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Proteo-Dendrimers Designed for Complementary Recognition of Cytochrome *c*: Dendrimer Architecture toward Nanoscale Protein Complexation

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Dedicated to the late Emeritus Professor Kazuhiro Maruyama of Kyoto University

Abstract: "Proteo-dendrimers" in which polyanionic hepta(glutamic acids), fluorescent zinc porphyrinate cores, hydrophilic polyether surfaces, and nonpeptide hydrophobic dendrons are combined, were developed as a new series of synthetic receptors for protein recognition. They have polyanionic "patch" structures on their surfaces and undergo complementary electrostatic interactions with a positively charged cytochrome *c* patch, as observed in biological protein–protein recognition systems. Stability constants of the resulting supramolecular complexes were determined in phosphate buffer (pH 7) by monitoring the fluorescence quenching of the zinc porphyrinates. These proteo-dendrimer receptors exhibited higher affinities with cytochrome *c* proteins in aqueous solutions than with biological cytochrome b_5. Furthermore, they effectively blocked complexation of biological cytochrome b_5 with cytochrome *c*, indicating that the proteo-dendrimers and cytochrome b_5 similarly occupy the polycationic patch of cytochrome *c*.

Keywords: cytochromes · dendrimers · fluorescence · protein recognition · supramolecular chemistry

Introduction

Biological electron-transfer reactions often involve highly specific complexation between protein redox partners. Cytochrome *c* and cytochrome b_5 typically form a stable 1:1 supramolecular complex, in which two porphyrin centers are arranged in a suitable geometry for efficient electron transfer.[1] As cytochrome *c* has a positively charged "patch" composed of four protonated lysines and cytochrome b_5 has several carboxylate anions on its surface, the two asymmetrically distributed patches effectively dock to undergo complementary electrostatic interactions for protein–protein complexation. Several synthetic receptors have recently been developed for cytochrome *c* complexation.[2–4] Hamilton et al. demonstrated that tetraphenylporphyrins with four $-CO_2^-$ groups matched up well with the polycationic patch of cytochrome *c*.[2] Hirota et al. and Ogawa et al. reported that polyanionic oligo(glutamic acids) and oligo(aspartic acids) strongly bound cytochrome *c*.[3] Dendrimers have received recent attention as more sophisticated receptors.[5] Kluger and Zhang attached hemoglobin clusters onto the dendrimer surface,[6] and Zimmerman et al. introduced the molecularly imprinted cavity into the interior domains.[7] Hirsch et al. first prepared polyanionic fullerene dendrimers for cytochrome *c* complexation.[8] Although a few dendrimers have been constructed from combinations of different dendritic blocks,[9] many biological examples suggest that the dendrimers with asymmetrically distributed patch structures on their surface can offer nanoscale protein recognition.

We present a new series of "proteo-dendrimer"-type receptors for recognition of biological proteins (Scheme 1). These dendrimers were designed to undergo complementary electrostatic interactions with cytochrome *c* by including several key features: 1) asymmetrically distributed polyanionic hepta(glutamic acids) responsible for the interaction with the polycationic patch on the cytochrome *c* surface, 2) a zinc porphyrinate core working as a fluorescence signaling device, 3) a hydrophilic polyether surface for high water solubility, and 4) nonpeptide dendritic components provid-

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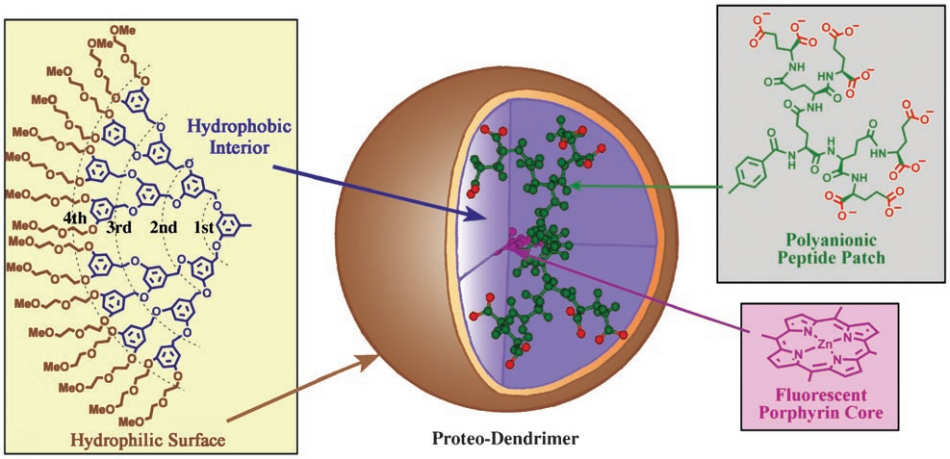


Scheme 1. Schematic illustration of proteo-dendrimers.

ing a peripheral, hydrophobic structure. As illustrated in Figure 1, we linked an oligopeptide composed of seven glutamic acid moieties to a zinc porphyrinate core. The resulting dendrimer has four pairs of $-CO_2^-$ anions on its surface, and provides a polyanionic patch to dock with the polycationic patch of cytochrome *c*. We also attached benzyl ether type dendritic components of different generations. Although the same polyanionic peptide was introduced, the generation of the nonpeptide dendritic component effectively regulated the supramolecular complexation with cytochrome *c*. Due to the size compatibility with proteins, the present type of proteo-dendrimers can work as novel supramolecular receptors of cytochrome *c*.

Results and Discussion

Synthesis and characteristics of proteo-dendrimers: The ester derivatives of proteo-dendrimers **1b**–**4b** were obtained by the coupling of peptide dendron **5** with zinc hepta-substituted tetraphenylporphyrinates **1c**–**4c** (Scheme 2). Scheme 3

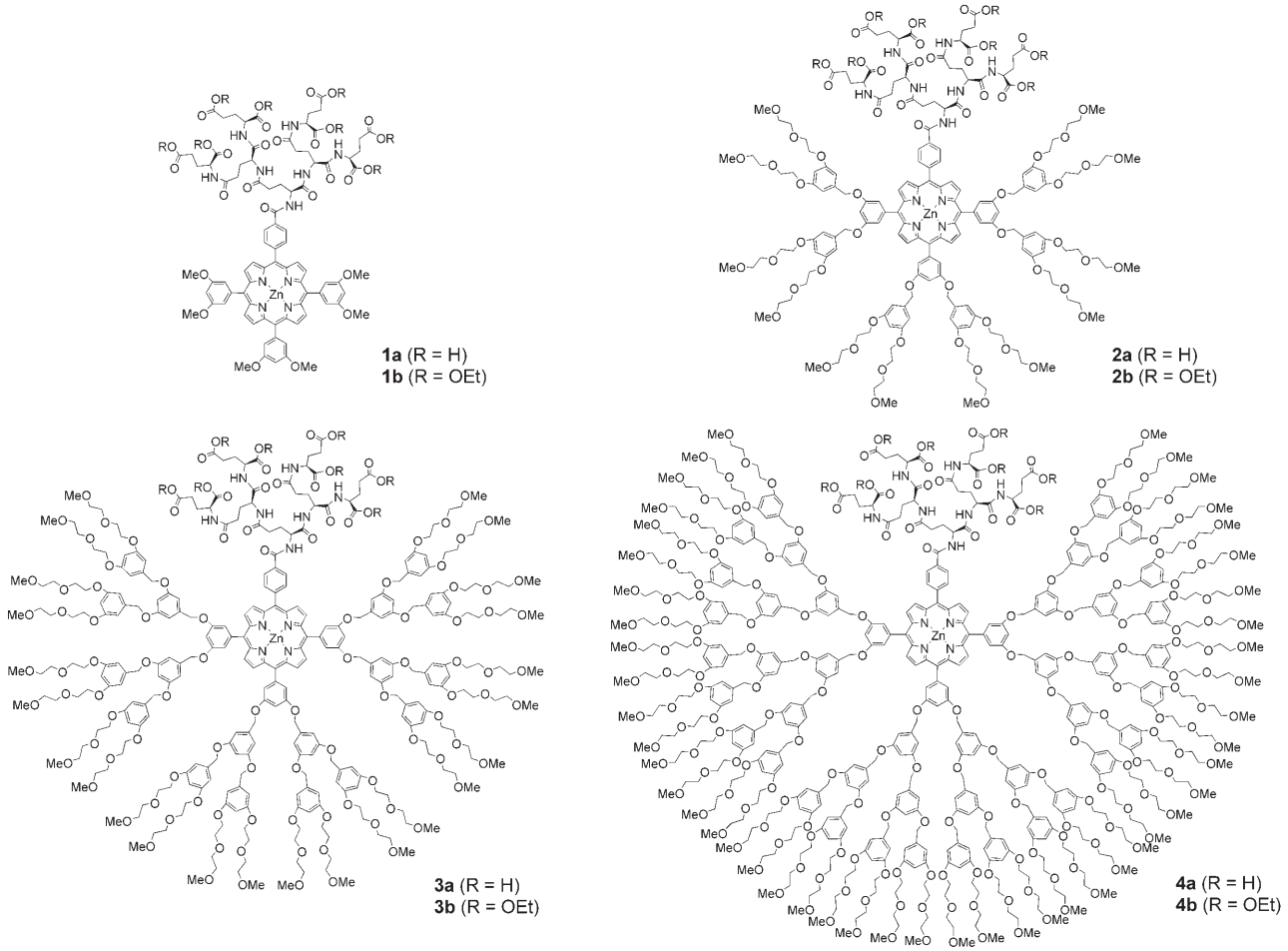
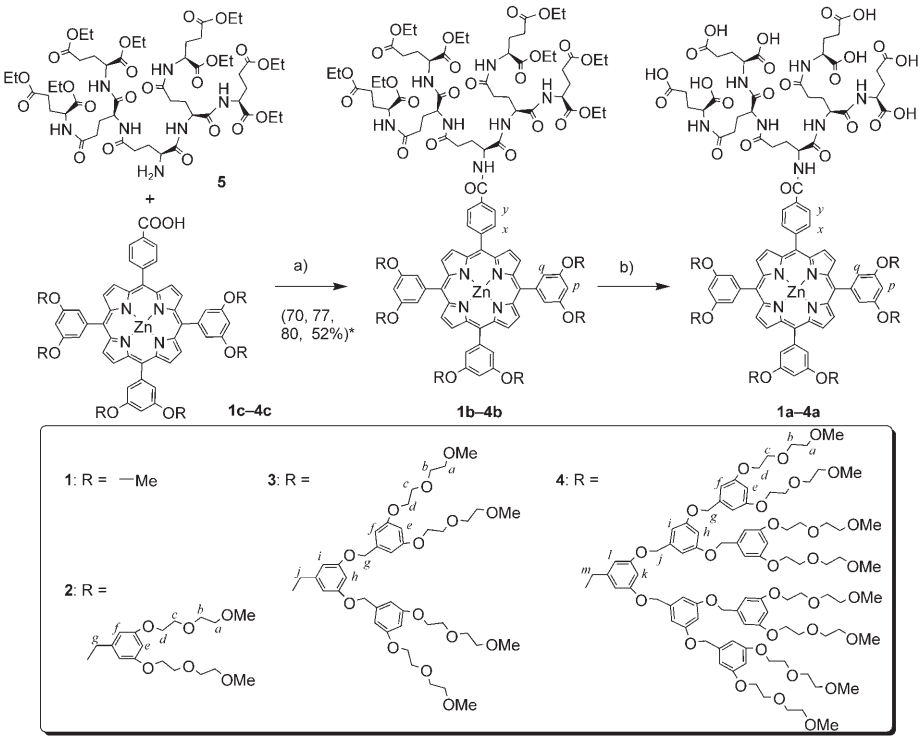
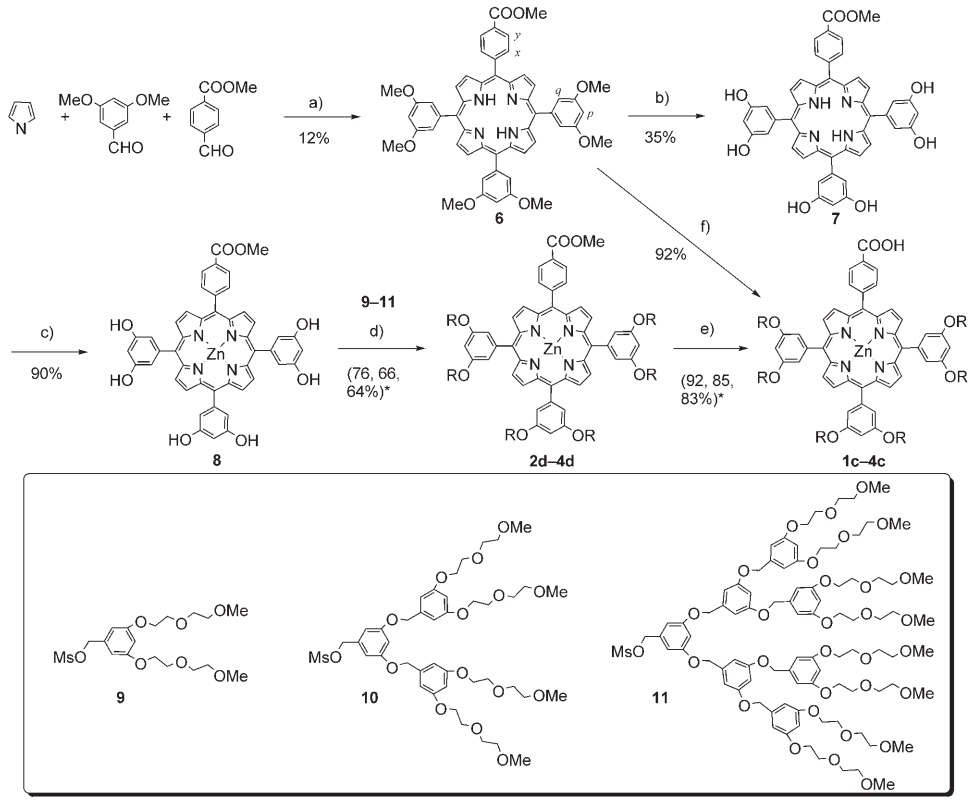


