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Synthesis of novel optical isomers of α-methylpolyamines

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Abstract—Earlier unknown (*R*)- and (*S*)-α-methylspermidine, (*R*)- and (*S*)-α-methylspermine, (*R*,*R*)-, (*S*,*S*)-, and (*R*,*S*)-α,ω-dimethylspermine were synthesized in gram scale from readily available (*R*)- and (*S*)-2-aminopropanols in high overall yields.
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1. Introduction

Biogenic polyamines spermidine (Spd, 1,8-diamino-4-azaoctane) and spermine (Spm, 1,12-diamino-4,9-diazadodecane) are present in millimolar concentration in mammalian cells and they are essential for normal cell growth and proliferation. The deficiency of Spm and Spd leads to an inhibition of cell growth and migration, whereas overaccumulation of polyamines induces cell death and transformation.[1]

The consequences of polyamine pool depletion are most complex in vivo.[2,3] For example, activation of polyamine catabolism in metallothionein I promoter driven spermidine/spermine-N^1-acetyltransferase (MT-SSAT) transgenic rats with zinc dramatically reduces Spd and Spm pools mainly in liver and pancreas, former delaying liver regeneration after partial hepatectomy[4] and the latter provokes acute pancreatitis.[5] Racemic 1,8-diamino-5-azanonane (α-methylspermidine, α-MeSpd), which is metabolically stable and able to fulfill crucial functions of Spd in cell culture,[6–8] was the first polyamine analogue shown to completely prevent acute pancreatitis when administered prior to activation of SSAT by zinc.[9] Racemic 2,13-diamino-5,10-diazatetradecane (α,ω-dimethylspermine, α,ω-Me_2Spm) also turned to be capable of preventing acute pancreatitis as such.[10] Furthermore, both the drugs have proved to be efficient in restoring the liver regeneration in MT-SSAT rats after partial hepatectomy.[11] Thus, Spd/α-MeSpd and Spm/α,ω-Me_2Spm seem to be equally potent in cellular processes associated with proper maintenance of pancreatic integrity and liver regeneration. Moreover, both the drugs improved the survival of MT-SSAT rats when given after the appearance of pancreatitis.[10] The metabolism of α,ω-Me_2Spm and 1,12-diamino-4,9-diazatridecane (α-methylspermine, α-MeSpm) has been recently investigated in detail both in vitro and in vivo.[11] However, it is still unclear which enantiomer of α-MeSpd, α-MeSpm, and which diastereomer of α,ω-Me_2Spm are the exact biochemical equivalents of Spd and Spm, respectively.

We have recently shown that chiral methylpolyamines are invaluable as tools for discovering and investigating the novel properties of well-known polyamine metabolizing enzymes. Thus, it was for the first time shown that human polyamine oxidase possesses dormant stereoselectivity,[12] which can be controlled with the aid of reversibly binding to the substrate with 'guide molecules'.[13] According to our observations in the case of FAD-dependent polyamine oxidases variation of the nature of alkyl substituent as well as introduction of acyl-, or aralkyl group at terminal amino group resulted in alteration of not only stereospecificity[12,13] but also of the regioselectivity of the enzymatic reaction.[14] As a result, this chemical regulation of enzyme catalysis offers a novel and convenient route to prepare optically active α-substituted derivatives of diaminopropanes, which are useful building blocks for the synthesis of other optically pure compounds, e.g., drug molecules. Chiral polyamine analogues are likely to interact differently in distinct cellular processes, which may involve macromolecules like enzymes and receptors not forgetting the interactions with DNA and RNA. Thus, they help to dissect physiological roles for individual polyamines. This is the scientific rationale to describe here an efficient synthetic strategy to prepare the required enantiomerically pure earlier unknown (*R*)- and (*S*)-α-MeSpd's, (*R*)- and (*S*)-α-MeSpm's, and also (*S*,*S*)-, (*R*,*R*)-, (*R*,*S*)-α,ω-Me_2Spm's in gram scale from readily available starting materials in high overall yields.

