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# Synthesis and antioxidant properties of dendritic polyphenols

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ABSTRACT

Three dendritic polyphenols (generation 1) were synthesized: a syringaldehyde-based dendrimer (**1**), a vanillin-based dendrimer (**2**), and an iodinated vanillin-based dendrimer (**3**). They all showed strong antioxidant activity according to the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. The syringaldehyde dendrimer was twice and 10 times stronger than quercetin and Trolox, respectively. The vanillin-based dendrimer and its more hydrophobic iodinated derivative were also more potent antioxidants than quercetin and Trolox. The DPPH order of potency was **1** > **2**, **3** > quercetin > Trolox. All three dendrimers also protected human LDL from free radical attack in a dose-dependent manner. Their order of free radical scavenging was **1** > **3** > **2** > quercetin > Trolox. The increased hydrophobic nature of the iodinated derivative may have contributed to its better LDL protection than **2**. Protection of linoleic acid oxidation was studied by the β-carotene–linoleate assay. Dendrimer **1** was clearly superior to the other antioxidants in protecting the fatty acid. In case of DNA protection against free radical damage, the order of activity was **1** > quercetin > **2** > **3**, Trolox. Pro-oxidant effect on copper-induced DNA oxidation showed the following order: quercetin, Trolox > **1** > **2** > **3**. Results of the study show that dendritic antioxidants, even at the generation 1 level, provide promising antioxidant properties for their potential use as drug candidates for diseases associated with oxidative stress.



Flavonoids, plant based-polyphenols, have drawn considerable attention due to their health benefits including anti-cancer,[1] anti-lipoperoxidation,[2] anti-ischemic,[3] anti-allergic and anti-inflammatory[4] properties. Quercetin, one of the most abundant flavonoids, is a potential chemo-preventive agent.[5] However, flavonoids may also exhibit carcinogenic and mutagenic effects.[6] Their adverse biological actions are suspected to result from their pro-oxidant action in the presence of transition metals like copper and iron, and are more prominent in flavonoids that contain catecholic or pyrogalloyl moiety.[7] Antioxidants with high antioxidant potential and low pro-oxidant activity will therefore be beneficial for clinical applications.

In this study we designed and synthesized three generation 1 (G1) dendritic polyphenols devoid of catechol and pyrogallyol moiety in order to reduce potential pro-oxidant effect. The dendritic architecture allows synthesis of precise molecules with increasing size via repetitive synthetic steps. Well-defined molecular structures are important for clinical applications. In addition, dendrimers may also exhibit unpredictable 'dendritic effects' which can enhance solubility and stability and provide other beneficial properties.[8] An important long-term objective is to design a dendrimer with strong antioxidant potential and diminished pro-oxidant activity. The antioxidant dendrimers were made from syringaldehyde, vanillin, and 5-iodovanillin as building blocks. Syringaldehyde and vanillin, plant-based phenolic aldehydes with weak antioxidant activities, are found in fruits, nuts, grains, and various food plants.[9] They are also lignin precursors[10] and are found in aged liquors.[11] Lignin is hydrolyzed during the liquor aging process in wooden barrels releasing variously substituted small phenolic antioxidants including syringaldehyde and vanillin, which impart smell and taste to the liquor.[12] The presence of aldehyde groups in these phenols allows their ready conversion into dendritic forms via covalent attachment to a core molecule with two or more amino groups. We used 4-aminomethylbenzylamine as the core for all three compounds, thus having the same core and branching points but differing in their terminal phenol rings. 5-Iodovanillin was used to study the effects of increased hydrophobicity of the G1 antioxidants in the oxidation of lipophilic biomolecules such as lipoproteins and fatty acids.

The antioxidant capacity of the three dendrimers was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. Their ability to protect fatty acid (linoleic acid), DNA (pBR322), and lipoprotein (human LDL) against free radical damage was studied using β-carotene bleaching, DNA and LDL electrophoresis, respectively. Their harmful pro-oxidant effect was also evaluated with copper(II)-induced DNA oxidation. The three dendritic antioxidants were compared to quercetin, which is one of the most widely studied flavonoids and reported to have all of the requirements for a potent antioxidant[13] and Trolox, a water-soluble vitamin E analog, often used as a comparative control in many antioxidant studies (Fig. 1).

