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Original article

# Synthesis, characterization and antimicrobial activity of water soluble dendritic macromolecules

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**Abstract**

Several families of water soluble dendrimers were synthesized based on poly(propyleneoxide) amines (Jeffamines®) (**$P_1$**). **$P_1$**-core and branched units were constructed from both methylacrylate and ethylenediamine (**$P_2$**–**$P_9$**, and generations 0–3 with $-NH_2$, $-COOH$ functionalities). They were characterized by elemental analysis (EA), gel permeation chromatography (GPC), FT-IR, $^1H$, and $^{13}C$ NMR. The antimicrobial activities of only water soluble compounds (**$P_1$**, **$P_3$**, **$P_4$**, **$P_6$**, **$P_7$** and **$P_9$**) were evaluated using disk diffusion method in water as well as the minimal inhibitory concentration (MIC) dilution method against 9 bacteria. The obtained results from disk diffusion method are assessed in side-by-side comparison with those of Penicillin-g, Ampicillin, Cefotaxime, Vancomycin, Oflaxacin, and Tetracyclin, well-known antibacterial agents. The results from dilution procedure are compared with Gentamycin as antibacterial and Nystatin as antifungal. The antifungal activities are reported on five yeast cultures namely, *Candida albicans*, *Kluyveromyces fragilis*, *Rhodotorula rubra*, *Debaryomyces hansenii*, and *Hanseniaspora guilliermondii*, and the results are referenced with Nystatin, Ketaconazole, and Clotrimazole, commercial antifungal agents. In most cases, the compounds show broad-spectrum (Gram-positive and Gram-negative bacteria) activities that are comparatively higher or equipotent to the antibiotic and antifungal agents in the comparison tests.
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*Keywords:* Antibacterial; Dendritic Jeffamines; Michael addition; Nystatin; PAMAM

## 1. Introduction

Dendrimers are relatively new class of macromolecules which have a regular branching structure. They consist of a central core and several generations of three-dimensional branches which result in a large number of functionalized end groups at the surface. The main advantage of these compounds compared to other conventional and natural polymers is the tremendous tolerance of their synthetic routes. They allow the precise control of size, shape and placement of functional groups and combine typical characteristics of small organic molecules and or polymers that result in special physical and chemical properties [1–3]. Dendrimers with multiple identical ligands are very attractive for pharmacochemists, since these structures can exhibit amplified substrate binding [3]. Their surfaces, however, can also be given a significant repertoire of tunable characteristics not found on natural or biological polymers such as nucleic acids and proteins [4]. Therefore, these features have greatly propelled efforts toward the development of practical applications for such molecules. For instance, some dendritic peptides as antimicrobial agents are reported using basic amino acids (lysine, arginine) and also amino acids containing aromatic residues such as tyrosine and phenylalanine [5]. Many dendrimers have also been reported and several of them are subjected as preclinical trial as useful additives in drug formulations for increasing the solubility, stability, bioavailability, cellular uptake, targeting ability and patient compliance of the administrated drugs, and for decreasing the drug resistance and irritation [6].

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PAMAM type dendrimers are one of the most studied dendritic polymer families today. They possess perfect solubility in a large number of solvents, particularly in water. Non-polar cavities in PAMAM dendrimers in combination with their hydrophilic exterior surface make them capable of encapsulating hydrophobic drug molecules and ensure their applications as solubility enhancers of these hydrophobic agents [6,7]. These non-covalent inclusions offer a variety of physicochemical advantages over the free drug molecules including the possibility of enhanced water solubility and drug stability [8]. Moreover, large numbers of functional groups such as amine, carboxyl and hydroxyl groups on the outer shell of PAMAM dendrimers are responsible for high reactivity and expected to conjugate with a series of biomolecules such as DNA and proteins or bioactive molecules such as drugs. These guest molecules can be loaded either in the functional groups on the surface or can be attached to the hydrophobic cavities. These specific features of dendrimers provide the availability of dendrimers to deliver bioactive agents to specific diseased sites, consequently enhancing bioactivity properties and possibility of minimizing drug systemic toxicity [6,9,10]. These features make dendrimers possible future reliable alternatives to traditional polymers as novel biocompatible drug enhancers and carriers. The additional characteristics have given impetus to their widespread use in medicinal chemistry, including diagnostic reagents, protein mimetics, anti-cancer and anti-viral agents, vaccines, drug and gene delivery systems as well as curing agents [11–20].

The unique architecture of dendrimers which offers a high local concentration of a given functionality, cooperative effects, polyvalent effects, and sometimes polycationic structures can be utilized to design both effective antimicrobial agents and efficient biocide delivery systems [21].

In the present report we focus upon the synthesis, physicochemical characterization and pharmacological investigation of the water soluble dendritic polychelatogens starting from $\mathbf{P_1}$ as an initial core which utilizes a combination of amide connectivity affording no internal hydrolytic cleavage. In order to functionalize the $\mathbf{P_1}$-core, we have followed the literature procedures [22–24].

