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Synthesis of novel vitamin C phosphodiesters: Stability and antioxidant activity

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Abstract

A novel series of hybrid L-ascorbic acid (vitamin C) phosphodiesters linked at the C-2 hydroxyl group with other biologically active substances, namely *myo*-inositol, arbutin, 4-hydroxy-L-proline, and glycolic acid were synthesized, and their thermal stability and reducing activity against free radicals were estimated in vitro. All of the phosphodiesters exhibited high thermal stabilities; however, their antioxidant activities in vitro were generally lower than that of vitamin C. © 1996 Elsevier Science Ltd.

Keywords: Vitamin C derivatives; Antioxidants; Ascorbic acid phosphodiesters

1. Introduction

It is known that L-ascorbic acid (vitamin C) scavenges active oxygen species and free radicals as a chain-breaking antioxidant [1]. Furthermore, there is considerable evidence that vitamin C plays an important role in the prevention of a large number of chronic diseases such as cancer, cerebral apoplexy, diabetes, atopic dermatitis, myocardial infarction, and AIDS [2]. These characteristic biological activities of vitamin C result from its enediol structure, which manifests a strong electron-donating ability. The well-known susceptibility of vitamin C to thermal and oxidative degradation has focused interest in derivatives of increased stability. The chemical modification of hydroxyl groups of vitamin C is of particular interest, and numerous stable derivatives of vitamin C have been reported [3–10]. In general, partial modification of the enediol system leads to two isomers, both of which have markedly lower reducing power and are therefore

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stabilized against oxidation. Additionally, vitamin C activities tend to decrease in proportion to the increase in the number of substituents in the molecule [11]. Among these derivatives, the phosphate esters of vitamin C serve as hydrophilic antioxidants, showing vitamin C activity through enzymic degradation to free vitamin C in vivo, and increased stability toward alkali, oxidation, and prolonged storage [4]. In particular, the magnesium salt of ascorbic acid 2-phosphate (APC-3) has been widely used as a bleaching ingredient for cosmetics [3,5,11]. Furthermore, APC-3 derivatives combined with α-tocopherol (vitamin E) [12] or 4-hydroxy-L-proline [13] have also been developed.

It is well known that *myo*-inositol phosphatides (IP) act in biomembranes as intracellular second messengers [14]. In addition, it is also known that 4-hydroxy-L-proline is a constituent of collagen. It has been reported that arbutin (hydroquinone β-D-glucopyranoside) exhibits marked inhibitory activity on melanogenesis in human skin [15]. Furthermore, α-hydroxy acids, especially glycolic acid, repair wrinkles and revitalize cells through proliferation of dermal fibroblast [16].

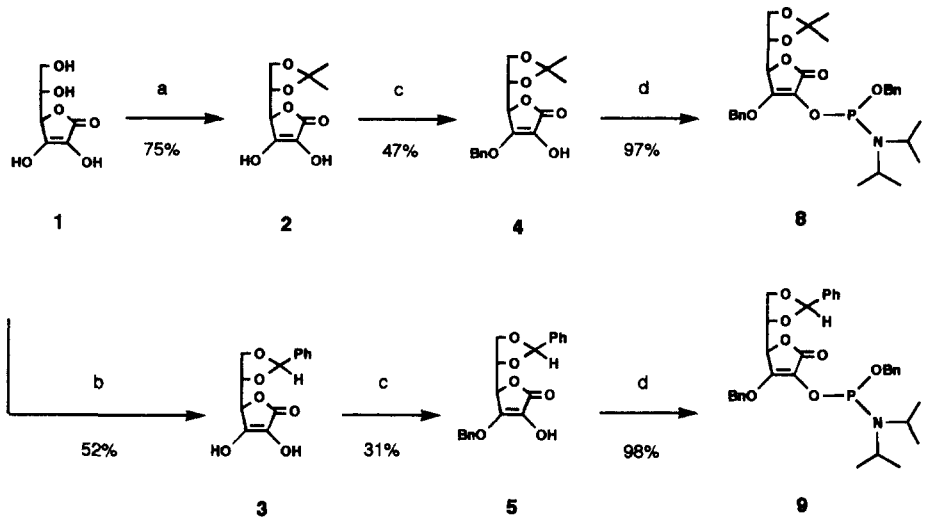
Based on this background, we designed and synthesized a novel series of hybrid vitamin C phosphodiesters linked with other biologically active substances (*myo*-inositol, arbutin, 4-hydroxy-L-proline, and glycolic acid) at the C-2 hydroxyl group with the aim to simultaneously improve the stability and prevent the diminution of activity, and estimated their thermal stability and reducing activity against free radicals in vitro.

2. Results and discussion

In general, phosphorylation of vitamin C with $POCl_3$ under basic conditions affords a mixture consisting of four phosphates, L-ascorbic acid 2-phosphate (APC-3), 3-phosphate, 2-pyrophosphate, and bis(L-ascorbic acid) 2,2′-phosphate [17]. However, the application of modified phosphoramidites has become the most popular method for syntheses of oligonucleotide and nucleopeptide analogues. In the phosphoramidite approach, benzyloxybis(*N*,*N*-diisopropylamino)phosphine (**7**) has been widely used as a bifunctional phosphorylating agent [18–20]. This method was considered suitable instead of the $POCl_3$ method for introducing the phosphate diester linkage between vitamin C and other biologically active substances having hydroxyl groups.

The diamidite **7** was prepared in two steps from $POCl_3$ by King's method [21] followed by Elie's method [18].

L-Ascorbic acid 2-phosphoramidites **8** and **9** were synthesized as shown in Scheme 1. The 5,6-*O*-isopropylidene derivative **8** was prepared in three steps from vitamin C (**1**). The hydroxyl groups at C-5 and C-6 were acetonated by Jung's method to yield 5,6-*O*-isopropylideneascorbic acid (**2**) in 75% yield [22]. The 5,6-*O*-isopropylidene derivative **2** was alkylated with benzyl bromide in the presence of $KHCO_3$ in DMF to give the 3-*O*-benzyl intermediate **4** in 47% yield. Subsequently **4** was condensed with the diamidite **7** in the presence of 1*H*-tetrazole in CH_2Cl_2 to yield the 2-phosphoramidite **8** almost quantitatively. The 5,6-*O*-benzylidene derivative **9** was synthesized in three steps from **1**. The hydroxyl groups at C-5 and C-6 were protected as the benzylidene acetal by Kochi's method to yield diastereomeric 5,6-*O*-benzylideneas-

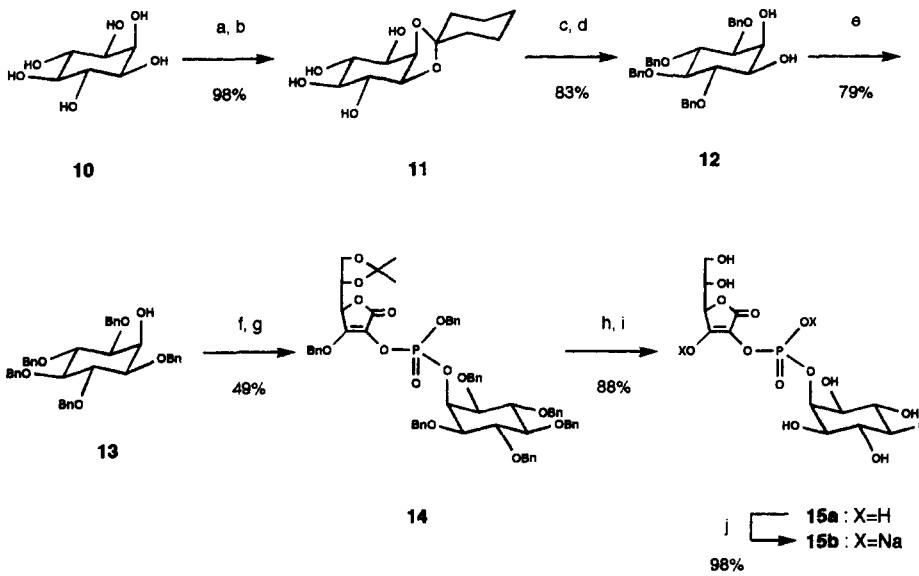


Scheme 1. (a) AcCl, 0.1 equiv; acetone, room temperature, 4 h. (b) $PhCH(OMe)_2$, 1 equiv; p-TsOH, 0.1 equiv; DMF, 55–65 °C, 5 h. (c) BnBr, 1 equiv; $KHCO_3$, 1 equiv; room temperature, 1 day. (d) $(i\text{-}Pr_2N)_2POBn$ (**7**), 1 equiv; 1*H*-tetrazole, 1 equiv; CH_2Cl_2, room temperature, 1 h.

corbic acids (**3**) in 52% yield [23]. Similarly **3** was alkylated with benzyl bromide to afford the 3-*O*-benzyl intermediate **5** in 31% yield, followed by condensation with **7** to give the 2-phosphoramidite **9** nearly quantitatively.

