Abstract

KIM, NAMJIN. Synthesis and Characterization of Amphiphilic Fe_4S_4-Core Dendrimers as Protein Models. (Under the direction of Professor Christopher B. Gorman.)

The ultimate goal of this research is to clarify the structural effects on the rate and driving force for electron transfer in amphiphilic iron-sulfur core dendrimers, which can eventually be used as protein models. These dendrimers were synthesized by ligand exchange reactions, including the synthesis of dendrons via a modular synthesis approach. Their structure-property relationships were then investigated using electrochemical methods. Three different routes were used to synthesize thiol dendrons with both cationic and anionic peripheral units. The first utilized the disulfide linkage as a focal and protecting group. However, due to difficulties associated with generating free thiol from the disulfide, only partially-substituted dendrimers were prepared in ligand exchange reaction. Similar problems were encountered with the next two methods, which used thiocarbamate and trityl protecting groups, respectively. First generation dendrimers were successfully prepared and characterized using the thiocarbamate group; however, low thiol concentration resulted in only partially substituted dendrimers for the second generation. All synthetic attempts using the trityl focal group resulted in only partial substitution. Because the concentration of free thiol was the factor limiting the

synthesis of these amphiphilic iron-sulfur core dendrimers, a protocol was established to measure the thiol concentration using Ellman's reagent test. However, this proved to be inaccurate due to oxygen dissolved in solution and then re-oxidized thiols during the course of the test. The electrochemical properties of these cationic and anionic dendrimers were measured and compared to G1-flexible dendrimer previously studied by the Gorman Group. G1-cationic dendrimer exhibited more effective attenuation of the electron transfer rate than G1-flexible dendrimer. This was attributed to the difference of molecular weight in both dendrimers. The redox potential in the cationic dendrimer was shifted more positively by about 100 mV due to polar microenvironment around the iron-sulfur core. Cyclic voltammogram of G1-anionic dendrimer exhibited an unexpected peak so other electrochemical properties could not be measured. Only an approximate redox potential was obtained and exhibited a positive shift by about 60 mV compared to G1-flexible dendrimer.

Synthesis and Characterization of Amphiphilic Fe_4S_4-Core Dendrimers as Protein Models

by

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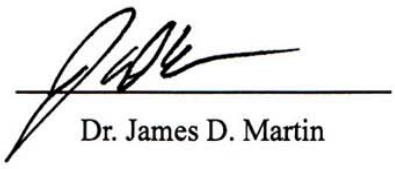

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Chair of Advisory Committee

To loving parents, wife, Gilsu, and daughter, Eunbi

Biography

Namjin Kim was born in Taejon, Korea on April 5, 1968 to Dongjun Kim and Aeja Cha. He received his primary and secondary education from the local schools. After graduating from the high school in 1987, Namjin Kim attended Chungnam National University and received his Bachelor of Science in Polymer Science & Engineering in 1994. He continued researches related to polymer synthesis at the graduate school of his alma mater and received his Master of Science in Polymer Science in 1996. He had an industrial works in Advanced Materials Research Division in Hanwha Chemical R & D Center in Taejon, Korea for about 6 years. He moved to Raleigh, North Carolina in July 2001 to attend graduate school in chemistry at North Carolina State University. Namjin Kim pursued Doctor of Philosophy Degree under the direction of Professor Christopher B. Gorman and received his Doctor of Philosophy Degree in August 2006.

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Chapter 1

Overview of Dendritic Encapsulation

1.1. Origin and Structure of Dendrimers

The chemistry of dendrimers, also known as cascade, arborols, cauliflower, or starburst polymers, was started with the study of highly branched macromolecules.[1-6] The field has attracted increased attention over the last two decades due to the fascinating structure and unique properties[7,8] of these new functional polymers. Since the first synthetic reports in 1985 by the Tomalia's group[1] and by Newkome and coworkers[5], a wide range of dendrimers have been generated resulting in the production of numerous research activities in various fields including synthesis, characterization and applications.[9-22]

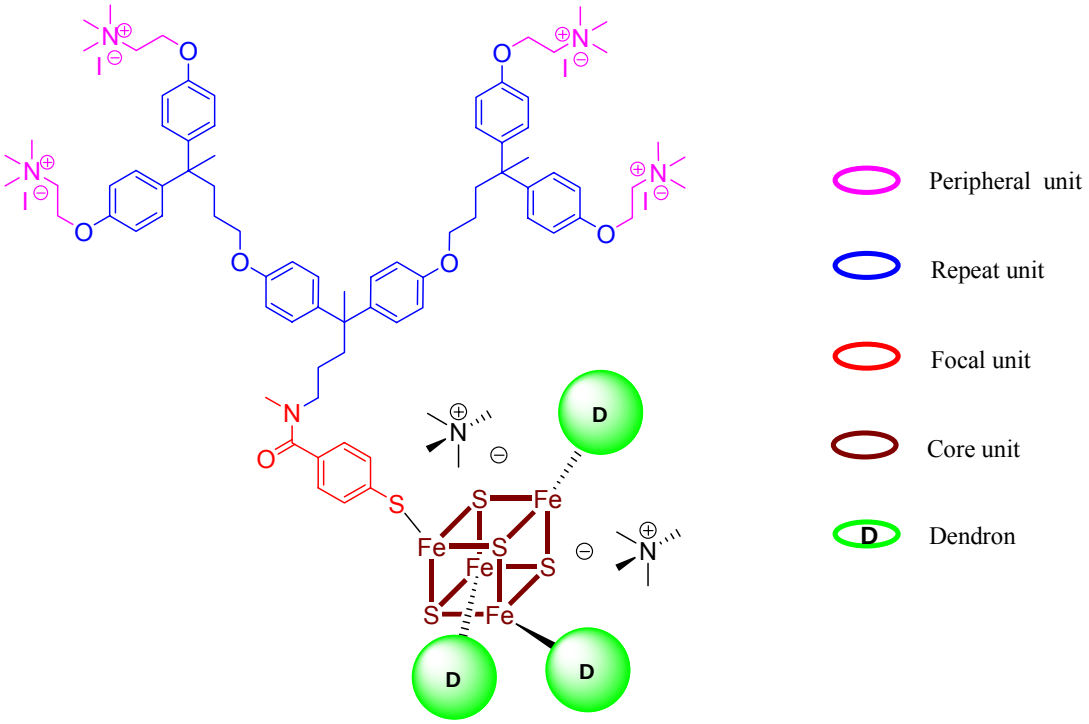


Figure1.1. The primary structure of a dendrimer (2nd generation).

Dendrimers are globular, highly branched, and three dimensional macromolecules. Their structure can be divided into an inner core unit which is surrounded by hyperbranched polymeric units called dendrons. The core can be composed of small molecules, single metals, metal clusters or polymers. Dendrons are consisted of focal units, layers of branched repeat unit, and peripheral units (Figure 1.1). By adequate selection of the core and focal units, a strength or type between the core and the dendrons can be determined. The repeat units are flexible branches that generate the topographic layers within dendrimers. The peripheral units are the outer layer of the dendrimers and have an effect on properties such as solubility or diffusion coefficient.[13,14,21,23-28]

1.2. Construction of Dendrimers: Divergent vs. Convergent

For the synthesis of dendrimers, two general approaches have been used: “divergent” and “convergent” (Figure 1.2). The divergent approach was developed from the research activities of Tomalia[1-3] and Newkome[4-6] in the mid of 1980s. This approach is initiated at the core and proceeds radially outward by repetitive coupling and activation steps. However, as the coupling and activation steps are repeated, the number of reactions at the periphery increases exponentially. Excessive reagents are required as the synthesis progress. In addition, incomplete reactions and side reactions can occur, causing structural flaws and polydispersity in the higher generations. To eliminate these problems, the convergent approach was developed

by Fréchet and coworkers in 1989-1990.[15,22] This approach starts growth at the periphery of the dendrimer and proceeds inward to the central core through repetition of coupling and activation steps. After synthesis and purification of the dendron is complete, the focal unit of the dendron is activated and attached to the central core, affording a globular mono-disperse dendrimer. However, in higher generations (above the 6th generation), dendrimers are difficult to prepare due to steric hindrance, resulting in low yields.

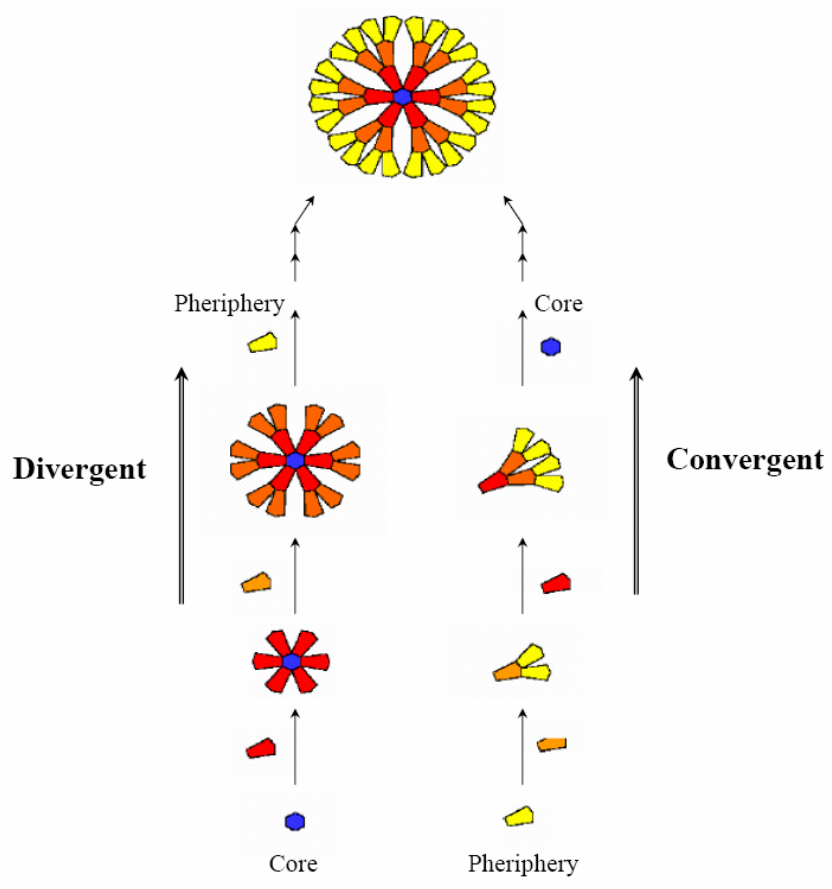


Figure1.2. General approaches for dendrimer synthesis. Graphic from Stoddart's group.[29]

The basic difference between the two approaches is the dendrimer assembly. Each method requires a different number of reactions and purification steps. In addition, the divergent approach yields poly-disperse dendrimers while products of the convergent method are generally mono-disperse.

1.3. Encapsulation of Dendritic Core

Recent interest in dendrimer research has been geared toward structures that exhibit specific functions as functional dendrimers.[8,29-44] Specifically, dendrimers with photo- and/or redox-active units at the core have potential applications in research areas such as electroluminescence and molecular biology.[13,17,19,21,24,45-54]

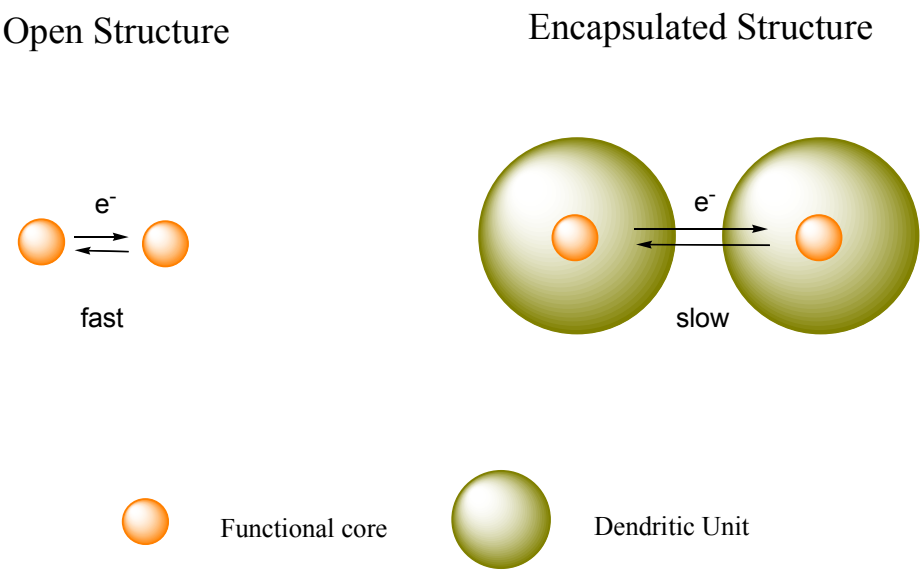


Figure1.3. General illustration of dendritic encapsulation.

Dendrons surrounding a functional core can change the microenvironment around it and thus modify its photochemical and/or electrochemical properties.[19,46-49,55-60] Encapsulation is the ability of a dendrimer to shield the core from a surrounding environment and is a function of dendrimer generation (Figure 1.3). As the dendrimer generation increases, the degree of dendritic shielding increases and thus limits the core's accessibility to the external environment, which includes small molecules, electrons, and solvents.[23,61,62] Encapsulation can result in the attenuation of luminescence quenching of photo-active cores[60] and electron transfer rate in electro-active cores[23,24].

In this section, the encapsulation of photo- and/or redox-active core dendrimers is described.

1.3.1. Cores in Dendrimers

Two kinds of cores can be used in dendrimer research: "non-functional" and "functional". Non-functional cores exhibit no intrinsic measurable properties and serve only as a polyvalent focal unit for dendrimer preparation. Dendrimers containing non-functional cores are involved in materials applications such as supramolecular construction[63], self-assembly[64-67], drug delivery[68,69], polyvalent scaffolds[70-72] or solid-state synthesis[73].

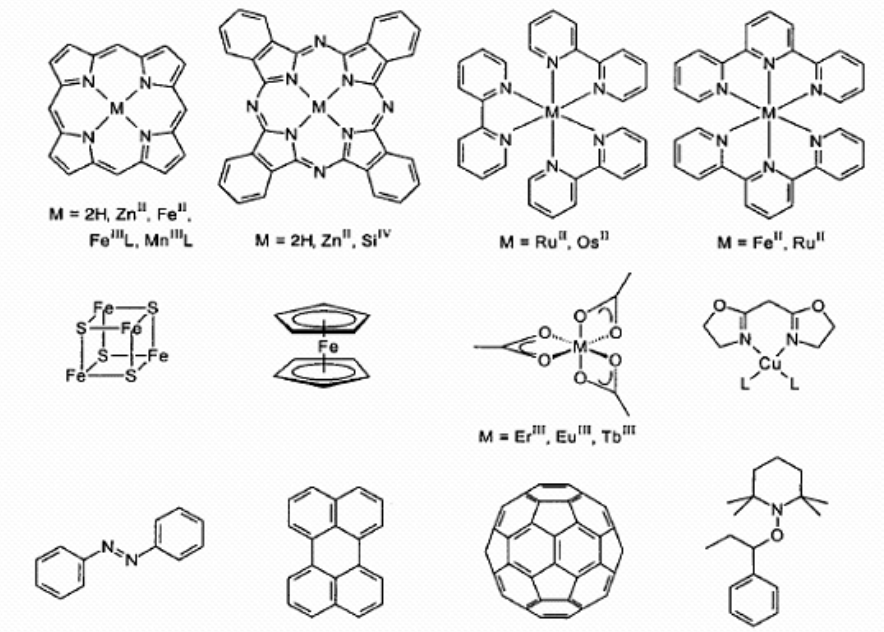


Figure1.4. Examples of core functionalities.[54]

Functional cores, on the other hand, possess observable physical and/or chemical properties in addition to serving as a polyvalent focal unit. Specifically, photo- and/or redox-active core dendrimers have been studied dominantly for studies of encapsulation effects. Cores can be classified into four groups: porphyrins/phthalocyanines, inorganic clusters, ionic complexes/organometallics, and fluorescent dyes (Figure 1.4). Porphyrins/phthalocyanines cores[54,74,75] have been the focus of many studies because they show various observable properties: photophysical, electrochemical, and ligand-binding. Inorganic clusters cores are used in attempt to mimic the ligand binding and catalytic properties of naturally-occurring enzymes[76,77]. Recently cores such as iron-sulfur, Fe_4S_4[14], rhenium

selenide, Re_6Se_8[78,79], oxymolybdo-sulfur, $MoOS_4$[76], and molybdenum chloride, Mo_6Cl_8[80], have been used in photophysical and electrochemical studies. Ionic and organometallic complexes cores[81,82] generally contain iron, ruthenium, iridium, osmium, and lanthanides and have been useful due to the establishment of various synthetic approaches for dendrimer assembly. Finally, fluorescent dyes cores[83] have been studied for organic light emitting diode applications.

1.3.2. Photo-active Core Dendrimers

The photophysical properties of dendrimers with photo-active cores can be affected by changes in the microenvironment around the core. Because the core is shielded from the external environment by encapsulation, changes in the core's absorption and emission properties can be seen with increasing dendrimer generation. For example, encapsulation can reduce an inflow of quenching agents such as solvent and oxygen to the photo-active core, resulting in higher luminescence efficiency.[51,53,83,84] The effects of encapsulation on the optical properties of dendrimers can be dependent on structure of dendron or size of quenching agent and examples to support this are presented.

Hawker and co-workers prepared the first photophysical probe to study the influences of a core's microenvironment on its absorption properties (Figure 1.5).[85] The absorption maxima of the probe using 4-(N-methylamino)-1-nitrobenzene core were shifted as a function of dendrimer generation. The absorption maximum in

carbon tetrachloride changed dramatically between generation 3 and generation 4 (372nm to 379nm). This observation indicated that the microenvironment around the core was had a high polarity generated by polyether moieties, resulting in shielding effectively the solvent in the higher generation dendrimer.

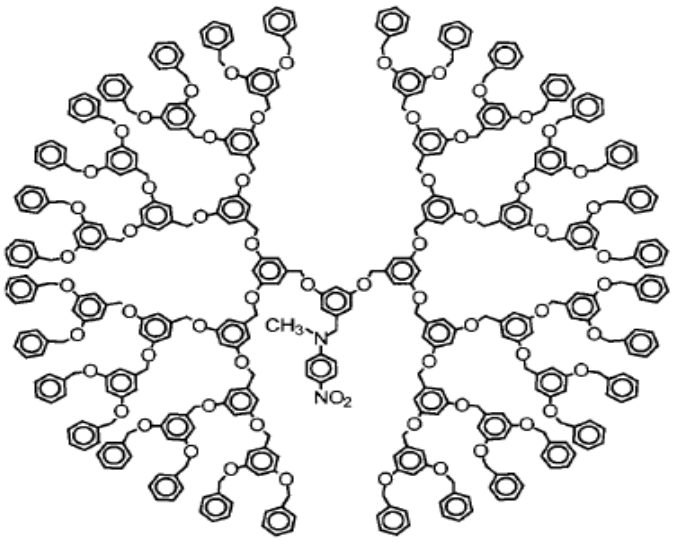


Figure1.5. The 1st dendritic encapsulation from Fréchet's group.[85]

Aida and co-workers also studied the encapsulation effects of photo-active cores. The first zinc-porphyrin core dendrimer up to generation 4 was prepared to study fluorescence quenching (Figure 1.6).[60] Introduction of a small quencher such as vitamin K_3 efficiently quenched the fluorescence of the 4th generation dendrimer. In contrast, introduction of larger quencher such as the first generation free base dendrimer indicated the opposite effect. This behavior indicates that while bulky

dendritic units act as a barrier against accessibility of large molecules to the core, they behave as a trap for small molecules.

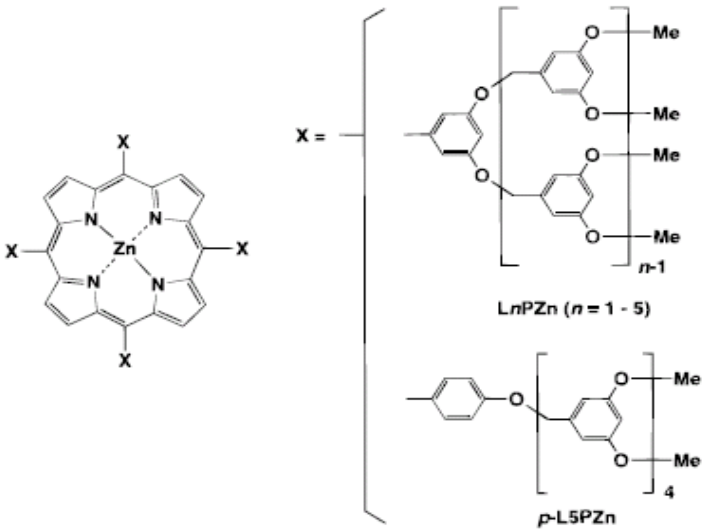


Figure1.6. Zinc-porphyrin core dendrimers from Aida's group.[86]

Kawa and Fréchet prepared self-assembled lanthanide-core dendrimers up to generation 4 (Figure 1.7).[17] Lanthanide cations (Er^{3+}, $Eu3^{3+}$, and Tb^{3+}) were encapsulated by Fréchet-type polyether dendritic ligands with carboxylate anion focal units which self-assembled around the core. The measurement of luminescence properties both in solution and in the bulk films showed an enhancement in luminescence with increasing generation. The result can be explained by the site isolation of the Ln^{3+} core, which prevented mutual interaction and thus reduced the rate of self-quenching. It can also be attributed to the antenna

effect; the excitation energy transfer from non-conjugated phenyl benzyl ether dendritic ligands to the core.

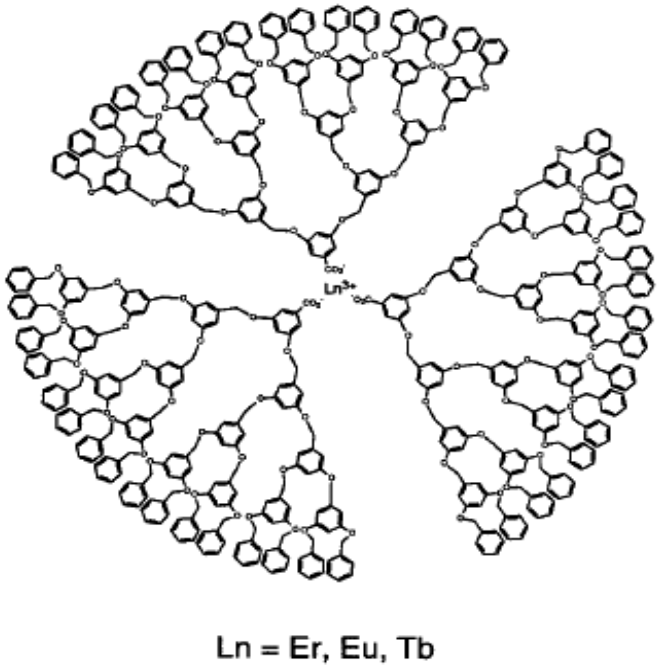


Figure1.7. Lanthanide-core dendrimer from Fréchet's group.[17]

Balzani, Vögtle, and co-workers studied encapsulation effects for dendrimers with a tris(bipyridine)ruthenium core[87]. The ruthenium metal as a core was encapsulated with Newkome-type poly(ether amide) dendrons containing bidentate bipyridine focal units up to generation 3 (Figure 1.8). Higher generations of dendrimers, in aerated solution, showed a more intense emission, enhanced quantum yields, and a longer excited-state life time. This behavior indicates that quenching

by molecular oxygen was limited due to effective encapsulation of the core by the bulky dendritic units.

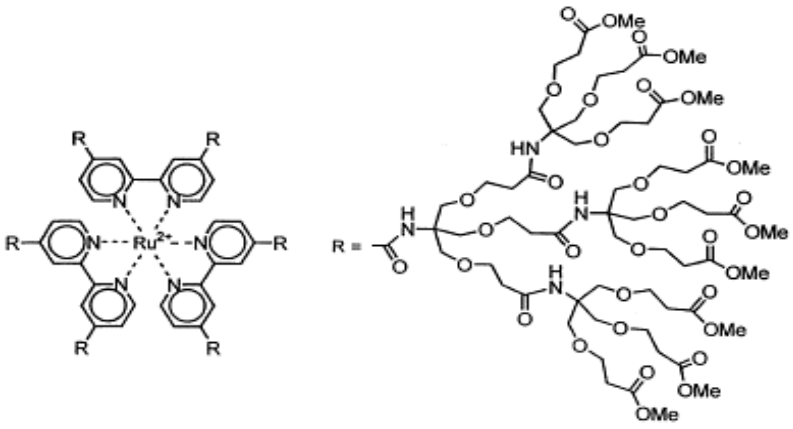
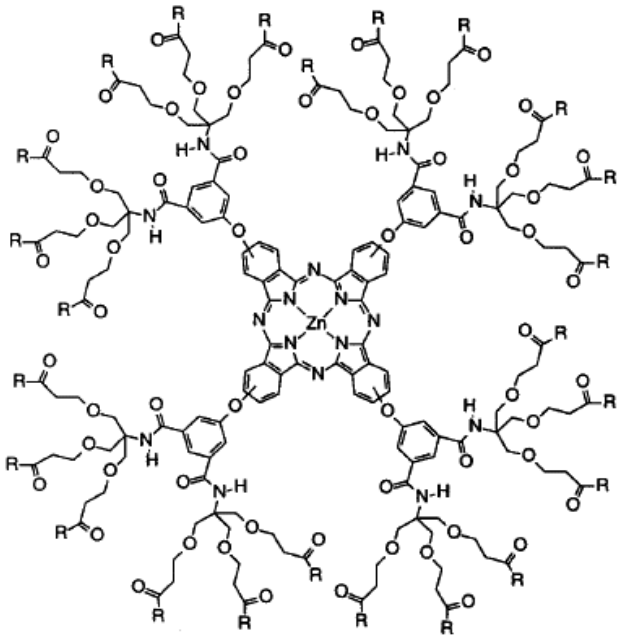


Figure1.8. Ru(II) bipyridine dendrimer from Vögtle and Balzani's group.[87]

Kimura and co-workers performed another study with zinc-phthalocyanine core dendrimers containing polyether-amide dendrons (Figure 1.9).[88] In this case, the dendrons served to prevent core aggregation which alters the optical properties of the core unit. The aggregation behavior of these hydrophilic dendrimers was monitored using UV and fluorescence spectroscopy. The UV spectra indicated that the bulky dendritic units effectively shielded the core and prevented aggregation. The absence of a peak in the fluorescence spectra of lower-generation dendrimer indicated aggregation had occurred while the strong fluorescence peak observed for higher generation dendrimers indicated the larger dendrons had successfully

prevented aggregation.



a) R = OLi

b) R = $NHC(CH_2OCH_2CH_2CO_2Li)_3$

Figure1.9. Phthalocyanine-based dendrimers from Kimura's group.[88]

1.3.3. Redox-active Core Dendrimers

As with photo-active core, the properties of redox-active core dendrimers can also be affected by changes in the microenvironment around the core. Thus, the electron transfer properties can significantly vary with increasing dendrimer generation. Metalloproteins in nature are good standards and they can control the rate and driving force of electron transfer to and from a redox core by encapsulating the core with polypeptide ligands.[89-95] Dendritic structures which mimic the

metalloproteins have been studied in order to better understand this process which occurs in nature. Electron transfer rate is governed by the distance of electron transfer and the properties of dendrons surrounding the core.[14,96] The distance can be adjusted by increasing the dendrimer generation and/or modifying the dendron architecture.

Dendritic encapsulation of redox-active core can be observed through variations in the kinetic and thermodynamic properties of electron transfer to the core.[13,14,17,19,21,23,24,27,28,57,59,97-114] Kinetic properties are evidenced by changes in the heterogeneous electron transfer rate, while thermodynamic properties are evidenced by shifts in the redox potential. Attenuation of the heterogeneous electron transfer rates between a redox core and an electrode surface via non-conducting branched dendritic shell has been studied extensively, affording a better understanding of the encapsulation of redox-active cores.[14,19,21,23,24,28,57,59,97-110,112-114] However, the properties governing the thermodynamics have not been studied thoroughly and there has been little understanding of how the dendritic structure affects redox potential.[13,17,24,27,57,98,111,113]

Numerous molecular encapsulation studies have been reported for redox-active core dendrimers. Fréchet and co-workers demonstrated the effect of the dendritic environment on the electrochemical properties of a zinc-porphyrin core encapsulated with poly(benzyl ether) dendrons (Figure 1.10).[102] As the size of the dendritic unit increased, the attenuation was observed in the heterogeneous electron

transfer rate. In the higher generation dendrimers, this was attributed to the increased distance between the zinc-porphyrin core and the electrode surface created by the encapsulating dendrons.

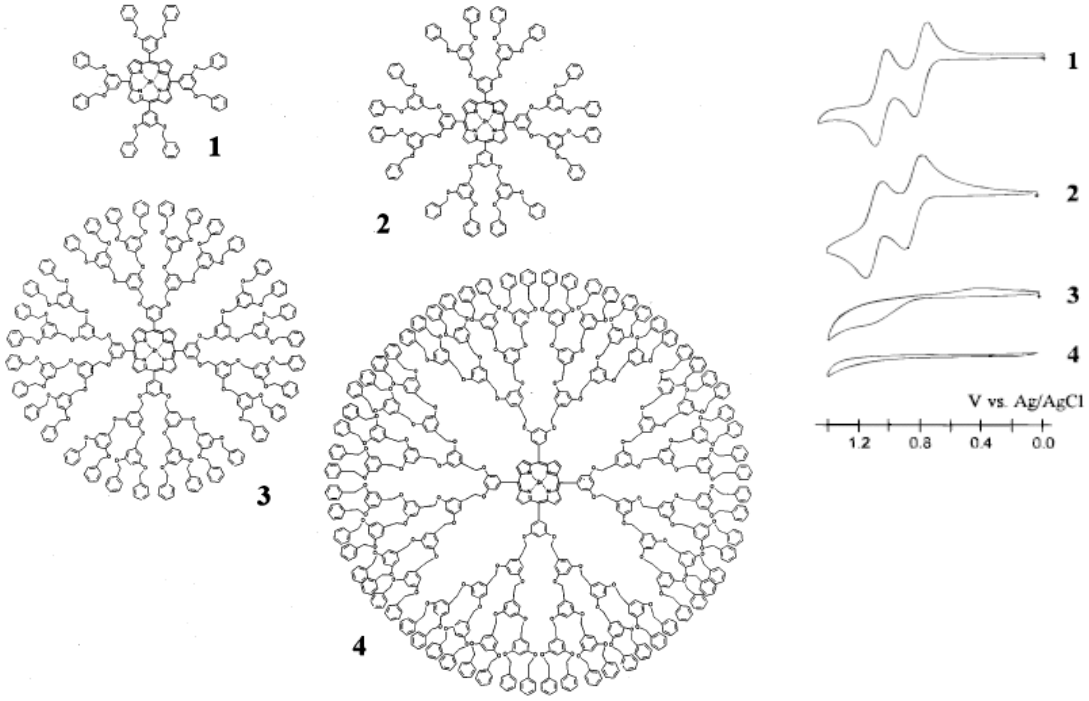


Figure1.10. A series of Zinc-porphyrin core dendrimers (left) and cyclic voltammograms (right) from Fréchet's group.[102]

Chow and co-workers also studied the effects of encapsulation on electron transfer using bis(terpyridine) iron(II) core dendrimers encapsulated with poly(ether) dendrons up to generation 4 (Figure 1.11).[55] Cyclic voltammograms indicated that the heterogeneous electron transfer rate was attenuated with

increasing dendrimer generation. This result can also be attributed to the increased distance from the core to the electrode surface in higher generation dendrimers.

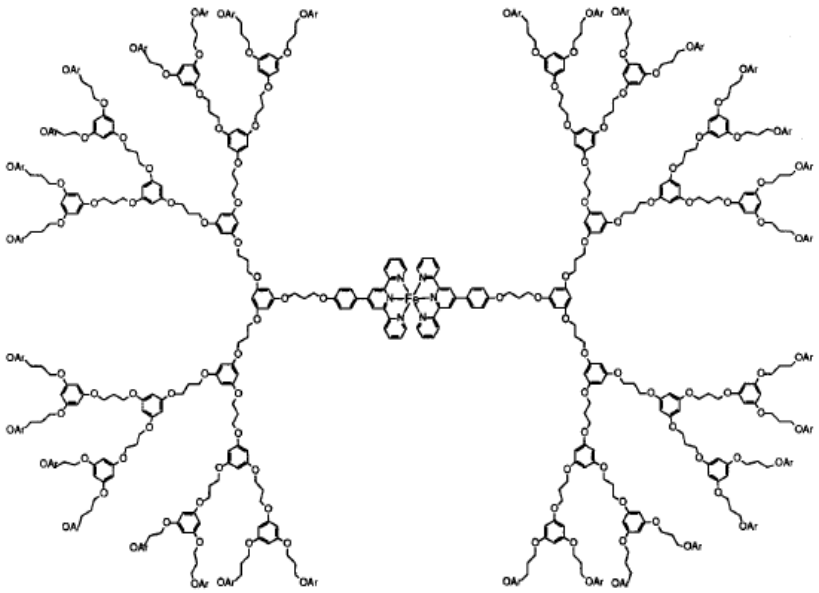


Figure1.11. Fe (II) –terpyridyl core dendrimer from Chow's group.[55]

Balzani[28] and Kaifer[110] reported studies on a series of 4,4,'-bipyridinium core dendrimers covalently encapsulated with Fréchet-type dendrons up to generation 3, respectively (Figure 1.12). Electrochemical measurements did not indicate any attenuation in the electron transfer rate with increasing generation. This was attributed to the loosely packed structure of these dendritic units which was not effective for encapsulating the bipyridinium core.

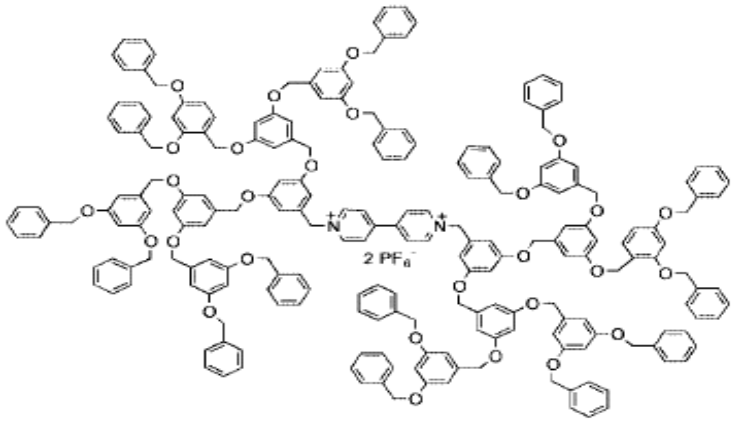


Figure1.12. Bipyridinium core dendrimer from Balzani's[28] and Kaifer's group.[110]

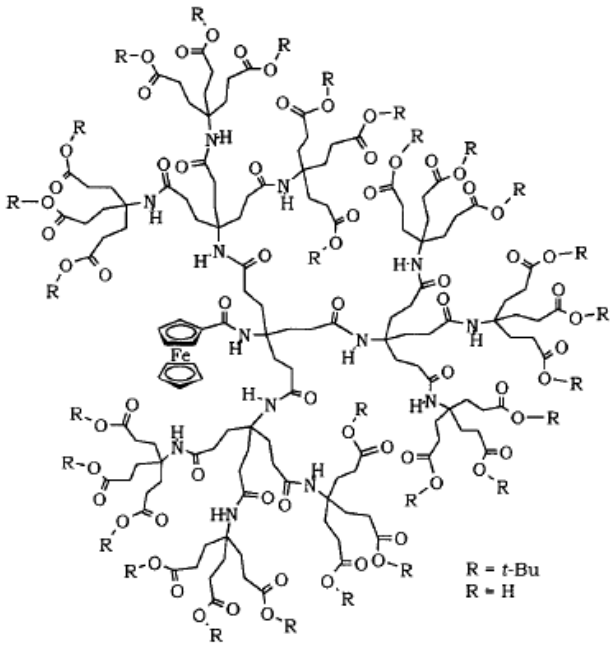


Figure1.13. Ferrocene core asymmetric dendrimers from Kaifer's group.[103]

Kaifer and co-workers prepared two series of dendrimers containing a single ferrocene core unsymmetrically located in their structures with Newkome-type dendrons up to generation 3 (Figure 1.13).[103] The electrochemical data for these dendrimers showed that heterogeneous electron transfer rates and diffusion coefficients decreased as the dendrimer generation increased. Diffusion coefficients could be reduced due to the increase in molecular weight and the effective radius with increasing dendrimer generation. The attenuation of electron transfer rates was related to the increase of the distance between the ferrocene core and the electrode surface.

Kaifer and co-workers studied the effects of molecular orientation on heterogeneous electron transfer rates with unsymmetrical ferrocene-core dendrimers (Figure 1.14).[21] This system exhibited a pH dependence on electron transfer. A positively charged ammonium-terminated alkyl monolayer was deposited on gold electrodes. The carboxylic acid periphery of the dendrimers was protonated or deprotonated with changing pH. At low pH, the carboxylic acid groups were protonated, the dendrimer exhibited a neutral state and could rotate freely with respect to the charged monolayer affording a fast electron transfer rate. However, at neutral pH, the carboxylic acid groups were deprotonated, negatively charged and interacted with the positively charged monolayer so that the ferrocene core was oriented away from the electrode. Because the distance between the core and the electrode surface was maximized under these conditions, slow electron transfer rates

were observed.

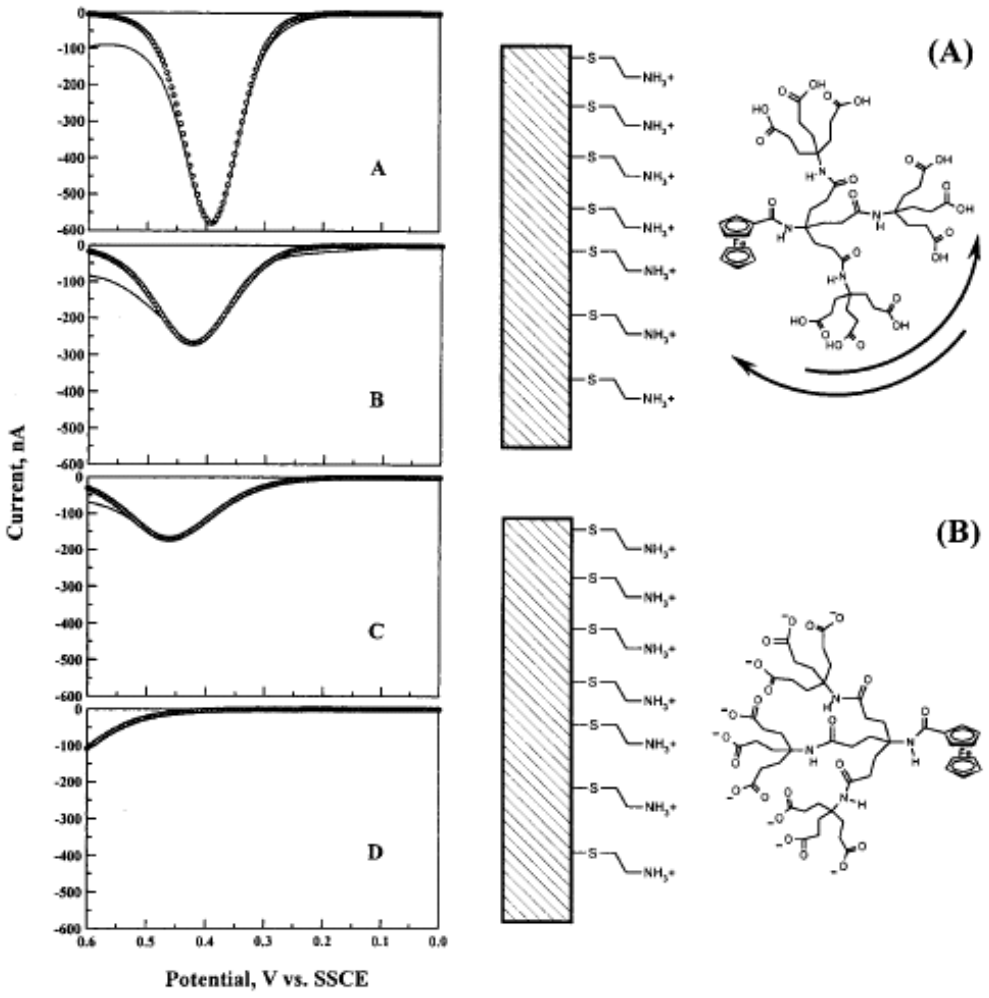
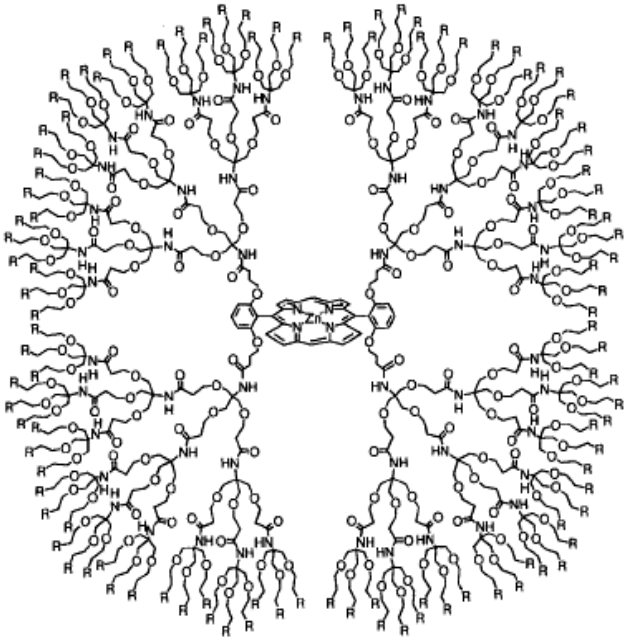


Figure1.14. Interfacial orientation of dendrimers (right) and their experimental and simulated square wave voltammetry (SWV) responses (right) from Kaifer's group: (A) pH 4.2, (B) pH 5.3, (C) pH 6.0, (D) pH 7.0.[21]



R = $COOCH_3$

Figure1.15. 3rd zinc-porphyrin core dendrimers from Diederich's group.[57]

Diederich and co-workers prepared dendrimers with zinc-porphyrin cores as synthetic models for electron transfer proteins such as cytochrome c (Figure 1.15).[57] These dendrimers were surrounded by polyether-amide dendrons and their electrochemical properties were investigated using cyclic voltammetry. The redox potential of these zinc-porphyrin core dendrimers was clearly affected by the bulky dendritic units. With increasing dendrimer generation, the redox potential associated with the first reduction process shifted to a more negative value. At the same time, the oxidation potential shifted to a less positive value indicating the presence of dendrons made the core easier to oxidize. This observation can be explained by the electron-rich nature of the dendrons. This increasing electron-rich

microenvironment around the porphyrin core with increasing dendrimer generation makes the addition of an electron to the core more difficult in reduction process, while the removal of electron is more favorable in oxidation process. Therefore, at higher generation, the favorable relationship between the electron-rich dendrimer and the positively charged zinc-porphyrin oxidation product is easier the oxidation process, while the reduction product is harder to be formed. In addition to the observed shifts in redox potential, these dendrimers also indicated attenuation in the electron transfer rate with increasing dendrimer generation.

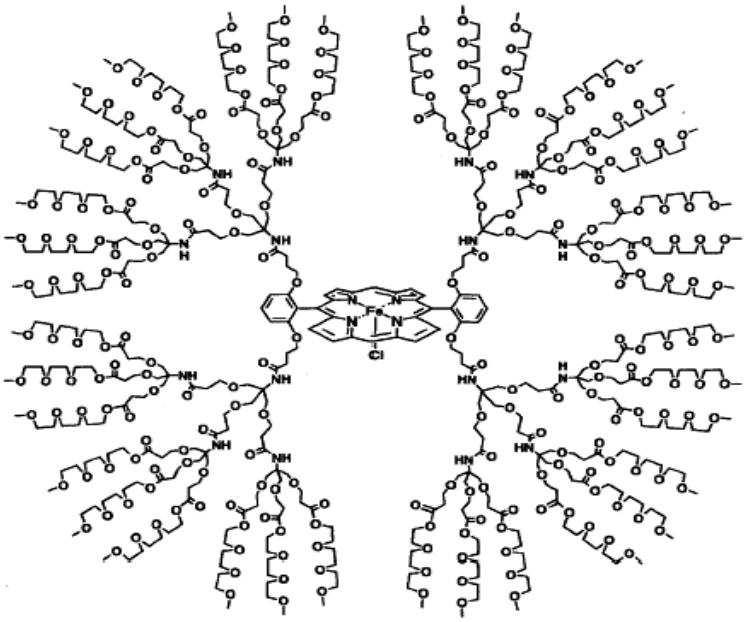


Figure1.16. Iron-porphyrin core dendrimers from Diederich's group.[56,57]

Diederich and co-workers later also prepared a series of iron-porphyrin core dendrimers with triethylene glycol monomethyl ether periphery units up to generation 2 (Figure 1.16).[56] These dendrimers were soluble in non-polar and polar solvents and electrochemical studies showed that solvent polarity strongly affected the redox potential of the porphyrin core. In a non-polar solvent such as CH_2Cl_2, the redox-potentials of iron-porphyrin core dendrimers did not exhibit a significant shift due to be exposed to similar microenvironments. However, in a polar solvent such as H_2O, the redox potential of these dendrimers shifted to a more positive value. The author suggested that, in the first generation dendrimer, the iron-porphyrin core was loosely packed with dendritic units, so that the accessibility of water molecules to the core was not hindered. However, in the second generation dendrimer, the core was densely packed, resulting in shielding of the core from solvent molecules. Thus, the effective encapsulation of the core reduced the stability of the oxidized form, leading to a shift of the redox potential to a more positive value. As with previous studies, attenuation of the electron transfer rate was observed with increasing generation.

1.4. Dendritic Encapsulation on Iron-Sulfur Core Dendrimers

Over the past few years, Gorman and co-workers have systematically studied molecular encapsulation effects with redox-active iron-sulfur core dendrimers.[13,14,23,49,58,59,96,105,115-117] In nature, proteins can control the electron transfer rates and redox potentials by encapsulating their redox-active cores with

polypeptide ligands. It has been suggested that iron-sulfur core complexes mimicking metalloproteins can be used as probes for studies of electron transfer via changes of a microenvironment around the core.[13,14,118-120] However, the application of dendrimers to biomimetic materials requires a better understanding of how encapsulation influences properties of the redox core. This requires the establishment of structure-property relationships, specifically the thermodynamic and kinetic properties of iron-sulfur clusters.

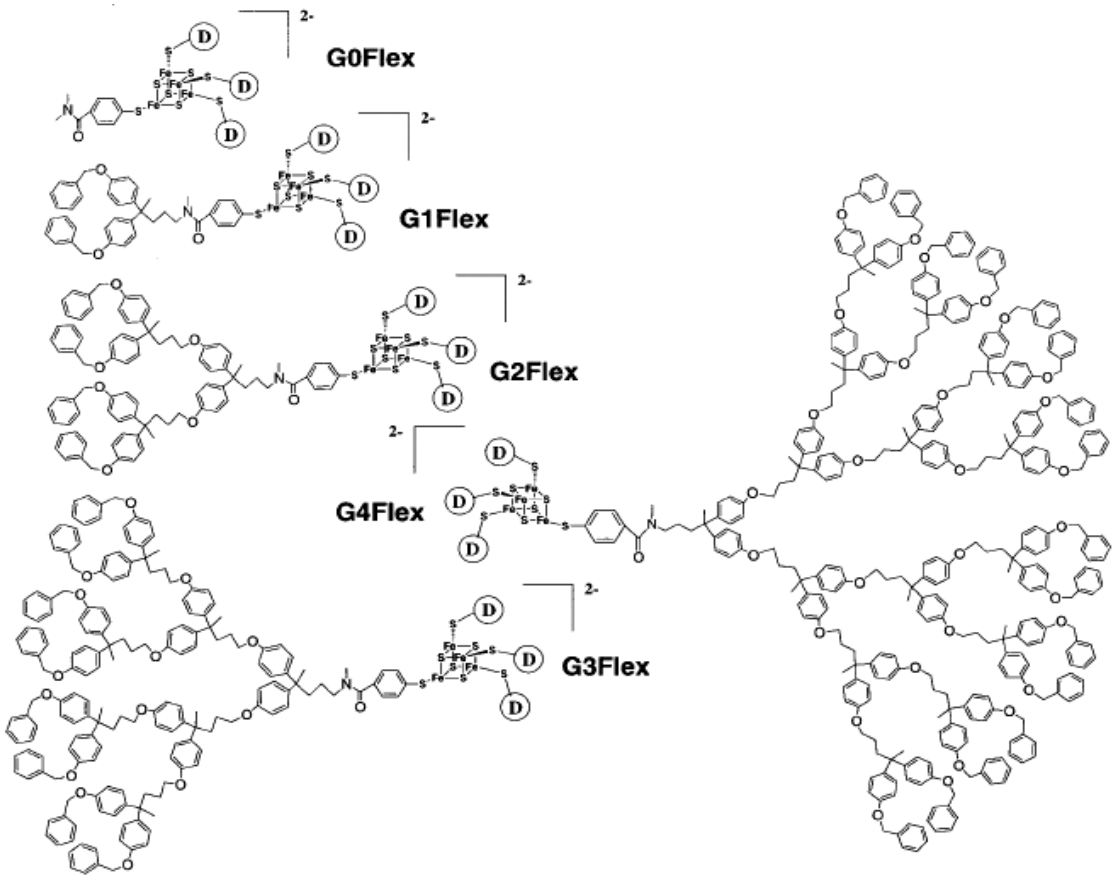


Figure1.17. Flexible iron-sulfur core dendrimers from Gorman's group.[14]

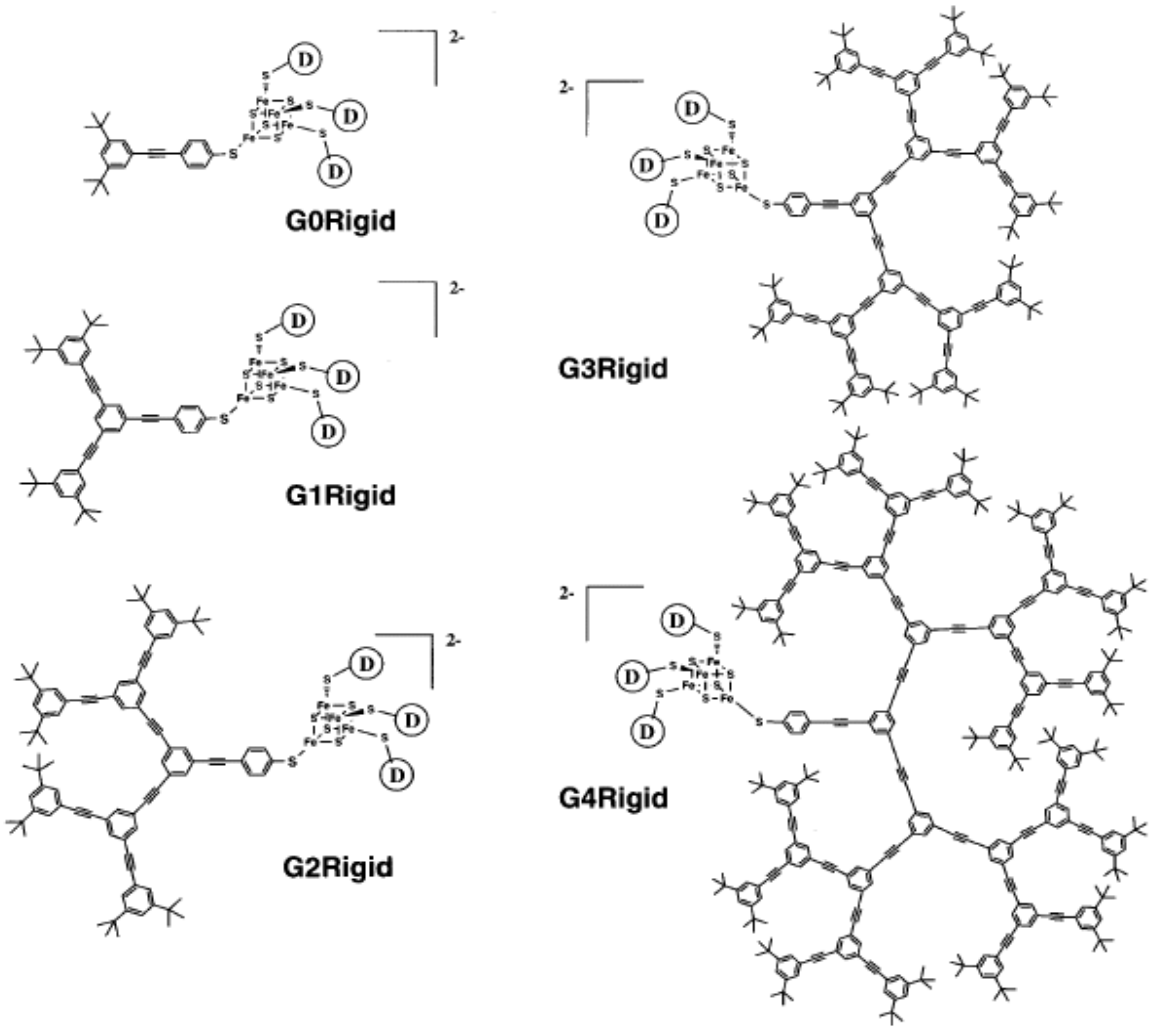


Figure1.18. Rigid iron-sulfur core dendrimers from Gorman's group.[14]

In attempt to explore some of these structure-property relationships, the Gorman group synthesized two different types of dendrimers via ligand exchange reactions: "flexible" (Figure 1.17) and "rigid" (Figure 1.18).[14] The effects of two dendritic architectures on the heterogeneous electron transfer rate of the redox-

active core were compared. The flexible architectures consisted of 4,4′-bis(hydroxyphenyl)pentanol repeat units of Fréchet type, whereas the rigid architectures were composed of stiff phenylacetylene units as used in Moore's group.[121,122]

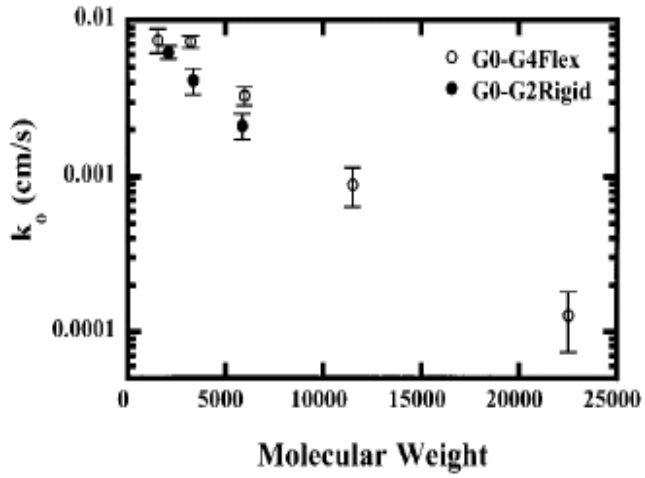


Figure1.19. Heterogeneous electron transfer rates vs. molecular weight (flexible vs. rigid).[14]

Both dendrimers exhibited significant differences in the heterogeneous electron transfer rates with increasing generation, as expected. The plot of the electron transfer rate constant vs. molecular weight shows the attenuation in electron transfer with increasing generation was more effective in rigid dendrimers than flexible dendrimers (Figure 1.19).[14] This observation was attributed to conformational differences between these dendrimers. Computer modeling showed that in flexible dendrimers, the iron-sulfur core was located at molecular edge, while in rigid

dendrimers, the core was at the center. This result can be explained by the core mobility with flexible dendrons compared to rigid dendrons. The differences in electron transfer properties between both dendrimers could be rationalized by the difference of the effective distance from the core to the electrode surface due to the core mobility. These results demonstrate the effects of these dendritic architectures on the electron transfer rate of the redox-active core.

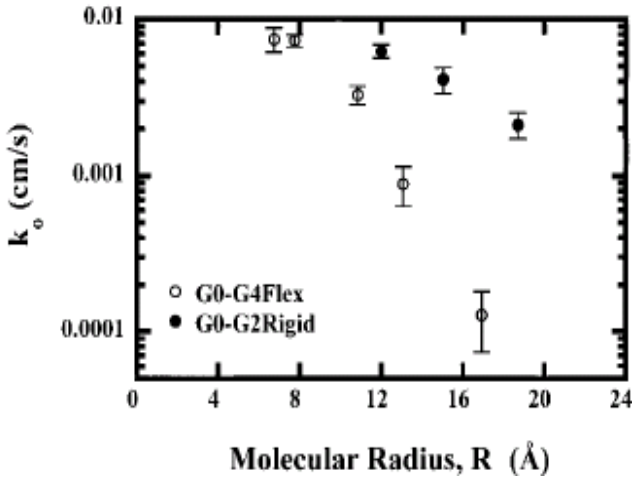


Figure1.20. Heterogeneous electron transfer rates vs. molecular size (flexible vs. rigid).[14]

However, when the electron transfer rate constants were plotted versus molecular radius, the attenuation in electron transfer rates showed an anomalous behavior (Figure 1.20).[14] The rigid dendrimers did not appear to be more effective than the flexible dendrimer in attenuating electron transfer. This observation indicates that the distance was not sole factor in the electron transfer. The

anomalous behavior may be explained by the large number of π-bonds in the cross-conjugated phenylacetylene units which more effectively shield the core and could also help to transfer electrons via through-bond electron tunneling from the core to the electrode surface. Therefore, it was concluded that the attenuation of the electron transfer rates was due to both the structure of the dendritic units and distance effects.

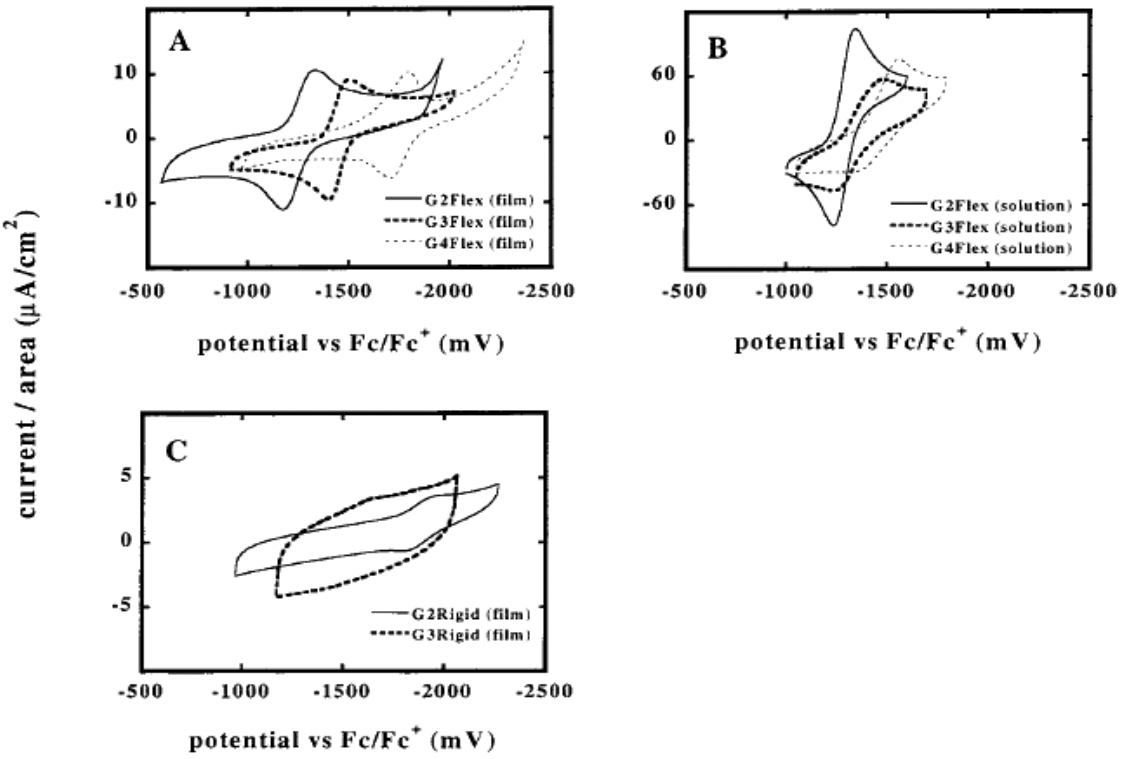


Figure1.21. Cyclic voltammograms of iron-sulfur core dendrimer in film (A, flexible / C, rigid) and in solution (B, flexible).[116]

While changes were observed in the kinetics of electron transfer with increasing generation, no significant changes were observed in the thermodynamic redox potential. This indicates that the iron-sulfur core in each dendrimer was solvated similarly in solution and the microenvironment was defined by this solvent shell. However, in film studies of iron-sulfur core dendrimers cast on a platinum electrode surface, significant differences in redox potential was observed with increasing dendrimer generation, indicating that the iron-sulfur core in these dendrimers was not solvated with solvent (Figure 1.21).[116] Specifically, solvent exclusion and generation of a less polar microenvironment by the hydrophobic dendritic units around the core in film studies led to negative shifts in the redox potential of higher generation dendrimers. However, film studies did not exhibit the differences in electron transfer kinetics observed in solution. This observation can be explained by mobility of the redox core. In films, the redox cores of neighboring dendrimers can closely approach each other over the time scale of electron hopping through the film even in higher generation dendrimers. Therefore, dendrimer size had not a strong effect on the effective distance for electron hopping.

