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ARTICLE TYPE

A fast track strategy toward highly functionalized dendrimers with different structural layers: "Onion peel approach"

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An accelerated strategy depending on different chemical building blocks between each layer, coined "onion peel", was used to construct a library of third generation dendrimers with 108, 180 and 252 hydroxyl surface groups using a combination of microwave assisted highly efficient CuAAC and thiol-ene reactions. These dendrimers were conveniently acquired with high purity and good yields in divergent manner using a variety of orthogonal and dense AB_3, AB_5, and AB_7 building blocks. The resulting polyhydroxylated dendrimers tested in several human cell types did not impair mitochondrial metabolic function or cell viability suggesting that they are good candidates for applications in biological investigations.

Introduction

The last three decades have marked the emergence of dendrimers with their manifold uses in diverse areas ranging from nanomedicine, drug delivery, pharmaceuticals, material sciences, catalysis, and gene therapy.[1-8] Due to the staggering growth and high demands for the rapid and efficient access to dendrimers, researchers were motivated to introduce new innovative strategies to produce these macromolecular entities in higher yields, fewer steps, and in more economical ways. Recent advances in synthetic methodologies have boosted the development of dendrimers in an efficient and rapid manner, but access to low generation dendrimers with large number of surface groups is still a challenge.

Since the first attempts toward accelerated construction of high generation dendrimers using double stage convergent method were described,[9] one of the major breakthrough was introduced wherein protection/deprotection steps were eliminated using orthogonal building blocks.[9,10] The concept of orthogonality was next exploited whereby successful sixth generation dendrimer could be achieved rapidly.[11] Higher generation dendrimers with large number of surface groups have gained great interest in recent years due to their potential use in electronics and nanomedicine.[12-18] However, only a handful approaches exist to synthesize dendrimers with large number of surface groups in few steps. One such example is a three-step synthesis of a POSS dendrimer having 392 end groups.[19] Hence, an additional clue for the rapid growth lies in the choice of highly functionalized cores.

In order to improve the art in the synthetic design of these well-defined macromolecular architectures, an "onion peel" approach was recently introduced by employing a combination of a variety of orthogonal building blocks and robust chemical reactions at each generation giving rise to structurally controlled smart dendrimers.[20] The versatility of this strategy was further demonstrated using both convergent and divergent routes to produce dendrimers with rationally programmed branching units.[21] Herein, a facile and accelerated "onion peel" dendrimer synthesis approach to provide large numbers of surface groups at low generations is reported. Thus, G3 dendrimers using AB_3, AB_5 and AB_7 orthogonal hypermonomers were generated to afford 108, 180, and 252 surface groups respectively in only 2 steps starting from a common G1 dendrimer (Figure 1). These hypermonomers were systematically scaffolded employing highly efficient atom economical chemical reactions such as Cu(I)-catalyzed alkyne-azide (CuAAC)[22] and thiol-ene reactions[23, 24] using microwave radiations.[25] Microwave-assisted reactions have been used in several instances in polymer synthesis to provide remarkable accessibility of reactive functionalities leading to higher yields.[26, 27] The present strategy also takes advantage of microwave radiations to enhance the rate of reaction and decrease the reaction time as we were attempting to conjugate bulky building blocks on large number of reactive surface functionalities. The syntheses were fast, convenient, and resulted in defect free monodisperse dendrimers in high yields.

Generally, the binding interactions between synthetic ligands and their cognate biological targets increase with increasing number of peripheral groups.[28-30] It is necessary to compare different generations of multivalent dendrimers to observe the effect of multivalency. The most attractive advantage of this accelerated approach is that by using orthogonal building blocks with different number of surface groups, it is possible to generate a library of dendrimers with different numbers of functional groups at the same generation.

To assess the dendrimers safety in biological systems, the cytotoxicity of the synthetic dendrimers with 108, 180 and 252-OH terminal groups in human liver carcinoma (HepG2), glioblastoma (U251), and breast adenocarcinoma (MCF-7) cells were evaluated.

