Carotenoids and related polyenes. Part 9.[1] Total synthesis of thermozeaxanthin and thermocryptoxanthin and the stabilizing effect of thermozeaxanthin on liposomes

Yumiko Yamano,[a] Yoshitsugu Sakai,[a] Masayuki Hara[b] and Masayoshi Ito*[a]

[a] *Kobe Pharmaceutical University, Motoyamakita-machi, Higashinada-ku, Kobe 658-8558, Japan*

[b] *Research Institute for Advanced Science and Technology, Osaka Prefecture University, 1-2 Gakuen-cho, Sakai, Osaka 599-8570, Japan*

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Thermozeaxanthin and thermocryptoxanthin are efficiently synthesized *via* β-selective glucosidation of (3*R*)-3-hydroxy-β-ionone **7**, and the stabilizing effects of zeaxanthin **5**, its glucoside **3** and thermozeaxanthin-15 **1a** on liposomes are also examined.

Introduction

Thermozeaxanthins (TZs: **1**) and thermocryptoxanthins (TCs: **2**) (Fig. 1) are novel carotenoid-glucoside-fatty acid esters isolated[2,3] from the thermophilic eubacterium, *Thermus thermophilus*. These characteristic "hydrophobic–hydrophilic–hydrophobic" structures are considered[2] to be preferred for stabilizing membranes even at high temperature. In membranes, the rigid conjugated hydrocarbon chains of these carotenoids are supposed[2] to be located in the hydrophobic core of lipid bilayers while the glucose moieties are anchored in the hydrophilic headgroup region, and the branched fatty acid moieties curl back into the hydrophobic region like a 'hook', to reinforce the membranes. Recently, Hara *et al.* showed[4] that TZs **1** have a stabilizing effect on liposomes of phospholipids. The lack of unity in the fatty acid moiety of natural TZs has prompted us to synthesize TZ in a pure form for better understanding of the stabilizing effect.

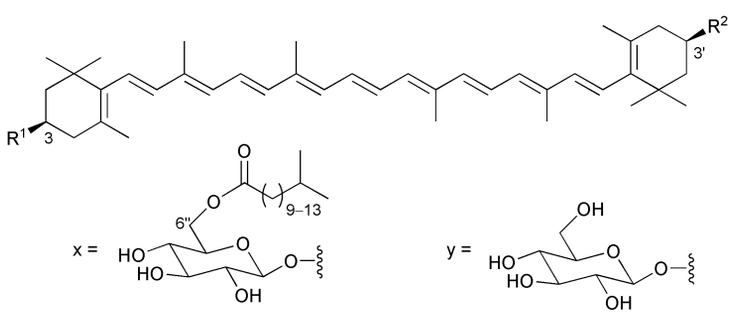
Partial synthesis of zeaxanthin-mono- and diglucosides from zeaxanthin by the Koenigs–Knorr method was reported by Pfander's group.[5] However, a satisfactory amount of glucosides was not obtained in the method, probably due to the instability of the carotenoid molecule. In a previous communication,[6] we reported the efficient synthesis of zeaxanthin-mono-β-D-glucopyranoside **3** and cryptoxanthin-β-D-glucopyranoside **4** starting from (3*R*)-3-hydroxy-β-ionone **7** (Scheme 1). Here we describe the first total synthesis of TZ **1** and TC **2** *via* the direct acylation of the primary hydroxy group on these glucosides **3** and **4**, including the full detail of the previous report. The stabilizing effects of zeaxanthin **5**, its glucoside **3** and TZ-15 **1a** on liposomes are also reported.

Results and discussion

β-Glucosidation of 3-hydroxy-β-ionone 7

We first examined the β-selective glucosidation of (3*R*)-3-hydroxy-β-ionone **7** using glucosides **9a**–**c** carrying participating acyl groups in the C-2 position as glycosyl donors as shown in Table 1. The compound **7** was obtained (quantitatively) by deprotection of previously synthesized[7] *tert*-butyldimethylsilyl (TBS) ether **6** (Scheme 1). According to the mild thioglycoside method,[8] compound **7** was treated with methyl tetra-*O*-acetyl- or methyl tetra-*O*-benzoyl-1-thio-β-D-glucopyranosides **9a** or **9b** using Se-phenyl selenotriflate† as an activator (entries 1,2). However, the former compound **9a** provided only the acetylated compound **8**, and although **9b**

† The IUPAC name for triflate is trifluoromethanesulfonate.



1 R^1=x, R^2=OH: thermozeaxanthins
2 R^1=x, R^2=H: thermocryptoxanthins
3 R^1=y, R^2=OH: (3*R*,3'*R*)-zeaxanthin-mono-β-D-glucopyranoside
4 R^1=y, R^2=H: (3*R*)-cryptoxanthin-β-D-glucopyranoside
5 R^1=R^2=OH: (3*R*,3'*R*)-zeaxanthin

Fig. 1

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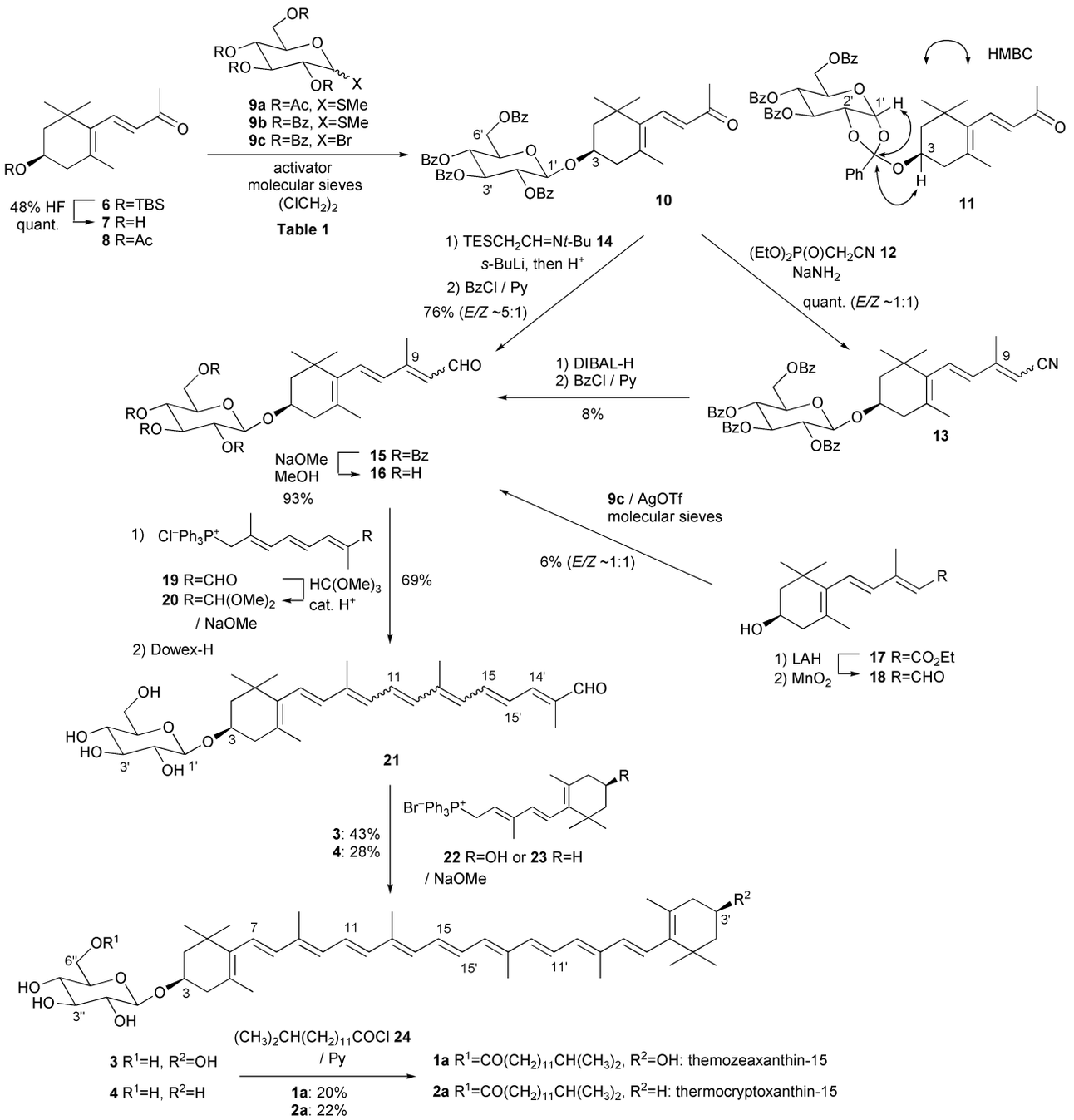
Table 1 Glucosidation of (3*R*)-3-hydroxy-β-ionone **7**

Entry	Glycosyl donor	Activator (equiv.)	Temp./Time	Product	Yield (%)
1	**9a**	PhSeOTf (1.4)	0 °C/50 min	**8**	46
2	**9b**	PhSeOTf (1.4)	0 °C/1 h	**10**	27
3	**9c**	AgOTf (2.0), $Me_2NC(O)NMe_2$ (3.0)	0 °C/30 min to rt/1 h	**11**	76
4	**9c**	AgOTf (2.0)	0 °C/2 h	**10**	66
5	**9c**	$HgBr_2$ (2.0)	rt/15 h	**10**	36

