Synthesis of a Novel Water-Soluble NMDA Receptor Antagonist

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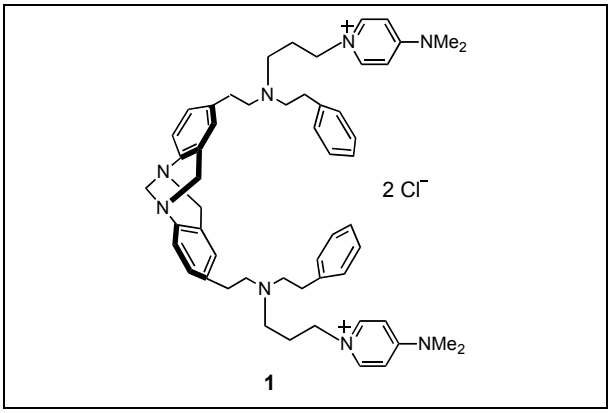

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A novel water-soluble *N*-methyl-D-aspartate (NMDA) receptor antagonist ATGDMAP **1** as synthesized and the effect of **1** on NMDA receptors was studied using voltage-clamp recordings of recombinant NMDA receptors expressed in *Xenopus* oocytes. The compound **1** inhibited macroscopic currents in NR1/NR2A, NR1/NR2B, NR1/NR2C and NR1/NR2D receptor subtypes in oocytes voltage-clamped at -70 mV. Mutant NR1/NR2B receptors containing NR1(T648A) or NR1(T648S) had very large holding currents when voltage-clamped at -70 mV compared to that of wild-type NMDA receptors, because these mutants generate constitutively open channels. ATGDMAP **1** and Mg^{2+}, a channel blocker of the NMDA receptor, reduced the large holding currents needed for mutant NMDA receptors. These data indicate that ATGDMAP **1** directly acts on the channel pores of the NMDA receptor.

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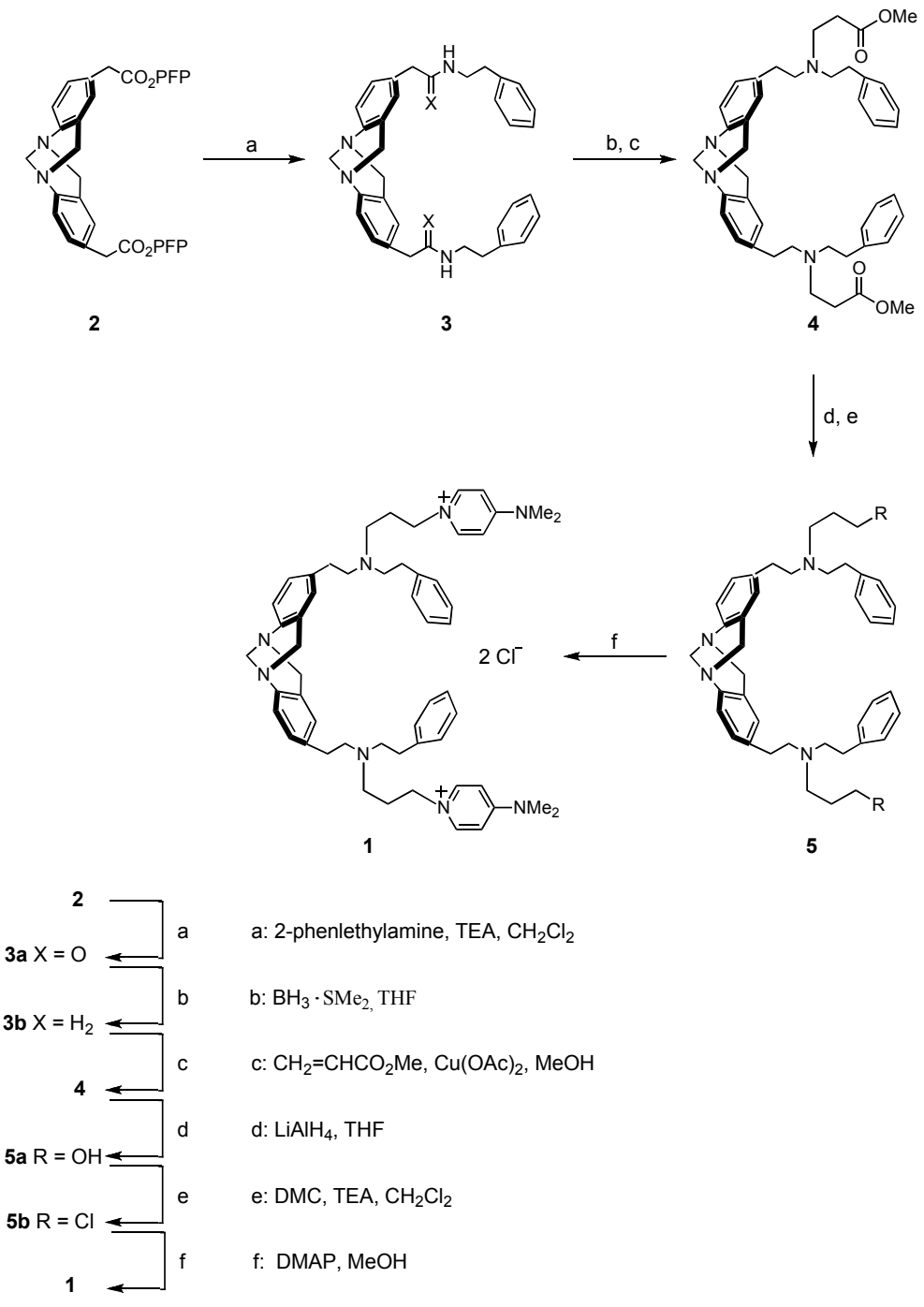
INTRODUCTION