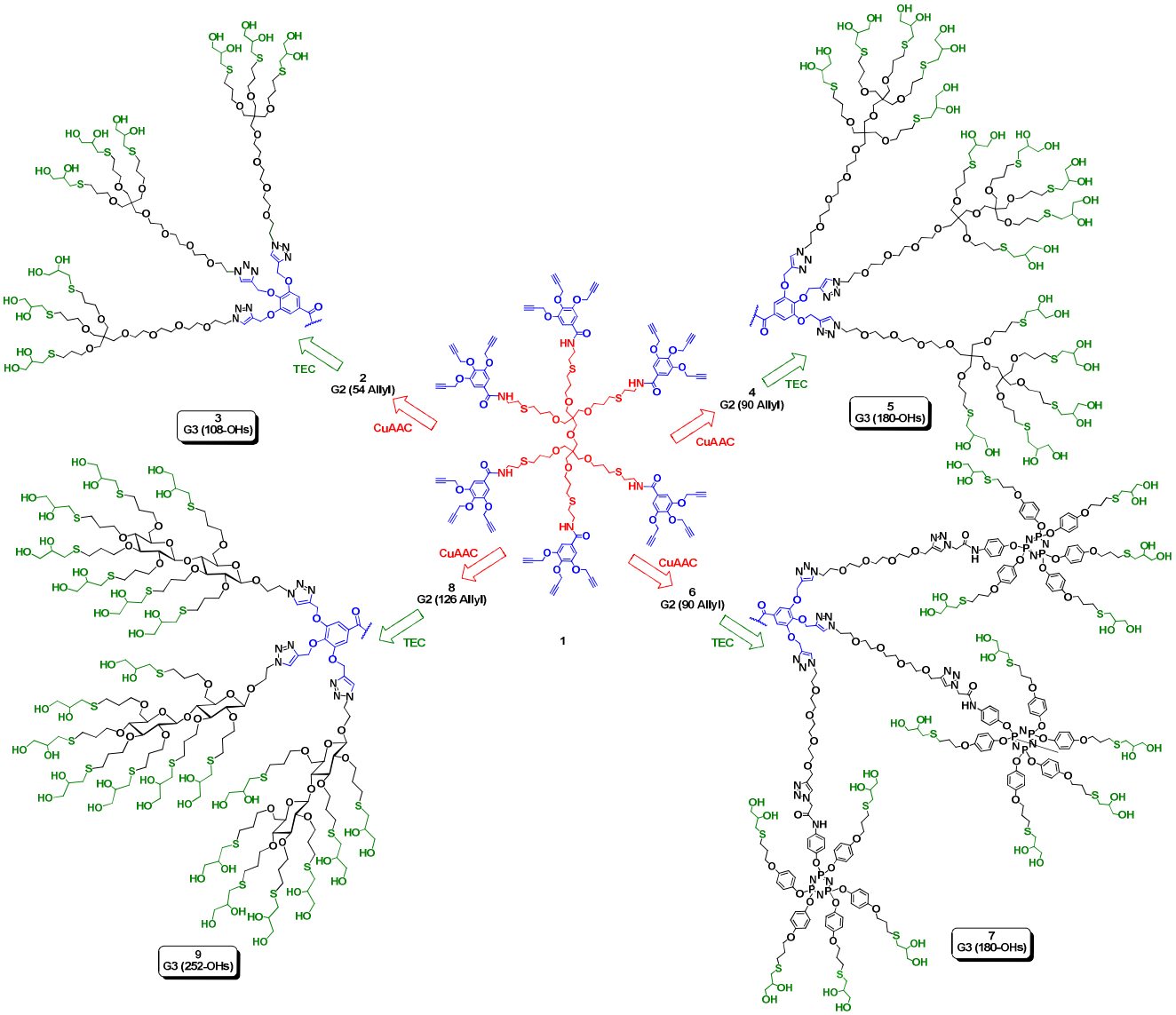


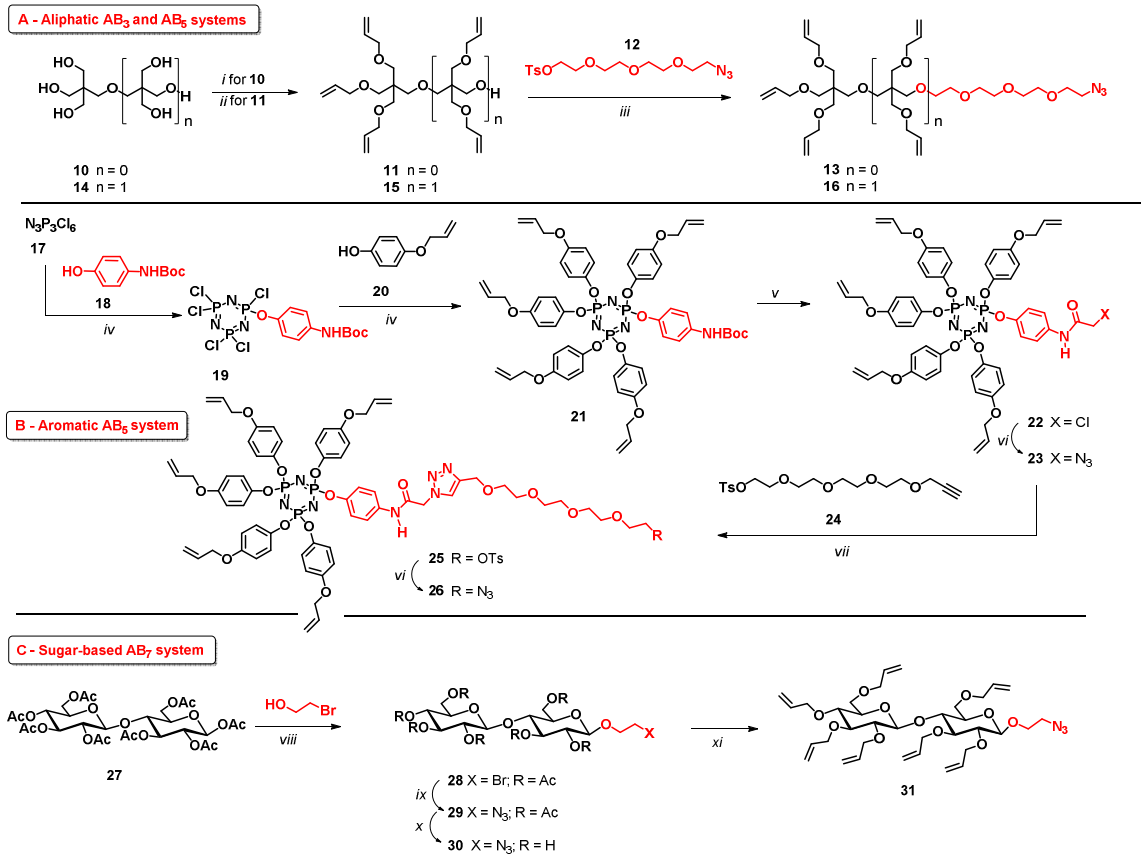
Fig. 1. Schematic illustration of accelerated divergent dendrimer synthesis from octadecavalent hypercore **1** *via* an "onion peel" approach using CuAAC and thiol-ene reactions with AB_3, AB_5, and AB_7 monomers giving rise to G(3)-dendrimers containing 108, 180, and 252 end groups, respectively.