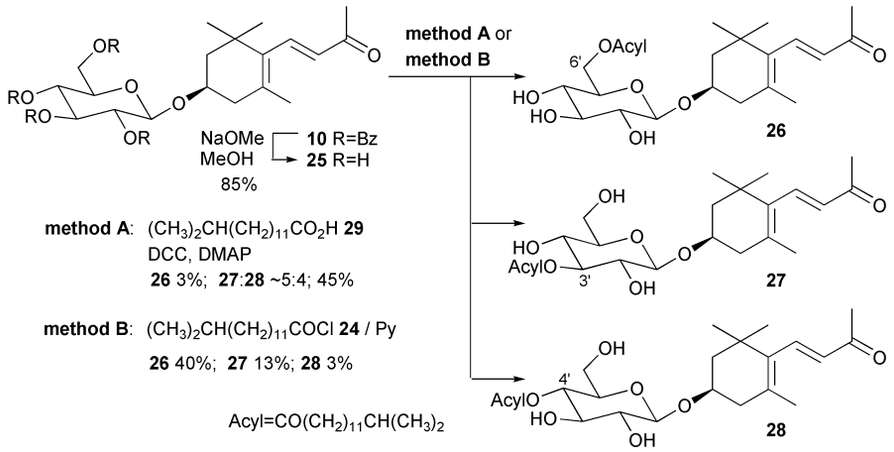


Scheme 1

afforded the desired β-glucoside **10** it was unfortunately in low yield.

Next, the glucosidation of **7** by use of glucosyl bromide **9c** as a glycosyl donor was investigated. Among activators tested (entries 3–5), the best yield (66%) of the β-glucoside **10** was obtained in the case of silver triflate (entry 4). It should be noted that combined use of *N*,*N*,*N*′,*N*′-tetramethylurea as a proton acceptor and silver triflate (entry 3) afforded the ortho ester **11** as a single product (76%). This finding is in marked contrast to the Banoub *et al.*'s report,[9] in which the glycosidation reaction between alcohol and acylated glycosyl halides under the same conditions provided 1,2-*trans*-glycosides.

The structures of compounds **10** and **11** were confirmed on the basis of their spectral data (see Experimental section). In secondary ion mass spectra (SIMS) of both compounds, quasimolecular ion peaks were observed at *m*/*z* at 809 $(M + Na)^+$ for both compounds. In the ^{13}C and ^1H NMR spectra of the compound **10**, four signals (*δ* 164.98, 165.28, 165.82, 166.07) based on carboxy carbons were observed and the anomeric proton signal appeared as a doublet at *δ* 4.98,



Scheme 2

showing a coupling constant of 8 Hz. Thus, compound **10** was confirmed to be a β-glucoside. On the other hand, only three carboxy carbon signals (δ 164.63, 165.17, 165.97) were observed in the ^{13}C NMR spectrum of compound **11**. A heteronuclear multiple-bond correlation (HMBC) experiment of **11** exhibited three-bond couplings between the quarternary carbon signal at δ 121.59 and both the anomeric proton signal at δ 6.06 and the signal at δ 3.71 (H-3) as shown in Scheme 1. These data indicated that **11** was an ortho ester.

Synthesis of zeaxanthin- and cryptoxanthin-β-D-glucosides 3 and 4

Glucosidation of (all-*E*)-(3*R*)-3-hydroxy-β-ionylideneacetaldehyde **18**, prepared from the previously synthesized[10] ester **17** in 2 steps (70%), was then carried out under optimum conditions as described above (Scheme 1). Nevertheless, the desired glucoside **16** was obtained only in poor yield. In addition, the isomerization of the 9,10-double bond occurred under the reaction conditions. Thus, the transformation of the ionone-glucoside **10** into the ionylideneacetaldehyde-glucoside **15** was examined. Horner–Emmons reaction of **10** with the cyanophosphonate **12** quantitatively afforded an isomeric mixture (*E*–*Z* ~1 : 1) of the nitrile **13**. However, conversion of **13** into the aldehyde **15** by reduction with DIBAL-H and subsequent benzoylation resulted in poor yield, probably due to the formation of organoaluminium complexes with the reducing intermediate. Thus, to avoid a troublesome reductive procedure, Peterson reaction of **10** with α-triethylsilyl (TES) imine **14**[11] was carried out. In this Peterson reaction, consumption of TES-imine **14** by benzoyl groups in **10** was inevitable, therefore an excess amount of **14** was required. The resulting mixture was subjected to acid hydrolysis followed by re-benzoylation to afford the desired aldehyde **15** (*E*–*Z* ~5 : 1) in good yield (76%).

Compound **15** (C_{15}) was then transformed into carotenoid-glucosides (C_{40}) **3** and **4** in 4 steps (C_{15} + C_{10} + C_{15}, Scheme 1). Methanolysis of the tetrabenzoate **15** provided the tetraol **16** (93%), which was condensed with the C_{10}-phosphonium salt **20**[12] in the presence of NaOMe as a base and then treated with ion-exchange resin, Dowex 50W-X8 (H^+), to furnish an isomeric mixture (all-*E*–other isomers ~3 : 1) of the C_{25}-apocarotenal-glucoside **21** in 69% yield. The Wittig condensation between this isomeric mixture **21** and C_{15}-phosphonium salt **22**[13] or **23**[14] using NaOMe as a base followed by purification of the condensed product employing preparative HPLC (PHPLC) provided (all-*E*)-zeaxanthin- or (all-*E*)-cryptoxanthin-β-D-glucopyranoside **3** (43%) or **4** (28%) accompanied by some isomers.

The structures of glucosides **3** and **4** were confirmed by comparison of their spectral data with those of TZ **1**,[2] TC **2**[3] and zeaxanthin **5**.[15] The visible absorption spectra of both compounds in ethanol (**3**: 427sh, 450 and 476 nm, **4**: 429sh, 452 and 472 nm) exhibited the chromophore such as β,β-carotene. In HRMS, **3** showed a molecular ion peak at *m*/*z* 730.4799 (calcd for $C_{46}H_{66}O_7$ 730.4804) and **4** at 714.4871 (calcd for $C_{46}H_{66}O_6$ 714.4856). Their ^{1}H NMR spectra were quite compatible with those of **1** and **2** respectively, except for the sugar and fatty acid moieties. Among the minor isomers of **3** and **4**, one isomer of **4** was isolated (10%) and could be characterized to be the (9′*Z*)-isomer by comparison of its ^{1}H NMR data with those of (9*Z*)-β-carotene.[16] However, other isomers could not be separated by HPLC.

This synthesis of the glucosides **3** and **4** consists of six steps with overall yields of 12% and 9%, respectively, from (3*R*)-3-hydroxy-β-ionone **7**.

Synthesis of thermozeaxanthin-15 1a and thermocryptoxanthin-15 2a

Towards synthesis of TZ **1** and TC **2**, the acylation of glucosides **3** and **4** was investigated. For a preliminary experiment, first we examined the acylation of β-ionone-glucoside **26**, which was obtained (85%) by methanolysis of the tetrabenzoate **10** (Scheme 2). The glucoside **25** was treated with the typical fatty acid **29**[17] in the presence of the dehydration reagent DCC to provide acylated products. However, the required 6′-acylate **26** (acylated compound of the primary hydroxy group on the glucoside) was unexpectedly a minor product and major products were 3′- and 4′-acylates **27** and **28**. On the other hand, treatment of the glucoside **25** with the corresponding acyl chloride **24** gave the 6′-acylate **26** predominantly. The structures of acylated products **26**, **27** and **28** were confirmed on the basis of ^{1}H NMR spectra, in which signals of the proton adjacent to an acyloxy moiety appeared at lower field (see Experimental section).

The glucosides **3** and **4** were then acylated by use of the acyl chloride **24** followed by purification with preparative TLC (PTLC) to afford TZ-15 **1a** (20%) and TC-15 **2a** (22%) in pure form, respectively (Scheme 1). Spectral data of synthetic **1a** and **2a** were in good agreement with those of natural specimens.[2,3] This is the first total synthesis of TZ and TC.

Stabilizing effects of thermozeaxanthin-15 1a, zeaxanthin-mono-β-glucoside 3 and zeaxanthin 5 on liposomes

The stabilizing effects of zeaxanthin **5**, its glucoside **3** and TZ-15 **1a** on liposomal lipid bilayers were investigated by measuring the extent of calcein released from liposomes according to the method shown in the literature.[4] The fluorescent dye, calcein was entrapped in liposomes composed of a small portion (1 mol%) of each sample and dipalmitoylphosphatidylcholine, and the leakage of calcein from the liposomes was determined by fluorescence measurement.

