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Sandra García-Gallego, Patrik Stenström, Pablo Mesa-Antunez, Yuning Zhang, and Michael Malkoch

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Synthesis of heterofunctional polyester dendrimers with internal and external functionalities as versatile multipurpose platforms

*Sandra García-Gallego, [a†§] Patrik Stenström, [a§] Pablo Mesa-Antunez, [a] Yuning Zhang, [a] and Michael Malkoch [*a]*

[a] Department of Fiber and Polymer Technology, School of Chemical Science and Engineering, KTH Royal Institute of Technology, Teknikringen 56-58, SE-100 44, Stockholm, Sweden.

E-mail: malkoch@kth.se

ABSTRACT: Heterofunctional dendrimers with internal and external representation of functionalities are considered as the ultimate dendritic frameworks. This is reflected by their unprecedented scaffolding, such as precise control over structure, molecular weight, number and location of different cargo across the whole dendritic skeleton. Consequently, these dendrimers with multipurpose character are the pinnacle of precision polymers and thereof are highly attractive by the scientific community as they can find use in a great number of cutting-edge applications, especially as discrete unimolecular carriers for therapeutic exploitation. Unfortunately, most established dendrimer families display external functionalities but lack internal scaffolding ability, which lead to inherent limitation for their full potential use as precision carriers. Consequently, we here embark on a novel synthetic strategy facilitating the introduction of internal functionalization of established dendrimers. As a Proof-of-Concept, a new class of internally and externally functionalized multipurpose dendrimers based on the established 2,2-bis(methylol)propionic acid (bis-MPA) were successfully accomplished by the elegant and simple design of AB_2C

monomers, amalgamated from two traditional AB_2 monomers. By utilizing Fluoride-Promoted Esterification (FPE), straightforward layer-by-layer divergent growth up to the fourth generation was successful in less than one day reaction time, with a molecular weight of 15 kDa and displaying 93 reactive groups divided by 45 internal and 48 external functionalities. The feasibility of post-functionalization through click reactions is demonstrated, where the fast and effective attachment of drugs, dyes and PEG chains is achieved, as well as crosslinking into multifunctional hydrogels. The simplicity and versatility of the presented strategy can easily be transferred to generate a myriad of functional materials such as polymers, surfaces, nanoparticles or biomolecules.

KEYWORDS. click chemistry, dendrimers, esterification, hydrogels, nanocarriers.

1. Introduction

The pursuit of multipurpose scaffolds has long been a topic of research in nanomedicine,[1-2] where the ability to transport several cargos such as therapeutic drugs, targeting agents and fluorescent tags, is highly desirable for cancer therapy, cardiovascular disease and

infections. For example, actively targeted nanocarriers increase the drug efficiency and minimize the side-effects;[3] or theranostics, which combine therapeutic and diagnostic actions in a single entity, emerge as a unique tool for predictive, preventive and personalized medicine.[4] Overall, multipurpose scaffolds increase the therapeutic efficacy by:[2] (i) enhancing the water-solubility and biodistribution; (ii) extending the blood circulation; (iii) improving stability; (iv) providing response to stimuli; (v) enabling an active or passive targeting; and (vi) enabling an *in situ* monitoring.

Despite ongoing nanomedicine research based on nano-based drug delivery systems (DDS), the journey to clinical approval is arduous.[5] Challenging obstacles such as the ease of preparation, safety and cost of the materials, scalability, batch-to-batch reproducibility, as well as the stability, biocompatibility and biodegradation of the final product need to be considered.[6-8] Since Doxil®, the first FDA-approved nano-drug, several formulations for passive and active targeting can be found in the market.[9] These DDS produce biologically reproducible activities, but lack a precise control over the type,

number and location of the biologically-relevant cargo within the carrier. This would provide critical information about the therapeutic response of such materials and enable a personalized treatment.[10]

To date, dendrimers are the only polymeric scaffolds capable of providing these features due to their monodispersity and multivalency,[2, 11] besides the influence of the branched architecture in drug delivery.[12-13] Successful examples, at pre-clinical or even clinical stage, have been reported for dendrimers comprising polylysine (e.g. VivaGel®),[14] poly(amidoamine) (PAMAM),[15] phosphorous[16] and bis-MPA based polyester scaffolds,[17] among others. To expand their usefulness, heterofunctional dendrimers (HFDs) were developed, which contain at least two distinctive functionalities typically attached to the dendritic surface in a statistical, alternating or block manner, but most often having dormant interiors.[6, 18-19]

It is inevitable that heterofunctional dendrimers full potential can only be capitalized on by displaying functionalities throughout the whole macromolecular skeleton e.g. to increase

carrying capacity and optimize its potential.[2, 11] The complex and tedious synthesis of dendrimers, however, has led to an extremely low number of reports proposing dendritic scaffolds with interior and exterior functionalities.[6, 20, 21] A viable method to render the dendritic interior more accessible is to incorporate highly selective and reactive pendant functional groups during the dendritic growth. This was accomplished by Malkoch *et al.*, who converted AB_3 monomer tris(hydroxymethyl)aminomethane (Trizma®) to its AB_2C derivative, where the A and B groups were used for dendrimer growth and the C groups were precisely functionalized through the copper-catalyzed azide-alkyne cycloaddition (CuAAC) click reaction.[22] Unfortunately, Trizma-based dendrimers were found susceptible toward fast hydrolytical degradation, limiting further exploitation.

