α-Methyl Polyamines: Efficient Synthesis and Tolerance Studies in Vivo and in Vitro. First Evidence for Dormant Stereospecificity of Polyamine Oxidase

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Received September 4, 2005

Efficient syntheses of metabolically stable α-methylspermidine **1**, α-methylspermine **2**, and bis-α,α′-methylated spermine **3** starting from ethyl 3-aminobutyrate are described. The biological tolerance for these compounds was tested in wild-type mice and transgenic mice carrying the metallothionein promoter-driven spermidine/spermine N^1-acetyltransferase gene (MT-SSAT). The efficient substitution of natural polyamines by their derivatives was confirmed in vivo with the rats harboring the same MT-SSAT transgene and in vitro with the immortalized fibroblasts derived from these animals. Enantiomers of previously unknown 1-amino-8-acetamido-5-azanonane dihydrochloride **4** were synthesized starting from enantiomerically pure (*R*)- and (*S*)-alaninols. The studies with recombinant human polyamine oxidase (PAO) showed that PAO (usually splits achiral substrates) strongly favors the (*R*)-isomer of **4** that demonstrates for the first time that the enzyme has hidden potency for stereospecificity.

Introduction

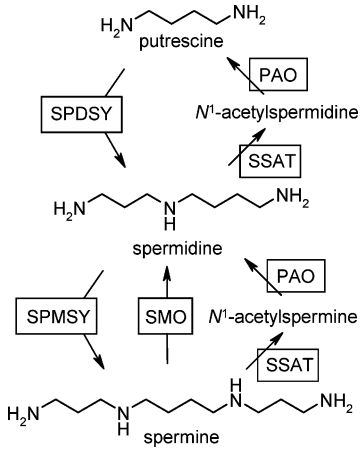
Under the physiological conditions, biogenic polyamines are polycations that interact with many cellular macromolecules and participate in several distinct functions in the mammalian physiology.[1] Precisely maintained polyamine homeostasis is imperative for cell proliferation as both the accumulation and depletion of polyamines can lead to disorders of cellular metabolism.[2] In many tumor cell lines, the polyamine levels are high compared to normal cells, supporting accelerated cell proliferation. On the other hand, the depletion of the polyamine pools with inhibitors of their biosynthesis or compounds activating the catabolism of the polyamines lead to cell growth inhibition and even to apoptosis.[3]

In mammalian cells (Scheme 1) spermine can be oxidized directly to spermidine by spermine oxidase.[4,5] Another possibility for the back-conversion of spermine to spermidine (and similarly spermidine to putrescine) is the concatenated actions of spermidine/spermine N^1-acetyltransferase (SSAT) and polyamine oxidase (PAO). SMO uses only spermine, whereas PAO strongly favors N^1-acetylated substrates; both singly or doubly acetylated spermine and N^1-acetylspermidine are excellent substrates for PAO.[6] This back-conversion of the polyamines produces one H_2O_2 molecule per each split carbon−nitrogen bond, thus causing oxidative stress. Drugs affecting polyamine catabolism may induce SSAT activity up to 1000-fold and cause the depletion of the higher polyamines spermidine and spermine. Such compounds are of great interest and are being studied as anticancer drugs.[7]

Alkylated polyamine derivatives are widely used for the investigation of the biochemistry of spermine and spermidine. Most of these studies have concentrated on the analogues of spermine and its homologues that are symmetrically (e.g. diethylnorspermine, DENSpm) or nonsymmetrically bis-alkylated at terminal amino groups. These compounds, similar to spermine, are actively transported into the cells but are incapable of fulfilling crucial cellular functions of polyamines.[8] The accumulation of DENSpm, or other compounds of similar nature, results in the down-regulation of the polyamine synthesis and/or the activation of the polyamine catabolism. Such terminally *N*-alkylated analogues are substrates for PAO[9] and after terminal dealkylation are prone to the SSAT−PAO pathway.

A less investigated group of polyamine derivatives comprises the analogues with an alkyl substituent(s) attached to carbon atom(s) of polyamine backbone. Contrary to terminally *N*-alkylated derivatives of spermine, some of these compounds are able to sustain cell viability with depleted polyamine pools.[10,11] The single methyl group in the α-position (Figure 1) prevents the acetylation of the adjacent amine of α-methylspermidine (**1**, α-MeSPD), α-methylspermine (**2**, α-MeSPM), and bis-α,α′-methylated spermine (**3**, α,α′-Me_2SPM) by SSAT,

Scheme 1. Polyamine Metabolism in Mammals[a]



[a] PAO, polyamine oxidase; SMO, spermine oxidase; SPDSY, spermidine synthase; SPMSY, spermine synthase; SSAT, spermidine/spermine N^1-acetyltransferase.

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10.1021/jm050872h CCC: $33.50
Published on Web 12/15/2005

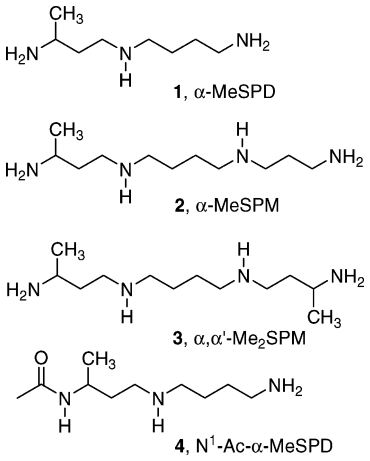


Figure 1. The structures of the α-methylated polyamine analogues used in this study: **1**, α-MeSPD, α-methylspermidine; **2**, α-MeSPM, α-methylspermine; **3**, α,α′-Me_2SPM, bis-α,α′-methylspermine; **4**, Ac-α-MeSPD, N^8-acetyl-α-methylspermidine.

which is the rate-limiting step in the interconversion of the natural polyamines. However, **1**−**3** turned out to be capable of inducing biosynthesis of SSAT similarly to spermine and spermidine.[12]

Analogues **1**−**3** are as effective as the natural polyamines in inducing the conversion of the right-handed B-DNA to left-handed Z-DNA.[13] Furthermore, **1** (similarly to spermidine) and **2** (similarly to spermine due to its conversion to spermidine) serve as substrates for deoxyhypusine synthase, which post-translationally modifies the eukaryotic initiation factor 5A.[14] Moreover, **1** and **3** proved to be metabolically quite stable, as they are not substrates for diamine or monoamine oxidases[15,16] and as such are auspicious tools to evaluate the roles of the polyamines in different cellular functions both in vivo and in vitro. Compound **2**, on the other hand, is more complicated, as one end of the spermine backbone is unmodified and this analogue can serve as a substrate for the catabolic enzymes.

We have recently published results covering the effects of these analogues in the transgenic rats.[17,18] The SSAT transgene under the control of the mouse metallothionein I promoter (MT) is greatly induced in the MT-SSAT transgenic rats by zinc administration. This leads to drastically reduced levels of the higher polyamines in the pancreas and results in a severe necrotizing pancreatitis.[18] In vitro studies have shown that, in the case of SSAT overinduction, exogenous supplementation of the natural polyamines does not restore cell viability due to their rapid intracellular catabolism.[19] However, treatment with **1** prevented the acute pancreatitis altogether in vivo[18] and both **1** and **3** restored the early liver regeneration in the partially hepatectomized transgenic rats.[17,18] Moreover, we showed that **1** and **3** are competitive SSAT inhibitors and rather poor substrates for the oxidases (especially **1**) involved in the catabolism of the polyamines in vitro.[17]

Here we present efficient synthetic protocols to prepare **1**−**3** in quantities sufficient for animal studies. Synthesis of previously unknown 1-amino-8-acetamido-5-azanonane (**4**, Ac-α-MeSPD), a mimic of N^1-acetylspermidine (an excellent substrate for PAO), is also described. This compound and its different enantiomers are essential to bypass the acetylation block of α-methylated polyamine analogues by SSAT and to study the substrate requirements of PAO. The biological tolerances of **1**−**3** were for the first time measured in MT-SSAT transgenic and syngenic mice in vivo. Furthermore, these analogues were also studied with MT-SSAT transgenic rats in vivo and with the immortalized MT-SSAT transgenic rat fibroblasts in vitro.