Figure 1. Proteo-dendrimers employed in this work.

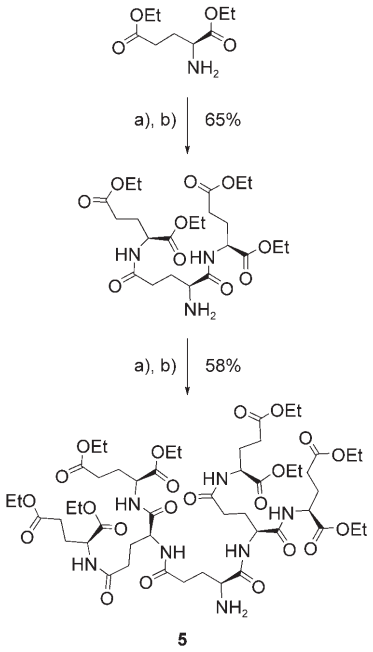


Scheme 2. Synthesis of proteo-dendrimers **1a**–**4a**. a) HOBt, HBTU, DIPEA, CH_2Cl_2; b) LiOH, H_2O, THF-MeOH. Italic letters have been attached to the structures for simplified descriptions of NMR assignments (see Experimental Section). * indicates the yields for (1st, 2nd, 3rd, and 4th generation).



Scheme 3. Synthesis of zinc porphyrinates **1c**–**4c**. a) BF_3•OEt_2, $CHCl_3$; then DDQ; b) BBr_3, CH_2Cl_2, 0 °C to RT; c) $Zn(OAc)_2$, $CHCl_3$/MeOH, reflux; d) K_2CO_3, [18]crown-6 ether, acetone, 50 °C; e) NaOH, H_2O, THF/MeOH; f) $Zn(OAc)_2$, $CHCl_3$/MeOH, reflux; NaOH, H_2O, THF/MeOH. Italic letters have been attached to the structures for simplified descriptions of NMR assignments (see Experimental Section). * indicates the yields for (2nd, 3rd, and 4th generation).

illustrates the synthesis of the zinc hepta-substituted tetraphenylporphyrinates. AB_3-type porphyrin **6** was synthesized by acid-catalyzed cross-condensation according to the Lindsey procedure.[10] After demethylation with BBr_3,[11] zinc porphyrinate **8** was obtained by treatment with $Zn(OAc)_2$ in $CHCl_3$/MeOH. Benzyl ether type dendritic branches with three different generations were prepared as mesylates **9**–**11** starting from methyl 3,5-dihydroxybenzoate according to the reported methods with some modifications.[11,12] Dendrimers **2d**–**4d** were obtained by reaction of zinc porphyrin **8** with 7–8 equivalents of the corresponding mesylate **9**–**11**, followed by hydrolysis to yield dendrimers **2c**–**4c**, whereas complex **1c** was derived from **6**. Peptide dendron **5** was synthesized according to the literature[13] (see Scheme 4). The ester dendrimer derivatives **1b**–**4b** were synthesized



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Scheme 4. Synthesis of peptide dendron **5**. a) CBZ-glutamic acid, HBTU, DIPEA, CH_2Cl_2, 0 °C; b) ammonium formate, Pd/C, H_2, MeOH.

by reactions of **1c**–**4c** with an excess of peptide dendron **5** using 1-hydroxybenzotriazole (HOBt), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and diisopropylethylamine (DIPEA). Esters **1b**–**4b** were fully characterized by elemental analysis, ^{1}H and ^{13}C NMR spectroscopy, IR spectroscopy, and TOF-MS methods (see Experimental Section). Hydrolysis of **1b**–**4b** with LiOH gave proteo-dendrimers **1a**–**4a** that exhibited broad ^{1}H NMR signals, and their purities were confirmed by capillary electrophoresis (see Figure S1 in the Supporting Information). As the two -CO_2H groups of parent glutamic acid have pK_a values of 2.19 and 4.25, all of the -CO_2H groups present in each proteo-dendrimer are expected to be deprotonated at neutral pH.

The proteo-dendrimers have comparable molecular weights (M_w) and diameters (d.) to those of the targeted cytochrome *c* (12173–12588 dalton and 4.0 nm, respectively): for **2a**, $M_w = 3680$ dalton, d. = 3.6 nm; for **3a**, $M_w = 6371$ dalton, d. = 5.0 nm; for **4a**, $M_w = 11753$ dalton, d. = 6.0 nm.[14] Table 1 summarizes absorption and fluorescence

Table 1. Spectroscopic profiles of proteo-dendrimers **2a**–**4a** in aqueous solution.[a] All values are λ_{max} in nm.

	UV		Fluorescence
	Soret band	Q band	
2a	429	559, 602	610, 658
3a	436 (br)	559, 599	610, 650
4a	434 (br)	559, 600	609, 648

[a] Conditions: dendrimer, 2.5×10^{-7} mol dm^{-3}; in 5×10^{-3} mol dm^{-3} sodium phosphate buffer, pH 7.0 (containing 10% DMF v/v); λ_{ex} = 558 nm.

spectral profiles of proteo-dendrimers **2a**–**4a**. The compounds exhibited almost the same spectra in aqueous DMF (at pH 7.0), which indicated that the zinc porphyrinate core of each dendrimer was located in a similar environment. The conformation search of dendrimer **1a** was carried out by using CONFLEX 5 and subsequent Mulliken population analysis was carried out by using Q-Chem (version 2.02, RHF/STO-3G, Q-Chem. Inc., USA).[15] As illustrated in Figure 2, the optimized structure reveals that three pairs of

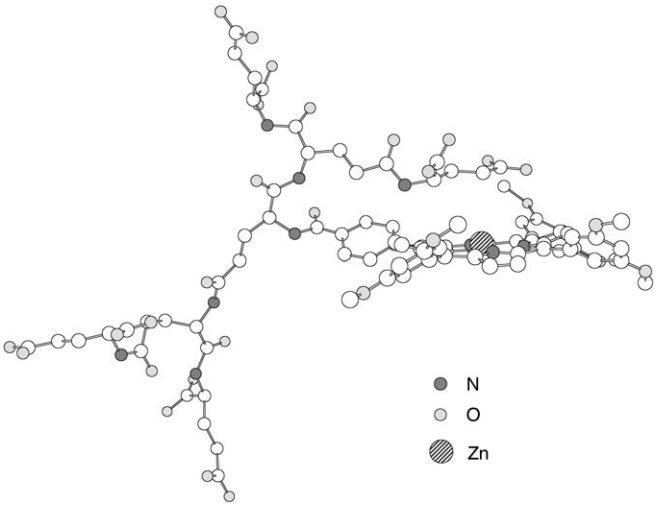

Figure 2. Optimized structure of zinc porphyrinate **1a**.

-CO_2^- anions point toward the outside and the remaining pair is located above the porphyrin plane. As one -CO_2^- anion of each pair can occupy the position matching the protonated lysine residue of the cytochrome *c*, the introduced oligopeptide is expected to provide a polyanionic "patch" suitable for complementary electrostatic interactions with cytochrome *c*.

Complexation with cytochrome *c*: Supramolecular complexation between cytochrome *c* and proteo-dendrimers **2a**–**4a**

was spectroscopically monitored in 5.0×10^{-3} mol dm^{-3} phosphate buffer solution containing 10% DMF (pH 7.0). As described above, their zinc porphyrin cores gave intense fluorescence bands around 610 and 652 nm upon photoexcitation at their Q bands (558 nm). Addition of horse heart cytochrome *c*, which contains a nonfluorescent heme, progressively decreased the fluorescence intensity of each zinc porphyrinate dendrimer (Figure 3). The relative fluorescence intensity of these dendrimers (F_x/F_t, for which F_x= fluorescence at molar ratio x and F_t=fluorescence of dendrimer alone) significantly decreased upon addition of 7 equivalents of cytochrome *c*, and their changes clearly depended on the dendrimer structures: 0.33 for **2a**, 0.53 for **3a**, and 0.72 for **4a**. As the generation of the nonpeptide component increased (**2a**→**4a**), the zinc porphyrinate of the dendrimer was confirmed to be positioned away from the bound cytochrome *c* heme. Lysine-acetylated horse heart cytochrome *c* and microperoxidase-8[16] have fewer protonated lysines than wild cytochrome *c*. When seven equivalents of one of these compounds were added to a solution of dendrimer **3a**, the fluorescence quenching efficiencies were modestly suppressed: $F_x/F_t=0.91$ with the acetylated one and 0.80 with microperoxidase-8 (the quenching curve was not included in Figure 3) relative to 0.53 with horse heart cytochrome *c*. Thus, the positively charged patch on cytochrome *c* was essentially involved in the complexation with the proteo-dendrimer.