Keywords: Polyamines; Methylspermidine; Methylspermine; Chiral synthesis.
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2. Results and discussion

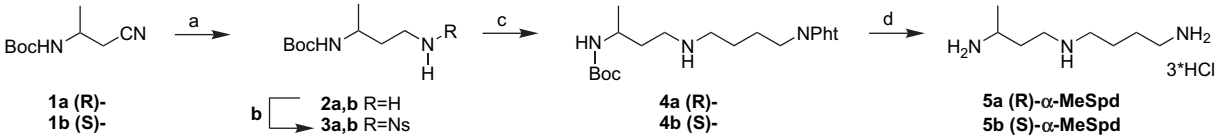
Recently we have developed convenient synthetic strategies for racemic α-MeSpd, α-MeSpm, and α,ω-Me_2Spm starting from readily available 3-aminobutanol in high overall yields.[15,16] To apply these methods for the syntheses of the stereoisomers of α-Me-polyamines, enantiomerically pure (*R*)- and (*S*)-3-aminobutanols are required. These isomers are prepared either by asymmetric synthesis[17] or from optically pure 3-aminobutyric acids,[18] which are synthesized either chemically or are obtained enzymatically from racemic esters, amides or acyl derivatives.[19]

However, in the present paper we have chosen another strategy and used commercially available (*R*)- and (*S*)-2-aminopropanols as starting chiral compounds. The aminoalcohols were converted into Boc-protected aminonitriles **1** (**a**=*R*-isomer and **b**=*S*-isomer) according to the published procedure.[19] The cyano group of **1** was smoothly reduced with $LiAlH_4$ at −5 °C to afford **2** without racemization (Scheme 1). The latter were protected as *o*-nitrophenylsulfonyl (Ns) derivatives **3**, which were the key intermediates in all syntheses.

Target hydrochlorides **5a** and **b** were obtained after removal of Pht- and Boc-groups with overall yields of 43 and 45%, as calculated for starting 2-aminopropanols.

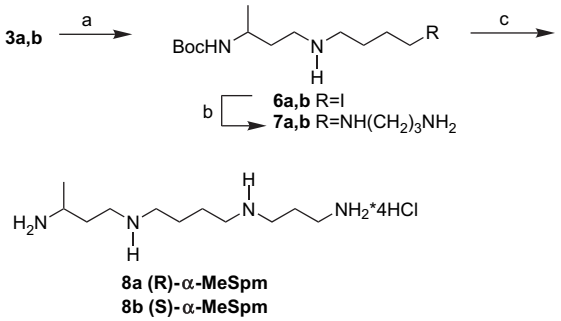
The backbones of (*R*)- and (*S*)-α-MeSpm were built from **3a**,**b** by consecutive introduction of iodobutyl and aminopropyl groups, as depicted in Scheme 2. Sulfamides **3** were alkylated with an excess of 1-bromo-4-chlorobutane in DMF with K_2CO_3 as a base and the obtained chlorides were converted into iodides **6** by refluxing with NaI in acetone.

Compounds **6** were treated with an excess of 1,3-diaminopropane in THF and diamines **7** were isolated by flash chromatography on silica gel. A small amount (~3%) of *o*-nitrophenyl-1,3-diaminopropane, formed, in all likelihood, by a S_NAr mechanism, was isolated from the reaction mixture. Besides, diamines **7** turned to be unstable on storage, most likely due to the similar intra- or intermolecular substitution of sulfamide group. This observation is in a good agreement with the efficient cleavage of Ns-group upon treatment with alkyl or arylsulfide anions, which are much more nucleophilic in comparison with aliphatic amines.



Scheme 1. Preparation of (*R*)- and (*S*)-enantiomers of α-methylspermidine. Reagents and conditions: (a) $LiAlH_4/Et_2O/-5$ °C; (b) $NsCl/Et_3N/CH_2Cl_2$; (c) $PhtN(CH_2)_4I/K_2CO_3$/DMF; $PhSH/K_2CO_3$/DMF; (d) $N_2H_4\cdot H_2O$/EtOH/Δ; HCl/MeOH.

For the preparation of enantiomers of α-MeSpd sulfamides, **3** was alkylated with *N*-(4-iodobutyl)phthalimide. Subsequent 'one-pot' removal of Ns protection led to bis-protected α-MeSpd's **4**, which were isolated by flash chromatography.

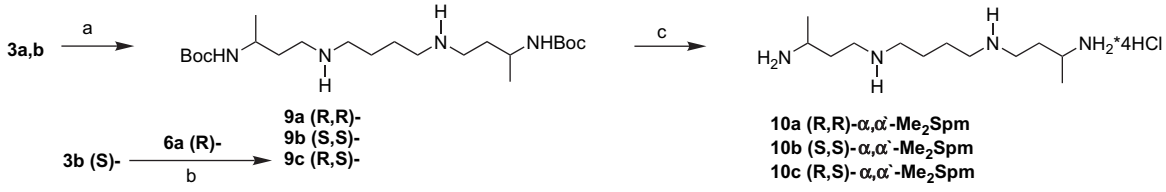


Scheme 2. Preparation of (*R*)- and (*S*)-enantiomers of α-methylspermine. Reagents and conditions: (a) $Br(CH_2)_4Cl/K_2CO_3$/DMF; NaI/acetone/Δ; (b) $H_2N(CH_2)_3NH_2$/THF; (c) $PhSH/Et_3N$/DMF; HCl/EtOH.