The *myo*-inositol derivative **15b** was synthesized in ten steps from *myo*-inositol (**10**) as shown in Scheme 2. The hydroxyl groups at C-1 and C-2 were protected as the cyclohexylidene ketal to yield **11** in 98% yield, and the C-3, C-4, C-5, and C-6 hydroxyl groups of **11** were protected as benzyl ethers. Acid hydrolysis by Massy's method [24] gave DL-1,4,5,6-tetra-*O*-benzylinositol (**12**) in 83% yield. Selective benzyl protection of the C-3 hydroxyl group was achieved by using benzyl chloride in the presence of NaOH in benzene under reflux [25] to give *meso*-1,3,4,5,6-penta-*O*-benzylinositol (**13**) in 79% yield plus a slight amount of 1,2,3,4,5,6-hexa-*O*-benzylinositol (**13a**, 0.7% yield). Angyal has reported that benzylation of **12** in the presence of KOH gave **13**, **13a**, and DL-1,2,4,5,6-penta-*O*-benzylinositol in 53, 4, and 0.5% yields, respectively [26]. Condensation of **13** with the amidite **8** in the presence of 1*H*-tetrazole in CH_2Cl_2, followed by oxidation by 4-chloroperoxybenzoic acid (mCPBA) in situ, gave the diastereomic phosphodiesters **14** in 49% yield. Condensation of **13** with **8** did not proceed smoothly, and resulted in a low yield of the coupling product even after extended reaction (4 days). The low reactivity indicates that the phosphorylation site is the thermodynamically unfavored axial hydroxyl group, which is restricted in conformational changes by five equatorial benzyloxy groups. Removal of the protecting groups by acid hydrolysis followed by catalytic hydrogenation afforded the 2-*O*-(*myo*-inositol-2-phosphoryl) derivative **15a** in 88% yield. Finally, **15a** was passed through an ion-exchange column to yield the corresponding disodium salt **15b** almost quantitatively.

The arbutin derivative **21b** was synthesized in eight steps from arbutin (**16**) as shown

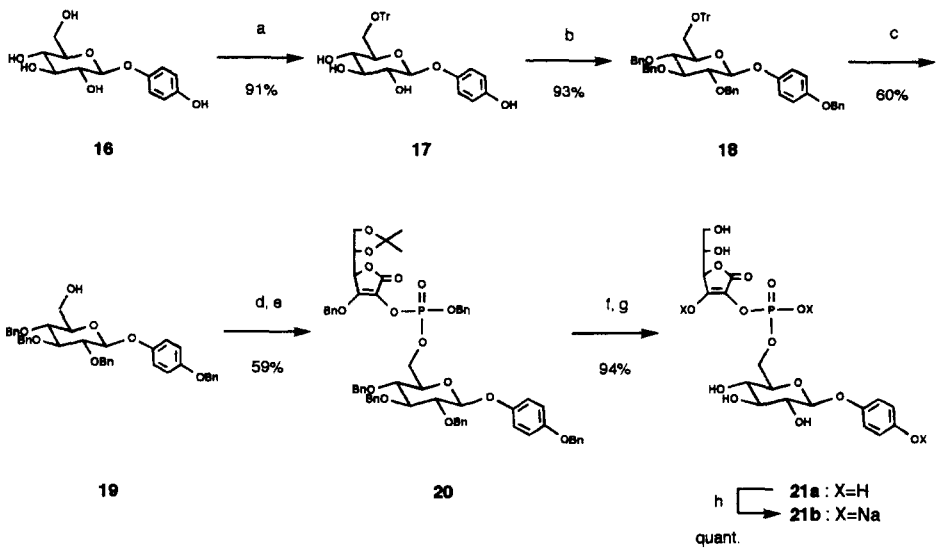


Scheme 2. (a) cyclohexanone, 1.5 equiv; p-TsOH, 0.1 equiv; DMF–toluene, 122–136 °C, 10 h. (b) p-TsOH, EtOH, room temperature, 2 days. (c) BnCl, 11 equiv; KOH, 13 equiv; 115–131 °C, 2 h. (d) 80% AcOH, 80–105 °C, 4 h. (e) BnCl, 1.6 equiv; NaOH, 14 equiv; benzene, reflux, 5 h. (f) **8**, 1 equiv; 1*H*-tetrazole, 2 equiv; CH_2Cl_2, room temperature, 4 days. (g) mCPBA, 2 equiv; CH_2Cl_2, 0 °C, 1 h. (h) 35% HCl, THF, room temperature, 1 h. (i) H_2, 5% Pd/C, MeOH, room temperature, 1.5 h. (j) DIAION SK1B (Na).

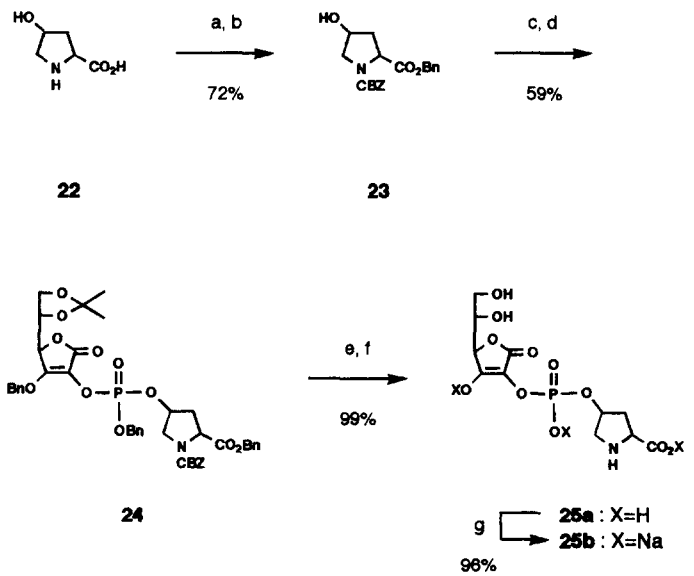
in Scheme 3. The hydroxyl group at C-6 was protected as the trityl ether to yield **17** in 91% yield. The C-2, C-3, C-4, and C-4′ hydroxyl groups of **17** were protected as benzyl ethers to afford **18** in 93% yield, and subsequent acid hydrolysis to give 2,3,4,4′-tetra-*O*-benzylarbutin (**19**) in 60% yield. Condensation of **19** with the amidite **8**, followed by oxidation by mCPBA in situ, gave the diastereomeric phosphodiesters **20** in 59% yield. Removal of the protecting groups by acid hydrolysis, followed by catalytic hydrogenation, afforded the 2-*O*-(arbutin-6-phosphoryl) derivative **21a** in 94% yield. Finally, **21a** was obtained as the corresponding trisodium salt **21b** in quantitative yield.

The 4-hydroxy-L-proline derivative **25b** was synthesized in seven steps from 4-hydroxy-L-proline (**22**) as shown in Scheme 4. The amino group was protected as benzyl carbamate (CBZ) and then the carboxyl group at C-2 was protected as its benzyl ester to give the 1-*N*-benzyloxycarbonyl-2-benzyl ester derivative **23** in 72% yield by Barrett's method [27]. Condensation of **23** with amidite **8**, followed by oxidation by mCPBA in situ, gave the diastereomeric phosphodiesters **24** in 59% yield. Removal of the protecting groups by acid hydrolysis, followed by a catalytic hydrogenation, afforded the 2-*O*-(L-proline-4-phosphonooxy) derivative **25a** almost quantitatively. Finally **25a** was converted nearly quantitatively into its to trisodium salt **25b** by passing it through ion-exchange resin.

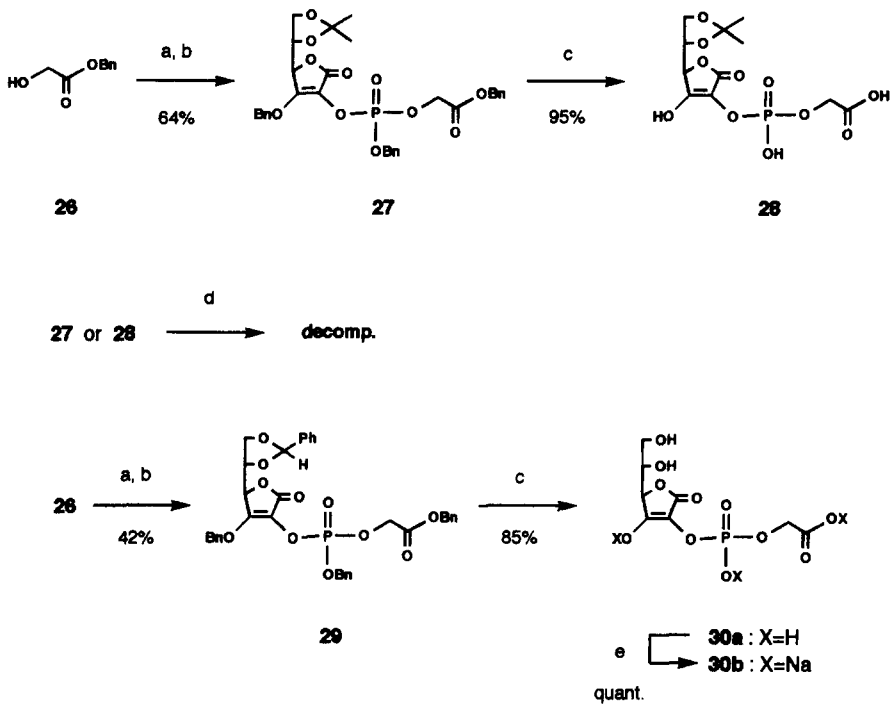
Synthesis of the glycolic acid derivative **30b** was attempted according to the same method by using glycolic acid benzyl ester (**26**), as shown in Scheme 5. However, the



Scheme 3. (a) Ph_3CCl, 1 equiv; Py, room temperature, 1 day. (b) BnCl, 4.4 equiv; NaOH, 5 equiv; Me_2SO, room temperature, 7 h. (c) 35% HCl, MeOH, room temperature, 23 h. (d) **8**, 1 equiv; 1*H*-tetrazole, 2 equiv; CH_2Cl_2, room temperature, 22 h. (e) mCPBA, 1.5 equiv; CH_2Cl_2, 0 °C, 1 h. (f) 35% HCl, THF, room temperature, 2 h. (g) H_2, 5% Pd/C, MeOH, room temperature, 4 h. (h) DIAION SK1B (Na).



