# Design and Synthesis of a Novel Water-Soluble NMDA Receptor Antagonist with a 1,4,7,10-Tetraazacyclododecane Group

Takashi MASUKO, Koichi METORI, Yasuo KIZAWA, Tadashi KUSAMA, and Muneharu MIYAKE*

*College of Pharmacy, Nihon University; Narashinodai, Funabashi, Chiba 274–8555, Japan.*
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**Polyamines, especially spermine, inhibit *N*-methyl-D-aspartate (NMDA) receptors as open channel blockers. Two types of water-soluble NMDA receptor antagonist, ACCn (1) and TGCn (2), with a 1,4,7,10-tetraazacyclododecane cyclic polyamine group, were synthesized and the effects of both compounds on NMDA receptors were studied using voltage-clamp recordings of recombinant NMDA receptors expressed in *Xenopus* oocytes. These compounds inhibited macroscopic currents in both NR1/NR2A and NR1/NR2B receptor subtypes in oocytes voltage-clamped at −70 mV. Inhibition by the compounds of NR1/NR2A receptors were more prominent than that of NR1/NR2B receptors. The inhibitory effects of ACCn (1) on both NMDA receptors were more potent than those of TGCn (2).**

**Key words** *N*-methyl-D-aspartate (NMDA) receptor; 1,4,7,10-tetraazacyclododecane; *Xenopus* oocytes

Glutamate receptors are classified into two major groups termed ionotropic and metabotropic glutamate receptors. The ionotropic receptors can be subdivided into three distinct types of receptors, the receptors for *N*-methyl-D-aspartate (NMDA), $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate, all of which contain glutamate-gated, cation-specific ion channels. Among ionotropic receptors, the NMDA receptor subtype has been found to play a key role in glutamate effects promoting synaptic plasticity, long-term potentiation, and neuronal cell death.[1] The NMDA receptor combines to form heteromeric complexes containing NR1 and NR2 subunits. The NR1 subunit is ubiquitous and assembles with a second family of subunits termed NR2, including NR2A, NR2B, NR2C and NR2D.

Overactivation of these receptors can lead to neuronal cell death, and the receptors also play a role in seizure activity. Thus NMDA receptors are potential targets for neuroprotective agents and anticonvulsants.[2,3] NMDA receptors have a complex pharmacology and are targets for antagonists acting at the glutamate and glycine coagonist sites, at a large number of modulatory sites, and at sites within the ion channel of the receptor. A number of organic polycations, including the endogenous polyamines spermine and spermidine, are antagonists at native and recombinant NMDA receptors.[4] In addition, a number of polyamine-conjugated spider and wasp toxins are more potent antagonists than spermine at glutamate receptors.[5] These toxins, which include the philanthotoxins, argiotoxins, and $\alpha$-agatoxins, are characterized structurally by the presence of an aromatic amino acid head group linked through a carbonamide bond to a polyamine tail such as spermine or a pentamine or hexamine.

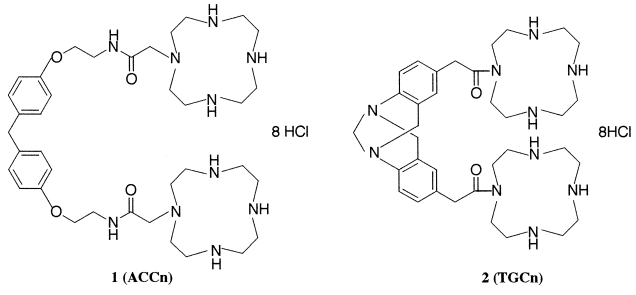
In this paper, we report the syntheses of two novel water-soluble compounds ACCn (**1**) and TGCn (**2**) with two 1,4,7,10-tetraazacyclododecane groups to determine whether these compounds have a role as NMDA receptor antagonists (Fig. 1).