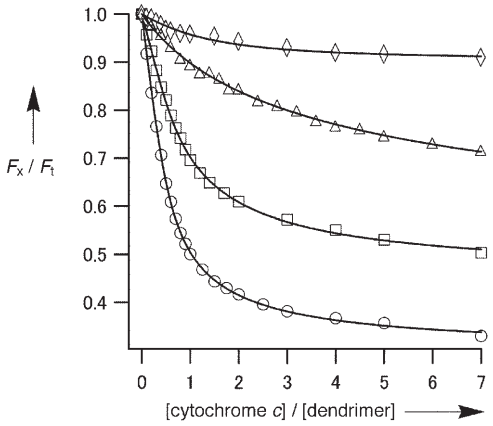

Figure 3. Fluorescence quenching of zinc porphyrinates with horse heart cytochrome *c* and its acetylated derivatives. Typical titration data for each dendrimer are shown. ○: **2a** + cytochrome *c*; □: **3a** + cytochrome *c*; △: **4a** + cytochrome *c*; ◇: **3a** + acetylated cytochrome *c*. Conditions: cytochrome *c*, 0–1.75×10^{-6} mol dm^{-3}; dendrimer, 2.5×10^{-7} mol dm^{-3}; in 5.0×10^{-3} mol dm^{-3} sodium phosphate buffer, pH 7.0 (containing 10% DMF v/v); $\lambda_{ex}=558$ nm; $\lambda_{em}=610$ nm. Each solid line was drawn based on nonlinear curve fitting.

Job plots for fluorescence quenching of dendrimers **2a**–**4a** with cytochrome *c* are illustrated in Figure 4.[17] Dendrimer **2a** appeared to form both 1:1 and 1:2 complexes (cytochrome *c*/dendrimer), whereas 3rd and 4th generation dendrimers **3a** and **4a**, respectively, exhibited roundly bent curves of around 1:1 stoichiometry. Thus, these plots gave

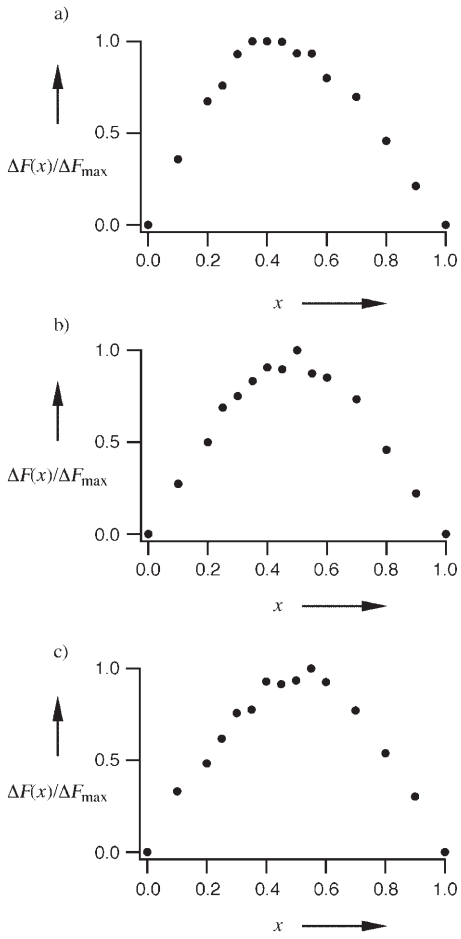

Figure 4. Job plots of proteo-dendrimers **2a**–**4a** versus cytochrome *c* in 5.0×10^{-3} mol dm^{-3} phosphate buffer, pH 7.0 (containing 10% DMF v/v); x=[cytochrome *c*]/([cytochrome *c*] + [dendrimer]); total concentration of two species was held at a) **2a**, 5.0×10^{-7} mol dm^{-3}, b) **3a**, 5.0×10^{-7} mol dm^{-3}, and c) **4a**, 2.5×10^{-6} mol dm^{-3}. The fluorescence intensity was monitored at 658 nm.

no clear indication of a single component mainly existing in each system. The supramolecular complexation behaviors of proteo-dendrimers with cytochrome *c* were analyzed by the fluorescence titration experiments (see Figure 3). The concentration of cytochrome *c* varied from 0 to 1.75×10^{-6} mol dm^{-3}, although that of the proteo-dendrimer was kept at 2.5×10^{-7} mol dm^{-3}. The titration curves with dendrimers **2a** and **3a** gave better fits for mixtures of 1:1 and 1:2 complexes than for solely 1:1 complexes, while dendrimer **4a** exhibited satisfactory results based on both assumptions. The stability constants were estimated by the nonlinear-curve-fit method and are listed in Table 2. Although the same hepta(glutamic acids) provided major binding sites for cytochrome *c*, dendrimers **2a** and **3a** exhibited higher stability (**2a**: $\log K_1=7.6$, $\log K_2=7.2$; **3a**: $\log K_1=7.7$, $\log K_2=7.2$) than dendrimer **4a** ($\log K_1=6.5$, $\log K_2=7.1$). The bulky nonpeptide dendritic component of dendrimer **4a** seems to sterically suppress the electrostatic interactions between the polyanionic dendrimer and the polycationic cytochrome *c* protein. Table 2 also lists fluorescence intensity ratios of cy-

Table 2. Stability constants between proteo-dendrimers **2a**–**4a** and cytochrome *c*[a] and calculated fluorescence intensity ratios of supramolecular complexes to free dendrimer.

	$\log K_1$	$\log K_2$	$I_{1:1}/I_o$[b]	$I_{1:2}/I_o$[c]
2a	7.6±0.3	7.2±0.3	0.29±0.02	1.10±0.20
3a	7.7±0.3	7.2±0.3	0.48±0.05	1.76±0.10
4a	6.5±0.1	7.1±0.2	0.45±0.14	1.96±0.03
4a[d]	6.3±0.1		0.59±0.06	

[a] Conditions: horse heart cytochrome *c*, 0–1.75×10^{-6} mol dm^{-3}; dendrimer, 2.5×10^{-7} mol dm^{-3}; in 5×10^{-3} mol dm^{-3} sodium phosphate buffer, pH 7.0 (containing 10% DMF v/v); $\lambda_{ex}=558$ nm; $\lambda_{em}=610$ nm. The averaged values of four or five independent experiments are shown. [b] Calculated fluorescence intensity ratio of a 1:1 complex to free dendrimer. [c] Calculated fluorescence intensity ratio of a 1:2 complex to free dendrimer. [d] The data was fitted considering only 1:1 complexation.

tochrome *c* bound dendrimer to free dendrimer. Each 1:2 complex contained two zinc porphyrinates, but its fluorescence was modestly quenched. The two zinc porphyrinates of the bound dendrimers were located under different environments. Because simple zinc porphyrinate **1a** gave a partially insoluble complex with cytochrome *c* in the employed neutral aqueous solution, nonpeptide dendritic substituents must play a significant role in the solubilization and complexation processes.

Proteo-dendrimers contained chiral oligopeptide moieties, but gave very small circular dichroism (CD) signals in the Soret region. They exhibited CD signals of the same sign at 432 and 442 nm upon cytochrome *c* complexation, which correspond to the zinc porphyrinate absorption bands. As cytochrome *c* itself displayed CD signals around 406 nm, the above-mentioned observed CD signals indicated asymmetric orientation of the zinc porphyrinate upon complexation with cytochrome *c*.[18] Proteo-dendrimer **2a** gave four times larger signals than dendrimer **3a**; this suggests that the generation of the nonpeptide component influenced the orientations and distances between the two porphyrinates.[19] The dense peripheral moieties of the higher generation dendrimer were thought to cause steric hindrance around the polyanionic patch and to prevent the docking with cytochrome *c*. Pigeon breast and yeast cytochromes *c* also have polycationic patches for complementary interactions with proteo-dendrimers, though the latter has lower solubility in the aqueous medium. Dendrimer **3a** typically formed a stable complex with the former ($\log K_1=7.5$, $\log K_2=6.5$), but insoluble material with the latter.

Competitive binding with cytochrome b_5: Proteo-dendrimers **2a**–**4a** formed stable complexes with horse heart cytochrome *c* at neutral pH. Their stability constants are larger than those reported with cytochrome b_5 ($\log K=4.8$)[20] and fullerene dendrimer ($\log K=5.2$),[8] though direct comparisons are difficult under different ionic strength and buffer conditions. The competitive binding of cytochrome *c* between proteo-dendrimer and cytochrome b_5 was carried out by monitoring the fluorescence of zinc porphyrinate dendrimer at 610 nm. It has already been established that complementary electrostatic interactions between cytochrome *c* and cytochrome b_5 play important roles in protein recognition.[16] The proteo-dendrimers competed well with cytochrome b_5 and blocked biologically important complexation with cytochrome *c*. Figure 5 illustrates the effects of cyto-

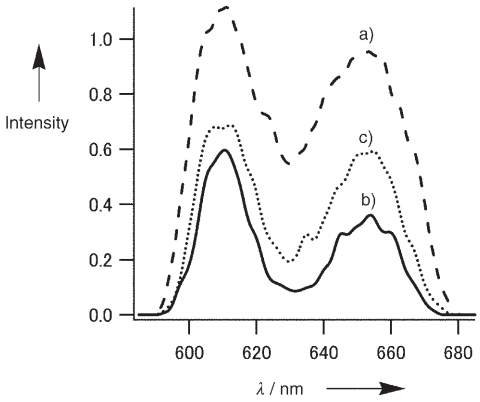

Figure 5. Competitive binding of cytochrome *c* between proteo-dendrimer **3a** and cytochrome b_5. a) – – –, **3a**; b) ——, **3a** + cytochrome *c*; c) •••••, **3a** + cytochrome *c* + cytochrome b_5). Conditions: dendrimer **3a**, 2.5×10^{-7} mol dm^{-3}; horse heart cytochrome *c*, 2.5×10^{-7} mol dm^{-3}; cytochrome b_5, 3.5×10^{-6} mol dm^{-3}; in 5.0×10^{-3} mol dm^{-3} phosphate buffer, pH 7.0 (containing 10% DMF v/v); $\lambda_{ex}=558$ nm.

chrome *c* and cytochrome b_5 on fluorescence of proteo-dendrimer **3a**. An equimolar addition of cytochrome *c* largely decreased the fluorescence intensity of dendrimer **3a** (see Figure 5a→b). When 14 equivalents of cytochrome b_5 were added to this mixture, the fluorescence signals partially recovered (see Figure 5c). This indicates that approximately 20% of the dendrimer-bound cytochrome *c* was released and then complexed with cytochrome b_5. Such competitive binding behavior supports the idea that proteo-dendrimer **3a** formed a more stable complex with cytochrome *c* than biological cytochrome b_5, and also that both dendrimer and cytochrome b_5 occupied the polycationic patch of cytochrome *c* in the same fashion. Supramolecular complexation with crown ethers, calixarenes, and other synthetic receptors have often been reported for modifying the structure and reactivity of cytochrome *c*.[4] Although resonance Raman and CD spectroscopic methods (in the α-helix region) were not available for the present systems, the cytochrome *c* complexes with proteo-dendrimers exhibited almost the same UV signal intensity at 690 nm as that of free cytochrome *c*. Thus, the methionine-coordinated heme center was accommodated in the protein matrix as observed in biological systems.[16]

Conclusion

A new series of proteo-dendrimers were successfully developed, in which polyanionic peptide binding sites, neutral dendritic structures, and zinc porphyrinate cores were combined in a nanosize three-dimensional skeleton. These struc-

tures undergo effective complementary electrostatic interactions with the polycationic surface of cytochrome *c*. The resulting supramolecular complexes were confirmed to be more stable than a biological protein pair of cytochrome *c* and cytochrome b_5 in aqueous solution. The asymmetrically distributed patch structures on the dendrimer surfaces efficiently offered sites for nanoscale protein recognition.

