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Synthesis and monoamine uptake inhibiting properties of perisubstituted tricyclic compounds

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The University of Arizona, 1988
SYNTHESIS AND MONOAMINE UPTAKE INHIBITING PROPERTIES
OF PERISUBSTITUTED TRICYCLIC COMPOUNDS

by

Jennifer Margaret Peters

A Thesis Submitted to the Faculty of the
DEPARTMENT OF PHARMACEUTICAL SCIENCES
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In the Graduate College
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1988
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7 July 1988
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ABSTRACT

The synthesis of 1-methyl-promazine, 4-hydroxymethyl-iminodibenzy1, and 4-bromo-5-trimethylsilyl-iminodibenzy1 via dilithiation and $^1$H-NMR's are described. Molecular modeling was done for the latter compound. The heat of dissociation was 30.6 kcal/mole for the lowest energy conformer. Rotational energies were examined for three bonds.

The $IC_{50}$ values for inhibition of neurotransmitter uptake by rat brain synaptosomes were determined for a series of 1-substituted promazines and 4-substituted imipramines. 1-Substituted promazines were fair inhibitors of serotonin uptake with an average $IC_{50}$ of 2000 nm. Their potency for inhibiting norepinephrine uptake was difficult to assess due to poor assay reproducibility, and the average $IC_{50}$ was estimated at 200 to 1700 nm. Serotonin, but not norepinephrine, uptake inhibition was increased with additional ring substitution at C(2) with a trifluoromethyl group. The 4-substituted imipramines were equal or slightly decreased in potency to unsubstituted imipramine for uptake inhibition of both neurotransmitters. $IC_{50}$'s were also reported for imipramine and desipramine.
INTRODUCTION

History of Tricyclic Drugs

The structural skeleton for imipramine was synthesized by Thiele and Holzinger in 1889, but more than fifty years would pass before its pharmacological significance would be discovered. Haefliger and Schindler worked in the late 1940's to synthesize more than forty derivatives of the skeleton, 10,11-dihydro-5-dibenz[b,f]azepine, or iminodibenzyl, in an effort to develop new antihistamines, sedatives, analgesics, and antiparkison drugs. Imipramine and a few other analogs were selected on the basis of animal studies for testing in humans for sedative/hypnotic properties. Since they were similar in structure to the antipsychotics, the phenothiazines, it was expected that they would have some value in the management of mental patients. However, the dibenzazepines exacerbated the agitation of psychotic patients. Kuhn, in 1958, found that these compounds had remarkable effects in elevating the mood of patients suffering from endogenous depressions and were particularly effective in retarded depressions [1].

The phenothiazines were developed in Germany in the late 1880's as part of an effort to synthesize aniline dyes. Although Ehrlich noted at that time that methylene blue
might be useful in treating psychoses, the phenothiazines were ignored until the 1940's when French drug manufacturer, Rhone Poulenc, began synthesizing and testing derivatives. The most promising were those with a dimethylaminopropyl group attached to the ring nitrogen. These compounds had strong anti-histaminic and sedative properties. Chlorpromazine, synthesized by Charpentier in 1950, was developed to potentiate the sedative effects of barbiturates. A French surgeon, Laborit, used it in combination with meperidine and external cooling of body temperature to produce a sort of artificial hibernation for surgery. During the early 1950's, chlorpromazine enjoyed extensive use in Europe and was named Largactil, meaning "many actions". Today it is known as Thorazine.

In 1952, Delay and Deniker took advantage of its potentiating effect with analgesics to treat hyperactive psychotic patients. Chlorpromazine was introduced into the United States in 1954, and was used as an antiemetic in addition to its uses as an antipsychotic and a potentiator of anesthetics, analgesics, and sedatives.

The varied uses of chlorpromazine inspired a search for similarly active derivatives. Over twenty of these derivatives are in use today and more than half of them are for the treatment of mental disorders [2].
Structure-Activity Relationship

Although phenothiazine and imipramine are similar in chemical structure (Figure 1), their modes of action are quite different.

Phenothiazine, when substituted with a dimethyamino-propyl group at the 10 position, becomes an active neuroleptic capable of blocking the actions of dopamine in the basal ganglia and limbic areas of the forebrain. Three carbons are required between the ring and side chain nitrogen for neuroleptic activity. Two-carbon side chains yield derivatives that have antihistaminic or anticholinergic properties. Derivatives with four carbon side chains have some antitussive activity, but are not therapeutically useful [1].

Branching at the beta carbon of the side chain (C-2') also alters the spectrum of activity. Substitution by a small, nonpolar group such as a methyl at this position increases antihistaminic, antitussive, and antipruritic properties. Unsubstituted phenothiazine generally assumes a conformation in which the rings are folded up on an axis that bisects S(5) and N(10) with the side chain bent slightly up and intra to the rings. (Figure 2a) [5,22]

Substitution at C-2' sterically interferes with the hydrogens at positions 1 and 9, and the rotation about the ring nitrogen and alpha-carbon bond is hindered [2].
Side chain substitution also slows metabolic attack, increasing the duration of action.

The tertiary amino group in the side chain is also critical for activity and normal metabolism to the secondary or primary amino reduces activity. Larger N-alkyl substitutions of the tertiary amine also decreases potency. [1]

There exists a group of phenothiazines with a piperidine in the side chain. These drugs have a greater potency than those with aliphatic sidechains and less extrapyramidal side effects. (Figure 1c) A third group of phenothiazines have a piperazine side chain. (Figure 1d) These compounds have optimal potency, and minimal autonomic side effects such as hypotension. However, acute extrapyramidal side effects are often experienced with these drugs [1].

The side chain of imipramine and its derivatives can be of either two or three carbons in length to retain antidepressant properties. These tricyclics inhibit the reuptake of norepineprine and serotonin into central neurons, and their effects are dependent on the type of substitution on the side chain nitrogen. A sidechain with a dimethylamino group yields a compound that tends to be more effective in inhibiting serotonin uptake and is useful in treating agitated depression. If the amino group is secondary in nature with only one methyl group bonded, such as
Figure 1. Structures and numbering of tricyclic compounds.

a. IMIPRAMINE
b. PROMAZINE
c. THIORIDAZINE (piperidine side chain)
d. FLUPHENAZINE (piperazine side chain)
desipramine, the compound tends to be more effective in inhibiting norepinephrine uptake and is useful in treating retarded depression [3,16]. It is thought that normal metabolism in vivo of imipramine yields the more potent desipramine.

