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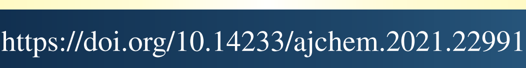

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Synthesis of Melamine Core Starburst Polyamide G_1 Dendrimer and its Antibacterial and Antioxidant Activities

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A novel polyamide first-generation dendrimer **7** was synthesized from new methodology under mild reaction conditions. The AB_2 adduct, ethyl 2-[*N*-[2,2-*bis*(hydroxymethyl)propionyl]amino]ethylate (**3**), was prepared from 2,2-*bis*(hydroxymethyl)propionic acid (*bis*-MPA) and glycine as a linker. Further, G_1 dendrimer **7** was synthesized by coupling melamine (**5**) with monomer **3**. The structural elucidations of all the compounds were confirmed by FTIR, ^{1}H & ^{13}C NMR and HRMS. The G_1 dendrimer was found to exhibit significant antibacterial activity against *S. aureus* and *E. cloacae* and antioxidant activity by DPPH scavenging method when compared to the standard, ascorbic acid.

Keywords: 2,2-*bis*(Hydroxymethyl)propionic acid, Glycine ethyl ester hydrochloride, Melamine, 2,2-Dimethoxypropane.

INTRODUCTION

Dendrimers are nano-sized macromolecules with a well-defined structure possessing three main units; a central core, interior repeating units and exterior functional groups [1]. Dendrimers are synthesized through two distinguished strategies; divergent and convergent methods. The hydroxyl functional groups terminated 2,2-*bis*(hydroxymethyl)propionic acid (*bis*-MPA) is a suitable monomer to synthesize dendrimers with adequate water solubility, biodegradability, biocompatibility and variety of biomedical applications such as drug delivery [2-7], imaging agents [8], gene transfection [9] and therapeutic agents [10,11]. Glycine is a suitable linker or spacer molecule which offers conjugative effect with the *bis*-MPA dendron [12]. Amide dendron for hydrolytic degradability of the H-bond forming ability of amide groups has proven very interesting for the design of synthetic polymers for drug and gene delivery [13,14].

On the other hand, the major advantage of melamine core was its hydrogen bonding capability has six H-donors (-NH_2) and three H-acceptors (=N-) and can form maximally nine H-bonds per molecule. The high functionality and the readiness of –NH_2 moiety to bond with an electronegative group are the structural advantages of melamine for using it as a core molecule in the dendrimer synthesis [15]. Fusing the melamine core with peripheral moiety through small molecules like glycine generates enough void space between the dendrimer arms and provides flexibility to accommodate/couple with various drug moieties [16-18]. Noteworthy, reports are existing in the literature reference on using melamine as a core with the different linkers and peripheral moieties. However, the combination of melamine core-glycine linker *bis*-MPA peripheral is not reported in the literature. Considering the structural, functional and reactivity features, a dendrimer is designed by using melamine (as core), glycine (as linker) and *bis*-MPA as a peripheral group and synthesized using a divergent method.

EXPERIMENTAL

Synthesis of ethyl 2-[*N*-[2,2-*bis*(hydroxymethyl)-propionyl]amino]ethylate (3): This product was synthesized by reported method [19]. Glycine ethyl ester hydrochloride (5 g, 35.82 mmol) was dissolved in DMF (20 mL) and was added triethylamine (4.99 mL, 35.82 mmol) into a round bottom flask. The reaction mixture was stirred at room temperature and the white precipitate formed, triethylammonium hydrochloride,

