Electronic Supplementary Information

Inhibitory Action of Macrocyclic Platiniferous Chelators on Metal-Induced A*β* Aggregation

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Contents

1 Experimental Section

1.1 Materials and Methods

5,5′-Dimethyl-2,2′-bipyridine was purchased from Alfa Aesar. Human Aβ40 was purchased from GL Biochem Ltd. (China) and verified by high-performance liquid chromatography and electrospray ionization mass spectrometry (ESI-MS). Zinc acetate dehydrate, copper chloride, 2′,7′-dichlorofluorescin diacetate (DCFH-DA), and tris(hydromethyl)aminomethane (Tris) were purchased from Sigma-Aldrich. 5-Monobromomethyl-5′-methyl-2,2′-bipyridine (**1**), 5,5′-bis(bromomethyl)-2,2′-bipyridine (**2**), and 1,4,7-tris-*tert*-butoxycarbonyl (Boc)-1,4,7,10-tetraazacyclododecane (cyclen) (**3**) were synthesized according to the literature methods.[1-3] Other common reagents used in the experiments were all of analytical grade. Stock solutions of Aβ40, Cu^{2+}, and Zn^{2+} were prepared according to the reported procedure;[4] those of **PC1**, **PC2**, cyclen, and CDDP were prepared by dissolving each compound in dimethyl sulfoxide (DMSO) to give a final concentration of 4 mM and filtered through a 0.22 μm filter (organic system). All the aqueous solutions used in this study were prepared with Milli-Q water and filtered through a 0.22 μm filter (Millipore).

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer. ESI-MS and MS/MS spectra were acquired on an LCQ Fleet electrospray mass spectrometer (Finnigan). Elemental analysis was performed on a CHN-O-Rapid analyzer (Heraeus, Germany). Turbidity, BCA, and H_2O_2 assays were conducted on a Thermo Scientific Varioskan Flash microplate reader. TEM images were obtained on a JEOL JEM-2100 LaB_6 (HR) transmission electron microscope.

1.2 Synthesis of Chelators

Synthesis of 5-(4,7,10-Tris(Boc)-1,4,7,10-tetraazacyclododecan-1-ylmethyl)-5′-methyl-2,2′-bipyridine (4) and 5,5′-Bis(4,7,10-tris(Boc)-1,4,7,10-tetraazacyclododecan-1-ylmethyl)-2,2′-bipyridine (5). They were synthesized by a modified literature procedure.[5] The dry CH_3CN solution (35 mL) of **3** (0.779 g, 1.65 mmol) and Na_2CO_3 (0.318 g, 3.0 mmol) was heated to 80 °C under N_2 for 20 min, and **1** (0.394 g, 1.5 mmol) or **2** (0.259 g, 0.75 mmol) in dry CH_3CN (15 mL) was added dropwise to the solution and allowed to reflux at 80 °C under stirring for 5 h. The insoluble product was filtered and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc, 1 : 3) to obtain a colorless solid. **4** (0.835 g, yield: 85%): ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 1.41−1.47 (s, 27H, -CH_3), 2.40 (s, 3H, CH_3-bipyridine), 2.69 (m, 4H, -CH_2-), 3.26−3.34 (m, 4H, -CH_2-), 3.44−3.49 (m, 4H, -CH_2-), 3.60 (brs, 4H, -CH_2-), 3.83 (s, 2H, -CH_2-bipyridine), 7.62 (d, 1H, bipyridine), 7.75 (d, 1H, bipyridine), 8.27−8.33 (d, 2H, bipyridine), 8.51 (s, 2H, bipyridine). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 18.27, 28.42, 47.83, 49.95, 53.97, 54.66, 55.33, 79.37, 120.28, 120.53, 132.08, 133.31, 137.37, 138.62, 149.52, 150.20, 153.35, 155.37, 156.10. IR (ν_{max}, cm^{-1}): 2974, 1607, 1462, 1415, 1365, 1249, 1168, 773. ESI-MS found (calcd) for $C_{35}H_{54}N_6O_6$ (m/z): 655.83 (655.42) $[M + H]^+$, 677.75 (677.40) $[M + Na]^+$. **5** (0.666 g, yield: 79%): ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 1.41−1.47 (s, 54H, -CH_3), 2.69 (m, 8H, -CH_2-), 3.34−3.44 (m, 16H, -CH_2-), 3.60 (brs, 8H, -CH_2-), 3.83 (s, 4H, -CH_2-bipyridine), 7.74 (d, 2H, bipyridine), 8.32 (d, 2H, bipyridine), 8.52 (s, 2H, bipyridine). ^{13}C NMR ($CDCl_3$, 125 MHz, δ, ppm): 28.43, 47.79, 49.97, 53.93, 54.63, 55.34, 79.45, 120.58, 132.51, 138.67, 150.25, 155.01, 155.36, 156.12. IR (ν_{max}, cm^{-1}): 2974, 1683, 1460, 1417,

1366, 1251, 1168, 1050, 618. ESI-MS found (calcd) for $C_{58}H_{96}N_{10}O_{12}$ (m/z): 1125.25 (1125.73) $[M + H]^+$, 1148.17 (1147.71) $[M + Na]^+$.

