A facile two-step synthesis of 8-arylated guanosine mono- and triphosphates (8-aryl GXPs)

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We report a simple and high-yielding two-step procedure for the preparation of 8-arylated guanosine mono- and triphosphates (8-aryl GXPs). The key step of our synthesis is the Suzuki–Miyaura coupling of unprotected 8-bromo GMP and 8-bromo GTP with various arylboronic acids in aqueous solution. The 8-bromoguanosine 5′-phosphates required as cross-coupling substrates were prepared from 8-bromoguanosine *via* an optimised Yoshikawa procedure.

Introduction

The 5′-mono- and triphosphates of the naturally occurring purine nucleosides adenosine and guanosine (AXPs and GXPs) serve numerous biological roles, from building blocks for nucleic acids to enzyme cosubstrates. Non-natural analogues of AXPs and GXPs are therefore sought after as chemical tools for the investigation of important biological processes. Base-modified purine nucleotides have been used as inhibitors of therapeutically relevant proteins,[1] as purine receptor antagonists,[2] and as probes for RNA structure and function.[3] Shokat and coworkers have employed N^6-modified ATP analogues for the engineering of orthogonal protein kinase–nucleoside triphosphate pairs.[4]

In order to explore further the usefulness of non-natural purine nucleotides as biological tools, robust synthetic methods for the rapid generation of structurally diverse analogues of AXPs and GXPs are required. While the direct structural modification of unprotected nucleotides represents the most elegant and efficient strategy towards that goal, this approach is complicated by three factors:

(a) The presence of multiple functional groups, including the free phosphate group(s), limits the choice of chemistry.

(b) The phosphate ester, pyrophosphate, and glycosidic bonds all represent sites for potential hydrolytic breakdown.

(c) The water-solubility of unprotected nucleotides necessitates the use of aqueous reaction media.

Consequently, limited precedence exists for the direct structural manipulation of unprotected nucleotides in aqueous solution. Important examples that have been reported to date concern the Sonogashira and Suzuki–Miyaura cross-coupling of unprotected pyrimidine[5] as well as purine[6] nucleotides, as Pd-catalysed cross-coupling chemistry is well suited to meet the synthetic challenges outlined above.

As part of an ongoing investigation into the biological activity of non-natural nucleotides, we are interested in GXP analogues bearing additional aryl substituents at position 8 of the guanine base (Fig. 1). The desired substituents can be installed *via* the Suzuki–Miyaura reaction. Previously, we and others have identified reaction conditions for the Pd-catalysed cross-coupling of unprotected halopurine *nucleosides* in aqueous media.[7,8] More recently, Hocek and coworkers have described the direct cross-coupling of unprotected 8-bromo AMP and 8-bromo ATP with boronophenylalanine in aqueous acetonitrile, using a catalytic system composed of $Pd(OAc)_2$ and TPPTS (triphenylphosphine trisulfonic acid).[6b] However, no examples have been reported to date for the Suzuki–Miyaura cross-coupling of 8-halo GMP and 8-halo GTP.

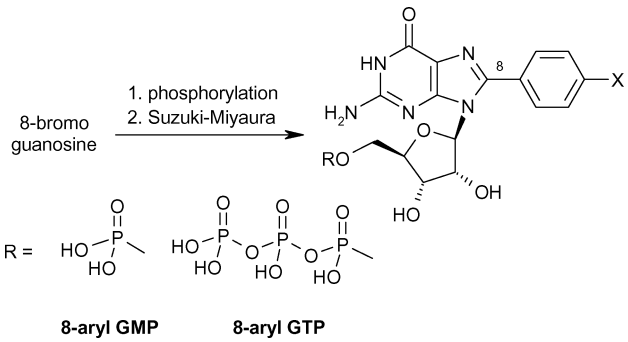

Fig. 1 General synthetic strategy towards the target 8-arylated guanosine mono- and triphosphates (8-aryl GXPs).

It is well known that guanosine analogues undergo Pd-catalysed cross-coupling reactions much less readily than the corresponding adenosine derivatives.[7b,9] It has been noted that there is indeed a noticeable lack of studies concerning the Pd-catalysed cross-coupling of 8-bromoguanosine, presumably due to this low reactivity.[10] The reduced reactivity of 8-bromoguanosine in the Suzuki–Miyaura reaction has been attributed to the coordination of the transition metal catalyst to the guanine base under basic conditions (Fig. 2),[9] and guanosine has been shown to inhibit the cross-coupling of both halopurine nucleosides and simple aryl bromides.[9] Because of these complications, the cross-coupling of guanosine and GXP derivatives is nontrivial. In addition to the low cross-coupling reactivity of guanosine derivatives, past attempts at the cross-coupling of guanine *nucleotides* may also have been hampered by difficulties in the preparation of the 8-bromoguanosine phosphates required as substrates for the cross-coupling reaction, resulting from their limited stability.[11]

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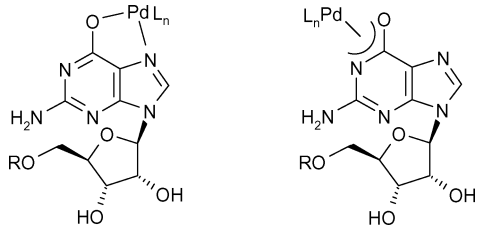
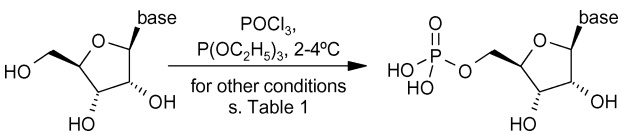


Fig. 2 Possible coordination of guanosine derivatives to Pd under basic conditions (after ref. 9).

