

Purine 5'-Ribonucleotide-L-Glutamate Hybrids As Potential Tools To Investigate The Mechanism Of *Umami* Taste Reception

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Abstract: *Umami* taste is elicited predominantly by monosodium glutamate (MSG) and purine 5'-ribonucleotides, in particular guanosine and inosine 5'-monophosphates (GMP and IMP). A significant peculiarity of *umami* compounds is their capacity to interact synergistically. A possible explanation of such phenomenon is that both L-glutamate and ribonucleotides may interact simultaneously with the "Venus flytrap" domain of T1R1/T1R3 *umami* receptor, but at different sites. Starting from this model, we reasoned that hybrid compounds, containing the two *umami* moieties covalently connected through flexible linkers of variable length, could be able to reach both *umami* receptor sites through a single molecule, thus giving an insight into the mechanism of synergism. MD simulations suggested that a chain of at least eight carbon atoms is requested to allow the interaction of both L-glutamate and 5'-ribonucleotide with their respective binding sites. We report here the synthesis of such hybrids starting from 2',3'-O-isopropylidene-5'-O-*t*-butyldimethylsilylguanosine.

Introduction

First identified in 1908 by K. Ikeda, *umami* has only recently been recognized as a basic taste quality after the discovery of specific *umami* receptors in the taste cell membranes.^[1,2]

Umami taste is an essential element in our appreciation of food and is mainly imparted by monosodium glutamate (MSG) and some purine 5'-ribonucleotides, in particular guanosine and inosine 5'-monophosphates (GMP and IMP). These compounds have been demonstrated to interact synergistically, and contribute to the attractive taste of many foods including vegetables (e.g. of tomato), by enhancing the sensory characteristics of other taste active components.^[1,2]

In recent years, many other substances that exhibit this taste have been identified in various natural sources^[1] or prepared by chemical synthesis, including free amino acids, organic acids and their derivatives, peptides, modified nucleotides etc.^[3,4,5,6]

However, at present only MSG, GMP and IMP are industrially relevant as they are currently used as food additives in particular as flavor enhancers in savory food manufacturing.^[3]

In the early 2000s, specific glutamate receptors were identified on taste buds: two metabotropic glutamate receptors 1 and 4 (mGluR1 and mGluR4) similar to the mGluR4 receptor usually present in the brain, and the heterodimer T1R1/T1R3.^[7,8,9] So far, eight candidate *umami* taste receptors were reported. They all, except for one, belong to the GPCRs superfamily (G protein-coupled receptors) and are distributed all over the tongue and even along the digestive tract.^[10]

Among them only one, the T1R1/T1R3 heterodimer in human is sensitive to both L-amino acids including glutamate, and ribonucleotides.^[10] Therefore it has been the most investigated for explaining the synergistic effect between glutamate and ribonucleotides, a hallmark of this taste quality, and it is today referred to as the *umami* receptor.

Using chimeric T1R receptors, site-directed mutagenesis and molecular modeling, Zhang *et al.* proposed that glutamate binds only on the T1R1 subunit of the heterodimer where the key ligand-binding residues of the mGluR1 metabotropic glutamate receptor are conserved. In particular, the glutamate binding site is present in an outer membrane N-terminal domain of the monomer T1R1 named as Venus flytrap (VFTD) that constitutes a characteristic motif of all T1R receptors belonging to the class C of GPCRs, along with metabotropic glutamate receptors (mGluRs). The VFTD consists of two lobes, which can remain open or close together in the open (inactive) or closed (active) conformations of the protein. Binding of L-glutamate causes the closure of the domain whereas binding of 5'-ribonucleotide would stabilize the closed conformation thus determining the synergistic effect.^[8,9]

Although this proposals has been supported by cell-based T1R1/T1R3 *umami* receptor assays performed on selected nucleotide derivatives,^[5,6] the molecular mechanism behind perception of the *umami* taste remain largely unclear.^[10]

Taking into account our previous experience on the synthesis of 5'-ribonucleotides with *umami*-enhancing activity,^[3,4] we

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reasoned that compounds containing the two *umami* moieties (L-glutamate and purine 5'-ribonucleotide) connected through a flexible spacer of suitable length (**Figure 1**) to interact with both *umami* receptor sites could help give an insight into the *umami* taste enhancement mechanism.

In this paper the synthesis of such hybrids compounds (**1**, **2**, **3** and **4**) is described.

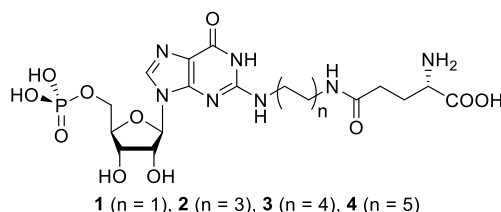


Figure 1. Target hybrid compounds **1**, **2**, **3** and **4**

Results and Discussion

Starting from the known structural data obtained through homology model of the T1R1 VFT domain,^[8] to explore the possibility to reach both the binding sites of *umami* ligands through a single molecule, as a preliminary approach aliphatic chains of different lengths were manually built connecting the exocyclic amino group of GMP to the γ -carboxyl group of MSG inside the receptor and the resulting complexes were subjected to molecular dynamics simulations. A number of methylene groups between 8 and 10 seems to allow an effective connection between the two moieties without strain. **Figure 2** reports the model of a GMP-MSG hybrid containing an 8-carbon linker after molecular dynamic simulations. All the key interactions of both MSG and GMP within the ligand-binding pocket were conserved.

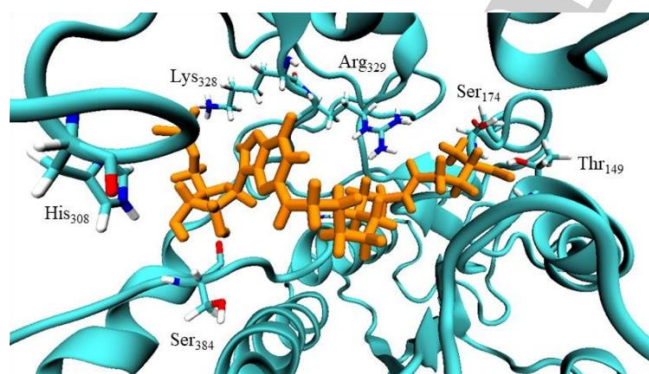
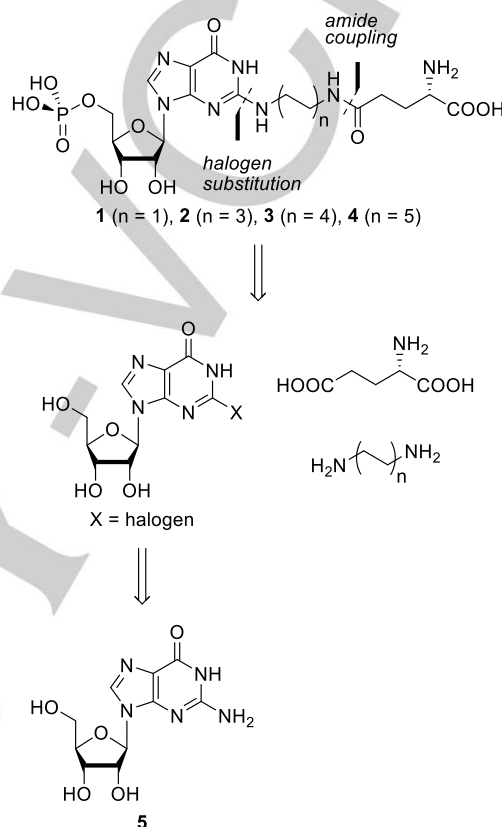


Figure 2. Molecular dynamics model of the guanosine-L-glutamate hybrid containing an 8-carbon linker bound in the ligand binding pocket.

