Table II. Chiral Modification of the Dolastatin 10 Amino Acid Dil Carbon 19a

no.	19a*R*-Dil sequence product	recryst solvent	mp, °C	$[\alpha]^{28}_D$, deg (*c*, $CHCl_3$)	yield, %
20[a]	*N*-Z-(*S*,*R*)-Ile		oil	−12 (4.9)	84
21[b]	*N*-Z-*N*-Me-(*S*,*R*)-Ile	ether	85–87	−64 (4.9)	92
22[c]	*N*-Z-*N*-Me-(*S*,*R*)-isoleucinol		oil	−1 (5.3)	93
23[d]	*N*-Z-*N*-Me-(*S*,*R*)-isoleucinal		oil	−63 (2.4)	80
24[e]	*tert*-butyl 3-hydroxy-4(*S*)-(*N*-Z-*N*-Me)-5(*R*)-methylheptanoate		oil	−37 (3.7)	76 (*R* and *S* isomers)
25[f]	*N*-Z-(3*R*,4*S*,5*R*)-dolaisoleucine-OBu^t		oil	−15 (2.8)	91
26[g]	(5*R*)-dolaisoleucine-OBu^t·HCl	ether	178–80	−2 (5.5)	79
27[h]	*N*-Z-*S*-Val-(3*S*,4*S*,5*R*)-Dil-OBu^t		glass	−27 (3.7)	69
28[i]	*S*-Dov-*S*-Val-(3*S*,4*S*,5*R*)-Dil-OBu^t		glass	−55 (2.2)	72
29[j,k]	*N*-Boc-(2*S*,2′*R*,3′*R*)-Dap-(*S*)-Doe	acetone–hexane	120–21	−68 (1.5)	69[l]

[a] *S*,*R*-Ile, benzyl chloroformate, 1.5 N NaOH. [b] NaH, CH_3I, THF. [c] BH_3·THF, THF. [d] SO_3–pyridine, DMSO, TEA. [e] *tert*-Butyl acetate, LDA, −78 °C. [f] $(CH_3O)_3OBF_4$, Proton Sponge, CH_2Cl_2. [g] Pd/C, H_2, Et_2O–HCl. [h] N-*Z*-(*S*)-Val, $(CH_3)_3CCOCl$, NMM, $CHCl_3$. [i] (*S*)-Dov-Opfp, H_2, Pd/C, dioxane. [j] TFA, CH_2Cl_2. [k] **28**·TFA + **29**·TFA, DEPC, TEA, DME, **2** (90%). [l] Coupling of *N*-Boc-(2*S*,2′*R*,3′*R*)-Dap, Doe·TFA, DEPC, TEA, DME.

(cf. **7**) this substance was 100-fold less active compared to the 18*R*,19*S* (cf. **2**).

For isodolastatins so far studied, modifications in the C-18 and C-19 (followed by C-10) stereochemistry results in profound effects on cytostatic activity. Conversion from the *S*-amino acid series to the *R* series at one to five chiral centers and substitution of amino acid units in the natural dolastatin 10 sequence are currently under study.

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Articles

Flexible *N*-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine Analogues: Synthesis and Monoamine Oxidase Catalyzed Bioactivation

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Eighteen analogues of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were synthesized and evaluated as substrates of monoamine oxidase. In general, the flexible analogues, characterized by the presence of a methylene (or ethylene) bridge between the aryl/heteroaryl and tetrahydropyridyl moieties, were better substrates of the enzyme than the conformationally restricted MPTP. It is suggested that the increased oxidative activity of these flexible analogues reflects enhanced binding due to the ability of the C-4-aryl/heteroaryl substituent to gain access to a hydrophobic pocket within the substrate binding site.

Introduction

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine, MPTP, is oxidized in vivo by monoamine oxidase (MAO; EC 1.4.3.4) to yield the cationic metabolite *N*-methyl-4-phenylpyridinium, MPP^+.[1-3] The latter selectively accumulates within catecholaminergic neurons via the catecholamine reuptake system[4-6] and subsequently induces a Parkinsonian syndrome in humans and nonhuman primates.[7-9] The discovery that MAO can convert relatively nontoxic molecules into potent neurotoxins has generated interest in the role of this enzyme in the bioactivation of potential neurotoxins present in the environment.

During the last few years, several MPTP analogues have been synthesized and tested for oxidative activity in MAO

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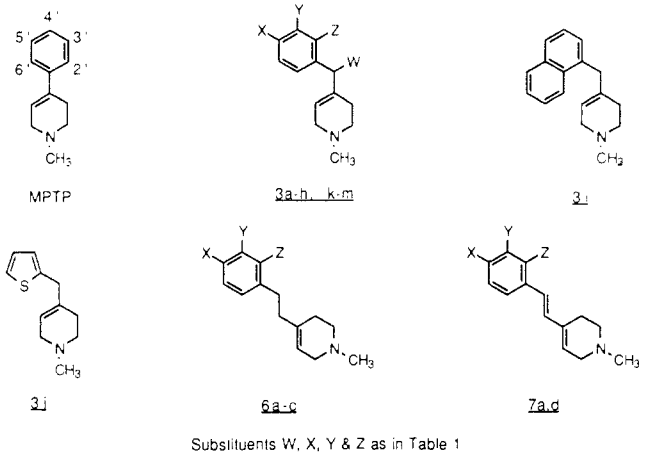