Results and Discussion

The new polyhydroxylated dendrimer series (**3**, **5**, **7**, and **9**) were constructed by a divergent manner around an octadecavalent hypercore **1**[21] equipped with propargyl groups and hypermonomers to generate large number of surface groups at very low generations (Fig. 1). Scaffold **1**, incorporating a dipentaerythritol and gallic acid moieties, was synthesized by employing a previously reported procedure to provide 18 terminal acetylene groups at the G1 stage.[21] Novel orthogonal AB_3, AB_5, and AB_7 dendrons having different branching subunits with a focal azide group and terminal alkenes functionalities were created to first participate into powerful Cu(I) catalyzed alkyne-azide cycloadditions (CuAAC) followed at the next layer by multiple thiol-ene reactions (Scheme **1**). The synthesis of the initial AB_3 building block **13** was initiated by treating pentaerythritol **10** with allyl bromide and sodium hydroxide that provided pentaerythritol triallyl ether **11** in 75% yield.[31] Compound **11** was next reacted with monotosylated tetraethylene glycol azide **12**[32] (NaH and DMF) to afford first intermediate **13** in 80% yield (Scheme 1A) clearly showing its characteristic stretching azide band at 2102 cm^{-1} by IR spectroscopy.

Analogous aliphatic AB_5 monomer **16** was similarly prepared using commercially available and inexpensive dipentaerythritol **14**, which upon treatment with allyl bromide (10 equivalents) in 40% solution of sodium hydroxide in DMSO gave pentakis allyl derivative **15** in 40% yield along with its partially tetrakis allylated intermediate in 49% yield. It is worth mentioning here that the use of NaH instead of NaOH resulted in the formation of fully allylated derivative as the major product with minor amount (15%) of the pentakis allyl derivative **15**. Compound **15** was transformed as above with **12** into azide **16** in 68% yield (NaH, DMF, 4 h, 0°C) after column chromatography (Scheme 1A).

Alternatively, subsequent aromatic AB_5 dendron **26**, possessing the analogous azido-alkene functionalities, was prepared starting from hexachlorocyclotriphosphazene **17** ($N_3P_3Cl_6$) (Scheme 1B). To this end, monofunctionalization of **17** was first carried out by treatment with 0.5 equivalent of *N*-Boc-protected 4-aminophenol (**18**) in the presence of dry cesium carbonate (THF, reflux, 18 h) to afford the expected compound **19** in a moderate yield (50%). The ^{31}P-NMR spectrum of **19** showed the characteristic triplet signal of the P-O-linked phosphorous at δ 12.8 ppm and a doublet signal at δ 22.4 ppm (P-Cl) due to the unsymmetrical environment of the molecule.[33] Using similar conditions, pentachloride **19** was treated with excess of *p*-allyloxyphenol **20** to provide pentakis-allylated dendron **21** in 88% yield, which showed identical phosphorous chemical shift at δ 9.89 ppm (triplet). *N*-Boc-deprotection of **21** (TFA, DCM, 0°C-rt) and subsequent *N*-chloroacetylation with chloroacetyl chloride and Hunig's base provided **22** in 76% yield. The chloride group in **22** was further substituted by an azide group using NaN_3 and NaI in DMF to give **23** in 81% yield. An upfield shift of the α-methylene protons from δ 4.18 to 4.12 ppm in its ^{1}H-NMR spectrum unequivocally confirmed the product formation.



