylation. In addition, formation of 2 is subject to genetic polymorphism in humans where it has been linked to debrisoquine hydroxylation and bufuralol hydroxylation, a process that is receiving considerable attention.[6-8] In this paper, we report results of work to establish the stereochemistry of the new benzylic hydroxylation center by synthesis and a method to examine the stereoselectivity of this hydroxylation process in the presence of the rat liver microsomal fraction.

Scheme I[a]



[a] Reagents: (a) NaOMe, MeOH; (b), NaOH, 6; (c) H_3O^+; (d) TsCl, C_5H_5N; (e) NaH; (f) $H_2NCH(CH_3)_2$, NH_4Cl; (g) 9-BH_3.



Chemistry. Our plan was to prepare a mixture of the benzylic hydroxylated metoprolols (2) enriched in one of the diastereoisomers by asymmetric reduction of acetophenone derivative 3 (Scheme I). Ketone 3 was synthesized from phenolic α-methoxyacetophenone 5,[9]

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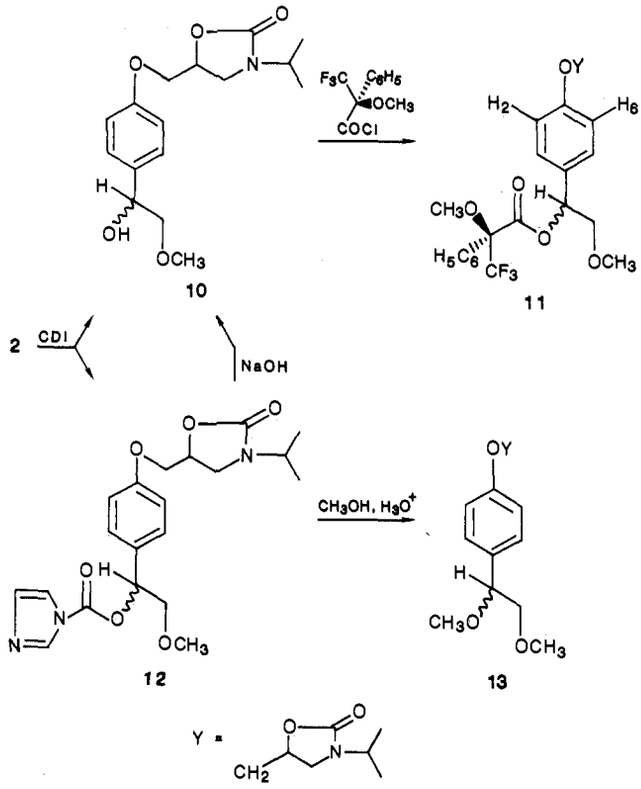
Scheme II

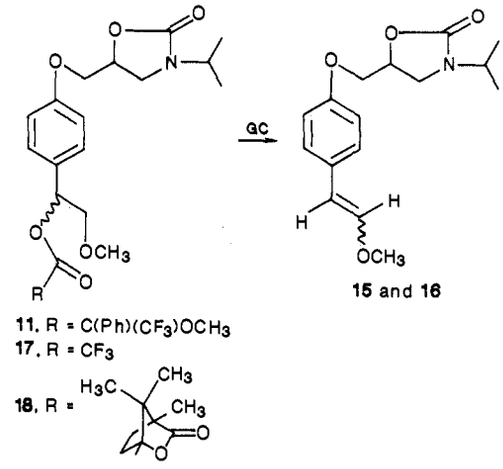


available from 2-bromo-4′-hydroxyacetophenone (**4**).[10] The side chain was elaborated by condensing **4** with (*RS*)-2,2-dimethyl-4-[(tosyloxy)methyl]-1,3-dioxolane (**6**). The dioxolane three-carbon electrophile was chosen because a deuteriated form was available[11] for subsequent planned work. Hydrolysis of the acetonide afforded diol **7**. Its conversion to **3** was effected via tosylation, followed by epoxide formation by adding sodium hydride. Subsequent opening of epoxide **8** with isopropylamine in the presence of a catalytic amount of NH_4Cl gave α-methoxyacetophenone **3**.

