Interaction of Tetrahydrostilbazoles with Monoamine Oxidase A and B⊥

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1-Methyl-1,2,3,6-tetrahydrostilbazole (MTHS) and its analogs are oxidized by monoamine oxidase (MAO) A at slow rates comparable to that for the structurally similar neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, but the rates of oxidation by MAO B vary over a wide range depending on the structure of the analog. MAO A oxidation of all of the analogs yielded nonhyperbolic kinetic patterns, with little difference between the *cis* and *trans* isomers. In contrast MAO B showed hyperbolic kinetics and distinct stereoselectivity for the *cis* isomers. The corresponding pyridinium forms of *trans*-MTHS and its analogs were more potent inhibitors of MAO A (K_i values between 0.3 and 5 μM) than of MAO B, for which the K_i values varied greatly. The data suggest that the stringency of the MAO A active site for the geometry of the substrate molecule is less strict than that of MAO B. With MAO B, any substitution on the phenyl ring can lead to dramatic changes in the substrate properties which may be explained by the different orientation of substrate at the active site of the enzyme. Molecular geometry but not the effects of the substituents was shown to be an important factor in determining the effectiveness of substrate oxidation by MAO B.

Each of the three laboratories which have cooperated in the present investigation has been engaged in systematic studies aimed at characterizing the main features of the substrate binding sites of monoamine oxidase (MAO) A and B based on information from the rates of oxidation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) analogs and their K_m values, from the mechanism-based inactivation of the two enzymes by tetrahydro- and dihydropyridines, and from the K_i values for reversible inhibition by their pyridinium forms.[1-9] These studies have brought to light several novel features of these two enzymes, e.g., that increasing the chain length of a 2′-alkyl substituent on MPTP progressively results in change from a MAO B to a MAO A substrate,[9] that 4′-alkyl substitution of 1-methyl-4-phenylpyridinium (MPP+) results in very tight binding to MAO A, but not to MAO B,[5] and that the oxidation of tetrahydrostilbazoles by MAO A, but not by MAO B, yields biphasic kinetics.[10]

The present study compares the relative rates of oxidation of semirigid 1-methyltetrahydrostilbazole (MTHS) analogs of MPTP (Figure 1) by highly purified MAO A and B and the inhibition of MAO A by the pyridinium oxidation products of MTHS analogs (Figure 2). In the course of this work an unexpected property of MAO B was discovered, namely, that the oxidation of MTHS analogs by that enzyme is stereoselective.

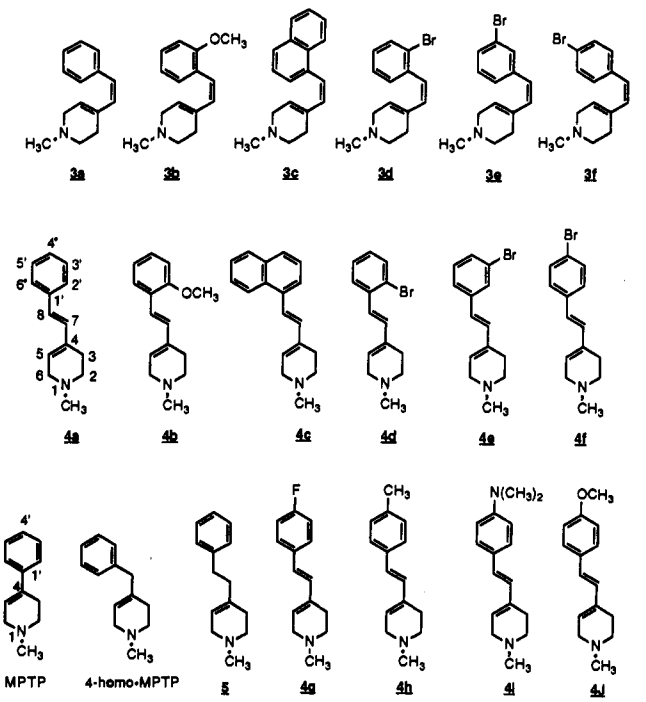

Figure 1. Structures of compounds in Table 1.

Chemistry

Compounds **2**, **4**, **6**, **10**, and **13–16** were reported previously.[8,13,19] The *trans*-(arylethenyl)pyridines were synthesized unambiguously, following a previously described method,[11] by reaction of the appropriate aldehydes with 4-picoline in acetic anhydride. The yields ranged between 40 and 70%. The *cis*-(arylethenyl)pyridines were obtained in 45–65% yield from mixtures of corresponding

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⊥ Abbreviations: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTHS, 1-methyl-1,2,3,6-tetrahydrostilbazole; MAO, monoamine oxidase; Me(4Bz)TP, 1-methyl-4-benzyl-1,2,3,6-tetrahydropyridine; MPP+, 1-methyl-4-phenylpyridinium; DHP, 2,3-dihydropyridinium; THP, 1,2,3,6-tetrahydropyridine.

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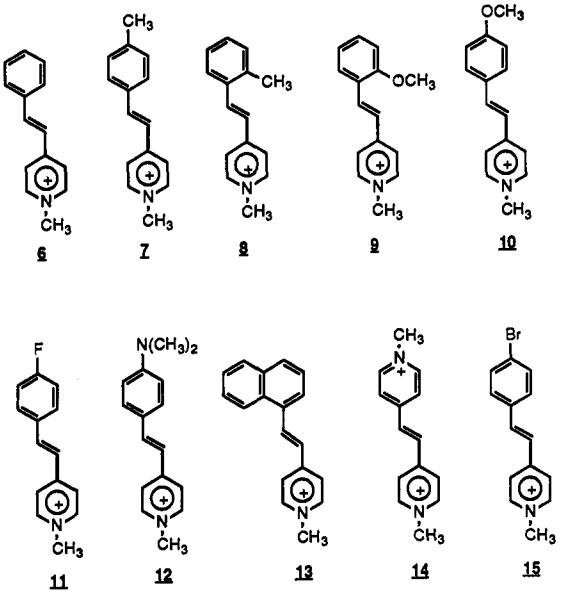


Figure 2. Structures of compounds in Table 2.

