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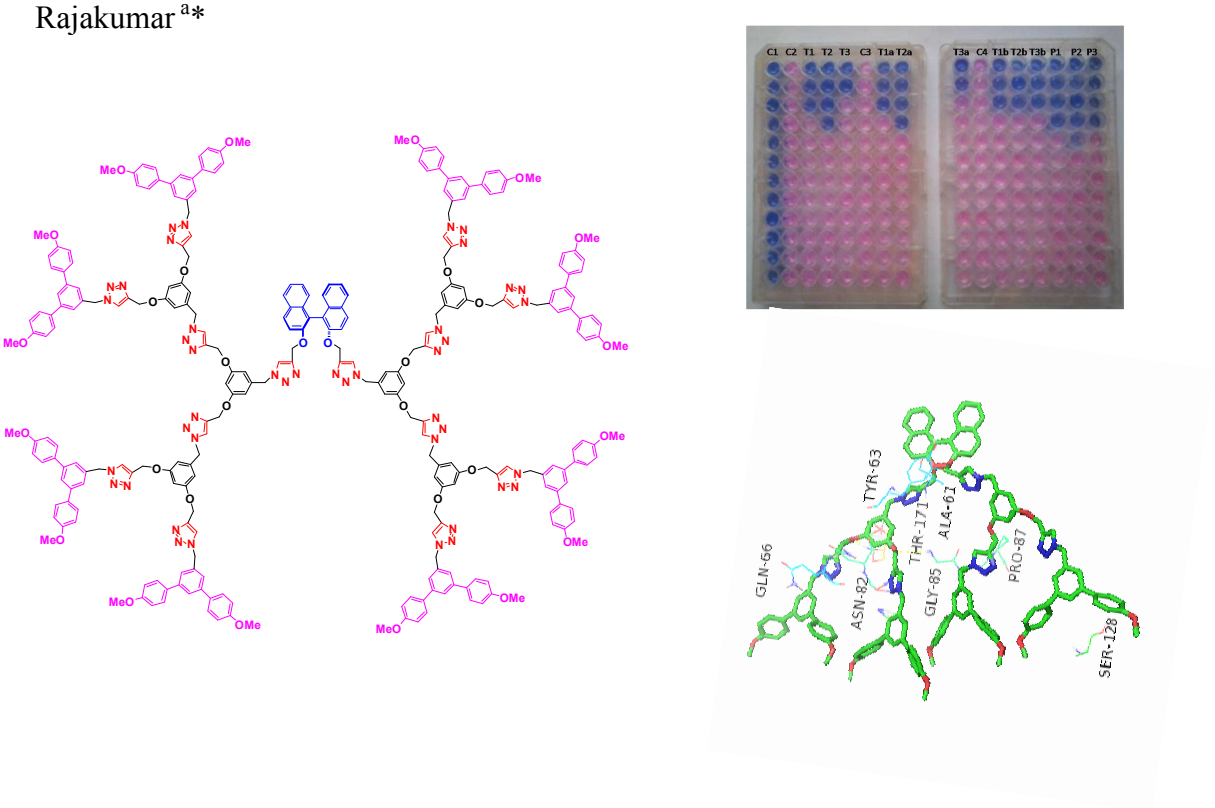
## Graphical Abstract

# Synthesis of chiral core based triazole dendrimers with *m*-terphenyl surface unit and their antibacterial studies

Ayyavu Thirunarayanan[a] Sebastian Raja[a] Gunasekaran Mohanraj[b] and Perumal Rajakumar [a]*

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**Ayyavu Thirunarayanan,[a] Sebastian Raja,[a] Gunasekaran Mohanraj[b] and Perumal Rajakumar[a]***

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## Introduction

The growing incidence of bacterial resistance to the existing antibiotics possess a serious medical problem in treating pathogenic infections.[1-2] Hence, there is an urgent need to search for the compounds which are more potent and less sensitive in developing resistance than the commercial antibiotics[3] are currently used. 1,2,3-Triazole is an important nitrogen containing heterocyclic system which shows a broad spectrum of biological and pharmacological applications.[4-5] Copper (I) catalyzed Huisgen dipolar cycloaddition of terminal alkynes to azides to give 1,4-disubstituted 1,2,3-triazole reported by Sharpless[6] is an example of click reaction.[7] Incorporation of 1,2,3-triazole into the supramolecular assemblies would be interesting and may generate promising candidates for biological applications.

