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Thiol−Disulfide Interchange Mediated Reversible Dendritic Megamer Formation and Dissociation

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ABSTRACT: We report the formation of dynamic, reversible cross-linked dendritic megamers and their dissociation to monomeric dendrimers, through a thiol−disulfide interchange reaction. For this study, poly(alkyl aryl ether) dendrimers up to three-generations presenting thiol functionalities, were prepared. The series from zero to three generations of dendrimers were installed with 3, 6, 12, and 24 thiol functionalities at their peripheries. Upon synthesis, cross-linking of the dendrimer was accomplished through disulfide bond formation. The cross-linking of dendrimers was monitored through optical density changes at 420 nm. Dense cross-linking led to visible precipitation of dendritic megamers and the morphologies of the megamers were characterized by transmission electron microscopy. The disulfide cross-links between megamer monomers could be dissociated readily upon reduction of disulfide bond by dithiothreitol reagent. Preliminary studies show that dendritic megamers encapsulate C_{60} and the efficiency of encapsulation increased with increasing generation of dendritic megamer.

Introduction

Dendritic macromolecules exhibit a degree of branching of 100% and as a result, these macromolecules present a large number of chain ends.[1] Fine tuning the structural features are important to interface dendrimers in varied studies and applications.[2] Core−shell dendrimers are elegant examples, wherein the core and the peripheral regions of dendritic structures are constituted with differing monomer branching units.[3] Another important direction in the studies of dendrimers is the cross-linking of dendrimers, in a manner analogues to the cross-linking exercized in polymers, that allow modifications, for example, in the viscoelastic properties of the resulting cross-linked dendrimers, and in increasing the water solubility behavior of otherwise water-insoluble dendrimers.[4,5] UV-curing of a functionalized dendrimer has also been reported previously, which provided a homogeneous net-worked dendrimer.[6] Covalent assembly of dendrimers, leading to structurally controlled megamers, are identified by Tomalia and co-workers, as a route to obtain large dendrimeric or megameric clusters that exhibit extraordinary topological features, characterized as dendri-catenanes, dendri-macrocycles, and dendri-clefts.[7] Cross-linking of dendrimers with the aid of bifunctional aldehyde reagents with amine-terminated dendrimers was adopted to generate dendritic networks, providing liquid crystalline, chemical and biological properties.[8] In an elegant demonstration, Zimmerman and co-workers exploited ring-closing metathesis reaction at the dendrimer peripheries, functionalized with allyl- and homo allyl-groups.[9] Furthermore, the cross-linking of the peripheries of dendrimers and removal of the hydrolytically unstable core led to isolate "cored" dendrimers that could find potential applications as drug-delivery agents and as molecularly imprinted dendrimers.[10] In the studies reported so far, covalent cross-linking of the dendrimers leads to stable, irreversible megameric clusters. Megameric dendrimer clusters capable of reversible cross-linking are unknown and yet to be realized.

We undertook an effort to conduct dendrimer cross-linking, which would allow dendritic cluster formation and dissociation of the cross-links, as a result of cleavage of the dynamic covalent bonds. In order to incorporate cleavable covalent bond cross-linking the dendrimers, the proto-typical thiol−disulfide interchange reaction was chosen. Thiol−disulfide interchange or exchange reaction provides a remarkable ability to form strong, covalent disulfide bond, yet allow a facile cleavage of the disulfide bond under a reducing environment. In this instance, the work of Caruso and co-workers is noteworthy, wherein the authors demonstrated the formation disulfide cross-linked polymeric capsules capable of retaining proteins and nucleotides, and releasing them under reducing environments.[11] Recent references can be seen further from the work of Armes and co-workers[12] on gelators, and Otto and co-workers[13] on dynamic libraries, both based on thiol−disulfide exchange reaction. With the interest to incorporate thiol−disulfide exchange reaction to assemble and disassemble dendrimers, we report herein the synthesis of three generations of thiol functionalized dendrimers, presenting up to 24 thiol groups at their peripheries, followed by a study of disulfide bond mediated cross-linking of the dendrimers and the reversible disulfide cleavage to the native thiol functionalized monomeric dendrimers. A preliminary study of a guest molecule encapsulation and release possibilities using megamer assembly disassembly is also presented.

Results and Discussion

Synthesis. In order to incorporate thiol−disulfide exchange reaction as the basis of reversible covalent bond formation, functionalizing the peripheries of dendrimers with thiol moieties was undertaken. The poly(alky aryl ether) series of dendrimers were used for the study. This series of dendrimers are constituted with 1,3,5-trihydroxybenzene (phloroglucinol) as the linker.[14] Varied types of fuctionalization were performed on this series of dendrimers, so as to identify the *exo*-and *endo*-receptor properties, emanating from these dendrimers.[15] Zero to three generation of dendrimers, with 3, 6, 12, and 24 peripheral functional groups, were subjected for modification with the thiol functional groups. Poly(alkyl aryl ether) dendrimers present

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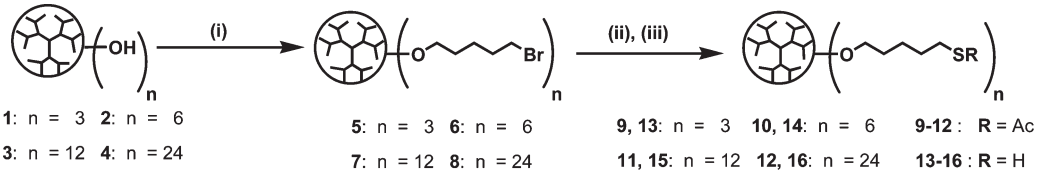
Scheme 1. Reagents and Conditions: (i) 1,5-Dibromopentane, K_2CO_3, 18-Crown-6 (Cat.), *N*,*N*-Dimethylformamide, RT; (ii) Potassium Thioacetate, 18-C-6 (Cat.), *N*,*N*-Dimethylformamide, RT; (iii) $LiAlH_4$, Tetrahydrofuran, RT



