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DESIGN AND SYNTHESIS OF INHIBITORS OF N\textsuperscript{8}-ACETYLSPERIDINE DEACETYLASE

Sasi Ahmed Dredar

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DESIGN AND SYNTHESIS OF INHIBITORS OF 
\(N^8\)-ACETYLSPERIMIDINE DEACETYLASE

by
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Master of Science
Bath University
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1980

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ABSTRACT

Ten N⁸-acetylspermidine analogues were synthesized. These compounds were used as active site directed agents to investigate the catalytic mechanism of N⁸-acetylspermidine deacetylase. This enzyme is present in the cytosolic fraction of rat liver homogenate.

The Ki values for all the compounds were determined from Dixon plots. The results of this study show that these compounds act as competitive inhibitors of N⁸-acetylspermidine deacetylase. The Ki values of these compounds range from 29.2 to 0.00018 mM.

The size of the active site was investigated by substitution of the acetyl group of N-acetyl-1,6-diaminohexane and N-acetyl-1,8-diamino-octane with larger groups. These modifications resulted in a decrease in the inhibitory activity. Compounds were made in which the length of the carbon chain between two terminal amino groups was varied. It was found that a six carbon separation is needed for optimal activity.

Bioisosteric replacement of N⁴ of N⁸-acetylspermidine with a -CH₂- group resulted in a decrease in the potency of the compound compared with N-acetyl-1,6-diaminohexane. On the other hand, bioisosteric replacement of the N⁸ of N⁸-acetylspermidine with a -CH₂- group resulted in a large increase in the inhibitory activity. The Ki value for this compound (Ki = 0.00018 mM) is lower than the Km of the enzyme (Km = 0.01m M).

Results obtained from this project and other results obtained in our laboratory were used to draw a hypothetical model for the active site of N⁸-acetylspermidine deacetylase.
DEDICATION

I dedicate this dissertation to my parents
for their never ending support
and encouragement.
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I would like to express my gratitude and appreciation to Dr. David Fries for his guidance, advice, and constructive criticism through all the stages of my study.

The author's thanks and appreciation are also shown to these committee members: Dr. James Blankenship, Dr. John Brown, Dr. Charles Matuszak, and Dr. Fuad Nahhas for their continuous support.

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INTRODUCTION

The aliphatic polyamines, putrescine, spermidine and spermine are normal cell constituents that play an important physiological role in living tissues. They are highly basic compounds (pKa range between 8 and 11) that influence the growth and differentiation of the cells.

The term polyamine applies to any compound that contains two or more amine groups in its structure. Structures of different polyamines are given in Figure 1.

Discovery of Polyamines

Antoni Van Leeuwenhoek (1) was the first to detect spermine phosphate in human semen in 1678. Spermidine and spermine derived their names from human semen from which they were first isolated (2). In the hundred years after its detection, spermine phosphate was detected in various body organs and fluids (such as liver, spleen, and sputum) and was reported under different names (3,4,5). In 1878 various derivatives of spermine were prepared by Schreiner (5).

The name putrescine was taken from the term putrefaction and is derived from the observations that it is rapidly formed in animal tissues under bacterial decomposition. The structure of spermine was obscure for many years in spite of the efforts of many scientists and only in 1924 was the right structure of spermine deduced (6). Once the structure was known, several methods were reported for the synthesis of the compound and its derivatives (7,8,9).

Rosenheim and co-workers (10) were the first to prove that spermine
A: \( \text{H}_2\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2 \)
1,3-Diaminopropane

B: \( \text{H}_2\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2 \)
Putrescine

C: \( \text{H}_2\text{N-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-NH}_2 \)
Cadaverine

D: \( \text{H}_2\text{N-}([\text{CH}_2])_3\text{-NH-}([\text{CH}_2])_4\text{-NH}_2 \)
Spermidine

E: \( \text{H}_2\text{N-}[\text{CH}_2]_3\text{-NH-}([\text{CH}_2])_4\text{-NH-}([\text{CH}_2])_3\text{-NH}_2 \)
Spermine

Figure 1. Structures of various polyamines
isolated from human semen was identical with the base isolated by similar methods from various tissues and organs. During the synthesis of spermine, Dudley et al. (11) obtained another basic product. The compound was identified as spermidine. After the elucidation of its structure, spermidine was found to be widely distributed in cells.

**Distribution of Polyamines**

Polyamines are constituents of every kind of prokaryotic and eukaryotic cell. They are also one of the components of many viruses. These substances are intracellular constituents and are found only in small amounts in the extracellular fluids.

Spermine and spermidine are more widely distributed in the tissues than putrecine (12). The concentrations of polyamines vary from one organ to another in a given species and from one species to another.

The first study concerning the distribution of polyamines in human tissues was presented by Hamalainen (13). In that study spermine was precipitated as a phosphate salt and the weight of the precipitate was determined gravimetrically. This study revealed that human prostate and bone marrow contain higher amounts of spermine than kidney, lung and muscle.

Neoplastic cells usually contain higher concentrations of polyamines than normal cells. Numerous studies have shown that polyamine levels are elevated in urine, serum, bone marrow cerebrospinal fluids and erythrocytes of many patients bearing tumors (14). It has been reported by many workers (15, 16) that the levels of polyamines in rapidly growing cells are much higher than the levels present in non-proliferating cells. Prokaryotic cells contain only spermidine and
putrescine. It has been found that putrescine is present in many organs and tissue fluids and these include liver (17), pancreas (18), and human semen (17). Siimes (19) had found that the level of polyamines in human organs ranges from 0.11 to 11.4 nmoles/mg protein depending on the organ used in the study. Cadaverine was found to be present in both bacterial and liver ribosomes (20).

Little effort has been devoted to the study of polyamines in plants compared with that given to their study in humans and animals. The reason for the lack of interest in the study of polyamines in plants is not clear.

In general, polyamines have been found to be present in many cell organelles such as ribosomes (21), mitochondria and chloroplast (21,22).

**Physiological Functions of Polyamines**

Polyamines are very important for the normal function of the body. The increase in polyamine concentrations is very important for cell proliferation. It is well documented that an increase in the ornithine decarboxylase activity and an increase in the level of putrescine and spermidine occurs in rat liver regenerating after partial hepatectomy (23). Polyamines have been implicated in the regulation of nucleic acid metabolism (24,25,26).

Aliphatic polyamines, putrescine, spermine and spermidine are strongly basic substances. At physiological pH, they are positively charged. Due to their cationic properties and structural characteristics, polyamines are able to bind with ionic groups on nucleic acids, proteins and phospholipids through electrostatic interactions (27,28). They can also bind to those molecules through hydrogen and
hydrophobic bonds. Tabor (29) has reported that polyamines protect DNA against heat denaturation. Many workers (30,31) have found that polyamines stabilize ribosomes and tRNA.

Sara et al. (32) have reported that the decrease in the basicity or hydrogen bonding capacity of putrescine resulted in a marked reduction in the stimulatory activity of putrescine on growth and protein biosynthesis of polyamine-depleted cultures. He suggested that two primary amino groups are needed in the polyamine structure for full activity.

There have been many studies on the effect of polyamines on DNA and tRNA. These studies include the effect of polyamines on the stability of nucleic acids (33), on transcription (34) and on translation (35). In many cases magnesium ions may be substituted for polyamines, but there are cases of biochemical reactions in which polyamines are specific in their reactions. In another study, it has been found that the affinity of ribosomes for polyamines is reduced in the presence of magnesium ions (36). Polyamines offer advantages in biological systems over cationic ions such as Ca\(^{++}\) and Mg\(^{++}\). One of their advantages is that polyamines are synthesized by the body and their level can be regulated and adjusted to the needed concentration. In contrast Mg and Ca ions have to be supplied in an absorbable form and they have to be transported across the cells' membranes. Recently, the effect of polyamines on the uptake of neurotransmitters has been studied (37). On the basis of these studies, it was concluded that polyamines inhibit the uptake of dopamine (DA) and choline by rat forebrain synaptosomes. The uptake of (GABA) is also inhibited but to a lesser extent than other neurotransmitters.
In the future, additional physiological functions of polyamines probably will be discovered and their role in life processes may be better understood.

**Pharmacological Aspects of Polyamines**

Polyamines, at physiological pH, are positively charged and for this reason they do not readily cross the blood brain barrier. In order to see their effects on the central nervous system (CNS) after intraperitoneal or intravenous injections, high doses have to be given.

The effect of spermine and spermidine on body temperature has been investigated (38,39,40). These compounds have been found to produce hypothermia when they are administered intraperitoneally or intracerebrally. In animals injected with 40 μg of spermine intracerebrally, the body temperature dropped, with a maximum decrease of about 1°C, 24 hours after the injection, and the hypothermic effect lasted for about 3 days (38).

The pharmacology and toxicology of the acetylated and nonacetylated polyamines have been investigated by Alshabanah and Blankenship (41). From those studies, they concluded that spermine is more toxic to mice than spermidine and putrescine. They also found the onset of toxicity of both N¹- and N⁸-acetyl spermidine is much more rapid than that of the spermine, spermidine, and putrescine. The reason for this difference in the onset could be explained on the basis that the acetylated form of polyamines may cross the blood brain barrier much better than the corresponding nonacetylated form of polyamines.

Both acetylated and nonacetylated polyamines produce hypothermia, sedation, muscle incoordination, decreased motor activity, clonic
convulsion and death which is due to respiratory arrest. In addition, polyamines (when they are injected at a lower dose level) produce anorexia and adipsia which last for about two days (42).

**Biosynthesis of Polyamines**

The biosynthetic pathway for polyamines is illustrated in Figure 2. Polyamines biosynthesis in plants as in bacteria is somewhat more complicated than that in mammalian cells in that two constitutive pathways lead to putrescine biosynthesis. Putrescine may be formed directly, through ornithine decarboxylation by ornithine decarboxylase (ODC), or indirectly, through a series of intermediates following arginine decarboxylation (43,44).

