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Reaction of guanosine with glucose, ribose, and glucose 6-phosphate

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Abstract

The reaction of guanosine with glucose, glucose 6-phosphate or ribose in the presence of propylamine leads to the formation of several guanosine derivatives. During this process sugar degradation products are attached to the amino group of the guanosine residue. N^2-(1-Carboxy-3,4-dihydroxybutyl)-guanosine, N^2-(1-carboxyethyl)-guanosine, N^2-[1-(*N*-propyl-carbamoyl)-ethyl]-guanosine, and N^2-[1-(*N*-propyl-carbamoyl)-methyl]-guanosine have been isolated as pure compounds. N^2-[1-(*N*-Propyl-carbamoyl)-3,4,5-trihydroxypentyl]-guanosine and N^2-[1-(*N*-propyl-carbamoyl)-3,4-dihydroxybutyl]-guanosine, which were synthesized, can be detected in glucose– and ribose–guanosine reaction mixtures. © 1996 Elsevier Science Ltd.

Keywords: D-Glucose; D-Glucose 6-phosphate; N^2-(1-Carboxyalkyl)-guanosine; N^2-(1-Carbamoylalkyl)-guanosine

1. Introduction

Reactions of glucose with proteins have been thoroughly investigated during the past decade by several groups. Nonenzymatic glycosylation of proteins is of considerable interest not only in food chemistry but also in medical biochemistry. However, only recently has it been suggested that the amino groups of DNA may interact with reducing sugars in a similar way [1]. In their pioneering work in this area, Cerami and coworkers [2–4] examined the reactions of glucose and glucose 6-phosphate with DNA. The nonenzymatic glucose-mediated DNA damage seems to be of considerable biological importance. This process can lead to DNA mutation, strand breaks, and crosslinks, as

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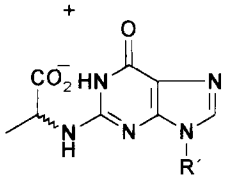
1,2,3,4

+



5,6,7

a,b = 2 (R) or 2 (S)

	R	R′
1	CH_2OH	β-D-ribofuranosyl
2	CH_2OH	2′-deoxy-β-D-ribofuranosyl
3	$CH_2OPO_3^{2-}$	2′-deoxy-β-D-ribofuranosyl
4	H	β-D-ribofuranosyl

	R′
5	β-D-ribofuranosyl
6	2′deoxy-β-D-ribofuranosyl
7	H

Scheme 1. Formation of the α-amino acids **1–7**.

has been derived from studies of in vitro and in vivo model systems [5]. Furthermore, it has been hypothesized that the modification of DNA with a reducing sugar may be involved in age related genetic dysfunctions [6]. Remarkably, the sugar–DNA interaction is accelerated by amino acids like lysine [3] or even simple primary amines, which serve as model compounds for the lysine side chains of proteins. Obviously a sugar-derived degradation product is formed by the catalytic action of the amine, which subsequently reacts with a purine or pyrimidine base of the DNA. Employment of radiolabelled lysine resulted in a time- and sugar concentration-dependent accumulation of radiolabel into DNA [3]. This result is in accordance with the finding that DNA–protein crosslinking does occur as a result of the aging process [7]. As far as we know, however, the chemical structure of the sugar-modified DNA is still unknown.

Recently, we have shown that guanosine and glucose react in the presence of lysine or another primary amine to give two diastereomeric guanosine substitution products with a trihydroxyhexanoic acid side chain of structure **1** (Scheme 1) [8]. Similar results were obtained with 2′-deoxyguanosine [9]. The stereoisomeric compounds **2a** and **2b** are formed as main products in neutral heated solution as well as at 40 °C after prolonged standing. Besides **2a,b**, minor amounts of the purine-substituted alanine derivatives **6a,b** have also been isolated [10]. In an analogous reaction, N^2-(1-carboxyethyl)-9-methylguanine was obtained from 9-methylguanine and glucose [11]. These model reactions are thought to give insight into the glucose–DNA interaction. Chemically, the

transformation of glucose into a trihydroxy–amino hexanoic acid is a previously unknown reaction.

Herein we report that other guanosine–glucose reaction products are formed in considerable quantity. Furthermore, compounds have been isolated which show a crosslink between guanosine and the primary amine by a sugar-derived moiety. The investigations have been extended to include glucose 6-phosphate and D-ribose.

2. Results and discussion

When glucose, propylamine, and guanosine are heated in concentrated phosphate buffer at neutrality for three days at 70 °C, a mixture of products is obtained which can be separated by HPLC. Prolonged standing of the reactants at 40 °C for several days leads to a similar result. The purine-substituted stereoisomeric amino acids **1a** and **1b** are formed as main products as we have shown previously [8]. On the other hand, when the reaction is performed in dilute buffered aqueous solution, several products besides **1** are obtained in remarkable quantity (Fig. 1). It has been estimated that about 60% of the guanosine has reacted. Two stereoisomeric substances were isolated by preparative HPLC. Interpretation of the spectral data leads to the structures **5a** and **5b**. Analogous 2′-deoxyguanosine derivatives have been obtained previously by a different route. When we investigated the reaction of 2′-deoxyguanosine with glyceraldehyde or methylglyoxal, a mutagenic sugar degradation product, we isolated the purine-substituted alanine derivatives **6a** and **6b** as main products [10]. Acid-catalyzed hydrolysis of **5a** and **5b** leads to a racemic mixture of the guanine derivative **7**, the structure of which has been established by an unequivocal synthesis [10]. A more detailed investigation of the reaction mixture revealed that, besides the guanosine derivatives **1** and **5**, the α-amino acid amides **10a** and **10b** were also formed (Scheme 2). The structures of **10a** and **10b** can be derived from spectral data (see Experimental), but one would expect similar data for a compound obtained by exchanging the guanosine and propylamine residues at the propionic acid moiety. A decision can be made on the basis of a cleavage reaction. Alkaline hydrolysis of the diastereomeric products **10a** and **10b** affords compounds **5a**

