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Studies on Umami Taste. Synthesis of New Guanosine 5′-Phosphate Derivatives and Their Synergistic Effect with Monosodium Glutamate

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A number of N^2-alkyl and N^2-acyl derivatives of guanosine 5′-phosphate (GMP) have been synthesized and tested for their synergistic effect with monosodium L-glutamate (MSG), the prototypical substance imparting umami taste to savory-based foods. Capacities to enhance the taste intensity of MSG (γ values) were estimated through subjective comparisons of MSG/nucleotide mixtures in water with appropriate solutions of MSG alone. Assuming $\beta = \gamma$[nucleotide]/γ[IMP], β values of the N^2-substituted GMPs were found in the range 1.2–5.7. Such values appear to be related to the chain length of the substituent in the 2-position of the purine nucleus and dependent on the replacement of a CH_2 group with an S atom and/or with an α-CO group. These findings indicate that the exocyclic NHR group of the guanine moiety is actively implicated in the synergism between GMP derivatives and MSG. Theoretical calculations suggest that an *anti* conformation is probably assumed by ribonucleotide molecules interacting with umami receptors.

KEYWORDS: Umami; guanosine 5′-phosphate; monosodium glutamate; 5′-ribonucleosides; 5′-ribonucleotides; MSG enhancement; sensory test; conformational analysis

INTRODUCTION

Umami, a Japanese term for delicious, is now well recognized as a fifth basic taste quality distinct from the other primary tastes of sweet, sour, salty, and bitter (*1, 2*). Monosodium glutamate (MSG) is considered to be the prototypical umami substance and has long been used in Asian cuisine as a flavor enhancer. Free L-glutamate is particularly abundant in many food items such as meat, fish, cheese, and some vegetables; its taste signals the presence of dietary proteins, and it can increase the palatability of food, thereby increasing the food intake (*3*).

Other compounds tasting umami and showing flavor enhancement properties have been identified in savory foods (*3, 4*). Among these, inosine 5′-monophosphate disodium salt (**1a**, IMP) and guanosine 5′-monophosphate disodium salt (**1b**, GMP) have been found to enhance the glutamate taste markedly. Systematic sensory studies on this synergism revealed that the umami intensity of aqueous solutions of MSG increased exponentially when IMP (or GMP) was added even in very low concentrations (*1*). This fact is of great relevance for the food industry, which uses ternary varying mixtures of MSG + IMP + GMP to enhance the flavor and mouthfulness of culinary products, snacks, soups, sauces, and seasonings (*4*).

It is well-known that many people consume more salt than their bodies's need, since the taste of salt added in foods is undoubtedly appealing. This behavior is especially pronounced in elderly people as we lose some of our sense of taste with age. On the other hand, nutritionists recommend a reduction of sodium chloride in the diet considering its link with coronary heart disease and hypertension. Thus, umami substances can be very useful when added in small amounts to foods with a low level of salt by making them more acceptable and appetizing (*5*).

Despite the wide human consumption of these food additives, relatively little is known about their involvement in the peripheral mechanism responsible for the perception of the umami taste (*6*). It has been hypothesized that several G protein-coupled receptors (GPCRs) are involved in the sense of umami taste. Using methods of molecular biology four L-glutamate receptors have been reported to function as umami receptors. The first to be discovered (termed *taste*-mGluR4) was a variant of the metabotropic glutamate receptor 4 having a truncated N-terminal extracellular protein portion (*7*). Next, a heterodimer built of receptors T1R1 and T1R3 was found to interact with L-glutamate, showing a response markedly enhanced by IMP. More recently, two variants of metabotropic glutamate receptor 1, i.e., mGluR1α and taste variant mGluR1, have been described as candidate receptors for umami taste sensation (*6, 8*).

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For practical applications and to investigate the structure–activity relationships (SARs) of 5′-nucleotide-related MSG enhancers, several mononucleotides have been synthesized and examined using sensory analysis (*9–12*). Data from such investigations indicated that the structural requirements for MSG-enhancing activity by nucleotide molecules are (*3*) (a) a purine nucleus as the base moiety, (b) a free phosphate group at the 5′-position (no activity was observed testing methyl esters and amidates of IMP and GMP as well as cyclic inosine 3′,5′-phosphate), (c) an electron-withdrawing group at the 6-position of the purine nucleus (*3, 11*), and (d) an unaltered ribose moiety (a strongly reduced activity occurs if the 2′-OH is replaced by H or the 2′- and 3′-positions are protected as in isopropylidene derivatives of IMP and GMP) (*11*).

A large variability of the synergistic effect between 5′-nucleotides and MSG was observed in 2-substituted derivatives of inosine 5′-phosphate (*3, 9*): in particular, 2-alkylthio-IMPs showed pronounced activities when compared with IMP and GMP (*9*). Thus, a receptor model was proposed containing three binding sites, A, B, and X (*3*). According to this model the umami-active 5′-nucleotide should interact with sites A and B by means of the 5′-phosphate group and the oxygen atom in the 6-position of the purine nucleus (which are about 8 Å far away in the solid state *anti* conformation of IMP) (*13*) and with site X through carbon 2 and its substituent.

It can be noted that, regardless of the relevance of the substitution at the 2-position in IMP to synergistic activity, as evidenced by SARs (*10*) and invoked in the receptor model mentioned above, only two N^2-substituted GMPs, i.e., **1c** and **1d**, have been submitted to sensory analyses showing an activity comparable to that of GMP (*3*). This prompted us to investigate the synthesis, the MSG enhancing activity, and the conformational features of N^2-substituted 5′-guanylic acids.

MATERIALS AND METHODS

Analytical TLC was performed on silica gel F_{254} precoated aluminum sheets (0.2 mm layer; Merck, Darmstadt, Germany) using the eluent CH_2Cl_2:MeOH = 9:1; components were detected under an UV lamp and by spraying either with a 1% solution of potassium permanganate or with a ceric sulfate/ammonium molybdate solution, followed by heating to ca. 150°. Silica gel 60, 40–63 μm (Merck), was used for flash chromatography. A Waters Model 600E liquid chromatograph (Waters Corp., Milford, MA) connected to a HP1050 diode-array detector (Hewlett-Packard GmbH, Waldbronn, Germany) was used for analytical HPLC. Analyses were performed using a LiChrosorb RP-18 (5 μm, 250 × 4.6 mm; Merck) at a flow rate of 0.8 mL/min, detection at λ 254 nm, and the following mobile phase: solvent A, 0.01 M acetic buffer, pH 5, and solvent B, MeOH, gradient elution from 10% to 100% solvent B in 20 min. Preparative HPLC was performed on an AKTA Basic100 instrument (Pharmacia, Uppsala, Sweden). Chromatographic conditions were as follows: column, LiChrosorb RP-18 (7 μm, 250 × 25 mm; Merck); flow rate, 10 mL/min; detector, λ 254 nm; mobile phase as reported above, gradient elution from 10% to 100% solvent B, the length of the gradient depending on the retention time in analytical HPLC.