1,3-dihydroxybenzene moiety at their peripheries, available readily for further fuctionalizations. Preparation of the 1,3-dihydroxybenzene moiety functionalized dendrimers are reported previously.[14] Reaction of excess 1,5-dibromopentane with 1,3-dihydroxybenzene-terminated dendrimers **1**−**4**, in the presence of K_2CO_3 and 18-crown-6 in *N*,*N*-dimethylformamide (DMF), provided bromide functionalized dendrimers **5**−**8** (Scheme 1). The *O*-alkylation of all available phenolic groups were ascertained through ^{1}H NMR spectrum. The bromide functionalized dendrimers **5**−**8** were transformed to thioacetyl functionalized dendrimers **9**−**12**, using potassium thioacetate (KSAc) in DMF. The pure products were isolated through column chromatography (SiO_2). The thioacetyl functionalized dendrimers **9**−**12** are brownish gums, freely soluble in organic solvents. The constitution and structural homogeneities of the functionalized dendrimers were confirmed through NMR spectroscopies and elemental analyses. From NMR characterizations, conversion of all available bromides to thioacetyl group was confirmed. Thus, resonance for methylene protons adjacent to the bromide group at ∼3.4 ppm was found to be absent for thioacetyl functionalized dendrimers **9**−**12**, for which the above protons resonated at ∼2.9 ppm. Similar observations were seen for the methylene carbon in ^{13}C NMR spectrum of each thioacetyl functionalized dendrimer (33.4 ppm **5**−**8** and 30.5 ppm for **9**−**12**). Characterizations through mass spectrometric analyses (MALDI−TOF−MS and ES−MS) could not be succeeded.

Subsequent deprotection of the acetyl groups in **9**−**12**, using $LiAlH_4$ and tetrahydrofuran (THF), led to isolation of free thiol-containing dendrimers **13**−**16** (Figure 1). The multivalent dendritic thiols were colorless gums and their constitutions were ascertained by NMR spectroscopies and elemental analyses. The purities of **13**−**16** were ascertained by high-performance liquid chromatography (HPLC) method (SiO_2, hexane−EtOAc eluant). As free thiol moieties might undergo oxidation upon storage, the acetyl group deprotections were performed short time before further studies.

Studies of Thiol−Disulfide Interchange Reaction. The disulfide bond formation at the peripheries of dendrimers was undertaken, under oxidative conditions, promoted by I_2. The thiol moieties at the peripheries of dendrimers were anticipated to be free of any steric and conformational constraints. The disulfide bond formation was conducted in THF and the solution concentrations were as follows: **13**, 2.3 mM; **14**, 0.75 mM; **15**, 0.32 mM and **16**, 0.15 mM. Addition of catalytic amount of a solution of I_2 in THF and few drops of water initiated the formation of disulfide bonds. Because of increased cross-linking of dendrimers through disulfide bond formation, the solution becomes progressively turbid. The turbidimetric measurements were followed by UV−vis spectroscopy, assessing the changes in the optical density at 420 nm. The reaction mixture contained typically dendritic thiol (4 mg), a solution of I_2 (2.75 mM, 200−600 μL), water (∼300 μL) in THF (4 mL). Progressive changes in the optical densities were read at 420 nm, against a blank without dendritic thiol. Figure 2a shows the turbidity formation as a function of time. The optical density increased with increasing turbidity, thereby allowing a qualitative assessment of cross-linking and megamer formation. From the plot, it is clear that all the dendrimers **13**−**16** undergo cross-linked megamer formation, resulting in a turbidity of the solution. The time required for maximal turbidity formation was as follows: **13**, 240 min; **14**, 300 min; **15**, 330 min; **16**, 360 min. Further significant changes in the optical densities were not observed beyond maximal precipitation. The presence of minute amounts of water was essential to initiate the cross-linking, and I_2 alone did not promote the disulfide bond formation. The turbid solutions were stable over a month. Also, increased additions of I_2 and water did not change the profile of the turbidity formation. Efforts were also undertaken to study concentration-dependent nature of turbidity formation. G3-SH monomer was taken for this purpose. Nearly no turbidity formation occurred below 0.15 mM solution concentration, and above this and higher concentrations, dense turbidity and attendant changes in the optical densities were observed. It was presumed that cross-linking at lower concentrations led to soluble oligomeric species. With increased turbidity as a function of increasing monomer concentration, the intermolecular nature of cross-linking of monomers was inferred.

Transmission electron microscopy (TEM) was employed, in order to assess the morphologies of cross-linked dendritic megamers. Figure 3 shows micrographs of the cross-linked dendritic megamers, derived from dendritic thiols **13** and **16**. Nearly spherical particles of disulfide cross-linked dendritic megamers were observed in general. The diameters of the megamers ranged between 20 and 160 nm. Histogram analyses showed that the mean-sizes were (with standard deviation in parentheses) as follows: **G0**-megamer, 49 nm (25.5); **G1**-megamer, 120 nm (15.8); **G2**-megamer, 131 nm (24.5) and **G3**-megamer, 150 nm (25.8).[16] Zero (Figure 3a), first, and to some extent second generation dendritic thiols provided disulfide cross-linked megamers, wherein the megamers formed nearly continuously. The higher propensity of such continuity in the lower generation dendritic cross-linking could be due to lesser crowding around thiol moieties, and the attendant facile disulfide bond formation. On the other hand, cross-linked megameric particles obtained from third generation dendritic thiol **16** were isolated particles, without cross-linking among the particles (Figure 3b). The inability to form cross-links between megameric particles in the latter case could be due to steric and kinetic volume effects. Many thiol moieties was presumed to participate in disulfide bond formation, although it was not clear how many free thiol moieties would have remained within a dendrimer after complete precipitation of dendritic megamers. Toward this, the supernatant, isolated from **G1**-megamer formation, was assessed and was found to contain the dendrimer component (< 5%), from the ^{1}H NMR spectrum. The dendrimer component in the supernatant was presumed to be soluble cross-linked oligomer.

