Supporting Information for

Gene Delivery by Aminofullerene: Structural Requirements for Efficient Transfection

Hiroyuki Isobe, Waka Nakanishi, Naoki Tomita, Shigeki Jinno, Hiroto Okayama, Eiichi Nakamura

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General

Aminofullerenes examined in the present study (Figure 2) were synthesized by reported procedures with some necessary modifications. Compounds are purified by recrystallization in appropriate solvent (CHCl₃, hexane and CH₃CN), flash chromatography (Silica; toluene, CHCl₃, MeOH, ethyl acetate and hexane) and/or preparative HPLC. The spectra of known compounds (6, 10, 13-17)¹,²,³ were identical with the reported ones, and those of new compounds are shown below.

Synthesis of aminofullerenes

Aminofullerene 3

Deprotection of Boc group of aminofullerene 13 (56 mg, 0.038 mol) was carried out in 10 % CF₃COOH/CHCl₃ (40 mL). After removal of solvent, the title compound was obtained quantitatively (59 mg, 100%). Compound 3 was obtained in cationic form with trifluoroacetate as a counterion and formed aggregates in both aqueous and organic phase. Similarly to other amphiphilic fullerenes,⁴ no signals appeared in the NMR spectra. IR (powder) 2493, 2250, 2016, 1167, 1121, 720; MS (MALDI: β-Carboline as a matrix) calcd for C₇₆H₃₇N₈O₁ [MH⁺] 1077, found 1077.

Aminofullerene 4

Deprotection of Boc group of 6,12,15,18-tetra[4-(tert-butoxycarbonylamino)piperidin-1-yl]-6,12,15,18-(tetrahydro)oxireno [2',3':1,9](C₆₀-I₄)-[5,6]fullerene¹ (30 mg, 19.6 µmol) was carried out in 10 %

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CF$_3$COOH/CHCl$_3$ (20 mL) for 3h at rt. After removal of solvent, the title compound was obtained in good yield (30 mg, 97%). Compound 4 was obtained in cationic form with trifluoroacetate as a counterion and formed aggregates in both aqueous and organic phase. Similarly to other amphiphilic fullerenes, no signals appeared in the NMR spectra.$^4$ IR (powder) 3446, 2869, 2358, 1653, 1456, 1349, 1300, 1113, 951, 847; MS (APCI) calcd for $C_{80}H_{45}N_8O_1$ [MH$^+$] 1133, found 1133.

