ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 329 (2000) 515–523

CARBOHYDRATE RESEARCH

# Synthesis of 1-deoxy-D-*erythro*-hexo-2,3-diulose, a major hexose Maillard intermediate

Marcus A. Glomb *, Christoph Pfahler

*Institute of Food Chemistry, Technical University of Berlin, Gustav-Meyer-Allee 25, TIB4/3-1, D-13355 Berlin, Germany*

Received 26 January 2000; accepted 14 July 2000

**Abstract**

1-Deoxy-D-*erythro*-hexo-2,3-diulose (1-DG) was prepared by the reaction of ethoxyvinyllithium with an erythronolactone derivative. Characterization by $^{1}H$ and $^{13}C$ NMR spectroscopy and NOE difference experiments revealed the $^{2}C_{5}$-chair β-pyranose as the major isomer in solution. Experiments assessing browning and polymerization reactivity proved 1-DG to be a much more potent protein modifier than 3-deoxy-D-*erythro*-hexos-2-ulose. © 2000 Elsevier Science Ltd. All rights reserved.

*Keywords:* 1-Deoxy-D-*erythro*-hexo-2,3-diulose; Maillard reaction; Protein modification; Browning; $^{2}C_{5}$ chair

## 1. Introduction

The reaction of reducing sugars with amines, also called the Maillard reaction, is of major interest within food chemistry. During food processing it provides positive aspects like the formation of flavor and color, but also quite negative consequences, such as loss of essential amino acids and generation of mutagenic and cancerogenic compounds. In addition the Maillard reaction has been established in vivo and is thought to have a considerable impact on diabetic complications and aging [1,2].

The Maillard reaction does not represent a single well-defined reaction step, but is a synonym for complicated reaction pathways including enolization, dehydration, cyclization, fragmentation and redox reactions, leading from the formation of a Schiff base adduct through highly reactive intermediates to stable modifications and polymers called advanced glycosylation end products (AGEs) and melanoidines, respectively. So far, mostly stable modifications have been isolated and identified, but for the understanding of the general reaction scheme and the underlying mechanisms, it is the reactive intermediates that are of fundamental importance. α-Dicarbonyl compounds represent a major group of these. Due to their high reactivity, they cannot be isolated from reaction mixtures or are destroyed during necessary work-ups. In the presence of trapping reagents like aminoguanidine and *o*-phenylenediamine, they can be detected as stable triazine or quinoxaline derivatives, respectively [3,4], but these represent only poor tools for mechanistic studies of the Maillard reaction.

In the classical view of the Maillard reaction the primarily formed Schiff base adduct rearranges to an aminoketose or Amadori

* Corresponding author.
*E-mail address:* glom1530@mailszrz.zrz.tu-berlin.de (M.A. Glomb).


PII: S0008-6215(00)00219-6

product via a 1,2-enaminol. For hexoses, allylic dehydration and cleavage of the amine results in 3-deoxy-2-hexosulose. Starting from the Amadori product 2,3-enolization leads to 1-deoxy-2,3-hexodiulose and 1-amino-1,4-dideoxy-2,3-hexodiulose. Other α-dicarbonyl compounds with intact carbon backbones include 2-hexosulose and 1,4-dideoxy-2,3-hexodiulose, which are formed within this reaction scheme by oxidation and reduction, respectively. While synthesis of 2-hexosulose and 3-deoxy-2-hexosulose are well established procedures, other structures like 1-deoxy-2,3-hexodiulose are not available as authentic reference material for detailed studies.

## 2. Results and discussion

Several attempts have been reported to synthesize the free 1-deoxy-2,3-hexodiulose of glucose [5–8]. They all ended up with stable derivatives, the protection groups of which could only be cleaved off under very rigid conditions, if at all. This results in immediate degradation of this highly reactive structure, and follow-up experiments with controllable well-defined reaction conditions are impossible. In addition, some of the syntheses resulted in mixtures of stereoisomers, whereas starting from glucose only the *erythro* isomer is of interest.

In our synthesis the absolute configuration is set with 2,3-*O*-isopropylidene-D-erythronolactone (**1**) as the educt (Fig. 1). Reaction with ethoxyvinyllithium as an acyl equivalent leads to formation of the $C_6$-carbon backbone. To avoid side reactions the molarities of the reactants were controlled and an excess of metallated vinylether was destroyed with ammonium chloride within a defined time frame. Extraction, high vacuum distillation and column chromatography resulted in pure 1-deoxy-4,5-*O*-isopropylidene-2-*O*-ethyl-D-*erythro*-hex-1-en-3-ulose (**2**). Vinylether **2** was extremely acid labile and could be transformed under weak acid conditions to the corresponding carbonyl derivative 1-deoxy-4,5-*O*-isopropylidene-D-*erythro*-hexo-2,3-diulose (**3**). The isopropylidene protection group was readily cleaved off under mild controllable conditions with a protonated ion-exchange resin. Filtration and evaporation of the solvent resulted in pure amorphous 1-deoxy-D-*erythro*-hexo-2,3-diulose (1-DG). In addition to standard structure verification we isolated the corresponding (1*S*,2*R*)-1-(3-amino-6-methyl-1,2,3-triazine-5-yl)-1,2,3-propanetriol (**4**) and (1*S*,2*R*)-1-(3-methyl-2-quinoxalinyl)-1,2,3-propanetriol (**5**) in high yields from incubation mixtures with aminoguanidine (AG) and *o*-phenylenediamine (OPD), respectively. The spectroscopic data complied with the literature [9,10].

