hexane/ether, R_f 0.66, $Ce(SO_4)_2$. To a stirred solution of 101 mg (0.27 mmol) of the above methyl ester in 12.9 mL of freshly distilled THF was added 2.5 mL of H_2O and 2.9 mL of 1 N aqueous lithium hydroxide solution. The reaction mixture was purged with argon vigorously for 15 min and stirred at room temperature for 8 h and 30 min. Another batch of 25 mg of methyl ester was hydrolyzed separately in the same manner and then combined for workup. The combined reaction mixtures were acidified to pH 3 by the addition of 1 N aqueous HCl solution and poured into 70 mL of brine. The resulting solution was saturated with NaCl and extracted with EtOAc (4 × 100 mL). The combined EtOAc extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo to give 200 mg of crude product as an oil. Purification was effected by flash chromatography on 30 g of silica gel 60 with 1:1 hexane/ether as eluant to give 42 mg (35%) of acid **24**. TLC: silica gel, 1:1 hexane/ether, R_f 0.26, I_2. ^1H NMR ($CDCl_3$, 270 MHz): δ 5.47–5.33 (m, 2 H), 4.45 (s, 1 H), 4.22 (s, 1 H), 2.96 (d, J = 9 Hz, 1 H), 2.51 (t, J = 8 Hz, 2 H), 2.35 (t, J = 8 Hz, 2 H), 0.88 (t, J = 7 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ 178.6, 129.9, 129.7, 82.9, 79.8, 53.5, 48.4, 33.4, 33.2, 31.7, 31.8, 29.6, 29.4, 29.2, 28.9, 28.0, 26.7, 24.6, 22.6, 14.0. Anal. ($C_{20}H_{34}O_3S$) H, S, C: calcd, 67.75; found, 67.30.

Acknowledgment. We thank M. B. Phillips, I. M. Michel, H. J. Goldenberg, T. E. Steinbacher, G. T. Allen, and E. C.-K. Lui (Pharmacology Department) for the biological evaluation of these compounds. Microanalyses, IR spectra, and mass spectra were kindly provided by the Squibb Analytical Department and 400-Mz ^1H NMR spectra were provided by Dr. M. Porubcan and A. Kahle. We also thank Drs. P. W. Sprague and M. F. Haslanger for their encouragement and support of this project. Finally, we thank Caroline Coleman for the careful and expedient preparation of this manuscript.

Registry No. 1, 57576-52-0; (±)-**6**, 104596-10-3; (9*R*)-**6**, 70120-34-2; (9*S*)-**6**, 94903-79-4; (±)-**7**, 104596-12-5; (±)-**8**, 104596-11-4; (±)-**9**, 104596-13-6; (±)-**10**, 119785-27-2; (+)-**10**, 110902-61-9; (−)-**10**, 119785-54-5; (±)-**10** (methyl ester), 119785-26-1; (±)-**10** (sulfoxide, isomer 1), 119785-43-2; (±)-**10** (sulfoxide, isomer 2), 119785-44-3; (±)-**10** (methyl ester; sulfoxide, isomer 1), 119785-40-9; (±)-**10** (methyl ester; sulfoxide, isomer 2), 119785-41-0; (±)-**11**, 119785-28-3; (±)-**12**, 119785-29-4; (±)-**13**, 119785-30-7; **14**, 119694-90-5; **14** (methylthiomethyl ester), 119694-89-2; **15**, 119694-84-7; **16**, 119785-38-5; (±)-**18**, 104596-27-2; (±)-**19**, 119785-50-1; (±)-**20**, 104506-57-2; (±)-**21**, 119694-94-9; (±)-**22**, 119785-51-2; (±)-**23**, 119785-52-3; (±)-**24**, 119785-53-4; (±)-**25**, 119785-49-8; (±)-**28**, 119694-93-8; (±)-**29**, 119785-46-5; (±)-**30** (R_2 = CH_3), 119785-47-6; (±)-**30** (R_2 = C_7H_{15}), 119785-48-7; (±)-**33**, 119785-45-4; (±)-**33** (methyl ester), 119785-42-1; (±)-**34**, 119785-31-8; (±)-**35**, 119785-32-9; (±)-**36**, 119785-33-0; (±)-**37**, 119786-24-2; (±)-**38**, 119785-34-1; (±)-**39**, 119785-35-2; **40**, 119694-80-3; **41**, 119785-36-3; (*R*)-**42**, 105616-83-9; (*S*)-**42**, 105551-88-0; **44**, 119694-81-4; **45**, 119694-85-8; **46**, 119694-86-9; **47**, 119694-87-0; **48**, 119694-88-1; **49**, 104108-78-3; **50**, 104108-86-3; **51**, 119694-91-6; **52**, 119694-92-7; **53**, 104108-76-1; **54**, 119785-39-6; **55**, 104154-31-6; **56**, 104108-71-6; **57**, 104154-29-2; (±)-**58**, 119785-25-0; **59**, 119694-82-5; **60**, 119694-83-6; $HS(CH_2)_5CH_3$, 111-31-9; $HS(CH_2)_4CH_3$, 110-66-7; $HS(CH_2)_6CH_3$, 1639-09-4; $HSCH_2$-c-C_6H_{11}, 2550-37-0; $HS(CH_2)_2Ph$, 4410-99-5; $HS(CH_2)_3Ph$, 24734-68-7; $HS(CH_2)_2OPh$, 6338-63-2; (*E*)-$HSCH_2CH{=}CHPh$, 95351-73-8; $HS(CH_2)_7CH_3$, 111-88-6; (*S*)-$HSCH(CH_3)(CH_2)_4CH_3$, 119785-37-4; (*R*)-$HSCH(CH_3)(CH_2)_4CH_3$, 119785-55-6; (±)-$HSCH_2CH(OH)(CH_2)_3CH_3$, 105551-87-9; $HSCH_2CONH(CH_2)_2CH_3$, 38042-20-5; $HSCH_3$, 74-93-1; $HSCH_2CH_3$, 75-08-1; $HS(CH_2)_2CH_3$, 107-03-9; $HS(CH_2)_3CH_3$, 109-79-5; $HSCH_2CH{=}CH_2$, 870-23-5; $HS(CH_2)_2CH{=}CH_2$, 5954-70-1; $ClCH_2SCH_3$, 2373-51-5; HSPh, 108-98-5; (*E*)-$HSCH_2CH{=}CHC_3H_7$, 89222-69-5; (*E*)-$HSCH_2C(CH_3){=}CHC_3H_7$, 104108-73-8; (*Z*)-$HSCH_2CH{=}CHC_3H_7$, 104108-89-6; (*Z*)-$HS(CH_2)_2CH{=}CHC_2H_5$, 89222-70-8; (*E*)-$HS(CH_2)_2CH{=}CHC_2H_5$, 104108-88-5; $ClCH_2S(CH_2)_3CH_3$, 42330-14-3; $Br(CH_2)_6CH_3$, 629-04-9.

