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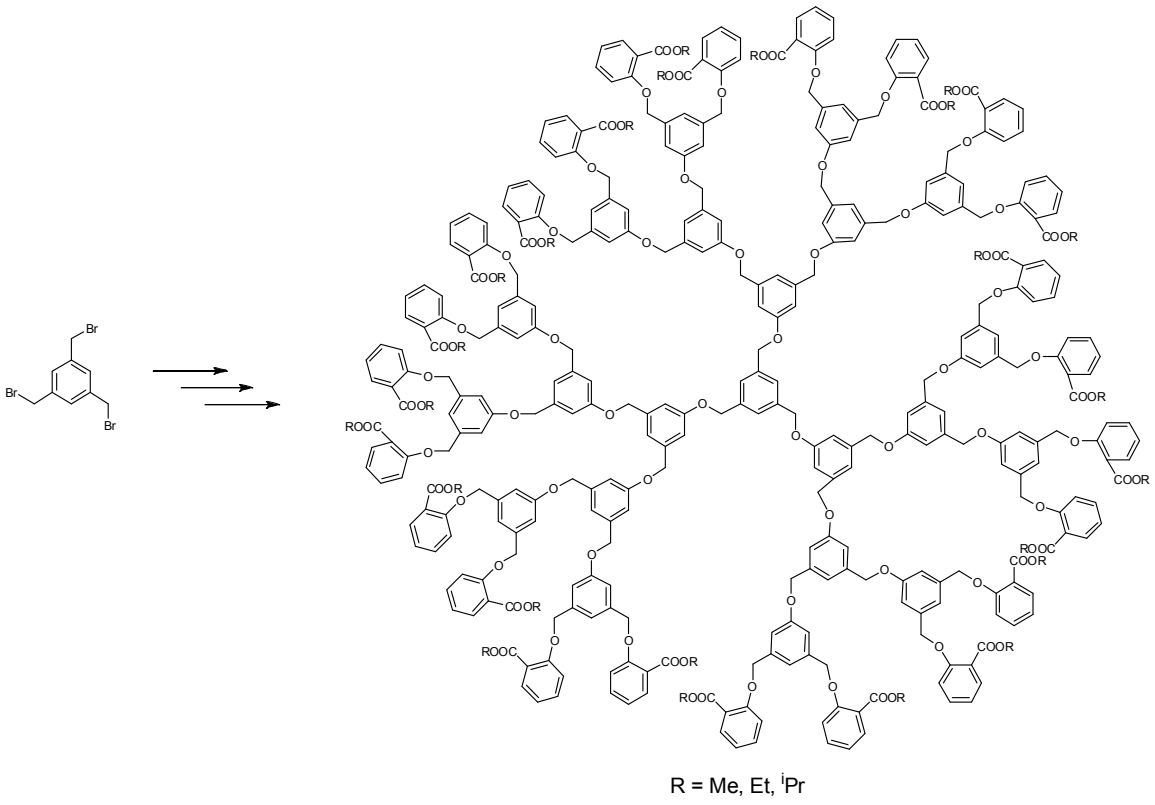
Graphical Abstract

Dendrimers containing methyl, ethyl and isopropyl salicylates at the surface were synthesized by divergent approach starting from a simple core unit benzene 1,3,5-tricarboxylic acid.



Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Synthesis of In-vitro anti-arthritic activity of dendrimers with methyl, ethyl and isopropyl salicylates as surface units

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

Fretchet type dendrimers with salicylates as surface groups have been synthesized by divergent approach and their In-vitro anti-arthritic acitivities were carried out by inhibition of protein denaturation method.

Introduction

For years, methyl salicylate (Wintergreen Oil) has been found to be the active ingredient of most of the topical analgesics; it is the major ingredient (about 30 %) in Bengay (pain relieving cream, Johnson & Johnson). Methyl salicylate is approved by the U.S. Food and Drug Administration (FDA) and finds many applications[1-3] along with the analgesic property such as stimulant or surrogant for sulfur mustard and also finds application in immunohistochemistry[3], triboluminescence[3]; hence the synthesis of dendrimers with methyl and other salicylate analogous were focused. Dendrimers[4-5] is one of the most exciting classes of macromolecules that have sparked significant interest in recent years from synthetic, structural and functional points of view. Recently, conjugated dendrimers and bioactive molecules show increased therapeutic efficacy and the delivery of bioactive molecules, is of great importance. Synthesis of dendrimers with anti-bacterial activity has been reported from our group[6]. Synthesis and In-vitro anti-arthritic[7] as well as anti-inflammatory activities of glycodendrimer bearing α-D-glycopyranosyl surface unit was reported from our laboratory[8].

In general salicylic acid and its derivatives find a versatile place in pharmaceutical chemistry because of their bioactivity in vitro system. Salicylate derivatives gains the tendency to build up electrical charge when crushed or rubbed with sugar, which can be observed by crushing wintergreen Life Savers candy in a dark room[9]. Further, metal binding properties of salicylate dendrimers has been also reported in 2001[10]. Hence it is of great interest to synthesize dendrimers with methyl, ethyl and isopropyl salicylate units at the periphery by means of O-alkylation methodology[11-12]. Herein, we report the synthesis and in-vitro anti-arthritic activity of Frechet type dendrimers **1-12 (Figure 1)**.

In order to study the anti-arthritic activity of salicylate dendrimers **1-12** inhibition of protein denaturation method (using BSA - Bovine Serum Albumin) was employed and diclofenac sodium was used as standard[13]. It was reported that the denaturation of protein is one of the causes for rheumatic arthritic and the production of auto-antigens in certain rheumatoid may be due to in vivo denaturation of proteins[14] and probably involves alteration in electrostatic and hydrophobic interactions, hydrogen and disulfide bonding[15].

Results and discussion

Chemistry

In order to synthesize the required dendrimers with methyl, ethyl and isopropyl salicylate surface units, a multifunctional core unit has to be synthesized. For which, 1,3,5-Tris(bromomethyl) benzene **16** was chosen as core unit and was synthesized from benzene 1,3,5- tricarboxylic acid **13** by esterification in ethanol in the presence of thionyl chloride to give the triester **14** followed by the reduction with LAH (Lithium Aluminium Hydride) in THF[15] to give the corresponding triol **15** and finally brominating the triol **15** with PBr_3 (**Scheme 1**).

