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Chemiluminescence Change of Polyphenol Dendrimers with Different Core Molecules

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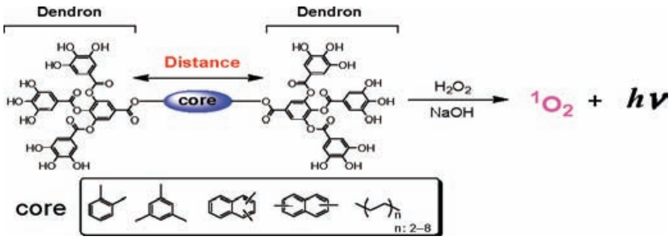
ABSTRACT



Second generation polyphenol dendrimers (PDs) with different core molecules were synthesized, and their chemiluminescence (CL) was measured by reacting the PDs with H_2O_2 under alkaline conditions. All of the PDs showed a strong CL, more than 120-fold greater than that of gallic acid. Various CL intensities of the PDs were obtained using different core molecules in the PDs. The distance between each dendron in the PD structure is crucial in the PD CL intensity.

Polyphenols, such as pyrogallol and gallic acid (3,4,5-trihydroxybenzoic acid, GA), produce singlet oxygen ($^1O_2$) in the presence of alkali and H_2O_2 and emit light (Figure 1).[1,2] The CL emission wavelength (634 nm) of $^1O_2$ is long compared to that of other CL compounds.[1] This indicates that polyphenols, a selective $^1O_2$ source, are useful as selective probes for CL assays. However, the CL intensities of the polyphenols are weak compared to that of well-known luminescent compounds, such as luminol,[3] acridinium ester,[4] and 1,2-dioxetane derivatives.[5] When a polyphenol with a strong and long-lasting CL is synthesized by a facile synthesis method, the polyphenol can be practically used as an analytical probe. We propose that polyphenols having many emitting units (GA units) can produce a strong CL and should be useful for the specific sources of $^1O_2$.

Dendrimers are constructed from the core, branch, and periphery moieties and can be designed into various molecules.[6] For example, biodegradable, self-assembled dendrimers and dendrimers as a host or catalyst have been

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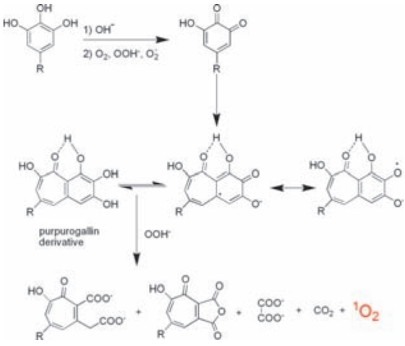
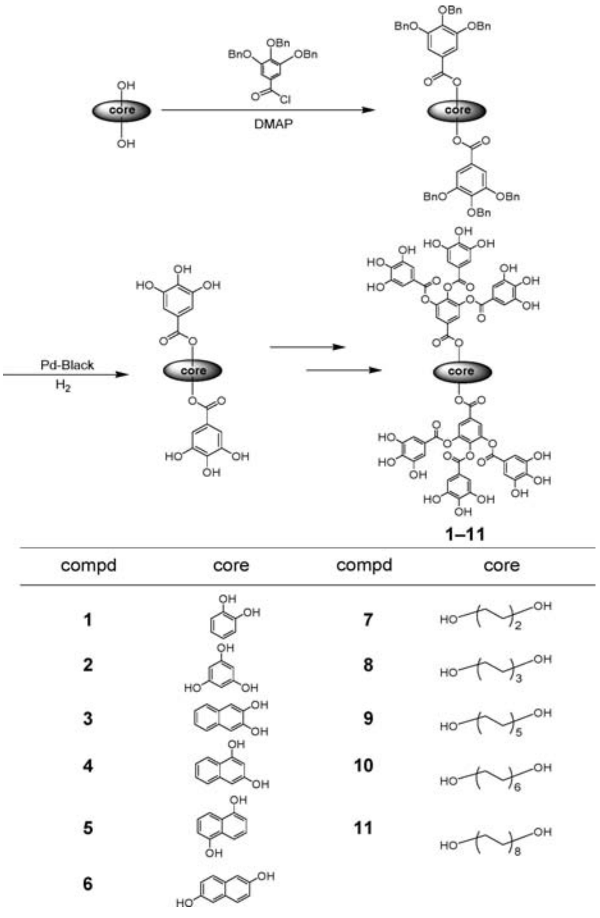


Figure 1. Generation process of singlet oxygen during polyphenol chemiluminescence.