Herein we report a simple two-step procedure for the preparation of novel 8-arylated GMP and GTP analogues (Fig. 1), addressing the twin issues of problematic precursor synthesis and low cross-coupling reactivity of 8-bromo GXPs. We have optimised the preparation of the 8-bromo GXPs required as cross-coupling substrates, and we have developed suitable reaction conditions for the Suzuki–Miyaura reaction of these unprotected 8-bromo GXPs in water. Our synthetic protocol is operationally simple, does not require any special precautions, and uses only commercially available reagents. The synthetic method reported herein provides fast and reliable access to a novel family of 8-substituted GXP analogues with potentially interesting biological activity.

Results and discussion

As substrates for the Suzuki–Miyaura reaction of GXPs, 8-bromo GMP (**1**) and 8-bromo GTP (**2**) were required. A commonly used method for the preparation of non-natural nucleoside 5′-monophosphates is Yoshikawa's procedure for the selective phosphorylation of unprotected nucleosides in position 5′ (Scheme 1).[12] While this approach circumnavigates the need for lengthy protection–deprotection sequences, it had previously been reported to be unsuccessful in the case of 8-bromoguanosine.[13] In our hands, treatment of 8-bromoguanosine under the original Yoshikawa conditions (excess $POCl_3$, H_2O, trialkylphosphate) led to cleavage of the glycosidic bond and to a partial exchange of the 8-bromo substituent with chlorine. A mixture of 8-bromo- and 8-chloroguanine was isolated as the sole product from this reaction (Table 1, entry 1).



Scheme 1 Optimisation of phosphorylation conditions.

The bromo–chloro exchange reaction, which is accompanied by a characteristic ^{13}C NMR downfield shift for the carbon atom in position 8, was also observed in control experiments carried out with 8-bromoadenosine ($\delta_{C\text{-}8}$ 127.9 ppm) and 8-bromoinosine ($\delta_{C\text{-}8}$ 126.7 ppm) under the same conditions. However, in these cases the mononucleotides 8-chloro AMP ($\delta_{C\text{-}8}$ 139.1 ppm) and 8-chloro IMP ($\delta_{C\text{-}8}$ 138.9 ppm) were isolated with the glycosidic bond intact (Table 1, entries 2 and 3). 8-Chloro AMP proved to be unreactive during the Pd-catalysed cross-coupling reactions (data not shown), suggesting that the more reactive bromo substituent is required for successful cross-coupling. Therefore, we sought to optimise the procedure for the phosphorylation of 8-bromoguanosine in order to avoid both halogen exchange and depurination.

Table 1 Optimisation of phosphorylation conditions[a]

Entry	Conditions[b]	Nucleoside[c] (substrate)	Nucleotide (product)	Yield (%)
1	H_2O	8-BrG	n/a	0
2	H_2O	8-BrA	8-Chloro AMP	46
3	H_2O	8-BrI	8-Chloro IMP	34
4	H_2O–pyridine	8-BrG	8-Bromo GMP (1)	23[d]
5	H_2O–pyridine	8-BrA	8-Bromo AMP	14
6	H_2O–pyridine	8-BrI	8-Bromo IMP	19
7	Proton sponge	8-BrG	1	75[d]
8	Proton sponge[e]	8-BrG	1	90

[a] For structures, see Scheme 1. [b] $POCl_3$: 4 equiv., H_2O: 0.5 equiv., pyridine: 4 equiv. [c] 8-BrG: 8-bromoguanosine; 8-BrA: 8-bromoadenosine; 8-BrI: 8-bromoinosine. [d] Contaminated with 2′,3′-cyclophosphate. [e] Solvent: acetonitrile instead of triethylphosphate.

We reasoned that both side reactions might be promoted by hydrogen chloride formed *in situ* under the aqueous Yoshikawa conditions, and that use of a base might therefore be beneficial. While attempts with other bases (*e.g.* lutidine, proton sponge) were unsuccessful, addition of pyridine to the reaction prevented both halogen exchange and, in the case of 8-bromoguanosine, depurination. However, the desired 8-bromopurine nucleoside 5′-monophosphates were isolated in only low yields under these conditions (Table 1, entries 4–6). In the case of **1** we attributed the low yield mainly to the formation of a bisphosphorylated side product, which was identified as the cyclic bisphosphate $O^{2'},O^{3'}$-hydroxyphosphoryl 8-bromoguanosine 5′-monophosphate (δ_P 7.43, 24.45 ppm). This observation is in agreement with earlier reports that the phosphorylation of unprotected nucleosides with $POCl_3$ is 5′-selective only in aqueous–acidic, but not in aqueous–basic medium.[12b,14] Finally, the use of proton sponge under *anhydrous* conditions[15] gave **1** in 75% yield, albeit still slightly contaminated with traces of the cyclic bisphosphate as indicated by ^1H and ^{31}P NMR (Table 1, entry 7).