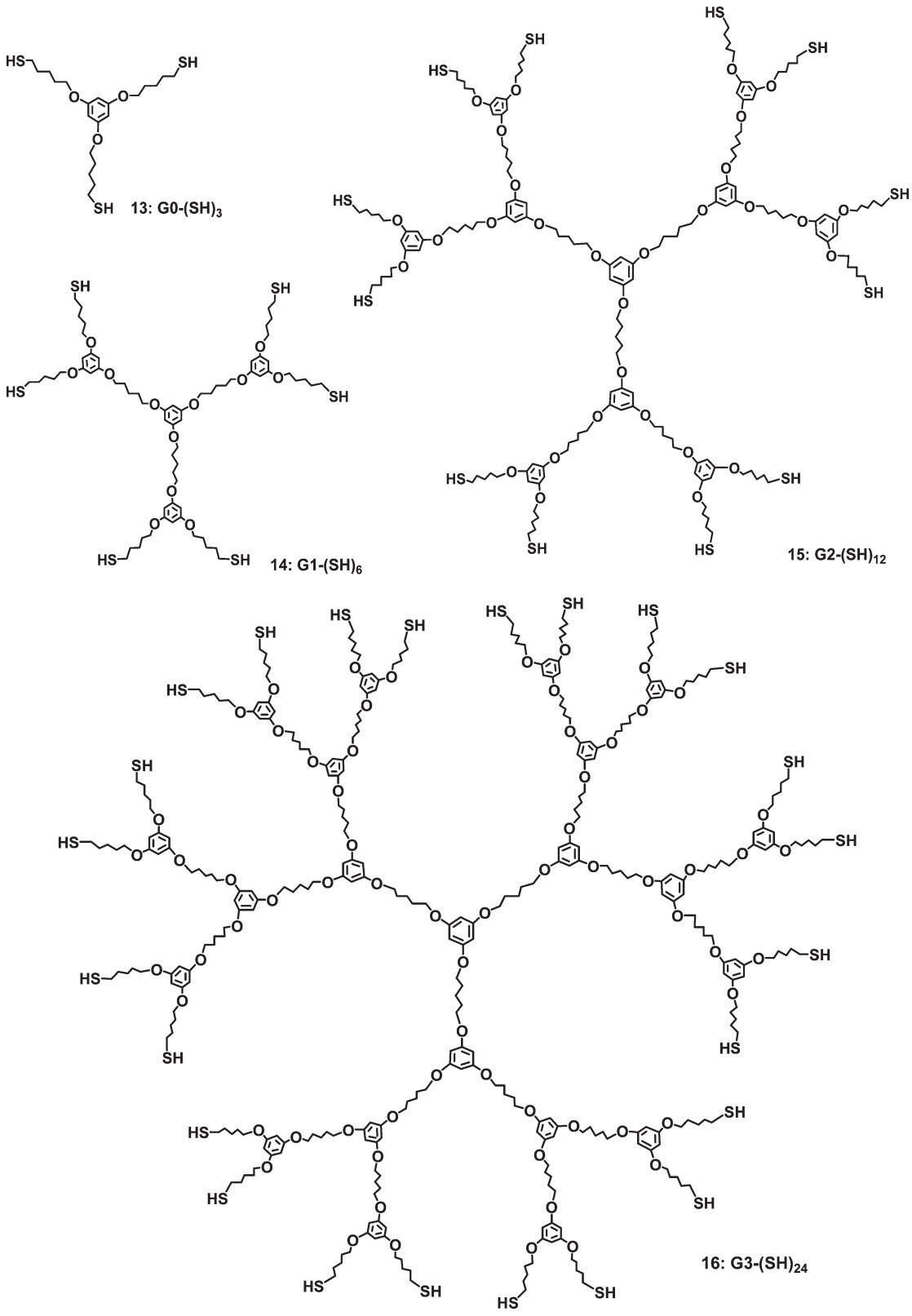


Figure 1. Molecular structures of the multivalent dendritic thiols **13-16**.

The thiol−disulfide interchange reaction was employed further, in order to assess the disassembly of the cross-linked dendritic megamers to individual dendritic molecules. The assembly disassembly process is well-known, involving the thiol−disulfide interchange reaction, for example, the elegant demonstration of disulfide-stabilized polymeric capsules, reported by Caruso and co-workers.[11] The cross-linked dendritic megamers were subjected to thiol−disulfide exchange, mediated by dithiothreitol (DTT). The exchange reaction was initiated by the addition of a solution DTT in THF (3.24 mM, 400−600 μL) to the turbid megamer solution. The disassembly was followed by changes in the optical density at 420 nm. A gradual change in the optical density was observed with all four dendritic megamers. Figure 2b shows

