Structure–Activity Relationship of Bis-Galloyl Derivatives Related to (−)-Epigallocatechin Gallate

Kosuke Dodo,*[,a,b] Taro Minato,[a] and Yuichi Hashimoto[a]

[a] *Institute of Molecular and Cellular Biosciences, University of Tokyo; 1–1–1 Yayoi, Bunkyo-ku, Tokyo 113–0032, Japan: and* [b] *RIKEN (The Institute of Physical and Chemical Research); 2–1 Hirosawa, Wako, Saitama 351–0198, Japan.*
Received July 22, 2008; accepted November 10, 2008

Green tea and (−)-epigallocatechin gallate (EGCG: one of main components of green tea) are well known to have preventive activities against human cancers. Previously, using a galloyl group as a core structure derived from EGCG, we developed alkyl gallate and gallamide derivatives, which showed strong antiproliferative activity towards human leukemia HL-60 cells by inducing apoptosis. Here, as a further structural development study, we planned to introduce an additional galloyl group into alkyl gallates and gallamides. According to this strategy, various bisgallate and bisgallamide derivatives were synthesized and tested for antiproliferative activity towards HL-60 cells. In gallamide derivatives having a short alkyl chain, the additional galloyl group enhanced the antiproliferative activity. In contrast, in the gallate derivatives, the additional galloyl group had no effect on the antiproliferative activity.

Key words (−)-epigallocatechin gallate; gallic acid; antiproliferative activity

Various epidemiological studies on the relationship between intake of green tea and risk of cancer indicate that green tea has preventive activities against human cancers.[1,2] (−)-Epigallocatechin gallate (EGCG), one of the major components of green tea,[3] was reported to have a range of activities, including cell growth inhibition, apoptosis induction, and cell cycle arrest.[4,5] Therefore, EGCG and related polyphenolic compounds were thought to contribute to the antitumor activity of green tea. To clarify the essential structure for the antitumor activity, EGCG and related compounds, such as epicatechin, epicatechin gallate (ECG), epigallocatechin (EGC), were tested for antiproliferative activity towards PC-9 human lung cancer cells (Fig. 1).[6] It was concluded that a galloyl group, which is composed of one benzoyl and three phenoxy groups, contributes significantly to the antiproliferative activity. Based on this concept, we previously developed various alkyl gallate and gallamide derivatives having a galloyl group and evaluated their cell growth-inhibitory activity towards HL-60 human leukemia cells.[7] In our previous studies, gallates **1** and gallamides **2** (Fig. 1) were found to have potent cell growth-inhibitory activity, and the hydrophobicity of their alkyl chains was thought to be important for the antiproliferative activity. On the other hand, the hydroxyl groups of the galloyl moiety were also discovered to be essential for cell growth inhibition, indicating that the galloyl group greatly contributes to the activity. Therefore, we planned to introduce an additional galloyl group into gallate and gallamide derivatives in an attempt to increase the activity (Fig. 2).

Results and Discussion

Initially, we synthesized bisgallate derivatives having various lengths of alkyl chain according to the previously reported method, as shown in Chart 1. To obtain bisgallate derivatives, benzyl-protected gallic acid **6**[7] was introduced at both ends of the alkyl chain *via* transformation of **6** to acyl chloride (**7**), followed by debenzylation using H_2/Pd–C to afford bisgallate derivatives **9a**—**c**. Considering the possibility of intracellular hydrolysis of the ester group, bisgallamide derivatives **11a**—**c** having amide bonds instead of ester bonds were also synthesized. As shown in Chart 2, bisgallamide derivatives having various lengths of alkyl chain were

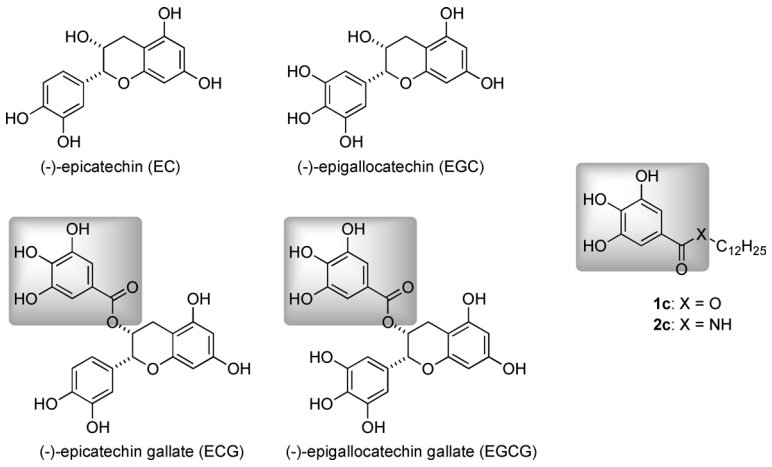


Fig. 1. Structures of EGCG and Related Compounds

* To whom correspondence should be addressed. e-mail: dodo@iam.u-tokyo.ac.jp; dodo@riken.jp