Besides the absence of base, two other experimental parameters have previously been regarded as critical for the 5′-selectivity of the Yoshikawa phosphorylation of unprotected nucleosides: low reaction temperature and use of trialkylphosphates as solvents.[12] We found that while maintaining the reaction temperature at 2–4 °C is indeed essential in order to suppress formation of the bisphosphate side product, use of triethylphosphate is not. We discovered that on the contrary, changing the solvent to acetonitrile completely restored the selectivity of the phosphorylation reaction for the 5′ position. 8-Bromoguanosine is not initially soluble in acetonitrile, but a clear solution forms within minutes upon addition of $POCl_3$ to the reaction at 2 °C. After 2 hours, TLC indicated complete phosphorylation, and **1** was isolated in 90% yield following purification by ion-pair chromatography (Table 1, entry 8). While initial experiments were carried out on a 50 mg scale, the reaction was subsequently scaled up to 500 mg without difficulty. The use of 0.2M TEAB (pH 7.2) during the aqueous work-up proved to be critical, as replacement of the buffer with H_2O led to depurination. The protocol was also successfully adapted for the synthesis of 8-bromo GTP (**2**) using a modification of Ludwig's procedure.[16] Instead of the aqueous work-up, the intermediate dichlorophosphate species resulting from treatment

Table 2 Optimisation of conditions for the preparation of **3** *via* Suzuki–Miyaura coupling of 8-bromoguanosine with phenylboronic acid[a]

Entry	Pd source	Ligand	Yield (%)	Time/h
1	$Pd(OAc)_2$	TPPTS	61	3
2	$Pd(NO_3)_2$	TPPTS	80	1
3	Na_2Cl_4Pd	TPPTS	77	0.3
4	Na_2Cl_4Pd	Buchwald ligand	69	17
5	$PdCl_2$	EDTA	0	24

[a] For structures, see Scheme 2; R = H, X = H.

of 8-bromoguanosine with $POCl_3$–proton sponge was reacted *in situ* with tri-*n*-butylammonium pyrophosphate in DMF[17] to give **2** in 72% yield.

Before attempting the potentially challenging Suzuki–Miyaura coupling of unprotected nucleotides **1** and **2**, we sought to optimise conditions for the cross-coupling of 8-bromoguanosine. Recently, we have reported that a catalytic system composed of $Pd(OAc)_2$ as the Pd source and the water-soluble ligand TPPTS[7b] is superior to $Pd(PPh_3)_4$ for the Suzuki–Miyaura coupling of unprotected halopurine nucleosides in neat water (Table 2, entry 1).[7d] Products from the reaction of 8-bromoguanosine with various arylboronic acids under these conditions were generally isolated by simple precipitation from the aqueous reaction. However, most of the 8-arylguanosine derivatives prepared in this fashion were obtained as black powders, whose poor solubility precluded further purification by either recrystallisation or column chromatography. The mass spectra of these samples frequently showed a molecular ion of $[M + H + 106]^+$ indicative of the presence of the aforementioned guanosine–Pd complex (Fig. 2), which presumably was responsible for the black colour.

In order to avoid this Pd contamination we tested various catalytic systems which have recently been reported to be effective for cross-coupling reactions in aqueous media.[6b,7b,18] The preparation of 8-phenylguanosine (**3**) *via* Suzuki–Miyaura coupling of 8-bromoguanosine and phenylboronic acid was chosen as a model reaction to identify optimum conditions (Table 2). Out of the catalytic systems tested, only those with a phosphine ligand were found to be active (Table 2, entries 1–4) while the $PdCl_2$–EDTA system was not (Table 2, entry 5). This result is in agreement with a recent report that Na_2Cl_4Pd–EDTA is not an efficient catalyst for the cross-coupling of 8-bromoadenosine,[6b] and emphasises the importance of the ligand for cross-coupling reactions of nucleosides in aqueous media.

The role of the Pd source was highlighted by the finding that replacement of $Pd(OAc)_2$ with a water-soluble Pd source ($Pd(NO_3)_2$ or Na_2Cl_4Pd) results in a cleaner product. In contrast to the $Pd(OAc)_2$–TPPTS system, all other catalysts that proved to be active in this study allowed the isolation of 8-phenylguanosine from the reaction as a white crystalline powder (Table 2, entries 2–4). The best results with regard to yield and reaction times were achieved with catalytic systems combining $Pd(NO_3)_2$ or Na_2Cl_4Pd with the water-soluble phosphine ligand TPPTS (Table 2, entries 2–3). Use of Na_2Cl_4Pd in combination with a different water-soluble ligand recently reported by the Buchwald group[18a] gave no advantage and led to longer reaction times (Table 2, entry 4).

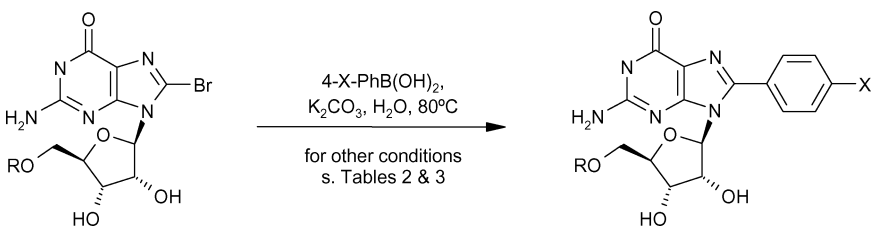
Because of the lower cost and higher stability of Na_2Cl_4Pd in comparison with $Pd(NO_3)_2$, we subsequently adopted the Na_2Cl_4Pd–TPPTS system as our standard catalytic system. In order to explore the scope of its applicability, the cross-coupling of 8-bromoguanosine with a range of electronically diverse phenylboronic acids was investigated. Under these conditions, all cross-coupling reactions afforded the product 8-phenylguanosine derivatives as white crystalline powders in high yields and with short reaction times (Table 3, **4–6**). Encouraged by these results, we next applied our optimised cross-coupling conditions to the Suzuki–Miyaura reaction of the nucleotides **1** and **2**. With Na_2Cl_4Pd–TPPTS as the catalyst, **1** was successfully reacted with various arylboronic acids (Table 3, **7–10**). All product 8-aryl GMP analogues were obtained in good to excellent yields, and most reactions were complete within 2 hours. Only the cross-coupling reaction of 8-bromo GMP with 4-methoxyphenylboronic acid could not be driven to completion under these conditions, even after prolonged reaction time. However, the resulting 8-methoxyphenyl GMP (**10**) was still obtained in 71% yield from this reaction. All cross-coupling reactions could be conveniently monitored by TLC (2-propanol–H_2O–35%NH_3 6 : 3 : 1), and the work-up of the reactions required only a single purification step.

