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# Synthesis, photophysical properties and anticancer activity of micro-environment sensitive amphiphilic bile acid dendrimers

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**Abstract**

Porphyrin-cored bile acid dendrimers containing deoxymethyl cholate and methyl cholate units at the periphery, have been synthesized by convergent methodology using click chemistry approach and characterized by $^{1}H$ and $^{13}C$ NMR and MALDI-TOF MS data. Absorption−emission behavior of dendrimers and its modulation under the influence of the dendritic environment is also investigated. The therapeutic efficacy of the bile acid dendrimers for the inhibition of the growth tumor cell (MIA PaCa-2) increases as the dendrimer generation increases.

**Keywords**: Porphyrin, Bile acid dendrimers, Click chemistry.

## Introduction

Dendrimers [1]are well-defined, hyper branched macromolecules with a wide range of applications in solar cells,[2] light harvesting systems,[3] catalysis,[4] molecular encapsulation[5] and also in medical fields.[6] Bile acids and their derivatives have considerable attention in supramolecular chemistry in recent years.[7] Bile acids are naturally occurring steroidal molecules biosynthesized in the liver.[8,9] Bile acids are rigid, chiral, biocompatible and facially amphiphilic



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molecules which function as biological surfactants with multiple physiological functions. Due to the unique properties such as structural rigidity and the distribution of hydrophobic and hydrophilic domains forming self-organized assemblies in water, bile acids are widely used in biomedical applications as adjuvant of liver specific drugs, a potential polyhydroxy initiator. Modified bile acids have pharmaceutical importance as novel drug delivery systems and have also been used in the excellent building blocks for dendritic construction of polymeric materials, which have been studied as potential drug releasing agents,[10] sensors for metal ions,[11] molecular containers,[12-15] hydrogelators,[16,17] and ion transporters.[18] More recently, bile acids has became attractive candidates in the construction of star-shaped derivatives called "molecular pockets" and form hydrophobic or hydrophilic nanocavities depending on the polarity of the surrounding solvents. Kun Zhang et al showed anti-cancer drug activity and bio availability of cholic acid moiety in polyamidoamine dendrimer.[19]

Porphyrin is an essential component which has been widely used as functional building blocks in supramolecular chemistry because of its biological representation in hemoglobin, cytochromes, and vitamin $B_{12}$. It plays important role in several biologically relevant processes on account of their distinct physical and chemical properties[20] and the ability to be used as donor/accepter in electron or energy transfer process. [21] In this context, large dendrimeric architectures have been developed and explored for various applications containing a porphyrin moiety surrounded by a variety of peripheral functional units[22]. One of the interesting application of the porphyrin is in the field of Photodynamic therapy (PDT) which is of paramount importance in cancer treatment.[23]

Linear, star-like, and hyperbranched polymers can be synthesized by the 1, 3-dipolar cycloaddition of azide and a terminal alkyne using Cu (I)-catalyst which is known as "click



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chemistry" [24] that occur under mild reaction conditions with high yields. Click reaction has been employed for the synthesis of triazoles which are widely employed for diverse biological activities such as anti-HIV, antibacterial, antimalarial, anticancer, herbicidal, antiallergic and antifungal[25-27] and further triazole nucleus has stability towards metabolic degradation and capable of exhibiting hydrogen bonding.

Recently, we have reported the synthesis of dendrimers with quinolone,[28] benzothiazole [29] and chalcone[30] as surface groups through Click chemistry. Herein, we report the synthesis of bile acid dendrimers **1-6** built on porphyrin core by a convergent synthetic strategy using a click Chemistry.

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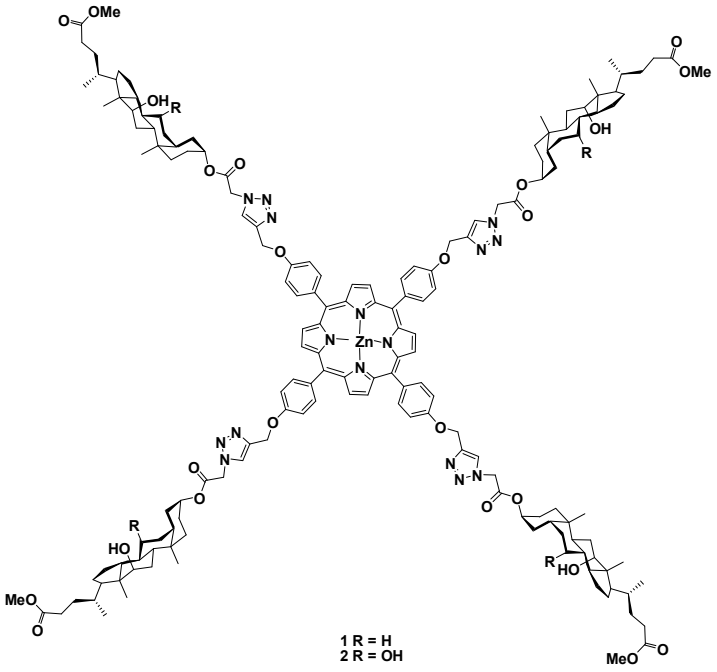

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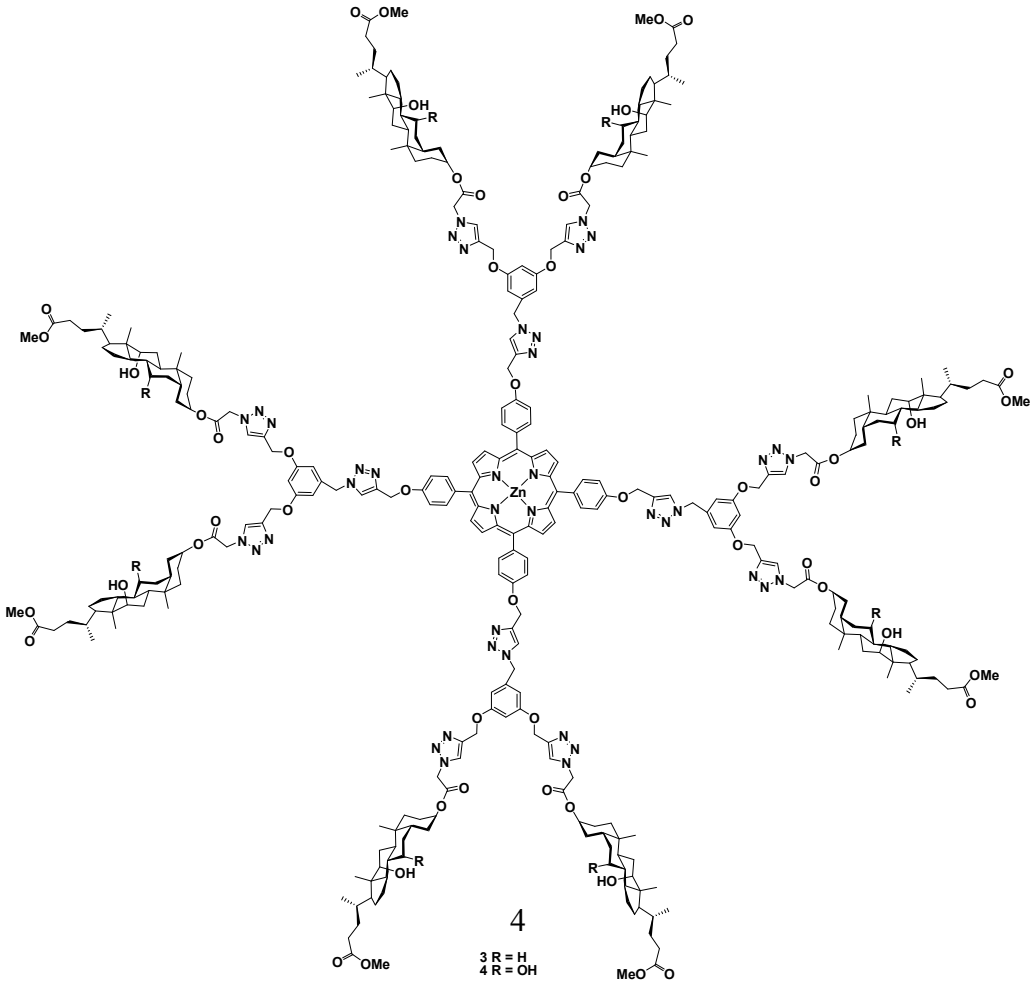

3 R = H
4 R = OH



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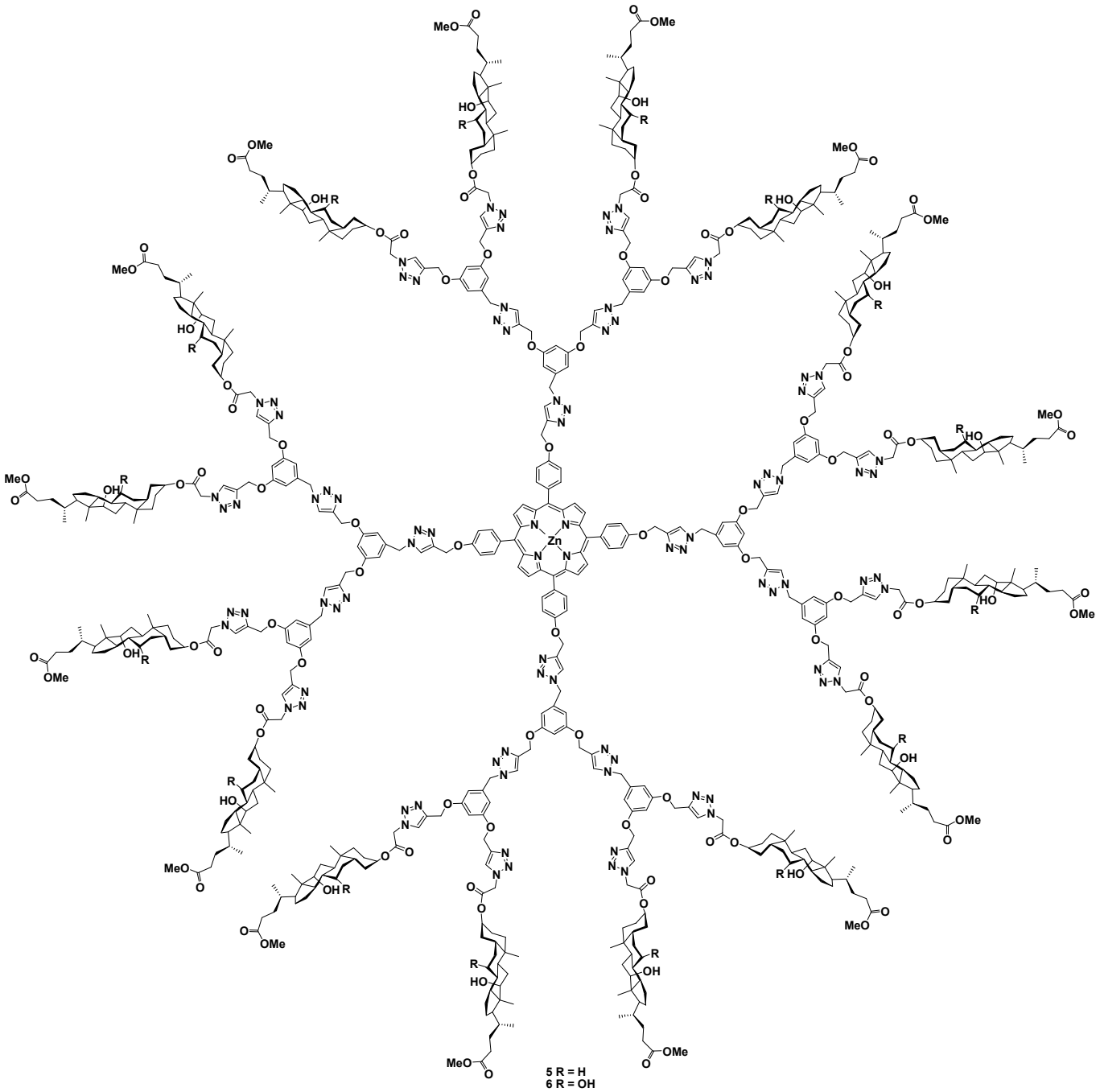