Design and Synthesis of Inhibitors of N^8-Acetylspermidine Deacetylase

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Analogues of N^8-acetylspermidine (1) were synthesized as potential inhibitors of the cytoplasmic enzyme N^8-acetylspermidine deacetylase. The compounds were assayed for their ability to inhibit the deacetylation of 1 in a cytosolic fraction from rat liver. The apparent K_i values were determined by Dixon plots. The apparent K_m of 1 for this enzyme is 11.0 μM. It was found that compounds which lacked the N1 or the N4 of spermidine were less effective at competing for the enzyme than the substrate. All compounds with acyl substituents larger than acetyl were less potent inhibitors than the corresponding acetylated derivatives. Thus, the enzyme's selectivity as a deacetylase seems to be attributable to steric hindrance which occurs with larger acyl groups. The N8 of the substrate is not essential for its binding to the enzyme. Replacement of N8 with a CH_2 group gives the ketone 14, which has an apparent K_i of 0.18 μM, 60-fold lower than the apparent K_m of 1. The inhibitory potency of 14 is retained in compounds substituted at the N1 position. The N^1,N^1-dimethyl and the N^1,N^1-diethyl analogues (**15** and **16**) of 14 have apparent K_i values of 0.096 and 0.10 μM, respectively. These agents are the most potent inhibitors of N^8-acetylspermidine deacetylase reported, and they are promising tools for use in determining the physiological function of N^8-acetylspermidine deacetylation.

The polyamines are a group of compounds present in all higher organisms.[1,2] The polyamines are deemed essential for cell growth and proliferation; however, their function in regulating cell metabolism remains unknown.[1-4] These substances are polycationic at physiological pH and are found closely associated with DNA in the nuclei of the cells. The concentrations of polyamines are greatly increased within cells that are undergoing rapid growth. In mammalian tissues, the concentrations of polyamines are governed primarily by the activity of the enzyme ornithine decarboxylase (ODC), the first and a rate-limiting enzyme in the biosynthesis of polyamines (Figure 1).[5] ODC converts ornithine to putrescine. Putrescine is then converted, by the action of spermidine synthase, to spermidine via the transfer of an aminopropyl group from decarboxylated

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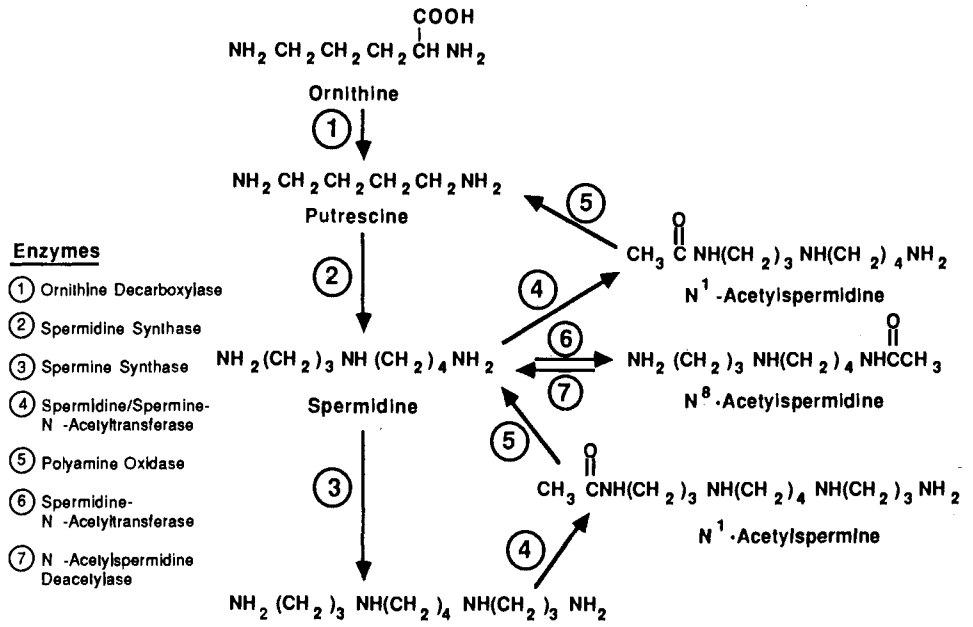

Figure 1. Metabolic pathways for the polyamines in mammals.

S-adenosylmethionine.[6,7] In a similar reaction, spermine synthase catalyzes the addition of an aminopropyl group to the N8 position of spermidine to yield spermine.[6,7]

The cell contains cytoplasmic enzymes capable of converting spermine to spermidine and spermidine to putrescine. This two-step process involves the acetylation of spermine or spermidine at the N1 position and subsequent oxidation at the C3–N4 position.[8-13] The importance of this polyamine interconversion process is not understood.