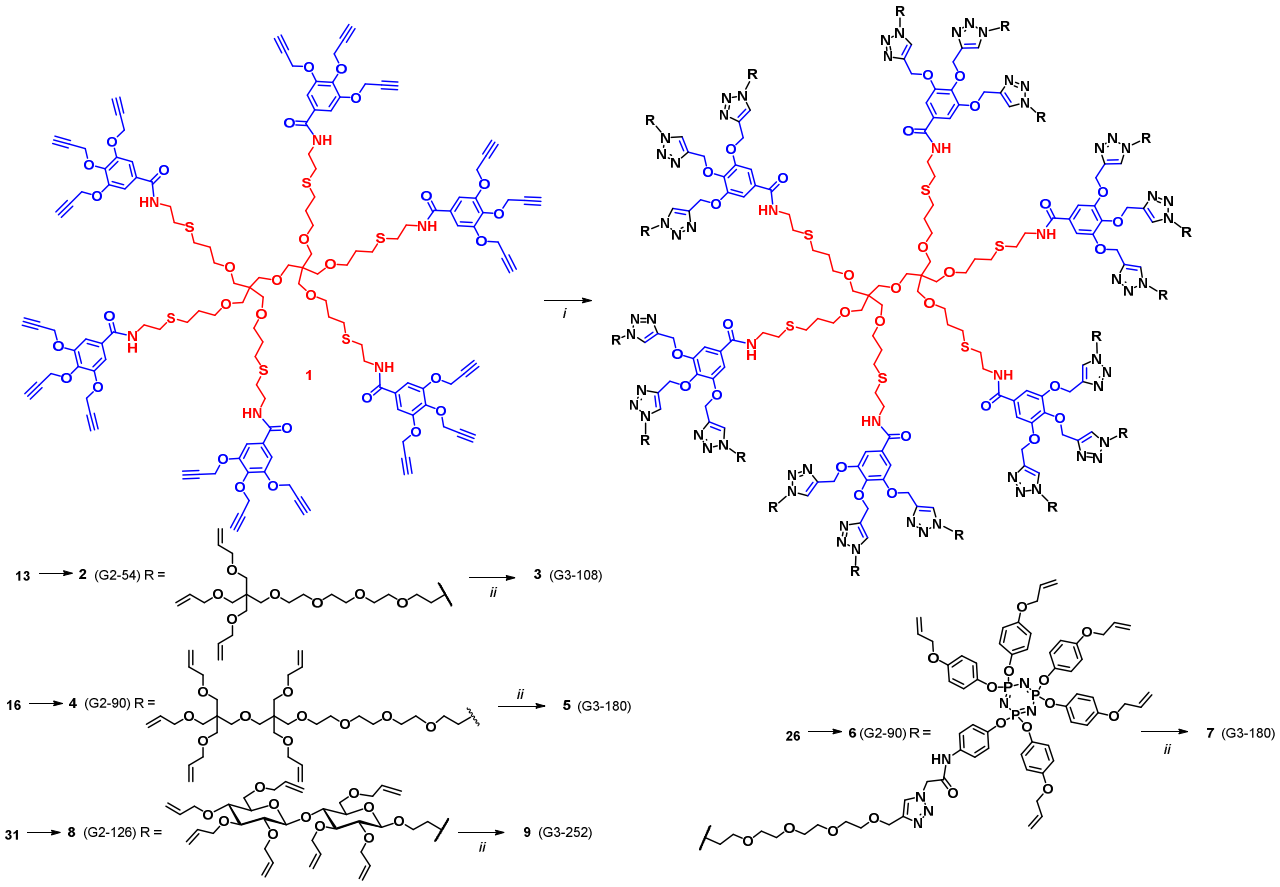
Scheme 1. A: Synthesis of aliphatic AB_3 (**13**) and AB_5 building blocks (**16**). *Reagents and conditions*: (*i*) AllylBr, NaOH, H_2O, rt, 75%; (*ii*) AllylBr, 40% NaOH, DMSO, 16h., 0°C−rt, 40%; (*iii*) NaH, DMF, 4h., 0 °C, 80% for **13** and 68% for **16**. **B:** Synthesis of AB_5 aromatic building block **26**. *Reagents and conditions*: (*iv*) Cs_2CO_3 anhy., dry THF, reflux, 18h., 50% (with 0.5 eq. of **18**) and 88% (with 10.0 eq. of **20**); (*v*) TFA, DCM, 0 °C−rt, 4h. then DIPEA, chloroacetyl chloride, DCM, rt, 4h., 76% (2 steps); (*vi*) NaN_3, NaI, DMF, 60°C 12h., 81% for **23** and 86% for **26**; (*vii*) $CuSO_4{\cdot}5H_2O$, Na Asc., THF/water (1:1), 55°C, overnight, 64%. **C:** Synthesis of AB_7 sugar-based building block **31**. *Reagents and conditions*: *(viii)* BF_3 etherate, DCM, 0°C, 4h., 50% ; *(ix)* NaN_3, DMF, 70°C,4h., 92%; *(x)* NaOMe/MeOH, rt, 3h., 90%; *(xi)* AllylBr, NaH, DMF, 0°C−rt, 2h., 85%.

To avoid the possibility of partial reactions due to the steric hindrance of this bulky AB_5 system with the dense G1 core, an extended linker was incorporated. Thus, azido alkene **23** was treated with monopropargylated tosyltetraethylene glycol (TEG **24**)[34] under classical click reaction conditions ($CuSO_4.5H_2O$, Na ascorbate in THF/H_2O) to afford dendron **25** in 64% yield. ^{1}H-NMR spectroscopy confirmed the completion of reaction as the sharp singlet for triazole proton appearing at δ 7.81 ppm integrated nicely with the NH (δ 8.73 ppm) and one of the allyl signal (δ 6.08-5.93 ppm). In the next step, the tosyl group of dendron **25** was substituted with an azide group using NaN_3 in DMF to afford **26** in 86% yield (Scheme 1B). In the ^{1}H NMR spectrum of the final pentaallylated azidodendron, the diagnostic signals related to tosyl group at δ 7.37, 7.26 and 2.38 ppm completely disappeared, thus confirming complete conversion.For the synthesis of the sugar-based AB_7 hypermonomer **31,** boron trifluoride etherate ($BF_3.Et_2O$) promoted glycosylation was performed between cellobiose octaacetate **27** and 2-bromoethanol which lead to the formation of **28** in 50% yield based on the isolated β-anomer. Bromide

substitution in **28** using sodium azide in DMF gave azido derivative **29** in 92% yield. Again, the characteristic frequency of the azide stretching was observed in the IR spectrum at 2105 cm^{-1}. Treatment of peracetate **29** under typical Zemplén conditions (NaOMe/MeOH) provided completely de-*O*-acetylated dendron **30** in 90% yield. Per-*O*-allylation with allyl bromide under usual conditions (NaH, DMF) provided cellobiose-based AB_7 derivative **31** in 85% yield (Scheme 1C). Complete allylation was confirmed by ^{1}H-NMR as distinct allyl signals appeared at δ 6.07-5.79 and 5.35-5.04 ppm.

All the orthogonal building blocks were thus successfully achieved in high purity with excellent to moderate yields. They were fully characterized by ^{1}H-, ^{13}C-, ^{31}P-NMR, COSY experiments, HRMS, and IR spectroscopy (see SI). These modular bifunctional building blocks were designed and synthesized to satisfy the ongoing quest to create complex dendritic scaffolds with high functionalities at low generation and using efficient ligation chemistry.

After building the hypermonomer subfragments **13**, **16**, **26**, and **31**, representing perallylated azides, key steps to construct the final dendrimers were followed. In order to construct the final dendrimers, a divergent "onion peel" route using CuAAC and thiol-ene reactions as synthetic tools were followed. Both these reactions have been successfully established for the synthesis of dendrimers and polymeric materials due to their desired features including simple execution, high reaction yields, few undesired side products, and easy purifications. The most attractive feature of these reactions is the introduction of orthogonality, which improves the synthetic route by dramatically decreasing the number of reaction steps. The synthesis of the next G2 dendrimer **2**, having 54 peripheral allyl groups, was achieved by ligating the AB_3 building block **13** with hypercore **1** employing standard CuAAC click reaction under microwave at 50°C (Scheme **2**). The complete conversion was obtained within 5 hours to yield the G2 dendrimer **2** easily purified using silica gel column chromatography in 78% yield.



