One-Pot Synthesis of Hyperbranched Polyamines Based on Novel Amino Glycidyl Ether

Gyunhyeok Ahn,[1†] Songa Kweon,[1†] Chungmi Yang,[2] Ji Eun Hwang,[2] Kyuseok Kim,[2] Byeong-Su Kim [1]

[1]Department of Chemistry, Ulsan National Institute of Science and Technology (UNIST), Ulsan 44919, Republic of Korea
[2]Department of Emergency Medicine, Seoul National University Bundang Hospital, Seongnam, Gyeonggi-do 13620, Republic of Korea
Correspondence to: B. S. Kim (E-mail: bskim19@unist.ac.kr)

Received 22 August 2017; accepted 12 September 2017; published online 00 Month 2017
DOI: 10.1002/pola.28865

ABSTRACT: We report a one-pot synthesis of hyperbranched polyglycerols possessing amino functionality by using a novel Boc-protected aminoethanol glycidyl ether monomer (BAG). A series of hyperbranched Boc-protected polyamino glycerols (PBAG) were prepared through a one-pot anionic ring opening multibranching polymerization to yield PBAG with controlled molecular weights (3500–17400 g/mol). Subsequent deprotection of PBAG yielded hyperbranched polyamino glycerols (PAG) with a globular polymeric structure that comprises a randomly branched structure with a large number of functional amine and hydroxyl groups. ^{1}H, ^{13}C, and ^{15}N-NMR, GPC, and MALDI-TOF measurements confirmed the successful polymerization of the hyperbranched PAG polymers. With its superior biocompatibility of PAG, we anticipate the prospective potentials for the applications in biological and biomedical fields. © 2017 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2017**, *00*, 000–000

KEYWORDS: polyglycerol; hyperbranched; ring-opening polymerization; synthesis design; cytotoxicity

INTRODUCTION Polyamines have attracted significant attention due to their wide range of functions in industry and biotechnology.[1–3] Polymers containing amine groups such as poly(ethylene imine) (PEI) and poly(propylene imine) are promising CO_2 absorbent due to their high amine content.[4,5] Polyamines are also effective chelating agents used to dissolve metal ions in organic solvents and used as a hardener with epoxy resin.

In nature, polyamines are essential molecules supporting the structure, conformation, and function of many key biological molecules including nucleic acids and proteins. Naturally occurring polyamines such as spermine and spermidine are involved in cell growth, maintenance of membrane stability, regulation of programmed cell death and free radical scavenging.[6,7] Additionally, with their cationic properties under physiological conditions, polyamines possess high potential as a vector for gene therapy. As a notable example, PEI is commonly used as a gene transfer vector with a high transfection efficiency due to the proton sponge effect;[8,9] however, this high charge density often leads to significant toxicity to cells.[10,11] Thus, various methods have been proposed to lower the cytotoxicity of cationic polyamines.[12] For example, poly(ethylene glycol) (PEG) has often been conjugated to reduce its toxicity by shielding the positive charges.[13,14]

As an alternate approach, novel amine-containing monomers are introduced to synthesize polyamines with a biocompatible polyether backbone. For example, Frey and coworkers have reported various amine containing monomers such as *N*,*N*-dibenzyl amino glycidol,[15] *N*,*N*-diallyl glycidyl amine,[16] *N*,*N*-diethyl glycidyl amine,[17] and epicyanohydrin.[18] Moller and coworkers also reported poly(glycidol-*co*-glycidyl amine), and poly(glycidol)-*b*-poly(glycidyl amine).[19] In another worthwhile effort, Satoh and co-workers have reported the preparation of polyethers with various pendant amine groups using *N*,*N*-disubstituted glycidyl amine derivatives.[20] Our group has also recently reported the use of protected butanolamine glycidyl ether for copolymerization with glycidol to enhance the biocompatibility of the resulting polyamines.[21] Many of the polymers developed thus far retained the primary amine groups and polymerized via the glycidyl amine monomer, leading to the synthesis of linear polyamine

†Gyunhyeok Ahn and Songa Kweon contributed equally to this work.

Additional Supporting Information may be found in the online version of this article.

