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Design, Synthesis, and Biochemical and Biological Evaluation of Novel 7-Deazapurine Cyclic Dinucleotide Analogues as STING Receptor Agonists

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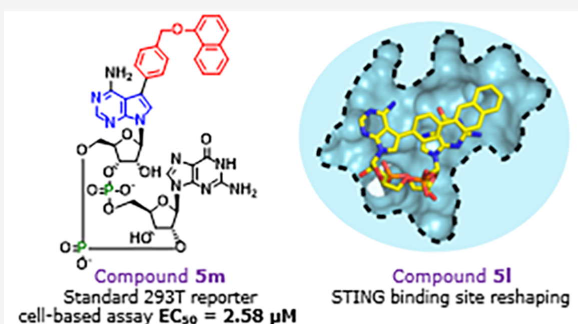
ABSTRACT: Cyclic dinucleotides (CDNs) are second messengers that activate stimulator of interferon genes (STING). The cGAS-STING pathway plays a promising role in cancer immunotherapy. Here, we describe the synthesis of CDNs containing 7-substituted 7-deazapurine moiety. We used mouse cyclic GMP−AMP synthase and bacterial dinucleotide synthases for the enzymatic synthesis of CDNs. Alternatively, 7-(het)aryl 7-deazapurine CDNs were prepared by Suzuki−Miyaura cross-couplings. New CDNs were tested in biochemical and cell-based assays for their affinity to human STING. Eight CDNs showed better activity than 2′3′-cGAMP, the natural ligand of STING. The effect on cytokine and chemokine induction was also evaluated. The best activities were observed for CDNs bearing large aromatic substituents that point above the CDN molecule. We solved four X-ray structures of complexes of new CDNs with human STING. We observed $\pi-\pi$ stacking interactions between the aromatic substituents and Tyr240 that are involved in the stabilization of CDN-STING complexes.



INTRODUCTION

The cyclic GMP−AMP synthase (cGAS)−stimulator of interferon genes (STING) pathway is an important player in detecting damage-associated (DAMPs) and pathogen-associated molecular patterns (PAMPs).[1−4] Upon dsDNA detection in cytosol, cGAS synthetizes a STING ligand 2′3′-cGAMP (**1a**, cyclic [G(2′,5′)pA(3′,5′)p] with mixed 2′−5′ and 3′−5′ phosphodiester linkages).[5,6] Besides eukaryotic 2′3′-cGAMP, STING can be also activated by bacterial cyclic dinucleotides (CDNs) such as 3′3′-cGAMP (**1b**), c-di-GMP (**1c**), and c-di-AMP (**1d**), containing two 3′−5′ phosphodiester bonds (Scheme 1).[7−10] Binding of CDNs to STING triggers a downstream response that results in the expression of proinflammatory cytokines (TNF-α, IL-1β) via a nuclear factor κ-light-chain enhancer of activated B-cells (NF-κB) and/or the expression of type I interferons (IFN-α, IFN-β) via an interferon regulatory factor 3 (IRF3).[11−15] When taking into consideration this response, STING plays a crucial role in defense against pathogen infections, immune surveillance of tumor cells, and maintenance of the normal immune functions of the body.[16−19]

Human STING agonists[21,22] can be divided into two major groups: (1) CDNs and their derivates (ADU-S100,[23] MK-1454,[24] TAK-676,[25] etc.[26]) and (2) synthetic non-nucleotide STING agonists (diABZI,[27] G10,[28,29] MSA-2,[30] etc.[31,32]). Due to the importance of the cGAS-STING pathway, there has been an increased interest in identifying new STING agonists with improved drug-like properties compared to the natural STING ligands. This attention is highlighted by the fact that seven different STING agonists are presently being investigated in different phases of clinical trials.[22]

Herein, we report the design, synthesis, and biochemical and biological evaluation of 7-substituted 7-deazapurine CDNs that activate STING signaling. As we reported in our previous study,[20] 7-deazapurine CDN (Scheme 1) can be tolerated when forming the STING-CDN complex. In an effort to further probe CDN−STING interactions, we decided to explore the effect of 7-substitutions on the activity of CDNs by preparing 24 new 7-substituted 7-deazapurine CDNs. Considering the results of the initial trials, we decided to focus mainly on 7-deazaadenine derivatives due to the fact that the

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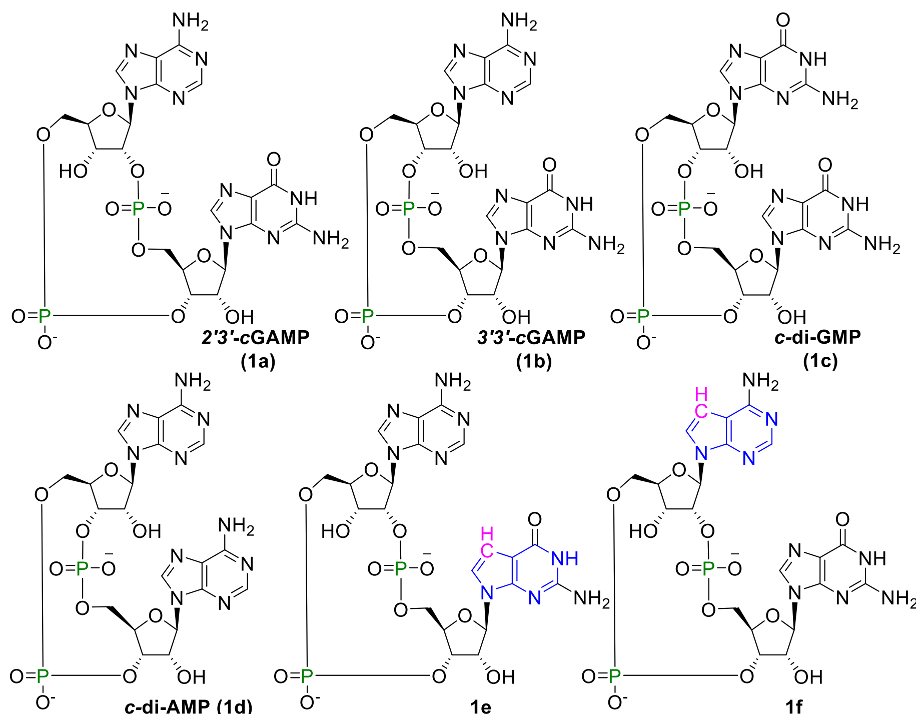
Scheme 1. Naturally Occurring CDNs (1a–d) and 7-Deaza Variants (1e,f) Reported in Our Previous Study[20]

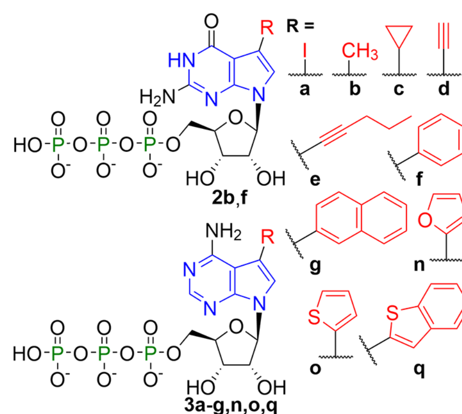


presence of 7-deazaguanine in compound **1e** caused a substantial drop in affinity to wt hSTING.[20] When possible, compounds were prepared enzymatically by mouse cGAS (mcGAS) as previously shown or with bacterial dinucleotide cyclase from *Vibrio cholerae* (DncV) and diadenylate cyclase from *Bacillus thuringiensis* (DisA).[20,33−35] Otherwise, the compounds were prepared by chemical synthesis, either by arylation of an enzymatically prepared precursor or by total synthesis. All compounds were tested by differential scanning fluorimetry (DSF) and in cell-based assays. For four of the compounds, we determined their structures in complex with STING by X-ray crystallography to better understand their binding mode.

RESULTS AND DISCUSSION

Enzymatic Synthesis. As shown in previous works, cGAS and bacterial dinucleotide cyclases DncV and DisA can be used for the synthesis of CDNs by employing various NTP analogues.[20,33,34] This allows for the substitution of a long and multi-step total synthesis of CDNs[36] with a one-step enzymatic reaction. Only the enzymatic synthesis of CDNs from nucleoside triphosphates (NTPs) bearing small nucleobase modifications has been studied so far.[33] Therefore, we decided to explore 7-substituted 7-deazaguanosine triphosphates **2** (G^RTPs) and 7-substituted 7-deazaadenosine triphosphates **3** (A^RTPs) as substrates for these enzymes (Scheme 2). Successful enzymatic synthesis of CDNs **4b** (2′3′-*c*G^RAMP), **5a-f,n,o** (2′3′-*c*GA^RMPs), and **6f** (2′3′-*c*G^RA^RMP) indicates that mcGAS tolerates small substituents in position 7 of 7-deazapurine NTPs including halogen, alkyl, cycloalkyl, alkynyl, small hetaryl, and phenyl groups (Schemes 3 and 4). Unfortunately, NTPs with aryls bigger than phenyl, that is, 2-naphthyl (**3g**) and 2-benzothienyl (**3q**),[37] caused a disruption of the enzymatic reaction, leaving triphosphates unreacted. In some cases, such as CDN **5a**, the conversions were quantitative; thus, we were able to enzymatically prepare

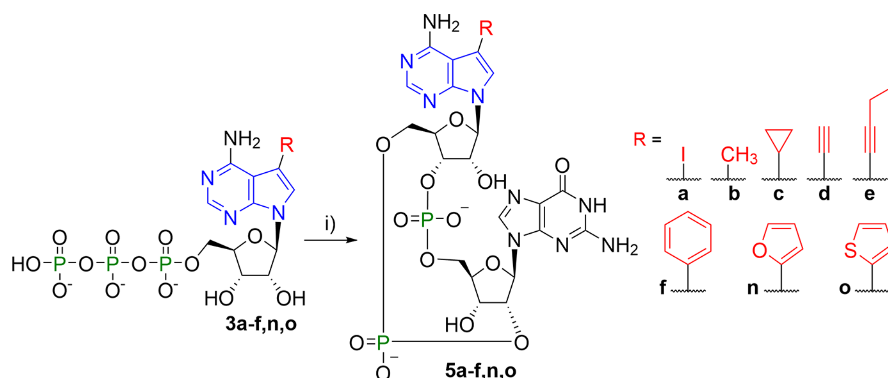
Scheme 2. 7-Deazapurine NTPs for Enzymatic Synthesis



more than 70 mg of this CDN in one batch, which was further arylated as described below. For the synthesis of the 7-substituted 3′3′-CDNs **7a** and **8a**, we used the bacterial enzymes DncV and DisA (Scheme 5).

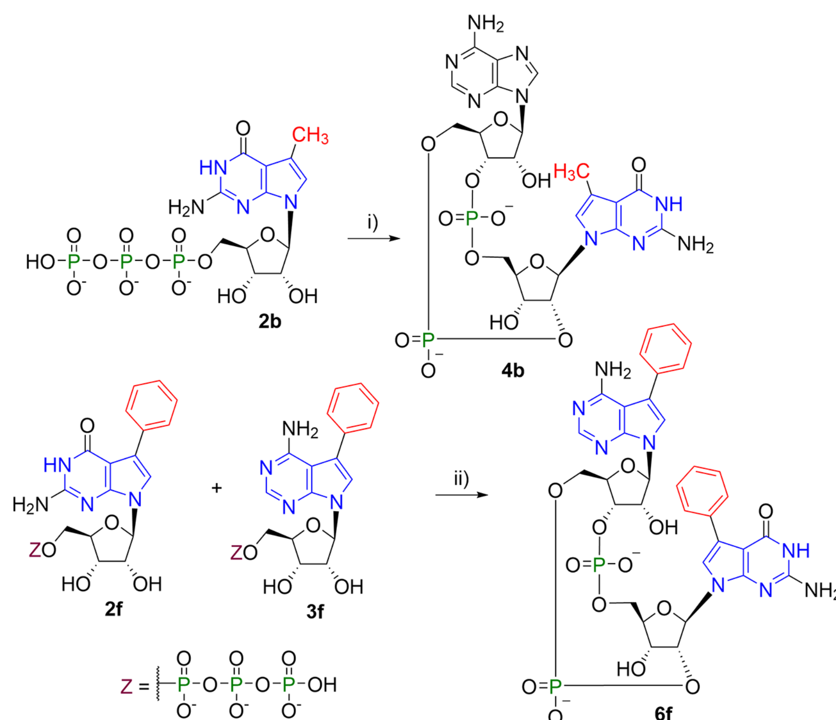
Chemistry. Chemical synthesis of 7-aryl-7-deazaadenine CDNs relied on a modular approach. The key intermediates, 7-iodo-7-deazaadenine CDNs **5a**, **7a**, and **8a**, were converted to the desired aryl-CDNs using Suzuki−Miyaura cross-coupling reactions. The iodinated CDNs can be prepared by chemical synthesis (i.e., **5a**) and/or enzymatic synthesis (i.e., **5a**, **7a**, and **8a**). The chemical synthesis of iodinated 7-deazaadenine CDN **5a** started from 7-iodo-7-deazaadenosine (**9**). First, H-phosphonate **12** had to be synthesized (Scheme 6). Iodinated nucleoside **9** was *N*-benzoylated using transient silyl protection.[38] The *N*-benzoylated intermediate was not isolated due to its poor solubility that complicated its chromatographic purification. The crude product was directly used in a tritylation step to obtain 5′-*O*-DMTr-protected nucleoside **10** (68% over two steps). Regioselective 2′-*O*-silylation using

Scheme 3. Enzymatic Synthesis of 2′3′-cGA^{R}MPs[a]



[a]Reagents and conditions: (i) GTP, Tris−HCl [pH 8.0], $MgCl_2$, dsDNA, mcGAS, 37 °C 16 h.

Scheme 4. Enzymatic Synthesis of 7-Deazaguanine Containing CDNs[a]

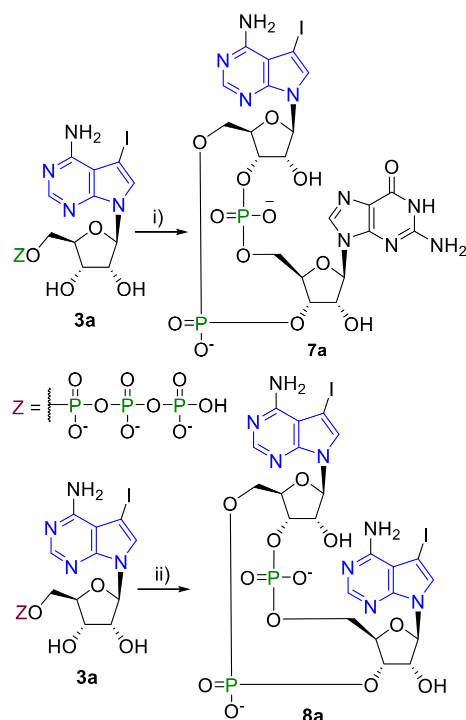


[a]Reagents and conditions: (i) ATP, Tris−HCl [pH 8.0], $MgCl_2$, dsDNA, mcGAS, 37 °C 16 h; (ii) Tris−HCl [pH 8.0], $MgCl_2$, dsDNA, mcGAS, 37 °C 16 h.

silver nitrate catalysis[39] provided nucleoside **11**. 3′-*O*-Silyl isomer was also formed during the reaction as a minor product, but it could not be efficiently separated from the 2′-*O*-silyl isomer **11**. However, the mixture of silylated products could be easily deprotected using TBAF in THF to regenerate the starting material **10**. 3′-*H*-Phosphonate moiety was installed by the reaction of **11** with diphenyl phosphite. The crude product was directly used for the next step in order to avoid a loss of the material during column chromatography. After the removal of the DMTr-group by DCA, *H*-phosphonate **12** was obtained as a triethylammonium salt (77% over two steps).