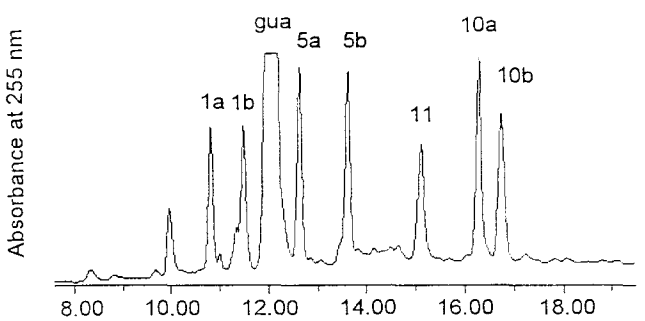
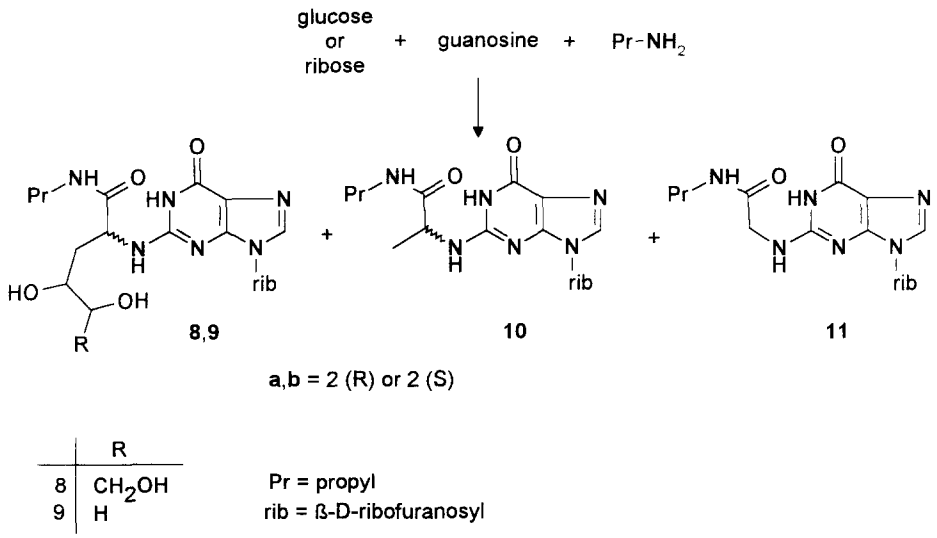


Fig. 1. High performance liquid chromatogram of the reaction mixture of glucose, guanosine, and propylamine in phosphate buffer (3 days, 70 °C, pH 7.0; system 1, $\lambda = 255$ nm; gua = guanosine).

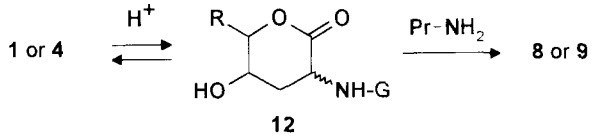


Scheme 2. Formation of the α-amino acid amides **8–11**.

and **5b**, which means that the aliphatic amine is connected to the acid group in accordance with the proposed structure. As a two-carbon sugar degradation product, the glycine derivative **11** (Scheme 2) can be isolated from the reaction mixture by preparative HPLC. The structure of this α-amino acid amide was established via spectral data (see Experimental).

It is well known that pentoses are more reactive than hexoses [12]. As expected, the interaction of D-ribose with guanosine leads to the formation of analogous products as described above for the glucose–guanosine reaction. For instance, compounds **4**, **5**, **10**, and **11** can be isolated from reaction mixtures. The structures of the products are deduced from spectral data (see Experimental), which can be compared to those of the identified glucose-derived products **1**, **5**, **10**, and **11**.

On the basis of the results described above, one might expect that crosslinking products should be formed as well from the α-amino acids **1–4**. Reference compounds **8a,b**, and **9a,b** have been obtained by a simple reaction sequence and their structures established by spectral data. In a reversible, pH-dependent process, cyclisation of the trihydroxy- (**1a,b**) or dihydroxy- (**4a,b**) α-amino acids afforded the lactones **12** which react further with propylamine to give the stereoisomeric amino acid amides **8a** and **8b**, or **9a** and **9b** (Scheme 3). At the same time this transformation confirms that the guanosine- and glucose- (or ribose) derived product **1** (or **4**) is a polyhydroxy amino acid and not a polyhydroxy acid amide. Indeed, a careful chromatographic separation of the guanosine–glucose (or ribose) –propylamine reaction mixture revealed that two stereoisomeric substances are formed which show the same chromatographic behaviour as the reference compounds **8a** and **8b**, and **9a** and **9b**, obtained by synthesis. Unfortunately the amount of these products is low and could not be isolated in pure form.



H_2N-G = guanosine

R = CH_2OH or H

Pr = propyl

Scheme 3. Synthesis of **8** and **9**.