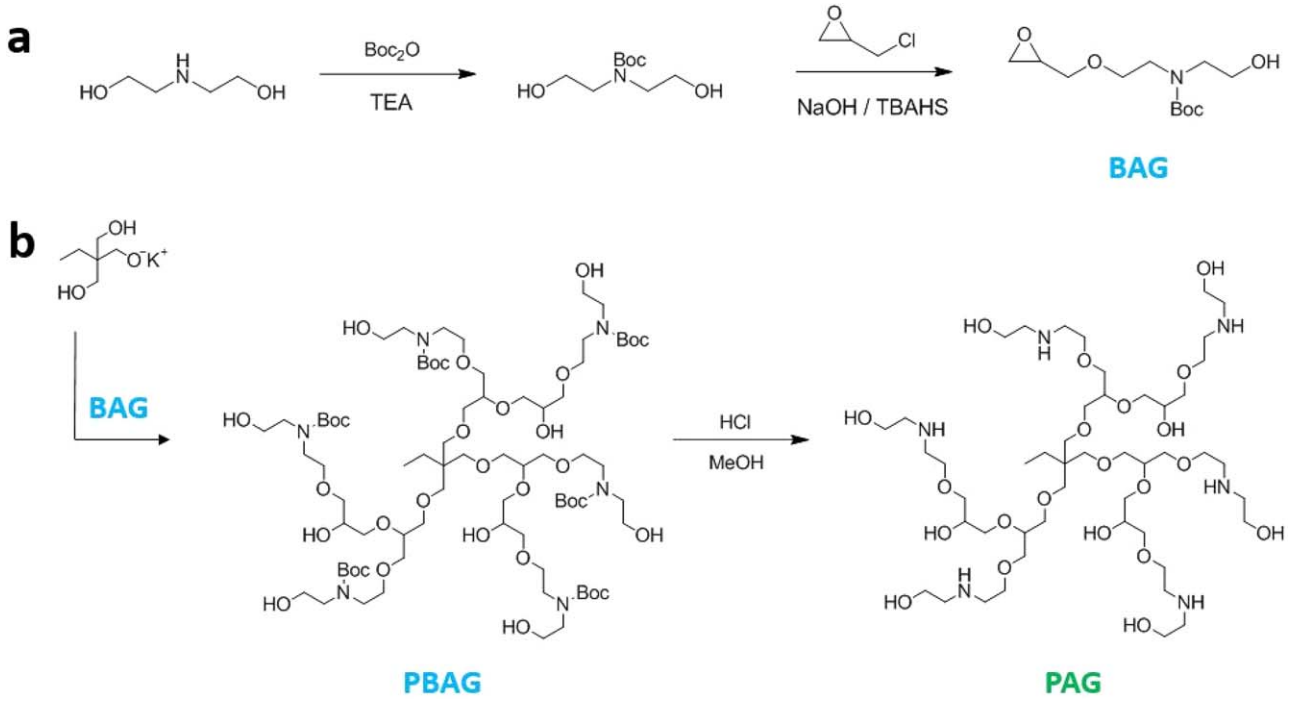

FIGURE 1 Synthetic scheme of (a) the BAG monomer and (b) the anionic ring-opening polymerization of PBAG and subsequent deprotection to yield PAG. [Color figure can be viewed at wileyonlinelibrary.com]

with a polyether backbone. In contrast to these previous reports, here, we focused on the synthesis of hyperbranched polyamines with a protected monomer approach.

In continuation of our endeavor to develop functional hyperbranched polyethers for biomedical applications, herein we report the one-pot synthesis of hyperbranched polyglycerols possessing amino functionality by using a Boc-protected aminoethanol glycidyl ether monomer (BAG; Fig. 1). Specifically, *t*-butyl (2-hydroxyethyl)(2-(oxiran-2-ylmethoxy) ethylcarbamate was designed and polymerized through an anionic ring-opening multibranching polymerization to yield a series of hyperbranched PBAG with controlled molecular weights (3500–17400 g/mol) and relatively low molecular weight distributions. Subsequent deprotection of PBAG yielded amino-containing hyperbranched polyamino glycerols (PAG) with a globular polymeric structure that comprises a randomly branched structure with a large number of amine and hydroxyl groups. We also demonstrated the superior biocompatibility of the prepared PAG via a cell viability assay.

EXPERIMENTAL

Materials

All reagents and solvents were purchased from Sigma-Aldrich and Acros unless otherwise stated. Deuterated NMR solvents such as $CDCl_3$ and D_2O were purchased from Cambridge Isotope Laboratory.

Measurements

^{1}H- and ^{13}C-NMR spectra were measured using a 400-MR DD2 (400 MHz) spectrometer with $CDCl_3$, D_2O and DMSO-d_6 as solvents, and chemical shifts were recorded in ppm units with TMS as an internal standard. The weight-averaged (M_w) molecular weights and molecular-weight distribution (M_w/M_n) were measured using gel permeation chromatography (GPC, Agilent Technologies 1200 series) with a poly(methyl methacrylate) (PMMA) standard and dimethylformamide (DMF) as an eluent at 30 °C with a flow rate of 1.00 mL/min. ^{15}N NMR spectra was measured using a Varian VNMRS 600 MHz NMR spectrometer with DMSO as solvents and formamide as a standard. Matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF) measurements were carried out on an Ultraflex III MALDI mass spectrometer. α-Cyano-4-hydroxycinnamic acid was used as the matrix. A 10 g/L solution of the polymer in acetonitrile and 10 g/L solution of the matrix solution were prepared separately. A 1.0 μL aliquot of the mixture was applied to a target plate, and the solvent was evaporated before measurement. Differential scanning calorimetry (DSC) was performed using a DSC (Q200 model, TA Instruments) in the temperature range from −80 to 20 °C at a heating rate of 10 K/min under nitrogen. The zeta potential was measured using a Malvern Zetasizer Nano-ZS (ZEN3600, Malvern, UK).

