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SYNTHESIS OF TWO NOVEL WATER-SOLUBLE CLEFT-TYPE CYCLOPHANES EFFECTIVE AS *N*-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST

Takashi Masuko, Yuta Nemoto, Tadashi Kusama, Koichi Metori, Yasuo Kizawa,
and Muneharu Miyake*

College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi-shi,
Chiba, 274-8555, Japan

E-mail: miyake.muneharu@nihon-u.ac.jp

Abstract – Novel cleft-type cyclophanes, 4,4'-bis[*N*-(5-dimethylamino-aphthalene-sulfonylaminoethyl)-*N*-(1,4,7,10-tetraazacyclododecane-1-ylacetyl)-2-aminoethoxy]diphenylmethane octahydrochloride (**1a**, DNCn) and 4,4'-bis[*N*-(*p*-toluene-sulfonylaminoethyl)-*N*-(1,4,7,10-tetraazacyclododecane-1-ylacetyl)-2-aminoethoxy]diphenylmethane octahydrochloride (**1b**, TsDCn) having an effective role as *N*-methyl-D-aspartate (NMDA) receptor antagonist were synthesized. Neuroprotective effects of cleft-type cyclophanes, DNCn (**1a**) and TsDCn (**1b**) against cell damage caused by NMDA were measured in cultured rat hippocampal neurons. DNCn (**1a**) and TsDCn (**1b**) reduced the neurotoxicity and acted as open channel blocker for NMDA receptor.

In the central nervous system (CNS), the *N*-methyl-D-aspartate (NMDA) receptor plays a critical role in a variety of neurophysiological phenomena, including neuronal development, synaptic plasticity, and excitotoxicity. Glutamate is known to be neurotoxic under certain circumstances, in particular when energy supply is compromised. Thus some researchers now believe that the neurodegeneration associated with a variety of acute and chronic disorders (including ischemic stroke, Parkinson's disease, Alzheimer's disease, and dementia) may be caused in part by overactivation of glutamate receptors. Alzheimer's disease is a neurodegenerative disorder characterized by irreversible, progressive loss of memory followed by complete dementia. The cognitive decline is accompanied by impaired

