ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 325 (2000) 157–168

CARBOHYDRATE RESEARCH

Independent synthesis of aminophospholipid-linked Maillard products

Carmen M. Utzmann, Markus O. Lederer *

Institut für Lebensmittelchemie (170), Universität Hohenheim, Garbenstraße 28, D-70593 Stuttgart, Germany

Received 26 July 1999; accepted 2 December 1999

Abstract

Phospholipid-linked glycation products are supposed to play an important role in lipid oxidation in vivo. Independent syntheses and unequivocal structural characterization are reported for the phosphatidyl ethanolamine (PE)-derived Amadori compound 4-hydroxy-4-oxo-1-[(palmitoyloxy)methyl]-9-(2,3,4,5-tetrahydroxytetrahydro-2*H*-pyran-2-yl)-3,5-dioxa-8-aza-4λ^5-phosphanon-1-yl palmitate, pyrrolecarbaldehyde 2-{[{2-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]ethoxy}(hydroxy)phosphoryl]oxy}-1-[(palmitoyloxy)methyl]ethyl palmitate, the carboxymethyl (CM) derivative 7-hydroxy-7,13-dioxo-10-(palmitoyloxy)-6,8,12-trioxa-3-aza-7λ^5-phosphaoctacosan-1-oic acid, and the carboxyethyl (CE) derivative 7-hydroxy-2-methyl-7,13-dioxo-10-(palmitoyloxy)-6,8,12-trioxa-3-aza-7λ^5-phosphaoctacosan-1-oic acid. With these reference compounds, a liquid chromatography–mass spectrometry (LCMS) method for the determination of such PE-linked Maillard products has been developed.

Keywords: Maillard reaction; Glycation; Phosphatidyl ethanolamine; Amadori compound; Advanced glycation endproducts (AGEs)

1. Introduction

Reactions of reducing carbohydrates with amino acids and proteins, generally known as Maillard reactions or glycation, play an important role in food chemistry and medicine [1,2]. The initial phase of such nonenzymatic processes is triggered by an amine adding to the carbonyl function of a reducing sugar with the formation of glycosylamines. These adducts can rearrange to the more stable aminoketoses (Amadori products) [3]. Once formed, Amadori compounds undergo further reactions, e.g., via dicarbonyl intermediates (deoxyosones), producing a heterogeneous group of amine-bound moieties termed 'advanced glycation endproducts' or AGEs [4]. In vivo, glycation reactions are involved in pathophysiology associated with hyperglycemia in diabetes and age-related dysfunction of tissues [5–7]. So far, the numerous studies addressing such subjects have been focussed on the Maillard reaction of proteins. However, several recent reports indicate that aminophospholipids may likewise be targets for glycation reactions. These membranous functional lipids are vital for the maintenance of cellular integrity and survival. The nonenzymatic glycation of membrane lipids could conceivably cause inactivation of receptors, cross-linking of aminophospholipids and proteins, membrane lipid peroxidation, and cell death.

* Corresponding author. Tel.: +49-711-4594095; fax: +49-711-4594096.

E-mail address: ledererm@uni-hohenheim.de (M.O. Lederer)

0008-6215/00/$ - see front matter
PII: S0008-6215(99)00330-4

Bucala et al. [8,9] have shown phosphatidyl ethanolamine (PE), a lipid component of low-density lipoprotein (LDL), to react with glucose and so to initiate advanced glycation reactions, forming phospholipid-linked AGEs. There is a significant correlation between the AGE levels, detected by ELISA, and the level of oxidized LDL; this finding supports the hypothesis that AGE-initiated oxidation plays an important role in lipid oxidation in vivo.

Pamplona et al. [10] have studied the liberation of 5-(hydroxymethyl)-2-furaldehyde, a stable follow-up product of Amadori compounds, from the acid-treated phospholipid fraction from rat liver. Ravandi et al. [11,12] further documented the existence of glycated PE (*g* PE) in human red blood cells. The analytical procedures employed by both groups could not distinguish, though, between glucosylamine **1** and aminoketose **2** (see Fig. 1). Recently, we have definitely established the formation of a PE-linked Amadori product in model reactions [13]. Lertsiri et al. [14] isolated *g* PE from incubation of egg-yolk PE and D-glucose; based on ^{1}H NMR data they assigned an aminoketose structure to the product obtained. These authors also isolated and identified *g* PE from human blood plasma and red blood cells employing a two-stage normal- and reversed-phase high-performance liquid chromatography (HPLC) procedure. Since the molar ratio *g* PE:PE was determined by measuring the phospholipid phosphorus and sugar contents of the combined (*g* PE + PE) fraction, i.e., a method that is not structure specific, the isolated *g* PE material might consist of both **1** and **2**.

Carboxymethyl (CM)-PE **3**, originating from either glycoxidation or lipoxidation processes, has been detected in red blood cells [15] and mitochondrial membranes [16]. Finally, we have identified the pyrrolecarbaldehyde **4** as an AGE from model reactions of PE with D-glucose and 3-deoxyglucosone [17].

So far, identification and quantification methods for phospholipid-linked Maillard products mostly rely on complex workup and/or derivatization procedures, and allow monitoring of one single compound only [13–16]. For an unequivocal identification and simultaneous quantification of such products by liquid chromatography–mass spectrometry (LCMS) techniques in biological matrices, the availability of authentic reference material is a prerequisite. We have therefore developed an independent synthesis for the Amadori product 4-hydroxy-4-oxo-1-[(palmitoyloxy)-methyl]-9-(2,3,4,5-tetrahydroxytetrahydro-2*H*-pyran-2-yl)-3,5-dioxa-8-aza-4λ^5-phosphanon-1-yl palmitate (**9**), the pyrrolecarbaldehyde derivative 2-{[{2-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]ethoxy}(hydroxy)phosphoryl]-oxy}-1-[(palmitoyloxy)methyl]ethyl palmitate (**15**), the carboxymethyl derivative 7-hydroxy-7,13-dioxo-10-(palmitoyloxy)-6,8,12-trioxa-3-aza-7λ^5-phosphaoctacosan-1-oic acid (**20**), and the carboxyethyl (CE) derivative 7-hydroxy-2-methyl-7,13-dioxo-10-(palmitoyloxy)-6,8,12-trioxa-3-aza-7λ^5-phosphaoctacosan-1-oic acid (**21**) (see Schemes 1–3). For all these new compounds, an unequivocal structural characterization is given, and an LCMS method for the determination of such Maillard products was developed.