As depicted in Fig. 2, in which calcein released is plotted against time, TZ-15 **1a** had the best stabilizing effect. Although

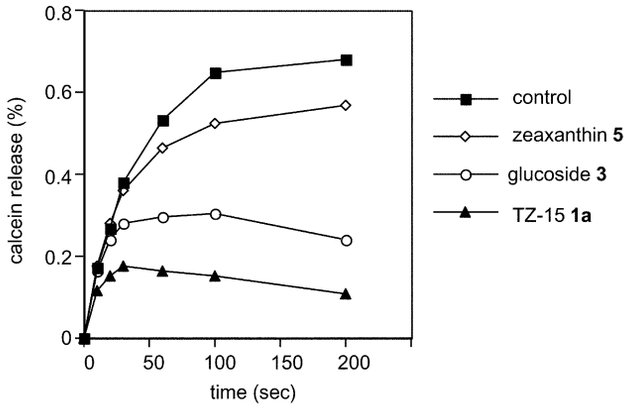


Fig. 2 Stabilizing effect of thermozeaxanthin-15 **1a**, zeaxanthin-mono-β-glucoside **3** and zeaxanthin **5** on liposomes.

further study on the exact structure of TZ in membrane is required, the distinct effect would indicate that TZ's unique "hydrophobic–hydrophilic–hydrophobic" structure is quite beneficial for stabilizing liposomal lipid bilayers.

Experimental

UV–VIS spectra were recorded on a JASCO Ubest-55 instrument. IR spectra were measured on a Perkin Elmer FT-IR spectrometer, model Paragon 1000, for chloroform solutions. ^{1}H NMR spectra at 200, 300 or 500 MHz were determined on a Varian Gemini-200, 300 or a Varian VXR-500 superconducting FT-NMR spectrometer, respectively, for deuteriochloroform solutions unless otherwise stated (tetramethylsilane as internal reference). ^{13}C NMR spectra at 125 MHz were measured on a Varian VXR-500 superconducting FT-NMR spectrometer in deuteriochloroform solutions using tetramethylsilane as an internal standard. *J*-Values are given in Hz. Mass spectra were taken on a Hitachi M-4100 spectrometer. Optical rotations were measured on a JASCO DIP-181 polarimeter ($[\alpha]_D$ values are in units of 10^{-1} deg cm^2 g^{-1}), and CD spectra on a Shimadzu-AVIN 62A DS circular dichroism spectrometer. Fluorescence intensity was measured on a Hitachi F-4500 spectrophotometer.

Column chromatography (CC) was performed on silica gel (Merck Art. 7734). Short-column chromatography (SCC) was conducted on silica gel (Merck Art. 7739) under reduced pressure. PTLC was performed on silica gel plate (Merck silica gel $60F_{254}$ precoated plate, 0.5 mm thickness). PHPLC was carried out on a Waters Model 510 instrument with a UV–VIS detector.

All operations were carried out under nitrogen or argon. Ether refers to diethyl ether, and hexane to *n*-hexane. NMR assignments are given using the carotenoid numbering system.

Glucosidation of 3-hydroxy-β-ionone 7 (Table 1)

General procedure. 3-Hydroxy-β-ionone **7** was treated with a glucosyl donor and an activator under the conditions shown in Table 1 and the reaction was quenched with saturated aq. $NaHCO_3$. The reaction mixture was diluted with AcOEt and filtered through Celite. The organic layer of the filtrate was washed with brine, dried and evaporated to give a residue, which was purified under the conditions described below.

Entry 1. To a stirred suspension of PhSeCl (130 mg, 0.68 mmol) and powdered molecular sieves 4 Å (1.5 g) in dry $(ClCH_2)_2$ (5 ml) was added AgOTf (180 mg, 0.70 mmol) at 0 °C and the mixture was stirred for a further 10 min. To this mixture was added the thioglycoside **9a**[8] (215 mg, 0.57 mmol) followed by dropwise addition of a solution of **7** (104 mg, 0.50 mmol) in dry $(ClCH_2)_2$ (20 ml) at 0 °C. After being stirred at 0 °C for 50 min, the reaction mixture was subjected to the general procedure to give a residue, which was purified by SCC (AcOEt–hexane, 1 : 4) to provide 3-acetoxy-β-ionone **8** (57 mg, 46%) as a colourless oil.

Entry 2. To a stirred suspension of PhSeCl (130 mg, 0.68 mmol) and powdered molecular sieves 4 Å (1.5 g) in dry $(ClCH_2)_2$ (5 ml) was added AgOTf (180 mg, 0.70 mmol) at 0 °C and the mixture was stirred for a further 10 min. To this mixture was added the thioglycoside **9b**[8] (380 mg, 0.61 mmol) followed by dropwise addition of a solution of **7** (110 mg, 0.53 mmol) in dry $(ClCH_2)_2$ (20 ml) at 0 °C. After being stirred at 0 °C for 1 h, the reaction mixture was subjected to the general procedure to give a residue, which was purified by CC (AcOEt–hexane, 3 : 7) to give the β-glucoside **10** (57 mg, 27%) as a pale yellow foam.

Entry 3. To a stirred suspension of glucosyl bromide **9c** (1.24 g, 1.88 mmol), **7** (300 mg, 1.44 mmol), *N*,*N*,*N*′,*N*′-tetramethylurea (0.52 ml, 4.34 mmol) and powdered molecular sieves 4 Å (5 g) in dry $(ClCH_2)_2$ (20 ml) was added AgOTf (740 mg, 2.88 mmol) at 0 °C. After being stirred at 0 °C for 30 min and 1 h at rt, the reaction mixture was subjected to the general procedure to give a residue, which was purified by CC (CH_2Cl_2–hexane–ether, 5 : 4 : 0.7) to give the ortho ester **11** (860 mg, 76%) as a pale yellow foam.

Entry 4. To a stirred suspension of glucosyl bromide **9c** (8.08 g, 12.3 mmol), **7** (1.50 g, 7.21 mmol) and powdered molecular sieves 4 Å (25 g) in dry $(ClCH_2)_2$ (75 ml) was added AgOTf (3.70 g, 14.4 mmol) at 0 °C. After being stirred at 0 °C for 2 h, the reaction mixture was subjected to the general procedure to give a residue, which was purified by CC (CH_2Cl_2–hexane–ether, 5 : 4 : 1) to yield the β-glucoside **10** (3.74 g, 66%) as a pale yellow solid.

Entry 5. To a stirred suspension of glucosyl bromide **9c** (1.45 g, 2.20 mmol), **7** (380 mg, 1.83 mmol) and powdered molecular sieves 4 Å (5 g) in dry $(ClCH_2)_2$ (20 ml) was added $HgBr_2$ (1.32 g, 3.66 mmol) at 0 °C. After being stirred at rt for 15 h, the reaction mixture was subjected to the general procedure to give a residue, which was purified by short CC (CH_2Cl_2–hexane–ether, 5 : 4 : 0.7) to afford the β-glucoside **10** (440 mg, 36%) as a pale yellow foam.

3-Acetoxy-β-ionone 8

λ_{max}(EtOH)/nm 287, 217; ν_{max}/cm^{-1} 1728 (OCO), 1668 (conj. CO), 1606 (C=C); δ_H (300 MHz) 1.06 and 1.10 (each 3H, s, *gem*-Me), 1.55 (1H, t, *J* 12, 2-H_{ax}), 1.71 (3H, s, 5-Me), 1.75 (1H, ddd, *J* 12, 3.5 and 2, 2-H_{eq}), 2.00 (3H, s, OAc), 2.10 (1H, dd, *J* 17.5 and 9.5, 4-H_{ax}), 2.25 (3H, s, 5-Me), 2.45 (1H, dd, *J* 17.5 and 5.5, 4-H_{eq}), 5.00 (1H, m, 3-H), 6.06 (1H, *J* 16.5, 8-H), 7.15 (1H, br d, *J* 16.5, 7-H) (Found: M$^+$, 250.1544. $C_{15}H_{22}O_3$ requires M, 250.1568).

β-Glucoside 10

$[\alpha]_D^{24}$ −3.99 (*c* 1.00, $CHCl_3$); λ_{max}(EtOH)/nm 287sh, 280, 275sh, 230; ν_{max}/cm^{-1}: 1732 (OCO), 1668 (conj. CO), 1603 (C=C); δ_H (500 MHz) 1.00 and 1.01 (each 3H, s, *gem*-Me), 1.56 (1H, t, *J* 12, 2-H_{ax}), 1.59 (3H, s, 5-Me), 1.88 (1H, ddd, *J* 12, 3 and 1.5, 2-H_{eq}), 2.00 (1H, br dd, *J* 18 and 10, 4-H_{ax}), 2.27 (3H, s, 9-Me), 2.29 (1H, br dd, *J* 18 and 5.5, 4-H_{eq}), 4.02 (1H, m, 3-H), 4.20 (1H, ddd, *J* 10, 6 and 3.5, 5′-H), 4.53 (1H, dd, *J* 12 and 6, 6′-H), 4.63 (1H, dd, *J* 12 and 3.5, 6′-H), 4.98 (1H, d, *J* 8, 1′-H), 5.51 (1H, dd, *J* 10 and 8, 2′-H), 5.63 (1H, t, *J* 10, 4′-H), 5.92 (1H, t, *J* 10, 3′-H), 6.00 (1H, *J* 16, 8-H), 7.10 (1H, br d, *J* 16, 7-H), 7.25–7.56 (12H, m, Ar–H), 7.84–8.02 (8H, m, Ar–H); δ_C (125 MHz) 21.39 (5-CH_3), 27.26 (CH_3CO), 28.49 (1-CH_3), 29.73 (1-CH_3), 36.48 (1-C), 39.29 (4-CH_2), 45.67 (2-CH_2), 63.46 (6′-CH_2), 69.99 (4′-CH), 72.05 (2′-CH), 72.29 (5′-CH), 72.90 (3′-CH), 73.15 (3-CH), 100.12 (1′-CH), 128.29, 128.39, 128.43, 129.70, 129.73, 129.76, 129.85, 133.19, 133.24 and 133.47 (Ar–CH), 128.75, 128.81, 129.37 and 129.58 (Ar–C), 131.17 (5-CH), 132.41 (8-CH), 135.89 (6-C), 142.10 (7-CH), 164.98

(2′-OCO), 165.28 (4′-OCO), 165.82 (3′-OCO), 166.07 (6′-OCO), 198.42 (9-C) [Found: (M + Na)$^+$, 809.2949. $C_{47}H_{46}O_{11}Na$ requires M + Na, 809.2935].

