ELSEVIER

Carbohydrate Research 299 (1997) 23–31

CARBOHYDRATE RESEARCH

Synthesis of α-tocopheryl oligosaccharides

Martina Lahmann, Joachim Thiem *

Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany

Received 30 September 1996; accepted 3 December 1996

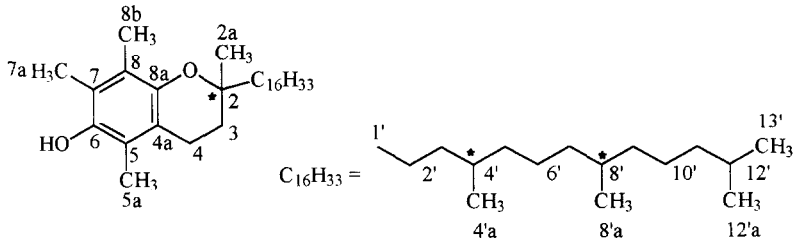
Abstract

As effective natural antioxidants, tocopherols and also their esters are frequently added to foodstuffs, pharmaceuticals and cosmetics. For increase of polarity, hence solubility in water, a series of α-tocopheryl oligosaccharides was synthesised using BF_3-etherate. The pure α-tocopheryl β-maltotetraoside as well as the higher homologues proved to be water-soluble.

Keywords: α-Tocopherol; Glycosylation; BF_3-etherate; Antioxidants

1. Introduction

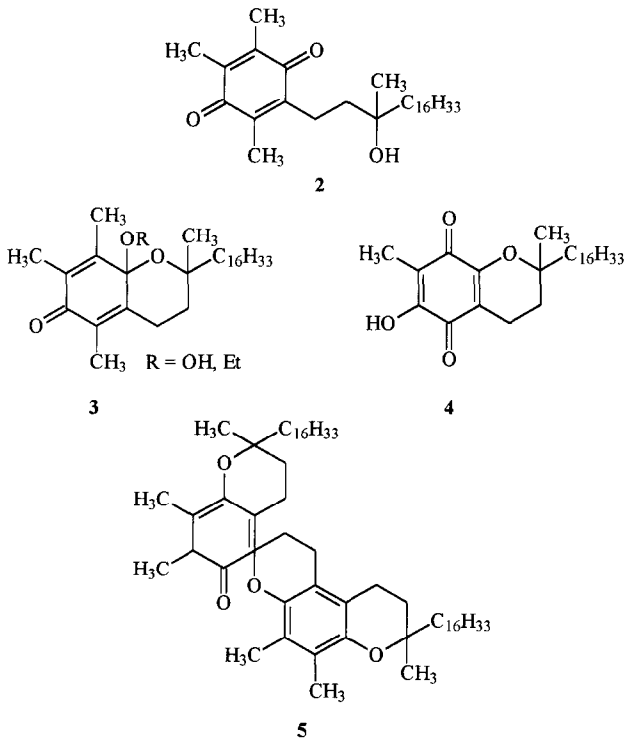
α-Tocopherol **1** (Scheme 1) [1] is one of the most important and essential natural antioxidants, which operates as a radical scavenger protecting biological membranes against lipid peroxidation [2,3]. It is the main component of Vitamin E, which consists of at least eight structurally related compounds: four tocopherols and four tocotrienols. Each of the tocopherols contains three chiral centres with the natural *R* configuration. Following the official nomenclature, the natural α-compound is called *RRR*-α-tocopherol [4]. Synthetic substances differ in their configuration depending on the procedure by which they were synthesised [5]. The most widely used and cheapest compound contains all eight stereoisomers and is called *all-rac*-α-tocopherol **1** and this was also employed in these glycosylation reactions. Tocopherols are added to many foodstuffs, pharmaceuticals and cosmetics to prevent them from becoming rancid [6,7]. Nowadays,



Scheme 1. α-Tocopherol **1**.

* Corresponding author.

0008-6215/97/$17.00
PII S0008-6215(96)00331-X



Scheme 2. Some oxidation products of **1**.

the general opinion is that naturally occurring antioxidants rather than novel synthetic substances should be added to the above mentioned products. A major disadvantage, however, of tocopherols is their limited solubility in aqueous solutions. Therefore, several attempts such as the preparation of tocopheryl phosphates were made to overcome this problem [8,9].

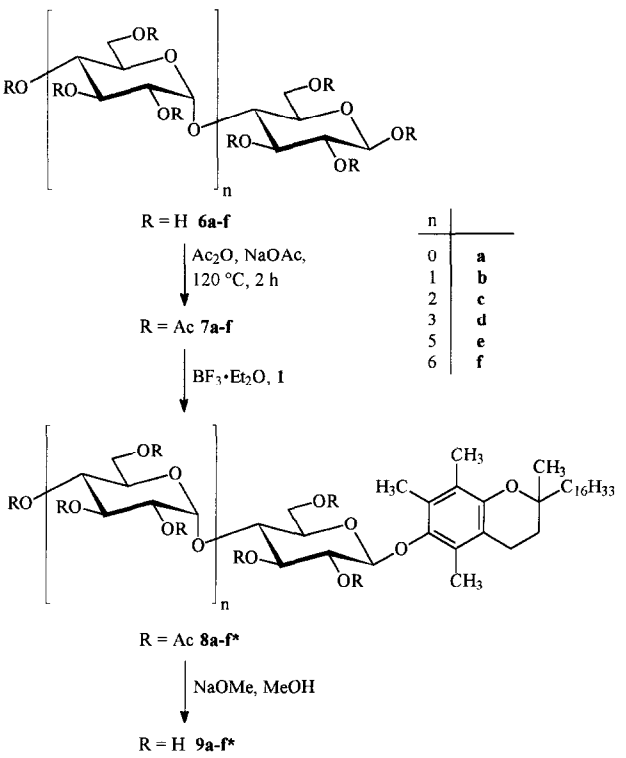
The idea of using carbohydrates as hydrophilic components in tocopheryl derivatives has not yet been investigated. However, the preparation of some tocopheryl mono- and di-saccharides has been reported in very poor yields (about 13%) and they were claimed to be novel derivatives of antiallergic activity [10].

The main problem in glycosylation of tocopherols is their easy oxidation ($E^{\circ} = +500$ mV, pH 7 [11]) to the corresponding open chain quinone **2** [12] and other oxidation products such as the cyclic paraquinones **3** [13,14] and **4** [15] or the dimeric spiro derivative **5** [16,17] under various conditions as shown in Scheme 2. In addition, many oxidation products with known and unknown structures have been reported [18,19]. Hence, for example, the often successfully applied Koenigs–Knorr glycosylation conditions employing silver salts were not suitable in this case.

