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Synthesis and evaluation of N^8-acetylspermidine analogues as inhibitors of bacterial acetylpolyamine amidohydrolase

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ABSTRACT

Polyamines are small essential polycations involved in many biological processes. Enzymes of polyamine metabolism have been extensively studied and are attractive drug targets. Nevertheless, the reversible acetylation of polyamines remains poorly understood. Although eukaryotic N^8-acetylspermidine deacetylase activity has already been detected and studied, the specific enzyme responsible for this activity has not yet been identified. However, a zinc deacetylase from *Mycoplana ramosa*, acetylpolyamine amidohydrolase (APAH), has been reported to use various acetylpolyamines as substrates. The recently solved crystal structure of this polyamine deacetylase revealed the formation of an 'L'-shaped active site tunnel at the dimer interface, with ideal dimensions and electrostatic properties for accommodating narrow, flexible, cationic polyamine substrates. Here, we report the design, synthesis, and evaluation of N^8-acetylspermidine analogues bearing different zinc binding groups as potential inhibitors of APAH. Most of the synthesized compounds exhibit modest potency, with IC_{50} values in the mid-micromolar range, but compounds bearing hydroxamate or trifluoromethylketone zinc binding groups exhibit enhanced inhibitory potency in the mid-nanomolar range. These inhibitors will enable future explorations of acetylpolyamine function in both prokaryotes and eukaryotes.

1. Introduction

Polyamines such as putrescine, spermidine, and spermine are ubiquitous in living organisms and implicated in numerous essential biological processes.[1] For instance, polyamine concentrations affect the cell cycle progression through tightly regulated biosynthetic pathways.[1,2] At the molecular level, since polyamines are polycations, they can bind to nucleic acids and modulate DNA–protein interactions.[2] Given the importance of polyamines in different cellular processes, various enzymes of polyamine metabolism have been studied as potential drug targets.[3,4] For example, since upregulation of polyamine biosynthesis is a hallmark of certain cancers,[5,6] inhibitors of ornithine decarboxylase (ODC), *S*-adenosylmethionine decarboxylase, spermidine synthase, and spermine synthase have been evaluated in approaches to cancer chemotherapy. Depletion of putrescine and spermidine was achieved in vitro by cell treatment with the irreversible ODC inhibitor α-difluoromethylornithine (DFMO),[7] leading to inhibition of cell growth.[8] Although DFMO failed in clinical trials as a cancer chemotherapeutic agent, it was approved by the FDA for the treatment of parasitic infections such as African sleeping sickness.[9]

While most of the enzymes of polyamine metabolism have been extensively studied, enzymes involved in the reversible acetylation of polyamines are less well understood. The acetylation of polyamines decreases their overall charge, which is believed to regulate their function in vivo. Indeed, acetylated polyamines destabilize nucleosome structure, whereas the corresponding free polyamines bind to DNA and facilitate condensation.[10,11] In eukaryotes, spermidine can be either N^1- or N^8-acetylated by two distinct enzymes: a cytoplasmic spermidine/spermine N^1-acetyltransferase[12] or a nuclear spermidine N^8-acetyltransferase.[13] Despite similar structures, N^1- and N^8-acetylspermidine are metabolized differently once formed: N^1-acetylspermidine is catabolized to putrescine by a cytosolic polyamine oxidase,[14] and N^8-acetylspermidine is hydrolyzed to generate spermidine by a specific cytosolic N^8-acetylspermidine deacetylase that is unable to use N^1-acetylspermidine as a substrate.[15]

Even though the N^8-acetylspermidine deacetylase activity has been characterized and studied in vivo and in subcellular fractions, no eukaryotic polyamine deacetylase has been identified to date.

Abbreviations: APAH, acetylpolyamine amidohydrolase; dppm, bis(diphenylphosphino)methane; 9-BBN, 9-borabicyclo[3.3.1]nonane; DMP, Dess–Martin periodinane; DFMO, α-difluoromethylornithine; Boc_2O, di-*tert*-butyl pyrocarbonate; ESI, electrospray ionization; HRMS, high-resolution mass spectrometry; HDAC, histone deacetylase; *m*-CPBA, *m*-chloroperbenzoic acid; NMO, *N*-methylmorpholine *N*-oxide; CDI, *N*,*N*′-carbonyldiimidazole; ODC, ornithine decarboxylase; rt, room temperature; Boc, *tert*-butoxycarbonyl; TBAF, tetra-*n*-butylammonium fluoride; $TMSCF_3$, trifluoromethyltrimethylsilane; PPh_3, triphenylphosphine.

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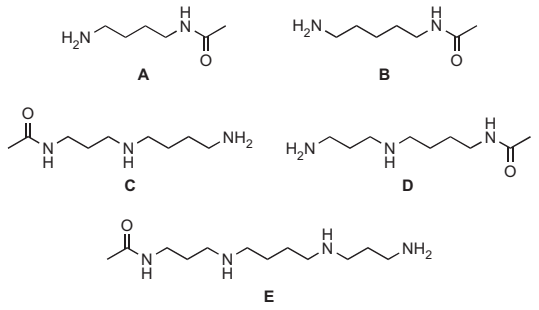


Figure 1. Substrates of APAH from *Mycoplana ramosa*: (A) acetylputrescine, (B) acetylcadaverine, (C) N^1-acetylspermidine, (D) N^8-acetylspermidine, and (E) N^1-acetylspermine.

However, a prokaryotic polyamine deacetylase has been reported: acetylpolyamine amidohydrolase (APAH) from *Mycoplana ramosa*.[16] Notably, APAH has broader substrate specificity in comparison with the mammalian enzyme. As shown in Figure 1, APAH substrates include both small and large acetylpolyamines such as acetylputrescine, acetylcadaverine, N^1- and N^8-acetylspermidine, and N^1-acetylspermine.[16,17] Bacterial APAH is a dimeric zinc-dependent hydrolase[16,17] as recently confirmed in crystal structure determinations of APAH from *M. ramosa*[18] and from *B. pseudomallei*.[19] As previously proposed,[20] APAH adopts the α/β fold first observed for the binuclear manganese metalloenzyme arginase[21] and also shared by the histone deacetylases (HDACs).[22–24] Key active site residues required for the chemical mechanism of deacetylation are conserved between APAH and HDACs.[25] In contrast with the HDACs, the dimerization of APAH results in the formation of a narrow 'L'-shaped active site at the dimer interface,[18] conferring specificity for slender, flexible substrates rather than the large peptide substrates typically processed by HDACs. This structural feature now guides the design of specific inhibitors of APAH.

Inhibitors of APAH will be helpful tools for the exploration of both prokaryotic and eukaryotic acetylpolyamine function in vivo. For instance, inhibitors of the mammalian N^8-acetylspermidine deacetylase have been described earlier, some of which exhibit low nanomolar inhibitory activity.[26,27] So far, the HDAC inhibitor M344[28] and the trifluoromethylketone analogue of L-arginine[29] are the only compounds reported to inhibit the bacterial polyamine deacetylase in the low and mid-micromolar range, respectively.[18,29] Here, we report the synthesis of new polyamine derivatives as well as new synthetic routes for some of the previously described mammalian N^8-acetylspermidine deacetylase inhibitors.[26,27] All compounds synthesized in the current study are analogues of N^8-acetylspermidine bearing different functional groups targeting Zn^{2+} coordination. We also report the inhibitory potency of these compounds against *M. ramosa* APAH.

2. Results and discussion

2.1. Inhibitor design

The X-ray crystal structure of inactive H159A APAH complexed with N^8-acetylspermidine (PDB accession code 3Q9C) illustrates the molecular details of substrate recognition in the enzyme active site.[18] Key interactions are made by the N4 secondary amino group of N^8-acetylspermidine, which donates a hydrogen bond to E117 and makes a cation–π interaction with F225; the N1 primary amino group, which donates a hydrogen bond to E106 in the other monomer of the homodimer; the amide NH group, which donates a hydrogen bond to the backbone carbonyl of G167; and the amide carbonyl group, which coordinates to the Zn^{2+} ion and accepts a hydrogen bond from Y323. Based on the mechanism of catalysis by the related HDACs,[30–32] a nucleophilic Zn^{2+}-bound water molecule is activated by metal coordination and general base H159 (Fig. 2). Nucleophilic attack at the scissile carbonyl group of the substrate results in the formation of a tetrahedral intermediate stabilized by metal coordination and hydrogen bond interactions with surrounding residues. The collapse of this intermediate is enabled by H159, which serves as a general acid catalyst in this step of the mechanism,[31] leading to the formation of products spermidine and acetate.[25]

Based on the structural features important for substrate recognition and catalysis, including the tetrahedral structure of the transition state flanking the tetrahedral intermediate, we designed and synthesized potential APAH inhibitors based on the N^8-acetylspermidine substrate-like scaffold. As shown in Figure 3, compounds **I–X** all share a common 1,3-diaminopropane moiety to preserve key enzyme–substrate hydrogen bond interactions with the polyamine N1 and N4 groups (Fig. 2). However, each compound differs in the nature of its head group designed to mimic substrate or tetrahedral transition state binding to the active site Zn^{2+} ion. Most of these Zn^{2+}-binding groups have been successfully incorporated into effective inhibitors of HDACs[29,33–39] and other metallohydrolases.[40–43]

2.2. Chemistry

Syntheses of compounds **I–X** are summarized in Schemes 1–4. Compounds **I–X** were each synthesized from key intermediates **3** or **4**. As shown in Scheme 1, **3** and **4** were obtained in two steps. In the first step, 1,3-diaminopropane was *N*-alkylated with an alkylbromide, either 5-bromopent-1-ene or 7-bromohept-1-ene, to yield alkylamines **1** and **2**, respectively. This reaction was performed with an excess of 1,3-diaminopropane (10 equiv, neat) to favor the monoalkylation of the unprotected diamine over polyalkylation. Monoalkylamines **1** and **2** were then quantitatively *N*-protected with *tert*-butoxycarbonyl (Boc) groups using di-*tert*-butyl pyrocarbonate (Boc_2O).