Zero-generation dendrimers (G_0) **1**, **2** and **3** were synthesized by reacting three equivalents of methyl, ethyl and isopropyl salicylate with one equivalent of 1,3,5-tris(bromomethyl)benzene **16** in the presence of potassium carbonate in dry acetone (**Scheme 1**). In the ^{1}H NMR spectrum of denerimer **1**, showed a sharp singlet at δ 3.88, 5.22 for the ester methyl and O-methylene protons respectively in addition to the signals for the aromatic protons. The ^{13}C NMR spectrum of dendrimer **1** showed the ester methyl, O-methylene carbon peaks at δ 52.0, 70.6 respectively. The carbonyl carbon appeared at δ 166.8 in addition to the signals for the aromatic carbons. The structure of zero-generation dendrimers **2** and **3** was also confirmed from the spectral and analytical data.

Initially 5-hydroxy isophthalic acid was chosen as building unit in order to synthesis dendrimers with higher generation by convergent approach. In this strategy, deprotection of hydroxyl group of the dendron (which contains the two salicylates groups at 1,3 positions and the hydroxyl group protected as acetyl/ trityl/ TBDMS) either by acidic or basic condition leads to complications as the reaction condition also hydrolyse the ester groups of salicylates. Hence, the O-alkylation method was adapted to synthesis dendrimers **1-12** which eliminates the protection and deprotection protocol in the divergent approach.

In order to synthesize the first-generation (G_1) dendrimer the hexaester **17** was synthesized by reacting three equivalents of diethyl 5-hydroxyisophthalate with one equivalent 1,3,5-tris(bromomethyl)benzene **16** in dry acetone in the presence of potassium carbonate. The alcohol **18** obtained by the LAH reduction of the ethyl hexaester **17** was converted into the

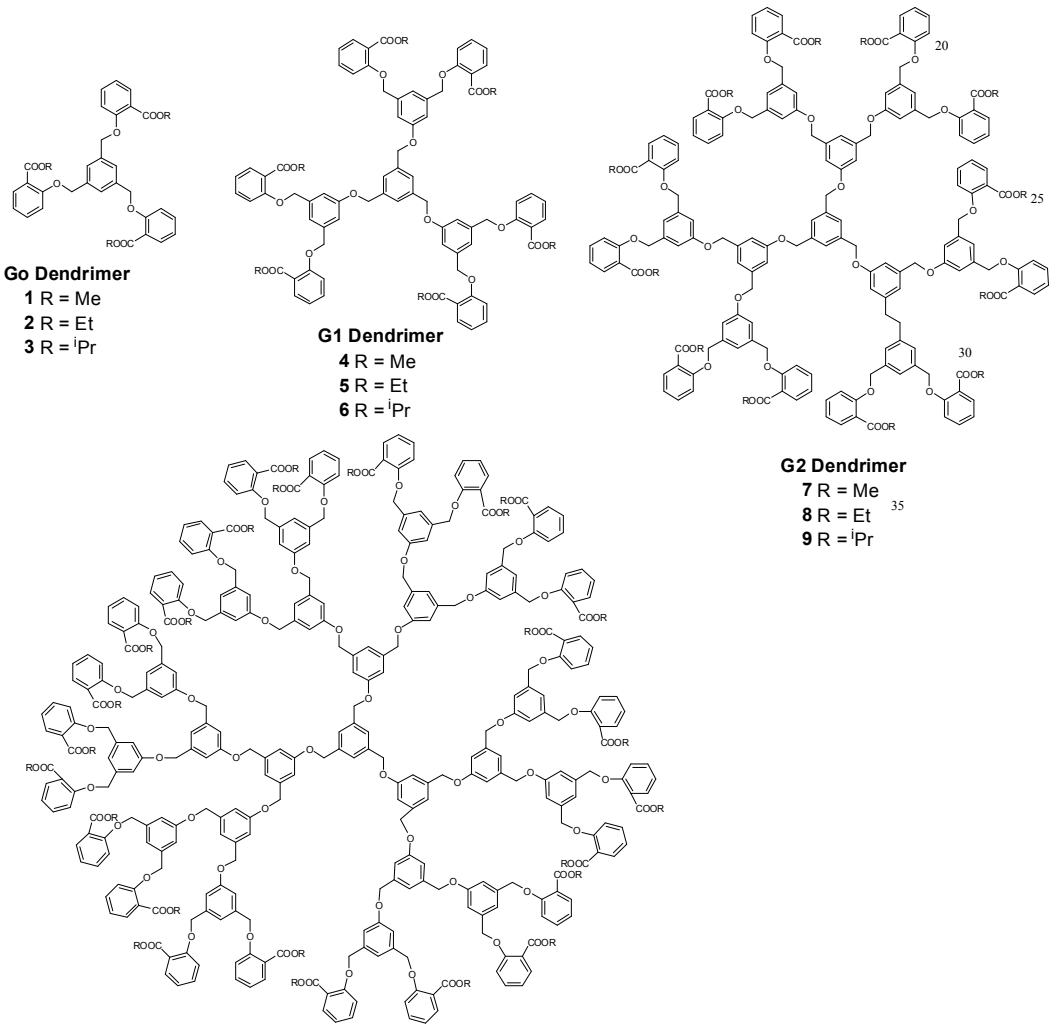
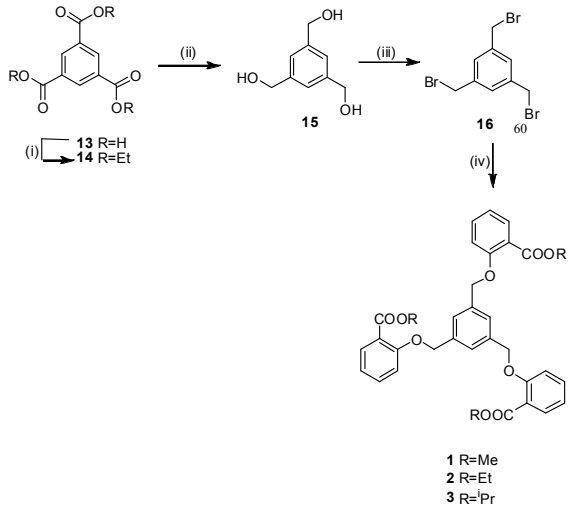


