Pergamon

Tetrahedron Letters 43 (2002) 8185–8189

TETRAHEDRON
LETTERS

Synthesis of poly (3,4,5-trihydroxybenzoate ester) dendrimers and their chemiluminescence

Manabu Nakazono, Li Ma and Kiyoshi Zaitsu*

Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Received 17 May 2002; revised 22 August 2002; accepted 23 August 2002

Abstract—Gallic acid (GA) and gallic acid methyl ester (GM), polyphenol chemiluminescence (CL) compounds, produces light in the presence of alkali and hydrogen peroxide. We synthesized first-generation polyphenol dendrimers with GA units in the periphery and measured their CL for developing a polyphenol compound which has a strong CL intensity. The CL intensities of the poly (3,4,5-trihydroxybenzoate ester) dendrimers **4** and **8** in MeOH are approximately 400- and 600-fold stronger than that of GA, respectively. © 2002 Elsevier Science Ltd. All rights reserved.

Polyphenol such as pyrogallol and gallic acid (GA) produces singlet oxygen in the presence of hydrogen peroxide in an alkaline medium and emits light.[1] GA was utilized for selective Co^{2+} detection using a chemiluminescence (CL) flow injection system.[2] The CL intensities of polyphenol are very weak compared to that of luminol and the acridinium ester, representative CL compounds. Thus, *trans*-4-(3-propionic acid) phenylboronic acid, 4-biphenylboronic acid in the presence of peroxidase[3] or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide[4] have been used as enhancer for highly sensitive polyphenol CL determination. However, the derivatization method for polyphenol that has a strong CL intensity has not yet been reported.

Recently, the functions of dendrimers have focused on the material engineering based on optics in the field of nanoscale science[5,6] since the poly (amidoamine) dendrimer as a new polymer was synthesized.[7] The poly (propylene amine) family functionalized in the periphery with fluorescent dansyl units interacts with Co^{2+}.[8] More recently, the acridinium dendrimers were conjugated with bovine serum albumin and its CL was estimated as six-fold compared to that of the acridinium salt.[9]