Consequently, novel synthetic strategies that can expand the scaffolding window of already established dendrimers and in parallel maintain the intrinsic features of the main building block is logical progression in dendrimer chemistry.[16-17] The new multipurpose HFDs would resemble most of their parent´s properties – e.g. biocompatibility,

degradability, solubility – while providing increased accessibility and carrying capability of the typically dormant interior. To accomplish this, traditional building blocks must be synthetically revisited and re-programmed to include orthogonal chemoselective groups that are dormant during the dendrimer growth.

Inspired by the "AB_2C approach", we here unlock a facile strategy to internally and externally functionalized HFDs synthesized using a new generation AB_2C monomers by combining two identical monomers. As proof-of-concept, the bis-MPA AB_2 monomer was selected – which is simple, versatile and commercially available-,[23] and generates biocompatible and biodegradable dendrimers.[17, 23] Nevertheless, the proposed strategy is a guiding approach which can be translated to other monomer/dendrimer platforms with other chemistries, where two individual building blocks with orthogonally complementary functionalities are combined into a single AB_2C monomer. Furthermore, the simplicity and versatility of the AB_2C approach could expand the library of high-value precursors with orthogonal functionalities, including monomers and dendrons as well as derived

functional materials such as polymers, surfaces, nanoparticles or biomolecules, in a straightforward way.

In contrast to the state-of-the-art in internally/externally functionalized HFDs, the current strategy benefits from the versatility of combining two traditional AB_2 building blocks to generate AB_2C monomers, as well as from the advantages of using the Fluoride-Promoted Esterification (FPE) with imidazolide-activated compounds. FPE chemistry has earlier been described by Malkoch and coworkers as a powerful synthetic tool to generate monomers,[24] dendrimers[24-25] and dendrons[26] with high fidelity and functionality. FPE relies on 1,1'-carbonyldiimidazole (CDI) as an activating agent, where it demonstrates clear advantages in terms of scalability, widespread utility and green chemistry,[27] generating CO_2 and imidazole as the only by-products, which can easily be removed through wash steps; as well as on catalytic Cesium Fluoride (CsF), exhibiting unprecedented efficacy in the synthesis of complex dendritic polyesters.[25] Consequently,

FPE chemistry was herein explored as a tool in the facile construction of internally/externally functionalized HFDs.

2. Experimental section

2.1. Synthetic procedures.

Synthesis and characterization details (^{1}H, ^{13}C-NMR; MALDI-TOF; FTIR; SEC) for the different monomers, dendrimers and click probes can be found in the Supporting Information. The most relevant general synthetic procedures are:

Dendrimer synthesis - General esterification procedure. The carboxylic-functionalized compound is slowly added over a suspension of CDI in EtOAc (1-2 M), while heating the mixture at 50°C. After 15 min stirring at 50°C, CsF (0.1 or 0.2 eq/OH, for monomers or dendrimers respectively) and the hydroxyl-functional compound is added and the reaction proceeds at 50°C. The reaction is monitored by NMR and MALDI-TOF. Upon completion the mixture is allowed to cool to room temperature, and the excess of imidazolide-

activated acid is quenched by stirring with water. The mixture is then diluted with EtOAc and washed repeatedly with aqueous solutions of 10% $NaHCO_3$, 10% $NaHSO_4$ and brine before being dried with $MgSO_4$, filtered and evaporated. Dendrimers are isolated in 70-98% yields.

Dendrimer synthesis - General deprotection procedure. The acetonide-protected dendrimer is dissolved in MeOH and *p*-TSA (10 wt.%) is added. The mixture is stirred 1 h at r.t. and then percolated over an Amberlyst A21 column. The solution is concentrated to dryness and washed thoroughly with DCM to remove methanol traces. Dendrimers are isolated in 89-98 % yields.

Dendrimer post-functionalization - General procedure for CuAAC click reaction. The heterofunctional dendrimer and the azide- or alkyne-functional probe are dissolved in DMSO. The flask is degassed and flushed with argon before adding CuBr and PMDETA. The flask is once again degassed and sealed. The solution is stirred for 1 h at r.t. under inert atmosphere. The purification protocol will depend on the nature of the final product.