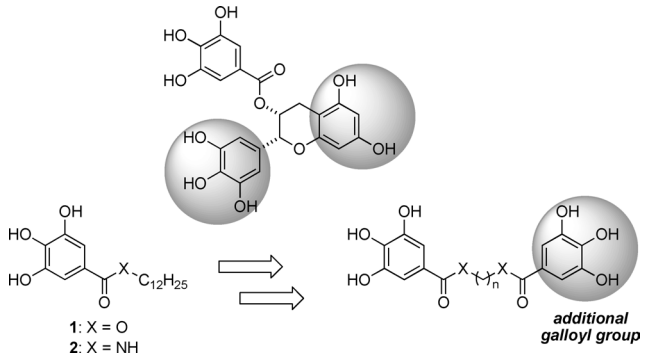
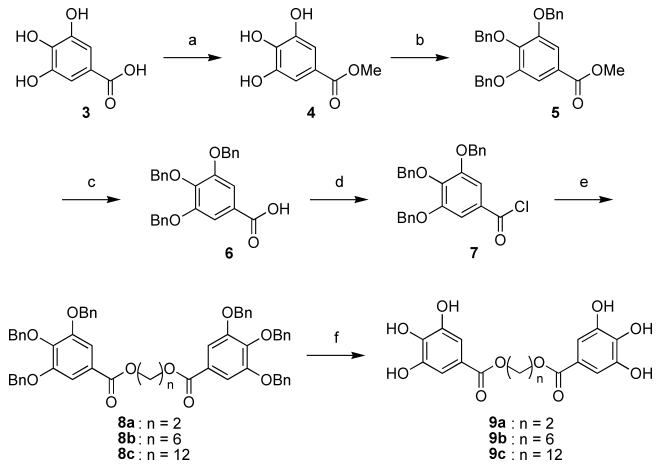
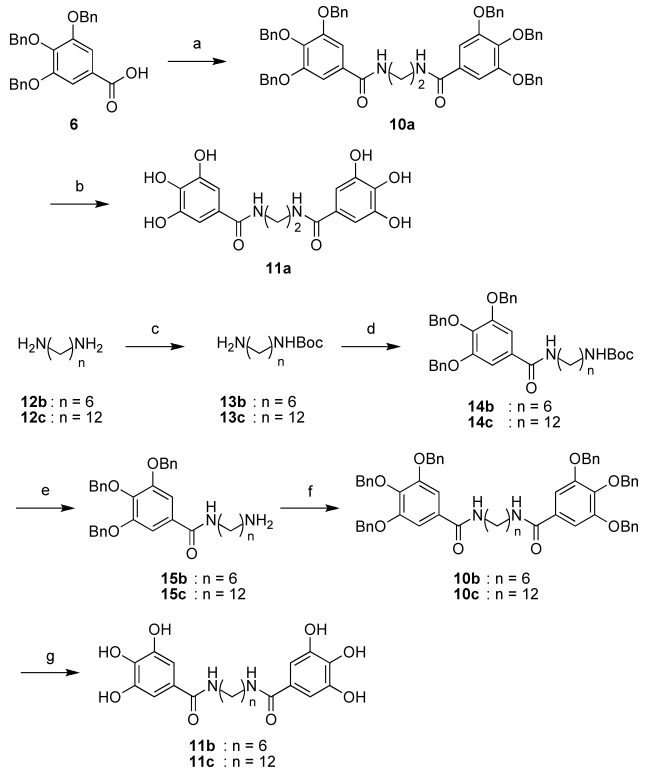


Fig. 2. Design of Bis-Galloyl Derivatives



Reagents and conditions: (a) MeOH, H_2SO_4, 100 °C, 2 h; (b) BnCl, K_2CO_3, DMF, 80 °C, 15 h; (c) NaOH, MeOH/H_2O/dioxane, 120 °C, 6 h; (d) $(COCl)_2$, DMF, toluene, rt; (e) $HO(CH_2)_nOH$, K_2CO_3, DMF, rt, overnight; (f) 10% Pd/C, H_2, AcOEt, rt.

Chart 1. Modification of Terminal Carbon of Alkylgallate



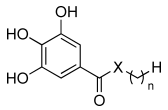
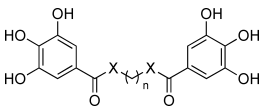
Reagents and conditions: (a) $H_2N(CH_2)_2NH_2$, EDCl, HOBt, CH_2Cl_2; (b) 10% Pd/C, H_2, AcOEt, rt; (c) Boc_2O, CH_2Cl_2/MeOH; (d) **6**, EDCl, HOBt, DMF; (e) TFA, CH_2Cl_2; (f) **6**, EDCl, HOBt, CH_2Cl_2; (b) 10% Pd/C, H_2, AcOEt, rt; (c) Boc_2O, CH_2Cl_2/MeOH; (d) **6**, EDCl, HOBt, DMF; (e) TFA, CH_2Cl_2; (f) **6**, EDCl, HOBt, DMF; (g) 10% Pd/C, H_2, AcOEt, rt.

Chart 2. Modification of Terminal Carbon of Alkylgallamide

synthesized by condensation of alkyldiamine with protected gallic acid **6** in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) and HOBt, followed by the debenzylation using H_2/Pd–C to give **11a**—**c**.