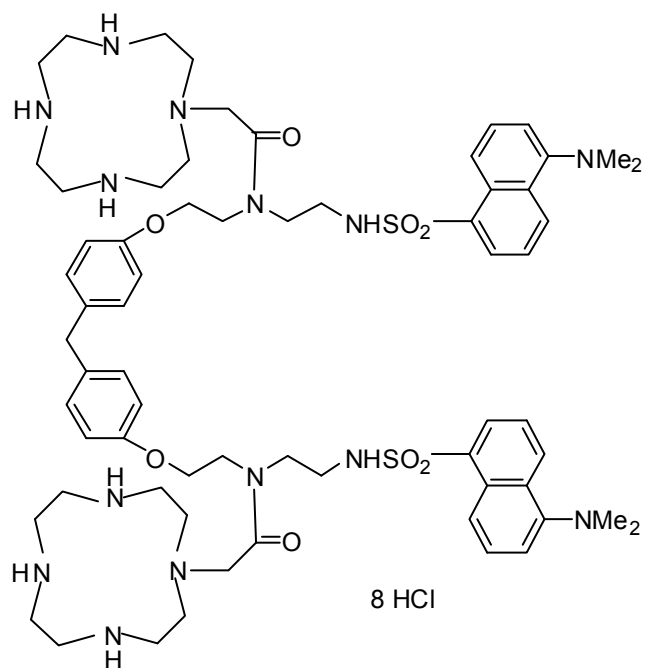
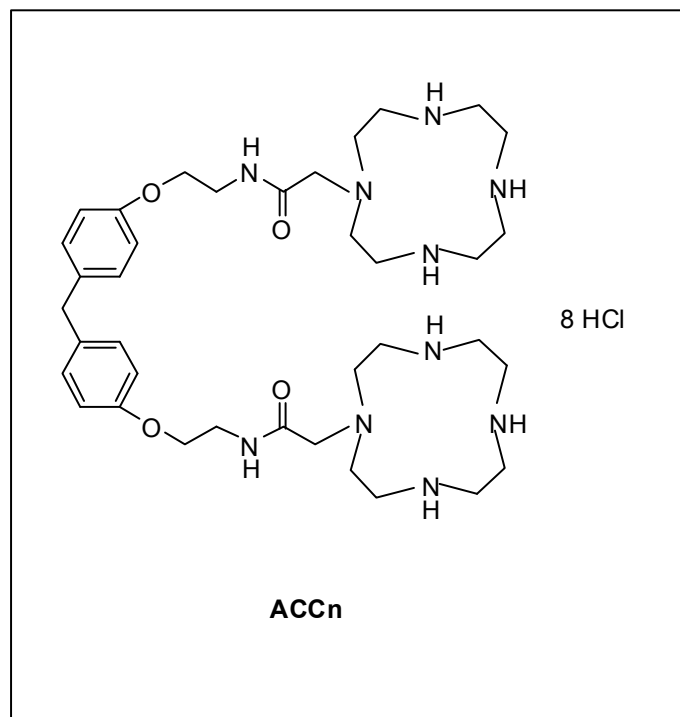
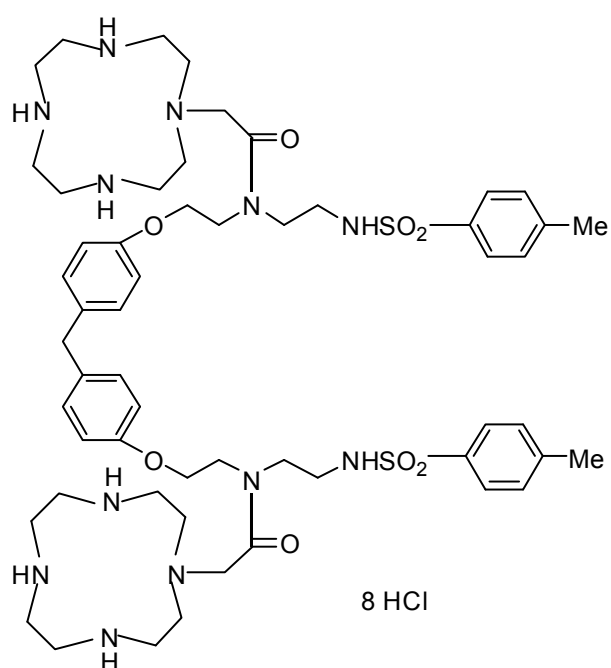
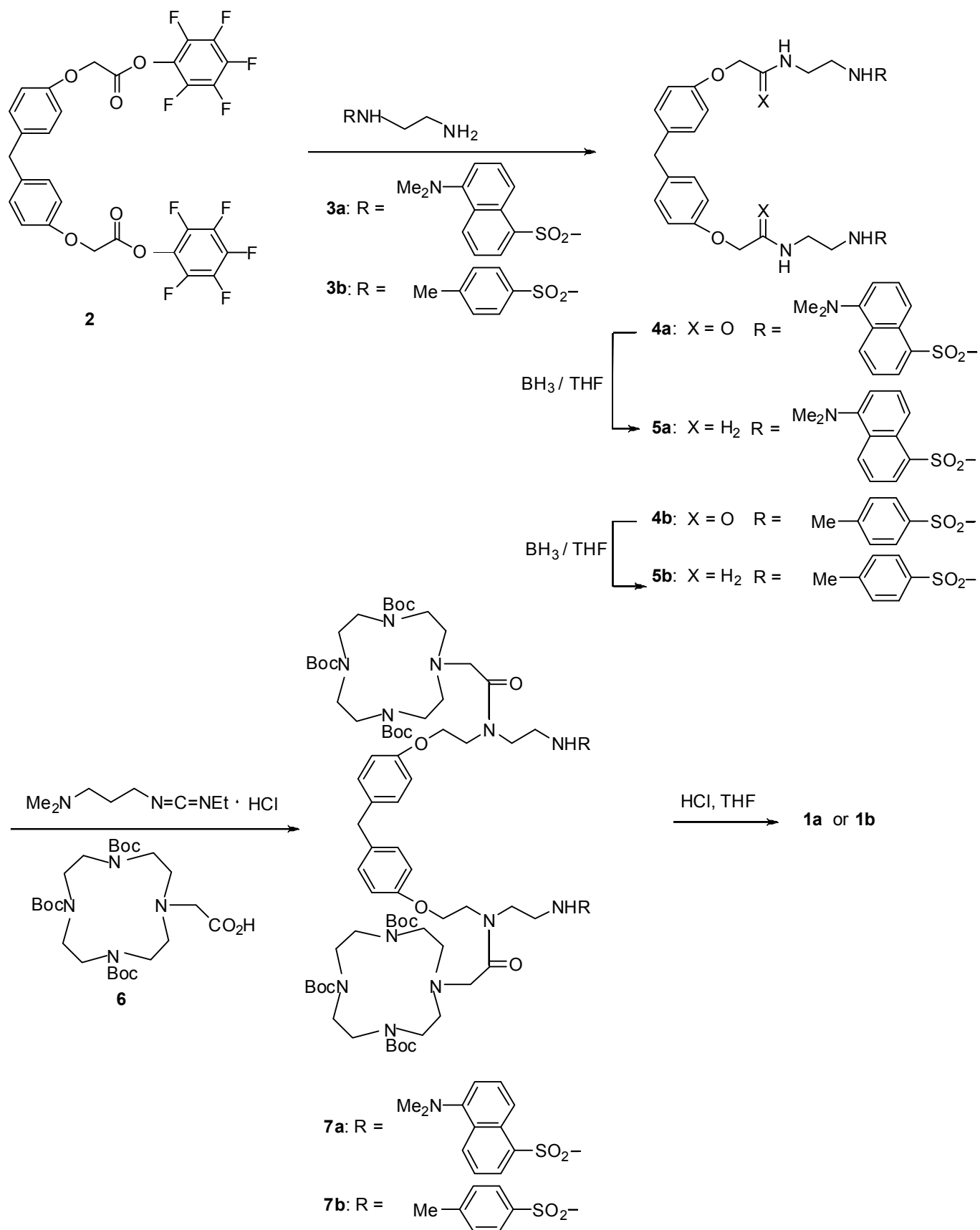
**1a (DNCn)****1b (TsDNCn)**