cis and *trans* isomers resulting from the reaction of an appropriately substituted benzylphosphonium halide and 4-pyridinecarboxaldehyde in ethanolic sodium ethoxide.[12] Separation of *cis* and *trans* isomers was accomplished either by radial flow chromatography on silica gel (hexane–acetone–Et_3N, 84:15:1) or by HPLC on silica gel (hexane–2-propanol–Et_3N, 89:10:1). All compounds were characterized by ^1H NMR and mass spectrometry. The assignment of *cis*/*trans* geometry was based on ^1H NMR data. Specifically, the coupling constant for the vinyl protons of the *cis* isomers was found to be 12–14 Hz, while the coupling constant for the *trans* isomers was 18 Hz. The tetrahydropyridines were synthesized, in 65–70% yield, from the corresponding pyridines by previously described methods.[13] Finally, the pyridinium methiodides were obtained as intermediates in the synthesis of the tetrahydrostilbazoles. All the tetrahydropyridines were subsequently converted to the corresponding hydrochlorides or oxalates which were used for biological evaluation. MTHS and its analogs are potentially neurotoxic, and so aerosols and skin contact must be avoided.

Results

Molecular Mechanics Calculations. Molecular mechanics calculations carried out with PCModel (Serena software) show that the most stable structure for *trans*-MTHS is one in which the benzene ring and the two double bonds are coplanar. Similar results were reported earlier.[7,8] In *cis*-MTHS, the barrier to rotation about C8–C1′ is about 1 kcal/mol. Hence rotation about this bond is relatively unhindered. However, substitution at the ortho position of the phenyl group leads to an increase in the rotational barrier attributable to unfavorable steric interactions between the substituent and the hydrogen atom on the vinyl bridge. Consequently, the phenyl group is rotated away from the plane of the vinyl bridge, thereby reducing the extent of conjugation within the system. Similar observations were reported in a previous study of styrylpyridinium cations with the semiempirical method MNDO.[16]

In contrast to the linear *trans*-MTHS, the adoption of a coplanar conformation by *cis*-MTHS is clearly disfavored, owing to unfavorable steric interactions. Steric congestion in this structure is relieved by the adoption of a conformation which contains minimal extended conjugation. Our calculations show that the phenyl group of *cis*-MTHS is rotated 63° out of the plane of the vinyl bridge while the endocyclic double bond is rotated 36° away from the plane of the bridge. Ortho substitution on the benzene ring increases the unfavorable steric interactions, and results in further rotation of the benzene ring away from the plane of the vinyl bridge. Hence, the most stable conformation for *cis*-MTHS is best described as U-shaped. In this structure, the planes of the endocylic double bond (C3–C4–C5–C6), the vinyl bridge (C4–C7–C8–C1′), and the phenyl group are neither coplanar nor parallel. These results are consistent with those reported earlier for styrylpyridinium cations.[16]

The *Trans* to *Cis* Isomerization. As noted in a recent paper,[10] the oxidation of **4a**, the unsubstituted *trans*-MTHS, by MAO A is characterized by nonlinear Lineweaver–Burke plots. Although this was eventually shown to be the consequence of the kinetic mechanism of MAO A, we initially considered the possibility that the biphasic kinetics represented the oxidation of the *cis* and *trans* isomers of MTHS (**3a** and **4a**). We based this hypothesis on the photolability of stilbazoles[17] and reasoned that exposure to light of the *trans* isomer in the course of use may have caused partial conversion to the *cis* form. However, no contamination with the opposite stereoisomer was found to be present in **3a** or **4a** and no *trans* to *cis* or *cis* to *trans* isomerization was observed during the catalysis (Figure 5, supplementary material). *Cis* isomers of MTHS analogs were oxidized into *cis*-dihydropyridinium (DHP) species, and *trans* isomers were oxidized into *trans*-DHPs. The rates of oxidation of MTHS and its analogs were measured spectrophotometrically following the accumulation of DHP product. No formation of the pyridinium products analogous to those produced during the oxidation of some MPTP analogs was observed during the steady-state assay. A blue shift of the absorption band of the product, indicating the beginning of the formation of pyridiniums, was observed after 1–1.5 h for the *cis* isomers and after about 15 h for the *trans* isomers.

In order to establish that *trans*–*cis* isomerization was not the reason for the biphasic kinetics and in order to obtain *cis* isomers for use as MAO substrates, we studied the photoconversion of *trans* to *cis* isomers as described in the Experimental Section. Since the rate of photoisomerization is a function of the concentration of the compound,[18] dilute solutions (50 μM) were used for the investigation of this process. After 30–40-min exposure to an intense light source in a quartz cuvette, a photostable equilibrium was reached, after which further exposure caused no spectral changes. The absorption band was markedly reduced in intensity and blue-shifted by 20–30 nm (Figure 3, supplementary material). The degree of conversion of the *trans* to the *cis* isomer varied depending on the presence and position of substituents in the phenyl ring of the molecule. For the compounds having a substituent at the ortho position, a 100% conversion was achieved. For the rest of the compounds, photoisomerization was incomplete.

The spectral changes observed were similar to those reported for a number of styrylpyridines,[17] but appeared to take place more slowly. These authors also noted that for that group of compounds the naphthyl derivatives were

Table 1. Oxidation of 1-Methyl-1,2,3,6-tetrahydrostilbazoles by Highly Purified MAO A and B

		MAO A				MAO B		
compound	isomer	TN[a]	K_{m1}[b] (mM)	K_{m2}[b] (mM)	TN/K_{m2}	TN[a]	K_m (mM)	TN/K_m
MPTP[c]		20	0.14		143	204	0.39	523
3a	*cis*	35	0.014	0.47	81	73	0.080	912
4a[e,f]	*trans*	35	0.010	0.40	88	35	1.8	19
3b	*cis*	37	0.025	0.49	75	25	0.27	93
4b[e]	*trans*	31	0.029	0.48	65	33	2.1	16
3c	*cis*	31	0.013	0.22	141	19	0.39	49
4c[e,g]	*trans*	31	0.015	0.24	129	13	0.63	21
3d	*cis*	27	0.010	0.28	96	30	0.28	107
4d	*trans*	11	0.009	0.23	48	74	0.12	617
3e	*cis*	150	0.012	0.63	238	169	0.16	1056
4e[f]	*trans*	132	0.018	0.70	189	25[d]	4.2[d]	6.0[d]
3f	*cis*	5	0.005	0.30	17	98	0.11	890
4f	*trans*	20	0.003	0.38	53	40	0.41	98
4g[e]	*trans*	47	0.005	0.30	157	83	0.60	138
4h[e]	*trans*	34	0.008	0.60	57	10	0.17	59
4i[e]	*trans*	27	0.008	0.35	77	7.4	0.11	67
4j[e]	*trans*	20	0.01	0.42	48	20	0.32	63