Regarding the structure of the chain connecting the two *umami* moieties, it was planned taking into account that: 1) several *N*²-alkylated and *N*²-acylated derivatives of guanosine 5'-monophosphate have been demonstrated to possess *umami*-enhancing activity thus indicating that the exocyclic NHR group of the nucleotide is actively implicated in the synergism between GMP derivatives and MSG; 2) the amide bond between L-glutamate and the remaining chain would involve the γ -carboxyl

group as the α -amino acid portion interacts with a greater number of residues, some of which are essential for glutamate recognition. **Scheme 1** reports the retrosynthetic analysis elaborated to synthesize the target compounds (**1**, **2**, **3** and **4**), based on the use of guanosine (**5**) as a starting material. Its key steps were the conversion of its exocyclic amino moiety into a proper leaving group (*i.e.* a halogen), followed by substitution with diamines and the condensation of the resulting intermediates with the γ -carboxylic group of L-glutamic acid.

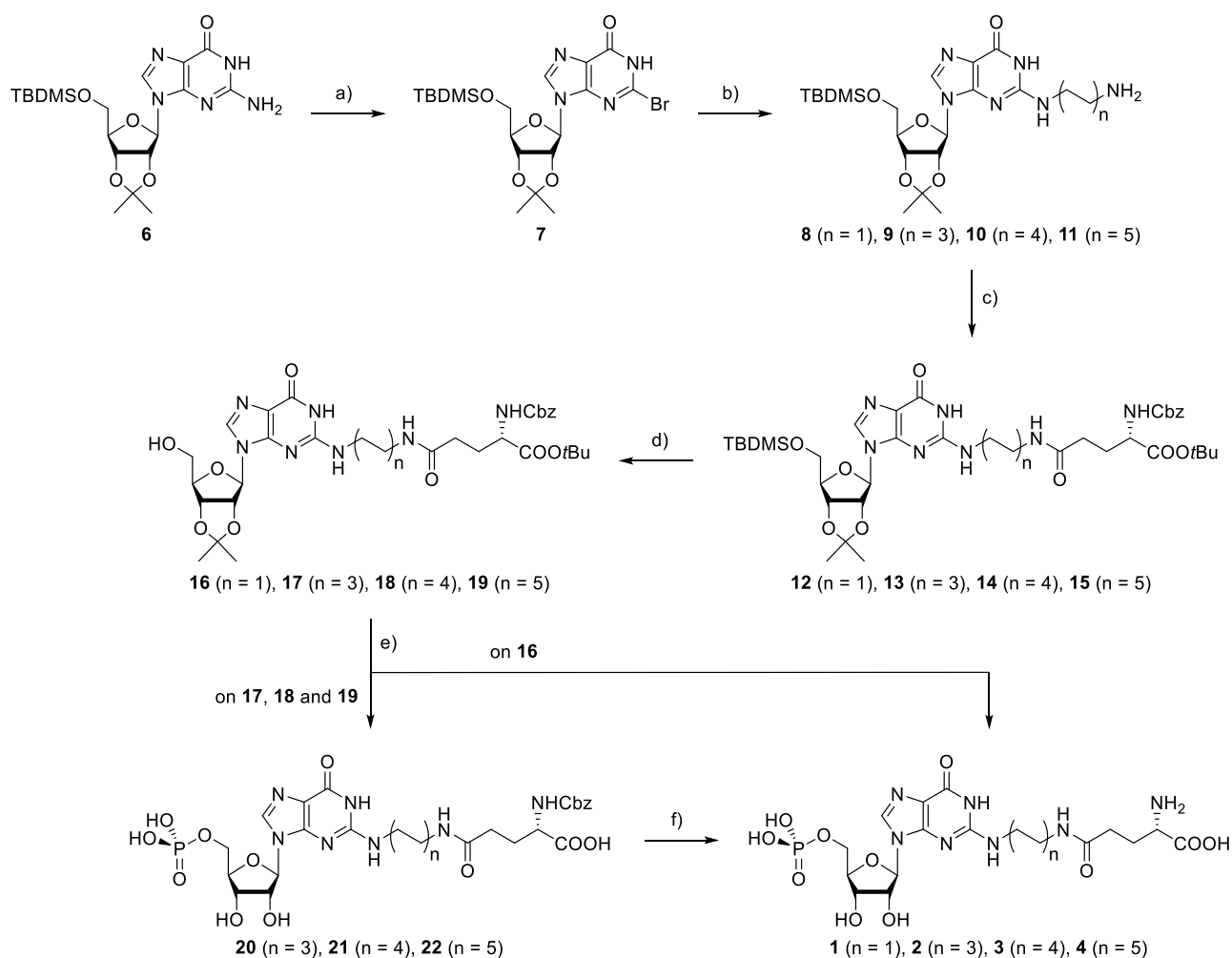


Scheme 1. Retrosynthetic analysis of target compounds **1-4**

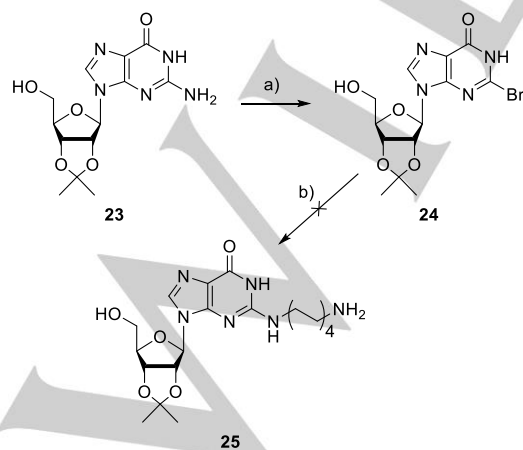
The overall reaction sequence is reported in **Scheme 2**.

A properly protected guanosine derivative, *i.e.* 2',3'-O-isopropylidene-5'-O-*t*-butyldimethylsilylguanosine (**6**) prepared according to a literature procedure,^[11] was used as starting material. As a result of a preliminary screening of halogenation reagents and conditions (see **Supporting Information S1.1**), bromine was selected as leaving group, because of the scarce yields, purity and stability of the iodo- and chloro derivatives. More in details, **6** was submitted to a nonaqueous diazotization/halodediazonation^[12] with a mixture of *t*-BuONO and TMSBr in CH_2Br_2 to obtain **7** in satisfactory yield (70%).

The introduction of a linker of variable length in position 2 was carried out by direct substitution of bromine with the proper diamines ($\text{H}_2\text{N}(\text{CH}_2\text{CH}_2)_n\text{NH}_2$, $n = 1, 3, 4$ and 5). After a solvent and temperature screening, the best results were obtained in 2-methoxyethanol at 85 °C, although these reactions required several days for completion (4-5 days). Furthermore, due to the low solubility of the products in organic solvents and of the diamines in water, an effective purification by standard methods was not feasible. Dilution of the reaction mixtures with EtOAc and



repeated washing with H₂O and Et₂O were therefore performed to remove the diamine excess, affording crude products **8**, **9**, **10** and **11** (detected by ESI-MS).