Figure 1. Previously (ACCn) and Currently (DNCn (**1a**) and TsDNCn (**1b**)) Reported NMDA Receptor Antagonists



Scheme 1

performance of daily activities, behavior, speech and visual-spatial perception. Glutamate excitotoxicity as a result of blockade of glutamate uptake into astrocytes by A β aggregates induces excessive Ca influx mainly through the NMDA receptors, followed by neuronal cell death.¹ The NMDA receptor consists of at least two types of subunits, NR1 and NR2.² One subunit includes only three transmembrane domains (M1, M3, and M4) plus a cytoplasm-facing re-entrant membrane loop (M2). The M2 loop region in NR1 and NR2 subunits is a critical determinant of divalent cation permeability and Mg²⁺ blockade. NR1 is a single gene product expressed as eight alternatively spliced mRNAs, and NR2A, NR2B, NR2C, and NR2D are distinct gene products.

We previously reported³ two cleft-type cyclophanes DNCn (**1a**) and TsDCn (**1b**) which inhibited the activity of NR1/NR2A and NR1/NR2B receptors in *Xenopus* oocytes voltage-clamped at -70 mV. The IC₅₀ values for DNCn (**1a**) and TsDCn (**1b**) were 0.36 and 0.2 μ M respectively on NMDA receptors. The inhibition of NR1/NR2 and NR1/NR2B receptor activity by DNCn and TsDCn, containing two sulfonamide groups, is stronger than that of 4,4'-bis[2-(1,4,7,10-tetraazacyclododecane-1-yl)-acethylaminoethoxy]diphenylmethane octahydrochloride (ACCn) (IC₅₀ = 7.0 μ M) possessing no sulfonamide group, given that the sulfonamide moiety is more effective to enhance the inhibitory effects of NMDA receptor.

In this paper, we report the first syntheses of two novel cleft-type cyclophanes DNCn (**1a**) and TsDCn (**1b**) (Figure 1) and their ability to act as an NMDA receptor antagonist and as an open channel blocker.

RESULTS AND DISCUSSION

The two cleft-type cyclophanes DNCn (**1a**) and TsDCn (**1b**) were synthesized as shown in Scheme 1. 4,4'-bis(pentafluorophenoxycarbonylmethoxy)diphenylmethane (**2**)⁴ was converted into corresponding amide (**4a**, 97%) by treatment with 5-dimethylaminonaphthalene-1-[*N*-(2-aminoethyl)]sulfonamide⁵ (**3a**) in the presence of triethylamine (TEA), followed sequentially by reduction with borane dimethyl sulfide complex (BH₃·SMe₂) to the corresponding amine (**5a**, 90%). The amine (**5a**) was converted into **7a** by treatment with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 1-carboxymethyl-1,4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclotetradecane⁶ (**6**) in the presence of TEA in dichloromethane. Finally, deprotection of **7a** with concentrated HCl in tetrahydrofuran (THF) resulted in the desired compound DNCn (**1a**) in quantitative yield. Likewise, TsDCn (**1b**) was synthesized from *N*-(2-aminoethyl)-4-methylbenzenesulfonamide (**3b**)⁷ by a method similar to that of DNCn (**1a**).

In cultured hippocampal neurons, treatment with nerve growth factor and 21 mM KCl for 7 days induced remarkable neuronal activity, dendritic elongation and branching, compared to controls.⁸ Cell damage caused by the activation of NMDA receptors was assessed by detecting lactate dehydrogenase (LDH) leakage from the damaged neuron. Exposure of cultured neurons to 1mM NMDA for 1h increased LDH

release to $201 \pm 4\%$ of control after 24 h. Addition of $5 \mu\text{M}$ DNCn and TsDCn reduced the neurotoxicity induced by 1 mM NMDA. Also, memantine, an open channel blocker of NMDA receptors;