Dendrimer post-functionalization - General procedure for thiol-ene click reaction. The heterofunctional dendrimer and the thiol-functional probe are dissolved in methanol and the photoinitiatior LAP (1 wt.%) is added. The solution is irradiated for 20 min with 365 nm UV light using a Black-Ray B-100AP UV-lamp, and then the solvent in removed by rotoevaporation. The purification protocol will depend on the nature of the final product.

Dendrimer post-functionalization - General procedure for FPE reaction. The functionalization through Fluoride-Promoted Esterification follows the same procedure as for dendrimer growth.

2.2. Hydrogels preparation.

Hydrogels preparation - CuAAC crosslinking. The heterofunctional dendrimer and dialkyne PEG (10 kDa) were dissolved in equal volumes of THF and deionized water respectively and mixed. Stoichiometric ratios of azides and alkynes were used. The water solution used to dissolve the PEG contained 0.8 equivalents of sodium ascorbate and 0.8

equivalents of $CuSO_4 \cdot 5H_2O$ per reactive functional group. The total mass of dendrimer and PEG in the final mixture was 20 wt.%. The mixture was sonicated and 15 µL droplets were added to a PTFE-plate. The mixture was allowed to gel at room temperature. All droplets solidified into discs that swelled when submerged in deionized water.

Hydrogels preparation - Thiol-Ene crosslinking. The heterofunctional dendrimer and dithiol PEG (10 kDa) were dissolved in a 1:1 ethanol and deionized water solution with 0.5 wt.% LAP at stoichiometric ratios of alkenes and thiols. The total mass of dendrimer and PEG was 20 wt.%. 15 µL droplets were added to a PTFE-plate that was cured with 365 nm UV light using a Black-Ray B-100AP UV-lamp for 5 min. All droplets solidified into discs that swelled when submerged in deionized water.

2.3. Cell culture and cytotoxicity assays.

Cell culture. The cell lines (mouse monocyte cell Raw 264.7 and human debris fibroblast cell (hDF)) were obtained from ATCC (American Tissue Culture Collection) and

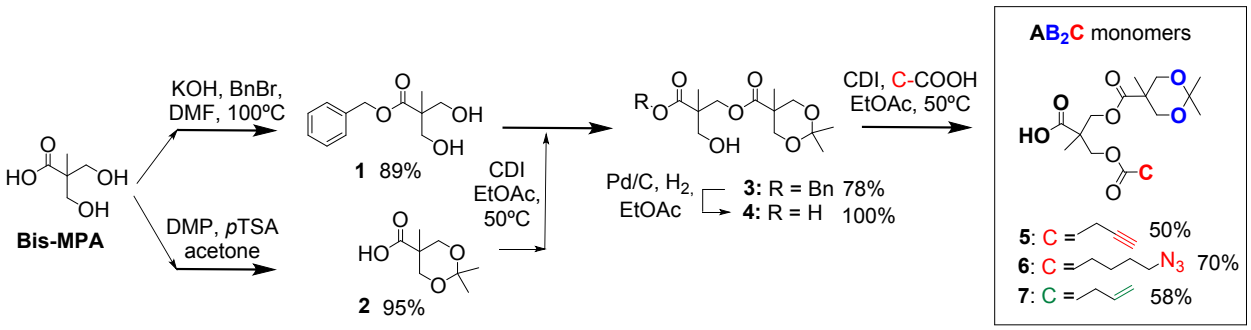
maintained in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum FBS and 100 units mL^{-1} penicillin plus 100 mg mL^{-1} streptomycin under 5% CO_2 at 37 °C.

Cytotoxicity assessment. The cytotoxicity produced by the different HFDs was evaluated through AlamarBlue assay. Cells were cultured into 96-well plates (1×10^5 cells/well in 100 μL DMEM). After 24 h, media were removed and fresh DMEM containing HFDs samples were added. Six parallel wells were set for each sample. After 72 h incubation, 10 μL of AlamarBlue agents were added into each well and fluorescent intensity were measured 4 h later with a plate reader (Tecan Infinite M200 Pro) at the wavelength of 560/590 (excitation/emission) nm.

3. Results and discussion

3.1. Multifunctional dendritic scaffolds preparation

Synthesis of AB_2C bis-MPA monomers. In this context, three bis-MPA derivatives with different chemical groups available for orthogonal reactions were synthesized. The targeted C groups (alkynes, azides and alkenes) are known to participate with high efficiency and selectivity in the CuAAC[28] and thiol-ene (TEC)[29] click reactions, and be dormant during FPE growth.[24-25] The AB_2C monomers **5**, **6** and **7** were synthesized using streamlined strategies (**Scheme 1**). In brief, bis-MPA undergoes sequential esterification, starting at one of the hydroxyl groups, with acetonide-protected bis-MPA, followed by a C-COOH molecule bearing the desired orthogonal moiety C on the other, both assisted by CDI. Detailed synthetic protocols and characterization (^1H, ^{13}C NMR) are described in the Supporting Information (**Figures S1-S4**), which confirm the purity of these molecules.