## Results and Discussion

The host ACCn (**1**) was synthesized as shown in Chart 1. Treatment of 4,4′-dihydroxydiphenylmethane (**3**) with methyl bromoacetate in *N*,*N*-dimethylformamide (DMF) in the presence of $K_2CO_3$ furnished **4** in 93% yield, and then hydrolysis of the methyl ester **4** with 5 N KOH in methanol provided dicarboxylic acid (**5**, 100%), which underwent smooth esterification upon treatment with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and pentafluorophenol to give the pentafluorophenyl ester **6** in 92% yield. The pentafluorophenyl ester function in compound (**6**) was converted into carboxamide (**7**, 98%) after treatment of pentafluorophenyl ester with 25% $NH_4OH$ in THF, followed sequentially by reduction with $BH_3$ to the corresponding primary amine (**8**, 85%), which was converted into **10** by treatment with 1-ethyl-3-(3-dimethylaminopropyl)cabodiimide hydrochloride (EDC) and 1-carboxymethyl-4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (**9**).[6] Finally, deprotection of **10** with concentrated HCl in THF resulted in the desired compound ACCn (**1**) in 91% yield.

On the other hand, TGCn (**2**) was synthesized as follows (Chart 2). Treatment of pentafluorophenyl ester (**11**)[7] with 4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane (**12**)[8] in the presence of triethylamine (TEA) gave **13** in 96% yield, and removal of the *tert*-butoxycarbonyl groups of **13** by treatment with concentrated HCl in THF synthesized the desired compound TGCn (**2**) in quantitative yield.

The effects of ACCn (**1**) and TGCn (**2**) on NMDA receptors were studied using voltage-clamp recordings of recombinant NMDA receptors expressed in *Xenopus* oocytes. We measured the effects of ACCn (**1**) and TGCn (**2**) (10 $\mu$M) on responses to glutamate (10 $\mu$M, with 10 $\mu$M glycine) or GABA 10 $\mu$M at NR1/NR2A, NR1/NR2B, or GABAc ($\rho$-1)



1 (ACCn) 2 (TGCn)