Reaction of glucose 6-phosphate (Glc 6-P) with DNA in the presence of lysine has been studied by Lee and Cerami [2,3]. It was not surprising when we observed that the amine-catalyzed interaction of Glc 6-P with 2′-deoxyguanosine affords the phosphorylated guanosine derivatives **3a** and **3b**. The identification was easily achieved by enzymatic hydrolysis of the phosphate ester leading to the known products **2a** and **2b**. Compared to glucose, Glc 6-P reacts considerably faster.

Reaction of guanosine with glucose or ribose in the presence of a primary amine leads to several products with a sugar-derived carbon chain at the amino group of the purine moiety. Most remarkably, the aldoses are transformed into α-amino acids by intramolecular redox reactions. This process may be accompanied by a cleavage reaction of the sugar-carbon chain. A reaction mechanism which would explain the formation of the products described above has not been established so far. Schiff bases of sugar-derived dicarbonyl compounds may be involved as intermediates [9]. Furthermore a retroaldol reaction of the hexose or pentose carbon chain affords the C_3-derivatives [10]. Further work is in progress to address this problem.

The isolation of the amide **11** is of special interest in connection with the previous report that N^{ε}-(carboxymethyl)-lysine [13] is a product of a Maillard reaction of glucose with a protein. The formation of **11** cannot be explained by a simple retroaldol reaction of the sugar carbon chain; an oxidation step is probably involved.

Several investigations have shown that simple primary aliphatic amines react in the same way as lysine side chains of proteins. If this is accepted, the formation of compounds **8**, **9**, **10**, and **11** would be a model for crosslinking of DNA with proteins. One might assume that analogous reactions are operating in the crosslinking of protein chains, a well documented reaction which is thought to be of considerable biological importance. Our studies of this problem will be reported in a separate paper.

3. Experimental

General methods.—Melting points were determined in open capillary tubes in a Büchi apparatus and are uncorrected. Optical rotations were obtained with a Perkin–Elmer 241 polarimeter in a 1 dm cell at 20 °C. ^{1}H NMR and ^{13}C NMR spectra were

recorded with a Jeol 400 GSX spectrometer. Chemical shifts are reported in ppm and referenced to $(CH_3)_4Si$ in CD_3OD, or to acetone in D_2O as the internal or external standard, respectively. Chemical shift correlations were made via $^1H-^1H$ COSY and $^1H-^{13}C$ COSY spectra. Positive FABMS data were obtained with a Kratos MS 80 RFA spectrometer. For analytical chromatography, a Merck L-6200 gradient pump, a Merck D-6500 photodiode array detector including Merck DAD-Manager software, and a Hewlett–Packard deskjet 850 C were used. UV spectra were directly taken from this system (λ in nm). Preparative chromatography was performed with a Merck L-6250 pump, a Merck L-4000 UV-detector, and a Merck D-2500 integrator. The water used for HPLC was distilled and filtered through a nylon 0.45 μm membrane. HPLC grade solvent (MeOH) was used without further purification. All solvents were degassed with helium. System 1 (analytical): column: Nucleosil RP 18, 250 × 4.6 mm i.d., 5 μm with guard cartridge (25 × 4.6 mm i.d.); UV-detection between 220 and 350 nm (DAD); eluent: gradient elution starting with 0.05 M triethylammonium acetate buffer, pH 7, ending within 25 min with 2:8 buffer–MeOH at a flow rate of 0.8 mL/min. System 2 (preparative): column: Hibar LiChrospher RP 18, 250 × 25 mm i.d., 10 μm; UV-detection at 255 nm; 2a, eluent: 97:3 5 mM ammonium formate, pH 7–MeOH at a flow rate of 12 mL/min; 2b, eluent: 80:20 water–MeOH at a flow rate of 12 mL/min; 2c, eluent: 85:15 water–MeOH at a flow rate of 12 mL/min. The absolute configurations of the diastereomeric compounds were not determined.

Reaction of guanosine with ribose in the presence of propylamine.—Ribose (11.3 g, 75 mmol), guanosine (4.2 g, 15 mmol), and propylamine (0.9 g, 15 mmol) were heated at 70 °C for 3 days in a mixture of 4.1 g potassium dihydrogen phosphate and 5.3 g disodium hydrogen phosphate dihydrate (30 mmol each) in 20 mL of water. The mixture was diluted with water (20 mL), filtered, and concentrated for chromatography. Separation of the two diastereomeric compounds **4a** and **4b** was achieved by preparative HPLC (system 2a). The products eluting after 16 min (**4a**) and 29 min (**4b**) were collected, lyophilized, and recrystallized from 3:1 EtOH–water.

Ammonium (2R/2S,4S)-2-(N^2-guanosyl)-4,5-dihydroxy-pentanoate (**4a**).—Yield, 348 mg (5.4%); mp 226–230 °C (dec); $[\alpha]_D^{20}$ +2.2° (*c* 0.2, H_2O); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^1H NMR (D_2O): δ 7.89 (s, 1 H, gua), 5.87 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1 rib), 4.98 (t, 1 H, *J* 5.6 Hz, H-2 rib), 4.40 (t, 1 H, *J* 5.6 Hz, H-3 rib), 4.31 (m, 1 H, H-2), 4.17 (m, 1 H, H-4 rib), 3.91–3.95 (m, 2 H, H-5a rib, H-4), 3.86 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 5.6 Hz, H-5b rib), 3.67 (dd, 1 H, J_{gem} 11.8, $J_{5,4}$ 3.6 Hz, H-5a), 3.57 (dd, 1 H, J_{gem} 11.8, $J_{5,4}$ 6.5 Hz, H-5b), 2.10 (m, 1 H, H-3a), 1.93 (m, 1 H, H-3b); ^{13}C NMR: 179.6 (C-1), 159.0 (C-6 gua), 152.1 (C-2 gua), 151.5 (C-4 gua), 139.1 (C-8 gua), 116.9 (C-5 gua), 88.7 (C-1 rib), 84.6 (C-4 rib), 72.4 (C-2 rib), 70.2 (C-3 rib), 70.0 (C-4), 65.2 (C-5), 61.5 (C-5 rib), 55.2 (C-2), 35.2 (C-3); FABMS (Xe, 7 KV, glycerol): *m/z* 416 $[M + H]^+$ for the free acid.

