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Novel N7- and N1- substituted cGMP derivatives are potent activators of cyclic nucleotide-gated channels^a

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Abstract

Cyclic nucleotide-gated (CNG) channels, key players in olfactory and visual signal transduction, generate electrical responses to odorant- and light-induced changes in cyclic nucleotide concentration. Previous work suggests that substitutions are tolerated solely at the C8 position on the purine ring of cGMP. Our studies with C8, 2'-OH, and 2-NH₂-modified cGMP derivatives support this assertion. To gain further insight into determinants important for CNG channel binding and activation, we targeted previously unexplored positions. Modifications at N7 of 8-SH-cGMP (**6**) are well tolerated by olfactory and retinal rod CNG channels. Tolerant of a very large substituent, a 3400 MW PEG, at either N7 or C8 argues for broad accommodation at these positions in the binding site. Modification at N1 of cGMP reduces the apparent affinity for the channel, however, when combined with 8-parachlorophenylthio-derivatization, the resulting cGMP analog is more potent than cGMP itself. These studies establish the N7 and N1 positions of cGMP as targets for modification in the design of novel CNG channel agonists.

Introduction

The essential role played by CNG channels in olfactory sensory neurons and retinal photoreceptor cells is well established; odorant- and light-induced changes in cyclic nucleotide concentrations gate these non-selective cation channels, thus generating electrical signals that are transmitted to the brain (reviewed in ¹⁻⁶). CNG channels are members of the voltage-gated K⁺ channel superfamily, however, despite global structural similarities, CNG channels are endowed with unique functional properties. They are voltage-independent, gated solely by cyclic nucleotide binding, and non-selective, with a significant fraction of the cationic current carried by Ca²⁺.⁴ Acting as a cyclic nucleotide-activated Ca²⁺ source, CNG channels are uniquely situated at the intersection of two second messenger signaling systems. Indeed, in both vision and olfaction, Ca²⁺ entry through CNG channels is crucial for sensory adaptation. Beyond these sensory systems, CNG channels have been detected in many other tissues including brain, heart, kidney, testis, liver, lung, skeletal muscle, adrenal gland, pancreas, and colon, yet their physiological roles are poorly understood⁷⁻⁹. Development of selective pharmacological agents will significantly accelerate the elucidation of CNG channel participation in cellular signaling. While progress has been made in generating potent activators and inhibitors, development of highly selective probes remains an important, yet elusive, goal¹⁰.

^aAbbreviations: COSY, COReletion SpectroscopY; CNG, cyclic nucleotide-gated; HMBC, heteronuclear multiple bond coherence; HSQC, heteronuclear single quantum correlation; pCPT, parachlorophenylthio.

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Native CNG channels are composed of different combinations of six pore-forming subunits, including both α (CNGA1-4) and β (CNGB1 & 3) subunits (reviewed in ^{2,4,6}). Many of the α subunits can be functionally expressed as homomultimeric channels, but co-expression of the β subunits confers distinct functional properties in terms of ion permeation, ligand sensitivity, gating mechanisms, and regulation. Rod channels are composed of two types of subunits: CNGA1 and CNGB1. Recent evidence suggests that the stoichiometry of the native rod photoreceptor channel is 3 CNGA1 to 1 CNGB1. The CNG channel in cone photoreceptors is composed of CNGA3 and CNGB3 subunits, and in contrast to rod channels, functional evidence suggests that the stoichiometry of the cone channel is 2:2. The native olfactory channel contains three subunit types, including CNGA2, CNGA4, and CNGB1b, in a ratio of 2:1:1.

The study of cyclic nucleotide analogs provides insight into how these second messengers bind and activate CNG channels, revealing a template for the development of selective agonists and antagonists. The earliest report of electrical recordings of a cGMP-stimulated cationic conductance in rod photoreceptors employed cyclic nucleotide derivatives; neither 5'-GMP nor 2', 3'-cGMP were active, demonstrating the importance of the cyclic phosphate¹¹. Another early study employing a Ca^{2+} -sensitive dye to report cGMP-stimulated cation flux in rod outer segment membranes, revealed that 250 μM 2'-deoxy-cGMP, 2'-O-butyryl-cGMP, and N2-butyryl cGMP were ineffectual¹². In contrast, 250 μM cGMP, 8-Br-cGMP, and 7-deaza-cGMP triggered identical rates of Ca^{2+} release. Further reports reinforced the importance of the 2'-OH for binding and showed that C8 is amenable to a wide range of substituents¹³⁻¹⁵. Brown et al. systematically studied the effect of C8 derivatives on native rod CNG channel activation, demonstrating an unfavorable impact of positive charge and a broad tolerance for apolar and negatively charged substituents¹⁶. Although many of the C8-substituted cGMP derivatives are more potent than cGMP itself, including the membrane permeant and widely used 8-parachlorophenylthio(pCPT)-cGMP, these compounds are also high-affinity activators of cGMP-dependent protein kinase^{17,18}. Some advances have been made in generating cyclic nucleotide-based channel antagonists by combining modifications at several positions on cGMP. Simultaneous derivatization of both 2-NH₂ and N1 positions of cGMP blocks the ability to activate native retinal CNG channels. However, in combination with C8 and phosphorothioate modifications, the resulting derivatives act as competitive antagonists^{10,19,20}, albeit with low affinity and uncertain specificity.

Here we have taken advantage of the enhanced reactivity of 8-thio-substituted cGMP analogs towards vinyl sulfones to examine the effect of modifications at N1 and N7 on heteromeric rod and olfactory CNG channel activation. These novel cGMP derivatives target positions previously unexplored with regard to activation of CNG channels. Additionally, we have investigated the impact of modifications at 2'-OH, 2-NH₂, and C8 relative to cGMP on heteromeric olfactory CNG channel activation. These studies provide new insight into the cyclic nucleotide determinants that are important for activation of CNG channels and identify two novel sites on the cyclic nucleotide that are amenable to modification.