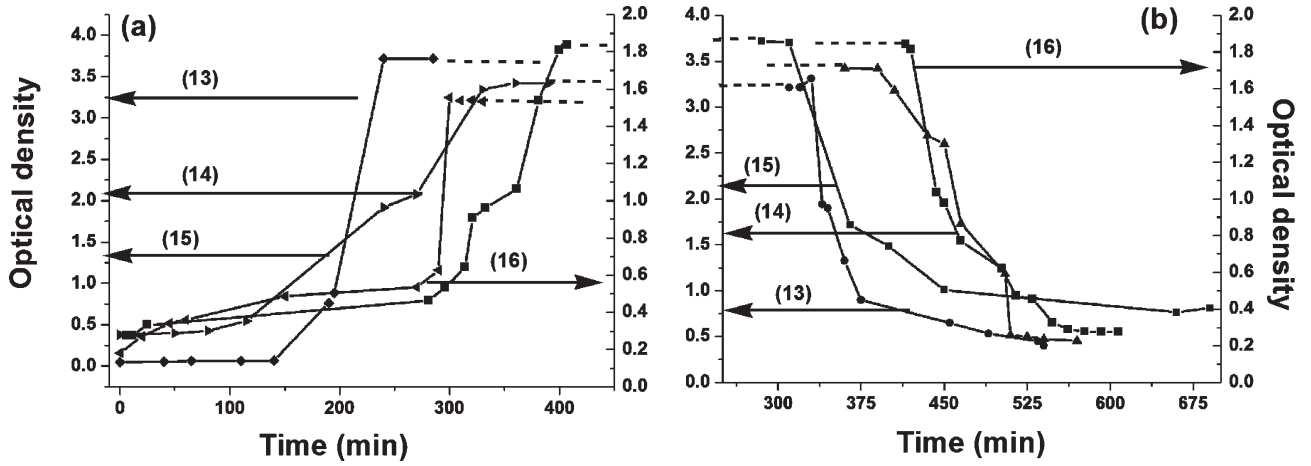


Figure 2. Changes in the optical density as a result of (a) disulfide bond mediated cross-linking of dendritic thiols and (b) disassembly of the megamers to the monomers.

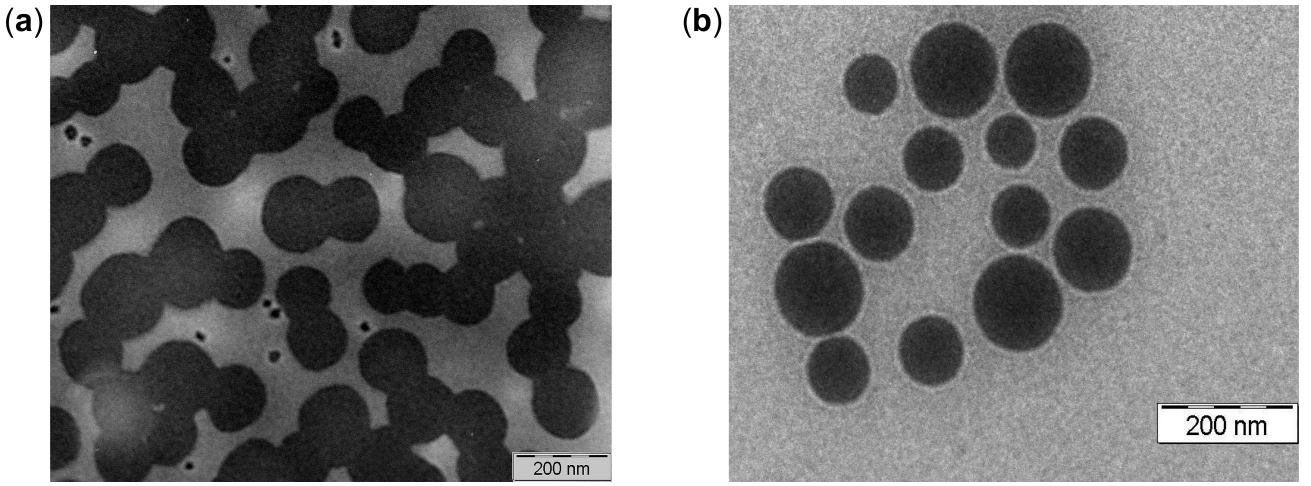


Figure 3. Transmission electron micrographs of dendritic megamers derived from (a) $G0(SH)_3$ (**13**) and (b) $G3(SH)_{24}$ (**16**).

the decreasing trend in the optical densities, as a function of time. The optical densities reached nearly that of clear homogeneous solution. The disassembly process was dependent on the concentration of DTT. Thus, higher the concentration of DTT solution, the faster the disassembly process was.

Upon complete disassembly to monomeric dendritic thiol molecules, reoxidation could be accomplished. This repeat oxidation of dendritic thiols, however, required purification of the components, wherein the solutions were passed through a short silica gel column, which facilitated the removal of oxidized and excess DTT, as well as, iodine used to initiate the oxidation process. The purified dendritic thiols were subjected to reoxidation, in the presence of catalytic amount of I_2/H_2O, and the formation of dendritic megamers was monitored through optical density changes. The megamer formation traced a similar optical density changes as that of the first cycle. The completely cross-linked megamers turbid solution could, in-turn, be disassembled in the presence of DTT. This repeat megamer−monomer assembly disassembly experiment demonstrated the facile and reversible formation of thiol−disulfide bonds that cross-links the dendritic molecules, and their dissociation to monomeric thiol upon reduction.

A preliminary study to explore the possibility of megamer assembly disassembly for encapsulation of a guest molecule was undertaken. In this instance, the ability of megamers to encapsulate C_{60} was explored, as C_{60} is insoluble in THF.[17] Dendritic megamer formation was conducted in THF, in the presence of C_{60}, for 12 h. After this period, insoluble megamer was isolated, washed with toluene (PhMe) exhaustively, followed by resuspension of the megamer in THF. Reducing agent DTT was added and the disassembly was allowed for 8−10 h, after which the solvent was removed and the residue extracted using PhMe. C_{60} isolated by the extraction process was considered to indicate the encapsulation. PhMe was chosen as the extraction solvent, as dendritic thiols and the reagents were insoluble, thereby allowing a facile isolation of C_{60}. The amounts of C_{60} encapsulated by megamers, formed from monomeric thiols (100 μM), were measured by UV−vis spectroscopy in PhMe (λ_{max} for C_{60} 335 nm; ε = 56 850 M^{-1} cm^{-1}). The amounts encapsulated were as follows: **G0**-megamer, 0.005 μM; **G1**-megamer, 0.007 μM; **G2**-megamer, 0.011 μM; **G3**-megamer, 0.024 μM. The effective strength of C_{60} solubilization increased with increasing generation of dendritic megamer. Further studies are required to characterize the megamer−C_{60} complexes in detail. The preliminary study at present allows identifying the transport properties of dendritic megamer assembly and disassembly.

