Supplementary Information

Precise Control of Nanoparticle Surface by Host-Guest Chemistry and in vivo Disposition

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SI-1. Synthesis of 16RD (Scheme S1, S2 and Figure S1)

Scheme S1. Synthetic scheme of 16RD

DMF: N,N-dimethylformamide
TEA: Triethylamine
DCC: N,N-Dicyclohexyl carbodiimide
HOBt: 1-Hydroxybenzotriazole
DMT-MM: 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinum Chloride·n·hydrate
Scheme S2. Synthetic scheme of AB-LP.

H — Ab — OH
2-1

Boc — Leu — OH

HCl — Ab — OMe
2-2 quant.

DCC, HOBt, TEA/DMF

Boc — Leu — Ab — OMe
2-3 80%

NaOH, 1,4-Dioxane, MeOH

HCl, 1,4-Dioxane
2-4 97%

DCC, HOBt, TEA/DMF

Boc — Leu — Ab — OMe
2-5 96%

88%

NaOH, 1,4-Dioxane, MeOH

HCl, 1,4-Dioxane
2-6 90%

DCC, HOBt, TEA/DMF

Boc — Leu — Ab — OMe
2-7 95%

87%

NaOH, 1,4-Dioxane, MeOH

Boc — Leu — Ab — OMe
2-8 89%

Sar — NCA/DMF
2-14

H_{2}N

H

Boc — Leu — Ab — N_{4}
2-9

NaOH, 1,4-Dioxane, MeOH

Boc — Leu — Ab — OH
2-10 98%

HCl, 1,4-Dioxane
2-11

Boc — Leu — Ab — N_{4}
2-12 92%

HCl, 1,4-Dioxane
2-13 42%

Boc — Leu — Ab — N_{6}

MeO

MeO

H

Boc — Leu — Ab — N_{6}
2-14 95%

MeO

MeO

H

Boc — Leu — Ab — N_{6}
2-15

HATU, HOAl, DIEA/DMF

MeO

MeO

H

Boc — Leu — Ab — N_{6}
2-16 76% (2 steps)

HBr, AuOAc, MeOH

Boc — Leu — Ab — N_{6}
2-17

HATU, DIEA/DMF

MeO

MeO

H

Boc — Leu — Ab — N_{6}
2-18 89% (2 steps)
Synthesis of 16RD in Scheme S1 and S2
Compounds 1-8 and 2-18 were synthesized according to our previous reports.\(^1\)

**Compound 1-9**
Compound 1-8 (170.3 µmol) was dissolved in EtOH/MeOH (2/1) (6 mL), and to this solution DIEA (1.42 mL, 7.83 mmol) was added. Compound 1-4 (1.06 g, 2.73 mmol) and DMT-MM (0.794 g, 2.73 mmol) was added in this order, and the solution was stirred for 10 days at 38 °C. The solvent was evaporated, and the residue was dried in vacuo for 1 h. The residue was purified with LH20 Sephadex column eluted by CHCl\(_3\)/MeOH (1/1) twice.
Yield: 0.499 g, 123 µmol (72 %)