**Fig. 1 The structure of bile acid dendrimers 1-6.**

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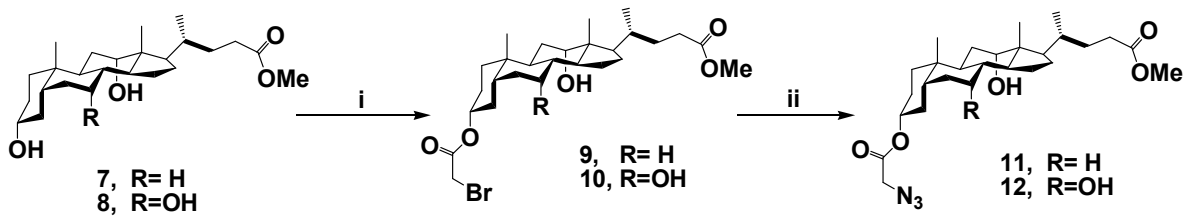
## Results and discussion

### Synthesis of Zeroth-Generation Azide-Functionalized Dendrons

Bile-acid-based dendrimers **1-6** were synthesized in high yields from cholic acid and deoxycholic acid as the starting material, using a straightforward synthetic strategy. The synthesis of dendritic azide **11** and **12** is outlined in the scheme 1. Deoxymethyl cholate **7**[31] and methyl cholate **8**[31] were treated with 1.0 equiv. of bromoacetylbromide at 0 ºC in the presence of $K_2CO_3$ in chloroform for 4h to give monobromoacetyl derivatives **9** and **10** in good yields. Treatment of **9 and 10** with sodium azide in DMF gave the corresponding dendritic azides **11** and **12** in 89% and 90% yield **(Scheme 1).**




**Scheme 1: Reagents and conditions:** (i) 1.0 equiv. of bromoacetyl bromide, $CHCl_3$, $K_2CO_3$, 4 h, **9** (64%), **10** (54%) (ii) $NaN_3$, DMF, 60 ºC, 12 h, **11** (89%), **12** (90%).

In the $^1$H NMR spectrum the azide **11** showed singlets at δ 0.69, 0.93, 3.67, and 3.83 for $C_{18}$-Me, $C_{19}$-Me, ester methyl and $–CH_2$-$N_3$ protons respectively in addition to other aliphatic protons. The $^{13}$C NMR spectrum of the azide **11** showed signals at δ 167.8 and δ 174.7 for the carbonyl carbons in addition to the aliphatic carbons. Similarly, the structure of the methyl cholate dendritic azide **12** was confirmed from spectral and analytical data.

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**Synthesis of First- and Second-Generation Dendritic Azide**

A variety of first- and second-generation of functionalized bile acid dendritic azides were prepared and coupled with alkyne-functionalized *meso*-positions of the porphyrin core using a click reaction to obtain the bile acid dendrimers. Bile acid dendritic azides **16, 17, 20,** and **21** were obtained as outlined in Scheme 2. The reaction of **13**[32] with dendritic azides **11** and **12** in the presence of $CuSO_4{\cdot}5H_2O$/sodium L-ascorbate (NaAsc) in THF/water afforded first-generation dendritic chlorides **14** and **15** in 95% and 92% respectively. The formation of **14** was confirmed from the $^1$H NMR spectrum, which showed the characteristic triazole proton as a singlet at δ 7.77 in addition to the aliphatic and aromatic protons. In $^{13}$C NMR, the peaks at δ 124.2 and 144.2 for **14** confirmed the presence of triazolyl carbons in addition to the aliphatic and aromatic carbons. Similarly, the structure of the methyl cholate dendritic chloride **15** was confirmed from spectral and analytical data.

In the next step, azidation of **14** and **15** was brought about in quantitative yields by the reaction of the dendritic chlorides **14** and **15** with $NaN_3$ in DMF at 60 °C to generate dendritic azides **16** and **17** in 92% and 89% yields respectively. In $^1$H NMR for the dendritic azide **16** the $CH_2$-$N_3$ proton appeared as a singlet at δ 4.26 ppm where as the $CH_2$-Cl proton in **14** appeared at δ 4.50 ppm. Similarly the structure of the dendritic azide **17** is also confirmed from the spectral data. After synthesizing the first-generation azides, we focused our attention on the synthesis of second-generation dendritic azides. The azide-functionalized dendrons **16** and **17** (2 equiv) were clicked with 3,5-bis(propargyloxy)benzyl chloride **13** to gave the respective dendritic chloride **18** and **19 (Scheme 2).** The appearance of a new triazolyl peak at δ 7.83 for **18** and at δ 8.24 for **19** with integrals corresponding to two protons confirmed the click transformation. Further, the dendritic chlorides **18** and **19** were quantitatively converted into their respective azides **20** and **21**



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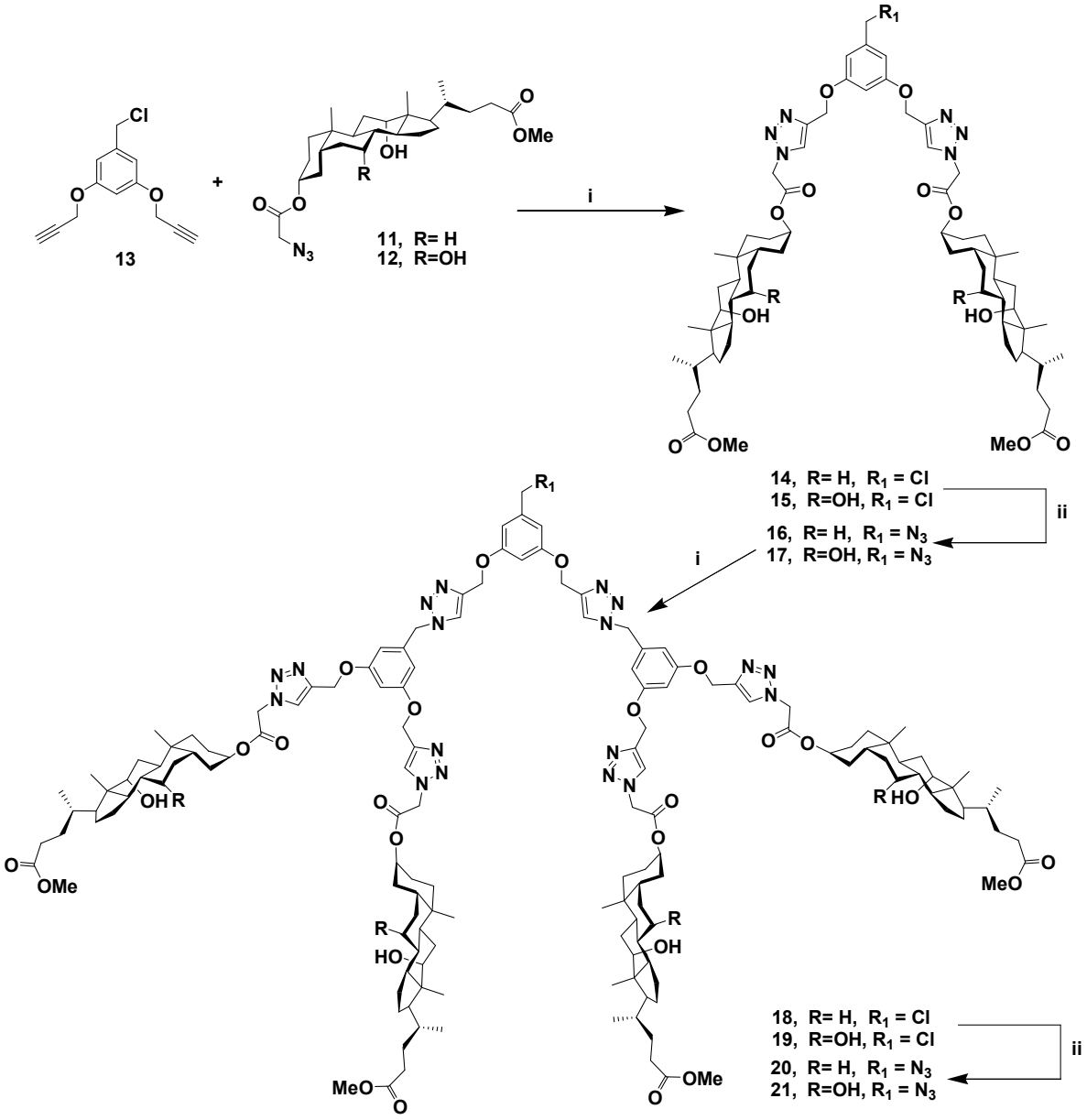
in 80% and 84% yields using $NaN_3$ in DMF at 60 °C for 12h and characterized from spectral data.




**Scheme 2: Reagents and conditions:** (i) $CuSO_4.5H_2O$ (5 mol %), sodium ascorbate (10 mol %), $H_2O$: THF (1:3), rt, 10 h, **14** (95%), **15** (92%), **18** (80%), **19** (78%) (ii) 2.5 equiv. $NaN_3$, DMF, 60 ºC, 12 h, **16**(92%), **17** (89%), **20** (80%), **21** (84%).



