Synthesis of Water-Soluble Polyamine Derivatives Effective as *N*-Methyl-D-aspartate Receptor Antagonists

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The novel water-soluble *N*-methyl-D-aspartate (NMDA) receptor antagonists, *N*-{4-[4-(4-Guanidinobutylamino)butylamino]butyl}-*p*-toluenesulfonamide trihydrochloride (1a, TsHSPMG), *N*-{4-[4-(4-Guanidinobutylamino)butylamino]butyl}butane-1-sulfonamide trihydrochloride (1b, BsHSPMG), *N*-{3-[4-(3-Guanidinopropylamino)butylamino]propyl}-*p*-toluenesulfonamide trihydrochroride (2a, TsSPMG) and *N*-{3-[4-(3-Guanidinopropylamino)butylamino]propyl}butane-1-sulfonamide trihydrochroride (2b, BsSPMG), were synthesized, and the effects of these polyamine derivatives on NMDA receptors were studied using voltage-clamp recordings of recombinant NMDA receptors expressed in *Xenopus* oocytes. Although spermine potentiates 153% and 310% of NMDA (NR1A/NR2B) receptors in the presence of saturated and unsaturated glycine, respectively, all the novel polyamine derivatives, TsHSPMG (1a), BsHSPMG (1b), TsSPMG (2a) and BsSPMG (2b), significantly inhibited NR1A/NR2B receptors in both conditions. The degree of NMDA receptor inhibition by TsHSPMG (1a) and BsHSPMG (1b) was stronger than that by TsSPMG (2a) and BsSPMG (2b).

Key words *N*-methyl-D-aspartate receptor; polyamine; *Xenopus* oocyte; voltage-clamp recording

Three pharmacologically-defined classes of ionotropic glutamate receptors were originally named according to their agonist selectivity: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate. The NMDA receptor consists of at least two distinct subunits, NR1 and NR2[1] (Fig. 1A). Each subunit has three transmembrane domains (M1, M3, and M4) plus a cytoplasm-facing re-entrant membrane loop (M2). The M2 loop region in the NR1 and NR2 subunits is a critical determinant of divalent cation permeability and Mg^{2+} block. NR1 is a single gene product expressed as eight alternatively spliced mRNAs, and NR2A, NR2B, NR2C and NR2D are distinct gene products. NMDA receptors probably consist of tetrameric subunit assemblies that have different physiological and pharmacological properties depending on the specific NR2 subunit. In the central nervous system, NMDA receptors play critical roles in a variety of neurophysiological phenomena, including neurodevelopment, synaptic plasticity, and excitotoxicity, because of their high permeability to Ca^{2+}. Neurodegeneration associated with a variety of acute and chronic disorders (*e.g.*, ischemic stroke, Parkinson's disease, Alzheimer's disease and dementia) is due in part to overactivation of NMDA receptors (Fig. 1B). Inhibitors of NMDA receptors have thus been developed as anticonvulsants and neuroprotective agents.

Polyamines (putrescine, spermidine and spermine) are ubiquitously present in prokaryotic and eukaryotic cells. In the central nervous system, specific interactions of polyamines with several structurally and functionally distinct types of cation channels have been reported previously.[2] Among these, the most striking are the blockade of some types of K^+ channels and the modulation of NMDA receptors. Spermine has complex effects on NMDA receptors, including two types of stimulation and one type of voltage-dependent blockade. One of the effects of spermine is "glycine-independent" stimulation, observed in the presence of saturating concentrations of glutamate and glycine. With recombinant NMDA receptors, this type of stimulation is observed only at receptors containing splice variants of NR1 that lack the exon-5 insert, expressed together with the NR2B subunit.[3] Inhibition by extracellular spermine is strongly voltage-dependent, being more pronounced at hyperpolarized

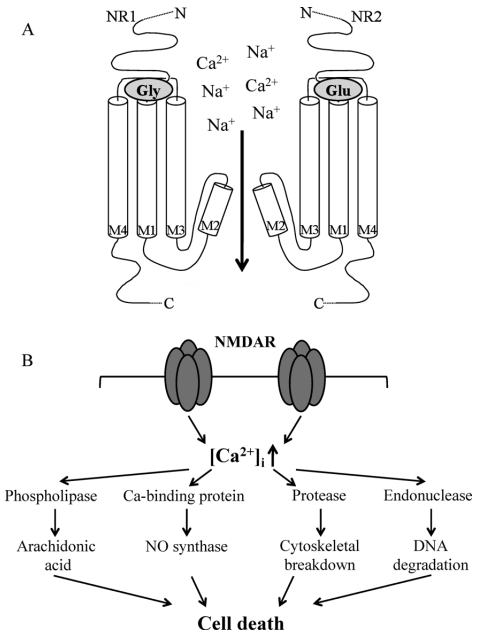

Fig. 1. Structures of NMDA Receptor and Model for NMDA Receptor Mediated Ca^{2+}-Dependent Neurotoxicity

(A) Schematic illustration of the NR1 and NR2B subunits, which contain three transmembrane segments (M1, M3, M4), a re-entrant loop (M2), an extracellular N-terminal domain, an extracellular loop between M3 and M4, an intracellular loop between M1 and M2, and an intracellular C-terminal domain. The glycine and glutamate binding pockets involving residues from the N-terminal domain and from the M3-M4 loop of NR1 and NR2 subunits, respectively. (B) Excitotoxicity is thought to be a major mechanism contributing to neurodegeneration during central nervous system, ischemia, trauma and other neurological disorders. Excitotoxicity is thought to result from an excessive synaptic release of glutamate. Also, it is generally accepted that the NMDA receptor plays a key role in mediating at least certain aspects of glutamate neurotoxicity, possibly owing to their high Ca^{2+} permeability. When Ca^{2+} overloaded, such processes including enzymes (*e.g.*, proteases, lipases, endonucleases) and other metabolic machinery directly damage neurons or lead to the formation of toxic reaction products which ultimately cause cell death.