Recently, Gorman and co-workers synthesized three pairs of constitutional isomeric dendrimers to probe the effects of structures on encapsulation: “extended” and “backfolded” (Figure 1.22).[96] Electrochemical data indicated that backfolded dendrimers exhibited more effective encapsulation due to the increase in steric congestion around the iron-sulfur core (Figure 1.23). Backfolded dendrimers

generated more hydrophobic environments surrounding the core, making the core more difficult in reduction and leading to a negative shift in redox potential. Backfolded dendrimers also maintained a smaller, more compact conformation limiting the mobility of the redox core. Dendrimers in the extended conformation allowed for more core mobility. The differences in the effective electron transfer distance between the varying architectures have a significant effect on the efficacy of encapsulation.

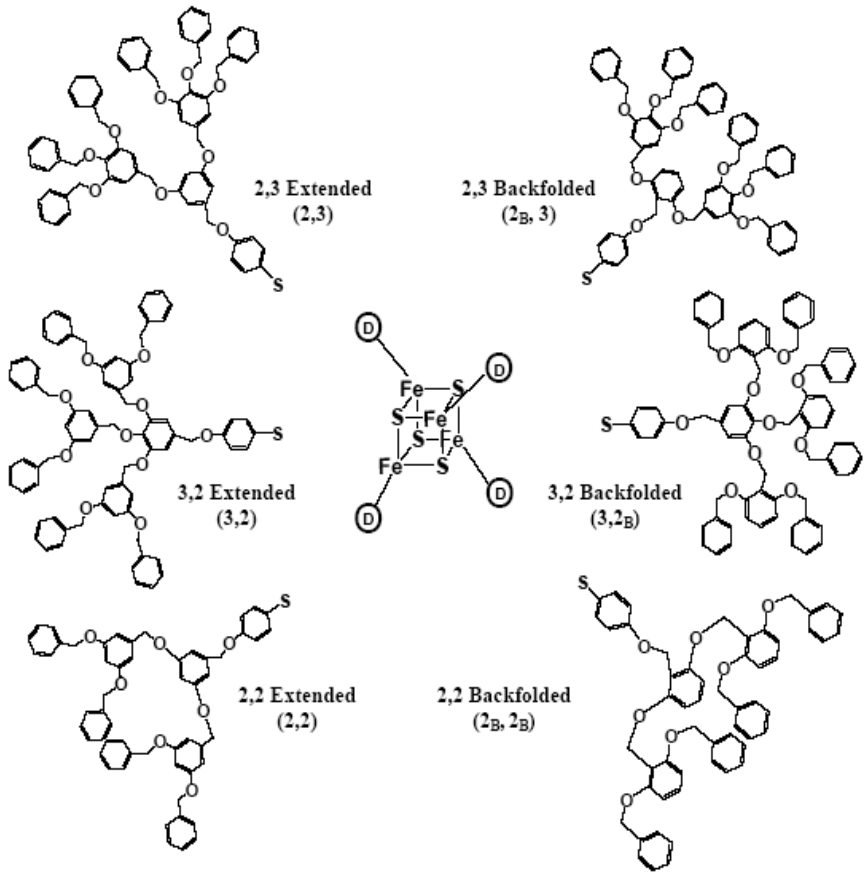


Figure1.22 Structures of constitutional iron-sulfur core dendrimer isomers from Gorman's group.[96]

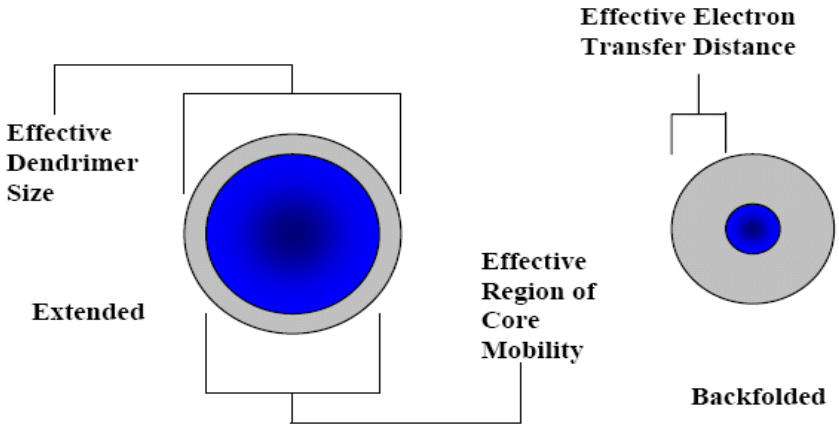


Figure1.23. Illustration of the role of core mobility and molecular size on the effective electron transfer distance.[96]

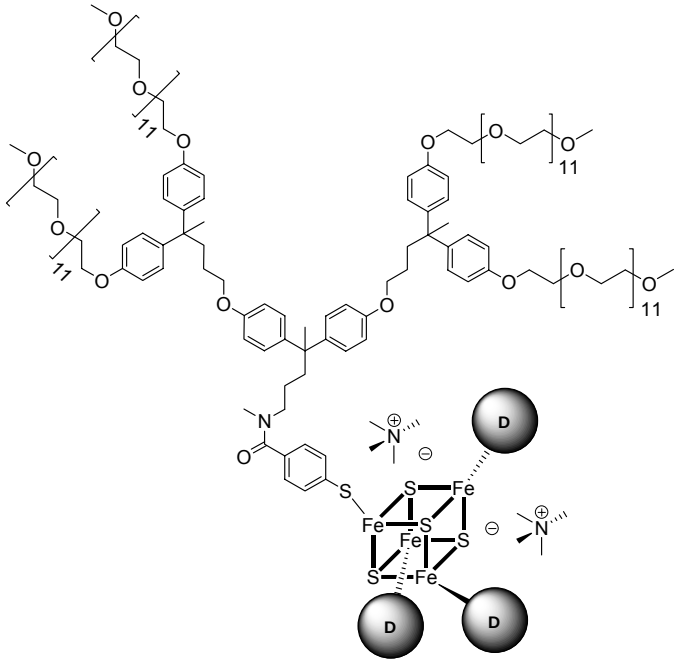


Figure1.24. Amphiphilic / water-soluble PEG12 iron-sulfur core dendrimer from Gorman's group.[123]

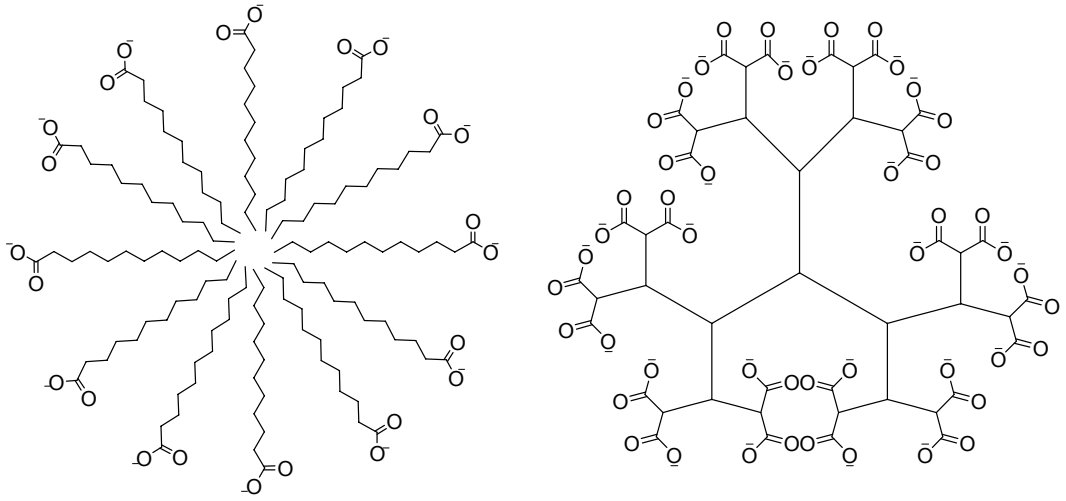
More recently, amphiphilic / water-soluble iron-sulfur core dendrimers using ethylene glycol group as periphery units were prepared to investigate structural effects on redox potential as an iron-sulfur protein model (Figure 1.24).[123]

1.5. Amphiphilic Dendrimers

The solubility properties of dendrimers can be changed by adequate selection of the peripheral groups. In this aspect, dendrimers are analogous to globular proteins. The periphery of water-soluble, globular proteins (e.g., hemoglobin) is tailored with amino acids containing polar side chains such as lysine and glutamate.[124] Similarly, hydrophobic dendrimers can be solubilized in water by introducing charged and/or polar peripheries such as triethylene glycol[56,125,126], carboxylate[21,24,50,56,103,121,127-133], and ammonium[134-138]. These water-soluble dendrimers may be a prerequisite for biochemical and pharmaceutical applications.

Amphiphilic dendrimers consist of the hydrophobic interiors and hydrophilic peripheral units. Hydrophilic polar end groups can impart water-solubility and hydrophobic non-polar interiors can solvate hydrophobic molecules.[5,6,121,127,128,137-145] The hydrophilic periphery prevents backfolding, isolating the dendritic interior. These dendrimers act as a microenvironment analogous to that which occurs in regular micelles and are therefore called “unimolecular micelles”.[127,143,146] Dendritic micelles resemble the Hartley model of the micelle (globular, with hydrophobic interior and hydrophilic exterior) (Figure 1.25)[127,128,139,141,147-150] and are able to solubilize organic

molecules in aqueous media like micelles. However, dendrimers differ from micelles in that they are a single molecule and can not be disintegrated due to covalent linkages, while the micelle is dependent on some time scale. Dendrimers maintain their structure without regard to the physical environment[129], whereas micellar structures vary with factors such as concentration, pH, temperature and ionic strength[151].



Hartley Micelle

Dendritic Unimolecular Micelle

Figure1.25. Illustration of Hartley micelle and dendritic unimolecular micelle from Moore's group.[121]

Hawker and Newkome prepared amphiphilic dendrimers of two different types and showed that the dendrimers exist as a single molecule without any aggregation. Thus, they act as unimolecular micelles and the concept of the critical micelle concentration has no meaning (Figure 1.26 and Figure 1.27).[127,128]

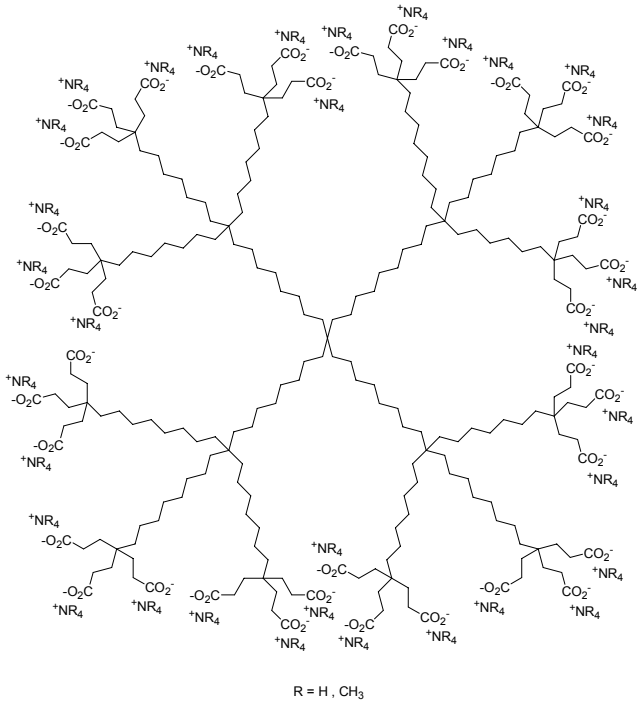


Figure1.26. Cascade polymers as unimolecular micelle from Newkome's group.[127]

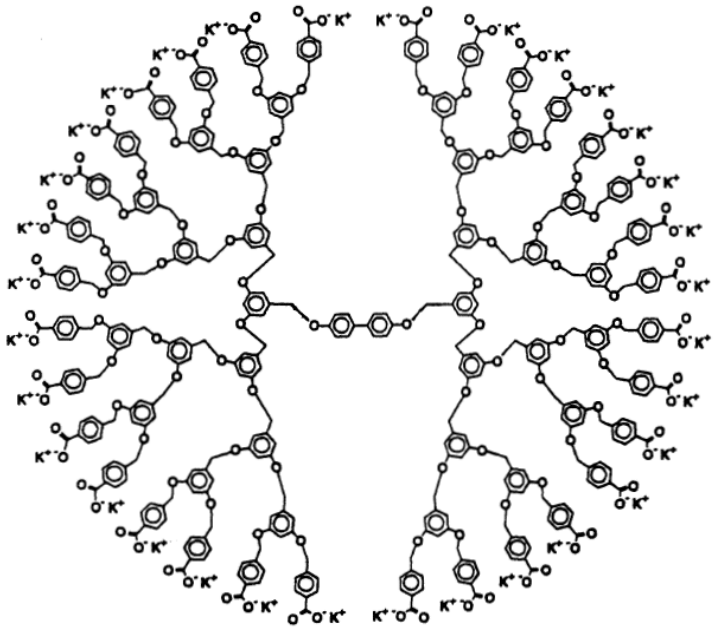


Figure1.27. Water-soluble unimolecular dendritic polyether micelle from Hawker's group.[128]

Vinogradov and coworkers have shown how fluorescence is affected by controlling the encapsulation of polyglutamic porphyrins in aqueous solvent by changing the pH. They also demonstrated that polyglutamic porphyrins shrink to form denser encapsulation around the central core in aqueous solvent restricting the diffusion of oxygen.[20,152] Similarly, Newkome and coworkers have shown that the hydrodynamic radii of amphiphilic dendrimers exhibit a pH dependence.[131] Majoral and coworkers have demonstrated that the addition of tetrahydrofuran to aqueous solutions of water-soluble dendrimers caused swelling and an increase in dendrimer size (Figure 1.28).[18]

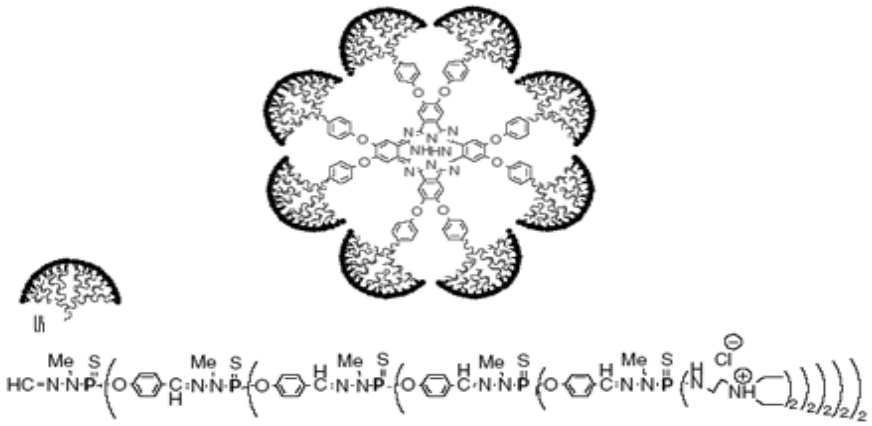


Figure1.28. Water-soluble hydrophobic dendrimer (5th generation) from Majoral's group.[18]

Iron-sulfur core dendrimers have been successfully prepared by ligand exchange reactions with hydrophobic dendrons.[13,14,25,58,59] With potential applications as an iron-

sulfur protein model, the peripheral units of hydrophobic dendrons are modified to hydrophilic end groups such as triethylene glycol[56,125,126], carboxylate[21,24,50,56,103,121,127-133], and ammonium[134-138]. These peripheral units may exhibit the effects of charge on the rate and/or driving force of electron transfer relative to non-ionic & hydrophobic flexible dendrimers.

Amphiphilic iron-sulfur core dendrimers may show changes in size and morphology such as swelling due to micellar encapsulation in aqueous / organic mixed solvent. These changes may be measured by electrochemical experiments to help the understanding on control of the rate and / or driving force of electron transfer. Thus, the effective electron transfer distance or microenvironment around the iron-sulfur core may be tuned to study various structure-property relationships. For example, electron transfer rate can be attenuated as the hydrodynamic radius is systematically altered.

1.6. Perspectives and Goals of Dissertation Research

There has been recent research interest in dendritic structures containing photo- and/or redox-active units at the core due to their many potential applications. In dendrimers containing functional cores, the bulky dendritic units are able to change a microenvironment around the cores by encapsulation and thus modify their intrinsic properties. The concept of encapsulation can be applied to research problems in molecular electronics and bio-mimic chemistry.

Before amphiphilic iron-sulfur core dendrimers can be applied to mimic iron-sulfur proteins, the structural effects on the rate and driving force of electron transfer need to be further established.

In chapter 2, the syntheses of two types of amphiphilic iron-sulfur core dendrimers are reported: cationic dendrimers containing a tetra-alkyl ammonium group as the peripheral units, and anionic dendrimers containing a carboxylate group as the peripheral units. The disulfide linkage is used as a focal unit and protecting group of the thiols. The disulfide linkage is divided into two types, symmetrical and unsymmetrical. Numerous attempts to cleave the disulfide linkage are carried out to regenerate the thiols. Attempts are reported to prepare the dendrimers by ligand exchange reaction with the resulting, deprotected dendrons.

In chapter 3, two types of dendrimers are prepared by ligand exchange reaction with new focal units and protecting groups, thiocarbamate group and trityl group. To quantify the thiol concentration, the Ellman's reagent test is introduced. To synthesize more efficiently the dendrons, a modular synthesis approach is applied following chapter 2.

In chapter 4, electrochemical experiments on two types of dendrimers, G1-cationic and G1-anionic, are performed in organic solvent to investigate the heterogeneous electron transfer rate and the redox potential, comparing with G1-flexible dendrimer. Then, the electrochemistry of G1-cationic dendrimer in organic/aqueous mixed solutions is studied to investigate how this mixed solvent

system may affect the stability and morphology of the molecule.

The long-term and ultimate purpose of this research is to explore and clarify the structural effects on the rate and driving force for electron transfer in redox-active core dendrimers as a model for metalloproteins. In this dissertation, as a part of the effort to achieve the goal, the focus is on the challenges in the synthesis of amphiphilic cationic and anionic iron-sulfur core dendrimers by ligand exchange reaction of the iron-sulfur core with the thiol dendrons, including the synthesis of dendrons via a modular synthesis approach. Also, a protocol to quantify the thiol concentration by the Ellman's reagent test after the removal of the protecting groups is established and assessed. Finally, the dendrimers resulting from any successful syntheses are characterized by electrochemical experiments.

1.7. References

(1) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117-132.

(2) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Macromolecules* **1986**, *19*, 2466-2468.

(3) Tomalia, D. A.; Hall, M.; Hedstrand, D. M. *J. Am. Chem. Soc.* **1987**, *109*, 1601-1603.

(4) Newkome, G. R.; Baker, G. R.; Saunders, M. J.; Russo, P. S.; Gupta, V. K.; Yao, Z. Q.; Miller, J. E.; Bouillion, K. *J. Chem. Soc., Chem. Comm.* **1986**, 752-753.

(5) Newkome, G. R.; Yao, Z. Q.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985**, *50*, 2003-2004.

(6) Newkome, G. R.; Yao, Z. Q.; Baker, G. R.; Gupta, V. K.; Russo, P. S.; Saunders, M. J. *J. Am. Chem. Soc.* **1986**, *108*, 849-850.

(7) Fréchet, J. M. J.; Tomalia, D. A. *Dendrimers and Other Dendritic Polymers*; 1st ed.; Wiley: Chichester, 2001.

(8) Newkome, G. R.; Moore, G. R.; Vögtle, F. *Dendritic Molecules: Concepts, Synthesis, Perspectives*; 1st ed.; WILEY-VCH: Weinheim, Germany, 1996.

(9) de Brabander-van den berg, E. M. M.; Meijer, E. W. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1308-1311.

(10) Amabilino, D. B.; Ashton, P. R.; Balzani, V.; Brown, C. L.; Credi, A.; Fréchet, J. M. J.; Leon, J. W.; Raymo, F. M.; Spencer, N.; Stoddart, J. F.; Venturi, M. *J. Am. Chem. Soc.* **1996**, *118*, 12012-12020.

(11) Campagna, S.; Denti, G.; Serroni, S.; Ciano, M.; Juris, A.; Balzani, V. *Inorg. Chem.* **1992**, *31*, 2982-2984.

(12) Cuadrado, I.; Casado, C. M.; Alonso, B.; Moran, M.; Losada, J.; Belsky, V. *J. Am. Chem. Soc.* **1997**, *119*, 7613-7614.

(13) Gorman, C. B.; Smith, J. C. *Acc. Chem. Res.* **2001**, *34*, 60-71.

(14) Gorman, C. B.; Smith, J. C.; Hager, M. W.; Parkhurst, B. L.; Sierzputowska-Gracz, H.; Haney, C. A. *J. Am. Chem. Soc.* **1999**, *121*, 9958-9966.

(15) Hawker, C.; Fréchet, J. M. J. *J. Chem. Soc., Chem. Comm.* **1990**, 1010-1013.

(16) Jiang, D. L.; Aida, T. *Nature* **1997**, *388*, 454-456.

(17) Kawa, M.; Fréchet, J. M. J. *Chem. Mater.* **1998**, *10*, 286-296.

(18) Leclaire, J.; Coppel, Y.; Caminade, A. M.; Majoral, J. P. *J. Am. Chem. Soc.* **2004**, *126*, 2304-2305.

(19) Newkome, G. R.; Guther, R.; Moorefield, C. N.; Cardullo, F.; Echegoyen, L.; Perezcordero, E.; Luftmann, H. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2023-2026.

(20) Vinogradov, S. A.; Lo, L. W.; Wilson, D. F. *Chem. Eur. J.* **1999**, *5*, 1338-1347.

(21) Wang, Y.; Cardona, C. M.; Kaifer, A. E. *J. Am. Chem. Soc.* **1999**, *121*, 9756-9757.

(22) Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638-7647.

(23) Cameron, C. S.; Gorman, C. B. *Adv. Func. Mater.* **2002**, *12*, 17-20.

(24) Cardona, C. M.; Mendoza, S.; Kaifer, A. E. *Chem. Soc. Rev.* **2000**, *29*, 37-42.

(25) Chasse, T. L.; Yohannan, J. C.; Kim, N.; Li, Q.; Li, Z. M.; Gorman, C. B. *Tetrahedron* **2003**, *59*, 3853-3861.

(26) Goldsmith, J. I.; Takada, K.; Abruna, H. D. *J. Phy. Chem.* **2002**, *106*, 8504-8513.

(27) Ong, W.; Kaifer, A. E. *J. Am. Chem. Soc.* **2002**, *124*, 9358-9359.

(28) Vicinelli, V.; Maestri, M.; Balzani, V.; Muller, W. M.; Muller, U.; Hahn, U.; Osswald, F.; Vögtle, F. *New J. Chem.* **2001**, *25*, 989-993.

(29) Matthews, O. A.; Shipway, A. N.; Stoddart, J. F. *Prog. Polym. Sci.* **1998**, *23*, 1-56.

(30) Archut, A.; Vögtle, F. *Chem. Soc. Rev.* **1998**, *27*, 233.

(31) Fréchet, J. M. J. *Science* **1994**, *263*, 1710.

(32) Frey, H. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2193.

(33) Frey, H.; Lach, C.; Lorenz, K. *Adv. Mater.* **1998**, *10*, 279.

(34) Issberner, J.; Moors, R.; Vögtle, F. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2413.

(35) Jiang, D. L.; Aida, T. *J. Am. Chem. Soc.* **1998**, *120*, 10895.

(36) Narayanan, V. V.; Newkome, G. R. *Top. Curr. Chem.* **1998**, *197*, 19.

(37) Newkome, G. R. *JAI Press: London* **1994-1996**, *1-3*.

(38) Peerlings, H. W. I.; Meijer, E. W. *Chem. Eur. J.* **1997**, *3*, 1563.

(39) Schlüter, A. D. *Top. Curr. Chem.* **1998**, *197*, 165.

(40) Seebach, D.; Rheiner, P. B.; Greiveldinger, G.; Butz, T.; Sellner, H. *Top. Curr.*

Chem. **1998**, *197*, 125.

(41) Tomalia, D. A.; Durst, H. D. *Top. Curr. Chem.* **1993**, *165*, 193.

(42) Voit, B. I. *Acta Polym.* **1995**, *46*, 87.

(43) Zeng, F.; Zimmerman, S. C. *Chem. Rev.* **1997**, *97*, 1681.

(44) Majoral, J. P.; Caminade, A. M. *Top. Curr. Chem.* **1998**, *197*, 79.

(45) Balzani, V.; Campagna, S.; Denti, G.; Juris, A.; Serroni, S.; Venturi, M. *Acc. Chem. Res.* **1998**, *31*, 26.

(46) Venturi, M.; Serroni, S.; Juris, A.; Campagna, S.; Balzani, V. *Top. Curr. Chem.* **1998**, *197*, 193.

(47) Ardoin, N.; Astruc, D. *Bull. Soc. Chim. Fr.* **1995**, *132*, 875.

(48) Constable, E. C. *Chem. Comm.* **1997**, 1073.

(49) Gorman, C. *Adv. Mater.* **1998**, *10*, 295-309.

(50) Tomioka, N.; Takasu, D.; Takahashi, T.; Aida, T. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1531-1534.

(51) Halim, M.; Pillow, J. N. G.; Samuel, I. D. W.; Burn, P. L. *Synth. Met.* **1999**, *102*, 922-923.

(52) Lupton, J. M.; Samuel, I. D. W.; Beavington, R.; Burn, P. L.; Bassler, H. *Adv. Mater.* **2001**, *13*, 258-262.

(53) Wang, P. W.; Liu, Y. J.; Devadoss, C.; Bharathi, P.; Moore, J. S. *Adv. Mater.* **1996**, *8*, 237-241.

(54) Hecht, S.; Fréchet, J. M. J. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 74-91.

(55) Chow, H. F.; Chan, I. Y. K.; Chan, D. T. W.; Kwok, R. W. M. *Chem. Eur.J.* **1996**, *2*, 1085-1091.

(56) Dandliker, P. J.; Diederich, F.; Gisselbrecht, J. P.; Louati, A.; Gross, M. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2725-2728.

(57) Dandliker, P. J.; Diederich, F.; Gross, M.; Knobler, C. B.; Louati, A.; Sanford, E. M. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1739-1742.

(58) Gorman, C. B. *Adv. Mater.* **1997**, *9*, 1117-1119.

(59) Gorman, C. B.; Parkhurst, B. L.; Su, W. Y.; Chen, K. Y. *J. Am. Chem. Soc.* **1997**, *119*, 1141-1142.

(60) Jin, R. H.; Aida, T.; Inoue, S. *J. Chem. Soc., Chem. Comm.* **1993**, 1260-1262.

(61) Chow, H. F.; Mong, T. K. K.; Nongrum, M. F.; Wan, C. W. *Tetrahedron*

1998, *54*, 8543-8660.

(62) Grayson, S. K.; Fréchet, J. M. J. *Chem. Rev.* **2001**, *101*, 3819-3867.

(63) Zimmerman, S. C.; Lawless, L. J. *Top. Curr. Chem.* **2001**, *217*, 95-120.

(64) Fischer, M.; Vögtle, F. *Angew. Chem. Int. Ed. Engl.* **1999**, *38*, 885-905.

(65) Percec, V.; Ahn, C. H.; Ungar, G.; Yeardley, D. J. P.; Moller, M.; Sheiko, S. S. *Nature* **1998**, *391*, 161-164.

(66) Hudson, S. D.; Jung, H. T.; Percec, V.; Cho, W. D.; Johansson, G.; Ungar, G.; Balagurusamy, V. S. K. *Science* **1997**, *278*, 449-452.

(67) Percec, V.; Chu, P. W.; Ungar, G.; Zhou, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 11441-11454.

(68) Liu, M. J.; Fréchet, J. M. J. *J. Pharm. Sci. Tech. Today* **1999**, *2*, 393.

(69) Chen, C. Z. S.; Cooper, S. L. *Adv. Mater.* **2000**, *12*, 843-846.

(70) Kircheis, R.; Wightman, L.; Wagner, E. *Adv. Drug Delivery Rev.* **2001**, *53*, 341-358.

(71) Esfand, R.; Tomalia, D. A. *Drug Discovery Today* **2001**, *6*, 427-436.

(72) Tomlinson, E.; Rolland, A. P. *J. Controlled Release* **1996**, *39*, 357-372.

(73) Kim, R. M.; Manna, M.; Hutchins, S. M.; Griffin, P. R.; Yates, N. A.; Bernick, A. M.; Chapman, K. T. *Proc. Nat. Acad. Sci. USA.* **1996**, *93*, 10012-10017.

(74) Brewis, M.; Hassan, B. M.; Li, H.; Makhseed, S.; McKeown, N. B.; Thompson, N. *J. Porphyrins Phthalocyanines* **2000**, *4*, 460-464.

(75) Diederich, F. *Chimia* **2001**, *55*, 821-827.

(76) Mondal, S.; Basu, P. *Inorg. Chem.* **2001**, *40*, 192-+.

(77) Hannon, M. J.; Mayers, P. C.; Taylor, P. C. *J. Chem. Soc. Perkin Trans. 1* **2000**, 1881-1889.

(78) Roland, B. K.; Carter, C.; Zheng, Z. P. *J. Am. Chem. Soc.* **2002**, *124*, 6234-6235.

(79) Wang, R. Y.; Zheng, Z. P. *J. Am. Chem. Soc.* **1999**, *121*, 3549-3550.

(80) Gorman, C. B. *Chem. Commun.* **1999**, 877-878.

(81) Serroni, S.; Campagna, S.; Puntoriero, F.; Di Pietro, C.; McClenaghan, N. D.; Loiseau, F. *Chem. Soc. Rev.* **2001**, *30*, 367-375.

(82) Balzani, V.; Ceroni, P.; Juris, A.; Venturi, M.; Campagna, S.; Puntoriero, F.; Serroni, S. *Coord. Chem. Rev.* **2001**, *219*, 545-572.

(83) Freeman, A. W.; Koene, S. C.; Malenfant, P. R. L.; Thompson, M. E.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2000**, *122*, 12385-12386.

(84) Setayesh, S.; Grimsdale, A. C.; Weil, T.; Enkelmann, V.; Mullen, K.; Meghdadi, F.; List, E. J. W.; Leising, G. *J. Am. Chem. Soc.* **2001**, *123*, 946-953.

(85) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1993**, *115*, 4375-4376.

(86) Tomoyose, Y.; Jiang, D. L.; Jin, R. H.; Aida, T.; Yamashita, T.; Horie, K. *Macromolecules* **1996**, *29*, 5236-5238.

(87) Issberner, J.; Vögtle, F.; Cola, L. D.; Balzani, V. *Chem. Eur. J.* **1997**, *3*, 706-712.

(88) Kimura, M.; Nakada, K.; Yamaguchi, Y.; Hanabusa, K.; Shirai, H.; Kobayashi, N. *Chem. Comm.* **1997**, 1215-1216.

(89) Beratan, D. N.; Onuchic, J. N.; Winkler, J. R.; Gray, H. B. *Science* **1992**, *258*, 1740-1741.

(90) Beratan, D. N.; Skourtis, S. S. *Curr. Opin. Chem. Biol.* **1998**, *2*, 235-243.

(91) Langen, R.; Chang, I. J.; Germanas, J. P.; Richards, J. H.; Winkler, J. R.; Gray, H. B. *Science* **1995**, *268*, 1733-1735.

(92) Risser, S. M.; Beratan, D. N.; Onuchic, J. N. *J. Phys. Chem.* **1993**, *97*, 4523-4527.

(93) Smith, D. K.; Diederich, F. *Chem. Eur. J.* **1998**, *4*, 1353-1361.

(94) Winkler, J. R.; Gray, H. B. *Chem. Rev.* **1992**, *92*, 369-379.

(95) Wuttke, D. S.; Bjerrum, M. J.; Winkler, J. R.; Gray, H. B. *Science* **1992**, *256*, 1007-1009.

(96) Chasse, T. L.; Sachdeva, R.; Li, C.; Li, Z. M.; Petrie, R. J.; Gorman, C. B. *J. Am. Chem. Soc.* **2003**, *125*, 8250-8254.

(97) Wyatt, S. R.; Hu, Q. S.; Yan, X. L.; Bare, W. D.; Pu, L. *Macromolecules* **2001**, *34*, 7983-7988.

(98) Stone, D. L.; Smith, D. K.; McGrail, P. T. *J. Am. Chem. Soc.* **2002**, *124*, 856-864.

(99) Huang, B. H.; Parquette, J. R. *J. Am. Chem. Soc.* **2001**, *123*, 2689-2690.

(100) Peerlings, H. W. I.; Trimbach, D. C.; Meijer, E. W. *Chem. Comm.* **1998**, 497-498.

(101) Recker, J.; Tomcik, D. J.; Parquette, J. R. *J. Am. Chem. Soc.* **2000**, *122*, 10298-10307.

(102) Pollak, K. W.; Leon, J. W.; Fréchet, J. M. J.; Maskus, M.; Abruna, H. D. *Chem. Mater.* **1998**, *10*, 30-38.

(103) Cardona, C. M.; McCarley, T. D.; Kaifer, A. E. *J. Org. Chem.* **2000**, *65*, 1857-1864.

(104) Chow, H. F.; Chan, I. Y. K.; Fung, P. S.; Mong, T. K. K.; Nongrum, M. F. *Tetrahedron* **2001**, *57*, 1565-1572.

(105) Gorman, C. B.; Smith, J. C. *Polymer* **2000**, *41*, 675-683.

(106) Moore, J. S.; Xu, Z. F. *Macromolecules* **1991**, *24*, 5893-5894.

(107) Pesak, D. J.; Moore, J. S. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1636-1639.

(108) Rosini, C.; Superchi, S.; Peerlings, H. W. I.; Meijer, E. W. *Eur. J. Org. Chem.* **2000**, 61-71.

(109) Scherrenberg, R.; Coussens, B.; van Vliet, P.; Edouard, G.; Brackman, J.; de Brabander, E.; Mortensen, K. *Macromolecules* **1998**, *31*, 456-461.

(110) Toba, R.; Quintela, J. M.; Peinador, C.; Roman, E.; Kaifer, A. E. *Chem. Comm.* **2001**, 857-858.

(111) Weyermann, P.; Diederich, F.; Gisselbrecht, J. P.; Boudon, C.; Gross, M. *Helv.Chim. Acta* **2002**, *85*, 571-598.

(112) Xu, Z. F.; Kahr, M.; Walker, K. L.; Wilkins, C. L.; Moore, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 4537-4550.

(113) Cardona, C. M.; Kaifer, A. E. *J. Am. Chem. Soc.* **1998**, *120*, 4023-4024.

(114) Gandhi, P.; Huang, B. H.; Gallucci, J. C.; Parquette, J. R. *Org. Lett.* **2001**, *3*, 3129-3132.

(115) Gorman, C. B.; Hager, M. W.; Parkhurst, B. L.; Smith, J. C. *Macromolecules* **1998**, *31*, 815-822.

(116) Gorman, C. B.; Smith, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 9342-9343.

(117) Gorman, C. B.; Smith, J. C.; Sachdeva, R.; Su, W. Y.; Jiang, H. W. *Macromol. Symp.* **2000**, *156*, 61-67.

(118) Depamphi, B. V.; Averill, B. A.; Herskovi, T.; Que, L.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4159-4167.

(119) Hill, C. L.; Renaud, J.; Holm, R. H.; Mortenson, L. E. *J. Am. Chem. Soc.*

1977, *99*, 2549-2557.

(120) Que, L.; Bobrik, M. A.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4168-4178.

(121) Pesak, D. J.; Moore, J. S.; Wheat, T. E. *Macromolecules* **1997**, *30*, 6467-6482.

(122) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1991**, *113*, 4252-4261.

(123) Cameron, C. S. Synthesis of Amphiphilic Iron-Sulfur Core Dendrimers and Investigations into Their Encapsulation Abilities in Different Solutions. Ph.D. Thesis, North Carolina State University, Raleigh, 2005.

(124) Stryer, L. *Biochemistry*; 4th ed.; Freeman: New York, USA, 1995.

(125) Smith, D. K.; Diederich, F. *Chem. Comm.* **1998**, 2501-2502.

(126) Smith, D. K. *J. Chem. Soc. Perkin Trans. 2* **1999**, 1563-1565.

(127) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Saunders, M. J.; Grossman, S. H. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1178-1180.

(128) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Chem. Soc. Perkin Trans. 1* **1993**, 1287-1297.

(129) Kim, Y. H.; Webster, O. W. *J. Am. Chem. Soc.* **1990**, *112*, 4592-4593.

(130) Pesak, D. J.; Moore, J. S. *Tetrahedron* **1997**, *53*, 15331-15347.

(131) Young, J. K.; Baker, G. R.; Newkome, G. R.; Morris, K. F.; Johnson, C. S. *Macromolecules* **1994**, *27*, 3464-3471.

(132) Cardona, C. M.; Alvarez, J.; Kaifer, A. E.; McCarley, T. D.; Pandey, S.; Baker, G. A.; Bonzagni, N. J.; Bright, F. V. *J. Am. Chem. Soc.* **2000**, *122*, 6139-6144.

(133) Laufersweiler, M. J.; Rohde, J. M.; Chaumette, J. L.; Sarazin, D.; Parquette, J. R. *J. Org. Chem.* **2001**, *66*, 6440-6452.

(134) Krska, S. W.; Seyferth, D. *J. Am. Chem. Soc.* **1998**, *120*, 3604-3612.

(135) Loup, C.; Zanta, M. A.; Caminade, A. M.; Majoral, J. P.; Meunier, B. *Chem. Eur. J.* **1999**, *5*, 3644-3650.

(136) Lee, J. J.; Ford, W. T.; Moore, J. A.; Li, Y. F. *Macromolecules* **1994**, *27*, 4632-4634.

(137) Pan, Y. J.; Ford, W. T. *J. Org. Chem.* **1999**, *64*, 8588-8593.

(138) Pan, Y. J.; Ford, W. T. *Macromolecules* **2000**, *33*, 3731-3738.

(139) Caminati, G.; Turro, N. J.; Tomalia, D. A. *J. Am. Chem. Soc.* **1990**, *112*, 8515-8522.

(140) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Johnson, A. L.; Behera, R. K. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1176-1178.

(141) Gopidas, K. R.; Leheny, A. R.; Caminati, G.; Turro, N. J.; Tomalia, D. A. *J. Am. Chem. Soc.* **1991**, *113*, 7335-7342.

(142) Ottaviani, M. F.; Cossu, E.; Turro, N. J.; Tomalia, D. A. *J. Am. Chem. Soc.* **1995**, *117*, 4387-4398.

(143) Stevelmans, S.; vanHest, J. C. M.; Jansen, J.; vanBoxtel, D.; vandenBerg, E.; Meijer, E. W. *J. Am. Chem. Soc.* **1996**, *118*, 7398-7399.

(144) Zhang, H.; Dubin, P. L.; Kaplan, J.; Moorefield, C. N.; Newkome, G. R. *J. Phys. Chem. B* **1997**, *101*, 3494-3497.

(145) Ashton, P. R.; Boyd, S. E.; Brown, C. L.; Nepogodiev, S. A.; Meijer, E. W.; Peerlings, H. W. I.; Stoddart, J. F. *Chem. Eur. J.* **1997**, *3*, 974-984.

(146) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665-1688.

(147) Hartley, G. S. *Aqueous solutions of paraffin-chain salts*; 1st ed.; Herman: Paris, 1936.

(148) Naylor, A. M.; Goddard, W. A.; Kiefer, G. E.; Tomalia, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 2339-2341.

(149) Morenobondi, M. C.; Orellana, G.; Turro, N. J.; Tomalia, D. A. *Macromolecules* **1990**, *23*, 910-912.

(150) Watkins, D. M.; SayedSweet, Y.; Klimash, J. W.; Turro, N. J.; Tomalia, D. A. *Langmuir* **1997**, *13*, 3136-3141.

(151) Moroi, Y. *Micelles, Theoretical and Applied Aspects*; 1st ed.; Plenum: New York, 1992.

(152) Vinogradov, S. A.; Wilson, D. F. *Chem. Eur. J.* **2000**, *6*, 2456-2461.

Chapter 2

Synthetic Approaches to Amphiphilic Iron-Sulfur Core Dendrimers using a Disulfide as a Focal Protecting Group

2.1. Introduction

The disulfide linkage is widespread in many artificial and natural products. It plays an important role in organic synthesis and biological systems. In organic synthesis the disulfide linkage is often useful as a protecting group for thiols. In biochemical process thiol-disulfide interchange reactions take place at room temperature in aqueous solution, including cleavage and formation of strong, covalent disulfide linkage.[1-7]

There are several approaches to protect / deprotect the thiol group. Examples include the thioether[8], thioacetate[9], and disulfide linkage[8-22]. The disulfide linkage is ubiquitous in biological systems and a useful protecting group of the thiols.[1-7] Moreover, because these molecules mimic naturally-occurring proteins, the disulfide linkage was selected naturally as a protecting group of the thiols like in biological systems. In small molecules and aliphatic thiols, the disulfide linkage has been used as a good protecting group of thiols. Also, there are many reducing agents to reduce the disulfide linkage to the corresponding thiol group.[6,10-24]

Basu & coworkers previously employed symmetrical disulfide dendrons to prepare metal-cluster core dendrimers. The thiol group or sulfydryl group, SH, was protected by oxidizing the thiols to the disulfides. The thiols were regenerated using a reducing agent, sodium borohydride. Using this method, dendrimers were synthesized successfully.[25-27]

In the synthesis of amphiphilic dendrons with more than one type of peripheral

group (carboxylate or ammonium) a "modular synthesis approach" is useful. This approach involves the synthesis of dendrons with methoxy methyl (MOM)-protected phenol groups at the periphery. The modular synthesis approach is an extension of the double-stage convergent approach first employed by Fréchet and coworkers.[28] Dendrons with MOM groups can be easily converted under acidic conditions to the phenolic dendrons. The advantages of this approach are rapid synthesis of various functionalized dendrons and excellent yield within short times. This modular synthesis approach will be employed in this study.

Iron-sulfur core molecules can be synthesized through a ligand exchange reaction first described by Holm and coworkers.[29-42] In this reaction a coordinated alkylthiolate (SR) in form $[Fe_4S_4(SR)_4][N(CH_3)_4]_2$ exchanges with added aryl thiols (R′SH). Gorman group members have previously successfully synthesized flexible and rigid iron-sulfur core dendrimers using this method.[43-54] However, these dendrimers were not amphiphilic or water-soluble but hydrophobic. Hydrophobic thiol dendrons are generally found to be relatively stable to oxidation. In this regard, they behave like cysteine, which is not oxidized to cystine with iodine very rapidly. On the other hand, amphiphilic or water-soluble thiol dendrons are rapidly oxidized by iodine.[55,56] Thus, new synthetic approaches to prepare amphiphilic iron-sulfur core dendrimers which can be used as protein models are required.

Throughout this chapter, all synthetic efforts for amphiphilic iron-sulfur core dendrimers (cationic and anionic) use a modular synthesis approach and the

disulfide linkage as a focal group and protecting group. These efforts include the synthesis of amphiphilic thiol dendrons using both symmetrical and unsymmetrical disulfides.

2.2. Results and Discussion

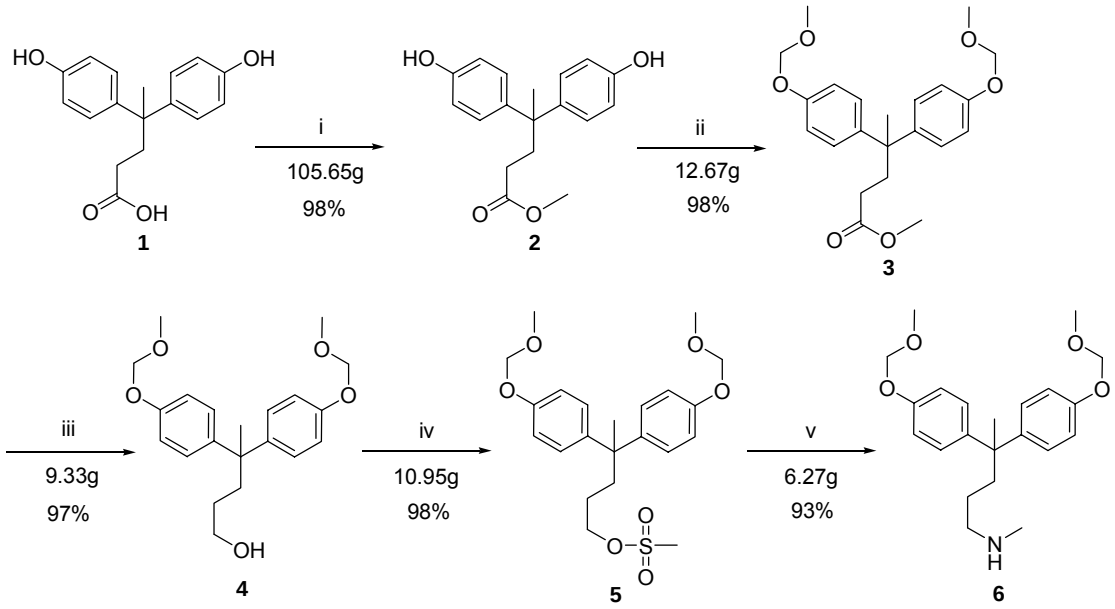
2.2.1. Synthesis from Symmetrical Disulfide

Two classes of amphiphilic iron-sulfur core dendrimers, cationic (ammonium) and anionic (carboxylate)), were synthesized using the symmetrical disulfide linkage as a focal group. With various reducing agents, the cleavage of the disulfide linkage was attempted and ligand exchange reactions were tried with the thiols generated.

2.2.1.1. Cationic Dendron containing Symmetrical Disulfide

The flexible dendritic unit, 4,4′-bis(4′-hydroxyphenylvaleric) acid (**1**), was used to prepare dendrons following previous studies in the Gorman group.[43,44,57] Dendrons were synthesized with slight modifications in the synthetic procedure.

Scheme 2.1.[a]



[a]Reagents and conditions: i) $SOCl_2$, MeOH; ii) MOMCl, DMAP, Hunig's base, CH_2Cl_2; iii) $LiAlH_4$, THF; iv) MsCl, DMAP, TEA, CH_2Cl_2; v) CH_3NH_2, THF.

By esterification with thionyl chloride and methanol, the commercially available 4,4′-bis(4′-hydroxyphenylvaleric) acid (**1**) was converted to the methyl ester, methyl 4,4-bis(4′-hydroxyphenyl) valerate (**2**), in high yield (Scheme 2.1). Before the reduction of the methyl ester, the two phenolic groups of **2** were protected with methoxymethyl (MOM) groups to give G1-Ester-MOM (**3**). This reaction is a starting point for the synthesis of the MOM-protected dendrons called the "modular synthesis approach". G1-Ester-MOM (**3**) was reduced to G1-OH-MOM (**4**) with lithium aluminum hydride (LAH) in high yield. G1-OH-MOM (**4**) was then activated to G1-OMs-MOM (**5**) in excellent yield using mesylate

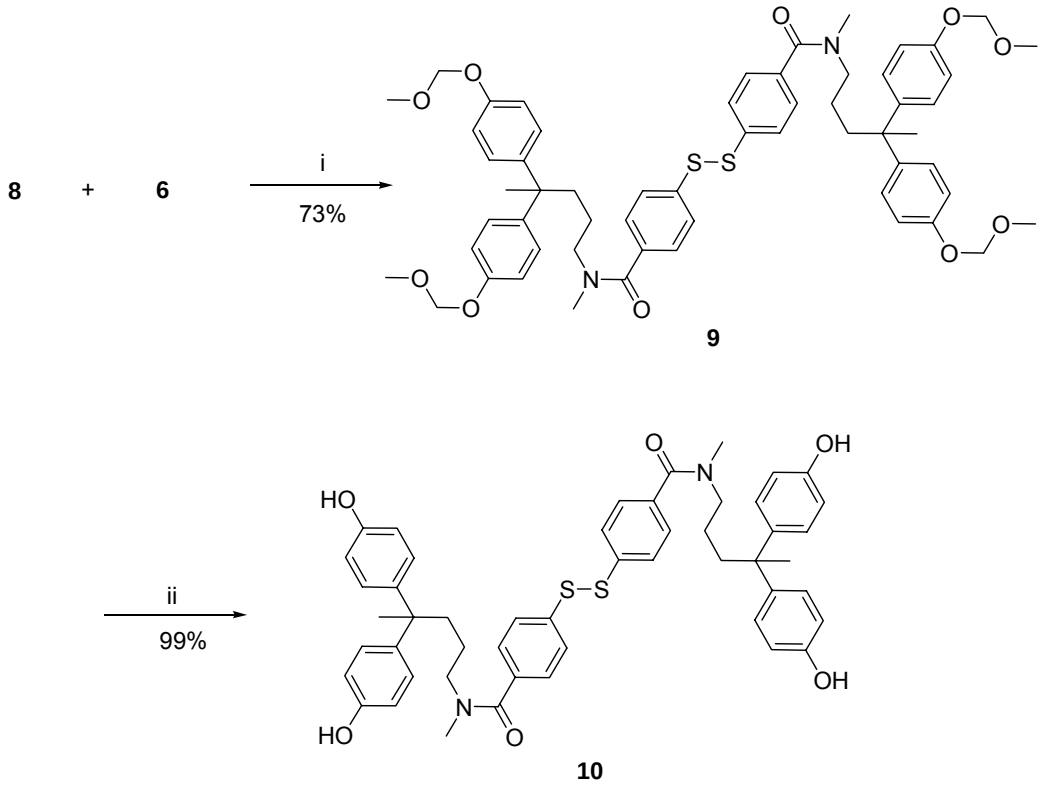
activating / leaving group for nucleophilic substitution reaction. This approach requires at least two protected functional groups and one reactive functional group in a dendritic unit like **5**. G1-OMs-MOM (**5**) was converted to G1-MA-MOM (**6**) by refluxing methylamine (40 wt% solution in H_2O) in THF in sealed tube. The reaction mixture was washed with H_2O. The resulting product was pure enough to be carried to the next step without further purification.

Scheme 2.2.[a]

[a]Reagents and conditions: i) I_2, EtOH.

The symmetrical disulfide, 4,4′-dithiobenzoic acid (**8**), focal group was prepared by the oxidation of 4-mercatpobenzoic acid (**7**) with iodine (Scheme 2.2).[25-27,58] Two dendritic fragments, G1-MA-MOM (**6**) was then coupled with the symmetrical disulfide (**8**) to form the amide, G1-Sym-Disulfide-MOM (**9**), in acceptable yield via a dicyclohexylcarbodiimide (DCC) mediated coupling reaction (Scheme 2.3). The DCC-mediated coupling reaction could be set up simply and gave the better yield than an acid chloride-based reaction used previously to prepare analogous amides.

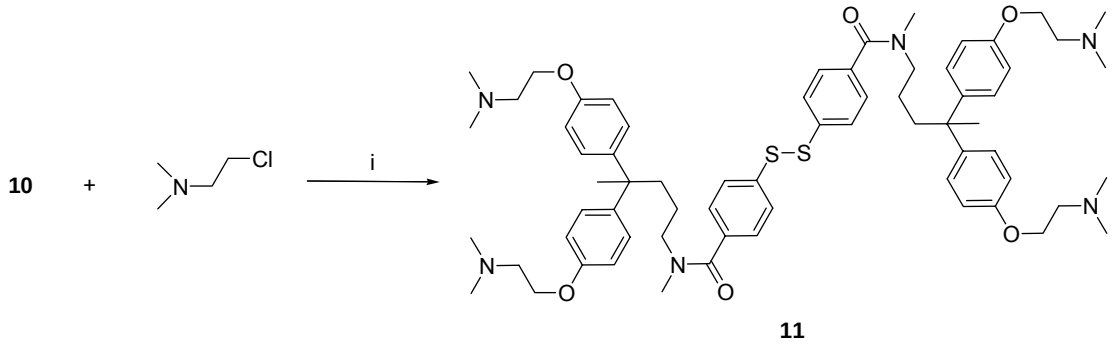
Scheme 2.3.[a]



[a]Reagents and conditions: i) DCC, DMAP, CH_2Cl_2; ii) MeOH, HCl.

The MOM group on G1-Sym-Disulfide-MOM (**9**) could be removed easily by refluxing methanol solution with a small amount of concentrated hydrochloric acid to give the phenolic dendron, G1-Sym-Disulfide-OH (**10**). The deprotection reaction of MOM should be performed in methanol because THF can cause polymerization reactions between the deprotected phenolic dendrons and THF. The solubility of G1-Sym-Disulfide-MOM (**9**) in methanol solvent is also satisfactory for the reaction.

Scheme 2.4.[a]



[a]Reagents and conditions: i) Base, solvent, see Table 2.1.

The concept of a modular synthesis approach is that the MOM group can be easily deprotected with acids and then the phenolic dendrons deprotected can be coupled to whatever peripheral unit is required. Two peripheral units were selected, ammonium[59-63] as a cationic periphery and carboxylate[64-76] as an anionic periphery. The hydrophobic dendrons prepared by the Gorman group consist of non-polar interiors and non-polar peripheries. In contrast, amphiphilic dendrons are composed of hydrophobic interiors and hydrophilic peripheries Charged and/or polar end groups such as ammonium and carboxylate can impart polarity to periphery units of dendrons. The amphiphilic dendrons using charged and/or polar end groups and hydrophobic interiors can be regarded as a "unimolecular micelle".[65,77,78] The properties from a unimolecular micelle such as a change of hydrodynamic radius in different solvents can be measured by electrochemistry experiments.

Table 2.1. Several attempts to prepare G1-Sym-Disulfide-MA (11) by reaction of G1-Sym-Disulfide-OH (10) with N,N-dimethylaminoethyl chloride·HCl.

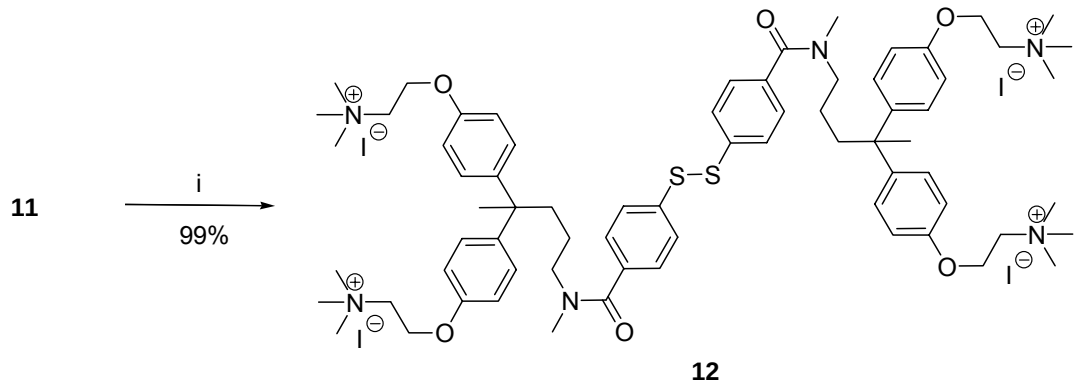
Reagents	Solvents	Temperature (°C)	Time (h)	Yields (%)
K_2CO_3/ 18-crown-6	acetone	reflux	24	x
K_2CO_3/ 18-crown-6	acetone/H_2O	reflux	34	x
K_2CO_3	acetone/DMF	60	22	x
K_2CO_3	DMF	70-80	24	x
KOH	ethanol/H_2O	85-90	3	30
NaH	DMF	60-70	12	91

x: no product as determined by ^{1}H NMR.

In the preparation of an amphiphilic cationic dendron, a precursor was required. This dendron could be prepared by the nucleophilic substitution reaction of the phenolic dendron, G1-Sym-Disulfide-OH (**10**), with the precursor of periphery unit, amine halide (Scheme 2.4). Several reactions used in the literature were attempted (Table 2.1). G1-Sym-Disulfide-OH (**10**) reacted with N,N-dimethylaminoethyl chloride·HCl by refluxing acetone suspension including potassium carbonate and a catalytic amount of 18-crown-6. However, ^{1}H NMR spectrum showed only starting material indicating that the desired product was not produced. In the next approach, co-solvents of acetone/water, acetone/DMF, and DMF solvent were used according to literature studies.[79-81] Nevertheless, ^{1}H NMR spectra showed the same result, giving the starting material.

As another possible route, the base catalyst was changed from potassium carbonate to stronger potassium hydroxide[82] and this approach gave the desired product, but with unacceptably low yield (30%).

Scheme 2.5.[a]



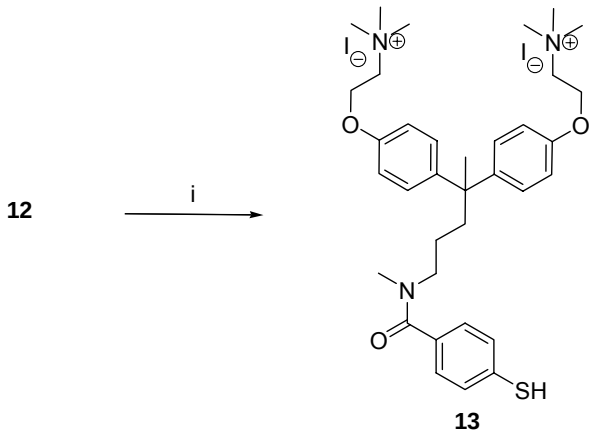
[a]Reagents and conditions: i) CH_3I, CH_2Cl_2.

To improve the yield, a strong base, NaH, was chosen.[83] Under these conditions, G1-Sym-Disulfide-MA (**11)** was prepared successfully with excellent yield (91%) (Scheme 2.4). G1-Sym-Disulfide-MA (**11**) was then methylated with methyl iodide[59,61,63,84,85] to give amphiphilic cationic dendron containing iodide counter ions. The desired product, G1-Sym-Disulfide-Ammonium (**12**), was prepared with excellent yield (99%) (Scheme 2.5).

2.2.1.2. Attempts to Reduce the Symmetrical Disulfide and Form the Cationic Dendrimer

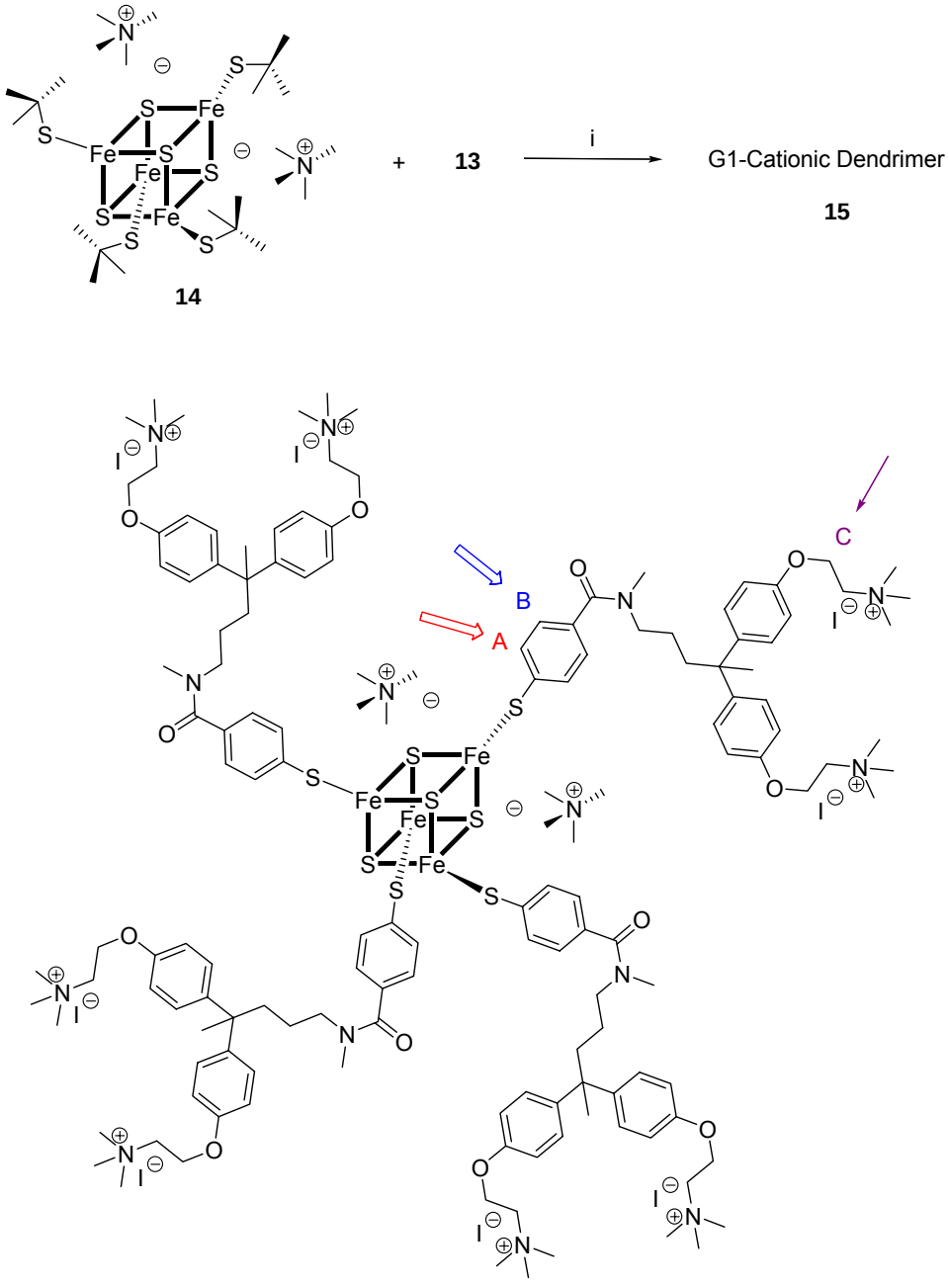
To be used as a dendritic ligand, the dendron containing the symmetrical disulfide linkage needed to be reduced to give the corresponding thiol (Scheme 2.6). The dendritic thiol ligands generated could then participate in the ligand exchange reaction with iron-sulfur core, $[Fe_4S_4(SR)_4][N(CH_3)_4]_2$ (**14**), generating the amphiphilic iron-sulfur core dendrimer (Scheme 2.7). The reaction was performed in the dry box because three of them, the thiol generated, the iron-sulfur core, and the dendrimer, are oxygen-sensitive.