Substitution about the benzene rings has been of particular interest in both systems. The 3-chloro derivative of imipramine, clomipramine, is used in Europe as antidepressant and sedative. [1] Other electron withdrawing substituents at this position have similar properties but none exceed the potency of imipramine. The addition of chlorine at the 4 position has been studied by our laboratory and the conformation of this compound was compared to that of 3-chloro-imipramine. [4] X-ray studies have shown that the tricyclic conforms to a structure in which the benzene rings are folded back and twisting occurs in the azepine ring. The protons of C(10) and C(11) are magnetically equivalent as the carbons to which they are bonded rapidly flip back and forth. Although little difference is seen in the ring conformations of imipramine and its 3- and 4-substituted derivatives, there is an effect on the position of the side chain. The side chains of imipramine and the 3-chloro derivative are normally pointed up towards the plane of the C(4) and C(6) carbons and form an angle of 13-35° to the C(4a)-N(5)-C(6a) plane. (Figure 2a) The angle about N(5)-C(4a) is
Figure 2. Three dimensional configurations of Cl-imipramine.

a. 3-Cl-Imipramine (13–35° intra)

b. 4-Cl-Imipramine (coplanar)
a. 2-Cl-Promazine (17° intra)

b. 1-Cl-Promazine (25° extra)

Figure 3. Three dimensional configurations of Cl-promazine.
such that C(l') of the side chain and C(4) are synperiplanar [23] to each other. Nitrogen is sp$^2$ hybridized and the sum of the bond angles about it is 348 to 355°. [4] The intra conformation [22] of the N-substituent is believed to be essential for antidepressant activity [5,10].

The 4-chloro derivative has a side chain that lies in the same plane as C(4a)-N(5)-C(6a), due to a sp$^2$ hybridized nitrogen that has a sum of angles about it of 360° instead of the usual 348-355°. This conformation is assumed to avoid steric interference with the peri chlorine on C(4). (Figure 2b) The lone electron pair in this p orbital does not appear to overlap with the p orbitals of C(6a) and C(4a).

A similar study was done on 1-chloro- and 2-chloro-promazine. Again, promazine assumes a conformation in which the benzo rings of the tricyclic are folded about a axis formed by a line bisecting S(5) and N(10) when nitrogen is tetragonal (sp$^3$). The angle between the benzo rings is 140°. In promazine and 2-chloro-promazine, the side chain is oriented intra to the C(9a)-N(10)-C(1a) plane, forming a 17° angle with it. (Figure 3a) The conformation about the bond N(10)-C(1a) is one in which C(l') of the side chain and C(l) of the benzene ring are synperiplanar. The 1-chloro derivative differs in that its side chain is extra [22] to this plane and forms a 25° angle with it. C(l') and C(l) are synclinal [23] to each other. This may occur in order
for the side chain to avoid steric interaction with the chlorine on C(1). Also, the central ring is somewhat flatter with an angle between the benzo rings of 148°. (Figure 3b)[5]

The tricyclic structure is nearly planar when nitrogen is trigonal (sp²), and the conformations of the N-substituent become nearly equivalent. The hybridization of N(10) appears to be sp² when it is substituted with the side chain, and the p character increases with the size of the substituent. The lone electron pair in nitrogen's p orbital can delocalize into the aromatic ring when the side chain is intra, but is unable to delocalize when the sidechain is extra to the rings. [13]

The significance of the position of the side chain relative to the ring system has been a matter of debate. Some believe that it may affect the fit of the tricyclic into the receptors that govern neurotransmitter uptake by more closely resembling the conformation of one neurotransmitter over another. There are numerous studies available on molecular modeling that attempt to superimpose various tricyclics over dopamine, serotonin, and norepinephrine to show similarities in conformation. [6,7] Kaiser and Setler reported that 1-chlorpromazine, with its side chain in an unfavorable conformation, lacks the neuroleptic activity typical of phenothiazines and has antidepressant activity in
the animal models. [8] Our laboratory found that the side chain of this compound assumes an extra conformation, and appears to resemble the conformation assumed by 4-chloroimipramine.

**Screening Tricyclic Compounds in Animal Models**

The myriad of drugs that are synthesized each year call for a quick method of screening for possible utility in humans. This requires an animal model that approximates the human. Although primates are our closest relatives, the expense of acquiring and housing them, and lack of availability makes them impractical to use as a screening model. Small rodents have become popular for use in screening because they meet the criteria of expense and availability. They are sensitive to the effects of tricyclics, although not in the same ways as humans, so that parallels between the two species are difficult to make. One can find numerous examples in the literature of tricyclic screening in the tissues of mice, rats, and guinea pigs. Generally, the tissues used to screen for norepinephrine uptake inhibition are various brain structures, either whole, in slices, or as synaptosomes. Screens for serotonin uptake inhibition employ the same tissues or human blood platelets. Again, the link in activity of a compound on serotonin uptake sites in platelets and in the CNS can be difficult to make. In vitro
animal tissue screens for uptake inhibitors have several disadvantages. Active compounds may have toxicities that are not apparent in an in vitro system. Also, compounds with activity in animal tissue may not be active in the human, or vice versa. One can only guess at how many valuable therapeutics may have been overlooked in animal screens.

A recent technique combines the animal model with the human model, by incubating rat synaptosomes in human plasma. The amount of labeled neurotransmitter taken up by the synaptosomes can be measured, and this reveals the quantity of inhibitor and its active metabolites in the human plasma. This technique has proven valuable in studying the in vivo effects of tricyclics and has suggested the presence of previously unknown hydroxylated metabolites. [17]

The theories on the mechanisms of depression and psychoses have been formed by inference on how tricyclic drugs act in animals. For example, it is known that amphetamine and methylphenidate increase the amount of dopamine at receptors and exacerbate symptoms of acute schizophrenia in humans. Phenothiazines block dopamine receptors and improve the symptoms of schizophrenia in milligram potency [9]. From this indirect evidence it has been concluded that some psychoses caused by faulty dopaminergic activity in the CNS.

However, there is evidence against the dopamine
hypothesis. Apomorphine also has dopamine-like actions, but does not exacerbate schizophrenia. Amphetamine does not worsen the symptoms of the chronic schizophrenic, which may point to different mechanisms in the chronic and acute disease states. Finally, defects in the CNS dopaminergic systems of psychotic patients have yet to be proven [9].