Then, mono-gallates **1a**—**c**, bis-gallates **9a**—**c**, mono-gallamides **2a**—**c**, and bis-gallamides **11a**—**c** were tested for antiproliferative activity by calculating the IC_{50} values from the viability of human leukemia HL-60 cells treated with each compound, as shown in Table 1. In all cases, disappointingly, an additional galloyl group did not elicit an improvement of antiproliferative activity. As for gallate derivatives (**1a**—**c** and **9a**—**c**), the relationship between antiproliferative activity and the length of the alkyl chain was similar in mono- and bis-gallates, *i.e.*, the longer the alkyl chain length, more potent the antiproliferative activity. But, interestingly, in gallamide derivatives (**2a**—**c** and **11a**—**c**), the relationship was different for mono- and bis-gallamides. In the case of monogallamide derivatives (**2a**—**c**), the longer the alkyl chain, the greater the antiproliferative activity. On the contrary, in the case of bisgallamide derivatives (**11a**—**c**), the relationship was reversed, *i.e.*, the shorter the alkyl chain length, the greater the antiproliferative activity. The results suggest that the effect of an additional galloyl moiety is different between gallate and gallamide derivatives. In the previous report,[7] we confirmed the apoptosis-inducing activity of galloyl derivatives based on the apoptotic morphological

Table 1. Antiproliferative Activity of Alkyl Mono- and Bis-Gallate and Gallamide Derivatives

Compound		X	*n*	IC_{50} (μM)
	**1a**	O	2	42
	1b	O	6	4.4
	1c	O	12	1.1
	2a	NH	2	16
	2b	NH	6	24
	2c	NH	12	3.0
	**9a**	O	2	29
	9b	O	6	14
	9c	O	12	1.4
	11a	NH	2	5.2
	11b	NH	6	7.1
	11c	NH	12	12
		EGCG		9.4

changes, the DNA fragmentation, and the activation of caspase-3. Therefore, to clarify the difference between gallate and gallamide derivatives, we examined the apoptosis-inducing activity of gallate and gallamide derivatives. As a result, a great difference between bisgallates and bisgallamides was found in the activation of caspase-3, a typical hallmark of apoptosis. As shown in Fig. 3, potently antiproliferative gallates, **1c** (IC_{50}=1.1 μM) and **9c** (IC_{50}=1.4 μM), efficiently induced the activation of caspase-3 to the extent of 350—510%. A moderately potent antiproliferative monogallamide,

2c (IC_{50}=3.0 μM), also induced the activation of caspase-3 by *ca.* 200%. However, no apparent activation of caspase-3 was found with bis-gallamide derivatives **11a**—**c** (IC_{50} values of 5.2—12 μM) (Fig. 3). These results indicate that bisgallamides may elicit the antiproliferative activity without inducing apoptosis.

Although the antiproliferative activity was not increased by introduction of an additional galloyl group, the additional galloyl group in bis-gallate derivatives might have some effect. To examine this possibility, we modified one of galloyl groups in the bis-gallate derivative **9c**. In the previous study, the hydroxyl group(s) of mono-galloyl derivatives was found to be essential for the antiproliferative activity.[7] Therefore, various **9c**-related gallate derivatives were synthesized as shown in Chart 3, and their antiproliferative activities were tested (Table 2). However there was little change in the IC_{50} values of compounds **20** (IC_{50} values of 0.84—1.7 μM) and **9c** (IC_{50}=1.4 μM), indicating that the second galloyl group had little effect on the antiproliferative activity. Moreover, the non-hydroxyl derivative **20d** showed the most potent activity among this series of compounds, which implies that the hydrophobicity of the alkyl chain is important for the activity.

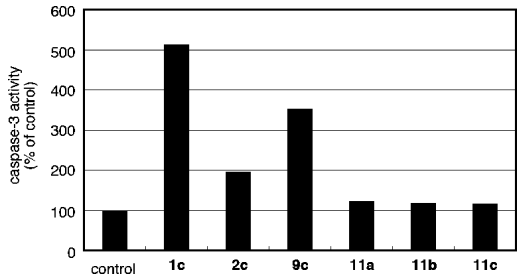
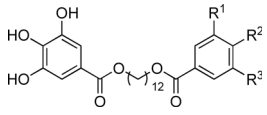


Fig. 3. Caspase-3 Activation Induced by Galloyl Derivatives (10 μM, 4 h)

Table 2. Antiproliferative Activity of Alkyl Bis-Gallate and Related Derivatives



Compound	R^1	R^2	R^3	IC_{50} (μM)
9c	OH	OH	OH	1.4
20a	OH	OH	H	1.7
20b	OH	H	H	1.2
20c	H	OH	H	1.3
20d	H	H	H	0.84

In this study, as a further structural development of alkyl gallate and gallamide derivatives derived from EGCG, we synthesized various bisgallate and bisgallamide derivatives having an additional galloyl group and tested them for antiproliferative activity in HL-60 cells. The effects of the additional galloyl group were different between gallate and gallamide derivatives. In gallamide derivatives having a short alkyl chain, the additional galloyl group enhanced the antiproliferative activity. In contrast, in the gallate derivatives, the additional galloyl group had no effect on the antiproliferative activity. Further structural development and biological studies of the molecular mechanisms involved are in progress.