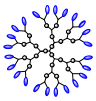
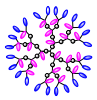


Scheme 1. Synthetic strategy towards bis-MPA based AB_2C monomers. Abbreviations: Bis-MPA, 2,2-Bis(hydroxymethyl)propionic acid; BnBr, benzyl bromide; DMF, dimethylformamide; DMP, 2,2-dimethoxypropane; pTSA, p-toluenesulfonic acid; CDI, 1,1´-carbonyldiimidazole; EtOAc, ethyl acetate.

Synthesis of heterofunctional dendrimers with internal and external functional groups. Considering the complexity of the dendrimers herein presented, the unified nomenclature for heterofunctional dendrimers will be employed.[23] It is based on the general formula HFD(*ie*)-Gb-*e*-$(B)_m$-*i*-$(C)_n$, which details: (i) the type of dendrimer (HFD) and location of the heterofunctionality (*ie* = internal and external); (ii) the generation of the dendrimer (Gb); and (iii) the number/type of functional groups in the exterior *e*-$(B)_m$ and the interior *i*-$(C)_n$. To verify the feasibility of the proposed strategy, a generation four bis-MPA dendrimer HFD(ie)-G4-*e*-$(OH)_{48}$-*i*-$(C)_{45}$ was targeted. This HFD is comparable to the traditional TMP-G4-$(OH)_{48}$ dendrimer, exhibiting the same core and peripheral groups, but differs in the distinct increase in mass due to the larger AB_2C monomer and the

additional 45 internal C groups. A comparison of these bis-MPA dendrimers is highlighted in **Table 1**.

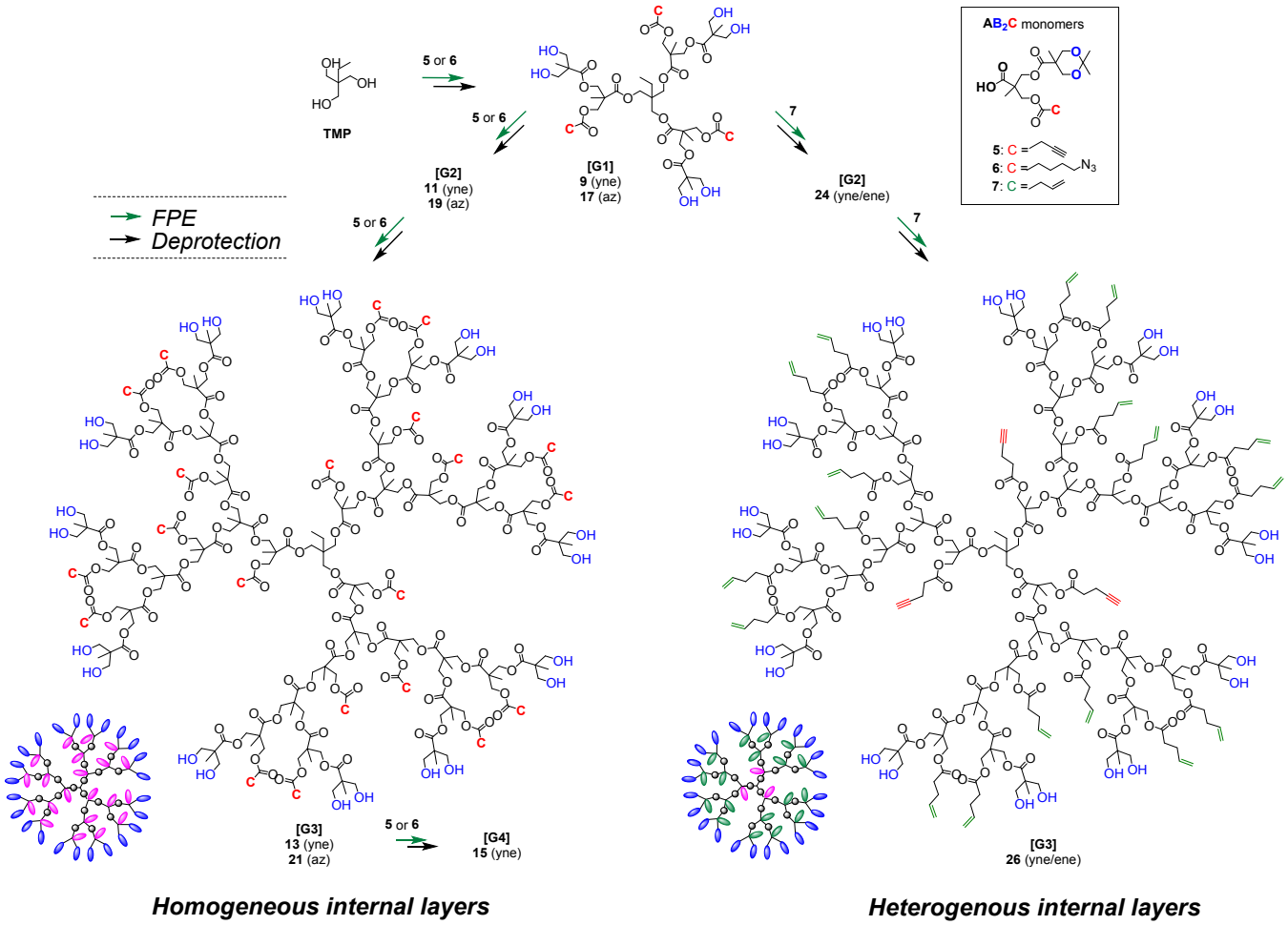
Table 1. Overview of strategies to fourth generation bis-MPA dendrimers using Fluoride-Promoted Esterification.