Figure 1. MPTP and its Analogues.

assays and for dopaminergic neurotoxicity in vitro and/or in vivo.[10-20] These studies have been useful both in identifying potent neurotoxins such as 2'-methyl-MPTP and in defining the optimum substrate requirements for MAO types A and B. However, the vast majority of MPTP analogues reported are semirigid, i.e., the tetrahydropyridine is directly bonded to an alicyclic or heterocyclic substitutent at C-4, thus providing little conformational mobility. Although such conformationally restricted analogues may be useful in defining optimum structural parameters for substrate binding, the combination of rigid and flexible analogues may provide a more complete picture. One notable flexible MPTP analogue is *N*-methyl-4-benzyl-1,2,3,6-tetrahydropyridine (4-homo-MPTP). This compound is a much better substrate for MAO than MPTP.[11,20] However, when tested in mice, 4-homo-MPTP failed to exhibit central dopaminergic toxicity,[11] suggesting that MAO-catalyzed bioactivation was necessary but not sufficient for dopaminergic toxicity. On the basis of these findings, we have used 4-homo-MPTP as the lead compound in our studies of potential MPTP-like substrates of MAO.

This paper describes the synthesis and monoamine oxidase catalyzed oxidation of a number flexible MPTP analogues and offers some insights into the substrate requirements of the enzyme active site.

Experimental Section

Materials. Synthetic intermediates were purchased from Aldrich, Inc. (Milwaukee, WI) and were used as received. Tetrahydrofuran (THF) was distilled from CaH_2 immediately prior to use. All other reagents and solvents were reagent grade and used without subsequent purification.

General. All air-sensitive reactions were carried out under nitrogen. Standard handling techniques for air-sensitive materials were employed throughout this study. Yields are not optimized.

Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. UV–vis absorbencies were recorded on a Beckman DU Series 70 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on an IBM-Brucker spectrometer at 200 and 50 MHz, respectively. Both types of spectra are referenced to the deuterium lock frequency of the spectrometer. With this condition, the chemical shifts (in ppm) of residual solvent in the ^1H and ^{13}C NMR were found to be, respectively: $CHCl_3$, 7.26, 77.08; DMSO, 2.25, 39.48; HOD, 4.81, not applicable. The following abbreviations are used to describe peak patterns when appropriate: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Both low- and high-resolution MS were performed on an AEI MS-30 instrument. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Column chromatography was performed with "Baker Analyzed" silica gel (60–200 mesh). Preparative chromatography was performed on a Harrison Research Chromatotron using Merck 60 PF_{254} silica gel. Analytical TLC was performed on Analtech glass TLC plates coated with silica gel GHLF and were visualized with UV light and/or methanolic iodine.

Comparison of molecules was accomplished by least-squares fitting with the software package PCMODEL (Serena Software).

Chemistry. 4-(α-Hydroxybenzyl)pyridine (1a). Sodium borohydride (4.73 g, 125 mmol) was added in portions to a solution of 4-benzoylpyridine (18.32 g, 100 mmol) in MeOH (250 mL) under cooling in an ice bath. The mixture was stirred for 3.5 h and the solvent subsequently removed under reduced pressure. The residue was taken up in H_2O (100 mL) and extracted with EtOAc (4 × 200 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was triturated with hexane and filtered. The filter cake was washed with hexane then dried in vacuo to give 17.4 g (94%) of a white, crystalline solid (mp 103–105 °C). ^1H NMR ($CDCl_3$) δ 5.55 (b s, 1 H), 5.71 (s, 1 H), 7.29 (m, 7 H), 8.26 (dd, 2 H, J = 1.55 Hz, J = 4.57 Hz).

General Procedure for the Synthesis of Phenyl Pyridyl Alcohols (1). 4-(α-Hydroxy-4-methylbenzyl)pyridine (1c). 4-Bromotoluene (4.1 mL, 33 mmol) was added to a suspension of dry magnesium turnings (0.80 g, 33 mmol) in dry THF (20 mL). The mixture was refluxed for 2 h and an additional amount of dry THF (30 mL) was added. A solution of 4-pyridinecarboxaldehyde (2.83 mL, 30 mmol) in dry THF (50 mL) was then added to this solution dropwise with vigorous stirring over 1 h. The mixture was refluxed for 16 h and the reaction quenched with saturated NH_4Cl (20 mL) and H_2O (100 mL). The pH was adjusted to 1 with 6 N HCl and the solution extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were discarded, and the pH of the aqueous layer was adjusted to 9 with 10% NaOH and extracted with EtOAc (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give a light yellow solid. This was recrystallized from CH_3CN to give 2.25 g (38%) of pale yellow prisms (mp 131–133 °C): ^1H NMR (DMSO-d_6) δ 2.25 (s, 3 H), 5.67 (d, 1 H, J = 4.00 Hz), 6.14 (d, 1 H, J = 4.12 Hz), 7.12 (d, 2 H, J = 8.00 Hz), 7.26 (d, 2 H, J = 8.04 Hz), 7.37 (d, 2 H, J = 6.02 Hz), 8.47 (dd, 2 H, J = 1.53 Hz, J = 4.57 Hz); MS (70 eV) m/e (intensity) 199.1 (M^+, 100.0).