Experimental Section

Materials: Cytochromes *c* from horse heart (Wako, $M_w = 12384$), pigeon breast muscle (Sigma-Aldrich, $M_w = 12173$), and *Saccaromyces cerevisiae* yeast (Sigma-Aldrich, $M_w = 12588$) are commercially available. As their absorbance at the Soret band region was only slightly enhanced by oxidation with $K_3[Fe(CN)_6]$, we used them as received. Acetylated horse heart cytochrome *c* was obtained from Sigma-Aldrich, in which 60% of the lysine residues were derivatized. Microperoxidase-8 was also obtained from Sigma-Aldrich. This is partially hydrolyzed material of the horse heart cytochrome *c* and has the heme portion with amino acids 14–21 attached.[16] Cytochrome b_5 was purchased from Wako Pure Chemical Industries and used after dialysis.

All the reaction mixtures containing porphyrin derivatives and dendrimers were protected from light by covering reaction flasks with aluminum foil. K_2CO_3 was dried in an oven at 150 °C for 5 h and cooled under a dry N_2 atmosphere. Dry acetonitrile and THF were freshly distilled over P_2O_5 and sodium benzophenone, respectively. Stabilizer-free THF was used in the hydrolysis of ester dendrimers. Chloroform was used as an eluting solvent for gel permeation chromatography (GPC) purification of dendrimers using JAIGEL 1H-2H or 2H-3H column combinations (Japan Analytical Industry Co., Ltd.). Phosphate buffer was prepared to adjust the pH to 7.0 by mixing 5.0×10^{-3} mol dm^{-3} solutions of mono- and dibasic sodium phosphate in deionized water containing 10% DMF.

^{1}H and ^{13}C NMR spectra were recorded on JEOL LA-300 and LA-400 spectrometers. The details of their assignments refer to the structures described in Schemes 2–4. IR and CD spectra were obtained on Perkin–Elmer SpectrumOne FTIR and Jasco J-720 spectrometers, respectively. FAB mass spectra were measured on a JEOL JMS-700T spectrometer using 3-nitrobenzyl alcohol as a matrix. MALDI-TOF mass experiments were carried out by the Shimadzu Corporation, Tsukuba, Japan, using Shimadzu AXIMA CFRplus apparatus: a *trans*-3-indoleacrylic acid matrix in THF for **2b**–**3b**; a sinapic acid matrix in acetonitrile/water for **1a**–**4a**. The calculated molecular masses are the average molecular masses. Capillary electrophoresis was carried out with Otsuka CAPI-3200 apparatus using a bare fused-silica capillary (75 μm (i.d.) × 45 cm).

Synthesis of zinc porphyrinate 1c

Compound ***6***: This AB_3-type porphyrin was synthesized by acid-catalyzed cross-condensation according to the Lindsey procedure[10] in a 12% yield. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = -2.83$ (s, 2H; NH), 3.96 (s, 18H; OMe), 4.11 (s, 3H; COOMe), 6.90 (brt, 3H; H^p), 7.39 (brd, 6H; H^q), 8.30 (d, $J = 8.2$ Hz, 2H; H^y), 8.44 (d, $J = 8.2$ Hz, 2H; H^x), 8.77 (d, $J = 4.7$ Hz, 2H; pyrrole-β), 8.95 (s, 4H; pyrrole-β) 8.96 ppm (d, $J = 4.7$ Hz, 2H; pyrrole-β); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 52.42$, 55.62, 100.15, 113.85, 118.61, 120.03, 120.09, 127.91, 129.56, 131.8, 134.52, 143.89, 147.00, 158.85, 167.33 ppm; MS (FAB): *m*/*z*: 853.4 $[M+H]^+$.

Compound ***7***: Demethylation of **6** was carried out by using BBr_3 by following the procedure similar to that reported in ref. [11] to give **7** (35%). ^{1}H NMR (400 MHz, CD_3OD): $\delta = 4.07$ (s, 3H; COOMe), 6.72–6.73 (m, 3H; H^p and H$^{p'}$), 7.15 (d, $J = 2.2$ Hz, 4H; H^q), 7.16 (d, $J = 2.2$ Hz, 2H; H$^{q'}$), 8.29 (d, $J = 8.3$ Hz, 2H; H^y), 8.41 (d, $J = 8.3$ Hz, 2H; H^x), 9.00 ppm (brm, 8H; pyrrole-β); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 52.90$, 103.23, 115.77, 119.51, 121.74, 121.88, 128.92, 130.82, 135.67, 144.96, 148.32, 157.93, 168.62 ppm; MS (FAB): *m*/*z*: 769.2 $[M+H]^+$.

Compound ***8***: Porphyrin metalation was carried out with $Zn(OAc)_2$ in a mixture of chloroform and MeOH by using a procedure similar to that reported in ref. [10]. Yield: 90%. ^{1}H NMR (400 MHz, CD_3OD): $\delta = 4.08$ (s, 3H; COOMe), 6.70 (m, 3H; H^p), 7.16 (m, 6H; H^q), 8.32 (d, $J = 8.0$ Hz, 2H; H^y), 8.42 (d, $J = 8.0$ Hz, 2H; H^x), 8.76 (d, $J = 4.4$ Hz, 2H; pyrrole-β), 8.98 (s, 4H; pyrrole-β) 8.99 ppm (d, $J = 4.4$ Hz, 2H; pyrrole-β); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 52.85$, 102.62, 115.87, 119.79, 121.99, 122.13, 128.55, 130.17, 131.72, 132.52, 132.57, 132.83, 135.77, 146.66, 150.27, 150.62, 151.09, 151.30, 151.37, 157.43, 168.92 ppm; MS (FAB): *m*/*z*: 830.2 $[M]^+$.

Compound ***1c***: Porphyrin **6** was metallated with $Zn(OAc)_2$ by using a procedure similar to that reported[10] in the literature, and the isolated product was dissolved in THF (5 mL) and MeOH (5 mL), and NaOH (50 mg in 0.5 mL water) was added to the reaction mixture. The progress of hydrolysis was monitored by TLC (silica plate, 2% EtOAc in dichloromethane). After completion of the reaction (5 h), the solvents were removed under vacuum and the residue was dissolved in dichloromethane and washed with water, 2% citric acid, water, and brine (with gentle shaking). The organic layer was separated, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel chromatography using 10% EtOAc in dichloromethane (to remove unhydrolyzed ester derivative) and then 10% MeOH in acetone, giving **1c** (92%). ^{1}H NMR (300 MHz, CD_3OD): $\delta = 3.94$ (s, 12H; OMe), 3.95 (s, 6H; OMe), 6.90–6.92 (m, 3H; H^p), 7.36–7.37 (m, 6H; H^q), 8.26 (d, $J = 8.0$ Hz, 2H; H^y), 8.40 (d, $J = 8.2$ Hz, 2H; H^x), 8.80 (d, $J = 4.7$ Hz, 2H; pyrrole-β), 8.91 ppm (m, 6H; pyrrole-β); MS (FAB): *m*/*z*: 900.3 $[M]^+$.

Synthesis of dendritic methanesulfonates 9–11[11,12]

Compound ***9***: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.91$ (s, 3H; SO_2Me), 3.40 (s, 6H; OMe), 3.58 (AA′BB′ multiplet, 4H; CH_2), 3.72 (AA′BB′ multiplet, 4H; CH_2), 3.85 (brt, $J = 4.8$ Hz, 4H; CH_2), 4.12 (brt, $J = 4.8$ Hz, 4H; CH_2), 5.15 (s, 2H; benzylic-CH_2), 6.51 (t, $J = 2.4$ Hz, 1H; ArH), 6.56 ppm (d, $J = 2.4$ Hz, 2H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 38.23$, 58.90, 67.42, 69.44, 70.57, 71.30, 71.74, 102.13, 107.19, 135.22, 160.05 ppm; MS (FAB): *m*/*z*: 422.2 $[M+H]^+$.

Compound ***10***: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.86$ (s, 3H; SO_2Me), 3.39 (s, 12H; OMe), 3.58 (AA′BB′ multiplet, 8H; CH_2), 3.72 (AA′BB′ multiplet, 8H; CH_2), 3.85 (brt, $J = 4.8$ Hz, 8H; CH_2), 4.12 (brt, $J = 4.8$ Hz, 8H; CH_2), 4.96 (s, 4H; benzylic-CH_2), 5.15 (s, 2H; benzylic-CH_2), 6.45 (t, $J = 2.4$ Hz, 2H; ArH), 6.57–6.60 ppm (m, 7H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 38.19$, 58.88, 67.29, 69.48, 69.79, 70.53, 71.24, 71.74, 100.92, 102.80, 105.82, 107.40, 135.36, 138.62, 159.89, 159.93 ppm; MS (FAB): *m*/*z*: 871.4 $[M+H]^+$.

Compound ***11***: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.86$ (s, 3H; OSO_2Me), 3.38 (s, 24H; OMe), 3.57 (AA′BB′ multiplet, 16H; CH_2), 3.71 (AA′BB′ multiplet, 16H; CH_2), 3.84 (brt, $J = 4.8$ Hz, 16H; CH_2), 4.12 (brt, $J = 4.8$ Hz, 16H; CH_2), 4.95 (brs, 8H; benzylic-CH_2), 4.98 (brs, 4H; benzylic-CH_2), 5.16 (s, 2H; benzylic-CH_2), 6.45 (brt, $J = 1.6$ Hz, 4H; ArH), 6.54 (brt, $J = 1.6$ Hz, 2H; ArH), 6.59 (s, 10H; ArH), 6.63–6.65 ppm (m, 5H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 37.75$, 58.56, 67.03, 69.20, 69.46, 69.51, 70.22, 71.00, 71.46, 100.64, 101.15, 102.39, 105.60, 105.91, 107.13, 135.24, 138.56, 138.65, 159.62, 159.64 ppm; MS (FAB): *m*/*z*: 1766.8 $[M]^+$.

