Development of a Novel Synthetic Method for Aliphatic Ester Dendrimers

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ABSTRACT: Aliphatic ester dendrimers based on 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) growth units were successfully synthesized as a form of dendritic hybrids up to G4. Linear polystyrene supports in the dendritic hybrids were obtained through atom transfer radical polymerization at 130 °C for 12 h using the CuCl/PMDETA catalyst/ligand system with 4-(chloromethyl)benzyl alcohol as the initiator. An optimized molecular weight of 8700 was chosen as the support, considering the difference in solubility between the dendritic hybrids and the growth units as well as the effective characterization of the end functional groups in the ^{1}H NMR analysis. The dendritic repeating units were grown from the benzyl alcohol end functional group of the support via a typical esterification process in methylene chloride for 12−24 h at 25 °C. The resulting hybrids were purified by a simple precipitation into methanol without any chromatographic separation, utilizing the difference in solubility between the poorly soluble dendritic hybrids and highly soluble growth units. At the final step, the dendrimers were obtained via the selective cleavage of the benzyl ester group connecting the polymeric supports and the dendrimers using a paladium(II) acetate catalyst in DMF (10 equiv volume of dendritic hybrid) for 24 h under the hydrogen pressure of 6 atm. The dendritic hybrids and dendrimers obtained with 100% conversion yield were characterized by ^{1}H NMR, ^{13}C NMR, and gel permeation chromatography. It is believed that this simplified purification procedure in the proposed synthesis will be suitable for the production of high-generation dendrimers on a large scale.

1. Introduction

Solid-phase peptide synthesis involves attaching a growing peptide to an insoluble solid support with any unreacted soluble reagents remaining at the end of a synthetic step being removed by a simple wash procedure.[1−3] The easy separation of the product from the reaction mixture using simple washing and filtration makes it possible to use reagents in excess, which allows the reactions to proceed to completion in a minimum time with a high level of conversion. Dendrimer synthesis have many aspects in common with peptide synthesis because the activation step can be achieved by the repetitive removal of the protection groups at the N-terminal of the amino acid for each elongation and the growth step of the peptide chain using a protected amino acid. These time and labor consuming reaction steps are generally associated with a low final yield and purity. Hence, there has been constant drive to develop new and improved strategies for dendrimer synthesis. Divergent and convergent approaches are two general routes for the synthesis of dendrimers.[4−6]

The development of aliphatic ester dendrimers are mainly limited by the synthetic difficulty caused by the degradable nature of the ester groups during the synthetic procedure. The Hult group first reported dendrimer synthesis up to the fourth generation (G4) using the divergent method with 2, 2-bis-(hydroxymethyl)propionic acid as the growth unit.[7] The overall yield for G4 dendrimers (approximately 91%) was very high compared with other dendrimer syntheses, even though each growth step required purification by column chromatography.

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The same dendrimer was grown up to G4 in the form of a dendritic hybrid. Dendritic hybrids are defined as a macromolecule that consists of dendrimers and chemically conjugated linear polymers.[8−11] The Fréchet and Gitsov group reported the rapid and convenient divergent synthesis of dendritic hybrids based on 2, 2-bis(hydroxymethyl)propionic acid up to G4 without the need for chromatographic purification using a simple precipitation employing the differences in solubility between poly(ethylene glycol) conjugated dendritic hybrids and growth units.[12] Even though the final product was not a dendrimer, this method suggests a new way for purifying compounds containing dendrimers.

This paper proposes a novel synthetic route for preparing high generation dendrimers in quantitative yield without any chromatographic purification steps. The dendrimers were grown up to G4 in the form of dendritic hybrids from the chain end of the polymeric support using the divergent method. Purification for each growth and activation step was completed by precipitation only according to Fréchet's method. The major difference from Fréchet's method was that the linear polymeric support was conjugated with the dendrimers using a labile group, which could be decomposed selectively without affecting the interior ester groups presented in the dendrimer. Biodegradable dendrimers up to G4, which were previously reported by Hult group, were successfully synthesized from a polystyrene support as prepared by ATRP with a controlled molecular weight. The synthetic strategy as well as the characterization of the dendrimers obtained is reported.