Scheme 4. (a) BnOCOCl, 1.1 equiv; 1 N Na_2CO_3, THF, 0 °C, 1 day. (b) BnBr, 1.1 equiv; Et_3N, 1.2 equiv; acetone, room temperature, 2 days. (c) **8**, 1 equiv; 1*H*-tetrazole, 2 equiv; CH_2Cl_2, room temperature, 22 h. (d) mCPBA, 2 equiv; CH_2Cl_2, 0 °C, 2 h. (e) 35% HCl, THF, room temperature, 2 h. (f) H_2, 5% Pd/C, MeOH, room temperature, 4 h. (g) DIAION SK1B (Na).



Scheme 5. (a) **8** or **9**, 1 equiv; 1 *H*-tetrazole, 2 equiv; CH_2Cl_2, room temperature, 2.5 h. (b) mCPBA, 2 equiv; CH_2Cl_2, 0 °C, 1 h. (c) H_2, 5% Pd/C, MeOH, room temperature, 4 h. (d) 1 N HCl, THF, room temperature, 5 h; or 80% AcOH, room temperature, 1 day; or DIAION SK1B (H), 80% MeOH, room temperature, 3 days. (e) DIAION SK1B (Na).

intermediates **27** and **28** decomposed during the process of acid-catalyzed deprotection (1 N HCl, 80% AcOH, or ion-exchange resin). Condensation of **26** was then achieved with the benzylidene compound **9** instead of the isopropylidene derivative **8**. Successive oxidation by mCPBA in situ gave the phosphodiester **29** in 42% yield as diastereomers. Removal of the protecting groups by a catalytic hydrogenation afforded the 2-*O*-(carboxymethylphosphonooxy) derivative **30a** in 85% yield. Finally, **30a** was obtained quantitatively as the corresponding trosodium salt **30b**.

The thermal stabilities of the former sodium salts **15b**, **21b**, **25b**, and **30b** were tested with 1% (w/v) of the test compounds in 1:1 (v/v) EtOH–H_2O at 60°C, and were compared with those of the reference compounds vitamin C (**1**) and ascorbic acid 2-phosphate magnesium salt (APC-3) under the same conditions. The stabilities of these compounds were estimated on the basis of the remaining ratio as measured by HPLC after 3 months, and the results are shown in Table 1. It was found that all of the phosphodiesters were more stable than **1** (37% remaining after 3 months) and that the stabilities of these compounds were almost equal to that of APC-3, more than 90% of which remained intact after 3 months.

The antioxidant activities of phosphodiesters **15a**, **21a**, **25a**, and **30a**, and the

Table 1
Stability of the vitamin C derivatives in aqueous solution

Vitamin C derivatives [a]	Remaining (%) [b]		
	1 month	2 months	3 months
15b	99.8	99.2	98.7
21b	99.7	99.0	98.4
25b	99.9	99.5	98.9
30b	99.1	95.1	91.7
APC-3 [c]	100	98.9	95.5
1 [c]	89.2	77.7	37.1

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

reference compounds **1** and vitamin E (α-tocopherol) were measured by use of a stable radical α,α-diphenyl-β-picrylhydrazyl (DPPH) in vitro [28], and the results are shown in Table 2. At a high concentration (10^{-3} M) corresponding to ten molar amounts to DPPH, all phosphodiesters exhibited almost the same reducing activity as **1** (inhibition > 90%). However, the IC_{50} (inhibition concn 50%) of all phosphodiesters was much higher ($IC_{50} > 10^{-4}$ M) than that of **1** and vitamin E ($IC_{50} < 2 \times 10^{-5}$ M). It was found that vitamin C derivatives modified by phosphates decreased the reducing activity of free radicals in vitro. These phosphodiesters appear to generate the inherent antioxidant activity through enzymatic cleavage to free vitamin C by phosphatase in vivo.

To improve the stability and prevent the simultaneous diminution of antioxidant activity of vitamin C, we synthesized a novel series of hybrid vitamin C phosphodiesters linked at the C-2 hydroxyl group with various biologically active substances (*myo*-inositol, arbutin, 4-hydroxy-L-proline, and glycolic acid) to alter their stability and biological activity. Although all phosphodiesters showed improved thermal stability in

Table 2
Reducing activity against α,α-diphenyl-β-picrylhydrazyl (DPPH)

Compounds [a]	IC_{50} (10^{-6} M) [b]
15a	102.9
21a	158.5
25a	149.6
30a	158.5
1 [c]	17.8
Vitamin E [c]	11.6

[a] The test compound dissolved 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. After the resulting solution had been stirred at 25 °C for 20 min, the absorbance (OD) of the reaction mixture was measured by HPLC at 517 nm.
[b] The inhibition concn 50% on DPPH was calculated from the plot of OD against $-\log M$.
[c] Reagents (Waco Pure Chemical Industries Ltd.) were used without purification.

comparison with the conventional stable vitamin C derivative APC-3, their antioxidant activities were lower than the inherent activity of vitamin C in vitro.

3. Experimental

General methods.—All solvents and reagents used were of reagent grade; where further purification was required, standard procedures were followed [27]. Thin-layer chromatography (TLC) was performed 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 30:1–100:1. Elemental analyses were performed by the Advanced Center for Chemical Analysis at Ehime University. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were obtained with JEOL GSX-270 and Varian Gemini-300 spectrometers. ^{1}H NMR spectra were recorded relative to internal tetramethylsilane ($\delta = 0.00$ in $CDCl_3$ or Me_2SO-d_6) or sodium 4,4-dimethyl 4-silapenfanoate-2,2,3,3-d_4 ($\delta = 0.00$ in D_2O). ^{13}C NMR spectra were recorded with $CHCl_3$ ($\delta = 77.00$) as the internal standard. ^{31}P NMR spectra were recorded with phosphoric acid as the external standard. IR spectra were recorded on a Shimadzu IR-460 spectrometer. Visible 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 are uncorrected. The HPLC analyses were performed on Shim-pack CLC-ODS columns (6 mm diam × 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 19:1 (v/v) MeOH–H_2O, the flow rate 1.0 mL/min, and the detection was at 254 nm.

5,6-O-Isopropylidene-L-ascorbic acid (**2**).—This was synthesized in 75% yield by Jung's method [22]; mp 208–210 °C (lit. mp 217–222 °C [22], 202–204 °C [7], 201–203 °C [8], 204–206 °C [9]).

5,6-O-Benzylidene-L-ascorbic acid (**3**).—This was synthesized in 52% yield by Kochi's method [23]; mp 162–164 °C (lit. mp 166–168 °C [23]).

Benzylation of **2** *and* **3**.—A mixture of **2** or **3** (10.0 mmol) and $KHCO_3$ (1.0 g, 10.2 mmol) in DMF (5 mL) was stirred for 10 min at room temperature. Benzyl bromide (1.7 g, 10.0 mmol) was added dropwise, and the mixture was vigorously stirred for 24 h at room temperature. The mixture was diluted with H_2O (5-fold) and extracted with EtOAc. The organic layer was thoroughly washed with brine, dried over anhyd Na_2SO_4, and evaporated in vacuo. The semisolid product was recrystallized from isopropyl ether to give **4** and **5**, respectively; *3-O-Benzyl-5,6-O-isopropylidene-L-ascorbic acid* (**4**): 1.4 g, 47% yield; mp 105–106 °C (lit. mp 105–106 °C [7,10]); ^{1}H NMR ($CDCl_3$): δ 5.52 (two d, 2 H, CH_2Ph), 4.57 (d, 1 H, $J_{4,5}$ 3.66 Hz, H-4), 4.26 (dt, 1 H, $J_{4,5}$ 3.66, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.54 Hz, H-5), 4.10 (dd, 1 H, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.54 Hz, H-6), 4.02 (dd, 1 H, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.54 Hz, H-6), 1.39 (s, 3 H, O_2CCH_3), and 1.36 (s, 3 H, O_2CCH_3); ^{13}C NMR ($CDCl_3$): δ 171.8 (s, C-1), 145.2 (s, C-3), 119.9 (s, C-2), 110.29 (s, O_2CCH_3), 75.9 (d, C-4), 74.2 (d, C-5), 73.5 (t, CH_2Ph), 65.3 (t, C-6), 25.9 (q, O_2CCH_3), and 25.6 (q, O_2CCH_3); ν_{max}^{KBr} 3500, 3000, 1764, 1695, 1120, and 1050 cm^{-1}; *3-O-Benzyl-5,6-*

O-*benzylidene*-L-*ascorbic acid* (**5**): 1.1 g, 31% yield; mp 112–115 °C; ^{1}H NMR ($CDCl_3$): δ 5.85 (two s, 1 H, *J* 22.3 Hz, O_2CHPh), 5.55 (m, 2 H, CH_2Ph), 4.69 (d, 1 H, $J_{4,5}$ 2.75 Hz, H-4), 4.51 (m, 1 H, $J_{4,5}$ 2.75, $J_{5,6}$ 7.02, $J_{5,6'}$ 8.24 Hz, H-5), 4.34 (m, 1 H, $J_{5,6}$ 7.02, $J_{5,6'}$ 8.24 Hz, H-6), and 4.13 (dd, 1 H, $J_{5,6}$ 7.32, $J_{5,6'}$ 7.63 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 171.1 (C-1), 148.8 (C-3), 119.6 (C-2), 105.0 (O_2CHPh), 75.8 (C-4), 74.7 (CH_2Ph), 73.6 (C-5), and 66.5 (C-6). Anal. Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.81; H, 5.15.