Protection of Diethanolamine

The precursor, *t*-butyl bis(2-hydroxyethyl)carbamate was synthesized similar to the literature protocol with slight modifications.[22] A solution of di-*tert*-butyl-dicarbonate (22.9 mL, 99.9 mmol) in CH_2Cl_2 (50 mL) was added to a solution of diethanolamine (10 g, 95.1 mmol) and triethylamine (TEA; 13.9 mL, 99.9 mmol) in CH_2Cl_2 (30 mL) dropwise over 1 h using a dropping funnel at room temperature. The mixture was stirred

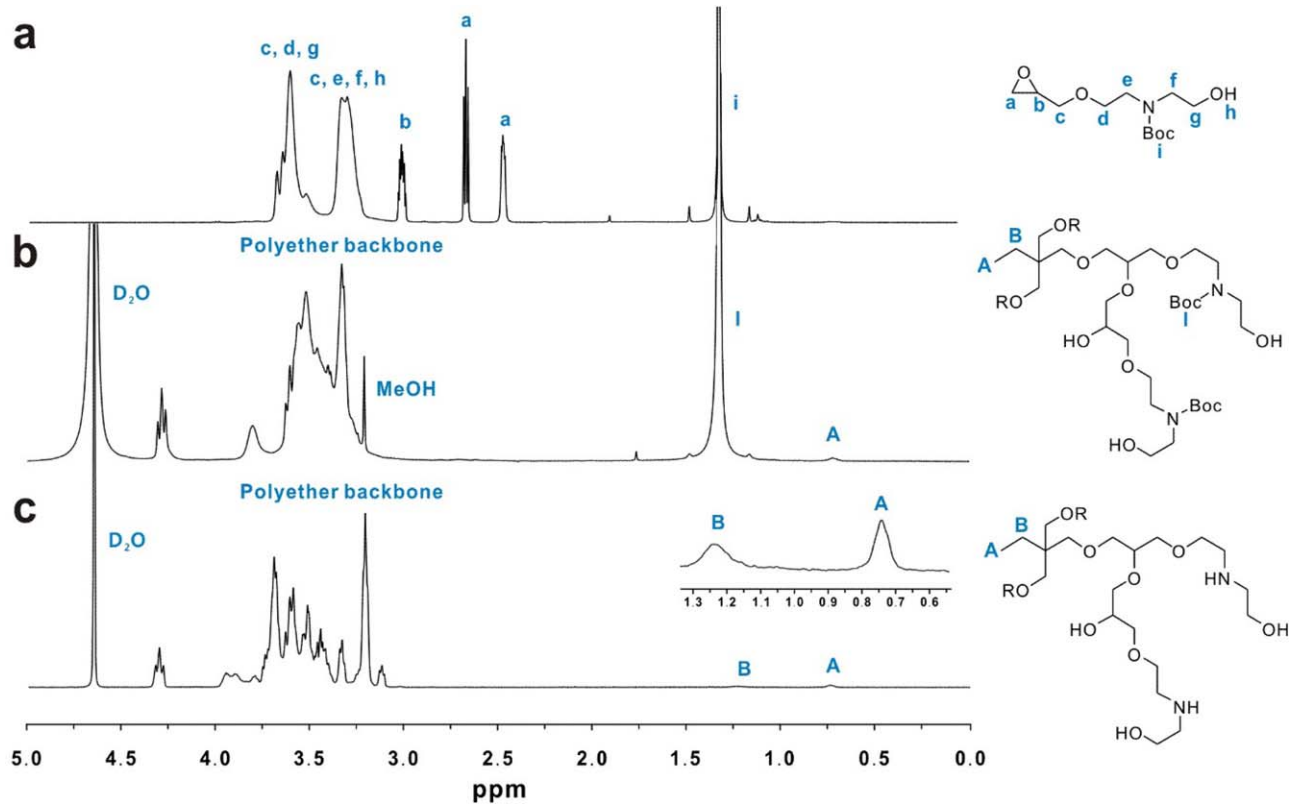

FIGURE 2 ^{1}H NMR spectra of (a) the BAG monomer measured in $CDCl_3$, (b) the $PBAG_{66}$ polymer, and (c) the deprotected PAG_{66} polymer (polymer 5) measured in D_2O. [Color figure can be viewed at wileyonlinelibrary.com]

at room temperature for 6 h, diluted with CH_2Cl_2, and extracted with water and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography with 10% hexane in ethyl acetate as the eluent to obtain a pure compound as pale-yellow oil (13.67 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 3.85 (d, 6H, $J = 55.9$ Hz), 3.42 (s, 4H), 1.46 (s, 9H).