Initially, the chiral 1,1′-bi-2-naphthol-modified lithium aluminum hydride reagent, used successfully to reduce several alkyl phenyl ketones with high enantiomeric excess, was tried.[12] It failed to reduce ketone **3**, even when a large excess of the reagent was added. However, use of the chiral complex of borane and (2*S*)-(−)-2-amino-3-methyl-1,1-diphenylbutan-1-ol (**9**),[13] prepared by the addition of phenylmagnesium bromide to (2*S*)-valine methyl ester hydrochloride, was successful. The reduction was carried out at room temperature, and the diastereoisomers of **2** were separated from **9** by chromatography. The diastereomerically enriched product **2** was optically active, $[\alpha]^{22}_D$ +24.6°. Ketone **3** was also reduced with $NaBH_4$ to yield **2**, but as an equal molar mixture of the two diastereoisomers.

The diastereomeric composition of **2** obtained from chiral reduction was determined by measuring intensities

Scheme III



of the ^1H NMR signals of the diastereotopic protons of the Mosher acid ester[14] of derivative **11** (Scheme II). The propanolamine side chain was converted to oxazolidone **10** and then into Mosher ester **11**. When excess carbonyldiimidazole was used in the first step, carbamate **12** was obtained, which could be cleaved with NaOH without altering the chiral center; however, aqueous methanolic HCl cleavage ether **13**, in which the benzylic chiral center was racemized. The ester of the diastereoisomers of **2**, obtained by $NaBH_4$ reduction, gave equally intense ^1H NMR signals for several sets of diastereotopic protons. Most clearly separated were the signals for H_2 and H_6 of the aromatic ring of metoprolol. The composition of the diastereomers of **2** obtained by chiral reduction was determined to be 23:77, on the basis of integration of the areas of their signals.

On the basis of reported reductions of several aromatic ketones with the reagents prepared from (2*S*)-(−)-2-amino-3-methyl-1,1-diphenylbutan-1-ol (**9**) and borane,[13] the absolute configuration would be expected to be *S* at the new chiral center. This assignment was also consistent with the circular dichroism spectrum, which showed a negative Cotton effect in the 210–240-nm region and a weaker positive Cotton effect in the 280-nm region.

A good correlation exists between the sign of the Cotton effect of the ^1L_a band (shorter wavelength transition) and the absolute configuration of chiral phenylcarbinols, which does not vary with the substituent in the aromatic ring; however, the sign of the Cotton effect associated with the ^1L_b band (longer wavelength transition) is highly dependent on the substituent attached to the aromatic ring.[15] The 1′*R* enantiomer of **14**, which is a very close analogue of **2**, exhibits a negative Cotton effect in the far-UV region (227 nm) and a positive Cotton effect in the 280-nm region.[16] The mixture of diastereoisomers of **2** obtained by chiral borane reduction showed Cotton effects of the opposite signs in both of these regions, respectively, supporting the assignment of absolute configuration as 1′*S*.

GC and GC/MS separation of **11** into its diastereoisomers was attempted. Unexpectedly, the diastereoisomers of **11** formed from the 1:1 mixture of diastereoisomers of **2** obtained by borohydride reduction, or from the 1:3.35 mixture of diastereoisomers obtained by chiral borane reduction, gave the same two peaks of unequal intensities

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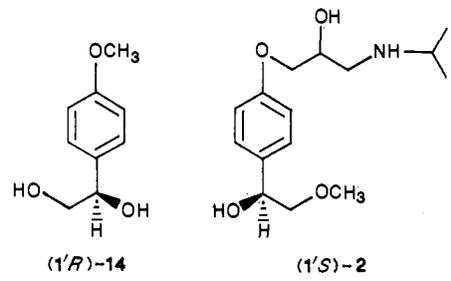

(ca. 2:3), when analyzed by GC. In the GC/mass spectrum, no molecular ion (m/z 525) was observed; instead, an intense ion at m/z 291 was noted, which was assigned to the isomeric methoxystyrenes **15** and **16**, arising from thermal elimination of the ester (Scheme III). Further evidence for these olefinic products was the observation that the related trifluoroacetic acid ester **17** and the camphanic acid ester **18** gave the same GC/mass spectra. We note, however, GC separation of components, probably diastereoisomers of **10** (as the TMS ethers), is thought to be due to the chiral center in the propanolamine side chain and was obtained only with considerable difficulty by using a chiral capillary column.[17] Thus, other methods were sought.

