of the log dose–response curves with use of the Linefit program by R. B. Barlow, ElsevierBiosoft, Cambridge, UK.[8]

Acknowledgment. This work was supported in part by Auburn University Grant-in-Aid 84-206 and a grant from the American Diabetes Association, Alabama Affiliate. We also thank James Kercher, Dave Ennis, and Syble McClellan for their excellent technical assistance and Pfizer Central Research, Groton, CT, for providing a sample of sorbinil.

Registry No. **5b**, 5263-87-6; **5c**, 2598-29-0; **6b**, 6563-13-9; **6c**, 103368-17-8; **7a**, 607-66-9; **7b**, 13676-00-1; **7c**, 15450-72-3; **8a**, 103368-18-9; **8b**, 103368-19-0; **8c**, 103368-20-3; **9a**, 103368-21-4; **9b**, 103368-22-5; **9c**, 103368-23-6; **9d**, 103368-24-7; **9e**, 103368-25-8; **11a**, 103368-26-9; **11b**, 103368-27-0; **11c**, 103368-28-1; **12**, 103368-29-2; **13**, 75371-21-0; **17**, 19315-93-6; **18**, 85770-30-5; $BrCH_2CO_2Et$, 105-36-2; $CH_3CH(Br)CO_2Et$, 535-11-5; $ClCH_2CH_2CO_2Et$, 623-71-2; aldose reductase, 9028-31-3.

Investigation of 4-(3-Hydroxyphenyl)-4-methylpipecolic Acid as a Conformationally Restricted Mimic of the Tyrosyl Residue of Leucine-Enkephalinamide[1]

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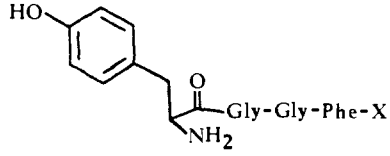
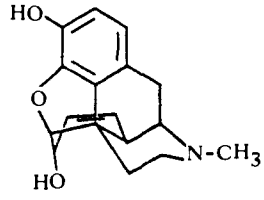
Received January 15, 1986

Analogues of leucine-enkephalinamide containing N-terminal *cis*- or *trans*-4-(3-hydroxyphenyl)-4-methylpipecolic acid were prepared to examine the conformational requirements of the N-terminal tyrosyl residue in opioid activity. The diastereomeric amino acids were prepared and purified by semipreparative HPLC before incorporation into the peptide. Spectroscopic analysis based on proton nuclear Overhauser enhanced differential spectroscopy (NOEDS) allowed assignment of the cis and trans stereochemistry. Despite spatial analogy between the trans isomer **5** and leucine-enkephalinamide, it possessed neither opioid agonist nor antagonist activity in the guinea pig ileal longitudinal muscle (GPI) or mouse vas deferens (MVD) preparations. Possible explanations for this inactivity are discussed.

Soon after characterization[2,3] of the enkephalins **1**, it was proposed[3,4] that the tyramine moiety was the key pharmacophore for both the opioid peptides and alkaloid opiates [e.g., morphine (**2**)]. This hypothesis was recently



1, X = Leu, Met



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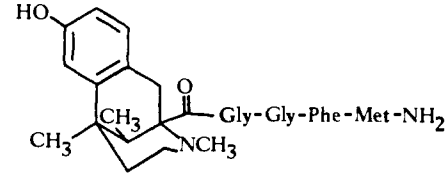
tested by the preparation of a hybrid opioid peptide **3** in which the tyrosyl residue was replaced by (−)-metazocine.[5] The absence of significant in vitro opioid agonist or antagonist activity with this analogue suggested that the peptidergic and alkaloid opioids differ in their recognition of opioid receptors, a concept that was reinforced by the characterization of multiple opioid receptors.[6,7] The tertiary amino terminus and conformational restriction of the phenolic moiety in **3** were considered among the possibilities contributing to the absence of receptor recognition for this ligand. Indeed, the more flexible aminotetralin analogue **4** was reported to be biologically active.[8]



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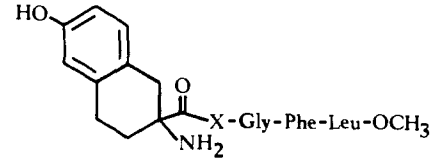
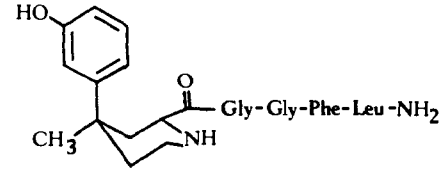


4, X = Gly, D-Ala

This report describes an effort to investigate further the conformational requirements for opioid receptor recognition of the tyrosyl side chain through the synthesis and in vitro biological evaluation of enkephalin analogue **5**.



trans-**5**

The use of C-4-substituted pipecolic acids allows predictable conformational restriction of the aromatic residue.[9] Thus, the aryl and amino moieties can be spatially superimposed upon those of the tyrosine residue in leu-

