idines > imidazoles.[2b] It has been also noted that aliphatic amines are expelled much faster than imidazoles of the same pK_a from phtalimidium addition compounds.[6]

The poor nucleofugality of pyridines compared to secondary alicyclic amines found in this work is consistent with a resonance stabilization by electron donation from the pyridine to the carbonyl group of the acetylpyridinium product and to the dinitrophenoxy oxygen in the transition state for the second step of eq 2.[2b]

The rate for amine expulsion from the zwitterionic tetrahedral intermediate formed in the pyridinolysis of aryl acetates (k_{-1}) is given by eq 7, where $pK_a(N)$ is the pK_a of

$$\log k_{-1} = 13.0 + 0.4pK_a(lg) - 0.7pK_a(N) \quad (7)$$

the protonated amine.[2,7] This equation was tested for pyridines only and together with eq 6 can predict reasonably well the $pK_a°$ values found in the pyridinolysis of aryl acetates.[7,24] According to our results, eq 7 cannot be applied to alicyclic amines, as was erroneously done,[7] nor to quinuclidines or imidazoles (see above),[2b] and since no information is available at the moment on nucleofugalities of other amines, this equation should only be valid for pyridines.

It is very likely that only the constant term of eq 7 should vary with the amine nature, in view that the sensitivity of the microscopic rate constants concerning $T^{\pm}$ to the basicity of the nucleophile and leaving group (β_N and β_{lg}) seems to be independent of the amine nature.[2a]

According to the $pK_a°$ value obtained in this work (9.1) and the discussion above, the leaving abilities of secondary alicyclic amines from $T^{\pm}$ in the aminolysis of aryl acetates is given by eq 8.[25] Comparison of eq 7 and 8 shows that

$$\log k_{-1} = 14.3 + 0.4pK_a(lg) - 0.7pK_a(N) \quad (8)$$

secondary alicyclic amines leave $T^{\pm}$ ca. 20-fold faster than pyridines of the same basicity, which is similar to the ratio of nucleofugalities of quinuclidines and isobasic pyridines from the $T^{\pm}$ formed in the aminolysis of *p*-nitrophenyl phenyl carbonate, as found by Gresser and Jencks.[2b,26]

The $pK_a°$ values found in the reactions of phenyl and *p*-nitrophenyl thiolacetates with secondary alicyclic amines are >11.5 and 10.5, respectively.[7] The $pK_a°$ values calculated by means of eqs 6 and 8 for the aminolysis of the *O*-aryl acetates with leaving aryl oxide anions isobasic with thiophenoxide and *p*-nitrothiophenoxide anions (pK_a 6.5 and 4.6, respectively) are 12.4 and 9.9, respectively. Therefore, the ratio k_{-1}/k_2 from the thio-$T^{\pm}$ is only a little larger than that from the isobasic oxy-$T^{\pm}$, and since it is known that the nucleofugalities of ArS^- are lower than those of isobasic ArO^-,[27] it is doubtful whether k_{-1} from the thio-$T^{\pm}$ should be larger than that from the isobasic oxy analogue, as stated.[7] Obviously, more data are needed to quantify the leaving abilities of ArS^- groups before evaluation of the "push" (to expel the amine) provided by ArS and isobasic ArO from the corresponding tetrahedral intermediates.

(24) The $pK_a°$ value for a given Brønsted-type correlation can be calculated as the $pK_a(N)$ value for which $k_{-1} = k_2$, i.e. eq 6 = eq 7.

(25) The constant term of eq 8 was obtained from: $11.5 - 0.5pK_a(lg) = X + 0.4pK_a(lg) - 0.7pK_a(N)$, where $pK_a(lg) = 4$ and $pK_a(N) = 9.1$.