Remarkably, both the starting nucleotide **1** as well as the product 8-aryl GMP analogues **7–10** withstood the basic cross-coupling

Table 3 Suzuki–Miyaura coupling of 8-bromoguanosine, 8-bromo GMP (**1**), and 8-bromo GTP (**2**) with various arylboronic acids[a]

Compound	R	X	Yield (%)	Time/h
4	H	Cl	84	0.3
5	H	CH_3	70	3
6	H	OCH_3	84	0.5
7	PO_3^{2-}	H	63	0.25
8	PO_3^{2-}	Cl	92	1.3
9	PO_3^{2-}	CH_3	90	1
10	PO_3^{2-}	OCH_3	71[b]	6
11	$(PO_3^-)_3^{4-}$	H	85	1
12	$(PO_3^-)_3^{4-}$	Cl	56	1
13	$(PO_3^-)_3^{4-}$	CH_3	65	2

[a] For structures, see Scheme 2; Pd source: Na_2Cl_4Pd; ligand: TPPTS. [b] Reaction does not go to completion.



Scheme 2 Suzuki–Miyaura coupling of 8-bromoguanosine, 8-bromo GMP (**1**), and 8-bromo GTP (**2**).

conditions and the reaction temperature of 80 °C without significant decomposition, despite the known instability of nucleoside phosphates.[11] This promising result prompted us to attempt the cross-coupling of the potentially even more unstable nucleoside triphosphate 8-bromo GTP (**2**) under the same conditions, using three different boronic acids. These reactions also proceeded smoothly, with short reaction times and good yields (Table 3, **11–13**). During the reaction, no decomposition of the triphosphates was detected by TLC, and the ^{31}P NMR spectra of all cross-coupling products showed the triade of signals characteristic for triphosphates. An important factor contributing to the astonishing stability of the guanosine triphosphate derivatives under our cross-coupling conditions certainly is the pronounced activity of the Na_2Cl_4Pd–TPPTS catalytic system, which allows short reaction times at relatively low temperature. In contrast, Hocek and coworkers found that cross-coupling of 8-bromo AMP and 8-bromo ATP requires a much higher temperature of 125 °C when $Pd(OAc)_2$–TPPTS is used as the catalyst, despite the higher cross-coupling reactivity of adenosine derivatives compared to guanosine.[6b]

Conclusions

We have identified suitable reaction conditions for the Suzuki–Miyaura coupling of unprotected 8-bromoguanosine, 8-bromo GMP, and 8-bromo GTP with a range of phenylboronic acids in water, using only commercially available reagents. A catalytic system composed of Na_2Cl_4Pd and TPPTS is suitable to overcome the generally reduced reactivity of guanine nucleosides and nucleotides in Pd-catalysed reactions. We have also optimised the synthesis of the 8-bromoguanosine 5′-phosphates required as cross-coupling substrates. The phosphorylation of 8-bromoguanosine *via* a modified Yoshikawa procedure cleanly gave 8-bromo GMP and 8-bromo GTP when carried out in acetonitrile and with proton sponge as the base. The novel 8-aryl GMP and GTP derivatives presented in this study are interesting biological tools, and investigations into their biological activity are currently underway. Our synthetic conditions may also be applicable to the direct structural modification of even more complex and sensitive biomolecules like sugar-nucleotides and oligonucleotides in aqueous solution.

Experimental

General

MeCN was distilled from CaH_2 prior to use. Dowex resin was washed with water before use. All other chemicals and solvents were of commercial quality and used as received unless stated otherwise. 8-Bromoguanosine was prepared as described by Sheu and Foote.[19] TLC was performed on precoated aluminium plates (Silica Gel 60 F_{254}, Merck). Compounds were visualized by exposure to UV light. NMR spectra were recorded at 298 K on a Varian VXR 400 S spectrometer at 400 MHz (^{1}H) or on a Bruker Avance DPX-300 spectrometer at 300 MHz (^{1}H). Prior to the recording of ^{31}P NMR spectra a drop of triethylamine was added to each sample to suppress line broadening and enhance resolution. Chemical shifts (δ) are reported in ppm and referenced to residual solvent resonances (for DMSO-d_6) or acetone (^{1}H δ 2.05, ^{13}C δ 30.83 for solutions in D_2O). Coupling constants (J) are reported in Hz. Resonance assignments are based on COSY experiments. Splitting patterns are abbreviated as follows: br, broad; s, singlet; d, doublet; t, triplet; m, multiplet. Accurate electrospray ionization mass spectra (HR ESI-MS) were obtained on a Finnigan MAT 900 XLT mass spectrometer. Preparative chromatography was performed on a Biologic LP chromatography system equipped with a peristaltic pump and a 254 nm UV Optics Module using the following conditions: Method A—stationary phase: LiChroprep RP-18 resin, equilibrated with 0.05M TEAB buffer (pH 6.0–6.7); gradient: 0–10% MeCN against 0.05M TEAB over 400 mL; flow rate: 3 mL min^{-1}. Method B—stationary phase: MacroPrep 25Q washed with H_2O; gradient: 0–100% 1M TEAB buffer (pH 7.1–7.6) against H_2O over 400 mL; flow rate: 5 mL min^{-1}. Product containing fractions were combined and reduced to dryness. The residue was co-evaporated with methanol to remove residual TEAB.