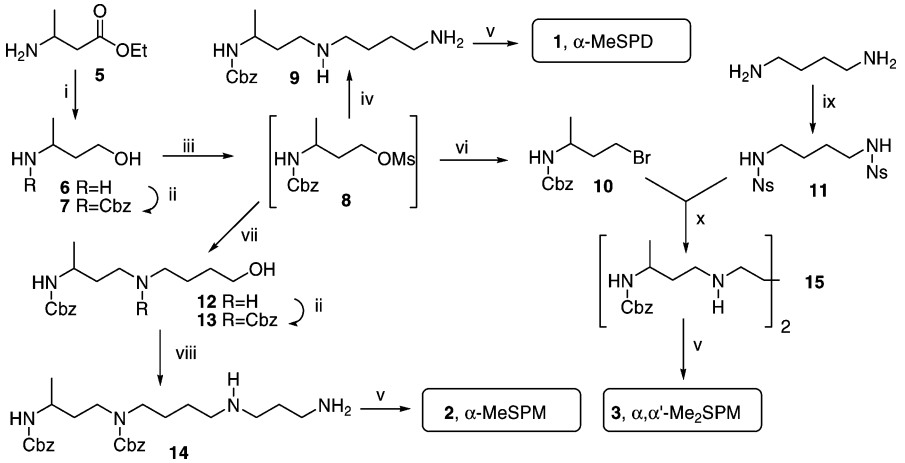
Chemical Synthesis

Scheme 2 outlines the synthesis of the studied α-methylated polyamine analogues **1**−**3**. Synthesis was started from commercially available ethyl 3-aminobutyrate (**5**), which was reduced by $LiAlH_4$ to amino alcohol **6**, protected with benzylchloroformate (CbzCl) to give **7**, and mesylated to our key intermediate **8**. This mesyl derivative, which was not further purified, was easily converted either to *N*-protected methylspermidine **9**, bromide derivative **10**, or amino alcohol **12**.

To prepare compound **1**, the intermediate **8** was first aminated with an excess of putrescine to **9** at 0−20 °C, which decreased the number of minor byproducts that were difficult to separate and enabled good yield (72%). The target trihydrochloride of **1** was obtained after catalytic hydrogenation and crystallization from aqueous acidic alcohol in 46% overall yield as calculated for starting **5**.

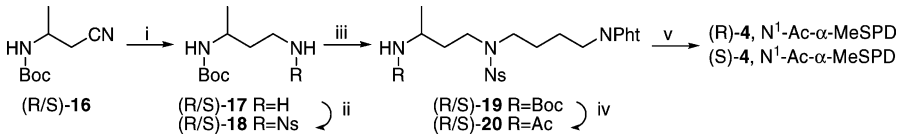
The synthesis of corresponding spermine analogue **2** was started from mesyl intermediate **8** by elongation of the protected polyamine backbone first with an excess of 4-aminobutanol at 0−37 °C to give **12**, which was then protected to **13**, mesylated, and treated with an excess of 1,3-diaminopropane in THF at 0−37 °C to give **14** in 70% yield. We also tried amination at

Scheme 2. Synthesis of Racemic α-Methylated Polyamines **1**−**3**[a]



[a] Reagents and conditions: (i) $LiAlH_4$, THF, reflux; (ii) Cbz-Cl, H_2O, $NaHCO_3$; (iii) MsCl, Et_3N, 0 °C; (iv) $H_2N(CH_2)_4NH_2$, THF, 0−20 °C; (v) (1) H_2/Pd, AcOH−MeOH 1:1; (2) HCl, MeOH; (vi) LiBr, THF; (vii) $H_2N(CH_2)_4OH$, THF, 0−37 °C; (viii) (1) MsCl, Et_3N, (2) $H_2N(CH_2)_3NH_2$, THF, 0−20 °C; (ix) NsCl, Et_3N, CH_2Cl_2, 0 °C; (x) (1) K_2CO_3, DMF; (2) PhSH, K_2CO_3, DMF.

Scheme 3. Synthesis of Enantiomers of Acetyl-α-methylspermidine, **4**[a]



[a] Reagents and conditions: (i) $LiAlH_4$, Et_2O, −5 °C; (ii) Ns-Cl, Et_3N, CH_2Cl_2, 0 °C; (iii) $PhtN(CH_2)_4I$, K_2CO_3, DMF; (iv) (1) HCl, EtOH, EtAc; (2) AcCl, Et_3N, CH_2Cl_2, 0 °C; (v) (1) PhSH, K_2CO_3, DMF; (2) $H_2NNH_2 \cdot H_2O$, EtOH, reflux; (3) HCl, MeOH.

20 °C, but this only increased the number of minor byproducts, which made purification of the compound **14** more laborious. The target tetrahydrochloride of **2** was obtained after deprotection similar to **1** in 41% overall yield calculated for *N*-Cbz-3-aminobutanol **7**.

The above linear strategy was ineffective for the synthesis of α,α′-Me_2SPM **3**. However, due to symmetry of the target molecule, a straightforward convergent strategy was developed starting from bis-nosyl protected putrescine **11**. This derivative was not readily soluble in most of organic solvents, except DMF and DMSO. The attempts to alkylate **11** with mesyl or tosyl derivatives of **7** gave low yields. However, the bromide derivative **10** reacted smoothly with **11** in the presence of K_2CO_3, and after selective removal of Ns-groups with a small excess of thiophenol, symmetrical **15** was obtained in a high yield. As previously, Cbz-groups were removed by catalytic hydrogenation and the tetrahydrochloride of **3** was isolated in 57% overall yield as calculated for **7**.

To prepare isomers (*R*)-**4** and (*S*)-**4**, the corresponding enatiomerically pure nitriles **16** were synthesized as described earlier[20] and were reduced by $LiAlH_4$ without racemization to give the key amines (*R*)-**17** and (*S*)-**17** (Scheme 3). These amines were converted into nosylates **18** and then smoothly alkylated with 4-iodophthalimidobutane to give enantiomers (*R*)-**19** and (*S*)-**19** in 89% and 91% yields, respectively. Removal of the Boc group with HCl/EtOH followed by acetylation and subsequent one-pot removal of nosyl and phthaloyl groups with PhSH and $H_2NNH_2 \cdot H_2O$, respectively, resulted in (*R*)−**4** and (*S*)−**4**, which were converted to hydrochlorides and crystallized. In conclusion, the earlier unknown (*R*)-**4** and (*S*)-**4** were prepared in seven steps with good overall yields 51% and 52%, respectively, as calculated for starting material **16**.

