Supplementary Material (ESI) for Chemical Communications

Supramolecular Dendrimer Capsules by Cooperative Binding

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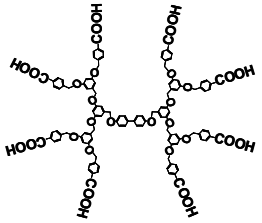
Supporting Information

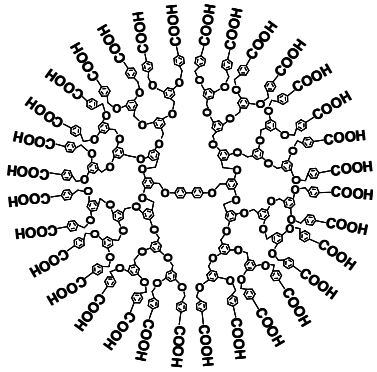
Materials and Methods

1. Materials

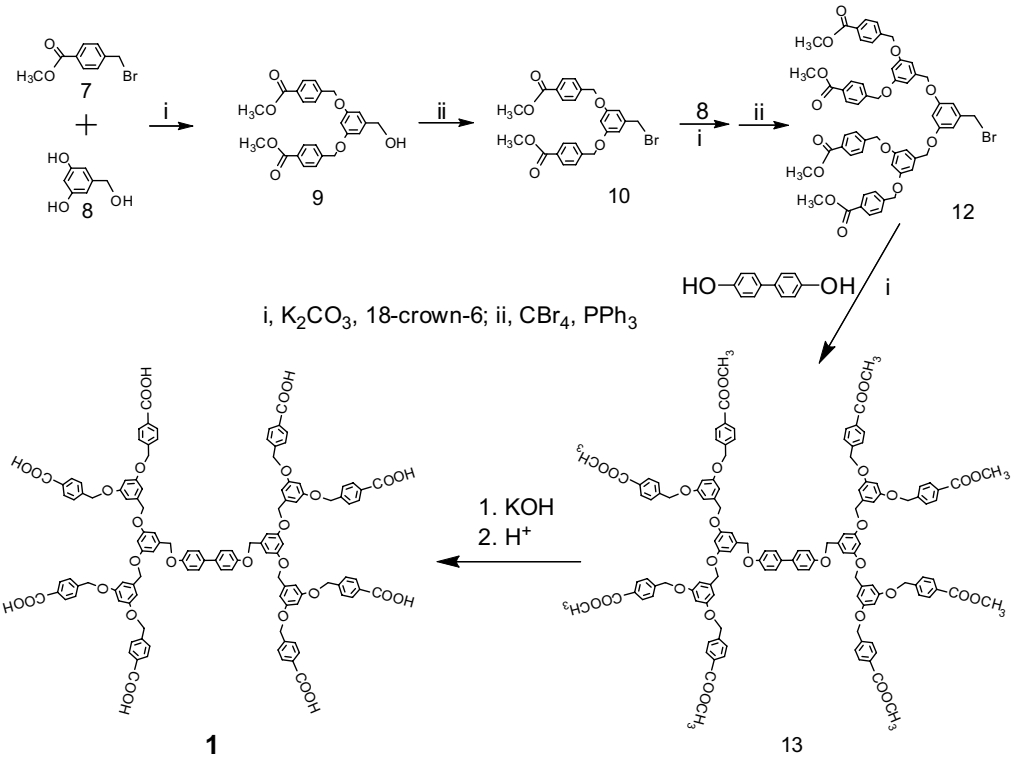
1.1.G2-Fréchet-8COOH and G4-Fréchet-32COOH were synthesized by the reported convergent route (*1*), using 3,5-dihydroxybenzyl alcohol as the monomer unit and step-wise growth process consisting of activation by bromination and coupling by alkylation. In the final step, two dendritic fragments were coupled with difunctional core, 4,4'-dihydroxy-biphenyl, and followed by hydrolysis of the methyl ester protecting groups. All synthesized dendrimers were characterized by NMR before use.