Bis(N,N-*diisopropylamino*)*chlorophosphine* (**6**).—This was synthesized in 55% yield by King's method [21]; mp 98–100 °C (lit. mp 98–99 °C [21], 94–96 °C [29]); bp 97–99 °C (0.1 mmHg) (lit. bp 95–100 °C (0.1 mmHg) [21], 76–79 °C (0.4 mmHg) [29]).

Benzyloxybis(N,N-*diisopropylamino*)*phosphine* (**7**).—This was synthesized in 99% yield by Elie's method [18]; mp 14–15 °C.

General procedure for the preparation of the phosphoramidites **8** *and* **9**.—To a suspension of **4** or **5** (0.5 mmol) in CH_2Cl_2 (3 mL) were added 1*H*-tetrazole (40.1 mg, 0.6 mmol) and **7** (0.2 mL, 0.6 mmol). The suspension was vigorously stirred for 1 h at room temperature. A saturated $NaHCO_3$ soln was added to the mixture and it was extracted with EtOAc. The organic layers were washed with brine and dried over anhyd $MgSO_4$. After the solvent had been evaporated in vacuo, the residue was chromatographed (SiO_2, 5:2:1 hexane–EtOAc–NEt_3) to afford **8** and **9**, respectively; *3*-O-*Benzyl*-*2*-O-*benzyloxy*-N,N-*diisopropylaminophosphine*-*5,6*-O-*isopropylidene*-L-*ascorbic acid* (**8**): 263.6 mg, 97% yield; ^{1}H NMR ($CDCl_3$): δ 5.49 (d, 2 H, *J* 2.44 Hz, $COCH_2Ph$), 4.7–5.0 (m, 2 H, $J_{P,H}$ 12.21 Hz, $POCH_2Ph$), 4.5–4.6 (m, 1 H, $J_{4,5}$ 3.36 Hz, H-4), 4.2–4.3 (m, 1 H, $J_{4,5}$ 3.36, $J_{5,6}$ 6.87, $J_{5,6'}$ 7.02 Hz, H-5), 4.0–4.1 (m, 2 H, $J_{5,6}$ 6.87, $J_{5,6'}$ 7.02, $J_{6,6'}$ 22.58 Hz, H-6), 3.7–3.8 (m, 2 H, *J* 8.54 Hz, NCH), 1.29 (two s, 6 H, *J* 8.57 Hz, O_2CCH_3), and 1.21 (dd, 12 H, *J* 8.54 Hz, $NCHCCH_3$); ^{13}C NMR ($CDCl_3$): δ 171.1 (C-1), 152.5 (C-3), 118.5 (C-2), 110.1 (O_2CCH_3), 74.9 (C-4), 74.2, 74.1 (CH_2Ph), 73.6 (C-5), 65.2 (C-6), 43.9, 43.7 (NCH), 25.9, 25.7 (O_2CCH_3), 24.6, 24.5, 24.4, and 24.3 ($NCHCH_3$); ^{31}P NMR ($CDCl_3$): δ 154.5 and 154.1. Anal. Calcd for $C_{29}H_{38}NO_7P$: C, 64.08; H, 7.05. Found: C, 64.12; H, 6.99; *3*-O-*Benzyl*-*5,6*-O-*benzylidene*-*2*-O-*benzyloxy*-N,N-*diisopropylaminophosphine*-L-*ascorbic acid* (**9**): 290.0 mg, 98% yield; ^{1}H NMR ($CDCl_3$): δ 5.85 (d, 1 H, *J* 5.8 Hz, O_2CHPh), 5.39–5.57 (m, 2 H, $COCH_2Ph$), 4.69–5.10 (m, 2 H, $J_{P,H}$ 12.21 Hz, $POCH_2Ph$), 4.63 (m, 1 H, $J_{4,5}$ 2.75 Hz, H-4), 4.48 (m, 1 H, $J_{4,5}$ 2.75, $J_{5,6}$ 7.02, $J_{5,6'}$ 8.24 Hz, H-5), 4.34 (m, 2 H, $J_{5,6}$ 7.02, $J_{5,6'}$ 8.24, $J_{6,6'}$ 21.06 Hz, H-6), 3.72 (m, 2 H, *J* 8.24 Hz, NCH), and 1.21 (m, 12 H, *J* 8.24 Hz, $NCHCH_3$); ^{13}C NMR ($CDCl_3$): δ 170.4 (C-1), 155.8 (C-3), 118.5 (C-2), 105.1 (O_2CHPh), 75.1 (C-4), 74.7, 74.6 (O_2CH_2Ph), 73.6 (C-5), 66.5 (C-6), 43.8, 43.7 (NCH), 24.5 (2 C, O_2CCH_3), 24.4 (2 C, $NCHCH_3$), and 24.3 (2 C, $NCHCH_3$); ^{31}P NMR ($CDCl_3$): δ 154.9 and 155.0. Anal. Calcd for $C_{33}H_{38}NO_7P$: C, 66.99; H, 6.47. Found: C, 67.02; H, 6.50.

DL-*1,2*-O-*Cyclohexylidene*-myo-*inositol* (**11**).—This was synthesized in 98% yield by Massy's method [24]; mp 176–177 °C (lit. mp 174–175 °C [30], 179–180 °C [31,32], 176–178 °C [33]).

DL-*1,4,5,6*-*tetra*-O-*benzyl*-myo-*inositol* (**12**).—This was synthesized in 83% yield by Massy's method [24]; mp 127–128 °C (lit. mp 127.5–128 °C [24], 127 °C [26], 126–128 °C [32]).

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 stirred vigorously for 5 h. Water was added to the mixture and the product extracted with ether. The organic layer was thoroughly washed with brine and dried (Na_2SO_4). After the solvent had been evaporated in vacuo, the residue was chromatographed (SiO_2, 10:1 hexane–EtOAc) to give **13** (1.0 g, 79% yield) as a colorless solid; mp 128–129 °C (lit. mp 128–129 °C [33], 128–130 °C [25,26]).

6-O-Triphenylmethylarbutin (**17**).—To a solution of **16** (13.6 g, 50.0 mmol) in pyridine (50 mL) was added chlorotriphenylmethane (15.3 g, 55.0 mmol) at room temperature. The mixture was stirred for 23 h at room temperature. After the solvent had been evaporated in vacuo, the residue was extracted with EtOAc. The organic layer was thoroughly washed with 0.5 M HCl followed by saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed (SiO_2, 1:1 hexane–EtOAc) to afford **17** (23.5 g, 91% yield) as a colorless solid; mp 104–105 °C; ^{1}H NMR (Me_2SO-d_6): δ 4.84 (d, 1H, $J_{1,2}$ 6.10 Hz, H-1), 3.80 (m, 1 H, H-3), 3.65 (m, 1 H, H-5), and 3.45 (m, 4 H, H-2, H-4, H-6). Anal. Calcd for $C_{31}H_{30}O_7$: C, 72.36; H, 5.88. Found: C, 72.33; H, 5.87.

2,3,4,4′-Tetra-O-benzyl-6-O-triphenylmethylarbutin (**18**).—A mixture of **17** (22.0 g, 42.8 mmol) and NaOH (8.66 g, 214.3 mmol) in Me_2SO (100 ml) was stirred for 10 min at room temperature. Benzyl chloride (23.8 g, 188.4 mmol) was added dropwise, and the mixture was vigorously stirred for 42 h at room temperature. After the mixture had been extracted with EtOAc, the organic layer was thoroughly washed with H_2O, brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed (SiO_2, 20:1 hexane–EtOAc) to afford **18** (34.6 g, 93% yield) as a colorless solid; mp 127–128 °C; ^{1}H NMR ($CDCl_3$): δ 4.78–5.07 (m, 8H, CH_2Ph), 4.67 (d, 1 H, $J_{1,2}$ 10.99 Hz, H-1), 3.89 (dd, 1 H, $J_{1,2} = J_{2,3} = 10.99$ Hz, H-2), 3.72 (m, 2 H, H-4, H-5), 3.58 (t, 1 H, $J_{2,3} = J_{3,4} = 6.41$ Hz, H-3), and 3.48 (m, 2 H, H-6). Anal. Calcd for $C_{59}H_{54}O_7$: C, 80.98; H, 6.22. Found: C, 81.00; H, 6.25.