The protecting groups in compounds **7** were removed in 'one-pot' by consecutive treatment with PhSH in DMF with Et_3N as a base and then with HCl in dry EtOH. The precipitates were recrystallized from H_2O/MeOH/EtOH to afforded target (*R*)- and (*S*)-α-MeSpm **8a** and **b** as their tetrahydrochloride derivatives with overall yields of 32 and 33%, as calculated from the 2-aminopropanol starting material.

To prepare (*R*,*R*)- and (*S*,*S*)-isomers of bis-α,ω-Me_2Spm the sulfamides **3a** and **b** were reacted with stoichiometrical amount of 1,4-diiodobutane (Scheme 3). The traces of unreacted **3** present were removed by adding BnBr (10 mol %) to the reaction mixture and incubation was continued for additional 14 h. This simplified the purification of the products after removal of Ns-groups, because of the rather close retention times of compounds **9** and **2**.

Ns-groups were removed by $PhSH/K_2CO_3$ treatment and di-Boc-derivatives **9a** and **b** were isolated by flash chromatography. The target tetrahydrochlorides of (*R*,*R*)- and



Scheme 3. Preparation of stereoisomers of α,ω-dimethylspermine. Reagents and conditions: (a) $I(CH_2)_4I/K_2CO_3$/DMF; $PhSH/K_2CO_3$/DMF; (b) **3a**/K_2CO_3/DMF; (c) HCl/EtOH.

(*S*,*S*)-bis-α,ω-Me_2Spm's **10a** and **b** were obtained after removal of Boc-groups by treatment with HCl/EtOH and crystallization from H_2O/MeOH/EtOH with overall yields 42 and 46%, as calculated for the 2-aminopropanol starting material.

meso-α,ω-Me_2Spm was prepared by reacting the iodide **6a** with stoichiometrical amount of sulfamide **3b**. The unreacted **3b** was again removed by using the BnBr method described above and the Ns-groups were cleaved with PhSH; (*R*,*S*)-di-Boc-α,ω-Me_2Spm **9c** was then isolated by flash chromatography. After the removal of Boc-groups with HCl/EtOH the target (*R*,*S*)-bis-α,ω-Me_2Spm **10c** was isolated as tetrahydrochloride with overall yield 40% as calculated for 2-aminopropanol.

3. Conclusion

The syntheses described in the present paper are convenient for the preparation of the previously unknown optical isomers of α-methylpolyamines from readily available enantiomers of 2-aminopropanol. (*R*)- and (*S*)-isomers of α-methylspermidine and α-methylspermine, (*R*,*R*)-, (*S*,*S*)-, and (*R*,*S*)-isomers of α,ω-dimethylspermine were prepared in seven to eight steps in overall yields from 32 to 46%. All optical isomers of α-methylpolyamines thus obtained were of 99+% purity, as determined by standard HPLC protocol for polyamines.[20] We propose that these optical isomers will be differently recognized by the enzymes of polyamine metabolism and may possess diverse biological activity in vivo.

4. Experimental

4.1. General

All reagents and solvents were used as received from manufacturers unless otherwise specified. (*R*)- and (*S*)-2-aminopropanols, 1-bromo-4-chlorobutane, 1,4-diiodobutane, *N*-(4-iodobutyl)phthalimide, *o*-nitrophenylsulfonyl chloride, and thiophenol were purchased from Aldrich, 1,3-diaminopropane, Boc_2O, Et_3N, and $N_2H_4 \cdot H_2O$ from Fluka, $LiAlH_4$ from Sigma, and SiO_2 (Kieselgel, 40–63 μm) from Merck. Nitriles **1a** and **1b** were prepared starting from enantiomers of 2-aminopropanol as described previously.[21]

1-Bromo-4-chlorobutane, 1,4-diiodobutane, and enantiomers of 2-aminopropanol were purified by distillation under reduced pressure. Anhydrous solvents were prepared by standard procedures. Melting points were measured in open capillary tubes and are uncorrected. Optical rotation angles were measured using a Perkin–Elmer 241 digital polarimeter at 589 nm. HPLC analysis of enantiomers was performed by using a Whelk-O1 (*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 α-methylated Spd's and Spm's after dansylation, and treatment was as described previously.[22] ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 DRX spectrometer operating at 500.1 and 125.8 MHz, respectively. Signals are referenced compared to TMS as an internal standard in $CDCl_3$ or sodium trimethylsilylpropanesulfonate (TSP) in D_2O.

