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# Regio-specific size, shape and surface chemistry designed dendrimers based on differentiated dendroid templates†

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Various generations of zero α,ω-alkylenediamine core poly(amidoamine) PAMAM dendrimers (tetra-amine terminated substrates, $A_4$) were allowed to react with sub-stoichiometric amounts (*i.e.*, 2.0 equivalents) of Boc anhydride to give product mixtures consisting of the symmetrical tetra-Boc product ($B_4$) and four partial Boc protected adducts, namely, (1) the tri-($AB_3$), (2) di-geminal ($A_2B_2$), (3) di-vicinal ($A_2B_2$) and (4) mono-Boc ($A_3B$) products. These unprecedented, regio-differentiated Boc cores possess emerging dendritic features and intrinsic ''protect–deprotect'' function. We refer categorically to these newly differentiated branched cores as differentiated dendroid templates (DDT's). These dendroid templates can be well resolved by thin layer chromatography (TLC). Gram quantities of these Boc adducts were purified using single pass silica gel column chromatography. These partial Boc protected, differentiated dendroid templates ($A_mB_n$, $m/n$ = 1–3) were used as ''protect–deprotect'' functionalized starting substrates (*i.e.*, regio-differentiated cores) for synthesizing higher generation libraries of regio-differentiated PAMAM dendrimers. In principle, the use of differentiated dendroid templates (*i.e.*, regio-functionalized star-branched cores) should be expected to offer broad options for the systematic engineering and control of many diverse organic nanostructures as a function of size, shape and regio-chemistry.

## 1. Introduction

It is now recognized that ''dendritic effects,''[1] as well as many other important nano-periodic property patterns observed for well defined, hard/soft nanoparticles are largely controlled by only six critical nanoscale design parameters (CNDPs).[2] These CNDP's include (a) size, (b) shape, (c) surface chemistry, (d) flexibility/rigidity, (e) architecture and (f) elemental composition. Many of these CNDP dependent property relationships define an emerging ''central paradigm'' for a recently proposed nano-periodic system/framework.[3] This concept provides a rational framework for embracing a growing list of literature examples describing facets of this emerging central paradigm such as (i) nanoscale atom mimicry,[4] (ii) discrete nano-building block (*i.e.*, nano-element type) features, well defined nano-stoichiometries,[2,3] and (iii) new emerging nano-periodic property patterns dependent on CNDP's.[1,3] Most profound are recent examples by Glotzer *et al.*,[5] Mirkin *et al.*,[6] and Percec *et al.*[7] These groups have demonstrated predictive nano-polyhedra building block shape dependency for self-assembly, predictive nano-rules for 3-D lattice formation and predictive Mendeleev-like nano-periodic tables for dendron self-assembly, respectively. Predictive CNDP dependent nano-periodic patterns/rules reported by these three groups each fulfill and validate this unifying nano-periodic system/framework. The important role that CNDP's play in determining nano-periodic assembly patterns for both hard and soft nanoparticles portends the need for developing broad strategies for controlling regio-differentiated CNDP's such as size, shape and surface chemistry for both hard and soft nanoparticles.

While homogeneous, spherical dendrimer[8] structures provide a useful platform for enabling many important applications,[9] attention is now turning to the synthesis of discrete regio-differentiated dendritic materials possessing non-spherical shapes[10] as well

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as differentiated size, shape and regio-chemistry within the same molecule. This is undoubtedly inspired by the vast libraries of natural biomolecules, wherein, regio-differentiated size, shape, surface chemistry, flexibility/rigidity are some of the most important determinants of biological function.[11] In this regard PAMAM di-dendron type structures possessing differentiated surface chemistries and/or different generational sizes have been reported. Differentiated PAMAM dendrimers derived from disulfide redox linkages,[12] the hybridization of complementary single stranded DNA,[13] or *via* copper(I) catalyzed click chemistry[14] have been developed. A three faced PAMAM dendrimer has been synthesized through click-chemistry.[15] Other asymmetrical dendrimer families possessing differentiated generations, surface chemistries, and branch cell types were also explored. For example, bow-tie dendrimers with differentiated generation numbers and surface functionalities were developed for drug delivery.[16] More recently, amphiphilic Janus type dendrimers have been reported by Percec *et al.*,[17] who linked chemically differentiated dendritic components possessing hydrophilic and hydrophobic elements. These Janus dendrimers have been shown to function as powerful structure-directing amphiphiles, with greater versatility than simple lipids, surfactants, or block copolymers.[17] An extraordinary example of regio-differentiated size and shape asymmetry around the central carbon atom of pentaerythritol was reported by Kremers and Meijer.[18] This group synthesized a chiral dendrimer possessing four dendrons/substituents differentiated as a function of generation levels. Although isolated examples of regio-differentiated dendrimers have been reported, a general synthetic strategy for regio-designing and controlling dendrimer structures as a function of size, shape and surface chemistry has not been available. This present work describes first steps toward the development of such an approach using divergent PAMAM amplification protocols, however, the general principles described should be applicable to many other dendrimer families.

Traditional divergent dendrimer growth generally begins with simple linear or point like cores. Using iterative amplification chemistry, these cores are first converted into star-branched intermediates, generally referred to as $G = 0$ type PAMAM intermediates,[19] followed by transformation into dendritic architectures (*i.e.*, higher generation dendrimer structures). It is at this star-branched intermediate stage that sub-stoichiometric reactions are performed to produce non-homogenous differentiated species bearing various degrees of functionalization. This functionalization step may be engineered to enhance independent physical separation of all species formed as well as to introduce appropriate protect–deprotect moieties for subsequent differentiation operations. These sub-stoichiometric functionalizations are chosen to allow physical separation and isolation of these differentiated star-branched species by standard solvent extraction, precipitation, complexation or chromatography protocols. Rapid screening for physical separation/isolation of these product mixtures usually involved elutions with appropriate solvent mixtures using thin layer chromatography (TLC) procedures to determine relative $R_f$ values. These TLC results were adapted to provide suitable chromatographic separation protocols for isolating laboratory quantities of these $G = 0$, differentiated star-branched structures which were used as starting templates for synthesizing higher generation differentiated dendrimers. These templates constitute a new and unprecedented structural category of precursors suitable for synthesizing higher generation, regio-differentiated dendrimers. As such, we refer to these highly adaptable precursors as differentiated dendroid templates (DDTs).

These differentiated dendroid templates are designed to possess ''protect–deprotect'' functionality which is robust and orthogonal to divergent dendritic amplification but can be physically separated into discrete species by chromatographic methods. These discrete, differentiated dendroid templates were used in a proof of concept strategy to synthesize higher generation, regio-differentiated PAMAM dendrimers. Thus selective synthesis of higher generation regio-differentiated PAMAM dendrimers involved the following three steps: (step-1) iterative advancement of traditional α,ω-alkylenediamine cores to Tomalia-type PAMAM starbranched ($G = 0$); tetra-amine structures (*i.e.*, [core: α,ω-alkylenediamine];{4 → 2}; *dendri*-{poly(amidoamine)-$(NH_2)_4$}); (step-2) simple sub-stoichiometric modification of these traditional starbranched, ($G = 0$) tetra-amine precursors ($A_4$) with ''protect–deprotect'' functionality to produce discrete *regio-functionalized dendroid templates*, ($A_nB_m$). In this present case, these PAMAM dendroid templates were appropriately differentiated to allow TLC analysis and simple physical separation by silica gel chromatography into discrete regio-differentiated template species. These differentiated dendroid templates possessed residual functionality (*i.e.*, A = amines) suitable for subsequent divergent dendritic iterations or convergent coupled generation growth. (step-3) These dendroid templates are divergently amplified and de-protected at any higher generation level to produce regio-differentiated dendritic constructs. Similarly these dendroid templates maybe de-protected and/or convergently coupled to produce differentiated dendrimers at any higher generation level. These differentiated dendroid templates constitute a unique class of regio-differentiated precursor cores suitable for traditional amplification by either divergent or convergent strategies to produce discrete, regio-differentiated higher generation nano-scale dendrimers. An overview of this three-step, ''dendroid template strategy'' is outlined in Fig. 1.