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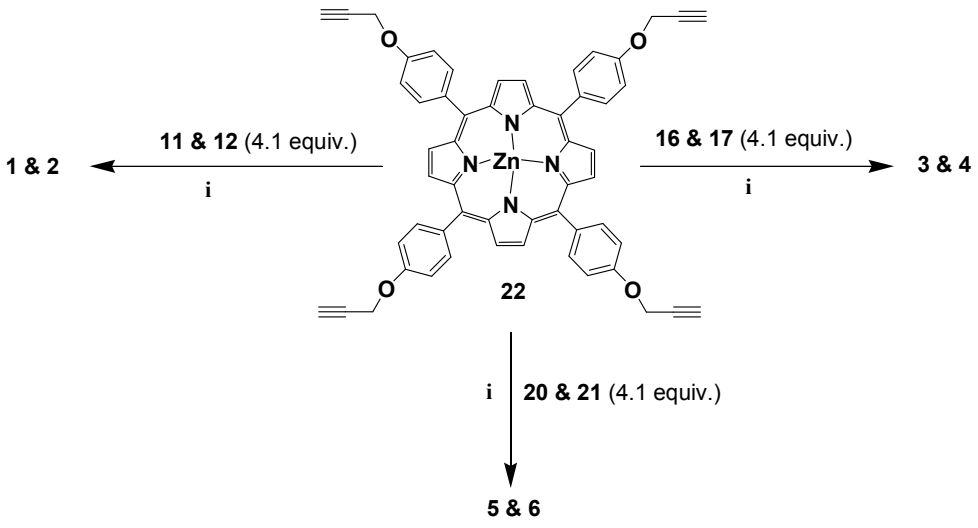
**Synthesis of porphyrin core**

We then focused on the synthesis of the click counterpart **22**. The porphyrin core was obtained by *Lindsey*-type condensation[33] and then functionalized with the propargyl bromide to give tetra-alkyne armed porphyrin macrocycle which was then metalated with Zn in the presence of zinc acetate in chloroform/methanol at reflux to give **22** in 98% yield. Protection of the porphyrin by Zn(II) complexation is important preceding the Cu(I)-catalyzed click reaction, since Zn insertion sufficiently stabilizes the system against any replacement by Cu(II) ions.[34]

**Synthesis of Zeroth, First- and Second-Generation Bile acid Dendrimers**

Finally, the tetrasubstituted porphyrin **22** was utilized as the starting material for coupling with each of the dendritic azides **11, 12, 16, 17, 20** and **21** using $CuSO_4.5H_2O$/NaAsc in THF/water to derive the respective bile acid dendrimers **1, 2, 3, 4, 5** and **6** in 69%, 78%, 58%, 54%, 69% and 57% yields respectively **(Scheme 3).** The bile acid dendrimers **1- 6** were purified by column chromatographic technique without any significant involvement of steric congestion imposed by large dendritic azides. The formation of dendrimers **1, 2, 3, 4, 5** and **6** was confirmed by the appearance of newly formed triazolyl protons at δ 7.79, 8.37, 7.75, 8.49, 8.47 and 8.42, respectively, in their $^1$H NMR spectrum.

The structure of the all the newly synthesized bile acid-dendrimers was confirmed by MALDI-TOF MS. In all the cases, most intense peak was observed for $[M + H - Zn]^+$ species along with $[M + H]^+$ peak suggesting the loss of Zn metal under ionization condition. Also, with increasing generations of dendrimers, a poor quality of the baseline, broadened peaks, and fragmentation in molecule was noticed due to the requirement of higher laser power in MALDI analysis.[35]



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**Scheme 3: Reagents and conditions:** (i) $CuSO_4.5H_2O$ (5 mol %), sodium ascorbate (10 mol %), $H_2O$: THF (1:3), rt, 12 h, **1** (69%), **2** (78%), **3** (58%), **4** (54%), **5** (69%) and **6** (57%).

**Photophysical studies**

Absorption and emission studies on bile acid dendrimers were carried out to understand the effect of amphiphilic dendritic micro-environments over the photophysical properties of the porphyrin core. UV-visible spectrum of the dendrimers **1-6** was recorded in THF which showed a typical metalloporphyrin behavior.[36] All the dendritic generations displayed the characteristic soret band at 426−428 nm along with two Q bands at 557-559 and 598-600 nm ranges (Figure 2 and Table 1).The amphiphilicity of bile acid moiety and polarity of the solvent used, substantially affected the photophysical properties of the porphyrin core. A pronounced change at Q1 and Q2 band in the absorption spectrum of all the dendrimers is lower than the absorption of porphyrin core except second generation of cholic acid dendrimeric systems. In cholic acid based dendrimer, on going from lower generation to higher generation the Q bands appearing at 558−559 and 598−600 significantly increases in intensity of extinction coefficient though the absorption maxima shows a small red shift of 2nm (Figure 2 and Table 1). This may be due to the presence of polar groups (OH groups) and self assembling nature of the bile acid moiety plays vital role over the hydrophobic porphyrin core. The same was also observed in the case of deoxy cholic acid dendrimers (Figure 2). Thus, comparatively deoxy cholic acid dendrimer shows higher hydrophobicity than cholic acid dendrimer. Owing to this assemblage behavior the $G_2$ cholic acid dendrimer shows higher hydrophilic shielding than its lower generations and deoxy cholic acid dendrimers.

Beer-Lambert linearity between the increase in the concentration and absorbance was then tested on dendrimers **1-6**. As the concentration increases absorbance also increases but the plot of concentration versus absorbance was not linear for all the dendrimers and hence the dendrimers **1-6** do not obey the Beer-Lambert linearity law (SI in Figure 2-7).

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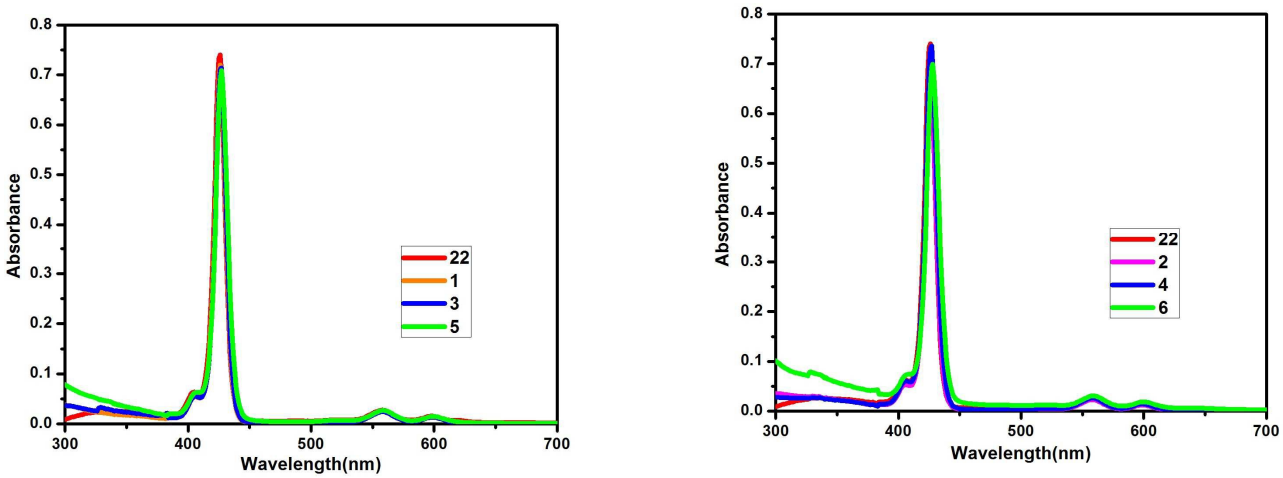


**Figure 2**. Absorption spectrum of dendrimers **1-6** recorded in THF at rt (conc. = $10^{-5}$ M).

| Compound | $\lambda_a^{max}$ | | | | | | $\lambda_f^{max}$ | $\Phi_f$ |
|---|---|---|---|---|---|---|---|---|
| | Soret band | $\varepsilon_{max}$ | $Q_1$ | $\varepsilon_{max}$ | $Q_2$ | $\varepsilon_{max}$ | | |
| **1** | 426 | 0.72 | 558 | 0.024 | 598 | 0.014 | 608, 659 | 0.032 |
| **2** | 426 | 0.66 | 558 | 0.023 | 598 | 0.012 | 609, 659 | 0.030 |
| **3** | 427 | 0.71 | 558 | 0.024 | 599 | 0.013 | 609, 660 | 0.033 |
| **4** | 427 | 0.74 | 558 | 0.026 | 598 | 0.014 | 609, 659 | 0.032 |
| **5** | 427 | 0.70 | 557 | 0.027 | 599 | 0.015 | 609, 659 | 0.030 |
| **6** | 428 | 0.69 | 559 | 0.030 | 600 | 0.018 | 609, 660 | 0.023 |
| **22** | 426 | 0.74 | 557 | 0.027 | 598 | 0.34 | 608, 659 | 0.033[37] |

**Table 1.** Photophysical studies of **1-6** and **22** (rt, in THF)

To study the polarity of the micro environment absorption study of **1-6** was done in THF/water mixed system of various compositions. On successive addition of water up to 70% a gradual decrease in absorption was noted for all the dendrimer with respect to increase in the generations and no significant change in spectra was observed upon successively increasing the percentage of water in THF (SI in Figure 1and Table 1-6). This result implies a single molecular dispersion of dendrimers in solvents. However, a gradual decrease in peak intensity and peak broadening was found when the water content goes beyond 50%. UV−vis spectra studied in water still exhibited the original soret band, which suggested a **5** and **6** arising due to effective



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shielding of the hydrophobic porphyrin and aromatic systems by hydrophilic peripheral bile acids. However, a small increase in peak intensity of Q bands was found for **5** and **6** at 70% of water which may be attributed to the presence of hydrophobic aggregation of the dendrimers (Table 2 and Figure 3).