2,3,4,4′-Tetra-O-benzylarbutin (**19**).—To a solution of **18** (32.0 g, 36.6 mmol) in THF (150 mL) was added 35% HCl (15 mL) at room temperature. The mixture was stirred for 6 h at room temperature. After the solvent had been evaporated in vacuo, the mixture was extracted with EtOAc. The organic layer was thoroughly washed with saturated $NaHCO_3$, brine, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed (SiO_2, 10:1 hexane–EtOAc) to give **19** (13.8 g, 60% yield) as a colorless solid; mp 111–113 °C; ^{1}H NMR ($CDCl_3$): δ 4.80–5.05 (m, 8 H, CH_2Ph), 4.67 (d, 1 H, $J_{1,2}$ 10.99 Hz, H-1), 3.89 (dd, 1 H, $J_{1,2} = J_{2,3} = 10.99$ Hz, H-2), 3.71 (m, 2 H, H-4, H-5), 3.58 (t, 1 H, $J_{2,3} = J_{3,4} = 6.41$ Hz, H-3), and 3.48 (m, 2 H, H-6); ^{13}C NMR ($CDCl_3$): δ 154.55 (C-4″), 151.35 (C-1″), 118.01 (2 C, C-3′, C-5′), 115.80 (2 C, C-2′, C-6′), 84.42, 82.03, 77.32, 75.72 (*C*H_2Ph), 75.28 (C-3), 75.11 (C-5), 75.07 (C-2), 70.53 (C-4), and 61.94 (C-6). Anal. Calcd for $C_{40}H_{40}O_7$: C, 75.93; H, 6.37. Found: C, 75.97; H, 6.41.

(2S,4R)-4-Hydroxy-1,2-pyrrolidinedicarboxylic acid 1,2-dibenzyl ester (**23**).—This was synthesized in 72% yield by Barrett's method [27]; ^{1}H NMR ($CDCl_3$): δ 5.17 (ABq, 1 H, *J* 12.0 Hz, CH_2Ph), 5.12 (s, 1 H, CH_2Ph), 5.00 (ABq, 1 H, *J* 12.4 Hz,

CH_2Ph), 4.98 (s, 1 H, H-4), 4.53 (m, 1 H, *J* 8.0 Hz, CH_2Ph), 4.40 (m, 1 H, H-2), 3.59 (m, 2 H, H-5), 2.25 (m, 1 H, H-3), and 2.02 (m, 1 H, H-3); ^{13}C NMR ($CDCl_3$): δ 172.5 (NCO), 172.3 (C-6), 69.9 (C-4), 67.2, 67.1 (*C*H_2Ph), 58.0 (C-2), 54.5 (C-5), and 38.2 (C-3); ν_{max}^{neat} 3440, 2950, 1745, 1700, 1420, 1360, 1280, 1190, 1170, 1125, 1085, 1005, 770, 740, and 700 cm^{-1}.

General procedure for the preparation of the phosphodiester **14**, **20**, **24**, **27**, *and* **29** *by the phosphoramidite method.*—To a suspension of **8** or **9** (2.7 g, 5.0 mmol) in CH_2Cl_2 (5 mL) were added 1*H*-tetrazole (0.7 g, 10.0 mmol) and alcohol **13**, **19**, **23**, or **26** (5.0 mmol). The suspension was vigorously stirred for 1–4 days at room temperature. The mixture was cooled to 0 °C and *m*-chloroperoxybenzoic acid (2.5 g, 10.0 mmol) was added. The stirring was continued 2 h–4 days at room temperature. The aqueous layer was extracted with EtOAc. The combined layers were washed with 10% Na_2SO_3, saturated $NaHCO_3$ soln, brine, and dried (Na_2SO_4). After the solvent had been evaporated in vacuo, the residue was chromatographed (SiO_2, 3:1 hexane–EtOAc) to afford **14**, **20**, **24**, **27**, and **29**, respectively; *3-O-Benzyl-5,6-O-isopropylidene-2-O-(1,3,4,5,6-penta-O-benzyl-*myo*-inositol-2-benzylphosphoryl)-*L*-ascorbic acid* (**14**): 2.5 g, 49% yield; ^{1}H NMR ($CDCl_3$): δ 5.81 (d, 2 H, *J* 11.60 Hz, COCH_2Ph), 5.49 (t, 1H, $J_{1,2} = J_{2,3} = 2.75$, $J_{P,H}$ 11.29 Hz, H-2′), 4.85–4.90 (m, 4 H, COCH_2Ph), 4.90 (ABq, 2 H, $J_{P,H}$ 10.68 Hz, POCH_2Ph), 4.85 (m, 2 H, COCH_2Ph), 4.79 (d, 2 H, *J* 11.60 Hz, COCH_2Ph), 4.60 (m, 2 H, COCH_2Ph), 4.55 (d, 1 H, $J_{4,5}$ 3.66 Hz, H-4), 4.22 (dd, 1 H, $J_{4,5}$ 3.66, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.96 Hz, H-5), 4.15 (t, 1 H, $J_{3,4} = J_{4,5} = 9.46$ Hz, H-4), 3.95 (t, 1 H, $J_{1,6} = J_{6,5} = 9.46$ Hz, H-6), 3.89 (m, 2 H, $J_{5,6}$ 6.71, $J_{5,6'}$ 8.64 Hz, H-6), 3.54 (dd, 1 H, $J_{2,3}$ 2.44, $J_{3,4}$ 9.46 Hz, H-3′), 3.52 (t, 1 H, $J_{4,5} = J_{5,6} = 9.46$ Hz, H-5′), 3.50 (dd, 1 H, $J_{1,2}$ 2.44, $J_{1,6}$ 9.46 Hz, H-1′), and 1.34 (two s, 6 H, *J* 6.41 Hz, O_2CCH_3); ^{31}P NMR ($CDCl_3$): δ −4.34. Anal. Calcd for $C_{64}H_{65}O_{14}P$: C, 70.58; H, 6.02. Found: C, 70.62; H, 5.99; *3-O-Benzyl-2-O-[4-(benzyloxy)phenyl-2,3,4-tri-O-benzyl-β-*D*-glucopyranoside-6-benzylphosphoryl]-5,6-O-isopropylidene-*L*-ascorbic acid* (**20**): 3.2 g, 59% yield; ^{1}H NMR ($CDCl_3$): δ 7.01 (m, 2 H, *J* 9.15 Hz, H-2″, H-6″), 6.88 (m, 2 H, *J* 9.15 Hz, H-3″, H-5″), 5.50 (t, 2 H, COCH_2Ph), 5.25 (ABq, 2 H, $J_{P,H}$ 10.60 Hz, POCH_2Ph), 4.77–5.08 (m, 8 H, COCH_2Ph), 4.67 (dd, 1 H, $J_{1,2}$ 10.99 Hz, H-1′), 4.55 (d, 1 H, $J_{4,5}$ 3.66 Hz, H-4), 4.24 (dd, 1 H, $J_{4,5}$ 3.66, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.96 Hz, H-5), 4.02 (t, 1 H, $J_{1,2} = J_{2,3} = 9.46$ Hz, H-2′), 3.89 (m, 2 H, $J_{5,6}$ 6.71, $J_{5,6'}$ 8.64 Hz, H-6), 3.66 (m, 2 H, H-4′, H-5′), 3.55 (m, 1 H, $J_{2,3} = J_{3,4} = 6.41$ Hz, H-3′), 3.47 (m, 2 H, $J_{P,H}$ 11.29 Hz, H-6′), and 1.27 (two s, 6 H, *J* 7.02 Hz, O_2CCH_3); ^{31}P NMR ($CDCl_3$): δ 4.72 and −4.81. Anal. Calcd for $C_{63}H_{63}O_{15}P$: C, 69.35; H, 5.82. Found: C, 69.31; H, 5.79; *3-O-Benzyl-5,6-O-isopropylidene-2-O-[(2S,4R)-1,2-pyrrolidinedicarboxylic acid 1,2-dibenzylester-4-benzylphosphonooxy]-*L*-ascorbic acid* (**24**): 2.4 g, 59% yield; ^{1}H NMR ($CDCl_3$): δ 5.38 (m, 2 H, $J_{P,H}$ 10.98 Hz, POCH_2Ph), 5.19 (m, 4 H, *J* 9.16 Hz, COCH_2Ph), 4.98 (m, 2 H, *J* 9.16 Hz, COCH_2Ph), 4.74 (m, 1 H, $J_{P,H}$ 2.44 Hz, H-4′), 4.45 (m, 1 H, $J_{4,5}$ 3.97 Hz, H-4), 3.96 (m, 1 H, $J_{2,3}$ 7.93 Hz, H-2′), 3.85 (ddd, 1 H, $J_{4,5}$ 3.66, $J_{5,6}$ 6.71 Hz, H-5), 3.75 (m, 2 H, $J_{5,6}$ 6.71 Hz, H-6), 3.64 (m, 2 H, H-5′), 2.64 (m, 1 H, m, H-3′), 2.47 (m, 1 H, H-3′), and 1.34 (two s, 6 H, *J* 6.73 Hz, O_2CCH_3),; ^{31}P NMR ($CDCl_3$): δ −5.85 and −5.90. Anal. Calcd for $C_{43}H_{44}NO_{13}P$: C, 63.46; H, 5.45. Found: C, 63.51; H, 5.43; *3-O-Benzyl-5,6-O-isopropylidene-2-O-(benzylbenzyloxycarbonylmethylphosphoryl)-*L*-ascorbic acid* (**27**): 2.0 g, 64% yield; ^{1}H NMR

