Synthesis and Bactericidal Efficacy of Novel Dendrimers

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Abstract: Synthesis of dendrimers with enones as the core unit and *m*-terphenyl as surface end group has been achieved. All the enones and the dendrimers were antibacterial against *Salmonella typhi*, *Salmonella paratyphi* and *Staphylococcus aureus.*

Key words: dendrimers, enone, *m*-terphenyl, antibacterial, *salmonella typhi*

The synthesis and applications of dendrimers have been the focal point of investigation during recent times.[1] Various synthetic approaches leading to varied functionality in the dendrimer are well documented.[2–5] Syntheses of dendrimers may be divergent[6] or convergent[7] approaches. Syntheses of photochromic,[8] photoluminescent,[9] fluorescence sensing,[10] photoresponsive and water-soluble dendrimers[11] have been reported recently. The presence of the *m*-terphenyl surface group would greatly assist the generation of hyperbranched dendritic structures[12] and permanent fluorescence sensing dendritic architectures.[13] Dendrimers have found utility as carrier molecules for drug delivery and as homogeneous catalysts with interesting biological applications.[14] One of the most attractive applications in the biomedical field is the use of functionalized dendrimers as antibacterial and antiviral agents.

Fuctionalized dendrimers are able to form stable complexes with bacterial or viral structure receptors at the cell surface, resulting in disruption of the bacterial cell interaction during the infection process. Enones and their related compounds are used extensively in biological systems especially against microbes. Another antimicrobial activity of enones has been established and proved.[15] Dendrimeric peptides selective for microbial surfaces have been developed to achieve both broad antimicrobial activity and hemolytic activity against human erythrocytes. The tetra- and octavalent R4 and R8 dendrimers exhibit relatively similar potency against bacteria and fungi.[16] A series of dendrimer quaternary ammonium compounds (QAC) exhibit antimicrobial activity against bacteria.[17a,b] More generally, compounds and materials that possess biocidal activity have commercial applicability where prevention of biofilms is desired, for example in the health care sector.

Bourne et al.[18] have studied antiviral activity of dendrimers against Herpes Simplex Virus (HSV). To the best of our knowledge, the work reported herein provides the only example of enone core based dendrimers using *m*-terphenyl as the surface end group as antibacterial agents. We report herein the synthesis and bactericidal efficacy of enone core based dendrimers **1a**, **1b**, **1c**, **2a**, **2b** and **2c** against human pathogenic bacteria.

5′-Methyl-1,1′:3′,1″-terphenyl (**3**) was prepared by the application of the Hart reaction[19] from 3,5-dibromo 4-iodotoluene. Radical bromination of **3** with NBS in CCl_4 in the presence of Bz_2O_2 afforded 5′-bromomethyl-

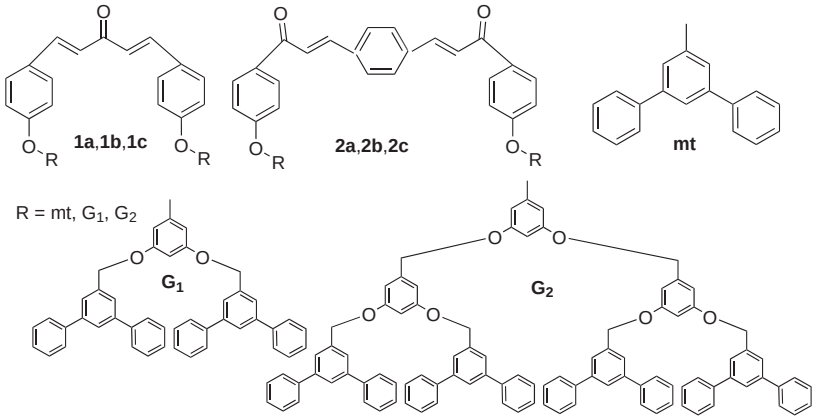

Figure 1

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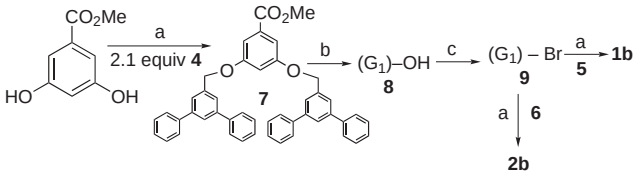
1,1′:3′,1″-terphenyl (**4**) in 84% yield.[20] Reaction of the dienone **5**[21] obtained from *p*-hydroxybenzaldehyde and acetone with 2.1 equivalents of 5′-bromomethyl-1,1′:3′,1″-terphenyl in the presence of K_2CO_3 in DMF gave the dendrimer **1a**[22,23] in 44% yield. Similarly, reaction of 2.1 equivalents of the 5′-bromomethyl-1,1′:3′,1″-terphenyl with 1 equivalent of the diketone **6**[24] derived from terephthaldehyde and *p*-hydroxyacetophenone gave **2a**[25] in 45% yield (Scheme 1).