2. Experimental Section

2.1. Materials. 2,2-bis(methoxy)propanoic acid (98.0%), 4-chloromethyl benzyl alcohol (99.0%), copper(I) chloride (99.9+ %), 1,3-dicyclohexyl carbodiimide (99.0%), *p*-toluenesulfonic acid monohydrate (98.5%), 4-dimethyl amino pyridine (99.0%), am-

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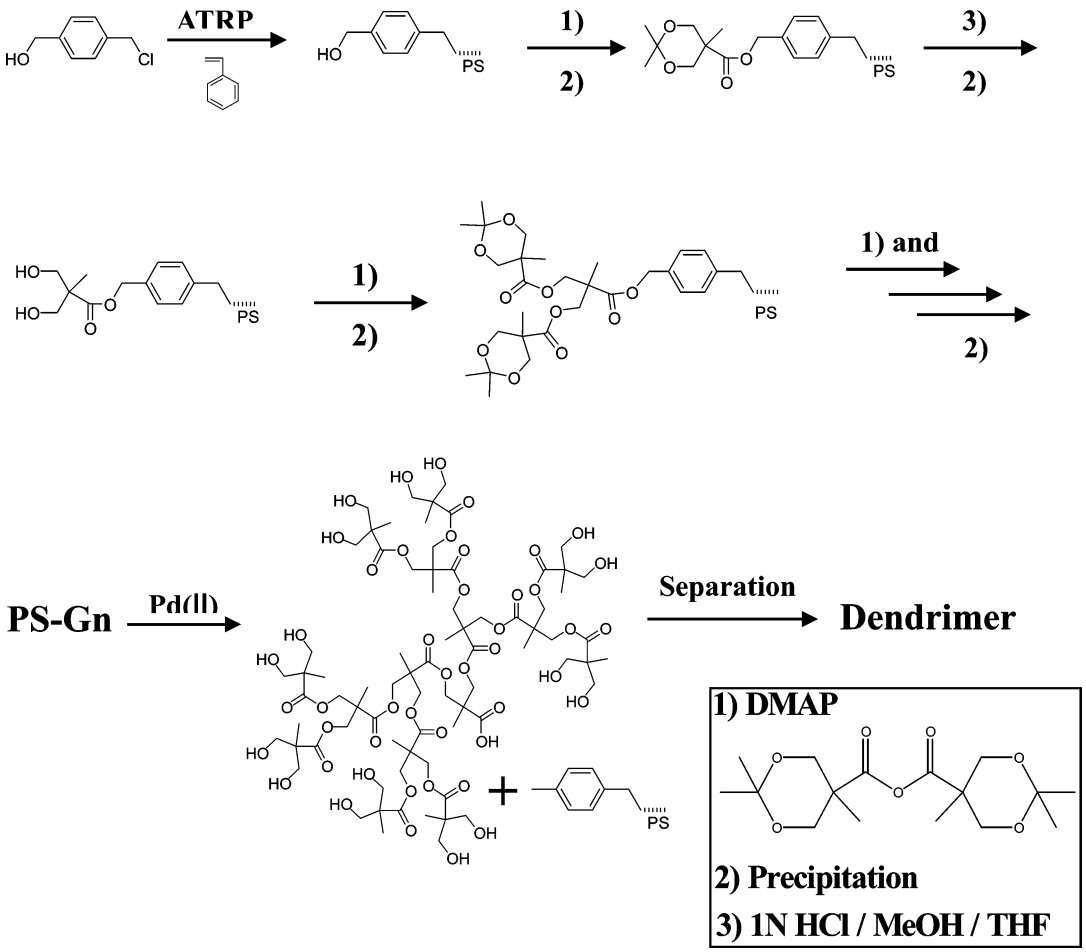
Scheme 1. Proposed Synthetic Procedure for the Synthesis of Dendrimers



monia 2.0 M solution in ethanol (99.0%), 2,2-dimethoxy propane (98%), *N*,*N*,*N*,*N*,*N*-pentamethyl dimethylenetriamine (PMDETA) (99%), palladium(II) acetate (98%), neutral Al_2O_3 (Standard grade, 150 mesh, 58 Å) and *N*,*N*-dimethylformamide (99.9+ %) were purchased from Aldrich. Styrene (99.0%) was obtained from ACROS.

Methylene chloride were dried over calcium hydride and distilled under N_2. Styrene was passed through a column of activated neutral Al_2O_3 to remove the inhibitor. All other chemicals were commercially available and were used as received.