\(^1\)H NMR (400MHz, CDCl\(_3\)): δ(ppm) 7.90−7.45 (m, 14H, CONHCH\(_2\)CH\(_2\)CH\(_2\)(G\(_0\)), CONHCH\(_2\)CH\(_2\)CH\(_2\)(G\(_1\)), CONHCH\(_2\)CH\(_2\)CH\(_2\)(G\(_2\))), 5.35−5.00 (m, 16H, urethane), 3.40−3.20 (m, 28H, NC\(_2\)H\(_2\)CONHCH\(_2\)CH\(_2\)(G\(_0\)), NC\(_2\)H\(_2\)CONHCH\(_2\)CH\(_2\)(G\(_1\)), NC\(_2\)H\(_2\)CONHCH\(_2\)CH\(_2\)(G\(_2\))), 3.20−2.95 (m, 60H, C\(_2\)H\(_2\)CH\(_2\)N(G\(_0\)), C\(_2\)H\(_2\)CH\(_2\)N(G\(_1\)), C\(_2\)H\(_2\)CH\(_2\)N(G\(_2\))), 2.60−2.40 (m, 56H, CH\(_2\)CH\(_2\)N(G\(_1\)), CH\(_2\)CH\(_2\)N(G\(_2\))), 1.95−1.55 (m, 58H, CH\(_2\)CH\(_2\)N(G\(_0\)), CH\(_2\)CH\(_2\)N(G\(_1\)), CH\(_2\)CH\(_2\)N(G\(_2\))), 1.45−1.30 (s, 144H, BocC\(_3\)H),
m/z: [M] calcd: 4071.851, found: 4071.822, [M+Na]\(^+\) calcd: 4094.841, found: 4093.818

**Compound 1-10**
Compound 1-9 (0.156 g, 38.0 µmol) was dissolved in MeOH (0.5 mL), and 4N HCl/dioxane (1.66 mL) was added to the solution. After stirring the solution for 3 h, diisopropyl ether (5 mL) was added. The solution was stirred for 1 h. When the white solid was deposited, the supernatant was decanted slowly. The residue was washed by diisopropyl ether and dissolved in MeOH. The solution was evaporated and dried in vacuo for 3 h.

\(^1\)H NMR (400MHz, CD\(_3\)OD): δ(ppm) 4.35−4.10 (m, 28H, NCH\(_2\)CONHCH\(_2\)CH\(_2\)(G\(_0\)), NCH\(_2\)CONHCH\(_2\)CH\(_2\)(G\(_1\)), NCH\(_2\)CONHCH\(_2\)CH\(_2\)(G\(_2\))), 3.55−3.35, 3.20−3.05 (m, 116H, CH\(_2\)CH\(_2\)CH\(_2\)N(G\(_0\)), CH\(_2\)CH\(_2\)CH\(_2\)N(G\(_1\)), CH\(_2\)CH\(_2\)CH\(_2\)N(G\(_2\))), 2.35−1.80 (m, 58H, CH\(_2\)CH\(_2\)CH\(_2\)N(G\(_0\)), CH\(_2\)CH\(_2\)CH\(_2\)N(G\(_1\)), CH\(_2\)CH\(_2\)CH\(_2\)N(G\(_2\))),
PAMAM G\(_3\)-(C≡CH)\(_{16}\) (1-11)
HCl salt of Compound 1-10 (38.0 µmol) was dissolved in EtOH (3 mL). DIEA (0.318 mL, 1.748 mmol) was added to the solution, and it was stirred in ice bath for 10 min. 5-pentynoic acid (0.120 g, 1.216 mmol) and DMT-MM (0.338 g, 1.216 mmol) was added to the solution, and it was stirred in ice bath for 10 min and at the room temperature for 12 h. After the solvent was evaporated, the residue
was dissolved in CHCl\textsubscript{3}/MeOH (1:1) and purified with Sephadex LH20 column for three times. Yield: 0.0630 g, 16.8 \textmu mol (44\%) (2steps)

\textsuperscript{1}H NMR (400MHz, CDCl\textsubscript{3}): \delta (ppm) 7.95–7.05 (m, 30H, CONH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}(G\textsubscript{0}), CONH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}(G\textsubscript{1}), CONH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}(G\textsubscript{2}), urethane), 3.40–3.20 (m, 60H, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{0}), CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{1}), CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{2}), NHCH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{3})), 3.15–2.95 (m, 28H, NCH\textsubscript{2}CONHCH\textsubscript{2}CH\textsubscript{2}(G\textsubscript{0}), NCH\textsubscript{2}CONHCH\textsubscript{2}CH\textsubscript{2}(G\textsubscript{1}), NCH\textsubscript{2}CONHCH\textsubscript{2}CH\textsubscript{2}(G\textsubscript{2}), NHCOC\textsubscript{H}\textsubscript{2}CONHCH\textsubscript{2}CH\textsubscript{2}(G\textsubscript{3})), 2.70–2.35 (m, 120H, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{0}), CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{1}), CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{2}), CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{3}), NHCOCH\textsubscript{2}CH\textsubscript{2}CC\textsubscript{H}(G\textsubscript{3})), 2.05–1.95 (m, 16H, NHCOCH\textsubscript{2}CH\textsubscript{2}CC\textsubscript{H}(G\textsubscript{3})), 1.90–1.50 (m, 58H, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{0}), CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{1}), CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(G\textsubscript{2}), NCH\textsubscript{2}CONHCH\textsubscript{2}CH\textsubscript{2}(G\textsubscript{3})