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was filtered out. To the filtrate reacts with 2,2-*bis*-(hydroxymethyl)propionic acid (4.8 g, 35.82 mmol) in the presence of *N*,*N*-dicyclohexylcarbodiimide (DCC) (8.13 g, 39.40 mmol) in (15 mL) of 1,4-dioxane. Again the reaction mixture was stirred for 12 h and filtered to remove dicyclohexyl urea. The filtrate was concentrated under vacuum by rotavapor to give a residue, which was column chromatography using SiO_2 (mesh size 100-200), eluting with a CH_2Cl_2:CH_3OH (95:5), to give a yellowish oil compound **3** (5.65 g 72%). ^1H NMR ($CDCl_3$): δ 1.07 (s, -CH_3, 3H), 1.26 (t, -CH_2CH_3, 3H), 3.72 (broad s, -CH_2, 4H), 3.99 (d, -$NHCH_2$, 2H), 4.18 (q, -CH_3CH_2, 2H), 7.77 (t, -$NHCH_2$, 1H); ^{13}C NMR δ ppm: 14.07 (CH_2CH_3), 17.45 (CCH_3), 41.58 (CH_2NH), 48.02 (CCH_3), 61.68 (CH_2CH_3), 67.38 $(CH_2O)_2$, 170.54 (NHC=O), 177.14 (OC=O); FTIR (KBr, ν_{max}, cm^{-1}): 3364 (N-H *str.*) 3364, 1644 (NHC=O *str.*). HRMS *m/z* calcd: 219.1107, $[M+H]^+$ = 220.1140, found $[M+H]^+$ = 220.1185, $[M+Na]^+$ = 242.1003.

Synthesis of ethyl 2-(2,2,5-trimethyl-1,3-dioxane-5-carboxamido)acetate (4): Ethyl 2-[*N*-[2,2-*bis*(hydroxymethyl)-propionyl]amino]ethylate (**3**) (3.5 g, 15.97 mmol) was added into a round-bottom flaks containing 2,2-dimethoxypropane (2.93 mL, 23.96 mmol) in the presence of *p*-toluenesulfonic acid monohydrate (*p*-TSA) (0.013 g, 0.079 mmol) in acetone (15 mL). The reaction mixture was stirred for 4 h at room temperature, after that *p*-TSA was neutralized by 0.5 mL of NH_3/EtOH (50:50, v/v) solution. The solvent was removed by rotavapor to give a residue, and dissolved in (50 mL) CH_2Cl_2 and washed with water (3 × 10 mL) and the organic phase was dried over $MgSO_4$ anhydrous. The solvent was concentrated under reduced pressure and purified by column chromatography using SiO_2 (mesh size 100-200) by eluting with hexane: EtOAc (95:5), to afford honey-like oil compound **4** (3.10 g 75%). ^1H NMR ($CDCl_3$, δ ppm): 1.21 (s, -CCH_3, 3H), 1.21 (t, J = 7.2 Mz, -CH_3-CH_2), 1.41 (s, -$C(CH_3)_2$, 6H), 3.70, 3.88 (dd, J = 12.4 Mz, CH_2OH, 4H), 4.02 (d, J = 5.2 Mz, CH_2NH, 2H), 4.15 (q, J = 6.8 Mz, CH_3CH_2, 2H); 7.63 ($NHCH_2$, 1H); ^{13}C NMR ($CDCl_3$, δ ppm): 14.15 (CH_3CH_2), 17.64, 18.37, (CCH_3), 28.61 $(CCH_3)_2$, 40.13 (CH_2NH), 41.69 (CCH_3),61.39 (CH_3CH_2), 66.06, 67.00 $(CH_2O)_2$, 98.60 ($OCCH_3$), 169.96 (NHC=O), 175.06 (OC=O); FTIR (KBr, ν_{max}, cm^{-1}): 3381 (N-H *str.*), 1630 (C=O *str.*) and 1221 (C-O-C *str.*). HRMS *m/z* calcd: 259.1420, $[M+H]^+$ = 260.1453, found $[M+H]^+$ = 260.1505, $[M+Na]^+$ = 282.1321.