^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at 400.13, 100.61, and 161.96 MHz, respectively, on a Bruker AVANCE 400 spectrometer equipped with a XWIN-NMR software package (Bruker, Karlsruhe, Germany) at 300 K, unless stated otherwise. ^{1}H and ^{13}C chemical shifts (δ) are given in parts per million and were referenced to the solvent signals [δ_H 7.25, δ_C 77.00; δ_H 2.50 and δ_C 39.50 ppm from TMS (tetramethylsilane) for $CDCl_3$ and DMSO-d_6, respectively]; in the case of D_2O solutions TSP [3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt, δ_{Me} 0 ppm] was used as internal standard. ^{31}P chemical shifts (ppm) are reported with respect to 85% H_3PO_4 (δ 0 ppm) as external standard. ^{13}C NMR signal multiplicities were based on APT (attached proton test) spectra. ^{13}C NMR signals were assigned with the aid of ^{1}H–^{13}C correlation experiments (heteronuclear multiple quantum correlation spectroscopy, HMQC, and heteronuclear multiple bond correlation spectroscopy, HMBC); ^{1}H signals were assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY). Electrospray ionization mass spectra (ESI-MS) were acquired on a ThermoFinnigan LCQ Advantage spectrometer (Hemel Hempstead, Hertfordshire, U.K.) and MALDI-TOF-MS on a Omniflex Bruker Daltonics (Bruker Daltonics, Billerica, MA).

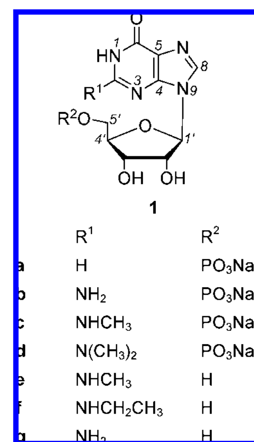


Figure 1. Purine ribonucleosides and ribonucleotides.

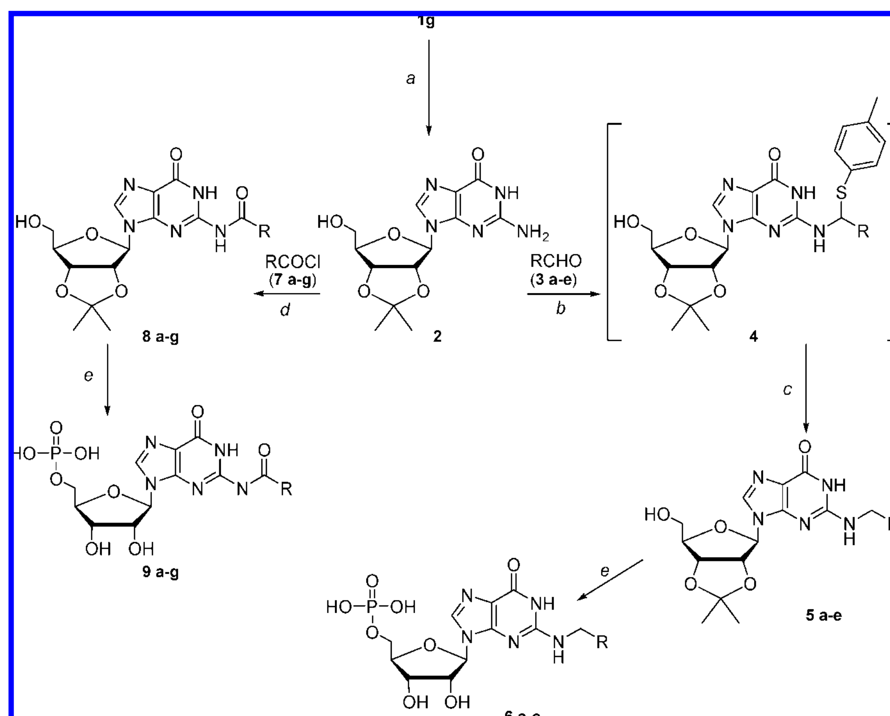
CH_2Cl_2 and THF were dried over $CaCl_2$ and $LiAlH_4$, respectively, and distilled before use; all other solvents were of HPLC grade. All reagents were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO) and were used without further purification; IMP and GMP were from Sigma-Aldrich.

2′,3′-*O*-isopropylideneguanosine (**2**) was prepared as reported by Defrancq et al. (*14*). Aldehydes **3a–e** and acyl chlorides **7a–g** were either commercially available or prepared according to the literature (see Supporting Information for details).

Synthesis of *N*²-Alkyl-2′,3′-*O*-isopropylideneguanosines (5). General Procedure. To a solution of **2** (2.3 g, 7.2 mmol) and *p*-thiocresol (2 g, 16.1 mmol) in ethanol (50 mL) and acetic acid (4 mL) was added the aldehyde (**3a–e**) (25 mmol), and the reaction mixture was heated under reflux for 24–48 h until TLC showed complete disappearance of **2**. After the resultant mixture was cooled to room temperature, the solvent was evaporated under reduced pressure to give a gummy residue which was triturated with petroleum ether. The resulting suspension was vacuum filtered, and the precipitate was dissolved in EtOH (25 mL) and treated with sodium borohydride (817 mg, 21.6 mmol). The reaction mixture was heated under reflux for 1–2 h, monitoring the progress of the reaction by TLC analysis. The solution was then cooled to room temperature and concentrated *in vacuo*. The residue was taken up in ethyl acetate (50 mL) and washed with water (2 × 25 mL). The organic phase was dried on Na_2SO_4 and evaporated under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2:MeOH = 10:1) to afford the N^2-alkyl-2′,3′-*O*-isopropylideneguanosine (**5a–e**) in 43–61% yields; see Supporting Information for NMR data.

