Nanoscale Advances

Electronic Supplementary Material

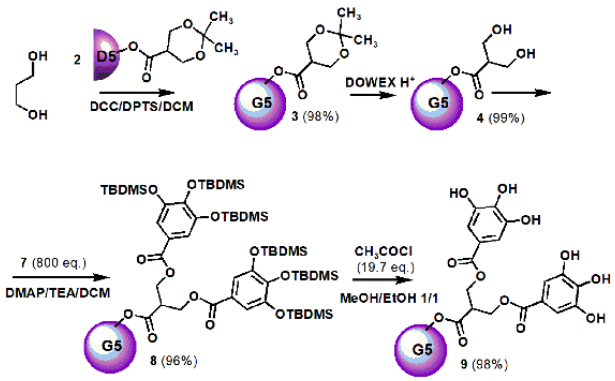
Reshaped as polyester-based nanoparticles, gallic acid inhibits platelet aggregation, reactive oxygen species production and multi-resistant Gram-positive bacteria with an ever-achieved efficiency

Silvana Alfei,*[,a] Maria Grazia Signorello,[a] Anna Schito,[b] Silvia Catena,[a] Federica Turrini[a]

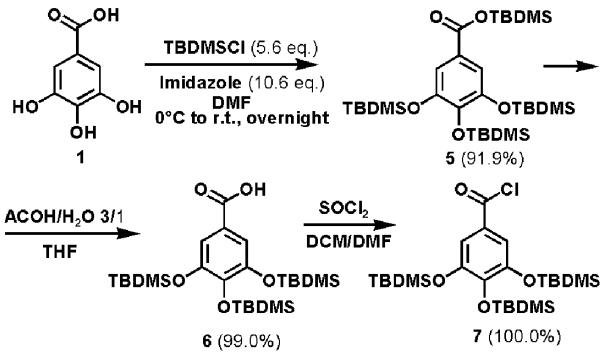
[a]*Dipartimento di Farmacia (DiFAR), Università degli studi di Genova, Viale Cembrano 4, I-16148 Genova (ITALY)*
[b]*Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate (DISC), Università degli studi di Genova, Viale Benedetto XV, 6, I-16132 Genova (ITALY)*

*Correspondence 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



Scheme S1. Synthetic pathway to prepare GAD (**9**)[1]



Scheme S2. Synthesis of GA derivative **7**[1]

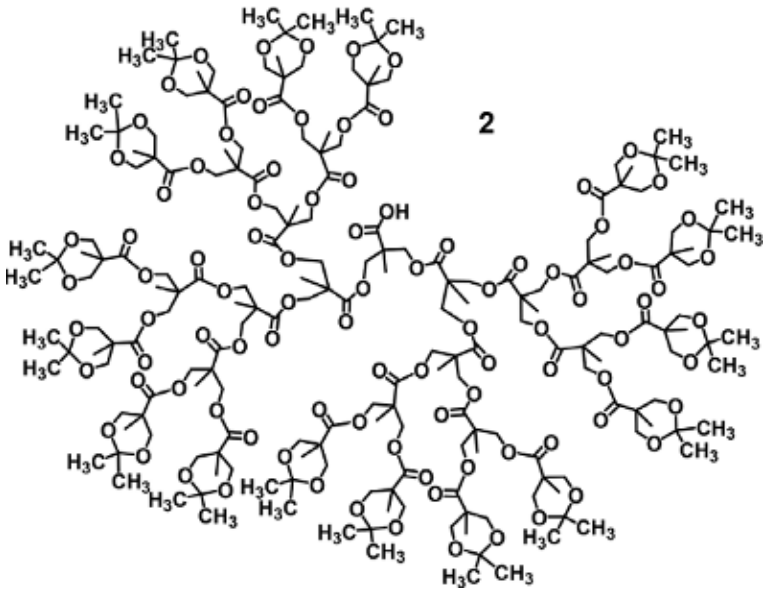


Figure S1. Structure of the fifth generation dendron **2**[2]

Section S1

Synthesis of dendrimer 3[1]

A solution made of a mixture of dendrimer **2** (2.64 g, 0.6199 mmol), 1, 3-propandiol (0.0214 g, 0.2818 mmol, 20.4 µl) and DPTS (0.1244 g, 0.4227 mmol) in CH_2Cl_2 (8 ml) was treated with dicyclohexylcarbodiimide (DCC) (0.1454 g, 0.7045 mmol) dissolved in DCM (3 ml) at room temperature under N_2 and magnetic stirring for 24 h. The precipitated dicyclohexylurea (DCU) was removed by filtration and washed with CH_2Cl_2. 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[1]
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 Et_2O, filtered to give **4** as a pink hygroscopic solid (2.40 g) which was further purified by dissolution in H_2O (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 P_2O_5.