## 2. Experimental

### 2.1. Chemistry

All chemicals and solvents were reagent grade and used without further purification. Purity of the compounds was tested on thin layer chromatography plates (silica gel 60 $F_{254}$ Merck). Elemental analyses were carried out by Perkin–Elmer Model 2400 Series II. FT-IR spectra were obtained as KBr discs on Mattson Satellite spectrophotometer in the range of 4000–400 $cm^{-1}$. Routine $^1H$ and $^{13}C$ NMR spectra are recorded at ambient temperature on a 500 MHz Inova-Varian NMR Spectrometer in $CDCl_3$. Chemical shifts ($\delta$) are expressed in units of ppm relative to TMS. Gel permeation chromatography (GPC) analyses were performed with a set-up consisting of a pump (Waters) and four ultrastyragel columns of different porosities. Tetrahydrofuran was used as the eluent (flow rate 3 mL $min^{-1}$) and the detection was carried out with the aid of a differential refractometer. The average molecular weights were determined using polystyrene standards.

The compounds are prepared by slight modification of literature procedures [22–24]. Full general procedures for preparation of these compounds are given in the experimental and the reaction sequence is shown in Scheme 1. The physical and spectroscopic data from FT-IR, $^{13}C$, $^1H$ NMR and GPC do provide useful information for their formation and structural characterization. These data and their prominent band assignments are reported in Tables 1 and 2 and Figs. 1 and 2. Table 2 contains the molecular weight distribution of the first generation dendrimers ($\mathbf{P_1}$, $\mathbf{P_2}$, $\mathbf{P_3}$ and $\mathbf{P_4}$). These values were obtained from GPC versus polystyrene (PS) standard. Required analytical data and physical properties are summarized in the end of each experimental.

