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New Antimuscarinic Agents for Improved Treatment of Poisoning by Cholinesterase Inhibitors

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This is to certify that the dissertation prepared by

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Doctor of Philosophy in Medicinal Chemistry

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Date

New Antimuscarinic Agents for Improved Treatment of Poisoning by Cholinesterase Inhibitors

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medicinal Chemistry at the Medical College of Virginia, Virginia Commonwealth University.

by

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B.S., Atlantic Christian College, 1976

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A special thanks to my parents for their enormous contribution to my career.

Finally, I wish to express my sincere gratitude to my wife, Diane, for her patience, encouragement and understanding. It is to her this work is dedicated.

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List of Abbreviations

ACh - acetylcholine

AChE - acetylcholinesterase

ChE - cholinesterase

DFP - diisopropylfluorophosphate

DMSO - dimethyl sulfoxide

DSS - 2,2-dimethyl-2-silapentane-5-sulfonate

IR - infrared

NMR - nuclear magnetic resonance

OMPA - octamethyl pyrophosphate

OP - organophosphate

2-PAM - pyridine-2-aldoxime methyl iodide

P2S - pyridine-2-aldoxime methyl methanesulfonate

QNB - quinuclidinyl benzilate

QSAR - quantitative structure-activity relationship

TEPP - tetraethyl pyrophosphate

THF - tetrahydrofuran

TMB-4 - 1,3-bis(pyridinium-4-aldoxime)propane dibromide

TMS - tetramethylsilane

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ABSTRACT

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medicinal Chemistry at the Medical College of Virginia, Virginia Commonwealth University.

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Advisor: James F. Stubbins, Ph.D.

Poisoning by organophosphate cholinesterase inhibitors results in a rapid rise in acetylcholine (ACh) in the synapse and many pronounced pharmacological effects in numerous tissues in the body. The treatment for organophosphorus poisoning involves administration of a cholinesterase-reactivating oxime to restore the activity of the cholinesterase and an antimuscarinic agent to block the response to the excess ACh. Presently atropine is the standard antimuscarinic agent used clinically as an antidote. This research was directed toward finding an antimuscarinic agent better than atropine as an antidote.

Since caramiphen and aprophen have been reported to be effective antidotes we synthesized numerous structural variations of these molecules with the aim of enhancing the antimuscarinic and antidotal properties. Many of these compounds showed enhanced antimuscarinic

properties. We synthesized analogs of caramiphen which have different substituents in the para position of the phenyl ring. The purpose of the set was to test the effect of different substituents on the antimuscarinic and antidotal activity and to look for any possible relationship of activity with substituent parameters such as Hammett's sigma (σ) or Hansch's pi (π) values. Four substituents were selected which have extreme values for sigma and pi in a positive or negative direction, in all combinations. The substituents chosen for use in this approach were the amino ($-\sigma$, $-\pi$); 1-tetrazolyl ($+\sigma$, $-\pi$); 1-pyrrolidinyl ($-\sigma$, $+\pi$); and the trifluoromethyl ($+\sigma$, $+\pi$).

Some N-substituted-1,6-hexanediamines were synthesized to examine the SAR for antimuscarinic and antidotal properties of this series. In a binding assay these compounds showed moderate affinity with a preference for the M2 receptor subtype. It was determined the bis-quaternary structure was not mandatory for muscarinic activity. The optimum compound of this series was N,N'-dimethyl-N,N'-bis[3-(2-phthalimido)propyl]-1,6-hexanediamine.

Also, a number of literature and currently manufactured antimuscarinics were obtained. All of the compounds are being screened in a number of *in vitro* and *in vivo* assays designed to give information on the SAR for the pharmacological properties which might be important as an antidote.

Current information from this research indicates the best antidotes are not the best antimuscarinics in the pharmacological assays. Good central antimuscarinic activity is the primary property

of a good antidote, although the antidotal effectiveness of an agent can not be described solely based on its antimuscarinic properties.

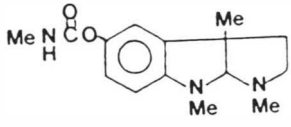
I. INTRODUCTION

A. Historical Background

Long before acetylcholine (ACh) was recognized to be a chemical transmitter, the presence in the blood of an enzyme capable of degrading ACh had been postulated by Sir Henry Dale[1]. The first existence of such an esterase hydrolyzing ACh was demonstrated in blood plasma by Staedman and coworkers in 1932[2]. Extensive studies during the following years contributed information on different types of acetylcholinesterases (AChEs), their kinetic properties, and distribution in excitable tissues.

Drugs that inhibit AChE are called anticholinesterase agents. They cause ACh to accumulate at cholinergic receptor sites and thus are capable of producing effects equivalent to excessive stimulation of cholinergic receptors throughout the central and peripheral nervous system.

Prior to World War II, only the "reversible" anticholinesterase agents were generally known, of which physostigmine, also called eserine, (1), is the most common example.

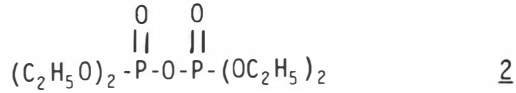


1

Physostigmine is an alkaloid obtained from the Calabar bean, the dried ripe seed of Physostigma venenosum Balfour, a perennial plant found in tropical West Africa. The Calabar bean, also called Esire nut, chop nut, or bean of Etu Esere, was once used by native tribes of West Africa as an "ordeal poison" in trials for witchcraft[1].

The Calabar bean was first brought to England in 1840 by a British medical officer. The earliest investigations of its pharmacological properties were conducted by Christiosen (1855)[3], Fraser (1863)[4], and Argyll-Robertson (1863)[5]. The first therapeutic use of the drug was in 1877 in the treatment of glaucoma, one of its few medical uses today. It was not until 1930 that Engelhart and Loewi[6], and then Matthes[7], first demonstrated that its action was that of an inhibitor of acetylcholinesterase.

Shortly before and during World War II, a new class of highly toxic compounds, the organophosphates, was developed by Schrader[8] of I.G. Farbenindustrie, first as agricultural insecticides and later as potential chemical warfare agents. The extreme toxicity of these compounds was determined to be due to their "irreversible" inactivation of AChE, thereby exerting a long lasting inhibition. It is of importance to note that the first account of the synthesis of a highly potent compound of the organophosphate series, tetraethylpyrophosphate (TEPP) (2), was in 1854 by Clermont[9], ten years prior to the isolation of physostigmine.



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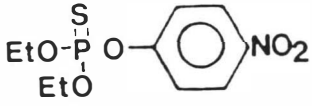
Lange and Kruegar[10] described the synthesis of dimethyl and diethyl fluorophosphate in 1932 and also mentioned some of their physiological effects, most notably their action in causing tightness of the chest and blurred vision. This information may have been instrumental in leading Schrader to explore this class for insecticidal activity. During the synthesis and investigation of approximately 2000 compounds, Schrader[8] described structural requirements for insecticidal (and subsequently anti-ChE) activity. One compound of this series, parathion (3), has become the most widely employed insecticide of this class.



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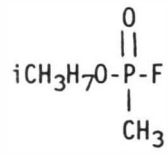
Prior to and during World War II, the efforts of Schrader's group were directed toward developing chemical warfare agents. Several compounds of much greater toxicity than parathion, such as sarin (4), soman (5), and tabun (6) were manufactured for the German government for this purpose.



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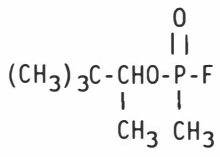

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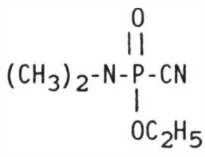

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In view of the widespread distribution of cholinergic neurons, it is not unusual that the anticholinesterase agents as a group have received more applications as toxic agents in the form of agricultural insecticides and potential chemical warfare "nerve gases" than as therapeutic agents.

B. Characteristics of Cholinesterases

Cholinesterases can be defined as a group of serine esterases which are capable of hydrolyzing esters of choline at a very high rate. All cholinesterases will hydrolyze noncholine esters. Other esterases in the body either do not hydrolyze choline esters or the rate of hydrolysis is very low. The majority of cholinesterases are completely inhibited by physostigmine or organophosphates in concentrations of 1-10 μM.

There are two types of ChE in vertebrates. They differ in substrate preference and specificity, kinetics of substrate hydrolysis and specificity to inhibitors. The terms acetylcholinesterase (AChE) and nonspecific cholinesterase are accepted and most frequently used for the two types of the enzyme. There is no generally

accepted name for nonspecific cholinesterase. The literature often uses the term butyrylcholinesterase or pseudocholinesterase[11]. Substantial differences in substrate preference and specificity as well as sensitivity towards inhibitors may exist among ChEs of different animal species[11,12].

Acetylcholinesterase hydrolyzes ACh faster than butyrylcholine; the reverse is true of butyrylcholinesterase. Also, acetyl-β-methyl choline (methachol) is not hydrolyzed as fast as ACh but is considered a specific substrate for AChE because it is practically not hydrolyzed by nonspecific AChE. The relationship between substrate concentration versus activity also differentiates the two types of ChE. AChE is inhibited by high concentrations of ACh whereas nonspecific cholinesterase is not inhibited by high substrate concentrations. Diisopropylfluorophosphate (DFP) appears to be a more potent inhibitor of nonspecific cholinesterase[13].

A significant amount of information has accumulated on the distribution of ChEs in various neuronal and nonneuronal tissues of different vertebrate species[12,14]. It is generally agreed that AChE is the only type of ChE playing a functional role in the nervous system. Neural tissue contains acetylcholinesterase while nonneuronal tissue usually contains nonspecific or butyrylcholinesterase. As pointed out, differences in the distribution and activity of AChE exist among various species[15], but a valid conclusion can be drawn that nervous structures whose function is based on cholinergic mechanisms exhibit the highest AChE activity[16]. AChE is not only associated with cholinergic structures, but is often present in other

transmitter systems as well as effector organs such as striated and smooth muscle or glands. AChE can also be found in nonexcitable tissues such as red blood cells and also in the liver, kidneys and placenta[13,14]. The predominant type of ChE in the skeletal muscles of vertebrates is AChE, but low activity of nonspecific ChE is often present[17].

Acetylcholinesterase is an essential part of the ACh system together with the cholinergic receptor, the ACh synthesizing enzyme, choline acetyltransferase, and the mechanisms controlling the choline uptake and the ACh storage and release. Based on the neurohumoral theory of transmission, the physiological function of the enzyme is to speed up the termination of the action of presynaptically released ACh at the postsynaptic receptor. The presence of AChE at a particular synapse indicates but does not necessarily mean that the synapse is cholinergic[14].

The essential role of AChE in excitable tissue is the hydrolysis of acetylcholine to acetic acid and choline (Eq. 1).

Eq. 1 $$CH_3COCH_2CH_2N^+(CH_3)_3 \xrightarrow[AChE]{H_2O} CH_3COOH + HOCH_2CH_2N^+(CH_3)_3$$

The enzymatic cleavage of ACh proceeds in several steps. An acetyl enzyme intermediate is formed from the Michaelis enzyme-substrate complex after which the release of choline occurs. Deacetylation occurs during the next step and the reaction can be viewed as

irreversible as long as the concentration of the hydrolytic products is so low that they cannot affect the reaction kinetics[18-20] (Eq. 2).

$$\text{Eq. 2} \qquad \text{ACh} + \text{E} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{ACh-E} \xrightarrow{k_2} \text{A-E} \xrightarrow[-\ \text{Ch}]{k_3} \text{A} + \text{E}$$

The active site of AChE consists of two subsites: an anionic and an esteratic subsite (Fig. 1)[18,22].

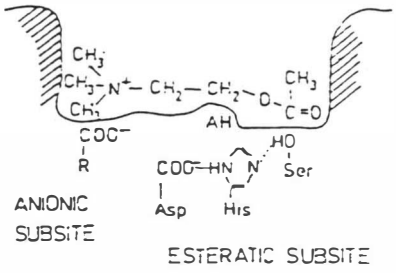


FIGURE 1

The Active Site of AChE. The quaternary nitrogen head of ACh is bound to the anionic subsite. The serine hydroxyl group, made a strong nucleophile by a charge-relay system, displaces choline from the substrate during the acetylation step. An acidic group (AH) is also involved in catalysis. The shaded areas represent the hydrophobic regions of the active site. Taken from Reference 197.

The quaternary ammonium group of ACh is bound to the anionic subsite. Ionic electrostatic forces enhance binding of charged molecules to the enzyme[18,22]. Hydrophobic interactions between the methyl groups of the cationic head and the immediate surroundings of the anionic site contribute significantly to the binding, and proper orientation of ACh may be even more important than coulombic forces[21,23].

It is at the esteratic site that the catalytic process takes place. The reaction is basically a nucleophilic substitution. The nucleophile in the esteratic site of the active center of AChE displaces choline from ACh. It is generally agreed that the hydroxyl group of serine in the esteratic site is the nucleophile and is finally acetylated, although other neighboring functional groups are also necessary for this step to occur.

An important feature of this enzyme is a system of hydrogen bonds in the active center, consisting of a serine hydroxyl group, an imidazole ring of histidine, and a carboxyl group of a dibasic amino acid[24] (Fig. 2).

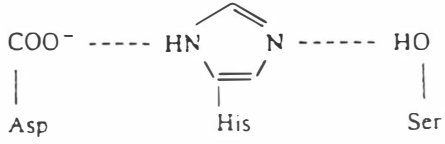


FIGURE 2

Charge Relay System in the Active Center of AChE.
Taken from Reference 198.

The function of the charge relay system is to deprotonate the serine hydroxyl group, thereby increasing its nucleophilicity, and in this way accelerating acylation of the enzyme.

During enzymatic attack on the ester, a tetrahedral intermediate is formed that collapses to an acetyl enzyme conjugate with the simultaneous release of choline. The acetyl enzyme is labile to hydrolysis, which results in the formation of acetate and active enzyme[25]. AChE is one of the most efficient enzymes known and has the capacity to hydrolyze 3 x 10^5 ACh molecules per molecule of enzyme per minute; this is equivalent to a turnover time of 150 microseconds[26].

C. Inhibitors of AChE

Choline esters are among the best substrates for AChE, but there are many other esters that are also hydrolyzed by this enzyme. In a homologous series of choline esters, ACh is hydrolyzed faster than propionylcholine, whereas butyrylcholine is hardly hydrolyzed at all. Butyrylcholine does bind to the active site and therefore can function as an inhibitor of AChE. Different compounds that bind either to the anionic or esteratic subsites of the active center of AChE inhibit the hydrolysis of ACh by the enzyme. Also, peripheral binding sites are thought to exist on AChE and several ligands are known to produce enzyme inhibition by binding to these sites[27].

1. Anionic Site Inhibitors

Many mono- and bisquaternary ammonium compounds were shown to reversibly inhibit AChE through binding to the anionic subsite of the enzyme's active center[20]. The best known among these are tetramethylammonium, tetraethylammonium, phenyltrimethylammonium and its derivative edrophonium, and decamethonium (Fig. 3).

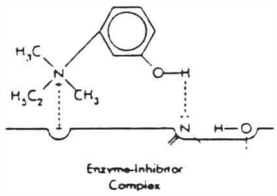


FIGURE 3

Interaction of Edrophonium with the Anionic Sub-Site of AChE. Taken from Reference 199.

Besides electrostatic forces, hydrophobic interactions are important for the binding of these substances to the anionic subsites and their affinity for AChE usually increases with bulkier hydrophobic substituents on quaternary nitrogen[28]. Bisquaternary agents with the proper interhead distance such as decamethonium (7) probably bridge the distance between the active center and a peripheral anionic center[29].

$$(CH_3)_3\overset{+}{N}\text{-}(CH_2)_{10}\text{-}\overset{+}{N}\text{-}(CH_3)_3 \qquad 7$$

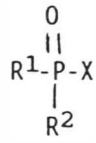
The kinetics of inhibition of AChE by anionic site inhibitors may be complicated, since beside binding to the free enzyme and thus preventing the access of the substrate to the active center, many of them often bind the acetyl-enzyme intermediate and slow or prevent deacetylation. This type of inhibition may therefore be competitive, or often of a mixed competitive-uncompetitive type.

2. Esteratic Site Inhibitors

Inhibitors of this type react with AChE by essentially the same mechanism as do substrates[30]. They are also called "acid transferring inhibitors"[29] because the serine residue in the esteratic subsite is acylated during the first step of the reaction[21]. In contrast to the substrates, the deacetylation step is usually much slower so that many of them are actually known as "irreversible" inhibitors of AChE. Organophosphorus compounds, carbamates and methanesulfonates are representative of this type of inhibitor and are widely used as insecticides, and because of their extreme toxicity some are synthesized and stored as potential chemical warfare agents.

a. Organophosphorus Compounds

The organophosphorus type AChE inhibitors can be represented by the following general formula.



A great variety of substituents are possible. R^1 and R^2 may be alkyl, alkoxy, aryloxy, amido, mercapto, or other groups, and X may represent a halide, cyanide, thiocyanide, phenoxy, thiophenoxy, phosphate or carboxylate group. A chemical classification of the compounds in this class that are of pharmacological or toxicological interest has been developed by Holmstedt[31,32] (Table 1). Extensive reviews of the organophosphorus compounds and their toxicity has been compiled by Frear[33] and Gaines[34].

The organophosphorus inhibitors serve as true hemisubstrates, since the resultant phosphorylated or phosphonylated enzyme is very stable (Figure 4).

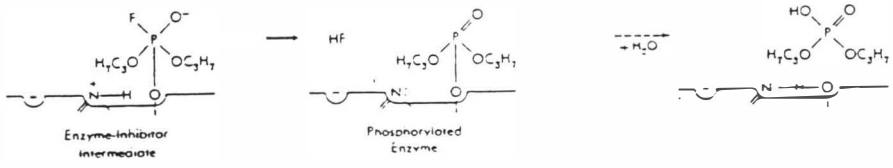


FIGURE 4

The Interaction of DFP with the Esteratic Site of AChE.
Taken from Reference 199.

TABLE 1

Chemical Classification of Representative Organophosphorus Compounds of Particular Pharmacological or Toxicological Interest Taken from Reference 200.

Group A, X = halogen, cyanide, or thiocyanate; group B, X = alkyl, alkoxy, or aryloxy; group C = thiol- or thionophosphorus compounds; group D, pyrophosphates and similar compounds; group E, quaternary ammonium compounds

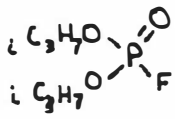
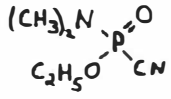
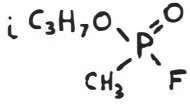
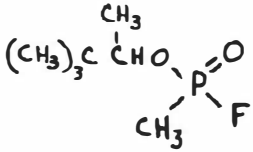
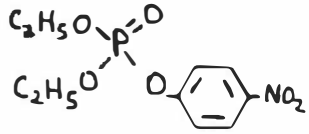
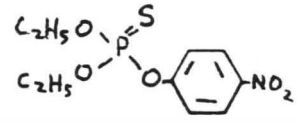
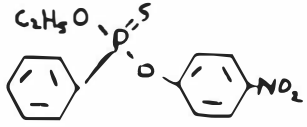
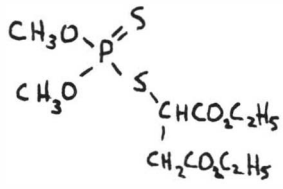
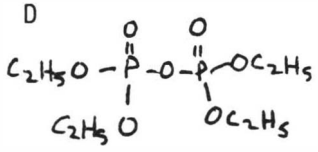
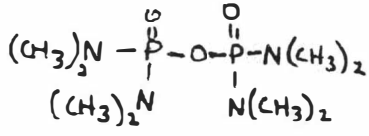
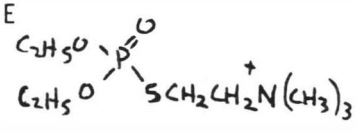
GROUP	STRUCTURAL FORMULA	COMMON, CHEMICAL, AND OTHER NAMES	COMMENTS
A		DFP Diisopropyl phosphoro-fluoridate	Potent, irreversible inactivator
		Tabun Ethyl N-dimethyl-phosphoramido-cyanidate	Extremely toxic "nerve gas"
		Sarin (GB) Isopropyl methyl-phosphonofluor-idate	Extremely toxic "nerve gas"
		Soman Pinacolyl methyl-phosphono-fluoridate	Extremely toxic "nerve gas"
B		Paraoxon, Mintacol, E 600 Diethyl 4-nitro-phenyl phosphate	Active metabolite of parathion

TABLE 1 (Continued)

GROUP	STRUCTURAL FORMULA	COMMON, CHEMICAL, AND OTHER NAMES	COMMENTS
C		Parathion Diethyl O-(4-nitrophenyl)-phosphorothionate	Widely employed agricultural insecticide, resulting in numerous cases of accidental poisoning
		EPN O-Ethyl O-(4-nitrophenyl) phenylphosphonothioate	Widely employed agricultural insecticide
		Malathion O,O-Dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate	Widely employed insecticide of greater safety than parathion or EPN because of rapid detoxication by higher organisms
D		TEPP Tetraethyl pyrophosphate	Early insecticide
		OMPA, Schradan Octamethyl pyrophosphoramide	Insecticide; inactive *in vitro*, but metablized by animals and plants to potent anti-ChE agent
E		Echothiophate, Phospholine, 217MI Diethoxyphosphinylthiocholine iodide	Extremely potent choline derivative; employed in treatof glaucoma; relatively stable in aqueous solution

The reaction with AChE is that of nucleophilic substitution by the serine hydroxy group from the esteratic subsite, the leaving group X being replaced and the phosphorylated enzyme results. The reaction is enhanced by the geometry of the tetrahedral phosphates, which resembles the transition state for acetyl ester hydrolysis. Certain quaternary organophosphorus compounds (echothiophate; Table 1) interact with both the esteratic and anionic subsites in the active center to produce a stable complex; this contributes to the high potency of these compounds[32]. If the alkyl groups in the phosphorylated enzyme are ethyl or methyl, a significant degree of spontaneous regeneration of the active enzyme requires several hours. Secondary (DFP) or tertiary alkyl groups enhance the stability of the phosphorylated enzyme and significant regeneration of active enzyme is not observed. Therefore, the return of AChE activity depends on synthesis of new enzyme[30].

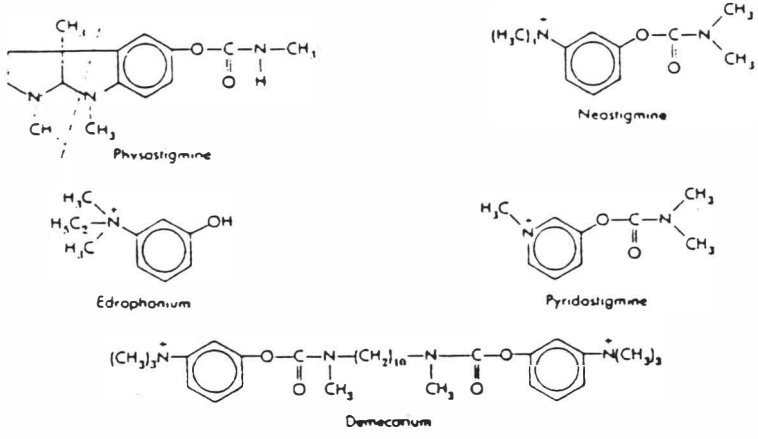
If the inhibited enzyme is stored for a period of time, it cannot be reactivated. The stability of the phosphorylated enzyme is further enhanced by a process called "aging" and is caused by the loss of an alkyl group from the organophosphate moiety of the inhibited enzyme[35] (see Fig. 6). The rate of aging depends on the electron donating capacity of the alkyl group[36]. This dealkylated enzyme-inhibitor complex is not susceptible to nucleophilic attack, and therefore cannot be reactivated.

b. Carbamates

Detailed information on these substances has been published[20,37]. Numerous carbamate cholinesterase inhibitors are used clinically (Figure 5).

FIGURE 5

Representative "Reversible" Anticholinesterase Agents
Employed Clinically.
Taken from Reference 201.



A carbamyl-enzyme intermediate is formed after the leaving group X is replaced (Fig. 6).

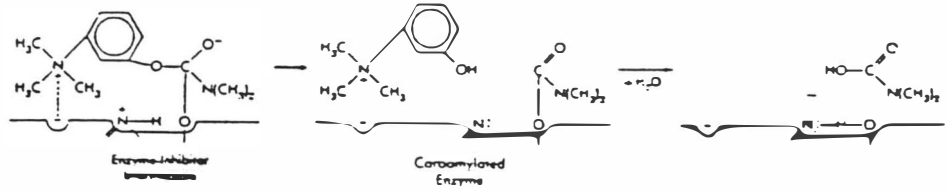


FIGURE 6

The Interaction of Neostigmine with AChE.
Taken from Reference 199.

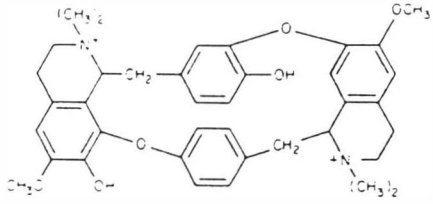
The decarbamylation of the enzyme is relatively fast, therefore, these type of AChE inhibitors are often called reversible[20,21]. There is no basic difference between the reversible and irreversible "acid transferring" inhibitors (organophosphates) as far as the reaction pathway of inhibition is concerned. The signs and symptoms of poisoning by carbamate type ChE inhibitors closely resembles those of the organophosphate type[38].

c. Miscellaneous.

Methanesulfonates (CH_3SO_2X) are strong irreversible inhibitors for AChE, which means that desulfonation is extremely slow. Methanesulfonyl fluoride is a useful probe for the esteratic site because of its small size[39,40].

The methanesulfonyl group also serves as a hemisubstrate (like the organophosphates) by forming a conjugate with the serine hydroxy group on AChE. Like the phosphorylated enzyme, methanesulfonyl AChE is resistant to hydrolytic reactivation[41].

The existence of peripheral anionic site ligands on AChE was first hypothesized from kinetic studies of the inhibition of AChE by d-tubocurarine (8) and gallamine (9)[42].



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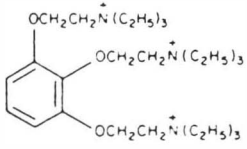

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These two compounds are bound to AChE outside its active center[43-45]. Several peripheral anionic sites appear to be located on each AChE subunit[46], or a broader anionic locus may possess several negative charges, each serving to bind several cationic ligands[41].

It is generally agreed that a second molecule of ACh is bound to the enzyme at high substrate concentrations and that the catalytic efficiency of such a complex is reduced, leading to substrate inhibition. Opinions differ as to where and when the second molecule is bound. The existence of two anionic subsites in the active center has been suggested, and the steric hindrance of two molecules of ACh at the esteratic site has been proposed to explain the catalytic inactivity of the ternary complex[47]. The second molecule of ACh may bind to the acetyl-enzyme intermediate and block deacetylation[48]. Involvement of a peripheral anionic site and of conformational changes has been suggested[49], as well as the binding of the second substrate molecule to the hydrophobic regions near the active site[50].

D. Cholinesterase Reactivators

While the phosphorylated esteratic site of AChE undergoes hydrolysis to regenerate active enzyme at a slow or negligible rate, Wilson[51] discovered that nucleophilic agents such as hydroxylamine (H_2NOH), hydroxamic acids (RCONHOH), and oximes (RCH=NOH) reactivate the enzyme more rapidly than spontaneous hydrolysis (Fig. 7).

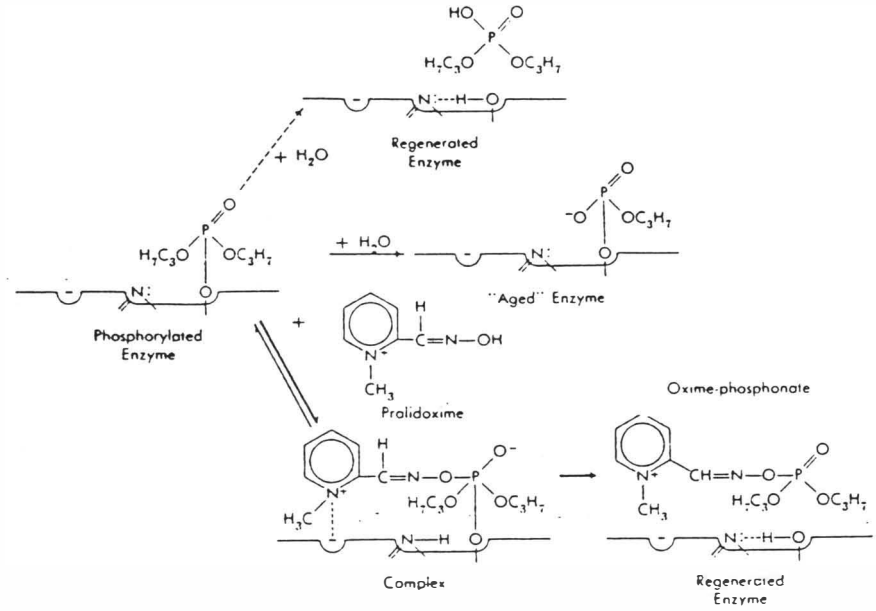
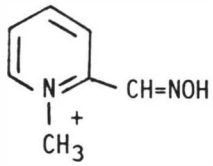


FIGURE 7

Reactivation of Alkylphosphorylated Acetylcholinesterase. Taken from Reference 52.

A selective regeneration is achieved by a site directed nucleophile wherein interaction of a quaternary nitrogen with the negative subsite of the active center places the nucleophile in close apposition to the phosphorus. Wilson and Ginsberg[53] demonstrated this selective regeneration on phosphorylated AChE with pyridine-2-aldoxime methyl chloride (pralidoxime; 2-PAM; 10).



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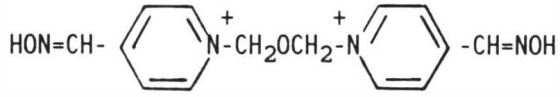
It was shown that reactivation with 2-PAM was one million times the rate of that with hydroxylamine. Certain bis-quaternary oximes such as obidoxime (11) were subsequently shown to be even more potent reactivators[54].



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With these type of agents the oxime is oriented proximally to render a nucleophilic attack on the phosphorus. The oxime-phosphonate is then hydrolyzed, leaving the regenerated enzyme[55] (see Fig. 7). The oximes are not as effective in antagonizing the toxicity of carbamyl ester inhibitors as with organophosphorus type agents.

E. Pharmacological and Toxicological Properties of Acetylcholinesterase Inhibitors

Compounds which inhibit AChE prevent the hydrolysis of ACh; therefore, ACh accumulates at cholinergic synapses throughout the central and peripheral nervous system. The properties of anti-ChE agents can be discussed by knowing the location where ACh is released physiologically by nerve impulses and the responses of the corresponding effector organs. In general, the sympathetic and parasympathetic systems are viewed as physiological antagonists. If one system inhibits a certain function, the other usually augments that function[56]. Since most viscera are innervated by both components of the autonomic nervous system, the level of activity represents the integration of the influence of the two divisions.

The integrating action of the autonomic nervous system is of importance for the wellbeing of the organism. In general, the autonomic nervous system regulates the activities of structures not under voluntary control. Thus, respiration, circulation, digestion, metabolism, body temperature, sweating, and the secretions of endocrine glands are regulated by the autonomic nervous system and its central mechanisms.

The parasympathetic system, in contrast to the sympathetic system, is organized mainly for discrete and localized discharge and not for a massive response. It is concerned primarily with the

functions of conservation and restoration of energy, rather than expenditure of energy. This system slows the heart rate, lowers blood pressure, stimulates gastrointestinal movements and secretions, aids absorption of nutrients, protects the eyes from excessive light and empties the bladder and rectum. There is no purpose for the parasympathetic system to discharge simultaneously.

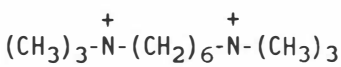
In contrast to other cholinergically innervated cells, smooth muscle and the cardiac system exhibit intrinsic electrical and mechanical activity which is modified, not initiated, by ACh. The addition of ACh to isolated intestinal smooth muscle causes a fall in the resting potential (membrane potential becomes more positive) and an increase in membrane conduction followed by a rise in tension. ACh initiates these effects probably by depolarization of the cell membrane by an increase in Na^+ conductance. ACh can produce contraction of smooth muscle when the membrane has been completely depolarized. Stimulation of cholinergic innervation or direct application of ACh causes inhibition of the cardiac conduction system associated with hyperpolarization of the fiber membrane and a decrease in the rate of depolarization. These effects are due to a selective increase in permeability of K^+ [57].