Synthesis of *N*²-Acyl-2′,3′-*O*-isopropylideneguanosines (8). General Procedure. To a stirred suspension of **2** (1 mmol, 323 mg) in dry CH_2Cl_2 (25 mL) and pyridine (5 mL) under N_2 at 0 °C was added dropwise trimethylchlorosilane (7 mmol, 760 mg, 0.9 mL), and the reaction mixture was stirred for 2–3 h at room temperature. The solution was then cooled again in an ice bath, and the acyl chloride (**7a–g**) (1.1 mmol) was added over 2 min. The mixture was stirred at room temperature for 3 h until complete disappearance of the starting material (TLC control). After being washed with 1 N HCl (20 mL) and with 5% $NaHCO_3$ (2 × 20 mL), the organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. When necessary the residue was treated with 1 N HCl/THF (10 mL, 1:1 v/v) for 15–30 min to hydrolyze the trimethylsilyl ether in the 5′-position and then diluted with CH_2Cl_2 (50 mL), washed with H_2O (30 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Final purification by flash chromatography (CH_2Cl_2:MeOH = 13:1) afforded the N^2-acyl-2′,3′-*O*-isopropylideneguanosine (**8a–g**) in 52–78% yields; see Supporting Information for NMR data.

Scheme 1[a]



[a] Reagents and conditions: (**a**) acetone, *p*-toluenesulfonic acid, 2,2′-dimethoxypropane, room temperature; (**b**) *p*-thiocresol, EtOH, AcOH, reflux; (**c**) $NaBH_4$, EtOH, reflux; (**d**) TMSCl, CH_2Cl_2, pyridine, room temperature; (**e**) $POCl_3$, H_2O, triethyl phosphate, 0 °C. See **Table 1** for substituents.

Table 1. Overall Yields of N^2-Substituted 5′-Guanylic Acids (**6**, **9**) from Guanosine (**1g**)[a]

3, 5, 6	R	yield of 6 (%)	7, 8, 9	R	yield of 9 (%)
a	$CH_2CH_2CH_3$	38	a	$CH_2CH_2CH_3$	40
b	$(CH_2)_6CH_3$	25	b	$(CH_2)_6CH_3$	42
c	CH_2CH_2Ph	30	c	CH_2SCH_3	39
d	CH_2SCH_3	29	d	$CH_2CH_2SCH_3$	40
e	$CH_2CH_2SCH_3$	52	e	$CH_2SCH_2CH_3$	43
			f	$CH_2CH_2SCH_2CH_3$	37
			g	CH_2-thien-2-yl	40

[a] Cf. **Scheme 1**.

Synthesis of N^2-Alkyl- (6) and N^2-Acyl-5′-guanylic Acids (9). General Procedure. Phosphoryl chloride (6 mmol) and H_2O (1 mmol) were added dropwise to a stirred solution of the N^2-alkyl-2′,3′-*O*-isopropylideneguanosine (**5a**−**e**) or N^2-acyl-2′,3′-*O*-isopropylideneguanosine (**8a**−**g**) (1 mmol) in triethyl phosphate (2.5 mL) at 0 °C. The mixture was stirred in an ice bath for a further 4–6 h, monitoring the progress of the reaction by HPLC. After dilution with water (5 mL), the solution was adjusted to pH 2 with 3 N NaOH, warmed to 70 °C under stirring for 0.5–1 h (HPLC control), then neutralized with 3 N NaOH, and lyophilized. The product was then purified by preparative HPLC. Fractions containing the nucleotide (**6a**−**e**, **9a**−**g**) were collected and lyophilized, and the residue was further dried at 70 °C for 4–6 h *in vacuo* to constant weight (67–81% yield). All compounds gave satisfactory elemental analyses (C, H, N). Spectral data (MS and ^1H, ^{13}C, and ^{31}P NMR) of compounds **6a**−**e** and **9a**−**g** are reported in Supporting Information.

Sensory Testing. Five screened and trained panelists, three females and two males, were selected. Distilled water was used for preparing the solutions and for oral rinsing. A solution named "fixed sample" containing MSG (10.0 mM) plus the test compound (0.4 mM) (pH 7.3) was compared, by the "sip and spit" procedure, with single solutions of MSG (five "reference samples" having concentrations determined in logarithmic equal steps at 40% intervals).

Five pairs of one fixed and one reference sample (40 mL in randomly coded glasses) were presented to each panelist. Panelists were asked to indicate which in each pair had the stronger umami taste (oral rinsing was strongly encouraged). Replications were 5 for each panelist, giving 25 responses for each reference sample. The response was considered as positive when the fixed sample had the stronger taste.

Statistical Analysis. According to the Probit method (*15*) for each fixed sample every percentage of positive responses was plotted as ordinate against the logarithm of the MSG concentration of the corresponding reference sample (plotted as abscissa) The best straight line fitting the five points on the coordinate plane was then calculated. For the percentage value of 50 read as ordinate, the abscissa of the line afforded the logarithm of the MSG concentration of a solution equivalent in taste intensity to the fixed sample. Using such concentration (y) and the concentrations of MSG (u) and of the test compound (v) in the fixed sample, the value of the constant γ (indicative of the MSG-enhancing ability of the specific nucleotide) was calculated from Yamaguchi's equation (*16*): $\gamma = (y - u)/(vu)$.

Conformational Studies. Conformational search was performed with the Monte Carlo multiple minimization algorithm (*17*) implemented in the MacroModel 9.0 program (Schrödinger, LLC, New York, 2005). The search was performed both *in vacuo* and in water. In the latter case, water solvent was described with the GB/SA model (*18*). Each molecule was submitted to 30000 cycles of random dihedral variation and subsequent geometrical optimization using the AMBER* force field. The structures obtained from this procedure were compared on a geometrical basis, and all unique structures whose energy fell in a 12 kcal/mol window from the global minimum were stored for subsequent analysis. Among these structures, the lowest energy *anti* and *syn* conformations for each molecule were selected, and their geometrical and energetic parameters were compared to each other and the experimental data.