Synthesis of ester dendrimers 2d–4d

Compound ***2d***: Zinc 5-(4-methoxycarbonylphenyl)-10,15,20-tris(3,5-dihydroxyphenyl)porphinate **8** (100 mg, 0.12 mmol), mesylate **9** (355 mg, 0.84 mmol), anhydrous K_2CO_3 (200 mg, 1.24 mmol) and [18]crown-6 (20 mg, 0.08 mmol) were added to dry acetone (20 mL). The reaction mixture was stirred at 50 °C under an N_2 atmosphere. The progress of the reaction was monitored by TLC by using acetone/dichloromethane (3:7). After completion of the reaction (20 h), the solvents were removed under vacuum and the residue was dissolved in dichloromethane (30 mL) and filtered. The separated solid was washed with dichloromethane. The filtrate and washings were collected and evaporated. The residue was dissolved in a minimum amount of dichloromethane (ca. 5 mL), and MeOH (ca. 30 mL) was added with stirring. The product precipitated as a thick gum and the solvents were separated by decantation. The residue was purified by silica gel column chromatography using EtOAc (to remove mesylate), dichloromethane, and then 30% acetone in dichloromethane as eluent, giving **2d** (76%). ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 3.12$ (s, 12H; OMe′), 3.13 (s, 24H; OMe), 3.28–3.31 (m, 24H; H^a and H$^{a'}$), 3.47–3.50 (m, 24H; H^b and H$^{b'}$), 3.73 (brt, $J = 4.4$ Hz, 24H; H^c and H$^{c'}$), 4.06 (brt,

J=4.8 Hz, 24H; H^{d} and $H^{d'}$), 4.11 (s, 3H; COOMe), 5.14 (s, 12H; H^{g} and $H^{g'}$), 6.42–6.44 (m, 6H; H^{e} and $H^{e'}$), 6.64–6.66 (m, 12H; H^{f} and $H^{f'}$), 7.02–7.04 (m, 3H; H^{p} and $H^{p'}$), 7.46 (d, J=2.4 Hz, 4H; H^{q}), 7.48 (d, J=2.4 Hz, 2H; $H^{q'}$), 8.30 (d, J=8.4 Hz, 2H; H^{y}), 8.43 (d, J=8.4 Hz, 2H; H^{x}), 8.81 (d, J=4.4 Hz, 2H; pyrrole-β), 8.94–8.97 ppm (m, 6H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=52.17, 58.65, 67.24, 69.36, 70.14, 70.30, 70.33, 71.47, 71.49, 101.10, 101.64, 106.08, 114.99, 119.17, 120.50, 120.54, 127.52, 128.96, 131.20, 131.89, 132.02, 134.38, 139.00, 144.65, 144.70, 147.93, 149.26, 149.63, 149.78, 149.81, 157.59, 157.61, 159.89, 167.19 ppm; IR (film between KBr discs): $\tilde{\nu}$=1718 cm^{-1} (ester C=O); UV/Vis ($CHCl_3$) : λ_{max} ($\log\varepsilon$)=427 (5.78), 555 (4.39), 595 nm (3.79); MS (MALDI-TOF): m/z: 2786.3 $[M]^+$; elemental analysis calcd (%) for $C_{148}H_{186}N_4O_{44}Zn$: C 63.70, H 6.72, N 2.01; found: C 63.53, H 6.72, N 1.94.

*Compound **3d***: Dendrimer **3d** was obtained by coupling of zinc porphyrinate **8** with mesylate **10**, by using the same procedure as that used for **2d** (66%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.18 (s, 24H; OMe′), 3.21 (s, 48H; OMe), 3.32–3.35 (m, 16H; $H^{a'}$), 3.36–3.39 (m, 32H; H^{a}), 3.46–3.49 (m, 16H; $H^{b'}$), 3.51–3.53 (m, 32H; H^{b}), 3.64 (brt, J=4.8 Hz, 16H; $H^{c'}$), 3.68 (brt, J=4.8 Hz, 32H; H^{c}), 3.96 (brt, J=4.8 Hz, 16H; $H^{d'}$), 4.00 (brt, J=4.8 Hz, 32H; H^{d}), 4.11 (s, 3H; COOMe), 4.92 (brs, 8H; $H^{g'}$), 4.93 (brs, 16H; H^{g}), 5.14 (s, 12H; H^{j} and $H^{j'}$), 6.34 (brt, J=2.4 Hz, 4H; $H^{e'}$), 6.36 (brt, J=2.4 Hz, 8H; H^{e}), 6.49 (d, J=2.0 Hz, 8H; $H^{f'}$), 6.51 (d, J=2.0 Hz, 16H; H^{f}), 6.53 (brs, 6H; H^{h} and $H^{h'}$), 6.73 (brd, J=2.0 Hz, 12H; H^{i} and $H^{i'}$), 7.06 (brt, J=2.0 Hz, 3H; H^{p} and $H^{p'}$), 7.49 (brd, J=2.0 Hz, 6H; H^{q} and $H^{q'}$), 8.24 (d, J=8.2 Hz, 2H; H^{y}), 8.39 (d, J=8.2 Hz, 2H; H^{x}), 8.80 (d, J=4.6 Hz, 2H; pyrrole-β), 8.98–9.03 ppm (m, 6H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=52.18, 58.69, 58.71, 67.14, 67.18, 69.32, 69.35, 69.79, 70.04, 70.29, 70.33, 71.53, 71.56, 100.88, 101.16, 101.62, 105.81, 106.35, 106.41, 115.13, 119.12, 120.41, 120.52, 127.50, 128.93, 131.18, 131.95, 134.38, 138.90, 139.02, 139.06, 144.78, 147.94, 149.23, 149.60, 149.74, 149.81, 157.59, 159.80, 159.81, 159.89, 167.22 ppm; IR (film between KBr discs): $\tilde{\nu}$=1719 cm^{-1} (ester C=O). UV/Vis ($CHCl_3$): λ_{max} ($\log\varepsilon$)=427 (5.78), 555 (4.38), 595 nm (3.77); MS (MALDI-TOF): m/z: 5476.5 $[M]^+$; elemental analysis calcd (%) for $C_{292}H_{378}N_4O_{92}Zn$: C 63.98, H 6.95, N 1.02; found: C 63.92, H 7.06, N 1.03.

*Compound **4d***: Dendrimer **4d** was obtained by coupling of zinc porphyrinate **8** with mesylate **11** (8.0 molar equiv) by using the same procedure as that for **2d** and was finally purified with 2H-3H JAIGEL GPC. Yield: 64%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.22 (s, 48H; OMe′), 3.26 (s, 96H; OMe), 3.36–3.39 (m, 32H; $H^{a'}$), 3.41–3.44 (m, 64H; H^{a}), 3.50–3.53 (m, 32H; $H^{b'}$), 3.56–3.58 (m, 64H; H^{b}), 3.67 (brt, J=4.8 Hz, 32H; $H^{c'}$), 3.72 (brt, J=4.8 Hz, 64H; H^{c}), 3.96 (brt, J=4.4 Hz, 32H; $H^{d'}$), 4.01 (brt, J=4.8 Hz, 64H; H^{d}), 4.06 (s, 3H; COOMe), 4.82 (s, 16H; $H^{g'}$), 4.87 (s, 32H; H^{g}), 4.91 (brs, 8H; $H^{j'}$), 4.92 (brs, 16H; H^{j}), 5.13 (brs, 12H; H^{m} and $H^{m'}$), 6.33 (brt, J=2.4 Hz, 8H; $H^{e'}$), 6.37 (brt, J=2.4 Hz, 16H; H^{e}), 6.44 (brt, J=2.4 Hz, 4H; $H^{h'}$), 6.46 (brd, J=2.4 Hz, 24H; $H^{f'}$ and H^{h}), 6.50 (d, J=2.4 Hz, 32H; H^{f}), 6.56 (brt, J=2.4 Hz, 6H; H^{k} and $H^{k'}$), 6.59 (brd, J=2.4 Hz, 8H; $H^{i'}$), 6.62 (brd, J=2.4 Hz, 16H; H^{i}), 6.77 (brm, 12H; H^{l} and $H^{l'}$), 7.08 (brs, 3H; H^{p} and $H^{p'}$), 7.54 (brs, 6H; H^{q} and $H^{q'}$), 8.23 (d, J=8.2 Hz, 2H; H^{y}), 8.36 (d, J=8.2 Hz, 2H; H^{x}), 8.81 (d, J=4.8 Hz, 2H; pyrrole-β), 9.02 (d, J=4.8 Hz, 2H; pyrrole-β), 9.07–9.10 ppm (m, 4H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=52.05, 58.63, 58.67, 67.07, 67.12, 69.26, 69.31, 69.54, 69.60, 69.71, 70.25, 70.30, 71.50, 71.54, 100.78, 101.25, 105.70, 105.75, 106.11, 106.25, 106.46, 114.92, 119.07, 120.37, 120.48, 127.42, 128.81, 131.12, 131.86, 134.30, 138.79, 138.91, 139.03, 144.75, 147.80, 149.14, 149.54, 149.67, 149.74, 157.59, 159.68, 159.72, 159.85, 167.05 ppm; IR (film between KBr discs): $\tilde{\nu}$=1718 cm^{-1} (ester C=O); UV/Vis ($CHCl_3$): λ_{max} ($\log\varepsilon$)=427 (5.78), 555 (4.38), 595 nm (3.78); MS (MALDI-TOF): m/z: 10863.8 $[M]^+$; elemental analysis calcd (%) for $C_{580}H_{762}N_4O_{188}Zn$: C 64.12, H 7.07, N 0.52; found: C 63.81, H 7.12, N 0.62.

Synthesis of proteo-dendrimers 2c–4c

*Compound **2c***: Dendrimer **2d** (200 mg, 0.072 mmol) was dissolved in stabilizer-free THF (5 mL) and MeOH (2 mL). Aqueous NaOH solution (40 mg, 1.0 mmol, in 1.0 mL water) was added, and the reaction mixture was stirred at room temperature. After 7 h, the solvents were removed under vacuum and the residue dissolved in dichloromethane was washed with water (2×10 mL), 2% citric acid (10 mL), and then with water (2×10 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel chromatography using acetone and then 10% MeOH in acetone to give **2c** (92%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.12 (s, 24H; OMe), 3.14 (s, 12H; OMe′), 3.27–3.33 (m, 24H; H^{a} and $H^{a'}$), 3.45–3.51 (m, 24H; H^{b} and $H^{b'}$), 3.69–3.74 (m, 24H; H^{c} and $H^{c'}$), 4.03–4.08 (m, 24H; H^{d} and $H^{d'}$), 5.14 (brs, 8H; H^{g}), 5.16 (brs, 4H; $H^{g'}$), 6.42 (brt, J=2.0 Hz, 4H; H^{e}), 6.44 (brt, J=2.0 Hz, 2H; $H^{e'}$), 6.64 (brd, J=2.0 Hz, 8H; H^{f}), 6.66 (brd, J=2.0 Hz, 4H; $H^{f'}$), 7.03–7.05 (brm, 3H; H^{p} and $H^{p'}$), 7.48 (brd, J=1.6 Hz, 4H; H^{q}), 7.49 (brd, J=1.6 Hz, 2H; $H^{q'}$), 8.34 (d, J=8.2 Hz, 2H; H^{y}), 8.49 (d, J=8.2 Hz, 2H; H^{x}), 8.84 (d, J=4.8 Hz, 2H; pyrrole-β), 8.95 ppm (m, 6H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=58.70, 58.72, 67.37, 67.39, 69.46, 69.47, 70.27, 70.39, 70.42, 71.57, 71.60, 101.26, 101.83, 106.22, 115.14, 119.19, 120.60, 120.65, 128.16, 128.61, 131.28, 131.92, 132.12, 134.57, 139.12, 144.73, 144.78, 148.60, 149.34, 149.74, 149.89, 149.91, 157.70, 157.72, 160.00, 160.01, 170.32 ppm; IR (film between KBr discs): $\tilde{\nu}$=1718 cm^{-1} (acid C=O); UV/Vis ($CHCl_3$): λ_{max} ($\log\varepsilon$)=427 (5.76), 555 (4.38), 595 nm (3.78); elemental analysis calcd (%) for $C_{147}H_{184}N_4O_{44}Zn$: C 63.59, H 6.68, N 2.02; found: C 63.76, H 6.82, N 2.00.