ACh has three main effects on the cardiovascular system: vasodilation, a decrease in cardiac rate (negative chronotropic effect), and a decrease in the force of cardiac contraction (negative ionotropic effect). The negative ionotropic effect is more pronounced in the atrial muscle than in the ventrical muscle. The intravenous injection of a small dose of ACh in an anesthetized animal

produces a fall in blood pressure due to vasodilation, accompanied by tachycardia. A larger dose will elicit bradycardia by block of the A-V node conduction from the direct action of ACh in the heart. Injection of ACh produces dilation of essentially all vascular beds including pulmonary and coronary[58].

ACh produces an increase in tone, amplitude of contractions and peristaltic activity of the stomach and intestines as well as enhanced secretory activity of the gastrointestinal tract. ACh stimulation increases urethral peristalsis, contracts the detrusor muscle of the urinary bladder, increases the maximum voluntary voiding pressure, and decreases the capacity of the bladder. At the neuromuscular junction ACh combines with the nicotinic cholinergic receptor to initiate an endplate potential and stimulate muscle contraction.

The pharmacology of ganglionic transmission is based on the modifications of the availability or the action of the primary neurotransmitter ACh. Neurotransmission in autonomic ganglia is more complex than can be described by a single neurotransmitter receptor system. The primary pathway involves the depolarization of postsynaptic sites by ACh. The receptors are classified as nicotinic and the pathway can be blocked by the classical non-depolarizing blocking agent hexamethonium (12).



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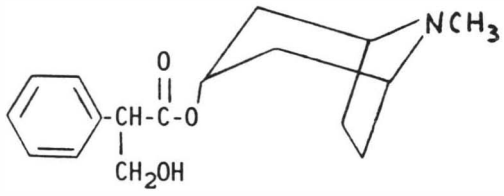
ACh stimulates the secretion of all glands that receive input from the parasympathetic nervous system, including lacrimal, tracheobronchial, salivary, digestive and exocrine sweat glands. The effects on the respiratory system in addition to increased bronchial secretion include bronchoconstriction and stimulation of the chemoreceptors in the carotid and aortic bodies.

The pharmacological effects of anti-AChE agents are due to the prevention of hydrolysis of ACh by AChE at sites of cholinergic transmission. The transmitter accumulates and the action of ACh which is liberated by cholinergic impulses is enhanced. With the organophosphorus agents all of the acute effects at moderate doses can be attributed to this action. The diverse number of cholinergic synapses increases the complexity of the pharmacological response. All anti-ChE agents are responsible for (1) stimulation of muscarinic receptor responses at autonomic effector organs; (2) stimulation, followed by depression or paralysis of all autonomic ganglia and skeletal muscle (nicotinic action); (3) stimulation followed by depression of cholinergic receptor sites (mainly muscarinic) in the CNS. The compounds containing a quaternary ammonium group do not readily penetrate the blood-brain barrier. The lipid soluble agents such as the tertiary amines and the organophosphorus compounds are well absorbed and have effects at both peripheral and central cholinergic receptor sites. The lipid soluble organophosphates can readily be absorbed through the skin. The actions of excess ACh in the peripheral and central nervous system where the receptors are muscarinic can be blocked by atropine (13).



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The main actions of anti-ChE agents that are of therapeutic importance are concerned with the eye, the intestine and the skeletal neuromuscular junction. Although anti-ChE agents have been recommended for the treatment of a wide variety of conditions, their acceptability over other drugs or therapeutic approaches is established only in four main areas: atony of the smooth muscle of the intestinal tract and the urinary bladder, glaucoma, myasthenia gravis, and termination of the effects of competitive neuromuscular blocking drugs. Other classes of drugs are often recommended as adjunct therapy or in preference to anti-AChE drugs because of the lack of selectivity and the toxicity associated with this class of compounds. The most extensive applications of AChE inhibitors are as toxic agents in the form of agricultural pesticides and insecticides and as chemical warfare "nerve gas agents".

Poisoning with a variety of inhibitors has been reported to produce essentially the same clinical picture[59]. The accumulation of ACh at sites where it functions as the chemical transmitter, that is, at postganglionic parasympathetic nerve endings (muscarinic sites), at autonomic ganglia and the skeletal neuromuscular junction (nico-

tinic sites) and in the central nervous system (predominantly central muscarinic sites) can be related to the signs and symptoms of poisoning by anti-ChE agents. The toxicological manifestations of the anti-ChE agents are of practical importance to the physician. In addition to the numerous cases of accidental intoxication from the use and manufacture of organophosphorus compounds as agricultural insecticides[60–62], these types of compounds have been employed frequently for homicidal and suicidal purposes, largely because of their ease of accessibility.

After local exposure to vapors or aerosols, ocular and respiratory effects generally are the earliest signs of poisoning by anticholinesterase agents. The ocular effects include miosis, ocular pain, conjunctival congestion, ciliary spasm, and brow ache along with nasal discharge[63]. Respiratory effects consist of tightness in the chest and wheezing due to a combination of bronchoconstriction and increased bronchial secretions. A generalized stimulation of smooth muscle results in contractions and increased peristalsis in the stomach and intestine, and contraction of the muscles in the walls of the urinary tract. There is stimulation of secretion by lachrimal, sweat, salivary, bronchial, gastric and pancreatic glands[65]. Excessive secretions of the salivary and bronchial glands lead to partial blockage of the airway and respiratory difficulty. Effects on the cardiovascular system include a slowing of the heart and a fall in blood pressure. Two factors seem to be operating to produce hypotension, decreased peripheral resistance due to vasodilation and a reduced cardiac output[64]. Severe intoxication is manifes-

ted by extreme salivation, lacrimation, bradycardia and hypotension[63].

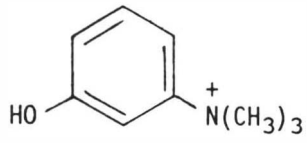
Skeletal muscles and autonomic ganglia are stimulated by low doses of anticholinesterase agents and depressed by higher doses[65]. There are fasiculations of the muscles of the eyelid. Difficulties in accommodation occur since there is contraction of the ciliary muscle, making the lens spherical. These effects, plus miosis, make vision difficult. The complex actions of anti-ChE agents at the autonomic ganglia have been reviewed by Zaimis[66]. At the neuromuscular junction, muscular fasciculations followed by muscular weakness and eventually neuromuscular blockade occur. The most serious effect on the neuromuscular junction is paralysis of the respiratory muscles[63].

The central actions of anticholinesterase agents can be explained by stimulation followed by inhibition. The stimulant effects, such as apprehension, dizziness, tremor, ataxia and convulsions, are succeeded by inhibitory effects which lead to coma. Effects on the respiratory center in the brain are characteristic of respiratory stimulation followed by respiratory depression and eventually respiratory failure. The central effects of anti-ChE agents are of significance because death from poisoning by these compounds is primarily due to central respiratory failure. Therapeutic or protective procedures are aimed primarily at reversing or preventing this action. Karczmar[67] has reviewed the acute and long-lasting central effects of the action of organophosphorus agents.

F. Treatment of Anticholinesterase Poisoning

Occasionally overdose with anticholinesterase agents may occur during their medical use for the conditions mentioned previously. Much more commonly, accidental over-exposure to anticholinesterase insecticides and the potential use of organophosphorus agents in chemical warfare as the "nerve gas agents" could constitute a considerable therapeutic or prophylactic problem. Aside from protective clothing or respirators, treatment of this situation must be directed at either protecting AChE from the inhibitor, releasing an active enzyme from the enzyme-inhibitor complex or minimizing the actions of accumulated acetylcholine which is responsible for all the main effects of anticholinesterases. In practice, metabolism or destruction of the inhibitor before it can inhibit significant proportions of the enzyme or inhibition of the release of acetylcholine or acceleration of its destruction have proven to be of little or no value. The main problems arise with the organophosphorus inhibitors where the effects are long (days or weeks) in comparison with the carbamates where the effects persist for no more than a few hours because of the relatively rapid rate at which spontaneous reactivation of the enzyme occurs[12].

The only way of protecting AChE from an irreversible inhibitor is to use another compound which will react with the enzyme. This will inhibit the enzyme to ensure that the inhibition is of a readily reversible type. Reversible inhibitors such as edrophonium (14) are not of practical importance since the length of time that they will



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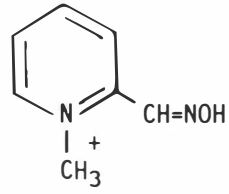
protect the enzyme from irreversible inhibition will be short. Koster[68] first proposed and demonstrated that carbamate type cholinesterase inhibitors were effective in protection against poisoning from organophosphorus compounds. To be effective in this approach, they must be given before poisoning by the organophosphorus compound because the inhibited enzyme would then not be available and could be reactivated.

Any drugs which effectively destroy the inhibitor before it can attack the enzyme or which protect the enzyme from inhibition need to be present when poisoning occurs. They demonstrate no therapeutic potential but rather presupposes some expectation that exposure to anticholinesterase agents is likely to occur (prophylactic treatment). A much more likely occurrence (accidental poisoning in agricultural or chemical warfare) is that poisoning will occur in an unprotected individual who will then require treatment. In this situation an enzyme inhibitor complex will already have been formed and thus will need to be broken down to release active enzyme. Conventional therapy for intoxication by organophosphate AChE inhibitors consists of coadministration of atropine (to antagonize

the effects of accumulated acetylcholine) and a pyridinium oxime to reactivate the inhibited enzyme[59,61,69].

Wilson[51] first demonstrated that chemical reactivation of organophosphate inhibited AChE with hydroxylamine could be achieved at a faster rate than with water alone. In this and in subsequent studies Wilson[53,70] found that the ease with which reactivation could be effected depended on the nature of the inhibitor. For example, diisopropylphosphorylacetylcholinesterase was much more difficult to reactivate than the diethylphosphorylated enzyme. Hydroxamic acids are not effective antidotes[73]. Studies found that oximes were even more effective reactivators[53,71]. The most extensively studied compounds of this class of agents in treating poisoning by AChE inhibitors have been pyridine-2-aldoxime methiodide (2-PAM) (10) and the corresponding methyl methanesulfonate (P2S).



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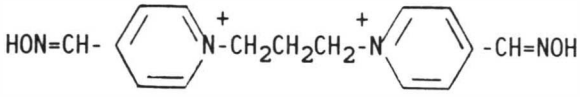
The bis-quaternary pyridine aldoximes constitute the most potent group of oxime reactivators known. Hobbinger et al.[71] showed the potent compound 1,3-bis-(pyridinium-4-aldoxime)propane dibromide (TMB-4) (15) was a more rapid reactivator of sarin-inhibited cholinesterase *in vitro* than either 2-PAM or P2S. Englehard and Erdman[72] reported 1,3-bis-(pyridinium-4-aldoxime) methyl ether bichloride (H-

6, toxogonin, 16) to be an even more effective reactivator than TMB-4.



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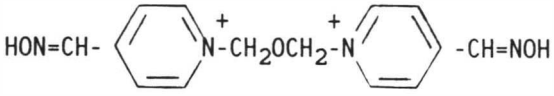

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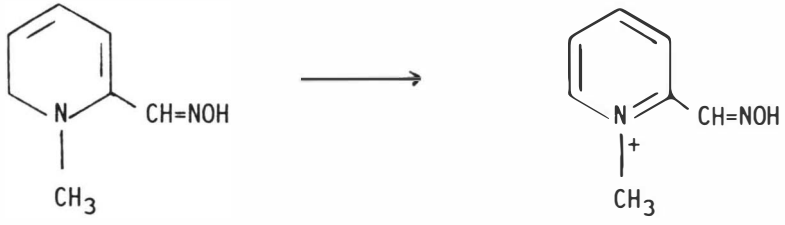
A vast amount of work has been carried out on the effectiveness of reactivators in protecting animals from the lethal effects of organophosphorus ChE inhibitors. There are differences between various reactivators and inhibitors and the experimental conditions used. The difference in species response makes the situation more complex. The oximes are the only class which are truly effective for this use.

It is known that the blood-brain barrier impedes the transport of the cationic pyridinium compounds into the CNS, but readily passes typical lipophilic organophosphorus anti-AChE agents. Treatment of anti-AChE poisoning with pyridinium oximes is highly effective for peripheral regions, but it fails to restore activity to a large fraction of inhibited brain AChE[74,75]. The issue of CNS activity of AChE reactivators is complicated because there are reports that pyridinium oximes devoid of CNS activity are effective antidotes[76].

Low concentrations of 2-PAM have been shown to penetrate into the brain[77].

Bodor et al.[78-81] applied the "prodrug" concept to the problem of anti-AChE agent therapy in an attempt to understand the CNS effects of AChE reactivators. 1-Methyl-1,6-dihydropyridine-2-carbaldoxime (proPAM, 17) rapidly oxidizes *in vitro* and *in vivo* to PAM (10)[80].



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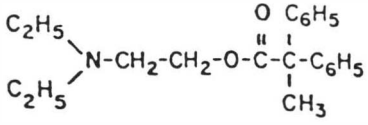
The tertiary amine can penetrate into the CNS and oxidize to PAM. Administration of proPAM in mice poisoned by DFP enhanced reactivation of brain AChE relative to an equivalent dose of PAM[79]. Studies have shown, though, that proPAM provides only a slight improvement over PAM in DFP poisoning and no improvement when given as an antidote against other organophosphorus anti-AChE agents[75,82,83]. The lack of correlation between reactivation of brain AChE and antidotal efficacy (survival rate) has raised the question of whether reactivation of CNS AChE is critical for survival[12,83]. Pro-PAM reaches the brain, reactivates AChE, but it is no more effective an antidote than 2-PAM with or without atropine. It has been suggested

that the only contribution toward protection from anti-AChE poisoning is peripheral[84] even though the main cause of death is central respiratory inhibition. No reactivator alone without the addition of atropine or other antimuscarinic drug is therapeutically successful after a lethal dose of the organophosphate.

During poisoning with anti-AChE agents ACh accumulates at peripheral nicotinic and muscarinic sites and also at various sites in the central nervous system. No one drug can antagonize the actions of ACh at all sites. The nicotinic actions can be antagonized at the autonomic ganglia by ganglionic blocking drugs and at the neuromuscular junction by curare-like agents. The predominant central actions can be antagonized by antimuscarinic drugs. In most reported studies ganglionic and neuromuscular blocking drugs have been used in conjunction with atropine. Their use alone would not be very significant since they act at only one site where ACh accumulates.

The first reported use of an antimuscarinic drug as an antidote of an anticholinesterase agent was in 1864 when Kleinwachter described the use of atropine for the treatment of poisoning with physostigmine in man[85]. Considerable emphasis has been placed on trials of oximes or combinations of oximes for improved antidotal effectiveness, but only a few studies have been undertaken to determine whether atropine sulfate is the most effective cholinolytic agent for combined treatment. Presently atropine sulfate is the standard antimuscarinic agent used clinically as an antidote. Atropine sulfate is a potent antimuscarinic agent with both peripheral and

central effects. However, atropine has two distinct disadvantages as an antidote for nerve gas poisoning. It is much less effective centrally than peripherally as an antimuscarinic (when administered peripherally) and it further promotes the release of ACh from cholinergic neurons by blocking inhibitory muscarinic receptors presynaptically. There is reason to believe that other antimuscarinic agents might be better than atropine, particularly for combatting the central effects of the excess ACh. Apparently the Russian Army has switched to a synthetic compound called aprophen (18) as its preferred antidote[86]. Because of its high antimuscarinic potency



18

and ability to penetrate the blood-brain barrier it is administered prophylactically and therapeutically as an antidote to nerve agents[87].

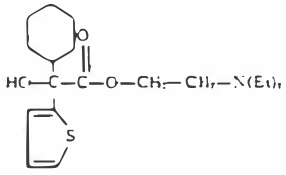
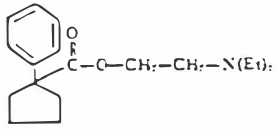
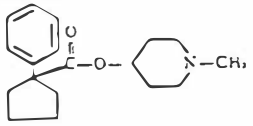
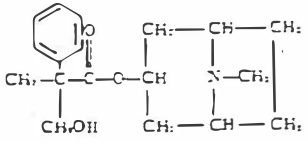
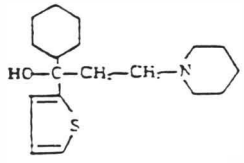
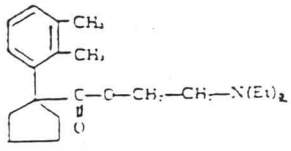
Coleman et al. reported a number of studies on the effectiveness of antimuscarinics as antidotes[88-92]. Initially 34 compounds were tested for their ability to protect mice and rats from lethal doses of sarin compared to atropine[88]. The effectiveness of the compounds was determined in a treatment utilizing the oxime P2S (30 mg/kg) plus 50 μmoles/kg of the antimuscarinic compound. The combination was

administered 15 min prior to injection of sarin. In mice and rats five of the compounds exhibited markedly enhanced protective potency compared to atropine. A comparison to atropine of the most effective compounds in the study for mice and rats are summarized in Table 2. A potency ratio, (PR-1) = LD_{50} of sarin in treated animals/LD_{50} of sarin in untreated controls. In this Table a PR-2 is the potency ratio of the LD_{50} of sarin in animals treated with a specific antimuscarinic divided by the LD_{50} of sarin in animals treated with atropine.

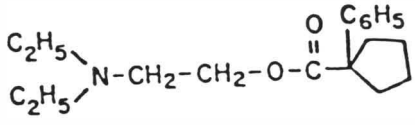
Selection of candidate compounds in this reported study was based on previously reported activity in antimuscarinic assays which indicated potency approximating that of atropine. The pharmacological tests included mydriatic action in mice and rabbits, inhibition of ACh-induced spasm in guinea pig ileum and rabbit ileum *in vitro*, and inhibition of gut motility *in vivo* in dogs. Comparison of results from these assays of the 34 compounds with their activity in conjunction with P2S in protecting mice or rats from sarin poisoning failed to reveal any correlation. All of the criteria mentioned are tests of inhibitory potency at peripheral cholinergically innervated smooth muscles or glands. These functions are not as significant in poisoning by anticholinesterase inhibitors as in central respiratory inhibition. Therefore, the comparison is not logical.

TABLE 2

Relative Effectiveness of Some Antimuscarinics in the Protection of Mice and Rats From Sarin Poisoning Taken from Reference 88.

Number	Compound	PR-2 Mouse	PR-2 Rat
60		1.8	4.8
19		1.6	1.4
20		2.3	4.1
		1.4	1.2
		1.09	1.7
21		1.5	—

Many of the compounds which gave more protection than atropine were tested in combination with atropine or with each other[90,92]. Ten of the compounds were shown to raise the LD_{50} of sarin by a factor of 4 to 20 when given in combination with atropine or each other plus an oxime reactivator. Of the 26 combinations where joint action exceeded in protection the sum of the activities of the individual components, caramiphen (19) and three of its analogs (20, 21, 22) were involved in 17. Two of the ten best compounds were the



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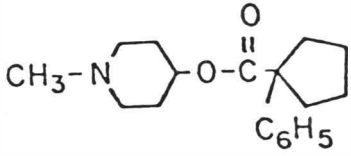

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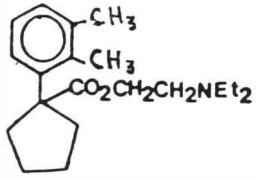

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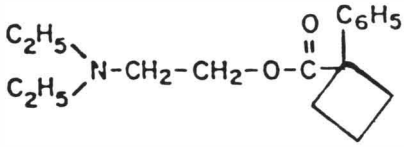

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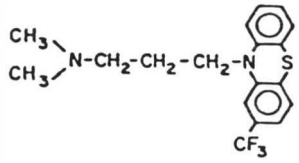
phenothiazine derivatives trifluprom azine (24) and diethazine (25), which are used clinically for other purposes.



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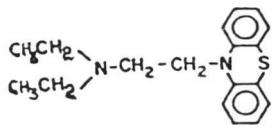

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The order of the activity of the compound or combination of compounds varied considerably with different test systems, and no one compound was best in mice and rats under every condition. Caramiphen was selected for intensive study with different AChE inhibitors, oxime reactivators and animal species in comparison with atropine. The mean activity of caramiphen relative to atropine taken from 102 separate trials indicated it to be 4.2 times as effective an antidote.

Coleman et al. further examined the effect on dose level, with protection by caramiphen and atropine poisoned with sarin[92]. As the dose of caramiphen was increased, the protection afforded also increased, and up to 150 mg/kg there was no indication of a limited protection level. Atropine increased in protection to a value of 2.1 at 20 mg/kg, but after this level was achieved little improvement was

demonstrated even at doses of 200 mg/kg. At 150 mg/kg caramiphen was over six times as active as atropine (Figure 8).

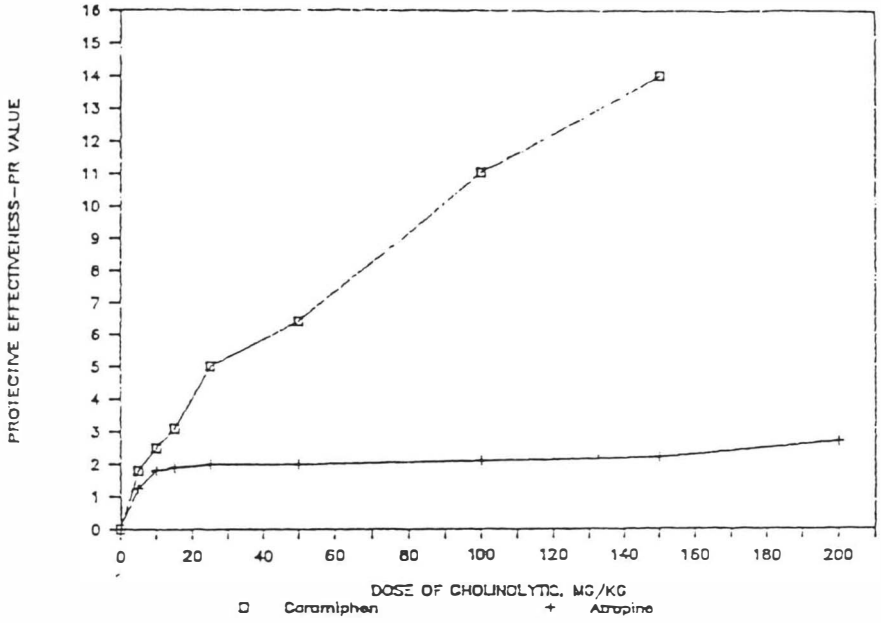
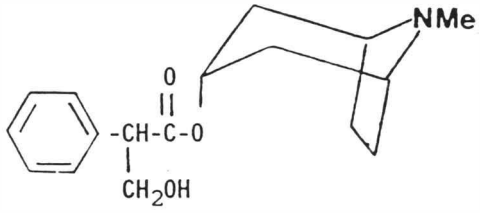


FIGURE 8

Protection Afforded Mice Exposed to Sarin With Increasing Dose of Caramiphen (19) or Atropine (13) in Combination With P2S (30.0 mg/kg). Taken from Reference 92.

$$PR = \frac{LD_{50} \text{ dose sarin in animals treated with oxime + cholinolytic}}{LD_{50} \text{ dose sarin in untreated animals}}$$

Jovic and Milosevic[93] conducted an extensive study employing twelve cholinolytics and nine different anticholinesterase inhibitors on poisoning in mice. Initially the doses of 12 cholinolytics which protected 50% of the animals poisoned by 1.3 x LD_{50} of armin (dose which caused 100% lethality in control untreated animals) were determined. The quaternary compounds, methylatropine and methylscopolamine, only protected 50% of animals poisoned by 1.3 x LD_{50} of armin in doses equal to their LD_{50}. This therapeutic value is very poor due to poor penetration into the CNS. The most active compounds were scopolamine (hyoscine; 26), atropine (13), caramiphen (19) and benactyzine (27).



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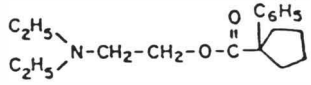

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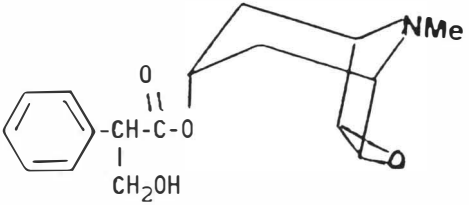

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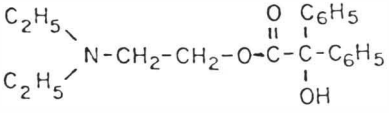

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On this basis these four compounds and methylatropine (as a representative quaternary compound) were selected for further study. The previous study looked at different cholinolytics against the same anticholinesterase inhibitor (armin). Since protection afforded by different cholinolytics was dependent on the organophosphate (OPC) used, it was important to see if similar results with the most active antagonists were obtained using other OPC. Nine different anticholinesterase inhibitors were used, and a summary of these results including the more important "nerve gas" agents sarin, soman and tabun is presented in Table 3. The results demonstrate that the order of protection is dependent on the antagonist and the OPC used. Caramiphen was the most active against sarin, soman and tabun, while atropine and scopolamine were better against amiton. Methylatropine was inactive against all three nerve gas agents. Benactyzine was approximately equal to atropine against the nerve agents. The most active cholinolytics collectively against sarin, soman and tabun were caramiphen and atropine, of which caramiphen was the most active.

TABLE 3

The Protective Doses (PD_{50}) and Protective Index (PI) of Some Antimuscarinic Agents in Mice Poisoned by OPC[93]

OPC	PD_{50} (mg/kg), (PI) Atropine	Hyoscine	Caramiphen	Benactyzine	Methylatropine
Armin	3.12 (27.2)	3.39 (45.1)	1.71 (21.3)	2.75 (11.0)	1.39 (5.0)
Sarin	4.12 (20.6)	10.00 (15.3)	3.45 (10.6)	5.20 (5.8)	Inactive
Soman	9.87 (8.6)	13.90 (11.0)	4.40 (8.3)	9.03 (3.3)	Inactive
Tabun	3.25 (26.4)	8.63 (17.7)	2.05 (17.8)	1.78 (17.1)	Inactive
Amiton	0.44	0.33	1.78	2.12	0.52

PI = LD_{50} of cholinolytic/PD_{50} of same cholinolytic.

It is well known that atropine potentiates the protective effects of oximes and that a combination of oxime and atropine gives the best protection and therapeutic effect against organophosphate poisoning. Therefore a further study compared the protection afforded by atropine and caramiphen with the oxime 2-PAM. The results (Table 4) show that caramiphen in combination with 2-PAM potentiated the protective effect. A combination of caramiphen (1 x PD_{50}), and 2-PAM (50 mg/kg i.p.) protected all animals poisoned by 10.4 x LD_{50} of armin and 50% of animals poisoned by 15 x LD_{50} of armin. The PD_{50} of atropine in combination with the same dose of 2-PAM (50 mg/kg) only gave 100% protection at 6 x LD_{50} of armin.

TABLE 4

Therapeutic Effects of Caramiphen and Atropine With 2-PAM[93]

Drug (mg/kg)	2-PAM (mg/kg)	Armin LD_{50}	Result Death/Tested	% Survival
Caramiphen				
1.71	0	1.0	0/6	100
1.71	0	1.3	3/6	50
0	50.0	1.3	2/6	66
1.71	50.0	1.3	0/6	100
1.71	50.0	2.6	0/6	100
1.71	50.0	5.2	0/6	100
1.71	50.0	10.4	0/6	100
1.71	50.0	15.0	3/6	50
1.71	50.0	20.8	6/6	0
Atropine				
3.12	0	1.0	0/6	100
3.12	0	1.3	2/6	66
3.12	50.0	1.3	0/6	100
3.12	50.0	6.0	0/6	100
3.12	50.0	11.5	4/6	33
3.12	50.0	14.3	6/6	0

Coleman et al. has stated that the ED_{50} of an antimuscarinic drug may not always be a good indication of its efficacy in treating animals poisoned by organophosphates[88]. It has been proposed that at high doses different mechanisms may be operating in the protective action against poisoning (although competition at central muscarinic sites is the most important mechanism for protection)[88,89,95]. Green et al.[94] proposed this additional mechanism to be due to an anticonvulsant effect at high doses, which additionally contributes to protection. Convulsant seizures caused by anticholinesterases are reversed by anticonvulsants[96]. In a further series of experiments, Jovic and Milosevic[93] examined caramiphen and atropine for the

protection afforded to mice when the dose of the cholinolytic was altered from 2.0 to 10.0 x PD_{50} in animals poisoned by a constant dose of 3.0 x LD_{50} of amiton. The resultant protection plotted against dosage is shown in Figure 9. It can be seen that atropine gave increased protection with increasing dosage until maximum protection was obtained with 4.0 x PD_{50}. In contrast, protection given by caramiphen was still increasing with 10 x PD_{50}.

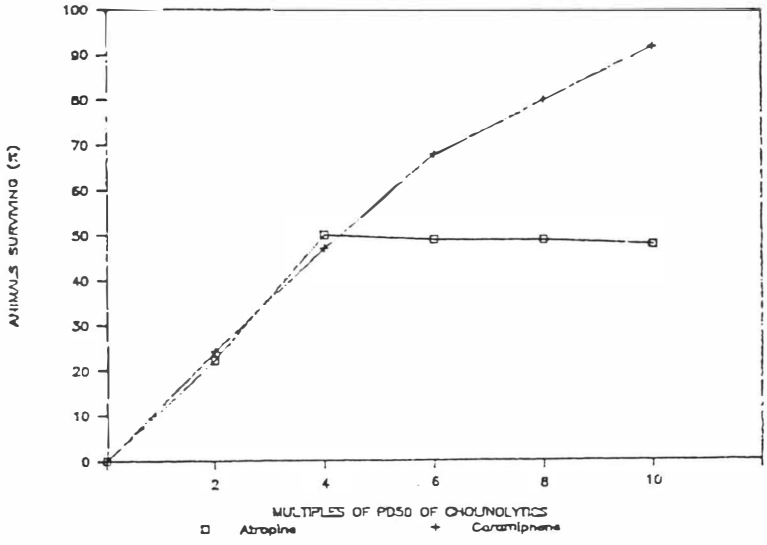


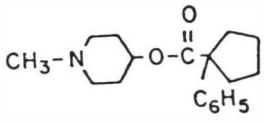
FIGURE 9

The Protective Effects of Caramiphen and Atropine as a Function of the Doses to Mice Poisoned by 3 x LD_{50} of Amiton[93]

The most effective compounds have many diverse structural features. Caramiphen and some of its derivatives do seem to constitute one structural type of agent with good protective activity. It

is not apparent why some agents which are more potent antimuscarinics are not as effective, but a report by Madill et al.[97] suggests that it depends on their CNS activity. It was found that for a series of nine compounds, ability to protect guinea pigs from sarin poisoning paralleled their CNS antimuscarinic activity, not their peripheral activity. For example, caramiphen was the most effective protecting agent, although it had only 1/100 the peripheral antimuscarinic action of atropine. High levels of therapeutic protection were directly related to high levels of central anticholinergic potency.

Green et al.[94] looked at the central and peripheral antimuscarinic activities of atropine and five related drugs. All five compounds were superior to atropine in protecting rats poisoned by sarin. The most effective compounds were G3063 (20) (analog of caramiphen) and PMCG (28).



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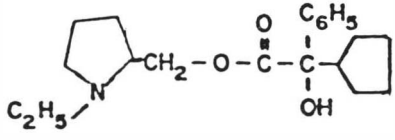

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At low doses of sarin all six drugs showed a direct relationship between central antimuscarinic activity (antagonism of oxotremorine induced tremors) and protection.

Brimblecombe et al.[95] compared the central and peripheral antiacetylcholine potencies of a number of drugs with their effectiveness when used alone and in conjunction with P2S in protecting mice, rats and guinea pigs. The compounds tested and the results of protective action are summarized in Table 5. The results demonstrate that the addition of the oxime markedly potentiated the protective action of all the anticholinergic drugs tested. The higher protection was obtained with caramiphen (19) and its analog G3063 (20). There was no statistically significant correlation between the peripheral anticholinergic potencies (measured either by production of mydriasis or by blockade of oxotremorine-induced salivation) or the central potencies (blockade of oxotremorine-induced tremors) and their ability to protect from the lethal effects of sarin.