Scheme 2.6.[a]



[a]Reagents and conditions: i) reducing agents.

Scheme 2.7.[a]



[a]Reagents and conditions: i) DMF.

Table 2.2. Various attempts to reduce the disulfide, G1-Sym-Disulfide-Ammonium (12), and form the Cationic Dendrimer (15).

Reagents	Solvents	Temperature (°C)	Time (h)	Results[e]
$NaBH_4$	EtOH	r.t.	12	x
$NaBH_4$	MeOH	r.t.	20	p
$NaBH_4$	MeOH	65	2	p
$NaBH_4$	MeOH	65	13	p
$NaBH_4$	H_2O	r.t.	13	x
$NaBH_4$	H_2O	65	12	x
Zn/HCl	H_2O	r.t.	12	x
Zn/acetic acid	acetic acid	reflux	13	p
DTT[a]	DMF	r.t.	12	p
ME[b]	DMF	100	24	p
TCEP·HCl[c]	H_2O	r.t.	16	p
H_3PO_2/DPDS[d]	MeOH + EtOH	60	12	x
NaHSe	MeOH + EtOH	r.t.	5	p
NaHSe	MeOH + EtOH	r.t.	16	p

[a]DTT: dithiothreitol; [b]ME: 2-mercaptoethanol; [c]DPDS: diphenyl diselenide; [d]TCEP·HCl: tri-(2-carboxyethyl)phosphine hydrochloride; e: the result was determined by ^{1}H NMR after ligand exchange reaction of iron-sulfur core with the thiol generated via reduction; x: no product; p: partially substituted dendrimer.

Many attempts were carried out with various reducing agents[6,10-27] to cleave the symmetrical disulfide linkage of the amphiphilic cationic dendron, G1-Sym-Disulfide-Ammonium (**12**) (Scheme 2.6 and Table 2.2). $NaBH_4$ was investigated first as a reducing agent as Basu and coworkers successfully employed this reagent to cleave the symmetrical disulfide dendrons, regenerating the corresponding thiols

successfully.[25-27] Three different solvents were used ethanol, methanol, and water. The Schlenk flasks containing the corresponding products put into the dry box. In the dry box, products were dissolved in DMF dried and degassed. Iron-sulfur cores, $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (**14**), were also dissolved in DMF in preparation for the ligand exchange reaction. The ratio of the dendritic thiol ligand to iron-sulfur core was 6 to 1, the optimum condition used in literature.[32,33,40] Upon addition, the red-wine color developed rapidly, indicating that the reaction was proceeding.[32,33,40]

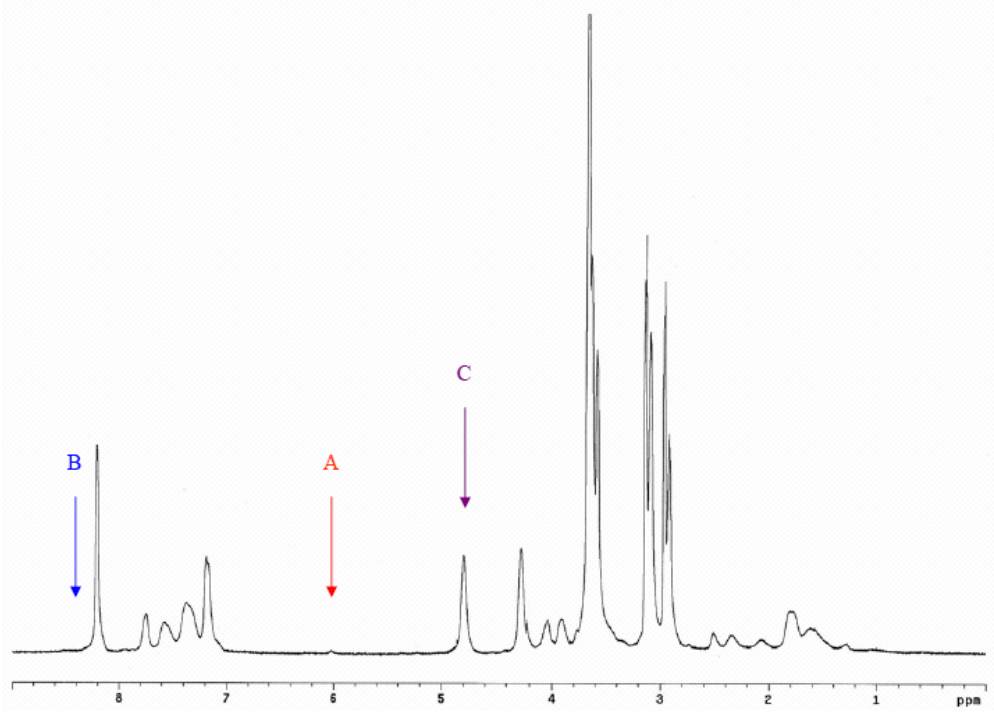


Figure 2.1. ^{1}H NMR of amphiphilic cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**15**), prepared using $NaBH_4$ / methanol / 65 $^\circ$C to reduce **12** followed by ligand exchange (Scheme 2.6 and 2.7) (solvent : DMF-d_7).

Each reaction to form G1-cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**15**), was characterized by ^1H NMR. The iron-sulfur cores used in these reactions are paramagnetic and thus, the thiolate aromatic ring protons (**A** and **B**) adjacent to the dendrimer core undergo a broadening and a change in chemical shift in the ^1H NMR spectrum.

The first attempt to form dendrimer (**15**) involved reduction of disulfide dendron (**12**) to thiol dendron (**13**) using $NaBH_4$/methanol followed by immediate reaction with cluster (**14**). The ^1H NMR spectrum exhibited only a partially substituted dendrimer in the reaction (Figure 2.1). The relative integration of two characteristic peaks, the aromatic protons in ortho and meta position (**A** and **B**), to signal C indicated a ratio of 2.1 : 16 instead of the expected 8 : 16, respectively.

The second and third attempt to form dendrimer (**15**) was similar but used $NaBH_4$/ethanol and $NaBH_4$/water. The ^1H NMR spectra of these reactions did not show the two characteristic peaks at all, indicating the ligand exchange reaction did not occur. These three results might be explained by the incomplete formation of the thiols because the disulfide linkage may be shielded by the relatively large dendritic unit, resulting in creating steric hindrance obstructing the generation of the thiols via the attack of reducing agent.[86] Also, the influence of oxygen may not be negligible because the thiols generated can be re-oxidized to the disulfides in the presence of oxygen.[56] To block the inflow of oxygen, all of them including solvent, reactant and glassware were degassed or dried in vacuum line before use and subsequently

maintained under N_2 purge. However, the reaction mixture in the disulfide cleavage might be exposed to oxygen while adding of $NaBH_4$ and HCl methanol solution during reaction. Specifically, a rotary evaporator was used to remove the solvent after reaction. It was a moment that the reaction mixture can be contacted with air. Thus, the exposure to oxygen might lead the formation of the disulfide.

Then, how could Basu and coworkers[25-27] mentioned earlier reduce successfully the symmetrical disulfide to the corresponding thiol? It may be explained by a hydrophobic dendritic unit used in this study. It is known that hydrophobic thiols are relatively stable like cysteine in comparison with amphiphilic or water-soluble thiols.[55,56] Also, disulfide mobility can be caused. Basu's dendritic unit was relatively more flexible than amphiphilic dendritic unit. Thus, because the disulfide focal group can move easily under steric hindrance from bulky dendritic unit, it can be easily reduced to the thiols from attack of reducing agent.

As another possible route, Zn / acid system was considered. Treatment of bis(4-trimethylammoniumphenyl)disulfide diiodide with Zn / aq. HCl reduced this polar disulfide efficiently. The product was collected with precipitation by HPF_6 in excellent yield.[32] Zn / acetic acid system also reduced dithiosalicylic acid to thiosalicylic acid in acceptable yield.[87] Thus, this reagent has precedent for reduction of aromatic thiols similar to G1-Sym-Disulfide-Ammonium (**12**). The ligand exchange reaction in the dry box was performed identically to the previous reactions. The red-wine color developed rapidly as observed previously.

Unfortunately, when the dendrimer was characterized by ^{1}H NMR, the same results were obtained, indicating a partially substituted dendrimer in the reaction. The relative integration of two characteristic peaks (**A** and **B**) to signal C indicated a ratio of 2.3 : 16 instead of the expected 8 : 16, respectively. As the chemical functionality on the bis(4-trimethylammoniumphenyl)disulfide diiodide is similar to that of G1-Sym-Disulfide-Ammonium (12), the failure of this method was puzzling. It is speculated that perhaps the steric hindrance due to the two dendrons around the disulfide group were to blame.

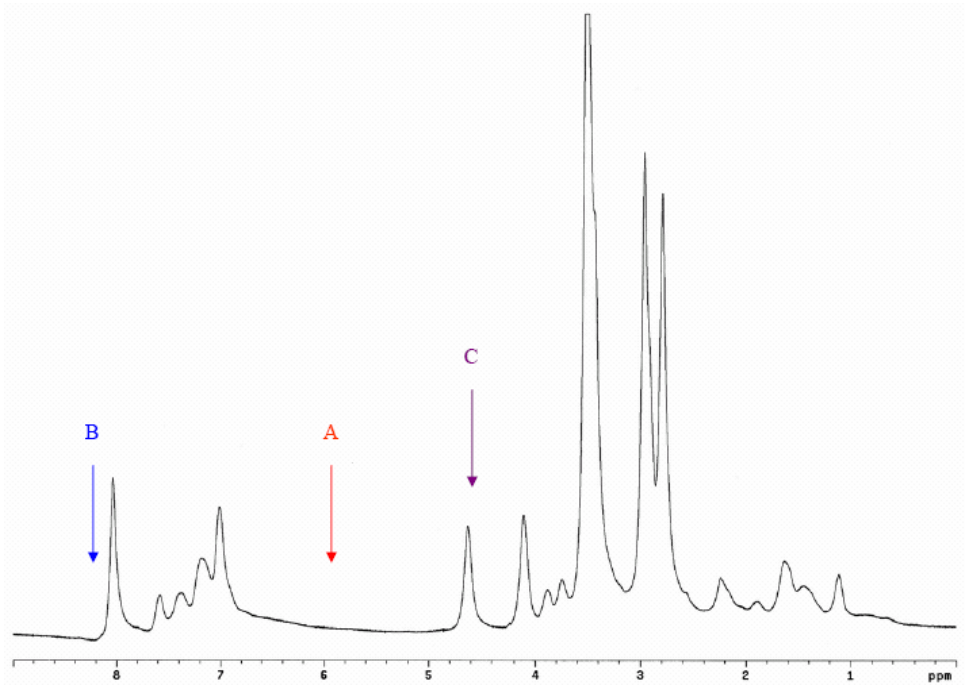


Figure 2.2. ^{1}H NMR of amphiphilic cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**15**), prepared using ME / DMF / 100 °C to reduce **12** followed by ligand exchange (Scheme 2.6 and 2.7) (solvent : DMF-d_7).

Other possible reducing agents including DTT[18,88], ME[89], and TCEP·HCl[19-21,90] were tried. These reducing agents are used in biochemistry, especially in reduction of cystine groups in proteins and in maintaining thiol groups in reduced states.[91-94] The ligand exchange reaction and the red-wine color phenomenon were identical to the previous reaction. However, in each case, the ^{1}H NMR spectrum of the reaction product after reduction and ligand exchange showed partially substituted dendrimers (Figure 2.2). The relative integration of two characteristic peaks (**A** and **B**) to Signal C indicated a ratio of 1.8 : 16 instead of the expected 8: 16, respectively. This result might be also rationalized by steric hindrance and oxygen like in previous reaction.

As another attempt, hypophosphorous acid / the diselenides system was attempted. The disulfides do not react with hypophosphorous acid alone, but the reduction to the corresponding thiols is achieved with a small amount of the diselenides.[15] The ligand exchange reaction and the red-wine color phenomenon were also identical to the previous reaction. However, the ^{1}H NMR spectrum after the reduction and ligand exchange sequence was carried out did not show two characteristic peaks at all, indicating that ligand exchange reaction was not achieved.

Finally, sodium hydrongen selenide was tried. It is an effective reagent for reduction of alkyl, aryl, and araalky disulfides to thiols under mild conditions.[14] Sodium hydrogen selenide was prepared according to the literature right before use.[13,14] After reduction and ligand exchange reaction, the development of red-wine color and ^{1}H NMR spectra were also identical to the previous reaction, showing

only partially substituted dendrimers (Figure 2.3). The relative integration of two characteristic peaks to signal C indicated a ratio of 2.0 : 16 instead of the expected 8 : 16, respectively.

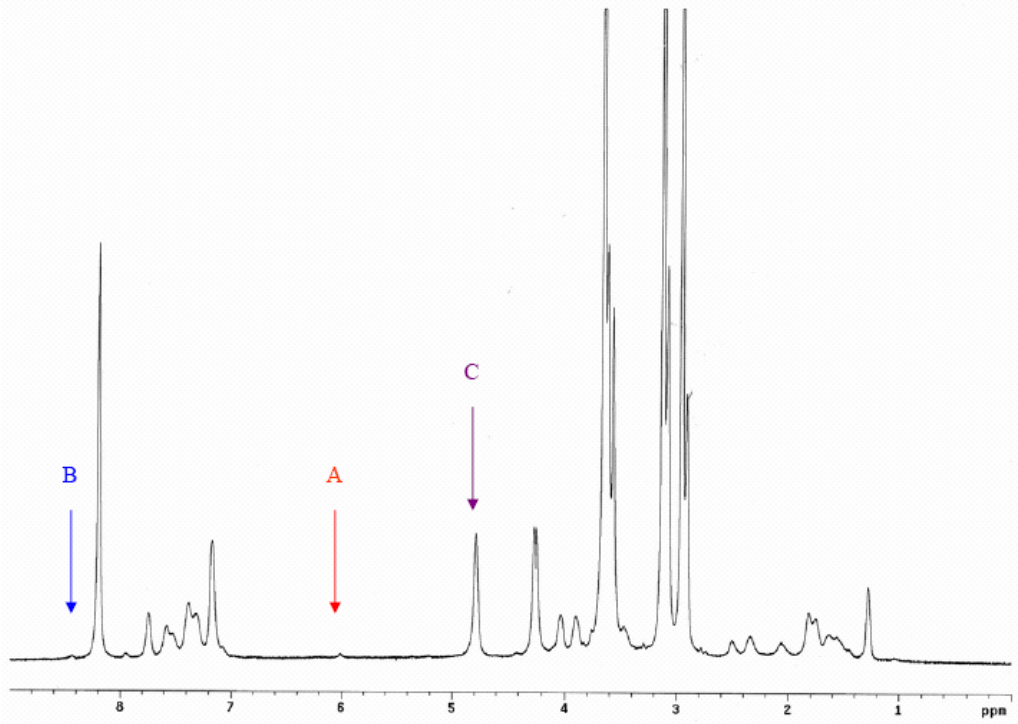


Figure 2.3. ^{1}H NMR of amphiphilic cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**15**), prepared using NaHSe / methanol – ethanol / 16hrs to reduce **12** followed by ligand exchange (Scheme 2.6 and 2.7) (solvent : DMF-d_7).

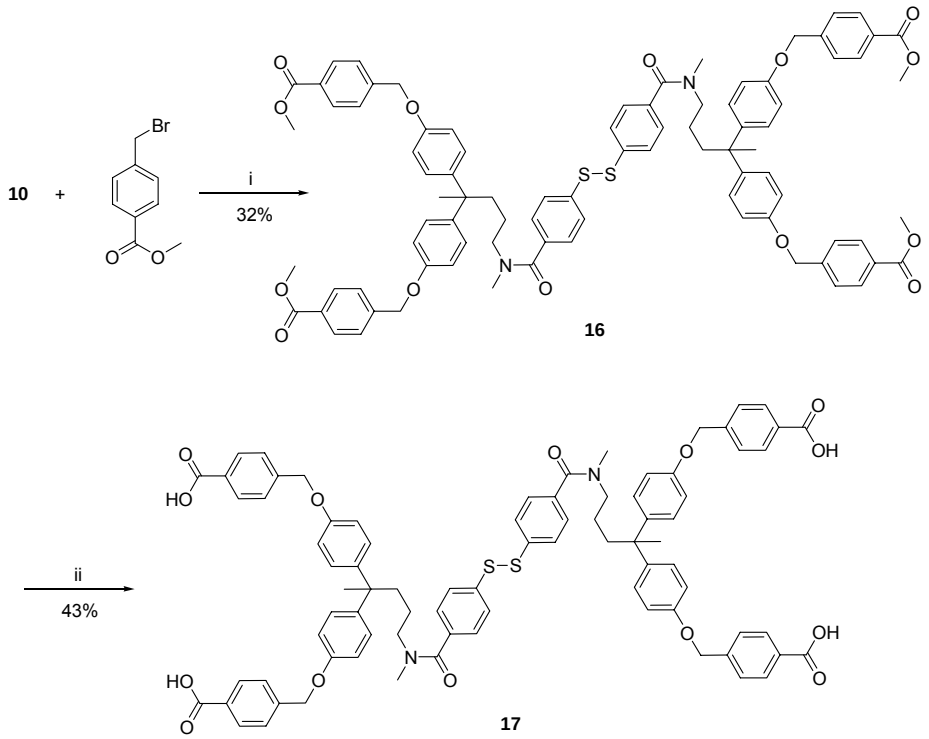
Thus, although various agents were used to reduce the disulfide to the corresponding thiol, all results showed that Fe_4S_4 clusters were partially and incompletely substituted in the ligand exchange reaction. It was suspected that only

some of the symmetrical disulfide linkage was reduced while some remained as the disulfide linkage. As stated above, this result is surprising. It might be rationalized that the shielding of disulfide linkage by the relatively large dendritic unit created a steric hindrance obstructing the generation of the thiols via the attack of reducing agents. A small amount of the reduced thiols participated in the ligand exchange reaction and the red-wine color phenomenon was evidence of the reaction. Also, the influence of oxygen may not be negligible. Oxygen should be excluded to block the re-oxidation of thiols generated to disulfides. All chemicals and glassware used were dried and degassed in vacuum line before use and subsequently maintained under positive pressure of N_2. However, the reaction did not proceed to completion successfully due to insufficient amount of thiol present.

2.2.1.3. Anionic Dendron containing Symmetrical Disulfide

Amphiphilic and/or water-soluble anionic dendrimers in many cases have been prepared with carboxylate as a peripheral unit.[64-76] The introduction of hydrophilic polar end groups such as carboxylate imparts water-solubility. The solubility is sensitive to pH. High pH values are required to deprotonate the carboxylic acid because carboxylate salts impart the water-solubility to them. If the pH value in an aqueous solution is acidic, the carboxylate is protonated and dendrimers generally will precipitate from water.

Scheme 2.8.[a]



[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone; ii) KOH, MeOH, THF, H_2O.

In the preparation of the amphiphilic anionic dendron, a precursor was also required. This dendron, G1-Sym-Disulfide-$COOCH_3$ (**16**) could be prepared by the nucleophilic substitution reaction of the phenolic dendron prepared by the modular synthesis approach, G1-Sym-Disulfide-OH (**10**), with the precursor of the periphery unit, 4-(bromomethyl) benzoate (Scheme 2.8).

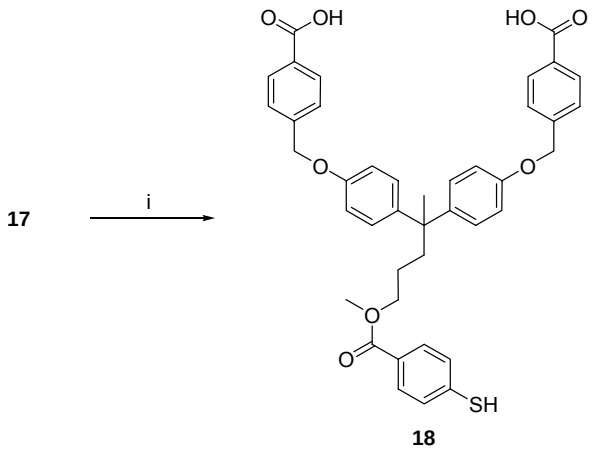
Subsequently, the methyl group in the precursor, G1-Sym-Disulfide-$COOCH_3$ (**16**), was removed with base (potassium hydroxide) in a mixture of methanol / THF to give G1-Sym-Disulfide-COOH (**17**) including polar end groups. However, in this

trial the ^{1}H NMR spectrum was not clean because THF initiated a polymerization reaction. In spite of containing impurities, the product was used for the next step.

2.2.1.4. Anionic Dendrimer from Symmetrical Disulfide

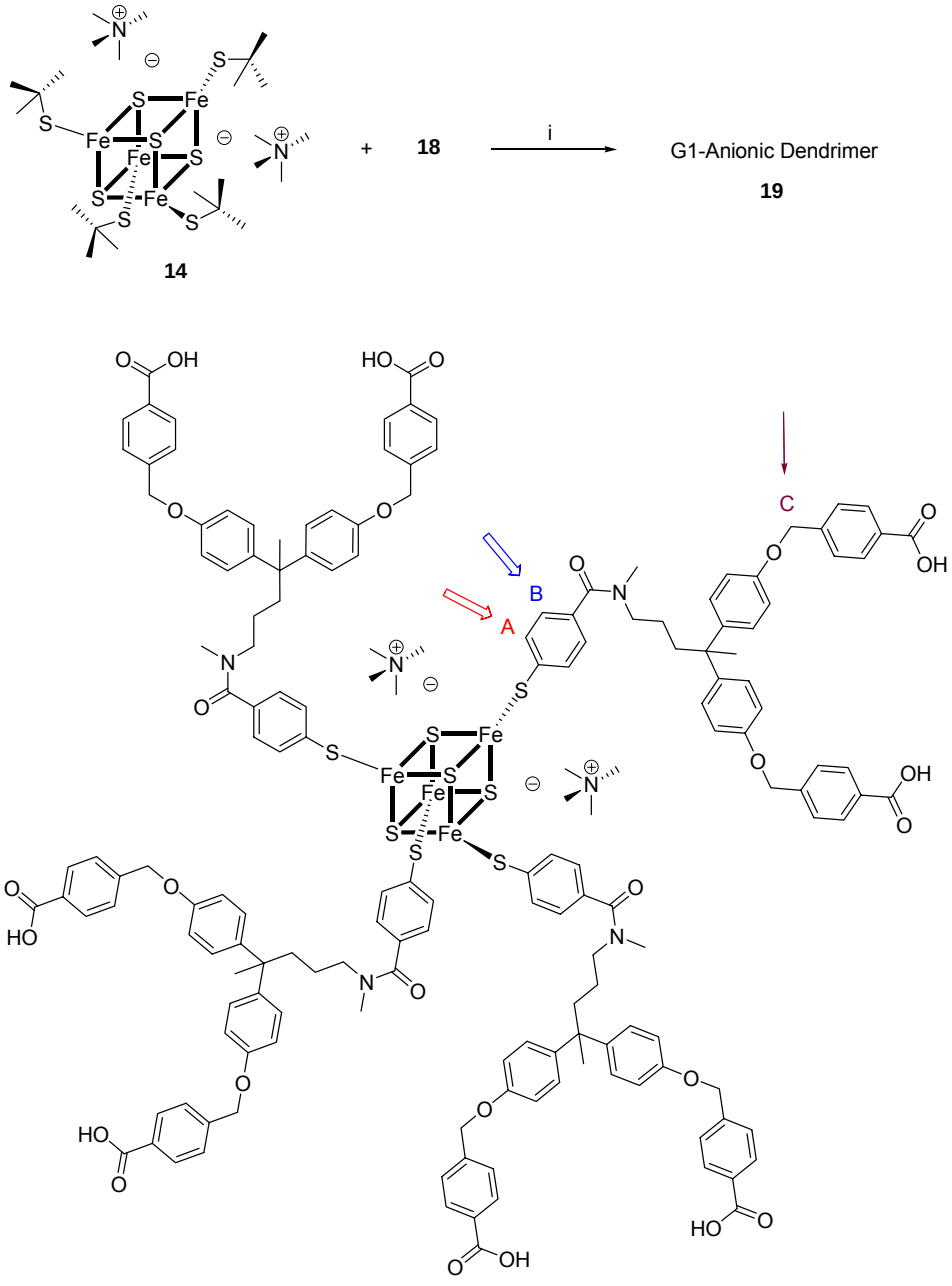
To be used as a dendritic thiol ligand, the anionic dendron containing the symmetrical disulfide linkage should also be reduced. A number of attempts were carried out with various reducing agents[6,10-27] previously explored for the cleavage of the symmetrical disulfide linkage in cationic dendron, G1-Sym-Disulfide-Ammonium (**12**) (Table 2.2). However, upon reaction of the disulfide (**17**) with these reagents followed by ligand exchange all results showed that dendrimers were partially and incompletely substituted. Thus, use of another reducing agent, tri-n-butyl phosphine,[95] was attempted (Scheme 2.9).

Scheme 2.9.[a]



[a]Reagents and conditions: i) tri-butylphosphine, acetone.

Scheme 2.10.[a]



[a]Reagents and conditions: i) DMF.

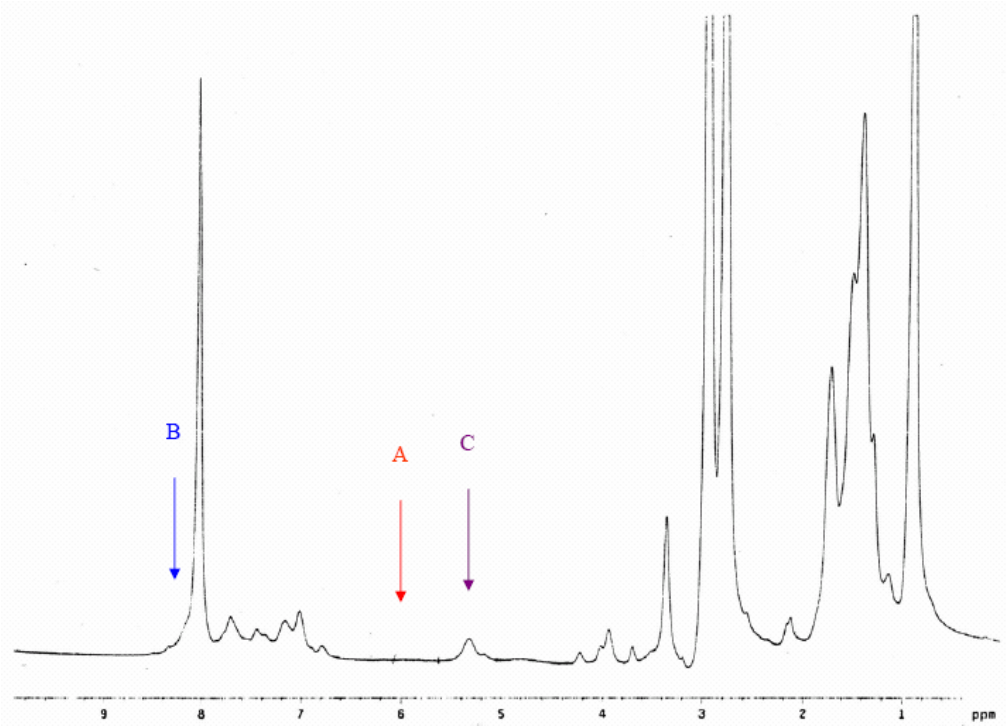


Figure 2.4. ^{1}H NMR of amphiphilic anionic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (**19**), prepared using tri-n-butylphosphine to reduce **17** followed by ligand exchange (Scheme 2.9 and 2.10) (solvent : DMF-d_7).

In the dry box, ligand exchange reaction of dendritic thiol ligand, G1-SH-COOH (**18**), with the iron-sulfur core, $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (**14**), was carried out (Scheme 2.10). The reaction and the red-wine color phenomenon were identical to in cationic dendrimer. The ^{1}H NMR spectrum of amphiphilic anionic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (**19**), showed that the dendrimer was incompletely substituted like in cationic dendrimer, exhibiting the relative integration of two characteristic peaks (**A** and **B**) to signal C indicated a ratio of

1.1 : 16 instead of the expected 8 : 16, respectively (Figure 2.4). This result might also be attributed on low thiol concentration due to steric hindrance obstructing the attack of reducing agent as postulated for the cationic dendrimer. Thus, unfortunately, first generation dendron disulfides with both tetra-alkyl ammonium and carboxylic acid peripheral groups could not successfully be reduced to thiols and employed in ligand exchange reactions.

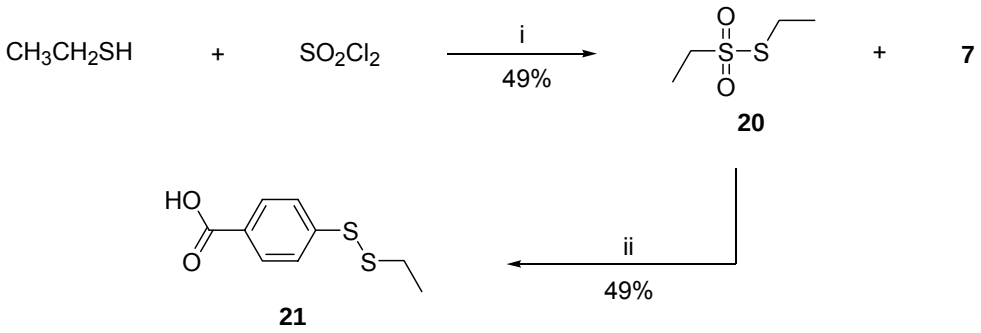
2.2.2. Synthesis from Unsymmetrical Disulfide

The use of the symmetrical disulfide linkage as a focal and protecting group was investigated but it could not be achieved because shielding of the disulfide linkage by bulky dendritic unit might obstruct the generation of the thiols. As another possible approach, the unsymmetrical disulfide linkage was tried to protect aromatic thiols via aliphatic thiols. Because the unsymmetrical disulfide is relatively less stable than symmetrical disulfide, the generation of the thiols by reducing agents is facile.[96-100] Also, it was hoped that any steric hindrance in the symmetrical disulfide that possibly obstructed the generation of the thiol would be avoided. Thus, the use of the unsymmetrical disulfide potentially has advantages relative to the use of the symmetrical disulfide.

2.2.2.1. Cationic Dendron containing Unsymmetrical Disulfide

The synthetic approach for the unsymmetrical disulfide was started from the preparation of the alkylthiosulfonate[97,101,102], ethyl ethanethiosulfonate (**20**) (Scheme 2.11). The unsymmetrical disulfide, p-carboxyphenyl ethyl disulfide (**21),** was prepared by the reaction of aromatic thiol (**7**) with alkylthiosulfonate (**20**).[96,97]

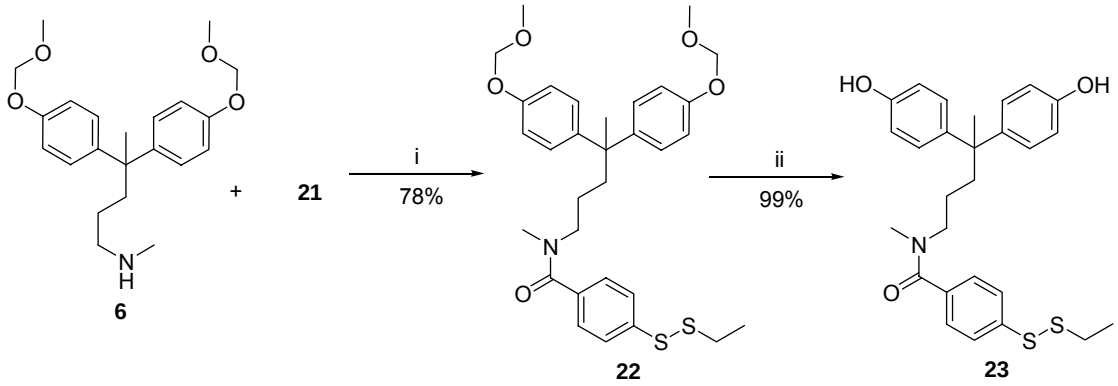
Scheme 2.11.[a]



[a]Reagents and conditions: i) CH_3COOH, H_2O; ii) EtOH, H_2O.

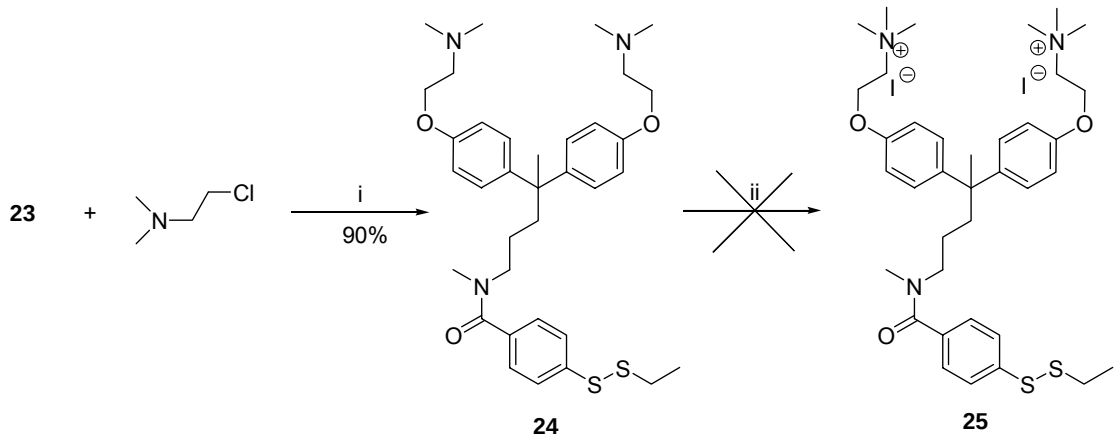
In the next step, the dendritic fragment, G1-MA-MOM (**6**), was coupled with the unsymmetrical disulfide linkage (**21**) to form the amide, G1-Unsym-Disulfide-MOM (**22**) in acceptable yield via DCC-mediated coupling reaction. The MOM groups on G1-Unsym-Disulfide-MOM (**22**) could be removed easily by refluxing the methanol solution with concentrated hydrochloric acid.

Scheme 2.12.[a]



[a]Reagents and conditions: i) DCC, DMAP, CH_2Cl_2; ii) HCl, MeOH.

Scheme 2.13.[a]



[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone; ii) CH_3I, CH_2Cl_2.

To introduce tetra-alkyl ammonium[59-63,84] as a peripheral unit to the phenolic dendron generated by a modular synthesis approach, G1-Unsym-Disulfide-MA (**24**), was prepared by nucleophilic substitution reaction of the phenolic dendron, G1-Unsym-Disulfide-OH (**23**), with N,N′-dimethylaminoethyl chloride·HCl (Scheme 2.13). The molecule G1-Unsym-Disulfide-MA (**24**) was then methylated with methyl iodides[59,61-63,84,85] in an attempt to prepare the amphiphilic cationic dendrons containing iodide counter ions, G1-Unsym-Disulfide-Ammonium (**25**). However, the ^{1}H NMR spectrum showed that the product generated was not G1-Unsym-Disulfide-Ammonium (**25**), but G1-Sym-Disulfide-Ammonium (**12**). Two characteristic peaks from the ethyl group of the unsymmetrical disulfide, CH_3 and CH_2, disappeared on ^{1}H NMR spectrum, indicating the product was G1-Sym-Disulfide-Ammonium (**12**). This result may be explained by disproportionation of the unsymmetrical disulfide to give the two corresponding symmetrical disulfides.[96-100] The unsymmetrical disulfide is less stable than the symmetrical disulfide and thus, the unsymmetrical disulfide may show a tendency toward disproportionation leading to the generation of the symmetrical disulfide. Disproportionation may be caused by heat, ultra violet radiation, alkali or thiolate ions. Thus, the unsymmetrical disulfide rearranged in one of the last 2 steps, dimethylaminoethylation and methylation, to the symmetrical disulfide. As this unsymmetrical disulfide could not be prepared, there was no opportunity to test its utility in the reduction/ligand exchange sequence.

2.3. Concluding Remarks

Modular synthesis approach was achieved successfully to prepare the phenolic dendrons and the peripheral unit desired could be introduced easily to the dendrons. The approach became a base to prepare amphiphilic dendrons.

Many attempts were made to prepare the amphiphilic cationic and anionic dendrimers with the symmetrical and unsymmetrical disulfide linkages as focal and thiol-protecting groups. However, in the symmetrical disulfide, only a part of the disulfide was reduced to the corresponding thiols and thus, the partially substituted amphiphilic cationic and anionic dendrimers were generated in ligand exchange reaction. This outcome might be explained as the result to the shielding of disulfide linkage by the relatively large dendritic unit and this steric hindrance obstructing the generation of the thiols via the attack of reducing agents. Also, the influence of oxygen may not be negligible. Oxygen should be excluded to block re-oxidation of thiols generated to disulfides. Thus, the reaction did not proceed to completion successfully due to low thiol concentration.

On the other hand, in the unsymmetrical disulfide, the amphiphilic dendron containing the disulfide could not be prepared. This result is explained by disproportionation leading to the generation of the symmetrical disulfide because the unsymmetrical disulfide is less stable than the symmetrical disulfide. Thus, the unsymmetrical disulfide might be rearranged to the symmetrical disulfide.

For the further step to prepare the amphiphilic dendrons, a modification to

protect/deprotect the thiols should be required and investigated.

2.4. Experimental Section

General considerations. All chemicals were purchased from Aldrich and Acros and were used without further purification unless otherwise indicated. All deuterated solvents were purchased from Cambridge Isotope Laboratories. All solvents used in the dry box were deoxygenated with a minimum of three freeze-pump-thaw cycles before use to remove any dissolved oxygen. Flash chromatography was carried out with silica gel 60 Å, 32 – 63 μm (Sorbent technologies). Nuclear Magnetic Resonance characterization was performed at 300MHz or 400MHz on Varian spectrometers.

G1-Ester-MOM (4,4-Bis-(4-methoxymethoxy-phenyl) pentanoic acid methyl ester) (3). To a solution of G1-Ester (**2**) (10.00 g, 33.3 mmol) and a catalytic amount of 4-dimethylaminopyridine in 150 mL of dry CH_2Cl_2 at 0 °C was added N, N-diisopropyl ethyl amine (Hunig's base) (34.8 mL, 199.8 mmol) with N_2. This mixture was stirred under N_2 for 1 h. Chloromethyl methyl ether (10.1 mL, 133.2 mmol) was then added dropwise via syringe and this solution was stirred for additional 20 min at 0 °C. The reaction mixture was then heated to 40 – 45 °C for 24 h. After the reaction mixture was cooled to room temperature, it was washed 3 times with saturated NaCl solution, dried with anhydrous magnesium sulfate, and then filtered. The filtrate was evaporated to dryness. The crude product was dissolved in diethyl ether and passed through basic alumina. The resulting product was pure enough to be carried out to the next step without further purification. Yield: 98 %

(12.67 g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.57 (s, 3H), 2.11 (m, 2H), 2.39 (m, 2H), 3.48 (s, 6H), 3.63 (s, 3H), 5.16 (s, 4H), 6.81 (d, 4H, J = 8 Hz), 7.01 (d, 4H, J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 27.90, 30.27, 36.72, 44.77, 51.75, 56.17, 94.60, 115.87, 128.44, 142.30, 155.40, 174.60.

G1-OH-MOM (4,4-Bis-(4-methoxymethoxy-phenyl) pentanol) (4). To a suspension of lithium aluminum hydride (3.04 g, 80.1 mmol) in 90 mL of dry THF at 0 °C was added dropwise a solution of G1-Ester-MOM (**3**) in 20 mL of dry THF. The reaction mixture was allowed to warm to room temperature and stirred for 12 h under N_2. 13 mL of ethyl acetate was added at 0 °C and stirred for 10 min. 24 mL of saturated ammonium chloride solution was then added dropwise. The mixture was filtered, and the filter cake was washed 2 times with diethyl ether. The organic layer was washed 3 times with saturated NaCl solution, dried with anhydrous magnesium sulfate, and then filtered. The filtrate was evaporated to dryness. The resulting product was pure enough to be carried out to the next step. Yield: 97 % (9.33 g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.30-1.40 (m, 2H), 1.58 (s, 3H), 2.05-2.11 (m, 2H), 3.47 (s, 6H), 3.63 (t, 2H), 5.14 (s, 4H), 6.91 (d, 4H, J = 8.8 Hz), 7.10 (d, 4H, J = 9.2 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 28.11, 28.48, 38.18, 56.22, 63.71, 94.69, 115.79, 128.49 143.22, 155.28.

G1-OMs-MOM (Methanesulfonic acid 4,4-bis-(4-methoxymethoxy-phenyl)

pentyl ester) (5). To a solution of G1-OH-MOM (**4**) (9.18 g, 25.5 mmol), triethylamine (10.6 mL, 76.4 mmol), and a catalytic amount of 4-dimethylaminopyridine in 100 mL of CH_2Cl_2 at 0 °C was added dropwise methanesulfonyl chloride (5.84 g, 51 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 12 h. Water was added to quench the reaction. The solution was extracted 3 times with CH_2Cl_2. The organic layer was combined, dried with anhydrous magnesium sulfate, and then filtered. The filtrate was evaporated to dryness. The crude product was purified by column chromatography, eluting with 15 % ethyl acetate in hexane. Yield: 98 % (10.95 g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.50-1.62 (m, 2H), 1.58 (s, 3H), 2.11-2.20 (m, 2H), 2.96 (s, 3H), 3.47 (s, 6H), 4.15 (t, 2H), 5.15 (s, 4H), 6.93 (d, 4H, J = 8.4 Hz), 7.08 (d, 4H, J = 8.8 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 25.07, 28.05, 37.44, 37.80, 44.88, 56.10, 70.66, 94.51, 115.75, 128.20, 142.34, 155.17.

G1-MA-MOM ([4,4-Bis-(4-methoxymethoxy-phenyl) pentyl] methyl amine) (6). Into a sealed tube with a solution of G1-OMs-MOM (7.94 g, 18.1 mmol) in 20 mL of THF was added methyl amine (40 wt% solution in H_2O) (31.3 mL, 36.2 mmol). The reaction mixture was then heated to 60 – 70 °C for 5 h. After the reaction mixture was cooled to room temperature, water was added to quench the reaction. The solution was extracted 3 times with CH_2Cl_2. The organic layer was combined, dried with anhydrous magnesium sulfate, and then filtered. The filtrate was evaporated to

dryness. The resulting product was pure enough to be carried out to the next step without further purification. Yield: 93 % (6.27 g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.20-1.34 (m, 2H), 1.58 (s, 3H), 2.03-2.10 (m, 2H), 2.37 (s, 3H), 2.53 (t, 2H), 3.47 (s, 6H), 5.14 (s, 4H), 6.91 (d, 4H, J = 9.2 Hz), 7.09 (d, 4H, J = 8.8 Hz).

4, 4′-dithiobenzoic acid (8). To a solution of 4-mercaptobenzoic acid (**7**) (2.00 g, 0.013 mol) in 100 mL of ethanol was slowly added a saturated solution of I_2 in 95 % ethanol. With addition of iodine solution, precipitates formed. The iodine solution was continued to add until the color of solution was a light yellow. The crude precipitate was collected with filtration and washed with ethanol. The crude product was dried *in vacuo* at 50 °C. Yield: 63 % (1.25 g); ^{1}H NMR (DMSO-D6) δ (ppm) 7.63 (d, 4H, J = 8.1 Hz), 7.93 (d, 4H, 8.4 Hz)

G1-Sym-Disulfide-MOM (9). A solution of 4, 4′-dithiobenzoic acid (**8**) (1.69 g, 5.51 mmol), G1-MA-MOM (**6**) (4.53 g, 12.13 mmol), 1,3-dicyclohexylcarbodiimide (DCC) (2.50 g, 12.13 mmol), and a catalytic amount of 4-dimethylaminopyridine in 45 mL of CH_2Cl_2 was stirred under N_2 at room temperature for 12 h. The solid residue was filtered and the filtrate was evaporated to dryness. The crude product was purified by column chromatography, eluting with 10 % ethyl acetate in CH_2Cl_2. Yield: 73 % (4.11 g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.30 (m, 2H), 1.43 (m, 2H), 1.51 (s, 3H), 1.60 (s, 3H), 1.79 (m, 2H), 2.09 (m, 2H), 2.75 (s, 3H), 2.92 (s, 3H), 3.13 (m, 2H), 3.47 (s,

12H), 3.50 (m, 2H), 5.14 (s, 8H), 6.92 (d, 8H, J = 7.2 Hz), 6.99 (d, 4H, J = 8.4 Hz), 7.10 (d, 4H, J = 8.0 Hz), 7.20 (d, 2H, J = 7.6 Hz), 7.31 (d, 2H, J = 7.6 Hz), 7.49 (d, 4H, J = 8.0 Hz).

G1-Sym-Disulfide-OH (10). To a solution of G1-Sym-Disulfide-MOM (**9**) (1.31 g, 1.28 mmol) in 10 mL of methanol was slowly added 10 drops of concentrated HCl. The solution was refluxed for 2 h. After the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure. The residue was dissolved in a minimum amount of THF and precipitated from hexane. The white precipitate was filtered, washed with hexane, and dried. Yield: 99 % (1.07 g); ^{1}H NMR (CD_3COCD_3) δ (ppm) 1.38 (m, 4H), 1.49 (s, 3H), 1.55 (s, 3H), 1.78 (m, 2H), 2.09 (m, 2H), 2.82 (s, 3H), 2.88 (s, 3H), 3.19 (m, 2H), 3.48 (m, 2H), 6.70 (d, 8H, J = 7.8 Hz), 6.93 (b, 4H), 7.02 (b, 4H), 7.29 (b, 2H), 7.39 (b, 2H), 7.59 (d, 4H, J = 7.5 Hz).

G1-Sym-Disulfide-MA (11). To a suspension of G1-Sym-Disulfide-OH (**10**) (0.5 g, 0.59 mmol) and sodium hydride (60 wt% in hexane) in 50 mL of DMF was slowly added N, N-dimethylaminoethyl chloride·HCl with N_2. The mixture was heated to 60 – 70 °C for 6hrs under N_2. After the reaction mixture was cooled to room temperature, 10 mL of ethanol was added to quench the reaction. The mixture was filtered, and the filter cake was washed 2 times with CH_2Cl_2. The organic layer was washed 3 times with saturated NaCl solution, dried with anhydrous magnesium sulfate, and then

filtered. The filtrate was evaporated to dryness. The resulting product was pure enough to be carried out to the next step. Yield: 91 % (0.61 g); ^{1}H NMR (CD_2Cl_2) δ (ppm) 1.34 (m, 2H), 1.41 (m, 2H), 1.53 (s, 3H), 1.61 (s, 3H), 1.81 (m, 2H), 2.10 (m, 2H), 2.25 (s, 8H), 2.29 (s, 16H), 2.57 (t, 2H), 2.68 (t, 6H), 2.75 (s, 3H), 2.89 (s, 3H), 3.08 (t, 2H), 3.17 (m, 2H), 3.46 (m, 2H), 4.02 (t, 6H), 6.80 (d, 8H, J = 8.0 Hz), 7.02 (b, 4H), 7.11 (b, 4H), 7.20 (b, 4H), 7.31 (d, 4H, J = 8.4 Hz).

G1- Sym-Disulfide-Ammonium (12). Into a sealed tube with a solution of G1-Sym-Disulfide-MA (**11**) (0.32 g, 0.28 mm0l) in 10 mL of CH_2Cl_2 was added CH_3I (1.41 mL, 22.6 mmol) dropwise via syringe. The tube was then wrapped with aluminum foil. The reaction mixture was stirred at room temperature for 24 h. The precipitated salt was filtered and dissolved in methanol to remove insoluble impurity. After micro-syringe filtration, the filtrate was evaporated to dryness. Yield: 99 % (0.47 g); ^{1}H NMR (CD_3OD) δ (ppm) 1.33 (m, 2H), 1.42 (m, 2H), 1.52 (s, 3H), 1.62 (s, 3H), 1.82 (m, 2H), 2.14 (m, 2H), 2.86 (s, 3H), 2.91 (s, 3H), 3.22 (s, 36H), 3.51 (m, 4H), 3.86 (b, 8H), 4.48 (b, 8H), 6.94 (d, 8H, J = 6.9 Hz), 7.07 (d, 4H, J = 8.4 Hz), 7.18 (d, 4H, J = 8.1 Hz), 7.29 (d, 2H, J = 8.1 Hz), 7.38 (d, 2H, J = 8.1 Hz), 7.56 (d, 4H, J = 8.4 Hz).

Attempts at *in situ* preparation of G1-SH-Ammonium (13). The reaction mixture containing G1-Sym-Disulfide-Ammonium (**12**), reducing agent and solvent distilled and/or degassed were stirred under N_2 as follows (see Table 2.2).

In the case of $NaBH_4$, into an oven-dried Schlenk flask with a solution of G1-Sym-Disulfide-Ammonium (**12**) in solvent distilled and/or degassed at 0 °C, $NaBH_4$ was added with N_2. Three different solvents were used, ethanol, methanol, and water. In the case of ethanol, the reaction mixture was stirred at room temperature for 12 h. In methanol, one reaction mixture was stirred at room temperature for 20 h, at 65 °C for 2 h, and another at 65 °C for 13 h. In water, the mixture was stirred at room temperature for 13 h and 65 °C for 12 h. Then, the reaction mixtures were acidified with 1N HCl methanol solution and the solvents were removed under reduced pressure. The Schlenk flasks containing the products put into dry box.

In the case of Zn/HCl and Zn/acetic acid, G1-Sym-Disulfide-Ammonium (**12**) was mixed with zinc dust and acetic acid degassed into an oven-dried Schlenk flask with N_2. The reaction mixture was stirred for 30 min at room temperature, refluxed for 13 h and cooled and filtered quickly. In the case of Zn/HCl, zinc dust was added to a solution of G1-Sym-Disulfide-Ammonium (**12**) in 3 N HCl and water degassed with N_2 and the mixture was stirred at room temperature for 12 h. Each solvent used in two reactions was removed *in vacuo* at 40 °C and the flasks put into the dry box.

In the case of DTT, ME and TCEP, DTT in the dry box was added directly to a solution of G1-Sym-Disulfide-Ammonium (**12**) in DMF degassed at room temperature to perform the ligand exchange reaction. In the case of ME, after addition ME into DMF solution degassed, the reaction mixture was heated at 100 °C for 24 h with N_2. In the TCEP·HCl reaction, water was used as a solvent and the reaction

mixture was stirred at room temperature for 16 h. Then, the solvents in the ME and TCEP·HCl reaction were removed and put them into the dry box.

In the case of H_3PO_2/DPDS, G1-Sym-Disulfide-Ammonium (**12**) was mixed with hypophosphorous acid and diphenyl diselenide in ethanol / methanol distilled, dried, and degassed into an oven-dried Schlenk flask with N_2. The mixture was stirred and heated at 60 °C for 12 h. The solvent was removed under reduced pressure and put it into the dry box.

In the case of NaHSe, G1-Sym-Disulfide-Ammonium (**12**) methanol solutions were added into sodium hydrogen selenide ethanol solutions with N_2. One reaction mixture was stirred at room temperature for 5 h and another for 16 h. Hydrochloric acid was added to the two reaction mixtures which were then stirred for 1 h more. The solvent was removed under reduced pressure and put them into the dry box.

Attempt at synthesis of $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (15). Into a oven-dried Schlenk flask with a solution of G1-SH-Ammonium (**18**) (6 eq.) in DMF dried and degassed was added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (1 eq.) in DMF dried and degassed in dry box. Upon addition, the red-wine color developed rapidly. The reaction mixture was stirred and the solvent was removed *in vacuo* at 40 °C.

G1-Sym-Disulfide-$COOCH_3$ (16). To a suspension of G1-Sym-Disulfide-OH (**10**) (0.13 g, 0.154 mmol), anhydrous potassium carbonate (0.085 g, 0.618 mmol), and a

catalytic amount of 18-crown-6 in 20 mL of dry acetone was added 4-(bromomethyl)benzoate (0.15 g, 0.68 mmol). The mixture was refluxed for 12 h and stirred vigorously under N_2. After the reaction mixture was cooled to room temperature, the mixture was filtered. The filtrate was evaporated to remove the solvent under reduced pressure. The crude product was purified by column chromatography, eluting with the solvent system of 50 % ethyl acetate, 25 % CH_2Cl_2, and 25 % hexane. Yield: 32 % (0.07 g); ^{1}H NMR (CD_2Cl_2) δ (ppm) 1.27 (m, 2H), 1.38 (m, 2H), 1.48 (s, 3H), 1.58 (s, 3H), 1.75 (m, 2H), 2.05 (m, 2H), 2.86 (s, 3H), 2.93 (s, 3H), 3.07 (m, 2H), 3.42 (m, 2H), 3.88 (s, 12H), 5.08 (s, 8H), 6.84 (d, 8H, J = 8.7 Hz), 6.99 (b, 4H), 7.10 (b, 4H), 7.44 (b, 4H), 7.50 (d, 8H, J = 8.4 Hz), 7.71 (b, 4H), 8.02 (d, 8H, J = 8.4 Hz).

G1-Sym-Disulfide-COOH (17). A suspension of G1-Sym-Disulfide-$COOCH_3$ (**16**) (0.76 g, 0.53 mmol) and potassium hydroxide (0.25 g, 2.1 mmol) in mixed solution of MeOH (33.8 mL), THF (33.8 mL), and H_2O (8 mL) was stirred and heated to 50 °C for 24 h. After the reaction mixture was cooled to room temperature, 100 mL of 1 N HCl solution was added to quench the reaction. The organic layer was washed 3 times with H_2O, dried with anhydrous magnesium sulfate, and then filtered. The filtrate was evaporated to dryness. The crude product was used for next reaction without further purification. (*NMR was not clean because there was a polymerization reaction aided by THF used as a solvent during the reaction.)

Attempt at *in situ* preparation of G1-SH-COOH (18). Into a oven-dried Schlenk flask with a solution of G1-Sym-Disulfide-COOH (**17**) (0.05 g, 0.04 mmol) in acetone and a small amount of aqueous methanol dried and degassed was added tri-butylphosphine (0.04 g, 0.2 mmol)with N_2. The reaction mixture was stirred for 12 h under N_2. The solvent was removed *in vacuo* and put it into dry box.

Attempt at preparation of $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (19). Into a oven-dried Schlenk flask with a solution of G1-SH-COOH (**18**) (0.05 g, 0.07 mmol) in 5 mL of DMF dried and degassed was added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (0.01 g, 0.01 mmol) in 8 mL of DMF dried and degassed in dry box. Upon addition, the red-wine color developed rapidly. The reaction mixture was stirred and the solvent was removed in vacuo at 30 – 40 °C.

Ethyl Ethanethiolsulfonate (20). To a solution of ethane thiol (15.0 mL, 200 mmol) and acetic acid (5.8 mL, 100 mmol) in 40 mL of CH_2Cl_2 at 0 °C was slowly added sulfuryl chloride (24.4 mL, 300 mmol) over 3 h. The reaction mixture was stirred vigorously for 1 h and allowed to warm to room temperature. Water (4.5 mL, 250 mmol) was then added dropwise via syringe for 15 min. The mixture was stirred overnight. The organic layer was washed 3 times with H_2O, dried with anhydrous magnesium sulfate, and then filtered. The filtrate was evaporated to dryness. The resulting product was pure enough to be carried out to the next step. Yield: 49 % (15.2

g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.42 (t, 3H), 1.46 (t, 3H), 3.15 (q, 2H), 3.31 (q, 2H).

p-Carboxyphenyl Ethyl Disulfide (21). To a solution of 4-mercaptobenzoic acid (**7**) (1.54 g, 9.99 mmol) in 50 mL of 95 % ethanol was added Ethyl ethanethiolsulfonate (**20**) (1.54 g, 9.98 mmol). The reaction mixture was stirred for 1 h at room temperature. Iced water was added to quench the reaction at 0 °C. Precipitate was filtered. The crude product was dissolved in methanol and filtered to remove insoluble impurity. The solution was precipitated from H_2O. The resulting product was filtered and dried *in vacuo* at 45 °C. Yield: 49 % (1.04 g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.33 (t, 3H), 2.81 (q, 2H), 7.67 (d, 2H, J = 8.4 Hz), 8.06 (d, 2H, J = 8.4 Hz).

G1-Unsym-Disulfide-MOM (22). A solution of p-Carboxyphenyl ethyl disulfide (**21**) (0.77 g, 3.61 mmol), G1-MA-MOM (**6**) (1.55 g, 4.15 mmol), 1,3-dicyclohexylcarbodiimide (0.86 g, 4.15 mmol), and a catalytic amount of 4-dimethylaminopyridine in 30 mL of CH_2Cl_2 was stirred under N_2 at room temperature for 12 h. The solid residue was filtered and the filtrate was evaporated to dryness. The crude product was purified by column chromatography, eluting with 10 % ethyl acetate in CH_2Cl_2. Yield: 78 % (1.58 g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.31(t, 3H), 1.35 (m, 1H), 1.44 (m, 1H), 1.52 (s, 1.5H), 1.61 (s, 1.5H), 1.80 (m, 1H), 2.10 (m, 1H), 2.73 (q, 2H), 2.78 (s, 1.5H), 2.94 (s, 1.5H), 3.17 (m, 1H), 3.47 (s, 6H), 3.60 (m, 1H), 5.15

(s, 4H), 6.93 (d, 4H, J = 6.3 Hz), 7.01 (d, 2H, J = 5.7 Hz), 7.11 (d, 2H, J = 5.7 Hz), 7.22 (d, 1H, J = 5.7 Hz), 7.33 (d, 1H, J = 4.8 Hz), 7.54 (d, 2H, J = 6.3 Hz).

G1- Unsym-Disulfide-OH (23). To a solution of G1-Unsym-Disulfide-MOM (**22**) (1.58 g, 2.77 mmol) in 30 mL of methanol was slowly added 30 drops of concentrated HCl. The solution was refluxed for 4 h. After the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure. The residue was dissolved in acetone and filtered to remove insoluble impurities. The solution was evaporated to dryness. The resulting product was pure enough to be carried out to the next step. Yield: 99 % (1.34 g); ^{1}H NMR (CD_3COCD_3) δ (ppm) 1.30(t, 3H), 1.41 (m, 2H), 1.51 (s, 1.5H), 1.56 (s, 1.5H), 1.83 (m, 1H), 2.09 (m, 1H), 2.82 (q, 2H), 2.85 (s, 1.5H), 2.87 (s, 1.5H), 3.33 (m, 1H), 3.48 (m, 1H), 6.73 (m, 4H), 6.98 (b, 2H), 7.10 (b, 2H), 7.30 (b, 1H), 7.38 (b, 1H), 7.59 (m, 2H).

G1-Unsym-Disulfide-MA (24). To a suspension of G1- Unsym-Disulfide-OH (**23**) (0.3 g, 0.62 mmol), anhydrous potassium carbonate (2.58 g, 18.6 mmol), and a catalytic amount of 18-crown-6 in 50 mL of dry acetone was slowly added N, N-dimethylaminoethyl chloride·HCl with N_2. The mixture was refluxed for 4 h and stirred vigorously under N_2. After the reaction mixture was cooled to room temperature, the mixture was filtered. The solution was washed 3 times with H_2O, dried with anhydrous magnesium sulfate, and then filtered. The filtrate was

evaporated to dryness. Yield: 99 % (0.39 g).

Attempt at preparation of G1-Unsym-Disulfide-Ammonium (25). Into a sealed tube with a solution of G1-Unsym-Disulfide-MA (**24**) (0.39 g, 0.63 mm0l) in 10 mL of CH_2Cl_2 was added CH_3I (1.63 mL, 26.3 mmol) dropwise via syringe. The tube was wrapped with aluminum foil. The reaction mixture was stirred at room temperature for 24 h. The precipitated salt was filtered and dissolved in methanol to remove insoluble impurity. After micro-syringe filtration, the filtrate was evaporated to dryness. (* NMR showed this product was not G1-Unsym-Disulfide-Ammonium, but G1-Sym-Disulfide-Ammonium)

2.5. References

(1) Whitesides, G. M.; Houk, J.; Patterson, M. A. K. *J. Org. Chem.* **1983**, *48*, 112-115.

(2) Uchiro, H.; Kobayashi, S. *Tet. Lett.* **1999**, *40*, 3179-3182.

(3) Puttaswamy; Jagadeesh, R. V. *Ind. & Engi. Chem. Res.* **2006**, *45*, 1563-1570.

(4) Pinchart, A.; Dallaire, C.; Van Bierbeek, A.; Gingras, M. *Tet. Lett.* **1999**, *40*, 5479-5482.

(5) Meienhofer, J.; Czombos, J.; Maeda, H. *J. Am. Chem. Soc.* **1971**, *93*, 3080-3081.

(6) Krishnamurthy, S.; Aimino, D. *J. Org. Chem.* **1989**, *54*, 4458-4462.

(7) Houk, J. W., G. M. *J. Am. Chem. Soc.* **1987**, *109*, 6825-6836.

(8) Greene, T. W.; Wuts, P. G. *Protective Groups In Organic Synthesis*; 2nd ed.; John Wiley & Sons, Inc.: 1991.

(9) Wallace, O. B.; Springer, D. M. *Tet. Lett.* **1998**, *39*, 2693-2694.

(10) DeCollo, T. V.; Lees, W. J. *J. Org. Chem.* **2001**, *66*, 4244-4249.

(11) Overman, L. E.; Matzinge.D; Oconnor, E. M.; Overman, J. D. *J. Am. Chem. Soc.* **1974**, *96*, 6081-6089.

(12) Lees, W. J.; Singh, R.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 7328-7331.

(13) Klayman, D. L.; Griffin, T. S. *J. Am. Chem. Soc.* **1973**, *95*, 197-200.

(14) Woods, T. S.; Klayman, D. L. *J. Org. Chem.* **1974**, *39*, 3716-3720.

(15) Gunther, W. H. H. *J. Org. Chem.* **1966**, *31*, 1202-1205.