Ammonium (2R/2S,4S)-2-(N^2-guanosyl)-4,5-dihydroxy-pentanoate (**4b**).—Yield, 335 mg (5.2%); mp 230–238 °C (dec); $[\alpha]_{20}^{D}$ +7.2° (*c* 0.2, H_2O); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^1H NMR (D_2O): δ 7.89 (s, 1 H, gua), 5.84 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1 rib), 4.79 (t, 1 H, *J* 5.6 Hz, H-2 rib), 4.42 (dd, 1 H, $J_{2,3a}$ 4.5, $J_{2,3b}$ 8.9 Hz, H-2), 4.33 (t, 1 H, *J* 5.6 Hz, H-3 rib), 4.08 (m, 1 H, H-4 rib), 3.75–3.81 (m, 2 H, H-5a rib, H-4), 3.69 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 5.6 Hz, H-5b rib), 3.53 (dd, 1 H, J_{gem} 11.4, $J_{5,4}$ 3.8

Hz, H-5a), 3.45 (dd, 1 H, J_{gem} 11.4, $J_{5,4}$ 6.8 Hz, H-5b), 1.80–1.94 (m, 2 H, H-3a, H-3b); ^{13}C NMR: 179.8 (C-1), 159.0 (C-6 gua), 155.1 (C-2 gua), 152.3 (C-4 gua), 138.3 (C-8 gua), 116.5 (C-5 gua), 88.3 (C-1 rib), 84.2 (C-4 rib), 73.1 (C-2 rib), 69.9 (C-3 rib), 68.9 (C-4), 65.6 (C-5), 61.4 (C-5 rib), 53.9 (C-2), 34.9 (C-3); FABMS (Xe, 7 KV, glycerol): m/z 416 $[M + H]^+$ for the free acid.

Reaction of glucose with guanosine and propylamine in dilute solution.—Glucose (8.1 g, 45 mmol), guanosine (2.5 g, 9 mmol), and propylamine (5.4 g, 90 mmol) were dissolved in 240 mL of 0.5 M phosphate buffer (pH 7.0) and stirred at 70 °C for 3 days. Samples were taken daily and assayed by analytical HPLC (system 1). The reaction mixture was filtered, concentrated, and the residue was diluted with 30 mL of water. Each time 10 μL (in portions of 1 mL) of this solution were subjected to preparative HPLC for the isolation of compounds **5a** and **5b** (system 2a), **10a** and **10b** (system 2b), and **11** (system 2b). Pure fractions with identical substances were collected and lyophilized.

Ammonium 2-(N^2-guanosyl)-propionate (**5**).—The absolute configuration at C-2 of **5** was not determined. Compounds eluting after 24 min (**5a**) and 37 min (**5b**) were collected. (+)-**5a**: Yield, 140 mg (4.4%); mp 183 °C (dec); $[\alpha]_D^{20}$ +2.6° (*c* 0.2, CH_3OH); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^{1}H NMR (D_2O): δ 7.89 (s, 1 H, gua), 5.84 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1 rib), 5.02 (t, 1 H, *J* 5.6 Hz, H-2 rib), 4.40 (t, 1 H, *J* 5.6 Hz, H-3 rib), 4.11–4.19 (m, 2 H, H-4 rib, H-2), 3.88 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 3.9 Hz, H-5a rib), 3.82 (dd, 1 H, J_{gem} 12.4, $J_{4,5}$ 5.6 Hz, H-5b), 1.41 (d, 3 H, $J_{3,2}$ 7.3 Hz, H-3); ^{13}C NMR: 180.7 (C-1), 158.4 (C-6 gua), 152.2 (C-2 gua), 151.0 (C-4 gua), 139.3 (C-8 gua), 116.0 (C-5 gua), 89.3 (C-1 rib), 85.0 (C-4 rib), 72.6 (C-2 rib), 70.2 (C-3 rib), 61.6 (C-5 rib), 51.1 (C-2), 16.8 (C-3); FABMS (Xe, 7 KV, glycerol): m/z 356 $[M + H]^+$ for the free acid. (−)-**5b**: Yield, 123 mg (3.8%); mp 190 °C (dec); $[\alpha]_D^{20}$ −2.6° (*c* 0.2, CH_3OH); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^{1}H NMR (D_2O): 7.95 (s, 1 H, gua), 5.92 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1 rib), 4.87 (t, 1 H, *J* 5.6 Hz, H-2 rib), 4.42 (t, 1 H, *J* 5.6 Hz, H-3 rib), 4.23 (q, 1 H, $J_{2,3}$ 7.3 Hz, H-2), 4.16 (m, 1 H, H-4 rib), 3.88 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 3.9 Hz, H-5a rib), 3.82 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 5.6 Hz, H-5b), 1.41 (d, 3 H, $J_{3,2}$ 7.3 Hz, H-3); ^{13}C NMR: 180.6 (C-1), 159.2 (C-6 gua), 152.1 (C-2 gua), 151.7 (C-4 gua), 138.6 (C-8 gua), 116.7 (C-5 gua), 88.8 (C-1 rib), 84.5 (C-4 rib), 73.4 (C-2 rib), 70.2 (C-3 rib), 61.7 (C-5 rib), 52.7 (C-2), 17.8 (C-3); FABMS (Xe, 7 KV, glycerol): m/z 356 $[M + H]^+$ for the free acid.