In addition to the cytoplasmic enzyme that acetylates spermidine at N1, there is a nuclear enzyme that acetylates spermidine selectively at the N8 position.[14-18] N^8-Acetylspermidine (N^8-AcSpd) is not detectable in most tissues probably due to the action of a cytoplasmic enzyme that rapidly and selectively deacetylates N^8-AcSpd.[19-22] It has been reported that spermidine binds to and stabilizes double-stranded DNA in vitro and that N^8-AcSpd has reduced binding affinity and ability to stabilize DNA.[23] It has been postulated that acetylation and deacetylation of spermidine at the N8 position may play a role in regulating DNA structure and function through effects on spermidine–DNA binding.[14] This idea has been difficult to test due to the lack of inhibitors of spermidine N^8-acetyltransferase or N^8-AcSpd deacetylase that possess activity in vivo.

Mamont et al.[24] reported 7-amino-2-heptanone to be an effective inhibitor (K_i = 2.2 μM) of N^8-AcSpd deacetylase. No other inhibitor for this enzyme has been reported. Two studies on inhibitors of histone and spermidine N^8-acetylation have been reported.[25,26] In both of these studies, the inhibitors are large, highly polar, multisubstrate analogues that would not be expected to have in vivo activity.

The ultimate goal of our research is to discover inhibitors of the acetylation and deacetylation of spermidine at its N8 position. New inhibitors are designed to have physicochemical properties that will allow activity in vitro and in vivo. In this paper we report our initial efforts to develop inhibitors of N^8-AcSpd deacetylase. An effective inhibitor of this enzyme will be a useful biochemical tool for elucidation of the importance of deacetylating N^8-AcSpd. In addition, an effective inhibitor might be useful, either alone or in combination with other inhibitors in the polyamine biosynthetic pathway, for the treatment of certain disease states.[27,28]

The discovery of an enzyme inhibitor is most effectively achieved when modern concepts of drug design are combined with knowledge of the mechanism of the enzymatic catalysis and the geometry of the enzyme. We have used active-site-directed protease inhibitors to investigate the catalytic mechanism of N^8-AcSpd deacetylase.[29] The

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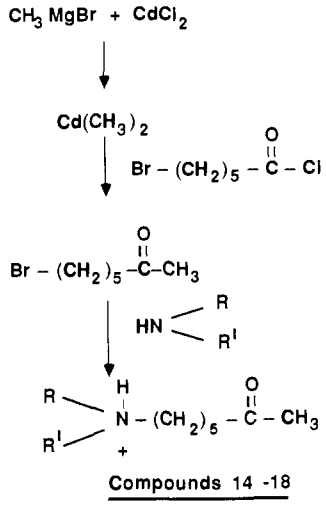
Scheme I



results from our preliminary studies suggest that (1) chelating agents inhibit the enzyme, indicating that it contains a transition metal in its active site, (2) sulfhydryl binding agents cause loss of activity in a manner similar to that seen with other thiol proteases,[30] (3) the low inhibitory potencies of diisopropyl fluorophosphate and phenylmethanesulfonyl fluoride indicate that the enzyme is not a serine-type amidase.[29] These results will be used in the present and in future studies to aid in the design of inhibitors to N^8-AcSpd deacetylase.

Chemistry

Monoacylated diamines **2–10** were prepared easily by reacting a molecular excess of the diamine with the appropriate anhydride or acyl halide.[31] Purification of the desired products was achieved by the use of ion-exchange chromatography. In preparing the chloroacetyl analogue **11**, it was necessary to use mono-Boc-blocked 1,6-diaminohexane to react with chloroacetyl chloride. The blocking group was removed by treatment with 3 N HCl in ethyl acetate. 7-Bromoheptan-2-one which was required for the synthesis of compounds **14–18** was prepared by reaction of 6-bromohexanoyl chloride with Me_2Cd (Scheme I). Reaction of the resulting ω-bromo ketone with the proper diamines and purification by absorption column chromatography gave the desired product.

Bioassay and Discussion

All compounds were assayed in a 100000*g* fraction of cytosol from rat liver for their ability to inhibit the deacetylation of [*acetyl*-^{3}H]-N^8-AcSpd.[19] The apparent K_i value for each compound was determined by using a Dixon plot.[32] An example of a typical Dixon plot is given in Figure 2, and the inhibitor results are listed in Table I. All compounds produced competitive inhibition kinetics.

While it is possible that the cytosolic fraction of rat liver contains more than one enzyme capable of deacetylating N^8-AcSpd, our results[19] and the results of others[20,21] show that possibility to be unlikely. Proteins (or other components) in the cytosolic fraction may affect the assay to give apparant K_i values higher than the true K_i values of the test compounds; however, the data obtained from the assay are highly reproducible and of sufficient quality to guide the design of inhibitors of the enzyme.

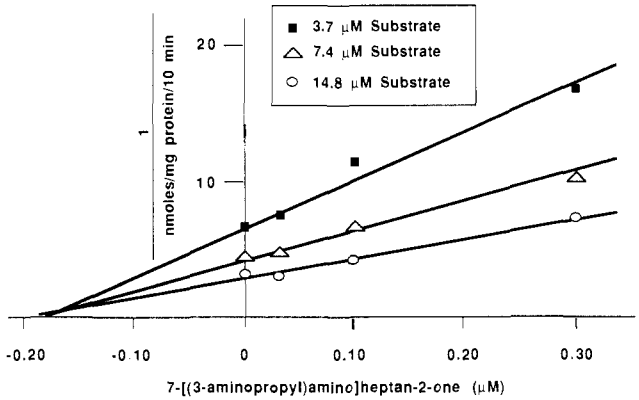
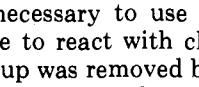
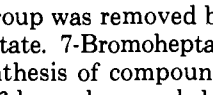


Figure 2. Dixon plots of the reciprocal velocity of N^8-acetylspermidine deacetylase versus 7-[(3-aminopropyl)amino]-heptan-2-one hydrochloride. Effects are shown in the presence of 3.7 μM (■), 7.4 μM (△), and 14.8 μM (○) substrate.