Free 1-DG was also reduced with sodium borohydride. The resulting four diastereomeric alcohols **6** verified the synthesis product

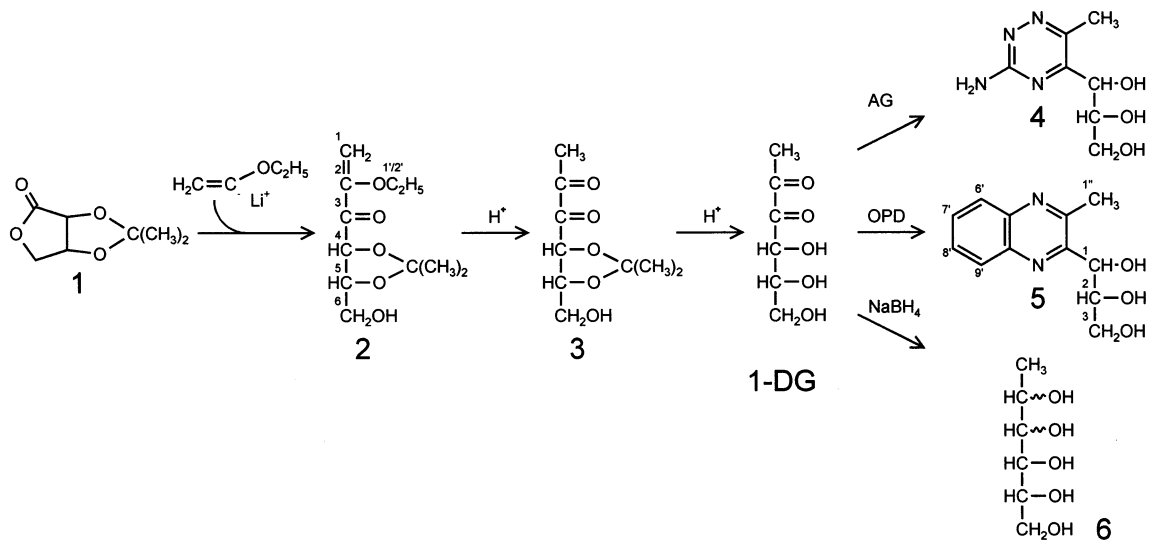


Fig. 1. Synthesis of 1-DG.

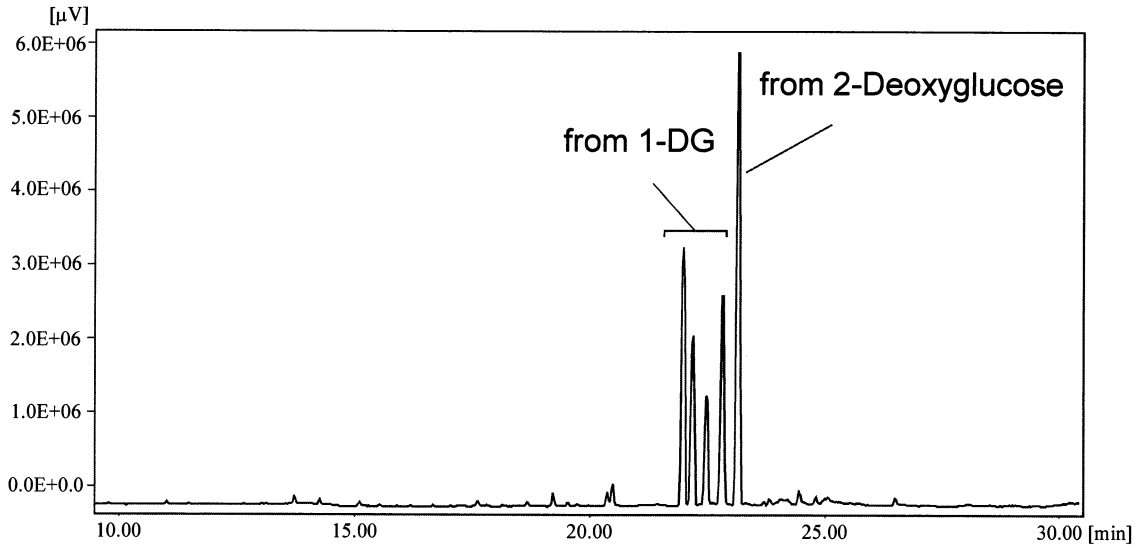


Fig. 2. GLC/FID of a synthesis purity determination after reduction and silylation; 1-DG results in four signals for persilylated 1-deoxy-D-allitol, 1-deoxy-D-altritol, 1-deoxy-D-mannitol and 1-deoxy-D-glucitol; 2-deoxy-D-glucose results in one signal for persilylated 2-deoxy-D-*arabino*-hexitol.

as an α-dicarbonyl. If the reduction is carried out in the presence of 2-deoxy-D-glucose as an internal standard, the purity of the product can be determined to be about 95%. Fig. 2 shows the gas chromatographic profile after reduction and silylation.

Structure **2** with the open-chain carbon backbone, as shown in Fig. 1, would require a resonance for the free carbonyl group at 190–200 ppm in the $^{13}C$ NMR data. Instead, a carbon at 106 ppm is found in addition to other quaternary signals at 113 ppm for the ketalic carbon of the isopropylidene group and at 160 ppm for the ethoxyvinyl configuration at C-2. This agrees well with a hemiketalic C-3 for the cyclic form and clearly shows compound **2** to exist as 1-deoxy-2-*O*-ethyl-4,5-*O*-isopropylidene-D-*erythro*-hex-1-en-3-ulofuranose.