Figure 1 Frechet type salicylate dendrimers

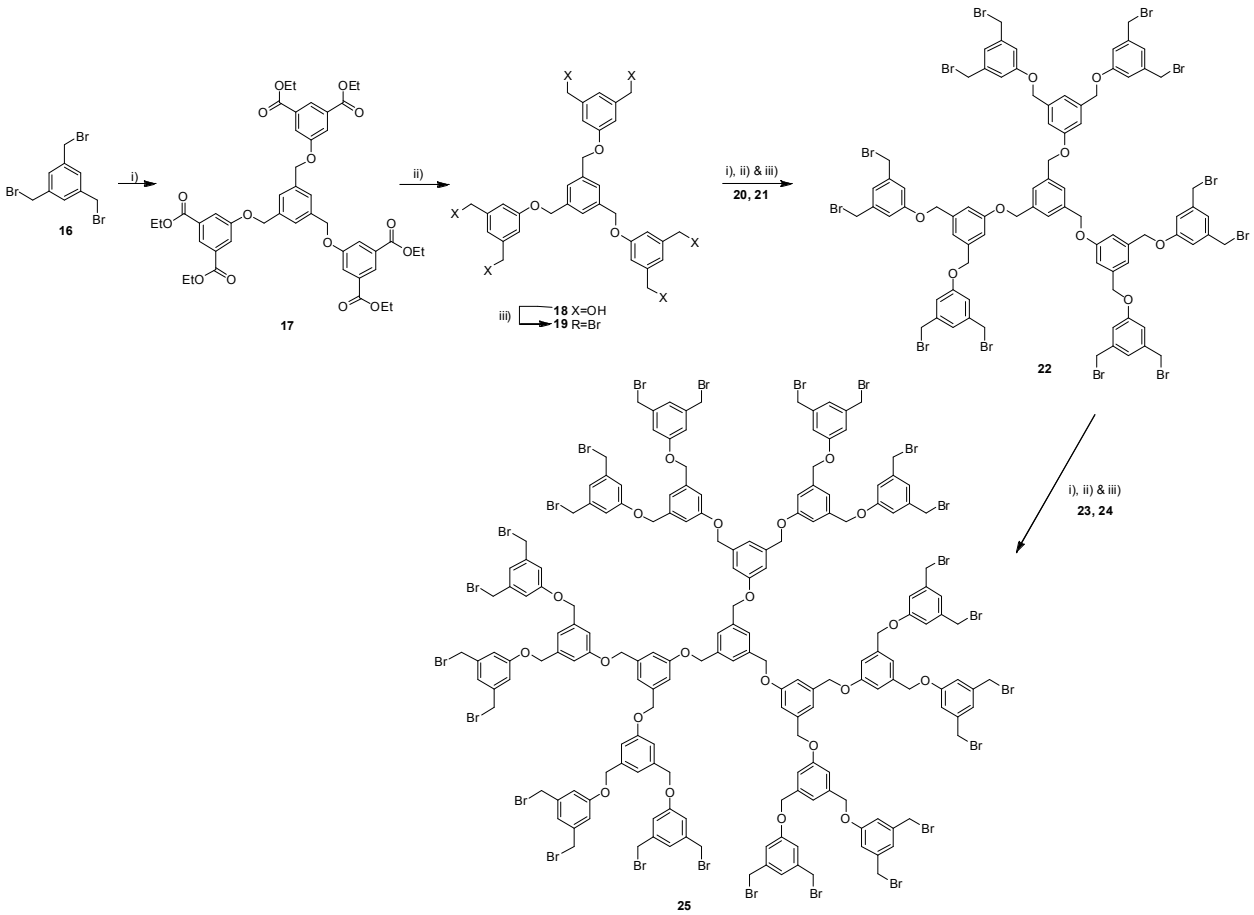


Scheme 1

Reagents and conditions: (i) EtOH, $SOCl_2$, Reflux, 12 h (ii) LAH, THF, 50 ^oC, 8 h (iii) PBr_3, CH_2Cl_2, 0 ^oC-RT, 6 h (iv) Methyl / ethyl / isopropyl salicylate, K_2CO_3, dry acetone, RT, 24h

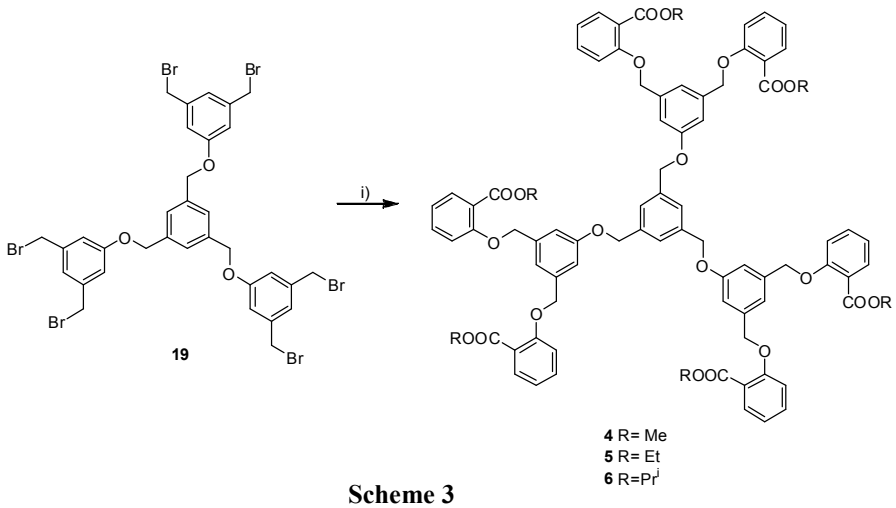
hexabromide **19** using PBr_3 in $CHCl_3$ (**Scheme 2**). A similar reaction procedure was repeated with the hexabromide **19** to give the dodeca bromide **22** and further the tetracosa bromide **25** in 75 % and 73 % yields respectively (**Scheme 2**).

The first-generation (G_1) dendrimers **4**, **5** and **6** were obtained in 71 %, 73% and 69 % respectively by the etherification of six equivalents of methyl, ethyl and isopropyl salicylates with one equivalent of the hexabromide **19** in dry acetone in the presence of potassium carbonate (**Scheme 3**). In the ^1H NMR spectrum, dendrimer **4** showed a sharp singlet at δ 3.89, 5.13 and 5.17 for ester methyl and for two distinct methylene protons respectively. The ^{13}C NMR spectrum of dendrimer **4** showed the ester methyl carbon at δ 52.0 and the two distinct methylene carbons at δ 69.8, 70.2 respectively and the carbonyl carbon appeared at δ 166.7. Similarly the structure of dendrimers **5** and **6** were also confirmed from the spectral and analytical data.



Scheme 2

Reagents and conditions: (i) Diethyl 5-hydroxy isophthalate, K_2CO_3, dry acetone, RT, 24 h (ii) LAH, THF, 50 ^oC, 8 h (iii) PBr_3, CH_2Cl_2, 0 ^oC-RT, 6 h.