Synthesis of 5-(1,4,7,10-Tetrazacyclododecan-1-ylmethyl)-5′-methyl-2,2′-bipyridine (L1) and 5,5′-Bis(1,4,7,10-tetrazacyclododecan-1-ylmethyl)-2,2′-bipyridine (L2). Concentrated HCl aq (10 mL) was added dropwise to a cold methanol solution (15 mL, 0 °C) of **4** (0.327 g, 0.5 mmol) or **5** (0.563 g, 0.5 mmol). The solution was warmed to room temperature, stirred overnight, and concentrated under vacuo. The resulting crude powder was crystallized from a mixture of methanol and 6 M HCl aq (2 : 1) to give a yellow solid as the hydrochloride of **L1** (0.281 g, yield: 98%) or **L2** (0.483 g, yield: 97%). **L1**: ^{1}H NMR (D_2O, 500 MHz, δ, ppm): 2.49 (s, 3H, CH_3-bipyridine), 2.83−2.85 (m, 4H, -CH_2-), 2.92 (brs, 4H, -CH_2-), 3.10−3.12 (m, 4H, -CH_2-), 3.22−3.24 (brm, 4H, -CH_2-), 3.95 (s, 2H, -CH_2-bipyridine), 8.09 (d, 1H, bipyridine), 8.21 (d, 1H, bipyridine), 8.37 (s, 2H, bipyridine), 8.60 (s, 1H, bipyridine), 8.71 (s, 1H, bipyridine). ^{13}C NMR (D_2O, 125 MHz, δ, ppm): 17.69, 41.66, 42.64, 43.22, 47.47, 53.16, 123.07, 124.02, 133.64, 139.30, 142.34, 142.65, 144.33, 146.01, 146.95, 149.81. IR (ν_{max}, cm^{-1}): 3427, 2975, 1639, 1542, 1450, 1081, 830, 669. ESI-MS found (calcd) for $C_{20}H_{30}N_6$ (m/z): 355.50 (355.26) $[M + H]^+$. Elemental analysis found (calcd) for $C_{20}H_{30}N_6 \cdot 5HCl \cdot 2H_2O$ (%): C, 42.40 (41.93); H, 6.88 (6.86); N, 14.42 (14.67). **L2**: ^{1}H NMR (D_2O, 500 MHz, δ, ppm): 2.87−2.88 (m, 8H, -CH_2-), 2.93 (brs, 8H, -CH_2-), 3.11−3.13 (m, 8H, -CH_2-), 3.24 (brs, 8H, -CH_2-), 4.03 (s, 4H, -CH_2-bipyridine), 8.35 (d, 2H, bipyridine), 8.41 (d, 2H, bipyridine), 8.79 (s, 2H, bipyridine). ^{13}C NMR (D_2O, 125 MHz, δ, ppm): 41.55, 42.59, 43.22, 47.42, 53.03, 124.11, 134.51, 145.04, 146.61, 146.99. IR (ν_{max}, cm^{-1}): 3404, 2966, 2640, 1631, 1452, 1074, 653. ESI-MS found (calcd) for $C_{28}H_{48}N_{10}$ (m/z): 263.50 (263.21) $[M + 2H]^{2+}$. Elemental analysis found (calcd) for $C_{28}H_{48}N_{10} \cdot 8HCl \cdot 10H_2O$ (%): C, 33.81 (33.75); H, 7.29 (7.69); N, 13.41 (14.05).

Synthesis of PC1 and PC2. The chelators were synthesized according to the reported method with some modifications.[6] Briefly, to a solution of **L1** (57.3 mg, 0.1 mmol) or **L2** (99.6 mg, 0.1 mmol) in HCl aq (3 M, 8 mL), K_2PtCl_4 (8 mL, 41.5 mg, 0.1 mmol) was added dropwise and refluxed at 100 °C for 3 h. After stirring overnight at room temperature, the yellow precipitate was collected, washed with dichloromethane and methanol, and dried in vacuo. **PC1** (54.5 mg, yield: 65%): ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 2.55 (s, 3H, CH_3-bipyridine), 2.77 (brs, 4H, -CH_2-), 2.93−3.21 (m, 12H, -CH_2-), 3.95 (s, 2H, -CH_2-bipyridine), 8.26 (d, 1H, bipyridine), 8.50 (s, 3H, bipyridine), 9.28 (s, 1H, bipyridine), 9.37 (s, 1H, bipyridine). ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 18.70, 42.12, 47.51, 52.84, 123.90, 124.04, 135.89, 138.69, 141.18, 142.33, 148.44, 148.59, 154.39, 156.21. IR (ν_{max}, cm^{-1}): 3414, 1641, 1462, 1401, 1153, 988. ESI-MS found (calcd) for $C_{20}H_{30}N_6Cl_2Pt$ (m/z): 621.08 (621.16) $[M + H]^+$. Elemental analysis found (calcd) for $C_{20}H_{30}N_6Cl_2Pt \cdot 3HCl \cdot 6H_2O$ (%): C, 28.74 (28.67); H, 4.81 (5.41); N, 9.77 (10.03). **PC2** (73.9 mg, yield: 57%): ^{1}H NMR (DMSO-d_6, 500 MHz, δ, ppm): 2.79 (brs, 8H, -CH_2-), 3.01−3.23 (brm, 24H, -CH_2-), 3.98 (s, 4H, -CH_2-bipyridine), 8.56 (d, 2H, bipyridine), 8.62 (d, 2H, bipyridine), 9.39 (s, 2H, bipyridine). ^{13}C NMR (DMSO-d_6, 125 MHz, δ, ppm): 42.02, 42.82, 47.71, 52.94, 124.48, 136.31, 142.61, 148.89, 156.15. IR (ν_{max}, cm^{-1}): 3414, 2974, 1641, 1449, 1132, 1050, 995, 830. ESI-MS found (calcd) for $C_{28}H_{48}N_{10}Cl_2Pt$ (m/z): 396.50 (396.16) $[M + 2H]^{2+}$, 827.17 (827.29) $[M + 2H + Cl]^+$. Elemental analysis found (calcd) for $C_{28}H_{48}N_{10}Cl_2Pt \cdot 6HCl \cdot 16H_2O$ (%): C, 25.48 (25.91); H, 6.03 (6.68); N, 10.97 (10.79).