Mammalian cells can synthesize putrescine through decarboxylation of ornithine. This conversion is catalyzed by ornithine decarboxylase (45). This step appears to be the rate limiting step in the polyamine biosynthesis. Putrescine is converted to spermidine by the addition of an aminopropyl group. This conversion is catalyzed by spermidine synthase. The aminopropyl group is derived from methionine, which is first converted into S-adenosylmethionine and then decarboxylated by the enzyme S-adenosylmethionine decarboxylase. Once it has been decarboxylated, decarboxylated S-adenosylmethionine is committed to polyamine synthesis (46).

To synthesize spermine an aminopropyl group must be added to spermidine. The source of this group is the same as that of the first aminopropyl group (decarboxylated S-adenosylmethionine). This step is catalyzed by an enzyme known as spermine synthase (47,48). Spermidine synthase, and spermine synthase are different enzymes and they are specific in their reactions.
Figure 2. The major pathway for the biosynthesis of polyamines in mammals.
**Ornithine Decarboxylase**

Ornithine decarboxylase is the first enzyme in the polyamine biosynthetic pathway. It is a unique enzyme because of its rapid induction and the very short half life.

The very rapid induction of ornithine decarboxylase has been described by many workers (49). The enzyme is studied mainly in rat liver, where great increases in the activity of the enzyme are observed. The enzyme can be induced by many drugs as well as by hormones. The maximum induction is reached within about four hours after the stimulation. Figure 3 shows the effect of administration of growth hormone on the induction of ornithine decarboxylase (50). The increase in the activity of the enzyme, under a variety of conditions, is accompanied by a corresponding increase in the amount of ornithine decarboxylase protein. The induction can be inhibited by administration of actinomycin which is a protein synthesis inhibitor. It has been reported (51) that this enzyme represents only between 0.01-0.05% of the total cellular protein, even when maximally inducted.

The half life of ornithine decarboxylase has been reported to be between 10-20 minutes (52). This short half life of ornithine decarboxylase has been attributed to its cellular function as the rate limiting step in the polyamine synthesis.

It has been found that addition of putrescine to mammalian cells in cultures causes a rapid decrease in the ornithine decarboxylase activity. This decrease is due to the synthesis of a protein inhibitor (antizyme) to ornithine decarboxylase (53,54). This antizyme can specifically interact with ornithine decarboxylase and form an inactive enzyme-antizyme complex. The interaction between ornithine decarboxylase
Figure 3. Induction of ornithine decarboxylase by growth hormone in adult (o) and weanling (•) rats.
and the antizyme might be the mechanism by which ornithine decarboxylase is regulated (55).

**Inhibition of Ornithine Decarboxylase**

The precise physiological role of polyamines is not clear, although many studies have shown that these amines may be involved in the control of cell division and growth as well as DNA synthesis. One of the best ways to investigate the function of the polyamines is through interference with their synthesis. This can be done by the inhibition of ornithine decarboxylase, which is the rate limiting step in their synthesis. The difficulties of this study are due to the fact that the enzyme is rapidly induced and has a very short half life. In addition, the activity of ornithine decarboxylase is partially regulated by the interaction between the enzyme and its antizyme. Substances which inhibit ornithine decarboxylase can be divided into two groups according to their mechanism of action.

**Competitive Inhibitors.** Several compounds which bind to ornithine decarboxylase in a reversible way have been synthesized. Alpha-methylornithine, and alpha-hydrazinoornithine are examples of this class (56,57). The major disadvantage of these compounds is that they increase the half life of ornithine decarboxylase (58,59). The half-life of ornithine decarboxylase in the presence and absence of alpha-methylornithine was found to be 57 and 19 minutes respectively. It has been reported that the decrease in the degradation of the enzyme might be due to the ability of the competitive inhibitor to enter the active site of ornithine decarboxylase and thus block or slow its degradation by proteolytic enzymes (58). Another disadvantage of the competitive
inhibitors is that the duration of inhibition of polyamine synthesis depends on the drug half-life in the body.

**Irreversible Inhibitors.** Alpha-difluoromethylornithine (DFMO) is an effective and specific inhibitor of ornithine decarboxylase (59,60,61). The inhibition has been reported to depend on the dose, time of contact with the enzyme, as well as on the type of the organ used in the study. An intraperitoneal dose of 100 mg/Kg of body weight of DFMO reduced the activity of ornithine decarboxylase by 90, 40, and 50% in the prostate, thymus, and testis respectively six hours after injection (60).

The acute oral median lethal dose (LD50) for DFMO has been found to be 5.0 g/Kg (46). Administration of total daily dose of 4 g/KG for 28 days to mice has been found to have no effect on the body weight and no organ toxicity was found. The mechanism of action of this compound is illustrated in Figure 4.

**Polyamine Metabolism**

There is more than one enzyme involved in polyamine metabolism (62). It has been reported (63) that polyamine oxidase catalyzes the oxidation of both spermine and spermidine. These molecules are cleaved at the secondary amino group to yield 3-aminopropionaldehyde and putrescine from spermidine or spermidine from spermine. Oxidative deamination of putrescine by diamine oxidase leads to the formation of 4-aminobutyraldehyde which on further oxidation yields gamma-amino butyric acid (GABA). Study of conversion of putrescine to GABA in mouse liver has shown that 2-3% of GABA was formed from putrescine (64). It was found (64) that addition of acetylCoA (2 mM) to the mouse brain
Figure 4. Mechanism of action of DFMO.
DFMO binds to pyridoxalphosphate, followed by loss of CO$_2$ and elimination of fluoride. The result of the enzyme catalyzed reaction is the formation of a highly reactive intermediate which alkylates the enzyme.

R = H$_2$N-(CH$_2$)$_3$-

Py = Pyridoxalphosphate ring system.
cortex homoginate enhanced conversion of putrescine to GABA. Seiler (65) has found that GABA which is formed from N-acetylputrescine was nearly twice as high as that formed from putrescine. These results indicate that monoacetylputrescine acts as a much better substrate for polyamine oxidase than putrescine.

Monoacetylated derivatives of polyamines have been shown to be normal constituents of human urine (66). Abdel Monem and Ohno (67) examined the acetylated derivatives of polyamines in the urine of normal subjects and cancer patients. Their study revealed that N¹-acetyl spermidine and N⁸-acetylspermidine were both present in all samples. However they were unable to detect N¹-acetylspermine, N⁴-acetylspermine and N⁴-acetylspermidine. This study also shows that cancer patients excrete higher amounts of N¹-acetylspermidine than N⁸-acetylspermidine.

Acetylation of spermidine leads to the formation of two isomers, N⁸-acetylspermidine and N¹-acetylspermidine. Spermidine was found to be converted to N⁸-acetylspermidine by an enzyme present in the chromatin of liver and kidney (68). On the other hand spermidine has been found to be converted to N¹-acetylspermidine by a cytosolic enzyme (69). Putrescine and histones were not acetylated by this enzyme (N¹-acetyl-spermidine transferase).

Spermidine N¹-acetyltransferase converts spermidine and spermine to N¹-acetylspermidine and N¹-acetylspermine respectively. In this conversion acetylCoA is the donor of the acetyl group. Spermidine/spermine N¹-acetyltransferase has been shown to be the rate limiting enzyme for biodegradation of polyamines (70,71). In vivo N¹-acetylspermidine is converted to putrescine and N¹-acetylspermidine and N¹-acetylspermine is converted to spermidine; both reactions are
catalyzed by polyamine oxidase.

The acetylated forms of polyamines are better substrates for polyamine oxidase than the nonacetylated polyamines (64). The enzymes spermidine synthase, spermidine N¹-acetyltransferase and polyamine oxidase form a cycle for the interconversion of polyamines (72). This cycle is shown in Figure 5.

Matsui et al. (73) studied the induction of the N¹-acetyltransferase and found the activity of this enzyme was 2.5 times higher than the control when 50 ng/mL of phorbal 12-myristate-13-acetate was added to the lymphocyte suspension. In another study Matsui and Pegg (74) have shown the maximum induction of spermidine/spermine N¹-acetyltransferase is reached after six hours of stimulation and this induction can be inhibited by addition of cycloheximide. The decrease in the activity of spermidine/spermine N¹-acetyltransferase by addition of cycloheximide depends on the method used to induce the enzyme (73). Cycloheximide at about 50 μM caused a 72% reduction in the activity of the enzyme which had been induced by addition of parathyroid hormone (75). On the basis of the above results it seems that the increase in the activity of N¹-acetyltransferase is regulated by changing the rate of the synthesis of the enzyme.

Further degradation of acetylated polyamine has been studied both in vitro (76) and in vivo (77). The results of these studies show that N⁸-acetylspermidine was deacetylated to spermidine by an enzyme present in the cytosol of the rat liver. However, N¹-acetylspermidine has been found to be not deacetylated by this enzyme, but it is good substrate for polyamine oxidase (63,78). The latter enzyme converts N¹-acetylspermidine to putrescine and 3-aminopropionaldehyde.
Figure 5. Route of the synthesis and metabolism of spermidine in rat liver. The enzymes involved are 1) ornithine decarboxylase; 2) spermidine N<sup>1</sup>-acetyltransferase; 3) polyamine oxidase; 4) spermidine synthase; 5) spermidine N<sup>8</sup>-acetyltransferase; 6) N<sup>8</sup>-acetyl-spermidine deacetylase.
Manneh and Blankenship (79) have found the Km for N⁸-acetyl-spermidine deacetylase to be 0.01 mM. Diisopropylfluorophosphate was tested and found to be a poor inhibitor (Ki=33 mM) for the enzyme. These results indicate that the active site of the enzyme does not have a serine hydroxyl group. On the other hand, the enzyme was found to be inhibited by 1,10-orthophenanthroline (Ki=1.25 mM) which is reported to be a specific inhibitor for enzymes containing zinc or copper in their active sites (80).