Chemistry

8-SH-cGMP (**6**) is a useful starting material for the synthesis of a plethora of C8-modified cGMP derivatives^{16,18,21}. We investigated the reaction of vinyl sulfones, regarded as thiol-selective electrophiles, with **6**, expecting a single C8-S- adduct. Surprisingly, we observed the formation of approximately equivalent amounts of two distinct products upon reaction at pH 7.9 (Scheme 1). To further explore this unanticipated reactivity, we tested reaction of phenyl vinyl sulfone with 8-pCPT-cGMP (**13**), an 8-thio-cGMP derivative lacking a free thiol, and observed the formation of a single main product (Scheme 2). While vinyl sulfones are highly selective for reaction with thiols, given elevated pH (>9) and long reaction time (days)

significant reaction with amines can be achieved²². In order to definitively determine the site of reaction of vinyl sulfones with **6** and **13**, we employed heteronuclear multiple bond correlation (HMBC) spectroscopy, a 2D NMR technique which yields cross-peaks for ¹H / ¹³C pairs connected by 2 and 3 bonds. The ¹H resonances were assigned by their chemical shifts, ¹H / ¹H COSY experiments, multiplicities, and comparison with published spectra²³. Assignment of ¹³C resonances was aided by heteronuclear single quantum coherence (HSQC) spectroscopy, HMBC, and comparison with published spectra²⁴. Figure 1 displays selected regions of HMBC spectra of phenyl vinyl sulfone derivatives, showing that **6** (panel A) forms an N7 adduct (**10**), while reaction with **13** (panel B) yields an N1 adduct (**14**) (Schemes 1 and 2, respectively). Modification of 8-thio-guanosine at N7 has been achieved by reaction with allyl bromide to form a C8-S adduct which is then transformed to the 7-allyl, 8-thioxo product via a [3,3] sigmatropic rearrangement²⁵. More recently, an acyclic 8-thio-guanosine derivative was reported to react with n-butyl vinyl ether directly at N7²⁶.

The equivalent reactivity of the C8 thio-group and N7 of compound **6** is indicative of a thioketo-thioenol tautomerization that favors the thioketo form (Scheme 1). In fact, ¹⁵N NMR experiments show that 8-thio-guanosine exists as the thioketo form²⁷. The N7 vinyl sulfone derivatives, **8**, **10** and **12**, also adopt the thioketo form (not the charged tautomer) based on several lines of evidence. First, the ¹³C chemical shift for the carbon at the 8-position is sensitive to whether C8-S is a single or a double bond. Compound **6** and the N7- phenyl vinyl sulfone derivative, **10**, give ¹³C shifts of 167.9 and 166.2 ppm respectively, while the ¹³C chemical shifts for compounds with a single bond at C8-S, **7**, **9**, **14**, and **15**, are on average >20 ppm upfield: 140.9, 143.8, 140.2, and 147.8 ppm respectively. The presence of a positive charge at N7, as in compound **15**, has a relatively small impact on the ¹³C chemical shift at C8. Second, the uv-vis spectra of **6** and the N7- derivative, compound **8**, share a characteristic two-peak shape: λ_{max} = 288/302 nm and 288/304 nm respectively. The slight red-shift for **8** relative to **6**, is reminiscent of the red-shift observed for 7-ethylguanosine relative to guanosine²⁸. In contrast, the λ_{max} for C8-S alkyl derivatives such as compound **7** at 276 nm is significantly blue-shifted from the parent, **6**. Third, N7 derivatives that impose a positive charge at N7 such as 7-ethyl guanosine²⁸ and compound **15** are brightly fluorescent; none of the other compounds described here are. Fourth, the N7 derivatives of **6**, elute after the analogous C8-S derivatives. If these N7 derivatives adopted the charged tautomeric form at N7-C8-S we would expect them to display significantly reduced retention times on RP-HPLC. Last, **10** does not participate in a second round of Michael addition even when the vinyl sulfone reactant is present in excess. Dual alkylation of C8-S and N7 can be achieved by reaction of **6** with 1,3 dibromopropane (Scheme 3). The reaction proceeds via a rapid alkylation of C8-S, followed by a slower intramolecular reaction at N7 to form a 6-membered ring and impart a permanent positive charge to the purine ring system.

The presence of sulfur at C8 activates the purine ring for reaction with vinyl sulfones. In contrast to the 8-thio derivatives (**6** and **13**), cGMP is resistant to reaction, requiring a strong base and a large excess of vinyl sulfone to yield a 2'-O adduct (compound **16**) in relatively low yield (Scheme 4). Similar conditions have been employed in the methylation of cyclic nucleotides; reaction with iodomethane in the presence of NaOH has been reported to result in methylation of the 2'-O (cAMP) when DMF was included in the reaction or at N1 (cGMP) when DMF was omitted^{29,30}. Our interest in an alternative route to N1-modified cGMP derivatives compelled us to try both methods. In our hands, reaction of cGMP with iodomethane in the presence of NaOH with or without DMF yields predominantly 2'-O- methyl- (**3**) or 1-,2'-O-dimethyl-cGMP (**4**), depending on the temperature and reaction time. The 1-methyl-cGMP derivative is a minor product that is difficult to isolate in purity greater than 90% and was therefore not tested further. The source of the discrepancy between our results and those of Miller et al. is unclear but may derive from the different purification methods employed³⁰. We used HSQC and HMBC NMR analysis in conjunction with mass spectrometry to ensure correct structural

characterization. Full HSQC and HMBC spectra for key compounds (**3**, **4**, **9**, **10**, **14** and **15**) are provided as supporting information.

Results

The unexpected reactivity of vinyl sulfones with 8-thio-substituted cGMP derivatives allowed us to study the effects of modifications at N1 and N7 on the activation of CNG channels. Our investigation of cGMP reactivity led us to synthesize additional derivatives at the C8 and 2'-O positions and to examine N2- and 2'-O-butyryl derivatives. We have included these in our functional analysis for comparison (Table 1). We initially focused our efforts on heteromeric olfactory CNG channels because they are more sensitive to cGMP than retinal channels, thus facilitating the study of derivatives that bind poorly. We co-expressed CNGA2, CNGA4 and CNGB1b in HEK-293 cells and measured the sensitivity of the resulting channels to cAMP, as this property is a major indicator of subunit composition. Bonigk et al., showed that CNG channels produced by coexpression of CNGA2 with CNGA4 and CNGB1b were as sensitive to cAMP as native channels from the olfactory sensory cilium; the $K_{1/2}$ values of the heterologously expressed and native channels are 4.8 and 4.1 μ M, respectively³¹. In contrast coexpression of CNGA2 with either of CNGA4 or CNGB1b individually results in channels with markedly decreased cAMP sensitivity. We observed an average $K_{1/2}$ for activation by cAMP of 3.8 μ M, a strong indication that the channels are indeed heteromeric.