(26) From ref 2b, p 6978, k_N/k_O (k_{-1}/k_2 in our work) = 4 for 4-(dimethylamino)pyridine/*p*-nitrophenoxide ion from $T^{\pm}$, in the pyridinolysis of *p*-nitrophenyl phenyl carbonate. Since $\beta_N = -0.7$ for k_{-1} in the aryl phenyl carbonate system,[2a] it follows that $k_{-1}/k_2 = 400$ for a pyridine of pK_a 7/*p*-nitrophenoxide ion. Since $k_N/k_O = k_{-1}/k_2 = 7800$ for a quinuclidine of pK_a 7/*p*-nitrophenoxide ion,[2b] it means that a quinuclidine of pK_a 7 leaves $7800/400 \approx 20$-fold faster from $T^{\pm}$ than an isobasic pyridine. For isobasic amines of pK_a 9.8, the ratio of k_1 values is ≈ 28.[2b]

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Registry No. 2,4-Dinitrophenyl acetate, 4232-27-3; piperidine, 110-89-4; piperazine, 110-85-0; 1-(β-hydroxyethyl)piperazine, 103-76-4; morpholine, 110-91-8; 1-formylpiperazine, 7755-92-2; piperazinium ion, 22044-09-3.

A Practical Synthesis of the [(2*R*)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidine Moiety (P_4–P_2) in Renin Inhibitors

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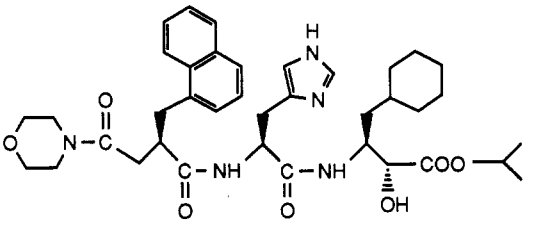
A large number of renin inhibitors have been investigated as therapeutic agents of hypertension.[1,2] The peptide inhibitors derived from renin substrate angiotensinogen have been considered to be unsuitable as drugs for oral administration due to proteolytic degradation by chymotrypsin, especially at the Phe–His amide bond (P_3–P_2)[3] of the inhibitors.[4] Recently, we have reported a novel class of low molecular weight renin inhibitor such as **1** which was stabilized against proteases by incorporating (−)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)-propionic acid [(−)-**2**] with a retro-inverso amide bond as the P_4–P_3 moiety.[1] In addition, the analysis of inhibitor–renin interaction[5] showed that the β-carbonyl group of (−)-**2** was at a suitable position to accept a hydrogen bond from the side chain OH of Ser-230 in human renin, the naphthyl group of P_3 was accommodated in the hydrophobic subsite S_3 of renin, and the imidazole of P_2 His was hydrogen bonded to the side chain OH of Ser-233.



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Thus, we considered a large amount of optically pure compound would be required for further evaluation of renin inhibitors as an antihypertensive drug. In this paper, we describe a convenient and practical method for synthesizing *N*-[(2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidine methyl ester (**3**), which is useful as a common precursor (P_4–P_2 moiety) for the syntheses of renin inhibitors such as **1**.[1,6] In addition, the absolute configuration of (−)-**2** was established from NMR spectra.

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Initially, we synthesized both isomers of the propionic acids, (−)-**2** and its isomer (+)-**2**, to define which isomer contributed more to the activity (Scheme I). The 2-(1-naphthylmethylidene)propionic acid **5** was prepared by condensation of 1-naphthaldehyde **4** with diethyl succinate in the presence of NaOMe followed by hydrolysis (91.5% yield). The acid **5** was activated with $SOCl_2$ and treated with morpholine, and the resulting amide was hydrogenated to give a racemic (±)-**2** (72% yield from **5**). Racemate resolution with many optically active amines was unsuccessful.[7] So, (±)-**2** was esterified with an optically active alcohol, and the resulting diastereoisomers were separated. Thus, (±)-**2** was esterified with (*S*)-methyl mandelate using 1,3-dicyclohexylcarbodiimide (DCC) in the presence of (*N*,*N*-dimethylamino)pyridine (DMAP) as a catalyst. A simple recrystallization from MeOH gave a pure diastereoisomer (28% yield) from the diastereoisomeric mixture. The other diastereoisomer (29% yield) was prepared by essentially the same method using (*R*)-methyl mandelate. Each diastereoisomer was hydrolyzed and chromatographed to give the corresponding optically pure propionic acid (−)-**2** {$[\alpha]^{22}_D$ −11.80° (*c* 2.00, methanol), 78% yield} and (+)-**2** {$[\alpha]^{26}_D$ +11.47° (*c* 1.29, methanol), 82% yield}. Subsequent renin inhibition assay revealed that isomer (−)-**2** has the preferred chiral center for accommodating in S_3 of human renin.[1] Compound (−)-**2** was coupled with L-histidine methyl ester by DCC and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) to give **3** (82% yield).