Scheme 1. Synthesis of **1**−**11**



reported.[7−10] We found that second generation poly(3,4,5-trihydroxybenzoate ester) dendrimers (PDs) with pyrocatechol and 1,3,5-trihydroxybenzene as core molecules (compounds **1** and **2**, Scheme 1), which have many GA units, showed a very strong CL compared to that of GA in the presence of alkali and H_2O_2.[11] There have been few studies of the PDs and their CL. Thus, we synthesized second generation PDs with different core molecules. The change in the CL intensities of the PDs with the change in the core molecules was evaluated. The CL spectrum of the PD was measured to identify the CL species. We tried to investigate the relationship between the structures of the PDs and the CL. The photophysical properties of the PDs were evaluated. These experimental results should provide a significant information for developing luminescent PDs.

Compounds **1**−**11** were synthesized by a divergent method (Scheme 1). In the divergent method, gradual esterification/deprotection is required. We used benzyl groups to protect the hydroxyl groups. The protecting groups can be easily cleaved by catalytic hydrogenation. The esterification successfully proceeded for all the tested core molecules and gave a protected first generation dendrimer (5−50% yields). The second step, debenzylation of the protected dendrimers, proceeded with good yields (80−100%). The two steps described above were repeated. Yields of the third and final steps were 15−50% and 60−100%, respectively.

We measured the CL of the second generation PDs.[12] The reaction conditions (NaOH and H_2O_2 conc) that gave the maximun CL intensity for each PD are shown in Table 1. The CL intensities were recorded under very different conditions for **1**−**11**. All of the synthesized PDs showed a strong and long-lasting CL (Figure 2).

These CLs lasted for more than 5 min, and their CL intensities were at least 120-fold stronger than that of GA (Table 1). The times to reach the maximum photon counts were 30−70 s after the addition of H_2O_2. It was also found that while the CL intensities of the PDs with pyrocatechol (**1**), 1,3,5-trihydroxybenzene (**2**), naphthalenediol (**3**−**6**), 1,4-butanediol (**7**), and 1,6-hexanediol (**8**) as core molecules were 120- to 360-fold stronger than that of GA, those of the PDs with 1,10-decanediol (**9**), 1,12-dodecanediol (**10**), and 1,16-hexadecanediol (**11**) as core molecules were 360-, 350-, and 300-fold stronger than that of GA (Table 1). Among the PDs with linear diols as the core molecule, the CL intensities

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(12) The CL measurements of **1**−**11** are as follows: To 200 μL of 10 μM **1**−**11** in CH_3OH was added 100 μL of 50−150 mM NaOH. After the solution stood for 25 s, the CL reaction was initiated by the addition of 100 μL of 50−1250 mM H_2O_2. The CL emission was measured for 5 min, and the integral photon counts were used for estimation of the CL intensities.

Table 1. Relative CL Intensities of **1**−**11**

compd^a	NaOH (mM)	H_2O_2 (mM)	integral photon count (× 10^4)	relative CL intensity^b	relative CL intensity per GA in periphery	no. of methylene groups in core molecule
GA	200	750	0.5	1	1	
1	100	750	115	230	38	
2	100	250	180	360	40	
3	75	1000	90	180	30	
4	75	1000	60	120	20	
5	67	100	65	130	22	
6	75	750	65	130	22	
7	75	500	70	140	23	4
8	125	500	75	150	25	6
9	100	250	180	360	60	10
10	100	250	175	350	58	12
11	67	250	150	300	50	16

^{a} 10 μM in CH_3OH. ^{b} CL intensity of GA in CH_3OH was taken as 1.

greatly changed with the change in the length of the core molecule, and the PD with 1,10-decanediol (**9**) as the core molecule showed the strongest CL (Table 1). The increase in the CL intensities was obtained by increasing the number of methylene groups (4−10) in the linear alkyl chain of the core molecule. The GA units in the periphery of **9** are three units smaller than that of **2**; however, the CL intensity is still similar in each case. On the basis of these results, the core molecule has a significant effect on the CL of the PDs.