Clinical Application of Polyamine Antimetabolites

The association of rapid cell growth with an increase in ornithine decarboxylase activity has been known for many years. Accumulation of putrescine, spermidine, and spermine usually follows the increase in the activity of ornithine decarboxylase. As a result of this, inhibition of polyamine biosynthesis via inhibition of ornithine decarboxylase might be a means for chemotherapy of disease states characterized by rapid cell multiplication.

Inhibition of ornithine decarboxylase by DFMO has a striking effect on the embryonic development (81). It has been shown that animals receiving DFMO (2% in drinking water) during 5-8 days of gestation arrested embryonic development. This arrest correlated with the decrease of ornithine decarboxylase activity and with putrescine and spermidine depletion in the uterus.

DFMO is also effective against Trypanosoma brucei brucei (T. b. brucei). Inoculation of mice with 5 x 10⁵ T. b. brucei killed the animal five days after the infection. However, treating the infected animals with 1 or 2% of DFMO solution in their drinking water resulted in
uring the animals (82). T. b. brucei starts to disappear from the blood of the infected animals five days after administration of DFMO. Injection of suspensions of the brain from cured animals failed to induce infection in the healthy animals. However, administration of putrescine, spermidine, and spermine along the DFMO abolished its effect (83). All these suggest that DFMO influences parasites' proliferation by the depletion of polyamines.

In order to cure groups of mice infected with 2-5 x 10^5 T. b. brucei, 3 mg/Kg of bleomycin (i.p.) or 1% of DFMO (in drinking water) was needed. However, coadministration of 0.25 mg/Kg of bleomycin with 0.25% DFMO resulted in cured animals (84). These values represent about 25 and 8% of the curative dose of DFMO and bleomycin respectively. These and other results suggest that there is synergism between DFMO and bleomycin (84).

DFMO at 5 mM has been shown to retard the growth rate of EMTS6, a murine mammary sarcoma in tissue culture (85). Combined treatment of this mammary sarcoma with DFMO and cyclophosphamide caused a reduction in the tumour weight greater than either treatment alone.

Sequential administration of DFMO and methylglyoxal bis (guanylhydrozone) (MGBG) resulted in an apparent synergism when tested in childhood leukemia (86). Even though there is an increase in the therapeutic response of the combined drugs, there was no increase in the side effects of those compounds. The increase in the therapeutic response has been shown to result from the enhancement of the cellular uptake of MGBG (86). The effect of combined treatment of DFMO and adriamycine on the L120 leukemia and EMT6 tumors in mice has been studied by Jacques et al. (87). The results of this investigation have
shown that combined treatment with these drugs caused a great reduction (60%) in tumor weight.

Interference with polyamine biosynthesis caused a decrease in the growth rate of *Escherichia coli* and *Pseudomonas aeruginosa*. Bacteria are capable of synthesizing polyamines through decarboxylation of ornithine by ornithine decarboxylase and through decarboxylation of arginine by arginine decarboxylase (88). The combined addition of alpha-monofluoromethylornithine (an irreversible inhibitor of arginine decarboxylase) to *E. coli* and *Ps. aeruginosa* cultures had been found to increase the mean doubling time of those organisms (88,89). Addition of 0.1 mM putrescine and 0.1 mM spermidine to the cultures of the above named organisms abolishes the effect of those inhibitors.

It is apparent that DFMO alone or in a combination with other drugs has strong potential for chemotherapy of many diseases. This new approach to treatment will give hope to those who suffer from *T. b. brucei*, leukemia, and many other diseases.

**Goal of the Project**

*N*-acetylspermidine has been shown to be synthesized in a chromatine catalyzed reaction (69,90). Analysis of human urine has indicated that this compound is present in the urine of cancer and noncancer individuals (67). Blankenship (77), in his investigation, has reported that *N*-acetylspermidine is present in spleen tissue at a concentration of only 1.0 ng/g. The absence of detectable level of *N*-acetylspermidine in the liver and kidney tissues does not rule out their presence in those tissues. The absence of detection might be due to the low concentrations relative to the sensitivity of the detection technique (77).
Study of the metabolism of N⁸-acetylspermidine has indicated that it is deacetylated in the cytosol fraction of the rat liver (76,78). Inhibition of the N⁸-acetylspermidine deacetylase enzyme will result in an increase in the level of N⁸-acetylspermidine. An inhibitor to this enzyme would provide a useful tool to find out the physiological functions of N⁸-acetylspermidine and deacetylation process.

The purpose of this project is to prepare competitive inhibitors for N⁸-acetylspermidine deacetylase. These compounds will be used to obtain information about the catalytic nature of the active site of the enzyme. Information gained from the activity of the above inhibitors will be used to design an irreversible inhibitor for the N⁸-acetylspermidine deacetylase. Finally these inhibitors will be used to inhibit N⁸-acetylspermidine deacetylase in Physarum polycephalum (slime mold) and in rats. These studies should give information on the function of N⁸-acetylspermidine in microorganisms and in mammalian cells.
MATERIALS AND METHODS

Chemicals. 1,6-Diaminohexane and 1,8-diaminoctane were purchased from the Sigma Chemical Company. Putrescine, butyryl chloride, acetic anhydride, triethylamine, methanesulfonyl chloride, cadmium chloride, 6-bromohexanoyl chloride, methylmagnesium bromide, 2-(tert-butoxy-carbonyloxyimino)-2-phenylacetonitrile (BOC-ON), and chloroacetic anhydride were purchased from the Aldrich Chemical Company.

All the solvents were ACS reagent grade and were used without further purification unless otherwise stated.

Instrumentation. Melting points were recorded in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 283 instrument. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-200 spectrometer, in a 5 mm probe using D_2O as a solvent and the water peak as a reference line.

Elemental analyses (C,H,N) were performed at the microanalytical laboratory of the National Institutes of Health (NIH), Bethesda, Maryland. Mass spectra were conducted on a Hitachi Perkin-Elmer RMS-4 mass spectrometer (CI,70 eV).

Glassware. All the glassware used was treated by the following procedure: Visually clean glassware was completely immersed at room temperature in a solution of the nonionic detergent (Alconox, INC) in tap-water for about 30 minutes. It was then washed and rinsed not less than five times in running tap water followed by two separate rinses.
with distilled water and one rinse with acetone. Following washing, rinsing and drying in air, all the glassware was dried in a hot air drying oven at 110°C (Radiant Heat oven, Lab Instruments, INC).

**Chromatography**

**Ion-Exchange Chromatography.** Amberlite (100 g) ion exchange resin CG-50 (wet mesh 100-200) was transferred into 100 mL flask. The resin was washed alternately with 350 mL of 3 N HCl and 3 N NH₄OH. The wash was repeated 3 times. The resin was then rinsed not less than two times with distilled water and its pH was brought to 9-9.5 with NH₄OH as measured on a pH meter (Corning Scientific Instruments). The washed resin was added to a column 65 x 2.5 cm and allowed to settle for about 2 hours. After packing the resin, it was washed with distilled water until the wash became clear. About 40 mL of the reaction mixture was transferred to the column, and the elution was carried out with 150 mL distilled water, followed by 250 mL of 0.5 N NH₄OH and 100 mL of 2 N NH₄OH. The elution flow rate was adjusted to 1-2 mL/minute. During the elution with 2 N NH₄OH, fractions of 10 mL were collected and analyzed by thin layer chromatography (TLC).

TLC plates were developed in a mixture of methanol, chloroform, and NH₄OH (10:10:1). Fractions which were found to contain the wanted compound were collected, combined and the solvent was removed by evaporation using a roto-evaporator (Brinkmann Instrument Company).

**Thin Layer Chromatography:**

TLC plates were developed in a mixture of methanol, chloroform and NH₄OH (10:10:1). All TLC plates were Merk E. Merk (60 F- 254), 5*10 cm, silica layer 0.25 mm thick. Visualization, unless otherwise mentioned,
was accomplished by ninhydrin.

Absorption Chromatography

Silica gel, (200 g, 230-400 mesh EM), was transferred into a 20 mL beaker. Approximately 600 mL of CH$_2$Cl$_2$ was added, and mixed well. The slurry of the silica gel was added to a column 65 x 3.5 cm and allowed to settle for about one hour. After packing the silica, 50 mL of the reaction mixture was transferred to the column and the elution was carried out with a mixture of methanol, CH$_2$Cl$_2$, and concentrated NH$_4$OH (7:5:1). Fractions (10 mL) were collected and those found to contain the desired compound were combined and the solvent was removed by evaporation using a rotary evaporator.

Crystallization

The oily residue was dissolved in 10-15 mL of distilled water and the pH was brought to 2 using 3 N HCl. This was evaporated on a rotary evaporator and the residue dried on vacuum pump (The Welch Scientific Company). The hydrochloride salt was dissolved in a hot 2-propanol. The solution was filtered, concentrated and allowed to crystallize at 40°C. The crystals were collected by filtration and stored in a desiccator.

