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Ph. D. Dissertation

Facile Synthesis of Polyamide Dendrimers

（簡便なポリアミドデンドリマーの合成）

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Contents

Chapter I

General Introduction

Dendrimers[1-9] stand for a key stage in the ongoing evolution of macromolecular chemistry. From the origins of polymer chemistry, a major focus had been the synthesis and characterization of linear polymers. In 1978, Vögtle and coworkers first reported an iterative cascade method for the synthesis of low molecular weight branched amines.[10] And the first dendrimers were synthesized by Tomalia and co-workers at Dow Chemical in the early 1980s in parallel with Newkome's "airbol" systems.[11,12] A great number of papers have been reported about the synthesis, properties, and applications of dendrimers because of their unique structures. The exponential growth and topicality of research into dendritic molecules is apparent not only from the large number of publications (presently totaling more than 10000, and increasing by more that 1000 per annum, plus about 150 patents), but also from the mere fact that more than 8000 reserchers are currently active in this area and more than 150 companies have already applied for patents relating to dendritic compounds.

Dendrimers are defined as highly ordered, regularly branched and globular macromolecules. Their structure is divided into three distinct architectural regions: (i) a core or focal moiety, (ii) layers of branched repeat units emanating from this core, and (iii) end groups on the outer layer of repeat units. These three regions can be independently designed according to our needs.

Dendrimers have at least following three characteristic features on its structure in contrast to those of conventional linear polymers.

(i) Dendrimers can be isolated as an essentially monodisperse single compounds, besides most linear polymers which have molecular weight distributions.

(ii) Whereas linear polymers contain only two terminal groups, terminal groups of dendrimers increase exponentially as a function of generation. Therefore, the properties of dendrimers such as solubility, chemical reactivity and glass transition temperature are dominated by the nature of the terminal groups.

(iii) Whereas linear polymer can infinitely continue the growth under ideal conditions except solubility and cyclization issues, a dendritic growth is mathematically limited due to the steric crowding of the terminal groups.

Their characteristic features give dendrons and dendrimersa number of interesting properties such as excellent solubility, low viscosities and encapsulation abilities. These distinctive properties make dendrimers attractive scaffolds for variety of high-end applications. For example,

1. Catalysis [13]
2. Drug delivery [14]
3. Gene delivery [15]
4. Light-harvesting [16]
5. Light-emitting diodes [17]
6. MRI agents [18]
7. Nonlinear optics [19]
8. Quantum dots [20]
9. Gelators [21]

1. General Synthetic Approach

Dendrimers are synthesized by two complementary general approaches, the divergent[22,23] and convergent[3,24,25] method.

1-1. Divergent Synthetic Method

The divergent method results from sequential monomer (AB_n-building block) addition beginning from a core and proceeding outward toward the macromolecular surface. This methodology is illustrated in Figure 1. Reaction of the peripheral functional groups of the core with the reactive group of the AB_n-building block gives a new latent branch point at each coupling site, and results in an increase in the number of peripheral functional groups. The peripheral functional groups (protecting group) on

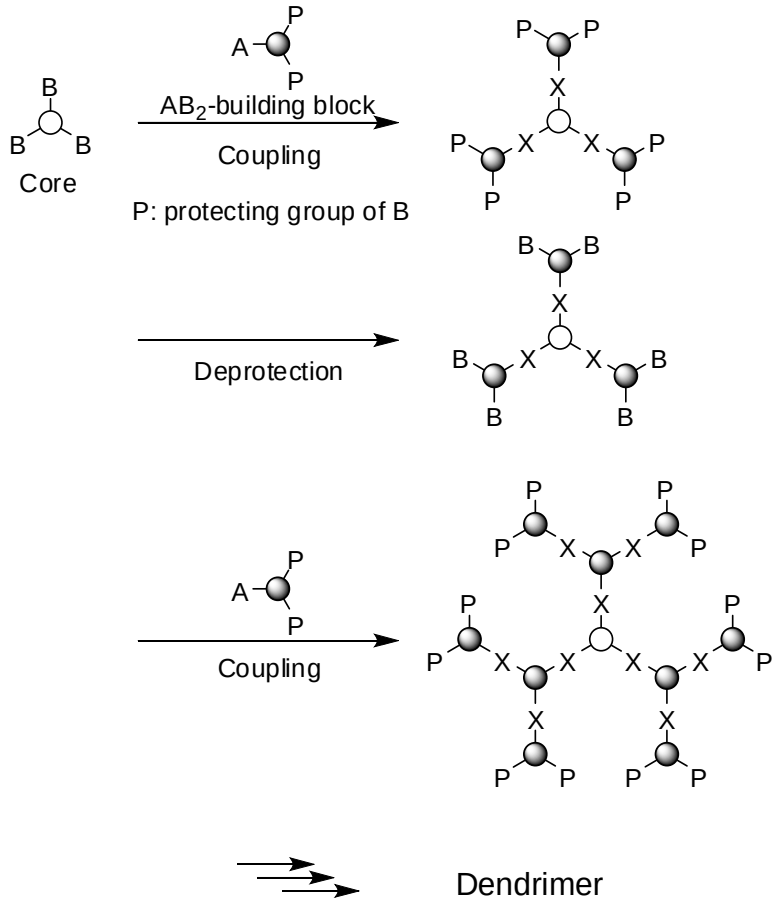


Figure 1 Divergent synthesis of dendrimer

each AB_n-building block should be inert to focal functional group to prevent self-condensation of AB_n-building block. After the complete coupling reaction, these protecting groups can be deprotected to afford a new layer of reactive end-groups capable of coupling with additional AB_n-building block.

A characteristic feature of the divergent method is the exponentially increasing number of reaction sites that require for providing each subsequent generation layer. Therefore, a large excess amount of reagents is generally required to afford a perfect growth of dendrimer structure. The divergent method is essentially suited for the large-scale synthesis of dendrimers, because each generation dendrimer could be isolated from the excess reagents by a simple distillation, precipitation, or ultra filtration due to the large difference in molecular weight. However, to ensure the integrity of the final product, every reaction must be very selective and quantitative. In addition, any defective growth resulting from side- or incomplete reaction cannot easily removed because of their very similar properties. As the result, the divergently prepared dendrimers frequently contain an appreciable number of structural defects.

1-2. Convergent Synthetic Method

The convergent synthetic method proceeds in the opposite direction compared to the divergent synthetic method. The method initiates from the periphery and growths inward as shown in Figure 2. The molecules that eventually becomes the exterior of the dendrimer, first reacts with the functional group B of AB_n-building block which possess chemically inert A goroups. After completion of the coupling reaction, the single functional group A located at the focal point of dendron can be deprotected or changed the functionality for the next coupling reaction. Coupling reaction of this

deprotected-dendron with additional AB_n-building block affords a higher generation dendron. Finally, these dendrons would be attached to a multifunctional core building block to form a globular architecture.

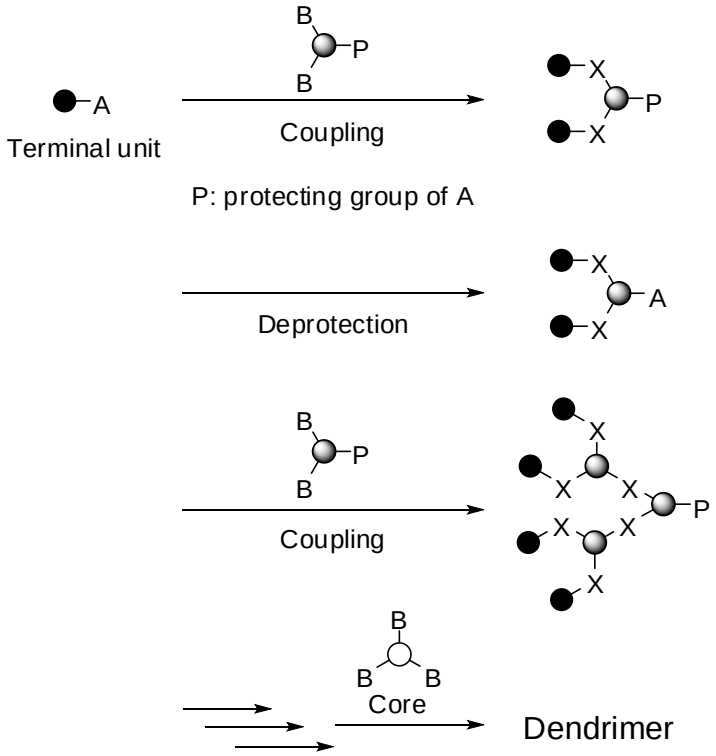


Figure 2. Convergent synthesis strategy of dendrimers

A notable advantage of convergent method is the requirement of a small number of reaction sites per a molecule during both coupling and deprotection steps. As the result, the reactions can be driven to completion with only a slight excess of reagent, besides the divergent method needs larger excess of reagent. In addition, purification of the product which obtained after the coupling step can be performed by chromatographic purification because the desired dendron and the defect molecules possess different properties. Thus, the convergent method can be a defect-free synthetic approach. However, since purification by chromatography results in some losses and difficulty in a large-scale synthesis, it is difficult to apply convergent method for industrial use.

Furthermore, the dendrons and dendrimers growth could be limited in higer-generation, due to the steric hindrance of the dendrons which caused by their periphery. The convergent synthetic methods are used mainly for the prepration of lower-generation dendrimers, then.

In both divergent and convergent synthetic methods, a tedious stepwise procedure including following steps is required: (i) attaching one generation to the proceeding one (ii) purification (iii) changing the functional groups for the next-stage reaction (iv) purification. Therefore, the development of the precise and facile synthetic methods for dendrimers is becoming one of the significant aspects of current work in this field.

2. Accelerated and Rapid Synthetic Approach

In response to these problems, many researchers have developed accelerated synthetic approaches and rapid synthetic methods of dendrimers.

2-1. Orthogonal synthesis

Spindler and Fréchet were first descreibed an accelerated approach for the dendrimer synthesis which is called an "orthogonal coupling strategy" in 1993.[26] The strategy involves the convergent growth with two different monomers, that is, AB_2- and CD_2-building blocks (Figure 3). These building blocks must be carefully selected such that the focal functional group of each individual building block will react only with the periphery of the other building block (A react only with D, and C only with B). Therefore, the total number of reactions is decreased to half of that for a conventional method. However, this strategy generally requires complicated reaction systems to perform the quantitative coupling in the presence of different types of functional groups,

especially for dendrimers having the same repeat unit of the linkage. And this prevents widely adoption of this method.

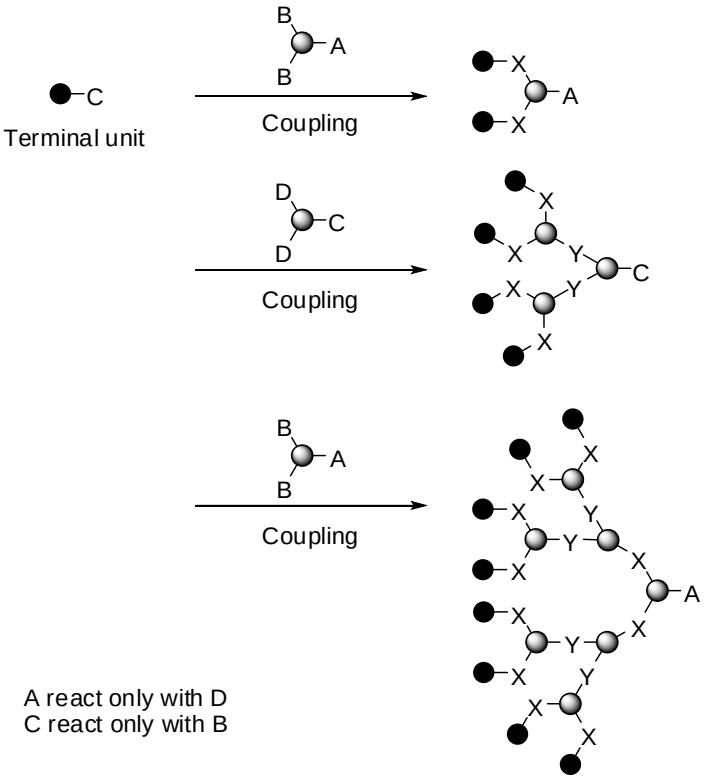


Figure 3. Orthogonal synthesis of dendrimer

2-2. Double-stage convergent method

In 1991, Fréchet and co-workers developed the "double-stage" approach which is a combination of the convergent and the divergent method.[27] In the method, dendrons synthesized via convergent method are grafted to the surface of small dendrimers called

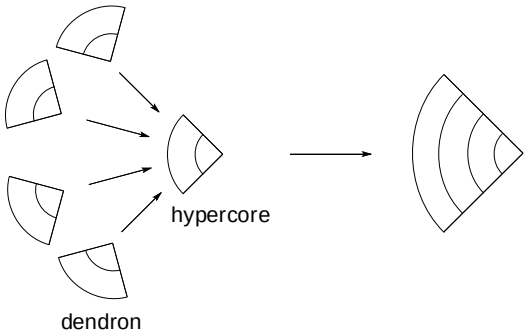


Figure 4. Double-stage synthesis of Dendrimer

"hypercores" (Figure 4). However, the total number of reactions required for preparing dendron and hypercore is the same as for the conventional synthesis of the final dendrimer.

2-3. Double-exponential method

The "double exponential growth" approach was proposed by Moore and co-workers in 1995 and takes direct advantage of both divergent and convergent techniques (Figure 5). [28] This procedure requires an AB_n-building block with different protecting groups for focal and peripheral functionalities. The first generation protected-dendron can be deprotected either at the focal point or at the periphery, resulting in the dendron possessing activated A group and the hypermonomer possessing activated B groups, respectively. Thus, coupling of the activated-dendron and hypermonomer yields a

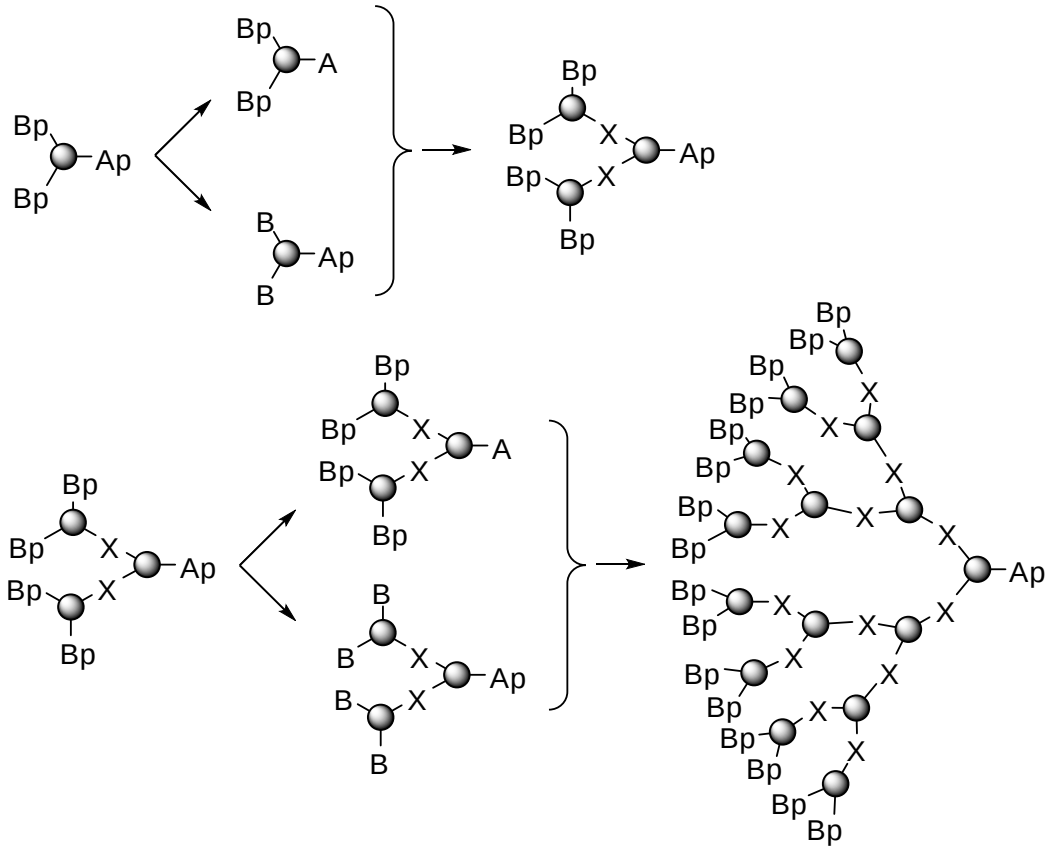


Figure 5. Double exponential growth synthesis of dendrimer

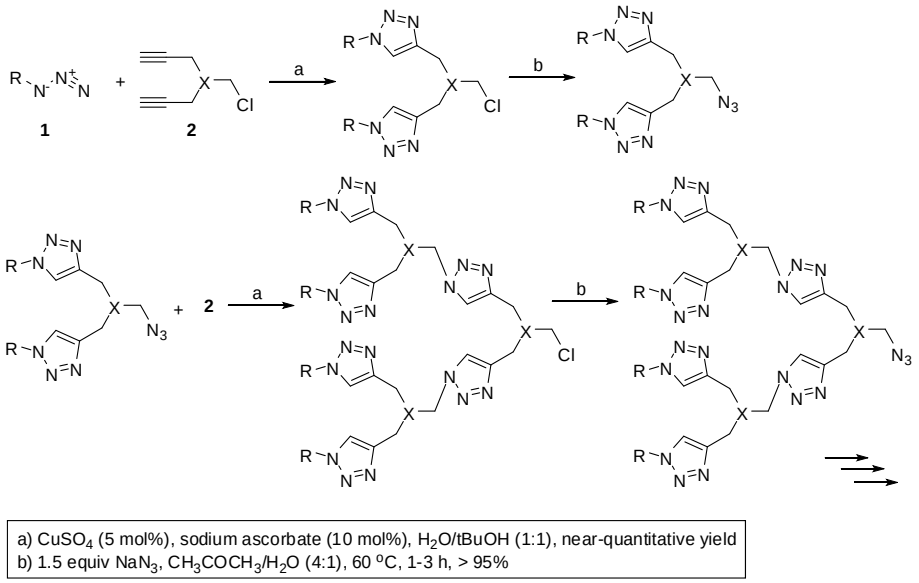
second generation dendron, which can be deprotected at either the focal point or the periphery again. Coupling of the resultant activated-second generation dendrons to the second generation hypermonomer provides a fourth generation dendron. Although this type of strategy could be interesting for the rapid synthesis of high-generation dendrimers, it is not effective for that of middle-sized dendrimers. In the case of the synthesis of fourth generation dendrons, it requires seven steps while eight steps by a conventional method.

2-4. Hypermonomer method

The rapid synthesis of dendrimer using "hypermonomer" such as a AB_4 or AB_8 building block instead of a AB_2 or AB_3 building block have been reported. [29] This strategy increases rapidly the number of end groups, however it does not improve the number of steps needed to obtain one generation because the coupling and deprotection steps may involve reactions identical to those used with a traditional denrimer synthesis.

2-5. Click Chemistry

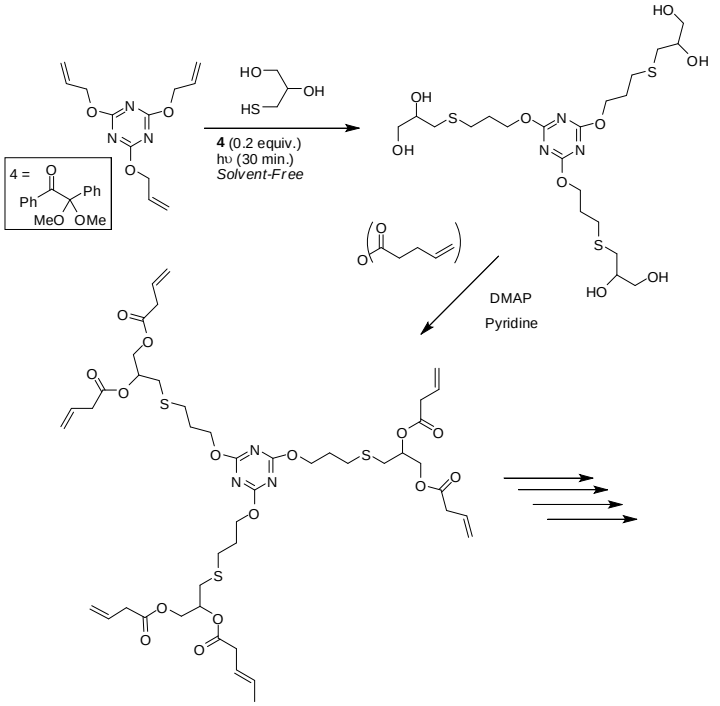
Hawker *et al.* have reported on an efficient and preparatively simple approach for the generation of diverse triazole-based dendrimers of high purity and excellent yield (Scheme 1). [30] In this approach, they utilized the click-chemistry transformation using copper-catalyzed ligation of azides and alynes. A variety of functional groups are compatible with this process, and the only major by-products formed in the reaction are NaCl. Although this approach utilizes the conventional stepwise convergent method, it is remarkable that, in some cases, filtration or solvent extraction is the only method required for purification in this highly efficient construction of the triazole units of the dendrimers.



Scheme 1 Effective synthesis of triazole dendrimers using click-chemistry

Furthermore, Hawker *et al.* recently reported another efficient and preparatively simple approach using thiol-ene coupling click methodology in 2008.[31](Scheme 2). In this methodology, they presented the facile and efficient synthesis of poly(thioether)dendrimers via orthogonal coupling reaction using thiol-ene addition reactions for construction of both the dendritic backbone as well as functionalization of the chain ends. Conducting the thiol-ene reactions in the absence of solvent under benign reaction conditions and without the use of any metal catalysts allows for an environmentally friendly process to be developed, further enhancing the attractive nature of the process.

More recently, hawker et al. reported the synthesis of a sixth generation dendrimer using the accelerated AB_2/CD_2 approach employing both CuAAC and thiol-ene coupling reactions. [32] The approach takes advantage of the time efficient and benign reaction conditions of those click reactions. And the simplified purification results in the preparation of a sixth generation dendrimer in a single day.

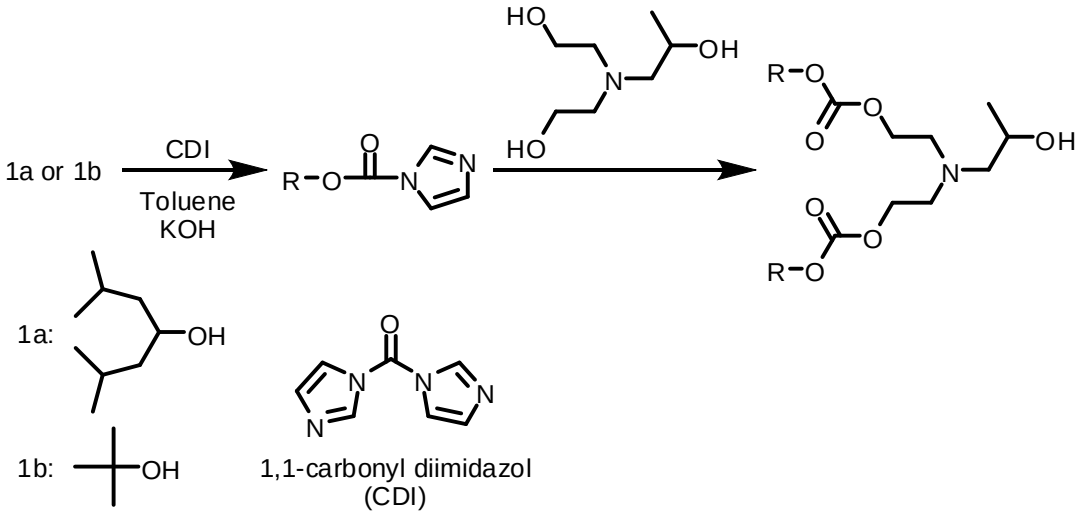


Scheme 2 Effective synthesis of dendrimers using "click"chemistry

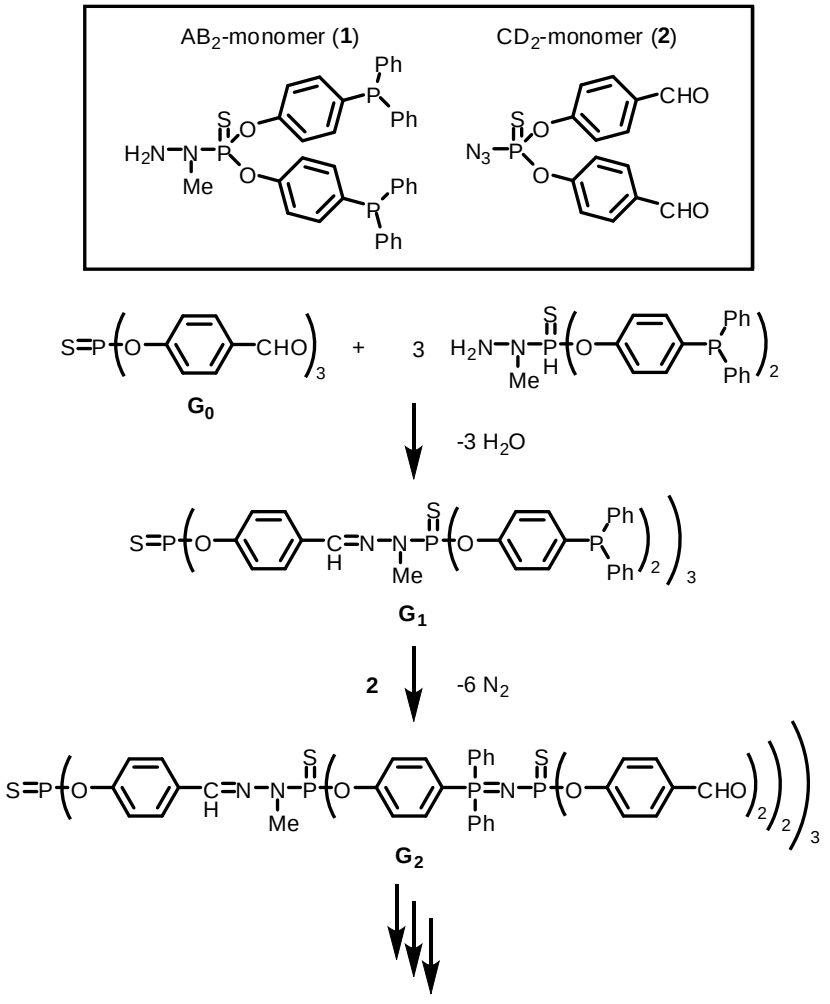
2-6. Other examples of rapid synthesis of dendrimers

There are other several recent reports that appear to be particularly noteworthy in the area of rapid synthesis of dendrimers.

Rannard *et al*. reported a one-pot multiple-addition convergent synthesis of polycarbonate dendrimers, in which the second generation dendrimer was obtained by sequential activation of an alcohol unit with 1,1-carbonyl diimidazole followed by addition of an unprotected-AB_2 triol (Scheme 3). [33] And Majoral et al reported the one-pot synthesis of a fourth generation dendrimer via divergent strategy using two different unprotected-AB_2 building blocks (Scheme 4). [34] In this method, to obtain phosphorus-containing dendrimers, they demonstrated that the condensation reaction between phophorhydrazides and aldehydes on one side, and the Staudinger reaction



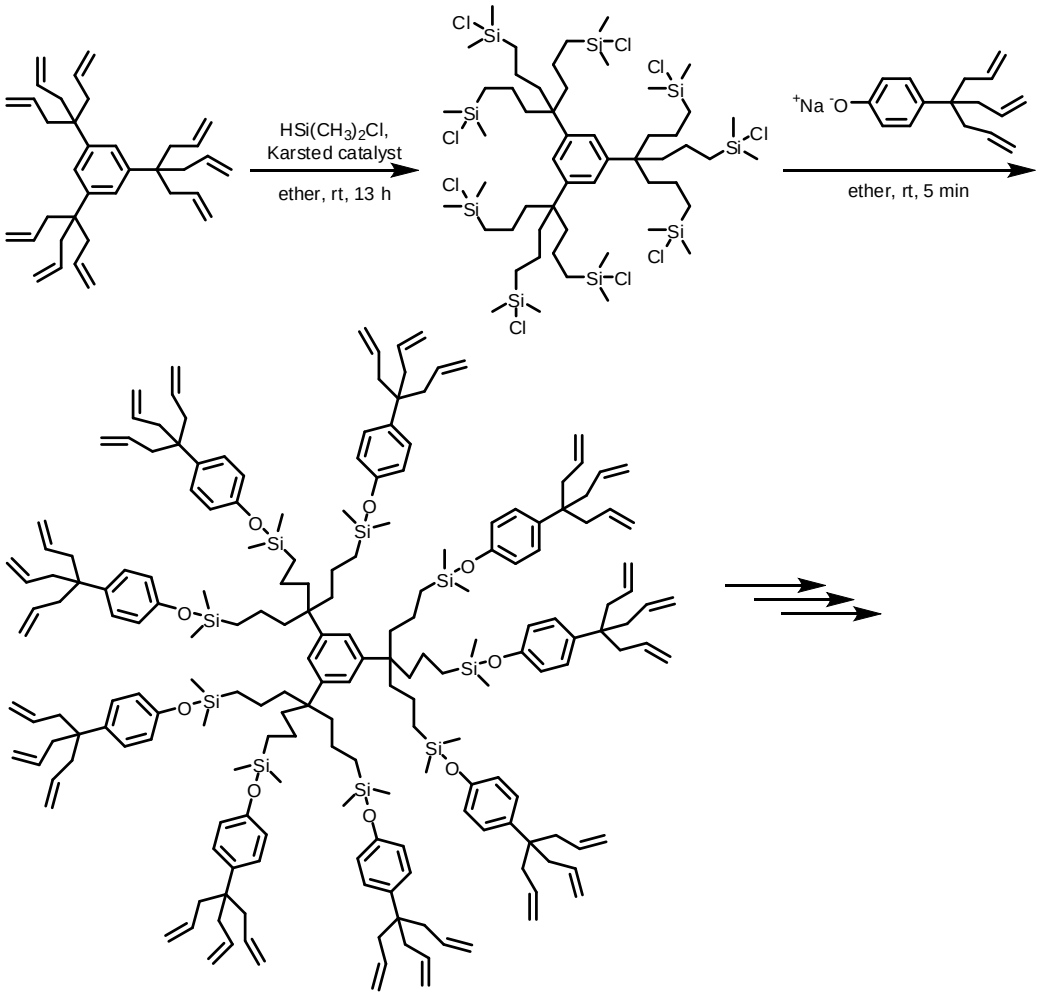
Scheme 3. One-pot convergent synthesis of polycarbonate dendrimer



Scheme 4. One-pot divergent synthesis of phosphorous dendrimer

between phosphines and azides on the other side. This sequence of reactions does not require any isolation, since the only by-products are H_2O and N_2. Although this method has poor versatility because the reactions used in this system cannot be applied for another structure, it is really attractive for industrial large-scale synthesis. In fact, this type of phosphorus dendrimers is now commercially available.

Eilbracht and Hagg's group has synthesized amino-functionalized dendritic architectures in a one-pot manner by hydroformylation-reductive amination sequences of dendritic polyglycerol with 30-40 amino termini at 75-80 ºC under 60 atm of CO/H_2 pressure. [35]

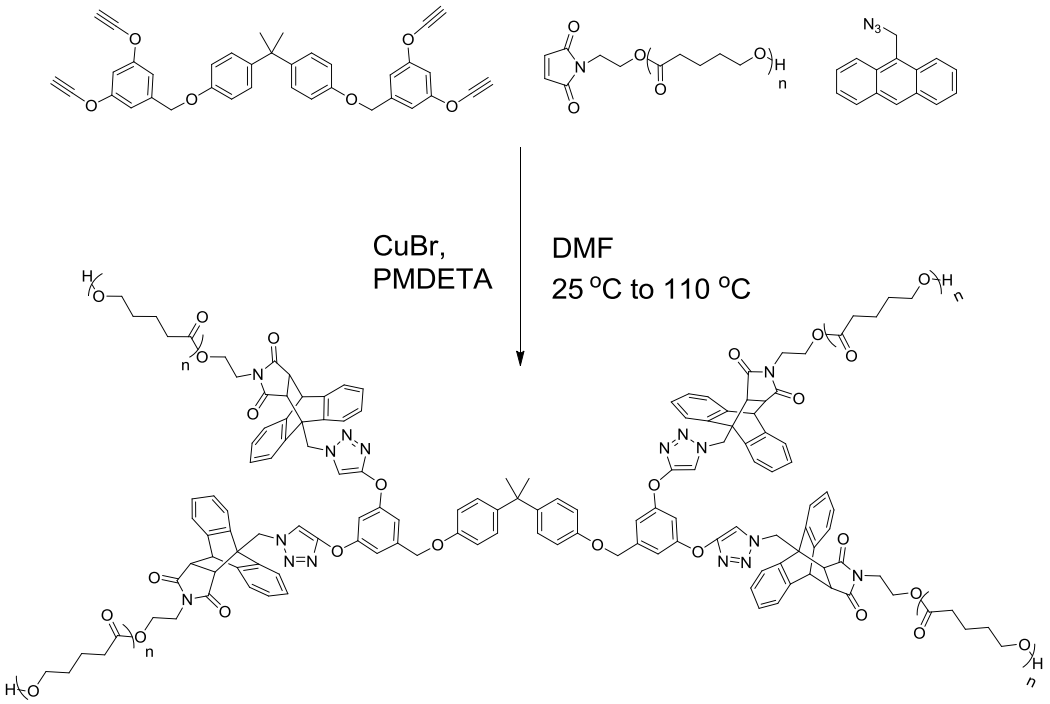


Scheme 5. One-pot allyl-terminated dendrimer synthesis

Also a one-pot synthesis of a 243-allyl-terminated dendrimer has been developed (Scheme 5). [36] Hydrosilylation of a nona-allyl dendritic core using $HSi(Me)_2Cl$ with an AB_3 building block possessing one phenolate and three allyl groups, followed by the repetition of this sequence of reactions twice, gives a 243-allyl-terminated dendrimer under ambient conditions.

All of the reports mentioned above utilize a one-pot synthetic approach to prepare dendrimers, in which one must add very precise amount of reagents at every step for performing the quantitative consumptions of the reactants. Therefore, formation of a small amount of defect molecules in final products is unavoidable, leading a molecular weight distribution.