**Aminofullerene 5**

Piperidine derivative bearing an aminoester functional group was first prepared as follows: To a solution of 4-(((3-dimethylamino-propyl)-methyl-amino)-acetoxy)-piperidine-1-carboxylic acid benzyl ester (890 mg, 2.27 mmol) in 10 mL of ethanol 5% Pd on activated carbon (484 mg) was added. The reaction mixture was stirred at room temperature under hydrogen pressure (1 atm) for 4 h. The catalyst was filtered and solvent was removed in vacuo to afford pure amine salt (colorless oil 114 mg). Neutralization using NaOH gave the desired product ([[(3-dimethylamino-propyl)-methyl-amino]-acetic acid piperidin-4-yl ester] as pale yellow oil (417 mg, 71%); IR (neat) 2994, 2861, 2815, 2769, 1698, 1430, 1273, 1227, 1175, 1135, 1071, 1027, 749, 697; $^1$H NMR (CDCl$_3$ 500 MHz) δ 1.56 (q, $J = 9.2$ Hz, 2H), 1.67 (m, 2H), 1.90 (br, 2H), 2.22 (s, 6H), 2.30 (t, $J = 7.5$ Hz, 2H), 2.38 (s, 3H), 2.52 (t, $J = 6.9$ Hz, 2H), 2.71 (t, $J = 10.9$ Hz), 3.07 (br, 2H), 3.25 (s, 2H) 4.90 (m, 1H); $^{13}$C NMR (CDCl$_3$ 125 MHz) δ 25.62, 32.16, 42.20, 44.00, 45.33, 54.93, 57.50, 58.53, 70.84, 170.15; LRMS (FAB; NBS as a matrix) calcd for $C_{21}H_{34}N_3O_4$ [MH$^+$] 258, found 258; HRMS (FAB; PEG400 as a matrix) calcd for $C_{13}H_{28}N_3O_2$ [MH$^+$]: 258.201; found: 258.201. The o xoamination of $C_{60}$ (40.0 mg, 55.6 µmol) was carried out in a similar manner as reported$^1$ to obtain aminofullerene 4 (55.7 mg, 57%) as an analytically pure brown powder: IR (powder) ν 3433, 2944, 2855, 2813, 1634, 1461, 1252, 1182, 1033; $^1$H NMR (CDCl$_3$ 500 MHz) δ 1.68 (q, $J = 6.3$ Hz, 2H), 1.84 (t, $J = 10$ Hz, 8H), 2.01 (br, 8H), 2.22 (s, 6H), 2.22 (s, 6H), 2.30 (m, 8H), 2.39 (m, 8H), 2.40 (m, 8H), 2.54 (q, $J = 9.2$ Hz, 2H), 3.07 (br, 2H), 3.25 (s, 2H) 4.90 (m, 1H); $^{13}$C NMR (CDCl$_3$ 125 MHz) δ 25.62, 32.16, 42.20, 44.00, 45.33, 54.93, 57.50, 58.53, 70.84, 170.15; LRMS (FAB; NBS as a matrix) calcd for $C_{21}H_{34}N_3O_4$ [MH$^+$] 258, found 258; HRMS (FAB; PEG400 as a matrix) calcd for $C_{13}H_{28}N_3O_2$ [MH$^+$]: 258.201; found: 258.201. The o xoamination of $C_{60}$ (40.0 mg, 55.6 µmol) was carried out in a similar manner as reported$^1$ to obtain aminofullerene 4 (55.7 mg, 57%) as an analytically pure brown powder: IR (powder) ν 3433, 2944, 2855, 2813, 1634, 1461, 1252, 1182, 1033; $^1$H NMR (CDCl$_3$ 500 MHz) δ 1.68 (q, $J = 6.3$ Hz, 2H), 1.84 (t, $J = 10$ Hz, 8H), 2.01 (br, 8H), 2.22 (s, 6H), 2.22 (s, 6H), 2.30 (m, 8H), 2.39 (m, 8H), 2.40 (m, 8H), 2.54 (q, $J = 9.2$ Hz, 2H), 3.07 (br, 2H), 3.25 (s, 2H) 4.90 (m, 1H); $^{13}$C NMR (CDCl$_3$ 125 MHz) δ 25.62, 32.16, 42.20, 44.00, 45.33, 54.93, 57.50, 58.53, 70.84, 170.15; LRMS (FAB; NBS as a matrix) calcd for $C_{21}H_{34}N_3O_4$ [MH$^+$] 258, found 258; HRMS (FAB; PEG400 as a matrix) calcd for $C_{13}H_{28}N_3O_2$ [MH$^+$]: 258.201; found: 258.201. The o xoamination of $C_{60}$ (40.0 mg, 55.6 µmol) was carried out in a similar manner as reported$^1$ to obtain aminofullerene 4 (55.7 mg, 57%) as an analytically pure brown powder: IR (powder) ν 3433, 2944, 2855, 2813, 1634, 1461, 1252, 1182, 1033; $^1$H NMR (CDCl$_3$ 500 MHz) δ 1.68 (q, $J = 6.3$ Hz, 2H), 1.84 (t, $J = 10$ Hz, 8H), 2.01 (br, 8H), 2.22 (s, 6H), 2.22 (s, 6H), 2.30 (m, 8H), 2.39 (m, 8H), 2.40 (m, 8H), 2.54 (q, $J =
Aminofullerene 7

The oxoamination reaction of C$_{60}$ (40.0 mg, 55.6 µmol) was carried out with 1-(N,N-dimethyl-3-aminopropyl) piperazine (Fluka) in a similar manner as reported$^1$ to obtain aminofullerene 7 (44.5 mg, 64%) as an analytically pure brown powder: IR (powder) 3437, 2940, 2811, 2763, 1652, 1456, 1373, 1277, 1097, 1041, 1004, 859; $^1$H NMR (CDCl$_3$ 125 MHz) δ 1.71 (br, 2H), 2.23 - 3.47 (m, 18H); $^{13}$C NMR (CDCl$_3$ 125 MHz) δ 25.22, 45.56, 50.48, 53.84, 53.99, 56.58, 56.72, 57.89, 57.93, 71.51, 72.30, 75.52, 76.29, 140.28, 141.63, 142.89, 143.05, 143.49, 143.60, 143.85, 144.03, 144.41, 144.68, 145.14, 146.14, 146.31, 146.84, 146.90, 147.10, 147.23, 147.42, 147.69, 149.06, 149.16, 149.16, 149.32, 149.36, 149.49, 151.46; MS (APCI) calcd for C$_{112}$H$_{104}$N$_{12}$O$_9^+$ [M$^+$] 1761 found, 1761.

