Electronic Supplementary Information (ESI)

Molecular Assembly Composed of Dendrimer Template and Block Polypeptide through Stereocomplex Formation

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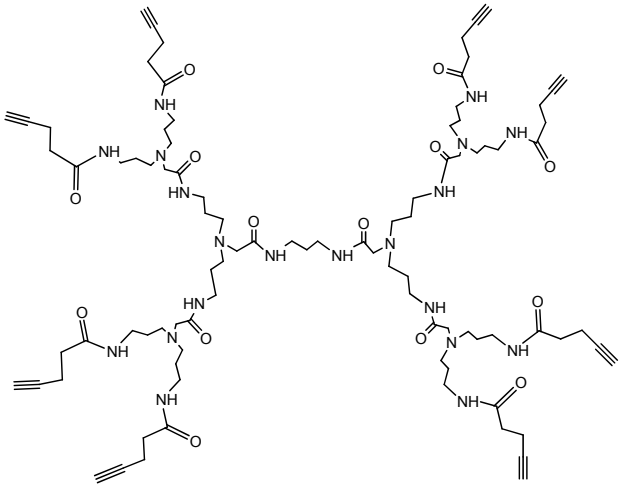
Chemical structures



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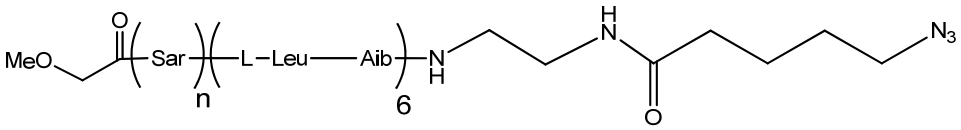
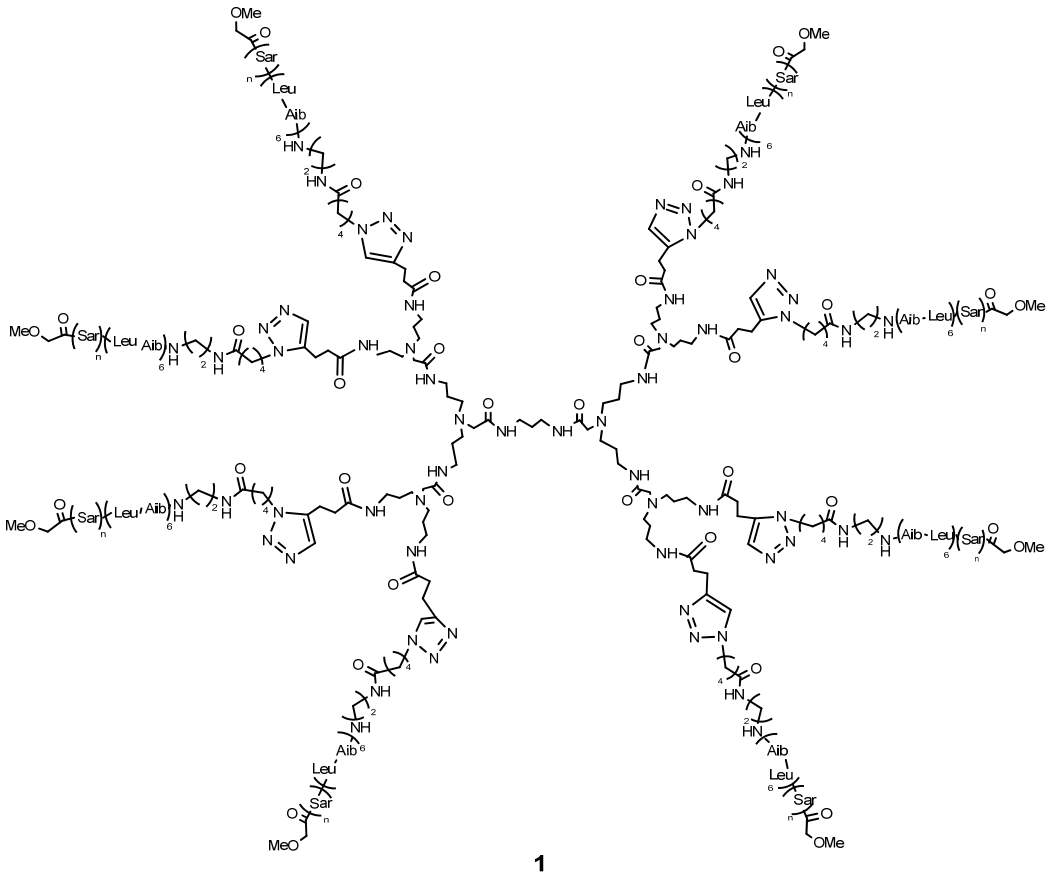
Hydrophobic core of 2nd generation dendrimer



Amphiphilic right-handed helical blockpolypeptide (n = 25) (RP)



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The TEM micrograph of molecular assemblies prepared from the left-handed helical blockpeptide (LP) (Ref. 12, 13)

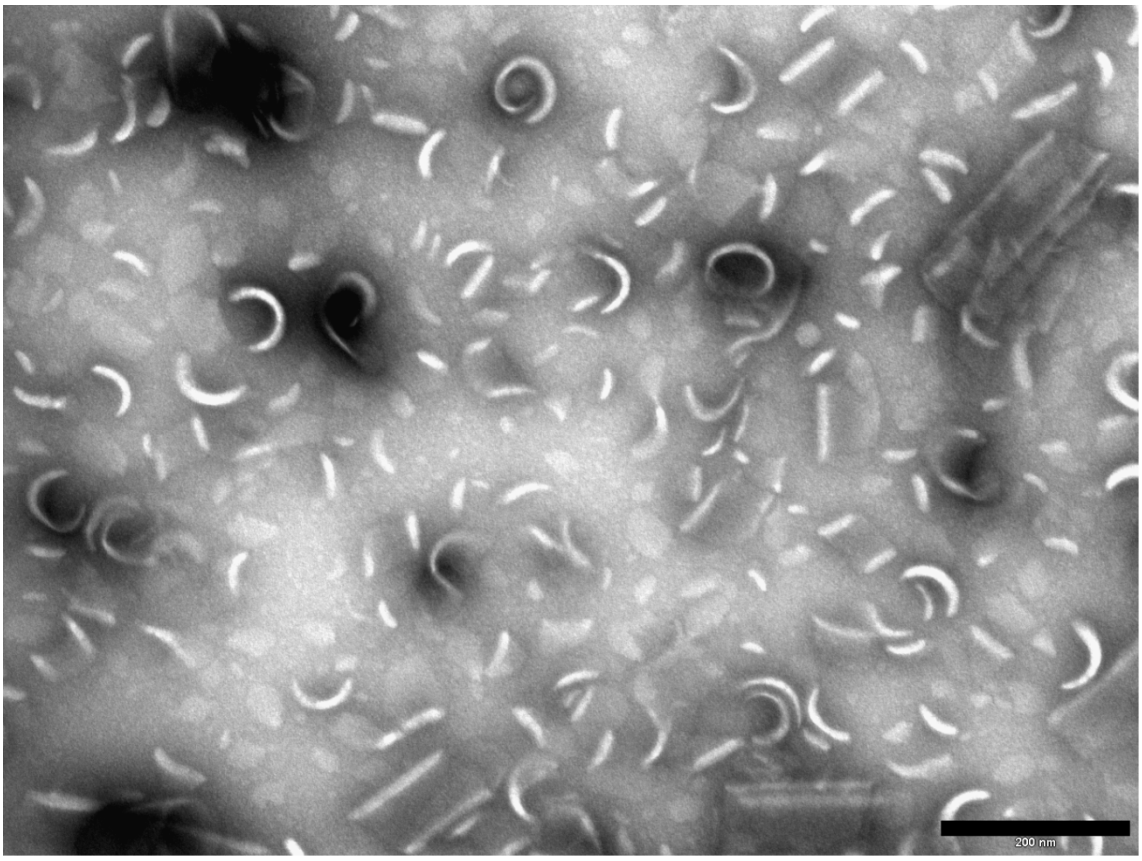


Fig. S1. Negative staining TEM micrograph of assemblies prepared from pure **LP**. The scale bar represents 200 nm.