(1) Stereochemical Studies on Medicinal Agents. 30. Part 29: Portoghese, P. S.; Larson, D. L.; Yim, C. B.; Sayre, L. M.; Ronsisvalle, G.; Lipkowski, A. W.; Takemori, A. E.; Rice, K. C.; Tam, S. W. *J. Med. Chem.* **1985**, *28*, 1140.
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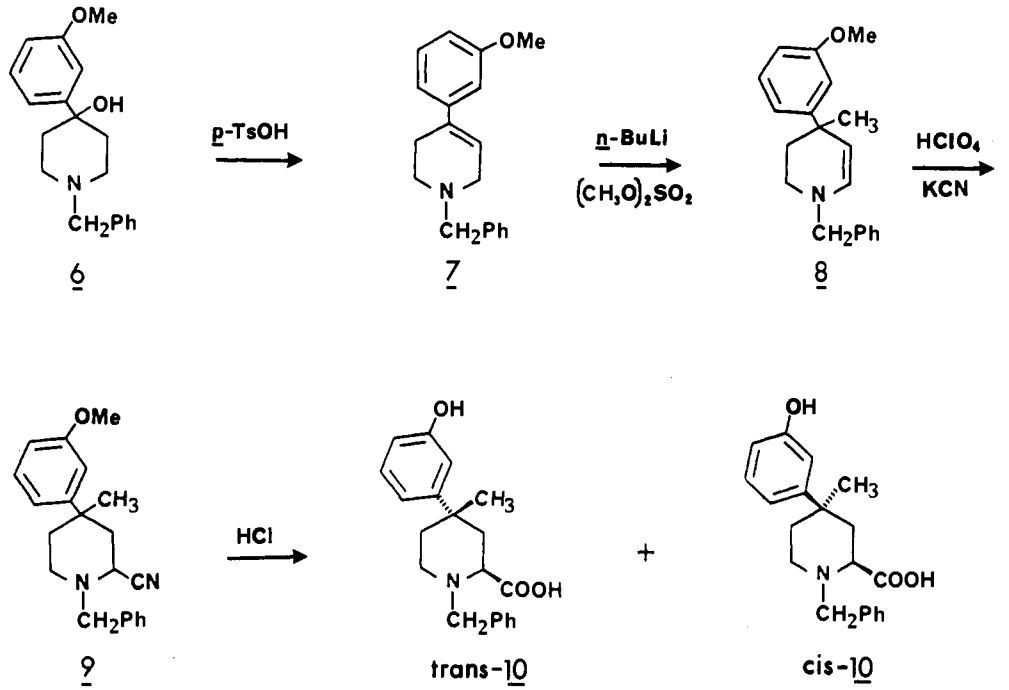
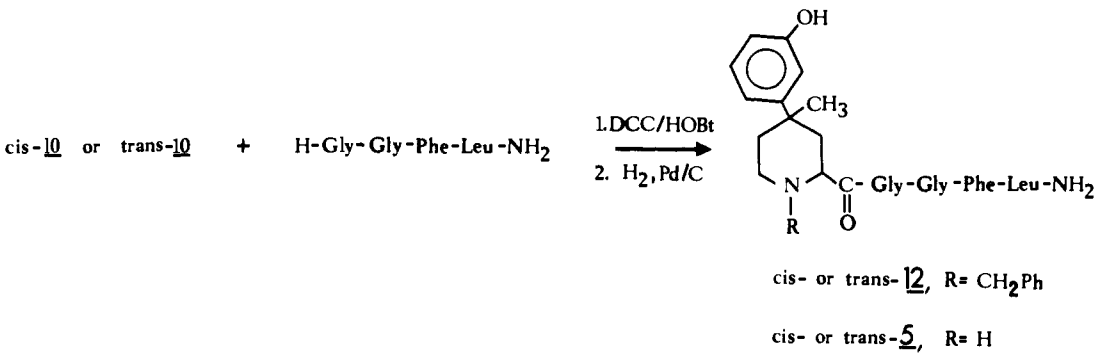
Scheme I



Scheme II



cine-enkephalinamide in the predominant gauche (−) rotamer ($\chi = -60°$). While structurally related to the metazocine-enkephalin hybrid **3**, the pentapeptide *trans*-**5** possesses greater conformational flexibility and a secondary amino terminus, two features that were presumed to favor opioid activity.

Results

Synthesis. The pipecolic acid stereoisomers were prepared according to the route outlined in Scheme I. Dehydration[10,11] of 4-arylpiperidinol **6** affored tetrahydropyridine **7**, which was converted to enamine **8** by regioselective methylation[12] with *n*-butyllithium and dimethyl sulfate. Acidification with aqueous perchloric acid and reaction with potassium cyanide in a biphasic (water/methylene chloride) reaction medium gave nitrile **9** as a mixture of diastereoisomers indicated by the presence of two C-4 methyl resonances at 1.4 ppm (trans) and 1.24 ppm (cis) in a ratio of 5:1. The diastereomeric nitriles were very labile under acidic[13-18] or basic[13-15] conditions. Chromatography on silica gel resulted in equilibration to a final diastereomeric ratio of 3:2. Attempts to isolate the hydrochloride salt resulted in selective precipitation of *cis*-**9**. The mother liquor contained both *trans*-**9** and enamine **8**. Despite this instability, the diastereomeric amino acids **10** were obtained in up to 38% yield by hydrolysis in concentrated hydrochloric acid. As expected,[19] the methoxy ether was readily cleaved under these conditions. The mixture of cis and trans racemates of **10** was separated by semipreparative HPLC, and the stereochemical assignment was based on proton NMR spectroscopy, as discussed below. Trans and cis refer to the geometric relationship between the aromatic and C-2 carbonyl substituents.

The target pentapeptides, *trans*-**5** and *cis*-**5**, were prepared by DCC/HOBt coupling of the racemic *N*-benzylpipecolic acids (*trans*-**10** or *cis*-**10**) with glycylglycylphenylalanylleucinamide **11** to give the corresponding *N*-benzyl pentapeptides, *trans*- or *cis*-**12**, followed by catalytic hydrogenolysis of the *N*-benzyl moiety (Scheme