4.1.1. (*R*)-N^3-(*tert*-Butyloxycarbonyl)-1,3-diaminobutane (2a). To a cooled (−5 °C) suspension of $LiAlH_4$ (10.5 g, 0.267 mol) in dry Et_2O (280 mL) a solution of **1a** (19.95 g, 0.108 mol) in dry Et_2O (70 mL) was added with stirring within 50 min and stirring was continued for 1 h at −5 °C. The reaction was quenched with 25% aq NaOH (90 mL) at −10 to −5 °C. The organic phase was separated, the residue was treated with Et_2O (4×100 mL), combined organic extracts were dried (Na_2SO_4), filtered, and the solvent was removed in vacuo. The residual colorless oil of **2a** (18.8 g, 92%) was >95% pure according to ^1H NMR and was used for further transformations without purification. An analytical sample was purified by flash chromatography (SiO_2, 1,4-dioxane/25% aq NH_3, 98:2); R_f 0.33 (1,4-dioxane/25% aq NH_3, 95:5); $[\alpha]_D^{20}$ −12.0 (*c* 2.0, $CHCl_3$, lit.[18] $[\alpha]_D^{20}$ −12.0); ^1H NMR ($CDCl_3$) δ 4.61 (br s, 1H, BocNH), 3.77 (br s, 1H, MeCH), 2.79–2.69 (m, 2H, CH_2N), 1.60–1.44 (m, 2H, $CHCH_2$), 1.43 (s, 9H, $C(CH_3)_3$), 1.08 (d, 3H, *J* 6.6 Hz, $CHCH_3$); ^{13}C NMR ($CDCl_3$) δ 155.51, 78.89, 44.26, 40.99, 38.81, 28.34, 21.43.

Starting from **1b** and following the same procedure **2b** was obtained as a colorless oil (91%); $[\alpha]_D^{20}$ +12.0 (*c* 2.0, $CHCl_3$, lit.[18] $[\alpha]_D^{20}$ +12.0).

4.1.2. (*R*)-N^1-(*o*-Nitrophenylsulfonyl)-N^3-(*tert*-butyloxycarbonyl)-1,3-diaminobutane (3a). To a cooled (0 °C) solution of **2a** (18.8 g, 0.1 mol) and Et_3N (18.1 mL, 0.13 mol) in dry CH_2Cl_2 (250 mL) a solution of NsCl (21.05 g, 95 mmol) in dry CH_2Cl_2 (7 mL) was added with stirring within 40 min and stirring was continued for 2 h at 0 °C. The reaction mixture was washed with H_2O (2×50 mL), 10% citric acid (4×50 mL), H_2O (30 mL), brine (20 mL), dried ($MgSO_4$), filtered, and solvent was removed in vacuo. The residue was crystallized (MeOH, 175 mL—H_2O, 140 mL) thus affording after drying **3a** (33.5 g, 90%) as a white solid, mp 115–116 °C; R_f 0.21 (EtOAc/hexane, 1:2); $[\alpha]_D^{20}$ −13.0 (*c* 5.0, $CHCl_3$); found, %: C 48.28, H 6.33, N 11.35; $C_{15}H_{23}N_3O_6S$, calcd, %: C 48.25, H 6.21, N 11.25; ^1H NMR ($CDCl_3$) δ 8.14–8.10 (m, 1H, Ar), 7.85–7.81 (m, 1H, Ar), 7.74–7.69 (m, 2H, Ar), 6.23 (br s, 1H, NsNH), 4.31 (br s, 1H, BocNH), 3.80–3.69 (m, 1H, MeCH), 3.33–3.22 (m, 1H, CH_2N), 3.05–2.95 (m, 1H, CH_2N), 1.78–1.69 (m, 1H, $CHCH_2$), 1.51–1.43 (m, 1H, $CHCH_2$), 1.37 (s, 9H, $C(CH_3)_3$), 1.11 (d, 3H, *J* 6.7 Hz, $CHCH_3$); ^{13}C NMR ($CDCl_3$) 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.

Starting from **2b** and following the same procedure **3b** was obtained (95%) as colorless crystals; mp 115–116 °C; $[\alpha]_D^{20}$ +13.0 (*c* 5.0, $CHCl_3$); found, %: C 48.17, H 6.26, N 11.28; $C_{15}H_{23}N_3O_6S$ calcd, %: C 48.25, H 6.21, N 11.25.