Conclusions

The work described herein explores thiol−disulfide interchange reaction as the source to form dendritic megamers.

For demonstrating the formation of megamers and their disassembly to monomeric thiols, poly(alkyl aryl ether) dendrimers of generations zero to three, presenting 3, 6, 12, and 24 thiols moieties, respectively, at their peripheries, were utilized. The formation of thiol-functionalized dendrimers was accomplished, through a nucleophilic substitution reaction, followed by a deprotection. Upon synthesis of thiol-functionalized dendrimers, an effort was undertaken to form dendritic megamers through disulfide bond formation. The cross-linking of individual dendrimer molecules could be accomplished under oxidative conditions, and the megameric particles of sizes between 20 and 160 nm were identified. The megameric particles, in turn, could be dissociated to monomeric thiol-functionalized dendrimers, in the presence of reducing agent DTT. The formation of the cross-linked dendritic megamers and their dissociation could be repeated. Further, the abilities of megamers to encapsulate C_{60} as the guest molecule were assessed. In the cross-linked dendritic megamers reported so far, the cross-linking of dendrimers involved highly stable covalent bonds, not amenable for facile cleavage.[3−10] On the other hand, the work reported herein could be related to the emerging area of "mendable" or "self-healing" or "reversible cross-linked" polymers, wherein an external stimulus mediates the facile and reversible cross-linking and dissociation of the constituent fragments of a polymer, whether covalent or noncovalent.[18,19] The reversible cross-linking and the disassembly process was achieved with the aid of dynamic covalent bond formation of disulfide bonds, under an oxidative condition, and their dissociation to the monomeric thiol, under a reducing condition. The thiol−disulfide interchange process mediated dendritic megamer formation, and their disassembly to monomers should be useful for the encapsulation and the release of various organic functional molecules as well as for the self-healing material properties.[18,19]

Experimental Section

Materials. All chemicals were reagent grade and were used without further purification. Phloroglucinol (98%, Spectrochem. Corp., India), 1,5-dibromopentane (98%, Spectrochem. Corp., India), K_2CO_3 (98%, Nice Chemicals, India), 18-crown-6 (98%, Nice Chemicals, India), KSAc (98%, Sigma-Aldrich, Inc., India), $LiAlH_4$ (98%, Sigma-Aldrich, Inc., India), I_2 (99.5%, Spectrochem. Corp., India), DTT (99%, Sigma-Aldrich, Inc.,) and C_{60} (99.5%, Sigma-Aldrich, Inc., India) were used as received. DMF (Merck, GR grade) was treated with CaH_2 for 6 h and then distilled over molecular sieves (4 Å), under reduced pressure. THF (99%, Merck) was treated with sodium wire and then distilled. Analytical TLC was performed on commercial Merck plates coated with silica gel GF254 (0.25 mm). Silica gel (100−200 mesh, SRL Ltd., India) was used for column chromatography. HPLC was performed using a Prodigy (250 mm × 10 mm) silica gel (5 μ) column (Phenomenex, USA).

Instrumentation. Microanalyses were performed on an automated Carlo-Erba C, H, and N analyzer. ^{1}H and ^{13}C NMR spectral analyses were performed either on a JEOL-JNM-LA spectrometer operating at 300 and 75 MHz, respectively, or on a Bruker-ARX400 spectrometer operating at 400 and 100 MHz, respectively. The residual solvent signal was used as the internal standard. The following abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; dd, doublet of a doublet; m, multiplet; band, several overlapping signals and br, broad. Precipitation experiments were monitored at 420 nm, using a Perkin-Elmer λ35 UV−vis spectrophotometer. Dendritic megamers were visualized using a JEOL 100 CX II TEM instrument, operating at 80 kV, with ammonium molybdate as the staining agent.

General Procedure. *Synthesis of Bromopentyloxy-Functionalized Dendrimers 5−8.* A solution of phloroglucinol **1** or hydroxyl group terminated dendrimer **2**, **3**, and **4**[14] (1.0 molar equiv) in DMF was added to a solution of 1, 5-dibromopentane (2.3 molar equiv per OH group) and K_2CO_3 (1.3 molar equiv. per OH group) and 18-crown-6 (cat.) in DMF. The reaction mixture was stirred at room temperature, filtered, washed with CH_2Cl_2 and the solvents removed *in vacuo*. The resulting residue was dissolved in CH_2Cl_2, washed with water, brine, dried (Na_2SO_4), concentrated, and purified (SiO_2, hexane−EtOAc gradient eluant system) to afford bromopentyloxy group terminated dendrimers **5**−**8**, as viscous oils.

5: A solution of **1** (1.0 g, 7.9 mmol) in DMF (6 mL) was added to a stirred solution of 1, 5-dibromopentane (8.2 g, 35.5 mmol), K_2CO_3 (4.3 g, 32 mmol) and 18-crown-6 (cat.) in DMF (20 mL), over a period of 30 min. The reaction mixture was stirred at room temperature for 48 h and worked-up further as given in the general procedure. Yield: 2.95 g (65%). TLC: R_f 0.35 (hexane/EtOAc 95/5). IR (neat, cm^{-1}): 2940.13, 2869.20, 1597.64, 1461.79, 1386.77, 1278.63, 1062.57. ^{1}H NMR ($CDCl_3$) δ: 1.54−1.64 (m, 6 H), 1.71−1.82 (m, 6 H), 1.85−1.96 (m, 6 H), 3.42 (t, J = 6.6 Hz, 6 H), 3.91 (t, J = 6.0 Hz, 6 H), 6.04 (s, 3 H). ^{13}C NMR ($CDCl_3$) δ: 24.7, 28.3, 32.3, 33.4, 67.5, 93.9, 160.7. Anal. Calcd for $C_{21}H_{33}Br_3O_3$: C, 44.00; H, 5.80. Found: C, 43.55; H, 5.84.