Synthesis of G_1 dendrimers with acetonide protected 6: Melamine (**5**) (0.2 g, 1.58 mmol), ethyl 2-(2,2,5-trimethyl-1,3-dioxane-5-carboxamido)acetate (**4**) (1.35 g, 5.23 mmol), K_2CO_3 (0.98 g, 7.135 mmol) and DMSO (10 mL) were charged into a reaction flask. The reaction mixture was stirred for 48 h at 25 ºC under N_2 atmosphere. After filtration, the filtrate was concentrated *in vacuo* to give a residual oil, water washed twice and centrifuged to get precipitate. The precipitate was dried in vacuum oven kept at 40 ºC overnight to afford a yellow precipitate, acetonide protected G_1 dendrimer without purification compound **6** (0.60 g 50%). ^1H NMR ($CDCl_3$, δ ppm): 1.25 (s, -$C(CH_3)_2$, 6H), 1.41 (s, -CCH_3, 3H), 3.65, 4.19 (dd, J = 12.0 Mz, $(CH_2O)_2$, 4H), 4.00 (d, J = 12.4 Mz, CH_2NH, 2H), 8.06 ($NHCH_2$, 1H), 9.18 (s, triazine-NHCO, 1H); ^{13}C NMR ($CDCl_3$, δ ppm): 18.53 (CCH_3), 25.44 $(CCH_3)_2$, 41.64 (CH_2NH), 49.50 (CCH_3), 65.27 $(CH_2O)_2$, 98.26 ($OCCH_3$), 158.08 (Ar-C), 165.66 (triazine-NHC=O), 175.86 (NHC=O); FTIR (KBr, ν_{max}, cm^{-1}): 3305 (N-H *str.*) and 1682, 1640 (NHC=O); ESI-MS *m/z* calcd: 765.3657, $[M+H]^+$ = 766.3691, found $[M+H]^+$ = 770.9492, $[M+Na]^+$ = 790.8112.

Synthesis of G_1 dendrimers with hydroxyl terminal 7: Melamine (**5**) (0.29 g, 2.29 mmol), ethyl 2-(3-hydroxy-2-(hydroxymethyl)-2-methylpropanamido)acetate (**3**) (1.66 g, 7.58 mmol), K_2CO_3 (1.28 g, 9.27 mmol) and DMSO (10 mL) were added into a dry flask. The reaction mixture was stirred for 48 h at 25 ºC under dry N_2 atmosphere. After the completion of the reaction, the solvent was concentrated *in vacuo* to give a residue, water washed, centrifuged to get precipitate. The precipitate was dried in a vacuum oven kept at 40 ºC overnight, and purified by column chromatography using silica gel (mesh size 100-200) and eluting with hexane:EtOAc (10:90), to get white solid compound **7** (0.75 g 51%). ^1H NMR ($CDCl_3$): δ 1.38 (s, -CCH_3, 3H), 3.57 (s, -OH, 2H), 3.94, 4.23 (dd, J = 12.0 Mz, CCH_2, 4H), 7.54 (s, NH, 1H); ^{13}C NMR δ ppm: 17.40 (CCH_3), 47.70 (CH_2NH), 50.14 (CCH_3), 65.04 $(CH_2O)_2$, 156.45 (Ar-C), 173.38 (NHC=O); FTIR (KBr, ν_{max}, cm^{-1}): 3306 (N-H *str.*), 1685, 1634 (NHC=O); HRMS *m/z* calcd: 645.2718, $[M+H]^+$ = 646.2752, found $[M+H]^+$ = 645.6196.

Antibacterial assessment: The antibacterial assessment of G_1 dendrimer **7** dissolved in DMSO, was screened against *Staphylococcus aureus* (Gram-positive) and *Enterobacter cloacae* (Gram-negative) using agar well diffusion method. A Muller Hinton agar solution (20 mL) was prepared and poured into sterile petri plates. Wells were cut on the agar using sterile cork borer and 24 h active culture of *S. aureus* and *E. cloacae* were spread over the agar plate using sterile L-rod. The bacterial suspensions were obtained by inoculating the active culture of the test organisms into 50 mL nutrient broth and incubated at 37 ºC for 24 h in a shaking incubator. The test sample of G_1 dendrimer was loaded into the well with varying concentrations such as 25, 50, 75 and 100 µg/mL, respectively. Ciprofloxacin was used as a standard. The plates were incubated aseptically at 37 ºC for 24 h.

