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Systematic Studies on the Chemical Structure and Umami Enhancing Activity of Maillard-Modified Guanosine 5′-Monophosphates

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Recent investigations on taste active principles in nucleotide rich yeast extracts led to the discovery of (*R*)- and (*S*)-N^2-(1-carboxyethyl)-guanosine 5′-monophosphate as previously not reported umami enhancing molecules formed upon the Maillard reaction of guanosine 5′-monophosphate (5′-GMP) with dihydroxyacetone and glyceraldehyde, respectively. In the present study, systematic Maillard-type model reactions were performed with 5′-GMP and a homologous series of monosaccharides exhibiting a C_3- to C_6-carbon skeleton as well as with the reducing disaccharide maltose in the presence of an amino acid. By preparative RP-HPLC, various (*R*)- and (*S*)-N^2-(1-carboxyalkyl)-guanosine 5′-monophosphates and (*R*)- and (*S*)-N^2-(1-alkylamino)carbonylalkyl)guanosine 5′-monophosphates were isolated and identified by means of LC-MS, LC-TOF-MS, and 1D/2D-NMR spectroscopy. Sensory evaluation of these Maillard products revealed β-values for umami enhancement between 0.06 and 7.0 and identified a strong influence of the stereochemistry as well as the chain length of the N^2-substituent on the umami enhancing activity. For all of the compounds evaluated, the (*S*)-configured isomers showed higher taste impact, whereas the (*R*)-isomers showed only marginal β-values, thus underlining the stereospecifity of the umami taste receptor binding site.

KEYWORDS: Taste; guanosine 5′-monophosphate; umami; taste enhancement; Maillard reaction

INTRODUCTION

Although the discovery of L-glutamate was reported as early as 1908 by Ikeda (*1*), the key taste stimulus in seaweed broth, the so-called umami taste, popularly referred to as savoriness (*1*, *2*), has been only recently recognized as the fifth basic taste since the cloning of a specific amino acid taste receptor in 2002 (*3*). The acidic amino acid L-glutamic acid is either naturally present in food (*4*) or added as its mono sodium salt (MSG) to savory food products to improve the umami taste impression.

A characteristic hallmark of umami taste perception is the strongly pronounced synergistic effect of selected 5′-ribonucleotides with L-glutamate (*5*, *6*). Referring to inosine 5′-monophosphate, **1** (**Figure 1**), as the reference, guanosine 5′-monophosphate (**2**) was found to be 2.3 times more active in enhancement of the umami taste of MSG solutions, while xanthosine 5′-monophosphate (**3**) and adenosine 5′-monophosphate (**4**) were less potent, showing only 0.53 and 0.13 times the impact of **1** (*7*).

Aimed at identifying the structural requirements for taste enhancement of 5′-ribonucleotides, a series of synthetic approaches were undertaken to obtain structural analogues with superior taste enhancement properties when compared to those of the parent nucleotides (*8*−*11*). For example, 2-mercaptoinosine 5′-monophosphate, **5** (**Figure 1**), and derivatives thereof were found to be promising, high-impact umami enhancing molecules (*8*, *10*, *11*), among which 2-furfuryl-thioinosine 5′-monophosphate (**6**) was reported as the most active candidate exceeding the umami enhancing activity of **1** by a factor of 17 (*8*). Moreover, various synthetic N^2-alkylated and N^2-acylated guanosine 5′-monophosphates, such as **7** and **8** (**Figure 1**), were recently found to exhibit umami enhancing activity, strongly depending on the chain length as well as the presence of a sulfur atom in the N^2-alkyl and N^2-alkanoyl substituent (*12*).

LC-MS studies on natural umami enhancers in processed foods led to the discovery of N^2-lactoyl-guanosine 5′-monophosphate, **9** (**Figure 1**), in dried bonito (*13*). This lactamide was proposed to be formed upon the reaction of GMP (**2**) with lactic acid and was reported to exhibit pronounced umami enhancing activity (*14*). Very recently, sensory-directed fractionation of commercial yeast extracts revealed (*R*)- and (*S*)-N^2-(1-carboxyethyl)-guanosine 5′-monophosphate, (*R*)-**10** and (*S*)-**10** (**Figure 1**), as previously not reported umami enhancing nucleotides (*15*). Model experiments confirmed the formation of these diastereomers by a Maillard-type glycation of guanosine 5′-monophosphate (**2**) with the reactive C_3-carbohydrates dihydroxyacetone and glyceraldehyde (*15*). Although some advanced glycation end products of the DNA base 2′-deoxyguanosine were identified in the past (*16*−*18*), the Maillard reaction products of the RNA base 5′-GMP (**2**) and, in particular, their putative umami modulating activity have not yet been investigated.

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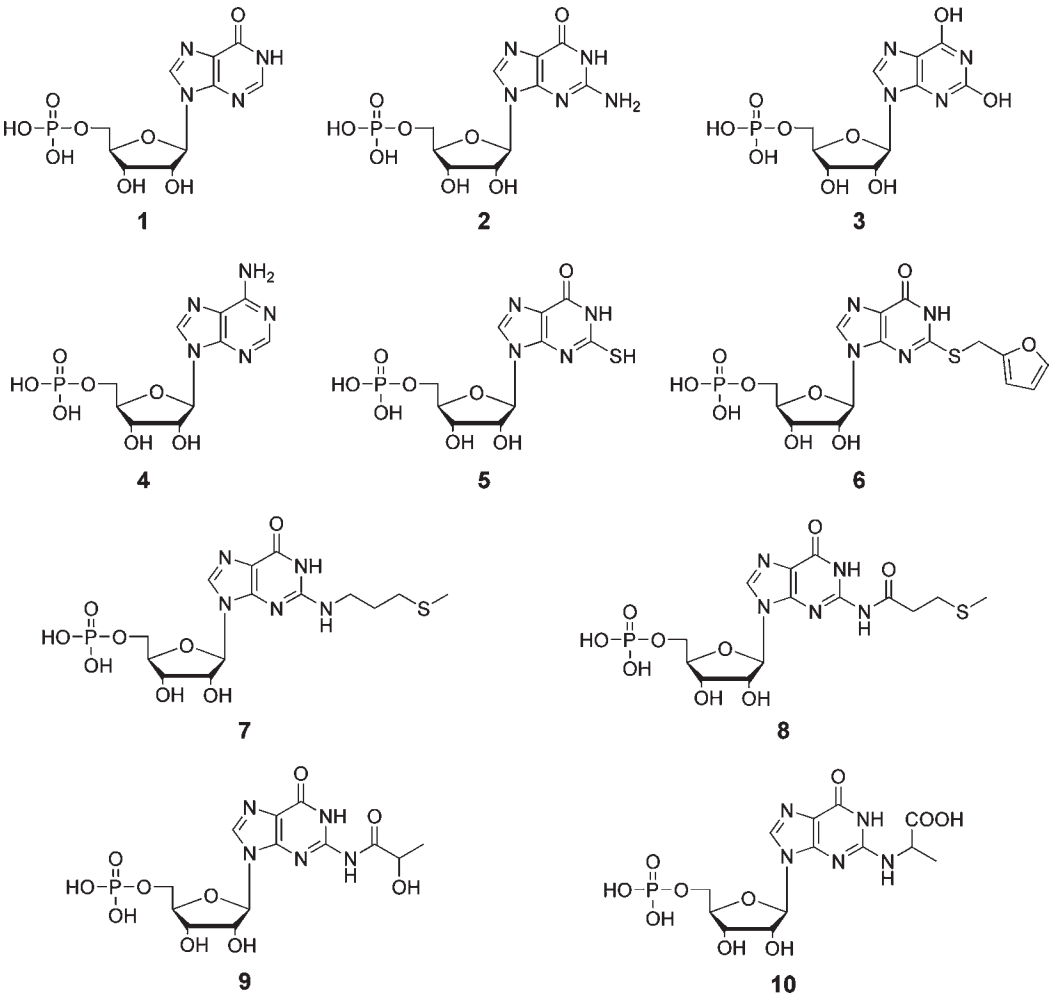

Figure 1. Chemical structures of umami enhancing purine ribonucleotides: inosine 5′-monophosphate (5′-IMP, **1**), guanosine 5′-monophosphate (5′-GMP, **2**), xanthosine 5′-monophosphate (5′-XMP, **3**), adenosine 5′-monophosphate (5′-AMP, **4**), 2-mercaptoinosine 5′-monophosphate (**5**), 2-furfuryl-thioinosine 5′-monophosphate (**6**), N^2-(3-methylthiopropyl)-guanosine 5′-monophosphate (**7**), N^2-(3-methylthiopropanoyl)-guanosine 5′-monophosphate (**8**), N^2-lactoyl-guanosine 5′-monophosphate (**9**), and N^2-(1-carboxyethyl)-guanosine 5′-monophosphate (**10**).

As a series of food-related model experiments led to the discovery of previously not reported taste compounds and taste modulators in processed food (*16*, *19*−*27*), the objective of the present study was to target the Maillard reaction toward the generation of glycation products of 5′-GMP (**2**) by the application of appropriate reaction conditions, to determine their chemical structure, and to evaluate their umami enhancing activity by means of human sensory experiments.

MATERIALS AND METHODS

Chemicals and Materials. Unless stated otherwise, all chemicals were obtained from Sigma-Aldrich (Steinheim, Germany) and were of puriss grade; formic acid (HCOOH), hydrochloric acid, and sodium hydroxide were from Grüssing GmbH (Filsum, Germany), disodium hydrogen phosphate dihydrate, potassium dihydrogen phosphate, and RP-18 bulk material (LiChroprep RP-18, 25−40 μm) were from Merck KGaA (Darmstadt, Germany). Deuterium oxide and sodium deuteroxide (40% w/w solution in D_2O) were obtained from Euriso-Top (Gif-Sur-Yvette, France); HPLC grade solvent acetonitrile (MeCN) was from Mallinckrodt Baker (Griesheim, Germany); and membrane filter discs (0.45 μm) were purchased from Satorius AG (Goettingen, Germany). Water used for chromatography was purified by means of a Milli-Q Advantage A10 water purification system (Millipore, Molsheim, France), and bottled water (Evian) was used for sensory analysis. The reference material of (*R*)- and (*S*)-N^2-(1-carboxyethyl)-guanosine 5′-monophosphate, (*R*)-**10** and (*S*)-**10**, was synthesized following the procedure reported recently (*15*).

Sensory Analyses. *Training of the Sensory Panel*. Thirteen subjects (11 women and 2 men, age 22−30 years), who were given the consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, were trained to evaluate the taste of aqueous solutions (pH 6.0) of sweet, sour, salty, bitter, umami, puckering astringent, and velvety astringent stimuli following the protocol reported recently (*15*). In addition, the panel was trained to differentiate the umami taste quality of aqueous solutions of monosodium L-glutamate (3.0 mmol/L), a binary mixture of monosodium L-glutamate (3.0 mmol/L) and guanosine 5′-monophosphate (0.1 mmol/L), and an aqueous solution of disodium succinate (5 mmol/L). The assessors had participated earlier at regular intervals for at least 18 months in sensory experiments and were, therefore, familiar with the techniques applied. To prevent cross-modal interactions with olfactory inputs, the panelists wore nose clips. Prior to the sensory analysis, the test compounds were confirmed by GC/MS and ion chromatographic analysis to be essentially free of the solvents and buffer compounds used (*15*). In order to minimize the uptake of any compound to the best of our knowledge, all of the sensory analyses were performed by using the sip-and-spit method, which means the test materials were not swallowed but expectorated.