Separation of diastereoisomers of **11**, on the basis of chirality at the benzylic position, was accomplished by HPLC with an ODS column, eluting with a reversed-phase solvent system, showing material eluting at 11.55 and 12.40 min, respectively. However, analysis of the metabolites produced by benzylic hydroxylation required addition of a second ODS column to effect separation of this metabolite from other components in the derivatized mixture obtained from the metabolic incubations. The chromatogram for separation of standards via the tandem ODS columns is given in Figure 1. The peak area ratio for the diastereoisomers of **11** obtained by borohydride reduction of **3** was 50:50 (retention times 18.13 and 19.50 min), while that for the diastereoisomers of **11** obtained by chiral reduction was 18:82 (retention times 18.14 and 19.52 min). This latter ratio was approximately the same as those obtained from NMR data, indicating that the separation is based on the new chiral center. Thus, the absolute configurations in **2** are assigned as 1'*R*,2*S* and 1'*R*,2*R* for the components eluting in the earlier peak, and 1'*S*,2*R* and 1'*S*,2*S* for those eluting in the later peak.

Metabolism. The benzylic hydroxylation of metoprolol (**1**) and its enantiomers was examined in the presence of the rat liver microsomal fraction to determine product stereoselectivity, i.e., whether the new chiral center was formed with a significant degree of stereoselectivity. Metabolites were extracted and then derivatized by addition of excess carbonyldiimidazole, followed by alkaline hydrolysis of the intermediate carbamate ester **12** and subsequent esterification with the (2*R*)-Mosher acid chloride in the presence of pyridine. The stereochemistry of the benzylic center is unaffected during this derivatization procedure as evidenced by the unchanged peak area ratios for the diastereomeric components when **2**, obtained by chiral reduction of **3**, was carried through these steps.

Benzylic hydroxylation metabolites were determined by HPLC with tandem ODS columns. The metabolite (peaks 1 and 2, Figure 1c) was found to coelute with the synthetic standard **12**, and their identity was further confirmed by GC and GC/MS analysis of the HPLC fractions.

Product stereoselectivity data obtained from metabolism of metoprolol in the presence of rat liver microsomes are

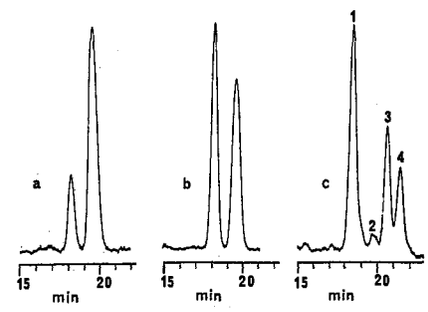

Figure 1. HPLC tracings: (a) **11** from chiral borane reduction, (b) **11** from $NaBH_4$ reduction, (c) metabolites extracted from the microsomal incubation. Peaks **1** and **2** are from diastereoisomers of **11** and peaks 3 (retention time 20.48 min) and 4 (21.30 min) are from the O-demethylation metabolite of metoprolol and from derivatizing agents, respectively.

Table I. Stereoselectivity Data on the Rat Liver Microsomal Metabolism of Metoprolol

substrate	% **2** with 1'*R* stereochemistry	ratio of 1'*R*/1'*S* products
racemic **1**	93.2% 1.3 (n = 4)	13.7[a]
(2*R*)-**1**	90.4% 0.97 (n = 4)	9.4[b]
(2*S*)-**1**	96.3% 0.61 (n = 4)	26.0[c]

[a] Ratio of [(1'*R*,2*S*) + (1'*R*,2*R*)]/[(1'*S*,2*S*) + (1'*S*,2*R*)] products. [b] Ratio of (1'*R*,2*R*)/(1'*S*,2*R*) products. [c] Ratio of (1'*R*,2*S*)/(1'*S*,2*S*) products.

summarized in Table I. The benzylic oxidation of metoprolol is highly product stereoselective, favoring formation of a new 1'*R* chiral center from both enantiomers of metoprolol. Hydroxylation of the related substrate, ethylbenzene, at its benzylic prochiral center in the presence of the rat liver microsomal fraction also shows a high degree of product stereoselectivity in the formation of a preponderance of (1*R*)-1-phenylethanol (R/S ratio 4.25).[18] However, in the presence of a purified rat liver cytochrome P-450 isozyme, obtained after phenobarbital induction, little stereoselectivity is noted. This process is a multistep, stereochemically complex one that includes hydrogen atom abstraction, followed by introduction of the hydroxyl group.[19]