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 N_2 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 Et_2O and the organic phase was washed with water (3 x 16 ml). Once separated from aqueous phase, the organic one was dried on $MgSO_4$. 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-H_2O (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 H_2O (80 ml) and the product was extracted with EtOAc (3 x 40 ml), washed with brine (2 x 40 ml), dried over $MgSO_4$ 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)[1]

Compound **6** (0.9300 g, 1.81 mmol) was dissolved in DCM (7.2 ml),cooled to 0°C and added with $SOCl_2$ (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[1]

*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% $KHSO_4$ (10 ml), extracted with DCM (3 x 15 ml) and dried over $MgSO_4$. 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***[1]

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 acethyl 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 $MgSO_4$. 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 P_2O_5 and under vacuum (0.4252 g, 0.0250, 97.6% yield). As early qualitative investigation, the $FeCl_3$ essay for phenols recognition was performed to confirm the presence of free phenol groups.

$FeCl_3$ 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% $FeCl_3$ prepared fresh. A strong green coloring indicating the presence of phenolic groups was observed (Figure S 3.1).

Fig. S3.1 Green coloring obtained in the $FeCl_3$ test performed on **9** solution

Section S4.1

Characterization data of compounds 3-9[1]

FT-IR and NMR spectra data of compounds 3-9

*Dendrimer **3**.* FTIR (KBr, cm^{-1}): 2992, 2939, 2878 (CH_3 and CH_2), 1748 (C=OO). ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 1.12, 1.17, 1.26 (s signals, 186H, CH_3 of generations), 1.33 (s, 96H, CH_3 acetonide), 1.40 (s, 96H, CH_3 acetonide), CH_2 propandiol, probably overlapped, 3.60 (d, 64H, *J* = 11.8 Hz, CH_2O acetonide), 3.71-3.91 (m, 4H, CH_2O propandiol), 4.13 (d, 64H, *J* = 11.7 Hz, CH_2O acetonide), 4.25-4.34 (m, 120H, CH_2O generations). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ (ppm): 173.48, 171.80 (C=O), 98.12 (quaternary C of acetonide), 67.29, 65.95, 65.90, 64.83 (CH_2O), 49.11, 46.82, 46.70 (quaternary C of three generations), 42.03 (quaternary C of fourth generation), 25.24 (CH_3 of acetonide), 22.00 (CH_3 of acetonide), 18.49, 17.68 (CH_3 of generation). Found: C, 57.05; H, 7.18. $C_{409}H_{637}O_{188}$ requires C, 57.41; H, 7.44%.

*Dendrimer **4**.* FTIR (KBr, cm^{-1}): 3433 (OH), 2933, 1733 (C=OO). ^{1}H NMR (300 MHz, 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), 3.52 (dd, 128H, CH_2OH), 3.56 (partially overlapped signal, 2H, CH_2O propandiol), 3.98 (partially overlapped signal, 2H, CH_2O propandiol), 4.08-4.18 (m, 120H, CH_2O of four generations), 4.37 (br s, 64H, OH). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ (ppm): 173.94, 171.73 (C=O), 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, 51.71; H, 7.01. $C_{313}H_{504}O_{188}$ requires C, 51.67; H, 6.98%.

*tert-Butyldimethylsilyl 3,4,5-tri[(tert-butyldimethylsilyl)oxy]benzoate (**5**).* TLC: Rf. = 0.84 (cyclohexane/EtOAc 4/1). FTIR (KBr, cm^{-1}): 2933, 2861 (CH_3 and CH_2), 1703 (C=O), 1258, 1092. ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): [0.017 and 0.10 (two s, CH_3Si, impurities)], 0.14 (s, 6H), 0.24 (s, 12H), 0.36 (s, 6H), [0.87 and 0.92 (two s, CH_3 t-Buthyl, impurities)], 0.95 (s, 18H), 0.99 (s, 9H), 1.01 (s, 9H), 7.23 (s, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 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. $C_{31}H_{62}O_5Si_4$ requires C, 59.39; H, 9.98; Si, 12.77%.

*3,4,5-tri[(tert-butyldimethylsilyl)oxy]benzoic acid (**6**).* TLC: Rf. = 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 (CH_3 and CH_2), 1687 (C=OOH), 1259, 1086. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.15 (s, 6H), 0.25 (s, 12H), 0.96 (s, 18H), 1.00 (s, 9H), 7.29 (s, 2H); ^{13}C-NMR (75.5 MHz, $CDCl_3$) 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. $C_{25}H_{48}O_5Si_3$ requires C, 58.56; H, 9.44; Si, 16.38%.