2. Results and discussion

Boron trifluoride diethyl etherate (Scheme 3) was chosen as glycosylation catalyst, because it does not oxidise the labile tocopherol. Glycosylation experiments using tin tetrachloride were also performed successfully, but the work-up of tin salts was difficult and tedious. Theoretically, glycosylation under phase transfer conditions should also be possible, however, solvents excluding oxygen must be used, otherwise the aglycon would be oxidised under such strongly basic conditions. Glycosylation of tocopherol employing 4-toluenesulfonic acid as catalyst gave only 13% [20] of the peracetylated α-tocopheryl β-D-glucopyranoside (**8a**). A more efficient method was claimed to yield about 80% of α-tocopheryl α-D-mannopyranoside, but this yield was dependent on the exact mixture of copper- and zinc-halides [21].

A major advantage of the glycosylation with BF_3-etherate as catalyst is the direct use of the β-anomer of the peracetylated sugars [22,23] without any further activation at the anomeric centre. This allowed the synthesis of the core molecule in only one step, by simple glycosylation of the β-peracetylated maltooligosaccharides with the tocopherol unit. The first attempt to glycosylate α-tocopherol **1** was performed with equimolar amounts of reactants and catalyst.



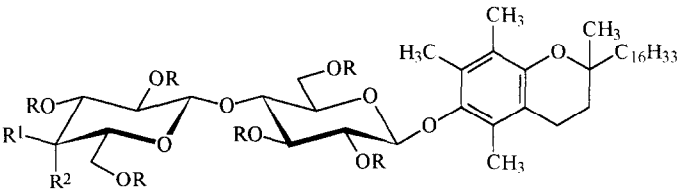
Scheme 3. * Locants as in Scheme 1.

After 45 min a new UV-active compound was detected by TLC, which was later shown to be the desired glycoside **8a**. The reaction was quenched after 2.5 h; with longer reaction times the formation of more decomposition and oxidation products was observed. The reaction mixture was worked-up and purified by column chromatography. The H-1 coupling constant in the ^{1}H NMR spectrum showed the presence of the pure α-tocopheryl β-glucoside **8a** as the main product in approximately 40% yield; no α-glucoside could be detected. Whereas the β-acetate **7a** reacted very well with α-tocopherol **1**, the α-anomer did not react under these conditions even after extended reaction times. Furthermore, no anomerisation could be detected within eight days using pure crystallised β-pentaacetylglucose **7a** as glycosyl donor. Generally, peracetylated maltooligomers crystallise with difficulty, however, the crude mixtures could be used, which could contain up to 20% of the α-anomer.

Attempts to optimise the reaction conditions showed the best results with 2.5 equivalents of the catalyst. Whereas one equivalent is used for formation of the oxocarbenium intermediate at the anomeric centre, another is assumed to coordinate the phenolic group of tocopherol. With a sixfold excess of the cheaper α-tocopherol, the equilibrium was shifted to the product side and, under these conditions, 67% of the α-tocopheryl β-D-glucoside **8a** could be isolated. Following this method, α-tocopheryl β-maltoside **8b** (57%), α-tocopheryl β-cellobioside **10** (53%) (Scheme 4), and α-tocopheryl β-lactoside **12** (65%) could be prepared, too. The highest yield for this type of reaction was obtained in the preparation of α-tocopheryl β-maltotrioside **8c** (69%). The glycosides of the higher maltooligomers were also synthesised in somewhat lower yields: α-tocopheryl β-maltotetraoside **8d** (39%), α-tocopheryl β-maltohexaoside **8e** (31%), and α-tocopheryl β-maltoheptaoside **8f** (44%) [24,25].

The peracetylated compounds were characterised by NMR spectroscopy (Tables 1 and 2). Up to the maltotrioside **8c**, the resolution of the ^{1}H NMR spectra was sufficient to assign all the signals. The use of *all-rac*-α-tocopherol did not affect the signals of the carbohydrate protons. For the higher maltooligosaccharides, it was possible to relate an unambiguous shift area to each proton by ^{1}H/^{1}H-COSY. Like in the free α-tocopherol, the glycosidically bound tocopherol showed badly resolved ^{1}H NMR signals. Fortunately, the shift of the signals relating to tocopherol are not influenced by the length of the carbohydrate chain. The resolution of the ^{13}C NMR of α-tocopherol is much better since all of the 29 carbon atoms display a defined signal [26,27]. Only C-2 of α-tocopherol has a chemical shift about 75 ppm and falls into the area of the signals of carbohydrates. Unfortunately, it was impossible to assign C-2, C-3 and C-5 signals of individual carbohydrates along the chain, therefore the ^{13}C NMR spectra did not give more information than the ^{1}H NMR spectra.

Unexpectedly, some problems occurred with the deacetylation of the glycosides. Following the Zemplén procedure [28], the α-tocopheryl β-D-glucoside **8a** could be deacetylated to **9a** in almost quantitative yield. However, the same procedure failed with the α-tocopheryl β-D-maltoside **8b** even at extended reaction time due to oxidative decomposition. Whereas several methods such as potassium carbonate in methanol [29] or 1:8:1 triethylamine–methanol–water were not successful. The best results were obtained with sodium methoxide in methanol in degassed solvents under argon atmosphere. The work-up of the α-tocopheryl β-D-glucoside **9a** and α-tocopheryl β-



Cellobiose → β-Cellobiose-octaacetate —1, BF_3·Et_2O→ **10*** —NaOMe→ **11***

Lactose → β-Lactose-octaacetate —1, BF_3·Et_2O→ **12*** —NaOMe→ **13***

	R	R1	R2
10	Ac	OAc	H
11	H	OH	H
12	Ac	H	OAc
13	H	H	OH

Scheme 4. * Locants as in Scheme 1.