(2*S*)-Metoprolol is transformed to the benzylic hydroxylation metabolite with a higher degree of product stereoselectivity than is the 2*R* enantiomer (Table I). Although distantly located, the chiral center of the propanolamine side chain of the substrate modifies the stereoselectivity of benzylic oxidation by nearly threefold (R/S ratio 26 vs 9.4). The high degree of stereoselectivity observed in the hydroxylation suggests the propanolamine side chain is a significant feature in the interaction of this substrate with the hydroxylation isozyme(s).

The determination of the stereochemistry of the benzylic hydroxyl group in **2** and the HPLC separation of diastereoisomers based on this chiral center provide the framework to examine aspects of this important hydroxylation process in other metabolic systems.

Experimental Section

Synthetic reactions were carried out under an argon atmosphere. Routine ^1H NMR spectra were recorded on a Varian T-60 (60 MHz) spectrometer and high-frequency ^1H NMR spectra were recorded on a Bruker WM-500 (500 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to TMS as an internal standard. Notations used in the descriptions are s = singlet, d = doublet, dd = doublet of doublets, t = triplet,

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and m = multiplet. Infrared (IR) spectra were recorded on a Perkin-Elmer 283 spectrophotometer. Circular dichroism (CD) spectra were recorded on a Jobin Yvon Dichrograph-III instrument. Optical rotations were determined on a JASCO DIP-4 Digital Polarimeter. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Kieselgel 60 (230–400-mesh ASTM) was used for flash column chromatography.[20] Gas chromatographic analysis was performed on a Hewlett-Packard 5840A gas chromatograph equipped with a flame-ionization detector (splitless) and utilizing a J & W DB-5 fused silica capillary column [30 m × 0.32 mm (i.d.), 0.25-μm film]. Chromatographic conditions were injector 250 °C, helium flow rate 60 mL/min, column head pressure 15 psi, temperature program 200 °C for 1 min and then increased to 280 °C at 10 °C/min. Electron-impact mass spectra were obtained on a VG 7070H mass spectrometer equipped with a VG 2050 data system. Gas chromatography/electron-impact mass spectrometric analysis was performed on the same setup, which was interfaced with a Hewlett-Packard 5710 gas chromatograph. Gas chromatography conditions were the same as above. Mass spectral conditions were source temperature 200 °C and ionizing voltage 70 eV. Chemical-ionization mass spectrometry was performed with methane as reagent gas. HPLC analysis was performed on a Micromeretics Model 700 liquid chromatograph equipped with a variable-wavelength UV detector (226 nm) and a Spectra Physics SP 4100 computing integrator.

2′-Methoxy-4-hydroxyacetophenone (5). To a solution of NaOMe (27.02 g, 500 mmol) in MeOH (100 mL), was added dropwise with continuous stirring 4[10] (10.75 g, 50 mmol) in MeOH (50 mL). The reaction mixture was refluxed for 30 min and cooled, and the MeOH was evaporated. The residue was suspended in H_2O (50 mL), acidified with aqueous HCl, and extracted with EtOAc (3 × 50 mL). The combined extracts were dried (Na_2SO_4), decolorized with charcoal, and evaporated, affording yellow needles, which were recrystallized from benzene and dried over P_2O_5, 7.08 g (85%) of **5**: mp 128–130 °C [lit.[9] mp 130–131 °C]; ^1H NMR (CD_3COCD_3) δ 3.41 (s, 3, OCH_3), 4.65 (s, 2, $COCH_2$), 6.93 (d, J = 9 Hz, 2, Ar H-2 and H-6), 7.88 (d, J = 9 Hz, 2, Ar H-3 and H-5).