The above synthetic method was unsuitable for large-scale preparation because of the troublesome procedure using optically active alcohol and column chromatography. Therefore, we investigated a more convenient method for synthesizing **3** (Scheme I). The racemic (±)-**2** was directly coupled with L-histidine methyl ester by DCC and HONB, and then the diastereoisomers were separated with salicylic acid by recrystallization from ethyl acetate to give **3** (36.2% yield). The optical purity of **3** was determined to be >99.9% by HPLC analysis. Thus, optically pure **3**, the P_4–P_2 moiety, was synthesized simply without complicated procedures. Compound **3** was identical with the product obtained by the condensation of (−)-**2** and L-histidine methyl ester. We have used this methodology for the mass production (kilogram scale) of **3** without any decrease in quality and yield of the product. Condensation of the hydrolyzed product of **3** using DCC–HONB gave optically pure **1**,[1] and the experimental details of synthesis of **1** from **3** will be reported elsewhere.

The stereochemistry of (−)-**2** and (+)-**2** was established by the MTPA (α-methoxy-α-(trifluoromethyl)phenylacetic acid) method[8] using NMR spectra. The proton NMR spectra of the both diastereoisomers of MTPA esters **6** (prepared as shown in Scheme I) with $Eu(fod)_3$ shift reagent clearly indicated that the configuration of (−)-**2** was *R* and that of (+)-**2** was *S*. The analysis of inhibitor-renin interaction supported that (−)-**2** having *R* configuration fitted to the subsite S_3 of human renin favorably.[5]

Experimental Section

Proton magnetic resonance spectra were measured on a JEOL JMX-GX270 (270 MHz) instrument. Chemical shifts are reported as δ values (parts per million) relative to Me_4Si as an internal standard. Mass spectra were obtained with JEOL JMX-DX300 (FAB) spectrometers having JMA-DA5000 (data processor). Infrared spectra (IR) were measured on JASCO IR-810 infrared spectrophotometer. HPLC analyses were performed on Shimadzu LC-6A liquid chromatograph instrument, Cosmosil $5C_{18}$ 4.6 × 100 mm with UV detection at 223 nm, and CHIRALCEL OC 4.6 × 250 mm with UV detection at 270 nm. Optical rotations were measured with Horiba SEPA-200 high-sensitive polarimeter. Melting points were measured on a Yamato micro melting point apparatus and are uncorrected. Preparative thin-layer chromatography was carried out using Merck precoated silica gel 60 F-254 plates (thickness 0.5 mm). Flash column chromatography was carried out using Merck silica gel 60 Art 9385 (230–400 mesh). Elemental analyses were performed by the Analytical Research Department, Central Research Laboratories, Kissei Pharmaceutical Co., Ltd.