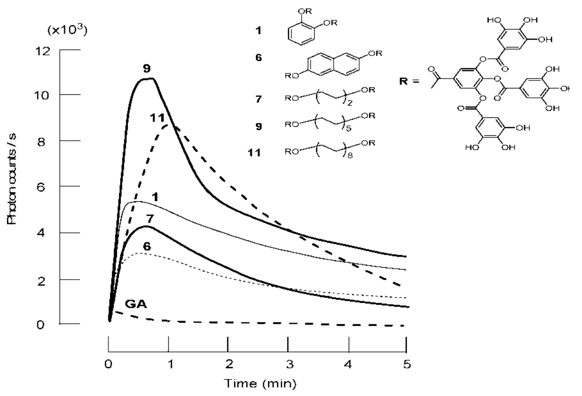

Figure 2. Time course of CL of GA, **1**, **6**, **7**, **9**, and **11** in the CL development. The final concentrations of GA, **1**, **6**, **7**, **9** and **11** were 5 μM. **1**: [NaOH] = 25 mM, [H_2O_2] = 188 mM. **6**: [NaOH] = 19 mM, [H_2O_2] = 188 mM. **7**: [NaOH] = 19 mM, [H_2O_2] = 125 mM. **9**: [NaOH] = 25 mM, [H_2O_2] = 63 mM. **11**: [NaOH] = 17 mM, [H_2O_2] = 63 mM. GA: [NaOH] = 50 mM, [H_2O_2] = 188 mM.

The CL spectrum of **2** was measured.[13] Compound **2** strongly emitted in the 560−660 nm range. The maximum emission wavelength was 630 nm. This maximum emission wavelength matched that of $^1O_2$. We measured the CL of **9** in the presence of 1,4-diazabicyclo[2,2,2]octane (DABCO) and D_2O (Figure 3).[14] DABCO is a selective quencher of $^1O_2$.[15] D_2O is a solvent which increases the lifetime of $^1O_2$.[16] DABCO apparently quenched the CL intensities of **9**. The CL intensities decreased with an increase in the concentration of DABCO. The addition of D_2O apparently increased the CL intensities of **9**. Thus, we concluded that the final CL species of the PDs was $^1O_2$.

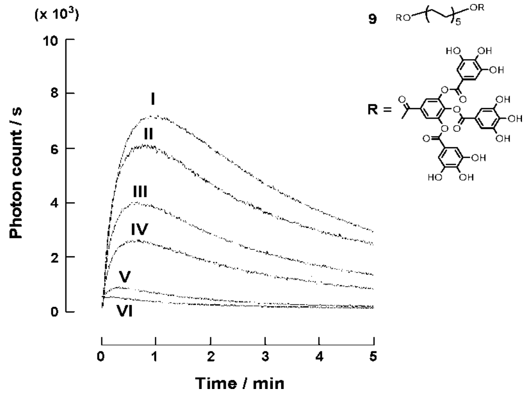

Figure 3. Time course of CL of **9** in CL development in the presence of DABCO and D_2O. The final concentaration of **9** was 4.7 μM. [NaOH] = 23 mM. [H_2O_2] = 58 mM. I: D_2O. II: [DABCO] = 0 (H_2O). III: [DABCO] = 3.5 mM. IV: [DABCO] = 7 mM. V: [DABCO] = 35 mM. VI: [DABCO] = 70 mM.

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(13) To 200 μL of 100 μM **2** in CH_3OH was added 100 μL of 75 mM NaOH. After the solution stood for 25 s, the CL reaction was initiated by the addition of 100 μL of 500 mM H_2O_2. Differential spectra were obtained from each photon count using 20 spectrofilters (350−720 nm).

(14) To 200 μL of 10 μM **9** in CH_3OH were added 30 μL of D_2O or 0.05−1 M DABCO and 100 μL of 100 mM NaOH. After allowing the solution to stand for 25 s, the CL reaction was initiated by the addition of 100 μL of 250 mM H_2O_2. The CL emission was measured for 5 min.

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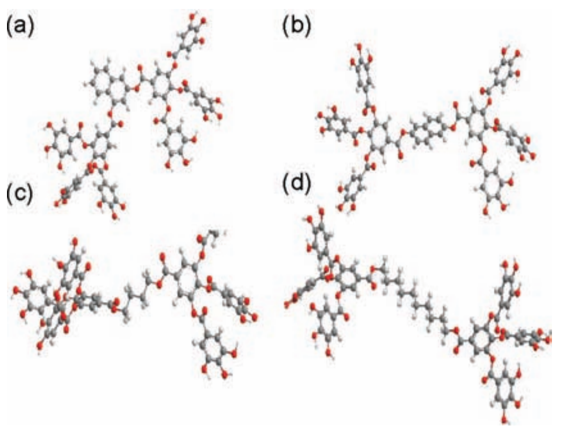

Figure 4. Optimized structures of **4** (a), **6** (b), **7** (c), and **9** (d) using density function theory (B3LYP, cc-pVDZ).