Biological Results and Discussion

The methylated polyamines accumulated in a dose-dependent manner in the three tissues studied: liver (Figure 2), pancreas (Tables S4, S9, and S14 of the Supporting Information), and kidney (Tables S5, S10, and S15 of the Supporting Information) of the MT-SSAT transgenic and syngenic mice. Target tissues were selected on the basis of our focus in current research: liver for toxicity and regeneration, pancreas due to a pancreatitis study, and kidney because higher polyamines are associated with nephrotoxicity. The dosages used in the studies, 50−500 mg/kg of **1** (equaling 1−10-fold of the dosage used for the liver regeneration or pancreatitis studies in rat), 12.5−50 mg/kg of **2**, and 12.5−50 mg/kg of **3** (equaling 0.25−1-fold of the dosage used in the pancreatitis studies in rat), were well-tolerated in mice. The total polyamine levels were not much affected in either mouse line by the treatments, with the exception of liver, where spermidine was markedly depleted (Figure 2, Tables S3−S5, S8−10, and S13−15 of the Supporting Information). In both mouse lines the SSAT induction was greatest with **1** (2−20-fold), whereas **3** resulted in practically no SSAT induction at all (Tables S2, S7, and S12 of the Supporting Information). As expected, the results from mice treated with **2** were more complicated. The induction of SSAT by the compounds

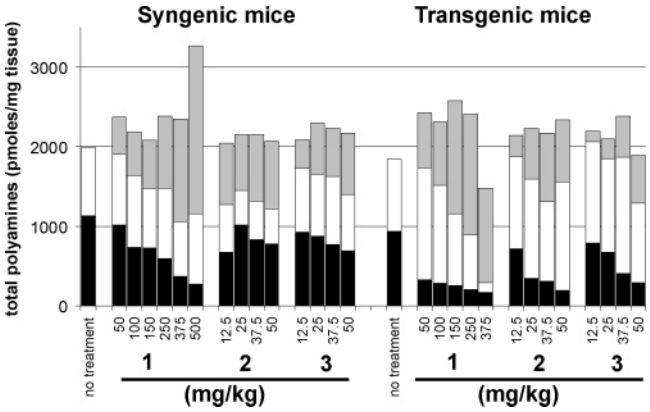


Figure 2. The distribution of polyamines and their α-methylated analogues in the liver of MT-SSAT transgenic mice and their syngenic littermates after 24 h treatment with the indicated dosages. $N = 3-5$; **1**, α-methylspermidine; **2**, α-methylspermine; **3**, bis-α,α′-methylspermine; black, spermidine; white, combined amounts of putrescine, N^1-acetylspermidine, and spermine; gray, α-methylated polyamine analogues.

pronounced in the transgenic mice, but only in the transgenic liver, **1** was detected as the degradation product of **2**. On the other hand, in the syngenic pancreas, **2** appeared to be completely degraded as shown by the large accumulation of spermidine. However, whether **2** degraded to **1** or spermidine was impossible to distinguish due to the large spermidine peak in the HPLC graphs that obscured the possible appearance of **1**. Interestingly, **2** appeared to be stable in the pancreas of the transgenic mice (Table S14 of the Supporting Information). The spermine levels remained mostly unaffected or decreased slightly in response to the treatments; only in the livers of the syngenic animals and in the kidneys of both mouse lines treated with **3** were spermine levels significantly depleted. The analogues did not affect plasma α-amylase or ALAT activities (Tables S1, S6, and S11 of the Supporting Information). All analogues were well-tolerated and the general tolerability was comparable to that of the natural polyamines in mice (LD_{50} values (ip, as hydrochloride) of 870 mg/kg for spermidine and 370 mg/kg for spermine).

The MT-SSAT transgenic rats were treated twice with **1**−**3** at 25 mg/kg dose, and none of the animals were lost during the study. However, at higher doses the mortality rate quickly rose (results not shown). While tissue SSAT activities were only marginally affected, the concentrations of spermidine and spermine were reduced, especially in the liver (Table S17 of the Supporting Information). As in the mouse, **1** appeared to be metabolically stable in the transgenic rat, whereas **2** was readily degraded in both the liver and the kidney. Furthermore, **3** degraded to **1** to some extent only in the liver (Table S17 of the Supporting Information). No degradation of spermine analogues was detected in the rat pancreas, but again low amounts of **1** could have remained undetected with the used HPLC system due to the spermidine peak. As a conclusion, it appears that there are some species-specific and/or the trans-

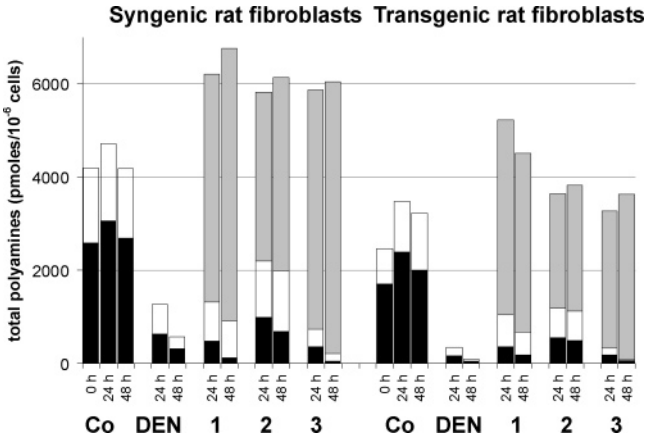


Figure 3. The distribution of polyamines and their α-methylated analogues in the immortalized rat fibroblasts during 48 h culture. Cells were plated in triplicates in six-well culture plates in Dulbecco's modified Eagle's medium supplemented with heat-inactivated 10% fetal bovine serum and gentamycin 50 μg/mL 24 h before the growth medium was replaced with fresh medium and the drugs. Co, untreated cells; DENSpm, 10 μM diethylnorspermine; **1**, 1 mM α-methylspermidine; **2**, 10 μM α-methylspermine; **3**, 1 mM bis-α,α′-methylspermine; black, spermidine; white, combined amounts of putrescine and spermine; gray, α-methylated polyamine analogues. No N^1-acetylspermidine was detected in either fibroblast cell lines.

genecity-related differences in the maintenance of the polyamine homeostasis in the studied animals.

The in vitro studies with immortalized rat fibroblasts derived from the MT-SSAT transgenic and syngenic rats showed that the treatment with **1**−**3** effectively reduced the pools of natural polyamines (Figure 3). Both **1** and **3** were well-tolerated at 1 mM, whereas concentrations higher than 10 μM of **2** were toxic. The supplementation of the cells with 1 mM aminoguanidine (AG) had insignificant effects on the polyamine contents of the fibroblasts. However, **2** was readily degraded in the absence of AG in both cell lines, while the degradation of **3** was detected only in the transgenic cells independent of the AG treatment (Tables S19A,B of the Supporting Information). Moreover, in the transgenic cells, **2** clearly inhibited the proliferation of the fibroblasts in the absence of AG, while in rodents both spermine analogues were equally well tolerated (Table S17 of the Supporting Information). Interestingly, the SSAT activity increased slightly (2−10-fold) in the transgenic cell lines upon exposure to **1**−**3** but remained unaffected in the control cells, whereas DENSpm induced SSAT 200−300-fold in the transgenic cells but only about 10-fold in the control cells (Table S18 of the Supporting Information). The maximal uptake of the analogues appeared to be reachable at low concentrations in vitro, whereas in vivo the analogue accumulation depended on the dose (Tables S3−S5, S8−S10, S13−S15, S17, and S19A,B of the Supporting Information).