PAMAM G\textsubscript{3}-(Leu-Aib)\textsubscript{6}-(Sar)\textsubscript{22} (1-12) 16RD

CH\textsubscript{2}Cl\textsubscript{2} and MeOH was bubbled by Ar gas for 45 min. Compound 1-11 (0.0108 g, 2.89 \textmu mol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2}/MeOH (1/1) (0.450 mL), and to this solution compound 2-18 (0.280 g, 92.3 \textmu mol) and Cu(I)OAc (7.07 mg, 57.7 \textmu mol) was added. The solution was stirred for 5 min in Ar atmosphere, and CH\textsubscript{2}Cl\textsubscript{2}/MeOH (1/1) (3.00 mL) was added to the solution. The solution was greenish colored like viridian. The solution was purged with Ar gas for four times and was stirred for 16 h in Ar atmosphere. After stirring, the color of the solution was changed to ultramarine color. The solvent was evaporated, and the residue was dried in vacuo separately for 3 h. The residue was dissolved in MeOH and purified with Sephadex LH20 column (1.0 m length, \phi 30 mm for three times and 1.2 m length, \phi 35 mm) for two times. Yield : 0.0285 g, 549 nmol (19%)
SI-2. Synthesis of A$_3$B-LP and ICG-LP (Scheme S3)

Scheme S3. Synthetic scheme of AB-LP, A$_3$B-LP and ICG-LP
Synthesis of ICG-LP and A\textsubscript{3}B-LP in Scheme S3

Compounds 3-13, 3-14 (AB-LP) and 3-16 were synthesized according to our previous reports.\textsuperscript{1-2}

**ICG-Sar\textsubscript{2}-(d-Leu-Aib\textsubscript{6})\textsubscript{2}-OMe (3-15) ICG-LP**

To the solution of Sar-NCA (6.19 mg, 53.8 μmol) in distilled DMF (150 μL), the solution of desalted compound 3-13 (6.57 mg, 5.38 μmol) in distilled DMF (50 μL) was added under Ar atmosphere, and the mixed solution was stirred at the room temperature for 15 h. After polymerization, ICG-sulfo-OSu (1.00 mg, 1.08 μmol) and DCC (0.444 mg, 2.15 μmol) were added to the solution in this order and stirred at the room temperature for 25 h under Ar atmosphere. The solvent was evaporated, and the residue was dissolved in DMF and purified by Sephadex LH20 column. The chain length of Sar was determined by Sar \textit{N-CH\textsubscript{2}} peak’s integrated value of \textit{1H} NMR spectrum. The yield was determined by the absorbed light intensity of ICG moiety (absorption wavelength : 794 nm)

Yield: 2.33 mg, 1.02 nmol (95 %) (2steps)

m/z : [M+Na]\textsuperscript{+} calcd : 2311.37, found : 2309.87, [M+K]\textsuperscript{+} calcd : 2327.47, found : 2324.83

**Fmoc-Sar-OCH\textsubscript{2}-C-NHCO-(CH\textsubscript{2})\textsubscript{2}-CO-(d-Leu-Aib\textsubscript{6})\textsubscript{2}-OMe (3-17)**

Compound 3-16 (0.150 g, 136 μmol) was dissolved in DMF (1.00 mL). To this solution, HATU (0.0748 g, 204 μmol), HOAt (0.0278 g, 204 μmol) was added. After stirring for 5 min, compound 2-12 (114 μmol) and DIEA (0.0680 mL, 420 μmol) were added, and the solution was stirred at the room temperature for 15 h under N\textsubscript{2} atmosphere. The solvent was evaporated, and the residue was dissolved in MeOH and purified with Sephadex LH20 column.