[a] TN, turnover number at 30 from double-reciprocal plots expressed as micromoles of substrate oxidized per minute per micromole of enzyme. [b] K_{m1} and K_{m2} derived from biphasic Lineweaver–Burke plots represent the values corresponding to the low and high substrate concentration ranges, respectively. [c] From ref 9. [d] From ref 15, with K_m values as expressed in this table. [e] Reference 8b. [f] Reference 13. [g] Reference 19. Kinetic constants were obtained from Lineweaver–Burke plots composed of more than six points, and the replicates differed by no more than 5%.

approximately 5 times as light sensitive as the corresponding phenyl derivatives.

Oxidation by MAO A and B. The oxidation of both the *trans* and *cis* isomers of MTHS and its analogs by MAO A follows nonhyperbolic kinetics, resulting in nonlinear double-reciprocal plots. This kinetic pattern has been shown to arise as a consequence of the branched pathway mechanism in which the reduced enzyme can be reoxidized slowly on its own or much more rapidly when complexed with substrate.[10] The dissociation constant for that complex is much higher than for the association of oxidized enzyme and substrate, so that the pathway via the ternary complex becomes significant only at high substrate concentrations. Because of the complex terms in the full kinetic equation and the clear separation of the two apparent K_m values, the steady-state data were fitted to a double hyperbola to give the parameters V, K_{m1}, and K_{m2}. K_{m1} estimates the concentration at which the slower oxidation pathway is half-maximal, the K_{m2} value is an estimate of the K_m for the faster pathway. Since K_{m2} is a measure of the dependence of the activity on substrate concentration when most of the flux is through the faster (ternary complex) pathway which is thought to predominate MAO B, TN/K_{m2} for MAO A is compared with TN/K_m for MAO B.

Not only do the *cis* and *trans* isomers both yield nonhyperbolic kinetic patterns, but as shown in Figure 6A (supplementary material) and Table 1, the stereoisomer pairs **3a** and **4a**, **3b** and **4b**, **3c** and **4c**, and **3e** and **4e** show very similar reactivities with MAO A. The *cis*/*trans* pair **3d** and **4d** (*o*-Br-MTHS) seems to be an exception to this for reasons which are not yet clear, but even for this pair the difference in TN/K_m for MAO A is lower than for MAO B.

In contrast, MAO B yields linear Lineweaver–Burke plots with all MTHS analogs studied, and its action is stereospecific since the K_m values are substantially lower for the *cis* than for the corresponding *trans* isomers, again, with the exception of the *o*-Br-MTHS pair (Figure 6B (supplementary material), Table 1). The differences in turnover numbers within pairs of stereoisomers are significant but not as great as the differences in their K_m values.

Table 2. Inhibition of MAO A and B by 1-Methyl-4-styrylpyridiniums

	MAO A		MAO B	
compound[a]	K_i (μM)	IC_{50}[b] (μM)	K_i (μM)	IC_{50}[b] (μM)
6	2.7		100	
7	0.40		9.0	
8	0.50		10.0	
9	5.0			2000
10	1.7		60.0	
11	0.80			480
12	0.80		62.0	
13	2.7		70.0	
14		340		>1200
15	0.25			87

[a] Trans isomer. [b] Secondary plots replotted from primary double-reciprocal plots were curved. Thus, true K_i values could not be determined. IC_{50} values for MAO A were determined at 50 μM kynuramine and for MAO B at 100 μM benzylamine. Inhibition constants were obtained from double-reciprocal plots composed of more than six points, and the replicates differed by no more than 5%. All assays were at 30 °C.

Stilbazole Analogs of MPP$^+$ as Inhibitors of MAO A and B. Table 2 compares the effectiveness of various 1-methyl-4-styrylpyridiniums as reversible competitive inhibitors of MAO A and B. As is true of the large number of MPP$^+$ analogs studied,[5,9,15] the K_i values of these nonflexible MPP$^+$ analogs for MAO A are 1 to nearly 2 orders of magnitude lower than for MAO B. The styrylpyridiniums tested (Table 2) are among the most potent inhibitors of MAO A, comparable to the 4′-alkyl-substituted MPP$^+$ analogs. The one exception is **14**, but this is not a stilbazole derivative. It bears the same relation to the unsubstituted 1-methyl-4-styrylpyridinium (**6**) as paraquat does to MPP$^+$, and like paraquat is not an effective inhibitor of MAO A and B. The reason in both cases seems to be the absence of a hydrophobic residue in the molecule needed for binding to the substrate site of MAO A.

Discussion

In previous studies of the oxidation of *trans*-MTHS analogs by a partially purified preparation from pig liver containing both MAO A and B,[7,8] biphasic kinetics were observed. These workers interpreted the two K_m and two TN values in Lineweaver–Burke plots as representing the reactions as catalyzed by MAO A and B, respectively. This notion had to be abandoned when similar behavior was observed with highly purified MAO A.[15]

When this investigation was undertaken, we considered the possibility that the *trans*-MTHS analogs had been contaminated with the *cis* stereoisomer, produced inadvertently during handling and storage on exposure to light and that, therefore, the two K_m values and TNs represented the reaction of the two stereoisomers with the enzyme. HPLC analysis of the *trans* isomers (Table 1) revealed, however, no trace of the *cis* isomers, and the absorption spectra, which would also clearly differentiate between the stereoisomers, confirmed this conclusion. All the *cis* isomers (**3a-f**) gave the same or very similar K_m values and turnover numbers with MAO A, except for **3f**, but even there the difference in turnover numbers may not be

as great as appears in Table 1 because the slow rate of oxidation made it difficult to obtain accurate values. As shown in a previous paper,[10] the biphasic kinetics was eventually shown to be the consequence of the operation of dual pathways in the kinetic mechanism of MAO A.