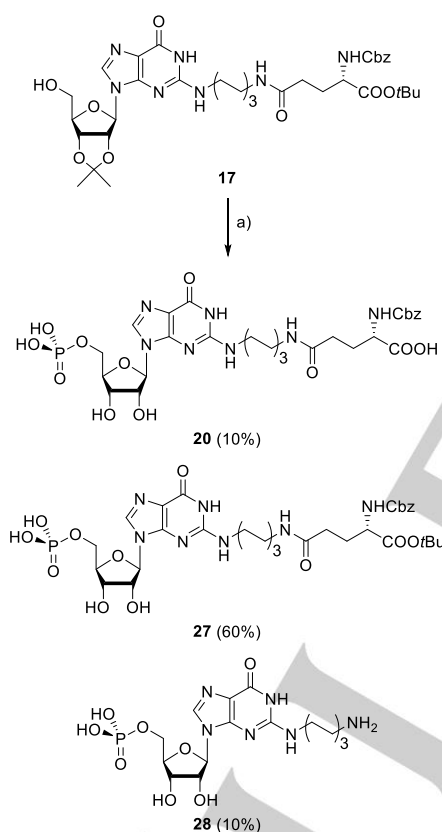
It is worth noting that the use of monoprotected diamines to simplify the purification step (e.g. *N*-Boc-1,4-diaminobutane) was not successful because of the concomitant removal of acid-labile protecting groups of the sugar moiety during the Boc deprotection. In addition, the amination product **25** was not achieved when partially protected 2-bromo-2',3'-O-isopropylideneinosine **24**^[13] (**Scheme 3**) was used as the substrate.

To determine the optimal conditions for the coupling of intermediates **8**, **9**, **10** and **11** with *N*-carbobenzyloxy-L-glutamic acid α-O-*t*-butyl ester (Z-L-Glu(α-OtBu)-OH, **26**), different solvents, condensing agents and temperatures were screened (see **Supporting Information S1.2**). The best results were obtained when DCC was selected as coupling agent at 25 °C, using EtOAc for **8-10** and 2-methoxyethanol for **11** as solvents to get **12-14** and **15**, respectively (42-64% yield). Subsequent treatment with TBAF to remove the *O*-*t*-butyldimethylsilyl group yielded intermediates **16**, **17**, **18** and **19**.

The last phosphorylation step was found to be the most challenging one due to a number of unexpected drawbacks. Phosphorylation was carried out following the procedure reported by Ikemoto *et al.*^[14] based on the use of triethylphosphate (TEP) as a solvent and POCl₃ as phosphorylating agent, involving the *in*

situ formation of an activated nucleoside-triethylphosphate complex. During the acidic hydrolysis of the intermediate dichlorophosphate, the complete removal of the 2',3'-O-isopropylidene was also observed in all compounds, as well as partial hydrolysis of the *t*-Bu ester (estimated around 10% by HPLC in the case of *n* = 3, **Scheme 4**). Furthermore, the same amount of the amine arising from removal of the γ -glutamyl residue was also detected (see **Supporting Information S1.3**). Therefore, in order to convert **17** into **20** and increase the overall yield, the reaction crude was desalted with Amberlite XAD-4® resin and the resulting mixture was deprotected by treatment with TFA to form **20**. The same procedure was applied to **18** and **19** to obtain **21** and **22**, respectively.

The final hybrids **2**, **3** and **4** were obtained by removing the *N*-Cbz protecting group by catalytic hydrogenolysis. In the case of hybrid **1**, this last step was not required since the reaction with TFA caused the simultaneous deprotection of both the amino and the carboxy groups of intermediate **16**.



Scheme 4. Phosphorylation of **17** (Reagents and conditions (yield): a) i) POCl_3 , triethylphosphate, 0 °C (3–4 h); iii) H_2O , 6 M NaOH to pH 2; iv) 70 °C (2–4 h), v) semi-preparative HPLC.

Compounds **1–4** were submitted to a cell-based taste receptor assay.^[5,6] In particular, their ability to enhance the L-glutamate-induced response of a functionally expressed T1R1/T1R3 *umami* receptor was tested. Unfortunately, the desired activity was not observed for any of them. A possible explanation is the influence of the whole hybrid (chain and portions of *umami* residues interacting within the binding pockets) on the conformational changes induced in the receptor upon binding, that were not examined in great enough detail. In fact, it has been proven that 5'-ribonucleotides operate as allosteric *umami* taste enhancers by

binding after at a different position of VFTD and further stabilizing the closed (active) state of the protein when activated by L-glutamate. However, other *umami* compounds (e.g. L-theanine and *umami* peptides) also elicit conformational changes of T1R1/T1R3 but to different extent and with a binding modality that remain largely unclear.^[10]

Conclusion

Herein we describe the synthesis of “*umami* hybrids”, which may represent useful tools to investigate the nucleotide-glutamate synergism, an extremely relevant phenomenon from both a biological and practical viewpoint. Hybrids **1–4** have been obtained by linking together the two taste-active molecules GMP and MSG through flexible linkers and subjected to T1R1/T1R3 receptor assays. The optimal length of the linkers has been refined by molecular dynamic simulations.

It is to note that the currently available model of the *umami* receptor was obtained by homology modeling and also the positions of the bound ligands inside the receptor were assessed by computational methods.^[8] Beside this, several factors can help explain why the *umami* enhancing properties of compounds **1–4** were not as expected. Recently, the idea that large ligands may bind differently from L-glutamic acid inside the *umami* receptor has been put forward.^[10] In this context, our hybrid compounds might be better assimilated to high-molecular weight ligands than to simple L-glutamic acid derivatives. In addition, it has been proposed that the binding of glutamate to the receptor should precede the stabilization of its closed conformation due to the binding of GMP.^[9] Connecting the two species with a linker in the hybrid compounds could then interfere with the proper temporal sequence of events required for the synergistic enhancement of receptor activation. It is anyway worth noting that our approach to “*umami* hybrids” is novel and has never been reported so far. Our results could give some hints for further investigations on the phenomenon of synergism and “*umami* hybrids”, possibly modified in the linker chain, could be still useful in studies on the *umami* receptor.

Experimental Section

Material and Methods

All reagents were purchased from Sigma-Aldrich (Milan, Italy) and/or from VWR International and were used without further purification. All the solvents were of HPLC grade. Analytical Thin Layer Chromatography TLC was performed on silica gel 60 F₂₅₄ or 60 RP-18 F254s precoated aluminum sheets (0.2 mm layer; Merck, Darmstadt, Germany); components were detected under an UV lamp (λ 254 nm), by spraying with a ceric sulfate/ammonium molybdate solution or with a ninhydrin solution [5% (w/v) ninhydrin in ethanol], followed by heating at about 150 °C. Hybrid compounds were detected by exposing the TLC sheets to iodine vapors. Amberlite XAD-4® was purchased from Supelco (Sigma-Aldrich). Product purification was accomplished either by flash chromatography (silica gel 60, 40–63 mm, Merck) or by semi-preparative HPLC using an Amersham pharmacia biotech (P900) liquid chromatographer equipped with a UV-vis detector. Chromatographic conditions were as follows: column, Jupiter® 10u Proteo 90A (10 μm , 250 x 4.6 mm (analytical HPLC) and 250 x 10.0 mm (semi-preparative HPLC), Phenomenex); flow rate, 0.5 mL·min⁻¹ (analytical HPLC) and 5 mL·min⁻¹ (semi-preparative HPLC); λ , 226 and 250 nm; mobile phase: A (0.1% TFA (v/v) in H_2O) and B

(acetonitrile-0.1% of TFA in H₂O, 8:2), gradient elution from 5% to 100% B in 45 min.