The commercially available monobutyryl cGMP derivatives, N2-butyryl- (**2**) and 2'-O-butyryl-cGMP (**5**), were full activators of the olfactory CNG channel, albeit with $K_{1/2}$ values 20- and 80- fold higher than cGMP, respectively (Table 1). Increasing the size of the substituent at 2'-O leads to decreasing apparent affinity for the channel; 2'-O-methyl (**3**), and 2'-O-butyryl (**5**) are 32- and 80-fold less potent than cGMP, respectively (Table 1). Preliminary experiments with 2'-O-methylsulfonyl-ethyl-cGMP (**16**) showed it to be a very poor activator with a $K_{1/2}$ at least 2.5 fold higher than **5** (data not shown). We also tested the impact of 2'-OH modification of cAMP by comparing the activation of olfactory CNG channels by 8-pCPT-cAMP (**18**) and 2'-O-methyl-8-pCPT-cAMP (**19**). Compound **19** was ~40 fold less potent than **18**, a decrease in apparent affinity similar to that observed with compound **3** (Table 2). The parachlorophenylthio group at the 8-position partially rescues the loss in affinity, leaving compound **19** with a $K_{1/2}$ of 22 μ M, only ~6-fold higher than cAMP (Table 2).

Consistent with prior work, our experiments with 8-substituted cGMP derivatives show that a wide range of substituents are well-tolerated^{10,13-16,32}. Addition of sulfur to cGMP at the 8-position (compound **6**) enhances the apparent affinity for the channel ~6-fold. Derivatization of the thio group of **6** has two consequences: addition of a new substituent and a shifting of the tautomeric form of the N7-C8-S system from predominantly thioketo- to the thioenol- form (Scheme 1). Among the C8-S- substituted derivatives, the $K_{1/2}$ varies somewhat depending on the substituent. 8-pCPT-cGMP (**13**), widely used as a membrane-permeant cGMP analog, is ~2.5-fold more potent than the precursor (**6**). The methyl vinyl sulfone derivative (**7**) is ~2-fold less potent than **6**, whereas the phenyl vinyl sulfone derivative (**9**) is essentially equipotent with **6**.

In contrast to C8-S- derivatives, N7 derivatives of **6** adopt the thioketo- form. While the contribution of the thioketo-thioenol tautomerization at N7-C8-S to activation is difficult to assess, we find that N7 derivatives of **6** are also well tolerated (Figure 2A). In fact, the methyl and phenyl vinyl sulfone derivatives at N7 (compounds **8** and **10**, respectively) are on average 3-fold more potent than the analogous C8-S- derivatives (compounds **7** and **9**) (Table 1). Compound **10** is a potent activator of olfactory CNG channels, equivalent to **13**. We extended these studies to test the impact of very large substituents at N7; a 3400 MW polyethylene glycol N7 derivative (**12**) remains a full agonist of heteromeric olfactory CNG channels and is at least

as potent as the analogous C8-S derivative (**11**) (Table 1). We were able to generate a N7-, C8-S- doubly alkylated compound (**15**) by reaction of **6** with 1,3 dibromopropane (Scheme 3). Compound **15** is a poor activator of the olfactory CNG channel, >50-fold less potent than **6** and 9-fold less potent than cGMP.

While **6** is primed for reaction with vinyl sulfones at N7, **13** reacts with phenyl vinyl sulfone at N1 to give compound **14** (Figure 1). In contrast to the N7 derivatives (**8** and **10**), **14** is less potent than its parent compound (**13**), requiring ~5-fold higher concentrations to yield similar activation (Table 1, Figure 2B). We also examined the effect of N1-methylation of cGMP for CNG channel activation. While 2'-O-methyl-cGMP (**3**) has a $K_{1/2}$ for activation of olfactory CNG channels of 35 μ M, 1-, 2'-O-dimethyl-cGMP (**4**) has a $K_{1/2}$ estimated to be 290 μ M (Table 1). This estimate was made with the assumption that **4** is a full agonist. Since the highest concentration of **4** tested was 242 μ M, it is unclear whether this assumption is correct. If, in the extreme case, 242 μ M of **4** is saturating, we have over-estimated the $K_{1/2}$ by ~2-fold. Thus, methylation of N1 reduces the apparent affinity for the channel 4-8 fold on a background of 2'-O-methylation (Table 1).

Numerous C8-S substituted cGMP derivatives have been tested for activation of retinal CNG channels^{10,12-16,32-35}. These studies demonstrate a broad tolerance for negatively charged and hydrophobic substituents at C8. To determine the effect of N1- and N7- modification of cGMP on activation of retinal rod CNG channels, we co-expressed CNGB1 and CNGB1 in *Xenopus* oocytes and measured activation of the heteromeric channels by a subset of cGMP derivatives. As with olfactory channels, derivatization of cGMP with sulfur at C8 (compound **6**) enhances the apparent affinity ~3-fold for the rod CNG channel (Table 2). Also, derivatization of **6** at N7 had a similar effect on activation of the rod CNG channel as on the olfactory channel. The phenyl vinyl sulfone derivative of **6** (compound **10**) was 3-fold more potent than the parent compound (Table 3). Modification of N1 was detrimental to activation; the loss of apparent affinity for the retinal channel was larger than observed for the olfactory channel. Compound **14** was ~12-fold less potent than the precursor **13** (Table 3).

Discussion

We were able to determine the impact of modification at N1 and N7 on CNG channel activation due to our serendipitous discovery of enhanced reactivity of 8-thio-substituted cGMP derivatives towards vinyl sulfones. Furthermore, examining the activation of heteromeric olfactory CNG channels by new mono- and di-methylated cGMP derivatives (**3** & **4**), as well as the commercially available monobutyl derivatives (**2** & **5**), allows us to assess the relative impact of derivatization at 2'-O- and 2-NH- positions of cGMP. In previous studies of native retinal CNG channels, cGMP derivatives modified at 2'-OH and 2-NH₂ were inactive^{12,15}. Here we show that for olfactory channels the N2-butyl derivative of cGMP (**2**), while 20-fold less potent than cGMP, is still 4-fold more potent than the 2'-O-butyl derivative (**5**). We further demonstrate a trend towards lower apparent affinity for the channel as the size of the 2'-O substituent is increased.

Our experiments with a 2'-O-methyl derivative of cAMP are noteworthy as compound **19** has found increasing utility as a membrane-permeant and selective activator of the guanine nucleotide exchange factor Epac³⁶⁻³⁸. Compound **19** is a very poor activator of the cAMP sensitive protein kinases, PKAI and PKAII, but is a potent Epac1 activator with an $K_{1/2}$ of 2.2 μ M^{36,39}. We find that the $K_{1/2}$ for activation of olfactory CNG channels by **19** is 22 μ M. This relatively modest difference in $K_{1/2}$ for Epac1 and CNG channel activation, and uncertainties regarding membrane permeability, highlight the need for improved activators of all cyclic nucleotide-binding proteins.