G2-Fréchet-8COO$^-$ dendrimer

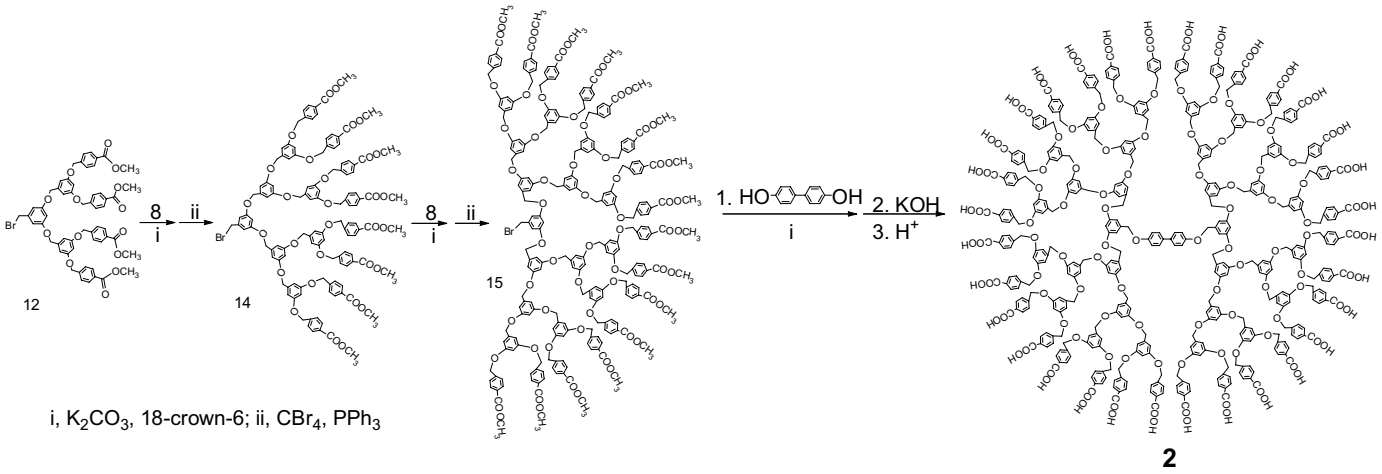


G4-Fréchet-32COO$^-$ dendrimer



Scheme S1. Reaction scheme for the preparation of G2-Fréchet-8COOH.

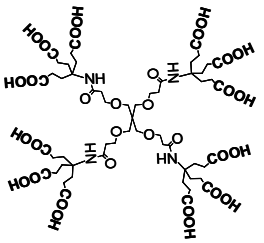
^{1}H NMR([H_6]-DMSO) of the synthesized G2-Fréchet-8COOH: δ(ppm) = 4.94 and 5.05 (s,24H,OCH_2), 6.4-6.7(m, 18H, ArH), 6.91(m,4H, core ArH) and 7.44 and 8.00(m, total 36H, 32 PhH and 4 core ArH)



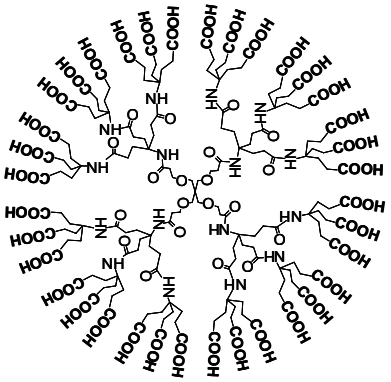
Scheme S2. Reaction scheme for the preparation of Frechet-G4-32COOH.