1.3 Binding of PC1 and PC2 to Aβ40

The sample (500 μL) for mass spectra was prepared by dissolving Aβ40 (100 μM) and **PC1** or **PC2** (100 μM) in Milli-Q water and incubating at 37 °C for 12 h. The solution was adjusted to pH 7.4 with NaOH aq (0.5 M) and HCl aq (0.5 M), centrifuged at 12000 rpm for 10 min, and the supernatant was subjected to ESI-MS and MS/MS analyses. In MS/MS analysis, the fragments of chelator-Aβ adduct were generated via collision-induced dissociation (CID) with an isolation width of 3 m/z and 30% collision energy. The samples for ^{1}H NMR spectra were prepared by adding **PC1** (5 μL, 10 mM in DMSO-d_6) to the solution of Aβ40 (0.2 mM, 500 μL) in 90% H_2O/10% D_2O and incubating at 37 °C for 12 h. The solution was adjusted to pH 7.4 with NaOD aq and HCl aq (0.5 M) after addition of DMSO-d_6 (5 μL). NMR spectra were recorded with water suppression.

1.4 Turbidity Assay

Aβ40 (20 μM) in buffer solution (20 mM Tris-HCl/150 mM NaCl, pH 7.4, 198 μL) was incubated with or without $Zn(Ac)_2$ or $CuCl_2$ (40 μM) for 5 min at room temperature. Metal chelator (2 μL, 40 μM) and DMSO (2 μL, final concentration 1%) were added to the solution and incubated at 37 °C for 24 h. Each sample was transferred to individual wells of a flat-bottomed 96-well plate (Corning Costar Corp). Turbidity of the solution was measured by absorbance at 405 nm. Data were expressed as mean ± standard deviations of at least three independent experiments.

1.5 BCA Protein Assay

Sample solutions were prepared as above except that the concentration of Aβ40 was changed to 40 μM. After incubation for 24 h, the solutions were centrifuged at 12000 rpm for 30 min and the peptide concentration in the supernatant was analyzed by the Micro BCA protein assay.

1.6 Transmission Electron Microscopy

Sample solutions were prepared in the same way as in the turbidity assay. An aliquot of each solution (5 μL) was spotted on the 300-mesh carbon-coated copper grids for 2 min at room temperature and excess sample was removed. Each grid was stained with uranyl acetate (1%, w/v) for 1 min and examined on a JEOL JEM-2100 LaB_6 (HR) transmission electron microscope.

1.7 H_2O_2 Assay

DCFH-DA stock solution (1 mM) was prepared in buffer (20 mM Tris-HCl/150 mM NaCl, pH 7.4) according to the reported procedures.[4] Horseradish peroxidase (HRP) stock solution (4 μM) was prepared with the same buffer. Sample solutions containing Aβ40 (0.8 μM) and $CuCl_2$ (0.6 μM) were incubated with or without chelators (0.6 μM) at 37 °C for 20 h. Ascorbate (10 μM) was added to each sample and incubated at 37 °C for 1 h. The sample was transferred to individual wells of a flat-bottomed 96-well black plate. HRP (2 μL, 0.04 μM) and DCFH-DA (20 μL, 100 μM) were added to each solution and incubated for 10 min in the dark at room temperature. Fluorescence spectra (λ_{ex} = 485 nm) in the range of 505–650 nm were measured by a Varioskan Flash microplate reader (Thermo Scientific).

1.8 Cytotoxicity Assay

Primary neuronal cultures and cell viability assay were prepared and carried out as described in the literature.[4] All the samples were treated with 0.5% DMSO.

1.9 Western Blot Analysis of Brain Homogenates

Brain extracts of 5-month-old APPswe Tg2576 transgenic mice were prepared according to the reported procedure.[7] The extracted tissue pieces were homogenized in lysis buffer (Tris-HCl, 50 mM; NaCl, 150 mM; NP-40, 1%; Na-deoxycholate, 0.25%; EDTA, PMSF, Na_3VO_4, NaF, 1 mM each; aprotinin, leupeptin, pepstatin, 1 μg/mL each). The homogenate solution was centrifuged at 12000 rpm for 10 min at 4 °C and the resulting supernatant was collected. **PC1**, **PC2** (2 μL, 20 μM), and DMSO (2 μL, final concentration 1%) was added respectively to the supernatant (200 μL). After incubation at 37 °C for 24 h, the samples were dissolved in loading buffer containing β-mercaptoethanol (5%) and boiled for 5 min. Each sample was separated by SDS polyacrylamide gel electrophoresis (PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked for 1 h at room temperature with non-fat milk powder (5%) and incubated at 4 °C overnight with monoclonal anti-Aβ antibody 6E10 (1 : 1000, Covance Inc). The bolts were then incubated with the HRP-conjugated goat anti-mouse anibody (1 : 10000) for 1 h at room temperature. Bands were visualized using SuperSignal (Thermo Scientific Inc.) and developed onto a Kodak film. The membranes were then developed using an enhanced chemiluminescence detection kit, stripped and reprobed with anti-glyceraldehyde-3-glyceraldehyde-3-phosphate dehydrogenase antibody (1 : 5000; Meridian Life Sciences, Brockville, ON, Canada) to ensure equal protein loading.[8]