Therefore, it is impossible to deduce the mechanism of phenothiazine in human psychotics from its effects in the animal model. The dopamine blockade may be the primary effect, or this action may only be on a condition that is secondary to the disease. [9]

Similar problems are encountered in determining the mechanism by which tricyclic antidepressants alleviate symptoms of depression. Imipramine and its derivatives block the reuptake of norepinephrine and serotonin into their respective nerve terminals. Although this effect is immediate, depressive symptoms take up to three weeks to disappear. Some speculate that the delayed therapeutic effect is due to a gradual change in receptor sensitivity in the CNS to the transmitters at the synaptic level. Also, two drugs with potent norepinephrine uptake inhibition, amphetamine and cocaine, are poor antidepressants. [1] If the present day theory were valid, one would expect all norepinephrine uptake inhibitors to relieve symptoms of depression.
Methods

Synthesis of 1-Alkyl-10-[3-(dimethylamino)propyl]phenothiazine

The 1-methyl phenothiazine had been previously synthesized by Mary Craven according to the method described by Hallberg, et al.[10] They were able to synthesize 1-alkyl-phenothiazines through a proposed benzyne intermediate. The phenothiazyne intermediate (10-lithio-1,2-dehydrophenothiazine) was formed by the metallation of 2-C1 phenothiazine with methyllithium. The mechanism is believed to be as follows:

1. N(10) exchanges its proton for a Li atom.
2. The electropositive lithium bonded to N(10) coordinates with alkyllithium to direct a second lithium-proton exchange at C(1).
3. Elimination of Li from C(1) and Cl from C(2) occurs spontaneously, leaving a triple bond between C(1) and C(2).
4. Excess alkyllithium nucleophilically attacks C(1). This ortho alkylation is again guided by the lithium bonded to N(10). A second lithiation occurs at C(2).
5. Quenching with H2O replaces the lithium atoms with protons.

Evidence for the above mechanism is given by the absence of the 2-alkylated isomer. Alkyl- and aryllithiums are known to be electron deficient and can behave as Lewis
Acids. This explains the readiness to coordinate with the N(10) amine which is a Lewis Base. The authors were unable to trap the phenothiazyne intermediate. The reaction has also been done with excess methyl-, n-butyl-, s-butyl-, t-butyl-, and phenyllithium to yield the corresponding 1-alkyl or aryl- derivatives. The strong reactivity of the higher substituted alkylolithiums requires them to be transferred under N₂ to a reaction flask at -70°.

The N-alkylation of the phenothiazine with 3-dimethylaminopropanol yields the corresponding promazine. This was done according to the method of Craig, et al.[11], with the exception that sodium hydride was used instead of sodamide for the base. (Figure 4)

**Synthesis of 4- and 5-substituted 10,11-dihydro-5H-dibenz[b,f]azepine**

The dilithiation of dibenzazepine at the 4 and 5 positions has been described by Dahlgren, et al. [12] The mechanism is believed to be very similar to that described for phenothiazine, where the lone electron pair on lithiated N(5) directs a second lithium to the adjacent carbon. Once lithiated, C(4) becomes a carbanion and readily reacts with dibromoethane to form the product in good yield. (Figure 5)

We found that the product could be easily substituted at N(5) once the proton had been abstracted. Initial
Figure 4. Synthesis of 1-methyl-phenothiazine.
Figure 5. Synthesis of 4-Br-5-TMS-iminodibenzyl.
attempts to deprotonate with NaH failed, but we were successful in using a stronger base such as lithium dialkylamine.

To form the 4-hydroxymethyl substituted product, the dilithiated intermediate was reacted with dimethylformamide. This reagent electrophilically attacks the C(4) carbanion to add a methanal which is easily converted to the alcohol by hydride reduction.

Elemental analysis gave a carbon content that was 0.8% lower than expected, which may be due to the presence of a slight amount of water in the crystals. The 90 mHz NMR confirmed the structure of the product. (Appendix II)

\[
\text{4-MeOH-10,11-dihydro-5-H-dibenz[b,f]azepine}
\]
**Serotonin and Norepinephrine Uptake Inhibition Assays**

The uptake inhibition assays for each of the two neurotransmitters are very similar. The following description is for both assays, and distinctions are made as needed. Descriptions of chemicals, animals, buffer and equipment can be found in the experimental section.

**Preparation of Synaptosomes**

Synaptosomes, an artifact of neuronal homogenates, are commonly used in uptake studies. When the membrane of a neuron is disrupted by homogenization, fragments can reseal themselves to form small spherical bodies or synaptosomes. Synaptosomes from nerve terminal membranes will be active as long as they have formed with their receptors facing outwards.

Male Sprague-Dawley rats, ranging in weight from 150-300 grams, were quickly decapitated and the brains were removed. Dissection and the following tissue preparation were done at 4°C. All instruments, solutions, and glassware were prechilled before coming into contact with the tissue. The technique for dissection was described by Glowinski and Iverson [14]. Midbrain with thalamus, subthalamus, and hypothalamus was retained for the serotonin assay. Cortex was retained for the norepinephrine assay.
The tissue was weighed and covered with 10 volumes of 0.32 M sucrose. The mixture was homogenized in a glass tube by ten up and down strokes with a rotating teflon pestle.

The method for isolation of P_2 fragments was that described by Gray and Whittaker with modifications [15]. The homogenate was centrifuged at 2750 rpm for 10 minutes. The supernatent (S_1) was separated from the pellet by (P_1) pipet and recentrifuged at 11250 rpm for 30 minutes. P_1 was discarded. The following the second spin, S_2 was decanted and P_2 was resuspended in 10 volumes to original weight of Krebs-Henseleit buffer (K-H buffer).

**Preparation of Assay Tubes**

The assay was done in 1.5 ml Eppendorf microtubes and each dilution of inhibitor was assayed in triplicate. If the synaptosomes were to be harvested by filtration, the assay was performed in 2 ml glass test tubes. The inhibitory compound dilutions were made the day prior to the assay and were 25 times more concentrated to account for dilution in the assay tubes by the synaptosome medium. On the morning of the assay, 20 microliters (ul) of each compound dilution and 410 ul of K-H buffer with 10^{-4}M pargyline were placed into the microtubes. The tubes were immersed in ice water and 50 ul of synaptosome preparation were added to each tube. The tubes were capped and kept on ice.
Measuring Uptake of Tritiated Neurotransmitter

To begin the uptake process, each tube was pre-warmed for 5 minutes in a 37°C shaker bath. Stock solutions of tritiated serotonin or norepinephrine were diluted in 10^{-4}M ascorbic acid to give a final dilution in the assay tube of 10^{-8}M. Following the pre-incubation, 20 μl of tritiated neurotransmitter were added, the tube was vortexed, and returned to the shaker bath to initiate the uptake process. The serotonin assay required 4 minutes incubation for optimal uptake and the norepinephrine assay required 6 minutes. Uptake was terminated by the addition of 0.8 ml of ice cold K-H buffer containing 10^{-6}M nontritiated or "cold" neurotransmitter. These diluted tubes were kept on ice until the assay was complete.