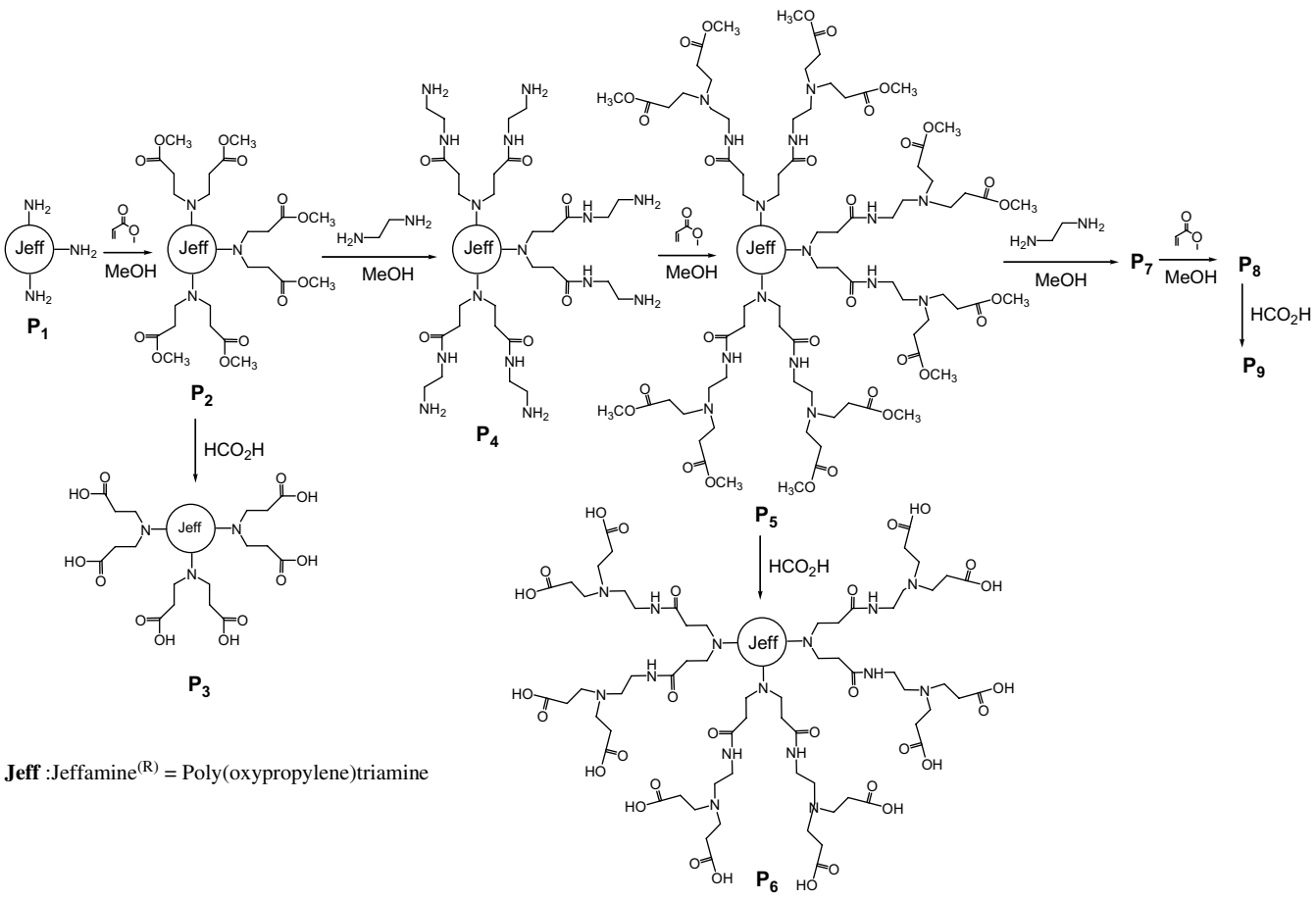
#### 2.1.1. Synthesis

***$P_2$–$P_9$ dendrimers***. The synthesis of dendritic polychelatogens ($\mathbf{P_2}$–$\mathbf{P_9}$) is outlined in Scheme 1. Esteric dendrimers ($\mathbf{P_2}$, $\mathbf{P_5}$, $\mathbf{P_8}$) were synthesized under mild condition modified from the literature [22–24]. Following purification they were hydrolyzed in the presence of formic acid to generate the yellowish dendritic carboxylic acids ($\mathbf{P_3}$, $\mathbf{P_6}$, $\mathbf{P_9}$). The amine functionalized dendrimers ($\mathbf{P_4}$, $\mathbf{P_7}$) were synthesized from the $\mathbf{P_2}$, $\mathbf{P_6}$ by using 10-fold excess amount of ethylenediamine under mild conditions.

*2.1.1.1. Amine esterification ($P_2$).* Methanolic solution (20 mL) of methylacrylate (20 g, 230 mmol) and a methanolic solution (100 mL) of $\mathbf{P_1}$ (100 g, 33 mmol) were mixed slowly with constant stirring under nitrogen for 48 h at ambient temperature. After this period of time the solution mixture was heated at 50 °C for further 1 h. The excess methylacrylate and the solvent were removed under reduced pressure to dryness. The residue was purified by dialysis using membrane filter (3 kDa) in methanol–water (1:1), resulting in a yellowish oily product (yield 100%). Elemental analyses found (calculated): C, 59.42 (62.18); H, 9.65 (10.23); N, 1.15 (1.20) [$C_{182}H_{357}N_3O_{58}$].

*2.1.1.2. Ester hydrolysis ($P_3$).* A solution of $\mathbf{P_2}$ (20 g, 5.70 mmol) in formic acid (30 mL) was stirred for 12 h. After removing the excess formic acid and hydrolyzed esteric groups under reduced pressure the oily ($\mathbf{P_3}$) product was obtained (yield 100%). Elemental analyses found (calculated) C, 58.72 (61.60); H, 8.65 (10.13); N, 1.17 (1.22) [$C_{176}H_{345}N_3O_{58}$].

*2.1.1.3. Ester aminolysis ($P_4$).* Methanolic solution (100 mL) of hexaester ($\mathbf{P_2}$) (10 g, 2.85 mmol) was added dropwise to a stirred methanolic solution (5 mL) of ethylenediamine (2.0 g, 33.33 mmol). The resulting solution was stirred at room temperature for 7 days. The excess ethylenediamine and solvent were removed under vacuum. Final traces of ethylenediamine was removed by dissolving the residue in 50 mL of *n*-butanol (a competitive hydrogen bonding solvent), the butanol was then removed under vacuum. The crude product was




Scheme 1. Synthesis of dendritic (**P2**–**P9**) macromolecules.

dialyzed (MWCO of 3.0 kDa) in water. After evaporation of water the oily **P4** was obtained (9.20 g, 83%). Elemental analyses found (calculated) C, 59.32 (61.29); H, 9.67 (10.42); N, 5.66 (5.70) [$C_{188}H_{381}N_{15}O_{52}$].

*2.1.1.4. Amine esterification (**P5**).* Methylacrylate (1.0 g. 11.62 mmol) was added to a solution of **P4** (1.5 g, 0.4 mmol) in methanol (30 mL). The solution mixture was stirred at room temperature for 5 days. The solution was then heated at 50 °C for further 24 h. Excess reagents and solvent were removed under vacuum. The product was dialyzed (MWCO of .5 kDa) in methanol (1.62 g. 85%). Elemental analyses found (calculated): C, 57.23 (60.09); H, 9.06 (9.68); N, 4.40 (4.45) [$C_{236}H_{453}N_{15}O_{76}$].

*2.1.1.5. Ester hydrolysis (**P6**).* **P6** was synthesized in a similar manner to dendrimer **P3**. A solution of **P5** (1 g. 0.21 mmol) in formic acid (5 ml) was stirred for 12 h. An oily product was obtained (yield 100%). Elemental analysis found (calculated): C, 56.14 (59.14); H, 8.88 (9.51); N, 4.98 (5.04) [$C_{224}H_{429}N_{15}O_{76}$].

*2.1.1.6. Ester aminolysis (**P7**).* **P7** was synthesized in a similar manner to dendrimer **P4** using 20 times excess amount of ethylenediamine to obtain an oily product, which was dialyzed in water (MWCO of 5 kDa), (0.71 g, 65%). Elemental analyses found (calculated): C, 57.62 (59.01); H, 9.12 (10.00); N, 10.71 (10.78), [$C_{249}H_{503}N_{39}O_{64}$].

*2.1.1.7. Amine esterification (**P8**).* **P8** was synthesized in a similar manner to dendrimer **P2** or **P5** using 30 times excess amount of methylacrylate to obtain an oily product, which was dialyzed in equal amount of water methanol mixture (MWCO of 5 kDa), (0.65 g, 80%). Elemental analyses found (calculated): C, 57.2 (58.08); H, 8.25 (9.14); N, 7.61 (7.66); [$C_{345}H_{647}N_{39}O_{112}$].