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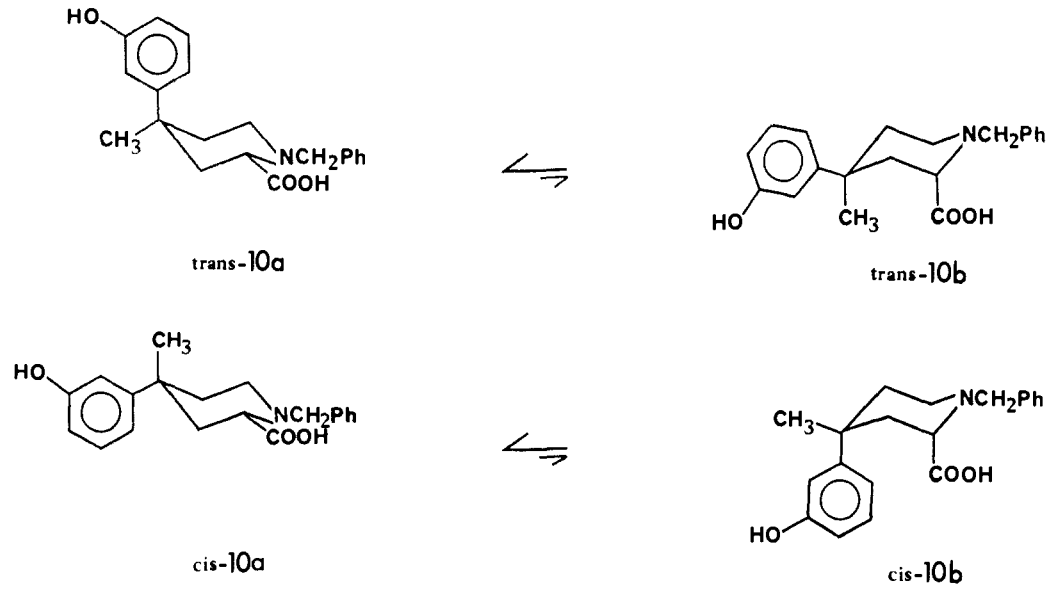
Scheme III



II). The pentapeptides, *trans*-**5** and *cis*-**5**, were not resolved.

Stereochemical Studies. Analysis of the 300-MHz proton NMR spectra of the diastereomeric *N*-benzylpipecolic acids, *trans*-**10** and *cis*-**10**, and of the pentapeptides, *trans*-**5** and *cis*-**5**, permitted determination of both the relative stereochemistry of the piperidine ring substituents and the conformational preference of the piperidine ring.

The chemical shift data for the piperidine ring protons of the diastereomeric *N*-benzylpipecolic acids **10** and pentapeptides **5** are presented in Table I. Preliminary assignment of trans and cis stereochemistry was based on the relative positions of the C-3 and C-5 methylene protons, which reflect the differential shielding/deshielding influences of the vicinal aromatic [20,21] and methyl[22] residues at C-4.[23] In both diastereomers of the *N*-benzylpipecolic acids **10**, the axial protons at C-3 and C-5 are shielded relative to the equatorial protons, but the chemical shift difference is greatest in *trans*-**10** (axial aromatic/equatorial methyl). The resonances for $H3_{eq}$ (2.68 ppm) and $H5_{eq}$ (2.2 ppm) in *cis*-**10** (axial methyl/equatorial aromatic) are shielded relative to the resonances for $H3_{eq}$ (3.1 ppm) and $H5_{eq}$ (2.64 ppm) in *trans*-**10**. The C-2 proton resonance was a doublet of doublets at 4.21 ppm (*trans*-**10**) or 4.42 ppm (*cis*-**10**). The vicinal coupling constants of 3 Hz (J_{ax-eq}) and 12 Hz (J_{ax-ax}) observed for this proton are consistent with an axial position. The predominant conformation of the piperidine ring in both isomers is that with an equatorial carboxyl group (*trans*-**10a** and *cis*-**10a**).

These same relative shielding patterns were observed for the piperidine ring protons in the *trans*-**5** and *cis*-**5** isomeric pentapeptides (Table I), suggesting that the piperidine ring conformation is the same for the isomeric pentapeptides as for the *N*-benzyl amino acids. It was not possible to obtain the vicinal coupling constants from the spectra for the *N*-benzyl pentapeptides, *trans*- and *cis*-**12**, or deprotected pentapeptides, *trans*- or *cis*-**5**, due to overlap of the diastereomeric proton resonances resulting from incorporation of racemic *trans*-**10** or *cis*-**10**. None of the spectra for the protected or deprotected pentapeptides showed contamination by the second isomer. The

Table I. Chemical Shift Data for the Piperidine Ring Protons of Trans and Cis Isomers of *N*-Benzyl-4-(3-hydroxyphenyl)-4-methylpipecolic Acid (**10**) and 4-(3-Hydroxyphenyl)-4-methylpipecolylglycylglycylphenylalanylleucinamide (**5**)

	chemical shift, ppm			
proton	*trans*-**10**[a]	*cis*-**10**[a]	*trans*-**5**[b]	*cis*-**5**[b]
H2	4.21	4.42	4.06	4.78
$H3_{ax}$	2.16	2.4	2.09	2.2
$H3_{eq}$	3.1	2.68	2.84	2.65
$H5_{ax}$	2.06	buried	1.98	2.07
$H5_{eq}$	2.64	2.2	2.56	2.07
$H6_{ax}$	3.1	3.53	3.1	3.6
$H6_{eq}$	3.65	3.84	3.56	3.6

[a] 300 MHz, in trifluoroacetic acid-*d*. [b] 300 MHz, in acetic acid-d_4.

Table II. Proton NOEDS Data for *trans*- and *cis*-*N*-Benzyl-4-(3-hydroxyphenyl)-4-methylpipecolic Acids

	NOED detected[a]	
proton irradiated	*trans*-**10**	*cis*-**10**
H2	ArH (1.1%)	methyl (2%) $H6_{ax}$ (4.7%)
$H6_{ax}$	overlaps $H3_{eq}$	methyl (2.4%) H2 (3.1%)
methyl	*b*	H6 (4.1%) H2 (3.5%)

[a] 300 MHz, in trifluoroacetic acid-*d*. [b] Only vicinal enhancements observed.