The next step was to separate the synaptosomes containing actively taken up neurotransmitter from the rest of the assay medium. Two methods were employed for this study. The first method entailed pelleting the tissue by centrifugation, washing the pellets, and then measuring the amount of radioactivity present. This method recovered a good yield of tissue, but was very slow since each tube was handled individually. This method was used for all of the norepinephrine assays and for 1/3 of the serotonin assays. The remainder of the assays were concluded by filtration, which provides a rapid separation of tissue from medium. A
comparison of the two methods revealed that roughly 50% of the tissue was lost in filtration. (Figure 6) This low yield did not have a significant effect in determining the IC$_{50}$ values of the compounds for serotonin uptake since the total uptake was good (0.7 pmoles/mg protein/4 minutes). However, the norepinephrine uptake was much less efficient (0.3 pmoles/mg protein/6 minutes) and the difference between total and nonspecific uptake following filtration was too small to allow for accurate determination of the IC$_{50}$ values. Descriptions of centrifugation and filtration techniques follow respectively.

The microcentrifuge was prechilled in a 4° C cold room. The assay tubes were spun for 2 minutes to pellet the synaptosomes. The supernatent was aspirated with a vacuum pump and the pellets were washed with 0.8 ml of K-H buffer. The tubes were vortexed and recentrifuged for 2 minutes. The first washes were aspirated and the washing procedure was repeated. After removal of the final wash, the pellets were ready for quantitation and could be handled at room temperature. The bottoms of the tubes holding the pellets were cut away with tin snips and transferred to 20 ml scintillation tubes. The organic material was dissolved in 12 ml scintillation cocktail, and the vials were shaken mechanically for 30 minutes to optimize the extraction of radioactivity. Radioactivity was then measured in a scintillation counter.
Figure 6. Comparison of centrifugation vs. filtration in the serotonin uptake inhibition assay.
Synaptosomes that were harvested by filtration could be handled in batches of 24 each, and were done on Whatman GF/B papers in a cell harvester. The filters were washed twice with 3 ml K-H buffer, allowed to dry, and then transferred to scintillation vials. Preparation for quantitation of radioactivity was the same as described above.

Nonspecific Binding
Nonspecific binding is the amount of radioactivity that is not actively taken up by the synaptosomes, but remains in measurable amounts at the conclusion of the assay. The radioligands have been shown to bind nonspecifically to the tissues in the assay tube, and to nonorganic components such as test tubes and filters. Excess radioligand may remain as a result of insufficient washing of tissues on separation filters or may be trapped within the interstitial spaces of centrifuged tissues.[16]

The amount of nonspecific binding in our experiments was measured by placing tubes without inhibitory compound at the beginning and end of each assay. The tubes were not pre-incubated, tritiated neurotransmitter and 0.8 ml cold buffer were added simultaneously, and the tubes remained on ice until the assay was complete.

We found that there was a greater amount of nonspecific binding in the tubes at the beginning of the
assay as opposed to those at the end. This indicated that nonspecific binding increased with time. Therefore, we added 100 fold excess of unlabeled neurotransmitter to the buffer used to stop active uptake by dilution.

Calculating IC\textsubscript{50}'s

Although several methods for calculating IC\textsubscript{50}'s were examined, we found greatest consistency in using the graphical method. Total uptake (A\textsubscript{0}) was defined as the amount of labeled neurotransmitter accumulated in the absence of inhibitory compound. It is customary to define specific uptake as the amount of labelled neurotransmitter accumulated minus the amount accumulated at 0\textdegree in the presence of excess "cold" neurotransmitter. However, nonspecific uptake uptake in our assays was small (about 3 x 10^{-15} moles/mg/min) and subtracting it from the specific uptakes did not change the IC\textsubscript{50} values. Therefore, we did not include it in the calculations.

A plot was made of uptake vs. inhibitor concentration and the distance on the Y axis at zero between nonspecific and total uptake was measured. A line is extrapolated from the midpoint of this distance to the curve. A line dropped from the intersection on the curve to the X axis gives the IC\textsubscript{50} concentration of inhibitor. Refer to Table I for the IC\textsubscript{50}'s for the compounds we tested.
RESULTS

PHARMACOLOGY

The pharmacological studies of 1-substituted promazines have shown us that substitution at this site does not increase the potency of these compounds in inhibiting neurotransmitter uptake. (Table 1) The 1-substituted promazines IC₅₀ for serotonin uptake was approximately \(2 \times 10^{-6}\) M, which is potent enough for therapeutic utility but 10 fold below the potency of imipramine \(2 \times 10^{-7}\) M. Molecular studies have shown that these compounds assume a side chain conformation in the extra position similar to that assumed by 4-Cl-imipramine. However, their inability to inhibit uptake of serotonin, and probably norepinephrine, at low concentrations may allow us to speculate that this conformational similarity to the antidepressants does not impart the same pharmacological activity. An extensive search of the literature failed to turn up similar testing of 1-substituted promazines by other laboratories within the last ten years. Their absence in the literature may be due to their less than promising performance as uptake inhibitors of the monoamines involved in depression. Although we did not examine their potency in blocking dopamine receptors, a comparison with their parent compounds would be interesting.
The disubstituted promazine, 1-Br-2-CF₃-promazine, appears to be somewhat more potent in inhibiting serotonin uptake with an IC₅₀ of about 5 x 10⁻⁷ M. Apparently, substitution at the 2-position with the very electron withdrawing and lipophilic trifluoromethyl group allows potency approaching that of imipramine. The dopamine blocking activity of 2-CF₃-promazine has been known since the mid-1950's, and substituents with similar Hansch pi values, such as the halogens, have similar activity. Groups containing fluorine, such as CF₃, SCF₃, and SO₂CF₃ are known for their strong lipophilic character which enhances a compound's ability to reach its site of action in the tissue. [2] This lipophicity, along with the electron attracting ability of the trifluoromethyl group, may partially improve the activity of the 1-substituted promazines. However, the combination of groups at both sites does not appear to increase the potency of these compounds over that of the parent compound. The 4-substituted imipramines had similar IC₅₀'s for serotonin uptake. The combined average for both the chloro- and bromo-substituted compounds was 463 ± 59 nm (mean ± standard deviation, n= 6). This is about three times greater than the IC₅₀ we found for unsubstituted imipramine. Therefore, we can assume that the halide substitution at the 4-position does not increase the potency of imipramine for inhibiting serotonin uptake.
Table 1. IC₅₀ values for 1-substituted promazines and 4-substituted imipramines.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Neurotransmitter</th>
<th>IC₅₀</th>
<th>Assay a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(nm)</td>
<td>Number</td>
</tr>
<tr>
<td>1-Me-Promazine</td>
<td>NA</td>
<td>141</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1259</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>8</td>
</tr>
<tr>
<td>1-Br-Promazine</td>
<td>NA</td>
<td>1995</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>398</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3162</td>
<td>9</td>
</tr>
<tr>
<td>1-Cl-Promazine</td>
<td>NA</td>
<td>1122</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1585</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>126</td>
<td>8</td>
</tr>
<tr>
<td>1-Br-2-CF₃-Promazine</td>
<td>NA</td>
<td>2239</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>708</td>
<td>5</td>
</tr>
<tr>
<td>4-Cl-Imipramine</td>
<td>NA</td>
<td>282</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>141</td>
<td>8</td>
</tr>
<tr>
<td>4-Br-Imipramine</td>
<td>NA</td>
<td>141</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56</td>
<td>8</td>
</tr>
<tr>
<td>Imipramine</td>
<td>NA</td>
<td>198 + 109 b (n= 3)</td>
<td></td>
</tr>
<tr>
<td>Desipramine</td>
<td>NA</td>
<td>12 + 3.6 b (n= 4)</td>
<td></td>
</tr>
</tbody>
</table>