Table 1
^{1}H NMR data (400 MHz, $CDCl_3$) for the acetylated α-tocopheryl glycosides

	Unit	Chemical shifts (δ)						
		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
8a	I	4.71, d	5.33, dd	5.24, dd	5.17, dd	3.53, ddd	4.19, dd	4.05, dd
8b	I	4.73, d	5.18, dd	5.30, dd	4.09, dd	3.50, ddd	4.43, dd	4.17, dd
	II	5.43, d	4.86, dd	5.34, dd	5.05, dd	3.91, ddd	4.25, dd	4.04, dd
10	I	4.52, d	4.67, d	5.19–5.26, dd	3.86, t	3.41, ddd	4.45, dd	4.08, m
	II	5.19–5.26, d	4.91, dd	5.14, t	5.06, t	3.66, ddd	4.37, dd	4.05, m
12	I	5.26–5.21, m, 2 H	4.67, m_c	5.26–5.21, m, 2 H	3.91–3.85, m, 2 H	3.42, ddd	4.42, ~ d	4.04–4.17, m, 3 H
	II	4.50, d	5.10, dd	4.96, dd	5.35, dd	3.91–3.85, m, 2 H	4.04–4.17, m, 3 H	4.04–4.17, m, 3 H
8c	I	4.74, d	5.2, dd	5.34, t	4.04, m	3.51, dt	4.40, dd	4.16, dd
	II	5.17, d	4.78, dd	5.46, t	5.09, t	4.03, m	3.70, dd	3.54, dd
	III	5.37, d	4.86, dd	5.30, t	5.06, t	3.90, dt	4.26, dd	4.08, dd
8d	I	4.71–4.77, m, 3 H	5.17, dd	5.25–5.42, m, 7 H	4.02–4.10, m, 2 H	3.54, ddd	5.42–4.51, m, 3 H	4.16, d
	II	5.25–5.42, m, 7 H	4.71–4.77, m, 3 H	5.25–5.42, m, 7 H	5.42–4.51, m, 3 H	4.21–4.31, m, 3 H	3.86–3.98, m, 5 H	3.86–3.98, m, 5 H
	III	5.25–5.42, m, 7 H	4.71–4.77, m, 3 H	5.25–5.42, m, 7 H	5.42–4.51, m, 3 H	4.21–4.31, m, 3 H	3.86–3.98, m, 5 H	3.86–3.98, m, 5 H
	IV	5.25–5.42, m, 7 H	4.85, dd	5.25–5.42, m, 7 H	5.06, t	4.02–4.10, m, 2 H	4.21–4.31, m, 3 H	3.86–3.98, m, 5 H
8e	I	4.66–4.78, m, 5 H	5.16, t	5.23–5.42, m, 11 H	4.00–4.11, m, 2 H	3.53, m	4.42–4.53, m, 5 H	4.17, d
	II	5.23–5.42, m, 11 H	4.66–4.78, m, 5 H	5.23–5.42, m, 11 H	4.42–4.53, m, 5 H	4.20–4.33, m, 5 H	3.86–3.98, m, 9 H	3.86–3.98, m, 9 H
	III	5.23–5.42, m, 11 H	4.66–4.78, m, 5 H	5.23–5.42, m, 11 H	4.42–4.53, m, 5 H	4.20–4.33, m, 5 H	3.86–3.98, m, 9 H	3.86–3.98, m, 9 H
	IV	5.23–5.42, m, 11 H	4.66–4.78, m, 5 H	5.23–5.42, m, 11 H	4.42–4.53, m, 5 H	4.20–4.33, m, 5 H	3.86–3.98, m, 9 H	3.86–3.98, m, 9 H
	V	5.23–5.42, m, 11 H	4.66–4.78, m, 5 H	5.23–5.42, m, 11 H	4.42–4.53, m, 5 H	4.20–4.33, m, 5 H	3.86–3.98, m, 9 H	3.86–3.98, m, 9 H
	VI	5.23–5.42, m, 11 H	4.86, dd	5.23–5.42, m, 11 H	5.07, t	4.00–4.11, m, 2 H	4.20–4.33, m, 5 H	3.86–3.98, m, 9 H
8f	I	4.67–4.78, m, 6 H	5.18, dd	5.26–5.44, m, 13 H	4.02–4.12, m, 2 H	3.54, ddd ~ dt	4.44–4.56, m, 6 H	4.17, ~ d
	II	5.26–5.44, m, 13 H	4.67–4.78, m, 6 H	5.26–5.44, m, 13 H	4.44–4.56, m, 6 H	4.19–4.34, m, 6 H	3.87–4.00, m, 11 H	3.87–4.00, m, 11 H
	III	5.26–5.44, m, 13 H	4.67–4.78, m, 6 H	5.26–5.44, m, 13 H	4.44–4.56, m, 6 H	4.19–4.34, m, 6 H	3.87–4.00, m, 11 H	3.87–4.00, m, 11 H
	IV	5.26–5.44, m, 13 H	4.67–4.78, m, 6 H	5.26–5.44, m, 13 H	4.44–4.56, m, 6 H	4.19–4.34, m, 6 H	3.87–4.00, m, 11 H	3.87–4.00, m, 11 H
	V	5.26–5.44, m, 13 H	4.67–4.78, m, 6 H	5.26–5.44, m, 13 H	4.44–4.56, m, 6 H	4.19–4.34, m, 6 H	3.87–4.00, m, 11 H	3.87–4.00, m, 11 H
	VI	5.26–5.44, m, 13 H	4.67–4.78, m, 6 H	5.26–5.44, m, 13 H	4.44–4.56, m, 6 H	4.19–4.34, m, 6 H	3.87–4.00, m, 11 H	3.87–4.00, m, 11 H
	VII	5.26–5.44, m, 13 H	4.88, dd	5.26–5.44, m, 13 H	5.07, dd ~ t	4.02–4.12, m, 2 H	4.19–4.34, m, 6 H	3.87–4.00, m, 11 H

Tocopherol-system: 2.51–2.60 (m, 2 H, CH_2-4), 1.96–2.19 (m, 9 H, CH_3-8b,7a,5a and OAc), 1.70–1.89 (m, 2 H, CH_2-3), 0.99–1.62 (m, 24 H, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.80–0.89 (m, 12 H, CH_3-4″a,8″a,12″a,13″).