TEM micrographs

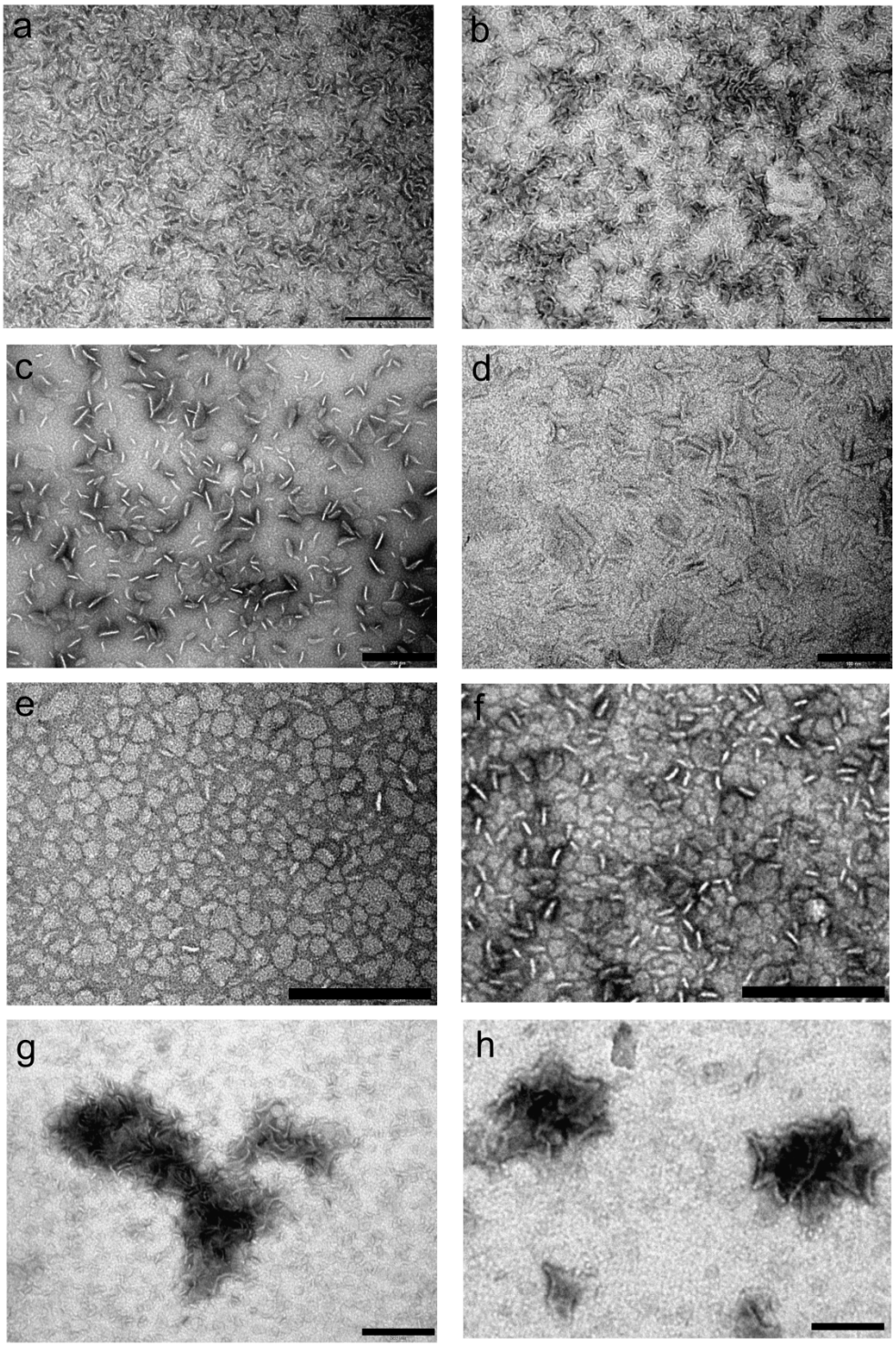


Fig. S2. Negative staining TEM micrographs : **LP/8RD =** (**a**) 0/1, (**b**) 1/1, (**c**) 2/1, (**d**) 4/1, (**e**) 8/1, (**f**) 16/1, (**g**) 24/1 and (**h**) 32/1. The scale bar represents (**a**-**c**, **g, h**) 200 nm, and (**d-f**) 100 nm.

DLS measurement (sonication effect)

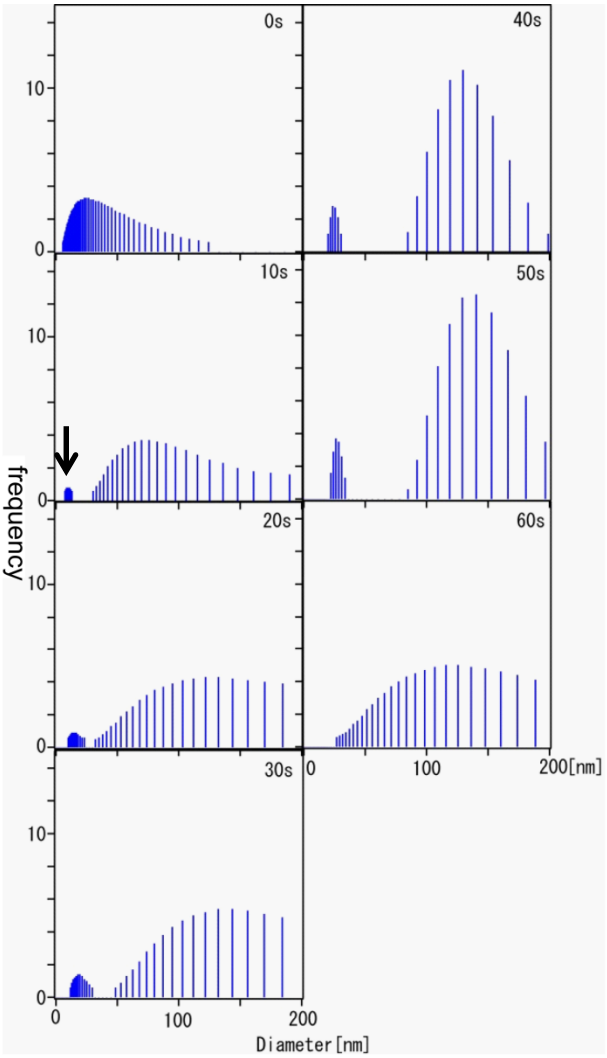


Fig. S3. DLS profiles of 8/1 **LP/8RD** assemblies with treatment of sonication with varying the sonication periods from 0 seconds to 60 seconds at 10 second interval. The arrow shows the minimum size among all the samples (19.9 ± 3.6 nm).