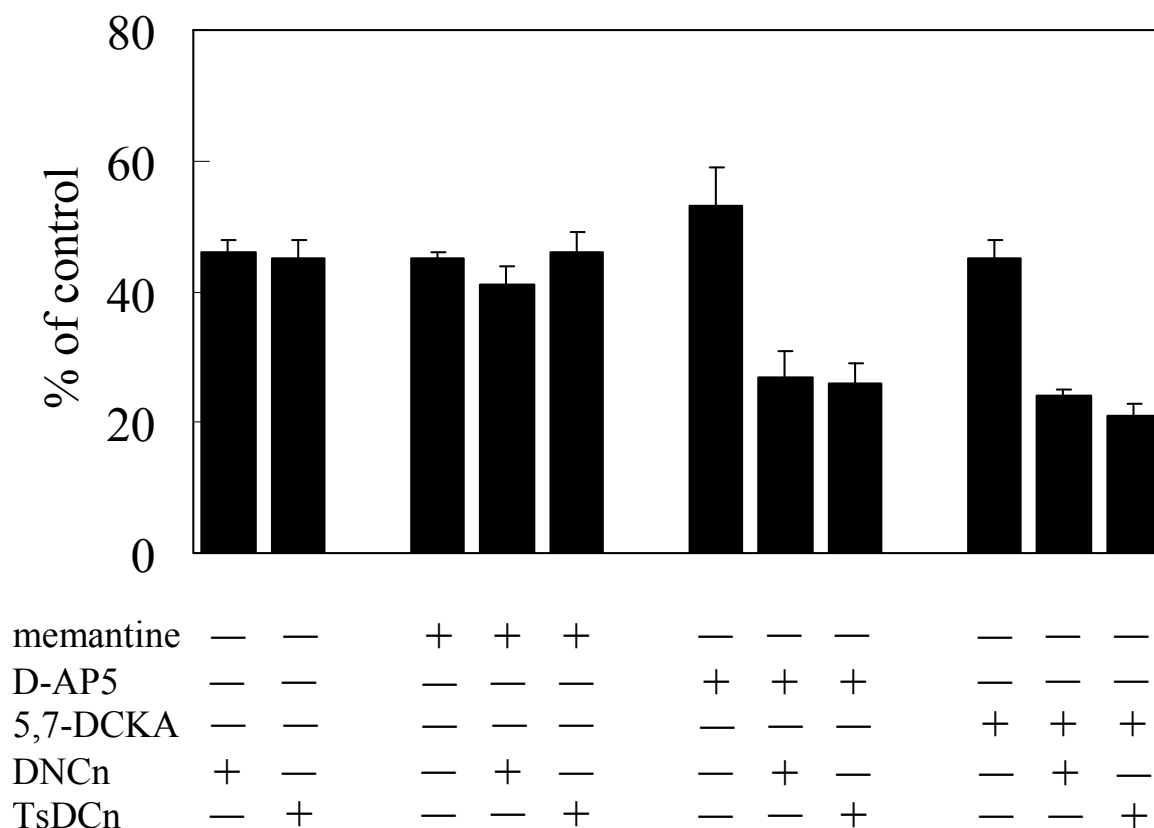


Figure 2. Neuroprotective effects of cleft-type cyclophane and NMDA receptor antagonists in rat cultured hippocampal neurons. Effects of NMDA on LDH-release from cultured hippocampal neurons, as index of neuronal cell death, were measured. Data were normalized to the activity of LDH-release from vehicle-treated cells (100%) and expressed as percentage of the control. LDH released from damaged hippocampal neurons into the cell culture medium was measured as described in Section 2. The value for 100% means LDH release in the presence of 1 mM NMDA in the absence of $5 \mu\text{M}$ DNCn, TsDCn, $20 \mu\text{M}$ memantine, $75 \mu\text{M}$ D-AP-5 and $10 \mu\text{M}$ 5,7-DCKA. Values are presented as mean $\pm$ S.E.M. from six to nine wells for each compound.

D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5), an antagonist of glutamate binding site; and 5,7-dichlorokynurenic acid (5,7-DCKA), an antagonist of glycine binding site of NMDA receptor, reduced the neurotoxicity under the same conditions. Moreover, cleft-type cyclophanes plus D-AP5 or 5,7-DCKA significantly reduced the toxicity more than the sum of each individual, whereas cleft-type cyclophanes plus memantine did not produce the same effect. If site of action of cleft-type cyclophane is the same as memantine, the combination of memantine and cleft-type cyclophane is not more different than a single administration. However, the effect becomes additive if memantine and cleft-type cyclophane act on the different site in NMDA receptors. These data indicate that cleft-type cyclophane

directly act on the channel pore of the NMDA receptor as an open channel blocker (Figure 2).