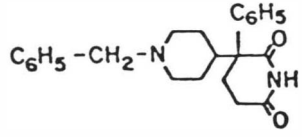
TABLE 5

The Protective Actions Against Sarin of Some Antiacetylcholine Drugs When Used Alone or in Combination With the Oxime P2S in Rats, Mice and Guinea Pigs[95]

Antiacetylcholine Drug (50 μmol/kg)	Protection Ratios: Rats Drug Alone	Rats Drug + P2S	Mice Drug Alone	Mice Drug + P2S	Guinea Pigs Drug Alone	Guinea Pigs Drug + P2S
Atropine	1.2	27.5	1.1	2.6	1.3	35.8
Hyoscine	1.2	9.3	1.3	2.0	1.4	21.4
Caramiphen	4.1	11.8	1.1	2.5	1.1	18.6
G3063	9.3	79.5	1.4	6.3	1.5	58.3
PMCG	2.0	62.3	1.5	3.0	1.5	84.2
Ditran	2.0	33.6	1.4	2.4	1.4	70.2
P2S	1.1	----	1.2	---	1.1	----

Antiacetylcholine drugs and/or P2S were given intramuscularly 15 min before sarin subcutaneously.
The dose of P2S given in conjunction with drug was 140 μmol/kg.

Lullman et al.[98] compared the therapeutic effectiveness of dexetimide (29) and atropine in poisoning. Dexetimide, the bio-



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logically active enantiomer of benzetimide, is a potent antimuscarinic drug[99]. Since it is more hydrophobic and possess a lower pK_a (8.7) than atropine (9.8-10.2), it readily penetrates lipid barriers and becomes highly accumulated in different tissues, including the

CNS as evident from the high central potency of the drug. Bertram et al.[100] initially showed that dexetimide applied prophylactically was superior to atropine in mice poisoned with DFP. Lullman et al.[98] showed **29** was better than atropine when applied after the onset of intoxication. Atropine (100 μmol) gave a protective factor of 12, while dexetimide (100 μmol) gave a factor of 90 against DFP poisoning in mice.

OMPA, an anticholinesterase agent with little CNS effects, was administered to induce a purely peripheral intoxication. No difference in protection of the toxicity of this anti-AChE agent could be noticed between atropine or dexetimide. In guinea pigs, the toxicity of the centrally acting organophosphates was estimated to be 20 μmol/kg for DFP, and 4 μmol for paraoxon (Table 6). The doses as shown could be increased drastically if the guinea pigs were treated with the antidote mixture at the onset of intoxication. They noted that with increasing dose, particularly with DFP, the intoxication developed very rapidly. The LD_{50} of DFP increased to 1300 μmol/kg when treated with dexetimide (a 65-fold increase) while the LD_{50} of paraoxon could be increased to approximately 300 μmol/kg (a 75-fold increase).

TABLE 6

Treatment of Organophosphate Intoxication in Guinea Pigs[98]

Drug	Atropine 100 μM/kg Obidoxim 100 μM/kg 24 Hr.	24 Hr.	Dexetimide 100 μM/kg Obidoxim 100 μM/kg 10 Min.	24 Hr.
DFP μM/kg				
17	0/3	---	---	---
21	2/3	---	---	---
28	3/3	---	---	---
1250	---	2/3	1/4	1/4
1320	---	2/3	1/4	1/4
1360	---	3/3	3/6	3/6
Paraoxon μM/kg				
3.7	0/3	---	---	---
4.5	2/3	---	---	---
5.2	3/3	---	---	---
272	---	1/2	---	---
309	---	0/3	1/4	1/4
363	---	2/3	2/4	2/4

Lullman et al.[98] additionally compared the distribution and accumulation of atropine and dexetimide in various central and peripheral tissues and organs. The compounds differed remarkably with respect to their pharmacokinetics. They indicated that dexetimide accumulated much more rapidly in tissues. Of particular significance in organophosphate poisoning is the accumulation rate in the CNS. Dexetimide attains its highest accumulation rate (tissue/plasma ratio of 4) within 5 minutes after application, whereas atropine requires 60 minutes to reach a tissue/plasma ratio of 0.4. The over proportional accumulation of dexetimide in comparison to atropine can be shown as the tissue/plasma ratios of dexetimide are expressed as the

multiples of atropine tissue/plasma ratio in various central and peripheral tissues (Table 7). This shows dexetimide rapidly penetrates the blood-brain barrier, therefore yielding a higher protection of the central muscarinic receptors.

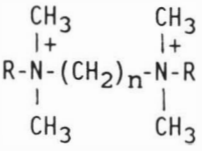
TABLE 7

Tissue/Plasma Ratios of Dexetimide Expressed as Multiples of the Tissue/Plasma Ratios of Atropine in Different Organs and At Different Times After Application[98]

Organ	Multiples of Atropine Tissue/Plasma Ratio (Time After Injection)			
	5 Min.	15 Min.	30 Min.	60 Min.
Liver	3.0	3.6	10.9	6.6
Kidney	3.0	2.1	4.7	4.1
Diaphragm	2.9	3.8	4.9	1.2
Cerebrum	12.5	15.0	19.4	5.5
Cerebellum	13.3	16.5	11.3	2.7
Brainstem	15.3	13.4	12.4	4.7

It is known that the reversible type AChE inhibitors could demonstrate some protective action in organophosphate intoxication when administered prophylactically. Lullman et al.[101] interestingly reported a new series of alkane-1,6-bis(alkyldimethylammonium) derivatives which showed good protection without any interference with the enzyme activity (30).

30



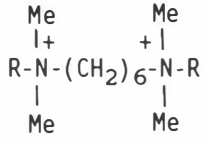
R = methyl-decyl
n = 4-9

The optimum distance between the cationic nitrogens for protection was six carbons. The active compounds did not exert ganglionic blocking activity. The substitution of one methyl group at each nitrogen atom by larger groups yielded compounds with reduced ganglionic or neuromuscular blocking activity[102]. Some additional hexane-bis-ammonium compounds were as effective as atropine in combatting the toxicity of DFP in mice[103]. The effect was found related to the antagonistic action of the compounds against ACh and carbachol. The mode of action of the most effective hexane-bis-ammonium compounds as antidotes differs from that of atropine in that they were found to be non-competitive antagonists[104]. Lullman et al. therefore proposed these compounds react with side receptors, producing an allosteric antagonism, and diminishing the accessibility of ACh for the receptor. The most powerful protective agent in this series was hexane-

bis-(dimethyl-phthalimidopropyl-ammonium bromide) (31). It had a protective ratio of 2.84 compared to 1.85 for atropine (Table 8).

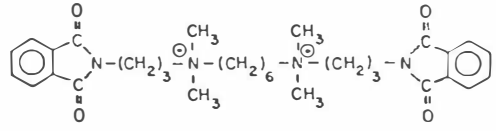
TABLE 8

Action of Some Hexane-1,6-Bis-Ammonium Derivatives on the Toxicity of DFP in Mice[103]



R	Dose (mole x 10^{-5}/kg)	LD_{50} of DFP (mole x 10^{-5}/kg)	F
----	----	2.65	----
Methyl (Hexamethonium; 12)	17.5	6.90	2.60
Ethyl	10.0	6.80	2.55
Propyl	8.4	4.90	1.83
Propenyl	4.8	2.80	1.05
Phenacyl	7.6	4.30	1.62
Phthalimidopropyl (31)	2.7	7.50	2.84
Atropine (13)	1.0	4.90	1.85
	5.0	5.60	2.11
	10.0	5.60	2.11

F = Protective Ratio = LD_{50} of DFP after pretreatment/LD_{50} of DFP.



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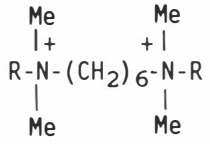
The combined pretreatment with atropine and a hexane-bis-ammonium derivative gave a more than additive protection against DFP intoxication. Hexane bis-(dimethyl-phthalimidopropyl-ammonium bromide) was the most powerful protective agent used alone. In

combination with atropine its protective factor was about 16 (Table 9).

TABLE 9

Influence of Pretreatment by a Combination of Atropine and Hexane-Bis-Ammonium Derivatives Upon the Lethal Dose of DFP in Mice[103]



R	Dose (mole x 10^{-5}/kg)	Atropine Dose (mole x 10^{-5}/kg)	LD_{50} DFP	F
Methyl (12) (Hexamethonium)	17.5	0.5	7.9	3.0
	17.5	5.0	37.0	14.0
Propyl	8.4	0.5	10.0	3.8
	8.4	5.0	12.0	4.5
Phenacyl	7.6	0.5	8.6	3.2
	7.6	5.0	13.0	4.9
Phthalimido-propyl (31)	2.7	0.5	38.5	14.5
	2.7	5.0	41.5	15.7

F = Protective Factor = $\frac{LD_{50} \text{ of DFP After Pretreatment}}{LD_{50} \text{ of DFP}}$

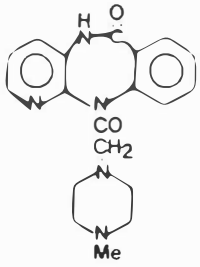
The most effective compounds as antidotes have many diverse structural features. Caramiphen does seem to constitute one structural type of agent with good protective activity. Of important concern in the key toxic effect for lethality is the action of ACh on postsynaptic muscarinic receptors in the brain to produce respiratory

paralysis. Therefore, there are three reasons which could influence the antidotal properties of an antimuscarinic for organophosphate poisoning.

One approach to an improved protective agent would thus involve a search for compounds with greater CNS activity compared to peripheral action. In whole animal tests different ED_{50} values are often found for peripheral (mydriatic) and central (block of oxotremorine-induced tremors) actions of a given muscarinic agent[105-107]. In man the dose of atropine required to produce peripheral effects is 0.25-2 mg/kg, whereas the dose to produce central effects which relieve symptoms of Parkinson's disease is 54 mg/day. A single dose of 30-50 mg of atropine is required to produce central psychotomimetic effects in man. It's not surprising that drugs significantly more potent than atropine and scopolamine *in vivo* in the peripheral nervous system have not been found. Usually for a given dose of agent the peripheral effect is more pronounced, and this has been attributed to a greater difficulty in obtaining the same brain as peripheral concentration rather than to differences in the receptors at different sites. The ratio of the potencies of the enantiomers of dimethylaminoethyl 2-cyclohexyl-2-hydroxy-2-phenylacetate was essentially constant when calculated from affinity constants determined on guinea pig ileum, from mydriatic potencies in mice, and from potencies for blockade of oxotremorine-induced salivation and tremor in mice[106]. Other studies using radiolabeled compounds have failed to find differences in specific binding of some antagonists to muscarinic receptors of brain compared to intestinal smooth muscle[157,158]. Only

recently has it been recognized that there are two or more subclasses of muscarinic receptors present in varying proportions in different organs and tissues. Nevertheless, the more lipophilic antagonist may be able to penetrate the brain to a greater extent and exert a better protective effect.

A second approach for gaining increased antidotal action would be to search for antagonists with a greater relative affinity for the relevant central muscarinic receptors. There is now substantial evidence suggesting the existence of pharmacologically and functionally distinct muscarinic receptor systems[108-111] (see Figure 10). Receptor classification is based on the differential sensitivity to pirenzepine (32), a muscarinic antagonist. Those receptors which display a high affinity for pirenzepine are classified as M1, whereas those that are less sensitive are considered M2 sites[108-112].



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While atropine fails to distinguish between these receptor systems, pirenzepine displays a much greater than the necessary ten-fold affinity for the M1 than the M2 site[109].

A distinguishing feature of these receptors is that they are unevenly distributed throughout various organs and tissues. Yamamura et al.[113] found that pirenzepine preferentially binds with high

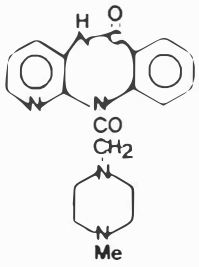
affinity in the rat cerebral cortex, hippocampus and corpus striatum and with low affinity in the heart, cerebellum and ileum. Application of autoradiographic visualization of the muscarinic receptors has revealed the M1 sites predominate in the basal ganglia, hippocampus, cortex and striatum, while the M2 sites predominate in the brainstem, medulla, cerebellum, the heart and smooth muscle[113-116].

In contrast to the M1 receptor, there are currently no selective M2 receptor radioligands for differentiating between these receptors in the brain. Therefore, another way to examine selectivity is to study the effect on functional responses that have been characterized on the basis of the M1-M2 classification. The muscarinic receptor-stimulated phosphatidyl inositol turnover in rat brain appears to be predominantly an M1 response, whereas cholinergic receptor-mediated inhibition of adenylate cyclase activity has been classified as an M2 receptor phenomenon[117].

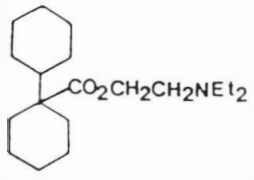
Several known muscarinic antagonists have been found to be selective compounds. Gibson et al. purported 3-quinuclidinyl xanthene-9-carboxylate (QNX) to be M1 selective[118]. Nilvebrand and Sparf reported pirenzepine (32), oxybutynine, trihexyphenidyl and dicyclomine had greater affinity for parotid gland receptors than ileum or bladder receptors in guinea pigs[119]. They found secoverine had the opposite selectivity. Secoverine, which is 0.6 times as active as atropine on intestinal smooth muscle, is more effective at inhibiting oxotremorine-induced tremors than lacrimation or salivation[120]. Additional reports have confirmed trihexyphenidyl, dicyclomine, and oxybutynin are M1 selective muscarinic antagonists[121-123].

FIGURE 10

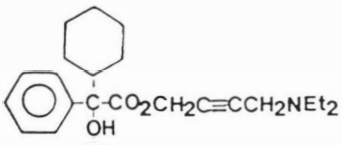
Structures of Selective Muscarinic Antagonists.



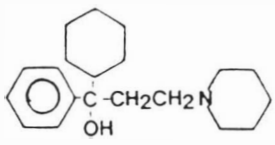
Pirenzepine



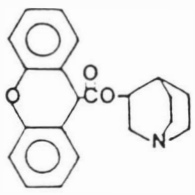
Dicyclomine



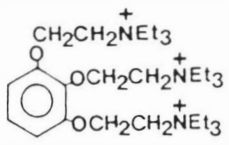
Oxybutynine



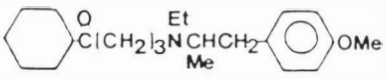
Trihexyphenidyl



QNX



Gallamine



Secoverine

A recent report has indicated secoverine may be a non-selective M2 muscarinic antagonist on rat heart and brain receptors[124]. A group of compounds which do exhibit an M2 selectivity are the bis- and tris-quaternary neuromuscular blocking agents[125]. They are more potent at antagonizing nicotinic effects, but they also inhibit muscarinic receptors. Gallamine has been extensively studied for its M2 selective activity[126,127]. If a difference exists in the type of receptors at the critical site in the respiratory center and at sites giving toxic effects (on heart or brain areas that produce convulsions), then an antagonist with proper receptor selectivity would be more beneficial.

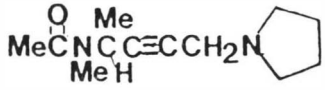
An additional possible approach for finding a better protective agent would be to search for antimuscarinics that are selective for postsynaptic receptors. It is well established that there are muscarinic presynaptic receptors that inhibit ACh release from both central and peripheral cholinergic neurons. It is possible that there may be differences between pre- and postsynaptic muscarinic receptors that could affect the affinity of different antagonists, just as has been established with other neurotransmitter receptor systems.

In the presence of a cholinesterase inhibitor, the potassium (K^+)- or electrically-stimulated release of ACh from rat cortical brain slices is potentiated by atropine[128-130]. This potentiation is reversed by the application of oxotremorine or methacholine. It has also been shown that K^+-stimulated release of ACh from rat brain hippocampal synaptosomes is inhibited by ACh, and atropine reverses

this action[131]. Kilbinger[132-134] reported similar experiments with strips of guinea pig ileum. It has also been shown that atropine increases and oxotremorine decreases release of ACh in the rat phrenic nerve-diaphragm preparation[135].

Kilbinger[136] has stated the function of presynaptic muscarinic receptors is to modulate the output of neurotransmitters such as dopamine and norepinephrine, in addition to ACh itself. Therefore it is felt that receptors located presynaptically may differ from those receptors located postsynaptically. Kilbinger et al.[137] found the pA_2 values of a range of muscarinic antagonists were similar at both pre- and postsynaptic muscarinic receptors using the rabbit isolated heart preparation. Several other studies of antagonists at pre- and postsynaptic receptors have shown the receptors to be similar at a given site[138,139]. It has yet to be established whether presynaptic receptors in the brain are M1, M2 or a different type receptor. Marchi and coworkers[140,141] have reported that presynaptic receptors which inhibit the release of ACh differ from those autoreceptors which inhibit the outflow of dopamine from dopaminergic terminals. This was based upon the ability of the M1 antagonists dicyclomine and pirenzepine to inhibit those receptors modulating dopamine release and not those mediating ACh release from superfused rat striatal synaptosomes. In contrast, the M2 antagonist gallamine has been shown more potent at autoreceptors inhibiting overflow of ACh than dopamine from rat neostriatal slices, whereas pirenzepine was not selective[142]. Therefore, presynaptic receptors may not be homogeneous; some may be M1 while some may be M2.

An analogue of oxotremorine, BM-5 (33), has been reported to enhance ACh outflow and also contract smooth muscle[143]. This compound has therefore been proposed to act as a presynaptic antagonist and a postsynaptic agonist[143,144].



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In cases of poisoning by cholinesterase inhibitors, the increased levels of ACh would help retard further release of ACh from nerve endings. However, if an antimuscarinic agent is administered which blocks presynaptic as well as postsynaptic receptors, then the autoinhibition is removed. Additional ACh will be released, and the increased levels in the synapses will tend to reverse the competitive inhibition of the antagonist.

G. Conclusion

The most effective muscarinic antagonists as antidotes have many diverse structural features. Caramiphen and aprophen appear to constitute types of agents with good protective action. It is important to recognize the key toxic effect for lethality is the action of ACh on postsynaptic receptors in the brain to produce respiratory paralysis. An improved antimuscarinic agent would not necessarily need to be more potent than atropine, but rather it would need to have a proper balance of central versus peripheral action with as little block of presynaptic receptors as possible. Increased antidotal action may be obtained with greater relative affinity for the relevant central muscarinic receptors. An ideal agent should effectively inhibit the centrally-mediated respiratory depression at a dose that is not too toxic. In cases of human poisoning with organophosphate insecticides, doses of atropine are given which would be lethal in the absence of excess ACh. The patient must, in effect, be titrated with an anticholinergic to combat the build up of ACh, but an excess of the anticholinergic drug must also be avoided. The military significance of having an effective antidote is obvious.

II. RESEARCH AIMS

Poisoning by organophosphate cholinesterase inhibitors results in a rise in ACh concentrations in synapses at numerous locations in the body, resulting in many pronounced pharmacologic effects. Although a fall in blood pressure, excessive secretions, bronchial constriction, and thoracic muscle weakness contribute to respiratory distress, the key toxic effect for lethality is the action of ACh on postsynaptic muscarinic receptors in the brain, producing respiratory paralysis[159]. The treatment for organophosphate poisoning involves administering an oxime reactivator to restore the activity of acetylcholinesterase and an antimuscarinic agent to block the response of many tissues and organs in the human body to the excess ACh. Oxime reactivators of cholinesterase can be useful in restoring enzyme activity, but even in the most favorable circumstances, an oxime reactivator which is a quaternary salt will not readily penetrate the CNS; therefore, reactivation will not occur at central synapses as effectively as it does at peripheral sites. Presently atropine sulfate is the standard antimuscarinic agent used clinically as an antidote. Atropine is a potent antimuscarinic agent. However, it has two distinct disadvantages as an antidote for nerve gas poisoning. It is less effective centrally than peripherally as an antimuscarinic (when administered peripherally) and it promotes the

release of ACh from cholinergic neurons by blocking inhibitory muscarinic receptors presynaptically. The unlimited use of atropine in treatment of men poisoned by organophosphate cholinesterase inhibitors could be ineffective in therapy and very dangerous. The results of Coleman et al.[92] and Jovic and Milosevic[93] demonstrate it is probably safer to give repeated therapeutic doses of caramiphen than atropine in the treatment of poisoning by organophosphates.

The aim of this research is to find a more effective antimuscarinic agent than atropine as an antidote for organophosphate cholinesterase inhibitors. An improved agent would not necessarily need to be a more potent antagonist than atropine, but rather it would need to have a proper balance of central versus peripheral action with as little block of presynaptic receptors as possible. Increased antidotal action may be obtained with greater relative affinity for the relevant central muscarinic receptors.

Correlations are to be sought between the relative ability of the compounds to protect against soman poisoning and activity in numerous pharmacological tests designed to give information on the structure-activity relationships for pharmacologic properties which might be important in a better antidote.

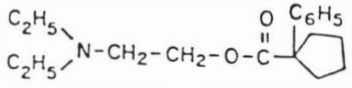
The chemistry involved in this project is a portion of a continuing program designed to discover a better antimuscarinic as an antidote. The rationale leading to the design of the compounds described and desired to be synthesized in this project will be discussed here in detail. Within the scope of the program was chosen a limited set of compounds containing many diverse structural

features for consideration among the hundreds of reasonably active antimuscarinic agents. Some of these compounds are currently marketed by various manufacturers and an authentic sample was requested. Numerous other compounds were either synthesized in other portions of the project or previously available from this laboratory. Structures of these compounds will also be shown, and the rationale for the compounds to be included in the program will be discussed.

A. Caramiphen and aprophen compounds.

Because caramiphen 19, and some of its analogs had previously been reported by Coleman et al.[88-92] to be effective antidotes against sarin poisoning, it would be important to make a number of additional structural variations of this molecule. Compounds 34-38 are standard variations in the amino-alcohol portion of caramiphen which may show changes in the activity of the parent compound. Compound 38 is the 1-phenylcyclohexanecarboxylate analog. This can not only be considered a caramiphen analog but also a variation of dicyclomine (the M1 selective antagonist) containing an aromatic phenyl ring instead of the reduced cyclohexyl moiety. Compounds 39-41 are similar variations of amide analogs of caramiphen.

Aprophen has been reported to be a potent antimuscarinic agent and an effective antidote[86,87]. Therefore it would be important to examine structural analogs of this molecule. Compounds 45-48 represent similar variations in the amino alcohol portion of aprophen as were described for caramiphen. Compounds 42-44 are variations in the propionic acid portion of aprophen. Compound 44 has a reduced



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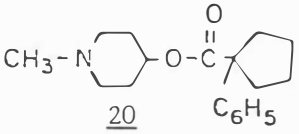

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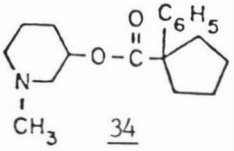

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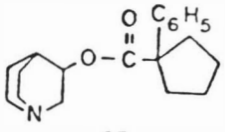

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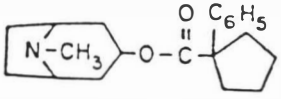

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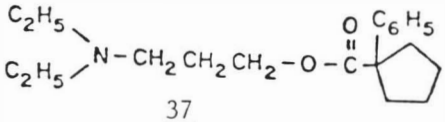

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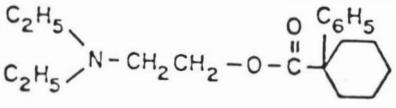

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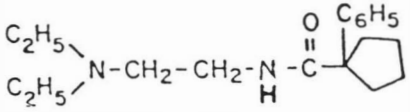

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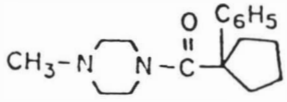

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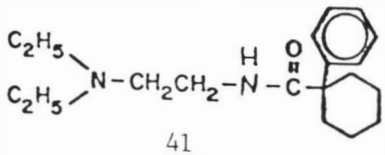

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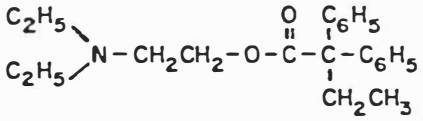
phenyl ring; 42 contains an additional methylene in the alkyl sidechain (ethyl instead of a methyl group). Compound 43 could be considered a rigid analog of 42 such that the ethyl is fused to one phenyl ring to form an indane ring system. This would also be a novel type of structure to examine because it may be considered to contain features of both caramiphen and aprophen. It may be considered to be an indane-aprophen analog (or a rigid analog of 18 and 42). Additionally it may be considered a caramiphen analog containing an additional phenyl ring fused to the cyclopentyl moiety of the lead compound caramiphen. Nevertheless this molecule which is a cross between aprophen and caramiphen could prove valuable in developing the structure-activity relationships for pharmacologic properties important in an antidote.

B. N-substituted-1,6-hexanediammonium compounds; N-substituted-1,6-hexanediamines; and N-(4-dialkylamino-2-butynyl)phthalimides.

Lullman et al.[101-103] reported a series of symmetrical N-substituted 1,6-hexanediammonium compounds which showed good antidotal protection. The effect was found to be an allosteric (non-competitive) antagonism against ACh and carbachol[104]. The best antidote in this series was N,N,N',N'-tetramethyl-N,N'-bis[3-(2-phthalimido)propyl)]-1,6-hexanediammonium bromide (31). These compounds contain more than one positive center like gallamine (see Figure 10) and other bis-quaternary neuromuscular blocking drugs which are known to demonstrate a non-competitive M2 selective antagonism[125-127]. The bis N-substituted 1,6-hexanediammonium compounds may also be acting in this fashion although this has never been determined.



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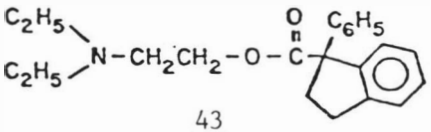

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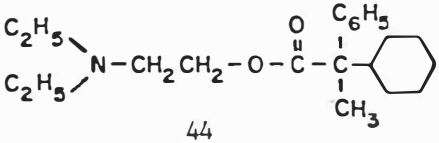

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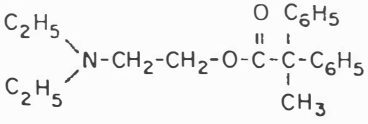

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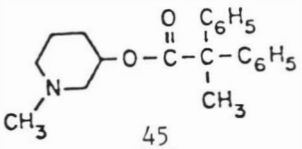

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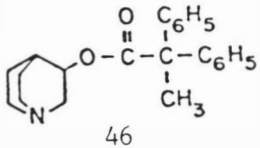

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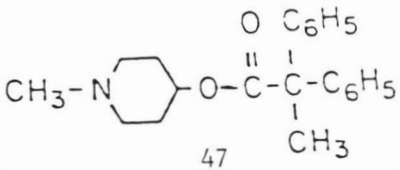

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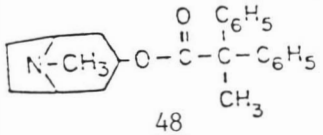

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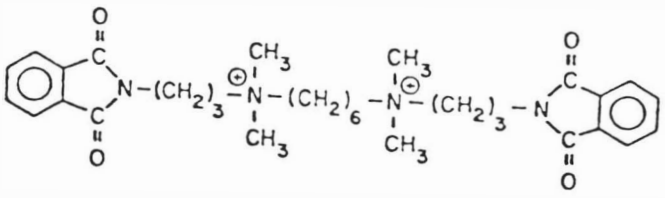
In the series of compounds studied by Lullman et al.[101,103] only the quaternary agents were ever reported or tested for antidotal or antimuscarinic properties. An important property of an antimuscarinic drug as an antidote is its ability to penetrate the brain and act on central muscarinic receptors to prevent centrally mediated respiratory paralysis. Since the quaternary ammonium compounds do not readily enter the brain it would be important to examine the tertiary amine compound of this series. If compound 49 retains the antimuscarinic properties observed with the N-substituted 1,6-hexanediammonium compound (31) it should be a more favorable antidote because it could act on the CNS.

Also, in the research described by Lullman et al.[101,103] only the symmetrically N-substituted 1,6-hexanediammonium compounds were synthesized and tested. It has not been determined if both 3-(2-phthalimido)propyl portions of compound 31 are necessary for activity or contribute in binding to the receptor. Therefore, the unsymmetrical compound 50 which is a partial structure of the parent compound 31 and contains only one 3-(2-phthalimido)propyl moiety would contribute information on the SAR of this series. For the same reasons as previously discussed for compound 49, the unsymmetrical tertiary amine should also be synthesized and examined for antimuscarinic and antidotal properties.



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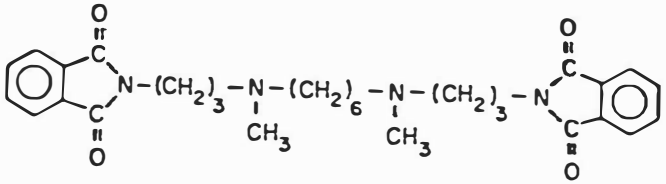

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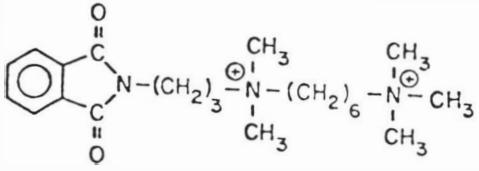

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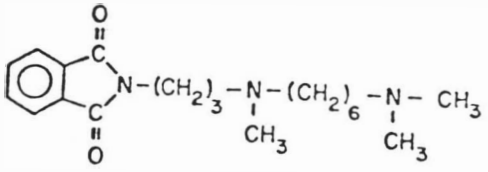

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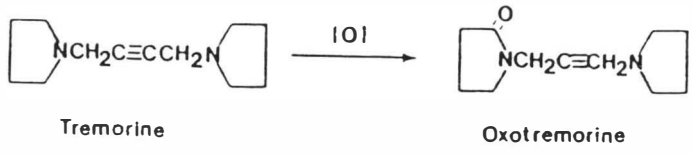
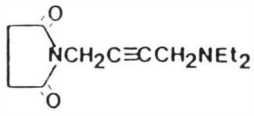
Oxotremorine, the metabolic oxidation product of tremorine[149], has been shown to be a potent muscarinic agonist in the central nervous system[150]. While the principal pharmacological properties of



Tremorine Oxotremorine

oxotremorine appear to be associated with its intense muscarinic activity, its actions upon the CNS are more marked than those of muscarinic tertiary amines such as arecoline, pilocarpine, and aceclidine. The high degree of potency led to the investigation of oxotremorine and structural analogs as a new class of CNS active muscarinic agonists[151-153]. It was observed while making structural variations that replacement of the 2-oxo-pyrrolidino moiety of the oxotremorine molecule by a succinimide group led to compounds with antagonistic properties[154,155]. N-(4-diethylamino-2-butynyl)-succinimide (52) was found to be quite potent in blocking the motor effects of oxotremorine, while the effect on peripheral cholinergic symptoms, such as ACh-induced spasms in guinea pig ileal strips, is of lower magnitude[154-156]. The relative specificity of oxotremorine on the CNS suggests that these type analogs might also be selectively



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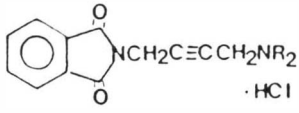
central in their blocking actions. Introduction of large substituents into agonist molecules frequently yields compounds possessing antagonistic properties. Additionally, incorporation of a lipophilic moiety such as a phenyl ring into the structure of a prototype compound should theoretically increase CNS activity provided the inherent activity of the parent compound is not destroyed. Therefore compounds 53 and 54 would be important to synthesize and examine with the aim of developing potent centrally active antagonists, which may show good antidotal properties.

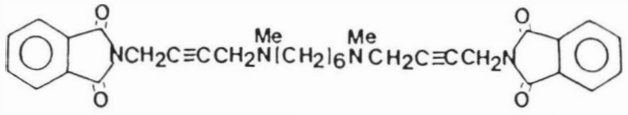


53 R = Methyl

54 R = Ethyl

It was previously reported that N,N,N′,N′-tetramethyl-N,N′-bis[3-(2-phthalimido)propyl]-1,6-hexanediammonium bromide (31) was an effective antidote and also displayed antimuscarinic activity[101,103]. The corresponding bis-tertiary amine (49) was designed with the aim

of retaining the antimuscarinic properties, but with good central activity, which is an important antidotal property. Based on these reasons and the fact that the oxotremorine-like antagonists (53 and 54) may show selective central antimuscarinic activity, it would be desirable to synthesize compound 55, which would incorporate features of the amino-alkyne compounds (oxotremorine-like antagonists 53-54) and the tertiary amine N,N′-dimethyl-N,N′-bis[3-(2-phthalimido)propyl]-1,6-hexanediamine, compound 49.