2.2. Instruments. ^{1}H−NMR analysis was performed by Bruker Avance 300 MHz spectrometer in $CDCl_3$ or MeOH-d_4 at room temperature. Molecular weights and distributions were determined by gel permation chromatography (GPC) using Shimadzu RID-10A refractometer detector with Styragel HR 3, HR 4, and HR 4E columns. DMF was used as an eluent with the flow rate of 1 mL/min and polystyrene standards were used for calibration.

2.3. Synthesis of Isopropylidene-2,2-bis(methoxy)propionic Acid Anhydride. 2,2-Bis(methoxy)propionic acid (100.0 g, 74.5 mmol), 2,2-dimethoxypropane (138.0 mL, 1.12 mol), and *p*-toluenesulfonic acid monohydrate (7.1 g, 37.3 mmol) were dissolved in 500 mL of acetone and introduced into a 1-neck round-bottom flask. The mixture was stirred at room temperature for 4 h. After *p*-toluenesulfonic acid was neutralized by 10 mL of NH_3/ethanol (50/50, v/v) solution, the solvent was evaporated under reduced pressure at room temperature. The residue was then dissolved in methylene chloride and extracted three times with water. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was evaporated to produce isopropylidene-2,2-bis(methoxy)propionic acid as white crystals (120.0 g, 93.0%).

Obtained isopropylidene-2,2-bis(methoxy)propionic acid (120.0 g, 688.9 mmol) was dissolved in 600 mL of anhydrous methylene chloride. 1,3-Dicyclohexylcarbodiimide (DCC) (71.1 g, 344.5 mmol) was added to the mixture, and the esterification reaction was continued at room temperature for 12 h under N_2. *N*,*N*-Dicyclohexylurea (DCU) byproduct was filtered off three times as the solvent was condensed and finally evaporated. The residue was further dried under vacuum to obtain isopropylidene-2,2-bis-(methoxy)propionic acid anhydride as a viscous oil (84.0 g, 74.0%).

^{1}H NMR ($CDCl_3$, δ, TMS): δ *1.20* (s, 3H, −C*H*$_3$), δ *1.39* (s, 3H, −C*H*$_3$), δ *1.42* (s, 3H, −C*H*$_3$), δ *3.65* (d, 2H, −C*H*$_2$O), δ *4.18* (d, 2H, −C*H*$_2$O).

2.4. Preparation of Styrene Support. Styrene support was prepared by atom transfer radical polymerization (ATRP) initiated by 4-(chloromethyl)benzyl alcohol. Styrene (63.0 mL, 579.1 mmol) was degassed three times by freeze−pump−thaw cycles in a 2 neck flask equipped with a septum. 4-(Chloromethyl)benzyl alcohol (2.0 g, 12.8 mmol) and copper(I) chloride catalyst (1.3 g, 12.8 mmol) were introduced into the flask under nitrogen atmosphere at room temperature, followed by the addition of *N*,*N*,*N*,*N*,*N*-pentamethyldimethylenetriamine (PMDETA) ligand (2.7 mL, 12.8 mmol) using a microsyringe. Polymerization was continued at 130 °C for 12 h. After the reaction was completed, copper catalyst was removed by passing through activated Al_2O_3 columns. The solution was precipitated three times in methanol and the product was dried under vacuum overnight to produce polystyrene support with the molecular weight of 8700 (40.0 g, 70.0%).

^{1}H NMR ($CDCl_3$, δ, TMS): δ *1.1 − 2.0* (br, −C*H*$_2$CHPh− in polystyrene), δ *4.61* (s, 2H, −OC*H*$_2$Ar), δ *6.2 − 7.4* (br, Ar*H* in polystyrene, Ar*H* in benzyl alcohol)

2.5. General Synthetic Procedure for Dendritic Hybrid of Each Generation. Dendritic hybrid polystyrene support was introduced into a flame-dried round-bottom flask equipped and dissolved in anhydrous methylene chloride, approximately 5 times volume of the polymer. Isopropylidene-2,2-bis(methoxy)propionic acid anhydride (3 equiv mole of hydroxyl groups of polymer