In conclusion, we have synthesized compound DNCn (**1a**) and TsDCn (**1b**) that have an inhibitory effect on the NMDA receptor. The synthesis of new chemicals with stronger inhibitory effects on the NMDA receptor is now underway in our laboratory and will be reported in due course.

EXPERIMENTAL

Melting points were determined using a Yanagimoto melting point apparatus, Yanaco MP, and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a JEOL JNM-ECA 600 spectrometer containing tetramethylsilane as standard. FAB-MS measurement was performed using a JEOL JMS-GC-mate instrument equipped with double-focusing mass analyzer.

4,4'-Bis(5-dimethylaminonaphthalenesulfonylaminoethylcarbamoylmethoxy)diphenylmethane (**4a**)

A mixture of **2** (245 mg, 0.38 mmol), **3a** (222 mg, 0.76 mmol), and TEA (81 mg, 0.8 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature. After 24 h, the reaction mixture was washed with H_2O and dried over MgSO_4 , and evaporated under reduced pressure. The residue was chromatographed on silica gel column with EtOAc as an eluent to give a yellow solid (320 mg, 97%). An analytical sample was obtained by recrystallizing this material from CH_2Cl_2 —hexane, yielding yellow fine needles, mp 132—133 °C. ^1H NMR (CDCl_3) δ : 2.85 (12H, s), 3.38 (4H, s), 3.05—3.08 (4H, m), 3.88 (2H, s), 4.32 (4H, s), 5.30 (2H, t, $J=5.94$ Hz), 6.78 (4H, d, $J=8.6$ Hz), 6.89 (2H, br t, $J=5.8$ Hz), 7.10 (4H, d, $J=8.6$ Hz), 7.15 (2H, d, $J=7.2$ Hz), 7.50 (1H, d, $J=7.56$ Hz), 7.51 (1H, d, $J=7.2$ Hz), 7.55 (2H, dd, $J=8.6$, 7.56 Hz), 8.219 (1H, d, $J=7.2$ Hz), 8.221 (1H, d, $J=7.56$ Hz), 8.23 (1H, d, $J=8.6$ Hz), 8.53 (2H, d, $J=8.6$ Hz). HRMS (FAB) (m/z) Calcd for $\text{C}_{45}\text{H}_{51}\text{N}_6\text{O}_8\text{S}_2$: 867.3209. Found 867.3208. Anal. Calcd for $\text{C}_{45}\text{H}_{50}\text{N}_6\text{O}_8\text{S}_2$: C, 62.34; H, 5.81; N, 9.69. Found: C, 62.57; H, 5.95; N, 9.48.

4,4'-Bis(*p*-toluenesulfonylaminoethylcarbamoylmethoxy)diphenylmethane (**4b**)

A mixture of **2** (790 mg, 1.21 mmol), **3b** (521 mg, 2.43 mmol), and TEA (267 mg, 2.64 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature. After 24 h, the reaction mixture was washed with H_2O and dried over MgSO_4 , and evaporated under reduced pressure. The residue was chromatographed on silica gel column with EtOAc as an eluent to give a colorless viscous oil (660 mg, 76%). ^1H NMR (CDCl_3) δ : 2.37 (6H, s), 3.38 (4H, s), 3.11—3.13 (4H, m), 3.42—3.44 (4H, m), 3.87 (2H, s), 4.40 (4H, s), 5.07 (2H, t, $J=5.82$ Hz), 6.82 (4H, d, $J=8.64$ Hz), 6.98 (2H, br t, $J=5.82$ Hz), 7.11 (4H, d, $J=8.58$ Hz), 7.28 (4H, d, $J=7.92$ Hz), 7.71 (4H, d, $J=8.22$ Hz). HRMS (FAB) (m/z) Calcd for $\text{C}_{35}\text{H}_{41}\text{N}_4\text{O}_8\text{S}_2$: 709.2365. Found 709.2365.

4,4'-Bis(5-dimethylaminonaphthalenesulfonylaminoethyliminoethoxy)diphenylmethane (**5a**)

A mixture of **4a** (303 mg, 0.35 mmol) in THF (10 mL) was stirred at room temperature under N_2 atmosphere. $\text{BH}_3\cdot\text{SMe}_2$ (0.42 mL, 4.42 mmol) was added. The reaction mixture was stirred for 24 h at