6: A solution of **2** (0.25 g, 0.35 mmol) in DMF (10 mL) was added to a stirred solution of 1, 5-dibromopentane (4.05 g, 17.63 mmol), K_2CO_3 (0.45 g, 3.1 mmol) and 18-crown-6 (cat.) in DMF (25 mL), over a period of 20 min. The reaction mixture was stirred at room temperature for 48 h and worked-up further as given in the general procedure. Yield: 0.31 g (55%). TLC: R_f 0.70 (PhMe/EtOAc 95/5). IR (neat, cm^{-1}): 2941.76, 2869.78, 1599.74, 1462.89, 1387.34, 1163.58, 1063.36. ^{1}H NMR ($CDCl_3$) δ: 1.58−1.64 (m, 18 H), 1.75−1.84 (m, 24 H), 1.90−1.96 (m, 12 H), 3.41 (t, J = 6.6 Hz, 12 H), 3.90−3.94 (m, 24 H), 6.05 (s, 12 H). ^{13}C NMR ($CDCl_3$) δ: 22.7, 24.8, 28.3, 28.9, 32.4, 33.4, 67.5−67.8, 93.9, 160.8. Anal. Calcd for $C_{69}H_{102}Br_6O_{12}$: C, 51.70; H, 6.41. Found: C, 51.32; H, 6.22.

7: A solution of **3** (0.25 g, 0.13 mmol) in DMF (10 mL) was added to a stirred solution of 1, 5-dibromopentane (1.64 g, 7.15 mmol), K_2CO_3 (0.35 g, 2.1 mmol) and 18-crown-6 (cat.) in DMF (15 mL), over a period of 20 min. The reaction mixture was stirred at room temperature for 72 h and worked-up further as given in the general procedure. Yield: 0.22 g (48%). TLC: R_f 0.72 (PhMe/EtOAc 99/1). IR (neat, cm^{-1}): 2941.09, 2869.94, 1703.88, 1599.53, 1462.89, 1386.25, 1255.48, 1162.67, 1062.91. ^{1}H NMR ($CDCl_3$) δ: 1.52 −1.68 (m, 42 H), 1.70−1.80 (m, 60 H), 1.88−1.93 (m, 24 H), 3.42 (t, J = 6.6 Hz, 24 H), 3.65−3.68 (m, 60 H), 6.05 (s, 30 H). ^{13}C NMR ($CDCl_3$) δ: 22.7, 24.8, 28.3, 28.9, 29.6, 32.4, 33.5, 67.5, 67.7, 93.8, 160.8. Anal. Calcd for $C_{165}H_{240}Br_{12}O_{30}$: C, 54.11; H, 6.60. Found: C, 53.42; H, 6.73.

8: A solution of **4** (0.24 g, 0.057 mmol) in DMF (10 mL) was added to a stirred solution of 1, 5-dibromopentane (0.64 g, 2.8 mmol), K_2CO_3 (0.20 g, 1.42 mmol) and 18-crown-6 (cat.) in DMF (20 mL), over a period of 25 min. The reaction mixture was stirred at room temperature for 72 h and worked-up further as given in the general procedure. Yield: 0.17 g (43%). TLC: R_f 0.84 (PhMe/EtOAc 96/4). IR (neat, cm^{-1}): 2939.85, 2869.68, 1597.94, 1462.47, 1386.04, 1258.11, 1161.98, 1062.25. ^{1}H NMR ($CDCl_3$) δ: 1.59−1.70 (m, 90 H), 1.79−1.93 (m, 180 H), 3.41 (t, J = 6.6 Hz, 48 H), 3.90 (br s, 132 H), 6.04 (br s, 66 H). ^{13}C NMR ($CDCl_3$) δ: 22.7, 24.7, 28.3, 28.9, 32.4, 33.4, 67.5, 67.7, 93.8, 160.8. Anal. Calcd for $C_{357}H_{516}Br_{24}O_{66}$: C, 55.70; H, 6.68. Found: C, 56.48; H, 6.65.

General Procedure. *Synthesis of Thioacetate-Functionalized Dendrimers 9−12.* A mixture of **5**−**8** (1 molar equiv), KSAc (2 molar equiv per bromide group) and 18-crown-6 (cat.) in DMF was stirred at room temperature, solvent removed *in vacuo*, the resulting residue dissolved in EtOAc,washed with water and brine, dried (Na_2SO_4), concentrated, and purified (SiO_2, hexane−EtOAc gradient eluant system) to afford thioacetate-terminated dendrimers **9**−**12**, as brownish gums.

9: A mixture of **5** (0.52 g, 0.92 mmol), KSAc (0.63 g, 5.52 mmol), and 18-crown-6 (cat.) in DMF (40 mL) was stirred for 24 h at room temperature and followed further as given in the general procedure. Yield: 0.47 g (91%). TLC: R_f 0.38 (hexane/EtOAc 95/5). IR (neat, cm^{-1}): 2933.77, 2861.08, 1691.10, 1598.48, 1462.26, 1353.87, 1160.66, 1066.02. ^{1}H NMR ($CDCl_3$) δ: 1.48−1.54 (m, 6 H), 1.57−1.67 (m, 6 H), 1.73−1.80 (m, 6 H), 2.33 (s, 9 H), 2.89 (t, J = 7.2 Hz, 6 H), 3.89 (t, J = 6.4 Hz, 6 H), 6.04 (s, 3 H). ^{13}C NMR ($CDCl_3$) δ: 25.2, 28.6, 28.9, 29.2, 30.5, 67.5, 93.8, 160.8, 195.7. Anal. Calcd for $C_{27}H_{42}O_6S_3$: C, 58.03; H, 7.58; S, 17.21. Found: C, 57.86; H, 7.12; S, 17.11.