Theoretically, structure **3** could exist in two cyclic and one open chain form (Fig. 3). In the $^{13}C$ NMR one signal at 206 ppm results from a free carbonyl function. The open-chain form would call for a second signal in this region. Instead, besides the signal for the quaternary carbon of the isopropylidene group at 114 ppm, a resonance is found at 107 ppm, which can only be explained by an anomeric ketalic carbon of one of the cyclic forms. It was possible to unequivocally decide for 1-deoxy-4,5-*O*-isopropylidene-D-*erythro*-hexo-2,3-diulo-3,6-furanose in favor of the pyranoic form based on GLC/MS analysis. The electron impact mass spectra showed a distinct M-43 signal, which can only be explained by loss of the acetyl group of the furanoic molecular ion. In addition, the chemical shift for the $CH_3$ group in $^1H$ NMR is 2.26 ppm, i.e., within the expected range for an acetyl moiety.

For 1-DG, three cyclic forms are possible beside the open-chain isomer (Fig. 3). Up to now an equilibrium of all three cyclic structures is assumed to exist in solution. However, the $^1H$ and $^{13}C$ NMR data of the synthesis product clearly show that one form is favored by a factor of about 10. For this major isomer the DEPT (distortionless enhancement by polarization transfer) NMR data verifies two quaternary carbon signals, one at 204 ppm for a free carbonyl function and one at 99 ppm for a ketalic carbon. The absence of any second signal in the 200 ppm region excludes the existence of the open-chain form. The $^1H$ NMR shows a signal for the methyl group of the major structure at 1.39 ppm, which excludes the furanoic form with an acetyl group. In that case the signal should be more low field at about 1.9–2.3 ppm. HMBC (hetero nuclear multiple bond correlation) experiments prove the major structure to be 1-deoxy-D-*erythro*-hexo-2,3-diulo-2,6-pyranose, showing $^3J$ correlations between the protons

H-6 and C-2. 1-DG was also subjected to NOE (nuclear overhauser effect) difference experiments. Presaturation of the proton H-4 resulted in significant enhancement for one of the signals for the protons at C-6, while presaturation of the C-1 methyl protons did not have any influence on the protons at C-6. The first experiment strongly suggested the major structure of 1-DG to be the $^2C_5$-chair conformer. The second experiment implied the molecule to have β-configuration, as only the α anomer would call for a correlation.

To study the quantitative role of 1-DG formation within the Maillard reaction, the degradation of the Amadori product of glucose and $N^{\alpha}$-*t*-BOC-lysine was followed in the presence of *o*-phenylenediamine (Fig. 4). The quinoxaline derivatives obtained were isolated from the reaction mixture by extraction, derivatized as trimethylsilylethers and analyzed by GLC/MS. Quantification was done with 2-ethyl-3-methylquinoxaline as the internal standard. Mild reaction conditions of 37 °C and 0.2 M phosphate buffer pH 7.4 were chosen to obtain a less complicated profile and, second, to mimic physiological conditions. Under these conditions the formation of D-*arabino*-hexos-2-ulose dominated. The 3-deoxy-D-*erythro*-hexos-2-ulose (3-DG) formation was independent of oxygen. In contrast, the generation of D-*arabino*-hexos-2-ulose was positively dependent on oxidative conditions, whereas 1-DG increased under deaerated conditions. When the oxidative conditions were further lowered by decreasing the phosphate concentration and using glycated