As shown in Fig. 1, various linear α,ω-alkylenediamine cores are converted into standard, homogenous PAMAM; $G = 0$; type, star-branched structures, **1** ($A_4$); where, A = amino groups. Sub-stoichiometric reaction of 2 equiv. of BOC anhydride with these tetra-functional amino substrates produced differentiated PAMAM; $G = 0$; star-branched structures (*i.e.*, DDT's-$A_mB_n$; where, A = amino groups, B = Boc-protected amino groups), including Boc differentiated; **2**, ($A_1B_3$), **3**, ($A_2B_2$, vicinal), **4**, ($A_2B_2$, geminal) and **5**, ($A_3B_1$), respectively. These Boc differentiated PAMAM; $G = 0$; (DDT's) can generally be physically separated and then independently further amplified to produce the respective PAMAM; $G = 1$; ($A_mB_n$; where, $m + 2n = 8$) and higher generation regio-differentiated PAMAM dendrimers, including Janus type dendrimers.

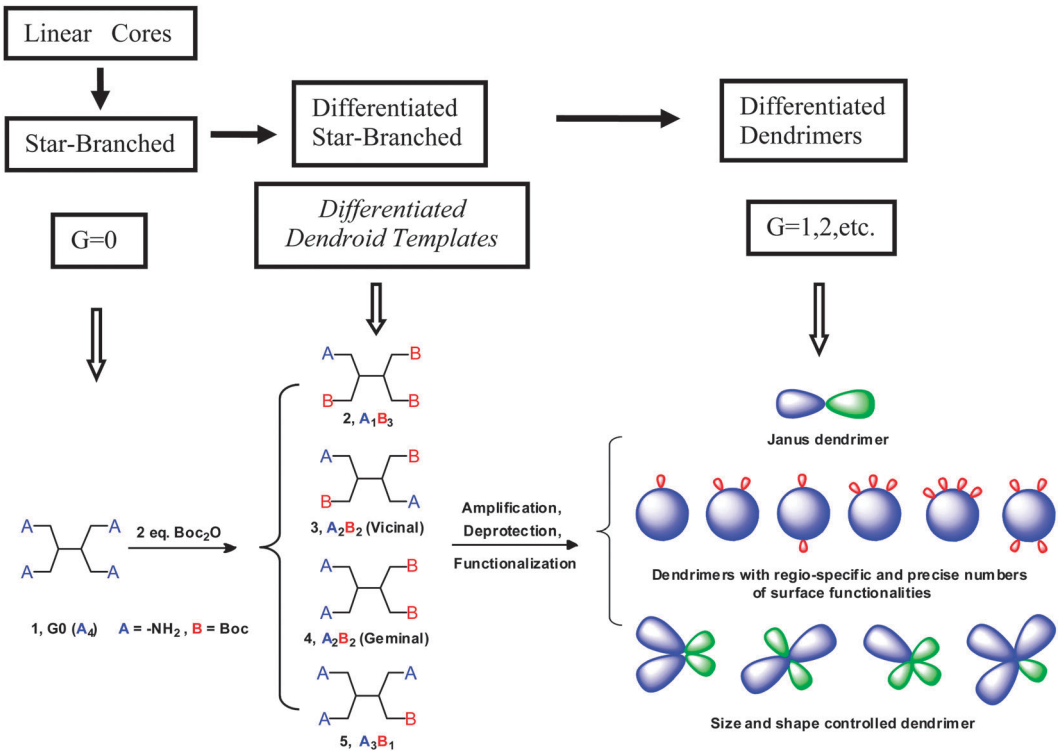



**Fig. 1** Illustration of the ''Differentiated Dendroid Template'' (DDT) strategy to produce higher generation regio-differentiated dendrimers.

This methodology offers broad implications for the systematic design and regio-control of size, shape and surface chemistry for organic nanostructures in general.

## 2. Experimental section

### 2.1. Materials and analytical techniques

Anhydrous solvents and chemicals were obtained from Acros Organics and Sigma-Aldrich. Various [core: α,ω-alkylenediamine]; (4 → 2); *dendri*-{poly(amidoamine)-$(NH_2)_4$; $G$ = 0; PAMAM dendrimers were obtained from Dendritic Nanotechnologies or Nano-Synthons LLC., Mt. Pleasant, MI. Silica gel 60, particle size of 0.040–0.063 mm and 230–400 mesh ASTM, was obtained from EM Sciences. Thin layer chromatography (TLC) was performed using Whatman Adsorption plates, 60 Å silica gel with 250 μm layer thickness. TLC plates were stained with iodine and scanned. $^1$H NMR and $^{13}$C NMR spectra were measured on a 500 Hz Varian NMR systems equipped with multinuclear 5 mm probes. $^1$H chemical shifts are reported in parts per million from TMS. Mass spectra were performed on a Waters 1525 mass spectrometer. A thermometer was used without further calibration.

### 2.2. Synthesis

**2.2.1. General procedures to produce regio-differentiated PAMAM; $G$ = 1; dendrimers.** Various [core: α,ω-alkylenediamine]; (4 → 2); *starbranched*-{poly(amidoamine)-$(NH_2)_4$; $G$ = 0; PAMAM structures (Dendritic Nanotechnologies/Nano-Synthons, LLC.) were dissolved in MeOH (20–30% w/w). Given equivalents of surface modification/protection reagents were used as described below. Appropriate equivalents of triethylamine were used with acetic anhydride, trifluoroacetic anhydride, and benzyl chloride. Both Boc anhydride and dimethyl itaconate were used without triethylamine. In all cases, the reactions were monitored by TLC as a function of time.

Specifically, [core: dodecylene diamine]; (4 → 2); *star-branched*-{poly(amidoamine)-$(NH_2)_4$; $G$ = 0; (PAMAM) structure (1.976 g, 3.007 mmol) was dissolved in anhydrous methanol (3.0 mL) and cooled to 0 °C. Boc anhydride (1.313 g, 6.014 mmol) in methanol (4.0 mL) was added dropwise. The reaction was stirred under nitrogen for 1 h at 0 °C, allowed to warm to room temperature and then stirred overnight. The solvent was removed using a rotary evaporator. The mixture was purified with a silica gel column using an eluent consisting of a 2 : 1 ratio (v/v) of $CHCl_3$ to MeOH and 0.93% ammonium hydroxide which was increased to 6.25% during the column chromatography elution process. The Boc protected, $G$ = 0; PAMAM dendroid templates were collected as fractions. Yields of the various dendroid template species were calculated from the original ($G$ = 0); star-branched PAMAM structure mole number and are described below.

*PAMAM; $G$ = 0; ($A_1B_3$);* differentiated dendroid template, **2**. Isolated yield: 725.2 mg, 25.3%, $R_f$ = 0.83 ($CHCl_3$/MeOH/$NH_4$·OH = 2 : 1 : 0.3). $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 27H), 1.46 (m, 4H), 2.32–2.37 (m, 8H), 2.44 (t, $J$ = 7.0 Hz, 4H), 2.71–2.76 (m, 10H), 3.14 (t, $J$ = 6.0 Hz, 6H), 3.22–3.26 (m, 8H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.85, 27.90, 28.72, 28.80, 30.78, 30.81, 30.87, 34.29, 34.39, 40.40, 40.93, 42.00, 42.81,

50.77, 54.51, 54.56, 80.11, 158.49, 175.28, 175.38 ppm; MS ($EI^+$) found $(M + H)^+$ 957.3, calc. ($C_{47}H_{92}N_{10}O_{10}$) 956.7.