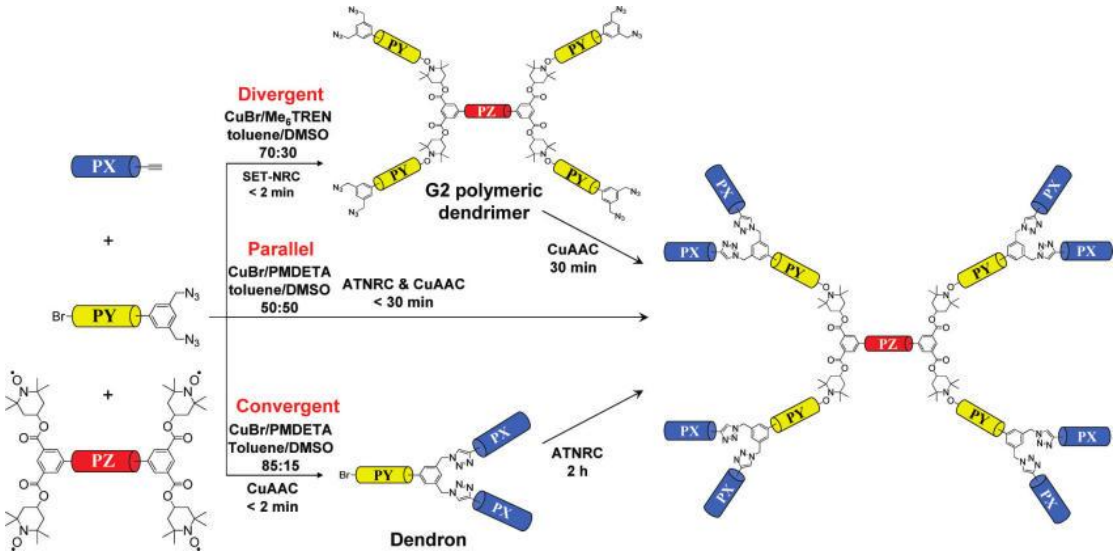
As another one-pot dendrimers synthetic method, "multiple click[37]" reactions have gained increasing attention. Xu et al. reported a synthesis of dendritic star polymers via double click reactions in 2010. [38] In this method, a class of well-defined dendritic star



Scheme 6. Synthesis of dendritic star polymers via double click reactions

polymers with poly (ε-caprolactone) (PCLs) on the periphery has been prepared via one-pot double click reactions (Cu-catalyzed azide/alkyne click chemistry, i.e., CuAAC and Diels–Alder reactions). (Scheme 6)

In 2011, Monteiro et al. reported the construction of third generation dendrimers using double click reactins. [39] Thruough modulating the Cu(I) activity for the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) and nitroxide radical coupling (NRC) reactions, the dendrimers could be formed divergently, convergently or in parallel.(Scheme 7) The parallel approach was the fastest and the third generation dendrimer could be synthesized at 25 °C in under 30 min.



Scheme 7. Synthesis of third generation dendrimers via double click reaction

However stringent structural requirements could prohibit the widely adoption, the "multiple click" strategy is attractive because in general they all promote a significantly greener approach with improve E-factor. In addition, a simple work-up due to the lack of any by-products and the use of benign solvents are attractive to reach commercialization.

3. Previous Work

Facile symthesis of aromatic polyamide dendrimers

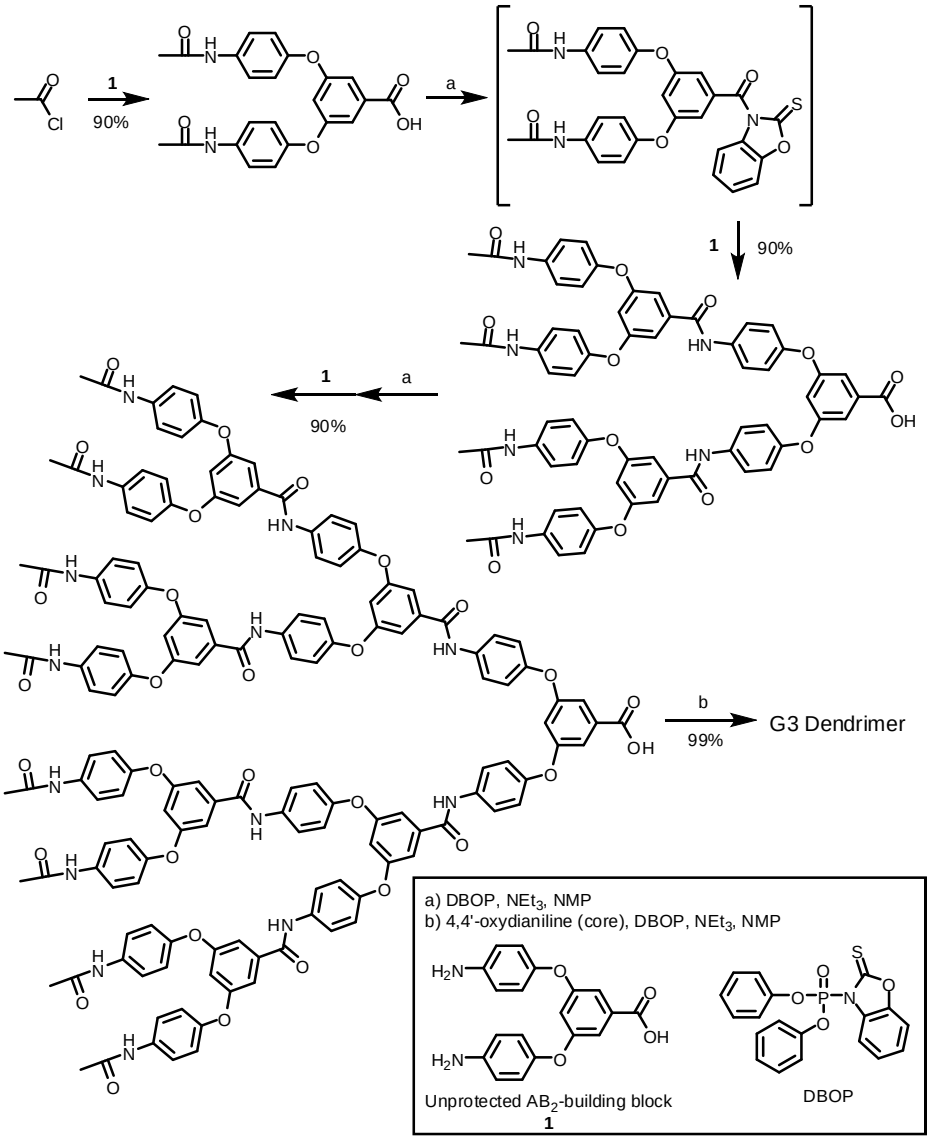
Our research group has focused on the synthesis of dendritic polyamide so far because polyamide is very attractive from the materials point of view. Linear polyamides are commonly available as engineering plastic materials with high modulus due to semicrystallinity or high glass transition temperatures. However the semicrystallinity and the strong tendency to form hydrogen bonding cause low solubility and high melting point, limiting the processing. A highly branched structure such as dendrimers, which is usually amorphous materials with excellent solubility and low viscosity, might improve the processing of polyamides. Thus, the introduction of dendritic structure into polyamides will result in new applications as an engineering plastic. Although various synthetic methods have been developed to prepare dendrimers containing amide functions, they have problems such as poor yield and tedious multi-synthetic steps. [40-46]

3-1. Convergent synthesis

① Using DBOP as activating agent[47]

We previously reported the rapid synthesis of a perfectly branched third generation polyamide dendrimer by the convergent method without repetitive protection-deprotection procedures, leading a decrease of number of steps to half of that required in conventional dendrimer formation (Scheme 8). This synthesis involved the direct condensation of a carboxylic acid and an unprotected AB_2-building block, 3,5-bis(4-aminophenoxy)benzoic acid, using the condensing agent diphenyl (2,3-dihydro-2-thioxo-3-benzoxazolyl)phosphonate (DBOP). Furthermore, in this

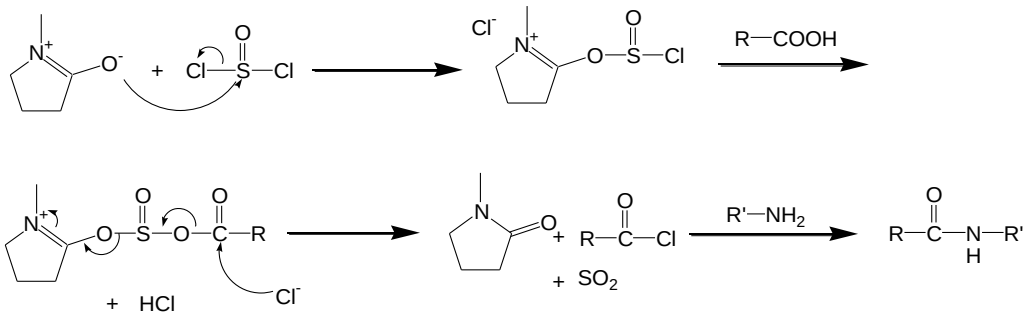
approach, all products were purified simply only by reprecipitation technique in excellent yields.



Scheme 8. Rapid synthesis of polyamide dendrimer from unprotected-AB_2-building block using DBOP

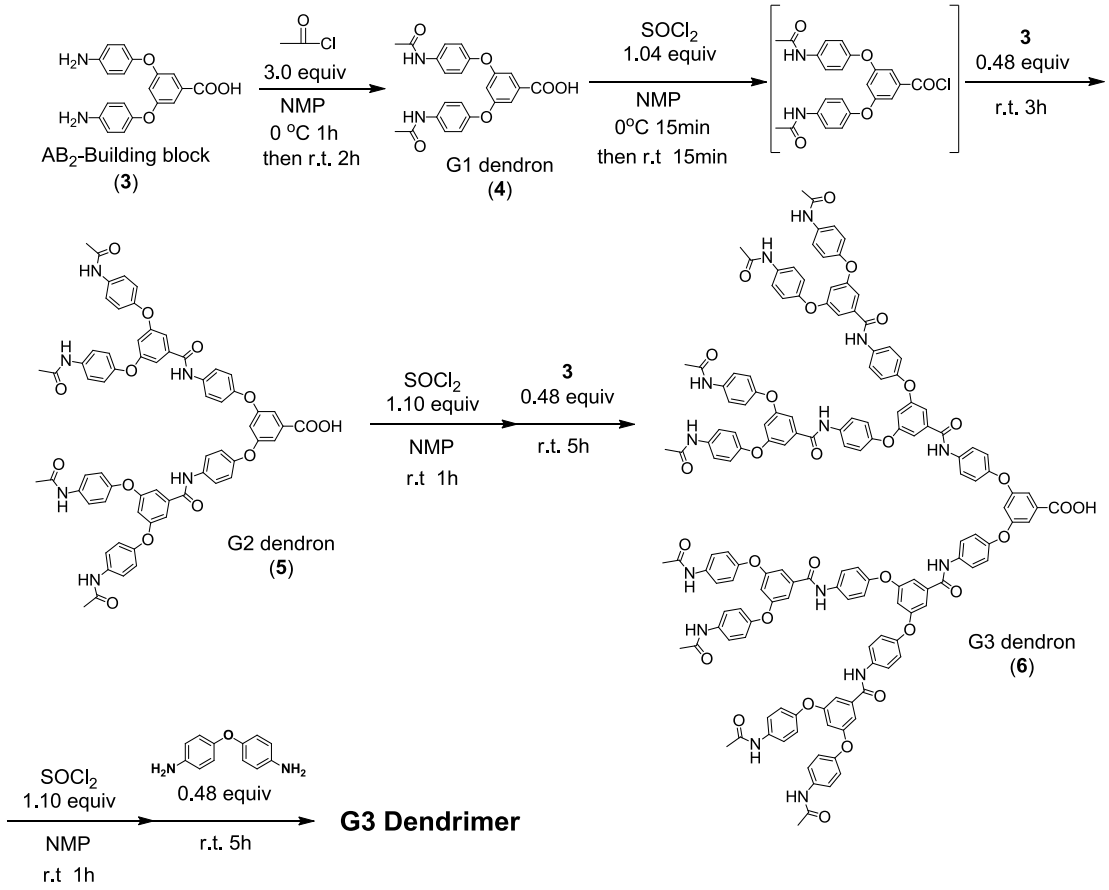
② Using $SOCl_2$ as activating agent[48,49]

Thionyl chloride, an inexpensive, commercially available reagent, is well known as an activating agent for the preparation of amides as well as acid chlorides from carboxylic acids.[14] (Scheme 9)



Scheme 9. Reaction mechanism by $SOCl_2$-NMP

The authors previously reported that thionyl chloride is effective for polyamide[15a] and polyester[15b] syntheses in amide solvents such as hexamethylphosphoric triamide and *N*-methyl-2-pyrrolidinone (NMP). Difficult purification procedures are not necessary in

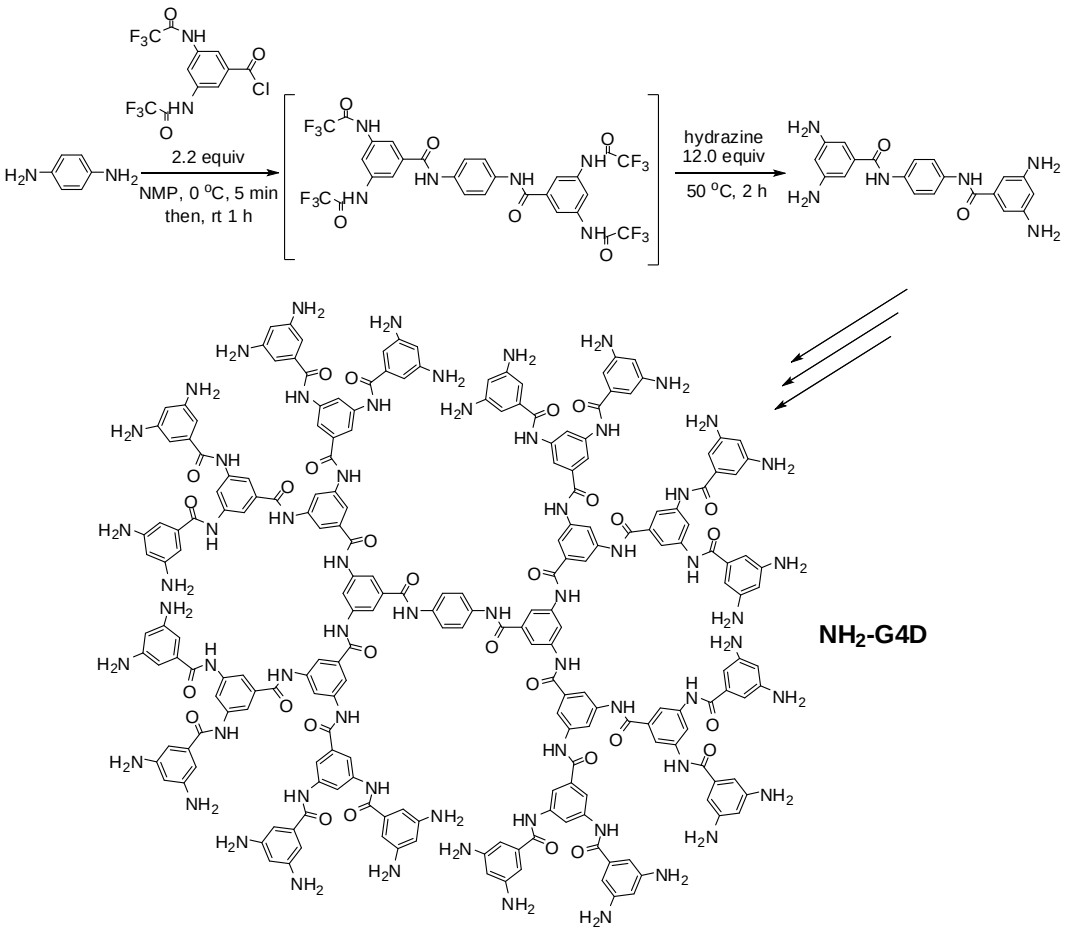


Scheme 10. Rapid synthesis of polyamide dendrimer from unprotected-AB_2-building block using $SOCl_2$

these syntheses, because the only by-products are gases such as SO_2 and HCl. Based on the knowledge, we developed a facile synthetic method of perfectly branched polyamide dendrimers from unprotected AB_2-building block by the convergent method using thionyl chloride as a versatile and common condensing reagent.(Scheme 10)

3-2. Divergent synthesis [50]

Our group has also developed facile synthetic method of polymide dendrimers via divergent synthesis. In this synthetic method, the authors have developed a two-step method for the facile synthesis of amine-terminated aromatic polyamide dendrimers using 3,5-bis(trifluoroacetamido)benzoyl chloride as an AB_2-building block via a



Scheme 11. Synthesis of aromatic polyamide dendrimer via divergent method

divergent approach in which the condensation and deprotection reactions are carried out rapidly in one-pot.(Scheme 11) Furthermore, the purification of every generation dendrimer only requires precipitation in alkaline water.

4. Purpose of This Work

Unique properties of dendrimers, which are a direct consequence of their regular structure, have significant interest in recent years. A large number of dendritic structures varying in size, solubility, and function have been prepared. However, the synthetic procedures used for their preparation detract from their widespread use. In this thesis, further studies on facile synthetic approaches of polyamide dendrimers and an example of the dendrimer's applications are constructed into six chapters.

General introduction is described in this Chapter I including representative rapid synthetic approaches described.

In Chapter II, the author describes a facile synthetic route for a tadpole-shaped polyamide dendrimer, based on the findings of the synthetic approach which previously developed in our group.

In Chapter III, based on the facile convergent synthetic method which previously developed in our group, water-soluble aromatic polyamide dendrimers are synthesized.

In Chapter IV, using the same concept of III, a novel synthetic method of aliphatic polyamide dendrimers is described.

In Chapter V, the auther describe the DNA sensory application of the water-soluble cationic polyamide dendrimers which synthesized via divergent method.

Finally, these research themes are concluded in the last chapter VI, and the prospects of these studies are described.

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Chapter II

Facile Synthesis of a Tadpole-Shaped Dendrimer Based on Aromatic Polyamides

Abstract

A novel, rapid, inexpensive, and highly accelerated approach for the synthesis of a tadpole-shaped dendrimer possessing monodisperse *N*-alkylated oligo(*p*-benzamide) as a rod block with a precise length and an amine-terminated dendritic block based on 3,5-diaminobenzoic acid has been developed. The *N*-alkylated oligo(*p*-benzamide)s were prepared by an rapid stepwise method using thionyl chloride as an activating agent and trifluoroacetamide as the protecting group for the end amine, in which the number of amide units dramatically propagated only through every deprotection and condensation. From this rod block, each generation of dendron-rod molecules were formed divergently using 3,5-bis(trifluoroacetamido)benzoyl chloride as an AB_2-buiding block.

Chapter II

1. Introduction

Dendrimers are characterized by their perfect branching, monodispersity, and three-dimensional structure with a large number of reactive end groups. Therefore, they have received considerable attention as new polymeric materials for applications in areas such as molecular light harvesting, catalysts, liquid crystals, molecular encapsulation, and drug-delivery systems.[1-6] However, the synthesis of dendrimers requires a tedious multistep procedure with repetitive protection-deprotection and purification processes, which interfere with their widespread use. To solve this problem, over the past several years, we have focused on the development of facile synthetic approaches to dendrimers,[7-11] and reported the rapid syntheses of aromatic polyamide dendrimers via both divergent and convergent methods, where the total number of reactions decreased to half of that required in the conventional approaches. In the convergent method, we utilized a two-step method comprising the activation of carboxylic acids with thionyl chloride and condensation with an unprotected AB_2-building block possessing diamine moieties. In the divergent method, we employed a novel protected AB_2-building block, 3,5-(bistrifluoroacetamido)benzoyl chloride, which enables a reduction in the deprotection reaction time of amines by a transamidation reaction with hydrazine, as well as one-pot condensation and deprotection reactions.[2d]

The development of an efficient approach to the synthesis of well-defined macromolecular architectures is also the central theme underlying a new strategy for the preparation of functional dendrimers. Recently, Stupp et al. reported the synthesis of dendron rodcoils comprising a series of novel block structures containing dendritic, rod-like, and coil-like segments, which yield interesting hierarchiral self-assembled nanostructures.[12] The synthesis is based on the combination of a catalyzed esterfication reaction and silyl protection/deprotection chemistry. Lee et al. synthesized tree-shaped molecules with octa-*p*-phenylene as the stem segment and oligoether dendrons as the flexible head by using a sequence of the Suzuki coupling reaction of 4-trimethylsilyl-biphenyl-4′-boronic acid with oligo-*p*-phenylene iodides.[13] Hammond et al. also reported an amphiphilic comb-dendritic block copolymer based on poly(γ-n-dodecyl-l-glutamate) as the hydrophobic comb block and a hydrophilic polyester dendron block modified with poly(ethylene glycol). [14] This comb-dendritic block copolymer was prepared from the polyester dendritic initiator, which initiated a ring-opening polymerization of the *N*-carboxyanhydride of γ-n-dodecyl-L-glutamate. Thus, the synthesis of dendrimers consisting of rod dendrons and monodisperse linear-rod segments is interesting to compare their hierarchiral self-assembled nanostructures to those of dendrimers from rod dendrons and coil-like segments.

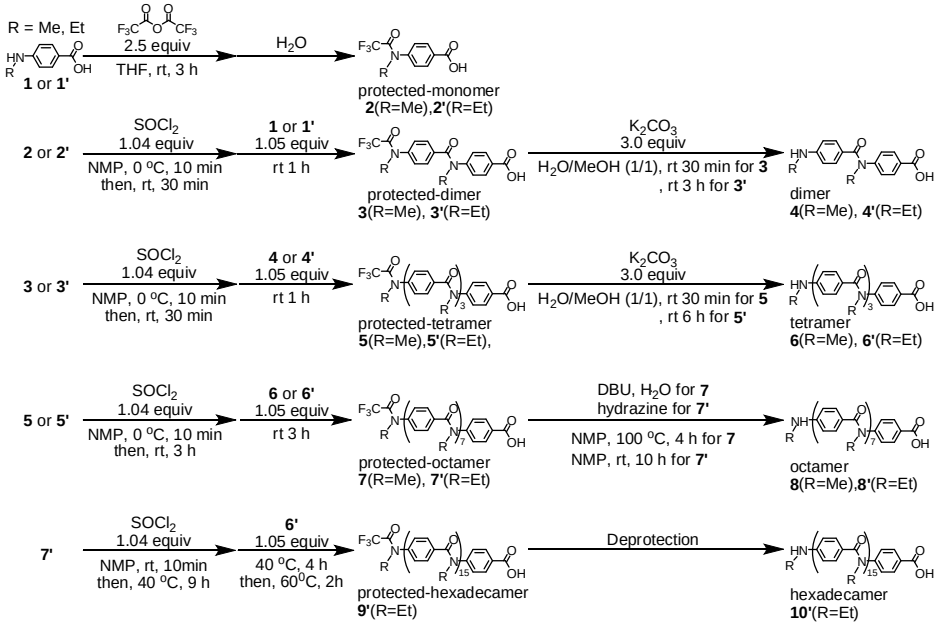
In this paper, we report a facile synthesis of a monodisperse dendron-rod block copolymer, termed as a tadpole-shaped dendrimer, possessing monodisperse *N*-alkylated oligo (*p*-benzamide) as a rod block with a precise length and an amine-terminated dendritic block based on 3,5-diaminobenzoic acid, where each block was prepared by fewer reaction steps using a protocol similar to that described above.

2. Results and Discussion

2-1 Synthesis of Rod Blocks

To synthesize rod blocks with precise lengths, 4-(*N*-alkylamino)benzoic acid **1** (methyl), **1'** (ethyl), and **1''**(butyl) were selected as unprotected AB-monomers and synthesized according to the literature.[15] As we previously reported,[11] a trifluoroacetamide group has high stability under acidic conditions and outstanding ability as a leaving group that enables the selective deprotection by hydrolysis under mild conditions or a transamidation with hydrazine. Thus, the AB-monomer **1** was first protected with excess trifluoroacetic anhydride to produce the protected monomer **2** in 94% yield after recrystallization from *i*-PrOH (Scheme 1). The coupling reaction was carried out by the two-step method involving the activation of the carboxylic acid of a protected-AB compound using thionyl chloride, followed by condensation with an unprotected-AB compound. The protected dimer **3** was successfully prepared from **2**

(protected) and **1** (unprotected) in 93% yield. The end trifluoroacetamide group was easily hydrolyzed by K_2CO_3 in H_2O/MeOH at room temperature for 30 min to yield the unprotected dimer **4** in a quantitative yield. Then, the carboxylic acid of **3** was activated using thionyl chloride and reacted with **4** to yield the protected tetramer **5** in one pot. The resulting protected tetramer **5** was quantitatively converted to the unprotected tetramer **6** by selective hydrolysis of the end group using K_2CO_3. We performed the reactions in the same manner using protected and unprotected tetramers to synthesize the protected octamer **7**. Since **7** was insoluble in H_2O/MeOH even under alkaline conditions, the hydrolysis of the end amide group in **7** was carried out in NMP using 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) as a base with a small amount of water at 100 ^oC.



Scheme 1 Synthesis of monodisperse *N*-alkylated oligo(*p*-benzamide)s

All the products were purified only by recrystallization or precipitation in almost quantitative yields, and characterized by ^{1}H-NMR. The ^{1}H-NMR spectrum of the protected-octamer **8** showed doublets at 7.87 and 6.13 ppm, integrated to two protons each. These signals are denoted as a and b, respectively, in Figure 1. The MALDI-TOF MS spectrum of **8** only shows signals attributed to the presumable MSs, indicating the formation and isolation of the desired octamer (Figure 2). However, due to the poor solubility of **7** in NMP, it was difficult to synthesize the protected hexadecamer. Nevertheless, this synthetic approach for monodisperse oligoamides is highly attractive since the number of amide units dramatically propagated only through every deprotection and condensation.

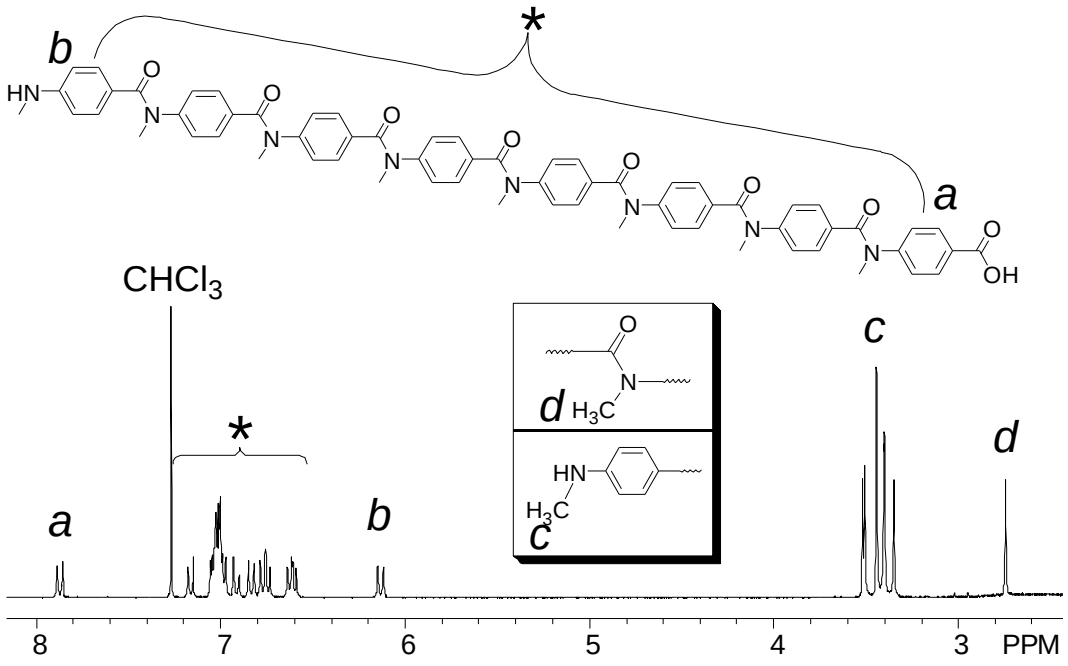


Figure 1 ^{1}H NMR spectrum of octamer **8**

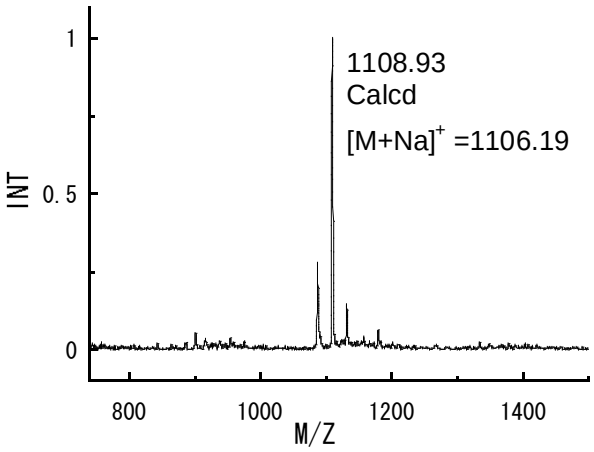


Figure 2 MALDI-TOF MS spectrum of octamer **8**

In the case of using 4-(*N*-ethylamino)benzoic acid **1'** as an another unprotected AB-monomer, the overall synthetic route was almost similar to that in the case of using the AB-monomer **1**. The resulting protected-octamer **7'** had good solubility in NMP as we expected; therefore, it could be reacted with octamer **8'** via a two-step method to provide the protected hexadecamer **9'** in 82% yield. However, protected-hexadecamer **9'** could not be converted to hexadecamer **10'** due to the poor solubility of the former in

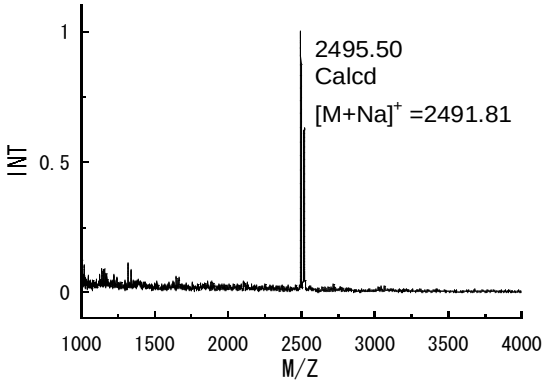
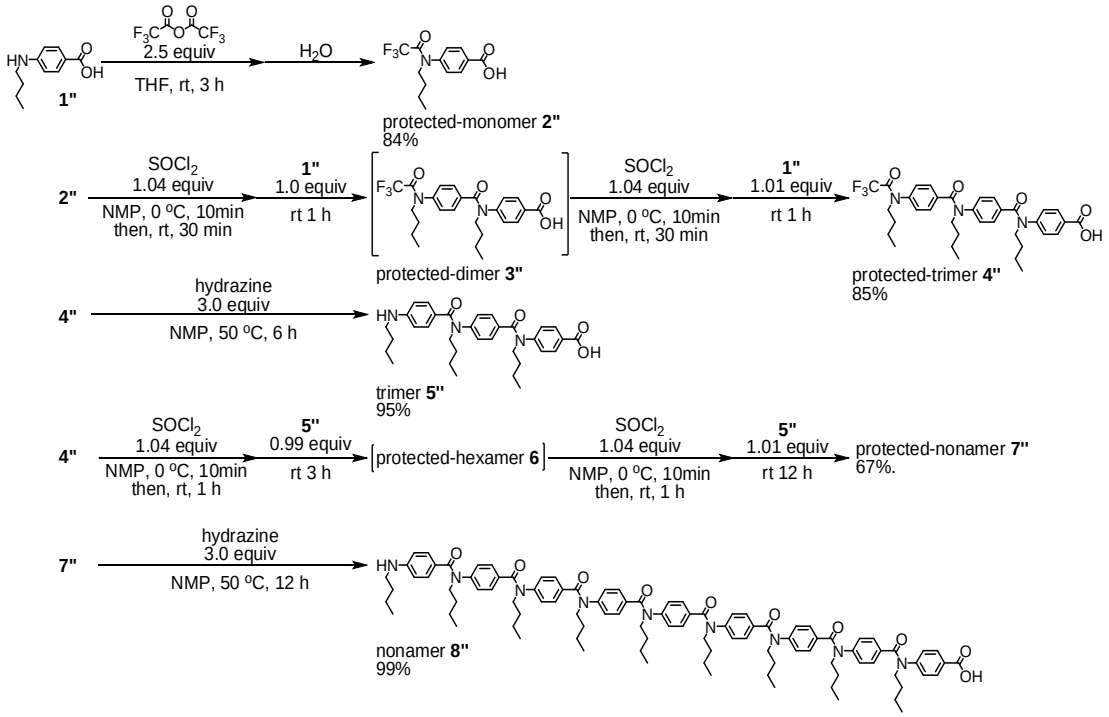


Figure 3 MALDI-TOF MS spectrum of protected-hexadecamer **9'**

NMP. Figure 3 shows the MALDI-TOF MS spectrum of protected-hexadecamer **9'**, which supports its formation.

Thus, we finally applied this approach to the preparation of monodisperse oligoamides with a butyl group in the side chain with better expected solubility in NMP (Scheme 2). In this case, the resulting oligoamides have low crystallinity and low melting points due to the relatively long alkyl-side chain, leading to difficulties in their purification by recrystallization or reprecipitation. Therefore, to reduce the number of purification steps, a one-pot multiple addition condensation reaction was applied. The protected-monomer **2''** was obtained from the reaction of AB-monomer **1''** and excess trifluoroacetic anhydride. **2''** was converted into the corresponding acid chloride by adding 1.04 equiv of thionyl chloride to it at 0 ºC for 10 min, and then at room temperature for 30 min in NMP. Then, the following condensation with **1''** at room temperature for 1 h yielded the corresponding protected dimer **3''** *in situ* almost quantitatively. To this solution, another 1.04 equiv of thionyl chloride was added; subsequently, **1''** was added in one-pot to afford the protected trimer **4''** in 85% yield after recrystallization from hexane/THF. **4''** was then successfully deprotected by a transamidation reaction with hydrazine at 50 ºC for 6 h to yield the trimer **5''** in 95% yield. In a similar manner, the protected nonamer **7''** was synthesized by the repetition

activation of the carboxylic acid of a protected-AB compound using thionyl chloride and sequential condensation with **5''**. The yield of the protected nonamer after reprecipitation with 2-butanone/hexane was 67%. The relatively low yield probably resulted from the incomplete condensation of the protected hexamer **6''** with **5''** due to the heterogeneous reaction system. Finally, the nonamer **8''** was quantitatively obtained by a transamidation of **7''** with hydrazine at 50 ºC for 12 h. Despite their moderate solubility in NMP (in weight percent), it was difficult to use 7" and 8" in the subsequent reaction because sufficient concentration of their solutions (in mole percent) were not achieved due to their relatively high molecular weights resulting from the butyl group in their side chains.



Scheme 2 Synthesis of monodisperse *N*-butylated oligo(*p*-benzamide)s

The ^{1}H NMR spectrum of **7''** shows two doublets at 7.24 and 7.85 ppm, integrated to two protons each. These signals are denoted by a and b, respectively, in Figure 4. The MALDI-TOF MS spectrum of **7''** (Figure 5) and **8''** (Figure 6) indicate their molecular weights $[M + Na]^{+}$ to be 1713 and 1616.9 Da, which are close to the calculated molecular weights of 1714 and 1614.9 Da, respectively. These findings clearly indicate the formation of the desired molecules.