Scheme 3

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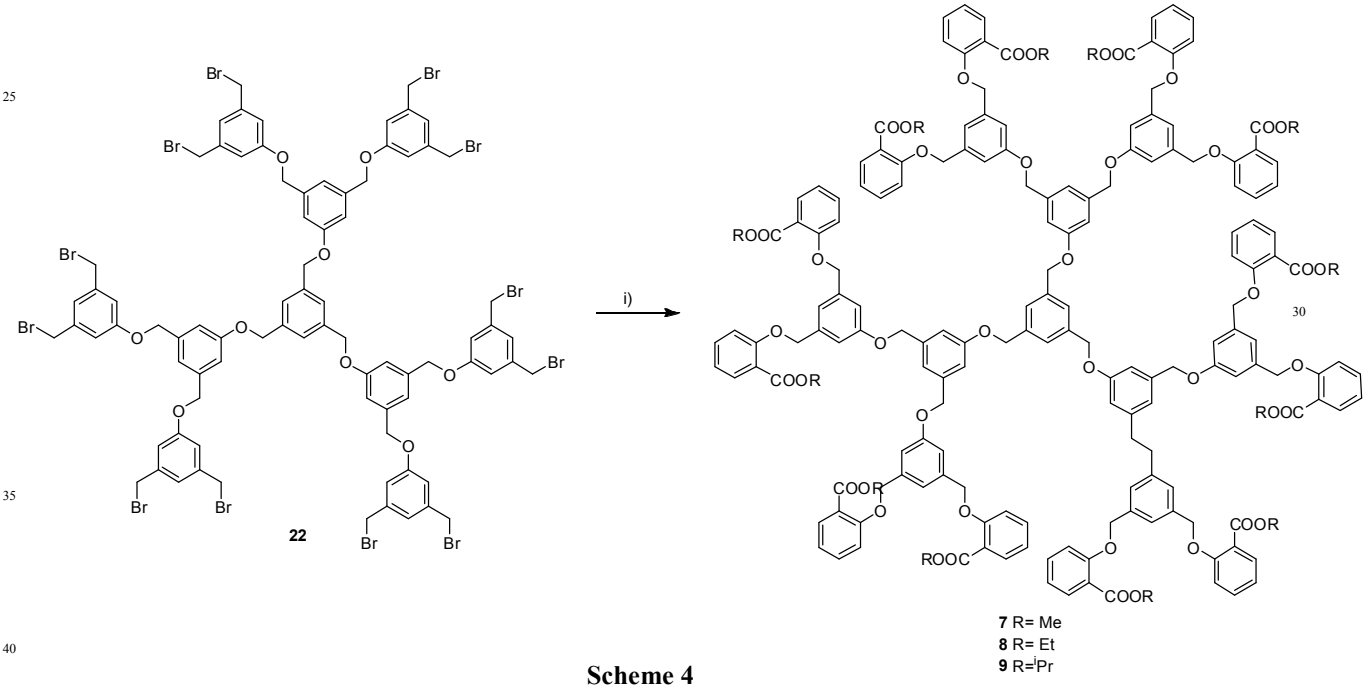
The second-generation dendrimers (G_2) **7**, **8** and **9** were obtained in 68 %, 69 % and 65 % yields respectively by the etherification of twelve equivalents of methyl, ethyl and isopropyl salicylate with one equivalents of the second-generation dodecabromide **22** in dry acetone in the presence of potassium carbonate **(Scheme 4)**. In the ^1H NMR spectrum, compound **7** showed sharp singlet at 3.89, 5.21 and 5.23 for ester methyl and two distinct O-methylene protons respectively in addition to the signals for the aromatic protons. The ^{13}C NMR spectrum of compound **7** showed ester methyl and two distinct

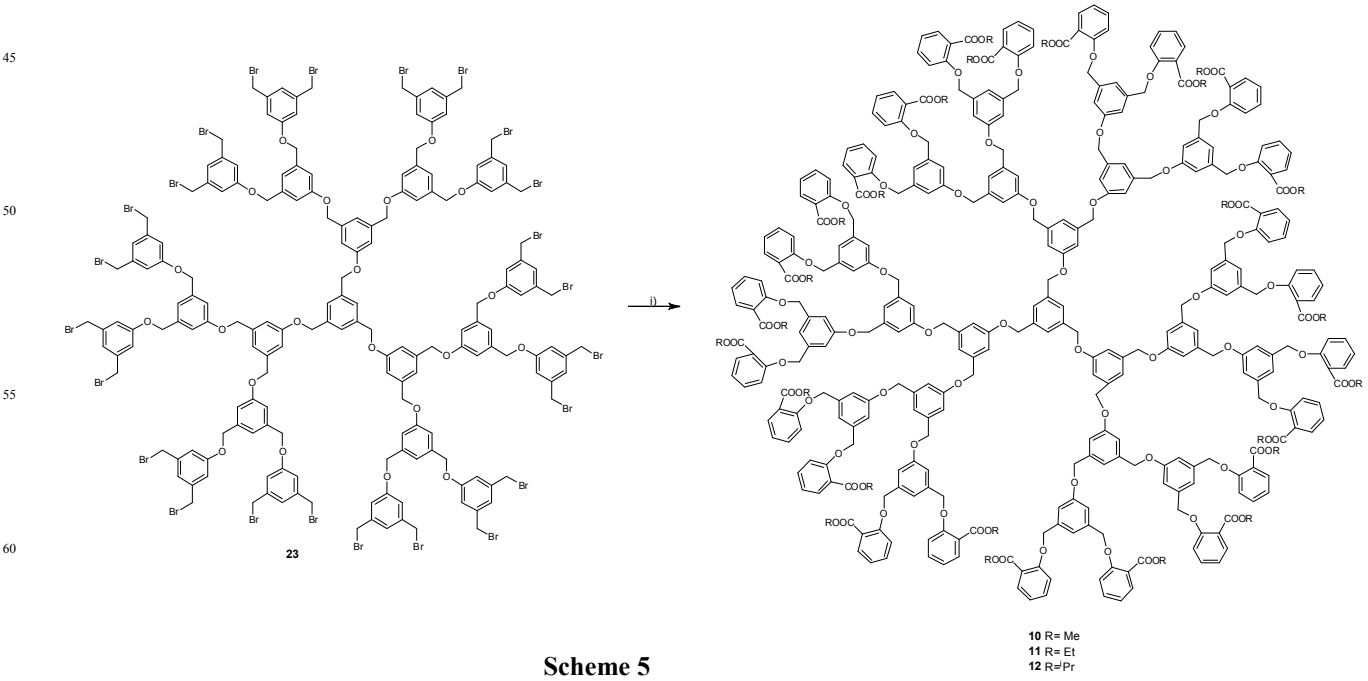
O-methylene carbons at δ 52.0, 70.4 and 70.6 respectively**.** The carbonyl carbon appeared at δ 165.7 in addition to the signals for other aromatic carbons. The structure of second-generation dendrimers (G_2) **8** and **9** was also confirmed from spectral and analytical data.