2 Supplementary Figures and Tables

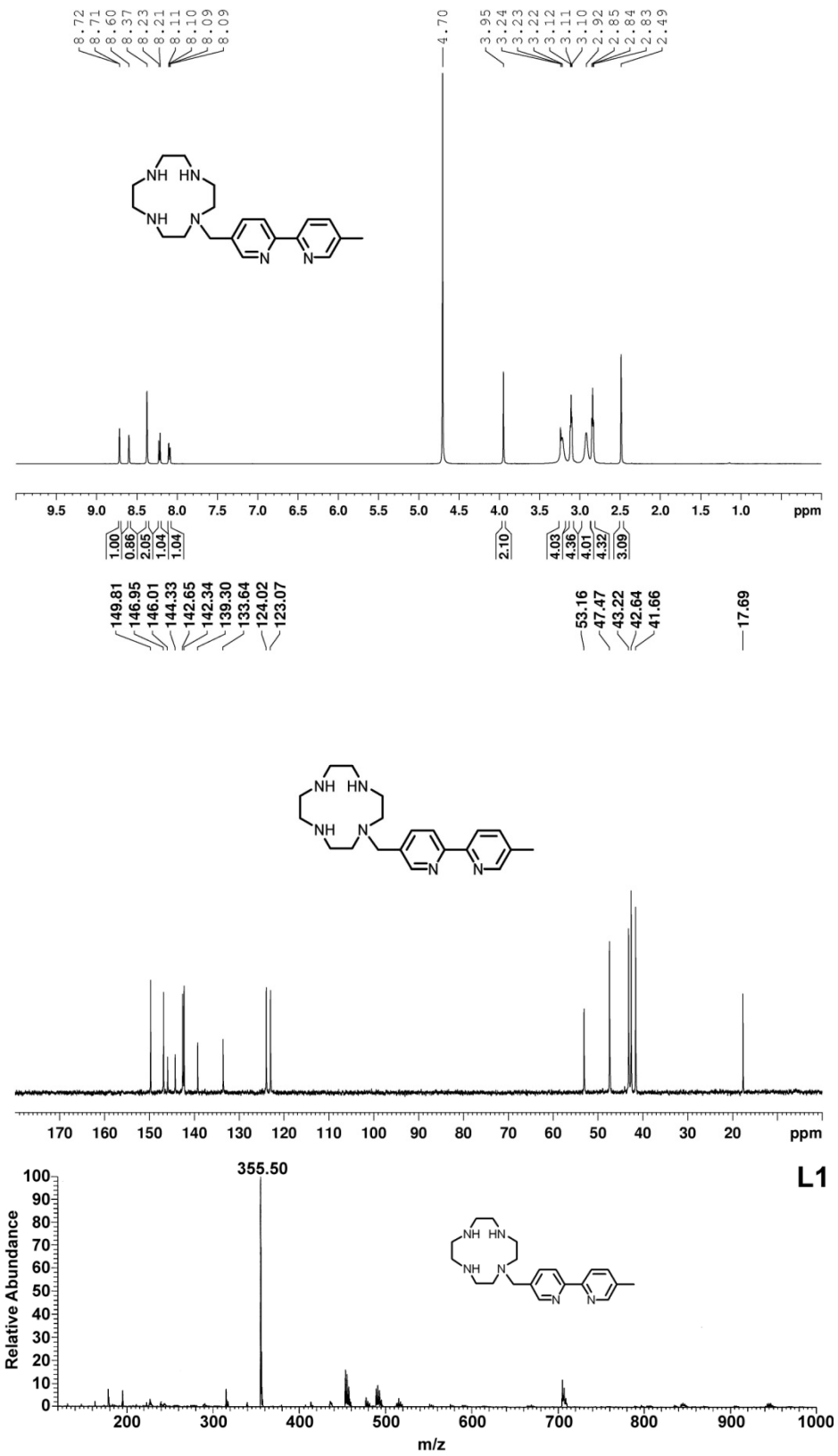

Fig. S1 ^{1}H NMR (D_2O), ^{13}C NMR (D_2O) and ESI-MS spectra for **L1**.