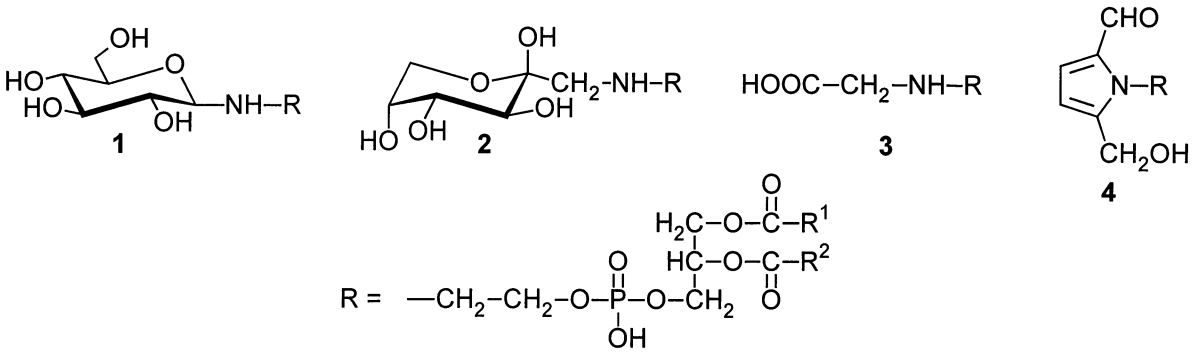
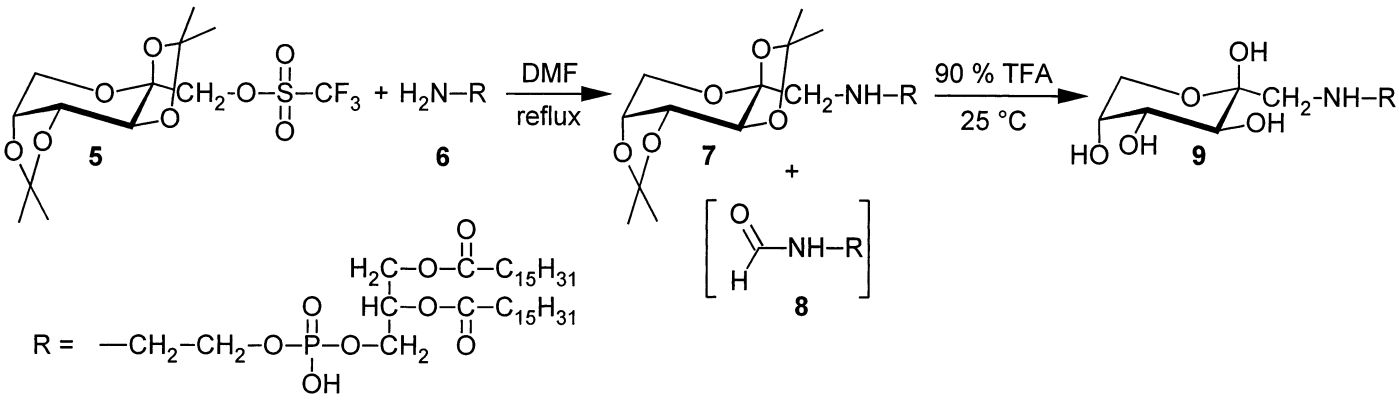
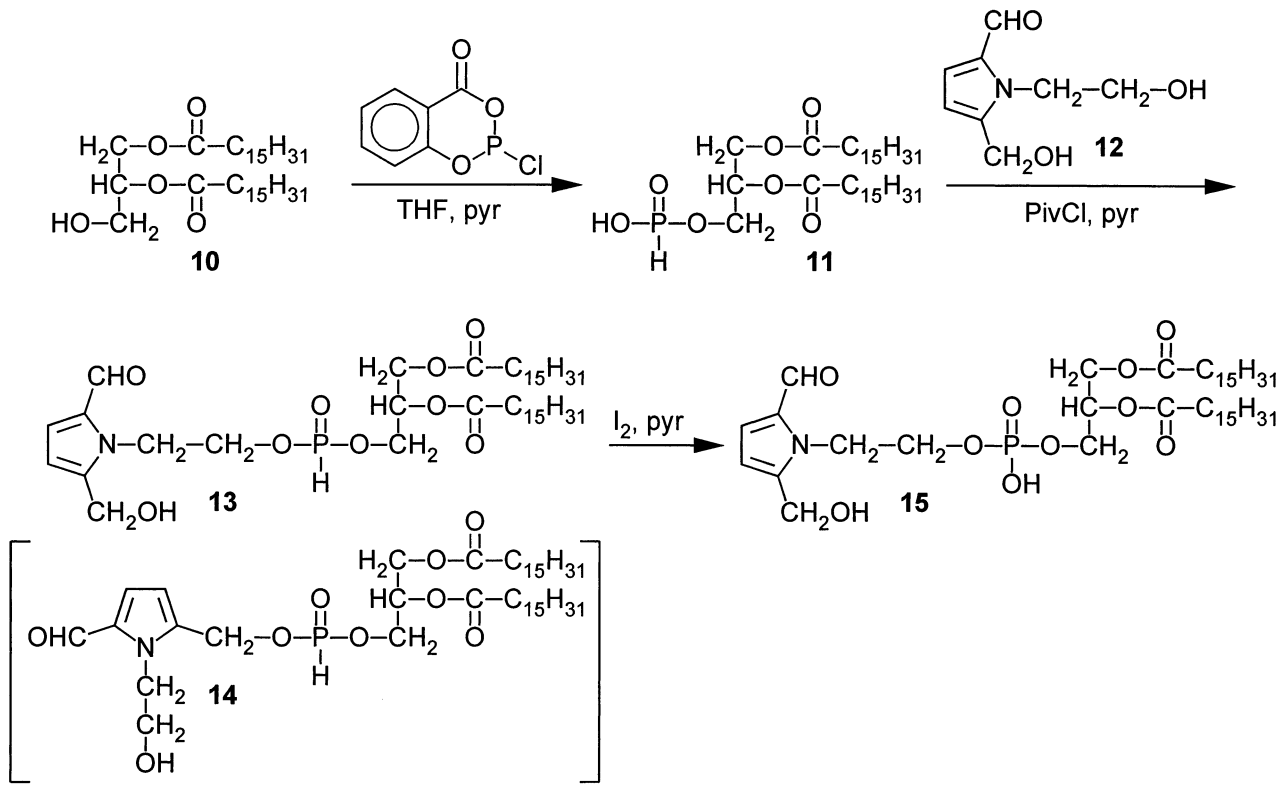


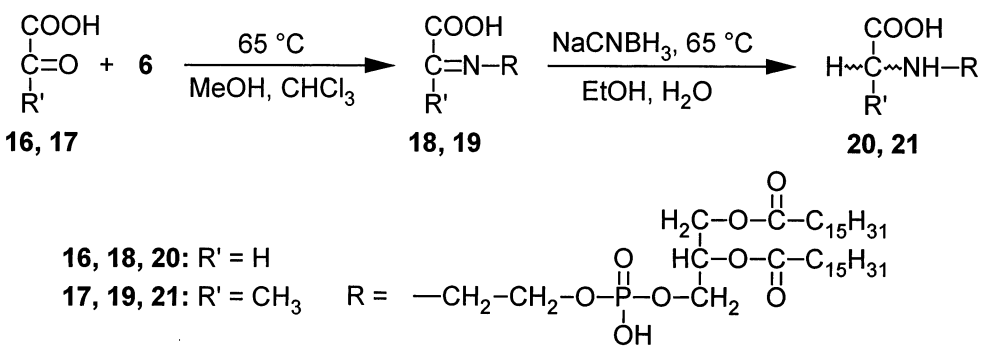
Fig. 1. General structure of PE-linked glucosylamine **1**, Amadori product **2**, carboxymethyl derivative **3**, and pyrrolecarbaldehyde **4**.



Scheme 1. Reaction pathway for the synthesis of Amadori-PE **9**; TFA = trifluoroacetic acid.



Scheme 2. Reaction pathway for the synthesis of Pyrrole-PE **15**; PivCl = pivaloyl chloride.



Scheme 3. Reaction pathway for the synthesis of CM-PE **20** and CE-PE **21**.