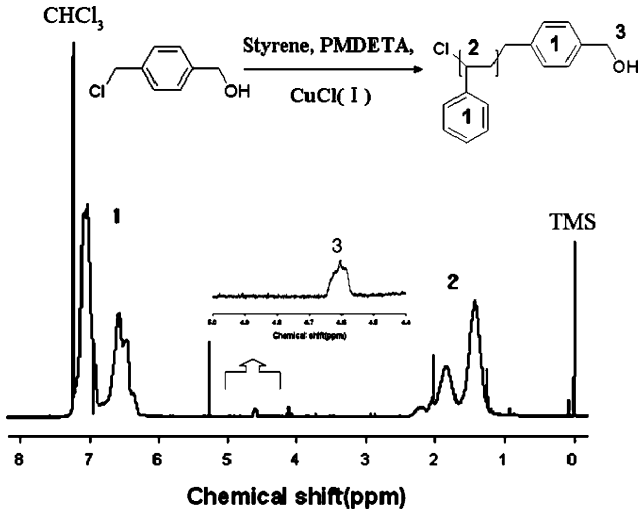


Figure 1. ^{1}H NMR spectrum of the polystyrene support prepared by ATRP.

support) and DMAP(0.2 equiv mole of acid anhydride monomers) were added into the flask to initiate dendritic growth from hydroxyl end group of the polystyrene supports under N_2 atmosphere. Esterification was continued for 12−24 h at 25 °C and the reaction mixture was poured into methanol to selectively precipitate dendritic hybrids as a white powder, while the excess monomer remained soluble in the solvent. The product was then filtered and dried under vacuum overnight at 40 °C.

The obtained dendritic hybrids, protected by dimethyl acetal groups at the periphery, were introduced into a round-bottom flask and dissolved in THF/methanol (80/20, v/v), which was approximately 20 times volume of the hybrids. 1 N HCl was added to the flask for the final concentration of 2 vol % and pH 3. The reaction was continued for 24 h and the mixture was poured into methanol to produce activated dendritic hybrids with the polystyrene molecular weight of 8700 as a white powder. The product were then filtered and dried under vacuum overnight at 40 °C.

2.6. General Synthetic Procedure for Dendrimers from Dendritic Hybrids. Dendritic hybrids and palladium(II) acetate (3 equiv mole of dendritic hybrid) were dissolved in DMF (10 equiv volume of dendritic hybrid) and the reaction mixture was stirred for 24 h under the hydrogen pressure of 6 atm. After the complete selective decomposition between dendrimers and polystyrene support was achieved, palladium(II) acetate catalyst was filtered off through a glass filter. The solution was poured into the methanol to precipitate dendrimer-free polystyrene support, while the dendrimers remained soluble in the solvent. The solvent was rotary-evaporated, and the obtained dendrimers were dried under vacuum overnight at 40 °C.

3. Results and Discussion

Biodegradable aliphatic ester dendrimers up to G4 were synthesized using the divergent method with the growing units of 2,2-bis(hydroxymethyl)propionic acid (bis-MPA). The dendrimer was grown from the polystyrene support in the form of dendritic hybrids and was obtained after the selective decomposition of the connecting groups between the dendrimers in the hybrids and the polymeric supports. During the growth and activation steps, there was no need for purification by column chromatography. Moreover, simple precipitation in cold methanol was sufficient to separate the one generation higher dendritic hybrids from the reaction mixture by employing the difference in solubility between the methanol-soluble low molecular weight