2-(1-Naphthylmethylidene)succinic Acid (5). Sodium metal (1.1 g, 48 mmol) was dissolved in absolute methanol (30 mL) at 0 °C under argon, followed by the addition of diethyl succinate (11.2 g, 64 mmol). To the solution under reflux was added 1-naphthaldehyde (5.0 g, 32 mmol) in absolute methanol dropwise over 30 min. After reflux for 2 h, 2 N NaOH solution (80 mL, 160 mmol) was added to the mixture, and it was refluxed for 6 h. The reaction mixture was concentrated under reduced pressure, and concentrated HCl (15 mL) was added to the residue. The aqueous layer was extracted with ethyl acetate (100 mL), and the combined organic layers were washed with saturated NaCl (50 mL × 2). The solution was dried over $MgSO_4$ and concentrated in vacuo to afford a viscous material as the crude product. To the crude material was added benzene (30 mL) and hexane (30 mL), and **5** (7.5 g, 91.5%) was collected by filtration as light yellow crystals: mp 181.5–182.5 °C; IR (KBr) 1690 cm^{-1}; ^1H NMR

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(7) The following optically active amines were used: dehydroabiethylamine, (−)-cinchonidine, (−)-ephedrine, (−)-norephedrine, (−)-α-phenethylamine, (−)-α-(1-naphthyl)ethylamine, several L-amino acid methyl esters, several L-amino alcohols.

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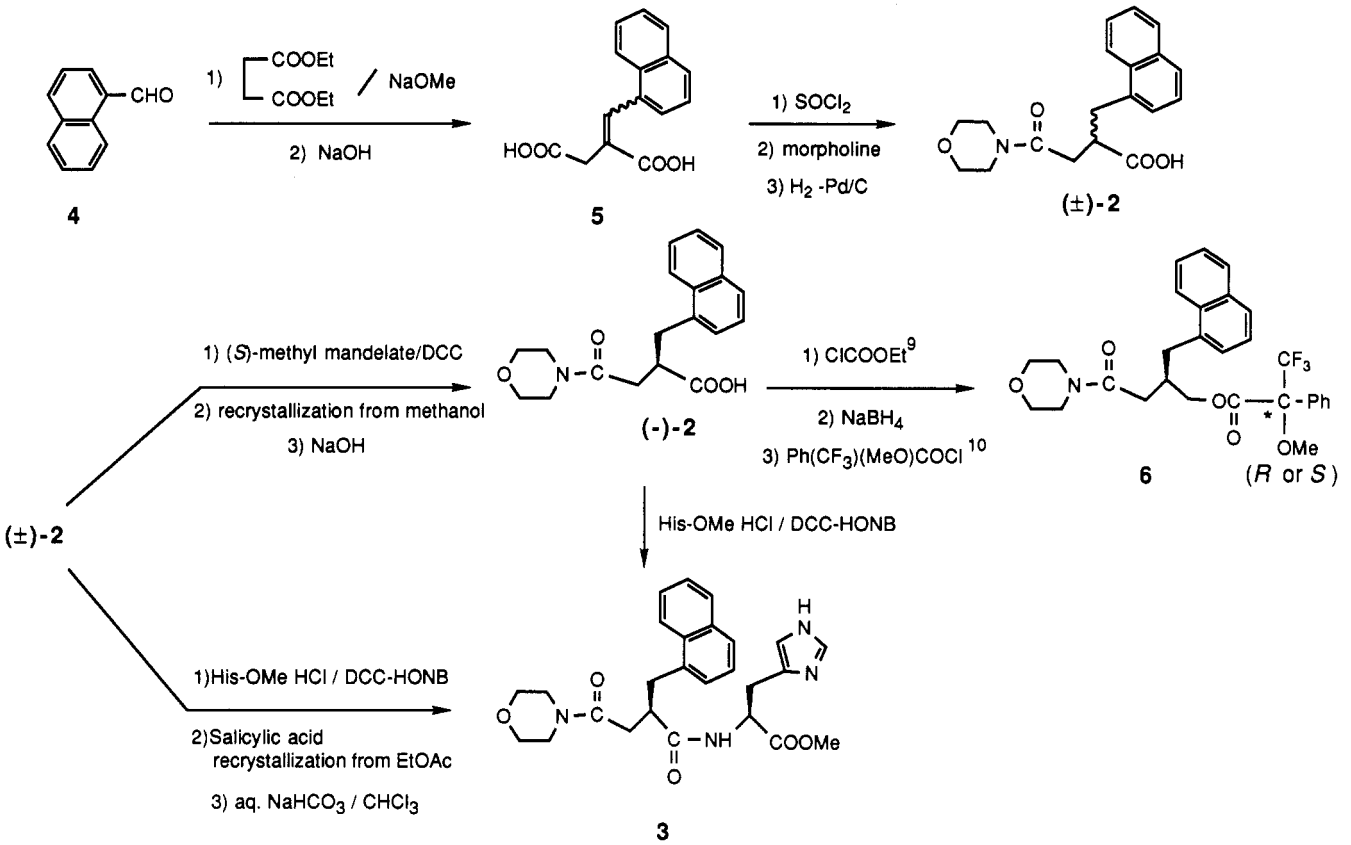
Scheme I



