Once the blood pressure was stabilized, the control values were taken and the compounds were injected intravenously (bolus) in cumulative and increasing doses. The effects were recorded continuously during 15 min. The dosages were 1, 3, 6, 10, 20, and 30 μg/kg when antihypertensive effects were recorded and 0.1 mg/kg when the hypertensive potency was evaluated. The compounds were dissolved in polyethylene glycol 400 (PEG-400; Merck, Germany) (0.1 mL/kg).

In *conscious* animals, systolic blood pressure was monitored by tail plethysmography. The animals were trained during 2 weeks prior to the monitoring procedure. On the day of the experiment the rats were placed at 31 ± 1 °C for 2 h, and blood pressure was recorded.

The measurement was the average of five determinations each time. Only animals with basal values over 170 mmHg systolic blood pressure were considered as hypertensive. After 24 h of fasting, the selected hypertensive animals were administered the compound and/or vehicle [20% PEG-400 in 0.5% aqueous methylcellulose (BDH) (10 mL/kg)] orally by gastric gavage. Data were expressed as percent change at 2 h post-treatment. Statistical analysis was performed by means of SPSS programs (ANOVA and Student's *t* test).

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# Inhibition of $N^8$-Acetylspermidine Deacetylase by Active-Site-Directed Metal Coordinating Inhibitors

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A number of substrate analogues of $N^8$-acetylspermidine ($N^8$-AcSpd) (16) and chemical modifying agents containing metal coordinating ligands were assayed as inhibitors of the cytoplasmic enzyme $N^8$-AcSpd deacetylase from rat liver. The enzyme is inhibited by metal chelators, several ω-amino-substituted carboxylic acids, and some thiol reagents. Inhibition by diisopropyl fluorophosphate was observed only at high concentrations. These results suggest that the catalytic mechanism of the enzyme requires a transition state metal and free sulfhydryl groups for activity. The most potent inhibitor synthesized 6-[(3-aminopropyl)amino]-*N*-hydroxyhexanamide (15), has an apparent $K_i$ of 0.001 μM. It binds to the target enzyme 11 000 times tighter than the substrate (apparent $K_m$ = 11 μM). These compounds and a previously reported series of compounds (Dredar, S. A.; Blankenship, J. W.; Marchant, P. E.; Manneh, V. A.; Fries, D. S. *J. Med. Chem.* **1989**, *32*, 984–989) are useful in mapping the active site and determining the physiological function of $N^8$-AcSpd deacetylase.

The naturally occurring polyamines, putrescine, spermidine, and spermine, play important roles in the regulation of DNA and RNA function and thereby affect cell growth and differentiation.[1-3] In previous publications from our laboratory,[4,5] the biochemical pathways of polyamine metabolism and interconversion were discussed. The focus of our work is the nuclear enzymatic acetylation of spermidine selectively at the N8 position[6,7] and the cytoplasmic deacetylation of this product to regenerate spermidine.[8,9] Polyamines, especially spermine and spermidine, are known to bind to and to stabilize DNA and chromosomal structure.[10-12] N-Acetylated polyamines have a reduced number of positive charges and are less efficient at stabilizing DNA than their nonacetylated precursors.[11] Thus, a simple hypothesis, that is depicted by the model shown in Figure 1, has been proposed to explain the function of the enzymatic nuclear acetylation and cytoplasmic deacetylation of spermidine. Inhibitors of the acetyltransferase and deacetylase enzymes are required to test the hypothesis. In this paper we describe the design, synthesis, and in vitro activity of potent inhibitors of $N^8$-acetylspermidine ($N^8$-AcSpd) deacetylase.

Care should be taken not to confuse the nuclear acetylation of spermidine with the cytoplasmic acetyltransferase which acetylates spermidine at the $N^1$ position.[13,14] $N^1$-Acetylspermidine is acted upon by polyamine oxidase to produce putrescine and 3-aminopropanal,[15,16] while $N^8$-AcSpd is deacetylated.