RESULTS

Synthesis of N^2-Alkyl and N^2-Acyl Derivatives of Guanosine 5′-Phosphate. Modified guanosine ribonucleosides and deoxyribonucleosides in which the exocyclic amino function is replaced with a methylamino (e.g., **1e**), dimethylamino, or ethylamino (e.g., **1f**) group have been found in transfer ribonucleic acids (tRNAs), in several types of small nuclear

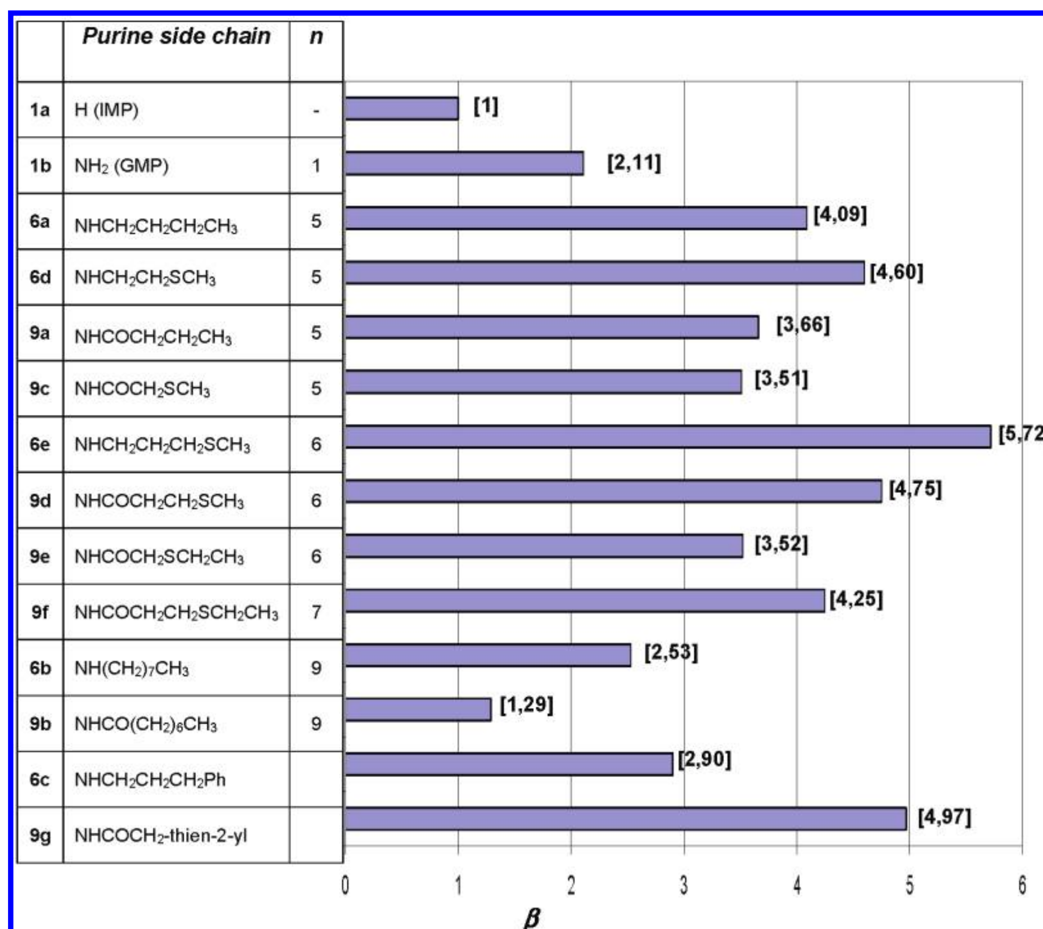


Figure 2. MSG-enhancing capacities of N^2-substituted 5′-guanylic acids referred to IMP. β values are in square brackets; *n* indicates the chain length of the substituent in the 2-position of the purine nucleus.

RNAs (snRNAs), and in granulocyte and limphocyte DNA of alcohol abusers (*19*) (**Figure 1**). These findings stimulated interest in the synthesis of such compounds; however, with regard to N^2-alkylated guanosines and their 5′-phosphates, the synthetic methods still developed appeared to be inadequate in overall yields and in the number of steps for our purpose (*19*).

In 1980 Kemal and Reese described the alkylation of the exocyclic amino group of guanosine (**1g**) in two steps by reaction of the nucleoside with an aldehyde and *p*-thiocresol, followed by reduction of the resulting *N*^{2}-[1-(*p*-tolylthio)alkyl] guanosine with $NaBH_4$ in dimethyl sulfoxide (*20*). According to this procedure, but starting from 2′,3′-*O*-isopropylideneguanosine (**2**) and reducing the thio adduct (**4**) without further purification (**Scheme 1**), we succeeded in obtaining compounds **5a**−**e** in satisfactory yields. The early introduced protecting group, which was suitable for the next phosphorylation (*21*), was then removed in the working up of the last reaction mixture, giving rise to nucleotides **6a**−**e**.

Acylation of the exocyclic amino group of cytidine, adenosine, and guanosine has been extensively investigated as a temporary protection of the correspondenting monomeric units to be assembled in oligonucleotide synthesis. The first procedure developed by Khorana consisted of the peracylation of the nucleoside and subsequent selective O-deacylation with controlled sodium hydroxide treatment. This method was simplified by Jones and co-workers by application of the concept of transient protection (*22*). In this case the nucleoside is first treated with trimethylchlorosilane (TMSCl) in anhydrous pyridine and immediately reacted with an acylating agent (acyl chloride or acid anhydride) to effect N-acylation; *O*-TMS groups are then hydrolyzed with pyridine and water during the workup, giving the *N*-acyl derivative of the starting nucleoside. Afterward, Jones' procedure, originally employed to synthesize N-acylated deoxyribonucleosides, was extended with improvement on the acylation step to prepare ribonucleosides bearing a variety of amino-protecting acyl groups. However, unlike cytidine and adenosine, acylation of guanine nucleotides was found to be problematic for some reasons: low solubility of the starting material, formation of colored side products, and unsatisfactory yields.

Recently, Jones and co-workers (*22*) showed that in CH_2Cl_2/pyridine and in excess trimethylsilyl chloride (TMSCl, 9 equiv) guanosine reacts with acyl chlorides under mild conditions, furnishing the corresponding N^2-acylated derivatives in high yield, also in the case of the labile amino-protecting group. These authors demonstrated that the silylation of the O6 and the amino group in a pentasilylated guanosine intermediate prevents O6 acylation, which leads to dark impurities, and also accelerates amino acylation. Considering that it was a great convenience for our synthetic strategy (**Scheme 1**) to have 2′-OH and 3′-OH protected in the N^2-acylated guanosine in order to perform phosphorylation (as said before), compound **2** was treated with different acyl chlorides (**7a**−**g**) under the conditions of transient protection and in the presence of excess TMSCl. In this way 2′,3′-*O*-isopropylidene-N^2-acylated guanosines (**8a**−**g**) were obtained in good yields and used for the final phosphorylation step (*21*). Both crude N^2-alkylated and N^2-acylated 5′-guanylic acids resulting from the phosphorylation reaction (**6a**−**e** and **9a**−**g**) were purified by preparative HPLC. Their overall yields from guanosine are reported in **Table 1**.