The ionotropic glutamate receptors are ligand-gated ion channels that mediate the vast majority of excitatory neurotransmission in the brain. The three pharmacologically defined classes of ionotropic glutamate receptor were originally named after the reasonably selective agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), and kainite. It turned out that NMDA, AMPA, and kainite receptor subunits are encoded by at least six gene families as defined by sequence homology: a single family for AMPA receptors, two for kainite, and three for NMDA [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.

In the central nervous system (CNS), the NMDA receptor plays a critically important role in a variety of neurophysiological phenomena, including neurodevelopment, 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 (ischemic stroke, Parkinson's disease, Alzheimer's disease, dementia *etc*) 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 performance of daily activities, behavior, speech and visual-spatial perception. Glutamate excitotoxicity as a result of blockade of glutamate uptake into astrocytes by Aβ aggregates induces excessive Ca influx through mainly the NMDA receptors, followed by neuronal cell death [2]. An NMDA receptor subtype has been found to play a key role in glutamate promotion of synaptic plasticity, long-term potentiation and neuronal cell death [1].

Recently, we reported that a half cyclophane 4,4'-bis[2-(1,4,7,10-tetraaza-cyclododecane-1-yl)acetylaminoethoxy]-diphenylmethane (ACCn) [3] and a cyclophane *N*,*N*'-bis(1,4,7,10-tetraazacyclododecane-1-ylacetyl)-10,26-diaza-7,13,23,29-tetraoxal[7.1.7.1]-paracyclophane (CPCn) [4] inhibited the activity of NR1/NR2A and NR1/NR2B receptors at -70 mV. The IC_{50} value of ATGDMAP **1** was determined to be 4.9 ± 0.5 μM. from the inhibitory curves for the NR1/NR2A receptors [4].

Scheme 1



Here we report the synthesis of a novel water-soluble half cyclophane, ATGDMAP **1** with two pyridinium groups and the ability to act as an NMDA receptor antagonist.

RESULTS AND DISCUSSION

The half cyclophane ATGDMAP **1** was synthesized as shown in Scheme 1.

The pentafluorophenyl ester [5] **2** was converted to carboxamide (**3a**, 96%) by treatment of pentafluorophenyl ester with 2-phenethylamine in CH_2Cl_2, followed sequentially by reduction with BH_3SMe_2 to the corresponding primary amine (**3b**, 96%). A Michael addition between **3b** and methyl acrylate took place smoothly in the presence of $Cu(OAc)_2$ as catalyst in MeOH at 100°C and gave the corresponding methyl ester