*PAMAM; G* = 0; ($A_2B_2$; vicinal); differentiated dendroid template, **3**. Isolated yield: 383.9 mg, 14.9%, $R_f$ = 0.73. $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 18H), 1.46 (m, 4H), 2.32–2.37 (m, 8H), 2.44 (t, $J$ = 7.0 Hz, 4H), 2.70–2.76 (m, 12H), 3.14 (t, $J$ = 6.0 Hz, 4H), 3.22–3.26 (m, 8H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 26.52, 27.32, 27.40, 29.39, 29.41, 29.48, 32.92, 33.01, 39.00, 39.53, 40.63, 41.57, 49.43, 53.17, 78.69, 157.09, 173.88, 173.95 ppm; MS ($EI^+$) found $(M + H)^+$ 857.3, calc. ($C_{42}H_{84}N_{10}O_8$) 856.6.

*PAMAM; G* = 0; ($A_2B_2$; geminal); differentiated dendroid template, **4**. Isolated yield: 368.6 mg, 14.3%, $R_f$ = 0.56. $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 18H), 1.46 (m, 4H), 2.32–2.37 (m, 8H), 2.44 (t, $J$ = 7.0 Hz, 4H), 2.71–2.76 (m, 12H), 3.14 (t, $J$ = 6.0 Hz, 4H), 3.22–3.26 (m, 8H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.85, 28.00, 28.72, 28.81, 30.79, 30.81, 30.87, 34.30, 34.45, 40.40, 40.93, 41.80, 42.04, 50.78, 50.85, 54.50, 54.63, 80.10, 158.49, 175.28, 175.32 ppm; MS ($EI^+$) found $(M + H)^+$ 857.3, calc. ($C_{42}H_{84}N_{10}O_8$) 856.6.

*PAMAM; G* = 0; ($A_3B_1$); differentiated dendroid template, **5**. Isolated yield: 392.5 mg, 17.2%, $R_f$ = 0.41. $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 9H), 1.46 (m, 4H), 2.32–2.37 (m, 8H), 2.44 (t, $J$ = 7.0 Hz, 4H), 2.71–2.76 (m, 14H), 3.14 (t, $J$ = 6.0 Hz, 2H), 3.22–3.26 (m, 8H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.89, 27.93, 28.71, 28.78, 30.78, 30.80, 30.87, 34.29, 34.40, 40.39, 40.91, 41.94, 42.61, 50.82, 54.54, 54.61, 80.08, 158.47, 175.25, 175.38 ppm; MS ($EI^+$) found $(M + H)^+$ 757.3, calc. ($C_{37}H_{76}N_{10}O_6$) 756.6.

**2.2.2. Advancement of partial Boc protected, PAMAM; *G* = 0; ($A_nB_m$) type dendroid templates to PAMAM; *G* = 0.5 ($E_mB_n$) and *G* = 1.0 ($A_mB_n$) type; differentiated dendrimers.** Various partial Boc protected, PAMAM; $G$ = 0;($A_mB_n$) type, dendroid templates above were dissolved in anhydrous methanol and cooled to 0 °C. Methyl acrylate (1.28 equiv. per –NH) was dissolved in anhydrous methanol and cooled to 0 °C. The $G$ = 0; dendroid template solutions were added dropwise to the methyl acrylate solution. After addition, the reactions were allowed to warm to room temperature and stirred under nitrogen for 24 h. The solvent was removed with rotary evaporation and the residues were flushed with nitrogen flow for several hours to remove excess methyl acrylate. The partial Boc protected, PAMAM; $G$ = 0.5; ($E_nB_m$) type, differentiated dendrimers were obtained as clear oils without further purification and are described below.

*Differentiated PAMAM; G* = 0.5; ($E_2B_3$) dendrimer, **6**. Isolated yield: 242.3 mg, 99.3%. $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 27H), 1.46 (m, 4H), 2.33–2.39 (m, 8H), 2.43–2.2.47 (m, 8H), 2.54 (t, $J$ = 6.5 Hz, 2H), 2.72–2.78 (m, 12H), 3.14 (t, $J$ = 6.0 Hz, 6H), 3.23–3.26 (m, 8H), 3.66 (s, 6H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.85, 27.91, 28.73, 28.81, 30.79, 30.82, 30.89, 33.62, 34.28, 38.47, 40.38, 40.41, 40.94, 50.50, 50.69, 50.78, 50.89, 52.17, 53.75, 54.52, 64.38, 80.11, 158.49, 174.78, 175.28 ppm; MS ($EI^+$) found $(M + H)^+$ 1129.3, calc. ($C_{55}H_{104}N_{10}O_{14}$) 1128.8.

*Differentiated PAMAM; G* = 0.5; ($E_4B_2$; vicinal), dendrimer, **7**. Isolated yield: 415 mg, 98.2%, $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 18H), 1.46 (m, 4H), 2.33–2.39 (m, 8H), 2.44–2.2.47 (m, 12H), 2.55 (t, $J$ = 6.0 Hz, 4H), 2.74–2.79 (m, 16H), 3.14 (t, $J$ = 6.0 Hz, 4H), 3.22–3.26 (m, 8H), 3.66 (s, 12H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.87, 28.71, 28.81, 30.78, 30.81, 30.89, 33.61, 34.22, 34.25, 38.46, 40.37, 40.95, 50.49, 50.68, 50.88, 52.18, 53.74, 54.51, 80.07, 158.43, 174.72, 174.76, 175.24 ppm; MS ($EI^+$) found $(M + H)^+$ 1201.3, calc. ($C_{58}H_{108}N_{10}O_{16}$) 1200.8.

*Differentiated PAMAM; G* = 0.5; ($E_4B_2$; geminal), dendrimer, **8**. Isolated yield: 445 mg, 99.1%, $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 18H), 1.46 (m, 4H), 2.32–2.39 (m, 8H), 2.44–2.2.47 (m, 12H), 2.55 (t, $J$ = 6.0 Hz, 4H), 2.72–2.79 (m, 16H), 3.14 (t, $J$ = 6.0 Hz, 4H), 3.23–3.26 (m, 8H), 3.66 (s, 12H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.86, 28.03, 28.73, 28.81, 30.81, 30.83, 30.90, 33.59, 34.33, 38.45, 40.41, 40.94, 50.49, 50.78, 50.81, 52.17, 53.76, 54.53, 80.11, 158.49, 174.75, 174.79, 175.28 ppm; MS ($EI^+$) found $(M + H)^+$ 1201.4, calc. ($C_{58}H_{108}N_{10}O_{16}$) 1200.8.

*Differentiated PAMAM; G* = 0.5; ($E_6B_1$); dendrimer, **9**. Isolated yield: 503 mg, 99.0%, $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 9H), 1.47 (m, 4H), 2.35–2.40 (m, 8H), 2.44–2.2.47 (m, 16H), 2.55 (t, $J$ = 6.0 Hz, 6H), 2.75–2.81 (m, 20H), 3.14 (t, PAMAM $J$ = 6.0 Hz, 2H), 3.22–3.26 (m, 8H), 3.66 (s, 18H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.81, 27.91, 28.69, 28.81, 30.77, 30.80, 30.88, 33.56, 34.14, 38.42, 40.36, 40.93, 50.46, 50.66, 50.79, 50.86, 52.18, 53.72, 54.50, 80.08, 158.48, 174.67, 174.75, 174.78, 175.20 ppm; MS ($EI^+$) found $(M + H)^+$ 1273.3, calc. ($C_{61}H_{112}N_{10}O_{18}$) 1272.8.