(DMSO-d_6) δ 3.3–3.7 (m, 2 H), 7.4–8.4 (m, 8 H), and 12.6 (br s, 2H). Anal. Calcd for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72. Found: C, 69.89; H, 4.75.

(±)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)-propionic Acid [(±)-2]. To a suspension of **5** (5.1 g, 19.9 mmol) in CH_2Cl_2 (50 mL) was added thionyl chloride (14.5 mL, 2 mol), followed by reflux until the suspension was homogenated (2–3 h). The solution was concentrated under reduced pressure, and benzene (12.5 mL) and hexane (37.5 mL) were added to the residue to afford 2-(1-naphthylmethylidene)succinic anhydride (4.2 g, 88%) as orange crystals by filtration. To a suspension of the anhydride (4.05 g, 17 mmol) in ethyl acetate (20 mL) was added morpholine (1.48 mL, 17 mmol), followed by stirring overnight to homogenate. Benzene (20 mL) and hexane (20 mL) were added to the mixture to give 3-(morpholinocarbonyl)-2-(1-naphthylmethylidene)propionic acid (4.6 g, 84%) as white crystals by filtration. A suspension of the acid (3.0 g, 9.2 mmol) and 10% Pd on activated carbon (0.3 g) in methanol (35 mL) was hydrogenated at atmospheric pressure overnight. After Pd on activated carbon was filtered out, the filtrate was concentrated in vacuo to afford (±)-**2** (2.95 g, 97.5%) as a white powder: mp 58–62 °C; IR (KBr) 1720, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.35–2.7 (m, 2 H), 3.05–3.85 (m, 11 H), 7.25–7.60 (m, 4 H), 7.77 (d, 1 H, J = 8.2 Hz), 7.87 (d, 1 H, J = 7.7 Hz), and 8.07 (d, 1 H, J = 8.2 Hz). Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.42; H, 6.43; N, 4.51.

In the large-scale synthesis, (±)-**2** was synthesized from **4** (2.5 kg) by the same method; 3.5 kg (overall yield 68%).