Dendrimers are regularly ordered, highly branched, monodispersed three dimensional architecture. Multifunctional nature of dendrimers offer countless applications [8] in biomedical field such as gene delivery vectors,[9] magnetic resonance imaging,[10] vaccine development,[11] drug delivery systems.[12-15] Click chemistry have been employed mainly to introduce 1,2,3-triazole in the dendritic structure either by convergent or divergent manner. Dendritic structures and assemblies composed of optically active moieties have also received much attention.[16] Chiral compounds have highly selective biological applications due to their specificity association with enzymatic structure hence synthesis of such compounds would be a challenge for synthetic organic chemists. Chiral supramolecular assemblies are very useful for enantioselective clathration, separation, catalysis,[17] and sensing[18] applications. Dendrimers with constitutionally different segments on a chiral core or chiral conformation due to molecular rigidity have also been reported.[19] Application of click reaction for the synthesis of chiral dendrimers and also some heteroatom containing macrocycles has been reported from our laboratory.[20]

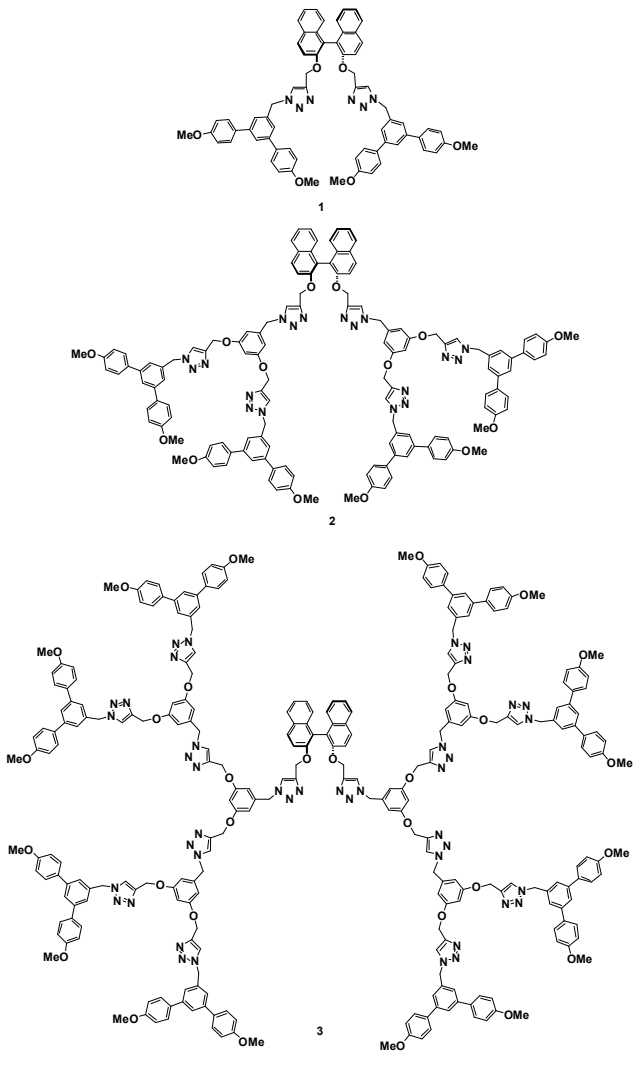



**Fig. 1**: Structural representation of chiral-triazole dendrimers **1**, **2** and **3**

[a]*Department of Organic Chemistry, University of Madras, Maraimalai campus (Guindy), Chennai– 600 025, INDIA*
*E-mail: perumalrajakumar@gmail.com; Fax: +91 44 2352494; Tel: +91 44 2220 2810*
[b]*Centre for Advanced Studies in Botany, University of Madras, Maraimalai Campus (Guindy), Chennai -600 025, INDIA*

The angular nature of *m*-terphenyl moiety gives rigidity to the overall structure and has been used in cyclophane chemistry[21] to synthesize molecules with concave structural geometry[22] and such molecules can be used as memory storage device. In the present study substituted *m*-terphenyl moiety has been used as the surface group in the dendrimers. In view of these observations, we report herein the synthesis of novel dentritic architectures **1**, **2** and **3** (Fig 1) and their antibacterial activity against the human pathogenic bacteria such as *Shigella dysenteriae, Staphylococcus aureus* and *Serratia marcescens.*

## 2. Chemistry

*S* (-) BINOL core based triazole dendrimers **1**, **2** and **3** were synthesized by stepwise synthetic methodology as shown in Scheme 1 and 2. Propargylation, azidation and click reactions were employed to achieve the target chiral triazole dendrimers. All the new dendrimers were characterized by $^{1}H$ and $^{13}C$ NMR, mass spectral and elemental analysis.

