# Design and Synthesis of 4,6-Di-*tert*-butyl-2,3-dihydro-5-benzofuranols as a Novel Series of Antiatherogenic Antioxidants

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*Received February 11, 2003*

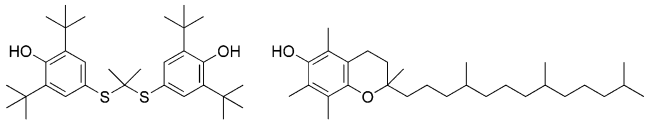
Antioxidants have been considered as potential antiatherogenic agents by inhibiting oxidation of low-density lipoprotein (LDL), albeit vitamin E, a natural antioxidant, has failed to show reduction on atherosclerosis in clinical trials. We have rationally designed and synthesized a novel series of antioxidants, 4,6-di-*tert*-butyl-2,3-dihydro-5-benzofuranols, to overcome the clinical limitation of vitamin E. In vitro, the compounds showed a potent inhibitory effect on lipid peroxidation detected as 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (MCLA)-dependent chemiluminescence in linoleic acid autoxidation. They also inhibited the LDL oxidation induced by $Cu^{2+}$, and the inhibition is more potent than that of vitamin E and probucol. In vivo, 4,6-di-*tert*-butyl-2,3-dihydro-2,2-dipentyl-5-benzofuranol (BO-653, **1f**), an optimal compound, showed the highest concentration in plasma and LDL fraction in Watanabe heritable hyperlipidemic rabbits, due to its high affinity to LDL. The isolated LDL samples from the **1f**-treated rabbits showed potent resistibility to LDL oxidation. Compound **1f** has been taken into clinical trials.

## Introduction

Many lines of evidence, based on atherosclerosis models, suggest that the oxidative modification of low-density lipoprotein (LDL) has a critical role in the development of atherosclerosis.[1−3] As a consequence of the oxidation hypothesis, antioxidants have been proposed as antiatherogenic agents, and lipophilic ones in many kinds of antioxidants have an advantage to suppress the LDL oxidation due to their affinity to lipid particles. For the last two decades, various lipophilic antioxidants have been intensively investigated to reduce the progression of atherosclerosis in animal models.[4−7]

Probucol, a lipophilic antioxidant, is well-known as a lipid-lowering agent (Chart 1), and its antiatherogenic effects in various animal models has been reported.[8−12] However, there was no demonstration of its antiatherogenic effect in a clinical trial.[13] Probucol has the untoward effects of lowering serum high-density lipoprotein (HDL) levels[14] and causing arrythmias.[15] The reduction of HDL has a contrary effect on atherosclerosis; that is, the concentration of HDL has been shown to correlate inversely with the risk of atherosclerosis,[16] and this leads to the controversial conclusion that lipophilic antioxidants do not reduce human atherosclerosis. To demonstrate the oxidation hypothesis, we need further study using the ideal antioxidant without such untoward effects.

**Chart 1.** Structures of Probucol and α-Tocopherol




Probucol α-Tocopherol

α-Tocopherol, the most active species of vitamin E, is a potent natural lipophilic antioxidant and is considered to play an important role in maintaining life processes against lipid peroxidation in the body. In general, α-tocopherol reduces the LDL oxidation 10- to 100-fold more potently than probucol.[17] It has neither of the untoward effects of HDL reduction and the causing of arrhythmias; however, the clinical intervention studies with vitamin E have afforded conflicting results. Rimm et al. reported that high intake of vitamin E reduces the incidence of coronary heart disease.[18] Meanwhile, three large-scale trials failed to demonstrate the effect of vitamin E on cardiovascular disease or cerebrovascular mortality.[19−21] We hypothesized that this discrepancy between high reactivity of vitamin E and the conflicting results in clinical trials would be based on the inability to exert its antioxidant action in the hydrophobic core of LDL particle where probucol is active. Furthermore, the properties of α-tocopherol that is not only an antioxidant but also a prooxidant may

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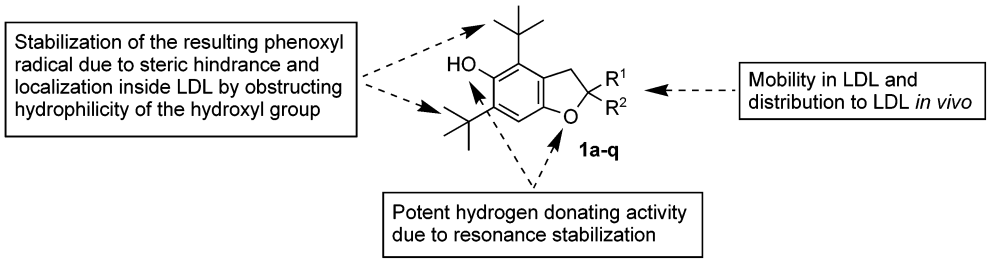
10.1021/jm030062a CCC: $25.00 
Published on Web 05/30/2003

**Chart 2.** Characteristics Required for Potent LDL Antioxidants




contribute to this discrepancy.[22,23] The evidence, that atherosclerotic lesions contain a certain amount of α-tocopherol even where the oxidized lipid was generated,[24,25] may support our consideration.

On the basis of the oxidation hypothesis, we commenced to design new antioxidants to overcome the clinical limitation of vitamin E. The points for the design are as follows:[26]

1. To have potent antioxidative reactivity that is equal to or greater than that of α-tocopherol.

2. To exert antioxidant action in the core of LDL particle.

3. To compensate for the prooxidant action of α-tocopherol by a synergic antioxidant action.

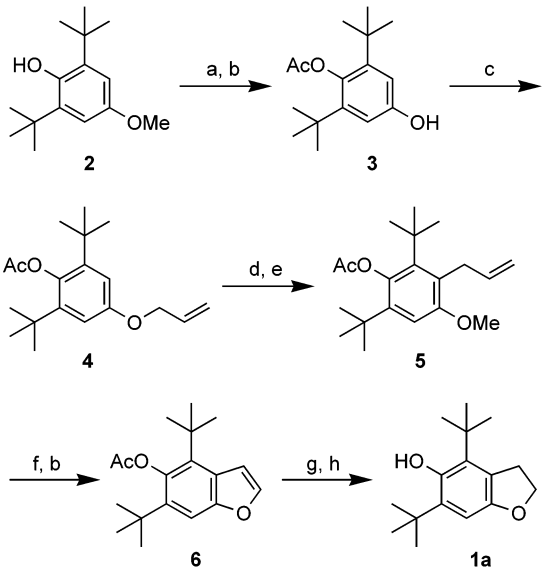
Our goal is to furnish a promising candidate for clinical usage. In this paper, we report novel antioxidants, which have been rationally designed to possess high reactivity and efficacy in LDL, which must be required for effective inhibition against LDL oxidation.

## Results and Discussion

**Drug Design.** Lipid peroxidation is considered to play a central role in the LDL oxidation.[27] Although endogenous antioxidants such as vitamin E and plasma antioxidative enzymes and substances act to prevent the surface of LDL from oxidation, the relevant oxidation for atherogenesis may happen in the hydrophobic core of LDL where the chain reaction of lipid peroxidation can be caused easily. First, we speculated that the point of the drug design was to realize a high reactivity like that of α-tocopherol compatible with the existence in the core of LDL like probucol. Next, we considered that the new antioxidants possessing high affinity to LDL may be delivered efficiently to LDL and then the vessel wall. Finally, we supposed that relative stability of the intermediate radicals of the new antioxidants would not lead to the prooxidant action such as α-tocopherol. The last property might make up for the disadvantage of α-tocopherol. Therefore, the drug design consists of the following characteristics: (i) high hydrogen-donating activity and low prooxidant activity of its intermediate, (ii) localization inside the core of the LDL particle, and (iii) efficient delivery to LDL and vascular vessels.

The first characteristic (i) is rationally achieved by lowering the dissociation energy of the phenolic O−H bond, which is determined in part by the resonance stabilization of the resulting phenoxyl radical. Ingold et al. reported that the p-type lone-pair orbital of the oxygen atom located in the *para* position of the phenolic hydroxyl group can stabilize the phenoxyl radical optimally when the oxygen atom belongs to a five-membered ring,[28,29] although vitamin E has a six-membered ring.

**Scheme 1**[a]




[a] Reagents: (a) $H_2SO_4$, $Ac_2O$; (b) TMSI, $CH_2Cl_2$; (c) $K_2CO_3$, allyl bromide, acetone; (d) *N*,*N*-dimethylaniline reflux; (e) $K_2CO_3$, MeI, acetone; (f) $OsO_4$, $NaIO_4$, THF, $H_2O$; (g) $LiAlH_4$, THF; (h) $H_2$, 10% Pd/C, AcOH.

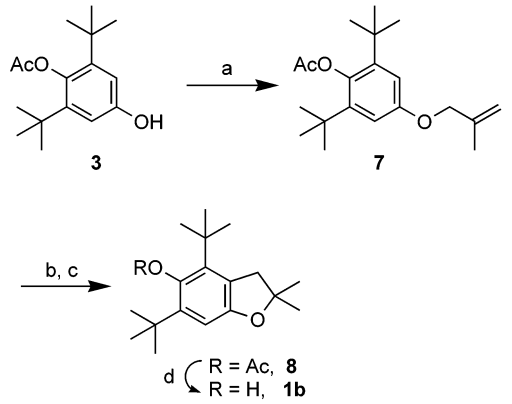
Thus, we designed a 2,3-dihydro-5-benzofuranol structure to achieve the former characteristic (Chart 2).