To study the fate of **4**, the use of recombinant human PAO was essential, as α-methylated polyamine analogues are not acetylated in vivo or in vitro. Furthermore, **4** is not effectively transported into the immortalized fibroblasts (unpublished observations), and in fact, acetylation is considered to facilitate the export of excess polyamines. In addition, **4** is an excellent compound to assess stereospecific substrate requirements for PAO. We found that PAO strongly preferred (Figure 4) the *R*-isomer of **4** (K_m = 95 μM, k_{cat} = 9.0 s^{-1}) versus the *S*-isomer (K_m = 170 μM, k_{cat} = 1.2 s^{-1}). Expectedly, the degradation of racemic **4** (K_m = 100 μM, k_{cat} = 1.3 s^{-1}) was strongly affected by the presence of (*S*)-**4**. Interestingly, the k_{cat} value for (*R*)-**4** was slightly higher than that for N^1-acetylspermidine (K_m = 14 μM, k_{cat} = 8.5 s^{-1}), suggesting that PAO could readily use acetylated *R*-isomers of α-substituted polyamine analogues. Thus, the polyamine analogues with other alkyl and/or aromatic substituents in the α-position are interesting compounds for further studies to determine the substrate requirements of PAO.

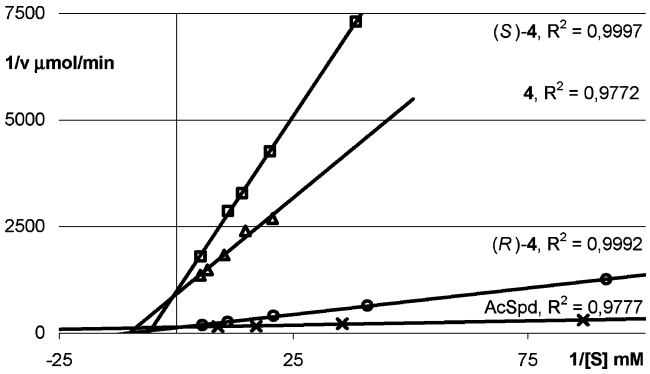


Figure 4. Lineweaver−Burk plot to calculate kinetic values of racemic and different isomers of N^8-acetyl-α-methylspermidine. The kinetic studies of human recombinant polyamine oxidase were performed in duplicates with four or five different substrate concentrations from 10 to 200 μM. Reactions were carried out in total volumes of 180 μL containing 100 mM glycine−NaOH (pH 9.5) and 5 mM DTT and were allowed to proceed for 10−60 min at 37 °C before HPLC analysis. No degradation of the substrates was detected in the absence of the enzyme. (*S*)-**4**, (*S*)-N^8-acetyl-α-methylspermidine; **4**, racemic N^8-acetyl-α-methylspermidine; (*R*)-**4**, (*R*)-N^8-acetyl-α-methylspermidine; AcSPD, N^1-acetylspermidine.

In summary, the synthetic schemes described in the present paper allow the preparation of **1**−**4** in gram quantities in more than 99% purity. Suggested synthetic schemes are also suitable for the preparation of (*R*)- and (*S*)-isomers of **1**−**3**, which may exhibit different biological activities. The strong stereoselectivity of PAO, which was demonstrated for the first time with the different isomers of **4**, may have biological implications. The presented animal experiments show that these polyamine analogues are well tolerated in mice. Furthermore, the studies in vitro confirm that these analogues are effectively transported into fibroblasts and can support cell proliferation when the natural polyamines are severely depleted. These compounds have proven to be useful tools in studies aimed at elucidating the functions of single polyamines both in vivo and in vitro.

Experimental Section

Chemistry. Ethyl 3-aminobutyrate, (*R*)-alaninol, (*S*)-alaninol, 1,4-diaminobutane, 4-(*N*-iodobutyl)phthalimide, thiophenol, methanesulfonyl chloride (MsCl), $LiAlH_4$, and *o*-nitrophenylsulfonyl chloride (NsCl) were purchased from Aldrich (United States), and the rest of the chemicals were from Fluka (Switzerland). (*R*)- And (*S*)-*N*-(*tert*-Butyloxycarbonyl)-3-amino-butyronitrile (**16**) were prepared following published procedures.[20] TLC was carried out on precoated Kieselgel 60 F254 plates and column chromatography with Kieselgel (40−63 μm, Merck, Germany) using the following elution systems: (A) 97:3 $CHCl_3$−MeOH, (B) 9:1 dioxane−25% ammonia, (C) 4:2:1:2 *n*-butanol−AcOH−pyridine−H_2O, (D) $CHCl_3$, (E) 200:1 $CHCl_3$−MeOH, (F) 97:3 dioxane−25% ammonia, (G) 95:5 $CHCl_3$−MeOH, (H) 96:4 dioxane−25% ammonia, (I) 98:2 dioxane−25% ammonia, (J) 95:5 dioxane−25% ammonia, (K) 1:2 EtOAc−hexane, and (L) 75:25 dioxane−25% ammonia. Melting points were determined in open capillary tubes and are uncorrected. Optical rotation angles were measured with Perkin-Elmer 241 digital polarimeter. Chiral-HPLC analysis was performed using a Whelk-O 1 (*R*,*R*) 25 cm × 4.6 mm column, with an isocratic run from 0 to 75 min with a flow rate of 0.5 mL/min of 60% ethanol to separate (*R*)- and (*S*)-isomers of Ac-α-MeSPD after dansylation, and treatment was as described previously.[21] ^1H and ^{13}C NMR spectra

were measured on a Bruker Avance 500 DRX using tetramethylsilane (TMS) in $CDCl_3$ or sodium 3-(trimethylsilyl)propanesulfonate (TSP) in D_2O as internal standards. Chemical shifts are given in ppm, and the letter *J* indicates normal $^3J_{HH}$ couplings and all *J* values are given in hertz.

3-Aminobutan-1-ol (6). A solution of freshly distilled ethyl 3-aminobutyrate (**5**, 13.5 g, 0.103 mol) in dry THF (50 mL) was added dropwise to the stirred suspension of $LiAlH_4$ (8 g, 0.21 mol) in dry THF (150 mL). The reaction mixture was refluxed with stirring for 3 h and kept at 20 °C overnight. The residue was quenched first with water (11.4 mL), 20% NaOH (10.6 mL), water (29.0 mL), and finally with 40% NaOH (34.2 mL). The organic phase was separated, and the solids were extracted with hot chloroform (4 × 80 mL). The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo, and the residue was distilled (bp 108−9 °C/42 mmHg, lit.[22] bp 73 °C/7 mmHg) to give **6** (7.3 g, 80%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 3.79−3.68 (2H, m, CH_2O), 3.12−3.03 (1H, m, MeCH), 2.58 (3H, bs, OH, NH_2), 1.62−1.54 (1H, m, CHCH_2), 1.50−1.40 [1H, m, CHCH_2 (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 1.09 (3H, d, $J = 6.5$, CH_3).

***N*-(Benzyloxycarbonyl)-3-aminobutan-1-ol (7)** was prepared from benzyl chloroformate (4.65 mL, 33 mmol), **6** (2.7 g, 30 mmol), THF (30 mL), water (5 mL), and $NaHCO_3$ (3.81 g, 45 mmol) using the known method.[23] The residue was triturated with a 1:2 ether−hexane mixture (80 mL), and the precipitate was filtered and dried in a vacuum over P_2O_5, to give **7** (6.03 g, 90%): mp 60 °C; R_f 0.38 (A); ^{1}H NMR ($CDCl_3$) δ 7.37−7.28 (5H, m, Ph), 5.09 (2H, s, CH_2Ph), 4.75 (1H, bs, NHCbz), 4.01−3.93 (1H, m, MeCH), 3.67−3.59 (2H, m, CH_2O), 2.98 (1H, bs, OH), 1.83−1.72 (1H, m), 1.45−1.35 [1H, m, CHCH_2 (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 1.20 (3H, d, $J = 6.5$, CH_3).