(16) Humphrey, R. E.; Hawkins, J. M. *Anal. Chem.* **1964**, *36*, 1812-1814.

(17) Lamoureux, G. V.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 633-641.

(18) Cleland, W. W. *Biochemistry* **1964**, *3*, 480-482.

(19) Getz, E. B.; Xiao, M.; Chakrabarty, T.; Cooke, R.; Selvin, P. R. *Anal. Biochem.* **1999**, *273*, 73-80.

(20) Cline, D. J.; Redding, S. E.; Brohawn, S. G.; Psathas, J. N.; Schneider, J. P.; Thorpe, C. *Biochemistry* **2004**, *43*, 15195-15203.

(21) Han, J. C.; Han, G. Y. *Anal. Biochem.* **1994**, *220*, 5-10.

(22) Lee, C. L.; Lam, Y. L.; Lee, S. Y. *Tet. Lett.* **2001**, *42*, 109-111.

(23) Lee, C. L.; Chan, K. P.; Lam, Y. L.; Lee, S. Y. *Tet. Lett.* **2001**, *42*, 1167-1169.

(24) Arnold, R. C.; Lien, A. P.; Alm, R. M. *J. Am. Chem. Soc.* **1950**, *72*, 731-733.

(25) Mondal, S.; Basu, P. *Inorg. Chem.* **2001**, *40*, 192-193.

(26) Sengar, R. S.; Nemykin, V. N.; Basu, P. *New J. Chem.* **2003**, *27*, 1115-1123.

(27) Basu, P.; Nemykin, V. N.; Sengar, R. S. *Inorg. Chem.* **2003**, *42*, 7489-7501.

(28) Wooley, K. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1991**, *113*, 4252-4261.

(29) Averill, B. A.; Herskovi.T; Holm, R. H.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 3523-3534.

(30) Bobrik, M. A.; Laskowski, E. J.; Johnson, R. W.; Gillum, W. O.; Berg, J. M.; Hodgson, K. O.; Holm, R. H. *Inorg. Chem.* **1978**, *17*, 1402-1410.

(31) Bobrik, M. A.; Que, L.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 285-287.

(32) Depamphi, B. V.; Averill, B. A.; Herskovi, T.; Que, L.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4159-4167.

(33) Dukes, G. R.; Holm, R. H. *J. Am. Chem. Soc.* **1975**, *97*, 528-533.

(34) Hill, C. L.; Renaud, J.; Holm, R. H.; Mortenson, L. E. *J. Am. Chem. Soc.* **1977**, *99*, 2549-2557.

(35) Holm, R. H. *Acc. Chem. Res.* **1977**, *10*, 427-434.

(36) Holm, R. H.; Averill, B. A.; Herskovi.T; Frankel, R. B.; Gray, H. B.; Siiman, O.; Grunthan.Fj *J. Am. Chem. Soc.* **1974**, *96*, 2644-2646.

(37) Holm, R. H.; Phillips, W. D.; Averill, B. A.; Mayerle, J. J.; Herskovi.T *J. Am. Chem. Soc.* **1974**, *96*, 2109-2117.

(38) Johnson, R. W.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5338-5344.

(39) Laskowski, E. J.; Frankel, R. B.; Gillum, W. O.; Papaefthymiou, G. C.; Renaud, J.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5322-5337.

(40) Que, L.; Bobrik, M. A.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4168-4178.

(41) Reynolds, J. G.; Coyle, C. L.; Holm, R. H. *J. Am. Chem. Soc.* **1980**, *102*, 4350-4355.

(42) Reynolds, J. G.; Laskowski, E. J.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5315-5322.

(43) Chen, K. Y.; Gorman, C. B. *J. Org. Chem.* **1996**, *61*, 9229-9235.

(44) Gorman, C. B.; Parkhurst, B. L.; Su, W. Y.; Chen, K. Y. *J. Am. Chem. Soc.* **1997**, *119*, 1141-1142.

(45) Gorman, C. B.; Smith, J. C.; Hager, M. W.; Parkhurst, B. L.; Sierzputowska-Gracz, H.; Haney, C. A. *J. Am. Chem. Soc.* **1999**, *121*, 9958-9966.

(46) Gorman, C. B.; Smith, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 9342-9343.

(47) Gorman, C. B.; Smith, J. C. *Acc. Chem. Res.* **2001**, *34*, 60-71.

(48) Cameron, C. S.; Gorman, C. B. *Adv. Funct. Mater.* **2002**, *12*, 17-20.

(49) Chasse, T. L.; Sachdeva, R.; Li, C.; Li, Z. M.; Petrie, R. J.; Gorman, C. B. *J. Am. Chem. Soc.* **2003**, *125*, 8250-8254.

(50) Gorman, C. B.; Smith, J. C. *Polymer* **2000**, *41*, 675-683.

(51) Gorman, C. B. *Adv. Mater.* **1997**, *9*, 1117-1119.

(52) Gorman, C. B.; Hager, M. W.; Parkhurst, B. L.; Smith, J. C. *Macromolecules* **1998**, *31*, 815-822.

(53) Gorman, C. *Adv. Mater.* **1998**, *10*, 295-309.

(54) Gorman, C. B.; Smith, J. C.; Sachdeva, R.; Su, W. Y.; Jiang, H. W. *Macromol. Symp.* **2000**, *156*, 61-67.

(55) Danehy, J. P.; Oester, M. Y. *J. Org. Chem.* **1967**, *32*, 1491-1495.

(56) Takada, T.; Barton, J. K. *J. Am. Chem. Soc.* **2005**, *127*, 12204-12205.

(57) Hong, Y. R.; Gorman, C. B. *J. Org. Chem.* **2003**, *68*, 9019-9025.

(58) Itoh, T.; Gotoh, K.; Ishikawa, N.; Hamaguchi, T.; Kubo, M. *J. Org. Chem.* **1996**, *61*, 1867-1869.

(59) Loup, C.; Zanta, M. A.; Caminade, A. M.; Majoral, J. P.; Meunier, B. *Chem. Eur. J.* **1999**, *5*, 3644-3650.

(60) Krska, S. W.; Seyferth, D. *J. Am. Chem. Soc.* **1998**, *120*, 3604-3612.

(61) Lee, J. J.; Ford, W. T.; Moore, J. A.; Li, Y. F. *Macromolecules* **1994**, *27*, 4632-4634.

(62) Pan, Y. J.; Ford, W. T. *J. Org. Chem.* **1999**, *64*, 8588-8593.

(63) Pan, Y. J.; Ford, W. T. *Macromolecules* **2000**, *33*, 3731-3738.

(64) Pesak, D. J.; Moore, J. S. *Tetrahedron* **1997**, *53*, 15331-15347.

(65) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Saunders, M. J.; Grossman, S. H. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1178-1180.

(66) Kim, Y. H.; Webster, O. W. *J. Am. Chem. Soc.* **1990**, *112*, 4592-4593.

(67) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1287-1297.

(68) Cardona, C. M.; Alvarez, J.; Kaifer, A. E.; McCarley, T. D.; Pandey, S.; Baker, G. A.; Bonzagni, N. J.; Bright, F. V. *J. Am. Chem. Soc.* **2000**, *122*, 6139-6144.

(69) Cardona, C. M.; McCarley, T. D.; Kaifer, A. E. *J. Org. Chem.* **2000**, *65*, 1857-1864.

(70) Cardona, C. M.; Mendoza, S.; Kaifer, A. E. *Chem. Soc. Rev.* **2000**, *29*, 37-42.

(71) Dandliker, P. J.; Diederich, F.; Gisselbrecht, J. P.; Louati, A.; Gross, M. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2725-2728.

(72) Laufersweiler, M. J.; Rohde, J. M.; Chaumette, J. L.; Sarazin, D.; Parquette, J. R. *J. Org. Chem.* **2001**, *66*, 6440-6452.

(73) Pesak, D. J.; Moore, J. S.; Wheat, T. E. *Macromolecules* **1997**, *30*, 6467-6482.

(74) Tomioka, N.; Takasu, D.; Takahashi, T.; Aida, T. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1531-1534.

(75) Wang, Y.; Cardona, C. M.; Kaifer, A. E. *J. Am. Chem. Soc.* **1999**, *121*, 9756-9757.

(76) Young, J. K.; Baker, G. R.; Newkome, G. R.; Morris, K. F.; Johnson, C. S. *Macromolecules* **1994**, *27*, 3464-3471.

(77) Bosman, A. W.; Janssen, H. M.; Meijer, E. W. *Chem. Rev.* **1999**, *99*, 1665-1688.

(78) Stevelmans, S.; vanHest, J. C. M.; Jansen, J.; vanBoxtel, D.; vandenBerg, E.; Meijer, E. W. *J. Am. Chem. Soc.* **1996**, *118*, 7398-7399.

(79) Gaster, L. M.; Blaney, F. E.; Davies, S.; Duckworth, D. M.; Ham, P.; Jenkins, S.; Jennings, A. J.; Joiner, G. F.; King, F. D.; Mulholland, K. R.; Wyman, P. A.; Hagan, J. J.; Hatcher, J.; Jones, B. J.; Middlemiss, D. N.; Price, G. W.; Riley, G.; Roberts, C.; Routledge, C.; Selkirk, J.; Slade, P. D. *J. Med. Chem.* **1998**, *41*, 1218-1235.

(80) Gauthier, S.; Mailhot, J.; Labrie, F. *J. Org. Chem.* **1996**, *61*, 3890-3893.

(81) Poirot, M.; De Medina, P.; Delarue, F.; Perie, J. J.; Klaebe, A.; Faye, J. C. *Bioorg. & Med. Chem.* **2000**, *8*, 2007-2016.

(82) Buuhoi, N. P.; Demerseman, P. *J. Org. Chem.* **1955**, *20*, 1129-1134.

(83) McCague, R.; Leclercq, G.; Jordan, V. C. *J. Med. Chem.* **1988**, *31*, 1285-1290.

(84) Pan, Y. J.; Ford, W. T. *Macromolecules* **1999**, *32*, 5468-5470.

(85) Richter, S. N.; Maggi, S.; Mels, S. C.; Palumbo, M.; Freccero, M. *J. Am. Chem. Soc.* **2004**, *126*, 13973-13979.

(86) Shaked, Z.; Szajewski, R. P.; Whitesides, G. M. *Biochemistry* **1980**, *19*, 4156-4166.

(87) Allen, C. F.; MacKay, D. D. *Organic Syntheses*; 1st ed.; Wiley: New York, 1943.

(88) Singh, R.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 2332-2337.

(89) Szajewski, R. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 2011-2026.

(90) Burns, J. A.; Butler, J. C.; Moran, J.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 2648-2650.

(91) Jocelyn, P. C. *Methods Enzymol.* **1987**, *143*, 246-256.

(92) Jocelyn, P. C. *Biochemistry of the SH group*; 1st ed.; Academic Press: London, 1972.

(93) Huxtable, R. J. *Biochemistry of sulfur*; 1st ed.; Plenum: New York, 1986.

(94) Gilbert, H. F. *Adv. Enzymol.* **1990**, *63*, 69-172.

(95) Humphrey, R. E.; Potter, J. L. *Anal. Chem.* **1965**, *37*, 164-165.

(96) Field, L.; Bryan, A. W.; Owen, T. C.; Crenshaw, R. R. *J. Am. Chem. Soc.* **1961**, *83*, 4414-4417.

(97) Field, L.; Giles, P. M. *J. Org. Chem.* **1971**, *36*, 309-313.

(98) Field, L.; Hanley, W. S.; McVeigh, I. *J. Org. Chem.* **1971**, *36*, 2735-2739.

(99) Field, L.; Kim, H. K. *J. Med. Chem.* **1966**, *9*, 397-402.

(100) Parsons, T. F.; Buckman, J. D.; Pearson, D. E.; Field, L. *J. Org. Chem.* **1965**, *30*, 1923-1926.

(101) Buckman, J. D.; Bellas, M.; Kim, H. K.; Field, L. *J. Org. Chem.* **1967**, *32*, 1626-1627.

(102) Douglass, I. B.; Farah, B. S. *J. Org. Chem.* **1959**, *24*, 973-975.

Chapter 3

Synthetic Approaches to Amphiphilic Iron-Sulfur Core Dendrimers through Alternate Thiol Protecting Groups

3.1. Introduction

The amphiphilic dendrons containing the disulfide linkage were successfully prepared to protect the thiols from oxidation in Chapter 2. Various agents to reduce the disulfides were considered and ligand exchange reactions to prepare dendrimers were performed. However, dendrimers could not be prepared due to incomplete reduction of the disulfide to form the thiol and, as a result a thiol to iron-sulfur cluster stoichiometry that was too low.

In Chapter 3, two alternate thiol protecting groups, the thiocarbamate group[1-6] and the trityl group[7-15], are explored. The thiocarbamate group has been used in previous work in the Gorman group to prepare successfully hydrophobic aromatic thiol dendrons.[2,16-20] These aryl thiocarbamates are stable in acidic and neutral solutions and are removed by basic hydrolysis with sodium hydroxide or potassium hydroxide.[21]

The trityl group was also explored as a protecting group. Wang and coworkers prepared hydrophilic, aliphatic thiol dendrons containing amide linkages as repeat units and hydroxyl groups as peripheral units with the deprotection of the trityl group.[10,14] The trityl group is stable in weak acid, neutral solution, and weak base in the range of approximately pH 2 – 12.[21] Deprotection occurs with strong acid such as trifluoroacetic acid containing trialkylsilanes.

As discussed in Chapter 2, a modular synthesis approach was taken to prepare the phenolic dendrons containing protecting groups. Using this method, peripheral

units including ammonium (cationic)[22-26] and carboxylate (anionic)[27-39] were easily introduced to the phenolic dendrons generated via the approach.

In the last chapter, it was speculated that formation of the thiol from the disulfide was incomplete and this result lead to the subsequent problems in ligand exchange and formation of the amphiphilic, iron-sulfur core dendrimers. Thus, it was of interest to quantitate the concentration of thiol. Two methods were employed here, the use of Ellman's reagent and integration of the proton on the free thiol using ^{1}H NMR.

3.2. Results and Discussion

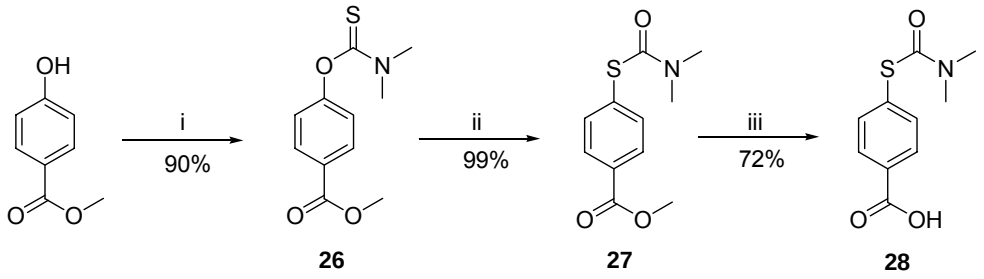
3.2.1. Synthesis from Thiocarbamate Protecting Group

Amphiphilic cationic and anionic dendrimers containing ammonium and carboxylate as peripheral units were prepared using the thiocarbamate group as a focal and protecting group. This work includes the synthesis of thiol dendrons and the measurement of thiol concentration by Ellman's reagent test.

3.2.1.1. Cationic Dendrons containing Thiocarbamate

The flexible dendritic unit, 4,4′-bis(4′-hydroxyphenylvaleric) acid (**1, Chapter 2**) was employed to prepare dendrons and the dendritic fragment (**6, Chapter 2**) was prepared identically to that shown Scheme 2.1.

Scheme 3.1.[a]



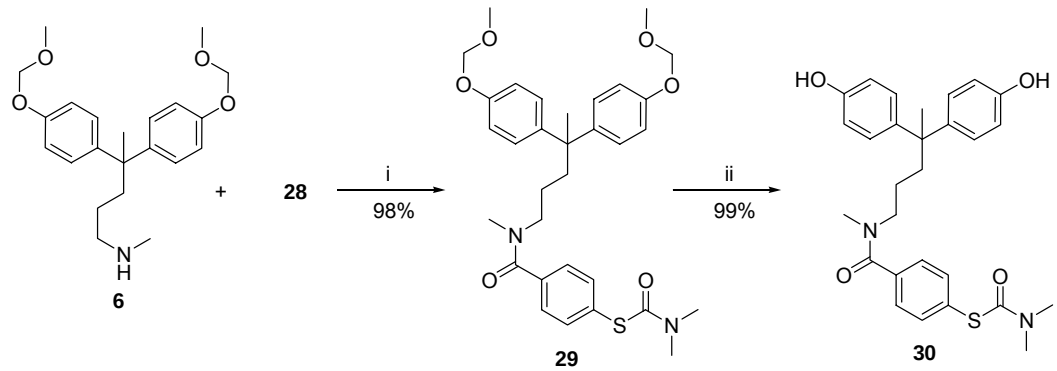
[a]Reagents and conditions: i) $ClC(S)N(CH_3)_2$, Dabco, DMF; ii) 220 - 230 ^oC; iii) $(CH_3)_3SiI$.

The thiocarbamate, 4-dimethylthiocarbamoylsulfanyl benzoic acid (**28**), was prepared in high yield via 3 reactions (Scheme 3.1). 4-dimethylthiocarbamoyloxy benzoic acid methyl ester (**26**) was previously prepared by former group members. 4-dimethylthiocarbamoyloxy benzoic acid methyl ester (**26**) was rearranged to 4-dimethylthiocarbamoylsulfanyl benzoic acid methyl ester (**27**) at high temperature. The methyl ester (**27**) was converted to the carboxylic acid (**28**) by trimethylsilyl iodide.

The dendritic fragment, G1-MA-MOM (**6, Chapter 2**) could be coupled with the thiocarbamate (**28**) as shown in Scheme 3.2. The Gorman group has previously shown the utility of the thiocarbamate group in the preparation of flexible thiol dendrons.[2,17,20] The thiocarbamate group was stable in the further steps when the compound was subjected to acidic and weakly basic conditions. This behavior indicated that the thiocarbamate group can be employed without decomposition in reactions for the preparation of amphiphilic dendrons using similar reaction

conditions.

Scheme 3.2.[a]



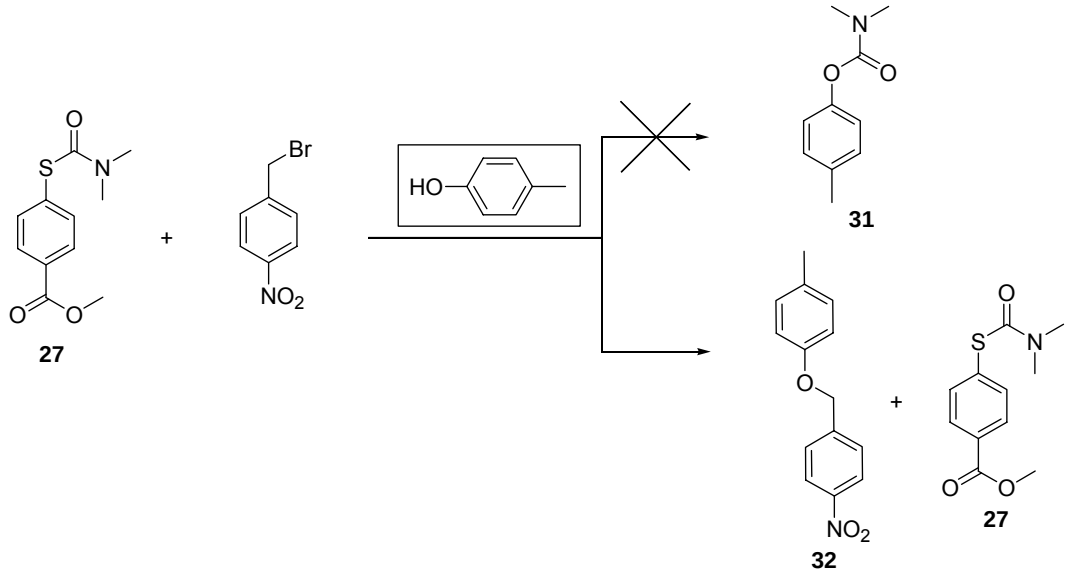
[a]Reagents and conditions: i) DCC, DMAP, CH_2Cl_2; ii) HCl, MeOH.

The dendritic fragment, G1-MA-MOM (**6, Chapter 2**) was coupled with the thiocarbamate (**28**) to give the amide, G1-Thiocarbamate-MOM (**29**), in excellent yield via DCC mediated coupling reaction. The MOM protecting groups were removed easily by refluxing methanol solution with concentrated hydrochloric acid to give G1-Thiocarbamate-OH (**30**) (Scheme 3.2)

Before introducing the tetra-alkyl ammonium groups to the molecule, a test reaction was performed to check the stability of the thiocarbamate group in the compound **27** in the presence of phenols (Scheme 3.3) as this group possibly could be susceptible to nucleophilic attack by phenolic alcohols. In the reaction mixture shown, the generation of dimethylcarbamic acid p-tolyl ester (**31**) would indicate

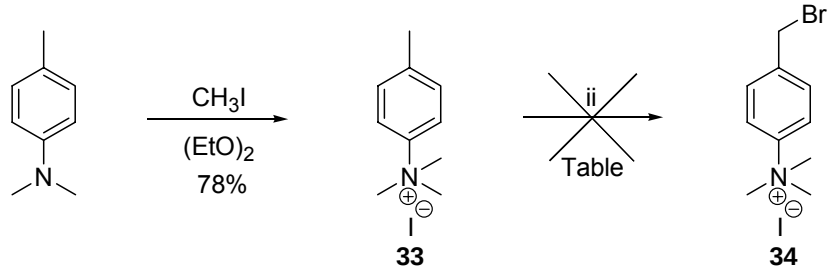
that 4-methylphenol reacted with the thiocarbamate group in compound **27** and that this sequence would not facilitate the introduction of tetra-alkyl ammonium peripheral unit onto the phenolic dendron. However, 4-methylphenol reacted with 1-bromomethyl-4-nitrobenzene to give **32** and 4-dimethylthiocarbamoylsulfanyl benzoic acid methyl ester (**27**) was recovered. This result indicated that the thiocarbamate group was stable against nucleophilic attack by phenolic alcohols. This outcome was determined via TLC and ^{1}H NMR spectroscopy. Thus, the preparation of benzylic bromine compound containing tetra-alkyl ammonium as the peripheral unit was attempted.

Scheme 3.3.[a]



[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone.

Scheme 3.4.[a]



[a]Reagents and conditions: i) CH_3I, $(EtO)_2$; ii) see Table 3.1.

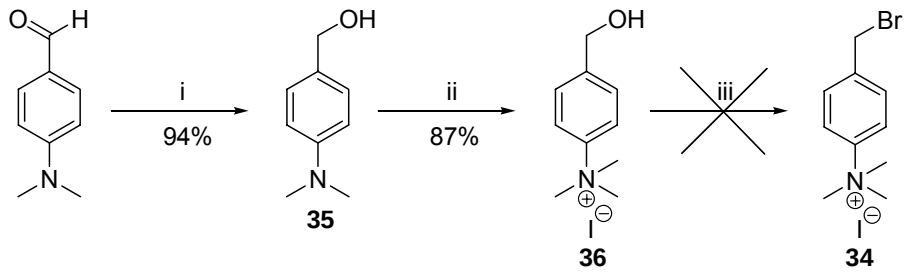
Table 3.1. Attempts to prepare (4-bromomethylphenyl) trimethylammonium iodide (34) by reaction of trimethyl-l-tolylammonium iodide (33) with bromine reagents.

Reagent	Solvent	Temperature (°C)	Method of Heating	Time (h)
[a]NBS / [b]AIBN	CH_2Cl_2	reflux	[e]o.b.	12
NBS / AIBN	$CHCl_3$	reflux	o.b.	12
NBS / AIBN	$CHCl_3$	reflux	o.b.	48
NBS / AIBN	DMF	r.t.	o.b.	12
NBS / AIBN	$CHCl_3$	*[d]	[f]s.l.	18
NBS	$CHCl_3$	100	[g]m.w.	3
NBS	-[c]	180	m.w.	3

[a]NBS: N-Bromosuccinimide; [b]AIBN: 2,2'-azobisisobutyronitrile; -[c]: no solvent; *[d] : no heating; [e]o.b.: oil bath; [f]s.l.: sun lamp; [g]m.w.: micro wave; the result of reactions was determined by ^1H NMR and no product was prepared in all reactions.

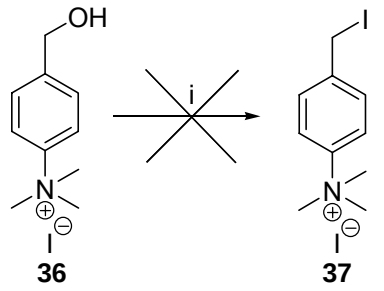
N,N-dimethyl-p-toluidine was methylated with methyl iodide to give trimethyl-l-tolylammonium iodide (**33**) containing iodide counter ions in an acceptable yield (Scheme 3.4). The free radical halogenation of ammonium iodide (**33**) was carried out with NBS under heat or light (Table 3.1).[40] An NBS / AIBN system was used as a bromine source and an oil bath as a heat source in the preparation of (4-bromomethylphenyl) trimethylammonium iodide (**34**) in CH_2Cl_2, $CHCl_3$, and DMF.[41,42] However, ^1H NMR spectra showed that the product, (4-bromomethylphenyl) trimethylammonium iodide (**34**), was not generated. No product was formed in four different sets of reaction conditions. In other attempts, the heat source was changed from an oil bath to a sun lamp[41] and a microwave reactor[43,44]. However, ^1H NMR spectra exhibited only the starting material (**33**). This observation could be explained by the quenching of the radicals with ionic species (tetra-alkyl ammonium).

Scheme 3.5.[a]



[a]Reagents and conditions: i) $NaBH_4$, MeOH; ii) CH_3I, CH_2Cl_2;
iii) PPh_3/NBS, CBr_4, or PBr_3 and DMF, THF, or CH_3CN.

Scheme 3.6.[a]



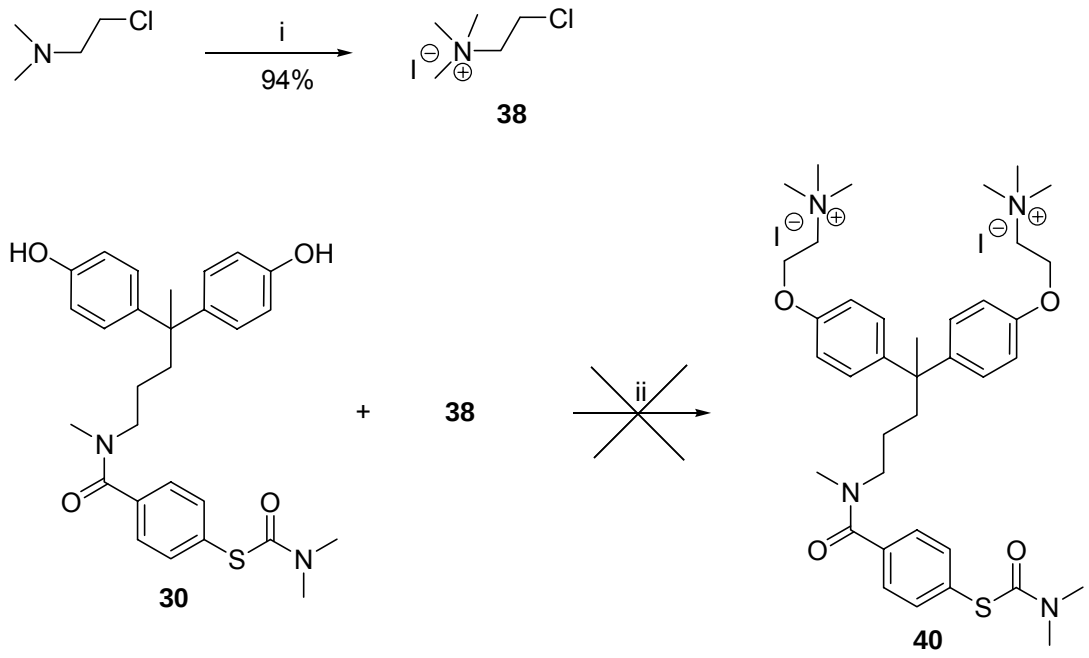
36 **37**

[a]Reagents and conditions: i) HI.

As a new approach to prepare compound **34**, the starting material was changed to 4-dimethylaminobenzaldehyde (Scheme 3.5). The benzaldehyde was reduced to the benzylic alcohol, (4-dimethylaminophenyl) methanol (**35**), with sodium borohydride.[45] The benzylic alcohol (**35**) was methylated with methyl iodide to give (4-hydroxymethylphenyl) trimethylammonium iodide (**36**) in an acceptable yield. The bromination of the ammonium iodide (**36**) was attempted using different bromine sources such as NBS[46], CBr_4[47-49], and PBr_3[50-55]. A NBS / PPh_3 system was tried to prepare (4-bromomethylphenyl) trimethylammonium iodide (**34**) in DMF and THF. ^{1}H NMR spectra displayed that the reactions were not successful and yielded the starting material (**36**). As other attempts, the bromine source was changed from NBS to CBr_4 and PBr_3. However, ^{1}H NMR spectra showed only the starting material (**36**), not the product (**34**). Finally, the halide source was changed from bromine to iodine, HI (Scheme 3.6).[56] However, the ^{1}H NMR spectrum of the

reaction mixture was identical to that obtained in the previous reaction and exhibited only starting material (**36**). This result could be explained by the increase in positive charge in the transition state via a strong electron-withdrawing group, tetra-alkyl ammonium.

Scheme 3.7.[a]

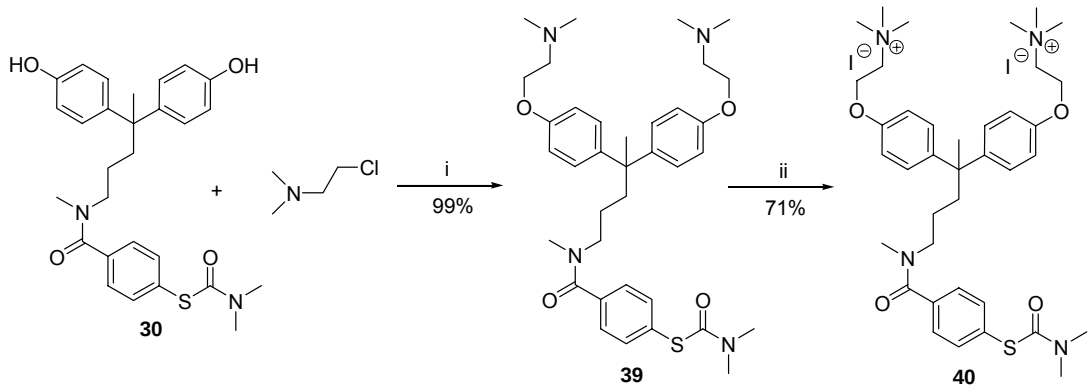


[a]Reagents and conditions: i) CH_3I, CH_2Cl_2; ii) K_2CO_3, 18-crown-6, acetone.

Another approach to the tetra-alkyl ammonium substituted dendron was attempted. In this case, the approach abandoned the aromatic ammonium and instead involved the preparation of an aliphatic, tetra-methyl ammonium group

(Scheme 3.7). N,N-dimethylaminoethyl chloride was methylated with methyl iodide to give (2-chloroethyl)trimethylammonium iodide (**38**). Nucleophilic substitution of the aliphatic ammonium iodide (**38**) with the phenolic dendron, G1-Thiocarbamate-OH (**30**), was performed and monitored by TLC. However, the product (**40**) was not generated, possibly because of the poor leaving group.[40,57]

Scheme 3.8.[a]

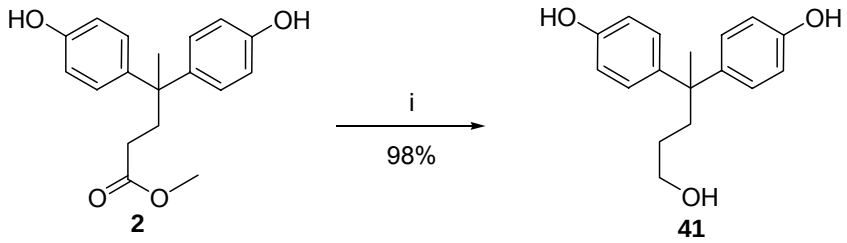


[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone; ii) CH_3I, CH_2Cl_2.

As an alternate approach to introduce the tetra-alkyl ammonium groups, the phenolic dendron, G1-Thiocarbamate-OH (**30**) reacted with the precursor of peripheral unit, N,N-dimethylaminoethyl chloride·HCl, using nucleophilic substitution reaction to give G1-Thiocarbamate-MA (**39**) (Scheme 3.8). G1-Thiocarbamate-MA (**39**), was then methylated with methyl iodides[23-26,58,59] to give amphiphilic cationic dendron containing iodide counter ions. The amphiphilic

cationic dendron, G1-Thiocarbamate-ammonium (**40**), was prepared successfully in acceptable yield.

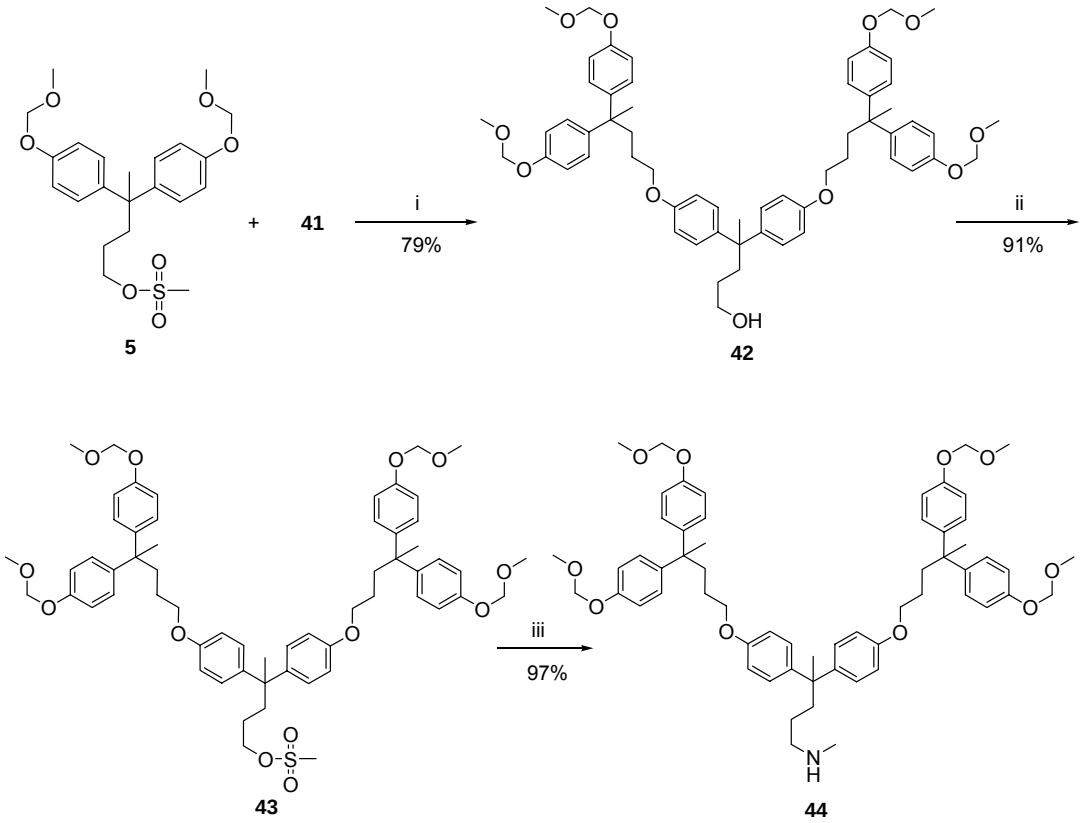
Scheme 3.9.[a]



[a]Reagents and conditions: i) $LiAlH_4$, THF.

With the successful preparation of the G1-cationic dendron, the G2 dendron was prepared by a convergent strategy and a modular synthesis approach using core unit. The core unit, 4,4-bis(4′-hydroxyphenyl)pentanol (**41**) was prepared from the reduction of the methyl ester, methyl 4,4-bis(4′-hydroxyphenyl) valerate (**2, Chapter 2**) (Scheme 3.9). Then, G2-OH-MOM (**42**) was prepared by the reaction of G1-OMs-MOM (**5, Chapter 2**) with triol (**41**) (Scheme 3.10). The remainder of the procedure to synthesize the G2-dendron was identical to that used for the G1-dendron. The dendritic alcohol (**42**) was activated to the dendritic mesylate, G2-OMs-MOM (**43**) with excellent yield. The dendritic mesylate (**43**) was converted to G2-MA-MOM (**44**) with refluxing methyl amine.

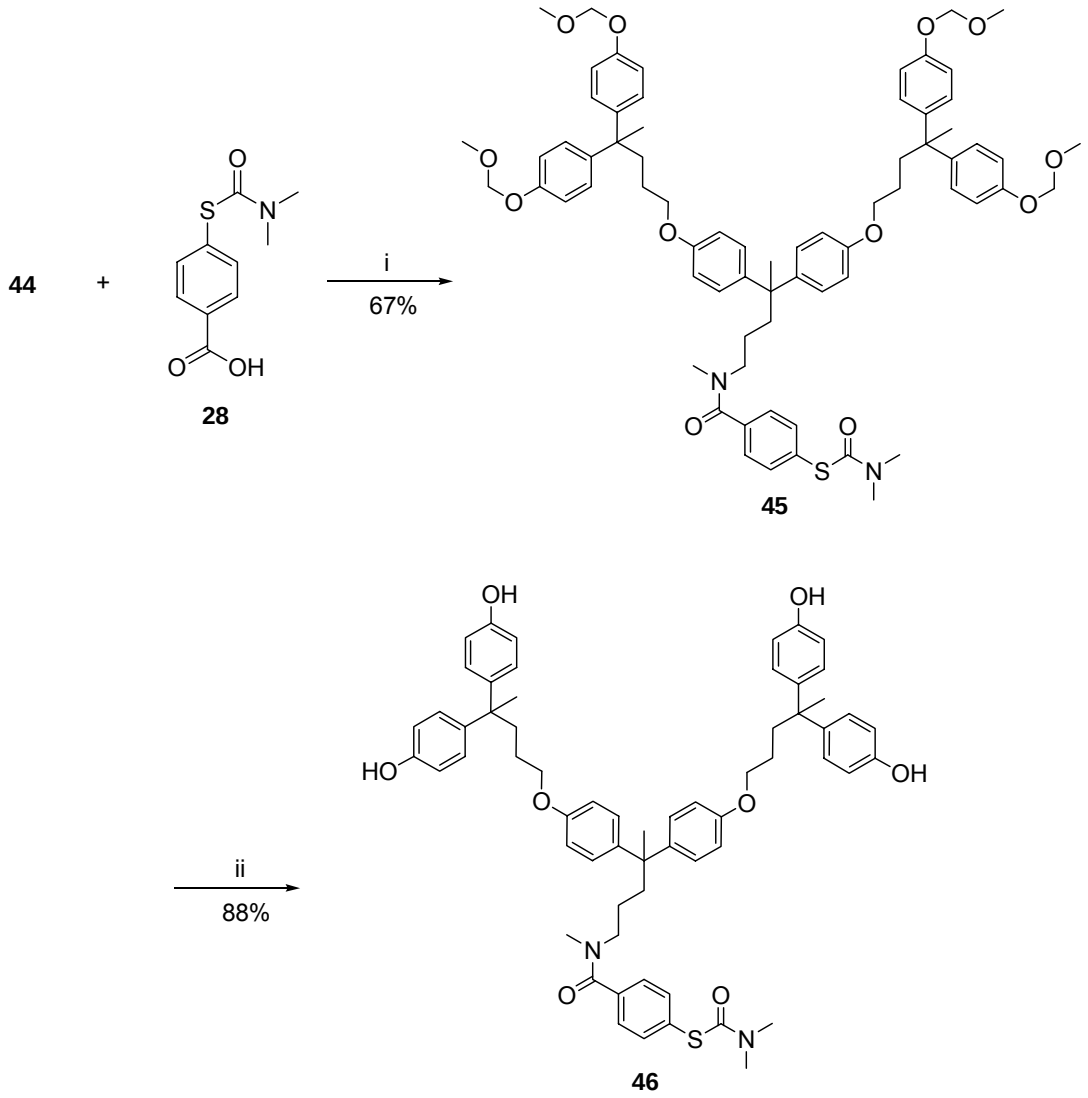
Scheme 3.10.[a]



[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone; ii) MsCl, DMAP, TEA, CH_2Cl_2; iii) CH_3NH_2, THF.

The dendritic fragment, G2-MA-MOM (**44**), was coupled with 4-dimethylthiocarbamoylsulfanylbenzoic acid (**28**) to give G2-Thiocarbamate-MOM (**45**) (Scheme 3.11). The phenolic dendron, G2-Thiocarbamate-OH (**46**) was prepared by the deprotection of G2-Thiocarbamate-MOM (**45**) with refluxing hydrochloric acid in methanol solution.

Scheme 3.11.[a]

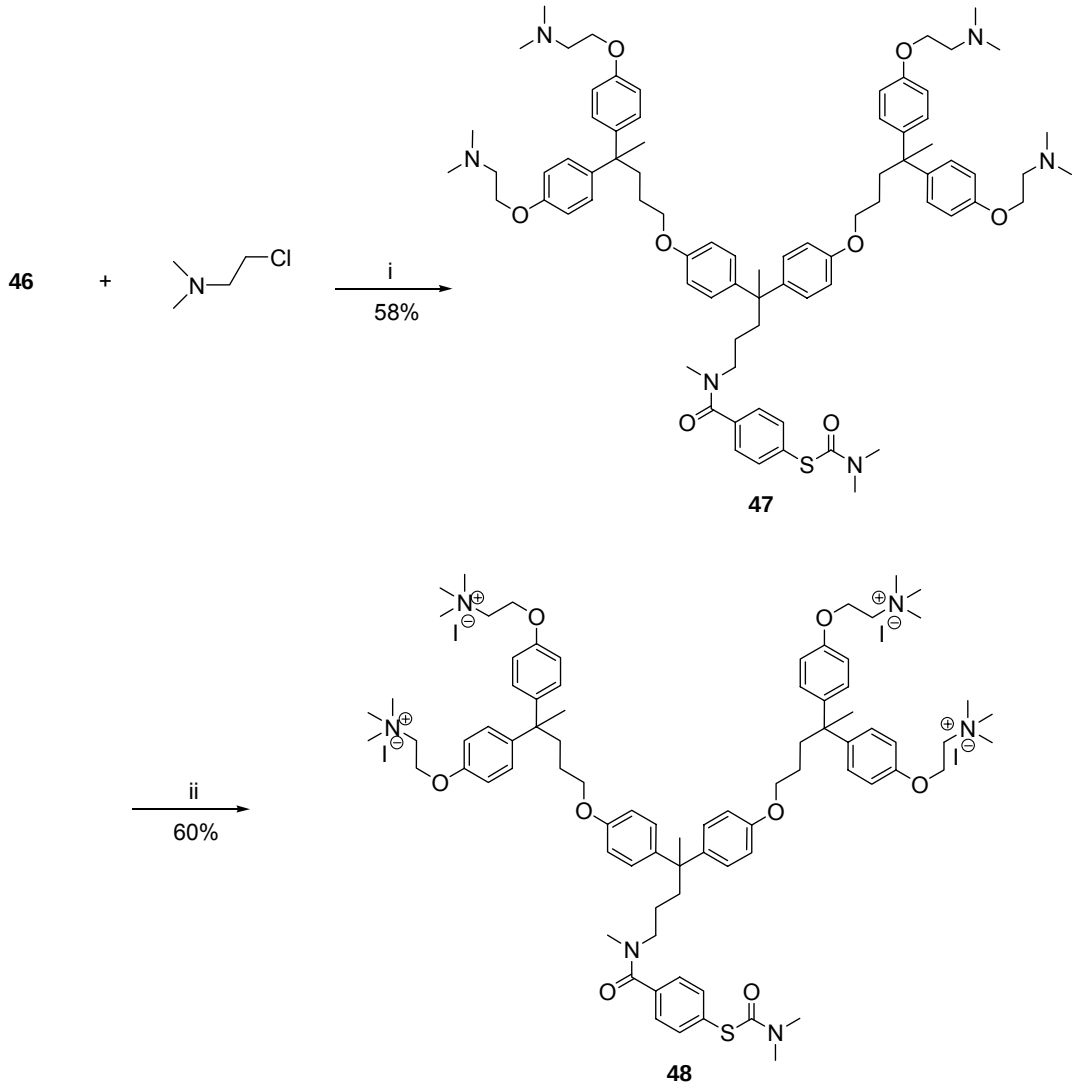


[a]Reagents and conditions: i) DCC, DMAP, CH_2Cl_2; ii) HCl, MeOH.

The phenolic dendron, G2-Thiocarbamate-OH (**46**) reacted with the precursor of peripheral unit, N,N-dimethylaminoethyl chloride·HCl, to give G2-Thiocarbamate-MA (**47**) (Scheme 3.12). G2-Thiocarbamate-MA (**47**), was then

methylated with methyl iodide[23-26,58,59]. Amphiphilic G2-cationic dendrons, G2-Thiocarbamate-ammonium (**48**), was also prepared in an acceptable yield.

Scheme 3.12.[a]



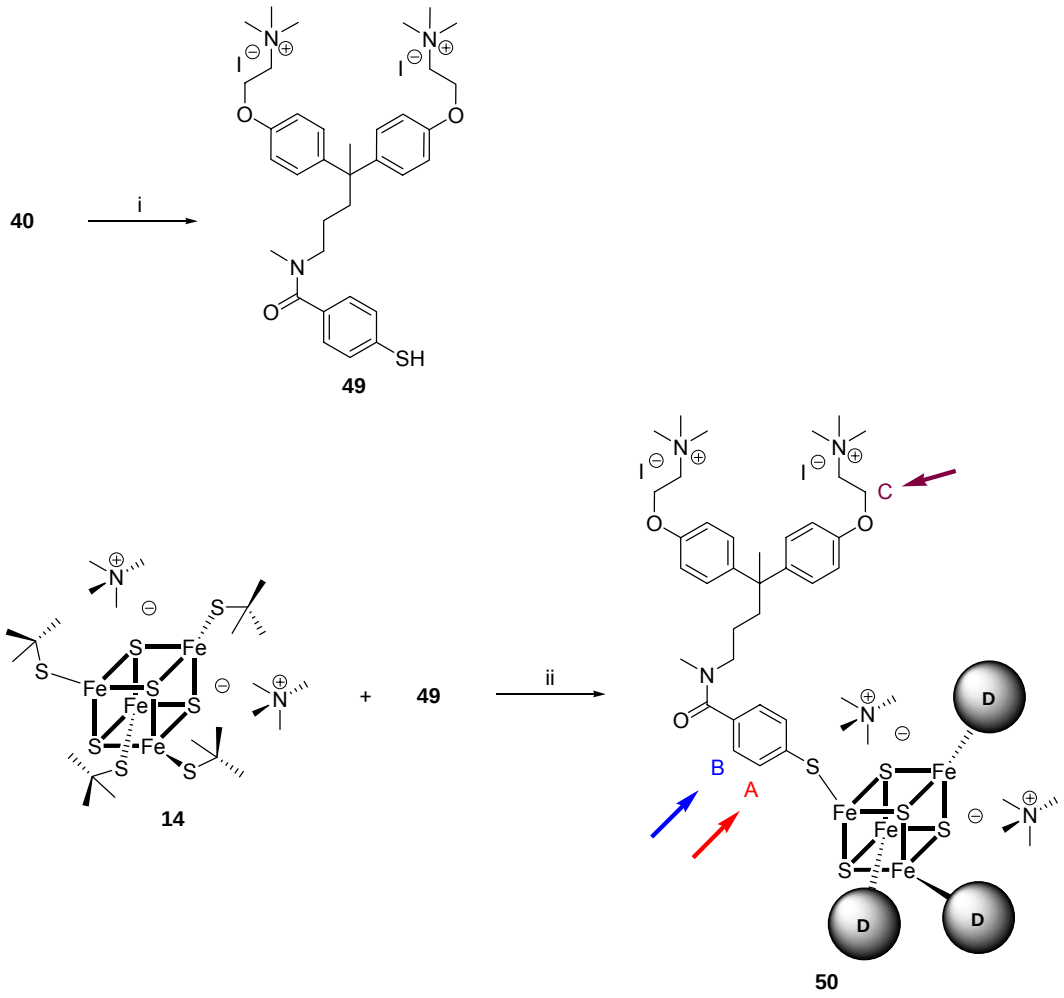
[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone; ii) CH_3I, CH_2Cl_2.

3.2.1.2. Cationic Dendrimer from Thiocarbamate

With the successful preparation of thiol protected dendrons (**40** and **48**), deprotection and dendrimer synthesis was attempted. Hydrophobic iron-sulfur core dendrimers were previously prepared by the Gorman group[2,16-20,60-65] via a ligand exchange reaction originally described by Holm and coworkers[66-79]. In the reaction, aromatic thiols added were exchanged with aliphatic thiols coordinated on the iron-sulfur core of the form $[Fe_4S_4(SR)_4]^{2-}$. With a slight modification of the reaction, the preparation of amphiphilic iron-sulfur core dendrimer was attempted.

Before its use as a dendritic ligand, the dendron containing the thiocarbamate group needed to be deprotected. This deprotection was performed by basic hydrolysis with sodium hydroxide to the corresponding thiol, G1-SH-Ammonium (**49**) (Scheme 3.13). As we were concerned about adventitious oxidation of **49** to the disulfide, the reaction mixture to form **49** was used directly without purification or in initial attempts, characterization. Amphiphilic G1-cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**50**), was prepared by the ligand exchange reaction of the cationic thiol ligand (**49**) with the iron-sulfur core, $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (**14**). Due to the oxygen-sensitivity of the thiol generated, the iron-sulfur core, and the dendrimer, the reaction was carried out in the dry box. The ratio of dendron ligand to iron-sulfur core was 6 to 1, optimum condition reported in the literature.[66,79,80] Upon addition of the core solution to the ligand solution, a red-wine color developed rapidly, indicating the reaction was being successfully achieved.

Scheme 3.13.[a]



[a]Reagents and conditions: i) NaOH, THF, MeOH; ii) DMF.

The product, G1-cationic dendrimer (**50**), was characterized by ^{1}H NMR spectroscopy and MALDI-TOF (Matrix-Assisted Laser Desorption Ionization-Time Of Flight) mass spectrometry. The iron-sulfur core is paramagnetic and thus, the thiolate aromatic ring protons (**A** and **B**) adjacent to the dendrimer core undergo a

broadening and change in chemical shift in ^{1}H NMR spectrum. Two characteristic peaks (**A** and **B**) of the protons on aromatic ring in ^{1}H NMR spectrum showed that the iron-sulfur cluster was fully substituted in the ligand exchange reaction, indicating the generation of G1-cationic dendrimer (Figure 3.1). The integration of two characteristic peaks showed the expected relative integration of 8 protons compared to signal **C** of 16 protons, respectively.

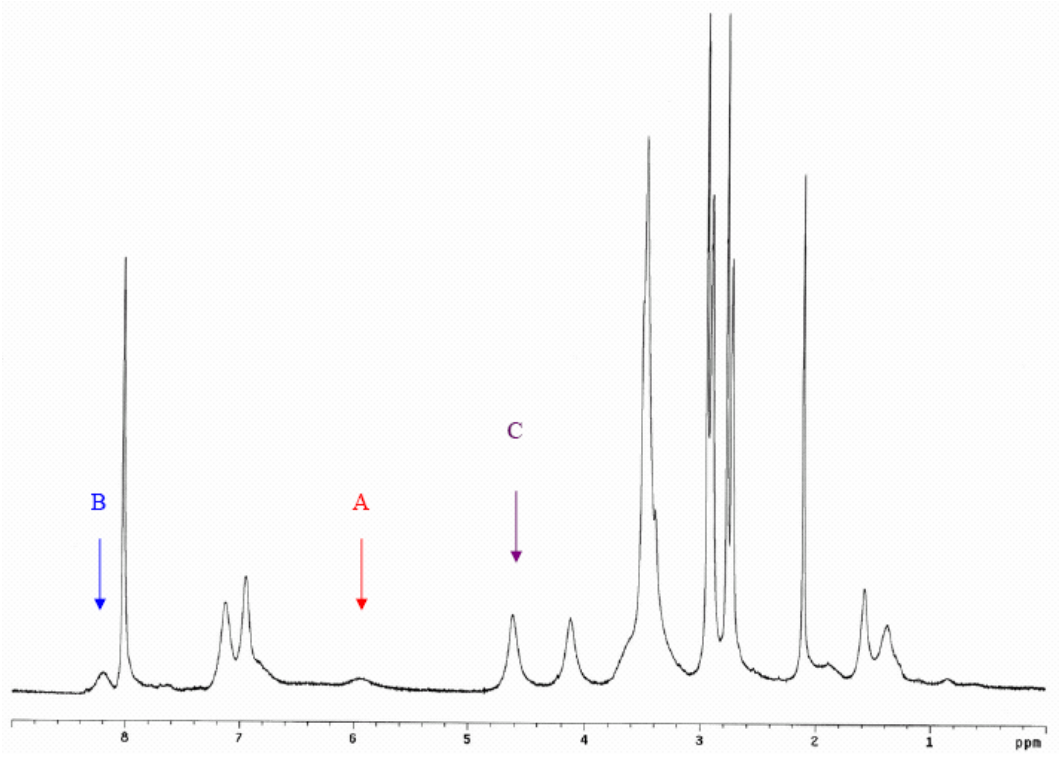


Figure 3.1. ^{1}H NMR spectrum of amphiphilic G1-cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**50**), prepared using NaOH / methanol / reflux to deprotect **40** followed by ligand exchange (Scheme 3.13) (Solvent : DMF-d_7).

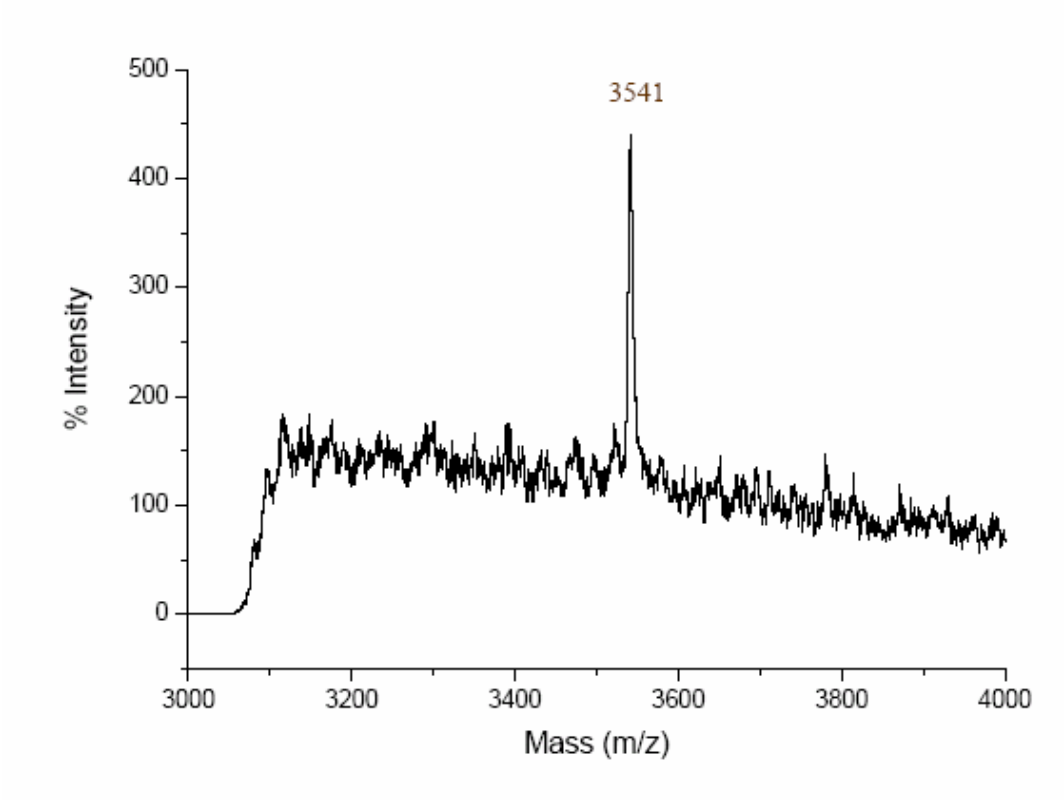
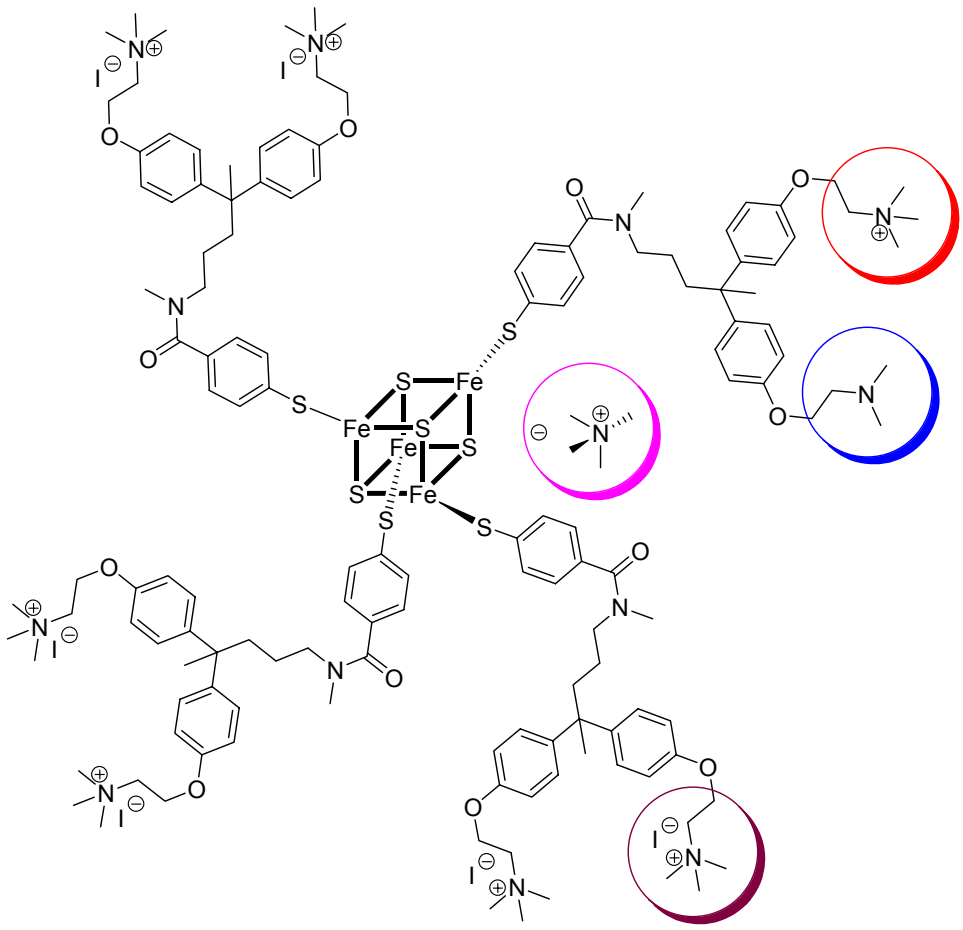


Figure 3.2. MALDI-TOF measurement of amphiphilic G1-cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**50**) (Matrix : dithranol).

MALDI-TOF measurements were obtained in positive mode and using dithranol as a matrix. These data indicated that the mass of the dendrimer was 3541 (Figure 3.2) and the mass calculated was 3884. A value of 3541 could be calculated and explained as the species: $[M + [N(CH_3)_4] - CH_3I - I^- + H]^+ = 3541$ (Figure 3.3). Counter ions (iodide and tetramethyl ammonium) forming weak bonds with substrate (dendron or dendrimer) and methyl group could be cleaved during sample ionization. Although this fragmentation pattern is unusual, the m/z ratio of the peak

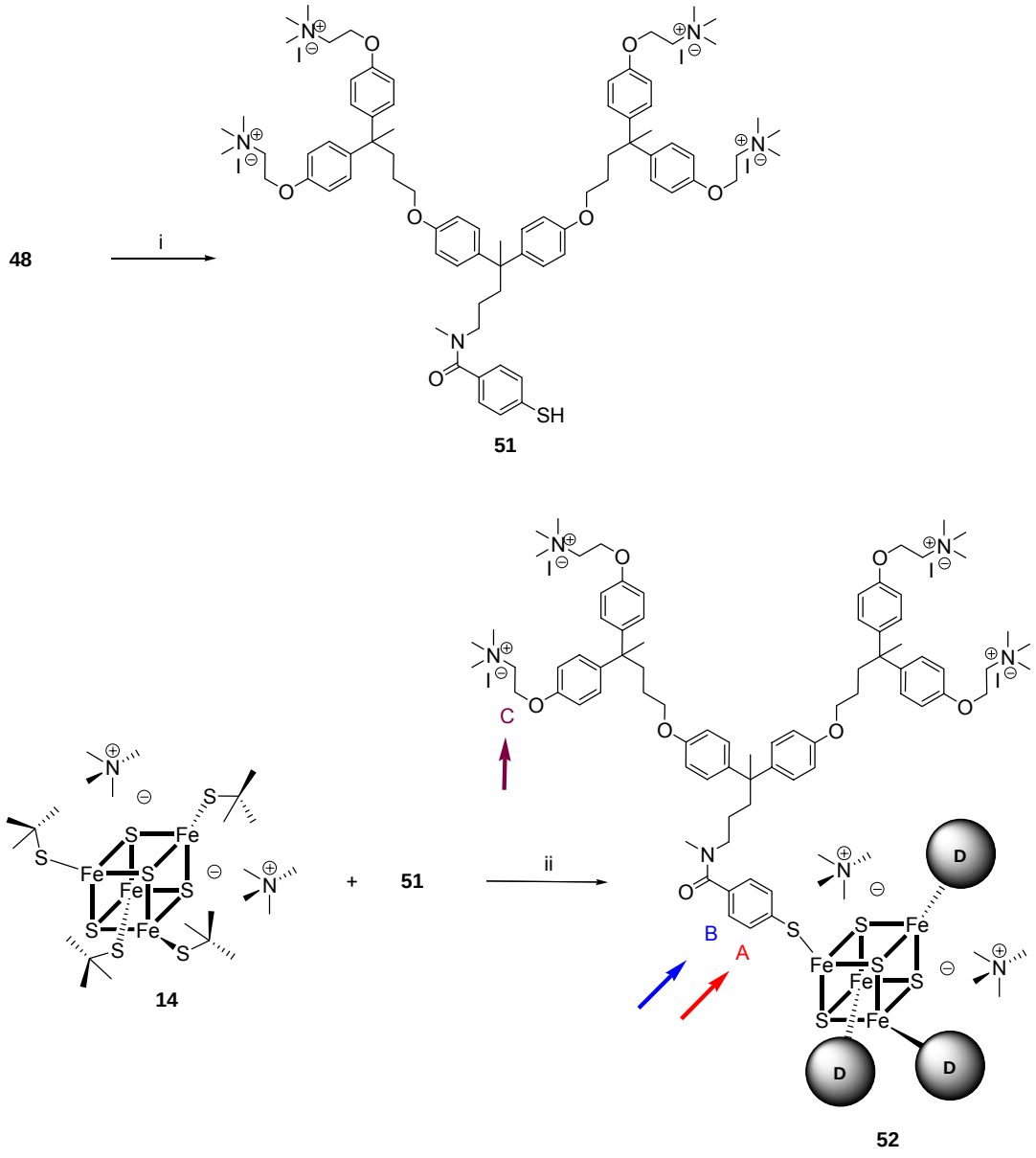
observed is inconsistent with the starting material or incompletely ligand substituted cluster as these species would have lower m/z values.