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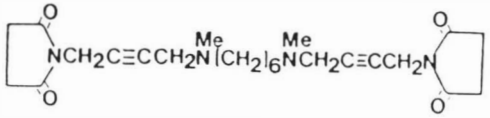
This would represent a novel type of compound designed to be a bis-type muscarinic antagonist (possibly M2) which may demonstrate good central activity. Since the succinimide analog 52 was the most potent in the series of oxotremorine-like antagonists[154,155], compound 56, a bis succinimide-alkyne, would also be important to synthesize and examine as an antimuscarinic for the same reason previously described.



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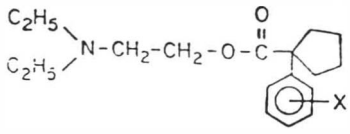
C. Para substituted caramiphen analogs (four corners approach).

Structure 57 represents a set of compounds that are derivatives of caramiphen, 19, which have different substituents in the para position of the phenyl ring. The purpose of the set is to test the effect of different substituents on the antimuscarinic activity and to look for any possible relationship of activity with substituent parameters such as Hammett's sigma (σ) constant or Hansch's pi (π) values. In the numerous cases of both the electronic (σ) and lipophilic (π) characters of aromatic ring substituents playing



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roles in the equation that relates structural properties to biological activity, both the sign and magnitude of the coefficients must be considered. A two-dimensional plot of Hammett sigma constants against Hansch pi values for aromatic substituents was proposed by Craig[148] to permit ready selection of novel compounds which may be expected to show improved biological activity in comparison to the lead compound in situations where these parameters can be directly correlated to activity. The use of two parameters in a multiple regression by the usual methods for determining quantitative structure-activity relationships (QSAR) would require 12 or more compounds in order to obtain a valid equation. In the current research, because the important factor was to determine if the parameters sigma

and/or pi had a significant influence on the antimuscarinic activity rather than to establish the quantitative relationship, it seemed this could be determined with only four properly chosen substituents. In this approach, a substituent is selected which has an extreme value for sigma and for pi in a positive or negative direction, in all combinations (Figure 11). Comparison of the activity of the four substituted derivatives and with the parent compound should reveal the importance of one or both of these parameters. The compounds chosen for use in this approach are the amino (-0.66 σ, -1.23 π) (57a); 1-tetrazolyl (+0.50 σ,, -1.04 π) (57b); 1-pyrrolidinyl (-0.83 σ, + 1.30 π) (57c); and trifluoromethyl (+0.54 σ, +0.88 π) (57d).

It is not known if the receptor can accomodate bulky substituents in this position. The lipophilic region where the phenyl ring binds may be a pocket only large enough for the phenyl ring itself. This has never been determined with structural analogs. This effect may be revealed with variations in the activity of the derivatives which can not be explained by the sigma or pi parameters.

D. Additional compounds chosen for testing.

A number of compounds were originally chosen for testing in this program so as to provide a very diverse group of structural features. Compounds 24, 58, 60 and 61 were chosen because of the previously reported effectiveness against the lethal effects of sarin and other cholinesterase inhibitors. Compound 29 is benzetimide; 41 is a dioxolane and 42 is a furan type antimuscarinic. The remaining members of the set were also chosen just to provide additional agents

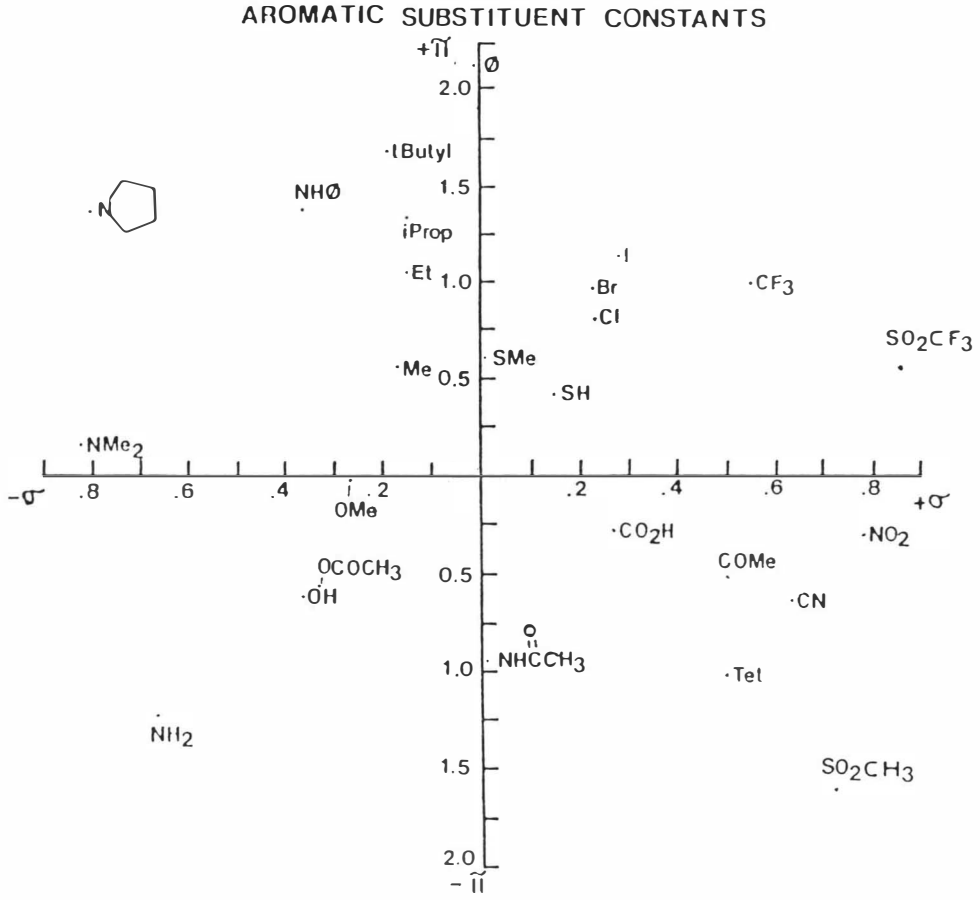
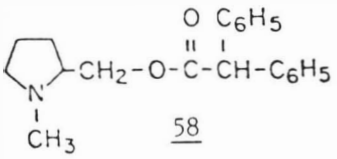


FIGURE 11

Two-Dimensional Craig Plot of Sigma Constants Versus Pi Values
for Aromatic Substituents
Information in this Table was compiled from Reference 147.

with diverse structural features. Compounds 24, 69-74, 76-78 and 82 are currently marketed antimuscarinics and an authentic sample was requested from the manufacturer. Compound 24 is triflupromazine (E.R. Squibb and Sons); 69 is cyclopentolate (Alcon Laboratories); 70 is the M1 selective compound oxybutynin (Marion Laboratories); 71 is chlorophenoxamine (Astra-Werke AG, Germany); 72 is benztropine (Merck, Sharp and Dohme); 73 is biperiden (Knoll Pharmaceutical); 74 is methixene (Dorset Laboratories); 76 is oxyphencyclimine (Beecham Laboratories); 77 is ethopropazine (Warner-Lambert Co.); 78 is orphenadrine (Riker Laboratories) and 82 is the M1 selective antagonist trihexyphenidyl (Lederle Laboratories). Compound 68 is the M2 selective antagonist secoverine and 75 is an ester analog of secoverine.

It was desired that pirenzepine also be included because of its selective M1 antagonist properties. However, it is known that pirenzepine, despite its affinity for brain muscarinic receptors, does not exhibit central activity when administered peripherally because it does not cross the blood-brain barrier[145]. Therefore an analog, 67, which lacks the polar amido group bridging the two aromatic rings, was chosen for inclusion rather than pirenzepine. Compounds 80 and 81 were previously available in this laboratory from another study and were designed as lipophilic analogs of the M1 selective antagonist pirenzepine. Compound 79 is the M1 selective antagonist QNX, and 83 is the M1 selective compound dicyclomine.



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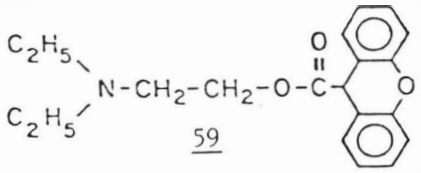

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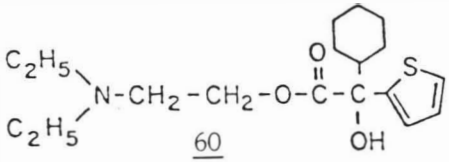

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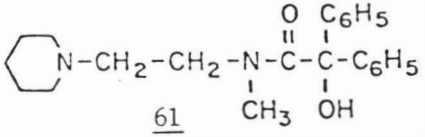

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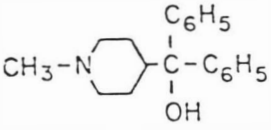

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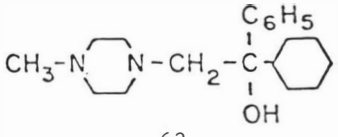

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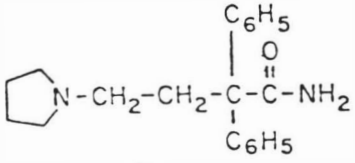

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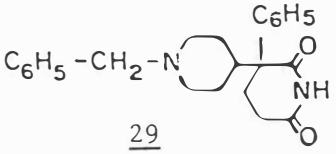

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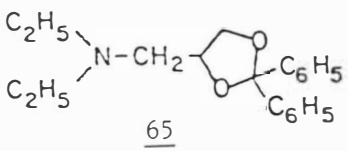

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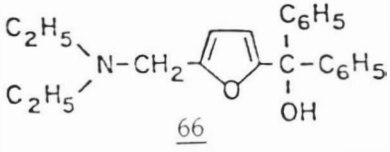

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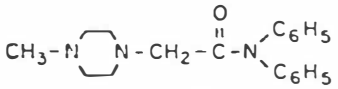

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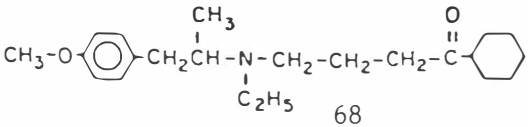

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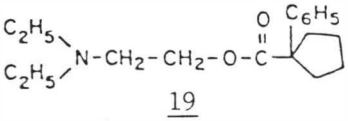

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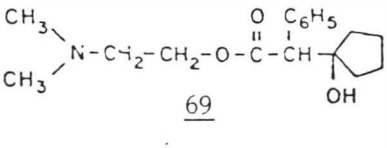

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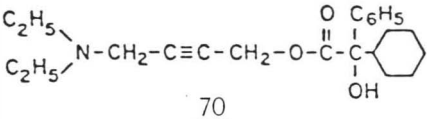

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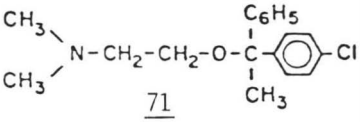

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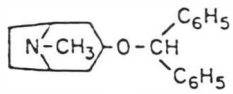

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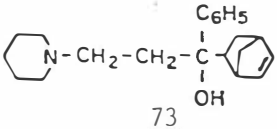

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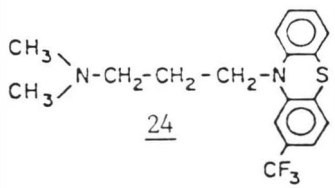

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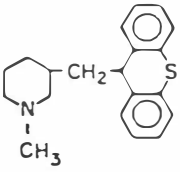

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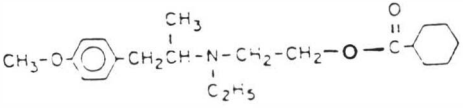

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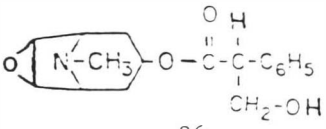

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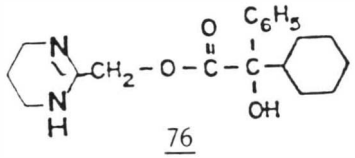

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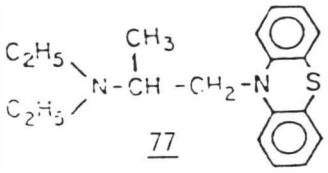

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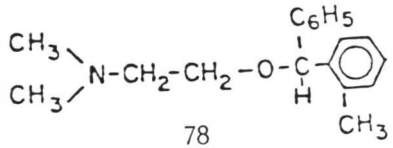

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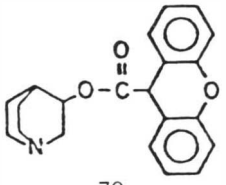

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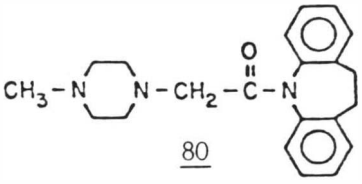

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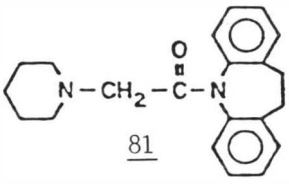

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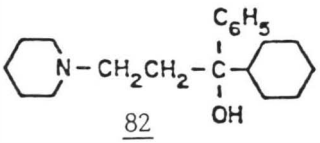

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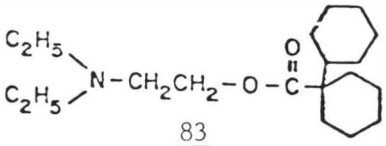

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From a purely theoretical consideration of the preferred conformation of a large set of antimuscarinic agents, Pauling[146] proposed that either one of two particular pharmacophoric patterns was necessary for binding to the receptor (Figure 12). Some similarities and differences can be determined from the chemical structure alone. Both contain a tertiary or quaternary amine (only the tertiary amines enter the CNS), a phenyl ring, and usually a hydroxyl and a large lipophilic group, such as a cyclohexyl or an additional phenyl ring, bonded to the carbon atom in which the phenyl ring is bonded. The two patterns differ in the distance between the nitrogen and an aromatic ring. While in the ester type anticholinergic substances the number of bonded atoms between the basic nitrogen atom and the phenyl ring is usually five, in anti-Parkinson's syndrome anticholinergic drugs it is usually three. It was suggested that agents which fit the pattern with the shorter distance between the

non-ester type ester type

R = aryl, cycloalkyl

FIGURE 12

Comparison of Pharmacophoric Patterns of Ester and Non-Ester Type Antimuscarinic Agents

nitrogen and phenyl ring might be slightly selective for central effects. Some non-ester type antimuscarinic agents which fit this

pattern are benzetimide (29), trihexyphenidyl (82), ethapropazine (77), orphenadrine (78), in addition to compounds such as 62, 72, 73, 74 and 24. It is not clear whether non-ester anti-Parkinson agents are useful because of a selectivity due to differences in distribution, receptor binding or other pharmacologic properties. Although no non-ester type antimuscarinics are to be synthesized in this portion of the project, these types of compounds are included because a comparison of the various structural types of antimuscarinics in testing for antidotal properties may prove fruitful.

The compounds in this project will be pharmacologically evaluated in comparison to atropine as antidotes for soman poisoning (in combination with 2-PAM) and also will be tested in numerous *in vitro* and *in vivo* tests designed to give information on the structure-activity relationships for pharmacologic properties which might be important in a better antidote. Thus, antimuscarinic activity will be measured for ability to block ACh-induced contractions of the smooth muscle of guinea pig ileum and potency to displace radiolabeled quinuclidinyl benzilate (QNB) from specific binding sites in mouse brain homogenates in order to compare central versus peripheral antimuscarinic activity. The compounds will also be examined in two *in vivo* tests, the block of oxotremorine-induced tremors and block of the CNS stimulus of oxotremorine or arecoline in a drug discrimination assay. These tests indicate a combination of ability to penetrate the blood-brain barrier, and also to act on central muscarinic receptors. Since the M1 and M2 subtypes are not evenly distributed in the brain, a preference or selectivity of these

receptors for one subtype may be a favorable property in a better antidote. Therefore the compounds will be evaluated for their potency to displace [3H]QNB from binding sites in homogenates prepared separately from rat forebrain (M1 sites) and medulla (predominantly M2 sites).

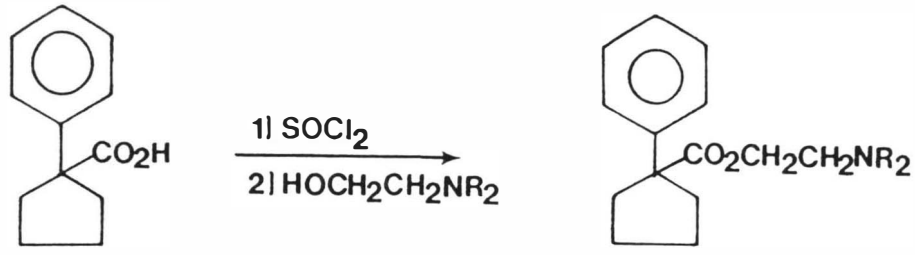
III. DISCUSSION

A. Chemistry

1. Caramiphen and aprophen compounds.

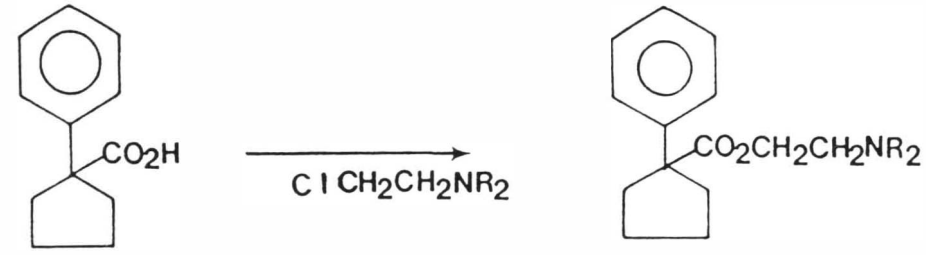
Three general procedures are most frequently used to synthesize amino ester and amide type antimuscarinics. Initially all three approaches were used to synthesize the lead compound caramiphen in order to determine which approach would be most favorable to use throughout this project. The three approaches are outlined in Scheme 1. Method A[160-163,168] involves reacting 1-phenylcyclopentanecarboxylic acid (84) with thionyl chloride to form the corresponding acid chloride. This intermediate is then reacted with 2-diethylaminoethanol in an aromatic solvent to give 2-(diethylamino)ethyl 1-phenylcyclopentanecarboxylate (19). In Method B[160,165,166,168] the carboxylic acid 84 is stirred in 2-propanol with freshly prepared 2-diethylaminoethyl chloride 86 to yield the HCl salt of the amino ester 19. Method C[160,167] involves making potassium 1-phenylcyclopentanecarboxylate (87) and simply stirring this at reflux with freshly prepared 2-diethylaminoethyl chloride (86) or its hydrochloride salt in ethanol to give 19. The transesterification method using the ethyl ester of 84 with an aminoalcohol and sodium in an aromatic solvent such as xylene or toluene is utilized much less frequently because of poor yields[160,168]. In this research yields for the synthesis of 19 were 68% for Method A, 52% for Method B and 56% for

Method A.



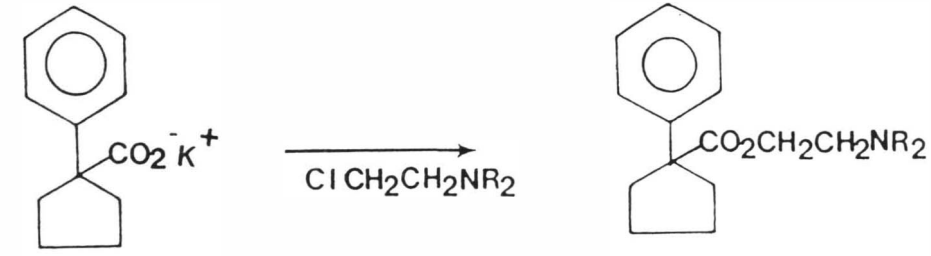
84 19

Method B.



84 19

Method C.



87 19

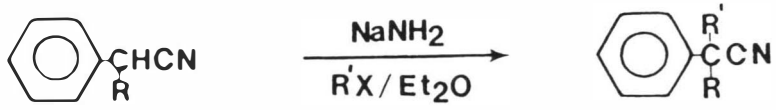
Scheme 1

Method C. Obviously there is not a significant difference in yields but it was decided that Method A was the most favorable and direct approach to make this type of compounds and was used primarily throughout the project. In no case was the intermediate acid chloride isolated and characterized. The acid chloride was formed using thionyl chloride as a solvent. After removal of the solvent and drying, the intermediate acid chloride was immediately reacted with the appropriate aminoalcohol using benzene as a solvent. Crystalline hydrochloride salts could readily be prepared and purified by recrystallization. Therefore the synthesis of the other derivatives of this series using this method was readily accomplished. Reacting freshly distilled 4-hydroxy-N-methylpiperidine with the acid chloride formed from 1-phenylcyclopentanecarboxylic acid (84) yielded compound 20; using freshly distilled 3-hydroxy-N-methylpiperidine gave compound 34; 3-quinuclidinol yielded compound 35; tropine yielded compound 36 and using freshly distilled 3-(diethylamino)-1-propanol gave compound 37. Benzene was used as solvent in all cases except for compound 35. Because of the poor solubility of 3-quinuclidinol in benzene either $CHCl_3$ or THF was used as a solvent, and a yield of 35% was obtained. Parkkari et al.[161] synthesized this compound in 28% yield by transesterification of methyl 1-phenylcyclopentanecarboxylate with 3-quinuclidinol in toluene with a catalytic amount of sodium. Transesterification with ethyl 1-phenylcyclopentanecarboxylate was reported to be unsuccessful.

Compound 38 was synthesized by the same procedure (Method A) using 1-phenylcyclohexanecarboxylic acid 85. The amide compounds 39-41 can also be readily synthesized by the same procedure. N,N-diethylethylenediamine gave compound 39 and N-methylpiperazine gave 40. Compound 41 was made by reacting N,N-diethylethylenediamine with the acid chloride from 1-phenylcyclohexanecarboxylic acid (85).

The aprophen analogs 45-48 which have similar variations in the aminoalcohol moiety of the molecule were not synthesized in this project. These compounds were synthesized in another project in this laboratory and were readily prepared by the same procedure[164] as described in Scheme 1 Method A.

The method of Larsen et al.[173] was used as a general method for making variations in the propionic acid portion (compounds 42 and 44) of aprophen (Scheme 2). Commercially available diphenylacetonitrile (88) reacts with exactly one equivalent of sodium amide followed by the addition of ethyl iodide in diethyl ether at low temperatures to yield 2,2-diphenylbutyronitrile (89)[173]. Alternatively, starting with cyclohexylphenylacetonitrile (90) and reacting it with methyl iodide under similar conditions gave 2-cyclohexyl-2-phenylpropionitrile (91)[174] in high yield. The next step involves hydrolysis of the hindered nitrile to the carboxylic acid. When the desired product is a carboxylic acid in the hydrolysis of a nitrile, the reagent of choice is most frequently aqueous alkali[170]. Although acid-catalyzed hydrolysis is also used, these conditions are most often used for stopping at the amide stage[171]. A review of the literature indicates the hydrolysis of hindered nitriles to the

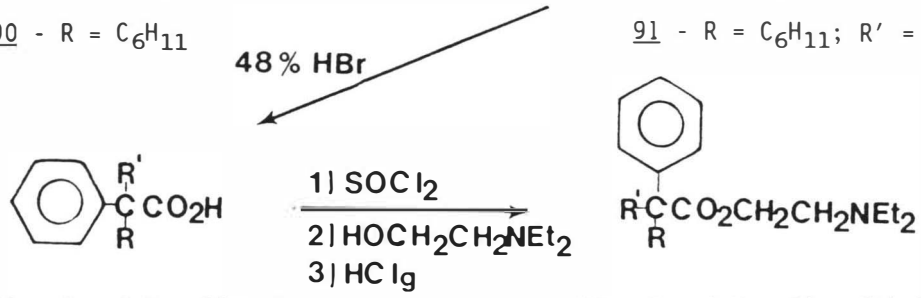


88 - R = C_6H_5

90 - R = C_6H_{11}

89 - R = C_6H_5; R′ = Et

91 - R = C_6H_{11}; R′ = Me



92 - R = C_6H_5; R′ = Ethyl

93 - R = C_6H_{11}; R′ = Methyl

42 - R = C_6H_5; R′ = Ethyl

44 - R = C_6H_{11}; R′ = Methyl

Scheme 2

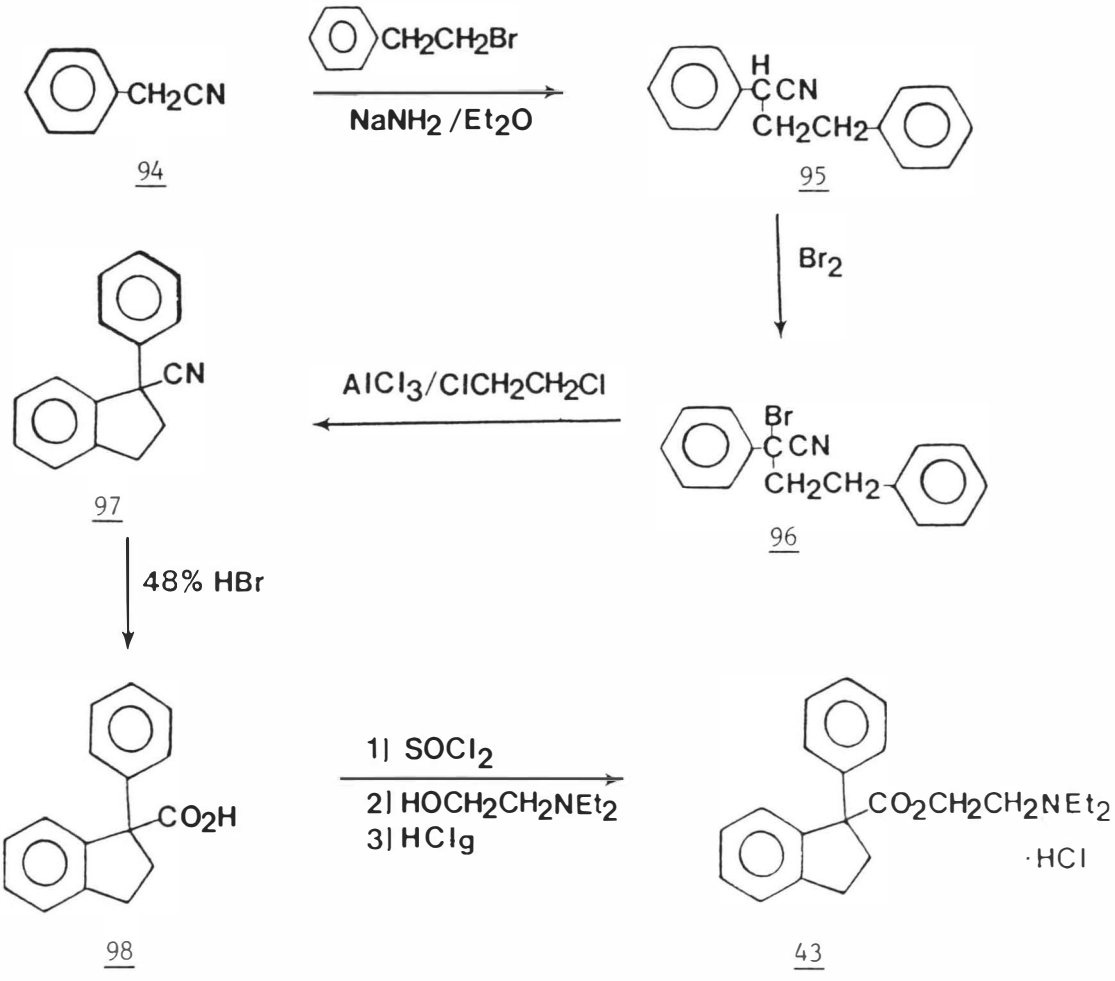
corresponding carboxylic acid often gives low yields by a number of methods and has been proven very difficult to accomplish. Case[172] reported that hindered nitriles containing the cyclopentane and cyclohexane rings on the a carbon atom were resistant to hydrolysis by alcoholic KOH. Larsen et al.[173] found trisubstituted acetonitriles were resistant to hydrolysis with alkali and water or alcohol. The principal product isolated was the amide.

A number of methods were attempted in this project using acidic conditions. Stirring the nitrile at reflux with concentrated HCl, or 6 N HCl was unsuccessful. Case[172] used concentrated HCl and sodium nitrite for the hydrolysis of 1-phenylcycloalkylcarbonitriles, which could not be hydrolyzed beyond the amide. Larsen et al.[173] reported low yields of acid by hydrolysis of the nitriles in a sealed tube with hydrochloric and sulfuric acids although the method required long reaction times and was generally difficult to run. They did report good yields of 2,2-diphenylbutyric acid using 70% sulfuric acid (d. 1.61) at high temperatures (reflux). When this method was attempted in this project only amide, water soluble products, tar-like material and a negligible amount of acid could be obtained. Varying the reaction temperature and reaction time (up to 7 days) did not improve the yields. Larsen et al.[173] reported conditions from 150 C to reflux and reaction times of 60 hours. Though good yields were reported they were always based on unrecovered starting material (nitrile) and not the final product (acid).

It was determined in this research that the required acid intermediates could be readily obtained in good yield by simply

stirring the nitrile in 48% HBr at vigorous reflux for periods of up to 10 days[167]. Hydrolysis of 89 under these conditions gives 2,2-diphenylbutyric acid (92) in 40% yield, and hydrolysis of 90 gives 2-methyl-2-phenylcyclohexaneacetic acid (93) in 86% yield. The reactions of acids 92 and 93 with thionyl chloride followed by esterification with 2-diethylaminoethanol yields the amino esters 42 and 44 respectively. All attempts to prepare a crystalline hydrochloride salt of 44 failed. Therefore a water soluble oxalate salt was made of this compound.

The synthesis of 2-(diethylamino)ethyl 1-phenyl-1-indanecarboxylate (43) is shown in Scheme 3. The necessary intermediate in the synthesis is 1-phenyl-1-indanecarboxylic acid (98). This compound has never been described in the literature but the synthesis of the corresponding nitrile (97) has been described by Philpott and Barltrop[176]. Phenylacetonitrile (94) was readily alkylated with phenylethyl bromide and exactly one equivalent of sodium amide in diethyl ether to give 2,4-diphenylbutyronitrile (95). Initially bromination was carried out as described by Philpott and Barltrop[176] in 1,2-dichloroethane, followed by immediate cyclization to the nitrile 97 in a one pot reaction. This approach gave poor yields of the desired product (approximately 20%) and a large amount of ether insoluble material. Therefore, bromination was carried out in methylene chloride and the crude bromo product 96 was isolated in 87% yield. Cyclization of 96 with $AlCl_3$ in 1,2-dichloroethane gave nitrile 97 in 58% yield, which could be readily purified by distillation. Hydrolysis of the nitrile 97 in 48% HBr gave carboxylic acid

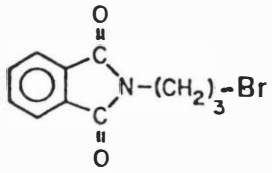

CH2CN
94
CH2CH2Br
NaNH2 /Et2O
H
CCN
CH2CH2
95
Br2
CN
97
AlCl3/ClCH2CH2Cl
Br
CCN
CH2CH2
96
48% HBr
CO2H
98
1| SOCl2
2| HOCH2CH2NEt2
3| HClg
CO2CH2CH2NEt2
·HCl
43

Scheme 3

98 in 75% yield. Precipitation of this acid from a cold dilute solution of NaOH gave a viscous brown semi-solid. All attempts to recrystallize the compound failed. Therefore this crude acid was reacted with thionyl chloride, then esterified with 2-diethylaminoethanol and purified as the hydrochloride salt to give the desired amino ester (43) in 46% yield.

2. N-Substituted-1,6-hexanediammonium compounds; N-substituted-1,6-hexanediamines; and N-(4-dialkylamino-2-butynyl)phthalimides.