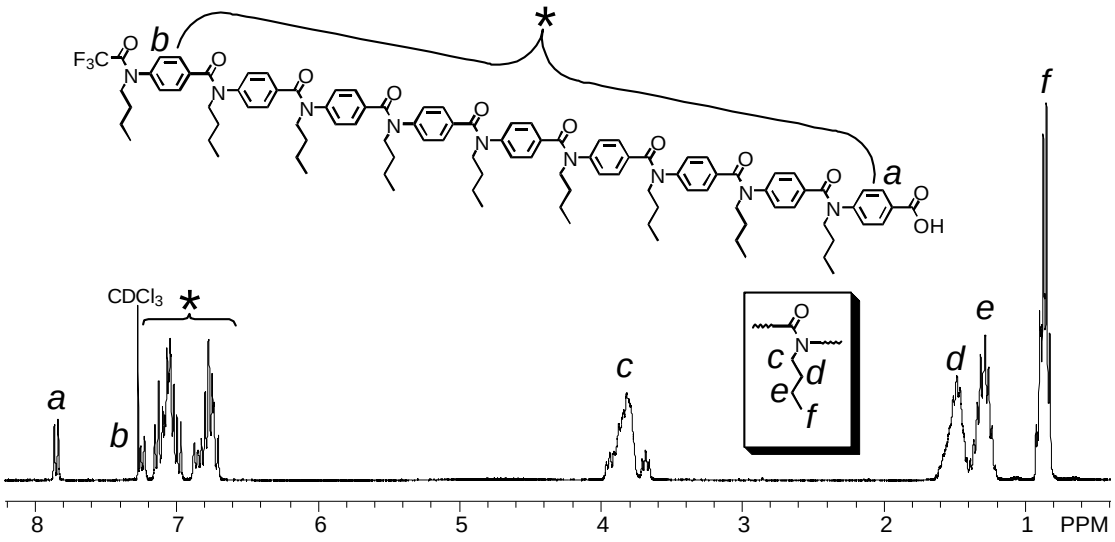


Figure 4 ^{1}H NMR spectrum of protected-nonamer **7"**

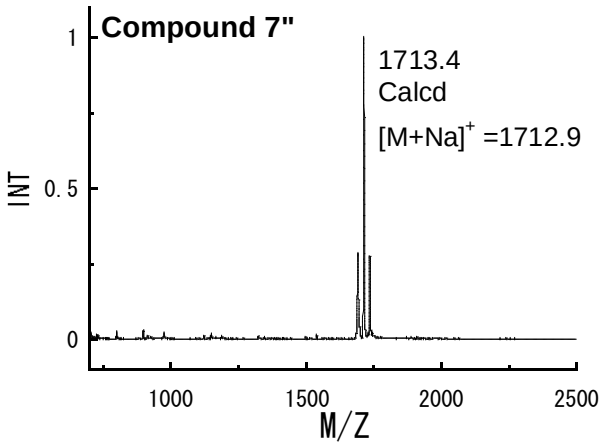


Figure 5 MALDI-TOF MS spectrum of protected-nonamer **7''**

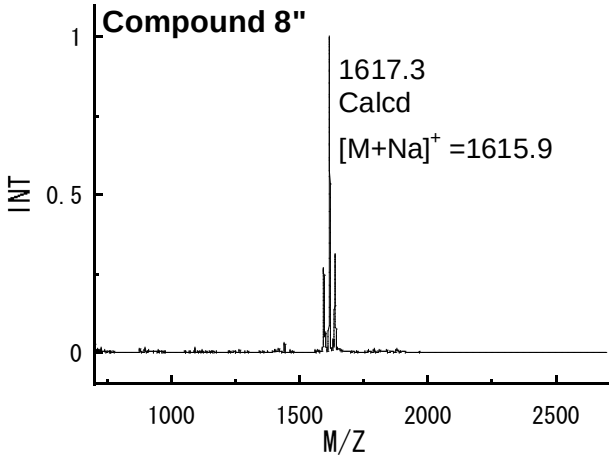
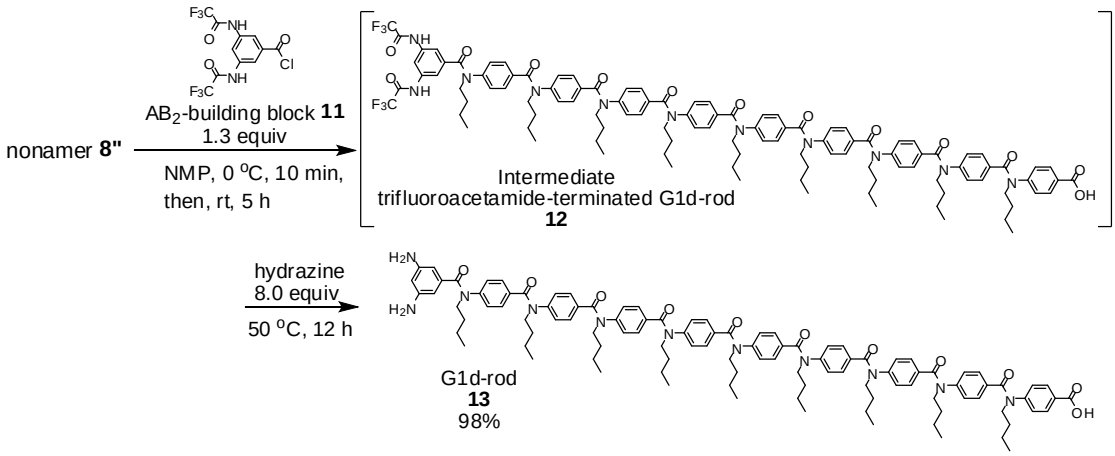


Figure 6 MALDI-TOF MS spectrum of nonamer **8"**

2-2 Synthesis of Dendrimers.

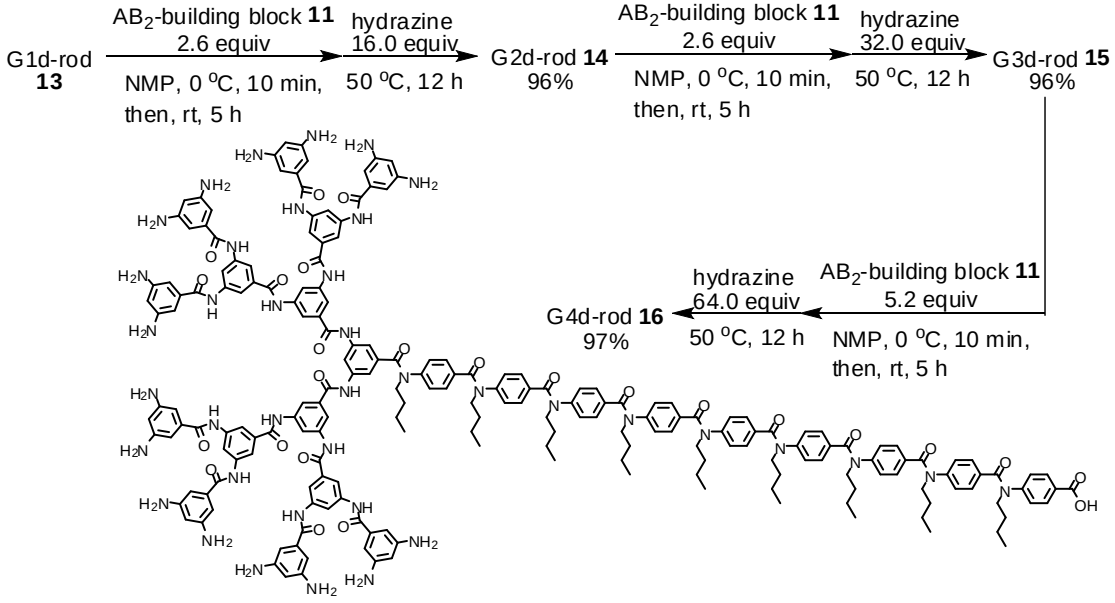
The dendron block was prepared by a divergent approach from the secondary amine group of the *N*-butylated nona(*p*-benzamide) **8"**. As reported previously[2d], the condensation and deprotection reactions of these growth processes can be performed in

one pot using 3,5-bis(trifluoroacetamido)benzoyl chloride **11** as an AB_2-building block. The reaction of **8''** with a slight excess of **11** quantitatively yields the intermediate, trifluoroactamide-terminated G1d-rod (1st generation dendron coupled with rod block) molecule **12***in situ*. After the hydrolysis of the acid chloride group of the unconverted-excess AB_2-building block, **12** was converted to the amine-terminated G1d-rod **13** by a transamidation reaction with excess hydrazine. Since all the by-products in the resulting final solution are soluble in alkaline water, G1d-rod **13** can be purified simply by precipitation in $NaHCO_{3aq}$ in a quantitative yield (Scheme 3). The



Scheme 3 Synthesis of G1d-rod **13**

larger amine-terminated rod-dendron molecules were synthesized using a similar protocol, and isolated in excellent yields after precipitation in alkaline water (Scheme 4). Finally, G4d-rod **16** was successfully obtained simply through a four-step process from **8''**.



Scheme 4 Synthesis of dendron-rod molecules

2-3 Characterization of Dendrimers.

The structures of the dendrimers were characterized by IR and ^{1}H NMR spectroscopies and elemental analyses. The IR spectrum of **16** showed strong absorptions at 3355 and 1628 cm^{-1}, characteristic of the N–H and C=O stretchings of the amino and amide carbonyl groups, respectively. The ^{1}H NMR spectrum of **16** showed signals corresponding to the amide protons (c, f, and i) at 9.98, 10.30, and 10.40 ppm and aromatic protons (a and b) of the end unit at 6.04 and 6.40 ppm, respectively (Figure 7). The MALI-TOF MS spectra of **13, 14**, and **15** indicate their molecular weights [M + Na]+ to be 1751.0, 2018.7, and 2553.5 Da, which are close to the calculated molecular weights of 1751.0, 2019.0 and 2554.2 Da, respectively (Figure 8). The G4d-rod could not be analyzed by MALDI-TOF MS spectrum probably because of

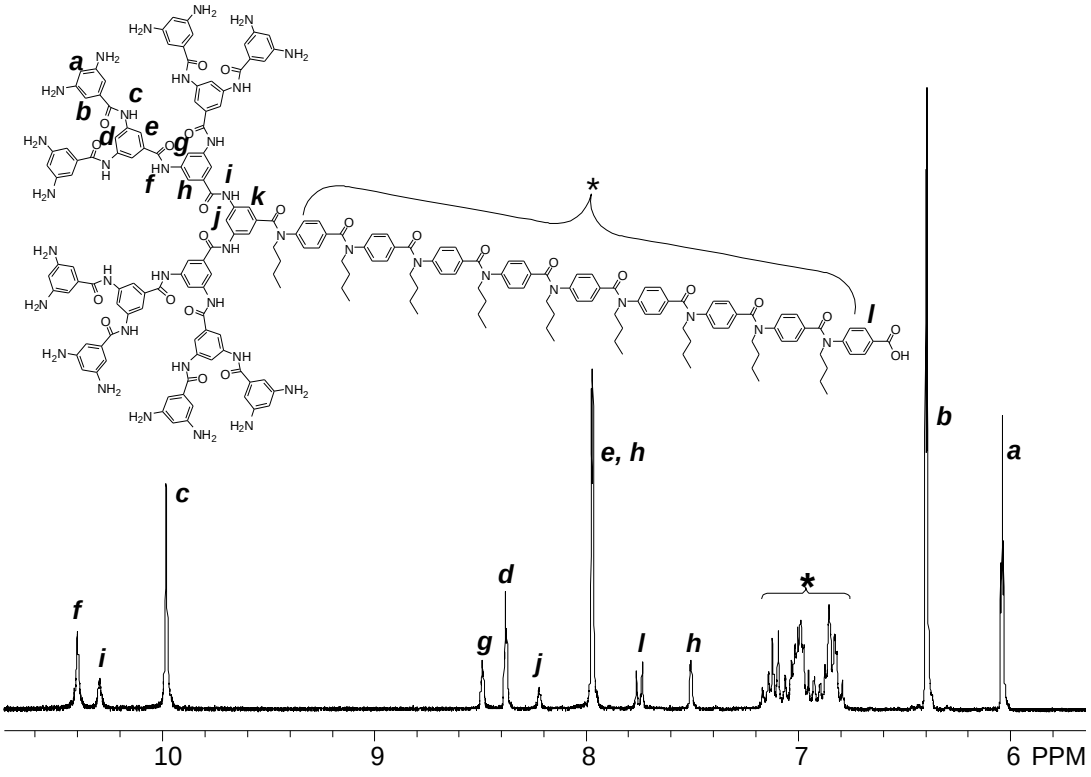


Figure 7 ^{1}H NMR spectrum of G4d-rod **16**

strong intermolecular interactions between the terminated amines and dendrimers. Therefore, the intermediate, trifluoroacetamide-terminated G4d-rod (CF_3-G4d-rod), which was expected to show weak interactions, was analyzed by MALDI-TOF MS spectroscopy (Figure 9). The spectrum exhibited the desired signals at M/Z $[M+Na]^+$ 5162.8, $[M-H+2Na]^+$ 5184.8, $[M-2H+3Na]^+$ 5206.8, and $[M-3H+4Na]^+$ 5228.5 Da. These findings clearly indicate the formation of the desired dendrimers.

The thermal property of **8"** and dendrimers **13, 14**, **15, and 16** were measured by DSC analysis. **8"** and **13** exhibited the melting point at 95 and 150 ^{o}C in the first scan, and the glass transition temperature (*Tg*) at 90 and 105 ^{o}C in the second and third scans,

respectively. On the other hand, no melting points and *Tg*s of **14, 15, and 16** were not observed.

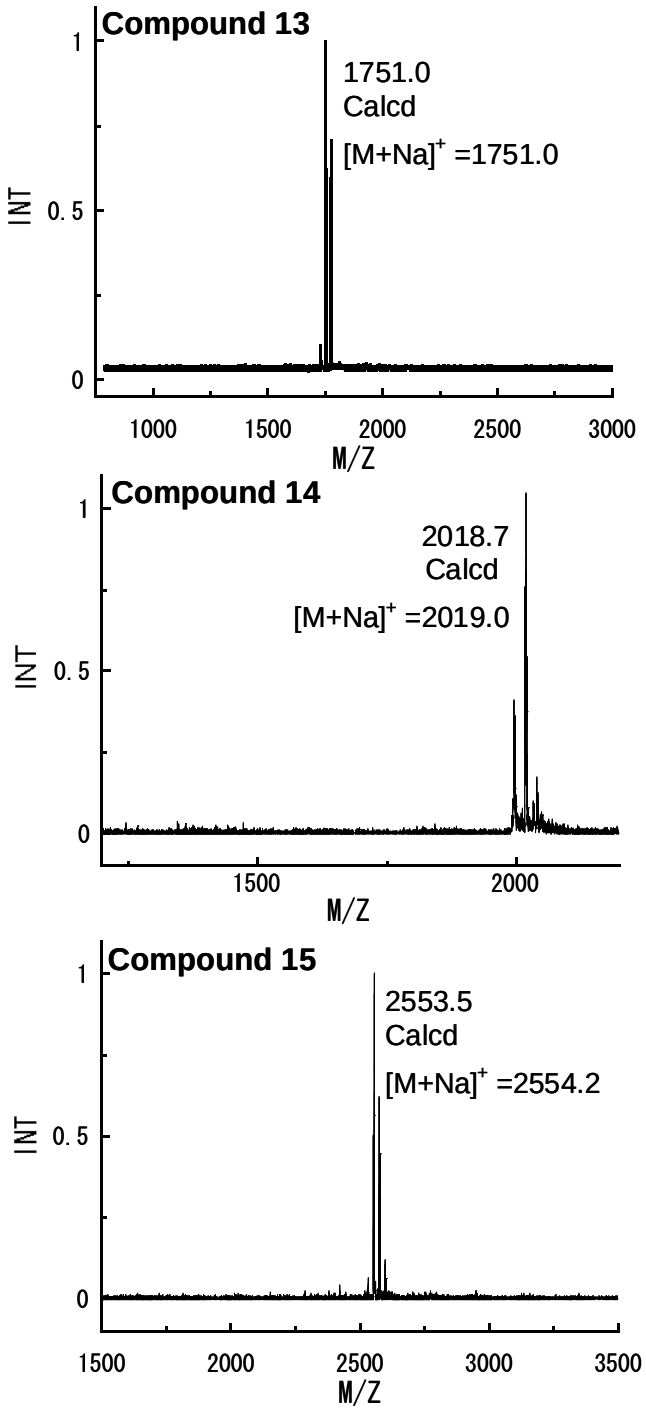


Figure 8 MALDI-TOF MS spectra of dendron-rod **13, 14, and 15**

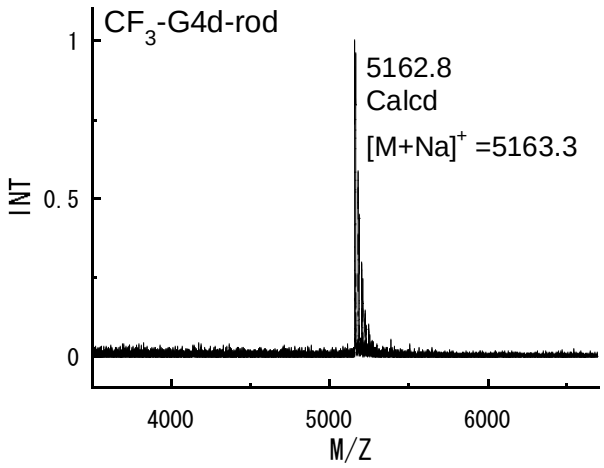


Figure 9 MALDI-TOF MS spectrum of CF_3-G4d-rod

3. Conclusions

We have developed a facile synthesis of a tadpole-shaped dendrimer possessing *N*-butylated nona(*p*-benzamide) as a rod block with a precise length and dendritic block based on 3,5-diaminobenzoic acid. In this method, the monodisperse *N*-alkylated oligoamides (R = methyl, ethyl, butyl) were prepared by an accelerated approach using thionyl chloride as an activating agent and trifluoroacetamide as a protecting group. Using the resulting nonamer **8''** as a core molecule, the dendritic block was successfully prepared in excellent yields by a divergent approach using a two-step method with the AB_2-building block **11**. Finally, the 4th generation tadpole-shaped dendrimer **16** was obtained *via* this efficient route. This novel structure is particularly attractive as a building block of a self-assembly because the strong hydrogen bonding between the polyamide dendritic blocks and interaction of alkyl pendant groups between the rod

blocks would enhance the stability of the resulting self-assembled architectures. Furthermore, since the rod moiety has an exact length and the oligomers/polymers of *N*-alkyl-*p*-benzamide show a helical conformation as reported by Yokozawa *et al.*,[16] this dendron-rod block molecule has a very persistent precise structure that would enable the direct application of the theory relating the molecular geometric shape to the final self-assembling form.[14] Modifying the functionalities either at the periphery or the focal point of this novel tadpole dendrimer would increase its promising applications for the fabrication of self-assembled architectures.

4. Experimental Section

Materials

N-Methyl-2-pyrrolidinone (NMP) was distilled under reduced pressure over calcium hydride and then stored under nitrogen. Thionyl chloride was distilled over triphenyl phosphite under nitrogen. THF was dried over sodium and distilled before use under nitrogen. The other reagents and solvents were obtained commercially and used as received

Synthesis of Rod Blocks.

Preparation of AB-monomer (1'')

To the solution of 4-aminobenzoic acid (33.0 g, 200 mol) in hexamethylphosphoric triamide (100 mL) was added butyl iodide (18.4 g, 100 mol) under nitrogen. The reaction solution was stirred at 120 ^{o}C for 10 h, allowed to cool to 25 ^{o}C, and then was poured into water. The deposit was filtered, dissolved in MeOH (400 mL) and then reprecipitated with water (500 mL). The precipitate was collected and dissolved in EtOH (150 mL). To this solution was added potassium hydroxide (22.0 g, 392 mmol) under nitrogen, and the resultant solution was refluxed for 6 h. The reaction mixture was poured into water, and then the pH of the resulting solution was adjusted around 4.0 using HCl_{aq}. The precipitate was filtered, dried and recrystallized from MeOH to give a slightly yellow crystal (77% yield calculated toward ethyl iodide), mp = 157 ^{o}C. IR (KBr, cm^{-1}): 1531, 1601 (Ar-H), 1662 (C=O), 2500~3300 (O-H), 2866, 2931, 2958 (C-H), and 3398 (N-H). ^{1}H NMR ($CDCl_3$, ppm, 25 ^{o}C): δ = 0.97 (t, 3H), 1.44 (m, 2H), 1.63 (m, 2H), 3.18 (t, 2H), 6.55 (d, 2H), 7.92 (d, 2H). ^{13}C NMR (DMSO, 40 ^{o}C): δ□= 13.6, 19.7, 30.6, 42.0, 110.6, 116.7, 131.1, 152.7, and 167.5. Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.33; H, 7.80; N, 7.14.

Preparation of protected-monomer (2'')

Trifluoroacetic anhydride (21.5 g, 102 mmol) was added to a solution of 4-(*N*-butylamino)benzoic acid (9.00 g, 46.6 mmol) in THF (72 mL) at 0 ^{o}C under

nitrogen. The solution was stirred at the temperature for 10 min, followed by 25 ^{o}C for 3 h. Then, water (150 mL) was added and stirring was continued for 6 h at the temperature. The reaction mixture was extracted with diethyl ether, and the organic layer was washed with water several times, dried over $MgSO_4$, and filtered. The filtrate was evaporated, and the residue was recrystallized from hexane at 50 ºC to give a white crystal (84% yield), mp = 85-87 ^{o}C. IR IR (KBr, cm^{-1}): 1211 (C-F), 1512, 1608 (Ar-H), 1701 (C=O), 2500~3300 (O-H), and 2877, 2935, 2962 (C-H). ^{1}H NMR ($CDCl_3$, ppm, 25 ^{o}C): δ = 0.92 (t, 3H), 1.34 (m, 2H), 1.56 (m, 2H), 3.78 (t, 2H), 7.36 (d, 2H), 8.21 (d, 2H). ^{13}C NMR ($CDCl_3$, ppm, 25 ^{o}C): δ = 13.5, 19.8, 28.9, 51.7, 116.3 (q, J = 286.7 Hz), 128.6, 129.9, 131.5, 144.0, 117.3, 156.4 (q, J = 35.3 Hz), and 171.1. Anal. Calcd for $C_{13}N_{14}F_3NO_3$: C, 53.98; H, 4.88; N, 4.84. Found: C, 54.06; H, 4.87; N, 4.65.

Preparation of protected-trimer (4'')

Thionyl chloride (1.91 mL, 26.2 mmol) was added to a solution of protected-monomer **2''** (7.29 g, 25.2 mmol) in NMP (30 mL) at 0 ^{o}C under nitrogen. The solution was stirred at the temperature for 10 min, followed by 25 ^{o}C for 30 min. To this solution, 4-(*N*-butylamino)benzoic acid **1''** (4.87 g, 25.2 mmol) was added and stirring was continued for 1 h at the temperature. Subsequently, the activation of terminal carboxylic acid using thionyl chloride (1.91 mL, 26.2 mmol) was performed again at 0 ºC for 10

min, followed by 25 ^{o}C for 30 min. Then, the condensation of it with another **1''** (4.87 g, 25.2 mmol) was conducted at the temperature for 1 h. The reaction mixture was poured into dilute HCl, and the precipitate was filtered and dried. The crude product was purified by recrystallization from hexane/THF (v:v=50:7) to give a white crystal (85% yield), mp = 112-113 ^{o}C. IR (KBr, cm^{-1}): 1207 (C-F), 1512, 1604 (Ar-H), 1651 (C=O(NH)), 1697 (C=O (trifluoroacetyl amide), C=O(OH)), 2500~3300 (O-H), and 2873, 2935, 2958 (C-H). ^{1}H NMR ($CDCl_3$, ppm, 25 ^{o}C): δ = 0.81-0.94 (m, 9H), 1.21-1.66 (m, 12H), 3.68 (t, 2H), 3.87 (t, 2H), 3.94 (t, 2H), 6.83 (d, 2H), 7.00 (d, 2H), 7.03 (d, 2H), 7.16 (d, 2H), 7.24 (d, 2H), 7.92 (d, 2H). ^{13}C NMR ($CDCl_3$, ppm, 25 ^{o}C): δ = 13.39, 13.47, 13.49, 19.59, 19.88, 19.93, 28.72, 29.58, 29.73, 50.00, 51.26, 116.23 (q, J = 287.0 Hz), 126.78, 127.17, 127.53, 127.88, 129.47, 129.72, 130.79, 134.40, 136.31, 140.15, 144.18, 147.74, 156.21 (q, J = 35.3 Hz), 168.80 and 169.4 ppm. Anal Calcd for $C_{35}H_{14}F_3N_3O_5$: C, 65.71; H, 6.30; N, 6.57. Found: C, 66.10; H, 6.39; N, 6.19.

Preparation of trimer (5'')

To a solution of protected-trimer **3'** (7.3 g, 11.4 mmol) in NMP (17 mL) was added hydrazine monohydrate (1.71 g, 34.2 mmol) at 25 ^{o}C under nitrogen. The reaction mixture was stirred at 50 ºC for 3 h. The resulting solution was diluted with water, and the pH of the solution was adjusted around 4.0 using HCl_{aq}. The precipitate was filtered

and dried at 60 ^{o}C under reduced pressure to give white glass (95% yield), mp = 174-176 ^{o}C. IR (KBr, cm^{-1}): 1512, 1604 (Ar-H), 1643 (C=O(NH)), 1716 (C=O(OH)), 2500~3300 (O-H), 2870, 2931, 2958 (C-H), and 3386 (N-H). ^{1}H NMR ($CDCl_3$, ppm, 25 ^{o}C): δ = 0.81-0.96 (m, 9H), 1.20-1.65 (m, 12H), 3.05 (t, 2H), 3.81 (t, 2H), 3.94 (t, 2H), 6.19 (d, 2H), 6.87 (d, 2H), 6.98 (d, 2H), 7.03 (d, 2H), 7.15 (d, 2H), 7.91 (d, 2H). ^{13}C NMR ($CDCl_3$, ppm, 25 ^{o}C): δ = 13.58, 13.70, 19.89, 19.92, 20.00, 29.54, 31.04, 42.87, 49.82, 50.18, 110.63, 121.95, 126.69, 127.06, 128.01, 129.15, 130.77, 130.92, 133.36, 145.54, 147.26, 149.82, 168.26, 169.90, and 170.78. Anal. Calcd for $C_{33}H_{41}N_3O_4$: C, 72.90; H, 7.60; N, 7.73. Found: C, 72.91;H, 7.65; N, 7.73.

Preparation of protected-nonamer (7'')

Thionyl chloride (65.0 uL, 0.895 mmol) was added to a solution of protected-trimer **4''** (0.550 g, 0.860 mmol) in NMP (1.5 mL) at 0 ^{o}C under nitrogen. The reaction mixture was stirred at the temperature for 10 min, followed by 25 ^{o}C for 1 h. To this solution trimer **5''** (0.463 g, 0.852 mmol) was added and stirring was continued for 3 h at the temperature. Subsequently, the activation of terminal carboxylic acid using thionyl chloride (65.0 uL, 0.895 mmol) was performed again at the temperature for 30 min. Then, the condensation of it with another **5''** (0.473 g, 0.869 mmol) was conducted at the temperature for 12 h. The reaction mixture was poured into dilute HCl, and the

precipitate was filtered and dried. The crude product was dissolved in 2-butanone, and reprecipitated with hexane. The precipitate was collected and dried at 100°C under reduced pressure to give white solid. (67% yield), mp = 212-214 °C. IR (KBr, cm^{-1}): 1215 (C-F), 1508, 1604 (Ar-H), 1651 (C=O(NH)), 1701 (C=O (trifluoroacetyl amide)), 1716 (C=O(OH)), and 2873, 2931, 2958 (C-H). ^1H NMR ($CDCl_3$, ppm, 25 °C): δ = 0.80-0.94 (m, 27H), 1.18-1.65 (m, 36H), 3.69 (t, 2H), 3.73-4.00 (m, 16H), 6.67-6.90 (m, 14H), 6.93-7.17 (m, 18H), 7.24 (d, 2H), 7.85 (d, 2H). Calcd.: $[M]^+$ m/z=1689.9. Found: MALDI-TOF-MS: $[M+H]^+$=1691.4, $[M+Na]^+$=1713.4, $[M-H+2Na]^+$=17365.5 Anal. Calcd for $C_{101}H_{118}F_3N_9O_{11}$: C, 71.73; H, 7.03; N, 7.45. Found: C, 71.53; H, 7.08; N, 7.18.

Preparation of nonamer (8'')

To a solution of protected-nonamer **7''** (0.951 g, 0.562 mmol) in NMP (2.8 mL) was added hydrazine monohydrate (84.3 mg, 1.69 mmol) at 25 °C under nitrogen. The reaction mixture was stirred at 50 °C for 12 h. Then, the resultant solution was diluted with water, and the pH of the solution was adjusted around 4.0 using HCl_{aq}. The precipitate was filtered and dried under reduced pressure at 100 °C to give white solid (99% yield), mp =95 °C IR (KBr, cm^{-1}): 1508, 1604 (Ar-H), 1643 (C=O(NH)), 1716

(C=O(OH)), and 2870, 2931, 2958 (C-H). ^{1}H NMR ($CDCl_3$, 25 ^oC): δ = 0.74-0.97 (m, 27H), 1.15-1.66 (m, 36H), 3.09 (t, 2H), 3.70-3.99 (m, 16H), 6.34 (d, 2H), 6.60-6.83 (m, 14H), 6.90-7.17 (m, 18H), 7.67 (d, 2H). Calcd.: $[M]^+$ m/z = 1593.9. Found: MALDI-TOF-MS: $[M+H]^+$ = 1593.0 $[M+Na]^+$ = 1614.9, $[M+2Na-H]^+$ = 1636.8. Anal. Calcd for $C_{99}H_{119}N_9O_{10}$: C, 74.55; H, 7.52; N,7.90. Found: C, 73.81; H, 7.46; N, 7.46.

Synthesis of Dendrimers

Preparation of G1d-rod (13)

AB_2-building block **11** (0.236 g, 0.650 mmol) was added to a solution of nonamer **8''** (0.798 g, 0.500 mmol) in NMP (1.0 mL) at 0 ^oC under nitrogen. The reaction mixture was stirred at the temperature for 5 min, subsequently at 25 ^oC for 5 h. Then, water (ca. 5 cc) was added and stirring was continued for 1 h at 50 ^oC. The resultant solution was treated with hydrazine monohydrate (0.200 g, 4.00 mmol) for another 12 h at the temperature. The reaction mixture was poured into 2 wt % of $NaHCO_{3aq}$. The precipitate was filtered and dispersed in water, then adjusted at pH~7 using diluted HCl_{aq}. The resulting precipitate was collected and dried at 60 ^oC under reduced pressure to give white solid (98% yield), mp = 150 ^oC. IR (KBr, cm^{-1}): 1508, 1604 (Ar-H), 1647 (C=O(NH)), 1716 (C=O(OH)), 2870, 2931, 2958 (C-H), and 3375 (N-H). ^{1}H NMR ($CDCl_3$, ppm, 25 ^oC): δ = 0.78-0.96 (m, 27H), 1.18-1.65 (m, 36H), 3.71-3.99 (m, 18H),

5.84 (t, 1H), 5.90 (d, 2H), 6.64-6.88 (m, 16H), 6.92-7.18 (m, 18H), 7.83 (d, 2H). Calcd.: $[M]^+$ m/z = 1728.0. Found: MALDI-TOF-MS: $[M+H]^+$ = 1729.0, $[M+Na]^+$ = 1751.0, $[M+2Na-H]^+$ = 1773.0. Anal. Calcd for $C_{106}H_{120}N_{11}O_{11}0.60H_2O$: C, 72.49; H, 7.18; N, 8.77. Found: C, 72.49; H, 7.01 N, 8.79.

Preparation of G2d-rod (14)

AB_2-building block **11** (0.236 g, 0.650 mmol) was added to a solution of G1d-rod **13** (0.432 g, 0.250 mmol) in NMP (1.0 mL) at 0 ^oC under nitrogen. The reaction mixture was stirred at the temperature for 5 min, subsequently at 25 ^oC for 5 h. Then, water (ca. 5 mg) was added and stirring was continued for 1 h at 50 ^oC. The resultant solution was treated with hydrazine monohydrate (0.200 g, 4.00 mmol) for another 12 h at the temperature. The reaction mixture was poured into 2 wt % of $NaHCO_{3aq.}$ The precipitate was filtered and dispersed in water, then adjusted at pH~7 using diluted HCl_{aq}. The resulting precipitate was collected and dried at 60 ^oC under reduced pressure to give white solid (96% yield). IR (KBr, cm^{-1}): 1508, 1601 (Ar-H), 1643 (C=O(NH)), 1716 (C=O(OH)), 2870, 2931, 2958 (C-H), and 1543, 3375 (N-H). ^1H NMR (DMSO-d_6, ppm, 40 ^oC): δ = 0.70-0.87 (m, 27H), 1.08-1.54 (m, 36H), 3.65-3.90 (m, 18H), 6.02 (t, 2H), 6.30 (d, 4H), 6.78-7.17 (m, 34H), 7.39 (d, 2H), 7.75 (d, 2H), 8.06 (t, 1H), 9.70 (s, 2H). Calcd.: $[M]^+$ m/z = 1997.0. Found: MALDI-TOF-MS: $[M+H]^+$ = 1997.0, $[M+Na]^+$

= 2018.7, $[M+2Na-H]^+$ = 2040.0. Anal. Calcd for $C_{120}H_{137}N_{15}O_{13}$ 0.65H_20: C,70.86; H, 6.80; N, 10.35. Found: C, 70.86; H, 6.83; N, 10.37.

Preparation of G3d-rod (15)

AB_2-building block **11** (0.236 g, 0.650 mmol) was added to a solution of G2d-rod **14** (0.252 g, 0.125 mmol) in NMP (1.0 mL) at 0 °C under nitrogen. The reaction mixture was stirred at the temperature for 5 min, subsequently at 25 °C for 5 h. Then, water (ca. 5 mg) was added and stirring was continued for 1 h at 50 °C. The resultant solution was treated with hydrazine monohydrate (0.200 g, 4.00 mmol) for another 12 h at the temperature. The reaction mixture was poured into 2 w t% of $NaHCO_{3aq}$. The precipitate was filtered and dispersed in water, then adjusted at pH~7 using diluted HCl_{aq}. The resulting precipitate was collected and dried at 60 °C under reduced pressure to give white solid (96% yield). IR (KBr, cm^{-1}): 1508, 1601 (Ar-H), 1635 (C=O(NH)), 1716 (C=O(OH)), 2870, 2931, 2958 (C-H), and 1543, 3375 (N-H). ^1H NMR (DMSO-d_6, ppm, 40 °C): δ = 0.68-0.87 (m, 27H), 1.08-1.54 (m, 36H), 3.65-3.90 (m, 18H), 6.04 (t, 4H), 6.39 (d, 8H), 6.77-7.18 (m, 34H), 7.49 (d, 2H), 7.75 (d, 2H), 7.89 (d, 4H), 8.14 (t, 1H), 8.36 (t, 2H), 9.94 (s, 4H), 10.17 (s, 2H). Calcd.: $[M]^+$ m/z = 2531.2. Found: MALDI-TOF-MS: $[M+Na]^+$ = 2553.5, $[M+2Na-H]^+$ = 2575.5, [M+3Na-2H]+ = 2598.3.

Anal. Calcd for $C_{148}H_{161}N_{23}O_{17}$ 0.84 H_2O: C, 67.56; H, 6.16; N, 12.24. Found: C, 67.56; H, 6.39; N, 12.33.