($CDCl_3$): δ 5.46 (d, 2 H, $J_{P,H}$ 10.07 Hz, POCH_2Ph), 5.32 (t, 2 H, J 6.71 and 7.33 Hz, COCH_2Ph), 5.19 (d, 2 H, J 3.66 Hz, COCH_2Ph), 4.85 (dd, 2 H, J 11.29 Hz, OCH_2CO), 4.56 (tt, 1 H, $J_{4,5}$ 3.05 Hz, H-4), 4.29 (ddd, 1 H, $J_{4,5}$ 3.05, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.55 Hz, H-5), 4.04 (m, 2 H, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.55 Hz, H-6), 1.34 (two s, 3 H, J 8.24 Hz, O_2CCH_3), and 1.31 (two s, 3 H, J 8.24 Hz, O_2CCH_3); ^{13}C NMR ($CDCl_3$): δ 167.4 (C-1), 167.3 (OCH_2CO), 158.2 (C-3), 114.4 (C-2), 110.4 (O_2CCH_3), 74.9 (C-4), 74.8, 74.4, 74.3 (CH_2Ph), 73.5 (C-5), 67.2 (OCH_2CO), 65.1 (C-6), 25.7, and 25.5 (O_2CCH_3); ^{31}P NMR ($CDCl_3$) δ −5.52 and −5.57. Anal. Calcd for $C_{32}H_{33}O_{11}P$: C, 61.54; H, 5.33. Found: C, 61.57; H, 5.29; *3-O-Benzyl-5,6-O-benzylidene-2-O-(benzylbenzyloxycarbonylmethylphosphoryl)-L-ascorbic acid* (**29**): 1.4 g, 42% yield; ^{1}H NMR ($CDCl_3$): δ 5.73 (d, 1 H, J 7.00 Hz, O_2CHPh), 5.45 (d, 2 H, $J_{P,H}$ 10.07 Hz, POCH_2Ph), 5.30 (t, 2 H, J 6.71 and 7.33 Hz, COCH_2Ph), 5.20 (d, 2 H, J 3.66 Hz, COCH_2Ph), 4.85 (dd, 2 H, J 11.29 Hz, OCH_2CO), 4.55 (tt, 1 H, $J_{4,5}$ 2.75 Hz, H-4), 4.25 (ddd, 1 H, $J_{4,5}$ 2.75, $J_{5,6}$ 7.02, $J_{5,6'}$ 8.24 Hz, H-5), and 3.89 (m, 2 H, $J_{5,6}$ 7.02, $J_{5,6'}$ 8.24 Hz, H-6); ^{31}P NMR ($CDCl_3$): δ 5.85 and −5.90. Anal. Calcd for $C_{36}H_{33}O_{11}P$: C, 64.28; H, 4.95. Found: C, 64.32; H, 4.91.

General procedure for removal of the protective group.—Method A (**15a**, **21a**, *and* **25a**). To a solution of **14**, **20**, or **24** (3.0 mmol) in THF (100 mL) was added 35% HCl (10 mL) at room temperature. The mixture was stirred for 2 h at room temperature. After the solvent had been evaporated in vacuo, the residue was dissolved in MeOH (200 mL) and 5% Pd/C (2.0 g) was added 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 extracted with H_2O, and the combined aqueous layer was washed with EtOAc. The solvent was evaporated in vacuo and the residue was dried under vacuum to yield **15a**, **21a**, and **25a**, respectively; *2-O-(*myo*-inositol-2-phosphoryl)-L-ascorbic acid* (**15a**): 1.1 g, 88% yield; mp 51–53 °C; ^{1}H NMR (Me_2SO-d_6): δ 5.49 (t, 1 H, $J_{1,2} = J_{2,3} = 2.75$, $J_{P,H}$ 11.29 Hz, H-2′), 4.55 (d, 1 H, $J_{4,5}$ 3.66 Hz, H-4), 4.22 (dd, 1 H, $J_{4,5}$ 3.66, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.96 Hz, H-5), 4.15 (t, 1 H, $J_{3,4} = J_{4,5} = 9.46$ Hz, H-4′), 3.95 (t, 1 H, $J_{1,6} = J_{5,6} = 9.46$ Hz, H-6′), 3.89 (m, 2 H, $J_{5,6}$ 6.71, $J_{5,6'}$ 8.64 Hz, H-6), 3.54 (dd, 1 H, $J_{2,3}$ 2.44, $J_{3,4}$ 9.46 Hz, H-3′), 3.52 (t, 1 H, $J_{4,5} = J_{5,6} = 9.46$ Hz, H-5′), and 3.50 (dd, 1 H, $J_{1,2}$ 2.44, $J_{1,6}$ 9.46 Hz, H-1′); ^{13}C NMR (Me_2SO-d_6): δ 170.6 (C-1), 152.8 (C-3), 118.0 (C-2), 75.1 (C-4), 74.6 (C-5′), 74.1 (C-6′), 74.1 (C-4′), 72.7 (C-3′), 72.7 (C-1′), 68.7 (C-2′), 68.4 (C-5), and 61.9 (C-6); ^{31}P NMR (Me_2SO-d_6): δ −1.45. Anal. Calcd for $C_{12}H_{19}O_{14}P$: C, 34.46; H, 4.58. Found: C, 34.42; H, 4.61; *2-O-(hydroquinone β-D-glucopyranoside-6-phosphoryl)-L-ascorbic acid* (**21a**): 1.4 g, 94% yield; mp 35–37 °C; ^{1}H NMR (D_2O): δ 6.76 (d, 2 H, J 8.85 Hz, H-2″, H-6″), 6.62 (d, 2 H, J 8.85 Hz, H-3″, H-5″), 4.68 (d, 1 H, $J_{1,2}$ 7.63 Hz, H-1′), 4.64 (dd, 1 H, $J_{4,5}$ 3.06 Hz, H-4), 4.19 (m, 1 H, $J_{4,5}$ 3.06, $J_{5,6}$ 6.41 Hz, H-5), 4.06 (t, 2 H, $J_{5,6}$ 6.41 Hz, H-6), 3.89 (dd, 1 H, $J_{1,2}$ 7.63, $J_{2,3}$ 8.24 Hz, H-2′), 3.47 (m, 2 H, J 8.85 Hz, H-4′, H-5′), 3.43 (t, 1 H, $J_{2,3} = J_{3,4} = 8.85$ Hz, H-3′), and 3.30 (ABq, 2 H, $J_{5,6}$ 8.85, $J_{P,H}$ 9.46 Hz, H-6′); ^{13}C NMR (D_2O): δ 172.3 (C-1), 160.4 (C-3), 151.9 (C-4″), 151.5 (C-1″), 118.8 (2 C, C-3″, C-5″), 117.0 (2 C, C-2″, C-6″), 115.2 (C-2), 101.8 (C-1′), 77.0 (C-3′), 76.9 (C-4), 76.4 (C-5′), 73.7 (C-2′), 70.2 (C-4′), 69.7 (C-6′), 69.6 (C-5), and 62.7 (C-6); ^{31}P NMR (D_2O): δ −0.56. Anal. Calcd for $C_{18}H_{23}O_{15}P$: C, 42.36; H, 4.54. Found: C, 42.39; H,

4.51; *2-O-[(2S,4R)-2-pyrrolidinecarboxylic acid 4-phosphonooxy]-L-ascorbic acid* (**25a**): 1.1 g, 99% yield; mp 32–34 °C; ^{1}H NMR (D_2O, pD 2.9): δ 5.02 (ddd, 1 H, $J_{P,H}$ 3.36 Hz, H-4′), 4.92 (d, 1 H, $J_{4,5}$ 3.66 Hz, H-4), 4.48 (dd, 1 H, $J_{2,3}$ 7.94 Hz, H-2′), 3.99 (ddd, 1 H, $J_{4,5}$ 3.66, $J_{5,6}$ 6.71 Hz, H-5), 3.63 (d, 2 H, $J_{5,6}$ 6.41 Hz, H-6), 3.53 (m, 1 H, $J_{4,5}$ 3.67 Hz, H-5′), 3.44 (t, 1 H, $J_{4,5}$ 3.67 Hz, H-5′), 2.62 (dd, 1 H, $J_{2,3}$ 7.94, $J_{3,4}$ 3.66 Hz, H-3′), and 2.23 (dd, 1 H, $J_{2,3}$ 7.94, $J_{3,4}$ 3.66 Hz, H-3′); ^{13}C NMR (D_2O, pD 2.9): δ 172.7 (C-1), 172.6 (C-6′), 162.2 (C-3), 114.9 (C-2), 77.2 (C-4), 76.5 (C-4′), 69.6 (C-5), 62.7 (C-6), 59.5 (C-2′), 53.3 (C-5′), and 37.1 (C-3′); ^{31}P NMR (D_2O, pD 2.9): δ −2.69. Anal. Calcd for $C_{11}H_{16}NO_{11}P$: C, 35.78; H, 4.37. Found: C, 35.81; H, 4.33.