The most direct approach to synthesize the quaternary ammonium compound N,N,N′,N′-tetramethyl-N,N′-bis[3-(2-phthalimido)propyl]-1,6-hexanediammonium bromide (31a) was to react two equivalents of N-(3-bromopropyl)phthalimide (102) and one equivalent of N,N,N′,N′-tetramethyl-1,6-hexanediamine (103) in an alcohol solvent[103] (Scheme 4). Multiple recrystallizations from methanol gave the desired bis-quaternary compound 31a in 45% yield. Since it was necessary to synthesize the corresponding tertiary amine, an alternate approach was also employed. In this method two equivalents of N-(3-bromopropyl)phthalimide (102) were reacted with one equivalent of N,N′-dimethyl-1,6-hexanediamine (104) (Scheme 4). This reaction gives a number of products including the desired N,N′-dimethyl-N,N′-bis[3-(2-phthalimido)propyl]-1,6-hexanediamine dihydrobromide (49c). This compound is more readily soluble in cold methanol whereas the quaternary compounds (by-products) are not. Multiple recrystallizations from methanol-ethyl acetate gave pure 49c. In addition to isolation of the dihydrobromide salt 49c by the method previously



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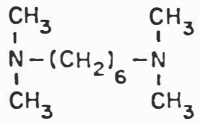

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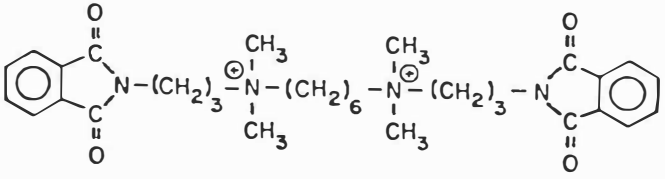
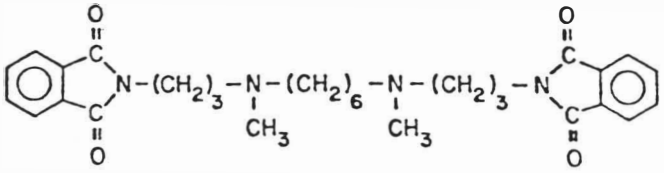


31a - 2 Br^-

31b - 2 I^-

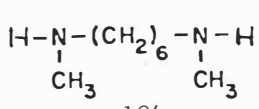
MeI



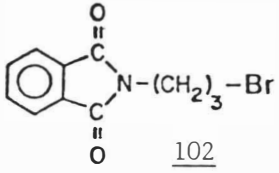
49a - Free base

49b - 2 HCl

49c - 2 HBr



104

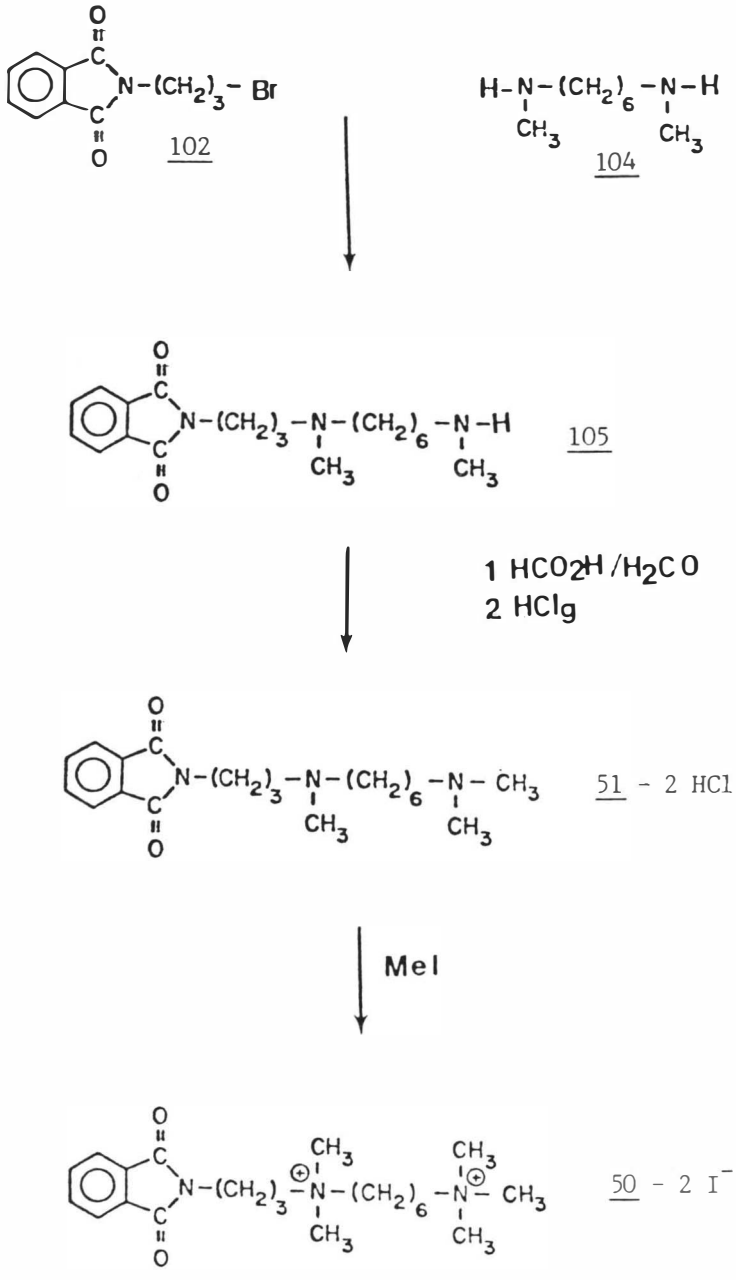


102

Scheme 4

described, by a similar method 49a, (as the free base) could be isolated by neutralization of the reaction followed by an extraction of the product into an organic solvent. This product was easily purified by recrystallization from methanol - water. After drying, 49a was readily converted to the dihydrochloride salt 49b by precipitation from a methanolic - gaseous HCl solution with ethyl acetate. Also, 49a was converted to the quaternary methiodide salt 31b by allowing 49a to react with an excess of methyl iodide in benzene[178,179]. Recrystallization of the dihydrochloride salt 49b and the dihydrobromide salt 49c of the tertiary N-substituted 1,6-hexanediamines proved to be very difficult and tedious. In numerous cases oils were obtained upon the addition of the co-solvent. Numerous recrystallizations were often necessary to obtain pure products. These types of compounds could not be purified or analyzed for purity by chromatography because extensive streaking occurs on silica. Reverse phase (C-18) chromatography also was unsuccessful. Kords et al.[103] reported chromatographic data using cellulose plates (phenol:benzene:water; 9:5:1), but R_f values of above 0.92 were reported. Accurate NMR data and CHN analysis confirmed the proposed structures in this research.

The synthesis of the unsymmetrical compounds N-[3-(2-phthalimido)propyl]-N,N′,N′-trimethyl-1,6-hexanediamine dihydrochloride and N,N,N′,N′,N′-pentamethyl-N-[3-(2-phthalimido)propyl]-1,6-hexanediammonium iodide is shown in Scheme 5. The reaction of N-(3-bromopropyl)phthalimide (102) and an excess of N,N′-dimethyl-1,6-hexanediamine (104) in THF gives N,N′-dimethyl-N-[3-(2-phthalimido)propyl]-

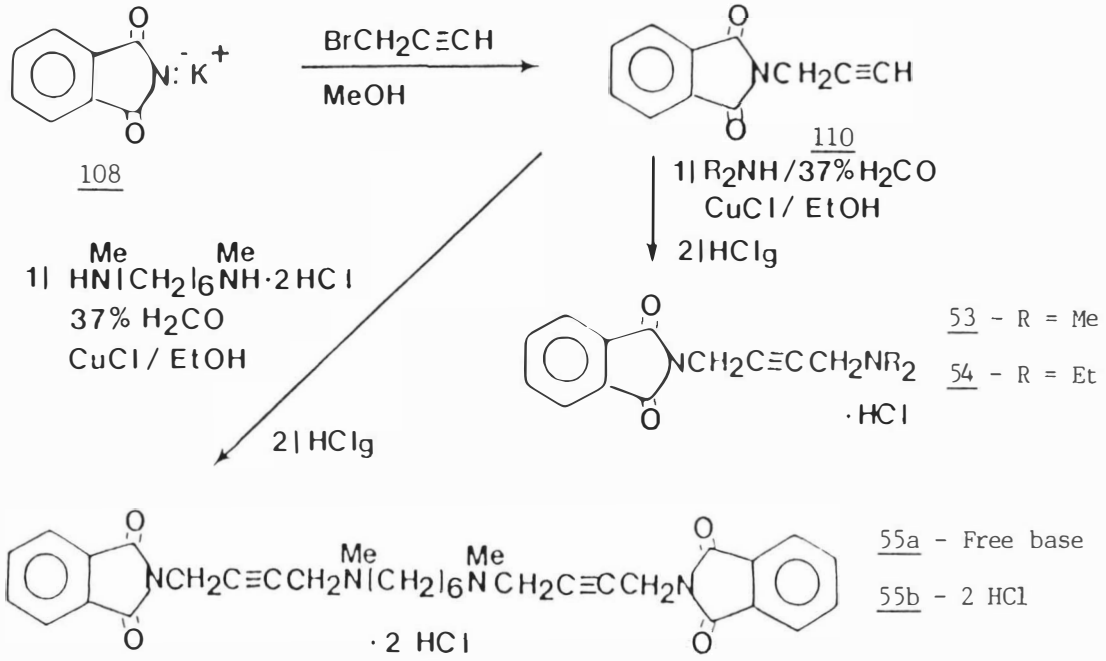


Scheme 5

1,6-hexanediamine (105) in 67% yield. After workup and purification this compound was immediately used in the next step. The formic acid - formaldehyde methylation of compound (105) (Eschweiler-Clarke reaction)[180] gave compound 106 in high yield. The dihydrochloride salt 51 was prepared in 43% yield by reacting an ether solution of the base with gaseous HCl and purified by recrystallization from methanol-ether.

The quaternary compound N,N,N′,N′,N′-pentamethyl-N-[3-(2-phthalimido)propyl]-1,6-hexanediammonium iodide (50) was prepared by a similar method as described for compound 31b[178,179]. The free base 106 of compound 51 was synthesized as previously described, then immediately allowed to react with an excess of methyl iodide in benzene to give 50 in 74% yield. The initial attempt to synthesize this compound was by exhaustive methylation of 105 by the method of Sommer et al.[178], using t-butylamine as a hindered base to react with the HI which is generated. This method proved unsatisfactory for the synthesis of the bis-quaternary amines in this project. Intermediate 105 which contains a secondary and a tertiary amine always yielded impure products by this method. An immediate precipitate would form which appeared by spectral data to be the mono-quaternary amine formed only from the tertiary amine.

The synthesis of the N-(4-dialkylamino-2-butynyl)phthalimides is shown in Scheme 6. Compounds 53 and 54 were prepared by a general Mannich reaction of a terminal alkyne with formaldehyde, a secondary amine and a small amount of cuprous chloride in either ethanol or dioxane[154-156]. The reaction of potassium phthalimide (108) and


BrCH2C≡CH
MeOH
108
110
1| R2NH / 37% H2CO
CuCl / EtOH
2| HCl g
1| HN|CH2|6NH·2HCl
37% H2CO
CuCl / EtOH
2| HCl g
NCH2C≡CCH2NR2
·HCl
53 - R = Me
54 - R = Et
·2 HCl
55a - Free base
55b - 2 HCl

Scheme 6

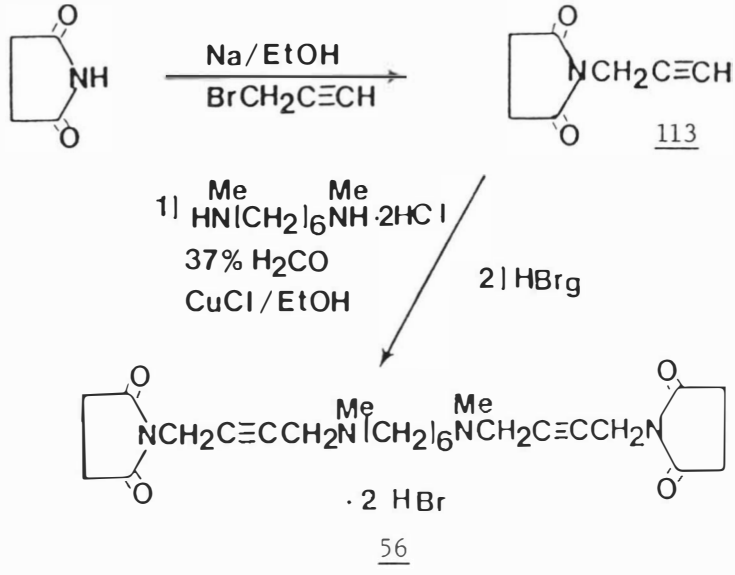
propargyl bromide in a methanol solution yielded N-(2-propynyl)phthalimide (110) in high yield[155]. The Mannich reaction of 110 with 37% formaldehyde and dimethylamine, followed by reaction with gaseous HCl gave 53 as the hydrochloride salt in 65% yield. Compound 54 was prepared using diethylamine by the same procedure in 45% yield. Bebbington et al.[151] originally reported this compound as the free base, which was synthesized in only 11% yield. Later, Dahlborn et al.[155] also only reported the free base of 54 (68% yield). The water soluble hydrochloride salt of 54, which would be more favorable for pharmacological testing, was prepared in this study.

The synthesis of the bis-amino-alkyne, N,N′-dimethyl-N,N′-bis[4-(2-phthalimido)-2-butynyl]-1,6-hexanediamine dihydrochloride (55b) is shown in Scheme 6. This compound was prepared by a double Mannich reaction at both secondary nitrogens of N,N′-dimethyl-1,6-hexanediamine dihydrochloride (111). Compound 111 was prepared by treating a methanol solution of N,N′-dimethyl-1,6-hexanediamine (104) with gaseous HCl, followed by precipitation of the salt with ether[181]. The reaction of compound 111 with two equivalents of 110, formaldehyde and a small amount of cuprous chloride yielded 55a in 53% yield. This compound was purified by recrystallization from methanol - water then analyzed. A water soluble dihydrochloride salt, 55b, was prepared by treating a solution of 55a in methanol with gaseous HCl. Recrystallization of this compound was achieved from methanol - ethyl acetate and was carried out with caution or only oils were obtained upon addition of the co-solvent.

The synthesis of N,N'-dimethyl-N,N'-bis[4-(1-succinimido)-2-butynyl]-1,6-hexanediamine (56) is shown in Scheme 7. Succinimide was reacted with an equivalent of sodium metal in ethanol followed by the addition of propargyl bromide to give N-(2-propynyl)succinimide (113) in 80% yield. Mannich reaction with 111 and two equivalents of 113 gave the free base of compound 56 as a viscous oil. Attempted preparation of a dihydrochloride salt of this compound resulted only in oils. Therefore the dihydrobromide salt of 56 was prepared.

3. Para substituted caramiphen analogs (four corners approach).

The purpose of this set of compounds is to test the effect on antimuscarinic activity of different substituents in the para position of the phenyl ring of caramiphen, and to look for any possible relationship of activity with substituent parameters such as Hammett's sigma (σ) or Hansch's pi (π) values. Substituents were chosen which have an extreme value for sigma and for pi in a positive and negative direction, in all combinations (see Figure 10). The substituents which were chosen for examination are the amino (57a); 1-tetrazolyl (57b); 1-pyrrolidinyl (57c); and trifluoromethyl (57d). This method is not limited only to these substituents. The only para substituted caramiphen analog which has been synthesized and examined as either an antimuscarinic or an antidote was the nitro derivative. Bannard et al.[160] reported the *p*-nitro derivative of caramiphen had a protective ratio in sarin-poisoned rats of 1.3. This is a much lower value than the unsubstituted parent compound caramiphen (3.2). The value for atropine was 2.03 in this assay. The nitro substituent is


Na/EtOH
BrCH2C≡CH
NH
NCH2C≡CH
113
1] HN[CH2]6NH·2HCl
Me Me
37% H2CO
CuCl/EtOH
2] HBrg
NCH2C≡CCH2N[CH2]6NCH2C≡CCH2N
Me Me
·2 HBr
56

Scheme 7

in the plus sigma (+0.78) and minus pi (-0.28) quadrant of the Craig plot (see Figure 10). The tetrazolyl (57b) was chosen from this quadrant in this research project.

The synthesis of the amino (57a) and 1-pyrrolidinyl (57c) compounds is shown in Scheme 8. The nitration of 1-phenylcyclopentanecarboxylic acid (87) with fuming nitric acid gave 1-(*p*-nitrophenyl)cyclopentanecarboxylic acid (114) in 87% yield. The corresponding acid chloride was formed using thionyl chloride, then reacted with 2-diethylaminoethanol. The hydrochloride salt was formed by reaction with gaseous HCl to give 2-(diethylamino)ethyl 1-(*p*-nitrophenyl)cyclopentanecarboxylate hydrochloride (115) in 78% yield. An alternate method to synthesize this compound was to react potassium 1-(*p*-nitrophenyl)cyclopentanecarboxylate (116) with 2-diethylaminoethyl chloride (86) in 2-propanol. The yield from this method was 58%. The nitro compound 115 was then catalytically reduced on a Parr apparatus with 10% platinum on carbon under a hydrogen atmosphere to give the corresponding amino compound 57a in high yield[182].

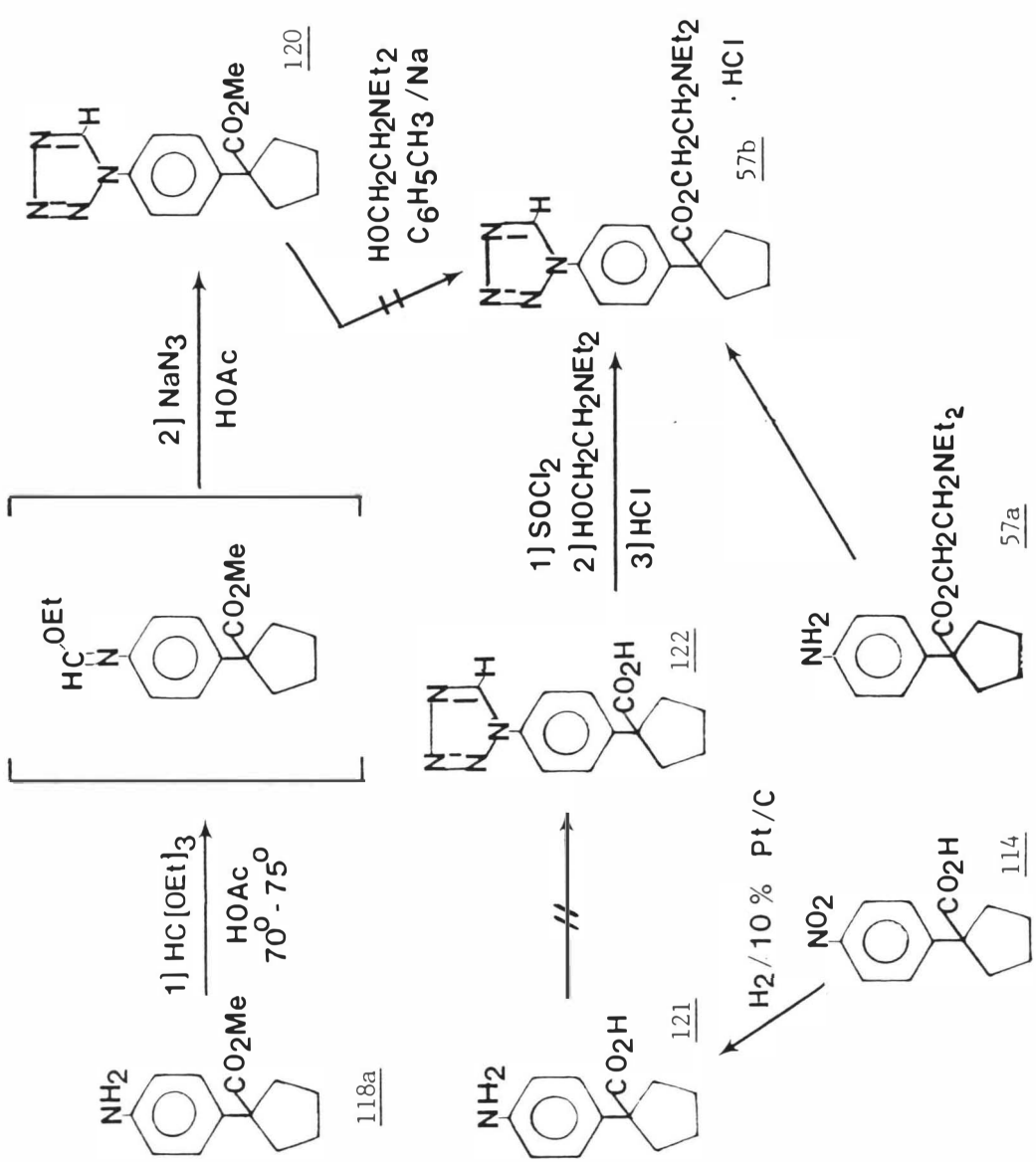
The route used to prepare the pyrrolidinyl derivative 57c is shown in Scheme 8. This compound was prepared by the transesterification method. The required intermediate in this approach is an alkylester of 1-[*p*-(1-pyrrolidinyl)phenyl]cyclopentanecarboxylic acid. The intermediate *p*-nitro-carboxylic acid 114 was converted to the methylester 117 via the acid chloride route (prepared from thionyl chloride)[161]. The nitro-methylester was then catalytically reduced to the *p*-amino-methylester 118 in 77% yield. The *p*-pyrrolidinyl ring was formed by using the method of Kalir et al.[183]. The

f HNO3
0°/ 4h
NO2
CO2H
114
1| SOCl2
2| MeOH
CO2CH3
117a
1| SOCl2
2| HOCH2CH2NEt2
3| HCl g
CO2CH2CH2NEt2
·HCl
115
H2
10%
Pt/C
NH2
118a
Br(CH2)4Br
K2CO3/DMF
80°/ 12 h
N
119a
H2/ 10% Pt/C
57a
1| HOCH2CH2NEt2
C6H5CH3/Na
2| HCl g
57c

Scheme 8

reaction of 118 with 1,4-dibromobutane in DMF gave methyl 1-[*p*-(1-pyrrolidinyl)phenyl]cyclopentanecarboxylate (119) in 63% yield. Initially in this project the ethyl esters of these intermediates were synthesized. The transesterification step using ethyl 1-[*p*-(1-pyrrolidinyl)phenyl]cyclopentanecarboxylate (119b) was unsuccessful. Therefore the methyl esters were then made of these intermediates. The transesterification using the methyl ester of the pyrrolidinyl compound 119a with 2-diethylaminoethanol and a catalytic amount of sodium in toluene gave the target compound 2-(diethylamino)ethyl 1-[*p*-(1-pyrrolidinyl)phenyl]cyclopentanecarboxylate (57c) in approximately 50% yield. This compound could be readily isolated and purified as the hydrochloride salt by recrystallization. Apparently the carbonyl is significantly hindered such that the addition of one extra methylene in the alcohol portion of the ester intermediate (methyl to ethyl) adds enough steric hindrance to prevent transesterification.

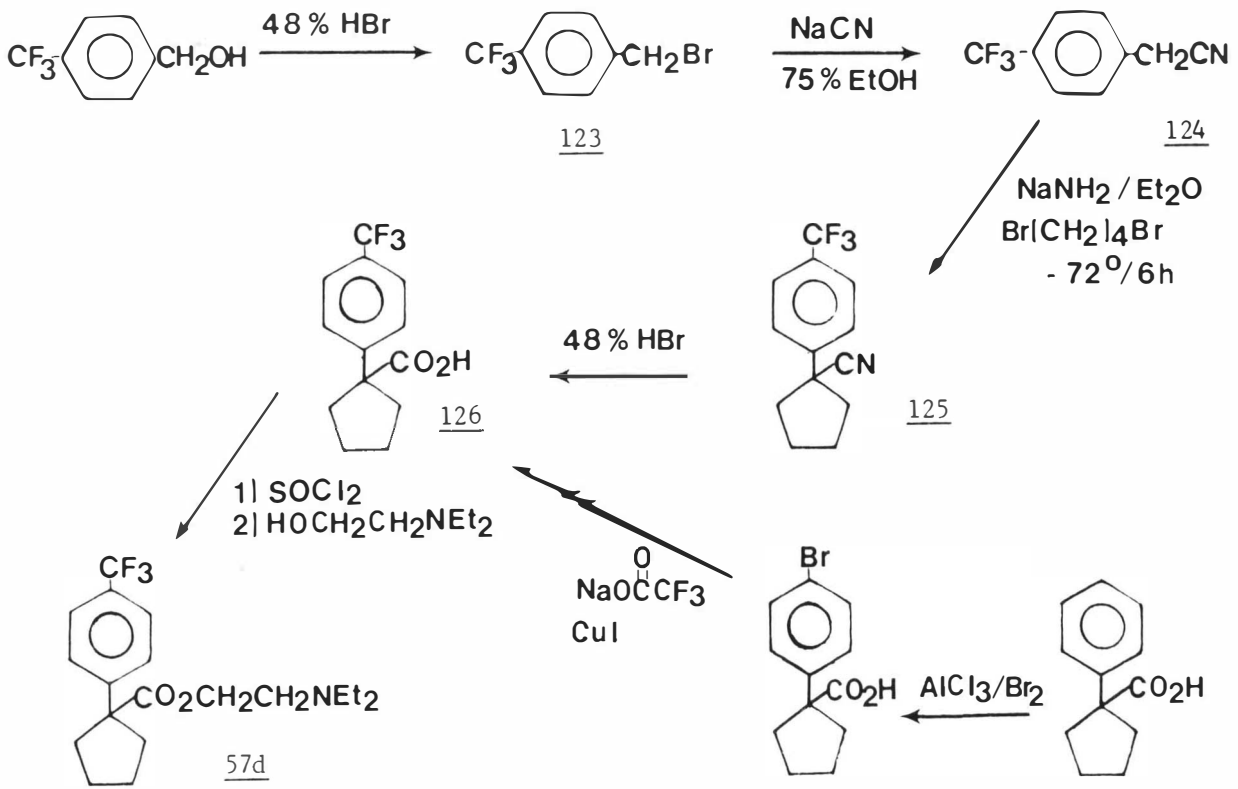
Although many 5-substituted tetrazoles are known, fewer 1-substituted tetrazoles have been described. 1-Aryltetrazoles have been prepared by addition of azide ion to isonitriles, by the reaction of diazonium salts with diformylhydrazine and by cyclization of N-aryl formamides with azide ion[185-187]. A more favorable general method to synthesize 1-aryltetrazoles from substituted anilines was reported by Kamitani and Saito[188]. In this approach the tetrazole is prepared by reacting an aniline or its salt with ethyl orthoformate and sodium azide in acetic acid. The approach to the 1-tetrazolyl compound 57b is outlined in Scheme 9. Methyl 1-[*p*-(1-tetrazolyl)-


1] HC(OEt)3
HOAc
70°-75°
2] NaN3
HOAc
HOCH2CH2NEt2
C6H5CH3/Na
1] SOCl2
2] HOCH2CH2NEt2
3] HCl
H2/10 % Pt/C
118a
120
121
122
114
57a
57b
·HCl

Scheme 9

phenyl]cyclopentanecarboxylate (120) was formed in 65% yield by reacting the aniline 118a with ethyl orthoformate and sodium azide in acetic acid at 70-75 C. All attempts to form the target compound 57b by transesterification of 120 with 2-diethylaminoethanol in toluene failed. The addition of a catalytic amount, or an equivalent of sodium metal to the reaction mixture resulted in massive decomposition. It was felt a more favorable route to form 57b could be from the tetrazolyl-acid 122. 1-(*p*-Aminophenyl)cyclopentanecarboxylic acid (121) was formed by catalytic reduction of the nitro-acid 114 in high yield[184]. All attempts to form a tetrazole 122 by the method previously described proved unsuccessful. A final approach was to form tetrazole 57b directly from the aniline 57a. This reaction gave low yields of a crude product which could not be purified by crystallization. Column chromatography of this crude product yielded a small quantity of pure 57b. Large quantities necessary for complete pharmacological testing could not be obtained by this method.

The synthesis of the trifluoromethyl compound 57d is outlined in Scheme 10. 4-(Trifluoromethyl)benzyl bromide (123) was synthesized in high yield from 4-(trifluoromethyl)benzyl alcohol[189]. The nitrile 124 was readily formed from 123 and sodium cyanide in 75% ethanol[190]. This nitrile was reacted with 1,4-dibromobutane and sodium amide (2 equivalents) at low temperature, in ether, to give a crude cyclopentyl compound 1-[*p*-(trifluoromethyl)phenyl]cyclopentanecarbonitrile (125)[162,172]. The crude reaction mixture contained a number of minor products. All attempts to obtain pure 125 (chromatography, distillation) were unsuccessful. Varying the conditions of the reaction


48 % HBr
NaCN
75 % EtOH
123
124
NaNH2 / Et2O
Br(CH2)4Br
- 72°/6h
48 % HBr
125
126
1| SOCl2
2| HOCH2CH2NEt2
57d
NaOCCF3
CuI
AlCl3/Br2

Scheme 10

(solvents, temperature, reaction times, and using various bases) did not afford a homogeneous product. The crude nitrile 125 was therefore hydrolyzed with 48% HBr to give acid 126. This product was reacted with thionyl chloride followed by reaction with 2-diethylaminoethanol to give 57d. A pure crystalline hydrochloride salt could not be prepared from this compound. Therefore a solid oxalate salt was made. A correct microanalysis of the elements could not be obtained on 57d. Because of the difficulty in the cyclization step to give pure 125, an alternate approach was attempted to synthesize 57d. Matsui et al.[191] reported aromatic halides could be trifluoromethylated with sodium trifluoroacetate and cuprous iodide in N-methyl-2-pyrrolidinone. This method was attempted, but proved unsuccessful in forming 126 directly from 1-(*p*-bromophenyl)cyclopentanecarboxylic acid.

B. Pharmacology

The pharmacological testing was conducted under the supervision of Dr. W.L. Dewey, Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University. The pharmacological data for all compounds involved in the program are included in the Tables. The compounds which were synthesized in this project are indicated with an asterisk (*) and the discussion is primarily involved with only these compounds. The aprophen analogs with variations in the amino-alcohol portion of the molecule are also included (*) for this purpose. Atropine and scopolamine (26) have been included as standards.