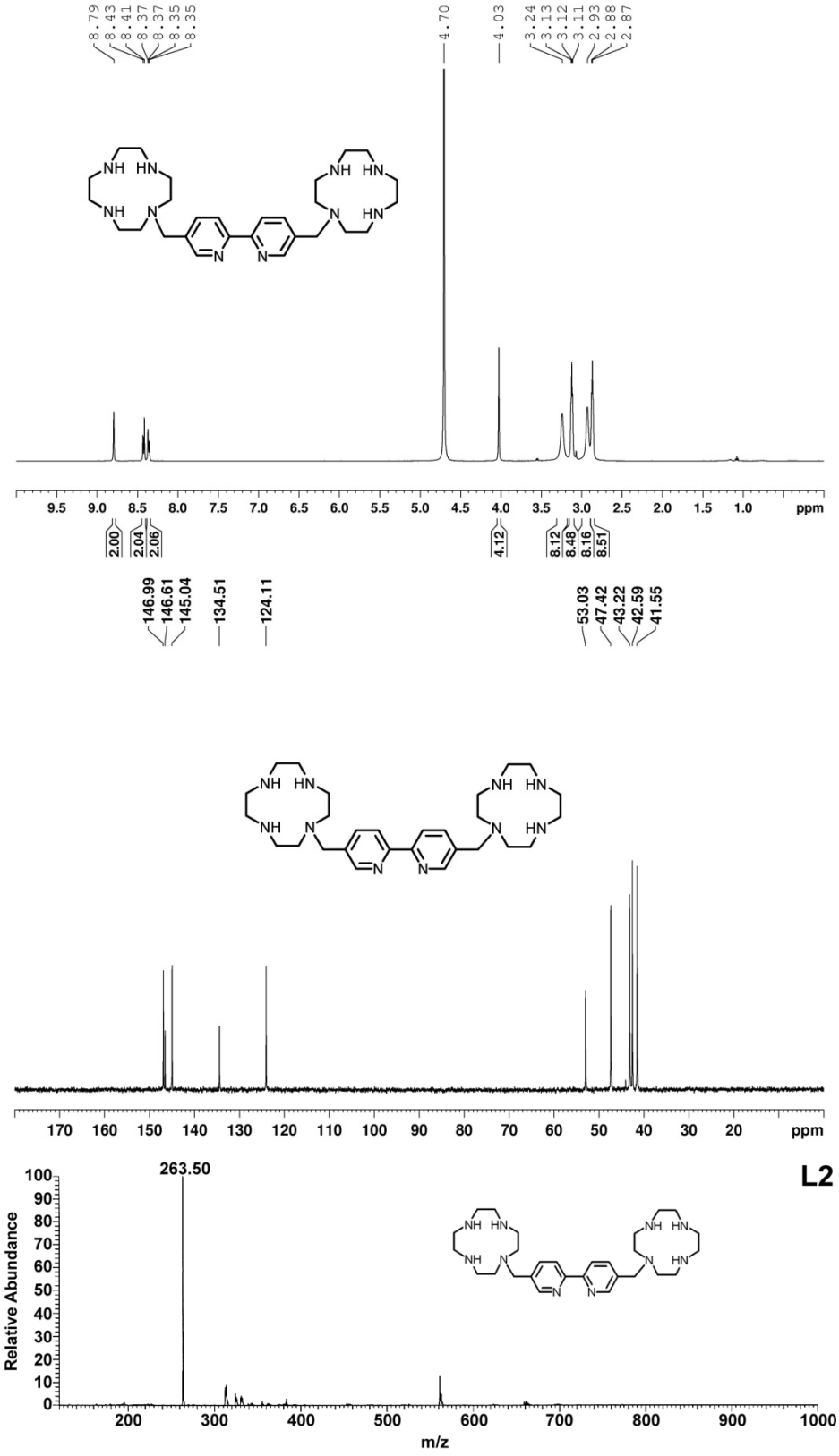

Fig. S2 ^{1}H NMR (D_2O), ^{13}C NMR (D_2O) and ESI-MS spectra for **L2**.