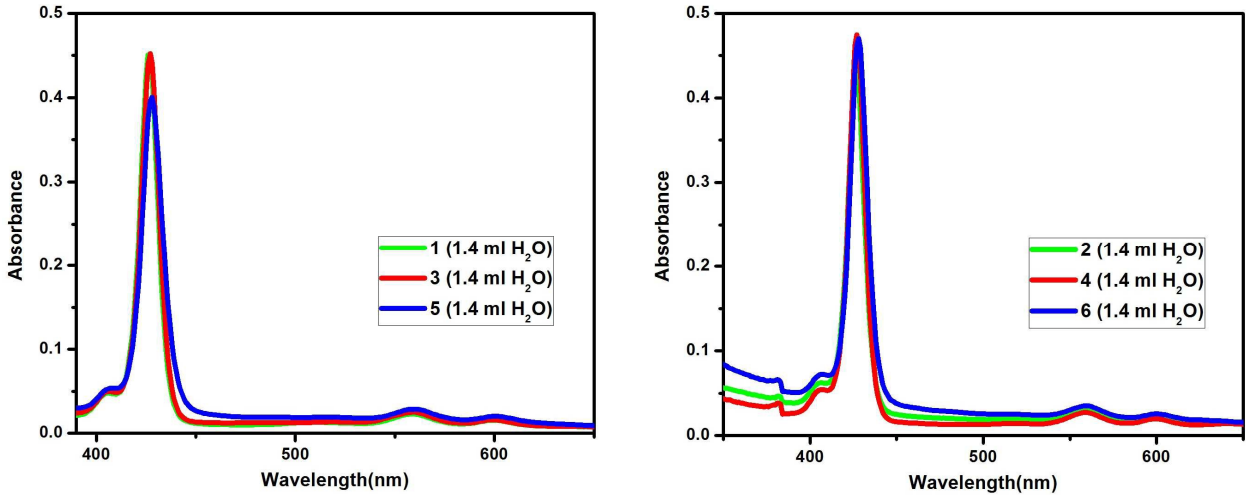


**Figure 3**. Absorption spectrum of dendrimers **1-6** recorded in 70% water in THF at rt (conc. = $10^{-5}$ M).

Fluorescence study of the dendrimers **1-6** was also carried out in THF at the excitation wave length of 560 nm, the dendrimers **1-6** showed fluorescence emission at 608, 609 and 659, 660 nm. The compounds **1**, **3** and **4** showed slightly higher fluorescence intensity in comparison to the core **22**, but in all, a negligible difference in emission behavior of dendrimers was observed under dendritic influences. The dendrimers **5** and **6** which showed slightly lower fluorescence intensity in comparison to the core **22**. There is a substantial decrease in fluorescence intensities along with 2 nm red shifts in comparison to the core **22** (Table 1). The results again highlighted the self assemblage of the bile acid moiety around the hydrophobic porphyrin core as discussed in UV studies. This shows clearly the synthesized dendrimers at higher generation exhibits strong hydrophilic shielding, which screens the emission property of the hydrophobic porphyrin core.

However, with increase of the generation of dendron, absorption and fluorescence of porphyrin-cored dendrimers do not significantly change within the experimental error. For example, fluorescence quantum yields of **1**, **2**, **3**, **4** and **5** lie in the range of 0.030-0.033 and the



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compound **6** the fluorescence quantum yields 0.023 which is compared to other low quantum yield (Table 1). In other words, absorption and emission properties were affected negligibly with dendritic environments.

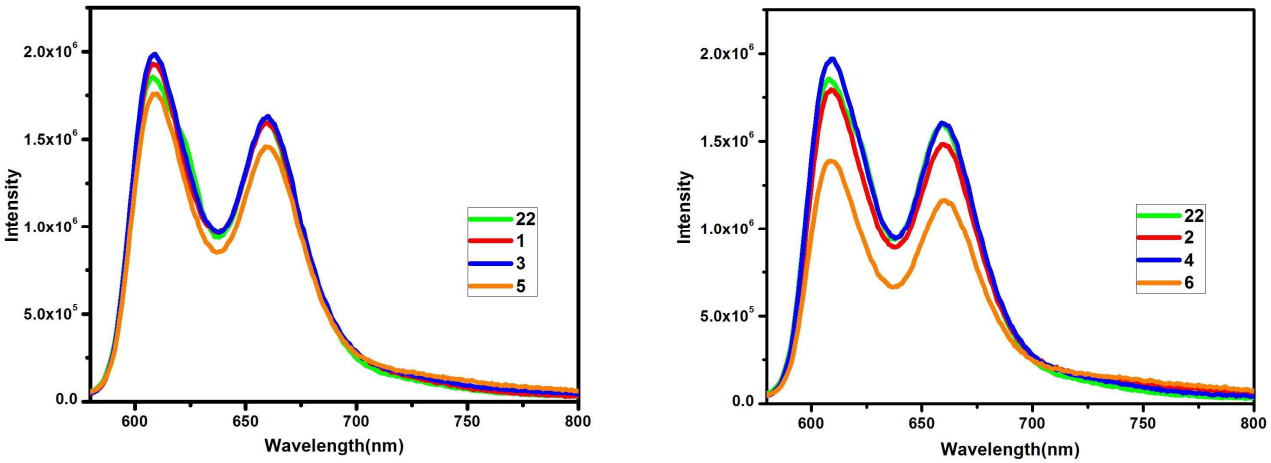


**Figure 4**.Emission spectrum ($\lambda_{exc}$ = 560nm) of dendrimers **1-6** recorded in THF at rt (conc. = $10^{-5}$ M).

| Compound | $\lambda_a^{max}$ | | | | | | $\lambda_f^{max}$ |
|---|---|---|---|---|---|---|---|
| | Soret band | $\varepsilon_{max}$ | $Q_1$ | $\varepsilon_{max}$ | $Q_2$ | $\varepsilon_{max}$ | |
| **1** | 426 | 0.45 | 559 | 0.023 | 600 | 0.015 | 610, 661 |
| **2** | 427 | 0.44 | 559 | 0.030 | 600 | 0.024 | 609, 660 |
| **3** | 427 | 0.45 | 559 | 0.025 | 600 | 0.016 | 610, 661 |
| **4** | 427 | 0.47 | 559 | 0.027 | 600 | 0.019 | 609, 661 |
| **5** | 428 | 0.40 | 560 | 0.028 | 600 | 0.020 | 611, 664 |
| **6** | 428 | 0.47 | 559 | 0.035 | 600 | 0.025 | 617, 664 |

**Table 2.** Photophysical studies of **1-6** and **22** in THF 70% water at rt.

Fluorescence study of all the dendrimers was also carried out in THF/water mixer system. With the addition of 70% water in THF the compounds **5** and **6** showed fluorescence emission at 611, 617 and 664 nm when compared with the compounds **1, 2, 3** and **4** showed fluorescence emission at 609-610 and 660-661nm (Table 2). The fluorescence intensity of **5** and **6** decreases when compared with the fluorescence intensity of **5** and **6** without water and also, a little red



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shift along with a strict peak broadening attributable to strong aggregations via hydrophobic interactions was observed in water. In a medium of 70% water in THF system the presence of hydrophilic shielding was enhanced for the compounds **5** and **6** and decreases the fluorescence intensity along with red shift of 5-7 nm (Table 2 and Figure 5).

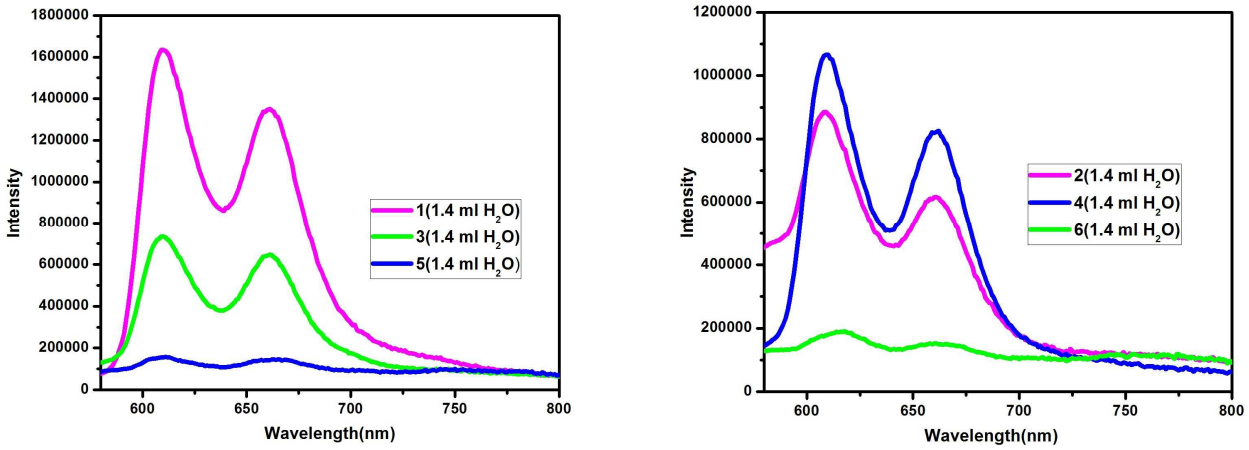


**Figure 5.** Emission spectrum of dendrimers **1-6** at 70% water in THF (conc. = $10^{-5}$ M).

**Biological activity**

To investigate the therapeutic efficacy of bile acid dendrimers by increasing dendritic generation, the inhibition of tumor cell (MIA PaCa-2) growth was evaluated. The results show that the cell growth inhibition was in deoxymethyl cholate dendrimers showed cancer inhibition activity was increasing along with dendritic generation with $IC_{50}$ 58.49µM in 300µM concentration treatment of **5** is comparatively more activity in cancer cell growth inhibition (Table 3 and Fig. 6). Methyl cholate dendrimers showed cancer inhibition activity which increases along with dendritic generation with $IC_{50}$ 75.51µM in 300 µM treatment of **6** is comparatively more activity in cancer cell growth inhibition (Table 3 and Fig. 7). From the study the bile acid dendrimers i-e compound **6** expressed is more activity then compound **5**.

| **Dendrimers** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| **$IC_{50}$ (µM)** | 32.03 | 46.33 | 41.11 | 55.02 | 58.49 | 75.51 |

**Table 3:** MIA PaCa-2 cell are treated for 24 hours in two different set of compound **1, 3, 5** and **2, 4, 6** were showing Inhibition of cell viability under different concentration of 100µM, 200µM and 300µM. values are shown in mean ± SD (n=3). Inhibition concentration 50 ($IC_{50}$) calculated and shown in µM.