2-(N^2-Guanosyl)-propionic acid N-propylamide (**10**).—The absolute configuration at C-2 of **10** was not determined. Compounds eluting after 36 min (**10a**) and 44 min (**10b**) were collected. (+)-**10a**: Yield, 140 mg (3.9%); mp 146–149 °C (dec); $[\alpha]_D^{20}$ +5.7° (*c* 0.2, CH_3OH); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^{1}H NMR (CD_3OD): δ 8.00 (s, 1 H, gua), 5.88 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1 rib), 4.62 (t, 1 H, *J* 5.6 Hz, H-2 rib), 4.46 (q, 1 H, $J_{2,3}$ 7.3 Hz, H-2), 4.35 (t, 1 H, *J* 5.6 Hz, H-3 rib), 4.07 (m, 1 H, H-4 rib), 3.86 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 3.9 Hz, H-5a rib), 3.76 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 5.6 Hz, H-5b), 3.19 (m, 2 H, CH_2N), 1.56 (m, 2 H, CH_2CH_3), 1.42 (d, 3 H, $J_{3,2}$ 7.3 Hz, H-3), 0.92 (t, 3 H, *J* 7.3 Hz, CH_3CH_2); ^{13}C NMR: 176.1 (C-1), 159.5 (C-6 gua), 152.9 (C-2 gua), 152.1 (C-4 gua), 139.9 (C-8 gua), 118.0 (C-5 gua), 89.3 (C-1 rib), 86.0 (C-4 rib), 74.8 (C-2 rib), 71.3 (C-3 rib), 62.6 (C-5 rib), 52.6 (C-2), 42.1 (CH_2N), 23.2 (CH_2CH_3), 18.5 (C-3), 11.6 (CH_3CH_2); FABMS (Xe, 7 KV, glycerol): m/z 397 $[M + H]^+$.

(−)-**10b**: Yield, 115 mg (3.2%); mp 180–190 °C (dec); $[\alpha]_D^{20}$ −21.3° (*c* 0.2, CH_3OH); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^1H NMR (CD_3OD): δ 8.03 (s, 1 H, gua), 5.91 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1 rib), 4.59 (t, 1 H, *J* 5.6 Hz, H-2 rib), 4.52 (q, 1 H, $J_{2,3}$ 7.3 Hz, H-2), 4.33 (t, 1 H, *J* 5.6 Hz, H-3 rib), 4.07 (m, 1 H, H-4 rib), 3.84 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 3.9 Hz, H-5a rib), 3.74 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 5.6 Hz, H-5b), 3.20 (m, 2 H, CH_2N), 1.56 (m, 2 H, CH_2CH_3), 1.46 (d, 3 H, $J_{3,2}$ 7.3 Hz, H-3), 0.93 (t, 3 H, *J* 7.3 Hz, CH_3CH_2); ^{13}C NMR: 175.9 (C-1), 158.1 (C-6 gua), 153.5 (C-2 gua), 151.6 (C-4 gua), 138.1 (C-8 gua), 115.2 (C-5 gua), 89.5 (C-1 rib), 85.9 (C-4 rib), 75.1 (C-2 rib), 71.0 (C-3 rib), 62.2 (C-5 rib), 52.4 (C-2), 42.1 (CH_2N), 23.2 (CH_2CH_3), 18.6 (C-3), 11.6 (CH_3CH_2); FABMS (Xe, 7 KV, glycerol): *m/z* 397 $[M + H]^+$.

2-(N^2*-Guanosyl)-acetic acid* N-*propylamide* (**11**).—Yield, 113 mg (3.3%); mp 132–145 °C (dec); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^1H NMR (D_2O): δ 7.98 (s, 1 H, gua), 5.90 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1 rib), 4.84 (t, 1 H, *J* 5.6 Hz, H-2 rib), 4.38 (t, 1 H, *J* 5.1 Hz, H-3 rib), 4.16 (m, 1 H, H-4 rib), 4.05 (s, 2 H, H-2), 3.87 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 3.9 Hz, H-5a rib), 3.78 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 5.6 Hz, H-5b), 3.18 (m, 2 H, CH_2N), 1.50 (m, 2 H, CH_2CH_3), 0.83 (t, 3 H, *J* 7.3 Hz, CH_3CH_2); ^{13}C NMR: 174.2 (C-1), 159.8 (C-6 gua), 152.9 (C-2 gua), 151.5 (C-4 gua), 137.1 (C-8 gua), 118.8 (C-5 gua), 88.8 (C-1 rib), 85.2 (C-4 rib), 73.2 (C-2 rib), 70.9 (C-3 rib), 62.1 (C-5 rib), 45.5 (C-2), 41.2 (CH_2N), 22.2 (CH_2CH_3), 10.9 (CH_3CH_2); FABMS (Xe, 7 KV, glycerol): *m/z* 383 $[M + H]^+$.

Hydrolysis of **5**.—A solution of **5a** and **5b** (20 mg, 0.06 mmol each) in 1 mL of 2 N HCl was heated at 100 °C for 2 h. After neutralization with 2 N NaOH, the hydrolysis product (**7**) was purified by preparative HPLC (system 2a). Chromatographic and spectroscopic data for racemic **7** were identical with those reported in the literature [10]. Yield, 22 mg (77%).