The N7 derivatives of **6** (compounds **8**, **10**, and **12**) combine N7-derivatization with a thioketone at C8 and represent a novel type of cyclic nucleotide derivative. Previously reported modification at N7 of cGMP is limited to the 7-deaza derivative in which N7 is replaced with C. While 7-deaza-cGMP was shown to activate cation efflux from lysed rod outer segments, its potency relative to cGMP is unclear, as both were employed at a concentration that elicited maximal Ca^{2+} release kinetics¹². Interestingly, while 7-deaza-cGMP displayed 16-fold lower affinity relative to cGMP for the rapidly exchanging site of cGMP-dependent protein kinase; the opposite was true of the type II cAMP-dependent protein kinase, where 7-deaza-cGMP bound with 50-fold higher affinity than cGMP^{17,40}.

Our results show that N7 modification of **6** is well tolerated by both heteromeric olfactory and retinal rod CNG channels and can increase the apparent affinity for the channel, as with **10**. While N7 derivatives are slightly more potent, N7 modification is broadly similar to C8 modification, as very large substituents, such as a 3400 MW PEG, can be accommodated at either position. This argues that, when bound to the channel, this face of the nucleotide is largely exposed. While dual alkylation of **6** at N7 and C8-S is not generally practical, we were able to accomplish this using 1,3-dibromopropane, where intramolecular reaction during the second alkylation step at N7 forms a 6-membered ring. The product (**15**) is a very poor activator of the olfactory channel relative to **6** and is ~10-fold less potent than cGMP. This is likely a result of the formation of a positive charge in the purine ring system and not steric clash due to the presence of the propyl group linking N7 and C8-S.

The finding that phenyl vinyl sulfone reacts with **13** to give an N1 adduct (**14**) led us to pursue alternative routes to modification of N1. While reaction of iodomethane with cGMP in NaOH was reported to yield 1-methyl-cGMP in 44% yield³⁰, we found 1-Me-cGMP to be a minor product compared with **3** and **4**. Comparing the activation of olfactory CNG channels by **3** and **4** indicates that methylation of N1 causes a 4-8-fold loss of affinity. This is similar to the 5-fold reduction in apparent affinity observed for phenylsulfonyl ethyl modification at N1 (compare compounds **13** and **14**). A similar decrease in potency for activation of cGMP-dependent protein kinase PKGI was reported for 1-methyl-cGMP relative to cGMP⁴¹. That substituents of such disparate size at N1, as in **4** and **14**, yield similar changes in apparent affinity for the olfactory CNG channel is in contrast with the size dependence observed at 2'-O with **3**, **5**, and **16**. This could indicate available space in this region of the binding site or that the N1 substituent of **14** makes favorable contacts with the protein that compensate for steric clashes. Despite the loss of affinity due to the N1-substituent of **14**, it remains ~3 fold more potent for activation of both heteromeric olfactory and retinal rod CNG channels than cGMP.

These studies introduce a new method for N7 and N1 modification of cGMP, enhance our understanding of the interaction between cGMP and CNG channels, and point to two additional sites on cGMP that are amenable to modification. Such modifications can be exploited for increasing membrane permeability, attachment of polymers and/or fluorescent probes, and future development of agonists selective for CNG channels as well as other cyclic nucleotide binding proteins.

Experimental section

Channel expression and electrophysiology

Inside-out excised patch recordings were made at room temperature using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). A RSC-100 rapid solution changer, (Molecular Kinetics, Pullman, WA) was used to control the solution bathing the patch. Electrodes pulled from borosilicate glass and heat polished had resistances between 1.5 and 3.2 M Ω . Currents were low pass filtered at 1 kHz with an eight-pole Bessel filter and digitized at 4 kHz. pCLAMP-8 software (Axon Instruments) was used for data acquisition and analysis, and

currents in the absence of cGMP were subtracted from all traces. All dose-response relations were fit with the Hill equation: $I/I_{\max} = 1/(1+(K_{1/2}/[\text{cyclic nucleotide}])^n)$ where $I/I_{\max}$ is the ratio of current elicited by a particular concentration of a cyclic nucleotide derivative divided by the current elicited by saturating cAMP or cGMP, n is the Hill coefficient, and $K_{1/2}$ is the concentration of cyclic nucleotide that gives half-maximal current.

Heteromeric retinal rod CNG channels were produced by coexpression of CNGB1 and CNGA1 in *Xenopus* oocytes. pGEM-HE expression plasmids containing CNGA1 or CNGB1 cDNA were linearized with NheI and used as a template for production of CNG channel cRNA using the mMESSAGE mMACHINE kit (Ambion, Austin, TX). *Xenopus* oocytes were micro-injected with 5–25 ng of cRNA (2:1 ratio of CNGB1 to CNGA1) and incubated at 18°C. Recordings were made within 1–6 days post-injection. Pipette and bath solutions were identical and consisted of (mM): 130 NaCl, 2 HEPES, 1 EGTA and 0.02 EDTA, pH 7.6. Formation of heteromeric channels was confirmed by testing for sensitivity to block by *l*-cis-diltiazem, and by measuring the fractional current elicited by 8 mM cAMP, as described previously⁴². Heteromeric olfactory CNG channels were expressed by transient transfection of HEK-293 cells with CNGA2, CNGA4 and CNGB1b cDNAs in the mammalian expression plasmid, pcDNA3. Mirus TransIT-293 (Mirus Corporation, Madison, WI) was used for transfection according to the manufacturer's instructions, employing a ratio of 1:1.75:1.75 CNGA2:CNGA4:CNGB1b. A CD4 expression plasmid was included (1/10 total DNA) to aid in the identification of transfected cells. Cells were incubated with anti-CD4 antibody-coated beads (Invitrogen, Carlsbad, CA) for two minutes prior to recording. Recordings were made 1–3 days post transfection. The extracellular solution consisted of (mM) 140 NaCl, 5 KCl, 10 Hepes, 1 EGTA pH to 7.4 with NaOH; and the intracellular solution consisted of (mM) 140 KCl, 5 NaCl, 10 Hepes, 1 EGTA pH to 7.4 with KOH. The production of heteromeric channels was confirmed by measuring the $K_{1/2}$ for activation by cAMP and current rectification under these bio-ionic conditions as described previously³¹.