The availability of both stereoisomers of a series of MTHS analogs enabled us to demonstrate that, in contrast to MAO A, MAO B readily distinguishes between the *cis* and *trans* isomers. Except for the *o*-Br pair (**3d** and **4d**), the TN/K_m function was much higher in each case for the *cis* than for the *trans* isomers, the difference being due primarily to lower K_m values for the former. The structure–function observations for each isozyme are discussed below.

MAO A. The oxidation of all MTHS analogs by MAO A followed kinetics that gave biphasic curves in a double-reciprocal plot. The factors underlying this nonlinearity are the subject of an earlier report.[10] This complex kinetic behavior with MAO A makes it difficult to compare the MTHS analogs with substrates studied previously, such as MPTP analogs and 4-homo-MPTP analogs on which the model[3] of the active site developed by one of us is based. The major problem is that the estimated kinetic parameters are complex constants. According to the mechanism proposed for MAO A,[10] K_{m1} includes terms for the binding of MTHS to the oxidized form of the enzyme only, whereas K_{m2} includes terms for the binding of MTHS to both the oxidized and reduced forms. If the rate-limiting step is reduction, the oxidized form predominates so that the ratio TN/K_{m1} might be used as an estimate of the effectiveness of the catalysis. However, the rate of substrate oxidation in the range of its concentration around K_{m1} is significantly less than V_{max}. Considerable apparent acceleration of catalysis is observed at much higher concentrations of substrate, near K_{m2}, due to diversion of the reoxidation of the reduced enzyme into the faster ternary complex pathway (i.e., oxidation of the reduced enzyme–substrate complex instead of the free enzyme). The parameter TN/K_{m2} reflects better the effectiveness of this faster pathway. However, this is still not equivalent to the TN/K_m parameter reported for MPTP analogs other than MTHS. We have chosen to consider the maximum velocity and K_{m2} as the estimate of catalytic efficacy.

In general, in terms of TN values, MTHS and its analogs are very poor MAO A substrates. In contrast to MPTP analogs where both ortho and para substitution led to significant changes of substrate properties towards MAO A,[9] neither molecular shape (*cis* and *trans* isomers) nor the nature and position of substituents seems to influence the kinetic parameters. Only the *m*-Br derivative (both *trans* and *cis* isomers) has a much higher TN value, whereas the rest of the compounds have TN values comparable to that of MPTP. In a previous report, *m*-halogen-substituted analogs of MPTP were found to be more effective substrates of both MAO A and B than the parent MPTP.[9] This observation was attributed to the electron-withdrawing character of these substituents; however, the nature of these influences remains unclear. Although we find a similar enhancement of effectiveness with *m*-halogenation for the MTHS series, we also note some differences: (1) the enhancement is limited to MAO A and (2) for MPTP analogs, the enhancement is attributed mainly to lower K_m values while for the MTHS analogs, higher TN values are observed. The foregoing observation suggests that the factors underlying these enhancements may not be the same for both series. Given the large spatial separation between the *m*-Br substituent and the reactive center (C6–N1), the observed rate enhancements cannot be attributed to inductive influences on the reactive center. Mesomeric influences are also excluded for two reasons: (1) there is no extended conjugated system which includes both the Br substituent and the reactive center and (2) the increase in the TN value is similar for *cis* and *trans* even though the nature of conjugation is different in the two molecules (*vide supra*). Additionally, since the *o*- and *p*-Br analogs do not manifest any increases over the parent MTHS, the observed increase in the TN value probably does not reflect a nonspecific effect of lipophilicity. Thus, the increase in the TN value observed for *m*-Br-MTHS may be attributed to specific interactions between this substituent and some functional groups of the enzyme. In this connection, one can envisage a small hydrophobic pocket which accomodates substituents at the meta but not the ortho and para positions. Since the phenyl groups of the *cis*- and *trans*-*m*-Br-MTHS isomers would bind to different regions of the substrate binding site, it would appear that this interaction is common to both regions.

Taken together these observations lead us to conclude that there is no obvious connection between the electronic or steric effects of substituents and the effectiveness of catalysis for this class of compounds.

MAO B. Most of the *trans*-MTHS analogs are poor MAO B substrates. However, in contrast to MAO A, MAO B is quite sensitive to the nature and position of the substituent. Even minor changes in substrate structure may lead to dramatic changes in kinetic parameters, as is evident for the K_m values (Table 1). In general, para substitution leads to a significant increase in TN/K_m value, mainly because of lower K_m values. However, electron-withdrawing substituents are more effective than electron-donating groups (**4f,g,h–j**). In addition, substituent size appears to be important since the *p*-fluoro-MTHS analog **4g** is the most effective of the para-substituted *trans*-MTHS compounds. Ortho substitution affects the substrate properties of *trans*-MTHS analogs in a slightly different manner. Whereas the kinetic properties of the *o*-methoxy and naphthyl analogs, **4b** and **4c**, are essentially unchanged, there is a dramatic increase in effectiveness with *o*-bromination, attributable to a 10-fold decrease in the K_m (**4a** vs **4d**). The increase in TN/K_m values observed for the halogenated analogs **4d,f,g** over the parent MTHS and other ortho- and para-substituted compounds suggests a preference for electron-withdrawing substituents at these positions. However, substituent size may limit the kinds of groups that are tolerated at each position. Such a limitation may be partly responsible for the inability of the meta-substituted analog **4e** to serve as an effective substrate for MAO B. Since the electronic effects of substituent on this ring are probably not transmitted to the reactive center (*vide supra*), the observed differences may be attributed to specific interactions between this region of the molecule and some functional groups of the enzyme.

Both the K_m and the TN values for the MTHS analogs with MAO B vary over a much wider range than was observed with MAO A. In contrast to the latter, MAO B is stereoselective, exhibiting a preference for the *cis* isomers. Five of the *cis*-MTHS analogs studied were found to be more effective substrates because of both higher TN

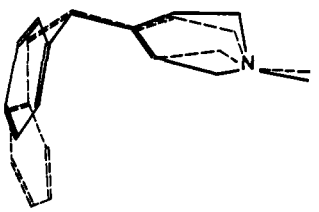


Figure 7. Overlay of the 3-D structures of *cis*-MTHS (dashed lines) and 4-homo-MPTP (solid lines). The geometries of the molecules were optimized with the PCModel program.

and lower K_m values compared to their corresponding *trans* isomers. One exception to this trend, the *cis*-*o*-Br derivative **3d**, is significantly less effective than the corresponding *trans* isomer **4d**. Compounds **3a,e,f** have very similar TN/K_m values, suggesting that substitution at the meta and para positions has little influence on effectiveness. However, substitution at the ortho position (**3b–d**) leads to a significant decrease in the efficacy of catalysis, suggesting a negative steric influence (*vide supra*).

