The acidic aqueous solution is stable for several weeks at room temperature. The observed chemical shifts and spin coupling patterns (see Table 1) strongly suggest the presence of a $(CF_3)_3BC$ fragment. The exact nature of these species and their concentrations in equilibria (2) and (3) will be the subject of a subsequent investigation. As reported for metal carbonyl cations,[5, 19] the carbonyl carbon atom in **1** is the electrophilic center. However, unlike in **1** traces of water induce irreversible hydrolysis in metal carbonyl cations.

To support the interpretation of **1**, we carried out calculations using the Gaussian 98 program package[23] B3LYP/6-31G*. According to these calculations, an equilibrium structure with C_3 symmetry is expected, in which the CF_3 groups are rotated by 13° relative to each other and the B–CO bond length is about 1.58 Å. A rather high value of 3.27 D is predicted for the dipole moment; hence the broad linewidth of the NMR signals is explained by the interaction with the quadrupolar nuclei ^{10}B and ^{11}B. The recorded IR spectrum of **1** agrees well with the calculated one. Using the same basis set to calculate the energies of CO and $B(CF_3)_3$, we obtain a bond dissociation energy of 112 kJ mol^{-1} for **1**, which is consistent with the observed low thermal stability of **1**. Finally, calculated partial atomic charges of $q_B = -0.002$, $q_C = +0.404$, and $q_O = -0.166$ e$^-$ are obtained for the B-C-O group, which support our claim that the C(O) atom is the electrophilic center in **1**.

Since the free Lewis acid $B(CF_3)_3$ is not stable, a dissociation equilibrium between the primary dissociation products is not observed. The calculated F^- ion affinity for the hypothetical $B(CF_3)_3$ molecule is similar to the so far strongest Lewis acids SbF_5 (502 kJ mol^{-1})[24] and AuF_5 (591 kJ mol^{-1}).[25]

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Unexpected Carbonyl Mobility in Aminoketoses: The Key to Major Maillard Crosslinks**

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Covalently crosslinked proteins are among the major modifications caused by Maillard processes (reactions of reducing sugars with amines) and belong to the so-called 'advanced glycation end products' (AGEs). Most AGEs are formed from aminoketoses **1** via highly reactive dicarbonyl intermediates such as **2**–**4** (Scheme 1).[1–3] In long-lived connective tissue and matrix components, AGEs accumulate with age and are generated to a greater extent in diabetes.[4] AGEs can activate cellular receptors[5] and contribute to pathophysiology associated with ageing in general as well as

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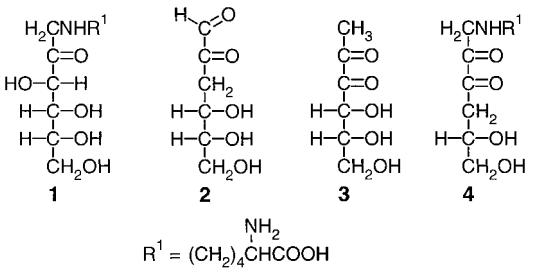