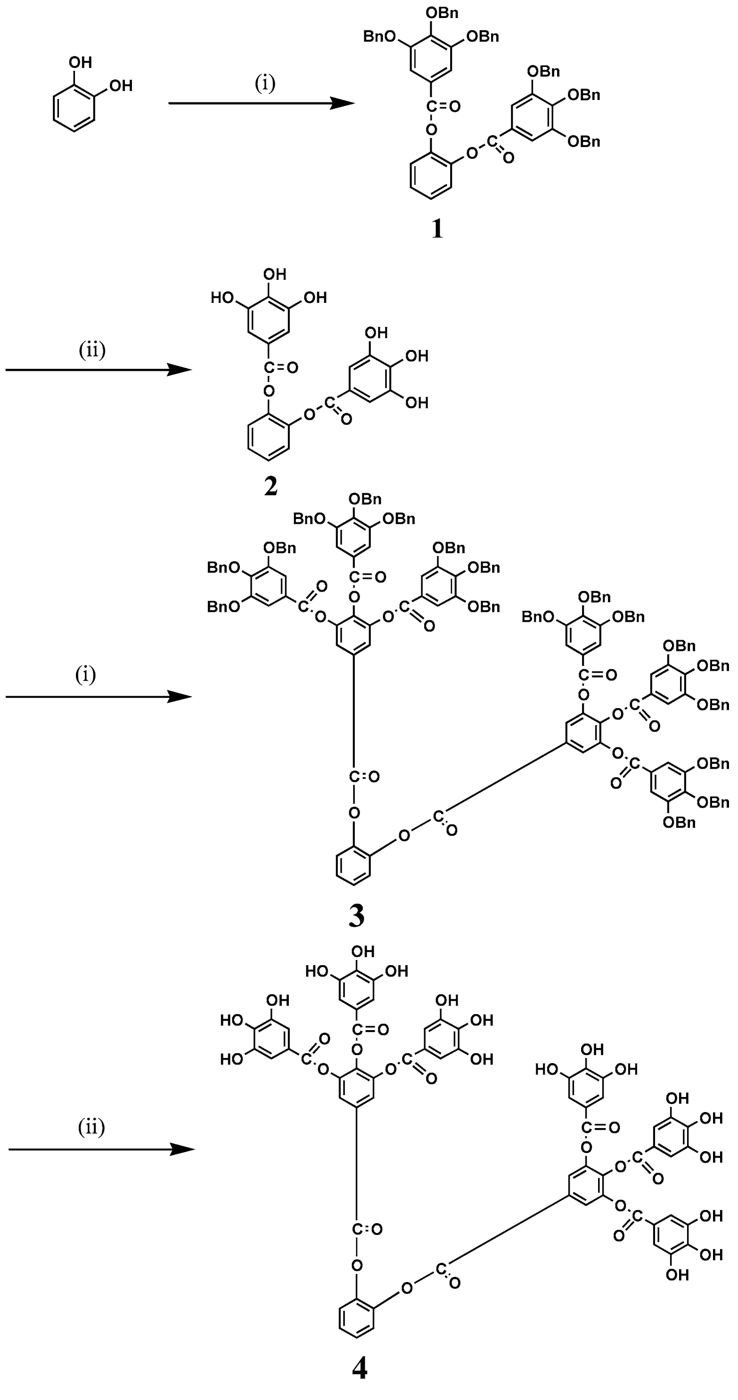
From our standpoint, we designed new types of polyphenol dendrimers having an increased number of chemiluminophores for high luminescence intensity and hydroxyl groups in the periphery to form hydrogen bonds with the analyte. The polyphenol dendrimers, which have GA in the periphery, were synthesized and their CL was measured.[10] We synthesized the first-generation polyphenol dendrimers with GA units in the periphery by a divergent method using pyrocatechol and 3,4,5-trihydroxybenzene as the core molecules (Schemes 1 and 2).[11] The esterification of 3,4,5-tribenzyloxybenzoyl chloride[12] with pyrocatechol gave **1** in 56.8% yield. After the debenzylation of **1**, we could not separate **2** and the byproduct by chromatographic purification. Therefore, the desired zero-generation ester **2** was not isolated. Compounds **3** and **4** were synthesized by repeating this esterification and debenzylation, but the yield of the first-generation ester **4** was a low yield. The esterification of 3,4,5-tribenzyloxybenzoyl chloride with 1,3,5-trihydroxybenzene gave **5** in 31% yield. The zero-generation ester **6** and first-generation **8** were synthesized as described above. The yields of the polyphenol dendrimers decreased with the increasing dendrimer generation. Compounds **3** and **7** were debenzylated in high yield by catalytic reduction under hydrogen gas in the presence of palladium-black at ambient temperature. Benzylation will be optimal for protecting the phenols. The mass spectra of **6** and **8** were obtained by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI TOF MS) using 2,5-dihydroxybenzoic acid as the matrix. **4** and **8** were very stable in MeOH. Compounds **4** and **8** showed maximum CL intensities when 75 mM sodium hydroxide and 500 mM hydrogen peroxide were added. Both the intensities of the maximum light emission and the length of the CL period of **4** and **8** were increased

---

Keywords: dendrimer; chemiluminescence; polyphenol.

* Corresponding author. Tel.: +81-92-642-6596; fax: +81-92-642-6601; e-mail: zaitsu@phar.kyushu-u.ac.jp