Scheme 2 Synthesis of hyperbranched dendrimers through a highly divergent accelerated approach. *Reagents and conditions*: (*i*) $CuSO_4 \cdot 5H_2O$, Na Asc., 50°C, 5h., microwave, G(2)-54 Allyl (**2**, from **13**): 78%, G(2)-90 Allyl (**4**, from **16**): 71%, G(2)-90 Allyl (**6**, from **26**): 50%, G(2)-126 Allyl (**8**, from **31**): 75%; (*ii*) 1-Thioglycerol, AIBN, methanol, 90°C, 6h., microwave, G(3)-108 OH (**3**): 86%, G(3)-180 OH (**5**): 82%, G(3)-180 OH (**7**): 63%, G(3)-252 OH (**9**): 85%.

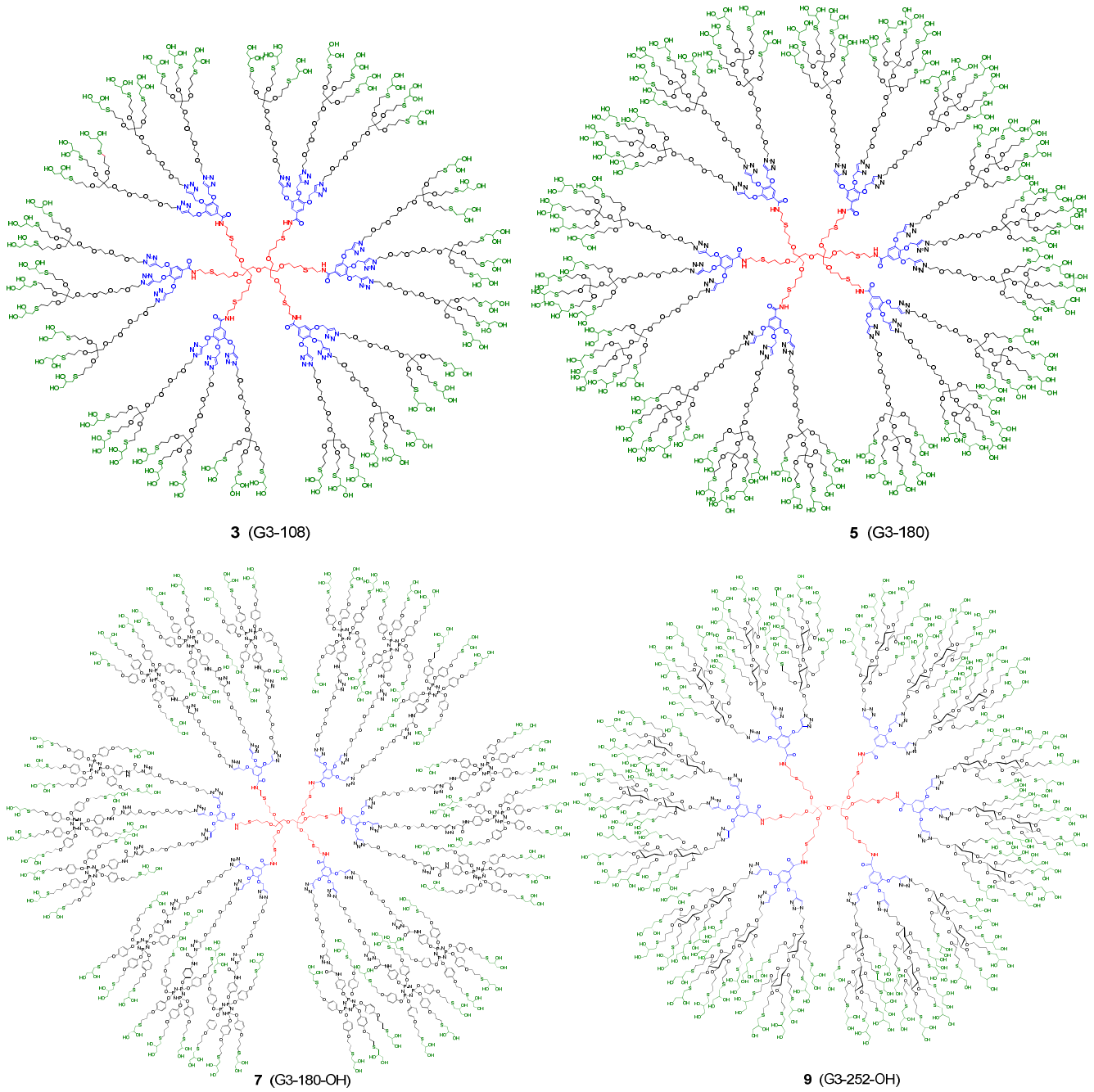


Figure 2 Molecular structures of dendrimers **3**, **5**, **7**, and **9** with 108, 180, and 252-OH end groups at third generation.

Completion of the reaction was clearly established by ^{1}H NMR spectroscopy which showed the complete disappearance of the propargylic C≡C*H* signals at δ 2.50 ppm and the expected appearance of two distinct triazole signals integrating in a 2:1 ratio at δ 7.92 and 7.84 ppm, respectively. In addition, characteristic signals for the allylic proton were also observed at δ 5.93-5.80 ppm.