CD measurements

The molecular assemblies were prepared by the injection method. An ethanol solution of **8RD** and **LP** at the specified feed ratio was injected into a 10 mM Tris buffer solution (pH 7.4, added 0.15 M NaCl) of 1 mL. The residue concentration at the each feed mol ratio was calculated with the following equation.

$$(C_1L_1N_1/M_1+C_2L_2N_2/M_2) \times 10^{-3} / 1.0\times10^{-3} \text{ [mol/L]}$$

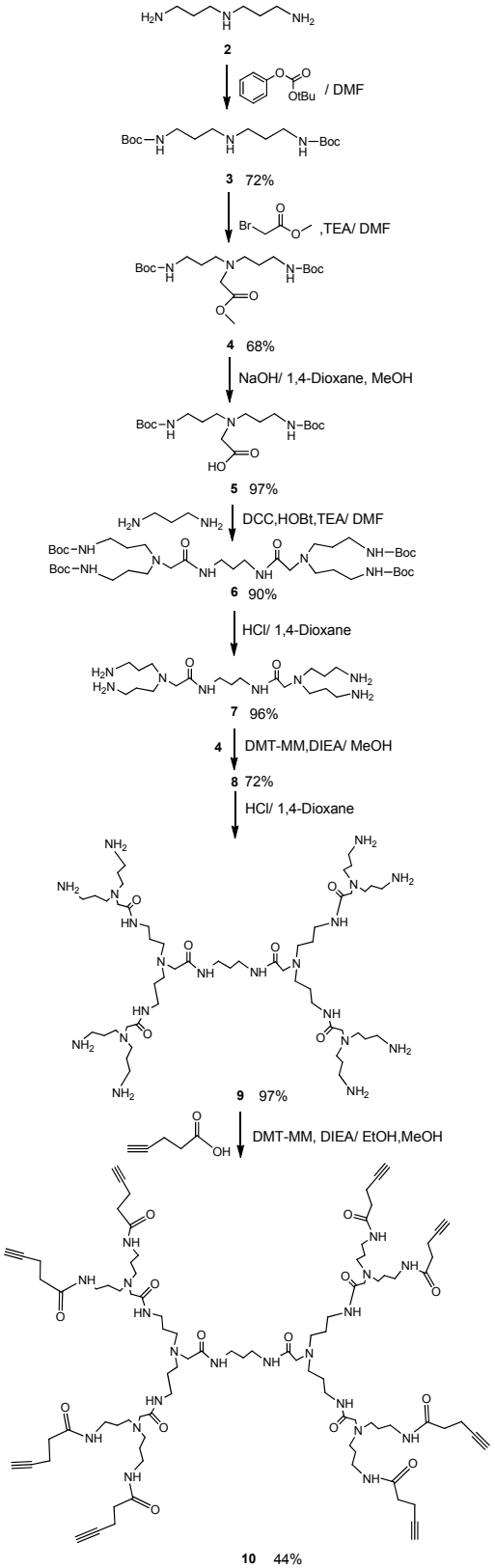
C : the concentration of **8RD** or **LP** ethanol solution (**8RD** : 0.1 mg/μL,
LP : 0.05 mg/μL)
L : the volume of **8RD** or **LP** ethanol solution (μL)
N : the residue number of peptides in **8RD** (N_1 = **96**, 12 mer×8) or **LP** (N_2 = **12**)
M : molecular weight of **8RD** or **LP** (**8RD** : about 27000 g/mol, **LP** : about 3000 g/mol)

The residue concentrations at CD measurements were as follows.
8RD/**LP** =

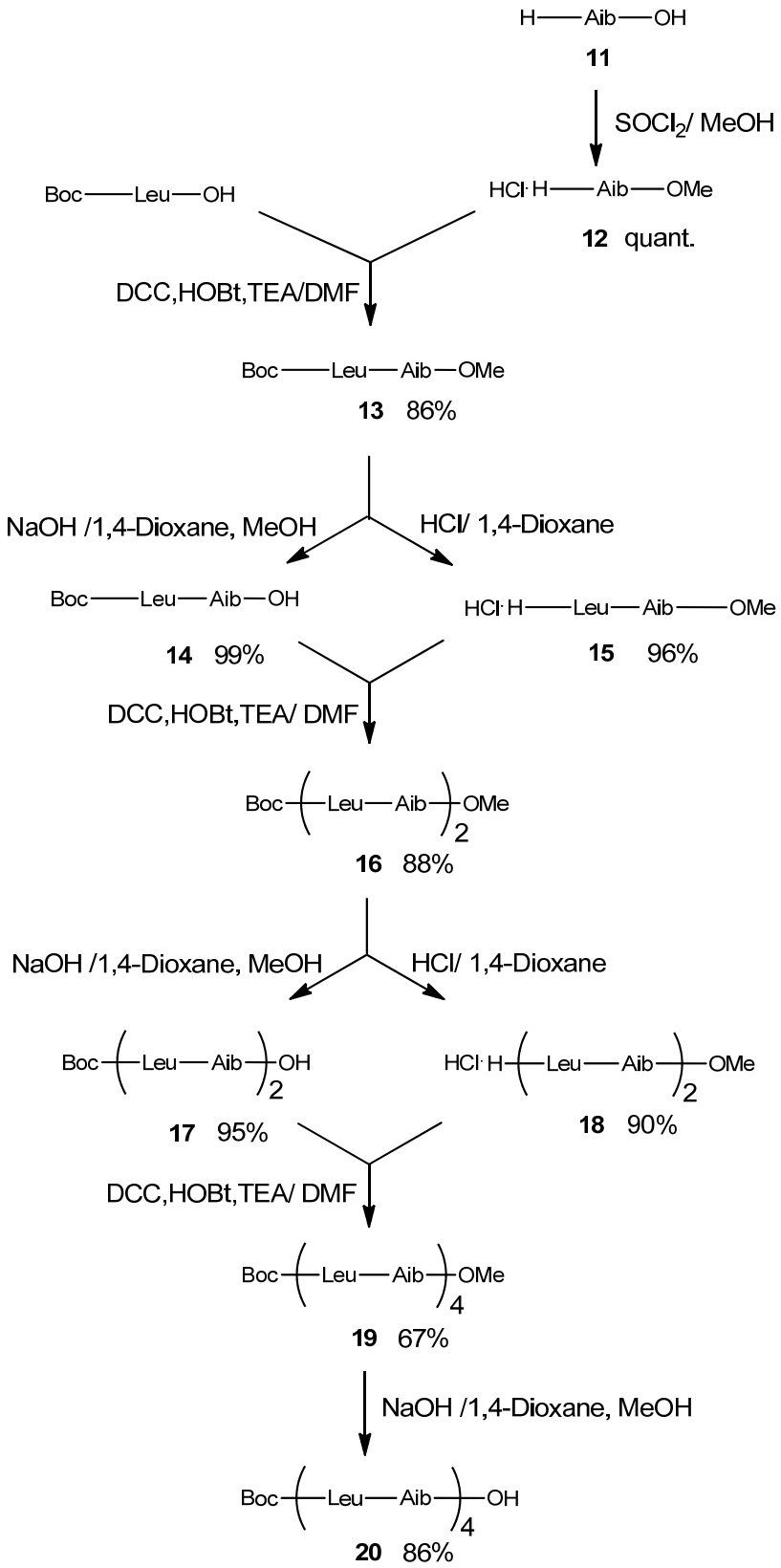
(1/0)	4.0×10^{-3} [mol/L]	(L1=11.25 μL, L2=0 μL)
(1/2)	2.5×10^{-3} [mol/L]	(L1=5.63 μL, L2=2.50 μL)
(1/4)	1.5×10^{-3} [mol/L]	(L1=2.81 μL, L2=2.50 μL)
(1/8)	1.0×10^{-3} [mol/L]	(L1=1.41 μL, L2=2.50 μL)
(1/16)	0.75×10^{-3} [mol/L]	(L1=0.703 μL, L2=2.50 μL)
(1/24)	0.67×10^{-3} [mol/L]	(L1=0.469 μL, L2=2.50 μL)
(1/32)	0.63×10^{-3} [mol/L]	(L1=0.352 μL, L2=2.50 μL)