We developed an alternative route for the synthesis of compounds **I**, **II**, and **III** compared with that previously published.[26,27] The new synthetic route allows for more flexibility in generating additional compounds from intermediate **4**. As depicted in Scheme 2, compounds **I**, **II**, and **III** were synthesized from carboxylic acid **5**. Oxidative cleavage of alkene **4** into **5** was performed by the Sharpless method with ruthenium chloride as catalyst and sodium periodate as oxidant[44,45] in solvent system H_2O/AcOEt/MeCN (3/2/2).[46] *N*,*N*′-Carbonyldiimidazole (CDI) mediated coupling of carboxylic acid **5** with *N*-*O*-dimethylamine quantitatively led to Weinreb amide **6**. *N*-Methoxy-*N*-methylamides are well-known

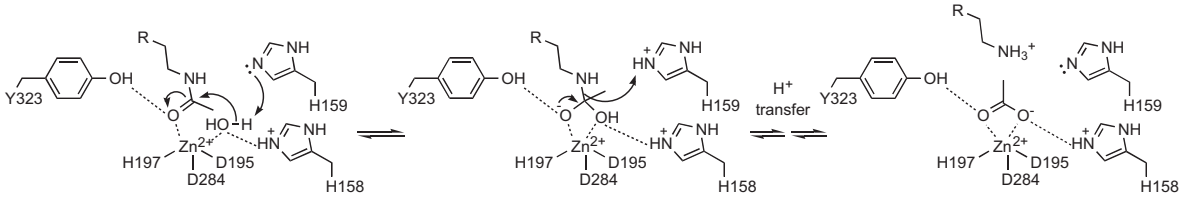


Figure 2. Mechanism of APAH.

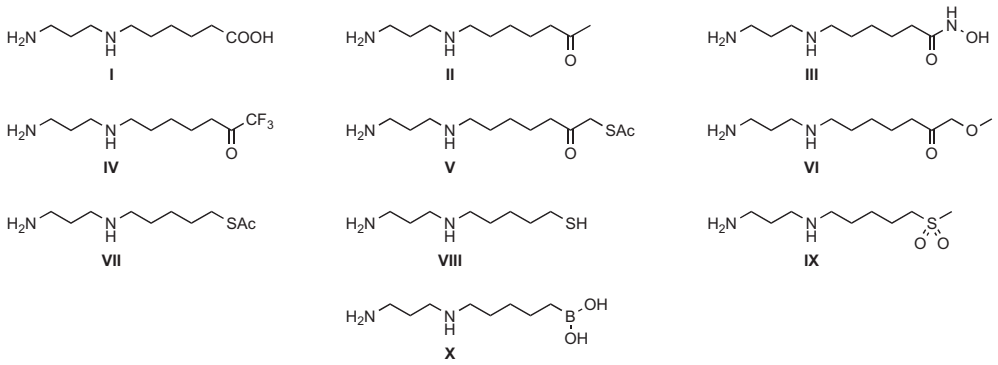
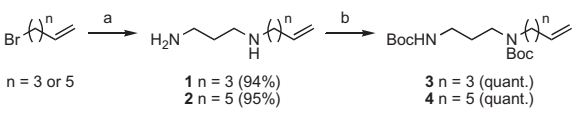


Figure 3. Structures of target compounds as potential APAH inhibitors. These compounds are analogues of the substrate N^8-acetylspermidine (Fig. 1D).

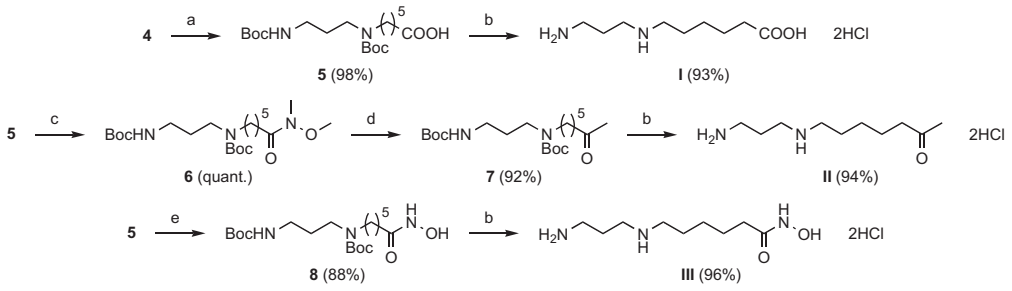


Scheme 1. Synthesis of intermediates **3** and **4**. Reagents and conditions: (a) 1,3-diaminopropane (neat, 10 equiv), 0 °C (1 h) then room temperature (RT) (2 h); (b) Boc_2O (3 equiv) in CH_2Cl_2, 0 °C then rt (overnight).

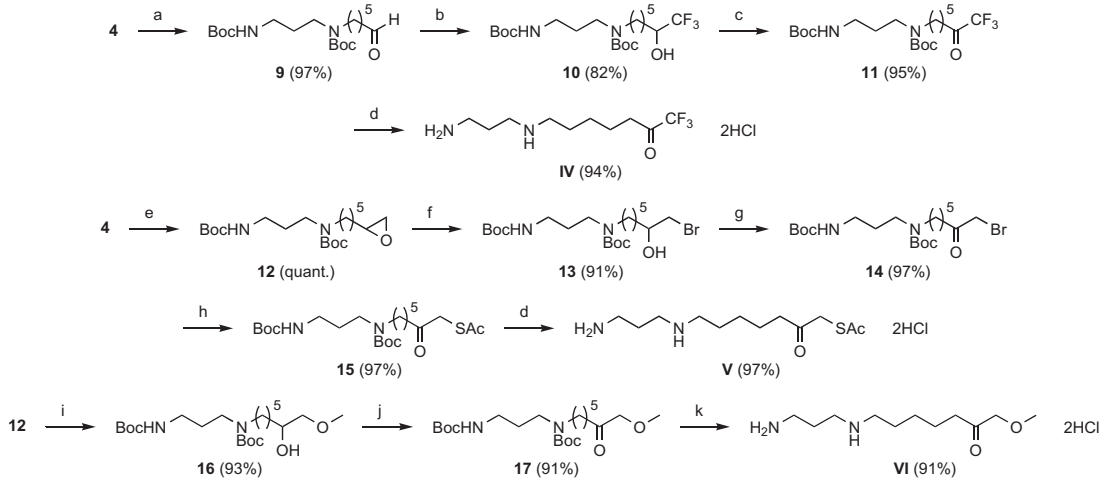
reagents for the synthesis of ketones from carboxylic acids in the presence of Grignard or organolithium reagents.[47] Using this strategy, methylketone **7** was synthesized from Weinreb amide **6** with an excess of methylmagnesium bromide (5 equiv). CDI-activated carboxylic acid **5** was reacted with unprotected hydroxylamine to form the corresponding hydroxamic acid **8**.[48] Deprotection of compounds **5**, **7**, and **8** with anhydrous HCl (4 N) in dioxane at room temperature led to target compounds **I**, **II**, and **III** as dihydrochloride salts.

Alkene **4** also served as a key common intermediate for the synthesis of compounds **IV**, **V**, and **VI** as shown in Scheme 3. As for the synthesis of carboxylic acid **5**, introduction of the aldehyde functionality was achieved by an oxidative cleavage of alkene **4**. This was done sequentially by first oxidizing **4** to the corresponding cis-diol with osmium tetroxide as catalyst in the presence of *N*-methylmorpholine *N*-oxide (NMO) in dioxane/H_2O (4/1) as solvent[49] until completion of the reaction (3 h). The cis-diol derived from **4** was not isolated, but directly cleaved to aldehyde **9** by reaction with sodium periodate. Nucleophilic trifluoromethylation of aldehyde **9** was achieved using Ruppert's reagent (trifluoromethyltrimethylsilane, $TMSCF_3$)[50] and a source of fluoride, tetra-*n*-butylammonium fluoride (TBAF), as initiator under usual conditions.[51,52] Trifluoromethyl carbinol **10** was then easily oxidized to the corresponding trifluoromethyl ketone **11** by Dess–Martin periodinane (DMP).[53,54] Alkene **4** was quantitatively oxidized to epoxide **12** using *m*-chloroperbenzoic acid (*m*-CPBA). Regioselective ring opening of unsymmetrically substituted epoxide **12** was achieved using lithium bromide and acetic acid yielding α-bromohydrin **13**.[55] Subsequent alcohol oxidation of **13** with DMP led to the formation of α-bromoketone **14**.[53] Bromide **14** was then treated with potassium thioacetate to afford thioester **15**. Oxirane **12** also served as a precursor for the synthesis of α-methoxyketone derivative **VI**. Ring opening of its epoxide moiety with sodium methoxide in MeOH regioselectively formed the corresponding α-methoxyalcohol **16**, after which oxidation with DMP afforded α-methoxyketone **17**.[53] Deprotection of compounds **11**, **15**, and **17** with anhydrous HCl (4 N in dioxane, or 1 N in AcOEt) at room temperature led to target compounds **IV**, **V**, and **VI** as dihydrochloride salts. Synthesis of the α-mercaptoketone from thioester **15** was attempted. Successful thioacetate group alcoholysis in MeOH under basic conditions (sodium methoxide) gave the corresponding *N*-Boc protected α-mercaptoketone, but no pure deprotected α-mercaptoketone could be isolated after deprotection under acidic conditions.