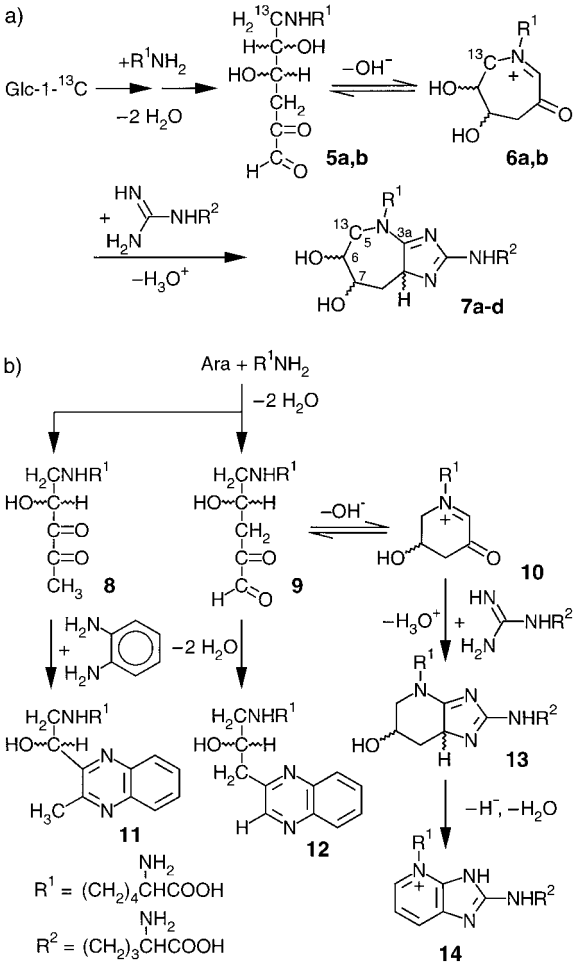
Scheme 1. The Amadori product 1-deoxy-1-(N^6-lysino)-D-fructose (**1**) and deoxyosones **2**–**4**, already described in the literature.

with long-term complications of diabetes, atherosclerosis, and Alzheimer's disease.[1, 6–8] To understand better the impact of the Maillard reaction on these processes and to develop effective therapeutic methods against AGE accumulation in tissues, it is an absolute prerequisite to elucidate the formation pathways of the major protein crosslinks.

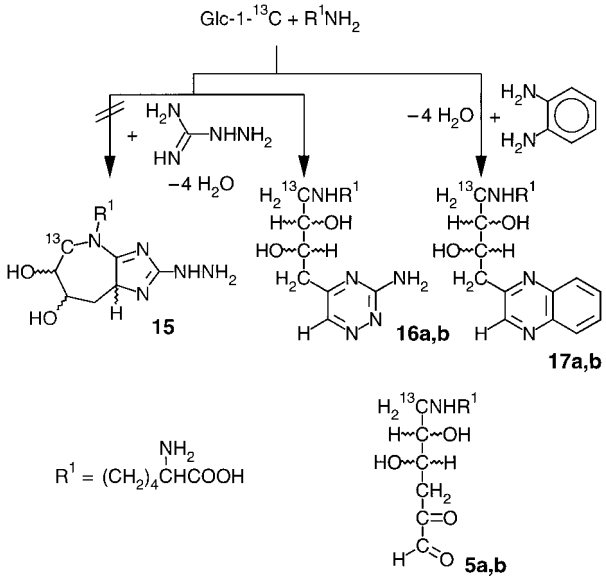
We recently reported the formation of the lysine–arginine crosslinks glucosepane (**7**) (Scheme 2a), pentosinane (**13**), and pentosidine (**14**) (Scheme 2b) from the respective carbohydrates and aminoketoses (Amadori products).[2] None of these crosslinks can be obtained from authentic 3-deoxyosones such as 3-deoxyglucosone **2** (3-DOG, Scheme 1), even though they are reasonable precursors. A novel pathway thus had to be postulated for generation of **7** from the aminoketose **1** to rationalize how the seven-membered ring in glucosepane is closed.[2]

In a painstaking study with improved chromatographic techniques, four stereoisomers **a**–**d** of glucosepane (**7**) could now be separated and unequivocally characterized by means of NMR spectroscopy,[9a] rather than a single pair **a**, **b** as reported previously.[10] This finding cannot be accommodated by the postulated formation pathway, which would give two glucosepane (**7**) isomers only, in which the stereochemistry at C6 and C7 is conserved from the native carbohydrate. Hence, the configuration of one or both of these stereogenic centers must have been lost in the formation of **7**, thus giving rise to four or eight possible diastereoisomers, respectively. As a result of the distance to the *S*-configured stereogenic centers of the amino acids, however, the 6,7,8a-stereoisomers with the same relative but opposite absolute configuration at these three chiral carbon atoms in **7** could neither be separated chromatographically nor resolved by means of NMR spectroscopy.[9b] With the stereochemical configuration variable at C6,7,8a, only four of the eight possible diastereoisomers of **7** can thus be differentiated.