Aminofullerene 8

Piperazine derivative bearing an ethoxyethyl group was first prepared as follows: To a solution of 1-benzyl-4-(2-ethoxy-ethyl)-piperazine (1.0 g 1.0mL) in MeOH (20 mL), 20%Pd(OH)$_2$ on activated carbon with 50% moisture (403 mg) was added. The reaction mixture was stirred at room temperature under hydrogen pressure (1 atm) for 24 h. The catalyst was filtered and solvent was removed in vacuo to afford pure amine salt (yellow oil 743 mg). Neutralization using NaOH gave the piperazine derivative (1-(2-ethoxy-ethyl)-piperazine) as pale yellow oil (339 mg, 45%): $^1$H NMR (CDCl$_3$ 500 MHz) δ 1.20 (t, $J$ = 7.0 Hz, 3H), 2.48 (br, 4 H), 2.57 (t, $J$ = 6.0 Hz, 4H), 3.50 (q, 2H), 3.57 (t, $J$ =
6.0 Hz, 2H); $^{13}$C NMR (CDCl$_3$ 500 MHz) δ 15.04, 54.93, 58.46, 66.32, 67.89; MS (FAB; NBS as a matrix) calcd for C$_8$H$_{19}$N$_2$O [MH$^+$] 159 found 159. The oxoamination of C$_{60}$ (40.0 mg, 55.6 µmol) was carried out in a similar manner as reported$^1$ to obtain aminofullerene 8 (38.4 mg, 54%) as an analytically pure brown powder: IR (powder) 3446, 2863, 2359, 1653, 1456, 1374, 1278, 1153, 1112, 1006, 527; $^1$H NMR (CDCl$_3$ 500 MHz) δ 1.22 (t, $J = 7.0$ Hz, 3H), 1.23 (t, $J = 7.0$ Hz, 3H), 1.97 (br, 2H), 2.63 – 2.70 (m, 6H), 3.27–3.64 (m, 6H); $^{13}$C NMR (CDCl$_3$ 125 MHz) δ 15.20, 15.24, 50.45, 50.78, 54.23, 54.51, 57.85, 57.95, 66.49, 66.50, 68.24, 68.35, 71.56, 71.98, 75.56, 76.31, 140.29, 141.75, 142.94, 143.09, 143.51, 143.63, 143.90, 144.07, 144.37, 144.72, 145.17, 145.34, 146.19, 146.37, 146.90, 146.96, 147.04, 147.13, 147.15, 147.23, 147.48, 147.44, 149.12, 149.38, 149.44, 151.45; MS (ESI) calcd for C$_{92}$H$_{68}$N$_8$O$_5$ [M$^+$] 1365 found 1365.

**Aminofullerene 9**

Piperazine derivative was first prepared as follows: To a solution of 1-benzyl-4-[2-(2-ethoxy-ethoxy)-ethyl]-piperazine (800 mg 2.74 mmol) in MeOH (16 mL), 20%Pd(OH)$_2$ on activated carbon with 50% moisture (274 mg) was added. The reaction mixture was stirred at room temperature under hydrogen pressure (1 atm) for 24 h. The catalyst was filtered and solvent was removed in vacuo to afford crude colorless oil. Neutralization using NaOH gave piperazine derivative (1-[2-(2-ethoxy-ethoxy)-ethyl]-piperazine) as pale yellow oil (354 mg, 64.0%): $^1$H NMR (CDCl$_3$ 500 MHz) δ 1.21 (t, $J = 7.0$ Hz, 3H), 1.62 (br, 1H), 2.48 (br, 4H), 2.60 (t, $J = 6.5$ Hz, 2H), 2.90 (t, $J = 4.5$ Hz, 4H), 3.52 (q, $J = 7.0$ Hz), 3.57 - 3.65 (m, 6H); $^{13}$C NMR (CDCl$_3$ 125 MHz) δ 15.05, 45.93, 54.92, 58.25, 66.51, 68.69, 69.69, 70.31; MS (FAB; NBS as a matrix) calcd for C$_{10}$H$_{23}$N$_2$O$_2$ [MH$^+$] 203, found 203. The oxoamination of C$_{60}$ (40.0 mg, 55.6 µmol) was carried out in a similar manner as reported$^1$ to obtain aminofullerene 9 (65.0 mg, 76%) as an analytically pure brown powder: IR (powder) 3447, 2867, 2817, 1653, 1457, 1278, 1109, 1033, 1005, 857, 537; $^1$H NMR (CDCl$_3$ 500 MHz) δ 1.18 (t, $J = 7.0$ Hz, 12H), 2.61 – 2.66 (m,
16H), 3.05–3.32 (br, 16H), 3.50 (q, \( J = 7.0 \text{ Hz} \), 8H); \(^{13}\text{C NMR (CDCl}_3, 125 \text{ MHz}) \delta 15.32, 50.32, 50.71, 54.19, 54.35, 57.60, 57.75, 66.59, 68.97, 69.06, 69.76, 71.44, 71.44, 71.83, 75.46, 76.23, 140.21, 141.58, 142.83, 142.99, 143.40, 143.54, 143.78, 143.97, 144.38, 144.62, 145.08, 145.22, 146.08, 146.25, 146.78, 146.84, 146.91, 147.04, 147.21, 147.59, 147.63, 147.73, 148.99, 149.25, 149.30, 149.48; MS (ESI) calcd for \( \text{C}_{100}\text{H}_{84}\text{N}_8\text{O}_9 \) [M+] 1541 found 1541.