a Each assay was designated a number for ease in comparing the various IC₅₀'s.

b Values represent the mean + the standard deviation.

c Norepinephrine
<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Neurotransmitter</th>
<th>IC$_{50}$ (nm)</th>
<th>Assay $^a$ Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Me-Promazine</td>
<td>5-HT$^c$</td>
<td>3162</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1259</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1514</td>
<td>E</td>
</tr>
<tr>
<td>1-Br-Promazine</td>
<td>5-HT</td>
<td>2512</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3981</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2492</td>
<td>H</td>
</tr>
<tr>
<td>1-Cl-Promazine</td>
<td>5-HT</td>
<td>1585</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1413</td>
<td>H</td>
</tr>
<tr>
<td>1-Br-2-CF$_3$-Promazine</td>
<td>5-HT</td>
<td>179</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>501</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td></td>
<td>891</td>
<td>H</td>
</tr>
<tr>
<td>4-Cl-Imipramine</td>
<td>5-HT</td>
<td>398</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>501</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>422</td>
<td>H</td>
</tr>
<tr>
<td>4-Br-Imipramine</td>
<td>5-HT</td>
<td>447</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>562</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>447</td>
<td>H</td>
</tr>
<tr>
<td>Imipramine</td>
<td>5-HT</td>
<td>150 + 54 $^b$</td>
<td>(n= 8)</td>
</tr>
</tbody>
</table>

$^a$ Each assay was assigned a letter for ease in comparing the various IC$_{50}$ values.

$^b$ Value represents the mean + the standard deviation.

$^c$ Serotonin
Generalizations about norepinephrine uptake are much more difficult, as an examination of Table 1 will reveal that reproducibility of our assay was poor. It is impossible to make any conclusions about the promazine compounds ability to inhibit norepinephrine uptake, except to state that they appear to be potent within a micromolar concentration. The results for the 4-substituted imipramines were somewhat better and we found a combined average IC$_{50}$ of 144 + 85 nm (mean + standard deviation, n=5). This is equivalent to the value we found for imipramine, and it appears that although the 4-halo-substitution does not improve potency, it does not impair the potency as it seems to do in serotonin uptake inhibition.

The problem of reproducibility in the uptake inhibition was one that we were not able to solve within a limited time frame. Our first suspicion was that the compounds were not pure, but analysis by thin layer chromatography and UV absorption failed to show the presence of any impurities.

Uptake by the synaptosomes was linear over time up to 6 minutes for tritiated serotonin and up to 7 minutes for tritiated norepinephrine. (Figure 7) Assay incubation times were chosen from points on the linear portions of the curve. Figure 8 compares total uptake in the absence of inhibitor with the amount of protein present in the synaptosomal
preparation. Each point on the graphs represents an individual assay. Uptake and protein concentration are shown to be proportionally related. Protein concentration was independent of brain area used to prepare the synaptosomes. Midbrain and cortical preparations yielded 2.5 to 5 mg protein/ml. Manipulation of the data revealed that the method used to separate tissue from medium may affect the results. The methods were initially compared using imipramine as a control, and the results obtained from both filtering the tissue away from the medium and pelleting the tissue by centrifugation were within experimental error. (Table 2) However, comparison of the IC50's for the other compounds reveals a trend in which filtration gave lower potencies.

There are problems inherent in both separation techniques, although it is difficult to explain why one technique gives consistently larger IC50's than the other. In figure 6, we showed that the curve obtained from the filtered assay is much flatter than the centrifugation curve. This may be a result of synaptosome lysis and loss of contents through the filters. Also, the steeper curves obtained with centrifugation may be due to excess labeled ligand in the medium becoming trapped within the interstitial spaces of the pellet. More needs to be done with this phase of the assay to improve reproducibility.
Table 2. Effect of tissue separation method on IC$_{50}$'s for serotonin uptake.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>IC$_{50}$ (Centrifuge)</th>
<th>IC$_{50}$ (Filter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipramine</td>
<td>138$^a$</td>
<td>163</td>
</tr>
<tr>
<td>1-Cl-Promazine</td>
<td>1961</td>
<td>2341</td>
</tr>
<tr>
<td></td>
<td>1207</td>
<td>2136</td>
</tr>
<tr>
<td>1-Br-Promazine</td>
<td>1362</td>
<td>3831</td>
</tr>
<tr>
<td></td>
<td>1287</td>
<td>2482</td>
</tr>
<tr>
<td>1-Br-2-CF$_3$-Promazine</td>
<td>181</td>
<td>468</td>
</tr>
<tr>
<td></td>
<td></td>
<td>717</td>
</tr>
<tr>
<td>4-Cl-Imipramine</td>
<td>303</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>342</td>
<td>436</td>
</tr>
<tr>
<td>4-Br-Imipramine</td>
<td>339</td>
<td>434</td>
</tr>
<tr>
<td></td>
<td>193</td>
<td>456</td>
</tr>
</tbody>
</table>

$^a$ Values are in nanomoles.
a. Serotonin uptake.

b. Norepinephrine uptake.