***N*^{8}-(Benzyloxycarbonyl)-1,8-diamino-5-azanonane (9).** To a stirred and cooled (0 °C) solution of **7** (5.51 g, 24.7 mmol) and Et_3N (5.22 mL, 37.5 mmol) in dry DCM (60 mL) was added MsCl (2.14 mL, 27.5 mmol) in dry DCM (10 mL) within 10 min. Stirring was continued for 1 h at 0 °C and 30 min at room temperature, and the reaction mixture was poured into 1 M $NaHCO_3$ (40 mL) solution. The organic layer was separated and washed with water (2 × 10 mL), 0.5 M H_2SO_4 (3 × 35 mL), water (2 × 10 mL), and 1 M $NaHCO_3$ (10 mL). The dried ($MgSO_4$) and evaporated residue of **8** was dissolved in anhydrous THF (40 mL) and cooled to 0 °C, a cold (0 °C) solution of 1,4-diaminobutane (35.1 g, 400 mmol) in dry THF (40 mL) was added in one portion, and the reaction mixture was kept for 6 h at 0 °C and 16 h at room temperature. THF and excess of 1,4-diaminobutane were evaporated in vacuo and the residue was purified on a silica gel column using eluent B to give **9** (5.3 g, 72%) as a viscous oil: R_f 0.15 (B); ^{1}H NMR ($CDCl_3$) δ 7.37−7.27 (5H, m, Ph), 5.57 (1H, bs, NHCbz), 5.08 (2H, s, CH_2Ph), 3.85−3.76 (1H, m, MeCH), 2.74−2.52 (6H, m, CH_2NH), 1.71−1.41 (6H, m, CCH_2C), 1.40 (3H, bs, NH, NH_2), 1.17 (3H, d, $J = 6.5$, CH_3); ^{13}C NMR δ 156.04 s, 136.85 s, 128.49 s, 128.01 s (2C), 66.39 t, 49.77 t, 46.43 t, 45.96 d, 42.11 t, 36.64 t, 31.57 t, 27.43 t, 21.25 q.

1,8-Diamino-5-azanonane Trihydrochloride (1, α-MeSPD). Pd black in methanol (∼1 mL) was added to a solution of **9** (5.2 g, 17.7 mmol) in a mixture of AcOH−MeOH (1:1, 40 mL), and hydrogenation was carried out at atmospheric pressure. Solids were filtered off and washed with MeOH, and the combined filtrates were evaporated in vacuo. The residue was dissolved in ethanol, diluted with 7 M HCl (6.6 mL), and evaporated to dryness in vacuo, and the residue was recrystallized from a H_2O−MeOH−EtOH mixture to give **1** (4.23 g, 89%) as colorless crystals: mp 191−2 °C (lit. mp 283 °C[16] for lyophilized powder); R_f 0.45 (C); ^{1}H NMR (D_2O) δ 3.53 (1H, m, MeCH), 3.21 (2H, m, NCH_2), 3.15 (2 H, m, NCH_2), 3.07 (2H, m, NCH_2), 2.16 [1H, m, CCH_2C (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 2.02 [1H, m, CCH_2C (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 1.86−1.74 (4H, m, CCH_2C), 1.36 (3H, d, $J = 6.5$, CH_3); ^{13}C NMR δ 50.03 t, 48.36 d, 46.89 t, 41.85 t, 33.34 t, 26.85 t, 25.69 t, 20.38 q. Anal. ($C_8H_{24}N_3Cl_3$) C, H, N.

***N*-(Benzyloxycarbonyl)-3-amino-1-bromobutane (10).** The mesyl intermediate **8** was prepared as in **9** from **7** (3.01 g, 13.5 mmol), Et_3N (2.52 mL, 18.1 mmol), and MsCl (1.16 mL, 15 mmol). The evaporated and dried residue was dissolved in THF (5 mL), and LiBr (3.5 g, 40 mmol) in THF (20 mL) was added in one portion. The reaction mixture was stirred overnight at 20 °C, chloroform (30 mL) was added, solids were filtered off, and the filtrate was evaporated in vacuo. The residue was dissolved in chloroform (50 mL); washed with water (2 × 30 mL), 1 M $NaHCO_3$ (2 × 15 mL), and brine (20 mL); and dried ($MgSO_4$). The evaporated residue was dried in vacuo over P_2O_5/KOH to give **10** (3.78 g, 95%) as a viscous oil: R_f 0.61 (D); ^{1}H NMR ($CDCl_3$) δ 7.37−7.26 (5H, m, Ph), 7.11 (1H, br s, N*H*Cbz), 5.03 (2H, s, CH_2-Ph), 3.78−3.72 (1H, m, MeC*H*), 3.46−3.41 (2H, t, $J = 7.4$, CH_2-Br), 2.10−1.99 [1H, m (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 1.95−1.85 [1H, m, CHCH_2 (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 1.12 (3H, d, $J = 6.6$, CH_3).

Bis-*N*^{1},*N*^{4}-*o*-nitrophenylsulfonyl-1,4-diaminobutane (11). To a cooled (0 °C) solution of freshly distilled 1,4-diaminobutane (1.1 g, 12.5 mmol) and Et_3N (5.2 mL, 37.5 mmol) in dry DCM (50 mL) was added NsCl (6.05 g, 27.3 mmol) in dry DCM (30 mL) within 40 min with vigorous stirring. After addition, stirring was continued for 1 h at 0 °C and 3 h at room temperature. The precipitate was filtered off, washed with MeOH (3 × 15 mL) and chloroform (3 × 10 mL), and dried in vacuo over P_2O_5 to give **11** (5.3 g, 91%) as a pale-yellow crystals: R_f 0.55 (E); mp 185−6 °C; ^{1}H NMR (DMSO-d_6) δ 7.98−7.91 (4 H, m, Ph), 7.86−7.81 (4H, m, Ph); 2.87−2.82 (4H, m, CH_2N), 1.44−1.37 (4H, m, CCH_2C).

***N*^{8}-(Benzyloxycarbonyl)-8-amino-5-azanonan-1-ol (12).** Mesyl intermediate **8** was prepared as in **9** from **7** (3.34 g, 15 mmol), Et_3N (2.61 mL, 19 mmol), and MsCl (1.24 mL, 16 mmol). The evaporated residue was dissolved in THF (10 mL) and cooled to 0 °C. To the resulting solution the cold (0 °C) mixture of 4-aminobutanol (12.3 g, 138 mmol) and THF (20 mL) was added in one portion, and reaction mixture was stirred at each three temperatures (0, 20, and 37 °C) for 12 h, totaling 36 h. The solvent and an excess of 4-aminobutanol were evaporated in vacuo, and the residue was dissolved in 2 M NaOH (25 mL) and extracted with DCM (2 × 45 mL). The organic layer was separated, washed with brine (15 mL), dried (Na_2SO_4), and evaporated in vacuo. The residue was purified on silica gel with eluent F, which resulted in **12** (3.75 g, 85%) as a viscous oil: R_f 0.45 (B); ^{1}H NMR ($CDCl_3$) δ 7.40−7.29 (5H, m, Ph), 5.09 (2H, s, CH_2Ph), 5.03 (0.5H, s, N*H*Cbz), 5.01 (0.5H, s, N*H*Cbz), 3.85−3.77 (1H, m, MeC*H*), 3.57 (2H, t, *J* 6.3, CH_2OH), 2.70−2.55 (4H, m, CH_2N), 1.76−1.51 (8H, m, NH, OH, CCH_2C), 1.18 (3H, d, $J = 6.5$, CH_3).