Synthesis of BAG Monomer

An aqueous solution of sodium hydroxide (3.90 g, 50 wt %), epichlorohydrin (10.7 g, 116 mmol) and tetrabutylammonium hydrogen sulfate (TBAHS, 1.65 g, 4.87 mmol) was stirred at 0 °C. Then, a solution of *t*-butyl bis(2-hydroxyethyl)carbamate (10 g, 48.7 mmol) in THF (30 mL) was slowly added dropwise over 30 min and stirred at room temperature for additional 15 h.[23] To this reaction mixture, CH_2Cl_2 was added to extract the product and washed with water and brine to neutrality. The organic layer was dried with anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash column chromatography with 17% hexane in ethyl acetate to give the BAG monomer as a pale-yellow viscous liquid (4.1 g, 32%). The synthesis of the BAG monomer was successfully confirmed via various spectroscopic and mass analyses, including ^{1}H and ^{13}C NMR and COSY spectroscopy (see Fig. 2 and Figure S1–S3 in the Supporting Information) and ESI-MS. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 3.88–3.21 (m, 11H), 3.21–3.06 (m, 1H), 2.88–2.73 (m, 1H), 2.62 (s, 1H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 156.03, 80.06, 77.11, 71.69, 70.34, 62.20, 52.21, 50.55, 48.97, 43.92, 28.32. MS ($m/z + Na^+$, ESI^+) calcd for $C_{12}H_{23}NO_2$ 284.3, found 283.9.

Synthesis of PBAG (Polymer 2)

1,1,1-Trimethylolpropane (TMP; 26.8 mg, 0.2 mmol) was placed in a one-neck round bottom flask. Potassium methoxide in methanol (25.0 wt %, 22.4 μL, 0.0758 mmol) was diluted with 0.70 mL of methanol and then added to the flask and stirred for 30 min at room temperature under an argon atmosphere. Methanol was removed with high vacuum for 4 h at 60 °C to yield a white salt, the initiator. The flask was purged with argon and heated to 90 °C. A *t*-butyl (2-hydroxylethyl)(2-oxiran-2-ylmethoxyl) ethyl carbamate (BAG) (1.0 g, 19.1 mmol) monomer was added slowly over 12 h via a syringe pump. After complete addition of the monomer, the solution was stirred for additional 36 h. The resulting homopolymer was quenched by adding 1.0 mL of methanol; the polymer solution was then precipitated into cold diethyl ether, and the precipitate was washed twice using diethyl ether. The resulting $PBAG_{17}$ polymer was dried under vacuum at 60 °C for 1 day. The M_n of $PBAG_{17}$ (polymer 2) was 4524 g/mol, as calculated from the NMR data (see Figure S4 in the Supporting Information) using the following equation: number of repeating units (BAG) = 218.39 (integration value of polyether backbone)/13 (number of protons of polyether backbone) = 16.80; M_n = 261.32 (molecular weight of the BAG monomer) × 16.80 + 134.17 (molecular weight of TMP) = 4524.35 g/mol.

TABLE 1 Characterization Data for PAG Polymers Synthesized

	M_n (NMR)		PBAG		T_g (°C) (DSC)		
Polymer Composition (NMR)[a]	PBAG	PAG	M_w[b]	M_w/M_n[b]	PBAG	PAG	Zeta Potential (mV)
PAG_{13}	3500	2300	3400	1.19	−28.4	−33.5	14.1 ± 1.0
PAG_{17}	4500	2800	4100	1.25	−15.4	−37.5	16.7 ± 0.7
PAG_{33}	8700	5300	8200	1.39	−14.0	−37.5	19.1 ± 2.2
PAG_{50}	13300	7700	9400	1.67	−21.0	−31.2	17.0 ± 0.9
PAG_{66}	17400	10800	10600	1.83	−25.6	−43.8	23.7 ± 1.4

[a] Composition is determined via ^{1}H NMR spectroscopy.

[b] Measured using GPC-RI in DMF with a PMMA standard.

Considering the error range of NMR integration, we used the 4500 g/mol as a M_n value determined from the NMR. Typical monomer conversion was determined to be between 87 and 95% for all polymers synthesized with isolated yields of around 85% after purification in ether.

Removal of the Boc Protecting Groups

The Boc-protected polyamino glycerols (PBAG) polymer (polymer 2) was dissolved in CH_2Cl_2 with 1.0 mL of 1.0 M hydrochloric acid (HCl) and stirred at room temperature for 2 h. The reaction mixture was removed under reduced pressure and the resulting deprotected polymer was dissolved in 1.0 mL of methanol; the homogeneous polymer solution was then precipitated into excess diethyl ether, and the precipitate was washed twice using diethyl ether. The resulting deprotected PAG_{17} polymer was dried under vacuum at 60 °C for 1 day, which gave a pale-yellow viscous liquid (yield 97%).