The monodisperse nature of dendrimer was also confirmed by MALDI-TOF data and GPC. MALDI-TOF spectrum showed the molecular ion peak corresponding to Na^+ adduct at 10814 (10791 M^+ + 23). The same (click) reaction was also carried out using an oil bath under similar conditions, but multiple spots were observed on TLC indicating partial conversions. Dendrimer **2** was further subjected to thermal thiol-ene reaction using 1-thioglycerol as an AB_2 monomer to afford G3 dendrimer **3** having 108-OHs surface groups. The purification was carried out by simply washing the reaction mixture with diethylether, followed by dialysing against distilled water using 1000-MW cut-off dialysis membrane. Complete disappearance of allylic signals and appearance of signals for protons corresponding to thioglycerol confirmed the product formation. HRMS (ESI^+) spectrum showed the expected molecular ion peak at 16631 (see Table 1 and SI).

After the successful execution of the above synthetic strategy with aliphatic AB_3 monomer **13**, the synthesis of the next higher order branching units was attempted. The synthesis of perallylated dendrimer **4** was carried out by treating hypercore **1** with penta-allylated AB_5 monomer **16** using the above CuAAC conditions under microwave to afford G2 dendrimer **4** harbouring 90 active alkene functions in 71% yield. The terminal alkenes of dendrimer **4** were next treated with 1-thioglycerol as above to provide G3 dendrimer **5** accommodating 180 terminal hydroxyl groups. The dendrimer was purified by precipitation with diethyl ether followed by dialysis against water to give the pure product in 86% yield. Once again, our accelerated "onion peel" approach proved to be efficient enough to introduce 180 surface groups at G3 stage only.

The next goal was to try even bulkier building blocks to test the potential of the strategy for complex dendrimers construction. In general, bulky hypermonomers are responsible for creating structural defects in dendritic scaffolds due to steric hindrance. Perpropargylated hypercore **1** was further reacted with a bulkier AB_5-based hypermonomer **26** (N_3P_3 core) via CuAAC click reaction using microwave radiations to generate G2 dendrimer **6** in an acceptable 50% yield after purification as above. The GPC chromatogram showed a narrow and symmetrical Gaussian pattern with a polydispersity index of 1.03, confirming the monodisperse nature of the dendrimer. Its ^{31}P-NMR spectrum showed a singlet for phosphorous indicating the symmetrical structure. In the next step, dendrimer **6** was subjected to the above thiol-ene conditions with 1-thioglycerol to afford dendrimer **7** with 180-OHs surface groups in 63% yield. The ^{1}H-NMR spectrum indicated the complete disappearance of all the allylic signals, again confirming the product formation.

Finally, the synthesis of perallylated dendrimer **8** using the novel glyconanosynthon,[8] constituting an AB_7 branching motif, was carried out. It has to be noted that the use of such an hyper functionalized synthons has been rarely described in the literature and reported only once for dendrimer synthesis.[19] The synthesis was achieved by treating hepta-allylated AB_7 monomer **31** with **1** using CuAAC click reaction under microwave to furnish G2 dendrimer **8** possessing 126 allyl groups at the periphery in 75% yield. Final coupling of excess 1-thioglycerol onto dendrimer **8** was achieved through thiol-ene coupling as mentioned in the general protocol described above. To test the reliability and sensitivity of NMR for complete conversion, the reaction conditions described above was used. As shown by ^{1}H-NMR spectroscopy (Figure 3), incomplete conversion (80%) was observed after 5 hours. The reaction was resubmitted for another 2 hours with the addition of more 1-thioglycerol (2 eq/alkene), which led to complete conversion as shown by the ^{1}H-NMR spectrum which indicated complete disappearance of all allyl signals. MALDI-TOF spectrometry also confirmed product formation by showing mass peaks corresponding to sodium adducts perfectly matching with the calculated value (SI). It is worth mentioning here that the resulting dendrimer **9** possesses 252 hydroxyl surface groups at the G3 stage only and can still be used as a precursor for further functionalization. There is still the possibility to generate an even greater number of peripheral groups at the G3 stage by using higher order AB_3 or AB_4 building blocks such as the AB_2 monomer (1-thiogycerol) used above. As a comparison, PAMAM dendrimer with ethylenediamine core (A_2 and AB_2 building blocks) bearing 256 surface groups requires generation 6 (G6) and approximately twelve steps, while the accelerated "onion peel" strategy allows us to acquire 252 surface groups at exactly half the number of generations and one third of the number of necessary reaction steps.

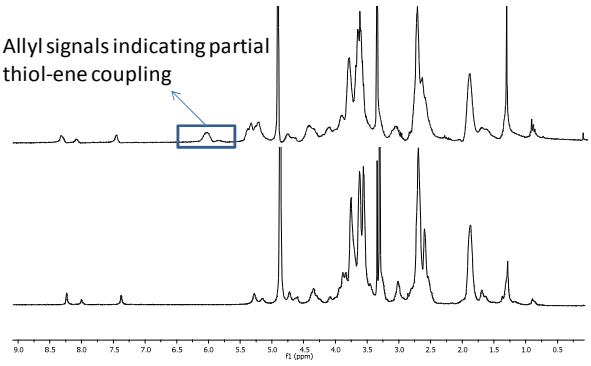


Figure 3. ^{1}H-NMR spectrum of **9** (G3-252-OHs) (Top) after 5 hours under microwave at 50°C indicating 80% conversion; (Bottom) After 7 hours under microwave at 50°C indicating 100% conversion.