3-[4-(Methoxyacetyl)phenoxy]-1,2-propanediol (7). A solution of **5** (3.32 g, 20 mmol), 2,2-dimethyl-4-[(tosyloxy)-methyl]-1,3-dioxolane (**6**)[11] (5.73 g, 20 mmol), and NaOH (0.80 g, 20 mmol) in DMF (50 mL) was heated at reflux for 2 h. The solvent was evaporated, and the residue was dissolved in EtOAc (50 mL) and washed successively with 5% NaOH solution (20 mL) and water (2 × 20 mL). After drying (Na_2SO_4), the solvent was evaporated, affording 4.5 g (80%) of the acetonide as an oil. The crude acetonide (2.8 g, 10 mmol) was dissolved in a mixture of acetone (30 mL) and aqueous 1 N HCl (10 mL) and refluxed for 2 h. The reaction mixture was cooled and neutralized by adding solid Na_2CO_3. Absolute EtOH (50 mL) was added, and the solvent was evaporated. The residue was suspended in EtOAc (50 mL) and filtered, the filtrate was concentrated, and the residue was flash chromatographed with EtOAc as the eluent. Evaporation of the solvent afforded an oily residue that crystallized as fine white needles upon cooling, yielding 1.60 g (67%) of **7**: mp 62–64 °C; ^1H NMR ($CDCl_3$) δ 3.10–4.30 (m, 8, OCH_2CHCH_2 and OCH_3), 4.58 (s, 2, $COCH_2$), 6.84 (d, J = 8 Hz, 2, Ar H-2 and H-6), 7.76 (d, J = 8 Hz, 2, Ar H-3 and H-5); HRMS (EI mode), m/z 240.1016 (240.0997 calcd for $C_{12}H_{16}O_5$).

3-[4-(Methoxyacetyl)phenoxy]-1-(isopropylamino)-2-propanol (3). Tosyl chloride (1.47 g, 7.7 mmol) in benzene (40 mL) was added dropwise into a solution of **7** (1.68 g, 7.0 mmol) in pyridine (10 mL) at 0 °C. The reaction mixture was maintained at 0 °C for 24 h and then 30% more tosyl chloride (0.40 g, 2.1 mmol) was added, stirred, and left at 0 °C for 3 more days. The starting material **7** disappeared at the end of this period. Then NaH (0.50 g, 21 mmol) was added, and the reaction mixture was refluxed for 90 min. The reaction mixture was cooled, MeOH (1 mL) was added dropwise, and the mixture was diluted with EtOAc (50 mL). The solution was washed with 1 N HCl (4 × 20 mL), and the combined acidic solutions were extracted with EtOAc (20 mL). The organic extracts were combined, dried (Na_2SO_4), and evaporated to dryness. The intermediate epoxide **8**, a pale yellow oil (1.50 g, 97%), was used without purification. Compound **8** (1.50 g, 6.8 mmol) was dissolved in isopropylamine (10 mL) and stirred with a catalytic amount of NH_4Cl (50 mg) for 24 h. Excess isopropylamine was evaporated, the residue was stirred with aqueous 5% HCl (20 mL) for 30 min, the supernatant was washed with ether (20 mL), and the aqueous layer was made alkaline with aqueous 10% NaOH. The precipitated product was extracted with EtOAc (3 × 50 mL), and the organic layers were combined, dried (Na_2SO_4), and evaporated to dryness. The crude product, a pale yellow solid, was subjected to flash chromatography, eluting with $CHCl_3$/MeOH/Et_3N, 95:5:0.5. The fractions containing the product were combined, the solvent was evaporated, and the residue was recrystallized from cyclohexane, affording 0.78 g (40%) of **3**[21] as long white needles: mp 85–86 °C; ^1H NMR ($CDCl_3$) δ 1.10 [d, J = 6 Hz, 6, $CH(CH_3)_2$], 2.31 to 3.05 [m, 3, CH_2NH and $CH(CH_3)_2$], 3.42 (s, 3, OCH_3), 4.02 (m, 3, OCH_2CH), 4.60 (s, 2, $COCH_2$), 6.93 (d, J = 9 Hz, 2, Ar H-2 and H-6), and 7.89 (d, J = 9 Hz, 2, Ar H-3 and H-5); HRMS (EI mode), m/z 281.1655 (281.1628 calcd for $C_{15}H_{23}NO_4$).