(-)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)-propionic Acid [(-)-2]. To a stirred 0 °C solution of (±)-**2** (10.33 g, 31.5 mmol) and (*S*)-methyl mandelate (4.77 g, 28.7 mmol) in dry $CHCl_3$ (50 mL, washed with water and then dried over $MgSO_4$) were added DCC (6.51 g, 31.5 mmol) and DMAP (0.70 g, 5.74 mmol) sequentially. After stirring at 0 °C for 2 h and at 25 °C overnight, the reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed with 10% citric acid, 5% $NaHCO_3$, and saturated NaCl. The solution was dried over $MgSO_4$ and concentrated in vacuo to yield a viscous material as a mixture of diastereomers. Crystallization from ether (40 mL) and then recrystallization from hot (60 °C) methanol (120 mL) gave pure [(1*S*)-1-(methoxycarbonyl)benzyl] (-)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionate (3.83 g, 28%) as white crystals: mp 141–142 °C; $[\alpha]^{22}_D$ +61.45° (*c* 0.96, $CHCl_3$); IR (KBr) 1740, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.33 (dd, 1 H, J_1 = 16.2, J_2 = 4.4 Hz), 2.78 (dd, 1 H, J_1 = 16.2, J_2 = 9.3 Hz), 3.19 (dd, 1 H, J_1 = 14.0, J_2 = 9.9 Hz), 3.2–3.7 (m, 9 H), 3.73 (s, 3 H), 3.92 (dd, 1 H, J_1 = 14.0, J_2 = 4.4 Hz), 5.99 (s, 1 H), 7.3–7.45 (m, 5 H), 7.5–7.7 (m, 4 H), 7.76 (d, 1 H, J = 8.2 Hz), 7.87 (d, 1 H, J = 7.8 Hz), and 8.23 (d, 1 H, J = 8.2 Hz). The isomer was hydrolyzed and chromatographed (silica gel, 96 g; eluent, $CHCl_3$/MeOH, 15/1) to give (-)-**2** (2.04 g, 78%) as a white powder: mp 58–61 °C; $[\alpha]^{22}_D$ -11.80° (*c* 2.00, methanol), $[\alpha]^{22}_D$ -35.36° (*c* 0.71, $CHCl_3$); HPLC 98% (analysis of the corresponding methyl ester: column; CHIRALCEL OC; eluent, hexane/2-propanol (4/1); flow rate, 0.9 mL/min; elution time, 32.7 min); other physical and spectral characteristics were identical to those of (±)-**2**. Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.48; H, 6.52; N, 4.48.

(+)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)-propionic Acid [(+)-2]. The synthesis of (+)-**2** was carried out as described above for (-)-**2** with (*R*)-methyl mandelate. [(1*R*)-1-(Methoxycarbonyl)benzyl] (+)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionate (29% yield) as white crystals: mp 140–141 °C; $[\alpha]^{22.5}_D$ -69.82° (*c* 0.57, $CHCl_3$); other physical and spectral characteristics were identical with those of [(1*S*)-1-(methoxycarbonyl)benzyl] (-)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionate. (+)-**2** (82% yield) as a white powder: mp 60–62 °C; $[\alpha]^{26}_D$ +11.47° (*c* 1.29, methanol), $[\alpha]^{22}_D$ +36.66° (*c* 0.12, $CHCl_3$); HPLC 98% (analysis of the corresponding methyl ester: elution time, 50.9 min); other physical and spectral characteristics were identical to those of (-)-**2**. Anal. Calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28. Found: C, 69.51; H, 6.41; N, 4.47.

***N*-[(2*R*)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)-propionyl]-L-histidine Methyl Ester (3).** To a stirred 0 °C solution of (±)-**2** (2.9 kg, 8.85 mol) and L-histidine methyl ester (dihydrochloride, 2.03 kg, 8.38 mol) in acetonitrile (18 L) were added triethylamine (2.46 L, 17.7 mol), HONB (1.59 kg, 8.87 mol) and DCC (1.83 kg, 8.85 mol) sequentially. After 2 h the mixture

was warmed to 25 °C, stirred overnight, and filtered, and the filtrate was evaporated in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed with 5% $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo to afford *N*-[(2*RS*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidine methyl ester (3.5 kg, 83%). The mixture of the isomers (3.5 kg, 7.31 mol) and salicylic acid (1.01 kg, 7.31 mol) was crystallized from ethyl acetate three times to give pure **3** salicylic acid salt (1.0 kg, 36.2%) as white crystals: mp 142–143 °C; $[\alpha]^{24.5}_D$ +38.10° (*c* 1.29, methanol); IR (KBr) 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (dd, 1 H, J_1 = 16.2, J_2 = 5.5 Hz), 2.60 (dd, 1 H, J_1 = 16.2, J_2 = 6.6 Hz), 2.87 (dd, 1 H, J_1 = 14.8, J_2 = 8.8 Hz), 3.00 (dd, 1 H, J_1 = 14.8, J_2 = 5.5 Hz), 3.1–3.45 (m, 11 H), 3.53 (s, 3 H), 4.54 (dd, 1 H, J_1 = 13.7, J_2 = 8.2 Hz), 6.7–6.85 (m, 2 H), 7.25–7.45 (m, 3 H), 7.45–7.6 (m, 2 H), 7.7–7.85 (m, 2 H), 7.91 (d, 1 H, J = 7.7 Hz), 8.20 (s, 1 H), 8.27 (d, 1 H, J = 8.2 Hz), and 8.36 (d, 1 H, J = 7.7 Hz).