*3,4,5-tri[(tert-butyldimethylsilyl)oxy]benzoyl chloride (**7**)*. TLC: Rf. = 0.80 (cyclohexane/EtOAc 10/1). FTIR (KBr, cm^{-1}): 2932, 2860 (CH_3 and CH_2), 1759 (C=OCl). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.15 (s, 6H), 0.26 (s, 12H), 0.96 (s, 9H), 0.99 (s, 18H), 7.32 (s, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) 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. $C_{25}H_{47}ClO_4Si_3$ requires C, 56.53; H, 8.93; Cl, 6.68; Si, 15.81%.

*TBDMS-GA-loaded dendrimer **8***

Not purified product. TLC: Rf. = 0.70 (cyclohexane/EtOAc 10/1). FTIR (KBr, cm^{-1}): 2958, 2932, 2897, 2860 (CH_3 and CH_2), 1741 (C=OO 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 (C=OO inner matrix), 1726 (peripheral conjugated C=OOGA). ^{1}H NMR (300 MHz, 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 CH=), 8.00-10.00 (br s, GA phenols OH). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ (ppm): 173.94, 171.73 (C=O of dendrimer scaffold), 167.11 (C=O of GA), 148.80, 145.94, 124.67 (quaternary C of phenyl), 117.41 (CH= 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. $C_{761}H_{760}O_{444}$ requires C, 53.72; H, 4.51%.

***Elemental analysis data of compounds 3-7 and 9*[1]**

Table S4.1.1
Elemental Analysis data and other physicochemical data of the isolated reported compounds

Compound	Formula	MW	Required (%)	Found (%)	Error (%)	Physical state
3	$C_{409}H_{632}O_{188}$[a]	8557.28[a]	C 57.41 H 7.44	C 57.05 H 7.18	C 0.36 H 0.26	Glassy Off-white solid
4	$C_{313}H_{504}O_{188}$[a]	7275.24[a]	C 51.67 H 6.98	C 51.71 H 7.01	C 0.04 H 0.03	Fluffy white hygroscopic solid
5	$C_{31}H_{62}O_5Si_4$	626.37	C 59.39 H 9.98 Si 12.77	C 59.00 H 10.02 Si 12.78	C 0.39 C 0.04 H 0.01	Viscous resin
6	$C_{25}H_{48}O_5Si_3$	512.90	C 58.56 H 9.44 Si 16.38	C 58.58 H 9.40 Si 16.00	C 0.02 H 0.04 Si 0.38	White crystals
7	$C_{25}H_{47}ClO_4Si_3$	531.35	C 56.53 H 8.93 Cl 6.68 Si 15.81	C 56.22 H 9.05 Cl 7.00 Si 16.02	C 0.31 H 0.12 Cl 0.32 Si 0.21	Pale yellow waxy solid
9	$C_{761}H_{760}O_{444}$[a]	17010.02[a]	C 53.72 H 4.51	C 54.03 H 4.89	C 0.31 H 0.38	Brownish glassy hygroscopic solid

[a] Estimated by ^{1}H NMR spectra and confirmed by Elemental Analysis in order to avoid the routine well known but very expensive MALDI-TOF technique not available in our laboratory.