Preparation of G4d-rod (16)

AB_2-building block **11** (0.236 g, 0.65 mmol) was added to a solution of G3d-rod **15** (0.158 g, 0.0625 mmol) in NMP (1.0 mL) at 0 ^oC under nitrogen. The reaction mixture was stirred at the temperature for 5 min, subsequently at 25 ^oC for 5 h. Then, water (ca. 5 mg) was added and stirring was continued for 1 h at 50 ^oC. The resultant solution was treated with hydrazine monohydrate (0.200 g, 4.00 mmol) for another 12 h at the temperature. The reaction mixture was poured into 2 wt % of $NaHCO_{3aq}$. The precipitate was filtered and dispersed in water, then adjusted at pH~7 using diluted HCl_{aq}. The resulting precipitate was collected and dried at 60 ^oC under reduced pressure to give white solid (97% yield). To obtain the validation of formation and isolation of the desired molecule using MALDI-TOF MS spectroscopy, the intermediate, trifluoroacetamide-terminated G4d-rod (CF_3-G4d-rod), was separately isolated and analyzed instead of amine-terminated G4d-rod **16**. IR (KBr, cm^{-1}): 1512, 1601 (Ar-H), 1628 (C=O(NH)), 2870, 2931, 2958 (C-H), and 1543, 3355 (N-H). ^1H NMR (DMSO-d_6, ppm, 40 ^oC): δ = 0.68-0.87 (m, 27H), 1.06-1.54 (m, 36H), 3.64-3.91 (m, 18H), 6.04 (t, 8H), 6.40 (d, 16H), 6.77-7.19 (m, 34H), 7.51 (d, 2H), 7.75 (d, 2H), 7.97 (d, 12H), 8.22

(t, 1H), 8.38 (t, 4H), 8.49 (t, 2H), 9.98 (s, 8H), 10.30 (s, 2H), 10.40 (s, 4H). Anal. Calcd for $C_{204}H_{209}N_{39}O_{25}$ 12.08 H_20. C, 64.06; H, 6.14; N, 14.28. Found: C, 63.72; H, 5.80; N, 13.98. Calcd. for CF_3-G4d-rod: $[M]^+$ m/z = 5140.3. Found: MALDI-TOF-MS: $[M+Na]^+$ = 5162.8, $[M+2Na-H]^+$ = 5184.8, [M+3Na-2H]+ = 5206.8.

Measurements

Infrared spectra were recorded on a Horiba FT-720 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a BRUKER DPX-300 spectrometer at 300 and 75 MHz, respectively. Deuterated chloroform ($CDCl_3$) and deuterated dimethylsulfoxide (DMSO-d_6) were used as a solvent with tetramethylsilane as an internal standard. Matrix-assisted laser desorption ionization with time of flight (MALDI-TOF) mass spectra were recorded on a Kratos Kompact MALDI instrument operated in linear detection mode to generate positive ion spectra using dithranol or 2,5-dihydroxybenzoic acid (DHBA) as a matrix, tetrahydrofuran (THF) or chloroform as a solvent, sodium trifluoroacetate as an additive agent.

5. References and Notes

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Chapter III

Synthesis of a Novel Water-Soluble Polyamide Dendrimer Based on a Facile Convergent Method

Abstract

A novel, rapid, inexpensive, and highly efficient convergent approach has been developed for the synthesis of a 32-amine-terminated G3 polyamide dendrimer by the hydrolysis of the dendrimer with trifluoroacetamide groups. The resulting dendrimer could be successfully modified with oligo(ethylene glycol) chains at its periphery to afford a novel water-soluble polyamide dendrimer. The structural homogeneity of the dendrimers was confirmed by NMR and MALDI-TOF mass spectroscopies.

Chapter III

1. Introduction

Dendrimers possess hierarchical three-dimensional structures consisting of a multifunctional core from which successive branched repeating units radiate outward. The large definite number of functionalities can be easily introduced at the periphery of dendrimers by exploiting the reactivity of multiple terminal groups[1-4]. Such structural specificity has encouraged in pursuit of new polymeric materials for applications in areas such as catalysts[5], liquid crystals[6], photonic devices[7], and drug delivery systems[8-10]. Dendrimers were synthesized based on a divergent[11 12] or convergent[13] methodology involving reiterative reaction sequences, which generally required tedious multi-step procedures such as repetitive protection-deprotection and purification in each generation. Several methods have been reported to shorten the synthetic pathways, including double-stage, double-exponential growth, hypermonomers, and orthogonal coupling approaches[4]. However, multi-step procedures are still essential to obtain high generation dendrimers.

Recently, we have demonstrated a rapid synthesis of a perfectly branched third generation polyamide dendrimer via a convergent method to eliminate

repetitive protection-deprotection procedures[14]. The method consists of an activation of carboxylic acid with thionyl chloride in *N*-methyl-2-pyrrolidinone (NMP), followed by condensation with the amino groups of an AB_2 building block in one-pot. The advantage is the employment of thionyl chloride which is a widely used and commercially available reagent for the preparation of acid chlorides. In addition, no difficult purification is required because the byproducts after condensation reactions with thionyl chloride are only SO_2 and HCl. Indeed, Normant et al. reported the effectiveness of thionyl chloride as a condensing agent for amide syntheses[15]. Polyamides and polyesters have been directly prepared by utilizing this condensing agent as well [16-18].

Unfortunately, the proposed convergent method has a limitation in modifying the terminal groups of polyamide dendrimer due to the stable acetamide moiety. Quite recently, we reported the facile synthesis of amine-terminated aromatic polyamide dendrimers via the divergent approach, where the trifluoroacetyl group in place of acetyl group was employed as a protecting group of amines[19]. The trifluoroacetyl moieties could be easily deprotected by nucleophiles; e.g. hydrazine to regenerate

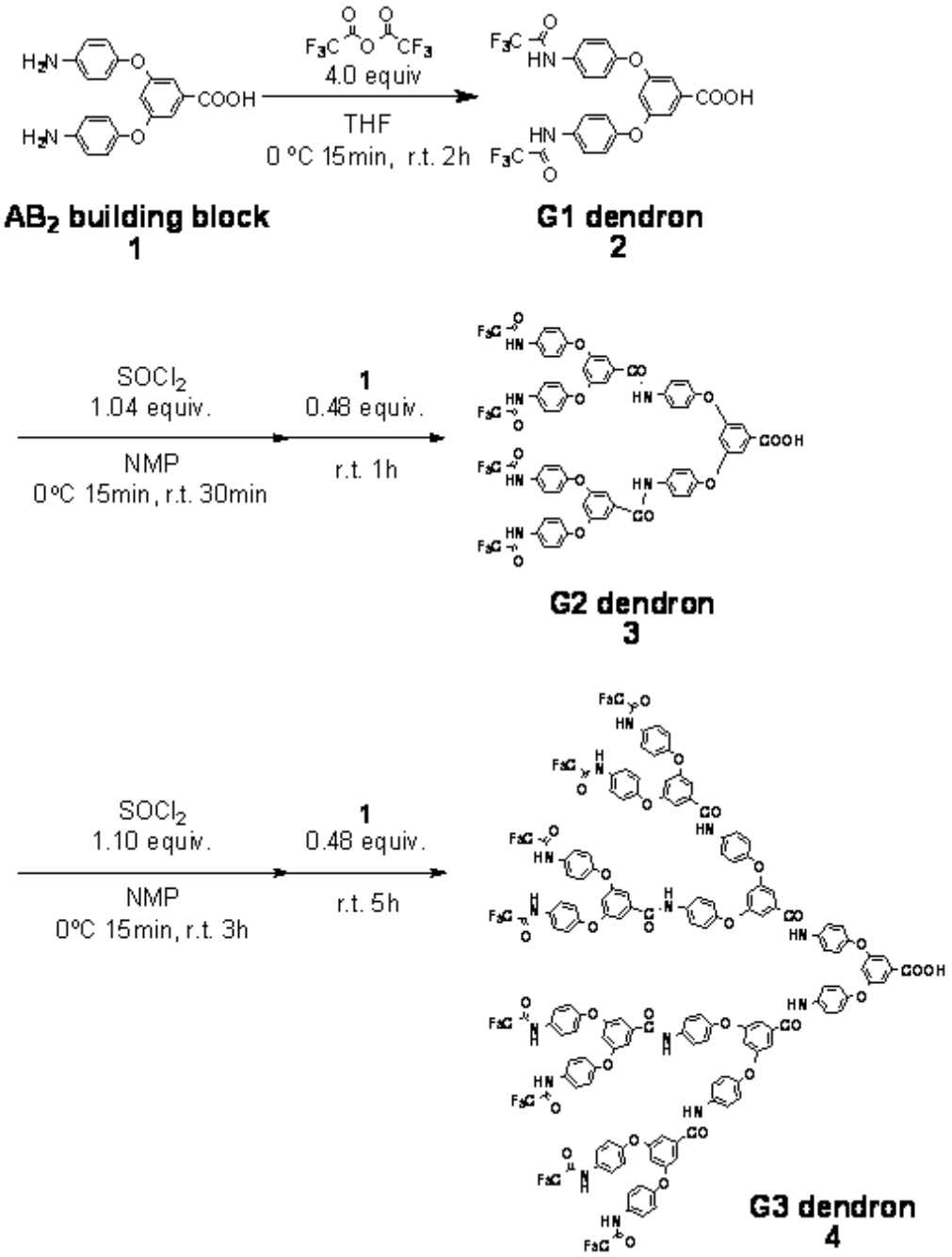
primary amino groups[20, 21]. Based on these findings, we report herein the convergent synthesis of amide dendrons and a third generation polyamide dendrimer which bear 32 trifluoroacetamide moieties at its periphery. The formation of the amino-terminated dendrimer prompts us to further synthesize a new water-soluble polyamide dendrimer with oligo(ethylene glycol) chains at the surface via the modification reaction of the amino groups. This motivation comes from the interest in water-soluble dendrimers showing their unique and specific properties; for example, unimolecular micellation behaviors[22-24]. In addition, potential applications of dendrimer-inorganic hybrid materials (solution-phase catalysis, additives for polymer blends) could benefit from the versatile solubility of the dendrimer

2. Results and Discussion

To achieve a convergent polyamide dendrimer synthesis using thionyl chloride as an activation agent, we selected an AB_2 building block, **1**, containing one carboxyl and two amino groups. The coupling reaction for the dendron synthesis was conducted by a successive reaction sequence involving 1) activation of carboxylic acids with thionyl chloride, and 2) condensation with **1**. The molar ratio of thionyl chloride and carboxyl group was very important for the quantitative activation and prevention of unfavorable reactions such as self condensation of **1** and the reaction of thionyl chloride was very important for the quantitative activation and with amines. Thus, we refer to the report[14] for the ratio of thionyl chloride and carboxyl groups, and determine the amount of thionyl chloride to be 1.04 equivalent toward carboxylic acid.

Synthetic routes for G1 (**2**), G2 (**3**), and G3 (**4**) dendrons are shown in Scheme 1. The AB_2 building block, **1**, reacted with trifluoroacetic anhydride to afford **2** in 95% yield after recrystallization from acetonitrile. The FT-IR spectrum of **2** showed strong absorptions at 1704 and 1157 cm^{-1} due to the characteristic absorptions derived from C=O stretching of carboxyl groups

and C-F stretching, respectively. Further evidence of the formation and isolation of **2** was obtained in the ^{1}H NMR spectrum.



Scheme 1 Synthesis of AB2 Building Block 1, G1 (2), G2 (3), and G3 (4) Dendron

Similarly, **3** and **4** were prepared by repeating the same reaction sequence, 1) and 2), using thionyl chloride asdescribed above. The activation time of the carboxylic acid and the time for condensation were different depending on dendrons as shown in Scheme 1. For the synthesis of **4**, drying **3** before the reaction and 1.10 equiv of thionyl chloride to **3** were required. We assume that thionyl chloride decomposed due to hygroscopic property of **3** possessing a number of amide groups. Each dendron was purified simply by fractional precipitation to remove the earlier generation dendron. The dendrons, **3** and **4**, were isolated in 97% and 71% yield, respectively, and characterized by ^{1}H NMR, MALDI-TOF mass spectroscopies, and elemental analysis.

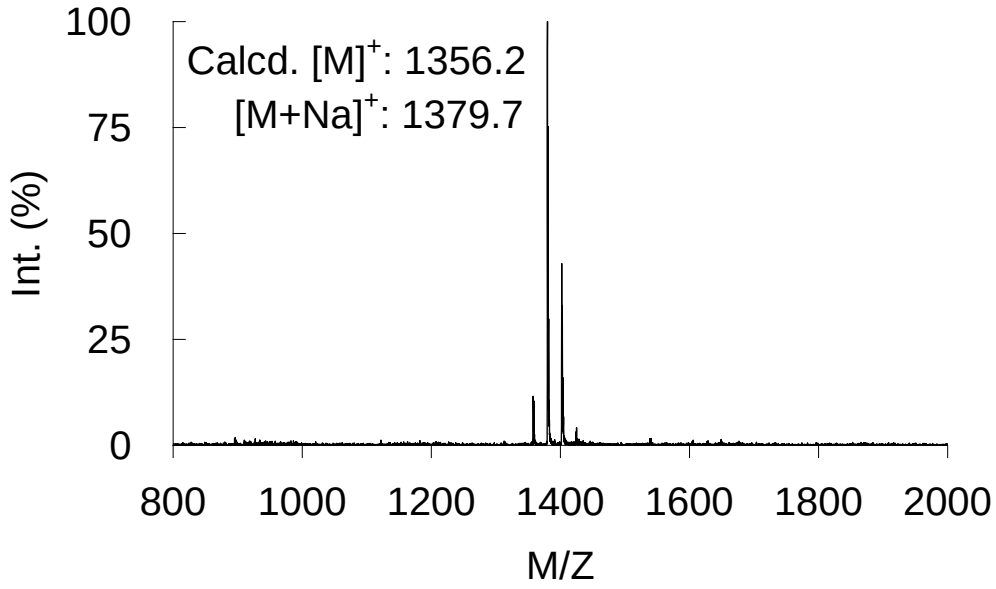


Figure 1 MALDI-TOF mass spectrum of G2 dendron, 3.

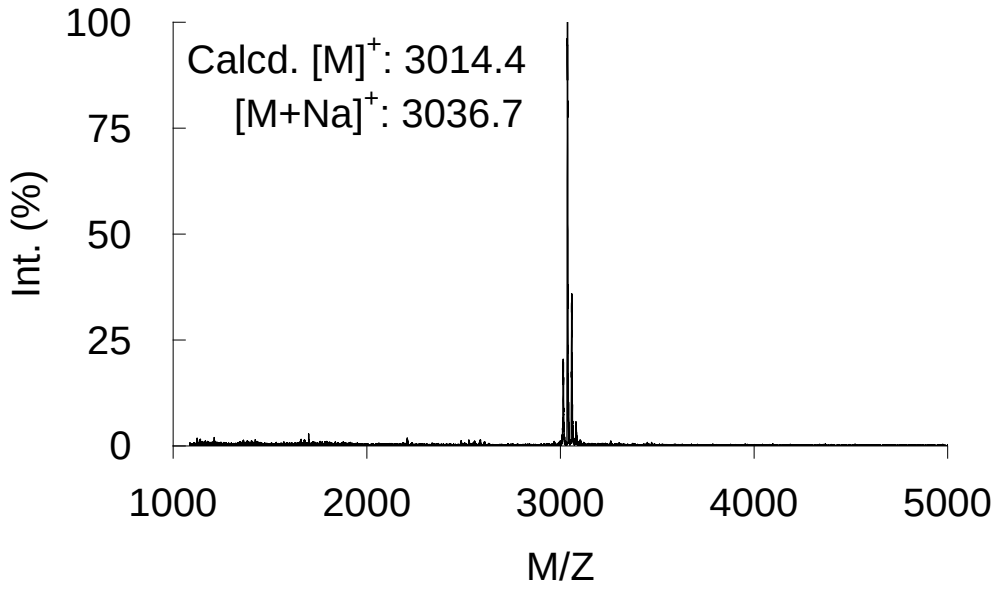


Figure 2 MALDI-TOF mass spectrum of G3 dendron, 4.

The MALDI-TOF mass spectra of **3** and **4** showed the expected peaks at 1379.7 and 3036.7 ($[M+Na]^+$), respectively (Fig. 1 and 2). These results clearly indicated the formation of the desired dendrons.

Next, **4** was activated with thionyl chloride, followed by condensing with 3,5-bis(3.5-diaminobenzoylamino)benzoic acid, **5**, to synthesize a G3 dendrimer (**6**) based on the reaction sequence, 1) and 2) (Scheme 2). Compared to the synthesis of **4**, a longer drying time and a larger amount of thionyl chloride were required in this reaction to go to completion. The resulting product contains only the target **6** and **4** used in slight excess. The G3 dendrimer, **6**, was easily isolated from the mixture by reprecipitation in

71% yield. Fig. 3 shows the MALDI-TOF mass spectrum of **6** (calc. mass 12398.4). Only a single signal was observed at M/Z([M+Na]$^+$) = 12420.5, indicating the successful preparation of the G3 dendrimer.

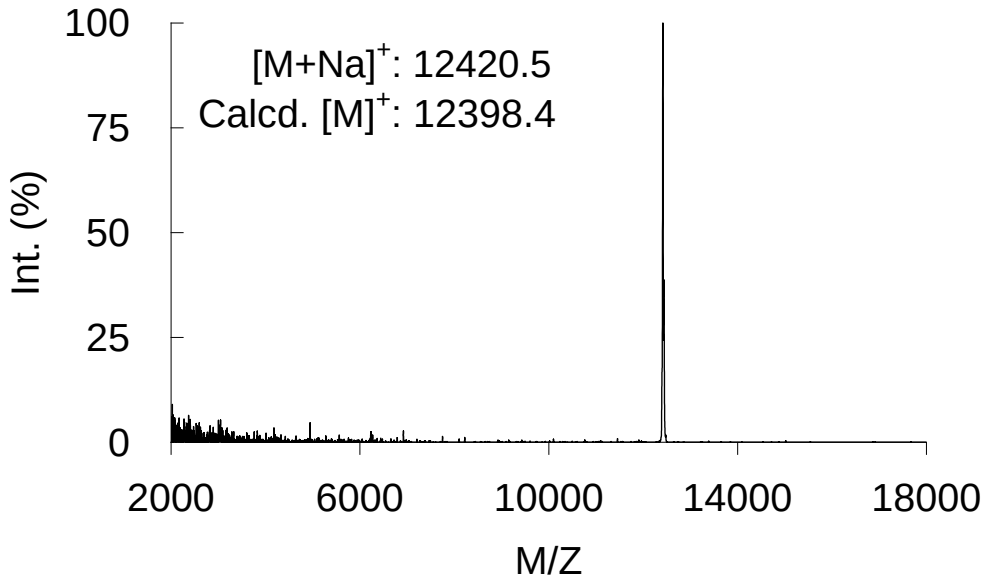
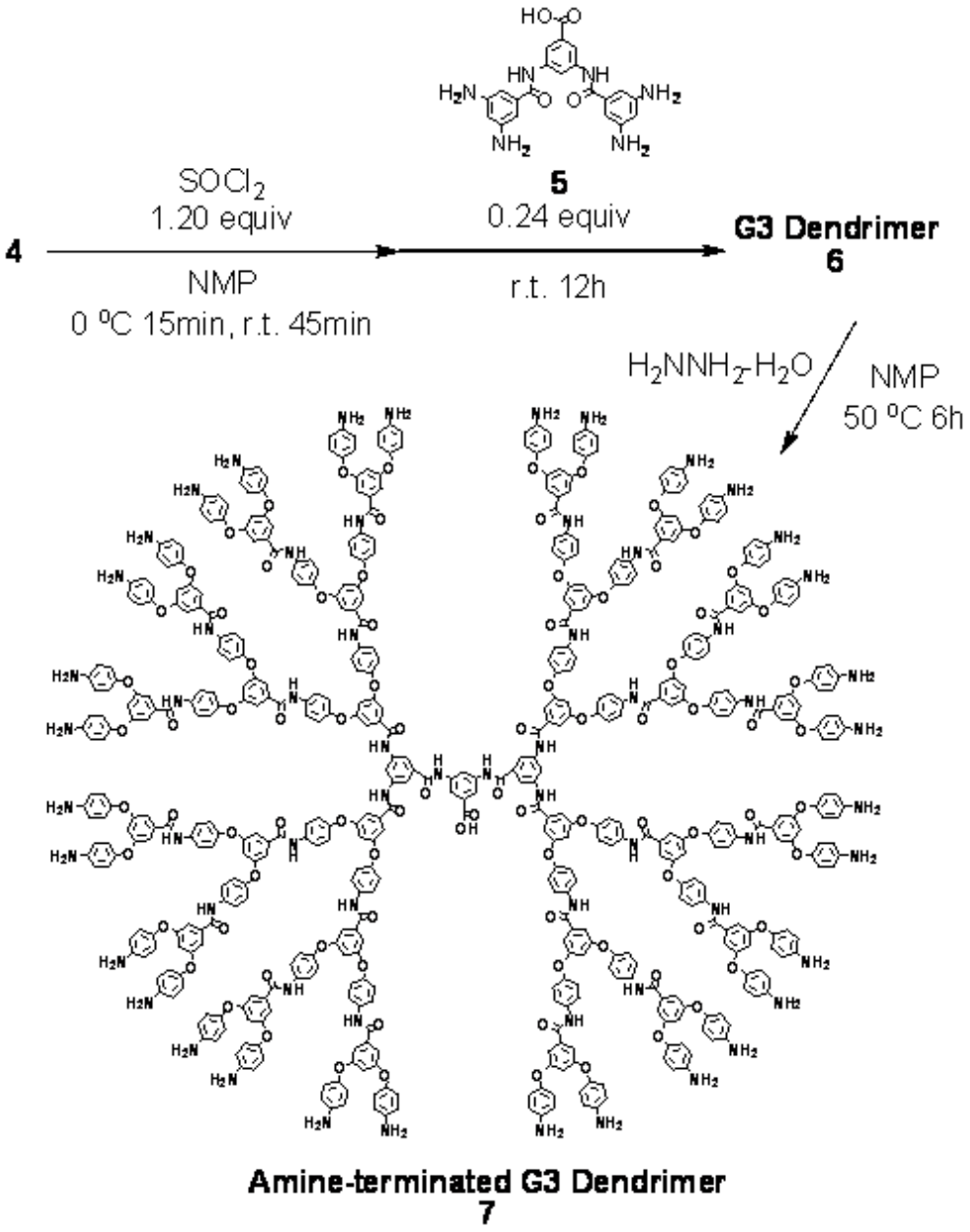


Figure 3 MALDI-TOF mass spectrum of G3 dendrimer, 6.

A functional G3 dendrimer with 32 amine groups at its periphery (**7**) could be easily obtained by the hydrolysis of **6** with excess hydrazine in NMP at 50 °C for 6 h (Scheme 2). As shown in Fig. 4, the ^{1}H NMR spectroscopy confirmed the complete hydrolysis by the absence of the characteristic signal at 11.19 ppm for the trifluoroacetamide protons (-N*H*COCF$_3$) and the appearance of a new signal at 5.03 ppm corresponded to amine protons (-N*H*$_2$) after the hydrolysis. Moreover, FT-IR spectra showed that the absorptions for the C=O

stretching at 1720 cm^{-1} and the C-F stretching at 1157 cm^{-1} of the trifluoroacetamide groups completely disappeared after the hydrolysis. It should be noted that the choice of trifluoroacetamide moieties is quite important and effective for the synthesis of **7**, perfectly filling the requirements



Scheme 2 Synthesis of G3 Dendrimer, 6, and Amine-Terminated Dendrimer, 7

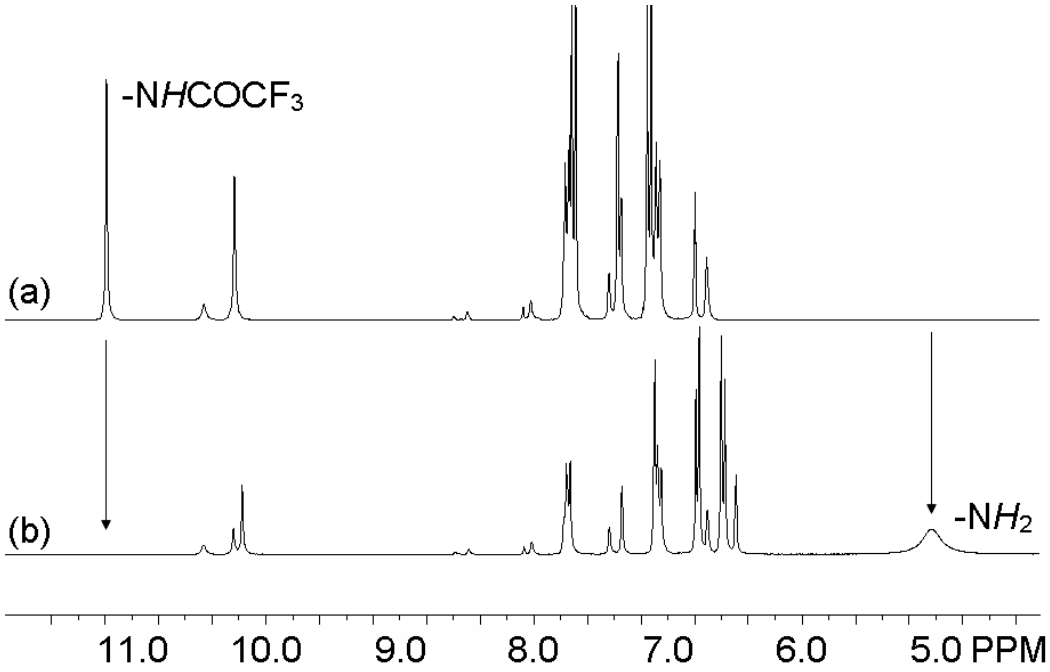
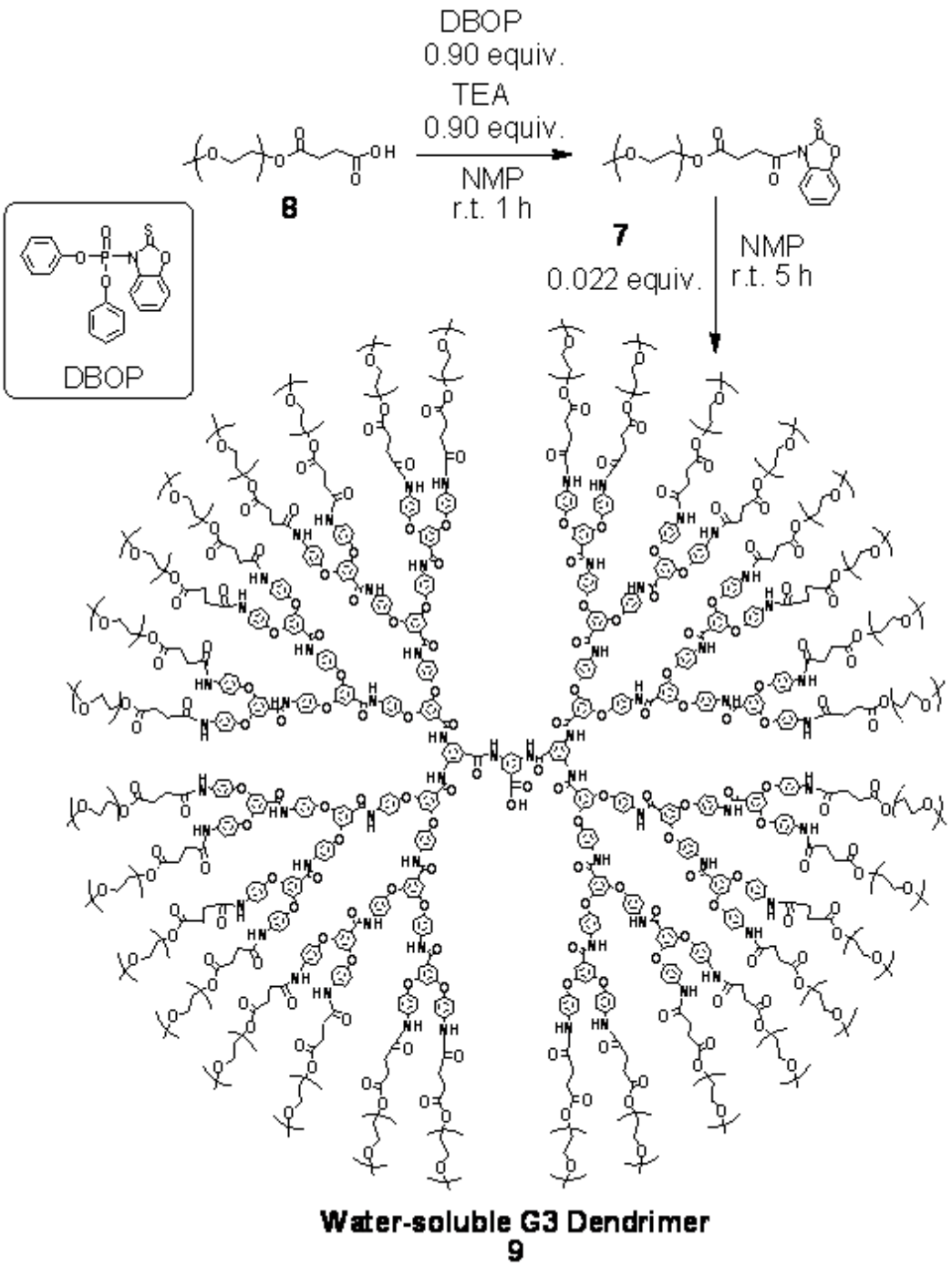


Figure 4 1H NMR spectra of (a) G3 dendrimer, 6 **and (b) amine-terminated G3 dendrimer,** 7

as follows; (1) high stability under acidic conditions, resulted from the condensation reaction of acid chlorides and amines and (2) selective hydrolysis while maintaining the amide bonds of the main chains.

The amine-terminated dendrimer, **7**, was then modified with carboxyl-terminated oligo(ethylene glycol) (**8**) to synthesize a new water-soluble G3 dendrimer (**9**) (Scheme 3). First, we attempted the activation of **8** with thionyl chloride, however it cleaved ester linkages in the structure of **8** unfortunately. Therefore, a highly efficient condensing agent, diphenyl 2,3-dihydro-2-thioxo-3-benzoxazolyl phosphonate (DBOP), was employed on behalf of thionyl chloride[25]. The activation of **8** with DBOP was

conducted in NMP at room temperature for 1 h in the presence of triethylamine without cleavage of the ester linkages. Subsequently, the activated **8** was reacted with **7** in such a way that the activated **8** is 1.28-fold excess toward the amine groups of **7**. After the reaction, the product was quenched with water, and then extracted with chloroform. The expected water-soluble G3 dendrimer, **9**, free of oligo(ethylene glycol) used in excess was obtained after the dialysis in water for 3 days, the isolation yield was 30 %. GPC curves shows that **8** used excessively was removed (Fig. 5). The ^{1}H NMR spectrum of **9** confirmed that the characteristic resonance at 5.03 ppm for the amine protons (-NH_2) was absent and the one for the amide protons (-$NHCOCH_2$-) at 9.92 ppm was newly observed after the modification with oligo(ethylene glycol). In addition, the signal at 6.78 and 6.59 ppm for the aromatic protons of the aminophenyl groups (-*Ar*NH_2) absolutely shifted to 7.58 and 7.00 ppm assignable to the aromatic protons of amidephenyl groups (-*Ar*-$NHCOCH_2$-). This result clearly indicates the successful synthesis of **9**. The dendrimer, **9**, was soluble in water as well as various common solvents such as methanol, acetone, ethyl acetate, tetrahydrofuran, chloroform, and dichloromethane.



Scheme 3 Synthesis of Water-Soluble Dendrimer, 9

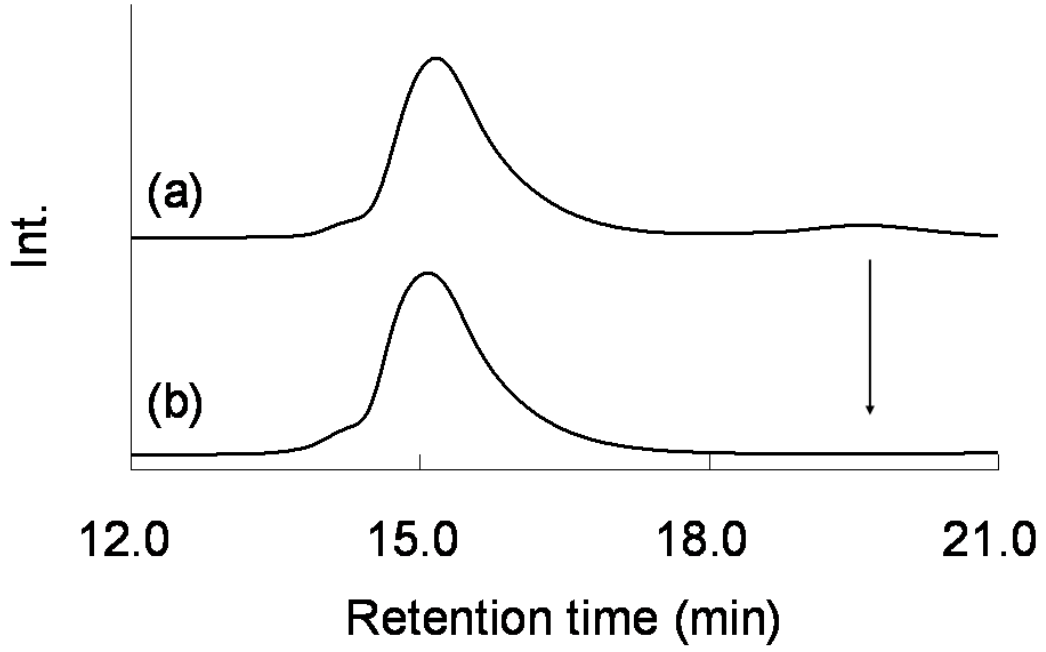


Figure 5 GPC curves of water-soluble dendrimer, 9, (a) before and (b) after dialysis.

The direct visualization of this dendrimer, **9**, has been conducted by the atom force micrograph (AFM) analysis in the tapping mode. Deposits of the dendrimer were obtained by spin casting of their acetone solutions (1 mg/mL) on a mica substrate. The dendrimers appear on topographic AFM images (Fig. 6(a)), as flattened objects in a spherical shape with a 10-20 nm diameter and a 2 nm height, considering a rough calculation[26] of the diameter (3.6-3.8 nm) of the precursory dendrimer, **7**. The aggregates of some dendrimers could also be observed in other regions (Fig. 6(b)).

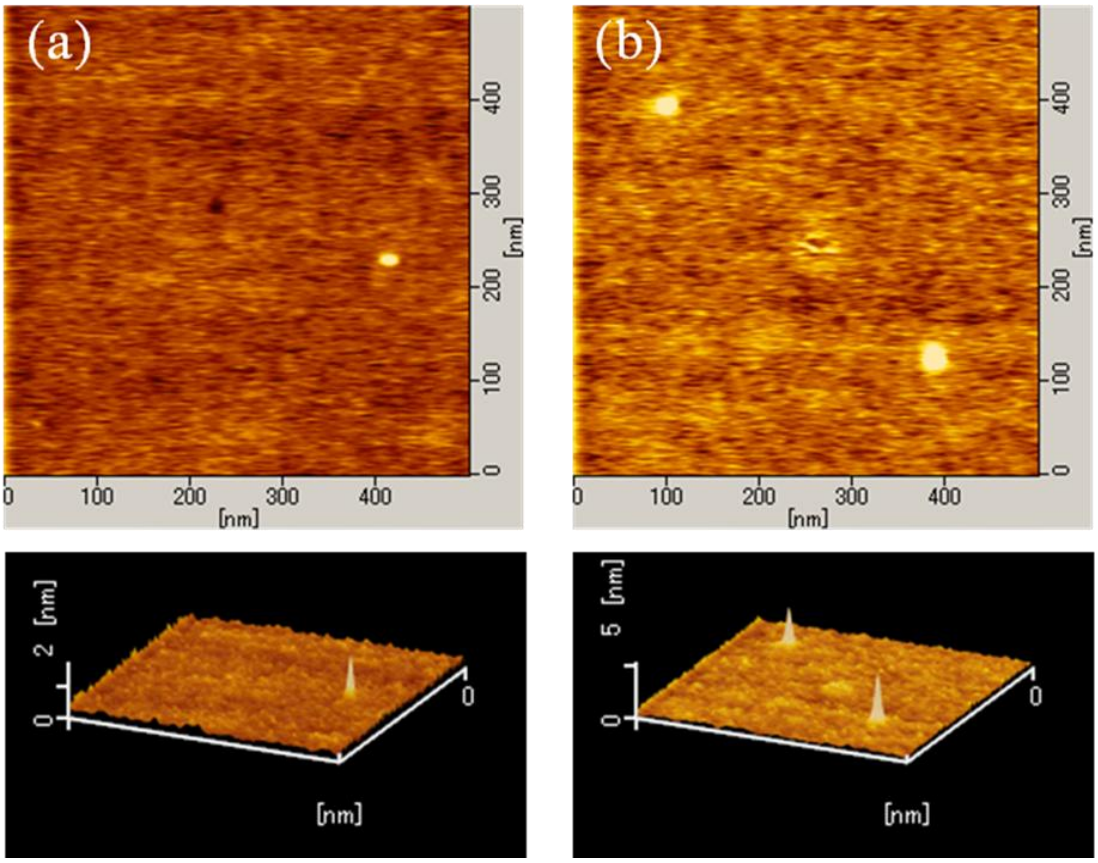


Figure 6 Topographic AFM images of water-soluble dendrimers, 9, obtained from deposits of their acetone solution on a mica substrate; (a) unimolecule and (b) aggregates.