^{1}H NMR([H_6]-DMSO) of the synthesized G4-Fréchet-32COOH: δ(ppm) = 4.95 and 5.10 (s,124H,OCH_2), 6.49-6.70 (m,90H,ArH), 6.91(m,4H,core ArH), 7.50 and 7.98 (m, total 132H, 128PhH and 4 core ArH)

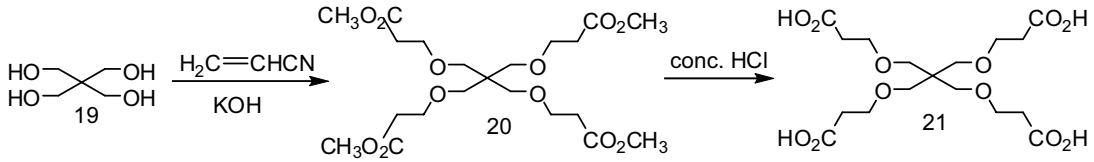
1.2. G1-Newkome-12COOH and G2-Newkome-36COOH were synthesized by the reported divergent approach, using a tetraacid core and stepwise growth consisting of DCC/1-HBT peptide coupling with the tri-branched amine monomer, and activation by facile removal of the t-Butyl protecting group.(*2, 3*) The tetraacid core was prepared by Michael addition of acrylonitrile to pentaerythritol(*3, 4*), and the tri-branched amine monomer was synthesized by the addition of nitromethane to t-butylacrylate, followed by Raney-nickel reduction(*5*)