Experimental

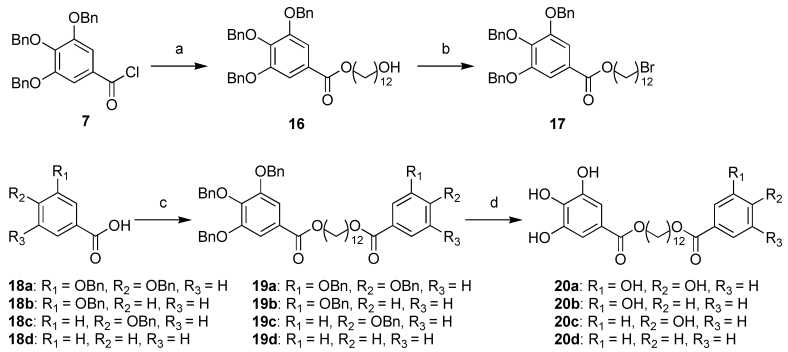
Biology. Cell Culture HL-60 cells were maintained in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal bovine serum (FBS). Cells were grown in a humidified incubator at 37 °C under 5% CO_2/95% air.

Cell Viability Assay HL-60 cells (1×10^5 cells/well) suspended in fresh medium in a 96-well plate (100 μl/well) were treated with test compounds (dimethyl sulfoxide (DMSO) solution, 0.5 μl/well). After 3-d incubation, 10 μl of Cell Counting Kit (Dojindo) was added to each well. The cell viability was determined based on the increase of absorbance (450 nm) during 4 h incubation.

Measurement of Caspase-3 Activation HL-60 cells (3×10^5 cells/ml) suspended in fresh medium in a 96-well plate (100 μl/well) were treated with test compounds (DMSO solution, 0.5 μl/well). After 4 h incubation, 100 μl of homogeneous caspase-3 assay kit (Roche) was added to each well. The activity of caspase-3 was determined based on the fluorescence of cleaved substrate (excitation 485 nm/emission 535 nm).

Chemistry. General ^{1}H-NMR (500 MHz) spectra were recorded on a JEOL JNM-α 500 spectrometer. The ^{1}H-NMR chemical shifts were reported in parts per million (ppm) relative to the singlet at 7.26 ppm for chloroform in deuteriochloroform or 2.49 ppm for DMSO in DMSO-d_6 and coupling constants were given in hertz (Hz). The following abbreviations are used for spin multiplicity: s=singlet, d=doublet, t=triplet, q=quartet, quint=quintet, sept=septet, m=multiplet, br=broad. Mass spectra were recorded on JEOL JMA-HX110 spectrometer with *m*-nitrobenzyl alcohol or JMS T100-LC spectrometer. Routine thin layer chromatography (TLC) was performed on silica gel 60 F254 plates (Merck, Germany). Flash column chromatography was performed on silica gel (spherical, particle size 40—100 μm, Kanto).

2-(3,4,5-Trisbenzyloxybenzoyloxy)ethyl 3,4,5-Trisbenzyloxybenzoate (8a) To a stirred solution of **7** (418 mg, 0.910 mmol), K_2CO_3 (254 mg, 1.84 mmol) in *N*,*N*′-dimethylformamide (DMF) (20 ml) was added ethylene glycol (30 μl, 0.538 mmol) and stirring was continued overnight. The reaction mixture was extracted with ethyl acetate three times and washed with sat $NaHCO_3$ aq. and brine. The organic layer was dried with $MgSO_4$ and filtered. The residue was purified by column chromatography (toluene : ethyl acetate=7 : 1) to afford **8a** (195 mg, 0.215 mmol 47%). ^{1}H-NMR (500 MHz,



Reagents and conditions: (a) $HO(CH_2)_nOH$, K_2CO_3, DMF, rt; (b) CBr_4, PPh_3, rt, overnight; (c) **17**, K_2CO_3, DMF, rt, overnight; (d) 10% Pd/C, H_2, AcOEt, rt.

Chart 3. Modification of Hydroxyl Groups of **9a**

$CDCl_3$) δ: 7.36—7.26 (m, 30H), 7.22 (s, 4H), 5.06 (s, 4H), 5.04 (s, 8H), 4.60 (s, 4H). MS (FAB, $[M+H]^+$) *m/z* 908.

6-(3,4,5-Trisbenzyloxybenzoyloxy)hexyl 3,4,5-Trisbenzyloxybenzoate (8b) According to the same procedure used for **8a**, starting from **7** (418 mg, 0.910 mmol), **8b** (133 mg, 0.138 mmol, 30%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.41—7.25 (m 30H), 7.21 (s, 4H), 5.10 (s, 8H), 5.09 (s, 4H), 4.28 (t, J=6.5 Hz, 4H), 1.77 (quint, J=6.5 Hz, 4H), 1.47 (br, 4H). MS (FAB, $[M+H]^+$) *m/z* 964.

12-(3,4,5-Trisbenzyloxybenzoyloxy)dodecyl 3,4,5-Trisbenzyloxybenzoate (8c) According to the same procedure used for **8a**, starting from **7** (646 mg, 1.41 mmol), **8c** (327 mg, 0.312 mmol, 44%) was obtained. MS (FAB, $[M+H]^+$) *m/z* 1048.

2-(3,4,5-Trihydroxybenzoyloxy)ethyl 3,4,5-Trihydroxybenzoate (9a) To a stirred solution of **8a** (99 mg, 0.109 mmol) in THF was added Pd/C (37 mg), and the reaction mixture was stirred at room temperature under a H_2 atmosphere for 1 h, then filtered and concentrated. The residue was recrystalized from ethanol and hexane. **9a** (31 mg, 0.085 mmol, 78%) was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 9.26 (s, 4H), 8.96 (s, 2H), 6.94 (s, 4H), 4.45 (s, 4H). HR-MS (FAB, $[M+H]^+$) Calcd for $C_{16}H_{14}O_{10}$ 367.0665, Found 367.0670.