Bis-*N*,*N*^{5}*N*^{8}-(benzyloxycarbonyl)-8-amino-5-azanonan-1-ol (13). Benzyl chloroformate (1.55 mL, 11 mmol) was added in three portions with 15-min intervals to a cooled (0 °C) and vigorously stirred mixture of **12** (2.94 g, 10 mmol), THF (20 mL), $NaHCO_3$ (0.84 g, 10 mmol), and 2 M Na_2CO_3 (6 mL). Stirring was continued for 1 h at 0 °C and 3 h at room temperature, organic layer was separated, and the residue was extracted with chloroform (15 mL). Organic layers were combined and evaporated to dryness in vacuo, and the residue was dissolved in chloroform (30 mL); washed with 0.5 M H_2SO_4 (2 × 10 mL), water (15 mL), and 1 M $NaHCO_3$ (2 × 10 mL); dried ($MgSO_4$); and evaporated in vacuo. The residue was purified on silica gel using first eluent E and then G to give **13** (3.94 g, 92%) a viscous oil: R_f 0.27 (A); ^{1}H NMR ($CDCl_3$) δ 7.34−7.27 (10H, m, Ph), 5.11 (2H, s, CH_2Ph), 5.07 (2H, s, CH_2-Ph), 4.96 (0.5H, s, N*H*Cbz), 4.57 (0.5H, s, N*H*Cbz), 3.73−3.55 (3H, m, MeC*H*, CH_2O), 3.30−3.18 (4H, m, CH_2N), 1.75−1.45 (7H, m, OH, CCH_2C), 1.17−1.10 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.16, 155.90, 136.82, 128.52, 128.07, 128.00, 127.85, 67.07, 66.59, 62.33, 47.44, 45.41, 44.78, 44.34, 36.22, 35.13, 29.66, 25.02, 24.70, 21.32.

Bis-*N*,*N*^{9}*N*^{12}-(benzyloxycarbonyl)-1,12-diamino-4,9-diazatridecane (14). The mesyl intermediate was prepared as in **9** from **13**

(3.85 g, 9 mmol), Et_3N (1.53 mL, 11 mmol), and MsCl (0.77 mL, 10 mmol). The evaporated and dried residue was dissolved in THF (15 mL) and cooled to 0 °C, and a cold (0 °C) solution of 1,3-diaminopropane (6.66 g, 90 mmol) in THF (5 mL) was added in one portion. After 6 h at 0 °C and overnight at room temperature, the reaction mixture was concentrated in vacuo. The residue was poured into 1 M NaOH (15 mL), extracted with DCM (2 × 15 mL), and evaporated in vacuo, and the residue was purified on silica gel using eluent H to give **14** (3.05 g, 70%) as a viscous oil: R_f 0.25 (F); ^{1}H NMR ($CDCl_3$) δ 7.34−7.29 (10H, m, Ph), 5.10 (2H, s, C*H*$_2$Ph), 5.07 (2H, s, C*H*$_2$Ph), 4.79 (1H, s, N*H*Cbz), 3.35−3.20 (5H, m, MeCH, CH_2N), 2.73 (2H, t, J = 6.8, C*H*$_2$N), 2.66−2.53 (4H, m, C*H*$_2$N), 1.70−1.35 (11H, m, NH, NH_2, CC*H*$_2$C), 1.16−1.10 (3H, m, CH_3); ^{13}C NMR ($CDCl_3$) δ 155.85, 136.88, 136.70, 128.48, 128.02, 127.92, 127.79, 67.07, 66.96, 66.48, 49.63, 47.85, 47.62, 47.17, 45.37, 44.77, 44.32, 40.54, 36.20, 35.06, 33.77, 27.25, 26.48, 25.95, 21.33.

1,12-Diamino-4,9-diazatridecane tetrahydrochloride (2,α-MeSPM) was prepared as **1** from **14** (3.0 g, 6.2 mmol) and recrystallized from a H_2O−MeOH−EtOH mixture to give **2** (1.97 g, 88%) as colorless crystals: mp 250−1 °C (dec) (lit.[16] mp 247 °C for lyophilized powder): R_f 0.13 (C); ^{1}H NMR (D_2O) δ 3.56−3.48 (1H, m, MeCH); 3.24−3.10 (10H, m, C*H*$_2$N); 2.18−2.08 (3H, m, NHCHC*H*$_2$, C*H*$_2$CH$_2$NH$_2$); 2.04−1.95 (1H, m, NHCHC*H*$_2$); 1.85−1.77 (4H, m, CCH_2C); 1.36 (3H, d, J = 6.6, CH_3); ^{13}C NMR (D_2O) δ 47.07, 45.41, 44.62, 44.00, 36.67, 30.47, 23.79, 22.83, 17.58, 17.34. Anal. ($C_{11}H_{32}N_4Cl_4$·0.25H_2O) C, H, N.

Bis-*N*^{2},*N*^{13}-(benzyloxycarbonyl)-2,13-diamino-5,10-diazatetradecane (15). Bromide **10** (3.78 g, 13.2 mmol), bis-sulfamide **11** (2.16 g, 4.7 mmol), and K_2CO_3 (4.3 g, 31 mmol) were stirred in DMF (30 mL) for 48 h at room temperature, followed by addition of a mixture of K_2CO_3 (3.7 g, 27 mmol) and PhSH (1.4 mL, 13.5 mmol) in DMF (10 mL). After stirring for an additional 12 h at 20 °C, salts were filtered off, the filtrate was evaporated to dryness in vacuo, the residue was suspended in chloroform (50 mL), and solids were filtered off and washed with chloroform (3 × 10 mL). Combined filtrates were washed with water (2 × 30 mL), dried ($MgSO_4$), and evaporated to dryness in vacuo, and the residue was purified on silica gel using eluent F, resulting in **15** (2.3 g, 70%) as a colorless solid: R_f 0.46 (F); mp 107−8 °C; ^{1}H NMR ($CDCl_3$) δ 7.36−7.26 (10H, m, Ph), 5.51 (2H, bs, N*H*Cbz), 5.08 (4H, s, C*H*$_2$Ph), 3.85−3.74 (2H, m, MeCH), 2.73−2.65 (2H, m, CH_2N), 2.64−2.54 (2H, m, CH_2N), 2.54−2.45 (4H, m, CH_2N), 1.72−1.40 (8H, m, CC*H*$_2$C), 1.16 (6 H, d, J = 6.5, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.03, 136.86, 128.53, 128.05, 67.14, 66.41, 49.82, 46.45, 46.01, 36.66, 27.87, 21.28.

2,13-Diamino-5,10-diazatetradecane tetrahydrochloride (3,α,α′-Me$_2$SPM) was prepared as **2** from **15** (2.3 g, 4.6 mmol) to give **3** (1.47 g, 85%) as colorless crystals: mp 224−5 °C (dec) (lit.[16] mp 180 °C for lyophilized powder): R_f 0.13 (C); ^{1}H NMR (D_2O) δ 3.55−3.46 (2H, m, MeCH); 3.21 (4H, t, J = 6.8, CH_2N); 3.17−3.11 (4H, m, CH_2N), 2.18−2.09 (2H, m, CCH_2C), 2.04−1.93 (2H, m, CCH_2C), 1.84−1.75 (4H, m, CCH_2C); 1.35 (6H, d, J = 6.6, CH_3); ^{13}C NMR (D_2O) δ 49.86, 48.14, 46.78, 33.27, 25.64, 20.09. Anal. ($C_{12}H_{34}N_4Cl_4$) C, H, N.