Table 2
Coupling constants for the acetylated α-tocopheryl glycosides

	Unit	Coupling constants (Hz)						
		$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
8a	I	7.7	9.4	9.4	9.4	4.6	2.5	12.2
8b	I	7.8	9.3	9.2	9.7	3.3	3.3	12.2
	II	4.1	10.1	9.9	10.2	2.5	2.5	12.5
10	I	7.9	9.4	9.8	9.9	2.4	4.7	11.2
	II	8.1	9.1	9.7	9.7	4.3	2.2	12.4
12	I	[a]	[a]	[a]	9.6	2.1	4.6	11.2
	II	7.6	10.4	3.6	1.1	[a]	[a]	[a]
8c	I	7.9	10.4	9.5	9.5	1.5	3.6	11.5
	II	3.9	10.3	9.9	9.9	3.9	2.2	11.5
	III	3.9	9.8	9.6	9.8	4.1	2.3	12.2
8d [b]	I	8.2	9.6	9.6	3.2	3.2	11.2	4.0
	IV	4.0	10.6	9.6	9.6	[a]	[a]	[a]
8e [b]	I	8.4	8.4	11.2	[a]	[a]	[a]	[a]
	VI	3.9	9.6	9.4	9.4	[a]	[a]	[a]
8f [b]	I	8.4	8.9	[a]	9.2	2.7	2.7	10.0
	VII	4.0	8.9	8.9	8.9	[a]	[a]	[a]

[a] Not assigned.
[b] Signals of the inner chain units not assigned.

maltoside **9b** was straightforward by neutralisation with ion exchange resin, filtration and evaporation of the solvent. During the deprotection of the α-tocopheryl β-lactoside **12**, the product **13** partially precipitated. A similar observation was also noted in the transfer of peracetylated α-tocopheryl β-cellobioside **10** to compound **11** and of peracetylated α-tocopheryl β-maltotrioside **8c** to compound **9c**. Therefore, after neutralisation, the ion exchange resin was removed and the products were precipitated by addition of water, filtrated and dried. Compounds **9d**, **9e** and **9f** were worked-up as described for **9a**, but after evaporation of the solvent the residue was dissolved in water and then lyophilised.

The characterisation of the deprotected tocopheryl glycosides such as **9a** was difficult due to a poorly resolved spectrum in $CDCl_3$. The maltoside **9b** was soluble in methanol and hence gave a well resolved spectrum in CD_3OD. All the other compounds were measured in Me_2SO-d_6. Glycosides **9c**, **9d**, **9e** and **9f** were also soluble in deuterium oxide, but it was not possible to record NMR spectra. Probably, these substances form supramolecular structures such as micelles. This is also assumed to favor the fast uptake of water which prevented correct elemental analysis.

One of the aims of the synthesis of the tocopheryl glycosides was a test of solubility properties. Therefore, small amounts (0.5 mg) of the derivatives were

Table 3
Solubility of the α-tocopheryl glycosides [a]

	H_2O	CH_3OH	CH_2Cl_2	Me_2SO	Pyridine
9a	−	−/+	+	+	+
9b	−	+/−	−	+	+
11	−	−	−	+	+
13	−	−	−	+	+
9c	−/+	+	−	+	+
9d	+/−	+/−	−	+	+
9e	+	−/+	−	+	+
9f	+	−/+	−	+	+

[a] − = unsoluble, −/+ = poor solubility (0.5 mg/0.5 ml), +/− = modest solubility (0.5 mg/0.2 ml), + = soluble (0.5 mg/0.1 ml).

put on a thin glass plate and treated in 0.1 mL steps with the chosen solvent at room temperature. The solvation process was observed under the microscope. With regard to the small number of glycosides with longer carbohydrate chains, the solubility tests were only qualitative. The results are depicted in Table 3.

Two tests were performed to examine the degradation of α-tocopheryl β-D-glycosides. Under acidic conditions (2 N HCl), the glycosidic bond was cleaved after some minutes as detected by the Emmerie–Engel reaction [30]. This colour detection of tocopherols is based on the easy oxidation of α-tocopherol with iron(III) chloride to give the corresponding quinone which in turn forms an intense red-coloured complex with bipyridine. For enzymatic degradation, the heptaoside **9f** was incubated with a mixture of β-glucosidase (almonds) and α-glucosidase (yeast) at 30 °C in buffer solution (pH 5.6) [31]. After five days, no reaction was observed as monitored by TLC. Further, β-amylase (potatoes) was added in order to cleave the α-(1 → 4)-maltose units [32]; however, the glucoside **9a** precipitated. Thus, degradation to the free α-tocopherol could not be achieved by these methods.

3. Experimental

General methods.—Optical rotations were determined with a Perkin–Elmer Model 241 Polarimeter (10 cm cells, Na–D-line: 589 nm). TLC was performed on precoated plates of silica gel (Kieselgel 60 F254, E. Merck), detection was by UV-absorption or/and spraying with 10% ethanolic H_2SO_4 followed by heating. Flash column chromatography was performed on silica gel (Kieselgel 60, 200–400 mesh, E. Merck). Ratios of solvent mixtures for chromatography are specified in terms of volume. ^1H NMR spectra and ^{13}C NMR spectra were recorded at 250 or 400 MHz on Bruker AC-250 and AMX-400 (100.67 MHz for ^{13}C) with Me_4Si (δ 0) as the internal standard. Lyophilisation was performed with Leybold-Heraeus Lyovac GT 2 or with Christ Alpha 1–4. Elemental analyses were determined by Mikroanalytische Abteilung, Institut für Organische Chemie, Universität Hamburg.

General procedure for acetylation (GM 1).—The β-peracetylated carbohydrates were prepared with sodium acetate and acetic anhydride according to the reported procedures [18]. The syrupy raw products were used without further purification.

General procedure for glycosylation (GM 2).—Boron trifluoride diethyl etherate was added to a solution of **1** and the β-peracetylated sugar in neat CH_2Cl_2. The mixture was kept in the dark and stirred for 3 h at room temperature (TLC control). The excess of BF_3-etherate mixture was decomposed with $NaHCO_3$. After dilution with CH_2Cl_2, the mixture was washed with water and NaCl. Following drying ($MgSO_4$), the solvents were removed under diminished pressure and the resulting syrup was purified by column chromatography.

General procedure for deprotection (GM 3).—The compound was dissolved in neat, degassed MeOH under argon atmosphere. Freshly prepared 0.1 M methanolic NaOMe was added dropwise until pH 8 was reached. After completion of the reaction, monitored by TLC, the mixture was neutralised by Amberlite IR 120 H^+ resin and filtrated. The material was concentrated at 40 °C under reduced pressure. In case of unpolar derivatives, traces of solvent were removed in highvacuum. Water-soluble compounds were dissolved in water and then lyophilised.