6-(3,4,5-Trihydroxybenzoyloxy)hexyl 3,4,5-Trihydroxybenzoate (9b) According to the same procedure used for **9a**, starting from **8b** (279 mg, 0.290 mmol), **9b** (97 mg, 0.230 mmol, 79%) was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.93 (s, 4H), 4.15 (t, J=6.5 Hz, 4H), 1.67 (quint, J=6.5 Hz, 4H), 1.42 (br, 4H). HR-MS (FAB, $[M+H]^+$) Calcd for $C_{20}H_{23}O_{10}$ 423.1291, Found 423.1300.

12-(3,4,5-Trihydroxybenzoyloxy)dodecyl 3,4,5-Trihydroxybenzoate (9c) According to the same procedure used for **9a**, starting from **8c** (275 mg, 0.263 mmol), **9c** (74 mg, 0.146 mmol, 56%) was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.92 (s, 4H), 4.13 (t, J=6.5 Hz, 4H), 1.63 (quint, J=6.5 Hz, 4H), 1.35 (br, 4H), 1.32—1.18 (m, 12H). HR-MS (FAB, $[M+H]^+$) Calcd for $C_{26}H_{35}O_{10}$ 507.2230, Found 507.2231.

***N*-(2-(3,4,5-Trisbenzyloxybenzamido)ethyl)-3,4,5-trisbenzyloxybenzamide (10a)** To a stirred solution of **4** (591 mg, 1.34 mmol), EDCI (382 mg, 1.99 mmol), DMAP (28 mg, 0.229 mmol) in dichloromethane was added ethylene diamine (90 μl, 0.896 mmol). The reaction mixture was stirred at room temperature under a N_2 atmosphere for 7 h, then filtered and concentrated. The residue was purified with column chromatography (dichloromethane : methanol=10 : 3) to afford **10a** (334 mg, 0.369 mmol, 55%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.66 (t, J=5.5 Hz, 2H), 7.45—7.24 (m, 34H), 5.13 (s, 8H), 4.96 (s, 4H), 3.43 (br, 4H). MS (FAB, $[M+H]^+$) *m/z* 906.

***N*-(6-(3,4,5-Trisbenzyloxybenzamido)hexyl)-3,4,5-trisbenzyloxybenzamide (10b)** To a stirred solution of **15b** (220 mg, 0.408 mmol), EDCI (124 mg, 0.647 mmol), DMAP (8 mg, 0.065 mmol) in CH_2Cl_2 (10 ml) was added **6** (218 mg, 0.495 mmol). The reaction mixture was stirred at room temperature under a N_2 atmosphere for 6 h, then filtered and concentrated. The residue was purified with column chromatography (hexane : ethyl acetate=3 : 2) to afford **10b** (151 mg, 0.157 mmol, 39%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 7.46—7.24 (m, 34H), 5.97 (t, J=5.5 Hz, 2H), 5.09 (s, 8H), 5.06 (s, 4H), 3.25 (q, J=6.0 Hz, 4H), 1.53 (quint, J=6.0 Hz, 4H), 1.34 (m, 4H). MS (FAB, $[M+H]^+$) *m/z* 962.

***N*-(12-(3,4,5-Trisbenzyloxybenzamido)dodecyl)-3,4,5-trisbenzyloxybenzamide (10c)** According to the same procedure used for **10b**, starting from **6** (485 mg, 1.10 mmol), **10c** (569 mg, 0.544 mmol, 49%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.46—7.25 (m, 30H), 7.03 (s, 4H), 5.94 (t, J=5.0 Hz, 2H), 5.09 (s, 4H), 5.06 (s, 4H), 3.36 (q, J=6.5 Hz, 4H), 1.42—1.27 (m, 16H). MS (FAB, $[M+H]^+$) *m/z* 1046.

***N*-(2-(3,4,5-Trihydroxybenzamido)ethyl)-3,4,5-trihydroxybenzamide (11a)** To a stirred solution of **10a** (306 mg, 0.338 mmol) in THF (10 ml) and methanol (3 ml) was added 10% Pd/C (54 mg). The reaction mixture was stirred at room temperature under a H_2 atmosphere for 3 h, then filtered and concentrated. **11a** (120 mg, 0.329 mmol, 97%) was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.84 (s 4H), 3.50 (s 4H). HR-MS (FAB, $[M+H]^+$) Calcd for $C_{16}H_{17}N_2O_8$ 365.0985, Found 365.0984.

***N*-(6-(3,4,5-Trihydroxybenzamido)hexyl)-3,4,5-trihydroxybenzamide (11b)** According to the same procedure used for **11a**, starting from **10b** (149 mg, 0.155 mmol), **11b** (62 mg, 0.147 mmol, 95%) was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.98 (br, 6H), 7.99 (t, J=6.0 Hz, 2H), 6.78 (s, 4H), 3.15 (br, 4H), 1.45 (quint, J=6.5 Hz, 4H), 1.29 (br, 4H). HR-MS (FAB, $[M+H]^+$) Calcd for $C_{20}H_{25}N_2O_8$ 421.1611, Found 421.1609.