4.1.3. (*R*)-N^1-(Phthaloyl)-N^8-(*tert*-butyloxycarbonyl)-1,8-diamino-5-azanonane (4a). A mixture of **3a** (8.2 g, 22 mmol), *N*-(4-bromobutyl)phthalimide (6.77 g, 24 mmol), and K_2CO_3 (10.12 g, 0.1 mol) in dry DMF (30 mL) was stirred for 20 h at 20 °C, then PhSH (3.36 mL, 33 mmol), K_2CO_3 (4.6 g, 33.0 mmol), and DMF (20 mL) were added

and stirring was continued for 20 h at 20 °C. The solvent was evaporated in vacuo, and the residue was treated with EtOAc (150 mL) and washed with H_2O (2×40 mL), brine (20 mL), and dried (Na_2SO_4). Solvent was evaporated in vacuo and the residue was purified by flash chromatography (SiO_2, $CHCl_3$/Et_3N 100:1.2 to 100:3) to afford **4a** (7.6 g, 89%) as an amorphous solid; R_f 0.37 ($CHCl_3$/MeOH/25% NH_4OH, 100:4:0.4); $[\alpha]_D^{20}$ −6.2 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.82–7.77 (m, 2H, Ar), 7.69–7.65 (m, 2H, Ar), 4.90 (br s, 1H, BocNH), 3.73–3.61 (m, 3H, MeCH+CH_2NPht), 2.67–2.55 (m, 4H, NHCH_2), 1.65 (m, 3H, CHCH_2+NH), 1.48 (m, 4H, CH_2CH_2), 1.38 (s, 9H, $C(CH_3)_3$), 1.09 (d, 3H, *J* 6.6 Hz, CHCH_3); ^{13}C NMR ($CDCl_3$) δ 168.31, 155.52, 133.78, 132.14, 123.09, 78.80, 49.35, 46.50, 45.03, 37.77, 37.11, 28.37, 27.26, 26.35, 21.34.

Starting from **3b** and following the same procedure **4b** was obtained (92%) as amorphous solid; $[\alpha]_D^{20}$ +6.4 (*c* 1.0, $CHCl_3$).

4.1.4. (*R*)-1,8-Diamino-5-azanonane trihydrochloride ((*R*)-α-MeSpd) (5a). A solution of **4a** (7.6 g, 19.5 mmol) and hydrazine monohydrate (3.55 g, 73 mmol) in 95% EtOH (85 mL) was refluxed for 3 h, then H_2O (30 mL) was added and the mixture was evaporated in vacuo. The residue was treated with KOH (4 M, 60 mL), extracted with CH_2Cl_2 (2×40 mL), and the combined extracts were dried (Na_2SO_4). The solvent was removed in vacuo, the residue was co-evaporated with DMF (30 mL) and dissolved in MeOH (25 mL), and 37% aq HCl (15 mL) was added. The reaction mixture was stirred for 15 h at 20 °C, the solvents were removed in vacuo and the residue was crystallized from H_2O/MeOH/EtOH to give (*R*)-1,8-diamino-5-azanonane trihydrochloride, (*R*)-α-MeSpd, **5a** (4.8 g, 91%) as a white solid, mp 191–192 °C; R_f 0.45 (Bu^{n}OH/AcOH/Py/H_2O, 4:2:1:2); $[\alpha]_D^{20}$ +5.6 (*c* 5.0, H_2O); ee 97%; found, %: C 35.90, H 9.12, N 15.80; $C_8H_{24}N_3Cl_3$ calcd, %; C 35.76, H 9.00, N 15.64; ^1H NMR ($CDCl_3$) δ 3.58–3.48 (m, 1H, MeCH), 3.24–3.18 (m, 2H, CH_2N), 3.18–3.12 (m, 2H, CH_2N), 3.10–3.05 (m, 2H, CH_2N), 2.20–2.12 (m, 1H, CHCH_2), 2.05–1.98 (m, 1H, CHCH_2), 1.86–1.74 (m, 4H, CH_2CH_2), 1.36 (d, 3H, *J* 6.6 Hz, CHCH_3); ^{13}C NMR ($CDCl_3$) δ 50.03, 48.36, 46.89, 41.85, 33.34, 26.85, 25.69, 20.38.

Starting from **4b** and following the same procedure **5b** was obtained (90%) as colorless crystals; $[\alpha]_D^{20}$ −5.6 (*c* 5.0, H_2O); ee 97%; found, %: C 35.88, H 9.10, N 15.63; $C_8H_{24}N_3Cl_3$ calcd, %: C 35.76, H 9.00, N 15.64.