Incubations with ^{13}C-labeled glucose ([1-^{13}C]D-glucose) were performed to clarify whether C3a or C5 in **7** derives from C1 of D-glucose. Contrary to our assumption, C5 turned out to be the labeled position, and the postulated pathway[2] was thus invalid. The results from the reactions of [1-^{13}C]D-glucose and lysine with aminoguanidine (AG) instead of arginine were even more surprising. The expected glucosepane analogue **15** (Scheme 3) could not be identified by liquid chromatography–electrospray ionization mass spectrometry (LC–ESI-MS); instead, a twin peak was detected with the quasimolecular ion $[M+H]^+$ at *m*/*z* 329 and a typical aminotriazine ultraviolet spectrum. NMR spectroscopic analysis



Scheme 2. a) Reaction pathway as established for the formation of the lysine–arginine crosslink glucosepane **7a**–**d**. b) Identification of the novel pentose-derived dideoxyosones **8** and **9**. Compound **9** very likely undergoes ring closure to form 6-(3-hydroxy-5-oxo-2,3,4,5-tetrahydro-1-pyridinium-yl)norleucine (**10**), which then reacts with an arginine side chain to form pentosinane (**13**) and finally pentosidine (**14**).



Scheme 3. Identification of the novel dideoxyosone N^6-(2,3-dihydroxy-5,6-dioxohexyl)lysine (**5a**,**b**).

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clearly proved that the compound was N^6-[4-(3-amino-1,2,4-triazin-5-yl)-2,3-dihydroxybutyl]lysine (**16a**,**b**; data not shown), and not the triazine derivative of 1,4-dideoxy-1-(N^6-lysino)-D-hexo-2,3-diulose (**4**). Since AG is an established trapping reagent for α-dicarbonyl compounds, we have clearly identified the new dideoxyosone **5a**,**b**, which is similar to 3-DOG **2**, with the terminal OH functional group substituted by N^ε of the lysine moiety. To validate this finding, another experiment was performed with *o*-phenylenediamine (OPD) instead of AG to trap **5a**,**b**. In fact, N^6-[2,3-dihydroxy-4-(2-quinoxalinyl)butyl]lysine (**17a**,**b**)[9c] was obtained as a pair of specifiable stereoisomers, analogously to **16a**,**b**; further diastereoisomers, a result of the *S*-configured stereogenic center of the lysine moiety, could not be differentiated for the reasons stated above. The ^{13}C label appears directly bonded to N^ε of the lysine moiety in both **16a**,**b** and **17a**,**b**. Hence, the formation of **5a**,**b** from aminoketose **1** requires carbonyl shifts along the entire carbohydrate backbone, thus leading to a loss of the original stereochemical configuration at the H–C–OH groups. This pathway is strongly supported by the results of experiments with D_2O as solvent. It was proven by means of LC–ESI-MS and NMR spectroscopy that isotopomers of **17a**,**b** that incorporate D-C-OH units are obtained; that is, both stereogenic centers have undergone H/D exchange in the course of keto-enol tautomerization. At which stage of the overall reaction sequence water is eliminated from **1** at C4, and whether **4** is an intermediate still needs to be clarified. Derivatives of **4** with primary amine substituents are rather unstable, even as quinoxalines.[11] Likewise, disaccharides such as lactose (*O*-β-D-gal*p*-(1$\rightarrow$4)-D-glc*p*) and maltose (*O*-α-D-glc*p*-(1$\rightarrow$4)-D-glc*p*) smoothly yield **5a**,**b**, since the 1$\rightarrow$4 glycoside bond is cleaved in this process by elimination of the *O*-substituent at C4.