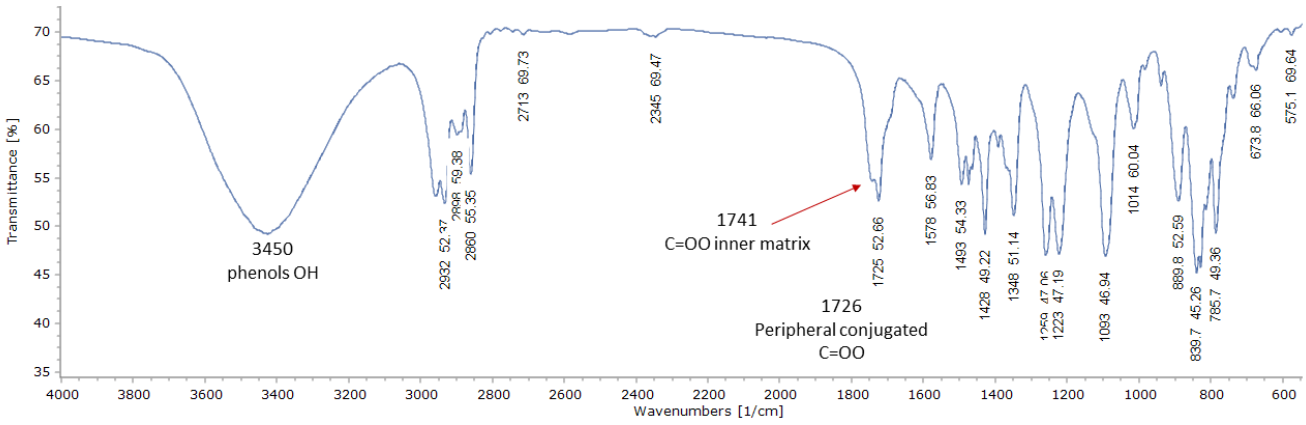


Fig. S4.1.1 FTIR spectrum (KBr) of GA-dendrimer **9**

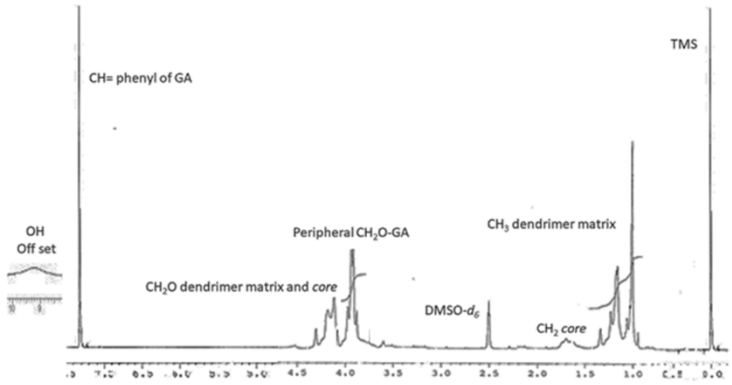


Fig. S4.1.2 ^{1}H NMR spectrum ($CDCl_3$, 300 MHz) of GA dendrimer **9**

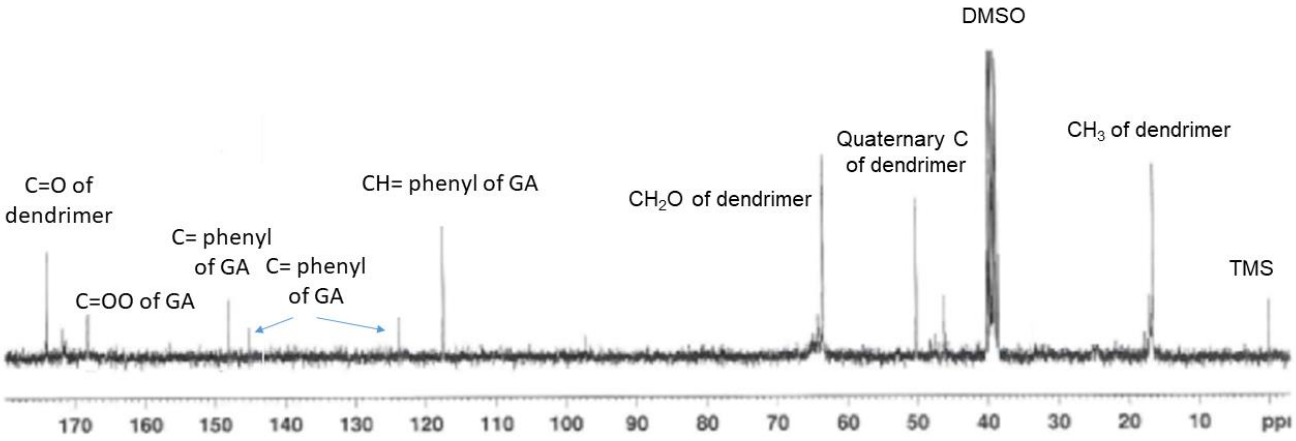


Fig. S4.1.3 ^{13}C NMR and DEPT-135 spectra ($CDCl_3$, 75.5 MHz) of GA dendrimer **9**

Section S4.2

Evaluation of cytotoxicity of GAD (9)[1]

Cell culture

B14 cells (Cricetulus griseus, ATCC, CCL-14.1, Sigma-Aldrich Inc.) were grown in high-glucose Dulbecco's modified Eagle medium with 10 % (v/v) fetal bovine serum (FBS) and 4 mM glutamine; BRL 3A (Rattus norvegicus, ATCC, CRL-1442, Sigma-Aldrich Inc.) were grown in Ham's F12 medium with 10% (v/v) FBS and 2 mM glutamine. All media were supplemented with 0.1% (w/v) penicillin and 0.1% (w/v) streptomycin. The cells were maintained in culture flasks in a 37 ∘ C humidified atmosphere of 5% CO_2/95 % air (incubator) and passaged every 2–3 days. Cells were harvested and used in experiments after obtaining 80–90 % confluence. The number of viable cells was determined by trypan blue exclusion with a haemo-cytometer. Then cells were suspended in media at a concentration of 1.0×10^{-5} cells per mL and plated in flat-bottom 96-well plates. Plates with cells were incubated for 24 h at 37 °C in a humidified atmosphere of 5% CO_2 to allow adherence of the cells before the administration of dendrimers.