Alkaline hydrolysis of **10a**.—A solution of **10a** (20 mg, 0.05 mmol) in 1 mL of 2 N NaOH was kept at 100 °C for 2 h. Samples were taken and analyzed by HPLC (system 1). After neutralization with 2 N HCl, a new compound was isolated and purified by preparative HPLC (system 2a). Chromatographic and spectroscopic data revealed the identity of the hydrolysis product with the isolated compound **5a**. Yield, 12 mg (67%).

Alkaline hydrolysis of **10b**.—Compound **10b** was treated in the same manner as described above. The chromatographic and spectroscopic data of the isolated product are identical with those of **5b**. Yield, 13 mg (73%).

Reaction of ribose with guanosine and propylamine in dilute solution.—Ribose (6.8 g, 45 mmol), guanosine (2.5 g, 9 mmol), and propylamine (5.4 g, 90 mmol) were dissolved in 240 mL of 0.5 M phosphate buffer (pH 7.0) and stirred at 70 °C for 3 days. Samples were taken daily and analyzed by HPLC (system 1). Compounds **5**, **10**, and **11** were isolated from this reaction mixture in the same way as described above for the glucose–guanosine reaction mixture.

Reaction of glucose 6-phosphate with 2′-deoxyguanosine.—Glucose 6-phosphate disodium salt dihydrate (340 mg, 1 mmol), 2′-deoxyguanosine monohydrate (285 mg, 1 mmol), and propylamine (59 mg, 1 mmol) were stored at 40 °C for one week in 20 mL of 2 M phosphate buffer, after which the reaction mixture was filtered and evaporated. The residue was dissolved in 20 mL of MeOH, placed into an ultrasonic bath for 15 min, and filtered to remove the buffer salt. The solvent was removed under reduced

pressure and the residue was dissolved in 0.05 M Tris HCl buffer (5 mL, pH 8.1). A 100 μL sample was incubated with 50 μL alkaline phosphatase suspension (Boehringer 108162) overnight. The mixture was diluted, filtered, and chromatographed (system 1). The detected peaks proved to be identical in all respects to the glucose-derived derivatives **2a,b** previously isolated from a glucose–2′-deoxyguanosine reaction mixture [9].

Synthesis of (2R/2S,4S,5R)-2-(N²-guanosyl)-4,5,6-trihydroxy-hexanoic acid N-propyl-amine **8**.—Glucose (4.5 g, 25 mmol), guanosine (1.4 g, 5 mmol), and propylamine (0.3 g, 5 mmol) were heated at 70 °C for 3 days in a mixture of 1.4 g potassium dihydrogen phosphate and 1.8 g disodium hydrogen phosphate dihydrate (10 mmol each) in 7 mL of water. The reaction mixture was filtered and the water evaporated. The residue was dissolved in 5:1 MeOH–HCl (36 mL) and the solution was stirred at room temperature for 2 h. After neutralization with saturated sodium hydrogen carbonate solution, an excess of propylamine (40 mL) was added, and the mixture was allowed to stand at room temperature for 2 h. The solvents were removed under reduced pressure and the brown syrup dissolved in MeOH (20 mL), filtered, and evaporated. The residue was dissolved in water (15 mL) for preparative HPLC (system 2c). The diastereomeric compounds eluted at 25 min (**8a**) and 32 min (**8b**). Pure fractions with identical substances were collected and lyophilized.

*(2R/2S,4S,5R)-2-(N²-Guanosyl)-4,5,6-trihydroxy-hexanoic acid N-propylamide (−)-***8a**.—Yield, 180 mg (7.4%); mp 130–140 °C (dec); $[\alpha]_D^{20}$ −0.6° (*c* 0.2, CH_3OH); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^1H NMR (CD_3OD): δ 7.95 (s, 1 H, gua), 5.87 (d, 1 H, $J_{1,2}$ 4.7 Hz, H-1 rib), 4.77 (dd, 1 H, $J_{2,3a}$ 9.4, $J_{2,3b}$ 3.4 Hz, H-2), 4.54 (m, 1 H, H-2 rib), 4.35 (m, 1 H, H-3 rib), 4.16 (m, 1 H, H-4 rib), 3.95 (dd, 1 H, J_{gem} 12.5, $J_{5,4}$ 3.3 Hz, H-5a rib), 3.70–3.81 (m, 3 H, H-5b rib, H-4, H-6a), 3.61 (dd, 1 H, J_{gem} 11.3, $J_{6,5}$ 5.9 Hz, H-6b), 3.51 (m, 1 H, H-5), 3.21 (m, 2 H, CH_2N), 2.18 (ddd, 1 H, $J_{3,4}$ 2.2, $J_{3,2}$ 9.6, J_{gem} 14.7 Hz, H-3a), 1.92 (ddd, 1 H, $J_{2,3}$ 3.62, $J_{3,4}$ 10.6, J_{gem} 14.7 Hz, H-3b), 1.56 (m, 2 H, CH_2CH_3), 0.92 (t, 3 H, *J* 7.3 Hz, CH_3CH_2); ^{13}C NMR: 175.8 (C-1), 161.0 (C-6 gua), 155.0 (C-2 gua), 152.5 (C-4 gua), 138.4 (C-8 gua), 118.6 (C-5 gua), 90.1 (C-1 rib), 86.6 (C-4 rib), 76.4 (C-2 rib), 75.5 (C-5), 71.9 (C-3 rib), 69.9 (C-4), 64.6 (C-6), 63.1 (C-5 rib), 54.2 (C-2), 42.4 (CH_2N), 37.3 (C-3), 23.7 (CH_2CH_3), 11.9 (CH_3CH_2); FABMS (Xe, 7 KV, glycerol): *m/z* 487 $[M + H]^+$.