To achieve the second characteristic (ii), we intended to utilize two *tert*-butyl groups on the *ortho* position of the hydroxyl group instead of the methyl groups of α-tocopherol. The two *tert*-butyl groups are expected to obstruct the hydrophilicity of the hydroxyl group and to result in the preferential localization to the hydrophobic region. Moreover, they make the resulting phenoxyl intermediate kinetically persistent due to their steric hindrance, and this persistence leads to a reduction in the prooxidant action of its phenoxyl intermediate. Thus, we realized the latter half of the first characteristic (i). Furthermore, accompanying its high reactivity, the relative stability of the intermediate also leads to a reduction in the coexisting prooxidant action of α-tocopherol.

The third characteristic (iii) is an essential point for LDL antioxidants, and we examined a series of compounds concerning their distribution to lipoproteins. We synthesized 2,3-dihydro-5-benzofuranol derivatives bearing different alkyl substituents on the 2-position and evaluated their distribution using Watanabe heritable hyperlipidemic (WHHL) rabbits in order to achieve this characteristic.

**Chemistry.** The synthesis of dihydrobenzofuranol **1a** was carried out as shown in Scheme 1. 3,5-Di-*tert*-butyl-4-hydroxyanisole **2** was acetylated with acetic anhydride

**Scheme 2**[a]




[a] Reagents: (a) NaH, DMF, 3-chloro-2-methylpropene; (b) *N*,*N*-dimethylaniline reflux; (c) 1*N* HCl aq, $Et_2O$; (d) $LiAlH_4$, THF.

and sulfuric acid, followed by demethylation with iodotrimethylsilane to give phenol **3** (71%). Treatment of **3** with allyl bromide in the presence of potassium carbonate yielded ether **4** (96%) which was converted into **5** via Claisen rearrangement in *N*,*N*-dimethylaniline and subsequent methylation (79% for two steps). Oxidation of **5** with sodium periodate and a catalytic amount of osmium tetroxide, followed by demethylation, afforded benzofuran **6** (83%). Benzofuran **6** was deprotected using lithium aluminum hydride in THF and then reduced catalytically to yield **1a** (71% for two steps).

Compound **1b** was prepared as shown in Scheme 2. Phenol **3** was alkylated with 3-chloro-2-methylpropene in the presence of sodium hydride to give ether **7** (90%). Claisen rearrangement of **7** and subsequent treatment with hydrochloric acid gave benzofuran **8**, which was then deprotected to yield **1b** (45% for three steps).

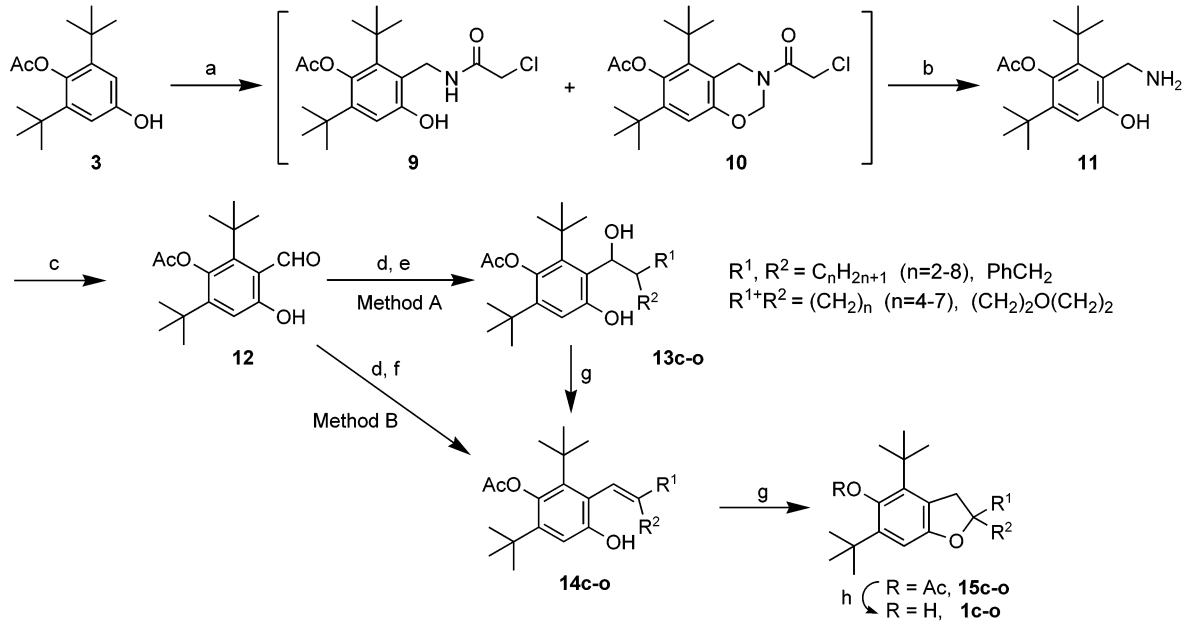
Dihydrobenzofuranols **1c**−**o** were prepared via addition of Grignard reagents to benzaldehyde **12** as a key reaction (Scheme 3). First, the synthesis of the key intermediate **12** was accomplished according to a procedure similar to that used by Stokker et al.[30] Treatment of phenol **3** with 2-chloro-*N*-(hydroxymethyl)-acetamide[31] in the presence of sulfuric acid afforded a mixture of **9** and **10**, which was hydrolyzed to give benzylamine **11**. Subsequently, **11** was treated with hexamethylenetetramine in aqueous acetic acid to yield **12**.[32] Next, conversion of **12** into **1c**−**o** was carried out as follows: Treatment of **12** with more than 2 equiv of Grignard reagents and following neutralization with an aqueous ammonium chloride solution gave intermediates **13c**−**o** (Method A). Subsequently, **13c**−**o** were converted to benzofurans **15c**−**o** via dehydrated compounds **14c**−**o** by treatment with boron trifluoride diethyl etherate. Alternatively, compounds **14c**−**o** were obtained by treatment with diluted hydrochloric acid after the Grignard reaction (Method B). Deprotection of **15c**−**o** afforded **1c**−**o**.

In the case of dihydrobenzofuranol **1p**, preparation of the corresponding Grignard reagent was not successful. However, the combination of lithium metal and alkylhalide **16** with aldehyde **12** (Barbier reaction) gave the addition compound **13p** (46%, Scheme 4). Compound **13p** was cyclized with boron trifluoride diethyl etherate into compound **15p**, which was deprotected with lithium aluminum hydride to afford **1p** (54% for two steps).

In the preparation of dihydrobenzofuranol **1q**, benzofuranol **17**[33] was *tert*-butylated with 2-methyl-2-propanol in the presence of methanesulfonic acid to give tri-*tert*-butylbenzofuranol **18**. Furthermore, **18** was reduced with triethylsilane in trifluoroacetic acid to yield **1q** (Scheme 5).

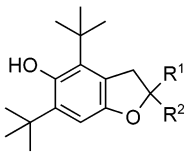
**Antioxidant Activity.** The antioxidant activity was measured as the inhibition of a *Cypridina* luciferin analogue, 2-methyl-6- (*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (MCLA)[34]-dependent chemiluminescence generated in linoleic acid autoxidation in which the scavenging activity of **1f** against linoleate peroxyl radicals was previously reported.[26] As shown

**Scheme 3**[a]




[a] Reagents: (a) *N*-hydroxymethyl-2-chloroacetamide, $H_2SO_4$, AcOH; (b) concentrated HCl, EtOH reflux; (c) $(CH_2)_6N_4$, AcOH, $H_2O$, then HCl aq; (d) $R^1R^2CHMgBr$, THF; (e) $NH_4Cl$ aq.; (f) HCl aq; (g) $BF_3{\cdot}OEt_2$, $CH_2Cl_2$; (h) $LiAlH_4$, THF.

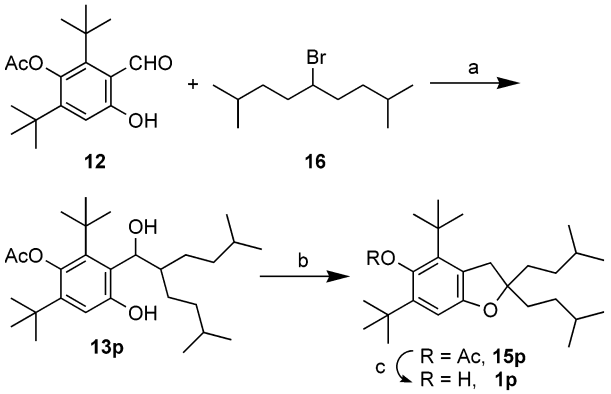
**Table 1.** Effects of Dihydrobenzofuranols on Chemiluminescence Generated by Linoleic Acid Autoxidation and LDL Oxidation Induced by Soybean Lipoxygenase or $CuSO_4$