Before CD measurements, each sample was filtered by disposable PD-10 column (packed Sephadex G-25 support) to purify the objective molecular assemblies.

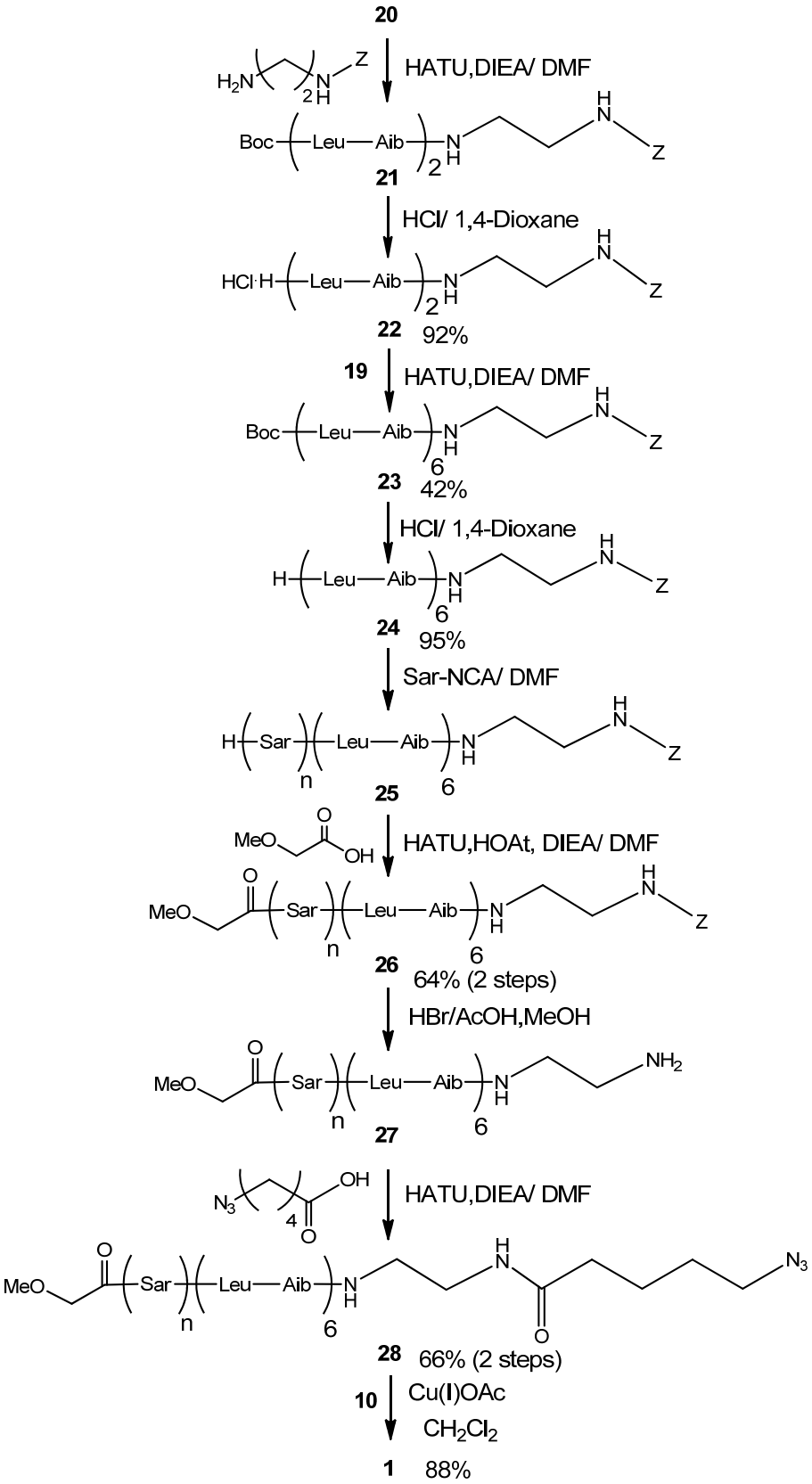
Synthetic Scheme



Scheme S1. Synthesis of the hydrophobic dendrimer core.



Scheme S2. Synthesis of the hydrophobic block polymer 8-mer.



Scheme S3. Synthesis of the amphiphilic dendrimer micelle.

Materials and Methods

Preparation of Molecular Assemblies. 8RD (Compound **1**) (10 mg) was dissolved in ethanol (100 μL). Then each mixed solution of the left-handed helical blockpeptides (0.05 mg/μL) and 2nd dendrimer (0.1 mg/μL) with the ratio of 1:0, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32 was injected into a buffer (0.5 mL, 10 mM Tris-HCl, pH 7.4) with stirring at 0 °C. On mixing the solutions, the volume of **8RD** was calculated as D-blockpolypeptides have consant volume (2.5 μL).

Dynamic Light Scattering (DLS). The hydrodynamic diameter of assemblies were measured by DLS-8000KS (Photal Otsuka Electronics) using He-Ne laser. Before DLS measurement, each prepared sample was filtered by 0.20 μm PVDF (polyvinylidene fluoride) syringe filter (GE Healthcare UK limited).

Transmission Electron Microscopy (TEM). TEM images were taken using a JEOL JEM-2000EXII at an accelerating voltage of 100 kV. For the observation, a drop of dispersion was mounted on a carbon-coated Cu grid and stained negatively with 2% uranyl acetate, followed by suction of the excess fluid with a filter paper.

Fourier Transform Infrared Spectroscopy (FT-IR). Infrared transmission spectroscopy of the assembly dispersion was performed on a Fourier transform infrared spectrometer (Nicolet 6700 FT-IR, Thermo Fisher Scientific, MA) at room temperature with a solution cell.

Circular Dichroism (CD). CD measurements were carried out on a JASCO J600 spectropolarimeter with an optical cell of 0.1 cm optical path length at room temperature.