In the ^{1}H NMR spectrum dendrimer **1a** displayed a sharp singlet at δ = 5.15 ppm for the methylene protons and the olefinic protons appeared as two doublets at δ = 6.88 ppm and δ = 6.98 ppm for four protons and two more doublets were observed at δ = 7.38 ppm and δ = 7.57 ppm along with other aromatic protons. In the ^{13}C NMR spectrum the methylene and carbonyl carbon appeared at δ = 70.2 ppm and δ = 188.8 ppm in addition to 14 aromatic carbons. The appearance of a molecular ion peak at 757 in the FAB mass spectrum also confirmed the structure of **1a**.

Dendrimer **2a** in the ^{1}H NMR spectrum displayed a sharp singlet at δ = 5.20 ppm and the olefinic proton appeared as a doublet at δ = 7.04 ppm and δ = 7.99 ppm for four protons along with aromatic protons. In the ^{13}C NMR spectrum the methylene and carbonyl carbon appeared at δ = 70.3 ppm and δ = 188.4 ppm in addition to the aromatic carbons. The proposed structure **2a** was further confirmed by the appearance of molecular ion peak at *m/z* = 855 in the FAB mass spectrum.

In order to synthesize the first generation dendrimer **1b**, methyl 3,5-dihydroxy benzoate was reacted with the 5′-bromomethyl-1,1′:3′,1″-terphenyl (**4**) and the resulting methyl ester **7** was reduced with $LiAlH_4$ in THF[26] to give dendritic alcohol **8** (G_1)-OH. Which, on further reaction with CBr_4/PPh_3 in THF,[26] afforded the dendritic bromide **9** (G_1)-Br in 53% yield. Reaction of 2.1 equivalents of the dendritic bromide **9** (G_1)-Br with one equivalent of dienone **5**/**6** in the presence of K_2CO_3 in DMF gave dendrimer **1b** and **2b**[27] in 51% and 48% yield (Scheme 2).

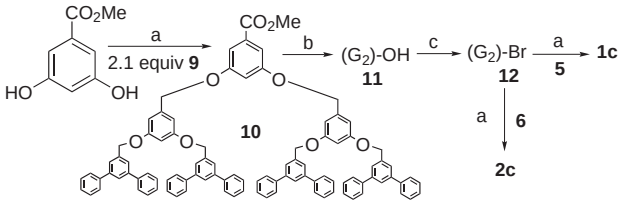
The ^{1}H NMR spectrum of dendrimer **1b** displayed four and eight proton singlets at δ = 4.90 ppm and δ = 5.01 ppm. The olefinic protons appeared at δ = 6.50 ppm and δ = 6.57 ppm as two doublets in addition to the aromatic protons. In the ^{13}C NMR spectrum the methylene and carbonyl carbons appeared at δ = 70.0, 70.3 and 188.1 ppm in addition to the aromatic carbons. The structure of the



Scheme 2 *Reagents and conditions*: a) K_2CO_3, DMF, 60 °C, 48 h; b) LAH, THF, reflux, 12 h; c) CBr_4, PPh_3, THF, r.t.

dendrimer **1b** was further supported by the appearance of a molecular ion at *m/z* = 1479 in the FAB mass spectrum. Similarly, dendrimer **2b** was characterized based on spectral and analytical data.

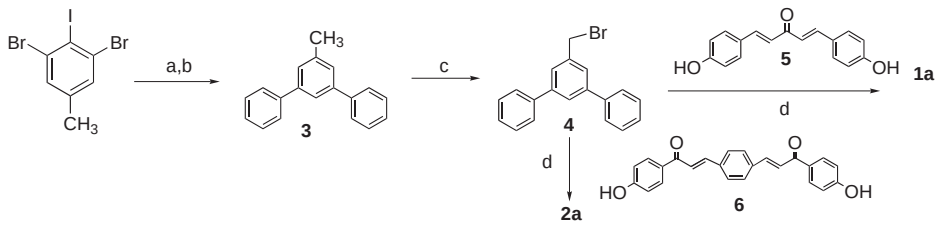
With a view to synthesize the next generation dendrimers **1c** and **2c**, methyl 3,5-dihydroxybenzoate was reacted with 2.1 equivalents of dendritic bromide **9** (G_1)-Br to give the methyl carboxylate **10** (G_2)-CO_2Me. The methyl ester **10** was reduced to give the corresponding alcohol **11** (G_2)-OH from which the dendritic bromide **12** (G_2)-Br was obtained as described earlier. Reaction of 2.1 equivalents of dendritic bromide **12** (G_2)-Br with one equivalent of **5**/**6** gave the dendrimers **1c**/**2c** in 58% and 51% yield, respectively (Scheme 3).