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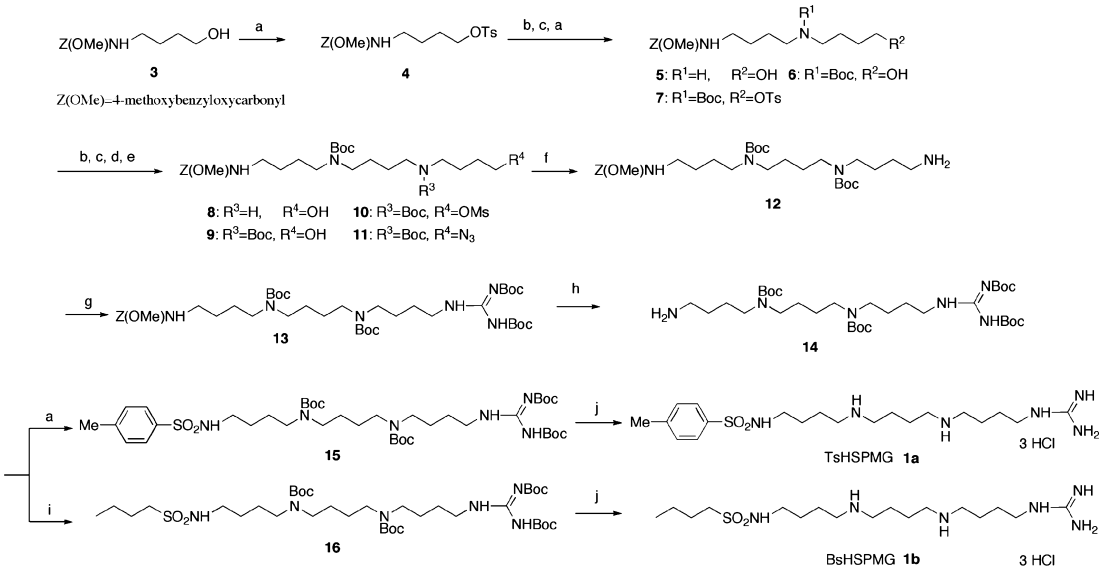
than depolarized membrane potentials. Single-channel patch-clamp recording has been used to study the mechanism of this effect. Spermine (10—1000 μM) was found to decrease both single-channel conductance and average channel open-time.

We previously reported that *N*-{4-[4-(4-guanidinobutylamino)butylamino]butyl}-*p*-toluenesulfonamide trihydrochloride (TsHSPMG) (**1a**) and *N*-{3-[4-(3-guanidinopropylamino)butylamino]propyl}-*p*-toluenesulfonamide trihydrochroride (TsSPMG) (**2a**) inhibited the activity of NMDA receptors expressed in *Xenopus* oocytes. Their antagonistic activities were more potent than that of memantine, an NMDA antagonist. TsHSPMG (**1a**), which has a longer polyamine tail than TsSPMG (**2a**), showed more potent antagonistic activity. We therefore concluded that the homospermine moiety and guanidyl group play an important role in neuroprotection against NMDA toxicity.[4] Pöhler *et al.* reported synthesis and pharmacological properties of polyamine derivatives and also exhibited that some analogs of 5-(4-aminobutyl)-2-thiophene-octylamine inhibited NMDA receptor binding using [^{3}H]MK-801 binding assay methods.[5]

In this paper, we report the syntheses of TsSPMG (**2a**) and *N*-{3-[4-(3-guanidinopropylamino)butylamino]propyl}butane-1-sulfonamide trihydrochroride (BsSPMG) (**2b**), which have a guanidyl group on the spermine moiety, and TsHSPMG (**1a**) and *N*-{4-[4-(4-guanidinobutylamino)butylamino]butyl}butane-1-sulfonamide trihydrochloride (BsHSPMG) (**1b**), which have a homospermine moiety instead of spermine. Through the addition of the guanidyl group or homospermine moiety we aimed at construct compounds which are less sensitive to polyamine oxidases (spermine oxidase and acetylpolyamine oxidase) and degrade spermine to produce acrolein, a toxic unsaturated aldehyde.[6] TsHSPMG (**1a**), TsSPMG (**2a**), BsHSPMG (**1b**) and BsSPMG (**2b**) (at concentrations up to 300 μM) were non-toxic to cells in the presence of fetal bovine serum (FBS) (data not shown). Neuronal cell death due to supranormal excitability of NMDA receptors is common under pathological depolarized conditions. The effects of these polyamine derivatives were investigated on NR1/NR2B receptors in the depolarized state in the presence of saturated or unsaturated concentrations of glycine, because spermine stimulates NMDA receptor activity under depolarized conditions. Also, N^4-benzylspermine and N^4-(3-phenylpropyl)spermine may stimulate NMDA receptor activities, because the low concentration of these polyamine derivatives promoted quantity of [^{3}H]MK-801 binding to NMDA receptor.[7]

Results and Discussion

The synthesis of polyamine compounds TsHSPMG (**1a**) and BsHSPMG (**1b**) is outlined in Chart 1. 4-(4-Methoxybenzyloxycarbonyl)amino-1-butanol (**3**) was synthesized from 4-methoxybenzyl *S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate and 4-amino-1-butanol in $CHCl_3$, which was converted to tosylate (**4**). Tosylate (**4**) was condensed with 4-amino-1-butanol in *N*,*N*-dimethylformamide (DMF) at 90 °C to yield **5**. Protection of the amino nitrogen in **5** with a *N*-*tert*-butoxycarbonyl (Boc) group, esterifcation of the resulting alcohol (**6**) as the tosylate, and condensation of the tosylate (**7**) and 4-amino-1-butanol gave the corresponding primary alcohol (**8**). After protecting the amino nitrogen of **8** with *N*-Boc, the terminal hydroxyl group was mesylated with methanesulfonyl chloride (MsCl) to yield **10**. Mesylate **10** was converted to azide compound (**11**) and subsequent hydrogenation using palladium-activated carbon ethylenediamine complex (Pd–C(en)) gave primary amino compound (**12**). Compound **13** was obtained by treatment of **12** with *N*, *N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea in CH_2Cl_2. The removal of the 4-methoxybenzyloxycarbonyl group in **13** gave **14**, then the terminal amino group in **14** was converted to *p*-toluenesulfonamide compound **15** and butanesulfonamide compound **16**. Finally, the Boc protecting groups of **15** and **16** were cleaved to give the desired compounds