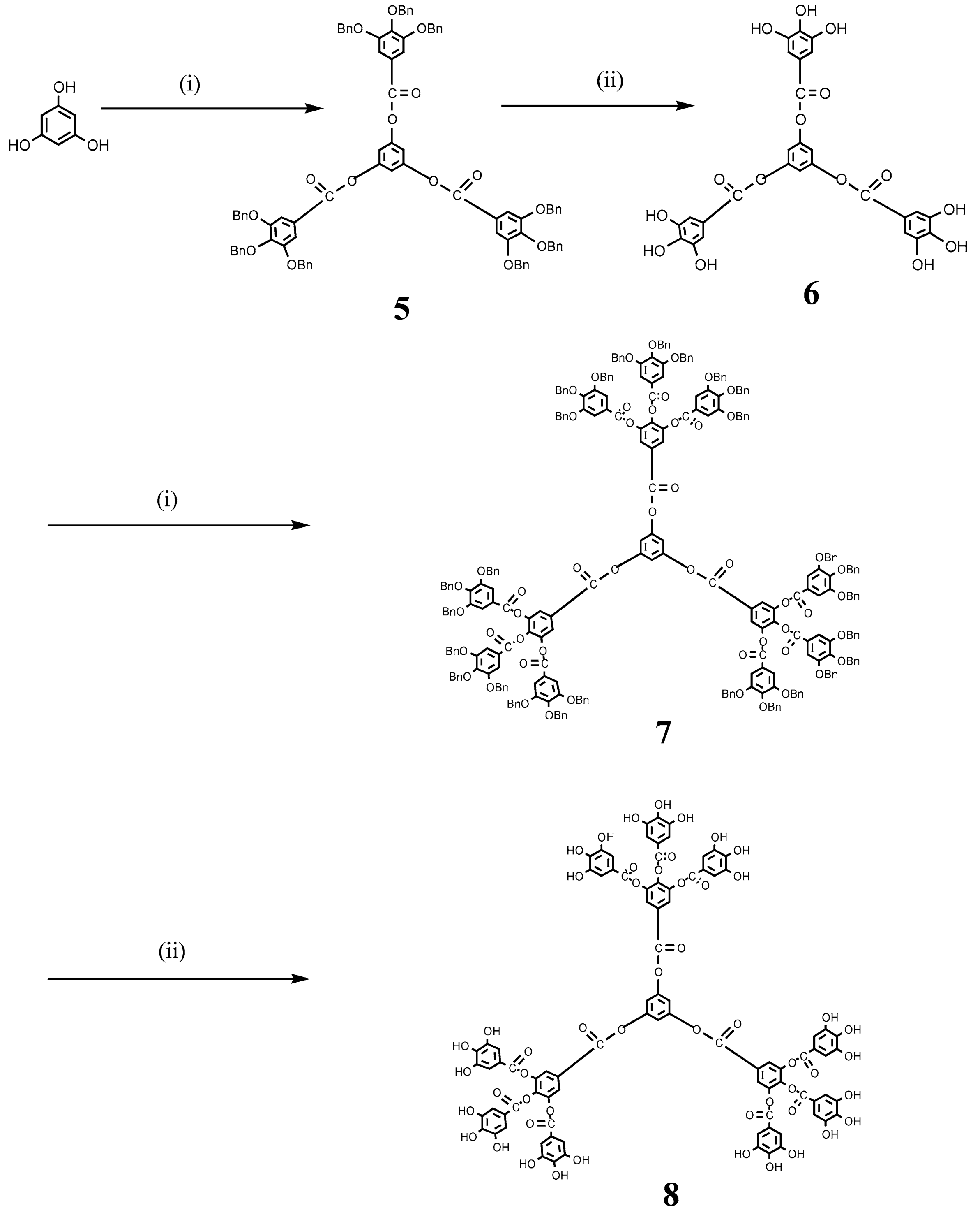
PII: S0040-4039(02)01959-7



Scheme 1. Synthetic route of **1**–**4**. *Reagents and conditions*: (i) $CHCl_3$, pyridine, 4-dimethylaminopyridine, 3,4,5-tribenzyloxybenzoyl chloride; (ii) Pd-black, H_2, $CHCl_3$–MeOH.

compared to that of GA. The time of maximum light emission of **4** and **8** were 50 and 60 s after the injection of hydrogen peroxide. The CL intensities in 5 min after injection of the hydrogen peroxide were approximately half the maximum CL intensities (Fig. 1). These kinds of polyphenol compounds, which emit light for such a long CL life, have not been found. The CL intensities of **4** and **8** in MeOH were approximately 400- and 600-fold stronger than that of GA, respectively. The CL intensities of **4** and **8** in MeOH were approximately 100- and 150-fold stronger than that of GM, respectively (Table 1). CL intensity per GA unit of **4** and **8** was approximately equal, it suggests that the increment of the number of chemiluminophores was effective to increase CL intensity of polyphenol dendrimers. An increase in the CL intensity of GA was accomplished by forming a dendric structure.