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(22) Booth, H. *Tetrahedron* **1966**, *22*, 615.
(23) In cyclohexane, an axial aromatic residue is reported[20,21] to deshield both axial (+0.12 ppm) and equatorial (+0.4 ppm) vicinal hydrogens. An equatorial aromatic residue exerts a minimal shielding influence on vicinal equatorial protons (−0.01 ppm), but a significant deshielding influence on the axial protons (+0.16 ppm). The influence of a methyl substituent has been similarly studied.[22] An equatorial methyl group induces a significant shielding effect on the resonances for both vicinal axial and equatorial protons (−0.3 to −0.5 ppm), while an axial methyl group shields the vicinal equatorial protons (−0.4 ppm) and deshields the vicinal axial protons (+0.2 ppm). In the absence of other influences, the resonances for the axial protons at C-3 and C-5 should be shielded relative to the equatorial protons in the axial aromatic/equatorial methyl isomer, and the reverse relationship is expected in the axial methyl/equatorial aromatic isomer. The resonances for $H3_{eq}$ and $H5_{eq}$ should be more shielded in the axial methyl/equatorial aromatic isomer than in the axial aromatic/equatorial methyl isomer.

relative positions of the C-4 methyl resonances in *trans*-**12** (1.22, 1.21 ppm) and *trans*-**5** (1.27 ppm) and in *cis*-**12** (1.44 ppm) and *cis*-**5** (1.47 ppm) were similar to the chemical shifts observed for these same resonances in *trans*-**10** (1.35 ppm) and *cis*-**10** (1.5 ppm).

Confirmation of the preliminary stereochemical assignment was provided by proton nuclear Overhauser enhanced differential (NOED) spectroscopic analysis[24,25] of the *N*-benzylpipecolic acids, *trans*-**10** and *cis*-**10**. This technique permits detection of cis-diaxial relationships within cyclic molecules.[26] By selective presaturation of H2, $H6_{ax}$, and C-4 methyl resonance signals, it was possible to examine diaxial relationships within the two isomers (Table II).

Presaturation of the H2 resonance induced an enhancement of the C-4 aromatic proton resonance (1.1%) in *trans*-**10**, while in *cis*-**10** the C-4 methyl resonance signal was enhanced (2%). Overlapping of the $H6_{ax}$ resonance by $H3_{eq}$ in the *trans*-**10** isomer complicated NOED spectral analysis. However, in *cis*-**10**, the $H6_{ax}$ resonance signal was well separated, and enhancements of both H2 (3.1%) and the C-4 methyl (2.4%) resonances were observed. Presaturation of the methyl resonance in *trans*-**10** resulted only in enhancements of the resonances for the vicinal protons at C-3 and C-5, while in *cis*-**10** enhancements of both $H6_{ax}$ (4.1%) and H2 (3.5%) resonances were observed.

Pharmacology. The target pentapeptides, **5**, as well as the *N*-benzyl pentapeptides, **12**, were tested in both the electrically stimulated guinea pig ileum (GPI)[27,28] and mouse vas deferens (MVD)[29,30] preparations for agonist and antagonist activities. Since the peptides possessed limited aqueous solubility, the compounds were tested as either hydrochloride or acetate salts in aqueous ethanolic solutions. This necessitated the use of a single-dose assay procedure, as high concentrations of ethanol were shown to significantly depress the twitch response of both smooth muscle preparations. At the maximal concentration that could be tested (3 μM), only the *N*-benzyl pentapeptide, *trans*-**12**, exhibited any biological activity, causing a 35% inhibition of the GPI. None of the compounds behaved as opioid antagonists at this concentration.

Discussion

The NMR studies permitted assignment of both the relative stereochemistry and preferred conformation of the pipecolyl residues of the trans and cis pentapeptide analogues, **5**. The data suggest that the trans analogue contains an axial aryl/equatorial carbonyl substitution pattern. This enables the phenolic moiety and basic nitrogen of the *trans*-**5** isomer to assume a conformation very similar to that of both morphine **2** and the spatially analogous tyrosyl residue of leucine-enkephalin in the gauche (−) rotamer conformation. A conformational feature of *trans*-**5** that distinguishes it from morphine is the rotational mobility of the phenolic moiety.

The lack of agonist or antagonist activity of *trans*-**5** on the GPI and MVD preparations suggests factors other than conformational rigidity of the aromatic residue may be responsible for the inactivity of the benzomorphan-containing peptide of **3**. This includes conformational restriction of the basic amine due to its inclusion in the piperidine ring system. The report that **4** is active while the closely related aminoindane analogue[31] is devoid of activity appears to be in harmony with this view.

Another possible explanation for inactivity is that when the pipecolyl residue of the *trans*-**5** pentapeptide is viewed through an imaginary methylene bridge connecting the C-2 carbon with the aromatic ring, with the spatial disposition of the aromatic, amino, and carboxyl residues equivalent to tyrosine, the C-3 carbon of the piperidine ring is similar to an α-methyl substituent. Since the α-methyl tyrosine derivative of (D-Ala^2)methionine-enkephalin was reported[32] to be inactive, it is conceivable that the C-3 methylene carbon of the piperidine ring could sterically interfere with opiate receptor binding.

That morphine is recognized by opioid receptors, while peptides containing a similar or identical conformational arrangement of groups are not, suggests different stereochemical requirements for alkaloid opiates and the opioid peptides.[5] This difference is probably, in part, a manifestation of the different stereochemical requirements for μ and δ opioid receptors.

Experimental Section

Melting points were determined with a Thomas–Hoover capillary apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 281 spectrometer. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ. Mass spectral analyses were performed on either an AE1 MS-30 or Finnigan 4000 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter using a 1-dm cell. TLC analyses utilized precoated silica gel (GF) plates, 0.25 mm thick, obtained from Analtech, Inc., Newark, DE. HPLC separations were performed on a Beckman Model 110-A with 5 μm (Lichrosorb Si 60, Altex) or 10 μm (Rsil, Alltech) semipreparative (10 mm × 250 mm) silica gel columns. HPLC-grade solvents were obtained from MCB, Cincinnati, OH. HPLC-grade chloroform (hydrocarbon stabilized) was passed over basic alumina to remove acidic decomposition products and used immediately.