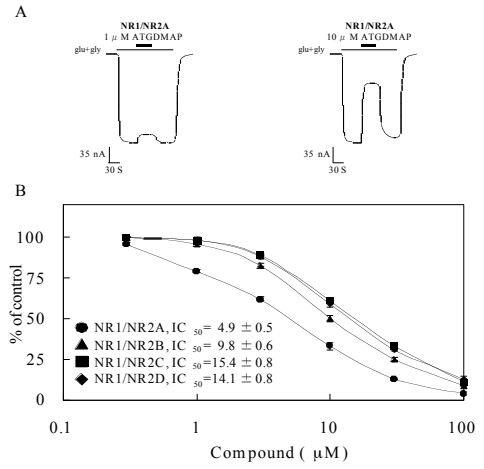


Figure 1. Inhibitory curves of ATGDMAP **1** against NMDA receptors at -70 mV. (a) Representative traces showing block by **1** and 10 μM ATGDMAP **1** at NR1/NR2A receptors at -70 mV. (b) Concentration -inhibition curves for ATGDMAP were determined at NR1 / NR2A and N R1 / NR2B receptors, voltage -clamped at -70 mV. Responces to 10 μM glutamate with 10 μM glycine measured in the presence of ATGDMAP **1** are expressed as a percentage of the control response at each receptor type. Data represent mean ± S.E.M. from four oocytes. Inhibitory curve of ATGDMAP **1** at the NR1 / NR2A receptor was quoted from a previous report [4].

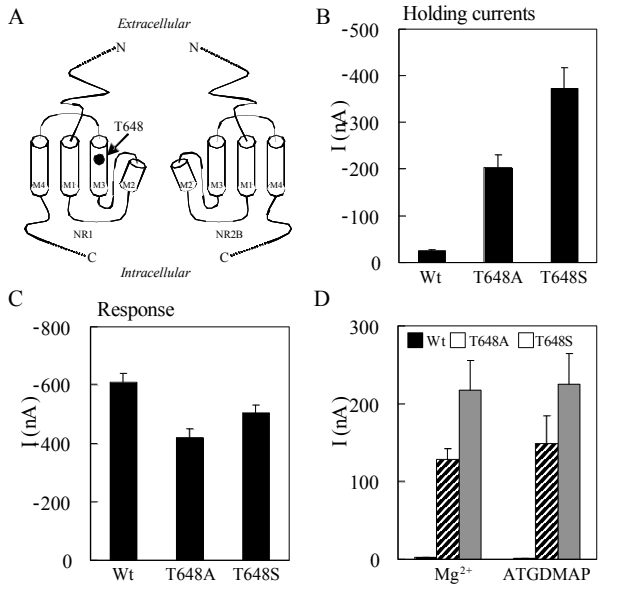


Figure 2. Holding currents (in oocytes voltage-clamped at -70 mV), and effects of Mg^{2+} and ATGDMAP **1** on holding currents in NR1 / NR2B receptors containing wild-type and mutant subunits. (a) Schematic showing the positions of NR1 T648 in NMDA receptors. (b) Holding currents (in oocytes voltage-clamped at -70 mV) in NR1 /NR2B receptors containing wild-type and mutant subunits. (c) Wild-type and mutant NMDA receptor-mediated inward currents in the presence of 10 μM glutamate with 10 μM glycine. (d) The effects of 100 μM Mg^{2+} and 30 μM ATGDMAP **1** on holding currents in NR1 / NR2B receptors containing wild-type and mutant subunits.

4 in 86% yield. The methoxycarbonyl function then underwent reduction with $LiAlH_4$ in THF to give a primary alcohol **5a** in 100% yield, followed sequentially by chlorination with 2-chloro-1,3-dimethylimidazolinium chloride (DMC) to (**5b**, 94%). Finally, treatment of **5b** with 4-dimethylaminopyridine (DMAP) in MeOH resulted in the formation of ATGDMAP **1** in 98% yield. The structure of ATGDMAP **1** was confirmed by ^{1}H- nmr and MS spectra, and elemental analysis [6].