Enzyme Assay

Male Wister rats, 140-180 g, were sacrificed by decapitation and a 100,000 x g supernatent fraction (cytosole) was prepared from 5 g of the rat liver as has been described by Blankenship (76). Tritium labeled N$^8$-acetyl spermidine ($^3$H-acetyl), (2.5-12.5 nM) was mixed with different concentrations of the potential inhibitor and with 100 uL of the cytosol
fraction in a final volume of 0.5 mL. The incubation mixture consisted of sucrose (0.125 mM), NaH₂PO₄ (29 mM), MgCl₂ (25 mM) at pH 7.0. The incubation was carried out for 10 minutes at 37°C in a shaking water bath (Dubnoff incubator). The reaction was stopped by addition of 0.5 mL of 1 N HCl containing 0.05 M acetic acid to the mixture. Ethyl acetate (3 mL) was added to each of the test tubes. The tubes were shaken for 10 minutes using an Eberbach shaker and then centrifuged for 5 minutes at 1500 x g using IEC Model 2 K centrifuge. The organic layer of the supernatents (2 mL) were transferred to counting vials to each of which was added 10 mL of scintillation cocktail which had been prepared by mixing 0.66 liter triton X-100, 1.34 liter toluene, 7.32 g of 2,5-di-phenyloxazole and 1.32 g of p-bis(2-(5-phenyloxazole))benzene. The radioactivity of the radiolabeled acetic acid released by deacetylase activity was measured with a liquid scintillation counter (Beckman CPM-100).

The control was run under the same conditions as the tested compounds and contained all the reaction components except the cytosol fraction. All the reactions were run in triplicate unless otherwise stated. The Ki of each inhibitor was determined by using a Dixon plot (90).

Synthesis

N-Butyryl-1,4-diaminobutane Hydrochloride. Putrescine (10 g, 0.113 mol) was dissolved in 50 mL of acetonitrile. Butyryl chloride (10.65 g, 0.099 mol) was mixed with 20 mL of acetonitrile and was added to the reaction flask in a dropwise manner. The reaction was stirred at room temperature and under nitrogen for about 3 hours. The organic
layer was evaporated and the residue was dissolved in 100 mL of distilled water. This solution was brought to pH 2 by using 3 N HCl and then extracted with CHCl₃ (3 x 50 mL). The water layer was added to an ion-exchange column and treated as has been described on p. 22-23. The yield was 4.0 g (25%), mp 139-141°C. IR (mineral oil) 1640 cm⁻¹ (C=O), broad (+NH₃) N-H stretch, 3300-2000 cm⁻¹ and asymmetric (+NH₃) N-H bend, 1610 cm⁻¹. NMR: ppm 0.85 (3H,t), 1.5 (6H,t), 2.1 (2H,t), 2.9 (2H,t), 3.1 (2H,t).

**Anal.** C₈H₁₉N₂OCl (M.W=194.70).

**Calculated.** C, 49.35; H, 9.83; N, 14.39.

**Found.** C, 49.60; H, 9.75; N, 14.67.

**Mass Spec.** CI, M + 1= 159.0 (for the base).

**N-Acetyl-1,8-diaminoctane Hydrochloride.** Five grams of 1,8-diaminoctane (0.035 mol) was dissolved in 40 mL of acetonitrile. Acetic anhydride (3.3 g, 0.035 mol) was mixed with 15 mL of acetonitrile and was added dropwise to the reaction flask. The reaction was stirred under a nitrogen atmosphere at room temperature for 3 hours. The organic layer was evaporated and the residue was dissolved in 150 mL of distilled water. The mixture was adjusted to pH 2 with 3 N HCl and then extracted with ether (2 x 30 mL). The aqueous layer was concentrated to about 50 mL and then transferred to an ion-exchange column. The compound was eluted with NH₄OH and crystallized as has been described on p. 23. The yield was 2.2 g (28%), mp 131-133°C, IR (mineral oil) 1640 cm⁻¹ (C=O), broad (+NH₃) NH stretch 3300-2000 cm⁻¹, and asymmetric (+NH₃) NH bend 1570 cm⁻¹. NMR: ppm 1.85 (3H,s), 2.85 (2H,t), 3.05 (2H,t).
Anal. \( \text{C}_2\text{H}_3\text{N}_2\text{OCl} \) (M.W = 222.76).

Calculated. C, 53.92; H, 10.40; N, 12.57.

Found. C, 53.82; H, 10.19; N, 12.23.

Mass Spec. CI, \( M + 1 = 187 \) (for the base).

**N-Acetyl-1,6-diaminohexane Hydrochloride.** Acetic anhydride (7 g, 0.07 mol) in 15 mL of benzene was added dropwise to 1,6-diaminohexane (8 g, 0.07 mol) in benzene (15 mL). The reaction was allowed to run under nitrogen atmosphere for 3 hours at room temperature and with continuous stirring. The solvent was removed in vacuo and the residue was dissolved in 200 mL of distilled water. This solution was brought to pH 2 by using 3 N HCl and extracted with CHCl\(_3\) (3 x 50 mL). The aqueous solution was concentrated and applied to an ion-exchange column. The compound was eluted with NH\(_4\)OH and crystallized as has been described on p. 22-23. The yield was 3.0 g (22%), mp 130-131°C. IR (mineral oil) 1650 \( \text{cm}^{-1} \) (C=O), broad (+NH\(_3\)) NH stretch 3300-2000 \( \text{cm}^{-1} \), and asymmetric (+NH\(_3\)) NH bend 1570 \( \text{cm}^{-1} \). NMR. ppm 1.85 (3H,s), 2.85 (2H,t), 3.05 (2H,t).

**Anal.** \( \text{C}_8\text{H}_{19}\text{N}_2\text{OCl} \) (M.W = 158.25)

**Calculated.** C, 49.30; H, 9.83; N, 14.38

**Found.** C, 49.11; H, 9.88; N, 14.17

**Mass Spec.** CI, \( M + 1 = 194.70 \) (for the base).

**N-Acetyl-1,10-diaminodecane Hydrochloride.** This compound was synthesized and isolated by the same procedure described on p. 25. In this synthesis, acetic anhydride (3.0 g; 0.035 mol) in 15 mL of benzene was added dropwise to the 1,10-diaminodecane (6 g; 0.035 mol) in 200 mL of benzene. The yield was 4.0 g (46%), mp 101-103°C. IR (mineral oil)
1650 cm\(^{-1}\) (C=O), broad (+NH\(_3\)) N-H stretch 3370-2000 cm\(^{-1}\), asymmetric (+NH\(_3\)) N-H bend 1540 cm\(^{-1}\). NMR. ppm 1.85 (3H,s), 2.85 (2H,t), 3.5 (2H,t).

**Anal.** C\(_{12}\)H\(_{27}\)N\(_2\)OCl (M.W. = 250.81).

**Calculated.** C, 57.46; H, 10.85; N, 11.16.

**Found.** C, 57.30; H, 10.69; N, 11.12.

**Mass Spec.** CI, M + 1 = 215 (for the base).

**N-Methanesulfonyl-1,8-diaminoctane Hydrochloride.** A stirred solution of 1,8-diaminoctane (3.0 g, 0.02 mol) in benzene (50 ml) was treated dropwise with methanesulfonyl chloride (2.2 g, 0.02 mol). The reaction was allowed to run for about 2 hours with continuous stirring at room temperature. The solvent was removed in vacuo and the residue was dissolved in 200 mL distilled water. To this was added 3 N HCl to pH=2 and the solution was filtered. The aqueous solution was concentrated, and applied to an ion-exchange column. The compound was eluted as usual with 2 N NH\(_4\)OH and crystallized as has been described on p. 22-23. The yield was 2.41 g (47%), mp 132-134° C. IR (mineral oil) asymmetric (S=O) stretch, 1380 cm\(^{-1}\), symmetric (S=O) stretch, 1155 cm\(^{-1}\), broad (+NH\(_3\)) N-H stretch, 3300-2000 cm\(^{-1}\), and asymmetric (+NH\(_3\)) N-H bend, 1600 cm\(^{-1}\). NMR: ppm 2.9 (2H,t), 2.95 (3H,s), 3.0 (2H,t).

**Anal.** C\(_9\)H\(_{23}\)N\(_2\)O\(_2\)SCl (M.W. = 258.81).

**Calculated.** C, 41.76; H, 8.95; N, 10.82.

**Found.** C, 41.85; H, 8.86, N, 10.72.

**Mass Spec.** CI, M + 1 = 223 (for the base).

**N-Methanesulfonyl-1,6-diaminoctane Hydrochloride.** This compound was prepared and isolated by the method described on p. 27. To a solution
of 1,6-diaminohexane (5.0 g, 0.04 mol) in benzene was added dropwise a
solution of methanesulfonyl chloride (3.0 g, 0.02 mol) in 15 mL of
benzene. The yield was 3.0 g (65.0%), mp 114-116°C. IR (mineral oil)
asymmetrical (S=O) stretch, 1380 cm⁻¹, symmetric (S=O) stretch, 1155
cm⁻¹, (+NH₃) N-H stretch, 3300-2000 cm⁻¹, and asymmetric (+NH₃) N-H
bend, 1600 cm⁻¹. NMR: ppm 2.9 (2H,t), 2.95 (3H,s), 3.0 (2H,t).

Anal. \( \text{C}_7\text{H}_{19}\text{N}_2\text{O}_2\text{SCl} \) (M.W. = 230.76).
Calculated. C, 36.43; H, 8.29; N, 12.13.
Found. C, 36.50; H, 8.31; N, 12.11.
Mass Spec. CI, M + 1 = 195 (for the base).

Methyl N-(8-Aminooctyl)carbamate Hydrochloride. A solution of
methyl chloroformate (1.9 g, 0.01 mol) in 15 mL of benzene was added
dropwise to a flask containing 1,8-diaminooctane (3.0 g, 0.01 mol) in
150 mL of benzene. The reaction was stirred at room temperature and
under nitrogen for 3 hours. The solvent was removed in vacuo and the
residue was dissolved in 150 mL of distilled water. The solution was
adjusted to pH 2 with 3 N HCl, filtered and extracted with CHCl₃. The
aqueous solution was concentrated to about 40 mL and then transferred to
an ion-exchange column. The compound was eluted with ammonium hydroxide
and crystallized as has been described on p. 22-23. The yield was 2.5 g
(52%), mp 131-133°C. IR (mineral oil) 1790 cm⁻¹, (C=O) broad (+NH₃)
N-H stretch, 3380-1000 cm⁻¹ asymmetric (+NH₃) N-H bend, 1530 cm⁻¹. NMR:
ppm 2.85 (2H,t), 2.95 (2H,t), 3.5 (3H,s).