A model proposed earlier[3] postulates that the phenyl group of 4-homo-MPTP projects into, and can be accommodated by the P3′–B pocket, thereby providing an explanation for the selectivity and effectiveness of this compound. Curiously, superposition of the *cis*-MTHS and 4-homo-MPTP structures indicates some similarity between the shape of the two compounds (Figure 7). This similarity suggests that the phenyl groups of both compounds may have access to the similar regions of the substrate binding site of MAO B. On the basis of this, we conclude that these two compounds may interact with the enzyme in a similar fashion. This conclusion is consistent with the effectiveness of both compounds as substrates for MAO B and their selectivity for this enzyme.

The ability of the rigid *cis*-MTHS to function as an effective substrate for MAO B also supports earlier claims[3] that the flexible ethylene-bridged analog, 1-Me-4-(phenylethyl)-THP, **5**, interacts with this enzyme in a folded form. Such a form can be achieved easily by the adoption of a *gauche* conformation about the ethylene bridge of this flexible analog.

Styrylpyridiniums as Inhibitors of MAO A and B. Previous studies have shown that MPP^+ and its analogs inhibit MAO A better than MAO B. Stilbazoles are no exception. In general, all of the compounds tested were better competitive inhibitors of MAO A than MPP^+. Some correlation between K_i for stilbazoles and K_{mi} for their corresponding tetrahydro forms is observed. The K_i value (2 μM) for inhibition of MAO A activity by **4a** is almost equal to the K_i value for the corresponding pyridinium compound. By extrapolation, we might expect similar correspondence between each THP/pyridinium pair. The positive charge on the N atom appears not to influence significantly the binding of ligand to the enzyme.

In contrast to MAO A, inhibition of MAO B by the styrylpyridinium compounds was of the mixed type (i.e., both the binding of substrate and the catalytic efficiency of the enzyme were affected). Values of K_i vary in a much wider range than those for MAO A, and there is no obvious correlation between the K_i for the stilbazoles and the K_m values for their corresponding THP forms. Earlier data suggested that charged MPP^+ and amine ligands may interact with MAO B by different mechanisms (i.e., different ionizable groups of the enzyme may take part in binding charged and uncharged ligands).[7,8] The same may be true for stilbazoles.

In conclusion, we have shown that both the molecular geometry and certain substitutions in the phenyl ring of the substrate molecule (but not electronic effects of the substituents) are important factors in the effectiveness of catalysis of the oxidation of the MTHS analogs by both MAO A and MAO B. In spite of the fact that the shape of the molecule is important for the catalysis of the oxidation of MPTP analogs by MAO A, the requirements of the enzyme for the geometry of the substrate molecule are less strict for MTHS analogs. In contrast, MAO B for the first time is shown to possess significant stereoselectivity toward the *cis* isomers. For MAO B, ortho substitution on the phenyl ring is unfavorable for catalysis. Indeed, with MAO B, any substitution on the phenyl ring may lead to dramatic changes in the substrate properties which may be explained by the different orientation of substrate in the active site of the enzyme.

Finally, *cis* isomers of MTHS analogs without substitution at the ortho position are much better substrates of MAO B than their corresponding *trans* isomers. Taking into account that dihydropyridinium metabolites of *cis* isomers are much less stable than those of the *trans* isomers, we may expect that the concentration of potentially neurotoxic pyridinium metabolites in brain tissues will be higher for *cis* than *trans* isomers. Therefore, the probability of the development of MPP^+-like neurotoxicity in the case of *cis*-MTHS analogs would be higher than in the case of those of *trans* configuration.

Experimental Section

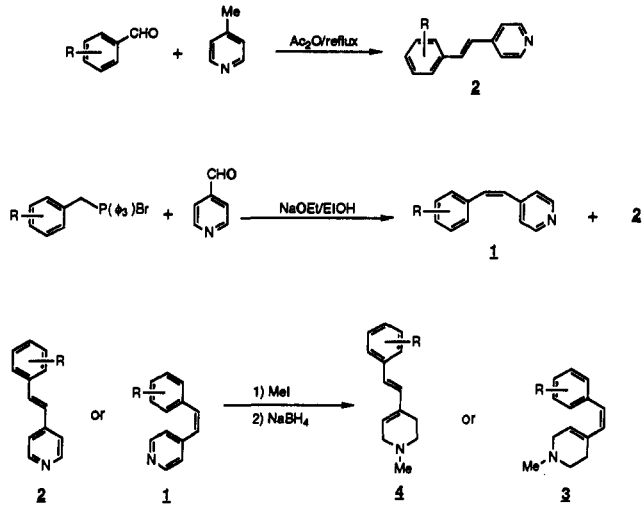
General Section. Synthetic intermediates were purchased from Aldrich, Inc. (Milwaukee, WI) and were used as received. Solvents were distilled immediately prior to use. Commercially available reagents were used without subsequent purification.

All air-sensitive reactions were carried out under nitrogen. Standard handling techniques for air-sensitive materials were employed throughout this study. Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. The specific rotation was determined on an automatic polarimeter (Autopol III, Rudolph Research, Flanders, NJ). ^1H NMR spectra were recorded on an IBM-Brucker spectrometer at 200 MHz. NMR spectra are referenced to the deuterium lock frequency of the spectrometer. Under these conditions, the chemical shifts (in ppm) of residual solvent in the ^1H NMR spectra were found to be as follows: $CHCl_3$, 7.26; DMSO, 2.56; HOD, 4.81. The following abbreviations are used to describe peak patterns when appropriate: br = 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. Unless otherwise indicated, these values are within ±0.4% of the theoretical.