To **3** salicylic acid salt was added chloroform, and the solution was washed with 5% $NaHCO_3$ and saturated NaCl and dried over $MgSO_4$. The solution was concentrated in vacuo and then crystallized from benzene to afford **3** (780 g, quant) as white crystals: mp 92–96 °C; $[\alpha]^{23}_D$ +35.7° (*c* 2.2, MeOH); IR (KBr) 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.3–2.5 (m, 1 H), 3.0–3.75 (m, 14 H), 4.55–4.65 (m, 1 H), 6.19 (d, 1 H, J = 6.6 Hz), 6.66 (s, 1 H), 7.25–7.6 (m, 7 H), 7.74 (d, 1 H, J = 8.2 Hz), 7.85 (d, 1 H, J = 7.7 Hz), 7.99 (d, 1 H, J = 8.2 Hz); HPLC >99.9% (column, Cosmosil $5C_{18}$ 4.6 × 100 mm; eluent, acetonitrile/0.05 M NH_4OAc (aqueous) (3/7); flow rate, 1 mL/min; elution time, 8.0 min); FABMS *m/z* 479 (M + 1). Anal. Calcd for $C_{26}H_{30}N_4O_5 \cdot C_6H_6$: C, 69.05; H, 6.52; N, 10.06. Found: C, 69.28; H, 6.58; N, 9.74.

Alternatively, **3** was obtained by the condensation of (−)-**2** and L-histidine methyl ester. To a stirred solution of (−)-**2** (0.33 g, 1 mmol) and L-histidine methyl ester (dihydrochloride, 0.24 g, 1 mmol) in acetonitrile (2 mL) were added triethylamine (0.28 mL, 2 mmol), HONB (0.18 g, 1 mmol), and DCC (0.21 g, 1 mmol) at 0 °C sequentially. After 2 h the mixture was warmed to ambient temperature gradually, stirred overnight, filtered, and evaporated in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed with 5% $NaHCO_3$ and saturated NaCl and dried over $MgSO_4$. The solution was concentrated in vacuo and then crystallized from benzene to afford **3** (0.38 g, 82%) as white crystals: physical and spectral characteristics were identical with those of **3** obtained above.

(2*R*)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)-propionyl (*R*)-α-Methoxy-α-(trifluoromethyl)phenylacetate (*R*,*R*-6). To a stirred 0 °C solution of (−)-**2** (0.1 g, 0.31 mmol) in dry THF were added triethylamine (0.047 mL, 0.34 mmol) and ethyl chloroformate (0.032 mL, 0.34 mmol) sequentially. After 1 h the reaction mixture was filtered, and the filtrate was added to a stirred 0 °C solution of $NaBH_4$ (0.058 g, 1.55 mmol) in water (0.3 mL). After 1 h the mixture was warmed to 25 °C for 15 min and evaporated in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed with 1 N HCl, 5% $NaHCO_3$, and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo to afford (2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propyl alcohol (0.05 g, 52%). To the alcohol (0.01 g, 0.032 mmol) in dry $CHCl_3$ (1 mL, washed with water, then dried over $MgSO_4$) at 0–5 °C (ice bath) were added triethylamine (0.06 mL, 0.038 mmol), DMAP (0.4 mg, 3.3 × 10^{-2} mmol), and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.01 g, 0.038 mmol) sequentially. After 2 h the mixture was warmed to 25 °C and stirred overnight. The mixture was washed with 5% $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed (silica gel plates; solvent, $CHCl_3$) to give *R*,*R*-**6** (15.8 mg, 93%) as colorless oil: $[\alpha]^{23}_D$ + 32.07° (*c* 6.61 chloroform); IR (neat) 1745 and 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.25–2.45 (m, 2 H), 2.75–2.9 (m, 1 H), 3.02 (dd, 1 H, J_1 = 13.7, J_2 = 8.2 Hz), 3.1–3.2 (m, 2 H), 3.27 (dd, 1 H, J_1 = 13.7, J_2 = 7.2 Hz), 3.56 (s, 3 H), 3.4–3.7 (m, 4 H), 4.25–4.4 (m, 2 H), 7.18 (d, 1 H, J = 7.1 Hz), 7.25–7.6 (m, 8 H), 7.74 (d, 1 H, J = 8.2 Hz), 8.85 (dd, 1 H, J_1 = 6.6, J_2 = 2.2 Hz), and 8.14 (d, 1 H, J = 9.9 Hz); FABMS *m/z* 530 (M + 1). The lanthanide-induced shift of the methoxy proton resonance vs molar ratio of $Eu(fod)_3$ for *R*,*R*-**6** was 0.72 ppm.