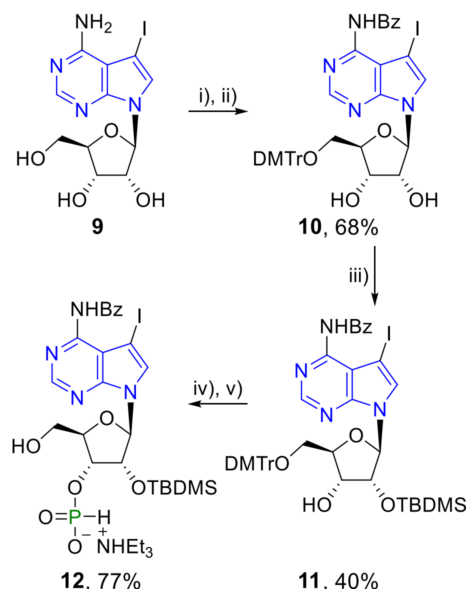
The reaction of *H*-phosphonate building blocks with phosphoramidites is typically performed in acetonitrile in the presence of a coupling activator,[40] such as py-TFA. However, due to the low solubility of **12** in acetonitrile, its reaction with guanosine phosphoramidite **13** proceeded poorly. Efforts to improve the reaction yield by increasing the amount of py-TFA or by the use of other coupling activators, such as ethylthio-1*H*-tetrazole (ETT), failed. However, when **12** was treated with DCA in order to convert the triethylammonium salt to a standard *H*-phosphonate, the solubility in acetonitrile improved, and the reaction with guanosine phosphoramidite **13** proceeded even without any coupling activator. After detritylation, linear dinucleotide **14** was partially purified using reverse phase flash chromatography. The crude linear product **14** was cyclized using 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide (DMOCP).[41] After oxidation with iodine and a partial purification on a reverse phase C18 column, the protected CDN **15** was obtained as a crude material. Deprotection of nucleobases and the phosphate using CH_3NH_2 and the removal of silyl groups by $Et_3N{\cdot}3HF$ provided 7-iodinated 7-deazaadenine CDN **5a** in an overall yield of 18% (starting from *H*-phosphonate **12**, Scheme 7).

Scheme 5. Enzymatic Synthesis with Bacterial Enzymes[a]



[a]Reagents and conditions: (i) GTP, HEPES [pH 8.0], $MgCl_2$, NaCl, DTT, DncV, 37 °C 16 h; (ii) HEPES [pH 8.0], $MgCl_2$, NaCl, DTT, DisA, 50 °C 16 h.

Scheme 6[a]



[a]Reagents and conditions: (i) TMSCl, BzCl/py, 0 °C—rt, 16 h; (ii) DMTrCl/py, rt, 16 h; (iii) TBDMSCl, $AgNO_3$, py/THF, rt 16 h; (iv) (1) $PO(OPh)_2$/py, rt, 1 h, (2) H_2O, rt, 5 min; (v) (1) DCA/DCM, rt, 15 min, (2) TES, rt, 30 min.

The Suzuki−Miyaura cross-coupling reaction is a generally used method for the introduction of aryl and hetaryl groups into the position 7 of 7-deazapurine nucleotides.[42] Hydrolysis of the phosphodiester backbone was observed during the synthesis of 8-arylguanosine-containing CDNs using Suzuki−Miyaura cross-coupling.[43] Therefore, we decided to use reaction conditions that were originally optimized for the synthesis of 7-deazapurine NTPs that also suffer from hydrolytic unstability.[44,45] A short reaction time (30 min) was crucial in order to avoid excessive hydrolysis. The cross-coupling reactions were performed under $Pd(OAc)_2$ catalysis in the presence of a water-soluble ligand, triphenylphosphan-3,3′,3″-trisulfonate (TPPTS), and Cs_2CO_3 in water−acetonitrile mixture (2:1). The reaction of iodinated CDN **5a** with 2 equiv of phenylboronic acid did not provide full conversion so that phenyl derivative **5f** was obtained in low yield (18%, Scheme 8). With 5 equiv of arylboronic acids (or arylboronic acid pinacol esters), the cross-couplings proceeded smoothly. Nevertheless, the yields were affected by the hydrolysis. Arylated 2′3′-*c*GA^{R}MPs **5f−m** and **p−r** were prepared in 30−59% yield (Scheme 8). From the reaction with dibenzofuran-4-ylboronic acid, isomerized side product **16** (Figure 1) was obtained in 13% yield. Similar byproducts were observed in all cross-coupling reactions, but the byproducts were not isolated. Due to hindered rotation at room temperature, CDNs **5h−j** were prepared as inseparable mixtures of diastereomers/atropoisomers. Hindered rotation of bulky aryl substituents has been reported among corresponding 7-aryl-7-deazaadenosines.[46]

The conditions for the Suzuki−Miyaura cross-coupling reaction were also applied for the synthesis of 3′3′-CDNs. Iodinated CDNs **7a** and **8a** were converted to corresponding phenyl derivatives **7f** (66%) and **8f** (78%), respectively (Scheme 9). In these reactions, higher yields were achieved because the hydrolysis rate of 3′3′-CDNs during the cross-coupling reaction was significantly lower compared to that of 2′3′-CDNs.

Biochemistry and Biology. In order to biochemically and biologically characterize prepared CDNs, all CDNs were tested using DSF and 293T cell-based reporter assays. The selected compounds that showed activity were further evaluated for the induction of cytokines using human peripheral blood mononuclear cells (PBMCs). DSF was performed using wild-type human STING protein (UniProt Q86WV6). This method provides a useful insight into the STING binding properties of CDNs by determining their ability to improve the thermal stability of the protein.[47,48] Digitonin 293T cell-based assays were performed using five major allelic variants (wild type, HAQ, AQ, Q, and REF)[49] in the presence of digitonin A that facilitates entry of CDNs into cells due to the permeabilization of cell membranes.[20] The standard format of the 293T cell assay was carried out only with cells expressing wild-type human STING and in the absence of the permeabilizing agent.[20]

Our initial experiments with 7-deaza substituted 2′3′-*c*GAMP showed that 7-methyl is much less tolerated on 7-deazaguanine (**4b**) than on 7-deazaadenine (**5b**) (Table 1). 7-Deazaguanine-modified **4b** had ΔT_m in a DSF assay of 3.3 °C while 7-deazaadenine-modified **5b** of 11.5 °C. This suggests that the noncovalent interactions of the NH group at position 7 of the nucleobases have a bigger impact on the CDN binding to STING for guanine than for adenine. The results are in agreement with the data for the 7-deaza modification that we reported on earlier.[20] These findings were further confirmed by improved activity of **5b** than of **4b** in the 293T cell-based reporter assay (Table 1). This led to focusing our efforts on the synthesis of 2′3′-*c*GA^{R}MP-derived CDNs. We prepared compounds **5c−r**, each containing a substituent of different size at position 7 of 2′3′-*c*GA^{R}MP. Surprisingly, STING turned

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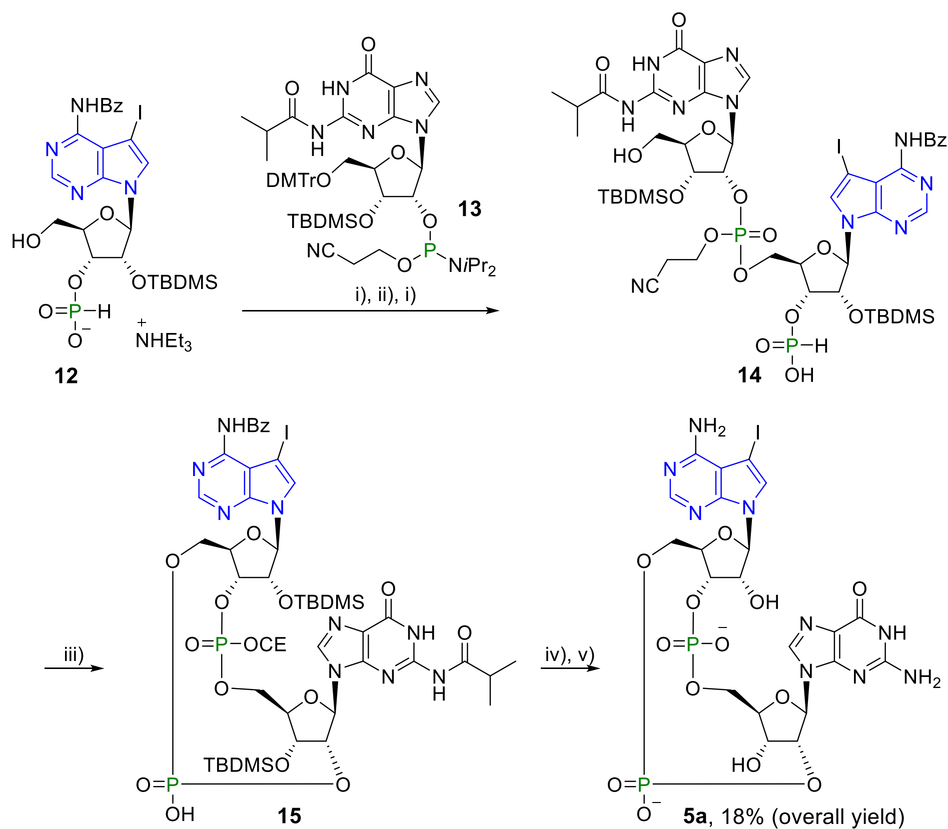
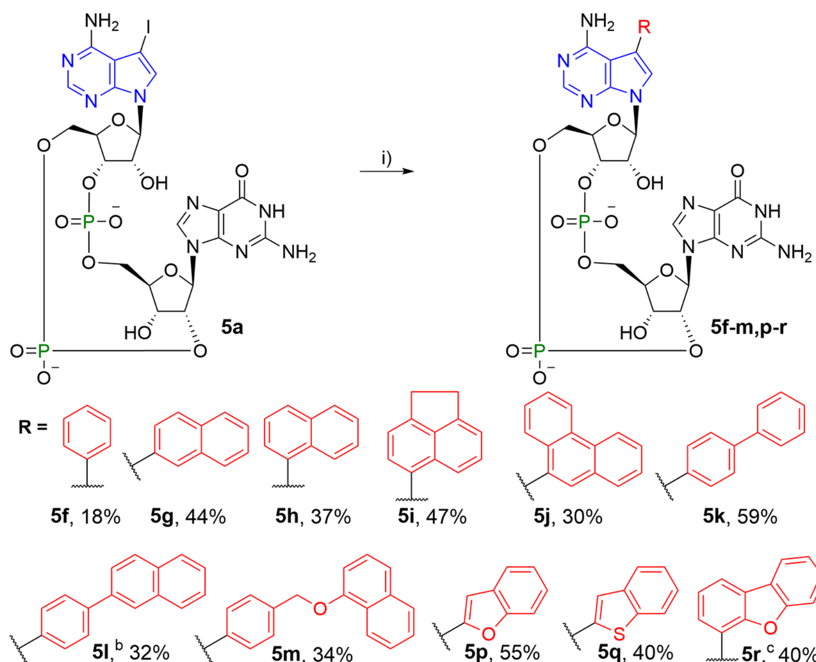
Scheme 7[a]



[a]Reagents and conditions: (i) DCA/DCM, rt, 10 min; (ii) (1) **13**/MeCN, rt, 10 min, (2) *t*BuOOH, rt, 30 min; (iii) (1) DMOCP/py, rt, 110 min, (2) I_2, H_2O, rt, 10 min; (iv) CH_3NH_2/EtOH, rt, 3 h; (v) $Et_3N{\cdot}3HF$/py, Et_3N, 50 °C, 3.5 h.

Scheme 8[a]



[a]Reagents and conditions: (i) $RB(OH)_2$, Cs_2CO_3, TPPTS, $Pd(OAc)_2$/H_2O−MeCN (2:1), 100 °C, 30 min. [b]RBpin was used instead of $RB(OH)_2$. [c]Open isomer **16** (13%) was also isolated.

out to tolerate not only relatively small substituents, that is, iodine (**5a**), cyclopropyl (**5c**), and ethynyl (**5d**), but also small aromatic substituents, that is, phenyl (**5f**), 2-furyl (**5n**), and 2-thienyl (**5o**) substituents, and even relatively large aromatic

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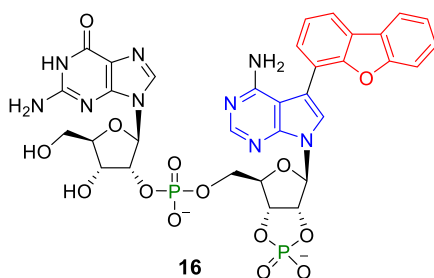


Figure 1. Open isomerized side product of a cross-coupling reaction.

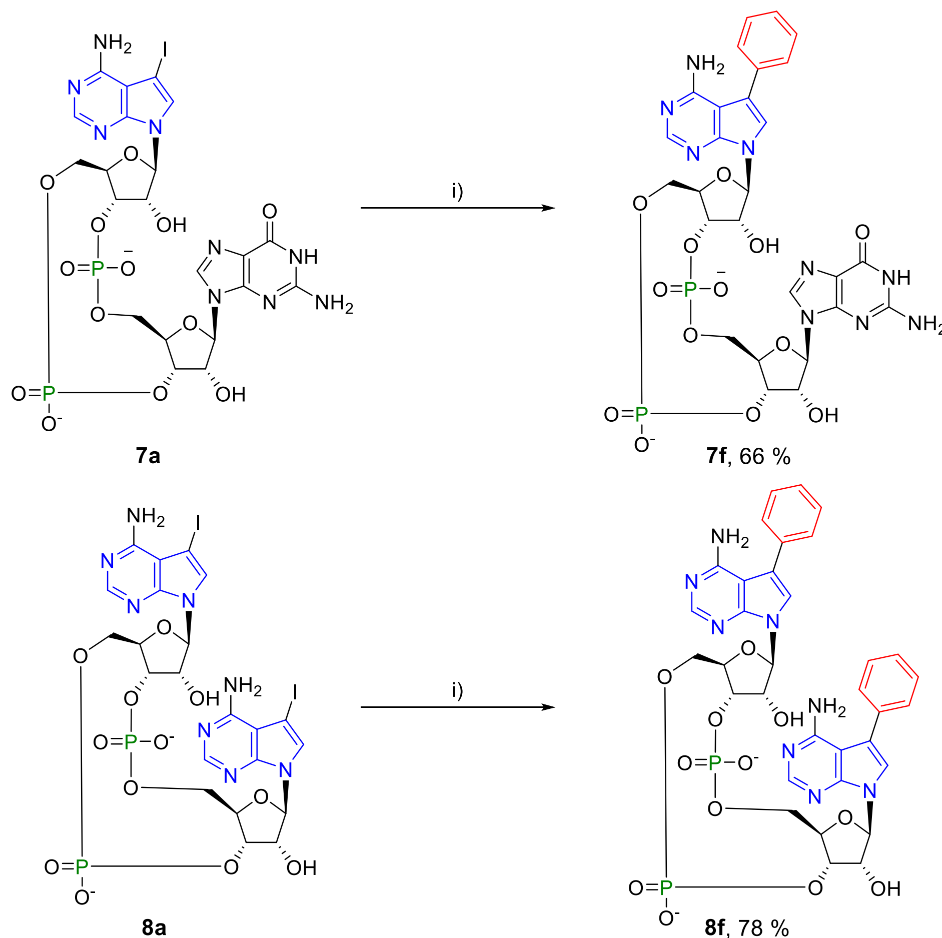
groups such as 2-naphthyl (**5g**), 4-biphenylyl (**5k**), 4-(2-naphthyl)phenyl (**5l**), 4-[(2-naphthyloxy)methyl]phenyl (**5m**), 2-benzofuryl (**5p**), and 2-benzothienyl (**5q**).