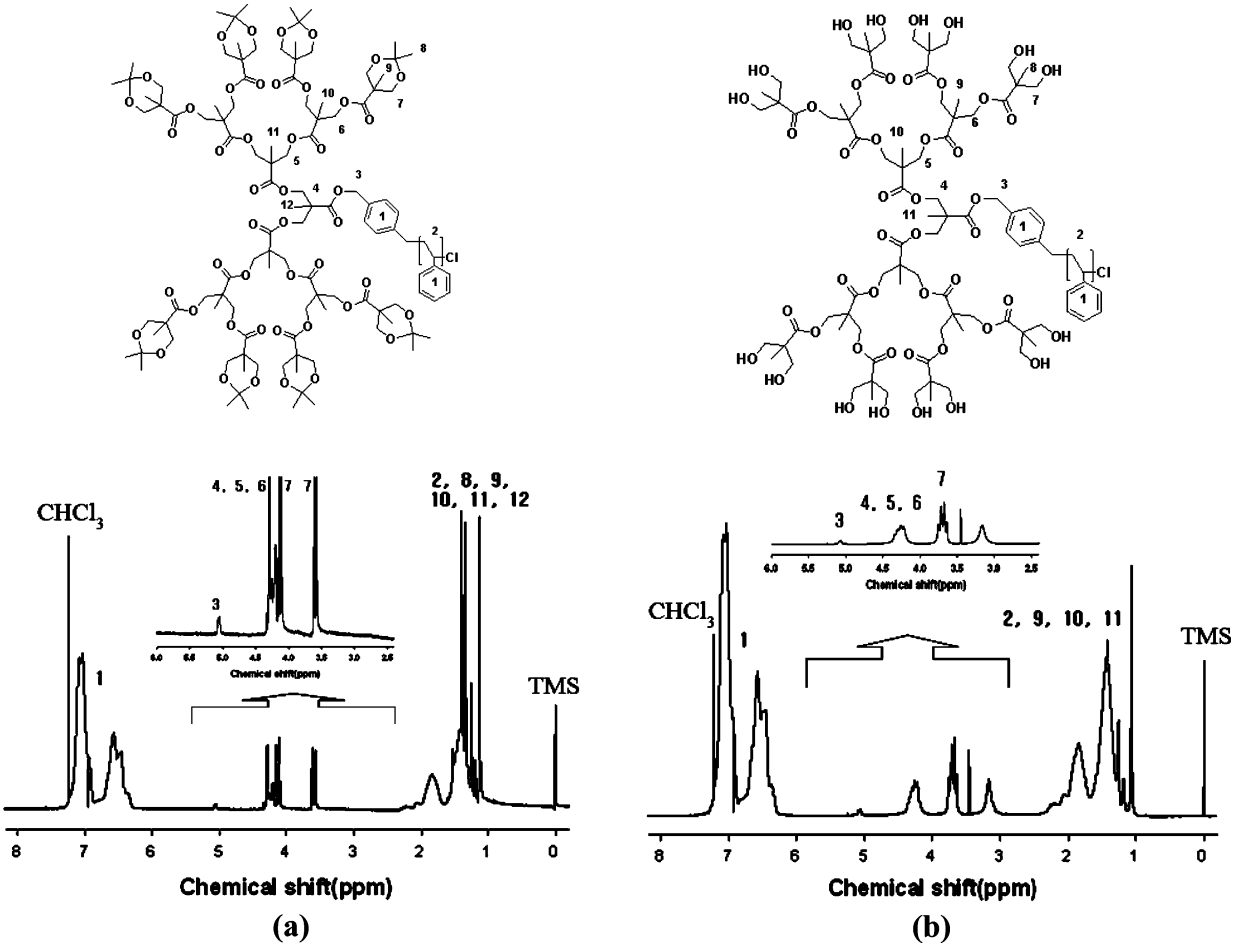


Figure 2. ^{1}H NMR spectra of G4 dendritic hybrid (a) before deprotection and (b) after deprotection.

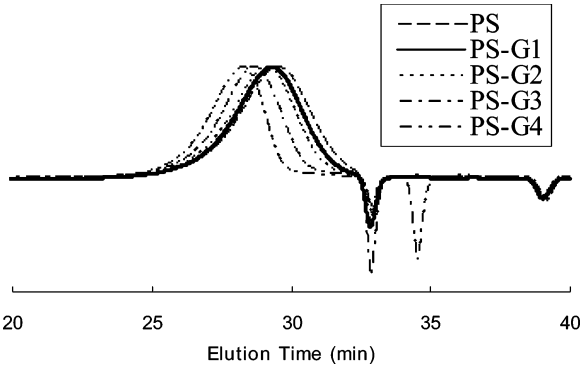


Figure 3. GPC traces of dendritic hybrids using DMF as an eluent.

Table 1. Gel Permeation Chromatography Data of Dendritic Hybrids

hybrids	M_n(calcd) (g/mol)	M_w(GPC)[a] (g/mol)	M_n(GPC)[a] (g/mol)	M_w/M_n[a]	yield[b] (%)
PS	8700[c]	11 500	9600	1.20	70
PS-G1	9600[d]	12 700	10 600	1.20	95
PS-G2	10 600[d]	14 900	12 400	1.20	94
PS-G3	12 500[d]	17 500	15 500	1.13	95
PS-G4	16 200[d]	24 300	21 700	1.12	93

[a] GPC with polystyrene standards and DMF as an eluent. [b] On the basis of isolation yield. [c] Calculated by ^{1}H NMR. [d] Calculated by the combination of the molecular weight of PS by ^{1}H NMR and formula weight of each generation dendrimer.

growing units and the methanol-insoluble high molecular weight dendrimer bound polystyrene supports. Scheme 1 gives an illustration of the synthetic procedure.