A single crystal of the PDs was not obtained for an X-ray crystallography study. The optimized structures of the PDs were calculated by the ab initio molecular orbital (MO) method. Our calculations started with the lower level method based on molecular mechanics (MM), and the sequential optimization was carried out by the Hartree−Fock (HF) method with the minimal basis set (STO-3G).[17,18] The obtained molecular conformation was employed to perform the final geometry optimization by the B3LYP level density functional theory[19,20] with Dunning's correlation-consistent, polarized-valence, double-ζ (cc-pVDZ) basis set.[21] Thus, all of the fully optimized structures for the second generation PDs were obtained. The general process for the generation of $^1O_2$ in polyphenol CL has been evaluated (Figure 1).[1,2] In this CL process, the purpurogallin derivative is produced. This CL process may occur both in intra- and inter-GA units in the periphery of the PD. When peripheral GA units have a $\pi-\pi$ stacking structure, the GA units are close to each other, and it is difficult to form the purpurogallin derivative. On the contrary, on the basis of the present results of the molecular modeling studies, the peripheral GA units of PDs do not stack their structures by a $\pi-\pi$ interaction of the benzene rings, and the GA units are not close to each other (Figure 4). This indicated that the formation of the purpurogallin derivative easily proceeded and $^1O_2$ should have been easily produced.

The absorption and fluorescence properties are shown in Table 2. Compounds **1** and **3**−**11** were fluorescent, but the quantum yield was very low. The absorption wavelengths of the PDs were 220−350 nm, and the emission wavelength of **2** was in the range of 550−660 nm. This indicated that the PDs did not absorb the CL of produced $^1O_2$. We confirmed that the CL intensities of **5** and **9** did not change in the presence of naphthalene or 1,5-naphthalenediol. Thus, naphthalene did not decrease the CL intensities of the PDs. The produced $^1O_2$ molecules were not quenched by naphthalene. From these results, it was confirmed that the CL intensities of PDs showed the amount of produced $^1O_2$, which should depend on the amount of purpurogallin produced (Figure 1). Compounds **9**−**11** gave approximately the maximum CL intensity (Table 1). There is an optimal distance between each dendron of PD to produce the maximum CL intensity. Focusing on compound **9**, which produced the maximum CL intensity, the distance between each dendron in optimized compound **9** was about 14 Å (Figure 4d). Thus, the optimal distance between each dendron of a second generation of PD is about 14 Å to give the maximum CL intensity. This distance should provide sufficient space for the production of purpurogallin by intermolecular reaction at the peripheral moiety in PD. We found that the distance between each dendron in the PD structure is a crucial factor in PD CL intensity.

Table 2. Absorption and Fluorescence properties of **1**, **3**, **5**, **8**, and **11**: Maximun Absorption Wavelength (λ_{abs}), Molar Absorption Coefficients (ε), Maximum Emission Wavelength (λ_{em}), and FL Quantum Yield (Φ_f)

compd[a]	λ_{abs} (nm)	ε ($\times 10^4$) (M^{-1} cm^{-1})	λ_{em}[b] (nm)	Stokes shift (nm)	Φ_f ($\times 10^{-3}$)[c]
1	283	8.4	369	86	2
3	283	6.1	345	62	1
5	287	6.8	369	82	1
8	285	6.8	379	94	4
11	285	7.7	368	83	1

[a] 1 μM in CH_3OH. [b] FL spectra were corrected. [c] Quantum yield (quinine sulfate in 0.05 M H_2SO_4, $\Phi = 0.51$).

In summary, we developed various PDs that have strong CL intensities. The CL intensity of the PD with 1,10-decanediol as the core molecule was the strongest among all the PDs. There is no report on the notable change in the CL intensities of the PDs by changing their core molecules. We showed that the core molecule was crucial for the CL of the PDs. Our study provided significant information for developing polyphenols which have strong CL intensities. Furthermore, these PDs are very interesting as compounds which selectively produce large amounts of $^1O_2$.

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Supporting Information Available: Experimental details and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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