Table I. Inhibitors of N^8-Acetylspermidine Deacetylase

no.	structure[a]	deacetylase inhibn[b] app K_i, μM
1	$H_2N(CH_2)_3NH(CH_2)_4NHCOCH_3$ (substrate)	11 (K_m)
2	$CH_3CONH(CH_2)_3NH(CH_2)_4NH_2$	1370
3	$H_2N(CH_2)_4NHCOCH_3$	800
4	$CH_3(CH_2)_2NH(CH_2)_4NHCOCH_3$	26
5	$H_2N(CH_2)_4NHCO(CH_2)_2CH_3$	29000
6	$H_2N(CH_2)_6NHCOCH_3$	31
7	$H_2N(CH_2)_8NHCOCH_3$	75
8	$H_2N(CH_2)_{10}NHCOCH_3$	110
9	$H_2N(CH_2)_6NHSO_2CH_3$	376
10	$H_2N(CH_2)_8NHSO_2CH_3$	650
11	$H_2N(CH_2)_6NHCOOCH_3$	NI[c]
12	$H_2N(CH_2)_8NHCOOCH_3$	260
13	$H_2N(CH_2)_6NHCOCH_2Cl$	130
14	$H_2N(CH_2)_3NH(CH_2)_5COCH_3$	0.18
15	$(Me)_2N(CH_2)_3NH(CH_2)_5COCH_3$	0.096
16	$(Et)_2N(CH_2)_3NH(CH_2)_5COCH_3$	0.10
17		0.63
18		1.00

[a] All compounds were prepared and tested as HCl or bis(HCl) salts. [b] Apparent K_i values were determined in a cytosolic fraction from rat liver as described previously.[19] [c] NI = no inhibition.

Compounds **3**, **4**, and **7** were made to investigate the relative importance of the basic nitrogens in the substrate. N-Acetylputrescine (**3**) is considerably poorer in binding the enzyme than is the substrate N^8-AcSpd (**1**). Surprisingly, **4**, which incorporates the propyl chain but not N1 of the substrate, regains much of the activity lost in **3**. Replacement of the N4 of the substrate with a CH_2 group gives a compound, **7**, that has an apparent K_i 7-fold higher than the K_m of the substrate. Thus, one can conclude that maximal binding to the enzyme's active site requires both N1 and N4 and that the hydrocarbon chain contributes considerably to the inhibitor–enzyme interaction.

The optimal distance for inhibitory activity between the terminal acetamido and the primary amino groups was investigated. Lengthening the separation from 8 to 10 carbon atoms resulted in a slight decrease in inhibitory activity as seen in **7** vs **8**. Shortening the distance to 6 carbon atoms gave **6**, a compound with increased inhibitory activity. A possible explanation for the increased activity of **6** vs **7** is that an unfavorable bonding interaction occurs between the enzyme surface and methylene group that replaces the positively charged N4. The shorter molecule **6** might be able to bridge between the N1 site and the deacetylation catalytic site while avoiding an unfavorable

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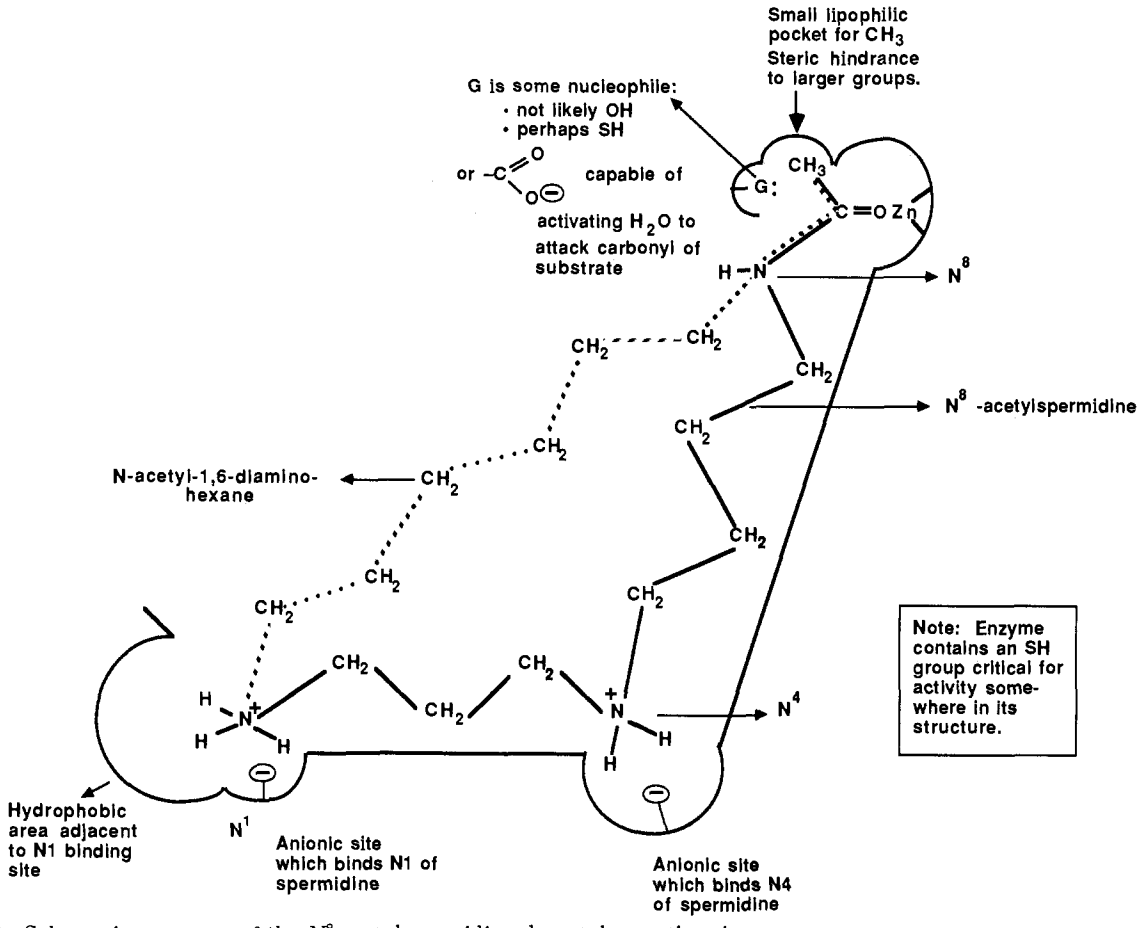