Linear polystyrene, instead of covalently cross-linked polystyrene beads in solid-phase peptide synthesis, was used as the support. The molecular weight of the polystyrene support was controlled by atom transfer radical polymerization (ATRP) at 130 °C using the CuCl/PMDETA catalyst/ligand system with 4-(chloromethyl)benzyl alcohol as the initiator.[13−15] Intact benzyl alcohol end groups were analyzed by ^{1}H NMR spectroscopy, as shown in Figure 1, where the methylene resonance of benzyl alcohol appeared at 4.6 ppm. The benzyl alcohol group was used as a reaction site on the polymeric support for esterification with the growth units. The degree of polymerization was determined using ^{1}H NMR spectroscopy based on the peak integration ratio between the aromatic protons of styrene at 6.2−7.1 ppm and the methylene peak of benzyl alcohol at 4.6 ppm.

Since the difference in solubility is a key factor in dendrimer synthesis, the molecular weight of the hydrophobic polystyrene support plays an important role. Separation of the dendritic hybrids from the excessively employed hydrophilic growth units by precipitation into a selective solvent became more efficient with increasing molecular weight of the polystyrene regardless of the generation number due to the increased difference in solubility between the two reactants. However, the qualitative characterization of the end groups by ^{1}H NMR to confirm the completion of the growth and the activation of the deprotection steps became more difficult with increasing molecular weight. After several trials, polystyrene with a molecular weight of 8700 was chosen as the support for the synthesis of dendritic hybrids up to G4.

Different types of growth units were used for dendritic growth. In the first place, bis-MPA acetal acid was used as the growth unit for esterification with the hydroxyl groups of the polystyrene support using DCC and DPTS (4-(dimethylamino)-pyridinium *p*-toluenesulfonate).[16] Dendritic growth up to G3 was

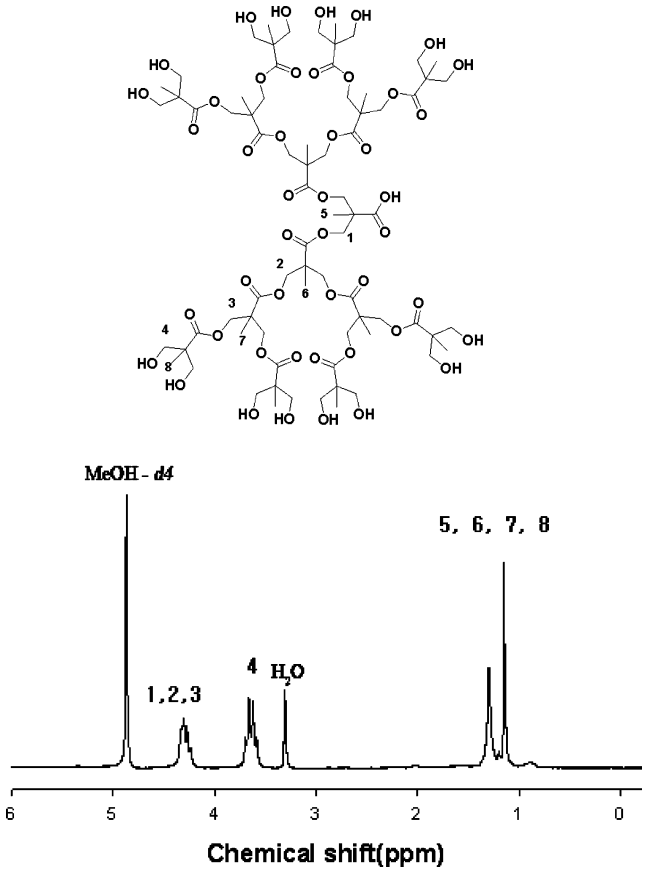


Figure 4. ^{1}H NMR spectrum of G4 dendrimer.

achieved in reasonable yield. However, the esterification required a long reaction time for complete conversion probably due to the decreasing reactivity between the carboxylic acid and hydroxyl groups of the dendritic hybrid with increasing size at each successive generation. It took more than 2 weeks for complete esterification in the case of growth from G3 to G4. In addition, qualitative separation yield was not achieved regardless of the high conversion yield, because of the unavoidable product loss through repeated precipitation to remove the DCU byproduct. Highly reactive carboxyl chloride was used in the esterification via an in-situ reaction between the bis-MPA acetal acid and thionyl chloride or oxallyl chloride. Esterification itself was successful with the carboxyl chloride but the HCl byproduct decomposed the acetal protecting group of the growth units, leading to the formation of uncontrolled hyperbranched polymers.