**2.2.3. General procedure for synthesis of differentiated PAMAM; *G* = 1.0; ($A_nB_m$) type, amine terminated dendrimers.** Various differentiated PAMAM ($G$ = 0.5); ($E_nB_m$) type; ester terminated dendrimers were dissolved in anhydrous methanol and cooled to 0 °C. These solutions were then added to ethylenediamine (EDA) (202 equiv. per ester) solutions at 0 °C. After addition, the reaction mixtures were stored at 4 °C for 72 h. The solvent was removed by rotary evaporation. Methanol (0.9 mL) and toluene (3.5 mL) were added to the residues and then removed by rotary evaporation. This step was repeated 6 times to remove trace EDA. The resulting differentiated PAMAM; $G$ = 1.0; ($A_nB_m$) type, amine terminated dendrimers were obtained as clear oils.

*Differentiated PAMAM; G* = 1; ($A_2B_3$) type, amine terminated dendrimer, **10**. Isolated yield: 248 mg, 97.5%, $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 27H), 1.46 (m, 4H), 2.32–2.37 (m, 12H), 2.43 (m, 4H), 2.57 (t, $J$ = 6.5 Hz, 2H), 2.72–2.80 (m, 16H), 3.14 (t, $J$ = 6.0 Hz, 6H), 3.23–3.26 (m, 12H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 26.46, 26.51, 27.33, 27.43, 29.37, 29.41, 29.48, 32.93, 33.42, 37.25, 39.01, 39.56, 40.62, 41.52, 49.40, 49.79, 52.10, 53.13, 78.71, 157.08, 173.52, 173.83, 173.87 ppm; MS ($EI^+$) found $(M + H)^+$ 1185.2, calc. ($C_{57}H_{112}N_{14}O_{12}$) 1184.9.

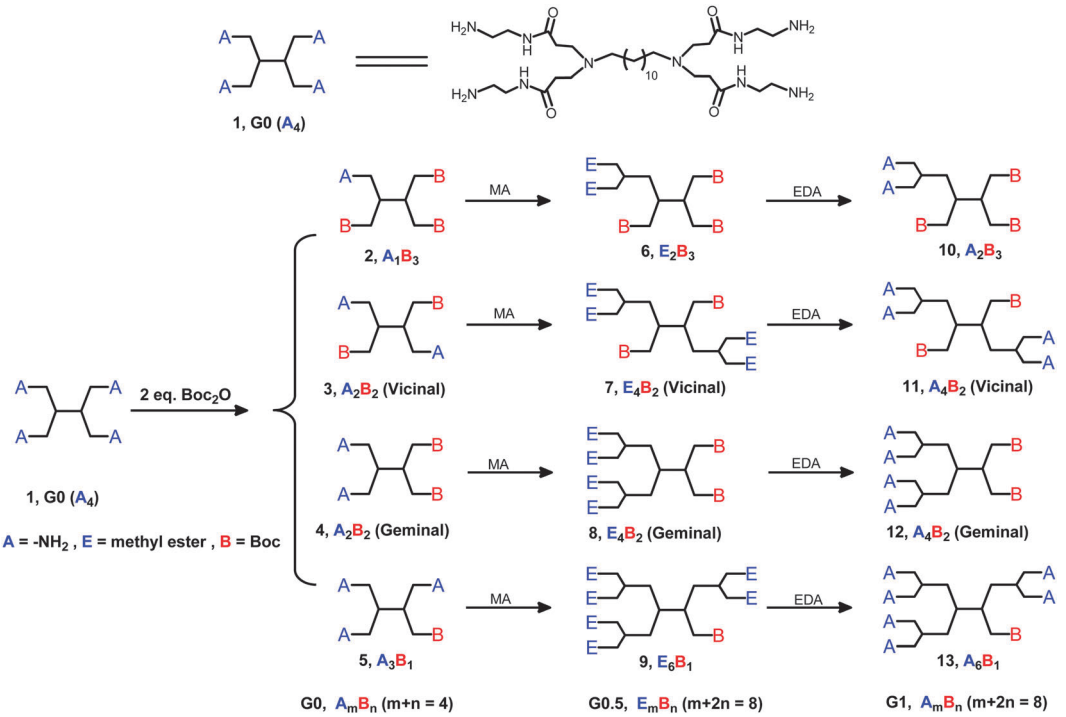



**Fig. 2** Sub-stoichiometric synthesis of differentiated dendroid templates followed by synthesis of differentiated *G* = 0.5 and 1.0 PAMAM dendrimers using standard Tomalia type, divergent dendrimer iteration protocols.

*Differentiated PAMAM; G* = 1; ($A_4B_2$ (vicinal)) type, amine terminated dendrimer, **11**. Isolated yield: 460 mg, 99.2%, $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 18H), 1.46 (m, 4H), 2.32–2.37 (m, 16H), 2.43–2.47 (m, 4H), 2.57 (t, $J$ = 7.0 Hz, 4H), 2.71–2.80 (m, 24H), 3.14 (t, $J$ = 6.5 Hz, 4H), 3.22–3.26 (m, 16H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.93, 28.74, 28.83, 30.81, 30.84, 34.90, 34.35, 34.83, 38.65, 40.41, 40.97, 42.04, 43.00, 50.78, 50.85, 51.20, 53.51, 54.56, 80.09, 158.47, 174.92, 175.21, 175.24 ppm; MS ($EI^+$) found $(M + H)^+$ 1314.1, calc. ($C_{62}H_{124}N_{18}O_{12}$) 1313.8.

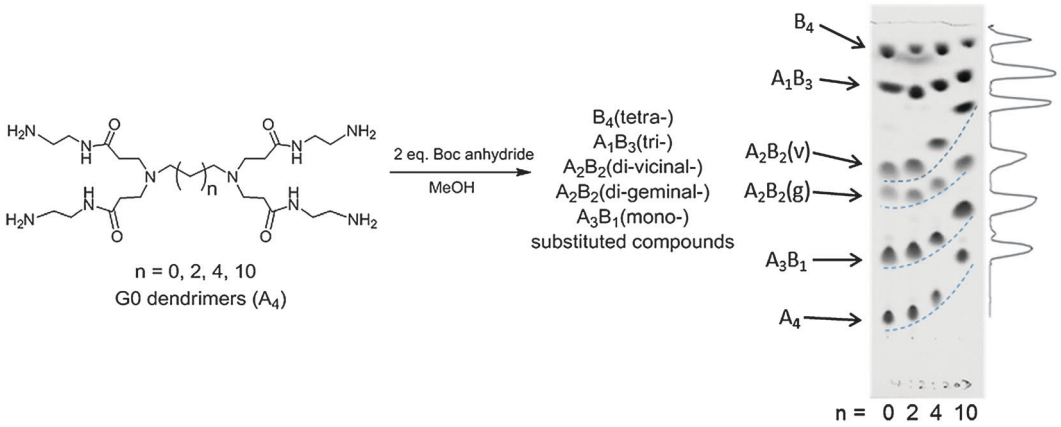
*Differentiated PAMAM; G* = 1; ($A_4B_2$(geminal)) type, amine terminated dendrimer, **12**. Isolated yield: 490 mg, 98.7%, $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 18H), 1.46 (m, 4H), 2.33–2.37 (m, 16H), 2.43–2.47 (m, 4H), 2.57 (t, $J$ = 7.0 Hz, 4H), 2.71–2.79 (m, 24H), 3.14 (t, $J$ = 6.5 Hz, 4H), 3.22–3.26 (m, 16H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.85, 27.97, 28.73, 28.82, 30.78, 30.83, 30.91, 34.28, 34.78, 38.60, 40.39, 40.92, 42.01, 42.98, 50.76, 51.15, 53.46, 54.49, 80.08, 158.45, 174.87, 175.18, 175.25 ppm; MS ($EI^+$) found $(M + H)^+$ 1314.1, calc. ($C_{62}H_{124}N_{18}O_{12}$) 1313.8.