3. Conclusions

The water-soluble aromatic polyamide G3 dendrimer with 32 oligo(ethylene glycol) chains could be successfully synthesized for the first time by modification of the amine-terminated G3 dendrimer which was easily generated by the hydrolysis of G3 dendrimer with 32 trifluoroacetamide groups at its periphery. This precursory dendrimer was prepared based on the novel, very simple, inexpensive, and highly efficient convergent approach using thionyl chloride. The NMR and MALDI-TOF mass spectroscopy supported the desired formation of G1, G2, and G3 dendrons and all G3 dendrimers. The isolated water-soluble G3 dendrimer, **9**, as well as their aggregates on the mica substrate could be directly visualized by AFM. The novel structure of this dendrimer may induce amphiphilic unimolecular micelle formations in aqueous media and should be a capable material in the wide range of biomedical applications.

4. Experimental Section

Materials

Thionyl chloride was distilled from triphenyl phosphite under nitrogen. *N*-methyl-2-pyrrolidinone (NMP) was distilled from calcium hydride under reduced pressure. Pyridine was distilled from calcium hydride under nitrogen. Tetrahydrofuran (THF) was distilled from sodium benzophenone under nitrogen. Diphenyl(2,3-dihydro-2-thioxo-3-benzoxazolyl)phosphonate (DBOP) was recrystallized from hexane. The other reagents and solvents were used as received. 3,5-Bis(4-aminophenoxy)benzoic acid (**1**) as an AB_2 building block and 3,5-bis(3,5-diaminobenzoylamino)benzoic acid (**5**) as a core were prepared by the reported procedure [26-27].

Instrumentation

Infrared spectra were recorded on a Horiba FT-720 spectrophotometer. ^{1}H NMR spectra were obtained in DMSO-d_6 on a BRUKER DPX-300 spectrometer at 300 MHz. Matrix-assisted laser desorption ionization with time of flight (MALDI-TOF) mass spectra were obtained on a Kratos Kompact MALDI instrument operated in linear detection mode to generate positive ion spectra. Dithranol as a matrix, sodium

trifluoroacetate as an additive agent, and the sample are dissolved in THF (10, 1, and 1 mg/mL, respectively). Then, they are mixed by the ratio of 5/1/1 (v/v/v) for G2 (**3**) and G3 (**4**) dendrons, and by the ratio of 1/1/5 (v/v/v) for G3 dendrimer (**6**).

Synthesis

Synthesis of G1 dendron (2)

AB_2 building block **1** (5.05 g, 15.0 mmol) was dissolved in 50 ml of THF, then 4.0 equivalent of trifluoroacetic anhydrate (8.28 ml, 60.0 mmol) toward **1** was added to the solution at 0 ^{o}C under nitrogen, and stirred for 15 min and for 2 h at room temperature. The reaction mixture was poured into pure water. The precipitate was collected by filtration and recrystallized from acetonitrile, and dried *in vacuo* at 120 ^{o}C to give a slightly pink powder (95% yield). IR (KBr): 725, 1157 (C-F), 1218 (Ar-O-Ar), 1704 (C=O), 3325 cm^{-1} (N-H, amide). ^{1}H NMR (DMSO-d_6, 40 ^{o}C): δ = 6.94 (t, 1H), 7.16 (d, 2H), 7.17 (d, 4H), 7.73 (d, 4H), 11.23 ppm (s, CF_3CONH-, 2H). Elemental analysis: Calcd.: C:52.28, H:2.67, N:5.30, and Found: C:52.29, H:2.91, N:5.13.

Synthesis of G2 dendron (3)

G1 dendron **2** (6.27 g, 11.9 mmol) was dissolved in 20 ml of NMP under nitrogen, and 1.04 equivalent of thionyl chloride (0.905 ml, 12.5 mmol) toward **2** was added to the solution at 0 ^{o}C, and stirred for 15 min and for 30 min at room temperature. Then, 0.48 equivalent of **1** (1.92 g, 5.70 mmol) toward **2** was added to the solution and the condensation was carried out for 1 h at room temperature. The reaction mixture was poured into pure water and the precipitate was collected and dried. The crude product was dissolved in acetone, and hexane (volume ratio was 1:3) was poured into this solution and stirred for 3 h. The precipitate was collected and dried *in vacuo* at 120 ^{o}C to give a brown powder (97% yield). IR (KBr): 725, 1157 (C-F), 1211 (Ar-O-Ar), 1658 (C=O, amide), 1712 (C=O, trifluoroacetamide), 3302 cm^{-1} (N-H, amide). ^{1}H NMR (DMSO-d_6, 40 ^{o}C): δ = 6.83 (t, 2H), 6.90 (t, 1H), 7.10 (t, 2H), 7.11 (d, 4H), 7.17 (d, 8H), 7.38 (d, 4H), 7.72 (d, 8H), 7.78 (d, 4H), 10.28 (s, -CON*H*-, 2H), 11.22 (s, CF_3CON*H*-, 4H). Elemental analysis: Calcd.: C:57.53, H:2.97, N:6.19, Found: C:57.16, H:3.35, N:6.13. MALDI-TOF MS: Calcd.: $[M]^+$ = 1356.2, Found: $[M+Na]^+$ = 1379.7.

Synthesis of G3 dendron (4)

G2 dendron **3** (5.86 g, 4.32 mmol) was dissolved in 30 ml of NMP under nitrogen after dried *in vacuo* for 5 h at 150 ^{o}C in a reaction pot. 1.10 equivalent of thionyl chloride

(0.345 ml, 4.75 mmol) toward **3** was added to the solution at 0 ^{o}C, and stirred for 15 min and for 3 h at room temperature. Then, 0.48 equivalent of **1** (0.683 g, 2.03 mmol) toward **3** was added to the solution and the condensation was carried out for 5 h at room temperature. The reaction mixture was poured into pure water and the precipitate was collected and dried. The crude product was dissolved in acetone, and hexane (volume ratio was 1:2) was poured into this solution and stirred for 3 h. The precipitate was collected and dried *in vacuo* at 120 ^{o}C to give a brown powder (71% yield). IR (KBr): 725, 1157 (C-F), 1211 (Ar-O-Ar), 1658 (C=O, amide), 1712 cm^{-1} (C=O, trifluoroacetamide), 3302 cm^{-1} (N-H, amide). ^{1}H NMR (DMSO-d_6, 40 ^{o}C): δ = 6.78 (t, 2H), 6.82 (t, 4H), 6.91 (t, 1H), 7.06 - 7.20 (m, 30H), 7.33, (d, 4H), 7.38 (d, 8H), 7.67 - 7.80 (m, 28H), 10.26 (s, -CON*H*-, 6H), 11.22 (s, CF_3CON*H*-, 8H). Elemental analysis: Calcd.: C:59.37, H:3.08, N:6.51, Found: C:59.07, H:3.42, N:6.41. MALDI-TOF MS: Calcd.: $[M]^+$ = 3014.4, Found: $[M+Na]^+$ = 3036.7.

Synthesis of G3 dendrimer (6)

G3 dendron **4** (2.94 g, 0.975 mmol) was dissolved in 9.7 ml of NMP under nitrogen after dried *in vacuo* for 6 h at 150 ^{o}C in a reaction pot. 1.20 equivalent of thionyl

chloride (0.085 ml, 1.17 mmol) toward **4** was added to the solution at 0 ^{o}C, and stirred for 15 min and for 45 min at room temperature. Then, 0.24 equivalent of **5** (0.0984 g, 0.234 mmol) toward **4** was added to the solution and the condensation was carried out for 12 h at room temperature. The reaction mixture was poured into pure water and the precipitate was collected and dried. The crude product was dissolved in ethanol. To this solution was added hexane, and the mixture was stirred for 6 h at room temperature. The precipitate was separated by centrifugation, and the solution was evaporated to give a brown powder (71% yield). IR (KBr): 725, 1157 (C-F), 1211 (Ar-O-Ar), 1658 (C=O, amide), 1720 (C=O, trifluoroacetamide), 3294 cm^{-1} (N-H, amide). ^{1}H NMR (DMSO-d_6, 40 ^{o}C): δ = 6.71 (t, 12H), 6.80 (t, 16H), 7.04 - 7.18 (m, 112H), 7.35, (d, 16H), 7.38 (d, 32H), 7.44 (d, 8H), 7.67 - 7.79 (m, 112H), 8.02 (s, 4H), 8.08 (s, 2H), 8.50 (s, 2H), 8.60 (s, 2H), 10.23 (s, -CON*H*-, 24H), 10.46 (s, -CON*H*-, 6H), 11.19 (s, CF_3CON*H*-, 32H). Elemental analysis: Calcd.: C:59.82, H:3.77, N:7.15, Found: C:59.73, H:3.09, N:7.00. MALDI-TOF MS: Calcd.: $[M]^+$ = 12398.4, Found: $[M+Na]^+$ = 12420.5

Synthesis of amine-terminated G3 dendrimer (7)

G3 dendrimer **6** (0.800 g, 0.0645 mmol) was dissolved in 2.0 ml of NMP under nitrogen. Excess amount of hydrazine monohydrate (more than 0.2 g) was added to the solution and stirred for 6 h at 50 ^{o}C. The reaction mixture was poured into brine and neutralized by diluted hydrochloric acid. Then the precipitate was collected and dried *in vacuo* at 80 ^{o}C to give a brown powder (93% yield). IR (KBr): 1211 (Ar-O-Ar), 1504, 1589 (C=C, aryl), 1658 (C=O, amide), 3355, 3417 cm^{-1} (N-H). ^{1}H NMR (DMSO-d_6, 40 ^{o}C): δ = 5.03 (br, -NH_2, 64H), 6.49 (t, 16H), 6.59 (d, 64H), 6.90 (t, 12H), 6.78 (d, 64H), 7.02 - 7.13 (m, 80H), 7.34 (d, 16H), 7.44 (d, 8H), 7.70 - 7.80 (m, 48H), 8.01 (s, 4H), 8.07 (s, 2H), 8.49 (s, 2H), 8.59 (s, 1H), 10,18 (s, -CON*H*-, 16H), 10.24 (s, -CON*H*-, 8H), 10.46 (s, -CON*H*-, 6H).

Synthesis of monocarboxyl-terminated oligo(ethylene glycol) (8)

Oligo(ethylene glycol) (M_n = 550 g/mol, 2.5 g) was dissolved in 25 ml of pyridine under nitrogen after dried *in vacuo* for 2 h at 60 ^{o}C in a reaction pot. After cooling at room temperature, excess succinic anhydride (1.00 g, 9.99 mmol) toward oligo(ethylene glycol) was added to the solution, and stirred for 10 h at 50 ^{o}C. Then, 1 ml of water was added in the reaction solution for quenching unreacted succinic anhydride, and poured into diluted hydrochloric acid. The product was extracted by dichloromethylene and

dried by $MgSO_4$. Then, the solution was evaporated to give a viscous liquid. IR (KBr): 1103 (CH_2-O-CH_2), 1727, (C=O, ester), 2884 cm^{-1} (C-H, -OCH_3). ^1H NMR (DMSO-d_6, 40 ^oC): δ = 2.51 (m, -COCH_2CH_2COOH, 4H), 3.26 (s, -OCH_3, 3H), 3.42 - 3.64 (m, -OCH_2CH_2O-), 4.14 (t, -COOCH_2CH_2O-, 2H)

Synthesis of water-soluble G3 dendrimer (9)

Carboxyl-terminated oligo(ethylene glycol) **8** (0.650 g, 1.00 mmol) was dissolved in 1 ml of NMP under nitrogen after dried *in vacuo* for 3 h at 70 °C in a reaction pot. After cooling at room temperature, 0.90 equivalent of DBOP (0.345 g, 0.900 mmol) and triethylamine (0.125 ml, 0.900 mmol) were added to the solution, and stirred for 1 h at room temperature. Then, 0.022 equivalent of **7** (0.204 g, 0.0219 mmol) was added to the solution and stirred for 6 h at room temperature. Water was added the solution and the product was extracted by chloroform. The crude compound was purified by dialysis with water for 3 days and the solution was extracted with chloroform, and the organic phase was concentrated *in vacuo* to give a slightly colored highly viscous liquid (30% yield). IR (KBr): 1103 (CH_2-O-CH_2), 1211 (Ar-O-Ar), 1674 (C=O, amide), 1735 (C=O, ester), 2877 (C-H, -OCH_3), 3425 cm^{-1} (amide). ^1H NMR (DMSO-d_6, 40 °C): δ = 2.59 (s,

-COC*H*$_2$C*H*$_2$CO-, 128H), 3.25 (s, -OC*H*$_3$, 96H), 3.37 - 3.66 (m, -OC*H*$_2$C*H*$_2$O-), 4.11 (t, -COOC*H*$_2$CH$_2$O-, 64H), 6.62 - 8.80 (derive from backbone of the dendrimer), 9.92 (s, -N*H*CO-PEG, 32H), 10.19 (s, -CON*H*-, 24H), 10.45 (s, -CON*H*-, 6H)

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26. Calculated by molecular mechanics (MM2) energy minimization.

Chapter IV

Synthesis of Aliphatic Polyamide Dendrimers via Facile Convergent Method

Abstract

A novel and rapid approach for the synthesis of an aliphatic polyamide dendrimer consisting of 3,4-dialkoxyhydrocinnamamide structure as a repeating unit, has been developed. Aliphatic polyamide dendrons and dendrimers were easily prepared by a convergent approach involving activation of a carboxylic acid at the focal point using (2,3-dihydro-2-thioxo-3-benzoxazolyl)phosphonate (DBOP) as an activating agent, followed by condensation with an unprotected AB_2 building block. A third generation dendrimer with a molecular weight of 5653 Da was prepared from the third generation dendron and *p*-xylylenediamine as the core molecule. All the above products could be purified only by precipitation, and their structures were confirmed by using ^{1}H-NMR, IR and Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass (MALDI-TOF-Ms) Spectroscopy and elemental analysis.

Chapter IV

1. Introduction

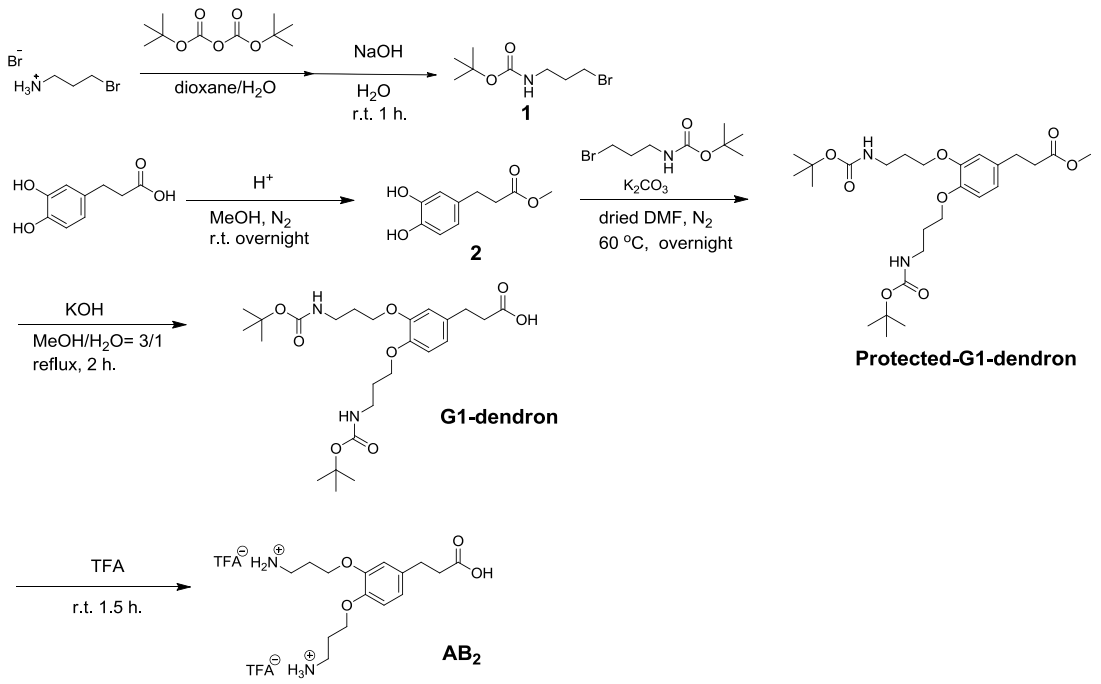
Dendrimers are characterized by their hierarchical three-dimensional structures consisting of a multifunctional core from which successive branched repeating units radiate outward[1-4]. The large definite number of functionalities can be easily introduced at the periphery of dendrimers by exploiting the reactivity of multiple terminal groups. Those structural specificities of dendrimers have encouraged in pursuit of new polymeric materials for applications in areas such as catalysts[5], liquid crystals[6], photonic devices[7], and drug delivery systems.[8-10] Although dendrimers have received much attention as new materials, their widespread use was interfered because the synthesis of dendrimers requires a tedious multistep procedure with repetitive protection-deprotection and purification processes. To solve this problem, we have focused on the development of facile synthetic approaches of dendrimers, and reported the rapid syntheses of aromatic polyamide dendrimers via both divergent and convergent methods[11-15], where the total number of reactions decreased to half of that required in the conventional approaches. Whereas a few aromatic polyamide dendrimers have been reported as functional materials, there are many reports on the development of aliphatic polyamide dendrimers[16, 17] as the functional materials such as polyamide amine (PAMAM) dendrimers, lysine dendrimers and airbol. These aliphatic polyamide dendrimers are getting attention especially as medical[18-21], biological[22, 23], catalytic[24],

and encapsulation materials[25, 26]. Those aliphatic polyamide dendrimers were preprared by repetitive conversion of the terminal groups or protection-deprotection, and purification processes, and a few papers were reported about the facile synthetic method of aliphatic polyamide dendrimers. Haridas et al. reported about the time-efficient synthesis of peptide dendrimers using 1, 3-dipolar cycloaddition (Click) reaction [27]. Al-Hamra et al. reported about the peptide dendrimers with a petaaminecobalt(III) complex at the core as a facile synthetic method [28]. However, those method still have following problems (i) the dendrimers synthesized by the former method contains triazole unit and that could change the property of the dendrimers (ii) though the latter synthetic method succeed to facilitate the purification steps, the number of reaction steps was not reduced and the protection-deprotection steps are still required. From this view point, it is valuable to develop of the facile synthetic method of aliphatic polyamide dendrimers. In this chapter, we report a facile synthesis of an aliphatic polyamide dendrimer having 3,4-dialkoxyhydrocinnamamide structure as a repeating unit via the convergent method, by extending the facile synthetic method of aromatic polyamide dendrimers.

2. Results and Discussion

2-1. Synthesis of G1 dendron and AB_2 building block

Scheme 1 shows the synthetic route for the first generation dendron (**G1 dendron**) and AB_2 building block (**AB_2**). 3-Bromo-propyl-1-NHBoc (**1**) was prepared according to a previous paper.[29] Methyl 3,4-dihydroxyhydrocinnamate (**2**) was prepared by the Fischer esterification of 3,4-dihydroxyhydrocinnamic acid with methanol.[30] **2** was reacted with 3-bromo-propyl-1-NHBoc in the presence of K_2CO_3 and molecular sieves to yield a protected G1 dendron (**Protected-G1-dendron**), which was converted into the G1dendron (**G1-dendron**) by the hydrolysis of the methyl ester group of **Protected-G1-dendron**. **Protected-G1-dendron** and **G1-dendron** were characterized by ^{1}H-NMR and IR spectorcopy and elemental analysis. The ^{1}H-NMR spectrum of the **G1-dendron** is shown in Figure 1. All signals are well assigned to the corresponding structure. The *N-tert*-Boc group of **G1-dendron** was deprotected using trifluoro acetic acid (TFA) to afford the AB_2 building block (**AB_2**). **AB_2** was also characterized by ^{1}H-NMR and IR spectroscopy and elemental analysis.



Scheme 1 Synthesis of first generation dendron (G1-dendron) and AB_2 building block (AB_2)

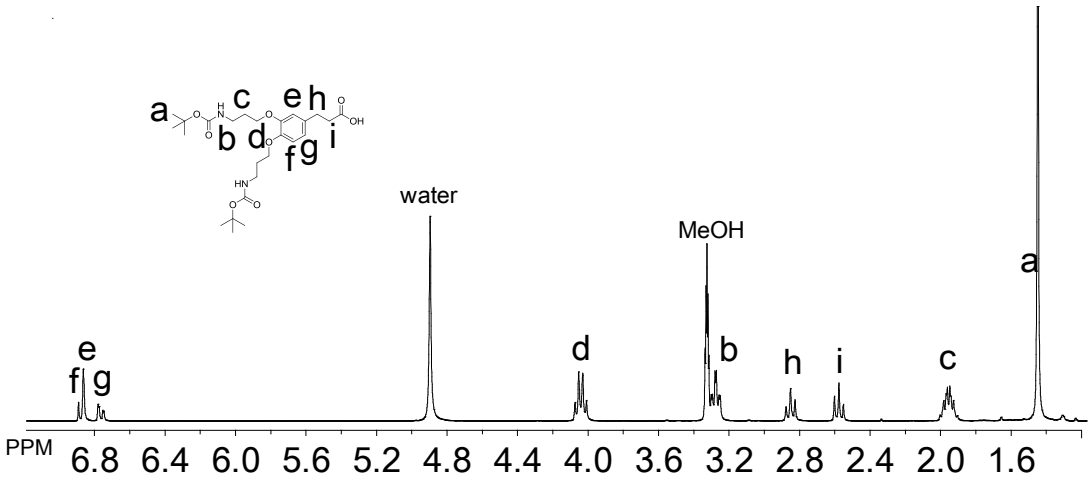
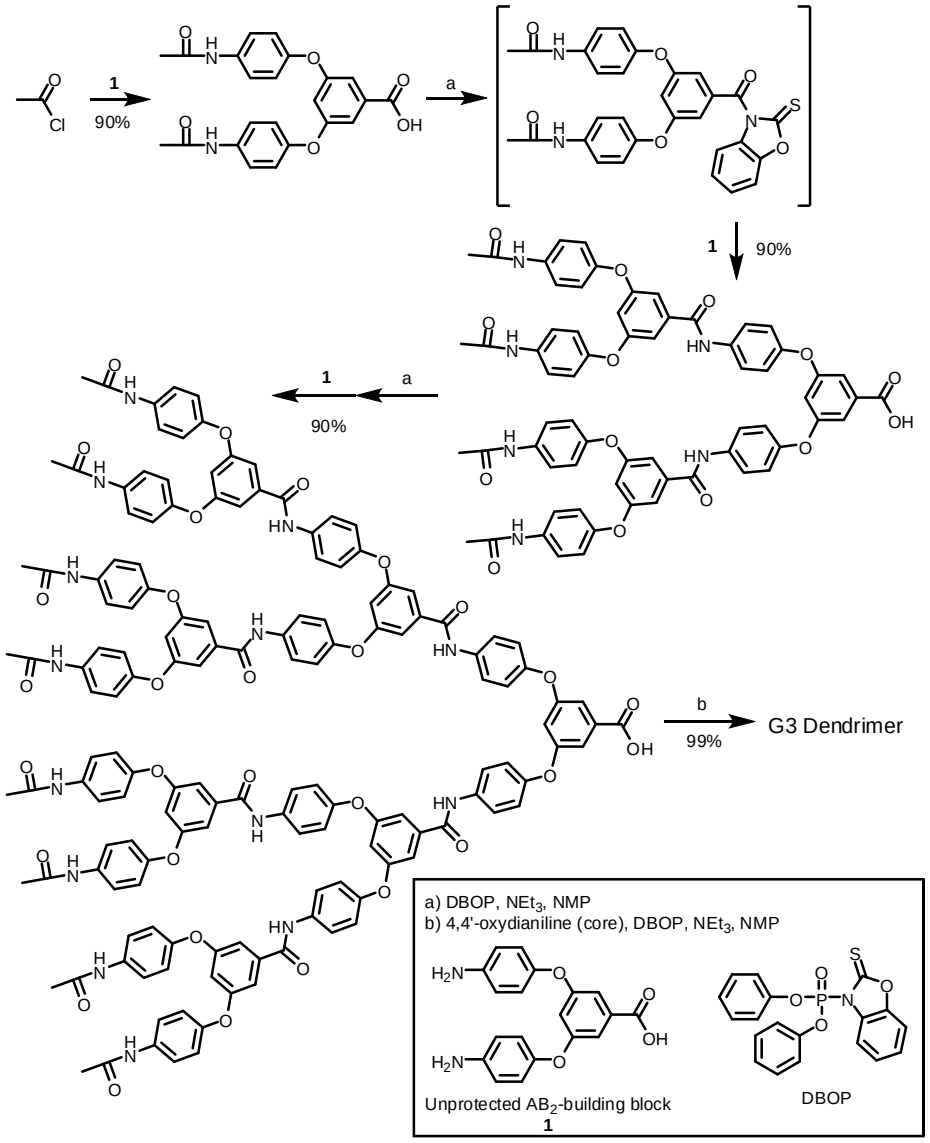


Figure 1 ^{1}H-NMR spectrum of G1 dendron (G1)

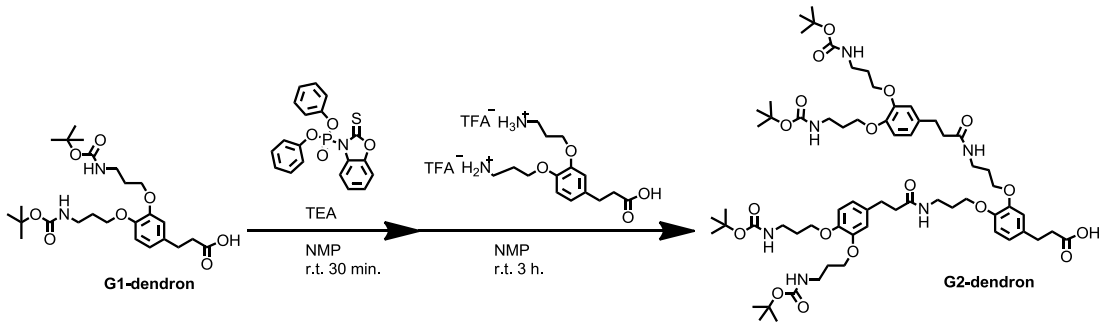
2-2. Synthesis of dendrons

As described in the introduction, we previously developed the facile synthetic method of aromatic polyamide dendrimers using diphenyl (2,3-dihydro-

2-thioxo-3-benzoxazolyl)phosphonate (DBOP) as the condensing agent (Scheme 2). In this method, coupling reactions for the synthesis of dendrons were conducted by a two step method consisting of (1) activation of a carboxylic acid by DBOP, i.e., generation of an active amide and (2) condensation of this active amide with an amino group by the addition of AB_2 building block.



Scheme 2 Facile Synthesis of aromatic polyamide dendrimers using DBOP



Scheme 3 Synthesis of G2-dendron

Then, we prepared the second generation dendron (**G2-dendron**) following the procedure described above (Scheme 3). The **G1-dendron** was activated with 0.98 equiv. of DBOP in the presence of TEA in NMP at room temperature for 30 min, and then reacted with $\mathbf{AB_2}$ in the presence of TEA in NMP at room temperature. for 3 h.

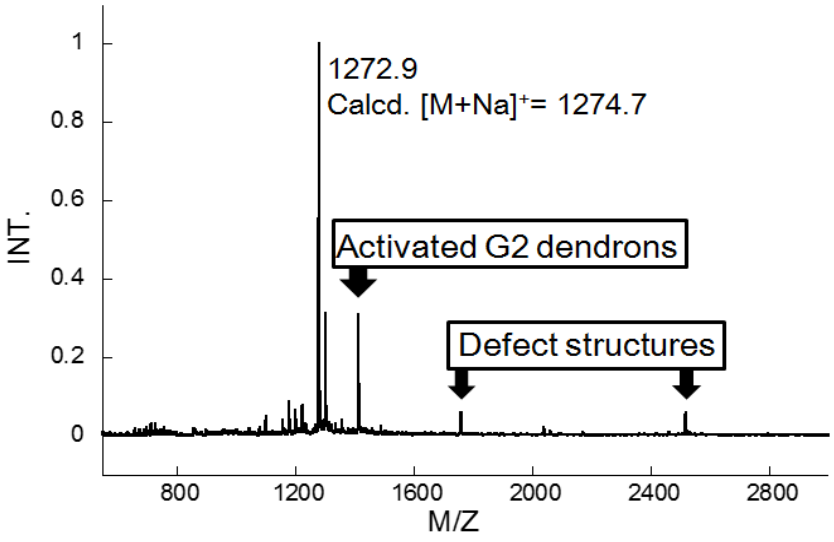
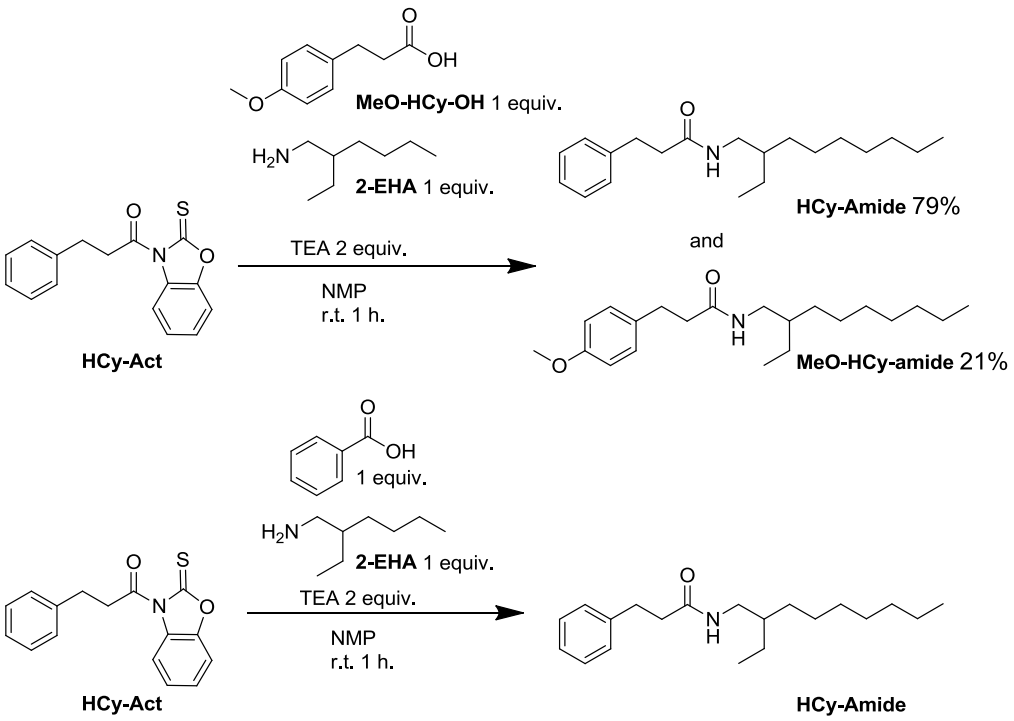


Figure 2 MALDI-TOF MS spectrum of G2 dendron

However, the MALDI-TOF MS spectrum of the isolated products showed strong signals corresponding to not only the **G2-dendron** but also higher molecular weight

products which were not observed in the synthesis of aromatic polyamide dendrimers (Figure 2).

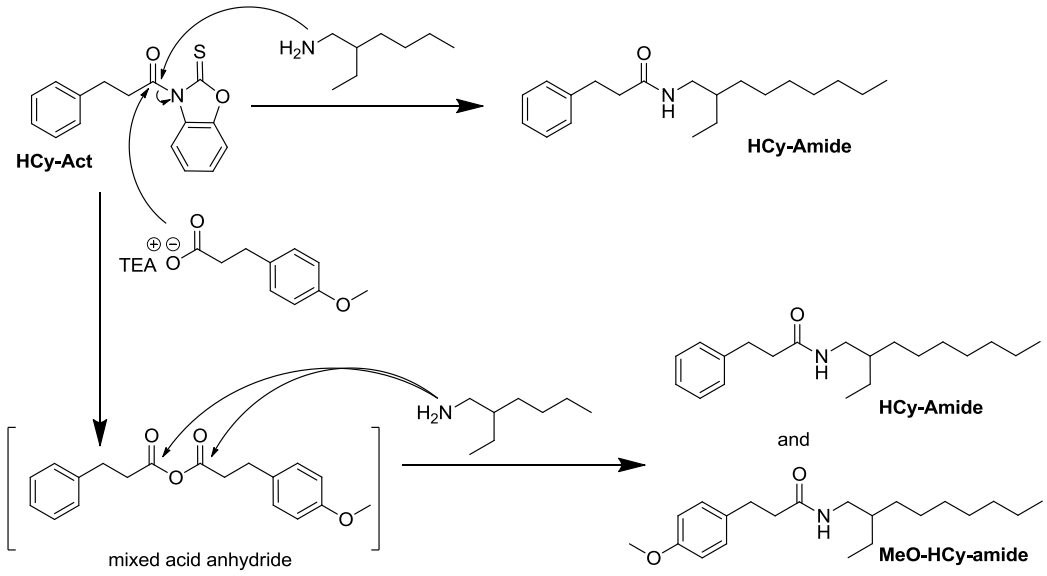
The presumable reasons for the presence of higher molecular weight products are (1) the presence of remained DBOP in the condensation step and (2) an undesired side reaction of the active amide with the aliphatic carboxy group of $\mathbf{AB_2}$. Thus, the molar ratio of DBOP to the substrate was decreased from 0.98 equiv. to 0.93 equiv. and the activation time was extended from 30 min. to 1.5 h. However, the signals of higher molecular weight products remained. Then, the following model reactions were carried out to confirm the reactivity of aliphatic carboxy group to the active amide (Scheme 4).