Method B (**30a** *and* **28**). To a solution of **29** or **27** (3.0 mmol) in MeOH (200 mL) was added 5% Pd/C (2.5 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 off, the filtrate was evaporated in vacuo to dryness. The residue was extracted with H_2O, and the combined aqueous layer was washed with EtOAc. The solvent was evaporated in vacuo and the residue was dried under vacuum to yield **30a** and **28**, respectively; *2-O-(carboxymethylphosphonooxy)-L-ascorbic acid* (**30a**): 0.8 g, 85% yield; ^{1}H NMR (D_2O): δ 4.84 (sd, 2 H, J 16.78 Hz, OCH_2CO), 4.55 (dd, 1 H, $J_{4,5}$ 3.36 Hz, H-4), 4.29 (ddd, 1 H, $J_{4,5}$ 3.36, $J_{5,6}$ 6.41 Hz, H-5), and 3.85 (t, 2 H, $J_{5,6}$ 6.41 Hz, H-6); ^{13}C NMR (D_2O): δ 174.3 (CO_2H), 174.1 (C-1), 172.1 (C-3), 118.9 (C-2), 77.1 (C-4), 64.3 (C-5), 63.1 (OCH_2CO), and 62.4 (C-6); ^{31}P NMR (D_2O): δ −3.32 and −3.38. Anal. Calcd for $C_8H_{11}O_{11}P$: C, 30.59; H, 3.53. Found: C, 30.62; H, 3.49; *5,6-O-isopropylidene-2-O-(carboxymethylphosphonooxy)-L-ascorbic acid* (**28**): 1.0 g, 95% yield; ^{1}H NMR (Me_2SO-d_6): δ 4.85 (dd, 2 H, J 11.29 Hz, OCH_2CO), 4.53 (tt, 1 H, $J_{4,5}$ 3.05 Hz, H-4), 4.29 (ddd, 1 H, $J_{4,5}$ 3.05, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.55 Hz, H-5), 4.06 (m, 2 H, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.55 Hz, H-6), 1.34 (d, 3 H, O_2CCH_3), and 1.31 (d, 3 H, O_2CCH_3); ^{13}C NMR (Me_2SO-d_6): δ 167.4 (C-1), 167.3 (OCH_2CO), 158.2 (C-3), 114.4 (C-2), 110.4 (O_2CCH_3), 74.9 (C-4), 74.8, 74.4, 74.3 (CH_2Ph), 73.5 (C-5), 67.2 (OCH_2CO), 65.1 (C-6), 25.7, and 25.5 (O_2CCH_3); ^{31}P NMR (Me_2SO-d_6): δ −5.53 and −5.59. Anal. Calcd for $C_{11}H_{15}O_{11}P$: C, 37.30; H, 4.27. Found: C, 37.36; H, 4.31.

General procedure for preparation of the sodium salts **15b**, **21b**, **25b**, *and* **30b**.—The phosphodiester **15a**, **21a**, **25a**, or **30a** was passed through a Na^+ cation-exchange column (DIAION SK1B). The solvent was evaporated and the residue was dried under vacuum to give **15b**, **21b**, **25b**, and **30b**, respectively; **15b** (1.2 g, 98%); mp 137–139 °C; ^{1}H NMR (D_2O): δ 5.52 (t, 1 H, $J_{1,2} = J_{2,3} = 2.75$, $J_{P,H}$ 11.29 Hz, H-2′), 4.53 (m, 1 H, H-4), 4.25 (dd, 1 H, $J_{4,5}$ 3.66, $J_{5,6}$ 6.72, $J_{5,6'}$ 8.96 Hz, H-5), 4.17 (t, 1 H, $J_{3,4} = J_{4,5} = 9.46$ Hz, H-4′), 3.97 (t, 1 H, $J_{1,6} = J_{6,5} = 9.46$ Hz, H-6′), 3.91 (m, 2 H, m, $J_{5,6}$ 6.71, $J_{5,6'}$ 8.64 Hz, H-6), 3.56 (dd, 1 H, $J_{2,3}$ 2.44, $J_{3,4}$ 9.46 Hz, H-3′), 3.54 (t, 1 H, $J_{4,5} = J_{5,6} = 9.46$ Hz, H-5′), and 3.52 (dd, 1 H, $J_{1,2}$ 2.44, $J_{1,6}$ 9.46 Hz, H-1′); ^{13}C NMR (D_2O): δ 170.0 (C-1), 155.7 (C-3), 118.3 (C-2), 76.0 (C-4), 75.3 (C-5′), 74.0 (C-6′), 74.0 (C-4′), 73.1 (C-3′), 73.1 (C-1′), 69.8 (C-2′), 69.1 (C-5), and 62.0 (C-6); ^{31}P NMR (D_2O): δ −1.00. Anal. Calcd for $C_{12}H_{17}Na_2O_{14}P \cdot H_2O$: C, 30.01; H, 3.99. Found: C, 30.03; H, 4.02; **21b** (1.7 g, quantitative); mp 142–144 °C; ^{1}H NMR (D_2O): δ 6.89 (d, 2 H, J 8.85 Hz, H-2″, H-6″), 6.74 (d, 2 H, J 8.85 Hz, H-3″, H-5″), 4.80 (d, 1 H, $J_{1,2}$ 7.63 Hz, H-1′), 4.55 (dd, 1 H, $J_{4,5}$ 3.06 Hz, H-4), 4.19 (m, 1 H, $J_{4,5}$ 3.06, $J_{5,6}$ 6.41 Hz, H-5), 4.00 (t, 2 H, $J_{5,6}$ 6.41 Hz, H-6), 3.84 (dd, 1 H, $J_{1,2}$ 7.63, $J_{2,3}$ 8.24 Hz, H-2′), 3.54 (m, 2

H, *J* 8.85 Hz, H-4′, H-5′), 3.45 (t, 1 H, $J_{2,3} = J_{3,4} = 8.85$ Hz, H-3′), and 3.40 (ABq, 2 H, $J_{5,6}$ 8.85, $J_{P,H}$ 9.46 Hz, H-6′); ^{13}C NMR (D_2O): δ 172.4 (C-1), 172.3 (C-3), 151.8 (C-4″), 151.5 (C-1″), 118.7 (2 C, C-3″, C-5″), 117.1 (2 C, C-2″, C-6″), 115.2 (C-2), 101.8 (C-1′), 77.0 (C-3′), 76.8 (C-4), 76.3 (C-5′), 73.7 (C-2′), 70.2 (C-4′), 69.7 (C-6′), 69.6 (C-5), and 62.1 (C-6); ^{31}P NMR (D_2O): δ −1.00. Anal. Calcd for $C_{18}H_{20}Na_3O_{15}P \cdot H_2O$: C, 36.38; H, 3.73. Found: C, 36.39; H, 3.71; **25b** (1.3 g, 96%); (lit. mp > 245 °C [13]); ^{1}H NMR (D_2O): δ 4.97 (ddd, 1 H, $J_{P,H}$ 3.36 Hz, H-4′), 4.66 (d, 1 H, $J_{4,5}$ 3.66 Hz, H-4), 4.35 (dd, 1 H, $J_{2,3}$ 7.94 Hz, H-2′), 3.95 (ddd, 1 H, $J_{4,5}$ 3.66, $J_{5,6}$ 6.72 Hz, H-5), 3.58 (d, 2 H, $J_{5,6}$ 6.72 Hz, H-6), 3.43 (m, 1 H, $J_{4,5}$ 3.67 Hz, H-5′), 3.38 (t, 1 H, $J_{4,5}$ 3.67 Hz, H-5′), 2.54 (dd, 1 H, $J_{2,3}$ 7.94, $J_{3,4}$ 3.66 Hz, H-3′), and 2.14 (m, 1 H, $J_{2,3}$ 7.94, $J_{3,4}$ 3.66 Hz, H-3′); ^{13}C NMR (D_2O): δ 173.1 (C-1), 172.4 (C-3), 162.5 (C-6′), 114.7 (C-2), 76.9 (C-4), 76.3 (C-4′), 69.3 (C-5), 62.4 (C-6), 59.6 (C-2′), 52.9 (C-5′), and 36.9 (C-3′); ^{31}P NMR (D_2O): δ −2.06. Anal. Calcd for $C_{11}H_{13}NNa_3O_{11}P \cdot H_2O$: C, 29.15; H, 3.34. Found: C, 29.19; H, 3.31; **30b** (1.2 g, quantitative); ^{1}H NMR (D_2O): δ 4.84 (sd, 2 H, *J* 16.78 Hz, OCH_2CO), 4.55 (dd, 1 H, $J_{4,5}$ 3.36 Hz, H-4), 4.29 (ddd, 1 H, $J_{4,5}$ 3.36, $J_{5,6}$ 6.41 Hz, H-5), and 3.85 (t, 2 H, $J_{5,6}$ 6.41 Hz, H-6); ^{13}C NMR (D_2O): δ 174.1 (CO_2H), 174.0 (C-1), 172.3 (C-3), 118.6 (C-2), 76.8 (C-4), 64.1 (C-5), 63.0 (OCH_2CO), and 62.6 (C-6); ^{31}P NMR (D_2O): δ −1.11 and −1.62. Anal. Calcd for $C_8H_8Na_3O_{11}P \cdot H_2O$: C, 24.14; H, 2.53. Found: C, 24.17; H, 2.50.