Yield : 0.162 g, 70.3 μmol (62%)

\textit{1H} NMR (400MHz, CD\textsubscript{3}OD): δ(ppm) 8.18~7.90, 7.80~7.70, 7.63~7.42, 7.40~7.20 (m, 37H, FmocbenzeneCH, CNHCOCH\textsubscript{2}CH\textsubscript{2}CO, LeuNH, AibNH), 4.70~4.67, 4.60~3.90 (m, 27H, FmocCH, FmocCH\textsubscript{2}, SarCH\textsubscript{2}, SarCOCH\textsubscript{2}C, LeuCH), 3.65 (s, 3H, OMe), 3.00~2.87 (m, 9H, SarCH\textsubscript{3}), 2.85~2.70, 2.54~2.35 (m, 4H, CNHCOCH\textsubscript{2}CH\textsubscript{2}CO), 1.95~1.40 (m, 54H, LeuCH\textsubscript{3}, LeuCH, AibCH\textsubscript{3}), 1.00~0.75 (m, 36H, LeuCH\textsubscript{3})

m/z : [M+Na]\textsuperscript{+} calcd. : 2326.76, found : 2326.17, [M+K]\textsuperscript{+} calcd : 2342.86, found : 2342.16

**H-Sar-OCH\textsubscript{2}-C-NHCO-(CH\textsubscript{2})\textsubscript{2}-CO-(d-Leu-Aib\textsubscript{6})\textsubscript{2}-OMe (3-18)**

Compound 3-17 (0.100 g, 43.4 μmol) was dissolved in dehydrated CH\textsubscript{3}CN (750 μL). To this solution, piperidine (215 μL), dehydrated CH\textsubscript{3}CN (1.00 mL) and CH\textsubscript{2}Cl\textsubscript{2} (0.500 mL) was added at the same time, and the solution was stirred for 25 min. After then, the main product was solidified by petroleum ether and hexane. The residue was dissolved in CH\textsubscript{2}Cl\textsubscript{2}, and the solvent was evaporated.

Yield : 0.0520 g, 31.8 μmol (73%)

m/z : [M+Na]\textsuperscript{+} calcd. : 1661.04, found : 1659.41, [M+K]\textsuperscript{+} calcd : 1677.14, found : 1675.38
(CH$_3$O-CH$_2$-CO-Sar$_2$OCH$_2$)-C-NHCO-(CH$_3$)$_2$-CO-(n-Leu-Aib)$_n$-OMe (3-20) $A3B$-LP

To the solution of Sar-NCA (0.274 g, 2.38 mmol) in distilled DMF (5.00 mL), the solution of compound 3-18 (0.0520 g, 31.8 μmol) in distilled DMF (1.50 mL) was added under Ar atmosphere, and the mixed solution was stirred at the room temperature for 15.5 h. After polymerization, methoxyacetic acid (36.3 μL, 476 μmol), HATU (0.254 g, 667 μmol) and HOAt (0.0910 g, 667 μmol) were added to the solution in this order. After stirring for 5 min at 0 °C, DIEA (0.166 mL, 0.953 mmol) was added to the solution, and the solution was stirred at the room temperature for 10 h under Ar atmosphere. The solvent was evaporated, and the residue was dissolved in MeOH and purified by Sephadex LH20 column for three times. The chain length of Sar was determined by Sar $N$-CH$_2$ peak’s integrated value of $^1$H NMR spectrum and the peak top of MALDI-TOF-MS spectrum.

