



Supporting Information

Biodegradable and biocompatible spherical dendrimer nanoparticles with a gallic acid shell and a double-acting strong antioxidant activity as potential device to fight diseases from «oxidative stress»

Silvana Alfei*, Silvia Catena and Federica Turrini

Department of Pharmacy, University of Genoa. Viale Cembrano, 4 I-16148 GENOA, ITALY

*Corresponding Author: Prof. Silvana Alfei
Department of Pharmacy, University of Genoa
Phone number: +39-010-3532296
Fax number: +39-010-3532684
Email: alfei@difar.unige.it
ORCID: 0000-0002-4630-4371

Table of Contents

Graphical Abstract for ESM

Figure S1: Structure of dendrons D4BnA, D4BnOH and D5BnA

Section S1: Table S1

Synthetic procedures for preparing dendrimers **3** and **4**

Section S2: Synthetic procedures for preparing compounds **5**, **6** and **7**

Section S3: Synthetic procedures for preparing GA-dendrimer (**9**) via dendrimer **8**

*FeCl₃ essay for phenols recognition for qualitative evaluation of GA-dendrimer (**9**) (Fig. S3.1)*

Section S4: Characterization of compounds **3-7** and GA-dendrimer (**9**)

Table S4.1: Molecular Weight (MW) and significant physicochemical data of isolated compounds

Section S5: Radical Scavenging Activity (RSA %) of GA-dendrimer (**9**)

Fig. S5.1: RSA % curves versus $\mu\text{g/mL}$ (a), $\mu\text{mol/mL}$ (b) and mM concentrations (c)

*Table S5.1: IC₅₀ values determined by us (GA-dendrimer (**9**), GA, AA and Trolox) or reported (a tocopherol) versus $\mu\text{g/mL}$, $\mu\text{mol/mL}$ and mM concentrations*

*In vitro investigation of GA-dendrimer (**9**) cytotoxicity by cell (%) viability evaluation*

Fig. S5.2: Cell viability (%) graph

Section S6: Qualitative evaluation of Pig Liver Esterase Hydrolyzed Product (PLEHP) **10**

FeCl₃ essay for phenols recognition (Fig. S6.1)

Thin Layer Chromatography (TLC) (Fig. S6.2)

Characterization data (m.p., FT-IR and NMR spectra peaks list and

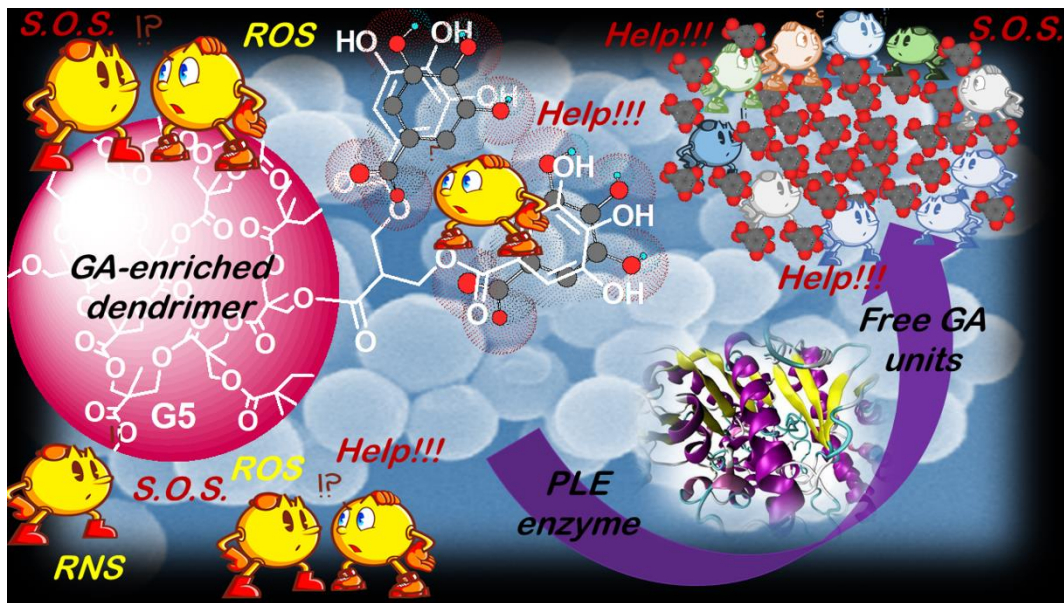
*Elemental Analysis results of a sample of **10** recrystallized from ethanol (**10c**)*

*Copies of NMR spectra of **10c** versus those ones of commercial GA (Fig. S6.3)*

Section S7: Copies of FT-IR and NMR spectra of compounds **3-8** and of GA-dendrimer (**9**) (Fig. S7.1-S7.21)

References

Graphical Abstract for ESM



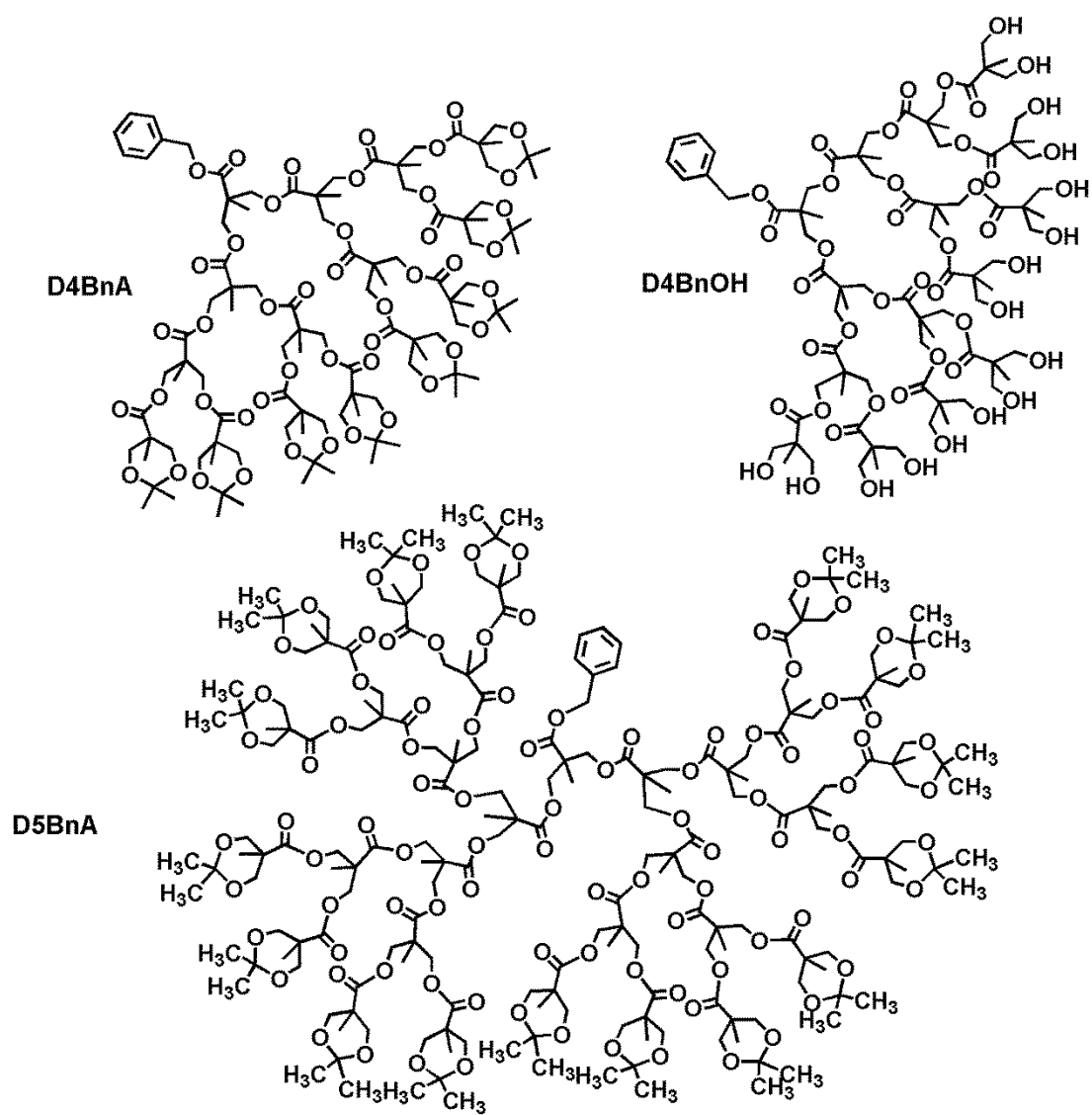


Fig. S1 Structure of dendrons D4BnA, D4BnOH and D5BnA

Section S1

Table S1 Experimental data for the preparation of compounds **3-9**

Entry (g; mmol)	Reagents (mg; mmol) ^a	Solvents (mL)	Time T (°C)	Product (g; mmol; %)
2 2.64; 0.600	DCC ^b 145.4; 0.70 DPTS ^c 124.4; 0.42 Diol 21.4; 0.28	DCM ^d 11	24h; r.t.	3 2.34; 0.27; 98.0
3 2.34; 0.270	Resins H ⁺ 500	MeOH 22	24h r.t.	4 1.94; 0.267; 99.0
1 398.7; 2.34	TBDMSCl 1.98; 13.1 Imidazole 1.60; 23.4	DMF ^e 6	19h r.t.	5 1.35 ^f ; 2.15; 91.9 ^f
5 1.20; 1.91	Acetic Acid 36 mL H ₂ O 12 mL	THF ^g 35	24h r.t.	6 0.94; 1.89; 99.0
6 0.93; 1.81	SOCl ₂ 861.3; 7.24; 526.1 μL	DCM ^d 7.2 DMF ^{e,h} 3ggt	2h; r.t.	7 0.96; 1.81; 100.0
4 159.3; 0.022	7 928.2; 1.75 DMAP ⁱ 2.7; 0.022 TEA ^l 207.4; 2.05	DCM ^d 5	24h; r.t.	8 0.82 ^m ; 0.02; 95.5 ^m
8 1.00; 0.0256	Acetyl Chloride 1540; 19.7; 1.4 mL	MeOH 10 EtOH 10	5h; r.t.	9 0.43; 0.025; 97.6

^aIf the unit of measurement is not specified. ^bDicyclohexylcarbodiimide. ^c4-(Dimethylamino)pyridinium 4-toluene-sulfonate. ^dDichloromethane. ^e*N,N*-Dimethylformamide. ^fWeight, mmol and yield have been calculated considering the impurities. ^gTetrahydrofuran. ^hCatalyst; ⁱ*N,N*-dimethylaminopyridine. ^l*N,N,N*-Triethylamine. ^mNot purified product.