(a) TsCl. TEA.CH_2Cl_2. (b) 4-amino-1-butanol. DMF; (c) $(Boc)_2O$. CH_2Cl_2; (d) MsCl. TEA. CH_2Cl_2; (e) NaN_3. DMF; (f) H_2, Pd-C(en). THF; (g) *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea . CH_2Cl_2; (h) H_2. 10% Pd-C. THF; (i) BsCl. TEA. CH_2Cl_2; (j) 36% HCl. THF

Chart 1

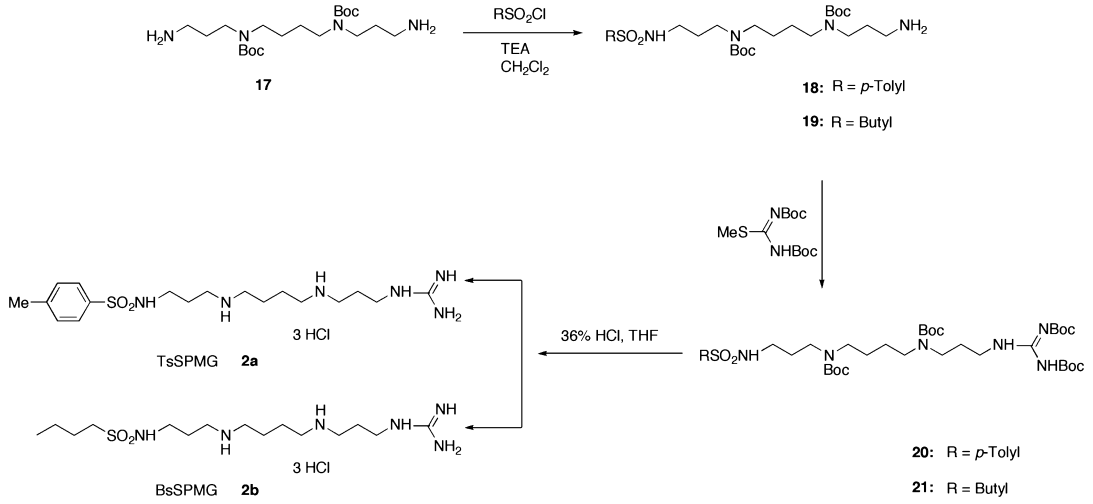


Chart 2

TsHSPMG (**1a**) and BsHSPMG (**1b**).

The synthesis of polyamine compounds TsSPMG (**2a**) and BsSPMG (**2b**) is outlined in Chart 2. Treatment of 4,9-diaza-4,9-di-(*tert*-butoxycarbonyl)dodecane-1,12-diamine[8] with *p*-toluenesulfonyl chloride (TsCl) in the presence of triethylamine (TEA) in CH_2Cl_2 gave *p*-toluenesulfonamide compound **18** in 49% yield, and then the terminal primary amino group in **18** was converted to guanidino compound (**20**) by treating **18** with *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea in CH_2Cl_2. Finally, the Boc protecting groups of **20** were removed using 36% HCl in tetrahydrofuran (THF) to give the desired compound TsSPMG (**2a**). BsSPMG (**2b**) was synthesized from butane-1-sulfonyl chloride (BsCl) by a method similar to that used to synthesize TsSPMG (**2a**).

The effects of the polymines TsHSPMG (**1a**), BsHSPMG (**1b**), TsSPMG (**2a**), BsSPMG (**2b**) and spermine on NMDA (NR1A/NR2B) receptors were studied using voltage-clamp recordings of recombinant NR1A/NR2B receptors expressed in *Xenopus* oocytes. We measured the effects of TsHSPMG (**1a**), BsHSPMG (**1b**), TsSPMG (**2a**), BsSPMG (**2b**) and spermine (100 μM) on responses to glutamate (10 μM, with 0.1 or 10 μM glycine) by NR1A/NR2B receptors in oocytes voltage-clamped at −20 mV. Spermine potentiates 153% and 310% of NMDA (NR1A/NR2B) receptors in the presence of saturated or unsaturated glycine, respectively. Spermine potentiates NMDA receptor currents in the presence of saturating concentrations of glycine (10 μM, glycine-independent stimulation), an effects that involves an increase in the frequency of channel opening and decrease in the desensitization of NMDA receptors. A second effect for the potentiation involves an increase in the affinity of NMDA receptors for glycine (0.1 μM, glycine-dependent stimulation). However, all the novel derivatives, TsHSPMG (**1a**), BsHSPMG (**1b**), TsSPMG (**2a**) and BsSPMG (**2b**) significantly inhibited NR1A/NR2B receptors in both conditions. The inhibition of NMDA receptors by BsSPMG (**2b**), BsHSPMG (**1b**), TsSPMG (**2a**) and TsHSPMG (**1a**) in the presence of saturated glycine (10 μM) was 26%, 83%, 62% and 82%, respectively. Comparable results were obtained in the presence of unsaturated glycine (0.1 μM) (Fig. 2). Inhibition by extracellular spermine is strongly voltage-dependent and may be due to fast open-channel block, similar to that by Mg^{2+}. Synthesized polyamine derivatives had voltage dependent inhibitory effects on NMDA receptor currents, but the facilitatory effects disappeared. NMDA receptor subtypes are thought to play a predominant role in triggering glutamate neurotoxicity in the central nervous system. Thus, polyamine derivatives may have neuroprotective effects towards glutamate neurotoxicity. Further pharmacological data regarding BsHSPMG and BsSPMG will be described elsewhere.