80 °C, and then was cooled to room temperature. 0.7 M hydrogen chloride-MeOH solution (3 mL) was added, and the reaction mixture was refluxed for 1 h, and evaporated under reduced pressure. The residue was made basic to pH 11 with excess 25% NH₄OH. The mixture was extracted with CH₂Cl₂ (30 mL), washed with brine and dried over Na₂SO₄. Removal of solvent under reduced pressure gave a yellow oil, which was chromatographed on a silica gel column with EtOAc:MeOH (9:1) and as an eluent to give a yellow amorphous solid (264 mg, 90%), which was used for the next reaction without further purification. ¹H NMR (CDCl₃) δ: 2.60 (4H, t, *J*=5.2 Hz), 2.62—2.64 (4H, m), 2.86 (12H, s), 2.92—2.94 (4H, s), 3.76 (4H, t, *J*=5.2 Hz), 3.86 (2H, s), 6.74 (4H, d, *J*=8.6, 1.7 Hz), 7.07 (4H, d, *J*=8.6), 7.12 (2H, d, *J*=6.9 Hz), 7.508 (2H, dd, *J*=8.6, 7.6 Hz), 7.514 (2H, dd, *J*=8.6, 7.2 Hz), 8.256 (1H, d, *J*=7.2 Hz), 8.258 (1H, d, *J*=7.2 Hz), 8.28 (2H, d, *J*=8.6 Hz), 8.52 (2H, d, *J*=8.6 Hz). HRMS (FAB) (*m/z*) Calcd for C₄₅H₅₅N₆O₆S₂: 839.3624. Found 839.3620. Anal. Calcd for C₄₅H₅₄N₆O₆S₂: C, 64.41; H, 6.49; N, 10.02. Found: C, 64.29; H, 6.64; N, 10.17.

4,4'-Bis(*p*-toluenesulfonylaminoethyliminoethoxy)diphenylmethane (5b) A mixture of **4b** (270 mg, 0.38 mmol) in THF (15 mL) was stirred at room temperature under N₂ atmosphere. BH₃·SMe₂ (0.5 mL, 5.26 mmol) was added. The reaction mixture was stirred for 24 h at 80 °C, and then was cooled to room temperature. 0.7 M hydrogen chloride-MeOH solution (5 mL) was added, and the reaction mixture was then refluxed for 1 h, and evaporated under reduced pressure. The residue was made basic to pH 11 with excess 25% NH₄OH. The mixture was extracted with CH₂Cl₂ (30 mL), washed with brine and dried over Na₂SO₄. Removal of solvent under reduced pressure gave a yellow oil, which was chromatographed on a silica gel column with CHCl₃:MeOH (9:1) as an eluent to give a colorless oil (210 mg, 81%), which was used for the next reaction without further purification. ¹H NMR (CDCl₃) δ: 2.40 (6H, s), 2.74—2.75 (4H, m), 2.84 (4H, t, *J*=5.5 Hz), 3.00—3.01 (4H, m), 3.86 (2H, s), 3.94 (4H, t, *J*=5.1 Hz), 5.01 (2H, br), 6.79 (4H, d, *J*=8.59 Hz), 7.07 (4H, d, *J*=8.93), 7.27 (4H, d, *J*=8.25 Hz), 7.74 (4H, d, *J*=8.25 Hz). HRMS (FAB) (*m/z*) Calcd for C₃₅H₄₅N₄O₆S₂: 681.2780. Found 681.2780.

4,4'-Bis[*N*-(5-dimethylaminonaphthalenesulfonylaminoethyl)-*N*-(4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane-1-ylacetyl)-2-aminoethoxy]diphenylmethane (7a) A mixture of **5a** (84 mg, 0.1 mmol), **6** (106 mg, 0.2 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (45 mg, 0.23 mmol) and TEA (23 mg, 0.23 mmol) in CH₂Cl₂ (5 mL) was stirred at room temperature for 24 h under N₂ atmosphere. The reaction mixture was diluted with CH₂Cl₂ (15 mL), washed with 2 N NaOH and dried over Na₂SO₄. The solvent was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc:MeOH (9:1) as an eluent to give a pale yellow powder (60 mg, 32%), which was used for the next reaction without further purification. mp 118—121 °C. ¹H NMR (CDCl₃) δ: 1.40—1.47 (54H, m), 2.87 (12H, s), 2.98—3.72 (50H, m),

3.83—3.85 (2H, m), 4.06 (4H, br s), 6.68—6.69 (2H, m), 6.76—6.78 (2H, m), 7.01—7.07 (4H, m), 7.15—7.17 (2H, m), 7.43—7.53 (4H, m), 8.14—8.21 (2H, m), 8.25—8.28 (2H, m), 8.49—8.52 (2H, m). HRMS (FAB) (m/z) Calcd for $C_{95}H_{143}N_{14}O_{20}S_2$: 1864.0043. Found 1864.0066. Anal. Calcd for $C_{95}H_{142}N_{14}O_{20}S_2$: C, 61.20; H, 7.68; N, 10.52. Found: C, 61.36; H, 7.95; N, 10.41.