Figure 7. Neurotransmitter uptake vs. time.
a. Serotonin uptake.

b. Norepinephrine uptake.

Figure 8. Neurotransmitter uptake vs. protein concentration.
Tuomisto, et al (1980), report good results with the use of 0.45 μm pore size Millipore filters in their assay. The Whatman filters that we used have a 1.0 μm pore size, which may be too large to effectively retain the synaptosomes.

Disrupting and rewashing the pellets of centrifuged synaptosomes would decrease the amount of labeled ligand that may become trapped within the interstitial spaces of the pellet. However, this would increase the working time in an assay that is already quite time consuming.

Another problem we encountered was that of biphasic curves. An ideal uptake inhibition curve should be sigmoid in shape with a single linear drop from total uptake to nonspecific uptake. However, we obtained biphasic curves for norepinephrine uptake inhibition by 1-methyl-promazine, 1-Br-promazine, imipramine, and desipramine. Ross and Renyi encountered a similar phenomena in their work with rat midbrain-hypothalamus slices and hypothesized the presence of two uptake sites for labeled norepinephrine with different sensitivity to the tested uptake inhibitors. [18] Javaid, et al., reported biphasic curves with synaptosomes prepared from whole rat brain when uptake was inhibited by imipramine, desipramine, and their 2-hydroxy metabolites. [19] It appears that two distinct uptake mechanisms were present in our cortical preparations that were affected by different concentrations of inhibitors. The second uptake
sites appear to be affected at concentrations of $10^{-5}$ to $10^{-4}$ M for all four of the above listed inhibitors. This shows a considerably greater resistance to the inhibitors than the sites we assumed to be associated with the actual presynaptic uptake mechanisms.

The biphasic curves for norepinephrine uptake inhibition presented a problem in calculating IC$_{50}$ concentrations since uptake was still occurring at fairly high concentrations of inhibitor in the second, more resistant sites (Appendix II-v,vi). Calculating IC$_{50}$'s by the method described on page 25 was impossible since the midpoint was often below the curve. We found greater consistency by extrapolating from a midpoint on the Y axis between the total uptake and the uptake at the highest concentration of inhibitor. Reproducibility may be improved by adding points of still higher concentrations of inhibitor to the curve until uptake approaches the level of nonspecific binding.

Assay reproducibility may also improve if the level of tritiated norepinephrine uptake can be increased. Comparison of the curves in Appendix I reveals that the level of total uptake in the serotonin assay was two to three times greater than the level seen for the norepinephrine assay. Higher total uptake yields a longer linear section to the sigmoid curve and improves accuracy in determining the IC$_{50}$ concentration. Pretreating rats 13
hours before decapitation with reserpine by i.p. injection depletes endogenous norepinephrine, therefore eliminating its competition for uptake with tritiated norepinephrine during the assay. Seidler, et al., found that the addition of 1mM of ATP to the assay medium gave a four-fold increase in norepinephrine uptake by storage vesicles prepared from whole rat brain [20]. They also found a more linear relationship between uptake vs. time at 30° as opposed to 37°C. Perhaps a shorter incubation time, such as 4 minutes, at a slightly lower temperature would improve our assay reproducibility.
Molecular Modeling for Tricyclic Drugs

Rotation about sigma bonds is of interest at three points in the iminodibenzyl molecule. The groups about the C(10)-C(11) sigma bond in the ethano bridge are capable of limited rotation between two "flip forms". (refer to Figure 1b for numbering) For 4-Br-5-TMS-iminodibenzyl, the barrier to inversion from one form to the other was calculated to be 7 kcal/mole, which is low enough to occur at room temperature. In this case, one flip form is lower in energy by 3 kcal/mole. (Figure 9) We were able to calculate the equilibrium constant, $K_{eq}$, for conversion between the two flip forms using the equation:

$$F = -RT\ln K_{eq}$$

$K_{eq}$ was calculated to be $5 \times 10^{-3}$, meaning that 99.5% of 4-Br-5-TMS-iminodibenzyl molecules are found in the low energy flip form at room temperature. Referral to a molecular model revealed that in the less stable flip form, steric interaction between the bromine and the substituent bonded to the azepine nitrogen causes this conformation to be energetically unfavorable.

We were also able to calculate the energy required to rotate about the N-Si bond of 4-Br-5-TMS-iminodibenzyl. Free rotation about this bond is inhibited by bromine and by the closest proton of the ethano bridge. Figure 10 compares the
Figure 9. C(10)-C(11) sigma bond rotation for 4-Br-5-TMS-iminodibenzyl.
Figure 10. N(5)-Si sigma bond rotation for 4-Br-5-TMS iminodibenzyl.
energetics of rotation for both of the ethano bridge flip forms. The lower curve represents rotation about the N-Si bond for the more stable flip form and the upper curve represents the least stable form. Since the trimethylsilyl group has a three-fold axis, the energy diagram would repeat itself every 120° as the groups about the N-Si bond are rotated a full 360°. Therefore the calculations were only done for a 120° rotation.

The high point for each diagram occurs when one of the TMS methyl groups approaches bromine. The lowest points on the energy diagrams occur when two of the TMS methyls are located on both sides of, and as far away as possible from, the bromine. The barriers to rotation about the N-Si bond are 1.4 and 1.8 kcal/mole for the high and low energy flip forms, respectively.

The most significant bond rotation is about N(5)-C(4a) for 4- and 5-substituted iminodibenzy1. The lowest energy conformation of imipramine has been calculated by our laboratory to have an angle between C(1')-N(5)-C(4a)-C(4) of 35°. The same angle for 4-substituted imipramine increase with the size of the substituent. The lowest energy conformation for 4-Cl- and 4-Br-imipramine have C(1')-N(5)-C(4a)-C(4) angles of 66° and 88°, respectively. The energy barrier to the eclipsed conformation of 4-substituted imipramines have been calculated to be greater than 25
kcal/mole and thus unlikely to occur at room temperature. The substituents are simply too large to pass by each other and this allows for stereoisomerism.