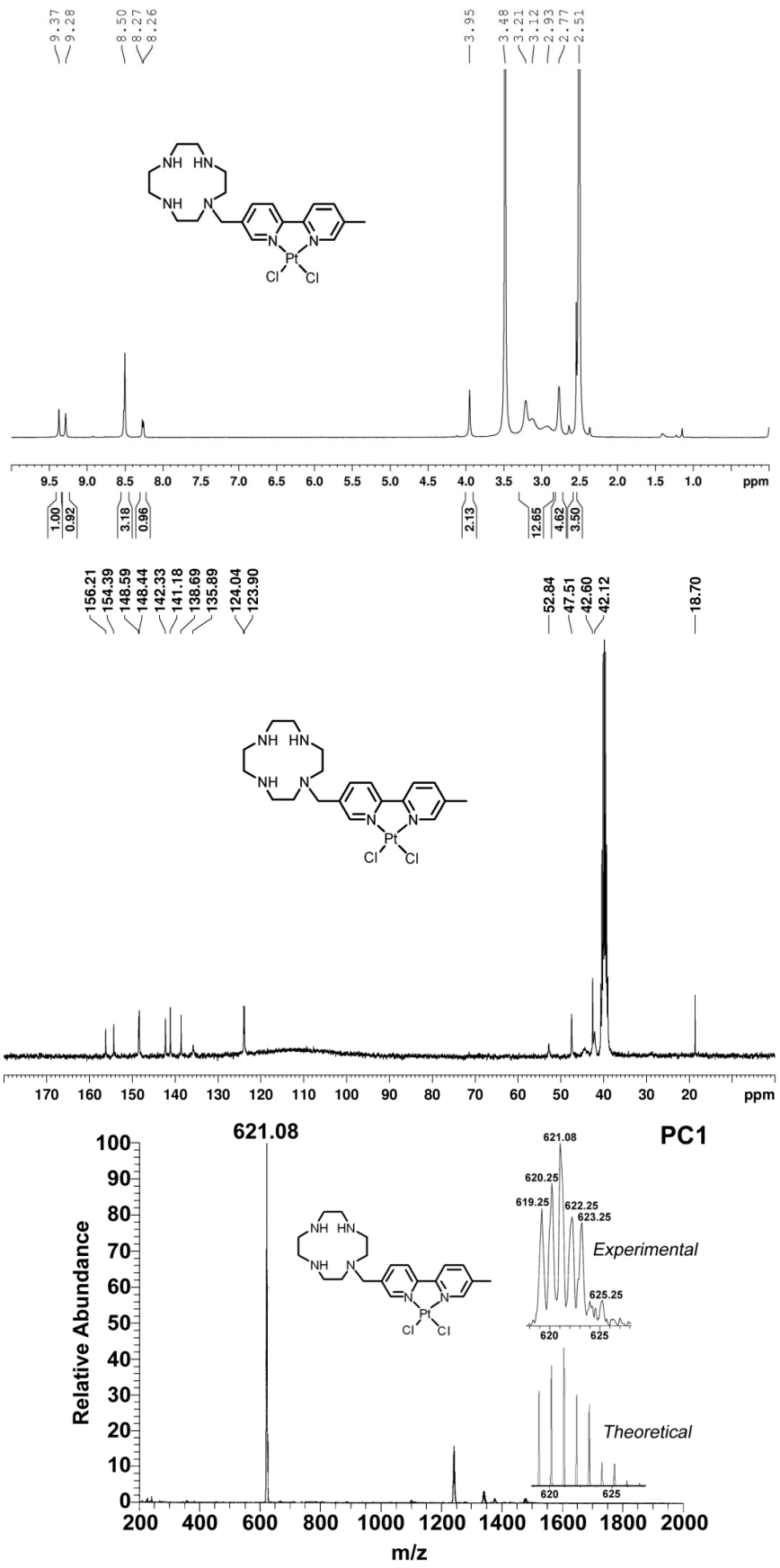

Fig. S3 ^{1}H NMR, ^{13}C NMR (DMSO-d_6) and ESI-MS spectra for **PC1**.

