Syntheses of Novel Hybrid Vitamin C Derivatives: Stability and Biological Activity

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A novel series of hybrid L-ascorbic acid (vitamin C) derivatives linking other biologically active substances glycolic acid, *myo*-inositol, and α-tocopherol (vitamin E) at the C-2 or C-3 hydroxyl group were synthesized, and their thermal stability and inhibitory activities against tyrosinase-catalyzed melanin formation, active oxygen species (AOS), and free radicals were evaluated *in vitro*. Among these derivatives, 2-*O*-carboxymethylascorbic acid had high thermal stability as well as moderate inhibitory activities against tyrosinase-catalyzed melanin formation, AOS, and free radicals compared to other typical inhibitors and scavengers. On the other hand, 3-*O*-carboxymethylascorbic acid was markedly unstable in aqueous solution. The 2-*O*-carbonylmethyl derivatives linking *myo*-inositol or vitamin E were susceptible to degrading, however the vitamin E derivative had stronger inhibitory activities against AOS and free radicals than free vitamin C.

The ozone layer in the stratosphere has been thinning significantly. With ozone depletion, the amount of solar ultraviolet (UV) radiation reaching the Earth's surface has been increasing. UV radiation has been shown to generate active oxygen species (AOS) such as superoxide anion radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen or free radicals in living organisms.[1] It has recently become clear that AOS and free radicals derived from the biological use of O_2 are responsible for oxidative injury to enzymes, lipid membranes, and DNA in living cells,[2] and tissues to participate in the development or exacerbation of various kinds of disease.[3] Furthermore it has been accepted that the oxidative stress or damage induced by free radicals is related to the aging process called dermatoheliosis.[4] Moreover, AOS accelerate tyrosinase activity to increase dopa and dopaquinone generation from tyrosine in melanocytes[5] resulting in excess melanin formation.[6] Under normal conditions, cells and tissues are protected from the attack of AOS and free radicals by various enzymes such as superoxide dismutase (SOD), catalase, and peroxidase, as well as α-tocopherol (vitamin E), glutathione, and L-ascorbic acid (vitamin C).[3,7]

It is known that vitamin C scavenges AOS and free radicals as a chain-breaking antioxidant[8] and inhibits melanin formation by reduction of intermediate dopaquinone and resulting eumelanin in melanogenesis.[9] There is considerable evidence that vitamin C is important in the prevention of a large number of chronic diseases such as cancer, cerebral apoplexy, diabetes, atopic dermatitis, myocardial infarction, and AIDS.[10] These characteristic biological activities of vitamin C are derived from the enediol structure, which has a strong electron-donating ability.

The well-known susceptibility of vitamin C to thermal and oxidative degradations has interested people in vitamin C derivatives that have shown increased stability *in vitro*, while being able to generate its inherent activity through enzymatic cleavage to free vitamin C *in vivo*. In particular the chemical modification of hydroxyl groups of vitamin C is of most interest, and numerous stable derivatives of vitamin C have been reported.[9,11—19,23,24] Generally, the partial modification of the enediol system would give rise to two isomers, both of which are markedly decreased in reducing power and therefore stabilized against oxidation, and additionally vitamin C activities tend to be lower in proportion to increase in the number of substituents in the molecule.[15c] Among these derivatives, the phosphate and sulfate esters of vitamin C are available as hydrophilic antioxidants due to their vitamin C activity through enzymic degradation to free vitamin C *in vivo* and the increased stability against treatment with alkali, oxidation, and prolonged storage.[14] Particularly the magnesium salt of ascorbic acid 2-phosphate (APC-3) has been widely used as a bleaching ingredient in cosmetics,[9,15] furthermore recently an APC-3 derivative linked with vitamin E (EPC) has been commercialized.[16] More recently, 2-*O*-α-D-glucopyranosylascorbic acid (AA-2G) has been developed, which is very stable and inhibits tyrosinase *in vivo*.[17] As to lipophilic antioxidants, ascorbic acid 6-stearate and 2,6-dipalmitate have been widely used,[18] and 2-*O*-octadecylascorbic acid (CV-3611)[19] and 3-*O*-(dodecylcarbomethyl)ascorbic acid (HX-0112)[13] have been developed as new lipophilic antioxidants.

It has been reported that α-hydroxy acids, especially glycolic acid, repair wrinkles and revitalize cells through dermal fibroblast proliferation.[20] In addition, one of the vitamin B family, *myo*-inositol, can be widely found in all biological systems, particularly associated with biomembranes. In biomembranes phosphatidylinositols act as a intracellular second messenger.[21] It is a well-known fact that vitamin E acts as a synergistic antioxidant in conjunction with vitamin C to suppress the lipid peroxidation in cell membrane.[22]

Based on this background, we designed and synthesized

a novel series of hybrid vitamin C derivatives linking these biologically active substances (glycolic acid, *myo*-inositol, and vitamin E) at the C-2 or C-3 hydroxyl group with a view to simultaneously improving the thermal stability and preventing the diminution of activity, and evaluated their stability and inhibitory activities against tyrosinase-catalyzed melanin formation, AOS, and free radicals *in vitro*.

Results and Discussion

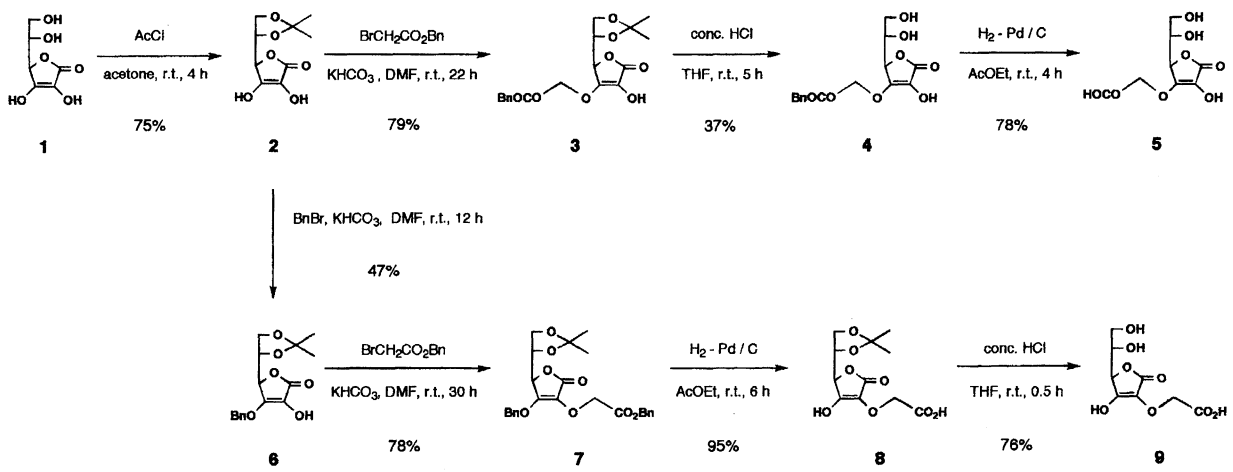
The 3-*O*- and 2-*O*-carboxymethyl derivatives **5** and **9** were synthesized as shown in Scheme 1. The 3-*O*-carboxymethyl derivative **5** was synthesized in four steps from ascorbic acid **1**. The hydroxyl groups at C-5 and C-6 were protected with acetone by Jung's method to yield 5,6-*O*-isopropylideneascorbic acid **2** in 75% yield.[23] The derivative **2** was alkylated with benzyl bromoacetate in the presence of $KHCO_3$ in DMF to give the 3-*O*-alkylated intermediate **3** in 79% yield. Removal of the protecting groups was done by acid hydrolysis followed by catalytic hydrogenation to yield 3-*O*-carboxymethyl derivative **5**. The 2-*O*-carboxymethyl derivative **9** was synthesized in five steps from ascorbic acid **1**. The C-3 hydroxyl group of 5,6-*O*-isopropylideneascorbic acid **2** was protected as benzyl ether by using benzyl bromide in the presence of $KHCO_3$ in DMF to give 3-*O*-benzyl-5,6-*O*-isopropylideneascorbic acid **6** in 47% yield. Alkylation of **6** with benzyl bromoacetate in the presence of $KHCO_3$ in DMF gave the 2-*O*-alkylated intermediate **7** in 78% yield. Removal of the protecting groups was done by catalytic hydrogenation followed by acid hydrolysis to yield the 2-*O*-carboxymethyl derivative **9**.