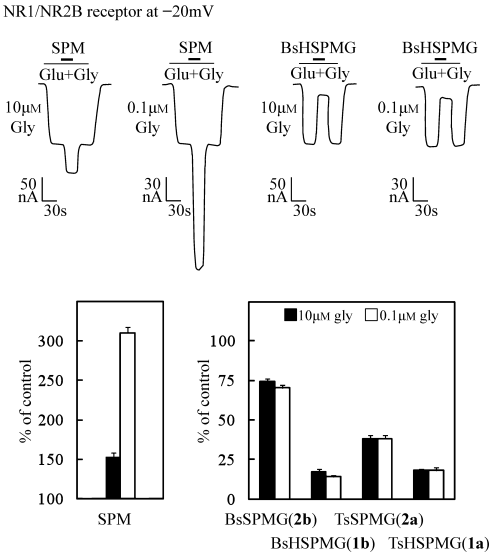


Fig. 2. Effects of Spermine (SPM) and Polyamine Derivatives on NR1/NR2B Receptors at −20 mV

Representative traces showing the effects of 100 μM spermine or TsHSPMG (**1a**) on the NR1/NR2B receptors. The effects of 100 μM spermine, TsHSPMG (**1a**), BsHSPMG (**1b**), TsSPMG (**2a**) and BsSPMG (**2b**) were determined in oocytes expressing NR1/NR2B receptors. Currents were evoked using 10 μM glutamate with 0.1 or 10 μM glycine and voltage-clamped at −20 mV. Macroscopic currents in the presence of spermine or polyamine derivatives were expressed as a percentage of the control response at the NMDA receptors. Data represent the mean±S.E.M. from 4 or 5 oocytes.

Experimental

Melting points were determined using a Yanagimoto Yanaco MP melting point apparatus and are uncorrected. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a JEOL JNM-ECA 600 spectrometer using tetramethylsilane as a standard. Mass spectra (MS) were measured on a JEOL LMS-GC mate instrument. IR spectra were recorded on a JASCO FT/IR300E spectrometer. Adult female *Xenopus laevis* were chilled on ice,

and the preparation and maintenance of oocytes were performed as described previously.[9] Capped cRNA was prepared from linearized cDNA templates using mMessage mMachine kits (Ambion, Austin, TX, U.S.A.). Oocytes were injected with NR1A and NR2B cRNAs at a ratio of 1 : 5 (0.2—1 ng of NR1A plus 1—5 ng of NR2B). Macroscopic currents were recorded with a two-electrode voltage-clamp using Dual Electrode Voltage Clamp Amplifier CEZ-1250 (Nihon Koden, Tokyo, Japan). Electrodes were filled with 3 M KCl. Oocytes were continuously superfused (*ca.* 5 ml/min) with a Mg^{2+}-free saline solution (96 mM NaCl, 2 mM KCl, 1.8 mM $BaCl_2$, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5). This solution contained $BaCl_2$ rather than $CaCl_2$, and in all experiments, oocytes were injected with K^+-1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-bis-tetraacetic acid (BAPTA; Sigma, St. Louis, MO, U.S.A.; 100 nl of 40 mM solution at pH 7.5) on the day of recording to eliminate Ca^{2+}-activated Cl-currents. Glutamate and glycine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

4-(4-Methoxybenzyloxycarbonyl)amino-1-butanol (3) A mixture of 4-amino-1-butanol (2.67 g, 30 mmol) and *p*-methoxybenzyl *S*-(4,6-dimethylpyrimidin-2-yl)thiocarbonate (9.12 g, 30 mmol) in $CHCl_3$ (160 ml) was stirred at room temperature overnight. The reaction mixture was washed successively with 4% aqueous $NaHCO_3$ solution and H_2O, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column. Elution with EtOAc : hexane (3 : 1) afforded a white solid (6.78 g, 89%). Recrystallization from EtOAc/hexane gave colorless fine needles, mp 74—75 °C. HR-FAB-MS *m*/*z*: 254.1391 $[M+H]^+$ (Calcd for C13H20NO4: 254.1392). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (1H, t, *J*=5.16 Hz), 1.59 (4H, br s), 3.21—3.26 (2H, m), 3.65—3.68 (2H, m), 3.81 (3H, s), 4.80 (1H, br s), 5.03 (2H, s), 6.88 (2H, d, *J*=8.58 Hz), 7.30 (2H, d, *J*=8.58 Hz). *Anal*. Calcd for $C_{13}H_{19}NO_4$: C, 61,64; H, 7.56; N, 5.53. Found: C, 61.62; H, 7.55; N, 5.57.

4-(4-Methoxybenzyloxycarbonyl)aminobutyl-1-*p*-toluenesulfonate (4) To a solution of **3** (7.24 g, 28.58 mmol) and TEA (12 ml, 85.7 mmol) in CH_2Cl_2 (150 ml), TsCl (5.43 g, 28.58 mmol) was added at 0—5 °C. The reaction mixture was stirred for 5 h at 0—5 °C and then at room temperature overnight. The mixture was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column. Elution with EtOAc : hexane (1 : 1) afforded a colorless oil (9.28 g, 80%). HR-FAB-MS *m*/*z*: 407.1402 $[M+H]^+$ (Calcd for $C_{20}H_{25}NO_6S$: 407.1402). ^{1}H-NMR ($CDCl_3$) δ: 1.53—1.55 (2H, m), 1.65—1.68 (2H, m), 2.44 (3H, s), 3.13—3.18 (2H, m), 3.80 (3H, s), 4.03 (2H, t, *J*=6.18 Hz), 4.66 (1H, br s), 5.00 (2H, s), 6.88 (2H, d, *J*=8.58 Hz), 7.28 (2H, d, *J*=8.58 Hz), 7.33 (2H, d, *J*=8.22 Hz), 7.77 (2H, d, *J*=8.22 Hz).

10-(4-Methoxybenzyloxycarbonyl)-5,10-diaza-1-decanol (5) A solution of **4** (9.28 g, 22.8 mmol) and 4-amino-1-butanol (82.59 g, 29 mmol) in DMF (14 ml) was stirred at 90 °C for 2.5 h. The reaction mixture was poured into ice water. The solution was made alkaline (pH 12) by adding 5% aqueous KOH solution and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4, and then the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$: MeOH : 25% NH_4OH=100 : 10 : 1) to give a colorless oil (4.43 g, 60%). HR-FAB-MS *m*/*z*: 325.2128 $[M+H]^+$ (Calcd for $C_{17}H_{28}N_2O_4$: 325.2127). ^{1}H-NMR ($CDCl_3$) δ: 1.53 (4H, br), 1.60—1.62 (4H, m), 1.65—1.67 (4H, m), 2.62—2.65 (4H, m), 3.18—3.19 (2H, m), 3.56 (2H, t, *J*=5.1 Hz), 3.80 (3H, s), 4.85 (1H, br s), 5.01 (2H, s), 6.88 (2H, d, *J*=8.58 Hz), 7.29 (2H, d, *J*=8.58 Hz).