Strategy	Properties
Homofunctional[25] 	• 8 steps • 76% total yield • 7.5 h • M_w [482-5356] g·mol^{-1} • 48 OH groups
Heterofunctional [a)] 	• 8 steps • 51% total yield • 22 h • M_w [1071-15150] [b)] g·mol^{-1} M_w [1248-17808] [c)] g·mol^{-1} • 48 OH and 45 C groups

[a)] This work; [b)] Alkyne-HFDs; [c)] Azide-HFDs

The divergent synthesis starts from a TMP core using FPE protocols (**Scheme 2**).[25] Alkyne-functional AB_2C monomer **5** was activated with CDI for 15 min at 50°C and then reacted *in situ* with TMP in the presence of the soft inorganic base CsF. After 1 h reaction

and subsequent washing, the first generation dendrimer HFD(ie)-G1-*e*-$(ac)_3$-*i*-$(yne)_3$ **8** was isolated in near-quantitative yields. Subsequent deprotection using *p*-TSA in MeOH for 1 h at 25ºC led to the bifunctional dendrimer HFD(ie)-G1-*e*-$(OH)_6$-*i*-$(yne)_3$ **9**. The iterative growth/deprotection steps of HFDs were conducted efficiently without chromatographic purifications, thereby synthesizing the monodisperse fourth-generation dendrimer HFD(ie)-G4-*e*-$(OH)_{48}$-*i*-$(yne)_{45}$ **15** with M_w 14188 g·mol^{-1} (**Figure S5-S20**).



Scheme 2. Synthesis of multipurpose bis-MPA dendrimers with homogeneous and heterogeneous internal layers. Abbreviations: Az, azide; ene, alkene; FPE, Fluoride-Promoted Esterification; TMP, trimethylolpropane; yne, alkyne.

The same synthetic sequence was employed to synthesize the analogous family containing azide groups from AB_2C monomer **6**. HFDs with internal azides were successfully isolated up to the third-generation HFD(ie)-G3-*i*-$(N_3)_{21}$-*e*-$(OH)_{24}$ **21**, but minor structural imperfections were obtained in the G4 counterpart **22** due to the higher steric hindrance (**Figure S21-S34**). The modularity of this strategy was further supported by combining two different AB_2C monomers, **5** and **7**, in the dendrimer growth (**Scheme 1**). Trifunctional dendrimers with heterogeneous internal layers HFD(ie)-G2-*e*-$(OH)_{12}$-*i*-$(yne)_3(ene)_6$ **23** and HFD(ie)-G3-*e*-$(OH)_{24}$-*i*-$(yne)_3(ene)_{18}$ **25** were isolated in high yields (**Figure S35-S42**). The compatibility between the internal alkyne and alkene groups enabled their selective post-functionalization, as it is later demonstrated. The structure and purity of all dendrimers was confirmed using MALDI-TOF, ^{1}H- and ^{13}C-NMR spectroscopy (**Figure 1**, Supporting Information).