Fig. 1. Structures of NMDA Receptor Antagonists

* To whom correspondence should be addressed. e-mail: miyake@pha.nihon-u.ac.jp

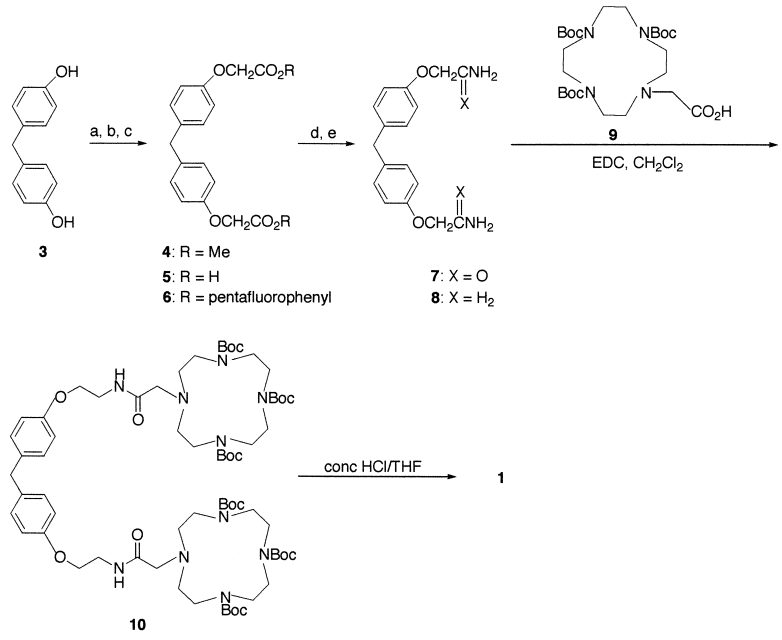



Chart 1

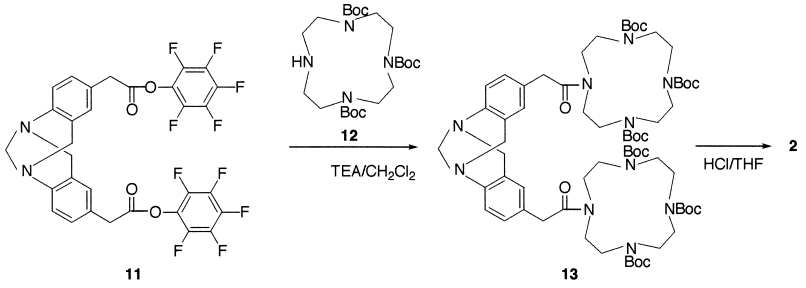



Chart 2

receptors in oocytes voltage-clamped at −70 mV. Both ACCn (**1**) and TGCn (**2**) inhibited macroscopic currents at both NMDA receptor subtypes (Fig. 2), but not at GABAc ($\rho$-1) receptors (data not shown). These results indicate that the compounds specifically block NMDA receptors. The inhibition by these compounds of NR1/NR2A receptors were more potent than those of NR1/NR2B receptors. An NMDA glutamate receptor subtype is thought to play a predominant role in triggering glutamate neurotoxicity in central nervous system, it is possible that ACCn (**1**), TGCn (**2**) or these derivatives have neuroprotective effects to glutamate neurotoxicity.

## Experimental

Melting points were determined using the Yanagimoto melting point apparatus Yanaco MP and are uncorrected. $^1$H-NMR spectra were recorded on a JEOL JNM-LA400 spectrometer containing tetramethylsilane as the standard. Mass spectra (MS) were measured on a JEOL JMS-GC mate instrument. Adult female *Xenopus laevis* were chilled on ice, and the preparation and maintenance of oocytes were carried out as described previously.[9,10] Capped cRNAs were prepared from linearized cDNA templates using mMessage mMachine kits (Ambion, Austin, TX, U.S.A.). Oocytes were injected with NR1A and NR2 cRNAs at a ratio of 1 : 5 (0.2—4 ng of NR1A plus 1—20 ng of NR2). Macroscopic currents were recorded with a two-electrode voltage clamp using a Dual Electrode Voltage Clamp Amplifier CEZ-1250 (Nihon Koden, Tokyo, Japan). Electrodes were filled with KCl 3 M. Oocytes were continuously superfused (*ca.* 5 ml/min) with $Mg^{2+}$-free saline solution (NaCl 96 mM, KCl 2 mM, $BaCl_2$ 1.8 mM, HEPES 10 mM, pH 7.5). This solution contained $BaCl_2$ rather than $CaCl_2$, and, in most experiments, oocytes were injected with $K^+$-1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA; 100 nl of 40 mM solution at pH 7.5) on the day of recording to eliminate $Ca^{2+}$-activated $Cl^-$ currents.[2,3] Glutamate and glycine were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). BAPTA was purchased from Sigma (St. Louis, MO, U.S.A.).

**4,4′-Bis(methoxycarbonylmethoxy)diphenylmethane (4)** A mixture of 4,4′-dihydroxydiphenylmethane (**3**) (1.0 g, 5 mmol), methyl bromoacetate (1.53 g, 10 mmol), and $K_2CO_3$ (1.38 g, 10 mmol) in DMF (20 ml) was stirred at room temperature. After 24 h, the reaction mixture was filtered. The filtrate was extracted with EtOAc (50 ml×3), washed with brine, and dried over $MgSO_4$. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel with EtOAc : $CHCl_3$ (1 : 9) as an eluent to give **4** (1.6 g, 93%) as a colorless solid. An analytical sample was obtained by recrystallizing this material from EtOAc–hexane, yielding colorless needles, mp 51—52 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 3.80 (6H, s); 3.85 (2H, s); 4.60 (4H, s); 6.82 (4H, d, $J$=8.8 Hz); 7.08 (4H, d, $J$=8.8 Hz). EI-MS *m*/*z*: 344 $[M]^+$. HR-EI-MS *m*/*z*: 344.1256 $[M]^+$ (Calcd for $C_{19}H_{20}O_6$: 344.1259). *Anal.* Calcd for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85. Found: C, 66.54; H, 5.91.