Synthetic Method and Measurement Result

(Blockpolypeptides)

・**CH_3O-CH_2-CO-(Sar)$_n$-(Leu-Aib)$_6$-NH-$(CH_2)_2$-NH-CO-$(CH_2)_4$-N_3 (28)**

Compound **27** (73.5μmol) was dissolved in corresponding amount of DMF. To this solution, N_3-$(CH_2)_4$-COOH (52.6 μL, 368 μmol), HATU (135 mg, 368 μmol) and DIEA (89.4 μL, 551 μmol) were added, and the solution was stirred at the room temperature for 12 h under N_2 atmosphere. The solvent was evaporated, and the residue was dissolved in MeOH and purified by Sephadex LH20. The presentence of N_3 was confirmed by FT-IR to monitor characteristic absorption (around 2100 cm^{-1}).

Yield: 0.201 g, 59.6 μmol (63 %) (2steps)

^{1}H NMR (400MHz, CD_3OD): δ(ppm) 4.55–3.90 (m, 69H, CH_3OCH_2CO, LeuC*H*, ethylenediamineCH_2, SarCH_2), 3.15–2.70 (m, 90H, CH_3OCH_2CO, SarNCH_3), 1.95-1.30 (m, 62H, LeuCH_2, LeuC*H*, AibCH_3, $CH_2CH_2CH_2CH_2N_3$), 1.15–0.75 (m, 36H, LeuCH_3)

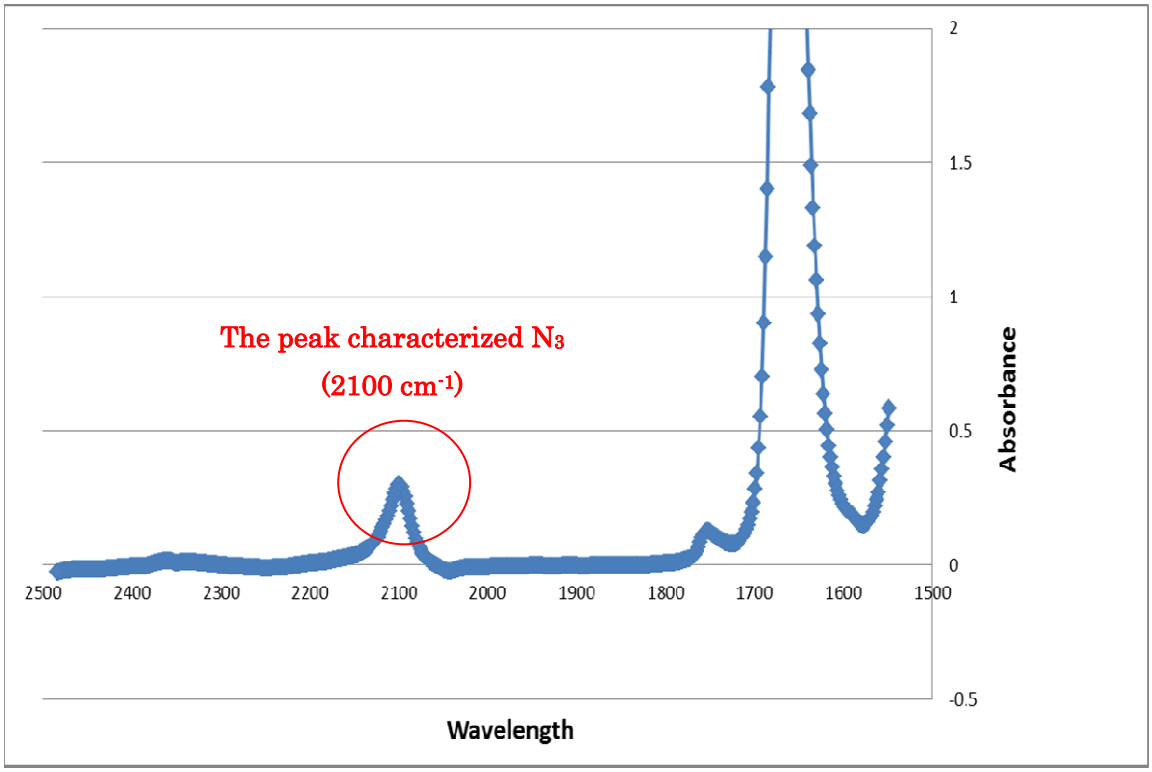


Fig. S4. FT-IR spectrum of compound **28**

(Dendrimer)

G0 means the part of nucleus compound, G1 means the one of 1st generation and G2 means the one of 2nd generation.

- **Boc-NH-$(CH_2)_3$-NH-$(CH_2)_3$-NH-Boc (3)**

Compound **2** (2.13 mL, 15.0 mmol) was dissolved in DMF (100 mL), and *tert*-butyl phenyl carbonate (6.94 mL, 37.5 mmol) was slowly added to the mixed solution. The solution was stirred at the room temperature for 22 h. The solvent was evaporated, and the residue was dissolved in CH_2Cl_2 (150 mL). After the solution's pH having been adjusted to 3 by adding 4% $KHSO_4$ aq., the product was collected into aqueous phase. After then, the aqueous phase's pH was adjusted to 10 by adding 2N NaOH aq., and the product was collected into the organic phase. The organic phase was washed by brine, dried over anhydrous Na_2SO_4 for 1 h and filtered. The solvent was evaporated, and the residue was dissolved in $CHCl_3$/MeOH (20:1) and chromatographed on silica gel with $CHCl_3$/MeOH (20:1, 10:1, 5:1) for two times.

Yield: 3.58 g, 10.8 mmol (72 %)

^{1}H NMR (400MHz, $CDCl_3$): δ(ppm) 5.19 (s, 2H, urethane), 3.24–3.23 (m, 4H, C*H*$_2$CH$_2$CH$_2$), 2.71 (m, 4H, CH$_2$CH$_2$C*H*$_2$), 1.74 (m, 5H, CH$_2$C*H*$_2$CH$_2$, CH$_2$N*H*CH$_2$), 1.43 (s, 18H, BocC*H*$_3$)

- **Boc-NH-$(CH_2)_3$-N(CH_2COOCH_3)-$(CH_2)_3$-NH-Boc (4)**

To the solution of Compound **3** (3.58 g, 10.8 mmol) dissolved in DMF (30.0 mL), methyl bromoacetate (1.88 mL, 16.2 mmol) and TEA (2.50 mL, 19.4 mmol) were slowly added in this order. The mixed solution was stirred for 17 h. After the solvent was evaporated, the residue was chromatographed on silica gel with $CHCl_3$.

Yield: 4.32 g, 10.7 mmol (99 %)

^{1}H NMR (400MHz, $CDCl_3$): δ(ppm) 5.29 (s, 2H, urethane), 3.71 (s, 3H, OMe), 3.27 (s, 2H, NC*H*$_2$COOMe), 3.20–3.18 (m, 4H, C*H*$_2$CH$_2$CH$_2$), 2.57–2.54 (m, 4H, CH$_2$CH$_2$C*H*$_2$), 1.63–1.61 (m, 5H, CH$_2$C*H*$_2$CH$_2$, CH$_2$N*H*CH$_2$), 1.43 (s, 18H, BocC*H*$_3$)

- **Boc-NH-$(CH_2)_3$-N(CH_2COOH)-$(CH_2)_3$-NH-Boc (5)**

Compound **4** (2.50 g, 6.20 mmol) was dissolved in the mixed solvent of MeOH (24.8 mL) and 1,4-dioxane (24.8 mL).1N NaOH aq. (12.4 mL) was slowly added to the solution, and the solution was stirred for 3 h. After the pH of the solution being 5 by adding 1N HCl aq., the solvent was evaporated. The residure was dissolved in hyperdehydrated MeOH and filtered for removing NaCl salt.