Cytotoxicity Assay

Murine macrophage cell line, RAW264.7, was purchased from the Korean Cell Line Bank (Seoul, Korea). Cytotoxicity assays were performed using the traditional WST-1 assay. Cells were seeded in 96-well plates at a density of 1×10^5 cells per well and incubated for 24 h in 5% CO_2 at 37 °C. RAW264.7 cells were cultured with Roswell Park Memorial Institute medium (RPMI; WELGENE) with 10% fetal bovine serum and 1% penicillin − streptomycin. Each well was then treated with various concentrations of PAG solutions (polymer 2 and 4) and incubated for an additional 24 h. For the WST-1 assays, each well was filled with 10 μL of EZ-Cytox (EZ-3000; Dogen bio). After incubation for 1 h, the plates were gently shaken for 1 min before the absorbance was measured. The absorbance of the solution was recorded at a wavelength of 450 nm using 600–650 nm as the reference.

RESULTS AND DISCUSSION

The synthesis of the BAG monomer and the PAG polymer was achieved according to the method described in Figure 1 (see also the section "Experimental"). In the first step, diethanolamine was protected with di-*t*-butyl dicarbonate and TEA in CH_2Cl_2. The converted *t*-butyl bis(2-hydroxyethyl)carbamate was then reacted with epichlorohydrin to obtain a Boc-protected aminoethanol glycidol (BAG), *t*-butyl (2-hydroxyethyl)(2-(oxiran-2-ylmethoxy) ethylcarbamate. The successful synthesis of the BAG monomer was confirmed via various spectroscopic and mass analyses, including ^{1}H and ^{13}C NMR and COSY spectroscopy (see Fig. 2 and Figure S1–S3 in the Supporting Information) and ESI-MS.

After the successful synthesis of the BAG monomer, we performed the anionic ring-opening multibranching polymerization using a potassium alkoxide initiator that was formed via the reaction of TMP and potassium methoxide solution. As demonstrated in previous studies,[24] we employed a slow monomer addition of BAG monomer to the deprotonated TMP initiator and polymerized at 90 °C for 48 h to synthesize the polymers in a controlled manner. The successful polymerization of PBAG polymers was characterized by ^{1}H NMR and GPC measurements (Fig. 2 and Table 1). As shown in Figure 2, the ^{1}H NMR spectra of the BAG monomer and the synthesized polymers revealed their corresponding characteristic proton peaks. Moreover, the M_n value was calculated by the ratio of the peak integrals between the methyl and methylene groups of the TMP initiator (peaks at 0.75 and 1.25 ppm, respectively) and polyether backbone (peaks at 3.0–4.0 ppm).

The Boc-protected PBAGs were treated with HCl for 2 h to yield the desired PAG. Deprotection of the Boc moiety could be clearly monitored in the ^{1}H NMR spectrum by the disappearance of the strong *t*-butyl group signal at 1.34 ppm [Fig. 2(c)]. The synthesized PBAG and deprotected PAG polymers were highly soluble in water as well as organic polar solvent such as methanol, DMSO, and DMF.

Interestingly, after the deprotection, the backbone peaks of the PAG (3.0–4.0 ppm) became sharper than those of PBAG. We postulate that the aqueous solubility of PAG is enhanced upon the removal of the bulky hydrophobic Boc protecting groups.[25]

The synthesized polymers were further characterized using GPC analysis. PBAG was used instead of PAG because the secondary amine group interacted strongly with the solid particles in the column. The GPC results showed a controlled molecular weight with a monomodal distribution (Table 1 and Supporting Information Figure S5). It proved that polymers

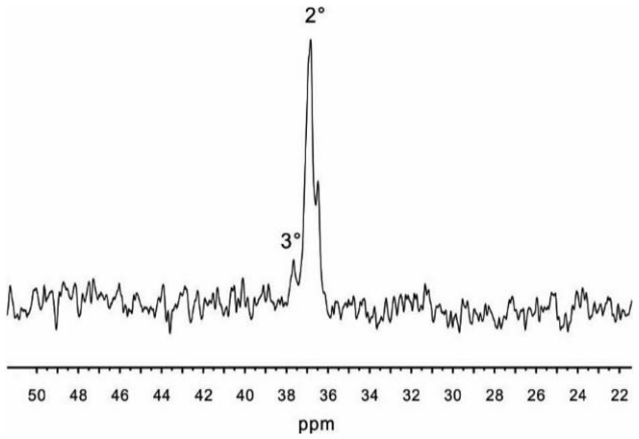

FIGURE 3 Detailed ^{15}N NMR spectrum of PAG_{66} (polymer 5) in DMSO-d_6.