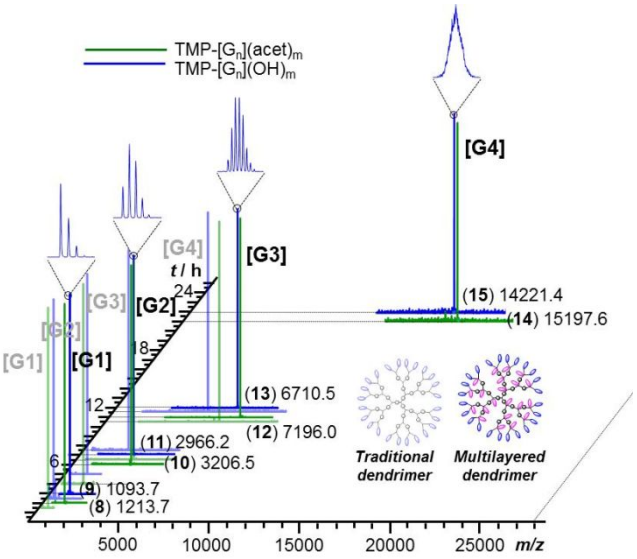


Figure 1. Comparison of MALDI-TOF MS spectra of "traditional" (homofunctional, depicted as shadowed lines) and "multilayered" (heterofunctional, depicted with intense colours) bis-MPA dendrimers with internal alkyne functionalities from the first (G1) to the fourth (G4) generation and the accumulative reaction time for their synthesis. For traditional dendrimers, *m/z* values have been previously reported by our group.[25] For HFDs, experimental *m/z* values for [M+Na$^+$] are shown in the figure while theoretical *m/z* values can be found in the Supporting Information.

The previously reported Trizma® AB_2C monomers were grown up to G3 dendrimers composed of 21 internal acetylene and 24 peripheral hydroxyl groups with M_w ~7300 g·mol^{-1}.[22] However, the protocol herein presented goes beyond state-of-the-art and delivers G4 dendrimers comprising 45 internal groups, 48 peripheral hydroxyls and M_w ~15000 g·mol^{-1}, including layered dendrimers with different internal groups for the first time. As previously demonstrated,[25] the importance of CsF catalysis is critical, enabling full conversion of the OH groups within a few hours, despite the steric hindrance of higher generations. In less than one day of total reaction time, FPE delivers a traditional G6 dendrimer with 192 peripheral hydroxyl groups and M_w 22080 g·mol^{-1} using traditional AB_2 monomers, or multifunctional G4 dendrimers with up to 93 functional groups, through the more sterically hindered AB_2C monomers.

2.2. *In vitro* biocompatibility of multifunctional dendritic scaffolds

Traditional bis-MPA dendrimers are highly biocompatible.[30] These novel HFDs, however, have relatively large interior compartments with hydrophobic pendant groups that may

cause cytotoxicity, as there is a proven affinity of amphiphilic entities towards cell membranes.[31] *In vitro* assays demonstrated that bis-MPA HFDs are biocompatible up to 10 μM in fibroblasts (hDF, **Figure S54**) and higher concentrations in monocytes (RAW 264.7, **Figure 2**). Unlike similar bis-MPA dendrimers decorated with ammonium groups,[32] HFDs' toxicity did not increase with succeeding dendritic generations, suggesting that the cytotoxicity is not as a result of the presence of higher concentrations of internal click groups or peripheral hydroxyl groups. A possible explanation to the low cell viability at high HFD concentration, 100 μM, could be related to the amphiphilic nature of the dendrimers, presumably similar among generations.

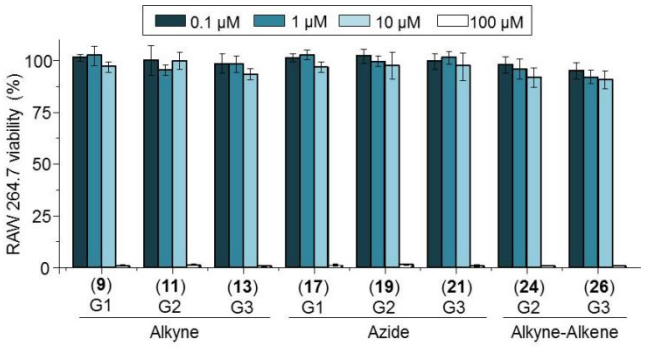


Figure 2. Viability of RAW 264.7 cells after 72 h incubation with different HFDs, measured through AlamarBlue assay.

2.3. Post-functionalization of multifunctional dendritic scaffolds

To position these dendritic scaffolds in application-driven research, two different routes were targeted (**Figure 3.a**). All synthetic protocols and characterization tools (NMR spectroscopy, MALDI-TOF, Size-Exclusion Chromatography (SEC) and FTIR analysis) are presented in the Supporting Information.