This observation is confirmed by the $^1$H-NMR of 4-Br-5-TMS-iminodibenzyl, in which the four protons of the ethane bridge are nonequivalent. Table 3 compares the chemical shifts for imipramine, which is a 4-substituted iminodibenzyl, and two 4-,5-substituted iminodibenzyls. Imipramine's dimethylaminopropyl side chain moves freely as rotation occurs about the N(5)-C(4a) bond and the four protons of the ethane bridge are chemically equivalent, forming a single peak at 3.06 ppm. Addition of a halide group at C(4) inhibits the azepine nitrogen's ability to invert. The $^1$H-NMR gives a separate multiplet for each of the bridge protons with first order splitting patterns. We were able to calculate coupling constants for the ethano bridge protons of 4-Br-5-TMS-iminodibenzyl after simplifying the spectra by decoupling experiments. Table 4 compares the coupling constants for two 4- and 5-substituted iminodibenzyls. Both compounds have geminal coupling of about 13 Hz between the protons of C(10). The larger geminal coupling of the C(11) protons of 17 Hz is due to an inductive effect by bromine. Both compounds had typically large axial-axial couplings, and small couplings for axial-equatorial and equatorial-equatorial protons.
Table 3. $^1$H-NMR chemical shifts for imipramine and derivatives.

![Chemical structure of imipramine](image)

<table>
<thead>
<tr>
<th>Proton</th>
<th>Imipramine [21]</th>
<th>4-Cl-Imipramine$^a$</th>
<th>4-Br-5-TMS-Iminodibenzyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 eq</td>
<td>3.06$^b$</td>
<td>2.72</td>
<td>2.68</td>
</tr>
<tr>
<td>10 ax</td>
<td>&quot;</td>
<td>3.66</td>
<td>3.59</td>
</tr>
<tr>
<td>11 eq</td>
<td>&quot;</td>
<td>3.25</td>
<td>3.27</td>
</tr>
<tr>
<td>11 ax</td>
<td>&quot;</td>
<td>2.87</td>
<td>2.86</td>
</tr>
</tbody>
</table>

$^a$ This unpublished data was determined previously by our laboratory.

$^b$ Chemical shifts are in ppm.
Table 4. $^1$H-NMR coupling constants for 4-Cl-imipramine and 4-Br-5-TMS-iminodibenzyl.

<table>
<thead>
<tr>
<th>Protons</th>
<th>4-Cl-Imipramine$^a$</th>
<th>4-Br-5-TMS Iminodibenzyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{10eq-10ax}$</td>
<td>13.0$^b$</td>
<td>13.0</td>
</tr>
<tr>
<td>$J_{10eq-1lleq}$</td>
<td>4.2</td>
<td>4.0</td>
</tr>
<tr>
<td>$J_{10eq-1lax}$</td>
<td>3.5</td>
<td>6.5</td>
</tr>
<tr>
<td>$J_{10ax-1lleq}$</td>
<td>3.7</td>
<td>4.5</td>
</tr>
<tr>
<td>$J_{10ax-1lax}$</td>
<td>13.7</td>
<td>13.4</td>
</tr>
<tr>
<td>$J_{1lleq-1lax}$</td>
<td>17.0</td>
<td>17.3</td>
</tr>
</tbody>
</table>

$^a$ This unpublished data was determined previously by our laboratory.

$^b$ Coupling constants are in Hz.
The stereoisomerism of 4- and 5-substituted iminodi-benzyl may have significance in the interaction of these compounds with serotonin and norepinephrine receptors. Resolution of the enantiomers on a chiral column and pharmacological testing of each one may reveal unique properties. One might expect to find that an enantiomer interacts with the receptors as an agonist, antagonist, or be void of activity.
EXPERIMENTAL

General

Since most of the syntheses described below are sensitive to water, all solvents have been dried by distillation over the appropriate reagents. The reactions were carried out in an atmosphere of dry nitrogen unless otherwise noted. Melting points were determined on an Electrothermal apparatus and were uncorrected. NMR (90 mHz) was done on a Jeol FX-90Q and NMR (250 mHz) was done on a Bruker WM-250. Elemental analysis was performed by Desert Analysis (Tucson, AZ).

For brevity, 10,11-dihydro-5-H-dibenz[b,f]azepine is referred to as "dibenzazepine" or "iminodibenzyl".

1-Methyl-10-(3-dimethylaminopropyl)-phenothiazine

3-Dimethylaminopropyl chloride hydrochloride, 1.16 g (7.1 mmoles), was dissolved in 10 ml of 5M KOH and stirred for several minutes. The resulting free base was extracted into toluene and stored overnight over 4A° molecular sieves (Union Carbide). 1-Methyl-phenothiazine, 0.96 g (4.5 mmole), was dissolved in 50 ml toluene. This solution was slowly dripped into a toluene solution containing 190 mg (4.8 mmole) of 60% NaH in mineral oil, which turned dark green.
The solution was refluxed for $3\frac{1}{2}$ hours. After refluxing, the 1-dimethylaminopropyl chloride was decanted from the sieves and slowly dripped into the phenothiazine solution. Refluxing was resumed overnight and the solution had become dark purple by morning. It was cooled to room temperature and quenched with 50 ml $H_2O$. The phases were separated and the $H_2O$ phase was extracted several times with toluene. All the toluenes were combined and extracted with 50% HCl. The HCl extracts were neutralized with 1 eq 50% KOH and further extracted with toluene. The toluene extracts were combined and dried over $Na_2SO_4$ for 2 days. Evaporation of the toluene yielded 700 mg of dark red oil. The oil was chromatographed twice on silica 60 (70-230 mesh) with 5% TEA: 45% EtOAc: 50% hexane. The pure 1-methyl-promazine was a purple oil. A final yield of 34% or 460 mg was obtained. Anal. Calcd. for $C_{18}H_{22}N_2S$: C, 72.44; H, 7.43; N, 9.39%. Found: C, 72.62; H, 7.47; N, 9.32%.

4-Br-10,11-Dihydro-5H-dibenz[b,f]azepine

28.5 ml (60 mmole) of n-butyllithium was slowly added to a solution of 3.9 g (20 mmole) 10,11-dihydro-5H-dibenz[b,f]azepine in diethyl ether at room temperature. The mixture was stirred for 24 hours and then cooled to 0°. Dibromoethane, 6 ml (70 mmole), was slowly added, and the
mixture was warmed to 25° and stirred for 4 hours. The reaction was quenched with 25 ml H$_2$O and the phases were separated. The H$_2$O phase was extracted several times with ether. The ethers were combined and dried over MgSO$_4$ for 1 hour. Evaporation of the ether left 900 mg of thin brown oil. The product was purified by column chromatography on silica 60 (70-230 mesh) with hexane. The yield was 2.92 g (60%) of a dark red oil.