Figure 3. Schematic summary of the N^8-acetylspermidine deacetylase active site.

bonding interaction at the N4 binding site. A diagram of this possible bonding interaction is shown in Figure 3. The observation that **6** approaches the substrate in affinity presents the possibility of using the smaller molecule as a starting point for making new inhibitors. We used the less complex 1,6-diaminohexane or 1,8-diaminooctane to make potential inhibitors **9–13**. All five of these compounds involved alterations of the molecule at the position expected to bind at the same site as the acetyl group of the substrate. On examining the data in Table I, it can be seen that in every case alteration of the acetyl group resulted in a decrease in inhibitory activity. Replacing the acetyl with a butyryl group (**5**) also resulted in a marked decrease in inhibitory activity.

The ability of some sulfhydryl binding agents to inhibit the enzyme's activity suggested that an active-site-directed irreversible inhibitor might be effective.[33] A successful active-site-directed irreversible inhibitor must have good affinity for the enzyme's active site, it must contain an electrophile in its structure that is properly aligned with a nucleophile on the enzyme, and the electrophile must be sufficiently strong to react with the nucleophile within a reasonable time period. Compound **13** contains the moderately reactive α-chloroacetamide group as an electrophile. The compound failed to show irreversible inhibition of the enzyme even after prolonged times of incubation at 37 °C. The decrease in the competitive inhibition of the enzyme by this compound (higher K_i than **6**) suggests that introduction of the Cl group is sterically hindering the enzyme–inhibitor complex formation. Thus, the possibility of increasing the irreversible bonding of this compound by increasing its electrophilicity through the substitution of a still larger Br or I group for Cl seems irrational.

With the knowledge that N^8-AcSpd deacetylase binds best to spermidine analogues with the N1 and N4 intact and that there is severe steric hindrance at the acetyl binding site, we synthesized compound **14**. This compound is the result of making the bioisosteric replacement of the N8 of N^8-AcSpd with a CH_2 group. The compound should retain high affinity for the enzyme and not be hydrolyzed. Mamont et al.[24] have prepared independently the putrescine analogue 7-aminoheptan-2-one. It is interesting to note that the spermidine analogue (**14**) is a significantly more active inhibitor than is the putrescine analogue. These results confirm the importance of both N1 and N4 in the enzyme–inhibitor bonding interaction.

Four additional analogues (**15–18**) of compound **14** were prepared. These compounds were made to investigate the nature of the enzyme's topography about N1. The *N,N*-dimethyl (**15**) and the *N,N*-diethyl (**16**) analogues showed slightly enhanced inhibitory potency compared to the primary amine **14**. Activity was decreased with the morpholino adduct **18**. Thus, it appears that binding of the substrate to the enzyme is enhanced when small hydrophobic groups are attached to the N1 position. The piperazinyl analogue **17** has the two nitrogens closer together than occurs in spermidine; thus, the decrease in inhibitory activity seen for this compound is consistent with a need for two nitrogens separated as in spermidine.

Experimental Section

Chemistry. 1,6-Diaminohexane and 1,8-diaminooctane were purchased from Sigma Chemical Co. All other chemicals were purchased from Aldrich Chemical Co. Solvents were ACS reagent grade or better quality and were used without further purification.

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Compounds 1–3 were prepared by the method of Tabor et al.[31] and physical properties were in agreement with the literature values.

Melting points were determined in open capillary tubes on a Thomas-Hoover Unimelt apparatus and are uncorrected. IR spectra were recorded on KBr disk or thin films of mineral oil dispersions by using a Perkin-Elmer 283 instrument. Proton NMR were recorded on Varian XL-200 spectrometer using D_2O as a solvent and the water peak as a reference line. Mass spectra were obtained by using a Hitachi Perkin-Elmer RMS-4 instrument (CI, 70 eV). Elemental analyses (C, H, N) were performed at the microanalytical laboratory of the National Institutes of Health, Bethesda, MD. All values for elemental analysis were within ±0.4% of the theoretical values.

Chromatography. Ion-exchange chromatography was performed by using Amberlite CG-50 (wet mesh 100–200) exchange resin. The resin (100 g) was washed three times each with 250 mL of 3 N HCl and 250 mL of 3 N NH_4OH. The resin was washed then two to three times with H_2O, and the pH was adjusted to 9–9.5 with concentrated NH_4OH. Finally, the resin was packed in a 65 × 2.5 cm column and eluted with H_2O until the eluant became clear. The reaction mixture to be purified (up to 40 mL) was transferred to the column and the elution was carried out at a flow rate of 1–2 mL/min with 150 mL of H_2O, followed by 250 mL of 0.5 N NH_4OH and 100 mL of 2 N NH_4OH. During the elution with 2 N NH_4OH, fractions (10 mL) were collected and analyzed by TLC. Fractions containing the desired product were combined and worked up by standard procedures.