When considering ΔT_m, for **5c**, it decreased by only 0.7 °C, whereas **5d** showed a nearly 3 °C drop compared to **5b**. This minor decrease suggests that in position 7 of 7-deazaadenine, there is a space for the introduction of larger modifications than just a methyl group. When tested in a digitonin cell-based reporter assay, modifications on both **5c** and **5d** were tolerated. However, for **5d**, the EC_{50} value obtained from an assay in cells expressing the REF allelic form increased by nearly 6-fold, whereas at all other tested allelic forms, the increase was not higher than 3-fold. This suggests a disruption of interactions between **5d** and STING when R232 is replaced by H232. In a standard cell-based reporter assay for compounds **5c** and **5d**, we observed a deterioration of cellular activity, which was not present when tested with a membrane-permeabilizing agent.

After proving that there might be space for the introduction of larger substituents in the binding site of STING, we introduced phenyl (**5f**), small heterocycles (**5n**, **5o**), and pent-1-ynyl (**5e**) into position 7 of 7-deazaadenine. Surprisingly, we found that ΔT_m for **5f**, **5n**, and **5o** decreased by less than 3 °C, and EC_{50} values in the digitonin assay for these compounds did not increase by more than 2-fold compared to **5c**. Moreover, for compounds **5f**, **5n**, and **5o**, we observed restored activity in the standard assay, which was deteriorated for **5c** and **5d** (Table 1). In fact, EC_{50} for **5o** in the assay was even slightly lower than that for 2′3′-*c*GAMP. We also solved the crystallographic structure for **5f** in complex with STING.

Considering the structure of **5f**, we hypothesized that there exists a possibility of intramolecular stabilization through $\pi-\pi$ stacking between phenyl and guanine at **5f** that might help to improve its activity. To prove this possibility, we prepared 2′3′-*c*G^{Phe}A^{Phe}MP (**6f**), 3′3′-*c*GA^{Phe}MP (**7f**), and 3′3′-*c*-diA^{Phe}MP (**8f**). Unfortunately, substitutions with phenyl in these compounds did not show an improvement at STING in DSF or cell-based reporter assays compared to **5f**. For **7f**, we found a decrease in ΔT_m by 3 °C, but in digitonin assay, EC_{50} values for allelic form HAQ and AQ remained nearly the same. For other tested allelic forms, EC_{50} did not increase more than

Scheme 9[a]



[a]Reagents and conditions: (i) $PhB(OH)_2$, Cs_2CO_3, TPPTS, $Pd(OAc)_2/H_2O-MeCN$ (2:1), 100 °C, 30 min.

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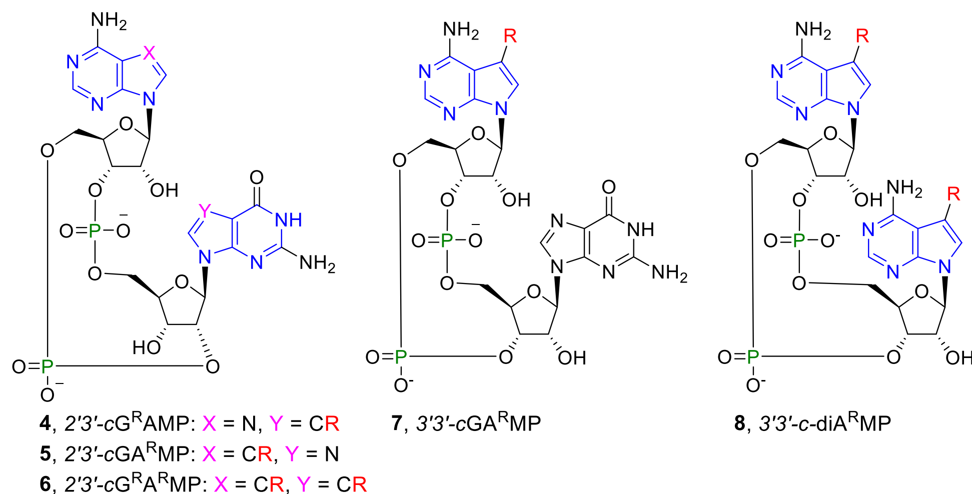
Table 1. Activities of Synthesized 7-Deazapurine CDNs in DSF and Cell-Based Assays



compound	R	DSF ΔT_m (°C)[a] wt	digitonin assay EC_{50} (μM)[b] wt	HAQ	REF	AQ	Q	standard assay EC_{50} (μM)[c] wt
4b	methyl	3.3	3.01	1.38	13.34	1.37	2.54	>150
5a	I	8.3	0.066	0.127	2.48	0.097	2.30	>150
5b	methyl	11.5	0.009	0.082	0.189	0.080	0.275	31.30
5c	cyclopropyl	10.8	0.064	0.403	0.379	0.291	0.302	>150
5d	ethynyl	8.7	0.020	0.132	1.09	0.111	0.885	>150
5e	pent-1-ynyl	6.7	0.116	0.243	6.77	0.181	2.71	112.9
5f	phenyl	8.1	0.063	0.385	5.90	0.307	1.70	40.7
5g	2-naphthyl	14.4	0.048	0.695	1.40	0.550	0.700	7.63
5h	1-naphthyl	4.4	1.10	0.497	18.90	0.470	7.23	42.45
5i	5-acenaphthenyl	2.5	1.16	1.63	>45	1.53	35.85	>150
5j	9-phenanthrenyl	4.4	0.061	0.900	>45	1.40	>45	65.45
5k	4-biphenylyl	12.3	0.013	0.173	0.346	0.092	0.235	2.95
5l	4-(2-naphthyl)phenyl	13.0	0.032	0.132	1.00	0.160	0.950	3.06
5m	4-[(2-naphthyloxy)methyl]phenyl	13.1	0.043	0.113	1.75	0.095	1.29	2.58
5n	2-furyl	8.9	0.025	0.163	0.816	0.115	0.469	41.30
5o	2-thienyl	9.8	0.028	0.174	0.647	0.184	1.85	27.30
5p	2-benzofuryl	12.1	0.076	0.060	0.334	0.085	0.176	10.94
5q	2-benzothienyl	13.5	0.035	0.195	0.350	0.330	0.250	8.69
5r	4-dibenzofuryl	7.1	0.367	0.503	8.97	0.869	7.07	19.10
6f	phenyl	1.0	6.41[d]	9.42[d]	19.49[d]	10.30[d]	0.271[d]	>150[d]
7a	I	4.4	0.101	0.896	>45	0.780	12.85	>150
7f	phenyl	5.1	0.110	0.367	10.37	0.280	4.480	>150
8a	I	0.5	3.488	>45	>45	>45	>45	>150
8f	phenyl	−0.6	>45	>45	>45	>45	>45	>150
2′3′-cGAMP		15.3	0.020	0.021	0.074	0.041	0.048	28.37
3′3′-cGAMP		5.1	0.121	0.123	4.26	0.260	2.06	68.37
2′2′-cGAMP		2.5	0.260	0.189	59.54	0.173	7.09	>150
ADU-S100		9.3[e]	0.08[e]	0.26[e]	1.64[e]	0.23[e]	1.01[e]	3.32[e]

[a]Values were obtained using DSF assay with wt hSTING as described in the Methods section. Measurements were performed as two independent experiments ($n = 2$). [b]Results of digitonin assay in 293T reporter cells expressing hSTING allelic variants were obtained as described in the Methods section. EC_{50} values are the mean of three independent experiments ($n = 3$) measured in triplicate with SD < 50% of EC_{50} values. [c]Results of standard assay in 293T reporter cells expressing wt hSTING were obtained as described in the Methods section. EC_{50} values are the mean of two independent experiments ($n = 2$) measured in triplicate with SD < 50% of EC_{50} values. [d]Data from one experiment only ($n = 1$). [e]Data from Dejmek et al.[50]

2-fold compared to **5f**. However, we observed a deterioration of activity in the standard cell-based reporter assay similar to compounds **5c** and **5d**. According to data from both DSF and cell-based reporter assays, substitutions in **6f** caused a significant drop of STING activity and led to the inactivity of **8f**. Substitutions in these compounds might potentially lead to the disruption of interactions with both R238 residues, which we have already reported to significantly affect the binding of CDNs.[47]

When we considered the fact that the results for **5n** and **5o** showed better activity than compounds with phenyl substituents **5–8f**, we focused on larger heterocycles like

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benzofuryl (**5p**), benzothienyl (**5q**), and dibenzofuryl (**5r**). For **5p** and **5q**, we observed an increase in ΔT_m by more than 3 °C when compared to **5n** and **5o**, respectively. Higher ΔT_m suggests increasing stabilization of complex STING–CDN.[47,48] We can only speculate that this stabilization could be related to the introduction of a benzofused heterocycle, which might be in a better position to form $\pi-\pi$ stacking with guanine. Consistent with DSF, we observed that **5p** showed lower EC_{50} values than **5n**, except for wild-type STING. Derivative **5q** showed lower EC_{50} for REF and Q than **5o**. Compound **5r** did not show any improvement in DSF or in digitonin assay when compared to **5n**, **5o**, **5p**, or **5q**. Nevertheless, all **5p**, **5q**, and **5r** compounds showed an improvement in a standard assay compared to **5n**, **5o**, and 2′3′-*c*GAMP. In the case of **5q**, it was 3-fold better than 2′3′-*c*GAMP.

We proved that there is a space for larger modifications without a reduction in activity in position 7 of 7-deazaadenine in 2′3′-*c*GA^{R}MP. When correlated with findings about improved activity in the standard assay for compounds with heterocyclic substituents, we designed compounds modified with large 2-naphthyl (**5g**), 1-naphthyl (**5h**), 5-acenaphthenyl (**5i**), and 9-phenanthrenyl (**5j**) substituents. Interestingly, derivatives **5h**, **5i**, and **5j** showed lower ΔT_m and considerably higher EC_{50}s compared to **5n**, **5o**, **5p**, **5q**, and **5r**. On the other hand, **5g** showed a ΔT_m of 14.4 °C and a nearly 4-fold lowered EC_{50} in a standard cell-based reporter assay when compared to 2′3′-*c*GAMP. Unfortunately, **5g** showed lower activation of HAQ, AQ, and REF allelic forms. These results show that interactions in the STING binding pocket are more likely to tolerate substitutions pointing above the adenine moiety rather than substitutions that need additional interactions and space around the *c*GAMP molecule.

We designed a 4-biphenylyl-substituted 2′3′-*c*GA^{R}MP (**5k**) by applying our findings about the preference of the STING protein in the site above AMP in 2′3′-*c*GAMP and with the knowledge of the relative flexibility of the lid above the binding.[51] The biphenylyl substituent brings additional lipophilicity to the compound, as in the case of **5g**, but with the substitution pointing above the *c*GAMP molecule, as in the case of **5f**. This substitution caused a decrease of only 3 °C in DSF, and EC_{50}s were comparable to those of 2′3′-*c*GAMP. Moreover, **5k** showed nearly 10 times better activity than 2′3′-*c*GAMP in the standard assay.

Considering the results for **5k**, we designed a substitution with 4-(2-naphthyl)phenyl (**5l**). This modification resulted in nearly the same ΔT_m and EC_{50}s despite the change from phenyl to naphthyl, which might cause more significant collisions with the lid of the STING binding pocket.

Compound **5m** contained the largest modification we introduced, 4-[(2-naphthyloxy)methyl]phenyl. Despite the introduction of such a large modification that was expected to largely clash with a lid of the STING binding pocket, we observed similar complex stability for **5m** and **5l** in DSF. Moreover, we observed comparable activities in cell-based reporter assays. When comparing the activities of derivatives **5k–m** with those of the clinical candidate ADU-S100,[23,50] similar or better activities were observed in digitonin assay. Moreover, in the standard 293T cell-based assay, compounds **5k–m** showed lower EC_{50} values than ADU-S100. To explain potent activities of compounds **5k–m**, we solved the X-ray crystallographic structure for the complex of STING and corresponding CDNs.

Structural Studies. To understand the interactions of large moieties in position 7 of 7-deazaadenine in 2′3′-*c*GA^{R}MPs, we crystallized human wild-type STING truncated to residues 140–379 in complexes with **5f**, **5k**, **5l**, and **5m** using our improved STING crystallization protocols.[52] Crystal structures were determined at resolution 2.69–1.89 Å, and the asymmetric unit consisted of two STING heterodimers (chain A/B) and one molecule of the ligand (Table S1). Protein residues were modeled into a well-defined electron density map, except for several regions belonging to flexible surface exposed loops that could not be resolved owing to their dynamic disorder (Table S2). All ligands were modeled into the binding site with full occupancy; however, some parts of the maps for substitutions of position 7 of 7-deazaadenine were not as well defined, suggesting some flexibility of this part of the molecule (Figure 2A).

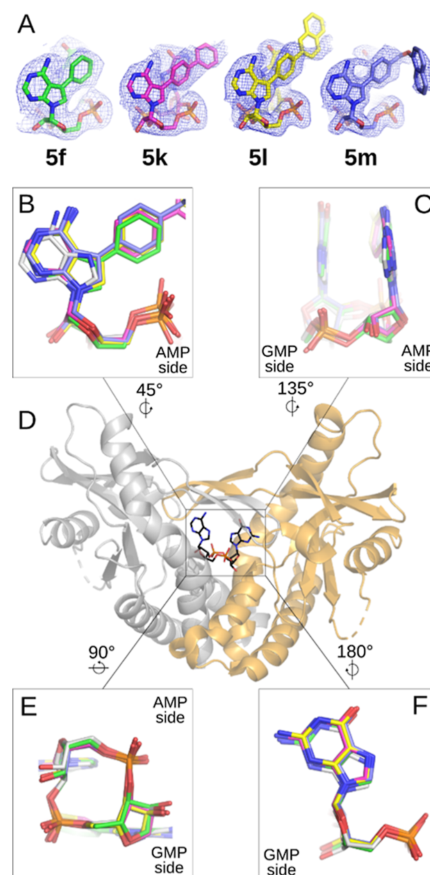

Figure 2. Crystal structures of human STING in complex with 2′3′-*c*GA^{R}MPs. (A) Binding poses of compounds are shown with $2Fo-Fc$ maps contoured at 1σ. Compounds are distinguished by carbon colors (**5f** in green, PDB 8A2H; **5k** in purple, PDB 8A2J; **5l** in yellow, PDB 8A2I; and **5m** in blue, PDB 8A2K), while nitrogen, oxygen, and phosphorus atoms are colored blue, red, and orange. (B) AMP side view of **5f**, **5k**, **5l**, and **5m** binding poses superposed with 2′3′-*c*GAMP (PDB 4KSY), showing the trend in positions of 7-deazaadenosine for all of our compounds, which differs from the position of adenosine in 2′3′-*c*GAMP (in white). (C) Side view superposition of ligands showing similarity of localization of nucleobases in this orientation. (D) Human STING dimer shown as a biological unit with differently colored monomers with 2′3′-*c*GAMP located in the binding site (PDB 4KSY). (E) Bottom view and (F) GMP side view of superposed ligands.