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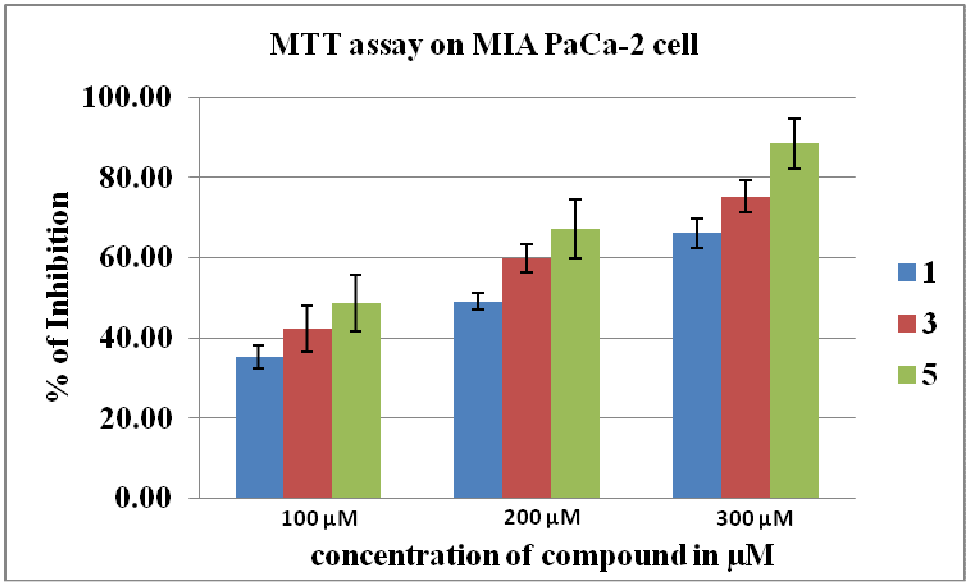


**Figure 6:** MIA PaCa-2 cell are treated for 24 hours in compound **1, 3** and **5** were showing Inhibition under different concentration of 100µM, 200µM and 300µM

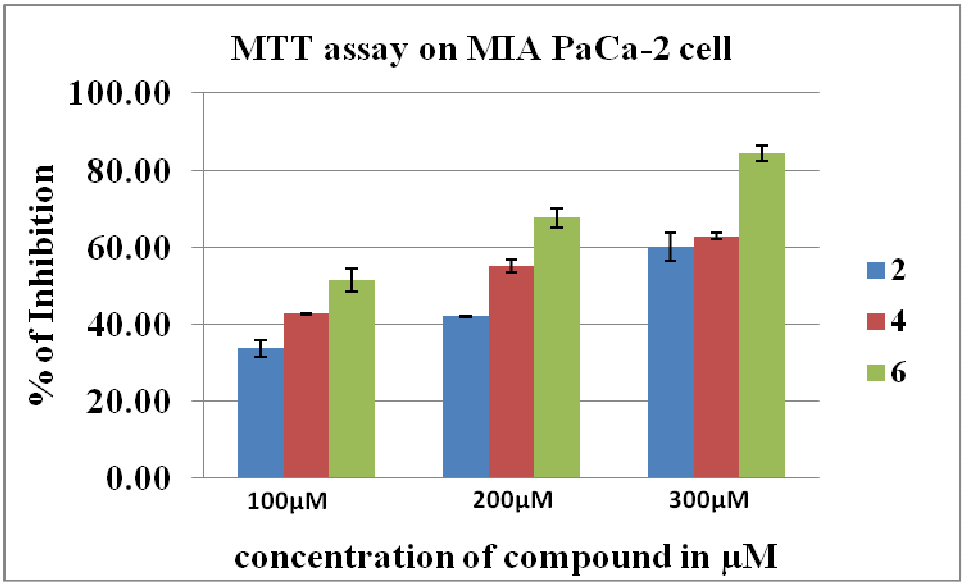


**Figure 7:** MIA PaCa-2 cell are treated for 24 hours in compound **2, 4** and **6** were showing Inhibition under different concentration of 100µM, 200µM and 300µM

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## Conclusion

In conclusion, the synthesis of zero, first and second generation bile acid dendritic structures with triazole as the building block and tetrafurcated propargylated porphyrin as core and with functionalized deoxymethyl cholate and methyl cholate wedges were obtained through 'click' chemistry approach. All the dendrimers were characterized by $^{1}H$ and $^{13}C$ NMR and MALDI-TOF MS analysis. Absorption-emission behavior of dendrimers and their modulation under the influence of the dendritic environment is also investigated. Fluorescence quantum yields of porphyrin dendrimers **1-5** show insignificant difference than that of **22** and the dendrimer **6** is very low compared to that of the other denrimers. Inhibition of growth of cancer cell was found to increase from lower generation to higher generation bile acid dendrimers. Especially the second-generation (G2) dendrimers **5** and **6** showed better activity than lower generation dendrimers.

## Experimental section

### General information

All chemicals and solvents were purchased commercially and used as such without further purification. All melting points were determined using a Toshniwal melting point apparatus by open capillary tube method and are uncorrected. ESI-MS spectra on JEOL DX-303 mass spectrometer. Elemental analyses were carried out using a Perkin-Elmer CHNS 2400 instrument. Column chromatography was performed on silica gel (ACME, 100-200 mesh). $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on Bruker 300 MHz instruments. Chemical shifts are given in parts per million, and *J* values are in hertz. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed with a 2,5-dihydroxy benzoic acid (DHB) matrix. UV-Vis absorption spectra were measured with a Perkin–Elmer Lambda 35 UV-vis spectrometer.

### Experimental Procedure for Azidation of Dendritic Chlorides (A)

To the solution of corresponding dendritic chloride (1 mmol) in dry DMF (20 mL), sodium azide (1.5 mmol) was added and stirring was continued at 75 ºC for 12h. The reaction mixture was allowed to cool room temperature. It was then poured into ice-cold water (30 mL) and extracted with $CH_3Cl$ (3 × 100 mL). The organic layer was washed with water (100 mL) and saturated NaCl (aq.) (3 × 100 mL), dried over $Na_2SO_4$. Solvent was evaporated under reduced



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pressure to afford crude product, which was purified by column chromatography ($SiO_2$), using the eluent as mentioned under each compound.

**Experimental Procedure for Cu (I)-Catalyzed Azide−Alkyne Cycloaddition (B)**

A mixture of Polypropargylated moieties (1.0 equiv) and azide functionalized compounds (1.2 equiv per azide functionalization), $CuSO_4 \cdot 5H_2O$ (0.3 equiv), and sodium L-ascorbate (0.3 equiv) was stirred at room temperature for 18 h in THF/water. After confirming the completion of reaction on TLC, chloroform was added to the reaction mixture and washed with water (10 mL), and brine (10 mL). The organic layer was separated, dried over $Na_2SO_4$ and evaporated to obtain the crude product, which was purified by column chromatography ($SiO_2$), using $CHCl_3$-MeOH as eluent to give the corresponding dendrimers.

**Synthesis of dendritic azide [$G_0$]-$CH_2$-$N_3$ 11:**

Following the general procedure **A**, the dendritic azide **11** was obtained as a white colored solid from the dendritic bromide **9** (1.0 g, 1.8 mmol) and sodium azide (0.34 g, 5.5 mmol) and by eluting the column with EtOAc:Hexane (1:9). Yield: 1.2g, 89%; M.p.: 130°C; $^1$H NMR(300 MHz, $CDCl_3$): $\delta_H$ 0.69 (s, 3H, 18-Me); 0.93 (s, 3H, 19-Me); 0.96- 2.41 (m, 30H, steroidal H); 3.67 (s, 3H); 3.83 (s, 2H); 4.00 (s, 1H); 4.80- 4.87 (m, 1H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta_C$ 12.7, 17.4, 23.1, 23.6, 26.0, 26.5, 26.9, 27.4, 28.7, 30.9, 31.1, 32.1, 33.7, 34.1, 34.8, 35.1, 36.0, 41.9, 46.5, 47.4, 48.3, 50.6, 51.5, 73.1, 76.3, 167.8, 174.7. MS (ESI): m/z = 490 [M + 1]. Anal. Calcd. For $C_{27}$ $H_{43}$ $N_3$ $O_5$: C, 66.23 ; H, 8.85; N, 8.58. Found: C, 66.15; H, 8.93; N, 8.67.

**Synthesis of dendritic azide [$G_0$]-$CH_2$-$N_3$ 12:**

Following the general procedure **A**, the dendritic azide **12** was obtained as a white colored solid from the dendritic bromide **10** (1.0 g, 1.8 mmol) and sodium azide (0.34 g, 5.5 mmol) and by eluting the column with EtOAc:Hexane (1:9). Yield: 0.83g, 90%; M.p.: 120°C; $^1$H NMR(300 MHz, $CDCl_3$): $\delta_H$ 0.69 (s,3H, 18-Me); 0.91 (s, 3H, 19-Me); 0.97-2.45 (m, 29H, steroidal H); 3.66 (s, 3H); 3.82 (s, 2H); 3.86 (s, 1H); 3.99 (s, 1H); 4.68-4.77 (m, 1H). $^{13}$C NMR: (75 MHz, $CDCl_3$) δc 12.5, 17.3, 22.4, 23.1, 26.5, 27.4, 28.2, 30.8, 31.0, 34.3, 34.6, 34.7, 34.9, 35.2, 39.4, 41.2, 41.9, 46.5, 47.2, 50.5, 51.5, 68.2, 72.9, 76.3, 167.9, 174.7. MS (ESI): m/z = 506 [M + 1]. Anal. Calcd. For $C_{27}$ $H_{43}$ $N_3$ $O_6$: C, 64.13 ; H, 8.57; N, 8.31. Found: C, 64.21; H, 8.68; N, 8.23.



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**First generation dendritic chloride [$G_1$]-$CH_2$-Cl 14:**

Following the general procedure **B**, the dendritic chloride **14** was obtained as a white colored solid from 3,5-bis(propargyloxy)benzyl chloride **13** (0.5 g, 2.1 mmol) and the dendritic azide **11** (2.19 g, 4.5 mmol) and by eluting the column with $CHCl_3$: MeOH (19:1). Yield: 2.45g, 95%; M.p.: 128 °C; $^1$H NMR(300 MHz, $CDCl_3$): $\delta_H$ 0.68 (s, 6H); 0.85-2.38 (m, 66H, steroidal H); 3.67 (s, 6H); 4.00 (s, 2H); 4.50 (s, 2H); 4.80- 4.83 (m, 2H); 5.14 (s, 4H); 5.21 (s, 4H); 6.59 (s, 1H); 6.64 (s, 2H); 7.80 (s, 2H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta_C$ 12.7, 17.4, 23.0, 23.6, 26.4, 26.9, 27.4, 28.7, 30.9, 31.1, 32.0, 33.7, 34.1, 34.7, 35.1, 36.0, 41.9, 46.1, 46.5, 47.4, 48.3, 51.2, 51.5, 62.2, 73.1, 102.0, 108.2, 124.2, 139.8, 144.2, 159.5, 165.7, 174.6. MS (ESI): m/z = 1213 [M + 1]. Anal. Calcd. For $C_{67}$ $H_{97}$ Cl $N_6$ $O_{12}$: C, 66.29; H, 8.05; N, 6.92. Found: C, 66.17; H, 7.96; N, 6.99.