*Differentiated PAMAM; G* = 1; ($A_6B_1$) type amine terminated dendrimer, **13**. Isolated yield: 611 mg, 99.1%, $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 1.29 (m, 16H), 1.43 (s, 9H), 1.46 (m, 4H), 2.32–2.37 (m, 20H), 2.45 (m, 4H), 2.57 (t, $J$ = 7.0 Hz, 6H), 2.71–2.80 (m, 32H), 3.14 (t, $J$ = 6.0 Hz, 2H), 3.22–3.26 (m, 20H); $^{13}$C NMR ($CD_3OD$, 125 MHz) 27.90, 27.98, 28.74, 28.83, 30.86, 30.92, 34.30, 34.78, 38.61, 40.39, 40.93, 41.99, 42.86, 50.79, 51.16, 53.47, 54.52, 80.05, 158.43, 174.86, 174.89, 175.19, 175.21 ppm; MS ($EI^+$) found $(M + H)^+$ 1142.2, $(M + 2H)^{2+}$ 721.6, calc. ($C_{67}H_{136}N_{22}O_{12}$) 1441.9.

## 3. Results and discussion

Beginning with various α,ω-alkylenediamine cores ($A_4$), Fig. 2 illustrates the general synthesis of partial Boc protected, PAMAM ($A_mB_n$) type; *G* = 0, differentiated dendroid templates and their transformation into differentiated PAMAM; *G* = 0.5 ($E_mB_n$) type, ester terminated and differentiated PAMAM (*G* = 1); ($A_mB_n$) type, amine terminated dendrimers, respectively. Screening for ideal combinations of surface modification/protection groups and dendrimer cores to produce optimal physical separation properties was performed. This study showed that Boc protected, differentiated dendroid templates (DDT's) (*i.e.*, $B_4$, $A_1B_3$, $A_2B_2$-vicinal, $A_2B_2$-geminal, and $A_3B_1$) derived from PAMAM; *G* = 0; dodecylene core star-branched structures exhibited the greatest $R_f$ value differences, according to TLC analyses. These DDT's were obtained by reacting two equivalents of Boc anhydride with PAMAM; *G* = 0; dodecylene core, star-branched structure in methanol. This reaction gave tetra-, tri-, di-(vicinal)-, di-(germinal)-, and mono-Boc protected products. These products were well resolved and characterized by TLC protocols. Based on this analysis, appropriate elution solvents and conditions were defined to separate gram quantities of purified DDT's from the reaction crude mixture by using standard silica gel column separation techniques. Five discrete, purified DDT's were isolated by means of a single column elution, using a tri-solvent system of chloroform–methanol–ammonium hydroxide. Next, using standard Tomalia-type divergent PAMAM




**Scheme 1** Sub-stoichiometric Boc anhydride reactions (*i.e.*, 2 equiv.) with various cores (*i.e.*, *n*); PAMAM star-branched; *G* = 0; amine terminated structures. TLC profiles of these reactions with densitometric evaluation for (*n* = 10) (last lane) are shown on the right.

synthesis protocols, methyl acrylate was allowed to react with the tri-, di(vicinal)-, di(geminal)-, and mono-Boc protected PAMAM; *G* = 0; dendroid templates. This step gave each PAMAM; *G* = 0.5; differentiated dendroid template in excellent yields. Finally, PAMAM; *G* = 1; differentiated dendroid templates were obtained quantitatively by amidation of these PAMAM; *G* = 0.5; dendroid templates in the presence of large excess of EDA as described in Fig. 2.

### 3.1. Physical separation screening for defining suitable surface modification/protection groups

In order to determine the product distribution/chromatographic separation potential of expected PAMAM; *G* = 0; differentiated dendroid templates (DDT's) by using sub-stoichiometric reaction conditions, various combinations of star-branched cores and surface modification/protection reagents were examined using TLC for rapid appraisal. Various α,ω-alkylenediamine cores ($A_4$) were examined with five different modification/protection reagents, namely, (1) Boc anhydride, (2) dimethyl itaconate, (3) acetic anhydride, (4) benzoyl chloride, and (5) trifluoroacetic acid. Typically, 2 equivalents of reagent were added to a methanol solution of the tetra-valent, PAMAM star-branched; (*G* = 0) structure. Amazingly, the two di-substituted isomers from each of these reactions exhibit fairly well distinguished $R_f$ values corresponding to the expected vicinal and a geminal species. For example, product distributions are illustrated for these five reagents using sub-stoichiometric reaction conditions on a tetra-amine, butylene core, star-branched structure. Based on these TLC analysis results, it was apparent that Boc anhydride modification offered the best chromatographic separation parameters required for the successful sub-stoichiometric modification of surface primary amino groups in the synthesis of differentiated dendroid templates and subsequent higher generation differentiated dendrimers (see ESI†).

### 3.2. Physical separation screening for differentiated PAMAM (*G* = 0); dendroid templates as a function of core length

The reactions of PAMAM; *G* = 0; star-branched intermediates with various cores; (*i.e.*, ethylene, butylene, hexylene, and dodecylene) using two equivalents of Boc anhydride were performed to further determine their products' distribution (Scheme 1, left panel). The $R_f$ value differences for the products possessing various degrees of substitution were evaluated. The optimized TLC eluent required to resolve all species was a tri-solvent system consisting of $CHCl_3$/MeOH/$NH_4OH$ (aq.) = 2 : 1 : 0.3. All TLC plates were stained in parallel by both iodine and ninhydrin. The TLC profiles of each reaction are shown in Scheme 1, right panel.

Five products, namely, $B_4$ (tetra-), $A_1B_3$ (tri-), $A_2B_2$ (di-vicinal), $A_2B_2$ (di-geminal), and $A_3B_1$ (mono-) Boc protected, differentiated dendroid template as well as unreacted PAMAM; (*G* = 0) star-branched structures ($A_4$) were obtained from each of the PAMAM; *G* = 0; star-branched cores. As shown in Scheme 1, the reaction products for all four cores can be well resolved under this TLC condition. The point groups for $A_2B_2$ (di-vicinal) and $A_2B_2$ (di-geminal) are $C_{2h}$ and $C_2$, respectively. We anticipate that the $A_2B_2$ (di-vicinal) structure may be less polar than the $A_2B_2$ (di-geminal) structure due to its more symmetrical structure thus exhibiting a bigger $R_f$ value. The $R_f$ values for the reaction products are summarized in Table S1 (see ESI†). The smallest $R_f$ value differences observed are associated with the two di-substituted isomeric products, *i.e.* $A_2B_2$ (di-vicinal), $A_2B_2$ (di-geminal). As shown in Table S1 (ESI†), the $R_f$ value differences derived for these isomeric products ethylene, butylene, hexylene and dodecylene cores are 0.08, 0.10, 0.13 and 0.17, respectively. These results indicate that the longer the core carbon chain, the greater the $R_f$ value difference between these isomeric di-substituted products.