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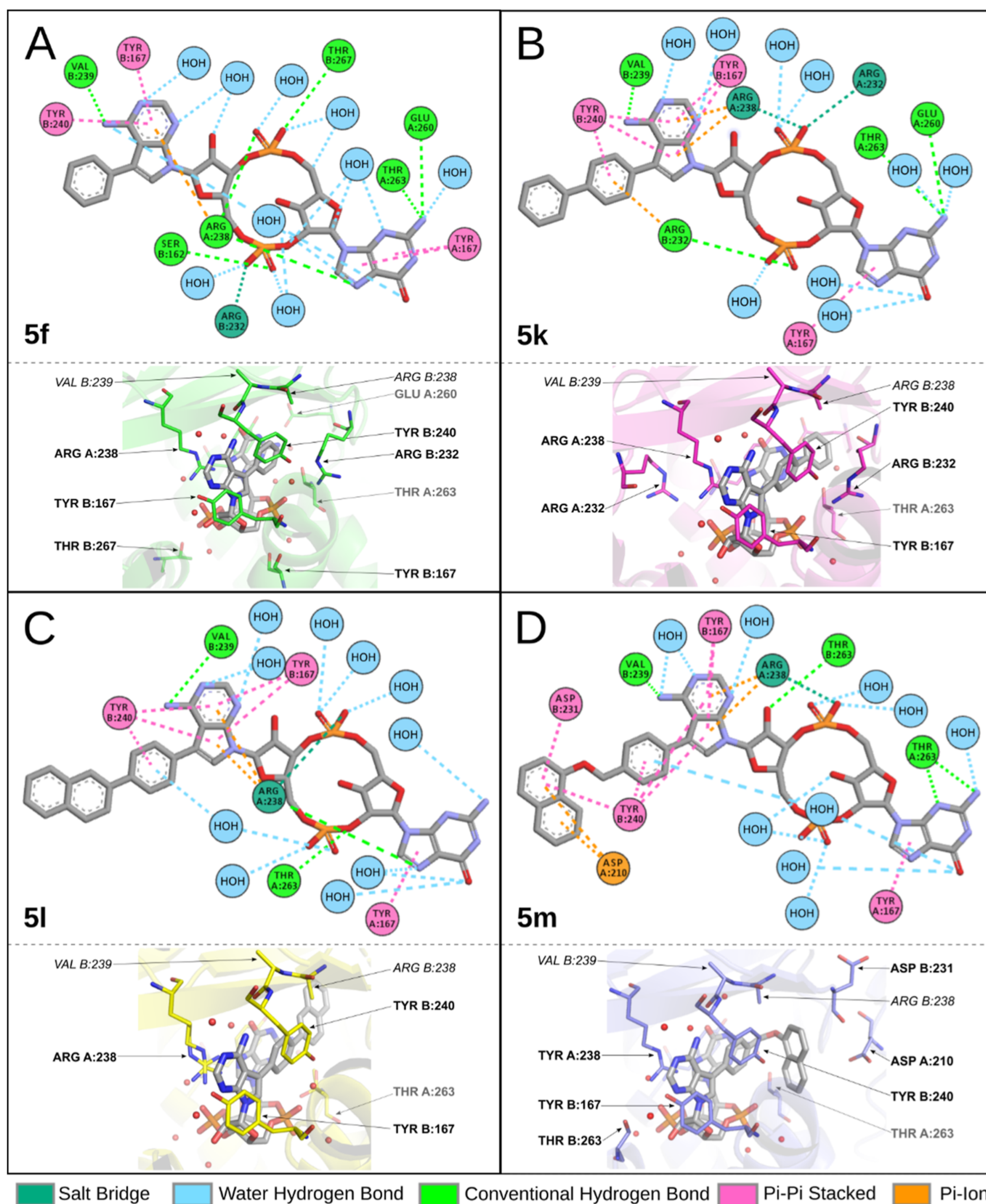

Figure 3. Interaction scheme of **5f** (A; PDB 8A2H), **5k** (B; PDB 8A2J), **5l** (C; PDB 8A2I), and **5m** (D; PDB 8A2K) in STING ligand-binding site, as seen in our structures.

The binding site is formed at the interface of two STING monomers and is covered by two "lids" (residues 227−241). The electron density for the lid region was mostly well defined, with some exceptions pointing to residues disordered by the binding of a voluminous ligand (residue Ile235 in **5l** complex; Arg232 in **5m** complex; and Tyr240 in **5k**, **5l**, and **5m**, Figure S1). Ligands are deeply buried in the binding site (Figure 3), with basic orientation resembling that of natural ligand 2′3′-

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*c*GAMP (PDB 4KSY).[5] Most of the interactions observed in the binding site were similar to those described for a natural ligand (Figure S2): Tyr167 π−π stacking with nucleobases, Arg232 forming a salt bridge with phosphates, a side chain of Thr263 forming a hydrogen bond with NH_2 of guanine, Arg238 interacting through a hydrogen bond with phosphates, and Arg238 cation−π stacking with nucleobases on the opposite of the binding site. Ribose moiety and the central part are linked to the protein residues through a network of water-mediated hydrogen bonds. In all our structures, we also observed Val239 carbonyl forming a hydrogen bond with NH_2 at 7-deazaadenosine and Tyr240 π−π stacking with introduced aromatic substituents (Figure 3).

When comparing the binding pose of the natural ligand with our compounds, the GMP side for all of the compounds shows only an insignificant shift in position (Figure 2C,E,F). For the AMP side, the ribose and nucleobase are slightly shifted upward, potentially because of our large substitutions at 7-deazaadenine. The substitutions in all of our structures displace the side chain of Arg238 from chain B and thus prevent interaction with guanosine (Figure 2B,C). This shift enables direct interaction with Tyr240 from chain B that was not observed for the natural ligand binding. This residue interacts with 7-deazaadenine through parallel displaced π−π stacking. This interaction is enabled by a change of AMP positioning, and the extent of its interaction with Tyr240 differs in our structures due to the different sizes of substituents. Additionally, for the bulky substituent **5m**, we observed unique interactions with Asp234 and Asp210, respectively (Figure 3D).

The main difference between the binding of the **5f** molecule and the binding of the natural ligand 2′3′-*c*GAMP that we observed is the interaction of **5f** with Tyr240, as described earlier (Figure 3A). By modifying 7-deazaadenine with phenyl, we displaced Arg238 from chain B; we also brought additional intramolecular stabilization of the ligand conformation through cation−π stacking of the phenyl moiety with guanosine on the opposite side of the molecule. However, this interaction was not sufficient and resulted in lower stability of STING-**5f** in DSF compared to 2′3′-*c*GAMP and **5b**.

Intramolecular interactions were further strengthened in compound **5k**, where the phenyl ring was replaced by biphenylyl. Similar to **5f**, this led to a stacking interaction between Tyr240 and nucleobase. Additionally, Tyr240 was involved in a stacking interaction with the phenyl closer to the nucleobase in biphenylyl substituent (Figure 3B), which resulted in a 1.5-fold higher stability of STING-**5k** complex in DSF when compared to **5f**.

For **5l** with a 4-(2-naphthyl)phenyl substitution, we preserved the interactions that were observed for **5k** (Figure 3C); however, naphthyl substitution clashed with Ile235 of the lid, making this residue disordered (Figure S1). Despite this clash, **5l** exhibited a slightly better stabilization of complex in DSF when compared to **5k** (Table 1).

Compound **5m** showed additional π-stacking interactions with Tyr240 and an unexpected change in the binding mode of the substituent. The structural reason for this is a change in the position of the naphthyl group, that is, in **5m**, observed in the position regularly occupied by the Arg232 side chain. This position change is allowed due to the flexibility of the longer methoxy linker connecting the naphthyl group that enables the formation of a T-shaped π-stacking between the naphthyl of **5m** and Tyr240, π−amide interaction with the backbone of Asp231 and π−anion interaction with Asp210 (Figure 3D). A substitution at **5m** resulted in just a slightly lower stabilization in DSF when compared to 2′3′-*c*GAMP.

PBMC Assay. Selected compounds were further tested for induction of IFNγ, TNFα, and IFNα secretion using PBMCs (Table 2). None of the tested compounds showed any cytotoxicity (Table S4). Most of the tested compounds induced higher levels of IFNγ, TNFα, and IFNα secretion when compared to 2′3′-*c*GAMP. EC_{50} obtained from testing at PBMCs correlated with data from a standard 293T cell-based reporter assay for most of the tested compounds.

Table 2. Cytokine Level Determination in PBMCs upon Treatment with Synthesized Compounds

compound	EC_{50} (μM)[a]		
	IFNγ	TNFα	IFNα
5b	7.8	50.60	30.60
5c	1.29	37.59	4.95
5f	6.84	52.45	7.36
5g	7.54	98.80	11.71
5h	34.51	>200	28.78
5i	85.20	>200	74.73
5j	47.54	>200	>200
5k	2.05	13.64	7.43
5l	1.77	53.86	5.09
5m	1.16	43.38	4.27
5n	2.24	6.72	2.80
5o	2.60	2.66	2.88
5p	8.33	24.56	12.84
5q	0.87	1.43	4.04
5r	5.63	99.70	26.98
7f	12.56	65.77	6.92
2′3′-*c*GAMP	7.10	36.03	8.46
3′3′-*c*GAMP	17.25	18.72	7.65
2′2′-*c*GAMP	11.75	44.54	7.21

[a] EC_{50} values are the mean of three independent experiments ($n = 3$), each of them performed on PBMCs from a different donor, measured in triplicate with SD < 50% of EC_{50} values.

CONCLUSIONS

Here, we report the design, synthesis, and biochemical and biological evaluation of a novel class of 7-substituted 7-deazaadenine containing CDNs capable of STING activation. During the preparation of this class of compounds, we demonstrated a mixed enzymatic−chemical synthetic approach for the preparation of CDNs. This approach allowed us to efficiently overcome the total synthesis of the whole CDN, a multistep and time-consuming process.[53] In the optimization process of biochemical binding potency of our compounds, SAR revealed an unexpected potential for modifications with large aromatic groups. This potential is applicable for 2′3′-*c*GAMP analogues with a preference for modifications that point above the CDN molecule. We observed π−π stacking interactions between the aromatic substituents and Tyr240 that are involved in stabilization of CDN−STING complexes. In the case of 3′3′-*c*GAMP and *c*-di-AMP, our modifications did not lead to increased binding potency. Substitutions with large aromatic moieties increased lipophilicity and thus potentially permeability into cells. They might also be more efficiently transported by an SLC19A1 receptor or an LRRC8A transporter, which would result in improved cellular activity.[54]

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■ EXPERIMENTAL SECTION

General Synthetic and Enzymatic Methods. Unless otherwise noted, all starting materials, solvents, and reagents were purchased from commercial suppliers and used as received. 7-Iodo-7-adenosine **9** and 7-deazapurine NTPs **2b,f, 3a–d,f,n**, and **o** were prepared according to the literature.[37,44,45,55] The synthesis of **3e** is described in the Supporting Information (Scheme S1). All chemical reactions were performed under an argon atmosphere. Reactions were monitored by thin layer chromatography (TLC) on TLC Silica gel 60 F_{254} (Merck) and detected by UV (254 nm). NMR spectra were measured on a Bruker AVANCE 500 MHz spectrometer (499.8 MHz for ^{1}H, 125.7 MHz for ^{13}C, and ^{31}P at 202.4 MHz) or on a Bruker 600 AVANCE III HD instrument (^{1}H at 600 MHz, ^{13}C at 150.9 MHz) equipped with 5 mm cryo-probe at 25 °C in D_2O (dioxane used as external standard, [δ(^{1}H) = 3.75 ppm, δ(^{13}C) = 67.19 ppm]) or in CD_3OD (referenced to the residual solvent signal, [δ(^{1}H) = 3.31 ppm, δ(^{13}C) = 49.0 ppm]). ^{31}P NMR spectra were referenced externally to the signal of H_3PO_4. Chemical shifts are given in ppm (δ-scale), and coupling constants (J) are given in Hz. The complete assignment of all NMR signals was performed using a combination of H,H-COSY, H,H-ROESY, H,C-HSQC, and H,C-HMBC experiments. Low resolution mass spectra were measured using electrospray ionization (ESI). High resolution mass spectra were measured on LTQ Orbitrap XL (Thermo Fisher Scientific) using ESI. High performance flash chromatography (HPFC) was performed with ISCO Combiflash Rf system on RediSep Rf Gold Silica Gel columns or Reverse Phase (C18) RediSep Rf Gold columns. Purification of CDNs was performed using HPLC (Waters modular HPLC system) on a column packed with 5 μm polar C18 reversed phase (Luna Omega 5 μm Polar C18 column, Phenomenex). The purity of all final compounds was determined by clean NMR spectra and by UPLC. The identification of CDNs was performed on ACQUITY UPLC HClass PLUS chromatographic system with MS SQ Detector 2 (Waters, Milford, USA) using Acquity UPLC BEH C18 column 50 mm × 2.1 mm, 1.7 μm (Waters, Milford, MA, USA), and 20 mM ammonium acetate buffer, pH 6.8, with a linear gradient of acetonitrile (0 to 50% in 4 min, additional 2 min at 50% acetonitrile; flow rate 0.5 mL/min). Column temperature was kept at 40 °C. Negative ESI method was used for ionization. ULPC purity (>95% with exception of enzymatically synthesized CDNs **4b**, **5b**, and **5e**) is shown in Table S3.

Enzymatic Synthesis. Enzymatically prepared 2′3′-CDNs (Schemes 3 and 4) were synthetized using mcGAS as described previously.[20] Briefly, the appropriate NTPs were mixed to the final 2 mM concentration with 5 μM of mcGAS and 0.1 mg/mL herring testes DNA in 1 mL buffer containing 20 mM Tris–HCl [pH 8.0] and 20 mM $MgCl_2$ and incubated for 16 h at 37 °C in a heating shaker. The synthesis of **7a** was performed by using 2 mM **3a**, 2 mM GTP, and 2 μM DncV in 1 mL of 50 mM HEPES buffer [pH 8.0] supplemented with 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM DTT. The whole mixture was incubated overnight at 37 °C in a heating shaker. The synthesis of **8a** was done overnight at 50 °C in a 1 mL reaction mixture containing 4 mM **3a**, 20 μM DisA, and 50 mM HEPES buffer [pH 8.0] supplemented with 100 mM NaCl, 10 mM $MgCl_2$, and 1 mM DTT. The next morning, the reactions were spun at 25,000g for 15 min and supernatants were passed through Nanosep 3K Omega (Pall Corporation, USA). The purification of CDNs was continued by adding 3 mL of ddH_2O to the flow-through fractions, and CDNs were purified on a semipreparative C18 column (Luna 5 μm C18 250 mm × 10 mm) using a 60 min gradient at a flow rate of 3 mL/min of 0–20% acetonitrile in 0.1 M TEAB buffer [pH 8.5]. TEAB was removed from the collected fractions by 3 cycles of evaporation/dissolution in 50% methanol.