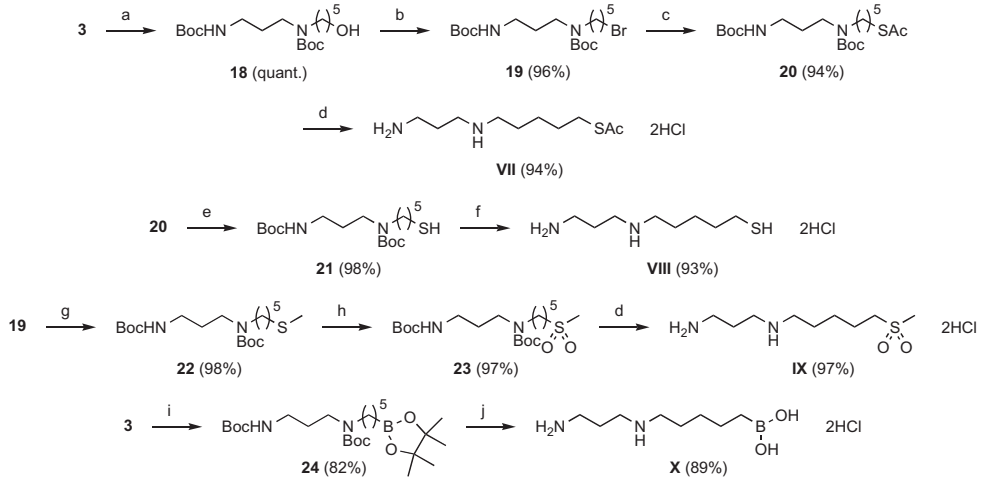
As shown in Scheme 4, alkene **3** was used as precursor for compounds **VII**–**X**. Alkene **3** hydroboration with 9-borabicyclo [3.3.1]nonane (9-BBN)[56] followed by oxidation with H_2O_2 under basic conditions (NaOH) quantitatively afforded alcohol **18**. Subsequent bromination of **18** was achieved using carbon tetrabromide (CBr_4) in the presence of triphenylphosphine (PPh_3). Corresponding alkyl bromide **19** was then treated with potassium thioacetate to afford **20**, as for the synthesis of thioesther **15**. Thioacetate group alcoholysis in MeOH under basic conditions (sodium methoxide) gave thiol **21**. Alkyl bromide **19** also served a precursor for the synthesis of sulfone derivative **IX**. Nucleophilic substitution of bromine by sodium thiomethoxide in EtOH afforded thioether **22**, after which oxidation with *m*-CPBA led to sulfone **23**. Hydroboration of alkene **3** with pinacolborane using $[Ir(cod)Cl]_2$ as catalyst and 1,1-bis(diphenylphosphino)methane (dppm) as ligand under usual conditions selectively gave terminal boronic ester **24**.[57] Complete



Scheme 2. Synthesis of compounds **I**–**III**. Reagents and conditions: (a) $RuCl_3$ (4 mol %), $NaIO_4$ (4.5 equiv) in H_2O/AcOEt/MeCN (3/2/2), rt (3 h); (b) anhydrous HCl (4 N) in dioxane, rt (2 h); (c) CDI (2 equiv) in CH_2Cl_2, rt (1 h), then MeNHOMe·HCl (2 equiv), rt (overnight); (d) MeMgBr (5 equiv) in THF, 0 °C (2 h) then rt (1 h); (e) CDI (1.5 equiv) in THF, rt (1 h), then NH_2OH·HCl (2 equiv), rt (overnight).



Scheme 3. Synthesis of compounds **IV**–**VI**. Reagents and conditions: (a) NMO (2.5 equiv), OsO_4 (2.5 mol %) in dioxane/H_2O (4/1), rt (3 h), then $NaIO_4$ (2.5 equiv), rt (20 min); (b) $TMSCF_3$ (3 equiv), TBAF (10 mol %) in THF, rt (2 h), then TBAF (in THF containing ca. 5% H_2O, 1.5 equiv), rt (45 min); (c) DMP (4 equiv) in CH_2Cl_2, rt (overnight); (d) anhydrous HCl (4 N) in dioxane, rt (2 h); (e) *m*-CPBA (2 equiv) in CH_2Cl_2, 0 °C then rt (21 h); (f) LiBr (3.2 equiv), AcOH (3 equiv) in THF, rt (overnight); (g) DMP (3 equiv), rt (3 h); (h) KSAc (6 equiv) in MeCN, rt (overnight); (i) NaOMe (6 equiv) in MeOH, rt (24 h); (j) DMP (6 equiv) in CH_2Cl_2, rt (24 h); (k) anhydrous HCl (1 N) in AcOEt, rt (6 h).



Scheme 4. Synthesis of compounds **VII**–**X**. Reagents and conditions: (a) 9-BBN (2.5 equiv) in THF, 0 °C then rt (20 h), then NaOH, H_2O_2, 0 °C then rt (30 min); (b) CBr_4 (2 equiv), PPh_3 (2 equiv) in THF, 0 °C then rt (overnight); (c) KSAc (6 equiv) in MeCN, rt (overnight); (d) anhydrous HCl (4 N) in dioxane, rt (2 h); (e) NaOMe (2 equiv) in MeOH, rt (2 h); (f) anhydrous HCl (1 N) in AcOEt, rt (5 h); (g) NaSMe (6 equiv) in EtOH, 60 °C (overnight); (h) *m*-CPBA (3 equiv) in CH_2Cl_2, 0 °C then rt (2 h); (i) $[Ir(cod)Cl]_2$ (5 mol %), dppm (10 mol %), pinacolborane (1.9 equiv), rt (24 h); (j) aqueous HCl (6 N), reflux (24 h).

deprotection of **24** was achieved in aqueous HCl (6 N) under reflux affording compound **X** as a dihydrochloride salt. Deprotection of compounds **20**, **21**, and **23** with anhydrous HCl (4 N in dioxane, or 1 N in AcOEt) at room temperature led to target compounds **VII**, **VIII**, and **IX** as dihydrochloride salts.

2.3. Enzyme inhibition

All N^8-acetylspermidine analogues were tested in vitro for APAH inhibition. Results are summarized in Table 1. Three compounds exhibit very poor inhibitory potency against APAH: carboxylic acid **I**, thioester **VII**, and sulfone **IX**, with IC_{50} values in the millimolar range. In contrast, sulfone and thioester analogues of SAHA are effective inhibitors of HDAC in the mid- or low micromolar range, respectively,[33,58] and carboxylic acid **I** was previously shown to be a potent inhibitor of the mammalian N^8-acetylspermidine deacetylase in the low micromolar range.[27]

A second set of compounds are modest inhibitors of APAH, with inhibitory potencies in the mid-micromolar range. In increasing order of potency, these compounds are: α-methoxyketone **VI**, boronic acid **X**, ketone **II**, thioester **V**, and thiol **VIII**. Ketone **II** is a modest inhibitor of the bacterial polyamine deacetylase but a

Table 1
Inhibitory potency of compounds **I**–**X** against *M. ramosa* APAH

Compound	Head-group	IC_{50} (μM)
I	–COOH	1800 ± 200
II	$-COCH_3$	160 ± 10
III	–CONHOH	0.39 ± 0.03
IV	$-COCF_3$	0.27 ± 0.03 38 ± 6
V	$-COCH_2SAc$	39 ± 10 4000 ± 1000
VI	$-COCH_2OCH_3$	380 ± 50
VII	–SAc	1900 ± 200
VIII	–SH	26 ± 3
IX	$-SO_2CH_3$	10000 ± 3000
X	$-B(OH)_2$	230 ± 40

mid-nanomolar selective inhibitor of the mammalian N^8-acetylspermidine deacetylase.[26] The efficacy and specificity of ketone **II** have also been demonstrated in vivo, and this compound was used to probe the function of the mammalian polyamine deacetylase.[59] In general, the incorporation of each of these functional groups in the design of HDAC inhibitors resulted in the generation of highly potent compounds with inhibitory potency in the nanomolar range.[33,35–39] However, in contrast to our N^8-acetylspermidine analogues, HDAC inhibitors are designed based on the combination of a metal-binding group, a linker, and an active site-capping group. For a given Zn^{2+}-binding group, optimization of the linker and the capping group to optimize interactions with the mouth of the active site cleft is usually required to achieve exceptional inhibitory potency.

The best two APAH inhibitors showing potency in the nanomolar range are hydroxamate **III** and trifluoromethylketone **IV** (Table 1). Hydroxamates have been extensively studied as metal-coordinating groups for the design of metalloenzyme inhibitors, such as the FDA-approved HDAC inhibitor SAHA for anti-cancer chemotherapy.[60] Hydroxamates form a stable bidentate five-membered ring complex with the catalytic Zn^{2+} ion that contributes to high affinity. Hydroxamate **III** exhibits an IC_{50} value of 390 nM against APAH. This compound is also a potent inhibitor of mammalian N^8-acetylspermidine deacetylase activity, with an apparent K_i of 1 nM measured with subcellular extracts.[27]

Trifluoromethylketones are well-known to exist as gem-diol hydrate in aqueous solution. This was further demonstrated for compound **IV** with ^{13}C and ^{19}F NMR in D_2O. Indeed, a single peak at −85.1 ppm for ^{19}F NMR and a quadruplet at 93.6 ppm for ^{13}C NMR clearly indicate that the trifluoromethylketone group is hydrated in water. This gem-diol form mimics the tetrahedral intermediate in zinc metalloenzyme-catalyzed hydrolytic reactions, as first demonstrated for carboxypeptidase A.[61,62] Surprisingly, as shown in Figure 4, the data obtained for compound **IV** fit better with a two-site binding model, whereas the concentration-response curve of most of the other compounds were typical of a one-site binding model as for hydroxamate **III**. The biphasic curve obtained for inhibition by trifluoromethylketone **IV** yields IC_{50} values of 270 nM and 38 μM. Similar behavior is also observed for compound **V**, with IC_{50} values of 39 μM and 4 mM. While such biphasic inhibition is also observed in other systems, for example, the inhibition of smooth muscle endothelin-converting enzyme by the metalloprotease inhibitor phosphoramidon,[63] the reason for this uncommon inhibition mode against APAH remains unclear. This type of dose–response curve is usually observed when a ligand binds to a receptor existing in two different affinity states,[64] or to a receptor or a transporter in two different sites.[65] However, the X-ray crystal structure of the APAH–**IV** complex reveals the binding of the gem-diol form of the inhibitor solely in the enzyme active site (study in progress), and we have not observed any other notable features, such as time-dependent inhibition, in our measurements. Thus, it is possible that monomers A and B of APAH exist in two different affinity states with regard to the binding of **IV**.

3. Conclusions

In summary, we have designed and synthesized a series of N^8-acetylspermidine analogues bearing different functional groups targeting Zn^{2+} coordination interactions in the active site of APAH. Most analogues studied are modest inhibitors, but two—compounds **III** and **IV**, bearing hydroxamate and trifluoromethylketone groups, respectively—exhibit nanomolar inhibitory potency. Future work on the optimization of these leads may facilitate the development of even better APAH inhibitors. Moreover, compounds **III** and **IV** may also be useful tools for probing the function of acetylpolyamines in both eukaryotes and prokaryotes, and in searching for the as-yet unidentified mammalian N^8-acetylspermidine deacetylase.