Scheme 4 Model reaction 1

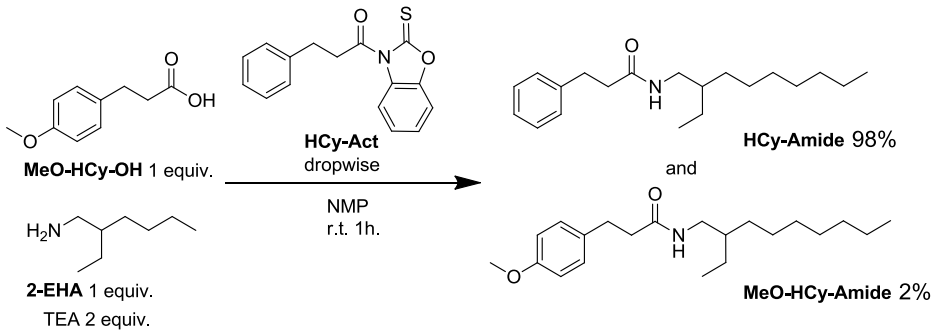
The active amide of hydrocinnamic acid (**HyC-Act**) was synthesized by the reaction of hycrocinnamic acid (**HyC-OH**) with DBOP. When a solution of 4-metoxyhydrocinnamic acid (**MeO-HyC-OH**), 2-ethylhexylamine (**2-EHA**), and TEA was added to a solution of **HyC-Act**, *N*-(2-ethylhexyl)-3-phenylpropanamide (**HyC-Amide**) (79 %) and *N*-(2-ethylhexyl)-3-(4-metoxyphenyl)propanamide (**MeO-HyC-Amide**) (21 %) were obtained as products. On the other hand, only **HyC-Amide** was obtained as the product when benzoic acid was used instead of **MeO-HyC-OH**. This result indicates that an aliphatic carboxylate anion possesses higher nucleophilicity than an aromatic carboxylate anion. In the former reaction, **MeO-HyC-OH** anion reacted with **HyC-Act** to form the mixed acid anhydride together with the formation of **HyC-Amide** from **HyC-Act** and **2-EHA**. The mixed acid anhydride reacted with **2-EHA** to yield two compounds of **HyC-Amide** and **MeO-HyC-Amide**. (Scheme 5) Formation of the mixed acid anhydride was investigated by the reaction of **HCy-Act** with **MeO-HyC-OH** in the presence of TEA. The reaction solution was diluted with ether, and the organic layer was washed with K_2CO_3 aq and brine to remove unreacted **MeO-HyC-OH**. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give yellow oil as product. The product was characterized by ^{1}H-NMR and IR spectroscopy. ^{1}H-NMR spectrum in $CDCl_3$ showed

decrease of the signal at 8.1 ppm which corresponding to active amide and appearance of the signal at 3.8 ppm which derived from methyl ether group of **MeO-HyC-OH**. The IR spectrum of the product showed peaks at 1727 and 1812 cm^{-1} which corresponding to C=O stretching of active amide and acid anhydride, respectively. These spectral evidences clearly indicate the formation of the mixed acid anhydride.



Scheme 5 Rection of HCy-Act with 2-EHA under the presence of MeO-HCy-OH

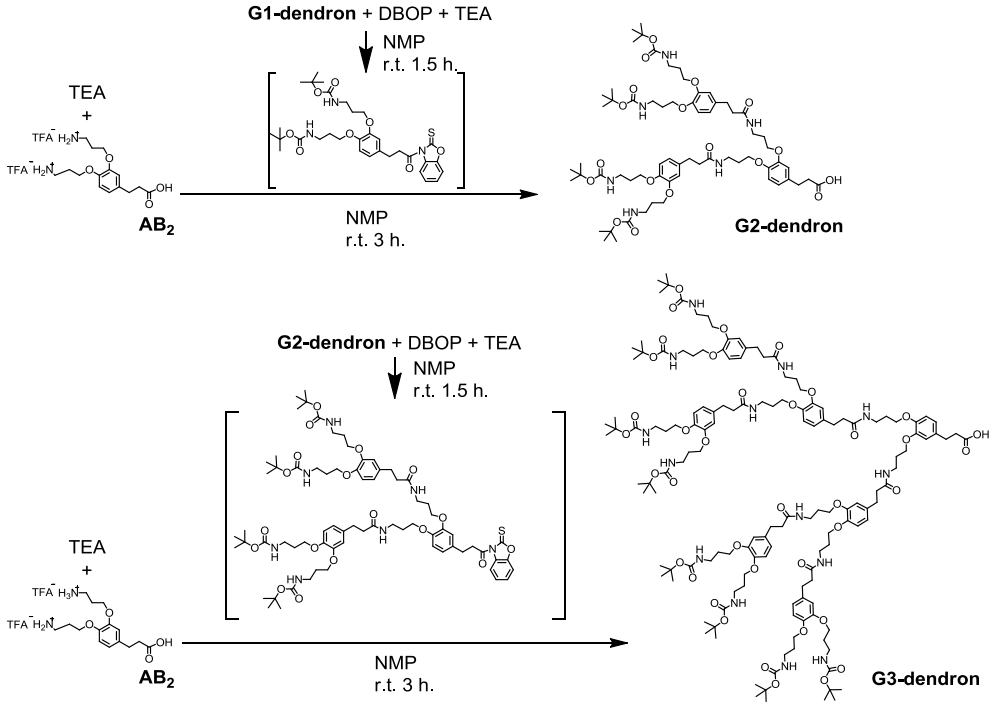
To avoid the formation of the mixed anhydride, the **HyC-Act** solution was added dropwise to a solution of **MeO-HyC-OH** and **2-EHA**, TEA in NMP. This procedure



Scheme 6 Model reaction 2

reduced the formation of **MeO-HyC-Amide** to 2 % (Scheme 6).

Based on these model reactions, the second and third generation dendrons (**G2-dendron**, **G3-dendron**) were prepared as shown in Scheme 7. The solution of activated **G1-dendron** or **G2-dendron** was added dropwise to the solution of $\mathbf{AB_2}$ and TEA in NMP. The **G2-dendron** and **G3-dendron** were purified simply by reprecipitation to remove **G1-dendron** or **G2-dendron**, and obtained in 75 and 74% yields, respectively, as white powder.



Scheme 7 Synthesis of G2-dendron and G3-dendron

The formation of the **G2-dendron** and **G3-dendron** was confirmed by ^{1}H-NMR and IR spectroscopy, elemental analysis, and MALDI-TOF MS. The IR spectrum of **G3-dendron** showed strong absorptions at 3401,3347,1697 and 1650 cm^{-1} due to the

characteristic N-H and C=O stretchings of the carbamate, amide and carboxy groups, respectively. Furthermore, the characteristic ether stretching was observed at 1172 cm^{-1}. The ^{1}H NMR spectrum of **G3-dendron** showed signals corresponding to the carbamate protons (A) at 6.13-6.25 ppm, amide protons (B and C) at 7.28-7.41 ppm, aromatic protons (e, f, g, m, n, o, u, v, w) at 6.61-6.79 ppm, and *tert*-butyl protons of the end unit (a) at 1.39 ppm, respectively (Figure 3).

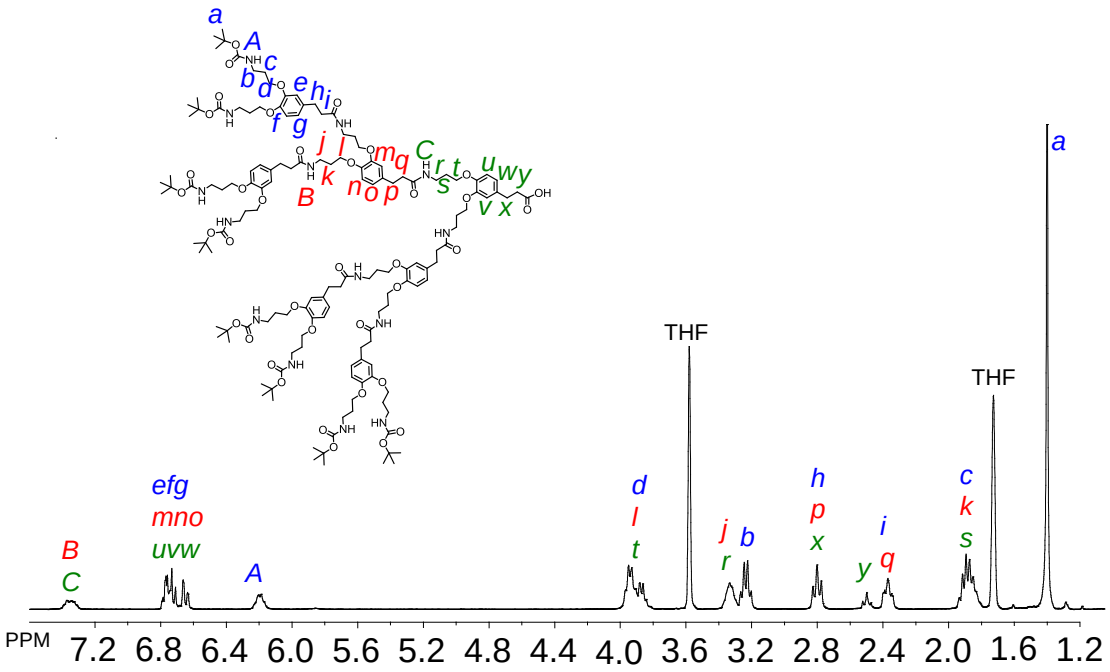


Figure 3 ^{1}H NMR spectrum of G3-dendron

Furthermore, MALDI-TOF MS spectra of the **G2-dendron** and **G3-dendron** showed peaks observed at M/Z ($[M+Na]^{+}$) = 1272.0 and 2787.6 and well agreed with the calculated mass (1274.7 and 2787.5), together with minor peaks which could be

assignable to partially de-protected dendrons (Figure 4 and Figure 5). The de-protection probably occurred during the measurement. Moreover, no signals derived from dendrons having lower or higher molecular weights were observed in Figure 4 and Figure 5.

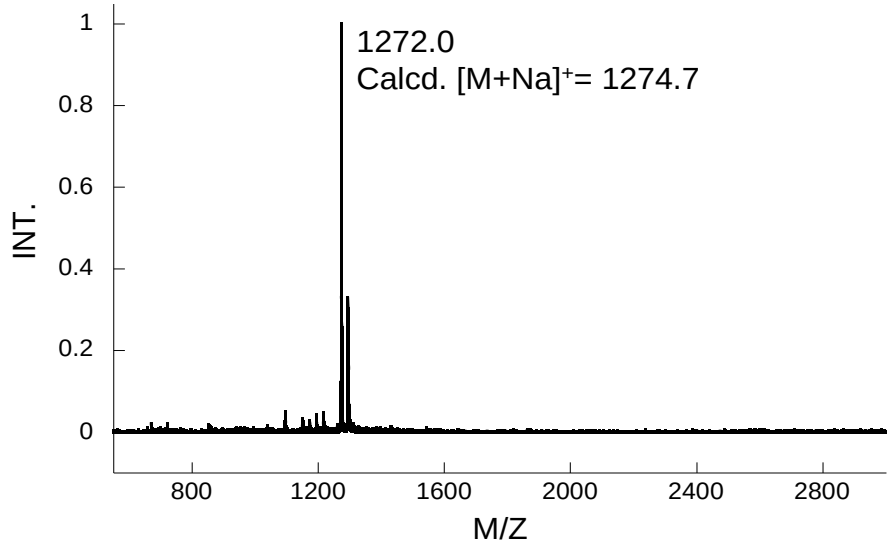


Figure 4 MALDI-TOF MS spectrum of G2-dendron

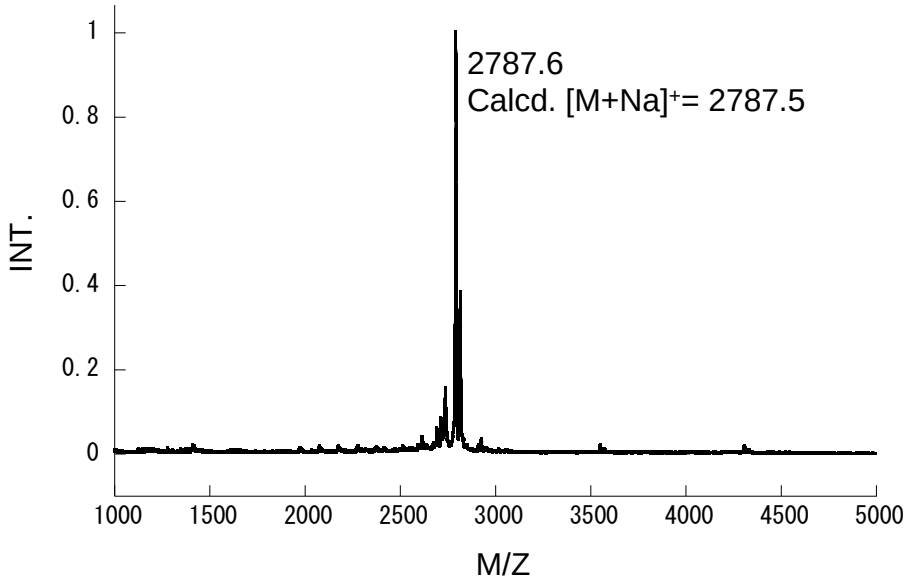
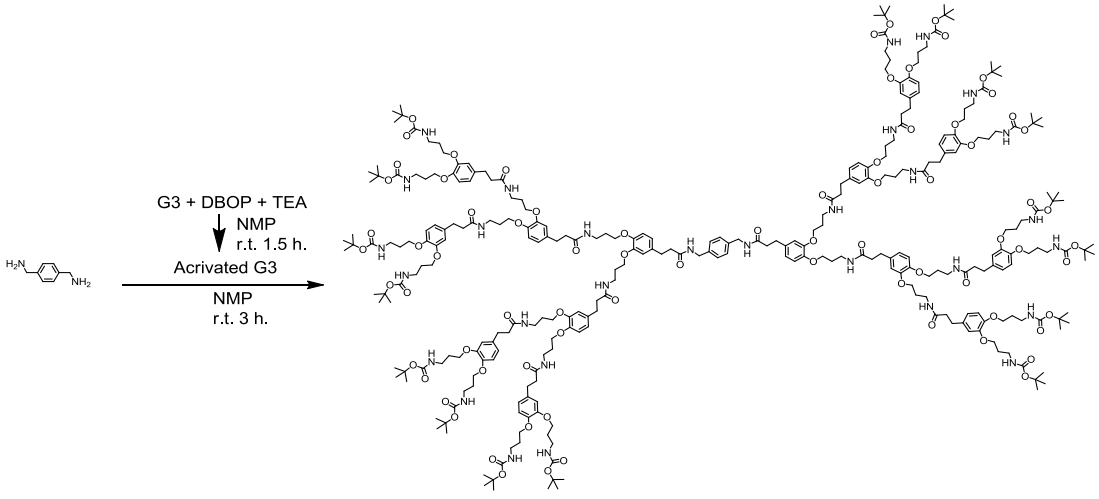


Figure 5 MALDI-TOF MS spectrum of G3-dendron

2-3. Synthesis of dendrimers

The third generation dendrimer (**G3-dendrimer**) was synthesized by a similar procedure with the dendron synthesis (Scheme 8). The activated **G3-dendron** was added dropwise to a solution of *p*-xylylenediamine in NMP. The **G3-dendromer** was purified by reprecipitation to remove the **G3-dendron** and obtained in 75% yield as white powder. The **G3-dendrimer** was characterized by ^{1}H-NMR and IR spectroscopy, elemental analysis, and MALDI-TOF MS. The IR spectrum of **G3-dendrimer** showed strong absorptions at 3347, 3309, 1697 and 1643 cm^{-1} due to the characteristic N-H and C=O stretchings of the carbamate and amide and groups, respectively. Furthermore, the characteristic ether stretching was observed at 1172 cm^{-1}.



Scheme 8 Synthesis of G3-dendrimers

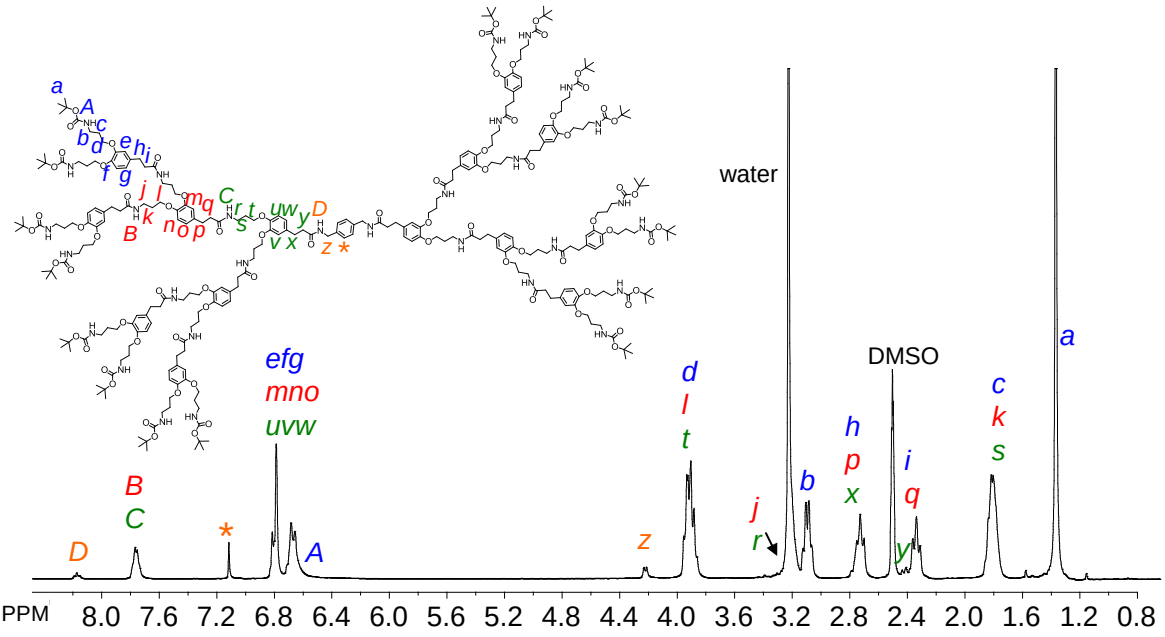


Figure 6 ^{1}H NMR spectrum of G3-dendrimer

The ^{1}H NMR spectrum of **G3-dendron** showed signals corresponding to the amide protons (B, C and D) at 7.69-7.91 and 8.17 ppm, aroatic protons of the core (*) at 7.11 ppm, carbamate and aromatic protons (A , e, f, g, m, n, o, u, v and w) at 6.50-6.89 ppm, benzyl protons of the core (z) at 4.22 ppm, and *tert*-butyl protons of the end unit (a) at 1.39 ppm, respectively. Furthermore, the MALDI-TOF MS spectrum of the **G3-dendrimer** showed the peak observed at M/Z ($[M+Na]^{+}$) = 5653.3, which well agreed with the calculated mass (5653.2), together with minor peaks which could be assignable to partially de-protected dendrimers (Figure 7). The de-protection probably occurred during the measurement. Moreover, no extra signals derived from dendrons having defect structures were observed in Figure 7.

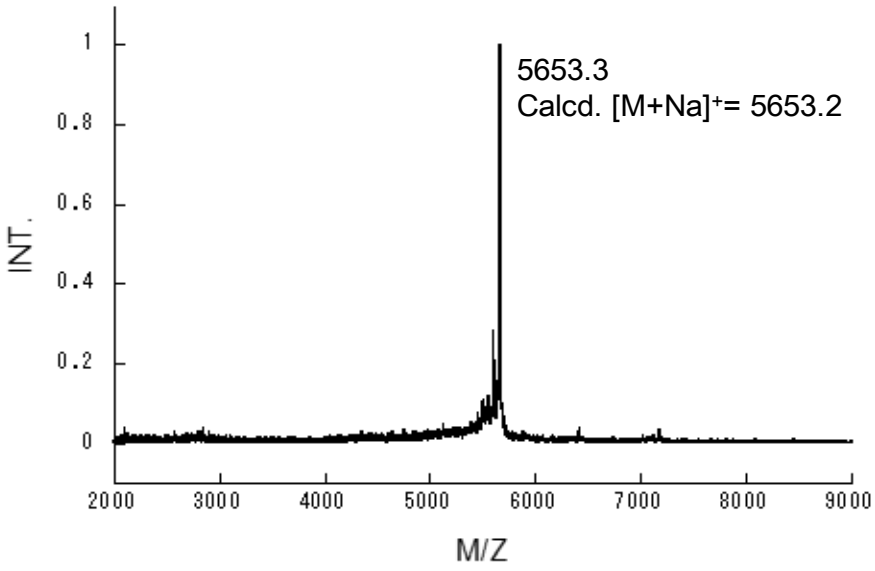


Figure 7 MALDI-TOF mass spectrum of G3-dendrimer

3. Conclusions

We have developed a facile synthetic method of aliphatic polyamide dendrons and dendrimer via convergent approach, which consists of direct condensation of carboxylic acid and unprotected AB_2 building block using DBOP as the condensing agent. In this method, each dendron and dendrimer are purified only by extraction and/or reprecipitation. The MALDI-TOF MS spectra supported the expected formation of each dendron and dendrimer. This novel convergent route of the aliphatic polyamide dendrimer is attractive for the preparation of dendrimers both for use in laboratories and industries, since this method could be expanded for the synthesis of other aliphatic dendrimers such as polyamide amine dendrimers and lysine dendrimers, which have already used as functional materials in many fields.

4. Experimental Section

Materials

N-Methyl-2-pyrrolidinone (NMP) was distilled under reduced pressure over calcium hydride, and then stored under nitrogen. Triethylamine (TEA) was distilled over calcium hydride under nitrogen, and then stored under nitrogen. Diphenyl(2,3-dihydro-2-thioxo-3-benzoxazolyl)phosphonate (DBOP) was supplied from KYOCERA Chemical Corporation and recrystallized from hexane, then stored under nitrogen in a refrigerator. Other reagents and solvents were obtained commercially and used as received unless otherwise noted.

Instrumentation

^{1}H NMR spectra were recorded in deuterated methanol (MeOH-d_4), tetrahydrofuran (THF-d_8) or dimethylsulfoxide (DMSO-d_6) on a BRUKER DPX-300 spectrometer at 300 MHz. Infrared spectra were recorded on a Horiba FT-720 spectrophotometer. Matrix-assisted laser desorption ionization with time of flight (MALDI-TOF) MS spectra were recorded on a Kratos Kompact MALDI instrument operated in linear detection mode to generate positive ion spectra using dithranol as a matrix, THF as a solvent, sodium trifluoroacetate as an additive.

Synthesis

Preparation of protected-first generation dendrons (Protected-G1-dendron)

To a mixture of 3-bromopropyl-1-NHBoc (9.00 g, 37.8 mmol), K_2CO_3 (7.84 g, 56.7 mmol) and molecular sieves (12 g) in DMF (25 ml) was added methyl 3,4-dihydroxyhydrocinnamate (2.47 g, 12.6 mmol) at room temperature under nitrogen. The reaction mixture was stirred at 60 ^oC overnight, cooled to r.t. and filtered. The filtrate was diluted with EtOAC/ether = 1/1, and the organic phase was washed with water and brine. The organic phase was dried over $MgSO_4$, concentrated in vacuo, and purified by column chromatography (MeOH/EtOAc/Hexane = 1/3/15). Recrystallization from (MeOH/water) gave white solid (4.36 g, 68%). M.p. 83-84 ^oC. IR (KBr, cm^{-1}): 1172 (Ar-O-alkyl), 1689, 1728 (C=O, amide and carbamate), 2938, 2977 (Ar-H), 3379 (N-H, carbamate). ^1H NMR (MeOH-d_4, δ, ppm): 1.43 (s, 18H), 1.87-1.99 (m, 4H), 2.60 (t, 2H, ^3J = 7.7 Hz), 2.84 (t, 2H, ^3J = 7.7 Hz), 3.21-3.29 (m, 4H) 3.64 (s, 3H) 4.02 (m, 4H) 6.73 (dd, 1H, ^3J = 8.0, ^4J = 2.0), 6.82 (d, 1H, ^4J = 2.0) 6.86 (d, 1H, ^3J = 8.0). Anal. Calcd for $C_{26}H_{42}N_2O_8$: C, 61.16; H, 8.29; N, 5.49. Found: C, 60.96; H, 8.15; N, 5.45.

Preparation of first generation dendrons (G1-dendron)

A mixture of compound **protected-G1-dendron** (4.05 g, 7.93 mmol) and KOH (0.620 g, 9.80 mmol) in methanol/water (45 ml/15 ml) was refluxed for 2 h. The reaction solution was cooled to room temperature and acidified with acetic acid. Then, the organic layer was diluted with DCM and washed with water three times, and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The first generation dendron was obtained as white solid after the recrystallization from toluene/hexane (3.32 g, 84%). M.p.102 -103 ^oC. IR (KBr, cm^{-1}): 1172 (Ar-O-alkyl), 1704, 1735 (C=O, carbamate and carboxylic acid), 2938, 2977 (Ar-H), 3332 (N-H, carbamate). ^1H NMR (MeOH-d_4, δ, ppm): 1.43 (s, 18H), 1.87-2.01 (m, 4H), 2.60 (t, 2H, ^3J = 7.7 Hz), 2.84 (t, 2H, ^3J = 7.7 Hz), 3.21-3.29 (m, 4H) 4.02 (m, 4H) 6.75 (dd, 1H, ^3J = 8.0, ^4J = 2.0), 6.83-6.88 (m, 2H). Anal. Calcd for $C_{25}H_{40}N_2O_8$: C, 60.47; H, 8.12; N, 5.64. Found: C, 60.38; H, 8.09; N, 5.63.

Preparation of AB_2 building blocks (AB_2)

The **G1-dendron** (50.0 mg, 0.1 mmol) was dissolved in trifluoroacetic acid (TFA) (0.5 mL), and the reaction solution was stirred at room temperature for 1.5 h. The solvent was evaporated to dryness to give white sticky oil. The oil was washed with ether three

times and dried under reduced pressure to give white stickly oil (51.1 mg, 97 %). IR (NaCl, cm^{-1}): 1133 (Ar-O-Alkyl), 1203 (C-F), 1681 (C=O, carboxylate), 2700-3300 (N-H, ammonium), ^{1}H NMR (MeOH-d_4, δ, ppm): 2.08-2.24 (m, 4H), 2.58 (t, 2H, 3J = 7.6 Hz) 2.86 (t, 2H, ^3J = 7.6 Hz), 3.13-3.23 (m, 4H) 4.09-4.21 (m, 4H), 6.81 (dd, 4H, ^3J =8.2 Hz, ^4J = 2.0 Hz), 6.59-6.82 (m, 9H) 7.16-7.29 (m, 2H) 6.65-6.85 (m, 9H). Anal. Calcd for $C_{19}H_{26}F_6N_2O_8$: C, 43.52; H, 5.00; N, 5.34. Found: C, 43.70; H, 4.91; N, 5.04.

Preparation of second generation dendrons (G2-dendron)

To a solution of **G1-dendron** (1.49 g, 3.00 mmol) in NMP (4.8 ml) were added DBOP (1.00 g, 2.85 mmol) and TEA (0.4 ml, 2.85 mmol) under nitrogen. The reaction solution was stirred at room temperature for 1.5 h. Then, the reaction solution was added dropwise to a NMP (4.8 ml) solution of TEA (1.20 ml, 8.10 mmol) and **AB_2** which was synthesized from 0.670 g (1.35 mmol) of **G1-dendron**, and the reaction solution was stirred at room temperature for 3h. The reaction solution was diluted with EtOAc/ether = 1/1. The organic layer was washed with 1 M HCl aq. and brine, and then dried with $MgSO_4$. After filtration, the solvent was removed under reduced pressure to give pale yellow oil. The oil was diluted with acetone (18 ml), and hexane (54 ml) was added to the solution. The precipitate was collected and dried in vacuo at 40 ^{o}C to give white

powder. (1.26 g, 75%) M.p.74 -75 ^{o}C. IR (KBr, cm^{-1}): 1172 (Ar-O-alkyl), 1643, 1689 (C=O, carbamate, carboxylic acid and amide), 2931, 2969 (Ar-H), 3363 (N-H, carbamate and amide). ^{1}H NMR (THF-d_8, δ, ppm): 1.39 (s, 36H), 1.80-1.95 (m, 12H), 2.30-2.39 (m, 4H), 2.57 (t, 2H, *J* = 7.8 Hz) 2.79 (t, 6H, J = 7.8 Hz), 3.17-3.40 (m, 12H) 3.86-3.99 (m, 12H), 6.07-6.24 (m, 4H), 6.59-6.82 (m, 9H) 7.16-7.29 (m, 2H) 6.65-6.85 (m, 9H). Anal. Calcd for $C_{65}H_{100}N_6O_{18}$: C, 62.28; H, 8.04; N, 6.70. Found: C, 62.52; H, 8.12; N, 6.62. MALDI-TOF MS: Calcd.: $[M]^+$ = 1274.7, Found: $[M+Na]^+$ = 1272.0.

Preparation of third generation dendrons (G3-dendron)

To a solution of **G2-dendron** (0.496 g, 0.400 mmol) in NMP (0.8 ml) were added DBOP (0.153 g, 0.400 mmol) and TEA (56.0 μl, 0.400 mmol) under nitrogen. The reaction solution was stirred at room temperature for 1.5 h. Then, the reaction solution was added dropwise to a NMP (0.6 ml) solution of TEA (154 μl, 1.10 mmol) and **AB_2** which was synthesized from 0.0913 g (0.184 mmol) of **G1-dendron**. The reaction solution was stirred at room temeperature for 3 h. The reaction solution was pured in water and acidified with 1 M HCl aq. The precipitate was collected and dried. The crude product was dissolved in MeOH (10 ml) and diluted with acetone (20 ml), and then hexane (80 ml) were added to the solution. The precipitate was collected and dried in

vacuo at 40 ^{o}C to give white powder (0.374 g, 74%). T_g: 45 ^{o}C. IR (KBr, cm^{-1}): 1172 (Ar-O-alkyl), 1650, 1697 (C=O, carbamate, carboxylic acid and amide), 2931, 2969 (Ar-H), 3347, 3402 (N-H, carbamate and amide). ^{1}H NMR (THF-d_8, δ, ppm): 1.39 (s, 72H), 1.78-1.95 (m, 28H), 2.31-2.41 (m, 12H), 2.49 (t, 2H, J = 7.8 Hz) 2.79 (t, 14H, J = 7.8 Hz), 3.17-3.40 (m, 28H) 3.79-4.01 (m, 28H), 6.13-6.25 (m, 8H), 6.59-6.82 (m, 21H) 7.16-7.29 (m, 6H) 6.65-6.85 (m, 9H). Anal. Calcd for $C_{145}H_{220}N_{14}O_{38}$: C, 62.93; H, 8.01; N, 7.09. Found: C, 63.26; H, 8.13; N, 6.98. MALDI-TOF MS: Calcd.: $[M]^+$ = 2787.5, Found: $[M+Na]^+$ = 2787.6.

Preparation of Third Generation Dendrimers (G3-dendrimer)

To a solution of **G3-dendron** (0.274 g, 0.100 mmol) in NMP (0.6 ml) were added DBOP (38.3 g, 0.100 mmol) and TEA (14.0 μl, 0.100 mmol) under nitrogen. The reaction solution was stirred at room temperature for 1.5 h. Then, the reaction solution was added dropwise to a solution of *p*-xylylenediamine (6.26 g, 0.0460 mmol) in NMP (0.6 ml), and the reaction solution was stirred at room temperature for 6 h. The reaction solution was poured in 1 wt% $NaHCO_3$ aq. The precipitate was collected and dried. The crude product was dissolved in DMF (1.2 ml) and diluted with MeOH (6.0 ml), and

then acetone (24 ml) and hexane (12 ml) was added to the solution. The precipitate was collected and dried in vacuo at 40 ^{o}C to give white powder (0.174 g, 75%). T_g: 52 ^{o}C. IR (KBr, cm^{-1}): 1172 (Ar-O-alkyl), 1643, 1697 (C=O, carbamate and amide), 2931, 2969 (Ar-H), 3309, 3347 (N-H, carbamate and amide). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.37 (s, 144H), 1.71-1.92 (m, 56H), 2.25-2.46 (m, 28H), 2.66-2.82 (m, 28H), 3.02-3.16 (m, 32H) 3.16-3.26 (m, 24H, overlapped with H_2O), 3.81-4.00 (m, 56H), 4.22 (d, 4H, ^{3}J = 5.8 Hz), 6.50-6.89 (m, 58H), 7.11 (s, 4H), 7.69-7.91 (m, 12H), 8.17 (t, 2H, ^{3}J = 5.8 Hz). Anal. Calcd for $C_{294}H_{448}N_{30}O_{74}$: C, 63.52; H, 8.01; N, 7.46. Found: C, 63.39; H, 8.02; N, 7.36. MALDI-TOF MS: Calcd.: $[M]^+$ = 5653.2, Found: $[M+Na]^+$ = 5653.3.

5. References and Notes

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Chapter V

Synthesis of New Cationic Water-Soluble Pyrene Containing Dendrons and their Multifunctional Sensory Applications

Abstract

A series of new water-soluble cationic pyrene-dendron derivatives, **G1**, **G2** and **G3,** was successfully synthesized and characterized. These new dendrons were designed with the quaternized amino moieties at the periphery of the dendrons for DNA detection and functionalized with pyrene as a fluorescent probe. The electrostatic interactions between the plasmid DNA (pDNA) and cationic charged dendrons in an aqueous solution resulted in a change in the photophysical properties of pyrene, which could be shown in the UV-vis and fluorescence spectra. Pyrene containing dendrons showed a high and rapid fluorescence response upon the addition of pDNA, which was strongly depending on the size and hydrophobicity of the dendrons. Furthermore, the new cationic charged dendrons also provided sensitivity to the pH value. The first and second generation amide dendrons (G1 and G2) formed hydrogels under basic conditions, but the hydrogel returned to the fluid state under acidic conditions, and this process was completely reversible. In addition, the

fluorescence of the diluted **G1**, **G2** and **G3** solutions also showed a high sensitivity to the pH value.