In conclusion, polyphenol dendrimers were synthesized in order to obtain a strong CL compound and their CL



Scheme 2. Synthetic route of **5**–**8**. *Reagents and conditions*: (i) $CHCl_3$, pyridine, 4-dimethylaminopyridine, 3,4,5-tribenzyloxybenzoyl chloride; (ii) Pd-black, H_2, $CHCl_3$–MeOH.

intensities were much stronger than that of GA. Esterification of the hydroxyl groups of GA in the dendrimer was very effective for developing a strong CL. The relationship between the CL intensity and structure of polyphenol dendrimers should be clarified to know the reason of the strong light emission of highperbranched compounds such as poly (3,4,5-trihydroxybenzoate ester) dendrimers. Polyphenol CL dendrimers will be used for a wide variety of CL assays utilizing hydroxyl groups of the polyphenol for forming a hydrogen bond with oxygen in the analyte structure.

Table 1. Relative CL intensities of GA, GM, **4** and **8** in MeOH

Compound[a]	NaOH (mM)	H_2O_2 (mM)	Integral photon count (×10^4)	Relative CL intensity[b]	CL intensity per GA unit[c] [photon count (×10^4)]
GA	50	500	4.8	1	
GM	300	5000	20	4	
4	75	500	2025	421	337.5
8	75	500	2986	622	331.7

[a] Concentration of each compound was 0.1 mM.

[b] CL intensity of GA obtained in MeOH was taken as 1.

[c] CL intensity per GA unit of **4** and **8** was calculated by dividing integral photon counts of **4** and **8** with the number of GA units in the periphery.

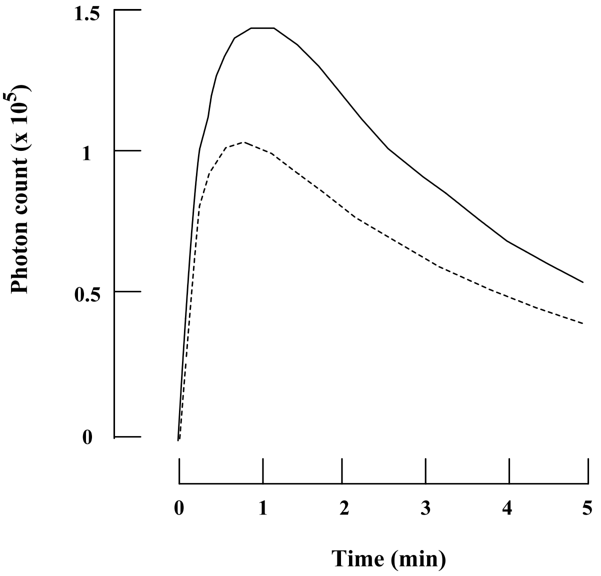


Figure 1. Time course of chemiluminescence of **4** and **8** in MeOH. The concentration was 0.1 mM in MeOH. The dashed line and solid line represent the CL development of **4** and **8**, respectively.