Yield: 0.0430 g, 5.50 μmol (17%) (2steps)

$^1$H NMR (400MHz, CD$_3$OD): δ(ppm) 8.25~7.70 (m, 13H, CNHCOCH$_2$CH$_2$CO, LeuNH, AibNH), 4.60~3.95 (m, 185H, CH$_3$OCH$_2$CO, SarCH$_2$), 3.65 (s, 3H, OMe), 3.50~3.40 (m, 6H, LeuCH), 3.20~2.85 (m, 261H, SarCH$_3$), 2.82~2.70, 2.62~2.40 (m, 4H, CNHCOCH$_2$CH$_2$CO), 1.95~1.50 (m, 54H, LeuCH$_2$, LeuCH, AibCH$_3$), 1.05~0.85 (m, 36H, LeuCH$_3$)

m/z: [M+Na]$^+$ calcd.: 7847.77, found: 7848.62
SI-3. Synthesis of A₃B-apLP (Scheme S4)

Scheme S4. Synthetic scheme of A₃B-apLP

\[ \text{Ac-DLeu-OH} \quad \text{HCl H-Alb-OMe} \quad \text{DCC, HOBT, TEA/DMF} \quad \text{Ac-DLeu-Alb-OMe} \]

\[ \text{Ac-DLeu-Alb-OH} \quad \text{NaOH /1,4-Dioxane, MeOH} \quad \text{Ac-DLeu-Alb-OH} \]

\[ \text{Ac-DLeu-Alb-OMe} \quad \text{DCC, HOBT, TEA/DMF} \quad \text{Ac(DLeu-Alb)OMe} \]

\[ \text{Ac-(DLeu-Alb)OH} \quad \text{NaOH /1,4-Dioxane, MeOH} \quad \text{Ac-(DLeu-Alb)OH} \]

\[ \text{Ac-(DLeu-Alb)N} \quad \text{HATU, HOA/I, D/EIA/DMF} \quad \text{Ac-(DLeu-Alb)N} \]

\[ \text{Ac-(DLeu-Alb)NH} \quad \text{25% HBr / AcOH} \quad \text{Ac-(DLeu-Alb)NH} \]

\[ \text{N₂CCO₂H} \quad \text{COMU, OxamaPure / DMF} \quad \text{N₂CCO₂H} \]

\[ \text{CuCl/CH₂Cl₂, MeOH} \quad \text{CuCl/CH₂Cl₂, MeOH} \]

\[ \text{Ac-(DLeu-Alb)N} \quad \text{HATU, HOA/I, D/EIA/DMF} \quad \text{A₃B-apLP} \]
Synthesis of A3B-apLP in Scheme S4

**Ac-**D-Leu-Aib-OMe (4-2)**
Ac-**D-Leu-OH (1.00 g, 5.77 mmol) was dissolved in DMF (3.00 mL). To this solution, DCC (1.43 g, 6.93 mmol) and HOBt (1.17 g, 8.66 mmol) dissolved in DMF (4.00 mL) were added, and compound 4-1 (1.06 g, 6.93 mol) and TEA (1.21 mL, 8.66 mmol) were added into the solution. It was stirred at 0 °C for 15 min and at the room temperature for 16 h. The solvent was evaporated, and chilled ethyl acetate was added to the residue. The solution was cooled for 1 h, filtered and washed with 4 wt% KHSO4 aq. and saturated NaHCO3 aq. for three times each. The organic phase was washed with brine, dried over anhydrous MgSO4 and filtered. The solvent was evaporated, and the residue was dried in vacuo. The residue was washed with diisopropyl ether for two times, and the residue was dissolved in chloroform. The solvent was evaporated, and the residue was dried in vacuo.
Yield : 1.18 g, 4.33 mmol (75%)
1H NMR (400MHz, CDCl3): δ(ppm) 6.76 (s, 1H, AibN), 6.11~6.05 (m, 1H, urethane), 4.45~4.40 (m, 1H, LeuC), 3.71 (s, 3H, OMe), 2.00 (s, 3H, AcC), 1.71~1.44 (m, 9H, LeuC, LeuC, AibC), 1.00~0.90 (m, 6H, LeuC)