10: A mixture of **6** (0.19 g, 1.2 mmol), KSAc (0.16 g, 1.4 mmol) and 18-crown-6 (cat.) in DMF (30 mL) was stirred for 24 h at room temperature and followed further as given in the general procedure. Yield: 0.18 g (89%). TLC: R_f 0.70 (PhMe/EtOAc 97/3). IR (neat, cm^{-1}): 2932.42, 2868.82, 1690.52, 1599.68, 1462.82, 1387.18, 1353.55, 1162.22, 1064.50. ^{1}H NMR ($CDCl_3$) δ: 1.48−1.55 (m, 12 H), 1.61−1.67 (m, 18 H), 1.73−1.86 (m, 24 H), 2.32 (s, 18 H), 2.89 (t, J = 7.2 Hz, 12 H), 3.88−3.94 (m, 24 H), 6.05 (s, 12 H). ^{13}C NMR ($CDCl_3$) δ: 22.6, 25.2, 28.6, 28.8, 28.9, 29.2, 30.6, 67.5, 67.7, 93.7, 160.7, 195.8. Anal. Calcd for $C_{81}H_{120}O_{18}S_6$: C, 61.80; H, 7.68; S, 12.22, Found: C, 61.61; H, 7.22; S, 12.21.

11: A mixture of **7** (0.14 g, 0.38 mmol), KSAc (0.18 g, 0.97 mmol) and 18-crown-6 (cat.) in DMF (30 mL) was stirred for 36 h at room temperature and followed further as given in the general procedure. Yield: 0.08 g (60%). TLC: R_f 0.51 (PhMe/EtOAc 98/2). IR (neat, cm^{-1}): 2938.63, 2869.38, 1690.66, 1598.43, 1462.58, 1386.62, 1354.01, 1162.29, 1063.31. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42−1.69 (m, 66 H), 1.70−1.80 (m, 60 H), 2.32 (s, 36 H), 2.88 (t, J = 7.2 Hz, 24 H), 3.87−3.92 (m, 60 H), 6.05 (s, 30 H). ^{13}C NMR ($CDCl_3$) δ: 22.7, 25.3, 28.6, 28.7, 28.9, 29.3, 30.6, 67.6, 67.7, 93.9, 160.8, 196.2. Anal. Calcd for $C_{189}H_{276}O_{42}S_{12}$: C, 62.97; H, 7.72; S, 10.67. Found: C, 63.29; H, 7.23; S, 10.31.

12: A mixture of **8** (0.14 g, 0.01 mmol), KSAc (0.10 g, 0.91 mmol) and 18-crown-6 (cat.) in DMF (35 mL) was stirred for q36 h at room temperature and followed further as given in the general procedure. Yield: 0.08 g (52%). TLC: R_f 0.67 (PhMe/EtOAc 95/5). IR (neat, cm^{-1}): 2937.43, 2869.64, 1691.76, 1599.62, 1463.08, 1386.61, 1353.95, 1162.89, 1063.71. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.50−1.70 (band, 138 H), 1.72−1.92 (band, 132 H), 2.33 (br s, 72 H), 2.83−2.95 (band, 48 H), 3.91 (br s, 132 H), 6.04 (br s, 66 H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 25.3, 28.7, 28.9, 29.1, 29.6, 30.6, 67.6, 67.7, 93.7, 161.1, 195.8. Anal. Calcd for $C_{405}H_{588}O_{90}S_{24}$: C, 63.45; H, 7.73; S, 10.04. Found: C, 62.97; H, 7.31; S, 9.72.

General Procedure. *Synthesis of Thiol-Functionalized Dendrimers **13**−**16***. A solution of dendritic thioacetates **9**−**12** (1 molar equiv) in THF was added dropwise to a slurry of $LiAlH_4$ (1.25 molar equiv. per thioacetate group) in THF, under N_2 atmosphere. After stirring for 21 h, the reaction mixture was quenched by careful addition of MeOH, added with aq. HCl (0.5 M) (25 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with water, dried (Na_2SO_4), filtered and concentrated *in vacuo* to afford free thiol containing dendrimers **13**−**16**, as colorless gums.

13: A solution of **9** (74 mg, 132 mmol) in THF (15 mL) was added dropwise to a slurry of $LiAlH_4$ (20 mg, 0.52 mmol) in THF (10 mL), under N_2 atmosphere. The reaction was followed further as given in the general procedure. Yield: 46 mg (82%). TLC: R_f 0.48 (PhMe/EtOAc 98/2). IR (neat, cm^{-1}): 2934.13, 2859.27, 2564.85, 1599.04, 1462.59. ^{1}H NMR ($CDCl_3$) δ: 1.35 (t, J = 7.8 Hz, 3 H), 1.53−1.59 (m, 6 H), 1.64−1.78 (m, 12 H), 2.53−2.58 (q, J = 6.0 Hz, 6 H), 3.91 (t, J = 6.0 Hz, 6 H), 6.04 (s, 3 H). ^{13}C NMR ($CDCl_3$) δ: 24.4, 24.9, 29.2, 33.1, 67.7, 93.9, 160.8. Anal. Calcd for $C_{21}H_{36}O_3S_3$: C, 58.29; H, 8.39; S, 22.23. Found: C, 58.62; H, 8.44; S, 22.10.