all-rac-*α-Tocopheryl 2,3,4,6-tetra*-O-*acetyl*-*β*-D-*glucopyranoside* (**8a**).—Compounds **7a** (0.78 g, 2 mmol) and **1** (0.86 g, 2 mmol), dissolved in CH_2Cl_2 (30 mL), were reacted in the presence of BF_3-etherate (0.60 mL, 4.8 mmol) according to **GM 2** procedure. After purification by flash chromatography (80:1 CH_2Cl_2–acetone,) the slightly yellow syrupy **8a** (1.00 g, 1.31 mmol, 66%) was obtained; $[\alpha]_D^{20}$ −7.7° (*c* 1, CH_2Cl_2); ^1H NMR: Tables 1 and 2. ^{13}C NMR ($CDCl_3$): δ 101.8 (C-1), 71.0 (C-2), 72.7 (C-3), 68.2 (C-4), 71.5 (C-5), 61.4 (C-6), 74.5 (C^T-2), 30.9 (C^T-3), 20.2 (C^T-4), 120.4 (C^T-5), 144.7 (C^T-6), 122.5 (C^T-7), 127.9 (C^T-8), 23.4 (C^T-2a), 117.0 (C^T-4a), 11.4 (C^T-5a), 12.2 (C^T-7a), 148.0 (C^T-8a), 12.9 (C^T-8b), 38.9 (C^T-1′. 11′), 20.9 (C^T-2′), 36.8–36.9 (C^T-3′, 5′, 7′, 9′), 32.3 (C^T-4′, 8′), 24.0 (C^T-6′), 24.4 (C^T-10′), 27.5 (C^T-12′), 19.3 (C^T-4′a, 8′a), 22.2, 22.3 (C^T-12′a, 13′), 168.8, 170.0 (*C*OCH$_3$), 20.3 (CO*C*H$_3$). Anal. Calcd for $C_{43}H_{68}O_{11}$ (761.0): C, 67.87; H, 9.00. Found: C, 67.74; H, 9.13.

all-rac-*α-Tocopheryl 2,3,6-tri*-O-*acetyl-4*-O-*(2,3,4,6-tetra*-O-*acetyl*-*α*-D-*glucopyranosyl)*-*β*-D-*glucopyranoside* (**8b**).—Compounds **7b** (1.24 g, 1.5 mmol relating to the β-acetate) and **1** (0.65 g, 1.5 mmol), dissolved in CH_2Cl_2 (20 mL), were reacted in the presence of BF_3-etherate (0.45 mL, 3.6 mmol) according to **GM 2** procedure. After purification by flash chromatography (20:1 CH_2Cl_2–acetone), the slightly yellow glass **8b** (0.89 g, 1.17 mmol, 57%) was obtained; mp 68 °C; $[\alpha]_D^{20}$ +72.8° (*c* 1.5, CH_2Cl_2); ^1H NMR: Tables 1 and 2. ^{13}C NMR

($CDCl_3$): δ 105.1 (C^I-1), 74.5 (C^I-2), 77.5 (C^I-3), 81.0 (C^I-4), 76.4 (C^I-5), 61.8 (C^I-6), 102.6 (C^{II}-1), 73.8 (C^{II}-2), 75.2 (C^{II}-3), 71.5 (C^{II}-4), 74.9 (C^{II}-5), 62.3 (C^{II}-6), 75.6 (C^T-2), 32.0 (C^T-3), 21.4 (C^T-4), 123.6 (C^T-5), 146.2 (C^T-6), 125.7 (C^T-7), 129.6 (C^T-8), 23.9 (C^T-2a), 117.0 (C^T-4a), 13.9 (C^T-5a), 13.0 (C^T-7a), 149.4 (C^T-8a), 11.8 (C^T-8b), 40.3 (C^T-1′, 11′), 21.4 (C^T-2′), 38.2 (C^T-3′, 5′, 7′, 9′), 33.6 (C^T-4′, 8′), 25.0 (C^T-6′), 25.6 (C^T-10′), 28.9 (C^T-12′), 19.9 (C^T-4′a, 8′a), 22.8 (C^T-12′a, 13′). Anal. Calcd for $C_{55}H_{84}O_{19}$ (1049.3): C, 62.96; H, 8.07. Found: C, 62.66; H, 8.17.

all-rac-*α-Tocopheryl (2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**8c**).—Compounds **7c** (724 mg, 0.6 mmol relating to the β-acetate) and **1** (1.55 g, 3.6 mmol), dissolved in CH_2Cl_2 (60 mL), were reacted in the presence of BF_3-etherate (0.18 mL, 1.44 mmol) according to **GM 2** procedure. After purification by flash chromatography (17:1 CH_2Cl_2–acetone), the slightly yellow glass **11** (552 mg, 0.41 mmol, 69%) was obtained; mp 78–79 °C; $[\alpha]_D^{20}$ +10.5° (*c* 1.28, CH_2Cl_2); ^{1}H NMR: Tables 1 and 2. Anal. Calcd for $C_{67}H_{100}O_{27}$ (1336.7): C, 60.17; H, 7.54. Found: C, 60.02; H, 7.58.

all-rac-*α-Tocopheryl (2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**8d**).—Compounds **7d** (278 mg, 0.17 mmol relating to the β-acetate) and **1** (83.0 mg, 0.2 mmol), dissolved in CH_2Cl_2 (20 mL), were reacted in the presence of BF_3-etherate (0.06 mL, 0.48 mmol) according to **GM 2** procedure. After purification by flash chromatography (15:1 CH_2Cl_2–acetone), the slightly yellow glass **9d** (109 mg, 0.17 mmol, 39%) was obtained; mp 94 °C; $[\alpha]_D^{20}$ −42.6° (*c* 0.56, CH_2Cl_2); ^{1}H NMR: Tables 1 and 2. Anal. Calcd for $C_{79}H_{116}O_{35}$ (1624.7): C, 58.36; H, 7.19. Found: C, 58.52; H, 7.40.

all-rac-*α-Tocopheryl (2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**8e**).—Compounds **7e** (343 mg, 0.15 mmol relating to the β-acetate) and **1** (388.0 mg, 0.9 mmol), dissolved in CH_2Cl_2 (30 mL), were reacted in the presence of BF_3-etherate (0.045 mL, 0.36 mmol) according to **GM 2** procedure. After purification by flash chromatography (8:1 CH_2Cl_2–acetone), the slightly yellow glass **9e** (102 mg, 0.046 mmol, 31%) was obtained; mp 108 °C; $[\alpha]_D^{20}$ +68.4° (*c* 0.68, CH_2Cl_2); ^{1}H NMR: Tables 1 and 2. Anal. Calcd for $C_{91}H_{132}O_{43}$(2200.7): C, 57.10; H, 6.95. Found: C, 57.31; H, 6.83.