were free from impurities or side products. Specifically, the M_w of the PBAG polymers was found to be 3400–10600 g/mol with a polydispersity index (M_w/M_n) of 1.19–1.83 determined by GPC using PMMA as a standard due to the presence of the hydrophobic Boc protecting group within the hyperbranched structure. In general, the molecular weights of polymers are in good agreement with those determined from ^{1}H NMR; however, there was a discrepancy in the case of the high molecular weight polymers $PBAG_{50}$ and $PBAG_{66}$ (polymer 4 and 5). As longer reaction times are required to synthesize high molecular weight polymers, the harsh reaction conditions (strong base and high temperature) can deprotect the Boc group

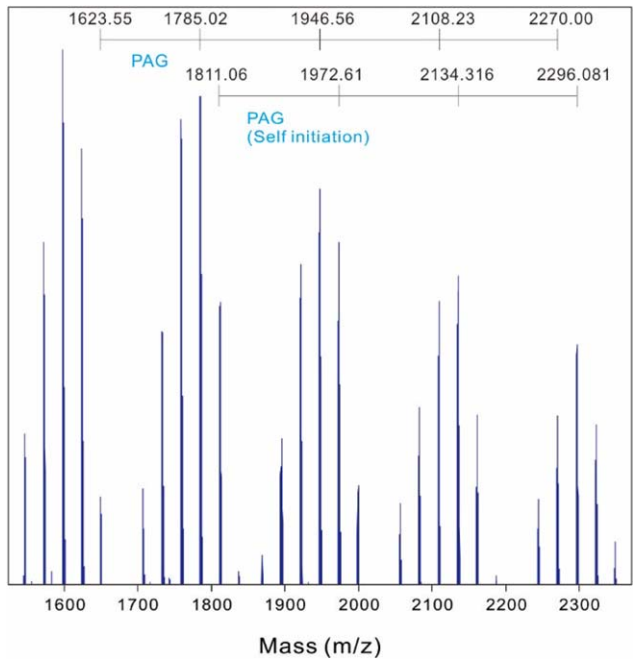

FIGURE 4 Expanded MALDI-TOF mass spectrum of the PAG_{13} (polymer 1) from 1600 to 2300 Da. The spacing of the signals corresponds to the mass of the respective monomers (AG: 161.2 g/mol). [Color figure can be viewed at wileyonlinelibrary.com]

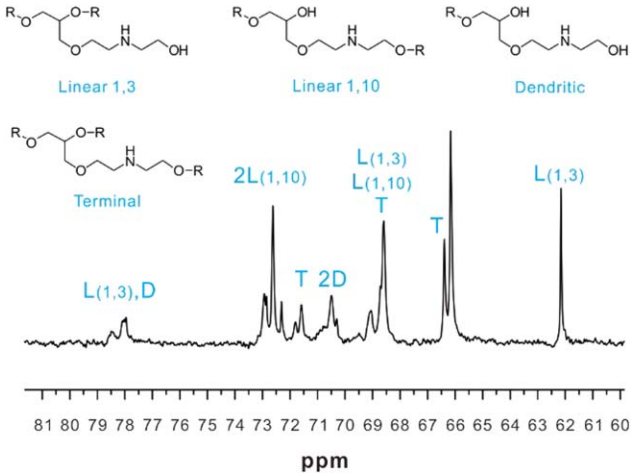

FIGURE 5 Detailed ^{13}C NMR spectrum of PAG_{66} (polymer 5) in DMSO with the assignment of its linear, dendritic, and terminal groups within the structure. [Color figure can be viewed at wileyonlinelibrary.com]

during polymerization. As a result, the secondary amine group might be involved as a reactive group during the polymerization, leading to a denser structure (Supporting Information Scheme S1). A similar phenomenon was observed in our previous report of the Boc-protected butanolamine glycidyl ether system.[16]

To confirm the existence of side reactions, we conducted a model experiment using a model compound, *tert*-butyl diethylcarbamate (Supporting Information Figure S6). Under identical reaction condition, ~5% of the Boc group was deprotected, revealing a potential side reaction during the polymerization.

Moreover, we could identify the presence of tertiary amine group in the polymeric backbone resulting from the potential side reaction of the deprotected Boc group during the polymerization by employing ^{15}N NMR (Fig. 3). As similarly determined in the structure of branched PEI in distinguishing between the secondary and tertiary amine groups,[26] we could monitor the side reaction during the polymerization. However, it should be noted that the fraction of the tertiary amine group is significantly lower than that of secondary amine groups in line with the model reaction conducted.