**4,4′-Bis(carboxymethoxy)diphenylmethane (5)** A mixture of **4** (1.19 g, 3.46 mmol) and 5 N KOH/MeOH (4 ml) in MeOH (40 ml) was refluxed for 2 h. After removal of the solvent under reduced pressure, the residue was dissolved in 100 ml of $H_2O$. The solution was extracted with EtOAc (100 ml). The aqueous solution was acidified to pH 1 with 10% HCl

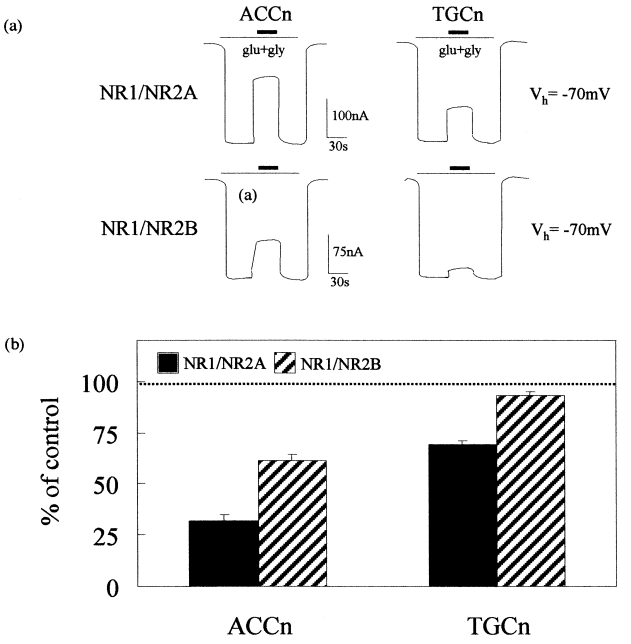



Fig. 2. Effects of ACCn and TGCn on NMDA Receptors at −70 mV

(a) Representative traces showing blocking by ACCn and TGCn 10 $\mu$M at NR1/NR2A and NR1/NR2B receptors at −70 mV. (b) Effects of ACCn and TGCn 10 $\mu$M were determined at NMDA receptors at −70 mV. Data are shown as percentage of control measured in the absence of the compounds. Values are mean±S.E.M. from four oocytes.

and extracted with EtOAc (300 ml). The EtOAc layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give **5** as a colorless powder (1.09 g, 100%). An analytical sample was obtained by recrystallizing this material from EtOAc–hexane, yielding colorless needles, mp 199—200 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.79 (2H, s); 4.59 (4H, s); 6.80 (4H, d, $J$=8.8 Hz); 7.10 (4H, d, $J$=8.8 Hz); 12.90 (2H, s). EI-MS *m/z*: 316 $[M]^+$. HR-EI-MS *m/z*: 316.0944 $[M]^+$ (Calcd for $C_{17}H_{16}O_6$: 316.0946). *Anal.* Calcd for $C_{17}H_{16}O_6$: C, 64.55; H, 5.10. Found: C, 64.60; H, 5.11.

**4,4′-Bis(pentafluorophenoxycarbonylmethoxy)diphenylmethane (6)** A mixture of **5** (2.95 g, 9.3 mmol), pentafluorophenol (3.46 g, 18.8 mmol) and DCC (3.88 g, 18.8 mmol) in THF (100 ml) was stirred at room temperature for 24 h. The mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with $CH_2Cl_2$ as an eluent to give **6** (5.56 g, 92%) as a colorless solid. An analytical sample was obtained by recrystallizing this material from EtOAc–hexane, yielding colorless needles, mp 135—136 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 3.90 (2H, s); 4.97 (4H, s); 6.89 (4H, d, $J$=8.4 Hz); 7.13 (4H, d, $J$=8.4 Hz). FAB-MS *m/z*: 648 $[M]^+$. HR-FAB-MS *m/z*: 648.0628 $[M]^+$ (Calcd for $C_{29}H_{14}F_{10}O_6$: 648.0630). *Anal.* Calcd for $C_{29}H_{14}F_{10}O_6$: C, 53.72; H, 2.18. Found: C, 53.73; H, 2.14.