¹H, ¹³C and ³¹P NMR spectra were recorded at 400.13, 100.61 and 161.98 Hz, respectively, on a Bruker AVANCE 400 spectrometer equipped with a TOPSPIN software package (Bruker, Karlsruhe, Germany) at 300 K, unless stated otherwise. ¹H and ¹³C chemical shifts (δ) are given in parts per million and were referenced to the solvent signals (δ_{H} 7.26 - δ_{C} 77.16, δ_{H} 2.50 - δ_{C} 39.52 and δ_{H} 4.79 ppm from tetramethylsilane (TMS) for CDCl₃, DMSO-*d*₆ and D₂O respectively). ³¹P chemical shifts are referred to 85% H₃PO₄ as external standard (δ_{P} 0.00 ppm).

¹H signals were assigned with the aid of ¹H-¹H correlation spectroscopy (¹H-¹H COSY). ¹³C NMR signal multiplicities were based on APT (attached proton test) spectra. ¹³C NMR signals were assigned with the aid of ¹H-¹³C correlation experiments (heteronuclear multiple quantum correlation spectroscopy, HMQC, and heteronuclear multiple bond correlation spectroscopy, HMBC).

Electrospray ionization mass spectra (ESI-MS) were recorded on a Thermo Finnigan LCQ Advantage spectrometer (Hemel Hempstead, Hertfordshire, U.K.).

The protein initial structure was supplied by Zhang *et al.* who obtained it through homology modeling.^[8] A binuclear compound comprising a MSG and a GMP moiety was manually docked in the binding pocket. The system was simulated in explicit water solvent with periodic boundary conditions. The protein was described using the amber99SB-ILDN force field^[15] while TIP3P model was adopted for water.^[16] The ligand was described with the General amber Force Field (GAFF).^[17] The system was submitted to geometry optimization using the steepest descent method (50000 steps). Then, a 200 ps equilibration in the NVT ensemble was carried out, followed by a second equilibration of further 200 ps in the NPT ensemble. During the equilibration, c_{α} were restrained to crystallographic positions. Equilibration phase was followed by a 100 ns long unrestrained production run in the NPT ensemble. Temperature and pressure were kept constant to their reference values (1 bar, 300 K respectively) through the velocity rescale algorithm^[18] and the Berendsen barostat.^[19] A 14 Å cutoff was applied for non-bonded interactions and the Particles Mesh Ewald algorithm was employed to calculate long range electrostatic interactions.^[20] During the MD simulations all bonds lengths were constrained to their equilibrium values with the LINCS algorithm.^[21] allowing time step of 2 fs. Simulations and subsequent analysis were performed with the GROMACS 5.0.7 program suite.^[22] Cell-based taste receptor assays were carried out according to literature.^[5,6]

Experimental procedures

2',3'-O-Isopropylidene-5'-O- β -butyldimethylsilylguanosine (6). The title product was synthesized according to a previously reported procedure.^[11]

¹H NMR (400 MHz, DMSO-*d*₆): δ = 12.05 (br s, 1H), 7.79 (s, 1H), 6.29 (br s, 2H), 6.02 (br d, J = 2.3 Hz, 1H), 5.19 (br dd, J = 6.0, 2.0 Hz, 1H), 4.94 (dd, J = 6.2, 2.8 Hz, 1H), 4.36 (dd, J = 7.0, 4.0 Hz, 1H), 3.87 (dd, J = 11.2, 4.0 Hz, 1H), 3.80 (dd, J = 11.3, 4.0 Hz, 1H), 1.64 (s, 3H), 1.43 (s, 3H), 0.90 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ = 159.0, 153.6, 151.3, 136.4, 117.4, 114.1, 90.3, 87.0, 84.7, 81.2, 63.5, 27.3, 25.5, 25.9, 18.4, -5.4, -5.5. MS (ESI⁺): m/z calcd for [C₁₉H₃₁N₅O₅Si]⁺: 437.21; found: 438.1 [M+H]⁺, 460.2 [M+Na]⁺, 897.2 [2M+Na]⁺.

2-Bromo-2',3'-O-isopropylidene-5'-O- β -butyldimethylsilylguanosine (7). TMSBr (1.20 mL, 9.09 mmol) and *t*-BuONO (2.40 mL, 20.18 mmol) were added dropwise at -10 °C to a solution of 6 (438 mg, 1.00 mmol) in CH₂Br₂ (7.0 mL) under inert atmosphere. The resulting dark brown solution was protected from light exposure and stirred at -10 °C for 2 h, allowed to warm and stirred at 5-10 °C for 5 h. The solution was added dropwise to a 1:1 CH₂Cl₂/saturated aqueous NaHCO₃ mixture (300 mL) and vigorously stirred at 0 °C for 10'. After separating the layers, the aqueous one was extracted with cold CH₂Cl₂ (1x70 mL), then the reunited organic phases were washed with H₂O (2x70 mL) and brine (1x70 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure and the resulting yellow foam was purified by flash chromatography (CH₂Cl₂-

MeOH, 95:5) to obtain 7 as a yellow crystalline solid (350 mg, 0.70 mmol, 70%).

¹H NMR (400 MHz, CDCl₃): δ = 12.85 (br s, 1H), 8.10 (s, 1H), 6.15 (d, J = 2.8 Hz, 1H), 5.06 (dd, J = 6.1, 2.6 Hz, 1H), 4.95 (dd, J = 6.1, 2.7 Hz, 1H), 4.41 (q, J = 3.4 Hz, 1H), 3.92 (dd, J = 11.4, 3.4 Hz, 1H), 3.83 (dd, J = 11.3, 3.7 Hz, 1H), 1.66 (s, 3H), 1.42 (s, 3H), 0.91 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ = 158.9, 148.3, 138.8, 133.1, 123.8, 114.4, 90.7, 87.1, 85.2, 81.1, 63.5, 27.3, 25.4, 25.9, 18.4, -5.4, -5.5. MS (ESI⁺): m/z calcd for [C₁₉H₂₉⁷⁹BrN₄O₅Si]⁺: 500.11; found: 523.0 [(⁷⁹Br)M+Na]⁺, 525.1 [(⁸¹Br)M+Na]⁺, 1023.0 [2(⁷⁹Br)M+Na]⁺, 1025.0 [(⁷⁹Br)M+(⁸¹Br)M+Na]⁺, 1027.0 [2(⁸¹Br)M+Na]⁺.

General procedure for the 2-amination of 7 (intermediates 8, 9, 10 and 11). A solution of 7 (1.30 mmol) and the proper diamine (6.50 mmol) in 2-methoxyethanol (5 mL) was stirred at 85 °C for 4-5 days. The reaction progress was monitored by TLC (CH₂Cl₂-MeOH, 95:5 or AcOH-BuOH-H₂O, 1:4:1). The mixture was then diluted with EtOAc (10 mL) and repeatedly washed with H₂O (4x5 mL). The organic phase was dried over Na₂SO₄ and the solvent was removed under reduced pressure to obtain 8, 9, 10 or 11 as crude products, which were not further purified.

N⁶-(2''-Aminoethyl)-2',3'-O-isopropylidene-5'-O- β -butyldimethylsilylguanosine (8). MS (ESI⁺): m/z calcd for [C₂₁H₃₆N₆O₅Si]⁺: 480.25; found: 481.3 [M+H]⁺, 504.3 [M+Na]⁺, 961.6 [2M]⁺, 983.6 [2M+Na]⁺.