***in vitro* Antioxidant activity:** The antioxidant ability of G_1 dendrimer was evaluated by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) method. Concisely, 1.8 mL of DPPH (1×10^{-4} M) in DMSO was added to 0.2 mL of dendrimers in different concentrations (10, 25, 50, 75 and 100 µg/mL) and used to determine the antioxidant ability using ascorbic acid as a positive control. The mixture was incubated in the static condition in the dark at 37 ºC for 20 min. Furthermore, incubation of the mixture was subjected to spectroscopic analysis using UV- visible spectrophotometry at 517 nm. The experiments were carried out in triplicate, and the radical scavenging activity of G_1 dendrimer was calculated by using the following formula:

$$\text{DPPH radical scavenging activity } (\%) = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

RESULTS AND DISCUSSION

The designed dendrimer G_1 was synthesized by an amidation procedure under mild reaction conditions using a diver-

gent approach. Initially, the synthesis of branching unit, ethyl 2-(2,2,5-trimethyl-1,3-dioxane-5-carboxamido)acetate (**4**) was started by condensing *bis*-MPA (**2**) with glycine ethyl ester hydrochloride (**1**) using DCC as a coupling agent to give ethyl 2-[*N*-[2,2-*bis*(hydroxymethyl)propionyl]amino]ethylate (**3**). Then, the 1,3-diol groups of compound **3** were protected using 2,2-dimethoxypropane in the presence of a catalyst, *p*-TSA, in dry acetone leaving the ester group as active to afford the branching unit **4**. Subsequently, triamino functionalized melamine **5** core was coupled with the branching unit **4** in the presence of K_2CO_3 catalyst in dry DMSO to afford the acetonide protected G_1 dendrimer **6**.

Deprotection of acetonide group was tried with reagents such as 1M HCl-THF [20,21], 6 M HCl-THF [22] and H_2SO_4-CH_2Cl_2/CH_3OH [23] reported in the literature. Unfortunately, in all such conditions, the starting material was recovered and the acetonide group was remained unaffected. This probably due to the proton delocalizing nature of amide functionality that prevented the protonation of acetonide oxygen. Alternatively, melamine **5** was directly reacted with ethyl ester **3** to afford G_1 dendrimer **7** with -OH terminal groups (**Scheme-I**).

Antibacterial activity: Antibacterial efficiency of dendrimer was evaluated against *S. aureus* and *E. cloacae* as test organisms. The zone of inhibition for G_1 dendrimer **7** at the