*2.1.1.8. Ester hydrolysis (**P9**).* **P9** was synthesized in a similar manner to dendrimer **P3** or **P6** obtaining an oily product, which was dialyzed in water (MWCO of 5 kDa), (yield 100%). Elemental analyses found (calculated): C, 55.20 (56.78); H, 8.41 (8.89); N, 7.93 (8.02), [$C_{322}H_{601}N_{39}O_{112}$].

### 2.2. Pharmacology

#### 2.2.1. Micro-organisms

The antimicrobial activities are evaluated against Gram-positive (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* La 2971) and Gram-negative (*Escherichia coli* ATCC 11230, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Enterobacter aerogenes* ATCC

Table 1
Prominent FT-IR, $^{1}H$ and $^{13}C$ band assignment for $\mathbf{P_1}$–$\mathbf{P_9}$ compounds

| Compound | IR ($cm^{-1}$) | $^{13}C$ NMR (ppm) | $^{1}H$ NMR (ppm) |
|---|---|---|---|
| $\mathbf{P_1}$ | 3296, 1110 | $CNH_2 = 46.17$ | $CNH_2 = 3.54$ (6H, br, s) |
| $\mathbf{P_2}$ | 1739 | $COOCH_3 = 173.06$ | $CH_2CH_2COOCH_3 = 2.39$ (12H, t) |
| | 1108 | $COOCH_3 = 51.35$ | $CH_2CH_2COOCH_3 = 2.75$ (12H, t) |
| | | $CNR_2 = 55.19$ | $COOCH_3 = 3.63$ (18H, s) |
| | | $CH_2CH_2COOCH_3 = 46.38$ | |
| | | $CH_2CH_2COOCH_3 = 34.46$ | |
| $\mathbf{P_3}$ | 3300–2650 | $COOH = 171.95$ | $CH_2CH_2COOH = 3.40$ (12H, t) |
| | 1671 | $CNR_2 = 56.90$ | $CH_2CH_2COOH = 3.55$ (12H, t) |
| | 1110 | $CH_2CH_2COOH = 46.59$ | $COOH = 8.05$ (6H, br, s) |
| | | $CH_2CH_2COOH = 31.28$ | |
| $\mathbf{P_4}$ | 3296 | $CONCH_2CH_2NH_2 = 173.02$ | $CNH_2 = 4.79$ (12H, br, s) |
| | 1653 | $CONCH_2CH_2NH_2 = 57.51$ | $CH_2CH_2NH_2 = 3.38$ (12H, m) |
| | 1551 | $CONCH_2CH_2NH_2 = 46.20$ | $CH_2CH_2NH_2 = 3.54$ (12H, m) |
| | 1108 | | $CONHR = 4.79$ (6H, br, s) |
| $\mathbf{P_5}$ | 3450 | $COOCH_3 = 172.91$ | $CH_2CH_2COOCH_3 = 2.40$ (24H, t) |
| | 1731 | $COOCH_3 = 75.25$ | $CH_2CH_2COOCH_3 = 2.72$ (24H, t) |
| | 1666 | $CH_2CH_2NR_2 = 51.49$ | $COOCH_3 = 3.63$ (36H, s) |
| | 1105 | $CH_2CH_2COOCH_3 = 49.69$ | |
| | | $CH_2CH_2COOCH_3 = 32.56$ | |
| $\mathbf{P_6}$ | 3300–2550 | $COOH = 172.06$ | $CH_2CH_2COOH = 2.63$ (24H, t) |
| | 1667 | $CNR_2 = 52.15$ | $CH_2CH_2COOH = 3.11$ (24H, t) |
| | 1105 | $CH_2CH_2COOH = 48.26$ | $COOH = 9.97$ (12H, br, s) |
| | | $CH_2CH_2COOH = 29.55$ | |
| $\mathbf{P_7}$ | 3421, 3349 | $CONCH_2CH_2NH_2 = 172.88$ | $CNH_2 = 5.10$–$4.60$ (24H, br, s) |
| | 3289, 1643 | $CONCH_2CH_2NH_2 = 55.67$ | $CH_2CH_2NH_2 = 3.39$ (24H, m) |
| | 1567, 1112 | $CONCH_2CH_2NH_2 = 46.29$ | $CH_2CH_2NH_2 = 3.55$ (24H, m) |
| | | | $CONHR = NO$ |
| $\mathbf{P_8}$ | 3309 | $COO\ CH_3 = 172.86$ | $CH_2CH_2COOCH_3 = 2.42$ (48H, t) |
| | 1737 | $COO\ CH_3 = 75.41$ | $CH_2CH_2COOCH_3 = 2.74$ (48H, t) |
| | 1650 | $CH_2CH_2NR_2 = 51.48$ | $COO\ CH_3 = 3.65$ (72H, s) |
| | 1544 | $CH_2CH_2COOCH_3 = 49.15$ | |
| | | $CH_2CH_2COOCH_3 = 32.63$ | |
| $\mathbf{P_9}$ | 3442 | $COOH = 172.67$ | $CH_2CH_2COOH = 2.50$ (48H, t) |
| | 1656 | $CNR_2 = 52.14$ | $CH_2CH_2COOH = 2.85$ (48H, t) |
| | 1590 | $CH_2CH_2COOH = 48.58$ | $COOH = 8.31$ (24H, br, s) |
| | 1101 | $CH_2CH_2COOH = 30.22$ | |

Br, broad; m, multiplet; s, singlet; t, triplet.