Determination of Taste Enhancing Activity (β-*Values*). The activity of the nucleotide derivatives to synergistically enhance the umami taste of MSG was determined by means of a paired choice comparison test as proposed by Yamaguchi (*7*). To achieve this, a binary solution (pH 6.0) containing the test nucleotide (0.05 mmol/L) and MSG (3 mmol/L) in water (fixed sample) was compared to a series of aqueous solutions containing constant levels of MSG (3 mmol/L) and logarithmically

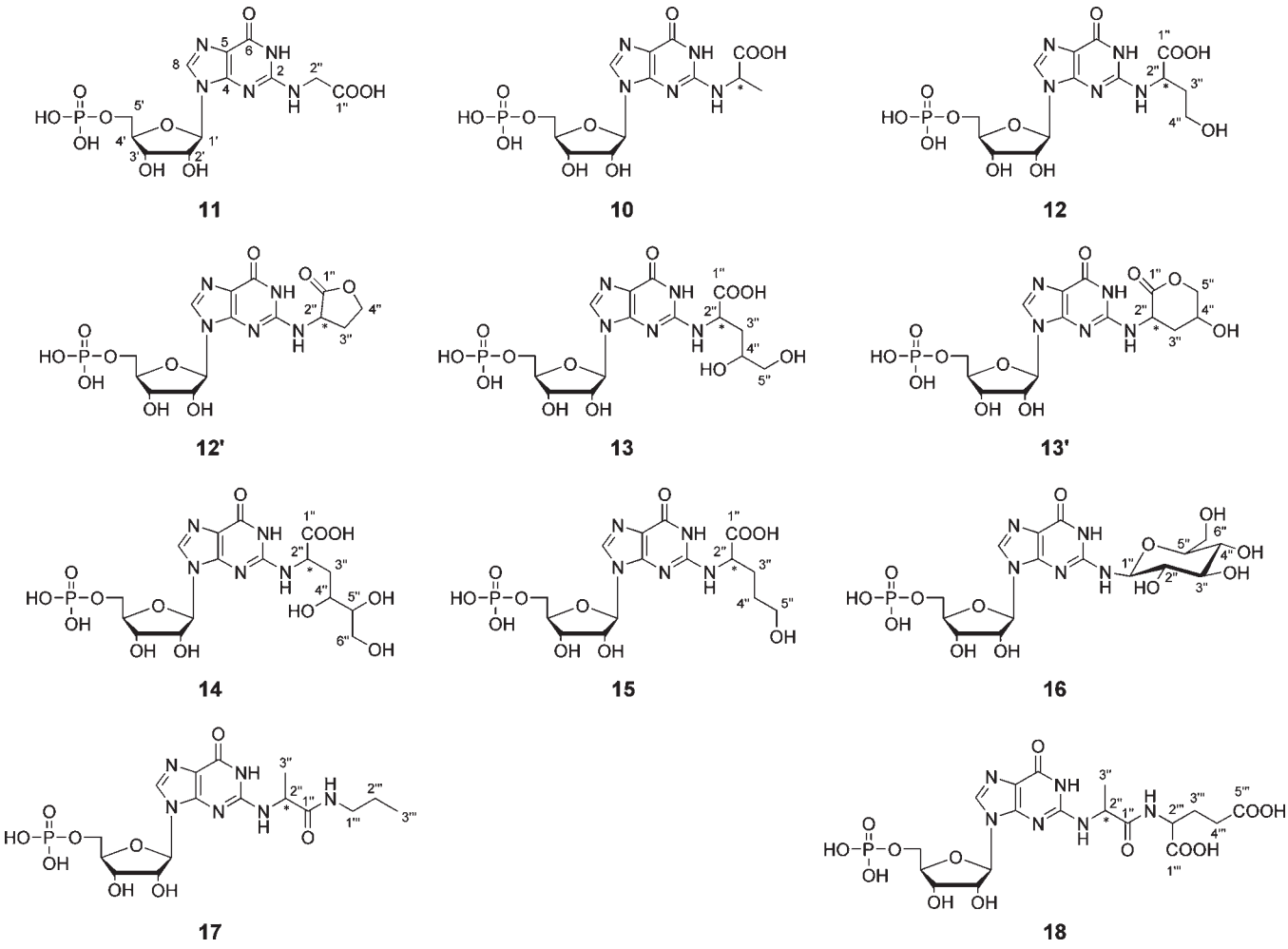

Figure 2. Structures of α-amino acids **11**−**16** isolated from Maillard reaction mixtures containing 5′-GMP (**2**) and reducing carbohydrates or degradation products thereof, and structures of α-amino acid amides **17** and **18** isolated from Maillard reaction mixtures containing 5′-GMP, dihydroxyacetone, and *n*-propylamine, and monosodium L-glutamate, respectively.

(30% intervals) increasing concentrations of inosine 5′-monophosphate (reference samples). In each sensory session, the assessors were asked to evaluate five sample pairs, presented in randomly coded cups, and to identify the sample exhibiting the stronger umami taste using a forced choice methodology. The data obtained were converted into the percentage of positive responses, whereas judgements were considered as positive, if the fixed sample had the stronger umami taste. Applying a probit analysis, the point of equivalent umami taste intensity (50% value) was determined and expressed in concentration of 5′-IMP (**1**). The so-called β-value of each test nucleotide, related to the reference 5′-IMP (**1**), was calculated according to the following equation: $v = \beta \cdot v'$, wherein v represents the 5′-IMP concentration at the point of umami taste equality [mmol/L] and v' the concentration of the test nucleotide [mmol/L].

Reaction of Guanosine 5′-Monophosphate (2) and Glyoxal. Glyoxal (2 mmol; 0.29 mL of a 40% solution in water) was added to a solution of guanosine 5′-monophosphate disodium salt hydrate (1 mmol) in phosphate buffer (10 mL; 1 mol/L, pH 8.0), and the mixture was heated in a sealed vessel for 24 h at 70 °C in a FD 115 lab oven (Binder, Tuttlingen, Germany). After cooling, the solution was diluted with water (10 mL) and filtered through a 0.45 μm membrane filter disk, and the target compound was isolated by means of preparative RP-HPLC with UV/vis detection at 260 nm. The major UV-absorbing peak was collected, solvents were separated in vacuum, followed by repeated lyophilization to yield N^2-carboxymethyl-guanosine 5′-monophosphate (**11**) as an amorphous white powder with a purity of more than 95% (HPLC-UV/vis, ^{1}H NMR).

N^2-Carboxymethyl-guanosine 5′-Monophosphate (***11***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 420 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 420.0562 (found), *m*/*z* 420.0562 (calculated for $[C_{12}H_{15}N_5O_{10}P]^-$). ^{1}H NMR (500 MHz, D_2O/NaOD, COSY): δ [ppm] = 3.96 [s, 2H, H−C(2″)], 3.97−4.06 [m, 2H, H−C(5′)], 4.31 [m, 1H, H−C(4′)], 4.48 [pt, 1H, *J* = 4.7 Hz, H−C(3′)], 4.77 [1H, overlapped by solvent signal, H−C(2′)], 6.01 [d, 1H, *J* = 5.4 Hz, H−C(1′)], 8.17 [s, 1H, H−C(8)]. ^{13}C NMR (125 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 47.8 [C(2″)], 66.6 [d, $^2J_{C,P}$ = 4.5 Hz, C(5′)], 73.3 [C(3′)], 76.8 [C(2′)], 87.0 [d, $^3J_{C,P}$ = 8.5 Hz, C(4′)], 90.4 [C(1′)], 118.9 [C(5)], 140.8 [C(8)], 154.8 [C(4)], 155.6 [C(2)], 162.1 [C(6)], 180.5 [C(1″)].

Reaction of Guanosine 5′-Monophosphate (2) and Erythrose. A mixture of guanosine 5′-monophosphate disodium salt hydrate (1 mmol) and erythrose (3 mmol) in phosphate buffer (2.5 mL, 1 mol/L, pH 7.0) was heated at 70 °C for 6 h in a closed pyrex tube. After cooling, we diluted the mixture with water (10 mL) and separated it by means of preparative RP-HPLC. Monitoring the effluent at 260 nm, the two major reaction products were collected and separated from solvents in vacuum, followed by lyophilization, to afford (*R*)- and (*S*)-N^2-(1-carboxy-3-hydroxypropyl)-guanosine 5′-monophosphate, (*R*)-**12** and (*S*)-**12** (**Figure 2**), with a purity of more than 95% (HPLC-UV/vis, ^{1}H NMR). Assignment of the stereochemistry at C(2″) was achieved by comparison of the elution order on RP-18 material as recently demonstrated for (*R*)- and (*S*)-**10** (*15*). (*R*)-**12** and (*S*)-**12** were found to be in a pH-dependent equilibrium with their corresponding lactones (*R*)-**12′** and (*S*)-**12′**.

(R)-N^2-(1-Carboxy-3-hydroxypropyl)-guanosine 5′-Monophosphate ((*R*)-***12***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 464 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 464.0828 (found), *m*/*z* 464.0824 (calculated for $[C_{14}H_{19}N_5O_{11}P]^-$). ^{1}H NMR (400 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.90 [m, 1H, H−C(3″$_a$)], 2.08 [m, 1H, H−C(3″$_b$)], 3.66 [m, 2H, H−C(4″)], 4.09−3.87 (m, 2H, H−C(5′)], 4.23 [m, 1H, H−C(4′)], 4.29 [dd, 1H, *J* = 4.5, 8.9 Hz,

H−C(2″)], 4.37 [pt, 1H, J = 4.9 Hz, H−C(3′)], 4.61 [pt, 1H, J = 5.2 Hz, H−C(2′)], 5.89 [d, 1H, J = 5.2 Hz, H−C(1′)], 8.07 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 32.8 [C(3″)], 52.1 [C(2″)], 58.0 [C(4″)], 63.5 [d, $^2J_{C,P}$ = 4.7 Hz, C(5′)], 69.0 [C(3′)], 74.0 [C(2′)], 83.4 [d, $^3J_{C,P}$ = 8.3 Hz, C(4′)], 89.7 [C(1′)], 109.6 [C(5)], 136.1 [C(8)], 149.2 [C(4)], 153.5 [C(2)], 163.6 [C(6)], 176.1 [C(1″)].

(*R*)-N^2-(*Tetrahydro-2-oxofuran-3-yl*)-guanosine 5′-Monophosphate ((*R*)-***12′***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): m/z (%) 446 (100) [M − H]$^-$. LC-TOF-MS: m/z 446.0727 (found), m/z 446.0719 (calculated for $[C_{14}H_{17}N_5O_{10}P]^-$). ^{1}H NMR (400 MHz, D_2O, COSY): TOF-MS. m/z 464.0828 (found), m/z 464.0824 (calculated for $[C_{14}H_{19}N_5O_{11}P]^-$); δ [ppm] = 2.55−2.74 [m, 2H, H−C(3″)], 4.08−4.16 [m, 1H, H−C(5′$_a$)], 4.20−4.28 (m, 1H, H−C(5′$_b$)], 4.35 [m, 1H, H−C(4′)], 4.43 [pt, 1H, J = 5.5 Hz, H−C(3′)], 4.49 [dd, 1H, J = 7.5, 9.7 Hz, H−C(4″$_a$)], 4.68 [m, 1H, H−C(2′)], 4.63 [dd, 1H, J = 2.9, 4.6 Hz, H−C(4″$_b$)], 4.88 [t, 1H, J = 10.2 Hz, H−C(2″)], 6.05 [d, 1H, J = 2.8 Hz, H−C(1′)], 8.92 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O, HMQC, HMBC): δ [ppm] = 26.1 [C(3″)], 50.7 [C(2″)], 63.3 [d, C(5′)], 74.0 [C(4″)], 74.1 [C(2′)], 83.2 [d, C(4′)], 89.5 [C(1′)], 109.6 [C(5)], 135.5 [C(8)], 148.5 [C(4)], 153.4 [C(2)], 160.4 [C(6)], 179.0 [C(1″)].

(*S*)-N^2-(*1-Carboxy-3-hydroxypropyl*)-guanosine 5′-Monophosphate ((*S*)-***12***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): m/z (%) 464 (100) [M − H]$^-$. LC-TOF-MS: m/z 464.0840 (found), m/z 464.0824 (calculated for $[C_{14}H_{19}N_5O_{11}P]^-$). ^{1}H NMR (400 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.86 [m, 1H, H−C(3″$_a$)], 2.06 [m, 1H, H−C(3″$_b$)], 3.65 [m, 2H, H−C(4″)], 4.02−3.78 (m, 2H, H−C(5′)], 4.22 [m, 1H, H−C(4′)], 4.27 [dd, 1H, J = 4.6, 9.0 Hz, H−C(2″)], 4.37 [pt, 1H, J = 4.9 Hz, H−C(3′)], 4.61 [pt, 1H, J = 5.2 Hz, H−C(2′)], 5.89 [d, 1H, J = 5.2 Hz, H−C(1′)], 8.07 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 32.8 [C(3″)], 52.1 [C(2″)], 58.2 [C(4″)], 63.5 [d, $^2J_{C,P}$ = 4.6 Hz, C(5′)], 69.0 [C(3′)], 74.0 [C(2′)], 83.5 [d, $^3J_{C,P}$ = 8.2 Hz, C(4′)], 89.8 [C(1′)], 109.7 [C(5)], 136.1 [C(8)], 149.4 [C(4)], 153.5 [C(2)], 163.6 [C(6)], 176.6 [C(1″)].