**Table I.** In Vitro Inhibition of $N^8$-AcSpd Deacetylase from Rat Liver Cytosol

| no. | structure or name | app $K_i$ (μM)[a] |
|---|---|---|
| 1 | diisopropyl fluorophosphate | 33000 |
| 2 | *p*-(hydroxymercurio)benzoate | 150 |
| 3 | *N*-ethylmaleimide | 860 |
| 4 | iodoacetamide | NI[b] |
| 5 | EDTA | 3500 |
| 6 | 2,2′-dipyridyl | 1900 |
| 7 | 1,10-phenanthroline | 1300 |
| 8 | 4,7-phenanthroline | NI[b] |
| 9 | sodium butyrate | 38500 |
| 10 | $H_2N(CH_2)_3COOH$ | 36500 |
| 11 | $H_2N(CH_2)_4COOH$ | 4100 |
| 12 | $H_2N(CH_2)_5COOH$ | 50 |
| 13 | $H_2N(CH_2)_4NHCOCH_3$ | 800 |
| 14 | $H_2N(CH_2)_3HN(CH_2)_5COOH$ | 11 |
| 15 | $H_2N(CH_2)_3HN(CH_2)_5CONHOH$ | 0.001 |
| 16 | $H_2N(CH_2)_3HN(CH_2)_4NHCOCH_3$ (substrate) | 11 ($K_m$) |

[a] The apparent $K_i$ values were determined from Dixon plots. [b] NI = no inhibition to $10^{-3}$ M.

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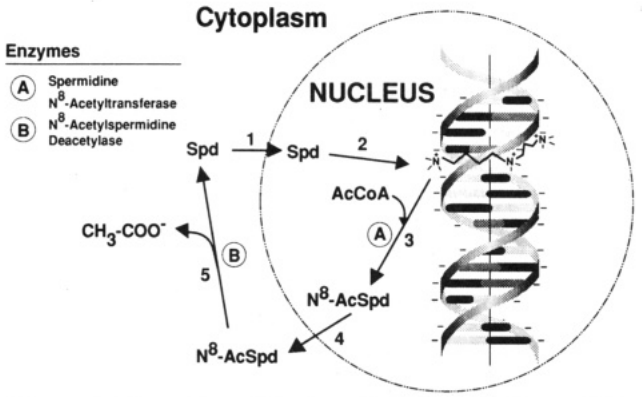


**Figure 1.** Illustration of the hypothesized cycle of spermidine acetylation and deacetylation. (1) Spermidine (Spd) enters the cell nucleus, (2) binds by ionic forces to sites on DNA, (3) is acetylated and dissociates from DNA, (4) diffuses to the cytoplasm where (5) it is deacetylated and the Spd released.

Our earlier efforts at finding inhibitors of $N^8$-AcSpd deacetylase were reasonably successful.[4] However, studies with active-site-modifying agents led us to believe that more potent and perhaps more selective inhibitors could be prepared for this enzyme. Thus, we describe in this article the preparation of compounds that contain a metal coordinating moiety and are potent inhibitors of $N^8$-AcSpd deacetylase.

## Inhibitor Design Rationale

A number of active-site chemical-modifying agents were tested in vitro for their effect on $N^8$-AcSpd deacetylase activity. The enzyme found in the 100 000*g* supernatant fraction from rat liver, was assayed as described in previous publications.[4,5] We have more recently verified the activities of the test compounds on a preparation of the purified enzyme.[17] The apparent $K_i$ values were determined by Dixon plots[18] and are presented in Table I.

Diisopropyl fluorophosphate (DFP; 1), a serine protease inhibitor, was relatively ineffective at inhibiting the deacetylase (apparent $K_i$ = 33 mM). DFP will inhibit most serine proteases at less than 1 mM.[19-21] This result indicates that serine is unlikely to be an essential constituent of the enzyme's active site.