| | | | | | LDL oxidation (% of control)[c] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | chemiluminescence[b] | SLO | | $CuSO_4$ | |
| compd | $R^1$ | $R^2$ | cLogP[a] | $IC_{50}$ ($\mu$M) | 0.5 $\mu$M | 5 $\mu$M | 1 $\mu$M | 10$\mu$M |
| **1a** | H | H | 5.21 | 7.2 | 36.0 | 6.9 | 53 | 11 |
| **1b** | $CH_3$ | $CH_3$ | 6.25 | 6.7 | 39.8 | 1.8 | 56 | 10 |
| **1c** | $C_2H_5$ | $C_2H_5$ | 7.31 | 4.3 | 9.4 | 1.3 | 77 | 10 |
| **1d** | *n*-$C_3H_7$ | *n*-$C_3H_7$ | 8.37 | 4.0 | 33.3 | 0 | 87 | 13 |
| **1e** | *n*-$C_4H_9$ | *n*-$C_4H_9$ | 9.42 | 7.9 | 51.5 | 0.5 | 82 | 12 |
| **1f** | *n*-$C_5H_{11}$ | *n*-$C_5H_{11}$ | 10.48 | 4.6 | 60.9 | 4.6 | 96 | 42 |
| **1g** | *n*-$C_6H_{13}$ | *n*-$C_6H_{13}$ | 11.54 | n.d.[d] | 75.5 | 2.7 | 86 | 23 |
| **1h** | *n*-$C_7H_{15}$ | *n*-$C_7H_{15}$ | 12.60 | n.d.[d] | 88.4 | 18.5 | 89 | 83 |
| **1i** | *n*-$C_8H_{17}$ | *n*-$C_8H_{17}$ | 13.66 | 5.6 | 89.8 | 11.3 | 85 | 72 |
| **1j** | $CH_2C_6H_5$ | $CH_2C_6H_5$ | 9.09 | 10.6 | 63.5 | 3.6 | 93 | 11 |
| **1k** | $(CH_2)_4$ | | 6.64 | 6.6 | 46.2 | 0.4 | 85 | 12 |
| **1l** | $(CH_2)_5$ | | 7.20 | 9.3 | 34.1 | 0 | 77 | 16 |
| **1m** | $(CH_2)_6$ | | 7.76 | 7.9 | 93.4 | 2.3 | 91 | 9 |
| **1n** | $(CH_2)_7$ | | 8.32 | 9.4 | 97.2 | 9.0 | 92 | 22 |
| **1o** | $(CH_2)_2O(CH_2)_2$ | | 4.73 | 8.2 | 71.8 | 4.7 | 87 | 22 |
| **1p** | $(CH_2)_2$*i*-Pr | $(CH_2)_2$*i*-Pr | 10.22 | 5.7 | 91.5 | 7.1 | 78 | 23 |
| **1q** | *tert*-$C_4H_9$ | H | 7.06 | 8.4 | 99.0 | 1.8 | 74 | 11 |
| probucol | | | 10.75 | 165 | 73.9 | 14.0 | 87 | 28 |

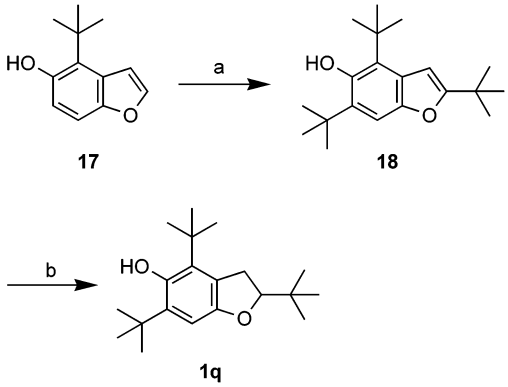
[a] cLogP for each compound was calculated with CLOGP v.3.54 (Daylight C.I.S.Inc., Claremont, CA). [b] Inhibition of MCLA chemiluminescence generated by autoxidation of linoleic acid. [c] Inhibitory activities against LDL oxidation induced by SLO or $CuSO_4$. [d] Not determined.

**Scheme 4**[a]




[a] Reagents: (a) Li, THF, then $NH_4Cl$ aq; (b) $BF_3{\cdot}OEt_2$, $CH_2Cl_2$; (c) $LiAlH_4$, THF.

**Scheme 5**[a]




[a] Reagents: (a) *t*-BuOH, MsOH, $CHCl_3$; (b) $Et_3SiH$, TFA.

in Table 1, the chemiluminescence was effectively quenched more than 10-fold by the compounds including **1f**, compared with probucol. That is, they have much more potent hydrogen donating activities compared with probucol. In addition to the chemical reactivity in the homogeneous solution, these compounds were also examined for their antioxidant activities on LDL oxidation induced by cupric ions ($CuSO_4$) or soybean lipoxygenase (SLO). The reason for using two kinds of initiators is to remove the initiator specific inhibition from the inhibition of lipid peroxidation. Thus, we could select the inhibitors against chain propagation process of lipid peroxidation. Rabbit's LDL accompanying each compound was oxidized with $CuSO_4$ and SLO for 24 h, and the antioxidant activity was evaluated as the resulting thiobarbituric acid reactive substances (TBARS) and the fluorescence generation from LDL fraction determined by gel-permeation chromatography, respectively. The TBARS represents the oxidation of lipids, and the fluorescence represents the oxidation of proteins. As shown in Table 1, the compounds inhibited the LDL oxidation induced by either $CuSO_4$ or SLO. Compounds possessing long alkyl groups (**1h** and **1i**) and probucol at 5 $\mu$M showed less potent inhibition in oxidation by SLO.

**Absorption in WHHL Rabbits.** The pharmacokinetic profile is important for antioxidants to achieve the clinical benefits. As our design for LDL antioxidants leads to highly lipophilic compounds, the design automatically leads to the realization that low solubility in water might cause low absorption. Furthermore, experimental animals show low plasma cholesterol levels in general and most of the plasma cholesterol in rodents comes from HDL. The animals fed normal diet show low absorption for lipophilic compounds. Normal animals should not be appropriate for evaluating the absorption

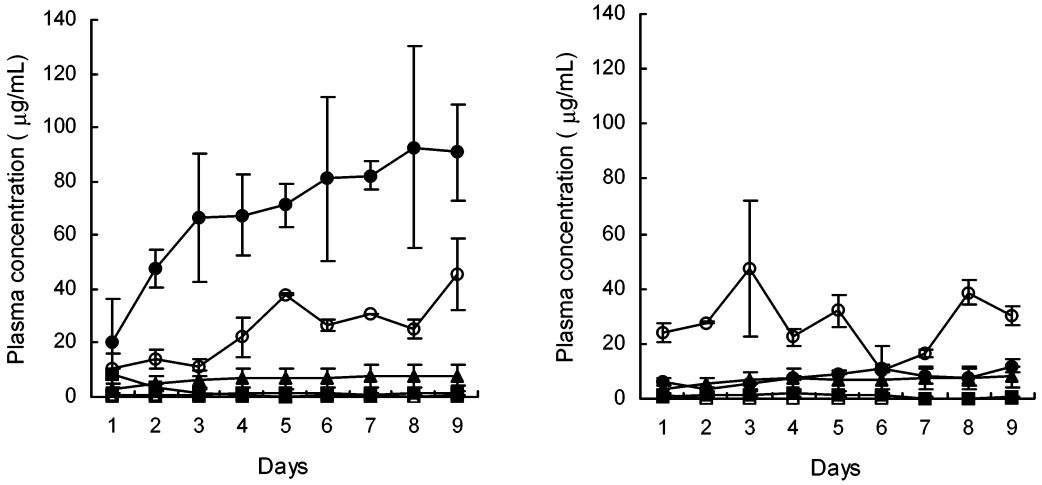



**Figure 1.** Plasma concentration of dihydrobenzofuranols in WHHL rabbits. Two rabbits were orally administered once a day with the test compound (250 mg/kg) or probucol (500 mg/kg) for 9 days and the plasma concentrations were evaluated at 24 h after daily administration. The results are shown as Mean ± Range: (left panel) **1b**, open squares; **1d**, closed squares; **1e**, open circles; **1f**, closed circles; **1i**, open triangles; probucol, closed triangles; (right panel) **1j**, open squares; **1m**, closed squares; **1p**, open circles; **1q**, closed circles; probucol, closed triangles.

in hyperlipidemia patients who will use the drugs against atherosclerosis. To overcome the discrepancy between patients and normal animals, we used WHHL rabbits of which the high LDL level allows us to assess the absorption in hyperlipidemia patients. WHHL rabbits were orally administered once a day with the dihydrobenzofuranols (250 mg/kg) or probucol (500 mg/kg) for 9 days, and the concentrations in plasma were measured. Figure 1 shows the serial changes in the plasma concentrations at 24 h after daily oral administration, in which the left panel shows the result of the dihydrobenzofuranols bearing two straight alkyl side chains, and the right panel shows the result of the other dihydrobenzofuranols. The compounds that possess the moderate length alkyl side chain (**1e** and **1f** in the left panel, and **1p** in the right panel) showed superior absorption in WHHL rabbits than probucol.

The above result suggests a correlation between the lipophilicity of the dihydrobenzofuranols and their absorption profiles in WHHL rabbits. Figure 2 shows the relationship between the calculated distribution coefficient (cLogP) of dihydrobenzofuranols and their plasma concentration in WHHL rabbits at 24 h after the final administration. The shape of the graph suggested that the dihydrobenzofuranols had an optimal cLogP value for the absorption and that the optimal length as a straight side chain could be determined from the graph (refer to closed squares in Figure 2). Thus, we selected as an optimal compound, compound **1f**, which has two pentyl groups as moderate length side chains from the straight chain type.