3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cytotoxicity of GAD **9** was assayed with MTT. After 24 h incubation when cells attached on 96-well plates they were treated with dendrimer GAD at a concentration from 0.5 to 20 µmol/L (dimethylsulfoxide). After 24 h incubation, a 20 µL solution of MTT in phosphate-buffered saline (5 mg/mL) was added to each well. Four hours later the medium was removed and the formazan precipitate dissolved in dimethylsulfoxide for absorbance measurement at 580 nm and reference at 700 nm. The cell viability was related to the control wells containing untreated cells with fresh cell culture medium and was calculated according to the following Equation (1):

$$\text{Cell viability (\%)} = \frac{\text{Absorption test}}{\text{Absorption control}} \times 100 \qquad (1)$$

The result is presented as the mean of three measurements (± standard deviation).

Statistical analyses

Data are expressed as means ± standard deviation. Statistical significance of differences was determined by one-way analysis of variances (ANOVA). $P < 0.05$ was considered statistically significant.

Table S4.2.1

GAD particle hydrodynamic size (DLS) at 25 °C and cell viability values (± standard deviation) from cytotoxicity essay at GAD concentration of 340 µg mL^{-1}.[1]

Z-AVE size (nm)[a]	Cell viability (%)[b]	
348.6±2.8	B14	BRL
	91.4±3.5	85.3±1.1

[a] N (degree of freedom) = 12; [b] N (degree of freedom) = 3

Section S5

Radical Scavenging Activity (RSA%) of dendrimer 9[1]

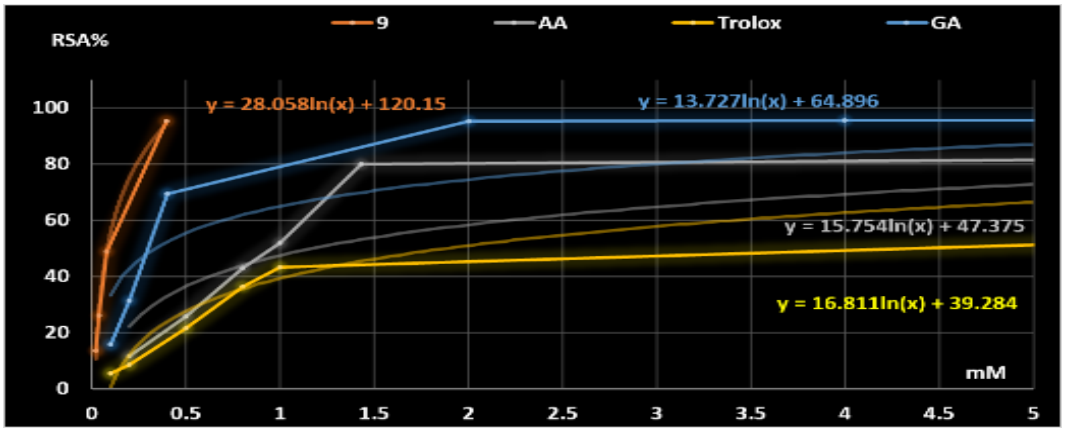


Fig. S5.1 RSA (%) curves recorded at different **9**, GA, AA and Trolox concentrations in ethanol or water solution: concentrations were given in mM. The corresponding exponential tendency curves and related equations used to derive the IC_{50} were also provided.

Table S5.1
Comparison between RSA (%) expressed as IC_{50} (mM), of **9**, GA, AA, Trolox[1] and α-Tocopherol.[3]

Entry (MW)	IC_{50} (μg ml^{-1})	IC_{50} (μmol ml^{-1}][e]	IC_{50} (mM)[e]
9[a,b] (17010.017)	1395.8	0.0821	0.08
GA[a,b] (170.12)	57.4	0.3378	0.34
AA[a,c] (176.12)	208.1	1.1813	1.18
Trolox[a,c] (250.29)	473.4	1.8916	1.89
α-Tocopherol[d] (430.7)	96.0	0.2230	0.22

[a] From DPPH test performed by us. [b] In ethanol. [c] In water. [d] Lit. [e] Calculated from concentration expressed in μg ml^{-1}.