The sulfhydryl binding agents *p*-(hydroxymercurio)-benzoate (**2**) and *N*-ethylmaleimide (**3**), but not iodoacetamide (**4**), inhibit the deacetylase. Thus, sulfhydryl groups may be important in the catalytic site (as nucleophiles or as ligands for holding metal cofactors) or in maintaining the enzyme in an active conformation. The activity of the larger and more lipophilic agents **2** and **3** may be due to their ability to react with a nonessential SH group (i.e. not located in the active site) and induce a conformational change, while the smaller compound **4** may react at the same site but does not induce the unfavorable conformational change. *N*-(Chloroacetyl)-1,6-diaminohexane was not an affinity label for the enzyme,[4] indicating that if the sulfhydryl group is in the active site it is not easily accessible by an affinity labeling agent.

We found the deacetylase activity to be inhibited by the metal chelators EDTA (**5**), 2,2′-dipyridyl (**6**), and 1,10-phenanthroline (**7**). The latter two compounds are reported to be selective inhibitors of enzymes containing transition metals.[22,23] In contrast to 1,10-phenanthroline, 4,7-phenanthroline (**8**) does not chelate transition metals and it is ineffective at inhibiting the enzyme. On the basis of the results of these chelating agents, the enzyme contains an essential divalent cation such as zinc or copper at its active site. Several studies have reported protease enzymes containing transition metals in their active sites and having sulfhydryl groups essential to their catalytic activity.[24,25]


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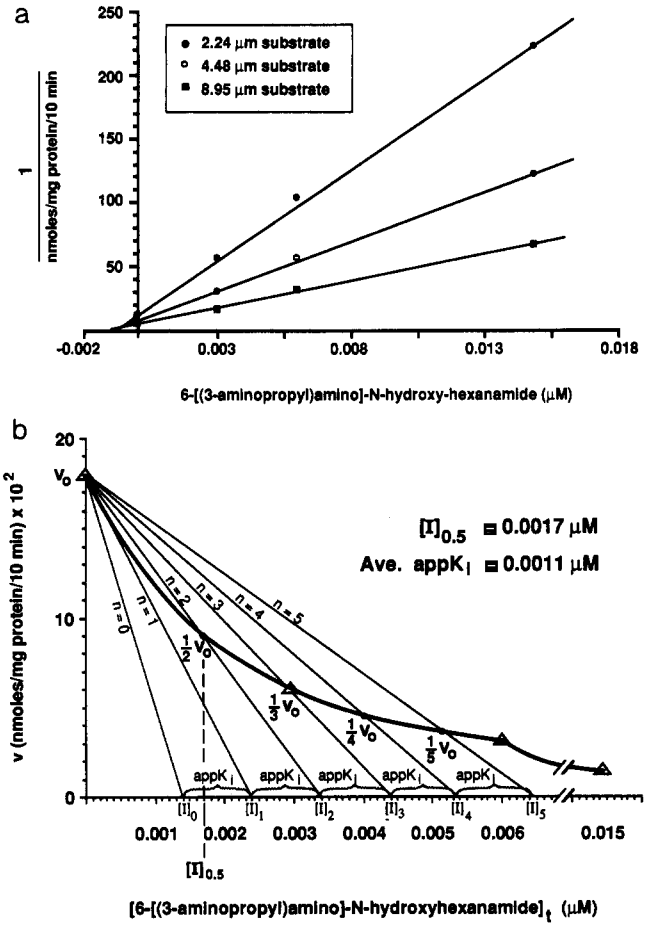


**Figure 2.** (A) Dixon plot of the reciprocal velocity of $N^8$-acetylspermidine deacetylation versus concentration of 6-[(3-aminopropyl)amino]-*N*-hydroxyhexanamide. The app $K_i$ from this plot is 0.0010 μM. (B) A modified Dixon plot (see ref 33) to investigate possible mutual depletion of the enzyme by the potent inhibitor. The affects are shown in the presence of 8.95 μM of the substrate and the average app $K_i$ is equivalent to that determined in graph A.