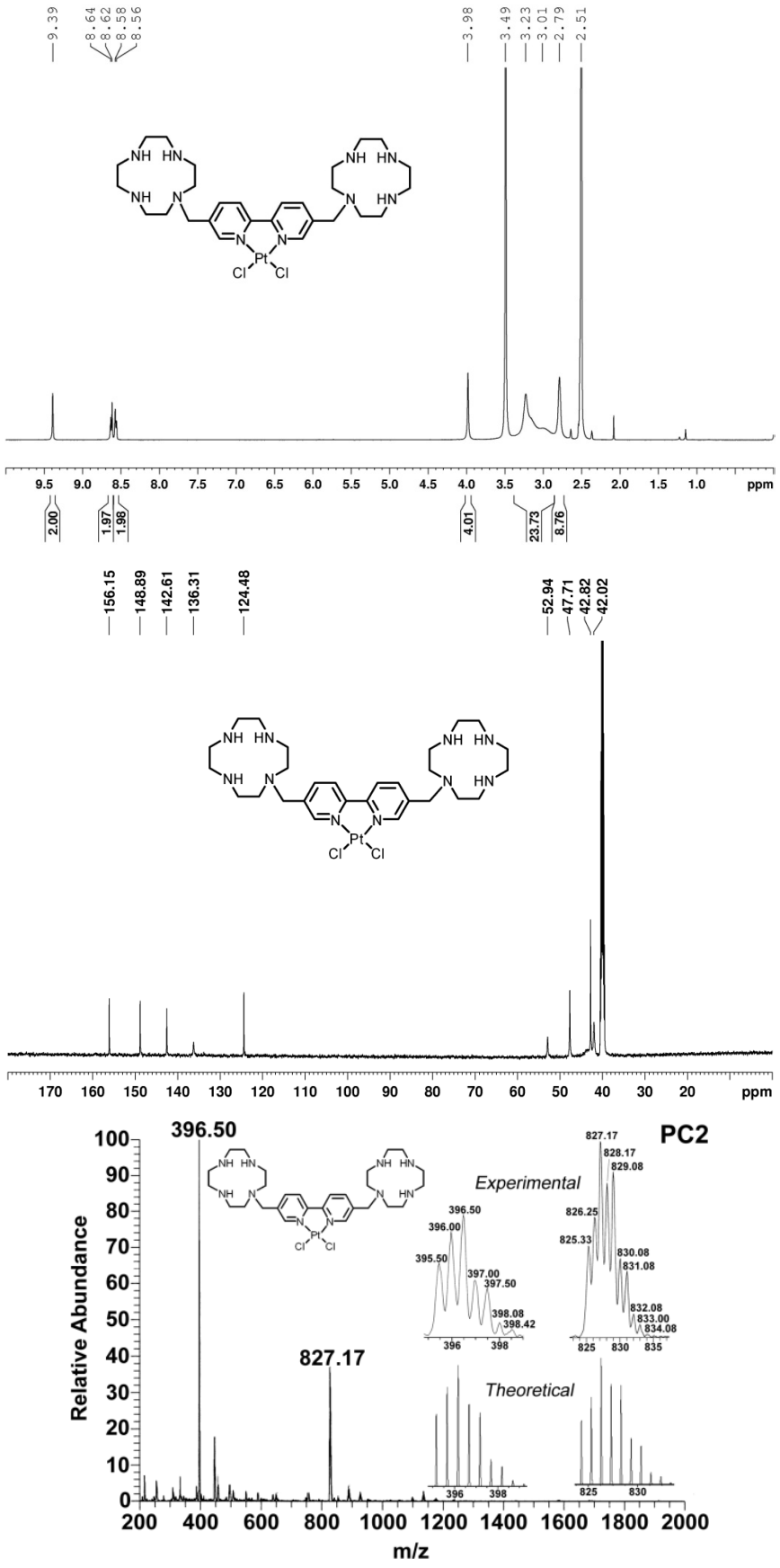

Fig. S4 ^{1}H NMR, ^{13}C NMR (DMSO-d_6) and ESI-MS spectra for **PC2**.

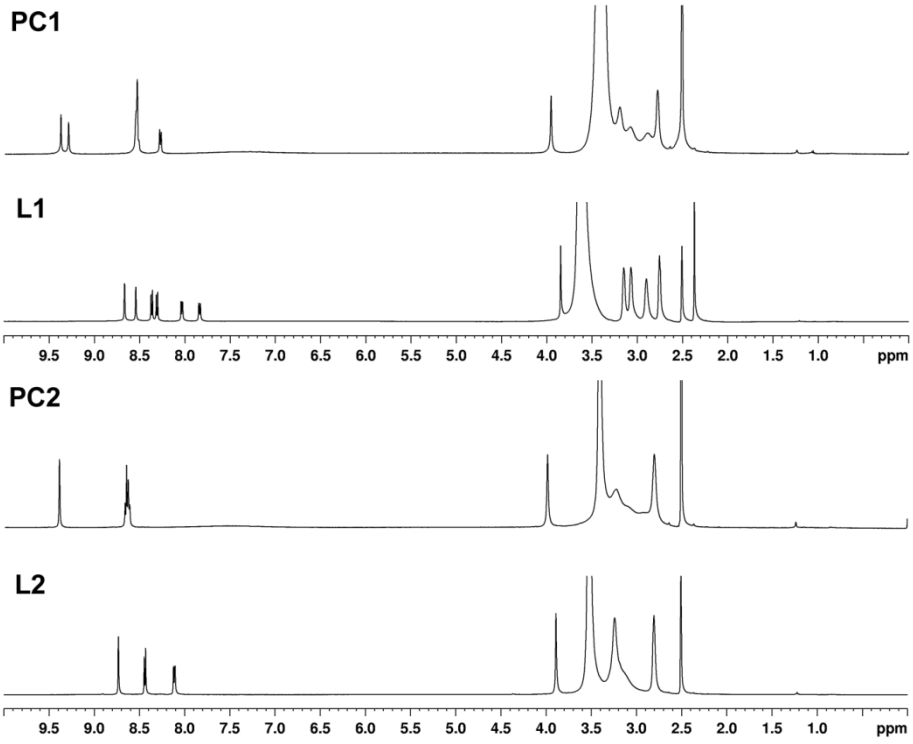

Fig. S5 Spectral comparison between ^{1}H NMR spectra of **PC1**, **L1**, **PC2** and **L2** in DMSO-d_6. The significant downfield shifts of pyridine signals and almost unchanged cyclen signals indicate that Pt^{II} ions coordinate with bipyridine moieties in **PC1** and **PC2**.

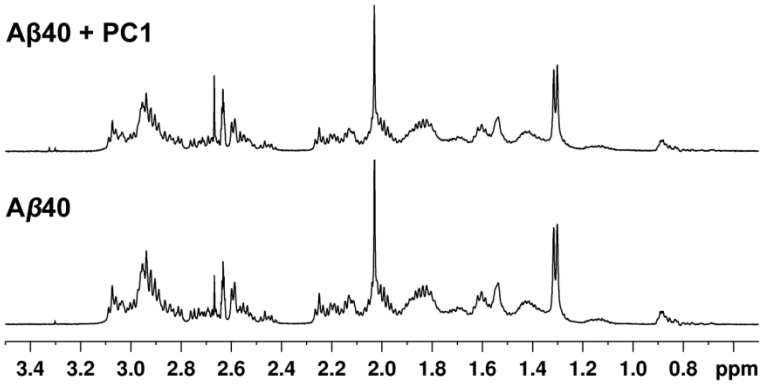

Fig. S6 ^{1}H NMR spectra of Aβ40 (0.2 mM) in the absence and presence of **PC1** (0.1 mM) at high magnetic field (0.5–3.5 ppm) and pH 7.4.