Section S6

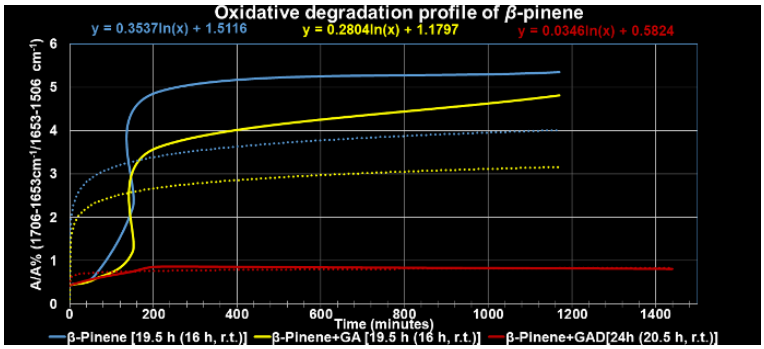


Fig. S6.1 *β*-pinene oxidative degradation profiles with and without additives as FTIR peaks areas ratio as function of time.[4]

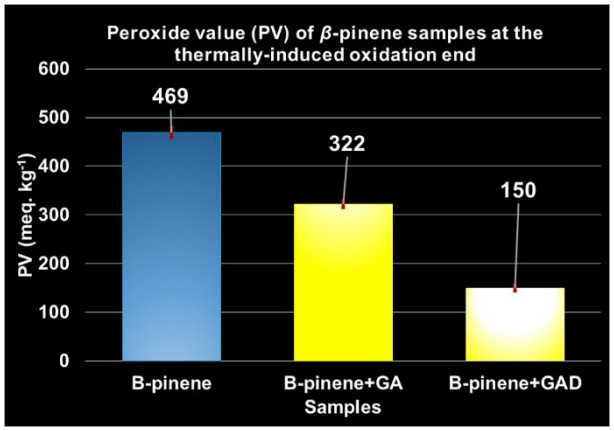


Fig. S6.2 PV obtained by iodometric titration of samples of *β*-pinene with and without additives at the end of thermally-induced oxidation process.[4]

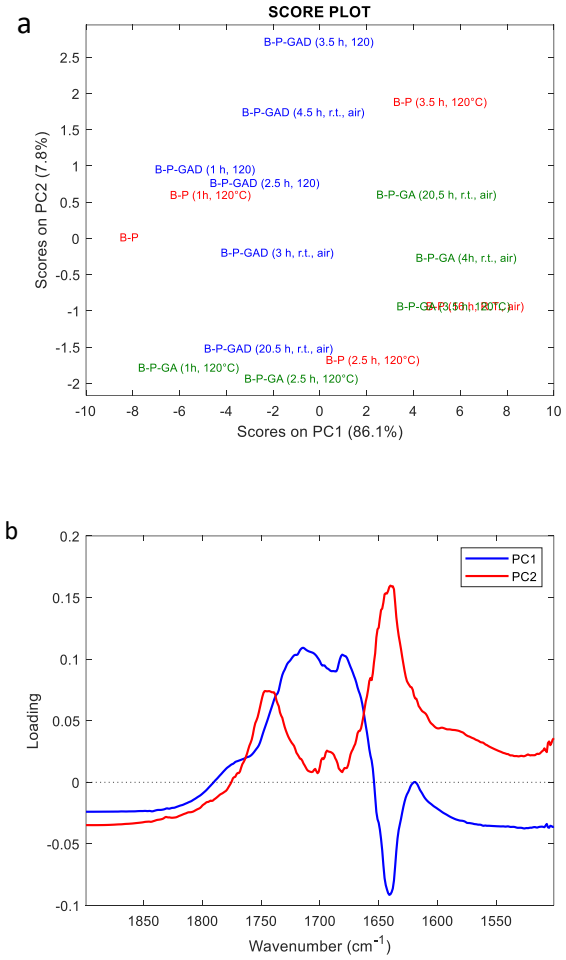


Fig. S6.3 PCA score plot (a) and loading plot (b) concerning β-P: PC1 vs. PC2. β-P samples in blue, GA samples in green, GAD samples in red.[4]