### 3.3. Thin layer chromatography (TLC) separation of differentiated dendroid templates (DDT's) as obtained under sub-stoichiometric reaction conditions

Thin layer chromatography (TLC) was used to characterize and assess the separation of Differentiated Dendroid Templates (DDT's) product distribution as a function of sub-stoichiometric Boc protection reactions. Specifically, reaction products from PAMAM; dodecyl core, (*G* = 0), star-branched structures with 1, 2, 3, and 4 equivalents of Boc reagent were examined. Facile separation of $B_4$ (tetra-), $A_1B_3$ (tri-), $A_2B_2$ (di-vicinal),

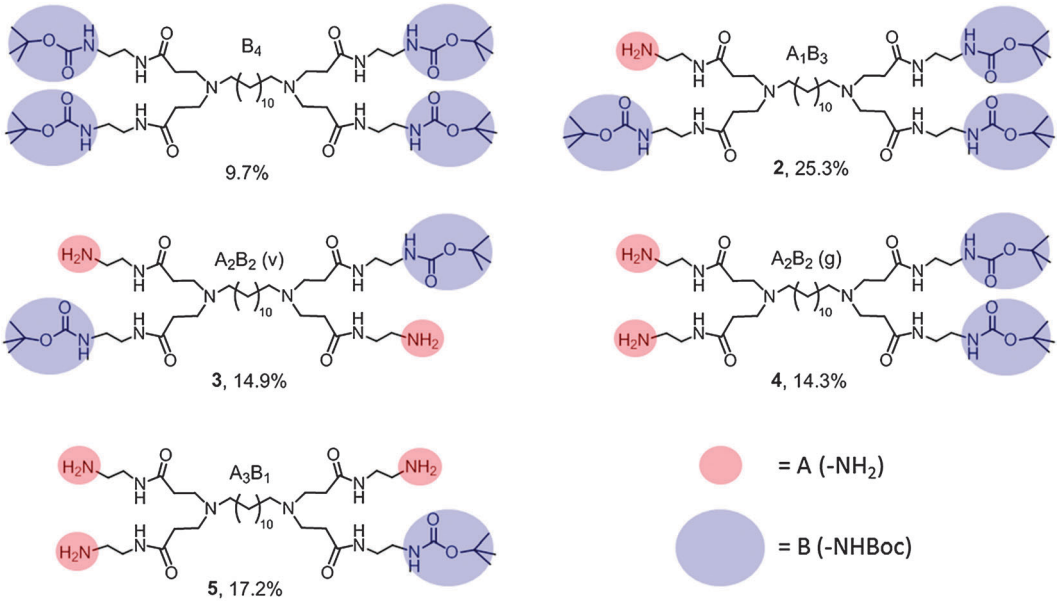



**Fig. 3** Structures of PAMAM; dodecylene core; $G = 0$; differentiated dendroid templates separated on a gram scale by silica gel column chromatography.

$A_2B_2$ (di-geminal), and $A_3B_1$ (mono-) Boc protected, ($G = 0$) dendroid templates was observed as illustrated in ESI† (Fig. S2). Sub-stoichiometric reaction of 1 equivalent of *i.e.*, Boc anhydride gave no tetra-substituted product and a higher portion of mono-substituted compound, however, yields of other species were fairly low. Reaction of three equivalents gave significant amounts of tetra-substituted products. Four equivalents of Boc anhydride reacted with PAMAM; $G = 0$, star-branched structure to give only tetra-substituted products. However, the 2× equivalent reaction gave a very nice distribution of all possible products

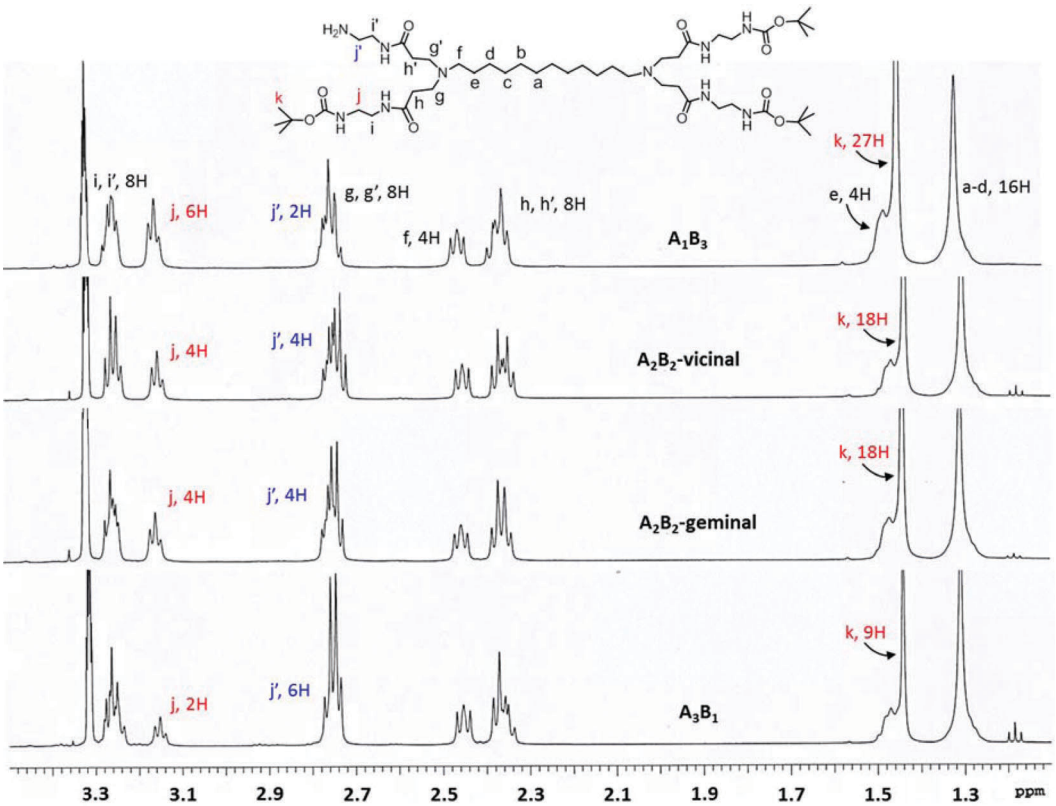



**Fig. 4** $^1$H NMR spectra of PAMAM; dodecylene core; $G = 0$; differentiated dendroid templates (DDT's).

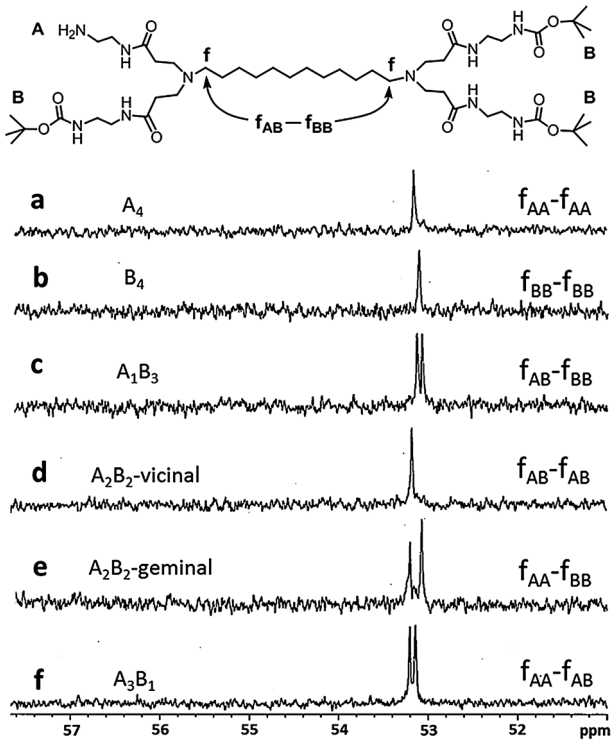
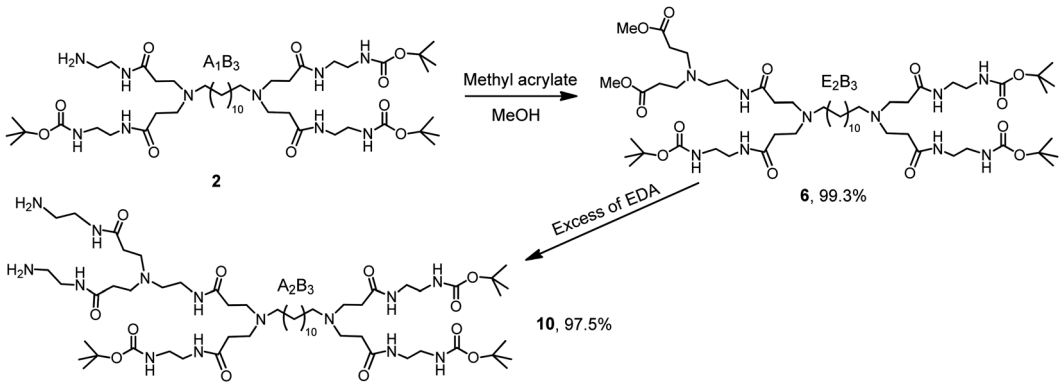