(*S*)-N^2-(*Tetrahydro-2-oxofuran-3-yl*)-guanosine 5′-Monophosphate ((***S*)-12′**, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): m/z (%) 446 (100) [M − H]$^-$. ^{1}H NMR (400 MHz, D_2O, COSY): δ [ppm] = 2.56−2.74 [m, 2H, H−C(3″)], 4.04−4.15 [m, 1H, H−C(5′$_a$)], 4.17−4.25 (m, 1H, H−C(5′$_b$)], 4.34 [m, 1H, H−C(4′)], 4.45 [pt, 1H, J = 5.4 Hz, H−C(3′)], 4.49 [dd, 1H, J = 7.1, 10.2 Hz, H−C(4″$_a$)], 4.68 [pt, 1H, J = 4.7 Hz, H−C(2′)], 4.71 [dd, 1H, J = 7.6, 9.3 Hz, H−C(4″$_b$)], 4.90 [t, 1H, J = 10.2 Hz, H−C(2″)], 5.99 [d, 1H, J = 4.1 Hz, H−C(1′)], 8.47 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O, HMQC, HMBC): δ [ppm] = 29.8 [C(3″)], 53.8 [C(2″)], 66.9 [d, C(5′)], 70.6 [C(4″)], 72.6 [C(3′)], 77.6 [C(2′)], 86.3 [d, $^3J_{C,P}$ = 8.6 Hz, C(4′)], 91.3 [C(1′)], 116.9 [C(5)], 139.9 [C(8)], 153.0 [C(4)], 154.8 [C(2)], 160.4 [C(6)], 182.1 [C(1″)].

Reaction of Guanosine 5′-Monophosphate (2) with Ribose, Glucose, and Maltose, Respectively, in the Presence of an Amino Acid. A ternary mixture of guanosine 5′-monophosphate disodium salt hydrate (1 mmol), L-alanine (1 mmol), and ribose (5 mmol), glucose (5 mmol), or maltose (5 mmol) in phosphate buffer (2.5 mL for ribose/glucose, 5.0 mL for maltose; 1 mol/L, pH 7.0) was heated at 70 °C in a pyrex tube for 5 days (ribose), 11 days (glucose), and 14 days (maltose), respectively. After cooling, the crude reaction mixtures were diluted with water (10 mL), filtered through a 0.45 μm membrane filter disk, and separated by means of preparative RP-HPLC. Monitoring the effluent at 260 nm, the major reaction products were collected and separated from solvents in vacuum, followed by lyophilization to afford (*R*/*S*)-N^2-(1-carboxy-3,4-dihydroxybutyl)-guanosine 5′-monophosphate, (*R*)-**13** and (*S*)-**13**, from the ribose reaction, (*R*/*S*)-N^2-(1-carboxy-3,4,5-trihydroxypentyl)-guanosine-5′-monophosphate, (*R*)-**14** and (*S*)-**14**, from the glucose reaction, and (*R*/*S*)-N^2-(1-carboxy-4-hydroxybutyl)-guanosine 5′-monophosphate, (*R*)-**15** and (*S*)-**15**, from the maltose-containing reaction system (**Figure 2**). The C(2″)-stereochemistry of these compounds was proposed by comparison of the elution order on RP-18 material as recently demonstrated for (*R*)- and (*S*)-**10** (*15*). As shown for (*R*)-**12** and (*S*)-**12**, (*R*)-**13** and (*S*)-**13** were found to be in a pH-dependent equilibrium with their corresponding lactones (*R*)-**13′** and (*S*)-**13′**.

N^2-(*1-Carboxy-3,4-dihydroxybutyl*)-guanosine 5′-Monophosphate ((*R*)-***13***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): m/z (%) 494 (100) [M − H]$^-$. LC-TOF-MS m/z 494.0938 (found), m/z 494.0930 (calculated for $[C_{15}H_{21}N_5O_{12}P]^-$). ^{1}H NMR (400 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.86 [m, 1H, H−C(3″$_a$)], 2.08 [m, 1H, H−C(3″$_b$)], 3.54 [dd, 1H, J = 6.5, 11.8 Hz, H−C(5″$_a$)], 3.65 [dd, 1H, J = 3.8, 11.8 Hz, H−C(5″$_b$)], 3.91 [m, 1H, H−C(4″)], 3.97−4.10 [m, 2H, H−C(5′)], 4.23−4.40 [m, 2H, H−C(2″), H−C(4′)], 4.46 [pt, 1H, J = 4.7 Hz, 1H, H−C(3′)], 4.83 [pt, 1H, J = 5.3 Hz, H−C(2′)], 5.96 [d, 1H, J = 5.4 Hz, H−C(1′)], 8.10 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 38.0 [C(3″)], 58.0 [C(2″)], 66.9 [d, $^2J_{C,P}$ = 4.6 Hz, C(5′)], 68.0 [C(5″)], 72.3 [C(4″)], 73.2 [C(3′)], 76.3 [C(2′)], 86.8 [d, $^3J_{C,P}$ = 8.5 Hz, C(4′)], 90.6 [C(1′)], 119.2 [C(5)], 141.3 [C(8)], 154.5 [C(4)], 155.0 [C(2)], 162.0 [C(6)], 182.6 [C(1″)].

N^2-(*Tetrahydro-5-hydroxy-2-oxo-2H-pyran-3-ylamino*)-guanosine 5′-Monophosphate ((*R*)-***13′***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): m/z (%) 476 (100) [M − H]$^-$. LC-TOF-MS m/z 476.0842 (found), m/z 476.0824 (calculated for $[C_{15}H_{19}N_5O_{11}P]^-$). ^{1}H NMR (500 MHz, D_2O, COSY): δ [ppm] = 2.53 [m, 1H, H−C(3″$_a$)], 2.69 [m, 1H, H−C(3″$_b$)], 3.75 [dd, 1H, J = 4.3, 12.8 Hz, H−C(5″$_a$)], 3.92 [dd, 1H, J = 2.5, 12.8 Hz, H−C(5″$_b$)], 4.06−4.12 [m, 1H, H−C(5′$_a$)], 4.12−4.19 [m, 1H, H−C(5′$_b$)], 4.32 [m, 1H, H−C(4′)], 4.44 [pt, 1H, J = 4.9 Hz, 1H, H−C(3′)], 4.72 [pt, 1H, J = 5.1 Hz, H−C(2′)], 4.81 [dd, 1H, J = 7.0, 14.0 Hz, H−C(2″)], 5.03 [m, 1H, H−C(4″)], 5.94 [d, 1H, J = 5.0 Hz, H−C(1′)], 8.16 [s, 1H, H−C(8)]. ^{13}C NMR (125 MHz, D_2O, HMQC, HMBC): δ [ppm] = 31.2 [C(3″)], 54.0 [C(2″)], 66.2 [C(5″)], 67.3 [d, $^2J_{C,P}$ = 4.9 Hz, C(5′)], 73.0 [C(3′)], 76.6 [C(2′)], 83.4 [H−C(4″)], 86.3 [d, $^3J_{C,P}$ = 8.6 Hz, C(4′)], 90.5 [C(1′)], 119.2 [C(5)], 140.5 [C(8)], 153.8 [C(4)], 154.0 [C(2)], 161.9 [C(6)], 182.3 [C(1″)].

N^2-(*1-Carboxy-3,4-dihydroxybutyl*)-guanosine 5′-Monophosphate ((*S*)-***13***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): m/z (%) 494 (100) [M − H]$^-$. LC-TOF-MS: m/z 494.0927 (found), m/z 494.0930 (calculated for $[C_{15}H_{21}N_5O_{12}P]^-$). ^{1}H NMR (400 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.74−1.90 [m, 2H, H−C(3″)], 3.46 [dd, 1H, J = 6.7, 11.7 Hz, H−C(5″$_a$)], 3.55 [dd, 1H, J = 4.0, 11.7 Hz, H−C(5″$_b$)], 3.79 [m, 1H, H−C(4″)], 3.83−3.97 [m, 2H, H−C(5′)], 4.23 [m, 1H, H−C(4′)], 4.38 [pt, 1H, J = 4.7 Hz, H−C(3′)], 4.41 [dd, 1H, J = 4.1, 9.5 Hz, H−C(2″)], 4.63 [pt, 1H, J = 5.2 Hz, H−C(2′)], 5.93 [d, 1H, J = 5.2 Hz, H−C(1′)], 8.14 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 35.0 [C(3″)], 54.0 [C(2″)], 63.5 [d, $^2J_{C,P}$ = 4.3 Hz, C(5′)], 65.5 [C(5″)], 68.9 [C(4″)], 70.4 [C(3′)], 74.3 [C(2′)], 84.1 [d, $^3J_{C,P}$ = 8.5 Hz, C(4′)], 86.8 [C(1′)], 115.9 [C(5)], 137.5 [C(8)], 151.8 [C(4)], 152.5 [C(2)], 159.2 [C(6)], 179.9 [C(1″)].

N^2-(*Tetrahydro-5-hydroxy-2-oxo-2H-pyran-3-ylamino*)-guanosine 5′-Monophosphate ((*S*)-***13′***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): m/z (%) 476 (100) [M − H]$^-$. LC-TOF-MS: m/z 476.0823 (found), m/z 476.0824 (calculated for $[C_{15}H_{19}N_5O_{11}P]^-$). ^{1}H NMR (500 MHz, D_2O, COSY): δ [ppm] = 2.36 [m, 1H, H−C(3″$_a$)], 2.71 [m, 1H, H−C(3″$_b$)], 3.84 [dd, 1H, J = 6.2, 13.0 Hz, H−C(5″$_a$)], 3.95 [dd, 1H, J = 2.7, 13.0 Hz, H−C(5″$_b$)], 4.05−4.19 [m, 2H, H−C(5′$_{a,b}$)], 4.35 [m, 1H, H−C(4′)], 4.48 [dd, 1H, J = 4.3, 4.8 Hz, 1H, H−C(3′)], 4.68 [pt, 1H, J = 5.3 Hz, H−C(2′)], 4.78 [1H, overlapped by solvent signal, H−C(4″)], 5.07 [dd, 1H, J = 9.1, 11.6 Hz, H−C(2″)], 6.02 [d, 1H, J = 5.5 Hz, H−C(1′)], 8.29 [s, 1H, H−C(8)]. ^{13}C NMR (125 MHz, D_2O, HMQC, HMBC): δ [ppm] = 31.7 [C(3″)], 54.7 [C(2″)], 65.6 [C(5″)], 67.3 [d, $^2J_{C,P}$ = 5.0 Hz, C(5′)], 73.3 [C(3′)], 77.4 [C(2′)], 82.7 [C(4″)], 86.7 [d, $^3J_{C,P}$ = 8.9 Hz, C(4′)], 90.0 [C(1′)], 118.2 [C(5)], 140.1 [C(8)], 153.8 [C(4)], 154.7 [C(2)], 161.4 [C(6)], 181.1 [C(1″)].

N^2-(*1-Carboxy-3,4,5-trihydroxypentyl*)-guanosine 5′-Monophosphate ((*R*)-***14***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): m/z (%) 524 (100) [M − H]$^-$. LC-TOF-MS: m/z 524.1028 (found), m/z 524.1035 (calculated for $[C_{16}H_{23}N_5O_{13}P]^-$). ^{1}H NMR (500 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.80−1.93 [m, 1H, H−C(3″$_a$)], 2.18 [ddd, 1H, J = 3.6, 6.3, 14.4 Hz, H−C(3″$_b$)], 3.56−3.63 [m, 1H, H−C(6′$_a$)], 3.63−3.71 [m, 1H, H−C(5″)], 3.72−3.81 [m, 1H, H−C(6′$_b$)], 3.82−3.89 [m, 1H, H−C(4″)], 3.89−4.03 [m, 2H, H−C(5′)], 4.23 [m, 1H, H−C(4′)], 4.29 [m, 1H, H−C(3′)], 4.38 [t, 1H, J = 6.8 Hz, H−C(2″)], 4.69 [pt, 1H, J = 5.6 Hz, H−C(2′)], 5.86 [d, 1H, J = 5.9 Hz, H−C(1′)], 8.03 [s, 1H, H−C(8)]. ^{13}C NMR (125 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 38.7 [C(3″)], 58.2 [C(2″)], 65.3 [C(6″)], 67.1 [d, $^2J_{C,P}$ = 5.0 Hz, C(5′)], 73.1 [C(4″)], 74.3 [C(3′)], 77.4 [C(5″)], 77.7 [C(2′)], 87.2 [d, $^3J_{C,P}$ = 8.7 Hz, C(4′)], 90.3 [C(1′)], 120.2 [C(5)], 138.8 [C(8)], 155.0 [C(4)], 163.2 [C(2)], 171.0 [C(6)], 184.2 [C(1″)].