With the limitations mentioned above, the growth units with the anhydride form were used based on the higher reactivity compared with the carboxylic acid without producing any acidic byproduct. Esterification with the anhydride resulted in the complete conversion within 24 h. Dendritic hybrids up to G4 were synthesized using the divergent approach in almost quantitative yield because there was no need to remove the DCU by repeated precipitation. The dimethoxy-protected groups were completely deprotected by acid-catalyzed hydrolysis at pH 3 without degrading the ester groups in the growth units, and the products were used for the reaction to obtain the next generation of dendrimers.

The complete conversion for the growth step was confirmed by the presence of methyl units at the peripheral acetal-protecting groups at 1.1−1.5 ppm in the ^{1}H NMR spectrum.[17] The vinyl protons of polystyrene overlap with the methyl peaks of the acetal-protecting group. Therefore, the methylene protons

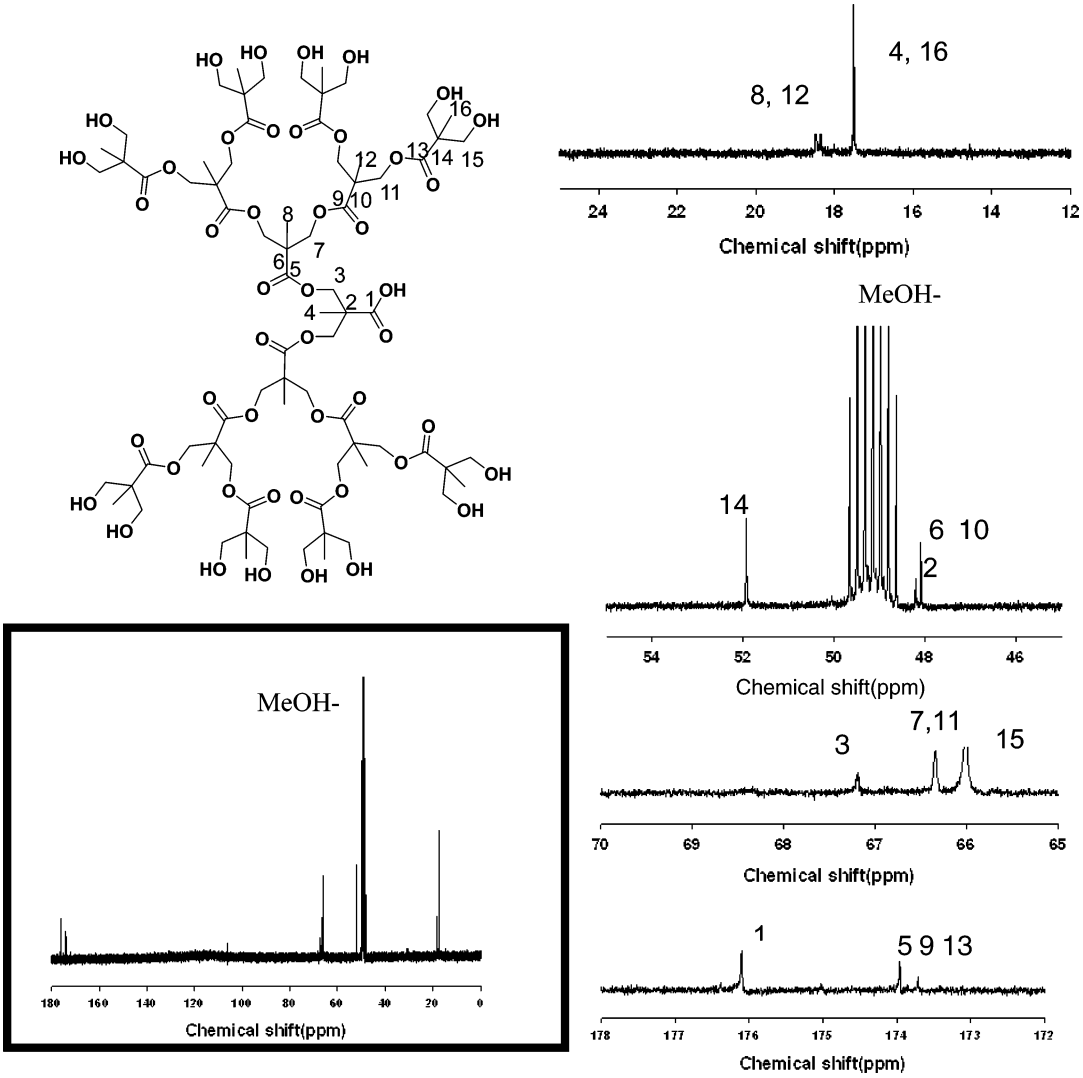


Figure 5. ^{13}C NMR spectrum of G4 dendrimer.