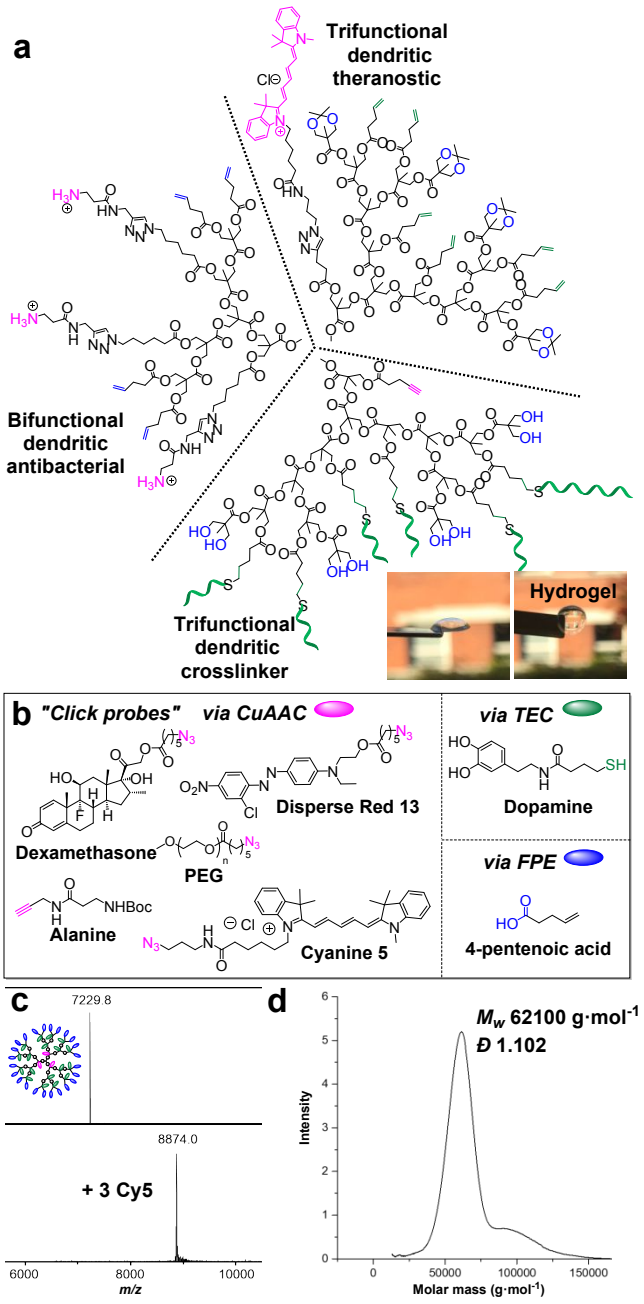


Figure 3. a. Examples of multipurpose HFDs, with potential applications as antibacterial agents, theranostic tools and crosslinkers for functional hydrogels. **b.** Library of "click

probes" successfully attached to the HFDs *via* CuAAC, TEC and FPE. **c.** MALDI-TOF spectra for HFD(ie)-G3-*e*-$(an)_{12}$-*i*-$(yne)_3(ene)_{18}$ **25** before and after reaction with Cy5. **d.** SEC spectrum for HFD(ie)-G3-*e*-$(OH)_{24}$-*i*-$(N_3)_{21}$ **21** after reaction with PEG-alkyne; the bimodal nature of the spectrum may be due to physical interaction of individual dendrimers.

Evaluation as precision polymer therapeutics. In the first route, selected HFDs were reacted with a library of "click probes" based on bulky and biologically-relevant substrates (**Figure 3.b**), as a mean to showcase the design criteria of new-generation, precision polymer therapeutics. To test the accessibility of the internal C moieties within the dendritic scaffolds, several modifications were performed based on click reactions. For example, HFD(ie)-G3-*e*-$(OH)_{24}$-*i*-$(yne)_{21}$ **13** was reacted *via* CuAAC with the azide-bearing dye Disperse Red 13 and the drug dexamethasone. After 1 h at 25 °C, ^{1}H-NMR spectroscopy and FTIR analysis confirmed the complete conversion of the 21 internal acetylene groups, even at the most internal layer (**Figure S43-44**). The alkyne groups in

the trifunctional HFD(ie)-G3-*e*-$(an)_{12}$-*i*-$(yne)_3(ene)_{18}$ **25** were also modified with azide-functional Cyanine 5 under the same conditions, as confirmed by MALDI-TOF, leading to a trifunctional potential theranostic agent (**Figure 3.a,c**, **S45**). The multivalent presence of fluorescent tags in the first internal layer could enable the monitoring with enhanced resolution, without interfering with the drug-loading capacity of the carrier.[33] All alkene groups in HFD(ie)-G3-*e*-$(OH)_{24}$-*i*-$(yne)_3(ene)_{18}$ **24** were also easily functionalized with bulky molecules such as thiol-functional dopamine in less than 20 min of UV-initiated TEC (**Figure S46**). The dual functionalization of HFD(ie)-G2-*e*-$(OH)_{12}$-*i*-$(N_3)_9$ **19** via external FPE with 4-pentenoic acid and internal CuAAC with modified β-alanine resulted in a bifunctional cationic dendrimer with potential antibacterial properties (**Figure 3.a**, **S47-52**).