N^2-(1-Carboxy-3,4,5-trihydroxypentyl)-guanosine 5′-Monophosphate ((*S*)-**14**, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 524 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 524.1028 (found), *m*/*z* 524.1035 (calculated for $[C_{16}H_{23}N_5O_{13}P]^-$). ^{1}H NMR (500 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.81−1.93 [m, 1H, H−C(3″$_a$)], 1.93−2.03 [m, 1H, H−C(3″$_b$)], 3.56−3.63 [m, 1H, H−C(6′$_a$)], 3.63−3.71 [m, 1H, H−C(5″)], 3.72−3.81 [m, 2H, H−C(4″), H−C(6′$_b$)], 3.89−4.03 [m, 2H, H−C(5′)], 4.23 [m, 1H, H−C(4′)], 4.29 [m, 1H, H−C(3′)], 4.50 [dd, 1H, *J* = 3.5, 10.9 Hz, H−C(2″)], 4.66 [pt, 1H, *J* = 5.6 Hz, H−C(2′)], 5.82 [d, 1H, *J* = 6.0 Hz, H−C(1′)], 8.07 [s, 1H, H−C(8)]. ^{13}C NMR (125 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 39.5 [C(3″)], 56.7 [C(2″)], 65.6 [C(6″)], 67.1 [d, $^2J_{C,P}$ = 4.5 Hz, C(5′)], 71.5 [C(4″)], 74.4 [C(3′)], 77.5 [C(5″)], 77.9 [C(2′)], 87.3 [d, $^3J_{C,P}$ = 8.7 Hz, C(4′)], 89.9 [C(1′)], 120.0 [C(5)], 138.5 [C(8)], 155.0 [C(4)], 163.7 [C(2)], 171.0 [C(6)], 184.2 [C(1″)].

N^2-(1-Carboxy-4-hydroxybutyl)-guanosine 5′-Monophosphate ((*R*)-***15***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 478 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 478.0974 (found), *m*/*z* 478.0981 (calculated for $[C_{15}H_{21}N_5O_{11}P]^-$). ^{1}H NMR (400 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.68 [m, 2H, H−C(4″)], 1.83 [m, 1H, H−C(3″$_a$)], 1.97 [m, 1H, H−C(3″$_b$)], 3.65 [t, 2H, *J* = 6.5 Hz, H−C(5″)], 3.93−4.06 [m, 1H, H−C(5′$_a$)], 4.06−4.12 [m, 1H, H−C(5′$_b$)], 4.28−4.34 [m, 2H, H−C(2″), H−C(4′)], 4.48 [pt, 1H, *J* = 4.8 Hz, 1H, H−C(3′)], 4.84 [pt, 1H, *J* = 5.3 Hz, H−C(2′)], 5.98 [d, 1H, *J* = 5.3 Hz, H−C(1′)], 8.11 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O/NaOD, HSQC, HMBC): δ [ppm] = 30.7 [C(4″)], 31.0 [C(3″)], 59.4 [C(2″)], 64.2 [C(5″)], 67.0 [d, $^2J_{C,P}$ = 4.6 Hz, C(5′)], 73.2 [C(3′)], 76.3 [C(2′)], 86.7 [d, $^3J_{C,P}$ = 8.3 Hz, C(4′)], 90.5 [C(1′)], 119.1 [C(5)], 141.1 [C(8)], 154.6 [C(4)], 155.0 [C(2)], 162.0 [C(6)], 182.8 [C(1″)].

N^2-(1-Carboxy-4-hydroxybutyl)-guanosine 5′-Monophosphate ((*S*)-***15***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 478 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 478.0981 (found), *m*/*z* 478.0981 (calculated for $[C_{15}H_{21}N_5O_{11}P]^-$). ^{1}H NMR (400 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.67 [m, 2H, H−C(4″)], 1.82 [m, 1H, H−C(3″$_a$)], 1.96 [m, 1H, H−C(3″$_b$)], 3.65 [t, 2H, *J* = 6.5 Hz, H−C(5″)], 3.97−4.10 [m, 2H, H−C(5′)], 4.32 [m, 2H, H−C(2″), H−C(4′)], 4.47 [pt, 1H, *J* = 4.8 Hz, 1H, H−C(3′)], 4.72 [pt, 1H, *J* = 5.2 Hz, H−C(2′)], 6.03 [d, 1H, *J* = 5.2 Hz, H−C(1′)], 8.18 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O/NaOD, HSQC, HMBC): δ [ppm] = 30.8 [C(4″)], 30.9 [C(3″)], 59.4 [C(2″)], 64.2 [C(5″)], 66.7 [d, $^2J_{C,P}$ = 4.5 Hz, C(5′)], 73.1 [C(3′)], 77.1 [C(2′)], 86.7 [d, $^3J_{C,P}$ = 8.5 Hz, C(4′)], 89.9 [C(1′)], 118.7 [C(5)], 140.4 [C(8)], 154.8 [C(4)], 155.2 [C(2)], 162.0 [C(6)], 182.3 [C(1″)].

Reaction of Guanosine 5′-Monophosphate (2) with Glucose in the Absence of an Amino Acid. A binary mixture of guanosine 5′-monophosphate disodium salt hydrate (1 mmol) and glucose (10 mmol) in phosphate buffer (1.25 mL, 1 mol/L, pH 7.0) was heated in a sealed vessel for 4 h at 100 °C. After cooling, we diluted the crude reaction mixture with water (10 mL) and separated it by means of RP-MPLC. A highly polar fraction, strongly absorbing at 260 nm, was collected and further purified by rechromatography by means of preparative RP-HPLC to remove residues of unreacted glucose. The major reaction product was collected, lyophilized twice, and analyzed by LC-MS and 1D/2D-NMR experiments to give *N^2*-(β-D-glucosyl)-guanosine 5′-monophosphate (**16**) as an amorphous white powder at a purity of more than 96% (HPLC-UV/vis, ^{1}H NMR).

N^2-(β-D-Glucosyl)-guanosine 5′-Monophosphate (***16***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 524 (100) [M − H]. LC-TOF-MS: *m*/*z* 524.1036 (found), *m*/*z* 524.1035 (calculated for $[C_{16}H_{23}N_5O_{13}P]^-$). ^{1}H NMR (500 MHz, D_2O/NaOD, COSY): δ [ppm] = 3.47 [t, 1H, *J* = 9.5 Hz, H−C(4″)], 3.50 [t, 1H, *J* = 9.2 Hz, H−C(2″)], 3.62−3.70 [m, 2H, H−C(3″), H−C(5″)], 3.72 [dd, 1H, *J* = 5.4, 12.5 Hz, H−C(6″$_a$)], 3.89 [dd, 1H, *J* = 2.2, 12.5 Hz, H−C(6″$_b$)], 3.96−4.07 [m, 2H, H−C(5′)], 4.31 [m, 1H, H−C(4′)], 4.48 [pt, 1H, *J* = 4.5 Hz, H−C(3′)], 4.84 [pt, 1H, *J* = 5.5 Hz, H−C(2′)], 5.31 [d, 1H, *J* = 9.1 Hz, H−C(1″)], 6.03 [d, 1H, *J* = 5.5 Hz, H−C(1′)], 8.22 [s, 1H, H−C(8)]. ^{13}C NMR (125 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 63.5 [C(6″)], 66.7 [d, $^2J_{C,P}$ = 4.5 Hz, C(5′)], 72.3 [C(4″)], 73.6 [C(3′)], 74.9 [C(2″)], 76.6 [C(2′)], 79.4 [C(3″)], 80.2 [C(5″)], 84.3 [C(1″)], 87.2 [d, $^3J_{C,P}$ = 8.1 Hz, C(4′)], 89.9 [C(1′)], 120.1 [C(5)], 141.2 [C(8)], 154.1 [C(4)], 155.0 [C(2)], 162.0 [C(6)].

Reaction of Guanosine 5′-Monophosphate (2) with Dihydroxyacetone and Excessive Amounts of an Amino Compound. Following a literature procedure (*28*) with some modifications, a mixture of guanosine 5′-monophosphate disodium salt hydrate (1 mmol) and dihydroxyacetone dimer (2 mmol) in phosphate buffer (1 mL, 1 mol/L, pH 7.0) was heated in a sealed vessel for 30 min at 100 °C. Thereafter, *n*-propylamine (8 mmol) and monosodium L-glutamate monohydrate (8 mmol), respectively, dissolved in phosphate buffer (2.5 mL, 1M, pH 7.4), was added, and the mixture was heated for an additional 4 h. After cooling, we diluted the reaction mixture with water (10 mL) and, after membrane filtration, preseparated it by means of RP-MPLC. The major reaction products, strongly absorbing at 260 nm, were collected and further purified by rechromatography by means of preparative RP-HPLC to give, besides (*R*)-**10** and (*S*)-**10**, the amides (*R*)- and (*S*)-*N^2*-((1-(*N*-propylamino)-carbonyl)-ethyl)-guanosine 5′-monophosphate, (*R*)-**17** and (*S*)-**17**, as the main products in the reaction system of *n*-propylamine and (*R*)- and (*S*)-*N^2*-((1-(*N*-(1′,3′-dicarboxypropylamino)-carbonyl)-ethyl)-guanosine 5′-monophosphate, (*R*)-**18** and (*S*)-**18**, as the main products in the reaction system of L-glutamate, each at a purity of more than 95% (HPLC-UV/vis, ^{1}H NMR).

(*R*)-*N^2*-((*1-(N-Propylamino)-carbonyl)-ethyl)-guanosine 5′-Monophosphate* ((*R*)-***17***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 475 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 475.1381 (found), *m*/*z* 475.1348 (calculated for $[C_{16}H_{24}N_6O_9P]^-$). ^{1}H NMR (400 MHz, CD_3OD/NaOD, COSY): δ [ppm] = 0.82 [t, 3H, *J* = 7.4 Hz, H−C(3‴), 1.39 [d, 3H, *J* = 7.1 Hz, H−C(3″)], 1.48 [sext., *J* = 7.4 Hz, 2H, H−C(2‴)], 3.13 [m, 2H, H−C(1‴)], 3.97−4.05 [m, 1H, H−C(5′$_a$)], 4.05−4.13 [m, 1H, H−C(5′$_b$)], 4.15 [m, 1H, H−C(4′)], 4.38−4.45 [m, 2H, H−C(3′), H−C(2″)], 4.58 [pt, 1H, *J* = 4.7 Hz, H−C(2′)], 5.87 [d, 1H, *J* = 4.3 Hz, H−C(1′)], 8.08 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, CD_3OD/NaOD, HMQC, HMBC, DEPT): δ [ppm] = 11.6 [C(3‴)], 18.9 [C(3″)], 23.6 [C(2‴)], 42.1 [C(1‴)], 52.9 [C(2″)], 65.0 [d, $^2J_{C,P}$ = 4.2 Hz, C(5′)], 72.1 [C(3′)], 76.5 [C(2′)], 85.5 [d, $^3J_{C,P}$ = 8.3 Hz, C(4′)], 89.9 [C(1′)], 119.6 [C(5)], 137.2 [C(8)], 152.9 [C(4)], 161.1 [C(2)], 169.7 [C(6)], 178.0 [C(1″)].

(*S*)-*N^2*-((*1-(N-Propylamino)-carbonyl)-ethyl)-guanosine 5′-Monophosphate* ((*S*)-***17***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 475 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 475.1372 (found), *m*/*z* 475.1348 (calculated for $[C_{16}H_{24}N_6O_9P]^-$). ^{1}H NMR (400 MHz, CD_3OD/NaOD, COSY): δ [ppm] = 0.83 [t, 3H, *J* = 7.4 Hz, H−C(3‴), 1.39 [d, 3H, *J* = 7.1 Hz, H−C(3″)], 1.48 [sext., *J* = 7.4 Hz, 2H, H−C(2‴)], 3.14 [m, 2H, H−C(1‴)], 3.96−4.04 [m, 1H, H−C(5′$_a$)], 4.05−4.12 [m, 1H, H−C(5′$_b$)], 4.17 [m, 1H, H−C(4′)], 4.38 [pt, 2H, H−C(3′)], 4.50 [q, 1H, *J* = 7.1 Hz, H−C(2″)], 4.54 [pt, 1H, *J* = 4.8 Hz, H−C(2′)], 5.88 [d, 1H, *J* = 4.3 Hz, H−C(1′)], 8.09 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, CD_3OD/NaOD, HMQC, HMBC, DEPT): δ [ppm] = 11.6 [C(3‴)], 19.0 [C(3″)], 23.6 [C(2‴)], 42.0 [C(1‴)], 52.7 [C(2″)], 65.1 [d, $^2J_{C,P}$ = 4.3 Hz, C(5′)], 72.2 [C(3′)], 76.9 [C(2′)], 85.7 [d, $^3J_{C,P}$ = 8.0 Hz, C(4′)], 89.8 [C(1′)], 119.5 [C(5)], 136.9 [C(8)], 153.0 [C(4)], 161.1 [C(2)], 169.8 [C(6)], 177.8 [C(1″)].