Anal. \( \text{C}_{10}\text{H}_{23}\text{N}_2\text{O}_2\text{Cl} \) (M.W. = 238.76).
Calculated. C, 50.30; H, 9.70; N, 11.73.
Found. C, 50.47; H, 9.66; N, 11.84.
Mass Spec. CI, M + 1 = 203 (for the base).
Methyl N-(6-Aminohexyl)carbamate Hydrochloride. This compound was obtained from 1,6-diaminohexane (5 g, 0.025 mol) and methyl chloroformate (2.36 g, 0.025 mol) by the same procedure described on p. 28. The yield was 2.5 g (48%), mp 140-141° C. IR (mineral oil) 1700 cm\(^{-1}\) (C=O), broad (+NH\(_3\)) stretch, 3370-2000 cm\(^{-1}\) and asymmetric (+NH\(_3\)) N-H bend, 1535 cm\(^{-1}\). NMR: ppm 2.85 (2H, t), 3 (2H, t), 3.5 (3H, s).

Anal. C\(_8\)H\(_{19}\)N\(_2\)O\(_2\)Cl (M.W. = 210.70).

Calculated. C, 45.56; H, 9.08; N, 13.29.

Found. C, 45.54; H, 9.13; N, 13.29.

Mass Spec. Cl, M + 2 = 175 (for the base).

N-Chloroacetyl-1,6-diaminohexane Hydrochloride. To 1,6-diamino-
hexane (4 g, 0.034 mol) in 100 mL of benzene was added a solution of BOC-ON (4 g, 0.016 mol) in 30 mL of benzene in a dropwise manner. The reaction was stirred under nitrogen atmosphere at room temperature for 3 hours. The solvent was evaporated in vacuo and the residue was dissolved in CH\(_2\)Cl\(_2\) and chromatographed over silica gel with a mixture CH\(_3\)OH, CH\(_2\)Cl\(_2\) and conc. NH\(_4\)OH (7:5:1). The eluent which appeared to contain only the product was collected and concentrated to dryness in vacuo. The residual oil (1.5 g) was dissolved in CH\(_2\)Cl\(_2\) (150 mL) and was mixed with triethylamine (7 g, 0.07 mol). To the above mixture a solution of 0.5 g (0.004 mol) of chloroacetyl chloride in 30 mL of CH\(_2\)Cl\(_2\) was dripped into the stirred reaction mixture. Stirring was continued for 3 hours at room temperature and then the reaction mixture concentrated to dryness in vacuo. The product was crystallized from a mixture of benzene and hexane (5:3). The crystals (1.3 g) were collected and dissolved in 3 N HCl-EtOAc (5 mL). After 2 hours the solution was filtered and the precipitate was dissolved in hot 2-propanol. The
mixture was concentrated to mL, and was placed in a refrigerator. The crystals were collected by filtration. The yield was 0.70 g (20%), mp 80° C. IR (mineral oil) 1650 cm⁻¹ (C=O), broad (+NH₃) N-H stretch, 3300-2000 cm⁻¹, asymmetric (+NH₃) bend, 1550 cm⁻² and C-Cl, 780 cm⁻¹.

NMR: ppm 2.85 (2H, t), 3.15 (2H, t), 4.0 (2H, s).

Anal. C₈H₁₈N₂OCl₂ (M.W. = 229.15).
Calculated. C, 41.94; H, 7.91; N, 12.22.
Found. C, 41.98; H, 7.95; N, 12.20.
Mass Spec. CI, M + 1 = 193 (for the base).

N-(3-aminopropyl)-7-aminooctane-2-one Hydrochloride. Methylmagnesium bromide (40 g, 2.80 mol) and 500 mL of anhydrous ether were mixed in a 1000 mL round bottom flask cooled in an ice-bath and stirred slowly under a nitrogen atmosphere while 55 g (0.31 mol) of anhydrous cadmium chloride was added in portions during 10 minutes. After stirring for 30 minutes, the ice-bath was removed and stirring continued an additional 4 hours. To the brown solution of the organocadmium compound was added, in about one hour, a solution of 55 g (0.31 mol) of 6-bromo-hexanoic chloride in 150 mL of benzene. Slow stirring was continued at room temperature for about 15 hours. The resulting grayish-brown solution was stirred for an additional hour with refluxing (during this period the solution becomes very viscous). Ice and 100 mL of 10% HCl were added and the solution was stirred for 2 hours. The organic layer was collected, dried over anhydrous sodium sulfate and distilled. The residue remaining after removing the organic solvents was distilled at 90-95° C (0.1-0.05 mm Hg). The yield of 7-bromoheptane-2-one was 35 g.

To 1,3-diaminopropylene (18.4 g, 0.25 mol) in 150 mL of tetrahydrofuran (THF), 7-bromoheptane-2-one (12 g, 0.062 mol) in 50 mL of THF
was added in a dropwise manner. The reaction was allowed to run for 10 hours with continuous stirring at room temperature. The solvent was removed in vacuo and the residue was dissolved in 120 mL of distilled water and applied to an ion-exchange column. The product was eluted with 2 N NH₄OH and crystallized as the hydrochloride salt salt from ethanol. The yield was 8.4 (52%), mp 210-215°C.

IR: broad (+NH₃) N-H stretch 3100-2000 cm⁻¹, asymmetric (+NH₃) N-H bend 1610 cm⁻¹ and 1720 cm⁻¹ (C=O). NMR: ppm 1.25 (m,2H), 1.4-1.7 (m,4H), 2.1 (s,3H), 2.5 (t,2H), 2.9-3.1 (m,6H).

Anal. C₁₀H₂₄N₂OCl₂ (M.W. = 259.22).
Calculated. C, 46.29; H, 9.33; N, 10.80.
Found. C, 46.37; H, 9.35; N, 10.78.
Mass Spec. CI, M + 1 = 187 (for the base).
DISCUSSION

Chemistry

All the 10 compounds were synthesized according to the outline in schemes 1, 2, 3 and 4. N-Butyrylputrescine was synthesized by modification of Taber synthesis of N-acetylputrescine (91). Butyryl chloride was added to the putrescine in a dropwise manner. This procedure gave two products, N-butyrylputrescine and N,N'-dibutyrylputrescine. Purification of N-butyrylputrescine from the impurities by crystallization from 2-propanol was unsuccessful. Ion-exchange chromatography was used to separate N-butyrylputrescine from the unreacted putrescine and from N,N'-dibutyrylputrescine. Putrescine binds much more strongly to the resin than the other two products. Binding of N-butyrylputrescine to the resin is much stronger than the binding of the N,N'-dibutyrylputrescine. The column was washed with water to remove all the salts and with 0.5 N NH₄OH to elute N,N'-dibutyrylputrescine. Ammonium hydroxide (2 N) was used to elute N-butyrylputrescine.

The IR absorptions for N-butyrylputrescine (Figure 6) are consistent with the expected structure showing most notably the C=O stretch at 1640 cm⁻¹ and broad (+NH₃) N-H stretch at 3300-2000 cm⁻¹. The NMR of this compound (Figure 7) exhibited all the expected absorptions. Protons A appeared as triplet at 0.9 ppm. Protons B appeared as a multiplet at 1.5 ppm. C, D and E protons appeared as triplets at 2.9, 3.1, and 2.1 ppm respectively. C and D protons was deshielded by the combination effect of both N and O. However, the downfield shift of E protons is mainly due to the effect of the N. In the NMR spectra of all
Scheme 1

**Compound 1**

\[
\begin{align*}
\text{H}_2 \text{N}-(\text{CH}_2)_4 \text{NH}_2 & \rightarrow \text{H}_2 \text{N}(\text{CH}_2)_4 \text{N}-\text{C}-\text{CH}_2-\text{CH}_2-\text{CH}_3 \\
\end{align*}
\]

**Compound 2**

\[
\begin{align*}
\text{H}_2 \text{N}-(\text{CH}_2)_8 \text{NH}_2 & \rightarrow \text{H}_2 \text{N}-(\text{CH}_2)_8 \text{NH}-\text{C}-\text{CH}_3 \\
\end{align*}
\]

**Compound 3**

\[
\begin{align*}
\text{H}_2 \text{N}-(\text{CH}_2)_6 \text{NH}_2 & \rightarrow \text{H}_2 \text{N}-(\text{CH}_2)_6 \text{NH}-\text{C}-\text{CH}_3 \\
\end{align*}
\]

**Compound 4**

\[
\begin{align*}
\text{H}_2 \text{N}-(\text{CH}_2)_{10} \text{NH}_2 & \rightarrow \text{H}_2 \text{N}-(\text{CH}_2)_{10} \text{NH}-\text{C}-\text{CH}_3 \\
\end{align*}
\]
Scheme 2