Synthesis of dendrimer **3**

A solution made of a mixture of dendrimer **2** (2.64 g, 0.6199 mmol), 1, 3-propanediol (0.0214 g, 0.2818 mmol, 20.4 μl) and DPTS (0.1244 g, 0.4227 mmol) in CH₂Cl₂ (8 ml) was treated with dicyclohexylcarbodiimide (DCC) (0.1454 g, 0.7045 mmol) dissolved in DCM (3 ml) at room temperature under N₂ and magnetic stirring for 24 h. The precipitated dicyclohexylurea (DCU) was removed by filtration and washed with CH₂Cl₂. Filtrate and washings were combined, concentrated at reduced pressure and taken with ethyl acetate (EtOAc) to make precipitate DPTS which was filtered and washed with EtOAc. The solvent was removed at reduced pressure to give the crude dendrimer **3** (2.78 g) which was submitted to a careful column chromatography to eliminate traces N-acylureic adduct of **2** highlighted by IR analysis and performed as follows. Dendrimer **3** was dissolved in the minimum quantity of a mixture petroleum ether/EtOAc=1:1 and passed through a short silica gel column (h=20 cm, ø=2 cm) using the same mixture of solvents (100 ml) collecting 1 mL fractions up to disappearance of IR bands at 2118 cm⁻¹ (DCC) and at 1648, 1527 cm⁻¹ (*N*-acylureic adduct of **2**). The chromatography was developed with petroleum ether/EtOAc=2:3 (100 ml) and completed with the mixture 1:4 (100 ml) and EtOAc 100% (200 ml) collected as a single fraction. The removal of the solvent at reduced pressure afforded **3** as glassy off-white solid which was brought to constant weight under vacuum (2.34 g, 0.2700 mmol, 98%).

Synthesis of dendrimer **4**

A solution of **3** (2.34 g, 0.2700 mmol) in MeOH (22 ml) was treated with four spatula tips of acid resin Dowex 50 WX2-200 at room temperature with magnetic stirring for 24 h.

The resins were removed by filtration and washed with fresh MeOH.

Filtrate and washings were combined, concentrated at reduced pressure to give a pink glassy solid which was left under magnetic stirring overnight in excess of dry Et₂O, filtered to give **4** as a pink hygroscopic solid (2.40 g) which was further purified by dissolution in H₂O (80 ml), centrifuged to remove insoluble residues and freeze-dried to obtain **4** as fluffy highly hygroscopic white solid (1.94 g, 0.270, 99 % isolated yield) which was stored in a dryer on P₂O₅.

Section S2

Synthesis of *tert*-Butyldimethylsilyl 3,4,5-tri[(*tert*-butyldimethylsilyl)oxy]benzoate (**5**) [1]

Commercial Gallic Acid (GA) **1** (0.3987 g, 2.34 mmol), imidazole (10 equiv., 1.60 g, 23.40 mmol) and recently bought TBDMSCl (5.6 equiv., 1.98 g, 13.10 mmol) were dissolved in DMF (6 mL) at room temperature under N₂ and maintained

under magnetic stirring overnight. The reaction was stopped when the GA spot was no longer found in TLC (cyclohexane/EtOAc 4/1). Rf. GA = 0; Rf. **5** = 0.84. The suspension was diluted with Et₂O and the organic phase was washed with water (3 x 16 mL). Once separated from aqueous phase, the organic one was dried on MgSO₄. After solvent removal **5** was obtained as sticky colourless resin (1.35 g, 2.15 mmol, 91.9 %, net of traces of impurities found with NMR analysis).

Synthesis of 3,4,5-tri[(tert-butyldimethylsilyl)oxy]benzoic acid (6) [1]

Compound **5** (1.20 g, 1.91 mmol) was dissolved in 35 ml of THF at room temperature. A 3:1 mixture of AcOH-H₂O (48 ml) was then added to the solution and the mixture was stirred at room temperature for 24 hours. The reaction was stopped when the **5** spot was no longer found in TLC (cyclohexane/EtOAc 4/1). The reaction mixture was poured into ice-cold H₂O (80 ml) and the product was extracted with EtOAc (3 x 40 ml), washed with brine (2 x 40 ml), dried over MgSO₄ and concentrated in vacuo to give crude **6** as white solid smelling of acetic acid. It was then recrystallized from MeOH obtaining in two successive precipitates the purified **6** as odorless white crystals (0.9400 g, 1.89 mmol, 99.0 % isolated yield).

Synthesis of 3,4,5-tri[(tert-butyldimethylsilyl)oxy]benzoyl chloride (7)

Compound **6** (0.9300 g, 1.81 mmol) was dissolved in DCM (7.2 ml), cooled to 0°C and added with SOCl₂ (0.8613 g, 7.24 mmol, 0.5211 ml), catalytic DMF (3 drops) and allowed to reach room temperature. The reaction was monitored by FTIR analysis and was stirred until the disappearance of C=OOH peak at 1680 cm⁻¹ with concomitant appearance of a peak at 1759 cm⁻¹ (C=OCl). After 2 h at room temperature, the solvent was removed obtaining a waxy brown solid with a pungent smell. It was washed first with toluene and then with *n*-hexane, eliminating from time to time insoluble dark pitches and obtaining **7** as a pale yellow waxy solid. Its level of purity was early tested by TLC (cyclohexane/EtOAc 10/1) and, since it was very good, **7** was used in the subsequent reaction without further purification (0.9600, 1.81 mmol, 100% yield).

Section S3

Synthesis of GA-dendrimer 9 via dendrimer TBDMS-GA-dendrimer 8

Esterification reaction of dendrimer 4 with 7

A solution of dendrimer **4** (0.1593 g, 0.0219 mmol), DMAP (0.0027 g, 0.0219 mmol) and TEA (0.2074 g, 2.05 mmol, 0.2860 ml) in DCM (3 ml) cooled to 0° C, was dropwise added with chloride acid **7** (0.9282 g, 1.75 mmol) in turn dissolved in DCM (2 ml). Once reached the room temperature the reaction mixture was stirred overnight. A TLC (cyclohexane/EtOAc 10/1) was performed to evaluate the reaction status. Only traces of unreact **7** were detected while the FTIR spectrum of a small hydrolyzed sample showed both the peak of the C=OO group (around 1733 cm⁻¹) of **4** scaffold and the C=OO peak of the conjugate ester type (1723 cm⁻¹) indicating the occurrence of peripheral functionalization. At the same time the band relative to the OH groups of **4** was strongly diminished. The reaction mixture was hydrolyzed with 10% KHSO₄ (10 ml), extracted with DCM (3 x 15 ml) and dried over MgSO₄. After evaporation of the solvent, the dendrimer functionalized with protected GA **8** was obtained as an orange glassy solid (0.8179, 0.021 mmol, 95.5 yield). Compound **8** was subjected to deprotection reaction without further purification.

Removal of TBDMS protecting groups from 8: synthesis of GA-loaded dendrimer 9

As previously reported [2,3], a solution of crude dendrimer **8** (1.00 g, 0.0256 mmol) in MeOH/EtOH 1/1 (20 ml) cooled to 0°C, was treated with a strong excess of acetyl chloride (4 eq./TBDMS group, 1.54 g, 19.7 mmol, 1.4 ml) and was stirred for 5 h at r.t. The reaction was stopped and the solvent removed. The obtained residue was dissolved in EtOAc (40 ml) and the organic solution was washed with 15% NaOH (3 x 30 ml) to remove low molecular weight phenolic compounds (mainly unbound GA residue) and the organic phase was dried over MgSO₄. Once removed the solvent, the dark glassy residue was taken up with EtOAc (q.s.), the neutral pH was lowered with HCl at pH = 2 observing a considerable solution clarification. The organic phase was washed with water and dried again. After removal of the solvent, **9** was obtained as a highly hygroscopic brownish glassy solid carefully stored in a dryer on P₂O₅ and under vacuum (0.4252 g, 0.0250, 97.6% yield). As early qualitative investigation, the FeCl₃ assay for phenols recognition was performed to confirm the presence of free phenol groups.

FeCl₃ essay

Approximately 1 ml of 5% ethanol solution of **9** pale yellow colored was placed in a test tube and was treated with 2-3 drops of 5% FeCl₃ prepared fresh. A strong green coloring indicating the presence of phenolic groups was observed (Fig. S 3.1).



Fig. S3.1 Green coloring obtained in the FeCl₃ test performed on GA-dendrimer (**9**) solution

Section 4

Characterization data of compounds 3-8 and of GA-dendrimer (**9**)

FT-IR, NMR spectra data and Elemental analysis results of compounds 3-8 and of GA-dendrimer (**9**)

Dendrimer 3. FTIR (KBr, cm⁻¹): 2992, 2939, 2878 (CH₃ and CH₂), 1748 (C=O). ¹H NMR (300 MHz, CDCl₃) δ (ppm): 1.12, 1.17, 1.26 (s signals, 186H, CH₃ of generations), 1.33 (s, 96H, CH₃ acetonide), 1.40 (s, 96H, CH₃ acetonide), CH₂ propandiol, probably overlapped, 3.60 (d, 64H, *J* = 11.8 Hz, CH₂O acetonide), 3.71-3.91 (m, 4H, CH₂O propandiol), 4.13 (d, 64H, *J* = 11.7 Hz, CH₂O acetonide), 4.25-4.34 (m, 120H, CH₂O generations). ¹³C NMR (75.5 MHz, CDCl₃) δ (ppm): 173.48, 171.80 (C=O), 98.12 (quaternary C of acetonide), 67.29, 65.95, 65.90, 64.83 (CH₂O), 49.11, 46.82, 46.70 (quaternary C of three generations), 42.03 (quaternary C of fourth generation), 25.24 (CH₃ of acetonide), 22.00 (CH₃ of acetonide), 18.49, 17.68 (CH₃ of generation). Found: C, 57.05; H, 7.18. C₄₀₉H₆₃₇O₁₈₈ requires C, 57.41; H, 7.44%.