of the dendritic unit were used to follow the complete growth reactions instead. The methylene resonances of the acetal-protecting groups on the surface of the dendrimers appeared at 3.65 and 4.18 ppm, which were quite different from the methylene peaks at the interior growing units of the dendrimers (4.3 ppm). In the activation step, the degree of peak shift of the methylene units from 4.38 and 4.18 ppm to 3.70 and 3.80 ppm indicated the degree of deprotection. Figure 2 shows the representative ^1H NMR spectra of the G4 dendritic hybrids before and after the activation step.

The dendritic hybrids were characterized by GPC with DMF as the eluent. Figure 3 shows the GPC traces and Table 1 summarizes the characterization results. The narrow M_w/M_n values in Table 1 and the monodisperse GPC traces along with the shift in the peak for the maximum molecular weight, which is dependent on the generation number, confirmed the successful formation of the dendritic hybrids.

The dendrimers were obtained by the selective cleavage of benzyl ester groups between the polystyrene supports and dendrimers without affecting the ester groups in the growth units.[18] The dendritic hybrid was deprotected before the cleavage reaction because the polystyrene and dimethoxy-protected dendrimers had a similar solubility, which made separation difficult by precipitation. Palladium, 10 wt % on activated carbon, and trifluoroacetic acid were initially used as a catalyst for the deprotection step, but no significant cleavage was obtained. Palladium(II) acetate, in the place of palladium, successfully cleaved the benzyl ester bond through catalytic hydrogenolysis in DMF at room temperature overnight under a medium pressure of H_2. The conversion yield was quantitative but the isolation yield was approximately 80% for each generation due to product loss during the workup process after the filtration step to remove the catalyst. The reaction mixture was precipitated into methanol and the precipitated polystyrene was filtered out. The solvent was evaporated and the final product was obtained by dialysis against methanol.

Figure 4 shows a representative ^1H NMR spectrum for the G4 dendrimer. Successful formation of each generation of dendrimer was confirmed by the peak integration ratio between the methylene peaks from the surface of the dendrimers at 3.70 ppm and those from the interior dendritic growth units at 4.20 ppm. The integration ratio of these two peaks also confirmed the intact interior ester groups.

Figure 5 shows a ^{13}C NMR spectrum of the G4 dendrimer. It is known that the ^{13}C NMR chemical shifts of the carbons in the dendrimers are strongly dependent on the generation number. The methyl carbons appeared at 17.51, 18.02, 18.35, and 18.49 ppm, and the quaternary carbons were observed at 48.09, 48.21, 49.60, and 51.92 ppm with each generation number, respectively, which also supported the successful formation of the G4 dendrimers. In contrast, the chemical shifts of the methylene (66.01, 66.34, and 67.19 ppm), carbonyl (173.71 and 193.93

ppm) and carboxyl carbons (176.10 ppm) were not dependent on the generation number.

4. Conclusions

Aliphatic ester dendrimers were successfully synthesized up to G4 in the form of dendritic hybrids. The optimized molecular weight of the polystyrene supports was approximately 8,700, which were prepared using atom transfer radical polymerization using the CuCl/PMDETA catalyst/ligand system with 4-(chloromethyl)benzyl alcohol as the initiator. The anhydride form of 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) was selected as the growth unit, considering the improved reactivity compared with the carboxylic acid form and the absence of an acidic byproduct that would degrade the acetal-protecting dimethoxy groups. Dendritic hybrids were grown from the benzyl alcohol end functional group of the support and were purified using a simple precipitation method without the need for chromatographic separation. The difference in solubility played an important role in separating the excess highly soluble growing units from the poorly soluble dendritic hybrids. The dendrimers were obtained by the selective cleavage of the benzyl ester group connecting the polymeric supports and dendrimers using palladium(II) acetate catalyst under a H_2 atmosphere. ^{1}H NMR, ^{13}C NMR, and gel permeation chromatography confirmed the successful formation of the G4 dendrimers. Overall, the proposed synthetic method opens a novel way for preparing high generation dendrimers without the need for time and labor consuming purification steps.

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