General Procedure for the Synthesis of Benzylpyridines (2). 4-(4-Methylbenzyl)pyridine (2c). Zinc (6.54 g, 100 mmol) was added to a solution of **1c** (1.99 g, 10 mmol) in formic acid (30 mL). The mixture was vigorously stirred under reflux for 16 h and then filtered. The filter cake was washed with formic acid

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and the filtrate concentrated under reduced pressure. The residue was taken up in H_2O (100 mL) and the pH adjusted to 9 with saturated Na_2CO_3. The solution was extracted with EtOAc (3 × 100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 1.88 g (quantitative) of a pale yellow oil: ^1H NMR ($CDCl_3$) δ 2.32 (s, 3 H), 3.90 (s, 2 H), 7.09 (m, 6 H), 8.48 (dd, 2 H, J = 1.54 Hz, J = 4.45 Hz); MS (70 eV) m/e (intensity) 183.1 (M^+, 100.0).

4-[α-Phenyl-α-(4-bromophenoxy)methyl]pyridine (2k). Diethyl diazenedicarboxylate (11.8 mL, 75 mmol) was added to a solution of 4-bromophenol (17.30 g, 100 mmol), triphenylphosphine (19.67 g, 75 mmol), and 1-phenyl-1-(4-pyridyl)methanol (9.26 g, 50 mmol) in Et_2O (500 mL) cooled in an ice bath. The mixture was stirred for 24 h, the triphenylphosphine oxide filtered off, and the solution concentrated under reduced pressure. The residue was placed on a 4 × 45 cm SiO_2 column and eluted with 4:1 hexane/acetone. The band containing the product was concentrated under reduced pressure to give 8.83 g (52%) of a yellow oil: ^1H NMR ($CDCl_3$) δ 6.10 (s, 1 H), 6.80 (d, 2 H, J = 8.99 Hz), 7.33 (m, 9 H), 8.56 (dd, 2 H, J = 1.57 Hz, J = 4.54 Hz); HRMS (30 eV) m/e (intensity) 339.0285 ($M^+ - 1$, 0.7).

4-(4-Iodobenzyl)pyridine (21). Chlorotrimethylsilane (1.1 mL, 8.4 mmol) was added to a stirred solution containing sodium iodide (1.68 g, 11.3 mmol) and triazine **2o** (1.5 g, 5.63 mmol) in dry acetonitrile (25 mL). The resulting mixture was heated to 50–60 °C under N_2 for 3.5 h, allowed to cool to room temperature, and treated with saturated $NaHCO_3$ (30 mL). The mixture was then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated to a residue which was purified by radial-flow chromatography (80 g of silica gel, 2.5–5% MeOH–CH_2Cl_2). The relevant fractions were collected, pooled, and concentrated to give 0.8 g (48%) of a dark brown residue which solidified on standing. This material was recrystallized from diisopropyl ether–hexane to give a yellow solid (mp 73.5–75.0 °C): ^1H NMR ($CDCl_3$) δ 3.65 (s, 2 H), 6.86 (d, 2 H, J = 8 Hz), 7.01 (d, 2 H, J = 8 Hz), 7.58 (d, 2 H, J = 8 Hz), 8.46 (2, 2 H, J = 8 Hz); CIMS (NH_3) m/e (intensity) 294.8 (M^+, 88.4).

4-(4-Hydroxybenzyl)pyridine (2m). A solution containing 4-(4-methoxybenzyl)pyridine (8.59 g, 43.3 mmol), glacial acetic acid (25 mL), and 48% HBr (25 mL) was refluxed for 6 h. At the end of this period, the solution was cooled and concentrated under reduced pressure to a solid residue which was taken up in cold, saturated $NaHCO_3$ to yield a solution of pH 8. The solution was stirred and filtered to yield a precipitate which was washed with a minimum volume of H_2O followed by ethyl acetate (3 × 10 mL). The filter cake was dried in vacuo at 45 °C to give 6.0 g (72%) of brown crystals (mp 176.3 °C): ^1H NMR δ 3.83 (s, 2 H), 6.74 (d, 2 H, J = 8.46 Hz), 7.04 (d, 2 H, J = 8.49 Hz), 7.19 (d, 2 H, J = 5.90 Hz), 8.45 (d, 2 H, J = 5.97 Hz), 9.47 (b s, 1 H); CIMS (NH_3) 185.9 (M^+, 100.0).

4-(4-Aminobenzyl)pyridine (2n). Concentrated HCl (20 mL) was added to a mixture of 4-(4-nitrobenzyl)pyridine (4.0 g, 18.6 mmol) and tin (3.52 g, 29.7 mmol) slowly with occasional cooling to avoid overheating. The solution was then heated in a boiling-water bath for 40–45 min, cooled down to room temperature, and neutralized by the addition of 15% NaOH. The resulting emulsion was extracted with ethyl acetate (3 × 80 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was crystallized from ethyl acetate to give 3 g (85%) of white plates (mp 164.2 °C): ^1H NMR (DMSO-d_6) δ 3.50 (s, 2 H), 4.97 (s, 2 H), 6.51 (d, 2 H, J = 8.13 Hz), 6.87 (d, 2 H, J = 8.15 Hz), 7.17 (d, 2 H, J = 4.34 Hz), 8.42 (d, 2 H, J = 4.30 Hz); HRMS (70 eV) m/e (intensity) 184.0997 (M^+, 88.2).