Dendrimer 4. FTIR (KBr, cm⁻¹): 3433 (OH), 2933, 1733 (C=O). ¹H NMR (300 MHz, DMSO-*d*₆) δ (ppm): 1.01, 1.16, 1.18, 1.23, 1.34 (five s signals, 186H, CH₃ of generations), 1.70 (m, 2H, CH₂ propandiol), 3.52 (dd, 128H, CH₂OH), 3.56 (partially overlapped signal, 2H, CH₂O propandiol), 3.98 (partially overlapped signal, 2H, CH₂O propandiol), 4.08-4.18 (m, 120H, CH₂O of four generations), 4.37 (br s, 64H, OH). ¹³C NMR (75.5 MHz, DMSO-*d*₆) δ (ppm): 173.94, 171.73 (C=O), 64.27, 63.55 (CH₂O), 50.13 (quaternary C of fifth generation), 46.12 (other generation detectable quaternary C), 17.05, 16.61 (CH₃ of generations). Found: C, 51.71; H, 7.01. C₃₁₃H₅₀₄O₁₈₈ requires C, 51.67; H, 6.98%.

tert-Butyldimethylsilyl 3,4,5-tri[(tert-butyldimethylsilyl)oxy]benzoate (5**) [1].** TLC: R_f = 0.84 (cyclohexane/EtOAc 4/1). FTIR (KBr, cm⁻¹): 2933, 2861 (CH₃ and CH₂), 1703 (C=O), 1258, 1092. ¹H NMR (300 MHz, CDCl₃) δ (ppm): [0.017 and 0.10 (two s, CH₃Si, impurities)], 0.14 (s, 6H), 0.24 (s, 12H), 0.36 (s, 6H), [0.87 and 0.92 (two s, CH₃ t-Buthyl, impurities)], 0.95 (s, 18H), 0.99 (s, 9H), 1.01 (s, 9H), 7.23 (s, 2H). ¹³C NMR (75.5 MHz, CDCl₃) δ (ppm): 166.31, 148.31, 143.18, 123.26, 115.95, 26.19, 26.12, [25.70 and 25.66 (impurities)], 25.60, 18.82, 18.48, [18.13 (impurity)], 17.70, [-2.94 (impurity)], -3.58, [-3.64 (impurity)], -3.89, -4.80. Found: C, 59.00; H, 10.02; Si, 12.78. C₃₁H₆₂O₅Si₄ requires C, 59.39; H, 9.98; Si, 12.77%.

3,4,5-tri[(tert-butyldimethylsilyl)oxy]benzoic acid (6**) [1].** TLC: R_f = 0.44 (cyclohexane/EtOAc 4/1). m.p. 237°C [lit.¹: m.p. 230°C (MeOH)]. FTIR (KBr, cm⁻¹): 3650-3100 (OH), 2933, 2900, 2861 (CH₃ and CH₂), 1687 (C=OOH), 1259, 1086. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 0.15 (s, 6H), 0.25 (s, 12H), 0.96 (s, 18H), 1.00 (s, 9H), 7.29 (s, 2H); ¹³C-NMR (75.5 MHz, CDCl₃) δ (ppm): 172.01, 148.49, 144.07, 121.07, 116.12, 26.19, 26.11, 18.82, 18.51, -3.63, -3.88. Found: C, 58.58; H, 9.40; Si, 16.00. C₂₅H₄₈O₅Si₃ requires C, 58.56; H, 9.44; Si, 16.38%.

3,4,5-tri[(tert-butyldimethylsilyl)oxy]benzoyl chloride (7**).** TLC: R_f = 0.80 (cyclohexane/EtOAc 10/1). FTIR (KBr, cm⁻¹): 2932, 2860 (CH₃ and CH₂), 1759 (C=OCl). ¹H-NMR (300 MHz, CDCl₃) δ (ppm): 0.15 (s, 6H), 0.26 (s, 12H), 0.96 (s, 9H), 0.99 (s, 18H), 7.32 (s, 2H). ¹³C NMR (75.5 MHz, CDCl₃) δ (ppm): 167.11, 148.76, 145.92, 124.65, 117.42, 26.14, 26.00, 18.86, 18.53, -3.67, -3.87. Found: C, 56.22; H, 9.05; Cl, 7.00; Si, 16.02. C₂₅H₄₇ClO₄Si₃ requires C, 56.53; H, 8.93; Cl, 6.68; Si, 15.81%.

TBDMS-GA-loaded dendrimer **8**

Not purified product. TLC: R_f = 0.70 (cyclohexane/EtOAc 10/1). FTIR (KBr, cm⁻¹): 2958, 2932, 2897, 2860 (CH₃ and CH₂), 1741 (C=O inner matrix), 1726 (peripheral conjugated C=OOGA), 1259, 1093.

GA-loaded dendrimer 9

FTIR (KBr, cm^{-1}): 2932, 2899, 2861 (CH_3 and CH_2 dendrimer matrix), 1741 ($\text{C}=\text{O}$ inner matrix), 1726 (peripheral conjugated $\text{C}=\text{OOGA}$). ^1H NMR (300 MHz, $\text{DMSO}-d_6$), δ (ppm): 1.01, 1.16, 1.18, 1.23, 1.34 (five s signals, 186H, CH_3 of generations), 1.70 (m, 2H, CH_2 propandiol), 3.95 (m, 128H, GA esterified CH_2O), 4.05-4.40 (m, 120H, CH_2O of four generations), 7.32 (s, 128H, GA phenyl $\text{CH}=\text{}$), 8.00-10.00 (br s, GA phenols OH). ^{13}C NMR (75.5 MHz, $\text{DMSO}-d_6$) δ (ppm): 173.94, 171.73 ($\text{C}=\text{O}$ of dendrimer scaffold), 167.11 ($\text{C}=\text{O}$ of GA), 148.80, 145.94, 124.67 (quaternary C of phenyl), 117.41 ($\text{CH}=\text{}$ of phenyl), 64.27, 63.55 (CH_2O), 50.13 (quaternary C of fifth generation), 46.12 (other generation detectable quaternary C), 17.05, 16.61 (CH_3 of generations). Found: C, 54.03; H, 4.89. $\text{C}_{761}\text{H}_{760}\text{O}_{444}$ requires C, 53.72; H, 4.51%.

Molecular Weight (MW) and Elemental analysis results of compounds 3-7 and of GA-dendrimer (9)

Table S4.1 Elemental Analysis data and other physicochemical data of the isolated reported compounds

Compound	Formula	MW	Required (%)	Found (%)	Error (%)	Physical state
3	$\text{C}_{409}\text{H}_{632}\text{O}_{188}^a$	8557.28 ^a	C 57.41	C 57.05	C 0.36	Glassy Off-white solid
			H 7.44	H 7.18	H 0.26	
4	$\text{C}_{313}\text{H}_{504}\text{O}_{188}^a$	7275.24 ^a	C 51.67	C 51.71	C 0.04	Fluffy white hygroscopic solid
			H 6.98	H 7.01	H 0.03	
5	$\text{C}_{31}\text{H}_{62}\text{O}_5\text{Si}_4$	626.37	C 59.39	C 59.00	C 0.39	Viscous resin
			H 9.98	H 10.02	C 0.04	
			Si 12.77	Si 12.78	H 0.01	
6	$\text{C}_{25}\text{H}_{48}\text{O}_5\text{Si}_3$	512.90	C 58.56	C 58.58	C 0.02	White crystals
			H 9.44	H 9.40	H 0.04	
			Si 16.38	Si 16.00	Si 0.38	
7	$\text{C}_{25}\text{H}_{47}\text{ClO}_4\text{Si}_3$	531.35	C 56.53	C 56.22	C 0.31	Pale yellow waxy solid
			H 8.93	H 9.05	H 0.12	
			Cl 6.68	Cl 7.00	Cl 0.32	
9	$\text{C}_{761}\text{H}_{760}\text{O}_{444}^a$	17010.02 ^a	Si 15.81	Si 16.02	Si 0.21	Brownish glassy hygroscopic solid
			C 53.72	C 54.03	C 0.31	
			H 4.51	H 4.89	H 0.38	

^aEstimated by ^1H NMR spectra and confirmed by Elemental Analysis

Section S5

Radical Scavenging Activity (RSA %) of GA-dendrimer (9)

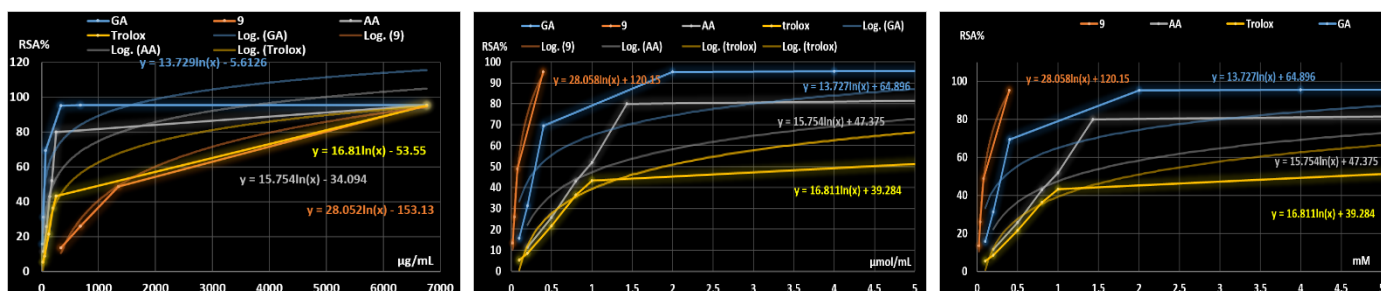


Fig. S5.1 RSA (%) curves recorded at different **9**, GA, AA and Trolox concentrations in ethanol or water solution: concentrations were given in $\mu\text{g/mL}$ (a), $\mu\text{mol/mL}$ (b) mM (c). The corresponding exponential tendency curves and related equations used to derive the IC_{50} were also provided

Table S5.1 Comparison between RSA (%) expressed as IC_{50} ($\mu\text{g/mL}$, $\mu\text{mol/mL}$, mM), of **9**, GA, AA, Trolox and α -Tocopherol [2].