$[M + [N(CH_3)_4] - CH_3I - I^- + H]^+ = 3541$

Figure 3.3. Calculation of a mass of G1-cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**50**) (M indicates the part that counter ions (tetramethylammonium) are excepted from dendrimer).

Scheme 3.14.[a]



[a]Reagents and conditions: i) NaOH, THF, MeOH; ii) DMF.

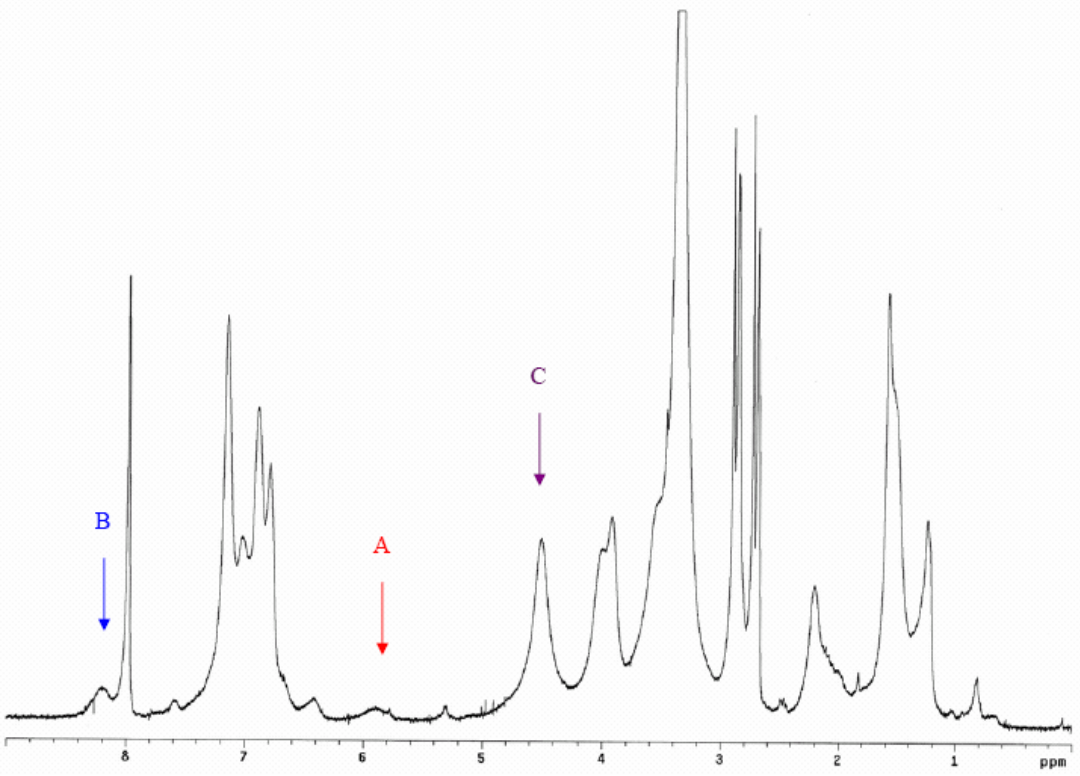


Figure 3.4. ^{1}H NMR spectrum of amphiphilic G2-cationic dendrimer, $[Fe_4S_4(S\text{-}G2\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**52**), prepared using NaOH / methanol / reflux to deprotect **48** followed by ligand exchange (Scheme 3.14) (Solvent : DMF-d_7).

With the successful preparation and characterization of the G1-cationic dendrimer (**50**), the ligand exchange reaction of the G2 dendron was tried. The reaction conditions were identical to those for the G1-dendrimer. G2-SH-Ammonium (**51**) generated by deprotection of G2-Thiocarbamate-ammonium (**48**) (Scheme 3.14) participated in the ligand exchange reaction, generating amphiphilic G2-cationic dendrimer, $[Fe_4S_4(S\text{-}G2\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**52**) with the red-

wine color phenomenon. However, the ^{1}H NMR spectrum exhibited only a partially substituted dendrimer in the reaction. The relative integration of two characteristic peaks to signal C indicated a ratio of 2.3 : 32 instead of the expected 4 : 32 (Figure 3.4). This result might be explained by the disulfides generated due to oxygen remaining in the reaction mixture during deprotection of the thiocarbamates, yielding low thiol stoichiometry. Also, the use of heat (refluxing of methanol solution) and base catalyst (sodium hydroxide) under such condition may be positive factor in the generation of the disulfide. Specifically, in order to remove the solvent, a rotary evaporator was used after deprotection. It was a moment that the reaction mixture can be exposed to air.[81-85]

Another possibility is that the G2-thiolate ions were formed in sufficient amount but were less reactive for ligand exchange. In the preparation of flexible G2 dendrimer, there was not a problem for hydrophobic dendrons as evidenced by our earlier results. However, in this case, perhaps the amphiphilic nature of the dendrons results in a conformational bias that shields the thiol. This can be occurred because the number of peripheral units increased exponentially from G1 to G2 dendron.

Another possibility is that the G2-thiolate ions were generated in insufficient amount in the deprotection due to the same reason above. The thiocarbamate group can be shielded by bulkier amphiphilic dendritic unit than G1-dendron. The attack of the thiocarbamate via base catalyst may be limited. Also, even if the thiols are generated, the exposure to oxygen mentioned above can increase the amount of the

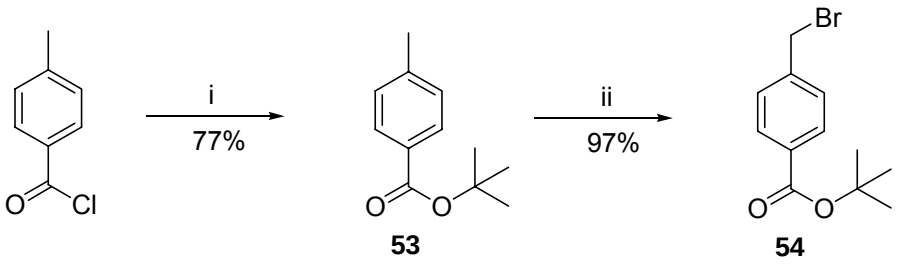
disulfide.

In addition to the concerns raised above, thiol oxidation involves proton loss, and is going to be facilitated in a hydrogen-bond accepting solvent. Solvation of nucleophilic anion (thiolate ions) influences its reactivity. The polar protic solvent (methanol) used in this deprotection solvate thiolate ions by hydrogen bonding interactions. These solvated species are more stable and less reactive than the unsolvated ions. Also, this solvent effect is more addressed for small basic anions than for large weakly basic anions.[40,57] Thus, as the generation of dendron increases, the anions are not solvated and thus are more reactive, resulting in forming the disulfides.

3.2.1.3. Anionic Dendron containing Thiocarbamate

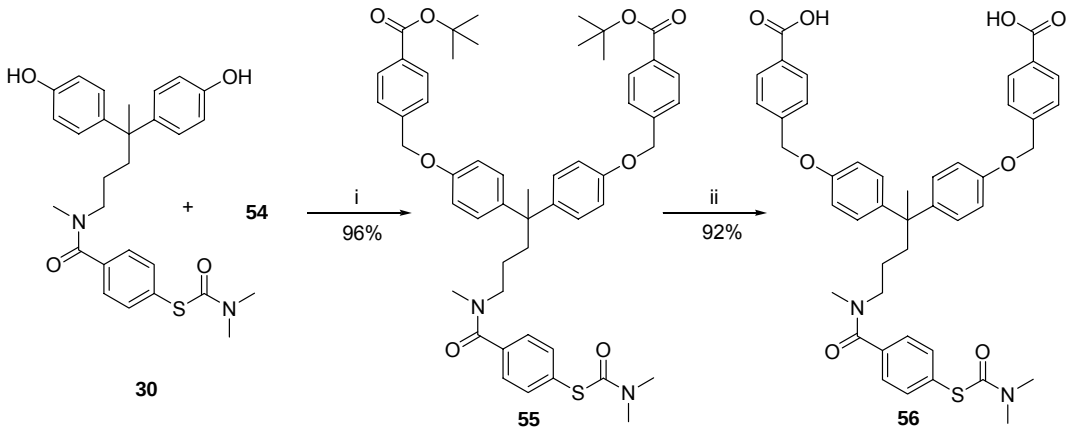
After the successful synthesis and characterization of amphiphilic G1-cationic dendrimer, the synthesis of anionic dendron and dendrimer was attempted. A carboxylate polar end group[27-39] was used as an anionic peripheral unit to impart water-solubility, which is sensitive to pH. High pH values are required to deprotonate the carboxylic acid to carboxylate salts which is water-soluble. At low pH, the carboxylate is protonated and dendrimers will precipitate from water.

Scheme. 3.15.[a]



[a]Reagents and conditions: i) t-BuOK, t-BuOH, THF; ii) NBS, AIBN, CH_2Cl_2.

Scheme 3.16.[a]



[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone; ii) TFA, CH_2Cl_2.

The benzylic bromide precursor of the peripheral unit was prepared (Scheme 3.15). The t-butyl group was used as a protecting group of the carboxylic acid.[86] 4-Methyl benzoic acid *tert*-butyl ester (**53**) was prepared with a coupling of p-toluoyl chloride with potassium t-butoxide in acceptable yield. Then, the benzyl bromide, 4-

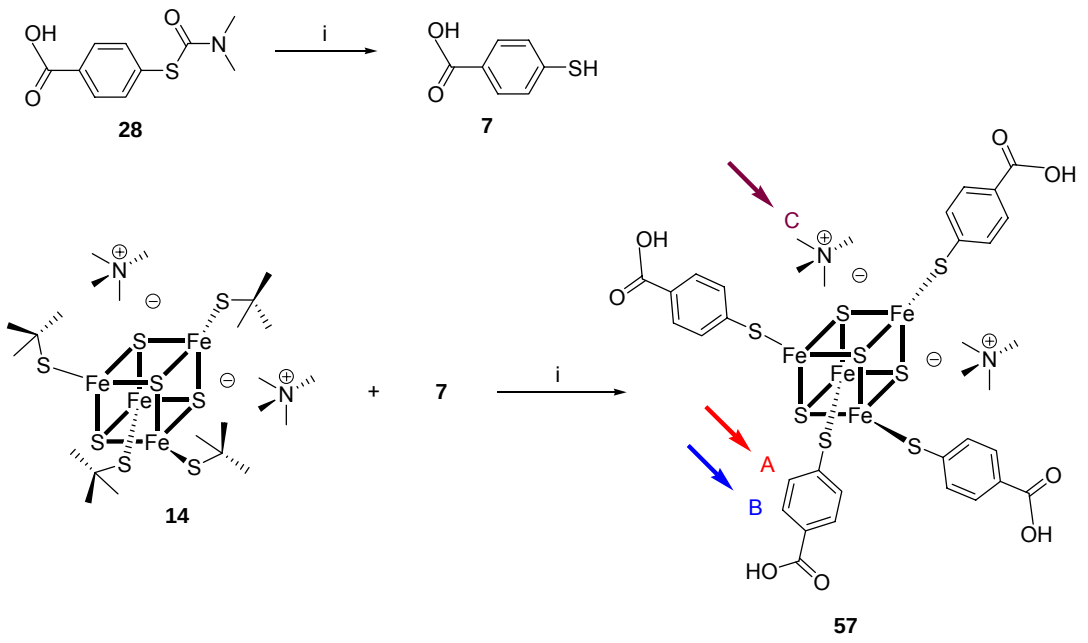
bromomethyl benzoic acid t-butyl ester (**54**), was synthesized by free radical bromination of the *t*-butyl ester (**53**) and NBS.[41]

To introduce the peripheral unit to the phenolic dendron prepared by a modular synthesis approach, G1-Thiocarbamate-OH (**30**) was reacted with the benzyl bromide (**54**) to give G1-Thiocarbamate-t-Bu (**55**), (Scheme 3.16). The t-butyl group was removed with an excess amount of trifluoroacetic acid[21,86], yielding amphiphilic G1-anionic dendron, G1-Thiocarbamate-COOH (**56**).

3.2.1.4. Anionic Dendrimers from Thiocarbamate

Prior to the synthesis of the G1-anionic dendrimer, G0-anionic model compound was prepared. 4-Mercaptobenzoic acid (**7**) was generated by basic hydrolysis of 4-dimethylthiocarbamoylsulfanyl benzoic acid (**28**) (Scheme 3.17). Ligand exchange of the iron-sulfur core, $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (**14**), with **28** yielded G0-anionic dendrimer, $[Fe_4S_4(S\text{-}G0\text{-}COOH)_4][N(CH_3)_4]_2$ (**57**). The ratio of dendron ligand to iron-sulfur core was 5 to 1. Also, using commercially obtained 4-mercaptobenzoic acid (**7**), G0-anionic dendrimer was prepared. The results of both ligand exchange reactions were compared. The results indicated problems which can occur in deprotection.

Scheme 3.17.[a]



[a]Reagents and conditions: i) NaOH, THF, MeOH; ii) DMF.

Dendrimers prepared by both routes were characterized by ^{1}H NMR spectroscopy and MALDI-TOF. The ^{1}H NMR spectra of the two G0-anionic dendrimers (**57**) were compared (Figure 3.5). Two characteristic peaks (**A** and **B**) indicated the expected 8 protons compared to signal **C** of 24 protons, respectively. However, in addition to the desired dendrimer, residual disulfide was observed when the dendrimer was prepared after the deprotection of the thiocarbamate group. Due to oxygen remaining in the reaction mixture during deprotection, disulfides may be generated. Also, in the presence of even a small amount of oxygen, the use of heat (refluxing of methanol solution) and base catalyst (sodium hydroxide) may generate

the disulfide. Specifically, in order to remove the solvent, the use of a rotary evaporator after deprotection did allow some exposure to air.[81-85] Because the disulfides present for G0, this can be a significant problem for G1 and G2. In this aspect, G2-cationic dendrimer might not be prepared due to high disulfide concentration.

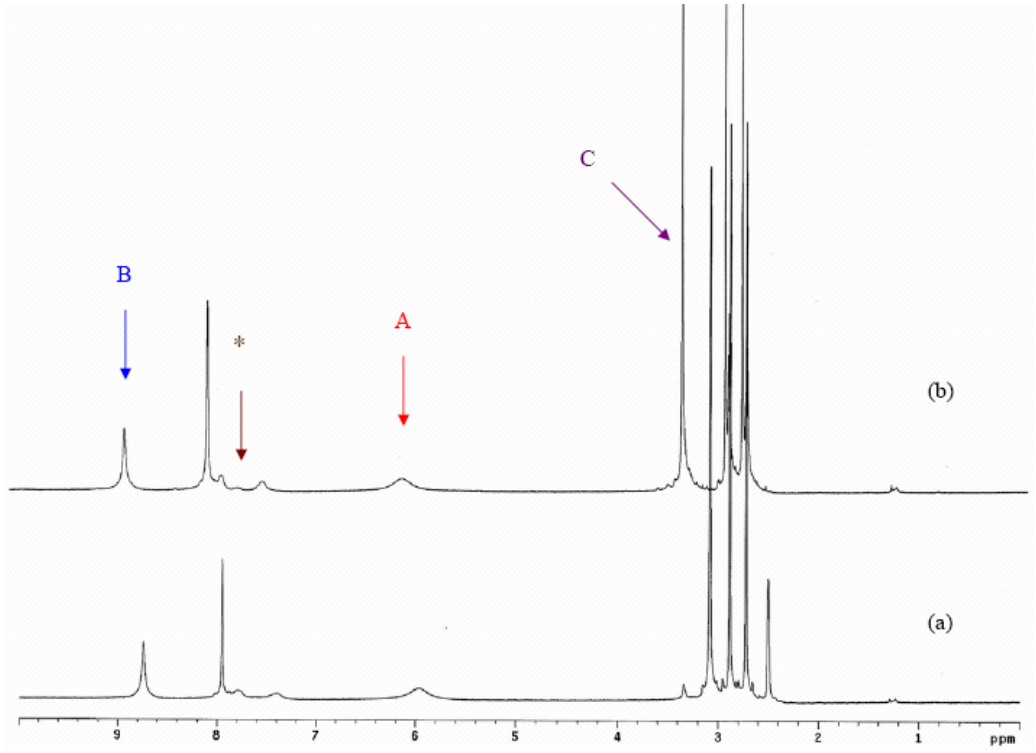


Figure 3.5. ^{1}H NMR spectra of G0-anionic dendrimers. (a) G0-dendrimer $[Fe_4S_4(S\text{-}G0\text{-}COOH)_4][N(CH_3)_4]_2$ (**57**) prepared via commercially obtained **7** (solvent : DMSO-d_6), (b) G0-dendrimer $[Fe_4S_4(S\text{-}G0\text{-}COOH)_4][N(CH_3)_4]_2$ (**57**) prepared via **7** generated by basic hydrolysis of the thiocarbamate (Scheme 3.17) (solvent : DMF-d_7), (* indicates a peak due to disulfide).

In MALDI-TOF mass spectrometry with dithranol used as a matrix, dendrimers (**57**) synthesized by both routes indicated a mass of 1037 in negative mode, while the calculated mass was 1111 (Figure 3.6). A value of 1037 could be calculated as the species: $[M + [N(CH_3)_4]]^-$ = 1037. However, there were several unknown peaks with m/z values of less than 950. These peaks might be generated by the reaction of dendrimer with matrix during measurement.

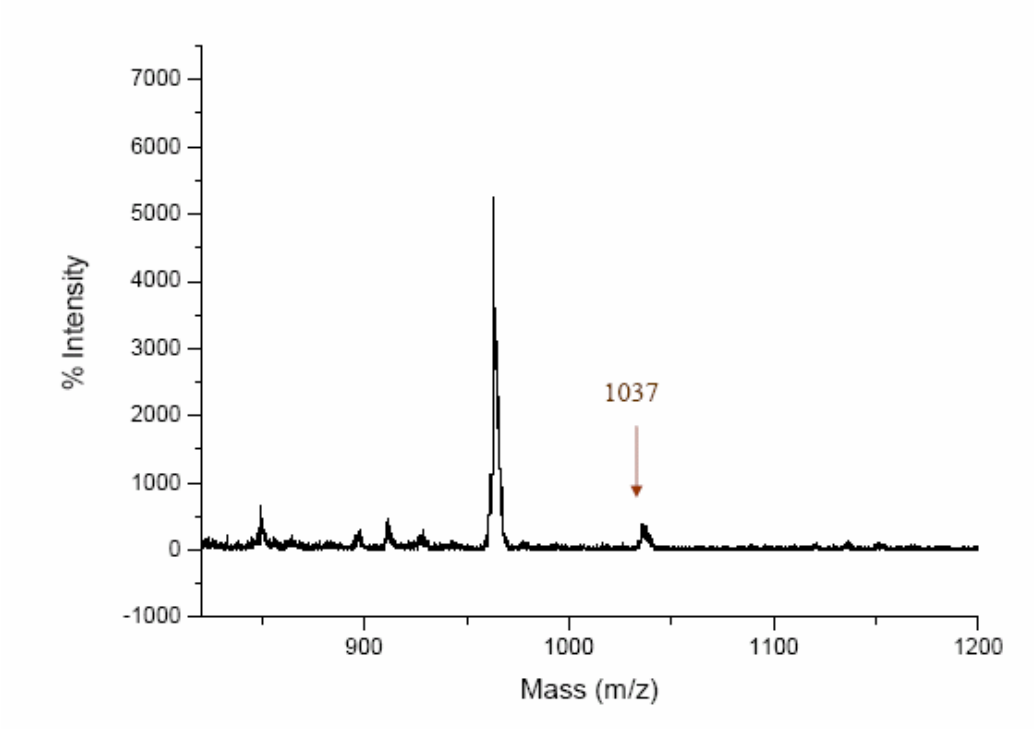
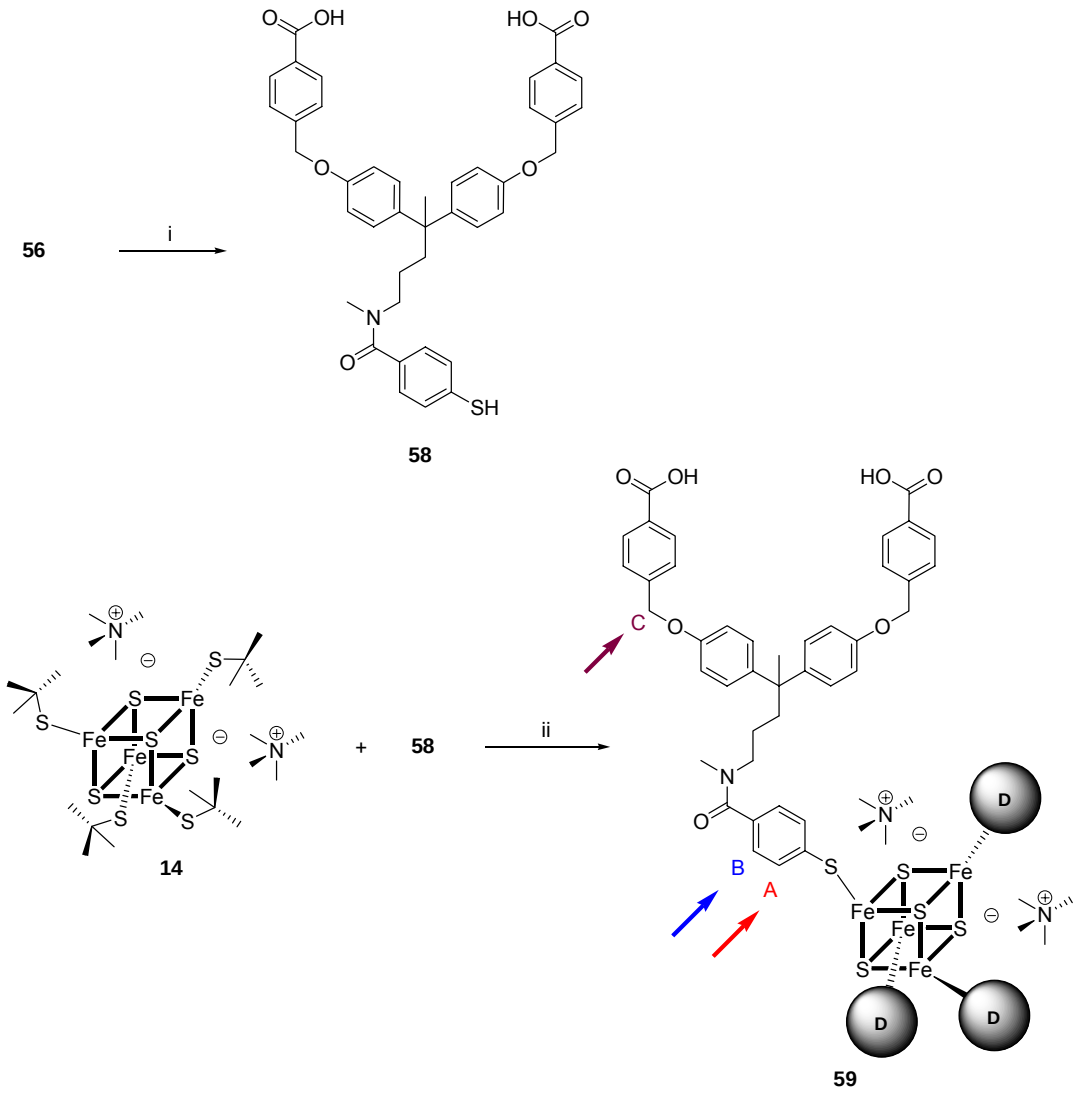


Figure 3.6. MALDI-TOF measurement of amphiphilic G1-anionic dendrimer, $[Fe_4S_4(S\text{-}G0\text{-}COOH)_4][N(CH_3)_4]_2$ (**57**) (Matrix : dithranol).

Scheme 3.18.[a]



[a]Reagents and conditions: i) NaOH, THF, MeOH; ii) DMF.

With the successful preparation of G0-anionic dendrimer, the synthesis of G1-anionic dendron was attempted. G1-Thiocarbamate-COOH (**56**) was deprotected by basic hydrolysis to the corresponding thiol, G1-SH-COOH (**59**), (Scheme 3.18). In

this deprotection, it was found that reaction temperature and the amount of sodium hydroxide were important. The product was not generated when the reaction mixture was refluxed with more than 3.5 equivalents of sodium hydroxide. Presumably due to the attack of the base catalyst on the benzyl linkages in the dendron, a fragmented product was obtained.

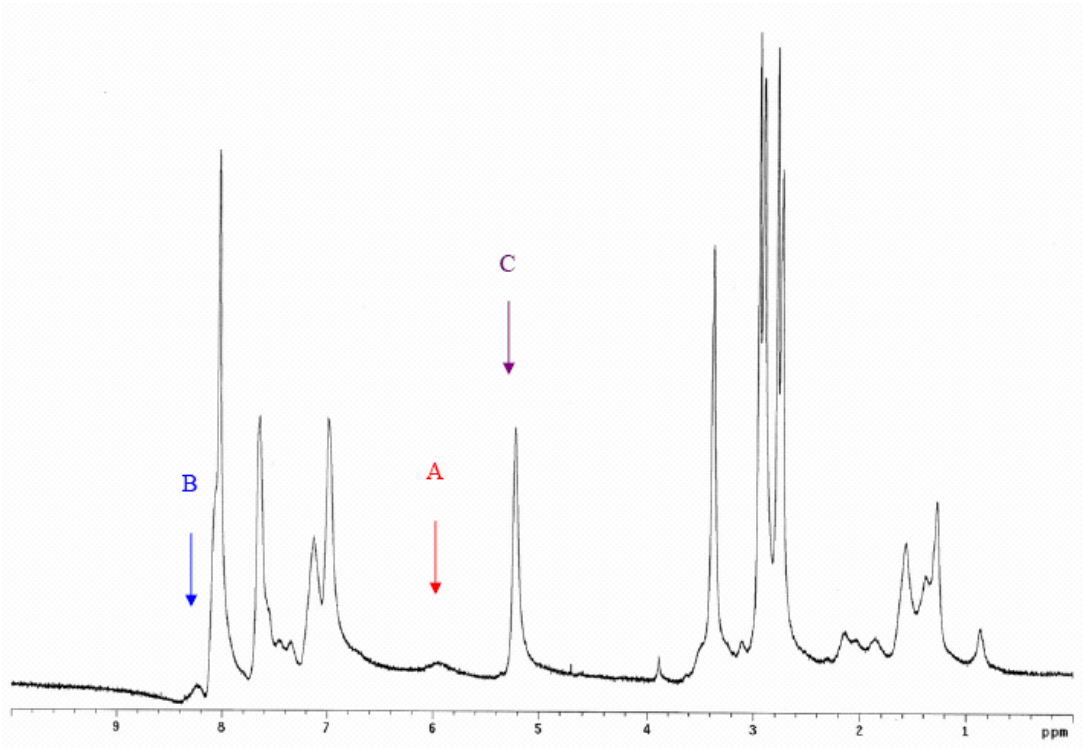


Figure 3.7. ^{1}H NMR spectrum of amphiphilic G1-anionic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (**59**), prepared using NaOH / methanol / reflux to deprotect **56** followed by ligand exchange (Scheme 3.18) (solvent : DMF-d_7).

After the formation of anionic thiol ligand (**58**), the ligand exchange reaction was performed. The red-wine color phenomenon was observed like with the G1-cationic dendrimer (**50**), generating amphiphilic G1-anionic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (**59**). The G1-anionic dendrimer (**59**) was also characterized by ^1H NMR spectroscopy and MALDI-TOF. The ^1H NMR spectrum indicated the fully substituted dendrimer, showing complete ligand exchange reaction occurred (Figure 3.7). The integration of two characteristic peaks (**A** & **B**) exhibited the expected relative integration of 8 protons compared to signal **C** of 16 protons, respectively.

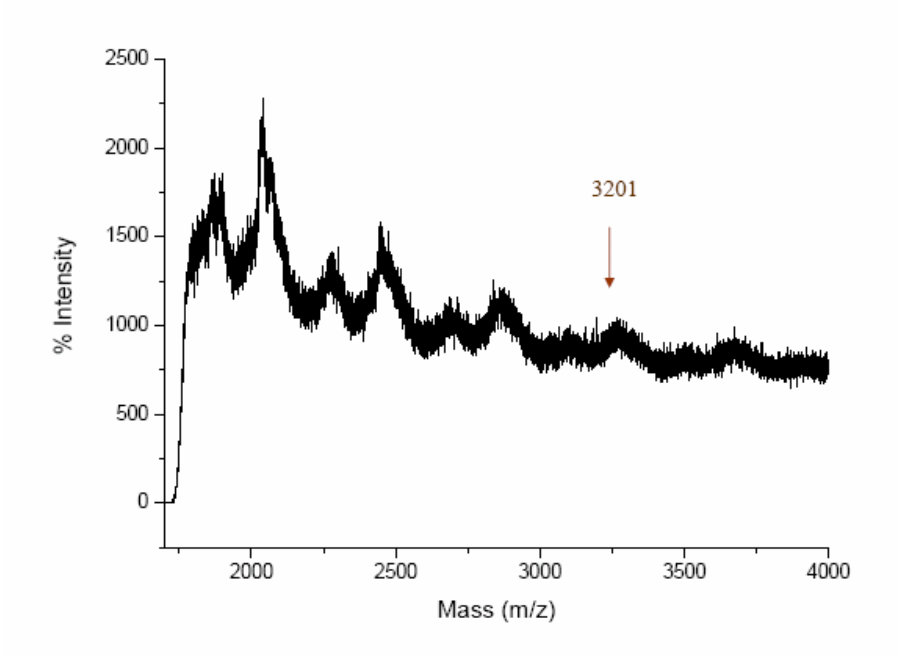


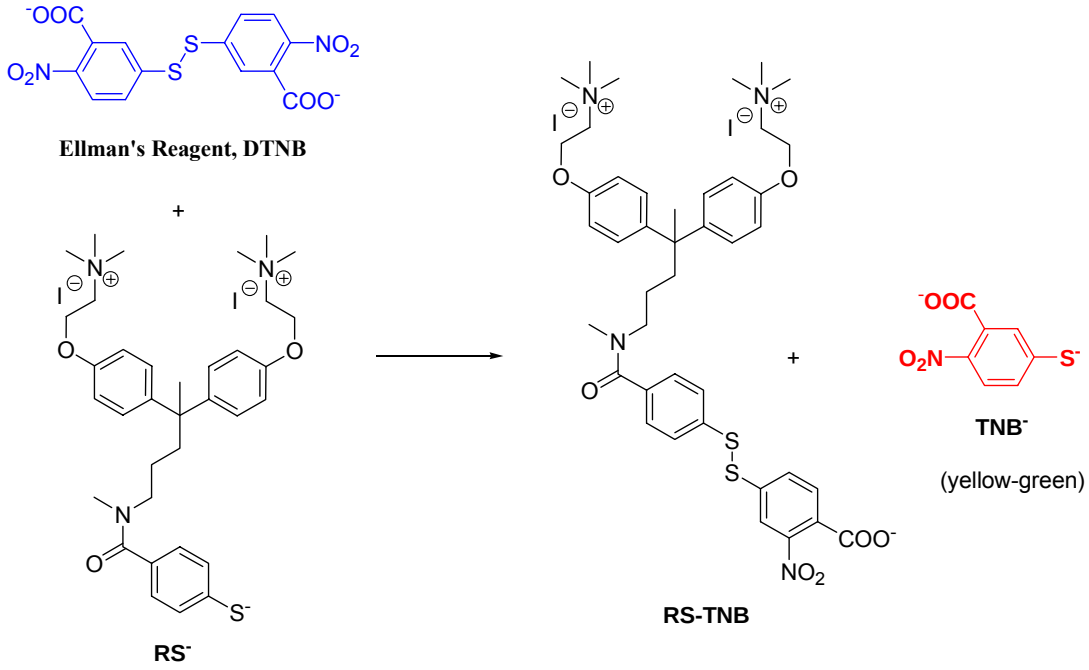
Figure 3.8. MALDI-TOF measurement of amphiphilic G1-anionic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (**59**) (Matrix : dithranol).

MALDI-TOF was measured in both positive mode and negative mode with dithranol as a matrix (Figure 3.8). However, multiple peaks as several unknown peaks seen in G0-anionic dendrimer (**57**) were observed. This could indicate that the dendrimer reacted with the matrix during measurement like in G0-anionic dendrimer. The matrix was then changed from dithranol into 2,5-dihydroxybenzoic acid (DHB), trans-3-indoleacrylic acid (IAA), and 2-(4-hydroxyphenylazo)benzoic acid (HABA), respectively. MALDI-TOF measurements were repeated but, the results were the same. In these spectra, a reoccurring peak was observed at 3201 in positive mode and the exact mass calculated was 3252. A value of 3201 could be calculated as the species: $[M + [N(CH_3)_4] + Na + H]^+ = 3201$ and thus, G1-anionic dendrimer (**59**) was also prepared and characterized successfully.

3.2.1.5. Ellman's Reagent Test

Disulfides are inevitably generated in the process of a deprotection reaction. The thiocarbamate group was deprotected by refluxing (heat) in methanol in the presence of sodium hydroxide (base catalyst). Under these conditions, disulfides might be generated if any oxygen is present as an oxidizing agent. Disulfides are less reactive with the iron-sulfur cluster for ligand exchange. Thus, the relative amount of disulfide present along with the thiols produced during deprotection is likely a critical variable in ligand exchange reactions to prepare dendrimer.

Scheme 3.19.



For this reason, it was considered important to find a way to determine the amount of thiol present after the deprotection was performed. The thiol concentration was measured using Ellman's reagent test.[87-89] Ellman's reagent test is the most popular method for quantitating both protein and non-protein thiols. The test is based on the thiol-disulfide interchange reaction.[89] The thiolate anion (RS^-) from thiols such as the G1-cationic thiol dendron (**49**) reacts with excessive Ellman's reagent [5,5′-dithiolbis(2-nitrobenzoic acid), DTNB] at pH 7 – 8. It rapidly forms a mixed disulfide (RS-TNB) and releases TNB thiolate anion (5-thio-2-nitrobenzoate, TNB^-) which is colored (Scheme 3.19). Ellman[87] determined the molar extinction coefficient of 4-nitrophenylthiolate (13.6×10^3 cm^{-1} M^{-1} at 412 nm

in 50 % aqueous acetone solution containing 20 mM phosphate, pH 8) and used this number for TNB thiolate anion. If 1.1 mL of solution is taken and mixed with 10 μL of 100 mM Ellman's reagent, the thiol concentration is calculated using the formula below:

$$\text{mM of Thiol} = \frac{\Delta A_{412} \times 1.1\text{mL}}{13{,}600 \times 0.4\text{mL}} \times 1000 \qquad \text{(Equation 3.1.)}$$

where ΔA_{412} is the corrected absorbance value, and 13,600 is the molar extinction coefficient ($cm^{-1}\ M^{-1}$) of TNB thiolate anion.

Table 3.2. Measurements of concentration of several thiols via Ellman's reagent tests.

Thiols	Expected concentration (mM)	Concentration determined using Ellman's reagent (mM)	Percentage (%)
cysteine	0.1	0.093	93
4-methoxythiophenol	0.1	0.0002	0
4-mercaptobenzoic acid (**7**)	0.1	0.090	90
G1-SH-Ammonium (**49**)	0.1	0.049	49
G1-SH-COOH (**58**)	0.1	0.046	46
G2-SH-Ammonium (**51**)	0.1	0.034	34

Tests of several thiols, particularly those generated during deprotection reactions, were performed with Ellman's reagent to quantitate the amount of free thiols present (Table 3.2). The first two compounds, cysteine and 4-methoxythiophenol, were measured to ensure that the test worked adequately on stock solutions of known concentration. In the case of cysteine, commercially available, 99% cysteine was used. The thiol concentration was calculated to 93%. The 6% reduction can be attributed to either the oxidation or some experimental error. In the case of commercially available 4-methoxythiophenol, this molecule was insoluble in Buffer A, an acetate solution of pH 4.7. The test was performed on the suspension with mixing, but no thiol was detected under these conditions, indicating that thiol must be dissolved. The 4-mercaptobenzoic acid dissolved in Buffer A after changing the hydrogen into potassium in 1N potassium carbonate aqueous solution. The molecule was reported to be 95 % pure by the supplier, and a reasonable value within experimental error of 90 % was obtained from the Ellman's test.

The G1-SH-Ammonium (**41**) and G1-SH-COOH (**58**) dendrons were deprotected with sodium hydroxide and Ellman's test on the resulting reaction products indicated percentage of thiol of 49% and 46%, respectively. In separate trials, G1-cationic and G1-anionic dendrimers were prepared successfully using the ratio of 6 to 1 (thiol ligand to iron-sulfur core) and characterized. However, a complete ligand exchange reaction could not occur with this ratio unless at least 65 % of the starting material was converted to the thiol. This result led to the

concern that some of the thiol (in this case ca. 20%) might have been oxidized during the Ellman's reagent test. Keeping this caveat in mind, when deprotection/Ellman's test was run to produce G2-SH-Ammonium (**51**), the percentage of thiol was determined to be 34%. This lower amount of thiol obtained explains why ligand exchange reaction for G2-cationic dendrimer was not complete. Even if 20% of the thiol was oxidized during the test, the original amount would not have been 65%.

After detecting disulfide using Ellman's reagent test, HPLC was used in attempt to separate the disulfide from the G1-SH-Ammonium (**41**). C8 and C18 reverse phase columns were used with methanol, H_2O, and CH_3CN. The chromatogram for G1-SH-Ammonium (**41**) was compared to that of G1-Sym-Disulfide-Ammonium (**12, Chapter 2**). However, the peaks associated with both the compounds were broad and overlapped, indicating the same retention time. Many attempts were made to improve the resolution. These included variations in the flow rate, solvent system, and buffer solution. DTT was added in attempt to prevent oxidation of the thiol. However, none of these conditions were effective in disulfide separation.

In the case of aliphatic thiols and small molecules, Ellman's tests were successfully carried out with the generation of only a small amount of disulfide. However, in amphiphilic G1 & G2 thiol dendrons, the thiol concentration was lower

than expected, leading to the suspicion that some of the thiol may have been oxidized to the disulfide during the course of the tests.[90,91] This result could be due to the large surface area of dendrons containing hydrophilic groups, which may show a high affinity for water. Oxygen dissolved in the water solution may oxidize thiols to disulfides. Also, because the test solutions exposed to air during measurement of UV, thiols might be oxidized. Thus, Ellman's reagent test may show a limitation in measurements of the amount of thiol produced during deprotection of polar dendrons.

3.2.2. Synthesis from Trityl Protecting group

G1-cationic and G1-anionic dendrimers were prepared successfully and characterized. However, G2 dendrimer could not be prepared due to a low amount of thiol during deprotection. The deprotection of the thiocarbamates using heat and base catalyst might be negative factors in the generation of amphiphilic thiol dendrons.[81-85] Thus, as a new approach, a trityl group was employed. Trityl thiols can be deprotected with acid catalyst and at room temperature.[7-13,15,21,34] Since thiol oxidation to disulfide involves loss of two protons and two electrons, this process should be less favorable as the pH is lowered. Thus, it was expected that the extent of thiol oxidation in acidic conditions would be reduced and compared to that observed in basic conditions.

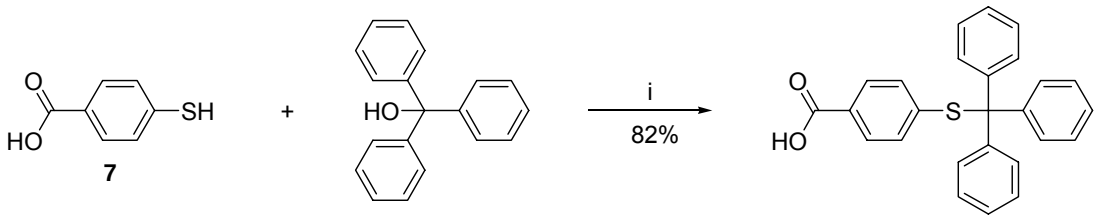
In this section, the preparation of amphiphilic cationic and anionic dendrimers

containing ammonium[22-26] and carboxylate[27-39] peripheral units and a trityl thiol focal group is described. This work includes the synthesis of amphiphilic thiol dendrons and the measurements of the amount of thiol produced during deprotection by ^{1}H NMR spectra and Ellman's reagent test[87-89].

3.2.2.1. Cationic Dendron containing Trityl

The synthesis of cationic dendrons containing the trityl group was started from the flexible dendritic unit, 4,4′-bis(4′-hydroxyphenylvaleric) acid (**1, Chapter 2**) and the dendritic fragment (**6, Chapter 2**) prepared identically as shown in Scheme 2.1.

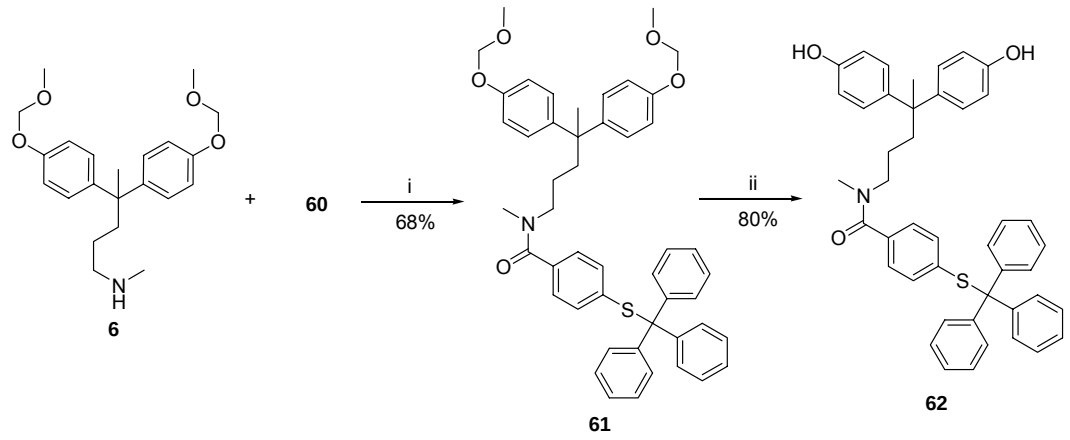
Scheme 3.20.[a]



[a]Reagents and conditions: i) TFA.

4-Tritylsulfanyl benzoic acid (**60**) was prepared by the reaction of 4-mercaptobenzoic acid (**7**) with triphenyl methanol in excess amount of trifluoroacetic acid in acceptable yield (Scheme 3.20).

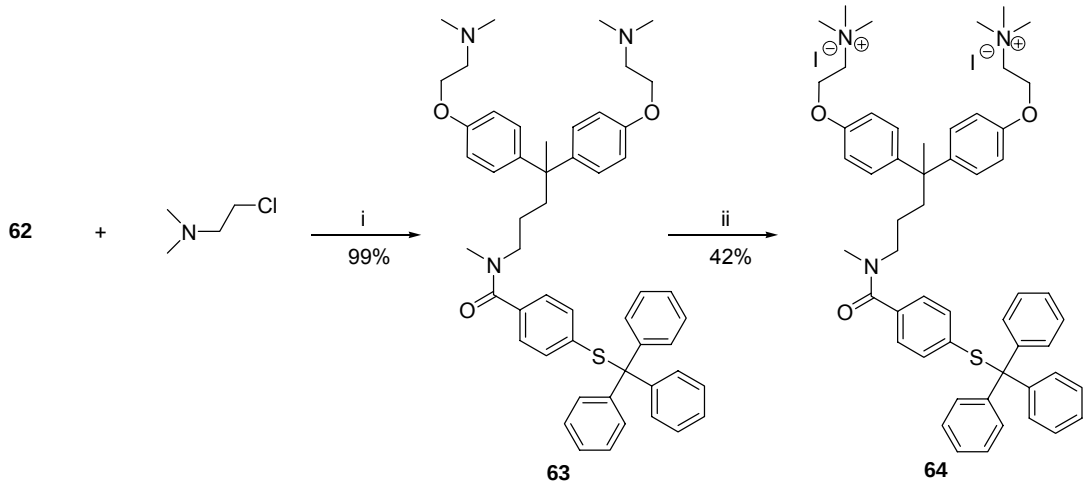
Scheme 3.21.[a]



[a]Reagents and conditions: i) DCC, DMAP, CH_2Cl_2; ii) HCl, MeOH.

The procedure to synthesize the G1-cationic dendron was identical to that for G1-dendron from the thiocarbamates. The dendritic fragment, G1-MA-MOM (**6, Chapter 2**) was coupled with 4-Tritylsulfanyl benzoic acid (**60**) using the convergent strategy (Scheme 3.21). The DCC mediated coupling reaction produced the amide, G1-STr-MOM (**61**), in excellent yield. The phenolic dendron, G1-STr-OH (**62**) was prepared by the deprotection of G1-STr-MOM (**61**) with hydrochloric acid in methanol solution. In this step, it was important to use gentle heat because the trityl group can be deprotected with hydrochloric acid.

Scheme 3.22.[a]



[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone; ii) CH_3I, CH_2Cl_2.

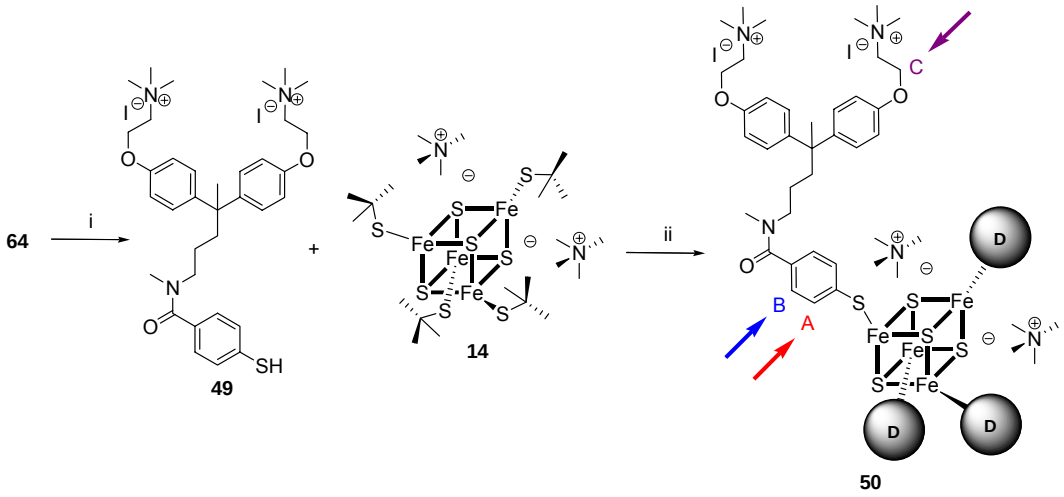
The phenolic dendron prepared by modular synthesis approach, G1-STr-OH (**62**) reacted with the precursor of peripheral unit, N,N-dimethylaminoethyl chloride·HCl, to give G1-STr-MA (**63**) (Scheme 3.22). G1-STr-MA (**63**) was then methylated with methyl iodide[23-26,58,59] to give the amphiphilic cationic dendron, G1-STr-ammonium (**64**).

3.2.2.2. Cationic Dendrimer from Trityl-protected Thiol Dendron

To be used as a dendritic ligand, the G1-STr-ammonium (**64**) containing the trityl group was deprotected in the mixture of an excessive amount of trifluoroacetic acid and a small amount of triethylsilane, affording the corresponding thiols, G1-SH-Ammonium (**49**) (Scheme 3.23).[7-15] The percentage of thiol formed was

measured by Ellman's reagent test[87-89] and found to be 40%. Unfortunately, the value was not higher than that of the thiocarbamates. It might suggest that the catalytic conditions were not important in the generation of disulfides.

Scheme 3.23.[a]



[a]Reagents and conditions: i) TFA, Et_3SiH; ii) DMF.

Despite the low yield of thiol in the deprotection of **64**, a ligand exchange reaction was run with this reaction mixture. In the dry box, amphiphilic G1-cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**50**), was prepared by the ligand exchange reaction of the cationic dendron ligand (**49**) with iron-sulfur core, $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (**14**). The red-wine color was observed. The ratio of dendron ligand to iron-sulfur core was 6 to 1. The ^1H NMR spectrum showed only partially substituted dendrimer in the ligand exchange reaction which is attributed to

insufficient thiol for complete exchange (Figure 3.9). The relative integration of two characteristic peaks (**A** and **B**) to signal C indicated a ratio of 2.6 : 16 instead of the expected 8 : 16.

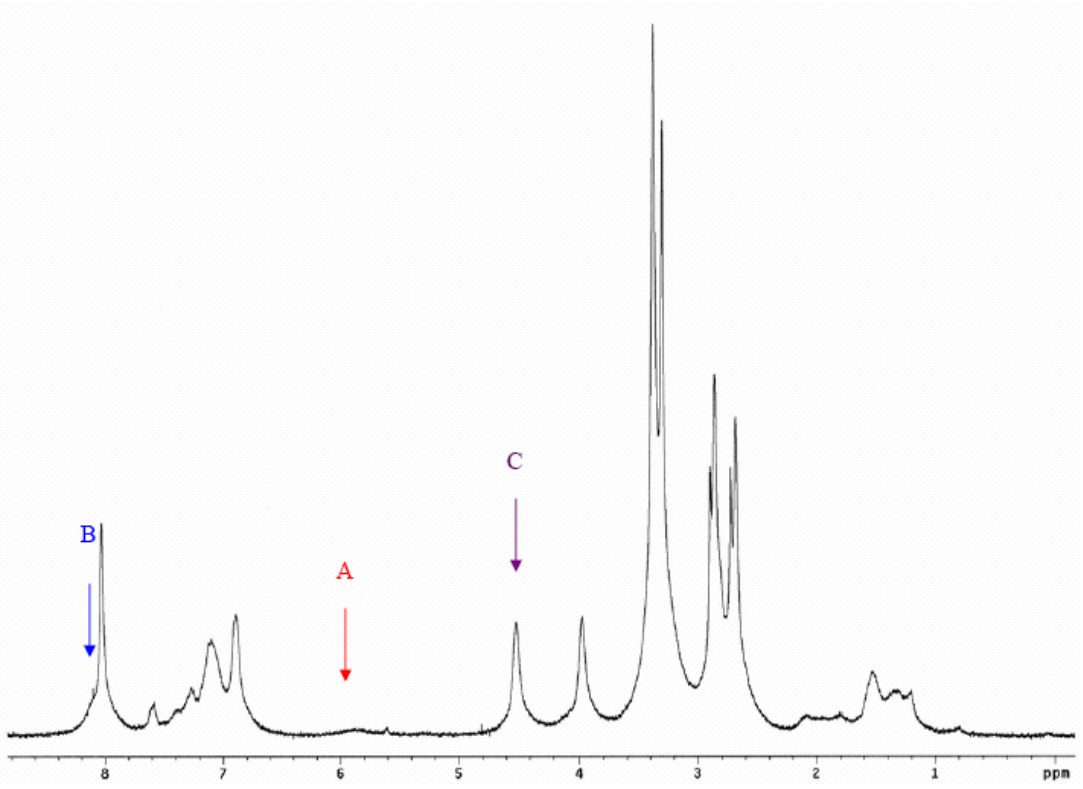
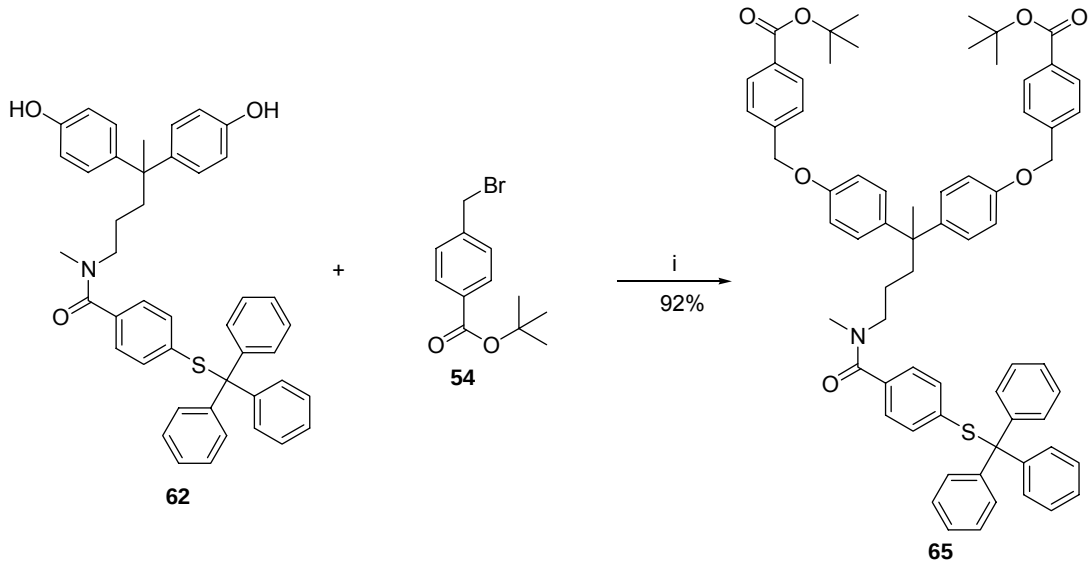


Figure 3.9. ^{1}H NMR spectrum of amphiphilic G1-cationic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (**50**), prepared using Et_3SiH / CF_3COOH / room temp. to deprotect **64** followed by ligand exchange (Scheme 3.23) (Solvent : DMF-d_7).

3.2.2.3. Anionic Dendron containing Trityl

The procedure to synthesize the G1-anionic dendron was similar to that used in the G1-cationic dendron. The phenolic dendron, G1-STr-OH (**62**), was reacted with the benzyl bromide (**54**) to give G1-STr-t-Bu (**65**) by nucleophilic substitution reaction (Scheme 3.24). This sequence nicely illustrates the efficiency of the modular synthesis approach used here.

Scheme 3.24.[a]



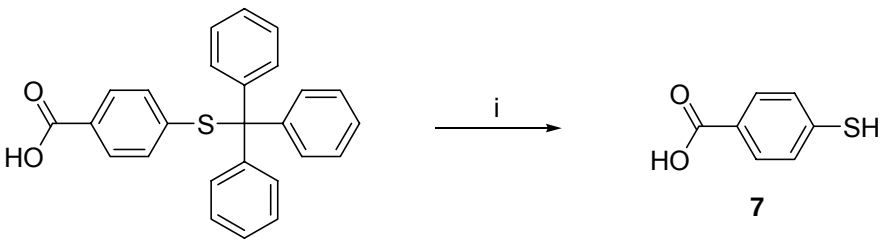
[a]Reagents and conditions: i) K_2CO_3, 18-crown-6, acetone.

3.2.2.4. Anionic Dendrimer from Trityl-protected Thiol Dendron

Before deprotection and dendrimer formation using molecule **65**, a G0-trityl compound was synthesized and deprotected as a model study for the preparation of

the G1-anionic thiol. The molecule 4-mercaptobenzoic acid (**7**) was prepared by the deprotection of 4-tritylsulfanyl benzoic acid (**60**) with trifluoroacetic acid and triethylsilane (Scheme 3.25). The thiol concentration in Ellman's reagent test[87-89] was 82% compared to 90% measured for commercially obtained 4-mercaptobenzoic acid (**7**). The difference of about 10% between them is attributed to disulfide formation during deprotection, impurities, and experimental error. On the other hand, the ^{1}H NMR spectrum in DMSO showed the thiol concentration of **7** was 88%. This suggests that ^{1}H NMR spectroscopy could be used as an alternative to the Ellman's reagent test to measure the thiol concentration. However, in this trial, there was no effort to eliminate water and oxygen present in the NMR solvent. These impurities should ideally be removed, adding some effort to this methodology.

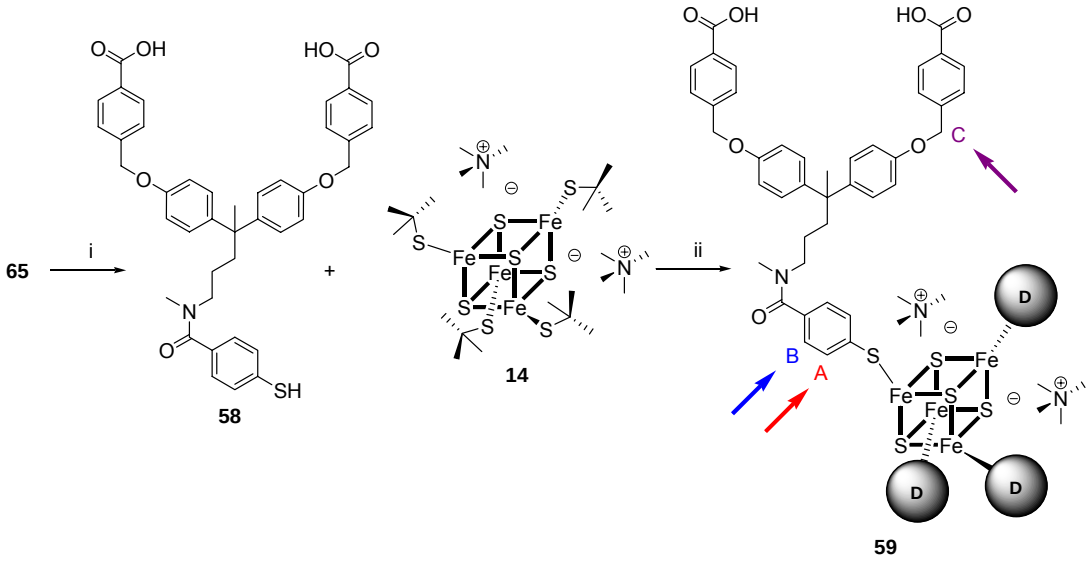
Scheme 3.25.[a]



60 **7**

[a]Reagents and conditions: i) TFA, Et_3SiH.

Scheme 3.26.[a]



[a]Reagents and conditions: i) TFA, Et_3SiH; ii) DMF.

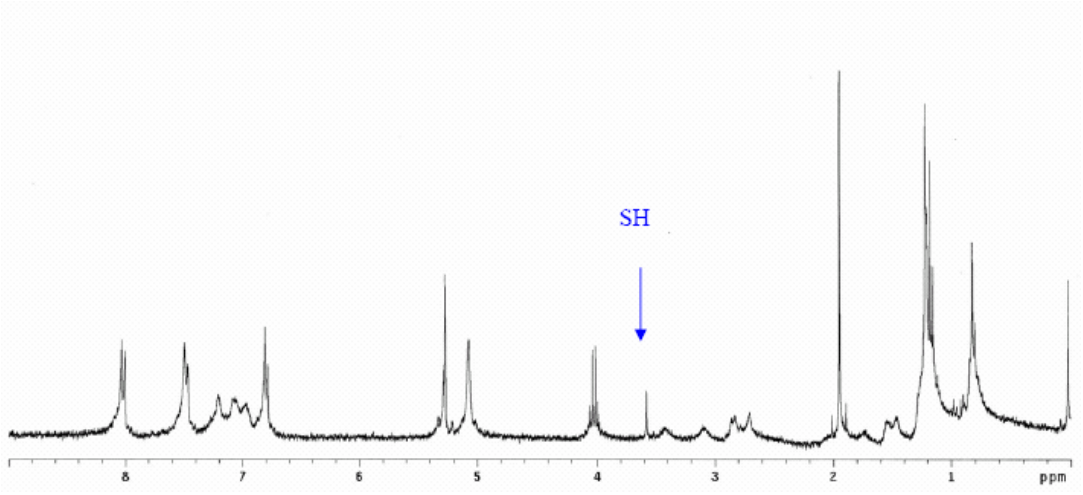


Figure 3.10. ^{1}H NMR spectrum of amphiphilic anionic thiol dendron, G1-SH-COOH (**58**), prepared using Et_3SiH / CF_3COOH / room temp. to deprotect **65** (Scheme 3.26) (solvent : CD_2Cl_2).