Yield: 2.34 g, 6.01 mmol (97 %)

^{1}H NMR (400MHz, $CDCl_3$): δ(ppm) 5.66 (s, 2H, urethane), 3.71–3.69 (m, 3H, NC*H*$_2$COO*H*), 3.21–3.19 (m, 8H, C*H*$_2$CH$_2$CH$_2$, CH$_2$CH$_2$C*H*$_2$), 1.94 (m, 4H, CH$_2$C*H*$_2$CH$_2$), 1.51–1.40 (s, 18H,

BocCH_3)

・**Compound 6 (1st generation)**

Compound **5** (0.839 g, 2.15 mmol) was dissolved in corresponding amount of DMF. DCC (0.555 g, 2.69 mmol), HOBt (0.436 g, 3.23 mmol) and TEA (0.451 mL, 3.23 mmol) were added in this order, and then H_2N-$(CH_2)_3$-NH_2 (76.0 μL, 898 μmol) was added to the mixed solution. The solution was stirred at the room temperature for 42 h. The solvent was evaporated, and the residue was dissolved in $CHCl_3$ and chromatographed on silica gel with $CHCl_3$ /MeOH (40:1, 30:1, 20:1).

Yield: 0.662 g, 810 μmol (90 %)

^{1}H NMR (400MHz, $CDCl_3$): δ(ppm) 5.50–5.10 (s, 4H, urethane), 3.35–3.07 (m, 16H, $CH_2CH_2CH_2N(G_1)$, $NCH_2CONHCH_2CH_2(G_0)$, $NCH_2CONHCH_2CH_2(G_0)$), 2.75–2.53 (m, 8H, $CH_2CH_2CH_2N(G_1)$), 1.80–1.64 (m, 10H, $CH_2CH_2CH_2N(G_1)$, $NCH_2CONHCH_2CH_2(G_0)$), 1.50–1.24 (s, 36H, BocCH_3)

・**Compound 7**

Compound **6** (0.351 g, 430 μmol) was dissolved in MeOH (0.835 mL). To this solution, 4N HCl/ dioxane (4.73 mL) was added, and the solution was stirred for 2 h. Then, diisopropyl ether 14 mL was added and stirred for 1 h. Then, white precipitation was produced around the flask. The solvent was decanted, and the residue was washed by MeOH for three times. The washed redidue was dissolved in MeOH and evaporated.

^{1}H NMR (400MHz, CD_3OD): δ(ppm) 3.45–3.38, 3.00–2.90 (m, 16H, $CH_2CH_2CH_2N(G_1)$, $NCH_2CONHCH_2CH_2(G_0)$, $NCH_2CONHCH_2CH_2(G_0)$), 2.85–2.75 (m, 8H, $CH_2CH_2CH_2N(G_1)$), 1.95–1.80 (m, 8H, $CH_2CH_2CH_2N(G_1)$), 1.75–1.65 (m, 2H, $NCH_2CONHCH_2CH_2(G_0)$)

・**Compound 8 (2nd generation)**

HCl salt of Compound **7** was dissolved in corresponding amount of MeOH. DIEA (0.624mL, 3.45 mmol), compound **5** (0.928 g, 2.30 mmol), and DMT-MM (0.948g, 3.45 mmol) were added to the solution in this order, and the solution was stirred at the room temperature for 18 h. After the solvent was evaporated, the residue was dissolved in $CHCl_3$ and washed with saturated $NaHCO_3$ aq. for four times. The organic phase was washed with brine twice, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated, and the residue was dissolved in $CHCl_3$ / MeOH (1:1) and purified with Sephadex LH20 column.

Yield: 0.585 g, 0.308 mmol (72%) (2steps)

^{1}H NMR (400MHz, $CDCl_3$): δ(ppm) 7.85–7.70 (m, 2H, $CONHCH_2CH_2CH_2(G_0)$), 7.60–7.40 (m, 4H, $CONHCH_2CH_2CH_2(G_1)$), 5.20–4.95 (m, 8H, urethane), 3.40–2.95 (m, 40H, $CH_2CH_2CH_2N(G_2)$, $CH_2CH_2CH_2N(G_1)$, $NCH_2CONHCH_2CH_2(G_0)$, $NCH_2CONHCH_2CH_2(G_1)$,

NC*H*$_2$CONHCH$_2$CH$_2$(G$_0$)), 2.60–2.40 (m, 24H, CH$_2$CH$_2$C*H*$_2$N(G$_2$), CH$_2$CH$_2$C*H*$_2$N(G$_1$)), 1.80–1.55 (m, 26H, CH$_2$C*H*$_2$CH$_2$N(G$_2$), CH$_2$C*H*$_2$CH$_2$N(G$_1$), NCH$_2$CONHCH$_2$C*H*$_2$(G$_0$)), 1.50–1.25 (s, 72H, BocC*H*$_3$)

・**Compound 9**

Compound **8** (0.200 g, 105.1 μmol) was dissolved in MeOH (0.4 mL). HCl/ Dioxane (2.26 mL, 9.04 mmol) was added to this solution, and the solution was stirred at the room temperature for 4h. Diisopropyl ether (10 mL) was added to the solution twice every 30 min. After removing the solvent by decantation, the residue was washed by diisopropyl ether twice. The residue was dissolved in MeOH, and the solution was evaporated and dried *in vacuo* for 2h.

Yield: 0.164 g, 102 μmoL (97%)

^{1}H NMR (400MHz, CD$_3$OD): δ(ppm) 3.75–3.65, 3.40–3.25, 3.10–2.95 (m, 64H, C*H*$_2$CH$_2$CH$_2$N(G$_0$), C*H*$_2$CH$_2$CH$_2$N(G$_1$), NCH$_2$CONHC*H*$_2$CH$_2$(G$_0$), NC*H*$_2$CONHCH$_2$CH$_2$(G$_1$), NC*H*$_2$CONHCH$_2$CH$_2$(G$_0$), CH$_2$CH$_2$C*H*$_2$N(G$_2$), CH$_2$CH$_2$C*H*$_2$N(G$_1$)), 2.10–1.75 (m, 26H, CH$_2$C*H*$_2$CH$_2$N(G$_2$), CH$_2$C*H*$_2$CH$_2$N(G$_1$), NCH$_2$CONHCH$_2$C*H*$_2$(G$_0$))

・**Compound 10**

HCl salt of Compound **9** (0.164 g, 101.7 μmol) was dissolved in the mixed solvent of EtOH (2 mL) and MeOH (1 mL). DIEA (0.404 mL, 2.237 mmol) was added to the solution, and it was stirred in ice bath for 5 min. 5-pentynoic acid (0.193 g, 1.627 mmol) and DMT-MM (0.474 g, 1.627 mmol) was added to the solution, and it was stirred in ice bath for 15 min and at the room temperature for 62 h. After the solvent was evaporated, the residue was dissolved in $CHCl_3$ and washed with saturated $NaHCO_3$ aq. for five times. The organic phase was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated, and the residue was dissolved in $CHCl_3$/ MeOH (1:1) and purified with Sephadex LH20 column.