The 2-*O*-glycolyl-*myo*-inositol derivative **17** was synthesized in nine steps from *myo*-inositol **10** as shown in Scheme 2. The hydroxyl groups at C-2 and C-3 were protected as cyclohexylidene ketal, and the C-1, C-4, C-5, and C-6 hydroxyl groups were protected as benzyl ethers followed by acid hydrolysis to give the DL-1,4,5,6-tetra-*O*-benzylinositol derivative **12** by Wyss's method.[25] Selective protection of the C-3 hydroxyl group with benzyl group was done by using benzyl chloride in the presence of NaOH in benzene under reflux to give the 1,3,4,5,6-penta-*O*-benzyl derivative **13** in 79% yield.[26] Esterification of **13** with bromoacetic acid in the presence of dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-dimethylaminopyridine (DMAP) in THF gave the 2-*O*-bromoacetyl derivative **14** quantitatively. Alkylation of the vitamin C derivative **6** with **14** in the presence of $KHCO_3$ and a catalytic amount of 18-crown-6 ether in DMF yielded the 2-*O*-alkylated intermediate of vitamin C **15** in 77% yield. Removal of the protecting groups was done by acid hydrolysis followed by catalytic hydrogenation to yield the 2-*O*-carbonylmethylascorbic acid derivative of inositol **17** quantitatively.

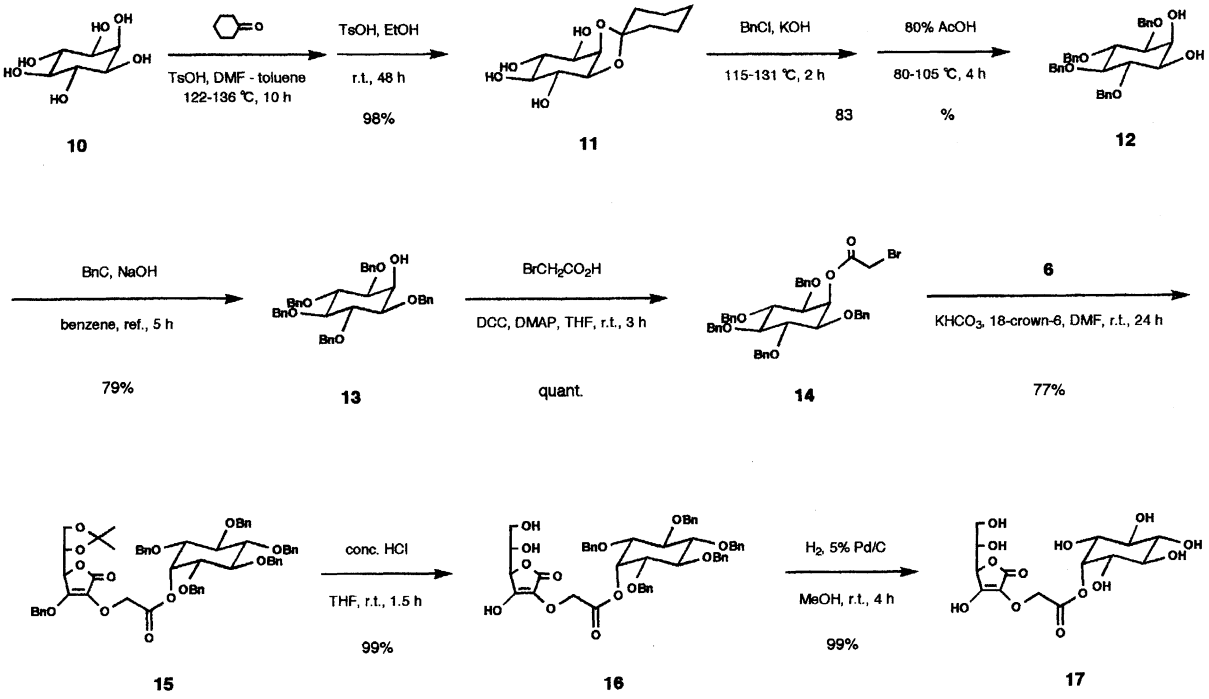
The 2-*O*-(α-tocopheryloxycarbonylmethyl) derivative **22** was synthesized in four steps from vitamin E **18** as shown in Scheme 3. Esterification of **18** with bromoacetic acid in the presence of DCC and a catalytic amount of DMAP in dioxane gave the bromoacetyl derivative **19** in 97% yield. Alkylation of the vitamin C derivative **6** with **19** in the presence of $KHCO_3$ and a catalytic amount of 18-crown-6 ether in DMF yielded the 2-*O*-alkylated intermediate **20** in 74% yield. Removal of the protecting groups was done by acid hydrolysis followed by catalytic hydrogenation to yield the 2-*O*-(α-tocopheryloxycarbonylmethyl) derivative **22** almost quantitatively.

The thermal stabilities of the above compounds **5**, **9**, **17**, **22**, and reference compounds vitamin C **1**, ascorbic acid 6-stearate, ascorbic acid 2,6-dipalmitate, and ascorbic acid 2-phosphate Mg-salt (APC-3) were tested under 1% (w/v) of the test compounds in a solution of EtOH/H_2O=1/1 (v/v) at 60 °C (Table 1). It was found that the 2-*O*-carboxymethyl derivative **9**, more than 90% of which remained after 3 months, was far more stable than **1** and the typical lipophilic derivatives. On the other hand, the 3-*O*-carboxymethyl derivative **5** decomposed almost completely within 2 months, although it was reported that the 3-*O*-alkylascorbic acids were far more stable than the 2-*O*-alkylascorbic acids.[13b] The 2-*O*-glycolylinositol and 2-*O*-(α-tocopheryloxycarbonylmethyl) derivatives **17** and **22** had nearly the same stability as **1**.

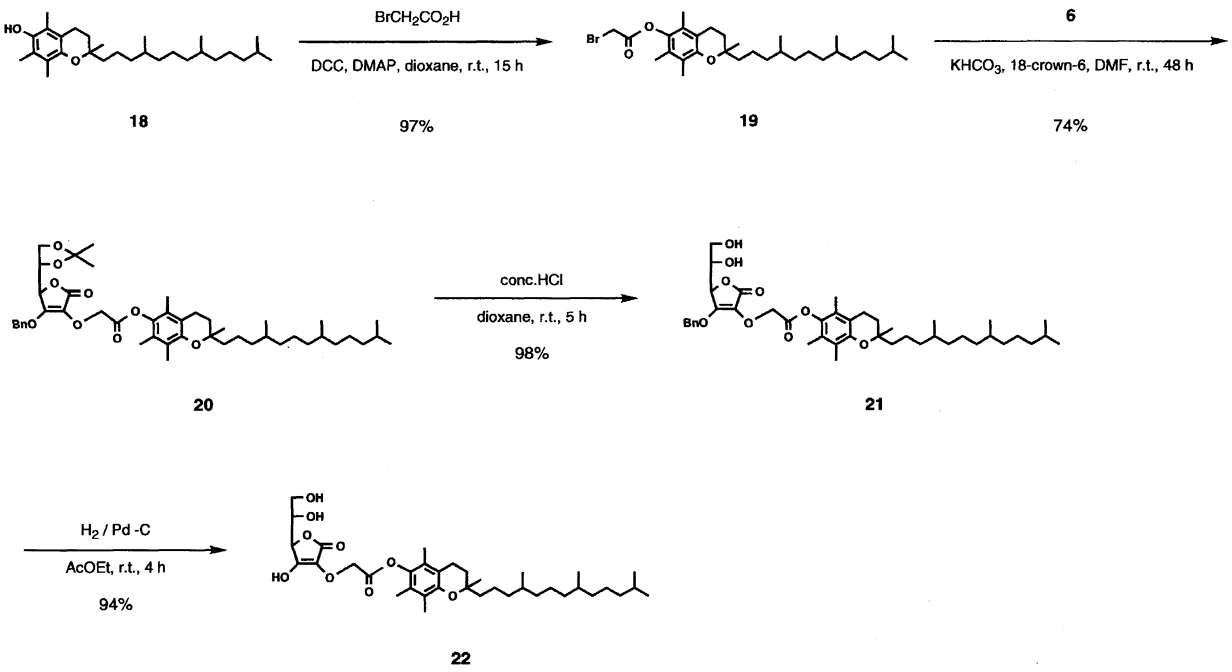
As described above, melanogenesis, melanin formation in melanocytes, is catalyzed by tyrosinase via the intermediates dopa and dopaquinone generation from tyrosine,[5] and vita-



Scheme 1.



Scheme 2.