8-Bromo GMP (1). A mixture of 8-bromoguanosine[19] (50 mg, 0.14 mmol) and proton sponge (180 mg, 0.84 mmol) was suspended in dry acetonitrile (3 mL). Under an atmosphere of N_2, the suspension was stirred for 30 min at room temperature. The suspension was cooled to 2 °C, and $POCl_3$ (51 μL, 0.56 mmol) was added dropwise while maintaining the temperature at 2–5 °C. After the addition of $POCl_3$ was complete, a clear, slightly yellow solution formed within minutes. The reaction was stirred at 2 °C until TLC indicated completion (2–3 h). Under cooling, the reaction was added dropwise to ice-cold 0.2M TEAB buffer (40 mL, pH 7.3). The aqueous solution was stored at 4 °C overnight to allow deprotonation of proton sponge, washed repeatedly with ethyl acetate (4 × 25 mL) and evaporated to dryness. The residue was purified by ion-pair chromatography (method A) to give **1** as a glassy solid in 90% yield (79 mg, 1.7 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 5.71 (d, J = 6.0, 1H, H-1′), 5.06 (t, J = 5.6/5.2, 1H, H-2′), 4.39–4.36 (m, 1H, H-3′), 4.04–4.00 (m, 1H, H-4′), 3.92–3.87 (m, 1H, H_a-5′), 3.84–3.78 (m, 1H, H_b-5′), 2.95 (q, J = 7.2, 9.9H, CH_2), 1.03 (t, J = 7.2, 15.7H, CH_3). δ_C (100 MHz, D_2O) 157.6, 153.6, 152.8, 124.1, 116.7, 89.9, 84.1 (d, $J_{C,P}$ = 8.4), 70.8, 70.4, 64.0, 46.6, 8.3. δ_P (162 MHz, D_2O) 7.48. HRMS (ES, negative) calcd. for $C_{10}H_{12}O_8N_5{}^{79}BrP$ 439.9612 (monoanion), found 439.9619.

8-Bromo GTP (2). A mixture of 8-bromoguanosine[19] (400 mg, 1.1 mmol) and proton sponge (1.44 g, 6.6 mmol) was suspended in dry acetonitrile (20 mL). Under an atmosphere of N_2, the suspension was stirred for 30 min at room temperature. The suspension was cooled to 2 °C, and $POCl_3$ (410 μL, 4.5 mmol) was added dropwise while maintaining the temperature at 2–5 °C. After the addition of $POCl_3$ was complete, a clear, slightly yellow solution formed within 10 min. The reaction was stirred at 2 °C until TLC indicated complete consumption of starting material (2.5 h). Under cooling, a cold mixture of Bu_3N (1.1 mL, 4.6 mmol) and 1.5M tri-*n*-butylammonium pyrophosphate in dry DMF[17] (5 mL, 7.5 mmol) was added in one portion. The reaction was allowed to stir at 4 °C for 15 min, and added dropwise to ice-cold 0.2M TEAB buffer (120 mL, pH 7.2). The aqueous solution was stored at 4 °C overnight to allow deprotonation of proton sponge, washed repeatedly with ethyl acetate (4 × 80 mL) and evaporated to dryness. The residue was purified consecutively by ion-pair (method A, to remove inorganics) and ion-exchange

(method B, to remove residual **1**) chromatography to give **2** as a glassy solid in 72% yield (709 mg, 2.9 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 5.78 (d, J = 6.2, 1H, H-1′), 5.18 (t, J = 5.9/6.0, 1H, H-2′), 4.51–4.48 (m, 1H, H-3′), 4.16–4.04 (m, 3H, H-4′, H-5′), 3.00 (q, J = 7.3, 17.8H, CH_2), 1.08 (t, J = 7.3, 25.3H, CH_3). δ_C (100 MHz, D_2O) 157.2, 153.1, 152.4, 123.4, 116.1, 89.1, 83.1, 70.1, 69.3, 64.5, 45.8, 7.5; δ_P (162 MHz, D_2O) −2.89 (d, J = 20.4), −7.68 (d, J = 19.3), −19.09 (t, J = 20.2). HRMS (ES, negative) calcd. for $C_{10}H_{14}O_{14}N_5{}^{79}BrP_3$ 599.8939 (monoanion), found 599.8942.