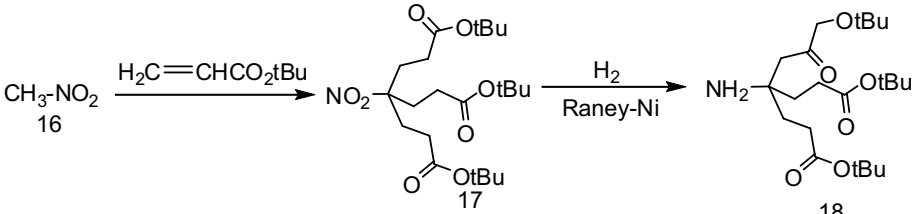
G1-Newkome-12COO$^-$ dendrimer



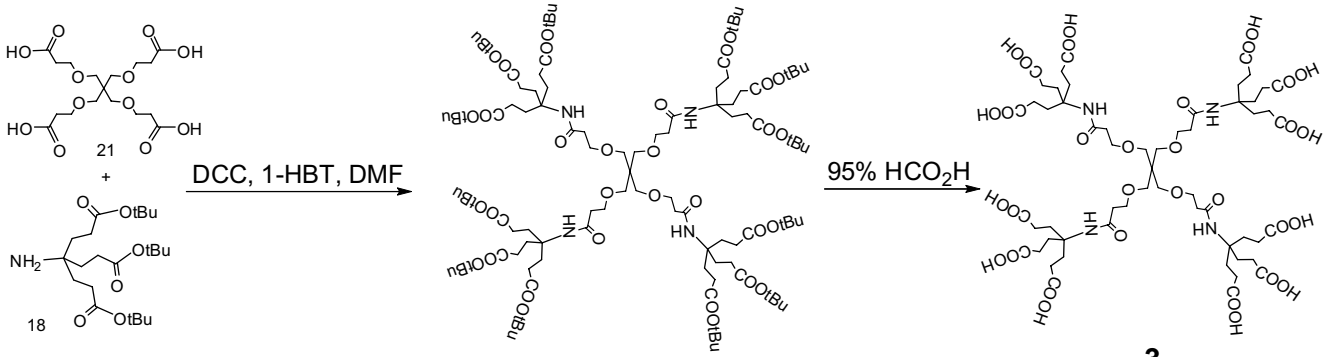
G2-Newkome-36COO$^-$ dendrimer



Scheme S3. *Reaction scheme for the preparation of tetraacid core.*

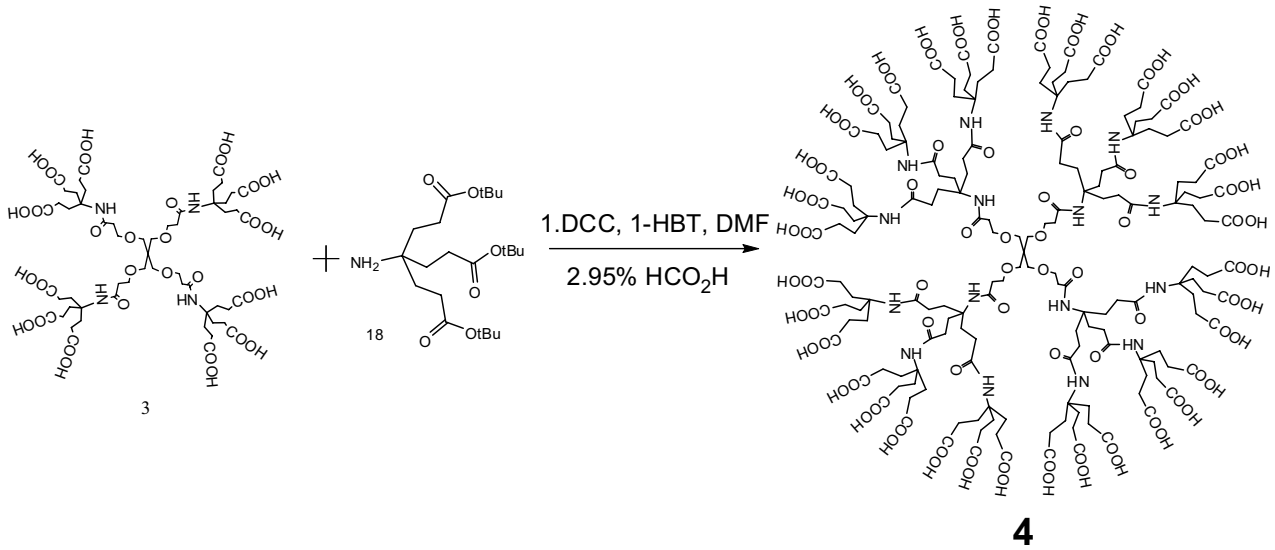


Scheme S4. Reaction scheme for the preparation of tri-branched amine monomer.



Scheme S5. Reaction scheme for the preparation of G1-Newkome-12COOH.

^{1}H NMR(D_2O) of the synthesized G1-Newkome-12COOH: δ(ppm) = 1.76 (t,24H,C**H**$_2$CH$_2$COO), 1.98 (t,24H,CH$_2$COO), 2.32(br,8H,CH$_2$CONH) and 3.15(br,8H,CH$_2$O) and 3.52(br,8H,OCH$_2$)



Scheme S6. Reaction scheme for the preparation of G2-Newkome-36COOH.

^{1}H NMR(D_2O) of the synthesized G2-Newkome-36COOH: δ(ppm) = 1.75 and 1.95 (br, 192H, CH_2CH_2CO), 2.38 (br,8H,CH_2CONH), 3.24(br,8H,CH_2O) and 3.50(br,8H,OCH_2)

2. **Methods**

2.1. Fluorescent and Light Microscopy Imaging.

A hydrophobic fluorophore dye (PKH 26) was used to label the Fréchet-type dendrimers in aqueous solution. The dipersed dendrimers and their self-assembled supramolecular capsules by the addition of Ca^{2+} in water were directly observed on an Olympus IX71 inverted fluorescence microscope with a 60X objective and a Cascade CCD camera. 2 μL samples were used in the chamber formed between glass slide and cover slip for imaging.

2.2. Cryo-TEM, TEM, negatively-stained TEM imaging.

For Cryo-TEM imaging on the dispersed carboxylate-decorated dendrimers in aqueous solutions, typically 3 μ L of the sample solution was pipetted onto a carbon coated copper grids. A piece of filter paper was then used to quickly remove the excess liquid, and the sample copper grid was quickly plunged into liquid ethane cooled by a reservoir of liquid nitrogen to ensure vitrification. The specimen was stored under liquid nitrogen, and then transferred to a cryogenic sample holder (Gatan 626) in a FET TECNAI 20G TEM operating at -177°C for imaging.