Ortho ester 11

$[\alpha]_D^{25}$ −13.74 (*c* 1.02, $CHCl_3$); λ_{max}(EtOH)/nm 285sh, 282, 275sh, 230; ν_{max}/cm^{-1}: 1725 (OCO), 1668 (conj. CO), 1603 (C=C); δ_H (500 MHz) 0.78 (3H, s, *gem*-Me), 0.99 (3H, s, *gem*-Me), 1.45 (1H, t, *J* 12, 2-H_{ax}), 1.51 (1H, ddd, *J* 12, 4 and 1.5, 2-H_{eq}), 1.69 (3H, s, 5-Me), 2.14 (1H, dd, *J* 17.5 and 9.5, 4-H_{ax}), 2.23 (1H, dd, *J* 17.5 and 6.5, 4-H_{eq}), 2.30 (3H, s, 9-Me), 3.71 (1H, m, 3-H), 4.11 (1H, ddd, *J* 8.5, 5 and 3, 5′-H), 4.36 (1H, dd, *J* 12 and 5, 6′-H), 4.52 (1H, dd, *J* 12 and 3, 6′-H), 4.79 (1H, ddd, *J* 5.5, 3 and 1, 2′-H), 5.50 (1H, dt, *J* 8.5 and 1, 4′-H), 5.78 (1H, dd, *J* 3 and 1, 3′-H), 6.02 (1H, d, *J* 16, 8-H), 6.06 (1H, d, *J* 5.5, 1′-H), 7.10 (1H, br d, *J* 16, 7-H), 7.24–7.65 (12H, m, Ar–H), 7.81–8.01 (8H, m, Ar–H); δ_C(125 MHz) 21.48 (5-CH_3), 27.30 (CH_3CO), 27.93 (1ax-CH_3), 29.79 (1eq-CH_3), 36.51 (1-C), 40.67 (4-CH_2), 45.74 (2-CH_2), 63.92 (6′-CH_2), 67.25 (3-CH), 67.44 (5′-CH), 68.55 (4′-CH), 69.16 (3′-CH), 71.98 (2′-CH), 97.59 (1′-CH), 121.59 (ortho ester-C), 126.43, 128.22, 128.46, 128.58, 129.70, 129.89, 130.04, 132.99, 133.53 and 133.69 (Ar–CH), 129.00, 129.13, 132.39 and 135.98 (Ar–C), 132.22 (8-CH), 132.39 (5-C), 135.56 (6-C), 142.05 (7-CH), 164.63 (3′-OCO), 165.17 (4′-OCO), 165.97 (6′-OCO), 198.38 (9-C) [Found: (M + Na)$^+$, 809.2940. $C_{47}H_{46}O_{11}Na$ requires M + Na, 809.2935].

(2*E*,4*E*)-5-[(4*R*)-4-Hydroxy-2,6,6-trimethylcyclohex-1-enyl]-3-methylpenta-2,4-dienal 18

A solution of the ester **17**[10] (1.30 g, 4.67 mmol) in dry ether (20 ml) was added dropwise to a stirred suspension of LAH (400 mg, 10.5 mmol) in dry ether (30 ml) at 0 °C and the mixture was stirred for a further 30 min. The excess of LAH was decomposed by dropwise addition of water and the mixture was extracted with ether. The extracts were washed with brine, dried and evaporated to afford a crude alcohol, which without purification was dissolved in ether–hexane (2 : 1) and shaken with active MnO_2 (10 g) at rt for 3 h. The mixture was filtered through Celite. Evaporation of the filtrate gave a residue, which was purified by SCC (acetone–hexane, 1 : 3) to give the aldehyde **18** (764 mg, 70% from **17**) as a colourless oil; $[\alpha]_D^{22}$ −96.1 (*c* 0.93, MeOH); λ_{max}(EtOH)/nm 318, 271sh; ν_{max}/cm^{-1} 3605 and 3453 (OH), 1658 (conj. CHO), 1609 (C=C); δ_H(300 MHz) 1.09 (6H, s, *gem*-Me), 1.50 (1H, t, *J* 12, 2-H_{ax}), 1.75 (3H, s, 5-Me), 1.81 (1H, ddd, *J* 12, 3.5 and 2, 2-H_{eq}), 2.07 (1H, br dd, *J* 17 and 9.5, 4-H_{ax}), 2.31 (3H, s, 9-Me), 2.42 (1H, br dd, *J* 17 and 5.5, 4-H_{eq}), 4.01 (1H, m, 3-H), 5.95 (1H, br d, *J* 8, 10-H), 6.21 (1H, *J* 16, 8-H), 6.68 (1H, br d, *J* 16, 7-H), 10.13 (1H, d, *J* 8, CHO) [Found: M$^+$, 234.1602. $C_{15}H_{22}O_2$ requires M, 234.1618].

Glucosidation of 3-hydroxy-β-ionylideneacetaldehyde 18

To a stirred suspension of glucosyl bromide **9c** (1.13 g, 1.32 mmol), **18** (330 mg, 1.71 mmol) and powdered molecular sieves 4 Å (5 g) in dry $(ClCH_2)_2$ (20 ml) was added AgOTf (678 mg, 2.46 mmol) at 0 °C. After being stirred at 0 °C for 1 h, the reaction was quenched with saturated aq. $NaHCO_3$. The reaction mixture was diluted with AcOEt and filtered through Celite. The organic layer of the filtrate was washed with brine, dried and evaporated to give a residue which was purified by CC (CH_2Cl_2–hexane–ether, 5 : 4 : 1) to afford an isomeric mixture of the ionylideneacetaldehyde-glucoside **15** (69 mg, 6%; all-*E*–9*Z* ~1 : 1). Purification of the isomeric mixture by CC (AcOEt–hexane, 1 : 3) provided each pure isomer as pale yellow foams.

(All-*E*) isomer. λ_{max}(EtOH)/nm 317, 273, 230; ν_{max}/cm^{-1} 1735 (OCO), 1657 (conj. CHO), 1603 (C=C); δ_H(300 MHz) 1.00 (6H, s, *gem*-Me), 1.57 (1H, t, *J* 12, 2-H_{ax}), 1.57 (3H, s, 5-Me), 1.88 (1H, ddd, *J* 12, 3.5 and 2, 2-H_{eq}), 1.97 (1H, br dd, *J* 17 and 9.5, 4-H_{ax}), 2.27 (3H, s, 9-Me), 2.29 (1H, br dd, *J* 17 and 5, 4-H_{eq}), 4.04 (1H, m, 3-H), 4.21 (1H, ddd, *J* 9.5, 6 and 3.5, 5′-H), 4.53 (1H, dd, *J* 12 and 6, 6′-H), 4.64 (1H, dd, *J* 12 and 3.5, 6′-H), 4.99 (1H, d, *J* 8, 1′-H), 5.52 (1H, dd, *J* 10 and 8, 2′-H), 5.64 (1H, t, *J* 10, 4′-H), 5.91 (1H, br d, *J* 8, 10-H), 5.93 (1H, t, *J* 10, 3′-H), 6.08 (1H, *J* 16, 8-H), 6.58 (1H, br d, *J* 16, 7-H), 7.26–7.60 (12H, m, Ar–H), 7.84–8.04 (8H, m, Ar–H), 10.12 (1H, d, *J* 8, CHO) [Found: (M + H)$^+$, 813.3262. $C_{49}H_{49}O_{11}$ requires M + H, 813.3272].