**Comparison of 1f with α-Tocopherol.** To confirm the antioxidant activity of the compound **1f**, we compared it with α-tocopherol and probucol. In addition to TBARS and fluorescence generation that was previously reported,[26] electrophoretic mobility is an important parameter in evaluating LDL oxidation as it reflects the surface charge of LDL that is relevant to binding with both LDL and scavenger receptors. We compared the changes of the electrophoretic mobility induced by $CuSO_4$ and SLO oxidation, as shown in Figure 3. The change in the LDL treated with **1f** was smallest for both

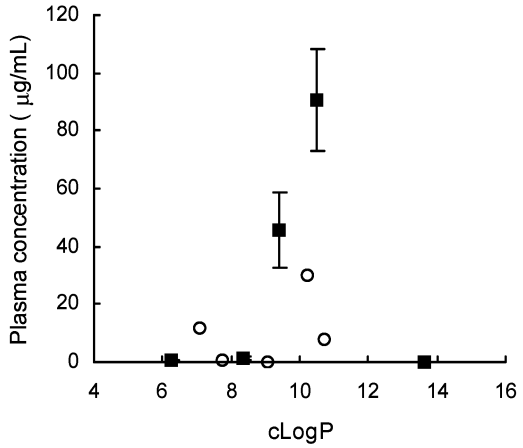



**Figure 2.** Relationship between cLogP and plasma concentration of dihydrobenzofuranols in WHHL rabbits. Two rabbits were orally administered once a day with test compound (250 mg/kg) for 9 days and the plasma concentrations were evaluated at 24 h after the final administration. Closed squares indicate the compounds bearing straight side chains and open circles indicate the other structures. The results are shown as mean ± range.

oxidations. In the case of $CuSO_4$ oxidation, the mobility of the LDL incubated with 1 $\mu$M of **1f** was 15.6 ± 0.8 mm (mean ± SD) where the control was 22.7 ± 0.8 mm and was similar to that of native LDL (13.3 ± 0.4 mm). The change with 1 $\mu$M of **1f** was much smaller than that with 10 $\mu$M of either probucol or α-tocopherol. Similar results were obtained for both parameters of TBARS and fluorescence generation (data not shown). The results confirm the antioxidant properties of these designed compounds that might be useful in their intended activity.

**Oxidizability of Isolated LDL from 1f-Treated Rabbits.** If the progress of atherosclerosis is closely related to oxidative modification of LDL, the practical target of antioxidants is to deliver them to LDL. Therefore, we examined the distribution to lipoproteins when a series of dihydrobenzofuranols were orally administered in WHHL rabbits. Table 2 shows the distribution of the compounds to the very low-density

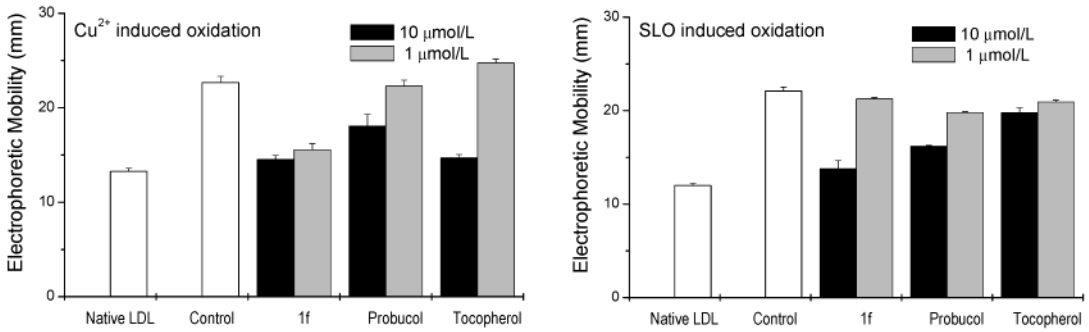



**Figure 3.** Inhibitory effects of **1f**, probucol and α-tocopherol on electrophoretic mobility change during LDL oxidation. Rabbit LDL at a concentration of 200 μg/mL was oxidized by incubating with either $CuSO_4$ ($Cu^{2+}$) or lipoxygenase (SLO) at 37 °C for 24 h in the presence of **1f**, probucol, or α-tocopherol (1 or 10 μM). The electrophoretic mobility after the LDL oxidation was evaluated by one-dimensional agarose gel electrophoresis. The results are shown as mean ± SD (triplicate).

**Table 2.** Distribution of Dihydrobenzofuranols into Lipoprotein Fractions of WHHL Rabbit's Plasma[a]

| compd | plasma (μg/mL) | VLDL (μg/mg) | LDL (μg/mg) | HDL (μg/mg) |
|---|---|---|---|---|
| **1e** | 0.89 ± 0.05 | 57.07 ± 1.69 | 11.04 ± 0.64 | 3.22 ± 0.78 |
| **1f** | 2.22 ± 0.41 | 119.25 ± 14.58 | 28.60 ± 6.43 | 2.98 ± 1.33 |
| **1i** | 0.01 ± 0.00 | 1.16 ± 0.26 | 0.36 ± 0.06 | n.d.[b] |
| **1p** | 0.59 ± 0.12 | 25.64 ± 2.53 | 9.34 ± 0.30 | n.d.[b] |
| **1q** | 0.44 ± 0.16 | 27.69 ± 9.28 | 5.08 ± 1.49 | n.d.[b] |
| probucol | 0.14 ± 0.07 | 5.48 ± 2.12 | 2.57 ± 1.20 | 0.44 ± 0.01 |

[a] Each group is composed of two rabbits. The data are expressed as mean ± range. [b] Not detected.

lipoprotein (VLDL), LDL, and HDL fractions in plasma. The results indicated that the orally administered dihydrobenzofuranols were distributed to the VLDL and LDL fractions rather than the HDL fraction, and that the concentration of **1f** in the VLDL and LDL fractions was higher than that of probucol. In general, lipophilic compounds such as **1f** would be absorbed via the pathway of lipid absorption. Therefore, we speculated that the absorption occurred at the intestine via chylomicron, and it was carried through the lymph to be utilized for the synthesis of VLDL and LDL. VLDL has the fate of being converted to LDL by lipoprotein lipase. Thus, the lipophilic compounds seem to possess a self-organized drug-delivery system, targeting by themselves the practical targets of the VLDL and LDL.

Finally, we assessed the inhibitory activity of **1f** by evaluating the oxidizability of isolated LDL from the **1f**-treated WHHL rabbits. The isolated LDL was oxidized by $CuSO_4$ or SLO, and the oxidation was evaluated as the fluorescence generation of LDL. As shown in Figure 4, the fluorescence increased due to oxidation by either $CuSO_4$ or SLO, and the LDL from the **1f**-treated animals showed significant resistance to oxidation more than that from probucol-treated animals. Together with the in vivo study,[26] the results described here imply that **1f** possesses potent inhibitory activity against LDL oxidation.

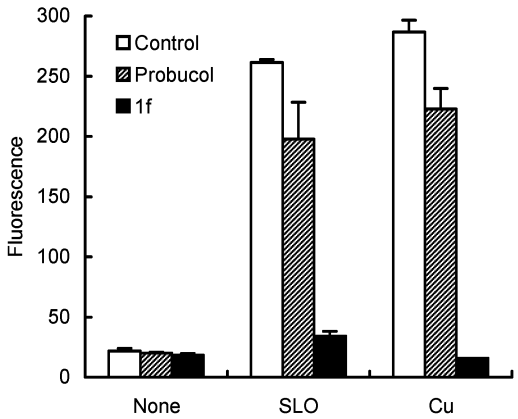



**Figure 4.** Oxidizability of LDL isolated from antioxidant-treated WHHL rabbits. Two rabbits were orally administered once a day with test compound for 9 days, and blood samples were collected at 24 h after the final administration. The isolated LDL was oxidized by incubating with either $CuSO_4$ (Cu) or lipoxygenase (SLO) at 37 °C for 24 h. The oxidizability was assessed as the generation of fluorescence induced by the LDL oxidation, integrating from the void to 150 kDa on gel-permeation chromatography. The results are shown as mean ± range.

## Conclusions

In this study, we demonstrated the design and synthesis of 4,6-di-*tert*-butyl-2, 3-dihydro-5-benzofuranols as a novel series of antioxidants. Furthermore, we demonstrated that the selected compound **1f** possesses a preferable distribution to LDL, the target molecule of our drug design for an antiatherogenic agent. Previously, Noguchi et al. described the radical scavenging activities of **1f** against lipid peroxidation[35] and the inhibitory action on LDL oxidation.[36] Furthermore, we demonstrated the antiatherogenic effects of **1f** in three different animal models including the WHHL rabbit.[26] Here, we have achieved the constructing of a strategy for screening LDL antioxidants to select the optimal dihydrobenzofuranol.

The western style of foods and the exposure to pollutants in the environment are increasing the risk of oxidative stress and the progression of atherosclerosis. The drugs that possess preferable antioxidative action could be beneficial to various diseases relevant to oxidative stress. Such drugs could prevent the oxidative modification of LDL and so could prevent coronary heart diseases such as angina and myocardial infarction. We concluded in this study that the rationally designed antioxidant **1f** (BO-653)[37] would be a promising candidate as a new antiatherogenic agent. The remaining question is the clinical efficacy of BO-653 that will be answered in coming clinical trials that will reveal the potential of antioxidants for cardiovascular diseases as atherosclerosis.

## Experimental Section

**Instruments and Analyses.** Column chromatography was carried out on Wako-gel C-200 (70−230 mesh) purchased from Wako Pure Chemical Industries (Osaka, Japan). Thin-layer chromatography was performed on Kieselgel $F^{254}$ plates purchased from E. Merck and Co. HPLC analyses were performed on a Hitachi L-4000 UV detector with a Hitachi L-6200 pump using a YMC−Pack C8 A-212 column eluted with $CH_3CN$−*i*-PrOH−water (8:1:1) at a flow rate of 1.0 mL/min. Melting points were recorded on a Yanagimoto micro melting point apparatus or a METTLER FP62 melting point apparatus and are uncorrected. $^1$H NMR spectra were recorded on a Hitachi R-24B spectrometer (60 MHz) or on a JEOL EX-270 spectrometer (270 MHz) with tetramethylsilane as an internal standard. Mass spectra were recorded on a Shimadzu GCMS-QP1000 spectrometer. Infrared spectra were recorded on a Hitachi 270−30 infrared spectrophotometer. High resonance mass spectra (HRMS) were measured at the Toray Research Center (Tokyo, Japan). All elemental analyses are within 0.4% of the calculated values unless otherwise specified.