**First generation dendritic chloride [$G_1$]-$CH_2$-Cl 15:**

Following the general procedure **B**, the dendritic chloride **15** was obtained as a white colored solid from 3,5-bis(propargyloxy)benzyl chloride **13** (0.5 g, 2.1 mmol) and the dendritic azide **12** (2.26 g, 4.5 mmol) and by eluting the column with $CHCl_3$: MeOH (19:1). Yield: 2.4g, 92%; M.p.: 130 °C; $^1$H NMR(300 MHz, $CDCl_3$): $\delta_H$ 0.69 (s, 6H); 0.85-2.42 (m, 66H, steroidal H); 3.66 (s, 6H); 4.00 (s, 2H); 4.50 (s, 2H); 4.78- 4.86 (m, 2H); 5.12 (s, 4H); 5.22 (s, 4H); 6.60 (s, 1H); 6.65 (s, 2H); 7.77 (s, 2H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta_C$ 12.7, 17.4, 23.0, 23.6, 26.4, 26.9, 27.4, 28.7, 30.9, 31.1, 32.0, 33.7, 34.1, 34.7, 35.1, 36.0, 41.9, 46.1, 46.5, 47.4, 48.3, 51.2, 51.5, 62.2, 73.1, 102.0, 108.2, 124.2, 139.8, 144.2, 159.5, 165.7, 174.6. MS (ESI): m/z = 1245 [M + 1]. Anal. Calcd. For $C_{67}$ $H_{97}$ Cl $N_6$ $O_{14}$: C, 64.59; H, 7.85; N, 6.74. Found: C, 64.49; H, 7.77; N, 6.83.

**First generation dendritic azide [$G_1$]-$CH_2$-$N_3$ 16:**

Following the general procedure **A**, the dendritic azide **16** was obtained as a white colored solid from dendritic chloride **14** (2.5 g, 1.4 mmol) and sodium azide (0.18 g, 2.7 mmol) and by eluting the column with $CHCl_3$: MeOH (19:1). Yield: 2.3g 92%; M.p.: 120 °C; $^1$H NMR: (300 MHz, $CDCl_3$): $\delta_H$ 0.69 (s, 6H); 0.92 (s, 6H); 0.97-2.38 (m, 66H, steroidal H); 3.67 (s, 6H); 4.00 (s, 2H); 4.26 (s, 2H); 4.79- 4.86 (m, 2H); 5.13 (s, 4H); 5.23 (s, 4H); 6.57 (s, 2H); 6.62 (s, 1H); 7.78 (s, 2H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta_C$ 12.7, 17.4, 23.0, 23.6, 26.0, 26.4, 26.9, 27.4, 28.7, 29.7, 30.9, 31.1, 32.0, 33.7, 34.1, 34.7, 35.1, 36.0, 41.9, 46.5, 47.4, 48.3, 51.1, 51.5, 54.7,



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62.1, 73.1, 101.8, 107.7, 124.2, 137.8, 144.2, 159.6, 165.7, 174.7. MS (ESI): m/z = 1221 [M + 1]. Anal. Calcd. For $C_{67}H_{97}N_9O_{12}$: C, 65.93; H, 8.01; N, 10.33. Found: C, 65.86; H, 8.11; N, 10.44.

**First generation dendritic azide [$G_1$]-$CH_2$-$N_3$ 17:**

Following the general procedure **A**, the dendritic azide **17** was obtained as a white colored solid from dendritic chloride **15** (2.5 g, 1.36 mmol) and the sodium azide (0.17 g, 2.72 mmol) and by eluting the column with $CHCl_3$: MeOH (19:1). Yield: 2.3g, 89%; M.p.: 135 °C; $^1$H NMR(300 MHz, $CDCl_3$): $\delta_H$ 0.69 (s, 6H); 0.85-2.42 (m, 66H, steroidal H); 3.67 (s, 6H); 4.00 (s, 2H); 4.26 (s, 2H); 4.79- 4.86 (m, 2H); 5.12 (s, 4H); 5.23 (s, 4H); 6.57 (s, 2H); 6.62 (s, 1H); 7.76 (s, 2H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta_C$ 12.7, 17.4, 23.0, 23.6, 26.0, 26.4, 26.9, 27.4, 28.7, 30.9, 31.1, 32.0, 33.7, 34.1, 34.7, 35.1, 36.0, 41.9, 46.5, 46.5, 47.4, 48.3, 51.2, 51.5, 54.7, 62.2, 73.1, 77.2, 101.9, 107.7, 124.2, 137.8, 144.3, 159.7, 165.7, 174.6. MS (ESI): m/z = 1253 [M + 1]. Anal. Calcd. For $C_{67}H_{97}N_9O_{14}$: C, 64.25; H, 7.81; N, 10.06. Found: C, 64.16; H, 7.88; N, 10.14.

**Second generation dendritic chloride [$G_2$]-$CH_2$-Cl 18:**

Following the general procedure **B**, the dendritic chloride **18** was obtained as a white colored solid from 3,5-bis(propargyloxy)benzyl chloride **13** (0.12 g, 0.51 mmol) and the dendritic azide **16** (1.31 g, 1.07 mmol) and by eluting the column with $CHCl_3$: MeOH (19:1). Yield: 1.1g, 80%; M.p.: 180 °C; $^1$H NMR(300 MHz, $CDCl_3$): $\delta_H$ 0.69 (s, 12H); 0.85-2.37 (m, 132H, steroidal H); 3.66 (s, 12H); 4.00 (s, 4H); 4.50 (s, 2H); 4.67- 4.70 (m, 4H); 4.85-4.91 (m, 4H); 5.18 (s, 16H); 5.45-5.54 (m, 4H); 6.64 (s, 5H); 6.72 (s, 4H); 7.46(s, 4H); 7.80 (s, 2H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta_C$ 11.4, 12.8, 14.1, 14.3, 17.3, 22.7, 23.0, 23.7, 25.3, 26.2, 26.9, 27.5, 27.7, 28.7, 29.1, 29.7, 30.9, 31.1, 31.6, 31.8, 33.6, 34.1, 34.5, 34.8, 35.1, 36.0, 41.8, 46.2, 46.6, 47.3, 48.1, 50.8, 51.5, 54.4, 61.5, 62.4, 73.1, 102.2, 107.4, 108.2, 124.9, 136.8, 139.9, 159.4, 159.6, 165.8, 174.7. MS (MALDI-TOP): m/z = 2697 $[M + Na]^+$. Anal. Calcd. For $C_{147}H_{205}ClN_{18}O_{26}$: C, 65.98; H, 7.72; N, 9.42. Found: C, 65.88; H, 7.80; N, 9.53.

**Second generation dendritic chloride [$G_2$]-$CH_2$-Cl 19:**

Following the general procedure **B**, the dendritic chloride **19** was obtained as a white colored solid from 3,5-bis(propargyloxy)benzyl chloride **13** (0.05 g, 0.21 mmol) and the dendritic azide **17** (0.56 g, 0.44 mmol) and by eluting the column with $CHCl_3$: MeOH (19:1). Yield: 0.45g, 78%; M.p.: 182 °C; $^1$H NMR(300 MHz, $CDCl_3$): $\delta_H$ 0.69-2.43 (m, 142H, steroidal



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H); 3.66 (s, 12H); 3.89 (s, 4H); 4.00 (s, 4H); 4.54 (m, 6H); 5.10 (s, 8H); 5.23 (s, 12H); 5.42-5.61 (m, 4H); 6.67 (s, 5H); 7.08(s, 4H); 7.16 (s, 4H); 8.24 (s, 2H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta_C$ 11.4, 12.5, 12.6, 12.6, 14.1, 17.3, 22.2, 22.5, 22.6, 23.2, 26.0, 26.6, 26.9, 27.5, 28.3, 29.7, 30.9, 31.1, 31.1, 31.6, 34.2, 34.5, 34.6, 34.7, 35.2, 35.3, 39.5, 41.0, 41.5, 41.9, 42.2, 46.5, 46.7, 47.1, 50.4, 51.5, 68.2, 68.4, 71.9, 73.0, 76.1, 77.5, 102.5, 106.3, 109.2, 122.8, 125.2, 136.4, 140.1, 159.2, 159.3, 165.8, 174.8. MS (MALDI-TOP): m/z = 2760 $[M + Na]^+$. Anal. Calcd. For $C_{147}$ $H_{205}$ Cl $N_{18}$ $O_{30}$: C, 64.44; H, 7.54; N, 9.20. Found: C, 64.33; H, 7.63; N, 9.31.

**Second generation dendritic azide [$G_2$]-$CH_2$-$N_3$ 20 :**

Following the general procedure **A**, the dendritic azide **20** was obtained as a white colored solid from dendritic chloride **18** (0.5 g, 0.19 mmol) and sodium azide (0.024 g, 0.37 mmol) and by eluting the column with $CHCl_3$-MeOH (19:1). Yield: 0.40g, 80%; M.p.: 125 °C; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta_H$ 0.69 (s, 12H); 0.83-2.41 (m, 130H, steroidal H); 3.66 (s, 12H); 4.00 (s, 4H); 4.26 (s, 2H); 4.69- 4.80 (m, 4H); 4.90 (s, 4H); 4.96 (s, 2H); 5.16 (s, 8H); 5.18 (s, 8H); 5.39-5.52 (m, 4H); 6.55 (s, 2H); 6.62 (s, 2H); 6.68 (s, 4H); 6.75 (s, 1H); 7.50 (s, 4H); 7.86 (s, 2H). $^{13}$C NMR: (75 MHz, $CDCl_3$) $\delta_C$ 11.4, 12.7, 17.3, 22.6, 22.9, 23.7, 25.3, 26.0, 26.9, 27.5, 28.7, 29.0, 30.9, 31.1, 31.6, 31.8, 33.6, 34.1, 34.7, 34.7, 35.1, 36.0, 36.1, 41.8, 46.6, 47.3, 48.1, 50.8, 51.5, 54.7, 61.5, 62.4, 73.1, 76.6, 102.2, 107.0, 108.2, 122.9, 124.8, 136.8, 138.0, 143.6, 159.6, 165.8, 174.7. MS (MALDI-TOP): m/z = 2704 $[M + Na]^+$. Anal. Calcd. For $C_{1497}H_{205}$ $N_{21}$ $O_{26}$: C, 65.82; H, 7.70; N, 10.97. Found: C, 65.92; H, 7.75; N, 11.05.