The compounds have been evaluated for ability to block ACh-induced contractions of the smooth muscle of the guinea pig ileum by Dr. J. Kachur, Department of Pharmacology, Medical College of Virginia according to the classical *in vitro* assay method of Magnus[192]. This demonstrates the drug's ability to act as a peripheral antimuscarinic agent. In this assay increasing concentrations of ACh are used in the presence of an antagonist to restore muscle contraction to a level produced in the absence of antagonist. Three concentrations of antagonist were tested in duplicate. From the relative quantity required (dose ratio) it is possible to calculate a measure of the affinity of the antagonist. If the antagonism is competitive then the dissociation constant (K_D) = [antagonist]/dose ratio - 1. The pA_2, a number used to measure relative affinity of different antagonists, can be calculated, ($pA_2 = -\log K_D$). The pA_2,

TABLE 10

The Dissociation Constant and Relative Potency (Atropine = 100) for a Number of Antimuscarinic Agents on Smooth Muscle of Guinea Pig Ileum

Compound	pA_2	K_D (nM)	Relative Potency
29	10.09	0.08	1600
79	9.80	0.16	810
46*	9.31	0.49	260
35*	9.29	0.51	250
47*	9.28	0.54	240
76	9.17	0.67	190
48*	9.03	0.93	140
60	9.00	1.0	130
Atropine	8.88	1.3	100
36*	8.77	1.7	76
20*	8.74	1.8	72
82	8.52	3.0	43
72	8.48	3.3	39
26	8.40	4.0	32
70	8.33	4.7	28
74	8.28	5.3	25
68	8.21	6.2	21
73	8.19	6.4	20
77	8.13	7.5	17
44*	7.99	10.3	13

TABLE 10 (Continued)

Compound	pA_2	K_D (nM)	Relative Potency
37*	7.96	11.0	12
45*	7.91	12.4	10
59	7.86	13.8	9
43*	7.80	16.0	8
69	7.76	17.4	8
18*	7.47	34.3	4
34*	7.46	34.6	4
19*	7.45	35.7	4
38*	7.42	38.5	3
65	7.37	43.0	3
63	7.20	62.5	2
66	6.94	116	1
42*	6.93	118	1
58	6.92	119	1
78	6.90	127	1
64	6.87	134	1
24	6.86	137	1
71	6.71	194	0.7
80	6.27	536	0.2
75	5.82	1500	0.08
61	5.69	2040	0.06
67	5.62	2380	0.05

TABLE 10 (Continued)

Compound	pA_2	K_D (nM)	Relative Potency
62	5.43	3700	0.03
49*	< 5.00	> 10,000	< 0.01
31*	< 5.00	> 10,000	< 0.01

K_D and the relative potency of the antagonists compared to atropine (100) are given in Table 10 in order of decreasing activity. As can be seen the affinity of the different antagonists for muscarinic receptors in the guinea pig ileum was quite variable. A number of the aprophen (46, 47, 48) and caramiphen (20, 35, 36) analogs were approximately equal to or better than atropine in this assay. It is interesting to note caramiphen (19) and aprophen (18), which are better antidotes than atropine, exhibited only 4% of the relative activity of atropine. Compound 43, the indane analog which contains features of 18 and 19, was a better antagonist than either compound, although still much weaker relative to atropine (8%). Breaking the bond in the indane ring system (compound 42) resulted in significant loss of activity. Also, compound 44, an aprophen analog with one hydrogenated aromatic ring, resulted in an increase in activity relative to atropine. N,N,N′,N′-tetramethyl-N,N′-bis[3-(2-phthal-

imido)propyl]-1,6-hexanediammonium bromide (31) and the corresponding tertiary amine 49 were not effective at the highest dose tested.

The compounds have been evaluated for affinity at CNS muscarinic receptors by examination for ability to displace tritiated quinuclidinyl benzilate (QNB) from specific binding sites in mouse brain homogenates[193]. Three concentrations of antagonists were run in duplicate. The concentration required in order to displace one-half (IC_{50}) of the specifically bound QNB (5.0 nM concentration) and relative affinity (atropine = 100) of the antagonists tested is shown in Table 11. Because QNB has a high affinity for muscarinic receptors, a high concentration of some antagonists is needed to displace it, and a number of compounds did not displace 50% of the QNB at the highest concentration used (1.0 μM). Therefore the concentration of QNB was reduced to 0.5 nM, which lowers the IC_{50} for the antagonist. The results of the compounds which were evaluated at this concentration are shown in Table 12. It is obvious that atropine and scopolamine (26) bind with high affinity from this binding assay. Caramiphen exhibited only 5% of the relative affinity of atropine for brain muscarinic receptors although it is a better antidote. Some of the analogs of caramiphen which were synthesized did improve on the affinity of the parent molecule. The best analog in binding was the 4-hydroxy-N-methylpiperdinyl derivative 20. The bis N-substituted 1,6-hexanediammonium compound 31 and the corresponding tertiary amine 49 were weak antagonists (0.3% and 0.4% respectively) relative to atropine.

TABLE 11

The IC_{50} and Relative Potency (Atropine = 100) of a Number of Antimuscarinic Agents in Displacing Radiolabeled Quinuclidinyl Benzilate from Specific Binding Sites in Mouse Brain Homogenates (QNB Concentration = 5.0 nM)

Compound	IC_{50} (nM)	Relative Affinity
26	36	125
Atropine	45	100
29	50	90
60	65	59
73	100	45
20*	111	41
70	133	34
35*	147	31
72	170	26
18*	273	16
38*	330	14
24	380	12
59	402	11
63	510	9
69	535	8
36*	645	7
37*	800	6
19*	915	5
65	930	5

TABLE 11 (Continued)

Compound	IC_{50} (nM)	Relative Affinity
64	1000	4
58	> 1000	< 4
61	> 1000	< 4
62	> 1000	< 4
66	> 1000	< 4
67	> 1000	< 4
71	> 1000	< 4
74	> 1000	< 4
24	> 1000	< 4

TABLE 12

The IC_{50} and Relative Potency (Atropine = 100) of a Number of Antimuscarinic Agents in Displacing Radiolabeled Quinuclidinyl Benzilate from Specific Binding Sites in Mouse Brain Homogenates (QNB Concentration = 0.5 nM)

Compound	IC_{50} (nM)	Relative Affinity
26	1.9	560
29	7.9	134
70	10.1	105
Atropine	10.6	100
46*	11.1	95
72	77	14
68	100	11
74	110	10
19*	124	9
45*	136	7
34*	192	6
58	228	5
49*	2380	0.4
31*	3550	0.3

Table 13 shows a comparison of relative activities of compounds in the guinea pig ileum (peripheral) and QNB binding (central) assays relative to atropine. If the relative affinities of antagonists are essentially the same for muscarinic receptors at both sites, then this may indicate that the difference in the *in vivo* effectiveness is a reflection of differences in distribution. As can be seen, for some of the compounds the ratio is near 1, showing the affinity of the compound in the different assays varies in the same way the affinity of atropine varies. Scopolamine (26), aprophen (18) and triflupromazine (24) appear to have a relatively stronger affinity for the brain muscarinic receptors than for those in the intestinal smooth muscle.

There is evidence that muscarinic receptors in the brain are not homogeneous. Both M1 and M2 receptors are present and unevenly distributed. The M1 receptors predominate in the forebrain and the M2 receptors are located primarily in the cerebellum and medulla. Therefore binding values of a compound obtained on a whole brain homogenate reflect a mixed affinity for both subtypes. To address this difference some of the antagonists have been evaluated for ability to displace QNB from binding sites prepared separately from rat forebrain (predominantly M1) or rat medulla (predominantly M2). The results are given in Table 14. It can be seen the IC_{50} values vary in the different brain regions. Both aprophen (18) and caramiphen (19) have a higher affinity for sites in the forebrain than in the medulla.

TABLE 13

Comparison of the Relative Potencies (Atropine = 100)
of Selected Antimuscarinic Agents
on Muscarinic Receptors of Mouse Brain and Guinea Pig Ileum

Compound	Mouse Brain	Guinea Pig Ileum	Ratio of Activities
26	125	32	3.91
Atropine	100	100	1.00
60	69	130	0.53
73	45	20	2.25
20*	41	72	0.57
70	34	28	1.21
72	26	39	0.67
18*	16	4	4.00
24	12	1	12.0
59	11	9	1.22
63	9	2	4.50
69	8	8	1.00
19*	5	4	1.25
65	5	3	1.67
64	4	1	4.00

TABLE 14

The IC_{50} Values and Relative Potency (Atropine = 100) of a Number of Antimuscarinic Agents in Displacing Radiolabeled Quinuclidinyl Benzilate from Specific Binding Sites in Homogenates of Rat Forebrain and Medulla

Compound	Forebrain IC_{50}	Forebrain Relative Potency	Medulla IC_{50}	Medulla Relative Potency
26	15	590	44	50
29	25	350	67	33
70	86	102	130	16
Atropine	88	100	22	100
18*	130	68	340	6
58	230	38	1000	2
19*	360	25	330	6

Inhibition of oxotremorine-induced tremors, which was conducted by Dr. U. Estrada, Department of Pharmacology, Medical College of Virginia, is a standard assay for *in vivo* antimuscarinic activity. Oxotremorine is a potent non-quaternary cholinergic agent that produces tremors as a result of stimulating central muscarinic receptors. In a preliminary study oxotremorine was injected subcutaneously into groups of 10 mice at doses ranging from 0.1-0.8 mg/kg. The number of mice with tremors at various time intervals was noted. The data are presented in Table 15. The peak effect of oxotremorine appeared to occur between 10 and 20 minutes. Effects of all doses had ceased in 90 minutes. From this data an optimum time and dose of oxotremorine were chosen for evaluating the inhibitory potency of the antimuscarinic agents.

TABLE 15

The Percentage of Mice Exhibiting Tremor at Different Times After Subcutaneous Injection of the Indicated Dose of Oxotremorine

Dose (mg/kg)*	Time (min) 5	10	20	30	60	90
0.1	10	10	10	0	0	0
0.2	10	40	50	10	0	0
0.4	40	80	100	80	10	0
0.6	50	90	100	80	10	0
0.8	70	100	100	100	60	0

*Dose is mg/kg of oxotremorine base. Ten mice were used at each dose level.

The method of Atkinson et al.[107] was used for assaying the muscarinic antagonists. In one series of experiments each of the antagonists was administered subcutaneously to groups of mice 30 minutes prior to challenge with a 0.48 mg/kg dose of oxotremorine. The number of mice with tremors at 10 and 20 minutes after the oxotremorine injection was observed. This was repeated using three different concentrations of the antagonist in groups of 6 animals until a graded inhibition of tremors was observed. From this data the effective dose (ED_{50}) reducing the number of mice with tremor by 50% was calculated at each time point. The results of the antagonists tested and the potency relative to atropine are shown in Table

16. This method demonstrates an antagonist's efficacy to protect in prophylactic treatment of organophosphate poisoning. In a chemical warfare situation, poisoning will have already occurred and treatment will then be required. Therefore in a parallel series of experiments the antagonists were administered one minute after the dose of oxotremorine. The number of mice with tremors at 10 and 20 minutes was observed, and an ED_{50} for antagonism of the tremors was calculated. These results are shown in Table 17.

For a compound to be an effective pretreatment agent for protecting against organophosphate poisoning, it will require a long duration of action. Results in Table 16 show that at 20 minutes (50 minutes after giving the antagonist) the effectiveness of most of the antagonists is declining (ED_{50} rises) compared to 10 minutes (40 minutes after administration). Atropine in contrast is about equally effective at both times, making the relative potency of most compounds even less favorable at 20 minutes than at 10 minutes. Analogs

TABLE 16

The ED_{50} Value and Relative Potency (Atropine = 100) for Antagonism of Tremors Induced in Mice When the Antagonist Compound is Administered 30 Minutes Before Oxotremorine

Compound	ED_{50} (mg/kg) At 10 Min	ED_{50} (mg/kg) At 20 Min	Potency (Atropine = 100) At 10 Min	Potency (Atropine = 100) At 20 Min
26	0.005	0.014	7560	2500
29	0.023	0.061	1640	574
35*	0.065	0.25	582	138
72	0.15	0.27	255	132
46*	0.22	0.62	173	56
69	0.25	0.67	149	52
20*	0.32	0.50	119	70
Atropine	0.38	0.35	100	100
24	0.43	0.60	88	58
79	0.46	0.67	82	53
68	0.95	0.84	40	42
18*	1.16	4.91	33	7
70	1.31	2.63	29	13
36*	1.54	1.99	25	18
45*	1.59	2.22	24	16
74	1.72	1.81	22	19
73	1.94	2.69	20	13
19*	2.12	3.34	18	10
65	2.43	6.10	16	6

TABLE 16 (Continued)

Compound	ED_{50} (mg/kg) At 10 Min	ED_{50} (mg/kg) At 20 Min	Potency (Atropine = 100) At 10 Min	Potency (Atropine = 100) At 20 Min
82	2.56	3.39	15	10
38*	2.89	5.44	13	6
34*	3.17	7.71	12	5
64	3.40	3.25	11	11
37*	3.42	3.36	11	10
77	3.52	5.62	11	6
66	4.96	8.18	8	4
59	6.32	8.17	6	4
63	6.44	10.6	6	3
78	7.52	11.7	5	3
61	10.8	25.6	4	1
58	> 20.0	> 20.0	< 2	< 2
62	> 20.0	> 20.0	< 2	< 2
67	> 20.0	> 20.0	< 2	< 2
71	> 20.0	> 20.0	< 2	< 2
80	> 20.0	> 20.0	< 2	< 2
49*	> 20.0	> 20.0	< 2	< 2
31*	> 20.0	> 20.0	< 2	< 2
76	> 20.0	> 20.0	< 2	< 2

TABLE 17

The ED_{50} Value and Relative Potency (Atropine = 100) for Antagonism of Tremors Induced in Mice When the Antagonist Compound is Administered 1 Minute After Oxotremorine

Compound	ED_{50} (mg/kg) At 10 Min	ED_{50} (mg/kg) At 20 Min	Potency (Atropine = 100) At 10 Min	Potency (Atropine = 100) At 20 Min
26	0.050	0.022	3910	2690
69	0.23	0.31	869	1091
29	0.30	0.16	641	381
72	1.08	0.35	181	167
70	1.27	0.59	154	100
82	1.54	1.15	127	51
59	1.79	2.01	109	29
Atropine	1.96	0.59	100	100
73	2.16	1.36	91	43
68	2.31	1.30	85	46
18*	2.49	1.87	78	32
74	2.93	1.33	67	44
19*	3.29	1.87	59	32
37*	3.81	2.90	51	20
65	3.83	3.17	51	19
45*	4.50	4.26	43	14
38*	4.86	5.32	40	11
61	5.10	3.39	38	17
24	6.29	2.14	31	28

TABLE 17 (Continued)

Compound	ED_{50} (mg/kg) At 10 Min	At 20 Min	Potency (Atropine = 100) At 10 Min	At 20 Min
77	7.89	6.22	25	10
34*	8.26	5.39	24	11
66	8.70	6.33	22	9
58	8.88	11.3	22	5
78	15.2	12.2	13	5
71	> 20.0	> 20.0	< 10	< 3
76	> 20.0	> 20.0	< 10	< 3

20, 35, and 46 are more effective than atropine at 10 minutes, and compound 35 is still significantly better than atropine at 20 minutes. Caramiphen 19 and aprophen 18 are both less effective than atropine. Compounds 31 and 49 did not antagonize the tremors even at the highest doses tested (20 mg/kg). A key factor is that an agent must have a protective effect at a dose that is not too toxic. Compounds with less antagonistic potency than atropine might be useful pretreatment agents if they can be given in higher doses without toxic effects.

For a compound to be an effective antidote for organophosphate poisoning when it is given after poisoning occurs it must have a rapid onset of action so as to block the muscarinic receptors before the rising levels of ACh are lethal. The effects of the inhibition

of tremors for the antagonists given one minute after oxotremorine are shown in Table 17. It can be seen in this situation the potency is generally rising between 10 and 20 minutes (ED_{50} declining). Caramiphen (19) and aprophen (18) are more effective in an antidote situation than in a pretreatment situation (Table 16) relative to atropine.

Another assay which was used to determine CNS activity of the antagonists was the discriminative stimulus assay which has been developed for detecting drug-induced subjective sensations in animals. This assay was conducted by Dr. J. Rosecrans, Department of Pharmacology, Medical College of Virginia. If a drug will produce a suitable cue upon administration, then an animal can be trained to recognize the cue and to distinguish among drugs which will or will not produce the same cue to the dose of a drug which the animal can detect. To carry out the assay a group of animals must be trained on a particular drug. One injects either the training drug or saline and places the animal in an operant chamber. The chamber contains two levers, one arbitrarily designated the drug lever and the other the saline lever. When the animal has been administered drug, a certain number of pushes on the drug lever results in the dispensing of a food pellet reward (saline lever produces no result). When saline has been administered, the opposite lever results in reward. If the drug is capable of stimulating detectable cues, the animals will in time learn to press the proper lever in the chamber 80% or more of the time. Once the animals are trained, different drugs can be used to substitute for or to block the learned cue.

This assay was used as a measure of central antimuscarinic activity by the method described by Glennon et al.[194]. Rats were trained on the muscarinic agonist arecoline (1.16 mg/kg) plus methylatropine as a cue. It was found rats learned to discriminate between arecoline and saline, but this was then blocked by both atropine and methylatropine. Since methylatropine is a quaternary compound that does not act centrally, the animals must have been detecting a peripheral cue. By administering methylatropine in the training session, any peripheral cue should be blocked. After training the animals on the agonist, the antagonists were administered along with the dose of arecoline. If the antagonist effectively blocks the cue, the rats should push the saline lever. The results for atropine and the other antagonists tested are shown in Table 18. A saline-like response (antagonism) is achieved when the animal responds less than 20% on the drug lever. An 80% or greater response on the drug lever is considered a drug-like response. That is, the animal still senses a cue, and the antagonism fails. Atropine blocked the arecoline cue at a dose of 12 mg/kg. Aprophen (18) effectively blocked the central cue at a dose of 45 mg/kg, whereas caramiphen (19) did not block at a dose of 75 mg/kg. An appropriate dose could not be found for all compounds examined. In general, the number of animals responding and the rate of response on the levers declined with increasing doses of antagonists. The antimuscarinic agents may be interfering with the memory processes necessary for responding. As can be seen in the results for many compounds, the higher doses seemed to interfere with responding

TABLE 18

Effect of Antimuscarinic Agents on Drug Discrimination in Arecoline-Trained Rats

Compound	Dose (mg/kg)	N/T*	Average % DL**	Average Rate***
Atropine	12	6/10	13	0.34
18*	30	3/6	53	0.03
	45	5/12	8	0.10
58	50	5/7	80	0.21
20*	12	5/8	39	0.06
59	30	3/10	33	0.07
	60	0/6	--	----
60	0.25	5/6	62	0.21
	0.50	5/8	60	0.01
	0.75	6/17	32	0.01
63	60	0/6	--	----
64	60	3/6	83	0.08
29	1	7/8	57	0.26
	2	5/7	63	0.16
	4	9/12	47	0.05
65	40	4/6	51	0.18
	75	8/17	43	0.09
	100	3/6	5	0.13

TABLE 18 (Continued)

Compound	Dose (mg/kg)	N/T*	Average % DL**	Average Rate***
68	30	7/8	33	0.11
	45	6/9	8	0.02
19*	50	8/15	54	0.14
	60	3/11	77	0.07
	75	3/13	78	0.008
69	8	5/16	2	0.006
70	40	4/6	77	0.08
	60	7/13	40	0.10
71	60	7/9	87	0.13
72	5	5/12	100	0.03
	8	1/6	50	0.008
24	0.25	7/7	100	0.03
	0.50	8/9	83	0.05
	0.75	6/9	32	0.02
34*	60	5/6	92	0.08
35*	2	6/17	63	0.01
	3	1/8	15	0.01
36*	60	2/7	67	0.02
37*	45	2/6	80	0.10
	60	1/6	0	0.03

TABLE 18 (Continued)

Compound	Dose (mg/kg)	N/T*	Average % DL**	Average Rate***
79	10	3/8	51	0.03
	15	3/10	39	0.02
45*	60	4/7	50	0.14
46*	8	8/15	25	0.03

*Number of rats responding of the number tested.
**Average % of responses on the drug lever.
***Average number of lever presses/second during the trial.

before it totally blocked the arecoline cue. The cholinergic effects on learning and memory have been well documented[202], although other factors could be responsible for the lack of responding. It has been reported animals trained on a learned task have been significantly impaired to perform the task after treatment with an anticholinergic agent such as scopolamine[196].

The N-substituted-1,6-hexanediammonium compounds, N-substituted-1,6-hexanediamines and N-(4-diethylamino-2-butynyl)phthalimide were evaluated for muscarinic receptor binding affinity and subtype selectivity by Dr. H.I. Yamamura, Department of Pharmacology, University of Arizona[195]. The results of the compounds' ability to separately displace radiolabeled pirenzepine from rat cortex (M1

receptor subtype) and radiolabeled QNB from rat heart (M2 receptor subtype) are given in Table 19. Each dose was run in triplicate. Atropine does not distinguish between these receptor subtypes. The bis-quaternary N,N,N′,N′-tetramethyl-N,N′-bis[3-(2-phthalimido)propyl]-1,6-hexanediammonium compound 31, though weaker than atropine showed moderate potency in binding to muscarinic receptors. Compound 31 had a two fold higher affinity for the M2 site than the M1 receptor subtype. The corresponding bis-tertiary amine 49 had approximately equal affinity for the M2 receptor subtype as the quaternary compound, but lost binding affinity for the M1 receptor subtype. Compound 49 showed nearly a five fold higher affinity for the M2 receptor. It is generally accepted for a compound to be classified as selective for one subtype it should demonstrate at least a ten fold excess in binding affinity for one receptor subpopulation. Although these compounds are not selective, they do show a preference for the M2 receptor. Since the quaternary compound 31 does not penetrate the brain, the corresponding tertiary amine 49 may function as a CNS active muscarinic agent. An examination of the data reveals information on the SAR of this series of compounds. As can be seen with the unsymmetrical quaternary compound (50), the removal of one 3-(2-phthalimido)propyl portion of the molecule results in loss of both affinity and receptor subtype selectivity (preference). The loss of binding affinity and receptor subtype preference is even more drastic with the corresponding tertiary amine (51). Thus in comparison of the symmetrical with the unsymmetrical compounds, it appears that for good antimuscarinic activity (possibly

TABLE 19

Binding Affinities and Subtype Selectivities
for Selected Muscarinic Antagonists

Compound	K_I (nM) M2 [3H]QNB (Rat Heart)	M1 [3H]Pirenzepine (Rat Cortex)	Ratio M1/M2
Atropine	0.70	0.25	0.4
31*	12	25	2.1
49*	18	87	4.8
55*	80	160	2.0
50*	73	95	1.3
51*	409	2B3	0.7
54*	69	29	0.4
56*	Not Tested		

antidotal activity) both 3-(2-phthalimido)propyl portions contribute in binding. When the 3-(2-phthalimido)propyl portion of the molecule is substituted with the bis-[4-(2-phthalimido)-2-butynyl] moiety (compound 55) a substantial loss of binding affinity is observed. N-(4-diethylamino-2-butynyl)phthalimide (54) showed a higher affinity for the M1 receptor than the M2 site.

The compounds are also being evaluated for ability to block the lethal effcts of soman in mice. This work is being done by the U.S. Army Medical Research and Development Command through the Division of Experimental Therapeutics, WRAIR, WRDMC, Washington, DC. The compounds are tested against 2 x LD_{50} of soman and survival rate noted after 24 h versus control. The antagonists are admininstered at 1/4 and 1/2 the LD_{50} of the antagonist, one minute after an i.m. injection of soman. The effectiveness of the antagonist as an antidote is first tested alone. It is then evaluated along with the oxime reactivator 2-PAM (25 and 50 mg/kg). Additionally, the novel antagonists are evaluated as antidotes in conjunction with atropine and 2-PAM. The results of this study have not yet been released.

IV. CONCLUSIONS

The goal of this research was to synthesize novel antimuscarinic agents for improved treatment of poisoning by organophosphate cholinesterase inhibitors. Correlations are to be sought between the relative ability of the compounds to protect against soman poisoning and activity in the other pharmacological tests. Antimuscarinic activity was measured on guinea pig ileum and on the ability to displace radiolabeled QNB from specific binding sites of mouse brain homogenates in order to compare central versus peripheral muscarinic activity. Information from the guinea pig ileum assay is a reliable test of a compound's ability to act as an antimuscarinic agent. The compounds in this assay are directly blocking effects of ACh. The QNB binding assay may be less reliable of a compound's affinity for muscarinic receptors because of the variability in the assay. Both M1 and M2 receptors are present in the brain, but they are not evenly distributed. Therefore, binding values of a compound obtained on a whole brain homogenate are a mixed function of the affinity for the separate subtypes. The compounds were examined for central muscarinic activity *in vivo* by ability to block oxotremorine-induced tremors and to block the arecoline cue in a drug discrimination assay. The block of an agonist cue with an antagonist in a drug discrimination paradigm has never been reported in the literature with the

muscarinic receptor system. Several compounds did show a dose-response relationship to block the cue. An appropriate dose could not be found for all compounds examined. The number of animals responding and the rate of response on the levers declined with increasing doses of antagonists. The results of the compounds' ability to protect in soman poisoning has not been released as yet. The reason a battery of pharmacological assays was employed instead of one representative assay of antimuscarinic effectiveness is because it is necessary to determine if there is a pharmacological property in an antagonist which correlates with its ability to act as an antidote. Aprophen and caramiphen have been reported to be significantly better antidotes than atropine, yet they were much weaker antagonists relative to atropine in the pharmacological assays. At this point it appears from this research that the antidotal properties can not be explained based solely on the antimuscarinic activity of the compounds from the various pharmacological assays. The best antidotes are not the best antagonists in the antimuscarinic assays. The primary pharmacological effect is the central antimuscarinic activity to block the response to the excess ACh. Beyond that point, compounds like caramiphen and aprophen may be acting by an additional mechanism to give added antidotal protection. Possibly the only reason atropine is a better antimuscarinic but a less effective antidote is because its only mechanism for protection is an antimuscarinic effect. This additional mechanism may be operating only with certain structural types of antimuscarinics at the extremely high doses which are used in this antidotal

situation. Even at high doses atropine may only be exhibiting antimuscarinic activity which would explain why its maximum protection levels off even with increasing doses. Caramiphen continues to give added protection at excessively increasing doses.

A. Caramiphen and aprophen compounds.

A number of structural variations of caramiphen and aprophen were synthesized. The purpose of this set of compounds was to determine if there was any parallel pattern of change in pharmacological activity in the various assays with the ability to protect animals poisoned by soman. Many of these analogs were better antagonists in the pharmacological assays than the parent compounds. In general, incorporation of a quinuclidinyl, tropinyl, or N-methyl-4-piperidinyl moiety in the amino-alcohol portion of the molecule resulted in more potent compounds. Modifications of the acid portion of aprophen also show changes in the activity of the compounds. Reducing one of the aromatic rings to the phenyl cyclohexyl moiety resulted in increased activity. Elongation of the alkyl portion of the acid (methyl to ethyl) resulted in decreased activity. However when the ethyl group was in a rigid conformation forming an indane ring system, enhanced activity was observed.

B. N-substituted-1,6-hexanediammonium compounds; N-substituted-1,6-hexanediamines; and N-(4-dialkylamino-2-butynyl)phthalimides.

N,N,N′,N′-tetramethyl-N,N′-bis[3-(2-phthalimido)propyl]-1,6-hexanediammonium was previously reported to be a more effective antidote than atropine. We determined using a binding assay that this compound binds with moderate affinity to the muscarinic receptor with a two fold higher affinity for the M2 site than the M1 receptor subtype. In order to determine if the quaternary structure was mandatory for the muscarinic binding affinity the corresponding tertiary amine was synthesized. The tertiary amine had approximately equal affinity for the M2 subtype as the quaternary compound, but lost binding affinity for the M1 receptor subtype. N,N′-dimethyl-N,N′-bis[3-(2-phthalimido)propyl]-1,6-hexanediamine shows nearly a five fold higher affinity for the M2 receptor. Since the quaternary compound does not penetrate the brain, the corresponding tertiary amine may function as a CNS active muscarinic agent. The unsymmetrical compounds were synthesized to determine if both 3-(2-phthalimido)propyl portions of the molecule are necessary for binding affinity. The removal of one 3-(2-phthalimido)propyl portion resulted in drastic loss of activity. The N-(4-dialkylamino-2-butynyl)phthalimide showed moderate affinity and a preference for the M1 receptor subtype. When the 4-(2-phthalimido)-2-butynyl moiety was incorporated into the 1,6-hexanediamine structure, drastic loss of binding affinity was observed. It has not yet been determined if these types of structures are effective as antidotes for soman poisoning.

C. Para substituted caramiphen analogs (four corners approach).

The purpose of this set was to determine if any possible relationship of antimuscarinic or antidotal activity exists with substituent parameters such as Hammett's sigma or Hansch's pi values. These compounds have not been tested as yet. Large quantities of compound are necessary for complete pharmacological testing. The amino ($-\sigma$, $-\pi$) and 1-pyrrolidinyl ($-\sigma$; $+\pi$) analogs were synthesized in 5 g quantities. The 1-tetrazolyl ($+\sigma$, $-\pi$) was synthesized, but because of the difficulty of the synthesis, could not be obtained in large enough quantity for complete whole animal testing. The trifluoromethyl ($+\sigma$, $+\pi$) derivative proved very difficult to obtain in analytically pure form. There are other substituents which might also fit into this particular series. For example, the methanesulfonyl ($+ 0.72\,\sigma$; $- 1.63\,\pi$) and the trifluoromethanesulfonyl ($+ 0.93\,\sigma$; $+ 0.55\,\pi$) moieties contain the required signs and sufficient values of sigma and pi to be used in combination with the amino and pyrrolidinyl compounds to test this SAR approach. The synthesis of the methanesulfonyl and trifluoromethanesulfonyl derivatives are currently in progress.

D. Additional compounds chosen for testing.

A number of literature compounds and currently manufactured antimuscarinics were chosen to be tested as antidotes and screened in the various *in vitro* and *in vivo* assays. Some of the compounds were chosen because of the previously reported effectiveness against the lethal effects of organophosphate cholinesterase inhibitors. Others

were chosen just to provide a set of agents with diverse structural features. Both ester and non-ester type antimuscarinics were selected. Other compounds were included which had been previously reported to be selective for muscarinic receptor subpopulations. When all testing is completed correlations can then be sought between the efficacy as an antidote and the potency in the various *in vitro* and *in vivo* tests that we have employed. It will be of interest to see if any particular pattern of structural features or pharmacological properties emerges as a key to an effective antidote.

V. EXPERIMENTAL

Proton nuclear magnetic resonance (NMR) spectra were recorded on either a Hitachi/Perkin-Elmer R-24 high resolution spectrometer or a Varian EM-360A-60 MHz spectrometer; chemical shifts are reported relative to tetramethylsilane (TMS) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Infrared (IR) spectra were obtained on either a Beckman Acculab 8 grating spectrophotometer or a Beckman FT 1300 spectrophotometer. Melting points were determined on a Thomas-Hoover melting point apparatus. All melting points and boiling points are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

2-(Diethylamino)ethyl 1-Phenylcyclopentanecarboxylate Hydrochloride (19). Method A. To a solution of 1-phenylcyclopentanecarboxylic acid (84) (5.0 g, 26 mmol) in 25 mL of sodium-dried benzene was added 25 mL of thionyl chloride, and the solution was stirred at reflux for 12 h. The solution was concentrated under reduced pressure, then reconcentrated with three 25 mL portions of benzene to remove any remaining thionyl chloride. The residue was dissolved in 50 mL of dry benzene and decolorized with charcoal. Then 6.29 g (52.6 mmol) of freshly distilled 2-diethylaminoethanol was added dropwise in 50 mL of benzene, and the mixture was stirred at reflux for 12 h. The solution was cooled to room temperature, then poured

into 250 mL of water and basified with 10% Na_2CO_3 solution. The layers were separated, and the aqueous phase was extracted twice with 50 mL of ether. The combined organic extracts were concentrated under reduced pressure. The residue was dissolved in about 100 mL of diethyl ether and extracted three times with 100-mL portions of water. The ether layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the hydrochloride salt was prepared by treating the ether solution of the base with gaseous HCl. Recrystallization from acetone yielded 5.8 g (68%) of 19 as white needles, mp 142-144 C (Lit[162] mp 142-144 C). IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.3 (t, 6, $-CH_2CH_3$); 1.85 (broad, 6, cpentyl); 2.3-2.8 (complex, 2, cyclopentyl); 3.2 (q, 4, $-N-CH_2CH_3$); 3.6 (t, 2, $-CH_2NEt_2$); 4.6 (t, 2, $-O-CH_2$); 7.6 (s, 5, Ar-H). Anal. Calculated for $C_{18}H_{28}NO_2Cl$: C, 66.55; H, 8.69; N, 4.31. Found: C, 66.39; H, 8.68; N, 4.26.

Method B. To a stirred solution of 1-phenylcyclopentanecarboxylic acid (84) (2.0 g, 10.5 mmol) in 25 mL of dry 2-propanol was added 1.63 g (12.0 mmol) of freshly-prepared 2-diethylaminoethyl chloride (86) in 10 mL of 2-propanol. The reaction was heated to reflux for 4 h. The white crystalline precipitate formed upon cooling on an ice bath was collected by filtration and washed with diethyl ether. Recrystallization from acetone yielded 1.77 g (52%) of 19 as white needles, mp 142-144 C. This compound was identical in its physical and spectral properties to 19 prepared from the acid chloride of 1-phenylcyclopentanecarboxylic acid (Method A).