(*R*)-*N^2*-((*1-(N-(1′,3′-Dicarboxypropylamino)-carbonyl)-ethyl)-guanosine 5′-Monophosphate* ((*R*)-***18***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 563 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 563.1159 (found), *m*/*z* 563.1144 (calculated for $[C_{18}H_{24}N_6O_{13}P]^-$). ^{1}H NMR (500 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.30 [d, 3H, *J* = 7.2 Hz, H−C(3″)], 1.60−1.70 [m, 1H, H−C(3‴$_a$)], 1.76−1.92 [m, 3H, H−C(3‴$_b$), H−C(4‴)], 3.79−3.75 [m, 1H, H−C(5′$_a$)], 3.80−3.86 [m, 1H, H−C(5′$_b$)], 3.97 [dd, 1H, *J* = 5.1, 8.3 Hz, H−C(2‴)], 4.10 [m, 1H, H−C(4′)], 4.19−4.26 [m, 2H, H−C(2″), H−C(3′)], 4.61 [pt, 1H, *J* = 5.4 Hz, H−C(2′)], 5.73 [d, 1H, *J* = 5.4 Hz, H−C(1′)], 7.90 [s, 1H, H−C(8)]. ^{13}C NMR (125 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 17.1 [C(3″)], 28.4 [C(3‴)], 33.5 [C(4‴)], 51.6 [C(2″)], 54.9 [C(2‴)], 64.0 [d, $^2J_{C,P}$ = 4.5 Hz, C(5′)], 70.3 [C(3′)], 73.1 [C(2′)], 83.9 [d, $^3J_{C,P}$ = 8.6 Hz, C(4′)], 87.8 [C(1′)], 116.6 [C(5)], 138.6 [C(8)], 151.1 [C(4)], 151.5 [C(2)], 158.9 [C(6)], 175.3 [C(1″)], 178.2 [C(1‴)], 181.7 [C(5‴)].

(*S*)-*N^2*-((*1-(N-(1′,3′-Dicarboxypropylamino)-carbonyl)-ethyl)-guanosine 5′-Monophosphate* ((*S*)-***18***, ***Figure 2***). UV/vis (1% aq. HCOOH/MeCN): λ_{max} = 256, 280 nm (sh). LC/MS (ESI$^-$): *m*/*z* (%) 563 (100) [M − H]$^-$. LC-TOF-MS: *m*/*z* 563.1145 (found), *m*/*z* 563.1144 (calculated for $[C_{18}H_{24}N_6O_{13}P]^-$). ^{1}H NMR (400 MHz, D_2O/NaOD, COSY): δ [ppm] = 1.38 [d, 3H, *J* = 7.2 Hz, H−C(3″)], 1.74−1.87 [m, 1H, H−C(3‴$_a$)], 1.88−1.98 [m, 1H, H−C(3‴$_b$)], 2.04−2.13 [m, 2H, H−C(4‴)], 3.83−3.96 [m, 2H, H−C(5′)], 4.01 [dd, 1H, *J* = 5.1, 8.3 Hz, H−C(2‴)], 4.19 [m, 1H, H−C(4′)], 4.34 [pt, 1H, *J* = 4.7 Hz, H−C(3′)], 4.43 [q, 1H, *J* = 7.2 Hz,

H−C(2″)], 4.54 [pt, 1H, J = 5.2 Hz, H−C(2′)], 5.90 [d, 1H, J = 5.2 Hz, H−C(1′)], 8.09 [s, 1H, H−C(8)]. ^{13}C NMR (100 MHz, D_2O/NaOD, HMQC, HMBC): δ [ppm] = 17.1 [C(3″)], 28.4 [C(3‴)], 34.0 [C(4‴)], 51.2 [C(2″)], 55.3 [C(2‴)], 63.7 [d, $^2J_{C,P}$ = 4.8 Hz, C(5′)], 70.4 [C(3′)], 74.3 [C(2′)], 83.7 [d, $^3J_{C,P}$ = 8.7 Hz, C(4′)], 86.6 [C(1′)], 116.2 [C(5)], 137.4 [C(8)], 151.6 [C(4)], 151.9 [C(2)], 159.1 [C(6)], 175.3 [C(1″)], 178.4 [C(1‴)], 182.1 [C(5‴)].

Medium Pressure Reversed Phase Chromatography (RP-MPLC). Medium pressure liquid chromatography was performed on a preparative Sepacore chromatography system (Büchi, Flawil, Switzerland) consisting of two C-605 pumps, a C-615 type pump manager, a C-660 type fraction collector, a manual injection port equipped with a 20 mL loop, and a C-635 type UV detector monitoring the effluent at 260 nm. Chromatography was performed on a 150 × 40 mm i.d. polypropylene cartridge (Büchi, Flawil, Switzerland) filled with a slurry of LiChroprep, 25−40 μm, RP-18 material (Merck, Darmstadt, Germany) as stationary phase and using a gradient of 1% aqueous formic acid (solvent A) and methanol (solvent B) as the mobile phase (flow rate: 40 mL/min). Starting with an isocratic elution with 100% A for 5 min, the content of solvent B was increased linearly to 100% within 55 min. The fractions showing UV absorption at 260 nm were collected individually, freed from solvent in vacuum, and lyophilized twice.

High Performance Liquid Chromatography (HPLC). The analytical HPLC system (Jasco, Gross-Umstadt, Germany) consisted of a PU-2080 Plus pump, a DG-2080-53 degasser, a LG-2080-02 gradient unit, an AS-2055 Plus autosampler with a 100 μL loop, and a MD-2010 Plus detector. Separations in analytical scale were performed on an Outstanding B C18 column, 250 × 4.6 mm i.d., 5 μm (Trentec, Rutesheim, Germany). Chromatography was performed with a gradient of 1% aqueous formic acid (solvent A) and acetonitrile (solvent B) as the mobile phase (flow rate: 1 mL/min). Monitoring the effluent at 260 nm, we started the separation with an isocratic elution of 100% A for 5 min, followed by an increase in the content of solvent B from 0 to 5% within 10 min, then to 10% B within an additional 5 min, then to 30% B within the next 10 min, and, finally, to 100% B within an additional 10 min.

For preparative liquid chromatography, the HPLC apparatus (Jasco, Gross-Umstadt, Germany) consisted of two PU-2087 pumps, a Degasys DG-1310 online degasser (Uniflows Co., Tokyo, Japan), a 1000 μL gradient mixer, a 7725 i injection valve (Rheodyne, Bensheim, Germany), and a MD-2010 Plus detector. Chromatographic separations were conducted on a Microsorb-MV C18, 250 × 21.2 mm i.d., 5 μm RP-18 column (Varian, Darmstadt, Germany) operated at a flow rate of 18 mL/min and applying the same solvent gradient as detailed above for analytical HPLC.

LC/Time-of-Flight Mass Spectrometry (LC/TOF-MS). Mass spectra of the target compounds were measured on a Bruker Micro-TOF-Q (Bruker Daltonics, Bremen, Germany) mass spectrometer with flow injection referenced on sodium formate. Data processing was performed by using Daltonics DataAnalysis software (version 3.4, Bruker Daltonics, Bremen, Germany).

Liquid Chromatography/Mass Spectrometry (LC/MS). Electrospray ionization (ESI) spectra were acquired on a API 3200 type LC/MS/MS system (AB Sciex Instruments, Darmstadt, Germany) coupled to an Agilent 1100 HPLC system operating at a flow rate of 200 μL/min with direct loop injection of the sample (2−20 μL). The spray voltage was set at −4500 V in ESI$^-$ mode. Zero grade air served as nebulizer gas (35 psi) and as turbo gas (350 °C) for solvent drying (45 psi). Nitrogen served as curtain (20 psi) and collision gas (4.5×10^{-5} Torr). Both quadrupols were set at unit resolution. The declustering potential was set at −10 to −40 V in ESI$^-$ mode. The mass spectrometer was operated in the full scan mode monitoring positive and negative ions. Fragmentation of $[M - H]^-$ molecular ions into specific product ions was induced by collision with nitrogen and a collision energy of −15 to −70 V. For instrumentation control and data aquisition, the Analyst software version 1.5.1 was used.

Nuclear Magnetic Resonance Spectroscopy (NMR). The ^{1}H, ^{13}C, COSY, DEPT, HMQC, and HMBC spectroscopic experiments were performed on either a DRX-400 or a 500 MHz Avance III NMR spectrometer from Bruker (Rheinstetten, Germany). Samples were dissolved in either a mixture (75/1, v/v) of D_2O and sodium deuteroxide (40% w/w solution in D_2O) using 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSP) as reference or in a mixture (75/1, v/v) of CD_3OD and sodium deuteroxide (40% w/w solution in D_2O) containing tetramethylsilane (TMS) as reference. While data processing was performed using Topspin, version 1.3 (Bruker, Rheinstetten, Germany), the individual data interpretation was done with MestReNova 5.1.0-2940 (Mestrelab Research S.L., Santiago de Compostela, Spain).

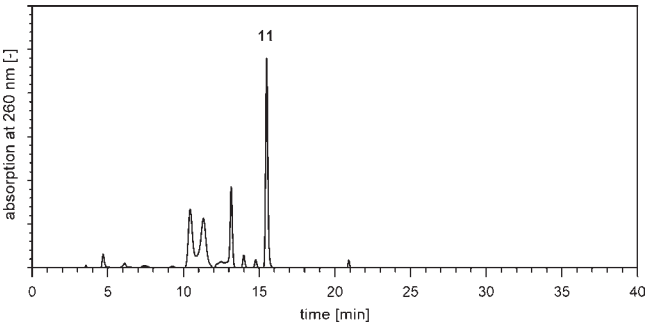

Figure 3. RP-HPLC chromatogram (λ = 260 nm) of a mixture of 5′-GMP (**2**) and glyoxal in phosphate buffer (1 mol/L, pH 8.0) heated for 24 h at 70 °C.

RESULTS AND DISCUSSION

Very recently, (*R*)- and (*S*)-N^2-(1-carboxyethyl)-guanosine 5′-monophosphate, (*R*)-**10** and (*S*)-**10**, were discovered as umami taste enhancers in commercial yeast extracts and were demonstrated to be generated upon Maillard-type reactions of guanosine 5′-monophosphate (5′-GMP, **2**) with the C_3-carbohydrates glyceraldehyde and dihydroxyacetone, respectively (*15*). As the formation of N^2-(1-carboxyethyl)-2′-deoxyguanosine, which is structurally related to **10**, from glyceraldehyde and dihydroxyacetone, respectively, is postulated to run via the common transient C_3-dehydration product methylglyoxal (*18*), the generation of structural homologues of **10** was to be expected from other dicarbonyls such as the C_2-dicarbonyl glyoxal.

Reaction of 5′-GMP (2) with Glyoxal. In order to extend the portfolio of putativly umami enhancing Maillard-modified nucleotides, 5′-GMP (**2**) was reacted with glyoxal in aqueous phosphate buffer (pH 8.0) for 24 h at 70 °C. RP-HPLC analysis of the reaction mixture revealed one major product (**11**) besides some earlier eluting, minor compounds (**Figure 3**). The major reaction product (**11**) was isolated and purified by means of preparative RP-HPLC and its chemical structure determined by means of LC-MS, LC-TOF-MS, and 1D/2D-NMR experiments.

NMR spectroscopy indicated the presence of 9 protons, which did not show H/D exchange, and 12 carbon atoms, as well as an intact phosphate moiety in the molecule. LC-TOF-MS analysis suggested an elemental composition of $C_{12}H_{16}N_5O_{10}P$ for **11**. The mass difference of 58 Da between compound **11** and the parent 5′-GMP (**2**) implied the addition of one glyoxal molecule to the parent nucleotide. Homo- and heteronuclear 2D-NMR experiments allowed the assignment of all signals of the intact nucleotide backbone of **2** as well as of an additional low-field shifted singlet of the methylene group H-C(2″), which showed heteronuclear couplings to the carbon atom C(2) of the heteroaromatic purine system as well as to the carboxy carbon C(1″) resonating at 180.5 ppm. Taking all the spectroscopic data into account, the previously not reported target compound was identified as N^2-carboxymethyl-guanosine 5′-monophosphate (**11**), differing from **10** by the lack of a methyl group (**Figure 2**).

Reaction of 5′-GMP with Reducing Carbohydrates. In order to further extend the discovery of umami enhancing Maillard-modified nucleotides, 5′-GMP (**2**) was reacted with the C_4- to C_6-aldoses erythrose, ribose, and glucose, as well as the reducing disaccharide maltose.