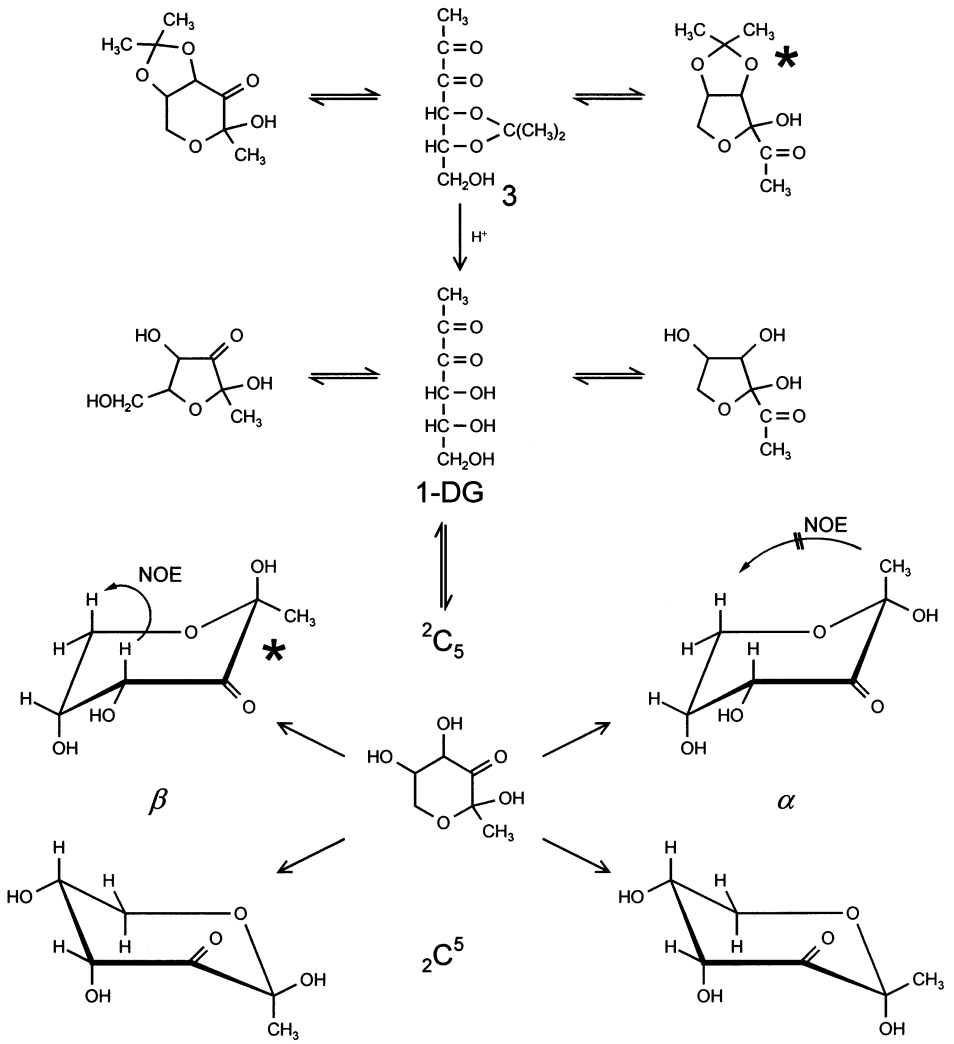


Fig. 3. Absolute configuration and conformation of 1-DG and precursor **3**. Structures of the main isomers verified by NMR and MS techniques are marked with an asterisk.

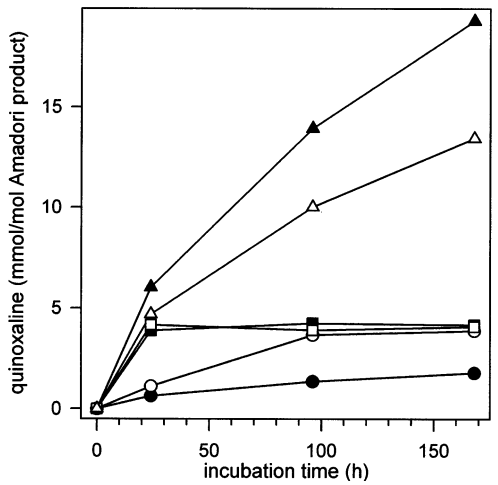


Fig. 4. Formation of α-dicarbonyls measured as the corresponding quinoxaline derivatives in incubations of the Amadori product of glucose/$N^{\alpha}$-*t*-BOC-lysine (42 mM) with *o*-phenylenediamine (5 mM); (●) 1-DG, (■) 3-DG, (▲) D-*arabino*-hexos-2-ulose, closed symbols indicate aerated, open symbols deaerated conditions.

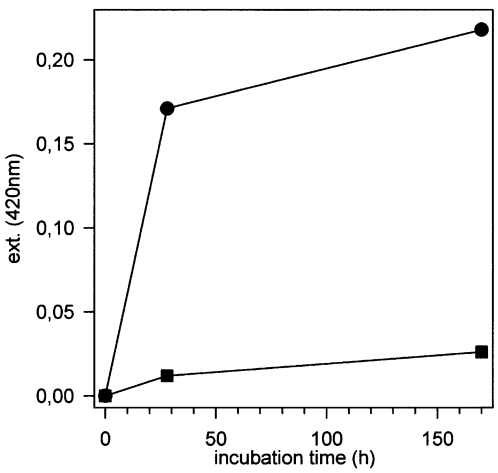


Fig. 5. Browning of incubations of RNase with 1-DG (●) vs. 3-DG (■) in 0.1 M phosphate buffer pH 7.4 at 37 °C.

proteins instead of the Amadori product, the yield of 1-DG reached 3 to 10 times that of 3-DG (data not shown). These results are expected. 1-DG not only reacts as an α-dicarbonyl, but due to its tautomeric α-oxo-endiol structure, also as a reductone. Thus, it will be easily degraded by oxidation and fragmentation even at neutral or mild acidic pH values.

Therefore, 1-DG readily should participate in redox reactions, and has to be considered as much more reactive than 3-DG. Nevertheless, it is 3-DG, which in the literature is judged as an extremely potent protein modifier, especially when it comes to formation of melanoidines [11,12]. Based on these considerations we undertook experiments to compare the protein modifying capabilities of both α-dicarbonyl compounds. Fig. 5 shows the results of browning experiments of 0.5 mM RNAse with 200 mM 1-DG and 3-DG, respectively; to assess browning intensity the extinction was recorded at 420 nm. Clearly, 1-DG leads to colored products about ten times faster. To study polymerization, 0.5 mM RNase was incubated with 40 mM of both α-dicarbonyl compounds. The reaction was stopped at 28 and 170 h by dialysis and subjected to SDS-PAGE (sodium dodecylpolyacrylamide gel electrophoresis, Fig. 6). The gel shows that polymerization increases with time and, more importantly, 1-DG dimerizes to a greater extent. Formation of a trimer is only visible with 1-DG. Compared with 3-DG the bands of the monomeric and dimeric region were broadened, indicating a larger variety of structurally different modifications. Thus, 1-DG is by far the more reactive Maillard intermediate. The new and convenient stereoselective synthesis will now facilitate in-depth studies of the role of 1-DG within the Maillard reaction and of resulting protein modifications on a molecular basis.

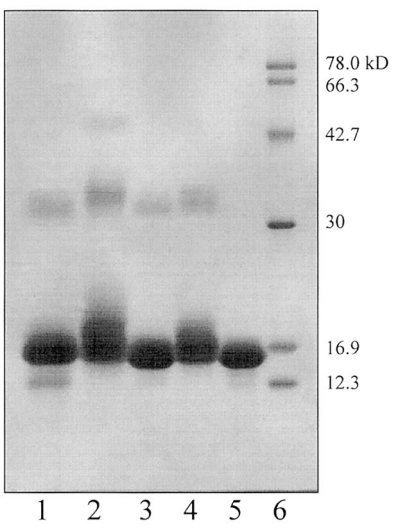


Fig. 6. Modification of RNase in 0.1 M phosphate buffer pH 7.4 at 37 °C followed by SDS-PAGE, traces 1,2: with 1-DG 28 and 170 h, traces 3,4: with 3-DG 28 and 170 h, trace 5: control incubation 170 h with no sugar, trace 6: molecular weight standard.