Scheme 3 *Reagents and conditions*: a) K_2CO_3, DMF, 60 °C, 48 h; b) LAH, THF, reflux, 12 h; c) CBr_4, PPh_3, THF, r.t.

Dendrimers **1c** and **2c** were characterized from spectral and analytical data.[28]

Bactericidal efficacy: The bactericidal efficacy of the parent **5**, **6**, dendrimer **1a**, **1b**, **1c**, **2a**, **2b** and **2c** was assayed against *Salmonella typhi*, *Salmonella paratyphi* and *Staphylococcus aureus* by disc diffusion method.[29] Parents **5** and **6** exhibited significantly activity towards *Salmonella typhi* and *Salmonella paratyphi* than *Staphylococcus aureus*. Dendrimers **1a**, **1b**, **2a** and **2b** showed good inhibition against the test bacteria when



Scheme 1 *Reagents and conditions*: a) 3.1 equiv PhMgBr, THF, reflux, 12 h; b) H_3O^+; c) NBS, Bz_2O_2, CCl_4, reflux; d) K_2CO_3, DMF, 60 °C, 48 h.

compared with **5** and **6**. Dendrimers **1c** and **2c** possessed more inhibitory activity than all other dendrimers and parent compounds. The standard antibiotic disc (*Streptomycin* 10 μg/disc) inhibited the growth of *Salmonella typhi* by 20 mm, *Salmonella paratyphi* by 21 mm and *Staphylococcus aureus* by 15 mm, respectively. The diameter of inhibition zone for each concentration against all the test bacteria is depicted in Table 1, Table 2, and Table 3.

Table 1 Effect of Enones and Dendrimers on the Growth of *Samonella typhi*

Inhibition zone (mm)			
Compounds	20 μg/mL	40 μg/mL	60 μg/mL
Parent **5**	4.2	7.2	9.8
Dendrimer **1a**	5.8	10.2	13.2
Dendrimer **1b**	7.2	11.0	14.4
Dendrimer **1c**	9.8	12.8	16.8
Parent **6**	3.6	5.8	8.2
Dendrimer **2a**	4.8	9.6	10.8
Dendrimer **2b**	6.8	10.4	12.2
Dendrimer **2c**	8.2	11.8	13.2

Table 2 Effect of Enones and Dendrimers on the Growth of *Samonella paratyphi*

Inhibition zone (mm)			
Compounds	20 μg/mL	40 μg/mL	60 μg/mL
Parent **5**	4.6	7.6	9.6
Dendrimer **1a**	6.2	10.6	13.6
Dendrimer **1b**	7.4	11.2	14.8
Dendrimer **1c**	10.2	13.0	17.2
Parent **6**	3.8	6.2	8.6
Dendrimer **2a**	5.2	10.4	12.8
Dendrimer **2b**	7.2	10.8	12.8
Dendrimer **2c**	8.8	12.0	14.6

All dendrimers other than parent compounds in the present study significantly inhibited the growth of *Salmonella typhi*, *Salmonella paratyphi* and *Staphylococcus aureus*. These dendrimers could be potential compounds for the eradication of typhoid caused by these pathogens.

Table 3 Effect of Enones and Dendrimers on the Growth of *Staphylococcus aureus*

Inhibition zone (mm)			
Compounds	20 μg/mL	40 μg/mL	60 μg/mL
Parent **5**	3.6	6.6	9.2
Dendrimer **1a**	5.4	9.6	12.6
Dendrimer **1b**	6.6	10.2	13.2
Dendrimer **1c**	8.8	11.8	15.8
Parent **6**	3.2	5.4	8.0
Dendrimer **2a**	4.4	9.2	10.2
Dendrimer **2b**	6.2	10.2	11.6
Dendrimer **2c**	8.0	10.8	12.6

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(22) **General Procedure for the Preparation of Dendrimer.** A mixture of dienone **6**/diketone **7** (1 mmol) and dendritic bromide **4**/**9**/**12** (2.1 mmol) was stirred with K_2CO_3 (5 mmol) in DMF (20 mL) at 60 °C for 48 h. The reaction mixture was poured into H_2O and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were washed with brine (80 mL), water (2 × 100 mL) and dried over $MgSO_4$. Evaporation of the organic layer gave a residue, which was purified by chromatography over SiO_2 gel using $CHCl_3$ as eluent to give the corresponding dendrimer.