For TEM imaging on the Ca^{2+} induced capsules, the sample copper grids were freeze dried before imaging. For negative staining, a droplet of 2% uranyl acetate was placed onto the freeze dried specimen for 60 seconds and the excess liquid was removed by filter paper before imaging. TEM images were obtained on a FET TECNAI 20G Transmission Electron Microscopy operating at an acceleration voltage of 200kV and at ambient temperature.

2.3. Encapsulation of Doxorubicin.

Doxorubicin, a spontaneously red-fluorescent anti-cancer drug, was dissolved in aqueous solution. Green fluorescently-labeled Fréchet-COO^- dendrimers and then Ca^{2+} were added to the Dox solution to form capsules and encapsulate Dox. The Fréchet-COO^-/ Ca^{2+} capsules were then collected by centrifuge and re-dispersed in water for FM imaging, which demonstrated the encapsulation of the red-fluorescent Dox within the cavity of the green-fluorescent Fréchet-COO^- dendrimer capsules.

2.4. Isothermal Titration Calorimetery (ITC) measurement.

ITC thermograms were recorded on a VP-ITC MicroCalorimeter (MicroCal, Inc). Aqueous solution of 0.344 mM G2-Newkome-36COO^- dendrimer at pH 7.4 was loaded into the titration cell, and the reference cell was filled with deionized water. Fifty successive injections of 50 mM Ca^{2+} were made into the dendrimer sample cell in 5 μL increments at 10 min interval with stirring at 300 rpm to ensure complete equilibration. Control experiments to determine the heat of dilution were carried out by making identical injections in the absence of dendrimers. The net binding reaction heat was obtained by subtracting the heat of dilution from the measured total heat of reaction. The titration data were then fitted using the MicroCal Origin software and least-square algorithm, and these data were best fit to a two independent binding site model. The binding enthalpy ΔH, binding constant K, and the binding stoichiometry n were permitted to float during the least-square minimization process and taken as the best-fit value.

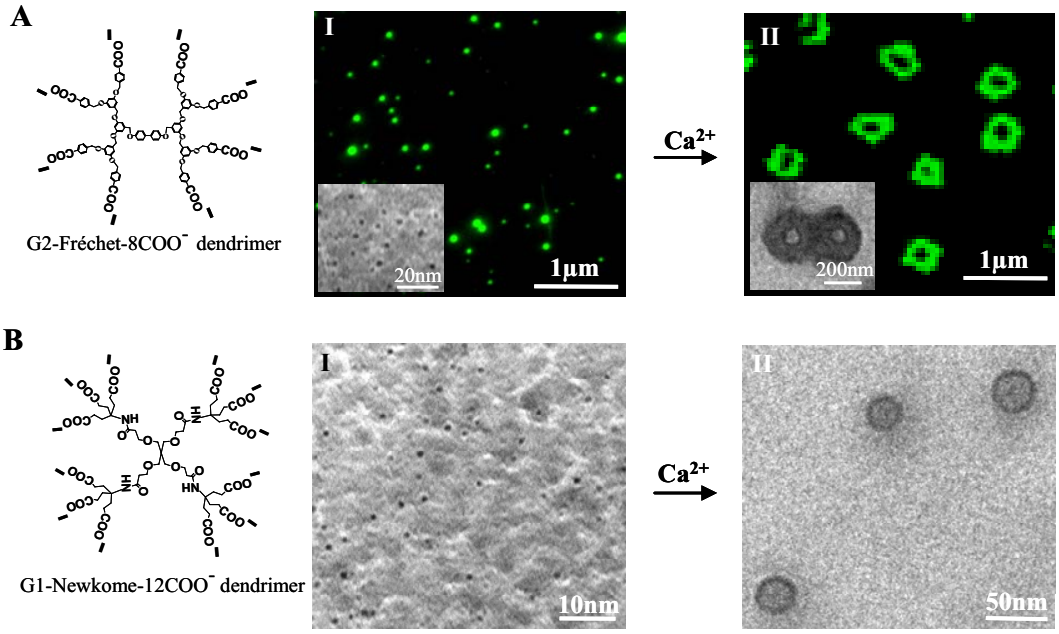
2.4. Computational simulations.