## 3. Experimental

*General methods.*—Unless otherwise noted, materials were of the highest quality available from commercial sources and used without further purification. TLC was performed on precoated plates (E. Merck Silica Gel 60 F254) and separated material visualized with the detection reagent given. Column chromatography was performed on silica gel (63–200 μm) and reverse phase C18 material (40–63 μm). Solvents were all chromatographic grade. From the individual chromatographic fractions, solvents were evaporated under reduced pressure. Gas liquid chromatography with flame ionization detection (GLC/FID) was performed on a GC 6000 Vega Series 2 (Carlo Erba), quartz capillary column (30 m, inner diameter 0.25 mm, J&W DB-1, 0.25 μm, He, 27.75 cm $s^{-1}$), injection port/detector 270 °C, samples were injected at 100 °C, the GC oven temperature was then raised to 200 °C at 5 °C $min^{-1}$, then to 270 °C at 10 °C $min^{-1}$ and held for 10 min. GLC/MS was performed on a HRGC 6160 Mega Series (Carlo Erba), quartz capillary column (30 m, inner diameter 0.25 mm, J&W DB-5, 0.25 μm, He, 26.55 cm $s^{-1}$), injection port 270 °C, transfer line 280 °C, samples were injected at 100 °C, the GC oven temperature was then raised to 200 °C at 5 °C $min^{-1}$, then to 270 °C at 10 °C $min^{-1}$ and held for 10 min; connected to a MAT ITD 700 (Finnigan), EI at 70 eV and positive MeOH–CI. High resolution mass spectrometry (HR-MS) was run on a VG 7070 with heptacosane as the internal standard. NMR spectra were recorded on a Bruker AC 400 instrument, except for the HMBC experiments and the NOE-difference spectra, which were recorded on a Bruker DRX 500 spectrometer. HMBC and NOE were performed both according to Ref. [16]. Chemical shifts are relative to residual non-deuterated solvent as the internal reference. Hydrogen and carbon numbering is according to Fig. 1.

*1-Deoxy-2-*O*-ethyl-4,5-*O*-isopropylidene-*D*-erythro-hex-1-en-3-ulose* (**2**).—*t*-Butyl lithium (4.5 mL, 1.5 M in pentane, 6.3 mmol) was added to a soln of ethylvinylether (0.96 mL; 10 mmol) in 6 mL THF at −65 °C. The temperature was allowed to rise to 0 °C until the solution was colorless and it was then recooled to −65 °C. After addition of 2,3-*O*-isopropylidene-D(−)-erythronolactone (1020 mg, 6.5 mmol) in 4 mL THF the reaction mixture was stirred at 0 °C for 30 min, quenched with 20 mL saturated ammonium chloride solution and extracted with diethylether. The organic layer was dried, the solvents removed and the residue subjected to high vacuum distillation (105–110 °C; 0.1 torr). The resulting yellowish oil was further purified by column chromatography (RP18, 3:7 MeOH–water). Fractions with material having $R_f$ 0.69 (TLC1, 3:7 EtOAc–hexane, triphenyltetrazoliumchloride (TTC)) were pooled, solvents evaporated to yield a colorless oil (738 mg, 50%); GLC/FID $t_R$ 9.9 min; GLC/MS $t_R$ 13.2 min; *m/z* 215 [M − 15, 5%], 187(4), 172(6), 159(2), 116(7), 99(9), 85(18), 73(11), 59(42), 43(100); HR-MS *m/z* 215.0915 (215.0920 calcd for $C_{10}H_{15}O_5$, M − 15); $^1$H NMR ($CD_3OD$): $\delta$ 1.27, 1.36 (2 s, each 3 H, isopropylidene group), 1.31 (t, 3 H, $J_{2',1'}$ 7.0 Hz, H-2′), 3.77 (q, 2 H, *J* 1.7 Hz, H-1′), 3.91 (d, 1 H, $J_{6A,6B}$ 10.5 Hz, H-6A), 4.02 (dd, 1 H, $J_{6B,5}$ 4.0 Hz, H-6B), 4.11 (d, 1 H, $J_{1A,1B}$ 2.0 Hz, H-1A), 4.48 (d, 1 H, $J_{4,5}$ 6.0 Hz, H-4), 4.50 (d, 1 H, H-1B), 4.85 (dd, 1 H, H-5); $^{13}$C NMR ($CD_3OD$): 14.7 (C-2′), 25.5 and 26.7 (isopropylidene group), 64.2 (C-1′), 71.9 (C-6), 81.6 (C-5), 83.7 (C-1), 86.8 (C-4), 105.8 (C-3), 113.5 (isopropylidene group), 160.5 (C-2).