*Cyclo-adenosine 5′-O-Phosphate (3′ → 5′) 2-Amino-5-methyl-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidin-4(3H)-one 5′-O-Phosphate (2′ → 5′) Sodium Salt (**4b**).* NTP **2b** (2 μmol) and ATP (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5–30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **4b** (73 nmol, 4%). ^{1}H NMR (600 MHz, D_2O): 1.81 (bs, 3H, CH_3); 4.14 (ddd, 1H, $J_{5'b,4'}$ = 1.7, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 3.4, H5′b-G); 4.23 (ddd, 1H, $J_{5'a,4'}$ = 2.3, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 2.7, H5′a-G); 4.23 (ddd, 1H, $J_{5'b,4'}$ = 1.4, $J_{5'b,5'a}$ = 12.1, $J_{5'b,P}$ = 3.6, H5′b-A); 4.37 (ddd, 1H, $J_{4',5'a}$ = 2.3, $J_{4',5'b}$ = 1.7, $J_{4',P}$ = 3.8, H4′-G); 4.47 (m, 1H, H5′a-A); 4.49 (dm, 1H, $J_{4',3'}$ = 8.7, H4′-A); 4.62 (d, 1H, $J_{3',4'}$ = 4.1, H3′-G); 4.75 (dd, $J_{2',1'}$ = 1.1, $J_{2',3'}$ = 4.3, H2′-A); 4.99 (ddd, 1H, $J_{3',2'}$ = 4.3, $J_{3',4'}$ = 8.7, $J_{3',P}$ = 6.9, H3′-A); 5.33 (um, 1H, H2′-G); 6.01 (d, 1H, $J_{1',2'}$ = 8.3, H1′-G); 6.19 (d, 1H, $J_{1',2'}$ = 1.1, H1′-A); 6.83 (q, 1H, J_{8,CH_3} = 1.3, H8-G); 8.23 (s, 1H, H8-A); 8.27 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 12.90 (5-CH_3); 65.61 (C5′-A); 68.75 (C5′-G); 73.55 (C3′-A); 74.20 (C3′-G); 76.54 (C2′-A); 79.46 (C2′-G); 83.02 (C4′-A); 85.95 (C4′-G); 87.70 (C1′-G); 92.47 (C1′-A); 103.55 (C5-G); 118.24 (C4a-G); 119.52 (C6-G); 121.67 (C5-A); 141.42 (C8-A); 150.64 (C4-A); 154.81 (C7a-G); 155.07 (C2-G); 155.40 (C2-A); 158.26 (C6-A); ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.20 and −1.02. ESI MS m/z (rel. %): 342 (100) $[M-2H]^{2-}$, 686 (37) $[M-H]^-$, 708 (8) $[M-2H + Na]^-$. HR MS (ESI): for $C_{22}H_{25}N_9O_{13}P_2$ $[M-H]^-$, calcd 686.11308; found, 686.11212.

*Cyclo-4-amino-5-iodo-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]-pyrimidine 5′-O-Phosphate (3′ → 5′) Guanosine 5′-O-Phosphate (2′ → 5′) Sodium Salt (**5a**).* Enzymatic synthesis: NTP **3a** (75 mg, 107 μmol) and GTP (107 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5–30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5a** (56 mg, 61%) as a white lyophilizate (water).

Chemical synthesis: Phosphonate **12** (161 mg, 0.21 mmol) was dissolved in DCM (2.5 mL). Water (38 μL, 2.11 mmol) and dichloroacetic acid in DCM (6%, 2.5 mL, 1.81 mmol) were added, and the solution was stirred for 10 min. Then, pyridine (298 μL, 3.70 mmol) was added, and the mixture was evaporated under reduced pressure and co-evaporated with anhydrous acetonitrile (3×). The residue was dissolved in anhydrous acetonitrile (480 μL), and a solution of phosphoramidite **13** (262 mg, 0.268 mmol) in anhydrous acetonitrile (1.45 mL) was added. The mixture was stirred at rt for 10 min, then *t*-butylhydroperoxide (5.5 M solution in decane, 113 μL, 0.621 mmol) was added, and the stirring continued for another 30 min. Then, the solution was cooled to 0 °C, and a solution of $NaHSO_3$ (33% wt, 627 μL, 2.46 mmol) was added. The mixture was stirred for 10 min at 0 °C and then for 5 min at rt. Then, solvent was removed in vacuo, and the residue was dissolved in DCM (3.22 mL). Water (38 μL, 2.11 mmol) and dichloroacetic acid in DCM (6%, 3.22 mL, 2.33 mmol) were added. The mixture was stirred for 10 min, then pyridine (670 μL, 8.32 mmol) was added, and the mixture was evaporated under reduced pressure and co-evaporated with water. Product **14** was partially purified by flash chromatography (C18 column, gradient 5–100% MeCN in 0.1 M TEAB).

Crude **14** was co-evaporated with anhydrous pyridine (3 × 2 mL) and dissolved in anhydrous pyridine (2.6 mL). DMOCP was added (85 mg, 0.46 mmol), and the mixture was stirred for 110 min at rt. Then, water (77 μL, 0.46 mmol) and iodine (44 mg, 0.46 mmol) were added, and the stirring continued for another 10 min at rt. The mixture was cooled to 0 °C, and an aqueous solution of $NaHSO_3$ (40% wt, 64 μL) was added. After stirring for 5 min at 0 °C, another portion of $NaHSO_3$ solution (40% wt, 40 μL) was added. The clear solution was evaporated under reduced pressure and co-evaporated with water. Crude **15** was partially purified by flash chromatography (C18 column, gradient 5–100% MeCN in 0.1 M TEAB).

Crude **15** was dissolved in ethanolic solution of CH_3NH_2 (33% wt., 4 mL, 32.1 mmol), and the solution was stirred for 3 h at rt. Then, the mixture was evaporated under reduced pressure, and the residue was co-evaporated with anhydrous pyridine (3 × 2 mL). A mixture of anhydrous pyridine, Et_3N (1:1 v/v, 4 mL), and $Et_3N \cdot 3HF$ (640 μL, 3.93 mmol) was added, and the mixture was stirred at 50 °C for 3.5 h. Then, aqueous ammonium acetate (1 M, 6 mL) was added, and the solvents were removed in vacuo. The residue was co-evaporated with water. After HPLC purification (0–15% MeCN in 0.1 M TEAB) and conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle), CDN **5a** (32 mg, 18% overall yield) was obtained as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.14 (ddd, 1H, $J_{5'b,4'}$

= 1.8, $J_{5'b,5'a}$ = 11.7, $J_{5'b,P}$ = 2.1, H5′b-G); 4.22 (ddd, 1H, $J_{5'a,4'}$ = 3.1, $J_{5'a,5'b}$ = 11.7, $J_{5'a,P}$ = 4.6, H5′a-G); 4.23 (ddd, 1H, $J_{5'b,5'a}$ = 12.0, $J_{5'b,4'}$ = 1.3, $J_{5'b,P}$ = 2.2, H5′b-A); 4.41 (ddd, 1H, $J_{4',5'a}$ = 3.1, $J_{4',5'b}$ = 1.8, $J_{4',P}$ = 3.6, H4′-G); 4.44 (dm, 1H, $J_{4',3'}$ = 9.3, H4′-A); 4.52 (dm, 1H, $J_{5'a,5'b}$ = 12.0, H5′a-A); 4.61 (d, 1H, $J_{3',2'}$ = 4.0, H3′-G); 4.67 (bd, 1H, $J_{2',3'}$ = 4.0, H2′-A); 4.92 (ddd, 1H, $J_{3',2'}$ = 4.0, $J_{3',4'}$ = 9.3, $J_{3',P}$ = 6.6, H3′-A); 5.62 (ddd, 1H, $J_{2',1'}$ = 8.6, $J_{2',3'}$ = 4.0, $J_{2',P}$ = 4.4, H2′-G); 5.94 (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); 6.10 (s, 1H, H1′-A); 7.71 (s, 1H, H6-A); 7.88 (s, 1H, H8-G); 8.09 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 51.15 (C5-A); 65.23 (d, $J_{C,P}$ = 4.5, C5′-A); 68.48 (d, $J_{C,P}$ = 5.2, C5′-G); 72.88 (d, $J_{C,P}$ = 5.6, C3′-A); 73.75 (C3′-G); 76.68 (C2′-A); 77.43 (d, $J_{C,P}$ = 5.4, C2′-G); 82.55 (t, $J_{C,P1}$ = $J_{C,P2}$ = 11.3, C4′-A); 85.14 (d, $J_{C,P}$ = 10.1, C4′-G); 89.10 (d, $J_{C,P}$ = 12.0, C1′-G); 92.50 (C1′-A); 106.80 (C4a-A); 120.60 (C5-G); 129.42 (C6-A); 143.38 (C8-G); 150.09 (C7a-A); 153.95 (C2-A); 154.71 (C4-G); 155.84 (C2-G); 159.59 (C4-A); 161.74 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.32 and −1.41. ESI MS m/z (rel. %): 398 (100) $[M-2H]^{2-}$, 798 (97) $[M-H]^-$, 820 (30) $[M-2H + Na]^-$. HR MS (ESI): for $C_{21}H_{23}O_{13}N_9IP_2$ $[M-H]^-$, calcd 797.99407; found, 797.99335.

*Cyclo-4-amino-5-methyl-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]-pyrimidine 5′-O-Phosphate (3′ → 5′) Guanosine 5′-O-Phosphate (2′ → 5′) Sodium Salt (**5b**).* NTP **3b** (2 μmol) and GTP (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5b** (354 nmol, 18%). ^{1}H NMR (600 MHz, D_2O): 1.98 (d, 3H, $J_{CH3,6}$ = 1.1, CH_3); 4.17 (m, 1H, H5′b-G); 4.18 (ddd, 1H, $J_{5'b,5'a}$ = 11.7, $J_{5'b,4'}$ = 2.4, $J_{5'b,P}$ = 1.8, H5′b-A); 4.26 (ddd, 1H, $J_{5'a,5'b}$ = 11.7, $J_{5'a,4'}$ = 3.2, $J_{3'P}$ = 4.7, H5′a-A); 4.43 (m, 2H, H4′-A and H4′-G); 4.44 (m, 1H, H5′a-G); 4.63 (d, 1H, $J_{3',4'}$ = 4.1, H3′-G); 4.71 (bd, 1H, $J_{2',3'}$ = 4.3, H2′-A); 5.06 (ddd, 1H, $J_{3',2'}$ = 4.3, $J_{3',4'}$ = 9.0, $J_{3',P}$ = 6.7, H3′-A); 5.62 (ddd, 1H, $J_{2',1'}$ = 8.5, $J_{2',3'}$ = 4.1, $J_{2',P}$ = 6.6, H2′-G); 5.96 (d, 1H, $J_{1',2'}$ = 8.5, H1′-G); 6.23 (s, 1H, H1′-A); 7.36 (q, 1H, $J_{6,CH3}$ = 1.1, H6-A); 7.92 (s, 1H, H8-G); 8.19 (bs, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O; chemical shifts obtained from 2D-HSQC and 2D-HMBC spectra; J(C,P) and shifts of C4-A, C2-G, and C6-G were not determined): 13.29 (CH_3); 64.98 (C5′-A); 68.33 (C5′-G); 73.32 (C3′-A); 73.85 (C3′-G); 77.08 (C2′-A); 77.49 (C2′-G); 82.64 (C4′-A); 86.11 (C4′-G); 88.85 (C1′-G); 92.20 (C1′-A); 105.17 (C4a-A); 114.97 (C5-A); 119.83 (C5-G); 123.61 (C6-A); 143.19 (C8-G); 147.84 (C2-A); 149.48 (C7a-A); 154.89 (C4-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.27 and −1.18. ESI MS m/z (rel. %): 342 (100) $[M-2H]^{2-}$, 353 (3) $[M-3H + Na]^{2-}$, 686 (36) $[M-H]^-$, 708 (8) $[M-2H + Na]^-$. HR MS (ESI): for $C_{22}H_{27}N_9O_{13}P_2$, calcd 686.11308; found, 686.11212.

*Cyclo-4-amino-5-cyclopropyl-7-β-D-ribofuranosyl-7H-pyrrolo-[2,3-d]pyrimidine 5′-O-Phosphate (3′ → 5′) Guanosine 5′-O-Phosphate (2′ → 5′) Sodium Salt (**5c**).* NTP **3c** (2 μmol) and GTP (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5c** (387 nmol, 19%). ^{1}H NMR (600 MHz, D_2O): 0.16 m and 0.74 m (2H, H3-cyclopropyl); 0.31 m and 0.47 m (2H, H2-cyclopropyl); 1.52 (m, 1H, H1-cyclopropyl); 4.18 (dt, 1H, $J_{5'b,4'}$ = 1.8, $J_{5'b,5'a}$ = 11.7, $J_{5'b,P}$ = 1.8, H5′b-G); 4.24 (ddd, 1H, $J_{5'b,5'a}$ = 11.8, $J_{5'b,4'}$ = 2.6, $J_{5'b,P}$ ∼ 1.0, H5′b-A); 4.26 (ddd, 1H, $J_{5'a,4'}$ = 3.2, $J_{5'a,5'b}$ = 11.7, $J_{5'a,P}$ = 4.3, H5′a-G); 4.41 (dm, 1H, $J_{4',3'}$ = 9.2, H4′-A); 4.44 (m, 1H, $J_{5'a,5'b}$ = 11.8, H5′a-A); 4.45 (m, 1H, H4′-G); 4.66 (d, 1H, $J_{3',4'}$ = 4.0, H3′-G); 4.70 (d, 1H, $J_{2',3'}$ = 4.2, H2′-A); 5.05 (ddd, 1H, $J_{3',2'}$ = 4.2, $J_{3',4'}$ = 9.2, $J_{3',P}$ = 6.7, H3′-A); 5.65 (ddd, 1H, $J_{2',1'}$ = 8.6, $J_{2',3'}$ = 4.0, $J_{2',P}$ = 4.8, H2′-G); 5.98 (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); 6.22 (s, 1H, H1′-A); 7.10 (s, 1H, H6-A); 7.96 (bs, 1H, H8-G); 8.18 (bs, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O, chemical shifts of quaternary carbons C2-G, C4-G, C5-G, C6-G, and C2-A were not determined): 6.49 (C2-cyclopropyl); 8.97 (C1-cyclopropyl); 10.80 (C3-cyclopropyl); 65.31 (d, $J_{C,P}$ = 5.0, C5′-A); 68.48 (d, $J_{C,P}$ = 4.7, C5′-G); 73.09 (d, $J_{C,P}$ = 5.6, C3′-A); 73.66 (C3′-G); 76.99 (C2′-A); 77.63 (d, $J_{C,P}$ = 5.7, C2′-G); 82.42 (dd, $J_{C,P1}$ = 11.3, $J_{C,P2}$ = 10.5, C4′-A); 86.08 (d, $J_{C,P}$ = 10.2, C4′-G); 89.02 (d, $J_{C,P}$ = 12.7, C1′-G); 91.94 (C1′-A); 105.76 (C5-A); 121.01 (C6-A); 121.77 (C4a-A); 143.31 (C8-G); 149.79 (C7a-A); 161.15 (C4-A). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.26 and −1.09. ESI MS m/z (rel. %): 356 (100) $[M-2H]^{2-}$, 367 (6) $[M-3H + Na]^{2-}$, 712 (7) $[M-H]^-$, 734 (8) $[M-2H + Na]^-$. HR MS (ESI): for $C_{24}H_{28}O_{13}N_9P_2$, calcd 712.12873; found, 712.12814.