The third-generation dendrimers (G_3) **10**, **11** and **12** were obtained in 67 %, 69 % and 64 % yields respectively by the etherification of twenty four equivalents of methyl, ethyl and isopropyl salicylate with one equivalent of the third generation tetracosa bromide **25** in dry acetone in the presence of potassium carbonate **(Scheme 5**). In the ^{1}H NMR spectrum, compound **10** showed signals at δ 3.85, and 5.09, 5.12 for ester methyl and two distinct O-methylene protons respectively in addition to the signals for the aromatic protons. The ^{13}C NMR spectrum of compound **10** shows ester methyl and two distinct O-methylene carbons at δ 51.8, 70.5 and 70.7 respectively. The carbonyl carbon appeared at δ 163.2 in addition to the signals for the aromatic carbons. The structure of third-generation dendrimers (G_3) **11** and **12** was also confirmed from spectral and analytical data.



Scheme 4

Reagents and conditions: i) Methyl / ethyl / isopropyl salicylate, K_2CO_3, dry acetone, RT, 24 h.



Scheme 5

Reagents and conditions: i) Methyl / ethyl / isopropyl salicylate, K_2CO_3, dry acetone, RT, 24 h

Biologicalactivity

Anti-Arthritic Activity

The anti-arthritic activities of all the synthesized dendrimers are concentration dependent and by adapting the standard protocol[13] the salicylate dendrimers **7-12** were found to possess the maximum anti-arthritic activity (85.4 %, 79.3 %, 70.1 %, 94.3 %, 94.0 % and 92.1 % at 400 µg/mL) when compared to the reference drug diclofenac sodium (58.4 % at 400µg/mL) which

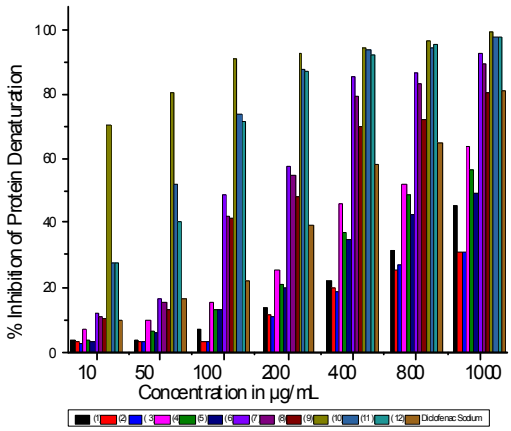


Figure 2 Anti-arthritic activities of salicylate dendrimers **1-12**

reveals that the salicylate dendrimers especially the dendrimer **10** (shows maximum activity even at a concentration of 10 µm/gL) shows better anti-arthritic activity than the reference drug diclofenac sodium. These results also reveal that the salicylate dendrimer are more stable in BSA than the diclofenac sodium.

The degree of anti-arthritic activity of salicylate dendrimers **10, 12, 11, 6, 7** and **8** at 1000 µg/mL are found to be 99.1 %, 97.9 %, 97.5 %, 92.3 %, 89.1 % and 80.6 % respectively **(Table 1** and **Figure 2)**.

Conclusion

In conclusion, methyl, ethyl and isopropyl salicylate dendrimers were synthesized by divergent approach in moderate to good yield. Those dendrimers synthesized were subjected to In-vitro anti-arthritic activity by inhibition of protein denaturation method. The second and third generation dendrimers **7-12** shows superior anti-arthritic activity than the reference drug diclofenac sodium at a concentration 100 µg/mL and above. Especially the third-generation (G3) dendrimer **10** shows better activity even at low concentration.

Experimental section

Chemistry

General: All chemicals and solvents were purchased commercially and used as such without further purification. All melting points of those synthesized compounds are uncorrected and the ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker 300-MHz instrument in $CDCl_3$ and DMSO-d_6 solvent with tetramethylsilane (TMS) as an internal reference. Elemental analyses were carried out on a 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 (TLC) developed on 0.25 mm glass plates coated with silica gel-G (ACME**)** and visualized with iodine.

Table 1 In vitro anti-arthritic activity of salicylate dendrimers **1–12** by inhibition of protein denaturation method (Bovine Serum Albumin)

Dendrimer	**Activity (% Inhibition of protein denaturation)**						
	10 µg/ml	**50 µg/ml**	**100 µg/ml**	**200 µg/ml**	**400 µg/ml**	**800 µg/ml**	**1000 µg/ml**
1	3.66±0.86	3.97±1.02	7.07±0.56	13.61±0.42	22.21±1.08	31.93±0.98	45.52±0.64
2	2.98±0.99	2.97±0.84	3.14±1.06	11.51±1.04	19.87±2.00	25.90±1.93	31.03±0.54
3	2.72±1.58	2.84±1.92	2.92±0.72	11.13±0.94	18.84±0.53	26.68±0.82	30.86±1.54
4	7.07±1.32	9.81±0.45	15.79±0.94	25.90±0.91	46.01±0.74	55.24±0.97	64.10±1.05
5	3.97±0.97	6.18±0.63	13.09±0.62	21.11±0.84	37.13±0.93	48.81±1.31	56.62±0.79
6	3.33±0.86	6.03±1.38	12.84±0.55	19.97±0.91	35.23±0.94	42.61±2.01	49.62±0.91
7	12.72±0.82	16.33±0.80	48.78±0.56	57.74±1.03	85.44±0.76	86.62±0.93	92.31±0.54
8	11.09±1.23	15.83±0.92	42.16±0.67	54.80±0.59	79.26±0.62	83.21±0.81	89.08±0.29
9	10.75±0.95	13.32±0.81	41.34±0.78	48.05±0.62	70.08±0.93	72.34±0.75	80.64±0.34
10	70.92±0.91	80.64±0.54	91.09±0.19	92.38±0.68	94.29±1.08	96.64±1.04	99.11±0.97
11	27.93±0.94	52.12±0.42	73.98±0.45	88.14±0.84	93.98±0.82	94.28±1.13	97.52±1.54
12	27.71±1.01	40.13±0.47	71.65±0.95	87.50±0.78	92.11±0.87	95.08±0.91	97.91±0.53
Diclofenac Sodium	9.50±1.21	16.32±0.64	22.20±0.95	39.37±0.64	58.42±0.87	64.49±1.08	81.42±0.98

General procedure for esterification

To a solution of the carboxylic acid (5 g) in ethanol (50 ml), $SOCl_2$ (about 1 ml) was added at 0 °C. The reaction mixture was then refluxed for 12 h. The solvent was removed under reduced pressure. The residue, thus obtained was dissolved in $CHCl_3$ (200 ml), washed with water (2x 100 ml), brine (100 ml) and dried over anhydrous Na_2SO_4. Removal of the solvent afforded the esters [17a-e] which was used as such without further purification.