(2*R*)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)-propionyl (*S*)-α-Methoxy-α-(trifluoromethyl)phenylacetate (*R*,*S*-6). The synthesis of *R*,*S*-**6** was carried out as described above for *R*,*R*-**6** with (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. *R*,*S*-**6** (94% yield) as a white powder: mp 86–88 °C; $[\alpha]^{23}_D$ +5.34° (*c* 0.72, chloroform); IR (KBr) 1750 and 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.2–2.4 (m, 2 H), 2.75–2.9 (m, 1 H), 3.03 (dd, 1 H, J_1 = 13.7, J_2 = 8.2 Hz), 3.1–3.3 (m, 3 H), 3.49 (t, 1 H, J = 4.6 Hz), 3.56 (s, 3 H), 3.6–3.7 (m, 2 H), 4.19 (dd, 1 H, J_1 = 11.0, J_2 = 4.4 Hz), 4.44 (dd, 1 H, J_1 = 11.0, J_2 = 4.4 Hz), 7.19 (d, 1 H, J = 7.2 Hz), 7.36–7.6 (m, 8 H, 7.74 (d, 1 H, J = 8.3 Hz), 7.85 (d, 1 H, J = 9.3 Hz), and 8.13 nd, 1 H, J = 9.3 Hz); FABMS *m/z* 530 (M + 1). The lanthanide-induced shift of the methoxy proton resonance vs molar ratio of $Eu(fod)_3$ for *R*,*S*-**6** was 0.64 ppm.

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Mutactin, a Novel Polyketide from *Streptomyces coelicolor*. Structure and Biosynthetic Relationshp to Actinorhodin

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In 1979 Rudd and Hopwood[1] reported the isolation of 75 mutants of *Streptomyces coelicolor* A 3(2) which were unable to synthesize the pigment antibiotic, actinorhodin (1). These were grouped into seven classes based on various phenotypic characteristics, particularly their ability to engage in cosynthesis of 1.[1] Subsequent chemical analysis, based on a modification of the cosynthesis assay, led to the isolation of several intermediates of 1 biosynthesis accumulated by these mutants.[2-4] The biosynthetic intermediate in extracts of mutant B_{40}, a member of class VII, the earliest class of mutants acting as secretors in the cosynthesis assay,[1] proved too unstable for isolation. However, another less polar material was uniquely prominent in chromatograms of these extracts, was identified as a novel 16-carbon polyketide, and was called mutactin.

Mutactin was purified from cultures of *S. coelicolor* mutant B_{40}[1] grown in CM medium[5] by extraction of the broth at pH 3.0 with EtOAc, chromatography on LH-20 (methanol), or partitioning between aqueous MeOH and organic solvents, followed by either preparative TLC or crystallization. The material, mp 192–193 °C, had UV absorptions at 222 (ε 24 300), 265 (ε 16 900), and 290 nm (sh, ε 9600) and showed no antibiotic activity (MIC > 100 μg/mL) against 19 strains of bacteria, fungi, and yeasts.

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