## 3. Biology

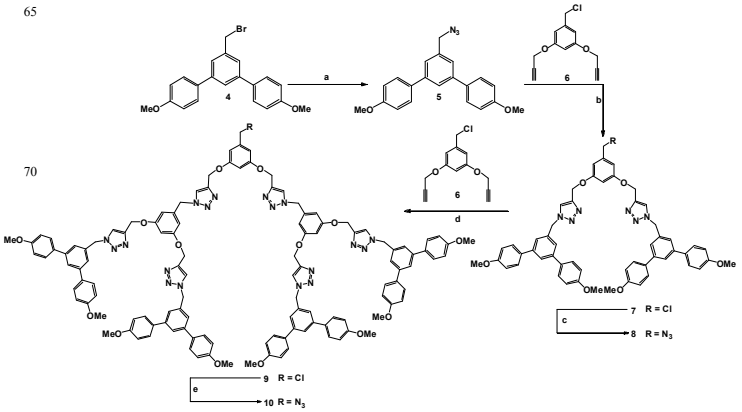
The antibacterial activity of the chiral triazole dendrimeric compounds **1**, **2** and **3** are evaluated against three human pathogens namely *Shigella dysenteriae, Staphylococcus aureus and Serratia marcescens,* by the resazurin reduction assay method (Fig 2). The observed data showing the antibacterial activity of the dendrimers and the standard drug with control experiments are shown in Table **1**. Docking studies reveal that the dendrimers show effective binding with the DNA gyrase enzyme of *Staphylococcus aureus* (Fig 3).

## 4. Results and discussion

In order to the synthesis the target chiral triazole dendrimers **1**, **2** and **3**, the *m*-terphenyl capped azido dendrons **5**, **8** and **10** were synthesized in good yields by using convergent synthetic strategy.[23] Hart's tandem aryne sequence reaction[24] followed by NBS bromination afforded the bromomethyl *m*-terphenyl **4** in moderate yield, which was further subjected to azidation with 1.1 equiv. of $NaN_3$ in DMF at room temperature for 48 h to give the *m*-terphenyl azide **5** in 55% yield. We then focused our attention on the synthesis of first generation dendritic azide **8.**

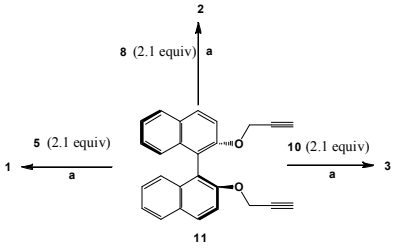
The treatment of 1.0 equivalent of the azide **5** with 0.5 equivalent of 3,5-bis(propargyloxy)benzylchloride **6**[25] under click reaction conditions *viz*., $CuSO_4.5H_2O$ and sodium ascorbate in a 1:1 mixture of water and *t*-BuOH at room temperature afforded the compound **7** in 92% yield. The structure of dendritic chloride **7** was confirmed from the spectral and analytical data. The dendritic chloride **7** was further converted into the first generation dendritic azide ($G_1$-$N_3$) **8** in 73% yield by the reaction of **7** with 1.1 equiv. of $NaN_3$ in DMF at room temperature. In $^{1}H$ NMR spectrum of the first generation dendritic azide **8** showed a singlet at δ 3.86, ppm for twelve methoxy protons, three singlets at δ 4.21, 5.18 and 5.78 ppm for azido methylene, *N*-methylene and *O*-methylene protons respectively, in addition to the signals for aromatic protons. The $^{13}C$ NMR spectrum of the compound **8** displayed the azido methyl, *N*-methylene, methoxy and *O*-methylene carbons at δ 54.7, and 55.0, 55.4, 62.2 ppm respectively, the triazole *CH* carbon appeared at δ 144.2 and in addition to the signals for aromatic carbons.

Similarly, reaction of 1.0 equivalent of dendritic azide **8** with 0.5 equiv. of 3,5-bis (propargyloxy)benzylchloride **6** under click reaction conditions as mentioned earlier afforded the dendritic chloride **9** in 79% yield, which undergoes azidation with 1.1 equiv. of $NaN_3$ in DMF at room temperature to give the corresponding second generation dendritic azide ($G_2$-$N_3$) **10** in 64% yield (Scheme 1). The structure of the azido dendron **10** was also confirmed by IR, $^{1}H$ NMR, $^{13}C$ NMR and EI-MS Spectral data.




**Scheme 1:** Reagents and conditions: a) 1.1, equiv. $NaN_3$, DMF, rt, 48 h. b) 0.5 equiv. of **6,** $CuSO_4.5H_2O$ (5 mol %), NaAsc (10 mol %), $H_2O$-*t*-BuOH (1:1), rt, 12 h. c) 1.1, equiv. $NaN_3$, DMF, rt, 48 h. d) 0.5 equiv. of **6**, $CuSO_4.5H_2O$ (5 mol %), NaAsc (10 mol %), $H_2O$-*t*-BuOH (1:1), rt, 12 h. e) 1.1 equiv. $NaN_3$, DMF, rt 48 h.