**4,4′-Bis(carbamoylmethoxy)diphenylmethane (7)** To a solution of **6** (4.0 g, 6.17 mmol) in THF (30 ml) was added 25% $NH_4OH$ (12 ml) at room temperature. After stirring for 12 h, saturated $NaHCO_3$ (200 ml) was added to the reaction mixture. The precipitate was collected by filtration, washed with $H_2O$, EtOH, and $Et_2O$, and dried under a vacuum at 60 °C to give **7** (1.9 g, 98%) as a colorless powder which was used in the next step without further purification, mp 233—234 °C. $^1$H-NMR (DMSO-$d_6$) $\delta$: 3.80 (2H, s); 4.36 (4H, s); 6.85 (4H, d, $J$=8.8 Hz); 7.11 (4H, d, $J$=8.8 Hz); 7.32 (2H, s); 7.43 (2H, s). FAB-MS *m/z*: 315 $[M+H]^+$. HR-FAB-MS *m/z*: 315.1346 $[M+H]^+$ (Calcd for $C_{17}H_{19}N_2O_4$: 315.1344). *Anal.* Calcd for $C_{17}H_{18}N_2O_4$: C, 64.96; H, 5.77; N, 8.91. Found: C, 64.74; H, 5.67; N, 8.69.

**4,4′-Bis(2-aminoethoxy)diphenylmethane (8)** A mixture of **7** (314 mg, 1 mmol) and $BH_3$·DMS (1.16 ml, 12 mmol) in THF (12 ml) was stirred for 24 h at 80 °C under a $N_2$ atmosphere, then was cooled to room temperature. Six milliliters of 0.7 M hydrogen chloride–MeOH solution was added, and the mixture was refluxed for 0.5 h and evaporated under reduced pressure. The residue was basified with 25% $NH_4OH$. The mixture was extracted with $CH_2Cl_2$, washed with brine, and dried over $Na_2SO_4$. Removal of the solvent under reduced pressure afforded a pale yellow oil, which was purified by column chromatography on silica gel with $CHCl_3$ : MeOH : 25% $NH_4OH$ (100 : 40 : 4) as an eluent to give **8** (243 mg, 85%) as a colorless amorphous powder that was used in the next step without further purification. $^1$H-NMR ($CD_3OD$) $\delta$: 2.99 (4H, t, $J$=5.6 Hz); 3.82 (2H, s); 3.98 (4H, t, $J$=5.6 Hz); 6.84 (4H, d, $J$=8.4 Hz); 7.07 (4H, d, $J$=8.4 Hz). FAB-MS *m/z*: 287 $[M+H]^+$. HR-FAB-MS *m/z*: 287.1757 $[M+H]^+$ (Calcd for $C_{17}H_{23}N_2O_2$: 287.1759). *Anal.* Calcd for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.51; H, 7.78; N, 9.52.

**4,4′-Bis{2-[4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane-1-yl]acethylaminoethoxy}diphenylmethane (10)** A mixture of **8** (123 mg, 0.43 mmol), **9** (456 mg, 0.86 mmol) and EDC (197 mg, 1.03 mmol) in $CH_2Cl_2$ (10 ml) was stirred at room temperature for 24 h. The reaction mixture was diluted with $CH_2Cl_2$ (10 ml), washed with 2 N NaOH, and dried over $Na_2SO_4$. The solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel with $CHCl_3$ : MeOH : 25% $NH_4OH$ (100 : 10 : 1) as an eluent to afford **10** (314 mg, 56%) as a white powder that was used in the next step without further purification, mp 118—120 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 143 (36H, s); 1.46 (18H, s); 2.70 (4H, br); 3.19 (4H, s); 3.35—3.52 (28H, m); 3.58—3.62 (4H, m); 3.83 (2H, s); 4.00 (4H, t, $J$=5.6 Hz); 6.81 (4H, d, $J$=8.5 Hz); 7.05 (4H, d, $J$=8.5 Hz). FAB-MS *m/z*: 1311 $[M+H]^+$. HR-FAB-MS *m/z*: 1311.8190 $[M+H]^+$ (Calcd for $C_{67}H_{111}N_{10}O_{16}$: 1311.8179). *Anal.* Calcd for $C_{67}H_{110}N_{10}O_{16}$: C, 61.35; H, 7.74; N, 8.45. Found: C, 61.20; H, 8.57; N, 10.46.