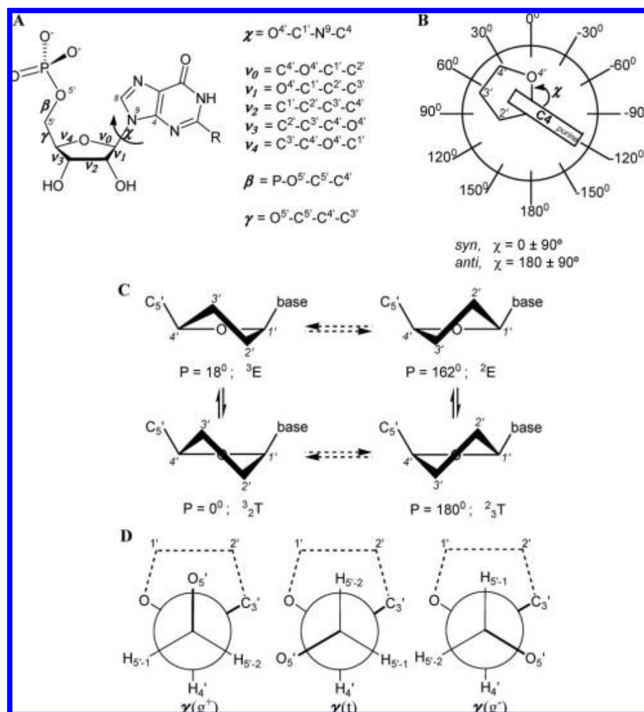


Figure 3. Essential geometric elements describing the conformation of purine 5′-ribonucleotides: (**A**) torsion angles; (**B**) representation of the N-glycosidic bond torsion angle and the *syn* and *anti* regions; (**C**) puckered forms of the ribose ring and their interconversion; (**D**) orientation of the OPO_3^{2-} group with reference to the sugar ring.

Synergistic Taste Effect of Guanosine 5′-Phosphate Derivatives with MSG. The umami potential of 5′-ribonucleotides **6a**−**e** and **9a**−**g** was estimated for their ability to enhance the taste intensity of MSG (*1, 4, 11*). We performed quantitative measurements of such enhancement according to the sensory testing procedure and the statistical treatment of resultant data developed by Yamaguchi (*16*). This author proposed a mathematical model to correlate the taste enhancement of MSG with its concentration and that of the enhancer present in the same aqueous solution. The relationship between the degree of taste enhancement and the two concentrations is expressed by eq 1, where u is the concentration of MSG, v is the concentration of the nucleotide, y is the concentration of MSG alone in a solution equivalent in taste intensity to the mixture ($u + v$), and γ is a constant (cf. Materials and Methods).

$$y = u + \gamma v u \qquad (1)$$

Equation 1 is now generally accepted (providing that u is not too far from the subjective threshold value of MSG and v is in the range 2–14% u) and has been validated by studies on the flavor-enhancing activity of compounds related to inosine 5′-phosphate (*9–12*). It must be noted that γ (conc^{-1}) is specific to the enhancer and is indicative of its synergistic capacity to increase the umami taste of MSG.

The γ value of each N^2-alkyl and N^2-acyl derivative of 5′-guanylic acid, obtained as the mean of three or more independent determinations, was then referred to the γ value of IMP (measured with the same procedure). Thus, the synergistic capacity of each nucleotide was expressed as β [β being equal to γ(nucleotide)/γ(IMP)]. β values are reported in **Figure 2**.

The data of **Figure 2** show that the synergistic potential of the N^2-substituted GMPs is higher, and for certain compounds much higher, than that of IMP. In addition, β values appear to be dependent on both the chain length of the substituent in the 2-position of the purine nucleus and the replacement of a CH_2 group with an S atom and/or with an α-CO group.

Conformational Analysis of Purine 5′-Mononucleotides. Extensive conformational studies of mononucleotides have been performed with the aim of relating molecular geometry to biological function. In the case of purine mononucleoside 5′-phosphates the problem of conformational preference has been approached with the aid of different techniques: X-ray analysis (*23*), NMR spectroscopy (*24, 25*), and theoretical calculations (*24–26*).

It is well established that purine 5′-ribonucleotides are flexible molecules which can be described in terms of geometrical parameters as follows (*27*): (a) A dihedral angle χ (**Figure 3A**,**B**) defines the rotation around the glycosidic bond of the planar purine nucleus; conformations having $0° < \chi < \pm 90°$ and $\pm 90° < \chi < 180°$ are called *syn* and *anti*, respectively. (b) P (phase angle of pseudorotation) and Ψ_m (maximum value of the endocyclic torsion angle ν_0) indicate the pseudorotational state of the ribose moiety in the conventional wheel (*28*): $P = 0$ (with $\nu_0 = \Psi_m = 40 \pm 5°$ in purine 5′-ribonucleotides) corresponds to a twist form of the puckered ring having C$^{3'}$-*endo*/C$^{2'}$-*exo* and is denoted by the symbol $^3{}_2$T (**Figure 3C**). For P going to $+18°$ the conformation turns into an envelope form denoted ^{3}E (C$^{3'}$-*endo*), for $P = +36°$ the conformation becomes $^3{}_4$T, and so on (for the interrelationship between the five torsion angles ν_{0-4} and P and Ψ_m in a nonplanar five-membered ring; see ref *28*). (c) Two torsional angles, β and γ, describe the orientation of the phosphate group: the gauche conformations corresponding to β and $\gamma = +60°$ or $-60°$ are usually designated as g^+ or g^-, respectively, and the trans conformation (β and $\gamma = \pm 180°$) as t (as shown in **Figure 3D** for γ). Of the above parameters the glycosyl torsion angle (χ) appears to be a major determinant of the molecular shape and volume of nucleosides and nucleotides.

Gas phase structural calculations on nucleotides at the molecular mechanics and semiempirical levels assessed that the *syn* conformation is preferred in dianionic 5′-GMP and that a hydrogen bond between NH_2 and the phosphate group is present (*25*). Higher level RHF and MP2 calculations in the gas phase were performed on 2′-deoxyguanine and adenine 5′-mononucleotides with a monoanionic phosphate group to mimic the situation in nucleic acid chains. When these nucleotides were energy minimized in B-DNA- or Z-DNA-like conformation with the basis in *anti* or in *syn* conformation, respectively, the energies of the Z-DNA-like structures were always higher than the B-DNA ones, and the energy difference was higher in the case of adenine nucleotide (*26*). This means that the *syn* conformation in the guanine nucleotide is less disfavored than in the adenine nucleotide.