It had been reported previously that butyrate ions inhibit the deacetylation of histones.[26] It seemed possible that the inhibition might be due to a chelating action by the carboxylate group of butyrate and that the inhibitory effect might cross to the deacetylation of $N^8$-AcSpd. Sodium butyrate (**9**; Table I) was a weak inhibitor of the enzyme. We reasoned that proper positioning of the carboxyl group at the active site, by adding components of the substrate, would enhance the activity of the compound. Thus, the series of ω-amino-substituted carboxylic acids **10**–**12** was tested for inhibitory activity. Not suprisingly, 6-aminohexanoic acid (**12**) (app $K_i$ = 50 μM), which positions the carboxyl group at the same distance from the basic nitrogen as the amide group of acetylputrescine (**13**, app $K_i$ = 800 μM), is the best inhibitor in this series. It seemed reasonable that substitution of a stronger chelating group for the carboxyl moiety and/or inclusion of the additional aminopropyl group, as in $N^8$-AcSpd (see Figure 3 for structure comparisons), would provide more active inhibitors.[24,27-30] The synthesis and

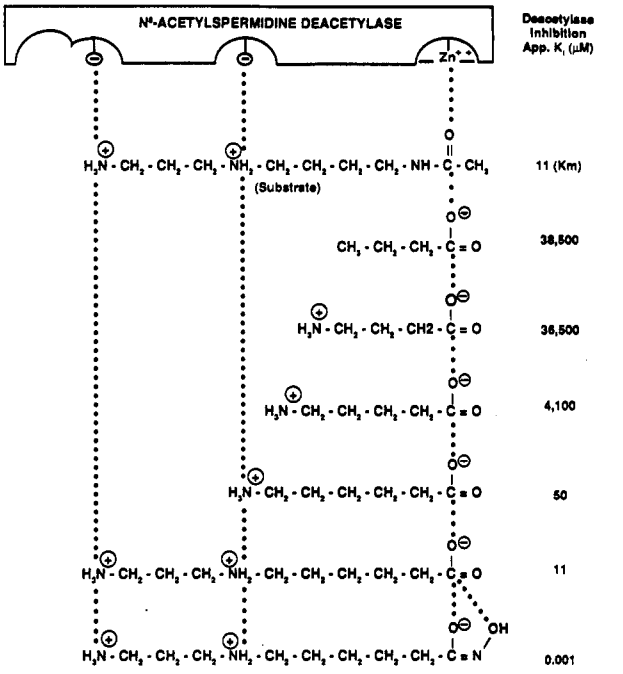


**Figure 3.** Proposed binding interactions of active-site-directed inhibitors to the active site of $N^8$-acetylspermidine deacetylase.

activities of such compounds are reported in this article.

## Chemistry

6-[(3-Aminopropyl)amino]hexanoic acid (**14**) and 6-[(3-aminopropyl)amino]-*N*-hydroxyhexanamide (**15**) were synthesized according to Scheme I. The mono-BOC-blocked diaminopropane **17** was obtained by reacting an excess of 1,3-diaminopropane with 2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile (BOC-ON) in tetrahydrofuran. Alkylation of **17** with ethyl 6-bromocaproate resulted in compound **18**. The protecting group (BOC) in **18** was removed in 3 N HCl–ethyl acetate followed by acid hydrolysis of the ester to yield **14**, as a dihydrochloride salt. Treatment of **18** with BOC-ON followed by alkaline hydrolysis resulted in the di-BOC acid derivative **19**. A coupling step with *O*-benzylhydroxylamine and the carboxylate group of **19**, in the presence of 1-hydroxybenzotriazole (HOBT) and dicyclohexylcarbodiimide (DCC), led to the fully protected inhibitor **20**. A sequential release of the protecting groups by acidic hydrolysis of the BOC groups and catalytic hydrogenolysis of the *O*-benzyl group led to the desired inhibitor **15**.