4.1.5. (*R*)-N^5-(*o*-Nitrophenylsulfonyl)-N^8-(*tert*-butyloxycarbonyl)-8-amino-5-aza-1-iodononane (6a). A mixture of **3a** (5.24 g, 14 mmol), K_2CO_3 (5.8 g, 42 mmol), and 1-bromo-4-chlorobutane (16 mL, 0.139 mol) in dry DMF (65 mL) was stirred for 20 h at 20 °C. The solvent and excess 1-bromo-4-chlorobutane were removed in vacuo, the residue was treated with EtOAc (200 mL), washed with H_2O (80 mL), brine (20 mL), and dried ($MgSO_4$). The solvent was removed in vacuo, the residual heavy colorless oil was evacuated at 90 °C/0.25 mbar for 3 h and then was refluxed with NaI (8.7 g, 58 mmol) in acetone (25 mL) for 27 h. The solvent was evaporated in vacuo, the residue was treated with EtOAc (170 mL), washed with H_2O (2×50 mL), brine (15 mL), and dried ($MgSO_4$). Solvent was evaporated in vacuo, and the residue was purified by flash chromatography (SiO_2, CH_2Cl_2 to CH_2Cl_2/EtOAc, 100:5) to afford **6a** (7.5 g, 96%) as a colorless oil; R_f 0.46 ($CHCl_3$/MeOH, 100:1); $[\alpha]_D^{20}$ −2.1 (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.99–7.96 (m, 1H, Ar), 7.69–7.65 (m, 2H, Ar), 7.63–7.60 (m, 1H, Ar), 4.37 (br s, 1H, BocNH), 3.64–3.56 (m, 1H, MeCH), 3.38–3.26 (m, 4H, $NsNCH_2$), 3.16 (t, 2H, *J* 6.6 Hz, CH_2I), 1.83–1.71 (m, 2H, CHCH_2), 1.72–1.63 (m, 4H, $CH_2CH_2CH_2I$), 1.42 (s, 9H, $C(CH_3)_3$), 1.11 (d, 3H, *J* 6.6 Hz, CHCH_3); ^{13}C NMR ($CDCl_3$) δ 155.31, 148.10, 133.45, 131.58 (2C), 130.70, 124.14, 79.45, 46.73, 44.82, 44.80, 36.12, 30.04, 28.95, 28.37, 21.29, 5.82.

Starting from **3b** and following the same procedure **6b** was obtained as a colorless oil (93%); $[\alpha]_D^{20}$ +2.0 (*c* 2, $CHCl_3$).

4.1.6. (*R*)-N^{12}-(*tert*-Butyloxycarbonyl)-N^9-(*o*-nitrophenylsulfonyl)-1,12-diamino-4,9-diazatridecane (7a). A cooled (+4 °C) solution of **6a** (1.61 g, 2.9 mmol) and 1,3-diaminopropane (2.51 mL, 30 mmol) in dry THF (12 mL) was stirred for 12 h. The solvent was evaporated in vacuo, NaOH (1 M, 10 mL) was added, and the mixture was extracted with $CHCl_3$ (2×15 mL). The combined extracts were washed with brine (15 mL), dried ($MgSO_4$), the solvent was evaporated in vacuo, and the residue was purified by flash chromatography (SiO_2, 1,4-dioxane/25% NH_4OH, 92:8) to afford **7a** (1.38 g, 92%) as a viscous yellow oil; R_f 0.25 (1,4-dioxane/25% NH_4OH, 90:10); $[\alpha]_D^{20}$ −2.3 (*c* 3.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.98–7.95 (m, 1H, Ar), 7.68–7.64 (m, 2H, Ar), 7.61–7.58 (m, 1H, Ar), 4.50 (br s, 1H, BocNH), 3.63–3.55 (m, 1H, MeCH), 3.35–3.24 (m, 4H, $NsNCH_2$), 2.72–2.66 (t, 2H, *J* 6.7 Hz, CH_2NH_2), 2.64–2.56 (m, 4H, CH_2NH), 1.69–1.57 (m, 7H, $CH_2CH_2CH_2CH_2NH$+$CH_2CH_2CH_2NH_2$+CHCH_2), 1.44–1.37 (m, 10H, $C(CH_3)_3$+CHCH_2), 1.11 (d, 3H, *J*=6.6 Hz, CHCH_3); ^{13}C NMR ($CDCl_3$) δ 155.31, 148.10, 133.45, 131.58 (2C), 130.70, 124.14, 79.45, 47.98, 47.64, 46.73, 44.82, 44.80, 40.60, 36.12, 33.98, 28.37, 26.26, 26.10, 21.29.

Starting from **3b** and following the same procedure **7b** was obtained as a viscous yellowish oil (90%); $[\alpha]_D^{20}$ +2.3 (*c* 3.0, $CHCl_3$).