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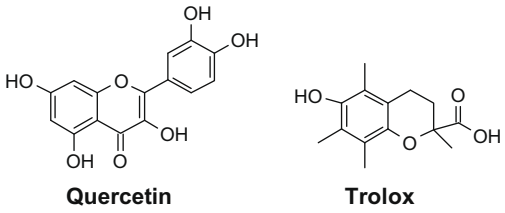



**Figure 1.** Antioxidants used for comparison.

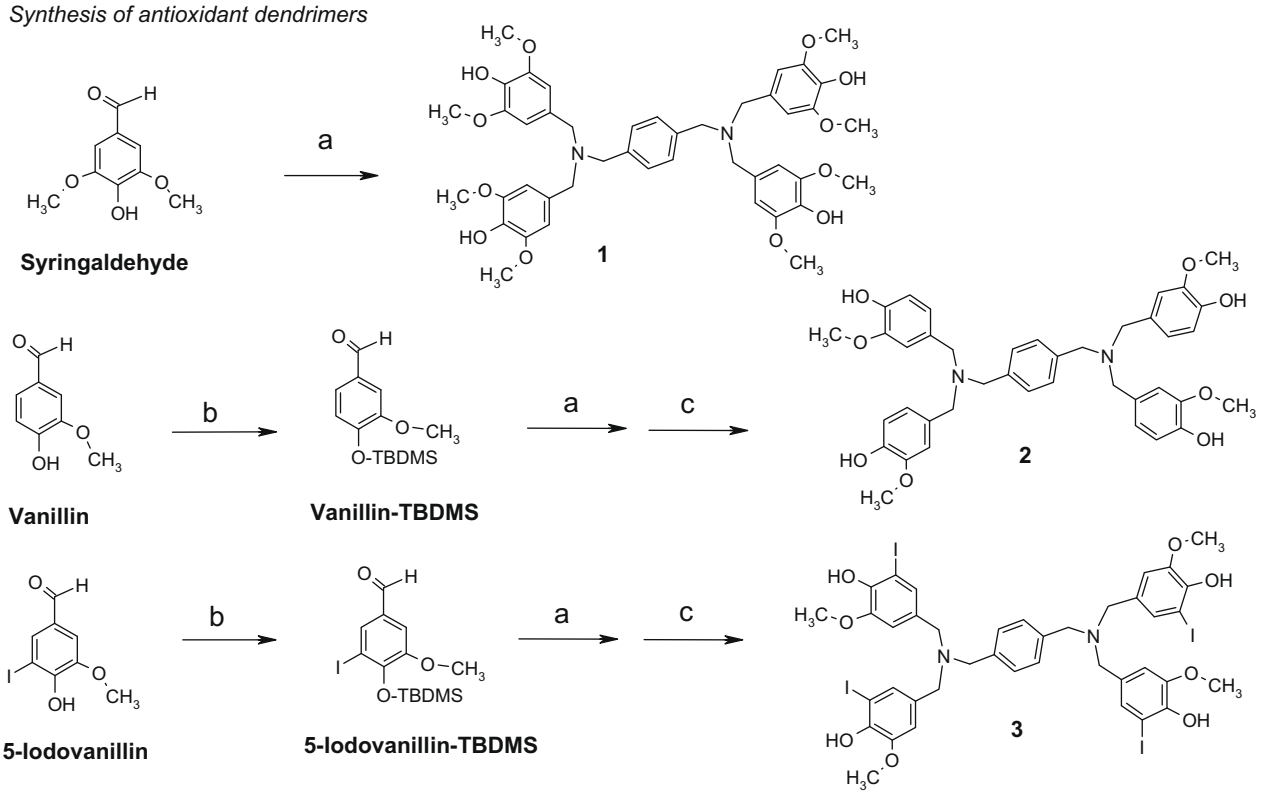
*Synthesis of antioxidant dendrimers*. Aldehyde groups of building blocks were reacted with the amino groups of the core, forming imines that were subsequently reduced to amines. Sodium triacetoxyborohydride ($Na(OAc)_3BH$) was found to be an appropriate reducing agent for amination. Although some aldehydes in the building block were reduced to an alcohol, it was far more efficient than other reducing agents like $NaCNBH_3$. In order to decrease aldehyde reduction, the building block and $Na(OAc)_3BH$ were added in a stepwise manner so that aldehyde exposure to $Na(OAc)_3BH$ was minimized. $Na(OAc)_3BH$ gave good reaction yields in the solvent, 1,2-dichloroethane. In compound **1** synthesis, both syringaldehyde and 4-aminomethylbenzylamine dissolved slowly in 1,2-dichloroethane and afforded high reaction yield (over 80%). However, vanillin and 5-iodovanillin were not very soluble in the solvent. They were therefore protected with *t*-butyldimethylsilyl chloride (TBDMS-Cl)[14] to increase their solubility in 1,2-dichloroethane, contributing to improved yields for both **2** and **3** (Scheme 1).[15]