## Bioassay and Discussion

Inhibitory activities of the newly synthesized compounds on $N^8$-AcSpd deacetylase are listed in Table I. Addition of an aminopropyl group to **12** resulted in compound **14**. Compound **14** has a 5-fold increase in inhibitory activity compared to **12**. The compound exhibited competitive

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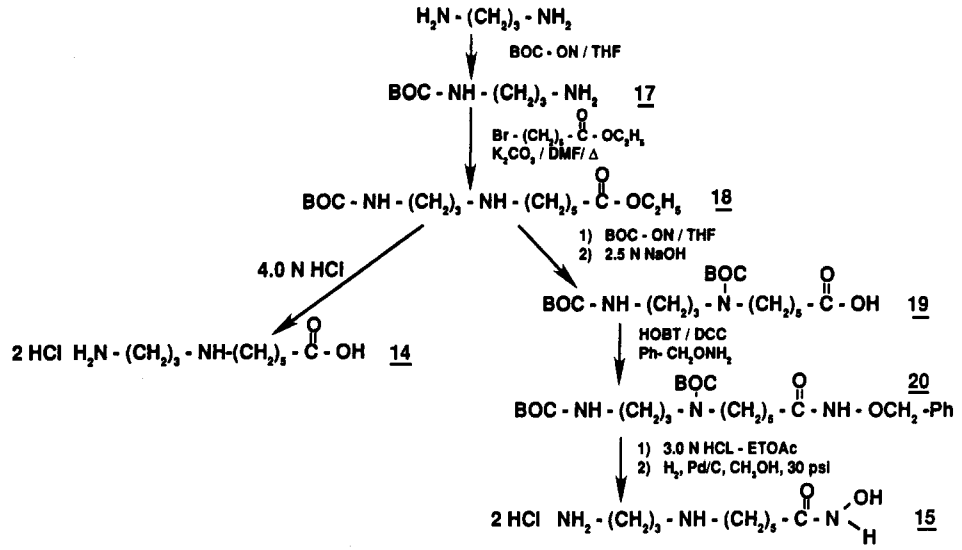
**Scheme I.** Synthesis of 6-[(3-Aminopropyl)amino]hexanoic Acid (14) and 6-[(3-Aminopropyl)amino]-*N*-hydroxyhexanamide (15)




inhibition kinetics. Inhibitory activity was greatly enhanced by addition of the bidentate hydroxamic acid ligand to **14**. The resultant compound (**15**) has an apparent $K_i$ = 1.0 nM, a value 4 orders of magnitude lower than the $K_m$ of the substrate and 2 orders of magnitude lower than the best inhibitor previously reported.[4] Analysis of the inhibition of **15**, using Dixon and Lineweaver–Burk plots, revealed noncompetitive inhibition kinetics. The high potency of **15** raised the question of "mutual depletion" occurring in the determination of its kinetic properties.[31] If mutual depletion were occurring, normal Michaelis–Menton graphical methods for determining inhibition constants may not be valid.[32] To verify the activity of **15**, a graphical method developed by Dixon et al.[33] for systems involving tightly bound inhibitors was used to analyze the kinetic data. Results of the analysis of the kinetic data are given in Figure 2. It can be seen that the two methods of Dixon analysis give essentially the same apparent $K_i$ value; thus, mutual depletion is not a factor in the kinetic analysis.

Figure 3 gives a representation of the activities of the various compounds versus their proposed binding to the active site of the enzyme. Compound **15**, which can form ionic interactions at $N^1$ and at $N^4$ and has a bidentate ligand to bind to the zinc in the inhibitor–enzyme complex, is the most potent inhibitor. These results validate our drug design rationale.