TLC was performed on plates purchased from EM Science (60F-254; 5 × 10 cm; 0.25-mm silica gel layer). Plates were developed in $MeOH/CHCl_3/NH_4OH$ (10:10:1). Visualization was accomplished with ninhydrin spray.

Absorption chromatography was done with silica gel (EM Science, 230–400 mesh) which was packed as a slurry in CH_2Cl_2 into a 65 × 3.5 cm column. The concentrated reaction mixtures (up to 50 mL) were added to the column, and elution was carried out with $MeOH/CH_2Cl_2/NH_4OH$ (7:5:1). Fractions (10 mL) were collected, and those found to contain the desired product were combined and the solvent was removed by using a rotary evaporator.

Enzyme Assay. Male Wister rats, 140–180 g, were sacrificed by decapitation, and a 100000*g* supernatant fraction (cytosol) was prepared from 5 g of the rat liver as has been described by Blankenship.[18] Tritium-labeled N^8-acetylspermidine (*acetyl*-^{3}H; 2.0–20.0 μM) was mixed with different concentrations of the potential inhibitor and with 100 μL of the cytosol fraction in a final volume of 0.5 mL. The incubation mixture consisted of sucrose (0.125 mM), NaH_2PO_4 (29 μM), and $MgCl_2$ (25 μM) at pH 7.4. The incubation was carried out for 10 min at 37 °C in a shaking water bath. The reaction was stopped by addition of 0.5 mL of a solution which was 1 N in HCl and 0.05 M in acetic acid. Ethyl acetate (3 mL) was added to each of the test tubes. The tubes were shaken for 10 min in an Eberbach shaker and then centrifuged for 5 min at 1500*g* by using a IEC Model 2K centrifuge. The ethyl acetate layers (2 mL) were transferred to counting vials, and 10 mL of scintillation cocktail [prepared by mixing 0.66 L of Triton X-100, 1.34 L of toluene, 7.32 g of 2,5-diphenyloxazole, and 1.32 g of *p*-bis(5-phenyl-2-oxazolyl)benzene] was added to each vial. The radioactivity of the radiolabeled acetic acid released by deacetylase activity and extracted into the ethyl acetate was measured with a liquid scintillation counter (Beckman CPM-100).

The controls were 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 apparent K_i of each inhibitor was determined by using a Dixon plot.[32]

N^8-Acetyl-N^1-propylputrescine Hydrochloride (4). *N*-Acetylputrescine (2 g, 0.015 mol) was dissolved in 300 mL of DMF containing 0.015 mol of triethylamine. 1-Iodopropane (1.3 g, 0.007 mol) in 50 mL of DMF was added to the amine in a dropwise manner. The reaction was stirred at 21 °C and kept under N_2 for 10 h. The mixture then was heated to 60 °C for 5 h. The solvent was removed in vacuo and the residue dissolved in H_2O. The pH of this solution was brought to 8–9 with NH_4OH, and then it was extracted with $CHCl_3$ (5 × 150 mL). The $CHCl_3$ extracts were combined, concentrated on the rotary evaporator, and applied to a 65 × 3.5 column that was wet packed ($CHCl_3$) with Florisil absorbant. The column was eluted with 300 mL of $CHCl_3$ containing 0.3 mL of NH_4OH followed by 600 mL of a mixture of $CHCl_3$, CH_3OH, and NH_4OH (3:0.6:0.1%). Fractions containing the desired compound were combined and evaporated, and the residue was dissolved in 20 mL of distilled H_2O. The pH of the solution was brought to 2 with 3 N HCl. The solvent was removed on a rotary evaporator and the residue dried in vacuo and then crystallized from 2-PrOH to yield 1.20 g (82%) of 4: mp 130 °C; ^{1}H NMR 0.85 (t, 3 H), 1.5–1.7 (m, 6 H), 2.0 (s, 3 H), 3.0 (m, 2 H), 3.22 ppm (t, 2 H); CIMS *m/e* 173 (M + 1). Anal. ($C_9H_{21}ClN_2O$) C, H, N.

***N*-Butyryl-1,4-diaminobutane Hydrochloride (5).** Putrescine (10 g, 0.113 mol) was dissolved in 50 mL of CH_3CN. Butyryl chloride (10.65 g, 0.099 mol) was mixed with 20 mL of CH_3CN and was added to the reaction flask in a dropwise manner. The reaction was stirred at room temperature under a N_2 atmosphere for 3 h. The organic layer was evaporated, and the residue was dissolved in 100 mL of H_2O. This solution was brought to pH 2 by adding 3 N HCl and then extracted with $CHCl_3$ (3 × 50 mL). The H_2O layer was subjected to ion-exchange chromatography. Fractions containing **5** as a free base were combined, and the solvent was removed by rotary evaporation. The pH of this oily residue was decreased to 2.0 by adding 3 N HCl. All solvent was removed from this fraction by evaporation in vacuo. The solid that remained was dissolved in hot 2-PrOH and, after storage at 4 °C for 2 days, yielded 4.0 g (25%) of **5**: mp 139–140 °C; IR (mineral oil) 1640 (C=O), 3300–2000 cm^{-1} (N–H$^+$); ^{1}H NMR 0.85 (t, 3 H) 1.5 (t, 6 H), 2.1 (t, 2 H), 2.9 (t, 2 H), 3.1 ppm (t, 2 H); CIMS *m/e* 159 (M + 1). Anal. ($C_8H_{19}ClN_2O$) C, H, N.