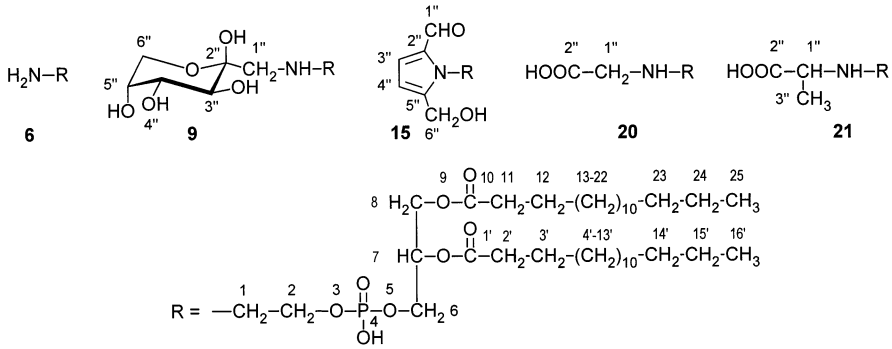
2. Results

The strategy for the synthesis of the Amadori compound **9** is based on a method described by Xenakis et al. [18]. According to this protocol, we have blocked D-fructose in the β-pyranose configuration by two isopropylidene protective groups [19]. Di-*O*-isopropylidene-2,3:4,5-β-D-fructopyranose was converted to the 1-*O*-triflate derivative **5** with trifluoromethanesulfonic anhydride at −15 °C. For a nucleophilic substitution at C-1, **5** was refluxed with 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine (**6**) in dimethylformamide (DMF) (see Scheme 1). The resulting 4-hydroxy-4-oxo-1-[(palmitoyloxy)methyl]-9-(2,2,7,7-tetramethyltetrahydro-3a*H*-di[1,3]dioxolo[4,5-*b*:4,5-*d*]pyran-3a-flyl)-3,5-dioxa-8-aza-4λ^5-phosphanon-1-yl palmitate (**7**) was purified by preparative thin-layer chromatography (PTLC) on silica gel. A by-product of the reaction, also isolated by PTLC, was identified as the formylation product of **6**, 5-hydroxy-5,10-dioxo-2-(palmitoyloxy)-4,6-dioxa-9-aza-5λ^5-phosphadec-1-yl palmitate (**8**). The protective groups of **7** were cleaved with 90% trifluoroacetic acid (TFA) at room temperature, yielding 4-hydroxy-4-oxo-1-[(palmitoyloxy)methyl]-9-(2,3,4,5-tetrahydroxytetrahydro-2*H*-pyran-2-yl)-3,5-dioxa-8-aza-4λ^5-phosphanon-1-yl palmitate (**9**, Amadori-PE). The crude Amadori product **9** was purified by column chromatography on silica gel, and the structure definitely established by nuclear magnetic resonance (NMR) spectroscopy and liquid secondary-ion high-resolution mass spectrometry (liquid SI-HRMS, analogous to FABHRMS). The accurate mass for the disodium adduct of **9** $[M - H + 2\ Na]^+$ was determined as m/z 898.5408, corresponding to an elemental composition of $C_{43}H_{83}N_1Na_2O_{13}P_1$, and thus clearly establishes the formation of a 1:1 reaction product between **6** and a C_6-monosaccharide, with elimination of one mole of water.

NMR data were acquired from a solution of **9** in 2:1 $CDCl_3$–CD_3OD. The spectra first showed very poor peak shapes, unsuitable for determining $^1H,^1H$ or $^{31}P,^{13}C$ coupling constants. This line broadening is due to H,H exchange reactions and can be resolved by adding a trace of DCl in D_2O to the solution, accelerating these processes enormously. However, since addition of acid to the mixture leads to a slow degradation of **9**, the spectra must be recorded immediately. ^1H and ^{13}C NMR data (chemical shifts, δ; coupling constants, J) of **9** are listed in Table 1. The predominant $^2C_5$ conformation of the pyranosyl ring in **9** is unequivocally proven by the spectroscopic evidence. The large vicinal coupling constant (^3J 9.6 Hz) between H-3″ and H-4″ can be reconciled only with a diaxial orientation of these protons. The ^3J value of 3.4 Hz between H-4″ and H-5″ is typical for the coupling of an axial with an equatorial proton. Furthermore, the small ^3J constants of H-5″ to both diastereotopic hydrogens H_a-6″ and H_b-6″ (1.4 and 1.9 Hz, respectively) clearly show H-5″ to be equatorial; the alternative axial orientation would require a value of 8–11 Hz for one of these coupling constants. The predominant formation of the β anomer can also be rationalized by a straightforward stereochemical argument; in a six-membered ring with chair conformation an equatorial position of the bulky PE substituent is preferred. Additionally, the polar NMR medium and the equatorial orientation of the 3″-OH group in **9** favor the β configuration due to the anomeric effect [20].

For the synthesis of 2-{[{2-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]ethoxy}(hydroxy)phosphoryl]oxy}-1-[(palmitoyloxy)-methyl]ethyl palmitate (**15**, Pyrrole-PE), 1,2-dipalmitoylglycerol (**10**) was transformed into the 3-*H*-phosphonate **11** by 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one [21] (see Scheme 2); crude **11** was purified by column chromatography on silica gel. Compound **11** was reacted with 1-(2-hydroxyethyl)-5-(hydroxymethyl)-1*H*-pyrrole-2-carbaldehyde (**12**), prepared according to the literature [17,22], using pivaloyl chloride as the condensing agent [23]. Since **12** has two reactive sites, formation of both regioisomers 2-{[{2-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]-ethoxy}(oxo)phosphoranyl]oxy}-1-[(palmitoyloxy)methyl]ethyl palmitate (**13**) and 2-{[{[5-formyl-1-(2-hydroxyethyl)-1*H*-pyrrol-2-yl]-methoxy}(oxo)phosphoranyl]oxy}-1-[(palmitoyloxy)methyl]ethyl palmitate (**14**) has to be

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **6**, **9**, **15**, **20**, and **21** [a]



	6 [b]	**9**	**15**	**20**	**21**
Solvent	$CDCl_3$–CD_3OD–DCl (trace)	$CDCl_3$–CD_3OD–DCl (trace)	$CDCl_3$	$CDCl_3$–CD_3OD–DCl (trace)	$CDCl_3$–CD_3OD–DCl (trace)
^{1}H NMR	δ (ppm)				
H-1	3.26	3.44	3.73	3.44	3.42
H-2	4.26	4.33	4.53	4.33	≈4.34
H-6	≈4.16	≈4.15	≈4.07	≈4.16	≈4.16
H-7	5.26	5.27	5.12	5.27	≈5.27
H-8_a	4.19	4.19	4.06	4.19	4.19
H-8_b	4.39	4.40	4.26	4.40	4.40
H-11/H-2′	2.33/2.36	2.34/2.36	2.27	2.34/2.36	2.33/2.36
H-12/H-3′	1.61	≈1.61	≈1.56	1.61	≈1.61
H-(13-24)/H-(4′-15′)	≈1.3	≈1.3	≈1.3	≈1.3	≈1.3
H-25/H-16′	0.89	0.89	0.88	0.89	0.89
H-1″			9.43	3.96	4.09
H-$1''_a$		3.32			
H-$1''_b$		3.38			
H-3″		3.76	6.88		1.67
H-4″		3.86	6.23		
H-5″		3.95			
H-6″			4.63		
H-$6''_a$		3.76			
H-$6''_b$		4.04			
J (Hz)					
$^2J_{8a,8b}$	(−)12.1	(−)12.1	(−)12.1	(−)12.1	≈(−)12.3
$^2J_{1''a,1''b}$		(−)12.8			
$^2J_{6''a,6''b}$		(−)12.6			
$^3J_{1,2}$	[c]	[c]	≈6.2	[c]	[c]
$^3J_{6,7}$	5.2	5.1	5.6	[d]	[d]
$^3J_{7,8a}$	3.5	3.4	3.0	3.4	3.3
$^3J_{7,8b}$	6.5	6.6	6.9	6.7	≈6.8
$^3J_{11,12/2',3'}$	7.6	7.6	≈7.7	7.6	7.6
$^3J_{24,25/15',16'}$	7.0	7.0	7.0	7.0	7.0
$^3J_{1'',3''}$					7.3
$^3J_{3'',4''}$		9.6	4.0		
$^3J_{4'',5''}$		3.4			
$^3J_{5'',6''a}$		1.4			
$^3J_{5'',6''b}$		1.9			
^{13}C NMR	δ (ppm)				
C-1	40.2	49.9	45.8	47.9	46.5
C-2	63.4	60.9	63.6	62.5	62.6

Table 1 (Continued)

C-6	65.4	64.4	65.3	65.4	65.4
C-7	70.0	70.6	70.2	70.1	70.0
C-8	62.4	62.8	62.6	62.4	62.4
C-10/C-1′	173.9/174.3	173.9/174.3	173.5/173.7	173.9/174.3	173.9/174.3
C-11/C-2′	34.3/34.4	34.4/34.5	34.1/34.3	34.3/34.5	34.3/34.4
C-12/C-3′	25.12/25.14	25.19/25.23	24.9/25.0	25.1/25.2	2 × 25.1
C-(13–22)/C-(4′–13′)	29.4–30.0	29.4–30.0	29.2–29.8	29.4–30.0	29.3–29.9
C-23/C-14′	32.2	32.3	32.0	32.2	32.2
C-24/C-15′	22.9	23.0	22.7	22.9	22.9
C-25/C-16′	14.2	14.2	14.1	14.2	14.2
C-1″		53.2	179.4	47.6	55.9
C-2″		95.6	132.0	167.7	170.8
C-3″		70.3	124.9		14.8
C-4″		70.2	110.6		
C-5″		69.7	143.5		
C-6″		63.9	55.5		
J (Hz)					
$^2J_{2,P}$	5.0	6.8	3.4	5.3	4.9
$^2J_{6,P}$	5.2	4.4	3.4	5.6	[e]
$^3J_{1,P}$	7.6	3.1	7.3	7.2	4.8
$^3J_{7,P}$	7.9	7.0	7.4	7.6	8.0

[a] δ, chemical shift for the indicated hydrogen/carbon; *J* (Hz), coupling constant between the indicated nuclei. Hydrogen/carbon assignment is validated by ^{1}H,^{1}H COSY, ^{1}H,^{13}C COSY and ^{13}C DEPT measurements.