4,4'-Bis[*N*-(*p*-toluenesulfonylaminoethyl)-*N*-(4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane-1-ylacetyl)-2-aminoethoxy]diphenylmethane (7b) A mixture of **4b** (136 mg, 0.2 mmol), **6** (212 mg, 0.4 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (88 mg, 0.46 mmol) and TEA (46 mg, 0.46 mmol) in CH_2Cl_2 (10 mL) was stirred at room temperature for 24 h under N_2 atmosphere. The reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with 2 N NaOH and dried over Na_2SO_4 . The solvent was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc:MeOH (9:1) and $CHCl_3$:MeOH (10:1) as an eluent to give **1b** (78 mg, 23%), which was used for the next reaction without further purification. mp 123—126 °C. 1H NMR ($CDCl_3$) δ : 1.43—1.46 (54H, m), 2.39 (3H, s), 2.40 (3H, s), 2.95—3.68 (48H, m), 3.83—3.84 (2H, m), 4.08—4.09 (4H, m), 6.73 (2H, dd, $J=8.64$, 3.1 Hz), 6.78 (2H, d, $J=8.58$ Hz), 7.04 (2H, d, $J=8.58$ Hz), 7.07 (2H, d, $J=8.64$ Hz), 7.25 (4H, d, $J=7.86$ Hz), 7.68 (2H, d, $J=8.22$ Hz). HRMS (FAB) (m/z) Calcd for $C_{85}H_{133}N_{12}O_{20}S_2$: 1705.9199. Found 1705.9210. Anal. Calcd for $C_{85}H_{132}N_{12}O_{20}S_2$: C, 59.83; H, 7.80; N, 9.85. Found: C, 59.98; H, 8.00; N, 9.63.

4,4'-Bis[*N*-(5-dimethylaminonaphthalenesulfonylaminoethyl)-*N*-(1,4,7,10-tetraazacyclododecane-1-ylacetyl)-2-aminoethoxy]diphenylmethane Octahydrochloride (1a, DNCn) A mixture of **7a** (56 mg, 0.03 mmol) and 36% HCl (0.3 mL) in THF (1 mL) was stirred at room temperature. After 24 h, the reaction mixture was concentrated under reduced pressure. Subsequently EtOAc (2 mL) was added to the residue, and the solid was collected by filtration. The hygroscopic solid was triturated with a mixture of THF and MeOH and concentrated under reduced pressure to give a white powder (46 mg, 100%). mp 246—248 °C. 1H NMR (D_2O) δ : 2.60—3.10 (32H, s), 3.13 (3H, s), 3.14 (3H, s), 3.15 (3H, s), 3.16 (3H, s), 3.22—3.26 (4H, m), 3.44—3.48 (6H, m), 3.53—3.56 (8H, m), 3.91—3.95 (4H, m), 6.59 (1H, d, $J=8.6$ Hz), 6.61 (1H, d, $J=8.9$ Hz), 6.64 (1H, d, $J=8.9$ Hz), 6.65 (1H, d, $J=8.9$ Hz), 6.97 (1H, d, $J=8.6$ Hz), 7.01 (1H, d, $J=8.6$ Hz), 7.04 (1H, d, $J=8.9$ Hz), 7.07 (1H, d, $J=8.9$ Hz), 7.53—7.59 (2H, m), 7.44—7.51 (2H, m), 7.68—7.71 (2H, m), 7.82—7.85 (2H, m), 8.13—8.19 (2H, m), 8.32—8.36 (2H, m). ^{13}C NMR (D_2O) δ : 42.2, 42.6, 44.2, 46.5, 46.9, 49.9, 50.0, 53.7, 54.6, 65.3, 114.5, 119.3, 119.4, 125.3, 126.7, 128.2, 129.6, 129.7, 129.8, 134.8, 135.0, 138.1, 155.6, 155.7, 173.4. HRMS (FAB) (m/z) Calcd for $C_{65}H_{95}N_{14}O_8S_2$: 1263.6898. Found 1263.6930. Anal. Calcd for $C_{65}H_{94}N_{14}O_8S_2 \cdot 8HCl$: C, 50.19; H, 6.61; N, 12.61. Found: C, 50.39; H, 6.37; N, 12.54.

4,4'-Bis[*N*-(*p*-toluenesulfonylaminoethyl)-*N*-(1,4,7,10-tetraazacyclododecane-1-ylacetyl)-2-amino-