Entry (MW)	IC_{50} ($\mu\text{g/mL}$)	IC_{50} ($\mu\text{mol/mL}$) ^a	IC_{50} [mM] ^a
9 ^{b,c} (17010.017)	1395.8	0.0821	0.08
GA ^{b,c} (170.12)	57.4	0.3378	0.34
AA ^{b,d} (176.12)	208.1	1.1813	1.18
Trolox ^{b,d} (250.29)	473.4	1.8916	1.89
α -Tocopherol ^e (430.7)	96.0	0.2230	0.22

^aCalculated from concentration expressed in $\mu\text{g/mL}$. ^bFrom DPPH test performed by us. ^cIn ethanol. ^dIn water. ^eLit.[2]

In vitro investigation of GA-dendrimer (9) cytotoxicity by cell (%) viability evaluation

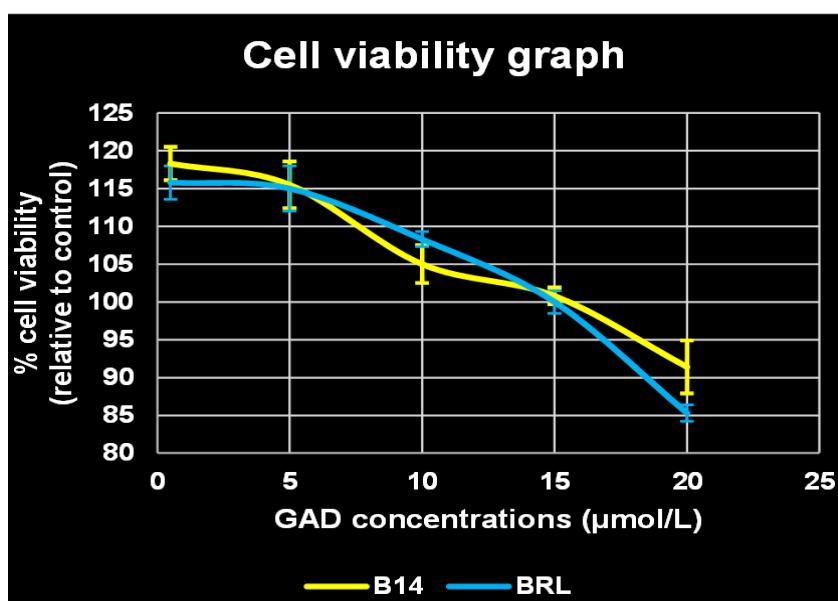


Fig. S5.2 Cell viability (%) graph

Section S6

Qualitative evaluation of Pig Liver Esterase hydrolyzed Product 10

FeCl₃ essay for phenols recognition

As early investigation, crude **10** was essayed with FeCl₃ test commonly used for phenols identification. Approximately 1 ml of 5% ethanol solution of **10** yellowish colored was placed in a test tube and was treated with 2-3 drops of 5% FeCl₃ freshly prepared. A strong blue coloring was observed. Then a commercial GA sample was essayed in the same conditions. A blue coloring identical to that observed for **10** it has been seen again (Fig. S6.1).

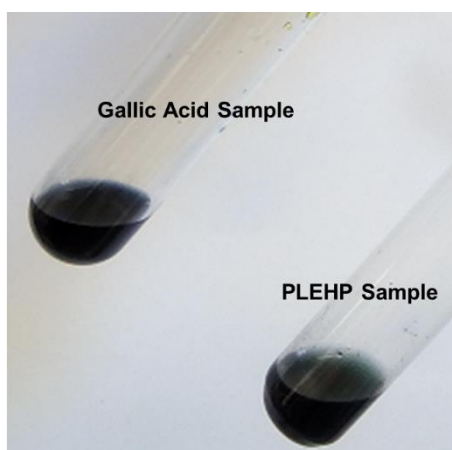


Fig. S6.1 FeCl₃ test coloring obtained from **10** sample versus commercial standard of GA

Thin Layer Chromatography (TLC)

As other early investigation a TLC (petrol ether/EtOAc/MeOH 5/5/1) was performed. A solution of **10** dissolved in acetone (q. s.) was seeded on TLC plate together with a sample of commercial GA in turn dissolved in acetone. Fig. S6.2 shows the elution profile.



Fig. S6.2 TLC of a sample of **10** versus GA

Characterization data (m.p., FT-IR and NMR spectra peaks list and Elemental analysis results) of 10c

10c. Withe crystals, m.p. 235-337°C [lit. [3] m.p. GA (ethanol): 238-240°C]. FTIR (KBr, cm⁻¹): 3400, 1689. ¹H NMR (300 MHz, CDCl₃) δ (ppm): 7.29 (s, 2H). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 172.01, 148.49, 144.07, 121.07, 116.12. Found: C, 49.15; H 3.89. C₇H₆O₅ requires C, 49.41; H, 3.56%.

Copies of NMR spectra of **10c** versus those ones of commercial GA (Fig. S6.3)

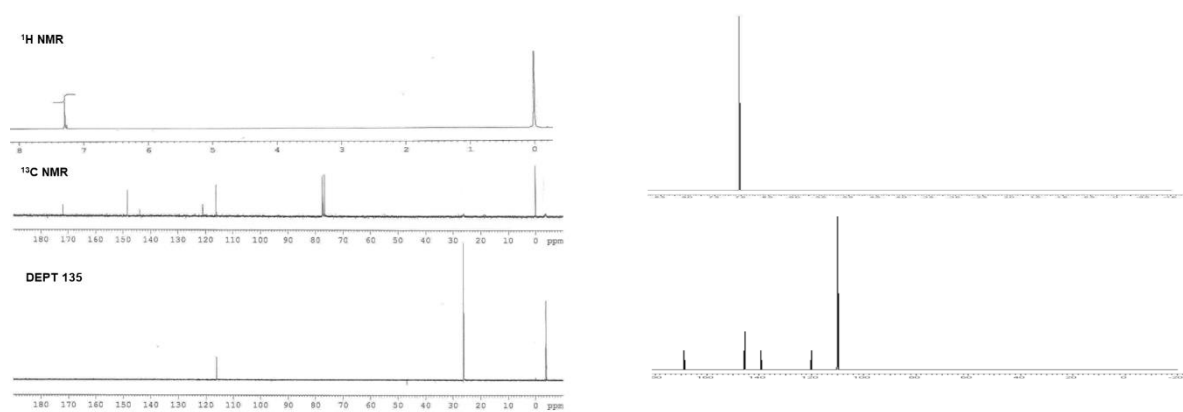


Fig. S6.3 NMR spectra of the sample recrystallized (left) and of a sample of commercial GA (right)

Section 7

Copies of FT-IR and NMR spectra of compounds 3-9 (Fig. S7.1-S7.21)