*1-Deoxy-4,5-*O*-isopropylidene-*D*-erythro-hexo-2,3-diulose* (**3**).—HCl (1 M, 0.5 mL) was added to a soln of **2** (1054 mg, 4.6 mmol) in 25 mL MeOH and stirred at rt for 90 min. After neutralization solvents were evaporated, the residue taken up in water and extracted with EtOAc. The organic layer was dried, the solvents evaporated and the residue subjected to column chromatography (RP18, 3:7 MeOH–water). Fractions with material having $R_f$ 0.56 (TLC1, 3:7 EtOAc–hexane, TTC) were pooled, solvents evaporated to yield colorless crystals (640 mg, 69%); GLC/FID $t_R$ 5.9 min; GLC/MS $t_R$ 7.5 min; *m/z* 187 [M − 15, 10%], 159(15), 144(10), 127(2), 113(1), 101(6), 85(15), 71(8), 59(100); HR-MS *m/z* 187.0609 (187.0607 calcd for $C_8H_{11}O_5$, M − 15); $^1$H NMR ($CD_3OD$): $\delta$ 1.27, 1.38 (2 s, each 3 H,

isopropylidene group), 2.26 (s, 3 H, H-1), 4.03 (d, 2 H, $J_{6,5}$ 2.0 Hz, H-6), 4.60 (d, 1 H, $J_{4,5}$ 6.0 Hz, H-4), 4.90 (dt, 1 H, H-5); $^{13}$C NMR ($CD_3OD$): 24.7 and 26.2 (isopropylidene group), 27.1 (C-1), 72.8 (C-6), 81.6 (C-5), 87.6 (C-4), 107.2 (C-3), 114.0 (isopropylidene group), 206.3 (C-2).

*1-Deoxy*-D-erythro-*hexo-2,3-diulose (1-DG).*—A soln of **3** (14 mg, 0.07 mmol) in 1 mL $H_2O$ was stirred with 1 mL Dowex 50 Wx8 ($H^+$-form, 50–100 mesh) under argon atmosphere. Completion of reaction was checked by TLC1 ($R_f$ 0.62, 3:7 MeOH–EtOAc, TTC). The resin was filtered off and washed with MeOH. Evaporation of the combined solvents yields a colorless amorphous product (9.9 mg, 88%); GLC/FID $t_R$ (min) 13.0, 13.4, 13.8, 14.0, 14.4, 14.5 (after trimethylsilylation); GLC/MS $t_R$ (min) 14.5, 15.2, 15.5, 16.0, 16.3, 16.5 (after trimethylsilylation); $m/z$ ($CH_3OH$–CI) 163 [M + 1, 7%], 145(22), 127(10), 119(62), 103(10), 91(18), 85(100), 73(36), 61(48), 55(50); HR-MS $m/z$ 145.0499 (145.0501 calcd for $C_6H_9O_4$, M-17); $^1$H NMR ($CD_3OD$): $\delta$ 1.39 (s, 3 H, H-1), 3.66 (dd, 1 H, $J_{6A,5}$ 2.0, $J_{6A,6B}$ 12.5 Hz, H-6A), 4.25 (ddd, 1 H, $J_{5,4}$ 4.5, $J_{5,6B}$ 1.0 Hz, H-5), 4.35 (dd, 1 H, H-6B), 4.77 (d, 1 H, H-4); $^{13}$C NMR ($CD_3OD$): 22.2 (C-1), 64.2 (C-6), 74.1 (C-4), 76.8 (C-5), 98.9 (C-2), 204.8 (C-3).

*Purity determination of 1-DG.*—A soln of 1-DG (2 mg, 0.01 mmol) and 2-deoxy-D-glucose (1.3 mg, 0.008 mmol) in 200 μL water was stirred with $NaBH_4$ (7 mg, 184 mmol) for 1 h, the reaction quenched with HOAc and volatiles evaporated. Three times, the still moist residue was taken up in 1% methanolic HCl and evaporated again. The residue was dried under high vacuum, dissolved in MeOH, filtered, the solvents removed and derivatized as trimethylsilyl derivatives.

*[(4R,5S) - 2,2 - Dimethyl - 5 - (3 - methyl - 2-quinoxalinyl) - 1,3 - dioxolan - 4 - yl]methanol* (**5′**).—A soln of **3** (680 mg, 3.37 mmol) and *o*-phenylenediamine (680 mg; 6.30 mmol) in 30 mL $CH_2Cl_2$ was stirred 12 h at rt in the presence of 3 Å molecular sieves. After filtration solvents were evaporated and the residue subjected to column chromatography (RP18, 1:1 MeOH–$H_2O$). Fractions containing material having $R_f$ 0.78 (TLC1, 1:9 MeOH–EtOAc, UV) were pooled, the solvents were evaporated and the residue was purified by column chromatography (silica gel, 1:1 EtOAc–hexane). Eluting material having $R_f$ 0.22 (TLC1, same solvent, UV) was pooled and the solvents were evaporated to yield colorless crystals (368 mg, 40%); GLC/FID $t_R$ 24.1 min; GLC/MS $t_R$ 26.6 min, $m/z$ 259 [M − 15, 4%], 217(7), 199(44), 187(24), 174(100), 157(30), 143(24), 130(6), 117(10), 102(34); HR-MS $m/z$ 259.1079 (259.1083 calcd for $C_{14}H_{15}N_2O_3$, M − 15); $^1$H NMR ($CDCl_3$): $\delta$ 1.53, 1.67 (2 s, each 3 H, isopropylidene group), 2.80 (s, 3 H, H-1″), 3.23 (dd, 1 H, $J_{3A,2}$ 7.5, $J_{3A,3B}$ 11.0 Hz, H-3A), 3.35 (dd, 1 H, $J_{3B,2}$ 6.0 Hz, H-3B), 4.71 (dt, 1 H, $J_{2,1}$ 6.0 Hz, H-2), 5.82 (d, 1 H, H-1), 7.76, 7.97, 8.13 (3 m, 2 H, 1 H, 1 H, H-7′/8′/6′/9′); $^{13}$C NMR ($CDCl_3$): 23.0 (C-1″), 25.4 and 27.2 (isopropylidene group), 62.1 (C-3), 77.5 (C-2), 78.6 (C-1), 109.6 (isopropylidene group), 128.4, 128.9, 129.4, 130.3, 139.8, 141.5, 150.3, 153.0 (quinoxaline).