*Evaluation of antioxidant activity*. Polyphenols with potent antioxidant activity are available in nature. For example, lignin, the most abundant natural biopolymer, is a strong antioxidant due to its numerous substituted phenols. Lignin is indigestible by human enzymes, thus limiting its bioavailability.[16] Smaller versions of organic polymers with potent antioxidant capacity or other therapeutic effects also exist.[17] Examples include proanthocyanidins that are oligomers of catechin/epicatechin. Like most potent antioxidants, they also show pro-oxidant activity in the presence of metal ions.[18] Strong antioxidants are generally associated with strong pro-oxidant activity. Therefore, it is important to investigate architectures that can separate the two activities.

Compound **1** has four peripheral phenols with two strong electron-donating methoxy groups per ring. Compound **2** is similar to **1**, but has only one electron-donating substituent on each phenol. Compound **3** resembles **2** except for the presence of an iodo group on each ring.

Antioxidant capacity of the compounds was assessed by the DPPH assay.[19] All three dendritic compounds (**1**–**3**) showed hyperbolic kinetic curves with rapid radical scavenging within 10 min followed by a slower phase (Fig. 2). Quercetin also displayed similar kinetics (data not shown). Trolox, on the other hand reached plateau values in less than a minute. The $IC_{50}$ values, determined at 120 min, were 3.7, 6.0, 6.0, 9.0, and 27.6 μM for compounds **1**, **2**, **3**, quercetin, and Trolox, respectively. All three dendritic antioxidants were stronger than quercetin and Trolox. Compound **1** was the most potent DPPH radical scavenger while compounds **2** and **3** showed identical antioxidant capacity. Compound **1** was stronger than **2** probably due to the presence of an additional electron-donating substituent (methoxy) *ortho* to the phenolic hydroxyl group. Based on $IC_{50}$ values of compounds **2** and **3**, the presence of iodo groups did not diminish the radical scavenging capacity. The starting materials used for syntheses of compounds **1–3** showed negligible DPPH activity ($IC_{50}$ values of syringaldehyde, vanillin, 5-iodovanillin and 4-aminomethylbenzylamine >100 μM).

The antioxidant activity of compounds **1–3** towards biomolecules was examined by studying their ability to protect LDL, fatty acid, and DNA from free radicals. For LDL, 2,2′-azobis(2-amidino-




**Scheme 1.** (A) Synthesis of dendritic antioxidants. Reagents and conditions: (a) 4-aminomethylbenzylamine, $Na(OAc)_3BH$, 1,2-dichloroethane; (b) TBDMS-Cl, triethylamine, $CH_2Cl_2$, 0 °C; (c) *n*-$Bu_4NF$, THF.

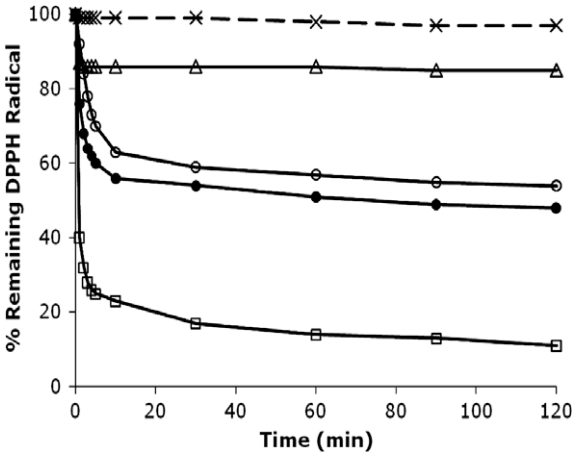



**Figure 2.** DPPH reaction kinetics of dendritic antioxidants: control with methanol (X, dashed), compound **1** (□), compound **2** (●), compound **3** (○), Trolox (Δ). All antioxidant concentrations are 5 μM.

propane) dihydrochloride (AAPH) was used to induce free radical damage. AAPH was used instead of copper ($Cu^{2+}$) ion for LDL oxidation so that the free radical scavenging activity of the antioxidants could be measured without any pro-oxidant interference caused by metal ions.[20,21]