In our model incubations, the novel dideoxyosone **5a**,**b** is formed in larger amounts than is 3-DOG **2**.[9d] Because **2** has thus far been considered the quantitatively most important deoxyosone both in vivo and in foodstuffs, most investigations hitherto have focused on this compound. The significance of **5a**,**b** for protein crosslinking in vivo is clear: On the one hand, this lysine-linked α-dicarbonyl compound, which was established first, yields the major crosslink glucosepane (**7a–d**) in the presence of arginine moieties. The formation pathway for **7a–d**, via the intramolecular aldimine **6a**,**b** as plausible intermediate, is given in Scheme 2a. The generation of four distinguishable diastereoisomers of **7** is now easily understood with **5a**,**b** as precursor. On the other hand, it is unlikely that protein-bound **5a**,**b** is detoxified by reductase catalysis in vivo in the same way as free 3-DOG **2**,[12] and thus represents a persistent glycating agent. This hypothesis is strengthened by our investigations into important crosslinks in human serum albumin and lens protein which clearly show that **7** is the dominant compound whereas N^6-{2-{[(4*S*)-4-ammonio-5-oxido-5-oxopentyl]amino}-5-[(2*S*,3*R*)-2,3,4-trihydroxybutyl]-3,5-dihydro-4*H*-imidazol-4-ylidene}-L-lysinate (DOGDIC),[2] which is derived from 3-DOG **2**, is present only in negligible amounts. The generation of **7a–d** under physiological conditions is remarkable, since this implies a whole sequence of mild enolization and dehydration steps, leading from **1** to **5a**,**b**. Though often postulated in Maillard chemistry for the formation of various AGEs,[1] such long-range shifts of carbonyl groups have not yet been proven. The dideoxyosone **5a**,**b** is thus not only pivotal in the formation of glucosepane (**7**) but may also be expected to represent the key intermediate for numerous other Maillard products.

The homology between the formation pathways of glucosepane (**7**) and pentosidine (**14**) has prompted us to look for the respective dideoxyosone N^6-(2-hydroxy-4,5-dioxopentyl)-lysine (**9**) in incubations of arabinose with lysine and OPD (Scheme 2b). N^6-[2-Hydroxy-3-(2-quinoxalinyl)propyl]lysine (**12**) was indeed obtained in good yield from these reaction mixtures, and **9** was thus established as a reasonable precursor of pentosinane (**13**). Further transformation of **13** into pentosidine (**14**) has already been studied in detail.[2] Unexpectedly, we have also isolated N^6-[2-hydroxy-2-(3-methyl-2-quinoxalinyl)ethyl]lysine (**11**) from these incubations, with a yield three times as high as that for **12**.[9e] Since no structural homologues to **11** could be detected for hexoses, the formation of dideoxyosones such as N^6-(2-hydroxy-3,4-dioxopentyl)lysine (**8**) seems to be specific for pentoses.

The results presented above unequivocally establish the novel dideoxyosones **5a**,**b** and **9** as crucial intermediates in the generation of prominent crosslinks. It is possible, thus, to rationalize the formation of such structures from hexoses or pentoses through a generalized common reaction pathway, paving the way for a better understanding of advanced Maillard processes.

Experimental Section

Chromatographic and spectroscopic procedures: The equipment and methods used for preparative high performance liquid chromatography (HPLC), LC–ESI-MS, and NMR spectroscopy are described in detail in ref. [2] Ammonium formate buffer (10 mM, pH 4.0)–MeOH gradients were employed for all LC separations. Accurate mass determinations were performed with a resolution of 1010 (10% valley definition) and poly(ethylene glycol) 350 monomethyl ether as mass reference. The following scan ranges and reference peaks were used for calibration: **17a**,**b** (*m/z* 335–435): *m/z* 341.2175, 358.2441, 385.2438, 402.2703, 429.2700; **11** and **12** (*m/z* 290–390): *m/z* 297.1913, 314.2179, 341.2175, 358.2441, 385.2438.