In order to verify that our results do not reflect interactions other than those with the deacetylase, we have purified $N^8$-AcSpd deacetylase and determined the activities of the inhibitors on the purified enzyme.[17] The enzyme from rat liver cytosol was purified 1375-fold by precipitation with $(NH_4)_2SO_4$, elution through a DEAE cellulose column, and final separation by HPLC on a TSK-G 3000 SW column. Details of the purification will be reported in a separate publication. Inhibitor $K_i$ values determined on the purified enzyme were similar to the results obtained on the crude cytosolic preparation of the enzyme. For example, the $K_m$ of **16** remained 11 $\mu$M, and the $K_i$ values for EDTA and **15** were 4.4 mM and 0.1 nM, respectively. The results indicate that for these compounds the results determined on the cytosolic fraction are indicative of the actions on the purified enzyme.

## Experimental Section

**Chemistry.** Sodium butyrate, $\gamma$-aminobutyric acid and 5-aminovaleric acid were purchased from Sigma Chemical Co. 6-Bromocaproate was purchased from Pfaltz and Bauer, Inc. All other chemicals were purchased from Aldrich Chemical Co. Solvents were American Chemical Society reagent grade or better quality and were used without further purification.

Melting points were determined in open capillary tubes on a Thomas-Hoover Unimelt apparatus and are uncorrected. The IR were recorded on thin films of mineral oil dispersions by using a Perkin-Elmer 457 spectrophotometer. Proton NMR were recorded on a Varian XL-200 or a Varian XL-300 spectrometer using $D_2O$ or $CDCl_3$ as solvents and the water or TMS peak as reference lines, respectively. Carbon-13 NMR were recorded on a Nicolet-360 spectrometer, using $D_2O$ as a solvent. Mass spectra were obtained by using a Hitachi Perkin-Elmer RMS-4 instrument (CI, 70 eV). Scintillation counting was done on a Packard Tri-Carb 300 instrument. 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.

Thin-layer chromatography was performed on plates purchased from Whatman (60F-254; 5 × 10 cm; 0.25-mm silica gel layer). Compounds were visualized by spraying with ninhydrin or stained with iodine vapor. For absorption chromatography, Whatman silica gel, grade 60, 230–400 mesh, was used. For the hydrogenation reactions, a standard 500-mL Parr shaker type hydrogenation apparatus was used.

**Enzyme Assays and Inhibition Studies.** Assays for rat liver $N^8$-AcSpd deacetylase activity followed procedures used previously in this laboratory.[4,5] Each assay mixture consisted of 0.25 M sucrose, 0.05 M $NaH_2PO_4$ buffer (pH 7.4), 0.005 M $MgCl_2$, 100 $\mu$L of the cytosol fraction (1.8–2.2 mg protein), different concentrations of the potential inhibitors, and 1.45–8.95 $\mu$M of $N^8$-[*acetyl*-$^3$H]acetylspermidine in a total volume of 0.5 mL. In cases where chelating agents were being tested, the assays were run with and without the $MgCl_2$ and identical results were obtained. The assay mixture was incubated for 10 min at 37 °C and halted on ice with addition of 500 $\mu$L of 1.0 N HCl–0.05 M acetic acid. The free [$^3$H]acetic acid was extracted with ethyl acetate (EtOAc), and the radioactivity was measured by liquid scintillation spectrometry.

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. The apparent $K_i$ of each inhibitor was determined by using a Dixon plot.[18] The protein content was determined by the method of Lowry et al.[34]

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**Synthesis. *N*-(*tert*-Butoxycarbonyl)-1,3-diaminopropane (17).** 1,3-Diaminopropane (10.0 g, 0.134 mol) was dissolved in tetrahydrofuran (THF) (75 mL). To the stirred solution was added 2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile (BOC-ON) (16.5 g, 0.067 mol) in THF (75 mL) slowly over a period of 4 h, and the reaction was allowed to proceed overnight at room temperature, and under an $N_2$ atmosphere. The solvent (THF) was evaporated on the rotary evaporator, and the crude product was purified by column chromatography on silica gel using $CH_2Cl_2$–$CH_3OH$–$NH_4OH$ (7:2:1) as eluent. The yield was 7.7 g (66%): $^1H$ NMR ($CDCl_3$) ppm 1.44 (s, 9 H), 1.64 (p, 2 H), 2.8 (t, 2 H), 3.2 (m, 2 H), 3.44 (s, 2 H), 5.17 (br s, 1 H).