Chemistry

All small-molecule reagents, including N2-butyryl-cGMP (**2**), 2'-O-butyryl-cGMP (**5**), 8-pCPT-cGMP (**13**) and 8-pCPT-cAMP (**18**), were obtained from Sigma-Aldrich and solvents were from Fisher Scientific. NHS-PEG₃₄₀₀-VS was obtained from Shearwater Polymers (Huntsville, AL). 2'-O-methyl-8-pCPT-cAMP (**19**) was from Biolog (Bremen, Germany). 8-SH-cGMP (**6**) was synthesized as described previously¹⁶. Reactions were followed by analytical reverse-phase (RP)-HPLC (Xterra RP8, 4.6 × 250 mm, 5 μm column, Waters, Milford, MA) or by thin layer chromatography (TLC) with visualization by uv shadowing. Preparative RP-HPLC was performed using an Xterra Prep RP8, 19 × 100 mm, 5 μm column (Waters, Milford, MA) with a water-methanol gradient (5 mM ammonium acetate, pH 5). Preparative anion exchange (AE) HPLC was performed using an RSIL ANION 10U, 22 × 250 mm, column (Alltech, Deerfield, IL). All NMR spectra were recorded on a 400 MHz Bruker Avance spectrometer. The chemical shifts for ¹H and ¹³C are reported in parts per million relative to external TMS via reference to signals from residual protons in the deuterated solvent. The purity of all compounds (except for those commercially available) was analyzed using two modes of analytical HPLC, and is presented in the supporting information.

General method for synthesis of methylated cGMP derivatives

This method follows closely the method of Tazawa et al., for preparation of 2'-O-methyl-cAMP, with some modifications²⁹. Briefly, 100 mg of cGMP (**1**) (272 μmol) was dissolved in 4 ml 4N NaOH and 10 ml of DMF was added. To this mixture was added 2 ml of iodomethane (32 mmol) with vigorous stirring. Rapid addition of iodomethane results in the evolution of heat and leads primarily to 1-,2'-O-dimethyl-cGMP (**4**) whereas slow drop-wise addition of iodomethane yields primarily 2'-O-methyl-cGMP (**5**). The reaction was diluted to 1 L with

H₂O and 5 g of charcoal was added. The charcoal was washed with H₂O on a Buchner funnel and the products were eluted with 2% NH₄OH in 50% ethanol. Alternatively, the method of Miller et al., was employed without modification³⁰. The products were purified on a DEAE-cellulose (DE-52) column, eluting with a gradient of 0 to 1N ammonium acetate, pH 7, followed by preparative RP-HPLC.

2-amino-9-[(4aR,6R,7R,7aR)-2-hydroxy-7-methoxy-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-1,9-dihydro-6H-purin-6-one (2'-O-Methyl-guanosine cyclic 3',5'-phosphate) (3)

White solid, yield 10%. ¹H NMR (400 MHz, D₂O): δ 3.53 (s, 3H, -CH₃), 4.16 (td, J=10.3, 4.4 Hz, 1H, H4'), 4.25 (td, J=10.0, 1.8 Hz, 1H, H5'b), 4.41-4.49 (m, 2H, H2', H5'a), 5.02 (ddd, J=9.9, 5.5, 2.3 Hz, 1H, H3'), 6.04 (s, 1H, H1'), 7.87 (s, 1H, H8). ¹³C NMR (100 MHz, D₂O): δ 58.9, 67.9 (d, ²J_{C5'P} 6.9 Hz), 72.6 (d, ³J_{C4',P} 4.0 Hz), 77.6 (d, ²J_{C3',P} 4.3 Hz), 81.4 (d, ³J_{C2',P} 7.9 Hz), 90.4, 117.2, 138.8, 151.8, 154.5, 159.6. MS (ESI) m/z 358.2 (MH)⁺.

2-Amino-9-[(4aR,6R,7R,7aR)-2-hydroxy-7-methoxy-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-1-methyl-1,9-dihydro-6H-purin-6-one (1-,2'-O-Dimethyl-guanosine cyclic 3',5'-phosphate) (4)

White solid, yield 36%. ¹H NMR (400 MHz, D₂O): δ 3.44 (s, 3H, -CH₃), 3.54 (s, 3H, -CH₃), 4.18 (td, J=10.0, 4.4 Hz, 1H, H4'), 4.26 (td, J=9.9, 1.6 Hz, 1H, H5'b), 4.42-4.50 (m, 2H, H2', H5'a), 4.98-5.02 (ddd, J = 9.8, 5.5, 2.4 Hz, 1H, H3'), 6.03 (s, 1H, H1'), 7.95 (s, 1H, H8). ¹³C NMR (100 MHz, D₂O): δ 29.21, 58.91, 67.9 (d, ²J_{C5'P} 7.0 Hz), 72.7 (d, ³J_{C4',P} 4.0 Hz), 77.6 (d, ²J_{C3',P} 4.4 Hz), 81.3 (d, ³J_{C2',P} 7.9 Hz), 90.5, 116.0, 138.7, 149.6, 155.6, 159.0.

Synthesis of methyl vinyl sulfone derivatives of 6

8.4 mg (21.4 μmol) of 8-SH-cGMP (7) was dissolved in 700 μL 100 mM sodium phosphate, pH 7.9, and 1.89 μL (21.5 μmol) of methyl vinyl sulfone was added. The reaction was stirred at 37°C for 24 hours. Products were purified by RP-HPLC; residual 6 eluted first followed by 8-methylsulfonylethylthio-GMP (7) and 7-methylsulfonylethyl-8-thio-cGMP (8).

2-Amino-9-[(4aR,6R,7R,7aS)-2,7-dihydroxy-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-8-[[2-(methylsulfonyl)ethyl]thio]-1,9-dihydro-6H-purin-6-one (8-[2-(Methylsulfonyl)ethyl]thio-guanosine cyclic 3',5'-phosphate) (7)

White solid, yield 24%. ¹H NMR (400 MHz, CD₃OD): δ 2.85 (s, 3H, -CH₃), 3.91 (td, J=10.1, 4.8 Hz, 1H, H4'), 4.02 (m, 1H, H5'b), 4.12 (ddd, J=21.5, 9.2, 4.7 Hz, 1H, H5'a), 4.64 (d, J=5.3 Hz, 1H, H2'), 5.18 (ddd, J=9.9, 5.3, 2.3 Hz, 1H, H3'), 5.63 (s, 1H, H1'). ¹³C NMR (100 MHz, d6-DMSO): δ 25.0, 40.5, 53.3, 65.3, 70.6, 72.3, 76.7, 92.2, 116.9, 140.9, 152.5, 153.6, 155.6. MS (ESI) m/z 482.1 (MH)⁺.