[b] The NMR data of native PE **6**, obtained under identical conditions, are included to allow direct comparison with the data of **9**, **15**, **20**, and **21**; they are consistent with those in the literature [23,25].

[c] Coupling constants could not be determined due to higher-order spin systems.

[d] Coupling constants could not be determined due to overlapping multiplets.

[e] The coupling constant could not be determined because the doublet was not resolved.

expected in principle. Without any attempt to isolate these intermediates, the reaction mixture was oxidized in situ with iodine [23]. After reduction of excess iodine, **15** was purified by preparative HPLC on silica gel. Unexpectedly, not even traces of the oxidation product of **14** were detected. This may be due to the fact that functional groups at the α-carbon of a pyrrole alkyl side-chain are especially susceptible to nucleophilic substitution [24]. With the phosphoric acid diester function, the oxidation product of **14** bears a potent leaving group at this position, and thus is expected to readily undergo hydrolysis during the workup procedure. The empirical formula of **15** was confirmed by FABHRMS, which gave *m*/*z* 822.5262 for the $[M + Na]^+$ peak, corresponding to $C_{43}H_{78}N_1Na_1O_{10}P_1$. The NMR data, compiled in Table 1, definitely prove the structure of **15**. The ^{13}C spectrum clearly displays a doublet (^2J 3.4 Hz) for C-2 at 63.6 ppm caused by the ^{31}P coupling, whereas the C-6″ signal at 55.5 ppm only shows singlet multiplicity (for the numbering see Table 1). In the formation of **15**, **12** must therefore have been bonded to the phospholipid by the 1-(2-hydroxyethyl)-substituent.

7-Hydroxy-7,13-dioxo-10-(palmitoyloxy)-6,8,12-trioxa-3-aza-7λ^5-phosphaoctacosan-1-oic acid (**20**, CM-PE) and 7-hydroxy-2-methyl-7,13-dioxo-10-(palmitoyloxy)-6,8,12-trioxa-3-aza-7λ^5-phosphaoctacosan-1-oic acid (**21**, CE-PE) were obtained from the reaction of glyoxylic acid **16** and pyruvic acid **17** with **6**, respectively, followed by reduction of the Schiff base intermediates **18** and **19** with sodium cyanoborohydride (see Scheme 3). Both products were purified by preparative HPLC on silica gel. Again, elemental composition and structure of **20** and **21** were established by FABHRMS and NMR (see Table 1). As in the case of **9**, a trace of DCl had to be added to the NMR solutions for obtaining well-resolved spectra.

With the reference compounds **9**, **15**, **20**, and **21** characterized unequivocally, we have elaborated a protocol for their determination by LCMS, equipped with an electrospray in-

terface (ESI). None of the various procedures described in the literature [12,26–28] for HPLC separation of phospholipids gave satisfactory signal resolution or peak shape for our compounds; some methods are a priori incompatible with an ESI source. We have therefore tested various stationary phases (silica, amino, polymer C18, endcapped C18) and solvent gradients for optimum chromatographic performance. The best results were obtained with a highly endcapped C18 material, which shows excellent stability to alkaline eluents, up to a pH of 9.0, and a water–ammonium hydroxide–methanol–tetrahydrofuran gradient. This basic medium additionally favors formation of negative ions, and thus enhances the MS sensitivity when operating in the negative ESI mode (ESI^-) for PE and the Maillard products derived therefrom.

Typical chromatograms for the analysis of the reference compounds are given in Fig. 2. The upper trace represents the total ion current (TIC); the lower chromatograms show $[M-H]^-$ ion traces of PE **6** (m/z 690.5), Pyrrole-PE **15** (m/z 798.5), Amadori-PE **9** (m/z 852.5), CE-PE **21** (m/z 762.5), and CM-PE **20** (m/z 748.5). Preliminary experiments with a phospholipid isolate from soy bean, containing PE derivatives with various combinations of fatty acid substituents, proved that our LCMS method is also suitable for separating and determine the different PE species as well as their respective Maillard products in a single run (not detailed in Section 4).

3. Discussion

Under hyperglycemic conditions (i.e., diabetes), many proteins in blood and tissue organelles are exposed to nonenzymatic glycation. Accordingly, aminophospholipids such as PE and phosphatidylserine are recognized to be prone to modification by Maillard processes, since both have a primary amino group.

Amadori compounds represent the initial stage of Maillard reactions; the formation of

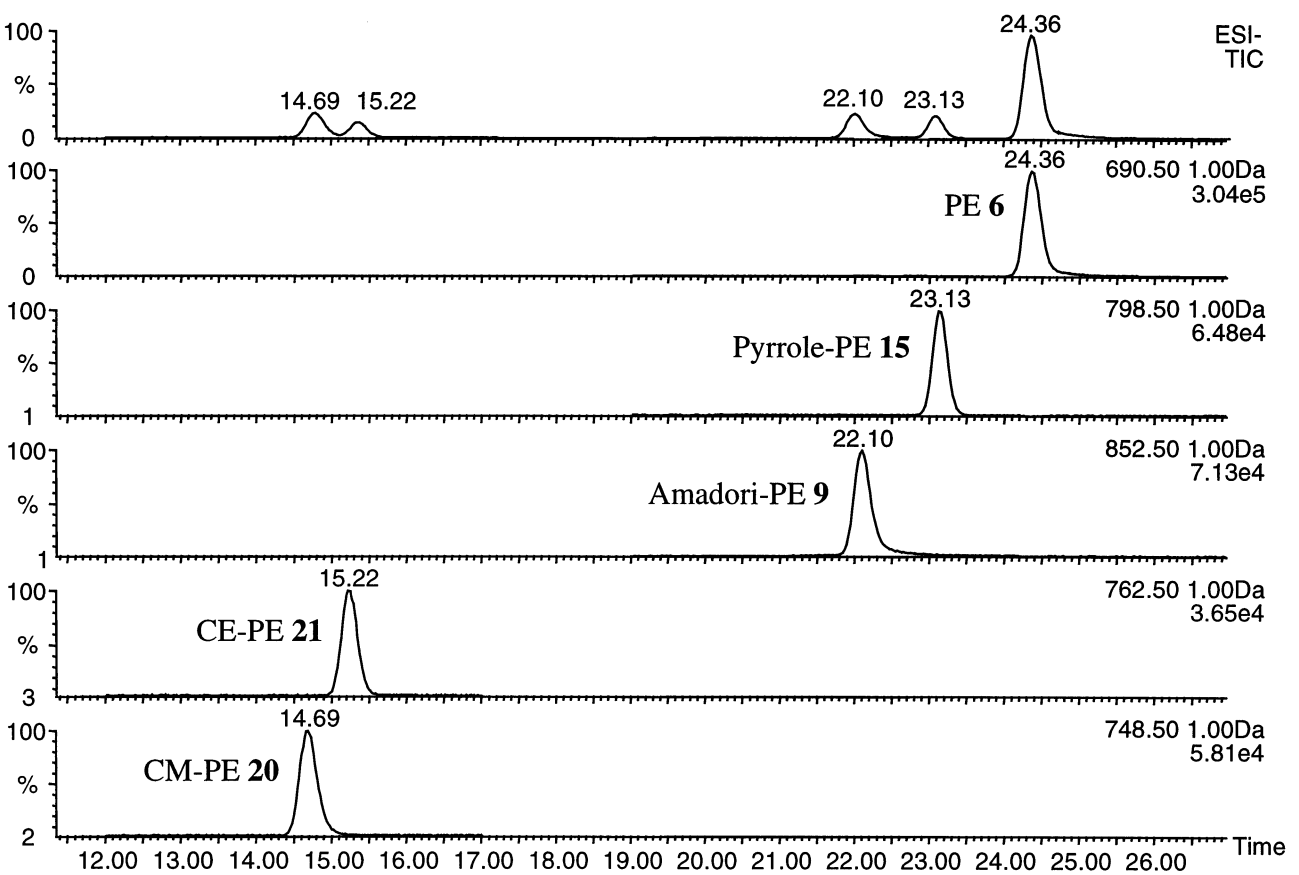


Fig. 2. LCMS chromatograms for the determination of compounds **6**, **9**, **15**, **20**, and **21**.