1-Pyrrolidinyl[4-(4-pyridylmethyl)phenyl]diazene (2o). The triazene was synthesized by following the method of Burns et al. Trifluoroacetic acid (1.9 mL, 24.4 mmol) was added to a stirring suspension of aniline **2n** (0.9 g, 4.9 mmol) in water (8 mL). The resulting solution was cooled in an ice bath to 0 °C. At this time, a solution of sodium nitrite (0.40 g, 5.9 mmol) in water (2 mL) was added dropwise. During the addition, the temperature of the reaction mixture was maintained below 5 °C. At the end of the addition, the solution was stirred for an additional 5 min and then carefully added to a precooled, stirred solution of pyrrolidine (0.5 mL, 24.4 mmol) in 10 N KOH (2.5 mL). During this addition, the temperature was kept below 5 °C. An off-white precipitate formed during the addition. After the addition, the reaction mixture was stirred for an additional 15 min and filtered. The product was recrystallized from ethanol–water to yield 0.94 g (72%) of light brown solid (mp 82–83 °C): ^1H NMR ($CDCl_3$) δ 1.98 (m, 4 H), 3.76 (b s, 4 H), 3.92 (s, 2 H), 7.09 (m, 4 H), 7.35 (d, 2 H), 8.46 (d, 2 H, J = 8 Hz); CIMS (NH_3) 267.1 (M^+, 100.00).

General Procedure for the Synthesis of Tetrahydropyridines. 1-Methyl-4-benzyl-1,2,3,6-tetrahydropyridine (3a). Methyl iodide (3.95 mL, 63 mmol) was added to a solution of 4-benzylpyridine (5.0 mL, 31.3 mmol) in acetone (50 mL). The solution was stirred at room temperature for 16 h and the solvent subsequently removed under reduced pressure. The residue was taken up in MeOH (50 mL), and $NaBH_4$ (4.73 g, 125 mmol) was added in portions. The solution was stirred at room temperature for 16 h and the solvent subsequently removed under reduced pressure. The residue was taken up in H_2O (100 mL) and the solution extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The oily residue was triturated with Et_2O (50 mL) and the insoluble material filtered off. The solvent was removed under reduced pressure and the free base distilled to yield 3.2 g (46%) of a colorless liquid. This was converted to the hydrochloride in cold methanol and crystallized from 2-propanol–ether (mp 149–151 °C): ^1H NMR ($CDCl_3$) δ 2.04 (b s, 2 H), 2.31 (s, 3 H), 2.46 (m, 2), 2.90 (b s, 2 H), 3.28 (s, 2 H), 5.38 (b s, 1 H), 7.14–7.30 (m, 5 H).

General Procedure for the Synthesis of Substituted Stilbazoles (4). (*E*)-4-(2-Phenylethenyl)pyridine (4a). Following the general method of Baker and Gibson,[21] acetic anhydride (50 mL) was added to a mixture of benzaldehyde (5.6 mL, 55 mmol) and 4-picoline (4.9 mL, 50 mmol). The mixture was refluxed for 24 h and the solution concentrated under reduced pressure. The residue was poured into an ice/water slush (100 g) and the pH adjusted to 1 with 6 N HCl. The solution was extracted with CH_2Cl_2 (5 × 100 mL). The organic layers were discarded, and the pH of the aqueous layer was adjusted to 7 with 10% NaOH. The solution was extracted with CH_2Cl_2 (5 × 100 mL), and the combined organic layers were passed through SiO_2. The eluate was concentrated under reduced pressure to give 4.45 g (49%) of yellowish crystals. An analytical sample was prepared by sublimation (120 °C/0.85 Torr) to give a white solid (mp 128.2–129.8 °C): ^1H NMR ($CDCl_3$) δ 7.01 (d, 1 H, J = 16.33 Hz), 7.30 (d, 1 H, J = 16.33 Hz), 7.35 (m, 5 H), 7.54 (d, 2 H, J = 6.38 Hz) 8.58 (d, 2 H, J = 6.00 Hz); MS (70 eV) m/e (intensity) 180.7 (M^+, 77.1).

General Procedure for the Synthesis of Phenethylpyridines (5). 4-(2-Phenylethyl)pyridine (5a). Palladium on carbon (50 mg) was added to a solution of *trans*-stilbazole (2.01 g, 10 mmol) in absolute EtOH (100 mL) in a hydrogenation bottle. The flask was attached to the hydrogenator and evacuated with an aspirator. Hydrogen was then allowed into the bomb. This process was repeated three times. The solution was shaken under 2–3 atm of hydrogen pressure for 21 h. The solution was filtered through diatomaceous earth and the filtrate concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 and passed through SiO_2 eluting with CH_2Cl_2. The filtrate was concentrated under reduced pressure to give 1.45 g (71%) of a yellow oil which crystallized under vacuum: ^1H NMR ($CDCl_3$) δ 2.93 (b s, 4 H), 7.20 (m, 7 H), 8.49 (dd, 2 H, J = 1.57 Hz, J = 4.53 Hz); MS (70 eV) m/e (intensity) 182.9 (M^+, 22.8).

Biological. Mitochondrial Preparation. Rat brain mitochondria were obtained by the method of Vyas et al.,[26] with the modifications of Clark and Nicklas.[22] Eight rats (Wistar, Harlan), weighing between 180 and 250 g, were stunned and decapitated.