Yield: 0.0780 g, 44.8 μmoL (44%)

^{1}H NMR (400MHz, $CDCl_3$): δ(ppm) 7.95–7.40 (m, 6H, CON*H*CH$_2$CH$_2$CH$_2$(G$_0$), CON*H*CH$_2$CH$_2$CH$_2$(G$_1$)), 7.20–6.80 (m, 8H,urethane), 3.40–3.20 (m, 28H, C*H*$_2$CH$_2$CH$_2$N(G$_0$), C*H*$_2$CH$_2$CH$_2$N(G$_1$), NHC*H*$_2$CH$_2$CH$_2$N(G$_2$)), 3.15–2.90 (m, 12H, NC*H*$_2$CONHCH$_2$CH$_2$(G$_1$), NC*H*$_2$CONHCH$_2$CH$_2$(G$_0$)), 2.70–2.30 (m, 56H, CH$_2$CH$_2$C*H*$_2$N(G$_2$), CH$_2$CH$_2$C*H*$_2$N(G$_1$), NHCOC*H*$_2$C*H*$_2$CCH(G$_2$)), 2.05–1.95 (m, 8H, NHCOCH$_2$CH$_2$CC*H*(G$_2$)), 1.85–1.60 (m, 26H, CH$_2$C*H*$_2$CH$_2$N(G$_2$), CH$_2$C*H*$_2$CH$_2$N(G$_1$), NCH$_2$CONHCH$_2$C*H*$_2$(G$_0$))

m/z : $[M+H]^+$ calcd. : 1743.13, found : 1743.128, $[M+Na]^+$ calcd : 1765.11, found : 1765.108, $[M+K]^+$ calcd : 1781.22, found : 1781.079

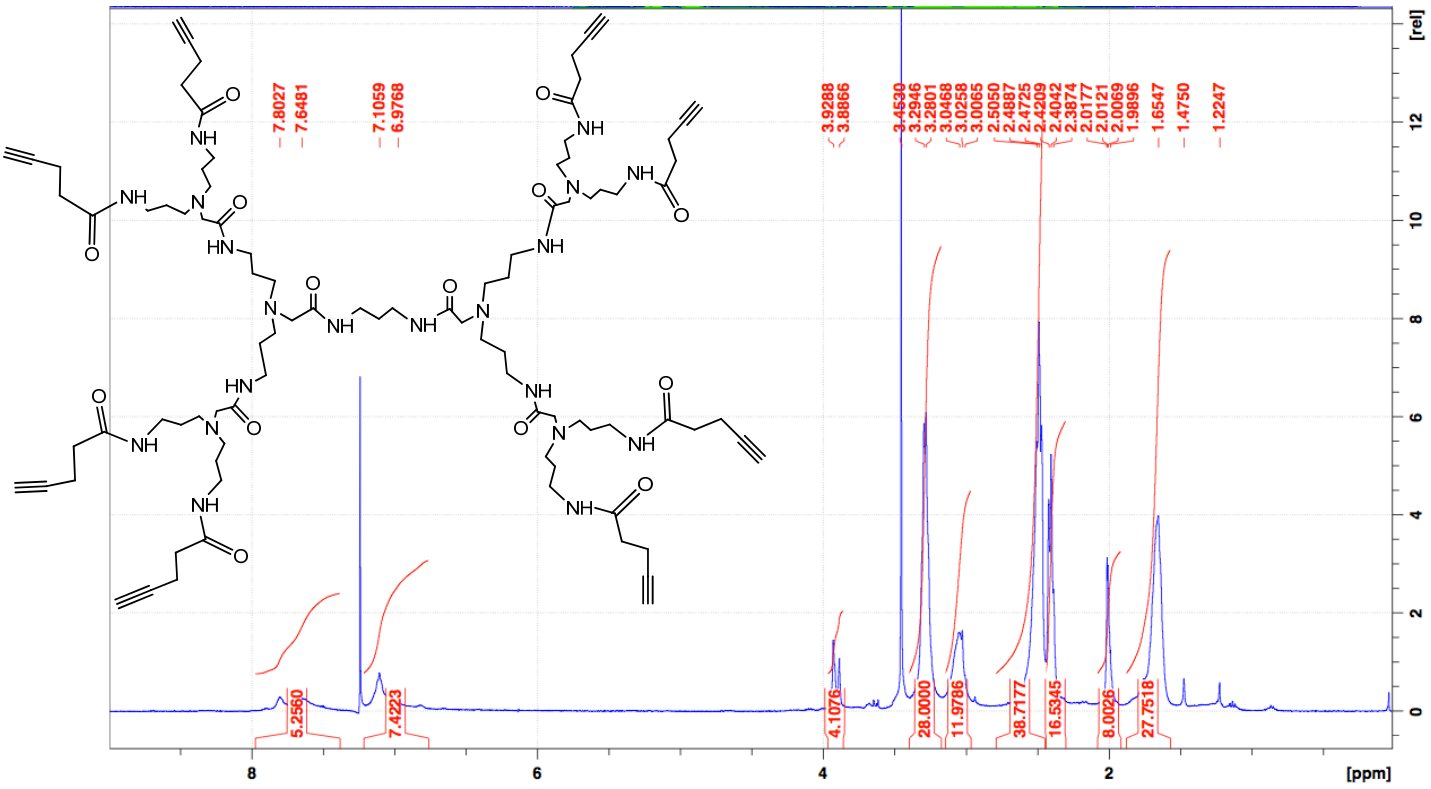


Fig. S5. ^{1}H NMR spectrum of compound **10** (G_2-(C≡CH)$_8$)

(click chemistry)

・PAMAM G_2-(Leu-Aib)$_6$-(Sar)$_{26}$ (1)

CH_2Cl_2 (20 mL) was bubbled by Ar gas for 30 min. Compound **10** (0.01 g, 5.743 μmol) was dissolved in CH_2Cl_2 (250 μL), and to this solution, Compound **28** (0.296 g, 91.89 μmol) and Cu(I)OAc (5.63 mg, 45.94 μmol) was added. The solution was stirred for 2 min, and CH_2Cl_2 (4mL) was added to the solution. The solution was purged with Ar gas for four times and was stirred for 49 h. The solvent was evaporated, and the residue was dried in vacuo for 2 h. The residue was dissolved in $CHCl_3$/MeOH (1:1) and purified with Sephadex LH20 column.