4. Experimental section

4.1. Chemistry

4.1.1. General procedures

All reagents were of at least 95% purity and purchased from Fisher Scientific, Alfa Aesar or Sigma Aldrich. All solvents were of HPLC grade and purchased from Fisher Scientific or Sigma Aldrich. For reactions requiring anhydrous conditions, solvents (THF, MeCN, and MeOH) were purchased as anhydrous grade from Fisher Scientific (except CH_2Cl_2, which was freshly distilled under N_2 from P_2O_5). Reactions were monitored by TLC with Sigma Aldrich aluminum plates (silica gel with fluorescent indicator, 60 Å, 200 μm) and visualized by staining with a ninhydrin solution or under UV light when necessary. Flash column chromatography was performed using Fisher Scientific silica gel 60 (230–400 mesh). Melting points were determined using a Mel-Temp Electrothermal apparatus and were uncorrected. High-resolution mass spectrometry (HRMS) was performed on a Waters LC-TOF mass spectrometer (model LCT-XE Premier) using electrospray ionization (ESI) in positive mode. For compound **X**, the boronic acid moiety was derivatized by adding (+)-pinanediol to enable analysis by mass spectrometry.

^1H and ^{13}C NMR spectra were recorded on Bruker DMX 360 and DRX 500 spectrometers operating at 360 and 500 MHz, respectively, for ^1H NMR and at 90.6 and 125.6 MHz, respectively, for ^{13}C NMR. ^{19}F NMR spectra were recorded at 282.4 MHz on a Bruker DMX 360 spectrometer, and ^{11}B NMR spectra at 128 MHz on a Bruker DMX 400 spectrometer. ^1H and ^{13}C NMR chemical shifts (δ) are reported in ppm relative to the residual solvent peak. NMR coupling constants (J) are reported in Hz, and multiplicities are denoted as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quadruplet; and m, multiplet.

4.1.2. N^1-(Pent-4-enyl)propane-1,3-diamine (1)

To 35.2 mL of 1,3-diaminopropane (422 mmol) at 0 °C and under argon was added dropwise 5-bromopent-1-ene (5.0 mL, 42.2 mmol). The solution was stirred at 0 °C one hour, and two additional hours at room temperature. The reaction mixture was

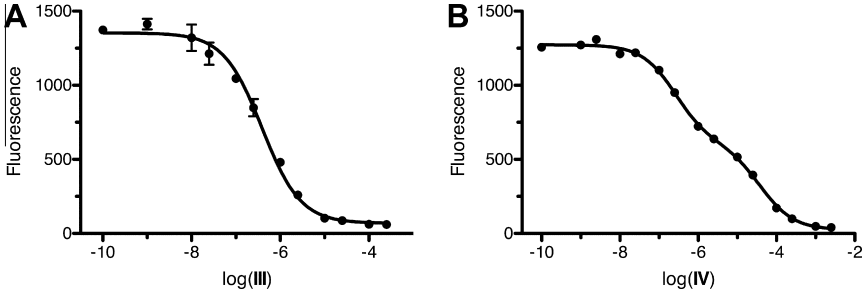


Figure 4. Inhibition of APAH by: (A) hydroxamate **III** and (B) trifluoromethylketone **IV**.

then partitioned between AcOEt (250 mL), brine (40 mL), saturated aqueous $NaHCO_3$ (40 mL), and H_2O (40 mL). The aqueous layer was extracted with AcOEt and the combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography on SiO_2 with $CHCl_3$/MeOH/NH_4OH gradients afforded pure alkyl diamine **1** (5.64 g, 94%) as a slightly yellow oil. ^1H NMR (360 MHz, $CDCl_3$) δ: 5.81–5.70 (m, 1H), 4.96 (dd, J = 1.8 Hz, J = 17.3 Hz, 1H), 4.90 (dd, J = 1.8 Hz, J = 10.1 Hz, 1H), 2.72 (t, J = 6.6 Hz, 2H), 2.63 (t, J = 6.8 Hz, 2H), 2.56 (t, J = 7.2 Hz, 2H), 2.06–2.00 (m, 2H), 1.63–1.41 (m, 7H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 138.2, 114.4, 49.3, 47.7, 40.3, 33.5, 31.3, 29.0. HRMS (ESI) calcd for $C_8H_{19}N_2$ $[M+H]^+$ 143.1548, found 143.1549.

4.1.3. N^1-(Hept-6-enyl)propane-1,3-diamine (2)

Alkylation of 1,3-diaminopropane (27.4 mL, 328 mmol) with 7-bromohept-1-ene (5 mL, 32.8 mmol) was performed under the same conditions as for **1**, and afforded after purification alkyl diamine **2** as a slightly yellow oil (5.31 g, 95%). ^1H NMR (360 MHz, $CDCl_3$) δ: 5.79–5.68 (m, 1H), 4.93 (dd, J = 1.1 Hz, J = 17.3 Hz, 1H), 4.87 (dd, J = 1.1 Hz, J = 10.1 Hz, 1H), 2.70 (t, J = 6.8 Hz, 2H), 2.60 (t, J = 6.8 Hz, 2H), 2.53 (t, J = 6.8 Hz, 2H), 1.99 (m, 2H), 1.57 (m, 2H), 1.45 (m, 2H), 1.36–1.24 (m, 4H), 1.15 (s, 3H). ^{13}C NMR (90.6 MHz, $CDCl_3$) δ: 139.0, 114.3, 50.2, 48.0, 40.7, 34.0, 33.7, 30.1, 28.9, 26.9. HRMS (ESI) calcd for $C_{10}H_{23}N_2$ $[M+H]^+$ 171.1861, found 171.1853.

4.1.4. N^1,N^3-Bis(*tert*-butoxycarbonyl)-N^1-(pent-4-enyl)propane-1,3-diamine (3)

To a solution of alkyl diamine **1** (5.40 g, 38.0 mmol) in dry CH_2Cl_2 (150 mL) at 0 °C and under argon was added dropwise Boc_2O (26.2 mL, 114 mmol). The solution was allowed to warm gradually to room temperature and was stirred overnight. The reaction mixture was concentrated in vacuo and the resulting residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford pure **3** as a colorless oil (12.7 g, quantitative). ^1H NMR (500 MHz, $CDCl_3$) δ: 5.78–5.70 (m, 1H), 5.10 (br s, 1H), 4.96 (dd, J = 2.0 Hz, J = 17.3 Hz, 1H), 4.91 (dd, J = 2.0 Hz, J = 10.0 Hz, 1H), 3.19 (t, J = 6.3 Hz, 2H), 3.08 (t, J = 7.5 Hz, 2H), 3.04 (t, J = 6.5 Hz, 2H), 1.97 (m, 2H), 1.62–1.52 (m, 4H), 1.40 (s, 9H), 1.38 (s, 9H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 156.1 (2C), 137.9, 115.0, 79.5, 79.0, 46.5, 44.0, 37.7, 31.0, 28.5 (7C), 27.6. HRMS (ESI) calcd for $C_{18}H_{34}N_2NaO_4$ $[M+Na]^+$ 365.2416, found 365.2410.

4.1.5. N^1,N^3-Bis(*tert*-butoxycarbonyl)-N^1-(hept-6-enyl)propane-1,3-diamine (4)

Boc-protection of alkyl diamine **2** (5.1 g, 29.9 mmol) with Boc_2O (20.6 mL, 89.7 mmol) was performed under the same conditions as for **3**, and afforded **4** as a colorless oil (11.1 g, quantitative). ^1H NMR (500 MHz, $CDCl_3$) δ: 5.63 (m, 1H), 5.26 (br s, 1H), 4.84 (dd, J = 1.6 Hz, J = 17.2 Hz, 1H), 4.79 (dd, J = 1.6 Hz, J = 10.1 Hz, 1H), 3.11 (t, J = 6.0 Hz, 2H), 3.00 (t, J = 6.0 Hz, 2H), 2.95 (t, J = 6.3 Hz, 2H), 1.91 (apparent q (dt), J = 7.2 Hz, 2H), 1.55–1.50 (m, 2H), 1.42–1.33 (m, 2H), 1.32 (s, 9H), 1.30 (s, 9H), 1.29–1.24 (m, 2H), 1.19–1.11 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 155.9 (2C), 138.5, 114.3, 79.2, 78.6, 46.8, 43.7, 37.6, 33.5, 28.4, 28.8 (7C), 28.2, 26.2. HRMS (ESI) calcd for $C_{20}H_{38}N_2NaO_4$ $[M+Na]^+$ 393.2729, found 393.2727.

4.1.6. N,N'-Bis(*tert*-butoxycarbonyl)-6-[(3-aminopropyl)amino]hexanoic acid (5)

To a solution of alkene **4** (4.00 g, 10.8 mmol) in 200 mL of a H_2O/AcOEt/MeCN (3/2/2) mixture were added successively ruthenium(III) chloride hydrate (35%, 256 mg, 432 μmol) and sodium periodate (10.4 g, 48.6 mmol). After stirring at room temperature for 3 h, the reaction mixture was quenched by the addition of isopropanol (80 mL), the suspension filtered over a celite pad, and the filtrate concentrated in vacuo. The aqueous phase was extracted with AcOEt and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was further purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford carboxylic acid **5** as a colorless oil (4.11 g, 98%). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.79 (br s, 1H), 5.37 (br s, 1H), 3.21 (t, J = 6.3 Hz, 2H), 3.12 (t, J = 6.6 Hz, 2H), 3.07 (t, J = 6.0 Hz, 2H), 2.32 (t, J = 7.4 Hz, 2H), 1.66–1.60 (m, 4H), 1.54–1.48 (m, 2H), 1.43 (s, 9H), 1.42 (s, 9H), 1.33–1.27 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 178.6, 156.5 (2C), 79.7, 79.4, 46.9, 44.2, 38.0, 34.0, 28.6 (7C), 28.2, 26.5, 24.5. HRMS (ESI) calcd for $C_{19}H_{36}N_2NaO_6$ $[M+Na]^+$ 411.2471, found 411.2473.