The chiral triazole dendrimers **1**, **2** and **3** were synthesized regioselectively in 82, 71 and 56 % yields, respectively by the treatment of 2.1 equiv of the azides **5**, **8** and **10** with 1.0 equiv. of bis(propargyloxy)*S*-BINOL[24a] **11** in the presence of $CuSO_4.5H_2O$, sodium ascorbate in the mixture of *t*-BuOH and water (1:1) at room temperature for 12 h (Scheme 2).




**Scheme 2:** Reagents and conditions: a) $CuSO_4.5H_2O$ (5 mol %), NaAsc (10 mol %), $H_2O$-*t*-BuOH (1:1), 12 h rt.

In $^{1}H$ NMR spectrum of the chiral triazole dendrimer **1** showed a singlet at δ 3.74 for methoxy protons, two doublets at δ 4.88 and 4.91 ppm for *O*-methylene protons attached to *S*-BINOL core unit and one singlet at δ 5.15 ppm for *N*-methylene protons, in addition to the signals for the aromatic protons. The $^{13}C$ NMR spectrum of compound **1** displayed the peaks at δ 53.4, 55.4, 63.9, and 65.9 ppm, for *N*-methylene, methoxy, and *O*-methylene carbons. The triazole *CH* carbon appeared at δ 145.2 and in addition to the signals for the aromatic carbons. The structure of the zero-generation dendrimer ($G_0$) **1** was confirmed from

spectral and analytical data. Similarly, structure of the first and second generation dendrimers **2** and **3** was also confirmed from spectral and analytical data.

## 4.1 Antibacterial activity

The chiral triazole dendrimers **1**, **2** and **3** showed antibacterial activity against the three pathogens viz., *Shigella dysenteriae, Serratia marcescens* and *Staphylococcus aureus*. The resazurin is a non-toxic oxidation-reduction indicator dye used in various cytotoxic assays to evaluate the cell growth. It is a blue non-fluorescent dye which changes to pink color (resorufin) by oxidoreductases enzymes in the viable cells. The resorufin will further reduce to hydroresorufin (uncolored or non-fluorescent). The change of color from blue to pink indicates the growth of bacteria and the blue color indicates the inhibition of bacteria in the assay plate wells. The antimicrobial activity is dose dependent and in the present assay, the compounds are added in different concentrations. Minimal inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after required incubation. In the test plates the control wells row (C1) without bacteria shows that the reagent does not reduce by exposure to air and also the control rows (C2, C3 and C4) with respective test bacteria shows the reduction and change of color from blue to pink confirms that the resazurin reagent was reduced by viable bacterial cells. Thus while adding reagent, bacteria and test materials together (T1 to T3, T1a to T3a, T1b to T3b) we can assess the antibacterial potential of the test material by visualizing the color change (Fig 2).

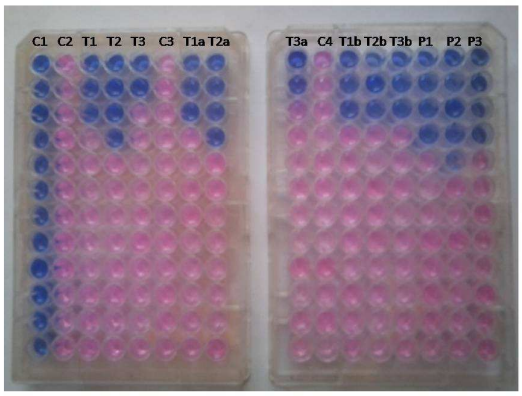



**Figure 2:** Minimum inhibitory concentration determined by Resazurin reduction assay.

**Figure 2**: Key for Photos

T1 – Dendrimer1 + reagent + *Shigella dysenteriae*
T2 – Dendrimer 2 + reagent + *Shigella dysenteriae*
T3 – Dendrimer 3 + reagent + *Shigella dysenteriae*
T1a – Dendrimer 1 + reagent + *Staphylococcus aureus*
T2a – Dendrimer 2 + reagent + *Staphylococcus aureus*
T3a – Dendrimer 3 + reagent + *Staphylococcus aureus*
T1b – Dendrimer 1 + reagent + *Serratia marcescens*
T2b – Dendrimer 2 + reagent + *Serratia marcescens*
T3b – Dendrimer 3 + reagent + *Serratia marcescens*
P1 – positive control – Streptomycin + reagent + *Shigella dysenteriae*
P2 - positive control – Streptomycin + reagent + *Staphylococcus aureus*
P3 - positive control – Streptomycin + reagent + *Serratia marcescens*
C1 - control 1- Test material + reagent + without bacteria
C2 - control 2 – reagent + *Shigella dysenteriae* + without test material
C3 - control 3– reagent + *Staphylococcus aureus* + without test material
C4 - control 4 – reagent + *Serratia marcescens* + without test material