With the deprotection of the G0-trityl compound, G1-STr-t-bu (**65**) was also deprotected with the addition of small amount of triethylsilane in an excess amount of trifluoroacetic acid (Scheme 3.26). This procedure yielded the corresponding thiol, G1-SH-COOH (**58**).[7-15] The percentage of thiol was measured by ^{1}H NMR and Ellman's reagent test.[87-89] The Ellman's reagent test indicated 29% thiol and the ^{1}H NMR spectrum indicated 43% thiol in CD_2Cl_2 solvent (Figure 3.10). The higher thiol concentration measured by ^{1}H NMR spectroscopy might be explained by the fact that the NMR solvent, CD_2Cl_2, was exposed to less oxygen than the samples that underwent Ellman's reagent test. As with the G1-cationic dendron (**49**), the measured thiol concentration for the anionic dendron prepared with the trityl group was less than that for dendrons protected by thiocarbamates. It might also indicate that catalytic conditions were not important in generation of the disulfides like mentioned in G1-cationic dendron (**49**).

The difference in the relative amount of thiol when comparing the deprotection of G0-SH-COOH (82%) and G1-SH-COOH (29%) can be explained by the same rationale suggested for the G2-SH- Ammonium (**51**). Due to oxygen remaining in the reaction mixture during deprotection of the trityl, the generated thiols may be re-oxidized. Also, by a larger amphiphilic dendritic unit than G0-dendron the trityl group can be shielded. The deprotection of the trityl group might be sterically hindered, yielding small amount of thiol.

Another possibility is that thiol oxidation is related proton loss, and that is

facile in a hydrogen-bond accepting solvent. Solvation of nucleophilic anion (thiolate ions) influences its reactivity. The thiolate ions are solvated by hydrogen bonding interactions in polar protic solvent (methanol) used in this deprotection. These solvated species are more stable and less reactive than the unsolvated ions. Also, this solvent effect is more pronounced for small basic anions than for large weakly basic anions.[40,57] Therefore, as the generation of dendron increases from G0 to G1, the anions are solvated a little and thus are more reactive, resulting in generating the disulfides.

In the dry box, amphiphilic G1-anionic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (**59**) was prepared with the red-wine color as was seen in the cationic dendrimer. The ^1H NMR spectrum exhibited partially substituted dendrimer which is attributed to low thiol concentration (Figure 3.11). The relative integration of two characteristic peaks (**A** and **B**) to signal C indicated a ration of 2.4 : 16 instead of the expected 8 : 16.

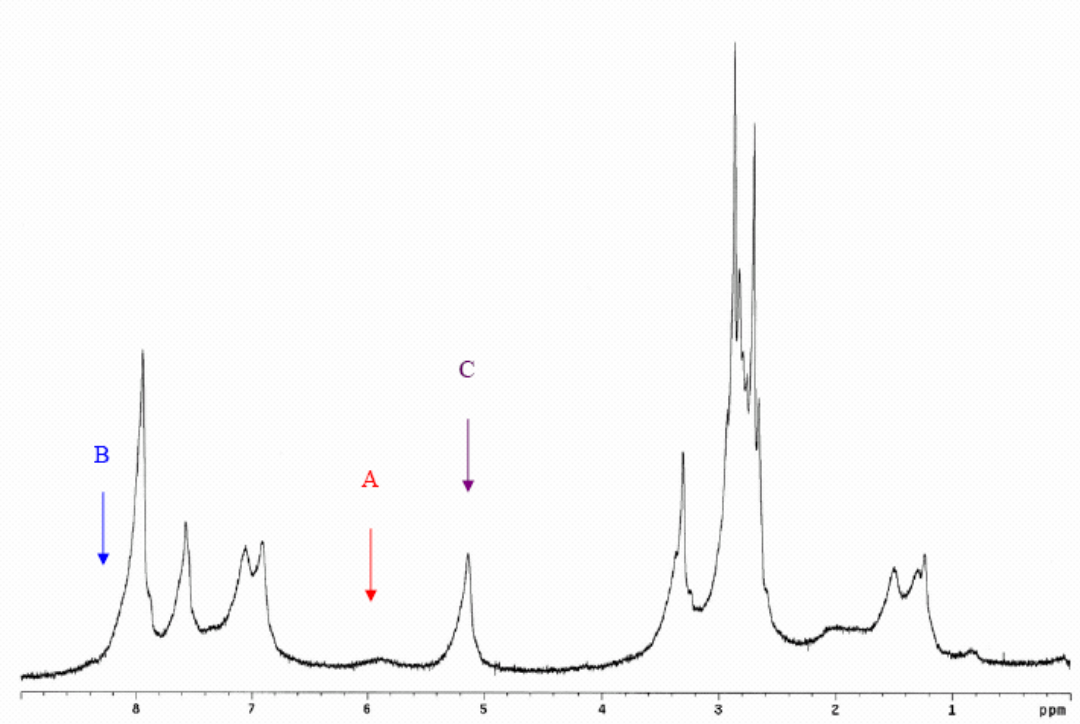


Figure 3.11. ^{1}H NMR spectrum of amphiphilic G1-anionic dendrimer, $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (**59**), prepared using Et_3SiH / CF_3COOH / room temp. to deprotect **65** followed by ligand exchange (Scheme 3.26) (solvent : DMF-d_7).

The protecting group was changed from the thiocarbamate group into the trityl group because the conditions for deprotection were predicted to yield a lower concentration of disulfide. However, this did not increase the percentage of thiol produced during deprotection. Ellman's reagent test indicated 40% for G1-SH-Ammonium dendron (**49**), 82% for G0-SH-COOH (**7**), and 29% for G1-SH- COOH (**58**). Also, the ^{1}H NMR spectrum showed 88% for G0-SH-COOH (**7**) and 43% for G1-SH-COOH (**58**). Due to low thiol concentration, G1-cationic and anionic

dendrimers (**50** and **59**) were incompletely substituted in their respective ligand exchange reactions. The new method using the trityl group, which can be deprotected in acidic condition and at room temperature, was not effective in the generation of the thiols.

3.3. Concluding Remarks

The modular synthesis approach was effective in the preparation of amphiphilic cationic and anionic dendrons. The ammonium and carboxylate as peripheral units could be easily introduced to the phenolic dendron in high yields.

Two different protecting groups, thiocarbamate and trityl, were used to synthesize amphiphilic dendrons and dendrimers.

Deprotection of thiocarbamate group required heat and base catalyst. Using this protecting group, amphiphilic G1-cationic dendrimer (**50**), G0-anionic dendrimer (**57**), and G1-anionic dendrimer (**59**) were prepared successfully and characterized with ^{1}H NMR spectroscopy and MALDI-TOF. However, G2-cationic dendrimer (**52**) could not be prepared successfully due to a low percentage of thiol produced from the deprotection. The thiol concentration was measured using Ellman's reagent test.

The protocol to quantitate the thiol concentration by Ellman's reagent test was established. In G1-SH-Ammonium (**49**), G2-SH-Ammonium (**51**) and G1-SH-COOH (**58**) dendrons, the measured thiol concentrations were 49%, 34%, and 46%,

respectively. However, it was speculated that in the amphiphilic G1 & G2 dendrons the thiols were oxidized rapidly to the disulfides in the process of Ellman's reagent test due to oxygen dissolved in solution. Thus, Ellman's reagent test exhibited limitations in measuring concentrations of polar dendrons.

The second protecting group, trityl, could be deprotected in acid catalyst at room temperature. After the deprotection, the thiol concentration measured in Ellman's reagent test was not higher than that the dendrons prepared with thiocarbamate. The result was 40% in G1-SH-Ammonium dendron (**49**), 82% in G0-SH-COOH (**7**), and 29% in G1-SH-COOH dendron (**58**). Also, the thiol concentration from the ^{1}H NMR spectrum showed 88% in **7** and 43% in **58**. It suggested that ^{1}H NMR spectroscopy could be used along with Ellman's reagent test to measure thiol concentrations.

In G1-cationic and anionic dendrimers (**50** and **59**), iron-sulfur clusters were incompletely substituted due to low yield of thiol as the result of deprotection, respectively. Neither protecting group was effective in regeneration of thiols.

3.4. Experimental Section

General considerations. All chemicals were purchased from Aldrich and Acros and were used without further purification unless otherwise indicated. 4-Mercaptobenzoic acid was purchased from TCI Chemicals. All deuterated solvents were purchased from Cambridge Isotope Laboratories. All solvents used in the dry box were deoxygenated with a minimum of three freeze-pump-thaw cycles before use to remove any dissolved oxygen. Flash chromatography was carried out with silica gel 60 Å, 32 – 63 μm (Sorbent technologies). Nuclear Magnetic Resonance characterization was performed at 300 MHz or 400 MHz on Varian spectrometers. UV-VIS spectra were recorded using a Hewlett Packard 8452A diode array spectrometer. MALDI-MS (Matrix Assisted Laser Desorption Ionization–Mass Spectrometry) Investigations were performed using a Bruker Proflex + (Bruker Daltonics, Billerica, MA) linear MALDI-TOF (Time Of Flight) instrument with 1.2 m flight tube. DHB (2,5-dihydroxy benzoic acid), trans-3-indole acrylic acid or dithranol was used as a matrix.

4-dimethylthiocarbamoylsulfanyl benzoic acid methyl ester (27). 4-dimethylthiocarbamoyloxy benzoic acid methyl ester (**26**) (27.94 g, 116.8 mmol) was put into a sealed tube and heated to 220 – 230 °C for 2 h. Yield: 99% (27.67g); ^{1}H NMR ($CDCl_3$) δ (ppm) 3.03 (s, 3H), 3.09 (s, 3H), 3.90 (s, 3H), 7.55 (d, 2H, J = 6.3 Hz), 8.00 (d, 2H, J = 6.3 Hz).

4-dimethylthiocarbamoylsulfanyl benzoic acid (28). 4-dimethylthiocarbamoylsulfanyl benzoic acid methyl ester (**27**) (20.0 g, 83.6 mmol) and iodotrimethyl silane (23.8 mL, 167.2 mmol) were stirred and heated to 100 $^{\circ}$C for 2 h under N_2. After the reaction mixture was cooled to room temperature, it was diluted with diethyl ether. To the reaction mixture was slowly added 10 % NaOH aq. solution and washed 3 times. The combined water layer was acidified with 6N HCl aq. solution and then extracted 3 times with CH_2Cl_2. The organic layer was combined, dried with anhydrous magnesium sulfate, and then filtered. The filtrate was evaporated to dryness. The resulting product was pure enough to be carried out to the next step without further purification. Yield: 72% (13.5g); ^{1}H NMR ($CDCl_3$) δ (ppm) 3.00 (s, 3H), 3.11 (s, 3H), 7.61 (d, 2H, J = 8.4 Hz), 8.04 (d, 2H, J = 8.4 Hz).

G1-Thiocarbamate-MOM (29). A solution of 4-dimethylthiocarbamoylsulfanyl benzoic acid (**28**) (1.90 g, 8.45 mmol), G1-MA-MOM (**6, Chapter 2**) (3.47 g, 9.30 mmol), 1,3-dicyclohexylcarbodiimide (1.92 g, 9.30 mmol), and a catalytic amount of 4-dimethylaminopyridine in 50 mL of CH_2Cl_2 was stirred under N_2 at room temperature for 12 h. The solid residue was filtered and the filtrate was evaporated to dryness. The crude product was purified by column chromatography, eluting with 20 % ethyl acetate in hexane. Yield: 98% (4.80g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.34 (m, 1H), 1.46 (m, 1H), 1.54 (s, 1.5H), 1.62 (s, 1.5H), 1.84 (m, 1H), 2.12 (m, 1H), 2.72 (q, 2H), 2.86 (s, 1.5H), 2.99 (s, 1.5H), 3.02 (s, 3H), 3.11 (m, 1H), 3.41 (s, 6H), 3.44 (m,

1H), 5.09 (s, 4H), 6.86 (d, 4H, J = 10.0Hz), 6.96 (d, 2H, J = 10.0 Hz), 7.05 (d, 2H, J = 10.0 Hz), 7.22 (d, 1H, J = 10.0 Hz), 7.31 (d, 1H, J = 10.0 Hz), 7.45 (d, 2H, J = 10.0 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 22.49 (23.96), 28.00, 32.77, 37.13 (37.39), 38.46 (38.96), 45.01, 47.69, 51.73, 56.17, 115.83, 127.20 (127.50), 128.43, 130.51, 135.58, 137.40, 142.69 (143.08), 155.30, 166.47, 171.31.

G1-Thiocarbamate-OH (30). To a solution of G1-Thiocarbamate-MOM (**29**) (5.67 g, 9.77 mmol) in 70 mL of methanol was slowly added 60 drops of concentrated HCl. The solution was refluxed for 4 h. After the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure. The crude product was purified by column chromatography, eluting with 20 % ethyl acetate in hexane. Yield: 99% (4.76g); ^{1}H NMR (CD_3OD) δ (ppm) 1.27 (m, 2H), 1.41 (s, 1.5H), 1.50 (s, 1.5H), 1.72 (m, 1H), 2.03 (m, 1H), 2.73 (s, 1.5H), 2.86 (s, 1.5H), 2.95 (s, 3H), 3.05 (s, 3H), 3.13 (t, 1H), 3.43 (t, 1H), 6.60 (d, 4H, J = 8.6 Hz), 6.86 (d, 2H, J = 8.7 Hz), 6.96 (d, 2H, J = 8.7 Hz), 7.19 (d, 1H, J = 7.8 Hz), 7.32 (d, 1H, J = 8.1 Hz), 7.47 (d, 2H, J = 8.1 Hz); ^{13}C NMR (CD_3COCD_3) δ (ppm) 22.51 (23.93), 27.55, 32.11, 36.29 (36.79), 38.52 (38.98), 44.54, 47.45, 51.46, 114.82, 127.19 (127.38), 128.26, 130.65, 135.39, 137.84, 140.68 (140.92), 155.30, 165.04, 170.20 (170.60).

Test Reaction (see Scheme 3. 3). 4-methylphenol (0.02 g, 0.16 mmol) was added to a solution of 4-dimethylthiocarbamoylsulfanyl benzoic acid methyl ester (**27**) (0.04 g,

0.16 mmol) and 1-bromethyl-4-nitrobenzene (0.04 g, 0.16 mmol) containing potassium carbonate (0.09 g, 0.65 mmol) and a catalytic amount of 18-crown-6 in acetone. The reaction mixture was stirred and refluxed for 12 h. Then, the mixture was cooled to room temperature and filtered. The solvent was removed under reduced pressure. The crude product was purified by column chromatography, eluting with 5 % ethyl acetate in hexane. The products were determined by TLC and ^{1}H NMR spectroscopy.

Trimethyl-l-tolylammonium iodide (**33**). Methyl iodide (31.5 g, 220 mmol) was added to N,N-dimethyl-p-toluidine (3.00 g, 22 mmol) in diethyl ether and stirred at 30 -35 $^{\circ}$C for 24 h. The crude product was filtered and washed with diethyl ether. The product was dissolved in methanol and precipitated from ethyl acetate. Yield: 78% (4.78g); ^{1}H NMR ($CDCl_3$) δ (ppm) 2.39 (s, 3H), 3.99 (s, 9H), 7.38 (d, 2H, J = 8.1 Hz), 7.84 (d, 2H, J = 8.1 Hz).

Attempt at synthesis of (4-bromomethylphenyl) trimethylammonium iodide (**34**) (see Table 3.1).

- Using an oil bath as heat source

A catalytic amount of AIBN was added to a solution of the ammonium iodide (**33**) (0.5 g, 1.8 mmol) and NBS (0.32 g, 1.8 mmol) in CH_2Cl_2. The reaction mixture was stirred and refluxed for 12 h in oil bath. Also, in $CHCl_3$ solvent, the same

reactions were performed for 12 h and 48 h, respectively. The mixture in DMF was stirred at room temperature for 12 h without heat or light.

- Using a sun lamp as heat source

The mixture in $CHCl_3$ was stirred for 18 h under sun lamp.

- Using a microwave reactor as heat source

The reaction mixtures were reacted in $CHCl_3$ at 100 °C for 3 h and without solvents at 180 °C for 3 h.

(4-dimethylaminophenyl) methanol (**35**). Into a solution of 4-dimethylaminobenzaldehyde (10.00 g, 67 mmol) in methanol was added $NaBH_4$ and stirred at 60 °C for 24 h. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure. The crude product was purified by column chromatography, eluting with 15 % ethyl acetate in hexane. Yield: 94% (9.51g); ^{1}H NMR ($CDCl_3$) δ (ppm) 2.95 (s, 6H), 4.57 (s, 2H), 6.73 (d, 2H, J = 8.8 Hz), 7.25 (d, 2H, J = 8.4 Hz).

(4-hydroxymethylphenyl) trimethylammonium iodide (**36**). Methyl iodide (45.6 g, 320 mmol) was added to (4-dimethylaminophenyl) methanol (**35**) (4.86 g, 32 mmol) in CH_2Cl_2 and stirred for 24 h. The reaction mixture was filtered and washed with CH_2Cl_2. The crude product was dissolved in methanol and precipitated from ethyl acetate. Yield: 87% (8.18g); ^{1}H NMR (CD_3OD) δ (ppm) 3.68 (s, 9H), 4.69 (s, 2H),

7.62 (d, 2H, J = 7.6 Hz), 7.89 (d, 2H, J = 9.2 Hz).

Attempt at synthesis of (4-bromomethylphenyl) trimethylammonium iodide (**34**) (see Scheme 3.5).

- NBS/PPh_3 system

NBS (0.45 g, 2.5 mmol) was added to a solution of ammonium iodide (**36**) (0.5 g, 1.7 mmol) and triphenylphosphine in DMF and THF dried under N_2, respectively. The reaction mixtures were stirred at room temperature for 12 h and the solvent was removed.

- CBr_4/PPh_3 system

CBr_4 (0.85 g, 2.5 mmol) was added to a solution of ammonium iodide (**36**) (0.5 g, 1.7 mmol) and triphenylphosphine in DMF and CH_3CN dried under N_2 at 0 °C, respectively. The reaction mixtures were stirred at room temperature for 12 h and the solvent was removed.

- PBr_3 system

PBr_3 (0.10 g, 0.4 mmol) was added to a solution of ammonium iodide (**36**) (0.3 g, 1.0 mmol) and triphenylphosphine in DMF dried under N_2 at 0 °C, respectively. The reaction mixtures were stirred at room temperature for 12 h and the solvent was removed.

Attempt at synthesis of (4-iodomethylphenyl) trimethylammonium iodide (**37**). Before using HI, the hydroiodic acid was purified 3 times by shaking with the

mixture of tributylphosphine and chloroform. The ammonium iodide (**36**) (1.0 g, 3.4 mmol) was added to HI (4.36 g, 3.4 mmol) and stirred at room temperature for 2 days. The product generated was filtered and washed with acetone.

(2-chloroethyl)trimethylammonium iodide (**38**). Methyl iodide (12.54 g, 88 mmol) was added to N,N-dimethylaminoethyl chloride (1.1 g, 10 mmol) in CH_2Cl_2 and stirred at room temperature for 24 h. The reaction mixture was filtered, washed with CH_2Cl_2, and dried in vacuo. Yield: 88% (2.23g); ^{1}H NMR (CD_3OD) δ (ppm) 3.24 (s, 9H), 3.81 (t, 2H), 4.06 (t, 2H).

Attempt at synthesis of G1-Thiocarbamate-Ammonium (40) (see Scheme 3.7). (2-chloroethyl)trimethylammonium iodide (**38**) (0.39 g, 1.6 mmol) was added to G1-Thiocarbamate-OH (**30**) (0.35 g, 0.5 mmol), anhydrous potassium carbonate (0.39 g, 2.8 mmol), and a catalytic amount of 18-crown-6 in 50 mL of dry acetone. The mixture was refluxed for 5 h and stirred vigorously under N_2. (According to TLC monitoring, the product was not generated, indicating starting material.)

G1-Thiocarbamate-MA (39). To a suspension of G1-Thiocarbamate-OH (**30**) (0.1 g, 0.20 mmol), anhydrous potassium carbonate (0.14 g, 1.01 mmol), and a catalytic amount of 18-crown-6 in 50 mL of dry acetone was slowly added N, N-dimethylaminoethyl chloride·HCl (73.1 mg, 0.51 mmol) with N_2. The mixture was

refluxed for 5 h and stirred vigorously under N_2. After the reaction mixture was cooled to room temperature, the mixture was filtered. The solution was washed 3 times with H_2O, dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. Yield: 99% (0.13g); ^{1}H NMR (CD_2Cl_2) δ (ppm) 1.34 (m, 1H), 1.44 (m, 1H), 1.53 (s, 1.5H), 1.62 (s, 1.5H), 1.84 (m, 1H), 2.12 (m, 1H), 2.28 (s, 12H), 2.67 (t, 4H), 2.79 (s, 1.5H), 2.91 (s, 1.5H), 3.02 (s, 3H), 3.08 (s, 3H), 3.17 (t, 1H), 3.49 (t, 1H), 4.02 (t, 4H), 6.81 (d, 4H, J = 7.2 Hz), 7.02 (d, 2H, J = 8.4 Hz), 7.12 (d, 2H, J = 8.1 Hz), 7.27 (d, 1H, J = 7.5 Hz), 7.37 (d, 1H, J = 7.8 Hz), 7.51 (d, 2H, J = 8.4 Hz). MALDI-TOF MS (matrix: dithranol); m/z Cald 634.36; found 635.85.

G1-Thiocarbamate-Ammonium (40). Into a sealed tube with a solution of G1-Thiocarbamate-MA (**39**) (0.39 g, 0.61 mm0l) in 10 mL of CH_2Cl_2 was added CH_3I (1.8 mL, 29.0 mmol) dropwise via syringe. The tube was wrapped with aluminum foil. The reaction mixture was stirred at room temperature for 24 h. The precipitated salts were filtered and dissolved in methanol to remove insoluble impurities. After micro-syringe filtration, the filtrates were evaporated to dryness. Yield: 71% (0.40g); ^{1}H NMR (CD_3OD) δ (ppm) 1.34 (m, 1H), 1.47 (m, 1H), 1.53 (s, 1.5H), 1.62 (s, 1.5H), 1.86 (m, 1H), 2.15 (m, 1H), 2.84 (s, 1.5H), 2.93 (s, 1.5H), 3.02 (s, 3H), 3.13 (s, 3H), 3.22 (t, 1H), 3.28 (s, 18H), 3.50 (t, 1H), 3.86 (s, 4H), 4.47 (s, 4H), 6.93 (d, 4H, J = 6.6 Hz), 7.08 (d, 2H, J = 8.4 Hz), 7.18 (d, 2H, J = 8.4 Hz), 7.28 (d, 1H, J = 8.1 Hz), 7.39

(d, 1H, J = 8.1 Hz), 7.54 (d, 2H, J = 5.4 Hz). MALDI-TOF MS (matrix: DHB); m/z Cald 918.21; found 791.35.

G1-SH-Ammonium (49) (See Scheme 3.13). G1-Thiocarbamate-Ammonium (**40**) (27.8 mg, 0.03 mmol) and solid NaOH (3.6 mg, 0.09 mmol) put into a flame-dried Schlenk flask and placed under vacuum for one hour. The flask was sealed under vacuum and transferred to a nitrogen line and placed under nitrogen for 1 h. The flask was fitted with a reflux condenser sealed with a rubber septum under N_2. Into the flask was added 5 mL of methanol and 0.1 mL of THF distilled, dried, and degassed via syringe. Nitrogen was purged for 30 min more using an outlet needle in the septum until sodium hydroxide dissolved. The septum was exchanged with a greased glass stopper and the flask fitted with a reflux condenser was sealed fully. The nitrogen purge was then stopped. The contents of the flask were refluxed as a sealed system for 12 h. After the reaction mixture was cooled to room temperature, 3 drops of concentrated HCl was added to quench the reaction and stirred for 5 min. The reflux condenser was dissembled and via a rotary evaporator the solvent was removed from the Schlenk flask directly. The residue was dried more *in vacuo* for 24 h and put it into the dry box for the next step.

$[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (50) (See Scheme 3.13). Into the Schlenk flask with a solution of G1-SH-Ammonium (**49**) (25.6 mg, 0.03 mmol) in 6 mL of

DMF dried and degassed was slowly added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (4.3 mg, 0.005 mmol) in 10 mL of DMF in dry box. Upon addition, the red-wine color developed rapidly. After the reaction mixture was stirred for 15 min, the flask was sealed and removed from the dry box. The solvent was then removed *in vacuo* at 40 °C for 5 h. The flask put into the dry box. The product was dissolved in 4 mL of DMF dried and degassed. The solution was transferred into a Falcon tube sealed with a rubber septum. The tube was centrifuged to remove insoluble parts including sodium chloride. The tube put into the dry box. Only the soluble parts was transferred into a new Falcon tube and precipitated from 8 mL of acetone distilled, dried, and degassed. The tube put into centrifugal separator in the outside and the solution was separated again. In the dry box the supernatant was removed and the precipitates were dried *in vacuo* at 40 °C for 2 days. Yield: 80% (15.6mg); ^{1}H NMR (DMF-d_7) δ (ppm) 1.38 (m, 8H), 1.58 (s, 12H), 1.89 (m, 8H), 3.47 (s, 96H), 4.12 (s, 16H), 4.61 (s, 16H), 5.95 (br, 8H), 6.95 (s, 16H), 7.12 (s, 16H), 8.20 (s, 8H). MALDI-TOF MS (matrix: dithranol); m/z Cald 3884; found 3541.

G2-OH-MOM (42). A suspension of G1-OMS-MOM (**5**) (11.73 g, 26.7 mmol), 4,4-bis(4'-hydroxyphenyl) pentanol (**41**) (3.39 g, 12.4 mmol), anhydrous potassium carbonate (8.60 g, 62.2 mmol), and a catalytic amount of 18-crown-6 in 100 mL of dry acetone was stirred under N_2. The mixture was refluxed for 12 h. After the reaction mixture was cooled to room temperature, the mixture was filtered. The

filtrate was evaporated to dryness. The crude product was dissolved in CH_2Cl_2. The solution was washed 3 times with H_2O, dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. The resulting product was pure enough to be carried out to the next step without further purification. Yield: 79% (9.57mg); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.31 – 1.55 (m, 6H), 1.58 (s, 3H), 1.62 (s, 6H), 2.09 (m, 2H), 2.19 (m, 4H), 3.48 (s, 12H), 3.59 (t, 2H), 3.86 (t, 4H), 5.15 (s, 8H), 6.73 (d, 4H, J = 8.4 Hz), 6.91 (d, 8H, J = 8.7 Hz), 7.06 (d, 4H, J = 8.4 Hz), 7.11 (d, 8H, J = 8.7 Hz).

G2-OMs-MOM (43). To a solution of G2-OH-MOM (**42**) (7.74 g, 7.95 mmol), triethylamine (3.32 mL, 23.9 mmol), and a catalytic amount of 4-dimethylaminopyridine in 80 mL of CH_2Cl_2 at 0 °C was added methanesulfonyl chloride (1.82 g, 15.9 mmol) dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. Water was added to quench the reaction. The solution was extracted 3 times with CH_2Cl_2. The organic layer was combined, dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. The crude product was purified by column chromatography, eluting with 25 % ethyl acetate in hexane. Yield: 91% (7.49g); ^{1}H NMR (CD_2Cl_2) δ (ppm) 1.40 – 1.52 (m, 9H), 1.85 (m, 2H), 2.15 (m, 4H), 2.88 (s, 3H), 3.39 (s, 12H), 3.82 (t, 4H), 4.08 (t, 2H), 5.08 (s, 8H), 6.70 (d, 4H, J = 8.4 Hz), 6.86 (d, 8H, J = 8.7 Hz), 7.01 (d, 4H, J = 8.4 Hz), 7.07 (d, 8H, J = 8.7 Hz).

G2-MA-MOM (44). Into a sealed tube with a solution of G2-OMs-MOM (**43**)(2.98 g, 2.88 mmol) in 10 mL of THF was added methyl amine (40 wt% solution in H_2O) (5.0 mL, 57.6 mmol). The reaction mixture was then heated to 60 – 70 $^{\circ}$C for 5 h. After the reaction mixture was cooled to room temperature, water was added to quench the reaction. The solution was extracted 3 times with CH_2Cl_2. The organic layer was combined, dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. The resulting product was pure enough to be carried out to the next step without further purification. Yield: 97% (2.70g); ^{1}H NMR (CD_2Cl_2) δ (ppm) 1.24 (m, 2H), 1.50 – 1.60 (m, 7H), 1.62 (s, 6H), 1.83 (m, 1H), 2.05 (m, 1H), 2.21 (m, 4H), 2.32 (s, 3H), 2.50 (t, 2H), 3.45 (s, 12H), 3.88 (t, 4H), 5.14 (s, 8H), 6.75 (d, 4H, J = 6.9 Hz), 6.93 (d, 8H, J = 6.6 Hz), 7.07 (d, 4H, J = 7.7 Hz), 7.14 (d, 8H, J = 6.6 Hz).

G2-Thiocarbamate-MOM (45). A solution of 4-dimethylthiocarbamoylsulfanyl benzoic acid (**28**) (0.60 g, 2.53 mmol), G2-MA-MOM (**44**) (2.70 g, 2.78 mmol), 1,3-dicyclohexylcarbodiimide (0.57 g, 2.78 mmol), and a catalytic amount of 4-dimethylaminopyridine in 50 mL of CH_2Cl_2 was stirred under N_2 at room temperature for 20 h. The solid residue was filtered and the filtrate was evaporated to dryness. The crude product was purified by column chromatography, eluting with 20 % ethyl acetate in hexane. Yield: 67% (1.99g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.26 (m, 1H), 1.33 (m, 1H), 1.42 – 1.62 (m, 13H), 1.74 (m, 1H), 2.08 (m, 1H), 2.13 (m, 4H), 2.70 (s,

1.5H), 2.84 (s, 1.5H), 3.00 (s, 6H), 3.09 (m, 1H), 3.41 (s, 12H), 3.46 (m, 1H), 3.80 (t, 3H), 5.08 (s, 8H), 6.68 (d, 4H, J = 8.1 Hz), 6.86 (d, 8H, J = 9.0 Hz), 6.92 (d, 2H, J = 7.2 Hz), 6.99 (m, 2H), 7.06 (d, 8H, J = 8.7 Hz), 7.25 (m, 1H), 7.29 (m, 1H), 7.45 (d, 2H, J = 7.8 Hz).

G2-Thiocarbamate-OH (46). To a solution of G2-Thiocarbamate-MOM (**45**) (3.31 g, 2.81 mmol) in 70 mL of methanol was slowly added 60 drops of concentrated HCl. The solution was refluxed for 6 h. After the reaction mixture was cooled to room temperature, the solvent was removed under reduced pressure. The crude product was purified by column chromatography, eluting with 20 % ethyl acetate in CH_2Cl_2. Yield: 88% (2.46g); ^{1}H NMR (CD_3COCD_3) δ (ppm) 1.35 – 1.70 (m, 15H), 1.84 (m, 1H), 2.12 (m, 1H), 2.20 (m, 4H), 2.78 (s, 1.5H), 2.93 (s, 1.5H), 3.00 – 3.08 (s, 6H), 3.20 (m, 1H), 3.46 (m, 1H), 3.88 (t, 4H), 6.70 (d, 4H, J = 9.0 Hz), 6.78 (d, 8H, J = 9.0 Hz), 6.95 – 7.05 (m, 12H), 7.20 - 7.33 (m, 1H), 7.34 - 7.44 (m, 1H), 7.48 (d, 2H, J = 9.0 Hz).

G2-Thiocarbamate-MA (47). To a suspension of G2-Thiocarbamate-OH (**46**) (0.44 g, 0.44 mmol), anhydrous potassium carbonate (1.22 g, 8.8 mmol), and a catalytic amount of 18-crown-6 in 50 mL of dry acetone was slowly added N, N-dimethylaminoethyl chloride·HCl (1.27 g, 8.8 mmol) under N_2. The mixture was refluxed for 5 h and stirred vigorously under N_2. After the reaction mixture was

cooled to room temperature, the mixture was filtered. The solution was washed 3 times with H_2O, dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. Yield: 58% (0.33g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.30 – 1.58 (m, 15H), 1.75 (m, 1H), 2.03 (m, 1H), 2.11 (m, 4H), 2.43 (s, 16H), 2.70 (s, 1.5H), 2.86 (m, 9.5H), 2.97 (s, 3H), 3.01 (s, 3H), 3.10 (m, 1H), 3.44 (m, 1H), 3.79 (t, 4H), 4.10 (t, 8H), 6.67 (d, 4H, J = 8.1 Hz), 6.73 (d, 8H, J = 9.0 Hz), 6.91 (d, 2H, J = 8.1 Hz), 7.00 (m, 2H), 7.03 (d, 8H, J = 9.0 Hz), 7.25 (m, 1H), 7.29 (m, 1H), 7.44 (d, 2H, J = 7.5 Hz).

G2-Thiocarbamate-Ammonium (48). Into a sealed tube with a solution of G2-Thiocarbamate-MA (**47**) (0.16 g, 0.12 mm0l) in 10 mL of CH_2Cl_2 was added CH_3I (0.39 mL, 6.2 mmol) dropwise via syringe. The tube was then wrapped with aluminum foil. The reaction mixture was stirred at room temperature for 24 h. The precipitated salts were filtered and dissolved in methanol to remove insoluble impurities. After micro-syringe filtration, the filtrates were evaporated to dryness. Yield: 60% (0.14g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.26 (m, 2H), 1.36 – 1.60 (m, 13H), 1.74 (m, 1H), 2.05 (m, 1H), 2.15 (m, 4H), 2.74 (s, 1.5H), 2.87 (s, 1.5H), 2.94 (s, 3H), 3.03 (s, 3H), 3.12 (m, 1H), 3.24 (s, 36H), 3.43 (m, 1H), 3.78 (m, 8H), 3.82 (m, 4H), 3.82 (m, 4H), 4.44 (m, 8H), 6.69 (m, 4H), 6.85 (d, 8H, J = 9.0 Hz), 6.91 (d, 2H, J = 8.4 Hz), 7.03 (d, 2H, J = 8.1 Hz), 7.11 (d, 8H, J = 8.4 Hz), 7.32 (d, 1H, J = 7.2 Hz), 7.38 (d, 1H, J = 7.8 Hz), 7.48 (d, 2H, J = 7.5 Hz).

Attempt at synthesis of G2-SH-Ammonium (51) (See Scheme 3.14). G2-Thiocarbamate-Ammonium (**48**) (0.14 g, 0.07 mmol) and solid NaOH (7.4 mg, 0.18 mmol) put into a flame-dried Schlenk flask and placed under vacuum for one hour. The flask was sealed under vacuum and transferred to a nitrogen line and placed under nitrogen for 1 h. The flask was fitted with a reflux condenser sealed with a rubber septum under N_2. Into the flask was added 10 mL of methanol and 0.5 mL of THF distilled, dried, and degassed via syringe. Nitrogen was purged for 30 min more using an outlet needle in the septum until the sodium hydroxide dissolved. The septum was exchanged for a grease glass stopper and the flask fitted with a reflux condenser was sealed fully. The nitrogen purge was then stopped. The contents of the flask were refluxed as a sealed system for 12 h. After the reaction mixture was cooled to room temperature, 4 drops of concentrated HCl was added to quench the reaction and the mixture was stirred for 5 min. The reflux condenser was dissembled and via a rotary evaporator the solvent was removed from the Schlenk flask directly. The residue was dried more *in vacuo* for 24 h and put it into the dry box for the next step.

Attempt at synthesis of $[Fe_4S_4(S\text{-}G2\text{-}Ammonium)_4][N(CH_3)_4]_2$ (52) (See Scheme 3.14). Into the Schlenk flask with a solution of G2-SH-Ammonium (**51**) (0.13 g, 0.07 mmol) in 6 mL of DMF dried and degassed was slowly added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (10.5 mg, 0.01 mmol) in 10 mL of DMF in dry box. Upon addition, the red-wine color developed rapidly. After the reaction mixture was stirred for 15

min, the flask was sealed and removed from the dry box. The solvent was then removed *in vacuo* at 40 °C for 5 h. The flask put into the dry box. The product was dissolved in 4 mL of DMF dried and degassed. The solution was transferred into a Falcon tube sealed with a rubber septum. The tube was centrifuged to remove insoluble parts including sodium chloride. In the dry box only the soluble parts were transferred into a new Falcon tube. The solvent was removed and dried *in vacuo* at 40 °C for 2 days.

4-Methyl benzoic acid *tert*-butyl ester (53). A solution of potassium t-butoxide (3.27 g, 29.14 mmol) in a mixed solvent of THF (35 mL) and t-butanol (35 mL) was stirred for 30 min. To the solution was added p-toluoyl chloride (2.6 mL, 19.40 mmol) dropwise. The reaction mixture was refluxed for 12 h under N_2. After the solution was cooled to room temperature, the solvents were removed under reduced pressure. The crude product was dissolved in ethyl acetate. The solution was washed 2 times with H_2O, washed 2 times with saturated Na_2CO_3 aqueous solution. The organic layer was combined and dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. The resulting product was pure enough to be carried out to the next step without further purification. Yield: 77% (2.82g); ^{1}H NMR (CD_3COCD_3) δ (ppm) 1.60 (s, 9H), 2.41 (s, 3H), 7.26 (d, 2H, J = 9.0Hz), 7.84 (d, 2H, J = 9.0Hz)).

4-Bromomethyl benzoic acid *tert*-butyl ester (54). To a solution of 4-Methyl benzoic acid *tert*-butyl ester (**53**) (3.94 g, 20.51 mmol) and N-Bromosuccinimide (3.29 g, 18.37 g) in 50 mL of CH_2Cl_2 was added a catalytic amount of 2, 2'-azobisisobutyronitrile to initiate free radical reaction. The reaction was refluxed for 48 h under N_2. After the solution was cooled to room temperature, the solvents were removed under reduced pressure. The crude product was dissolved in ethyl acetate. The solution was washed 2 times with H_2O, washed 2 times with saturated Na_2CO_3 aqueous solution. The organic layer was combined and dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. The resulting product was pure enough to be carried out to the next step. Yield: 97% (4.84g); ^{1}H NMR (CD_3COCD_3) δ (ppm) 1.60 (s, 9H), 4.70 (s, 2H), 7.58 (d, 2H, J = 9.0Hz), 7.95 (d, 2H, J = 9.0Hz)).

G1-Thiocarbamate-t-Bu (55). To a suspension of G1-Thiocarbamate-OH (**30**) (0.10 g, 0.2 mmol), anhydrous potassium carbonate (0.17 g, 1.22 mmol), and a catalytic amount of 18-crown-6 in 20 mL of dry acetone was added 4-Bromomethyl benzoic acid *tert*-butyl ester (**54**) (0.12 g, 0.45 mmol). The mixture was refluxed for 12 h and stirred vigorously under N_2. After the reaction mixture was cooled to room temperature, the mixture was filtered. The filtrates were evaporated to remove the solvent under reduced pressure. The crude product was purified by column chromatography, eluting with the solvent system of 30 % ethyl acetate in hexane.

Yield: 96% (0.17g); ^{1}H NMR (CD_2Cl_2) δ (ppm) 1.35 (m, 1H), 1.44 (m, 1H), 1.55 (s, 1H), 1.60 (s, 18H), 1.63 (s, 1H), 1.85 (m, 1H), 2.14 (m, 1H), 2.79 (s, 1.5H), 2.92 (s, 1.5H), 3.01 (s, 3H), 3.08 (s, 3H), 3.18 (m, 1H), 3.50 (m, 1H), 5.12 (s 4H), 6.79 (d, 4H, J = 7.8 Hz), 7.00 (d, 2H, J = 8.1 Hz), 7.04 (d, 2H, J = 7.8 Hz), 7.23 (d, 1H, J = 8.1 Hz), 7.31 (d, 1H, J = 7.8 Hz), 7.41 (d, 4H, J = 7.8 Hz), 7.46 (br s, 2H), 7.93 (d, 4H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 22.50 (23.92), 27.94, 28.37, 32.76, 37.12, 38.45 (38.58), 44.96, 47.64, 51.72, 69.52, 81.21, 114.41, 127.02, 127.23 (127.48), 128.38, 129.85, 130.47, 131.68, 135.56, 137.41, 141.99 (142.39), 156.62, 165.09, 170.77 (171.35). MALDI-TOF MS (matrix: DHB); m/z Cald 872.41; found 895.51.

G1-Thiocarbamate-COOH (56). To a solution of G1-Thiocarbamate-t-Bu (**55**) (0.62 g, 0.71 mmol) in 7 mL of CH_2Cl_2 at 0 °C was slowly added trifluoroacetic acid (7.5 mL, 97.4 mmol) via syringe. The reaction mixture was allowed to warm to room temperature and stirred for 8 h. The solvent and trifluoroacetic acid were removed under reduced pressure. The crude product was dissolved in CH_2Cl_2. The solution was washed 3 times with H_2O. The organic layer was combined and dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. Yield: 92% (0.5g); ^{1}H NMR (CD_2Cl_2) δ (ppm), 1.54 (s, 1.5H), 1.58 (s, 1.5H), 1.84 (m, 1H), 2.06 (m, 1H), 2.92 (s, 1.5H), 2.95 – 3.14 (m, 7.5H), 3.18 (t, 1H), 3.38 (t, 1H), 5.15 (s 4H), 6.86 (d, 4H, J = 8.0 Hz), 7.04 (d, 2H, J = 8.0 Hz), 7.11 (d, 2H, J = 8.8 Hz), 7.27 (d, 1H, J = 7.6 Hz), 7.33 (d, 1H, J = 8.4 Hz), 7.51 (d, 4H, J = 7.2 Hz), 7.55 (d, 4H, J =

9.6 Hz), 8.03 (d, 2H, J = 8.4 Hz), 8.09 (d, 2H, J = 8.0 Hz); ^{13}C NMR (CD_2Cl_2) δ (ppm) 22.26 (23.90), 27.65, 32.79, 37.00, 38.65 (38.78), 45.01, 47.90, 51.82, 69.30, 114.45, 127.08, 127.25, 128.41, 129.19, 130.36, 130.78, 135.50, 137.31, 142.21 (142.47), 143.49, 156.38 (156.59), 166.53, 170.42, 170.85. MALDI-TOF MS (matrix: dithranol); m/z Cald 760.28; found 761.23.

G0-SH-COOH (4-mercaptobenzoic acid) (7) (See Scheme 3.17). 4-dimethylthiocarbamoylsulfanyl benzoic acid (**28**) (26.5 g, 0.12 mmol) and solid NaOH 12.4 mg, 0.31 mmol) put into a flame-dried Schlenk flask and placed under vacuum for one hour. The flask was sealed under vacuum and transferred to a nitrogen line and placed under nitrogen for 1 h. The flask was fitted with a reflux condenser sealed with a rubber septum under N_2. Into the flask was added 8 mL of methanol and 0.2 mL of THF distilled, dried, and degassed via syringe. Nitrogen was purged for 30 min more using an outlet needle in the septum until sodium hydroxide dissolved. The septum was exchanged into a glass stopper pasted with grease and the flask fitted with a reflux condenser was sealed fully. Nitrogen purge was then stopped. The flask with a fully sealed system was heated to 55 $^{\circ}C$ for 12 h. After the reaction mixture was cooled to room temperature, 6 drops of concentrated HCl was added to quench the reaction and stirred for 5 min. The reflux condenser was dissembled and via a rotary evaporator the solvent was removed from the Schlenk flask directly. The residue was dried more *in vacuo* for 24 h and put it into the dry box for the next step.

$[Fe_4S_4(S\text{-}G0\text{-}COOH)_4][N(CH_3)_4]_2$ (57) (See Scheme 3.17). Into the Schlenk flask with a solution of G0-SH-COOH (**7**) (18.1 mg, 0.12 mmol) in 5 mL of DMF dried and degassed was slowly added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (16.8 mg, 0.02 mmol) in 10 mL of DMF in dry box. Upon addition, the red-wine color developed rapidly. After the reaction mixture was stirred for 15 min, the flask was sealed and removed from the dry box. The solvent was then removed *in vacuo* at 40 °C for 5 h. The flask put into the dry box. The product was dissolved in 4 mL of DMF dried and degassed. The solution was transferred into a Falcon tube sealed with a rubber septum. The tube was centrifuged to remove insoluble parts including sodium chloride. In the dry box only the soluble parts were transferred into a new Falcon tube. The solvent was removed and dried *in vacuo* at 40 °C for 2 days. ^{1}H NMR (DMF-d_7) δ (ppm) 3.38 (s, 24H), 6.16 (br s, 8H), 8.86 (br s, 8H); MALDI-TOF MS (matrix: dithranol); m/z Cald 1111.83; found 1037.11.

$[Fe_4S_4(S\text{-}G0\text{-}COOH)_4][N(CH_3)_4]_2$ (57) (See Scheme 3.17). Into the Schlenk flask with a solution of G0-SH-COOH (4-mercaptobenzoic acid from TCI Chemicals) (**7**) (50.0 mg, 0.32 mmol) in 5 mL of DMF dried and degassed was slowly added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (56.0 mg, 0.06 mmol) in 10 mL of DMF in the dry box. Upon addition, the red-wine color developed rapidly. After the reaction mixture was stirred for 15 min, the flask was sealed and removed from the dry box. The solvent was then removed *in vacuo* at 40 °C for 5 h and dried more *in vacuo* at

40 °C for 2 days. ^{1}H NMR (DMSO-d_6) δ (ppm) 3.08 (s, 24H), 5.97 (br s, 8H), 8.74 (br s, 8H); MALDI-TOF MS (matrix: dithranol); m/z Cald 1111.83; found 1036.48.

G1-SH-COOH (58) (See Scheme 3.18). G1-Thiocarbamate-COOH (**56**) (0.03 g, 0.04 mmol) and solid NaOH (4.7 mg, 0.12 mmol) put into a flame-dried Schlenk flask and placed under vacuum for one hour. The flask was sealed under vacuum and transferred to a nitrogen line and placed under nitrogen for 1 h. The flask was fitted with a reflux condenser sealed with a rubber septum under N_2. Into the flask was added 4 mL of methanol and 0.1 mL of THF distilled, dried, and degassed via syringe. Nitrogen was purged for 30 min more using an outlet needle in the septum until the sodium hydroxide dissolved. The septum was exchanged for a grease glass stopper and the flask fitted with a reflux condenser was sealed fully. The nitrogen purge was then stopped. The contents of the flask were heated as a sealed system to 55 °C for 12 h. After the reaction mixture was cooled to room temperature, 4 drops of concentrated HCl was added to quench the reaction and stirred for 5 min. The reflux condenser was dissembled and via a rotary evaporator the solvent was removed from the Schlenk flask directly. The residue was dried more *in vacuo* for 24 h and put it into the dry box for the next step.

$[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (59) (See Scheme 3.18). Into the Schlenk flask with a solution of G1-SH-COOH (**58**) (27.2 mg, 0.03 mmol) in 5 mL of DMF dried

and degassed was slowly added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (5.6 mg, 0.006 mmol) in 8 mL of DMF in dry box. Upon addition, the red-wine color developed rapidly. After the reaction mixture was stirred for 15 min, the flask was sealed and removed from the dry box. The solvent was then removed *in vacuo* at 40 °C for 5 h. The flask put into the dry box. The product was dissolved in 4 mL of DMF dried and degassed. The solution was transferred into a Falcon tube sealed with a rubber septum. The tube was centrifuged to remove insoluble parts including sodium chloride. In the dry box only the soluble parts were transferred into a new Falcon tube. The solvent was removed and dried *in vacuo* at 40 °C for 2 days. (* Attempts to purify the dendrimer was performed by precipitation, dialysis, and diafiltration. However, these works changed significantly solubility of the dendrimer. After purification, the dendrimer was partially-soluble. ^{1}H NMR spectrum of this molecule purified was totally different from that of the G1-anionic dendrimer. Thus, the G1-anionic dendrimer was employed without purification.)

4-Tritylsulfanyl benzoic acid (60). To a solution of 4-mercaptobenzoic acid (**7**) (0.5 g, 3.24 mmol) in 10 mL of trifluoroacetic acid was added a solution of triphenyl methanol (1.01 g, 3.88 mmol) in 10 mL of trifluoroacetic acid. The reaction mixture was stirred and heated at 40 °C for 2.5 h. After the mixture was cooled to room temperature, trifluoroacetic acid was removed under reduced pressure. The residual solid was dissolved in ethyl acetate and filtered to remove insoluble parts. The

filtrates were evaporated to dryness. The crude product was purified by column chromatography, eluting with the solvent system of 12 % ethyl acetate in hexane. Yield: 82% (1.05g); ^{1}H NMR (CD_3COCD_3) δ (ppm) 7.01 (d, 2H, J = 8.4 Hz), 7.12 – 7.29 (m, 9H), 7.30 – 7.42 (m, 6H), 7.60 (d, 2H, J = 8.4 Hz); ^{13}C NMR (CD_2Cl_2) δ (ppm) 71.17, 127.75, 128.67, 129.50, 129.55, 130.05, 131.80, 141.40, 144.10, 167.38.

G1-STr-MOM (61). A solution of 4-Tritylsulfanyl benzoic acid (**60**) (1.05 g, 2.65 mmol), G1-MA-MOM (**6**) (1.14g, 3.05 mmol), 1,3-dicyclohexylcarbodiimide (0.63 g, 3.05 mmol), and a catalytic amount of 4-dimethylaminopyridine in 100 mL of CH_2Cl_2 was stirred under N_2 at room temperature for 12 h. The solid residue was filtered and the filtrate was evaporated to dryness. The crude product was purified by column chromatography, eluting with 20 % ethyl acetate in hexane. Yield: 68% (1.36g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.37 (m, 2H), 1.51 (s, 1.5H), 1.58 (s, 1.5H), 1.72 (m, 1H), 2.05 (m, 1H), 2.65 (s, 1.5H), 2.87 (s, 1.5H), 3.03 (m, 1H), 3.44 (m, 1H), 3.48 (s, 6H), 5.15 (s, 4H), 6.86 - 7.02 (m, 8H), 7.04 – 7.10 (m, 3H), 7.14 – 7.29 (m, 12H), 7.40 (d, 4H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 22.47 (24.04), 32.79, 38.62, 44.96, 47.65, 51.70, 71.12, 94.66, 126.49 (126.76), 127.02, 127.94, 128.29, 130.15, 133.57, 135.57, 136.79, 142.66 (143.09), 144.43, 155.46, 171.47.

G1-STr-OH (62). To a solution of G1-STr-MOM (**61**) (1.36 g, 1.80 mmol) in 50 mL of methanol was slowly added 15 drops of concentrated HCl. The solution was gently heated to 55 °C for 6 h. After the reaction mixture was cooled to room

temperature, the solvent was removed under reduced pressure. The crude product was purified by column chromatography, eluting with 20 % ethyl acetate in hexane. Yield: 80% (0.92g); ^{1}H NMR (CD_3OD) δ (ppm) 1.31 (m, 2H), 1.40 (s, 1.5H), 1.47 (s, 1.5H), 1.63 (m, 1H), 1.94 (m, 1H), 2.58 (s, 1.5H), 2.80 (s, 1.5H), 2.99 (m, 1H), 3.33 (m, 1H), 3.48 (s, 6H), 6.59 (d, 4H, J = 8.7 Hz), 6.74 – 6.86 (m, 4H), 6.88 – 7.02 (m, 3H), 7.04 – 7.24 (m, 12H), 7.33 (d, 4H, J = 7.8 Hz); ^{13}C NMR (CD_3COCH_3) δ (ppm) 22.53 (23.19), 27.53, 31.91, , 38.72, 44.50, 47.26, 51.29, 71.20, 114.71, 126.62, 127.04, 127.61, 127.96, 128.23, 130.00, 134.03, 135.73, 136.79, 140.71, 144.58, 155.23, 170.40. MALDI-TOF MS (matrix: dithranol); m/z Cald 663.28; found 665.62.

G1- STr -MA (63). To a suspension of G1-STr-OH (**62**) (0.3 g, 0.45 mmol), anhydrous potassium carbonate (1.87 g, 13.6 mmol), and a catalytic amount of 18-crown-6 in 50 mL of dry acetone was slowly added N, N-dimethylaminoethyl chloride·HCl (0.98 g, 6.78 mmol) with N_2. The mixture was refluxed for 4 h and stirred vigorously under N_2. After the reaction mixture was cooled to room temperature, the mixture was filtered. The solution was washed 3 times with H_2O, dried with anhydrous magnesium sulfate, and then filtered. The filtrates were evaporated to dryness. Yield: 99% (0.36g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.41 (m, 2H), 1.54 (s, 1.5H), 1.61 (s, 1.5H), 1.77 (m, 1H), 2.10 (m, 1H), 2.38 (s, 12H), 2.68 (s, 1.5H), 2.77 (t, 4H), 2.90 (s, 1.5H), 3.07 (m, 1H), 3.48 (m, 1H), 4.08 (t, 4H), 6.84 (d, 4H, J = 8.1 Hz), 6.98 – 7.06 (m, 4H), 7.07 – 7.16 (m, 3H), 7.18 – 7.34 (m, 12H), 7.45

(d, 4H, J = 6.9 Hz). MALDI-TOF MS (matrix: dithranol); m/z Cald 805.43; found 807.00.

G1- STr -Ammonium (64). Into a sealed tube with a solution of G1- STr -MA (**63**) (0.39 g, 0.61 mm0l) in 12 mL of CH_2Cl_2 was added CH_3I (1.4 mL, 22.49 mmol) dropwise via syringe. The tube was wrapped with aluminum foil. The reaction mixture was stirred at room temperature for 24 h. The precipitated salts were filtered and dissolved in methanol to remove insoluble impurities. After micro-syringe filtration, the filtrates were evaporated to dryness. Yield: 42% (0.21g); ^{1}H NMR (CD_3OD) δ (ppm) 1.22 (m, 2H), 1.44 (s, 1.5H), 1.54 (s, 1.5H), 1.70 (m, 1H), 2.04 (m, 1H), 2.80 (s, 1.5H), 2.86 (s, 1.5H), 3.11 (m, 1H), 3.22 (s, 18H), 3.38 (m, 1H), 3.79 (s, 4H), 4.40 (s, 4H), 6.76 – 6.88 (m, 4H), 7.00 – 7.02 (m, 4H), 7.04 – 7.20 (m, 9H), 7.24 – 7.38 (m, 6H), 7.54 (d, 4H, J = 8.4 Hz). MALDI-TOF MS (matrix: dithranol); m/z Cald 1089.28; found 963.34.

Attempt at synthesis of G1-SH-Ammonium (49) (See Scheme 3.23). G1- STr -Ammonium (**64**) (50.0 mg, 0.05 mmol) put into a flame-dried Schlenk flask and placed under vacuum for 1 h. The flask was sealed under vacuum and transferred to a nitrogen line and placed under nitrogen for 1 h. To the flask at 0 °C was added 7 mL of trifluoroacetic acid distilled, dried, and degassed via syringe. The reaction mixture was stirred for 30 min under N_2 and allowed to warm to room temperature (But, G1-

STr -Ammonium (**64**) was not dissolved yet). To the flask was then slowly added triethyl silane degassed (0.5 mL, 3.13 mmol). The reaction mixture started to dissolve in 10 min. The flask was sealed and was stirred for 2 h. Trifluoroacetic acid was removed under reduced pressure. The residual solid was washed 3 times with hexane distilled, dried, and degassed. The resulting product was dried *in vacuo* for 12 h and put into the dry box for the next step.

Attempt at synthesis of $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4][N(CH_3)_4]_2$ (50) (See Scheme 3.23). Into the Schlenk flask with a solution of G1-SH-Ammonium (**49**) (38.9 mg, 0.05 mmol) in 6 mL of DMF dried and degassed was slowly added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (6.6 mg, 0.007 mmol) in 10 mL of DMF in dry box. Upon addition, the red-wine color developed rapidly. After the reaction mixture was stirred for 15 min, the flask was sealed and removed from the dry box. The solvent was then removed *in vacuo* at 40 $^{\circ}$C for 5 h and the resulting product was dried more *in vacuo* at 40 $^{\circ}$C for 2 days.

G1- STr -t-Bu (65). To a suspension of G1-STr-OH (**62**) (0.30 g, 0.45 mmol), anhydrous potassium carbonate (0.37 g, 2.71 mmol), and a catalytic amount of 18-crown-6 in 30 mL of dry acetone was added 4-Bromomethyl benzoic acid t-butyl ester (**44**) (0.27 g, 0.99 mmol). The mixture was refluxed for 12 h and stirred vigorously under N_2. After the reaction mixture was cooled to room temperature, the mixture was

filtered. The filtrates were evaporated to remove the solvent under reduced pressure. The crude product was purified by column chromatography, eluting with the solvent system of 20 % ethyl acetate in hexane. Yield: 92% (0.44g); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.32 (m, 2H), 1.44 (s, 1.5H), 1.53 (s, 18H), 1.63 (s, 1.5H), 1.85 (m, 1H), 1.98 (m, 1H), 2.57 (s, 1.5H), 2.80 (s, 1.5H), 3.00 (m, 1H), 3.37 (m, 1H), 5.02 (s, 4H), 6.77 (d, 4H, J = 7.5 Hz), 6.86 – 6.96 (m, 5H), 6.98 – 7.06 (m, 2H), 7.08 – 7.25 (m, 12H), 7.33 (d, 4H, J = 6.9 Hz), 7.40 (d, 4H, J = 8.1 Hz), 7.93 (d, 4H, J = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ (ppm) 22.49 (24.03), 28.03, 32.82, 38.62 (38.89), 44.91, 47.66, 51.70, 69.53, 71.12, 81.24, 126.50 (126.76), 127.02, 127.94, 128.10, 128.32, 128.43, 129.05, 129.87, 130.14, 133.51, 135.53, 136.82, 141.94 (142.37), 144.41, 156.67, 165.68, 170.92 (171.46). MALDI-TOF MS (matrix: DHB); m/z Cald 1043.48; found 1066.37.

G0-SH-COOH (4-mercaptobenzoic acid) (7) (See Scheme 3.25). 4-Tritylsulfanyl benzoic acid (**60**) (46.4 mg, 0.12 mmol) put into a flame-dried Schlenk flask and placed under vacuum for 1h. The flask was sealed under vacuum and transferred to a nitrogen line and placed under nitrogen for 1 h. To the flask at 0 °C was added 10 mL of trifluoroacetic acid distilled, dried, and degassed via syringe. The reaction mixture was stirred for 10 min under N_2 and allowed to warm to room temperature. To the flask was then slowly added triethyl silane degassed (0.2 mL, 1.25 mmol). The flask was sealed and stirred for 2 h. Trifluoroacetic acid was removed under reduced pressure. The residual solid was washed 4 times with hexane distilled, dried, and

degassed. The resulting product was dried *in vacuo* for 12 h and put into the dry box. ^{1}H NMR (DMSO-d_6) δ (ppm) 5.93 (s, 1H), 7.39 (d, 2H, J = 6.6 Hz), 7.78 (d, 2H, J = 6.9 Hz).

Attempt at synthesis of G1-SH-COOH (58) (See Scheme 3.26). G1- STr -t-Bu (**65**) (43.6 mg, 0.04 mmol) was put into a flame-dried Schlenk flask and placed under vacuum for 1h. The flask was sealed under vacuum and transferred to a nitrogen line and placed under nitrogen for 1 h. To the flask at 0 °C was added 10 mL of trifluoroacetic acid distilled, dried, and degassed via syringe. The reaction mixture was stirred for 8 h under N_2 and allowed to warm to room temperature. To the flask was then slowly added triethyl silane degassed (0.5 mL, 3.13 mmol). The flask was sealed and stirred for 12 h. Trifluoroacetic acid was removed under reduced pressure. The residual solid was dissolved in ethyl acetate distilled, dried, and degassed and precipitated from hexane distilled, dried, and degassed. The precipitates were washed 3 times with hexane. The resulting product was dried *in vacuo* for 12 h and put into the dry box for the next step.

Attempt at synthesis of $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4][N(CH_3)_4]_2$ (59) (See Scheme 3.26). Into the Schlenk flask with a solution of G1-SH-COOH (**58**) (28.8 mg, 0.04 mmol) in 6 mL of DMF dried and degassed was slowly added a solution of $[Fe_4S_4(S\text{-}t\text{-}Bu)_4][N(CH_3)_4]_2$ (6.0 mg, 0.007 mmol) in 10 mL of DMF in dry box. Upon addition,

the red-wine color developed rapidly. After the reaction mixture was stirred for 15 min, the flask was sealed and removed from the dry box. The solvent was then removed *in vacuo* at 40 °C for 5 h and the resulting product was dried more *in vacuo* at 40 °C for 2 days.

Ellman's Reagent Test.

Ellman's reagent test consists of three parts: reagent preparation, measurement, and calculation.

The first step is the preparation of four different solutions, Buffer A, Buffer B, Ellman's reagent solution, DTNB, and stock solution of the thiol. In Buffer A, sodium acetate (5 mM, 41 mg) and sodium chloride (50 mM, 292 mg) were dissolved in deionized water (80 mL). 1 mL of EDTA (50 mM, pH 8.0) was added and the pH was adjusted to 4.7 with 1N HCl. Deionized water was added more to bring the volume to 100 mL. In Buffer B, sodium phosphate monohydrate (40 mM 0.55 g) was dissolved in deionized water (80 mL). 4 mL of EDTA (50 mM, pH 8.0) was added and the pH was adjusted to 7.6 with 1N NaOH. Deionized water was also added more to set the volume to 100 mL. In Ellman's reagent solution preparation, DTNB (100 mM, 10 mg) was dissolved in 250 μL in DMSO. In the preparation of stock solution, the thiol (100 mM) was dissolved in Buffer A. All solutions generated should be degassed thoroughly before use. Otherwise, the thiols added can be oxidized to the disulfides by oxygen dissolved in solutions. The solutions were degassed on ice with a vacuum for

30 minutes and then degassed for an additional 30 minutes right before use.