3-[4-(1-Hydroxy-2-methoxyethyl)phenoxy]-1-(isopropylamino)-2-propanol (2). $NaBH_4$ Reduction. To a solution of **3** (281 mg, 1.0 mmol) in MeOH (5 mL), cooled to 0 °C was added in small portions $NaBH_4$ (189 mg, 5.0 mmol), was stirred for 90 min. The solvent was evaporated, and the residue was partitioned between aqueous 1% NaOH solution (10 mL) and $CHCl_3$ (20 mL). The organic layer was separated, and the aqueous layer was saturated with NaCl and extracted with $CHCl_3$ (2 × 20 mL). The $CHCl_3$ extracts were combined, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography eluting with EtOAc/MeOH/Et_3N, 95:5:1. The fractions containing the product were combined and evaporated, affording 270 mg of **2** as a colorless oil (95%): ^1H NMR ($CDCl_3$) δ 1.10 [d, J = 6 Hz, 6, $CH(CH_3)_2$], 2.34–3.02 [m, 3, CH_2NH and $CH(CH_3)_2$], 3.40 (s, 3, OCH_3), 3.48 (d, J = 6 Hz, 2, CH_2OCH_3,), 3.95 (m, 3, OCH_2CH), 4.80 (t, J = 6 Hz, 1, ArC*H*), .85 (d, J = 9 Hz, 2, Ar H-2 and H-6) and 7.27 (d, J = 9 Hz, 2, Ar H-3 and H-5); HRMS (EI mode), m/z 283.1785 (283.1784 calcd for $C_{15}H_{25}NO_4$).

Asymmetric Reduction. (2*S*)-(−)-2-Amino-3-methyl-1,1-diphenylbutan-1-ol (**9**) was prepared by the method of Itsuno et al.[13] To 1.02 g (4.0 mmol) of **9** in dry THF (4 mL) cooled to −78 °C was added dropwise during ca. 20 min, with stirring, a solution of borane in THF (8.0 mL of a 1 M solution). The resulting solution was gradually warmed to room temperature, stirring was continued for 10 h, and then a solution of **3** (0.281 g, 1.0 mmol) in THF (2 mL) was added dropwise. The reaction mixture was then stirred at room temperature for 4 h. The mixture was cooled to 0 °C, aqueous NH_3 (5 mL) was added dropwise, and the resultant mixture was stirred for an additional 4 h at room temperature. Water was added, the reaction mixture was extracted with EtOAc (3 × 20 mL), and the organic extracts were combined, dried (Na_2SO_4), and evaporated. Flash chromatography, eluting with EtOAc/MeOH/Et_3N, 95:5:1, separated **2** from chiral amino alcohol **9**. Fractions containing **9** were combined, the solvent was evaporated, and the residue was recrystallized from cyclohexane, affording 0.74 g (73% recovery) of **9**. Evaporation of the fractions containing the mixture of diastereomers of **2** gave a colorless oil: 0.220 g (78%); $[\alpha]^{22}_D$ +24.6° (*c* 0.68, $CHCl_3$); 500-MHz ^1H NMR ($CDCl_3$) δ 1.10 [d, J = 6.0 Hz, 6, $CH(CH_3)_2$], 2.70 (dd, J_{gem} = 11.3 Hz, J_{vic} = 7.5 Hz, 1, CH_2NH), 2.82 [m, 1, $CH(CH_3)_2$], 2.86 (dd, J_{gem} = 11.3 Hz, J_{vic} = 3.8 Hz, 1, CH_2NH), 3.42 (s, 1, OCH_3), 3.42 (dd, J_{gem} = 10.5 Hz, J_{vic} = 5.3 Hz, 1, CH_2OCH_3), 3.50 (dd, J_{gem} = 10.5 Hz, J_{vic} = 3.0 Hz, 1, CH_2OCH_3), 3.96 (m, 3, OCH_2CH), 4.84 (dd, J = 3 Hz and 9 Hz, 1, ArC*H*), 6.90 (d, J = 9 Hz, 2, Ar H-2 and H-6) and 7.30 (d, J = 9 Hz, 2, Ar H-3 and H-5); CD (*c* 0.5 for 250–300 nm, *c* 0.04 for 200–250 nm, MeOH) $[\theta]_{300}$ 0, $[\theta]_{290}$ +45, $[\theta]_{285}$ +180, $[\theta]_{280}$ +165, $[\theta]_{274}$ +75, $[\theta]_{270}$ +105, $[\theta]_{260}$ +37, $[\theta]_{250}$ 0, $[\theta]_{235}$ −935, $[\theta]_{229}$ −5985, $[\theta]_{224}$ −3930, $[\theta]_{222}$ −6735, $[\theta]_{217}$ −2060, $[\theta]_{210}$ −7015.