(9*Z*)-Isomer. λ_{max}(EtOH)/nm 316, 272, 227; ν_{max}/cm^{-1} 1732 (OCO), 1659 (conj. CHO), 1603 (C=C); δ_H(300 MHz) 1.00 (6H, s, *gem*-Me), 1.57 (1H, t, *J* 12, 2-H_{ax}), 1.60 (3H, s, 5-Me), 1.90 (1H, ddd, *J* 12, 3.5 and 2, 2-H_{eq}), 1.99 (1H, dd, *J* 17 and 9, 4-H_{ax}), 2.08 (3H, s, 9-Me), 2.29 (1H, dd, *J* 17 and 5, 4-H_{eq}), 4.05 (1H, m, 3-H), 4.23 (1H, ddd, *J* 10, 6 and 3.5, 5′-H), 4.54 (1H, dd, *J* 12 and 6, 6′-H), 4.66 (1H, dd, *J* 12 and 3.5, 6′-H), 5.02 (1H, d, *J* 8, 1′-H), 5.54 (1H, dd, *J* 10 and 8, 2′-H), 5.66 (1H, t, *J* 10, 4′-H), 5.87 (1H, br d, *J* 8, 10-H), 5.96 (1H, t, *J* 10, 3′-H), 6.48 (1H, br d, *J* 16, 7-H), 6.98 (1H, d, *J* 16, 8-H), 7.20–7.60 (12H, m, Ar–H), 7.80–8.20 (8H, m, Ar–H), 10.11 (1H, d, *J* 8, CHO).

Synthesis of the ionylideneacetaldehyde-glucoside 15 *via* reduction of the ionylideneacetonitrile 13

A solution of diethylphosphonoacetonitrile **12** (338 mg, 1.9 mmol) in dry THF (5 ml) was added dropwise to a suspension of $NaNH_2$ (74 mg, 1.9 mmol) in dry THF (3 ml) at 0 °C and the mixture was stirred at rt for 20 min. To this mixture was added dropwise a solution of the ionone-glucoside **10** (440 mg, 0.56 mmol) in dry THF (8 ml) at 0 °C and the mixture was stirred for a further 15 min. After being quenched with saturated aq. NH_4Cl, the mixture was extracted with AcOEt. The extracts were washed with brine, dried and evaporated to give a residue, which was purified by SCC (ether–hexane, 2 : 1) to provide an isomeric mixture of the ionylideneacetonitrile-glucoside **13** (453 mg, quant.; all-*E*–9*Z* ~1 : 1) as a pale yellow foam; λ_{max}(EtOH)/nm 297, 273, 230; λ_{max}/cm^{-1} 2213 (CN), 1732 (OCO), 1602 (C=C); δ_H (300 MHz) 0.96, 0.97 and 0.99 (6H, each s, *gem*-Me), 1.55 [$\frac{3}{2}$H, s, (*E*)-5-Me], 1.60 [$\frac{3}{2}$H, s, (*Z*)-5-Me], 2.01 [$\frac{3}{2}$H, d, *J* 1.5, (*Z*)-9-Me], 2.16 [$\frac{3}{2}$H, d, *J* 1, (*E*)-9-Me], 4.03 (1H, m, 3-H), 4.22 (1H, ddd, *J* 10, 6 and 3.5, 5′-H), 4.53 (1H, dd, *J* 12 and 6, 6′-H), 4.64 (1H, dd, *J* 12 and 3.5, 6′-H), 4.99 [½H, d, *J* 8, (*E*)-1′-H], 5.01 [½H, d, *J* 8, (*Z*)-1′-H], 5.11 [½H, br s, (*Z*)-10-H], 5.13 [½H, br s, (*E*)-10-H], 5.51 [½H, dd, *J* 10 and 8, (*E*)-2′-H], 5.52 [½H, dd, *J* 10 and 8, (*Z*)-2′-H], 5.64 (1H, t, *J* 10, 4′-H), 5.93 [½H, t, *J* 10, (*E*)-3′-H], 5.94 [½H, t, *J* 10, (*Z*)-3′-H], 6.01 [½H, d, *J* 16, (*E*)-8-H], 6.41 [½H, br d, *J* 16, (*E*)-7-H], 6.45 [½H, br d, *J* 16, (*Z*)-7-H], 6.62 [½H, d, *J* 16, (*Z*)-8-H], 7.25–7.58 (12H, m, Ar–H), 7.81–8.07 (8H, m, Ar–H) [Found: (M + Na)$^+$, 832.3102. $C_{49}H_{47}NO_{10}Na$ requires M + Na, 832.3095].

Subsequently, a solution of DIBAL-H (1.0 M in hexane; 7.5 ml, 7.5 mmol) was added dropwise to a solution of the isomeric mixture of **13** (453 mg, 0.56 mmol) in dry CH_2Cl_2 (10 ml) at −20 °C and the mixture was stirred for a further 15 min. The excess DIBAL-H was destroyed by an addition of moist silica gel (SiO_2–H_2O, 5 : 1) and the mixture was filtered through Celite. The filtrate was dried and evaporated to give a residue, which was dissolved in pyridine (Py) (5 ml) and BzCl (1 ml) was added to it. This mixture was stirred at rt for 1.5 h, poured into ice water and extracted with ether. The extracts were washed with aq. 5% HCl, saturated aq. $NaHCO_3$ and brine. Evaporation of the dried extracts afforded a residue, which was purified by CC (CH_2Cl_2–hexane–ether, 5 : 4 : 0.7) to give an isomeric mixture of the ionylideneacetaldehyde-glucoside **15** (35 mg, 8% from **13**; all-*E*–9*Z* ~1 : 1).

Synthesis of the ionylideneacetaldehyde-glucoside 15 by Peterson reaction of the ionone-glucoside 10 with TES-imine 14

To a solution of TES-imine **14**[11] (4.57 g, 21.5 mmol) in dry THF (30 ml) was added *s*-BuLi (0.97 M in hexane; 22.4 ml, 21.7 mmol) at −78 °C and the mixture was stirred for a further 20 min. To this mixture was added dropwise a solution of the ionone-glucoside **10** (2.11 g, 2.68 mmol) in dry THF (25 ml) at −78 °C and the mixture was stirred at −78 °C for 20 min. After being quenched with saturated aq. oxalic acid, the mixture was extracted with AcOEt. The extracts were washed with saturated aq. $NaHCO_3$ and brine. Evaporation of the dried extracts gave a residue, which was dissolved in Py (20 ml). To the solution was added BzCl (5 ml) and the mixture was stirred at rt for 2 h, poured into ice water and extracted with ether. The extracts were washed with aq. 5% HCl, saturated aq. $NaHCO_3$ and brine. Evaporation of the dried extracts afforded a residue, which was purified by CC (CH_2Cl_2–hexane–ether, 5 : 4 : 1) followed by SCC (AcOEt–hexane 1 : 3) to give an isomeric mixture of the ionylideneacetaldehyde-glucoside **15** (1.65 g, 76% from **10**; all-*E*–9*Z* ~5 : 1).

(2*E*/*Z*,4*E*)-5-[(4*R*)-4-(β-D-Glucopyranosyloxy)-2,6,6-trimethylcyclohex-1-enyl]-3-methylpenta-2,4-dienal 16

To a solution of the isomeric mixture of benzoate **15** (923 mg, 1.14 mmol; all-*E*–9*Z* ~5 : 1) in MeOH (40 ml) was added NaOMe (1 M in MeOH; 1.5 ml, 1.5 mmol) and the mixture was stirred at rt for 40 min. To this mixture was added Dowex 50W-X8 (H^+) (3 g) and stirring continued at rt for a further 30 min. After Dowex was filtered off, the filtrate was evaporated to give a residue which was purified by CC (CH_2Cl_2–MeOH, 9 : 1) to yield an isomeric mixture of the tetraol **16** (420 mg, 93%; all-*E*–9*Z* ~5 : 1) as a yellow foam; λ_{max}(EtOH)/nm 316, 275sh, 231; ν_{max}/cm^{-1} 3406 (OH), 1660 (conj. CHO), 1609 (C=C); δ_H(300 MHz) 1.07 (6H, br s, *gem*-Me), 1.71 [$\frac{5}{2}$H, s, (*E*)-5-Me], 1.74 [½H, s, (*Z*)-5-Me], 2.11 [½H, s, (*Z*)-9-Me], 2.29 [$\frac{5}{2}$H, s, (*E*)-9-Me], 3.37 (1H, m, 5′-H), 3.46 (1H, br t-like, *J* 7.5, 2′-H), 3.61 (1H, br t, *J* 9, 4′-H), 3.71 (1H, br t, *J* 9, 3′-H), 3.82–3.95 (2H, m, 6′-H_2), 4.05 (1H, m, 3-H), 4.51 (1H, br d, *J* 7.5, 1′-H), 5.88 [$\frac{1}{6}$H, br d, *J* 7.5, (*Z*)-10-H], 5.91 [$\frac{5}{6}$H, br d, *J* 8, (*E*)-10-H], 6.16 [$\frac{5}{6}$H, d, *J* 16, (*E*)-8-H], 6.53 [$\frac{1}{6}$H, br d, *J* 16, (*Z*)-7-H], 6.63 [$\frac{5}{6}$H, br d, *J* 16, (*E*)-7-H], 7.07 [$\frac{1}{6}$H, d, *J* 16, (*Z*)-8-H], 10.10 [$\frac{1}{6}$H, d, *J* 7.5, (*Z*)-CHO], 10.12 [$\frac{5}{6}$H, d, *J* 8, (*E*)-CHO] [Found: $(M + H)^+$, 397.2240. $C_{21}H_{33}O_7$ requires M + H, 397.2224].