After optimization of the reaction parameters, a mixture of guanosine 5′-monophosphate and erythrose in a ratio of 1:3 was

thermally treated in phosphate buffer (pH 7.0) at 70 °C for 6 h. RP-HPLC analysis revealed two main reaction products, (*R*)-**12** and (*S*)-**12** (**Figure 4A**), which were isolated and purified by preparative RP-HPLC. Purity control of the isolated compounds (*R*)-**12** and (*S*)-**12** revealed each compound to be in equilibrium with an additional unknown compound, (*R*)-**12′** and (*S*)-**12′**, showing different retention behavior than the parent nucleotide **2**. HPLC analysis of (*S*)-**12** after additional rechromatography verified an equilibrium between the parent compound (*S*)-**12** and its transformation product (*S*)-**12′** (**Figure 4B**). To check as to whether (*S*)-**12′** was generated as an artifact during the lyophilization process, a sample of purified (*S*)-**12** was maintained in 1% aqueous formic acid (pH 2.1) and in an aqueous NaOH solution (0.1 mmol/L). Analysis by means of analytical RP-HPLC revealed that the equilibrium between (*S*)-**12** and (*S*)-**12′** is not influenced under acidic conditions (**Figure 4C**), whereas (*S*)-**12′** was found to be unstable and to regenerate (*S*)-**12** under alkaline conditions (**Figure 4D**).

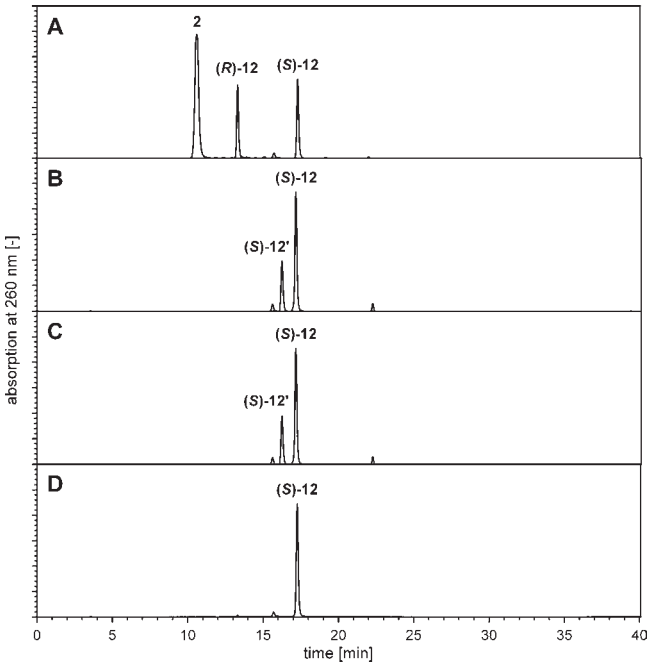

Figure 4. RP-HPLC chromatograms (λ = 260 nm) of (**A**) the crude reaction mixture of 5′-GMP (**2**) and erythrose heated for 6 h at 70 °C, (**B**) a purified and lyophilized sample of (*S*)-N^2-(1-carboxy-3-hydroxypropyl)-5′-GMP, (*S*)-**12**, dissolved in water, (**C**) a sample of lyophilized (*S*)-**12** dissolved in 1% aqueous formic acid (pH 2.1), and (**D**) a sample of lyophilized (*S*)-**12** dissolved in aqueous sodium hydroxide solution (0.1 mmol/L).

The isolates of (*R*)-**12** and (*S*)-**12** revealed similar exact mass data, determined by means of LC-TOF-MS, thus suggesting an elemental composition of $C_{14}H_{20}N_5O_{11}P$ for both molecules and indicating the addition of one erythrose molecule to the purine nucleotide. In order to fully assign the chemical structure of (*R*)-**12** and (*S*)-**12** and to gain more detailed information on the transformation of (*R*/*S*)-**12** into (*R*/*S*)-**12′**, (*S*)-**12′** was dissolved in D_2O, resulting in acidic conditions due to the presence of the highly acidic phosphate moiety in (*S*)-**12′**, and ^{1}H NMR spectra were recorded before (**Figure 5A**) and after the addition of 10 μL of sodium deuteroxide solution (40% NaOD in D_2O) (**Figure 5**). The ^{1}H NMR analysis shown in **Figure 5A** revealed the presence of two compounds which could be easily detected by the presence of a double signal set for the proton H-C(8) of the guanine moiety. The addition of NaOD affected the chemical shifts of the resonance signals observed and, most interestingly, reduced the ^{1}H NMR spectrum to a single set of proton signals as visualized by the sole singlet observed for H−C(8) (**Figure 5B**). To unequivocally determine the chemical structures of the isolated reaction products, additional 1D/2D-NMR experiments were performed in D_2O/NaOD as this spectrum was not complicated by a double signal set.

The ^{1}H NMR spectrum of (*S*)-**12** displayed the signals of the 5′-GMP moiety with the characteristic aromatic proton H-C(8)

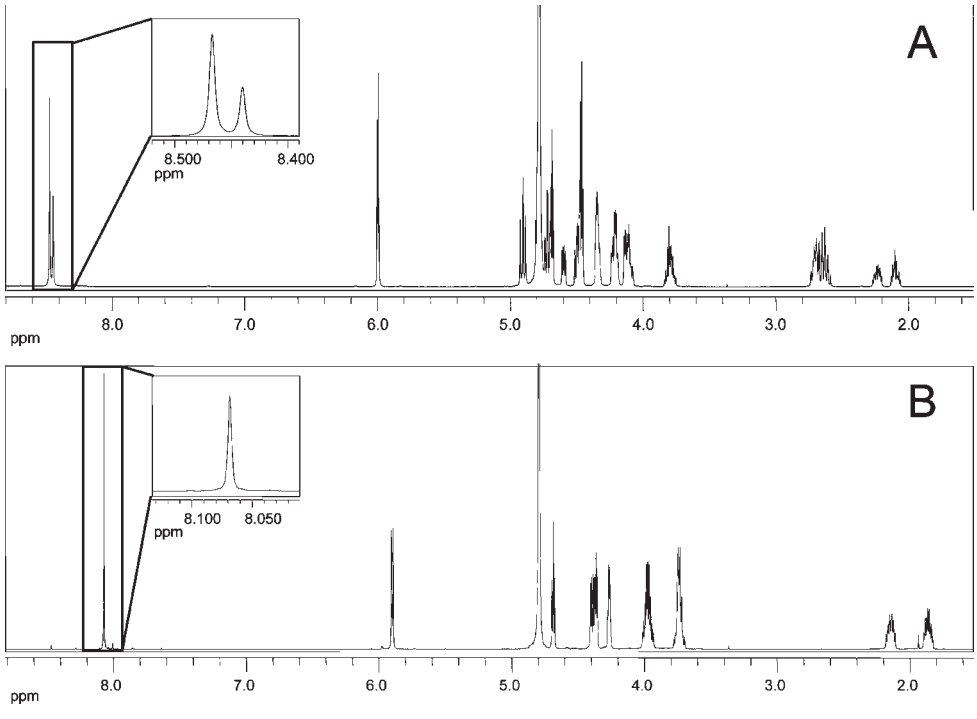

Figure 5. ^{1}H NMR spectra (400 MHz) of (*S*)-N^2-(1-carboxy-3-hydroxypropyl)-5′-GMP, (*S*)-**12**, in D_2O before (**A**) and after (**B**) the addition of 10 μL of sodium deuteroxide (40% NaOD in D_2O). Enlarged excerpts highlight the resonance signal of H-C(8) of the guanine moiety.

resonating at 8.07 ppm, the anomeric proton H-C(1′) resonating as a doublet at 5.89 ppm, and the residual protons of the ribose moiety showing chemical shifts between 3.78 and 4.61 ppm. In addition, two multiplets integrating for one proton each were observed between 1.86 and 2.06 ppm, a complex multiplet integrating for two protons showed resonance at 3.65 ppm, and an additional proton signal was detected at 4.27 ppm showing a doublet of a doublet multiplicity with coupling constants of 4.6 and 9.0 Hz, respectively (**Figure 5B**). The corresponding ^{13}C NMR revealed the presence of 14 carbon atoms, 2 of which were split to a doublet multiplicity with coupling constants of 4.6 and 8.2 Hz and were, therefore, assigned to the ribose carbons C(5′) and C(4′). Application of 2-dimensional homo- and heteronuclear correlation experiments (HMQC, HMBC) allowed the assignment of the signals at 1.86−2.06 ppm as the diastereomeric methylene group H-C(3″), which showed homonuclear ^{2}J-couplings to H-C(4″) and H-C(2″) resonating at 3.66 and 4.29 ppm, respectively. The HMBC experiment revealed H-C(2″) to show heteronuclear couplings to C(3″) observed at 32.8 ppm as well as to C(4″) resonating at 58.2 ppm. In addition, C,H-couplings were recorded between H-C(2″) and the aromatic guanine carbon C(2) as well as the carboxy carbon C(1″) with chemical shifts of 153.5 and 176.6 ppm, respectively. Taking all spectroscopic data into account, (*S*)-**12** and (*R*)-**12**, both isomers showing rather similar spectroscopic data, were unequivocally identified as (*R*)- and (*S*)-N^2-(1-carboxy-3-hydroxypropyl)-guanosine 5′-monophosphate (**Figure 2**). While the structurally related N^2-(1-carboxy-3-hydroxypropyl)-guanosine were already reported in the literature (*29*), the glycated nucleotide derivatives (*S*)-**12** and (*R*)-**12** were to the best of our knowledge not previously identified. The stereochemistry of the chiral center C(2″) was assigned by comparison of the retention order (RP-HPLC) to that of the corresponding N^2-(1-carboxyethyl)-derivatives (*S*)-**10** and (*R*)-**10** recently reported in the literature (*15*).

In order to elucidate the chemical structures of the transformation product (*S*)-**12′**, the NMR spectra were recorded in D_2O at a pD-value of about 3.0. Integration of the proton signals revealed a 2:1 equilibrium ratio for (*S*)-**12′** to (*S*)-**12**. The ^{1}H NMR analysis revealed downfield shifted signals for H-C(3″) (**Figure 5A**), while the corresponding ^{13}C signal was high-field shifted. Moreover, the signal of H-C(2″) observed as a double−doublet in (*S*)-**12** was found as a triplet exhibiting a coupling constant of 10.2 Hz in (*S*)-**12′**. Integration of the remaining resonance signals implied also a strong downfield shift of the methylene group H-C(4″) and signal splitting to show resonance at at 4.49 and 4.71 ppm, respectively. These data led to the assignment of the chemical structure of (*S*)-**12′** as the corresponding lactone of (*S*)-**12**, thus being well in line with the observed hydrolysis of (*S*)-**12′** into (*S*)-**12** under alkaline conditions (**Figure 4**). In summary, the reaction of **2** with erythrose leads to the formation of the α-amino acid derivatives (*R*)-**12** and (*S*)-**12**, which are in equilibrium with the corresponding α-amino acid lactones (*R*)- and (*S*)-N^2-(tetrahydro-2-oxofuran-3-yl)-guanosine 5′-monophosphate, (*R*)-**12′** and (*S*)-**12′**, respectively.

After successful identification of the reaction products generated from **2** and a tetrose, 5′-GMP should be reacted with a pentose, a hexose, and a reducing disaccharide. To achieve this, binary mixtures of 5′-GMP and ribose, glucose, and maltose, varying in the concentration ratio, were heated for up to 14 days at 70 °C in aqueous phosphate buffer at pH-values between 5.0 and 11.0. However, none of these reaction mixtures yielded significant amounts of candidate glycation products, which were expected to elute later than the parent nucleotide on RP-18 material (data not shown). As pentoses, hexoses, and disaccharides are well known to be less reactive when compared to trioses

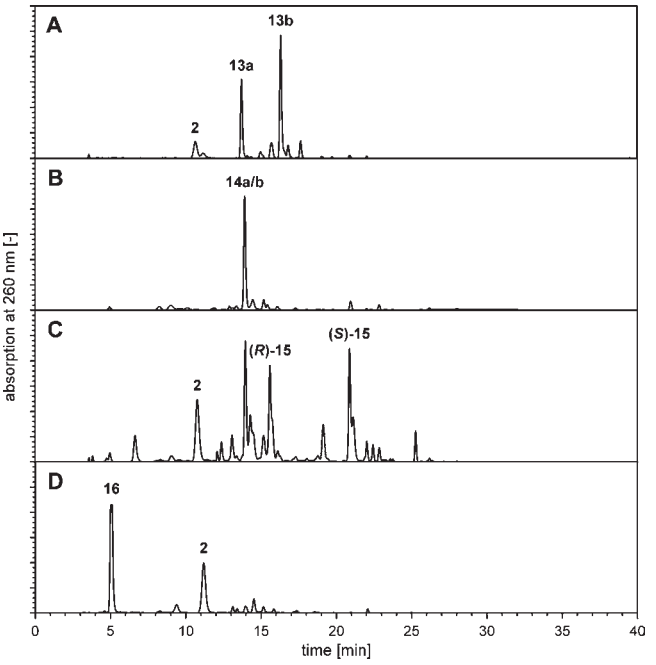

Figure 6. RP-HPLC chromatograms (λ = 260 nm) of phosphate-buffered solutions (pH 7.0, 1.0 mol/L) of (**A**) 5′-GMP (**2**)/ribose/L-alanine (1:5:1) after heating for 5 days at 70 °C, (**B**) 5′-GMP (**2**)/glucose/L-alanine (1:5:1) after heating for 11 days at 70 °C, (**C**) 5′-GMP (**2**)/maltose/L-alanine (1:5:1) after heating for 14 days at 70 °C, and (**D**) 5′-GMP (**2**)/glucose (1:10) after heating for 4 h at 100 °C in the absence of an amino acid.

and tetroses, the reactions were repeated in the presence of an amino acid in order to induce an amino-catalyzed degradation of carbohydrates to the corresponding deoxyosones. After optimization of the reaction parameters, ternary mixtures of guanosine 5′-monophosphate, L-alanine, and the carbohydrate in a ratio of 1:1:5 were thermally treated in phosphate buffer (pH 7.0) at 70 °C for 5 days (ribose), 11 days (glucose), and 14 days (maltose) to achieve a quantitative reaction.