Measurement of the stability in aqueous solution.—The test compound was dissolved in 1/1 (v/v) EtOH–H_2O to give a 1% (w/v) soln. The resulting solution was stored at 60 °C, and 0.05-mL aliquots 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 reducing activity of the stable radical α,α-diphenyl-β-picryl-hydrazyl (DPPH) [28].—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}, 10^{-5}, 10^{-4}, and 10^{-3} M sample solutions, respectively. After reaction for 20 min at 25 °C, the absorbance at 517 nm was measured. The difference in absorbance from control, in which the test compound was absent, was taken as the reducing activity. The results are shown in Table 2.

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References

[1] A.A. Frimer and P.G. Sharon, *J. Org. Chem.*, 60 (1995) 2796–2801; A. Bendich, L.J. Machlin, O. Scandurra, G.W. Burton, and D.D.M. Wayner, *Adv. Free Radical Biol. Med.*, 2 (1986) 419–444; B. Frei, R. Stocker, and B.N. Ames, *Proc. Natl. Acad. Sci. U.S.A.*, 85 (1988) 9748–9752.

[2] R.C. Rose and A.M. Bode, *FASEB J.*, 7 (1993) 1135–1142; B.N. Ames, M.K. Shigenaga, and T.M. Hagen, *Proc. Natl. Acad. Sci. U.S.A.*, 90 (1993) 7915–7922.
[3] Y. Ishida, *Fragrance J.*, (1983) 28–34.
[4] S.F. Quadri, P.A. Seib, and C.W. Deyoe, *Carbohydr. Res.*, 29 (1973) 259–264; C.H. Lee, P.A. Seib, Y.T. Liang, R.C. Hoseney, and C.W. Deyoe, *Carbohydr. Res.*, 67 (1978) 127–138; H. Nomura, M. Shimomura, and S. Morimoto, *Chem. Pharm. Bull.*, 19 (1971) 1433–1437.
[5] R. Hata and H. Senou, *J. Cell. Physiol.*, 138 (1989) 8–16; E. Niki, J. Tsuchiya, R. Tanimura, and Y. Kamiya, *Chem. Lett.*, 1982, 789–792; A.J. Kirby and A.G. Varvoglis, *J. Am. Chem. Soc.*, 89 (1967) 415–423.
[6] See for review P.A. Seib, *Int. J. Vitam. Nutr. Res., Suppl.*, 27 (1985) 259–306; G.C. Andrews and T. Crawford, *Adv. Chem. Ser.*, 200 (1982) 59–79; B.M. Tolbert, M. Downing, R.W. Carlson, M.K. Knight, and E.M. Baker, *Ann. N.Y. Acad. Sci.*, 258 (1975) 48–69; M.B. Davies, *Polyhedron*, 11 (1992) 285–321.
[7] K. Kato, S. Terao, N. Shimamoto, and M. Hirata, *J. Med. Chem.*, 31 (1988) 793–798.
[8] Y. Nihro, H. Miyataka, T. Sudo, T. Miki, H. Matsumoto, and T. Satoh, *J. Med. Chem.*, 34 (1991) 2152–2157.
[9] K. Wimalasena and M.P.D. Mahindaratne, *J. Org. Chem.*, 59 (1994) 3427–3432.
[10] K. Wimalasena and M.P.D. Mahindaratne, *J. Org. Chem.*, 59 (1994) 3427–3432; J. Cabral and P. Haake, *J. Org. Chem.*, 53 (1988) 5742–5750; M. Sekine, T. Futatsugi, and T. Hata, *J. Org. Chem.*, 47 (1982) 3453–3456; Y. Nihro, S. Sogawa, T. Sudo, T. Miki, H. Matsumoto, and T. Satoh, *Chem. Pharm. Bull.*, 39 (1991) 1731–1735; M. Tanaka, N. Muto, and I. Yamamoto, *Biochim. Biophys. Acta*, 1078 (1991) 127–132; H. Nomura and K. Sugimoto, *Chem. Pharm. Bull.*, 14 (1966) 1039–1044; H. Tanaka and R. Yamamoto, *J. Pharm. Soc. Jpn.*, 86 (1966) 376–383; J.M. Grisar, G. Marciniak, F.N. Bolkenius, J. Verne-Mismer, and E.R. Wagner, *J. Med. Chem.*, 38 (1995) 2880–2886.
[11] H. Takashima, H. Nomura, Y. Imai, and H. Mima, *Am. Perfum. Cosmet.*, 86 (1971) 29-36.
[12] K. Tojo and A.-R.C. Lee, *J. Soc. Cosmet. Chem.*, 38 (1987) 333–339.
[13] K. Sano, K. Shinho, Y. Oonishi, and Y. Ishimura, *Jpn. Kokai Tokkyo Koho* 0559,075 (1991); *Chem. Abstr.*, 119 (1991) 226343b.
[14] B.V.L. Potter and D. Lampe, *Angew. Chem., Int. Ed. Engl.*, 34 (1995) 1933–1972; L.R. Stephens, P.T. Hawkins, A.F. Stanley, T. Moore, D.R. Poyner, P.J. Morries, M.R. Hanley, R.R. Kay, and R.F. Irvine, *Biochem. J.*, 275 (1991) 485–499; H. Streb, R.F. Irvine, M.J. Berridge, and I. Schulz, *Nature*, 306 (1983) 67–69.
[15] S. Akiu, Y. Suzuki, Y. Fujinuma, T. Asahara, and M. Fukuda, *Proc. Jpn. Soc. Invest. Dermatol.*, 12 (1988) 138–139.
[16] E.J. Van Scott, *Cutis*, 43 (1989) 222–228; S. Sakaki, T. Oyama, Y. Okano, and H. Masaki, *J. Soc. Cosmet. Chem. Jpn.*, 27 (1993) 166–169; M. Takahashi and Y. Machida, *J. Soc. Cosmet. Chem.*, 36 (1985) 177–187.
[17] H. Nomura, T. Ishiguro, and S. Morimoto, *Chem. Pharm. Bull.*, 17 (1969) 381–386; H. Nomura, T. Ishiguro, and S. Morimoto, *Chem. Pharm. Bull.*, 17 (1969) 387–393; H. Nomura, M. Shimomura, and S. Morimoto, *Chem. Pharm. Bull.*, 19 (1971) 1433–1437; C.H. Lee, P.A. Seib, Y.T. Liang, R.C. Hoseney, and C.W. Deyoe, *Carbohydr. Res.*, 67 (1978) 127–138.
[18] C.J.J. Elie, C.E. Dreef, R. Verduyn, G.A. Van der Marel, and J.H. Van Boom, *Tetahedron*, 45 (1989) 3477–3486.
[19] C.M. Dreef-Tromp, A.W.M. Lefeber, G.A. Van der Marel, and J.H. Van Boom, *Synthesis*, (1992) 1269–1272.
[20] W. Bannwarth and A. Trzeciak, *Helv. Chim. Acta*, 70 (1987) 175–186.
[21] R.B. King and P.M. Sundaram, *J. Org. Chem.*, 49 (1984) 1784–1789.
[22] M.E. Jung and T.J. Shaw, *J. Am. Chem. Soc.*, 102 (1980) 6304–6311.
[23] M. Kochi, T. Ito, and T. Hisamatsu, *Eur. Pat.* 148094 (1985); *Chem. Abstr.*, 103 (1985) 215728z.
[24] D.J.R. Massy and P. Wyss, *Helv. Chim. Acta*, 73 (1990) 1037–1057.
[25] M.A. Nashed and L. Anderson, *Tetrahedron Lett.*, (1976) 3503–3506.
[26] S.J. Angyal and M.E. Tate, *J. Chem. Soc.*, (1965) 6949–6955.
[27] A.G.M. Barrett and D. Pilipauskas, *J. Org. Chem.*, 56 (1991) 2787–2800.
[28] M.S. Blois, *Nature*, 181 (1958) 1199–1200.

[29] J.B. Lambert and J.H. So, *J. Org. Chem.*, 56 (1991) 5960–5962.
[30] S. Ozaki, Y. Kondo, N. Shiotani, T. Ogasawara, and Y. Watanabe, *J. Chem. Soc., Perkin Trans. 1*, (1992) 729–737.
[31] C. Jiang and D.C. Baker, *J. Carbohydr. Chem.*, 5 (1986) 615–620.
[32] D.E. Kiely, G.J. Abruscato, and V. Baburao, *Carbohydr. Res.*, 34 (1974) 307–313.
[33] P.J. Garegg and B. Lindberg, *Carbohydr. Res.*, 173 (1988) 205–216.