N⁶-(6''-Aminoheptyl)-2',3'-O-isopropylidene-5'-O- β -butyldimethylsilylguanosine (9). MS (ESI⁺): m/z calcd for [C₂₅H₄₄N₆O₅Si]⁺: 536.31; found: 537.3 [M+H]⁺, 559.3 [M+Na]⁺.

N⁶-(8''-Aminooctyl)-2',3'-O-isopropylidene-5'-O- β -butyldimethylsilylguanosine (10). MS (ESI⁺): m/z calcd for [C₂₇H₄₈N₆O₅Si]⁺: 564.35; found: 565.6 [M+H]⁺.

N⁶-(10''-Aminodecyl)-2',3'-O-isopropylidene-5'-O- β -butyldimethylsilylguanosine (11). MS (ESI⁺): m/z calcd for [C₂₉H₅₂N₆O₅Si]⁺: 592.38; found: 593.8 [M+H]⁺, 1185.9 [2M+H]⁺.

General procedure for the coupling with Z-L-Glu(α -O β Bu)-OH (intermediates 12, 13, 14 and 15). Z-L-Glu(α -O β Bu)-OH (0.96 mmol) and DCC (1.20 mmol) were added to a mixture of crude products 8, 9, 10 or 11 (0.80 mmol) in either EtOAc or 2-methoxyethanol (10 mL) and the resulting reaction was stirred at RT for 5 days. The precipitate was filtered off and the filtrate solution was diluted with EtOAc (10 mL), then washed with 1M HCl (2x5 mL), saturated aqueous NaHCO₃ (5 mL) and brine (5 mL). The organic phase was dried over Na₂SO₄, evaporated under reduced pressure and the resulting crude was purified by flash chromatography (CH₂Cl₂-MeOH, 95:5) to obtain 12, 13, 14 or 15 as pale yellow solids (42-64% yield).

N⁶-(2''-(N-Carbobenzyloxy-O- β -butyl- γ -L-glutamyl)aminoethyl)-2',3'-O-isopropylidene-5'-O- β -butyldimethylsilylguanosine (12, n = 1). The title compound was obtained in 64% yield.

¹H NMR (400 MHz, CDCl₃): δ = 11.05 (br s, 1H), 8.08 (s, 1H), 7.51 (br s, 1H), 7.29-7.31 (m, 5H), 6.06 (d, 1H), 5.67-5.69 (br s, 1H), 5.25-5.23 (br s, 1H), 5.02-5.12 (m, 2H), 4.97-4.94 (m, 1H), 4.36-4.33 (m, 1H), 4.15-4.20 (m, 1H), 3.78-3.95 (m, 2H), 3.45-3.66 (m, 4H), 2.24-2.37 (m, 2H), 2.12-2.20 (m, 1H), 1.95-2.04 (m, 1H), 1.64 (s, 3H), 1.41 (s, 3H), 0.91 (s, 3H), 0.89 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ = 173.2, 171.1, 158.3, 156.2, 153.0, 151.6, 136.4, 128.4, 128.2, 127.9, 116.7, 114.1, 90.4, 87.1, 84.5, 81.3, 81.2, 66.7, 63.6, 54.4, 41.2, 38.6, 32.0, 28.3, 27.9, 24.9, 27.3, 25.9, 25.6, 18.4, 1.0, -5.4. MS (ESI⁺): m/z calcd for [C₃₈H₅₇N₇O₁₀Si]⁺: 799.39; found: 800.7 [M+H]⁺, 822.9 [M+Na]⁺, 1622.1 [2M+Na]⁺.

N⁶-(6''-(N-Carbobenzyloxy-O- β -butyl- γ -L-glutamyl)aminoheptyl)-2',3'-O-isopropylidene-5'-O- β -butyldimethylsilylguanosine (13, n = 3). The title compound was obtained in 50% yield.

¹H NMR (400 MHz, CDCl₃): δ = 11.74 (br s, 1H), 7.73 (s, 1H), 7.30-7.33 (m, 5H), 6.53 (br s, 1H), 6.02 (br s, 1H), 5.80 (m, 1H), 5.21 (m, 1H), 5.08 (m, 2H), 4.92 (m, 1H), 4.29 (m, 1H), 4.17-4.22 (m, 1H), 3.83-3.74 (m, 2H), 3.34-3.45 (m, 2H), 3.20-3.24 (m, 2H), 2.21-2.28 (m, 2H), 2.14-2.25 (m, 1H), 1.89-1.97 (m, 1H), 1.89-1.97 (m, 2H), 1.61-1.65 (m, 2H), 1.66 (s, 3H), 1.53-1.47 (m, 2H), 1.43 (s, 9H), 1.37 (s, 3H), 1.26-1.29 (m, 2H), 0.86 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ = 171.3, 170.1, 158.1, 155.5, 151.8, 150.2, 135.3, 127.0, 127.1, 127.5, 116.2, 112.9, 88.9,

85.9, 83.5, 80.4, 65.9, 62.6, 53.1, 41.0, 39.2, 32.6, 31.7, 28.9, 27.0, 26.3, 26.9, 26.3, 24.9, 24.5, 17.4, -6.4, -6.5. MS (ESI⁺): *m/z* calcd for [C₄₂H₆₅N₇O₁₀Si]⁺: 855.46; found: 878.4 [M+Na]⁺.

N⁶-(8''-(N-Carbobenzyloxy-O-*t*-butyl- γ -L-glutamyl)aminoethyl)-2',3'-O-isopropylidene-5'-O-*t*-butyldimethylsilylguanosine (14, n = 4). The title compound was obtained in 42% yield.

¹H NMR (400 MHz, CDCl₃): δ = 11.97 (s, 1H), 7.84 (s, 1H), 7.31-7.36 (m, 5H), 6.04 (d, *J* = 2.3, 1H), 5.81 (m, 1H), 5.25 (dd, *J* = 2.4, 6.1 Hz, 1H), 5.11 (m, 2H), 4.94 (dd, *J* = 2.8, 6.1 Hz, 1H), 4.36 (dd, *J* = 4.2, 6.1 Hz, 1H), 4.20-4.23 (m, 1H), 3.86 (dd, *J* = 4.2, 11.1 Hz, 1H), 3.77-3.81 (dd, *J* = 4.3, 11.1 Hz, 1H), 3.39-3.45 (m, 4H), 2.30-2.32 (m, 2H), 2.20-2.25 (m, 1H), 1.93-1.98 (m, 1H), 1.63 (s, 3H), 1.46 (s, 9H), 1.40 (s, 3H), 1.29-1.38 (m, 14H), 0.88 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H). ¹³C NMR (100 MHz, CDCl₃): δ = 172.3, 171.1, 159.5, 153.1, 152.9, 136.3, 135.6, 128.0, 128.1, 128.5, 113.9, 90.4, 87.1, 84.6, 81.5, 66.9, 63.6, 54.1, 41.4, 32.7, 29.3, 28.9, 28.0, 27.2, 26.4-26.5, 25.9, 25.4, 18.4, -5.4. MS (ESI⁺): *m/z* calcd for [C₄₄H₆₉N₇O₁₀Si]⁺: 883.49; found: 884.6 [M+H]⁺.

N⁶-(10''-(N-Carbobenzyloxy-O-*t*-butyl- γ -L-glutamyl)aminodecyl)-2',3'-O-isopropylidene-5'-O-*t*-butyldimethylsilylguanosine (15, n = 5). The title compound was obtained in 45% yield.