pyrrolecarbaldehydes, carboxymethyl (CM) and carboxyethyl (CE) derivatives is characteristic for advanced glycation processes. These three AGE derivatives have significantly different formation pathways. Pyrrolecarbaldehydes are derived directly from the reaction of 3-deoxyosones with amines under conservation of the complete carbon backbone [29]. CE derivatives [30] are follow-up products of the C-3 unit methylglyoxal, which can be formed, e.g., in vitro by reverse aldol reaction of 3-deoxyosones, and in vivo either enzymatically from dihydroxyacetone phosphate by methylglyoxal synthase and other pathways, or nonenzymatically by elimination of phosphate from glyceraldehyde phosphate or dihydroxyacetone phosphate [31,32]. Both pyrrolecarbaldehydes and CE derivatives are formed without an oxidation process being involved. Generation of CM derivatives, in contrast, requires an oxidation step, and can proceed via oxidative degradation of Amadori products [33], termed as glycoxidation, or reaction of amines with glyoxal, which can stem from D-glucose autoxidation [34] as well as lipid peroxidation [35]. Compounds like Pyrrole-PE **15** and CE-PE **21** may therefore serve as biomarkers for 'carbonyl stress', whereas CM-PE **20** is an indicator for 'oxidative stress' [36].

Hence, we have independently synthesized these three AGEs **15**, **20**, and **21**, as well as the Amadori compound **9**, to evaluate phospholipid glycation in general on one hand, and to distinguish between oxidative and nonoxidative pathways on the other hand. Since 1,2-dipalmitoyl - 3 - *sn* - phosphatidylethanolamine (**6**) is not a naturally occurring PE derivative, our products **9**, **15**, **20**, and **21** can be used as internal standards for the quantification of the respective Maillard products derived from other PE species. Unfortunately, compounds like **9**, **20**, and **21** have no chromophore and thus do not allow sensitive detection by UV spectroscopy; only pyrrolecarbaldehyde **15** shows an intense absorption with a maximum at 298 nm. For both sensitive and specific detection of **9**, **15**, **20**, **21**, as well as PE **6**, LCMS is therefore the method of choice. Since all these compounds bear highly polar functional groups, the electrospray interface can be employed for ionization. The PE-nitrogen in **15** is incorporated in a pyrrole ring and has lost its basic character; the yield of positively charged ions from **15** is thus very low. For the carboxy derivatives **20** and **21**, formation of negative ions is a priori favored. The ESI–MS was therefore operated in the negative-ion mode. The suitability of our compounds **9**, **15**, **20**, and **21** as internal standards is emphasized even more by the fact that ion generation in ESI exclusively depends on the polar functions of the molecule. Structures analogous to our synthesized products differing only in the fatty acid composition can therefore be assumed to give a molar response almost identical to that of the respective reference compound.

Based on these findings, we now try to quantify *g* PE from human erythrocytes–LDL and to prove an eventual correlation between atherosclerotic complications in diabetes and 'carbonyl' as well as 'oxidative stress'. Increased levels of CM-PE in diabetics with extensive vascular complications are likely, as they are predicted to suffer from enhanced oxidative stress. The formation of PE-linked glycation products in foodstuffs will also be investigated. In vitro studies with Amadori-PE will also unravel whether such compounds can generate reactive oxygen species, and thus induce peroxidation of unsaturated fatty acids as already described for protein-bound Amadori products [37–40].

4. Experimental

General methods.—Melting points were determined on an Electrothermal (Southend-on-Sea, UK) 8100 digital melting point apparatus and are not corrected. ^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded on a Bruker (Karlsruhe, Germany) ARX 500 spectrometer. Chemical shifts (δ) are given in ppm relative to internal Me_4Si, coupling constants (J) in Hz. Hydrogen and carbon numbering, except for compound **11**, is always according to the order given in Table 1. For the 2D NMR experiments, ^{1}H,^{1}H COSY and ^{1}H,^{13}C COSY, Bruker standard software (X-WIN-NMR 2.0) was employed. Liquid secondary-ion high-resolution mass

spectra (analogous to FABHRMS) were obtained on a Finnigan (Bremen, Germany) MAT 95 instrument. LCMS was run on an HP1100 HPLC system, which comprised an HP1100 autosampler, HP1100 gradient pump, HP1100 column thermoregulator, and HP1100 diode array detector (DAD) module (Hewlett–Packard, Waldbronn, Germany), coupled to a Micromass (Manchester, UK) VG platform II quadrupole mass spectrometer equipped with an electrospray (ESI) interface. Chromatographic conditions: YMC-Pack Pro C18, 120 Å, 5 μm (YMC Europe, Schermbeck, Germany; guard column 10 × 4.6 mm, column 150 × 4.6 mm); column temperature 25 °C; flow rate 1.0 mL/min; injection volume 10 μL; eluent mixtures: (A) 80:20:0.5 MeOH–water–NH_3 (25%); (B) 65:25:10:0.5 MeOH–THF–water–NH_3 (25%), gradient: % B (*t* [min]) 0(0)–100(30–40)–0(43–50); post-column splitting ratio 1:20. MS parameters: ESI^-; source temperature 120 °C; capillary 3.0 kV; HV lens 0.5 kV; cone voltage 60 V. The MS system was operated either in full scan (*m*/*z* 200–1000) or single ion mode (SIM; span 0.5 Da, dwell time 0.5 s). For data acquisition and processing, MassLynx 3.2 software was used. The preparative HPLC system consisted of a Kronlab (Sinsheim, Germany) HD 2-200 gradient pump system combined with a Kronlab SpectraFlow 500 variable wavelength detector and a Kronlab HPLC column (20 × 260 mm, YMC silica gel, 60 Å, 20 μm); flow rate 20 mL/min; injection volume 2.0 mL; eluent mixtures: (A) 84:15.5:0.5 $CHCl_3$–MeOH–NH_3 (25%); (B) 60:34:5.5:0.5 $CHCl_3$–MeOH–H_2O–NH_3 (25%); gradient 1: % B (*t* [min]) 0(0)–40(13)–0(16–21), gradient 2: % B (*t* [min]) 0(0)–100(30–35)–0(38–43). Silica Gel 60 F_{254}, 0.2 mm (E. Merck, Darmstadt, Germany) was used for analytical thin-layer chromatography (TLC), Silica Gel 60 F_{254}, 2 mm for preparative TLC (PTLC), and Silica Gel, 63–200 μm (Baker, Gross-Gerau, Germany) for column chromatography.

Materials.—For LC, ultrapure water from a Milli-Q 185 plus apparatus (Millipore, Eschborn, Germany), HPLC grade methanol, HPLC grade tetrahydrofuran (Fluka, Neu-Ulm, Germany), and aqueous ammonia (NH_3, 25%, E. Merck) were used. 1,2-Dipalmitoyl-3-*sn*-phosphatidylethanolamine, 1,2-dipalmitoyl-3-*sn*-glycerol, and pivaloyl chloride were purchased from Sigma–Aldrich (Steinheim, Germany), ethanolamine, sodium cyanoborohydride, glyoxylic acid monohydrate, trifluoroacetic acid, triethylammonium hydrogen carbonate buffer (1 M), trifluoromethanesulfonic anhydride, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and 2-chloro-4*H*-1,3,2-benzodioxaphosphinin-4-one from Fluka, sodium pyruvate from E. Merck. D-Glucose and D-fructose were generously supplied by Südzucker AG (Mannheim, Germany).