all-rac-*α-Tocopheryl (2,3,4,6-tetra*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-(2,3,6-tri*-O-*acetyl-α*-D-*glucopyranosyl)-(1 → 4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranoside* (**8f**).—Compounds **7f** (210 mg, 0.08 mmol relating to the β-acetate) and **1** (62 mg, 0.14 mmol), dissolved in CH_2Cl_2 (10 mL), were reacted in the presence of BF_3-etherate (0.025 mL, 0.19 mmol) according to **GM 2** procedure. After purification by flash chromatography (13:2 CH_2Cl_2–acetone), the slightly yellow glass **9f** (87 mg, 0.035 mmol, 44%) was obtained; mp 110.5 °C; $[\alpha]_D^{20}$ +50.8° (*c* 0.49, CH_2Cl_2); ^{1}H NMR: Tables 1 and 2. Anal. Calcd for $C_{115}H_{164}O_{59}$(2488.7): C, 55.46; H, 6.64. Found: C, 54.96; H, 6.86.

all-rac-*α-Tocopheryl β*-D-*glucopyranoside* (**9a**).—Compound **8a** (0.9 g, 1.2 mmol) was deprotected according to **GM 3** procedure yielding the colourless syrup **9a** (686 mg, 1.16 mmol, 97%); $[\alpha]_D^{20}$ −5.9 (*c* 1.53, MeOH +5% CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 4.56 (d, 1 H, $J_{1,2}^I$ 6.1 Hz, H^I-1), 3.65–3.82 (m, 5H, H^I-2, H^I-3, H^I-4, H^I-6a, H^I-6b), 3.81 (~ d, 1 H, $J_{4,5}^I$ 9.2 Hz, H^I-5), 2.44–2.53 (m, 2 H, CH_2-4), 2.13, 2.09, 2.01 (s, 9 H, CH_3-8b,7a,5a), 1.66–1.80 (m, 2 H, CH_2-3), 0.98–1.60 (m, 24 H, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.81–0.89 (m, 12 H CH_3-4″a,8″a,12″a,13″). Anal. Calcd for $C_{35}H_{60}O_7$(592.9): C, 70.91; H, 10.20. Found: C, 70.51; H, 10.34.

all-rac-*α-Tocopheryl 4*-O-*(α*-D-*glucopyranosyl)-β*-D-*glucopyranoside* (**9b**).—Compound **8b** (840 mg, 0.78 mmol) was deprotected according to **GM 3** procedure yielding the colourless powder **9b** (591 mg, 0.78 mmol, 100%); $[\alpha]_D^{20}$ +37.8 (*c* 0.91, MeOH +5% CH_2Cl_2); ^{1}H NMR (CD_3OD): δ 4.44 (d, 1 H, $J_{1,2}^I$ 6.8 Hz, H^I-1), 3.43–3.62 (m, 6H, H^I-2, H^I-3, H^I-4, H^{II}-3, H^{II}-5, H^{II}-6b,), 3.08–3.24 (m, 2H, H^I-5, H^{II}-4), 3.64–3.73 (m, 3H, H^I-6a, H^I-6b, H^{II}-6a,), 5.10 (d, 1 H, $J_{1,2}^{II}$ 4.2 Hz, H^{II}-1), 3.34 (dd, 1 H, $J_{2,3}^{II}$ 9.4 Hz, H^{II}-2) 2.34–2.46 (m, 2 H, CH_2-4), 2.11, 2.04, 1.95 (s, 9 H, CH_3-8b,7a,5a), 1.62–1.88 (m, 2 H, CH_2-3), 0.91–1.55 (m, 24 H, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.70–0.83 (m, 12 H CH_3-4″a,8″a,12″a,13″). $C_{41}H_{70}O_{12}$ (755.0).

all-rac-*α-Tocopheryl 4-O-[4-O-(α-D-glucopyranosyl)-α-D-glucopyranosyl]-β-D-glucopyranoside* (**9c**).—Compound **8c** (162 mg, 0.121 mmol) was deprotected according to **GM 3** procedure yielding the colourless powder **9c** (85.0 mg, 0.093 mmol, 77%); $[\alpha]_D^{20}$ +60.7 (*c* 0.99, MeOH); ^{1}H NMR (Me_2SO): δ 4.41 (d, 1 H, H^I-1, $J_{1,2}^I$ 7.6 Hz), 4.62 (d, 1 H, H^{II}-1, $J_{1,2}^{II}$ 3.5 Hz), 4.97 (d, 1 H, H^{III}-1, $J_{1,2}^{III}$ 3.5 Hz), 5.40–5.80 (m_c, 2 H, O*H*), 4.55–5.20 (m, 8 H), 4.20–4.45 (m, 3 H), 2.99–3.84 (m, 18 H) 1.65–1.88 (m, 2 H, CH_2-4), 2.16, 2.14, 1.98 (s, 9 H, CH_3-8b,7a,5a), 0.95–1.58 (m, 26 H, CH_2-3, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.81–0.85 (m, 12 H CH_3-4″a,8″a,12″a,13″). $C_{47}H_{80}O_{17}$ (917.14).

all-rac-*α-Tocopheryl β-maltotetraoside* (**9d**).—Compound **8d** (45 mg, 0.028 mmol) was deprotected according to **GM 3** procedure yielding the colourless powder **13** (25.0 mg, 0.023 mmol, 83%); $[\alpha]_D^{20}$ +6.2 (*c* 0.11, H_2O); ^{1}H NMR (Me_2SO): δ 4.39 (d, 1 H, $J_{1,2}^I$ 7.6 Hz, H^I-1), 4.98 (d, 1 H, $J_{1,2}^{II}$ 4.1 Hz, H^{II}-1), 4.99 (d, 1 H, $J_{1,2}^{III}$ 4.1 Hz, H^{III}-1), 5.04 (d, 1 H, $J_{1,2}^{IV}$ 3.5 Hz, H^{IV}-1), 5.38–5.87 (m, 6 O*H*), 5.10 (m_c, 1 H, O*H*), 4.44–4.72 (m_c, 3 H, O*H*), 4.21–4.34 (m, 2 H, O*H*), 3.00–3.69 (m, 24 H), 1.68–1.77 (m, 2 H, CH_2-4), 2.13, 2.11, 1.96 (s, 9 H, CH_3-8b,7a,5a), 0.98–1.57 (m, 26 H, CH_2-3, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.80–0.85 (m, 12 H CH_3-4″a,8″a,12″a,13″). $C_{53}H_{90}O_{22}$ (1079.3)

all-rac-*α-Tocopheryl β-maltohexaoside* (**9e**).—Compound **8e** (32.0 mg, 0.015 mmol) was deprotected according to **GM 3** procedure yielding the colourless powder **9e** (16.0 mg, 0.011 mmol, 76%); $[\alpha]_D^{20}$ +12.5 (*c* 0.14, H_2O); ^{1}H NMR (Me_2SO): δ 4.40 (m, 1 H, $J_{1,2}^I$ 7.6 Hz, H^I-1), 5.06 (d, 1 H, $J_{1,2}^{VI}$ 3.5 Hz, H^{VI}-1), 4.19–5.89 (m, 19 H, O*H*), 4.97–5.04 (m , 4 H, H^{II}-1, H^{III}-1, H^{IV}-1, H^V-1), 4.44–4.72 (m, 35 H), 1.69–1.76 (m, 2 H, CH_2-4), 2.16, 2.15, 2.14, 2.13 (s, 6 H, CH_3-7a,5a), 1.98 (s, 3 H, CH_3-8b), 0.97–1.57 (m, 26 H, CH_2-3, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.79–0.86 (m, 12 H CH_3-4″a,8″a,12″a,13″). $C_{65}H_{110}O_{32}$ (1403.6).