***N*-(12-(3,4,5-Trihydroxybenzamido)dodecyl)-3,4,5-trihydroxybenzamide (11c)** According to the same procedure used for **11a**, starting from **10c** (301 mg, 0.288 mmol), **11c** (144 mg, 0.285 mmol, 99%) was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.96 (s, 4H), 8.58 (s, 2H), 7.98 (t, J=5.5 Hz, 2H), 6.78 (s, 4H), 3.14 (q, J=6.5 Hz, 4H), 1.44 (quint, J=6.5 Hz, 4H), 1.35 (br, 4H), 1.27—1.19 (m, 16H). HR-MS (ESI, $[M+Na]^+$) Calcd for $C_{26}H_{36}N_2Na_1O_8$ 527.2369, Found 527.2363.

***tert*-Butyl 6-Aminohexylcarbamate (13b)** Boc_2O (1900 mg, 8.71 mmol) was dissolved in CH_2Cl_2 (20 ml) and methanol (30 ml) and cooled to 0 °C. This solution was dropped into a stirred solution of hexyldiamine **12b** (2187 mg, 10.9 mmol) in CH_2Cl_2 (200 ml) at 0 °C for 2 h, and stirring was continued overnight at room temperature. The reaction mixture was concentrated *in vacuo*, water was added, and the whole was extracted with ethyl acetate three times. The organic layer was washed with half-saturated NaCl aq., dried with $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2 : methanol : NH_3 aq.=100 : 20 : 1) to afford **13b** (1112 mg, 5.14 mmol, 59%). MS (FAB, $[M+H]^+$) *m/z* 217.

***tert*-Butyl 12-Aminododecylcarbamate (13c)** According to the same procedure used for **13b**, starting from dodecyldiamine **12c** (2690 mg, 13.4 mmol) and Boc_2O (633 mg, 2.90 mmol), **13c** (502 mg, 1.67 mmol, 58%) was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 6.75 (t, J=5.5 Hz, 1H), 2.87 (q, J=6.5 Hz, 2H), 2.52 (t, J=6.5 Hz, 2H), 1.35 (s, 9H), 1.24—1.18 (m, 20H). MS (FAB, $[M+H]^+$) *m/z* 301.

***N*-(6-(*tert*-Butyl-carbamoyloxy)hexyl)-3,4,5-trisbenzyloxybenzamide (14b)** To a stirred solution of **6** (616 mg, 1.40 mmol), EDCI (402 mg, 2,10 mmol), DMAP (41 mg, 0.336 mmol) in CH_2Cl_2 (30 ml) was added **13b** (412 mg, 1.96 mmol). The reaction mixture was stirred at room temperature under Ar atmosphere for 2 h, acidified with HCl aq., washed with brine, dried with $MgSO_4$, and concentrated *in vacuo*. **14b** (894 mg, 1.40 mmol 99%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.41—7.27 (m, 15H), 7.09 (s, 2H), 6.20 (br, 1H), 5.12 (s, 4H), 5.07 (s, 2H), 3.38 (q, J=7.0 Hz, 2H), 3.11 (br, 2H), 1.47—1.36 (m, 17H).

***N*-(12-(*tert*-Butyl-carbamoyloxy)-dodecyl)-3,4,5-trisbenzyloxybenzamide (14c)** According to the same procedure used for **14b**, starting from **6** (439 mg, 0.997 mmol), **14c** (688 mg, 0.952 mmol, 95%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.41—7.34 (m, 15H), 7.02 (s, 2H), 5.12 (s, 4H), 5.07 (s, 2H), 3.38 (q, J=6.5 Hz, 2H), 3.14—3.02 (br, 2H) 1.46—1.41 (m, 12H), 1.34—1.23 (m, 17H). MS (FAB, $[M+H]^+$) *m/z* 723.

***N*-(6-Aminohexyl)-3,4,5-trisbenzyloxybenzamide (15b)** To a stirred solution of **14b** (940 mg, 1.47 mmol) in CH_2Cl_2 (20 ml) was added TFA (5 ml). The reaction mixture was stirred at room temperature for 1 h, then concentrated. The residue was taken up in ethyl acetate (50 ml) and washed with $NaHCO_3$ aq., water, and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (CH_2Cl_2 : methanol=10 : 2) to afford **15b** (487 mg, 0.904 mmol, 62%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.12 (br, 1H), 7.41—7.25 (m, 15H), 7.05 (s, 2H), 5.04 (s, 4H), 5.02 (s, 2H), 3.30 (br, 2H), 2.86 (br, 2H) 1.64 (br, 2H), 1.51 (br, 2H), 1.35 (br, 2H), 1.28 (br, 2H). MS (FAB, $[M+H]^+$) *m/z* 539.

***N*-(12-Aminododecyl)-3,4,5-trisbenzyloxybenzamide (15c)** According to the same procedure used for **15b**, starting from **14c** (202 mg, 0.279 mmol), **15c** (172 mg, 0.276 mmol, 99%) was obtained. ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 8.40 (t, J=5.5 Hz, 2H), 7.47—7.25 (m, 17H), 5.15 (s, 4H), 4.98 (s, 2H), 3.23 (q, J=6.5 Hz, 2H), 2.73 (quint, J=6.5 Hz, 2H) 1.52—1.44 (m, 4H), 1.30—1.22 (m, 16H). MS (FAB, $[M+H]^+$) *m/z* 623.