13-[(4*R*)-4-(β-D-Glucopyranosyloxy)-2,6,6-trimethylcyclohex-1-enyl]-2,7,11-trimethyltrideca-2,4,6,8,10,12-hexaenal 21

An acidic solution (0.8 ml) prepared from toluene-*p*-sulfonic acid (*p*-TsOH) (500 mg) and H_3PO_4 (725 mg) in MeOH (38 ml) and methyl orthoformate (0.8 ml) were added to a solution of the C_{10}-phosphonium chloride **19**[12] (790 mg, 1.77 mmol) in MeOH (5 ml). The reaction mixture was stirred at rt for 2 h and neutralized with NaOMe (1 M in MeOH) until just before the red colour of an ylide appeared to give a solution of the Wittig salt **20**. To this solution were added a solution of the isomeric mixture of C_{15}-aldehyde **16** (140 mg, 0.35 mmol, all-*E*–9*Z* ~5 : 1) in CH_2Cl_2 (4 ml) and NaOMe (1 M in MeOH; 2 ml, 2 mmol) at 0 °C. After being stirred at 0 °C for 30 min and then at rt for 30 min, Dowex 50W-X8 (H^+) (3 g) was added to the reaction mixture and this was stirred at rt for 15 min. After Dowex was filtered off, the filtrate was evaporated. The resulting residue was purified by SCC (CH_2Cl_2–MeOH, 93 : 7) and then additional SCC (CH_2Cl_2–ether–MeOH, 4 : 5 : 1) to yield an isomeric mixture of the apocarotenal-glucoside **21** (128 mg, 69% from **16**; all-*E*–other isomers ~3 : 1) as an orange foam; λ_{max} (EtOH)/nm 423; ν_{max}/cm^{-1} 3407 (OH), 1659 (conj. CHO), 1610 and 1547 (C=C); δ_H(300 MHz: protons corresponding to the all-*E* isomer were assigned) 1.09 (6H, s, *gem*-Me), 1.75 (3H, s, 5-Me), 1.87 (3H, s, 9-Me), 1.97 (3H, s, 13-Me), 2.02 (3H, s, 13′-Me), 3.35–3.53 (2H, m, 2′-H and 5′-H), 3.62 (1H, br t, *J* 8.5, 4′-H), 3.71 (1H, br t, *J* 8.5, 3′-H), 3.91 (2H, m, 6′-H_2), 4.08 (1H, m, 3-H), 4.53 (1H, d, *J* 7, 1′-H), 4.71, 5.13 and 5.49 (each 1H, br s, OH × 3), 6.05–6.21 (3H, m, 7-H, 8-H and 10-H), 6.27 (1H, br d, *J* 11, 14-H), 6.35 (1H, br d, *J* 14.5, 12-H), 6.66 (1H, dd, *J* 14 and 12, 15′-H), 6.81 (1H, br dd, *J* 14.5 and 12, 11-H), 6.95 (1H, br d, *J* 11, 14′-H), 7.01 (1H, dd, *J* 14 and 12, 15-H), 9.44 (1H, s, CHO) (Found: M^-, 528.3082. $C_{31}H_{44}O_7$ requires M, 528.3085).

(3*R*,3′*R*)-3′-(β-D-Glucopyranosyloxy)-β,β-caroten-3-ol 3

To a solution of the phosphonium salt **22**[13] (383 mg, 0.68 mmol) and the isomeric mixture of apocarotenal-glucoside **21** (120 mg, 0.23 mmol; all-*E*—other isomers ~3 : 1) in CH_2Cl_2–MeOH (1 : 1; 10 ml) was added NaOMe (1 M in MeOH; 1 ml, 1 mmol) at 0 °C. After being stirred at rt for 3 h, Dowex 50W-X8 (H^+) (3 g) was added to the reaction mixture and this was stirred at rt for 20 min. After Dowex was filtered off, the filtrate was evaporated. The resulting residue was purified by SCC (CH_2Cl_2–ether–MeOH, 4 : 5 : 1) and then PHPLC [CHEMCOSORB 7-ODS-H, 10 × 30 cm; MeOH–H_2O (95 : 5)] to provide the all-*E* zeaxanthin-mono-β-glucoside **3** (71 mg, 43%) as a red solid; λ_{max}(EtOH)/nm 476, 450, 427sh, 277; λ_{max}(acetone)/nm 479, 453, 427sh; ν_{max}/cm^{-1} 3632 and 3440 (OH); δ_H($CDCl_3$ + CD_3OD, 300 MHz) 1.08 (12H, s, 1-*gem*-Me and 1′-*gem*-Me), 1.46 (1H, t, *J* 12, 2′-H_{ax}), 1.57 (1H, t, *J* 12, 2-H_{ax}), 1.74 (6H, s, 5-Me and 5′-Me), 1.75 (1H, m, 2′-H_{eq}), 1.86 (1H, m, 2-H_{eq}), 1.97 (12H, s, 9-Me, 9′-Me, 13-Me and 13′-Me), 1.98–2.16 (2H, m, 4-H_{ax} and 4′-H_{ax}), 2.36 (1H, br dd, *J* 17 and 4.5, 4′-H_{eq}), 2.46 (1H, br dd, *J* 17 and 5, 4-H_{eq}), 3.26 (1H, br dd, *J* 9 and 8, 2″-H), 3.32 (1H, m, 5″-H), 3.43–3.50 (2H, m, 3″-H and 4″-H), 3.79 (1H, dd, *J* 12 and 4.5, 6″-H), 3.87 (1H, dd, *J* 12 and 3, 6″-H), 3.96 (1H, m, 3′-H), 4.08 (1H, m, 3-H), 4.47 (1H, d, *J* 8, 1″-H), 6.02–6.16 (4H, m, 7-H, 7′-H, 8-H and 8′-H), 6.16 (2H, br d, *J* 11.5, 10-H and 10′-H), 6.26 (2H, br d-like, *J* 9.5, 14-H and 14′-H), 6.37 (2H, d, *J* 15, 12-H and 12′-H), 6.58–6.71 (4H, m, 11-H, 11′-H, 15-H and 15′-H); CD[ether–2-methylbutane–EtOH (5 : 5 : 2)] nm (Δε) 212 (0), 222 (−13.1), 235 (0), 248 (+8.5), 262 (0), 284 (−17.6), 315 (0), 342 (+3.8), 380 (0) (Found: M^+, 730.4799. $C_{46}H_{66}O_7$ requires M, 730.4804).

(3*R*)-3-(β-D-Glucopyranosyloxy)-β,β-carotene 4

In the same manner as described for the preparation of zeaxanthin-mono-β-glucoside **3**, Wittig reaction between the phosphonium salt **23**[14] (1.2 g, 22 mmol) and the apocarotenal-glucoside **21** (195 mg, 0.37 mmol) produced crude products, which were purified by SCC (CH_2Cl_2–ether–MeOH, 4 : 5 : 1.3) and then PHPLC [CHEMCOSORB 7-ODS-H, 10 × 30 cm; MeOH–EtOH (95 : 5)] to provide the (all-*E*)-cryptoxanthin-glucoside **4** (74 mg, 28%) and its 9′Z isomer (27 mg, 10%) as red solids, respectively.

(All-*E*) isomer 4. λ_{max}(EtOH)/nm 472, 452, 429sh, 275; λ_{max}(acetone)/nm 481, 455, 430sh; ν_{max}/cm^{-1} 3631 and 3423 (OH); δ_H($CDCl_3$ + CD_3OD, 300 MHz) 1.03 and 1.07 (each 6H, s, 1-*gem*-Me and 1′-*gem*-Me), 1.47 (2H, m, 2′-CH_2), 1.57 (1H, br t, *J* 12, 2-H_{ax}), 1.62 (2H, m, 3′-H_2), 1.72 and 1.74 (each 3H, s, 5-Me and 5′-Me), 1.86 (1H, br d, *J* 12, 2-H_{eq}), 1.97 (12H, s, 9-Me, 9′-Me, 13-Me and 13′-Me), 2.02 (2H, m, 4′-H_2), 2.11 (1H, br dd, *J* 16.5 and 9, 4-H_{ax}), 2.46 (1H, br dd, *J* 16.5 and 5, 4-H_{eq}), 3.20–3.39 (2H, m, 2″-H and 5″-H), 3.48 (2H, m, 3″-H and 4″-H), 3.80 (1H, br dd, *J* 12 and 4.5, 6″-H), 3.87 (1H, br dd, *J* 12 and 3, 6″-H), 4.07 (1H, m, 3-H), 4.47 (1H, d, *J* 7.5, 1″-H), 6.05–6.21 (6H, m, 7-H, 7′-H, 10-H, 10′-H, 8-H and 8′-H), 6.26 (2H, br d, *J* 8, 14-H and 14′-H), 6.35 and 6.36 (each 1H, br d, *J* 15, 12-H and 12′-H), 6.57–6.71 (4H, m, 11-H, 11′-H, 15-H and 15′-H); CD[ether–2-methylbutane–EtOH (5 : 5 : 2)] nm (Δε) 213 (0), 222 (−9.3), 233 (0), 247 (+8.8), 262 (0), 284

(−13.5), 316 (0), 340 (+2.7), 380 (0) (Found: M^+, 714.4871. $C_{46}H_{66}O_6$ requires M, 714.4856).