¹H NMR (400 MHz, CDCl₃): δ = 11.84 (s, 1H), 7.66 (s, 1H), 7.29-7.36 (m, 5H), 6.02 (m, 1H), 5.77-5.79 (m, 1H), 5.28 (m, 1H), 5.11 (m, 2H), 4.93-4.95 (dd, *J* = 2.7, 5.9 Hz, 1H), 4.33 (m, 1H), 4.20-4.23 (m, 1H), 3.84 (dd, *J* = 3.7, 11.1 Hz, 1H), 3.79 (dd, *J* = 4.0, 11.1 Hz, 1H), 3.39-3.47 (m, 2H), 3.22 (m, 2H), 2.27-2.30 (m, 2H), 2.19 (m, 1H), 1.92-1.99 (m, 1H), 1.63 (s, 3H), 1.46 (s, 9H), 1.40 (s, 3H), 1.27-1.34 (m, 16H), 0.88 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 172.1, 171.2, 159.4, 152.8, 151.4, 136.3, 136.1, 128.5, 128.1, 128.0, 113.9, 90.2, 87.0, 84.5, 81.5, 66.9, 63.6, 54.2, 39.6, 32.6, 30.9, 29.4, 28.0, 27.3, 26.9, 25.9, 25.4, 24.8, 18.4, -5.4. MS (ESI⁺): *m/z* calcd for [C₄₆H₇₃N₇O₁₀Si]⁺: 911.52; found: 912.3 [M+H]⁺, 934.6 [M+Na]⁺.

General procedure for 5'-deprotection (intermediates 16, 17, 18 and 19). TBAF·3H₂O (161 mg, 0.51 mmol) was added under inert atmosphere to a solution of **12**, **13**, **14** or **15** (0.26 mmol) in THF (8 mL) and the reaction was stirred at RT for 3 h. The mixture was diluted with EtOAc (5 mL) and washed with H₂O (2x10 mL) and brine (1x10 mL). The organic phase was dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The resulting crude was purified by flash chromatography (CH₂Cl₂/MeOH 9:1) to obtain **16**, **17**, **18** or **19** as pale yellow solids (55-85% yield over two steps from **7**).

N⁶-(2''-(N-Carbobenzyloxy-O-*t*-butyl- γ -L-glutamyl)aminoethyl)-2',3'-O-isopropylideneguanosine (16, n = 1). The title product was obtained in 84% yield.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.72 (br s, 1H), 7.92 (s, 1H), 7.58 (br s, 1H), 7.32 (m, 5H), 6.48 (br s, 1H), 5.98 (br s, 1H), 5.29 (m, 1H), 5.02 (m, 2H), 4.91 (m, 1H), 4.12 (m, 1H), 3.89 (br s, 1H), 3.47-3.56 (m, 2H), 3.86-3.91 (m, 2H), 3.86-3.91 (m, 2H), 2.14-2.18 (m, 2H), 1.89-1.98 (m, 1H), 1.70-1.79 (m, 2H), 1.52 (s, 3H), 1.38 (s, 9H), 1.32 (s, 3H). ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 172.1, 171.8, 157.11, 156.6, 153.1, 151.6, 137.5, 128.8, 128.3, 128.2, 117.4, 113.5, 89.1, 86.9, 83.7, 81.0, 81.7, 65.9, 62.0, 54.7, 41.1, 38.5, 32.1, 28.1, 27.5, 27.5, 27.1, 25.6. MS (ESI⁺): *m/z* calcd for [C₃₂H₄₃N₇O₁₀]⁺: 685.31; found: 686.8 [M+H]⁺; 708.9 [M+Na]⁺; 1393.8 [2M+Na]⁺.

N⁶-(6''-(N-Carbobenzyloxy-O-*t*-butyl- γ -L-glutamyl)aminoethyl)-2',3'-O-isopropylideneguanosine (17, n = 3). The title product was obtained in 58% yield.

¹H NMR (400 MHz, CDCl₃): δ = 11.74 (br s, 1H), 7.73 (s, 1H), 7.33-7.30 (m, 5H), 6.53 (br s, 1H), 6.02 (br s, 1H), 5.80 (m, 1H), 5.21 (m, 1H), 5.08 (m, 2H), 4.92 (m, 1H), 4.29 (m, 1H), 4.17-4.22 (m, 1H), 3.83-3.74 (m, 2H), 3.34-3.45 (m, 2H), 3.20-3.24 (m, 2H), 2.21-2.28 (m, 2H), 2.14-2.25 (m, 1H), 1.89-1.97 (m, 1H), 1.89-1.97 (m, 2H), 1.61-1.65 (m, 2H), 1.66 (s, 3H), 1.53-1.47 (m, 2H), 1.43 (s, 9H), 1.37 (s, 3H), 1.26-1.29 (m, 2H). ¹³C NMR (100 MHz, CDCl₃): δ = 171.3, 170.1, 158.1, 155.5, 151.8, 150.2, 135.3, 127.5, 127.1, 127.0, 116.2, 112.9, 88.9, 85.9, 83.5, 80.4, 65.9, 62.6, 53.1, 41.0, 39.2, 32.6, 31.7, 28.9, 27.0, 26.3, 26.9, 26.3, 24.5. MS (ESI⁺): *m/z* calcd for [C₃₆H₅₁N₇O₁₀]⁺: 741.37; found: 742.4 [M+H]⁺; 764.4 [M+Na]⁺.

N⁶-(8''-(N-Carbobenzyloxy-O-*t*-butyl- γ -L-glutamyl)aminoethyl)-2',3'-O-isopropylideneguanosine (18, n = 4). The title product was obtained in 85% yield.

¹H NMR (400 MHz, CDCl₃): δ = 11.62 (s, 1H), 7.78 (s, 1H), 7.39-7.33 (m, 5H), 5.93 (m, 1H), 5.20 (m, 1H), 5.08 (m, 2H), 5.04 (m, 1H), 4.41 (m, 1H), 4.09-4.13 (m, 1H), 3.84-3.86 (m, 1H), 3.74-3.77 (m, 1H), 3.31 (m, 2H), 3.18 (m, 2H), 2.30-2.32 (m, 2H), 2.12-2.17 (m, 1H), 1.93-1.99 (m, 1H), 1.59 (s, 3H), 1.44 (s, 9H), 1.36 (s, 3H), 1.24-1.38 (m, 14H). ¹³C NMR (100 MHz, CDCl₃): δ = 172.3, 171.3, 157.0, 156.8, 153.9, 141.4, 139.8, 128.4, 127.9, 113.6, 91.6, 86.9, 84.4, 81.6, 66.7, 62.5, 54.5, 41.1, 39.6, 32.7, 28.9, 29.0, 28.0, 27.3, 26.7, 25.3. MS (ESI⁺): *m/z* calcd for [C₃₈H₅₅N₇O₁₀]⁺: 769.40; found: 770.4 [M+H]⁺, 792.5 [M+Na]⁺, 1539.4 [2M+H]⁺, 1561.4 [2M+Na]⁺.

N⁶-(10''-(N-Carbobenzyloxy-O-*t*-butyl- γ -L-glutamyl)aminodecyl)-2',3'-O-isopropylideneguanosine (19, n = 5). The title product was obtained in 55% yield.