**4,4′-Bis[2-(1,4,7,10-tetraazacyclododecane-1-yl)acethylaminoethoxy]-diphenylmethane Octahydrochloride (1, ACCn) 10** (255 mg, 0.19 mmol) was dissolved in THF (5 ml), to which concentrated HCl (0.5 ml) was added. The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with THF (5 ml) and the precipitate was collected by filtration, washed with THF, and dried to give **1** (ACCn) (173 mg, 91%) as a white powder, mp 250 °C (decomp). $^1$H-NMR ($D_2O$) $\delta$: 2.74—2.89 (32H, m); 3.17 (4H, s); 3.37 (4H, t, $J$=5.4 Hz); 3.65 (2H, s); 3.94 (4H, t, $J$=5.4 Hz); 6.73 (4H, d, $J$=8.6 Hz); 7.03 (4H, d, $J$=8.6 Hz). FAB-MS *m/z*: 711 $[M-8HCl+H]^+$. HR-FAB-MS *m/z*: 711.5029 $[M-8HCl+H]^+$ (Calcd for $C_{37}H_{63}N_{10}O_4$: 711.5033). *Anal.* Calcd for $C_{37}H_{62}N_{10}O_4$·8HCl: C, 44.32; H, 7.04; N, 13.97. Found: C, 44.19; H, 7.27; N, 13.92.

**2,8-Bis{[4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecane-1-yl]oxomethyl}-6*H*,12*H*-5,11-mefhanodibenzo[b,f][1,5]diazocine (13)** A mixture of **11** (67 mg, 0.1 mmol), **12** (95 mg, 0.2 mmol), and TEA (84 $\mu$l, 0.6 mmol) in $CH_2Cl_2$ (3 ml) was stirred at 60 °C for 24 h under a $N_2$ atmosphere. The mixture was evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc and EtOAc : MeOH (9 : 1) to give **13** (120 mg, 96%) as a white powder that was used in the next step without further purification, mp 136—138 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.41 (18H, s); 1.45 (18H, s); 1.47 (18H, s); 3.51—3.37 (36H, m); 4.12 (2H, d, $J$=16.8 Hz); 4.25 (2H, s); 4.66 (2H, d, $J$=16.8 Hz); 6.86 (2H, s); 7.06 (4H, s). FAB-MS *m/z*: 1247 $[M+H]^+$. HR-FAB-MS *m/z*: 1247.7654 $[M+H]^+$ (Calcd for $C_{65}H_{103}N_{10}O_{14}$: 1247.7656). *Anal.* Calcd for $C_{65}H_{102}N_{10}O_{14}$: C, 62.58; H, 8.24; N, 11.23. Found: C, 62.57; H, 8.06; N, 11.39.

**2,8-Bis[(1,4,7,10-tetraazacyclododecane-1-yl)oxomethyl]-6*H*,12*H*-5,11-mefhanodibenzo[b,f][1,5]diazocine Octahydrochloride (2, TGCn)** A mixture of **13** (80 mg, 0.064 mmol) and concentrated HCl (0.5 ml) in THF (1 ml) was stirred at room temperature. After 36 h, the resulting precipitate was collected by filtration, washed successively with THF and $Et_2O$, and dried to give (**2**, TGCn) (60 mg, 100%) as a white solid, mp >290 °C. $^1$H-NMR ($D_2O$) $\delta$: 2.64—2.89 (24H, m); 3.35—3.37 (4H, m); 3.43—3.46 (4H, m); 3.54 (4H, s); 4.00 (2H, d, $J$=16.8 Hz); 4.12 (2H, s); 4.45 (2H, d, $J$=16.8 Hz); 6.69 (2H, d, $J$=1.44 Hz); 6.91 (2H, dd, $J$=8.28, 1.96 Hz); 6.98 (2H, d, $J$=8.44 Hz). FAB-MS *m/z*: 647 $[M-8HCl+H]^+$. HR-FAB-MS *m/z*: 647.4509 $[M-8HCl+H]^+$ (Calcd for $C_{37}H_{63}N_{10}O_4$: 647.4509). *Anal.* Calcd for $C_{35}H_{54}N_{10}O_2$·8HCl: C, 44.79; H, 6.66; N, 14.92. Found: C, 44.82; H, 6.75; N, 14.76.

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