**4-Acetoxy-3,5-di-*tert*-butylanisole.** A mixture of 3,5-di-*tert*-butyl-4-hydroxyanisole **2** (23.6 g, 0.1 mol) and concentrated $H_2SO_4$ (0.5 mL) in acetic anhydride (150 mL) was stirred at 70 °C for 2 h. The reaction mixture was concentrated under reduced pressure, and saturated aqueous $NaHCO_3$ was added to the concentrate. After the mixture was extracted with EtOAc, the extract was dried over anhydrous $MgSO_4$ and concentrated. The residue was recrystallized from MeOH−water (2:1) to give the title compound (24.5 g, 88%) as a white solid, mp 97 °C: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$1.06 (s, 18H), 2.02 (s, 3H), 3.47 (s, 3H), 6.53 (s, 2H); MS *m*/*e* 278 ($M^+$).

**4-Acetoxy-3,5-di-*tert*-butylphenol (3).** To a solution of 4-acetoxy-3,5-di-*tert*-butylanisole (0.50 g, 1.8 mmol) in $CH_2Cl_2$ (2 mL) was added dropwise at 0 °C iodotrimethylsilane (0.31 mL, 2.2 mmol). The mixture was allowed to warm to room temperature and was stirred for 2 days. A saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture, and the mixture was extracted with $Et_2O$. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (15% EtOAc in *n*-hexane) to give **3** (0.38 g, 80%) as a white solid, mp 157 °C: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.27 (s, 18H), 2.27 (s, 3H), 5.22 (br, 1H), 6.67 (s, 2H); MS *m*/*e* 264 ($M^+$).

**4-Acetoxy-3,5-di-*tert*-butyl-1-(2-propenyloxy)benzene (4).** To a mixture of **3** (10 g, 37.8 mmol) and $K_2CO_3$ (15.6 g, 0.11 mol) in acetone (300 mL) was added allyl bromide (6.55 mL, 75.6 mmol). The mixture was heated to reflux for 24 h and then cooled to room temperature and concentrated under reduced pressure. After addition of water, the mixture was extracted with $Et_2O$. The extract was washed with water and saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to give **4** (11 g, 96%) as a colorless oil: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.30 (s, 18H), 2.27 (s, 3H), 4.47 (d, $J$ = 5.0 Hz, 2H), 5.05−5.57 (m, 2H), 5.68−6.37 (m, 1H), 6.81 (s, 2H); MS *m*/*e* 304 ($M^+$).

**4-Acetoxy-3,5-di-*tert*-butyl-2-(2-propenyl)phenol.** A solution of **4** (11 g, 36 mmol) in *N*,*N*-dimethylaniline (50 mL) was heated to reflux for 18 h under a $N_2$ atmosphere. After being cooled to room temperature, the reaction mixture was concentrated under reduced pressure. Purification of the concentrate by silica gel column chromatography (15% EtOAc in *n*-hexane) gave the title compound (8.84 g, 80%) as a white solid, mp 104 °C: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.30 (s, 9H), 1.42 (s, 9H), 2.28 (s, 3H), 3.52−3.84 (m, 2H), 4.88−5.42 (m, 3H), 5.68−6.45 (m, 1H), 6.79 (s, 1H); MS *m*/*e* 304 ($M^+$).

**4-Acetoxy-3,5-di-*tert*-butyl-2-(2-propenyl)anisole (5).** To a mixture of 4-acetoxy-3,5-di-*tert*-butyl-2-(2-propenyl)phenol (30 g, 99 mmol) and $K_2CO_3$ (13.8 g, 0.1 mol) in acetone (300 mL) was added iodomethane (28 g, 0.2 mol). The mixture was heated to reflux for 24 h and then cooled to room temperature and concentrated under reduced pressure. After addition of water, the mixture was extracted with EtOAc. The organic layer was washed with water and saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to yield **5** (31 g, 99%) as a colorless oil: $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.34 (s, 9H), 1.34 (s, 9H), 2.30 (s, 3H), 3.63−3.68 (m, 2H), 3.78 (s, 3H), 4.88−5.02 (m, 2H), 5.89−6.02 (m, 1H), 6.83 (s, 1H); MS *m*/*e* 318 ($M^+$).

**4-Acetoxy-3,5-di-*tert*-butyl-2-formylmethylanisole.** Compound **5** (19 g, 60 mmol) was dissolved in a 1:1 mixture (200 mL) of THF and water. $OsO_4$ (0.12 g, 0.5 mmol) and $NaIO_4$ (26.9 g, 126 mmol) were added to the solution, and the resulting mixture was stirred at room temperature for 48 h. After addition of saturated aqueous $Na_2S_2O_3$, the mixture was extracted with EtOAc. The organic layer was washed with water and saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (25% EtOAc in *n*-hexane) to afford the title compound (16 g, 84%) as a colorless oil: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.36 (s, 9H), 1.40 (s, 9H), 2.30 (s, 3H), 3.77 (s, 3H), 3.87 (bs, 2H), 6.89 (s, 1H), 9.63 (bs, 1H); MS *m*/*e* 320 ($M^+$).

**5-Acetoxy-4,6-di-*tert*-butylbenzofuran (6).** To a solution of 4-acetoxy-3,5-di-*tert*-butyl-2-formylmethylanisole (16 g, 50 mmol) in $CH_2Cl_2$ (100 mL) was added dropwise at 0 °C iodotrimethylsilane (7.1 mL, 50 mmol). The reaction mixture was stirred at room temperature for 1 h, and then a saturated aqueous $Na_2S_2O_3$ solution was added. The mixture was extracted with $CHCl_3$, and the organic layer was washed with water and saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to yield **6** (14.3 g, 99%) as a white solid, mp 88 °C: $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.39 (s, 9H), 1.51 (s, 9H), 2.35 (s, 3H), 6.98 (d, $J$ = 2.3 Hz, 1H), 7.46 (s, 1H), 7.55 (d, $J$ = 2.3 Hz, 1H); MS *m*/*e* 288 ($M^+$).

**4,6-Di-*tert*-butyl-5-benzofuranol.** Lithium aluminum hydride ($LiAlH_4$) (1.31 g, 34.5 mmol) was suspended in dry THF (150 mL) under a $N_2$ atmosphere. A solution of **6** (10 g, 34.7 mmol) in dry THF (100 mL) was added dropwise to the suspension at 0 °C, and then the mixture was heated to reflux for 3 h. After the mixture was cooled to room temperature, water was added dropwise to quench the excess $LiAlH_4$. After addition of 10% aqueous HCl (100 mL), the mixture was extracted with EtOAc. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to give the title compound (8.3 g, 97%) as a fine-grained pale yellow crystal, mp 60 °C: IR (KBr) 3648, 2960 $cm^{-1}$; $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.49 (s, 9H), 1.62 (s, 9H), 5.11 (s, 1H), 7.02 (d, $J$ = 2.4 Hz, 1H), 7.35 (s, 1H), 7.43 (d, $J$ = 2.4 Hz, 1H); MS *m*/*e* 246 ($M^+$), 231, 57.

**4,6-Di-*tert*-butyl-2,3-dihydro-5-benzofuranol (1a).** 4,6-Di-*tert*-butyl-5-benzofuranol (6.0 g, 24 mmol) was dissolved in acetic acid (50 mL). After addition of 10% Pd/C (5.0 g), the solution was stirred under a $H_2$ atmosphere (4 atm.) for 15 h. The Pd catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to yield **1a** (4.4 g, 73%) as a fine-grained white crystal, which was recrystallized from *n*-hexane, mp 117−118 °C: IR (KBr) 3623, 2969 $cm^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.41 (s, 9H), 1.51 (s, 9H), 3.42 (t, $J$ = 8.6 Hz, 2H), 4.39 (t, $J$ = 8.6 Hz, 2H), 4.75 (s, 1H), 6.70 (s, 1H); MS *m*/*e* 248 ($M^+$), 233, 191, 57. Anal. ($C_{16}H_{24}O_2$) C, H.

**4-Acetoxy-3,5-di-*tert*-butyl-1-(2-methyl-2-propenyloxy)benzene (7).** To a suspension of 60% oily NaH (0.18 g, 4.5 mmol) in dry DMF (10 mL) was added dropwise at 0 °C a solution of **3** (1.0 g, 3.8 mmol) in dry DMF (5 mL). The mixture was stirred for 30 min at the same temperature. Subsequently, the mixture was allowed to warm to room temperature, and 3-chloro-2-methylpropene (0.45 mL, 4.5 mmol) was added. After stirring at room temperature for 2 h, saturated aqueous $NH_4Cl$ (15 mL) was added to the reaction mixture, and the mixture was extracted with $Et_2O$. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column

chromatography (10% EtOAc in *n*-hexane) to afford **7** (1.08 g, 90%) as a colorless oil: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.30 (s, 18H), 1.83 (s, 3H), 2.30 (s, 3H), 4.37 (br, $J$ = 6.6 Hz, 2H), 6.83 (s, 2H); MS *m/e* 318 ($M^+$).