References

1. Slawinska, D.; Slawinski, J. *Anal. Chem.* **1975**, *47*, 2101.
2. Nakahara, S.; Yamada, M.; Suzuki, S. *Anal. Chim. Acta* **1982**, *141*, 255.
3. Nozaki, O.; Ji, X.; Kricka, L. J. *J. Biolum. Chemilum.* **1995**, *10*, 151.
4. Nakazono, M.; Ijichi, J.; Ohba, Y.; Zaitsu, K. *Anal. Sci.* **1998**, *14*, 853.
5. Fréchet, J. M. J. *Science* **1994**, *263*, 1710.
6. Oosterom, E. G.; Reek, N. H. J.; Kamer, C. J. P.; van Leeuwen, W. N. M. P. *Angew. Chem., Int. Ed.* **2001**, *40*, 1828.
7. Tomaria, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Martin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117.
8. Vögtle, F.; Gestermann, S.; Kauffmann, C.; Ceroni, P.; Vicinelli, V.; Balzani, V. *J. Am. Chem. Soc.* **2000**, *122*, 10398.
9. Adamczyk, M.; Fishpaugh, J.; Mattingly, P. G.; Shreder, K. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3595.
10. The CL measurements were performed using a photon-counting luminometer, Lumat LB 9501 (Berthold, Wildbad, Germany); its operation and data processing were performed using a personal computer (PC 9801 ES2, NEC Co., Tokyo, Japan) with a luminometer program, LB 9501/9801 Ver. 1.41 (Japan Berthold, Tokyo, Japan). Procedure for the CL development of GA, **4** or **8**: To 200 μl of a 0.1 mM MeOH solution of GA, **4** or **8** was added 100 μl of 10–250 mM NaOH. After standing for 25 s, the CL reaction was initiated by the addition of 100 μl of 500 mM H_2O_2 using an automatic injection system in the luminometer. The CL emission was measured for 5 min and the integral photon counts were used.
11. Spectroscopic data: Compound **1**: ^{1}H NMR ($CDCl_3$): 4.91 (s, 8H, benzyl H), 4.99 (s, 4H, benzyl H), 7.19 (m, 34H, ArH), 7.43 (s, 4H, ArH). Anal. calcd for $C_{62}H_{50}O_{10}$: C, 77.97; H, 5.28. Found: C, 77.58; H, 5.33. Compound **3**: ^{1}H NMR ($CDCl_3$): 4.69 (m, 36H, benzyl H), 7.11 (m, 110H, ArH), MALDI-TOF MS; 2974.94 $[M+Na]^+$. Compound **4**: ^{1}H NMR (CD_3OD): 6.98 (s, 4H, ArH), 7.08 (s, 8H, ArH), 7.41 (m, 4H, ArH), 8.00 (s, 4H, ArH). Anal. calcd for $C_{62}H_{38}O_{34}\cdot 8H_2O$: C, 50.62; H, 3.7. Found: C, 50.29; H, 3.60. Compound **5**: ^{1}H NMR ($CDCl_3$): 5.15 (s, 18H, benzyl H), 7.07–7.50 (m, 54H, ArH). Compound **6**: ^{1}H NMR (CD_3OD): 7.02 (s, 3H, ArH), 7.20 (s, 6H, ArH). MALDI-TOF MS; 605.303 $[M+Na]^+$. Anal. calcd for $C_{27}H_{28}O_{15}\cdot 5H_2O$: C, 48.22; H, 4.2. Found: C, 48.51; H, 4.24. Compound **7**: ^{1}H NMR ($CDCl_3$): 4.78–5.02 (m, 54H, benzyl H), 7.12–7.45 (m, 162H, ArH). Compound **8**: ^{1}H NMR (DMSO-d_6): 6.89–8.11 (m, 27H, ArH), 9.15–9.36 (m, 27H, Phenol OH). MALDI-TOF MS; 1975.19 $[M+Na]^+$. Anal. calcd for $C_{90}H_{54}O_{51}\cdot 12H_2O$: C, 49.87; H, 3.63. Found: C, 49.73; H, 3.91.
12. 3,4,5-Tribenzyloxybenzoyl chloride was prepared as follows. To a stirred solution of methyl-3,4,5-trihydroxybenzoate (22 g, 0.1 mol) and benzylchloride (46 ml, 0.4 mmol) in DMSO (200 ml) was added potassium carbonate (50 g, 0.36 mol). The mixture was refluxed for 2 h and then H_2O (400 ml) was added. The reaction solution was cooled in ice and then filtered. The crude product was dried and then recrystallized by *n*-hexane–benzene (1:2, v/v) to give methyl-3,4,5-tribenzyloxybenzoate as a colorless powder (16.07 g, 35.4% yield, mp 87°C). To a stirred solution of methyl-3,4,5-tribenzyloxybenzoate (15.9 g, 35 mmol) and NaOH (4.2 g, 105 mmol) in MeOH (300 ml) was added H_2O (15 ml). The mixture was refluxed for 1 h and then H_2O (300 ml) was added. The reaction solution was acidified by 36% HCl. The crude product was washed with H_2O, dried and then recrystallized by

EtOH to give 3,4,5-tribenzyloxybenzoic acid as a colorless powder (12.32 g, 80% yield, mp 187°C). To a stirred solution of 3,4,5-tribenzyloxybenzoic acid (12.32 g, 28 mmol) and 95% thionylchloride (42 ml, 560 mmol) in benzene (200 ml) was added pyridine (0.5 ml). The mixture was refluxed for 1.5 h at 73°C. To the resulting solution was added petroleum ether (150 ml) and then cooled in ice. The crude precipitate was recrystallized by *n*-heptane to give 3,4,5-tribenzyloxybenzoyl chloride as a colorless powder (9.78 g, 61% yield, mp 113°C). FAB-MS *m*/*z*; 458 [M$^+$].