The blue color in the test solutions shows the inhibition of the growth of bacteria and change of pink color shows the reduction of resazurin by viable bacteria. In this present assay, the chiral triazole dendrimer **2** shows excellent antibacterial activity than other dendrimers **1** and **3**. The MIC value of dendrimer **2** against the pathogen *Shigella dysenteriae* and *Staphylococcus aureus* was found to be 12.5µg/mL and its active is comparable to that of the standard *viz*., Streptomycin. The commercial antibiotic shows lowest MIC value of 6.25µg/ml against the pathogen *Staphylococcus aureus*. The triazole dendrimer **1** showed antibacterial activity against *Shigella dysenteriae, Serratia marcescens* and *Staphylococcus aureus* bacteria to the same exist of minimum inhibition of 25µg/ml but less effective when compared to the dendrimer **2**. Even though the dendrimer **3** showed antibacterial activity it is less effective for *Shigella dysenteriae* and *Staphylococcus aureus* than the other dendrimers (Table 1). The MIC values of all the dendrimer are equal with respect the bacteria *Serratia marcescens* and the method adopted here is very easy to follow and accurate in measuring. As described by Sarker[26] *et al*, this method helps in giving accurate MIC value, which can be compared to the existing antibiotics and helps the scientist to decide whether the compounds are worthy for further investigation in terms of their antimicrobial property.

The second-generation dendrimer ($G_2$) **3** was less effective than the zero generation dendrimer ($G_0$) **1** and the first generation dendrimer ($G_1$) **2** shows better antibacterial properties than dendrimers **1** and **3**. The second generation dendrimer ($G_2$) **3** show less activity against all the tested bacterial pathogens. The comparison of anti-bacterial efficacy of the dendrimers **1, 2** and **3** in the assay plate is reveal in (Fig. 2).

**Table 1:** MIC values (µg/ml) of dendrimers determined by the Resazurin reduction assay.

| Tested compounds | MIC (µg/ml) | | |
|---|---|---|---|
| | Bacterial pathogens | | |
| | *Shigella dysenteriae* | *Staphylococcus aureus* | *Serratia marcescens* |
| Dendrimer **1** | 25 | 25 | 25 |
| Dendrimer **2** | 12.5 | 12.5 | 25 |
| Dendrimer **3** | 50 | 50 | 25 |
| Streptomycin (**Standard drug**) | 12.5 | 6.25 | 12.5 |
| **Control** | NA | NA | NA |

**NA** = Not active

## 4.2 Docking studies

The first generation ($G_1$) chiral triazole dendrimer **2** shows strong binding with DNA gyrase enzyme of *Staphylococcus aureus*. It binds to the amino acid residues in the active pocket of the enzyme and thus inhibits its function. It shows interactions (Fig 3) with Proline 87 (PRO 87), Serine 128 (SER 128), Glysine 85 (GLY 85), Alanine 61 (ALA 61), Threonine 171 (THR 171), Tyrosine 63 (TYR 63), Asparagine 82 (ASN 82) and Glysine 66 (GLN 66). The interactions of triazole dendrimer **2** with the enzyme may inhibit its activity and hence could decreases the value of MIC wich is infact observed in the wet laboratory experiments.

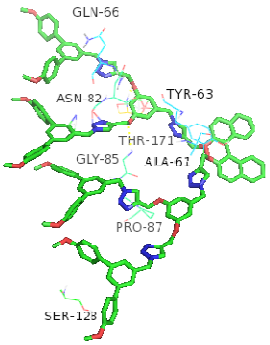



**Figure 3**: Molecular docking of chiral triazole dendrimer **2** with DNA gyrase (PDB: 3G7B)

## 5. Conclusion

Chiral triazole dendrimers **1**, **2** and **3** have been synthesized in good yields by click chemistry and all the dendrimers are evaluated for their antibacterial activity against three human pathogenic bacteria such as *Shigella dysenteriae, Staphylococcus aureus* and *Serratia marcescens*. The study reveals that the chiral dendrimer **2** showed excellent activity against the human pathogenic bacteria than the other dendrimers. Dendrimer **2** was as effective as that of streptomycin against the bacteria *Shigella dysenteriae*, and hence it could be further developed as a good lead in the clinical chemistry.

The docking study also showed the effective binding of chiral dendrimer **2** with the amino acids of the DNA gyrase enzyme as supports the wet laboratory experiments.