**Boc-(**D-Leu-Aib)3-NH-(CH2)2-NH-Z (4-6)**
Compound 3-10 (0.506 g, 555 μmol) was dissolved in DMF (3.00 mL). To this solution, HATU (0.317 g, 833 μmol), HOAt (0.113 g, 833 μmol) was added. HCl • H2N-(CH2)2-NH-Z (0.154 g, 666 μmol) and DIEA (0.291 mL, 1.67 mmol) were added to the solution, and the solution was stirred at 0 °C for 15 min and the room temperature for 17 h under N2 atmosphere. The solvent was evaporated, and the residue was dissolved in CHCl3 and filtered. The solution was washed with 4 wt% KHSO4 aq. and saturated NaHCO3 aq. for four times each. The organic phase was washed with brine and dried over anhydrous Na2SO4 for 1 h. After the solution was filtered, the solvent was evaporated, and the residue was dried in vacuo.
Yield : 0.562 g, 0.517 mmol (93%)
1H NMR (400MHz, CDCl3): δ(ppm) 7.64~7.62, 7.50, 7.40~7.28, 6.69, 6.37~6.30 (m, 14H, LeuN, AibN, NHCH2CH2NH, ZCH), 5.18~4.98 (m, 3H, urethane, ZCH), 4.28~4.20, 4.02~3.84 (m, 4H, LeuCH), 3.48~3.25 (m, 4H, NHCCH2CH2NH), 1.85~1.40 (m, 45H, BocCH, LeuCH, LeuCH, AibCH3), 1.05~0.80 (m, 24H, LeuCH3)

**Ac-(**D-Leu-Aib)6-NH-(CH2)2-NH-Z (4-8)**
Compound 4-5 (0.470 g, 1.03 mmol) was dissolved in DMF (4.00 mL). To this solution, HATU (0.844 g, 2.22 mmol), HOAt (0.302 g, 2.22 mmol) was added. After stirring for 5 min, compound 4-7 (1.46 g, 1.48 mmol) and DIEA (0.722 mL, 4.14 mmol) were added, and the solution was stirred at the room
temperature for 16 h under N₂ atmosphere. The solvent was evaporated, and the residue was dissolved in CHCl₃ and filtered. The solution was washed with 4 wt% KHSO₄ aq. for four times and saturated NaHCO₃ aq. for three times. The organic phase was washed with brine and dried over anhydrous Na₂SO₄ for 1 h. After the solution was filtered, the solvent was evaporated, and the residue was dried in vacuo, dissolved in DMF and purified with Sephadex LH20 column.

Yield : 0.112 g, 78.6 μmol (8%)

¹H NMR (400MHz, CDCl₃): δ(ppm) 7.67~7.50, 7.35~7.27, 7.22, 6.86, 6.41 (m, 16H, LeuN₄H, AibN₄H, N₃CH₂CH₂N₃, ZCH), 5.12~4.99 (m, 3H, urethane, ZCH₂), 4.20~4.05, 4.00~3.90 (m, 6H, LeuC₂H), 3.45~3.20 (m, NHC₂H₂CH₂NH), 2.04 (s, 3H, AcC₃H), 1.90~1.30 (m, 54H, LeuC₂H, LeuC₂H, AibC₃H), 1.10~0.75 (m, 36H, LeuC₂H)  
m/z : [M+Na]⁺ calcd : 1447.8, found : 1448

Ac-(D-Leu-Aib)₂-NH-(CH₂)₂-NHCO-(CH₂)₄-N₃ (4-10)

Compound 4-9 (32.9 mg, 25.5 μmol) was dissolved in DMF (1.40 mL) at 0 °C, and COMU (19.7 mg, 45.9 μmol) and Oxyma Pure (6.52 mg, 45.9 μmol) were added to the solution. 5-Azido pentanoic acid (5.47 mg, 38.2 μmol) and DIEA (7.44 μL, 45.9 μmol) were added, and the solution was stirred under N₂ atmosphere at 0 °C for 20 min. After then, the solution was stirred at the room temperature for 16 h. The solvent was evaporated, and the residue was dried in vacuo for 4 h. The residue was dissolved in DMF, and purified by Sephadex LH20 column.