The effects of ATGDMAP **1** on NMDA receptors were examined using voltage-clamp recording of recombinant NMDA receptors expressed in *Xenopus* oocytes. We studied dose-dependency of the inhibition by ATGDMAP **1** of the NMDA receptors at -70 mV (Fig. 1). The IC_{50} values of ATGDMAP (**1**) for NR1 / NR2A, NR1 / NR2B, NR1/NR2C and NR1/NR2D receptors were 4.9 ± 0.5, 9.8 ± 0.6, 15.4 ± 0.8 and 14.1 ± 0.8 μM, respectively. We previously reported that ATGDMAP **1** acts on the channel pores of NMDA receptor as an open channel blocker [4]. We then demonstrated that ATGDMAP**1** directly interacts on the channel pores of NMDA receptors using mutations in the NR1 subunits, NR1(T648A) and NR1(T648S) (Fig. 2A) [7]. Oocytes expressing NR1 / NR2B receptors with a T-to-A or T-to-S mutation at T648 (located in M3) had very large holding currents when voltage-clamped at -70 mV compared to that of wild-type NMDA receptors (Figs. 2A, B). The mutant NMDA receptors containing NR1 T648A or T648S formed functional receptors, because application of glutamate and glycine to these mutants induced inward currents (Fig. 2C). To confirm that these mutations generate constitutively open channels, the effects of the channel blocker Mg^{2+} on mutant NMDA receptors containing NR1(T648A) or NR1(T648S) were measured in the absence of agonist, glutamate and glycine. Application of Mg^{2+} reduced the large holding currents in both mutant NMDA receptors, when voltage-clamped at -70 mV (Fig. 2D). ATGDMAP **1** also reduced the holding currents, similar to Mg^{2+}. These results indicate that ATGDMAP **1** directly acts on the channel pores of NMDA receptors. Because an NMDA receptor subtype is thought to play a predominant role in triggering glutamate neurotoxicity in the CNS, ATGDMAP **1** or its derivatives appear to be neuroprotective against glutamate neurotoxicity.

In conclusion, we have synthesized a compound ATGDMAP **1** that has 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 the Yanagimoto Melting point Apparatus Yanaco MP and were uncorrected. ^{1}H-

NMR was recorded on a JEOL JNM-GSX 400 spectrometer containing tetramethylsilane as the standard. Mass spectra were taken on a JEOL JMS-GCmate instrument. Adult female *Xenopus laevis* were chilled on ice, and the preparation and maintenance of oocytes performed as described previously [8,9]. Capped cRNAs were prepared from linearized cDNA templates using mMessage mMachine kits (Ambion, Austin, TX). 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 the Dual Electrode Voltage Clamp Amplifier CEZ-1250 (Nihon Koden, Tokyo, Japan). Electrodes were filled with 3M 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 HEPES, 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 [8,9]. Glutamate and glycine were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). BAPTA were purchased from Sigma (St. Louis, MO).

2,8-Bis(phenethylcarbonylmethyl)-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]-diazocine (3a). A mixture of 6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine-2,8-diylaceticacid pentafluorophenyl ester **2** (670 mg, 1 mmol), 2-phenethylamine (242 mg, 2 mmol) and TEA (0.28 mL, 2 mmol) in CH_2Cl_2 (40 ml) was stirred at room temperature. After 15 h, the reaction mixture was diluted with CH_2Cl_2 (150 ml). The organic layer was washed with H_2O and dried over $MgSO_4$, and evaporated under reduced pressure to afford a white solid, which was chromatographed on a silica gel column with $CHCl_3$:MeOH (10:1) as the eluent to give a white solid. This white solid was then washed with EtOAc to give a white powder (0.52 g, 96%). An analytical sample was obtained by recrystallizing this material from $CHCl_3$-hexane, cottony colorless needles (mp 243—244 °C). ^{1}H nmr ($CDCl_3$) δ : 2.71 (4H, t, J =6.8 Hz), 3.38 (s, 4H), 3.42 (4H, q, J =6.8 Hz), 4.11 (2H, d, J =16.8 Hz), 4.28 (2H, s), 4.64 (2H, d, J =16.8 Hz), 5.35 (2H, br s), 6.72 (2H, s), 6.97 (2H, dd, J =6.8 Hz, 1.7 Hz), 7.02—7.04 (4H, m), 7.08 (2H, d, J =8.1 Hz), 7.19—7.23 (6H, m). HR-ms (FAB) *m*/*z*: 545.2916 (Calcd for $C_{35}H_{37}N_4O_2$: 545.2916). *Anal*: Calcd for $C_{35}H_{36}N_4O_2$: C, 77.18; H, 6.66; N, 10.29. Found: C, 76.98; H, 6.63; N, 10.23.