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The brains were rapidly removed and suspended in 40 mL of cold isolation medium (225 mM mannitol/75 mM sucrose/1.0 mM EDTA/5 mM 3-*N*-morpholinopropanesulfonic acid (MOPS–KOH), pH 7.4). All subsequent steps were carried out on ice or at 2–4 °C. The brains were finely chopped with scissors and then homogenized in a Potter–Elvehjem homogenizer by four up and down strokes with a Teflon pestle attached to a drill. The homogenate was transferred to a centrifuge tube, and the homogenizer and pestle were rinsed with an additional 20 mL of cold isolation medium which was added to the homogenate. The suspension was centrifuged for 3 min at 2000*g* and the resultant supernatant was subsequently centrifuged for 8 min at 12500*g*. The pellet was resuspended in 3-*N*-morpholinopropanesulfonic acid (MOPS) buffer (3% Ficol/0.12 M mannitol/0.03 M sucrose/25 μM K-EDTA/5 mM MOPS–KOH, pH 7.4) and diluted to a final volume of 10 mL with an additional amount of buffer. This was layered onto 20 mL of 6% Ficoll in MOPS buffer (6% Ficoll/0.24 M mannitol/0.06 M sucrose/50 μM K–EDTA/5 mM MOPS–KOH, pH 7.4) and the resulting suspension centrifuged for 30 min at 11500*g*. The supernatant was decanted, the pellet resuspended in 40 mL of isolation medium, and the suspension subsequently centrifuged for 10 min at 12500*g*. Finally, the pellet was resuspended in 100 mL of lysing buffer (0.05 M Na_2HPO_4/0.05 M NaH_2PO_4/0.31 mM sodium azide, pH 7.4). Aliquots taken from this suspension were frozen at −70 °C and stored for later use. Protein content was determined by a commercial dye-binding assay (Bio-Rad).

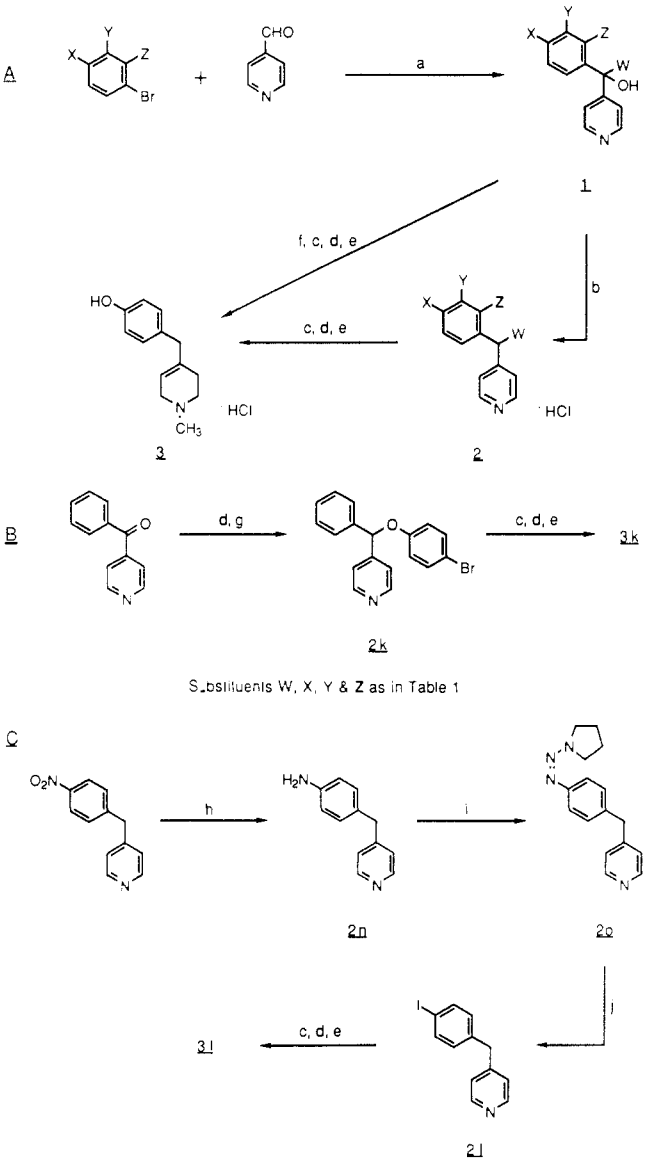
MAO Assay. The monoamine oxidase assay was performed by the method of Szutowicz et al.,[25] with minor modifications. Rat brain mitochondrial preparations were diluted with additional lysing buffer to a concentration of 400–800 μg of protein/mL. An aliquot of 375 μL of the diluted mitochondrial preparation was added to each reaction tube and blank. A volume of 75 μL of a 10 mM solution of the substrate in lysing buffer was then added to each reaction tube (75 μL of lysing buffer was added to the blanks). Pargyline hydrochloride in lysing buffer was added to appropriate tubes to obtain final concentrations of 2.5 μM, 0.5 μM, and 0.1 μM and to appropriate blanks. Additional lysing buffer was added to obtain a final reaction volume of 750 μL. Reaction mixtures were prepared in duplicate while on ice, and the substrate was added last. The reaction tubes were then vortexed briefly and incubated at 37 °C for 30 min. To each tube, 0.375 mL of ABTS–peroxidase reagent (0.05 M phosphate–citrate buffer/1.8 mM 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) with 10 units of horseradish peroxidase per milliliter of ABTS reagent were added immediately before use) was added. After 15 s, 0.375 mL of SDS–HCl (0.75 M HCl with 5% sodium dodecyl sulfate) was added, the tubes were vortexed briefly, and the absorbance was read at 414 nm. Standard curves were prepared daily with urea hydrogen peroxide, a stable crystalline form of H_2O_2, to yield final H_2O_2 amounts of 2.5–20 nmol per incubation. Specific activity is expressed as nanomoles of H_2O_2 produced per minute per microgram of protein, as determined from the standard curve and Bio-Rad protein determination.