(9′*Z*)-Isomer. λ_{max}(EtOH)/nm 473, 446, 420sh, 340, 265; δ_H(500 MHz) 0.98 and 1.04 (each 6H, s, 1-*gem*-Me and 1′-*gem*-Me), 1.42 (2H, m, 2′-H_2), 1.52–1.60 (3H, m, 2-H_{ax} and 3′-H_2), 1.67 and 1.73 (each 3H, s, 5-Me and 5′-Me), 1.84 (1H, br d, *J* 13, 2-H_{eq}), 1.91, 1.92 and 1.93 (12H, each s, 9-Me, 9′-Me, 13-Me and 13′-Me), 1.97 (2H, br t, *J* 6, 4′-H_2), 2.08 (1H, br dd, *J* 16.5 and 9, 4-H_{ax}), 2.44 (1H, br dd, *J* 16.5 and 5, 4-H_{eq}), 3.30 (2H, m, 2″-H and 5″-H), 3.49 (1H, br t, *J* 8.5, 4″-H), 3.52 (1H, br t, *J* 8.5, 3″-H), 3.79 (1H, br dd, *J* 12 and 3.5, 6″-H), 3.84 (1H, br d, *J* 12.5, 6″-H), 4.06 (1H, m, 3-H), 4.45 (1H, d, *J* 7.5, 1″-H), 6.02 (1H, br d, *J* 12, 10′-H), 6.08–6.12 (2H, m, 7′-H and 10-H), 6.09 (1H, d, *J* 16, 8-H), 6.13 (1H, br d, *J* 16, 7-H), 6.20 (2H, br d-like, *J* 9.5, 14-H and 14′-H), 6.25 (1H, d, *J* 15, 12′-H), 6.30 (1H, d, *J* 15, 12-H), 6.59 (3H, m, 11-H, 15-H and 15′-H), 6.61 (1H, d, *J* 16.5, 8′-H), 6.68 (1H, dd, *J* 15 and 12, 11′-H).

(3*E*)-4-[(4*R*)-4-(β-D-Glucopyranosyloxy)-2,6,6-trimethylcyclohex-1-enyl]but-3-en-2-one 25

According to the procedure described in the preparation of the compound **16**, methanolysis of the tetrabenzoate **10** (1.83 g) followed by purification by SCC (CH_2Cl_2–MeOH, 9 : 1) gave the tetraol **25** (733 mg, 85%) as a pale yellow foam; λ_{max}(EtOH)/nm 291, 218; ν_{max}/cm^{-1} 3631 and 3406 (OH), 1670 (conj. CO), 1606 (C=C); δ_H ($CDCl_3$ + D_2O, 300 MHz) 1.06 and 1.09 (each 3H, s, *gem*-Me), 1.53 (1H, t, *J* 12, 2-H_{ax}), 1.73 (3H, s, 5-Me), 1.86 (1H, br d, *J* 12, 2-H_{eq}), 2.13 (1H, br dd, *J* 18 and 9, 4-H_{ax}), 2.28 (3H, s, 9-Me), 2.48 (1H, br dd, *J* 18 and 4.5, 4-H_{eq}), 3.32 (2H, m, 2′-H and 5′-H), 3.49 (1H, t, *J* 9, 4′-H), 3.57 (1H, t, *J* 9, 3′-H), 3.82 (2H, m, 6′-H_2), 4.03 (1H, m, 3-H), 4.46 (1H, d, *J* 8, 1′-H), 6.05 (1H, d, *J* 16.5, 8-H), 7.15 (1H, br d, *J* 16.5, 7-H) [Found: $(M + H)^+$, 371.2069. $C_{19}H_{31}O_7$ requires M + H, 371.2068].

Acylation of the glucoside 25 (Scheme 2)

Method A. To an ice-cooled solution of the glucoside **25** (180 mg, 0.49 mmol), the fatty acid **29**[17] (121 mg, 0.50 mmol) and DMAP (61 mg, 0.50 mmol) in dry CH_2Cl_2 (15 ml) was added DCC (103 mg, 0.50 mmol). After being stirred at rt for 3 h, the reaction mixture was diluted with AcOEt. The organic layer was washed successively with aq. 5% HCl, saturated aq. $NaHCO_3$ and brine. Evaporation of the dried extracts provided a residue, which was purified by SCC (CH_2Cl_2–MeOH, 97 : 3) and then PTLC (CH_2Cl_2–ether–MeOH, 4 : 4 : 1) to afford the 6′-acylate **26** (8 mg, 3%) and a mixture of the 3′-acylate **27** and the 4′-acylate **28** (130 mg, 45%; **27**–**28** ~5 : 4) as yellow foams.

Method B. A solution of the acyl chloride **24** prepared from the corresponding acid **29**[17] (53 mg, 0.22 mmol) in CH_2Cl_2 (1 ml) was added to a solution of the glucoside **25** (200 mg, 0.22 mmol) and Py (1.5 ml) in CH_2Cl_2 (1.5 ml). After being stirred at rt for 30 min, the reaction mixture was diluted with AcOEt. The organic layer was washed successively with aq. 5% HCl, saturated aq. $NaHCO_3$ and brine. Evaporation of the dried solution provided a residue, which was purified by SCC (CH_2Cl_2–MeOH, 95 : 5) to afford the 6′-acylate **26** (129 mg, 40%) and a mixture of the 3′-acylate **27** and the 4′-acylate **28**. This mixture was then purified by PTLC (CH_2Cl_2–MeOH, 94 : 6) to afford the 3′-acylate **27** (42 mg, 13%) and the 4′-acylate **28** (9 mg, 3%).

Compound 26

λ_{max}(EtOH)/nm 291, 218; ν_{max}/cm^{-1} 3590 and 3434 (OH), 1732 (OCO), 1669 (conj. CO), 1606 (C=C); δ_H(300 MHz) 0.86 (6H, d, *J* 6.5, CH*Me*$_2$), 1.10 and 1.12 (each 3H, s, *gem*-Me), 1.10–1.36 (18H, m, CH_2 × 9), 1.51 (1H, nonet, *J* 6.5, C*H*Me_2), 1.57 (1H, t, *J* 12, 2-H_{ax}), 1.62 (2H, m, C$H_2$$CH_2$CO), 1.77 (3H, s, 5-Me), 1.93 (1H, br d, *J* 12, 2-H_{eq}), 2.16 (1H, br dd, *J* 17 and 9, 4-H_{ax}), 2.30 (3H, s, 9-Me), 2.33 (2H, t, *J* 7.5, CH_2CO), 2.47 (1H, dd, *J* 17 and 6, 4-H_{eq}), 3.39 (2H, br t-like, *J* 9.5, 2′-H and 4′-H), 3.50 (1H, ddd, *J* 9.5, 6 and 2.5, 5′-H), 3.57 (1H, t, *J* 9, 3′-H), 4.02 (1H, m, 3-H), 4.32 (1H, dd, *J* 12 and 6, 6′-H), 4.39 (1H, dd, *J* 12 and 1.5, 6′-H), 4.43 (1H, d, *J* 7.5, 1′-H), 6.10 (1H, d, *J* 16, 8-H), 7.20 (1H, br d, *J* 16, 7-H) [Found: $(M + Na)^+$, 617.4011. $C_{34}H_{58}O_8Na$ requires M + Na, 617.4026].

Compound 27

λ_{max}(EtOH)/nm 291, 218; ν_{max}/cm^{-1} 3602 and 3436 (OH), 1726 (OCO), 1668 (conj. CO), 1606 (C=C); δ_H(300 MHz) 0.86 (6H, d, *J* 6.5, CH*Me*$_2$), 1.10 and 1.12 (each 3H, s, *gem*-Me), 1.13–1.40 (18H, m, CH_2 × 9), 1.51 (1H, nonet, *J* 6.5, C*H*Me_2), 1.64 (1H, br t, *J* 12, 2-H_{ax}), 1.66 (2H, m, C$H_2$$CH_2$CO), 1.77 (3H, s, 5-Me), 1.89 (1H, br ddd, *J* 12.5, 3.5 and 2, 2-H_{eq}), 2.14 (1H, br dd, *J* 17.5 and 9.5, 4-H_{ax}), 2.30 (3H, s, 9-Me), 2.42 (2H, t, *J* 7.5, CH_2CO), 2.49 (1H, br dd, *J* 17.5 and 5.5, 4-H_{eq}), 2.56 (1H, br s, OH), 3.44 (1H, m, 5′-H), 3.47 (1H, t-like, *J* 8, 2′-H), 3.68 (1H, br t, *J* 9, 4′-H), 3.84 (1H, br dd, *J* 12 and 4.5, 6′-H), 3.93 (1H, br dd, *J* 12 and 4, 6′-H), 4.07 (1H, m, 3-H), 4.54 (1H, d, *J* 8, 1′-H), 4.93 (1H, t, *J* 9, 3′-H), 6.10 (1H, d, *J* 16.5, 8-H), 7.19 (1H, br d, *J* 16.5, 7-H) [Found: $(M + Na)^+$, 617.4014. $C_{34}H_{58}O_8Na$ requires M + Na, 617.4026].