4.1.7. N',N''-Bis(*tert*-butoxycarbonyl)-6-[(3-aminopropyl)amino]-*N*-methoxy-*N*-methylhexanamide (6)

To a solution of carboxylic acid **5** (2.08 g, 5.35 mmol) in dry CH_2Cl_2 (80 mL) under argon was added CDI (1.74 g, 10.7 mmol). After one hour at room temperature, *N*,*O*-dimethylhydroxylamine hydrochloride (1.04 g, 10.7 mmol) was added to the solution. After stirring overnight, the reaction mixture was diluted CH_2Cl_2 (100 mL), washed with 0.1 M HCl (3 × 50 mL), water, and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was further purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford Weinreb amide **6** (2.31 g, quantitative) as a colorless oil. ^1H NMR (360 MHz, $CDCl_3$) δ: 5.21 (br s, 1H), 3.65 (s, 3H), 3.22 (t, J = 6.1 Hz, 2H), 3.15 (s, 3H), 3.14–3.03 (m, 4H), 2.39 (t, J = 7.2 Hz, 2H), 1.67–1.58 (m, 4H), 1.56–1.46 (m, 2H), 1.43 (s, 9H), 1.41 (s, 9H), 1.34–1.26 (m, 2H). ^{13}C NMR (90.6 MHz, $CDCl_3$) δ: 174.6, 156.1 (2C), 79.6, 79.1, 61.3, 46.9, 44.1, 37.8, 32.3, 31.9, 28.6 (7C), 28.3, 26.8, 24.5. HRMS (ESI) calcd for $C_{21}H_{42}N_3O_6$ $[M+H]^+$ 432.3074, found 432.3091.

4.1.8. N,N'-Bis(*tert*-butoxycarbonyl)-7-[(3-aminopropyl)amino]heptan-2-one (7)

To a solution of Weinreb amide **6** (1.36 g, 3.15 mmol) in dry THF (25 mL) at 0 °C and under argon was added dropwise methylmagnesium bromide (1.0 M in THF, 15.8 mL, 15.8 mmol). The solution was stirred 2 h at 0 °C and then 1 h at room temperature. The reaction mixture was cooled down to 0 °C, quenched by the addition of saturated aqueous NH_4Cl (20 mL), diluted with AcOEt (100 mL), and the aqueous phase was extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was further purified by flash column chromatography with hexanes/AcOEt gradients to afford ketone **7** as a colorless oil (1.12 g, 92%). ^1H NMR (500 MHz, $CDCl_3$) δ: 5.21 (br s, 1H), 3.17 (t, J = 6.5 Hz, 2H), 3.06 (t, J = 6.5 Hz, 2H), 3.03 (t, J = 6.5 Hz, 2H), 2.36 (t, J = 7.5 Hz, 2H), 2.06 (s, 3H), 1.61–1.56 (m, 2H), 1.55–1.49 (m, 2H), 1.48–1.41 (m, 2H), 1.39 (s, 9H), 1.37 (s, 9H), 1.22–1.16 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 208.9, 156.1 (2C), 79.5, 79.0, 46.8, 43.9, 43.6, 37.7, 29.9, 28.5 (7C), 28.3, 26.4, 23.5. HRMS (ESI) calcd for $C_{20}H_{38}N_2O_5Na$ $[M+Na]^+$ 409.2678, found 409.2670.

4.1.9. N',N''-Bis(*tert*-butoxycarbonyl)-6-[(3-aminopropyl)amino]-*N*-hydroxyhexanamide (8)

To a solution of carboxylic acid **5** (600 mg, 1.54 mmol) in dry THF (20 mL) under argon was added CDI (376 mg, 2.32 mmol). After 1 h at room temperature, hydroxylamine hydrochloride (215 mg, 3.09 mmol) was added to the solution. After stirring overnight, the reaction mixture was diluted with a 5% $KHSO_4$ aqueous solution (60 mL) and extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was further purified by flash column chromatography on SiO_2 with AcOEt/MeOH gradients to

afford hydroxamic acid **8** (550 mg, 88%) as a slightly yellow oil. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.29 (br s, 1H), 8.61 (br s, 1H), 6.72 (br s, 1H), 3.08 (apparent q (2t), J = 7.5 Hz, 4H), 2.88 (apparent q (dt), J = 6.5 Hz, 2H), 1.93 (t, J = 7.4 Hz, 2H), 1.58–1.54 (m, 2H), 1.52–1.46 (m, 2H), 1.44–1.39 (m, 2H), 1.38 (s, 9H), 1.37 (s, 9H), 1.21–1.15 (m, 2H). ^{13}C NMR (125.6 MHz, DMSO-d_6) δ: 169.0, 155.6, 154.6, 78.2, 77.4, 46.3, 44.3, 37.6, 32.2, 28.2, 28.1 (6C), 27.6, 25.9, 24.9. HRMS (ESI) calcd for $C_{19}H_{37}N_3O_6Na$ [M+Na]$^+$ 426.2580, found 426.2576.

4.1.10. *N,N′*-Bis(*tert*-butoxycarbonyl)-6-[(3-aminopropyl)amino]hexanal (9)

To a solution of alkene **4** (2.71 g, 7.31 mmol) in 150 mL of a dioxane/H_2O (4/1) mixture were successively added NMO monohydrate (2.47 g, 18.3 mmol) dissolved in 6 mL of water, and osmium tetroxide (47 mg, 185 μmol). TLC monitoring showed completion after 3 h at room temperature. Sodium periodate (3.91 g, 18.3 mmol) was added to the solution. After stirring for 20 min, the reaction mixture was quenched with isopropanol (50 mL), the suspension filtered over a celite pad, and the filtrate concentrated in vacuo. The aqueous phase was extracted with AcOEt and the combined organic extracts were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was further purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford aldehyde **9** as a colorless oil (2.64 g, 97%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.63 (s, 1H), 5.25 (br s, 1H), 3.11 (t, J = 7.0 Hz, 2H), 3.02 (t, J = 6.7 Hz, 2H), 2.96 (t, J = 7.4 Hz, 2H), 2.31 (t, J = 6.9 Hz, 2H), 1.55–1.49 (m, 4H), 1.42–1.37 (m, 2H), 1.32 (s, 9H), 1.30 (s, 9H), 1.21–1.15 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 202.1, 155.9 (2C), 79.3, 78.7, 46.6, 43.8, 43.6, 37.5, 28.3 (7C), 27.8, 26.2, 21.6. HRMS (ESI) calcd for $C_{19}H_{36}N_2O_5Na$ [M+Na]$^+$ 395.2522, found 395.2509.

4.1.11. *N,N′*-Bis(*tert*-butoxycarbonyl)-7-[(3-aminopropyl)amino]-1,1,1-trifluoroheptan-2-ol (10)

To a solution of aldehyde **9** (1.53 g, 4.11 mmol) in dry THF (20 mL) at room temperature and under argon were added successively $TMSCF_3$ (1.82 mL, 12.3 mmol) and anhydrous TBAF (1.0 M in THF, 410 μL, 410 μmol). After 2 h at room temperature, TBAF (1.0 M in THF containing ca. 5% H_2O, 6.2 mL, 6.20 mmol) was added dropwise and the solution was stirred for 45 min and concentrated in vacuo. The corresponding residue was partitioned between AcOEt (100 mL) and saturated aqueous $NaHCO_3$ (50 mL) and the aqueous phase was extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash column chromatography on SiO_2 with hexanes/AcOEt gradients afforded trifluoromethylalcohol **10** as a colorless oil (1.49 g, 82%). ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.32 (br s, 1H), 4.46 (br s, 1H), 3.79–3.73 (m, 1H), 3.11 (t, J = 6.5 Hz, 2H), 3.03 (t, J = 6.5 Hz, 2H), 2.97 (t, J = 6.5 Hz, 2H), 1.58–1.48 (m, 6H), 1.46–1.38 (m, 2H), 1.34 (s, 9H), 1.32 (s, 9H), 1.25–1.14 (m, 2H). ^{13}C NMR (90.6 MHz, $CDCl_3$) δ: 156.3 (2C), 125.5 (q, J = 281.8 Hz), 79.6, 79.1, 69.7 (q, J = 29.0 Hz), 46.9, 43.9, 37.6, 29.5, 28.3 (7C), 28.1, 26.4, 24.6. ^{19}F NMR (338.8 MHz) δ: −79.8. HRMS (ESI) calcd for $C_{20}H_{37}N_2NaO_5F_3$ [M+Na]$^+$ 465.2552, found 465.2553.

4.1.12. *N,N′*-Bis(*tert*-butoxycarbonyl)-7-[(3-aminopropyl)amino]-1,1,1-trifluoroheptan-2-one (11)

To a solution of trifluoromethylalcohol **10** (1.40 g, 3.16 mmol) in dry CH_2Cl_2 (50 mL) at room temperature and under argon was added DMP (5.37 g, 12.7 mmol). The reaction mixture was stirred overnight at room temperature, then cooled down to 0 °C, quenched with the addition of an aqueous sodium thiosulfate solution (0.5 M) saturated with $NaHCO_3$ (150 mL), and the aqueous layer was extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford trifluoromethylketone **11** as a slightly yellow oil (1.32 g, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) (ketone/hydrate : 1/0) δ: 5.26 (br s, 1H), 3.11 (t, J = 6.7 Hz, 2H), 3.03 (t, J = 6.5 Hz, 2H), 2.96 (t, J = 6.0 Hz, 2H), 2.60 (t, J = 7.1 Hz, 2H), 1.60–1.53 (m, 4H), 1.45–1.39 (m, 2H), 1.33 (s, 9H), 1.30 (s, 9H), 1.23–1.17 (m, 2H). ^{13}C NMR (500 MHz, $CDCl_3$) δ: 191.2 (q, J = 35.2 Hz), 156.1 (2C), 115.5 (q, J = 291.4 Hz), 79.5, 78.8, 46.6, 43.4, 37.6, 36.1, 28.3 (7C), 28.1, 25.9, 22.0. ^{19}F NMR (338.8 MHz) δ: −79.4. HRMS (ESI) calcd for $C_{20}H_{35}N_2O_5F_3Na$ [M+Na]$^+$ 463.2396, found 463.2397.

4.1.13. N^1,N^3-Bis(*tert*-butoxycarbonyl)-N^1-[5-(oxiran-2-yl)pentyl]propane-1,3-diamine (12)

To a solution of alkene **4** (2.89 g, 7.80 mmol) in dry CH_2Cl_2 (150 mL) under argon at 0 °C was added dropwise *m*-CPBA (77%, 3.50 g, 15.6 mmol) in 40 mL dry CH_2Cl_2. The solution was allowed to reach room temperature and stirred until completion of the reaction (21 h) as shown by TLC. Saturated aqueous $NaHCO_3$ (50 mL) was added to the reaction mixture and the aqueous layer was extracted with AcOEt. Combined organic extracts were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford epoxide **12** (3.01 g, quantitative) as a colorless oil. ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.28 (br s, 1H), 3.05 (t, J = 7.2 Hz, 2H), 2.97–2.88 (m, 4H), 2.71–2.67 (m, 1H), 2.54–2.52 (m, 1H), 2.26–2.24 (m, 1H), 1.51–1.46 (m, 2H), 1.38–1.26 (m, 6H), 1.27 (s, 9H), 1.24 (s, 9H), 1.18–1.14 (m, 2H) . ^{13}C NMR (90.6 MHz, $CDCl_3$) δ: 155.8 (2C), 79.0, 78.4, 51.8, 46.6, 46.5, 43.6, 37.3, 32.1, 28.3, 28.2 (7C), 26.4, 25.5. HRMS (ESI) calcd for $C_{20}H_{39}N_2O_5$ [M+H]$^+$ 387.2859, found 387.2854.