We performed conformational calculations on IMP, GMP, and N^2-alkyl and N^2-acyl derivatives of GMP as dianionic molecules (nucleoside-5′-OPO_3^{2-}), both in gas phase and in water. This search was carried out using the MACROMODEL program as described in Materials and Methods. For each nucleotide two minima were found corresponding to the *anti* and *syn* conformations. The values of geometrical parameters (χ, P, β, and γ) and the *syn*/*anti* energy difference of each pair of conformers are listed in **Table 2**. In **Figure 4** the structures of two 5′-ribonucleotides, i.e., GMP and N^2-methylthioethyl-5′-guanylic acid (**6d**), in gas phase and in water, are reported to show the most apparent differences in their minimum conformation.

DISCUSSION

Of the five senses, the molecular mechanism underlying taste perception remains the most mysterious. It is generally assumed

Table 2. Calculated Geometrical Parameters and Energy Differences of *syn*/*anti* Minimum Conformers of Purine Ribonucleotides (as Dianions) in Gas Phase and in Water

	nucleotide		*anti*				*syn*				
			χ	*P*	γ	β	χ	*P*	γ	β	ΔE (*syn*/*anti*), kcal/mol
1a	IMP	*in vacuo*	−142.97	126	−57.40	−56.10	43.07	126	−57.44	−56.08	1.982
		H_2O	−176.48	136	178.46	178.63	47.67	145	−179.17	174.89	1.905
1b	GMP	*in vacuo*	−143.49	125	−57.45	−56.47	81.00	302	−79.97	69.68	−6.627
		H_2O	−174.93	136	178.53	178.43	44.19	167	179.71	178.12	3.363
9c	$NHCOCH_2SCH_3$	*in vacuo*	−157.33	126	−57.55	−57.76	33.30	124	−56.35	−57.07	1.803
		H_2O	−173.96	138	178.28	178.65	36.03	120	−57.47	−59.75	1.761
9d	$NHCOCH_2CH_2SCH_3$	*in vacuo*	−149.83	123	−58.76	−56.26	47.11	125	−91.24	62.02	2.122
		H_2O	−152.01	147	178.17	178.49	48.41	118	−59.18	−59.11	1.630
6d	$NHCH_2CH_2SCH_3$	*in vacuo*	−152.30	126	−57.42	−56.12	41.54	130	−57.13	−55.44	0.211
		H_2O	−177.40	137	178.46	178.49	38.57	121	−58.08	−58.81	1.847
6a	$NHCH_2CH_2CH_2CH_3$	*in vacuo*	−144.53	126	−57.54	−56.04	41.45	128	−56.91	−55.85	1.119
		H_2O	−177.85	136	178.49	178.51	40.55	120	−58.05	−59.07	1.952
9a	$NHCOCH_2CH_2CH_3$	*in vacuo*	−157.14	126	−57.51	−55.72	55.42	124	−56.70	−56.79	2.239
		H_2O	−151.33	146	178.30	178.45	49.03	118	−58.12	−59.72	1.606

that a chemical stimulus combines with a receptor in such a way that the stimulus−receptor combination leads to neural activity and eventually to taste sensations. In the case of the enhancement of savory flavors in food due to the presence of umami components such as MSG and purine mononucleotides, the data so far accumulated (*29*) indicate (a) the effectiveness of MSG as a flavor enhancer without the contribution of 5′-ribonucleotides; (b) the flavor potentiating effect of IMP and GMP in the absence of MSG; (c) the unique ability of these flavor enhancers to bring about an improvement in the flavors of foods containing distinctly different attributes (e.g., beef vs chicken) and to modify such attributes; (d) the synergism

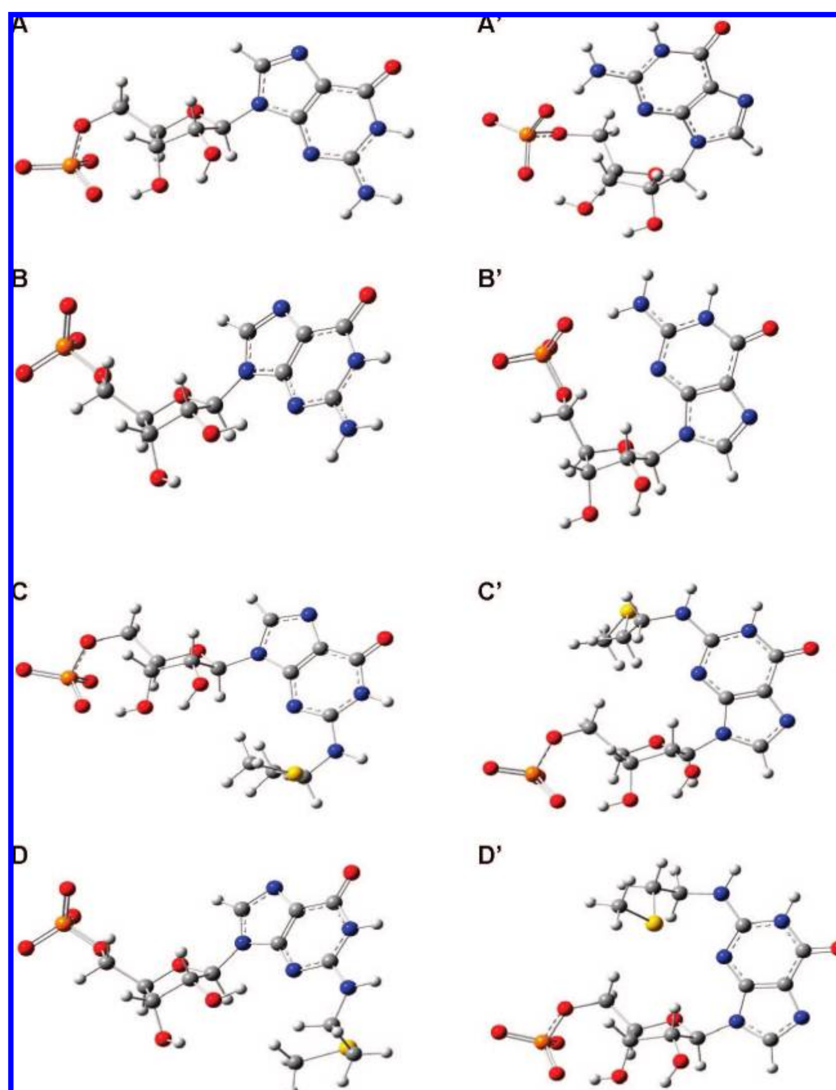


Figure 4. Calculated geometries of guanine ribonucleotides. GMP: *in vacuo* (**A**, *anti*; **A′**, *syn*); in water (**B**, *anti*; **B′**, *syn*). Dianion of compound **6d**: *in vacuo* (**C**, *anti*; **C′**, *syn*); in water (**D**, *anti*; **D′**, *syn*).

between MSG and ribonucleotides and, as a consequence, the possibility of L-glutamate concentrations being reduced in foods by addition of small amounts of IMP and/or GMP (*4*).