General procedure for LAH reduction

To a stirred suspension of LAH (1.2 eq.,/ester group) in dry THF, the ester compound in THF was added slowly under N_2 atmosphere at 0-5 °C. The above reaction mixture was then allowed to reach room temperature and heated at 50 °C for 8 h. It was then cautiously quenched with 10% NaOH solution at 0 °C. The reaction mixture was then filtered, and the residue obtained was agitated with THF (3x50ml) and the combined THF layers were evaporated. The residue obtained was dissolved in $CHCl_3$ and extracted with $CHCl_3$ (3x100ml), washed with brine (100 ml), dried over Na_2SO_4 and evaporated to give the hydroxyl compound as crude product, which was purified using silica gel 100-200 using $CHCl_3$:hexane (3:5) as eluent.

*1,3,5-tris(Hydroxy methyl) benzene (**15**)*
White solid. mp: 123-125 °C; Yield: 73 %; ^{1}H NMR (300 MHZ, DMSO-d_6): δ_H 4.47-4.49 (d, J=5.7 Hz, 6H), 5.18-5.22 (m, 3H), 7.13 (s, 3H); ^{13}C NMR (75 MHZ, DMSO-d_6): δ_C 62.9, 122.9, 142.0

*(5,5',5''-(benzene-1,3,5-triyltris(methylene)) tris(oxy)tris(benzene-5,3,1-triyl))hexamethanol (**18**)*
Off-white solid. mp: 138-142 °C; Yield: 71 % ; ^{1}H NMR (300 MHz, DMSO-d_6): δ_H 3.38 (s, 12H), 4.39-4.49 (m, 6H), 6.37-6.38 (m, 9H), 6.56-6.60 (m, 3H); ^{13}C NMR (75 MHZ, DMSO-d_6): δ_C 61.9, 68.9, 114.5, 122.2, 125.8, 137.3, 138.7, 153.7; Anal. calcd for $C_{33}H_{36}O_9$: C, 68.74; H, 6.29; found: C, 68.72; H, 6.18.

General procedure for bromination

To a solution of triol / hexa / dodeca / tetracosa hydroxy compound (9.4 mmol) in $CHCl_3$ (50 ml) was added an excess of PBr_3 at 0 °C and stirred for 1 h. The reaction mixture was then allowed to reach room temperature and stirred for further 6 h. After the completion of the reaction, it was extracted with $CHCl_3$ (3x100 ml), washed with aq., $NaHCO_3$ (100 ml), brine (100 ml), dried over Na_2SO_4 and evaporated. The residue obtained was purified by column chromatography using $CHCl_3$/hexane (2:3) as eluent.

*1,3,5-tris(Bromomethyl)benzene (**16**)*
White solid. mp: 79-81 °C; Yield: 88 %; ^{1}H NMR (300 MHZ, $CDCl_3$): δ_H 4.46 (s, 6H), 7.36 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 32.2, 129.6, 139.1; Anal. calcd for $C_9H_9Br_3$: C, 30.27; H, 2.54; found: C, 30.21; H, 2.48; m/z: 357 [M+1]

*1,3,5-tris((3,5-bis(Bromomethyl)phenoxy)methyl)benzene (**19**)*
White solid. mp: 135-138 °C; Yield : 85 % ; ^{1}H NMR (300 MHZ, $CDCl_3$): δ_H 4.43 (s, 12H), 5.11 (s, 6H), 6.96 (s, 6H), 7.03 (s, 3H), 7.48 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 69.5, 69.7, 115.4, 122.2, 126.1, 137.4, 139.7, 158.9; Anal. calcd for $C_{33}H_{30}Br_6O_3$: C, 41.55; H, 3.17; found: C, 40.98; H, 3.10.

*1,3,5-Tris((3,5-bis((3,5-bis(bromomethyl)phenoxy)methyl) phenoxy)methyl) benzene (**22**)*
Off-white solid. mp: 148-152 °C; Yield: 85 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.04 (s, 30H), 5.09 (s, 12H), 6.87-6.90 (m, 12H), 7.10-7.13 (m, 3H), 7.40-7.47 (m, 6H), 7.86 (s, 3H), 7.87-7.88 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 58.4, 58.5, 115.6, 121.3, 124.2, 139.6, 139.7, 156.9.

*1,3,5-Tris((3,5-bis((3,5-bis((3,5-bis(bromomethyl)phenoxy) methyl)phenoxy)methyl) phenoxy)methyl)benzene (**25**)*
Off-white solid. mp: 172-174 °C; Yield: 83 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 5.02 (s, 48H), 5.03 (s, 42H), 6.79 (s, 24H), 6.87-6.90 (m, 12H), 7.02-7.13 (m, 12H), 7.40-7.46 (m, 12H), 7.80-7.86 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 69.4, 69.7, 115.4, 115.5, 121.8, 126.1, 136.4, 137.4, 157.4

General Procedure for etherification

A solution containing the diethyl 5-hydroxy isophthalate / salicylates (2.1 mmol/4.2 mmol/8.4 mmol/16.8 mmol) and the tribromide **16**/ hexabromide **19**/ dodecabromide **22**/ tetracosabromide **25** (0.7 mmol) was stirred with K_2CO_3 (5.1 mmol/10.1 mmol/15.1 mmol/ 20.1 mmol) in dry acetone at room temperature for 24 h after which the reaction mixture was filtered-off and the solvent was evaporated to dryness to give the multifunctional ester compound / dendrimer as residue which was then purified by column chromatography using hexane: $CHCl_3$ (3:2) as eluent.

*Hexaethyl5,5',5''-(benzene-1,3,5-triyltris(methylene))tris(oxy) triisophthalate (**17**)*
White solid. mp: 122-124 °C; Yield: 83 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.39-1.44 (m, 18H), 4.37-4.44 (m, 12H), 5.21 (s, 6H), 7.57 (s, 3H), 7.85 (d, J=0.9 Hz, 6H), 8.31 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 14.4, 61.5, 70.1, 119.9, 123.4, 126.4, 132.2, 137.4, 158.6, 165.7

*Dodecaethyl 5,5',5'',5''',5'''',5'''''-(5,5',5''-(benzene-1,3,5-triyltris (methylene)) tris(oxy)tris(benzene-5,3,1-triyl))hexakis(methylene) hexakis(oxy)hexaisophthalate (**20**)*
Off-white solid. mp: 174-177 °C; Yield: 84 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.36-1.43 (m, 36H) , 4.34-4.43 (m, 24H), 5.09 (s, 12H), 5.12 (s, 6H), 6.95 (s, 6H), 7.07 (s, 3H), 7.14 (s, 3H), 7.81 (s, 12H), 8.21-8.28 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 16.5, 59.3, 69.5, 69.7, 115.0, 115.4, 115.5, 112.2, 126.1, 137.4, 139.7, 158.9; Anal. calcd for $C_{105}H_{108}O_{33}$: C, 66.45; H, 5.74; found: C, 66.30; H, 5.63.