Kinetic constants were determined by running the assay at several substrate concentrations. Seven substrate concentrations (0.0125, 0.025, 0.05, 0.10, 0.25, 0.5, and 1 mM), the same as those used by Sonsalla et al.,[24] were generally employed to determine the kinetic constants K_m and V_{max}. For poor substrates of MAO, two additional substrate concentrations (2 and 4 mM) were included to improve the quality of the data. Each compound was evaluated three times on three separate days, and each experiment was run in duplicate. The kinetic constants are reported as the mean of six values ± the standard deviation. In all cases, the kinetic constants, K_m and V_{max}, were determined by the Lineweaver–Burk double-reciprocal plot.[23] The pargyline inhibition data were obtained from two experiments, each of which was run in duplicate.

Chemistry

The target compounds (Table II) were synthesized as shown in Scheme I. With few exceptions, 4-pyridinecarboxaldehyde was reacted with a Grignard or organolithium reagent to yield the corresponding secondary alcohol in good to excellent yield. Zinc-mediated reduction

Scheme I.[a] Synthesis of MPTP Analogues



[a] (a) Mg, THF, Δ, pyridine-4-carboxaldehyde or 4-acetylpyridine; (b) Zn, HCO_2H, Δ; (c) MeI, acetone; (d) $NaBH_4$, MeOH, 0 °C to room temperature; (e) HCl; (g), MeOH, 0 °C; (f) HBr, HOAc, Δ; (g) DEAD, Ph_3P, *p*-bromophenol; (h) Sn, HCl, Δ; (i) $NaNO_2$, CF_3CO_2H, 0 °C then pyrrolidine, NaOH, 0 °C; (j) Me_3SiCl, NaI, acetonitrile, Δ.

of the benzylic alcohols yielded the corresponding 4-arylalkylpyridines **2** in good yields. N-methylation and subsequent reduction of the pyridinium salt with sodium borohydride yielded tetrahydropyridines **3**. These were obtained as the crystalline hydrochlorides in most cases, but others were simply collected as precipitates from diethyl ether. Compound **2k** was obtained from the Mitsunobu reaction of 4-bromophenol and 4-(α-hydroxybenzyl)pyridine in 52% yield (Scheme I, part B). On the other hand, 4-(4-nitrobenzyl)pyridine was converted to **2l** in 12% overall yield as shown on Scheme I, part C. The *trans*-stilbazoles **4a–d**, obtained by the method of Baker et al.,[21] were reduced to the corresponding 4-phenethylpyridines **5** (Scheme II). The latter were subsequently converted to the corresponding tetrahydropyridines in good to moderate yields. The trans stereochemistry was assigned to **7a** and **7d** on the basis of the large coupling constant (16 Hz) observed for the two exocyclic vinyl protons. In most cases, conversion of the chromatographically pure tetrahydropyridines to their correspond-

Table I. Oxidative Activity of MPTP Analogues in Rat Brain Mitochondrial MAO Assay

compd	W	X	Y	Z	$V_{max} \times 10^5$ [a]	K_m [b]	$V_{max} \times 10^5/K_m$
benzylamine					336 ± 69.76	168.83 ± 65.62	2.14 ± 0.60
MPTP	H	H	H	H	270.33 ± 84.57	134.0 ± 76.44	2.20 ± 0.47
3a	H	H	H	H	398.0 ± 141.29	44.37 ± 18.94	9.36 ± 1.68
3b	H	Cl	H	H	239 ± 75.72	49.40 ± 15.29	5.15 ± 2.05
3c	H	CH_3	H	H	81 ± 24.88	15.90 ± 1.61	5.17 ± 1.77
3d	H	OCH_3	H	H	180 ± 50.0	24.8 ± 5.8	7.87 ± 3.96
3e	H	H	CH_3	H	455.33 ± 89.46	77.10 ± 45.14	6.95 ± 2.87
3f	H	H	H	CH_3	219.33 ± 77.68	58.83 ± 19.19	3.71 ± 0.10
3g	H	H	H	OCH_3	49.7 ± 17.54	32.17 ± 13.82	1.79 ± 0.92
3h	CH_3	H	H	H	41.87 ± 9.28	70.23 ± 81.31	1.26 ± 0.90
3i					382.33 ± 92.42	76.53 ± 5.74	5.03 ± 1.31
3j					381.33 ± 94.52	42.17 ± 13.04	9.86 ± 4.16
3k	*p*-bromophenoxy	H	H	H	31.37 ± 7.74	57.20 ± 47.96	0.74 ± 0.35
3l	H	I	H	H	62.9 ± 32.13	36.1 ± 17.98	2.38 ± 2.28
3m		OH	H	H	98.5 ± 22.95	363.0 ± 206.13	0.38 ± 0.32
6a		H	H	H	385.33 ± 64.75	119.27 ± 60.53	3.74 ± 1.64
6b		H	Cl	H	64.77 ± 3.52	20.79 ± 14.23	4.44 ± 3.12
6c		Cl	Cl	H	ND	ND[c]	ND
7a		H	H	H	ND	ND[c]	ND
7d		H	Br	H	26.9 ± 14.63	187.33 ± 203.37	0.565 ± 0.68

[a] nmol of H_2O_2/min per μg of protein. [b] μM. [c] Kinetic constants not determined due to low substrate activity.