The second step of the Ellman's reagent test is measurement. The thiol working solution (0.1 mM, 5.0 mL) was prepared by a dilution of degassed stock solution (100 mM, 5 μL) into 5 mL of degassed Buffer A. Degassed Buffer B (0.69 mL) was added to each of two clean vials capped with rubber septum. The thiol working solution (0.1 mM, 0.4 mL) was added to one of the vials and Buffer A (0.4 mL) was added to the other vial to serve as a control. Ellman's reagent (100 mM, 10 μL) was added to each vial and the solutions were mixed well at room temperature for 5 minutes. The above procedures were performed under nitrogen. The absorbance of the two solutions at 412 nm was measured. The absorbance of the control at 412 nm was subtracted from the absorbance of the thiol solution. This is the corrected absorbance value (ΔA_{412}).

The last step was the calculation of thiol concentration. The thiol concentration of the thiol working solution was calculated using the equation 3.1.

3.5. References

(1) Brown, S.; Bernardo, M. M.; Li, Z. H.; Kotra, L. P.; Tanaka, Y.; Fridman, R.; Mobashery, S. *J. Am. Chem. Soc.* **2000**, *122*, 6799-6800.

(2) Chen, K. Y.; Gorman, C. B. *J. Org. Chem.* **1996**, *61*, 9229-9235.

(3) Delaigue, X.; Hosseini, M. W. *Tet. Lett.* **1993**, *34*, 8111-8112.

(4) Meier, H.; Rumpf, N. *Tet. Lett.* **1998**, *39*, 9639-9642.

(5) Sebok, P.; Timar, T.; Eszenyi, T.; Patonay, T. *J. Org. Chem.* **1994**, *59*, 6318-6321.

(6) Wolfers, H.; Kraatz, U.; Korte, F. *Synthesis-Stuttgart* **1975**, 43-44.

(7) Bryson, N.; Dewan, J. C.; Listerjames, J.; Jones, A. G.; Davison, A. *Inorg. Chem.* **1988**, *27*, 2154-2161.

(8) Fernandes, C.; Correia, J. D. G.; Gano, L.; Santos, I.; Seifert, S.; Syhre, R.; Bergmann, R.; Spies, H. *Bioconjugate Chem.* **2005**, *16*, 660-668.

(9) Glaser, M.; Karlsen, H.; Solbakken, M.; Arukwe, J.; Brady, F.; Luthra, S. K.; Cuthbertson, A. *Bioconjugate Chem.* **2004**, *15*, 1447-1453.

(10) Guo, W. H.; Li, J. J.; Wang, Y. A.; Peng, X. G. *J. Am. Chem. Soc.* **2003**, *125*, 3901-3909.

(11) Hiskey, R. G.; Mizoguchi, T.; Igeta, H. *J. Am. Chem. Soc.* **1966**, *31*, 1188-1192.

(12) Kramer, D. J.; Davison, A.; Davis, W. M.; Jones, A. G. *Inorg. Chem.* **2002**, *41*, 6181-6183.

(13) Pearson, D. A.; Blanchette, M.; Baker, M. L.; Guindon, C. A. *Tet. Lett.* **1989**, *30*, 2739-2742.

(14) Wang, Y. A.; Li, J. J.; Chen, H. Y.; Peng, X. G. *J. Am. Chem. Soc.* **2002**, *124*, 2293-2298.

(15) Zervas, L.; Photaki, I. *J. Am. Chem. Soc.* **1962**, *84*, 3887-3897.

(16) Gorman, C. B. *Adv. Mater.* **1997**, *9*, 1117-1119.

(17) Gorman, C. B.; Parkhurst, B. L.; Su, W. Y.; Chen, K. Y. *J. Am. Chem. Soc.* **1997**, *119*, 1141-1142.

(18) Gorman, C. B.; Smith, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 9342-9343.

(19) Gorman, C. B.; Smith, J. C. *Acc. Chem. Res.* **2001**, *34*, 60-71.

(20) Gorman, C. B.; Smith, J. C.; Hager, M. W.; Parkhurst, B. L.; Sierzputowska-

Gracz, H.; Haney, C. A. *J. Am. Chem. Soc.* **1999**, *121*, 9958-9966.

(21) Greene, T. W.; Wuts, P. G. *Protective Groups In Organic Synthesis*; 2nd ed.; John Wiley & Sons, Inc.: 1991.

(22) Lees, W. J.; Singh, R.; Whitesides, G. M. *J. Org. Chem.* **1991**, *56*, 7328-7331.

(23) Loup, C.; Zanta, M. A.; Caminade, A. M.; Majoral, J. P.; Meunier, B. *Chem. Eur. J.* **1999**, *5*, 3644-3650.

(24) Pan, Y. J.; Ford, W. T. *Macromolecules* **1999**, *32*, 5468-5470.

(25) Pan, Y. J.; Ford, W. T. *J. Org. Chem.* **1999**, *64*, 8588-8593.

(26) Pan, Y. J.; Ford, W. T. *Macromolecules* **2000**, *33*, 3731-3738.

(27) Cardona, C. M.; Alvarez, J.; Kaifer, A. E.; McCarley, T. D.; Pandey, S.; Baker, G. A.; Bonzagni, N. J.; Bright, F. V. *J. Am. Chem. Soc.* **2000**, *122*, 6139-6144.

(28) Cardona, C. M.; McCarley, T. D.; Kaifer, A. E. *J. Org. Chem.* **2000**, *65*, 1857-1864.

(29) Cardona, C. M.; Mendoza, S.; Kaifer, A. E. *Chem. Soc. Rev.* **2000**, *29*, 37-42.

(30) Dandliker, P. J.; Diederich, F.; Gisselbrecht, J. P.; Louati, A.; Gross, M. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2725-2728.

(31) Hawker, C. J.; Wooley, K. L.; Fréchet, J. M. J. *J. Chem. Soc. Perkin Trans. 1* **1993**, 1287-1297.

(32) Pesak, D. J.; Moore, J. S. *Tetrahedron* **1997**, *53*, 15331-15347.

(33) Pesak, D. J.; Moore, J. S.; Wheat, T. E. *Macromolecules* **1997**, *30*, 6467-6482.

(34) Wang, Y.; Cardona, C. M.; Kaifer, A. E. *J. Am. Chem. Soc.* **1999**, *121*, 9756-9757.

(35) Young, J. K.; Baker, G. R.; Newkome, G. R.; Morris, K. F.; Johnson, C. S. *Macromolecules* **1994**, *27*, 3464-3471.

(36) Newkome, G. R.; Moorefield, C. N.; Baker, G. R.; Saunders, M. J.; Grossman, S. H. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1178-1180.

(37) Kim, Y. H.; Webster, O. W. *J. Am. Chem. Soc.* **1990**, *112*, 4592-4593.

(38) Laufersweiler, M. J.; Rohde, J. M.; Chaumette, J. L.; Sarazin, D.; Parquette, J. R. *J. Org. Chem.* **2001**, *66*, 6440-6452.

(39) Tomioka, N.; Takasu, D.; Takahashi, T.; Aida, T. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 1531-1534.

(40) Carey, F. A. *Organic Chemistry*; 2nd ed.; McGRAW-HILL, INC.: 1992.

(41) Gruter, G. J. M.; Akkerman, O. S.; Bickelhaupt, F. *J. Org. Chem.* **1994**, *59*, 4473-4481.

(42) Xia, C. J.; Fan, X. W.; Locklin, J.; Advincula, R. C. *Org. Lett.* **2002**, *4*, 2067-2070.

(43) Campbell, L. J.; Borges, L. F.; Heldrich, F. J. *Bioorg. & Med. Chem. Lett.* **1994**, *4*, 2627-2630.

(44) Goswami, S.; Dey, S.; Jana, S.; Adak, A. K. *Chem. Lett.* **2004**, *33*, 916-917.

(45) Brummond, K. M.; Lu, J. L. *J. Org. Chem.* **1999**, *64*, 1723-1726.

(46) Jayakumar, K. N.; Bharathi, P.; Thayumanavan, S. *Org. Lett.* **2004**, *6*, 2547-2550.

(47) Bo, Z. S.; Schafer, A.; Franke, P.; Schluter, A. D. *Org. Lett.* **2000**, *2*, 1645-1648.

(48) Dvornikovs, V.; House, B. E.; Kaetzel, M.; Dedman, J. R.; Smithrud, D. B. *J. Am. Chem. Soc.* **2003**, *125*, 8290-8301.

(49) Bhattacharya, S.; Haldar, S. *Proc. Indian Acad. Sci. (Chem. Sci.)* **2002**, *114*, 197-201.

(50) Kimura, T.; Takase, Y.; Hayashi, K.; Tanaka, H.; Ohtsuka, I.; Saeki, T.; Kogushi, M.; Yamada, T.; Fujimori, T.; Saitou, I.; Akasaka, K. *J. Med. Chem.* **1993**, *36*, 1630-1640.

(51) Woo, L. W. L.; Sutcliffe, O. B.; Bubert, C.; Grasso, A.; Chander, S. K.; Purohit, A.; Reed, M. J.; Potter, B. V. L. *J. Med. Chem.* **2003**, *46*, 3193-3196.

(52) Pettit, G. R.; Grealish, M. P.; Jung, M. K.; Hamel, E.; Pettit, R. K.; Chapuis, J. C.; Schmidt, J. M. *J. Med. Chem.* **2002**, *45*, 2534-2542.

(53) Naudin, E.; Ho, H. A.; Bonin, M. A.; Breau, L.; Belanger, D. *Macromolecules* **2002**, *35*, 4983-4987.

(54) Lenihan, B. D.; Shechter, H. *J. Org. Chem.* **1998**, *63*, 2072-2085.

(55) Kucerovy, A.; Li, T. Q.; Repic, O.; Blacklock, T. J. *Org. Proc. Res. & Dev.* **1997**, *1*, 287-293.

(56) Fraenkel, G.; Kim, J. P. *J. Am. Chem. Soc.* **1966**, *88*, 4203-4211.

(57) Morrison, R. T.; Boyd, R. N. *Organic Chemistry*; 5th ed.; Allyn and Bacon, Inc.: 1987.

(58) Lee, J. J.; Ford, W. T.; Moore, J. A.; Li, Y. F. *Macromolecules* **1994**, *27*, 4632-4634.

(59) Richter, S. N.; Maggi, S.; Mels, S. C.; Palumbo, M.; Freccero, M. *J. Am. Chem. Soc.* **2004**, *126*, 13973-13979.

(60) Chasse, T. L.; Sachdeva, R.; Li, C.; Li, Z. M.; Petrie, R. J.; Gorman, C. B. *J. Am. Chem. Soc.* **2003**, *125*, 8250-8254.

(61) Gorman, C. *Adv. Mater.* **1998**, *10*, 295-309.

(62) Gorman, C. B. *Chem. Commun.* **1999**, 877-878.

(63) Gorman, C. B.; Hager, M. W.; Parkhurst, B. L.; Smith, J. C. *Macromolecules* **1998**, *31*, 815-822.

(64) Gorman, C. B.; Smith, J. C. *Polymer* **2000**, *41*, 675-683.

(65) Gorman, C. B.; Smith, J. C.; Sachdeva, R.; Su, W. Y.; Jiang, H. W. *Macromol. Symp.* **2000**, *156*, 61-67.

(66) Dukes, G. R.; Holm, R. H. *J. Am. Chem. Soc.* **1975**, *97*, 528-533.

(67) Holm, R. H. *Acc. Chem. Res.* **1977**, *10*, 427-434.

(68) Holm, R. H.; Averill, B. A.; Herskovi.T; Frankel, R. B.; Gray, H. B.; Siiman, O.; Grunthan, F. J. *J. Am. Chem. Soc.* **1974**, *96*, 2644-2646.

(69) Holm, R. H.; Phillips, W. D.; Averill, B. A.; Mayerle, J. J.; Herskovi.T *J. Am. Chem. Soc.* **1974**, *96*, 2109-2117.

(70) Johnson, R. W.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5338-5344.

(71) Reynolds, J. G.; Coyle, C. L.; Holm, R. H. *J. Am. Chem. Soc.* **1980**, *102*, 4350-4355.

(72) Reynolds, J. G.; Laskowski, E. J.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5315-5322.

(73) Averill, B. A.; Herskovi.T; Holm, R. H.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 3523-3534.

(74) Bobrik, M. A.; Laskowski, E. J.; Johnson, R. W.; Gillum, W. O.; Berg, J. M.; Hodgson, K. O.; Holm, R. H. *Inorg. Chem.* **1978**, *17*, 1402-1410.

(75) Bobrik, M. A.; Que, L.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 285-287.

(76) Depamphi.Bv; Averill, B. A.; Herskovi.T; Que, L.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4159-4167.

(77) Hill, C. L.; Renaud, J.; Holm, R. H.; Mortenson, L. E. *J. Am. Chem. Soc.*

1977, *99*, 2549-2557.

(78) Laskowski, E. J.; Frankel, R. B.; Gillum, W. O.; Papaefthymiou, G. C.; Renaud, J.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1978**, *100*, 5322-5337.

(79) Que, L.; Bobrik, M. A.; Ibers, J. A.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4168-4178.

(80) Depamphi, B. V.; Averill, B. A.; Herskovi, T.; Que, L.; Holm, R. H. *J. Am. Chem. Soc.* **1974**, *96*, 4159-4167.

(81) Field, L.; Bryan, A. W.; Owen, T. C.; Crenshaw, R. R. *J. Am. Chem. Soc.* **1961**, *83*, 4414-4417.

(82) Field, L.; Giles, P. M. *J. Org. Chem.* **1971**, *36*, 309-313.

(83) Field, L.; Hanley, W. S.; McVeigh, I. *J. Org. Chem.* **1971**, *36*, 2735-2739.

(84) Field, L.; Kim, H. K. *J. Med. Chem.* **1966**, *9*, 397-402.

(85) Parsons, T. F.; Buckman, J. D.; Pearson, D. E.; Field, L. *J. Org. Chem.* **1965**, *30*, 1923-1926.

(86) Bryan, D. B.; Hall, R. F.; Holden, K. G.; Huffman, W. F.; Gleason, J. G. *J. Am. Chem. Soc.* **1977**, *99*, 2353-2355.

(87) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70-77.

(88) Eyer, P.; Worek, F.; Kiderlen, D.; Sinko, G.; Stuglin, A.; Simeon-Rudolf, V.; Reiner, E. *Anal. Biochem.* **2003**, *312*, 224-227.

(89) Singh, R.; Blattler, W. A.; Collinson, A. R. *Anal. Biochem.* **1993**, *213*, 49-56.

(90) Danehy, J. P.; Oester, M. Y. *J. Org. Chem.* **1967**, *32*, 1491-1495.

(91) Takada, T.; Barton, J. K. *J. Am. Chem. Soc.* **2005**, *127*, 12204-12205.

Chapter 4

Electrochemical Behaviors of Amphiphilic Iron-Sulfur Core Dendrimers

4.1. Introduction

Dendritic encapsulation of the iron-sulfur core has been investigated as variations in the kinetics and thermodynamics of electron transfer to the redox-active iron-sulfur cluster core.[1-29] Variation in the kinetics of electron transfer is evidenced as changes in the heterogeneous electron transfer rate, while variations in the thermodynamics of electron transfer are evidenced as shifts of the redox potential of the iron-sulfur cluster. Attenuation of the electron transfer rate between a redox core and an electrode surface via non-conducting branched dendritic shell has been studied extensively. These studies have indicated that the electron transfer rate can be governed by the distance of electron transfer and the properties of dendrons surrounding the core.[1-9,11,12,14,15,17-26,28-30] However, the properties governing the thermodynamics have not been studied thoroughly and there has been little understanding of how the dendritic structure affects redox potential.[2,4,6,10,13,16,23,27]

The electrochemical behavior of iron-sulfur core dendrimers containing flexible and rigid structures was studied by the Gorman group.[11,31] Attenuation of the electron transfer rate in solution and the shifts of the redox potential to more negative potential in film study were also observed with increasing generation. This research contributed to better understanding structure-property relationship affecting electron transfer reactions in dendrimer encapsulated redox centers.

Synthesis and study of amphiphilic iron-sulfur core dendrimers is an extension

of flexible dendrimer studied as a protein model. The hydrophobic peripheral units in the flexible series of dendrimers were modified into the hydrophilic charged and polar end groups including ammonium and carboxylate. The resulting amphiphilic iron-sulfur core dendrimers were essentially unimolecular micelles. These peripheral units may indicate the effects of charge on the rate and/or driving force of electron transfer relative to non-ionic and hydrophobic flexible dendrimers.

Amphiphilic iron-sulfur core dendrimers may undergo changes in size and morphology by swelling via micellar encapsulation of small, non-polar molecules in aqueous or aqueous/organic mixed solvent. These changes can be measured by electrochemical experiments in attempt to understand the effects of dendritic structure on the rate and driving force of electron transfer. Thus, effective electron transfer distance or the microenvironment surrounding the iron-sulfur core of this dendrimer model may be tuned. In this way, structure-property relationships may be studied via micellar encapsulation. Attenuation of electron transfer rates due to the change of hydrodynamic radius or relationship of the effective electron transfer distance and molecular size may be observed.

In this chapter, electrochemical experiments of two types of dendrimers in organic solvent, G1-cationic and G1-anionic, are described and compared with the G1-flexible dendrimer. Then, the effects on the morphology and the stability of G1-cationic dendrimer in an organic/aqueous mixed solution are studied.

4.2 Results and Discussion

4.2.1 Cationic Dendrimer

Both cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV) were measured to investigate the electrochemical properties of G1-cationic dendrimer. In cyclic voltammetry, peak separation (ΔE_p) provided qualitative kinetic information and redox potential ($E_{1/2}$), determined from the half wave potential of both cathodic and anodic peaks, provided thermodynamic information.

Table 4.1. Diffusion coefficient (D_o) obtained from Chronoamperometry (CA), corresponding Stokes-Einstein Radius (R_H), heterogeneous electron-transfer rate constant (k_0), reduction potential ($E_{1/2}$), and transfer coefficient (α) for the one-electron redox couple $[Fe_4S_4(S\text{-}G1\text{-}Flex)_4]^{2-/3-}$, $[Fe_4S_4(S\text{-}G1\text{-}Ammonium)_4]^{2-/3-}$ (50), and $[Fe_4S_4(S\text{-}G1\text{-}COOH)_4]^{2-/3-}$ (59) in DMF.

	CA		CV		OSWV		
Structure	D_o ($\times 10^6$ cm^2/s)	R_H (Å)	k_o ($\times 10^3$ cm/s)	$E_{1/2}$ (mV)	k_o ($\times 10^3$ cm/s)	$E_{1/2}$ (mV)	α
G1-Flex	3.47	7.75	8.36	-1360	6.15	-1359	0.54
G1-Cat	3.44	8.07	4.72	-1261	3.42	-1256	0.55
G1-Ani	-	-	-	-1320	-	-	-

* G1-Flex: G1-Flexible dendrimer; G1-Cat: G1-Cationic dendrimer (**50**); G1-Ani: G1-Anionic dendrimer (**59**).

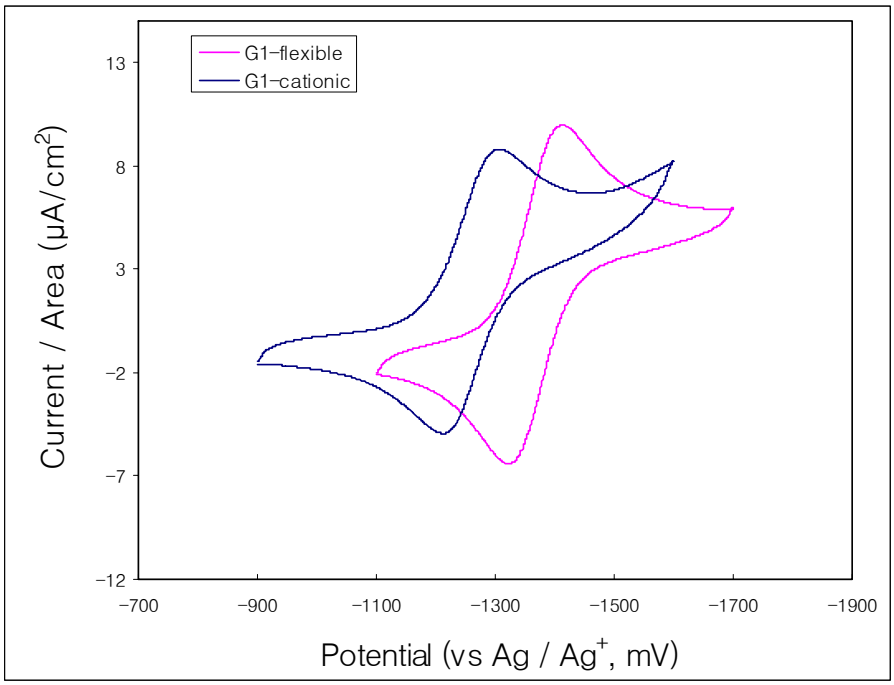


Figure 4.1. Cyclic voltammograms for G1-cationic dendrimer (**50**) and flexible dendrimer (1mM dendrimer solution, scan rate 100mV/s, 100mM tetraethylammonium tetrafluoroborate supporting electrolyte, platinum as working electrode).

Moreover, the quantitative heterogeneous electron transfer rate (k_0) for quasi-reversible system could be calculated using the diffusion coefficient measured from chronoamperometry (CA) and the Nicholson analysis relating peak separation (ΔE_p) to a kinetic parameter, Ψ.[32-35]

The electrochemical behavior measured for G1-cationic dendrimer (**50**) was compared with that of G1-flexible dendrimer (Figure 4.1 and Table 4.1). A plot of peak current obtained from CV versus the square root of the scan rate showed linear

behavior, indicating no adsorption of the macromolecular species onto the electrode and freely diffusing molecules (Figure 4.2).

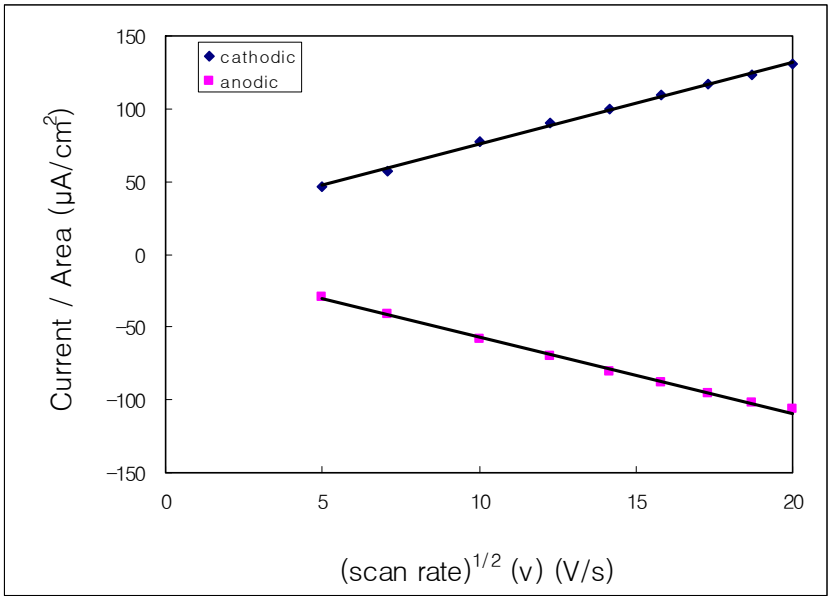


Figure 4.2. Plots of current versus the square root of scan rate. This linear relationship indicates a freely diffusing species.

The Nicholson analysis is adequate for quasi-reversible systems. However, in very fast and slow electron-transfer regimes, the electron transfer rate can not be accurately calculated. The kinetic parameter, Ψ, varies too greatly with ΔE for peak separations of $\Delta E_p < 70$ mV and varies very little for peak separations of $\Delta E_p > 150$ mV. Thus, use of ΔE_p in these regimes to compute a rate is not desiable as the error in the determination is large in each case.

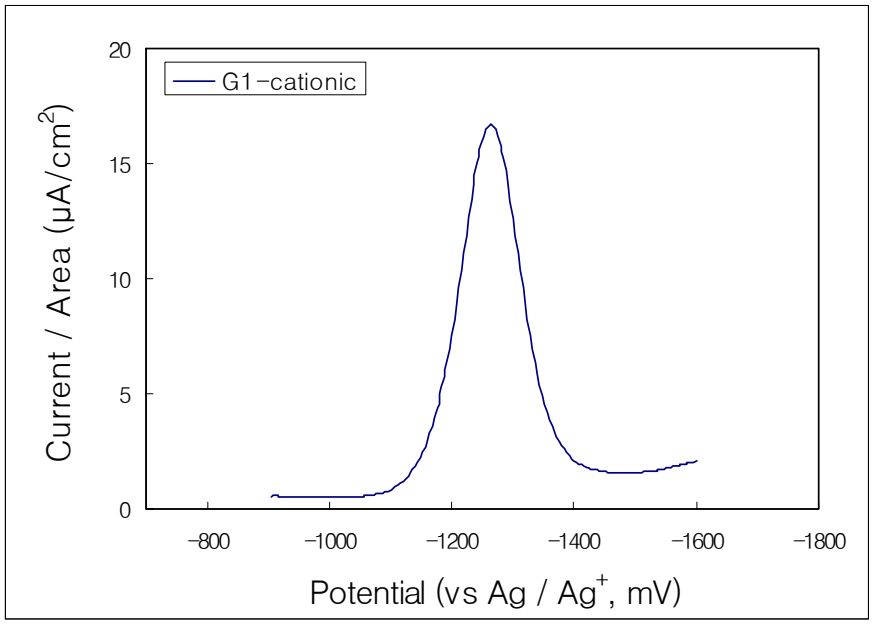


Figure 4.3. Osteryoung square wave voltammogram for G1-cationic dendrimer (**50**).

OSWV is highly sensitive and useful in slow rate regime and thus, could be used to determine accurate electron-transfer rate constants. This method uses a square wave superimposed on a staircase waveform to reduce the background charging currents, affording enhanced sensitivity in comparison with CV. Its current-voltage response is able to be fitted iteratively to display heterogeneous electron transfer rate constant. The results for G1-cationic dendrimer (**50**) are shown in Table 4.1 and Figure 4.3.

Diffusion coefficients (D_0) for dendrimers were measured by CA and used to calculate their heterogeneous electron transfer rate constants (Table 4.1). This method uses a potential step technique and the current response is monitored as a

function of time. By plotting the time-dependent current versus $1 / t^{1/2}$, the diffusion coefficient was calculated using the Cottrell equation.[32] Moreover, diffusion coefficients could be used to determine the effective size of the dendrimers in solution. By the Stokes-Einstein equation, the hydrodynamic radii (R_H) of the dendrimers were calculated (Table 4.1) [11,36]

$$R_H = \frac{k_b T}{6\pi\eta D_0} \qquad \text{(Equation 4.1)}$$

where k_b is the Boltzmann constant, T is temperature, and η is the viscosity of the medium.

According to Table 4.1, the heterogeneous electron transfer rate constant of G1-cationic dendrimer measured with both CV and OSWV was 1.8 times smaller than that of the G1-flexible dendrimer. Initially it was thought that perhaps the big molecular size of G1-cationic dendrimer could explain the smaller rate constant. However, diffusion coefficients measured via CA exhibited only a slight difference between the two dendrimers. Hydrodynamic radius calculations based on the diffusion coefficients also showed only slight differences. The only structural difference between the dendrimers was the peripheral units (Figure 4.4). In DMF, the salvation of these peripheral units presumably is similar and thus the hydrodynamic radii of them are similar. Because the differences in diffusion

coefficients and hydrodynamic radii are small, the difference in relative electron transfer rates between these molecules could not be explained by differences in molecular size.

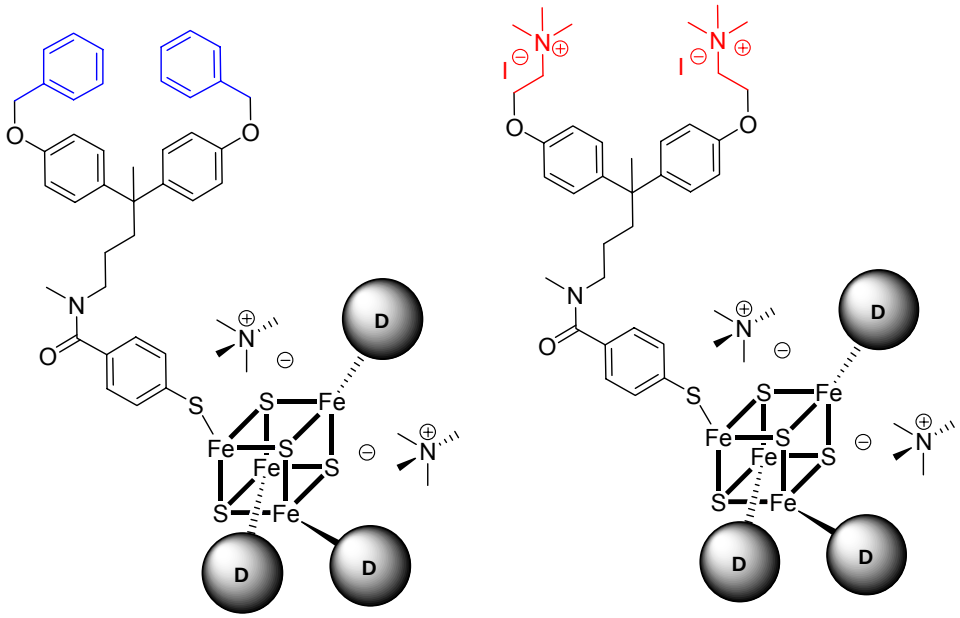


Figure 4.4. Structures of G1-flexible dendrimer (left) and G1-cationic dendrimer (right). Circled D's represent additional arms identical to that fully drawn out.

Because the differences in electron transfer rates could not be attributed to differences in dendrimer size, the electrochemical data was examined more closely. The peak separation between the anodic and cathodic peaks was different for the two dendrimers. Quantitative heterogeneous electron transfer rate constants for quasi-reversible system can be calculated by the Nicholson analysis. This analysis is based on a relationship between peak separation and a kinetic parameter. In a quasi-

reversible system, the rate of electron transfer is too slow to maintain the surface concentrations of redox couple at the equilibrium values required by Nernst equation as the potentials are changed. The slow electron transfer is indicated by the increase in peak separation in CV as the scan rate is increased. Slow electron transfer can be due to intramolecular structural changes and/or variations in the solvent coordination sphere accompanying electron transfer.[37]

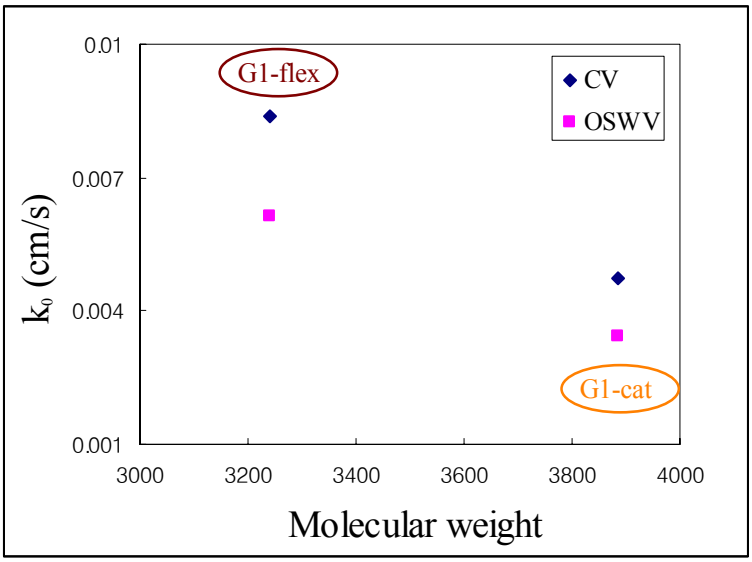


Figure 4.5. Heterogeneous electron transfer rates vs. molecular weight (flexible vs. cationic).

Also, another possibility is that the molecular weight of the dendrimers affects the electron transfer rate (Figure 4.5). While the size of both dendrimers is approximately the same, the G1-cationic dendrimer has a larger molecular weight. This difference could contribute to the attenuation in the heterogeneous electron

transfer rate.

On the other hand, redox potential of the G1-cationic dendrimer was positively shifted around 100mV compared to that of G1-flexible dendrimer. The only structural difference between cationic and flexible dendrimers was the peripheral units, strong electron-withdrawing groups (NMe_3^+) and weak electron-donating groups (phenyl), respectively. According to the work of Holm and co-workers, the introduction of an electron withdrawing group to synthetic iron-sulfur clusters could lead to positive shift in redox potential.[38] This result indicated that the electron withdrawing character of these substituents impeded the oxidation process. Also, in the case of these molecules, the electron withdrawing groups could only affect the redox potential inductively as no direct resonance interaction between these groups and the iron-sulfur cluster exists. However, the inductive effect may not be relevant in the G1-cationic dendrimer because the effect weakens continuously with increasing distance from the substituents. Probably, the positive shift in redox potential of the G1-cationic dendrimer is because the iron-sulfur core is encapsulated in dendrimer microenvironment that is more polar than the solvent due to polar peripheral units (NMe_3^+). This change around the core makes reduction more favorable and the electron-releasing tendency of the ligand more difficult, resulting in showing positive shift in redox potential.

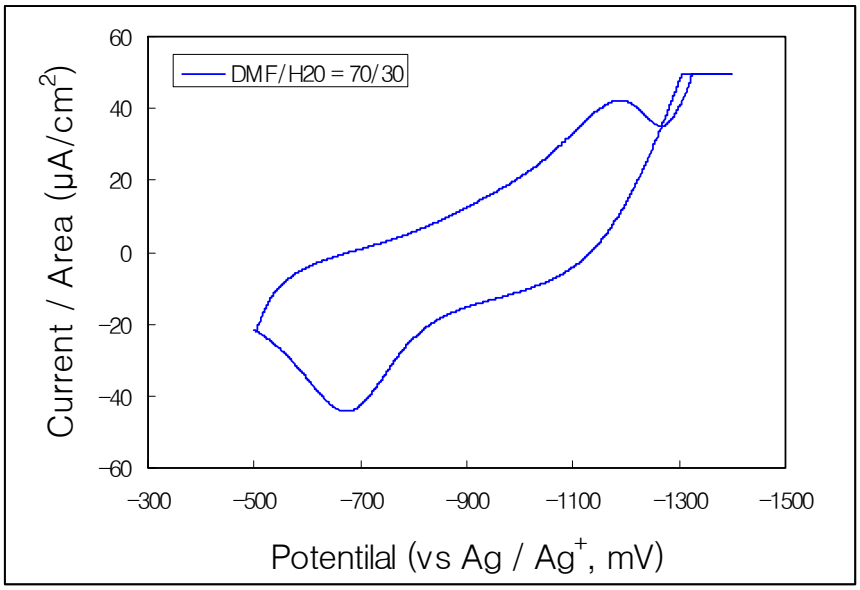


Figure 4.6. Cyclic voltammogram of G1-cationic dendrimer in DMF/H_2O = 70/30 solution. (1mM dendrimer solution, scan rate 100mV/s, 100mM tetraethylammonium tetrafluoroborate supporting electrolyte added to both the DMF and H_2O, platinum as working electrode).

After the electrochemical properties of G1-cationic dendrimer were measured in DMF solution, the addition experiment was performed in a solution of H_2O and DMF. This experiment was done to study the effects of water on the electrochemical properties of the dendrimer. The water could possibly have effects on the dendrimer morphology as a unimolecular micelle and also the stability of the iron-sulfur cluster. This electrochemical experiment was carried out in a nitrogen-filled Vacuum Atmospheres glovebox at room temperature. Distilled water was used after three cycles of boiling and cooling with nitrogen followed by vacuum for 1 hr with stirring. This procedure was performed to remove oxygen dissolved in water.

However, the CV of G1-cationic dendrimer for 70% DMF / 30% H_2O solution indicated that other chemical reactions might be occurring in solution (Figure 4.6). It appeared that the iron-sulfur core dendrimer was decomposed upon electrochemical oxidation or reduction in the presence of water.

Generally, the iron-sulfur core in electron transfer proteins is found in hydrophobic cavities including bulky surrounding materials. These bulky hydrophobic units shield the core from water, preventing decomposition during oxidation and reduction. The fact that the G1-cationic dendrimer did decompose could be attributed to its size. The G1-cationic dendron was probably not big enough to encapsulate the iron-sulfur core. The core was more accessible to water compared to cores shielded by bulkier dendritic units, resulting in decomposition. Therefore, in order to observe the redox behavior of iron-sulfur core dendrimer in water, the synthesis of higher generation dendrimers is required.

4.2.2 Anionic Dendrimer

Cyclic voltammetry was measured to investigate the electrochemical properties of the G1-anionic dendrimer compared to the G1-cationic dendrimer. However, an unexpected cathodic peak was observed at around -1700mV in the cyclic voltammogram of this molecule (Figure 4.7). This peak was attributed to impurities in the dendrimer. Attempts to purify the dendrimer included precipitation, dialysis, and diafiltration and followed by vacuum drying. These procedures drastically

changed the dendrimer solubility. After the first purification, the dendrimer was partially-soluble and after the second, it was completely insoluble. Therefore, the CV of G1-anionic dendrimer was measured without purification. The peak at -1700 mV might be attributed to impurities. Ellman's reagent test indicated the less than the expected amount of thiol during deprotection and ligand exchange reactions used to form the dendrimer. Presumably, some of the thiol formed could have formed disulfide upon thiol-deprotection, and this disulfide impurity was not removed. The peak at -1700 mV in Figure 4.8 could correspond to the reduction of the disulfide in the small molecule, disulfide dicarboxylic acid (**8**). This molecule (**8**) is the core of the disulfide, G1-anionic dendron (**17, Chapter 2**). Cyclic voltammogram of this disulfide molecule was measured and compared to G1-anionic dendrimer. The peak at 1700 mV was observed in both small molecule (**8**) and G1-anionic dendrimer. This peak leads to uncertainty regarding the electrochemical properties of the dendrimer. Only an approximate value of the redox potential was obtained (Table 4.1). It appears that the G1-anionic dendrimer exhibited a positive shift of 60mV compared to that of G1-flexible dendrimer[11]. As with the G1-cationic dendrimer, this result is most likely explained by the properties of the peripheral units. The carboxylate is also polar peripheral unit. Perhaps, the positive shift in redox potential is because the iron-sulfur core is encapsulated in dendrimer microenvironment that is more polar than the solvent due to polar peripheral units, COOH. This is supported by the fact that the G1-cationic

dendrimer contained a strong polar group (NMe_3^+) and was shifted more positively than G1-anionic dendrimer.

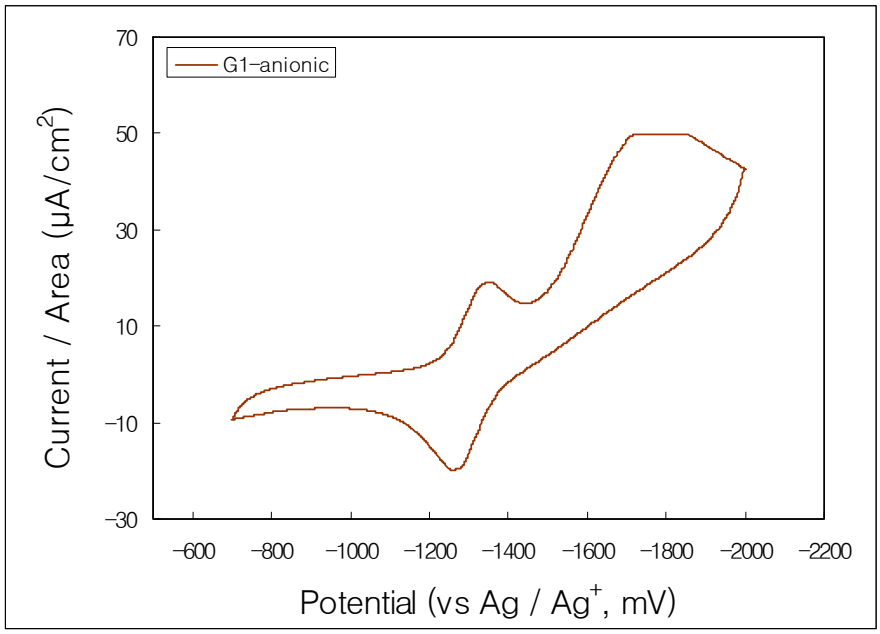


Figure 4.7. Cyclic voltammograms for G1-anionic dendrimer (**59**) (1mM dendrimer solution, scan rate 100mV/s, 100mM tetraethylammonium tetrafluoroborate supporting electrolyte, platinum as working electrode).

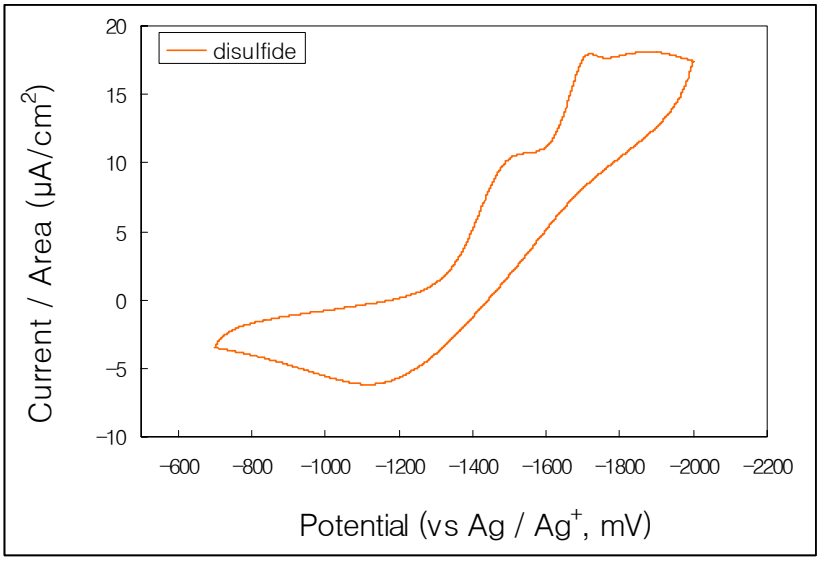


Figure 4.8. Cyclic voltammograms for disulfide dicarboxylic acid (**8**) (1mM solution, scan rate 100mV/s, 100mM tetraethylammonium tetrafluoroborate supporting electrolyte, platinum as working electrode).

4.3. Concluding Remarks

The attenuation of heterogeneous electron transfer rate in G1-cationic dendrimer compared with G1-flexible dendrimer was observed. This observation could be explained by the difference of molecular weight in both dendrimers. The increase in molecular weight of the G1-cationic dendrimer compared to the G1-flexible dendrimer contributed to the attenuation of the electron transfer rate in the G1-cationic dendrimer.

The redox potential in the G1-cationic dendrimer was positively shifted by about 100mV compared to that of the G1-flexible dendrimer. This difference could

be attributed to the differing properties of the peripheral units in each molecule. Probably, the positive shift is because the iron-sulfur core is encapsulated in dendrimer microenvironment that is more polar than the solvent due to polar peripheral units (NMe_3^+). This change around the core makes reduction more favorable and the electron-releasing tendency of the ligand more difficult, resulting in showing positive shift in redox potential.

Cyclic voltammograms of the G1-cationic dendrimer in 30:70 H_2O/DMF solution indicated that iron-sulfur core dendrimer was decomposing upon electrochemical oxidation or reduction. This result can be explained by the dendrimer size; unlike the situation found in proteins, the G1-cationic dendron was not big enough to encapsulate the iron-sulfur core. In order to observe the redox behavior in water, higher generation dendrimers capable of shielding the iron-sulfur core from water likely are required.

Cyclic voltammograms of the G1-anionic dendrimer showed an unexpected cathodic peak at around -1700mV. This peak might be explained by the reduction of disulfide dendron impurities in solution. Due to the existence of this peak, other electrochemical properties could not be measured. Only an approximate redox potential was obtained and exhibited a positive shift of 60mV compared to that of the G1-flexible dendrimer. This shift could also be explained by the differing properties of peripheral units containing a polar group. Also, by polarity nature of these substituents, G1-cationic dendrimer containing strong electron-withdrawing

group (NMe_3^+) was shifted more positively than G1-anionic dendrimer.

These studies provide into on how dendritic structure affects electron transfer rate. However, amphiphilic dendrimers for eventual use as protein model will require; (a) higher generation dendrimers not including disulfide impurities and (b) purification methods that do not affect dendrimer solubility.

4.4. Experimental Section

General considerations.[11,36] Electrochemical experiments were performed on a Bioanalytical Systems CV-50W Voltammetric Analyzer. The three-electrode cell was composed of a platinum disk working electrode with a geometric area of 0.0201 cm^2, a Pt auxiliary electrode, and a homemade non-aqueous $Ag/AgNO_3$ reference electrode (Ag wire contacting a DMF solution of 10 mM $AgNO_3$ and 100 mM supporting electrolyte, tetraethylammonium tetrafluoroborate ($TEABF_4$)). Millimolar concentrations of the analytes were dissolved in DMF, which contained 100 mM $TEABF_4$ supporting electrolyte. Pt disk working electrodes were polished, conditioned, and referenced to a dendrimer standard solution previously studied in Gorman group. All electrochemical experiments except the addition experiment of H_2O to dendrimer solution were performed in a nitrogen-filled Vacuum Atmospheres drybox at room temperature. The electrochemical experiment on the addition of H_2O was carried out in a nitrogen-filled Vacuum Atmospheres glovebox at room temperature.

Cyclic Voltammetry. Heterogeneous electron transfer rate constants (k_0) were calculated using the Nicholson analysis based on relationships between the peak separation (ΔE_p) and scan rate.[32-35] The rate constant was determined by the following equation:

$$k_0 = \Psi\left[D_0 \pi \nu \left(\frac{nF}{RT}\right)\right]^{1/2} \qquad \text{(Equation 4.2)}$$

where Ψ is the kinetic parameter related to ΔE_p which is tabulated elsewhere,[35] D_0 is the diffusion coefficient (cm^2/s), ν is the scan rate (V/s), n is the number of electrons passed, F is Faraday's constant, R is the gas constant, and T is the cell temperature. Scan rates ranged from 25 to 400 mV/s for analytes exhibiting quasi-reversible electrochemical behavior.

Osteryoung Square Wave Voltammetry. Osteryoung Square Wave Voltammetry was carried out using a step height of 4 mV, a sweep width amplitude of 25 mV, and a frequency of 15 Hz. Difference current was iteratively fit using FSQPLT software[39] to determine the transfer coefficient α and log $(k / (t_d)^{1/2})$. Electron transfer rate constant was calculated from the relation,

$$\log\left(k\sqrt{t_d}\right) = \log\left(\left(\frac{k_0}{D_0}\right)\sqrt{t_d}\right) \qquad \text{(Equation 4.3)}$$

where t_d is the experimental pulse period, D_0 is the diffusion coefficient, and k is the reduced rate constant.[40]

Chronoamperometry. Chronoamperometry was performed using a pulse width of 800, 900, and 1000 ms. This technique employs a potential step to generate a diffusion limited current response as a function of time. Time dependent current is plotted versus $t^{-1/2}$ by BAS CW 50 software. In pulse width of 800 ms, the time range of 160 – 800 ms was used. In pulse width of 900 ms and 1000 ms, 180 – 900 ms and 200 – 1000 ms were used, respectively. A diffusion coefficient was calculated from the slope of this plot (Cottrell plot).[32]

$$i(t) = \frac{nFAD_0^{1/2}C_0}{\pi^{1/2}t^{1/2}} \qquad \text{(Equation 4.4)}$$

where n is the number of electron, F is the Faraday's constant, A is the area of the electrode, and C is the concentration of the measured molecule.

4.5. References

(1) Cameron, C. S.; Gorman, C. B. *Adv. Func. Mater.* **2002**, *12*, 17-20.

(2) Cardona, C. M.; Kaifer, A. E. *J. Am. Chem. Soc.* **1998**, *120*, 4023-4024.

(3) Cardona, C. M.; McCarley, T. D.; Kaifer, A. E. *J. Org. Chem.* **2000**, *65*, 1857-1864.

(4) Cardona, C. M.; Mendoza, S.; Kaifer, A. E. *Chem. Soc. Rev.* **2000**, *29*, 37-42.

(5) Chow, H. F.; Chan, I. Y. K.; Fung, P. S.; Mong, T. K. K.; Nongrum, M. F. *Tetrahedron* **2001**, *57*, 1565-1572.

(6) Dandliker, P. J.; Diederich, F.; Gross, M.; Knobler, C. B.; Louati, A.; Sanford, E. M. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1739-1742.

(7) Gandhi, P.; Huang, B. H.; Gallucci, J. C.; Parquette, J. R. *Org. Lett.* **2001**, *3*, 3129-3132.

(8) Gorman, C. B. *Adv. Mater.* **1997**, *9*, 1117-1119.

(9) Gorman, C. B.; Smith, J. C. *Polymer* **2000**, *41*, 675-683.

(10) Gorman, C. B.; Smith, J. C. *Acc. Chem. Res.* **2001**, *34*, 60-71.

(11) Gorman, C. B.; Smith, J. C.; Hager, M. W.; Parkhurst, B. L.; Sierzputowska-Gracz, H.; Haney, C. A. *J. Am. Chem. Soc.* **1999**, *121*, 9958-9966.

(12) Huang, B. H.; Parquette, J. R. *J. Am. Chem. Soc.* **2001**, *123*, 2689-2690.

(13) Kawa, M.; Fréchet, J. M. J. *Chem. Mater.* **1998**, *10*, 286-296.

(14) Moore, J. S.; Xu, Z. F. *Macromolecules* **1991**, *24*, 5893-5894.

(15) Newkome, G. R.; Guther, R.; Moorefield, C. N.; Cardullo, F.; Echegoyen, L.; Perezcordero, E.; Luftmann, H. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2023-2026.

(16) Ong, W.; Kaifer, A. E. *J. Am. Chem. Soc.* **2002**, *124*, 9358-9359.

(17) Peerlings, H. W. I.; Trimbach, D. C.; Meijer, E. W. *Chem. Comm.* **1998**, 497-498.

(18) Pesak, D. J.; Moore, J. S. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1636-1639.

(19) Pollak, K. W.; Leon, J. W.; Fréchet, J. M. J.; Maskus, M.; Abruna, H. D. *Chem. Mater.* **1998**, *10*, 30-38.

(20) Recker, J.; Tomcik, D. J.; Parquette, J. R. *J. Am. Chem. Soc.* **2000**, *122*, 10298-10307.

(21) Rosini, C.; Superchi, S.; Peerlings, H. W. I.; Meijer, E. W. *Eur. J. Org. Chem.* **2000**, 61-71.

(22) Scherrenberg, R.; Coussens, B.; van Vliet, P.; Edouard, G.; Brackman, J.; de Brabander, E.; Mortensen, K. *Macromolecules* **1998**, *31*, 456-461.

(23) Stone, D. L.; Smith, D. K.; McGrail, P. T. *J. Am. Chem. Soc.* **2002**, *124*, 856-864.

(24) Toba, R.; Quintela, J. M.; Peinador, C.; Roman, E.; Kaifer, A. E. *Chem. Comm.* **2001**, 857-858.

(25) Vicinelli, V.; Maestri, M.; Balzani, V.; Muller, W. M.; Muller, U.; Hahn, U.; Osswald, F.; Vögtle, F. *New J. Chem.* **2001**, *25*, 989-993.

(26) Wang, Y.; Cardona, C. M.; Kaifer, A. E. *J. Am. Chem. Soc.* **1999**, *121*, 9756-9757.

(27) Weyermann, P.; Diederich, F.; Gisselbrecht, J. P.; Boudon, C.; Gross, M. *Helv.Chim. Acta* **2002**, *85*, 571-598.

(28) Wyatt, S. R.; Hu, Q. S.; Yan, X. L.; Bare, W. D.; Pu, L. *Macromolecules* **2001**, *34*, 7983-7988.

(29) Xu, Z. F.; Kahr, M.; Walker, K. L.; Wilkins, C. L.; Moore, J. S. *J. Am. Chem. Soc.* **1994**, *116*, 4537-4550.

(30) Wuttke, D. S.; Bjerrum, M. J.; Winkler, J. R.; Gray, H. B. *Science* **1992**, *256*, 1007-1009.

(31) Gorman, C. B.; Smith, J. C. *J. Am. Chem. Soc.* **2000**, *122*, 9342-9343.

(32) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; 2nd ed.; Wiley: New York, 2001.

(33) Heinze, J. *J. Phys. Chem. Chem. Phys.* **1981**, *85*, 1096-1103.

(34) Heinze, J. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 831-847.

(35) Nicholson, R. S. *Anal. Chem.* **1965**, *37*, 1351-1355.

(36) Chasse, T. L.; Sachdeva, R.; Li, C.; Li, Z. M.; Petrie, R. J.; Gorman, C. B. *J. Am. Chem. Soc.* **2003**, *125*, 8250-8254.

(37) Gennett, T.; Geiger, W. E.; Willett, B.; Anson, F. C. *J. Electroanal. Chem.* **1987**, *222*, 151-160.

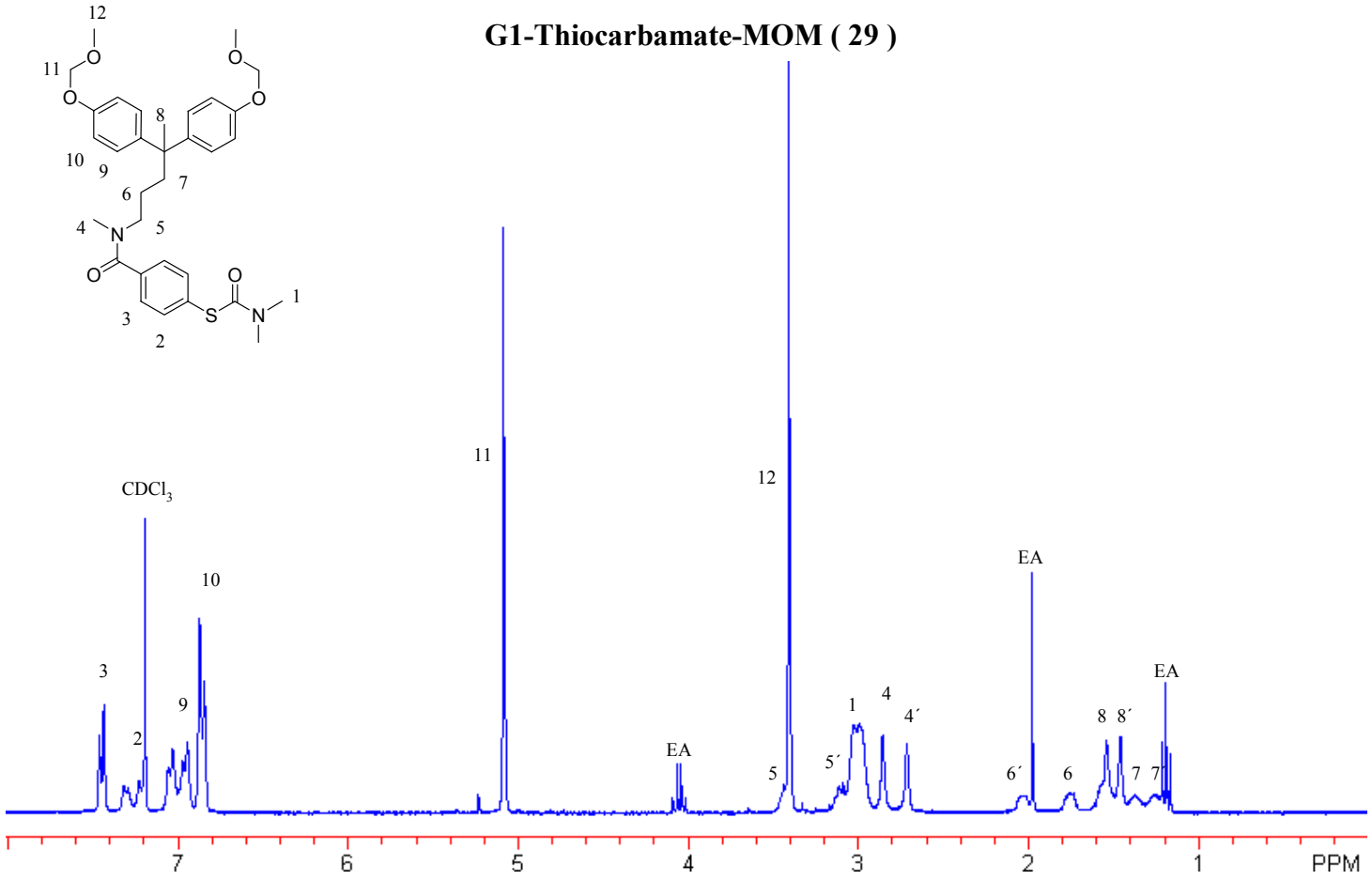
(38) Zhou, C. Y.; Raebiger, J. W.; Segal, B. M.; Holm, R. H. *Inorg. Chim. Acta* **2000**, *300-302*, 892-902.

(39) *FSQPLT software was provided by John J. O'Dea of the J. Osteryoung group.*

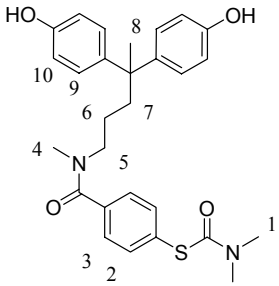
(40) Odea, J. J.; Osteryoung, J.; Osteryoung, R. A. *Anal. Chem.* **1981**, *53*, 695-701.