**Fig. 5** Expanded region of $^{13}$C-NMR spectra of PAMAM; dodecylene core; $G = 0$; star-branched substrates.

as determined by the separation and intensity of the TLC spots. Based on the above screening studies, a 2 gram scale reaction of PAMAM, dodecylene core; $G = 0$; star-branched structure with 2× equivalents of Boc anhydride (*i.e.*, methanol) was performed for further separation of these unsymmetrical DDT structures. The crude reaction products were loaded onto a silica gel column and fine separation of $B_4$ (tetra-), $A_1B_3$ (tri-), $A_2B_2$ (di-vicinal), $A_2B_2$ (di-geminal), and $A_3B_1$ (mono-) species was achieved using a tri-solvent eluent system (see Experimental section for a detailed description). After the collection of the mono-Boc protected $A_3B_1$ type (DDT), the chromatographic separation was stopped and the unreacted PAMAM; $G = 0$; star-branched substrate was not collected. Isolated yields for these compounds were found to be 9.7% ($B_4$), 25.3% ($A_1B_3$), 14.9% ($A_2B_2$, di-vicinal), 14.3% ($A_2B_2$, di-geminal), and 17.2% ($A_3B_1$), respectively. These yields matched the respective TLC spot intensities very well. The total recovery of the two equivalents of Boc anhydride used in the reaction is 95.1%. The isolated DDT structures are shown in Fig. 3.

$^1$H-NMR spectral analyses of each product are in good agreement with their structures, by comparing the $^1$H-NMR of PAMAM; $G = 0$, dodecylene core, star-branched structure ($A_4$) with all five isolated products. In each case, the proton signals are assigned for each PAMAM; $G = 0$; differentiated dendroid template (Fig. 4). The signals of protons a–d within the core are overlapped at 1.29 ppm and integration for this broad peak is 16, wherein, this integral value is set as an internal standard for integration of the other peaks in the spectra. Protons on the branches with amine terminal groups are marked with prime signs (*i.e.*, g′, h′, i′, and j′). Proton pairs h–h′, g–g′, and i–i′ have slight chemical shift differences within each pair and their peaks split into multiplets compared with the PAMAM ($G = 0$) star-branched precursor which has only h′, g′, and i′ protons thus showing simple triplets (data not shown). However, chemical shifts for protons j (3.14 ppm) and j′ (around 2.74 ppm, overlapped with g–g′) are well separated due to their close proximity to the terminal groups thus having the most chemical environment differences. The full peak assignment of compound **2** ($A_1B_3$) is shown in the first panel of Fig. 4. The spectra are similar with a few exceptions in terms of peak integrations highlighted with red and blue colors. Integrations of Boc protons (k) at 1.43 ppm are 27, 18, 18, and 9 for $A_1B_3$, $A_2B_2$ (vicinal), $A_2B_2$ (geminal), and $A_3B_1$, respectively. Integrations of proton j at 3.14 ppm are 6, 4, 4, and 2 for the four above-mentioned compounds and correspond to 3, 2, 2, and 1 Boc substituted branches.

These compounds are further characterized using $^{13}$C-NMR. Specifically, we observed that the chemical shifts of the two f carbon atoms adjacent to the branching tertiary amines are slightly different if the outer branches attached to each tertiary amine group are not identical. As shown in the upper structure ($A_1B_3$ as an example) of Fig. 5, the f carbon adjacent to the tertiary amine with one amine terminal group (A) and one Boc protected amine group (B) is marked $f_{AB}$, and the f carbon adjacent to the tertiary amine with two Boc protected amine groups is marked $f_{BB}$. The f carbons of the rest of the compounds




**Scheme 2** Synthesis of a differentiated PAMAM; $G = 1$; $A_2B_3$ type dendrimer using iterative alkylation and amidation reactions.

are marked in the same manner. The expanded (51–58 ppm) region of the $^{13}$C-NMR spectra of PAMAM; $G$ = 0; star-branched ($A_4$), tetra-Boc substituted PAMAM; ($G$ = 0) ($B_4$), and compounds **2–5** are illustrated in Fig. 5. The two f carbons of three species, namely $G$ = 0 dendrimer ($f_{AA}$–$f_{AA}$), tetra-Boc substituted $G$ = 0 ($f_{BB}$–$f_{BB}$), and $A_2B_2$-vicinal ($f_{AB}$–$f_{AB}$) have identical chemical environments thus only single peaks are presented in their $^{13}$C NMR spectra (Fig. 5a, b and d). On the other hand, the spectra of the rest of the three compounds, namely $A_1B_3$ ($f_{AB}$–$f_{BB}$), $A_2B_2$-geminal ($f_{AA}$–$f_{BB}$), and $A_3B_1$ ($f_{AA}$–$f_{AB}$) show two peaks in this region indicating that the two f carbons in these structures are not identical (Fig. 5c, e and f). The $^{13}$C NMR spectra of the two di-substituted isomers confirmed the assignment of these two structures with molecular symmetry analysis in our previous discussion.

### 3.4. Synthesis of differentiated PAMAM ($G$ = 1); dendrimers

Following the standard divergent PAMAM dendrimer synthesis protocol,[19] these four PAMAM; $G$ = 0; differentiated dendroid templates were subjected to further generation growth to produce differentiated PAMAM; ($G$ = 1) dendrimers. Scheme 2 illustrates an example of this two-step iterative synthesis.

Briefly, compound **2** ($A_1B_3$) was first reacted with methyl acrylate in methanol to give differentiated PAMAM; $G$ = 0.5; ($E_2B_3$) type dendrimer, **6**. After characterization, compound **6** was allowed to react with a large excess of EDA to give differentiated PAMAM ($G$ = 1); $A_2B_3$ type dendrimer, **10** (ESI,† Fig. S3) in excellent yield. The other three differentiated PAMAM; ($G$ = 1) dendrimers (compounds **11–13**, for structures see Fig. S3, ESI†) were synthesized in a similar manner. The four differentiated PAMAM; ($G$ = 1) dendrimers exhibit well distinguished $R_f$ values by TLC (ESI,† Fig. S4). The $R_f$ values which are directly related to compound polarities are correlated to the relative number of Boc groups and amino groups presented in the structures. For example, compound **10** ($A_2B_3$) has three Boc groups and two amino groups thus its $R_f$ value (0.77) is much greater than the $R_f$ value (0.43) of compound **13** ($A_6B_1$) which has only one Boc group and six amino groups. The two di-Boc compounds, **11** ($A_4B_2$-vicinal) and **12** ($A_4B_2$-geminal), have $R_f$ values of 0.56 and 0.51, respectively. Moreover, compound **11** ($A_4B_2$-vicinal) has a greater $R_f$ value than compound **12** ($A_4B_2$-geminal) due to its more symmetrical structure ($C_{2h}$) than that of compound **12** ($C_2$). These G1 dendrimers are fully characterized using NMR and mass spectroscopy.