*(1S,2R)-1-(3-Methyl-2-quinoxalinyl)-1,2,3-propanetriol* (**5**).—To a soln of **5′** (352 mg, 1.28 mmol) in a mixture of 5 mL MeOH and 1 mL water, 1 mL of Dowex 50 Wx8 ($H^+$-form, 50–100 mesh) was added and stirred for 2 h at rt. Completion of the reaction was checked by TLC1 (3:7 MeOH–EtOAc, UV, $R_f$ 0.75). The resin was filtered off, solvents removed and the residue subjected to column chromatography (silica gel, EtOAc). Fractions containing material with $R_f$ 0.18 (TLC1, EtOAc, UV) were combined and solvents evaporated to yield colorless crystals (91 mg, 31%); GLC/FID $t_R$ 24.4 min (after trimethylsilylation); GLC/MS $t_R$ 26.4 min (after trimethylsilylation), $m/z$ (after trimethylsilylation) 450 [$M^{+\bullet}$, 3%], 435(10), 345(2), 271(6), 246(42), 231(3), 205(8), 172(2), 147(30), 133(4), 117(18), 103(5), 73(100); HR-MS (after trimethylsilylation) $m/z$ 435.1965 (435.1955 calcd for $C_{20}H_{35}N_2O_3Si_3$, M-15); $^1$H NMR ($CD_3OD$): $\delta$ 2.86 (s, 3 H, H-1″), 3.86 (dd, 1 H, $J_{3A,2}$ 6.0, $J_{3A,3B}$ 11.5 Hz, H-3A), 3.93 (dd, 1 H, $J_{3B,2}$ 3.5 Hz, H-3B), 4.09 (m, 1 H, $J_{2,1}$ 8.5 Hz, H-2), 5.09 (d, 1 H, H-1), 7.75, 7.97, 8.09 (3 m, 2 H, 1 H, 1 H, H-7′/8′/6′,9′); $^{13}$C NMR ($CD_3OD$): 22.5 (C-1′), 64.5 (C-3), 71.8 (C-2), 76.0 (C-1), 128.4, 129.6, 130.5, 131.3, 141.7, 141.8, 155.2, 157.7 (quinoxaline).

*[(4*R,*5*S*)-5-(3-Amino-6-methyl-1,2,4-triazin-5-yl)-2,2-dimethyl-1,3-dioxolan-4-yl]-methanol* (**4′**).—HCl (0.5 mL, 1 M) was added to a soln of **3** (2753 mg; 2.0 mmol) in 25 mL MeOH. After complete reaction (about 40 min, TLC1, $R_f$ 0.91, 3:7 MeOH–EtOAc, charring) the reaction mixture was neutralized with 1 M NaOH. Aminoguanidine (1630 mg, 12.0 mmol) was added and the solution stirred for 4 days at 37 °C. Solvents were removed and the residue subjected to column chromatography (silica gel, 1:9 MeOH–EtOAc). Fractions with material having $R_f$ 0.49 (TLC1, same solvent, UV) were combined and the solvents were evaporated to yield a yellowish powder (609 mg, 21%); GLC/FID $t_R$ 24.8 min (after trimethylsilylation); GLC/MS $t_R$ 26.4 min (after trimethylsilylation), $m/z$ (after trimethylsilylation) 385 [M + 1, 43%], 369(28), 309(10), 280(30), 253(18), 206(35); HR-MS (after trimethylsilylation) $m/z$ 384.2004 (384.2015 calcd for $C_{16}H_{32}N_4O_3Si_2$); NMR data consistent with Ref. [9].

*(1*S,*2*R*)-1-(3-Amino-6-methyl-1,2,4-triazin-5-yl)-1,2,3-propanetriol* (**4**).—A soln of **4′** (160 mg, 0.67 mmol) in 10 mL methanolic HCl (5%) was stirred for 30 min at rt, the pH was adjusted to 7 with NaOH and the solvents were removed. The residue was subjected to column chromatography (silica gel, 3:7 MeOH–EtOAc). Fractions with material having $R_f$ 0.40 (TLC1, same solvent, UV) were pooled, the solvents were evaporated and the residue was subjected to column chromatography (silica gel, 2:8 MeOH–$CHCl_3$). Fractions containing material with $R_f$ 0.18 (TLC1, same solvent, UV) were pooled, the solvents were removed to yield yellowish crystals (39 mg; 29%); GLC/FID $t_R$ 25.0 min (after trimethylsilylation); GLC/MS $t_R$ 27.1 min (after trimethylsilylation), $m/z$ (after trimethylsilylation) 488 [$M^{+\bullet}$, 23%], 473(5), 284(55), 269(10), 187(2), 147(12), 117(8), 73(100); HR-MS (after trimethylsilylation) $m/z$ 488.2487 (488.2490 calcd for $C_{19}H_{44}N_4O_3Si_4$); NMR data consistent with [9].

*3-Deoxy*-D-erythro-*hexos-2-ulose (3-DG).*—Synthesis was carried out mainly according to Ref. [13].