14: A solution of **10** (42 mg, 0.21 mmol.) in THF (10 mL) was added dropwise to a slurry of $LiAlH_4$ (8 mg, 0.26 mmol) in THF (5 mL), under N_2 atmosphere. The reaction was followed further as given in the general procedure. Yield: 27 mg (78%). TLC: R_f 0.46 (PhMe/EtOAc 98/3). IR (neat, cm^{-1}): 2933.29, 2867.95, 2566.61, 1598.00, 1462.38; ^{1}H NMR ($CDCl_3$) δ: 1.35 (t, J = 7.5 Hz, 6 H), 1.50−1.60 (m, 18 H), 1.63−1.81 (m, 36 H), 2.52−2.59 (q, J = 6.0 Hz, 12 H), 3.91 (t, J = 6.0 Hz, 24 H), 6.05 (s, 12 H). ^{13}C NMR ($CDCl_3$) δ: 22.3, 24.4, 24.9, 29.1, 29.6, 32.4, 67.7, 67.9, 93.9, 160.9. Anal. Calcd for $C_{69}H_{108}O_{12}S_6$: C, 62.69; H, 8.23; S, 14.55. Found: C, 62.74; H, 8.54; S, 14.38.

15: A solution of **11** (40 mg, 0.01 mmol) in THF (15 mL) was added dropwise to a slurry of $LiAlH_4$ (8 mg, 0.20 mmol) in THF (5 mL), under N_2 atmosphere. The reaction was followed further as given in the general procedure. Yield: 24 mg (70%). TLC: R_f 0.52 (PhMe/EtOAc 98/4). IR (neat, cm^{-1}): 2933.34, 2867.89, 2566.59, 1598.22, 1462.18. ^{1}H NMR ($CDCl_3$) δ: 1.35 (t, J = 7.8 Hz, 12 H), 1.56−1.69 (m, 60 H), 1.76−1.89 (m, 66 H), 2.54−2.68 (br, 24 H), 3.91−3.97 (br 60), 6.05 (br s, 30 H). ^{13}C NMR ($CDCl_3$) δ: 22.7, 24.4, 24.8, 29.0, 29.6, 32.2, 32.4, 67.6, 67.8, 93.9, 160.9. Anal. Calcd for $C_{165}H_{252}O_{30}S_{12}$: C, 63.92; H, 8.19; S, 12.41. Found: C, 63.41 ; H 8.35; S, 12.84.

16: A solution of **12** (66 mg, 0.008 mmol) in THF (20 mL) was added dropwise to a slurry of $LiAlH_4$ (9 mg, 0.23 mmol) in THF (10 mL), under N_2 atmosphere. The reaction was followed further as given in the general procedure. Yield: 32 mg (63%). TLC: R_f 0.42 (PhMe/EtOAc 98/5). IR (neat, cm^{-1}): 2924.52, 2856.06, 2564.86, 1595.81, 1462.74. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34 (t, J = 7.7 Hz, 24 H), 1.55−1.65 (band, 138 H), 1.73−1.90 (band, 132 H), 2.51−2.57 (band, 48 H), 3.83−3.92 (band, 132 H), 6.04 (band, 66 H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 22.7, 24.4, 24.9, 28.9, 29.6, 32.7, 67.6, 67.7, 93.3, 161.0. Anal. Calcd for $C_{357}H_{540}O_{60}S_{24}$ C, 64.40; H, 8.18; S, 11.56. Found: C, 63.92 ; H 8.35; S, 11.34.

Molecular Modeling. Molecular modeling calculations on monomer thiols **13**−**16** were performed on the Macromodel 8.5 program.[20] The 3D structures from the Chem3D Ultra 7.0 were used as starting structures. Structures were optimized using the AMBER* force field. Energies of the molecule were minimized when the algorithm was Polak-Ribiere and the termination condition was rms gradient of 0.05 kcal mol. The minimum energy conformer was obtained after converging rms gradient. In a typical procedure, the structural preference of the dendrimers was determined by simulation using the above protocol with a minimum of 20,000 iterations at 300 K and the dielectric constant was set at 1.4.

C_{60} Encapsulation. A suspension of dendritic thiol (100 μM) and C_{60} (0.5 mg) in THF (5 mL) was added with I_2 (~3 mg) and water (~0.5 mL). After the reaction was stirred for 12 h, solvents were removed *in vacuo*, and the resulting residue was triturated with PhMe several times, until PhMe solution was devoid of C_{60}, as judged from UV−vis spectroscopy (λ_{max} 335 nm). The residue was dried *in vacuo*, resuspended in THF (5 mL), a solution of DTT in THF (3.24 mM) (0.6 mL) was added and left for 8−10 h at room temperature. After evaporation off of solvents *in vacuo*, the residue was triturated with PhMe (~(6−8) × 3 mL), combined PhMe solutions filtered, and the filtrate evaporated, and a standard solution of the resulting residue was prepared in PhMe. The amount of C_{60} present in the solution was determined by UV−vis spectroscopy.

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(16) An effort was undertaken to estimate the number of monomers that would constitute the megamer, assuming megamers as speherical particles. The estimation represented the ratio of megamer volume to that of monomer volume. The mean diameter for each megamer was obtained from TEM measurements and was used in the calculation of volume ($^4/_3\pi r^3$). The volume of each megamer was (mean diameter in parentheses): **G0**-megamer, 61 600 nm^3 (49 nm); **G1**-megamer, 904 778 nm^3 (120 nm); **G2**-megamer, 1 177 097 nm^3 (131 nm) and **G3**-megamer, 1 763 438 nm^3 (150 nm). The volume of each monomer was (average diameter, deduced from molecular modeling method, in parentheses): **13**, 3.1 nm^3 (1.8 nm); **14**, 26.0 nm^3 (3.7 nm); **15**, 57.9 nm^3 (4.8 nm); **16**, 107.5 nm^3 (5.9 nm). Accordingly, the number of monomers constituting each megamer would be as follows: **G0**-megamer, 19 870; **G1**-megamer, 34 800; **G2**-megamer, 20 330; **G3**-megamer, 16 404. A deviation of the number of monomers in **G0**-megamer is seen, which is attributed to a high standard deviation in the TEM particle sizes.

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