5-*tert*-Butoxycarbonyl-10-(4-methoxybenzyloxycarbonyl)-5,10-diaza-1-decanol (6) A mixture of **5** (2.4 g, 7.4 mmol) and $(Boc)_2O$ (1.77 g, 8.1 mmol) in CH_2Cl_2 (36 ml) was stirred at 0—5 °C for 4 h and then at room temperature overnight. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluted with $CHCl_3$: MeOH=10 : 1) to give a colorless oil (3.11 g, 99%). HR-FAB-MS *m*/*z*: 425.2653 $[M+H]^+$ (Calcd for $C_{22}H_{37}N_2O_6$: 425.2651). ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.45—1.57 (8H, m), 3.17—3.20 (6H, m), 3.64—3.66 (2H, m), 3.62 (3H, s), 5.02 (2H, s), 6.88 (2H, d, *J*=8.22 Hz), 7.29 (2H, d, *J*=8.22 Hz).

5-*tert*-Butoxycarbonyl-10-(4-methoxybenzyloxycarbonyl)-5,10-diazadecyl *p*-Toluenesulfonate (7) To a solution of **6** (6.14 g, 13.75 mmol) and TEA (6.1 ml, 43.57 mmol) in CH_2Cl_2 (150 ml), TsCl (2.75 g, 14.42 mmol) was added at 0—5 °C. The reaction mixture was stirred for 5 h at 0—5 °C and then at room temperature overnight. The mixture was washed with brine, dried over $MgSO_4$, and then concentrated under reduced pressure. The residue was chromatographed on a silica gel column. Elution with EtOAc : hexane (1 : 1) afforded a colorless oil (4.85 g, 61%). HR-FAB-MS *m*/*z*: 579.2739 $[M+H]^+$ (Calcd for $C_{29}H_{43}N_2O_8S$: 579.2739). ^{1}H-NMR ($CDCl_3$) δ: 1.41 (9H, s), 1.42—1.63 (8H, m), 2.45 (3H, s), 3.12 (4H, br s), 3.18—3.21 (2H, m), 3.80 (3H, s), 4.03 (2H, t, *J*=6.18 Hz), 5.02 (2H, s), 6.88 (2H, d, *J*=8.58 Hz), 7.29 (2H, d, *J*=8.58 Hz), 7.34 (2H, d, *J*=8.22 Hz), 7.78 (2H, d, *J*=8.22 Hz).

10-*tert*-Butoxycarbonyl-15-(4-methoxybenzyloxycarbonyl)-5,10,12-triaza-1-pentadecanol (8) A solution of **7** (7.66 g, 13.2 mmol) and 4-amino-1-butanol (1.42 g, 15.9 mmol) in DMF (8 ml) was stirred at 90 °C for 2.5 h. The reaction mixture was poured into ice water. The solution was made alkaline (pH 12) by adding 5% aqueous KOH solution and then extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4, and then the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$: MeOH : 25% NH_4OH (100 : 20 : 2) to give a colorless oil (3.86 g, 59%). HR-FAB-MS *m*/*z*: 496.3385 $[M+H]^+$ (Calcd for $C_{26}H_{45}N_3O_6$: 496.3386). ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.45—1.54 (8H, m), 1.58—1.68 (4H, m), 2.61—2.65 (4H, m), 3.12—3.21 (6H, m), 3.55—3.57 (2H, m), 3.80 (3H, s), 5.02 (2H, s), 6.88 (2H, d, *J*=8.58 Hz), 7.29 (2H, d, *J*=8.58 Hz).

5,10-Di-*tert*-butoxycarbonyl-15-(4-methoxybenzyloxycarbonyl)-5,10,15-triaza-1-pentadecanol (9) A mixture of **8** (1.3 g, 2.62 mmol) and $(Boc)_2O$ (0.628 g, 2.88 mmol) in CH_2Cl_2 (15 ml) was stirred at 0—5 °C for 9 h. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using EtOAc : hexane (3 : 1) to give a colorless oil (1.48 g, 95%). HR-FAB-MS *m*/*z*: 596.3911 $[M+H]^+$ (Calcd for $C_{31}H_{54}N_3O_8$: 596.3910). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.44 (9H, s), 1.45—1.56 (12H, m), 3.16—3.20 (10H, m), 3.62—3.67 (2H, m), 3.80 (3H, s), 5.02 (2H, s), 6.88 (2H, d, *J*=8.22 Hz), 7.29 (2H, d, *J*=8.22 Hz).

5,10-Di-*tert*-butoxycarbonyl-15-(4-methoxybenzyloxycarbonyl)-5,10,15-triaza-1-pentadecanol Methanesulfonate (10) To a solution of **9** (1.45 g, 2.43 mmol) and TEA (6.1 ml, 43.57 mmol) in CH_2Cl_2 (45 ml), MsCl (0.334 g, 2.92 mmol) was added at 0—5 °C. The reaction mixture was stirred for 2 h at 0—5 °C and then at room temperature overnight. The mixture was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column. Elution with EtOAc : hexane (2 : 1) afforded a colorless oil (1.55 g, 95%). HR-FAB-MS *m*/*z*: 674.3688 $[M+H]^+$ (Calcd for $C_{32}H_{56}N_3O_{10}S$: 674.3686). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.44 (9H, s), 1.47—1.55 (8H, m), 1.60—1.65 (2H, m), 1.71—1.74 (2H, m), 3.00 (3H, s), 3.15—3.20 (10H, m), 3.80 (3H, s), 4.24 (2H, t, *J*=6.54 Hz), 5.02 (2H, s), 6.88 (2H, d, *J*=8.58 Hz), 7.29 (2H, d, *J*=8.58 Hz).