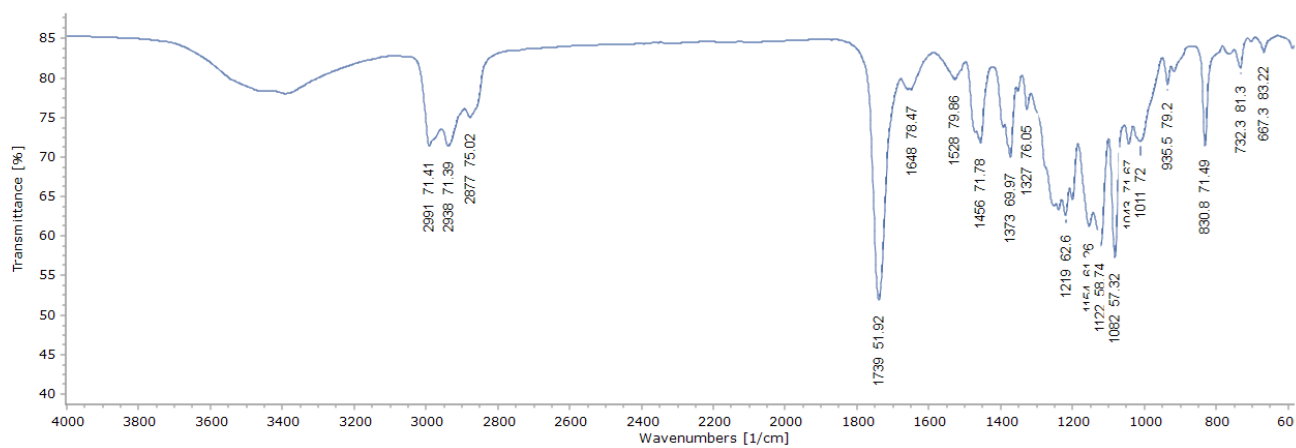


Fig. S7.1 FT-IR spectrum (KBr) of dendrimer **3**

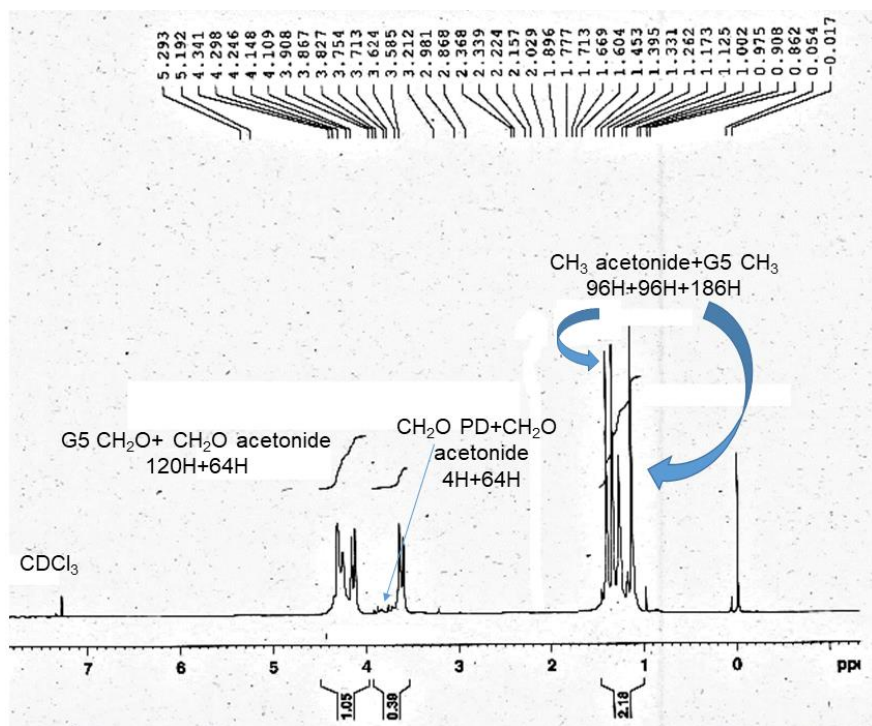


Fig. S7.2 ¹H NMR spectrum (CDCl₃, 300 MHz) of dendrimer 3

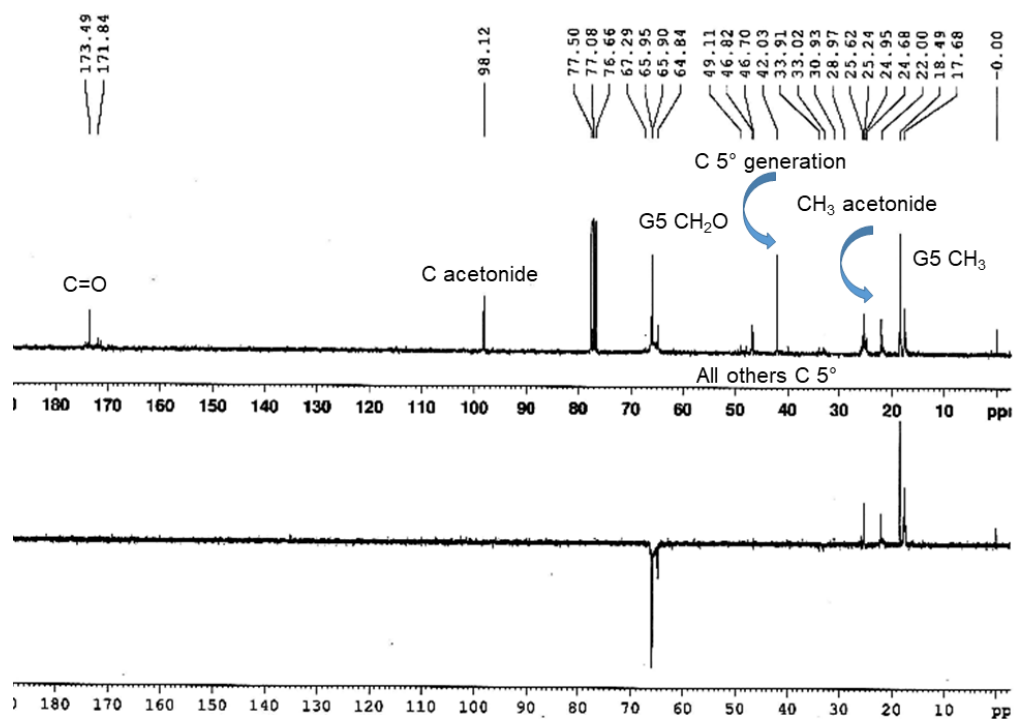


Fig. S7.3 ¹³C NMR and DEPT-135 spectra (CDCl₃, 75.5 MHz) of dendrimer 3

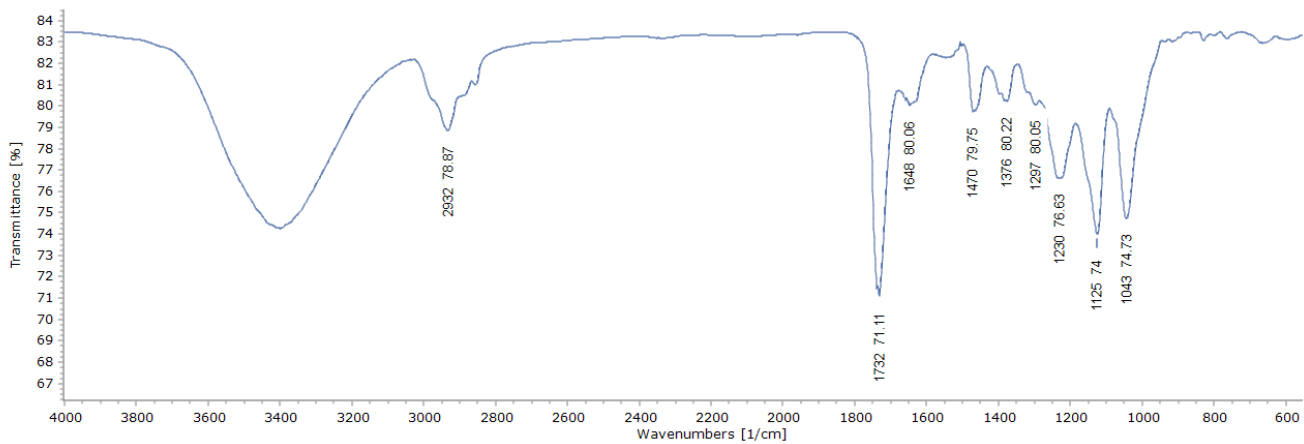


Fig. S7.4 FT-IR spectrum (KBr) of dendrimer 4

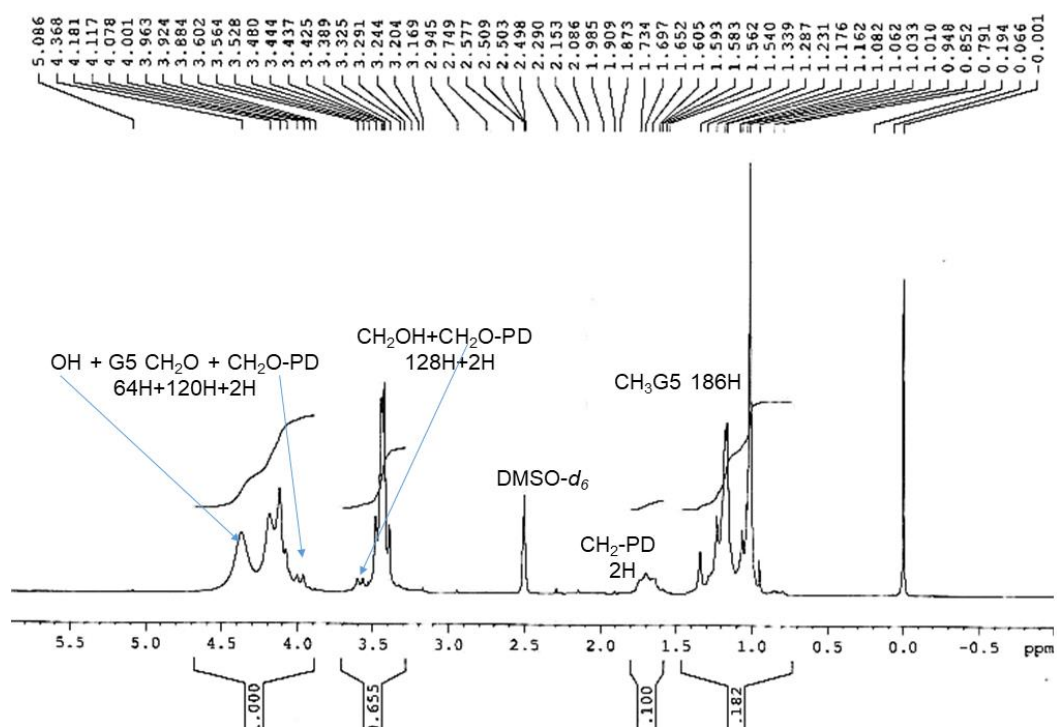


Fig. S7.5 ^1H NMR spectrum ($\text{DMSO-}d_6$, 300 MHz) of dendrimer 4

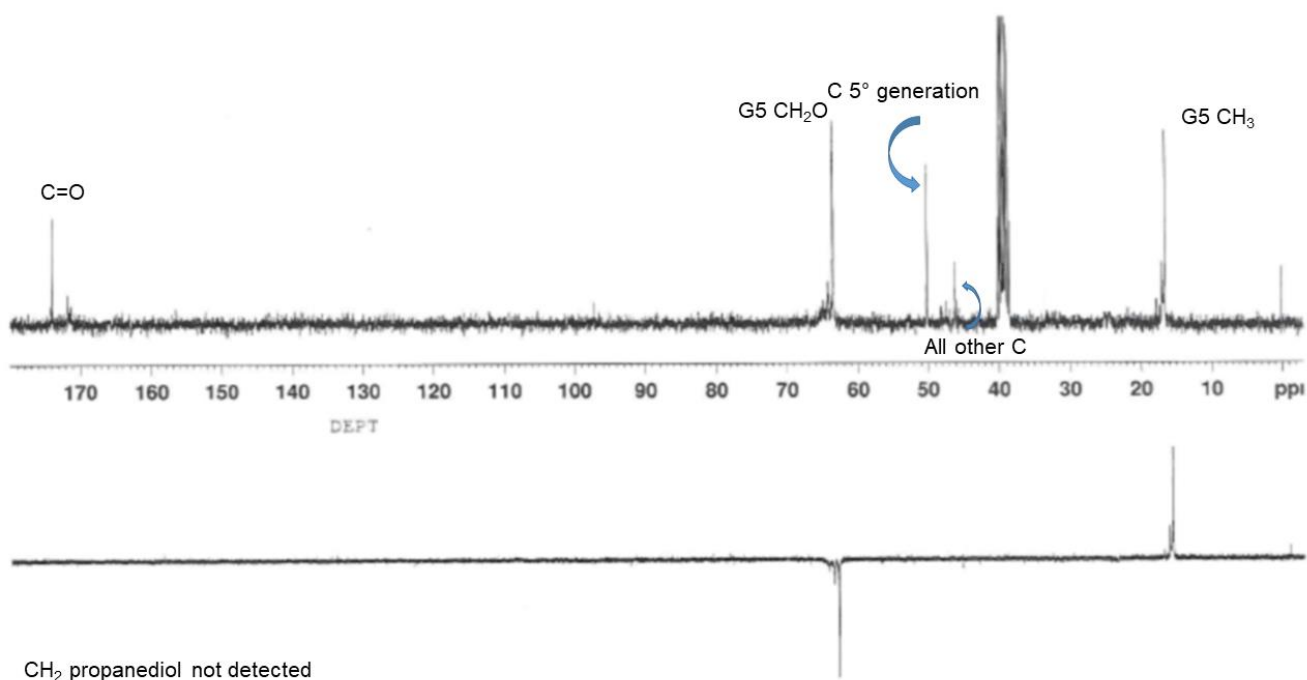


Fig. S7.6 ¹³C NMR and DEPT-135 spectra (DMSO-*d*₆, 75.5 MHz) of dendrimer **4**