*(2R/2S,4S,5R)-2-(N²-Guanosyl)-4,5,6-trihydroxy-hexanoic acid N-propylamide (+)-***8b**.—Yield, 175 mg (7.2%); mp 130–140 °C (dec); $[\alpha]_D^{20}$ 0.6° (*c* 0.2, CH_3OH); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^1H NMR (CD_3OD): δ 8.03 (s, 1 H, gua), 5.89 (d, 1 H, $J_{1,2}$ 5.1 Hz, H-1 rib), 4.60 (t, 1 H, $J_{1,2}$ 5.1 Hz, H-2 rib), 4.54 (m, 1 H, H-2), 4.35 (m, 1 H, H-3 rib), 4.06 (m, 1 H, H-4 rib), 3.87 (dd, 1 H, J_{gem} 12.2, $J_{5,4}$ 3.4 Hz, H-5a rib), 3.70–3.78 (m, 3 H, H-5b rib, H-4, H-6a), 3.61 (dd, 1 H, J_{gem} 11.4, $J_{6,5}$ 6.2 Hz, H-6b), 3.51 (m, 1 H, H-5), 3.20 (m, 2 H, CH_2N), 2.24 (ddd, 1 H, *J* 2.8, *J* 7.0, J_{gem} 14.2 Hz, H-3a), 1.89 (m, 1 H, H-3b), 1.56 (m, 2 H, CH_2CH_3), 0.92 (t, 3 H, *J* 7.3 Hz, CH_3CH_2); ^{13}C NMR: 175.5 (C-1), 156.0 (C-6 gua), 154.0 (C-2 gua), 152.8 (C-4 gua), 138.9 (C-8 gua), 118.9 (C-5 gua), 90.2 (C-1 rib), 87.0 (C-4 rib), 76.6 (C-5), 76.2 (C-2 rib), 72.1 (C-3 rib), 71.8 (C-4), 64.9 (C-6), 63.2 (C-5 rib), 56.0 (C-2), 42.8 (CH_2N), 37.2 (C-3), 24.0 (CH_2CH_3), 12.2 (CH_3CH_2); FABMS (Xe, 7 KV, glycerol): *m/z* 487 $[M + H]^+$.

Synthesis of (2R/2S,4S)-2-(N²-guanosyl)-4,5-dihydroxy-pentanoic acid N-*propylamide* **9**.—Compounds **9a** and **9b** were synthesized and separated according to the method described above for **8a** and **8b**. Ribose (3.75 g, 25 mmol), guanosine (1.4 g, 5 mmol), and propylamine (0.3 g, 5 mmol) were heated at 70 °C for 3 days in a mixture of 1.4 g potassium dihydrogen phosphate and 1.8 g disodium hydrogen phosphate dihydrate (10 mmol each) in 7 mL of water. The compounds eluting at 24 min (**9a**) and 32 min (**9b**) were collected and lyophilized.

(2R/2S,4S)-2-(N²-Guanosyl)-4,5-dihydroxy-pentanoic acid N-*propylamide (−)*-**9a**.—Yield, 167 mg (7.3%); mp 125–135 °C (dec); $[\alpha]_D^{20}$ −22.7° (*c* 0.2, CH_3OH); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^1H NMR (CD_3OD): δ 7.98 (s, 1 H, gua), 5.87 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1 rib), 4.72 (dd, 1 H, $J_{2,3a}$ 9.1, $J_{2,3b}$ 3.8 Hz, H-2), 4.62 (m, 1 H, H-2 rib), 4.30 (t, 1 H, *J* 4.9 Hz, H-3 rib), 4.05 (m, 1 H, H-4 rib), 3.77–3.84 (m, 2 H, H-5a rib, H-4), 3.71 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 4.2 Hz, H-5b rib), 3.50 (m, 2 H, H-5a, H-5b), 3.18 (m, 2 H, CH_2N), 1.91 (m, 2 H, H-3a, H-3b), 1.53 (m, 2 H, CH_2CH$_3$), 0.89 (t, 3 H, *J* 7.3 Hz, CH_3CH$_2$); ^{13}C NMR: 175.2 (C-1), 160.2 (C-6 gua), 154.4 (C-2 gua), 152.4 (C-4 gua), 138.1 (C-8 gua), 118.5 (C-5 gua), 89.7 (C-1 rib), 86.7 (C-4 rib), 75.6 (C-2 rib), 71.7 (C-3 rib), 70.0 (C-4), 67.7 (C-5), 62.9 (C-5 rib), 53.9 (C-2), 42.1 (*C*H$_2$N), 37.2 (C-3), 23.5 (*C*H$_2$CH$_3$), 11.6 (*C*H$_3$CH$_2$); FABMS (Xe, 7 KV, glycerol): *m/z* 457 $[M + H]^+$.