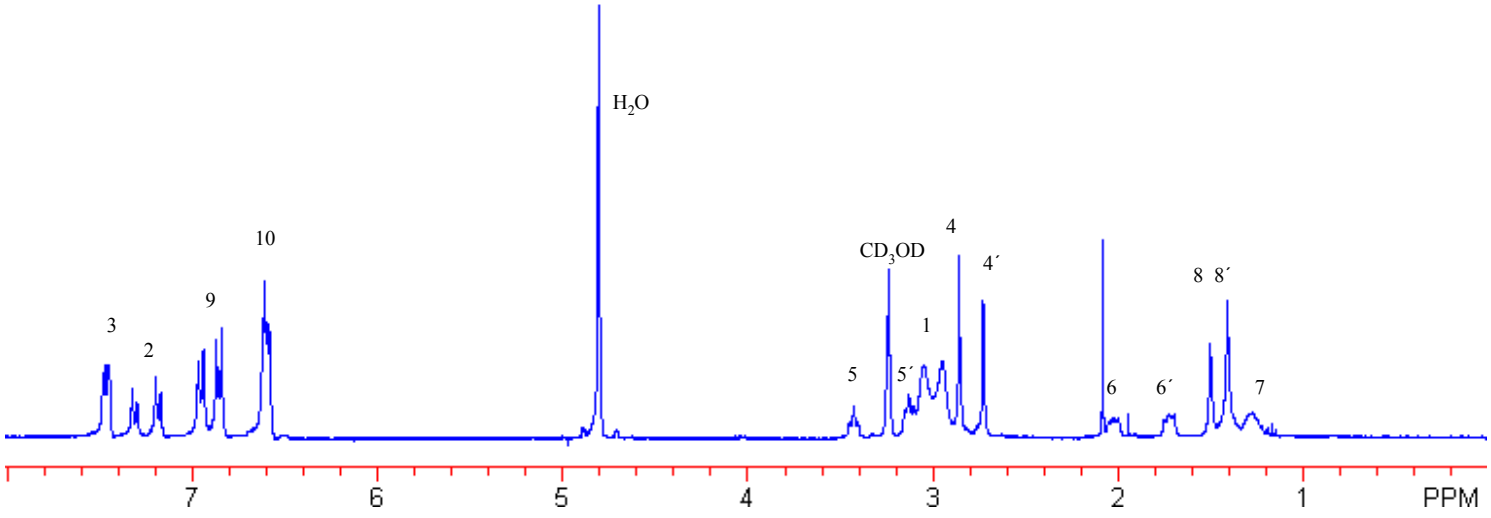
Appendix

The selected ^{1}H and ^{13}C NMR Spectra

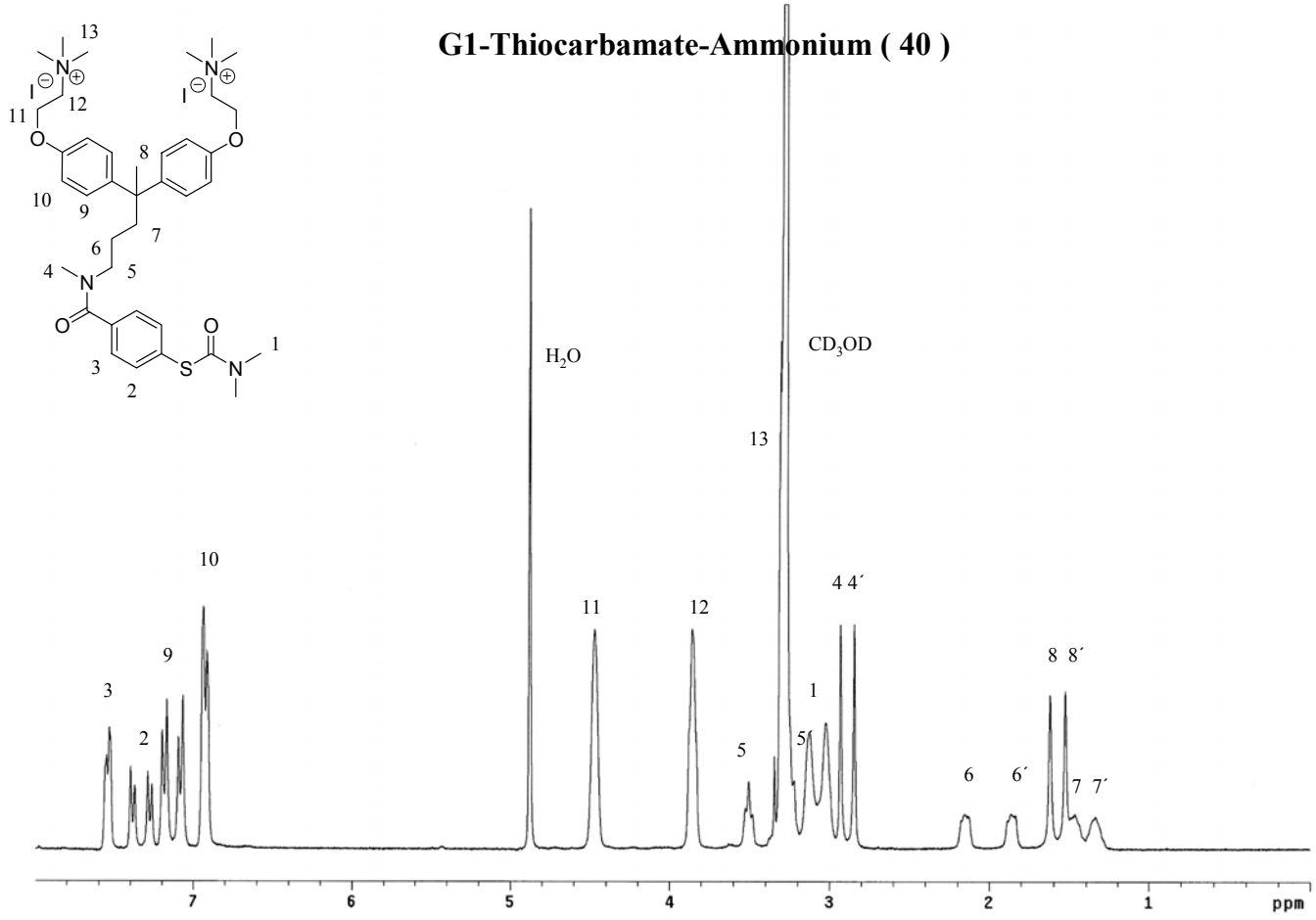

G1-Thiocarbamate-MOM (29)
12
11
O
O
O
O
8
10
9
6
7
4
N
5
O
O
1
3
S
N
2
CDCl3
11
12
EA
EA
EA
10
3
9
2
1
4
4´
8
8´
5
5´
6´
6
7
7´
7
6
5
4
3
2
1
PPM

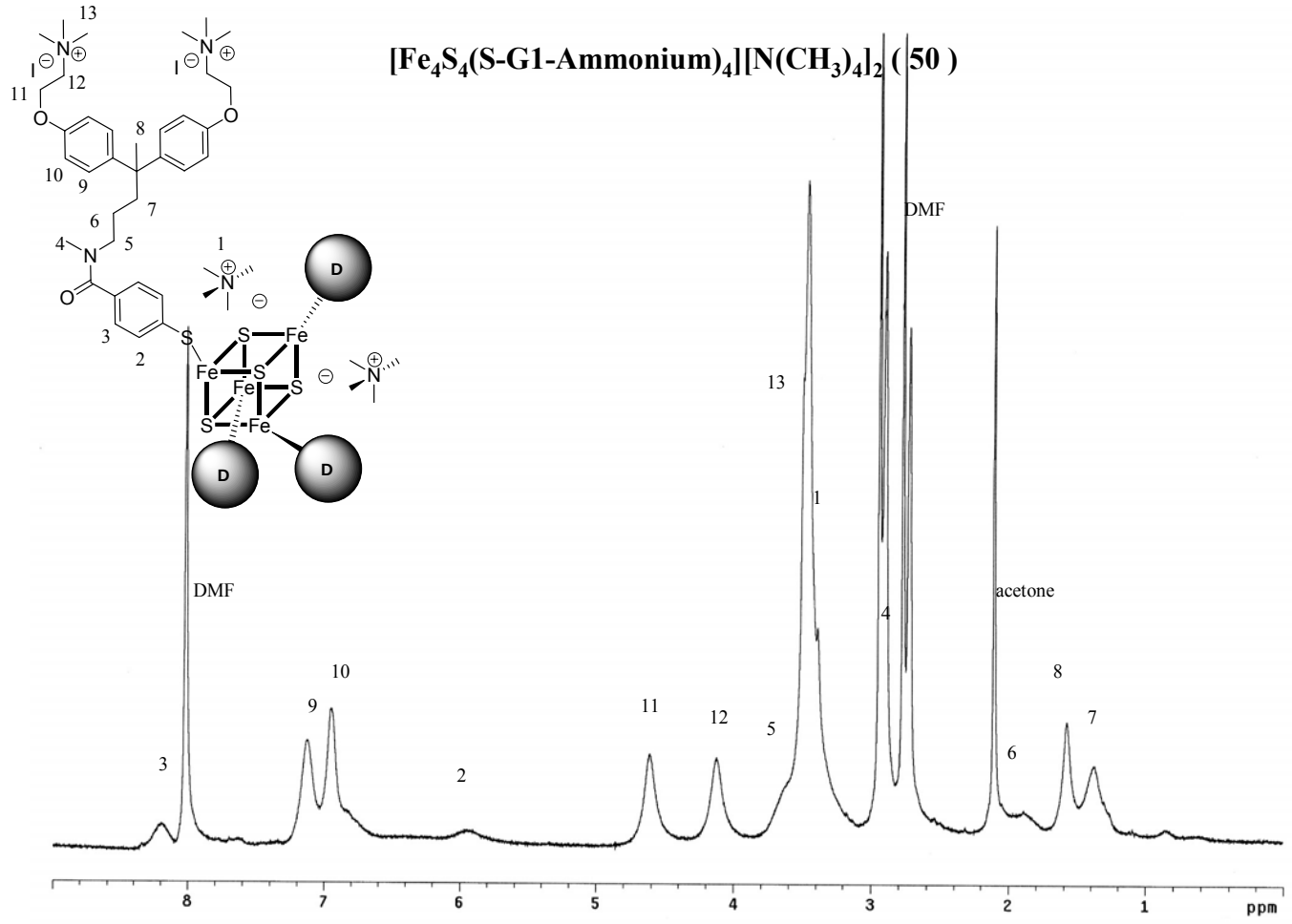
G1-Thiocarbamate-OH (30)

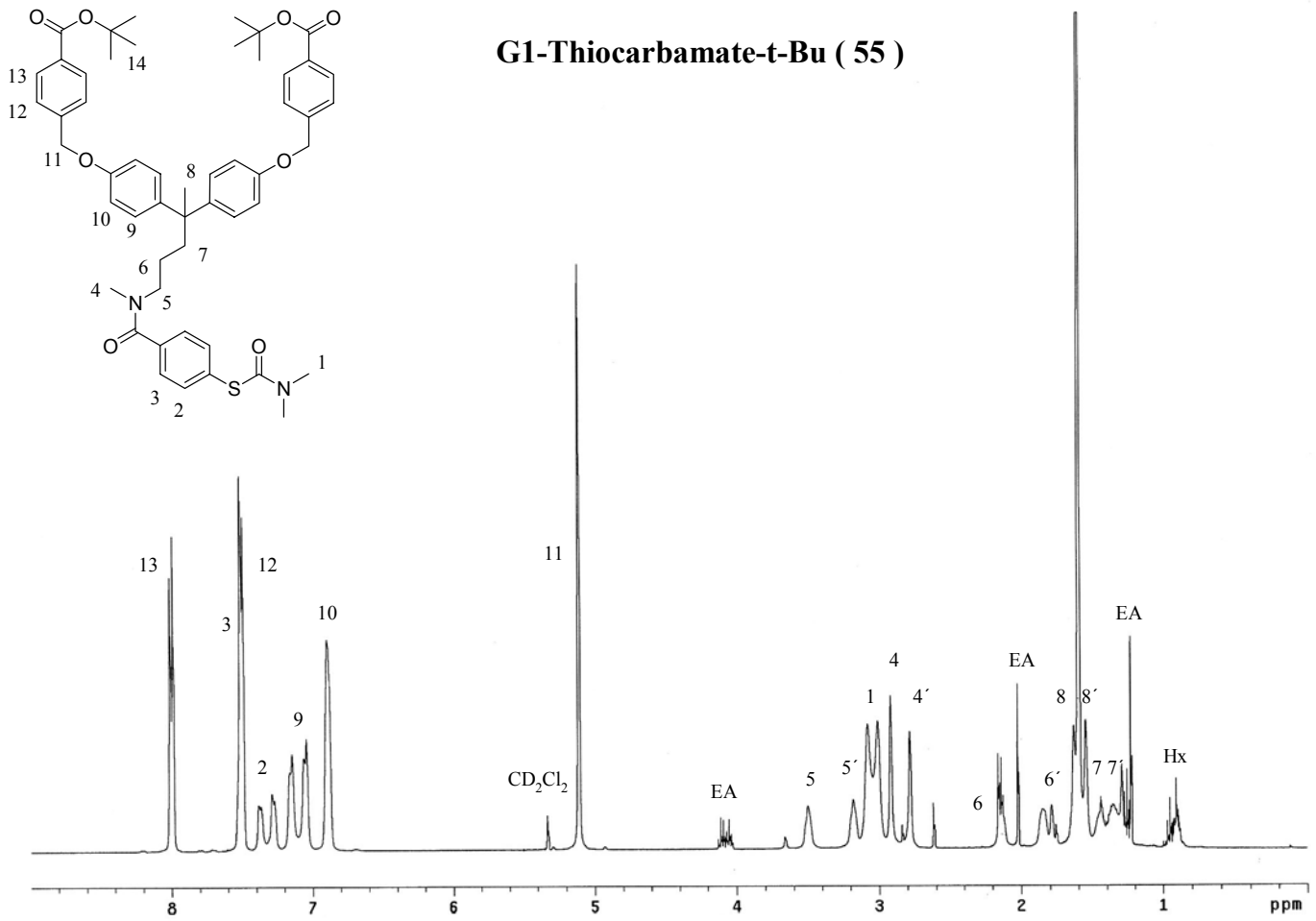


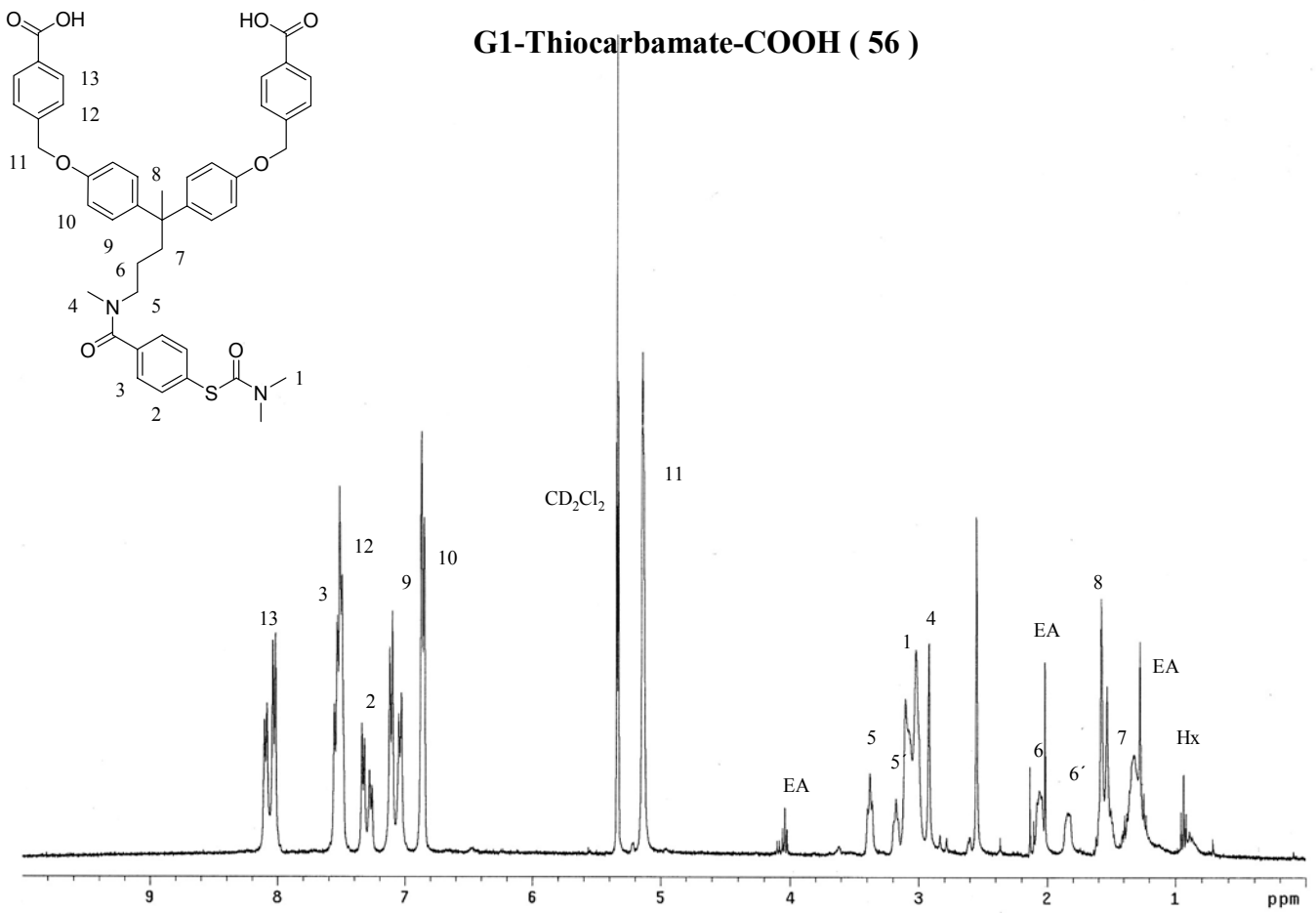


G1-Thiocarbamate-Ammonium (40)

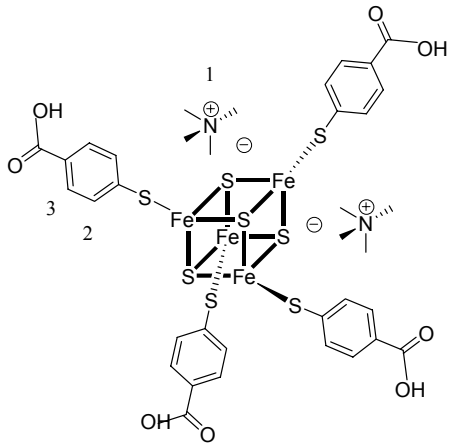


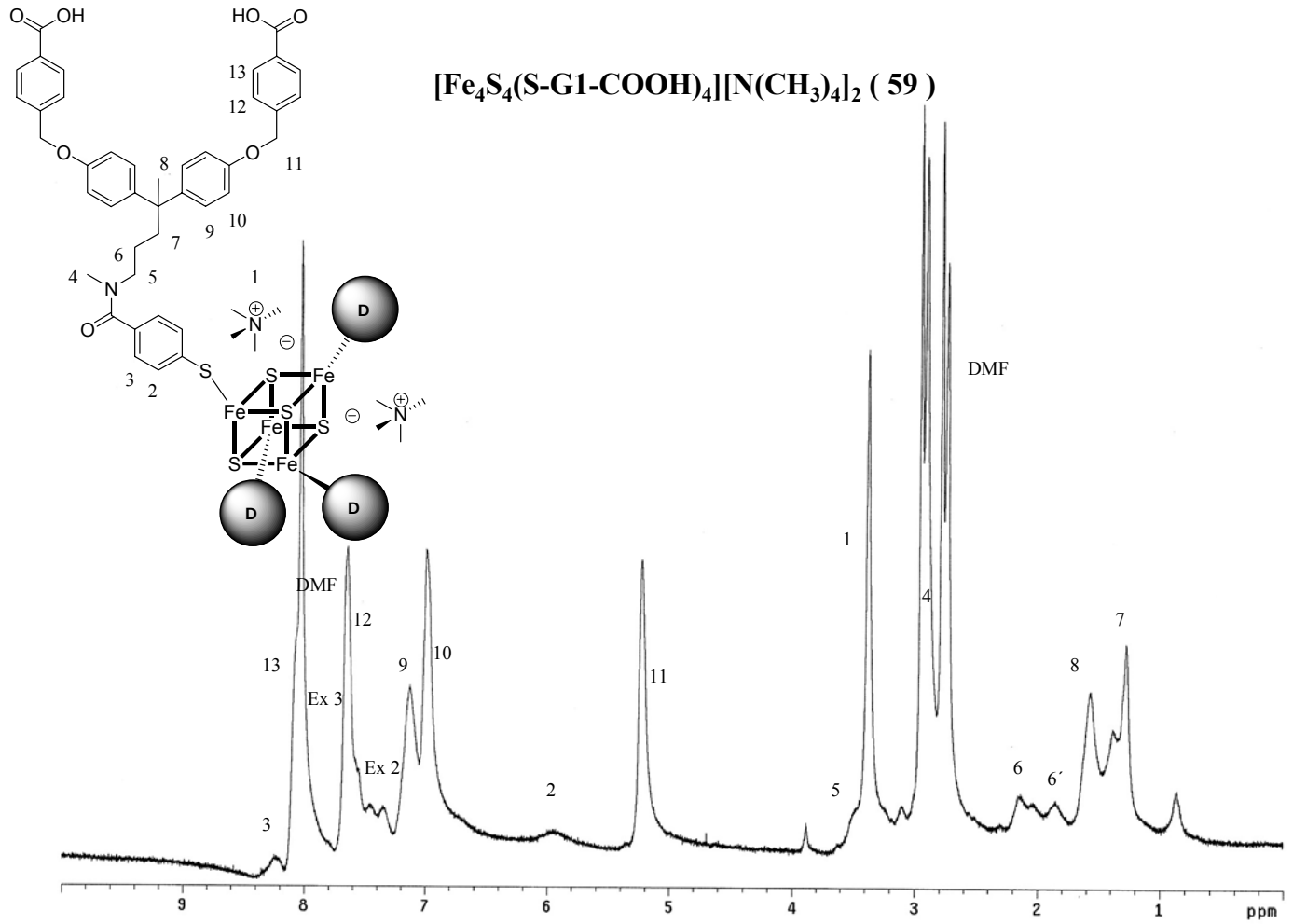

[Fe4S4(S-G1-Ammonium)4][N(CH3)4]2 (50)
DMF
DMF
acetone
ppm

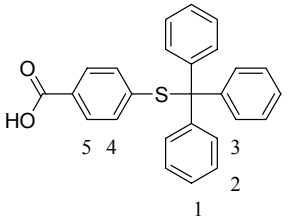

G1-Thiocarbamate-t-Bu (55)
13
12
11
10
9
8
7
6
5
4
3
2
1
14
CD2Cl2
EA
Hx
ppm


G1-Thiocarbamate-COOH (56)
CD2Cl2
EA
Hx
ppm

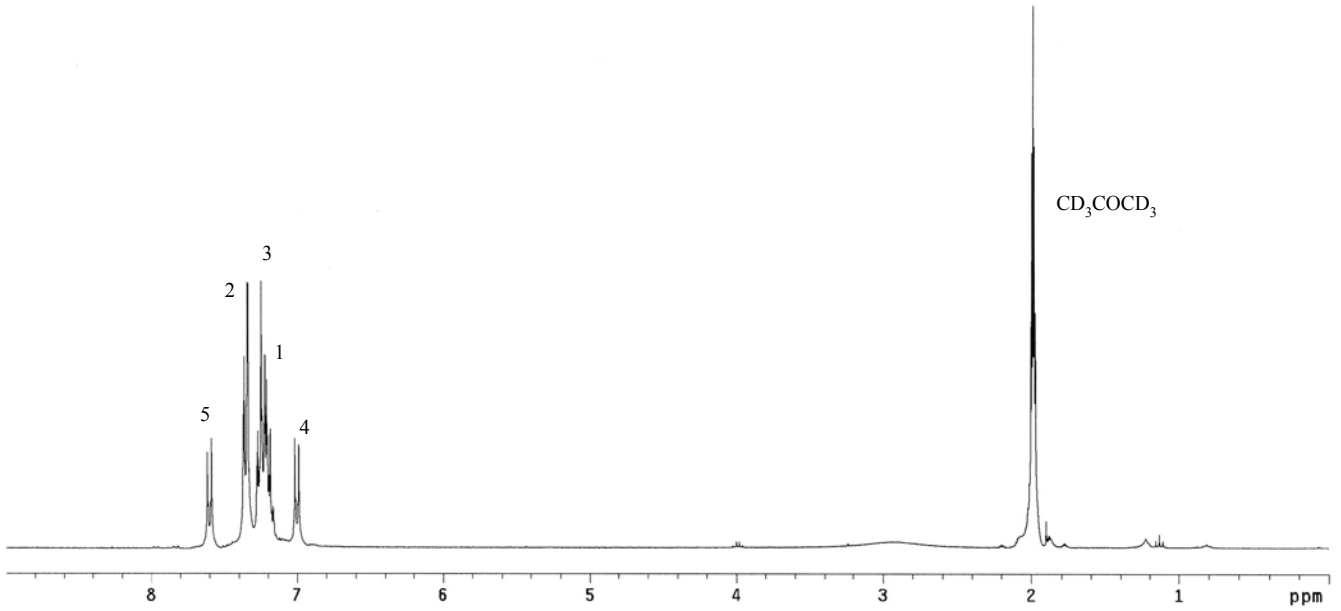
$[Fe_4S_4(S\text{-}G0\text{-}COOH)_4][N(CH_3)_4]_2$ (57)

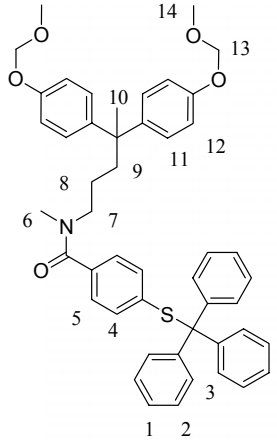



[Fe4S4(S-G1-COOH)4][N(CH3)4]2 (59)
DMF
DMF
Ex 3
Ex 2
ppm

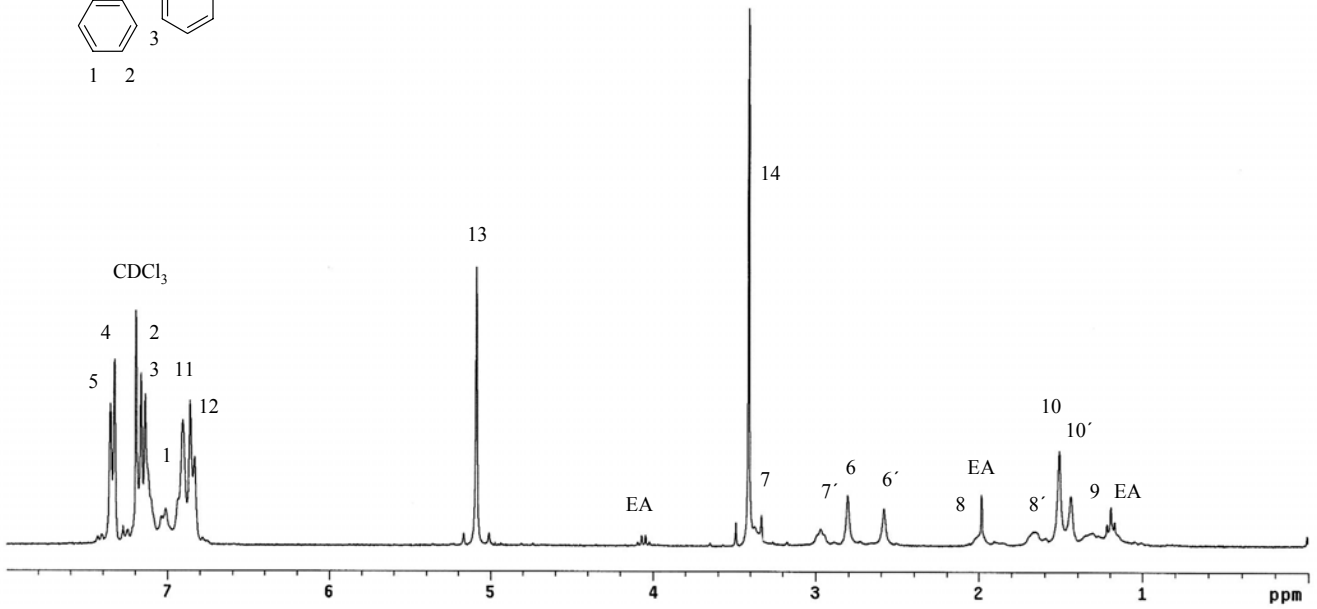


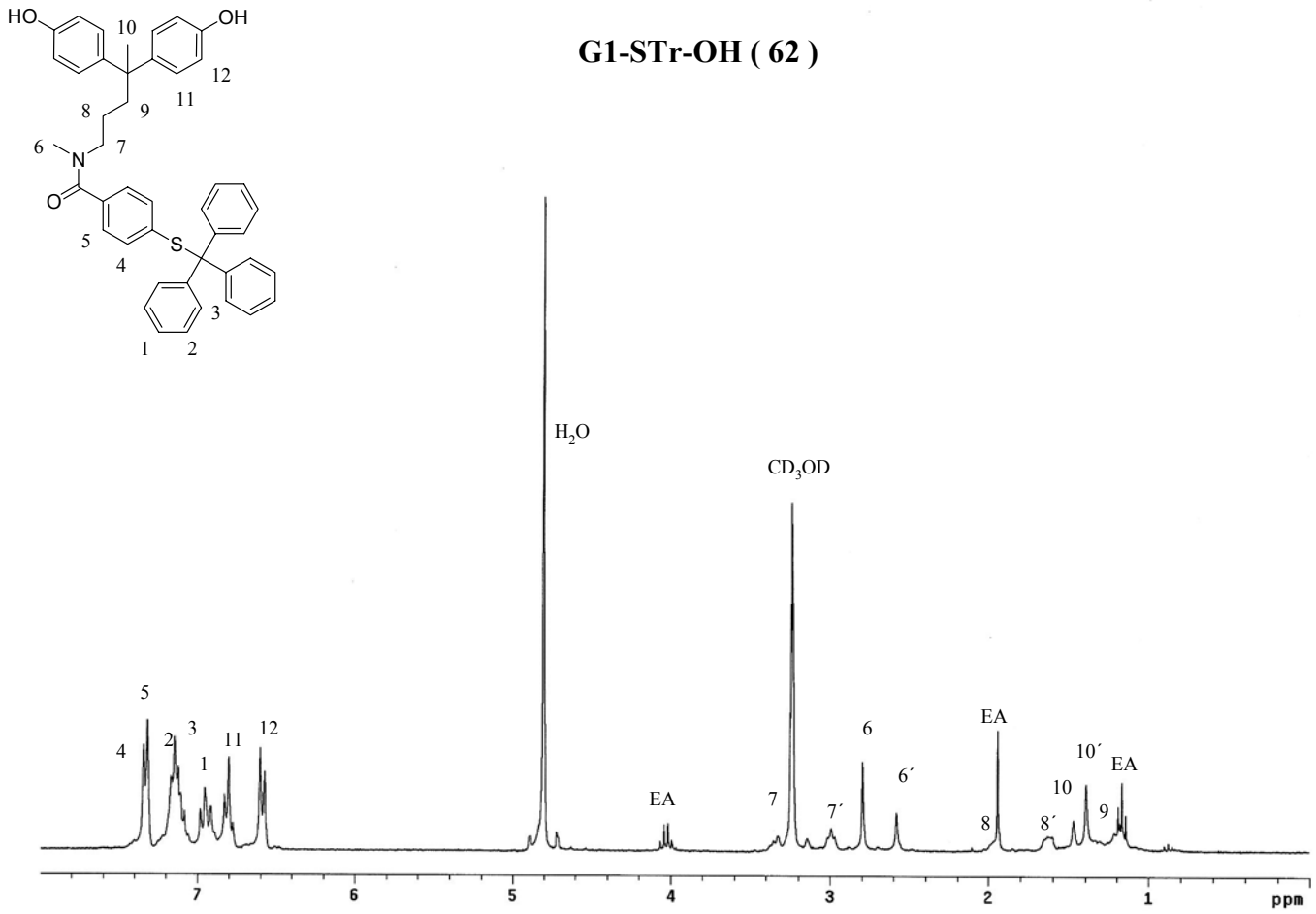
4-Tritylsulfanyl benzoic acid (60)

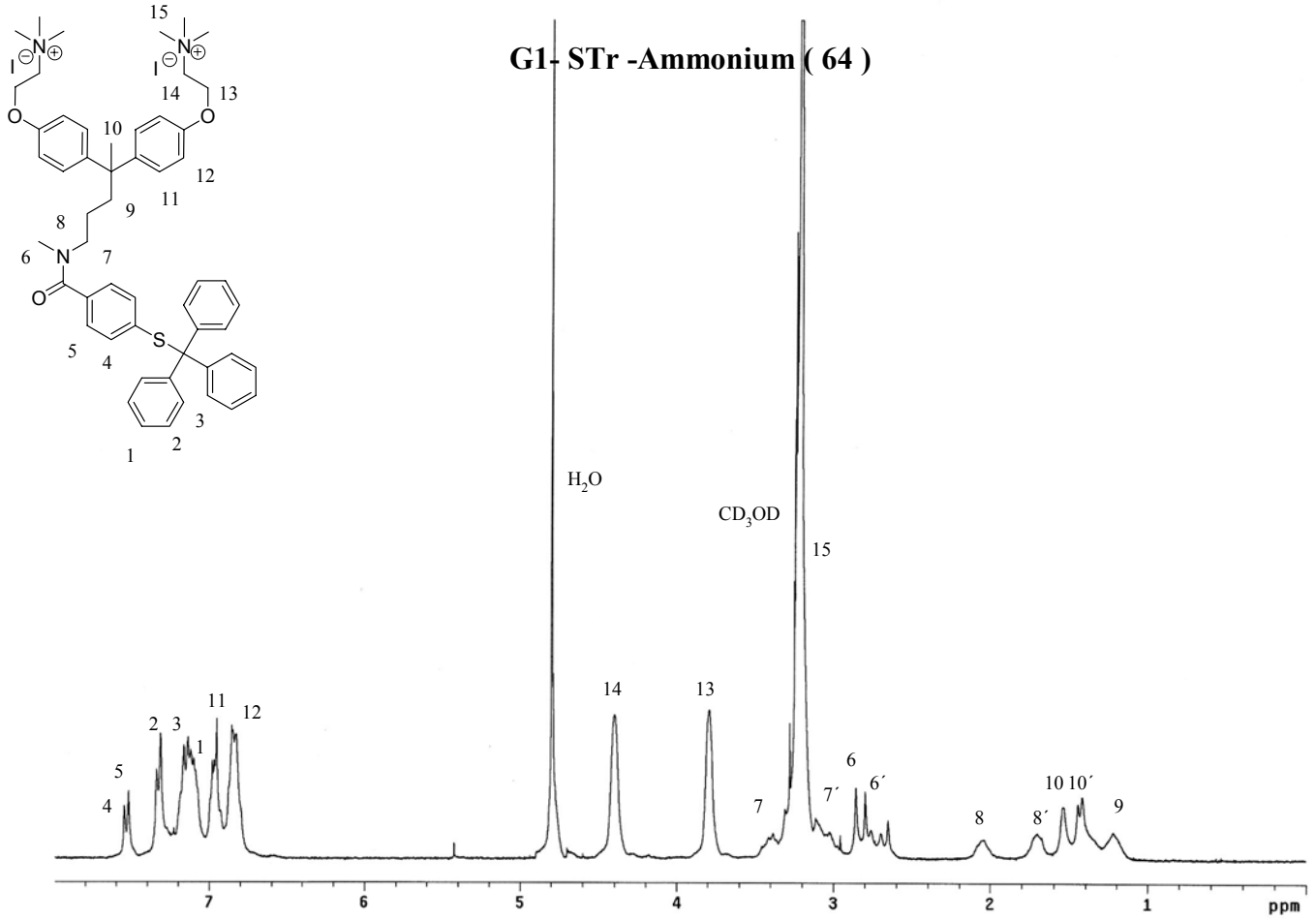


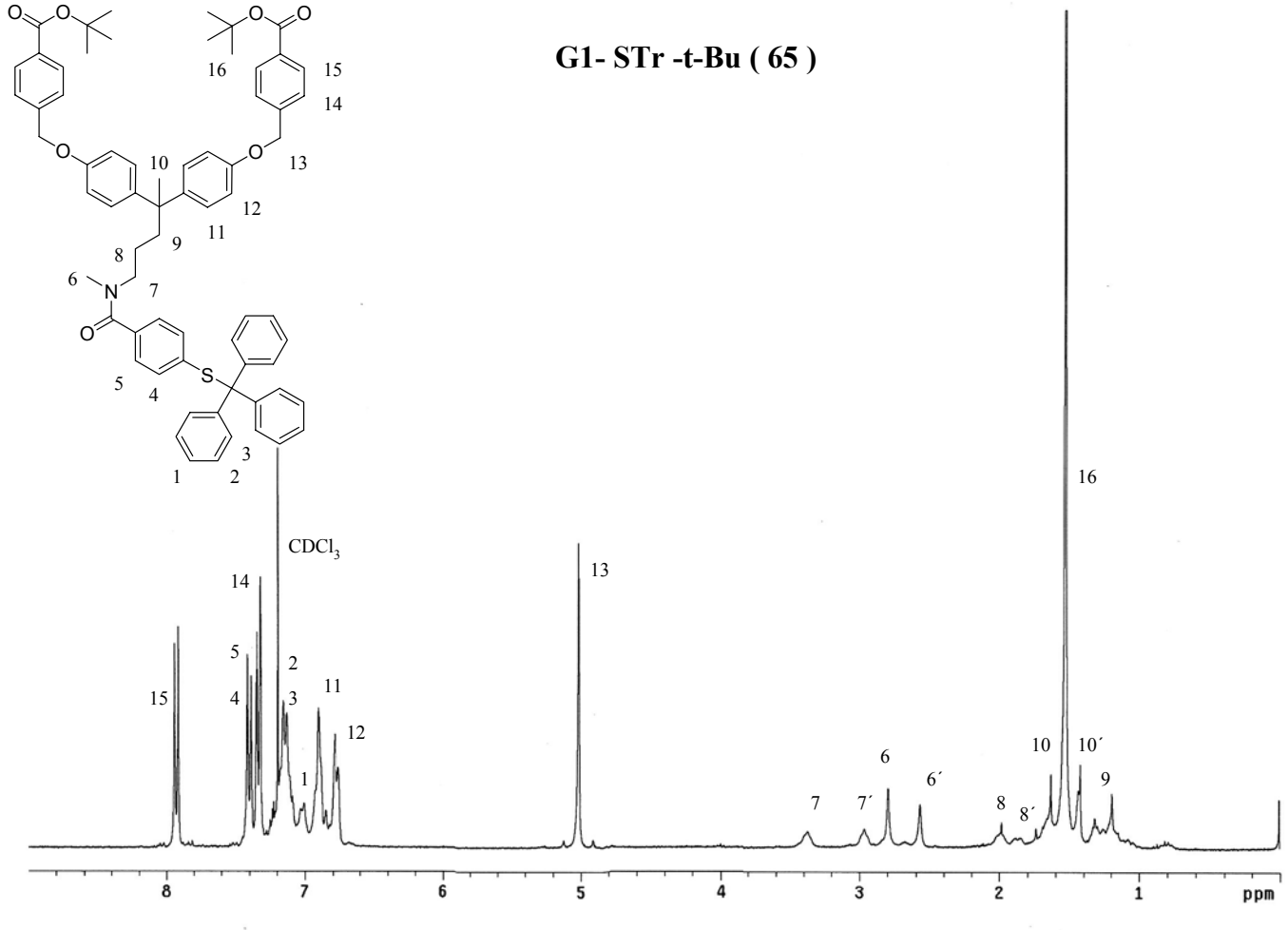

14
13
10
12
11
9
8
6
7
5
4
3
1
2

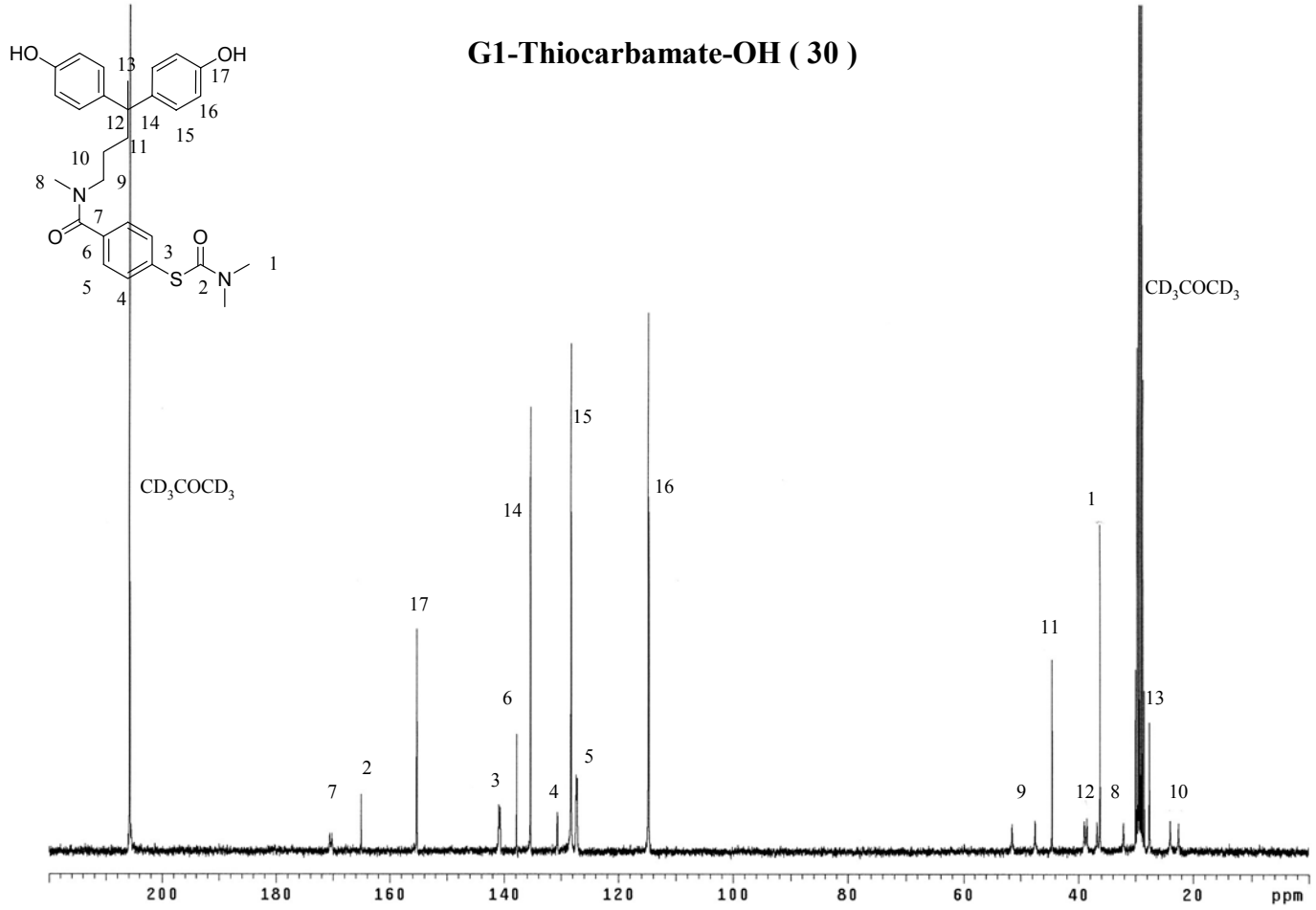
G1-STr-MOM (61)

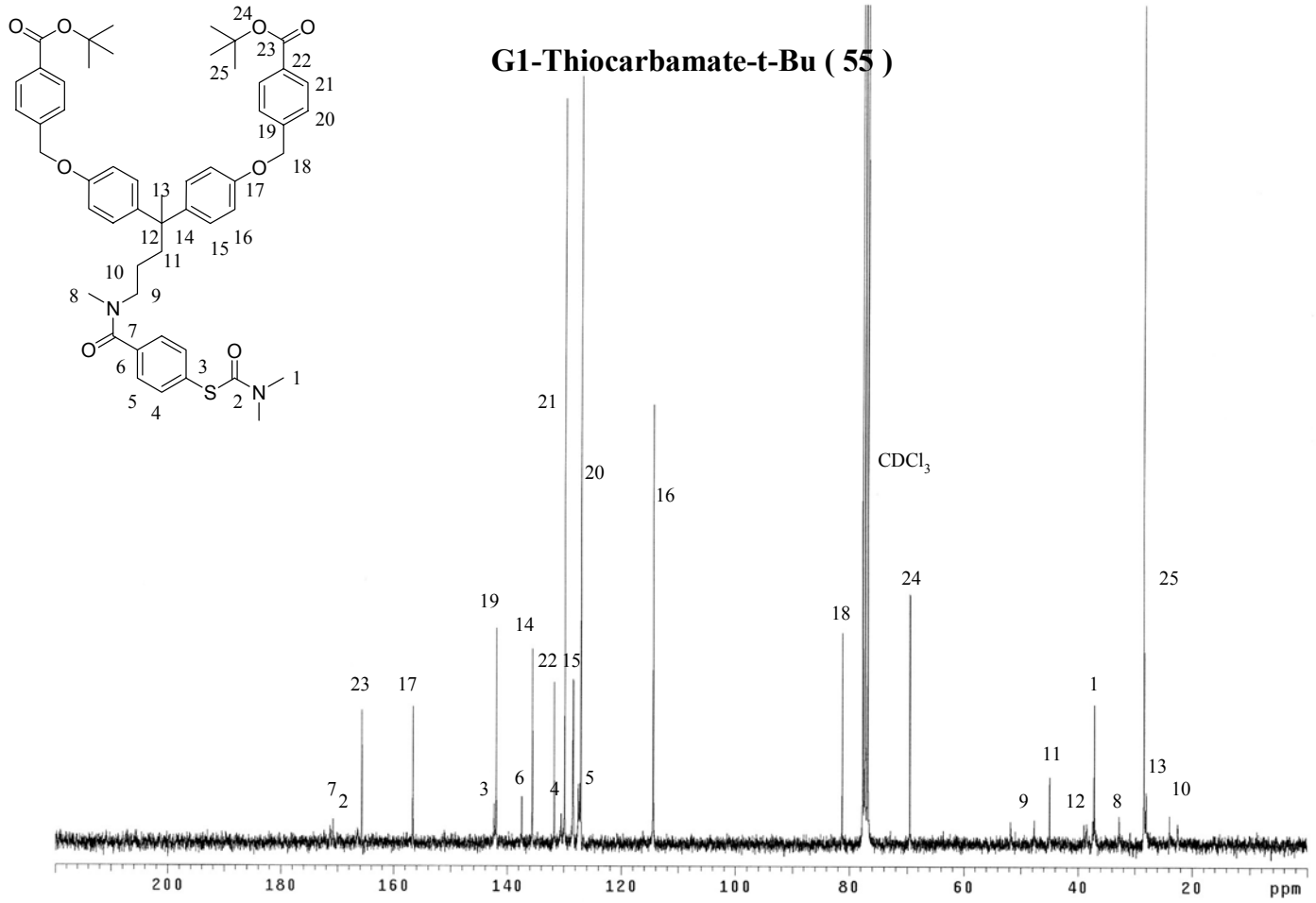

CDCl3
4
2
5
3
11
12
1
13
EA
14
7
7´
6
6´
EA
8
10
10´
8´
9
EA
7
6
5
4
3
2
1
ppm

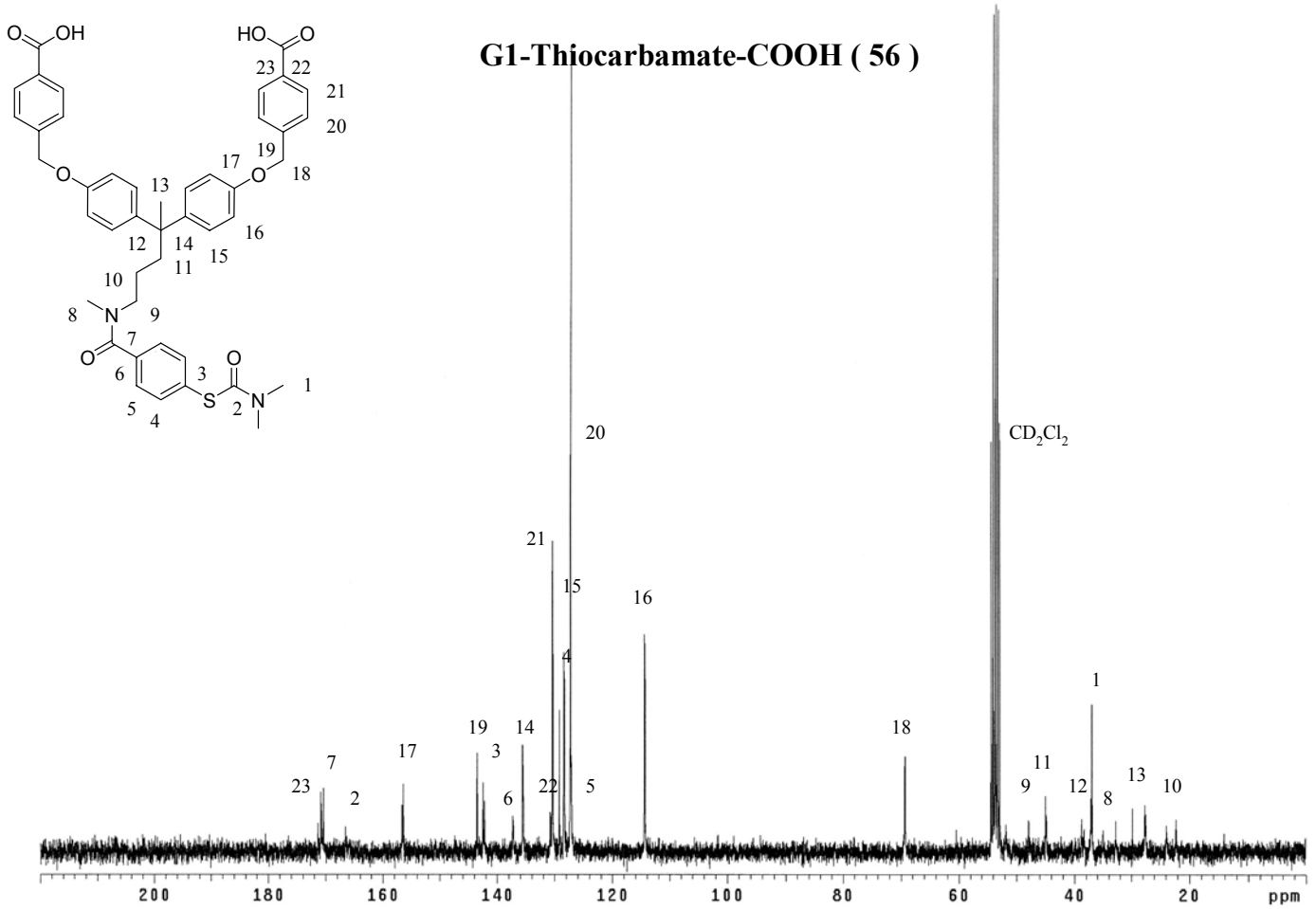

G1-STr-OH (62)
HO
OH
10
12
11
8
9
6
7
N
O
5
4
S
3
1
2
H2O
CD3OD
EA
EA
EA
5
4
2
3
1
11
12
7
7´
6
6´
8
8´
10
10´
9
7
6
5
4
3
2
1
ppm

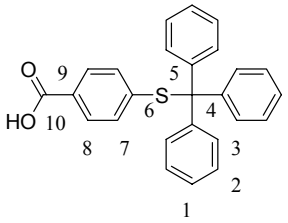

G1- STr -Ammonium (64)
H2O
CD3OD
ppm


G1- STr -t-Bu (65)
CDCl3
ppm

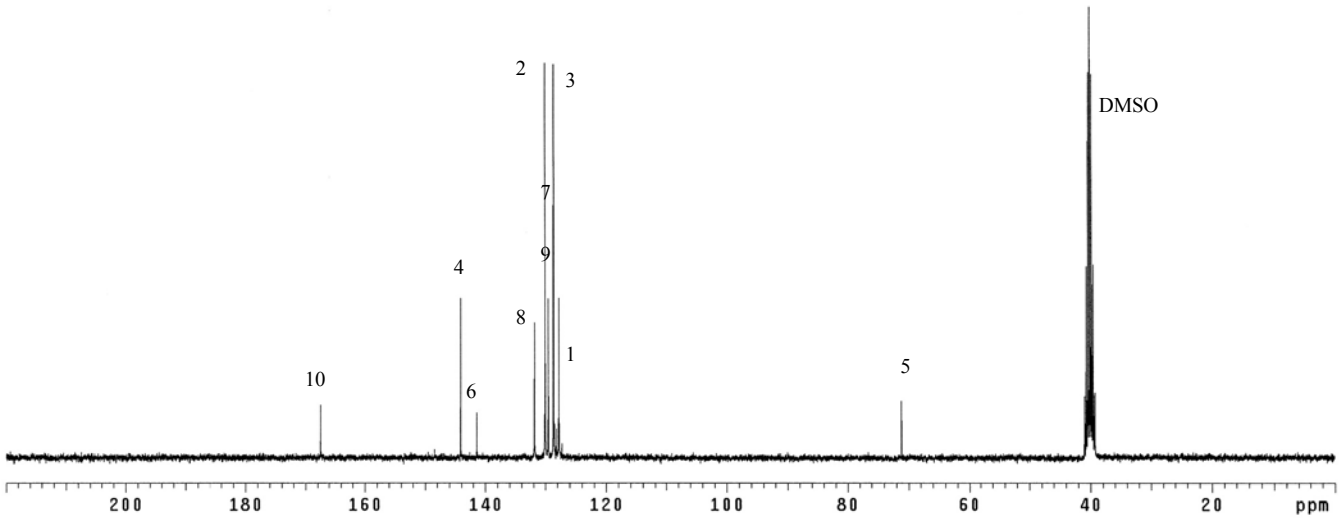

G1-Thiocarbamate-OH (30)
HO
OH
CD3COCD3
CD3COCD3
200
180
160
140
120
100
80
60
40
20
ppm

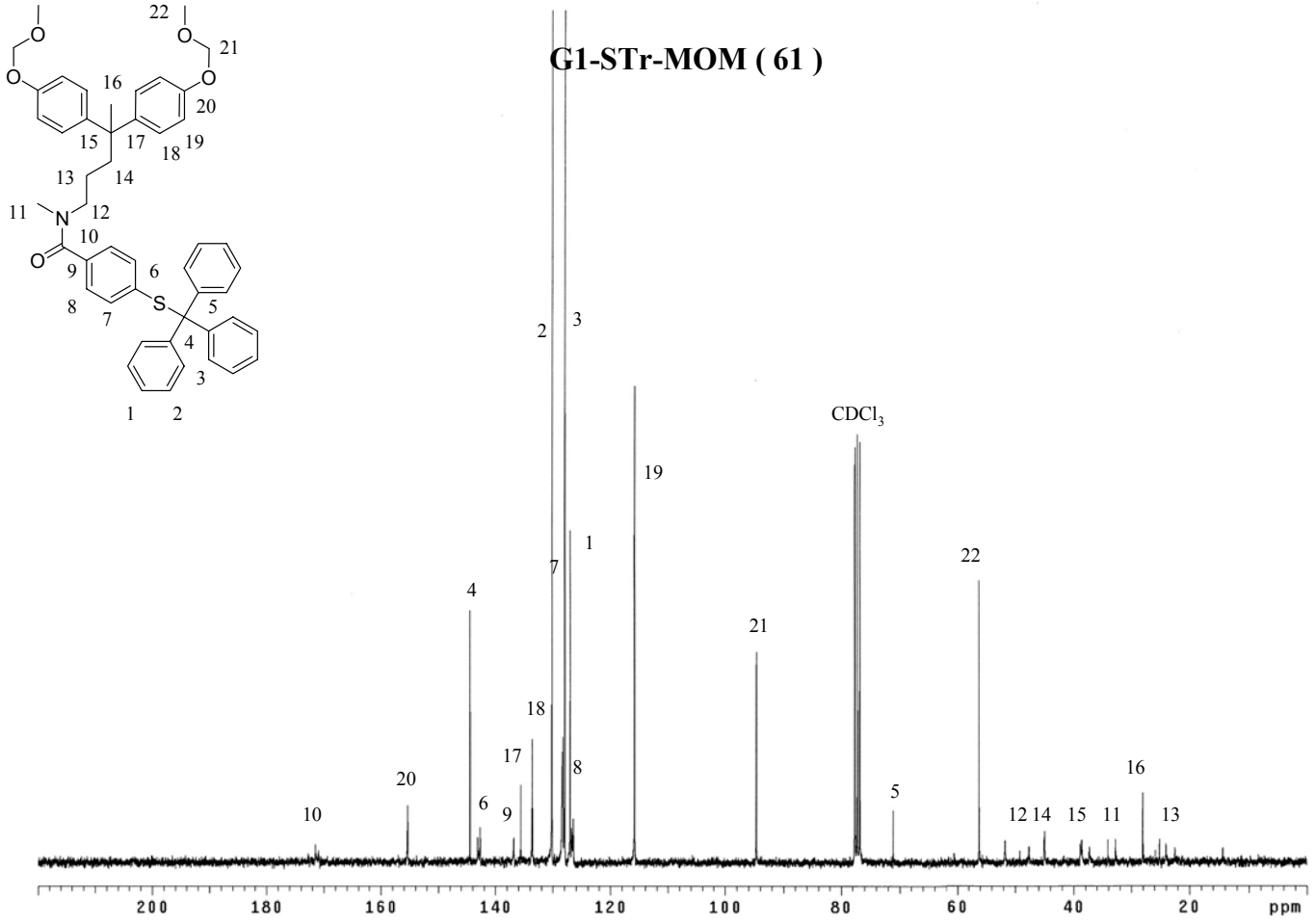

G1-Thiocarbamate-t-Bu (55)
CDCl3
200
180
160
140
120
100
80
60
40
20
ppm

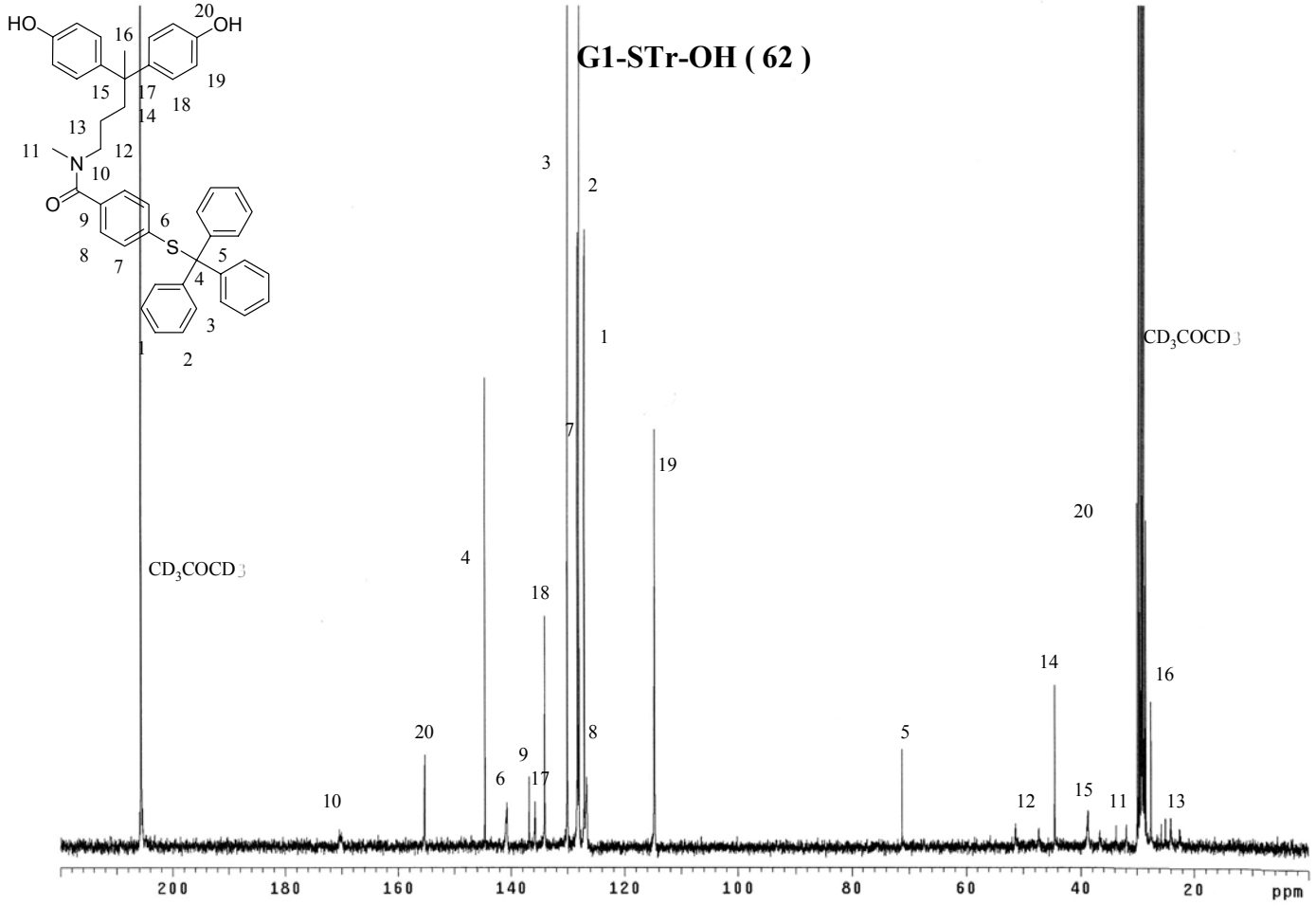

G1-Thiocarbamate-COOH (56)
CD2Cl2
ppm
200
180
160
140
120
100
80
60
40
20

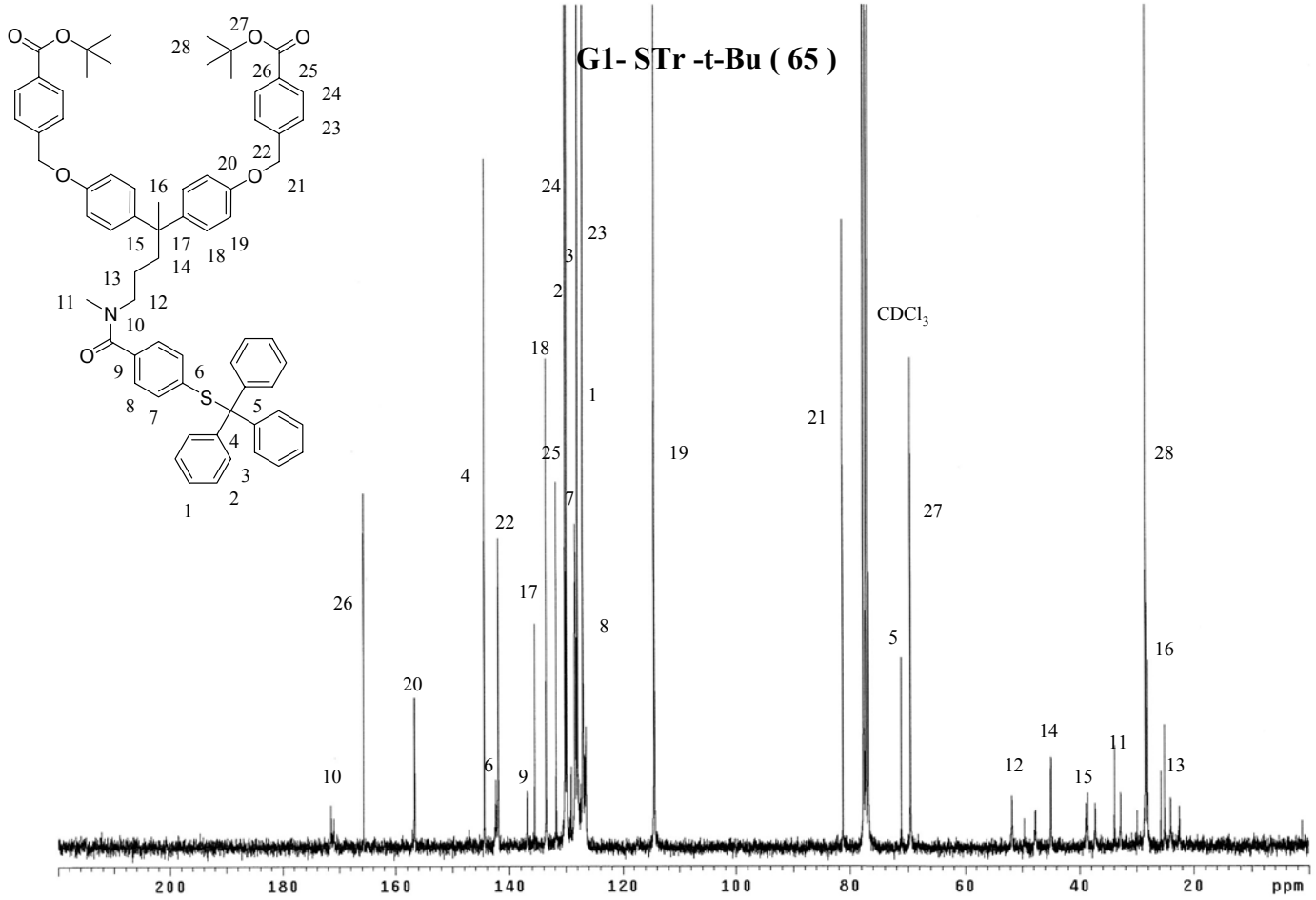


4-Tritylsulfanyl benzoic acid (60)




G1-STr-MOM (61)
CDCl3
200
180
160
140
120
100
80
60
40
20
ppm


G1-STr-OH (62)
HO
OH
20
16
19
15
17
18
14
13
11
12
N
10
O
9
6
S
5
8
7
4
3
1
2
CD3COCD3
CD3COCD3
200
180
160
140
120
100
80
60
40
20
ppm


G1- STr -t-Bu (65)
CDCl3
200
180
160
140
120
100
80
60
40
20
ppm