**5-Acetoxy-4,6-di-*tert*-butyl-2,3-dihydro-2,2-dimethylbenzofuran (8).** Compound **7** (2.22 g, 7.0 mmol) was dissolved in *N*,*N*-dimethylaniline (8 mL), and the solution was refluxed under a $N_2$ atmosphere for 36 h. After being cooled to room temperature, the reaction solution was concentrated under reduced pressure. To the concentrate were added 1 N HCl (5 mL) and $Et_2O$ (10 mL), and the mixture was stirred for 15 min. The organic layer was separated, and the aqueous layer was extracted with $Et_2O$. The combined extracts were washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (5% EtOAc in *n*-hexane) to give **8** (1.19 g, 54%) as a white solid, mp 98 °C: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.16−1.60 (m, 24H), 2.25 (s, 3H), 3.18 (s, 2H), 6.63 (s, 1H); MS *m/e* 318 ($M^+$).

**4,6-Di-*tert*-butyl-2,3-dihydro-2,2-dimethyl-5-benzofuranol (1b).** $LiAlH_4$ (0.10 g, 2.7 mmol) was suspended in dry THF (5 mL) under a $N_2$ atmosphere. A solution of **8** (0.86 g, 2.7 mmol) in dry THF (6 mL) was added dropwise to the suspension, and the mixture was refluxed for 4 h. After the mixture was cooled to room temperature, water was added dropwise to quench the excess $LiAlH_4$. After addition of 1 N NaOH (5 mL), the mixture was extracted with $Et_2O$. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (5% EtOAc in *n*-hexane) to give **1b** (0.62 g, 83%) as a white solid, mp 109−110 °C: IR (KBr) 3632, 2964, 1404, 1386, 1134 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 1.41 (s, 9H), 1.42 (s, 6H), 1.49 (s, 9H), 3.24 (s, 2H), 4.70 (s, 1H), 6.64 (s, 1H); MS *m/e* 276 ($M^+$). Anal. ($C_{18}H_{28}O_2$) C, H.

**4-Acetoxy-3,5-di-*tert*-butyl-2-(chloroacetylaminomethyl)phenol (9) and 6-Acetoxy-5,7-di-*tert*-butyl-3-chloroacetyl-2,3-dihydro-4*H*-1,3-benzoxazine (10).** Phenol **3** (29.0 g, 0.11 mol) was dissolved in a 9:1 mixed solution (200 mL) of acetic acid and $H_2SO_4$. After addition of 2-chloro-*N*-(hydroxymethyl)acetamide[31] (34.0 g, 0.28 mol), the mixture was stirred at room temperature for 48 h. Subsequently, the reaction mixture was poured into water, neutralized with 1 N NaOH, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The concentrate was used in the subsequent reaction without further purification. When a portion of the concentrate was purified by silica gel column chromatography (20% EtOAc in *n*-hexane), the title compounds were obtained: **9** as a colorless oil; $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.30 (s, 9H), 1.43 (s, 9H), 2.28 (s, 3H), 4.00 (s, 2H), 4.73 (d, $J$ = 6.0 Hz, 2H), 6.88 (s, 1H), 7.54 (t, $J$ = 6.0 Hz, 1H); MS *m/e* 369 ($M^+$), 327, 234, 57; and **10** as a colorless oil; $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.30 (s, 9H), 1.47 (s, 9H), 2.30 (s, 3H), 4.17 (s, 2H), 5.00 (s, 2H), 5.33 (s, 2H), 6.83 (s, 1H); MS *m/e* 381 ($M^+$), 339, 304, 57.

**4-Acetoxy-2-aminomethyl-3,5-di-*tert*-butylphenol (11).** The concentrate obtained in the above reaction was dissolved in a 10:3 mixed solution (550 mL) of EtOH and concentrated HCl, and the reaction solution was heated to reflux for 2 h. After being cooled, the solution was poured into water, and the mixture was neutralized with 1 N NaOH, followed by extraction with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The concentrate was used in the subsequent reaction without further purification. When a portion of the concentrate was purified by silica gel column chromatography (20% MeOH in $CHCl_3$), phenol **11** was obtained: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.27 (s, 9H), 1.37 (s, 9H), 2.25 (s, 3H), 4.22 (s, 2H), 5.18 (bs, 3H), 6.85 (s, 1H); MS *m/e* 293 ($M^+$), 234, 191, 57.

**5-Acetoxy-4,6-di-*tert*-butyl-2-hydroxybenzaldehyde (12).** The concentrate obtained in the above reaction was dissolved in a 11:3 mixed solution (636 mL) of acetic acid and water. After addition of hexamethylenetetramine (19.3 g, 0.14 mol), the mixture was heated to reflux for 4 h. Subsequently, 4.5 N HCl (85 mL) was added to the mixture, and the resulting mixture was heated to reflux for additional 20 min. After being cooled, the reaction mixture was poured into water, neutralized with 1 N NaOH, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The concentrate was purified by silica gel column chromatography ($CHCl_3$) to afford **12** (19.0 g, 59% for three steps) as a fine-grained pale yellow crystal, mp 79 °C: IR (KBr) 2976, 1758 cm$^{-1}$; $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 1.35 (s, 9H), 1.54 (s, 9H), 2.35 (s, 3H), 6.92 (s, 1H), 10.67 (s, 1H), 12.32 (s, 1H); MS *m/e* 292 ($M^+$), 250, 235, 217, 57.

**4-Acetoxy-3,5-di-*tert*-butyl-2-(2-ethyl-1-butenyl)phenol (14c).** To a mixture of Mg (0.21 g, 8.6 mmol) and dry THF (10 mL) was added dropwise under a $N_2$ atmosphere a solution of 3-bromopentane (1.3 g, 8.6 mmol) in dry THF (10 mL) to prepare a Grignard reagent. A solution of **12** (1.0 g, 3.4 mmol) in dry THF (5 mL) was added dropwise to the Grignard reagent, and the mixture was stirred for 30 min at room temperature. A 5:2 mixed solution (7 mL) of water and concentrated HCl was added to the reaction mixture, followed by stirring at room temperature for 30 min and subsequent extraction with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The concentrate was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to give **14c** (0.85 g, 72%) as a yellow oil: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 0.78−1.57 (m, 6H), 1.33 (s, 9H), 1.37 (s, 9H), 1.73−2.48 (m, 4H), 2.27 (s, 3H), 5.38 (d, $J$ = 9.6 Hz, 1H), 6.17 (s, 1H), 6.87 (s, 1H); MS *m/e* 346 ($M^+$), 304, 289, 57.

**5-Acetoxy-4,6-di-*tert*-butyl-2,2-diethyl-2,3-dihydrobenzofuran (15c).** To a solution **14c** (0.85 g, 2.5 mmol) in $CH_2Cl_2$ (10 mL) was added dropwise under a $N_2$ atmosphere $BF_3$ diethyl etherate (0.4 mL, 3.2 mmol). After the mixture was stirred at room temperature for 3 h, water was added to the reaction mixture, and the mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated. The concentrate was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to afford **15c** (0.45 g, 53%) as a pale yellow oil: $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 0.80−1.79 (m, 10H), 1.29 (s, 9H), 1.37 (s, 9H), 2.26 (s, 3H), 3.10 (s, 2H), 6.71 (s, 1H); MS *m/e* 346 ($M^+$), 304, 57.

**4,6-Di-*tert*-butyl-2,2-diethyl-2,3-dihydro-5-benzofuranol (1c).** $LiAlH_4$ (76 mg, 2.0 mmol) was suspended in dry THF (5 mL) under a $N_2$ atmosphere. A solution of **15c** (0.17 g, 0.5 mmol) in dry THF (5 mL) was added dropwise to the suspension at 0 °C. After heated to reflux for 3 h, the reaction mixture was allowed to cool to room temperature, and water was added dropwise to quench the excess $LiAlH_4$. After addition of 1 N aqueous NaOH (5 mL), the mixture was extracted with $Et_2O$. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to afford **1c** (0.13 g, 87%) as a pale yellow oil: IR (film) 3663, 2975 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 0.92 (t, $J$ = 7.4 Hz, 6H), 1.40 (s, 9H), 1.49 (s, 9H), 1.65−1.75 (m, 4H), 3.18 (s, 2H), 4.66 (s, 1H), 6.63 (s, 1H); MS *m/e* 304 ($M^+$), 289, 163, 57; HPLC analysis ($CH_3CN$−*i*-PrOH-water (8:1:1)) $t_R$ = 6.9 min (90.7%). HRMS Calcd for $C_{20}H_{32}O_2$: 304.2402. Found: 304.2419.

The procedure used for the preparation of **1c** (Method B) was repeated with the following compounds using compound **12** and the corresponding Grignard reagents.

**4,6-Di-*tert*-butyl-2,3-dihydro-2,2-dipropyl-5-benzofuranol (1d).** 23% yield from **12**: IR (film) 3662, 2971 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 0.92 (t, $J$ = 7.3 Hz, 6H), 1.20−1.35 (m, 4H), 1.40 (s, 9H), 1.49 (s, 9H), 1.60−1.70 (m, 4H), 3.19 (s, 2H), 4.66 (s, 1H), 6.61 (s, 1H); MS *m/e* 332 ($M^+$); HPLC analysis ($CH_3CN$−*i*-PrOH-water (8:1:1)) $t_R$ = 8.9 min (97.5%). HRMS Calcd for $C_{22}H_{36}O_2$: 332.2715. Found: 332.2730.