*Compound **23***
Off-white solid. mp: 185-187 °C; Yield: 81 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.38-1.41 (m, 72H), 4.36-4.43 (m, 48H), 5.08 (s, 24H), 5.12 (s, 18H), 6.99 (s, 6H), 7.09 (s, 12H), 7.12 (s, 6H), 7.75 (s, 6H), 7.81 (s, 24H), 8.28 (s, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 14.3, 61.5, 69.9, 70.1, 114.4, 120.0, 120.9, 122.1, 123.2, 132.1, 138.3, 158.7, 165.9.

*Dendrimer **1***
Off-white solid. mp: 75-77 °C; Yield: 74 % ; ^{1}H NMR (300 MHZ, $CDCl_3$): δ_H 3.88 (s, 9H), 5.22 (s, 6H), 6.98-7.02 (m, 6H), 7.39-7.47 (m, 3H), 7.59 (s, 3H), 7.81-7.85 (m, 3H); ^{13}C NMR (75 MHZ, $CDCl_3$): δ_C 52.0, 70.6, 114.1, 120.8, 124.8, 125.9, 131.8, 133.5, 137.7, 158.0, 166.8; Anal. calcd for $C_{33}H_{30}O_9$: C, 69.45; H, 5.30; found: C, 69.23; H, 5.22; m/z: 571 [M+1]

Dendrimer **2**

Off-white solid. mp: 81-83 ^{o}C; Yield: 75 % ; ^{1}H NMR (300 MHZ, $CDCl_3$): δ_H 1.26-1.31 (m, 9H), 4.30-4.37 (m, 6H), 5.19 (s, 6H), 6.97-7.02 (m, 6H), 7.38-7.43 (m, 3H), 7.58 (s, 3H), 7.79-7.82 (m, 3H); ^{13}C NMR (75 MHZ, $CDCl_3$): δ_C 14.3, 60.9, 70.6, 114.0, 120.7, 121.3, 125.1, 131.7, 133.3, 137.6, 157.9, 166.4; Anal. calcd for $C_{36}H_{36}O_9$: C, 70.57; H, 5.92; found: C, 70.13; H, 5.67; m/z: 613 [M+1]

Dendrimer ***3***

Off-white solid. mp: 67-69 ^{o}C; Yield : 73 % ; ^{1}H NMR (300 MHZ, $CDCl_3$): δ_H 1.27-1.29 (d, *J*= 6.0 Hz, 18H), 5.19 (s, 6H), 5.21-5.27 (m, 6H), 6.97-7.02 (m, 6H), 7.37-7.42 (m, 3H), 7.58 (s, 3H), 7.76-7.79 (m, 3H); ^{13}C NMR (75 MHZ, $CDCl_3$): δ_C 21.9, 68.2, 70.6, 113.9, 120.7, 121.9, 125.4, 131.4, 133.0, 137.6, 157.8, 166.0; Anal. calcd for $C_{39}H_{42}O_9$: C, 71.54; H, 6.47; found: C, 71.46; H, 6.42.

Dendrimer ***4***

Off-white solid. mp: 110-112 ^{o}C; Yield : 71 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.87 (s, 18H), 5.13 (12 H, s), 5.17 (s, 6H), 6.96 (br, 6H), 6.99 (br, 6H) , 7.14 (s, 6H), 7.18 (s, 3H), 7.38-7.43 (m, 6H), 7.48 (s, 3H), 7.80-7.82 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 51.4, 69.5, 69.7, 111.8, 113.9, 115.4, 122.2, 126.1, 137.4, 139.7, 147.8, 158.9, 167.4; Anal. calcd for $C_{81}H_{72}O_{21}$: C, 70.42; H, 5.25; found: C, 70.36; H, 5.18; m/z: 1382 [M+1]

Dendrimer ***5***

Off-white solid; Yield : 73 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.24-1.33 (m, 18H), 4.32-4.36 (m, 12H), 5.10 (s, 12H), 5.14 (s, 6H), 6.95 (s, 6H), 6.98 (s, 6H), 7.11 (s, 3H), 7.16 (s, 6H), 7.39 (s, 6H), 7.48 (s, 3H), 7.78-7.81 (d, *J*= 7.5 Hz, 6H) ; ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 14.3, 60.9, 70.2, 70.4, 112.8, 113.8, 113.9, 120.7, 120.8, 121.3, 131.6, 131.7, 133.3, 138.3, 157.7, 166.4; Anal. calcd for $C_{87}H_{84}O_{21}$: C, 71.30; H, 5.78; found: C, 70.89; H, 5.42.

Dendrimer ***6***

Off-white solid; Yield : 69 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.24-1.33 (m, 36H), 4.32-4.44 (m, 6H), 5.09 (s, 12H), 5.14 (s, 6H), 5.21-5.27 (m, 6H), 6.95-6.98 (m, 6H), 7.11 (s, 6H), 7.16 (s, 3H), 7.39 (s, 6H),7.48 (s, 3H), 7.79-7.81 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 21.9, 68.2, 69.8, 70.6, 113.3, 120.1, 122.2, 125.4, 131.4, 133.8, 136.9, 158.8, 166.6; Anal. calcd for $C_{93}H_{96}O_{21}$: C, 72.08; H, 6.24; found: C, 71.84; H, 6.12.

Dendrimer ***7***

Pale Brown Gum; Yield: 68 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H; 3.89 (s, 36H), 5.21 (s, 24), 5.23 (s, 18H), 6.96 (s, 6H), 6.99-7.01 (m, 24H), 7.36-7.39 (m, 12H), 7.42-7.43 (m, 12H), 7.57-7.76 (m, 12H), 7.78-7.79 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 52.0, 70.4, 70.6, 109.4, 114.1, 120.8, 124.8, 125.9, 131.8, 133.5, 137.7, 142.1, 158.0, 165.7; Anal. calcd for $C_{177}H_{156}O_{45}$: C, 70.79; H, 5.24; found: C, 70.64; H, 5.13.

Dendrimer ***8***

Pale Brown Gum; Yield: 69 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.21-1.32 (m, 36H), 4.31-4.39 (m, 24H), 6.98 (s, 24H), 70.2 (s, 18H), 7.04-7.11 (m, 12H), 7.14 (s, 6H), 7.20-7.31 (m, 24H), 7.78-7.88 (m, 12H), 8.12-8.17 (m, 24H); Anal. calcd for $C_{189}H_{180}O_{45}$: C, 71.58; H, 5.72; found: C, 71.34; H, 5.48.