The functions of natural macromolecules, such as proteins and oligonucleotides, are defined by their finely-tuned molecular sizes, shapes and precise controlled numbers of functionalities.[20] These unique structures manifest the minimal level of 'ordered complexity' necessary to support, sustain and amplify the many familiar life forms of higher complexity.[21] Since we have successfully isolated hundreds of milligrams of differentiated PAMAM; ($G$ = 1) dendrimers, it is pretty straightforward to achieve size, shape and regio-chemical controlled higher generation dendrimer levels. Using DDT's one simply follows the standard PAMAM dendrimer synthesis protocol to produce discrete higher generation differentiated dendrimers. For example, starting from structure **12**, a full series of asymmetrical dendritic materials with two different sized dendrons can be made (Fig. 6). Compound **12**, differentiated dendrimer ($A_4B_2$) and the later differentiated PAMAM ($A_8B_2$, $A_{16}B_2$, *etc.*)

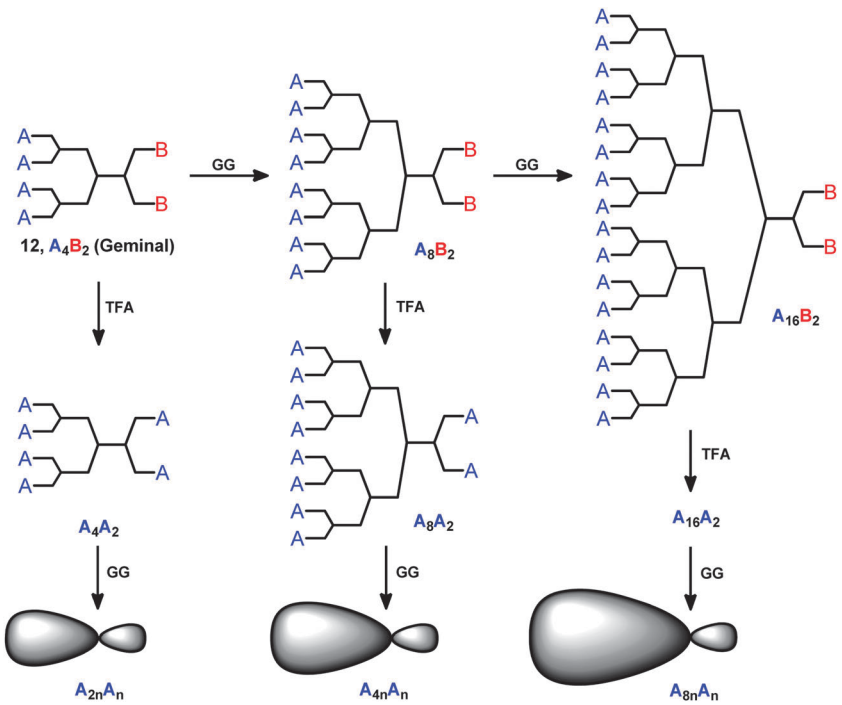



**Fig. 6** The synthesis schemes for Janus type, dendron size differentiated dendrimers derived from compound **12**. GG = Generation Growth; A = –$NH_2$; B = –NHBoc; $n$ = 4, 8, 16. . ..

dendrimers synthesized from **12** *via* the PAMAM dendrimer generation growth process are subjected to TFA treatment to expose the two primary amino groups to give $A_{2n}A_2$ type asymmetrical dendrimers. Further generation growth (GG) from these structures will give an array of asymmetrical dendritic materials (Fig. 6, $A_{2n}A_n$, $A_{4n}A_n$, $A_{8n}A_n$, *etc.*). In the same manner, other differentiated PAMAM; $G = 1$; dendrimers (*i.e.* compounds **10**, **11**, and **13**) can be further developed. For example, from compound **10**, a series of $A_{2n}A_{3n}$, $A_{4n}A_{3n}$, $A_{8n}A_{3n}$, and from compound **13**, a series of $A_{12n}A_{2n}$, $A_{24n}A_{2n}$, $A_{48n}A_{2n}$ differentiated dendrimers will be generated. Due to their differentiated branch length and the varying number of surface functional groups, the molecular sizes and shapes would be varied leading to the achievement of size and shape controlled regio-chemically controlled asymmetric dendritic materials.

The conjugation of small molecule ligands onto PAMAM dendrimers possessing large numbers of reactive sites produces materials with a wide range of surface distributions.[22] Efforts have been made to resolve and purify discrete dendrimer conjugates with precise numbers of ligands attached using HPLC.[23] The PAMAM; ($G = 0$) (DDT's) and differentiated PAMAM; ($G = 1$) dendrimers reported in this paper clearly illustrate the feasibility of manufacturing nanomaterials with precise numbers of regio-positioned ligands. The exposed primary amino groups on these DDT's can first be conjugated with a desired ligand in precise numbers. Subsequent deprotection of Boc protected primary amino groups with trifluoroacetic acid (TFA), followed by generation growth, will lead to higher generation regio-functionalized dendritic materials. Moreover, subtle manipulation of ligand geometry on the surface of the nanoparticle is also possible due to the original vicinal–geminal structural differences preserved in the starting DDT's. Starting from these lower generation asymmetrical DDTs (*i.e.*, geminal DDT's), it is possible to produce Janus type dendrimers with differentiated surface functional groups, as illustrated in Fig. 6. For example, the PAMAM; $G = 1$; $A_4B_2$ type, di-geminal (*i.e.*, compound **12**) can first be advanced by generation growth to a certain generation and the surface amino groups then modified to a desired kind of functionality. The two primary amino groups are then exposed, and subjected to dendrimer generation growth to the same generation number, and all of the surface amino groups are modified to another kind of functionality. This process will generate Janus type dendrimers with two different kinds of surface functionalities. Furthermore, the numbers of these two kinds of functionalities can also be controlled by differentiating the generation growth of each half of the structure.

## 4. Conclusion

Sub-stoichiometric surface protection/modification reactions (*i.e.*, using 2×- equiv. of reagents with tetravalent substrates) were performed on tetra-amine functionalized, PAMAM; $G = 0$; ($A_4$) type star-branched substrates derived from various α,ω-alkylenediamine cores. Five different protection/modification reagents were examined to define optimal sub-stoichiometric product properties suitable for chromatographic separation and subsequent dendritic amplification. The resulting product libraries, derived as a function of protection/modification reagent and α,ω-alkylenediamine core length, were rapidly screened for optimal physical isolation parameters using TLC protocols. It was found that sub-stoichiometric Boc anhydride modification of a tetra-amine functionalized, PAMAM; (dodecylene core); $G = 0$; ($A_4$) type star-branched substrate produced differentiated dendroid templates (*i.e.*, DDT's) possessing the most highly differentiated $R_f$ values for each of the DDT's formed. Based on these observations, five products, namely $B_4$ (tetra-), $A_1B_3$ (tri-), $A_2B_2$ (di-vicinal), $A_2B_2$ (di-geminal), and $A_3B_1$ (mono-) were successfully purified in gram quantities by one pass through a silica gel column in good yields, using a tri-solvent elution system. Four differentiated, PAMAM; $G = 1$; dendrimers were then synthesized from the four partial Boc protected, PAMAM; $G = 0$; differentiated dendroid template precursors. Using standard divergent PAMAM dendrimer synthesis protocols these differentiated dendrimers were obtained in excellent yields. This indicates that the Boc protected intermediates were robust and stable throughout the dendrimer generation growth process. These results clearly demonstrate that this differentiated dendroid template strategy may be used as a versatile method for the construction of discrete, regio-specific; size, shape and surface chemistry differentiated PAMAM dendrimers and will undoubtedly be adaptable to other dendrimer families and macromolecules.

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