13048) bacteria and the yeast cultures *Candida albicans* ATCC 10231, *Kluyveromyces fragilis* NRRL 2415, *R. rubra* DSM 70403, *Debaryomyces hansenii* DSM 70238 and *Hanseniaspora guilliermondii* DSM 3432 using both disk diffusion method [25,26] and measuring the MIC determined by the broth dilution method [27].

### 2.2.2. Methods

*2.2.2.1. Disk diffusion method.* Screening for antibacterial and antifungal activities are carried out using sterilised antibiotic discs (6 mm), following the procedure performance standards for Antimicrobial Disk Susceptibility Tests, outlined by the National Committee for Clinical Laboratory Standards – NCCLS [25,26].

Fresh stock solutions (30 μg $mL^{-1}$) of the ligands are prepared in freshly deionised water according to the needed concentrations for experiments. Sterilised antibiotic discs having a diameter of 6 mm (Schleicher & Schull No. 2668, Germany) are impregnated with 20 μL of these solutions. All the bacteria are incubated and activated at 30 °C for 24 h inoculation into Nutrient Broth (Difco), and the yeasts are incubated in Malt Extract Broth (Difco) for 48 h. Inoculums containing $10^6$ bacterial cells or $10^8$ yeast cells per mL are spread on Mueller–Hinton Agar (Oxoid) plates (1 mL inoculum for each plate). The discs injected with solutions are placed on the inoculated agar by pressing slightly and incubated at 35 °C (24 h) and at 25 °C (72 h) for bacteria and yeast, respectively. On each plate an appropriate reference antibiotic disc is applied depending on the test micro-organisms. In each case triplicate tests are performed and the average is taken as final reading.

Table 2
The number average and weight average molecular weights ($M_w$, $M_n$) and polydispersity index, ($M_w/M_n$) of the dendrimer samples

| Sample | Expected $M_w$ | $M_n$ (g/mol) | $M_w$ (g/mol) | $M_w/M_n$ |
|---|---|---|---|---|
| **P1** | 3000 | 469 | 505 | 1.07 |
| **P2** | 3511 | 470 | 512 | 1.09 |
| **P3** | 3427 | 418 | 451 | 1.08 |
| **P4** | 3679 | 462 | 498 | 1.08 |

*2.2.2.2. Dilution method.* Screening for antibacterial and antifungal activities was carried out by preparing a broth

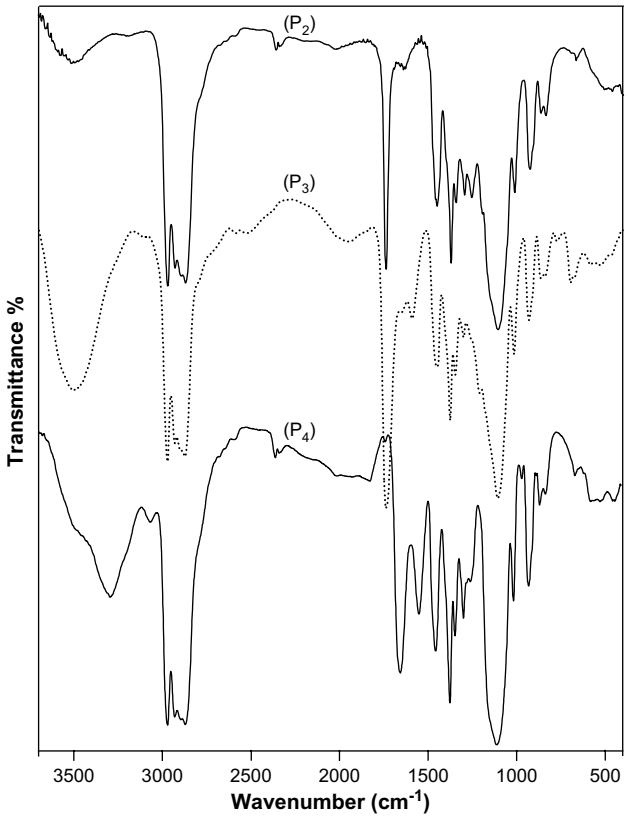



Fig. 1. IR spectrum of dendritic macromolecules (**P2**–**P4**) range in 3700–400 $cm^{-1}$ region.

micro-dilution, following the procedure outlined in Manual of Clinical Microbial [27]. All the bacteria were incubated and activated at 30 °C for 24 h inoculation into Nutrient Broth, and the yeasts were incubated in Malt Extract Broth for 48 h. The compounds were dissolved in water (2 mg $mL^{-1}$) and then diluted using caution adjusted Mueller–Hinton Broth (Oxoid). Two-fold serial concentrations of the compounds were employed to determine the (MIC) ranging from 200 to 1.56 μg $mL^{-1}$. Cultures were grown at 37 °C (20 h) and the final inoculation (inoculums) was approximately $10^6$ cfu $mL^{-1}$. Test cultures were incubated at 37 °C (24 h). The lowest concentrations of antimicrobial agents that result in complete inhibition of the micro-organisms were represented as MIC (μg $mL^{-1}$). In each case triplicate tests were performed and the results are expressed as means.