Column chromatography was performed using "Baker Analyzed" silica gel (60–200 mesh). Preparative chromatography was performed on either a Harrison Research Chromatotron using Merck 60 PF_{254} silica gel or a preparative HPLC (Rainin Instrument Co.) using a 41.1-mm-i.d. Dynamax silica gel column (at a solvent delivery rate of 80 mL/min). Analytical TLC was performed on Analtech glass TLC plates coated with silica gel GHLF and were visualized with UV light and/or methanolic iodine. Representative procedures for the steps shown on Scheme 1 are provided below as procedures A–F.

Photochemical Synthesis of 3b and 3c. The *cis* isomers, **3b** and **3c**, were prepared by photochemical conversion of the corresponding *trans* isomers, **4b** and **4c**, as follows. A 10 mM aqueous solution of the MTHS derivative was placed in a 1-mL quartz spectrophotometer cell and irradiated with a Blak-Ray ultraviolet light source (115 V, 0.18 A), Ultraviolet Products, San Gabriel, CA, at 10-cm distance at 25. The rate of conversion was monitored periodically by scanning the spectrum in a Hewlett-

Scheme 1. Synthesis of Tetrahydrostilbazoles



Packard 8451A diode array spectrophotometer, as in Figure 3 (supplementary material). At the same time 10-μL aliquots were analyzed by HPLC on a Beckman Ultrasil SCX column (4.6 mm × 250 mm). The mobile phase was 90% 50 mM triethanolamine-phosphate buffer, pH 2.7, and 10% acetonitrile. Absorbance was monitored at 210 nm. All compounds were chromatographically homogeneous on HPLC (15% isopropyl alcohol–hexanes and 10% acetonitrile–50 mM triethanol-phosphate buffer). Figure 4 (supplementary material) illustrates the clear separation of stereoisomers **3b** and **4b**. Complete conversion of **4b** to **3b** and of **4c** to **3c** required 150–180 min. Under the same conditions the isomerization of **4a** to **3a** was only 5%, of **4g** to the corresponding *cis* compound 25%, and of **4j** about 50%. Continued irradiation did not increase the yield but led to the formation of breakdown products.

Molecular modeling studies were performed on a Macintosh II computer with PC Model version 2.0 (Serena Software, Bloomington, IN). Each structure was analyzed several times to obtain optimum convergence on the energy minimum.

MAO A from human liver, expressed in yeast, was isolated in homogeneous form, as described earlier.[14] MAO B was isolated, and both forms of MAO were assayed as in previous work.[15] All assays were performed at air-saturation oxygen concentration as in previous studies.[15]

General Procedure for the Synthesis of Substituted Stilbazoles. Method A: (*Z*)- and (*E*)-4-[2-(2-Bromophenyl)-ethenyl]pyridine (1d and 2d). A solution of triphenylphosphine (10.17 g, 38.8 mmol) and 2-bromobenzyl bromide (9.68 g, 38.8 mmol) in toluene (100 mL) was refluxed for 17 h. The product was filtered upon cooling of the reaction mixture to room temperature, washed with toluene, and dried in vacuum oven at 50 °C to provide (18.9 g, 95.2%) of a white solid.

NaOEt was prepared by adding Na (0.96 g, 41.7 mmol), to absolute EtOH (150 mL). (2-Bromobenzyl)triphenylphosphonium bromide (18.0 g, 35.1 mmol) was added to the solution of NaOEt in EtOH, and pyridine-4-carboxaldehyde (3.76 g, 35.1 mmol) was added to the resulting bright yellow reaction mixture. Stirring was continued for 16 h under N_2. The reaction mixture was concentrated under reduced pressure to a residue. The latter was triturated with Et_2O and filtered. The precipitate was washed several times with Et_2O and discarded. The filtrate was concentrated under reduced pressure to a residue which was subjected to HPLC (i-PrOH–hexane–Et_3N, 10:90:trace, hexane) to obtain 2.14 g (23%) of the trans isomer, **2d**, and 4.1 g (44.8%) of the cis isomer, **1d**.

1d: ^{1}H NMR ($CDCl_3$) δ 6.57 (d, 1H, *J* = 12 Hz, C*H*-Ph), 6.80 (t, 1H, *J* = 12 Hz, CH-pyr), 7.07 (d, 2H, *J* = 4 Hz, pyridyl), 7.09–7.69 (m, 4H, phenyl), 8.39 (d, 2H, *J* = 4 Hz, pyridyl).

2d: ^{1}H NMR ($CDCl_3$) δ 6.95 (d, 1H, *J* = 16 Hz, C*H*-Ph), 7.17–7.71 (m, 4H, phenyl), 7.41 (d, 2H, *J* = 4 Hz, pyridyl), 8.60 (d, 2H, *J* = 4 Hz, pyridyl).

(*Z*)-4-[2-(4-Bromophenyl)ethenyl]pyridine (1f): yield 34%; ^{1}H NMR ($CDCl_3$) δ 6.50 (d, 1H, *J* = 12 Hz, C*H*-THP), 6.67 (d, 1H, *J* = 12 Hz, CH-phenyl), 7.05 (d, 2H, *J* = 8 Hz, phenyl), 7.08 (d, 2H, *J* = 4 Hz, pyridyl), 7.36 (d, 2H, *J* = 8 Hz, phenyl), 8.45 (d, 2H, *J* = 4Hz, pyridyl).

(*Z*)- and (*E*)-4-[2-(3-Bromophenyl)ethenyl]pyridine (1e and 2e). **1e**: yield 63%; ^{1}H NMR ($CDCl_3$) (cis) δ 6.97 (d, 1H, *J* = 16 Hz, C*H*-Ph), 7.15–7.67 (m, 7H, phenyl, pyridyl, CH-pyr), 8.57 (d, 2H, *J* = 6 Hz, pyridyl). **2e**: yield 19%; ^{1}H NMR ($CDCl_3$) δ 6.58 (d, 1H, *J* = 18 Hz, C*H*-Ph), 7.01–7.69 (m, 7H, phenyl, pyridyl, CH-pyr), 9.56 (d, 2H, *J* = 6 Hz, pyridyl).