4.1.7. (*R*)-1,12-Diamino-4,9-diazatridecane tetrahydrochloride ((*R*)-α-MeSpm) (8a). A mixture of **7a** (1.24 g, 2.48 mmol), PhSH (0.36 mL, 3.5 mmol), and Et_3N (0.74 mL, 5.3 mmol) in dry DMF (8 mL) was stirred for 5 h at 40 °C. The solvent was evaporated in vacuo, the residue was dissolved in 20% EtOH (30 mL), acidified to pH 2 with 1 M HCl, and extracted with $CHCl_3$ (2×20 mL). The water layer was evaporated to dryness in vacuo, the residue was dissolved in MeOH (8 mL), and HCl/EtOH (10 M, 4 mL) was added. The reaction mixture was stirred for 2 h at 20 °C, the solvents evaporated in vacuo and the residue recrystallized twice from H_2O/MeOH/EtOH to afford **8a** (0.58 g, 65% for two steps) as a white solid, mp 253–254 °C; R_f 0.13 (Bu^{n}OH/AcOH/Py/H_2O, 4:2:1:2); $[\alpha]_D^{20}$ +4.5 (*c* 2.0, H_2O); ee 97%; found, %: C 36.51, H 8.92, N 15.18; $C_{11}H_{32}N_4Cl_4$, %: C 36.48, H 8.90, N 15.46; ^1H NMR (D_2O) δ 3.56–3.48 (m, 1H, MeCH), 3.24–3.10 (m, 10H, CH_2N), 2.18–2.08 (m, 3H, NHCHCH_2+C$H_2CH_2NH_2$), 2.04–1.95 (m, 1H, NHCHCH_2), 1.85–1.77 (m, 4H,

$CH_2CH_2CH_2CH_2$), 1.36 (d, 3H, *J* 6.6 Hz, $CHCH_3$); ^{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.

Starting from **7b** and following the same procedure **8b** was obtained as colorless crystals (63%); $[\alpha]_D^{20}$ −4.5 (*c* 2.0, H_2O); ee 97%; found, %: C 36.40, H 9.00, N 15.35; $C_{11}H_{32}N_4Cl_4$ calcd, %; C 36.48, H 8.90, N 15.47.

4.1.8. (*R*,*R*)-N^2,N^{13}-di-(*tert*-Butyloxycarbonyl)-2,13-diamino-5,10-diazatetradecane (9a). A mixture of **3a** (8.95 g, 24 mmol), K_2CO_3 (11.6 g, 84 mmol), and 1,4-diiodobutane (3.72 g, 12 mmol) in dry DMF (50 mL) was stirred for 24 h at 30 °C before the addition of BnBr (0.34 mL, 2.9 mmol). The mixture was then stirred for a further 14 h, PhSH (3.67 mL, 36 mmol) and K_2CO_3 (4.97 g, 36 mmol) were added followed by stirring for 20 h at 30 °C. The reaction mixture was evaporated in vacuo to dryness and the residue was treated with CH_2Cl_2 (250 mL), washed with H_2O (2×50 mL), brine (20 mL), and dried (K_2CO_3). The solvent was evaporated in vacuo, and the residue was purified by flash chromatography (SiO_2, 1,4-dioxane/25% NH_4OH, 100:2.5) to give **9a** (4.7 g, 91%) as an amorphous colorless solid; R_f 0.28 (1,4-dioxane/25% NH_4OH, 95:5); $[\alpha]_D^{20}$ −11.6 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.91 (br s, 2H, BocNH), 3.76–3.68 (m, 2H, CH_3CH), 2.72–2.55 (m, 8H, NCH_2), 1.70–1.62 (m, 2H, $CHCH_2$), 1.54–1.47 (m, 6H, $CH_2CH_2CH_2CH_2$+$CHCH_2$), 1.44 (s, 18H, CMe_3), 1.14 (d, 6H, *J* 6.6 Hz, $CHCH_3$); ^{13}C NMR ($CDCl_3$) δ 155.22, 78.52, 49.77, 46.44, 44.91, 37.03, 28.45 (6C), 27.78, 21.42.

Starting from **3b** and following the same procedure **9b** was obtained as amorphous colorless solid (90%); R_f 0.28 (1,4-dioxane/25% NH_4OH, 95:5); $[\alpha]_D^{20}$ +11.8 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.90 (br s, 2H, BocNH), 3.75–3.67 (m, 2H, MeCH), 2.72–2.54 (m, 8H, NCH_2), 1.71–1.62 (m, 2H, $CHCH_2$), 1.54–1.46 (m, 6H, $CH_2CH_2CH_2CH_2$+CH_3CHCH_2), 1.43 (s, 18H, CMe_3); 1.13 (d, 6H, *J* 6.6 Hz, $CHCH_3$); ^{13}C NMR ($CDCl_3$) δ 155.53, 78.82, 49.83, 46.51, 45.08, 37.17, 28.40 (6C), 27.86, 21.36.