2-Amino-9-[(4aR,6R,7R,7aS)-2,7-dihydroxy-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-7-[2-(methylsulfonyl)ethyl]-8-thioxo-1,7,8,9-tetrahydro-6H-purin-6-one (7-[2-(Methylsulfonyl)ethyl]-8-thioxo-guanosine cyclic 3',5'-phosphate) (8)

White solid, yield 14%. ¹H NMR (400 MHz, CD₃OD): δ 2.99 (s, 3H, -CH₃), 3.53 (td, J=7.3, 2.4 Hz, 2H, -CH₂-), 3.92 (td, J=10.2, 4.6 Hz, 1H, H4'), 4.06 (td, J=9.8, 1.6 Hz, 1H, H5'b), 4.16 (ddd, J=21.8, 9.0, 4.8 Hz, 1H, H5'a), 4.59 (d, J=5.56 Hz, 1H, H2'), 4.70 (t, J=7.5 Hz, 2H, -CH₂-), 5.21 (ddd, J=9.9, 5.4, 2.2 Hz, 1H, H3'), 6.39 (s, 1H, H1'). MS (ESI) m/z 482.1 (MH)⁺.

Synthesis of phenyl vinyl sulfone derivatives of **6**

14.2 mg (35.9 μ mol) of 8-SH-cGMP (**6**) was dissolved in 100 mM sodium phosphate, pH 7.9, and 13.4 mg (79.7 μ mol) of phenyl vinyl sulfone was dissolved in methanol. The two solutions were combined (final solvent composition: 38% methanol and 63 mM sodium phosphate) and stirred at 37°C for 24 hours. Products were purified by RP-HPLC; residual **6** eluted first followed by 8-phenylsulfonylethylthio-GMP (**9**) and 7-phenylsulfonylethyl-8-thio-cGMP (**10**).

2-Amino-9-[(4aR,6R,7R,7aS)-2,7-dihydroxy-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-8-[[2-(phenylsulfonyl)ethyl]thio]-1,9-dihydro-6H-purin-6-one (8-[2-(Phenylsulfonyl)ethyl]thio-guanosine cyclic 3',5'-phosphate) (**9**)

White solid, yield 45%. ^1H NMR (400 MHz, CD_3OD): δ 3.23 (m, 2H, $-\text{CH}_2-$), 3.59 (m, 2H, $-\text{CH}_2-$), 3.98 (td, $J=10.0$, 4.9 Hz, 1H, H_4'), 4.11 (m, 1H, $\text{H}_5'\text{b}$), 4.20 (ddd, $J=21.4$, 9.2, 4.8 Hz, 1H, $\text{H}_5'\text{a}$), 4.69 (d, $J=5.6$ Hz, 1H, H_2'), 5.26 (ddd, $J=9.9$, 5.3, 2.3 Hz, 1H, H_3'), 5.71 (s, 1H, H_1'), 7.52 (t, $J=7.6$ Hz, 2H, phenyl), 7.62 (tt, $J=7.5$, 1.4 Hz, 1H, phenyl), 7.81 (m, 2H, phenyl). ^{13}C NMR (100 MHz, CD_3OD): δ 28.0, 56.6, 68.5 (d, $^2J_{\text{C}_5'\text{P}}$ 6.9 Hz), 73.1 (d, $^3J_{\text{C}_2'\text{P}}$ 8.3 Hz), 73.6 (d, $^3J_{\text{C}_4'\text{P}}$ 3.9 Hz), 78.9 (d, $^2J_{\text{C}_3'\text{P}}$ 4.2 Hz), 94.0, 118.8, 129.4, 130.8, 135.4, 140.2, 143.8, 154.4, 155.4, 158.8. MS (ESI) m/z 544.1 (MH) $^+$.

2-Amino-9-[(4aR,6R,7R,7aS)-2,7-dihydroxy-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-7-[2-(phenylsulfonyl)ethyl]-8-thioxo-1,7,8,9-tetrahydro-6H-purin-6-one (7-[2-(Phenylsulfonyl)ethyl]-8-thioxo-guanosine cyclic 3',5'-phosphate) (**10**)

White solid, yield 40%. ^1H NMR (400 MHz, D_2O): δ 4.08 (td, $J=10.2$, 4.7 Hz, 1H, H_4'), 4.16-4.41 (m, 3H, $\text{H}_5'\text{b}$, $-\text{CH}_2-$), 4.45 (ddd, $J=21.6$, 9.4, 4.7 Hz, 1H, $\text{H}_5'\text{a}$), 4.70-4.73 (m, 3H, H_2' , $-\text{CH}_2-$), 5.11 (ddd, $J=9.9$, 5.6, 2.2 Hz, 1H, H_3'), 6.29 (s, 1H, H_1'), 7.42 (t, $J=8.0$ Hz, 2H, phenyl), 7.60 (t, $J=7.6$ Hz, 1H, phenyl), 7.68 (dd, $J=8.5$, 1.1 Hz, 2H, phenyl). ^{13}C NMR (100 MHz, D_2O): δ 41.2, 53.0, 68.1 (d, $^2J_{\text{C}_5'\text{P}}$ 7.0 Hz), 71.3 (d, $^3J_{\text{C}_2'\text{P}}$ 8.3 Hz), 72.2 (d, $^3J_{\text{C}_4'\text{P}}$ 4.2 Hz), 77.7 (d, $^2J_{\text{C}_3'\text{P}}$ 4.2 Hz), 92.7, 105.4, 127.5, 130.0, 135.2, 138.8, 149.8, 153.2, 154.5, 166.2. MS (ESI) m/z 544.1 (MH) $^+$.

Synthesis of PEG vinyl sulfone derivatives of **6**

100 mg (28.3 μ mol) of a 3400 MW heterobifunctional PEG derivative, NHS-PEG₃₄₀₀-VS, (N-hydroxy-succinimide-PEG₃₄₀₀-vinyl sulfone), was dissolved in 1.85 ml of 100 mM sodium phosphate, pH 7.4, and 10 mg (22.7 μ mol) of the tetracaine derivative, (3-amino-*N*-(2-ethyl)-*N,N*-dimethylpropan-1-aminium acetate) was added⁴³. Reaction of the primary amine of the tetracaine derivative with the NHS ester was complete within 60 minutes. 60 mg (15.4 μ mol) of this intermediate product was isolated by RP-HPLC and mixed with 11 mg (27.7 μ mol) of **6** in 300 μ L of 100 mM sodium phosphate, pH 7.9. The reaction was stirred for 24 hours at 37°C and the products were purified by AE-HPLC.