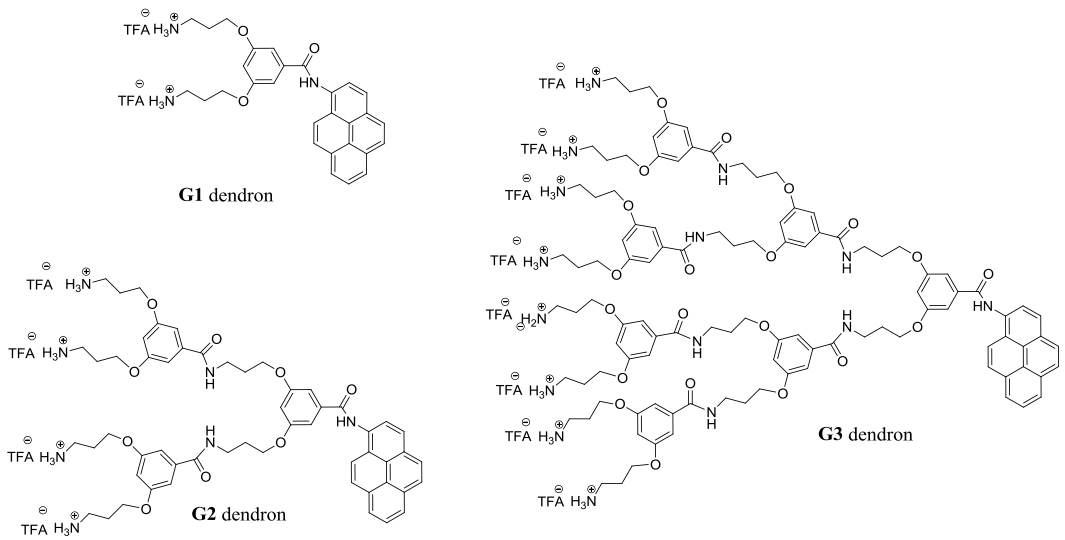
1. Introduction

Dendrimers are well-defined, three-dimensional hyperbranched macromolecules with a large number of terminal groups that can be easily utilized for introducing functionalities at the periphery of the dendrimers. Such a structural specificity has received great interests as new polymeric materials for applications in the fields of molecular light harvesting, [1] catalysts,[2] liquid crystals, [3,4] molecular encapsulation, [5, 6] and drug delivery systems.[7-10] Dendrimers containing fluorescent components not only facilitate the detailed investigation of the self-assembly process and molecular interaction in the dendrimers, but also extend their applications in fluorescence-based sensing materials.[11-15] Pyrene is a good candidate as a fluorescent probe because its fluorescence properties are well-known and highly sensitive to the microenvironment. [16, 17] In addition, it has a strong tendency to form excimers via intermolecular π-π stacking, which exhibit a broad and structure-less fluorescent emission red-shifted with respect to that of monomeric pyrene. In the past decades, several pyrene-labeled dendrimers have been developed by a noncovalent incorporation of pyrene into the cavities of the dendrimers, or covalent attachment of pyrene to the core or periphery of the dendrimers.[18-25] However, few of them have been reported for use in sensory applications. [21]

For sensory applications, electrostatic interactions between cationic moieties and negatively charged analyte targets (e.g., DNA or RNA) are commonly coordinated when designing fluorescence-based sensors. Various cationic fluorescence-based sensors, such as cationic amphiphilic molecules, [26, 27] cationic conjugated polymers, [28-30] or cationic charged dendrimers, [12] have been designed and synthesized for DNA or RNA detection. Recently, we also reported the aligned electrospun nanofibers formed from cationic polyfluorene that showed an enhanced sensitivity to plasmid DNA. [31] Although cationic fluorescent conjugated polymers have been proven to have a high sensitivity due to the excitation energy transfer mechanism resulting in amplification of the fluorescent signal, their rigid conformation led to its poor response to various secondary structures naturally present in biological macromolecules, such as the double helices of DNA. [32] In addition, the uncontrolled molecular weight variation of conjugated polymers caused reproduction of their sensitivity difficult with different batches of conjugated polymers. Dendrimers have more conformational freedom and precise molecular structures that allows them to be more suitable for DNA detection. However, the synthesis of cationic dendrimers might require a tedious multistep procedure with a repetitive protection-de protection and purification process for each

generation. Recently, we demonstrated the simple and rapid synthesis of dendritic polyamides and successfully obtained third generation dendrimers. [18, 33-35]

Hence, we now report the synthesis and investigation of a different generation of new cationic charged dendrons with functional pyrene as a fluorescent probe (first generation dendrons (**G1)**, second generation dendrons (**G2**) and third generation dendrons (**G3**) dendrons) for sensing DNA molecules (Scheme 1).



Scheme 1 Chemical structures of **G1**, **G2**, and **G3.**

The quaternized amino moieties at the periphery of dendrons were designed for favorable electrostatic attraction with negatively charged nucleic acids. The number of charged moieties, the distance between the cationic groups and fluorescent units, as well as the hydrophobicity of the dendron generation were also examined to reveal their

sensitivity to a plasmid DNA (pDNA), since it has been reported that the binding affinity of the cationic molecules to pDNA was strongly dependent on the structures of the cationic molecules. [36, 37]

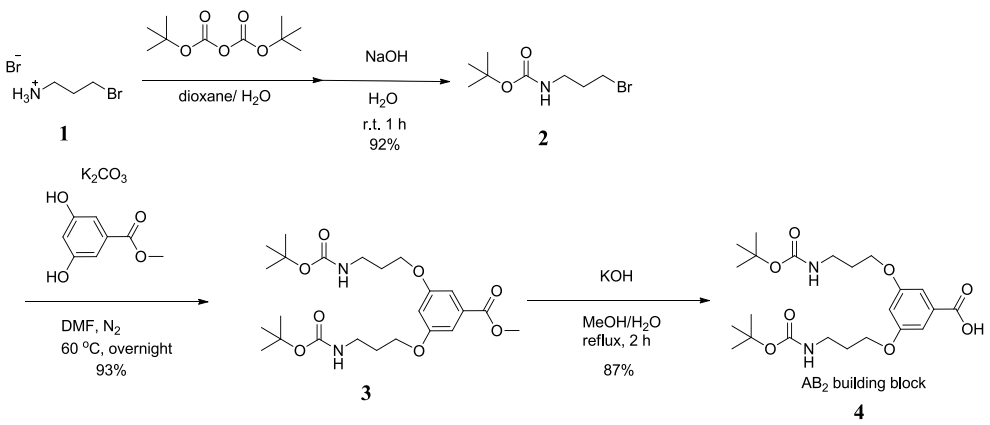
In addition, this new series of cationic charged dendrons also provided sensitivity to the pH value, and that result in the formation of pH-responsive hydrogels and pH-responsive photophysical propertiy. This is because the quaternized amino moieties were deprotonated to form amino group by the addition of acid, which was favorable for the formation of hydrogen-bonding. The study of the morphologies of the hydrogels were investigated by scanning electron microscope (SEM).

Our experimental results suggested that the prepared fluorescent cationic charged dendrimers were demonstrated to have pH- and pDNA-sensing ability by means of UV-vis and fluorescent spectrometers.

2. Results and Discussion

2-1. Synthesis of AB_2 Building Block and Dendrons

Scheme 2 shows the synthetic route for an AB_2 building block. Compound **2** was prepared according to a previous paper. [38] Methyl 3,5-dihydroxybenzoate reacted with **2** in the presence of K_2CO_3 to yield a protected AB_2 building block precursor (**3**), which was converted into an AB_2 building block (**4**) by the hydrolysis of the methyl ester group of **3**. The ^{1}H-NMR spectrum of the AB_2 building block **4** is shown in the supporting information (Figure 1). All peaks are well assigned to the corresponding structure.



Scheme 2 Synthesis of AB_2 building block (**4**).

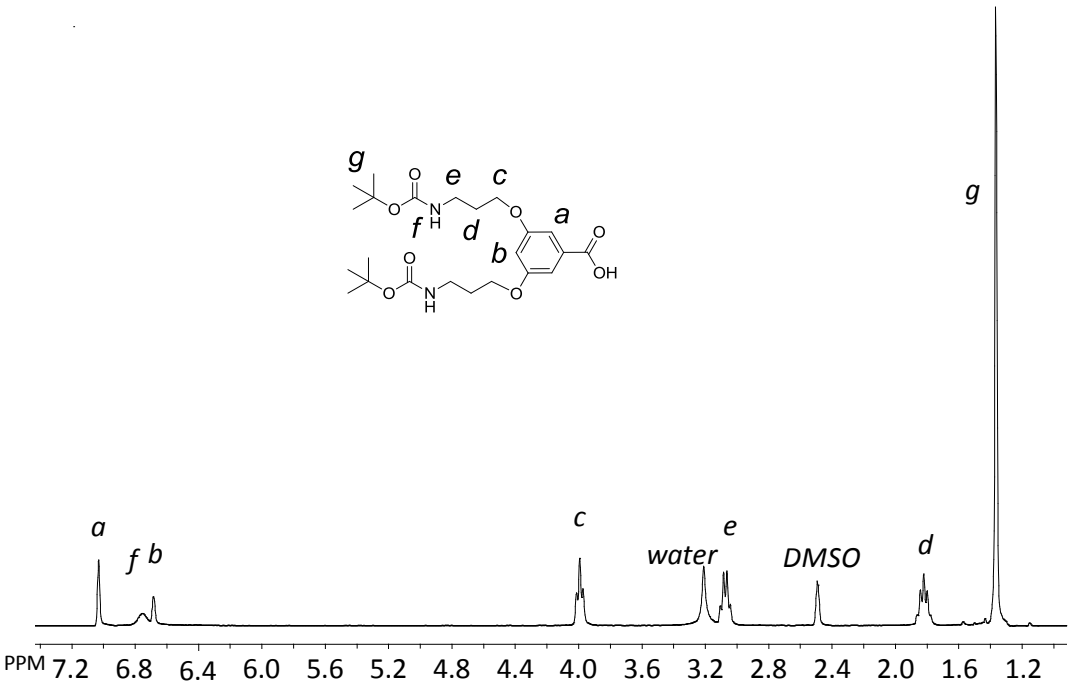
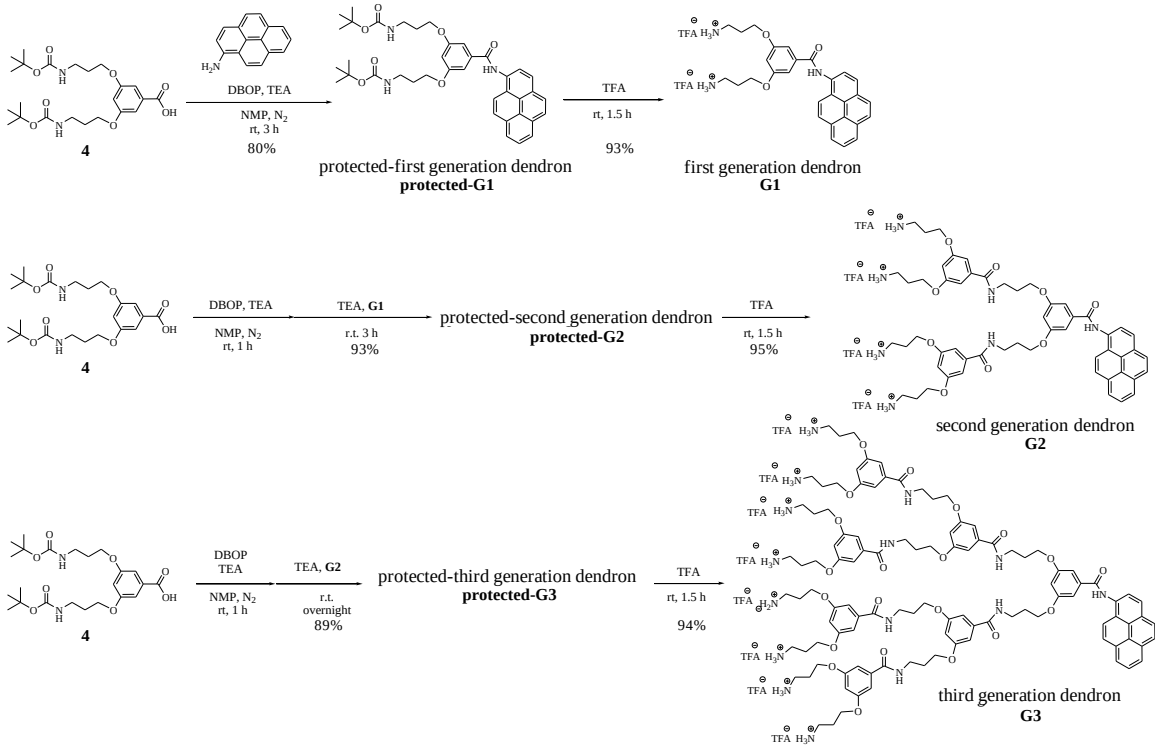


Figure 1 ^{1}H NMR spectrum of AB_2 building block (**4**)



Scheme 3 Synthesis of first – third generation water-soluble polyamide dendrons (**G1**, **G2**, **G3**).

The dendrons were grown from the 1-aminopyrene core by a divergent approach using DBOP as the condensing agent. [39] Coupling reactions for the synthesis of the **protected-G1**, **protected-G2** and **protected-G3** were conducted by a two-step method[34] consisting of (i) the activation of a carboxylic acid moiety of the AB_2 building block by DBOP to generate an active amide moiety, and (ii) the condensation of the active amide with 1-aminopyrene, **G1** or **G2**. The *tert*-butyl carbamate group of the **protected-G1**, **protected-G2** and **protected-G3** was deprotected with trifluoroacetic acid to afford the cationic charged dendrons (**G1**, **G2** and **G3**) (Scheme 3). It should be noted that this new synthetic method is the first example of the synthesis of hemi-aliphatic polyamide dendrons by two-step method using DBOP. All products were characterized by IR, ^{1}H-NMR spectroscopy and elemental analysis. The IR spectrum of **G3** showed strong absorptions at 3440 and 1681 cm^{-1} due to the characteristic N-H and C=O stretchings of the amide groups, respectively. Furthermore, the characteristic carboxylate and ether stretching were observed at 1592 and 1172 cm^{-1}, respectively. The ^{1}H NMR spectrum of **G3** showed signals corresponding to the amide protons (f, l, and r) at 8.42-8.55 and 10.69 ppm, pyrene protons (s) at 8.04-8.40 ppm, and alkyl protons of the end unit (a) at 2.96 ppm, respectively (Figure 2).

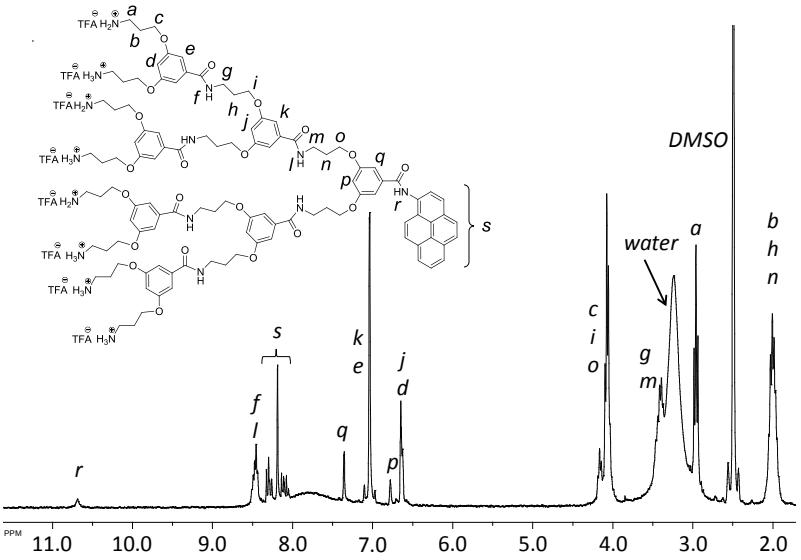


Figure 2 ^{1}H NMR spectrum of third generation dendron (**G3**).

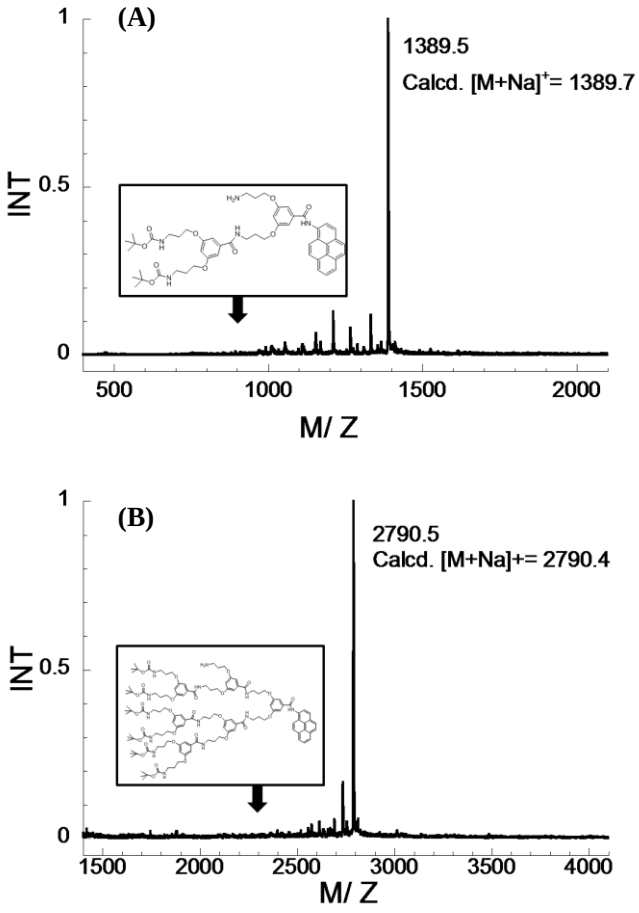


Figure 3 MALDI-TOF-MS spectrum of (A) protected-second generation dendron (**protected-G2**), and (B) protected-third generation dendron (**protected-G3**).

Moreover, Figure 3 shows the MALDI-TOF MS spectra of the **protected-G2** and **protected-G3**, which exhibited peaks observed at M/Z ($[M+Na]^+$) =1389.5 and 2790.5 and well agreed with the calculated mass (1389.7 and 2790.4), together with minor peaks which could be assignable to partially de-protected dendrons. The de-protection probably occurred during the measurement. Moreover, no peaks derived from dendrons having defect structures were observed in Figure 3. The SEC curves for **protected-G1**, **protected-G2** and **protected-G3** showed quite narrow polydispersities (Figure 4). These findings clearly indicated the formation of the target dendrons.

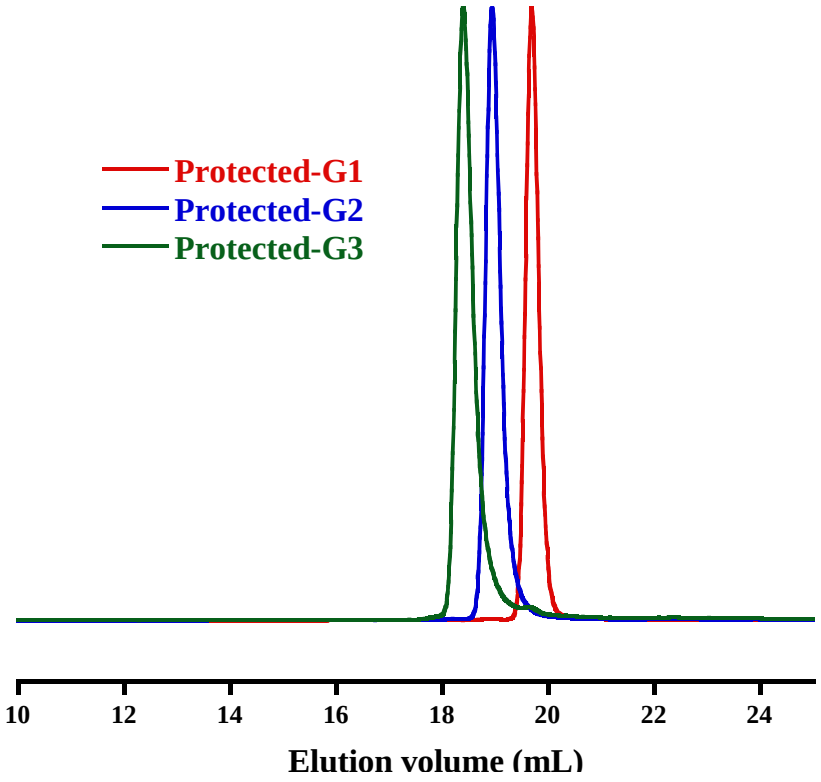


Figure 4 SEC profiles of Protected-G1, G2 and G3.

2-2. Photophysical Properties of Plasmid DNA Sensibility

The sensing ability of this new series of cationic charged dendrons as a function of the pDNA molar concentration was investigated in an aqueous solution. Figure 5 shows the UV-vis spectra of **G1**, **G2** and **G3** with various concentrations of pDNA in an aqueous solution. The dendron concentration was fixed at 5.0 μM. The absorption peak attributed to pyrene at 340 nm was observed in the UV-vis spectra of **G1**, but it gradually decreased and red-shifted when pDNA was added to the **G1** aqueous solution (Figure 5A). The absorption spectra of **G2** and **G3** showed the absorption peak of pyrene at 347 nm and slightly red-shifted to 350 nm with the increasing pDNA concentration (Figure 5B and3C). The red shift due to pyrene groups absorption may be caused by the formation of the dendron/pDNA complex. While the pyrene absorption peak of **G2** kept increasing during the addition of pDNA, the pyrene absorption peak of **G3** increased up to a 15 mM pDNA solution addition and then became saturated. This is probably because the larger molecular size and more charged terminal groups of **G3** compared to the **G2** ones induced a higher affinity with pDNA and formed a stiffer aggregation. The formation of the **G3**/pDNA complex aggregation did not change with the further addition of pDNA.

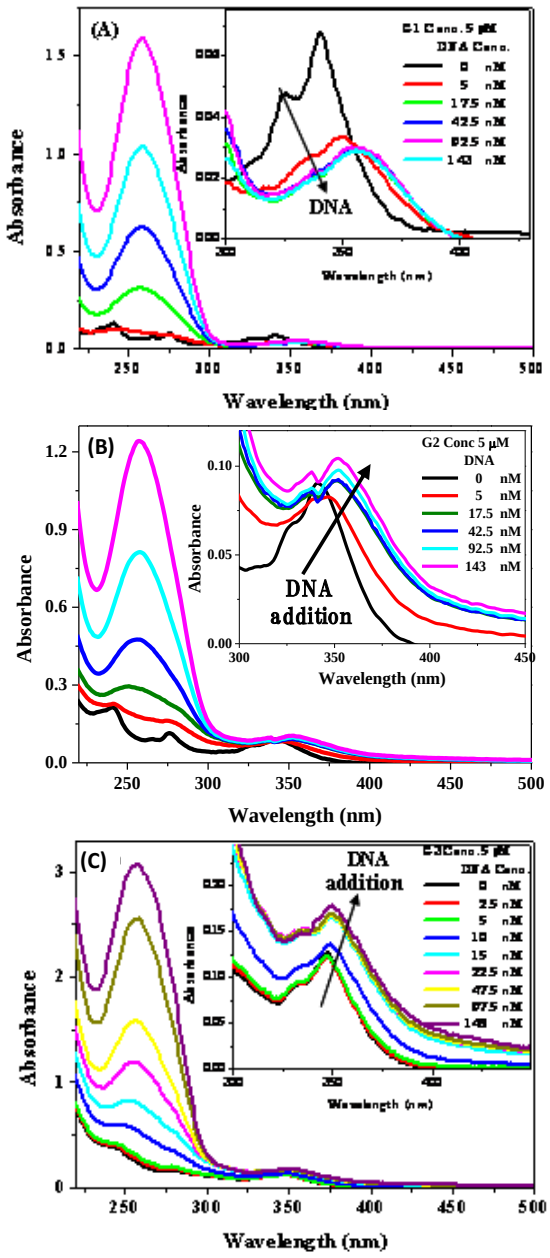


Figure 5 UV-vis spectra of (A) **G1**, (B) **G2** and (C) **G3** as a function of added plasmid DNA.

The fluorescence emission spectra of the **G1**, **G2** and **G3** with the increasing pDNA concentration are shown in Figure 6. The emission at 385 nm corresponds to the monomeric pyrene emission. Without pDNA, **G1** showed a strong excimer fluorescence

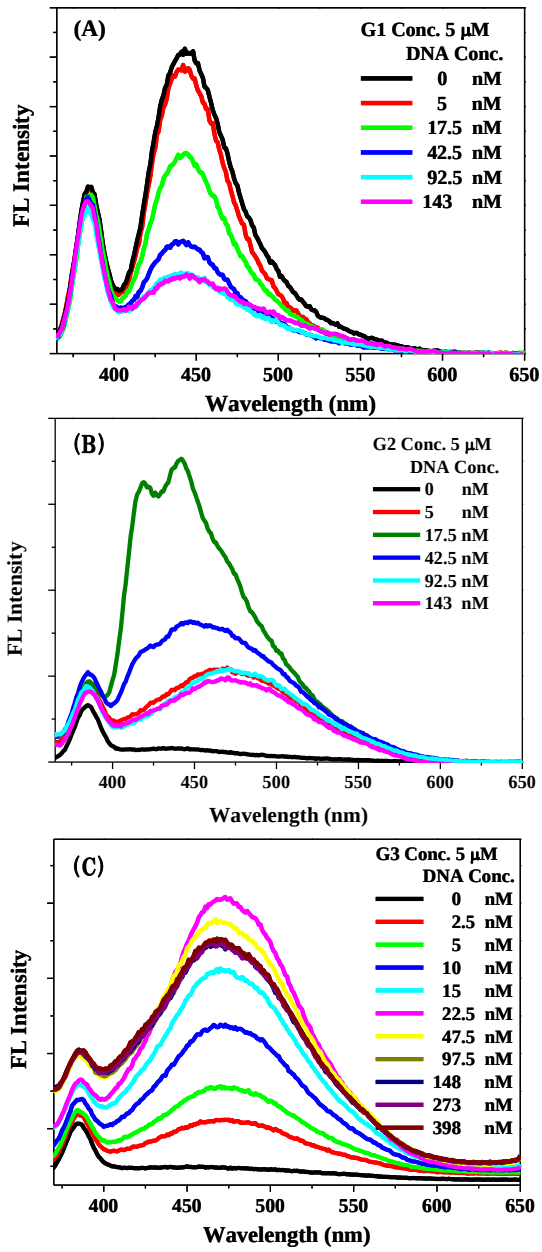


Figure 6 Fluorescence spectra of (A) **G1**, (B) **G2** and (C) **G3** as a function of added plasmid DNA.

at 442 nm due to intermolecular π-π stacking, however, no excimer emission was observed for **G2** and **G3**. This is attributed to the large dendron size and more highly charged groups of **G2** and **G3** that resulted in the increased steric hindrance and charge

repulsion to form the pyrene excimer. When the pDNA concentration increases, **G1** gradually showed a decrease in the fluorescent emission from the pyrene excimer. However, **G2** and **G3** initially showed an enhancement of the fluorescent emission, and then a decrease in its fluorescence intensity with an increase in the pDNA concentration, which is different from the behavior of the **G1**/pDNA complexes. This result indicates that the excimer formation of pyrene might be strongly dependent on the size and hydrophobicity of the dendrons because **G2** and **G3** have a larger size and more hydrophobic units than **G1**. [26] In addition, the formation of the **G2**/pDNA and **G3**/pDNA complexes reduces the electrostatic repulsion between the cationic charges of **G2** and **G3**, which gives rise to the formation of more pyrene excimers. To illustrate the

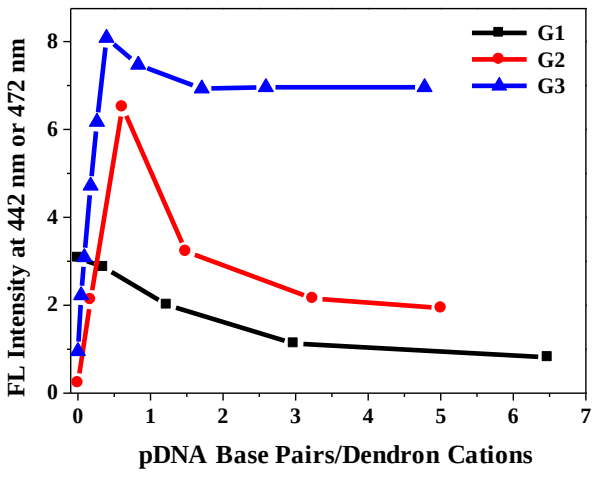


Figure 7 Fluorescence emissions at 442 nm for the **G1** and 472 nm for **G2** and **G3** with concentration of 5 μM and various ratios of the pDNA base pairs (negative charge)/dendron cations in aqueous solution.

effect of the ratios of the positive charge to negative charge on the emission of the pDNA/dendrons in detail, the fluorescence intensities for the **G1**, **G2** and **G3** are plotted versus the various ratios of the pDNA/dendrons (Figure 7).

As shown in the figure, **G1, G2** and **G3** exhibited a different fluorescence tendency and maximum emission intensities at the different pDNA/dendrons charge ratios. Initially, with the increasing pDNA/dendrons ratios, pDNA plays a role in either the disruption or helps with the formation of the pyrene excimers, which gradually decrease or increase the emission intensities of dendrons. This result also indicates the interactions between the pDNA and cationic dendrons are significantly dependent on the cationic charged moieties and hydrophobicity of the dendron generation. Actually, the fluorescent emission intensities of **G1** kept decreasing after the addition of pDNA, suggesting that the pyrene excimer formation was disrupted by the electrostatic interactions between the negatively charged pDNA and positively charged amino groups in the dendrons. When excess pDNA was added (charge ratio > 1) to the **G2** aqueous solution, the fluorescence intensities decreased and reached saturation. This might be because an excessive amount of pDNA reduces the number of dendrons attached to each pDNA strand and thus prevents formation of the pyrene excimers. On the other hand, **G3** showed only a slight decrease in the fluorescence intensities after the

charge ratio of 1. **G3**, which possesses a greater number of charged terminal groups probably has a stronger interaction with pDNA and formed aggregates during the addition. The aggregates might not collapse with the further addition of pDNA. These results are in agreement with the results of UV-vis spectra (Figure 5B and C).

2-3. Morphologies of Self-assembled Pyrene Dendrons and pDNA Complexes

The morphologies of first to third generation dendrons (**G1**, **G2** and **G3)** and dendron/pDNA complexes were also investigated by TEM with a fixed concentration of dendrons and the pDNA/dendrons ratio of 3.0. (Figure 8). Without addition of pDNA, interesting morphologies of **G1**, **G2** and **G3** are observed. **G1** showed the aggregation of the sphere-like nanostructures with diameter around 20 to 60 nm (Figure 8A). **G2** and **G3** exhibit entangled fibrous network with widths around 20 to 100 nm for **G2** and 15 to 80 nm for **G3**, respectively (Figure 8B and 6C). On the other hand, when addition of the pDNA into the dendron solutions, the large lamellar-like sheet of **G1/**pDNA complexes is observed in Figure 8D, however, the small lamellar aggregations of **G2/**pDNA and **G3/**pDNA complexes are shown in Figure 8E and 6F, respectively. The morphologies of the dendron/ pDNA complexes are significantly different from those of **G1**, **G2** and **G3**, which indicated the existence of electrostatic interactions between the

negatively charged pDNA and positively charged amino groups in the dendrons. The larger lamellar aggregation of **G1**/pDNA complexes compared to those of **G2**/pDNA and **G3**/pDNA complexes might be attributed to the number of the cationic charged moieties in the dendron. Since **G1** has the less cationic charges, the more **G1** may interact on each pDNA strand. In addition, **G2** and **G3** with large size may reduce the number of dendrons attached to each pDNA strand due to steric hindrance effect and prevent the formation of large aggregates.

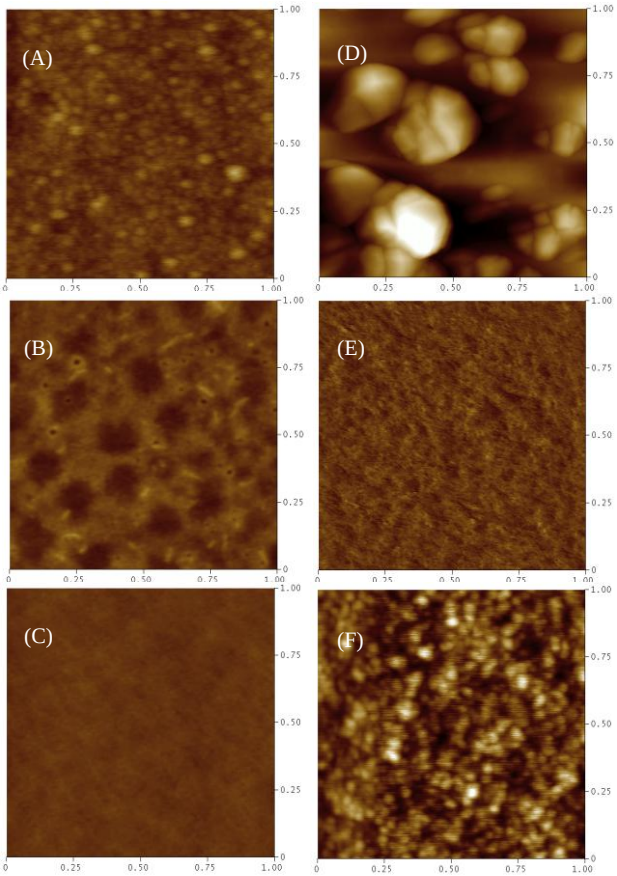


Figure 8 AFM morphologies of self-assembled (A-C) **G1**, **G2** and **G3**, respectively, and (D-F) **G1**, **G2** and **G3**/pDNA complexes, respectively.

2-4. Fabrication and Morphology of pH-responsive Supramolecular Gels

Supramolecular gels generated through the self-assembly of small gelator molecules via physical interactions like hydrogen bonding, π-π stacking, hydrophobic interactions, donor-acceptor interactions, or van der Waals interactions form entangled fibril networks. [39-41] Based on these characteristics of the gel formations, we expected that the π-π interactions between the pyrene moieties and hydrogen bonding between the amine and amide groups under basic conditions both provide a sufficient force required for the generation of extended fibril nanostructures. After the addition of a stoichiometric ratio of a NaOH aqueous solution, **G1** and **G2** show excellent abilities to form gels at the gelator concentration of ~ 14×10^{-3} or 3.5×10^{-3} M, respectively. However, **G3** failed to form gels under similar conditions probably due to its relatively large size, which caused an increase of the steric hindrance and reduction of its flexibility between intermolecular interactions to form hydrogels. Figure 9 shows the optical images of the supramolecular gels of **G1** and **G2** with the addition of a stoichiometric amount of base. The hydrogel of **G1** appeared opaque, indicating the presence of insoluble microparticles in the hydrogel. This might be due to the limited solubility of **G1**. On the contrary, the hydrogel of **G2** was transparent. The gelations of **G1** and **G2** could be immediately changed back to fluid state by appropriately adjusting the pH value to protonation of the basic sites of the gelators.

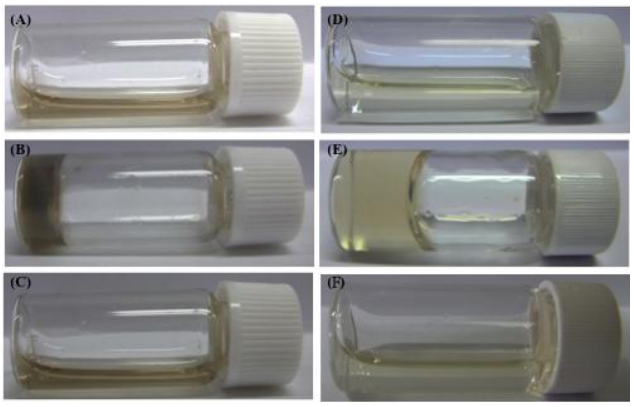


Figure 9 Optical images of supramolecular gels of **G1** and **G2** before [(A) and (D)] and after adding stoichiometric amount of NaOH [(B) and (E)] or HCl [(C) and (F)] aqueous solution, respectively.

This sol-gel phase transition can be reversibly switched without affecting the gelation ability. An SEM analysis was also performed to study the morphologies of the hydrogels of **G1** and **G2** (Figure 10).

G1 exhibits an entangled irregular fibrous matrix with widths around 200 to 500 nm. This irregular morphology might suggest that there should be more than one type of aggregation process for the hydrogel network. The morphology of **G2** shows fine nanofibers with widths from 100 to 300 nm, which significantly differs from that of the fibrous matrix formed by **G1**. These well-developed fibrous networks indicate that supramolecular interactions (hydrogen bonding and π-π stacking) promote the self-assembly of the small molecular gelator to form long and polymer-like fibrous networks that mainly trap the water by surface tension.