Inspection of **Figure 2** suggests that the synergistic activity of N^2-substituted guanylic acids is related to the chain length of the alkyl or acyl substituent. In both of these series such activity reaches a maximum, i.e., about 6 times the activity of IMP, for a number of terms equal to six (including NH and S as an equivalent of CH_2). It can be noted that the synergistic efficacy decreases slightly by replacing the CH_2 group in the α-position of the aliphatic chain with a carbonyl group (cf. **6a** vs **9a**, **6e** vs **9d**, and **6b** vs **9b**). An opposite effect was observed when the CH_2 group in the γ- or δ-position of the alkyl chain was substituted with an S atom (cf. **6a** vs **6d** and **6e**), while in the case of acyl chains the effect appears to be noticeable only for the sulfur substitution in the δ-position (cf. **9e** and **9c** vs **9f** and **9d** and also **9g**). The contribution of S atoms present in the N^2-substitutent of GMPs to the synergistic effect is understandable, considering the importance of sulfur compounds in the formation of flavors (*12, 30*), in particular of meat flavors. This fact, together with the above SAR data, indicates that the substituent linked to the exocyclic NH of the guanine moiety is actively implicated in the sensory perception of MSG, in agreement with Kuninaka's hypothesis (*3*) on the binding mode of 5′-ribonucleotides to the taste receptor.

A number of theories have been proposed to explain the taste potentiating phenomenon (*29*). Most of them are interrelated, and all invoke combined allosteric interactions between receptor proteins and flavor enhancement molecules. In particular, it has been suggested that the function of GMP is to unmask the receptor site for the L-glutamate ion (as well as that of other chemical stimuli), making it more available for the attachment of its specific ligand (*29*). It is known that thermodynamic and conformational parameters are of key importance in describing the structure and stability of nucleotide/protein (enzyme) complexes. For instance, it was found that the activation of glycogen phosphorylase *b* by purine nucleotides requires the latter to be as an *anti* conformer (*31*). Taking account of these considerations, we have carried out theoretical calculations to estimate conformational preferences and related energies of IMP, GMP, and some of the GMP derivatives we have synthesized. Calculations were performed assuming the mononucleotide to be present as a dianion both in gas phase and in aqueous solution, the former condition being useful to gain insight into the intrinsic features of the molecule.

The data of **Table 2** show that the *anti* conformation is energetically favored in all of the purine ribonucleotides examined, both in aqueous solution and *in vacuo*, with the exception of GMP *in vacuo*. In addition, the *anti* conformers are characterized by very small differences in the geometrical parameters of the nucleoside moiety. So, a common global geometry can be assigned to IMP, GMP, compounds **6a**,**d** and **9a**,**c**,**d** both in gas (χ, ca. $-150°$; P, ca. 125°) and in condensed phase (χ, ca. $-170°$; P, ca. 139°), apart from the orientation of the 5′-phosphoryl group (β and γ are g^- *in vacuo* but *t* in water) (see **Figures 3** and **4A−D**). By contrast, structural dissimilarities are evident among the 5′-nucleotides in the two sets of *syn* conformers. In particular, we can note a marked difference in the orientation of the phosphoryl group between the couple IMP/GMP and the other compounds in water and also an anomalous behavior of GMP *in vacuo*. In the latter condition *syn*-GMP shows a lower energy than the *anti* conformer and a unique global geometry. The intrinsically more stable *syn* conformation of the GMP dianion (cf. **Figure 4A′**) may be explained as due to electrostatic interaction between the positively charged exocyic NH_2 (*26, 32*) and the phosphate group as well as to a hydrogen bond between the same amino group (weakly pyramidalized) (*(26)*) and one oxygen atom of PO_3^{2-} (N^2---O distance = 2.68 Å; N^2—H---O angle = 163.5°) Such factors of stabilization do not occur in *syn* conformers of N^2-substituted GMPs either *in vacuo* or in water (see compound **6d** in **Figure 4C′**,**D′**): in fact, in these compounds hydrogen bonding is precluded by a preferred orientation of the N^2HR group coplanar with the pyrimidine ring (R being an alkyl or acyl substituent). Such orientation may be the result of steric repulsion ($A^{(1,3)}$ strain) (*33*) and/or hydrophobic attractions.

If we assume that the continuity in the synergistic effect we observed going from IMP to **9b** (**Figure 2**) implies a substantial similarity in the conformation adopted by all of the nucleotides while interacting with the receptor protein, then the *anti* conformation appears to be the most probable one.