8-[2-(PEG₃₄₀₀sulfonyl)ethyl]thio-guanosine cyclic 3',5'-phosphate (**11**)

White solid, 19% yield. ^1H NMR: (For simplicity, only the cyclic nucleotide and aromatic tetracaine resonances are listed.) (400 MHz, CD_3OD): δ 3.95 (td, $J=10.2$, 4.8 Hz, 1H, H_4'), 4.07 (td, $J=9.9$, 1.5 Hz, 1H, $\text{H}_5'\text{b}$), 4.18 (ddd, $J=21.1$, 9.4, 4.7 Hz, 1H, $\text{H}_5'\text{a}$), 4.68 (d, $J=5.1$ Hz, 1H, H_2'), 5.23 (ddd, $J=9.9$, 5.3, 2.0 Hz, 1H, H_3'), 5.68 (s, 1H, H_1'), 6.45 (d, $J=8.8$ Hz, 2H, H-tetracaine), 7.64 (d, $J=8.8$ Hz, 2H, H-tetracaine). MS (MALDI) m/z 4413.25 * (MH) $^+$.

^{*}PEG derivatives are polydisperse, and mass spectrometry gives a gaussian distribution of peaks with 44 mass unit spacing. The mass listed is for the central mass peak.

7-[2-(PEG₃₄₀₀sulfonyl)ethyl]-8-thioxo-guanosine cyclic 3',5'-phosphate (12)

White solid, 26% yield. ¹H NMR: (For simplicity, only the cyclic nucleotide and aromatic tetracaine resonances are listed.) (400 MHz, CD₃OD): δ 3.95 (td, *J*=10.0, 4.9 Hz, 1H, H4'), 4.10 (t, *J*=9.6 Hz, 1H, H5'b), 4.21 (ddd, *J*=21.6, 9.0, 4.8 Hz, 1H, H5'a), 5.24 (ddd, *J*=9.7, 5.3, 1.9 Hz, 1H, H3'), 6.42 (s, 1H, H1') 6.49 (d, *J*=9.1 Hz, 2H, H-tetracaine), 7.68 (d, *J*=8.8 Hz, 2H, H-tetracaine). MS (MALDI) *m/z* 4413.24⁺ (MH)⁺.

2-amino-9-[(4aR,6R,7R,7aS)-2,7-dihydroxy-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-8-[(4-chlorophenyl)thio]-1-[2-(phenylsulfonyl)ethyl]-1,9-dihydro-6H-purin-6-one (8-(4-Chlorophenyl)thio-1-[2-(phenylsulfonyl)ethyl]-guanosine cyclic 3',5'-phosphate) (14)

24 mg (47 μmol) of 8-pCPT-cGMP (13) was dissolved in 1.6 ml of 37.5% methanol, 62.5 mM sodium phosphate, pH 7.9, and 46.3 mg (276 μmol) phenyl vinyl sulfone was added. The reaction was stirred at 37°C for 20 hours. Excess phenyl vinyl sulfone was removed by chromatography on DEAE-cellulose (DE-52). 14 was isolated on RP-HPLC. White solid, 61% yield. ¹H NMR (400 MHz, CD₃OD): δ 3.68 (td, *J*=6.7, 1.5 Hz, 2H, -CH₂-), 3.98 (td, *J*=10.1, 4.8 Hz, 1H, H4'), 4.14 (td, *J*=9.8, 1.6 Hz, 1H, H5'b), 4.24 (ddd, *J*=21.5, 9.1, 4.8 Hz, 1H, H5'a), 4.37 (t, *J*=6.7 Hz, 2H, -CH₂-), 4.64 (d, *J*=5.6 Hz, 1H, H2'), 5.22 (ddd, *J*=9.8, 5.4, 2.0 Hz, 1H, H3'), 5.98 (s, 1H, H1'), 7.30 (m, 4H, pCPT), 7.41 (m, 3H, phenyl), 7.82 (m, 2H, phenyl). ¹³C NMR (100 MHz, CD₃OD): δ 37.3, 53.1, 68.7 (d, ²J_{C5'P} 7.3 Hz), 73.0 (d, ³J_{C2'P} 8.1 Hz), 73.5 (d, ³J_{C4'P} 3.9 Hz), 79.0 (d, ²J_{C3'P} 4.2 Hz), 94.3, 118.4, 128.9, 130.6, 130.8, 132.8, 133.0, 135.0, 135.5, 140.5, 141.8, 151.9, 155.6, 157.8. MS (ESI) 654.1 (MH)⁺.

2-amino-10-[(4aR,6R,7R,7aS)-2,7-dihydroxy-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-4-oxo-3,4,6,7,8,10-hexahydro[1,3]thiazino[2,3-f]purin-5-ium (2-Amino-10-(β-D-ribofuranosyl)-4-oxo-3,4,6,7,8,10-hexahydro-[1,3]thiazino[2,3-f]purin-5-ium cyclic 3',5' phosphate) (15)

11.7 mg (29.6 μmol) of 8-SH-cGMP (6) was dissolved in 1.6 ml methanol and 90.5 μL of 1,3-dibromopropane (888 μmol) was added. The reaction was stirred at RT for 48 hours. The solvent and excess 1,3-dibromopropane were removed *in vacuo* and the resulting residue was dissolved in H₂O. The product was purified by RP-HPLC. White solid, 91% yield. ¹H NMR (400 MHz, D₂O): δ 2.39 (bm, 2H, -CH₂-), 3.40 (t, *J* = 5.6 Hz, 2H, -CH₂-), 4.13 (td, *J*=10.2, 4.7 Hz, 1H, H4'), 4.26 (td, *J*=10.0, 1.5 Hz, 1H, H5'b), 4.36-4.50 (m, 3H, H5'a, -CH₂-), 4.78 (d, *J* = 5.5 Hz, 1H, H2') 5.02 (ddd, *J*=9.9, 5.5, 2.3 Hz, 1H, H3'), 5.81 (s, 1H, H1'). ¹³C NMR (100 MHz, D₂O): δ 21.5, 26.8, 46.5, 68.0 (d, ²J_{C5'P} 7.2 Hz), 72.6 (d, ³J_{C2'P} 8.3 Hz), 73.5 (d, ³J_{C4'P} 4.1 Hz), 77.7 (d, ²J_{C3'P} 4.1 Hz), 93.4, 109.4, 147.8, 150.8, 155.2, 156.1. MS (ESI) *m/z* 418.1 (MH)⁺.