RP-HPLC analysis of the model reaction of ribose revealed two main reaction products eluting after the parent nucleotide (**Figure 6A**). After isolation by means of preparative RP-HPLC, the chemical structure of the two major compounds was determined by means of LC-MS and NMR experiments. As found for (*R*)/(*S*)-**10** and (*R*)/(*S*)-**12**, the isolates exhibited rather similar spectroscopic data, thus indicating the presence of the diastereomeric pair (*R*)/(*S*)-**13**. LC-MS and LC-TOF-MS studies on the earlier eluting isomer (*R*)-**13** suggested an elemental composition of $C_{15}H_{22}N_5O_{12}P$, corresponding to the addition of one ribose molecule to the nucleotide. The ^{1}H NMR spectrum of the isolate showed the signals expected for the guanylic acid and five additional proton signals. Two high-field shifted multiplets, integrated for one proton each, were identified as the diastereomeric methylene group H-C(3″), for which homonuclear couplings were observed to H-C(2″) showing heteronuclear connectivity with carboxyl function C(1″). Furthermore, two double doublets with coupling constants of 11.8 Hz for a geminal coupling and 6.5 and 3.8 Hz for ^{3}J couplings, respectively, were found to resonate at 3.54 and 3.65 ppm, respectively, thus implying an additional diastereomeric methylene group H-C(5″). This methylene group showed homonuclear coupling to the multiplet resonating in low field at 3.91 ppm (H-C(4″)) as well as heteronuclear couplings to C(4″) at 72.3 ppm and C(3″) resonating at 38.0 ppm. Finally, H-C(2″) showed $^{1}H,^{13}C$ coupling to the purine carbon C(2) verifying the connection of the substituent to the exocyclic

amino function. The presence of the phosphate moiety was confirmed by the ^{13}C-split signals detected for C(4′) and C(5′). Taking all these spectroscopic data into account, the reaction products (*R*)-**13** and (*S*)-**13** were unequivocally identified as N^2-(1-carboxy-3,4-dihydroxybutyl)-guanosine 5′-monophosphate (**Figure 2**). In analogy to the observed equilibrium between (*R*)/(*S*)-**12** and (*R*)/(*S*)-**12′**, the ribose reaction products (*R*)-**13** and (*S*)-**13** were found to undergo lactonization to give (*R*)-**13′** and (*S*)-**13′** under acidic conditions. This was confirmed by ^{1}H NMR spectroscopy of these lactones demonstrating the downfield shift of the resonance signals of the ribose side chain and the resolution of the complex multiplet observed for H-C(3″) into a split signal resonating at 2.36 and 2.71 ppm, respectively.

Analytical RP-HPLC separation of the reaction mixture of guanosine 5′-monophosphate, L-alanine, and glucose revealed only a single peak (**Figure 6B**). After isolation by means of preparative HPLC, LC-TOF-MS showed an elemental composition of $C_{16}H_{24}N_5O_{13}P$, and ^{1}H and ^{13}C NMR studies demonstrated that the effluent of this peak contained two coeluting isomers, namely, (*R*)-**14** and (*S*)-**14**. On the basis of the spectroscopic data, (*R*)/(*S*)-**14** were identified as (*R*)/(*S*)-N^2-(1-carboxy-3,4,5-trihydroxypentyl)-guanosine 5′-monophosphate. Depending on the pH-value of the solution used for NMR spectroscopy, the candidate lactones (*R*)/(*S*)-**14′** were observed besides (*R*)/(*S*)-**14**. The complexity of these mixed NMR spectra did not allow an unequivocal signal assignment of these lactones.

The reaction of guanosine 5′-monophosphate and maltose in the presence of L-alanine resulted in a variety of products (**Figure 6C**). LC-MS and LC-TOF-MS revealed an elemental composition of $C_{15}H_{22}N_5O_{11}P$ for the reaction products (*R*)-**15** and (*S*)-**15** eluting after 15.5 and 21.0 min, thus indicating the addition of a C_5-carbohydrate backbone to the nucleotide moiety of the target molecules. 1D/2D-NMR studies on (*R*)-**15** verified the presence of five additional carbon atoms in comparison to those of the parent nucleotide. Besides the carboxy carbon C(1″), application of a HSQC experiment verified the presence of three additional methylene groups, two of which showed diastereomeric signal splitting. The high-field shifted signal at 1.68 ppm integrating for two protons was assigned as H-C(4″) showing heteronuclear ^{1}H,^{13}C couplings to the carbon atoms C(2″), C(3″), and C(5″) resonating at 59.4, 31.0, and 64.2 ppm, respectively. The downfield shift of C(5″) and H-C(5″) indicated this carbon atom to be substituted with an electronegative heteroatom and suggested the attachment of a hydroxyl substituent. An HMBC experiment demonstrated the proton H-C(2″) resonating between 4.28 and 4.34 ppm to show couplings to C(3″) and the carboxy function C(1″) at 182.8 ppm, as well as the aromatic proton C(2) at 155.0 ppm, thus verifying C(2″) as the carbon atom directly attached to the exocyclic amino function of the nucleotide. Taking all of the spectroscopic data into consideration, (*R*)-**15** and (*S*)-**15** were identified as (*R*)- and (*S*)-N^2-(1-carboxy-4-hydroxybutyl)-guanosine 5′-monophosphate, which to the best of our knowledge has not been reported earlier. The stereochemistry of the chiral center C(2″) was assigned by comparison of the retention order (RP-HPLC) to that of the corresponding N^2-(1-carboxyethyl)-derivatives (*S*)-**10** and (*R*)-**10** recently reported in the literature (*15*).

Whereas thermal treatment of 5′-GMP with ribose, glucose, and maltose, respectively, did only lead to N^2-1-carboxy-alkylated guanosine 5′-monophosphate at 70 °C when the reaction was performed in the presence of an amino acid, the reaction of 5′-GMP with excess amounts of glucose under more severe conditions (4 h, 100 °C) led surprisingly to the formation of an early eluting, highly polar product (**16**) as shown in **Figure 6D**. After purification by means of RP-MPLC and preparative RP-HPLC, LC-TOF-MS proposed an elemental composition of $C_{16}H_{24}N_5O_{13}P$, and 1D/2D-NMR experiments led to the identification of **16** as N^2-(β-D-glucosyl)-guanosine 5′-monophosphate (**Figure 2**), thus confirming the structure of the compound proposed earlier in the literature (*16*).

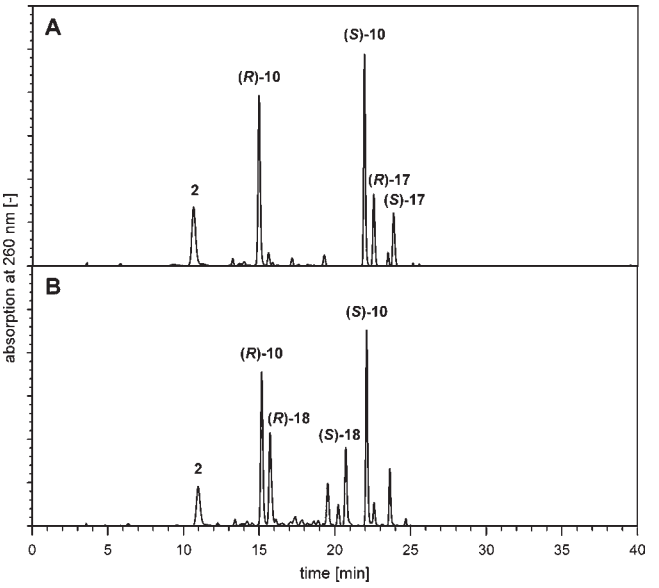

Figure 7. RP-HPLC chromatograms (λ = 260 nm) of mixtures (1:2:8) of 5′-GMP (**2**), dihydroxyactone, and *n*-propylamine (**A**) or mono sodium L-glutamate (**B**) heated in phosphate buffer (pH 7.0, 1.0 mol/L) for 4.5 h at 100 °C.

Reaction of Guanosine 5′-Monophosphate (2) with Amino Compounds and Dihydroxyacetone or Glycerinaldehyde. As earlier model studies on potential DNA−protein cross-link products suggested the formation of amides of N^2-(1-carboxyethyl)-modified DNA bases (*28*, *30*), the following experiments were done in order to check whether structural modifications of the umami enhancing α-amino acids (*S*)-**10** and (*R*)-**10** can be further extended by forming the corresponding amides when performing the reaction in the presence of excess amounts of an amine and amino acid, respectively. After optimization of the reaction conditions, a binary mixture of guanosine 5′-monophosphate and dihydroxyacetone or glyceraldehyde, respectively, was thermally treated in phosphate buffer (pH 7.0) for 30 min at 100 °C, then *n*-propylamine was added in excess amounts, and heating was continued for additional 4 h.

RP-HPLC of the *n*-propylamine reaction mixture revealed the formation of four reaction products besides trace amounts of the unreacted educt (**2**) (**Figure 7A**). Comparison of the retention times (RP-HPLC) and LC-MS data, followed by cochromatography with the reference compounds, led to the identification of the N^2-(1-carboxyethyl)-5′-GMP diastereomers (*S*)-**10** and (*R*)-**10**. As the two less polar compounds (*R*)-**17** and (*S*)-**17**, showing UV/vis absorption data similar to those of (*S*)-**10** and (*R*)-**10**, could not be assigned as one of the other Maillard-modified nucleotides identified above, these compounds were isolated by preparative HPLC and analyzed by means of LC-MS and 1D/2D-NMR experiments. LC-TOF-MS revealed an elemental composition of $C_{16}H_{25}N_6O_9P$ for both isomers, thus indicating the incorporation of one molecule of the amine into the target compounds. In order to verify the amide bond formed between the carboxy function of (*S*)/(*R*)-**10** and the *n*-propylamine, an HMBC experiment was performed demonstrating the heteronuclear coupling of the α-protons (H-C(1‴)) of *n*-propylamine and C(1″) of the α-amino acid moiety in the target molecules. Taking all of the spectroscopic