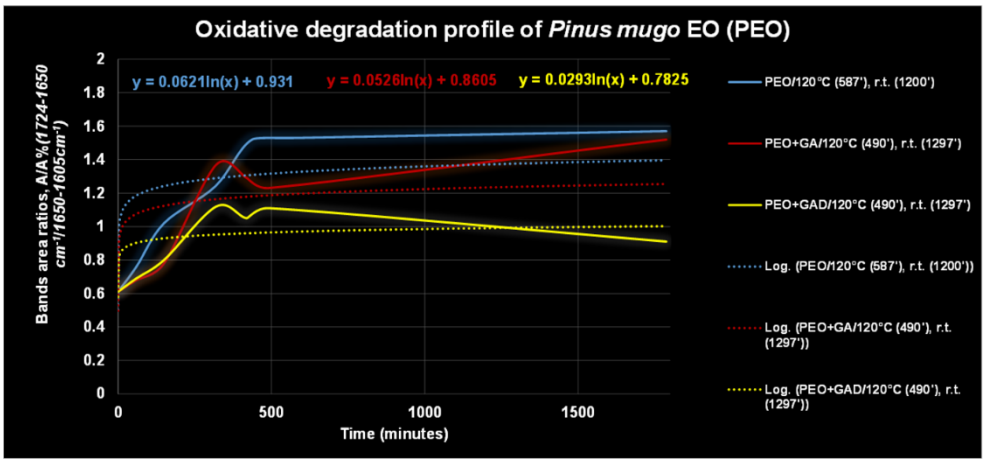


Fig. S6.4 PEO oxidative degradation profiles as FTIR peaks areas ratio (range 1700-1506 cm^{-1}) in function of time.[4]

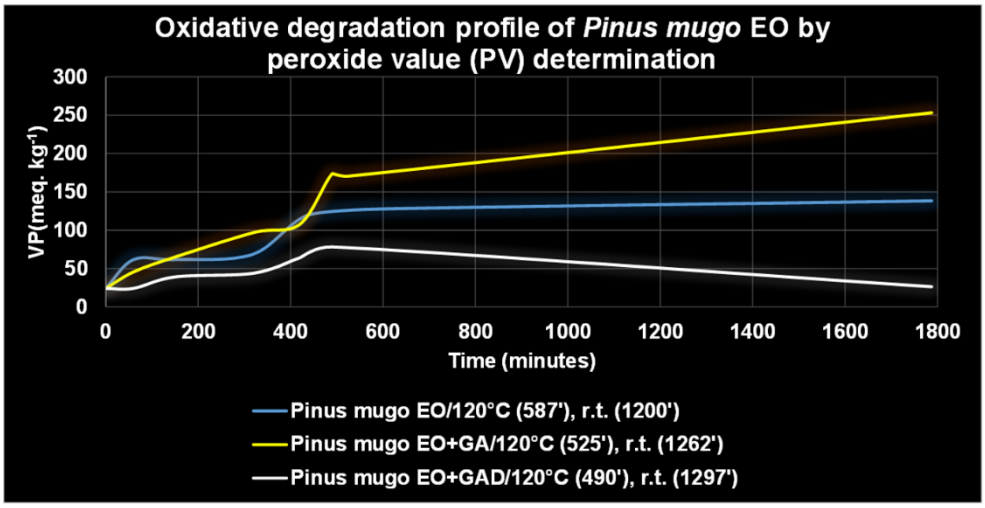


Fig. S6.5 PEO Oxidative degradation profiles as Peroxide Value (PV) in function of time.[4]

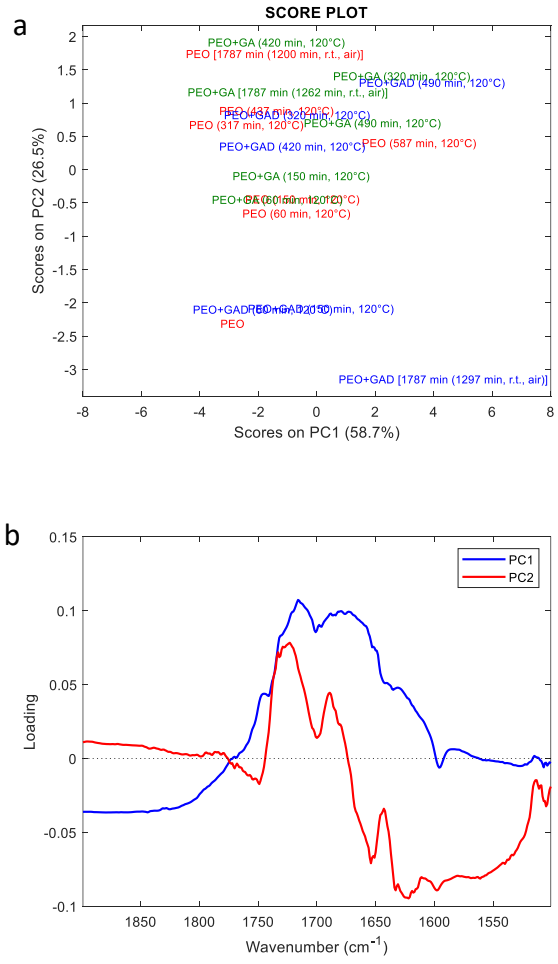


Fig. S6.6 PCA score plot (a) and loading plot (b) concerning PEO: PC1 vs. PC2. PEO samples in red, GA samples in green, GAD samples in blue.[4]