***N*-Acetyl-1,6-diaminohexane Hydrochloride (6).** 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 a N_2 atmosphere for 3 h at room temperature and with continuous stirring. The solvent was removed in vacuo, and the residue was dissolved in 200 mL of distilled H_2O. This solution was brought to pH 2 with 3 N HCl and extracted with $CHCl_3$ (3 × 50 mL). The aqueous solution was concentrated and the product separated on an ion-exchange column.

The eluant containing **6** was treated as described for compound **5**. Crystallization from 2-PrOH gave **6**, 3.0 g (22%): mp 130–131 °C; ^{1}H NMR 1.85 (s, 3 H), 2.85 (t, 2 H), 3.05 ppm (t, 2 H); CIMS *m/e* 195 (M + 1). Anal. ($C_8H_{19}ClN_2O$) C, H, N.

***N*-Acetyl-1,8-diaminooctane Hydrochloride (7).** Five grams of 1,8-diaminooctane (0.035 mol) was dissolved in 40 mL of CH_3CN. Acetic anhydride (3.3 g, 0.035 mol) was mixed with 15 mL of CH_3CN and was added dropwise to the reaction flask. The reaction was stirred under a N_2 atmosphere at room temperature for 3 h. The organic layer was evaporated, and the residue was dissolved in 150 mL of H_2O. The mixture was adjusted to pH 2 with 3 N HCl and then extracted with ether (2 × 30 mL). The aqueous layer was concentrated to 50 mL and then transferred to an ion-exchange column. The eluant was treated as described for compound **5**. Compound **7** was obtained as white needle crystals from 2-PrOH, 2.2 g (28%): mp 131–133 °C; IR (mineral oil) 1640 cm^{-1} (C=O); ^{1}H NMR 1.85 (s, 3 H), 2.85 (t, 2 H), 3.05 ppm (t, 2 H): CIMS *m/e* 187 (M + 1). Anal. ($C_{10}H_{23}ClN_2O$) C, H, N.

***N*-Acetyl-1,10-diaminodecane Hydrochloride (8).** This compound was synthesized and isolated by the same procedure described for compound **6**. 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 of **8** was 4.0 g (46%): mp 101–103 °C; ^{1}H NMR 1.85 (s, 3 H), 2.85 (t, 2 H), 3.5 ppm (t, 2 H); CIMS *m/e* 215 (M + 1). Anal. ($C_{12}H_{27}ClN_2O$) C, H, N.

***N*-(Methylsulfonyl)-1,6-diaminohexane Hydrochloride (9).** 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. Purification and crystallization were done as described for **5**. The yield of **9** was 3.0 g (65%): mp 114–116 °C; IR (mineral oil) 1380 (S=O), 1155 (S=O), 3300–2000 cm^{-1} (N–H$^+$); ^{1}N NMR 2.9 (t, 2 H), 2.95 (s, 3 H), 3.0 ppm (t, 2

H); CIMS m/e 195 (M + 1). Anal. ($C_7H_{19}ClN_2O_2S$) C, H, N.

N-(Methylsulfonyl)-1,8-diaminooctane Hydrochloride (10). A stirred solution of 1,8-diaminooctane (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 2 h with continuous stirring at room temperature. The reaction mixture was worked up as described for compound **5**. The yield of **10** was 2.41 g (47%): mp 132–134 °C; IR (mineral oil) 1380 (S=O), 1155 (S=O), 3300–2000 cm^{-1} (N–H^+); ^1H NMR 2.9 (t, 2 H), 2.95 (s, 3 H), 3.0 ppm (t, 2 H); CIMS m/e 223 (M + 1). Anal. ($C_9H_{23}ClN_2O_2S$) C, H, N.

Methyl N-(6-Aminohexyl)carbamate Hydrochloride (11). This compound was obtained from 1,6-diaminohexane (5 g, 0.025 mol) and methyl chloroformate (2.36 g, 0.026 mol) by the same procedure described for previous compounds. The yield was 2.5 g (48%): mp 140–141 °C; IR (mineral oil) 1700 (C=O), 3370–2000 cm^{-1} (N–H^+); ^1H NMR 2.85 (t, 2 H), 3 (t, 2 H), 3.5 ppm (s, 3 H); CIMS m/e 175 (M + 1). Anal. ($C_8H_{19}ClN_2O_2$) C, H, N.

Methyl N-(8-Aminooctyl)carbamate Hydrochloride (12). 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 under N_2 for 3 h. Purification and recrystallization were achieved as described for **5**. The yield of **12** was 2.5 g (52%): mp 131–133 °C; IR (mineral oil) 1790 cm^{-1} (C=O); ^1H NMR 2.85 (t, 2 H), 2.95 (t, 2 H), 3.5 ppm (s, 3 H); CIMS m/e 203 (M + 1). Anal. ($C_{10}H_{23}ClN_2O_2$) C, H, N.

N-(Chloroacetyl)-1,6-diaminohexane Hydrochloride (13). To 1,6-diaminohexane (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 a N_2 atmosphere at room temperature for 3 h. The solvent was evaporated in vacuo, and the residue was dissolved in $CHCl_3$ and chromatographed over silica gel. The eluted fractions that contained the product were collected and concentrated to dryness in vacuo. The residual oil (1.5 g) was dissolved in $CHCl_3$ (150 mL) and was mixed with Et_3N (7 g, 0.07 mol). To the above mixture was added a solution of 0.5 g (0.004 mol) of chloroacetyl chloride in 30 mL of $CHCl_3$, which was dripped into the stirred reaction mixture. The reaction was continued for 3 h at room temperature under N_2. The reaction mixture was 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 h the solution was filtered and the precipitate was dissolved in hot 2-PrOH. The mixture was concentrated to 5 mL and was cooled to 5 °C. The crystals which formed in 24 h were collected by filtration. The yield of **13** was 0.70 g (20%): mp 80 °C; IR (mineral oil) 1650 (C=O), 3300–2000 (N–H^+), 780 cm^{-1} (C–Cl); ^1H NMR 2.85 (t, 2 H), 3.15 (t, 2 H), 4.0 ppm (s, 2 H); CIMS m/e 193 (M + 1). Anal. ($C_8H_{18}Cl_2N_2O$) C, H, N.