Aminofullerene 11

The oxoamination of \( \text{C}_{60} \) (40.0 mg, 55.6 \( \mu \text{mol} \)) was carried out with \( \text{syn-2,6-dimethylpiperazine (Wako)} \) in a similar manner as reported\(^1\) to obtain aminofullerene 11 (55.4 mg, 84%) as an analytically pure brown powder: IR (powder) 3420, 2959, 2813, 1454, 1324, 1150, 1055, 873, 752; \(^1\text{H NMR (500 MHz, CDCl}_3) \delta 1.11 (d, \( J = 6.5 \text{ Hz} \), 6H), 1.19 (d, \( J = 6.5 \text{ Hz} \), 6H), 1.21 (d, \( J = 6.5 \text{ Hz} \), 6H), 1.24 (d, \( J = 6.5 \text{ Hz} \), 6H), 1.65 (br, 4H), 2.34 (t, \( J = 10.5 \text{ Hz} \), 2H), 2.43 (t, \( J = 10.5 \text{ Hz} \), 2H), 2.48 (t, \( J = 11.0 \text{ Hz} \), 2H), 2.52 (t, \( J = 10.5 \text{ Hz} \), 2H), 3.06 (m, 4H), 3.11 (m, 4H), 3.53 (d, \( J = 10.0 \text{ Hz} \), 2H), 3.57 (d, \( J = 11.5 \text{ Hz} \), 2H), 3.65 (d, \( J = 11 \text{ Hz} \), 2H), 3.72 (d, \( J = 11.0 \text{ Hz} \), 2H); \(^{13}\text{C NMR (125 MHz, CDCl}_3) \delta 20.13, 20.35, 20.53, 20.64, 51.11, 51.14, 57.54, 57.90, 58.05, 58.45, 71.51, 72.01, 75.70, 76.43, 140.30, 141.88, 142.86, 143.09, 143.54, 143.75, 143.83, 143.90, 144.04, 144.24, 144.65, 145.22, 145.35, 146.11, 146.33, 146.88, 146.91, 146.97, 147.12, 147.21, 147.43, 147.71, 147.77, 149.05, 149.33, 149.54, 149.61, 151.33; MS (APCI) calcd for \( \text{C}_{84}\text{H}_{52}\text{N}_8\text{O} \) [M+] 1188 found, 1188.

Aminofullerene 12

Piperazine derivative was first prepared as follows: To a solution of dimethylamino-acetic acid 2-(4-benzyl-piperazin-1-yl)-ethyl ester (1.80 g, 5.89 mmol) in EtOH (29.5 mL), 20% \( \text{Pd(OH)}_2 \) on activated carbon with 50% moisture (1.66 g, 1.18 mmol) was added. The reaction mixture was stirred at room temperature under hydrogen pressure (4 atm) for 4 h. The catalyst was filtered off and solvent was removed \textit{in vacuo} to afford crude material (1.60 g). Purification of the crude product was performed by column chromatography.
(neutral Al₂O₃, 80 g) to afford the piperazine derivative (2-piperazin-1-yl-ethyl ester; 500 mg, 59% yield). ¹H NMR (400 MHz, CD₃OD) δ 2.29 (s, 6H), 2.47 (m, 4H), 2.60 (t, J = 6.0 Hz, 2H), 2.80 (m, 4H), 3.19 (s, 2H), 4.23 (t, J = 6.0 Hz, 2H); ¹³C NMR (100 MHz, CD₃OD) δ 45.34, 46.11, 54.96, 58.25, 60.59, 62.26, 171.51. The oxamination of C₆₀ (30.0 mg, 41.7 µmol) was carried out in a similar manner as reported¹ to obtain aminofullerene 12 (50.1 mg, 76%) as an analytically pure brown powder: IR (powder) 3432, 2924, 2852, 2819, 1735, 1635, 1455, 1385, 1280, 1152, 1062, 1005; ¹H NMR (400 MHz, CDCl₃) δ 2.36 (br s, 24H), 2.71 (m, 24H), 3.20 (m, 24H), 4.30 (m, 8H); ¹³C NMR (100 MHz, CDCl₃) δ 45.34, 50.39, 50.79, 54.00, 54.18, 56.54, 56.63, 60.44, 61.78, 71.50, 71.76, 75.41, 76.20, 140.05, 141.50, 142.72, 142.83, 143.32, 143.38, 143.67, 143.87, 144.11, 144.43, 144.89, 145.09, 145.84, 146.12, 146.64, 146.72, 146.78, 146.87, 146.91, 147.23, 147.50, 147.59, 148.88, 149.15, 149.37, 151.18, 170.29.