**Second generation dendritic azide [$G_2$]-$CH_2$-$N_3$ 21 :**

Following the general procedure **A**, the dendritic azide **21** was obtained as a white colored solid from dendritic chloride **19** (0.6 g, 0.21 mmol) and sodium azide (0.03 g, 0.43 mmol) and by eluting the column with $CHCl_3$-MeOH (19:1). Yield: 0.5g, 84%; M.p.: 170 °C; $^1$H NMR: (300 MHz, $CDCl_3$) δ 0.68-2.38 (m, 140H, steroidal H); 3.66 (s,12H); 3.88 (s, 4H); 4.0 (s, 4H); 4.34 (s, 2H); 4.43-4.46 (m, 4H); 5.14-5.28 (m, 20H); 5.48-5.60 (m, 4H); 6.60 (s, 2H); 6.70 (s, 2H); 7.00 (s, 4H); 7.11 (s, 1H); 7.27 (4H); 8.34 (s, 2H). $^{13}$C NMR: (75 MHz, $CDCl_3$) δ 9.6, 10.7, 10.9, 12.3, 15.5, 20.4, 20.4, 20.8, 20.8, 21.4, 23.5, 24.1, 25.1, 25.6, 26.5, 27.2, 29.1, 29.3, 29.7, 32.4, 32.5, 32.8, 32.9, 33.4, 33.5, 37.7, 37.7, 39.1, 40.2, 40.5, 44.7, 44.9, 45.3, 48.4, 49.7, 53.1, 66.5, 70.1, 71.2, 74.0, 75.4, 123.5, 143.5, 141.5, 143.9, 157.3, 157.6, 163.9, 172.9. MS (MALDI-TOP): m/z = 2769 $[M + Na]^+$. Anal. Calcd. For $C_{147}$ $H_{205}$ $N_{21}$ $O_{30}$: C, 64.29; H, 7.52; N, 10.71. Found: C, 64.18; H, 7.59; N, 10.78.



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**Preparation of 5,10,15,20-Tetrakis(4'-propargyloxyphenyl)-Zn(II)-porphyrin. 22.**

5,10,15,20-Tetrakis(4-hydroxyphenyl)-porphyrin (1.0 g, 1.474 mmol, 1.0 equiv) was dissolved in anhydrous DMF under argon atmosphere, $K_2CO_3$ (2.0 g, 14.748 mmol, 10 equiv) and propargyl bromide (0.80 mL, 8.84 mmol, 6 equiv) were then added. The reaction was allowed to stir at room temperature for overnight. After completion of reaction, DMF was evaporated under reduced pressure and extracted thrice with the $CH_2Cl_2$ (100 mL) and washed with water system at least three times to remove excess $K_2CO_3$. The organic layer was dried over $Na_2SO_4$, filtered, and concentrated to obtain a purple solid. The crude mixture was further purified by flash chromatography over $SiO_2$ (hexane/ethyl acetate) to give 5, 10, 15, 20-tetrakis (4-propargyloxyphenyl)-porphyrin; Yield 1.10 g, 95%; A solution of the tetrapropargyl functionalized porphyrin (1.10 g, 1.325 mmol, 1.0 equiv) in $CHCl_3$ (7 mL) and a solution of zinc acetate (1.50 g, 6.618 mmol, 5 equiv) in methanol (7 mL) were mixed and refluxed under $N_2$ for 2−3 h. The reaction mixture was concentrated by vacuum evaporation, and $CH_2Cl_2$ (150 mL) was added. The organic layer was washed with water (3 × 20 mL), dried over $Na_2SO_4$, filtered, and concentrated. The crude mixture was further purified by flash chromatography over $SiO_2$ (hexane/ethyl acetate) to give pure compound **22** as a purple solid; Yield 0.61 g, 98%; $^1$H NMR ($CDCl_3$, 300 MHz) δ 8.97 (s, 8 H), 8.13 (d, 8 H, $J$ = 8.7 Hz), 7.34 (d, 8 H, $J$ = 8.7 Hz), 4.98 (d, 8 H, $J$ = 2.4 Hz), 2.69 (t, 4 H, $J$ = 2.4 Hz).

**Synthesis of $G_0$ dendrimer 1**

Compound **22** (0.1 g, 0.12 mmol, 1.0 equiv) was reacted with **11** (0.24 g, 0.50 mmol, 4.2 equiv) in the presence of $CuSO_4 \cdot 5H_2O$ (0.009 g, 0.03 mmol, 0.3 equiv), and sodium L-ascorbate (0.007 g, 0.03 mmol, 0.3 equiv) in THF/water (30 mL/10 mL) according to procedure **B**. Pure compound **1** was isolated by silica gel column chromatography as a purple solid with $CHCl_3$-MeOH (19:1). Yield: 0.22g, 69%; M.p.: 168 °C; $^1$H NMR: (300 MHz, $CDCl_3$+ DMSO-$d_6$): $\delta_H$ 0.67 (s, 12H); 0.92 (s, 12H); 0.95-2.41 (m, 123H, steroidal H); 3.64 (s, 12H); 3.96 (s, 4H); 4.81-4.84 (m, 4H); 5.14 (s, 8H); 5.35 (s, 8H); 7.30(d, 8H, $J$ = 7.8Hz); 7.79 (s, 4H); 8.10 (d, 8H, $J$ = 7.5Hz); 8.88 (s, 8H). $^{13}$C NMR: (75 MHz, $CDCl_3$+ DMSO-$d_6$): $\delta_C$ 12.7, 14.0, 17.2, 23.0, 23.6, 26.0, 26.3, 26.9, 27.4, 28.7, 29.3, 29.6, 30.9, 31.0, 32.0, 33.6, 34.1, 34.7, 35.1, 35.9, 41.8, 46.4, 47.1, 48.1, 51.1, 51.4, 62.0, 72.6, 77.4, 77.6, 112.7, 124.5, 131.6, 135.5, 144.3, 150.2, 157.7,



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165.8, 176.4. MS (MALDI-TOF): m/z = 2806 $[M + H - Zn]^+$. Anal. Calcd. For $C_{165}$ $H_{211}$ $N_{16}$ $O_{24}$ Zn: C, 69.04; H, 7.35 ; N, 7.86. Found: C, 68.96; H, 7.40; N, 7.94.

**Synthesis of $G_0$ dendrimer 2**

Compound **22** (0.1 g, 0.20 mmol, 1.0 equiv) was reacted with **12** (0.25 g, 0.50 mmol, 4.2 equiv) in the presence of $CuSO_4 \cdot 5H_2O$ (0.009 g, 0.03 mmol, 0.3 equiv), and sodium L-ascorbate (0.007 g, 0.03 mmol, 0.3 equiv) in THF/water (30 mL/10 mL) according to procedure **B**. Pure compound **2** was isolated by silica gel column chromatography as a purple solid with $CHCl_3$-MeOH (19:1). Yield 0.25g, 78%; M.p.: 195 °C; $^1$H NMR: (300 MHz, DMSO-$d_6$): $\delta_H$ 0.56 (s, 12H); 0.83 (s, 12H); 0.90-2.31 (m, 119H, steroidal H); 3.78 (s, 12H); 4.19 (d, 8H, *J* = 7.5Hz); 4.57-4.64 (m, 4H); 5.46 (s, 16H); 7.45 (d, 8H, *J* = 7.8Hz); 8.08 (d, 8H, *J* = 7.2Hz); 8.39 (s, 4H); 8.80 (s, 8H). $^{13}$C NMR: (75 MHz, DMSO-$d_6$): $\delta_C$ 12.9, 17.5, 22.9, 23.4, 26.8, 27.9, 29.0, 31.1, 31.4, 34.9, 35.1, 35.6, 41.7, 42.0, 46.4, 46.7, 51.3, 51.9, 61.9, 66.8, 71.7, 76.9, 79.7, 113.6, 120.5, 126.9, 132.1, 135.9, 136.0, 143.5, 150.2, 158.2, 167.4, 174.6. MS (MALDI-TOF): m/z = 2852$[M + H\text{-}Zn\text{-}NH_4]^+$. Anal. Calcd. For $C_{165}$ $H_{211}$ $N_{16}$ $O_{28}$ Zn: C, 67.53; H, 7.19 ; N, 7.68. Found: C, 67.44; H, 7.27; N, 7.75.

**Synthesis of $G_1$ dendrimer 3**

Compound **22** (0.06 g, 0.067 mmol, 1.0 equiv) was reacted with **16** (0.34 g, 0.27 mmol, 4.2 equiv) in the presence of $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.020 mmol, 0.3 equiv), and sodium L-ascorbate (0.004 g, 0.020 mmol, 0.3 equiv) in THF/water (30 mL/10 mL) according to procedure **B**. Pure compound **3** was isolated by silica gel column chromatography as a purple solid with $CHCl_3$-MeOH (19:1). Yield: 0.22g, 58%; M.p.: 170 °C; $^1$H NMR: (300 MHz, $CDCl_3$+ DMSO-$d_6$): $\delta_H$ 0.64 (s, 24H); 0.87 (s, 24H); 0.92-2.37 (m, 247H, steroidal H); 3.64 (s, 24H); 3.93 (s, 8H); 4.71-4.74 (m, 8H); 4.98 (s, 16H); 5.10 (s, 16H); 5.35 (s, 8H); 5.49 (s, 8H); 6.53 (s, 8H); 6.61 (s, 4H); 7.29 (d, 8H, *J* = 6.9Hz); 7.61 (s, 4H); 7.75 (s, 4H); 8.04 (d, 8H, *J* = 7.5Hz); 8.82 (s, 8H). $^{13}$C NMR: (75 MHz, $CDCl_3$+ DMSO-$d_6$): $\delta_C$ 12.6, 17.2, 22.9, 23.6, 25.9, 26.2, 26.9, 27.4, 28.7, 30.8, 31.0, 31.9, 33.5, 34.0, 34.7, 35.1, 35.9, 39.1, 39.4, 39.7, 39.9, 40.2, 40.5, 40.8, 41.8, 46.4, 47.0, 48.0, 50.9, 51.4, 61.8, 62.1, 72.6, 76.8, 76.9, 101.9, 107.5, 112.9, 123.3, 124.6, 135.4, 137.0, 143.5, 144.3, 150.1, 157.6, 159.8, 165.7, 174.7. MS (MALDI-TOF): m/z = 5814 $[M + Na]^+$. Anal. Calcd. For $C_{325}$ $H_{427}$ $N_{40}$ $O_{52}$ Zn: C, 67.37; H, 7.40; N, 9.70. Found: C, 67.45; H, 7.48; N, 9.77.