All chemicals and solvents are reagent grade unless otherwise specified. Tetrahydrofuran (THF) was freshly distilled over lithium aluminum hydride or sodium prior to use. Cbz-Gly-Gly-Phe and leucineamide were obtained from Sigma Chemical Co., St. Louis, MO. All other chemicals were obtained from Aldrich Chemical Co., Milwaukee, WI. Extracts of materials in organic solvents were dried over anhydrous potassium carbonate, and the solvent evaporated under aspirator pressure with a rotary flash evaporator at a bath temperature between 25 and 45 °C.

Morphine sulfate was obtained from Merck and Co., Inc., Rahway, NJ., naloxone hydrochloride from Endo Laboratories, Inc., Garden City, NY, and chlorpheniramine maleate from Schering Corp., Kenilworth, NJ. [D-Ala^2,D-Leu^5]enkephalin was obtained from Sigma Chemical Co., St. Louis, MO. All other chemicals were obtained either from Mallinckrodt Chemical Works, St. Louis, MO, or Fisher Scientific Company, Fair Lawn, NJ.

NMR spectra were recorded on a JEOL FX-90 or Nicolet NT 300 spectrometers. Chemical shifts are reported in ppm downfield from Me_4Si. Steady-state NOE differential spectra were acquired by low-power selective presaturation for 3 s. Four accumulations were acquired at each resonance frequency, and the sequence was repeated until an adequate S/N ratio was achieved. A control spectrum was acquired simultaneously at a frequency at which there was no resonance absorption.

***N*-Benzyl-4-(3-methoxyphenyl)piperidin-4-ol (6).** To a stirred solution of *m*-bromoanisole (23.6 g, 0.127 mol) dissolved in freshly distilled THF (100 mL) under a nitrogen atmosphere was added *n*-butyllithium (90 mL, 1.4 M in hexane) such that

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the temperature was maintained at -55 °C. The solution was stirred 2 h at -55 °C. *N*-Benzyl-4-piperidinone (15 g, 0.08 mol) in tetrahydrofuran (100 mL) was added, and the final solution was allowed to warm to room temperature and stirred 2 hours. The reaction mixture was neutralized by the addition of aqueous 1 N hydrochloric acid and extracted once with ether (100 mL), basified with 1 N ammonium hydroxide, and extracted twice with ether (200 mL). The basic extract was dried and evaporated in vacuo to give 22.6 g of an oil that contained approximately 15–20% starting ketone. The crude product was used without further purification: R_f 0.28 (chloroform/ammonia, 99:1); IR (neat) 3460 (OH, br) cm^{-1}; *m/e* 297 (M^+); NMR (300 MHz, Me_2SO-d_6, HCl salt) δ 7.63, 7.46 (m, 5 H, benzyl Ar*H*), 7.26, 7.0, 6.83 (m, 4 H, C-4 Ar*H*), 5.43 (s, 1 H, OH), 4.35 (s, 2 H, benzyl C*H*), 3.74 (s, 3 H, OCH_3), 3.26 (s, 4 H, H2 and H6), 2.49 (q, 2 H, $H5_{ax}$ and $H3_{ax}$), 1.76 (d, 2 H, $H5_{eq}$ and $H3_{eq}$).

***N*-Benzyl-4-(3-methoxyphenyl)-1,2,5,6-tetrahydropyridine (7).** A solution of alcohol **6** (22.6 g, 0.76 mol) and *p*-toluenesulfonic acid monohydrate (18.4 g, 0.107 mol) in toluene (500 mL) was refluxed for 6 h with a Dean–Stark trap to remove water. Upon cooling, the product precipitated as the *p*-toluenesulfonic acid salt, mp 150–151 °C. The precipitate was collected, converted to the free base with aqueous 1 N ammonium hydroxide, and extracted into ether (200 mL). The organic layer was dried and evaporated to give 12.9 g (0.046 mol, 58%) of **7** as an oil: R_f 0.62 (chloroform/ammonia, 99:1); IR (neat) 1660 (C=C, weak) cm^{-1}; *m/e* 279 (M^+); NMR (300 MHz, acetone-d_6) δ 7.32 (s, 5 H, benzyl Ar*H*), 7.22, 6.98, 6.8 (m, 4 H, C-4 Ar*H*), 6.13 (m, 1 H, *J* = 1.6, 3.6, 5.2 Hz, H3), 3.79 (s, 3 H, OCH_3) 3.61 (s, 2 H, benzyl C*H*), 3.11 (dd, 2 H, *J* = 2.8, 6.3 Hz, H2), 2.68 (t, 2 H, *J* = 5.3, 5.6 Hz, H6), 2.52 (m, 2 H, H5). Anal. ($C_{19}H_{21}NO{\cdot}C_7H_8SO_3$) C, H, N.

***N*-Benzyl-4-(3-methoxyphenyl)-4-methyl-1,4,5,6-tetrahydropyridine (8).** To a solution of **7** (12.9 g, 0.046 mol) dissolved in THF (150 mL) at -20 °C under a nitrogen atmosphere was added *n*-butyllithium (33 mL, 1.4 M in hexane) such that the temperature was maintained at less than -10 °C. After the addition, the reaction mixture was cooled to -30 °C and added by transfer loop to a solution of dimethyl sulfate (5.87 g, 0.046 mol) in tetrahydrofuran (100 mL) at -40 °C. This final solution was warmed to 0 °C, stirred an additional 0.5 h, and quenched by the addition of cold 1 N ammonium hydroxide (100 mL) and cold saturated saline (100 mL). The product was extracted into ether (500 mL), and the organic extract was washed five times with a mixture of saline and 1 N ammonium hydroxide (100 mL, 50:50), then dried, and evaporated in vacuo to give 13.02 g (0.044 mol) of an oil, R_f 0.33 (methylene chloride/hexane/ammonia, 1:3:0.04). The product was purified by column chromatography on alumina (activity grade II, hexane/methylene chloride, 4:1) to give 8.33 g (65%) of **8** as an oil that was promptly employed in the next synthetic step: IR (neat) 1645 (C=CN, str) cm^{-1}; *m/e* 294 (M^+); NMR (300 MHz, acetone-d_6) δ 7.29 (s, 5 H, benzyl Ar*H*), 7.15, 6.98, 6.68 (m, 4 H, C-4 Ar*H*), 6.23 (d, 1 H, *J* = 8 Hz, H2), 4.37 (dd, 1 H, *J* = 7.9, 1.5 Hz, H3), 4.04 (s, 2 H, benzyl CH), 3.74 (s, 3 H, OCH_3), 2.78 (m, 1 H, *J* = 0.51, 3.6, 5.8, 5.8, 11.6 Hz, $H6_{eq}$), 2.58 (m, 1 H, *J* = 3.2, 10.2, 11.6 Hz, $H6_{ax}$), 1.99 (m, *J* = 3.2, 5.7, 13, $H5_{eq}$), 1.83 (m, 1 H, *J* = 3.6, 10.2, 13.1, $H6_{ax}$), 1.34 (s, 3 H, CH_3).