Compound 28

λ_{max}(EtOH)/nm 291, 218; ν_{max}/cm^{-1} 3598 and 3489 (OH), 1731 (OCO), 1668 (conj. CO), 1603 (C=C); δ_H(300 MHz) 0.86 (6H, d, *J* 6.5, CH*Me*$_2$), 1.11 (6H, s, *gem*-Me), 1.12–1.40 (18H, m, CH_2 × 9), 1.51 (1H, nonet, *J* 6.5, C*H*Me_2), 1.60 (1H, br t, *J* 12.5, 2-H_{ax}), 1.64 (2H, m, C$H_2$$CH_2$CO), 1.77 (3H, s, 5-Me), 1.91 (1H, br ddd, *J* 12.5, 3.5 and 2, 2-H_{eq}), 2.15 (1H, br dd, *J* 17 and 9, 4-H_{ax}), 2.30 (3H, s, 9-Me), 2.33–2.42 (2H, m, CH_2CO), 2.48 (1H, m, 4-Heq), 3.45 (2H, m, 2′-H and 5′-H), 3.60 (1H, dd, *J* 12.5 and 5, 6′-H), 3.73 (1H, dd, *J* 12.5 and 2.5, 6′-H), 3.74 (1H, t, *J* 9, 3′-H), 4.06 (1H, m, 3-H), 4.48 (1H, d, *J* 8, 1′-H), 4.87 (1H, t, *J* 9, 4′-H), 6.10 (1H, d, *J* 16.5, 8-H), 7.19 (1H, d, *J* 16.5, 7-H) [Found: $(M + Na)^+$, 617.4011. $C_{34}H_{58}O_8Na$ requires M + Na, 617.4026].

Synthesis of thermozeaxanthin-15 1a

In the same manner as described for the acylation of the glucoside **25** by method B, zeaxanthin-mono-β-glucoside **3** (28 mg) was treated with the acyl chloride **24** to give crude products, which were purified by PTLC (CH_2Cl_2–ether–MeOH, 4 : 5 : 1) to provide TZ-15 **1a** (8 mg, 22%) as a red solid. Spectral properties of the synthetic TZ-15 **1a** were in agreement with those[2] of a natural specimen; λ_{max}(acetone)/nm 479, 453, 429sh; ν_{max}/cm^{-1} 3580 and 3487 (OH), 1728 (OCO); δ_H(300 MHz) 0.86 (6H, d, *J* 6.5, CH*Me*$_2$), 1.07 (12H, s, 1-*gem*-Me and 1′-*gem*-Me), 1.10–1.40 (18H, m, CH_2 × 9), 1.42–1.71 (5H, m, 2-H_{ax}, 2′-H_{ax}, C$H_2$$CH_2$CO and C*H*$Me_2$), 1.78 (1H, m, 2′-$H_{eq}$), 1.92 (1H, m, 2-$H_{eq}$), 1.97 (12H, s, 9-Me, 9′-Me, 13-Me and 13′-Me), 2.04 (1H, br dd, *J* 17 and 8.5, 4′-H_{ax}), 2.14 (1H, br dd, *J* 17 and 8, 4-H_{ax}), 2.36 (2H, t, *J* 7.5, CH_2CO), 2.40 (2H, m, 4-H_{eq} and 4′-H_{eq}), 3.37 (1H, dd, *J* 9 and 8, 2″-H), 3.40 (1H, t, *J* 9, 4″-H), 3.49 (1H, ddd, *J* 9, 4.5 and 2, 5″-H), 3.59 (1H, t, *J* 9, 3″-H), 4.03 (2H, m, 3-H and 3′-H), 4.30 (1H, dd, *J* 12 and 2, 6″-H), 4.44 (1H, d, *J* 8, 1″-H), 4.49 (1H, dd, *J* 12 and 4.5, 6″-H), 6.04–6.14 (4H, m, 7-H, 7′-H, 8-H and 8′-H), 6.15 (2H, br d, *J* 11, 10-H and 10′-H), 6.25 (2H, br d-like, *J* 10, 14-H and 14′-H), 6.36 (2H, d, *J* 15, 12-H and 12′-H), 6.57–6.70 (4H, m, 11-H, 11′-H, 15-H and 15′-H); CD[ether–2-methylbutane–EtOH (5 : 5 : 2)] nm (Δε) 213 (0), 224 (−9.8), 238 (0), 250 (+5.5), 263 (0), 285 (−11.9), 343 (0) (Found: M^+, 954.6953. $C_{61}H_{94}O_8$ requires M, 954.6944).

Synthesis of thermocryptoxanthin-15 2a

In the same manner as described for the acylation of the glucoside **25** by method B, cryptoxanthin-glucoside **4** (32 mg) was treated with the acyl chloride **24** to give crude products, which

were purified by PTLC (CH_2Cl_2–ether–MeOH, 4 : 5 : 1) to provide TC-15 **2a** (8.3 mg, 20%) as a red solid. Spectral properties of the synthetic TC-15 **2a** were in agreement with those[3] of a natural specimen; λ_{max}(acetone)/nm 480, 454, 428sh; ν_{max}/cm^{-1} 3593 and 3468 (OH), 1729 (OCO); δ_H(500 MHz) 0.84 (6H, d, *J* 6.5, CH*Me*$_2$), 1.01 (6H, s, 1′-*gem*-Me), 1.05 and 1.06 (each 3H, s, 1-*gem*-Me), 1.13–1.31 (18H, m, CH_2 × 9), 1.45 (2H, m, 2′-CH_2), 1.49 (1H, m, C*H*Me$_2$), 1.54 (1H, 2-H$_{ax}$), 1.57–1.64 (4H, m, C*H*$_2$CH$_2$CO, 3′-H_2), 1.70 (3H, s, 5′-Me), 1.72 (3H, s, 5-Me), 1.89 (1H, br d, *J* 12, 2-H$_{eq}$), 1.94 (3H) and 1.95 (9H) (each s, 9-Me, 9′-Me, 13-Me and 13′-Me), 2.00 (2H, t, *J* 6, 4′-H_2), 2.09 (1H, dd, *J* 17 and 10, 4-H$_{ax}$), 2.34 (2H, t, *J* 7.5, CH_2CO), 2.40 (1H, br dd, *J* 17 and 6, 4-H$_{eq}$), 2.41 (1H, d, *J* 2, 2″-OH), 2.72 (1H, d, *J* 2, 3″-OH), 2.96 (1H, d, *J* 3, 4″-OH), 3.36 (1H, ddd, *J* 9.5, 8 and 2, 2″-H), 3.38 (1H, td, *J* 9.5 and 3, 4″-H), 3.46 (1H, ddd, *J* 10, 5 and 2, 5″-H), 3.57 (1H, td, *J* 9 and 2, 3″-H), 4.03 (1H, m, 3-H), 4.27 (1H, dd, *J* 12 and 2, 6″-H), 4.42 (1H, d, *J* 8, 1″-H), 4.48 (1H, dd, *J* 12 and 5, 6″-H), 6.06 (1H, br d, *J* 16.5, 7-H), 6.11 (2H, d, *J* 16.5, 8-H and 8′-H), 6.13 (2H, br d, *J* 11, 10-H and 10′-H), 6.16 (1H, br d, *J* 16.5, 7′-H), 6.23 (2H, m, 14-H and 14′-H), 6.33 and 6.34 (each 1H, d, *J* 14.5, 12-H and 12′-H), 6.59–6.66 (4H, m, 11-H, 11′-H, 15-H and 15′-H); CD[ether–2-methylbutane–EtOH (5 : 5 : 2)] nm ($\Delta\varepsilon$) 212 (0), 223 (−6.2), 235 (0), 248 (+4.0), 261 (0), 285 (−8.0), 318 (0), 343 (+1.5), 370 (0) (Found: M$^+$, 938.7005. $C_{34}H_{58}O_8Na$ requires M, 938.6994).

Stabilizing effects of thermozeaxanthin-15 1a, zeaxanthin-mono-β-glucoside 3 and zeaxanthin 5 on liposomes

Calcein-entrapped large unilamellar liposomes composed of a small portion (1 mol%) of each sample and dipalmitoylphosphatidylcholine were prepared by the method as described before.[4] Leakage of calcein from the liposomes was determined by fluorescence measurement with an excitation at 488 nm and emission at 517 nm. The calcein-entrapped liposomes were diluted 1000-fold with buffer (50 mM Tris-HCl, pH 7.5) in a cuvette and kept for a few minutes at rt (25 °C); release of the calcein from the liposomal interiors was monitored as a function of time. The percentage of the released calcein was calculated as follows:% release = $(F' - F_0)/(F_t - F_0) \times 100$.

F' is the fluorescence intensity determined under various time periods, F_0 and F_t are the initial and total fluorescence intensity defined as before and after addition of Triton X-100 to a final concentration of 0.03% (w/v).

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