LDL oxidized with 10 mM AAPH (18 h, 37 °C) showed increased anodic migration on agarose gels (expressed as 100% migration, Fig. 3) compared to un-oxidized native lipoprotein (dashed line, ~50% migration of oxidized LDL, Fig. 3). Protection of LDL from AAPH free radicals by the antioxidant decreased the anodic shift in a dose-dependent manner (Fig. 3). Compound **1** was the most efficient in protecting LDL followed by **3**, **2**, quercetin, and Trolox. Although **2** and **3** showed identical DPPH values, the iodinated dendrimer (**3**) displayed better LDL protection than compound **2**. The increased hydrophobicity of **3** may be partly responsible for its improved LDL protection over **2**. The starting materials for compounds **1–3** showed no LDL protection under these conditions.

The ability of the dendritic antioxidants **1–3** to protect linoleic acid against free radical damage was examined by the β-carotene–linoleate model.[22] In the absence of an antioxidant, hydroperoxides formed from linoleic acid oxidation bleach the highly unsaturated β-carotene by a free-radical-mediated phenomenon. The bleaching of the reddish-orange color of β-carotene was monitored at 470 nm in the presence of various concentrations of antioxidant. The % absorbance (corrected for control without antioxidant) was determined at each antioxidant concentration after incubation at 50 °C for 180 min.[23] Results shown in Figure 4 demonstrate that all three compounds **1–3** were effective in protecting linoleic acid oxidation. Compound **1** was the most effective. Compounds **2** and **3** were about twice weaker than compound **1**. Under the concentrations used in the experiment, **2** and **3** showed identical activities with one another and were quite similar to quercetin. In comparison, Trolox was more effective than compounds **2** and **3** (and quercetin) at lower concentrations. Among the starting materials, 4-aminomethylbenzylamine and syringaldehyde showed 16% and 12% A 470 nm at 12 μM after 180 min incubation, respectively while vanillin and 5-iodovanillin exhibited <5%.

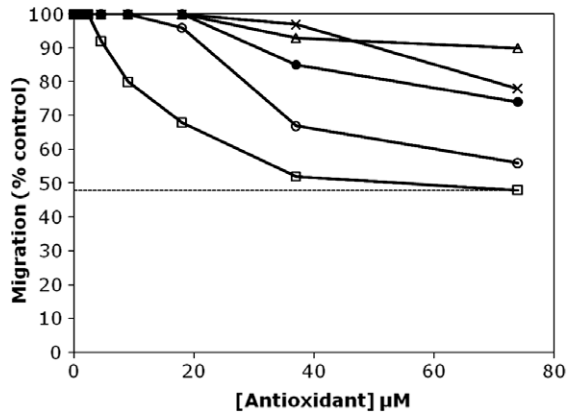



**Figure 3.** Migration of oxidized LDL in electrophoresis gels. AAPH oxidized LDL without any added antioxidant is expressed as 100%. Native LDL showed a mobility of ~50% that of oxidized lipoprotein (dashed line). Migration of oxidized LDL in the presence of various antioxidants including compound **1** (□), compound **2** (●), compound **3** (○), Trolox (Δ), and quercetin (X) is shown.

The effectiveness of the three compounds to protect DNA from AAPH damage was also determined. The plasmid DNA pBR 322 was incubated with AAPH (final concentration 10 mM) at 37 °C for 4 h with or without antioxidants (final concentrations, 0.35–45 μM), after which the samples were subjected to agarose electrophoresis.[24] An example of the gel obtained with compound **1** is shown in Figure 5. In the absence of AAPH, pBR 322 was mostly in its supercoiled (S) form (small amount of open circular, O, form was also visible, lane 1 Fig. 5). After reaction with AAPH, the DNA was transformed almost entirely into its open circular form (lane 2, Fig. 5). Compound **1** showed the best protection. In the presence of 11–45 μM of **1**, DNA was well protected (the S band intensity was similar to control without AAPH, lanes 3–5, Fig. 5). Quercetin showed similar protection (data not shown). Some protection was also obtained for **1** at 5 μM (lane 6, Fig. 5) but not with quercetin (data not shown). No protection was obtained for both **1** and quercetin below 5 μM (lanes 7–10, Fig. 5). In case of compound **2**, some protection was only observed at ⩾45 μM. Lower concentrations did not show any protection. Compound **3** as well as Trolox were ineffective at all concentrations used in the experiment (0.35–45 μM). The starting materials for dendrimer synthesis did not show any DNA protection under these conditions.