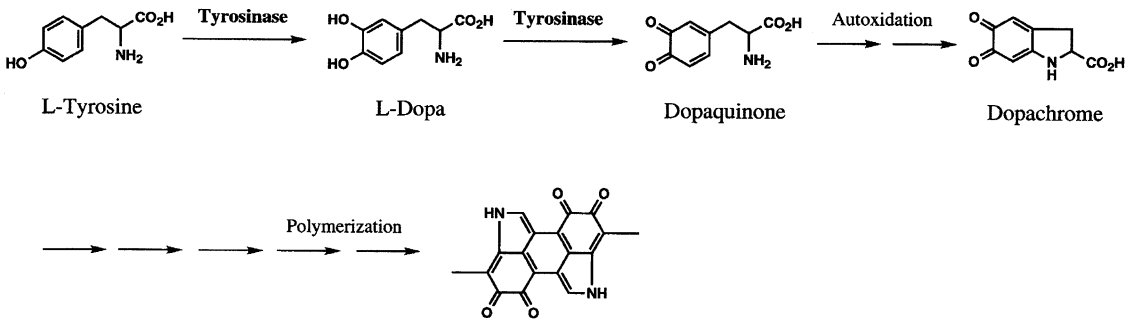


Scheme 3.

min C inhibits melanogenesis by the reduction of dopaquinone to dopa.[9] These processes are summarized in Scheme 4. Therefore we evaluated the inhibitory activity of compounds **5**, **9**, **17**, **22**, and reference compounds vitamin C **1**, ascorbic acid 6-stearate, ascorbic acid 2,6-dipalmitate, APC-3, kojic acid, and arbutin on tyrosinase from mushrooms by using both substrates L-tyrosine and L-dopa *in vitro*.[27] The results of inhibitory activity against tyrosinase-catalyzed oxidation of L-tyrosine are shown in Table 2.

The 2-*O*-glycolylinositol and 2-*O*-(α-tocopheryloxycarbonylmethyl) derivatives **17** and **22** had almost the same inhibitory activity as **1**, APC-3, and arbutin (inhibition > 50%). On the other hand, the inhibitory activity of 2-*O*- and 3-*O*-carboxymethyl derivatives **9** and **5** as well as 2,6-dipalmitate and 6-stearate were slightly lowered, in particular the inhibitory activity of **9** halves (inhibition = 25.2%). Kojic acid was an excellent inhibitor among the test compounds (inhibition = 100%).

The results of inhibitory activity against tyrosinase-catalyzed oxidation of L-dopa are shown in Table 3. The in-



Scheme 4.

Table 1. Stability of the Vitamin C Derivatives in the Aqueous Solution

Vitamin C derivatives[a]	Remaining/%[b]		
	1 month	2 months	3 months
5	50.7	< 1	< 1
9	98.1	95.2	91.4
17	90.8	75.5	39.7
22	90.0	74.7	37.3
Vitamin C **1**[c]	89.2	77.7	37.1
6-Stearate[c]	25.5	17.3	15.5
2,6-Dipalmitate[c]	42.6	22.3	21.7
2-Phosphate Mg-salt[c]	100	98.9	95.5

a) The test compound was dissolved in a solution of $EtOH/H_2O$=1/1 (v/v) to prepare concentration of 1% (w/v). b) The resulting solution was stored at 60 °C, and decrease in concentration was measured by HPLC. c) Reagents (Waco Pure Chemical Industries Ltd.) were used without purification.

Table 2. Inhibitory Activity against Tyrosinase-Catalyzed Oxidation of L-Tyrosine

Compounds[a]	Inhibition/%[b] (10^{-3} M)
5	39.9
9	25.2
17	53.4
22	51.8
Vitamin C **1**[c]	50.1
6-Stearate[c]	31.4
2,6-Dipalmitate[c]	39.7
2-Phosphate Mg-salt[c]	57.1
Kojic acid[d]	100
Arbutin[d]	52.4

a) A 10^{-3} M sample solution was added to 0.3 mg ml^{-1} L-tyrosine aqueous solution in buffer solution (pH 6.8). b) The resulting solution was added to 1.0 mg ml^{-1} tyrosinase aqueous solution, and incubated at 37 °C for 20 min. The absorbance of reaction mixture was measured at 475 nm, and the inhibition was calculated. c) Reagents (Waco Pure Chemical Industries Ltd.) were used without purification. d) Reagents (Tokyo Chemical Industry Co., Ltd.) were used without purification.

hibitory activity was estimated from inhibition at 3×10^{-4} M (M=mol dm^{-3}) and the inhibition concentration 50% (IC_{50}).

Table 3. Inhibitory Activity against Tyrosinase-Catalyzed Oxidation of L-Dopa

Compounds[a]	Inhibition/%[b] (3×10^{-4} M)	$IC_{50}/10^{-3}$ M[c]
5	26.4	7.33
9	19.7	10.00
17	18.5	11.06
22	6.5	>100
Vitamin C **1**[d]	38.2	0.47
6-Stearate[d]	31.5	0.93
2,6-Dipalmitate[d]	18.9	11.68
2-Phosphate Mg-salt[d]	5.5	>100
Kojic acid[e]	59.2	0.18
Arbutin[e]	12.8	20.00

a) 10^{-4}, 3×10^{-4}, and 10^{-3} M sample solutions were added to 0.5 mg ml^{-1} L-dopa aqueous solution in buffer solution (pH 6.8) respectively. b) The resulting solution was added to 1.0 mg ml^{-1} tyrosinase aqueous solution, and incubated at 37 °C for 1 min. The absorbance of reaction mixture was measured at 475 nm, and the inhibition was calculated. c) The inhibition concentration 50% on tyrosinase activity was calculated from the plot of inhibition against log *M*. d) Reagents (Waco Pure Chemical Industries Ltd.) were used without purification. e) Reagents (Tokyo Chemical Industry Co., Ltd.) were used without purification.

All vitamin C derivatives and arbutin had lower inhibitory activity than **1** (inhibition = 38.2%, $IC_{50}=4.7\times10^{-4}$ M), in particular the inhibitory activity of **22** and APC-3 were very low *in vitro* (inhibition < 7%, $IC_{50} > 10^{-1}$ M), although it was reported that APC-3 was a potent inhibitor of tyrosinase through phosphatase catalyzed degradation to free vitamin C *in vivo*,[15d] and arbutin was also a prominent inhibitor of melanogenesis not only in cultured B16 melanoma cells but also in human skin.[28] From these results, it is apparent that vitamin C derivatives modified at either the 2-*O* or 3-*O* position decrease in reduction activity of dopaquinone. On the other hand, kojic acid was an excellent inhibitor among the test compounds (inhibition = 59.2%, $IC_{50}=1.8\times10^{-4}$ M) as reported in the literature.[29]

The reducing activity of compounds **5**, **9**, **17**, **22**, and reference compounds vitamin C **1**, ascorbic acid 6-stearate, ascorbic acid 2,6-dipalmitate, and vitamin E **18** was measured by use of a stable radical, α,α-diphenyl-β-picrylhydr-

azyl (DPPH),[30] and the results are shown in Table 4. At a high concentration (10^{-3} M) corresponding to ten molar amounts to DPPH, all derivatives had almost the same reducing activity as **1** except for 2,6-dipalmitate. But the IC_{50} of compounds **5**, **9**, **17**, and **22** were higher than that of **1**, 6-stearate, and **18** ($IC_{50} < 1.9\times10^{-7}$ M), that is, the reducing activity fell. Especially the 3-*O*-carboxymethyl derivative **5** decreased in reducing activity ($IC_{50} = 8.1\times10^{-7}$ M). Among these derivatives, the 2-*O*-(α-tocopheryloxycarbonylmethyl) derivative **22** maintained the intrinsic activity of vitamin C ($IC_{50} = 2.3\times10^{-7}$ M) caused by the vitamin E effect, however the active site of vitamin E, the hydroxyl group at C-6, was shielded by the ester bond.

The AOS scavenging activities of compounds **5**, **9**, **17**, **22**, and reference compounds vitamin C **1**, ascorbic acid 6-stearate, ascorbic acid 2,6-dipalmitate, APC-3, vitamin E **18**, and SOD were measured by the nitro blue tetrazolium (NBT) method depending on enzymatic superoxide anion generation *in vitro*,[31] and the results are shown in Table 5. SOD (MW≒32000) scavenged AOS generated by the xanthine–xanthine oxidase (XOD) system at 10^{-6} M almost completely (inhibition = 95.6%, $IC_{50} = 8\times10^{-10}$ M). However, the AOS scavenging activity of the other compounds was too low to calculate the IC_{50} at a concentration of less than 10^{-3} M, the AOS scavenging activity of even the most effective scavenger, **18**, was only 11% of that of SOD (inhibition = 10.8%). The AOS scavenging activity of the 2-*O*-(α-tocopheryloxycarbonylmethyl) derivative **22** was slightly higher than **1**, 2,6-dipalmitate (inhibition = 6.1%), which is also caused by the vitamin E effect, though the active site was shielded by the ester bond. Although the 2-*O*-carboxymethyl derivatives, **9** had the same scavenging activity as 6-stearate (inhibition = 4.1%), the AOS scavenging activity of **5**, **17**, and APC-3 was very low (inhibition<1%).