Table S6.1
PV determination at fixed time points for all samples and comparison estimate efficiency of GA and GAD[4]

	PV (meq. Kg^{-1})					
Time (min)	**PEO**	**PEO+GA**	**PEO+GAD**	***β*-pinene**	***β*-pinene +GA**	***β*-pinene +GAD**
0	24±0.78	24±0.78	24±0.78			
26						
50						
60	61±0.70	45. ±0.55	24±0.50			
140						
150	61±0.75	65±0.82	39±0.60			
206						
240						
312						
317	68±0.65					
320		97±0.54	44±0.54			
330						
420		107±0.75	64±0.80			
437	119±0.90					
456						
490		173±0.88	78±0.85			
525		170±0.95				
587	127±0.88					
1170				469±2.05	322±1.54	
1440						150±0.98
1787	138±0.72	253±1.21	26±0.45			

Section S7

Comparison between ROS and platelet aggregation inhibition activity of GAD and GA

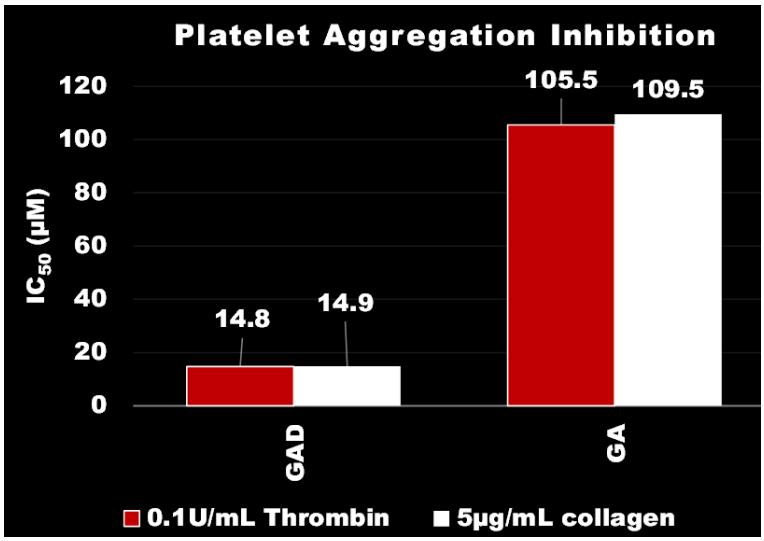


Fig. S7.1 Platelet aggregation inhibition activity of GAD and GA expressed as IC_{50} (µM).

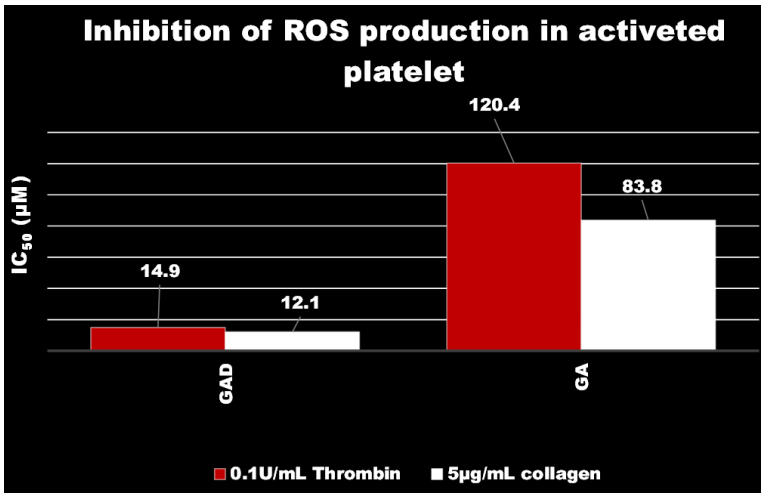


Fig. S7.2 ROS production inhibition activity of GAD and GA expressed as IC_{50} (μM).

References

1 S. Alfei, S. Catena and F. Turrini, *Drug Deliv. Transl. Res.,* 2019, ID: DDTR-D-19-00157, under review.
2 S. Alfei and S. Castellaro, *Macromol. Res.,* 2017, **25**, 1172.
3 H. Mohamada, F. Abasa, D. Permanaa, N. H. Lajisa, A. M. Alib, M. A. Sukaric, T. Y. Y. Hin, H. Kikuzaki and N. Nakatani, (Zingiberaceae). *Zeitschrift fur Naturforschung*, 2004, **59**, 811.
4 S. Alfei, P. Oliveri and C. Malegori. *ChemistrySelect*, 2019, **4**, 8891 –8901.