***N*-Benzyl-2-cyano-4-(3-methoxyphenyl)-4-methylpiperidine (9).** A solution of **8** (8.33 g, 0.03 mol) dissolved in methylene chloride (80 mL) was cooled to 10 °C and acidified with 30% aqueous perchloric acid (10 mL). Potassium cyanide (1.95 g, 0.03 mol) in water (10 mL) was added with vigorous stirring. A total of 3 equiv of potassium cyanide was added, alternating with portions of aqueous perchloric acid until the solution was adjusted to pH 6. The final solution was stirred 1 h at room temperature and then basified with cold saturated sodium bicarbonate (100 mL). Additional methylene chloride (200 mL) was added, and the organic layer was separated, dried, and evaporated in vacuo to give 9.35 g (0.029 mol, 97%) of **9** as an oil. Conversion to the HCl salt provided a minor crop (15%) of the *cis* isomer of **9**. The mother liquor (ethanol) contained both the *trans* isomer and starting enamine, **8**: R_f 0.21 cis, 0.12 (trans) (hexane/methylene chloride/ammonia, 3:1:0.04); IR (neat) 2220, 2240 (weak) cm^{-1}; *m/e* 320 (M^+); NMR (300 MHz, $CDCl_3$) δ 7.26, 6.83 (m, 9 H, Ar*H*), 3.92 (d 1 H, *J* = 13.1 Hz, benzyl C*H*), 3.67 (t, major 1 H, *J* = 4.95 Hz, H2), 3.62 (t, minor 1 H, *J* = 4.9 Hz, H2), 3.52 (d, major 1 H, *J* = 13.1 Hz, benzyl C*H*), 3.49 (d, minor 1 H, *J* = 13.1, benzyl C*H*), 2.77 (m, 1 H, *J* = 2.8, 10.2, 12.7, $H6_{ax}$), 2.62 (m, 1 H, $H6_{eq}$), 2.23 (m, 1 H, *J* =5.2, 13.8, $H3_{ax}$), 2.12 (m, 1 H, *J* = 1.9, 4.6, 13.8, $H5_{eq}$), 2.04 (dd, 1 H, *J* = 4.2, 13.4 Hz, $H3_{ax}$), 1.8 (obscured by water peak), 1.45 (s, major 3 H, CH_3), 1.22 (s, minor 3 H, CH_3). Anal. ($C_{21}H_{24}N_2O{\cdot}HCl$) C, H, N.

***N*-Benzyl-4-(3-hydroxyphenyl)-4-methylpipecolic Acid (*trans*- and *cis*-10).** A. **From *cis*-9·HCl.** Nitrile *cis*-**9**·HCl (0.24 g, 0.0006 mol) was dissolved in concentrated hydrochloric acid (40 mL) and refluxed 48 h. After evaporation in vacuo, the residue was dissolved in ethanol (2 mL) and passed over an ion-exchange column (Dowex 50). Residual salts were removed by elution with water (100 mL), and the amino acid was eluted with 2 N ammonium hydroxide (500 mL). Evaporation of the ammonium hydroxide and trituration of the residue with a 3:1 mixture of ethyl acetate/ethanol induced precipitation of the product that was collected by filtration and dried to give *cis*-**10** in a yield of 0.0809 g (38%): IR (crude HCl salt, KBr) 1720 cm^{-1}, (zwitterion, KBr) 1625 cm^{-1}; *m/e* 281 (M^+ - 44). The product was purified further by HPLC. Retention time (t_0 = 3.3 min, 5 mL/min, chloroform/methanol/glacial acetic acid, 95:5:0.5), 18.8 min.

B. From a Mixture of *cis*- and *trans*-9. Concentrated hydrochloric acid (50 mL) cooled to 0 °C was added to **9** (2.2 g, 0.0069 mol), and the solution was refluxed 48 h. After purification, 0.47 g (21%) of zwitterionic phenolic amino acids were obtained. HPLC separation (as above) provided both isomers with retention times of 10.2 min [(±)-*trans*-**10**, major] and 18.8 min [(±)-*cis*-**10**, minor]. A separation of 0.350 g provided 0.141 g of (±)-*trans*-**10** and 0.02 g of (±)-*cis*-**10**.

NMR (300 MHz, CF_3COOD) for *trans*-**10**: δ 7.32 (m, 5 H, benzyl Ar*H*), 7.13, 6.77, 6.6 (m, 4 H, C-4 Ar*H*), 4.62 (d, 1 H, *J* = 12.8 Hz, benzyl C*H*), 4.21 (dd, 1 H, *J* = 2.8, 12.9 Hz, H2), 4.04 (d, 1 H, *J* = 12.8 Hz, benzyl C*H*), 3.65 (m, 1 H, $H6_{eq}$), 3.18 (m, 2 H, $H6_{eq}$ and $H3_{eq}$), 2.64 (d, 1 H, *J* = 16.7 Hz, $H5_{eq}$), 3.18 (m, 2 H, $H6_{eq}$ and $H3_{eq}$), 2.64 (d, 1 H, *J* = 16.7 Hz, $H5_{eq}$), 2.16 (q, 1 H, *J* = 13, 15.1 Hz), 2.06 (br t, 1 H, *J* = 16.7 Hz), 1.349 (s, 3 H, CH_3). Anal. ($C_{20}H_{22}NO_3{\cdot}CH_3COOH$) C, H, N.