General procedure A for the Suzuki–Miyaura coupling of 8-bromoguanosine

8-Bromoguanosine[19] (1 equiv.), Na_2Cl_4Pd (2.5 mol%), TPPTS (2.5 equiv. to Pd), K_2CO_3 (2 equiv.), and arylboronic acid (1.2 equiv.) were placed in a flask and purged with N_2. Degassed water (6 mL) was added *via* a syringe, and the reaction was stirred at 80 °C under N_2 for the given time. Upon completion, the aqueous reaction was diluted with H_2O (20 mL) and the pH adjusted to 7 using 10% HCl. The resulting precipitate was collected by filtration and dried *in vacuo.*

8-Phenylguanosine (3). The target compound was obtained from 8-bromoguanosine (103.1 mg, 0.285 mmol) and phenylboronic acid according to general procedure A (0.3 h) as a white crystalline powder in 77% yield (78.7 mg). δ_H (400 MHz, DMSO-d_6) 10.75 (s, 1H, N–H), 7.65–7.63 (m, 2H, 2Ph), 7.53–7.48 (m, 3H, 3Ph), 6.38 (br s, 2H, NH_2), 5.61 (d, J = 6.4, 1H, H-1′), 5.37 (d, J = 6.4, 1H, OH-2′), 5.05–4.97 (m, 3H, H-2′, OH-3′, OH-5′), 4.07–4.03 (m, 1H, H-3′), 3.82–3.79 (m, 1H, H-4′), 3.68–3.62 (m, 1H, H_a-5′), 3.55–3.49 (m, 1H, H_b-5′). δ_C (100 MHz, DMSO-d_6) 157.3, 153.8, 152.7, 148.2, 130.7, 130.1, 129.9, 129.3, 117.8, 89.6, 86.5, 71.3, 70.9, 62.8. HRMS (ES, positive) calcd. for $C_{16}H_{18}O_5N_5$ 360.1302 $[M + H]^+$, found 360.1301.

8-(4-Chlorophenyl)guanosine (4). The target compound was obtained from 8-bromoguanosine (100.5 mg, 0.278 mmol) and 4-chlorophenylboronic acid according to general procedure A (0.5 h) as a white crystalline powder in 84% yield (91.9 mg). δ_H (400 MHz, DMSO-d_6) 10.78 (s, 1H, N–H), 7.66 (d, J = 8.5, 2H, 2Ph), 7.58 (d, J = 8.6, 2H, 2Ph), 6.42 (br s, 2H, NH_2), 5.58 (d, J = 6.6, 1H, H-1′), 5.35 (d, J = 6.4, 1H, OH-2′), 5.05–4.94 (m, 3H, H-2′, OH-3′, OH-5′), 4.06–4.03 (m, 1H, H-3′), 3.83–3.80 (m, 1H, H-4′), 3.67–3.62 (m, 1H, H_a-5′), 3.55–3.50 (m, 1H, H_b-5′). δ_C (100 MHz, DMSO-d_6) 157.2, 153.9, 152.8, 146.9, 135.0, 131.5, 129.6, 129.4, 117.8, 89.5, 86.5, 71.1, 70.9, 62.6. HRMS (ES, positive) calcd. for $C_{16}H_{17}O_5N_5{}^{35}Cl$ 394.0913 $[M + H]^+$, found 394.0913.

8-(4-Methylphenyl)guanosine (5). The target compound was obtained from 8-bromoguanosine (101.5 mg, 0.280 mmol) and 4-methylphenylboronic acid according to general procedure A (3 h) as a white crystalline powder in 70% yield (73.1 mg). δ_H (400 MHz, DMSO-d_6) 10.73 (s, 1H, N–H), 7.52 (d, J = 8.1, 2H, 2Ph), 7.31 (d, J = 8.2, 2H, 2Ph), 6.36 (br s, 2H, NH_2), 5.59 (d, J = 6.6, 1H, H-1′), 5.34 (d, J = 6.4, 1H, OH-2′), 5.05–4.95 (m, 3H, H-2′, OH-3′, OH-5′), 4.05–4.03 (m, 1H, H-3′), 3.80–3.78 (m, 1H, H-4′), 3.65–3.62 (m, 1H, H_a-5′), 3.54–3.51 (m, 1H, H_b-5′), 2.36 (s, 3H, CH_3). δ_C (100 MHz, DMSO-d_6) 157.3, 152.7, 152.6, 140.3, 139.8, 129.8, 129.8, 127.9, 117.7, 89.6, 86.4, 71.3, 70.9, 62.7, 21.6. HRMS (ES, positive) calcd. for $C_{17}H_{20}O_5N_5$ 374.1459 $[M + H]^+$, found 374.1463.

8-(4-Methoxyphenyl)guanosine (6). The target compound was obtained from 8-bromoguanosine (98.8 mg, 0.273 mmol) and 4-methoxyphenylboronic acid according to general procedure A (0.5 h) as a white crystalline powder in 84% yield (88.6 mg). δ_H (400 MHz, DMSO-d_6) 10.72 (s, 1H, N–H), 7.56 (d, J = 8.5, 2H, 2Ph), 7.06 (d, J = 8.5, 2H, 2Ph), 6.35 (br s, 2H, NH_2), 5.59 (d, J = 6.5, 1H, H-1′), 5.35 (d, J = 6.3, 1H, OH-2′), 5.07–4.96 (m, 3H, H-2′, OH-3′, OH-5′), 4.07–4.03 (m, 1H, H-3′), 3.80 (br s, 4H, H-4′, CH_3), 3.67–3.62 (m, 1H, H_a-5′), 3.55–3.49 (m, 1H, H_b-5′). δ_C (100 MHz, DMSO-d_6) 160.8, 157.2, 153.6, 152.5, 148.2, 131.3, 123.0, 117.6, 114.7, 89.6, 86.4, 71.3, 70.9, 62.8, 56.0. HRMS (ES, positive) calcd. for $C_{17}H_{19}O_6N_5$ 412.1228 $[M + Na]^+$, found 412.1228.