*2-Ethyl-3-methylquinoxaline.*—A soln of 2,3 pentanedione (400 mg, 4 mmol) and *o*-phenylenediamine (440 mg, 4.1 mmol) in phosphate buffer (67 mM, pH 7) was stirred at 37 °C overnight and subsequently extracted with EtOAc. The organic layer was dried, evaporated to dryness and the residue subjected to column chromatography (silica gel, 3:7 EtOAc–hexane). Fractions with material having $R_f$ 0.53 (TLC 1, same solvent, UV) were pooled, solvents evaporated to yield yellowish crystals (369 mg; 54%); GLC/FID $t_R$ 11.2 min; GLC/MS $t_R$ 14.0 min, $m/z$ 172 [$M^{+\bullet}$, 100%], 157(5), 144(13), 130(15), 117(8), 103(8), 89(3), 76(18), 63(5), 50(14); HR-MS $m/z$ 172.0999 (172.1001 calcd for $C_{11}H_{12}N_2$); $^1$H NMR ($CD_3OD$): $\delta$ 1.39 (t, 3 H, $J$ 8.0 Hz, ethyl-$CH_3$), 2.73 (s, 3 H, –$CH_3$), 3.35 (q, 2 H, ethyl-$CH_2$), 7.70, 7.93, 8.01 (3 m, 2 H, 1 H, 1 H, quinoxaline); $^{13}$C NMR ($CD_3OD$): 12.0 (ethyl-$CH_3$), 22.4 (–$CH_3$), 29.5 (ethyl-$CH_2$), 128.7, 129.1, 130.1, 130.2, 141.5, 142.1, 154.7, 159.1 (quinoxaline).

*Incubations of* $N^{\alpha}$-t-*BOC-*$N^{\varepsilon}$*-(1-deoxyfructos-1-yl)lysine.*—Incubations were conducted in 0.1 M phosphate buffer (pH 7.4) at 37 °C in a shaker incubator after sterile filtration. Deaerated conditions were achieved by presence of 1 mM diethylenetriaminepentaacetic acid and gassing with argon. Aliquots of the incubation were taken at various time points and 2-ethyl-3-methylquinoxaline was added as the internal standard. Quinoxalines were extracted with EtOAc, organic layers were combined and dried with anhydrous $CaSO_4$. After filtration solvents were removed, the residue was derivatized with a 1:1 mixture of *N*,*O*-bis-(trimethylsilyl)-acetamide and pyridine and subjected to GLC/FID and GLC/MS. $N^{\alpha}$-*t*-BOC-$N^{\varepsilon}$-(1-deoxyfructos-1-yl)lysine and the quinoxalines of 3-deoxy-D-*erythro*-hexos-2-ulose and D-arabino-hexos-2-ulose were synthesized mainly according to Refs. [4,14,15], respectively.

*SDS-polyacrylamide gel electrophoresis.*—For an estimation of the extent of cross-linking mediated by 1-DG/3-DG at various time points, RNase (0.5 mM, Sigma) incubations were dialyzed overnight at 4 °C against phosphate buffered saline, pH 7.4, and analyzed by SDS-gel electrophoresis on a 12% acrylamide gel. The amount of RNase loaded onto the gel

was 16 μg in each lane. The gel was stained with Coomassie Blue for 1 h and destained in a mixture of 30% MeOH and 10% AcOH in water.

## Acknowledgements

This work was supported by a grant of the Deutsche Forschungsgemeinschaft. The authors like to thank Dr Jakupovic from the institute of organic chemistry TU Berlin for recording the NMR spectra.

## References

[1] F. Ledl, E. Schleicher, *Angew. Chem., Int. Ed. Engl.*, 29 (1990) 565–594.
[2] V.M. Monnier, M.A. Glomb, A. Elgawish, D.R. Sell, *Diabetes*, 45 (suppl. 3) (1996) 67–72.
[3] J. Hirsch, V.V. Mossine, M.S. Feather, *Carbohydr. Res.*, 273 (1995) 171–177.
[4] J. Beck, F. Ledl, Th. Severin, *Z. Lebensm. Unters. Forsch.*, 188 (1989) 118–121.
[5] H.O.L. Fischer, E. Baer, *Helv. Chim. Acta*, 16 (1933) 534–547.
[6] A. Ishizu, B. Lindberg, O. Theander, *Carbohydr. Res.*, 5 (1967) 329–334.
[7] B.E. Fisher, H.B. Sinclair, J.C. Goodwin, *Carbohydr. Res.*, 116 (1983) 209–215.
[8] M.S. Feather, S.J. Eitelman, *J. Carbohydr. Chem.*, 7 (1988) 251–262.
[9] J. Hirsch, E. Petrakova, M.S. Feather, *J. Carbohydr. Chem.*, 14 (1995) 1179–1186.
[10] J. Beck, F. Ledl, Th. Severin, *Carbohydr. Res.*, 177 (1988) 240–243.
[11] R. Tressl, E. Kersten, G. Wondrag, D. Rewicki, R.P. Krüger, *The Maillard reaction in Foods and Medicine*, Spec. Pub.-R. Soc. Chem. No. 223, 1998, pp 69–75.
[12] F. Hayase, H. Hinuma, M. Asano, H. Kato, S. Arai, *Biosci. Biotech. Biochim.*, 58 (1994) 1936–1937.
[13] M.A. Madson, M.S. Feather, *Carbohydr. Res.*, 94 (1981) 183–191.
[14] M.A. Glomb, V.M. Monnier, *J. Biol. Chem.*, 270 (1995) 10017–10026.
[15] N. Morita, K. Inoue, M. Takagi, *Agric. Biol. Chem.*, 45 (1981) 2665–2668.
[16] S. Brown, H.-O. Kalinowski, S. Berger, *150 and More Basic NMR Experiments*, second ed., Wiley-VCH, New York, 1998.