7a–d: The synthesis with native D-glucose and the isolation of the *tert*-butoxycarbonyl (*t*Boc) derivatives of **7** as well as the removal of the *t*Boc groups was performed as described in ref. [10] The two crude products obtained were purified by means of preparative HPLC (gradient: % MeOH (*t* [min]) 0(0)-5(5–7)-0(8–13); detection at 254 nm). Fractions with $t_R = 5.4$, 6.2, 7.7, and 8.9 min yielded, after lyophilization, **7c**·3HCOOH (7.0 mg, 0.012 mmol, 0.3%), **7a**·3HCOOH (24.3 mg, 0.043 mmol, 1.1%), **7b**·3HCOOH (14.9 mg, 0.026 mmol, 0.7%), and **7d**·3HCOOH (4.7 mg, 0.008 mmol, 0.2%), respectively. Preparation (scaled down to one quarter) was repeated with [1-^{13}C]D-glucose (Cambridge Isotope Laboratories Inc., Andover, MA, USA), and the products were isolated analogously.

17a,**b**, **11**, and **12**: D-Glucose (360 mg, 2 mmol) or D-arabinose (300 mg, 2 mmol), N^α-*t*Boc-L-lysine (1.47 g, 6 mmol), *o*-phenylenediamine (430 mg, 4 mmol), diethylenetriaminepentaacetic acid (5 mg, 0.013 mmol), and phosphate buffer (1.6 g, 1 mmol, pH 7.4) were dissolved in water (10 mL). Each mixture was flushed with argon, kept at 70 °C for 2 d, and purified by means of preparative HPLC (gradient: % MeOH (*t* [min]) 5(0)-70(20)-100(22–25)-5(28–35); detection at 318 nm). Fractions with $t_R = 19.9$ min (incubation with D-glucose), $t_R = 20.1$ and 20.9 min (incubation with D-arabinose) were lyophilized, dissolved in HCl (3N, 2 mL), and kept at ambient temperature for 30 min. The pH value was adjusted to 7 by slowly adding solid $NaHCO_3$, the volume was finally filled up to 4 mL, and the solution was subjected to preparative HPLC (gradient: % MeOH (*t* [min]) 5(0)-55(12)-100(15–18)-5(20–25); detection at 318 nm). For the D-glucose

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incubation, the fraction with $t_R = 10.0$ min yielded, after lyophilization, **17a,b** · HCOOH (unlabeled, 36.5 mg, 0.089 mmol, 4.5 %), LC–ESI-MS (gradient: % MeOH (t [min]) 5(0)-95(30–35)-5(40–45), cone voltage 40 V). **17a**: $t_R = 8.3$ min; m/z (%): 401 (3) $[M\mathrm{K}]^+$, 385 (4) $[M\mathrm{Na}]^+$, 363 (100) $[M\mathrm{H}]^+$, 219 (8), 187 (17); **17b**: $t_R = 8.5$ min; m/z (%): 401 (4) $[M\mathrm{K}]^+$, 385 (6) $[M\mathrm{Na}]^+$, 363 (100) $[M\mathrm{H}]^+$, 219 (12), 187 (14); accurate mass (mean of 11 measurements ± standard deviation): **17a,b**: calculated for $C_{18}H_{27}N_4O_4$: 363.2032, found: m/z 363.2034 ± 0.0007 $[M\mathrm{H}]^+$. Preparation (scaled down to one seventh) was repeated with [1-^{13}C]D-glucose, and the products were isolated analogously. For the D-arabinose incubation, fractions with $t_R = 10.5$ and 11.3 min yielded, after lyophilization, **11** · HCOOH (12.3 mg, 0.033 mmol, 1.6 %) and **12** · HCOOH (4.8 mg, 0.013 mmol, 0.6 %), respectively, LC–ESI-MS (for gradient and cone voltage, see above). **11**: $t_R = 7.5$ min; m/z (%): 371 (1) $[M\mathrm{K}]^+$, 355 (2) $[M\mathrm{Na}]^+$, 333 (45) $[M\mathrm{H}]^+$, 175 (100), 159 (30); **12**: $t_R = 8.3$ min; m/z (%): 371 (1) $[M\mathrm{K}]^+$, 355 (1) $[M\mathrm{Na}]^+$, 333 (100) $[M\mathrm{H}]^+$, 315 (5), 189 (5), 187 (4); accurate mass (mean of 10 measurements ± standard deviation): **11**:, calculated for $C_{17}H_{25}N_4O_3$: 333.1927, found: m/z 333.1931 ± 0.0007 $[M\mathrm{H}]^+$; **12**: calculated for $C_{17}H_{25}N_4O_3$: 333.1927, found: m/z 333.1935 ± 0.0010 $[M\mathrm{H}]^+$.