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**Synthesis of $G_1$ dendrimer 4**

Compound **22** (0.07 g, 0.078 mmol, 1.0 equiv) was reacted with **17** (0.4 g, 0.32 mmol, 4.2 equiv) in the presence of $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.023 mmol, 0.3 equiv), and sodium L-ascorbate (0.002 g, 0.023 mmol, 0.3 equiv) in THF/water (30 mL/10 mL) according to procedure **B**. Pure compound **4** was isolated by silica gel column chromatography as a purple solid with $CHCl_3$-MeOH (19:1). Yield 0.25g, 54%; M.p.: 240 °C; $^1$H NMR: (300 MHz, DMSO-$d_6$): $\delta_H$ 0.55 (s, 24H); 0.78 (s, 24H); 0.90-2.34 (m, 227H, Steroidal H); 3.56 (s, 24H); 3.78 (s, 8H); 4.12 (d, 16H, $J$ = 4.2Hz); 4.51-4.56 (m, 8H); 5.20 (s, 16H); 5.38 (s, 16H); 5.44 (s, 8H); 5.64 (s, 8H); 6.69 (s, 8H); 6.81 (s, 4H); 7.46 (d, 8H, $J$ = 8.1Hz); 8.09 (d, 8H, $J$ = 7.5Hz); 8.24 (s, 8H); 8.49 (s, 4H); 8.81 (s, 8H). $^{13}$C NMR: (75 MHz, DMSO-$d_6$): $\delta_C$ 11.4, 16.1, 21.3, 21.9, 25.3, 26.4, 27.6, 28.1, 29.6, 29.9, 33.4, 33.6, 33.9, 34.1, 40.1, 40.5, 44.9, 45.2, 49.7, 50.3, 52.1, 60.4, 60.6, 65.3, 70.1, 75.3, 78.3, 100.1, 106.6, 111.9, 119.0, 124.2, 125.2, 130.6, 134.3, 134.6, 137.4, 141.6, 142.3, 148.7, 156.9, 158.6, 165.8, 172.9. MS (MALDI-TOF): m/z = 5910$[M + H]^+$. Anal. Calcd. For $C_{325}$ $H_{427}$ $N_{40}$ $O_{60}$ Zn: C, 65.91; H, 7.24; N, 9.49. Found: C, 65.99; H, 7.30; N, 9.40.

**Synthesis of $G_2$ dendrimer 5**

Compound **22** (0.02 g, 0.024 mmol, 1.0 equiv) was reacted with **20** (0.28 g, 0.10 mmol, 4.2 equiv) in the presence of $CuSO_4 \cdot 5H_2O$ (0.003 g, 0.007 mmol, 0.3 equiv), and sodium L-ascorbate (0.002 g, 0.007 mmol, 0.3 equiv) in THF/water (30 mL/10 mL) according to procedure **B**. Pure compound **5** was isolated by silica gel column chromatography as a purple solid with $CHCl_3$-MeOH (19:1). Yield: 0.18g, 69%; M.p.: 168 °C; $^1$H NMR: (300 MHz, DMSO-$d_6$): $\delta_H$ 0.54 (s, 48H); 0.81-2.29 (m, 513H, steroidal H); 3.55 (s, 48H); 3.75 (s, 16H); 4.20 (s, 16H); 4.68 (br s, 16H); 5.14 (s, 42H); 5.37 (s, 42H); 5.52 (s, 22H); 5.60 (s, 8H); 6.60 (s, 16H); 6.67 (s, 8H); 6.76 (s, 12H); 7.43 (br s, 8H); 8.08 (br s, 8H,); 8.21 (s, 24H); 8.47 (s, 4H); 8.79 (s, 8H). $^{13}$C NMR: (75 MHz, DMSO-$d_6$): $\delta_C$ 12.3, 16.8, 22.6, 23.4, 29.9, 26.5, 27.1, 28.4, 28.7, 29.0, 29.4, 30.4, 30.7, 31.3, 31.5, 31.6, 32.7, 33.6, 34.3, 34.9, 35.4, 41.1, 45.9, 46.1, 47.3, 50.5, 51.1, 61.1, 71.0, 75.6, 79.1, 100.9, 107.3, 124.8, 126.1, 138.1, 142.4, 149.5, 159.3, 166.6, 173.7. MS (MALDI-TOF): m/z = 116340 $[M + H- Zn]^+$. Anal. Calcd. For $C_{645}$ $H_{859}$ $N_{88}$ $O_{108}$ Zn: C, 66.54; H, 7.42; N, 10.60. Found: C, 66.48; H, 7.48; N, 10.53.

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**Synthesis of $G_2$ dendrimer 6**

Compound **22** (0.02 g, 0.024 mmol, 1.0 equiv) was reacted with **21** (0.28 g, 0.10 mmol, 4.2 equiv) in the presence of $CuSO_4 \cdot 5H_2O$ (0.003 g, 0.007 mmol, 0.3 equiv), and sodium L-ascorbate (0.002 g, 0.007 mmol, 0.3 equiv) in THF/water (30 mL/10 mL) according to procedure **B**. Pure compound **6** was isolated by silica gel column chromatography as a purple solid with $CHCl_3$-MeOH (19:1). Yield 0.15g, 57%; M.p.: 160 °C; $^1$H NMR: (300 MHz, DMSO-$d_6$): $\delta_H$ 0.50-2.42 (m, 563H, steroidal H); 3.53 (s, 48H); 4.09(s, 16H); 4.14 (s, 16H); 4.50 (br s, 16H); 5.12 (s, 48H); 5.32 (s, 48H); 5.50 (s, 16H); 6.57 (s, 16H); 6.65 (s, 8H); 6.72 (s, 12H); 7.40 (br s, 8H); 8.03 (br s, 8H,); 8.17 (s, 24H); 8.42 (s, 4H); 8.77 (s, 8H). $^{13}$C NMR: (75 MHz, DMSO-$d_6$): $\delta_C$ 12.1, 16.8, 22.1, 22.6, 26.0, 27.1, 28.3, 30.4, 30.6, 34.1, 34.3, 34.6, 34.9, 40.9, 41.2, 45.7, 46.0, 50.4, 51.1, 52.8, 61.0, 66.1, 71.0, 76.2, 79.0, 100.9, 107.2, 124.8, 126.0, 138.0, 142.4, 159.3, 166.6, 173.9. MS (MALDI-TOF): m/z = 11896 $[M + H- Zn]^+$. Anal. Calcd. For $C_{645}$ $H_{859}$ $N_{88}$ $O_{124}$ Zn: C, 65.11; H, 7.26; N, 10.38. Found: C, 65.02; H, 7.37; N, 10.30.

**Inhibition of cancer Cell Growth.**

The MIA PaCa-2 cells were cultured in DMEM media containing 10% fetal bovine serum at 37° C and 5% $CO_2$. 5000 cells were seeded in each well containing 200µl of DMEM medium in 96 well plates. After 24h dendrimers **1, 2, 3, 4, 5** and **6** were added in triplicate. Three different test concentrations 100µM, 200µM and 300µM were added and cell viability was assessed; after 24 h of treatment, 20µl per well of MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; 5 mg/ml; stock solution, Sigma) was added. The plates were incubated at 37°C for additional four hours. The medium was discarded and the formazan blue, which formed in the cells, was dissolved with 200 µl of DMSO. Cell viability and $IC_{50}$ value was calculated for compound efficacy on cancer cell.

**Acknowledgements**

The authors thank Department of Science and technology (DST) and Council of Industrial and Scientific Research (CSIR), New Delhi, for financial assistance and DST-FIST for providing NMR facilities to the department. DA thanks UGC-UPE Phase II for fellowship.

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## Graphical Abstract

# Synthesis, photophysical properties and anticancer activity of micro-environment sensitive amphiphilic bile acid dendrimers

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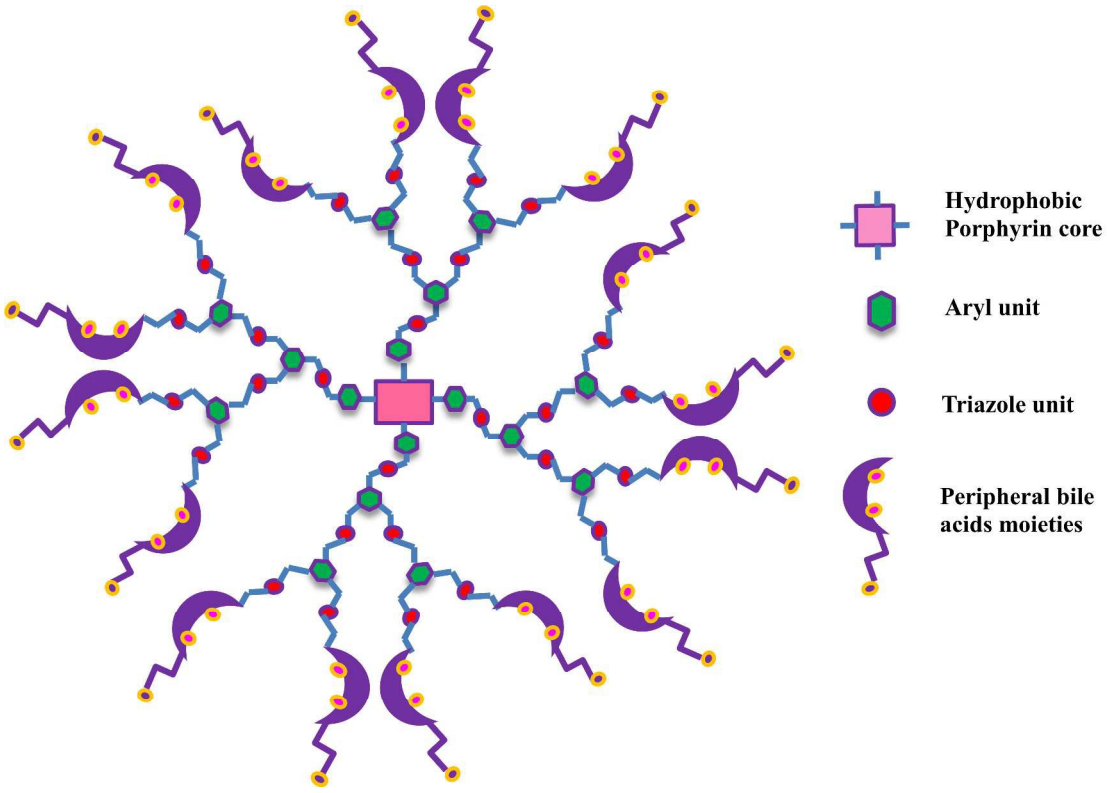


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