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Supporting Information Available: Synthesis of aldehydes **3a−e** and acyl chlorides **7a−g**; ^{1}H and ^{13}C NMR data of nucleosides **5a−e** and **8a−g**; MS, ^{1}H, ^{13}C, and ^{31}P NMR data of nucleotides **6a−e** and **9a−g**; and calculated minimum geometries of **1a**, **6a**, and **9a**,**c**,**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Yamaguchi, S. Basic properties of umami and its effects on food flavor. *Food Rev. Int.* **1998**, *14* (2 and 3), 139–176.
(2) Yamaguchi, S.; Ninomiya, K. What is umami? *Food Rev. Int.* **1998**, *14* (2 and 3), 123–138.
(3) Kuninaka, A. Taste and flavor enhancers. In *Flavor Research—Recent Advances*; Teranishi, R., Flath, R. A., Sugisawa, H., Eds.; Marcel Dekker: New York, 1981; pp 305–353, and references cited therein.
(4) Nagodawithana, T. W. *Savory Flavors*; Esteekay Associates: Milwaukee, WI, 1995; pp 297−333.
(5) Roininen, K.; Lähteenmäki, L.; Tuorila, H. Effect of umami taste on pleasantness of low-salt soups during repeated testing. *Physiol. Behav.* **1996**, *60*, 953–958.
(6) Maruyama, Y.; Pereira, E.; Margolskee, R. F.; Chaudhari, N.; Roper, S. D. Umami responses in mouse taste cells indicate more than one receptor. *J. Neurosci.* **2006**, *26*, 2227–2234.
(7) Chaudari, N.; Landin, A. M.; Roper, S. D. A metabotropic glutamate receptor variant functions as a taste receptor. *Nat. Neurosci.* **2000**, *3*, 113–119.
(8) Toyono, T.; Seta, Y.; Kataoka, S.; Kawano, S.; Shigemoto, R.; Toyoshima, K. Expression of metabotropic glutamate receptor group I in rat gustatory papillae. *Cell Tissue Res.* **2003**, *313*, 29–35, and references cited therein.
(9) Imai, K.; Marumoto, R.; Kobayashi, K.; Yoshioka, Y.; Toda, J.; Honjo, M. Synthesis of compounds related to inosine 5′-phosphate and their flavor enhancing activity. IV. 2-Substituted inosine 5′-phosphate. *Chem. Pharm. Bull.* **1971**, *19*, 576–586.
(10) Mizuta, E.; Toda, J.; Suzuki, N.; Sugibayashi, H.; Imai, K.; Nishikawa, M. Structure-activity relationship in the taste effect of ribonucleotide derivatives. *Chem. Pharm. Bull.* **1972**, *20*, 1114–1124.
(11) Yamaguchi, S.; Yoshikawa, T.; Ikeda, S.; Ninomiya, T. Measurement of the relative taste intensity of some L-α-amino acids and 5′-nucleotides. *J. Food Sci.* **1971**, *36*, 846–849.
(12) Kuninaka, A.; Kumagai, M.; Fujiyama, K.; Ogura, M.; Sakata, S.; Yonei, S. Flavor activity of sulfur-containing compounds

related to flavor nucleotides. Agric. Biol. Chem. **1980**, *44*, 1437–1439, and references cited therein.
(13) Nagashima, N.; Iitaka, Y. The crystal structures of barium inosine-5′-phosphate and disodium inosine-5′-phosphate. *Acta Crystallogr.* **1968**, *B24*, 1136–1138.
(14) Defrancq, E.; Leterme, A.; Pelloux, N.; Lhomme, M.; Lhomme, J. Arylamidation de la guanosine par un agent cancérogène, le 2-aminofluorène. Approche intramoléculaire. *Tetrahedron* **1991**, *47*, 5725–5736.
(15) Natrella, M. G. *Experimental Statistics*; Dover Publications: Mineola, NY, 2005.
(16) Yamaguchi, S. The synergistic taste effect of monosodium glutamate and disodium 5′-inosinate. *J. Food Sci.* **1967**, *32*, 473–478.
(17) Chang, G.; Guida, W. C.; Still, W. C. An internal-coordinate Monte Carlo method for searching conformational space. *J. Am. Chem. Soc.* **1989**, *111*, 4379–4386.
(18) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. Semianalytical treatment of solvation for molecular mechanics and dynamics. *J. Am. Chem. Soc.* **1990**, *112*, 6127–6129.
(19) Sako, M.; Kawada, H.; Hirota, K. A convenient method for the preparation of N^2-ethylguanine nucleosides and nucleotides. *J. Org. Chem.* **1999**, *64*, 5719–5721, and references cited therein.
(20) Kemal, O.; Reese, C. B. Sodium borohydride reduction of adducts of primary amines with aldehydes and *p*-thiocresol. The alkylation of heterocyclic and aromatic amino-compounds. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1569–1573.
(21) Yoshikawa, M.; Kato, T.; Takenishi, T. Studies of phosphorylation. III: Selective phosphorylation of unprotected nucleosides. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 3505–3508.
(22) Fan, Y.; Gaffney, B. L.; Jones, R. A. Transient silylation of the guanosine O6 and amino groups facilitates *N*-acylation. *Org. Lett.* **2004**, *6*, 2555–2557, and references cited therein.
(23) Sundaralingam, M. Structure and conformation of nucleosides and nucleotides and their analogues as detemined by X-ray diffraction. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 3–42, and references cited therein.
(24) Meier, C.; Boche, G. ^{1}H und^{13}C-NMR-Konformationsanalysen und minimal-potential-energie-rechnungen an desoxyguanosin-, guanosin- und 5′-guanosinmonophosphat-addukten des grenzcarcinogens 4-methylanilin. *Chem. Ber.* **1990**, *123*, 1707–1713, and references cited therein.
(25) West, R. T.; Garza, L. A., II; Winchester, W. R.; Walmsley, J. A. Conformation, hydrogen bonding and aggregate formation of guanosine 5′-monophosphate and guanosine in dimethylsulfoxide. *Nucleic Acids Res.* **1994**, *22*, 5128–5134.
(26) Foloppe, N.; Hartmann, B.; Nilsson, L.; MacKerell, A. D., Jr. Intrinsic conformational energetics associated with the glycosyl torsion in DNA: a quantum mechanical study. *Biophys. J.* **2002**, *82*, 1554–1569, and references cited therein.
(27) IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Abbreviations and symbols for the description of conformations of polynucleotide chains. *Eur. J. Biochem.* **1983**, *131*, 9–15.
(28) Altona, C.; Sundaralingam, M. Conformational analysis af the sugar ring in nucleosides and nucleotides. A new description using the concept of psudorotation. *J. Am. Chem. Soc.* **1972**, *94*, 8205–8212.
(29) Nagodawithana, T. Flavor enhancers: their probable mode of action. *Food Technol.* **1994**, *7*, 9–85, and references cited therein.
(30) Widder, S.; Lüntzel, C. S.; Dittner, T.; Pickenhagen, W. 3-Mercapto-2-methylpentan-1-ol, a new powerful aroma compound. *J. Agric. Food Chem.* **2000**, *48*, 418–423, and references cited therein.
(31) Chachaty, C.; Forchioni, A.; Morange, M.; Buc, H. Conformations of purine ribosyl 5′-nucleotides bound to glycogen phosphorylase *b*. *Eur. J. Biochem.* **1978**, *82*, 363–372.
(32) Blackburn, G. M. DNA and RNA structure. In *Nucleic Acids in Chemistry and Biology*; Blackburn, G. M., Gait, M. J., Eds.; Oxford University Press: Oxford, U.K., 1996; pp 15–82.
(33) Eliel, E. L.; Wilen, S. H. *Stereochemistry of organic compounds*; John Wiley & Sons: New York, 1994; pp 737–740.

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