Method C. To a stirred solution of potassium 1-phenylcyclopentanecarboxylate (87) (2.0 g, 8.8 mmol) in 25 mL of absolute ethanol was added in one portion 1.42 g (10.5 mmol) of freshly prepared 2-diethylaminoethyl chloride (86) in 25 mL of ethanol. The reaction immediately became turbid, and a precipitate began to separate. The mixture was heated at reflux for 12 h. After the solution was cooled, the precipitated inorganic salt was removed by filtration and discarded. The filtrate was evaporated to dryness at reduced pressure, and the resulting oil was dissolved in 75 mL of diethyl ether. The solution was washed with three 50 mL portions of water, then dried over anhydrous magnesium sulfate. The hydrochloride salt was prepared by treating an ethereal solution of the base with gaseous HCl. Recrystallization from acetone yielded 1.6 g (56%) of 19 as colorless needles, mp 142-144 C. This compound was identical in its physical and spectral properties to 19 prepared from Method A or Method B.

2-Diethylaminoethyl Chloride (86). The method described by Breslow et al.[169] was used. An ice-cold aqueous (25 mL) solution of 2-diethylaminoethylchloride hydrochloride (5.0 g, 29.4 mmol) was made basic with 10% sodium hydroxide and stirred vigorously with ice-cold ether (50 mL) for 5 min. The layers were separated, and the aqueous phase was extracted three times with 25 mL of diethyl ether. The combined extracts were dried 4 h at 5-10 C over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the ether layer was concentrated at reduced pressure, yielding 3.22 g (82%) of

a colorless oil. No attempt was made to characterize the product, and it was used immediately for esterification.

Potassium 1-Phenylcyclopentanecarboxylate (87). A solution of 1.7 g (10 mmol) 1-phenylcyclopentane carboxylic acid (84) in 50 mL of absolute ethanol was titrated with 10% ethanolic potassium hydroxide using phenophthalein as external indicator. The solvent was evaporated under reduced pressure followed by recrystallization from ethanol - ether, yielding 2.2 g (97%) as colorless needles, mp > 350 C (Lit[160] mp > 350 C).

N-Methyl-4-piperidyl 1-Phenylcyclopentanecarboxylate Hydrochloride (20). This compound was prepared by the same general procedure as 19 (Method A) using 5.0 g (26.3 mmol) of 84 and 6.05 g (52.6 mmol) of 4-hydroxy-N-methylpiperidine. The hydrochloride salt was prepared by treating an ethereal solution of base with gaseous HCl. Recrystallization from acetone yielded 4.45 g (52%) of 20 as white needles, mp 175-176 C (Lit[161] mp 177.5-178.5 C). IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.8 (broad, 6, cpentyl); 2.6 (broad, 2, N-CH); 2.9 (s, 3, $N-CH_3$); 3.25 (broad, 2, -N-CH-); 5.0 (broad, s, 1, -O-CH); 7.5 (s, 5, Ar-H). Anal. Calculated for $C_{18}H_{26}NO_2Cl$: C, 66.75; H, 8.09; N, 4.33. Found: C, 66.75; H, 8.10; N, 4.32.

N-Methyl-3-piperidyl 1-Phenylcyclopentanecarboxylate Hydrochloride (34). This compound was prepared by the same general procedure as 19 (Method A) using 5.0 g (26.3 mmol) of 84 and 6.05 g (52.6 mmol) of 3-hydroxy-N-methylpiperidine. The hydrochloride salt was prepared by treating an ethereal solution of the base with gaseous HCl.

Recrystallization from acetone yielded 4.9 g (58%) of 34 as white needles, mp 184-185 C. IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.8 (broad, 10, $-CH_2-$); 2.4-2.8 (complex m, 2, cpentyl); 3.1 (s, 3, $-N-CH_3$); 3.5 (broad m, 4, $N-CH_2$); 5.2 (s, 1, -OCH); 7.5 (s, 5, Ar-H). Anal. Calculated for $C_{18}H_{26}NO_2Cl$: C, 66.75; H, 8.09; N, 4.33. Found: C, 66.59; H, 8.10; N, 4.24.

3-Quinuclidinyl 1-Phenylcyclopentanecarboxylate Hydrochloride (35). To a solution of 5.0 g (26.2 mmol) of 1-phenylcyclopentanecarboxylic acid in 25 mL of sodium-dried benzene was added 25 mL of thionyl chloride, and the solution was stirred at reflux for 12 h. The solution was concentrated under reduced pressure, then reconcentrated with three 25 mL portions of benzene to remove any remaining thionyl chloride. The resulting oil was dissolved in 50 mL of dry chloroform. Then, 6.7 g (52.8 mmol) of 3-quinuclidinol in 50 mL of dry chloroform was added, and the solution was stirred at reflux for three days. After the reaction was cooled to room temperature, the solid precipitate was collected by filtration and discarded. The organic layer was evaporated at reduced pressure, then dissolved in 100 mL diethyl ether and washed three times with 100 mL of water. The ether layer was then dried over anhydrous magnesium sulfate. The hydrochloride salt was prepared by treating the ethereal solution of the base with gaseous HCl. Recrystallization from methanol - ethyl acetate yielded 3.1 g (35%) of 35 as white flakes, mp 205-207 C (Lit[161] mp 206-207 C). IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.8 (broad s, 6, cpentyl); 1.8-2.35 (m, 5, $-CH_2-$); 2.4-2.8 (complex m, 2, cpentyl); 3.3 (m, 6, $-CH_2N$); 5.1 (q, 1, O-CH); 7.4 (s,

5, Ar-H). Anal. Calculated for $C_{19}H_{26}NO_2Cl$: C, 68.35; H, 7.55; N, 4.20. Found: C, 68.33; H, 7.81; N, 4.94.

3-Tropinyl 1-Phenylcyclopentanecarboxylate Hydrochloride (36). This compound was prepared by the same general procedure as 19 (Method A) using 5.0 g (26 mmol) of 1-phenylcyclopentanecarboxylic acid, and 7.42 g (52.6 mmol) of tropine. The hydrochloride salt was prepared by treating an ethereal solution of the base with gaseous HCl. Recrystallization from methanol - ethyl acetate yielded 3.9 g (43%) of 36 as a white solid, mp 226-228 C. IR (nujol): 1710 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.8 (broad s, 6, cpentyl); 2.05 (broad, 4, $-CH_2-$); 2.3-2.7 (complex m, 6, $-CH_2-$, cpentyl); 2.8 (s, 3, $N-CH_3$); 3.8 (p, 2, -N-CH-); 5.1 (m, 1, -O-CH); 7.4 (s, 5, Ar-H). Anal. Calculated for $C_{20}H_{28}NO_2Cl$: C, 68.65; H, 8.07; N, 4.00. Found C, 68.75; H, 8.10; N, 3.98.

3-(Diethylamino)-1-propyl 1-Phenylcyclopentanecarboxylate Hydrochloride (37). This compound was prepared by the same general procedure as 19 (Method A) using 5.0 g (26.3 mmol) of 1-phenylcyclopentanecarboxylic acid and 6.94 (52.6 mmol) of freshly distilled 3-(diethylamino)-1-propanol. The hydrochloride salt was prepared by treating an ethereal solution of the base with gaseous HCl. Recrystallization from acetone yielded 4.3 g (48%) of 37 as white needles, mp 139-140 C (Lit[161] mp 140-140.5 C). IR (nujol); 1710 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.2 (t, 6, $-CH_2CH_3$); 1.7 (broad, 6, cpentyl); 1.9 (p, 2, $-CH_2-$); 2.4-2.8 (complex m, 2, cpentyl); 3.1 (complex m, 6, $-CH_2-N$); 4.2 (t, 2, $O-CH_2-$); 7.4 (s, 5, Ar-H). Anal. Calculated for

$C_{19}H_{30}NO_2Cl$: C, 67.14; H, 8.90; N, 4.12. Found: C, 67.22; H, 8.90; N, 4.07.

2-(Diethylamino)ethyl 1-Phenylcyclohexanecarboxylate Hydrochloride (38). This compound was prepared by the same general procedure as 19 (Method A) using 4.0 g (20 mmol) of 1-phenylcyclohexanecarboxylic acid and 4.64 g (39.0 mmol) of freshly distilled 2-diethylaminoethanol. The hydrochloride salt was prepared by treating an ethereal solution of the free base with gaseous HCl. Recrystallization from acetone yielded 4.3 g (66%) of 38 as a white solid, mp 158-160 C (Lit[162] mp 159-161 C). IR (nujol): 1720 cm^{-1} (C=0). ^1H NMR (D_2O): δ 1.2 (t, 6, $-CH_2CH_3$); 1.4-1.8 (broad, 10, chexyl); 3.2 (q, 4, $N-CH_2CH_3$); 3.6 (t, 2, CH_2N); 4.6 (t, 2, $-O-CH_2-$); 7.4 (s, 5, Ar-H). Anal. Calculated for: $C_{19}H_{30}NO_2Cl$: C, 67.33; H, 8.92; N, 4.13. Found: C, 67.09; H, 8.94; N, 4.10.

N-[2-(Diethylamino)ethyl] 1-Phenylcyclopentanecarboxamide Hydrochloride (39). This compound was prepared by the same general procedure as 19 (Method A) using 4.0 g (21 mmol) of 1-phenylcyclopentanecarboxylic acid and 4.9 g (42 mmol) of N,N-diethylethylenediamine. The hydrochloride salt was prepared by treating an ethereal solution of the free base with gaseous HCl. Recrystallization from methanol - ether yielded 2.8 g (41%) 39 as a white solid, mp 138-140 C. IR (neat, base): 3310 cm^{-1} (-NH); 1700 cm-1 (C=0). ^1H NMR ($CDCl_3$, base): δ 1.1 (t, 6, $-CH_2CH_3$); 1.8 (broad, 6, cpentyl); 2.6 (q, 6, $-CH_2$); 3.2 (broad, 2, $-CH_2-N$); 3.8 (t, 2, $-NH-CH_2$); 7.0 (s, 1,

NH); 7.4 (s, 5, Ar-H). Anal. Calculated for: $C_{18}H_{28}N_2OCl$: C, 66.35; H, 9.0; N, 8.62. Found: C, 66.11; H, 8.96; N, 8.62.

1-Methyl-4-(1-phenylcyclopentanecarbonyl) piperazine Hydrochloride (40). This compound was prepared by the same general procedure as 19 (Method A) using 2.5 g (13.2 mmol) of 1-phenylcyclopentanecarboxylic acid 84 and 2.64 g (26.4 mmol) of freshly distilled N-methylpiperazine. The hydrochloride salt was prepared by treating an ethereal solution of the base with gaseous HCl. Recrystallization from methanol - ethyl acetate yielded 2.3 g (58%) of 40 as white needles, mp 242-244 C. IR (nujol): 1700 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.8 (broad, 6, cpentyl; 2.2-2.6 (complex m, 2, cyclopentyl); 3.0 (s, 3, NCH_3); 3.3 (m, 4, CH_2NMe); 3.85 (m, 4, N-CH_3); 7.5 (s, 5, Ar-H). Anal. Calculated for $C_{17}H_{25}N_2OCl$. 0.5 H_2O: C, 64.23; H, 8.25; N, 8.81. Found: C, 64.19; H, 8.12; N, 8.73.

N-[2-(Diethylamino)ethyl] 1-Phenylcyclohexanecarboxamide Hydrochloride (41). This compound was prepared by the same general procedure as 19 (Method A) using 5.0 g (24.4 mmol) of 1-phenylcyclohexanecarboxylic acid and 5.7 g (48.8 mmol) N,N-diethylethylenediamine. The hydrochloride salt was prepared by treating an ethereal solution of the base with gaseous HCl. Recrystallization from methanol - ethyl acetate yielded 4.9 g (59%) of 41 as a white solid, mp 166-168 C (Lit[167] mp 166.5-167 C). IR (neat, base): 3320 cm^{-1} (-NH); 1700 cm^{-1} (C=O). ^1H NMR ($CDCl_3$, base): δ 1.0 (t, 6, -CH_2CH_3; 1.4-1.8 (broad, 10, chexyl); 2.1-2.6 (m, 6, CH_2N-); 3.3 (t, 2, -NH-CH_2); 6.2 (s, 1, -NH); 7.4 (s, 5, Ar-H).

2,2-Diphenylbutyronitrile (89). To a stirred suspension of 4.02 (103 mmol) of $NaNH_2$ in 150 mL of anhydrous ether, maintained under nitrogen at about -50 C, was added dropwise 20.0 g (103 mmol) of diphenylacetonitrile in 100 mL of ether. The mixture was stirred for one hour. Then 24.0 g (155 mmol) of ethyl iodide in 75 mL of ether was added dropwise over a 30 min period. The reaction mixture was allowed to warm to room temperature, then stirred at reflux for 4 h. The solution was then poured into 250 mL of crushed ice and water. The layers were separated, and the aqueous phase was extracted twice with 50 mL of diethyl ether. The combined extracts were washed with 200 mL water, then dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent removed under reduced pressure, to give an oil. This oil was distilled under vacuum to yield 21.2 g (93%) of 89 as a colorless oil, bp 120-125 C/0.2 mm (Lit[173] bp 145-147 C/0.3 mm). IR (neat): 2210 cm^{-1} (-C≡N). ^1H NMR ($CDCl_3$): δ 1.0 (t, 3, $-CH_2CH_3$); 2.35 (q, 2, $-CH_2CH_3$); 7.3 (s, 10, Ar-H).

2-Cyclohexyl-2-phenylpropionitrile (91). To a stirred suspension of 4.4 g (113 mmol) of $NaNH_2$ in 150 mL of anhydrous ether, maintained under nitrogen at about -30 C, was added dropwise 19.0 g (75.4 mmol) of cyclohexylphenylacetonitrile in 100 mL of ether. The mixture was stirred for 1 h. Then 15.9 g (113 mmol) of methyl iodide in 75 mL of ether was added dropwise. The mixture was allowed to warm to room temperature, then brought to reflux for 4 h. The solution was poured onto 250 mL of crushed ice water. The layers

were separated and the aqueous phase extracted twice with diethyl ether (50 mL). The combined extracts were washed with 200 mL of water, then dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, giving an oil. The oil was distilled under vacuum to yield 13.7 g (86%) of 91 as a colorless oil, bp 122-124 C/0.25 mm (Lit[174] bp 166 C/11 mm). IR (neat): 2220 cm^{-1} (-C≡N). ^1H NMR ($CDCl_3$): δ 1.2-1.6 (broad, 11, chexyl); 1.65 (s, 3, $-CH_3$); 7.45 (s, 5, Ar-H).

2,2-Diphenylbutyric acid (92). A suspension of 10.0 g (45.3 mmol) of 89 in 75 mL of 48% HBr was stirred vigorously at reflux for ten days. The reaction mixture was cooled to room temperature, then poured onto 300 mL of crushed ice. The solid material which precipitated was collected by filtration and washed repeatedly with water to leave a white product. The crude acid was dissolved in a cold 5% NaOH solution. The solution was filtered to remove any insoluble material. The crude acid was precipitated by the slow addition of a 5% HCl solution. The product was collected by filtration. Reprecipitation from a 5% NaOH solution with a 5% HCl solution yielded 4.3 g (40%) of 92 as a white solid, mp 172-174 C (Lit[173] mp from benzene 174-175 C). IR (nujol): 1680 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.0 (t, 3, $-CH_2CH_3$); 3.1 (q, 2, $-CH_2CH_3$); 7.3 (s, 10, Ar-H); 12.2 (s, 1, -COOH).

2-Methyl-2-phenylcyclohexaneacetic acid (93). A suspension of 10.0 g (46.9 mmol) of 90 in 75 mL of 48% HBr was stirred vigorously at reflux for ten days. The reaction mixture was cooled to room temperature, then poured onto 300 mL of crushed ice and water. The

solid material which precipitated was collected and triturated repeatedly with water to leave a white product. The crude acid was dissolved in a cold 5% NaOH solution. The solution was filtered to remove any insoluble material. The crude acid was precipitated by the slow addition of a 5% HCl solution. The product was collected by filtration and reprecipitated from a cold 5% NaOH solution with a 5% HCl solution, yielding 9.4 g (86%) of 93 as a white solid, mp 128-130 C (Lit[175] mp 130-131 C). IR (nujol): 1670 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.1-1.4 (broad, 11, chex); 1.5 (s, 3, $-CH_3$); 7.3 (s, 5, Ar-H); 12.15 (s, 1, COOH).

2-(Diethylamino)ethyl 2,2-Diphenylbutyrate Hydrochloride (42). To a solution of 3.9 g (16.3 mmol) 92 in 25 mL of sodium dried benzene was added 25 mL of thionyl chloride and the solution stirred at reflux under nitrogen for 12 h. The reaction was cooled to room temperature, then concentrated under reduced pressure. The resulting oil was washed with three 25-mL portions of benzene to remove any remaining thionyl chloride. The residue was dissolved in 50 mL of dry benzene and the solution was decolorized with charcoal. Then 3.8 g (32.5 mmol) of freshly distilled 2-diethylaminoethanol in 50 mL of dry benzene was added dropwise and the solution stirred at reflux for 12 h. The reaction mixture was cooled to room temperature, poured into 250 mL of water and basified with a 10% Na_2CO_3 solution. The layers were separated and the aqueous phase extracted twice with 50 mL of diethyl ether. The combined organic layers were concentrated under reduced pressure. The residue was dissolved in 100 mL diethyl ether and extracted with four 100 mL portions of water. The

ether layer was separated and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration. The hydrochloride salt was prepared by treating the ethereal solution of the base with gaseous HCl. Recrystallization from ethyl acetate yielded 4.1 g (66%) 42 as a white solid, mp 118-120 C (Lit[173] mp 124-126 C). IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR (D_2O): δ 0.8 (t, 3, $-CH_2CH_3$); 1.15 (t, 6, $-NCH_2CH_3$); 2.5 (q, 2, $-CH_2CH_3$); 3.0 (q, 4, $-NCH_2CH_3$); 3.4 (t, 2, $-CH_2NEt_2$); 4.5 (t, 2, $-OCH_2-$); 7.35 (s, 10, Ar-H). Anal. Calculated for $C_{22}H_{30}NO_2Cl$: C, 70.28; H, 8.04; N, 3.73. Found: C, 70.00; H, 8.11; N, 3.70.

2-(Diethylamino)ethyl 2-Cyclohexyl-2-phenylpropionate Hydrogen Oxalate (44). To a solution of 5.85 g (25.2 mmol) 93 in 25 mL of sodium dried benzene was added 25 mL of thionyl chloride and the solution was stirred at reflux under nitrogen for 12 h. The mixture was cooled to room temperature, then concentrated under reduced pressure. The resulting oil was washed with three 25-mL portions of benzene to remove the remaining thionyl chloride. The residue was dissolved in 50 mL of dry benzene, and the solution was decolorized with charcoal. Then 6.0 g (50.4 mmol) of freshly distilled 2-diethylaminoethanol was added dropwise in 75 mL of benzene, and the mixture was stirred at reflux for 12 h. The solution was cooled to room temperature, poured into 250 mL water and basified with 10% Na_2CO_3 solution. The layers were separated and the aqueous phase washed twice with 50 mL of diethyl ether. The combined organic extracts were concentrated under reduced pressure, giving an oil. This oil was dissolved in about 100 mL of diethyl ether and extracted

with four 100-mL portions of water. The ether layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the solvent was removed under reduced pressure, yielding 6.65 g (80%) of 44 as a golden oil. The oxalate salt was prepared by treating an ethereal solution of oxalic acid with an ethereal solution of the base. Recrystallization of the crude salt from methanol - diethyl ether yielded a white solid, mp 117-119 C. IR (base, neat): 1720 cm^{-1} (C=O). ^1H NMR (d6DMSO): δ 1.1 (t, 6, $-CH_2CH_3$); 1.1-1.6 (broad, 11, chexyl); 1.2 (s, 3, $-CH_3$); 3.05 (q, 4, $-CH_2CH_3$); 3.3 (m, 2, $-CH_2NEt_2$); 4.3 (t, 2, $-OCH_2$); 7.3 (s, 5, Ar-H). Anal. Calculated for $C_{23}H_{34}NO_6 \cdot 0.5\ H_2O$: C, 64.16; H, 8.43; N, 3.25. Found: C, 64.36; H, 8.41; N, 3.26.

2,4-Diphenylbutyronitrile (95). To a stirred suspension of 4.17 g (107 mmol) of sodium amide in 200 mL of anhydrous ether maintained at -50 C under nitrogen was added dropwise 12.5 g (107 mmol) of phenylacetonitrile in 100 mL of diethyl ether over a 30 min period. The mixture was stirred for an additional 2 h. Then 19.9 g (107 mmol) of phenethyl bromide in 100 mL of diethyl ether was added dropwise. The reaction was continued for an additional 2 h. This solution was allowed to warm to room temperature, then brought to slow reflux for 4 h. The mixture was then poured over about 400 mL of ice-water, and the layers were separated. The aqueous phase was extracted twice with diethyl ether (50 mL). The extracts were combined and dried over anhydrous magnesium sulfate. The ether solution was concentrated under reduced pressure, yielding a clear oil. This oil was distilled under vacuum to give 17.25 g (74%) 95 as

a colorless oil, bp 120-122 C/0.25 mm (Lit[176] bp 132 C/0.16 mm). IR (neat): 2220 cm^{-1} (-C≡N). ^1H NMR ($CDCl_3$): δ 2.2 (m, 2, HC-CH_2-CH_2-); 2.7 (t, 2, Ar-CH_2-); 3.75 (t, 1, Ar-CH-CN); 7.4 (d, 10, Ar-H).

2-Bromo-2,4-diphenylbutyronitrile (96). To a stirred solution of 9.25 g (41.9 mmol) of 95 in 100 mL of methylene chloride was added dropwise 7.15 g (44.7 mmol) of bromine in 50 mL CH_2Cl_2 over a 2 h period. The solution was brought to slow reflux for an additional 2 h, then poured into 250 mL of a cold 10% Na_2CO_3 solution. The layers were separated, and the aqueous phase was extracted twice with CH_2Cl_2 (50 mL). The extracts were dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, giving 10.85 g (87%) of a crude dark oil. IR (neat): 2200 cm^{-1} (-C≡N). ^1H NMR ($CDCl_3$): 2.8 (m, 4, -CH_2-); 7.3 (d, 10, Ar-H). This compound was not further analyzed, but was immediately used in the next step.

1-Cyano-1-Phenylindane (97). To a stirred solution of 10.85 g (36.2 mmol) of 96 at -10 C in 500 mL of dry 1,2-dichloroethane under a nitrogen atmosphere was added 6.0 g of pulverized solid aluminum chloride portionwise over 1 h. The mixture was stirred at -10 C for an additional 6 h, then allowed to warm to room temperature and kept for 36 h. This mixture was then poured onto 300 mL of cold 10% HCl solution and the layers were separated. The aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, giving an oil. The oil was distilled under vacuum

giving 4.55 g (58%) of 97 as a golden colored oil, bp 150-155 C/0.6 mm (Lit[176] bp 122 C/0.2 mm). IR (neat): 2210 cm^{-1} (-C≡N). ^1H NMR ($CDCl_3$): δ 2.3-3.1 (complex m, 4, $-CH_2$); 7.3 (broad s, 9, Ar-H).

1-Phenylindane-1-carboxylic acid (98). A suspension of 4.5 g (20.6 mmol) of 97 in 40 mL of 48% HBr was stirred at vigorous reflux for five days. The reaction mixture was cooled to room temperature, then poured onto 250 mL of crushed ice. The resulting semi-solid material was collected by filtration and washed repeatedly with water. The crude acid was dissolved in a cold 5% NaOH solution, then filtered to remove the insoluble material. The acid was precipitated by the slow addition of a 5% HCl solution, to yield 3.7 g (75%) of a thick light brown oil. IR (neat): 1680 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 2.5 (broad, 3, -CH-); 3.5 (broad s, 1, -CH-); 7.3 (d, 9, Ar-H); 12.1 (s, 1, COOH). All attempts to obtain a crystalline material were unsuccessful. Therefore this compound was used immediately in the next step.

2-(Diethylamino)ethyl 1-Phenylindane-1-carboxylate Hydrochloride (43). To a solution of 4.0 g (16.8 mmol) 98 in 25 mL of sodium dried benzene was added 25 mL of thionyl chloride and the solution stirred at reflux under a nitrogen atmosphere for 12 h. The reaction was cooled to room temperature, then concentrated under reduced pressure. The resulting oil was washed with three 25-mL portions of benzene to remove any remaining thionyl chloride. The dark residue was dissolved in about 50 mL of dry benzene, and the solution was decolorized with charcoal. Then 4.0 g (33.6 mmol) of freshly distilled 2-

diethylaminoethanol in 50 mL of dry benzene was added dropwise and the mixture was stirred at reflux for 24 h. The reaction mixture was cooled to room temperature, poured into 250 mL of water and basified with a 10% sodium carbonate solution. The layers were separated, and the aqueous phase was extracted twice with 50 mL of diethyl ether. The combined organic phases were concentrated under reduced pressure, giving an oil. This oil was dissolved in about 100 mL of diethyl ether and extracted with four 100-mL poritons of water. The ether layer was separated and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration. The hydrochloride salt was prepared by treating the ethereal solution of the base with gaseous HCl. Recrystallization from methanol - ethyl acetate yielded 2.88 g (46%) 43 as a white solid, mp 145-147 C. IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.2 (t, 6, $-CH_2CH_3$); 2.3 (broad s, 2, $-CH_2-$); 3.0 (broad, m, 6, $-N-CH_2CH_3$, $ArCH_2-$); 3.4 (broad, 2, $-CH_2NEt_2$); 4.5 (broad s, 2, $-OCH_2$); 7.3 (s, 8, Ar-H); 7.5 (s, 1, Ar-H). Anal. Calculated for $C_{22}H_{28}NO_2Cl$: C, 70.66; H, 7.55; N, 3.75. Found: C, 70.43; H, 7.60; N, 3.71.

1-Phenyl-1-indanol (100). The phenyl Grignard reagent was prepared by treating bromobenzene (7.06 g, 45 mmol) in 150 mL of anhydrous ether under a nitrogen atmosphere with 1.07 g (45 mmol) of magnesium. A crystal of iodide was added and the mixture was allowed to stand until all the magnesium reacted. 1-Indanone (5.0 g, 37.8 mmol) in 75 mL of ether was added dropwise and the reaction was stirred for 4 h. Saturated ammonium chloride solution (200 mL) was then added and the mixture was allowed to stir for 30 min. After the

layers were separated, the aqueous phase was extracted twice with 50 mL of ether. The combined ether extracts were dried over anhydrous magnesium sulfate, then evaporated at reduced pressure to yield a colorless oil. The yield of crude 100 was 7.8 g (98%). IR (neat): 3450 cm^{-1} (-OH). ^1H NMR ($CDCl_3$): δ 2.25 (t, 2, $-CH_2-$); 2.8 (t, 2, $Ar-CH_2$); 2.75 (s, 1, -OH); 7.15 (s, 9, Ar-H).

N,N,N',N'-Tetramethyl-N,N'-bis[3-(2-phthalimido)propyl]-1,6-hexanediammonium Bromide (31a). A solution of 10.0 g (58 mmol) of N,N,N',N'-tetramethyl-1,6-hexanediamine and 34.3 g (128 mmol) of N-(3-bromopropyl)phthalimide in 350 mL of dry methanol was stirred at reflux under a nitrogen atmosphere for 24 h. The reaction was then cooled to room temperature and the heavy white precipitate which formed was collected by filtration. Multiple recrystallizations from methanol - diethyl ether yielded 18.5 g (45%) of 31a as a white solid, mp 266-268 C (Lit[103] mp 266-268 C). IR (nujol): 1710-1760 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.5-2.3 (broad m, 12, $-CH_2-$); 3.1 (s, 12, $-NCH_3$); 3.1-3.4 (complex m, 8, $-NCH_2-$); 3.75 (t, 4, $(CO)_2N-CH_2$); 7.8 (s, 8, Ar-H). Anal. Calculated for $C_{32}H_{44}N_4O_4Br_2 \cdot H_2O$: C, 52.86; H, 6.38; N, 7.75. Found: C, 52.85; H, 6.42; N, 7.67.

N,N,N',N'-Tetramethyl-N,N'-bis[3-(2-phthalimido)propyl]-1,6-hexanediammonium Iodide (31b). To a solution of 1.5 g (2.9 mmol) of 49a in 15 mL of sodium dried benzene was added 3 mL of methyl iodide. The reaction was stoppered and allowed to sit at room temperature for 24 h. The heavy precipitate which formed was collected by filtration, washed with benzene, and dried under vacuum. Recrystallization

from dilute methanol yielded 2.1 g (90%) of 31b as a light yellow solid, mp 274-275 C. IR (nujol): 1720, 1760 cm^{-1} (C=O). Anal. Calculated for $C_{32}H_{44}N_4O_4I_2$: C, 47.89; H, 5.53; N, 6.98. Found: C, 47.74; H, 5.45; N, 6.91.

N,N'-Dimethyl-N,N'-bis[3-(2-phthalimido)propyl]-1,6-hexanediamine (49a). To a solution of N-(3-bromopropyl)phthalimide 102 (8.0 g, 30 mmol) in 50 mL dry acetone was added 1.96 g (15 mmol) of N,N'-dimethyl-1,6-hexanediamine 104 in 20 mL acetone. The mixture was stirred at reflux under nitrogen for 4 h, then cooled to room temperature. Triethylamine was then rapidly added (3.02 g, 30 mmol) and the heating continued for 12 h. After cooling the mixture on an ice bath, the solid precipitate which formed was removed by filtration. The mother liquor was concentrated at reduced pressure, yielding a yellowish viscous oil. This oil was triturated repeatedly with benzene, resulting in a semi-solid white product which was discarded. The benzene solution was washed with three 50 mL portions of water. The benzene was then evaporated at reduced pressure, leaving a white solid. Multiple recrystallizations from methanol-water yielded 4.2 g (27%) of 49a as a white solid, mp 80-82 C. IR (nujol): 1720, 1760 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.4 (broad s, 8, $-CH_2-$); 1.85 (m, 4, $-CH_2-$); 2.2 (s, 6, $-N-CH_3$); 2.35 (t, 8, $-N-CH_2$); 3.8 (t, 4, $(CO)_2-N-CH_2$); 7.8 (d, 8, Ar-H). Anal. Calculated for $C_{30}H_{38}N_4O_4 \cdot 0.5\ H_2O$: C, 68.29; H, 7.44; N, 10.62. Found: C, 68.51; H, 7.34; N, 10.61.

N,N'-Dimethyl-N,N'-bis[3-(2-phthalimido)propyl]-1,6-hexanediamine Dihydrochloride (49b). The dihydrochloride salt of 49a was prepared by dissolving 2.0 g (3.9 mmol) of 49a in about 20 mL of dry methanol and treating with gaseous HCl. The methanoic solution was concentrated under vacuum to a thick white precipitate. Multiple recrystallizations from methanol - ethyl acetate yielded 1.65 g (72%) of 49b as a fine white powder, mp 248-250 C. IR (nujol): 1720, 1760 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.6-2.4 (complex m, 12, $-CH_2-$); 3.1 (s, 6, NCH_3); 3.35 (q, 8, $N-CH_2$); 3.9 (t, 4, $(CO)_2-N-CH_2$); 7.9 (s, 8, Ar-H). Anal. Calculated for $C_{30}H_{40}N_4O_4Cl_2$: C, 60.90; H, 6.82; N, 9.47. Found: C, 60.55; H, 6.86; N, 9.24.

N,N'-Dimethyl-N,N'-bis[3-(2-phthalimido)propyl]-1,6-hexanediamine Dihydrobromide (49c). To a solution of 11.1 g (41.7 mmol) of N-(3-bromopropyl)phthalimide (102) in 100 mL of dry methanol was added 3.0 g (20.8 mmol) of N,N'-dimethyl-1,6-hexanediamine in 25 mL of methanol. The reaction mixture was stirred at reflux under nitrogen for 24 h. The solution was then chilled on an ice bath and filtered to remove any insoluble products. The solvent was removed at reduced pressure to give a viscous oil. This oil was triturated repeatedly with ethyl acetate until a white precipitate formed. The crude product was collected by filtration and washed with ethyl acetate. Multiple recrystallizations from methanol - diethylether yielded 6.5 g (46%) of 49c as a white solid, mp 117-119 C. IR (nujol): 1710, 1760 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.4-2.3 (broad, 12, $-CH_2-$); 3.0 (s, 6, $-N-CH_3$); 3.1-3.4 (m, 8, $N-CH_2$); 3.6-4.0 (m, 4, $(CO)_2-N-CH_2$); 7.9 (s, 10, ArH). Anal. Calculated for

$C_{30}H_{40}N_4O_4Br_2 \cdot 2\ H_2O$: C, 50.27; H, 6.18; N, 7.82. Found: C, 50.05; H, 6.22; N, 8.01.