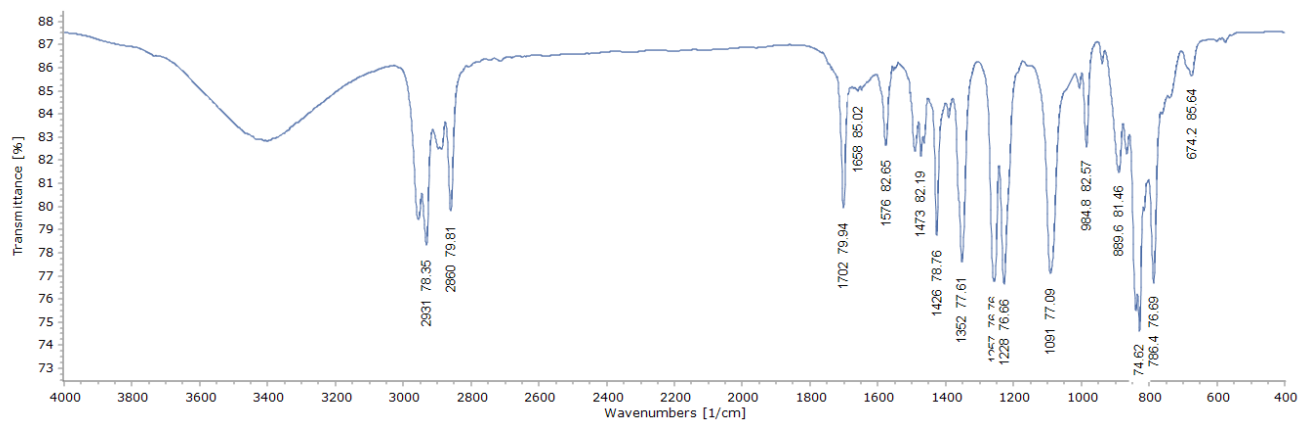


Fig. S7.7 FT-IR spectrum (KBr) of compound **5**

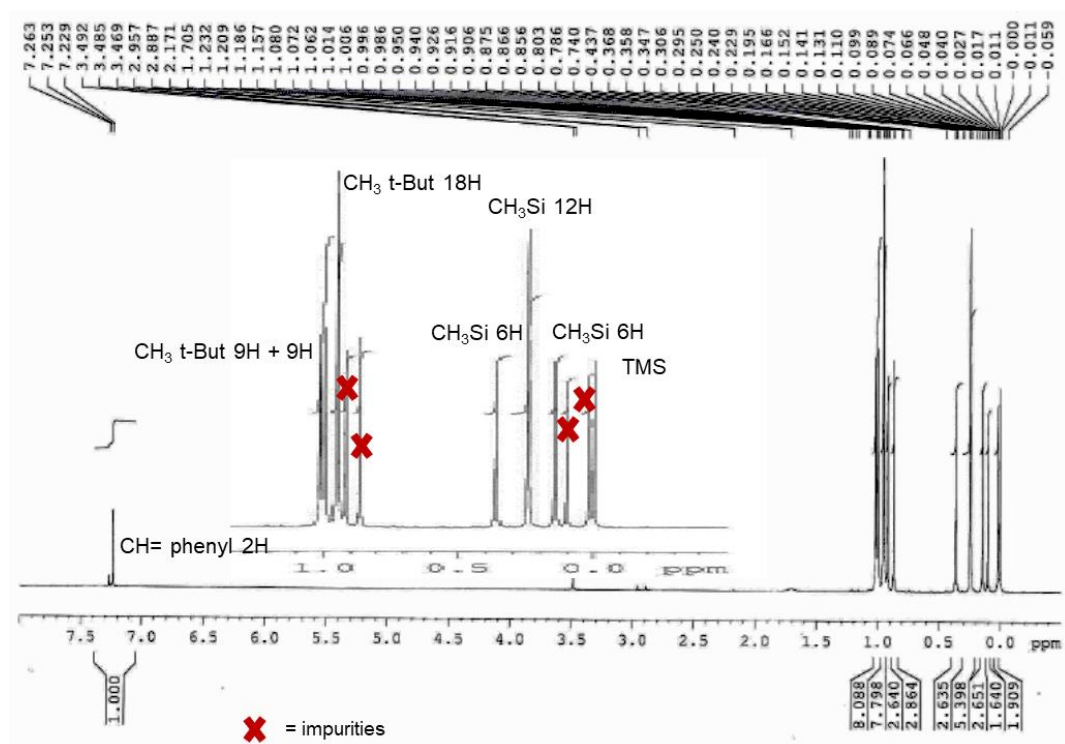


Fig. S7.8 ^1H NMR spectrum (CDCl_3 , 300 MHz) of compound **5**

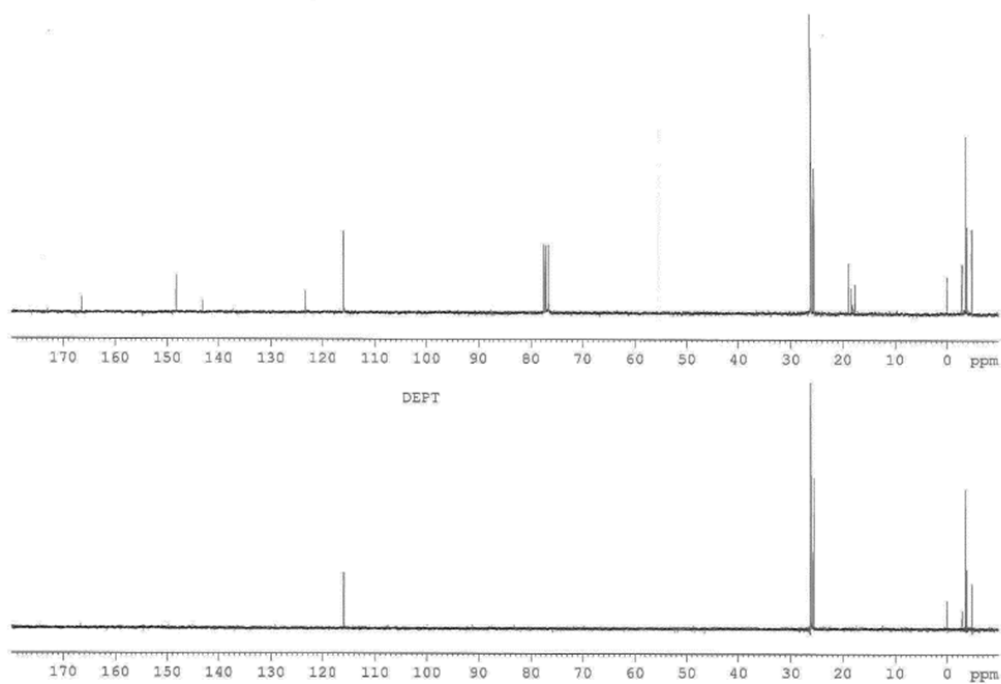


Fig. S7.9 ^{13}C NMR and DEPT-135 spectra (CDCl_3 , 75.5 MHz) of compound **5**

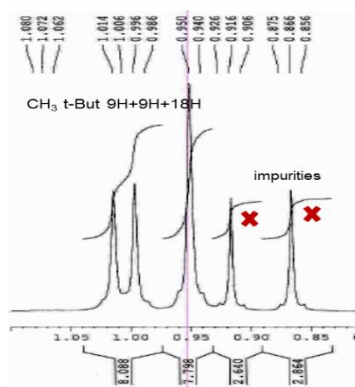


Fig. S7.10 Expansion of a significant part of ^1H NMR spectrum (CDCl_3 , 300 MHz) of compound **5**

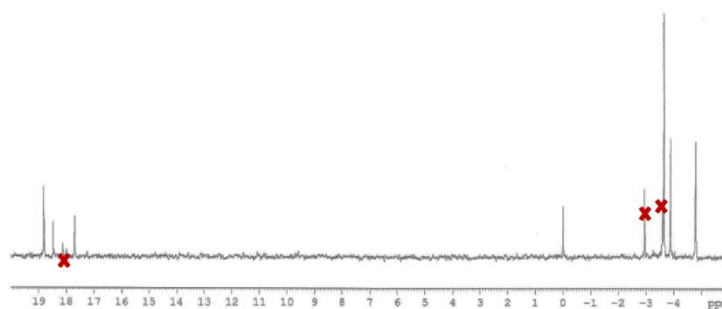


Fig. S7.11 Expansion of a significant part of ^{13}C NMR spectrum (CDCl_3 , 75.5 MHz) of compound **5**