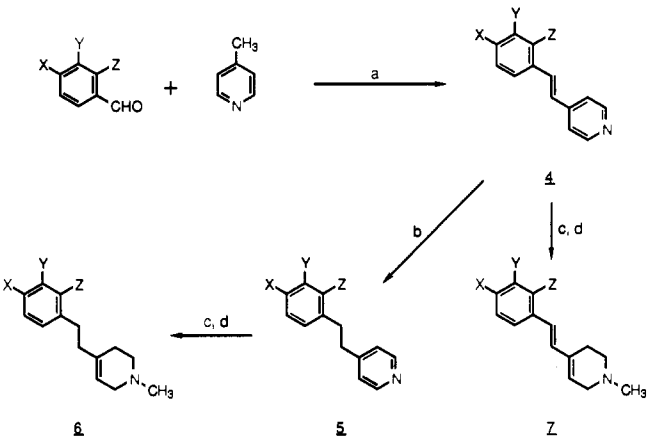
Table II. Physical Properties, Yields, and Elemental Analyses

no.	method	% yield	mp/bp, °C	formula	anal.
1a	A	94	103–105		
1c	A	38	131–133[c]		
1d	A	58	135–137[d]		
1e	A	76	136–138[e]		
1f	A	72	133–135[c]		
1g	A	46	152–154[d]		
1h	A	53	129–131[c]		
1i	A	70	160–163[i]		
1j	A	31	104–106[e]		
2c	B	quant[a]	*b*		
2d	B	86	112–131 (0.55 Torr)		
2e	B	91	*b*		
2f		50	*b*		
2g		80	120–140 (1.5 Torr)		
2h		98	*b*		
2i		97	73–75[f]		
2j		25	*b*		
3a	C	46	149–151[g]		
3b	C	37	184–186[h]	$C_{13}H_{17}Cl_2N$	C, H, N
3c	C	93	194.3–195.5[g]	$C_{14}H_{20}ClN$	C, H, N
3d	C	84	159–161[h]	$C_{14}H_{20}ClNO$	C, H, N
3e	C	70	168.8–170[i]	$C_{14}H_{20}ClN$	H, N, C[m]
3f	C	42	166.4–167.8[g]	$C_{14}H_{20}ClN$	C, H, N
3g	C	69	122–125[g]	$C_{14}H_{20}ClNO$	C, H, N
3h	C	quant	67–69	$C_{14}H_{20}ClN$	H, N, C[n]
3i	C	30	204–205[h]	$C_{17}H_{20}ClN$	C, H, N
3j	C	41	138–139[g]	$C_{11}H_{16}ClNS$	C, H, N
3k	C	49	*b*	$C_{19}H_{21}BrClNO$	
3l	C	41	208–211[h]	$C_{13}H_{17}ClIN$	C, H, I, N
3m	C	81	121–122[j]	$C_{13}H_{17}NO$	H, N, C[o]
6a	C	quant	161–163[g]	$C_{14}H_{20}ClN$	
6b	C	69	134–136[g]		
6c	C	73	*b*		
7a	C	42	276–278[h]	$C_{14}H_{16}Cl_3N$	C, H, N
7d	C	60	239–242[j]	$C_{14}H_{17}BrClN$	C, H, N
4a	D	49	128.2–129.8[k]		
4b	D	64			
4c	D	62			
4d	D	42			
5a	E	71			
5b	E	80	100 (0.45 Torr)		
5c	E	85	125 (0.65 Torr)		

[a] Quantitative. [b] Viscous oil. [c] Acetonitrile. [d] Methylene chloride. [e] Ethyl acetate–hexane. [f] Diethyl ether–hexane. [g] 2-Propanol–diethyl ether. [h] 2-Propanol. [i] Diethyl ether. [j] Acetone. [k] Obtained by sublimation. [l] Ethyl acetate. [m] C: calcd, 70.72; found 69.72. [n] C: calcd, 70.72; found, 67.01. [o] C: calcd, 76.81; found, 76.03.

ing hydrochlorides yielded crystalline products. However, a few of these hydrochlorides (**3h**, **3k**, **6c**) could not be crystallized, and poor elemental analyses were obtained for them.

Scheme II.[a] Synthesis of MPTP Analogues



[a] (a) Ac_2O; (b) H_2/Pd/C; (c) MeI, acetone; (d) $NaBH_4$, MeOH, 0 °C to room temperature. Substituents W, X, Y, and Z as in Table I.