## 6. Experimental part

### *6.1. General considerations in chemistry*

All melting points are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded on BRUKER 300 MHz instruments in $CDCl_3$ solvent with TMS as a standard. All chemical shifts values are reported in δ ppm relative to internal standard tetramethylsilane (TMS, δ 0.00). $^{13}$C chemical shifts are reported in δ relative to $CDCl_3$ (center of triplet, δ 77.23) The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The coupling constants J are reported in Hertz (Hz). Mass spectra (MS) were recorded using Voyager-DE PRO mass spectrometer by either Fast Atom Bombardment or MALDI-TOF technique. Elemental analyses were carried out by Perkin-Elmer CHNS 2400 instrument. Column chromatography was performed on silica gel (ACME, 100 -200 mesh). Routine monitoring of the reaction was made using thin layer chromatography developed on glass plates coated with silica gel-G (ACME) of 25 mm thickness and visualized with iodine.

### *6.2. Synthesis of dendron 5, 8 and 10*

*General Procedure (A)*: A mixture of dendritic halide (1.0 eq.) and $NaN_3$ (1.5 eq.) in DMF (20 mL) was stirred at room temperature for 48 h. After completion of the reaction, the reaction mixture was poured into water and extracted with $CHCl_3$ (3X100 mL). The organic layer was washed with saturated brine solution (100 mL) and dried with anhydrous sodium sulphate and filtered. The solvent was evaporated in vaccum and the residue was column chromatographed with $CHCl_3$: MeOH (9:1) to give the pure azide dendron.

#### *6.2.1. Zero generation dendritic azide 5:*

Colourless solid; Yield 70%; mp: 106-108 $^o$C; $^1$H NMR (300 MHz, $CDCl_3$): δ 3.76 (s, 6H); 4.34 (s, 2H); 6.90 (d, 4H, $J$ = 9.0 Hz); 7.33 (s, 2H); 7.48 (d, 4H, $J$ = 9.0 Hz); 7.60 (s, 1H). $^{13}$C

NMR (75 MHz, $CDCl_3$): δ 53.9, 54.3, 113.3, 123.8, 124.2, 127.3, 132.2, 135.3, 141.0, 158.4. (ESI-MS): *m/z* = 345.1 [$M^+$]. Elemental Anal. Calcd for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17%; Found: C, 72.95; H, 5.45; N, 12.11%.

### *6.2.2. First generation dendritic azide 8:*

Colourleess solid; Yield 73%; mp: 124 $^oC$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.86 (s, 12H); 4.22 (s, 2H); 5.18 (s, 4H); 5.79 (s, 4H); 6.55 (s, 2H); 6.61 (s, 1H); 6.93 (d, 4H, *J* = 8.7 Hz); 6.98 (d, 4H, *J* = 8.7 Hz); 7.31 (s, 2H); 7.33 (d, 4H, *J* = 8.7 Hz); 7.44 (d, 4H, *J* = 8.7 Hz); 7.47-7.48 (m, 4H); 7.76 (s, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 54.7, 55.0, 55.4, 62.2, 101.7, 107.5, 114.5, 122.5, 123.4, 127.2, 128.1, 130.6, 131.4, 133.6, 135.1, 137.8, 140.5, 144.2, 159.4, 159.7. (FAB-MS): *m/z* = 932.0 [$M^+$]. Elemental Anal. Calcd for $C_{55}H_{49}N_9O_6$: C, 70.88; H, 5.30; N, 13.53%. Found: C, 70.76; H, 5.23; N,13.48%.

### *6.2.3. Second generation dendritic azide 10:*

Colourless solid; Yield 64%; mp: 162 $^oC$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 24H); 4.43 (s, 4H), 4.59 (s, 2H), 5.1 (s, 8H); 5.39 (s, 4H); 5.60 (s, 8H); 6.42-6.65 (m, 12H); 6.71 (d, 16H, *J* = 8.4 Hz); 7.33 (s, 9H); 7.54 (d, 16H, *J* = 8.7 Hz); 7.61 (s, 6H), 7.74 (s, 2H ): $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 54.1, 54.4, 54.7, 55.4, 55.9, 62.1, 102.1, 107.8, 108.0, 114.4, 123.1, 124.6, 125.6, 128.1, 132.1, 132.5, 135.4, 136.9, 140.2, 142.1, 142.6, 144.0, 158.9, 159.4, 159,6, 159.7. (MALDI-TOF- MS): *m/z* = 2105.3 [$M^+$]. Elemental Anal. Calcd for $C_{123}H_{109}N_{23}O_{14}$: C, 70.17; H, 5.22; N, 13.97%. Found: C, 70.31; H, 5.13; N, 13.90%.