12-Hydroxydodecyl 3,4,5-Trisbenzyloxybenzoate (16) To a stirred solution of **7** (646 mg, 1.41 mmol), K_2CO_3 (393 mg, 2.85 mmol) in DMF was added 1,12-dodecanediol (168 mg, 0.833 mmol) at room temperature. The reaction mixture stirred overnight. Water was added, and the whole was extracted with ethyl acetate three times and washed with $NaHCO_3$ aq. and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel to afford white solid **16** (429 mg, 0.687 mmol, 49%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.42—7.29 (m, 17H), 5.12 (s, 4H), 5.09 (s, 2H), 4.25 (t, J=6.7 Hz, 2H), 3.38 (t, J=6.7 Hz, 2H), 1.82 (quint, J=6.7 Hz, 2H), 1.72 (quint, J=6.7 Hz, 2H), 1.39—1.27 (m, 16H). MS (FAB, $[M+H]^+$) *m/z* 625.

12-Bromododecyl 3,4,5-Trisbenzyloxybenzoate (17) To a stirred solution of **16** (409 mg, 0.655 mmol), triphenylphosphine (352 mg, 1.34 mmol) in THF (10 ml) was added tetrabromomethane (441 mg, 1.33 mmol). The reaction mixture was stirred overnight, then evaporated. The residue was taken up in water and extracted with ethyl acetate three times. The organic layer was washed with water and brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (hexane : ethyl acetate=15 : 1) to afford white solid **17** (400 mg, 0.582 mmol, 89%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.42—7.29 (m, 17H), 5.12 (s, 4H), 5.09 (s, 2H), 4.25 (t, J=6.7 Hz, 2H), 3.38 (t, J=6.7 Hz, 2H), 1.82 (quint,

J=6.7 Hz, 2H), 1.72 (quint, J=6.7 Hz, 2H), 1.39—1.27 (m, 16H). MS (FAB, $[M+H]^+$) m/z 688.

12-(3,4-Trisbenzyloxybenzoyloxy)dodecyl 3,4,5-Trisbenzyloxybenzoate (19a) To a stirred solution of **17** (81 mg, 0.119 mmol), K_2CO_3 (30 mg, 0.217 mmol) in DMF (10 ml) was added **18a** (59 mg, 0.134 mmol). The reaction mixture was stirred at room temperature overnight under a N_2 atmosphere. Water was added and the whole was extracted with ethyl acetate three times. The organic layer was washed with water, sat $NaHCO_3$ aq. and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel chromatography (hexane : ethyl acetate=10 : 1) to afford **19a** (110 mg, 0.105 mmol, 84%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.62—7.29 (m 28H), 6.90 (d, J=8.55 Hz, 2H), 5.20 (s, 2H), 5.17 (s, 2H), 5.12 (s, 4H), 5.09 (s, 2H), 4.25 (t, J=6.7 Hz, 2H), 4.23 (t, J=6.7 Hz, 2H), 1.71 (quint, J=6.7 Hz, 4H) 1.38—1.24 (m, 16H). MS (FAB, $[M+H]^+$) m/z 941.

12-(3-Benzyloxybenzoyloxy)dodecyl 3,4,5-Trisbenzyloxybenzoate (19b) According to the same procedure used for **19a**, starting from **17** (71 mg, 0.105 mmol), **19b** (78 mg, 0.093 mmol, 89%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.64—7.62 (m, 2H), 7.43—7.31 (m, 20H), 7.15—7.13 (m, 2H), 5.12 (s, 4H), 5.09 (s, 2H), 5.08 (s, 2H), 4.28 (t, J=6.7H z, 2H), 4.25 (t, J=6.7 Hz, 2H), 1.76—1.69 (m, 4H), 1.43—1.24 (m, 16H). MS (FAB, $[M+H]^+$) m/z 835.

12-(4-Benzyloxybenzoyloxy)dodecyl 3,4,5-Trisbenzyloxybenzoate (19c) According to the same procedure used for **19a**, starting from **17** (70 mg, 0.103 mmol), **19c** (67 mg, 0.080 mmol, 78%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.97 (d, J=9.2 Hz, 2H), 7.42—7.31 (m, 22H), 6.96 (d, J=8.5Hz, 2H), 5.11 (s, 4H), 5.09 (s, 4H), 4.25 (t, J=6.7 Hz, 4H), 1.74—1.69 (m, 4H), 1.40—1.23 (m, 16H). MS (FAB, $[M+H]^+$) m/z 835.

12-Benzoyloxydodecyl 3,4,5-Trisbenzyloxybenzoate (19d) According to the same procedure used for **19a**, starting from **17** (63 mg, 0.093 mmol), **19d** (64 mg, 0.088 mmol, 94%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.02 (d, J=7.9 Hz, 2H), 7.54—7.29 (m, 20H), 5.12 (s, 4H), 5.09 (s, 2H), 4.29 (t, J=6.7 Hz, 2H), 4.25 (t, J=6.7 Hz, 2H), 1.75—1.70 (m, 4H), 1.43—1.24 (m, 16H). MS (FAB, $[M]^+$) m/z 729.