MALDI-TOF spectrometry was performed to confirm the presence of the TMP initiator and the functional monomer segment in the PAG polymers. As shown in Figure 4, the spacing of the signals corresponds to the mass of the respective monomers in the PAG polymer, which are present to varying degrees, unambiguously demonstrating the successful polymerization of PAG. For example, the mass peak at 1946.56 m/z corresponded to the polymer with TMP as an initiator, 11 units of monomer, and K^+ as a counterion [TMP (134.17) + monomer of PAG (161.2) $\times$ 11 + K^+ (39.1)]. However, the peaks corresponding to polymers of self-initiated PAG was also observed (see Scheme S2 in the Supporting Information). During the polymerization with potassium methoxide, a fraction of the monomer acts as an

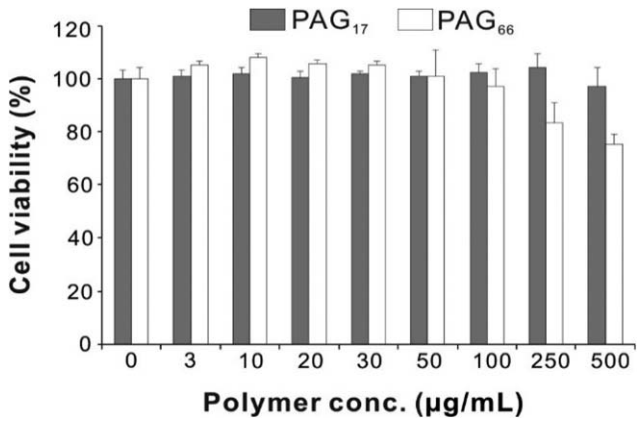

FIGURE 6 *In vitro* cell viability assay of the polymers. (Gray) PAG_{17} (polymer 2) and (white) PAG_{50} (polymer 4) determined by WST-1 assays using RAW264.7 cell lines.

initiator and the polymer formed as a side reaction may have a cyclic form, albeit the use of the slow monomer addition to keep the concentration of the monomers low during the reaction.

Furthermore, the hyperbranched nature of the PAG polymers was assessed by measuring the degree of branching (DB) via a detailed analysis of the ^{13}C NMR spectra (Fig. 5, Supporting Information Figure S7, S8, and Table S1) based on a previously reported equation.[27] The resulting DB indicated the ratio of the branched segment within the PAG backbones. The DB of the selected polymer $PBAG_{66}$ was determined to be $\sim$0.41, which was slightly lower than the conventional hyperbranched polymers (0.4–0.6). We postulated that the longer spacer unit in the BAG monomer limited the branching of terminal hydroxyl group compared with a glycidol monomer.

The thermal properties of the prepared PBAG and PAG polymers were investigated with DSC (Table 1 and Supporting Information Figure S9). According to the DSC measurements, the range of the glass transition temperature, T_g, for PAG polymers was between -31.2 °C and -43.8 °C. Furthermore, the DSC results of the PBAG polymers correlated well with the presence of bulkier substituents, which reduces the rotational freedom of the polymer chains, leading to a higher T_g.

Moreover, upon deprotection to release the free amino groups within the polymers, we confirmed the charge density by measuring the zeta potential. As expected, the synthesized PAG polymers displayed highly positive charges between 14.1 and 23.7 mV, which suggested potential applications as prospective gene delivery carriers (Table 1).

Encouraged by the successful synthesis and the sufficient charge density of the polymers, we evaluated the cytotoxicity of PAGs, such as PAG_{17} and PAG_{50} to investigate their potential in biomedical application. Each polymer was treated with the murine macrophage cell line, RAW264.7 as a model normal cell line. The cytotoxicity of the polymers was examined using WST-1 assay, which are commonly used for in vitro cytotoxicity testing of polymers and nanomaterials. Unlike MTT assay, which requires a solubilizing step, the WST-1 assays have the advantage of higher sensitivity and wider measurement range. As shown in Figure 6, the cell viability of each cell line treated with various concentrations of PAG_{17} was $>$90% up to a concentration of 500 μg/mL. In the case of PAG_{50}, which has more content of amine moieties, the study indicated a moderate toxicity due to its free amine groups; thus the cell viability decreased gradually up to a concentration of 250 μg/mL. Although many polyamines are reported to display significant cytotoxicity due to their free amine groups associated with tight cell binding,[28,29] our PAG polymers exhibited considerably lower cellular toxicity; this is attributable to the amine groups being sheathed by the hyperbranched polyglycerol shell, yielding optimum cell viability.

CONCLUSIONS

In summary, we present a one-pot synthesis of amine-functionalized hyperbranched polyglycerols. A novel protected aminoethanol glycidyl ether monomer (BAG) was designed and polymerized through anionic ring-opening multibranching polymerization to yield a well-defined PBAG with controlled molecular weights and relatively low molecular weight distributions. Subsequent deprotection of PBAG yielded the desired hyperbranched PAG. The polymerization was successfully characterized by ^{1}H, ^{13}C and ^{15}N-NMR, GPC, MALDI-TOF, and DSC measurements. The high zeta-potential together with the high biocompatibility of PAG clearly demonstrate its significant potential for use in biological and biomedical applications, which takes advantage of the free amine groups sheathed in the hyperbranched polyglycerols. We anticipate that the new class of functional epoxide monomer and polymers developed in this study will contribute to the advancement of polyglycerol-based polymers and will be promising candidates for emerging materials and biomedical applications.