NMR (300 MHz, CF_3COOD) for *cis*-**10**: δ 7.57 (m, 5 H, benzyl Ar*H*), 7.32, 6.97, 6.9 (m, 4 H, C-4 Ar*H*), 4.77 (d, 1 H, *J* = 12.9 Hz, benzyl C*H*), 4.42 (dd, 1 H, *J* = 3.5, 12.1 Hz, H2), 4.4 (d, 1 H, *J* = 12.9 Hz, benzyl C*H*), 3.82 (m, 1 H, $H6_{eq}$), 3.53 (m, 1 H, $H6_{ax}$), 2.68 (d, 1 H, *J* = 14.2 Hz, $H3_{eq}$), 2.4 (dd, 1 H, *J* = 12.3, 14.5 Hz, $H3_{ax}$), 2.2 (d, 1 H, 14.8 Hz, $H5_{eq}$), 1.495 (s, 3 H, CH_3). Anal. ($C_{20}H_{22}NO_3{\cdot}0.5H_2O$) C, H, N.

Glycylglycylphenylalanylleucinamide (11). To a solution of *N*-Cbz-glycylglycylphenylalanine (1 g, 0.0024 mol) and *N*-methylmorpholine (0.243 g, 0.0024 mol) dissolved in freshly distilled THF (5 mL) at -20 °C was added isobutyl chloroformate (0.326 g, 0.0024 mol) dissolved in the THF (2 mL). The solution was stirred 10 min at -20 °C. A solution of leucinamide hydrochloride salt (0.403 g, 0.0024 mol) dissolved in water (2 mL) and *N*,*N*-dimethylformamide (3 mL) was added. The final solution was allowed to warm to room temperature and stirred an additional 1 h. The addition of water (50 mL) caused the formation of a precipitate that was collected by filtration, washed sequentially with portions (50 mL) of 1 N hydrochloric acid, water, and saturated sodium bicarbonate, and dried to give 1.26 g (96%) of the protected tetrapeptide: R_f 0.6 (ethanol/ethyl acetate/ammonia, 1:3:0.08), 0.21 (chloroform/methanol, 10:1); NMR (90 MHz, Me_2SO-d_6) δ 8.05 (m, 3 H, peptide bond NH), 7.46 (m, 1 H, urethane NH), 7.33 (s, 5 H, Ar*H*), 7.22 (s, 5 H, Phe Ar*H*), 7.06, 6.96 (s, 2 H, $LeuNH_2$), 5.02 (s, 2 H, $C_6H_5CH_2O$), 4.5 (m, 1 H, α-CH), 4.16 (m, 1 H, α-CH), 3.66 (s, 2 H, Gly C*H*), 3.60 (s 2 H, Gly C*H*), 3.04–2.68 (m, 2 H, Phe β-CH), 1.5 (m, 3 H, Leu C*H*), 0.85 (t, 6 H, Leu CH_3). The product, Cbz-Gly-Gly-Phe-Leu-NH_2, was used without further purification.

A solution of the protected tetrapeptide (1.26 g, 0.0024 mol), 10% Pd/C (0.15 g), and glacial acetic acid (1 mL) in methanol (50 mL) was stirred at room temperature under a hydrogen atmosphere for 8 h. The reaction mixture was filtered through Celite, and the filtrate was washed with methanol. The methanol was evaporated in vacuo, and excess glacial acetic acid was removed by an azeotrope with carbon tetrachloride. Trituration of the residue with ethyl acetate and ethanol induced precipitation of Gly-Gly-Phe-Leu-NH_2, which was filtered and dried to give 0.68 g (63% for two steps) of the acetate salt: R_f 0.09 (ethyl acetate/ethanol/ammonia, 1:3:0.08); $[\alpha]^{25}_D$ -20° (*c* 1, MeOH);

NMR (90 MHz, CD_3OD) δ 8.22, 8.15 (m, 3 H, peptide bond NH), 7.24 (s, 5 H, Phe Ar*H*), 7.16, 7.00 (s, 1 H, $CONH_2$), 4.45 (m, 1 H, α-CH), 4.22 (m, 1 H, α-CH), 3.69 (m, 2 H, Gly^2 CH_2), 3.1 (s, 2 H, Gly^3 CH_2), 3.04–2.6 (m, 2 H, Phe β-CH), 1.84 (s, 3 H, acetate CH_3), 1.54 (m, 3 H, Leu β- and γ-CH), 0.86 (m, 6 H, Leu CH_3). The sample was converted to the hydrochloride salt for the coupling reaction and for elemental analysis. Anal. ($C_{19}H_{29}N_5O_4$·HCl·0.75H_2O) C, H, N.

***N*-Benzyl-4-(3-hydroxyphenyl)-4-methylpipecolylglycylglycylphenylalanylleucinamide Hydrochloride (*trans*- and *cis*-12).** To a mixture of (±)-*trans*-**10**·HCl or (±)-*cis*-**10**·HCl (40 mg, 0.123 mmol), tetrapeptide **11** (57.6 mg, 0.135 mmol), hydroxybenztriazole (18.2 mg, 0.135 mmol), and triethylamine (13.7 mg, 0.135 mmol) dissolved in *N*,*N*-dimethylformamide (1.5 mL) was added dicyclohexylcarbodiimide (27.9 mg, 0.135 mmol), and the final solution was stirred either 24 (trans) or 48 (cis) h. The solvent was evaporated in vacuo, and the residue was passed through a silica gel column (ethyl acetate/ethanol/ammonia, 20:2:0.4). The material obtained from evaporation in vacuo of the column eluant was triturated with aqueous 10% hydrochloric acid/ethanol (10:1, 4 mL), and some residual dicyclohexylurea was filtered. Evaporation in vacuo of solvent gave 49.5 mg (55%) of *cis*-**12**·HCl or 75.2 mg (83%) of *trans*-**12**. Both compounds had the same R_f (0.36, ethyl acetate/ethanol/ammonia, 20:5:0.5).