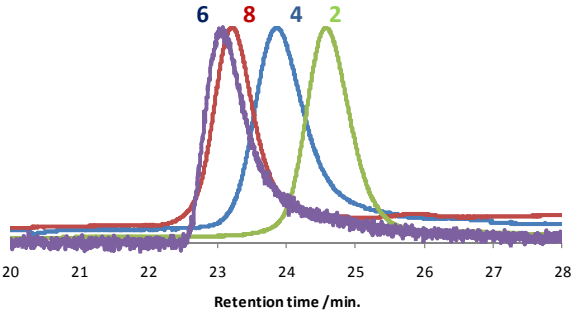


Figure 4 GPC traces of the perallylated G2 dendrimers: **2** (54-Allyl), **4** (90-Allyl), **6** (90-Allyl), and **8** (126-Allyl)

All dendrimers described herein were fully characterized using NMR (^{1}H, ^{13}C, ^{31}-P, COSY), IR, mass spectrometry and were shown to be in full agreement with the structures presented. Gel permeation chromatography (GPC) were performed at the penultimate steps and all the chromatograms showed narrow peaks with low polydispersity indexes (PDI) indicating the monodisperse nature of the products (Figure 4 and Table 1). The *Mn* values obtained from GPC exhibited very good correlation with theoretical molecular weights as well as molecular weights acquired from mass spectrometric data. The dendrimer diameters in solution were calculated with the help of dynamic light scattering (DLS) and diffusion NMR spectroscopy experiments (Table 1). Diffusion NMR experiments were carried out in methanol at 25°C to measure diffusion coefficients D.[35] The corresponding solvodynamic diameters ($D_s = 2 \times R_s$) were calculated using the Stokes–Einstein equation and the viscosity of pure CD_3OD (Table 1). The sizes of the dendrimers were also obtained using DLS technique in methanol. Hydrodynamic

diameters calculated from both methods were remarkably close and were in the range of approximately 2-8 nm for the G3 dendrimers. Interestingly, dendrimer **7**, having a dense N_3P_3 scaffold, appears as an unusually packed structure by both DLS and DOSY-NMR, Table 1). This could be rationalized on the basis of its 3D structure having 3-up/3-down substituent orientations.[3,33]

Table 1. Summary of characterization of dendrimers.

Entry	Dendrimer	Theoretical MW	Exp. Mass [MALDI/MS]	*Mn* [a] (g/mole)	PDI	D^b (m^2s^{-1})	D_s^c (nm)	D_H^d (nm)
1	**2 (G2-54)**	10791.1392	10814.3920 $[M+Na]^+$	10770	1.08	-	-	-
2	**3 (G3-108)**	16631.7479	16631.7480	-	-	1.15×10^{-10}	6.30	5.70
3	**4 (G2-90)**	14359.7980	14385.1000 $[M+Na]^+$	14490	1.2	-	-	-
4	**5 (G3-180)**	24110.1882	24124.9040	-	-	1.05×10^{-10}	6.90	8.22
5	**6 (G2-90)**	26481.1320	26249.7870	26350	1.03	-	-	-
6	**7 (G3-180)**	36215.4798	37226.6720	-	-	2.54×10^{-10}	2.85	1.95
7	**8 (G2-126)**	15007.6007	15011.0180	15140	1.07	-	-	-
8	**9 (G3-252)**	28667.7725	28690.0370 $[M+Na]^+$	-	-	1.23×10^{-10}	5.90	6.41

[a] Determined from GPC.
[b] Diffusion coefficient measured in CD_3OD at 25 °C[36].
[c] Solvodynamic diameter from diffusion NMR experiment calculated using the Stokes-Einstein equation. The error on the measurement can be estimated from repeated calculations of the diffusion coefficients to be below 5%.
[d] Hydrodynamic diameter determined from DLS experiment in methanol.

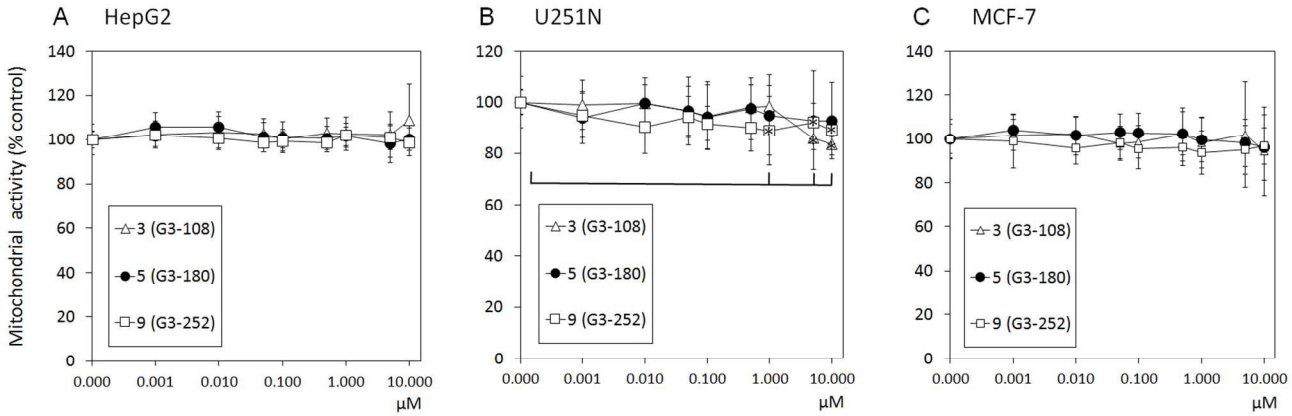


Figure 5. Low cytotoxicity of dendrimers in human cells. Dendrimers **3** (G3-108), **5** (G3-180) and **9** (G3-252) were tested in **(A)** HepG2 liver carcinoma, **(B)** U251N glioblastoma and **(C)** MCF-7 breast adenocarcinoma cells. Increasing concentrations of dendrimers (1 nM – 10 µM) were incubated with the cells for 24h. Mitochondrial metabolic activity was assessed using the MTT assay. Values are presented as mean percentages ± S.D relative to untreated controls (set as 100%).The data is reported for six measurements for each concentration. Three independent experiments performed *($p < 0.01$).