H₂N-(CH₂)₆-NH₂ + CH₃-S-Cl → H₂N-(CH₂)₆-NH-S-CH₃

Compound 5

H₂N-(CH₂)₆-NH₂ + CH₃-S-Cl → H₂N-(CH₂)₆-NH-S-CH₃

Compound 6

H₂N-(CH₂)₆-NH₂ + CH₃-O-C-Cl → H₂N-(CH₂)₆-NH-C-O-CH₃

Compound 7

H₂N-(CH₂)₆-NH₂ + CH₃-O-C-Cl → H₂N-(CH₂)₆-NH-C-O-CH₃

Compound 8
\[ \text{Scheme 3} \]

\[ \begin{align*}
\text{H}_2 \text{N} & - (\text{CH}_2)_6 - \text{NH}_2 \\
\downarrow & \\
(\text{CH}_3)_3 \text{COCONH} & - (\text{CH}_2)_6 - \text{NH}_2 \\
\downarrow & \\
(\text{CH}_3)_3 \text{COCONH} & - (\text{CH}_2)_6 - \text{NH}_2 - \text{C} - \text{CH}_2 - \text{Cl} \\
\downarrow & \\
\text{H}_2 \text{N} & - (\text{CH}_2)_6 - \text{NH} - \text{C} - \text{CH}_2 - \text{Cl}
\end{align*} \]
\[
\begin{align*}
\text{CH}_3\text{MgBr} + \text{CdCl}_2 \\
\downarrow \\
\text{Cd}(\text{CH}_3)_2 \\
\downarrow \\
\text{Br-}(\text{CH}_2)_5-\text{C-Cl} \\
\downarrow \\
\text{Br-}(\text{CH}_2)_5-\text{C-CH}_3 \\
\downarrow \\
\text{H}_2\text{N-}(\text{CH}_2)_3-\text{NH}_2 \\
\downarrow \\
\text{H}_2\text{N-}(\text{CH}_2)_3-\text{N-}(\text{CH}_2)_5-\text{C-CH}_3 \\
\end{align*}
\]

Compound 10

Scheme 4
Figure 6. Infrared Spectrum of N-butyrylputrescine hydrochloride (in mineral oil).
Figure 7. Nuclear magnetic resonance spectrum of N-butyrylputrescine hydrochloride (in D$_2$O), 200 MHz.
the compounds the solvent peak appeared at 4.75 ppm. Duhr et al. (92) has found the HDO resonance peak at 4.8 ppm. The integration was in agreement with the above assignments. The mass spectrum (Figure 8) is also consistent with the assigned structure showing the M + 1 peak at 159 (M.W. = 158.25).

Compound 2 was synthesized to determine the importance of the nitrogen in the 4-position for the binding of N-acetylsperrmidine to the N-acetylsperrmidine deacetylase. The N was replaced with the bioisosteric -CH- group. If this replacement does not affect the affinity of 2 for the enzyme, inhibitors with simpler structures could be designed and synthesized.

Compounds 5 and 7 were designed to investigate the size of the active site of the enzyme. The acetyl group on the N8-position was replaced with methanesulfonyl and with carbamyl groups. If the size of the active site of N8-acetylsperrmidine deacetylase is not critical and can accommodate these bioisosteric groups, these compounds should serve as inhibitors of the enzyme.

Compounds 2, 5 and 7 were synthesized according to the schemes 1 and 2. A solution of 1,8-diaminoctane in acetonitrile or in benzene was treated with an appropriate reagent. The reactions were run at room temperature and with continuous stirring. As in the synthesis of compound 1, this procedure gave two products in each reaction. The wanted compounds were purified by an ion exchange chromatography. The impurities were eluted with H2O and with 0.5 N NH4OH. The wanted compound was eluted with 2 N NH4OH. The yield of both compounds 5 and 7 (47, 52%) was higher than that of compound 2 (28.21%). Crystallization of compounds 5 and 7 was easier than compound 2.
Figure 8. Chemical ionization (NH$_3$) mass spectrum of N-butyrylputrescine hydrochloride.
The IR absorption of compound 2 (Figure 9) showed strong absorption at 1640 cm\(^{-1}\) \((\text{C=O})\) and broad \((+\text{NH}_3)\) N-H stretch at 3300-2000 cm, asymmetric \((+\text{NH}_3)\) NH bend at 1610 cm\(^{-1}\). The spectrum showed a weak absorption at about 720 cm\(^{-1}\). This absorption is caused by a bending vibration of the \((-\text{CH}_2-)\) groups. The NMR of compound 2 is shown in Figure 10. The protons of the acetyl group appear as a singlet at 1.85 ppm. Protons B and D appear as triplets at 3.1 and 2.9 ppm. The C protons appear as a multiplet between 1 and 2.1 ppm. The mass spectrum (Figure 11) of this compound is also consistent with the assigned structure, showing a molecular ion peak at \(M + 1 = 187\) (M.W. = 186.31).

The IR for compound 5 (Figure 12) showed a strong asymmetrical stretching absorption at 1380 cm\(^{-1}\) \((\text{S=O})\) symmetric (SO) stretch at 1155 cm, a broad absorption \((+\text{NH}_3)\) N-H stretch at 3300-2000 cm\(^{-1}\) and an asymmetric \((+\text{NH}_3)\) N-H bend at 1610 cm. A weak absorption appears at about 720 cm\(^{-1}\), and is characteristic for a \((\text{CH}_2)\) group where \(n > 4\). The NMR spectrum of compound 5 (Figure 13) showed the characteristic peaks expected of the compound. The A protons appear as a singlet at 2.95 ppm. The downfield shift of these protons is due to the effect of N,0 and S (N-SO). B and D protons appear as triplets at 3 and 2.9 ppm respectively. C protons appeared as a multiplet between 1.2 and 2.1 ppm. The mass spectrum of this compound (Figure 14) showed the \(M + 1\) peak at 223 (M.W. = 222.35).

The IR for compound 7 (Figure 15) showed clearly the presence of the carbonyl group at 1690 cm. Major absorptions are observed for \((+\text{NH}_3)\) N-H stretch at 3300-2000 cm\(^{-1}\), and asymmetric \((+\text{NH}_3)\) N-H stretch at 1610 cm\(^{-1}\). The spectrum also showed a weak absorption at about 722 cm\(^{-1}\). This absorption is caused by bending vibrations of the \((\text{CH}_2)\)
Figure 9. Infrared spectrum of N-acetyl-1,8-diaminoctane hydrochloride (in mineral oil).
Figure 10. Nuclear magnetic resonance spectrum of N-acetyl-1,8-diaminoctane hydrochloride (in D$_2$O), 200 MHz.
Figure 11. Chemical ionization (NH$_3$) mass spectrum of N-acetyl-1,8-diaminoocctane hydrochloride.
Figure 12. Infrared spectrum of N-methanesulfonyl-1,8-diaminoctane hydrochloride (in mineral oil).
Figure 13. Nuclear magnetic resonance spectrum of N-methanesulfonyl-1,8-diaminooctane hydrochloride (in D₂O), 200 MHZ.
Figure 14. Chemical ionization (NH$_3$) mass spectrum of N-methanesulfonyl-1,8-diaminoctane hydrochloride.
Figure 15. Infrared spectrum of methyl N-(8-aminooctyl)carbamate hydrochloride (in mineral oil).
Figure 16. Nuclear magnetic resonance spectrum of methyl N-(8-aminooctyl)carbamate hydrochloride (in D$_2$O), 200 MHz.
Figure 17. Chemical ionization (NH$_3$) mass spectrum of methyl N-(8-aminoctyl) carbamate hydrochloride.
group. The strong absorption of \( \text{C-O} \) stretching appeared at about 1115 cm\(^{-1}\).

The NMR of compound 7 (Figure 16) showed the characteristic peaks of the compound. Protons A appear as singlet at 3.5 ppm. The downfield shift of these protons is due to the deshielding effect of the O. B and D protons appear as triplet at 2.95 and 2.85 ppm respectively. C protons appear as multiplet between 1.2 and 2.2 ppm. The mass spectrum of this compound (Figure 17) is also consistent with the assigned structure showing a molecular ion peak at \( M + 1 = 203 \) (M.W. = 202.31).

As part of this study compounds 3 and 4 were designed to explore further the structure-activity relationships associated with changing the length of the \(-\text{CH}_2-\) group between the two nitrogens. The results of this investigation will give an idea about the distance between the cationic binding sites of the enzyme. Furthermore, the results of this study, along with those of compounds 2, 5, and 7, could be used to design inhibitors with an optimum \(-\text{CH}_2-\) length and with an optimum substituent group on the nitrogen.

Compounds 3 and 4 were synthesized according to scheme 1. A solution of acetic anhydride in benzene was added in a dropwise manner to stirred solutions of 1,6-diaminohexane or 1,10-diaminododecane (in benzene). Separation of both products from impurities was achieved by ion-exchange chromatography.

The yield of compound 4 (46%) was double that of compound 3 (22%). The IR (Figures 18, 19) and NMR (Figures 20, 21) spectra of compounds 3 and 4 were similar to those of compound 2 and all major peaks were accounted for as previously mentioned. Integration of the NMR spectra
Figure 18. Infrared spectrum of N-acetyl-1,6-diaminohexane hydrochloride (in mineral oil)
Figure 19. Infrared spectrum of N-acetyl-1,10-diaminodecane hydrochloride (in mineral oil).
Figure 20. Nuclear magnetic resonance spectrum of N-acetyl-1,6-diaminohexane hydrochloride (in D$_2$O), 200 MHz.
Figure 21. Nuclear magnetic resonance spectrum of N-acetyl-1,10-diaminodecane hydrochloride (in D$_2$O), 200 MHz.
of compounds 2, 3 and 4 was run and was in agreement with the assigned structures. The masses of compound 3 and 4 (Figures 22, 23) are also consistent with the assigned structures showing molecular ion peaks at $M + 1 = 159$ (M.W. = 158.21) and 215 (M.W. = 214.31) respectively.

Compounds 6 and 8 were synthesized and purified by the same procedure used for synthesis and purification of compounds 5 and 7 except 1,6-diaminohexane was used instead of 1,8-diaminooctane. The IR spectra of compounds 6 and 8 (Figures 24, 25) exhibited all the peaks found in the IR spectra of compounds 5 and 7 respectively. The NMR spectra (Figures 26, 27) of compounds 6 and 8 appear very similar to those of compounds 5 and 7 respectively. The mass spectra of compounds 6 and 8 (Figures 28, 29) also were consistent with the assigned structures and showed molecular ion peaks at $M + 1 = 195$ (M.W. = 194.30) and 175 (M.W. = 174.24) respectively. In addition to the peak at 195, compound 8 shows another peak at 144 (M - O-CH$_3$).