¹H NMR (400 MHz, CDCl₃): δ = 11.44 (s, 1H), 7.55 (s, 1H), 7.29-7.39 (m, 5H), 6.08 (d, *J* = 4.1 Hz, 1H), 5.44 (m, 1H), 5.10 (m, 2H), 4.99 (m, 1H), 4.43 (m, 1H), 4.20 (m, 1H), 4.04 (m, 1H), 3.96 (m, 1H), 3.41 (m, 2H), 3.20 (m, 2H), 2.27 (m, 2H), 2.19 (m, 1H), 1.94 (m, 1H), 1.65 (m, 3H), 1.46 (m, 9H), 1.38-1.40 (m, 3H), 1.23-1.34 (m, 16H). ¹³C NMR (100 MHz, CDCl₃): δ = 172.2, 171.1 (C-6), 160.8, 153.3, 152.4, 137.9, 136.4, 128.5, 128.0, 127.6, 114.1, 89.7, 86.5, 83.2, 81.4, 66.9, 63.2, 54.2, 41.4, 39.7, 33.6, 31.6, 29.4, 28.0, 27.4, 26.7, 25.4. MS (ESI⁺): *m/z* calcd for [C₄₀H₅₉N₇O₁₀]⁺: 797.43; found: 798.1 [M+H]⁺; 820.5 [M+Na]⁺; 1617.4 [2M+Na]⁺.

General procedure for 5'-phosphorylation (product 1 and intermediates 20, 21 and 22). A suspension of **16**, **17**, **18** or **19** (0.13 mmol) in TEA (6.5 mL) was stirred at 50 °C for 15'. POCl₃ (75 μ L, 0.80 mmol) was added at 0 °C and the resulting transparent colourless mixture was stirred at 0 °C for 3-4 h. The reaction progress was monitored by RP-TLC (MeOH-H₂O, 4:1; R_f \approx 0.7-0.9). After adding H₂O (2 mL), pH was adjusted to 2 with 6M NaOH and the resulting solution was stirred at 70 °C for 3 h. The mixture was neutralized with 6M NaOH, diluted with H₂O (15 mL) and freeze-dried. The resulting crude was dissolved in H₂O (20 mL) and Amberlite XAD-4® resin was added (200 g/substrate mmol). The mixture was kept under mild stirring and the product adsorption was monitored by RP-TLC (MeOH-H₂O, 4:1; R_f \approx 0.7-0.9). The suspension was transferred in a column and washed with H₂O, monitoring the elution of Cl⁻ ions with a 1% AgNO₃ solution in H₂O. The column was then eluted with MeOH to recover the desired products mixture and the solvent was removed under reduced pressure. The resulting crude was dissolved in dry TFA (1.8 mL) and the solution was stirred for 15' at 0 °C. The reaction progress was monitored by RP-TLC (MeOH-0.1% TFA in H₂O, 1:1; R_f \approx 0.3-0.5). TFA was removed under reduced pressure and the residue was resuspended in Et₂O (5 mL), collected by filtration, washed with Et₂O (3x5 mL) and dried to get **1**, **20**, **21** or **22** as off-white solids (38-74% yield).

N⁶-(2''-(γ -L-Glutamyl)aminoethyl)-5'-guanylic acid (1, n = 1). The title product was obtained in 74% yield.

¹H NMR (400 MHz, CD₃OD): δ = 7.92 (s, 1H), 5.80 (br s, 1H), 4.53 (m, 1H), 4.12 (m, 1H), 4.06 (br s, 1H), 3.90-3.76 (m, 1H), 3.89-3.77 (m, 2H), 2.89-3.21 (m, 2H), 2.14-2.17 (m, 2H), 2.02-2.09 (m, 1H), 1.67-1.83 (m, 1H). ¹³C NMR (100 MHz, CD₃OD): δ = 183.1, 176.9, 168.5, 159.0, 151.6, 141.9, 121.0, 92.4, 85.6, 80.0, 72.4, 67.0, 57.1, 46.0, 33.2, 28.8, 27.2. ³¹P NMR (161 MHz, CD₃OD): δ = -0.39. MS (ESI⁺): *m/z* calcd for [C₁₇H₂₆N₇O₁₁P]⁺: 535.14; found: 535.2 [M]⁺; 558.1 [M+Na]⁺.

N⁶-(6''-(N-Carbobenzyloxy- γ -L-glutamyl)aminoethyl)-5'-guanylic acid (20, n = 3). The title product was obtained in 66% yield.

¹H NMR (400 MHz, D₂O): δ = 7.98 (s, 1H), 7.23-7.28 (m, 5H), 5.87 (br s, 1H), 4.90-5.02 (m, 2H), 4.64 (m, 1H), 4.41 (m, 1H), 4.22 (m, 1H), 3.95-4.05 (m, 2H), 3.81-3.86 (m, 1H), 3.19-3.30 (m, 2H), 3.05-3.15 (m, 2H), 2.96-3.05 (m, 2H), 2.15-2.27 (m, 2H), 2.00-2.11 (m, 1H), 1.75-1.82 (m, 1H), 1.46-1.56 (m, 2H), 1.35-1.45 (m, 2H), 1.18-1.34 (m, 4H). ³¹P NMR (161 MHz, D₂O): δ = 1.03. MS (ESI⁺): *m/z* calcd for [C₂₉H₄₀N₇O₁₃P]⁺: 741.725.24; found: 726.3 [M+H]⁺, 748.4 [M+Na]⁺.

N⁶-(8''-(N-Carbobenzyloxy- γ -L-glutamyl)aminoethyl)-5'-guanylic acid (21, n = 4). The title product was obtained in 38% yield.

¹H NMR (400 MHz, CD₃OD): δ = 8.88 (s, 1H), 7.25 (m, 5H), 5.99 (d, *J* = 3.4 Hz, 1H), 5.33 (m, 1H), 5.19 (m, 1H), 5.01 (m, 2H), 4.33-4.43 (m, 1H), 4.09-4.22 (m, 1H), 3.87-3.96 (m, 1H), 3.29-3.38 (m, 2H), 3.11-3.15 (m, 2H), 2.27 (m, 2H), 2.17 (m, 1H), 1.90 (m, 1H), 1.10-1.40 (m, 12H). ³¹P NMR (161 MHz, CD₃OD): δ = 1.93. MS (ESI⁺): *m/z* calcd for [C₃₁H₄₄N₇O₁₃P]⁺: 753.27; found: 752.2 [M-H]⁻.

N⁶-(10''-(N-Carbobenzyloxy-γ-L-glutamyl)aminodecyl)-5'-guanylic acid (22, n = 5).

The title product was obtained in 72% yield.

¹H NMR (400 MHz, CD₃OD): δ = 8.95 (s, 1H), 7.33 (5H), 6.01 (d, J = 3.1 Hz, 1H), 5.11 (m, 2H), 4.62 (m, 1H), 4.37 (m, 1H), 4.19 (dd, J = 4.6, 9.5 Hz, 1H), 4.07 (m, 1H), 3.82 (m, 1H), 3.45 (m, 2H), 3.15 (m, 2H), 2.32 (m, 2H), 2.19 (m, 1H), 1.97 (m, 1H), 1.66 (m, 2H), 1.33-1.42 (m, 16H). ³¹P NMR (161 MHz, CD₃OD): δ = 0.90. MS (ESI⁺): m/z calcd for [C₄₀H₆₀N₇O₁₃P]⁺: 781.30; found: 781.5 [M+H]⁺.

General procedure for removal of N-Cbz (2, 3 and 4). HCOONH₄ (58 mg, 0.92 mmol) was added under inert atmosphere to a suspension of **20**, **21** or **22** (0.06 mmol) and 10% Pd-C (23 mg) in a H₂O-MeOH mixture (1:1 v/v, 10 mL). The mixture was stirred at 95 °C for 3-4 h. The reaction progress was monitored by RP-TLC (MeOH-0.1% TFA in H₂O, 1:1; R_f ≈ 0.7-0.9). The reaction was filtered on celite, concentrated under reduced pressure and freeze-dried. The resulting crude was purified by semi-preparative RP-HPLC to get **2**, **3** or **4** as off-white solids (62-95% yield).