(*R*)-*N*^{3}-(*tert*-Butyloxycarbonyl)-1,3-diaminobutane ((*R*)-17). To a cooled (−5 °C) suspension of $LiAlH_4$ (0.96 g, 25 mmol) in Et_2O (20 mL) was added a solution of (*R*)-**16**[20] (1.7 g, 9.2 mmol) in Et_2O (15 mL) with stirring within 20 min, and stirring was continued for 45 min at −5 °C, followed by quenching of the reaction mixture with 20% (w/w) aq NaOH. The organic phase was separated, the residue was extracted with Et_2O (3 × 20 mL), and the combined organic phases were washed with brine (20 mL). Ether was evaporated in vacuo and the residue was purified on silica gel using eluent I to give (*R*)-**17** (1.56 g, 90%) as a colorless oil: R_f 0.33 (J); $[\alpha]^{20}_D$ −12.0° (*c* 2.0, $CHCl_3$) (lit.[20] $[\alpha]^{20}_D$ −12.0°); ^{1}H NMR ($CDCl_3$) δ 4.61 (1H, bs, NH), 3.77 (1H, bs, MeCH), 2.79−2.69 (2H, m, CH_2N), 1.60−1.44 (m, 2H), 1.43 (s, 9H), 1.08 (3H, d, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 155.51, 78.89, 44.26, 40.99, 38.81, 28.34, 21.43.

(*S*)-*N*^{3}-(*tert*-Butyloxycarbonyl)-1,3-diaminobutane ((*S*)-17) was prepared as (*R*)-**17** starting from (*S*)-**16**[20] to give (*S*)-**17** as a colorless oil (91%): $[\alpha]^{20}_D$ +12.0° (*c* 2.0, $CHCl_3$) (lit.[20] $[\alpha]^{20}_D$ +12.0°); ^{1}H NMR shifts were identical to those of (*R*)-**17**.

(*R*)-*N*^{1}-(*o*-Nitrophenylsulfonyl)-*N*^{3}-(*tert*-butyloxycarbonyl)-1,3-diaminobutane ((*R*)-18). To the cooled (0 °C) solution of (*R*)-**17** (1.43 g, 7.6 mmol) and Et_3N (1.21 mL, 8.8 mmol) in dry DCM (15 mL) was added the solution of NsCl (1.77 g, 8.0 mmol) in dry DCM (7 mL) with stirring within 30 min. Stirring was continued for 1 h at 0 °C, followed by washing the reaction mixture with H_2O (10 mL), 10% citric acid (15 mL), 1 M $NaHCO_3$ (10 mL), and brine (10 mL) and drying ($MgSO_4$). The solvent was removed in vacuo to afford (*R*)-**18** (2.78 g, 98%) as a colorless solid: mp 115−6 °C (H_2O−MeOH); R_f 0.21 (K); $[\alpha]^{20}_D$ −13.0° (*c* 5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.14−8.10 (1H, m, Ar), 7.85−7.81 (1H, m, Ar), 7.74−7.69 (2H, m, Ar), 6.23 (1H, bs, NsNH), 4.31 (1H, d J = 6.5, NH), 3.80−3.69 (1H, m, MeCH), 3.33−3.22 (1H, m, CH_2N), 3.01 (1H, m, CH_2N), 1.78−1.69 [1H, m, CHC*H*$_2$ (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 1.51−1.43 [1H, m, CHC*H*$_2$ (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 1.37 (9H, s, CMe_3), 1.11 (3H, d, J = 6.7); ^{13}C NMR δ 155.91, 148.07, 134.24, 133.28, 132.49, 130.70, 125.06, 79.59, 43.81, 40.81, 38.10, 28.26, 21.37. Anal. ($C_{15}H_{23}N_3O_6S$) C, H, N.

(*S*)-*N*^{1}-(*o*-Nitrophenylsulfonyl)-*N*^{3}-(*tert*-butyloxycarbonyl)-1,3-diaminobutane ((*S*)-18) was prepared as (*R*)-**18** starting from (*S*)-**17** to give (*S*)-**18** as a colorless solid (99%): mp 115−6 °C (H_2O−MeOH); $[\alpha]^{20}_D$ +13.0° (*c* 5, $CHCl_3$); ^{1}H NMR shifts were identical to those of (*R*)-**18**. Anal. ($C_{15}H_{23}N_3O_6S$) C, H, N.

(*R*)-*N*^{1}-Phthaloyl-*N*^{5}-(*o*-nitrophenylsulfonyl)-*N*^{8}-(*tert*-butyloxycarbonyl)-1,8-diamino-5-azanonane ((*R*)-19). The mixture of (*R*)-**18** (0.75 g, 2.0 mmol), *N*-(4-iodobutyl)phthalimide (0.725 g, 2.2 mmol), and K_2CO_3 (0.85 g, 6.2 mmol) in dry DMF (4 mL) was stirred for 24 h at 20 °C. Solvent was evaporated in vacuo, the residue was treated with EtOAc−H_2O (2:1, 30 mL), and the organic layer was separated, washed with H_2O (10 mL) and brine (20 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue was crystallized from EtOAc−*n*-hexane to give (*R*)-**19** as pale-yellow crystals (1.03 g, 89%): mp 109−110 °C (EtOAc−*n*-hexane); $[\alpha]^{20}_D$ −7.0° (*c* 2, EtOAc); ^{1}H NMR ($CDCl_3$) δ 8.00−7.96 (1H, m, Ar), 7.86−7.80 (2H, m, Ar), 7.73−7.68 (2H, m, Ar), 7.66−7.61 (2H, m, Ar), 7.58−7.53 (1H, m, Ar), 4.48 (1H, bs, NH), 3.66 (2H, t, J = 6.5, CH_2NPht), 3.62−3.55 (1H, m, MeCH), 3.40−3.21 (4H, m, CH_2N); 1.76−1.52 (6H, m, CCH_2C), 1.42 (9H, s), 1.11 (3H, d, J = 6.5 Hz); ^{13}C NMR δ 168.35, 133.99, 133.49, 133.33, 132.09, 131.54, 130.68, 124.10, 123.28, 78.80, 47.40, 45.14, 37.11; 36.18, 28.41, 25.70, 25.53, 21.47.

(*S*)-*N*^{1}-Phthaloyl-*N*^{5}-(*o*-nitrophenylsulfonyl)-*N*^{8}-(*tert*-butyloxycarbonyl)-1,8-diamino-5-azanonane ((*S*)-19) was prepared as (*R*)-**19** starting from (*S*)-**18** to give (*S*)-**19** as a pale-yellow crystals (91%): mp 109−110 °C (EtOAc−*n*-hexane); $[\alpha]^{20}_D$ +7.0° (*c* 2, EtOAc); ^{1}H NMR shifts were identical to those of (*R*)-**19**.

(*R*)-*N*^{1}-Phthaloyl-*N*^{5}-(*o*-nitrophenylsulfonyl)-*N*^{8}-acetyl-1,8-diamino-5-azanonane ((*R*)-20). To the solution of (*R*)-**19** (0.97 g, 1.69 mmol) in EtOAc (8 mL) was added the solution of dry HCl in EtOH (10 M, 4 mL) and the mixture was stirred for 30 min at 20 °C. Solvents were evaporated in vacuo, the residue was coevaporated with DMF (2 × 15 mL) in vacuo, and the residual oil was dissolved in dry DCM (8 mL) and Et_3N (1.41 mL, 10.2 mmol). After cooling (0 °C), AcCl (1.17 mL, 2.38 mmol) in dry DCM (3 mL) was added with stirring over 10 min, and stirring was continued for an additional 15 min at 0 °C, followed by adding MeOH (3 mL). The solvents were evaporated in vacuo, the residue was dissolved in EtOAc (40 mL); washed with H_2O (20 mL), 10% citric acid (10 mL), and brine (20 mL); dried ($MgSO_4$); and evaporated in vacuo. The residue was crystallized from (EtOAc−*n*-hexane, 1:2) to give (*R*)-**20** as a pale-yellow crystals (0.8 g, 92%): R_f 0.54 (J); mp 125−6 °C (EtOAc−*n*-hexane, 1:2); $[\alpha]^{20}_D$ −6.7° (*c* 2, EtOAc). ^{1}H NMR ($CDCl_3$) δ 7.96−7.92 (1H, m, Ar), 7.86−7.80 (2H, m, Ar), 7.74−7.69 (2H, m, Ar), 7.66−7.61 (2H, m, Ar), 7.57−7.52 (1H, m, Ar), 5.58 (1H, d, J = 8.1, NH); 4.00−3.89