### 2.2.3. Biological data

Standardised samples of Penicillin-g (blocking the formation of bacterial cell walls, rendering bacteria unable to multiply and spread); Ampicillin (penetrating and preventing the growth of Gram-negative bacteria); Cefotaxime (used against most Gram-negative bacteria); Vancomycin (acting by interfering with the construction cell walls in bacteria), Ofloxacin (entering the bacterial cell and inhibiting DNA-gyrase, which is involved in the production of genetic material, preventing the bacteria from reproducing); Tetracyclines (exerting their antimicrobial effect the inhibition of protein synthesis); Nystatin (binding to sterols in the fungal cellular membrane altering the permeability to allow leakage of the cellular contents and destroying the fungus); Ketoconazole (inhibiting the growth of fungal organisms by interfering with the formation of the fungal cell wall) and Clotrimazole (interfering with their cell membranes and causing essential constituents of the fungal cells leakage). Mueller–Hinton media, Nutrient Broth and Malt Extract Broth are purchased from Difco and yeast extracts is obtained from Oxoid.

## 3. Results and discussion

### 3.1. Spectral deconvolution

There were three distinctive pathways for the formation of these compounds namely: (a) amine esterification, (b) ester hydrolysis and (c) ester aminolysis such as shown in Scheme 1. Amine esterification pathway (formation of ester functionalized **$P_2$**, **$P_5$** and **$P_8$** dendrimers) could easily be confirmed by IR and NMR spectroscopic data. The characteristic $\nu(NH_2)$ and $\delta(NH_2)$ modes of primary amines (**$P_1$**, **$P_4$** and **$P_7$**) were observed in the regions 3400–3300 and 1650–1550 $cm^{-1}$, respectively. The formation of esters (**$P_2$**, **$P_5$** and **$P_8$**) has been verified by the appearance of very characteristic (C=O) stretching vibrations in the 1740–1720 $cm^{-1}$ region. These results were supported with $^1H$ and $^{13}C$ spectral data. The $^1H$ NMR spectra pattern has changed significantly due to esterification of amines by appearance of several new signals and disappearance of broad unresolved amine protons. The $^{13}C$ NMR data also tend to support ester formation by appearance of several new peaks, particularly for carboxyl (C=O) chemical shifts at approximately 173 ppm, typical for ester compounds (Table 1).

Ester hydrolysis pathway (formation of carboxylic function dendrimers **$P_3$**, **$P_6$** and **$P_9$**) could also be confirmed by IR and NMR spectroscopic data. The band corresponding to $\nu$(O–H) of the COOH group is observed as a broad band at ca. 3440 $cm^{-1}$, this may be taken as evidence that the ester groups fully hydrolyzed to carboxylic acid groups. This was also confirmed by lower frequency shift of $\nu$(C=O) characteristic mode from ester to acid from ca. 1740 to ca. 1650 $cm^{-1}$. The $^1H$ NMR spectra pattern have changed significantly due to hydration of the ester groups to corresponding carboxylic acid groups. This was confirmed by the appearance of a singlet in the 8–10 ppm region for the COOH proton chemical shift and by disappearance of singlet-methoxy proton chemical shift at 3.63 ppm. The formation of COOH can also be supported by absence of $^{13}C$ NMR signal for the methoxy groups from the ester as well as appearance of a new band at lower chemical shift values for COOH groups. The formation of amines from esters via aminolysis (formation of amine function dendrimers **$P_4$**, **$P_7$**) could be confirmed by similar strategic manner using vibrational and nuclear magnetic resonance spectral data. Appreciable band assignments are presented in Table 1.

GPC results show that first generation dendrimers (**$P_1$**–**$P_4$**) were relatively monodisperse. However, measured $M_w$s were smaller than expected value. This may be attributed to the