4.1.14. *N,N′*-Bis(*tert*-butoxycarbonyl)-7-[(3-aminopropyl)amino]-1-bromoheptan-2-ol (13)

To a solution of epoxide **12** (2.10 g, 5.43 mmol) in dry THF (25 mL) under argon were added successively lithium bromide (1.51 g, 17.4 mmol) and glacial acetic acid (930 μL, 16.2 mmol) dropwise. After stirring at room temperature overnight, saturated aqueous $NaHCO_3$ (30 mL) was added to the reaction mixture. The aqueous layer was extracted with AcOEt and the combined organic extracts were dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by flash column chromatography on SiO_2 with hexanes/AcOEt gradients afforded α-bromohydrin **13** (2.31 g, 91%) as a colorless oil. ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.26 (br s, 1H), 3.71–3.64 (m, 1H), 3.38 (dd, J = 4.0 Hz, J = 10.1 Hz, 1H), 3.30 (dd, J = 6.5 Hz, J = 10.1 Hz, 1H), 3.13 (t, J = 6.5 Hz, 2H), 3.05–2.96 (m, 5H), 1.58–1.53 (m, 2H), 1.48–1.39 (m, 4H), 1.37–1.31 (m, 2H), 1.36 (s, 9H), 1.34 (s, 9H), 1.22–1.15 (m, 2H). ^{13}C NMR (90.6 MHz, $CDCl_3$) δ: 156.0 (2C), 79.4, 78.9, 70.7, 46.9, 43.9, 39.8, 37.6, 34.9, 28.4 (7C), 27.9, 26.6, 25.2. HRMS (ESI) calcd for $C_{20}H_{39}N_2O_5BrNa$ [M+Na]$^+$ 489.1940, found 489.1931.

4.1.15. *N,N′*-Bis(*tert*-butoxycarbonyl)-7-[(3-aminopropyl)amino]-1-bromoheptan-2-one (14)

To a solution of α-bromohydrin **13** (2.10 g, 4.49 mmol) in dry CH_2Cl_2 (50 mL) at room temperature and under argon was added DMP (5.72 g, 13.5 mmol). After 3 h, the reaction mixture was cooled down to 0 °C, quenched with the addition of an aqueous sodium thiosulfate solution (0.5 M) saturated with $NaHCO_3$ (100 mL), and the aqueous layer was extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford α-bromoketone **14** as a colorless oil (2.03 g, 97%). ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.21 (br s, 1H), 3.78 (s, 2H), 3.12 (t, J = 6.5 Hz, 2H), 3.04–2.95 (m, 4H), 2.54 (t, J = 7.2 Hz, 2H), 1.56–

1.47 (m, 4H), 1.45–1.36 (m, 2H), 1.33 (s, 9H), 1.31 (s, 9H), 1.21–1.12 (m, 2H). ^{13}C NMR (90.6 MHz, $CDCl_3$) δ: 201.7, 155.9 (2C), 79.3, 78.7, 46.6, 43.8, 39.5, 37.6, 34.2, 28.3, 27.8 (7C), 26.1, 23.4. HRMS (ESI) calcd for $C_{20}H_{37}N_2O_5BrNa$ $[M+Na]^+$ 487.1784, found 487.1794.

4.1.16. S-{*N*,*N*′-Bis(*tert*-butoxycarbonyl)-7-[(3-aminopropyl)amino]-2-oxoheptyl}thioacetate (15)

To a solution of α-bromoketone **14** (1.72 g, 3.70 mmol) in dry MeCN (25 mL) at room temperature under argon was added potassium thioacetate (2.54 g, 22.2 mmol). After stirring overnight, the reaction mixture was diluted with water and the aqueous layer was extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography on SiO_2 with hexanes/AcOEt gradients afforded thioester **15** (1.65 g, 97%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.21 (br s, 1H), 3.68 (s, 2H), 3.18 (t, J = 6.5 Hz, 2H), 3.08–3.02 (m, 4H), 2.50 (t, J = 7.5 Hz, 2H), 2.32 (s, 3H), 1.62–1.54 (m, 4H), 1.49–1.43 (m, 2H), 1.40 (s, 9H), 1.38 (s, 9H), 1.25–1.18 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 203.8, 194.3, 156.1 (2C), 79.5, 79.0, 46.7, 43.9, 41.5, 39.0, 37.7, 30.2, 28.5 (7C), 28.4, 26.3, 23.4. HRMS (ESI) calcd for $C_{22}H_{40}N_2O_6SNa$ $[M+Na]^+$ 483.2505, found 483.2517.

4.1.17. *N*,*N*′-Bis(*tert*-butoxycarbonyl)-7-[(3-aminopropyl)amino]-1-methoxyheptan-2-ol (16)

Epoxide **12** (1.33 g, 3.44 mmol) was dissolved in a 0.5 M solution of sodium methoxide in MeOH (41.2 mL, 20.6 mmol). The reaction mixture was stirred under argon at room temperature until completion of the reaction (24 h) as shown by TLC. After removal of the solvent in vacuo, the residue was partitioned between AcOEt (100 mL) and water (25 mL), and the aqueous layer was extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by flash column chromatography on SiO_2 with hexanes/AcOEt gradients afforded alcohol **16** (1.34 g, 93%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.33 (br s, 1H), 3.66–3.60 (m, 1H), 3.27–3.24 (m, 4H), 3.15–3.10 (m, 3H), 3.02–2.96 (m, 4H), 2.72 (br s, 1H), 1.55–1.50 (m, 2H), 1.43–1.35 (m, 4H), 1.33 (s, 9H), 1.31 (s, 9H), 1.29–1.23 (m, 2H), 1.20–1.14 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 156.0 (2C), 79.2, 78.7, 77.1, 69.9, 58.8, 46.9, 43.7, 37.4, 33.1, 28.3 (7C), 28.2, 26.8, 25.2. HRMS (ESI) calcd for $C_{21}H_{43}N_2O_6$ $[M+H]^+$ 419.3121, found 419.3124.

4.1.18. *N*,*N*′-Bis(*tert*-butoxycarbonyl)-7-[(3-aminopropyl)amino]-1-methoxyheptan-2-one (17)

To a solution of alcohol **16** (1.19 g, 2.84 mmol) in dry CH_2Cl_2 (50 mL) at room temperature and under argon was added DMP (7.24 g, 17.1 mmol). After 24 h, the reaction mixture was cooled down to 0 °C, quenched with the addition of an aqueous sodium thiosulfate solution (0.5 M) saturated with $NaHCO_3$ (150 mL), and the aqueous layer was extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford α-methoxyketone **17** as a colorless oil (1.08 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.21 (br s, 1H), 3.94 (s, 2H), 3.35 (s, 3H), 3.18 (t, J = 6.5 Hz, 2H), 3.08–3.02 (m, 4H), 2.38 (t. J = 7.5 Hz, 2H), 1.61–1.52 (m, 4H), 1.49–1.43 (m, 2H), 1.39 (s, 9H), 1.37 (s, 9H), 1.29–1.23 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 208.5, 156.24 (2C), 79.5, 79.0, 77.6, 59.3, 46.8, 43.9, 38.6, 37.7, 28.4 (7C), 28.3, 26.4, 23.0. HRMS (ESI) calcd for $C_{21}H_{40}N_2O_6Na$ $[M+Na]^+$ 439.2784, found 439.2772.

4.1.19. *N*,*N*′-Bis(*tert*-butoxycarbonyl)-5-[(3-aminopropyl)amino]pentan-1-ol (18)

To a solution of alkene **3** (3.00 g, 8.76 mmol) in dry THF (150 mL) at 0 °C and under argon was added dropwise 9-BBN (0.5 M in THF, 43.8 mL, 21.9 mmol). The solution was allowed to warm up to room temperature and stirred for 20 h. The reaction mixture was then cooled down to 0 °C, and aqueous NaOH (6 M, 30 mL) was added, followed by the dropwise addition of H_2O_2 (30%, 15 mL). After stirring 30 min at room temperature, the solution was concentrated in vacuo. The aqueous phase was diluted with 50 mL of water, and extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford alcohol **18** as a colorless oil (3.15 g, quantitative). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.35 (br s, 1H), 3.54 (t, J = 6.5 Hz, 2H), 3.16 (t, J = 6.5 Hz, 2H), 3.06 (t, J = 6.5 Hz, 2H), 3.01 (t, J = 6.5 Hz, 2H), 2.74 (br s, 1H), 1.61–1.55 (m, 2H), 1.53–1.43 (m, 4H), 1.38 (s, 9H), 1.36 (s, 9H), 1.29–1.23 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ:156.2 (2C), 79.5, 79.0, 62.4, 47.0, 43.9, 37.6, 32.3, 28.4 (7C), 27.8, 23.0. HRMS (ESI) calcd for $C_{18}H_{36}N_2O_5Na$ $[M+Na]^+$ 383.2522, found 383.2508.

4.1.20. N^1,N^3-Bis(*tert*-butoxycarbonyl)-N^1-(5-bromopentyl)propane-1,3-diamine (19)

To a solution of alcohol **18** (3.00 g, 8.32 mmol) in dry THF (100 mL) at 0 °C were added successively CBr_4 (5.52 g, 16.6 mmol) and PPh_3 (4.37 g, 16.7 mmol). The reaction mixture was allowed to reach room temperature and stirred overnight. After removal of the solvent in vacuo, purification of the residue by flash column chromatography on SiO_2 with hexanes/AcOEt gradients afforded alkyl bromide **19** (3.38 g, 96%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.21 (br s, 1H), 3.23 (t, J = 6.5 Hz, 2H), 3.17 (t, J = 6.5 Hz, 2H), 3.08 (t, J = 6.5 Hz, 2H), 3.02 (t, J = 6.5 Hz, 2H), 1.83–1.77 (m, 2H), 1.61–1.56 (m, 2H), 1.51–1.44 (m, 2H), 1.39 (s, 9H), 1.37 (s, 9H), 1.38–1.31 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 156.0 (2C), 79.5, 78.9, 46.7, 43.8, 37.7, 33.6, 32.4, 28.4 (7C), 27.7, 25.4. HRMS (ESI) calcd for $C_{18}H_{36}N_2O_4Br$ $[M+H]^+$ 423.1858, found 423.1866.