Table III. Pargyline-Induced Inhibition of MAO-Catalyzed Oxidation of MPTP Analogues

compd	specific activity[a] control	specific activity[a] with inhibitor[b]	% inhibn
benzylamine	16.68	2.20	87
MPTP	16.58	0	100
3a	31.40	3.71	88
3b	18.87	10.30	45
3e	30.65	9.49	69
3f	19.20	9.24	52
3i	28.60	26.37	8
3j	33.85	10.94	68
6a	17.95	8.22	54

[a] nmol of H_2O_2/min per μg of protein. [b] Pargyline hydrochloride was present at a concentration of 2.5 μM.

Results and Discussion

The kinetic constants, obtained from the MAO assay, are reported in Table I. The parameter V_{max}/K_m has been used to probe the effects of structural modification on substrate oxidative activity.

In agreement with earlier reports,[11,20] we find that the interposition of a methylene group between the tetrahydropyridyl and phenyl moieties of MPTP yields comparable or better substrates of MAO than the parent

MPTP (Table I). The oxidation of these compounds was inhibited by pargyline, an inhibitor of monoamine oxidase (Table III). At the highest concentration of pargyline tested (2.5 μM), **3i** was noticeably, but inexplicably, insensitive to inhibition. Most substitutions on the phenyl ring do not significantly alter the substrate effectiveness of these homologues (Table I, **3a–g**, **31**). In addition, the replacement of the phenyl groups with a heterocyclic (**3j**) or naphthyl substituent (**3i**) yields better substrates than MPTP. However, hydrophilic substituents are poorly tolerated (**3m**). Furthermore, substitution at the benzylic methylene bridge is clearly disfavored (**3h**, **3k**). The latter observation is consistent with the reduced oxidative activity of MPTP relative to 4-homo-MPTP since the Cl′-position of MPTP may be regarded as a substituted benzylic carbon. Although the ethylene-bridged compounds **6a** and **6b** are fairly good substrates, the vinyl-bridged analogues **7a** and **7d** showed poor activity. Given that **6a** and **7a** are of similar length, this disparity, possibly a reflection of the ridigity of **7a**, suggests that **6a** may not bind to the enzyme in its linear conformation. This view is consistent with earlier reports which indicate that substitution along the long axis of MPTP (either at C4′ or at the *N*-methyl group) invariably leads to relatively poor substrates of MAO.[11,20] Alternatively, some unfavorable electronic effects may be involved.

In addition to increasing flexibility, the methylene bridge in **3a** and its congeners should further project the phenyl ring into regions of the substrate binding site which would otherwise be out of reach. In earlier studies of MAO,[11,20] MPTP displayed significant bulk tolerance at the meta position of the phenyl group. This observation suggests the existence of a hydrophobic pocket in this region. It is also noteworthy that, in these earlier studies, 3′-bromo-MPTP and **3a** were found to be the best substrates of monomaine oxidase B. By superimposing models of these two structures (data not shown), it becomes apparent that both the phenyl group of **3a** and the bromine atom of 3′-bromo-MPTP can occupy the same region on the enzyme. Thus, the increased oxidative activity of these flexible MPTP analogues may be attributed, in part, to their ability to bind to hydrophobic regions such as this. In addition, the data suggest that the optimum separation between the amine and the lipophilic moiety is defined by the 4-pyridylmethyl fragment.

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Supplementary Material Available: NMR data of all compounds synthesized (10 pages). Ordering information is given on any current masthead page.

Hemoglobin S Antigelation Agents Based on 5-Bromotryptophan with Potential for Sickle Cell Anemia

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5-Bromotryptophan (5-BrTrp) is the most potent amino acid derivative reported in the literature to inhibit the gelation of hemoglobin S (from sickle cell anemia patients). Trp-Trp is also more potent than Trp as an antigelation agent. Therefore, we have prepared a series of dipeptides containing 5-BrTrp and evaluated the antigelation activity. 5-BrTrp-5-BrTrp is the most potent, i.e., 5.9 times the activity of Trp, followed by 5-BrTrp-Trp and then Trp-5-BrTrp. This improved antigelation potency for 5-BrTrp-5-BrTrp and 5-BrTrp-Trp is very significant and will be pursued further as lead compounds with potential for sickle cell anemia.

Sickle cell anemia is a genetic disease that results from the substitution of a valine residue for glutamic acid at the β6-position in the hemoglobin (Hb) molecule. Deoxygenated hemoglobin S (HbS) molecules aggregate together to form long helical fibers that deform and rigidify the red blood cells.[1-3]

The basic unit of the sickle hemoglobin fiber appears to be a pair of monofilament strands, with the two strands approximately in half-register with each other.[4-6] The arrangement of the individual HbS molecules within these strands has been suggested by Love and co-workers from X-ray studies of HbS crystals and involves two axial and two lateral intermolecular contact regions within the deoxy-HbS double strand.[7,8] In the deoxy-HbS double-strand structure, the β6-Val residue from a hemoglobin molecule in one strand fits into a hydrophobic pocket formed by EF helices of a β-chain in a hemoglobin molecule from the neighboring strand. That molecule, in turn, has the β6-Val from its other β-chain inserted into the hydrophobic EF pocket in the next molecule up or down the chain in the original strand. Thus, each hemoglobin molecule contributes one β6-mutation site and one hydrophobic pocket to the double-strand structure.

A variety of noncovalent inhibitors of deoxyhemoglobin aggregation, presumably acting by competitive binding at one of the important HbS–HbS intermolecular contact sites, have been investigated. In vitro studies utilizing

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