(1H, m, MeCH); 3.67 (2H, t, $J = 6.9$, CH_2NPht), 3.40−3.17 (4H, m, CH_2N), 1.97 (s, 3 Í), 1.85−1.54 (6H, m, CCH_2C), 1.14 (3H, d, $J = 6.5$ Hz); ^{13}C NMR δ 169.94, 168.55, 148.37, 134.21, 133.60, 133.36, 132.22, 131.77, 130.63, 124.21, 123.43, 48.02, 45.47, 43.60, 37.28, 36.29, 25.85, 25.81, 23.60, 21.32.

(*S*)-N^1-Phthaloyl-N^5-(*o*-nitrophenylsulfonyl)-N^8-acetyl-1,8-diamino-5-azanonane ((*S*)-20) was prepared as (*R*)-**20** starting from (*S*)-**19** to give (*S*)-**20** as a pale-yellow crystals (91%): mp 125−6 °C (EtOAc−*n*-hexane, 1:2); $[\alpha]^{20}_D$ +6.7° (*c* 2, EtOAc); ^{1}H NMR shifts were identical to those of (*R*)-**20**.

(*R*)-N^8-Acetyl-1,8-diamino-5-azanonane ((*R*)-4). A mixture of (*R*)-**20** (0.75 g, 1.45 mmol), PhSH (0.23 mL, 2.25 mmol), and K_2CO_3 (0.62 g, 4.5 mmol) in DMF (3 mL) was stirred for 16 h at 20 °C, the solvent was removed in vacuo, and the residue was dissolved in the mixture of DCM−H_2O (3:1, 50 mL). The water layer was separated and extracted with DCM, the combined organic phases were washed with brine (15 mL) and dried ($MgSO_4$), and solvent was evaporated in vacuo. The residue was dissolved in EtOH (6 mL) containing N_2H_4·H_2O (0.06 g, 1.83 mmol) and refluxed for 3 h, and the solvent was evaporated in vacuo. The residue was treated with ann EtOH−water (1:1) mixture (5 mL), an insoluble oil was separated, and the solution was applied to Dowex 1 × 8 (200−400 mesh, HO^-form, 10 mL) and eluted with EtOH−H_2O (1:1) to get rid of phthalyl hydrazide. Fractions containing crude (*R*)-**4** were evaporated to dryness in vacuo, and the residue was purified on a silica gel column using eluent L, resulting in pure (*R*)-**4** as a base. After treatment with HCl in EtOH and recrystallization from a MeOH−EtOH mixture, (*R*)-**4** (0.21 g, 72%) was obtained as colorless crystals: R_f 0.39 (C); ee 95%; mp 200−1 °C (dec) (MeOH−EtOH); $[\alpha]^{20}_D$ +4.0° (*c* 2, H_2O); ^{1}H NMR (D_2O) δ 3.98−3.89 (1H, m, MeC*H*), 3.14 (6H, m, CH_2N), 2.00 (3H, s, $COCH_3$) 1.97−1.87 [1H, m, CHCH_2 (due to chiral center on the next carbon, these CH_2-protons have different chemical shifts)], 1.83−1.72 (5H, m, 5H, CCH_2C), 1.19 (3H, d, $J = 6.6$, CH_3); ^{13}C NMR δ 174.00, 47.04, 44.91, 43.05, 38.96, 32.56, 24.02, 22.85, 22.05, 19.58. Anal. ($C_{10}H_{25}N_3Cl_2O$) C, H, N.

(*S*)-N^8-Acetyl-1,8-diamino-5-azanonane ((*S*)-4) was prepared as (*R*)-**4** starting from (*S*)-**20** to give (*S*)-**4** (70%): ee 95%; mp 200−1 °C (dec) (MeOH−EtOH); $[\alpha]^{20}_D$ −4.0° (*c* 2, H_2O); ^{1}H NMR shifts were identical to those of (*R*)-**4**. Anal. ($C_{10}H_{25}N_3Cl_2O$) C, H, N.

Transgenic Animal Studies. The production of the transgenic mice and rats has been described earlier in detail.[17] Both animal lines were produced by the standard pronuclear injection technique using the same transgene construct. The methylated polyamine analogues were administered in saline. Three to four month old male mice were injected once with 0, 50, 100, 150, 250, 375, or 500 mg/kg (**1**) or with 0, 12.5, 25, 37.5, or 50 mg/kg (**2** and **3**) intraperitoneally 24 h before sacrifice. Ten week old transgenic male rats were treated twice with 25 mg/kg (**1**−**3**) intraperitoneally 22 and 16 h before sacrifice. Tissue samples were frozen in liquid nitrogen and homogenized in the standard buffer (25 mM Tris-HCl pH 7.4, 0.1 mM EDTA, 1 mM DTT). An aliquot of the homogenates was used for the polyamine assays. The homogenates were centrifuged (at 13 000*g*, for 30 min, at 4 °C) and the supernatant fractions were used for the enzyme activity assays. The Institutional Animal Care and Use Committee of the University of Kuopio and the Provincial Government approved the animal experiments.

Immortalized Rat Fibroblast Studies. The production of the immortalized fibroblasts was explained earlier in detail.[17] The cells were plated in triplicates in six-well culture plates in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with heat-inactivated 10% fetal bovine serum and gentamycin (50 μg/mL; Gibco). The cells were allowed to adhere for 24 h before the growth medium was replaced with fresh medium and the drugs. After the incubation, the cells were washed with PBS, detached with trypsin, and counted, and the polyamines were determined after sulfosalicylic acid precipitation from the supernatant fractions with the aid of the HPLC.

Recombinant Protein Studies. The human recombinant PAO was produced according to the Qiagen Qiaexpressionist manual, and the protein was purified under native conditions using Ni−NTA His Bind Resin (Novagen) as described in ref 17, except without affinity purification. PAO was estimated to be 80% pure according to SDS−PAGE. However, the contaminating bacterial protein did not have any PAO-like activity (data not shown). The kinetic studies of PAO were performed in duplicates with four or five different substrate concentrations from 10 to 200 μM. The total volumes of reactions were 180 μL containing 100 mM glycine−NaOH (pH 9.5), 5 mM DTT, and appropriate amount of PAO. The reactions were allowed to proceed for 10−60 min at 37 °C before addition of 20 μL of 100 μM diaminohexane as internal standard in 50% w/v sulfosalicylic acid prior to HPLC analysis.

Assays Related to Biological Studies. The SSAT activity was assayed as described earlier.[24] HPLC was used to determine the concentrations of the polyamines and their α-methylated analogues essentially as described by Hyvönen et al.[25] α-Amylase and alanine amino transferase (ALAT) were determined from heparinized plasma of the mice using a Microlab 200 from Merck.

Acknowledgment. We thank Sisko Juutinen, Anne Karppinen, and Tuula Reponen for skillful technical assistance. This work was supported by the Academy of Sciences of Finland (project no. 108092) and the Russian Foundation for Basic Research, project no. 03-04-49080.

Supporting Information Available: Biological tolerance data and elementary analysis results of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM050872H