2-amino-9-[(4aR,6R,7R,7aR)-2-hydroxy-7-[2-(methylsulfonyl)ethoxy]-2-oxotetrahydro-4H-furo[3,2-d][1,3,2]dioxaphosphinin-6-yl]-1,9-dihydro-6H-purin-6-one (2'-O-[2-(Methylsulfonyl)ethyl]-guanosine cyclic 3',5'-phosphate) (16)

110 mg (300 μmol) of cGMP (2) was dissolved in 200 μL of 4N NaOH and 72 μL (800 μmol) of methyl vinyl sulfone was added. The mixture was stirred vigorously at RT for 1 hour, diluted 7-fold with H₂O and neutralized with HCl. The major product (17) was separated from unreacted 2 by RP-HPLC. White solid, yield 10%. ¹H NMR (400 MHz, D₂O): δ 3.13 (s, 3H, -CH₃), 3.44-3.57 (m, 2H, -CH₂-), 4.16 (m, 4H, H4', H5'b, -CH₂-), 4.39 (ddd, *J*=21.4, 8.7, 4.0 Hz, 1H, H5'a), 4.55 (d, *J*=5.3 Hz, 1H, H2'), 4.96 (ddd, *J*=9.2, 5.22, 2.0 Hz, 1H, H3'), 6.01 (s, 1H, H1'), 7.82 (s, 1H, H8). ¹³C NMR (100 MHz, D₂O): δ 42.8, 54.9, 65.0, 67.8 (d, ²J_{C5'P} 6.9 Hz), 72.8 (d, ³J_{C4'P} 4.2 Hz), 77.8 (d, ²J_{C3'P} 4.3 Hz), 80.4 (d, ³J_{C2'P} 8.0 Hz), 90.7, 117.1, 138.8, 151.9, 154.6, 159.6. MS (ESI) *m/z* 450.2 (MH)⁺.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.

HMBC spectroscopy of compound **10** in D₂O (A) and compound **14** in CD₃OD (B). A select region from each 2D spectrum is shown to highlight the relevant signals. The ¹³C 1D projections are on the left, while the ¹H 1D projections are at the top in both A and B. An abbreviated structure of each derivative is shown above with arrows indicating the three-bond ¹H / ¹³C couplings for the methylene protons of interest. In panel A the 2' and -CH₂- ¹H resonances are overlapping, and in B the 5'a ¹H resonance is labeled for clarity.

Figure 2. Inside-out patch recording. Activation of heteromeric olfactory CNG channels by compound **10** (A) and compound **14** (B). Traces on the left were recorded at a holding potential of +60 mV with 3-fold increasing concentrations of each compound applied to the patch as indicated by arrows above. The dose response curve is shown to the right. A: **10**, (filled circles), **6** (open circles), and **1** (diamonds). B: **13** (open squares), **14** (filled squares), and **1** (diamonds). The dashed line indicates the zero current level.

Scheme 1.
 Vinyl sulfone reaction with 8-SH-cGMP[†].

[†]The ribose and cyclic phosphate portion is abbreviated rcp

Scheme 2.
Vinyl sulfone reaction with 8-pCPT-cGMP[†].

Scheme 3.
1,3 dibromopropane reaction with 8-SH-cGMP[†].

Scheme 4.
Vinyl sulfone reaction with cGMP.

Table 1
Activation of olfactory CNG channels by cGMP derivatives *

#	R ₁	R ₂	R _{2'}	K _{1/2} (μM)	n	
1	-H	-H	-H	1.1 ± 0.3	2.2 ± 0.3	(7)
2	-H	-H	-H	22 ± 5.3	2.2 ± 0.2	(7)
3	-H	-H	-CH ₃	35 ± 8.1	2.0 ± 0.3	(8)
4	-CH ₃	-H	-CH ₃	290 ± 77 [†]	ND	(7)
5	-H	-H	-H	88 ± 28	2.6 ± 0.2	(6)
#	R ₁	R ₂	R _{2'}	K _{1/2}	n	
6	-H			0.18 ± 0.04	1.8 ± 0.3	(8)
7	-H			0.37 ± 0.08	2.1 ± 0.1	(9)
8	-H			0.11 ± 0.03	1.5 ± 0.3	(5)
9	-H			0.21 ± 0.04	2.5 ± 0.5	(9)
10	-H			0.082 ± 0.024	2.2 ± 0.3	(8)
11	-H			1.9 ± 0.3	1.9 ± 0.2	(6)
12	-H			1.3 ± 0.5	2.2 ± 0.3	(5)

#	R ₁	R ₂	K _{1/2} (μM)	n
13	-H		0.073 ± 0.021	2.3 ± 0.6 (10)
14			0.38 ± 0.16	1.7 ± 0.5 (10)
15	-H		10 ± 2	2.1 ± 0.2 (8)

* Excised patch recordings from HEK-293 cells expressing heteromeric olfactory CNG channels (CNGA2, CNGB4, and CNGB1b). K_{1/2} and n (Hill equation coefficients) are defined in the experimental section, and the values shown are the mean ± the standard deviation. The number of experiments is given in parentheses in the right-hand column. ND = not determined.

[†] The K_{1/2} was estimated assuming **5** to be a full agonist. The average I/I_{max} for 242 μM **5** is 0.44.

[‡] N7 and C8 are shown in addition to the substituent(s) for clarity.

Table 2

Activation of olfactory CNG channels by cAMP derivatives*

#	compound	$K_{1/2}$ (μM)	n	
17	cAMP	3.8 ± 1.2	2.0 ± 0.4	(75)
18	8-pCPT-cAMP	0.55 ± 0.13	2.4 ± 0.6	(7)
19	8-pCPT-2'-O-Me-cAMP	22 ± 4	2.4 ± 0.7	(8)

* Excised patch recordings from HEK-293 cells expressing heteromeric olfactory CNG channels (CNGA2, CNGB1b). $K_{1/2}$ and n (Hill equation coefficients) are defined in the experimental section, and the values shown are the mean $\pm$ the standard deviation. The number of experiments is given in parentheses in the right-hand column.

Table 3
Activation of retinal rod CNG channels by cGMP derivatives*

#	R ₁	R _{7/8} [†]		K _{1/2} (μM)	n
1	-H			41 ± 8	2.2 ± 0.5 (6)
6	-H			14 ± 4	1.9 ± 0.3 (4)
10	-H			4.1 ± 0.7	1.9 ± 0.4 (6)
13	-H			1.0 ± 0.2	1.7 ± 0.22 (5)
14				12 ± 2	2.1 ± 0.2 (6)

* Excised patch recordings from *Xenopus* oocytes expressing heteromeric retinal rod CNG channels (CNGB1 and CNGB1). K_{1/2} and n (Hill equation coefficients) are defined in the experimental section, and the values shown are the mean ± the standard deviation. The number of experiments is given in parentheses in the right-hand column. The ribose and cyclic phosphate rings are abbreviated with rcp.

[†] N7 and C8 are shown in addition to the substituent(s) for clarity.