### *6.3 Synthesis of dendritic chloride 7, 9 and dendrimer 1, 2 and 3*

### *6.3.1. General Procedure for the Cu-catalyzed Huisgen click reaction: (B)*

A mixture of propargyl derivative (1.0 eq.), dendritic azide (2.1 eq.), sodium ascorbate (0.4 eq.) and $CuSO_4.5H_2O$ (0.2 eq.) was added in the mixture of water - *t*-butanol (1:1) solution (20 mL). The reaction was stirred for 12 h at room temperature. After completion of the reaction the solvent was evaporated under reduced pressure and the resulting residue was extracted with $CHCl_3$ (2x100 mL), and saturated brine solution (100 mL). The combined organic layer was dried with anhydrous sodium sulphate, concentrated to give the crude triazole. This was purified by column chromatography using $CHCl_3$: MeOH (9:1) as eluent to afford the desired product.

### *6.3.2. First generation dendritic chloride 7:*

Colourless solid; Yield 84%; mp 116 $^oC$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.83 (s, 12H); 4.42 (s, 2H), 5.12 (s, 4H); 5.58 (s, 4H); 6.57 (s, 3H); 6.97 (s, 8H); 7.36-7.68 (m, 16H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 46.0, 54.4, 55.4, 62.1, 101.8, 107.9, 113.8, 114.3, 122.8, 124.7, 125.6, 128.2, 132.7, 135.3, 142.4, 144.2, 159.5. (FAB-MS): *m/z* = 925.5 [$M^+$]. Elemental Anal. Calcd for $C_{55}H_{49}ClN_6O_6$: C, 70.88; H, 5.30; N, 13.53%. Found: C, 70.76; H, 5.23; N, 13.48%.

### *6.3.3. Second generation dendritic chloride 9:*

Colourless solid; Yield 78%; mp 132-134 $^oC$; $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 24H); 4.45 (s, 4H); 4.61 (s, 2H); 5.09 (s, 8H); 5.36 (s, 4H); 5.59 (s, 8H); 6.45-6.60 (m, 12H); 6.96 (d, 16H, *J* = 8.4 Hz); 7.36 (s, 9H); 7.49 (d, 16H, *J* = 8.7 Hz); 7.60 (s, 4H); 7.68 (s, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 46.0, 54.0, 54.3, 55.9, 56.0, 62.0, 102.0, 107.5, 108.1, 114.3, 123.1, 124.7, 125.6, 128.2, 132.7, 135.4, 135.6, 136.7, 139.6, 142.3, 144.0, 158.8, 159.5, 159.6, 159.8. (MALDI-TOF- MS): *m/z* = 2098.8 [$M^+$]. Elemental Anal. Calcd for $C_{123}H_{109}ClN_{18}O_{14}$: C, 70.39; H, 5.23; N, 12.01%. Found: C, 70.30; H, 5.11; N, 12.10%.

### *6.3.4. Dendrimer 1:*

Colourless solid; Yield 82%; mp 128 $^oC$; $[\alpha]^{25}D$ -47.3 (*c* 0.01, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.74 (s, 12H); 4.88 (d, 2H, *J* = 12.3 Hz); 4.91 (d, 2H, *J* = 12.6 Hz); 5.15 (s, 4H); 6.33 (s, 2H); 6.87 (d, 8H, *J* = 7.5 Hz); 6.96 (s, 4H); 7.06 (s, 2H); 7.10 (s, 4H); 7.21(d, 2H, *J* = 9.0 Hz); 7.40 (d, 8H, *J* = 7.5 Hz); 7.54 (s, 2H); 7.58 (d, 4H, *J* = 9.3 Hz). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 53.4, 55.4, 63.9, 65.9, 114.4, 115.7, 119.1, 120.5, 122.4, 123.8, 124.7, 125.3, 125.4, 126.3, 127.9, 128.3, 129.4, 132.8, 133.8, 135.4, 142.2, 145.2, 153.5,159.6. (ESI-MS): *m/z* 1053 [$M^+$]. Elemental Anal. Calcd for $C_{68}H_{56}N_6O_6$: C, 77.55; H, 5.36; N, 7.98%. Found: C, 77.46; H, 5.28; N, 7.85%.