*Compound **3c***: Dendrimer **3d** was hydrolyzed to dendrimer **3c** by using the same procedure as that used for **2c**. Yield: 85%; ^{1}H NMR ($CDCl_3$, 400 MHz): δ=3.19 (s, 24H; OMe′), 3.20 (s, 48H; OMe), 3.34–3.38 (m, 48H; H^{a} and $H^{a'}$), 3.48–3.52 (m, 48H; H^{b} and $H^{b'}$), 3.64–3.69 (m, 48H; H^{c} and $H^{c'}$), 3.96–4.01 (m, 48H; H^{d} and $H^{d'}$), 4.93 (s, 24H; H^{g} and $H^{g'}$), 5.13–5.21 (m, 12H; H^{j} and $H^{j'}$), 6.35 (brt, J=2.4 Hz, 4H; $H^{e'}$), 6.37 (brt, J=2.4 Hz, 8H; H^{e}), 6.49 (brd, J=2.0 Hz, 8H; $H^{f'}$), 6.50 (brd, J=2.0 Hz, 16H; H^{f}), 6.54 (brt, J=2.0 Hz, 6H; H^{h} and $H^{h'}$), 6.74 (brd, J=1.6 Hz, 12H; H^{i} and $H^{i'}$), 7.04–7.06 (m, 3H; H^{p} and $H^{p'}$), 7.46 (brd, J=2.4 Hz, 4H; H^{q}), 7.48 (brd, J=2.0 Hz, 2H; $H^{q'}$), 8.14 (d, J=7.8 Hz, 2H; H^{y}), 8.29 (d, J=7.8 Hz, 2H; H^{x}), 8.70 (d, J=4.8 Hz, 2H; pyrrole-β), 8.80 (d, J=4.4 Hz, 2H; pyrrole-β), 9.00 (d, J=4.8 Hz, 2H; pyrrole-β), 9.05 ppm (d, J=4.4 Hz, 2H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=58.74, 67.21, 69.37, 69.40, 69.83, 70.06, 70.33, 70.50, 71.60, 100.92, 101.57, 101.66, 105.78, 105.85, 106.43, 115.20, 115.41, 119.24, 120.45, 120.51, 127.85, 128.86, 131.27, 131.97, 134.44, 138.97, 139.12, 139.19, 144.73, 144.86, 147.98, 149.24, 149.65, 149.73, 149.81, 157.56, 157.63, 159.85, 159.86, 159.94, 159.98, 168.62 ppm; IR (film between KBr discs): $\tilde{\nu}$=1716 cm^{-1} (acid C=O); UV/Vis ($CHCl_3$): λ_{max} ($\log\varepsilon$)=427 (5.75), 555 (4.35), 595 nm (3.77); elemental analysis calcd (%) for $C_{291}H_{376}N_4O_{92}Zn$: C 63.93, H 6.93, N 1.02; found: C 63.75, H 7.08, N 0.99.

*Compound **4c***: Ester dendrimer **4d** was hydrolyzed to dendrimer **4c** (83%) by using the same procedure as that used for dendrimer **2c**. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=3.23 (s, 48H; OMe′), 3.27 (s, 96H; OMe), 3.38–3.40 (m, 32H; $H^{a'}$), 3.43–3.45 (m, 64H; H^{a}), 3.51–3.54 (m, 32H; $H^{b'}$), 3.57–3.59 (m, 64H; H^{b}), 3.68 (brt, J=4.2 Hz, 32H; $H^{c'}$), 3.73 (brt, J=5.1 Hz, 64H; H^{c}), 3.97 (brt, J=5.1 Hz, 32H; $H^{d'}$), 4.02 (brt, J=5.1 Hz, 64H; H^{d}), 4.83 (brs, 16H; $H^{g'}$), 4.87 (s, 32H; H^{g}), 4.91 (brs, 8H; $H^{j'}$), 4.93 (brs, 16H; H^{j}), 5.13 (brs, 12H; H^{m} and $H^{m'}$), 6.34 (brt, J=2.1 Hz, 8H; $H^{e'}$), 6.38 (brt, J=2.1 Hz, 16H; H^{e}), 6.45–6.48 (brm, 28H; $H^{h'}$, H^{f} and H^{h}), 6.52 (brd, J=2.1 Hz, 32H; H^{f}), 6.57 (brs, 6H; H^{k} and $H^{k'}$), 6.60 (brd, J=1.8 Hz, 8H; $H^{i'}$), 6.62 (brd, J=1.8 Hz, 16H; H^{i}), 6.77 (brd, J=1.8 Hz, 12H; H^{l} and $H^{l'}$), 7.06–7.08 (brm, 3H; H^{p} and $H^{p'}$), 7.54 (brs, 6H; H^{q} and $H^{q'}$), 8.20 (d, J=8.0 Hz, 2H; H^{y}), 8.35 (d, J=8.0 Hz, 2H; H^{x}), 8.81 (brd, J=4.8, 2H; pyrrole-β), 8.99 (d, J=4.8 Hz, 2H; pyrrole-β), 9.06 (brd, J=4.8 Hz, 2H; pyrrole-β), 9.10 ppm (brd, J=4.8 Hz, 2H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 100 MHz): δ=58.76, 58.79, 67.24, 67.27, 69.40, 69.45, 69.71, 69.76, 69.87, 70.39, 70.43, 71.63, 71.68, 100.91, 100.96, 101.40, 105.87, 105.93, 106.27, 106.50, 106.61, 115.15, 119.42, 120.48, 120.54, 127.83, 129.18, 131.39, 132.01, 134.42, 138.94, 139.03, 139.10, 139.14, 144.89, 147.73, 149.32, 149.73, 149.86, 157.74, 159.83, 159.86, 159.88, 160.00, 167.72 ppm; IR (film between KBr discs): $\tilde{\nu}$=1718 cm^{-1} (ester C=O); UV/Vis ($CHCl_3$): λ_{max} ($\log\varepsilon$)=427 (5.78), 555 (4.38), 595 nm (3.76); MS (MALDI-TOF): m/z: 10849.9 $[M]^+$; elemental analysis calcd (%) for $C_{579}H_{760}N_4O_{188}Zn$: C 64.10, H 7.06, N 0.52; found: C 63.68, H 7.04, N 0.56.

Synthesis of oligopeptide ester 5:[13] Oligopeptide ester **5** was synthesized by repeated condensation of *N*-CBZ-glutamic acid (CBZ=carbobenzyloxy) and glutamic acid diethyl ester followed by deprotection as shown

in Scheme 4. $[\alpha]_D^{25} = -1.2$ ($c = 2.5$ in $CHCl_3$), -19.9 ($c = 0.26$ in MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.21$–1.32 (m, 24H; ester CH_3), 1.77–2.68 (m, 28H; CH_2), 2.96 (dd, 1H; CH), 4.05–4.28 (m, 18H; ester CH_2 and CH), 4.63–4.71 (m, 2H; CH), 4.81–4.88 (m, 2H; CH), 7.78 (d, $J =$ 8.8 Hz, 1H; amide NH), 7.81 (d, $J = 8.8$ Hz, 1H; amide NH), 8.21 (d, $J =$ 6.4 Hz, 1H; amide NH), 8.24 (d, $J = 9.2$ Hz, 1H; amide NH), 8.29 (d, $J =$ 9.2 Hz, 1H; amide NH), 8.41 ppm (d, $J = 6.4$ Hz, 1H; amide NH); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.01$, 14.13, 14.20, 26.45, 26.51, 27.00, 27.21, 27.23, 27.34, 30.03, 30.18, 30.70, 31.89, 31.97, 32.02, 33.15, 51.41, 51.46, 51.63, 51.67, 53.01, 53.30, 53.40, 60.46, 60.52, 60.60, 62.13, 62.17, 62.32, 172.37, 172.45, 172.52, 172.59, 172.67, 173.03, 173.92, 173.99, 174.11, 174.20, 175.59 ppm; MS (FAB): m/z: 1146.5 $[M+H]^+$; elemental analysis calcd (%) for $C_{51}H_{83}N_7O_{22}$: C 53.44, H 7.30, N 8.55; found: C 53.33, H 7.29, N 8.44.

Synthesis of ester dendrimers 1b–4b

*Compound **1b***: Acid dendrimer **1c** (25.0 mg, 0.028 mmol), HOBt (6.0 mg, 0.039 mmol), HBTU (15 mg, 0.039 mmol), and DIPEA (10 mg, 0.078 mmol) were added to dry dichloromethane (10 mL) and stirred at 0°C for 20 min. Peptide dendron **5** (45 mg, 0.039 mmol) was added and the reaction mixture was stirred at 0°C for 45 min and the temperature was allowed to rise to room temperature slowly. The progress was monitored by TLC (20% EtOAc in dichloromethane). The reaction was found to be complete after stirring for 36 h. The solvent was removed under vacuum and the residue was dissolved in dichloromethane and washed with 2% citric acid (10 mL), water (2×10 mL), aqueous $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by alumina column chromatography, followed by 1H-2H JAIGEL GPC, giving **1b** (70%). M.p. 117–118°C; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.20$–1.34 (m, 24H; Glu-ester CH_3), 1.85–2.85 (m, 28H; Glu-CH_2), 3.94 (s, 18H; OMe), 4.10–4.30 (m, 17H; Glu-ester OCH_2 and Glu-CH), 4.32–4.38 (m, 1H; Glu-CH), 4.44–4.50 (m, 1H; Glu-CH), 4.65–4.73 (m, 2H; Glu-CH), 4.83–4.89 (m, 1H; Glu-CH), 5.01–5.07 (m, 1H; Glu-CH), 6.82 (d, $J = 8.4$ Hz, 1H; CONH), 6.88 (brt, 3H; H^p), 7.39 (brd, 6H; H^q), 7.77 (d, $J = 9.0$ Hz, 1H; CONH), 7.90 (d, $J = 8.8$ Hz, 1H; CONH), 8.15 (d, $J = 8.4$ Hz, 2H; H^x), 8.21–8.34 (m, 5H; 3×CONH and H^y), 8.85 (d, $J = 4.6$ Hz, 2H; pyrrole-β), 8.87 (brd, 1H; CONH), 9.04 ppm (brs, 6H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 14.02$, 14.06, 14.13, 14.14, 14.20, 14.21, 26.44, 26.61, 27.00, 27.20, 27.44, 29.62, 30.15, 30.39, 30.59, 30.65, 31.39, 31.91, 32.01, 51.51, 51.62, 51.71, 51.79, 52.95, 53.29, 55.50, 60.27, 60.55, 60.57, 62.07, 62.26, 99.90, 113.76, 119.65, 120.80, 125.35, 131.45, 131.95, 132.10, 133.10, 134.34, 144.58, 146.31, 149.66, 149.83, 149.97, 158.50, 158.55, 166.51, 171.71, 172.34, 172.42, 172.51, 172.53, 172.61, 173.26, 173.36, 173.49, 173.74, 173.78, 173.92, 174.05 ppm; IR (film between KBr discs): $\tilde{\nu} = 1657$ (amide C=O), 1736 cm^{-1} (ester C=O); UV/Vis ($CHCl_3$): $\lambda_{max}(\log\varepsilon) = 425$ (5.77), 553 (4.37), 593 nm (3.68); HRMS (FAB): m/z calcd for $C_{102}H_{121}N_{11}O_{29}Zn$: 2027.7623 $[M]^+$; found: 2027.7596; elemental analysis calcd (%) for $C_{102}H_{121}N_{11}O_{29}Zn + H_2O$: C 59.80, H 6.05, N 7.52; found: C 59.80, H 6.05, N 7.44.