***N*,*N*'-Bis(phenethyl)-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]-diazocine-2,8-diethanamine (3b)**. A mixture of **3a** (200 mg, 0.37 mmol) in THF (10 ml) was stirred at room temperature under N_2 atmosphere. BH_3·SMe_3 (0.44 mL, 4.4 mmol) was added and the reaction mixture then stirred for 24 h at 80 °C and cooled to room temperature. Hydrogen chloride-MeOH (0.7M) solution (2.2 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 using an excess of 25% NH_4OH. The mixture was then extracted with CH_2Cl_2 (10 ml ×3). The combined organic phases were washed with brine and dried over Na_2SO_4. Removal of solvent under reduced pressure afforded a yellow oil, which was chromatographed on a silica gel column with $CHCl_3$:MeOH (10:1) and $CHCl_3$:MeOH:25% NH_4OH (100:20:2) as the eluent to give a viscous oil (182 mg, 96%), which was used for the next reaction without further purification. ^{1}H-nmr ($CDCl_3$) δ : 2.65 (4H, t, J =7.1 Hz), 2.75-2.88 (12H, m), 4.09 (4H, d, J =16.8 Hz), 4.27 (2H, s), 4.63 (2H, d, J =16.8 Hz), 6.69 (2H, d, J =1.7 Hz), 6.95 (2H, dd, J =8.0, 1.7 Hz), 7.13—7.19 (6H, m), 7.23—7.26 (4H, m). HR-ms (FAB) *m*/*z*: 517.3331 (Calcd for $C_{35}H_{41}N_4$: 517.3331).

***N*,*N*'-(6*H*,12*H*-5,11-Methanodibenzo[*b*,*f*][1,5]diazocine-2,8-diyldi-2,1-ethanediyl)-bis[*N*-(2-methoxycarbonylethyl)phenethylamine] (4)**. A mixture of **3b** (200 mg, 0.38 mmol), methyl acrylate (196 mg, 2.28 mmol) and $Cu(OAc)_2$·H_2O (8 mg, 0.04 mmol) in MeOH (5 ml) was stirred for 24 h at 100 °C under N_2 atmosphere. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$:MeOH (10:1) and EtOAc:MeOH (9:1) as the eluent to give 230 mg, 88% as a pale yellow oil, which was used for the next reaction without further purification. ^{1}H nmr ($CDCl_3$) δ : 2.44 (4H, t, J =7.1 Hz), 2.59-2.72 (16H, m), 2.89 (4H, t, J =7.1 Hz), 3.64 (6H, s), 4.11 (2H, d, J =16.8 Hz), 4.29 (2H, s), 4.65 (2H, d, J =16.8 Hz), 6.69 (2H, d, J =1.7 Hz), 6.96 (2H, dd, J =8.0, 1.7 Hz), 7.04 (2H, d, J =8.0 Hz), 7.13—7.20 (6H, m), 7.24—7.28 (4H, m). HR-ms (FAB) *m*/*z*: 689.4074 (Calcd for $C_{43}H_{53}N_4O_4$: 689.4066).

***N*,*N*'-(6*H*,12*H*-5,11-Methanodibenzo[*b*,*f*][1,5]diazocine-2,8-diyldi-2,1-ethanediyl)-bis[*N*-(3-hydroxyxycarbonylethyl)-phenethylamine] (5a)**. Compound **4** (170 mg) in THF (5 ml) was added dropwise to a stirred suspension of $LiAlH_4$ (57 mg, 1.5 mmol) in THF (5 ml) at room temperature. After 12 h, H_2O (57 ml), 15% NaOH (57 ml), and H_2O (171 ml) was added slowly in that order under stirring at room temperature, and then K_2CO_3 was added and stirring was continued for 0.5 h at room temperature. The mixture was filtered and the filtrate then evaporated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$:MeOH (10:1) as the eluent to give 158 mg, 100% as a pale yellow oil, which was used for the next reaction without further purification. ^{1}H nmr ($CDCl_3$) δ : 1.66—1.71 (6H, m), 2.64—2.76 (18H, m), 3.68—3.70 (2H, m), 3.86 (4H, t, J =5.6 Hz), 4.12 (2H, d, J =16.8 Hz), 4.29 (2H, s), 4.65 (2H, d, J =16.8 Hz), 6.70 (2H, d, J =1.4 Hz), 6.97 (2H, dd, J =8.0, 1.4 Hz), 7.05 (2H, d, J =8.0 Hz), 7.15—7.21 (6H, m), 7.26—7.29(4H, m). HR-MS (FAB) *m*/*z*: 633.4168 (Calcd for $C_{41}H_{53}N_4O_2$: 633.4168).