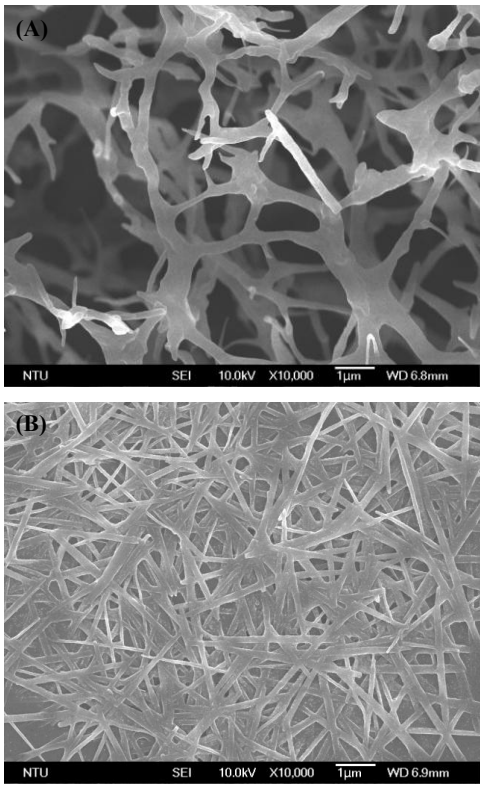


Figure 10 SEM images of hydrogels (A) **G1** and (B) **G2**

2-5. Photophysical Properties of pH Responses

In addition, the fluorescent responses of the **G1** – **G3** to various NaOH concentrations under dilute conditions (0.5×10^{-4} M) were investigated and shown in Figure 11A and Figure 12.

During titration with a NaOH aqueous solution, the **G1** – **G3** solutions showed a broad emission band of pyrene at 375 to 610 nm with an excitation at 340 nm and gradual increase in their fluorescence intensities. With the addition of NaOH at the equivalent

concentration ([OH]/[NH_3^+] = 1), **G3** was completely deionized and showed a large fluorescence enhancement and saturation, which suggests that the pyrene moiety is reorganized to form excimers (Figure 12B).

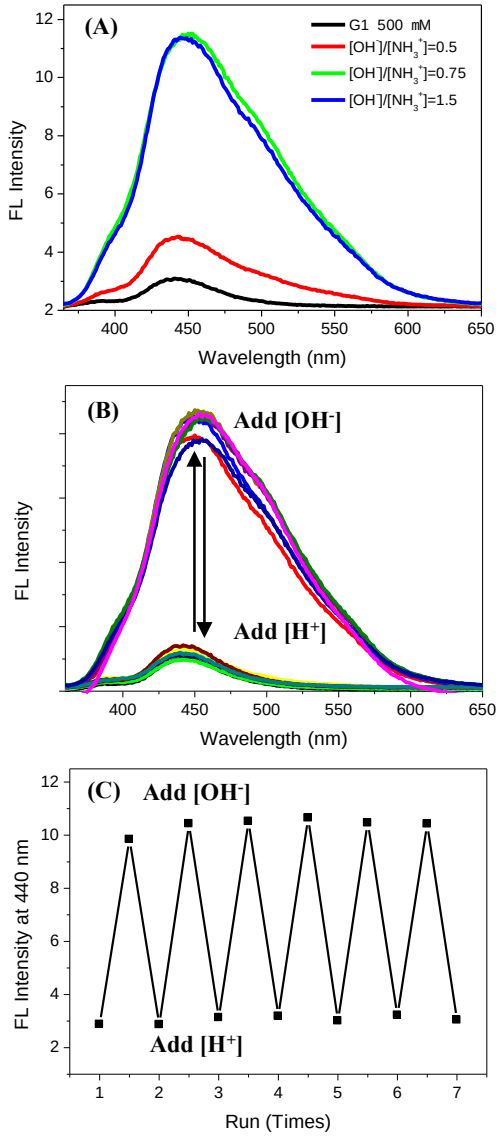


Figure 11 (A) Fluorescence emission of **G1** atconcentration of 5 × 10^{-4} M and at various NaOH concentrations. Excitation wavelength is at 340 nm. (B) Fluorescence emission of **G1** with repeating addition of equivalent concentration of NaOH or HCl aqueous solution. (C) Reversible response of **G1** by the addition of NaOH or HCl aqueous solution illustrated with its fluorescence intensity at 440 nm.

The increase in fluorescence might be due to the strong tendency of the pyrene group to form dimers or oligomers in an aqueous solution after deionization of the dendrons, which eliminated the cationic repulsion and increased the molecular interaction (e.g., hydrogen bonding, etc.). On the other hand, **G1** and **G2** showed large fluorescence enhancements and reached a fluorescence saturation at $[OH]/[NH_3^+] = 0.75$, indicating that **G1** and **G2** more favorably form pyrene excimers in the aqueous phase, despite the existence of a partial cationic repulsion. This might be because **G1** and **G2** have less steric hindrance compared to that of **G3**.

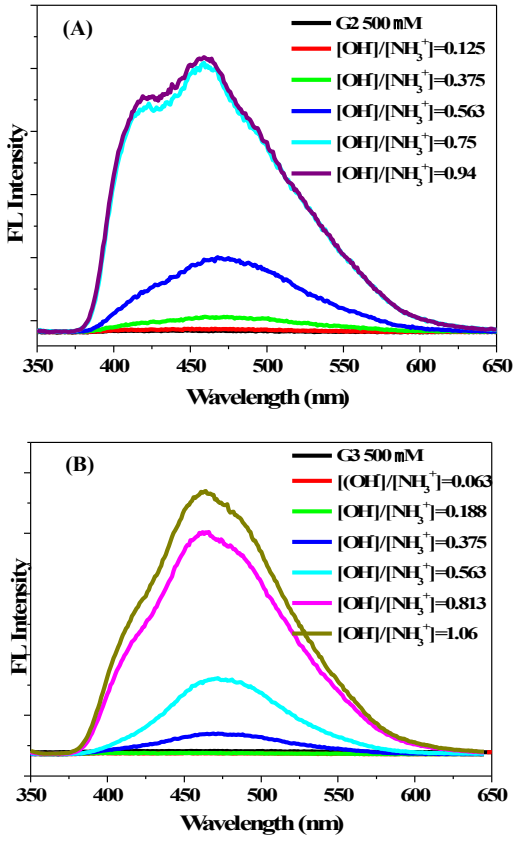


Figure 12 Fluorescence emission of (A) **G2** and (B) **G3** at various NaOH concentrations. The concentration of the dendron solution is 5×10^{-4} M.

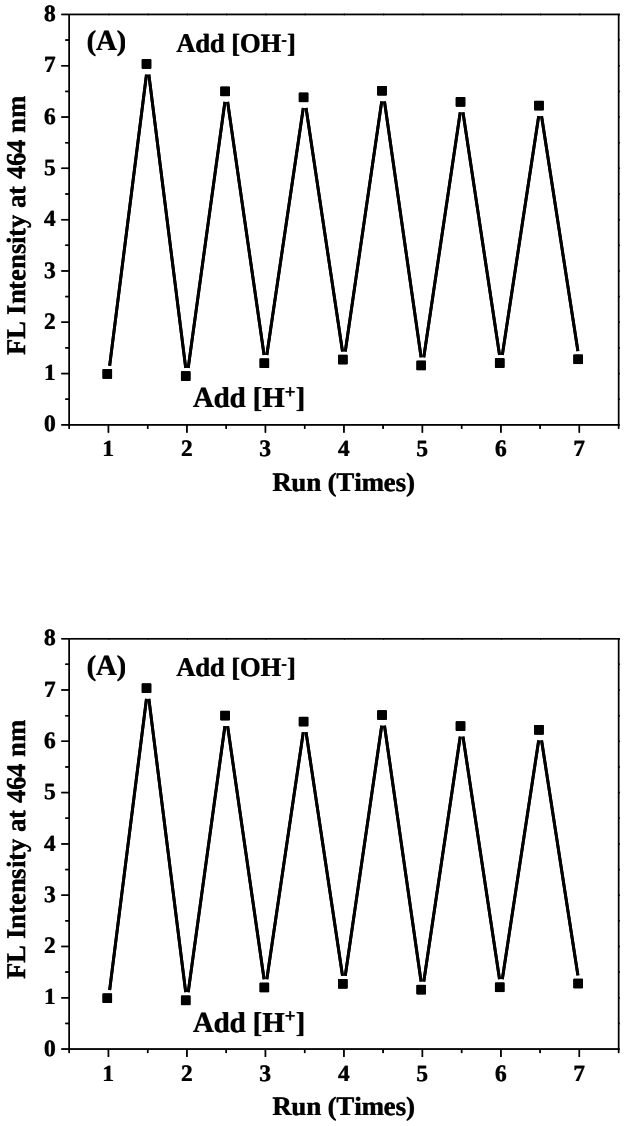


Figure 13 (A) Reversible response of **G2** with respect to addition of NaOH or HCl aqueous solution illustrated with its fluorescence intensity at 464 nm. (B) Reversible response of **G3** with respect to addition of NaOH or HCl aqueous solution illustrated with its fluorescence intensity at 462 nm.

Thus, the excimer formations of **G1** and **G2** are more pronounced with less added NaOH. These results are in agreement with the results of the pH-responsive hydrogel fabrication. The addition of an equivalent amount of acid (HCl aqueous solution) to the

deionized dendron solution, which was slightly cloudy, turned it into a clear solution. Moreover, the addition of an equivalent amount of acid caused a decrease in their fluorescence intensities, indicating the disappearance of the excimer band of pyrene (as shown in Figure 11B). Their pH responses of the **G1**, **G2** and **G3** solution are completely reversible as shown in Figure 11C and Figure 13.

3. Conclusions

We have designed and successfully synthesized a new series of water-soluble cationic pyrene-dendrons, **G1**, **G2** and **G3**. The UV-vis and fluorescence spectral investigation demonstrated that the electrostatic interactions between the plasmid DNA (pDNA) and cationic charged dendrons in an aqueous solution caused a change in the photophysical properties of pyrene due to the formation or disassembly of the pyrene excimers. The fluorescence responses of the pyrene-dendrons upon addition of pDNA were strongly dependent on the size, hydrophobicity and number of cationic charges of the dendrons. The TEM images of dendron/ pDNA complexes also demonstrated the existence of electrostatic interactions between the negatively charged pDNA and positively charged amino groups in the dendrons. In addition, this new series of cationic charged dendrimers also provided sensitivity to the pH value. **G1** and **G2** also showed their potential as the gelators for formation of supramolecular gels with entangled fibrous structures by appropriately adjusting the pH of the dendrimer solution. This indicated that physical interactions, such as π-π stacking of pyrene moieties and hydrogen bonding from the amine and amide groups in basic condition, are the dormant force for self-assembly of the extended fibrous nanostructures. However, **G3** did not form

hydrogel due to its higher steric hindrance. It was also found that all generation dendrons (**G1**, **G2** and **G3**) showed reversible pH-responsive fluorescent properties.

4. Experimental Section

Materials

N-Methyl-2-pyrrolidinone (NMP) was distilled under reduced pressure from calcium hydride, and then stored under nitrogen. Triethylamine (TEA) was distilled from calcium hydride under nitrogen, and then stored under nitrogen. Diphenyl(2,3-dihydro-2- thioxo-3-benzoxazolyl) phosphonate (DBOP) was supplied from KYOCERA Chemical Corporation and recrystallized from hexane, then stored under nitrogen in a refrigerator. The plasmid DNA molecules (base pair: 700) used for studying the responsive properties of the pyrene-dendrimers were purchased from Sigma (Milwaukee, USA). The stock solutions of pDNA were prepared by dissolving certain amount of solid pDNA in double distilled water and stored at 4 ^{o}C in the dark. Other reagents and solvents were obtained commercially and used as received unless otherwise noted.

Instrumentation

^{1}H NMR spectra were recorded in deuterated dimethylsulfoxide (DMSO-$_{d6}$) or chloroform ($CDCl_3$) on a BRUKER DPX-300 spectrometer at 300 MHz. Infrared

spectra were recorded on a Horiba FT-720 spectrophotometer. Matrix-assisted laser desorption ionization with time of flight (MALDI-TOF) mass spectra were recorded on a Kratos Kompact MALDI instrument operated in linear detection mode to generate positive ion spectra using dithranol as a matrix, THF as a solvent, sodium trifluoroacetate as an additive agent. Size exclusion chromatography (SEC) was performed on a Jasco GULLIVER 1500 system equipped with two polystyrene gel columns (Plgel 5 mm MIXED-C) eluted with $CHCl_3$ at a flow rate of 1.0 mL min^{-1} calibrated by standard polystyrene samples. Absorption and photoluminescence (PL) spectra were measured with a Jasco V- 550 spectrophotometer. Transmission electron microscopy (TEM) images were obtained with a Philips TEM (CM 100) instrument operating at a voltage of 80kV with a Morada CCD camera. The samples of G1-G3 dendrons were prepared with concentration of 100 μM. Images of hydrogel film were taken using a scanning electron microscope operated at an accelerating voltage of 15 kV after sputtering with a thin layer of gold.

Synthesis

The pyrene-dendrons (G1, G2 and G3) were synthesized by a divergent method as shown in Schemes 2 and 3.

Preparation of 3-Bromo-propyl-1-NHBoc (2)[38]

1M NaOH aq. (10 ml) was added to a solution of 3-bromopropylamine (1.00 g, 4.56 mmol) and di-*tert*-butyl dicarbonate (0.945g, 4.10 mmol) in dioxane/water (20 ml/10 ml). The mixture was stirred at room temperature for 1 h, and then diluted with ethyl acetate and water. The organic layer was washed successively with 1N HCl aq., 5 wt% of $NaHCO_3$ aq., and brine, and then dried with $MgSO_4$. After filtration, the solvent was removed under reduced pressure. The product was obtained as colorless oil (0.898 g, 92 % yield). ^{1}H NMR ($CDCl_3$, δ, ppm): 1.44 (s, 9H), 2.05 (triplet-triplet appearing as a quintet, 2H, *J* = 6.6 Hz), 3.27 (triplet-doublet appearing as a quartet, 2H, *J* = 6.6 Hz), 3.45 (t, 2H, *J* = 6.6 Hz), 4.67 (s, 1H).

Preparation of Protected-AB_2 Building Block (3)

To a mixture of compound **2** (3.63 g, 15.0 mmol) and K_2CO_3 (2.07 g, 15.0 mmol) in DMF (10 ml) was added methyl 3, 5-dihydorxybenzoate (0.84 g, 5.00 mmol) at room temperature under nitrogen. The reaction mixture was stirred at 60 ^{o}C overnight, and then poured into water. The precipitate was filtered and dried under reduced pressure to give a white solid (2.24 g, 93% yield). M.p. 123-124 ^{o}C. ^{1}H NMR ($CDCl_3$, δ, ppm): 1.44 (s, 18H), 1.99 (triplet-triplet appearing as a quintet, 4H, *J* = 6.6 Hz), 3.32

(triplet-doublet appearing as a quartet, 4H, *J* = 6.6 Hz), 3.90 (s, 3H), 4.04 (t, 4H, *J* = 6.6 Hz) 4.73 (s, 2H), 6.64 (t, 1H), 7.17 (d, 2H).

Preparation of AB_2 Building Block (4) [41]

A mixture of compound **3** (3.38 g, 7.00 mmol) and KOH (0.550 g, 9.80 mmol) in methanol/water (42 ml/14 ml) was refluxed for 2 h. The reaction solution was cooled to room temperature and acidified with acetic acid. Then, the organic layer was diluted with ethyl acetate and washed with water three times, and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure to afford a white solid (2.85 g, 87% yield). M.p. 128-129 ^oC. ^1H NMR (DMSO-$_{d6}$, δ, ppm, 40 ^oC): 1.37 (s, 18H), 1.82 (triplet-triplet appearing as a quintet, 4H, *J* = 6.5 Hz), 3.08 (triplet-doublet appearing as a quartet, 4H, *J* = 6.5 Hz), 4.00 (t, 4H, *J* = 6.5 Hz), 6.69 (t, 1H, *J* = 2.2 Hz), 6.77 (s, 2H), 7.04 (d, 2H, *J* = 2.2 Hz).

Synthesis of Protected-First Generation Dendron (protected-G1)

To a solution of **4** (1.48 g, 3.15 mmol) and 1-aminopyrene (0.652 g, 3.00 mmol) in NMP (3 ml) were added DBOP (1.21 g, 3.15 mmol) and TEA (0.440 ml, 3,15 mmol) under nitrogen. The reaction solution was stirred at room temperature for 3 h, and then poured into water. The precipitate was collected, washed with methanol and dried *in*

vacuo at 80 ^{o}C to give a gray powder (1.60 g, 80% yield). M.p. 161-162 ^{o}C. IR (KBr, cm^{-1}): 1180 (Ar-O-alkyl), 1585 (C=O, amide and carbamate), 3039, 3058 (Ar-H), 3421, 3532 (N-H, amide and carbamate). ^{1}H NMR (DMSO-$_{d6}$, δ, ppm, 40 ^{o}C): 1.38 (s, 18H), 1.89 (triplet-trople appearing as a quintet, 4H, *J* = 6.5 Hz), 3.13 (triplet-doublet appearing as a quartet, 4H, *J* = 6.5 Hz), 3.22 (s, 3H), 4.10 (t, 4H, *J* = 6.5 Hz), 6.74 (t, 1H, *J* = 2.0 Hz), 6.80 (s, 2H), 7.32 (d, 2H, *J* = 2.0 Hz), 8.05- 8.35 (m, 9H), 10.65 (s, -CON*H*-, 1H). Anal. calcd for $C_{39}H_{45}N_3O_7$: C, 70.14; H, 6.79; N, 6.29. Found: C, 70.04; H, 6.68; N, 6.37.

Synthesis of First Generation Dendron (G1)

Protected-G1 (0.500 g, 0.749 mmol) was dissolved in trifluoroacetic acid (TFA) (5 ml) a stirred at room temperature for 1.5 h. The solvent was evaporated to dryness and ether was added. The precipitate was collected and dried *in vacuo* at 80 ^{o}C to give a gray powder (0.485 g, 93% yield). M.p. 176-185 ^{o}C. IR (KBr, cm^{-1}): 1176 (Ar-O-Alkyl), 1203 (C-F), 1592 (C=O, carboxylate), 1677 (C=O, amide), 2700-3200 (N-H, ammonium), 3404, 3432 (N-H, amide). ^{1}H NMR (DMSO-$_{d6}$, δ, ppm, 40 ^{o}C): 2.07(triplet-triplet appering as a quintet, 4H, *J* = 6.9 Hz), 3.20 (t, 4H, *J* = 6.9 Hz), 4.19 (t, 4H, *J* = 6.9 Hz), 6.79 (s, 1H), 7.38 (s, 2H), 8.04- 8.40 (m, 9H), 10.68 (s, -CON*H*-,

1H). Anal. calcd for $C_{33}H_{31}F_6N_3O_7$ 1.08H_2O: C, 55.43; H, 4.67; N, 5.88. Found: C, 55.20; H, 4.54; N, 6.11.

Synthesis of Protected-Second Generation Dendrons (protected-G2)

To a solution of **4** (0.539 g, 1.15 mmol) in NMP (5 ml) were added DBOP (0.422 g, 1.10 mmol) and TEA (0.154 ml, 1.10 mmol) under nitrogen. The reaction solution was stirred at room temperature for 1 h. Then, **G1** (0.347 g, 0.500 mmol) and TEA (0.280 ml, 2.00 mmol) were added to the solution and the solution was stirred at room temperature for 6 h. The reaction solution was poured into water and the precipitate was collected and dried. The crude product was dissolved in methanol (100 ml) and water (25 ml) was added to this solution. The precipitate was collected and dried *in vacuo* at 80 ^oC to give a gray powder (0.636 g, 93% yield). M.p. 118-121 ^oC. IR (KBr, cm^{-1}): 1164 (Ar-O-alkyl), 1689 (C=O, amide and carbamate), 2935, 2973 (Ar-H), 3313, 3355 (N-H, amide and carbamate). ^1H NMR (DMSO-$_{d6}$, δ, ppm, 40 ^oC): 1.36 (s, 36H), 1.81 (triplet-triplet appearing as a quintet, 8H, J = 6.8 Hz), 2.03 (triplet-triplet appearing as a quintet, 4H, J = 6.8 Hz), 3.07 (triplet-doublet appearing as a quartet, 8H, J = 6.8 Hz), 3.45 (triplet-doublet appearing as a quartet, 4H, J = 6.8 Hz), 3.99 (t, 8H, J = 6.8 Hz), 4.16 (t, 4H, J = 6.8 Hz), 6.59 (s, 2H), 6.69- 6.80 (m, 5H), 7.00 (s, 4H), 7.35 (s, 2H),

8.03-8.37 (m, 9H), 8.43 (t, 2H, J= 5.0 Hz), 10.7 (s, 1H). Anal. calcd for $C_{75}H_{97}N_7O_{17}$: C,65.82; H, 7.14; N, 7.16. Found: C, 65.38; H,6.93; N,7.05. MALDI-TOF MS: Calcd.: $[M]^+$ = 1367.7, Found: $[M+Na]^+$ = 1389.5.

Synthesis of Second Generation Dendron (G2)

Protected-G2 (0.550 g, 0.402 mmol) was dissolved in 5 ml of TFA and stirred for 1.5 h. The solvent was evaporated to dryness and ether was added. The precipitate was collected and dried *in vacuo* at 80^{o}C to give a gray powder (0.544 g, 95% yield). IR (KBr, cm^{-1}): 1172 (Ar-O-Alkyl), 1203 (C-F), 1592 (C=O, carboxylate), 1677 (C=O, amide), 2700-3200 (N-H, ammonium), 3370, 3432 (N-H, amide). ^{1}H NMR (DMSO-$_{d6}$, δ, ppm, 40 ^{o}C): 1.94-2.12 (m, 12H), 2.96 (t, 8H, *J* = 6.6 Hz), 3.46 (triplet-triplet appearing as a quintet, 4H, *J* = 6.8 Hz), 4.08 (t, 8H, *J* = 6.6 Hz), 4.16 (t, 4H, *J* = 6.8 Hz), 6.65 (s, 2H), 6.77 (s, 1H), 7.07 (s, 4H), 7.36 (s, 2H) 8.04- 8.36 (m, 9H), 8.48 (t, 2H, *J* = 4.9 Hz) 10.70 (s, -CON*H*-, 1H). Anal. calcd for $C_{63}H_{69}F_{12}N_7O_{17}$ 1.18H_2O: C, 52.35; H,4.98; N, 6.78. Found: C, 51.89; H, 4.90; N, 7.24.

Synthesis of Protected-Third Generation Dendron (protected-G3)

DBOP (0.248 g, 0.644 mmol) and TEA (90.0 μl, 0.644 mmol) were added to a solution of **4** (0.316 g, 0.672 mmol) in NMP (1 ml) under nitrogen and the solution was stirred at

room temperature for 1 h. To this solution were added **G2** (0.200 g, 0.140 mmol) and TEA (0.240 ml, 1.68 mmol) and the solution was stirred at room temperature overnight. The reaction solution was poured into water and the precipitate was collected and dried. The crude product was dispersed in methanol (20 ml) and water (5 ml) was added to this mixture. The precipitate was collected and dried *in vacuo* at 80 ^{o}C to give a gray powder (0.345 g, 89% yield). IR (KBr, cm^{-1}): 1164 (Ar-O-alkyl), 1693 (C=O, amide and carbamate), 2935, 2973 (Ar-H), 3343 (N-H, amide and carbamate). ^{1}H NMR (DMSO-$_{d6}$, δ, ppm, 40 ^{o}C): 1.25 (s, 72H), 1.71 (triplet-triplet appearing as a quintet, 16H, *J* = 6.6 Hz), 1.79-1.99 (m, 12H), 2.97 (triplet-doublet appearing as a quartet, 16H, *J* = 6.6 Hz), 3.21-3.40 (m, 12H), 3.87 (t, 16H, *J* = 6.6 Hz), 3.94 (t, 8H, *J* = 6.3 Hz), 4.06 (t, 4H, *J* = 6.6 Hz), 6.48 (s, 4H), 6.52 (s, 2H), 6.56-6.71 (m, 7H), 6.88 (s, 8H), 6.94 (s, 4H) 7.92-8.24 (m, 9H), 8.24-8.39 (m, 6H), 10.6 (s, 1H). Anal. calcd for $C_{147}H_{261}N_{15}O_{37}$: C, 63.73; H,7.31; N,7.58. Found: C,63.40; H,7.25; N,7.59. MALDI-TOF MS: Calcd.: $[M]^{+}$ = 2768.4, Found: $[M+Na]^{+}$ = 2790.5.

Synthesis of Third Generation Dendron (G3)

Protected-G3 (0.260 g, 0. 0939 mmol) was dissolved in TFA (5 ml) and stirred at room temperature for 1.5 h. The solvent was evaporated to dryness and ether was added. The

precipitate was collected and dried *in vacuo* at 80 ^{o}C to give a gray powder (0.254 g, 94% yield). IR (KBr, cm^{-1}): 1172 (Ar-O-Alkyl), 1203 (C-F), 1592 (C=O, carboxylate), 1681 (C=O, amide), 2700-3200 (N-H, ammonium), 3440 (N-H, amide). ^{1}H NMR (DMSO-$_{d6}$, δ, ppm, 40 ^{o}C): 1.88-2.12 (m, 28H), 2.96 (t, 16H, *J* = 6.7 Hz), 3.36-3.52 (m, 12H), 4.00-4.20 (m, 28H), 6.60-6.66 (m, 6H), 6.78 (s, 1H), 7.04 (s, 12H), 8.03-8.34 (m, 9H), 8.42-8.55 (m, 6H), 10.70 (s, -CON*H*-, 1H). Anal. calcd for $C_{123}H_{145}F_{24}N_{15}O_{37}$ 2.39 H_2O: C,50.51; H, 5.16; N,7.18. Found: C,50.07; H, 5.09; N, 7.63.

Preparation of G1 - G3 Solutions and pDNA Sensing Studies

The sample solutions were prepared by dissolving certain amount of pyrene-dendrons in double distilled water with gentle shaking and stored at 4 ^{o}C in the dark before titration. For the pDNA titration, the sample solutions were prepared to 5 μM. To 2 mL of sample solution was added 0.5 to 5 μL aliquots of pDNA stock solutions. Upon each addition, the solution was stirred for 2 minutes to reach equilibrium. UV-vis spectrum and fluorescence spectrum were subsequently monitored. The fluorescence spectra were excited at 340 nm for G1 and G2 and 347 nm for G3. No obvious influence on both UV-vis and fluorescence spectra was observed due to the limited water volume added.

Procedure of pH Responses of G1 - G3 Solutions

The sample solutions were prepared to 5×10^{-4} M by dissolving certain amount of pyrene-dendrimers in double distilled water with gentle shaking. To 2 mL of sample solution was added 0.5 to 10 μL aliquots of NaOH or HCl aqueous solutions (concentration 5×10^{-3} M and 0.5 M). Upon each addition, the solution was stirred for 2 minutes to reach equilibrium. Fluorescence spectra were subsequently monitored

Procedure to Generate Hydrogel and Preparation of SEM Samples

10 mg of **G1** was dissolved in 1 mL double distilled water with gentle shaking to obtain a clear solution. Then, 57.5 μL of 0.5 M NaOH aqueous solution was added to the mixture to produce the hydrogel immediately. **G2** hydrogel was also obtained using similar procedure. The SEM samples were prepared by dropping 10 μL of sample solution on a pre-cleaned glass substrate and then, added certain amount of NaOH solution on the top of the sample. Keep the SEM samples at room temperature for 30 minutes to generate the hydrogel. Then, the samples were frozen in liquid nitrogen and then freezed dried until complete removal of water.

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Chapter VI

General Conclusion

In Chapter I, the background and outline of this thesis is described.

The results of present studies in this dissertation are summarized as follows.

In Chapter II, a facile synthetic route for a tadpole-shaped polyamide dendrimer, which consists of *N*-alkylated nona (p-benzamide) as a rod block and the amine-terminated dendritic block based on 3,5-diaminobenzoic acid, was developed based on the findings of the synthetic approach which was previously developed in our group. The rod block was prepared by an accelerate approach using thionyl chloride as an activating agent and trifluoroacetamide as the protecting group which could be deprotected selectively. The dendritic block was synthesized by a facile divergent method in which condensation and deprotection were carried out in one-pot. The tadpole shaped dendrimers were easily purified only by extraction, recrystallization and precipitation.

In Chapter III, the end functionable aromatic polyamide dendrimers with trifluoroacetamide groups at periphery were synthesized by the facile convergent

synthetic method which was previously developed in our group. In addition to the simplicity of the synthetic route, the purification of each dendron and dendrimer was performed only by extraction, recrystallization and precipitation. The trifluoroacetamide groups were easily deprotected by the transamidation reaction with hydrazine to give amine-terminated dendrimers. The end functionalizations of the amine-terminated dendrimers with PEG produced water-soluble aromatic polyamide dendrimers.

In Chapter IV, the convenient convergent synthesis of aliphatic polyamide dendrimers from unprotected AB_2-building blocks using (DBOP) as activating agent was described. In order to prepared each generation dendron without a protection-deprotection procedure, this approach used a two-step method that consists of the activation of carboxylic acid of former generation dendron into the corresponding active amide using DBOP, followed by condensation with AB_2 building block. All the resulting dendrons and dendrimers were purified by using reprecipitation technique.

In Chapter V, the DNA sensory application of the water-soluble cationic polyamide dendrons that were prepared via the divergent method was described. In the method, coupling reactions for the synthesis of the dendrons were conducted by a two-step

method consisting of (i) the activation of a carboxylic acid moiety of the AB_2 building block by DBOP to generate an active amide moiety, and (ii) the condensation of the active amide. All the resulting dendrons could be purified only using reprecipitation technique. The dendrimers show not only DNA sensitivity but also pH-responsibility. Furthermore, the dendrimer form pH-responsive hydrogels.

As described above, we succeeded to develop the rapid and highly efficient synthetic approaches for both aromatic and aliphatic polyamide dendrimers. For aromatic polyamide dendrimers, facile synthetic methods via both divergent and convergent methods have been developed. On the other hand, the facie synthetic method via convergent method has been developed for the synthesis of aliphatic polyamide dendrimers.

Dendrimers are attractive scaffolds for a variety of high-end applications because of their well-defined, unique macromolecular structures. Especially, PAMAM and peptide dendrimers consisting of aliphatic polyamide main chains are one of the most expected materials. However, those dendrimers are still synthesized via tedious multistep procedures including repetitive conversion of the terminal groups or protection-deprotection steps. Thus, those tedious multistep synthetic procedures interfere the widespread usage of dendrimers.

Therefore, it should be applied the facile synthetic method of aliphatic polyamide dendrimers for the synthesis of well-studied aliphatic polyamide dendrimers such as PAMAM and peptide dendrimers. We hope that our facile synthetic method would encourage the widespread usage of dendrimers.

On the other hand, not only facile but also low-cost and environmental friendly synthetic methods of dendrimers would be necessary for the further widespread usage of dendrimers. Atom economical synthetic methods of dendrimers including click-chemistry are getting attention, then. Thermal amidation reactions of carboxylic acids and amines are recently studied by many groups, as low-cost and atom economical synthetic method of amides[1-6]. Therefore, it would be valuable as the future work to develop the facile synthetic method of polyamide dendrimers via thermal amidation.

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Appendix

List of publications (concerning this thesis)

1. "Facile Synthesis of a Tadpole-Shaped Dendrimer Based onN-Alkylated Oligo(p-benzamide)"

   Ito, Y.; Washio, I.; Ueda, M., *Macromolecules,* **2008,** *41* (8), 2778-2784.

2. "Synthesis of a novel water-soluble polyamide dendrimer based on a facile convergent method"

   Endo, K.; Ito, Y.; Higashihara, T.; Ueda, M., *European Polymer Journal,* **2009,** *45* (7), 1994-2001.

3. "Design and synthesis of new cationic water-soluble pyrene containing dendrons for DNA sensory applications"

   Chen, C.-Y.; Ito, Y.; Chiu, Y.-C.; Wu, W.-C.; Higashihara, T.; Ueda, M.; Chen, W.-C., *Journal of Polymer Science Part A: Polymer Chemistry,* **2011,** *50* (2), 297-305. (co-first author)

4. "pH-responsive Dendritic Gelators"

   Chen, C.-Y.; Ito, Y.; Chiu, Y.-C.; Wu, W.-C.; Higashihara, T.; Ueda, M.; Chen, W.-C., *Chemistry Letters*, **2011**, *41*, 92-94. (co-first author)

Appendix

Other Publications

1. "Positive-Working Photoresist Based on a First-Generation Dendrimer Consisting of 3,5-Bis(tetrahydropyranyloxy)benzyl Units"
   Ito, Y.; Higashihara, T.; Ueda, M., *Journal of Photopolymer Science and Technology*, **2008**, 21(6), 799-803.

2. "Synthesis of a linear polymer from an AB2 monomer of 1-(3-phenoxypropyl)piperidine-4-one in trifluoromethanesulfonic acid"
   Tamura, Y.; Ito, Y.; Segawa, Y.; Higashihara, T.; Ueda, M., *Polymer Chemistry,* **2011,** *2* (8), 1644-1647.

List of Presentation (concering this thesis)

1. "Facile Synthesis of Tadpole-Shapted Dendrimer Based on N-Alkylated Oligo(p-benzamide)"
   Ito, Y.; Washio, I.; Ueda, M., The 234rd ACS National Meeting, USA, Boston, August 2007 (poster).

2. "Facile Synthesis of Tadpole-Shapted Dendrimer Based on N-Alkylated Oligo(p-benzamide)"
   Ito, Y.; Washio, I.; Ueda, M., 56th Symposium on Macromolecules, Japan, Nagoya, September 2007 (poster).

3. "Facile Synthesis of Tadpole-Shapted Dendrimer Based on N-Alkylated Oligo(p-benzamide)"

Ito, Y.; Washio, I.; Ueda, M., International Symposium on Advanced Macromolecules and Nano-materials with Precisely Designed Architectures 07'", Japan, Hokkaido, October 2007 (poster).

4. "Synthesis of water-soluble Polyamide Dendrimers for DNA Sensors"

   Ito, Y.; Chen, C. –Y.; Higashihara, T.; Chen, W. –C.; Ueda, M. 58th *Annual Meeting of Society of Polymer Science*, *Japan*, Osaka, May 2011 (poster).

5. "Synthesis of water-soluble Polyamide Dendrimers for DNA Sensors"

   Ito, Y.; Chen, C. -Y.; Higashihara, T.; Chen, W. -C.; Ueda, M. European Polymer Congress 2011, Spain, Granada, June 2011

Other Presentations

1. "Development of Dendrimer Resist for Microlithography"

   Ito, Y.; Shibasaki, Y.; Ueda, M., *56th Annual Meeting of Society of Polymer Science*, *Japan*, Kyoto, May 2007 (poster).

2. "Self-Assembly of Aromatic Amide Dendrons"

   Ito, Y.; Endo, K.; Higashihara, T.; Ikeda, Y.; Nojima, S.; Ueda, M., 57th *Annual Meeting of Society of Polymer Science*, *Japan*, Yokohama, May 2008 (poster).

3. "Synthesis of Dendrimer for Diagnostic Agent"

   Ito, Y.; Higashihara, T.; Ueda, M. 2009 Taiwan-Japan Bilateral Polymer

Symposium, Taiwan, Taipei, April 2009 (poster).

4. “Synthesis of Dendrimer for Diagnostic Agent”

Ito, Y.; Higashihara, T.; Ueda, M. 2010 Tokyo Tech – Asia Materials week, Japan, Fujiyoshida, November 2010 (oral).

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