General procedure B for the Suzuki–Miyaura coupling of 8-bromo GMP (2) and 8-bromo GTP (3)

Na_2Cl_4Pd (2.5 mol%), TPPTS (2.5 equiv. to Pd), K_2CO_3 (1.5 equiv.), and arylboronic acid (1.2 equiv.) were placed in a flask and purged with N_2. A solution of 8-bromo nucleotide (1 equiv.) in degassed water (3 mL) was added *via* a syringe. The pale yellow solution was stirred at 80 °C under N_2 for the given time. Upon completion, the reaction turned dark red/brown. The reaction was cooled to room temperature, filtered and the pH adjusted to 7 using Dowex 50WX-8 100 resin (H^+ form). The resin was filtered off and washed with water, and the combined filtrate was evaporated to dryness. The crude product was dissolved in 0.05M TEAB buffer and purified by ion-pair chromatography (method A).

8-Phenylguanosine monophosphate (7). The target compound was prepared from **1** (43.4 mg, 0.072 mmol) and phenylboronic acid according to general procedure B (15 min). After purification by ion-pair chromatography (method A, fractions 43–53), **7** was obtained as a glassy solid in 63% yield (25.0 mg, 1.2 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 7.38–7.29 (m, 5H, 5Ph), 5.56 (d, J = 6.0, 1H, H-1′), 5.02 (t, J = 5.9, 1H, H-2′), 4.28 (dd, J = 3.6/5.7, 1H, H-3′), 4.02–3.90 (m, 3H, H-4′ + H_2-5′), 2.97 (q, J = 7.3, 7H, CH_2), 1.05 (t, J = 7.3, 10H, CH_3). δ_C (100 MHz, D_2O) 159.2, 153.8, 153.3, 150.9, 131.1, 129.8, 129.5, 128.3, 116.6, 89.6, 84.1 (d, $J_{C,P}$ = 8.4), 71.0, 70.7, 65.2 (d, $J_{C,P}$ = 4.6), 47.2, 8.8. δ_P (162 MHz, D_2O) 7.43. HRMS (ES, negative) calcd. for $C_{16}H_{17}O_8N_5P$ 438.0813 (monoanion), found 438.0820.

8-(4-Chlorophenyl)guanosine monophosphate (8). The target compound was prepared from **1** (50.0 mg, 0.079 mmol) and 4-chlorophenylboronic acid according to general procedure B (1.3 h). After purification by ion-pair chromatography (method A, fractions 59–70), **8** was obtained as a glassy solid in 92% yield (43.9 mg, 1.2 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 7.18 (s, 4H, 4Ph), 5.51 (d, J = 5.1, 1H, H-1′), 4.94 (t, J = 5.5, 1H, H-2′), 4.44–4.41 (m, 1H, H-3′), 4.09–3.94 (m, 3H, H-4′ + H_2-5′), 2.99 (q, J = 7.3, 8H, CH_2), 1.08 (t, J = 7.3, 12H, CH_3). δ_C (100 MHz, D_2O) 158.8, 153.6, 153.0, 149.0, 136.4, 130.6, 129.4, 126.4, 116.4, 90.1, 83.7 (d, $J_{C,P}$ = 8.2), 71.0, 70.8, 65.2 (d, $J_{C,P}$ = 4.3), 47.2, 8.8. δ_P (162 MHz, D_2O) 7.40. HRMS (ES, negative) calcd. for $C_{16}H_{16}O_8N_5ClP$ 472.0430 (monoanion), found 472.0423.

8-(4-Methylphenyl)guanosine monophosphate (9). The target compound was prepared from **1** (49.6 mg, 0.078 mmol) and 4-methylphenylboronic acid according to general procedure B (1 h). After purification by ion-pair chromatography (method A, fractions 59–72), **9** was obtained as a glassy solid in 90% yield (41.5 mg, 1.4 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 7.28 (d, $J = 7.2$, 2H, 2Ph), 7.19 (d, $J = 8.0$, 2H, 2Ph), 5.72 (d, $J = 5.2$, 1H, H-1′), 5.05 (t, $J = 5.6/5.2$, 1H, H-2′), 4.57–4.55 (m, 1H, H-3′), 4.26–4.11 (m, 3H, H-4′ + H_2-5′), 3.15 (q, $J = 7.6/7.2$, 8.6H, CH_2), 2.34 (s, 3H, CH_3–Ph), 1.24 (t, $J = 7.2/7.6$ Hz, 12.9H, CH_3). δ_C (100 MHz, D_2O) 158.9, 153.4, 152.0, 150.4, 141.5, 129.9, 129.2, 124.9, 116.3, 90.1, 83.7 (d, $J_{C,P} = 8.6$), 71.0, 70.9, 65.2 (d, $J_{C,P} = 4.3$), 47.2, 21.1, 8.8. δ_P (162 MHz, D_2O) 7.41. HRMS (ES, negative) calcd. for $C_{17}H_{19}O_8N_5P$ 452.0980 (monoanion), found 452.0977.