3-(2-Propyl)-5-[[4-(1-hydroxy-2-methoxyethyl)phenoxy]-methyl]-2-oxazolidone (10). To a solution of the diastereoisomers of **2** (71 mg, 0.25 mmol) in dry THF (1 mL) was added

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dropwise at 0 °C carbonyldiimidazole (41 mg, 0.25 mmol) in 0.5 mL THF. The mixture was allowed to warm to room temperature and then stirred for an additional 2 h. Ether (10 mL) was added, and the mixture was washed with H_2O (2 × 2 mL) and dried (Na_2SO_4). The residue obtained by the evaporation of the solvent was flash chromatographed, eluting with EtOAc. All fractions containing the product were combined and evaporated, affording 41 mg (53%) of **10** as a colorless oil: IR (CCl_4) 1782 cm^{-1} (s); ^1H NMR ($CDCl_3$) δ 1.10 [d, J = 7 Hz, 6, $CH(CH_3)_2$], 3.19–3.8 (m, 7, CH_2N, CH_2OCH_3), 3.8–4.4 (m, 3, OCH_2CH), 4.82 (m, 2, ArC*H* and OCH_2CH), 6.86 (d, J = 9 Hz, 2, Ar H-2 and H-6), and 7.30 (d, J = 9 Hz, 2, Ar H-3 and H-5).

Esterification of 10 with (2*R*)-2-Methoxy-2-(trifluoromethyl)phenylacetyl Chloride To Form 11. To a solution of **10** (16.0 mg, 0.05 mmol) in dry benzene (500 μL) was added Et_3N (10 μL) followed by (2*R*)-2-methoxy-2-(trifluoromethyl)phenylacetyl chloride (15.0 mg, 0.06 mmol) in benzene (100 μL). The reaction mixture was maintained overnight at room temperature. After evaporation of the solvent, the residue was dissolved in $CHCl_3$ and flash chromatographed, eluting with $CHCl_3$. The fractions containing ester **11** were combined and evaporated, affording a colorless oil: 24 mg (91%); 500-mHz ^1H NMR ($CDCl_3$) δ 1.23 [d, J = 6 Hz, 6, $CH(CH_3)_2$], 3.30 and 3.40 (2 s, 3, CH_2OCH_3), 3.48 and 3.58 (2 s, 3, OCH_3), 3.45–3.76 (m, 4, CH_2OCH_3 and CH_2N), 4.07–4.13 [m, 3, OCH_2CH and $CH(CH_3)_2$], 4.80 (m, 1, OCH_2CH), 6.15 (2 dd, 1, ArC*H*), 6.82 and 6.90 (2 d, J = 9 Hz, 2, OAr H-2 and H-6), and 7.1–7.5 (m, 7, OAr H-3 and H-5, ArH).

Diastereomeric Composition of 2. Compound **2** obtained by chiral reduction was derivatized first with carbonyldiimidazole and then with (2*R*)-2-methoxy-2-(trifluoromethyl)phenylacetyl chloride to yield a mixture of ester **11** by using the procedure described above for the preparation of **11**. A 500-MHz ^1H NMR spectrum was obtained. The integration ratio of the intensities of the diastereotopic protons, OAr H-2 and H-6, in the 500-MHz ^{1}NMR spectrum was 23:77 (1:3.35).

GC and GC/MS Analysis of 11. A solution of **11** in EtOAc (100–200 ng/μL) was prepared, and 1-μL quantities were injected onto the GC column. Retention times of the peaks at 12.1 and 14.0 min were assigned to β-methoxystyrenes **15** and **16**. GC/EI-MS, m/z 291 (100, $M^{\bullet+}$), 149 (27), 135 (9), 100 (14), 56 (18).