1-Azido-5,10-di-*tert*-butoxycarbonyl-15-(4-methoxybenzyloxycarbonyl)-5,10,15-triazapentadecane (11) A mixture of **10** (4.38 g, 6.5 mmol) and NaN_3 (0.62 g, 9.5 mmol) in DMF (30 ml) was stirred at room temperature overnight. The mixture was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc : hexane (1 : 1) to give a colorless oil (3.91 g, 97%). HR-FAB-MS *m*/*z*: 621.3973 $[M+H]^+$ (Calcd for $C_{31}H_{53}N_6O_7$: 621.3975). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.44 (9H, s), 1.45—1.55 (12H, m), 3.16—3.20 (10H, m), 3.12—3.21 (10H, m), 3.29—3.30 (2H, m), 3.80 (3H, s), 5.02 (2H, s), 6.88 (2H, d, *J*=8.88 Hz), 7.29 (2H, d, *J*=8.88 Hz).

5,10-Di-*tert*-butoxycarbonyl-15-(4-methoxybenzyloxycarbonyl)-5,10,15-triazapentadecylamine (12) A solution of **11** (3.9 g, 6.28 mmol) in THF (36 ml) was hydrogenated over 3.5—6.5% palladium-activated carbon ethylenediamine complex (Pd–C(en)) (0.65 g) at room temperature for 2 h under H_2 atmosphere. The catalyst was filtered through a pad of celite, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$: MeOH : 25% NH_4OH (100 : 20 : 2) to give a colorless oil (3.14 g, 84%). HR-FAB-MS *m*/*z*: 595.4070 $[M+H]^+$ (Calcd for $C_{31}H_{55}N_4O_7$: 595.4070). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.44 (9H, s), 1.45—1.56 (12H, m), 2.70 (2H, t, *J*=7.26 Hz), 3.16—3.20 (12H, m), 5.02 (2H, s), 6.88 (2H, d, *J*=8.58 Hz), 7.29 (2H, d, *J*=8.58 Hz).

(4-{[4-(4-Methoxybenzyloxycarbonylamino)butyl]-*tert*-butoxycarbonylamino}butyl)-{4-[*N*′,*N*″-bis(*tert*-butoxycarbonyl)guanidinobutyl]}carbamic acid *tert*-Butyl Ester (13) A mixture of **12** (315 mg, 0.53 mmol) and *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (154 mg, 0.53 mmol) in CH_2Cl_2 (8 ml) was stirred at room temperature for 2 d. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc : hexane (2 : 1) to give a colorless oil (295 mg, 66%). HR-FAB-MS *m*/*z*: 837.5336 $[M+H]^+$ (Calcd for $C_{42}H_{72}N_6O_{11}$: 837.5332). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.44 (9H, s), 1.47 (6H, br s), 1.49 (9H, s), 1.50 (9H, s), 3.16—3.22 (10H, m), 3.40—3.45 (2H, m), 3.80 (3H, s), 5.02 (2H, s), 6.88 (2H, d, *J*=8.58 Hz), 7.29 (2H, d,

J=8.58 Hz).

{4-[(4-Aminobutyl)-*tert*-butoxycarbonylamino]butyl}-{4-[*N*′,*N*″-bis-(*tert*-butoxycarbonyl)guanidinobutyl]}carbamic Acid *tert*-Butyl Ester (14) A solution of **13** (220 mg, 0.145 mmol) in THF (4 ml) was hydrogenated over 10% Pd–C (60 mg) at room temperature for 2 d under H_2 atmosphere. The catalyst was filtered through a pad of celite, and the filtrate was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$: MeOH : 25% NH_4OH (100 : 10 : 1) to give a colorless oil (80 mg, 49%). HR-FAB-MS *m*/*z*: 673.4863 $[M+H]^+$ (Calcd for $C_{33}H_{65}N_6O_8$: 673.4864). ^{1}H-NMR ($CDCl_3$) δ: 1.44—1.59 (48H, m), 3.16 (10H, br s), 3.41—3.22 (2H, m).

(4-{[4-(*p*-toluenesulfonylamino)butyl]-*tert*-butoxycarbonylamino}butyl)-{4-[*N*′,*N*″-bis(tert-butoxycarbonyl)guanidinobutyl]}carbamic Acid *tert*-Butyl Ester (15) To a solution of **14** (67 mg, 0.1 mmol) and TEA (42 μl, 0.3 mmol) in CH_2Cl_2 (2 ml), TsCl (19 mg, 0.1 mmol) was added at 0—5 °C. The reaction mixture was stirred for 2 h at 0—5 °C and then at room temperature overnight. The mixture was diluted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column. Elution with EtOAc : hexane (1 : 1) afforded a colorless oil (66 mg, 80%). HR-FAB-MS *m*/*z*: 827.4967 $[M+H]^+$ (Calcd for $C_{40}H_{71}N_6O_{10}S$: 827.4951). ^{1}H-NMR ($CDCl_3$) δ: 1.41—1.59 (48H, m), 2.43 (3H, s), 2.94—2.96 (2H, m), 3.10—3.21 (8H, m), 3.41—3.44 (2H, m), 7.30 (2H, d, J=8.22 Hz), 7.75 (2H, d, J=8.22 Hz).

(4-{[4-(Butane-1-sulfonylamino)butyl]-*tert*-butoxycarbonylamino}butyl)-{4-[*N*′,*N*″-bis(tert-butoxycarbonyl)guanidinobutyl]}carbamic Acid *tert*-Butyl Ester (16) To a solution of **14** (76 mg, 0.11 mmol) and TEA (46 μl, 0.33 mmol) in CH_2Cl_2 (2 ml), BsCl (17 mg, 0.11 mmol) was added at 0—5 °C. The reaction mixture was stirred for 2 h at 0—5 °C and then at room temperature overnight. The mixture was diluted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed on a silica gel column. Elution with EtOAc : hexane (1 : 1) afforded a colorless oil (70 mg, 80%). HR-FAB-MS *m*/*z*: 793.5109 $[M+H]^+$ (Calcd for C37H72N6O10S: 793.5108). ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7.56 Hz), 1.44—1.60 (50H, m), 1.78—1.80 (2H, m), 2.99—3.02 (2H, m), 3.12—3.25 (12H, m).