*Cyclo-4-amino-5-ethynyl-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]-pyrimidine 5′-O-Phosphate (3′ → 5′) Guanosine 5′-O-Phosphate (2′ → 5′) Sodium Salt (**5d**).* NTP **3d** (2 μmol) and GTP (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5d** (196 nmol, 10%). ^{1}H NMR (600 MHz, D_2O): 3.41 (s, 1H, −C≡CH); 4.17 (dt, 1H, $J_{5'b,4'}$ = 2.0, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.2, H5′b-G); 4.22 (bdd, 1H, $J_{5'b,4'}$ = 2.5, $J_{5'b,5'a}$ = 12.0, H5′b-A); 4.24 (ddd, 1H, $J_{5'a,4'}$ = 3.1, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 4.6, H5′a-G); 4.42 (dt, 1H, $J_{4',5'a}$ = 3.1, $J_{4',5'b}$ = 2.0, $J_{4',P}$ = 3.1, H4′-G); 4.46 (bdt, 1H, $J_{4',3'}$ = 9.4, $J_{4',5'a}$ = 2.5, $J_{4',5'b}$ = 2.5, H4′-A); 4.50 (dm, 1H, $J_{5'a,5'b}$ = 12.0, H5′a-A); 4.63 (d, 1H, $J_{3',4'}$ = 4.1, H3′-G); 4.66 (bd, 1H, $J_{2',3'}$ = 4.0, H2′-A); 4.99 (ddd, 1H, $J_{3',2'}$ = 4.0, $J_{3',4'}$ = 9.4, $J_{3',P}$ = 6.6, H3′-A); 5.69 (ddd, 1H, $J_{2',1'}$ = 8.5, $J_{2',3'}$ = 4.1, $J_{2',P}$ = 5.7, H2′-G); 5.96 (d, 1H, $J_{1',2'}$ = 8.5, H1′-G); 6.26 (s, 1H, H1′-A); 7.86 (s, 1H, H6-A); 7.91 (s, 1H, H8-G); 8.23 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O; chemical shifts obtained from 2D-HSQC and 2D-HMBC spectra; J(C,P) and shifts of C2-G and C6-G not determined): 61.36 (-C≡**C**H); 65.02 (C5′-A); 68.40 (C5′-G); 72.82 (C3′-A); 73.82 (C3′-G); 76.91 (C2′-A); 77.38 (C2′-G); 82.47 (C4′-A); 84.18 (-**C**≡CH); 85.94 (C4′-G); 88.89 (C1′-G); 92.31 (C1′-A); 97.30 (C5-A); 105.67 (C4a-A); 120.22 (C5-G); 130.16 (C6-A); 143.10 (C8-G); 149.06 (C7a-A); 154.62 (C4-G); 151.83 (C2-A); 157.90 (C4-A). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.18 and −1.14. ESI MS m/z (rel. %): 348 (100) $[M-2H]^{2-}$, 696 (21) $[M-H]^-$, 718 (16) $[M-2H + Na]^-$. HR MS (ESI): for $C_{23}H_{23}O_{13}N_9P_2$, calcd 696.09743; found, 696.09637.

*Cyclo-4-amino-5-(pent-1-yn-1-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-Phosphate (3′ → 5′) Guanosine 5′-O-Phosphate (2′ → 5′) Sodium Salt (**5e**).* NTP **3e** (2 μmol) and GTP (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5e** (84 nmol, 4%). ^{1}H NMR (600 MHz, D_2O): 0.91 (t, 3H, H5-pentynyl); 1.47 (m, 2H, H4-pentynyl); 2.29 (m, 2H, H3-pentynyl); 4.15 (ddd, 1H, $J_{5'b,4'}$ = 2.0, $J_{5'b,5'a}$ = 11.9, $J_{5'b,P}$ = 2.0, H5′b-G); 4.23 (ddd, 1H, $J_{5'a,4'}$ = 3.0, $J_{5'a,5'b}$ = 11.9, $J_{5'a,P}$ = 4.7, H5′a-G); 4.25 (dm, 1H, $J_{5'b,5'a}$ = 12.0, H5′b-A); 4.41 (td, 1H, $J_{4',5'a}$ = 3.0, $J_{4',5'b}$ = 2.0, $J_{4',P}$ = 3.2, H4′-G); 4.45 (dm, 1H, $J_{4',3'}$ = 9.5, H4′-A); 4.51 (dm, 1H, $J_{5'a,5'b}$ = 12.0, H5′a-A); 4.62 (d, 1H, $J_{3',4'}$ = 4.3, H3′-G); 4.63 (d, 1H, $J_{2',3'}$ = 4.1, H2′-A); 4.98 (ddd, 1H, $J_{3',2'}$ = 4.1, $J_{3',4'}$ = 9.5, $J_{3',P}$ = 6.6, H3′-A); 5.75 (dt, 1H, $J_{2',1'}$ = 8.6, $J_{2',3'}$ = 4.3, $J_{2',P}$ = 4.3, H2′-G); 5.95 (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); 6.22 (s, 1H, H1′-A); 7.67 (s, 1H, H6-A); 7.88 (bs, 1H, H8-G); 8.18 (bs, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O; chemical shifts obtained from 2D-HSQC and 2D-HMBC spectra; J(C,P) and shifts of C2-G, C4-G, C5-G, and C6-G not determined): 15.55 (C5-pentynyl); 23.44 (C3-pentynyl); 24.04 (C4-pentynyl); 65.09 (C5′-A); 68.48 (C5′-G); 72.73 (C3′-A); 73.80 (C3′-G); 74.50 (C1-pentynyl); 76.83 (C2′-A); 77.11 (C2′-G); 82.28 (C4′-A); 85.73 (C4′-G); 89.00 (C1′-G); 92.02 (C1′-A); 96.60 (C2-pentynyl); 98.09 (C5-A); 105.93 (C4a-A); 127.64 (C6-A); 143.12 (C8-G); 149.52 (C7a-A); 154.41 (C2-A); 159.83 (C4-A); ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.15 and −0.83. ESI MS m/z (rel. %): 368 (100) $[M-2H]^{2-}$, 738 (15) $[M-H]^-$, 760 (9) $[M-2H + Na]^-$. HR MS (ESI): for $C_{26}H_{30}O_{13}N_9P_2$, calcd 738.14438; found, 738.14313.

*Cyclo-4-amino-5-phenyl-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]-pyrimidine 5′-O-Phosphate (3′ → 5′) Guanosine 5′-O-Phosphate (2′ → 5′) Sodium Salt (**5f**).* Enzymatic synthesis: NTP **3f** (2 μmol) and GTP (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5f** (100 nmol, 5%).

Chemical synthesis: CDN **5a** (15 mg, 17.8 μmol), phenylboronic acid (4.3 mg, 35.6 μmol), and cesium carbonate (17.4 mg, 53.4 μmol)

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were mixed with MeCN−H_2O (1:2 v/v, 600 μL) in an argon purged vial. In a separate vial, $Pd(OAc)_2$ (1.0 mg, 4.45 μmol) and TPPTS (12.6 mg, 22.2 μmol) were dissolved in MeCN−H_2O (1:2 v/v, 1.0 mL), and the solution was sonicated under an argon atmosphere for 30 s. Then, 200 μmol (i.e., 1/5) of this solution was transferred into the mixture containing the CDN **5a**, and the reaction was stirred at 100 °C for 30 min. Then, the reaction mixture was cooled to rt, diluted with water to approx. 3 mL, and filtered through a 5 μm nylon syringe filter. The filtrate was directly applied on HPLC for purification. After two HPLC separations (0−15% MeCN in 0.1 M TEAB) and conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle), CDN **5f** (2.5 mg, 18%) was obtained as a white lyophilizate (water). ^1H NMR (600 MHz, D_2O): 4.15 (ddd, 1H, $J_{5'b,4'}$ = 1.8, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.5, H5′b-G); 4.25 (ddd, 1H, $J_{5'a,4'}$ = 2.8, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 5.0, H5′a-G); 4.29 (m, 1H, H5′b-A); 4.43 (ddd, 1H, $J_{4',5'a}$ = 2.8, $J_{4',5'b}$ = 1.8, $J_{4',P}$ = 3.6, H4′-G); 4.49 (dm, 1H, $J_{4',3'}$ = 9.1, H4′-A); 4.51 (m, 1H, H5′a-A); 4.66 (d, 1H, $J_{3',2'}$ = 4.1, H3′-G); 4.79 (bd, 1H, $J_{2',3'}$ = 4.1, H2′-A); 5.02 (ddd, 1H, $J_{3',2'}$ = 4.1, $J_{3',4'}$ = 9.1, $J_{3',P}$ = 6.9, H3′-A); 5.73 (dt, 1H, $J_{2',1'}$ = 8.6, $J_{2',3'}$ = 4.1, $J_{2',P}$ = 4.1, H2′-G); 6.01 (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); 6.29 (s, 1H, H1′-A); 7.09 (m, 2H, *o*-Ph-A); 7.24 (m, 2H, *m*-Ph-A); 7.25 (m, 1H, *p*-Ph); 7.63 (s, 1H, H6-A); 7.89 (s, 1H, H8-G); 8.22 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.53 (d, $J_{C,P}$ = 4.6, C5′-A); 68.66 (d, $J_{C,P}$ = 5.4, C5′-G); 73.07 (d, $J_{C,P}$ = 5.5, C3′-A); 73.93 (C3′-G); 76.96 (C2′-A); 77.87 (d, $J_{C,P}$ = 4.6, C2′-G); 82.61 (t, $J_{C,P}$ = 11.3, C4′-A); 86.04 (d, $J_{C,P}$ = 9.7, C4′-G); 88.70 (d, $J_{C,P}$ = 12.5, C1′-G); 92.27 (C1′-A); 103.79 (C4a-A); 119.40 (C5-A); 119.88 (C5-G); 122.74 (C6-A); 129.47 (*p*-Ph-A); 130.27 (o-Ph-A); 131.61 (*m*-Ph-A); 135.65 (*i*-Ph-A); 142.42 (C8-G); 151.35 (C7a-A); 153.88 (C2-A); 154.58 (C4-G); 155.99 (C2-G); 160.08 (C4-A); 161.17 (C6-G). ^{31}P NMR (^1H-dec, 202.4 MHz, D_2O): −0.19 and −0.90. ESI MS *m*/*z* (rel. %): 373 (100) $[M-2H]^{2-}$, 385 (5) $[M-3H + Na]^{2-}$, 748 (2) $[M-H]^-$, 770 (6) $[M-2H + Na]^-$. HR MS (ESI): for $C_{27}H_{28}O_{13}N_9P_2$ $[M-H]^-$, calcd 748.12873; found, 748.12862.

*Cyclo-4-amino-5-(naphthalen-2-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5g**).* CDN **5a** (13 mg, 15.4 μmol), naphthalene-2-boronic acid (13.3 mg, 77.1 μmol), and cesium carbonate (15 mg, 46.2 μmol) were mixed with MeCN−H_2O (1:2 v/v, 520 μL) in an argon purged vial. In a separate vial, $Pd(OAc)_2$ (1.0 mg, 4.45 μmol) and TPPTS (12.6 mg, 22.2 μmol) were dissolved in MeCN−H_2O (1:2v/v, 1.0 mL), and the solution was sonicated under argon atmosphere for 30 s. Then, 173 μmol of this solution was transferred into the mixture containing the CDN **5a**, and the reaction mixture was stirred at 100 °C for 30 min. Then, the reaction mixture was cooled to rt, diluted with water to approx. 3 mL, and filtered through a 5 μm nylon syringe filter. The filtrate was directly applied on HPLC for purification (5−25% MeCN in 0.1 M TEAB). After HPLC repurification (9−24% MeCN in 0.1 M TEAB) and conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle), CDN **5g** (5.7 mg, 44%) was obtained as a white lyophilizate (water). ^1H NMR (600 MHz, D_2O): 4.13 (ddd, 1H, $J_{5'b,4'}$ = 1.6, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.6, H5′b-G); 4.26 (ddd, 1H, $J_{5'a,4'}$ = 2.7, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 4.5, H5′a-G); 4.36 (ddd, 1H, $J_{5'b,4'}$ = 1.3, $J_{5'b,5'a}$ = 12.1, $J_{5'b,P}$ = 2.9, H5′b-A); 4.45 (ddd, 1H, $J_{4',5'a}$ = 2.7, $J_{4',5'b}$ = 1.6, $J_{4',P}$ = 3.8, H4′-G); 4.53 (dm, 1H, $J_{4',3'}$ = 9.6, H4′-A); 4.57 (ddd, 1H, $J_{5'a,4'}$ = 2.3, $J_{5'a,5'b}$ = 12.1, $J_{5'a,P}$ = 1.1, H5′a-A); 4.73 (d, 1H, $J_{3',4'}$ = 4.0, H3′-G); 4.81 (d, $J_{3',2'}$ = 3.9, H2′-A); 5.03 (ddd, 1H, $J_{3',2'}$ = 3.9, $J_{3',4'}$ = 9.6, $J_{3',P}$ = 6.6, H3′-A); 5.67 (um, 1H, H2′-G); 6.07 (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); 6.34 (s, 1H, H1′-A); 7.32 (dd, 1H, $J_{3,2}$ = 1.8, $J_{3,4}$ = 8.3, H3-naphth); 7.53 (m, 1H, H7-naphth); 7.545 (m, 1H, H6-naphth); 7.64 (dm, 1H, $J_{1,3}$ = 1.8, H1-naphth); 7.74 (s, 1H, H8-G); 7.75 (s, 1H, H6-A); 7.77 (dm, 1H, $J_{4,3}$ = 8.3, H4-naphth); 7.82 (m, 1H, H5-naphth); 7.90 (m, 1H, H8-naphth); 8.25 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.66 (d, $J_{C,P}$ = 4.7, C5′-A); 68.76 (d, $J_{C,P}$ = 5.2, C5′-G); 73.16 (d, $J_{C,P}$ = 5.4, C3′-A); 73.91 (C3′-G); 76.98 (C2′-A); 79.00 (C2′-G); 82.58 (t, $J_{C,P}$ = 11.6, C4′-A); 86.13 (d, $J_{C,P}$ = 9.6, C4′-G); 87.80 (C1′-G); 92.32 (C1′-A); 103.93 (C5-A); 119.40 (C4a-A); 119.61 (C5-G); 123.09 (C6-A); 128.19 (C1-naphth); 128.51 (C3-naphth); 128.70 (C7-naphth); 129.24 (C6-naphth); 130.26 (C8-naphth); 130.89 (C5-naphth); 131.18 (C4-naphth); 133.19 (C2-naphth); 134.43 (C4a-naphth); 135.96 (C8a-naphth); 141.25 (C8-G); 151.60 (C7a-A); 153.89 (C2-A); 154.34 (C4-G); 155.90 (C2-G); 160.32 (C4-A); 160.72 (C6-G). ^{31}P NMR (^1H-dec, 202.4 MHz, D_2O): −0.12 and −0.96. ESI MS *m*/*z* (rel. %): 398 (100) $[M-2H]^{2-}$, 798 (47) $[M-H]^-$, 820 (35) $[M-2H + Na]^-$ HR MS (ESI): for $C_{31}H_{29}O_{13}N_9P_2$ $[M-2H]^{2-}$, calcd 398.56855; found, 398.56823.