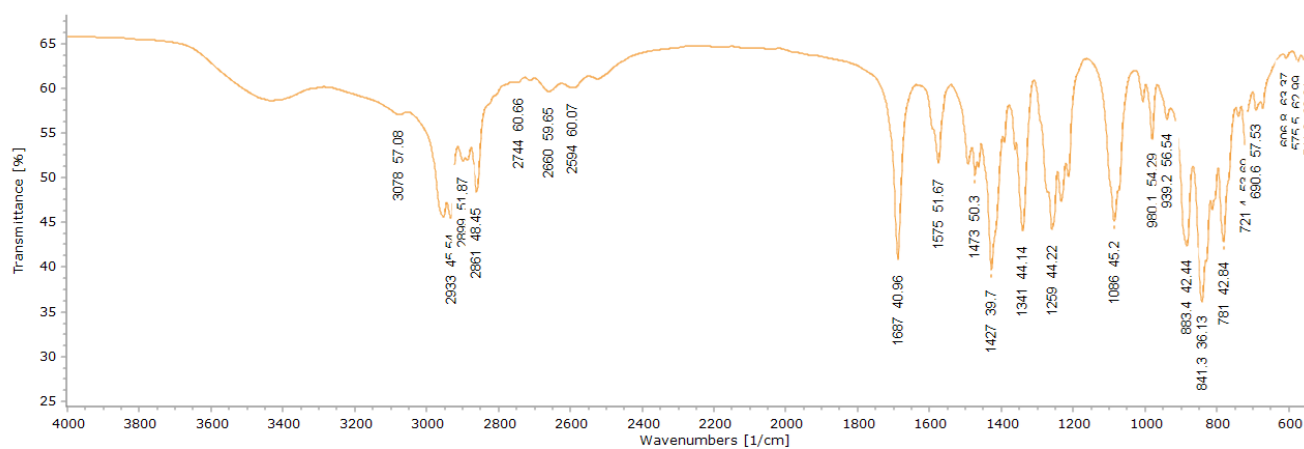


Fig. S7.12 FT-IR spectrum (KBr) of compound **6**

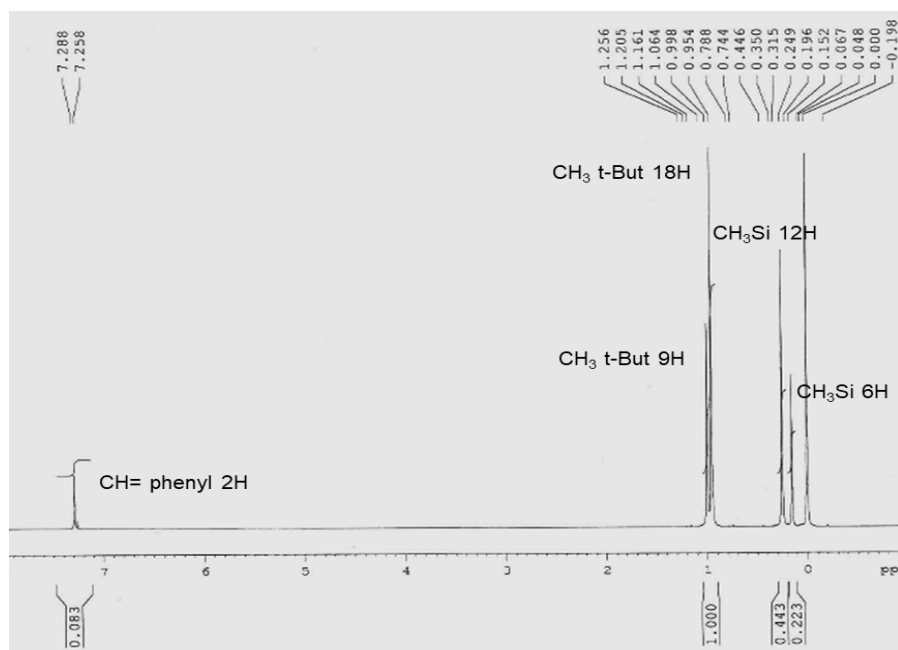


Fig. S7.13 ^1H NMR spectrum (CDCl_3 , 300 MHz) of compound **6**

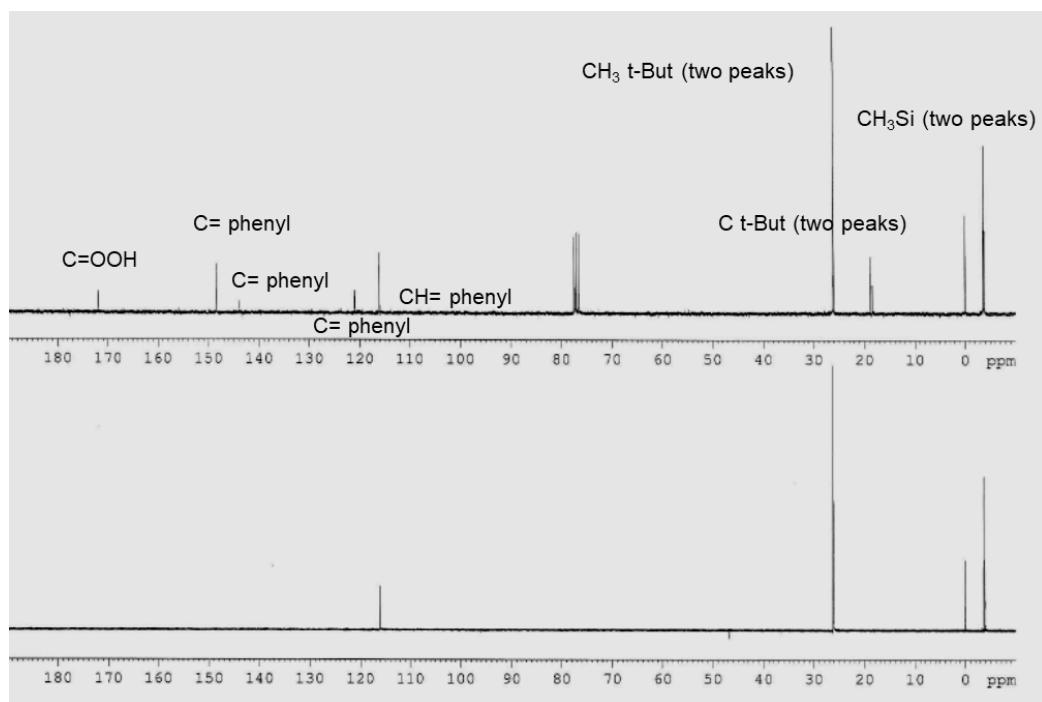


Fig. S7.14 ^{13}C NMR and DEPT-135 spectra (CDCl₃, 75.5 MHz) of compound **6**

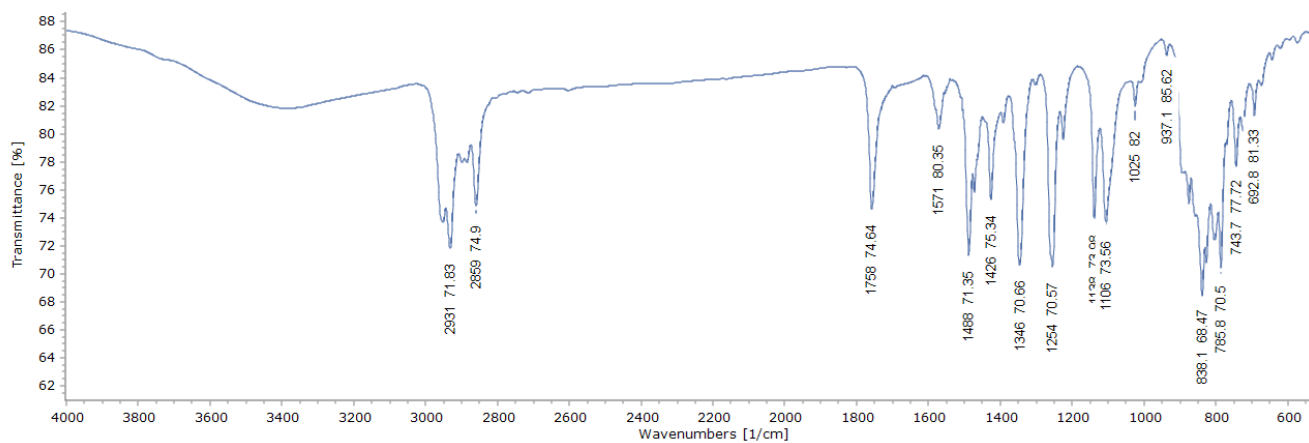


Fig. S7.15 FT-IR spectrum (KBr) of compound **7**

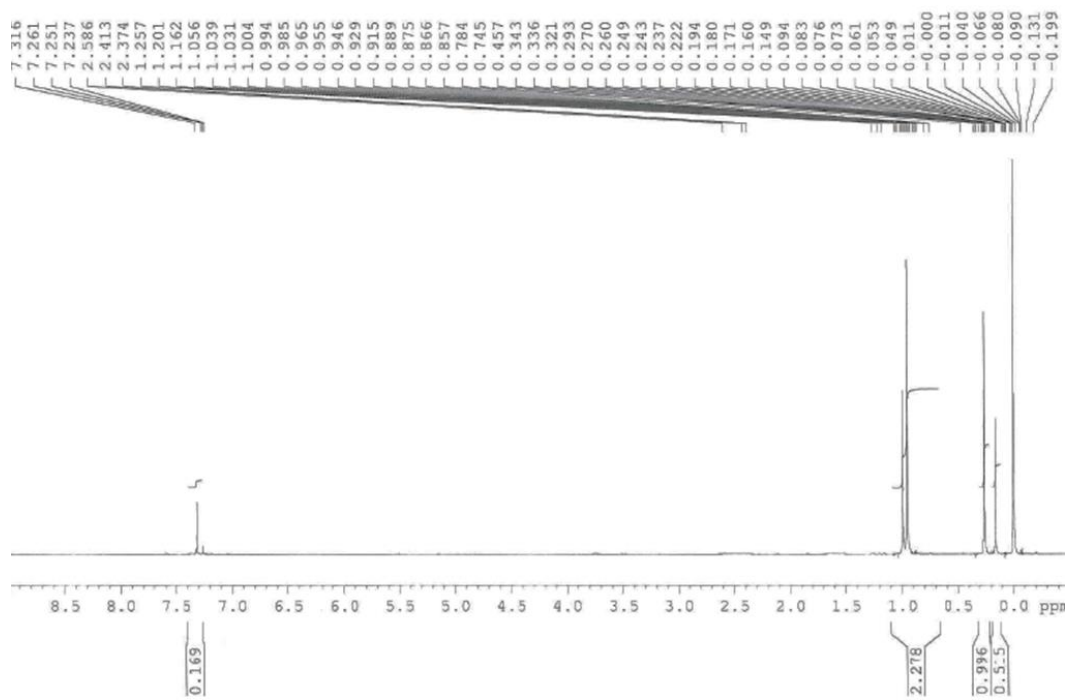


Fig. S7.16 ^1H NMR spectrum (CDCl_3 , 300 MHz) of compound **7**

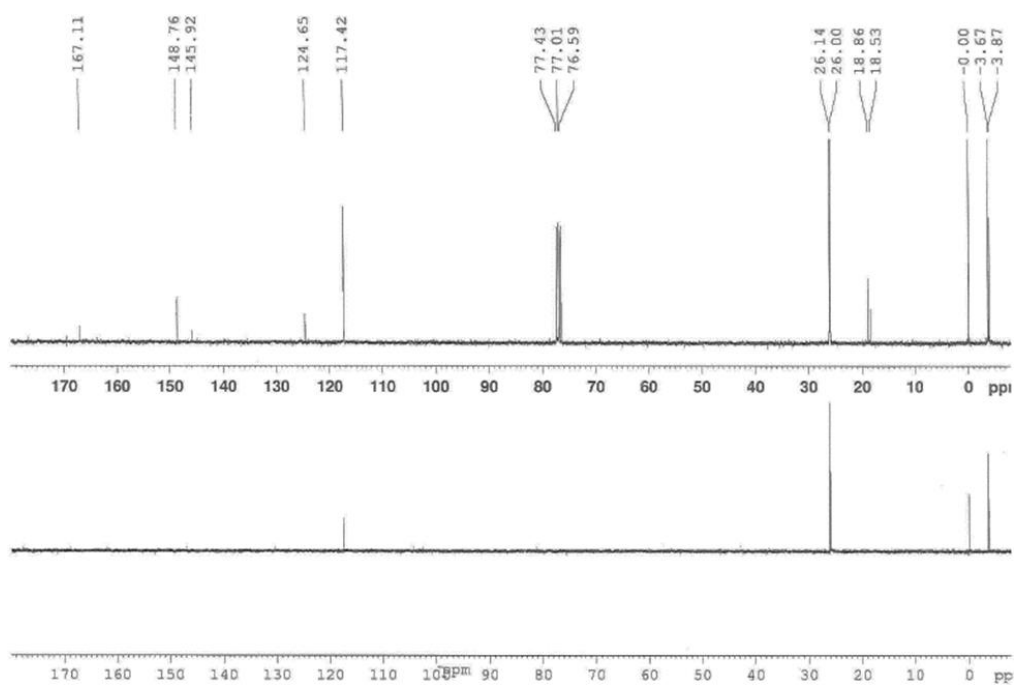


Fig. S7.17 ^{13}C NMR and DEPT-135 spectra (CDCl_3 , 75.5 MHz) of compound **7**

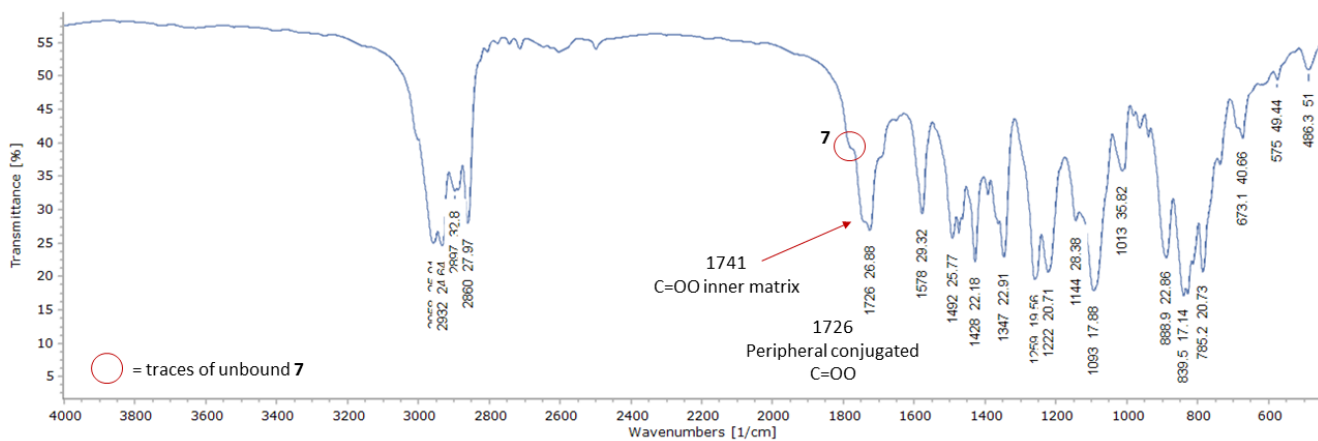


Fig. S7.18 FT-IR spectrum (KBr) of TBDMS-GA-dendrimer 8