N⁶-(6''-(γ-L-Glutamyl)aminohexyl)-5'-guanylic acid (2, n = 3).

The title product was obtained in 93% yield.

¹H NMR (400 MHz, D₂O): δ = 7.92 (s, 1H), 5.69 (br s, 1H), 4.46 (m, 1H), 4.13 (m, 1H), 4.07 (m, 1H), 3.90-3.81 (m, 1H), 3.89-3.71 (m, 2H), 3.10-3.19 (m, 2H), 2.87-3.11 (m, 2H), 2.14-2.17 (m, 2H), 2.00-2.07 (m, 1H), 1.72-1.79 (m, 1H), 1.41-1.52 (m, 2H), 1.53-1.46 (m, 2H), 1.25-1.31 (m, 4H). ¹³C NMR (100 MHz, D₂O): δ = 181.7, 176.9, 167.2, 159.0, 152.2, 137.3, 117.1 88.0, 85.2, 76.9, 70.2, 64.2, 57.5, 41.0, 39.3, 24.1, 29.1, 28.8, 28.5, 28.2, 25.9. ³¹P NMR (161 MHz, D₂O): δ = 0.27. MS (ESI⁺): m/z calcd for [C₂₁H₃₄N₇O₁₁P]⁺: 591.21; found: 592.5 [M+H]⁺; 614.3 [M+Na]⁺.

N⁶-(8''-(γ-L-Glutamyl)aminoctyl)-5'-guanylic acid (3, n = 4).

The title product was obtained in 95% yield.

¹H NMR (400 MHz, D₂O): δ = 8.60 (s, 1H), 5.99 (d, J = 3.8 Hz, 1H), 4.63-4.65 (m, 1H), 4.37-4.39 (m, 1H), 4.27-4.28 (d, J = 0.28 Hz, 1H), 4.12-4.16 (m, 1H), 3.99-4.04 (m, 1H), 3.85-3.88 (m, 1H), 3.25-3.31 (m, 2H), 3.02-3.06 (m, 2H), 2.26-2.37 (m, 2H), 2.04-2.12 (m, 2H), 1.51 (m, 2H), 1.36 (m, 2H), 1.19-1.22 (m, 2H). ¹³C NMR (100 MHz, D₂O): δ = 174.1, 135.9, 88.9, 83.8, 74.3, 69.6, 63.9, 53.4, 41.0, 39.4, 31.4, 26.2, 28.1. ³¹P NMR (161 MHz, D₂O): δ = 0.11. MS (ESI⁺): m/z calcd for [C₂₃H₃₈N₇O₁₁P]⁺: 619.24; found: 618.4 [M+H]⁺.

N⁶-(10''-(γ-L-Glutamyl)aminodecyl)-5'-guanylic acid (4, n = 5).

The title product was obtained in 62% yield.

¹H NMR (400 MHz, D₂O): δ = 8.22 (s, 1H), 5.97 (d, J = 5.1 Hz, 1H), 4.71 (m, 1H), 4.43 (m, 1H), 4.27 (m, 1H), 4.06 (m, 1H), 3.72 (m, 1H), 3.36 (m, 2H), 3.06 (m, 2H), 2.32 (m, 1H), 2.08-2.05 (m, 2H), 1.38-1.43 (m, 2H), 1.16-1.35 (m, 16H). ¹³C NMR (100 MHz, D₂O): δ = 87.5, 83.2, 74.3, 69.9, 64.5, 53.9, 40.2, 39.1, 30.8, 27.4, 28.6. ³¹P NMR (161 MHz, D₂O): δ = 1.24. MS (ESI⁺): m/z calcd for [C₂₅H₄₂N₇O₁₁P]⁺: 647.27; found: 648.4 [M+H]⁺.

2',3'-O-Isopropylideneinosine (23).

The title product was synthesized according to literature.^[13]

¹H NMR (400 MHz, DMSO-d₆): δ = 10.55 (br s, 1H), 7.92 (s, 1H), 6.52 (br s, 2H), 5.93 (d, J = 2.8 Hz, 1H), 5.19 (dd, J = 6.3, 2.8 Hz, 1H), 5.11 (t, J = 5.4 Hz, 1H), 4.97 (dd, J = 6.3, 3.0 Hz, 1H), 4.12 (td, J = 5.1, 3.2 Hz, 1H), 3.56 (dd, J = 11.2, 4.8 Hz, 1H), 3.51 (dd, J = 11.6, 5.2 Hz, 1H), 1.51 (s, 3H), 1.31 (s, 3H). ¹³C NMR (100 MHz, DMSO-d₆): δ = 157.2, 154.2, 151.2, 136.3, 117.2, 113.5, 88.9, 87.1, 84.0, 81.6, 62.1, 27.5, 25.7. MS (ESI⁺): m/z calcd for [C₁₃H₁₇N₅O₅]⁺: 323.12; found: 345.9 [M+Na]⁺, 669.0 [2M+Na]⁺.

2-Bromo-2',3'-O-isopropylideneinosine (24). Under inert atmosphere, TMSBr (3.17 mL, 24.02 mmol) and t-BuONO (5.95 mL, 50.03 mmol) were added dropwise at -10 °C to a suspension of **23** (646 mg, 2.00 mmol) in CH₂Br₂ (10 mL). The resulting dark brown solution was protected from light exposure and allowed to warm to 5-10 °C for 6 h under stirring. The solution was added dropwise to a AcOEt/sat. NaHCO₃ mixture (1:1 v/v, 300 mL) and vigorously stirred at 0 °C for 10'. After separating the layers, the aqueous one was extracted with AcOEt (100 mL). The reunited organic phases were washed with H₂O (1x50 mL) and brine (1x50 mL) and dried over Na₂SO₄. The solvent was removed under reduced pressure and the resulting yellow foam was purified by flash column chromatography (CH₂Cl₂-MeOH, 95:5) to obtain **24** as a yellow powder (518 mg, 67%).

¹H NMR (400 MHz, DMSO-d₆): δ = 13.37 (br s, 1H), 8.30 (s, 1H), 6.05 (d, J = 2.7 Hz, 1H), 5.24 (dd, J = 6.2, 2.7 Hz, 1H), 5.08 (br t, J = 4.8 Hz, 1H),

4.92 (dd, J = 6.2, 2.7 Hz, 1H), 4.22 (td, J = 4.8, 2.8 Hz, 1H), 3.57-3.51 (m, 2H), 1.54 (s, 3H), 1.33 (s, 3H). ¹³C NMR (100 MHz, DMSO-d₆): δ = 157.6, 148.2, 139.2, 134.6, 123.9, 113.6, 89.9, 87.4, 84.3, 81.7, 61.9, 27.5, 25.6. MS (ESI⁺): m/z calcd for [C₁₃H₁₅BrN₄O₅]⁺: 386.02; found: 409.2 [(⁷⁹Br)M+Na]⁺, 411.0 [(⁸¹Br)M+Na]⁺, 795.1 [2(⁷⁹Br)M+Na]⁺, 797.1 [(⁷⁹Br)M+(⁸¹Br)M+Na]⁺, 799.1 [2(⁸¹Br)M+Na]⁺.

Conflict of interest

The Authors have no conflict of interest to declare.

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