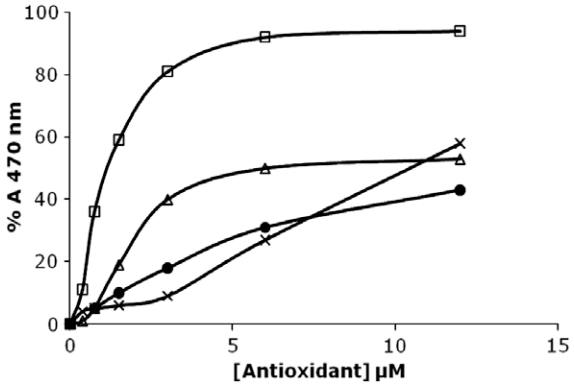



**Figure 4.** β-Carotene–linoleate assay for antioxidants. Protection of linoleic acid from oxidation by various antioxidants is shown for compound **1** (□), compounds **2** and **3** (●), quercetin (×), and Trolox (Δ).

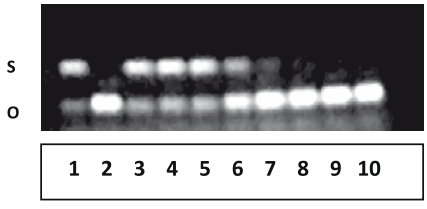



**Figure 5.** Protection against AAPH-induced DNA oxidation by compound **1**. Lane 1 (native DNA); lanes 2–10 (AAPH-oxidized DNA with 0, 45, 23, 11, 5, 3, 1.5, 0.7, and 0.35 μM antioxidant, respectively).

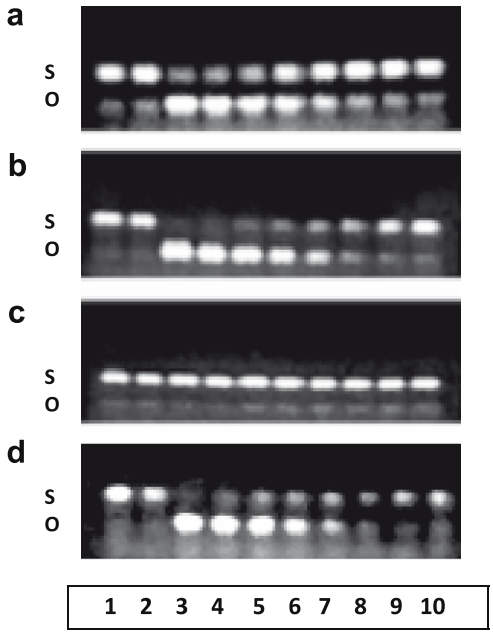



**Figure 6.** Pro-oxidant activity of (a) compound **1**; (b) quercetin; (c) compound **3**; (d) Trolox. Lane 1 (native DNA); lanes 2–10 ($Cu^{2+}$-oxidized DNA with 0, 45, 23, 11, 5, 3, 1.5, 0.7, and 0.35 μM antioxidant, respectively).

Antioxidants may also exhibit pro-oxidant activity in the presence of metal ions, which can lead to damage of biomolecules. The pro-oxidant effect of compounds **1–3** was examined by oxidation of DNA with copper(II) ion. pBR 322 was incubated with 10 μM $Cu^{2+}$ at 37 °C for 1 h in the presence of **1–3** (final concentrations between 0.35 and 45 μM). Examples of gels obtained for compound **1**, **3**, quercetin, and Trolox are shown in Figure 6. DNA damage was clearly observed with compound **1** and quercetin at concentrations between 11 and 45 μM (lanes 3–5, Fig. 6). However, quercetin exhibited a more pronounced pro-oxidant effect than dendrimer **1** between 3 and 5 μM (lanes 6 and 7, Fig. 6). For compound **2**, a slight pro-oxidant effect was seen at 23 and 45 μM (data not shown). Compound **3**, under similar conditions did not show any appreciable pro-oxidant effects at the concentrations tested. Trolox under these conditions also showed a strong pro-oxidant effect equivalent to quercetin. The starting materials did not show any pro-oxidant effect on DNA under these conditions.