**2,2-Dibutyl-4,6-di-*tert*-butyl-2,3-dihydro-5-benzofuranol (1e).** 15% yield from **12**: IR (film) 3663, 2964 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 0.90 (t, $J$ = 6.9 Hz, 6H), 1.30−1.38 (m, 8H), 1.40 (s, 9H), 1.49 (s, 9H), 1.60−1.71 (m, 4H), 3.18 (s, 2H), 4.66 (s, 1H), 6.62 (s, 1H); MS *m/e* 360 ($M^+$); HPLC analysis ($CH_3CN$−*i*-PrOH-water (8:1:1)) $t_R$ = 11.7 min (95.8%). HRMS Calcd $C_{24}H_{40}O_2$: 360.3028. Found: 360.3005.

**4,6-Di-*tert*-butyl-2,3-dihydro-5-benzofuranol-2-spiro-1′-cyclopentane (1k).** 23% yield from **12**: mp 105−106 °C; IR (KBr) 3644, 2979 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 1.41 (s, 9H), 1.49 (s, 9H), 1.62−2.08 (m, 8H), 3.40 (s, 2H), 4.69 (s, 1H), 6.64 (s, 1H); MS *m/e* 302 ($M^+$). Anal. ($C_{20}H_{30}O_2$) C, H.

**4-Acetoxy-3,5-di-*tert*-butyl-2-(1-hydroxy-2-pentylheptyl)phenol (13f).** To a Grignard reagent, which was prepared with Mg (30 g, 1.23 mol), 6-bromoundecane (290 g, 1.23 mol) prepared in a usual manner, and dry THF (1.2 L) under a $N_2$ atmosphere, was added dropwise a solution of **12** (120 g, 0.41 mol) in dry THF (400 mL). After the reaction mixture was stirred at room temperature for 2 h, a saturated aqueous $NH_4Cl$ solution was added to the mixture, followed by extraction with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated. The concentrate was purified by silica gel column chromatography (5% EtOAc in *n*-hexane) to give **13f** (74.3 g, 40%) as a white solid, mp 126 °C: IR (KBr) 3493, 1761, 1369, 1190, 908 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.74 (t, $J$ = 6.8 Hz, 3H), 0.91 (t, $J$ = 6.6 Hz, 3H), 0.95−1.63 (m, 16H), 1.29 (s, 9H), 1.40 (s, 9H), 2.12 (m, 1H), 2.28 (s, 3H), 2.50 (d, $J$ = 2.6 Hz, 1H), 5.22 (dd, $J$ = 2.6, 9.9 Hz, 1H), 6.77 (s, 1H), 7.89 (s, 1H); MS *m/e* 448 ($M^+$).

**5-Acetoxy-4,6-di-*tert*-butyl-2,3-dihydro-2,2-dipentylbenzofuran (15f).** To a solution **13f** (74.3 g, 0.166 mol) in $CH_2Cl_2$ (500 mL) was added dropwise under a $N_2$ atmosphere $BF_3$ diethyl etherate (20 mL, 0.16 mol). After being stirred at room temperature for 4.5 h, the reaction mixture was added to ice water, neutralized with 2 N KOH, and extracted with $CH_2Cl_2$. The extract was washed with saturated aqueous NaCl, dried over anhydrous $MgSO_4$, and concentrated to give **15f** as a pale yellow oil. The concentrate was used in the subsequent reaction without further purification: IR (film) 1760, 1567, 1365, 1214, 1172, 943 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.6 Hz, 6H), 1.22−1.39 (m, 12H), 1.30 (s, 9H), 1.37 (s, 9H), 1.55−1.75 (m, 4H), 2.29 (s, 3H), 3.10 (d, $J$ = 15.3 Hz, 1H), 3.21 (d, $J$ = 15.3 Hz, 1H), 6.72 (s, 1H); MS *m/e* 430 ($M^+$).

**4,6-Di-*tert*-butyl-2,3-dihydro-2,2-dipentyl-5-benzofuranol (1f).** $LiAlH_4$ (6.3 g, 0.166 mol) was suspended in dry THF (200 mL) under a $N_2$ atmosphere. A solution of **15f** in dry THF (400 mL) was added dropwise to the suspension at 0 °C. After being heated to reflux for 3 h, the reaction mixture was allowed to cool to room temperature, and EtOAc was added dropwise to quench the excess $LiAlH_4$. After addition of 10% HCl, the mixture was extracted with EtOAc. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane) to afford **1f** (55.0 g, 85% for two steps) as a colorless oil: IR (film) 3652, 2956 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.9 Hz, 6H), 1.30 (br, 12H), 1.40 (s, 9H), 1.49 (s, 9H), 1.58−1.70 (m, 4H), 3.18 (s, 2H), 4.66 (s, 1H), 6.62 (s, 1H); MS *m/e* 388 ($M^+$); HPLC analysis ($CH_3CN$−*i*-PrOH−water (8:1:1)) $t_R$ = 15.7 min (95.1%). HRMS Calcd for $C_{26}H_{44}O_2$: 388.3341. Found: 388.3339.

The procedure used for the preparation of **1f** (Method A) was repeated with the following compounds using compound **12** and the corresponding Grignard reagents.

**4,6-Di-*tert*-butyl-2,2-dihexyl-2,3-dihydro-5-benzofuranol (1g).** 16% yield from **12**: IR (film) 3650, 2920 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.87 (t, $J$ = 6.9 Hz, 6H), 1.28 (br, 16H), 1.40 (s, 9H), 1.49 (s, 9H), 1.60−1.70 (m, 4H), 3.18 (s, 2H), 4.66 (s, 1H), 6.61 (s, 1H); MS *m/e* 416 ($M^+$); HPLC analysis ($CH_3CN$−*i*-PrOH-water (8:1:1)) $t_R$ = 23.0 min (94.6%). HRMS Calcd for $C_{28}H_{48}O_2$: 416.3654. Found: 416.3637.

**4,6-Di-*tert*-butyl-2,2-diheptyl-2,3-dihydro-5-benzofuranol (1h).** 3% yield from **12**: IR (film) 3656, 2928 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.87 (t, $J$ = 7.3 Hz, 6H), 1.27 (br, 20H), 1.40 (s, 9H), 1.49 (s, 9H), 1.57−1.70 (m, 4H), 3.18 (s, 2H), 4.66 (s, 1H), 6.62 (s, 1H); MS *m/e* 444 ($M^+$); HPLC analysis ($CH_3CN$−*i*-PrOH−water (8:1:1)) $t_R$ = 34.5 min (98.3%). HRMS. Calcd for $C_{30}H_{52}O_2$: 444.3967C. Found: 444.3958.

**4,6-Di-*tert*-butyl-2,3-dihydro-2,2-dioctyl-5-benzofuranol (1i).** 2% yield from **12**: IR (film) 3652, 2928, 2856, 1412 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.88 (t, $J$ = 6.9 Hz, 6H), 1.26 (br, 24H), 1.40 (s, 9H), 1.49 (s, 9H), 1.59−1.70 (m, 4H), 3.17 (s, 2H), 4.65 (s, 1H), 6.61 (s, 1H); MS *m/e* 472 ($M^+$); HPLC analysis ($CH_3CN$−*i*-PrOH−water (8:1:1)) $t_R$ = 52.3 min (90.5%). HRMS Calcd for $C_{32}H_{56}O_2$: 472.4280. Found: 472.4301.

**2,2-Dibenzyl-4,6-di-*tert*-butyl-2,3-dihydro-5-benzofuranol (1j).** 9% yield from **12**: mp 132−133 °C; IR (KBr) 3661, 2970 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 1.37 (s, 9H), 1.39 (s, 9H), 2.97 (s, 4H), 3.22 (s, 2H), 4.59 (s, 1H), 6.63 (s, 1H), 7.23 (s, 10H); MS *m/e* 328 ($M^+$). Anal. ($C_{30}H_{36}O_2$) C, H.

**4,6-Di-*tert*-butyl-2,3-dihydro-5-benzofuranol-2-spiro-1′-cyclohexane (1l).** 56% yield from **12**: mp 129−131 °C; IR (KBr) 3650, 2934 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 1.41 (s, 9H), 1.49 (s, 9H), 1.37−1.63 (m, 6H), 1.75 (m, 4H), 3.18 (s, 2H), 4.68 (s, 1H), 6.65 (s, 1H); MS *m/e* 316 ($M^+$). Anal. ($C_{21}H_{32}O_2$) C, H.

**4,6-Di-*tert*-butyl-2,3-dihydro-5-benzofuranol-2-spiro-1′-cycloheptane (1m).** 30% yield from **12**: mp 94−95 °C; IR (KBr) 3646, 2927 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 1.40 (s, 9H), 1.49 (s, 9H), 1.57−2.02 (m, 12H), 3.22 (s, 2H), 4.67 (s, 1H), 6.63 (s, 1H); MS *m/e* 330 ($M^+$). Anal. ($C_{22}H_{34}O_2$) C, H.

**4,6-Di-*tert*-butyl-2,3-dihydro-5-benzofuranol-2-spiro-1′-cyclooctane (1n).** 13% yield from **12**: mp 96.8 °C; IR (film) 3660, 2933 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 1.40 (s, 9H), 1.49 (s, 9H), 1.56−1.76 (m, 10H), 2.03 (m, 4H), 3.18 (s, 2H), 4.67 (s, 1H), 6.63 (s, 1H); MS *m/e* 344 ($M^+$). Anal. ($C_{23}H_{36}O_2$) C, H.