(23) **Dendrimer 1a**: yield 44%; $CHCl_3$; mp 88–92 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.14 (s, 4 H), 6.86–6.98 (d, 4 H), 7.17–7.70 (m, 34 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 70.1, 115.3, 123.6, 125.2, 126.0, 127.7, 127.6, 128.0, 128.8, 130.1, 137.5, 140.7, 142.3, 142.6, 160.7, 188.8. MS (FAB, 70 eV): *m/z* = 750 [M$^+$]. Anal. Calcd for $C_{55}H_{42}O_3$: C, 88.00; H, 5.60. Found: C, 87.98; H, 5.56.

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(25) **Dendrimer 2a**: yield 45%; $CHCl_3$; mp 174–176 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.20 (s, 4 H), 7.04–7.05 (d, 4 H), 7.17–8.00 (m, 38 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 70.2, 76.7, 114.8, 122.7, 125.2, 126.1, 127.2, 127.6, 128.8, 129.2, 130.9, 131.2, 137.2, 140.7, 142.3, 142.8, 188.4. MS (FAB, 70 eV): *m/z* = 854 [M$^+$]. Anal. Calcd for $C_{62}H_{46}O_4$: C, 87.11; H, 5.38. Found: C, 87.05; H, 5.32.

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(27) **Dendrimer 1b**: yield 51%; $CHCl_3$; mp 114–116 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.90 (s, 4 H), 5.00 (s, 8 H), 6.49 (d, 2 H), 6.57 (d, 2 H), 7.18–7.59 (m, 66 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 69.9, 70.2, 101.8, 106.4, 115.3, 125.3, 125.9, 127.3, 127.6, 127.9, 128.8, 130.0, 137.7, 139.1, 140.8, 142.2, 142.6, 143.6, 160.2, 160.5,188.8. MS (FAB, 70 eV): *m/z* = 1478 [M$^+$]. Anal. Calcd for $C_{107}H_{82}O_7$: C, 86.87; H, 5.54. Found: C, 88.76; H, 5.46.
Dendrimer 2b: yield 48%; $CHCl_3$; mp 106–108 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.19 (s, 4 H), 5.22 (s, 8 H), 6.73–6.77 (d, 4 H), 7.39–7.79 (m, 70 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 65.4, 70.1, 70.2, 70.3, 101.4, 101.9, 105.9, 106.5, 114.9, 125.4, 126.0, 127.4, 127.7, 128.9,130.9, 137.8, 140.9, 142.3, 160.3, 160.4, 162.6, 188.4. MS (FAB, 70 eV): *m/z* = 1582 [M$^+$]. Anal. Calcd for $C_{114}H_{86}O_8$: C, 86.47; H, 5.43. Found: C, 86.40; H, 5.36.

(28) **Dendrimer 1c**: yield 58%; $CHCl_3$; yellow colored liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.98 (s, 4 H), 5.01 (s, 8 H), 5.13 (s, 16 H), 6.70 (d, 2 H), 6.78 (d, 2 H), 7.18–7.81 (m, 130 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 68.1, 70.1, 101.2, 101.8, 106.6, 115.3, 125.4, 127.4, 127.7, 128.9, 137.9, 139.4, 140.9, 142.3, 160.2, 160.3, 160.6, 188.6. MS (MALDI-TOF): *m/z* = 2934 [M$^+$]. Anal. Calcd for $C_{211}H_{162}O_{15}$: C, 86.29; H, 5.52. Found: C, 86.20; H, 5.42.
Dendrimer 2c: yield 51%; $CHCl_3$; yellow colored liquid. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.00 (s, 4 H), 5.02 (s, 8 H), 5.14 (s, 16 H), 6.67–6.70 (dd, 2 H), 6.75 (d, 2 H), 7.34–7.64 (m, 134 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 70.1, 70.2, 101.7, 106.5, 114.8, 125.4, 126.0, 127.4, 127.6, 128.9, 137.8, 139.4, 140.9, 142.2, 160.2, 160.3, 188.4. MS (MALDI-TOF): *m/z* = 3038 [M$^+$]. Anal. Calcd for $C_{218}H_{166}O_{16}$: C, 86.10; H, 5.46. Found: C, 86.06; H, 5.40.

(29) Antibacterial activity: All the test bacteria were subcultured in nutrient broth from which 1 mL of cell suspension was taken and was adjusted to 0.5 OD. This was spread as a thin film over the nutrient agar plates. Concentrations of 60, 40 and 20 μg/mL of the compound were loaded into a disc, which was placed over the inoculated plates. All the plates were incubated for 48 h at 37 °C and growth was measured. A streptomycin disc from Hi-media (10 μg/disc) was used as the standard.