4.1.9. (*R*,*S*)-2,13-di-(*tert*-Butyloxycarbonyl)-2,13-diamino-5,10-diazatetradecane (9c). Prepared as **9a** starting from **6a** (7.5 g, 13.5 mmol) and **3b** (4.85 g, 13 mmol), compound **9c** (5.3 g, 95%) was obtained as an amorphous colorless solid; R_f 0.28 (1,4-dioxane/25% NH_4OH, 95:5); $[\alpha]_D^{20}$ 0 (*c* 2.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.91 (br s, 2H, BocNH), 3.75–3.67 (m, 2H, MeCH), 2.73–2.54 (m, 8H, NCH_2), 1.70–1.62 (m, 2H, $CHCH_2$), 1.54–1.45 (m, 6H, $CH_2CH_2CH_2CH_2$+$CHCH_2$), 1.44 (s, 18H, CMe_3), 1.13 (d, 6H, *J* 6.6 Hz, $CHCH_3$); ^{13}C NMR ($CDCl_3$) δ 155.57, 78.91, 49.75, 46.47, 44.94, 37.11, 28.38, 27.73, 21.41.

4.1.10. (*R*,*R*)-2,13-Diamino-5,10-diazatetradecane tetrahydrochloride (10a). A mixture of **9a** (4.7 g, 10.9 mmol) in MeOH (30 mL) and 37% aq HCl (17 mL) was stirred for 14 h at 20 °C and then evaporated in vacuo. The residue was recrystallized from MeOH/EtOH to afford target **10a** (3.8 g, 92%) as a white solid, mp 224–225 °C; R_f 0.13 (Bu^nOH/AcOH/Py/H_2O, 4:2:1:2); $[\alpha]_D^{20}$ +8.2 (*c* 2.0, H_2O); ee 97%; found, %: C 38.17, H 9.24, N 14.82; $C_{12}H_{34}N_4Cl_4$ calcd, %; C 38.31, H 9.11, N 14.89; ^1H NMR (D_2O) δ 3.55–3.46 (m, 2H, MeCH), 3.20 (t, 4H, *J* 6.8 Hz, NCH_2), 3.17–3.11 (m, 4H, NCH_2), 2.18–2.09 (m, 2H, CH_2CHNH_2), 2.04–1.93 (m, 2H, CH_2CHNH_2), 1.84–1.75 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.35 (d, 6H, *J* 6.6 Hz, $CHCH_3$); ^{13}C NMR (D_2O) δ 49.86, 48.14, 46.78, 33.27, 25.64, 20.09.

Starting from **9b** and following the same procedure **10b** was obtained (92%) as a white solid, mp 224–225 °C (MeOH/EtOH); R_f 0.13 (Bu^nOH/AcOH/Py/H_2O, 4:2:1:2); $[\alpha]_D^{20}$ −8.4 (*c* 3.0, H_2O); ee 97%; found, %: C 38.20, H 9.28, N 14.69; $C_{12}H_{34}N_4Cl_4$ calcd, %: C 38.31, H 9.11, N 14.89; ^1H NMR (D_2O) δ 3.56–3.47 (m, 2H, MeCH), 3.20 (t, 4H, *J* 6.8 Hz, CH_2NH), 3.18–3.12 (m, 4H, NCH_2), 2.19–2.10 (m, 2H, CH_2CHNH_2), 2.05–1.94 (m, 2H, CH_2CHNH_2), 1.84–1.75 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.35 (d, 6H, *J* 6.6 Hz, $CHCH_3$); ^{13}C NMR (D_2O) δ 49.86, 48.14, 46.78, 33.27, 25.64, 20.09.

Starting from **9c** and following the same procedure **10c** was obtained (94%) as a white solid, mp 224–225 °C (H_2O/MeOH/EtOH); R_f 0.13 (Bu^nOH/AcOH/Py/H_2O, 4:2:1:2); $[\alpha]_D^{20}$ 0 (*c* 2.0, H_2O); found, %: C 38.13, H 9.20, N 14.86; $C_{12}H_{34}N_4Cl_4$ calcd, %: C 38.31, H 9.11, N 14.89; ^1H NMR (D_2O) δ 3.55–3.46 (m, 2H, MeCH), 3.21 (t, 4H, *J* 6.8 Hz, NCH_2), 3.17–3.11 (m, 4H, NCH_2), 2.18–2.09 (m, 2H, CH_2CHNH_2), 2.04–1.94 (m, 2H, CH_2CHNH_2), 1.84–1.75 (m, 4H, $CH_2CH_2CH_2CH_2$), 1.35 (d, 6H, *J* 6.6 Hz, $CHCH_3$); ^{13}C NMR (D_2O) δ 49.8, 48.14, 46.78, 33.27, 25.64, 20.09.

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