(2R/2S,4S)-2-(N²-Guanosyl)-4,5-dihydroxy-pentanoic acid N-*propylamide (+)*-**9b**.—Yield, 138 mg (6.0%); mp 125–130 °C (dec); $[\alpha]_D^{20}$ +7.9° (*c* 0.2, CH_3OH); UV (H_2O, pH 7): λ_{max} 255.6, 278.5 (sh); ^1H NMR (CD_3OD): δ 7.96 (s, 1 H, gua), 5.87 (d, 1 H, $J_{1,2}$ 5.6 Hz, H-1 rib), 4.63 (t, 1 H, $J_{1,2}$ 5.6 Hz, H-2 rib), 4.53 (m, 1 H, H-2), 4.33 (m, 1 H, H-3 rib), 4.06 (m, 1 H, H-4 rib), 3.86 (dd, 1 H, J_{gem} 12.4, $J_{5,4}$ 3.3 Hz, H-5a rib), 3.73–3.80 (m, 2 H, H-4, H-5b rib), 3.51 (m, 2 H, H-5a, H-5b), 3.17 (m, 2 H, CH_2N), 2.05 (m, 1 H, H-3a), 1.85 (m, 1 H, H-3b), 1.53 (m, 2 H, CH_2CH$_3$), 0.88 (t, 3 H, *J* 7.3 Hz, CH_3CH$_2$); ^{13}C NMR: 175.7 (C-1), 162.3 (C-6 gua), 155.5 (C-2 gua), 152.7 (C-4 gua), 138.5 (C-8 gua), 119.0 (C-5 gua), 90.3 (C-1 rib), 86.8 (C-4 rib), 75.7 (C-2 rib), 72.1 (C-3 rib), 71.2 (C-4), 67.3 (C-5), 63.1 (C-5 rib), 55.5 (C-2), 42.5 (*C*H$_2$N), 37.4 (C-3), 23.8 (*C*H$_2$CH$_3$), 12.0 (*C*H$_3$CH$_2$); FABMS (Xe, 7 KV, glycerol): *m/z* 457 $[M + H]^+$.

References

[1] (a) J.W. Baynes and V.M. Monnier (Eds.), *The Maillard Reaction in Aging, Diabetes, and Nutrition*, Liss, New York, 1989; (b) T.P. Labuza, G.A. Reineccius, V.M. Monnier, J. O'Brian, and J.W. Baynes (Eds.), *Maillard Reactions in Chemistry, Food, and Health*, The Royal Society of Chemistry, Cambridge, 1994; (c) P.A. Finot, H.U. Aeschbacher, R.F. Hurrell, and R. Liardon (Eds.), *The Maillard Reaction in Food Processing, Human Nutrition, and Physiology*, Birkhäuser, Basel, 1990.

[2] A.T. Lee and A. Cerami, *Nonenzymatic Glycosylation of DNA by Reducing Sugars*, in J.W. Baynes and V.M. Monnier (Eds.), *The Maillard Reaction in Aging, Diabetes, and Nutrition*, Liss, New York, 1989, pp 291–299.

[3] A.T. Lee and A. Cerami, *Mut. Res.*, 179 (1987) 151–158.

[4] (a) R. Bucala, P. Model, and A. Cerami, *Proc. Natl. Acad. Sci. USA*, 81 (1984) 105–109; (b) R. Bucala, P. Model, M. Russel, and A. Cerami, *Proc. Natl. Acad. Sci. USA*, 82 (1985) 8439–8442.

[5] (a) G.B. Price, S.P. Modak, and T. Makinodan, *Science*, 171 (1971) 917–920; (b) K. Hiramoto, K. Kido, and K. Kikugawa, *J. Agric. Food Chem.*, 42 (1994) 689–694.
[6] (a) A. Cerami, *The Role of the Maillard Reaction* In Vivo, in T.P. Labuza, G.A. Reineccius, V.M. Monnier, J. O'Brian, and J.W. Baynes (Eds.), *Maillard Reactions in Chemistry, Food, and Health*, The Royal Society of Chemistry, Cambridge, 1994, pp 1–10; (b) A.T. Lee, A. Cerami, and R. Bucala, *Glucose-mediated DNA Damage and Mutations*: In Vitro *and* In Vivo, in T.P. Labuza, G.A. Reineccius, V.M. Monnier, J. O'Brian, and J.W. Baynes (Eds.), *Maillard Reactions in Chemistry, Food, and Health*, The Royal Society of Chemistry, Cambridge, 1994, pp 249–253; (c) G.D. Berdyshev and S.M. Zhelabovaskaya, *Exp. Geront.*, 7 (1972) 321–330; (d) H.L. Gensler and H. Berstein, *Q. Rev. Bio.*, 56 (1981) 279–303.
[7] A.T. Lee and A. Cerami, *Nonenzymatic Reaction of Reducing Sugars with DNA*, in P.A. Finot, H.U. Aeschbacher, R.F. Hurrell, and R. Liardon (Eds.), *The Maillard Reaction in Food Processing, Human Nutrition, and Physiology*, Birkhäuser, Basel. 1990, pp 415–423.
[8] T. Knerr and T. Severin, *Tetrahedron Lett.*, 34 (1993) 7389–7390.
[9] S. Ochs and T. Severin, *Carbohydr. Res.*, 266 (1995) 87–94.
[10] S. Ochs and T. Severin, *Liebigs Ann. Chem.*, (1994) 851–853.
[11] A. Papoulis, Y. Al-Abed, and R. Bucala, *Biochemistry*, 34 (1995) 648–655.
[12] H.F. Bunn and P.J. Higgins, *Science*, 213 (1981) 222–224.
[13] (a) M.U. Ahmed, S.R. Thorpe, and J.W. Baynes, *J. Biol. Chem.*, 261 (1986) 4889–4894; (b) M.A. Glomb and V.M. Monnier, *J. Biol. Chem.*, 270 (1995) 10017–10026.