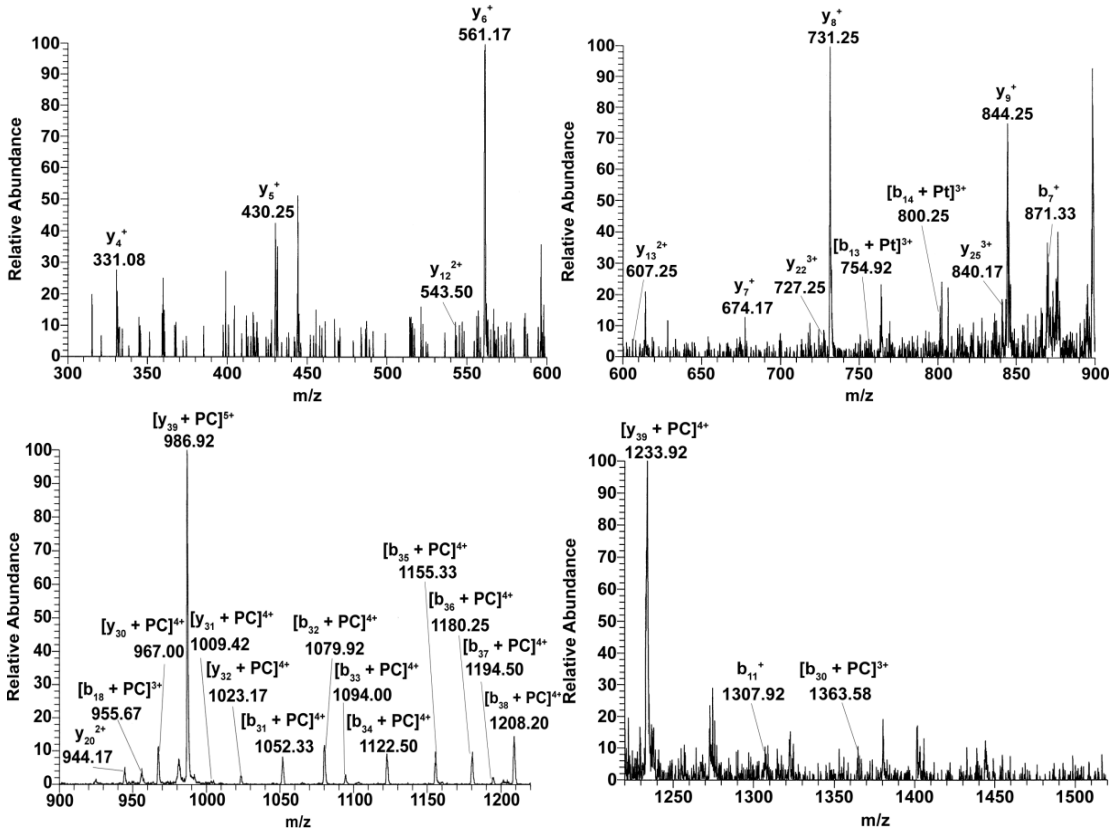

Fig. S7 MS/MS spectra for Aβ-**PC2** adduct species [Aβ40 + (**PC2** – 2Cl) + 3H]$^{5+}$ (m/z 1010.25) [PC stands for (**PC2** – 2Cl)$^{2+}$].

Table S1. MS/MS analysis for $[C_{222}H_{346}N_{63}O_{58}SPt]^{5+}$ (*m/z* 1010.25).

Assignment	Obsd *m/z*	Calcd *m/z*	Assignment	Obsd *m/z*	Calcd *m/z*
y_4^+	331.08	331.19	b_6^+	756.25	756.33
y_5^+	430.25	430.26	b_7^+	871.33	871.36
y_6^+	561.17	561.30	b_{11}^+	1307.92	1307.52
y_7^+	674.17	674.38	$[b_{13} + PC]^{3+}$	754.92	754.48
y_8^+	731.25	731.40	$[b_{14} + PC]^{3+}$	800.25	800.17
y_9^+	844.25	844.49	$[b_{18} + PC]^{3+}$	955.67	956.27
y_{12}^{2+}	543.50	543.31	$[b_{19} + PC]^{4+}$	754.92	754.22
y_{13}^{2+}	607.25	607.36	$[b_{21} + PC]^{3+}$	1077.08	1077.99
y_{17}^{2+}	786.33	785.95	$[b_{23} + PC]^{3+}$	1159.58	1159.35
y_{18}^{2+}	843.50	843.46	$[b_{24} + PC]^{3+}$	1192.67	1192.38
y_{20}^{2+}	944.17	943.50	$[b_{25} + PC]^{4+}$	909.42	908.79
y_{22}^{3+}	727.25	727.38	$[b_{30} + PC]^{3+}$	1363.58	1363.79
y_{24}^{3+}	798.25	798.09	$[b_{31} + PC]^{4+}$	1052.33	1051.37
y_{25}^{3+}	840.17	840.79	$[b_{32} + PC]^{4+}$	1079.92	1079.64
$[y_{27} + PC]^{3+}$ [a]	1169.17	1168.43	$[b_{33} + PC]^{4+}$	1094.00	1093.89
$[y_{28} + PC]^{4+}$	910.67	910.84	$[b_{34} + PC]^{4+}$	1122.50	1122.16
$[y_{30} + PC]^{4+}$	967.00	967.87	$[b_{35} + PC]^{4+}$	1155.33	1154.92
$[y_{31} + PC]^{4+}$	1009.42	1008.63	$[b_{36} + PC]^{4+}$	1180.25	1179.69
$[y_{32} + PC]^{4+}$	1022.17	1022.89	$[b_{37} + PC]^{4+}$	1194.50	1193.95
$[y_{35} + PC]^{5+}$	886.58	886.33	$[b_{38} + PC]^{4+}$	1208.83	1208.20
$[y_{39} + PC]^{5+}$	986.92	986.98	$[b_{39} + PC]^{4+}$	1232.92	1232.97

[a] PC represents the $(\mathbf{PC2} - 2Cl)^{2+}$ moiety.

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