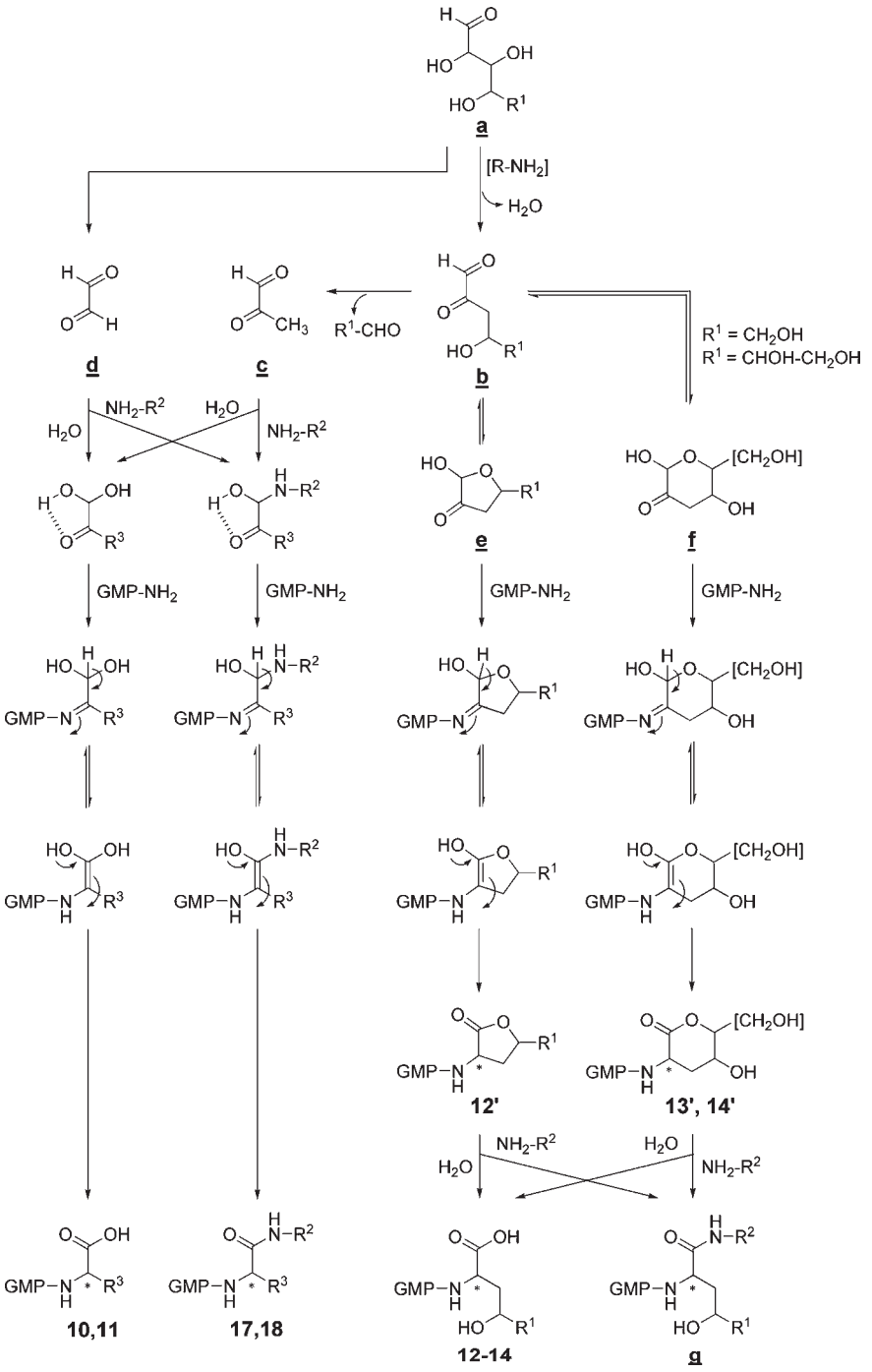

Figure 8. Reaction pathway leading to the formation of N^2-(1-carboxyalkyl)-guanosine 5′-monophosphates and N^2-(1-alkylamino)carbonylalkyl)-guanosine 5′-monophosphates by Maillard-type glycation of 5′-GMP.

data into consideration, (*R*)- and (*S*)-**17** were unequivocally identified as (*R*)- and (*S*)-N^2-((1-(*N*-propylamino)-carbonyl)-ethyl)-guanosine 5′-monophosphate (**Figure 2**).

In order to check as to whether similar amides are formed between (*S*)/(*R*)-**10** and food-related amino acids, guanosine 5′-monophosphate and dihydroxyacetone or glyceraldehyde, respectively, were reacted in the presence of L-glutamate. RP-HPLC analysis of the thermally treated reaction mixture revealed again (*R*)-**10** and (*S*)-**10** as the main reaction products besides the additional products (*R*)-**18** and (*S*)-**18** (**Figure 7B**). The elemental composition of $C_{18}H_{25}N_6O_{13}P$ found for (*R*)-**18** and (*S*)-**18** on the basis of LC-TOF-MS data as well as the 1D/2D-NMR data were well in line with the expected amide structure of these molecules. The most characteristic couplings were observed between H-C(2″) to C(2) of the aromatic system and between H-C(2‴) to C(1″), thus verifying the coupling of the amino function of glutamate to the carboxy function of the carboxyethyl moiety and confirming the structure of (*R*)-**18** and (*S*)-**18** as the previously not reported (*R*)- and (*S*)-N^2-((1-(*N*-(1′,3′-dicarboxypropylamino)-carbonyl)-ethyl)-guanosine 5′-monophosphate (**Figure 2**).

Proposed Formation Pathways Leading to Maillard-Modified Nucleotides. On the basis of the structures identified above, reaction pathways leading to the formation of **10**, **12**−**15**, and **18** are proposed in **Figure 8**. Thermal degradation of reducing carbohydrates (**a**) leads to the formation of 3-deoxyosones (**b**), methylglyoxal (**c**), and glyoxal (**d**) as highly reactive, transient dicarbonyl intermediates. Addition of a molecule of water or an amino compound at the aldehyde function of the short-chain

C_2- and C_3-dicarbonyls and the formation of an imine with the exocyclic amino group of 5′-GMP, followed by an imine-enamine and a keto−enol tautomerism, give rise to the generation of the α-amino acids *N*-phosphoguanosinyl glycine (**10**) and *N*-phosphoguanosinyl alanine (**11**) as well as their corresponding amides such as **17** and **18** (**Figure 8**). In contrast, the 3-deoxyosone of tetroses, pentoses, and hexoses undergo cyclization to give the corresponding furanoid (**e**) and pyranoid hemi acetals (**f**). In the presence of 5′-GMP, these transient intermediates do form an imine at position 2 and, after imine−enamine and a keto−enol tautomerism, give rise to γ-lactones as found for the tetrose reaction product **12′** and δ-lactones as found for **13′** and **14′** in the reaction system of ribose and glucose, respectively. Depending on the pH value, these lactones were found to be hydrolyzed into the corresponding α-amino acids (**12**−**14**). In addition, the formation of the corresponding amides (**g**) is to be expected in the presence of excess amounts of amino acids (**Figure 8**). By means of the same type of Maillard reaction chemistry, (*R*)- and (*S*)-**15** can be explained to be formed from 5′-GMP and 3,4-dideoxypentosulose, which has been recently demonstrated to be released from maltose as a transient intermediate (*31*).

Sensory Studies on the Umami Enhancement of Maillard-Modified 5′-GMP Derivatives. Prior to sensory analysis, the purity of each nucleotide derivative was checked by ^{1}H NMR spectroscopy as well as HPLC-MS. In order to investigate the umami enhancing activity of the Maillard-modified nucleotides, each compound was subjected to sensory evaluation by means of a paired choice comparison test (*7*). While direct comparison of the umami intensity of the test nucleotides to pure MSG solutions was not suitable due to different umami taste qualities, binary mixtures containing constant levels of MSG and increasing concentrations of 5′-IMP served as references to determine the so-called β-values, representing the potency of a test compound to enhance the umami taste of the L-glutamate containing matrix in relation to 5′-IMP as the reference (*7*). As a positive control, the β-value was determined for 5′-GMP to be 2.4, being well in line with previously published data (*11*, *12*). In order to overcome problems induced by partial lactonization of (*R*)/(*S*)-**12**, (*R*)/(*S*)-**13**, and (*R*)/(*S*)-**14**, the sodium salts of all the test nucleotides were used for sensory evaluation.

As summarized in **Table 1**, N^2-carboxymethylation of the exocyclic amino function of 5′-GMP was found to decrease the umami enhancement activity of 5′-GMP from 2.4 to a β-value of 1.2 (**11**). In comparison, N^2-carboxyethylation had a huge impact on the umami taste enhancement, strongly depending on the stereochemistry. While the (*R*)-configured isomer, (*R*)-**10**, was nearly inactive with a very low β-value of 0.08, the corresponding (*S*)-configured analogue, (*S*)-**10**, was found to exhibit a pronounced taste enhancement activity evaluated with a β-value of 7.0. Even a racemic 1:1 mixture of (*R*)- and (*S*)-**10** was rated by the panellists to be superior in umami enhancement when compared to that of the parent nucleotide **2** (2.9 → 2.4).

Further increase of the carbon skeleton of the N^2-substituent resulted in a decrease of the sensory activity of the nucleotide derivatives. Regarding the erythrose reaction products (*R*)-**12** and (*S*)-**10**, again the (*R*)-isomer exhibited only weak activity with an β-value of 0.10, whereas the (*S*)-configumer showed a comparable activity to that of the parent nucleotide **2** (**Table 1**). The single isomers of (*R*)/(*S*)-**15** and (*R*)/(*S*)-**13**, as well as the racemic mixture of (*R*)/(*S*)-**14**, all sharing a C_5- or a C_6-carbon skeleton as the N^2-substituent, showed rather low median β-values between 0.06 and 0.28, although the sensory impact of the later eluting (*S*)-configumer was always judged as slightly higher when compared to that of the corresponding (*R*)-isomer. Also the N^2-glycosylamine was found with a marginal β-value (0.14) only, thus implying that bulky hydrophilic substituents with more than four carbon atoms induce a drastic drop in the taste impact of 5′-GMP derivatives.

Table 1. Umami Enhancing Activity (β-Value) of Maillard-Modified 5′-GMP Derivatives, Related to Inosine 5′-Monophosphate (**1**)

test nucleotide[a]	β-value
inosine 5′-monophosphate[b] (5′-IMP), **1**	1.0
guanosine 5′-monophosphate[b] (5′-GMP), **2**	2.4
N^2-carboxymethyl-5′-GMP[c], **11**	1.2
(*R*)-N^2-(1-carboxyethyl)-5′-GMP[c], (*R*)-**10**	0.08
(*S*)-N^2-(1-carboxyethyl)-5′-GMP[c], (*S*)-**10**	7.0
(*R/S*)- N^2-(1-Carboxyethyl)-5′-GMP[c,d], (*R/S*)-**10**	2.9
(*R*)-N^2-(1-Carboxy-3-hydroxypropyl)-5′-GMP[c], (*R*)-**12**	0.10
(*S*)-N^2-(1-Carboxy-3-hydroxypropyl)-5′-GMP[c], (*S*)-**12**	2.0
(*R*)-N^2-(1-carboxy-4-hydroxybutyl)-5′-GMP[c], (*R*)-**15**	0.06
(*S*)-N^2-(1-carboxy-4-hydroxybutyl)-5′-GMP[c], (*S*)-**15**	0.12
N^2-(1-carboxy-3,4-dihydroxybutyl)-5′-GMP[c], (*R*)-**13**	0.08
N^2-(1-carboxy-3,4-dihydroxybutyl)-5′-GMP[c], (*S*)-**13**	0.28
N^2-(1-carboxy-3,4,5-trihydroxypentyl)-5′-GMP[c,e], (*R*)/(*S*)-**14**	0.19
N^2-(β-D-glucosyl)-5′-GMP[b], **16**	0.14
(*R*)-N^2-((1-(*N*-propylamino)-carbonyl)-ethyl)-5′-GMP, (*R*)-**17**	0.14
(*S*)-N^2-((1-(*N*-propylamino)-carbonyl)-ethyl)-5′-GMP, (*S*)-**17**	6.0
(*R*)-N^2-((1-(*N*-(1′,3′-dicarboxypropylamino)-carbonyl)-ethyl)-5′-GMP, (*R*)-**18**	0.10
(*S*)-N^2-((1-(*N*-(1′,3′-dicarboxypropylamino)-carbonyl)-ethyl)-5′-GMP, (*S*)-**18**	1.6

[a] The chemical structures of the nucleotides are displayed in **Figures 1** and **2**. [b] Applied as its disodium salt. [c] Applied as its trisodium salt. [d] Applied as a racemic mixture (1:1). [e] Applied as the 1:2 mixture of both diastereomers as determined by ^{1}H-NMR.

When comparing the α-amino acids (*R*)/(*S*)-**10** with the corresponding *n*-propyl amides (*R*)/(*S*)-**17**, no remarkable differences were observed for their taste enhancement activity (**Table 1**). Once again, the (*R*)-configured isomer exhibited only marginal activity, whereas (*S*)-**17** was evaluated with a high β-value of 6.0. However, in contrast to (*S*)-**10**, the sensory panel described the umami taste profile of the binary mixture of MSG and (*S*)-**17** to exhibit a later onset and a much more long-lasting taste sensation when compared to those of a binary mixture of MSG and 5′-GMP or even MSG and (*S*)-**10**. In contrast to (*S*)-**17**, the L-glutamyl amide **18** imparted only a minor umami enhancement activity, e.g., the (*R*)- and the (*S*)-isomer was evaluated with a β-value of 0.10 and 1.6, respectively (**Table 1**).

In conclusion, the present study demonstrates that 5′-GMP is able to undergo Maillard-type glycation reactions giving rise to umami enhancing molecules. Among the compounds identified, (*S*)-N^2-(1-carboxyethyl)-5′-GMP ((*S*)-**10**) and (*S*)-N^2-((1-propylaminocarbonyl)ethyl)-5′-GMP ((*S*)-**17**) were found as highly potent umami enhancers with 6−7 times higher activity when compared to that of the reference 5′-IMP. For the entire series of Maillard-modified nucleotides, the higher sensory activity was found for the (*S*)-configumer, whereas the (*R*)-isomer showed only marginal activity, thus emphasizing the stereospecifity of the umami taste receptor binding site. Cell-based umami taste receptor studies are currently ongoing in order to gain more detailed insight into the way of action of these compounds.

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