***N*-{4-[4-(4-Guanidinobutylamino)butylamino]butyl}-*p*-toluenesulfonamide Trihydrochloride (1a, TsHSPMG)** A mixture of **15** (116 mg, 0.14 mmol) and 36% HCl (0.5 ml) in THF (2 ml) was stirred at room temperature. After 36 h, the mixture was concentrated under reduced pressure. The residue was washed successively with THF and Et_2O, and dried to give a white solid (75 mg, 100%). HR-FAB-MS *m*/*z*: 427.2854 $[M-3HCl+H]^+$ (Calcd for $C_{20}H_{39}N_6O_2S$: 427.2855). ^{1}H-NMR (D_2O) δ: 1.31—1.36 (2H, m), 1.44—1.57 (10H, m), 2.24 (3H, s), 2.73 (2H, t, J=6.9 Hz), 2.80—2.82 (2H, m), 2.85—2.88 (6H, m), 3.02 (2H, t, J=6.9 Hz), 7.28 (2H, d, J=8.22 Hz), 7.56 (2H, d, J=8.22 Hz). 13C-NMR (D_2O) δ: 20.85, 22.82, 22.93, 22.98, 23.06, 25.29, 25.86, 40.67, 42.08, 46.82, 46.99, 47.14, 47.34, 126.90, 130.26, 135.08, 145.23, 156.94. IR (KBr) cm^{-1}: 3386, 3162, 2956, 2800, 1675, 1644, 1452, 1328, 1155. *Anal.* Calcd for $C_{20}H_{38}N_6O_2S \cdot 3HCl$: C, 44.81; H, 7.71; N, 15.68. Found: C, 45.08; H, 7.93; N, 15.81.

***N*-{4-[4-(4-Guanidinobutylamino)butylamino]butyl}butane-1-sulfonamide Trihydrochloride (1b, BsHSPMG)** A mixture of **16** (50 mg, 0.063 mmol) and 36% HCl (0.2 ml) in THF (0.5 ml) was stirred at room temperature. After 36 h, the mixture was concentrated under reduced pressure. The residue was washed successively with THF and Et_2O, and dried to give a white solid (31 mg, 100%). HR-FAB-MS *m*/*z*: 393.3012 $[M-3HCl+H]^+$ (Calcd for $C_{17}H_{41}N_6O_2S$: 393.3011). ^{1}H-NMR (D_2O) δ: 0.72 (3H, t, J= 7.56 Hz), 1.25 (2H, sex, J=7.56 Hz), 1.40—1.49 (4H, m), 1.50—1.59 (10H, m), 2.86—2.89 (8H, m), 2.92 (2H, t, J=6.9 Hz), 3.00—3.04 (4H, m). ^{13}C-NMR (D_2O) δ: 12.97, 20.98, 22.82, 22.95, 22.98, 23.05, 25.05, 25.29, 26.57, 40.66, 41.93, 46.87, 47.00, 47.23, 47.33, 51.03, 156.95. IR (KBr) cm^{-1}: 3307, 3160, 2950, 2871, 2794, 1664, 1444, 1305, 1133. *Anal.* Calcd for $C_{17}H_{41}N_6O_2S \cdot 3HCl$: C, 40.67; H, 8.63; N, 16.74. Found: C, 40.70; H, 8.88; N, 16.80.

4,9-Di-*tert*-butoxycarbonyl-13-(*p*-toluenesulfonyl)-4,9,13-triazatridecylamime (18) 4,9-Diaza-4,9-di-(*tert*-butoxycarbonyl)dodecane-1,12-diamine (**17**) (201 mg, 0.5 mmol) and TEA (0.07 ml 0.5 mmol) were dissolved in CH_2Cl_2 (10 ml), and then a solution of TsCl (95 mg, 0.5 mmol) in CH_2Cl_2 (5 ml) was added in 0.25-ml aliquots every 10 min while stirring at room temperature. After the addition was complete, the mixture was stirred at room temperature for 2 d, and then the reaction mixture was washed with water and dried over Na_2SO_4. Evaporation of the solvent yielded the crude compound as an oil, which was purified by column chromatography on silica gel with $CHCl_3$: MeOH : 25% NH4OH (100 : 20 : 2) to give a colorless oil (138 mg, 49%). HR-FAB-MS *m*/*z*: 557.3376 $[M+H]^+$ (Calcd for $C_{27}H_{49}N_4O_6S$: 557.3372). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (9H, s), 1.44 (9H, s), 1.53 (4H, br s), 1.64 (4H, quin, J=6.9 Hz), 2.41 (3H, s), 2.69 (2H, br s), 2.87 (2H, br s), 3.05 (2H, br s), 3.10—3.24 (6H, m), 7.26 (2H, br s), 7.74 (2H, d, J=8.0 Hz).

4,9-Di-*tert*-butoxycarbonyl-13-(butane-1-sulfonyl)-4,9,13-triazatridecylamime (19) 4,9-Diaza-4,9-di-(*tert*-butoxycarbonyl)dodecane-1,12-diamine (630 mg, 1.57 mmol) and TEA (0.219 ml, 1.57 mmol) were dissolved in CH_2Cl_2 (30 ml), and then a solution of BsCl (245 mg, 1.57 mmol) in CH_2Cl_2 (15 ml) was added in 0.25-ml aliquots every 10 min while stirring at room temperature. After the addition was complete, the mixture was stirred at room temperature for 2 d, then the reaction mixture was washed with water and dried over Na_2SO_4. Evaporation of the solvent yielded the crude compound as an oil, which was purified by column chromatography on silica gel with $CHCl_3$: MeOH : 25% NH_4OH (100 : 20 : 2) to give a colorless oil (406 mg 50%). HR-FAB-MS *m*/*z*: 523.3532 $[M+H]^+$ (Calcd for $C_{24}H_{51}N_4O_6S$: 523.3528). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, t, J=7.5 Hz), 1.45—1.46 (24H, m), 1.64 (2H, quin, J=6.9 Hz), 1.71—1.81 (4H, m), 2.69 (2H, m), 2.97 (2H, t, J=7.9 Hz), 3.06—3.34 (10H, m).