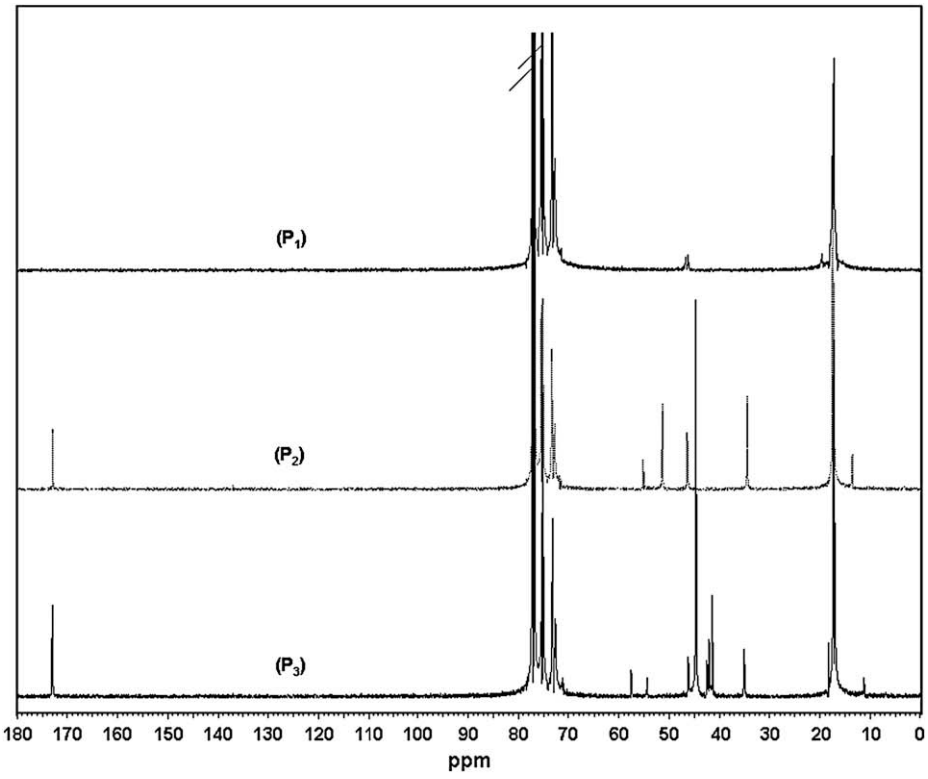



Fig. 2. $^{13}$C NMR spectrum of **P1–P3**.

reference molecules (polystyrene) which eluded much faster than our molecules. The dendrimers are expected to exhibit significantly larger chain stiffness than the calibration standard polystyrene, because the steric repulsion of the voluminous dendritic side chains should stretch the polymeric backbone considerably. This chain stiffness leads to an increased hydrodynamic volume which causes the GPC molar mass to become much smaller than the true molar mass. These results are consistent with many literatures [28,29].

### 3.2. Antimicrobial activity

The results concerning *in vitro* antimicrobial activities of the water soluble dendrimers together with the inhibition zone (mm) and (MIC) values of compared antibiotic and antifungal reagents are listed in Tables 2 and 3. All the compounds tested exhibit moderate antimicrobial activities. Among the test compounds attempted, amine carrying functional group particularly $\mathbf{P_1}$ and $\mathbf{P_4}$ showed slightly higher activities against

Table 3
*In vitro* antimicrobial activity of the compounds and the standard reagents (inhibition zone mm)

| M/P | $P_1$ | $P_3$ | $P_4$ | $P_6$ | $P_7$ | $P_9$ | $P_{10}$ | AMP | CTX | VA | OFX | TE | NY | KET | CLT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 18 | 11 | 15 | 13 | 12 | 12 | 18 | 12 | 10 | 22 | 30 | 28 | – | – | – |
| **B** | 20 | 13 | 16 | 14 | 13 | 12 | 13 | 16 | 12 | 13 | 24 | 26 | – | – | – |
| **C** | 18 | 12 | 14 | 12 | 12 | 10 | 18 | 14 | 13 | 22 | 28 | 30 | – | – | – |
| **D** | 16 | 12 | 13 | 11 | 11 | 12 | 8 | 10 | 54 | 10 | 44 | 34 | – | – | – |
| **E** | 20 | 15 | 15 | 16 | 14 | 14 | 10 | 16 | 18 | 20 | 28 | 26 | – | – | – |
| **F** | 22 | 14 | 18 | 13 | 15 | 12 | 14 | 12 | 14 | 18 | 30 | 25 | – | – | – |
| **G** | 14 | 12 | 14 | 11 | 12 | 12 | 15 | 21 | 11 | 20 | 32 | 24 | – | – | – |
| **H** | 16 | 15 | 14 | 12 | 14 | 14 | 10 | 12 | 16 | 26 | 30 | 28 | – | – | – |
| **I** | 14 | 11 | 13 | 10 | 10 | 11 | 36 | 32 | 32 | 34 | 28 | 22 | – | – | – |
| **J** | 21 | 15 | 17 | 16 | 14 | 15 | – | – | – | – | – | – | 20 | 21 | 15 |
| **K** | 24 | 16 | 18 | 16 | 15 | 14 | – | – | – | – | – | – | 18 | 16 | 18 |
| **L** | 20 | 14 | 16 | 14 | 14 | 15 | – | – | – | – | – | – | 18 | 22 | 16 |
| **M** | 22 | 15 | 18 | 16 | 15 | 14 | – | – | – | – | – | – | 21 | 24 | 22 |
| **N** | 23 | 16 | 17 | 15 | 15 | 16 | – | – | – | – | – | – | 16 | 14 | 18 |

**M**, micro-organisms; **A**, *Escherichia coli*; **B**, *Staphylococcus aureus*; **C**, *Klebsiella pneumoniae*; **D**, *Bacillus cereus*; **E**, *Micrococcus luteus*; **F**, *Proteus vulgaris*; **G**, *Mycobacterium smegmatis*; **H**, *Listeria monocytogenes*; **I**, *Pseudomonas aeruginose*; **J**, *Kluyveromyces fragilis*; **K**, *Rhodotorula rubra*; **L**, *Candida albicans*; **M**, *Hanseniaspora guilliermondii*; **N**, *Debaryomyces hansenii*. **P10**, Penicillin-G (10 Units); **AMP**, Ampicillin 10 μg; **CTX**, Cefotaxime 30 μg; **VA**, Vancomycin 30 μg; **OFX**, Ofloxacin 5 μg; **TE**, Tetracycline 30 μg; **NY**, Nystatin 100 μg; **KET**, Ketaconazole 20 μg; **CLT**, Clotrimazole 10 μg; **P**, polychelategons ($\mathbf{P_1}$, $\mathbf{P_2}$, $\mathbf{P_3}$, $\mathbf{P_4}$, $\mathbf{P_5}$, $\mathbf{P_6}$).