*4-Hydroxy-4-oxo-1-[(palmitoyloxy)methyl]-9-(2,2,7,7-tetramethyltetrahydro-3a*H*-di[1,3]-dioxolo[4,5-b:4,5-*d*]pyran-3a-yl)-3,5-dioxa-8-aza-4λ⁵-phosphanon-1-yl palmitate* (**7**).—Di-*O*-isopropylidene-2,3:4,5-β-D-fructopyranose was prepared according to Ref. [19]. The ^1H and ^{13}C NMR data are fully consistent with those given in Refs. [41,42]. This compound was transformed into its triflate derivative **5** as described by Xenakis et al. [18].

To compound **5** (100 mg, 0.25 mmol), dissolved in DMF (2 mL, dried over 4 Å molecular sieve), 1,2-dipalmitoyl-3-*sn*-phosphatidylethanolamine (**6**, 275 mg, 0.40 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (85 μL, 0.57 mmol) were added, and the solution was refluxed for 30 min. The solvent was removed in high vacuum (5×10^{-2} mbar), the residue taken up in a 2:1 $CHCl_3$–MeOH mixture (3 mL), and purified by PTLC (mobile phase: 60:20:0.8 $CHCl_3$–MeOH–NH_3 (25%)). The plates were covered except for 2 cm on each side and sprayed with 2,7-dichlorofluorescein solution (0.1% in MeOH). Two bands at R_f 0.5 and 0.3 showing fluorescence on the margins were scraped out, and the silica gel extracted with MeOH (2 × 50 mL) and 2:1 MeOH–$CHCl_3$ (60 mL). The filtrate was concentrated to dryness, the residue dissolved in MeOH (15 mL), passed through a membrane filter (0.45 μm), the solvent stripped off, and the remainder dried in high vacuum. The residue was taken up in $CHCl_3$, the solvent evaporated, and the product dried in high vaccuum. The extract from the band at R_f 0.3 yielded 5-hydroxy-5,10-dioxo-2-(palmitoyloxy)-4,6-dioxa-9-aza-5λ⁵-phosphadec-1-yl palmitate (**8**) as amorphous product (106 mg,

0.147 mmol): ^{1}H NMR (CD_3OD): δ 8.07 (s, 1 H, H-1″), 5.22 (ddt, 1 H, $J_{7,8a}$ 3.2, $J_{7,8b}$ 6.7, $J_{6,7}$ 5.2 Hz, H-7), 4.44 (dd, 1 H, $J_{8a,8b}$ (−)12.0 Hz, H-8$_b$), 4.18 (dd, 1 H, H-8$_a$), 3.98 (dd, 2 H, $J_{6,P}$ 6.0 Hz, H-6), 3.91 (dt, 2 H, $J_{1,2}$ 5.4, $J_{2,P}$ 6.9 Hz, H-2), 3.45 (m, 2 H, H-1), 2.33 and 2.31 (2 t, 2 H each, $J_{11,12/2',3'}$ 7.4 Hz, H-11 and H-2′), 1.59 (m, 4 H, H-12 and H-3′), ≈1.3 (m, 48 H, H-13–H-24 and H-4′–H-15′), 0.90 (t, 6 H, $J_{24,25/15',16'}$ 7.0 Hz, H-25 and H-16′); ^{13}C NMR (CD_3OD): δ 174.9 and 174.6 (2 s, C-10 and C-1′), 163.9 (s, C-1″), 71.9 (d, $J_{7,P}$ 8.6 Hz, C-7), 65.1 (d, $J_{2,P}$ 5.6 Hz, C-2), 64.7 (d, $J_{6,P}$ 5.3 Hz, C-6), 63.7 (s, C-8), 39.9 (d, $J_{1,P}$ 7.6 Hz, C-1), 35.2 and 35.0 (2 s, C-11 and C-2′), 33.2 (s, C-23 and C-14′), 30.3–30.9 (s, C-13–C-22 and C-4′–C-13′), 26.09 and 26.08 (2 s, C-12 and C-3′), 23.8 (s, C-24 and C-15′), 14.5 (s, C-25 and C-16′); FABMS (*m*-nitrobenzyl alcohol): *m*/*z* 718.6 $[M-H]^-$.

The extract from the band at R_f 0.5 gave crystalline 4-hydroxy-4-oxo-1-[(palmitoyloxy)methyl]-9-(2,2,7,7-tetramethyltetrahydro-3a*H*-di[1,3]dioxolo[4,5-*b*:4,5-*d*]pyran-3a-yl)-3,5-dioxa-8-aza-4λ^5-phosphanon-1-yl palmitate (**7**) (98 mg, 0.105 mmol, 42.0%): mp 44 °C; ^{1}H NMR (CD_3OD): δ 4.60 (dd, 1 H, $J_{3'',4''}$ 2.6, $J_{4'',5''}$ 7.9 Hz, H-4″), 4.28 (d, 1 H, H-3″), 4.22 (ddd, 1 H, $J_{5'',6''a}$ 0.8, $J_{5'',6''b}$ 2.0 Hz, H-5″), 3.97 (dt, 2 H, $J_{1,2}$ 5.1, $J_{2,P}$ 6.3 Hz, H-2), 3.88 (dd, 1 H, $J_{6''a,6''b}$ (−)13.0 Hz, H-6$''_b$), ≈3.88 (m, 2 H, H-6), 3.76 (quintuplet, 1 H, $J_{6,7}$ 5.3, $J_{7,8}$ 5.3 Hz, H-7), 3.64 (dd, 1 H, H-6$''_a$), 3.62 (dd, 1 H, $J_{8a,8b}$ (−)11.3 Hz, H-8$_b$), 3.56 (dd, 1 H, H-8$_a$), 2.95 (d, 1 H, $J_{1''a,1''b}$ (−)12.5 Hz, H-1$''_b$), 2.87 (m, 2 H, H-1), 2.83 (d, 1 H, H-1$''_a$), 2.31 (t, 4 H, $J_{11,12/2',3'}$ 7.4 Hz, H-11 and H-2′), 1.60 (quintuplet, 4 H, $J_{12,13/3',4'}$ 7.4 Hz, H-12 and H-3′), 1.49, 1.44, 1.40, 1.33 (4 s, 3 H each, isopropylidene groups), ≈1.3 (m, 48 H, H-13–H-24 and H-4′–H-15′), 0.90 (t, 6 H, $J_{24,25/15',16'}$ 7.0 Hz, H-25 and H-16′); ^{13}C NMR (CD_3OD): δ 176.1 (s, C-10 and C-1′), 110.2 and 109.5 (2 s, isopropylidene groups), 104.5 (s, C-2″), 73.3 (s, C-3″), 72.7 (d, $J_{7,P}$ 7.1 Hz, C-7), 72.4 (s, C-5″), 71.7 (s, C-4″), 67.6 (d, $J_{6,P}$ 5.9 Hz, C-6), 65.5 (d, $J_{2,P}$ 5.7 Hz, C-2), 63.9 (s, C-8), 62.2 (s, C-6″), 57.1 (s, C-1″), 51.3 (d, $J_{1,P}$ 7.8 Hz, C-1), 34.9 (s, C-11 and C-2′), 33.1 (s, C-23 and C-14′), 30.2–30.8 (s, C-13–C-22 and C-4′–C-13′), 26.7 and 26.3 (2 s, isopropylidene groups), 26.1 (s, C-12 and C-3′), 25.7 and 24.3 (2 s, isopropylidene groups), 23.8 (s, C-24 and C-15′), 14.5 (s, C-25 and C-16′); LCMS: t_R 25.4 min, *m*/*z* 932.6 $[M-H]^-$; FABMS (*m*-nitrobenzyl alcohol): *m*/*z* 932.6 $[M-H]^-$.