To improve the thermal stability and prevent the diminution of activity of vitamin C simultaneously, we designed and synthesized a novel series of hybrid vitamin C derivatives linking these biologically active substances (glycolic acid, *myo*-inositol, and vitamin E) at C-2 or C-3 hydroxyl group dominating both stability and activity. Among these derivatives, 2-*O*-carboxymethylascorbic acid **9** was thermally stable and was a moderate inhibitor of tyrosinase-catalyzed melanin formation, AOS, and free radicals *in vitro* compared to other typical inhibitors and scavengers. On the other hand, 3-*O*-carboxymethylascorbic acid **5** was very unstable in aqueous solution. The 2-*O*-carbonylmethyl derivatives linking *myo*-inositol or vitamin E were susceptible to degradation, although the vitamin E derivative was a noteworthy inhibitor of AOS and free radicals compared with free vitamin C.

Table 4. Reducing Activity against α,α-Diphenyl-β-picrylhydrazyl (DPPH)

Compounds[a]	Inhibition/%[b] (10^{-3} M)	$IC_{50}/10^{-6}$ M[c]
5	93.8	81.1
9	96.5	44.7
17	94.7	54.6
22	95.1	23.1
Vitamin C **1**[d]	97.2	17.8
6-Stearate[d]	97.0	18.8
2,6-Dipalmitate[d]	68.6	281.8
Vitamin E **18**[d]	97.0	11.6

a) The test compound in DMF was added to 10^{-4} M DPPH in EtOH to prepare 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M sample solutions respectively. b) The resulting solution was stirred at 25 °C for 20 min. The absorbance (OD) of reaction mixture was measured at 517 nm, and the inhibition was calculated. c) The inhibition concentration 50% on DPPH was calculated from the plot of OD against $-\log M$. d) Reagents (Waco Pure Chemical Industries Ltd) were used without purification.

Table 5. Scavenging Activity against Active Oxygen by Nitro Blue Tetrazolium (NBT) Method

Compounds[a]	Inhibition/%[b] (10^{-6} M)
5	0.6
9	4.1
17	<0.1
22	6.1
Vitamin C **1**[c]	5.0
6-Stearate[c]	3.6
2,6-Dipalmitate[c]	5.9
2-Phosphate Mg-salt[c]	0.7
Vitamin E **18**[c]	10.8
SOD (Cu,Zn-form)[c]	95.6

a) 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M sample solutions were added to 3×10^{-3} M xanthine buffer solution (pH 10.2), 7.5×10^{-4} M NBT buffer solution, and 3×10^{-3} M ethylenediaminetetraacetic acid disodium salt aqueous solution respectively. b) The resulting solution was added 1% (v/v) xanthine oxidase suspension in 0.15% (w/v) albumin aqueous solution, and incubated at 25 °C for 20 min. The enzymatic reaction was stopped with 6×10^{-3} M $CuCl_2$ aqueous solution. The absorbance of reaction mixture was measured at 560 nm, and the inhibition was calculated. c) Reagents (Waco Pure Chemical Industries Ltd.) were used without purification.

Experimental

All of the solvents and reagents used were of reagent grade; in cases where further purification was required, the standard procedures were followed.[32] Thin-layer chromatography (TLC) was done on precoated silica-gel $60F_{254}$ plates (Art. 5554, E. Merck). Silica gel (300—200 mesh, Wakogel C-300) was used for silica-gel chromatography, and the ratio of silica gel to compound was in the range of 30 : 1—100 : 1. Elemental analyses were done by the Advanced Center for Chemical Analysis at Ehime University. ^{1}H NMR spectra and ^{13}C NMR spectra were obtained on a JEOL GSX-270 and Varian Gemini-300. ^{1}H NMR spectra were recorded relative to internal tetramethylsilane ($\delta = 0.00$ in $CDCl_3$ or DMSO-d_6) or sodium 3-(trimethylsilyl) propionate-2,2,3,3-d_4 ($\delta = 0.00$ in D_2O). ^{13}C NMR spectra were recorded with $CDCl_3$ ($\delta = 77.00$) as an internal standard. IR spectra were recorded on a Shimadzu IR-460 spectrometer. UV-vis spectra were measured on a Shimadzu UV-1200 spectrometer using a plastic cell of 1 mm pathlength. The melting points were recorded on a Yanaco MP-

500V micro melting point apparatus and were uncorrected. The HPLC analysis was done on a Shim-pack CLC-ODS columns (6 mmϕ × 150 mm length, Shimadzu) with a system consisting of a Shimadzu LC-6A pump, SPD-6A UV spectrophotometric detector, SCL-6A system controller, and C-R4A Chromatopac. The eluent was MeOH/H_2O=95/5 (v/v), flow rate 1.0 ml min^{-1}, and detection was at 254 nm.

5,6-*O*-Isopropylidene-L-ascorbic Acid (2). This was synthesized in 75% yield according to the procedure of Jung et al.,[23] mp 208—210 °C (lit, mp 217—222 °C,[23] 201—203 °C,[13b] 202—204 °C,[19] 204—206 °C[24]); ^{1}H NMR (D_2O) δ = 1.37 (6H, s, acetonide), 4.17 (1H, dd, J_{65} = 5.0, 9.1 Hz), 4.31 (1H, dd, J_{65} = 7.2, 9.1 Hz), 4.59 (1H, ddd, J_{54} = 2.3 Hz, J_{56} = 5.0, 7.3 Hz), 4.91 (1H, d, J_{45} = 2.4 Hz); ^{13}C NMR (D_2O) δ = 26.7 (q), 27.5 (q), 67.8 (t), 75.7 (d), 78.5 (d), 113.5 (s), 120.5 (s), 158.4 (s), 176.1 (s); IR (KBr) 3300, 3000, 1757, 1667, 1100 cm^{-1}.

3- *O*- Benzyloxycarbonylmethyl- 5, 6- *O*- isopropylidene- L-ascorbic Acid (3). A mixture of **2** (19.6 g, 90.7 mmol) and $KHCO_3$ (9.3 g, 92.8 mmol) in DMF (40 ml) was stirred for 10 min at room temperature. Benzyl bromoacetate (20.8 g, 90.6 mmol) was added dropwise, and the mixture was vigorously stirred for 22 h at room temperature. The reaction mixture was diluted with H_2O (5-fold) and extracted with AcOEt. The organic layer was thoroughly washed with brine and dried over anhydrous Na_2SO_4. After the solvent was evaporated in vacuo, the residue was chromatographed (SiO_2, hexane/AcOEt=5/1) to give **3** (26.0 g, 79% yield) as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ = 1.34 (6H, s, acetonide), 4.05 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.17 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.26 (1H, dt J_{54} = 3.7 Hz, J_{56} = 6.7, 8.6 Hz), 4.57 (2H, s, CH_2CO), 4.78 (1H, d, J_{45} = 3.7 Hz), 5.18 (2H, s, benzyl), 7.10—7.40 (5H, m, aromatic). Found: C, 59.99; H, 5.49%. Calcd for $C_{18}H_{20}O_8$: C, 59.34; H, 5.53%.

3-*O*-Benzyloxycarbonylmethyl-L-ascorbic Acid (4). To a solution of **3** (26.0 g, 71.4 mmol) in THF (100 ml) was added 35% HCl (10 ml) at room temperature. The mixture was stirred for 5 h at room temperature. The reaction was quenched by the addition of saturated $NaHCO_3$ solution. An aqueous layer was extracted with AcOEt. The combined layers were washed with brine and dried over anhydrous Na_2SO_4. After the solvent was evaporated in vacuo, the residue was chromatographed (SiO_2, hexane/AcOEt=1/1) to give **4** (8.5 g, 37% yield) as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ = 4.05 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.17 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.21 (1H, d, J_{54} = 3.7 Hz, J_{56} = 6.7, 8.6 Hz), 4.47 (2H, s, CH_2CO), 4.79 (1H, d, J_{45} = 3.7 Hz), 5.18 (2H, s, benzyl), 7.30 (5H, s, aromatic). Found: C, 55.50; H, 5.01%. Calcd for $C_{15}H_{16}O_8$: C, 55.56; H, 4.97%.