Table 4
*In vitro* antimicrobial activity (MIC, $\mu g\,mL^{-1}$) of the compounds

| *M/P* | $P_1$ | $P_3$ | $P_4$ | $P_6$ | $P_7$ | $P_9$ | GEN | NYS |
|---|---|---|---|---|---|---|---|---|
| **A** | 3.13 | 25.00 | 6.25 | 12.50 | 12.50 | 12.50 | 6.25 | – |
| **B** | 1.56 | 12.50 | 6.25 | 12.50 | 6.25 | 12.50 | 25.00 | – |
| **C** | 6.13 | 12.50 | 12.50 | 12.50 | 12.50 | 25.00 | 6.25 | – |
| **D** | 6.25 | 12.50 | 12.50 | 25.00 | 25.00 | 12.50 | 6.25 | – |
| **E** | 1.56 | 6.25 | 6.25 | 6.25 | 12.50 | 12.50 | 25.00 | – |
| **F** | 1.56 | 12.50 | 3.13 | 6.25 | 6.25 | 12.50 | 6.25 | – |
| **G** | 12.50 | 12.50 | 12.50 | 25.00 | 12.50 | 12.50 | 12.50 | – |
| **H** | 6.25 | 6.25 | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 | – |
| **I** | 6.25 | 12.50 | 6.25 | 25.00 | 25.00 | 12.50 | 6.25 | – |
| **J** | 1.56 | 6.25 | 3.13 | 6.25 | 6.25 | 6.25 | – | 6.25 |
| **K** | 0.78 | 6.25 | 3.13 | 6.25 | 6.25 | 6.25 | – | 6.25 |
| **L** | 1.56 | 12.50 | 6.25 | 12.50 | 12.50 | 6.25 | – | 3.13 |
| **M** | 1.56 | 6.25 | 3.13 | 6.25 | 6.25 | 6.25 | – | 3.13 |
| **N** | 0.78 | 6.25 | 3.13 | 6.25 | 6.25 | 6.25 | – | 12.50 |

**M**, micro-organisms; **A**, *Escherichia coli*; **B**, *Staphylococcus aureus*; **C**, *Klebsiella pneumoniae*; **D**, *Bacillus cereus*; **E**, *Micrococcus luteus*; **F**, *Proteus vulgaris*; **G**, *Mycobacterium smegmatis*; **H**, *Listeria monocytogenes*; **I**, *Pseudomonas aeruginose*; **J**, *Kluyveromyces fragilis*; **K**, *Rhodotorula rubra*; **L**, *Candida albicans*; **M**, *Hanseniaspora guilliermondii*; **N**, *Debaryomyces hansenii*. **GEN**, Gentamycin; **NYS**, Nystatin; **P**, polychelategons ($P_1$, $P_3$, $P_4$, $P_6$, $P_7$, $P_9$).

certain bacteria and are definitely more potent on all the yeast cultures (Table 3). The MIC values in Table 4 also indicate that all the compounds tested exhibit moderate antimicrobial activity on the tested micro-organisms. Once again the data indicate that $P_1$ and $P_4$ compounds have stronger activity against some bacteria such as *P. vulgarise* ($P_1 = 1.56$ and $P_4 = 3.13\ \mu g\,mL^{-1}$) and *Micrococcus luteus* ($P_1 = 1.56$ and $P_4 = 6.25\ \mu g\,mL^{-1}$) compared with Gentamycin on these micro-organisms 6.25 and 25.00 $\mu g\,mL^{-1}$, respectively. These compounds also have strong activity against the yeast cultures such as *Rhodotorula rubra* ($P_1 = 0.78$ and $P_4 = 3.13\ \mu g\,mL^{-1}$) and *D. hansenii* ($P_1 = 0.78$ and $P_4 = 3.13\ \mu g\,mL^{-1}$) compared with Nystatin antifungal agent on these micro-organisms which are 6.25 and 25.00 $\mu g\,mL^{-1}$, respectively (Table 4). The results are not surprising, because it was well-known that PAMAM type dendrimers with amine surface functional groups particularly primary amine functional group could penetrate through the bacterial cell membrane, mainly due to their strong hydrogen bond donor characteristic properties toward biomolecules [30,31]. In this respect the inhibition activity is expected to be governed in certain degree by the presence of the amino groups in the compounds. If this is the case, one should expect that the amino groups must be free of interamolecular hydrogen bonding or other hindrance effects. Among these compounds $P_1$, $P_4$ and $P_7$ are not expected to have intramolecular hydrogen bonding characteristics, while the other compounds may be able to form intra- or even intermolecular hydrogen bonds, due to the presence of carboxyl or amide groups. The results of our study indicate that the compounds $P_1$, $P_4$ and $P_7$ have the potential to generate novel antimicrobial properties by displaying moderate to high affinities for most of the receptors, while the remaining coumpounds have lower activity against the microbial species, which could be used as a drug enhancer or drug delivery agent.

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