*4-Hydroxy-4-oxo-1-[(palmitoyloxy)methyl]-9-(2,3,4,5-tetrahydroxytetrahydro-2*H*-pyran-2-yl)-3,5-dioxa-8-aza-4λ^5-phosphanon-1-yl palmitate* (**9**).—A solution of **7** (70 mg, 0.075 mmol) in trifluoroacetic acid–water (2 mL, 9:1 (v/v)) was kept at room temperature (rt) for 4 h and the solvent removed in high vacuum. The residue was taken up in $CHCl_3$ (3 mL) and purified by column chromatography (2 × 8 cm, 285:100:5 $CHCl_3$–MeOH–water, 390 mL). Fractions (7 mL each) were collected and tested for **9** by TLC (R_f 0.33, 95:35:2 $CHCl_3$–MeOH–water, detection: 2,7-dichlorofluorescein (0.1 % in MeOH)). From the fractions containing **9**, the solvent was evaporated, the residue dissolved in MeOH (10 mL), passed through a membrane filter (0.45 μm), MeOH stripped off, and the remainder taken up in $CHCl_3$. Removal of the solvent and drying in high vacuum gave **9** as crystalline product (40 mg, 0.047 mmol, 63.7%): mp 120–130 °C decomp.; ^{1}H and ^{13}C NMR (2:1 $CDCl_3$–CD_3OD, with a trace of DCl (37% in D_2O) added): see Table 1; LCMS: t_R 22.1 min, *m*/*z* (%) 852.5 ($[M-H]^-$; 100), 784.5 (9); FABHRMS (*m*-nitrobenzyl alcohol–glycerol, with NaI added): *m*/*z* 898.5408 $[M-H+2\ Na]^+$ (898.5397, Calcd for $C_{43}H_{83}N_1Na_2O_{13}P_1$).

Preparation of 2-{[hydroxy(oxo)phosphoranyl]oxy}-1-[(palmitoyloxy)methyl]ethyl palmitate (**11**) *[21].*—Crude **11** was purified on a silica gel column (2 × 8 cm, 4:1 EtOAc–MeOH, 500 mL). Fractions (7 mL each) were collected and tested for **11** by TLC (R_f 0.13, solvent mixture as above, detection: $K_2Cr_2O_7$ (0.5% in 1 N H_2SO_4, 10 min 105 °C). The solvent was removed from the fractions containing **11**, the residue dissolved in hot EtOAc and passed through a membrane filter (0.45 μm). Evaporating the solvent yielded **11** as crystalline powder, containing traces of triethylamine (176 mg, 0.28 mmol, 75.7%): mp 99 °C; ^{13}C NMR data were consistent with those given in Ref. [23]. In this case we have numbered the glycerol moieties in **11** from 1 to 3, the fatty acids from 1′ to 16′ and 1″ to

16″, respectively. ^{1}H NMR (2:1 $CDCl_3$–CD_3OD, with a trace of DCl (37% in D_2O) added): δ 6.79 (d, 1 H, $J_{H,P}$ 642.5 Hz, lit. 626 Hz [23], H-P), 5.23 (ddt, 1 H, $J_{2,1a}$ 3.6, $J_{2,1b}$ 6.5, $J_{2,3}$ 5.2 Hz, H-2), 4.39 (dd, 1 H, $J_{1a,1b}$ (−)12.0 Hz, H-1_b), 4.19 (dd, 1 H, H-1_a), 4.03 (dd, 2 H, $J_{3,P}$ 8.1 Hz, lit. 6.8 Hz [23], H-3), 3.13 (q, J 7.3 Hz, –N–CH_2– (triethylamine)), 2.34 and 2.33 (2 t, 2 H each, $J_{2',3'/2'',3''} \approx 7.9$ Hz, H-2′ and H-2″), $\approx$1.62 (m, 4 H, H-3′ and H-3″), 1.34 (t, –N–CH_2–CH_3 (triethylamine)), $\approx$1.3 (m, 48 H, H-4′–H-15′ and H-4″–H-15″), 0.89 (t, 6 H, $J_{15',16'/15'',16''}$ 7.0 Hz, H-16′ and H-16″); LCMS: t_R 23.0 min, m/z (%) 631.5 ([M − H]$^-$; 100), 393.3 (9), 375.4 (22), 255.3 (46).

2-{[{2-[2-Formyl-5-(hydroxymethyl)-1H-pyrrol1-yl]ethoxy}(hydroxy)phosphoryl]oxy}-1-[(palmitoyloxy)methyl]ethyl palmitate (**15**). —Compound **15** was synthesized following a procedure given by Lindh and Stawinski [23]. Compound **11** (50 mg, 0.08 mmol) and 1-(2-hydroxyethyl)-5-(hydroxymethyl)-1*H*-pyrrole-2-carbaldehyde (**12**) (26 mg, 0.15 mmol, prepared according to Refs. [17,22]) were dissolved in pyridine (1 mL, dried over 4 Å molecular sieve) under gentle heating. Pivaloyl chloride (29 μL, 0.24 mmol) was added and the reaction mixture kept at rt for 1 h. The solution was tested for **13** by TLC (R_f 0.65, 2:1 EtOAc–hexane, detection: $K_2Cr_2O_7$ (0.5% in 1 N H_2SO_4, 10 min 105 °C)). Iodine was added (53 mg, 0.21 mmol, dissolved in 2 mL 49:1 pyridine–water) and the oxidation monitored by TLC; turnover to **15** (R_f 0.35, 60:20:0.8 $CHCl_3$–MeOH–NH_3 (25%), detection as above) being almost quantitative within 1 h. Chloroform (30 mL) was added, the organic layer washed with aq sodium bisulfite solution (5% (w/v), 30 mL) and triethylammonium hydrogen carbonate buffer (TEAB, 0.1 M, 3 × 15 mL), and dried over anhyd Na_2SO_4. The filtrate was evaporated to dryness, the residue taken up in eluent mixture A (5 mL, 84:15.5:0.5 $CHCl_3$–MeOH–NH_3 (25%)), and purified by preparative HPLC (gradient 1) using 298 nm as detection wavelength. Fractions at t_R 10 min were collected, the solvent stripped off, and the residue dried in high vacuum to afford **15** as crystals (10 mg, 0.0125 mmol, 15.6 %): mp 88 °C; ^{1}H and ^{13}C NMR ($CDCl_3$): see Table 1; LCMS: t_R 23.1 min, m/z 798.5 [M − H]$^-$; FABHRMS (*m*-nitrobenzyl alcohol–glycerol, with NaI added): m/z 822.5262 [M + Na]$^+$ (822.5261, Calcd for $C_{43}H_{78}N_1Na_1O_{10}P_1$).

7-Hydroxy-7,13-dioxo-10-(palmitoyloxy)-6,8,12-trioxa-3-aza-7λ^5-phosphaoctacosan-1-oic acid (**20**) *and 7-hydroxy-2-methyl-7,13-dioxo-10-(palmitoyloxy)-6,8,12-trioxa-3-aza-7λ^5-phosphaoctacosan-1-oic acid* (**21**).—1,2-Dipalmitoyl-3-*sn*-phosphatidylethanolamine (**6**, 200 mg, 0.29 mmol) and glyoxylic acid monohydrate (275 mg, 2.99 mmol) or sodium pyruvate (163 mg, 1.48 mmol), respectively, were suspended in 2:1 $CHCl_3$–MeOH (5 mL) and kept for 1 h at 65 °C. The mixture was allowed to cool to rt, the solvent removed in vacuo, and the residue taken up in 3:1 EtOH–water (6 mL). The reaction mixture was heated to 65 °C and, after addition of sodium cyanoborohydride (60 mg, 0.95 mmol), kept at 65 °C for 2 h. The solution was diluted with water (25 mL), the pH adjusted to 2.5 with glacial AcOH, and the mixture extracted with $CHCl_3$ (30 mL). The organic layer was dried over anhyd Na_2SO_4, the filtrate evaporated to dryness, and the residue dried in high vacuum. Crude **20** and **21** were dissolved in eluent mixture A (2.5 mL each, 84:15.5:0.5 $CHCl_3$–MeOH–NH_3 (25%)) and purified by preparative HPLC (gradient 2). Fractions (10 mL each) were collected between 14 and 31 min and tested for **20** and **21** by TLC (R_f 0.27 and 0.36, respectively, 60:34:5.5:0.5 $CHCl_3$–MeOH–water–NH_3 (25%), detection: 2,7-dichlorofluoresceine (0.1% in MeOH)). Fractions containing **20** and **21**, respectively, were combined, the eluent removed in vacuo, the residue taken up in $CHCl_3$ (5 mL), the solvent stripped off, and the product dried in high vacuum. Compounds **20** and **21** were obtained as crystals (**20**: 16 mg, 0.021 mmol, 7.2%; **21**: 12 mg, 0.16 mmol, 5.5%): mp 190–195 °C decomp. (**20**), 185–190 °C decomp. (**21**); ^{1}H and ^{13}C NMR (2:1 $CDCl_3$–CD_3OD, with a trace of DCl (37% in D_2O) added): see Table 1; LCMS: **20**, t_R 14.7 min, m/z (%) 770.5 ([M − 2 H + Na]$^-$; 8), 748.5 ([M − H]$^-$; 100), 255.3 (12); **21**, t_R 15.2 min, m/z (%) 784.5 ([M − 2 H + Na]$^-$; 10), 762.5 ([M − H]$^-$; 100), 255.2(8); FABHRMS (*m*-nitrobenzyl