The size of these nanocarriers, which influences the biodistribution and bioavailability through the Enhanced Permeation and Retention effect,[34] can also be tuned through the attachment of poly(ethylene glycol) (PEG) chains. Azide-functional dendrimers HFD(ie)-G2-*e*-$(OH)_{12}$-*i*-$(N_3)_9$ **19** and HFD(ie)-G3-*e*-$(OH)_{24}$-*i*-$(N_3)_{21}$ **21** were therefore reacted with

mPEG-alkyne (5 kg·mol^{-1}) through CuAAC. The M_w values, measured through SEC, increased approximately 36.6 and 55.4 kg·mol^{-1} for G2 and G3, respectively, with $Đ$ values below 1.1 (**Figure 3.d**, **S53**). These values correlate to 81% and 53% of the reacted internal groups, which are considered successful in view of the steric hindrance of the long PEG chains.

Evaluation as crosslinkers towards functional hydrogels. In the second route, the HFDs were tested as sophisticated crosslinkers to generate functional hydrogels. To achieve this, HFD(ie)-G2-*e*-$(OH)_{12}$-*i*-$(N_3)_9$ **19** and HFD(ie)-G3-*e*-$(OH)_{24}$-*i*-$(N_3)_{21}$ **21** were crosslinked with dialkyne PEG (10 kg·mol^{-1}) using CuAAC. The gelation process took 30-60 min to deliver solid networks with hydroxyl groups available for further modification. Similarly, the trifunctional HFD(ie)-G2-*e*-$(OH)_{12}$-*i*-$(yne)_3(ene)_6$ **24** and HFD(ie)-G3-*e*-$(OH)_{24}$-*i*-$(yne)_3(ene)_{18}$ **26** were reacted with dithiol PEG (10 kg·mol^{-1}) through TEC. Here, it required only 5 min of UV irradiation to form solid networks (**Figure 3.a**) which presented both alkyne and hydroxy groups available for the attachment of different agents.

4. Conclusions

Many of the established dendrimer families lack the capacity of internal functionalities. Here, we have successfully synthesized heterofunctional dendrimers with internal and external functionalities based on the bis-MPA building block. This was accomplished by careful design of novel class of AB_2C monomers being amalgamated from two traditional AB_2 building blocks. FPE was demonstrated as a powerful tool that can be used to streamline the synthesis of complex HFDs by avoiding time-consuming purifications, being more sustainable from an economic and environmental perspective. Through FPE chemistry, the functional group density of the dendrimer can be optimized by taking advantage of the entire dendritic skeleton. This led to fourth generation HFDs with 93 functional groups (45 internal moieties and 48 peripheral hydroxyl groups) and M_w ~15000 g·mol-1 within less than one day of reaction time. The resultant HFDs are biocompatible and can be used as polymeric “stem-cells” for an array of cutting-edge applications, such as nanocarriers, theranostic tools or in network formation. The structural perfection of

these HFDs allows for the control of parameters that would otherwise have caused discrepancies in vivo using established nanocarriers, and furthermore provides valuable information on structure-to-property relationships.

ASSOCIATED CONTENT

Supporting Information. Experimental details, structure and synthetic procedures of monomers and dendrimers. Analytical data including ^{1}H, ^{13}C-NMR, SEC traces, MALDI-TOF-MS, FTIR and *in vitro* assays.

This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

* Prof. Michael Malkoch

Royal Institute of Technology, School of Chemical Science and Engineering, Fibre and Polymer Technology, Teknikringen 56-58, SE-100 44 Stockholm, Sweden

E-mail: malkoch@kth.se.

Present Addresses

† Current address: Department of Organic and Inorganic Chemistry, Faculty of Sciences, University of Alcalá, UAH Campus, 28871, Alcalá de Henares, Madrid, Spain

Author Contributions

§ These authors contributed equally.

Notes

The authors declare no competing financial interest.

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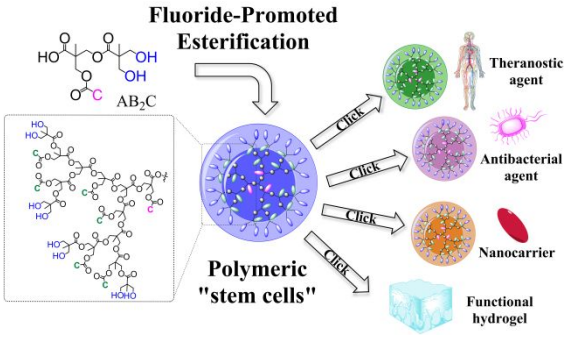
Table of Contents Graphic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