*Cyclo-4-amino-5-(naphthalen-1-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5h**).* CDN **5h** was prepared as described for **5g** from iodinated CDN **5a** (15 mg, 17.8 μmol) and naphthalene-1-boronic acid (17.6 mg, 88.9 μmol). The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). After HPLC repurification (5−50% MeOH in 0.1 M TEAB) and conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle), CDN **5h** (5.6 mg, 37%) was obtained as a white lyophilizate (water). The final product was a mixture of two diastereomers (63:37) due to hindered rotation (at 298 K). Chemical shifts of the resolved signals of minor isomer are given in italics. ^1H NMR (600 MHz, D_2O): 4.125 (dt, 1H, $J_{5'b,4'}$ = 2.1, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.2, H5′b-G); *4.13* (ddd, 1H, $J_{5'b,4'}$ = 2.3, $J_{5'b,5'a}$ = 11.9, $J_{5'b,P}$ = 3.5, H5′b-G); 4.23 (dd, 1H, $J_{5'a,4'}$ = 2.6, $J_{5'a,5'b}$ = 11.8, H5′a-G); *4.24* (dd, 1H, $J_{5'a,4'}$ = 2.6, $J_{5'a,5'b}$ = 11.9, H5′a-G); *4.25* (m, 1H, H5′b-A); 4.28 (ddd, 1H, $J_{5'b,4'}$ = 1.5, $J_{5'b,5'a}$ = 12.1, $J_{5'b,P}$ = 2.8, H5′b-A); *4.385* (m, 1H, H4′-G); 4.39 (m, 1H, H4′-G); 4.44 (dm, 1H, $J_{5'a,5'b}$ = 12.1, H5′a-A); *4.47* (dm, 1H, $J_{4',3'}$ = 8.4, H4′-A); 4.50 (dm, 1H, $J_{4',3'}$ = 9.0, H4′-A); 4.615 (d, 1H, $J_{3',4'}$ = 4.1, H3′-G); *4.64* (d, 1H, $J_{3',4'}$ = 4.1, H3′-G); 5.025 (d, 1H, $J_{2',3'}$ = 4.3, H2′-A); *5.04* (dd, 1H, $J_{2',3'}$ = 4.4, $J_{2',1'}$ = 1.1, H2′-A); 5.09 (ddd, 1H, $J_{3',2'}$ = 4.3, $J_{3',4'}$ = 9.0, $J_{3',P}$ = 6.2, H3′-A); *5.34* (ddd, 1H, $J_{3',2'}$ = 4.4, $J_{3',4'}$ = 8.4, $J_{3',P}$ = 6.6, H3′-A); 5.79 (ddd, 1H, $J_{2',1'}$ = 8.6, $J_{2',3'}$ = 4.1, $J_{2',P}$ = 3.7, H2′-G); *5.87* (dt, 1H, $J_{2',1'}$ = 8.6, $J_{2',3'}$ = 4.1, $J_{2',P}$ = 3.9, H2′-G); 5.97 (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); *5.98* (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); 6.38 (s, 1H, H1′-A); 6.55 (d, 1H, $J_{1',2'}$ = 1.1, H1′-A); 6.895 (dd, 1H, $J_{2,3}$ = 7.0, $J_{2,4}$ = 1.3, H8-naphth); *7.18* (dd, 1H, $J_{3,4}$ = 8.3, $J_{3,2}$ = 7.0, H3-naphth); *7.21* (s, 1H, H6-A); 7.36 (dd, 1H, $J_{3,4}$ = 8.4, $J_{3,2}$ = 7.0, H3-naphth); 7.38 (ddd, 1H, $J_{6,7}$ = 8.2, $J_{6,5}$ = 6.8, $J_{6,8}$ = 1.5, H6-naphth); *7.38* (dd, 1H, $J_{2,3}$ = 7.0, $J_{2,4}$ = 1.5, H8-naphth); 7.41 (ddd, 1H, $J_{7,8}$ = 6.8, $J_{7,6}$ = 8.2, $J_{7,5}$ = 1.4, H7-naphth); *7.485* (ddd, 1H, $J_{6,7}$ = 8.5, $J_{6,5}$ = 6.8, $J_{6,8}$ = 1.3, H6-naphth); 7.52 (s, 1H, H6-A); *7.59* (ddd, 1H, $J_{7,8}$ = 6.8, $J_{7,6}$ = 8.5, $J_{7,5}$ = 1.3, H7-naphth); *7.64* (dd, 1H, $J_{5,6}$ = 6.8, $J_{5,7}$ = 1.3, H5-naphth); 7.70 (dd, 1H, $J_{5,6}$ = 6.8, $J_{5,7}$ = 1.4, H5-naphth); *7.86* (dd, 1H, $J_{4,3}$ = 8.3, $J_{4,2}$ = 1.5, H4-naphth); 7.90 (dd, 1H, $J_{8,7}$ = 6.8, $J_{8,6}$ = 1.5, H8-naphth); 7.905 (dd, 1H, $J_{4,3}$ = 8.4, $J_{4,2}$ = 1.3, H4-naphth); 7.91 (s, 1H, H8-G); *7.995* (dd, 1H, $J_{8,7}$ = 6.8, $J_{8,6}$ = 1.3, H8-naphth); *8.20* (s, 1H, H2-A); 8.235 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.48 (d, $J_{C,P}$ = 4.5, C5′-A); *68.92* (d, $J_{C,P}$ = 5.5, C5′-G); *65.98* (d, $J_{C,P}$ = 5.0, C5′-A); 68.79 (d, $J_{C,P}$ = 5.1, C5′-G); 73.59 (d, $J_{C,P}$ = 5.4, C3′-A); *73.71* (d, $J_{C,P}$ = 5.3, C3′-A); 74.19 (C3′-G); *74.32* (C3′-G); 76.70 (C2′-A); *76.84* (C2′-A); 77.34 (d, $J_{C,P}$ = 5.2, C2′-G); *77.50* (d, $J_{C,P}$ = 5.5, C2′-G); 82.55 (t, $J_{C,P}$ = 11.3, C4′-A); *82.83* (t, $J_{C,P}$ = 11.2, C4′-A); *85.72* (d, $J_{C,P}$ = 9.1, C4′-G); 85.75 (d, $J_{C,P}$ = 9.4, C4′-G); *88.63* (d, $J_{C,P}$ = 12.5, C1′-G); 89.02 (d, $J_{C,P}$ = 13.0, C1′-G); *90.32* (C1′-A); 91.67 (C1′-A); 105.81 (C4a-A); *106.86* (C4a-A); 117.04 (C5-A); *118.96* (C5-A); 119.75 (C5-G); *119.79* (C5-G); 123.68 (C6-A); *123.73* (C6-A); *127.65* (C5-naphth); 127.82 (C5-naphth); *128.11* (C3-naphth); 128.24 (C3-naphth); 128.92 (C7-naphth); *129.03* (C7-naphth); *129.64* (C6-naphth); 129.69 (C6-naphth); 130.82 (C2-naphth); 130.97 (C4-naphth); 131.06 (C8-naphth); *131.26* (C4-naphth); *131.45* (C2-naphth); *131.48* (C8-naphth); *132.48* (C4a-naphth); 132.73 (C4a-naphth); 134.11 (C8a-naphth); *134.28* (C8a-naphth); 136.12 (C1-naphth); 136.20 (C1-naphth); *142.91* (C8-G); 142.91 (C8-G); 151.38 (C7a-A); *152.67* (C7a-A); 154.21 (C2-A); 154.40 (C4-G); *154.40* (C4-G); *154.59* (C2-A); *156.04* (C2-G); 156.89 (C2-G); 159.90 (C4-A); *160.06* (C4-A); *160.28* (C6-G); 160.94 (C6-G). ^{31}P NMR (^1H-dec, 202.4 MHz, D_2O): *−0.08*; −0.25; *−0.32*; −0.61. ESI MS *m*/*z* (rel. %): 398 (100) $[M-2H]^{2-}$, 798 (25) $[M-H]^-$. HR MS (ESI): for $C_{31}H_{30}O_{13}N_9P_2$ $[M-H]^-$, calcd 798.114438; found, 798.14472.

Cyclo-4-amino-5-(1,2-dihydroacenaphthylen-5-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′)

*Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5i**)*. CDN **5i** was prepared as described for **5g** from iodinated CDN **5a** (15 mg, 17.8 μmol) and acenaphthene-5-boronic acid (17.6 mg, 88.9 μmol). The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5i** (7.3 mg, 47%) as a white lyophilizate (water). The final product was a mixture of two diastereomers (78:22) due to hindered rotation (at 298 K). The hindered rotation resulted in the line broadening of most NMR signals. Only a few resolved proton signals of minor isomer could be detected, and their chemical shifts are given in italics. ^{1}H NMR (600 MHz, D_2O): 3.38 (um, 2H, 2 × H1-acenaphtylene); 3.46 (um, 2H, 2 × H2-acenaphtylene); 4.11 (dm, 1H, $J_{5'b,5'a}$ = 11.9, H5′b-G); 4.23 (ddd, 1H, $J_{5'a,4'}$ = 2.4, $J_{5'a,5'b}$ = 11.9, $J_{5'a,P}$ = 6.0, H5′a-G); 4.30 (dm, 1H, $J_{5'b,5'a}$ ≈ 12.0, H5′b-A); 4.38 (dt, 1H, $J_{4',5'a}$ = 2.4, $J_{4',5'b}$ = 2.2, $J_{4',P}$ = 3.4, H4′-G); 4.43 (dm, 1H, $J_{5'a,5'b}$ ≈ 12.0, H5′a-A); 4.48 (dm, 1H, $J_{4',3'}$ ≈ 9.0, H4′-A); 4.62 (bd, 1H, $J_{3',4'}$ = 3.9, H3′-G); 4.99 (br, 1H, H2′-A); 5.13, *5.30* (um, 1H, H3′-A); 5.85 (um, 1H, H2′-G); 5.96, *5.90* (bd, 1H, $J_{1',2'}$ ≈ 8.0, H1′-G); 6.44, *6.52* (s, 1H, H1′-A); 7.03, *6.96* (bd, 1H, $J_{3,4}$ = 6.9, H3-acenaphtylene); 7.15 (bd, 1H, $J_{6,7}$ = 6.8, H6-acenaphtylene); 7.24 (bd, 1H, $J_{4,3}$ = 6.9, H4-acenaphtylene); 7.26 (bd, 1H, $J_{8,7}$ = 8.0, H8-acenaphtylene); 7.29 (bt, 1H, $J_{7,8}$ = 8.0, $J_{7,6}$ = 6.8, H7-acenaphtylene); 7.44 (bs, 1H, H6-A); 7.85 (s, 1H, H8-G); 8.21 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 32.48 (C1-acenaphtylene); 32.83 (C2-acenaphtylene); 65.48 (C5′-A); 68.89 (d, $J_{C,P}$ = 5.3, C5′-G); 73.69 (C3′-A); 74.29 (C3′-G); 76.93 (C2′-A); 77.37 (d, $J_{C,P}$ = 4.8, C2′-G); 82.27 (C4′-A); 85.62 (d, $J_{C,P}$ = 9.3, C4′-G); 88.92 (d, $J_{C,P}$ = 13.9, C1′-G); 91.06 (C1′-A); 105.17 (C4a-A); 117.20 (C5-A); 119.55 (C5-G); 121.73 (C4-acenaphtylene); 122.41 (C6-acenaphtylene); 122.58 (C8-acenaphtylene); 123.48 (C6-A); 127.73 (C5a-acenaphtylene); 131.66 (C7-acenaphtylene); 132.01 (C3-acenaphtylene); 132.25 (C5-acenaphtylene); 141.70 (C8b-acenaphtylene); 142.56 (C8-G); 148.94 (C8a-acenaphtylene); 149.81 (C2a-acenaphtylene); 151.60 (C7a-A); 154.28 (C2-A); 154.24 (C4-G); 155.89 (C2-G); 160.11 (C4-A); 160.68 (C6-G); ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.22 and −0.45. ESI MS *m/z* (rel. %): 411 (100) $[M-2H]^{2-}$, 824 (22) $[M-H]^-$, 846 (26) $[M-2H + Na]^-$. HR MS (ESI): for $C_{33}H_{32}O_{13}N_9P_2$ $[M-H]^-$, calcd 824.16003; found, 824.15900.

*Cyclo-4-amino-5-(phenanthren-9-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5j**)*. CDN **5j** was prepared as described for **5g** from iodinated CDN **5a** (15 mg, 17.8 μmol) and phenanthrene-9-boronic acid (19.4 mg, 88.9 μmol). The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5j** (4.7 mg, 30%) as a white lyophilizate (water). The final product was a mixture of two diastereomers (63:37) due to hindered rotation (at 298 K). Chemical shifts of the resolved signals of minor isomer are given in italics. ^{1}H NMR (600 MHz, D_2O): 4.07 (dt, 1H, $J_{5'b,4'}$ = 2.1, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.1, H5′b-G); *4.125* (dt, 1H, $J_{5'b,4'}$ = 2.1, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.1, H5′b-G); 4.22 (ddd, 1H, $J_{5'a,4'}$ = 2.4, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 5.1, H5′a-G); *4.25* (ddd, 1H, $J_{5'a,4'}$ = 2.6, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 5.5, H5′a-G); *4.30* (ddd, 1H, $J_{5'b,4'}$ = 1.6, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.8, H5′b-A); 4.33 (m, 2H, H5′a-A and H5′b-A); 4.35 (dt, 1H, $J_{4',5'a}$ = 2.4, $J_{4',5'b}$ = 2.1, $J_{4',P}$ = 3.6, H4′-G); *4.415* (dt, 1H, $J_{4',5'a}$ = 2.6, $J_{4',5'b}$ = 2.1, $J_{4',P}$ = 3.4, H4′-G); *4.47* (dm, 1H, $J_{5'a,5'b}$ = 11.8, H5′a-A); 4.49 (dm, 1H, $J_{4',3'}$ = 8.6, H4′-A); *4.53* (dm, 1H, $J_{4',3'}$ = 9.0, H4′-A); *4.66* (d, 1H, $J_{3',2'}$ = 4.0, H3′-G); 4.67 (d, 1H, $J_{3',2'}$ = 3.9, H3′-G); *5.09* (ddd, 1H, $J_{3',2'}$ = 4.3, $J_{3',4'}$ = 9.0, $J_{3',P}$ = 6.3, H3′-A); 5.10 (d, 1H, $J_{2',3'}$ = 4.5, H2′-A); *5.12* (d, 1H, $J_{2',3'}$ = 4.3, H2′-A); 5.47 (ddd, 1H, $J_{3',2'}$ = 4.5, $J_{3',4'}$ = 8.6, $J_{3',P}$ = 6.8, H3′-A); *5.80* (ddd, 1H, $J_{2',1'}$ = 8.6, $J_{2',3'}$ = 4.0, $J_{2',P}$ = 3.5, H2′-G); 5.86 (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); 5.92 (um, 1H, H2′-G); *6.06* (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); *6.35* (s, 1H, H1′-A); 6.62 (s, 1H, H1′-A); *7.305* (s, 1H, H10-phen); 7.32 (s, 1H, H6-A); 7.34 (br, 1H, H8-G); *7.515* (ddd, 1H, $J_{2,1}$ = 8.3, $J_{2,3}$ = 6.9, $J_{2,4}$ = 1.2, H2-phen); 7.61 (m,1H, H2-phen); *7.63* (m, 1H, H6-phen); 7.635 (m, 1H, H7-phen); *7.65* (s, 1H, H6-A); 7.725 (m, 2H, H6 and H8-phen); 7.75 (m, 1H, H1-phen); *7.765* (m, 1H, H3-phen); *7.77* (m, 1H, H7-phen); 7.785 (s, 1H, H10-phen); 7.79 (m, 1H, H3-phen); *7.805* (m, 1H, H1-phen); *7.90* (br, 1H, H8-G); 8.21 (s, 1H, H2-A); *8.25* (s, 1H, H2-A); 8.76 (dm, 1H, $J_{4,3}$ = 8.5, H4-phen); *8.79* (dm, 1H, $J_{5,6}$ = 8.5, H5-phen); *8.795* (dm, 1H, $J_{4,3}$ = 8.5, H4-phen); 8.84 (dm, 1H, $J_{5,6}$ = 8.5, H5-phen). ^{13}C NMR (150.9 MHz, D_2O): *65.52* (C5′-A); 66.17 (C5′-A); *68.79* (C5′-G); 69.00 (C5′-G); *73.73* (C3′-A); *74.17* (C3′-G); 74.29 (C3′-G); 74.66 (C3′-A); *76.43* (C2′-A); 76.89 (C2′-A); *77.91* (C2′-G); 77.91 (C2′-G); *82.66* (C4′-A); 82.73 (C4′-A); 85.34 (C4′-G); *85.88* (C4′-G); 88.17 (C1′-G); *88.67* (C1′-G); 90.19 (C1′-A); *91.83* (C1′-A); 105.86 (C4a-A); *106.05* (C4a-A); *116.93* (C5-A); 118.78 (C5-G); 119.24 (C5-A); *119.48* (C5-G); *123.85* (C6-A); 124.16 (C6-A); *125.23* (C4-phen); 125.34 (C4-phen); *125.60* (C5-phen); 126.24 (C5-phen); 128.53 (C8-phen); *128.79* (C8-phen); *129.70* (C7-phen); 129.76 (C7-phen); 129.84 (C3-phen); 129.84 (C6-phen); *130.00* (C2-phen); 130.03 (C2-phen); 130.88 (C8a-phen); *131.18* (C10-phen); 131.48 (C10-phen); *131.49* (C8a-phen); *131.74* (C1-phen); *132.33* (C4a-phen); 132.35 (C1-phen); 132.45 (C4a-phen); *132.84* (C4b-phen); *133.02* (C9-phen); 133.09 (C4b-phen); 133.18 (C9-phen); 141.58 (C8-G); *142.58* (C8-G); *151.23* (C7a-A); 152.86 (C7a-A); 153.68 (C4-G); *154.05* (C2-A); 154.37 (C4-G); 154.57 (C2-A); 155.96 (C2-G); *155.98* (C2-G); 159.90 (C4-A); 159.68 (C6-G); *159.74* (C6-G); *160.72* (C4-A). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.07; *−0.23*; −0.44; *−0.68*. ESI MS *m/z* (rel. %): 423 (100) $[M-2H]^{2-}$, 856 (9) $[M-2H + Na]^-$. HR MS (ESI): for $C_{35}H_{32}O_{13}N_9P_2$ $[M-H]^-$, calcd 848.16003; found, 848.15924.