The size of the active site was investigated further by replacement of the acetyl group with a chloroacetyl group. If the active site of the enzyme is large enough and can accommodate this group, this compound might inhibit the enzyme irreversibly. Irreversible inhibition would be dependent upon the presence of a nucleophile at a suitable position on the enzyme and the reactivity of the leaving group. Although Cl is less reactive than Br and I, it was chosen because its size is smaller than the other groups.

Compound 9 was synthesized according to scheme 3. Direct chloroacetylation was tried for the synthesis of compound 9 but was unsuccessful. The reason for this is that the chloro group which is alpha to the carbonyl group is very reactive. The result of this is
Figure 22. Chemical ionization (NH₃) mass spectrum of N-acetyl-1,6-diaminohexane hydrochloride.
Figure 23. Chemical ionization (NH$_3$) mass spectrum of N-acetyl-1,10-diaminodecane hydrochloride.
Figure 24. Infrared spectrum of N-methanesulfonyl-1,6-diaminohexane hydrochloride (in mineral oil).
Figure 25. Infrared spectrum of methyl N-(6-aminohexyl)carbamate hydrochloride (in mineral oil).
Figure 26. Nuclear magnetic resonance spectrum of N-methnesulfonyl-1,6-diaminohexane hydrochloride (in D₂O), 200 MHz.
Figure 27. Nuclear magnetic resonance spectrum of methyl N-(6-aminohexyl)carbamate hydrochloride (in D$_2$O), 200 MHz.
Figure 28. Chemical ionization ($\text{NH}_3$) mass spectrum of N-methanesulfonyl-1,6-diaminohexane hydrochloride.
Figure 29. Chemical ionization (NH₃) mass spectrum of methyl N-(6-aminohexyl) carbamate hydrochloride.
that the product will react with the amino group of another or the same molecule.

The key to the successful synthesis of N-chloroacetyl-1,6-diaminohexane is the protection of one of the amino group of 1,6-diaminohexane by some readily removable group. It is desired that the protecting group also favorably alters the solubility properties of the product so that its separation from the unsubstituted and disubstituted by-product is readily accomplished. The Boc group [2(tert-butoxycarbonyloxyimino)-2-phenylacetonitril] was chosen because it is removable and renders N-Boc-1,6-diaminohexane separable from the unreacted diamine and from bis(Boc)-1,6-diaminohexane. Introduction of Boc group is accomplished by the addition of a solution of BOC-ON in benzene to a solution of 1,6-diaminohexane in benzene. N-Boc-1,6-diaminohexane was purified by using absorption chromatography. N-Boc-1,6-diaminohexane is then treated with chloroacetyl chloride in the presence of triethylamine. N-Boc-N-chloroacetyl-1,6-diaminohexane was crystallized from a mixture of benzene and n-hexane (5:3). The Boc group was then removed with 3 M HCl in ethyl acetate to give N-chloroacetyl-1,6-diaminohexane hydrochloride. Finally the product was crystallized from 2-propanol.

The IR (Figure 30) of compound 9 showed the characteristic absorption of the carbonyl group at 1650 cm\(^{-1}\). Broad absorptions are observed for (\(+\text{NH}_3\)) N-H stretch at 3300-2000 cm\(^{-1}\) and asymmetric (\(+\text{NH}_3\)) N-H bend at 1610 cm\(^{-1}\). The spectrum also showed a weak absorption at about 725 cm\(^{-1}\), which is caused by bending vibrations of (CH\(_2\)) groups. The absorption at 780 cm\(^{-1}\) is due to C-Cl stretching vibrations. The NMR of compound 9 (Figure 31) showed a singlet at 4 ppm for CH\(_2\)-Cl. The A protons are deshielded by the Cl and by the N and O. B and D protons
Figure 30. Infrared spectrum of N-chloroacetyl-1,6-diaminohexane hydrochloride (in mineral oil).
Figure 31. Nuclear magnetic resonance spectrum of N-chloroacetyl-1,6-diaminoheXane hydrochloride (in D₂O), 200 MHz.
showed as triplets at 3.15 and 2.85 ppm respectively. The C protons appear as a multiplet between 1.2 and 1.6 ppm. The mass spectrum of this compound (Figure 32) is also consistent with the assigned structure, showing the M + 1 peak at 193 (M.W. = 192.69) and a peak at 158 (M = Cl).

Compound 10 was designed in order to investigate the importance of the nitrogen in the 8-position for spermidine binding. Bioisosteric replacement of the N with -CH₂- group makes the compound stable toward hydrolysis by N-acetylspemidine deacetylase. In addition it is reasonable to expect substitution of -CH₂- for -NH- to cause no/or small change in the affinity of 10 for the enzyme. For these reasons, compound 10 was expected to be a potent competitive inhibitor for the enzyme. This compound, along with the others, will give a good picture of the shape and the binding characteristics of the enzyme active site.

Compound 10 was synthesized by using the modified methods of Cason, Cole and others (93-96) according to scheme 4. The use of the proper solvent in the reaction of the acid chloride with the organocadmium compounds is very important. Several advantages are found in the use of the benzene as solvent compared to ether. First, the precipitate which forms in the reaction mixture after addition of the acid chloride is less likely to become too thick for efficient stirring if benzene is used. Second, the use of the benzene allows a higher reflux temperature and accordingly the reaction requires a shorter time in benzene. The reaction was run under nitrogen atmosphere in order to avoid degradation of Grignard reagent. The ether should be absolute and as a precaution it should be dried on molecular sieves before its use.
Figure 32. Chemical ionization (NH₃) mass spectrum of N-chloroacetyl-1,6-diaminoxhexane hydrochloride.
The IR absorption of 10 (Figure 33) showed strong absorption at 1720 cm\(^{-1}\) (C=O), a broad \((^{+}\text{NH}_3\text{)}\) N-H stretch at 3100-2000 cm\(^{-1}\), and an asymmetric \((^{+}\text{NH}_3\text{)}\) N-H bend at 1610 cm\(^{-1}\). The spectrum showed a weak absorption at 720 cm\(^{-1}\) which is caused by bending vibrations of the -CH\(_2\)- groups.

The NMR spectrum of 10 is shown in Figure 34. Protons of the methyl group (A protons) appear as a singlet at 2.1 ppm and B protons appear as a triplet at 2 ppm. C protons appear as a multiplet centered at 3 ppm and their integration corresponds to 6 protons. D, E, and F protons appeared as multiplet at 2, 1.3, and 1.9-3.1 ppm respectively. The mass spectrum (Figure 35) of this compound is also consistent with the assigned structure, showing a molecular ion peak at M + 1 = 187 (M. W. = 186.2) and at 169 (M = HO + 1).

**Pharmacology**

Inhibitory activity determined for all the compounds was performed by V. Manneh and P. Marchant in the Department of Physiology and Pharmacology at the University of the Pacific.

Our objective in this study was to design and synthesize potent inhibitors to N\(^8\)-acetyllyspermidine deacetylase. Inhibition of this enzyme in vitro should increase the level of N\(^8\)-acetyllyspermidine and may be useful to clarify the physiological functions of N\(^8\)-acetyllyspermidine and the deacetylation process. In order to do this we have studied N\(^8\)-acetyllyspermidine deacetylase, its substrate specificity and the nature of its active site.

A most important requirement for the work was to determine the Ki for the active site probes and the newly synthesized inhibitors. In these studies, the Ki values for all the inhibitors was calculated from
Figure 33. Infrared spectrum of N-(3-aminopropyl)-7-aminoheptane-2-one hydrochloride (in mineral oil).
Figure 34. Nuclear magnetic resonance spectrum of N-(3-aminopropyl)-7-aminooctane-2-one hydrochloride (in D₂O), 200 MHZ.
Figure 35. Chemical ionization (NH$_3$) mass spectrum of N-(3-aminopropyl)-7-aminohexane-2-one hydrochloride.
Dixon plots. In this graph inhibitor concentrations are plotted versus the reciprocal of the initial velocity at a constant substrate concentration. A vertical line from the intersection point of the substrate concentrations on the X axis equals the $K_i$ value. By using this plot, the nature of the inhibitors (competitive or noncompetitive) can be determined, and the $K_i$ values can be directly determined. The in vitro $K_i$ values for all the compounds are given in Table 1.

Manneh et al. have reported that the $K_i$ for acetylputrescine is 0.80 mM (80). Replacement of the acetyl group in this compound with the butyryl group caused a dramatic decrease in the inhibitory activity of the compound ($K_i = 29.2$ mM) (Figure 36). The change in the $K_i$ value corresponds to an about 36-fold decrease in the inhibitory activity compared to acetylputrescine. To investigate the importance of the nitrogen at the 4-position for the binding of $N^8$-acetylsperrmidine to the enzyme, the nitrogen was replaced by isosteric (-CH$_2$-) group and 1,8-diaminoctane was synthesized. On the basis of the results obtained from the Dixon plot (Figure 37), this compound was found to be a competitive inhibitor with a $K_i$ value of 0.076 mM. Although the $K_i$ value of this compound is still higher than the $K_m$ value for the substrate ($K_m = 0.01$ mM), N-acetyl-1,8-diaminoctane is 10.5 times more potent than N-acetylputrescine.