In conclusion, three dendritic polyphenols were synthesized, all of which showed strong antioxidant capacity. Among the three dendrimers, quercetin and Trolox, the syringaldehyde-based dendrimer (**1**) was the most potent and displayed the best protection for LDL, linoleate, and DNA against free radical attack. Its pro-oxidant activity was stronger than the other two dendrimers (**2** and **3**) but weaker than quercetin and Trolox. All three dendrimers showed far superior DPPH radical scavenging activity compared to their individual as well as sum of their starting materials (core and building blocks), suggesting potential dendritic effect. In addition, they were also more effective in protecting LDL, linoleic acid, and DNA from free radical damage than their starting materials. Detailed activity of this antioxidant will be reported in forthcoming publication. The study shows that dendritic polyphenols, even at the G1 level, offer a novel and exciting class of molecules with beneficial antioxidant properties.

## Acknowledgments

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14. General procedure for TBDMS protection of phenolic aldehyde:Vanillin (5 g, 32.86 mmol) and triethylamine (10 mL, 71.74 mmol) were mixed in $CH_2Cl_2$ (200 mL). TBDMS-Cl (14 mL, 40.41 mmol) was added dropwise at 0°C. Reaction was run for 3 h and purified on a silica gel column with hexane.TBDMS-protected vanillin: Yield 96%; $R_f$ = 0.59 (hexane/ethyl acetate = 5:1); $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.17 (s, 6H, 2 × $CH_3$), 0.98 (s, 9H, C($CH_3$)$_3$), 3.84 (s, 3H, O$CH_3$), 6.93 (d, $J$ = 8.1 Hz, 1H, $C_5$-$H$), 7.33 (dd, 1H, $J$ = 7.95 Hz and $J$ = 1.95 Hz, $C_2$-$H$), 7.37 (d, 1H, $J$ = 1.5 Hz, $C_6$-$H$), 9.82 (s, 1H, $CHO$); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ −4.3 (2 × $CH_3$), 18.7 (C($CH_3$)$_3$), 25.8 (C($CH_3$)$_3$), 55.6 (O$CH_3$), 110.2 ($C_2$), 120.9 ($C_5$ and $C_6$), 126.4 ($C_1$), 131.1($C_4$), 151.5 ($C_3$), 191.2 ($CHO$).TBDMS-protected 5-iodovanillin: Yield 93%; $R_f$ = 0.62 (hexane/ethyl acetate = 3:1); $^1$H (300 MHz, $CDCl_3$) $\delta$ 0.24 (s, 6H, 2 × $CH_3$), 1.0 (s, 9H, C($CH_3$)$_3$), 3.82 (s, 3H, O$CH_3$), 7.31 (d, $J$ = 2.1 Hz, 1H, $C_2$-$H$), 7.81 (d, $J$ = 1.8 Hz, 1H, $C_6$-$H$), 9.73 (s, 1H, $CHO$); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ −2.9 (2 × $CH_3$), 19.3 (C($CH_3$)$_3$), 26.2 (C($CH_3$)$_3$), 55.3 (O$CH_3$), 89.9 ($C_5$), 109.7 ($C_2$), 131.5 ($C_1$ and $C_6$), 136.2 ($C_4$), 150.0 ($C_3$), 189.8 ($CHO$).
15. Formation of dendrimer using 4-aminomethylbenzylamine as a core and syringaldehyde, vanillin, or 5-iodovanillin as a building block. To a flask were added molecular sieves (50 g), 4-aminomethylbenzylamine (0.38 g, 2.79 mmol), two equivalents of TBDMS-protected vanillin (1.50 g, 5.64 mmol), and 1,2-dichloroethane (200 mL). The reaction was run for 12 h at room temperature. Na(OAc)$_3$BH (1.27 g, 5.99 mmol) was then added and run for another 24 h. To the reaction were added another 2 equivalents of TBDMS-protected vanillin (1.81 g, 6.80 mmol) and fresh molecular sieves (20 g). The reaction was gently stirred for 15 h, followed by Na(OAc)$_3$BH (1.28 g, 6.04 mmol) addition. The reaction was monitored with MALDI-TOF and stopped after 72 h. After filtration of molecular sieves, 1,2-dichloroethane was removed under reduced pressure. The resulting residue was dissolved in THF, followed by n-$Bu_4NF$ (2 mL) addition (TBDMS deprotection). The reaction was stirred overnight and condensed under reduced pressure. The resulting residue was partitioned between $CH_2Cl_2$ and water. The $CH_2Cl_2$ layer was dried with $MgSO_4$. After removal of $MgSO_4$, the solvent was removed on the rotorvap. The oily substance was purified via silica gel column chromatography using a gradient hexane/ethyl acetate solvent system (8:1→1:1).Compound **1** (dendrimer derived from syringaldehyde): Yield: 81%; mp: 89–92 °C, off-white powder; $R_f$ = 0.126 (hexane/acetone = 1:1); $^1$H (300 MHz, $CDCl_3$) $\delta$ 3.45 (s, 8H, 4 × periphery Ph-$CH_2$-N), 3.55 (s, 4H, 2 × core Ph-$CH_2$-N), 3.9 (s, 24H, 8 × O$CH_3$), 5.4 (s, 4H, 4 × O$H$), 6.6 (s, 8H, 4 × periphery Ph), 7.3 (s, 4H, 4 × core Ph-$H$); $^{13}$C NMR (75 MHz, $CDCl_3$) 56.5 (8 × O$CH_3$), 57.9 (2 × core Ph-$CH_2$-N), 58.2 (4 × periphery Ph-$CH_2$-N), 105.6 (8 × periphery Ph $C$-H), 129 (4 × core Ph $C$-H), 131 (4 × periphery Ph $C$-$CH_2$), 133.5 (4 × periphery Ph $C$-OH), 138.2