**Ethyl 6-[[[3-(*tert*-Butoxycarbonyl)amino]propyl]amino]hexanoate (18).** A mixture of 17 (13.5 g, 0.077 mol), ethyl 6-bromocaproate (10.0 g, 0.045 mol), anhydrous $K_2CO_3$ (32.1 g, 0.233 mol), and 140 mL of sieve-dried dimethylformamide was stirred at 60 °C for 18 h. The reaction mixture was cooled, and 500 mL of distilled $H_2O$ was added. The mixture was then extracted with EtOAc (3 × 300 mL), and the combined organic extracts were washed with saturated NaCl solution (300 mL) and dried over anhydrous $Na_2SO_4$. Evaporation of the solvent left a crude product which was purified by column chromatography on silica gel using $CH_2Cl_2$–$CH_3OH$–$NH_4OH$ (8.0:1.5:0.5) as eluent. The yield was 6.9 g (48%): $^1H$ NMR ($CDCl_3$) ppm 1.25 (t, 3 H), 1.45 (s, 11 H), 1.65 (m, 6 H), 2.3 (t, 2 H), 2.64 (m, 4 H), 3.2 (m, 2 H), 4.12 (q, 2 H), 5.3 (br s, 1 H).

**6-[(3-Aminopropyl)amino]hexanoic Acid Dihydrochloride (14).** A solution of 2.0 g of 18 (0.006 mol) in 3 N HCl–EtOAc (25 mL) was allowed to stand at room temperature for 1 h. The solution was filtered, and the precipitate was rinsed with cold EtOAc to yield 1.8 g of white solid in which the BOC group had been removed. Ester hydrolysis was carried out by dissolving 206 mg of the white solid in 15 mL of 4 N HCl and stirring at room temperature for 2 days. The aqueous solution was evaporated under reduced pressure, and the product was purified by recrystallization from 2-propanol to yield 110 mg (59%) of 14: TLC ($MeCl_2$–MeOH–$NH_4OH$; 6:5:2) of 14 revealed only one spot ($R_f$ = 0.3); mp 169–171 °C; IR (mineral oil) 1700 (C=O), 3300–2600 ($N^+$–H) $cm^{-1}$; $^1H$ NMR ($D_2O$) ppm 1.41 (p, 2 H), 1.67 (m, 4 H), 2.08 (p, 2 H), 2.39 (t, 2 H), 3.1 (m, 6 H); $^{13}C$ NMR ($D_2O$) 23.50, 24.95, 33.28, 36.40, 44.20, 47.40, 178.59; CIMS *m/e* 189 (M + 1). Anal. ($C_9H_{22}O_2N_2Cl_2$) C, H, N.

***N*,*N*′-Bis(*tert*-butoxycarbonyl)-6-[(3-aminopropyl)amino]hexanoic Acid (19).** A solution of BOC-ON (3.113 g, 0.013 mol) in THF (20 mL) was added dropwise to a solution of 18 (4.0 g, 0.013 mol) in THF (30 mL), and the mixture was stirred for 4 days at room temperature under a $N_2$ atmosphere. The solvent was then evaporated, and the residue was dissolved in 100 mL of ether, washed with 5% NaOH (4 × 40 mL), and dried over anhydrous $Na_2SO_4$. The solvent was evaporated, and the crude product was purified by column chromatography using silica gel and EtOAc as the eluent to yield 5.2 g (96%) of product. To a solution of this product (4.0 g, 0.01 mol) in EtOH (20 mL) and $H_2O$ (10 mL) cooled to 0 °C was added 25 mL of 2.5 N NaOH. The mixture was stirred at 0 °C for 1 h and at room temperature for 4 h. The solution was concentrated in vacuo, diluted with 30 mL of $H_2O$, washed with ether (4 × 10 mL), acidified to pH 2.0 with 2 N HCl, and extracted with ether (4 × 25 mL). The combined extracts were dried over anhydrous $Na_2SO_4$ and evaporated in vacuo to yield 19 (3.4 g, 91%) as a pale yellow oil: IR (neat) 1700 (C=O), 3500–2600 (O—H) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) ppm 1.20 (t, 3 H), 1.40 (d, 20 H), 1.55 (m, 6 H), 2.24 (t, 2 H), 3.1 (m, 6 H), 4.1 (q, 2 H).