***N*,*N*'-(6*H*,12*H*-5,11-Methanodibenzo[*b*,*f*][1,5]diazocine-2,8-diyldi-2,1-ethanediyl-bis[*N*-(3-chloropropyl)phenethylamine] (5b)**. A mixture of **5a** (141 mg, 0.22 mmol), 25% DMC-CH_2Cl_2 solution (0.4 ml, 0.6 mmol) and TEA (0.1 ml, 0.34 mmol) in CH_2Cl_2 (5 ml) was stirred at room temperature for 24 h under N_2 atmosphere. The mixture was washed with H_2O, dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. Purification by column chromatography on silica gel with $CHCl_3$:MeOH (10:1) as an eluent afforded 138 mg, 94% as a pale yellow oil, which was used for the next reaction without further purification. ^{1}H nmr ($CDCl_3$) δ : 1.57 (4H, br s), 1.76—2.72 (4H, br s), 2.61—2.78 (16H, m), 3.33—3.37 (4H, m), 4.10 (2H, d, J =16.6 Hz), 4.29 (2H, s), 4.65 (2H, d, J =16.6 Hz), 6.66 (2H, d, J =1.8 Hz), 6.94 (2H, dd, J =8.0, 1.8 Hz), 7.04 (2H, d, J =8.0 Hz), 7.13—7.20 (6H, m), 7.24—7.28 (4H, m). HR-ms (FAB) *m*/*z*: 669.3491$(M+1)^+$, 671.3461 $(M+1)^{+37}$Cl, 673.3382 $(M+1)^{+37}Cl_2$ (Calcd for $C_{41}H_{51}N_4Cl_2$: 669.3490 $(M+1)^+$, 671.3461 $(M+1)^{+37}$Cl, 673.3431 $(M+1)^{+37}Cl_2$).

***N*,*N*'-(6*H*,12*H*-5,11-Methanodibenzo[*b*,*f*][1,5]diazocine-2,8-diyldi-2,1-ethanediyl)-bis[*N*-(3-(4-dimethylaminopyridinium)propyl)phenethylamine] dichloride (1)**. A mixture of **5b** (90 mg, 0.134 mmol) and 4-dimethylaminopyridine (33 mg, 0.27 mmol) in MeOH (3 ml) was stirred at 80 °C for 24 h. After removal of the solvent under reduced pressure, the residue was triturated with EtOAc, and the resulting hygroscopic solid was

collected by filtration. The solid was dissolved in MeOH and purified by gel filtration on a Sephadex LH-20 column with MeOH as the eluent to give a colorless hygroscopic amorphous powder (120 mg, 98%). ^{1}H-nmr (D_2O) δ : 1.62-1.66 (4H, m), 2.19—2.31 (20H, m), 2.90 (12H, s), 3.60 (4H, t, J =6.8 Hz), 3.78 (2H, d, J =17 Hz), 4.00 (2H, s), 4.30 (2H, d, J =17 Hz), 6.36 (2H, s), 6.56 (4H, d, J =7.8 Hz), 6.64 (4H, d, J =7.8 Hz), 6.80 (4H, d, J =7.8 Hz), 6.84 (2H, d, J =7.8 Hz), 6.98—7.06 (6H, m), 7.51 (4H, d, J =7.8 Hz). HR-ms (FAB) *m/z*: 841.5648 (Calcd for $C_{55}H_{69}N_8$: 841.5645 (M-HCl-Cl)).

***N*,*N*'-(6*H*,12*H*-5,11-Methanodibenzo[*b*,*f*][1,5]diazocine-2,8-diyldi-2,1-ethanediyl)-bis[*N*-(3-(4-dimethylaminopyridinium)-propyl)phenethylamine] dihexafluorophos-phate (6)**. A mixture of ATGDMAP (13 mg, 0.014 mmol) and NH_4PF_6 (4.6 mg, 0.028 mmol) in CH_2Cl_2 (1 ml) was stirred at room temperature for 24 h. After removal of the solvent under reduced pressure, the residue was triturated with H_2O, and the resulting solid was collected by filtration to afford a white powder (12 mg, 76%). HR-ms (FAB) *m/z*: 1133.5078 (Calcd for $C_{55}H_{71}N_8F_{12}P_2$: 1133.5085). *Anal*: Calcd for $C_{55}H_{70}N_8 \cdot 2PF_6$: C, 58.30; H, 6.23; N, 9.89. Found: C, 58.23; H, 6.05; N, 9.89.

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