all-rac-*α-Tocopheryl β-maltoheptaoside* (**9f**).—Compound **8f** (37.0 mg, 0.015 mmol) was deprotected according to **GM 3** procedure yielding the colourless powder **9f** (21.0 mg, 0.013 mmol, 80%); $[\alpha]_D^{20}$ +16.3 (*c* 0.19, H_2O); ^{1}H NMR (Me_2SO): δ 4.40 (m, 1 H, $J_{1,2}^I$ 7.6 Hz, H^I-1), 4.10–5.75 (m, 22 H, O*H*, H^{VII}-1, 4.93–5.04 (m , 4 H, $J_{1,2}^{II-VI}$ 3.6 Hz, H^{II}-1, H^{III}-1, H^{IV}-1, H^V-1, H^{VI}-1), 2.74–3.72 (m, 42 H), 1.64–1.72 (m, 2 H, CH_2-4), 2.11, 2.10, 2.09, 2.08 (s, 6 H, CH_3-7a,5a), 1.93 (s, 3 H, CH_3-8b), 0.95–1.51 (m, 26 H, CH_2-3, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.78–0.81 (m, 12 H CH_3-4″a,8″a,12″a,13″). $C_{71}H_{120}O_{37}$ (1565.7).

all-rac-*α-Tocopheryl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside* (**10**).—1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-β-D-glucopyranoside (1.02 g, 1.5 mmol) and **1** (0.65 g, 1.5 mmol), dissolved in CH_2Cl_2 (60 mL), were reacted in the presence of BF_3-etherate (0.45 mL, 3.6 mmol) according to **GM 2** procedure. After purification by flash chromatography (30:1 CH_2Cl_2–acetone), the slightly yellow glass **10** (0.84 g, 0.80 mmol, 53%) was obtained; mp 61.5 °C; $[\alpha]_D^{20}$ −5.9° (*c* 1.1, CH_2Cl_2); ^{1}H NMR: Tables 1 and 2. Anal. Calcd for $C_{55}H_{84}O_{19}$ (1049.3): C, 62.96; H, 8.07. Found: C, 62.92; H, 8.17.

all-rac-*α-Tocopheryl 2,3,6-tri-O-acetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside* (**11**).—1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (1.00 g, 1.5 mmol) and **1** (0.65 g, 1.5 mmol), dissolved in CH_2Cl_2 (60 mL), were reacted in the presence of BF_3-etherate (0.45 mL, 3.6 mmol) according to **GM 2** procedure. After purification by flash chromatography (20:1 CH_2Cl_2–acetone), the slightly yellow glass **11** (1.02 g, 0.97 mmol, 64%) was obtained; mp 72 °C; $[\alpha]_D^{20}$ +10.5° (*c* 0.53, CH_2Cl_2); ^{1}H NMR: Tables 1 and 2. Anal. Calcd for $C_{55}H_{84}O_{19}$ (1049.3): C, 62.96; H, 8.07. Found: C, 62.61; H, 8.12.

all-rac-*α-Tocopheryl 4-O-(β-D-glucopyranosyl)-β-D-glucopyranoside* (**12**).—Compound **10** (143 mg, 0.136 mmol) was deprotected according to **GM 3** procedure yielding the colourless powder **12** (74.3 mg, 0.098 mmol, 72%); $[\alpha]_D^{20}$ −13.2 (*c* 1.0, Me_2SO); ^{1}H NMR (Me_2SO) δ 4.41 (d, 1 H, $J_{1,2}^I$ 7.1 Hz, H^I-1), 3.27–3.47 (m, 4H, H^I-2, H^I-3, H^I-4, H^{II}-6b), 3.12–3.24 (m, 3H, H^I-5, H^{II}-3, H^{II}-5), 3.54–3.66 (m, 2H, H^I-6a, H^I-6b), 4.30 (d, 1 H, $J_{1,2}^{II}$ 8.2 Hz, H^{II}-1), 3.00 (ddd, 1 H, $J_{2,3}^{II}$ 8.2 Hz, H^{II}-2), 4.06 (ddd ~ dt, 1 H, $J_{3,4}^{II}$ 9.1 Hz, $J_{4,5}^{II}$ 9.1 Hz, H^{II}-4), 3.71 (ddd, 1 H, $J_{5,6a}^{II}$ 6.9 Hz, $J_{6a,6b}^{II}$ 11.8 Hz, H^{II}-6a), 5.55 (d, 1 H, $J_{2,OH}^I$ 4.5 Hz, OH^I-2), 4.71 (s, 1 H, OH^I-3), 4.33 (dt, 1 H, OH^I-6), 5.22 (d, 1 H,, $J_{2,OH}^{II}$ 5.0 Hz, OH^{II}-2), 4.98 (d, 1 H,, $J_{3,OH}^{II}$ 5.6 Hz, OH^{II}-3), 4.96 (d, 1 H,, $J_{4,OH}^{II}$ 5.6 Hz, OH^{II}-4), 4.59 (t, 1 H,, $J_{6a,OH}^{II}$ 5.3 Hz, $J_{6b,OH}^{II}$ 5.3 Hz, OH^{II}-6), 1.68–1.77 (m, 2 H, CH_2-4), 2.15, 2.02, 1.98 (s, 9 H, CH_3-8b,7a,5a), 0.98–1.60 (m, 26 H, CH_2-3, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.81–0.85 (m, 12 H CH_3-4″a,8″a,12″a,13″). $C_{41}H_{70}O_{12}$ (755.0).