Method B: (*E*)-4-[2-(2-Bromophenyl)ethenyl]pyridine (2d). Via the method of Baker, a mixture of 2-bromobenzaldehyde (2.0 g, 10.8 mmol) and 4-picoline (1.0 g, 10.8 mmol) in acetic anhydride (25 mL) was refluxed for 22 h. The resulting mixture was cooled and concentrated to a residue which was treated with saturated $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The organic extracts were dried over Na_2SO_4 and concentrated to a residue. The crude product was passed through a short column of silica gel (acetone–hexane–Et_3N, 9:90:1) to yield 1.1 g (39%) of **2d** as a yellow liquid: ^{1}H NMR ($CDCl_3$) δ 6.95 (d, 1H, *J* = 16 Hz, CH-Ph), 7.17–7.71 (m, 4H, phenyl), 7.41 (d, 2H, *J* = 4 Hz, pyridyl), 8.60 (d, 2H, *J* = 4 Hz, pyridyl).

General Procedure for the Synthesis of Tetrahydrostilbazoles. (*Z*)-*N*-Methyl-4-[2-(2-bromophenyl)ethenyl]-1,2,3,6-tetrahydropyridine (3d). Methyl iodide (2.18 g, 0.96 mL, 15.38 mmol) was added to a solution of **1d** (2.0 g, 7.69 mmol) in acetone (30 mL), and the resulting solution was stirred wtih exclusion of light for 16 h. The reaction mixture was concentrated under reduced pressure. The residue was redissolved in MeOH (60 mL), and the solution was cooled in an ice bath. $NaBH_4$ (1.44 g, 54.8 mmol) was added portionwise. Following the addition, the reaction mixture was slowly allowed to warm up to room temperature. After 18 h, the mixture was concentrated under reduced pressure, and the residue was partitioned between water (20 mL) and CH_2Cl_2 (40 mL). The aqueous layer was reextracted with CH_2Cl_2 (20 mL) and discarded. The combined organic extracts were dried over Na_2SO_4 and concentrated to a residue which was chromatographed using 1:1 hexane–acetone (+1% Et_3N) to yield (1.4 g, 65%) of a pale yellow liquid: ^{1}H NMR ($CDCl_3$) δ 1.90 (br s, 2H, CH=CCH_2), 2.28 (s, 3H, CH_3), 2.36 (t, 2H, *J* = 6 Hz, $CH_3NCH_2CH_2$), 2.98 (broad d, 2H, *J* = 4 Hz, CH_3NCH_2CH), 5.70 (br s, 1H, CH_2CH=C), 6.23 (d, 1H, *J* = 12 Hz, C*H*-THP), 6.29 (d, 1H, *J* = 12 Hz, CH-phenyl), 7.07–7.66 (m, 4H, phenyl). The product was converted to a hydrochloride and recrystallized in i-PrOH–ether to obtain 0.87 g of a pale yellow solid: mp 189–191 °C; EIMS *m/e* (intensity) 277.0 (M^+, 13.6). Anal. ($C_{14}H_{16}BrN$·HCl) C, H, N.

(*Z*)-*N*-Methyl-4-[2-(3-bromophenyl)ethenyl]-1,2,3,6-tetrahydropyridine (3e): yield (free base) 69%; mp 240–242 °C; ^{1}H NMR ($CDCl_3$) δ 2.04 (br s, 2H, CH=CCH_2), 2.33 (s, 3H, CH_3), 2.44 (t, 2H, *J* = 4 Hz, $CH_3NCH_2CH_2$), 3.01 (br s, 2H, CH_3NCH_2-CH), 5.70 (br s, 1H, CH_2CH=C), 6.10 (d, 1H, *J* = 12 Hz, C*H*-THP), 6.30 (d, 1H, *J* = 12 Hz, CH-phenyl), 7.16–7.71 (m, 4H, phenyl); EIMS *m/e* (intensity) 277.0 (M^+, 84.8). Anal. ($C_{14}H_{16}$-BrN·HCl) C, H, N.

(*Z*)-*N*-Methyl-4-[2-(4-bromophenyl)ethenyl]-1,2,3,6-tetrahydropyridine (3f): yield 65%; mp 189–191 °C; ^{1}H NMR ($CDCl_3$) δ 2.05 (br s, 2H, CH=CCH_2), 2.31 (s, 3H, CH_3), 2.41 (t, 2H, *J* = 6 Hz, $CH_3NCH_2CH_2$), 2.98 (d, 2H, *J* = 4 Hz, CH_3NCH_2-CH), 5.69 (br s, 1H, CH_2CH=C), 6.08 (d, 1H, *J* = 12 Hz, C*H*-THP), 6.27 (d, 1H, *J* = 12 Hz, C*H*-phenyl), 7.14 (d, 2H, *J* = 10 Hz, phenyl), 7.37 (d, 2H, *J* = 10 Hz, phenyl); EIMS *m/e* (intensity) 277.1 (M^+, 100.0). Anal. ($C_{14}H_{16}BrN·C_2H_2O_4$) C, H, N.

(*E*)-*N*-Methyl-4-[2-(2-bromophenyl)ethenyl]-1,2,3,6-tetrahydropyridine (4d): yield 69%; mp 240–242 °C; ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3), 2.49 (br s, 2H, CH=CCH_2), 2.64 (t, 2H, *J* = 6 Hz, $CH_3NCH_2CH_2$), 3.10 (d, 2H, CH_3NCH_2CH), 5.86 (br s, 1H, CH_2CH=C), 6.69 (d, 1H, *J* = 16 Hz, C*H*-THP), 6.80 (d, 1H, *J* = 16 Hz, CH-phenyl), 7.01–7.57 (m, 4H, phenyl); EIMS *m/e* (intensity) 276.9 (M^+, 17.70). Anal. ($C_{14}H_{16}BrN$·HCl) C, H, N.

(*E*)-*N*-Methyl-4-[2-(3-bromophenyl)ethenyl]-1,2,3,6-tetrahydropyridine (4e): yield 75%; mp 258–261 °C; ^{1}H NMR ($CDCl_3$) δ 2.40 (br s, 2H, CH = CCH_2), 2.40 (s, 3H, CH_3), 2.64 (t, 2H, *J* = 6 Hz, $CH_3NCH_2CH_2$), 3.10 (d, 2H, CH_3NCH_2CH), 5.8 (br s, 1H, CH_2CH=C), 6.35 (d, 1H, *J* = 16 Hz, C*H*-THP), 6.77

(d, 1H, J = 16 Hz, CH-phenyl), 7.11–7.54 (m, 4H, phenyl); EIMS m/e (intensity) 277.1 (M^+, 100.0). Anal. ($C_{14}H_{16}BrN$·HCl) C, H, N.