3-*O*-Carboxymethyl-L-ascorbic Acid (5). To a solution of **4** (8.5 g, 26.2 mmol) in AcOEt (100 ml) was added 5% Pd/C (0.8 g) at room temperature. The mixture was vigorously stirred for 4 h at room temperature under a hydrogen atmosphere. After the catalyst had been filtered, the filtrate was evaporated in vacuo to dryness. The residue was recrystallized from AcOEt to give **5** (4.8 g, 78% yield) as a colorless solid. Mp 142—144 °C; ^{1}H NMR (DMSO-d_6) δ = 3.42 (1H, dd, J_{65} = 6.7, 8.6 Hz), 3.48 (1H, dd, J_{65} = 6.7, 8.6 Hz), 3.73 (1H, dt, J_{54} = 3.7 Hz, J_{56} = 6.7, 8.6 Hz), 4.81 (2H, s, CH_2CO), 4.89 (1H, dd, J_{45} = 3.7 Hz), 8.70—9.30 (1H, m, CO_2H); ^{13}C NMR (DMSO-d_6) δ = 61.7 (t), 66.7 (t), 68.8 (d), 74.6 (d), 120.1 (s), 149.6 (s), 169.8 (s), 170.2 (s); IR (KBr) 3500, 3000, 1765, 1695, 1120, 1050 cm^{-1}. Found: C, 41.17; H, 4.36%. Calcd for $C_8H_{10}O_8$: C, 41.03; H, 4.30%.

3-*O*-Benzyl-5,6-*O*-isopropylidene-L-ascorbic Acid (6). A mixture of **2** (21.6 g, 99.9 mmol) and $KHCO_3$ (10.2 g, 101.9 mmol) in DMF (50 ml) was stirred for 10 min at room temperature. Benzyl bromide (17.0 g, 99.9 mmol) was added dropwise, and the mixture was vigorously stirred for 12 h at room temperature. The reaction mixture was diluted with H_2O (5-fold) and extracted with AcOEt. The organic layer was thoroughly washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The semisolid product was recrystallized from isopropyl ether to give **6** (14.3 g, 47% yield) as a colorless solid. Mp 105—106 °C (lit,[19,24] mp 105—106 °C); ^{1}H NMR ($CDCl_3$) δ = 1.36 (3H, s, acetonide), 1.39 (3H, s, acetonide), 4.02 (1H, dd, J_{65} = 6.72, 8.54 Hz), 4.10 (1H, dd, J_{65} = 6.72, 8.54 Hz), 4.26 (1H, dt, J_{54} = 3.66, Hz, J_{56} = 6.72, 8.54 Hz), 4.57 (1H, d, J_{45} = 3.66 Hz), 5.52 (2H, two d, benzyl), 7.35—7.42 (5H, m, aromatic); ^{13}C NMR ($CDCl_3$) δ = 25.6 (q), 25.9 (q), 65.3 (t), 73.5 (t), 74.2 (d), 75.9 (d), 110.3 (s), 119.9 (s), 128.2 (d), 128.6 (d), 128.7 (d), 129.7 (d), 130.2 (d), 145.2 (s), 171.8 (s); IR (KBr) 3500, 3000, 1764, 1695, 1120, 1050 cm^{-1}.

3-*O*-Benzyl-2-*O*-benzyloxycarbonylmethyl-5,6-*O*-isopropylidene-L-ascorbic Acid (7). A mixture of **6** (10.0 g, 32.6 mmol) and $KHCO_3$ (3.6 g, 36.0 mmol) in DMF (15 ml) was stirred for 10 min at room temperature. Benzyl bromoacetate (7.0 g, 30.6 mmol) was added dropwise, and the mixture was vigorously stirred for 30 h at room temperature. The reaction mixture was diluted with H_2O (5-fold) and extracted with AcOEt. The organic layer was thoroughly washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was chromatographed (SiO_2, hexane/AcOEt=5/1) to give **7** (10.8 g, 78% yeild) as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ = 1.34 (6H, s, acetonide), 4.05 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.17 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.26 (1H, dt, J_{54} = 3.7 Hz, J_{56} = 6.7, 8.6 Hz), 4.49 (2H, s, CH_2CO), 4.77 (1H, d, J_{45} = 3.7 Hz), 5.18 (2H, s, benzyl), 5.59 (2H, s, benzyl), 7.10—7.40 (10H, m, aromatic). Found: C, 65.99; H, 5.73%. Calcd for $C_{25}H_{26}O_8$: C, 66.07; H, 5.77%.

2-*O*-Carboxymethyl-5,6-*O*-isopropylidene-L-ascorbic Acid (8). To a solution of **7** (10.8 g, 23.8 mmol) in AcOEt (100 ml) was added 5% Pd/C (1.0 g) at room temperature. The mixture was vigorously stirred for 6 h at room temperature under a hydrogen atmosphere. After the catalyst had been filtered, the filtrate was evaporated in vacuo to dryness. The residue was recrystallized from AcOEt to give **8** (6.2 g, 95% yield) as a colorless solid. Mp 170—172 °C; ^{1}H NMR (DMSO-d_6) δ = 1.26 (6H, s, acetonide), 3.92 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.11 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.32 (1H, dt, J_{54} = 3.7 Hz, J_{56} = 6.7, 8.6 Hz), 4.54 (2H, s, CH_2CO), 4.79 (1H, d, J_{45} = 3.7 Hz), 9.0—11.0 (1H, m, CO_2H); ^{13}C NMR (DMSO-d_6) δ = 25.5 (q), 25.9 (q), 62.0 (t), 68.6 (d), 66.9 (t), 73.3 (d), 119.5 (s), 158.3 (s), 169.1 (s), 170.3 (s); IR (KBr) 3500, 3000, 1764, 1695, 1120, 1050 cm^{-1}. Found: C, 48.06; H, 5.21%. Calcd for $C_{11}H_{14}O_8$: C, 48.18; H, 5.15%.

2-*O*-Carboxymethyl-L-ascorbic Acid (9). To a solution of **8** (6.2 g, 22.6 mmol) in THF (50 ml) was added 35% HCl (5 ml) at room temperature. The mixture was stirred for 0.5 h at room temperature. After the solvent was evaporated in vacuo, the residue was recrystallized from MeCN to give **9** (4.2 g, 76% yield) as a colorless solid. Mp 163—165 °C; ^{1}H NMR (DMSO-d_6) δ = 4.02 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.14 (1H, dd, J_{65} = 6.7, 8.6 Hz), 4.49 (1H, dt, J_{54} = 3.7 Hz, J_{56} = 6.7, 8.6 Hz), 4.63 (2H, s, CH_2CO), 4.78 (1H, dd, J_{45} = 3.7 Hz), 10.0—12.0 (1H, m, CO_2H); ^{13}C NMR (DMSO-d_6), δ = 62.8 (t), 63.4 (d), 64.9 (t), 73.1 (d), 117.5 (s), 155.8 (s), 166.5 (s), 168.3 (s); IR (KBr) 3500, 3000, 1764, 1695, 1120, 1050 cm^{-1}. Found: C, 41.21; H, 4.18%. Calcd for $C_8H_{10}O_8$: C, 41.03; H, 4.30%.

DL-2,3-*O*-Cyclohexylidene-*myo*-inositol (11). This was synthesized in 98% yield by the procedure of Wyss et al.,[25] mp 176—

177 °C (lit, mp 174—175 °C,[33] 179—180 °C,[34,35] 181—183 °C,[36] 176—178 °C[37]); ^{1}H NMR (D_2O) δ = 1.20—1.70 (10H, m, cyclohexylidene), 3.24 (1H, t, $J_{54} = J_{56} = 9.45$ Hz), 3.57 (1H, t, $J_{61} = J_{65} = 9.45$ Hz), 3.63 (1H, t, $J_{43} = J_{45} = 9.45$ Hz), 3.85 (1H, dd, $J_{32} = 2.75$ Hz, $J_{34} = 9.45$ Hz), 4.04 (1H, dd, $J_{12} = 2.75$ Hz, $J_{16} = 9.45$ Hz), 4.46 (1H, t, $J_{21} = J_{23} = 2.75$ Hz); ^{13}C NMR (D_2O) δ = 70.4 (C-3), 73.0 (C-4), 73.4 (C-5), 75.9 (C-6), 76.5 (C-2), 78.8 (C-1), 112.0 (acetal).