N,N'-Dimethyl-N-[3-(2-phthalimido)propyl]-1,6-hexanediamine (105). A solution of 8.5 g (59.0 mmol) of N,N'-dimethyl-1,6-hexanediamine (104) and 3.16 g (11.8 mmol) of N-(3-bromopropyl)phthalimide (102) in 75 mL of dry THF was stirred at reflux under nitrogen for 20 h. The solution was cooled to room temperature. The solid which precipitated was removed by filtration and washed with 20 mL of THF. The combined THF solutions were concentrated at reduced pressure to yield a crude oil. The remaining N,N'-dimethyl-1,6-hexanediamine was distilled from this oil under vacuum (60 C/0.2 mm). The viscous oil which remained was dissolved in 50 mL of ether and extracted with three 50-mL portions of water. The ether layer was concentrated at reduced pressure to yield 2.61 g (67%) of 105 as a viscous golden oil. IR (neat): 1710, 1760 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.1-1.5 (broad, 8, $-CH_2-$); 1.6-2.0 (m, 2, $-CH_2-$); 2.1-2.6 (m, 6, $-CH_2$); 2.2 (s, 3, $-N-CH_3$); 2.45 (s, 3, $-N-CH_3$); 3.0 (s, 1, N-H); 3.5-3.9 (m, 2, $-N-CH_2$); 7.8 (s, 4, ArH). This compound was not further analyzed, but used immediately in the next step.

N-[3-(2-Phthalimido)propyl]-N,N',N'-trimethyl-1,6-hexanediamine Dihydrochloride (51). To a solution of 3.5 g (10.6 mmol) of 105 dissolved in 5 mL of formic acid was added 5 mL of 37% formaldehyde. The mixture was heated on a steam bath for 6 h, then poured onto crushed ice. This solution was made strongly basic with solid sodium hydroxide, then extracted with three 100-mL portions of ether. The

combined ether layers were dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the ether was removed at reduced pressure to yield 2.8 g (77%) of 106 as an amber oil. The dihydrochloride salt (51) was prepared by treating an ether solution of the base with an ether solution saturated with HCl gas. Multiple recrystallizations of the salt from methanol - ether yielded 1.9 g (43%) of 51 as a white solid, mp 176-178 C. IR (nujol): 1710, 1760 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.5-2.4 (m, 8, $-CH_2-$); 3.0 (s, 9, $-N-CH_3$); 3.1-3.5 (m, 8, $-N-CH_2$); 3.7-4.1 (m, 2, $-N-CH_2$); 7.8 (s, 4, ArH). Anal. Calculated for $C_{20}H_{33}N_3O_2Cl$: C, 57.41; H, 7.95; N, 10.04. Found: C, 57.32; H, 7.96; N, 9.99.

N,N,N',N',N'-Pentamethyl-N-[3-(2-phthalimido)propyl]-1,6-hexanediammonium Iodine (50). To a solution of 1.5 g (4.4 mmol) of 106 in 15 mL of sodium dried benzene was added 3 mL of methyl iodide. The reaction mixture was stoppered and allowed to sit at room temperature for 24 h. The heavy precipitate which formed was collected by filtration and then washed with benzene. Recrystallization of the product from methanol yielded 1.85 g (74%) of 50 as a light yellow solid, mp 206-208 C. IR (nujol): 1710, 1760 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.4-2.3 (m, 8, $-CH_2-$); 3.1 (s, 15, $N-CH_3$); 2.8-3.4 (m, 8, $-N-CH_2$); 3.6-3.9 (m, 2, $N-CH_2$); 7.8 (s, 4, ArH). Anal. Calculated for $C_{22}H_{37}N_3O_2I_2$: C, 41.98; H, 5.93; N, 6.68. Found: C, 42.08; H, 5.93; N, 6.63.

N-(2-Propynyl)phthalimide (110). To a solution of 20.0 g (108 mmol) of potassium phthalimide in 250 mL of dry methanol was added

dropwise 25.7 g (216 mmol) of propargyl bromide in 75 mL of methanol. The mixture was stirred at reflux under nitrogen for 24 h. After cooling to room temperature, the solution was filtered and the solvent was removed under reduced pressure to give a crude tan solid. Multiple recrystallizations from dilute methanol yielded 16.8 g (84%) of 110 as a white solid, mp 148-149 C (Lit[155] mp 147-150 C). IR (nujol): 3260 cm^{-1} (C≡CH); 2130 cm^{-1} (-C≡C-); 1700, 1760 cm^{-1} (C=O). ^1H NMR (d6DMSO): δ 2.9 (s, 1, -C≡CH); 4.2 (s, 2, N-CH_2); 7.7 (s, 4, Ar-H).

N-(4-Dimethylamino-2-butynyl)phthalimide Hydrochloride (53). To a solution of 6.0 g (32.4 mmol) of N-(2-propynyl)phthalimide (110) in 60 mL of ethanol was added 20 mL of 37% formaldehyde and 5.23 g (65 mmol) of dimethylamine hydrochloride. Cuprous chloride (0.3 g) was added, and the solution was stirred at reflux for 12 h. The mixture was cooled to room temperature and 250 mL of water was added. The mixture was acidified with a 10% HCl solution and extracted twice with 50 mL of diethyl ether. The aqueous phase was made alkaline with a 10% Na_2CO_3 solution with cooling and extracted exhaustively with six 50 mL portions of $CHCl_3$. The combined $CHCl_3$ extracts were dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent was evaporated under reduced pressure, yielding an oil. The hydrochloride salt was prepared by treating an ethereal solution of the oil with gaseous HCl. Multiple recrystallizations from acetone - ether yielded 4.7 g (65%) of 53 as a white solid, mp 185-187 C. IR (nujol): 1700, 1760 cm^{-1} (C=O). ^1H NMR (D_2O): δ 3.1 (s, 6, -NMe_2); 4.3 (s, 2, -CH_2C≡C-); 4.6 (s, 2,

-C≡C-CH_2-N); 7.8 (s, 4, Ar-H). Anal. Calculated for $C_{14}H_{15}N_2O_2Cl$: C, 60.33; H, 5.42; N, 10.05. Found: C, 60.42; H, 5.43; N, 10.05.

N-(4-Diethylamino-2-butynyl)phthalimide Hydrochloride (54). To a solution of 110 (5.5 g, 30 mmol) in 60 mL of ethanol was added 20 mL of 37% formaldehyde and 6.51 g (60 mmol) of diethylamine hydrochloride. Cuprous chloride (0.3 g) was added, and the solution was stirred at reflux for 12 h. The mixture was cooled to room temperature, and 250 mL of water was added. The mixture was acidified with a 10% HCl solution and extracted twice with 50 mL of diethyl ether. The aqueous phase was made alkaline with a 10% Na_2CO_3 solution with cooling and extracted exhaustively with six 50-mL portions of $CHCl_3$. The combined $CHCl_3$ extracts were dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the solvent was evaporated at reduced pressure, yielding an oil. The hydrochloride salt was prepared by treating an ethereal solution of the oil with gaseous HCl. Multiple recrystallizations from acetone yielded 4.12 g (45%) of 54 as a white solid, mp 174-175.5 C. IR (nujol): 1700, 1760 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.45 (t, 6, -CH_2CH_3); 3.5 (q, 4, -CH_2CH_3); 4.3 (s, 2, -CH_2C≡C); 4.6 (s, 2, C≡C-CH_2-N); 7.8 (s, 4, Ar-H). Anal. Calculated for $C_{16}H_{19}N_2O_2Cl$: C, 62.64; H, 5.93; N, 9.16. Found: C, 62.65; H, 6.03; N, 9.07.

N,N'-Dimethyl-1,6-hexanediamine Dihydrochloride (111). This compound was prepared by treating 2.3 g (16.0 mmol) of N,N'-dimethyl-1,6-hexanediamine in 20 mL of methanol with gaseous HCl and precipitating the salt with diethyl ether. The solid precipitate was

collected by filtration, washed with diethyl ether and then dried. Recrystallization from methanol - diethyl ether yielded 3.3 g (97%) of 111 as a white solid, mp 240-242 C (Lit[181] mp 240-241 C).

N,N'-Dimethyl-N,N'-bis[4-(2-phthalimido)-2-butynyl]-1,6-hexanediamine (55a). To a solution of 7.1 g (38.0 mmol) of N-(2-propynyl)phthalimide (110) in 50 mL of ethanol was added 25 mL of 37% formaldehyde and 2.75 g (19.1 mmol) of N,N'-dimethyl-1,6-hexanediamine dihydrochloride (111). Cuprous chloride (0.6 g) was added, and the solution was stirred at reflux for 6 h. The mixture was cooled to room temperature, and 250 mL of water was added. The mixture was acidified with a 10% HCl solution and extracted twice with 50-mL portions of ether. The aqueous phase was made alkaline with a 10% Na_2CO_3 solution, then extracted exhaustively with six 50 mL portions of $CHCl_3$. The combined $CHCl_3$ extract was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent was removed under reduced pressure to yield an oil, which solidified upon standing. The product was recrystallized from methanol - water to yield 5.45 g (53%) of 55a as a tan solid, mp 121-124 C. IR (nujol): 1710, 1760 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.3 (broad, 8, $-CH_2-$); 2.1-2.6 (broad, 10, -N-CH); 3.3 (broad, 4, $N-CH_2-C\equiv C$); 4.5 (s, 4, $(CO)_2-N-CH_2$); 7.85 (s, 8, ArH). Anal. Calculated for $C_{32}H_{34}N_4O_4 \cdot H_2O$: C, 69.05; H, 6.51; N, 10.07. Found: C, 69.30; H, 6.41; N, 10.04.

N,N'-Dimethyl-N,N'-bis[4-(2-phthalimido)-2-butynyl]-1,6-hexanediamine Dihydrochloride (55b). The dihydrochloride salt was

prepared by treating a solution of 55a in methanol with a methanol-gaseous HCl solution and precipitating the salt with ethyl acetate. Recrystallization from methanol - ethyl acetate yielded 55b as a light tan solid, mp 114-115 C. Anal. Calculated for $C_{32}H_{36}N_4O_4Cl_2 \cdot 2\ H_2O$: C, 59.35; H, 6.07; N, 8.65. Found: C, 59.48; H, 6.10; N, 8.68.

N-(2-Propynyl)succinimide (113). To a solution of 2.3 g of sodium (0.1 mol) in 200 mL absolute ethanol was added 10.0 g (0.1 mol) of solid succinimide portionwise. Propargyl bromide (12.0 g, 101 mmol) in 50 mL ethanol was added dropwise, and the mixture was stirred at reflux under a nitrogen atmosphere for 15 h. The reaction mixture was then cooled to room temperature, and the salt which precipitated was removed by filtration. The solvent was evaporated under reduced pressure to give a crude yellow oil. The residue was distilled under vacuum to yield 10 g (79%) of 113 as a golden oil, bp 95-100 C/0.25 mm (Lit[155] bp 95 C/0.5 mm). IR (neat): 3260 cm^{-1} (C≡CH); 2130 cm^{-1} (-C≡C-); 1710, 1770 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 2.65 (s, 1, C≡CH); 2.85 (s, 4, $-CH_2-$); 4.3 (d, 2, $(CO)_2N-CH_2-$).

N,N'-Dimethyl-N,N'-bis[4-(1-succinimido)-2-butynyl]-1,6-hexanediamine Dihydrobromide (56). To a solution of 5.0 g (36.4 mmol) of N-(2-propynyl)succinimide (113) in 40 mL of dioxane was added 20 mL of a 37% formaldehyde solution and 3.84 g (18.2 mmol) of N,N'-dimethyl-1,6-hexanediamine dihydrochloride (111). Cuprous chloride (0.2 g) was added, and the solution was stirred at reflux for 6 h. The mixture was cooled to room temperature, and 250 mL of

water was added. The mixture was acidified with a 10% HCl solution and extracted twice with 50 mL of ether. The aqueous phase was made alkaline with a 10% Na_2CO_3 solution, then extracted exhaustively with six 50-mL portions of $CHCl_3$. The combined $CHCl_3$ extract was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration, and the solvent was removed under reduced pressure to yield an oil. This oil was dissolved in 20 mL of methanol saturated with gaseous HBr. The dihydrobromide salt was precipitated with ethyl acetate. Multiple recrystallizations from methanol - ethyl acetate yielded 1.5 g (14%) of 56 as a tan solid, mp 168-170 C. IR (nujol): 1707, 1770 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.2-1.9 (broad, 8, $-CH_2-$); 2.8 (s, 6, $-N-CH_3$); 2.9 (s, 8, $-CO-CH_2$); 3.1-3.35 (m, 4, $-N-CH_2$); 4.05 (m, 4, $-(CO)_2N-CH_2-C\equiv C-$); 4.4 (m, 4, $-C=C-CH_2-N$). Anal. Calculated for $C_{24}H_{36}N_4O_4Br_2$: C, 47.69; H, 6.00; N, 9.27. Found: C, 47.50; H, 6.10; N, 9.21.

1-(*p*-Nitrophenyl)cyclopentanecarboxylic acid (114). 1-Phenylcyclopentanecarboxylic acid (5.0 g, 26.3 mmol) 84 was added portionwise with stirring to 75 mL of fuming nitric acid at -10 C. Stirring was continued at 0 C for 2 h. The mixture was allowed to warm to room temperature for 1 h, then poured onto crushed ice. The resultant precipitate was collected by filtration and recrystallized from methanol, yielding 5.15 g (87%) of 114 as light tan platelets, mp 179-181 C (Lit[160] mp 179-182 C). IR (nujol): 1695 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.85 (s, 6, $-CH_2-$); 2.5-2.8 (complex m, 2, cyclopentyl); 7.6-8.2 (complex m, 4, Ar-H); 11.55 (s, 1, -COOH).

2-(Diethylamino)ethyl 1-(*p*-Nitrophenyl)cyclopentanecarboxylate Hydrochloride (115). Method A. To a solution of 5.0 g (22.6 mmol) of 114 in 25 mL of sodium-dried benzene was added 25 mL of thionyl chloride. The solution was stirred at reflux for 12 h. The solution was concentrated under reduced pressure, then reconcentrated with three 25-mL portions of benzene to remove the remaining thionyl chloride. The residue was dissolved in 40 mL of dry benzene and decolorized with charcoal. Then, 5.38 g (45.3 mmol) of freshly distilled 2-diethylaminoethanol was added in 75 mL of dry benzene, and the mixture was heated under reflux for 12 h. The solution was cooled to room temperature, poured into 250 mL water and made basic with 10% Na_2CO_3 solution. The layers were separated, and the aqueous phase was washed twice (50-mL portions) with diethyl ether. The combined organic layers were concentrated under reduced pressure, dissolved in about 100 mL of diethyl ether and extracted four times with 100-mL portions of water. The ether layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the hydrochloride salt was prepared by treating the ethereal solution of the base with gaseous HCl. Recrystallization from methanol - diethyl ether yielded 6.53 g (78%) of 115 as a white solid, mp 180-182 C (Lit[160] mp 182-182.5 C). IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.35 (t, 6, $-CH_2CH_3$); 1.8 (broad s, 6, cpentyl); 2.4-2.8 (complex m, 2, cyclopentyl); 3.2 (q, 4, $-CH_2CH_3$); 3.5 (m, 2, CH_2NEt_2); 4.5 (m, 2, $-OCH_2-$); 7.55 (d, 2, Ar-H); 8.05 (d, 2, Ar-H).

Method B. Potassium 1-(*p*-nitrophenyl)cyclopentanecarboxylate (116) (2.3 g, 8.4 mmol) dissolved in 25 mL of absolute ethanol was added in one portion to freshly prepared 2-diethylaminoethyl chloride (86) (1.68 g, 12.4 mmol) dissolved in 10 ml of absolute ethanol. The solution immediately became turbid, and a precipitate began to separate. The mixture was then stirred at reflux for 16 h. After the solution was cooled, the precipitated inorganic salt was removed by filtration and discarded. The filtrate was evaporated to dryness at reduced pressure. The resulting oil was dissolved in diethyl ether (100 mL), washed with three 50-mL portions of water and dried over anhydrous magnesium sulfate. The hydrochloride salt was prepared by treating the ethereal solution of the base with gaseous HCl. Recrystallization from methanol - diethyl ether yielded 1.8 g (58%) of 115 as a white solid, mp 182-184 C (Lit[160] mp 182-182.5 C). This compound was identical in its physical and spectral properties to 115 prepared from 1-(4-nitrophenyl)cyclopentanecarboxylic acid (Method A).

Potassium 1-(*p*-Nitrophenyl)cyclopentanecarboxylate (116). A solution of 2.6 g (11.1 mmol) of 114 in 50 mL of absolute ethanol was titrated with 10% ethanolic potassium hydroxide using phenolphthalein as an external indicator. The solvent was evaporated under reduced pressure, followed by recrystallization of the product from ethanol - ether, yielding 2.95 g (97%) of 116 as white needles, mp 222-224 C (Lit[160] mp 225 C).

2-(Diethylamino)ethyl 1-(*p*-Aminophenyl)cyclopentanecarboxylate Hydrochloride (57a). A dry methanol solution (60 mL) of 5.95 g (16.1 mmol) 115 was catalytically reduced with 10% platinum on carbon under a hydrogen atmosphere on a Parr apparatus. When the theoretical quantity of hydrogen had been absorbed, the catalyst was removed by filtration. The solvent was evaporated under reduced pressure, yielding a crude yellow solid. Recrystallization from methanol - ethyl acetate yielded 4.35 g (80%) of 57a as a light tan solid, mp 135-137 C. IR (nujol): 3460, 3360, 3200 cm^{-1} (-NH2); 1720 cm^{-1} (C=O). ^1H NMR (D_2O): δ 1.2 (t, 6, $-CH_2CH_3$); 1.75 (broad s, 6, cpentyl); 2.2-2.7 (complex m, 2, cyclopentyl); 3.05 (q, 4, $-CH_2CH_3$); 3.4 (broad, 2, CH_2NEt_2); 4.4 (broad, 2, $-OCH_2-$); 6.85 (d, 2, Ar-H); 7.25 (d, 2, Ar-H). Anal. Calculated for $C_{18}H_{29}N_2O_2Cl \cdot 0.5\ H_2O$: C, 61.78; H, 8.64; N, 8.01. Found: C, 61.76; H, 8.48; N, 7.97.

Methyl 1-(*p*-Nitrophenyl)cyclopentanecarboxylate (117a). To a solution of 6.25 g (26.6 mmol) of 1-(*p*-nitrophenyl)cyclopentanecarboxylic acid (114) in 25 mL of sodium-dried benzene was added 25 mL of thionyl chloride and the solution was heated at reflux for 12 h. The solution was concentrated under reduced pressure, then reconcentrated with three 25-mL portions of benzene. The residue was dissolved in 40 mL of benzene and decolorized with charcoal. The solution was then heated at reflux with 75 mL of dry methanol for 12 h. After the solution was cooled, the solvent was removed at reduced pressure. The resulting crude tan solid was recrystallized from methanol yielding 5.14 g (78%) of 117a as light tan needles, mp 72-73 C. IR (nujol): 1710 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.8 (broad, s,

6, cpentyl); 2.5-2.8 (complex m, 2, cyclopentyl); 3.55 (s, 3, $-CH_3$); 7.45 (d, 2, Ar-H); 8.1 (d, 2, Ar-H). Anal. Calculated for $C_{13}H_{15}NO_4$: C, 62.62; H, 6.07; N, 5.64. Found: C, 62.63; H, 6.11; N, 5.60.

Ethyl 1-(*p*-Nitrophenyl)cyclopentanecarboxylate (117b). To a solution of 3.5 g (14.2 mmol) of 1-(*p*-nitrophenyl)cyclopentanecarboxylic acid (114) in 25 mL sodium-dried benzene was added 25 mL of thionyl chloride, and the solution was heated at reflux for 12 h. The mixture was concentrated at reduced pressure and then reconcentrated with three 25 mL portions of benzene. The residue was dissolved in 40 mL of benzene and decolorized with charcoal. The solution was then heated at reflux with 75 mL of anhydrous ethanol for 12 h. The solution was then cooled to room temperature. Evaporation of the solvent under reduced pressure yielded an amber oil. The oil was distilled under vacuum yielding 2.8 g (75%) of an amber oil, bp 110-115 C/0.25 mm. IR (neat): 1720 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.2 (t, 3, $-OCH_2CH_3$); 1.85 (broad, 6, cpentyl); 2.4-2.8 (complex m, 2, cyclopentyl); 4.1 (q, 2, OCH_2CH_3); 7.45 (d, 2, Ar-H); 8.15 (d, 2, Ar-H). Anal. Calculated for: $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32. Found: C, 63.97; H, 6.56; N, 5.28.

Methyl 1-(*p*-Aminophenyl)cyclopentanecarboxylate (118a). A methanol solution (60 mL) of 2.25 g (9.0 mmol) of 117a was catalytically reduced with 10% platinum on carbon under a hydrogen atmosphere on a Parr apparatus. When the theoretical quantity of hydrogen had been absorbed, the catalyst was removed by filtration. The solvent

was evaporated under reduced pressure. Recrystallization of the residue from methanol yielded 1.45 g (77%) of 118a as white needles, mp 129.5-131 C. IR (nujol): 3210 cm^{-1}, 3360 cm^{-1}, 3430 cm^{-1} ($-NH_2$); 1710 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.7 (broad, 6, $-CH_2-$); 2.3-2.7 (complex m, 2, cyclopentyl); 3.5 (s, 3, $-OCH_3$); 6.6 (d, 2, Ar-H); 7.15 (d, 2, Ar-H). Anal. Calculated for $C_{13}H_{17}NO_2$: C, 71.20; H, 7.82; N, 6.39. Found: C, 71.26; H, 7.88; N, 6.34.

Ethyl 1-(*p*-Aminophenyl)cyclopentanecarboxylate (118b). An ethanol solution (60 mL) of 2.2 g (8.4 mmol) of 117b was catalytically reduced with 10% platinum on carbon under a hydrogen atmosphere on a Parr apparatus. When the theoretical quantity of hydrogen had been absorbed, the catalyst was removed by filtration. The solvent was evaporated under reduced pressure, yielding a yellow oil. The oil was distilled under vacuum, yielding 1.46 g (75%) of a yellow oil, bp 128-132 C/0.2 mm, which solidified on standing to yellow prisms, mp 62-63 C. IR (neat): 3210, 3360, 3430 cm^{-1} ($-NH_2$); 1710 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.1 (t, 3, $-OCH_2CH_3$); 1.7 (broad s, 6, $-CH_2-$); 2.3-2.7 (complex m, 2, cyclopentyl); 4.0 (q, 2, $-OCH_2CH_3$); 6.5 (d, 2, Ar-H); 7.05 (d, 2, Ar-H). Anal. Calculated for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.17; H, 8.22; N, 5.99.

Methyl 1-[*p*(1-Pyrrolidinyl)phenyl]cyclopentanecarboxylate (119a). To a solution of 2.7 g (12.3 mmol) 118a in 40 mL of dry DMF, containing 3.0 g of anhydrous potassium carbonate, under a nitrogen atmosphere, was added 2.66 g (12.3 mmol) of 1,4-dibromobutane dropwise over a 15 min period. The reaction mixture was then stirred

at 75-80 C for 12 h. The solution was poured over crushed ice, and the precipitate was collected by filtration. Recrystallization from methanol yielded 2.1 g (63%) 119a as light tan needles, mp 115-117 C. IR (nujol): 1710 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.7 (broad, 6, cpentyl); 1.9 (q, 4, N-CH_2-); 2.3-2.8 (complex m, 2, cyclopentyl); 3.2 (t, 4, N-CH_2CH_2); 3.55 (s, 3, -OCH_3); 6.5 (d, 2, Ar-H); 7.2 (d, 2, Ar-H). Anal. Calculated for $C_{17}H_{23}NO_2$: C, 74.69; H, 8.48; N, 5.12. Found: C, 74.55; H, 8.52; N, 5.06.

Ethyl 1-[*p*-(1-Pyrrolidinyl)phenyl]cyclopentanecarboxylate (119b). To a solution of 1.6 g (6.9 mmol) of 118b in 25 mL of dry DMF, containing 2.0 g anhydrous potassium carbonate, was added dropwise 1.5 g (6.9 mmol) of 1,4-dibromobutane in 5 mL of DMF over a 15 min period. The reaction mixture was stirred under nitrogen at 75-80 C for 12 h. The solution was then poured over crushed ice, and the precipitate was collected by filtration. Recrystallization from methanol yielded 1.3 g (66%) of 119a as a white solid, mp 78-79 C. IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.25 (t, 3, -CH_2CH_3); 1.7-2.2 (m, 10, -CH_2-); 2.5-2.8 (m, 2, -CH_2-); 3.3 (t, 4, -N-CH_2); 4.1 (q, 2, -CH_2CH_3); 6.5 (d, 2, Ar-H); 7.2 (d, 2, Ar-H). Anal. Calculated for $C_{18}H_{25}NO_2$: C, 75.22; H, 8.77; N, 4.87. Found: C, 75.34; H, 8.79; N, 4.86.

2-(Diethylamino)ethyl 1-[*p*-(1-Pyrrolidinyl)phenyl]cyclopentanecarboxylate Hydrochloride (57c). A mixture of 4.0 g (14.7 mmol) of 119a, 3.43 g (29.3 mmol) of freshly distilled 2-diethylaminoethanol and 0.1 g sodium in 125 mL of dry toluene was heated under reflux

with stirring for 20 h, during which massive precipitation occurred. After the reaction was cooled to room temperature, the solid was collected by filtration, washed with benzene and dissolved in water. The toluene solution was extracted three times with 100-mL portions of 10% HCl solution. The aqueous layer was made basic with the addition of solid sodium carbonate in portions, combined with the aqueous solution of the precipitate, and extracted four times with diethyl ether (50 mL portions). The ether extract was dried over anhydrous magnesium sulfate. The hydrochloride salt was prepared by the slow addition of an ethereal solution of gaseous HCl to the base in diethyl ether. Recrystallization from chloroform - ether yielded 2.75 g (49%) of 57c as a tan solid, mp 208-211 C. IR (nujol): 1720 cm^{-1} (C=O). ^1H NMR (D_2O · CF_3COOD): δ 1.2 (t, 6, $-CH_2CH_3$); 1.8 (broad, 6, -cpentyl); 2.0-2.4 (m, 6, $-CH_2$); 3.1 (q, 4, $-CH_2CH_3$); 3.2-3.8 (m, 6, $-N-CH_2$); 4.2-4.5 (m, 2, $-O-CH_2$); 7.4 (s, 4, ArH). Anal. Calculated for $C_{22}H_{35}N_2O_2Cl$: C, 66.90; H, 8.93; N, 7.09. Found: C, 66.78; H, 8.95; N, 7.07.

Methyl 1-[*p*-(1-Tetrazolyl)phenyl]cyclopentanecarboxylate (120). To a stirred solution of 2.1 g (9.6 mmol) of 118a in 20 mL of glacial acetic acid, at 70-75 C under nitrogen, was added 1.42 g (9.6 mmol) of freshly distilled ethyl orthoformate in 5 mL of glacial acetic acid. The mixture was stirred for 4 h at 70-75 C. Then, 1.87 g (28.8 mmol) of sodium azide was added portionwise, and the reaction was continued for an additional 24 h. After cooling to room temperature, the solution was poured into 250 mL of ice water. The precipitate was collected by filtration and recrystallized from methanol,

yielding 1.7 g (65%) of 120 as light yellow needles, mp 115-118 C. IR (nujol): 1710 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 1.7 (broad s, 6, cpentyl); 2.3-2.6 (complex m, 2, cyclopentyl); 3.5 (s, 3, $-OCH_3$); 7.2-7.8 (complex m, 4, Ar-H); 9.7 (s, 1, Tet-H). Anal. Calculated for $C_{14}H_{16}N_4O_2$: C, 61.74; H, 5.92; N, 20.58. Found: C, 61.64; H, 6.13; N, 20.53.

1-(*p*-Aminophenyl)cyclopentanecarboxylic acid (121). A methanol solution (60 mL) of 5.1 g (22.4 mmol) of 114 was catalytically reduced with 10% platinum on carbon under a hydrogen atmosphere on a Parr apparatus. When the theoretical quantity of hydrogen had been absorbed, the catalyst was removed by filtration. The solvent was removed under reduced pressure. Recrystallization of the residue from methanol yielded 4.2 g (91%) of 121 as light tan needles, mp 195-198 C (Lit[184] mp 196-199 C). ^1H NMR ($CDCl_3$-CF_3COOD): δ 1.85 (broad s, 6, $-CH_2-$); 2.4-2.7 (complex m, 2, cyclopentyl); 3.2 (broad flat, 2, $-NH_2$); 7.55 (d, 2, Ar-H); 7.9 (d, 2, Ar-H); 11.6 (broad, 1, -COOH).

2-(Diethylamino)ethyl 1-[*p*-(1-Tetrazolyl)phenyl]cyclopentane carboxylate Hydrochloride (57b). To a stirred solution of 3.8 g (11.2 mmol) of 57a in 40 mL of glacial acetic acid, at 70-75 C under nitrogen, was added 1.7 g (11.2 mmol) of freshly distilled ethyl orthoformate in 10 mL of glacial acetic acid. The mixture was stirred for 4 h. Then 2.2 g (33.6 mmol) of solid NaN_3 was added portionwise, and the reaction was continued for 48 h. After cooling the solution on an ice bath, the mixture was filtered. The collected

solid was washed with 20 mL of ether. The solvents were removed under reduced pressure to yield an orange oil. This oil was dissolved in 50 mL of diethyl ether and extracted with three 50-mL portions of water. The ether layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration. The hydrochloride salt was prepared by treating an ether solution of the base with gaseous HCl. Recrystallization from chloroform - diethyl ether yielded a crude yellow solid. The crude product was twice chromatographed on a column of silica gel, $CHCl_3$:methanol, 9:1, as eluent, to give a yellow compound, mp 154-156 C. IR (nujol): 1720 cm^{-1} (C=O) no NH abs. ^1H NMR (D_2O): δ 1.1-1.4 (m, 6H); 1.6-2.2 (broad m, 8H); 2.5-3.5 (m, 6H); 4.3-4.5 (m, 2H); 7.3 (d, 2H); 8.0 (d, 2H). Anal. Calculated for $C_{19}H_{28}N_5O_2Cl \cdot H_2O$: C, 55.40; H, 7.34; N, 17.00. Found: C, 55.14; H, 7.41; N, 17.04.

p-(Trifluoromethyl)benzyl Bromide (123). Five grams (28.4 mmol) of 4-(trifluoromethyl)benzyl alcohol in 10 mL of 48% HBr was stirred at reflux for 12 h. The solution was cooled to room temperature, poured over 50 mL of crushed ice and extracted with three 50 mL portions of ether. The ether layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the solvent concentrated at reduced pressure to yield an oil. The oil was distilled under reduced pressure to yield 6.42 g (96%) of 123 as a clear oil, bp 35-38 C/0.5 mm (Lit[189] bp 65-66 C/5 mm). IR (neat) cm^{-1} no OH abs. ^1H NMR ($CDCl_3$): δ 4.6 (s, 2, CH_2); 7.2 (d, 2, ArH); 7.9 (d, 2, ArH).

p-(Trifluoromethyl)phenylacetonitrile (124). To a solution of 6.8 g (28.4 mmol) of 123 in 40 mL of 75% ethanol was added 4.2 g (85.6 mmol) of NaCN in 10 mL of 75% ethanol. The reaction mixture was stirred for 12 h at room temperature, poured into about 200 mL of ice water and extracted with three 50 mL portions of $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous magnesium sulfate. The drying agent was removed by filtration. The solvent was removed at reduced pressure to yield a crude solid. Recrystallization of the product from petroleum ether yielded 4.8 g (92%) of 124 as a white solid, mp 40-42 C (Lit[190] mp 40-41 C). IR (nujol): 2220 cm^{-1} (C≡N). ^1H NMR ($CDCl_3$): δ 3.8 (s, 2, $-CH_2$); 7.4 (d, 2, Ar-H); 8.0 (d, 2, ArH).

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