Scheme-I: Schematic presentation of polyamide G1 dendrimer synthesis

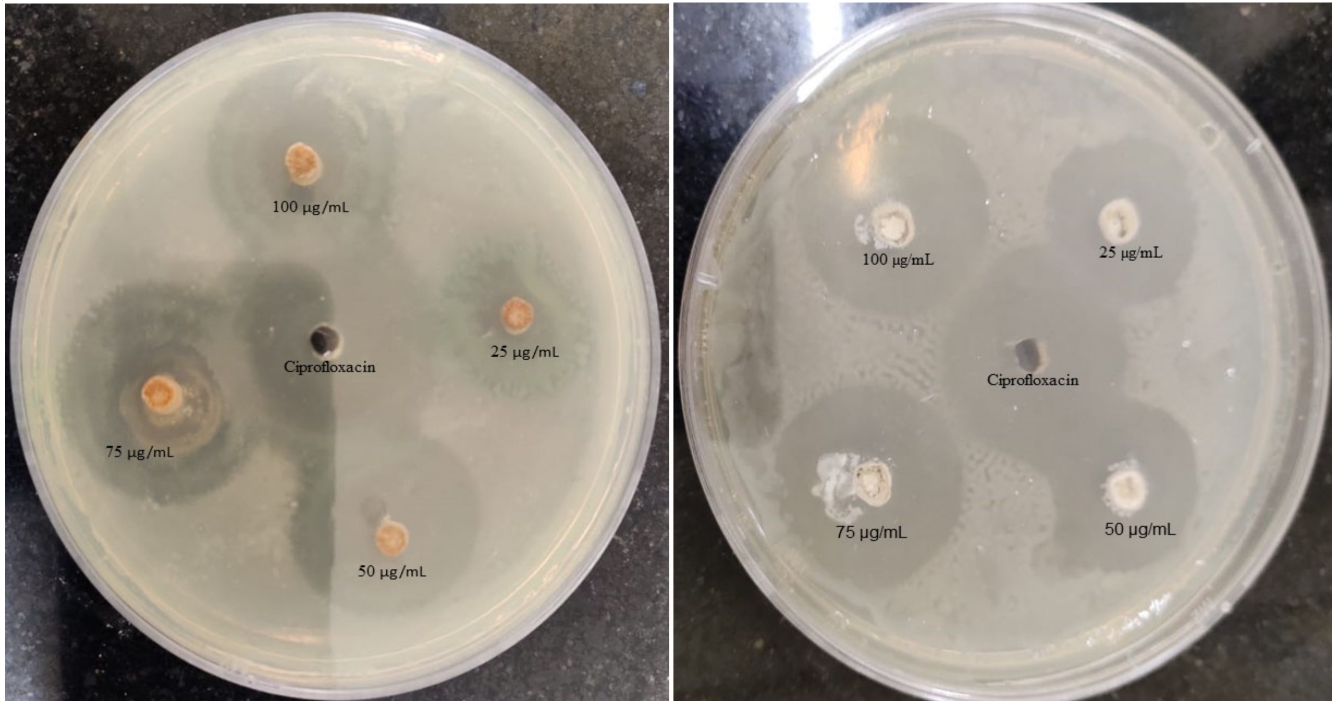

Fig. 1. Antibacterial activity of polyamide G_1 dendrimer

concentrations of 100, 75, 50 and 25 µg/mL against *S. aureus* was found to be 19, 18, 17 and 16 mm, respectively and that of the standard, ciprofloxacin, was observed to be 22 mm at the concentration of 100 µg/mL.

Similarly, G_1 dendrimer **7** exhibited the zones of inhibition of 21, 19, 18 and 17 mm at the concentrations of 100, 75, 50 and 25 µg/mL, respectively against *E. clocae* and the standard drug has shown 24 mm at the concentration of 100 µg/mL. The inhibition zones of the test compounds at varying concentrations are shown in Fig. 1. The results showed that the test compound has the potential to inhibit the growth of the pathogenic strains ably.

***in vitro* Antioxidant activity:** The antioxidant ability of the compound was determined efficiently using DPPH radical scavenging method. In the present study, the percentage of inhibition of the dendrimer **7** was studied and compared with the standard, ascorbic acid. The maximum inhibition of 66.21% was obtained at 100 µg/mL concentration of G_1 dendrimer **7** revealing a higher antioxidant activity, whereas 26.47% inhibition was achieved for a lower concentration of 10 µg/mL Similarly, the standard ascorbic acid shows better antioxidant activity at 100 µg/mL with 75.97% of inhibition, where as 30.91% inhibition was achieved for a lower concentration of 10 µg/mL (Fig. 2). The overall findings showed that G_1 dendrimer has significant radical scavenging when compared with standard antioxidant. The same line of observations were also reported by several researchers [24,25].

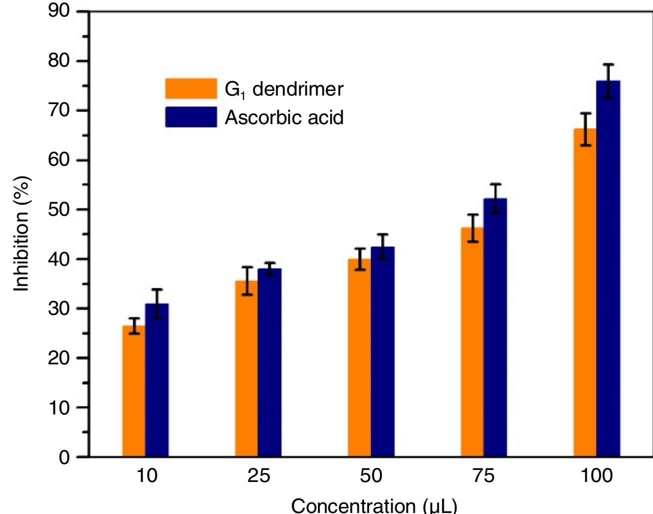

Fig. 2. Antioxidant activity of polyamide G_1 dendrimer

Conclusion

In this work, a novel polyamide G_1 dendrimer was synthesized using new synthetic approach and amidation reactions. The dendrimer has shown good antibacterial activity against Gram-positive *S. aureus* and Gram-negative *E. cloacae* bacteria. *in-vitro* Antioxidant activity of the G_1 dendrimer with amide functional group was studied by DPPH radical scavenging assay, which exhibited stronger antioxidant activity when compared to the standard ascorbic acid.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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