Yield : 10.6 mg, 7.49 μmol (29 %)

m/z : [M+H]⁺ calcd : 1416.97, found : 1431.16

(Fmoc-Sar-OCH₂)₂-C-NHCO-(CH₂)₂-COH-(CH₂)₂-C≡CH (4-11)

Compound 3-16 (0.332 g, 301 μmol) was dissolved in DMF (2.00 mL). To this solution, HATU (0.199 g, 543 μmol), HOAt (0.0739 g, 543 μmol) was added at 0 °C. After stirring for 5 min at 0 °C, H₂N-(CH₂)₂-C≡CH (29.6 μL, 362 μmol) and DIEA (0.132 mL, 814 μmol) were added, and the solution was stirred at 0 °C for 30 min and at the room temperature for 22 h under N₂ atmosphere. The solvent was evaporated, and the residue was dissolved in CHCl₃/MeOH (30:1) and chromatographed on silica gel with CHCl₃/MeOH (30:1).

Yield : 0.355 g, 3.08 μmol (quant.)

¹H NMR (400MHz, CDCl₃): δ(ppm) 7.78~7.70, 7.64~7.45, 7.40~7.27, 6.52~6.05 (m, 26H, FmocbenzeneCH, CNHCOCH₂CH₂CO), 4.50~4.30, 4.28~4.17, 4.10~3.90 (m, 15H, FmocCH, FmocCH₂, SarCH₂), 3.05~2.92 (m, 9H, SarC₃H), 2.50~2.30 (m, 4H, CNHCOC₂H₂C), 1.92 (s, 1H, NH₂CH₂CH₂CCCH), 1.57 (s, 6H, SarCOCH₂C)

m/z : [M+H]⁺ calcd : 1152.46, found : 1152, [M+Na]⁺ calcd : 1174.45, found : 1174
\((\text{CH}_3\text{O-CH}_2\text{CO-Sar}_n\text{OCH}_2\text{-CO-Sar}_n\text{-OCH}_2\text{-CO-NHCO-(CH}_2\text{)}_2\text{-CONH-(CH}_2\text{)}_2\text{-C≡CH (4-14)})\)

Compound 4-11 (0.100 g, 86.8 μmol) was dissolved in dehydrated CH₃CN (1.50 mL), and to this solution, the mixed solution of piperidine (0.429 mL, 4.34 mmol), dehydrated CH₃CN (2.00 mL) and super-dehydrated CH₂Cl₂ (1.00 mL) was added. The solution was stirred at room temperature for 25 min. As soon as the reaction was finished, super-dehydrated CH₂Cl₂ (about 10 mL) was added, the main product was solidified by hexane for four times. The residue was dissolved in CH₂Cl₂, the solvent was evaporated. The residue (compound 4-12) was dried in vacuo for 7 h.

To the solution of Sar-NCA (0.533 g, 4.63 mmol) in distilled DMF (10.0 mL), the solution of compound 4-12 (30.0 mg, 61.8 μmol) in distilled DMF (6.00 mL) was added under Ar atmosphere, and the mixed solution was stirred at the room temperature for 14 h. After the NCA polymerization, HATU (0.494 g, 1.30 mmol), HOAt (0.177 g, 1.30 mmol), methoxy acetic acid (70.7 μL, 0.927 mmol) and DIEA (0.323 mL, 1.85 mmol) was added to the solution in this order and stirred at 0 °C for 15 min under Ar atmosphere. After then, the solution was stirred at the room temperature for 7 h under Ar atmosphere. The solvent was evaporated, and the residue was dissolved in MeOH and purified by Sephadex LH20 column for two times.