ethoxy]diphenylmethane Octahydrochloride (1b, TsDCn) A mixture of **7b** (68 mg, 0.04 mmol) and 36% HCl (0.3 mL) in THF (1 mL) was stirred at room temperature. After 24 h, the reaction mixture was concentrated under reduced pressure. Subsequently EtOAc (2 mL) was added to the residue, and the solid was collected by filtration. The hygroscopic solid was triturated with a mixture of THF and MeOH and concentrated under reduced pressure to give a white powder (55 mg, 100%). mp 233—235 °C ^1H NMR (D_2O) δ : 2.11—2.13 (6H, m), 2.62—3.02 (36H, s), 3.23—3.28 (4H, m), 3.49—3.52 (8H, m), 3.70 (2H, t, $J=7.9$ Hz), 3.92—3.94 (2H, m), 3.98 (2H, t, $J=5.1$ Hz), 6.61—6.66 (4H, m), 7.01—7.09 (8H, m), 7.29 (4H, d, $J=8.24$ Hz). ^{13}C NMR (D_2O) δ : 20.6, 39.8, 41.7, 42.3, 42.6, 44.3, 47.1, 49.8, 54.6, 65.5, 114.4, 126.3, 129.7, 129.8, 134.3, 135.3, 144.1, 155.9, 173.1. HRMS (FAB) (m/z) Calcd for $\text{C}_{55}\text{H}_{85}\text{N}_{12}\text{O}_8\text{S}_2$: 1105.6054. Found 1105.6051. Anal. Calcd for $\text{C}_{55}\text{H}_{84}\text{N}_{12}\text{O}_8\text{S}_2 \cdot 8\text{HCl}$: C, 47.28; H, 6.64; N, 12.03. Found: C, 47.07; H, 6.89; N, 11.83.

Primary hippocampal cell culture. Hippocampi were dissected out from the brains of 19-day fetuses of Wistar rat and placed in Brooks-Logan solution (137 mM NaCl, 2.7 mM KCl, 6 mM Na_2HPO_4 , 1.7 mM KH_2PO_4 , 44 mM sucrose, 25 mM glucose, 10 mM HEPES, pH 7.4).⁹ Then, the hippocampal tissues were dissociated in Brooks-Logan solution containing 0.2% trypsin solution at 37 °C for 20 min. Subsequently, tissues were triturated by repeated passage through a constricted Pasteur pipette. Dispersed tissues were allowed to settle for 3 min, and the supernatant was transferred to a fresh tube and centrifuged at 1000 rpm for 1 min. The pellet was resuspended in a modified D-MEM containing 21 mM KCl, 10% fetal bovine serum, 100 U/mL penicillin, and 100 $\mu\text{g/mL}$ streptomycin. Viable cells were counted, and cells were then plated onto 24-well plate coated with polyethyleneimine (Sigma) at 1×10^5 per well. Cell cultures were kept in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. After 2 days, the medium was replaced with fresh medium containing 10% horse serum (Gibco) instead of fetal bovine serum and 3 μM cytosine arabinoside (Nacalai tesque). After every 2 days of culture, half of the medium was replaced with fresh medium containing 100 ng/mL nerve growth factor.

NMDA-induced toxicity in rat hippocampal neurons. After 7 days of culture, hippocampal neuronal cells were washed with a modified HBS (HEPES-buffered saline) buffer (146 mM NaCl, 21 mM KCl, 2 mM CaCl_2 , 10 mM D-glucose, 10 mM HEPES, pH 7.4), and exposed to 1 mM NMDA with 10 μM glycine at 37 °C for 1 h in the presence and absence of cleft-type cyclophane in a modified HBS buffer. Cells were washed with a modified HBS buffer, and cultured in a fetal bovine serum-free modified D-MEM medium. After 24 h, in order to quantify cell death, LDH released from damaged cells into the cell culture medium was estimated by measuring production of diformazane at A_{570} . Data were normalized to the activity of LDH released from vehicle-treated cells (100%) and expressed as percentage of the control.

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REFERENCES

1. S. K. Sonkusare, C. L. Kaul, and P. Ramarao, *Pharmacol. Res.*, 2005, **51**, 1.
2. R. Dingledine, K. Borges, D. Bowie, and S. F. Traynelis, *Pharmacol. Rev.*, 1999, **51**, 7.
3. T. Masuko, Y. Nemoto, H. Nagaoka, M. Miyake, Y. Kizawa, K. Kusama-Eguchi, K. Kashiwagi, and T. Kusama, *Neuropharmacology*, 2007, **53**, 515.
4. T. Masuko, K. Metori, Y. Kizawa, T. Kusama, and M. Miyake, *Chem. Pharm. Bull.*, 2005, **53**, 444.
5. E. L. Doyle, C. A. Hunter, H. C. Phillips, S. J. Webb, and N. H. Williams, *J. Am. Chem. Soc.*, 2003, **125**, 4593.
6. W. J. Joong, J. S. Sang, E. Y. Chang, S. H. In, B. S. Jung, and S. Junghun, *Org. Lett.*, 2002, **4**, 4155.
7. M. T. Barros and F. Siñeriz, *Tetrahedron*, 2000, **56**, 4759.
8. G. A. Wayman, S. Impey, D. Mark, T. Saneyoshi, W. F. Grant, V. Derkach, and T. R. Soderling, *Neuron*, 2006, **50**, 897.
9. L. V. Dawson, M. T. Dawson, D. E. London, S. D. Brecht, and H. S. Snyder, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 6368.