(2 × core Ph *C*-$CH_2$), 147 (8 × periphery Ph *C*-$OCH_3$); MS: *m*/*z* 801 $[M+H]^+$.Compound **2** (dendrimer derived from vanillin): Yield: 87%; $R_f$ = 0.18 (hexane/ethyl acetate = 1:1); $^1H$ (300 MHz, $CDCl_3$) $\delta$ 3.49 (s, 8H, 4 × periphery Ph-C$H_2$-N), 3.55 (s, 4H, 2 × core Ph-C$H_2$-N), 3.87 (s, 12H, 4 × OC$H_3$), 6.87 (d, *J* = 0.9 Hz, 8H, 4 × periphery Ph $C_2$-*H* and $C_6$-*H*), 6.91 (s, 4H, 4 × periphery Ph $C_5$-*H*), 7.34 (s, 4H, 4 × core Ph-*H*); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 56.0 (4 × $OCH_3$), 57.5 (2 × core Ph-C$H_2$-N), 57.7 (4 × periphery Ph-C$H_2$-N), 111.5 (4 × periphery Ph $C_2$-H), 114.2 (4 × periphery Ph $C_5$-H), 121.7 (4 × periphery Ph $C_6$-H), 128.8 (4 × core ph *C*-H), 131.7 (4 × periphery Ph $C_1$-$CH_2$), 138.4 (2 × core ph *C*-$CH_2$), 144.6 (4 × periphery ph $C_4$-OH), 146.6 (4 × periphery ph $C_3$-$OCH_3$); MS: *m*/*z* 681 $[M+H]^+$.Compound **3** (dendrimer derived from 5-iodovanillin): $R_f$ = 0.2 (hexane/ethyl acetate = 1:1); yield = 76%; $^1H$ (300 MHz, $CDCl_3$) $\delta$ 3.41 (s, 8H, 4 × periphery Ph-C$H_2$-N), 3.53 (s, 4H, 2 × core Ph-C$H_2$-N), 3.88 (s, 12H, 4 × OC$H_3$), 6.82 (d, *J* = 1.5 Hz, 4H, 4 × periphery Ph $C_2$-*H*), 7.28 (s, 8H, 4 × core Ph-*H* and 4 × periphery Ph $C_6$-*H*); $^{13}C$ NMR (75 MHz, $CDCl_3$) $\delta$ 56.4 (4 × $OCH_3$), 57.0 (4 × periphery Ph-C$H_2$-N), 57.8 (2 × core Ph-C$H_2$-N), 81.1 (4 × periphery Ph *C*-I), 111.4 (4 × periphery Ph $C_2$-H), 128.9 (4 × core ph *C*-H), 130.6 (4 × periphery Ph $C_1$-$CH_2$), 133.2 (4 × periphery Ph $C_6$-H), 138.1 (2 × core ph *C*-$CH_2$), 144.7 (4 × periphery Ph *C*-OH), 146.1(4 × periphery Ph *C*-$OCH_3$); MS: *m*/*z* 1184.78 $[M+H]^+$.


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