Metabolic Studies. Male Sprague–Dawley rats (approximately 150 g) were decapitated, and the livers were removed and rinsed with 0.01 M (pH 7.4) sodium phosphate buffer containing 1.15% KCl. The livers were then homogenized in 4 volumes of the same buffer with a Potter–Elvehjem Teflon pestle homogenizer. The homogenate was centrifuged at 9000*g* for 30 min, and the supernatant fraction was recentrifuged at 100000*g* for 1 h. The microsomal pellet was resuspended in the same buffer at a protein concentration of 20–30 mg/mL. The entire operation was performed at 0–4 °C. Microsomal protein was determined by the method of Lowry et al.[22]

Incubation mixtures contained racemic or individual enantiomers of metoprolol (0.201 mg, 250 μM), $MgCl_2$ (2.850 mg, 10 mM), glucose 6-phosphate (16.90 mg, 10 mM), $NADP^+$ (2.295 mg, 1 mM), and microsomal protein (3 mg) in 0.2 M pH 7.4 phosphate buffer. The incubation volume was 3 mL. Metabolic incubations were performed at 37 °C for 30 min, the mixtures were then made alkaline by the addition of saturated Na_2CO_3 solution (0.5 mL) and then extracted with EtOAc (2 × 5 mL). The organic layer was separated by centrifugation, combined, and evaporated to dryness by a stream of N_2. The residue was further dried in a desiccator over P_2O_5 and then subjected to derivatization.

Derivatization of Metabolites. The metabolites extracted from the incubation mixtures were treated with a solution of carbonyldiimidazole in dry THF (2% solution, 0.5 mL), mixed, and left standing for 30 min. Then, 1 M NaOH solution (0.5 mL) was added, the mixture was vortexed, and following the addition of saturated NaCl solution (1 mL), the entire mixture was extracted with ether (2 × 5 mL). The combined ether extracts were washed with H_2O (3 × 1 mL), dried (Na_2SO_4), and evaporated to dryness in Reacti-Vials. The vials were dried over P_2O_5 and then capped with serum caps. A solution of dry pyridine, distilled from NaH and stored over molecular sieves (3A) under argon, in dry benzene (10% solution, 100 μL) was introduced into each vial by means of a syringe. After vortex mixing, (2*R*)-2-methoxy-2-(trifluoromethyl)phenylacetyl chloride (5 mL) prepared from the (2*S*)-acid by the procedure of Dale et al.[14] was added. After the solution was allowed to stand 1 h at room temperature, MeOH (10 μL) was added, and the contents of the vials were evaporated to complete dryness with a stream of N_2 at 45 °C. The samples for the HPLC analysis were prepared by dissolving the residue in 200 μL of mobile phase.

HPLC Analysis. For the separation of diastereomeric components of **11**, an Ultrasphere ODS 5-μm column, 4.6 mm × 15 cm (Beckman), with a Co:Pell ODS (Whatman) guard column was used. The components were eluted isocratically with a mixture of CH_3CN/MeOH/phosphate buffer (0.01 M, pH 7.0), 20:47:33, flow rate 1 mL/min. The retention times are 11.55 and 12.40 min, respectively, ratio 50:50 for the diastereomers of **11**, obtained from borohydride reduction of **3**, and 11.57 and 12.42 min, ratio 18:82, for the diastereoisomers of **11** obtained from chiral borane reduction of **2**.

For the analysis of metabolites isolated from the microsomal incubation, the above column was connected to a second column, a Microsorb C_{18} (Rainin) column, 4.6 mm × 10 cm, in that order. Usually, 20 μL of the metabolites dissolved in mobile phase was injected and eluted with the solvent system listed above. The retention times were 18.28 and 19.54 min, respectively. These fractions were collected, diluted with H_2O, and extracted with EtOAc. After drying (Na_2SO_4), the extract was evaporated, and the residue was subjected to GC and GC/MS analysis as described before.

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Registry No. (±)-**1**, 37350-58-6; (1′*S*,2*R*)-**2**, 110458-46-3; (1′*S*,2*S*)-**2**, 110458-47-4; (1′*R*,2*S*)-**2**, 110458-48-5; (1′*R*,2*R*)-**2**, 110458-52-1; (±)-**3**, 110458-45-2; **4**, 2491-38-5; **5**, 32136-81-5; (±)-**6**, 23737-51-1; (±)-**7**, 110458-43-0; (±)-**7** acetonide, 110458-42-9; (±)-**8**, 110458-44-1; (*R**,*R**)-**10**, 110458-49-6; (*R**,*S**)-**10**, 110458-50-9; **11**, 110458-51-0.

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