7-[(3-Aminopropyl)amino]heptan-2-one Dihydrochloride (14). Methylmagnesium bromide (40 g, 0.33 mol) and 500 mL of slowly stirred anhydrous Et_2O were mixed in a 1-L round-bottom flask, which was cooled in an ice bath under N_2 while 55 g (0.30 mol) of anhydrous $CdCl_2$ was added in portions during 10 min. After 30 min, the ice bath was removed and stirring was continued for an additional 4 h. To the brown solution of the organocadmium compound was added, in about 1 h, a solution of 50 g (0.23 mol) of 6-bromohexanoyl chloride in 150 mL of benzene. Slow stirring was continued at room temperature for 15 h. The resulting grayish brown solution was stirred for an additional 1 h 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 h. The organic layer was collected, dried (Na_2SO_4), and filtered and the solvent removed by rotary evaporation. The residue remaining was vacuum distilled to yield 35 g of 7-bromoheptan-2-one, bp 90–95 °C (0.1–0.05 mmHg).

To 1,3-diaminopropane (18.4 g, 0.25 mol) in 150 mL of THF was added in a dropwise manner 7-bromoheptan-2-one (12 g, 0.062 mol) in 50 mL of THF. The reaction was allowed to run for 10 h with continuous stirring at room temperature. The solvent was removed in vacuo, and the residue was dissolved in 120 mL of distilled H_2O and applied to an ion-exchange column. The product was eluted with 2 N NH_4OH and crystallized as the HCl salt from EtOH. The yield of **14** was 8.4 g (52%): mp 210–215 °C; IR (mineral oil) 1720 cm^{-1} (C=O); ^1H NMR 1.25 (m, 2 H), 1.4–1.7 (m, 4 H), 2.1 (s, 3 H), 2.5 (t, 2 H), 2.9–3.1 ppm (m, 6 H); CIMS m/e 187 (M + 1). Anal. ($C_{10}H_{24}Cl_2N_2O$) C, H, N.

7-[N-[3-(Dimethylamino)propyl]amino]heptan-2-one Dihydrochloride (15). A solution of 7-bromoheptan-2-one (6.25 g, 0.032 mol) in 20 mL of THF was added dropwise to a flask containing 3-(dimethylamino)propylamine (10 g, 0.097 mol), Et_3N (0.097 mol), and 100 mL of benzene. The reaction mixture was heated to 60 °C and stirred under a N_2 atmosphere for 10 h. Purification of the product was accomplished as described for **14**. Final crystallization of **15** was from 2-PrOH at 4 °C to yield 3.5 g (38%): mp 164–165 °C; IR (mineral oil) 1720 cm^{-1} (C=O); ^1H NMR 1.25 (m, 2 H), 1.4–1.7 (m, 4 H), 2.1 (s, 3 H) 2.5 (t, 2 H), 2.8 ppm (s, 6 H); CIMS m/e 215 (M + 1). Anal. ($C_{12}H_{28}Cl_2N_2O\cdot 0.5H_2O$) C, H, N.

7-[N-[3-(Diethylamino)propyl]amino]heptan-2-one Dihydrochloride (16). In this synthesis 7-bromoheptan-2-one (5 g, 0.026 mol) and 3-(diethylamino)propylamine (10 g, 0.07 mol) were reacted and the product isolated as described for **15**. The yield of **16** was 1.5 g (18%): mp 102–103 °C; IR (mineral oil) 1720 cm^{-1} (C=O); ^1H NMR 1.1 (t, 6 H), 2.1 (s, 3 H), 2.5 (t, 2 H), 3.1 (m, 4 H), 3.2 ppm (m, 2 H); CIMS m/e 244 (M + 1). Anal. ($C_{14}H_{32}Cl_2N_2O\cdot 0.5H_2O$) C, H, N.

7-(4-Methyl-1-piperazinyl)heptan-2-one Dihydrochloride (17). This compound was synthesized and isolated by the same procedures as described for compound **14**. 7-Bromoheptan-2-one (5.0 g, 0.026 mol) and 1-methylpiperazine (25.0 g, 0.025 mol) were reacted to yield 2.0 g (28%) of **17**: mp 231–232 °C; ^1H NMR 1.5 (m, 2 H), 1.7 (m, 2 H), 2.1 (s, 3 H), 2.5 (t, 2 H), 3.0 (s, 3 H), 3.2 ppm (t, 10 H); CIMS m/e 213 (M + 1). Anal. ($C_{12}H_{26}Cl_2N_2O$) C, H, N.

7-[(3-Morpholinopropyl)amino]heptan-2-one Dihydrochloride (18). A solution of 7-bromoheptan-2-one (5.0 g, 0.026 mol) and 4-(3-aminopropyl)morpholine (5.0 g, 0.031 mol) in THF was reacted and the product isolated as described for **15**. The reaction gave 1.2 g (14%) of **18**: mp 180 °C; ^1H NMR 1.7 (m, 8 H), 2.1 (s, 3 H), 2.5 (t, 2 H), 2.9 (t, 4 H), 3.1 (m, 6 H), 3.3 ppm (t, 4 H); CIMS m/e 257 (M + 1). Anal. ($C_{14}H_{30}Cl_2N_2O_2\cdot H_2O$) C, H, N.

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