Molecular dynamics (MD) simulations on the conformations of the Newkome-carboxylate(COO^-) dendrimers in water were performed with the GLYCAM06 force field with the Sander module of the AMBER 9 simulation package (*6*). Detailed simulation procedures can be found in the following webpage: http://www.glycam.com/supporting_info.html and will be published elsewhere.

Briefly, Newkome-COO^- dendrimers were built from the angles and torsions obtained from HF/6-31++g** optimized fragments which were used to generate the partial charge models. These initial structures were then subjected to a gas-phase minimization using a 5,000 step minimization with a dielectric of 1.0 and a non-bonded cutoff of 12.0 Å. Before solvating the dendrimer molecule, it was neutralized by 18 Ca^{2+} ions (7) to allow for Particle Mesh Ewald simulations (8). The optimized dendrimer models were then solvated using a minimum of edge to solute distance of 8.0 Å to form a cubic box of water. Each solvated complex was minimized using 5,000 steps of steepest descent and up to 15,000 steps of conjugate gradient minimization with a 8.0 Å non-bonded cutoff and a dielectric constant of 1.0. Following minimization, the dendrimer was subjected to a series of constant pressure (NPT) simulations using a 2 fs timestep, non-bonded scaling factors set to unity, an 8.0 Å non-bonded cutoff, a pressure of 1.0 atm, and a compressibility equivalent to water, 44.6 10^{-11} m kg^{-1} s^2. Since the dendrimers were developed from small fragments, it was necessary to equilibrate the system using 100 ps of heating from 5 K to 300 K followed by 10,000 ps at 300 K and annealing from 300 K back to 5 K over 600 ps. A standard heat/cooling scheme was used to disorder and relax the dendrimers into different conformations. The first heating/cooling scheme consists of heating from 5 K to 300 K over 50 ps followed by 50 ps of heating to 1,000 K and 500 ps of simulation time at 1,000 K. The system was then cooled to 300 K over 100 ps and 50 ps of simulation time was obtained at 300 K and re-heated once more from 300 K to 1000 K over 50 ps and a 1000 K simulation time of 500 ps followed by cooling back to 300 K over 100 ps. In subsequent heating schemes, the simulation is heated from 5 K to 300 K for 50 ps and the simulation is not annealed to 5 K between cycles. Ten sequential heating/cooling schemes are performed where structures are extracted after each scheme finishes and these structures were each subjected to a 10 ns production run at 300 K yielding ten distinct production simulations of 10 ns each. The solvent accessible surface area (SASA) was obtained from the

MM_PBSA module in AMBER. An estimation of the diameter of the solvated dendrimers was also performed using the average maximum cross-distance from the heavy atoms at the ends of each finger to each finger on the branch opposite it. The two maximum distances from both pairs of opposite branches are then averaged together to get an approximate diameter. The average diameter for G2- and G1-Newkome-COO^- dendrimers were calculated as 4.1 nm and 2.7 nm respectively, consistent with the cryo-TEM measurements.

Figure S1. Ca^{2+} induced supramolecular capsule formation from the smaller lower generation of carboxylate-dendrimers. **A.** Capsules from G2-Fréchet-8COO^- dendrimers; The average cavity size is around 70nm, and the membrane thickness can grow up to 80nm. **B.** Capsules from G1-Newkome-12COO^- dendrimers; The average cavity size is around 30nm, and the membrane thickness can grow up to 12nm.



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