**4,6-Di-*tert*-butyl-2,3-dihydro-5-benzofuranol-2-spiro-4′-tetrahydropyran (1o).** 52% yield from **12**: mp 185−186 °C; IR (KBr) 3365, 2972 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 1.41 (s, 9H), 1.49 (s, 9H), 1.71−1.89 (m, 4H), 3.24 (s, 2H), 3.72−3.80 (m, 2H), 3.86−3.94 (m, 2H), 4.73 (s, 1H), 6.67 (s, 1H); MS *m/e* 318 ($M^+$). Anal. ($C_{20}H_{30}O_3 \cdot 0.25H_2O$) C, H.

**4-Acetoxy-3,5-di-*tert*-butyl-2-[1-hydroxy-5-methyl-2-(3-methylbutyl)hexyl]phenol (13p).** Lithium (2.8 g, 0.40 mol) was added portionwise to a solution of **12** (24.3 g, 83 mmol) and 5-bromo-2,8-dimethylnonane (47.0 g, 0.20 mol) in dry THF (200 mL) at 0 °C under a nitrogen atmosphere, and the mixture was stirred for 24 h. The reaction mixture was carefully poured into ice water, neutralized with saturated aqueous $NH_4Cl$, and extracted with $Et_2O$. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (10% EtOAc in *n*-hexane) to afford **13p** (17.0 g, 46%) as a colorless oil: $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ 0.80−1.81 (m, 11H), 0.87 (d, $J$ = 7.0 Hz, 12H), 1.25 (s, 9H), 1.37 (s, 9H), 2.24 (s, 3H), 3.51 (br, 1H), 5.19 (d, $J$ = 10.0 Hz, 1H), 6.72 (s, 1H), 7.92 (s, 1H); MS *m/e* 448 ($M^+$).

**5-Acetoxy-4,6-di-*tert*-butyl-2,3-dihydro-2,2-di-(3-methylbutyl)benzofuran (15p).** To a solution of **13p** (17.0 g, 38 mmol) in $CH_2Cl_2$ (200 mL) was added $BF_3$ etherate (4.7 mL, 37 mmol) dropwise at 0 °C under a nitrogen atmosphere. After stirring the reaction mixture overnight at room temperature, a saturated aqueous $NaHCO_3$ was added to the mixture. The mixture was extracted with EtOAc, and the organic layer was washed with saturated aqueous NaCl, and dried over anhydrous $MgSO_4$. The solvent was distilled off to afford **15p** (15.5 g, 95%) as a colorless oil: IR (film) 2956, 1764 $cm^{-1}$; $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ 0.87 (d, $J$ = 5.8 Hz, 12H), 1.04−1.93 (m, 10H), 1.28 (s, 9H), 1.35 (s, 9H), 2.25 (s, 3H), 3.14 (s, 2H), 6.67 (s, 1H); MS *m/e* 430 ($M^+$).

**4,6-Di-*tert*-butyl-2,3-dihydro-2,2-di-(3-methylbutyl)-5-benzofuranol (1p).** $LiAlH_4$ (1.90 g, 50 mmol) was suspended in dry THF (200 mL) under a nitrogen atmosphere. A solution of **15p** (17.4 g, 40 mmol) in dry THF (60 mL) was added dropwise to the suspension at 0 °C. After being heated to reflux overnight, the reaction mixture was allowed to cool to room temperature. To the mixture were added water and a saturated aqueous $NH_4Cl$ dropwise to quench the excess $LiAlH_4$. The resulting insolubles were filtered off on Celite, and the filtrate was extracted with $Et_2O$. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane) to afford **1p** (9.0 g, 57%) as a colorless oil: IR (film) 3652, 2956 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.89 (d, $J$ = 6.6 Hz, 12H), 1.19−1.69 (m, 10H), 1.41 (s, 9H),

1.49 (s, 9H), 3.17 (s, 2H), 4.66 (s, 1H), 6.62 (s, 1H); MS *m/e* 388 ($M^+$); HPLC analysis ($CH_3CN-i$-PrOH−water (8:1:1)) $t_R$ = 14.6 min (92.9%). HRMS Calcd for $C_{26}H_{44}O_2$: 388.3341. Found: 388.3342.

**2,4,6-Tri-*tert*-butyl-5-benzofuranol (18).** To a mixture of 4-*tert*-butyl-5-benzofuranol **17**[33] (35 g, 0.18 mol) and 2-methyl-2-propanol (70 g, 0.94 mol) in $CHCl_3$ (50 mL) at 0 °C was added methanesulfonic acid (14 mL, 0.22 mol) dropwise. After being stirred at 0 °C for 20 min, the mixture was poured into ice−water. Subsequently, the mixture was neutralized with 1 N NaOH and extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane) to give **18** (10.87 g, 20%) as a fine-grained, pale yellow crystal, mp 116 °C: IR (KBr) 3641, 2969 $cm^{-1}$; $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ 1.33 (s, 9H), 1.47 (s, 9H), 1.62 (s, 9H), 5.06 (s, 1H), 6.58 (s, 1H), 7.29 (s, 1H); MS *m/e* 302 ($M^+$), 287, 57.

**2,4,6-Tri-*tert*-butyl-2,3-dihydro-5-benzofuranol (1q).** To a mixture of **18** (24.7 g, 81.7 mmol) and triethylsilane (76 mL, 0.48 mol) at 0 °C was added trifluoroacetic acid (38 mL) dropwise. The mixture was stirred first at 0 °C for 15 min, then at room temperature for 15 min. After being poured into ice water, the mixture was neutralized with 1 N NaOH and extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$) to afford **1q** (19.0 g, 76%) as a white needle, which was recrystallized from *n*-hexane, mp 90−92 °C: IR (KBr) 3669, 2973 $cm^{-1}$; $^1H$ NMR (270 MHz, $CDCl_3$) $\delta$ 0.97 (s, 9H), 1.41 (s, 9H), 1.50 (s, 9H), 3.14 (dd, $J$ = 9.9, 15.7 Hz, 1H), 3.29 (dd, $J$ = 8.9, 15.7 Hz, 1H), 4.23 (dd, $J$ = 8.9, 9.9 Hz, 1H), 4.70 (s, 1H), 6.67 (s, 1H); MS *m/e* 304 ($M^+$), 289, 57. Anal. ($C_{20}H_{32}O_2$) C, H.

**Chemiluminescence Assay.** Linoleic acid peroxyl radicals were generated by autoxidation and detected by chemiluminescence using a *Cypridina* luciferin analogue, 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-*a*]pyrazin-3-one (MCLA).[34] A solution of 1 mM linoleic acid in 1-butanol containing 10 $\mu$M MCLA was incubated for 10 min at 37 °C under air. The chemiluminescence was induced by the fast reaction of MCLA with singlet oxygen generated by linoleic acid peroxyl radicals. The quenching of the peroxyl radicals was evaluated as the change in chemiluminescence intensity after addition of the compound. All determinations were performed in duplicate or more.

**Oxidation of LDL and Analysis.** LDL (density 1.019−1.063 g/mL) was isolated by sequential ultracentrifugation from rabbit plasma.[38] A test compound was added to the rabbit's LDL at 200 $\mu$g/mL, and subsequently a soybean lipoxygenase type-IS (SLO) or cupric sulfate ($CuSO_4$) was added to a final concentration shown in Table 1. Oxidation of LDL was carried out by incubation at 37 °C in a $CO_2$ incubator for 24 h.

Fluorescence of LDL was analyzed by gel permeation chromatography (GPC) using gel filtration column (TOSOH G5000PW). LDL solution (100 mL) was applied to GPC and eluted with 0.1 M NaCl + 0.05 M Tris buffer (pH 7.4). The eluent was monitored using a fluorophotometric detector (Ex; 360 nm, Em; 430 nm, JASCO FP-210), and the intensity was integrated with a molecular weight greater than 150 kDa.[39] Lipid peroxides were measured as thiobarbituric acid reactive substances (TBARS) by the Yagi method using a determination kit (Lipid Peroxide Test Wako, Wako Pure Chemical). Electrophoretic mobility was evaluated by one-dimensional agarose gel electrophoresis for 35 min in a 90 V field using universal electrophoresis film (Ciba Corning). Electrophoretic mobility was determined by measuring the distance between the point where LDL was applied and the center point of LDL band stained with Fat Red 7B. All determinations were performed in duplicate or more.

**Absorption in WHHL Rabbits.** Each test compound (250 mg/kg) and probucol (500 mg/kg) was administered orally to WHHL rabbits for 9 days. After 24 h of daily administration, plasma was prepared and extracted using EtOAc, and then plasma concentrations of compounds were determined by reverse-phase HPLC. Plasma lipoproteins were isolated according to the procedure of Havel et al.[38] from the WHHL rabbits treated with compounds at 24 h after the final administration. The concentrations in the lipoproteins were determined as mentioned above. The data are expressed as mean ± range ($n$ = 2).

**Oxidizability of the Isolated LDL from Antioxidant Treated WHHL Rabbits.** The LDL (200 $\mu$g/mL) isolated from WHHL rabbits treated with **1f** or probucol was incubated at 37 °C for 24 h with 10 $\mu$M $CuSO_4$ or 40 $\mu$g/mL SLO. The extent of LDL oxidation was measured by the fluorescence intensity as mentioned above. The data are expressed as mean ± range ($n$ = 2).

**Acknowledgment.** We gratefully thank Drs. Yasuhiro Ohba and Jun-ichiro Aono for their help with the in vivo experiments and valuable discussions and Mrs. Atsuko Higashida for her help on the determination of the compound's plasma concentrations. We also acknowledge Dr. Paul Langman for his useful advice in the preparation and language editing of this paper.

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JM030062A