DL-1,4,5,6-Tetra-*O*-benzyl-*myo*-inositol (12). This was synthesized in 83% yield by the procedure of Wyss et al.,[25] mp 127—128 °C (lit, mp 127.5—128 °C,[25] 127 °C,[38] 126—128 °C[34]); ^{1}H NMR ($CDCl_3$) δ = 3.43 (1H, dd, $J_{32} = 2.75$ Hz, $J_{34} = 9.45$ Hz), 3.46 (1H, dd, $J_{12} = 2.75$ Hz, $J_{16} = 9.45$ Hz), 3.47 (1H, t, $J_{54} = J_{56} = 9.45$ Hz), 3.83 (1H, t, $J_{43} = J_{45} = 9.45$ Hz), 3.97 (1H, t, $J_{61} = J_{65} = 9.45$ Hz), 4.20 (1H, t, $J_{21} = J_{23} = 2.75$ Hz), 4.67—4.97 (8H, m, benzyl), 7.25—7.35 (20H, m, aromatic); ^{13}C NMR ($CDCl_3$) δ = 69.0 (C-2), 71.5 (C-3), 72.5, 75.4, 75.5, 75.8, 79.8 (C-1), 81.1 (C-4), 81.4 (C-6), 83.0 (C-5), 127.5, 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 127.9, 128.0, 128.0, 128.1, 128.3, 128.3, 128.4, 128.4, 128.4, 128.6, 128.8, 135.6, 137.4, 137.5, 137.9, 138.5, 138.5.

1,3,4,5,6-Penta-*O*-benzyl-*myo*-inositol (13). To a mixture of **12** (1.1 g, 2.0 mmol) and benzyl chloride (0.38 g, 3.0 mmol) in benzene (20 ml) was added finely powdered NaOH (1.1 g, 27.0 mmol). The mixture was heated under reflux, and vigorously stirred for 5 h. The reaction mixture was added to H_2O and extracted with ether. The organic layer was thoroughly washed with brine and dried over anhydrous Na_2SO_4. After the solvent had been evaporated in vacuo, the residue was chromatographed (SiO_2, hexane/AcOEt=10/1) to give **13** (1.0 g, 79% yield) as a colorless solid. Mp 128—129 °C (lit, mp 128—129 °C,[37] 128—130 °C[38,39]); ^{1}H NMR ($CDCl_3$) δ = 3.38 (1H, dd, $J_{12} = 2.75$ Hz, $J_{16} = 9.45$ Hz), 3.38 (1H, dd, $J_{32} = 2.75$ Hz, $J_{34} = 9.45$ Hz), 3.45 (1H, t, $J_{54} = J_{56} = 9.45$ Hz), 4.00 (1H, t, $J_{43} = J_{45} = 9.45$ Hz), 4.00 (1H, t, $J_{61} = J_{65} = 9.45$ Hz), 4.22 (1H, t, $J_{21} = J_{23} = 2.75$ Hz), 4.70—4.92 (10H, m, benzyl), 7.15—7.38 (20H, m, aromatic); ^{13}C NMR ($CDCl_3$) δ = 67.3 (C-2), 72.6, 75.8, 79.6 (C-1), 79.6 (C-3), 81.0 (C-4), 81.0 (C-6), 83.0 (C-5), 127.5, 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 127.9, 128.0, 128.0, 128.1, 128.3, 128.3, 128.4, 128.4, 128.4, 128.6, 128.8, 135.6, 137.4, 137.5, 137.9, 138.5, 138.5.

1,3,4,5,6-Penta-*O*-benzyl-2-*O*-bromoacetyl-*myo*-inositol (14). To a mixture of **13** (1.6 g, 2.5 mmol), bromoacetic acid (0.9 g, 6.2 mmol), and 4-dimethylaminopyridine (0.3 g, 2.5 mmol) in THF (10 ml) was added dicyclohexylcarbodiimide (1.3 g, 6.1 mmol). The mixture was vigorously stirred for 3 h at room temperature. After the filtration, the filtrate was added to saturated $NaHCO_3$ solution and extracted with AcOEt. The combined layers were washed with brine and dried over anhydrous Na_2SO_4. After the solvent had been evaporated in vacuo, the residue was chromatographed (SiO_2, hexane/AcOEt=10/1) to give **14** (1.9 g, 99.6% yield) as a colorless solid. Mp 89—90 °C; ^{1}H NMR ($CDCl_3$) δ = 3.42 (1H, dd, $J_{12} = 2.75$ Hz, $J_{16} = 9.45$ Hz), 3.42 (1H, dd, $J_{32} = 2.75$ Hz, $J_{34} = 9.45$ Hz), 3.45 (1H, t, $J_{54} = J_{56} = 9.45$ Hz), 3.80 (1H, t, $J_{43} = J_{45} = 9.45$ Hz), 3.80 (1H, t, $J_{61} = J_{65} = 9.45$ Hz), 4.56 (2H, d, $J = 7.50$ Hz), 4.65—4.92 (10H, m, benzyl) 5.78 (1H, t, $J_{21} = J_{23} = 2.75$ Hz), 7.15—7.36 (20H, m, aromatic), ^{13}C NMR ($CDCl_3$) δ = 66.6 (acetyl), 68.1 (C-2), 72.3, 72.4, 74.0, 74.0, 74.7, 75.9, 75.9, 76.1, 77.9, 78.0 (C-1), 78.0 (C-3), 81.3, (C-4), 81.3 (C-6), 82.8 (C-5), 127.5, 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 127.9, 128.0, 128.0, 128.1, 128.3, 128.3, 128.4, 128.4, 128.4, 128.6, 128.8, 135.6, 137.4, 137.5, 137.9, 138.5, 138.5, 168.2 (C=O).

3-*O*-Benzyl-5,6-*O*-isopropylidene-2-*O*-carbonylmethyl-L-ascorbic Acid Derivative of 1,3,4,5,6-Penta-*O*-benzyl-*myo*-inositol (15). To a mixture of **14** (2.3 g, 3.1 mmol), **6** (1.4 g, 4.6 mmol), and 18-crown-6 ether (1.0 g, 3.7 mmol) in DMF (20 ml) was added $KHCO_3$ (0.5 g, 4.6 mmol). The mixture was vigorously stirred for 24 h at room temperature. The reaction mixture was diluted with H_2O (5-fold) and extracted with AcOEt. The organic layer was thoroughly washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was chromatographed (SiO_2, hexane/AcOEt=5/1) to give **15** (2.3 g, 77% yield) as a colorless semisolid. ^{1}H NMR ($CDCl_3$) δ = 1.33 (3H, s, acetonide), 1.37 (3H, s, acetonide), 3.46 (1H, dd, $J_{12} = 2.44$ Hz, $J_{16} = 9.45$ Hz, inositol), 3.46 (1H, dd, $J_{32} = 2.44$ Hz, $J_{34} = 9.45$ Hz, inositol), 3.48 (1H, t, $J_{54} = J_{56} = 9.45$ Hz, inositol), 3.80 (1H, t, $J_{61} = J_{65} = 9.45$ Hz, inositol), 3.82 (1H, t, $J_{43} = J_{45} = 9.45$ Hz, inositol), 3.97 (2H, dq, $J_{65} = 6.71$, 8.64 Hz), 4.22 (1H, dd, $J_{54} = 3.66$ Hz, $J_{56} = 6.71$, 8.64 Hz), 4.48 (1H, d, $J_{45} = 3.66$ Hz), 4.52 (2H, dd, $J = 4.58$ Hz, CH_2CO), 4.71—4.88 (10H, m, benzyl), 5.64 (2H, d, $J = 7.63$ Hz, benzyl), 5.88 (1H, t, $J_{21} = J_{23} = 2.44$ Hz, inositol), 7.02—7.22 (30H, m, aromatic); ^{13}C NMR ($CDCl_3$) δ = 25.6 (acetonide), 25.9 (acetonide), 65.2 (C-6), 66.6 (acetyl), 68.1 (inositol C-2), 68.2 (C-5), 72.3, 72.4, 74.0, 74.0, 74.7, 75.8 (C-4), 75.9, 76.1, 77.9, 78.0 (inositol C-1), 78.0 (inositol C-3), 81.3 (inositol C-4), 81.3 (inositol C-6), 82.8 (inositol C-5), 110.3 (acetonide), 120.9 (C-2), 127.5, 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 127.9, 128.0, 128.0, 128.1, 128.3, 128.3, 128.4, 128.4, 128.4, 128.6, 128.8, 135.6, 137.4, 137.5, 137.9, 138.5, 138.5, 155.6 (C-3), 168.2 (C=O), 168.6 (C-1). Found: C, 72.71; H, 6.22%. Calcd for $C_{59}H_{60}O_{13}$: C, 72.52; H, 6.19%.