12-(3,4-Dihydroxybenzoyloxy)dodecyl 3,4,5-Trihydroxybenzoate (20a) To a stirred solution of **19a** (97 mg, 0.103 mmol), 10% Pd/C (43 mg) in AcOEt (10 ml) was added at room temperature under a H_2 atmosphere. Stirring was continued for 6 h. The reaction mixture was filtered and concentrated. The residue was purified by silica gel chromatography (hexane : ethyl acetate=1 : 1) and recrystallized to afford white solid **20a** (51 mg, 0.102 mmol, 99%). ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 9.34 (br, 5H), 7.41 (s, 1H), 7.36 (d, J=8.5 Hz, 1H), 7.00 (s, 2H), 6.86 (d, J=8.5 Hz, 1H), 4.23 (t, J=6.7 Hz, 2H), 4.21 (t, J=6.7 Hz, 2H), 1.74—1.67 (m, 4H), 1.43—1.32 (m, 16H). MS (FAB, MH^+) m/z 491. HR-MS (FAB, $[M+H]^+$) Calcd for $C_{26}H_{35}O_9$ 491.2281, Found 491.2296.

12-(3-Hydroxybenzoyloxy)dodecyl 3,4,5-Trihydroxybenzoate (20b) According to the same procedure used for **20a**, starting from **19c** (73 mg, 0.087 mmol), **20b** (23 mg, 0.048 mmol, 56%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.34 (br, 3H), 7.45—7.35 (m, 3H), 7.07 (d, J=7.3 Hz, 1H), 7.00 (s, 1H), 4.29 (t, J=6.7 Hz, 2H), 4.20 (t, J=6.7 Hz, 2H), 1.73 (quint, J=6.7 Hz, 2H), 1.69 (quint, J=6.7 Hz, 2H), 1.43—1.31 (m, 16H). MS (FAB, MH^+) m/z 475. HR-MS (FAB, $[M+H]^+$) Calcd for $C_{26}H_{35}O_8$ 475.2332, Found 475.2344.

12-(4-Hydroxybenzoyloxy)dodecyl 3,4,5-Trihydroxybenzoate (20c) According to the same procedure used for **20a**, starting from **19d** (66 mg, 0.079 mmol), **20c** (35 mg, 0.074 mmol, 93%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 10.37 (s, 1H), 9.31 (s, 2H), 8.99 (s, 1H), 7.85 (d, J=7.3 Hz, 1H), 6.99 (s, 1H), 6.90 (d, J=7.3 Hz, 1H), 4.24 (t, J=6.7 Hz, 2H), 4.20 (t, J=6.7 Hz, 2H), 1.74—1.67 (m, 4H), 1.41—1.31 (m, 16H). MS (FAB, MH^+) m/z 475. HR-MS (FAB, $[M+H]^+$) Calcd for $C_{20}H_{35}O_8$ 475.2332, Found 475.2361.

12-Benzoyloxydodecyl 3,4,5-Trihydroxybenzoate (20d) According to the same procedure used for **20a**, starting from **19e** (62 mg, 0.085 mmol), **20d** (37 mg, 0.081 mmol, 94%) was obtained. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.24 (s, 2H), 8.92(s, 1H), 7.94 (d, J=6.1 Hz, 2H), 7.64 (t, J=6.1 Hz, 1H), 7.52 (t, J=6.1 Hz, 2H), 6.93 (s, 2H), 4.25 (t, J=6.7 Hz, 2H), 4.13 (t, J=6.7 Hz, 2H), 1.69 (quint, J=6.7 Hz, 2H), 1.61 (quint, J=6.7 Hz, 2H), 1.40—1.24 (m, 16H). MS (FAB, MH^+) m/z 459. HR-MS (FAB, $[M+H]^+$) Calcd for $C_{26}H_{35}O_7$ 459.2383, Found 459.2402.

Acknowledgment We thank K. Harata and Y. Hongo for recording mass spectra at RIKEN. The work described in this paper was partially supported by Grants-in-Aid for Scientific Research from The Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Japan Society for the Promotion of Science.

References

1) Nakachi K., Matsuyama S., Miyake S., Suganuma M., Imai K., *Biofactors*, **13**, 49—54 (2000).
2) Fujiki H., Suganuma M., Imai K., Nakachi K., *Cancer Lett.*, **188**, 9—13 (2002).
3) Fujiki H., Okuda T., *Drugs Future*, **17**, 462—464 (1992).
4) Komori A., Yatsunami J., Okabe S., Abe S., Hara K., Suganuma M, Kim S.-J., Fujiki H., *Jpn. J. Clin. Oncol.*, **23**, 186—190 (1993).
5) Fujiki H., Suganuma M., *Proc. Jpn. Acad.*, **78**, 263—270 (2002).
6) Okabe S., Suganuma M., Hayashi M., Sueoka E., Komori A., Fujiki H., *Jpn. J. Cancer Res.*, **88**, 639—643 (1997).
7) Dodo K., Minato T., Noguchi-Yachide T., Suganuma M., Hashimoto Y., *Bioorg. Med. Chem.*, **16**, 7975—7982 (2008).
8) Ren Y., Himmeldirk K., Chen X., *J. Med. Chem.*, **49**, 2829—2837 (2006).