4-Br-5-TMS-10,11-dihydro-5H-dibenz[b,f]azepine

Lithium-diisopropylamide was generated by combining 2.6 ml (5.5 mmole) n-butyllithium and 0.8 ml diisopropylamine (5.5 mmole) in diethyl ether at 0°. The reaction was warmed to room temperature and stirred for 1 hour. The reaction was cooled to -78° with a dry ice/acetone bath and 1.40 g (5 mmole) of 4-Br-dibenzazepine dissolved in 20 ml ether was added. The solution changed from colorless to yellow. The reaction was stirred at -78° for 1 hour. Next, 1.3 ml (10 mmole) of Cl-TMS was added to the reaction and no color change was evident. The reaction was warmed to room temperature and stirred for 19 hours. The solution had turned olive and contained a precipitate. Upon quenching with 20 ml H$_2$O, the precipitate redissolved, a gas was generated, and the solution turned orange. The phases were
separated and the ether was extracted several times with H$_2$O. The H$_2$O phase and extracts were combined and washed several times with ether. The ethers were combined and dried over MgSO$_4$. Product was isolated by chromatographing several times on silica 60 (230-400 mesh) with hexane. A clear oil was obtained in a yield of 550 mg (21%).

Anal. Calcd. for C$_{17}$H$_{20}$NSiBr: C, 58.96; H, 5.78; N, 4.05%.

Found: C, 59.38; H, 5.83; N, 3.94%.

4-Hydroxymethyl-10,11-dihydro-5H-dibenzo[b,f]azepine

Dibenazepine, 7.8 g (40 mmole), was dissolved in 200 ml diethyl ether at room temperature. n-Butyllithium, 57 ml of a 2.1 M solution (120 mmole), was slowly added. The mixture was stirred for 24 hours. The mixture was cooled to -78° with a dry ice/acetone bath and 9.3 ml (120 mmole) of DMF was added. The mixture was refluxed for 1 hour, using an oil bath, and then quenched with 100 ml H$_2$O.

The phases were separated, and the ether was extracted once with 0.5 N HCl and twice with H$_2$O saturated with NaCl. The H$_2$O phase was extracted twice with ether. The ethers were combined and dried overnight on MgSO$_4$.

The resulting aldehyde was reduced with 1.5 g (40 mmole) LiAlH$_4$ which was added at 0°. The reaction was warmed to room temperature and stirred for 3 hours. The reduction
was quenched with 100 ml H₂O. The solution was acidified to pH 3 with 0.5 N HCl, and the phases were separated. The H₂O phase was extracted several times with ether, and the ether phase was extracted several times with H₂O. The ethers were combined and dried over MgSO₄ for several days.

The ether was evaporated and 8.55 g of a green/brown oil remained. The product was isolated by chromatography on silica 60 (70-230 mesh) with 15% EtoAc:85% hexane. The yellow powder was recrystallized in cyclohexane to form 3.5 g (39%) of sparkling white crystals (mp 80-82°).

Anal. Calcd. for C₁₅H₁₅NO: C, 79.97; H, 6.71; N, 6.22%.
Found: C, 79.20; H, 6.68; N, 6.13%.

**Neurotransmitter Uptake Inhibition**

Levo-[7-³H]-Norepinephrine, 14.2 Ci/m mole, and 5-[1,2-³H(N)]-hydroxytryptamine, 29.2 Ci/m mole, were purchased from New England Nuclear Products. 5-Hydroxytryptamine creatinine sulfate and (-)-arterenol bitartrate salt were obtained from Sigma Chemical Co. Safety-Solve scintillation cocktail and plastic vials were purchased from Research Products International Corp.

The rats were purchased from Harlan Sprague-Dawley Co. Krebs-Henseleit buffer contained 127.8 mM NaCl, 5.8 mM KCl, 1.1 mM MgCl₂·6H₂O, 0.3 mM CaCl₂·2H₂O, 1.27 mM NaH₂PO₄·H₂O, 27.2 mM NaHCO₃, and 12.1 mM glucose. The NaHCO₃ was added last.
and the final solution was saturated with 95\% O_2:5\% CO_2 just before use. The pH was 7.2 to 7.4.

A Beckman JR-21 centrifuge was used for the synaptosome preparation. An Eppendorf 5413 microcentrifuge was used to pellet tissues at the end of the uptake experiments. It had a single speed that was preset at the factory. Experiments that were concluded by filtration were done on a Brandel M-24R cell harvester. Radioactivity was measured in a Packard Tri-Carb 460C scintillation counter.

**Molecular Modeling**

The strain energies for 4-Br-5-TMS-iminodibenzyl were calculated by using the MMPMI Program from the Quantum Chemistry Program Exchange [24] on an IBM clone computer. The nitrogen-silicon bond length was approximated to be 1.772 Å. The X-ray crystallography coordinates for 4-Cl-imipramine were entered into the program in lieu of determining the X-ray coordinates for 4-Br-5-TMS-iminodibenzyl, and the coordinates for the position of the 5-TMS group were approximated by hand.

The total energy for 4-Br-5-TMS-iminodibenzyl was calculated to be 32.4 kcal/mole.
APPENDIX I

Serotonin and Norepinephrine Uptake Inhibition by Perisubstituted Tricyclics

i. 1-Cl-promazine
ii. 1-Br-promazine
iii. 1-methyl-promazine
iv. 1-Br-2-CF₃-promazine
v. 4-Cl-imipramine
vi. 4-Br-imipramine
i. 1-Cl-promazine
ii. 1-Br-promazine
iii. 1-methyl-promazine
iv. 1-Br-2-CF$_3$-Promazine
v. 4-Cl-imipramine
vi. 4-Br-imipramine
APPENDIX II

$^1$H-NMR Chemical Shifts for 1-Methyl-Promazine and 4-MeOH-Iminodibenzyl

For 4-MeOH-iminodibenzyl, the hydroxyl proton was a broad peak at 1.75 ppm. The four protons of the ethano-bridge formed a singlet at 3.08 ppm. The methylene of 4-MeOH was a singlet at 4.75 ppm. The seven aromatic protons were in a multiplet ranging from 6.71 to 7.07 ppm. The proton of the azepine nitrogen formed a broad peak at 7.65 ppm.

For 1-methyl-promazine, the sidechain protons' chemical shifts were 3.74 ppm for $H_1$, (triplet), 1.75 ppm for $H_2$, (quintet), and 2.2 ppm for $H_3$. We were unable to determine the shape of the $H_3$ peaks as they were closely flanked by two other proton peaks, although a triplet would be expected. The two methyils bonded to the side chains' tertiary amine formed a single peak at 2.1 ppm. The methyl at position 1 absorbed as a singlet at 2.33 ppm, and the seven aromatic protons formed a multiplet ranging from 6.94 to 7.24 ppm. Refer to page 5 for atom numbering.
REFERENCES