### *6.3.5. Dendrimer 2:*

Colourless solid; Yield 71%; mp 144-146 $^oC$; $[\alpha]^{25}$ D -29.7 (*c* 0.01, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 24H); 4.94 (t, 4H, *J* = 12 Hz); 5.00 (s, 8H); 5.10 (s, 4H); 5.53 (s, 8H); 6.20 (s, 4H); 6.53 (s, 2H); 6.91 (d, 16H, *J* = 8.4 Hz); 6.95 (d, 4H, *J* = 8.7 Hz); 6.98 (s, 2H); 7.02 (s, 2H); 7.05 (d, 2H, *J* = 6.9 Hz); 7.26 (s, 2H); 7.35 (s, 4H); 7.46 (d, 16H, *J* = 8.4 Hz); 7.49 (d, 4H, *J* = 8.4 Hz); 7.55 (s, 4H); 7.64 (s, 2H); 7.71 (s, 2H); 7.76 (d, 2H, *J* = 9.0 Hz). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 53.5, 54.3, 55.3, 61.9, 63.8, 101.6, 107.3, 114.3, 115.8, 120.5, 122.5, 123.2, 123.9, 124.8, 125.3, 125.5, 126.4, 127.9, 128.3, 129.4, 132.7, 133.8, 135.5, 136.9, 142.2, 142.3, 143.9, 144.5, 153.5,159.5, 159.7. (MALDI-TOF- MS): *m/z* 2226.3 [$M^+$]. Elemental Anal. Calcd for $C_{136}H_{116}N_{18}O_{14}$: C, 73.36; H, 5.25; N, 11.32 %. Found: C, 73.48; H, 5.16; N, 11.21 %.

### *6.3.6. Dendrimer 3:*

Colourless solid; Yield 56%; mp 184 $^oC$; $[\alpha]^{25}$ D -18.5 (*c* 0.01, $CHCl_3$); $^1H$ NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 48H); 4.90 (t, 4H, *J* = 12 Hz), 5.03 (s, 4H); 5.37 (s, 16H); 5.48 (s, 8H); 5.61 (s, 16H); 5.67 (s, 8H); 6.41 (s, 8H); 6.63 (s, 4H); 6.65 (s, 2H); 6.91 (d, 32H, *J* = 8.4 Hz); 7.08-7.27 (m, 22H); 7.41 (d, 32H, *J* = 8.7 Hz); 7.48 (s, 16H); 7.63 (s, 8H); 7.68 (s, 4H); 7.73 (s, 2H); 7.74 (s, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): δ 53.4, 54.2, 54.4,

55.3, 55.9, 61.8, 65.4, 101.8, 107.4, 108.6, 114.7, 115.8, 120.5, 122.6, 123.5, 123.9, 124.7, 125.1, 125.6, 126.2, 127.9, 128.4, 129.4, 130.8, 132.1, 133.6, 135.2, 136.8, 138.1, 142.6, 143.7, 144.3, 153.5, 158.4, 159.1, 159.7, 159.9. (MALDI-TOF- MS): *m/z* 4595.6 [M+$Na^+$]. Elemental Anal. Calcd for $C_{272}H_{236}N_{42}O_{30}$: C, 71.44; H, 5.20; N, 12.86 %. Found: C, 71.57; H, 5.11; N, 12.98%.

### *6.4. Biological activity*

### *6.4.1. Minimum Inhibitory Concentration:*

The minimum inhibitory concentration of the dendrimer **1, 2** and **3** was done by Resazurin reduction assay (Sarker[26] et *al.,* 2007).

### *6.4.2. Bacterial culture:*

The bacterial pathogens *Staphylococcus aureus*, *Shigella dysenteriae*, *Serratia marcescens* were obtained from CAS in Botany, University of Madras.

### *6.4.3. Preparation of Resazurin solution:*

The Resazurin solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water. A vortex mixer was used to ensure the homogenous of the solution.

### *6.4.4. Preparation of the plates:*

The Resazurin reduction assay was done according to S. D. Sarker[26] *et al.,* method, 2007. Plates were prepared under aseptic conditions. A volume of 200µL of test material in 10% (v/v) DMSO was pipetted into the first row of the sterile 96 wells plate. To all other wells 100µL of nutrient broth was added. Serial dilutions were performed using a pipette. Tips were discarded after use such that each well had 100µL of the test material in serially descending concentrations. To each well 10µL of resazurin indicator solution was added. Finally, 10µL of bacterial suspension ($5 \times 10^6$ cfu/mL) was added to each well to achieve a concentration of $5 \times 10^5$ cfu/mL. The broad-spectrum antibiotic Streptomycin in serial dilution was used as positive control. The plates were placed in an incubator set at 37 °C for 18–24 h. The colour change was then assessed visually. Any colour changes from blue to pink or colourless were recorded as reduction of dye by the viable bacteria. The lowest concentration at which no colour change occurred was taken as the MIC value.

### *6.4.5. Molecular docking:*

The auto dock software version 4 was used to study the molecular binding of the dendrimer to the bacterial enzyme DNA gyrase from *Staphylococcus aureus* (PDB: 3G7B).[27]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