ACKNOWLEDGMENTS

This work was supported by the Samsung Research Foundation (SRFC-MA1602-07).

REFERENCES AND NOTES

1 R. A. Casero Jr., L. J. Marton, *Nat. Rev. Drug Discov.* **2007**, *6*, 373.

2 J. Kothandaraman, A. Goeppert, M. Czaun, G. A. Olah, G. K. S. Prakash, *J. Am. Chem. Soc.* **2016**, *138*, 778.

3 R. K. Pinschmidt, *J. Polym. Sci. Part A: Polym. Chem.* **2010**, *48*, 2257.

4 S. H. Pang, L. C. Lee, M. A. Sakwa-Novak, R. P. Lively, C. W. Jones, *J. Am. Chem. Soc.* **2017**, *139*, 3627.

5 X. Xu, J. M. Andresen, C. Song, B. G. Miller, A. W. Scaroni, *Energy Fuels.* **2002**, *16*, 1463.

6 H. C. Ha, N. S. Sirisoma, P. Kuppusamy, J. L. Zweier, P. M. Woster, R. A. Casero, *Proc. Natl. Acad. Sci. USA.* **1998**, *95*, 11140.

7 P. N. Moschou, K. A. Roubelakis-Angelakis, *J. Exp. Bot.* **2013**, *65*, 1285.

8 D. W. Pack, A. S. Hoffman, S. Pun, P. S. Stayton, *Nat. Rev. Drug Discov.* **2005**, *4*, 581.

9 J. Behr, *Int. J. Chem.* **1997**, *2*, 34.

10 D. Fischer, Y. Li, B. Ahlemeyer, J. Krieglstein, T. Kissel, *Biomaterials* **2003**, *24*, 1121.

11 D. Fischer, T. Bieber, Y. Li, H. P. Elsässer, T. Kissel, *Pharm. Res.* **1999**, *16*, 1273.

12 D. Wang, T. Zhao, X. Zhu, D. Yan, W. Wang, *Chem. Soc. Rev.* **2015**, *44*, 4023.

13 J. M. Harris, R. B. Chess, *Nat. Rev. Drug Discov.* **2003**, *2*, 214.

14 H. Petersen, P. M. Fechner, D. Fischer, T. Kissel, *Macromolecules* **2002**, *35*, 6867.

15 B. Obermeier, F. Wurm, H. Frey, *Macromolecules* **2010**, *43*, 2244.

16 V. S. Reuss, B. Obermeier, C. Dingels, H. Frey, *Macromolecules* **2012**, *45*, 4581.

17 V. S. Reuss, M. Werre, H. Frey, *Macromol. Rapid Commun.* **2012**, *33*, 1556.

18 J. Herzberger, H. Frey, *Macromolecules* **2015**, *48*, 8144.

19 J. Meyer, H. Keul, M. Möller, *Macromolecules* **2011**, *44*, 4082.

20 T. Isono, S. Asai, Y. Satoh, T. Takaoka, K. Tajima, T. Kakuchi, T. Satoh, *Macromolecules* **2015**, *48*, 3217.

21 S. Song, J. Lee, S. Kweon, J. Song, K. Kim, B. S. Kim, *Biomacromolecules* **2016**, *17*, 3632.

22 C. Nguyen, G. F. Ruda, A. Schipani, G. Kasinathan, I. Leal, A. Musso-Buendia, M. Kaiser, R. Brun, L. M. Ruiz-Pérez, B.-L. Sahlberg, N. G. Johansson, D. González-Pacanowska, I. H. Gilbert, *J. Med. Chem.* **2006**, *49*, 4183.

23 W. Wang, J. Ding, C. Xiao, Z. Tang, D. Li, J. Chen, X. Zhuang, X. Chen, *Biomacromolecules* **2011**, *12*, 2466.

24 S. Son, H. Park, E. Shin, Y. Shibasaki, B. S. Kim, *J. Polym. Sci. Part A: Polym. Chem.* **2016**, *54*, 1752.

25 S. G. Roy, R. Acharya, U. Chatterji, P. De, *Polym. Chem.* **2013**, *4*, 1141.

26 D. R. Holycross, M. Chai, *Macromolecules* **2013**, *46*, 6891.

27 D. Hölter, A. Burgath, H. Frey, *Acta Polym.* **1997**, *48*, 30.

28 Y. Deng, J. K. Saucier-sawyer, C. J. Hoimes, J. Zhang, Y. Seo, J. W. Andrejecsk, W. M. Saltzman, *Biomaterials.* **2014**, *35*, 6595.

29 F. S. Mehrabadi, O. Hirsch, R. Zeisig, P. Posocco, E. Laurini, S. Pricl, R. Haag, *RSC Adv.* **2015**, *5*, 78760.