3-*O*-Benzyl-2-*O*-carbonylmethyl-L-ascorbic Acid Derivative of 1,3,4,5,6-Penta-*O*-benzyl-*myo*-inositol (16). To a solution of **15** (1.8 g, 1.8 mmol) in THF (15 ml) was added 35% HCl (1.5 ml) at room temperature. The mixture was stirred for 1.5 h at room temperature. The reaction was quenched by the addition of saturated $NaHCO_3$ solution. An aqueous layer was extracted with AcOEt. The combined layers were washed with brine and dried over anhydrous Na_2SO_4. After the solvent had been evaporated in vacuo, the residue was chromatographed (SiO_2, hexane/AcOEt=1/2) to give **16** (1.7 g, 99% yield) as a colorless solid. Mp 40—42 °C; ^{1}H NMR ($CDCl_3$) δ = 3.49 (1H, dd, $J_{12} = 2.44$ Hz, $J_{16} = 9.45$ Hz, inositol), 3.50 (1H, t, $J_{54} = J_{56} = 9.45$ Hz, inositol), 3.50 (1H, dd, $J_{32} = 2.44$ Hz, $J_{34} = 9.45$ Hz, inositol), 3.62 (2H, d, $J_{65} = 6.72$ Hz), 3.81 (1H, dt, $J_{54} = 3.66$ Hz, $J_{56} = 6.72$ Hz), 3.84 (1H, t, $J_{61} = J_{65} = 9.45$ Hz, inositol), 3.85 (1H, t, $J_{43} = J_{45} = 9.45$ Hz, inositol), 4.52 (1H, d, $J_{45} = 2.44$ Hz), 4.59 (2H, dd, $J = 2.75$ Hz, CH_2CO), 4.71—5.01 (10H, m, benzyl), 5.64 (2H, s, benzyl), 5.89 (1H, t, $J_{21} = J_{23} = 2.44$ Hz, inositol), 7.12—7.18 (30H, s, aromatic); ^{13}C NMR ($CDCl_3$) δ = 62.8 (C-6), 66.5 (acetyl), 68.4 (inositol C-2), 69.7 (C-5), 72.3, 72.4, 74.1, 75.7, (C-4), 75.9, 76.1, 76.5, 78.0 (inositol C-1), 78.0 (inositol C-3), 81.2 (inositol C-4), 81.2 (inositol C-6), 82.9 (inositol C-5), 120.7 (C-2), 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 128.1, 128.3, 128.4, 128.5, 128.7, 128.7, 135.5, 137.4, 137.5, 138.4, 138.5, 138.5, 156.5 (C-3), 168.4 (C=O), 169.0 (C-1). Found: C, 71.71; H, 6.00%. Calcd for $C_{56}H_{56}O_{13}$: C, 71.78; H, 6.02%.

2-*O*-Carbonylmethyl-L-ascorbic Acid Derivative of *myo*-Inositol (17). To a solution of **16** (1.9 g, 2.0 mmol) in MeOH (100 ml) was added 5% Pd/C (0.2 g) at room temperature. The mixture was vigorously stirred for 4 h at room temperature under a hydrogen atmosphere. After the catalyst had been filtered, the filtrate was evaporated in vacuo to dryness and the residue was chromatographed (SiO_2, MeOH) to give **17** (0.8 g, 99% yield) as a colorless solid. Mp 137—139 °C; ^{1}H NMR (DMSO-d_6) δ = 3.49 (1H, dd, $J_{12} = 2.44$ Hz, $J_{16} = 9.45$ Hz, inositol) 3.50 (1H, t, $J_{54} = J_{56} = 9.45$ Hz, inositol), 3.50 (1H, dd, $J_{32} = 2.44$ Hz, $J_{34} = 9.45$ Hz, inositol), 3.62 (2H, d, $J_{65} = 6.72$ Hz), 3.81 (1H, dt, $J_{54} = 3.66$ Hz, $J_{56} = 6.72$ Hz), 3.84 (1H, t,

$J_{61}=J_{65}=9.45$ Hz, inositol), 3.85 (1H, t, $J_{43}=J_{45}=9.45$ Hz, inositol), 4.52 (1H, d, $J_{45}=2.44$ Hz), 4.59 (2H, dd, $J=2.75$ Hz, CH_2CO), 5.89 (1H, t, $J_{21}=J_{23}=2.44$ Hz, inositol); ^{13}C NMR (DMSO-d_6) $\delta=63.4$ (C-6), 67.5 (acetyl), 68.7 (inositol C-2), 70.0 (C-5), 75.2 (C-4), 78.6 (inositol C-1), 78.6 (inositol C-3), 78.7 (inositol C-4), 78.7 (inositol C-6), 79.2 (inositol C-5), 115.6 (C-2), 155.7 (C-3), 169.7 (C=O), 170.5 (C-1). Found: C, 42.51; H, 4.98%. Calcd for $C_{14}H_{20}O_{13}$: C, 42.43; H, 5.09%.

6-*O*-Bromoacetyl-DL-α-tocopherol (19). To a mixture of **18** (12.9 g, 30.0 mmol), bromoacetic acid (4.2 g, 30.0 mmol), and 4-dimethylaminopyridine (0.4 g, 3.0 mmol) in dioxane (50 ml) was added dicyclohexylcarbodiimide (6.2 g, 30.0 mmol). The mixture was vigorously stirred for 15 h at room temperature. After the filtration, the filtrate was added to saturated $NaHCO_3$ solution and extracted with AcOEt. The combined layers were washed with brine and dried over anhydrous Na_2SO_4. After the solvent was evaporated in vacuo, the residue was chromatographed (SiO_2, hexane/AcOEt=50/1) to give **19** (16.0 g, 97% yield) as a yellow liquid. ^{1}H NMR ($CDCl_3$) $\delta=0.86$ (15H, d, $J=6.00$ Hz, CH_3), 1.00—1.71 (23H, m, CH_2, CH), 2.01 (6H, d, benzyl), 2.10 (3H, s, benzyl), 2.62 (2H, t, benzyl), 4.02 (2H, s, CH_2CO); IR (neat) 2925, 1748, 1462, 1241, 1101 cm^{-1}.

3-*O*-Benzyl-5,6-*O*-isopropylidene-2-*O*-(DL-α-tocopheryloxycarbonylmethyl)-L-ascorbic Acid (20). To a mixture of **19** (25.0 g, 45.3 mmol), **6** (25.0 g, 81.6 mmol), and 18-crown-6 ether (2.7 g, 10.0 mmol) in DMF (160 ml) was added $KHCO_3$ (5.0 g, 50.2 mmol). The mixture was vigorously stirred for 48 h at room temperature. The reaction mixture was diluted with H_2O (5-fold) and extracted with AcOEt. The organic layer was thoroughly washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo. The residue was chromatographed (SiO_2, hexane/AcOEt=50/1) to give **20** (26.1 g, 74% yield) as a yellow liquid. ^{1}H NMR ($CDCl_3$) $\delta=0.86$ (15H, d, $J=6.00$ Hz, CH_3), 1.00—1.71 (23H, m, CH_2, CH), 1.35 (6H, s, acetonide), 2.01 (6H, d, benzyl), 2.10 (3H, s, benzyl), 2.62 (2H, t, benzyl), 3.98 (2H, dq, $J_{65}=6.71$, 8.64 Hz), 4.18 (1H, dd, $J_{54}=3.66$ Hz, $J_{56}=6.71$, 8.64 Hz), 4.51 (1H, d, $J_{45}=3.66$ Hz), 5.12 (2H, dd, $J=4.58$ Hz, CH_2CO), 5.66 (2H, s, benzyl), 7.29 (5H, s, aromatic); IR (neat) 1773, 1686, 1461, 1379, 1331, 1251, 1151 cm^{-1}. Found: C, 72.73; H, 8.90%. Calcd for $C_{47}H_{68}O_9$: C, 72.65; H, 8.82%.