4.1.21. *S*-{*N*,*N*′-Bis(*tert*-butoxycarbonyl)-5-[(3-aminopropyl)amino]pentyl} thioacetate (20)

Reaction of alkyl bromide **19** (1.70 g, 4.02 mmol) and potassium thioacetate (2.75 g, 24.1 mmol) under the same conditions as for compound **15** afforded after purification thioester **20** (1.58 g, 94%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.21 (br s, 1H), 3.07 (t, J = 6.5 Hz, 2H), 2.96 (t, J = 6.5 Hz, 2H), 2.91 (t, J = 6.0 Hz, 2H), 2.68 (t, J = 7.0 Hz, 2H), 2.14 (s, 3H), 1.51–1.47 (m, 2H), 1.44–1.38 (m, 2H), 1.38–1.32 (m, 2H), 1.28 (s, 9H), 1.26 (s, 9H), 1.19–1.13 (m, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 195.3, 155.8 (2C), 79.1, 78.5, 46.5, 43.6, 37.5, 30.3, 29.0, 28.7, 28.2 (7C), 27.8, 25.7. HRMS (ESI) calcd for $C_{20}H_{38}N_2O_5SNa$ $[M+Na]^+$ 441.2399, found 441.2406.

4.1.22. *N*,*N*′-Bis(*tert*-butoxycarbonyl)-5-[(3-aminopropyl)amino]pentane-1-thiol (21)

To a solution of thioester **20** (550 mg, 1.31 mmol) in dry MeOH (10 mL) under argon was added sodium methoxide (0.5 M solution in MeOH, 5.3 mL, 2.65 mml). The solution was stirred at room temperature for 2 h, quenched by the addition of 10% aqueous citric acid (100 mL), and the aqueous phase was extracted with AcOEt. Combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford thiol **21** as a colorless oil (485 mg, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.19 (br s, 1H), 3.07 (t, J = 6.0 Hz, 2H), 2.98 (t, J = 6.3 Hz, 2H), 2.92 (t, J = 6.3 Hz, 2H), 2.35 (apparent q (dt), J = 7.3 Hz, 2H), 1.51–1.41 (m, 4H), 1.37–1.32 (m, 2H), 1.29 (s, 9H), 1.26 (s, 9H), 1.23–1.17 (m, 2H), 1.16 (t, J = 7.3 Hz, 1H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 155.8 (2C), 79.1, 78.5, 46.6, 43.7, 37.5,

33.4, 28.2 (7C), 27.7, 25.3, 24.2. HRMS (ESI) calcd for $C_{18}H_{36}N_2O_4S$-Na $[M+Na]^+$ 399.2293, found 399.2293.

4.1.23. N^1,N^3-Bis(*tert*-butoxycarbonyl)-N^1-[5-(methylthio)pentyl]propane-1,3-diamine (22)

To a solution of alkyl bromide **19** (1.22 g, 2.88 mmol) in EtOH (15 mL) was added sodium thiomethoxide (1.21 g, 17.3 mmol). The reaction mixture was stirred overnight at 60 °C under argon. After removal of the solvent under vacuo, the residue was partitioned between CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (25 mL), and the aqueous layer was extracted with CH_2Cl_2. Combined organic extracts were washed with water, brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography on SiO_2 with hexanes/AcOEt gradients afforded thioether **22** (1.10 g, 98%) as a colorless oil. ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.21 (br s, 1H), 3.20 (t, J = 6.5 Hz, 2H), 3.13–3.05 (m, 4H), 2.46 (t, J = 7.4 Hz, 2H), 2.06 (s, 3H), 1.66–1.54 (m, 4H), 1.53–1.46 (m, 2H), 1.43 (s, 9H), 1.41 (s, 9H), 1.38–1.29 (m, 2H). ^{13}C NMR (90.6 MHz, $CDCl_3$) δ: 156.1 (2C), 79.6, 79.1, 46.9, 44.1, 37.8, 34.3, 29.0, 28.5 (7C), 28.2, 26.1, 15.6. HRMS (ESI) calcd for $C_{19}H_{39}N_2O_4S$ $[M+H]^+$ 391.2631, found 391.2633.

4.1.24. N^1,N^3-Bis(*tert*-butoxycarbonyl)-N^1-[5-(methylsulfonyl)pentyl]propane-1,3-diamine (23)

To a solution of thioether **22** (500 mg, 1.28 mmol) in dry CH_2Cl_2 (10 mL) under argon at 0 °C was added dropwise *m*-CPBA (77%, 861 mg, 3.84 mmol) in 10 mL dry CH_2Cl_2. The solution was allowed to reach room temperature and stirred until completion of the reaction (2 h) as shown by TLC. Saturated aqueous $NaHCO_3$ (10 mL) was added to the reaction mixture and the aqueous layer was extracted with AcOEt. Combined organic extracts were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford sulfone **23** (524 mg, 97%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.14 (br s, 1H), 3.07 (t, J = 7.0 Hz, 2H), 3.01 (t, J = 6.5 Hz, 2H), 2.92 (t, J = 6.5 Hz, 2H), 2.87 (t, J = 7.0 Hz, 2H), 2.75 (s, 3H), 1.73–1.67 (m, 2H), 1.53–1.48 (m, 2H), 1.44–1.40 (m, 2H), 1.31–1.26 (m, 2H), 1.30 (s, 9H), 1.27 (s, 9H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 155.9 (2C), 79.3, 78.6, 54.3, 46.3, 43.6, 40.2, 37.5, 28.2 (7C), 28.0, 25.4, 22.0. HRMS (ESI) calcd for $C_{19}H_{38}N_2O_6SNa$ $[M+Na]^+$ 445.2348, found 445.2344.

4.1.25. N^1,N^3-Bis(*tert*-butoxycarbonyl)-N^1-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pentyl]propane-1,3-diamine (24)

To a solution of $[Ir(cod)Cl]_2$ (196 mg, 292 μmol) and dppm (225 mg, 585 μmol) in dry CH_2Cl_2 (150 mL) under argon was added pinacolborane (1.65 mL, 11.3 mmol) and alkene **3** (2.00 g, 5.84 mmol) in 30 mL dry CH_2Cl_2. After stirring 24 h at room temperature, the reaction mixture was cooled to 0 °C, quenched with the addition of 60 mL of water, and the aqueous phase extracted with Et_2O. Combined organic extracts were dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on SiO_2 with hexanes/AcOEt gradients to afford **24** as a colorless oil (2.25 g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.24 ppm (br s, 1H), 3.16 (t, J = 6.6 Hz, 2H), 3.04–2.99 (m, 4H), 1.59–1.54 (m, 2H), 1.45–1.39 (m, 2H), 1.37 (s, 9H), 1.35 (s, 9H), 1.35–1.30 (m, 2H), 1.21–1.15 (m, 2H), 1.15 (s, 12H), 0.68 (t, J = 7.8 Hz, 2H). ^{13}C NMR (125.6 MHz, $CDCl_3$) δ: 156.0 (2C), 82.6 (2C), 79.3, 78.8, 47.0, 43.8, 37.7, 29.5, 28.5 (7C), 28.3, 24.8 (4C), 23.8, 11.2 (br s). ^{11}B NMR (128 MHz, $CDCl_3$) δ: 33.0 ppm. HRMS (ESI) calcd for $C_{24}H_{48}N_2O_5B$ $[M+H]^+$ 471.3605, found 471.3615.

4.1.26. 6-[(3-Aminopropyl)amino]hexanoic acid dihydrochloride (I)

A solution of compound **5** (300 mg, 772 μmol) in anhydrous 4 N HCl in dioxane (20 mL) was stirred at room temperature under argon for 2 h. The reaction mixture was cooled down to 0 °C and, diluted with Et_2O (20 mL). The precipitate was filtered, washed with cold Et_2O, and dried in vacuo to afford carboxylic acid **I** (dihydrochloride salt) as a white solid (188 mg, 93%). mp: 172–174 °C. ^{1}H NMR (500 MHz, D_2O) δ: 3.20 (t, J = 8.0 Hz, 2H), 3.17–3.11 (m, 4H), 2.45 (t, J = 7.4 Hz, 2H), 2.17–2.11 (m, 2H), 1.79–1.72 (m, 2H), 1.71–1.65 (m, 2H), 1.49–1.43 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 178.8, 47.6, 44.4, 36.6, 33.5, 25.2, 25.1, 23.8, 23.7. HRMS (ESI) calcd for $C_9H_{21}N_2O_2$ $[M+H]^+$ 189.1603, found 189.1604.

4.1.27. 7-[(3-Aminopropyl)amino]heptan-2-one dihydrochloride (II)

Deprotection of compound **7** (320 mg, 828 μmol) was performed under the same conditions as for **I** to afford ketone **II** (dihydrochloride salt) as a white powder (202 mg, 94%). mp: 206–209 °C (dec.). ^{1}H NMR (500 MHz, D_2O) δ: 3.14 (t, J = 8.0 Hz, 2H), 3.10 (t, J = 8.0 Hz, 2H), 3.06 (t, J = 7.8 Hz, 2H), 2.58 (t, J = 7.5 Hz, 2H), 2.19 (s, 3H), 2.11–2.05 (m, 2H), 1.71–1.65 (m, 2H), 1.60–1.54 (m, 2H), 1.38–1.31 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 216.9, 47.5, 44.3, 42.7, 36.5, 29.2, 25.2, 25.0, 23.6, 22.5. HRMS (ESI) calcd for $C_{10}H_{23}N_2O$ $[M+H]^+$ 187.1810, found 187.1813.