*Compound **2b***: Dendrimer **2b** was prepared by coupling **2c** with peptide dendron **5** using the same procedure as that for **1b** (reaction time = 24 h). Yield: 77%; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.21$–1.35 (m, 24H; Glu-ester CH_3), 1.80–2.85 (m, 28H; Glu-CH_2), 3.13 (s, 12H; OMe′), 3.15 (s, 24H; OMe), 3.29–3.33 (m, 24H; H^a and $H^{a'}$), 3.48–3.52 (m, 24H; H^b and $H^{b'}$), 3.72–3.76 (m, 24H; H^c and $H^{c'}$), 4.06–4.08 (m, 24H; H^d and $H^{d'}$), 4.09–4.49 (m, 19H; Glu-ester OCH_2 and Glu-CH), 4.67–4.74 (m, 2H; Glu-CH), 4.80–4.86 (m, 1H; Glu-CH), 5.00–5.06 (m, 1H; Glu-CH), 5.15 (brs, 12H; H^g and $H^{g'}$), 6.44 (brt, $J = 2.4$ Hz, 6H; H^e and $H^{e'}$), 6.65 (brd, $J = 2.4$ Hz, 12H; H^f and $H^{f'}$), 6.94 (d, $J = 7.6$ Hz, 1H; CONH), 7.04 (brs, 3H; H^p and $H^{p'}$), 7.46–7.48 (m, 6H; H^q and $H^{q'}$), 7.78 (d, $J = 10.4$ Hz, 1H; CONH), 7.89 (d, $J = 8.4$ Hz, 1H; CONH), 8.17 (d, $J = 8.4$ Hz, 2H; H^y), 8.24–8.27 (m, 3H; CONH and H^x), 8.34 (d, $J = 9.6$ Hz, 1H; CONH), 8.42 (d, $J = 6.4$ Hz, 1H; CONH), 8.80 (d, $J = 4.6$ Hz, 2H; pyrrole-β), 8.89 (d, $J = 6.8$ Hz, 1H; CONH), 8.94 (brs, 4H; pyrrole-β), 8.97 ppm (d, $J =$ 4.6 Hz, 2H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.02$, 14.05, 14.15, 14.19, 14.24, 26.50, 26.66, 27.00, 27.24, 27.30, 27.56, 29.77, 30.17, 30.47. 30.67, 30.72, 31.45, 32.06, 32.16, 51.60, 51.64, 51.74, 51.79, 51.85, 52.97, 53.44, 58.73, 58.75, 60.30, 60.55, 60.60, 62.06, 62.09, 62.32, 67.39, 69.49, 70.29, 70.44, 70.45, 71.60, 71.61, 101.27, 101.84, 101.92, 106.23, 114.99, 115.07, 119.45, 120.55, 125.30, 131.36, 131.91, 132.02, 133.05, 134.40, 139.12, 139.14, 144.73, 146.49, 149.53, 149.72, 149.86, 149.88, 157.71, 160.01, 166.57, 171.75, 172.38, 172.47, 172.53, 172.62, 172.67, 173.27, 173.36, 173.66, 173.81, 173.97, 174.06, 174.15 ppm; IR (film between KBr discs): $\tilde{\nu} = 1651$ (amide C=O), 1733 cm^{-1} (ester C=O); UV/Vis ($CHCl_3$): λ_{max} ($\log\varepsilon$) = 427 (5.79), 555 (4.38), 595 nm (3.76); MS (MALDI-TOF): m/z: 3903.2 $[M]^+$; elemental analysis calcd (%) for $C_{198}H_{265}N_{11}O_{65}Zn + 4H_2O$: C 59.80, H 6.92, N 3.87; found: C 59.81, H 6.94, N 3.85.

*Compound **3b***: Dendrimer **3b** was prepared by coupling acid dendrimer **3c** with peptide dendron **5** using the same procedure as that for **1b** (reaction time = 60 h). Yield: 80%; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 1.19$–1.34 (m, 24H; Glu-ester CH_3), 1.90–2.79 (m, 28H; Glu-CH_2), 3.17 (s, 24H; OMe′), 3.20 (s, 48H; OMe), 3.32–3.34 (m, 16H; $H^{a'}$), 3.36–3.38 (m, 32H; H^a), 3.46–3.48 (m, 16H; $H^{b'}$), 3.50–3.53 (m, 32H; H^b), 3.63 (brt, $J =$ 4.8 Hz, 16H; $H^{c'}$), 3.68 (brt, $J = 4.8$ Hz, 32H; H^c), 3.96 (brt, $J = 5.2$ Hz, 16H; $H^{d'}$), 4.00 (brt, $J = 5.2$ Hz, 32H; H^d), 4.07–4.48 (m, 19H; Glu-ester OCH_2 and Glu-CH), 4.66–4.73 (m, 2H; Glu-CH), 4.79–4.85 (m, 1H; Glu-CH), 4.92 (s, 8H; $H^{g'}$), 4.94 (s, 16H; H^g), 5.00–5.05 (m, 1H; Glu-CH), 5.14 (brs, 12H; H^j and $H^{j'}$), 6.34 (brt, $J = 2.4$ Hz, 4H; $H^{e'}$), 6.36 (brt, $J =$ 2.4 Hz, 8H; H^e), 6.49 (brd, $J = 2.4$ Hz, 8H; $H^{f'}$), 6.52 (brd, $J = 2.4$ Hz, 16H; H^f), 6.53 (brt, $J = 2.0$ Hz, 6H; H^h and $H^{h'}$), 6.73–6.75 (m, 12H; H^i and $H^{i'}$), 6.94 (d, $J = 7.2$ Hz, 1H; CONH), 7.05–7.07 (m, 3H; H^p and $H^{p'}$), 7.50 (brm, 6H; H^q and $H^{q'}$), 7.75 (d, $J = 9.6$ Hz, 1H; CONH), 7.88 (d, $J =$ 9.6 Hz, 1H; CONH), 8.15 (d, $J = 8.0$ Hz, 2H; H^y), 8.21–8.24 (m, 3H; H^x and CONH), 8.31 (d, $J = 10.0$ Hz, 1H; CONH), 8.42 (d, $J = 7.2$ Hz, 1H; CONH), 8.81 (d, $J = 4.4$ Hz, 2H; pyrrole-β), 8.86 (d, $J = 6.6$ Hz, 1H; CONH), 9.01 (s, 4H; pyrrole-β), 9.02 ppm (d, $J = 4.4$ Hz, 2H; pyrrole-β); ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.02$, 14.12, 14.16, 14.19, 26.48, 26.63, 26.93, 27.23, 27.61, 29.59, 30.15, 30.42, 30.60, 30.69, 31.39, 32.00, 32.14, 51.57, 51.68, 51.72, 51.76, 52.00, 52.83, 53.41, 58.77, 58.79, 60.29, 60.53, 60.55, 60.59, 61.98, 62.07, 62.29, 67.26, 67.30, 69.41, 69.45, 69.91, 70.14, 70.38, 70.42, 71.63, 71.66, 101.02, 101.73, 105.92, 105.95, 106.51, 115.04, 115.20, 119.38, 120.44, 120.55, 125.28, 131.32, 131.99, 132.83, 134.43, 139.02, 139.07, 139.15, 144.93, 144.98, 146.61, 149.50, 149.70, 149.85, 149.90, 157.69, 157.73, 159.90, 159.91, 159.98, 166.66, 172.72, 172.36, 172.47, 172.50, 172.55, 172.62, 172.66, 173.17, 173.60, 173.64, 173.95, 170.01, 174.06 ppm; IR (film between KBr discs): $\tilde{\nu} = 1651$ (amide C=O), 1732 cm^{-1} (ester C=O) ; UV/Vis ($CHCl_3$): λ_{max} ($\log\varepsilon$) = 427 (5.79), 555 (4.39), 595 nm (3.79); MS (MALDI-TOF): m/z: 6594.8 $[M]^+$; elemental analysis calcd (%) for $C_{342}H_{457}N_{11}O_{113}Zn + 6H_2O$: C 61.27, H 7.05, N 2.30; found: C 61.27, H 7.14, N 2.20.

*Compound **4b***: Dendrimer **4b** was prepared by coupling acid dendrimer **4c** with peptide dendron **5** using the same procedure as that for **1b** (reaction time = 85 h; purified by 2H-3H JAIGEL GPC followed by silica gel flash chromatography). Yield: 52%; ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta =$ 1.17–1.33 (m, 24H; Glu-ester CH_3), 1.80–2.80 (m, 28H; Glu-CH_2), 3.22 (s, 48H; OMe′), 3.25 (s, 96H; OMe), 3.36–3.39 (m, 32H; $H^{a'}$), 3.41–3.43 (m, 64H; H^a), 3.50–3.53 (m, 32H; $H^{b'}$), 3.55–3.58 (m, 64H; H^b), 3.66 (brt, $J = 4.8$ Hz, 32H; $H^{c'}$), 3.71 (brt, $J = 4.8$ Hz, 64H; H^c), 3.96 (brt, $J =$ 4.8 Hz, 32H; $H^{d'}$), 4.01 (brt, $J = 4.8$ Hz, 64H; H^d), 4.05–4.27 (m, 17H; Glu-ester OCH_2 and Glu-CH), 4.38–4.45 (m, 2H; Glu-CH), 4.62–4.71 (m, 2H; Glu-CH), 4.75 (m, 1H; Glu-CH), 4.82 (s, 16H; $H^{g'}$), 4.87 (s, 32H; H^g), 4.90 (brs, 8H; $H^{j'}$), 4.93 (brs, 16H; H^j), 5.00 (m, 1H; Glu-CH), 5.13 (brs, 12H; H^m and $H^{m'}$), 6.33 (brt, $J = 2.0$ Hz, 8H; $H^{e'}$), 6.37 (brt, $J =$ 2.0 Hz, 16H; H^e), 6.44 (brt, $J = 1.6$ Hz, 4H; $H^{h'}$), 6.46 (brd, $J = 2.0$ Hz, 24H; $H^{f'}$ and H^b), 6.50 (d, $J = 2.0$ Hz, 32H; H^f), 6.55 (brm, 6H; H^k and $H^{k'}$), 6.59 (brd, $J = 2.0$ Hz, 8H; $H^{i'}$), 6.61 (brd, $J = 2.0$ Hz, 16H; H^i), 6.76 (brd, $J = 2.0$ Hz, 4H; $H^{l'}$), 6.78 (brd, $J = 2.0$ Hz, 8H; H^l), 7.03 (brd, $J =$ 7.2 Hz, 1H; CONH), 7.07 (brs, 1H; $H^{p'}$), 7.10 (brs, 2H; H^p), 7.55 (brs, 6H; H^q and $H^{q'}$), 7.72 (d, $J = 6.8$ Hz, 1H; CONH), 7.84 (d, $J = 6.8$ Hz, 1H; CONH), 8.15–8.28 (m, 6H; H^y, H^x, and CONH), 8.46 (brd, $J =$ 6.8 Hz, 1H; CONH), 8.82 (brm, 3H; CONH and pyrrole-β), 9.05–9.08 ppm (m, 6H; pyrrole-β); IR (film between KBr discs): $\tilde{\nu} = 1652$ (amide C=O), 1734 cm^{-1} (ester C=O); UV/Vis ($CHCl_3$): λ_{max} ($\log\varepsilon$) = 427 (5.78), 555 (4.39), 595 nm (3.75); MS (MALDI-TOF): m/z: 10967.86 $[M]^+$; elemental analysis calcd (%) for $C_{630}H_{841}N_{11}O_{209}Zn + 8H_2O$: C 62.42, H 7.13, N 1.27; found: C 62.38, H 7.19, N 1.22.