***N*-(Benzyloxy)-6-[*N*′-[*N*″-(*tert*-butoxycarbonyl)-3-aminopropyl]-*N*′-(*tert*-butoxycarbonyl)amino]hexanamide (20).** To a solution of 19 (1.0 g, 2.57 mmol) in THF (10 mL), cooled to 0 °C, was added successively *O*-benzylhydroxylamine hydrochloride (0.4102 g, 2.57 mmol) and triethylamine (0.2631 g, 2.6 mmol) in $CHCl_3$ (7 mL), 1-hydroxybenzotriazole (0.3473 g, 2.57 mmol) in THF (7 mL), and dicyclohexylcarbodiimide (0.5842 g, 2.83 mmol) in $CHCl_3$ (7 mL). After 1 h at 0 °C, the mixture was stirred at room temperature for 2 days. After filtration of the dicyclohexylurea and evaporation of the solvents, the residue was dissolved in EtOAc and washed successively with $H_2O$ (2 × 25 mL), 10% citric acid (3 × 25 mL), $H_2O$ (25 mL), 10% $NaHCO_3$ (3 × 25 mL), $H_2O$ (25 mL), and finally with saturated NaCl (25 mL). The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated in vacuo to yield 0.70 g (55%) of 20 as a colorless oil. TLC ($CHCl_3$–MeOH; 9.5:0.5) of the oil showed a single spot with $R_f$ = 0.30. The product was used in the next step without further purification: $^1H$ NMR ($CDCl_3$) ppm 1.36 (d, 20 H), 1.55 (m, 6 H), 1.8 (t, 2 H), 3.1 (m, 6 H), 4.8 (s, 2 H), 7.3 (s, 5 H).

**6-[(3-Aminopropyl)amino]-*N*-hydroxyhexanamide Dihydrochloride (15).** A solution of 1.376 g of 20 (2.79 mmol) in 3 N HCl–EtOAc was allowed to stand at room temperature for 1 h. The solution was filtered, and the precipitate was rinsed with cold EtOAc to yield 0.80 g of white solid from which the BOC groups had been removed. The white solid (0.5 g; 1.365 mmol) in MeOH (10 mL) was added to a suspension of 10% Pd on activated carbon (0.175 g) in MeOH (10 mL), and the mixture was shaken at room temperature under hydrogen (30 psi) in a Parr hydrogenation apparatus for 4 h. After filtration, the solvent was evaporated in vacuo. A yellowish solid was obtained which was purified by recrystallization from absolute EtOH. The yield of 15 was 0.291 g (77% yield): mp 131–134 °C; TLC ($MeCl_2$–MeOH–$NH_4OH$; 7.0:2.5:1.5) $R_f$ = 0.43; IR (mineral oil) 1650 (C=O), 3300–2600 ($N^+$ – H) $cm^{-1}$; $^1H$ NMR ($D_2O$) ppm 1.4 (p, 2 H), 1.67 (m, 4 H), 2.08 (p, 2 H), 2.2 (t, 2 H), 3.1 (m, 6 H); $^{13}C$ NMR ($D_2O$) 23.55, 24.15, 24.83, 24.99, 31.79, 36.39, 44.20, 47.36, 172.86. Anal. ($C_9H_{23}N_3O_2Cl_2$) C, H, N.

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