(4-{[3-(*p*-Toluenesulfonylamino)propyl]-*tert*-butoxycarbonylamino}-butyl)-{3-[*N*′,*N*″-bis(*tert*-butoxycarbonyl)guanidinopropyl]}carbamic Acid *tert*-Butyl Ester (20) A mixture of **18** (126 mg, 0.23 mmol) and *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (66 mg, 0.23 mmol) in CH_2Cl_2 (5 ml) was stirred at room temperature for 2 d. Evaporation of the solvent yielded the crude compound as an oil, which was purified by column chromatography on silica gel with EtOAc : hexane (1 : 1) to give a colorless oil (136 mg, 74%). HR-FAB-MS *m*/*z*: 799.4639 $[M+H]^+$ (Calcd for $C_{38}H_{67}N_6O_{10}S$: 799.4638). ^{1}H-NMR ($CDCl_3$) δ: 1.38 (9H, s), 1.43 (18H, s), 1.49 (9H, s), 1.62 (6H,s), 1.78 (2H, quin, J=7.5 Hz), 2.40(3H, s), 2.87 (2H, br s), 3.04 (2H, br s), 3.18—3.24 (6H, m), 3.41 (2H, q, J=6.9 Hz), (6.14, 1H, br), 7.26 (2H, br s), 7.74 (2H, d, J=8.0 Hz), 8.35 (1H, br), 11.48 (1H, s).

(4-{[3-(Butane-1-sulfonylamino)propyl]-*tert*-butoxycarbonylamino}-butyl)-{3-[*N*′,*N*″-bis(*tert*-butoxycarbonyl)guanidinopropyl]}carbamic Acid *tert*-Butyl Ester (21) A mixture of **19** (230 mg, 0.44 mmol) and *N*,*N*′-bis(*tert*-butoxycarbonyl)-*S*-methylisothiourea (128 mg, 0.44 mmol) in CH_2Cl_2 (5 ml) was stirred at room temperature for 2 d. Evaporation of the solvent yielded the crude compound as an oil, which was purified by column chromatography on silica gel with EtOAc : hexane (1 : 1) to give a colorless oil (293 mg, 87%). HR-FAB-MS *m*/*z*: 765.4794 $[M+H]^+$ (Calcd for $C_{35}H_{69}N_6O_{10}S$: 765.4795). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, t, J=7.5 Hz), 1.44 (20H, m), 1.49 (18H, m), 1.62—1.71 (6H, m), 1.76—1.81 (4H, m), 2.97 (2H, t, J=7.5 Hz), 3.06—3.20 (8H, m), 3.33 (2H, br s), 3.40—3.44 (2H, m), 5.82 (1H, br s), 8.36 (1H, br s), 11.48 (1H, s).

***N*-{3-[4-(3-Guanidinopropylamino)butylamino]propyl}-*p*-toluenesulfonamide Trihydrochroride (2a, TsSPMG)** To a solution of **20** (80 mg, 0.1 mmol) in THF (2 ml), 36% HCl (1 ml) was added at room temperature. The reaction mixture was stirred for 48 h, and then concentrated under reduced pressure to give a white solid which was washed with Et2O to give a white solid (50 mg, 100%), mp 188—192 °C. HR-FAB-MS *m*/*z*: 399.2542 $[M-3HCl+H]^+$ (Calcd for $C_{18}H_{35}N_6O_2S$: 399.2541). ^{1}H-NMR (D_2O) δ: 1.55—1.58 (4H, m), 1.64—1.69 (2H, m), 1.76—1.81 (2H, m), 2.24 (3H, s), 2.80 (2H, t, J=6.9 Hz), 2.86—2.93 (8H, m), 3.10 (2H, t, J=6.9 Hz), 7.28 (2H, d, J=8.22 Hz), 7.56 (2H, d, J=8.22 Hz). ^{13}C-NMR (D_2O) δ: 20.86, 22.90, 22.97, 25.19, 25.84, 38.38, 39.92, 45.09, 45.18, 47.01, 47.13, 126.92, 130.31, 134.86, 145.34, 156.98. IR (KBr) cm^{-1}: 3357, 3160, 3064, 2964, 2803, 1654, 1459, 1321, 1155. *Anal.* Calcd for $C_{18}H_{34}N_6O_2S \cdot 3HCl$: C, 42.56; H, 7.34; N, 16.55. Found: C, 42.29; H, 7.44; N, 16.48.

***N*-{3-[4-(3-Guanidinopropylamino)butylamino]propyl}butane-1-sulfonamide Trihydrochroride (2b, BsSPMG)** To a solution of **21** (108 mg, 0.14 mmol) in THF (2 ml), 36% HCl (1 ml) was added at room temperature. The reaction mixture was stirred for 48 h, and then concentrated under reduced pressure to give a white solid which was washed with Et_2O to give a white solid (66 mg, 100%), mp 178—180 °C. HR-FAB-MS *m*/*z*: 365.2696 $[M-3HCl+H]^+$ (Calcd for $C_{15}H_{37}N_6O_2S$: 365.2698). ^{1}H-NMR (D_2O) δ: 0.74 (3H, t, J=7.5 Hz), 1.26 (2H, sex, J=7.5 Hz), 1.52—1.60 (6H, m), 1.74—1.77 (2H, m), 1.78—1.82 (2H, m), 2.92—2.99 (8H, m), 3.00—3.06 (4H, m), 3.12 (2H, t, J=7.5 Hz). ^{13}C-NMR (D_2O) δ: 12.96, 20.96, 22.93, 22.99, 25.01, 25.20, 26.53, 38.38, 39.73, 45.10, 45.15, 47.06, 47.14, 51.05, 157.01. IR (KBr) cm^{-1}: 3301, 3153, 2952, 2796, 1664, 1454, 1309, 1135. *Anal.* Calcd for $C_{15}H_{36}N_6O_2S \cdot 3HCl$: C, 38.01; H, 8.29; N, 17.73. Found: C, 38.00; H, 8.51; N, 17.62.

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