The cytotoxicity of the polyhydroxylated dendrimers **3**, **5** and **9** was evaluated *in vitro* using three different human cell lines. Human liver, glioblastoma and breast cancer cells were selected as models commonly used for the screening of polymeric biomaterials. Mitochondrial metabolic activity was determined using the MTT assay (*i.e.* formazan production by mitochondrial dehydrogenases).[37] Cell viability upon dendrimer treatment was also determined by nuclear labelling with Hoechst 33342 (10 µM, 10 mins), and cells were imaged with a fluorescence microscope. The concentration range used in both assays (1 nM to 10 µM) was delimited based on previous dendrimer cytotoxicity studies.[38] Concentration-dependant effects of dendrimers on mitochondrial activity in human cells exposed for 24h is shown (Figure 5).

No significant decrease in metabolic activity was observed in HepG2 and MCF-7 cells at any treatment concentration. In turn, dendrimers 3 and 9 were mildly cytotoxic in the highly proliferative U251N cells at 5-10 μM and 1-10 μM concentrations, respectively. Similar results were obtained in the viability assay based on nuclear labelling with Hoechst 33342 and subsequent cell counting (Figure 6).

The suitability of dendrimers for drug delivery or other biomedical applications is dependent on the number and the nature of their end groups.[7] Here, the hydroxyl groups found on the surface of dendrimers **3**, **5**, and **9** provide a neutral outer shell that reduces toxicity.[39, 40] In contrast, cationic end groups, such as the primary amines in poly(amidoamine) dendrimers, tend to induce concentration-dependent and generation-dependent toxicity both *in vitro* and *in vivo*.[41-43] Overall, dendrimers **3**, **5**, and **9** displayed very low toxicity in the human cell lines and concentration range tested, rendering them suitable for biomedical applications.

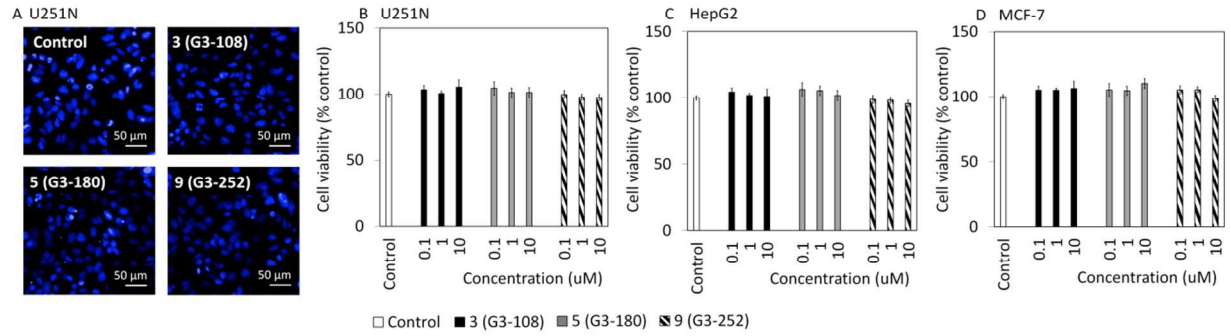


Figure 6 Concentration-dependent effect of dendrimers on human cell viability. Fluorescence micrograph (A) U251N glioblastoma treated with dendrimers **3**, **5**, and **9** (10 μM, 24h) and labelled with Hoechst 33342 (10 μM, 10 mins) (scale = 50 μm). The cytotoxicity of dendrimers **3** (G3-108), **5** (G3-180) and **9** (G3-252) was tested in (B) U251N glioblastoma, (C) HepG2 liver carcinoma, and (D) MCF-7 breast adenocarcinoma cells. Dendrimers in increasing concentrations (up to 10 μM) were incubated with the cells for 24h. Cell viability was assessed by high-throughput imaging (Operetta, Perkin Elmer) of Hoechst 33342 labelled cells. Values are presented as means ± S.D relative to untreated controls (set as 100%). The data is reported for three independent experiments performed in six replicates. *($p < 0.01$).

Conclusions

The syntheses of G3 dendrimers bearing 108, 180, and 252 hydroxyl surface groups using AB_3, AB_5, and AB_7 hypermonomers were successfully achieved. The dendrimers were constructed using highly efficient and facile accelerated "onion peel" approach without requiring any protection/deprotection steps. The use of hypercore and hypermonomers along with the combination of highly efficient chemical reactions (CuAAC and thiol-ene) provided rapid access to introduce high number of precise surface groups at low generations. Click reactions turned out to be highly efficient in ligating large number of functional groups and producing monodisperse dendrimers. The outer hydroxyl terminal groups provide a reactive platform for further growth and attachment of several other types of functionalities.[44-51] Hydroxylated dendrimers presented here and their derivatives could be easily synthesized and used for diverse range of applications. A particular attraction of these dendrimers for applications in biology is their low toxicity. Future studies should include the investigations of dendrimer pharmacokinetics and pharmacodynamics as well as their cellular uptakes. Relative ease of tailoring dendrimer chemistry to best fit physical and chemical properties of selected biologically active agents makes these dendrimers attractive candidates for further biological investigations in primary human cell cultures and experimental animal models mimicking different pathologies. In addition, the strategy described herein nicely complements the one using self-assembling Janus dendrimersomes,[52-53] including glycodendrimersomes.[54,55]

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Notes and references

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A fast track strategy toward highly functionalized dendrimers with different structural layers: “Onion peel approach”

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A novel strategy is described for the rapid syntheses of polyhydroxylated dendrimers in which the layer by layer building blocks are different from one another. The resulting dendrimers showed no cytotoxicity.