all-rac-*α-Tocopheryl 4-O-(β-D-galactopyranosyl)-β-*

D-*glucopyranoside* (**13**).—Compound **11** (179 mg, 0.171 mmol) was deprotected according to **GM 3** procedure yielding the colourless powder **13** (87.0 mg, 0.115 mmol, 67%); $[\alpha]_D^{20}$ +4.4 (*c* 1.0, Me_2SO); ^1H NMR (Me_2SO): δ 4.43 (d, 1 H, $J^{I}_{1,2}$ 7.6 Hz, H^{I}-1), 3.29–3.43 (m, 5H, H^{I}-2, H^{I}-3, H^{II}-2, H^{II}-3, H^{II}-5), 3.62–3.70 (m, 2H, H^{I}-4, H^{I}-6a), 3.18 (m_c, 1 H, H^{I}-5), 3.45–3.62 (m, 4H, H^{I}-6b, H^{II}-4, H^{II}-6a, H^{II}-6b), 4.26 (d, 1 H, $J^{II}_{1,2}$ 7.2 Hz, H^{II}-1), 5.10 (d, 1 H, $J^{I}_{2,OH}$ 4.0 Hz, O*H*^{I}-2), 4.74 (s, 1 H, O*H*^{I}-3), 4.33 (t. 1 H, $J^{II}_{6a,OH}$ 5.1 Hz, $J^{II}_{6b,OH}$ 5.1 Hz, O*H*^{I}-6), 5.29 (d, 1 H, $J^{II}_{2,OH}$ 4.6 Hz, O*H*^{II}-2), 4.78 (d, 1 H, $J^{II}_{3,OH}$ 5.1 Hz, O*H*^{II}-3), 4.52 (d, 1 H, $J^{II}_{4,OH}$ 4.5 Hz, O*H*^{II}-4), 4.59 (m_c, 1 H, O*H*^{II}-6).71–1.78 (m, 2 H, CH_2-4), 2.17, 2.15, 1.99 (s, 9 H, CH_3-8b,7a,5a), 1.00–1.58 (m, 26 H, CH_2-3, CH_3-2a, CH-4″,8″,12″, CH_2-⟨1″–11″⟩), 0.81–0.87 (m, 12 H CH_3-4″a,8″a,12″a,13″). $C_{41}H_{70}O_{12}$, (755.0).

Acknowledgements

Support of this work by the Fonds der Chemischen Industrie including a Doktorandenstipendium to M. L. and the Beiersdorf AG is gratefully acknowledged.

References

[1] A. Kamal-Eldin and L.-Å. Appelqvist, *Lipids*, 31 (1996) 671–701.
[2] P. Di Mascio, M.E. Murphy, and H. Sies, *Am. J. Clin. Nutr.*, 53 (1991) 194–200.
[3] H.J. Kayden and M.G. Traber, *J.Lipid Res.*, 34 (1993) 343–358.
[4] IUPAC-IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.*, 123 (1982) 473–475.
[5] O. Isler and G. Brubacher, *Vitamine I*, G. Thieme, Stuttgart, (1982) 126–151.
[6] G.W. Burton and K.U. Ingold, *Acc. Chem. Res.*, 19 (1989) 194–201.
[7] Y.D. Kim, C.K. Kim, C.N. Lee, B.J. Ha, and W.Y. Lee, *Cosmetics and Toil.*, 108 (1993) 63–78.
[8] T. Nakamura and S.Kujima, *Chem. Pharm. Bull.*, 20 (1972) 794–799.
[9] Y. Kuribayashi, K. Yoshida, T. Sakaue, and A. Okumura, *Drug Res.*, 42 (1992) 1072–1074.
[10] T. Satoh, H. Miyataka, Y. Masamoto, T. Asai, K. Hasegawa, and H. Kakegawa (Sunstar Inc.) EP 169 716 (1986); *Chem. Abstr.*, 104 (1986) 213266u.
[11] G.R. Buettner, *Arch. Biochem. Biophys.*, 300 (1993) 535–543.
[12] C. Martius and H. Eilingsfeld, *Liebigs Ann. Chem.*, 607 (1957) 159–168.
[13] D.C. Liebler and J.A. Burr, *Biochemistry*, 31 (1992) 8278–8284.
[14] C. Martius and H. Eilingsfeld, *Biochem. Z.*, 328 (1957) 507–508.
[15] V.L. Frampton, W.A. Skinner, P. Cambour, and P.S. Bailey, *J. Am. Chem. Soc.*, 82 (1960) 4632–4634.
[16] D.R. Nelang and C.D. Robeson, *J. Am. Chem. Soc.*, 84 (1962) 2963–2965.
[17] P. Schudel, H. Mayer, J. Metzger, P. Rüegg, and O. Isler, *Helv. Chim. Acta.*, 46 (1963) 636–649.
[18] C. Suarna and P.T. Southwell-Keely, *Lipids*, 26 (1991) 187–190.
[19] R. Yamauchi, K. Kato, and Y. Ueno, *Lipids*, 23 (1988) 779–783.
[20] Sunstar Inc., Jpn. Kokai Tokkyo Koho, JP 60 56 994 (1985); *Chem. Abstr.*, 104 (1986) 34290a.
[21] Y. Sano and H. Takagaki, Jpn. Kokai Tokkyo Koho, JP 02 144 151 (1990); *Chem. Abstr.*, 113 (1990) 152962e.
[22] M.L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, 2 (1962) 211–215.
[23] V. Vill, T. Böcker, J. Thiem, and F. Fischer, *Liq. Cryst.*, 6 (1989) 349–356.
[24] J. Thiem, M. Lahmann, and G. Schneider, Beiersdorf AG, DE 195 04 398 A1 (1996).
[25] G. Schneider, J. Thiem, and M. Lahmann, Beiersdorf AG, EP 0 726 273 A1 (1996).
[26] S. Matsumoto, M. Matsuo, and Y. Iitaka, *J. Org. Chem.*, 51 (1986) 1435–1440.
[27] S. Brownstein, G.W. Burton, L. Hughes, and K.U. Ingold, *J. Org. Chem.*, 54 (1989) 560–569.
[28] G. Zemplén and E. Pascu, *Ber. Dtsch. Chem. Ges.*, 62 (1929) 1613–1614.
[29] J. Conchie, G.A. Levy, and C.Y. Marsh, *Adv. Carbohydr. Chem.*, 12 (1957) 157–187.
[30] A. Emmerie and C. Engel, *Rec. Trav. Chim. Pays-Bas*, 58 (1939) 895–902.
[31] H.U. Bergmeyer, *Methoden der enzym. Analyse*, VCH, Weinheim (1977).
[32] B. Evers, *Dissertation*, Univ. Hamburg (1996).