Synthesis of proteo-dendrimers 1a–4a

*Compound **2a***: Dendrimer **2b** (25 mg, 0.0064 mmol) was dissolved in THF (1 mL) and MeOH (1 mL). LiOH (8.8 molar equiv, 0.056 mmol) dissolved in water (0.5 mL) was added to reaction mixture and stirred at room temperature. The progress of hydrolysis was followed by capillary electrophoresis by withdrawing small aliquots (see Figure S1 in the Supporting Information). After 36 h the solvents were removed under vacuum and the residue was dissolved in water (1 mL) and LiOH (1.6 molar equiv) was added. The reaction mixture was stirred at room temperature for a further 24 h. The solvents were removed under vacuum and the residue was dried under high vacuum, offering **2a** as a hygroscopic semisolid. ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.90–2.55 (brm, 28H; Glu CH_2), 3.04 (s, 12H; OMe′), 3.12 (s, 24H; OMe), 3.13–3.16 (m, 8H; CH_2O), 3.21–3.27 (brm, 24H; CH_2O), 3.30–3.40 (brm, 24H; CH_2O), 3.51 (brt, 16H; CH_2O), 3.62 (brs, 8H; CH_2O), 3.84 (brs, 16H; CH_2O), 4.25–4.74 (4brm + 1brs, total 11H; benzylic CH_2 and Glu-CH), 5.05 (brs, 8H; benzylic CH_2), 6.20 (s, 2H; ArH), 6.29 (s, 4H; ArH), 6.35 (s, 4H; ArH), 6.53 (brs, 8H; ArH), 6.68 (brs, 1H; ArH), 6.96 (brs, 2H; ArH), 7.37 (s, 2H; ArH), 7.42 (s, 4H; ArH), 8.31 (brs, 4H; ArH), 8.75 (brs, 6H; pyrrole-β), 8.86 ppm (brd, 2H; pyrrole-β); IR (between KBr discs): $\tilde{\nu}$ = 1596 cm^{-1} (broad with shoulder at 1656 cm^{-1}); UV/Vis (5.0×10^{-3} mol dm^{-3} sodium phosphate buffer containing 10% DMF, pH 7.0): λ_{max} (log ε) = 429 (5.52), 558 (4.34), 599 nm (3.81); MS (MALDI-TOF, linear): *m*/z calcd for $C_{182}H_{233}N_{11}O_{65}Zn$: 3680.3 $[M]^+$; found: broad peak centered at 3682.4.

*Compound **1a***: Dendrimer **1b** was hydrolyzed to proteo-dendrimer **1a** by using a procedure similar to that used for **2a** using 9.6 molar equivalent of LiOH. M.p.: >300 °C; ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.9–2.5 (brm, 28H; Glu CH_2), 3.94 (s, 18H; OMe), 4.24–4.63 (4brm, 7H; Glu-CH), 6.89 (brt, 3H; ArH), 7.36 (brt, 6H; ArH), 8.26 (brq, 4H; ArH), 8.75 (d, *J* = 5.4 Hz, 2H; pyrrole-β), 8.91 ppm (m, 6H; pyrrole-β); IR (KBr): $\tilde{\nu}$ = 1590 cm^{-1} (broad with shoulder at 1654 cm^{-1}); UV/Vis (5.0×10^{-3} mol dm^{-3} sodium phosphate buffer containing 10% DMF, pH 7.0): λ_{max} (log ε) = 425 (5.78), 557 (4.33), 597 nm (3.90); MS (MALDI-TOF, reflectron): *m*/*z* calcd for $C_{86}H_{89}N_{11}O_{29}Zn$: 1806.1 $[M]^+$; found: 1807.7.

*Compound **3a***: Dendrimer **3b** (25 mg) was dissolved in THF (1 mL) and MeOH (1 mL). LiOH (9.6 molar equiv, dissolved in 0.5 mL of water) was added to the reaction mixture and stirred at room temperature. The progress of the hydrolysis was followed by capillary electrophoresis by withdrawing small aliquots (see Figure S1 in the Supporting Information). After 48 h the solvents were removed under vacuum and the residue was dissolved in water (1 mL) and LiOH (3.2 molar equiv) was added. The reaction mixture was stirred at room temperature for another 48 h and the reaction was completed. The excess of LiOH was removed by ultrafiltration using a 3000 molecular weight cut-off membrane using distilled water three times. Finally, the residue was dried under high vacuum, offering **3a** as a hygroscopic semisolid. ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.90–2.55 (brm, 28H; Glu CH_2), 3.09 (s, 24H; OMe′), 3.13 (s, 48H; OMe), 3.22 (brm, 16H; CH_2O), 3.25–3.31 (brm, 48H; CH_2O), 3.36 (brm, 48H; CH_2O), 3.47 (brm, 32H; CH_2O), 3.60 (brm, 16H; CH_2O), 3.75 (brm, 32H; CH_2O), 4.27–4.70 (3brm + brs, 35H; benzylic CH_2 and Glu-CH), 5.01 (brs, 8H; benzylic CH_2), 6.13–6.60 (6brs, 54H; ArH), 6.80 (brs, 1H; ArH), 6.96 (brs, 2H; ArH), 7.37 (brs, 2H; ArH), 7.42 (brs, 4H; ArH), 8.26 (brs, 4H; ArH), 8.76–8.80 (2brs, 6H; pyrrole-β), 8.93 ppm (brd, 2H; pyrrole-β); IR (film between KBr discs): $\tilde{\nu}$ = 1598 cm^{-1} (broad with shoulder at 1655 cm^{-1}); UV/Vis (5.0×10^{-3} mol dm^{-3} sodium phosphate buffer containing 10% DMF pH 7.0): λ_{max} (log ε) = 436 (5.53), 558 (4.34), 599 nm (3.82); MS (MALDI-TOF, linear): *m*/*z* calcd for $C_{326}H_{425}N_{11}O_{113}Zn$: 6371.4 $[M]^+$; found: broad peak centered at 6388.7.

*Compound **4a***: Dendrimer **4b** (25 mg) was dissolved in THF (1 mL) and MeOH (1 mL). LiOH (14.4 molar equiv, dissolved in 0.5 mL of water) was added to the reaction mixture and stirred at room temperature. The progress of the hydrolysis was followed by capillary electrophoresis by withdrawing small aliquots (see Figure S1 in the Supporting Information). After 48 h the solvents were removed under vacuum and the residue was dissolved in water (1 mL) and LiOH (3.2 molar equiv) was added. The reaction mixture was stirred at room temperature for another 48 h. More LiOH (3.2 molar equiv) was added and stirred, and the reaction was found to be completed after 24 h. The excess of LiOH was removed by ultrafiltration using a 3000 molecular weight cut-off membrane using distilled water three times. Finally, the residue was dried under vacuum, giving **4a** as a hygroscopic semisolid. ^{1}H NMR (CD_3OD, 400 MHz): δ = 1.90–2.55 (brm, 28H; Glu CH_2), 3.16 (brs, 144H; OMe′ and OMe), 3.29–3.31 (brs, 96H; CH_2O), 3.41 (brs, 96H; CH_2O), 3.51 (brm, 96H; CH_2O), 3.78 (brs, 96H; CH_2O), 4.28–4.70 (brm + 2brs, 189H; benzylic CH_2 and Glu-CH), 6.13–6.60 (brm, 126H; ArH), 6.91 (brs, 3H; ArH), 7.43 (brs, 6H; ArH), 8.26 (brs, 4H; ArH), 8.77, 8.99 ppm (2brs, 8H; pyrrole-β); IR (film between KBr discs): $\tilde{\nu}$ = 1595 cm^{-1} (broad with shoulder at 1656 cm^{-1}); UV/Vis (5.0×10^{-3} mol dm^{-3} sodium phosphate buffer containing 10% DMF, pH 7.0): λ_{max} (log ε) = 434 (5.53), 558 (4.34), 599 nm (3.81); MS (MALDI-TOF, linear): *m*/*z* calcd for $C_{614}H_{809}N_{11}O_{209}Zn$: 11753.6 $[M]^+$; found: broad peak centered at 11774.7.

Log*K* determination: The complexation experiments were carried out at pH 7.0 (5.0×10^{-3} mol dm^{-3} sodium phosphate buffer containing 10% DMF v/v). A dendrimer solution (2.5×10^{-7} mol dm^{-3}, 2.5 mL) was added to a 1 cm cuvette and its fluorescence spectra was recorded by excitation at 558 nm. Cytochrome *c* solution (1.25×10^{-5} mol dm^{-3} and 6.25×10^{-5} mol dm^{-3}) prepared in the same buffer was added in small aliquots of 5–30 μl. After each addition, the sample was stirred for 5 min before the fluorescence spectrum was measured. The emission intensity at 610 or 650 nm was plotted against the molar ratio *x* [cytochrome *c*]/[dendrimer]. The stability constants were calculated by using nonlinear curve fitting as described below. We use the following abbreviations: R = proteo-dendrimer; S = cytochrome *c*; $[R]_t$ and $[S]_t$ = total concentrations of the proteo-dendrimer and cytochrome *c*, respectively. Total concentration of proteo-dendrimer $[R]_t$ was kept constant (2.5×10^{-7} mol dm^{-3}) during titration and the concentration of cytochrome was varied from 0 to 17.5×10^{-7} mol dm^{-3}. The molar ratio *x* of two species is defined as

$$x = [S]_t/[R]_t \tag{1}$$

Stepwise formation constants for 1:1 and 1:2 (cytochrome *c*/proteo-dendrimer) complexes are defined in Equations (2) and (3):

$$R + S = RS \qquad K_1 = \frac{[RS]}{[R][S]} \tag{2}$$

$$RS + R = R_2S \qquad K_2 = \frac{[R_2S]}{[RS][R]} \tag{3}$$

Total concentrations of proteo-dendrimer and cytochrome *c* are expressed as Equations (4) and (5):

$$[R]_t = [R] + [RS] + 2[R_2S] \tag{4}$$

$$[S]_t = [S] + [RS] + [R_2S] \tag{5}$$

From Equations (1)–(5), the concentration of free dendrimer [R] can be obtained by solving the following equation [Eq. (6)]:

$$\begin{aligned} &K_1K_2[R]^3 + (K_1 - K_1K_2[R]_t + 2K_1K_2[R]_t x)[R]^2 \\ &\quad + (1 - K_1[R]_t + K_1R_t x)[R] - [R]_t = 0 \end{aligned} \tag{6}$$

The concentration of other species can then be expressed as shown in Equations (7)–(9):

$$[S] = \frac{[R]_t x}{1 + K_1[R] + K_1K_2[R]^2} \tag{7}$$

$$[RS] = K_1[R][S] \tag{8}$$

$$[R_2S] = K_1K_2[R]^2[S] \tag{9}$$

The observed fluorescence can be expressed as shown in Equation (10):

$$F_x = I_0[R] + I_{1:1}[RS] + I_{1:2}[R_2S] \tag{10}$$

In the absence of cytochrome *c*, the fluorescence can be written as shown in Equation (11):

$$F_t = I_0[R]_t \quad (11)$$

From Equations (10) and (11), Equation (12) can be formed:

$$\frac{F_x}{F_t} = \frac{[R]}{[R]_t} + \frac{I_{1:1}}{I_0}\frac{[RS]}{[R]_t} + \frac{I_{1:2}}{I_0}\frac{[R_2S]}{[R]_t} \quad (12)$$

in which $I_{1:1}/I_0$ and $I_{1:2}/I_0$ are the fluorescence intensity ratios of complexes RS and R_2S to free dendrimer, respectively.

For fitting the titration curve, F_x/F_t was plotted against molar ratio x, and K_1, K_2, $I_{1:1}/I_0$, and $I_{1:2}/I_0$ were treated as variables to be determined. These parameters were determined by applying the least-square method using the curve-fit function of IGOR Pro software (version 4.08). The equations used for 1:1 complexation between **4a** and cytochrome *c* were obtained similarly.

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