8-(4-Methoxyphenyl)guanosine monophosphate (10). The target compound was prepared from **1** (49.7 mg, 0.078 mmol) and 4-methoxyphenylboronic acid according to general procedure B (6 h). After purification by ion-pair chromatography (method A, fractions 48–60), **10** was obtained as a glassy solid in 71% yield (32.1 mg, 1.1 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 7.20 (d, $J = 8.7$, 2H, 2Ph), 6.76 (d, $J = 8.8$, 2H, 2Ph), 5.56 (d, $J = 5.4$, 1H, H-1′), 4.96 (t, $J = 5.6$, 1H, H-2′), 4.40–4.37 (m, 1H, H-3′), 4.09–3.93 (m, 3H, H-4′ + H_2-5′), 3.68 (s, 3H, CH_3–O), 3.00 (q, $J = 7.4$, 6.5H, CH_2), 1.08 (t, $J = 7.4$, 10H, CH_3). δ_C (100 MHz, D_2O) 160.9, 158.9, 153.4, 153.0, 150.2, 131.1, 120.6, 116.2, 114.6, 89.9, 83.7 (d, $J_{C,P} = 8.4$), 70.9, 70.8, 65.3 (d, $J_{C,P} = 5.1$), 56.0, 47.2, 8.8. δ_P (162 MHz, D_2O) 7.45. HRMS (ES, negative) calcd. for $C_{17}H_{19}O_9N_5P$ 468.0926 (monoanion), found 468.0930.

8-Phenylguanosine triphosphate (11). The target compound was prepared from **2** (60.5 mg, 0.068 mmol) and phenylboronic acid according to general procedure B (1 h). After purification by ion-pair chromatography (method A, fractions 33–52), **11** was obtained as a glassy solid in 85% yield (53.3 mg, 3.1 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 7.45–7.37 (m, 5H, 5Ph), 5.61 (d, $J = 6.1$, 1H, H-1′), 5.19 (t, $J = 5.8$, 1H, H-2′), 4.46–4.44 (m, 1H, H-3′), 4.18–4.06 (m, 3H, H-4′ + H_2-5′), 2.99 (q, $J = 7.3$, 18H, CH_2), 1.08 (t, $J = 7.3$, 28H, CH_3). δ_C (100 MHz, D_2O) 153.8, 153.6, 151.0, 131.2, 129.9, 129.6, 128.6, 128.4, 116.6, 89.5, 84.0 (d, $J_{C,P} = 9.5$), 71.0, 70.7, 65.9 (d, $J_{C,P} = 4.9$), 47.2, 8.8. δ_P (162 MHz, D_2O) −2.87 (d, $J = 20.4$), −7.58 (d, $J = 19.6$), −19.02 (t, $J = 19.8/20.0$). HRMS (ES, negative) calcd. for $C_{16}H_{19}O_{14}N_5P_3$ 598.0147 (monoanion), found 598.0145.

8-(4-Chlorophenyl)guanosine triphosphate (12). The target compound was prepared from **2** (59.2 mg, 0.066 mmol) and 4-chlorophenylboronic acid according to general procedure B (1 h). After purification by ion-pair chromatography (method A, fractions 53–72), **12** was obtained as a glassy solid in 56% yield (35.8 mg, 3.2 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 7.35 (s, 4H, 4Ph), 5.57 (d, $J = 6.0$, 1H, H-1′), 5.18 (t, $J = 5.8$, 1H, H-2′), 4.48–4.46 (m, 1H, H-3′), 4.19–4.06 (m, 3H, H-4′ + H_2-5′), 3.00 (q, $J = 7.3$, 19H, CH_2), 1.08 (t, $J = 7.3$, 30H, CH_3). δ_C (100 MHz, D_2O) 159.2, 153.8, 153.5, 149.7, 136.7, 131.2, 129.7, 126.8, 116.6, 89.6, 84.0 (d, $J_{C,P} = 8.5$), 70.9, 70.7, 65.9, 47.2, 8.8. δ_P (162 MHz, D_2O) −2.86 (d, $J = 20.9$), −7.58 (d, $J = 19.6$), −19.00 (t, $J = 20.2/20.3$). HRMS (ES, negative) calcd. for $C_{16}H_{18}O_{14}N_5ClP_3$ 631.9757 (monoanion), found 631.9763.

8-(4-Methylphenyl)guanosine triphosphate (13). The target compound was prepared from **2** (59.2 mg, 0.066 mmol) and 4-methylphenylboronic acid according to general procedure B (2 h). After purification by ion-pair chromatography (method A, fractions 41–54), **13** was obtained as a glassy solid in 65% yield (40.4 mg, 3.3 equiv. of triethylammonium as determined by NMR). δ_H (400 MHz, D_2O) 7.24 (d, $J = 7.9$, 2H, 2Ph), 7.14 (d, $J = 8.0$, 2H, 2Ph), 5.60 (d, $J = 5.7$, 1H, H-1′), 5.10 (t, $J = 5.6$, 1H, H-2′), 4.49–4.47 (m, 1H, H-3′), 4.23–4.06 (m, 3H, H-4′ + H_2-5′), 2.99 (q, $J = 7.3$, 20H, CH_2), 2.23 (s, 3H, CH_3–Ph), 1.08 (t, $J = 7.3$, 30H, CH_3). δ_C (100 MHz, D_2O) 159.0, 153.7, 153.3, 150.8, 141.9, 130.1, 129.6, 125.0, 116.1, 89.8, 83.9 (d, $J_{C,P} = 8.7$), 71.0, 70.7, 65.9 (d, $J_{C,P} = 3.2$), 47.2, 21.2, 8.8. δ_P (162 MHz, D_2O) −2.87 (d, $J = 20.7$), −7.58 (d, $J = 19.7$), −19.00 (t, $J = 20.1$). HRMS (ES, negative) calcd. for $C_{17}H_{21}O_{14}N_5P_3$ 612.0303 (monoanion), found 612.0310.

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