alcohol–glycerol, with NaI added): **20**, m/z 772.5105 $[M+Na]^+$ (772.5115, Calcd for $C_{39}H_{76}N_1Na_1O_{10}P_1$); **21**, m/z 786.5266 $[M+Na]^+$ (786.5261, Calcd for $C_{40}H_{78}N_1Na_1O_{10}P_1$).

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft (DFG) for funding this research, Priv.-Doz. Dr P. Fischer, Institut für Organische Chemie, Universität Stuttgart, for many helpful discussions, Professor Dr Wolfgang Schwack for the excellent working conditions at his Institute, Dr J. Opitz, Dipl.-Ing. J. Trinkner, J. Rebell, and H.-U. Höhn, Institut für Organische Chemie, Universität Stuttgart, for the recording of the FABHRMS and NMR spectra.

References

[1] F. Ledl, E. Schleicher, *Angew. Chem.*, 102 (1990) 597–626.
[2] M. Friedman, *J. Agric. Food Chem.*, 44 (1996) 631–653.
[3] M. Amadori, *Atti R. Acad. Naz. Lincei Mem. Cl. Sci. Fis. Mat. Nat.*, 13 (1931) 72–77.
[4] V.M. Monnier, A. Cerami, *Science*, 211 (1981) 491–493.
[5] D.G. Dyer, J.A. Blackledge, B.M. Katz, C.J. Hull, H.D. Adkisson, S.R. Thorpe, T.J. Lyons, J.W. Baynes, *Z. Ernährungswiss.*, 30 (1991) 29–45.
[6] V.M. Monnier, *Prog. Clin. Biol. Res.*, 304 (1989) 1–22.
[7] D.R. Sell, M.A. Lane, W.A. Johnson, E.J. Masoro, O.B. Mock, K.M. Reiser, J.F. Fogarty, R.G. Cutler, D.K. Ingram, G.S. Roth, V.M. Monnier, *Proc. Natl. Acad. Sci. USA*, 93 (1996) 485–490.
[8] R. Bucala, Z. Makita, T. Koschinsky, A. Cerami, H. Vlassara, *Proc. Natl. Acad. Sci. USA*, 90 (1993) 6434–6438.
[9] R. Bucala, Z. Makita, G. Vega, S. Grundy, T. Koschinsky, A. Cerami, H. Vlassara, *Proc. Natl. Acad. Sci. USA*, 91 (1994) 9441–9445.
[10] R. Pamplona, M.J. Bellmunt, M. Portero, D. Riba, J. Prat, *Life Sci.*, 57 (1995) 873–879.
[11] A. Ravandi, A. Kuksis, L. Marai, J.J. Myher, *Lipids*, 30 (1995) 885–891.
[12] A. Ravandi, A. Kuksis, L. Marai, J.J. Myher, G. Steiner, G. Lewisa, H. Kamido, *FEBS Lett.*, 381 (1996) 77–81.
[13] M.O. Lederer, C.M. Dreisbusch, R.M. Bundschuh, *Carbohydr. Res.*, 301 (1997) 111–121.
[14] S. Lertsiri, M. Shiraishi, T. Miyazawa, *Biosci. Biotech. Biochem.*, 62 (1998) 893–901.
[15] J.R. Requena, M.U. Ahmed, C.W. Fountain, T.P. Degenhardt, S. Reddy, C. Perez, T.J. Lyons, A.J. Jenkins, J.W. Baynes, S.R. Thorpe, *J. Biol. Chem.*, 272 (1997) 17473–17479.
[16] R. Pamplona, J.R. Requena, M. Porterootin, J. Prat, S.R. Thorpe, M.J. Bellmunt, *Eur. J. Biochem.*, 255 (1998) 685–689.
[17] M.O. Lederer, M. Baumann, *Bioorg. Med. Chem.*, 8 (2000) 115–121.
[18] D. Xenakis, N. Moll, B. Gross, *Synthesis*, (1983) 541–543.
[19] R.F. Brady, *Carbohydr. Res.*, 15 (1970) 35–40.
[20] S.J. Angyal, Conformation of sugars, in W. Pigman, D. Horton (Eds.), *The Carbohydrates, Chemistry and Biochemistry*, Vol. 1A, second ed., Academic Press, New York, 1972, pp. 204–205.
[21] A. Crossman, J.S. Brimacombe, M.A.J. Ferguson, *J. Chem. Soc. Perkin Trans. 1*, (1994) 2769–2774.
[22] H. Kato, M. Fujimaki, *Lebensm.-Wiss. Technol.*, 5 (1972) 172–174.
[23] I. Lindh, J. Stawinski, *J. Org. Chem.*, 54 (1989) 1338–1342.
[24] E. Klein, F. Ledl, W. Bergmüller, T. Severin, *Z. Lebensm.-Unters. Forsch.*, 194 (1992) 556–560.
[25] M.L. Alcaraz, L. Peng, P. Klotz, M. Goeldner, *J. Org. Chem.*, 61 (1996) 192–201.
[26] W.W. Christie, *Z. Lebensm.-Unters. Forsch.*, 181 (1985) 171–182.
[27] K. Aitzetmüller, *Fette Seifen Anstrichm.*, 86 (1984) 318–322.
[28] K. Aitzetmüller, D. Handt, *Fette Seifen Anstrichm.*, 86 (1984) 322–325.
[29] G.R. Jurch, J.H. Tatum, *Carbohydr. Res.*, 15 (1970) 233–239.
[30] U. Büttner, S. Ochs, T. Severin, *Carbohydr. Res.*, 291 (1996) 175–181.
[31] P.J. Richard, *Biochemistry*, 30 (1991) 4581–4585.
[32] S.A. Phillips, P.J. Thornalley, *Eur. J. Biochem.*, 212 (1993) 101–105.
[33] M.A. Glomb, V.M. Monnier, *J. Biol. Chem.*, 270 (1995) 10017–10026.
[34] K.J. Wellsknecht, D.V. Zyzak, J.E. Litchfield, S.R. Thorpe, J.W. Baynes, *Biochemistry*, 34 (1995) 3702–3709.
[35] A. Loidl-Stahlhofen, G. Spiteller, *BBA-Lipids Lipid Metab.*, 1211 (1994) 156–160.
[36] J.W. Baynes, S.R. Thorpe, *Diabetes*, 48 (1999) 1–9.
[37] S. Kawakishi, Y. Okawa, K. Uchida, *J. Agric. Food Chem.*, 38 (1990) 13–17.
[38] S. Kawakishi, K. Uchida, Autoxidation of Amadori compounds in the presence of copper ion and its effects on the oxidative damage to protein, in P.A. Finot, H.U. Aeschbacher, R.F. Hurrell, R. Liardon (Eds.), *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*, Birkhäuser Verlag, Basel, 1990, pp. 475–480.
[39] S.P. Wolff, R.T. Dean, *Biochem. J.*, 245 (1987) 243–250.
[40] J.V. Hunt, M.A. Bottoms, M.J. Mitchinson, *Biochem. J.*, 291 (1993) 529–535.
[41] T. Maeda, K. Tori, S. Satoh, K. Tokuyama, *Bull. Chem. Soc. Jpn.*, 42 (1969) 2635–2647.
[42] A. Couto, R.M. DeLederkremer, *Carbohydr. Res.*, 126 (1984) 313–320.