Yield : 0.154 g, 29.7 μmol (34 %) (3steps)
m/z : [M+Na]⁺ (n = 22) calcd : 5199.64, found : 5199.69

\(\text{Boc-}\text{(D-Leu-Alb)}_2\text{-NH-(CH}_2\text{)}_2\text{-NHCO-(CH}_2\text{)}_2\text{-NHCO-(CH}_2\text{)}_2\text{-CONH-C-(CH}_2\text{O-Sar}_n\text{CO-CH}_2\text{OCH}_3\text{)3 (4-15) A,B-apLP}\)

CH₂Cl₂ and MeOH was bubbled by Ar gas for 50 min. Compound 4-10 (0.0660 g, 44.8 μmol) was dissolved in CH₂Cl₂/MeOH (1/1) (0.400 mL), and to this solution compound 4-14 (0.0860 g, 16.6 μmol) and Cu(I)OAc (5.49 mg, 44.8 μmol) was added. The solution was stirred for 5 min under Ar atmosphere, and CH₂Cl₂/MeOH (1/1) (0.600 mL) was added to the solution. The solution was purged with Ar gas for five times and was stirred for 23 h in Ar atmosphere with protection from light. The solvent was evaporated, and the residue was dried in vacuo separately for 5 h. The residue was dissolved in MeOH and purified with Sephadex LH20 column (1.0 m length, φ 30 mm for three times and 1.2 m length, φ 35 mm) for two times. After Sephadex LH20 column purification, the product was purified with Asahipak GS-310 20F GPC column.

Yield : 0.0203 g, 2.89 μmol (17%)
m/z : [M+Na]⁺ (n = 24) calcd : 7102.87, found : 7093.42

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Figure S2. The self-assemblies were analyzed by circular dichroism (CD) measurements. The self-assemblies of pure 16RD showed double minima at 208 and 222 nm indicating α-helical structure. The self-assemblies prepared at the feed molar ratios of 2:1 and 4:1, which were purified by a PD-10 column (packed Sephadex G-25, GE Healthcare), decreased the intensities of the Cotton effects upon increasing the amounts of AB-LP, and no signal was observed with the self-assemblies prepared at the feed molar ratio of 16:1. The self-assemblies prepared at the feed molar ratios of 24:1 and 32:1, which were purified by a PD-10 column and a cut-off filter of 0.1 mm to obtain self-assemblies of several tens of nm, show no signal at all. These results also support the interpretation that no more than sixteen molecules of AB-LP are inserted into 16RD even in the presence of excess amounts of AB-LP.
SI-5. Size of molecular assemblies of 16RD/A₃B-LP, 16RD/A₃B-apLP (Figure S3)

Figure S3. Hydrodynamic diameters vs feed molar ratios of A₃B-LP/16RD (up) and A₃B-apLP/16RD (bottom) by DLS measurement.
SI-6. Rational size of molecular assembly with using 16RD and A$_3$B-apLP (Figure S4)

**3G dendrimer core**

![Diagram showing the schematic illustration and rational size of dendrimer 16RD](image)

Figure S4. The schematic illustration and rational size of dendrimer 16RD
SI-7. *In vivo* NIRF imaging (Figure S5)

Figure S5. The time-lapsed NIRF images of nanocarrier from A3B-apLP/16RD/ICG-LP after 1st (left column) and 2nd administrations (right column).

Figure S5. The time-lapsed NIRF images of nanocarrier from A3B-apLP/16RD/ICG-LP after 1st (left column) and 2nd administrations (right column).
SI-8. 2nd administration of A₃B-LP/16RD (Figure S6)

Figure S6. The time profiles of ROI at tumor site, liver site and background (left-leg site).

Reference in supporting information