Dendrimer ***9***

Brown Gum; Yield: 64 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.23-1.29 (m, 72H), 5.12 (s, 24H), 5.16 (s, 18H), 5.48-5.52 (m, 12H), 6.87-6.89 (m, 6H), 6.93-7.09 (m, 12H), 7.13-7.28 (m, 24H), 7.68-7.85 (m, 12H), 7.98-8.13 (m, 24H); Anal. calcd for $C_{201}H_{204}O_{45}$: C, 72.29; H, 6.16; found: C, 72.12; H, 6.02.

Dendrimer ***10***

Brown Gum; Yield: 67 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.85 (s, 72H), 5.09 (s, 48H), 5.12 (s, 42H), 6.95 (s, 36H), 7.07 (s, 6H), 7.74 (s, 24H), 7.81 (s, 24H), 8.12-8.21 (m, 48H), 8.28 (s, 24H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 51.8, 70.5, 70.7, 116.4, 117.5, 117.7, 118.9, 121.7, 126.4, 131.3, 132.9, 133.7, 133.9, 137.0, 148.5, 161.7, 163.2; Anal. calcd for $C_{393}H_{372}O_{93}$: C, 71.70; H, 5.70; found: C, 71.30; H, 5.56.

Dendrimer ***11***

Brown Gum; Yield: 69 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.21-1.26 (m, 72H), 4.34-4.45 (m, 24H), 5.04 (s, 48H), 5.09 (s, 42H), 6.88 (s, 48H), 6.96-6.99 (m, 36H), 7.10-7.13 (m, 24H), 7.39-7.48 (m, 24H), 7.79 (s, 8H), 7.84-7.87 (m, 22H); Anal. calcd for $C_{393}H_{372}O_{93}$: C, 71.70; H, 5.70; found: C, 71.30; H, 5.56.

Dendrimer ***12***

Dark Brown Gum; Yield: 64 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.34-1.36 (m, 144H), 5.21 (s, 48 H), 5.22 (s, 42H), 5.66-5.78 (m, 24H), 6.64-6.66 (m, 23H), 7.01 (s, 34H), 7.09-7.12 (m, 32H), 7.26-7.38 (m, 22H), 7.54 (s, 31H), 7.85-7.88 (m, 12H), 7.94-7.97 (m, 8H) ; ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 21.8, 67.6, 68.9, 116.2, 116.7, 117.5, 117.6, 118.9, 120.7, 126.1, 131.3, 133.0, 133.7, 133.8, 137.0, 150.4, 161.7, 163.2; Anal. calcd for $C_{417}H_{420}O_{93}$: C, 72.38; H, 6.12; found: C, 72.30; H, 6.03.

Anti-Arthritic Studies

Test solutions consist of 0.45 ml of bovine serum albumin (5% w/v aqueous) and 0.05 ml of dendrimers **1-12** in various concentrations were prepared and the pH was adjusted to 6.3 by adding a small amount of 1 N HCl. In a similar manner test control and standard solutions were prepared by adding 0.05 ml of distilled water and 0.05 ml diclofenac sodium respectively to 0.45 ml of bovine serum albumin. Product control was prepared by adding 0.05 ml of test solutions in various concentrations to 0.45 ml of distilled water. The samples prepared were incubated at 37 ^{o}C for 20 minutes and then heated to 57 ^{o}C for 3 minutes. After cooling, 2.5 ml of phosphate buffer (pH 6.3) was added to each solution. The optical densities (OD) of the samples were measured from a spectrophotometer at 660 nm. Each experiment was done in triplicate and taken the average.

The percentage inhibition of Protein denaturation was calculated as follows.

$$\text{Percent Inhibition} = \frac{100 - [\text{OD of test solution} - \text{OD of product control}]}{\text{OD of test control}} \times 100$$

The control represents 100 % protein denaturation and the result was compared with diclofenac sodium treated samples.

Acknowledgements

The authors thank DST-PURSE, New Delhi for financial assistance, DST-FIST for providing NMR facility to the department and Faculty of Pharmacy, Sri Ramachandra University, Chennai-600 116 for in-vitro anti-arthritic studies.

References and notes

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† Electronic Supplementary Information (ESI) available: The ^{1}H and ^{13}C spectra of all the synthesized compounds and dendrimers. See DOI: 10.1039/b000000x/

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17. (a) *Methyl Salicylate:* Yield: 93 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 3.95 (s, 3H), 6.86-6.91 (dt, J_1=5.2 Hz, J_2=1.2 Hz, 1H), 6.97-7.0 (dd, J_1=7.2 Hz, J_2=1.2 Hz, 1H), 7.43-7.49 (dt, J_1=5.2 Hz, J_2=1.2 Hz, 1H), 7.82-7.86 (dd, J_1=4.8 Hz, J_2=1.8 Hz, 1H), 10.77 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 52.3, 112.4, 117.6, 119.2, 129.9, 135.7, 161.6, 170.6 ;
(b) *Ethyl Salicylate:* Yield: 95 % ; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.30-1.34 (t, *J*= 7.2 Hz, 3H), 4.27-4.35 (q, *J1*= 7.2 Hz, *J2*=14.4 Hz, 2H), 6.75-6.81 (m, 1H), 6.87-6.90 (dd, *J1*=0.9 Hz, *J2*=8.4 Hz, 1H), 7.32-7.38 (m, 1H), 7.74-7.77 (dd, *J1*=3.6 Hz, *J2*=8.1 Hz, 1H), 10.78 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 13.4, 61.7, 111.6, 115.4, 117.9, 128.8, 134.2, 160.9, 170.4;
(c) *Isopropyl Salicylate:* Yield: 93 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.34-1.36 (d, *J*=6.0 Hz, 6H), 5.19-5.26 (m, 1H), 6.61-6.66 (m, 2H), 7.22-7.28 (m, 1H), 7.85-7.88 (m, 1H), 10.50 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 22.0, 67.6, 111.6, 116.2, 116.6, 131.3, 133.49, 150.4, 167.7;
(d) *Diethyl 5-hydroxy isophthalate:* Yield: 92 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.39-1.44 (t, *J*=7.2 Hz, 6H), 4.37-4.45 (q, *J*1=7.2 Hz, *J*2=14.4 Hz, 4H), 7.15 (s, 1H), 7.81 (d, *J*=1.5 Hz, 2H), 8.24 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 14.2, 61.7, 121.0, 122.6, 132.0, 156.5, 166.2; MS: m/z 239 (M^+);
(e) *Triethyl benzene-1,3,5-tricarboxylate(14):* Yield: 93 %; ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 1.42-1.47 (t, *J*=7.2 Hz, 9H), 4.42-4.49 (q, *J*1=7.2 Hz, *J*2=14.4 Hz, 6H), 8.85 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 14.3, 61.7, 131.4, 134.4, 165.1