3-*O*-Benzyl-2-*O*-(DL-α-tocopheryloxycarbonylmethyl)-L-ascorbic Acid (21). To a solution of **20** (26.3 g, 33.8 mmol) in dioxane (150 ml) was added 35% HCl (15 ml) at room temperature. The mixture was stirred for 5 h at room temperature. The reaction was quenched by the addition of saturated $NaHCO_3$ solution. An aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4. After the solvent had been evaporated in vacuo, the residue was chromatographed (SiO_2, hexane/AcOEt=4/1) to give **21** (24.4 g, 98% yield) as a colorless solid. Mp 40—41 °C; ^{1}H NMR ($CDCl_3$) $\delta=0.82$—0.84 (15H, m, CH_3), 0.95—1.87 (23H, m, CH_2, CH), 2.01 (6H, d, benzyl), 2.10 (3H, s, benzyl), 2.62 (2H, t, benzyl), 3.78 (2H, dq, $J_{65}=6.71$, 8.64 Hz), 4.00 (1H, dd, $J_{54}=3.66$ Hz, $J_{56}=6.71$, 8.64 Hz), 4.75 (1H, d, $J_{45}=3.66$ Hz), 5.18 (2H, d, CH_2CO), 5.69 (2H, s, benzyl), 7.40 (5H, s, aromatic); ^{13}C NMR ($CDCl_3$) $\delta=11.9$ (q, tocopherol C-5-Me), 12.2 (q, tocopherol C-7-Me), 13.1 (q, tocopherol C-8-Me), 19.8 (q, tocopherol C-4′-Me), 19.8 (q, tocopherol C-8′-Me), 20.7 (t, tocopherol C-4), 21.1 (t, tocopherol C-2′), 22.7 (q, tocopherol C-13′), 22.8 (q, tocopherol C-12′-Me), 23.9 (q, tocopherol C-2-Me), 24.5 (t, tocopherol C-6′), 24.9 (t, tocopherol C-10′), 28.1 (d, tocopherol C-12′), 31.1 (t, tocopherol C-3), 32.8 (d, tocopherol C-4′), 32.9 (d, tocopherol C-8′), 37.4 (t, tocopherol C-11′), 37.5 (t, tocopherol C-3′, 5′, 7′, 9′), 39.5 (t, tocopherol C-1′), 63.0 (t, C-6), 66.2 (t, acetyl), 70.1 (d, C-5), 75.3 (s, tocopherol C-2), 76.0 (d, C-4), 117.7 (s, tocopherol C-7), 120.6 (s, C-2), 123.4 (s, tocopherol C-8), 124.9 (s, tocopherol C-5), 126.6 (s, tocopherol C-4a), 128.2 (d, Ph), 128.8 (d, Ph), 128.9 (d, Ph), 135.6 (s, Ph), 139.8 (s, tocopherol C-6), 149.9 (s, tocopherol C-8a), 156.5 (s, C-3), 168.4 (s, C=O), 168.7 (s, C-1); IR (KBr) 3240, 2925, 1769, 1670, 1461, 1329, 1156 cm^{-1}. Found: C, 71.83; H, 8.79%. Calcd for $C_{44}H_{64}O_9$: C, 71.71; H, 8.75%.

2-*O*-(DL-α-Tocopheryloxycarbonylmethyl)-L-ascorbic Acid (22). To a solution of **21** (17.1 g, 23.3 mmol) in AcOEt (300 ml) was added 5% Pd/C (7.5 g) at room temperature. The mixture was vigorously stirred for 4 h at room temperature under a hydrogen atmosphere. After the catalyst had been filtered, the filtrate was evaporated in vacuo to dryness. The residue was chromatographed (SiO_2, benzene/AcOEt=5/1) to give **22** (14.1 g, 94% yield) as a colorless solid. Mp 105—107 °C; ^{1}H NMR ($CDCl_3$) $\delta=0.82$—0.84 (15H, m, CH_3), 0.95—1.82 (23H, m, CH_2, CH), 1.95 (6H, d, benzyl), 2.09 (3H, s, benzyl), 2.58 (2H, t, benzyl), 3.79 (2H, dq, $J_{65}=6.71$, 8.64 Hz), 4.03 (1H, dd, $J_{54}=3.66$ Hz, $J_{56}=6.71$, 8.64 Hz), 4.75 (1H, d, $J_{45}=3.66$ Hz), 4.94 (2H, d, CH_2CO); ^{13}C NMR ($CDCl_3$) $\delta=11.9$ (q, tocopherol C-5-Me), 12.1 (q, tocopherol C-7-Me), 13.0 (q, tocopherol C-8-Me), 19.7 (q, tocopherol C-4′-Me), 19.8 (q, tocopherol C-8′-Me), 20.6 (t, tocopherol C-4), 21.1 (t, tocopherol C-2′), 22.7 (q, tocopherol C-13′), 22.8 (q, tocopherol C-12′-Me, 2-Me), 24.5 (t, tocopherol C-6′), 24.9 (t, tocopherol C-10′), 28.0 (d, tocopherol C-12′), 31.0 (t, tocopherol C-3), 32.8 (d, tocopherol C-4′), 32.9 (d, tocopherol C-8′), 37.4 (t, tocopherol C-11′), 37.5 (t, tocopherol C-3′, 5′, 7′, 9′), 39.4 (t, tocopherol C-1′), 63.3 (t, C-6), 68.1 (t, acetyl), 70.0 (d, C-5), 75.3 (s, tocopherol C-2), 76.1 (d, C-4), 117.8 (s, tocopherol C-7), 121.1 (s, C-2), 123.4 (s, tocopherol C-8), 124.8 (s, tocopherol C-5), 126.4 (s, tocopherol C-4a), 139.8 (s, tocopherol C-6), 150.0 (s, tocopherol C-8a), 160.1 (s, C-3), 169.4 (s, C=O), 171.3 (s, C-1); IR (KBr) 3380, 2920, 1755, 1673, 1462, 1205, 1150 cm^{-1}. Found: C, 69.01; H, 9.13%. Calcd for $C_{37}H_{58}O_9$: C, 68.70; H, 9.04%.

Physicochemical and Biological Experiments. Measurement of the Thermal Stability in Aqueous Solution. The test compound was dissolved in a solution of EtOH/H_2O=1/1 (v/v) to prepare concentration of 1% (w/v) solution. The resulting solution was stored at 60 °C, and 0.05 ml samples were taken at 1 month intervals for 3 months. The concentration of the test compounds was measured by HPLC. The difference from initial concentration was taken as the remaining ratio. The results are shown in Table 1.

Measurement of the Inhibitory Activity against Tyrosinase-Catalyzed Oxidation of L-Tyrosine.[27] A mixture containing 10^{-3} M test compound aqueous solution (0.5 ml), 0.3 mg ml^{-1} L-tyrosine (0.5 ml) aqueous solution, and potassium phosphate buffer solution (pH 6.8, 2.0 ml) was heated for 10 min at 37 °C. Tyrosinase (1.0 mg ml^{-1}) from mushrooms (2000 units/mg) aqueous solution (0.02 ml) was added to the above mixture, and incubated at 37 °C. After 20 min, the absorbance of reaction mixture was measured at 475 nm, the maximum absorbance of produced dopachrome. The ratio of inhibitory activity was estimated with following equation. The results are shown in Table 2.

$$\text{Inhibition}(\%) = [(\text{control OD}) - (\text{sample OD})]/[(\text{control OD}) - (\text{blank OD})] \times 100$$

Measurement of the Inhibitory Activity against Tyrosinase-Catalyzed Oxidation of L-Dopa.[27] To the mixture of L-dopa (0.5 mg ml^{-1}) aqueous solution (0.5 ml), and potassium phosphate buffer solution (pH 6.8, 2.0 ml) was added 10^{-4}, 3×10^{-4}, and 10^{-3}

M aqueous solution of the test compound (0.2 ml) respectively, and the mixture was heated for 5 min at 37 °C. Tyrosinase (1.0 mg ml^{-1}) from mushrooms (2000 units/mg) aqueous solution (0.05 ml) was added to the above mixture, and incubated at 37 °C. After 1 min, the absorbance of reaction mixture was measured at 475 nm, the maximum absorbance of produced dopachrome. The ratio of inhibitory activity was estimated with the above equation. The results are shown in Table 3.

Measurement of the Reducing Activity of the Stable Radical α,α-Diphenyl-β-picrylhydrazyl (DPPH).[30] The test compound in DMF (3 ml) was added to a solution of DPPH (10^{-4} M) in EtOH to prepare concentrations of 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M sample solutions respectively. After being reacted for 20 min at 25 °C, the absorbance at 517 nm was measured. The difference in absorbance from control, which the test compound was absent, was taken as the reducing activity. The results are shown in Table 4.

Measurement of the Active Oxygen Scavenging Activity. Nitro Blue Tetrazolium (NBT) Method.[31] To a mixture containing 3×10^{-3} M xanthine solution in sodium carbonate buffer solution (pH 10.2, 0.1 ml), 7.5×10^{-4} M NBT aqueous solution (0.1 ml), sodium carbonate buffer solution (pH 10.2, 0.1 ml) and 3×10^{-3} M ethylenediaminetetraacetic acid disodium salt (EDTA) aqueous solution (0.1 ml) was added 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M aqueous solution of the test compound (0.1 ml) respectively. Further, a mixture containing 1% (v/v) xanthine oxidase (XOD) suspension from buttermilk (≧0.5 units/mg protein) in 0.15% (w/v) albumin from bovine serum (BSA) aqueous solution (1.6×10^{-2} ml) was added to the above mixture, and incubated at 25 °C. The enzymatic reaction was stopped at the end of 20 min with 6 mM $CuCl_2$ aqueous solution (0.1 ml), and the absorbance of the reaction mixture was measured at 560 nm, the maximum absorbance of produced diformazan. The difference in absorbance from control, which the test compound was absent, was taken as the scavenging activity. The results are shown in Table 5.

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