NMR (300 MHz, CD_3OD) for *trans*-**12**: δ 7.44 (s, 5 H, benzyl Ar*H*), 7.25 (s, 6 H, Phe Ar*H* and C-4 Ar*H*), 6.93 (d, 1 H, J = 7.8 Hz, C-4 Ar*H*), 6.87 (s, 1 H, C-4 Ar*H*), 6.87 (d, 1 H, J = 7.95 Hz, C-4 Ar*H*), 4.61 (m, 1 H, α-CH Phe), 4.46 (d, 1 H J = 12.8, benzyl CH), 4.34 (m, 1 H, α-CH Leu), 3.15 (q, 1 H, J = 14.03, 5.75 Hz, β-CH Phe), 1.73–1.55, (m, 3 H, β- and γ-CH Leu), 1.22, 1.21 (s 3 H, C-4 CH_3), 0.87 (m, 6 H, Leu CH_3). Anal. ($C_{39}H_{50}N_6O_6$·HCl·2H_2O) C, H, N.

NMR (300 MHz, CD_3OD) for *cis*-**12**: δ 7.55, 7.5 (m, 5 H, benzyl Ar*H*), 7.32 (s, 5 H, Phe Ar*H*), 7.2 (m, 1 H, C-4 Ar*H*), 6.78 (m, 2 H, C-4 Ar*H*), 6.65 (d, 1 H, J = 8 Hz, C-4 Ar*H*), 4.64 (m, 1 H, α-CH Phe), 2.4 (d, 1 H, J = 13.9 Hz, $H3_{eq}$), 1.57 (m, 3 H, β- and γ-CH Leu), 1.44 (s, 3 H, C-4 CH_3), 0.86 (m, 6 H, Leu CH_3). Anal. ($C_{39}H_{50}N_6O_6$·HCl·1.5H_2O) C, H, N.

4-(3-Hydroxyphenyl)-4-methylpipecolylglycylglycylphenylalanylleucinamide Hydrochloride (5). A mixture of *trans*-**12**·HCl (58 mg, 0.79 mmol) and 10% Pd/C (15 mg) in methanol (5 mL) was shaken overnight at room temperature under a hydrogen atmosphere. The reaction mixture was filtered through Celite and the solvent evaporated in vacuo to give 36.6 mg (0.057 mmol, 72%) of the deprotected pentapeptide **5**·HCl: R_f 0.08 (ethyl acetate/ethanol/ammonia, 20:5:0.5); NMR (*trans*-**5**, 300 MHz, CD_3COOD) δ 7.26 (s 6 H, Phe Ar*H* and C-4 Ar*H*), 6.98 (d, 1 H, J = 7.8 Hz, C-4 Ar*H*), 6.91 (s, 1 h, C-4 Ar*H*), 6.73 (q, 1 H, J = 1.9, 7.97 Hz, C-4 Ar*H*), 4.83 (t, J = 7.2, α-CH Phe), 4.56, 4.54 (t, 1 H, J = 6.5, 6.8, α-CH Leu), 4.17 (d, $^1/_2$ H, J = 17 Hz), 4.16 (d, $^1/_2$ H, J = 17 Hz), 4.10 (s, 1 H), 4.03 (s, 1 H), 3.96 (d, $^1/_2$ H, J = 17 Hz), 3.95 (d, $^1/_2$ H, J = 17 Hz) (all Gly^2 and Gly^3 protons), 4.06 (buried, H2), 3.56 (br d, 1 H, J = 15.1 Hz, $H6_{eq}$), 3.1 (m, 3 H, $H3_{ax}$ and β-CH Phe), 2.84 (br d, 1 H, J = 13 Hz, $H3_{eq}$), 2.56 (br d, 1 H, J = 1.51 Hz), 2.09 (partially buried, $H3_{ax}$), 1.98 (partially buried, $H5_{ax}$), 1.6 (m, 3 H, β- and γ-CH Leu), 1.28 (s, 3 H, C-4 CH_3), 0.87 (m, 6 H, Leu CH_3). Anal. ($C_{32}H_{44}N_6O_6$·HCl·2H_2O) C, H, N.

Intermediate *cis*-**12** (40 mg, 0.054 mmol) was converted in a similar manner to cis pentapeptide **5** (26.5 mg, 76%): R_f 0.08 (ethyl acetate/ethanol/ammonia, 20:5:0.5); NMR (*cis*-**5**, 300 MHz, CD_3COOD) δ 7.26 (s, 5 H, Phe Ar*H*), 7.19 (t, 1 H, J = 8.02, C-4 Ar*H*), 6.85 (m, 2 H, C-4 Ar*H*), 6.67 (q, 1 H, J = 1.94, 8.01, C-4 Ar*H*), 4.85 (t, 1 H, J = 7 Hz, α-CH Phe), 4.78 (buried, 1 H, H2), 4.56 (t, J = 7.5 Hz, α-CH Leu), 4.29 (s, 2 H, Gly), 4.09 (dd, 2 H, J = 17 Hz, Gly), 3.6 (br m, 2 H, $H6_{ax}$ and $H6_{eq}$), 3.1 (m, 2 H, β-CH Phe), 2.65 (br d, 1 H, J = 13.2, $H3_{eq}$), 2.2 (dd, 1 H, J = 13.2, 15.04 Hz, $H3_{ax}$), 2.07 (m, 2 H, $H5_{ax}$ and $H5_{eq}$), 1.6 (m,3 H, β- and γ-CH Leu), 1.48 (s, 3 H, C-4 CH_3), 0.9 (m, 6 H, Leu CH_3). Anal. ($C_{32}H_{44}N_6O_6$·2HCl·$C_2H_4O_2$) C, H, N.

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