4.1.28. 6-[(3-Aminopropyl)amino]-N-hydroxyhexanamide dihydrochloride (III)

Deprotection of compound **8** (350 mg, 867 μmol) was performed under the same conditions as for **I** to afford hydroxamic acid **III** (dihydrochloride salt) as a white powder (230 mg, 96%). mp: 135–139 °C. ^{1}H NMR (500 MHz, D_2O) δ: 3.19 (t, J = 8.0 Hz, 2H), 3.16–3.10 (m, 4H), 2.23 (t, J = 7.3 Hz, 2H), 2.16–2.10 (m, 2H), 1.77–1.71 (m, 2H), 1.70–1.64 (m, 2H), 1.45–1.39 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 173.1, 47.6, 44.4, 36.6, 32.0, 25.2, 25.0, 24.3, 23.7. HRMS (ESI) calcd for $C_9H_{22}N_3O_2$ $[M+H]^+$ 204.1712, found 204.1722.

4.1.29. 7-[(3-Aminopropyl)amino]-1,1,1-trifluoroheptan-2-one dihydrochloride (IV)

Deprotection of compound **11** (350 mg, 795 μmol) was performed under the same conditions as for **I** to afford trifluoromethylketone **IV** (dihydrochloride salt) as a white powder (234 mg, 94%). mp: 228–231 °C (dec.). ^{1}H NMR (500 MHz, D_2O) (ketone/hydrate : 0/1) δ: 3.20 (t, J = 8.0 Hz, 2H), 3.17–3.12 (m, 4H), 2.18–2.11 (m, 2H), 1.88 (t, J = 8.2 Hz, 2H), 1.80–1.74 (m, 2H), 1.62–1.55 (m, 2H), 1.51–1.45 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 123.6 (q, J = 286.4 Hz), 93.6 (q, J = 31.4 Hz), 47.7, 44.4, 36.6, 33.7, 25.7, 25.3, 23.7, 20.7. ^{19}F NMR (338.8 MHz) δ: −85.1. HRMS (ESI) calcd for $C_{10}H_{20}N_2OF_3$ $[M+H]^+$ 241.1528, found 241.1534.

4.1.30. 7-{[(3-Aminopropyl)amino]-2-oxoheptyl} thioacetate dihydrochloride (V)

Deprotection of compound **15** (300 mg, 651 μmol) was performed under the same conditions as for **I** to afford thioacetate derivative **V** (dihydrochloride salt) as a white powder (210 mg, 97%). mp: 211–213 °C (dec.). ^{1}H NMR (500 MHz, D_2O) δ: 4.00 (s, 2H), 3.23 (t, J = 8.0 Hz, 2H), 3.19 (t, J = 7.8 Hz, 2H), 3.16 (t, J = 7.8 Hz, 2H), 2.81 (t, J = 7.2 Hz, 2H), 2.49 (s, 3H), 2.21–2.15 (m, 2H), 1.82–1.76 (m, 2H), 1.74–1.68 (m, 2H), 1.50–1.43 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 209.8, 200.1, 47.7, 44.5, 41.1, 39.3, 36.7, 29.5, 25.3, 25.1, 23.8, 22.7. HRMS (ESI) calcd for $C_{12}H_{25}N_2O_2S$ $[M+H]^+$ 261.1637, found 261.1626.

4.1.31. 7-[(3-Aminopropyl)amino]-1-methoxyheptan-2-one dihydrochloride (VI)

A solution of compound **17** (240 mg, 576 μmol) in anhydrous 1 N HCl in AcOEt (30 mL) was stirred at room temperature under argon for 6 h. The suspension was diluted with hexanes (20 mL). The precipitate was filtered, washed with AcOEt and hexanes,

and dried in vacuo to afford α-methoxyketone **VI** (dihydrochloride salt) as an off-white solid (151 mg, 91%). mp: 216–218 °C (dec.). ^{1}H NMR (500 MHz, D_2O) δ: 4.35 (s, 2H), 3.44 (s, 3H), 3.19 (t, J = 8.0 Hz, 2H), 3.15 (t, J = 7.9 Hz, 2H), 3.11 (t, J = 7.8 Hz, 2H), 2.55 (t, J = 7.3 Hz, 2H), 2.16–2.10 (m, 2H), 1.77–1.71 (m, 2H), 1.68–1.62 (m, 2H), 1.45–1.39 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 212.8, 76.5, 58.7, 47.6, 44.4, 37.8, 36.6, 25.3, 25.2, 23.7, 22.3. HRMS (ESI) calcd for $C_{11}H_{25}N_2O_2$ $[M+H]^+$ 217.1916, found 217.1925.

4.1.32. *S*-{5-[(3-Aminopropyl)amino]pentyl} thioacetate dihydrochloride (VII)

Deprotection of compound **20** (300 mg, 717 μmol) was performed under the same conditions as for **I** to afford thioacetate derivative **VII** (dihydrochloride salt) as a white powder (196 mg, 94%). mp: 260–262 °C (dec.). ^{1}H NMR (500 MHz, D_2O) δ: 3.19 (t, J = 8.0 Hz, 2H), 3.15 (t, J = 7.9 Hz, 2H), 3.11 (t, J = 7.8 Hz, 2H), 2.94 (t, J = 7.2 Hz, 2H), 2.41 (s, 3H), 2.16–2.10 (m, 2H), 1.78–1.72 (m, 2H), 1.69–1.64 (m, 2H), 1.52–1.46 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 202.2, 47.6, 44.4, 36.6, 30.1, 28.4, 28.1, 25.0, 24.7, 23.7. HRMS (ESI) calcd for $C_{10}H_{23}N_2OS$ $[M+H]^+$ 219.1531, found 219.1532.

4.1.33. 5-[(3-Aminopropyl)amino]pentane-1-thiol dihydrochloride (VIII)

A solution of compound **21** (208 mg, 552 μmol) in anhydrous 1 N HCl in AcOEt (40 mL) was stirred at room temperature under argon for 5 h. The reaction mixture was concentrated in vacuo and resuspended in hexanes (20 mL). The precipitate was filtered, washed with hexanes, and dried in vacuo to afford thiol derivative **VIII** (dihydrochloride salt) as a white solid (129 mg, 93%). mp: 284–286 °C (dec.). ^{1}H NMR (500 MHz, D_2O) δ: 3.24 (t, J = 8.0 Hz, 2H), 3.21–3.15 (m, 4H), 2.65 (t, J = 7.1 Hz, 2H), 2.21–2.15 (m, 2H), 1.82–1.76 (m, 2H), 1.76–1.70 (m, 2H), 1.59–1.52 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 47.8, 44.5, 36.7, 32.4; 25.0, 24.5, 23.8, 23.5. HRMS (ESI) calcd for $C_8H_{21}N_2S$ $[M+H]^+$ 177.1425, found 177.1424.

4.1.34. N^1-[5-(Methylsulfonyl)pentyl]propane-1,3-diamine dihydrochloride (IX)

Deprotection of compound **23** (300 mg, 710 μmol) was performed under the same conditions as for **I** to afford sulfone **IX** (dihydrochloride salt) as a white powder (202 mg, 97%). mp: 202–204 °C. ^{1}H NMR (500 MHz, D_2O) δ: 3.28 (t, J = 7.8 Hz, 2H), 3.15 (t, J = 7.8 Hz, 2H), 3.11–3.07 (m, 7H), 2.12–2.05 (m, 2H), 1.89–1.82 (m, 2H), 1.78–1.71 (m, 2H), 1.58–1.51 (m, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 53.1, 47.3, 44.3, 39.5, 36.4, 24.9, 24.3, 23.6, 20.9. HRMS (ESI) calcd for $C_9H_{23}N_2O_2S$ $[M+H]^+$ 223.1480, found 223.1483.

4.1.35. 5-[(3-Aminopropyl)amino]pentylboronic acid dihydrochloride (X)

The protected compound **24** (300 mg, 638 μmol) was stirred to reflux with 6 N aqueous HCl (10 mL) for 24 h. After cooling to room temperature, the reaction mixture was washed with Et_2O (20 mL) and the aqueous phase was concentrated in vacuo to give boronic acid **X** as an off-white solid (148 mg, 89%). mp: 288–290 °C (dec.). ^{1}H NMR (500 MHz, D_2O) δ: 3.18 (t, J = 8.0 Hz, 2H), 3.15 (t, J = 7.9 Hz, 2H), 3.10 (t, J = 7.8 Hz, 2H), 2.16–2.10 (m, 2H), 1.76–1.70 (m, 2H), 1.50–144 (m, 2H), 1.43–1.37 (m, 2H), 0.83 (t, J = 7.6 Hz, 2H). ^{13}C NMR (125.6 MHz, D_2O) δ: 47.8, 44.4, 36.6, 28.3, 25.3, 23.7, 23.1, 13.8 (br s). ^{11}B NMR (128 MHz, D_2O) δ: 32.4. HRMS (ESI) calcd for $C_{18}H_{36}N_2O_2B$ [M+(+)-pinanediol−$2H_2O$+H]$^+$ 323.2870, found 323.2869.

4.2. APAH expression, purification, and activity assay

APAH was expressed in *Escherichia coli* BL21(DE3) cells (Novagen Inc.) and purified as previously described.[18] The inhibition of APAH by the synthesized diamine derivatives was analyzed using a fluorimetric assay, as previously described.[18] Activity was measured using the commercially available Fluor-de-Lys deacetylase fluorogenic substrate (BML-KI104, Enzo Life Sciences). Briefly, deacetylation of the substrate molecule allows a protease developer to cleave the peptide bond linking the C-terminal fluorophore, resulting in a fluorescence shift. Assays were run at 25 °C and contained 250 nM enzyme, 150 μM substrate, and 0–100 mM inhibitor in assay buffer (25 mM Tris (pH 8.2), 137 mM NaCl, 2.7 mM KCl and 1 mM $MgCl_2$ (100 μM tris-(2-carboxyethyl)phosphine was added only for the assay of thiol compound **VIII**) in a final volume of 50 μL. After 30 min, reactions were quenched by adding 100 μM M344 (Sigma Aldrich) and the appropriate Fluor-de-Lys developer (BML-KI105, Enzo Life Sciences, 50 μL). Fluorescence was measured after 45 min using a Fluoroskan II plate reader (excitation = 355 nm, emission = 460 nm). Assays for each concentration of inhibitor were performed in triplicate in separate experiments. IC_{50} values for each compound were determined using the software Graphpad Prism (2008).

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Supplementary data

Supplementary data (NMR spectra for compounds **I**–**X**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.05.045.

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