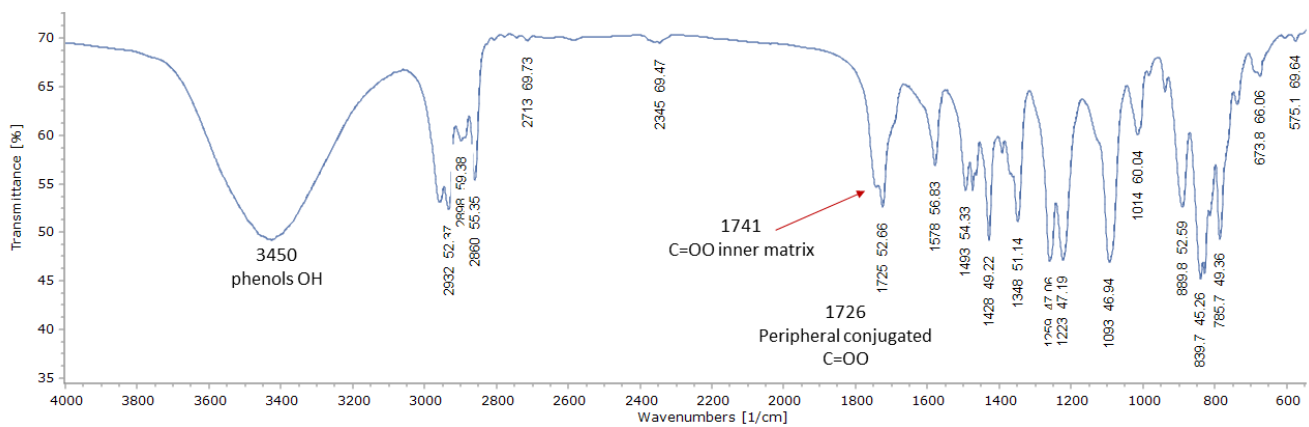


Fig. S7.19 FT-IR spectrum (KBr) of 192OH-GA-dendrimer 9

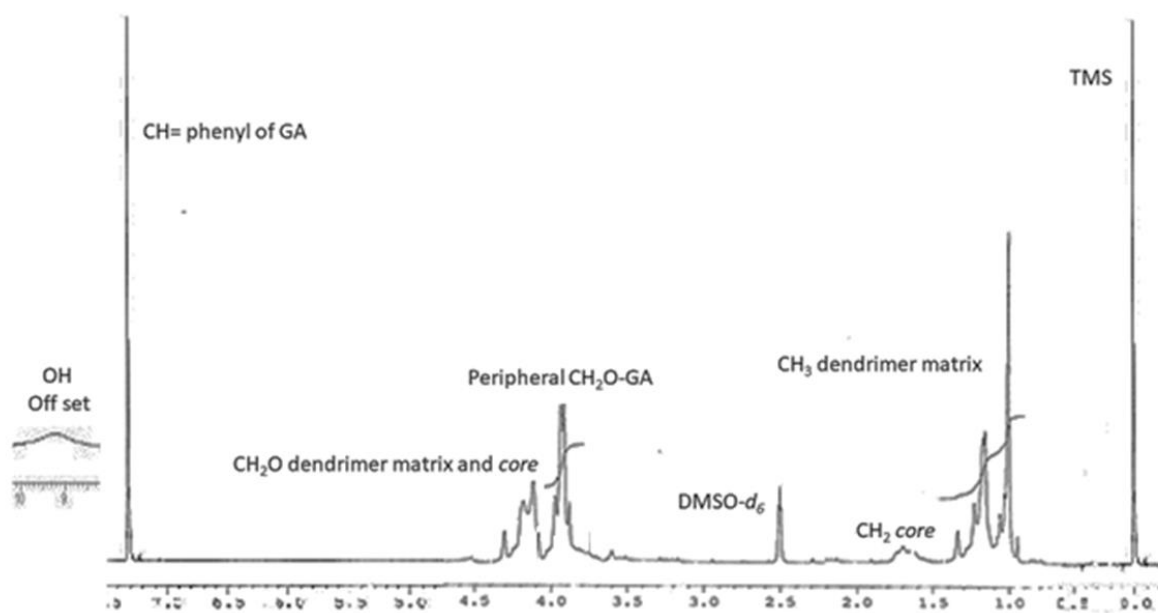


Fig. S7.20 ¹H NMR spectrum (DMSO-*d*₆, 300 MHz) of dendrimer 9

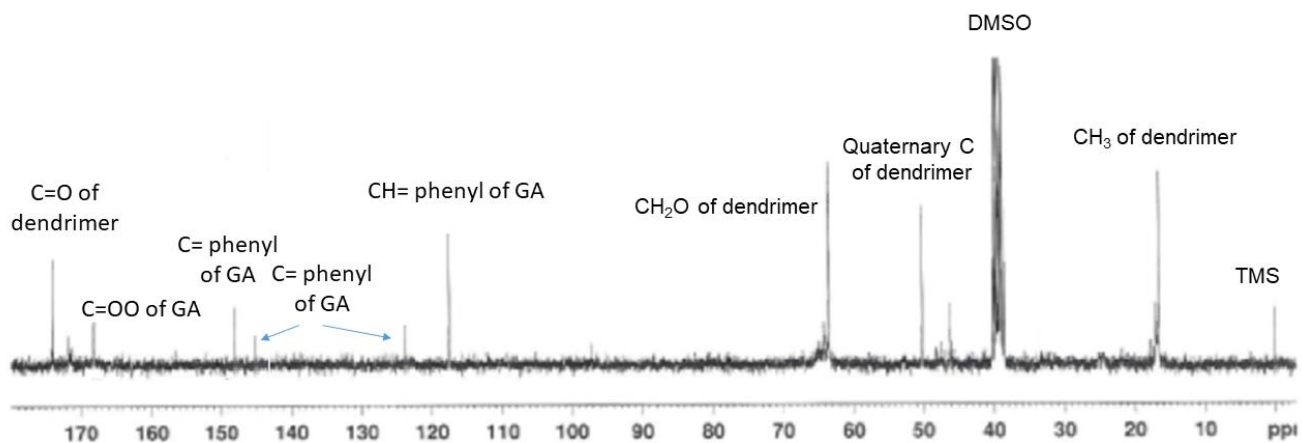


Fig. S7.21 ^{13}C NMR and DEPT-135 spectra ($\text{DMSO-}d_6$, 75.5 MHz) of compound **9**

References

1. Malik G, Natangelo A, Charris J, Pouysegue L, Manfredini S, Cavagnat D, Buffeteau T, Deffieux D, Quideau S. *Chem Eur J.* 2012; 18: 9063.
2. Mohamada H, Abasa F, Permana D, Lajisa NH, Alib AM, Sukaric MA, Hin TYY, Kikuzaki H, Nakatani N. (Zingiberaceae). *Zeitschrift fur Naturforschung.* 2004; 59: 811.
3. Olennikov DN. *Chem Nat Comp.* 2005; 41: 222.