For further exploration of the active site of the enzyme, methyl N-(8-aminoctyl)carbamate and N-methylsulfonyl-1,8-diaminoctane were synthesized and their $K_i$ values were determined. These compounds were found to be less active than the N-acetyl-1,8-diaminoctane. To our surprise methyl N-(8-aminoctyl)carbamate ($K_i = 0.26$ mM) is 3.5 times less active than the N-acetyl-1,8-diaminoctane (expected to act as
Table 1. Inhibition of N<sup>8</sup>-acetylsperrmidinedeacetylase by structural analogs of N-acetylpolyamines.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Ki (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N&lt;sup&gt;8&lt;/sup&gt;-Acetylspermidine</td>
<td>0.01 (Km)</td>
</tr>
<tr>
<td>N-Butyrylputrescine</td>
<td>29.2</td>
</tr>
<tr>
<td>N-Acetyl-1,6-diaminohexane</td>
<td>0.031</td>
</tr>
<tr>
<td>N-Acetyl-1,8-diaminoctane</td>
<td>0.075</td>
</tr>
<tr>
<td>N-Acetyl-1,10-diaminododecane</td>
<td>0.11</td>
</tr>
<tr>
<td>Methyl N-(8-aminooctyl) carbamate</td>
<td>0.26</td>
</tr>
<tr>
<td>N-Methyisulfonyl-1,6-diaminohexane</td>
<td>0.37</td>
</tr>
<tr>
<td>N-Methyisulfonyl-1,8-diaminoctane</td>
<td>0.65</td>
</tr>
<tr>
<td>Methyl N-(6-aminohexyl) carbamate</td>
<td>None</td>
</tr>
<tr>
<td>N-Chloroacetyl-1,6-diaminohexane</td>
<td>0.13</td>
</tr>
<tr>
<td>N-(3-aminopropl)-7-aminoheptane-2-one</td>
<td>0.00018</td>
</tr>
</tbody>
</table>
Figure 36. Dixon plots of the reciprocal velocity of N^8-acetylspermidine deacetylase versus N-butrylputrescine hydrochloride. Effects are shown in the presence of 5 μM (■), 10 μM (△), and 20 μM (○) of substrate concentrations.
Figure 37. Dixon plots of reciprocal velocity of N\textsuperscript{8}-acetylspermidine deacetylase versus N-acetyl-1,8-diaminoctane hydrochloride. Effects are shown in the presence of 5 μM (■), 10 μM (△), and 20 μM (○) of substrate concentrations.
pseudo-irreversible inhibitor). As can be seen from Figure 38, this compound is a weak competitive inhibitor for the enzyme.

Steric hindrance caused by bulky methylsulfonyl and methycarbamate groups might be the reason for the decrease in the activity of these compounds. The bulkier the substituents are, the larger is the decrease of the affinity of the corresponding compound. These results are in agreement with that obtained from testing of N-butyrylputrescine. These results suggest that the size of the active site of the enzyme is critical and is too small to accommodate groups larger than the acetyl group.

As part of the study, N-acetyl-1,6-diaminohexane and N-acetyl-1,10 diaminodecane were synthesized to explore the structure-activity relationship with changing the length of the -CH- group, separating the two basic nitrogens. Our results revealed that separation of the terminal amines with 10 carbons caused a decrease in the inhibitory activity of the compound ($K_i = 0.11 \text{ mM}$). On the other hand, separation of the terminal amines with 6 carbons, resulted in 2.5-fold increase in the activity of this compound (Figure 39, $K_i = 0.031 \text{ mM}$) compared with that of N-1,8-diaminoctane.

Replacement of the acetyl group of the N-acetyl-1,6-diaminohexane with methylsulfonyl and with methyl carbamate groups caused a decrease in the activity of those compounds. These results are in agreement with those of N-methylsulfonyl-1,8-diaminoctane and methyl N-(8-aminooctyl)-carbamate.

Substitution of the acetyl group of N-acetyl-1,6-diaminohexane with a chloroacetyl group accounted for about a 4-fold decrease in the activity of this compound ($K_i = 0.13 \text{ mM}$). The reason for this decrease
Figure 38. Dixon plots of the reciprocal velocity of N$^{8}$-acetylspermidine deacetylase versus methyl N-(8-aminooctyl) carbamate hydrochloride. Effects are shown in the presence of 3.7 µM (■), 7.4 µM (△), and 14.8 µM (○) of substrate concentrations.
Figure 39. Dixon plots of the reciprocal velocity of \( N^9 \)-acetyl spermidine deacetylase versus N-acetyl-1,6-diaminohexane hydrochloride. Effects are shown in the presence of 3.7 \( \mu \text{M} \) (■), 7.4 \( \mu \text{M} \) (▲), and 14.8 \( \mu \text{M} \) (○) of substrate concentrations.
may be due to the decrease in the affinity of the compound for the active site of the enzyme caused by the increased bulk on the acetyl group. No irreversible inhibition of the enzyme was observed even after longer (30 min) incubation times were used. It is possible that the failure of the chloroacetyl compound to alkylate the enzyme is due to the low reactivity of this group.

It is well known that Br and I are better leaving groups than the Cl group. However, it is not rational to synthesize bromo or iodo derivatives since these derivatives are much more bulky than the chloro derivatives, thus they would be expected to have a greater decrease in affinity for the enzyme active site. The results of this study support our conclusion in that any substituent on the terminal nitrogen with groups larger than the acetyl group is detrimental for the activity of the compound. As indicated earlier, it seems that the binding site of the enzyme is small and cannot sterically accommodate any added bulk.

To investigate the importance of the N⁸ for the binding of the N⁸-acetylspermidine to the enzyme, the nitrogen was replaced with a -CH₂- group. As predicted this compound was found to be the most effective inhibitor for the enzyme among the compounds studied in this project. The Ki value determined for this compound (Ki = 0.18 uM; Figure 40) is 55 times less than the Km value of the substrate (Km = 0.01 mM). It is apparent that bioisosteric replacement of the nitrogen with -CH₂- group has a profound effect on the activity of compound 10. The large increase in the activity is due to the following reasons. First, the affinity of the compound is not decreased by such substitution. Second, the bioisosteric replacement makes the compound resistant to the action of the enzyme. Third, the compound binds much more
Figure 40. Dixon plots of the reciprocal velocity of N⁸-acetylspermidine deacetylase versus N-(3-aminopropyl)-7-aminohexane-2-one hydrochloride. Effects are shown in the presence of 3.7 μM (★), 7.4 μM (△), and 14.8 μM (○) of substrate concentrations.
tightly to the enzyme (through the carbonyl group and through the nitrogens at 1 and 4 positions) than N-acetyl-1,6-diaminohexane and N-acetyl-1,8-diaminooctane.

From the above results, it is clear that the active site of N8-acetylspermidine deacetylase is not large enough to accommodate groups bigger than an acetyl group. This study also shows that the number of the carbons separating the two nitrogens influences the affinity of the inhibitors for the enzyme.

On the basis of the results of this investigation, and other results obtained in our laboratory, a hypothetical model for the active site of N8-acetylspermidine deacetylase is shown in Figure 41. The distance between the binding sites of N1-N4, N4-N8 and N1-N8 is about 5, 6.2 and 8.9 Å respectively. This model explains why N-acetyl-1,6-diaminohexane is a more potent inhibitor than N-acetyl-1,8-diaminooctane. According to this model, compound 10 binds more tightly and has higher affinity than all the compounds tested in this project. Compound 10 is the most potent and specific inhibitor for the N8-acetylspermidine deacetylase reported. This compound will be used as a lead compound to design and synthesize more potent inhibitors for N8-acetylspermidine deacetylase.
Figure 41. Hypothetical model for $N^8$-acetyl spermidine deacetylase
CONCLUSION

The polyamines putrescine, spermidine and spermine play an essential role in cell growth and differentiation. Spermidine is metabolized to N¹/N⁸-acetyl spermidine by two different enzymes (spermidine N¹-acetyltransferase and spermidine N⁸-acetyltransferase). The concentrations of N⁸-acetyl spermidine in liver, kidney and spleen are lower than those of spermidine or N¹-acetyl spermidine. N⁸-Acetyl spermidine is deacetylated by an enzyme in the 100,000 x g supernatant of rat liver.

One approach for determining the physiological functions of N⁸-acetyl spermidine is to increase its concentration in the tissues. In order to do this, a series of N⁸-acetyl spermidine analogues were designed and synthesized to inhibit the N⁸-acetyl spermidine deacetylase.

The nature of the inhibition and the Ki values for the inhibitors were determined from Dixon plots. N-Butyryl putrescine was the least effective inhibitor (Ki = 29.2 mM). Bioisosteric replacement of N⁴ of N⁸-acetyl spermidine with a -CH₂- group resulted in an increase in the inhibitory activity (Ki = 0.075 mM).

Separating the terminal nitrogens with 10 carbons resulted in a decrease in the potency of the compound (Ki = 0.11 mM). In contrast, decreasing the distance between the terminal amines to 6 carbons resulted in 2.5 fold increase in the inhibitory activity compared with N-acetyl-1,8-diamino octane.

The active site of the enzyme was investigated by substitutions of the acetyl group with larger groups (methane sulfonyle, carbamyl and chloroacetyl). These substitutions caused a decrease in the potency of
the inhibitors. These results are in agreement with those of N-Butyrylputrescine. It seems that the active site of the enzyme is small and it is not large enough to accommodate groups larger than the acetyl group.

Results obtained from the above studies were used to design and synthesize compound 10. In this inhibitor the bioisosteric replacement of the N8 of N8-acetylspermidine with a -CH2- group was made. In vitro studies showed this compound to be the most potent inhibitor for N8-acetylspermidine deacetylase yet to be reported (Ki = 0.00018 mM).

Based on these results and others obtained from Dr. Blankenship's laboratory, a hypothetical model for the enzyme is drawn. The active site of the enzyme is shown to contain zinc. The distances between the sites on the enzyme which are important for binding the substrate are estimated. Useful information obtained from this study about the catalytic mechanism and about the binding sites of the enzyme will be used to synthesize more potent inhibitors for the enzyme.
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