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Supplementary Material Available: Figure 3, absorbance changes accompanying the photochemical conversion of **4b** to **3b**; Figure 4, HPLC analysis of the photochemical conversion of **4b** to **3b**; Figure 5, spectral scans during the oxidation of **3a** and **4a** by MAO B; and Figure 6, Lineweaver–Burke plots for the oxidation of MTHS analogs of MAO A and B (6 pages). Ordering information is given on any current masthead page.

References

(1) Bachurin, S. O.; Sablin, S. O.; Grishina, G. V.; Gaydarova, E. L.; Dubova, L. G.; Zubov, N. D. Monoamine oxidase catalysis of bioconversion of physiologically active 1-methyl-4-aryl-1,2,3,6-tetrahydropyridines. *Bioorgancheskaya Khimia* **1989**, *15*, 620–626.

(2) Booth, R. G.; Trevor, A.; Singer, T. P.; Castagnoli, N., Jr. Studies on semirigid tricyclic, analogues of the nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *J. Med. Chem.* **1989**, *32*, 473–477.

(3) Efange, S. M. N.; Boudreau, R. J. Molecular determinants in the bioactivation of the dopaminergic neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *J. Comput.-Aided Mol. Des.* **1991**, *5*, 405–417.

(4) Heikkila, R. E.; Kindt, M. V.; Sonsalla, P. K.; Youngster, S. K.; McKeown, K. A.; Singer, T. P. Importance of monoamine oxidase in the bioactivation of neurotoxic analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6172–6176.

(5) Jin, Y.-Z.; Ramsay, R. R.; Youngster, S. K.; Singer, T. P. A new class of powerful inhibitors of monoamine oxidase A. *Biochem. Biophys. Res. Commun.* **1990**, *172*, 1338–1341.

(6) Krueger, M. J.; McKeown, K. A.; Ramsay, R. R.; Youngster, S. K.; Singer, T. P. Mechanism-based inhibition of monoamine oxidases A and B by tetrahydropyridines and dihydropyridines. *Biochem. J.* **1990**, *268*, 219–224.

(7) Sablin, S. O. Oxidative-reductive biotransformation of physiologically active compounds by monoamine oxidases. (1991) Ph.D. dissertation, pp. 63–65, M. Gorky Library at the M. V. Lomonosov Moscow State University, Moscow.

(8) (a) Sablin, S. O.; Tkachenko, S. E.; Bachurin, S. O. Structure-activity study of MAO-catalyzed dehydrogenation of MPTP analogs. *Doklady Acad. Sci. USSR* **1991**, *318*, 228–231. (b) Sablin, S. O.; Bachurin, S. O.; Tkachenko, S. E. Stylbasole analogues of MPTP as monoamine oxidase (MAO) substrates. *J. Neur. Transm. [Suppl.]* **1990**, *32*, 119–122.

(9) Youngster, S. K.; McKeown, K. A.; Jin, Y.-Z.; Ramsay, R. R.; Heikkila, R. E.; Singer, T. P. Oxidation of analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by monoamine oxidases A and B and the inhibition of monoamine oxidases by the oxidation products. *J. Neurochem.* **1989**, *53*, 1837–1842.

(10) Ramsay, R. R.; Sablin, S. O.; Bachurin, S. O.; Singer, T. P. Oxidation of tetrahydrostilbazole by monoamine oxidase A demonstrates the effect of alternative pathways in the kinetic mechanism. *Biochemistry* **1993**, *32*, 9025–9030.

(11) Cavallito, C. J.; Yun, H. S. Choline acetyltransferase inhibitors. Configurational and electronic features of styrylpyridine analogs. *J. Med. Chem.* **1969**, *12*, 134–138.

(12) Baker, B. R.; Doll, M. H. Irreversible enzyme inhibitors. 183. Proteolytic enzymes. 18. Inhibition of guinea pig complement derived by quarternization of phenylalkylpyridines with α-bromomethylbenzenesulfonyl fluorides. *J. Med. Chem.* **1971**, *14*, 793–799.

(13) Efange, S. M. N.; Michelson, R. H.; Remmel, R. P.; Dutta, A. K.; Freshler, A. Flexible N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogues: synthesis and monoamine oxidase catalyzed bioactivation. *J. Med. Chem.* **1990**, *33*, 3133–3138.

(14) Tan, A. K.; Weyler, W.; Salach, J. I.; and Singer, T. P. Differences in substrate specificities of monoamine oxidase A from human liver and placenta. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 1084–1088.

(15) Krueger, M. J.; Efange, S. M. N.; Michelson, R. H.; Singer, T. P. Interaction of flexible analogs N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and of N-methyl-4-pyridinium with highly purified monoamine oxidase A and B. *Biochemistry* **1992**, *31*, 5611–5615.

(16) Chweh, A. Y.; DeBernardis, J. F.; Siuda, J. F.; Rondan, N. G.; Abola, J. E.; Abraham, D. E. Structural correlations of choline acetyltransferase inhibitors: trans-N-(carboxymethyl)-4-(β-1-naphthylvinyl)pyridinium bromide and cis-N-(2-aminoethyl)-4-(β-1-naphthylvinyl)-3-methylpyridinium bromide hydrobromide. *J. Med. Chem.* **1984**, *27*, 825–830.

(17) White, H. L.; Cavallito, C. J. Photoisomerization of styrylpyridine analogues in relation to choline acetyltransferase and cholinesterase inhibition. *Biochim. Biophys. Acta* **1970**, *206*, 242–251.

(18) Cauzzo, G.; Mazzucato, U.; Aloisi, G. Acid-base equilibria of isomeric styrylpyridines and some of their derivatives. Spectrophotometric study. *Gazz. Chim. Ital.* **1966**, *96*, 721–732.

(19) Gray, A. P.; Platz, R. D.; Henderson, T. R.; Chong, T. C. P.; Takahashi, K.; Dretchen, K. L. Approaches to protection against nerve agent poisoning. (Naphthylvinyl)pyridine derivatives as potential antidotes. *J. Med. Chem.* **1988**, *31*, 807–814.