Yield: 0.260 g, 9.44 μmoL (88%)

^{1}H NMR (400MHz, CD_3OD): δ(ppm) 8.30–7.15 (m, 142H, CON*H*$CH_2CH_2CH_2(G_0)$, CON*H*$CH_2CH_2CH_2(G_1)$, triazole cycle C=CH-N, LeuN*H,* AibN*H* ,urethane), 4.55–3.90 (m, 480H, CH_3OCH_2CO, LeuC*H*, ethylenediamineCH_2, SarCH_2), 3.75–3.70 (m, 24H, CH_3OCH_2CO), 3.50–3.20 (m, 60H, ethylenediamineCH_2, $CH_2CH_2CH_2N(G_0)$, $CH_2CH_2CH_2N(G_1)$, NHC$H_2CH_2CH_2N(G_2)$), 3.20–2.85 (m, 636H, SarNCH_3, $CH_2CH_2CH_2N(G_0)$, $CH_2CH_2CH_2N(G_1)$, NHC$H_2CH_2CH_2N(G_2)$), 2.65–2.25 (m, 56H, $CH_2CH_2CH_2N(G_2)$, $CH_2CH_2CH_2N(G_1)$, NHCOCH_2CH_2CCH(G_2)), 2.00–1.45 (m, 522H, LeuCH_2, LeuC*H*, AibCH_3, $CH_2CH_2CH_2CH_2N_3$, $CH_2CH_2CH_2N(G_2)$, $CH_2CH_2CH_2N(G_1)$, $NCH_2CONHCH_2CH_2(G_0)$), 1.05–0.85 (m, 288H, LeuCH_3)

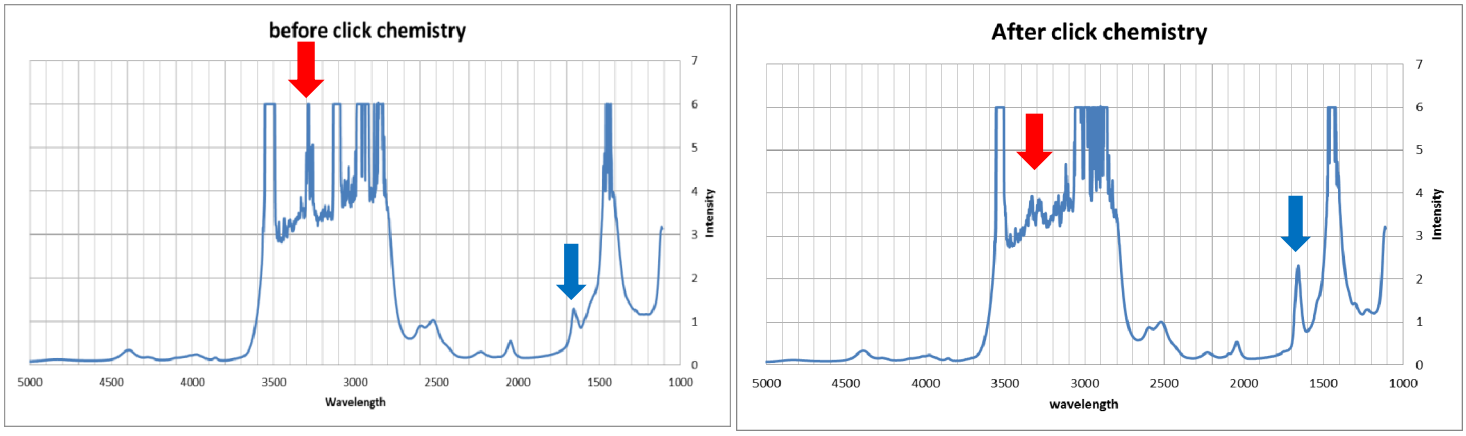


Fig. S6. FT-IR spectrum of compound **10** (left) and compound **1** (right). The unit of horizontal axis is cm^{-1}.1675 cm^{-1} shows C=C bond (blue arrow)and 3300 cm^{-1} shows C≡C bond (red arrow). After click chemistry, the intensity of C≡C bond decreases and one of C=C bond increases.

To confirm the combination of hydrophobic dendrimer core and **RP** at the ratio of 1:8 and the nonexistence of other ratio combination, GPC measurement was performed (apparatus: JASCO GULLIVER 970 model, column: Shodex Asahipak GS-310 20F, solvent: CH_2Cl_2). The only correlative spectrum of UV and RI was existed, and this result suggested the production of 1:8 dendrimer template (**8RD**).

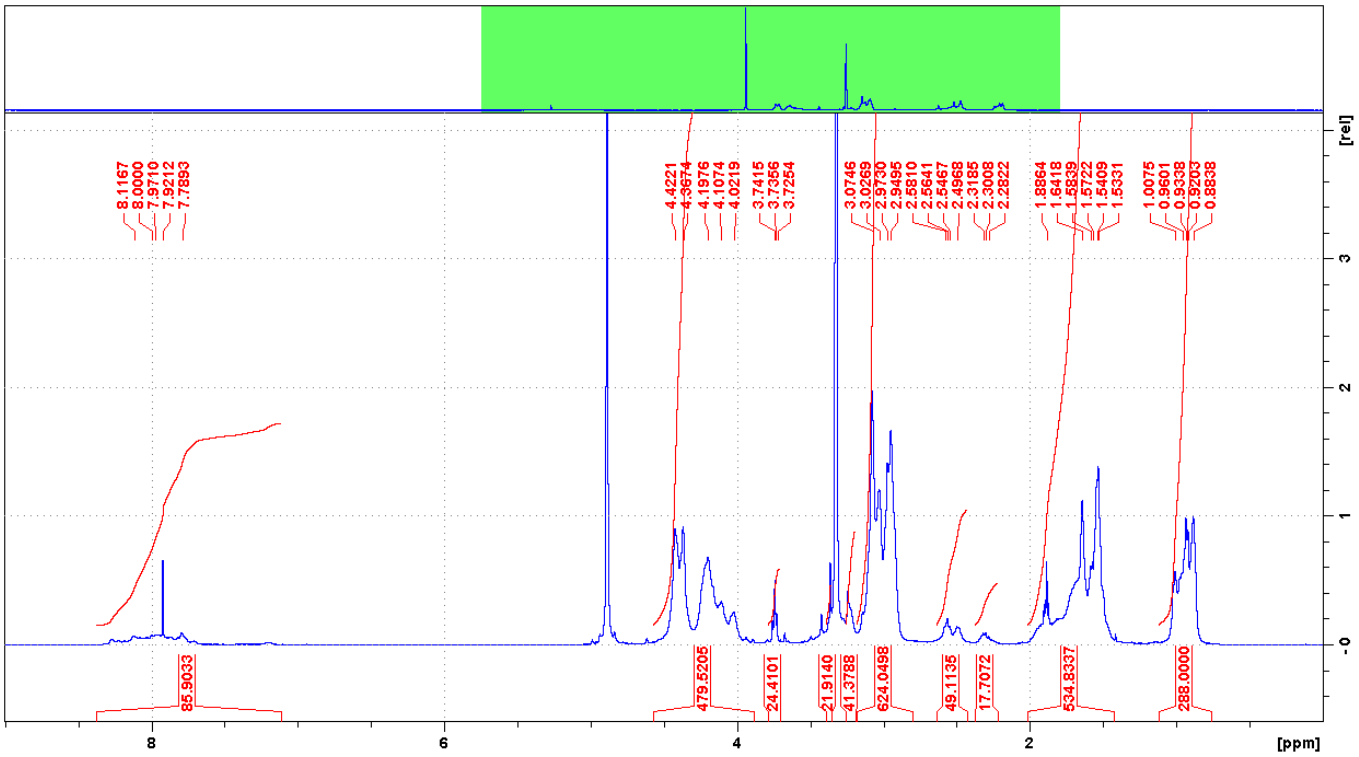


Fig. S7. ^{1}H NMR spectrum of compound **1**