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[9] See Supporting Information for: a) ^{1}H and ^{13}C NMR data for **7a–d**, (Table 1); b) reasoning for the number of observable diastereoisomers for **7** and assignment of their relative configuration (Tables 1 and 2 and Figure 1); c) ^{1}H and ^{13}C NMR data for **17a,b** (Table 3); d) LC chromatograms (Figure 2); e) ^{1}H and ^{13}C NMR data for **11** and **12** (Table 3).

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A Method for Thermal Generation of Aryloxyl Radicals at Ambient Temperatures: Application to Low-Density Lipoprotein (LDL) Oxidation**

Thomas Paul* and Keith U. Ingold*

Antioxidant phenols (ArOH) react with peroxyl radicals (ROO•) and form relatively unreactive aryloxyl radicals (ArO•) [Eq. (1)] which, in homogeneous solutions, then trap a second peroxyl [Eq. (2)].[1, 2] α-Tocopherol (TocH, vitamin E) is the most active lipid-soluble antioxidant in

$$\mathrm{ArOH} + \mathrm{ROO}^{\bullet} \longrightarrow \mathrm{ArO}^{\bullet} + \mathrm{ROOH} \quad (1)$$

$$\mathrm{ArO}^{\bullet} + \mathrm{ROO}^{\bullet} \longrightarrow \text{nonradical products} \quad (2)$$

mammals[2] but, surprisingly, it acts as a prooxidant in human low-density lipoproteins (LDL).[3] Oxidatively modified LDL may initiate atherosclerosis.[4] Various agents (e.g., enzymes, transition metals) have been suggested to be responsible for this modification of LDL in vivo.[5] The free radical initiated oxidation of LDL in which TocH transfers radical character from water-soluble peroxyls into the LDL has been studied extensively. The resulting tocopheroxyl radical (Toc•) then carries a lipid peroxidation chain within the LDL in a process christened tocopherol-mediated peroxidation (TMP).[6] The aryloxyl radical, tyrosyl, which is formed (in 25 % yield) by reaction of myeloperoxidase with tyrosine during the immune response, can also initiate LDL peroxidation.[7] These two examples of aryloxyl radical-induced biological damage highlight the need for quantitative, in vitro studies of their reactions using thermolabile compounds which would provide "clean" and well-defined ArO• fluxes. To design an aryloxyl radical thermal source (ARTS) which would generate *any* ArO• and *only* that ArO• radical is therefore a worthwhile and exciting challenge.[8]

Hyponitrites, which are not subject to metal ion- or radical-induced decomposition,[9, 10] decompose at ambient temperatures to give N_2 and alkoxyl radicals. It appeared probable that aryloxyalkoxyl radicals would undergo very fast β-scission[11] to yield aryloxyl radicals [Eq. (3)]. A synthetic route to aryloxyalkyl hyponitrites suitable for many different

$$[\mathrm{ArOCR_2ON{=}}]_2 \xrightarrow[-N_2]{\Delta} \mathrm{ArOCR_2O}^{\bullet} \xrightarrow{\beta\text{-scission}} \mathrm{ArO}^{\bullet} + \mathrm{R_2C{=}O} \quad (3)$$

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