*Cyclo-4-amino-5-(biphenyl-4-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5k**)*. CDN **5k** was prepared as described for **5g** from iodinated CDN **5a** (15 mg, 17.8 μmol) and 4-biphenylboronic acid (17.6 mg, 88.9 μmol). The final product was purified by HPLC (5−25% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5k** (9.1 mg, 59%) as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.13 (ddd, 1H, $J_{5'b,4'}$ = 1.7, $J_{5'b,5'a}$ = 11.7, $J_{5'b,P}$ = 2.5, H5′b-G); 4.24 (ddd, 1H, $J_{5'a,4'}$ = 2.7, $J_{5'a,5'b}$ = 11.7, $J_{5'a,P}$ = 4.7, H5′a-G); 4.32 (ddd, 1H, $J_{5'b,4'}$ = 2.9, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 1.2, H5′b-A); 4.42 (ddd, 1H, $J_{4',5'a}$ = 2.7, $J_{4',5'b}$ = 1.7, $J_{4',P}$ = 3.7, H4′-G); 4.50 (dm, 1H, $J_{4',3'}$ = 9.3, H4′-A); 4.56 (bdd, 1H, $J_{5'a,4'}$ = 2.1, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = <1, H5′a-A); 4.68 (d, 1H, $J_{3',4'}$ = 4.1, H3′-G); 4.74 (d, 1H, $J_{2',3'}$ = 4.0, H2′-A); 4.97 (ddd, 1H, $J_{3',2'}$ = 4.0, $J_{3',4'}$ = 9.3, $J_{3',P}$ = 6.5, H3′-A); 5.64 (um, 1H, H2′-G); 6.02 (d, 1H, $J_{1',2'}$ = 8.5, H1′-G); 6.25 (s, 1H, H1′-A); 7.11 (m, 2H, H3-phenylene + H5-phenylene); 7.41 (m, 1H, H4′-Ph); 7.48 (m, 2H, H2-phenylene + H6-phenylene); 7.51 (m, 2H, H3′-Ph + H5′-Ph); 7.66 (s, 1H, H6-A); 7.71 (m, 2H, H2′-Ph + H6′-Ph); 7.82 (s, 1H, H8-G); 8.18 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.54 (d, $J_{C,P}$ = 4.8, C5′-A); 68.72 (d, $J_{C,P}$ = 5.2, C5′-G); 73.09 (d, $J_{C,P}$ = 6.5, C3′-A); 73.80 (C3′-G); 76.97 (C2′-A); 78.50 (C2′-G); 82.55 (t, $J_{C,P1}$ = $J_{C,P2}$ = 11.3, C4′-A); 86.10 (d, $J_{C,P}$ = 9.2, C4′-G); 88.07 (C1′-G); 92.24 (C1′-A); 103.65 (C4a-A); 119.08 (C5-A); 119.67 (C5-G); 122.79 (C6-A); 129.37 (C2′-Ph + C6′-Ph); 129.67 (C2-phenylene + C6-phenylene); 130.31 (C4′-Ph); 130.49 (C3-phenylene + C5-phenylene); 131.82 (C3′-Ph + C5′-Ph); 134.81 (C4-phenylene); 140.79 (C1-phenylene); 141.33 (C8-G); 142.49 (C1′-Ph); 151.25 (C7a-A); 153.39 (C2-A); 154.49 (C4-G); 155.98 (C2-G); 159.95 (C4-A); 160.98 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.14 and −0.93. ESI MS *m/z* (rel. %): 411 (100) $[M-2H]^{2-}$, 824 (43) $[M-H]^-$, 846 (17) $[M-2H + Na]^-$. HR MS (ESI): for $C_{33}H_{32}O_{13}N_9P_2$ $[M-H]^-$, calcd 824.16003; found, 824.16012.

*Cyclo-4-amino-5-(4-(naphthalen-2-yl)phenyl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5l**)*. CDN **5l** was prepared as described for **5g** from iodinated CDN **5a** (15 mg, 17.8 μmol) and 4-(naphthalene-2-yl)phenylboronic acid pinacol ester (29.4 mg, 88.9 μmol). The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5l** (5.3 mg, 32%) as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.14 (ddd, 1H, $J_{5'b,4'}$ = 1.8, $J_{5'b,5'a}$ = 11.7, $J_{5'b,P}$ = 2.4, H5′b-G); 4.22 (ddd, 1H, $J_{5'a,4'}$ = 2.5, $J_{5'a,5'b}$ = 11.7, $J_{5'a,P}$ = 4.8, H5′a-G); 4.35 (ddd, 1H, $J_{5'b,5'a}$ = 11.9, $J_{5'b,4'}$ = 1.1, $J_{5'b,P}$ = 2.7, H5′b-A); 4.44 (ddd, 1H, $J_{4',5'a}$ =

2.5, $J_{4',5'b}$ = 1.8, $J_{4',P}$ = 3.7, H4′-G); 4.52 (dm, 1H, $J_{4',3'}$ = 9.3, H4′-A); 4.58 (bdd, 1H, $J_{5'a,5'b}$ = 11.9, $J_{5'a,4'}$ = 2.0, $J_{5'b,P}$ = <1, H5′a-A); 4.70 (d, 1H, $J_{3',2'}$ = 4.0, H3′-G); 4.76 (overlap, H2′-A); 4.98 (ddd, 1H, $J_{3',2'}$ = 4.0, $J_{3',4'}$ = 9.3, $J_{3',P}$ = 6.6, H3′-A); 5.61 (um, 1H, H2′-G); 6.03 (d, 1H, $J_{1',2'}$ = 8.5, H1′-G); 6.30 (s, 1H, H1′-A); 7.16 (m, 2H, H2 + H6-phenylene); 7.53 (m, 1H, H7-naphth); 7.54 (m, 1H, H6-naphth); 7.57 (m, 2H, H3 + H5-phenylene); 7.65 (s, 1H, H6-A); 7.81 (s, 1H, H8-G); 7.84 (dd, 1H, $J_{3,1}$ = 1.9, $J_{3,4}$ = 8.6, H3-naphth); 7.90 (m, 1H, H8-naphth); 7.91 (m, 1H, H5-naphth); 7.94 (d, 1H, $J_{4,3}$ = 8.6, H4-naphth); 8.12 (bd, 1H, $J_{1,3}$ = 1.9, H1-naphth); 8.12 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.71 (d, $J_{C,P}$ = 3.2, C5′-A); 68.78 (d, $J_{C,P}$ = 4.8, C5′-G); 73.19 (d, $J_{C,P}$ = 6.5, C3′-A); 73.96 (C3′-G); 76.98 (C2′-A); 78.87 (C2′-G); 82.58 (t, $J_{C,P1}$ = $J_{C,P2}$ = 11.4, C4′-A); 86.12 (d, $J_{C,P}$ = 9.5, C4′-G); 87.90 (d, $J_{C,P}$ = 13.9, C1′-G); 92.18 (C1′-A); 103.64 (C4a-A); 119.16 (C5-A); 119.61 (C5-G); 122.71 (C6-A); 127.76 (C1-naphth); 127.81 (C3-naphth); 128.93 (C7-naphth); 129.24 (C6-naphth); 129.89 (C3 and C5-phenylene); 130.24 (C8-naphth); 130.57 (C2 and C6-phenylene); 130.82 (C5-naphth); 131.15 (C4-naphth); 134.88 (C1-phenylene); 134.99 (C4a-naphth); 136.05 (C8a-naphth); 139.85 (C2-naphth); 140.55 (C4-phenylene); 141.08 (C8-G); 151.23 (C7a-A); 153.51 (C2-A); 154.49 (C4-G); 156.00 (C2-G); 159.89 (C4-A); 160.98 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.14 and −0.93. ESI MS m/z (rel. %): 436 (100) $[M-2H]^{2-}$, 874 (17) $[M-H]^-$, 896 (14) $[M-2H + Na]^-$. HR MS (ESI): for $C_{37}H_{34}O_{13}N_9P_2$ $[M-H]^-$, calcd 874.17568; found, 874.17462.

*Cyclo-4-amino-5-[4-{(naphthalen-1-yloxy)methyl}phenyl]-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5m**).* CDN **5m** was prepared as described for **5g** from iodinated CDN **5a** (15 mg, 17.8 μmol) and [(1-naphthyloxy)methyl]phenylboronic acid (24.7 mg, 88.9 μmol). The final product was purified by HPLC (5−32.5% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5m** (5.7 mg, 34%) as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.13 (dt, 1H, $J_{5'b,4'}$ = 1.8, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 1.8, H5′b-G); 4.24 (ddd, 1H, $J_{5'a,4'}$ = 2.7, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 4.8, H5′a-G); 4.30 (dm, 1H, $J_{5'b,5'a}$ = 11.7, H5′b-A); 4.42 (m, 1H, H4′-G); 4.49 (dm, 1H, $J_{4',3'}$ = 9.5, H4′-A); 4.53 (dm, 1H, $J_{5'a,5'b}$ = 11.7, H5′a-A); 4.66 (d, 1H, $J_{3',4'}$ = 4.0, H3′-G); 4.72 (bd, 1H, $J_{2',3'}$ = 4.0, H2′-A); 4.97 (ddd, 1H, $J_{3',2'}$ = 4.0, $J_{3',4'}$ = 9.5, $J_{3',P}$ = 6.5, H3′-A); 5.26 (d, 1H, J_{gem} = 11.5, O-CHaHb); 5.30 (d, 1H, J_{gem} = 11.5, O-CHaHb); 5.68 (dt, 1H, $J_{2',1'}$ = 8.5, $J_{2',3'}$ = 4.0, $J_{2',P}$ = 4.0, H2′-G); 5.97 (d, 1H, $J_{1',2'}$ = 8.5, H1′-G); 6.11 (s, 1H, H1′-A); 7.09 (bd, 1H, $J_{2,3}$ = 7.6, H2-naphth-A); 7.12 (m, 2H, H2 and H6-phenylene); 7.34 (m, 2H, H3 and H5-phenylene); 7.47 (m, 2H, H3 and H7-naphth-A); 7.54 (s, 1H, H6-A); 7.54 (m, 2H, H4 and H6-naphth-A); 7.76 (s, 1H, H8-G); 7.87 (bd, 1H, $J_{5,6}$ = 8.3, H5-naphth-A); 8.03 (s, 1H, H2-A); 8.17 (bd, 1H, $J_{8,7}$ = 8.5, H8-naphth-A). ^{13}C NMR (150.9 MHz, D_2O): 65.51 (d, $J_{C,P}$ = 4.0, C5′-A); 68.67 (d, $J_{C,P}$ = 5.1, C5′-G); 73.02 (CH_2O); 73.15 (d, $J_{C,P}$ = 5.6, C3′-A); 73.91 (C3′-G); 76.88 (C2′-A); 78.06 (d, $J_{C,P}$ = 5.8, C2′-G); 82.56 (t, $J_{C,P1}$ = $J_{C,P2}$ = 11.2, C4′-A); 85.95 (d, $J_{C,P}$ = 9.4, C4′-G); 88.51 (d, $J_{C,P}$ = 12.2, C1′-G); 92.09 (C1′-A); 103.57 (C4a-A); 109.29 (C2-naphth); 118.94 (C5-A); 119.81 (C5-G); 122.80 (C6-A); 123.49 (C4-naphth); 124.15 (C8-naphth); 127.72 (C4a-naphth); 128.35 (C7-naphth); 129.04 (C3-naphth); 129.41 (C6-naphth); 130.23 (C5-naphth); 130.46 (C2 and C6-phenylene); 130.98 (C3 and C5-phenylene); 136.61 (C1-phenylene); 136.86 (C8a-naphth-A); 137.84 (C4-phenylene); 142.06 (C8-G); 150.92 (C7a-A); 153.37 (C2-A); 154.50 (C4-G); 155.92 (C2-G); 156.43 (C1-naphth-A); 159.61 (C4-A); 161.05 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.21 and −0.96. ESI MS m/z (rel. %): 451 (100) $[M-2H]^{2-}$, 462 (5) $[M-3H + Na]^{2-}$, 904 (6) $[M-H]^-$. HR MS (ESI): for $C_{38}H_{36}O_{14}N_9P_2$ $[M-H]^-$, calcd 904.18624; found, 904.18469.

*Cyclo-4-amino-5-(furan-2-yl)-7-β-D-ribofuranosyl-7H-pyrrolo-[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5n**).* NTP **3n** (2 μmol) and GTP (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5n** (299 nmol, 15%). ^{1}H NMR (600 MHz, D_2O): 4.13 (ddd, 1H, $J_{5'b,4'}$ = 1.9, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.0, H5′b-G); 4.23 (ddd, 1H, $J_{5'a,4'}$ = 3.1, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 4.5, H5′a-G); 4.34 (ddd, 1H, $J_{5'b,5'a}$ = 12.0, $J_{5'b,4'}$ = 2.7, $J_{5'b,P}$ = 1.1, H5′b-A); 4.42 (ddd, 1H, $J_{4',5'a}$ = 3.1, $J_{4',5'b}$ = 1.9, $J_{4',P}$ = 3.6, H4′-G); 4.50 (dm, 1H, $J_{4',3'}$ = 9.7, H4′-A); 4.61 (bdd, 1H, $J_{5'a,5'b}$ = 12.0, $J_{5'a,4'}$ = 2.2, $J_{5'a,P}$ < 1.0, H5′a-A); 4.66 (d, 1H, $J_{3',4'}$ = 4.0, H3′-G); 4.66 (d, 1H, $J_{2',3'}$ = 4.0, H2′-A); 5.01 (ddd, 1H, $J_{3',2'}$ = 4.0, $J_{3',4'}$ = 9.7, $J_{3',P}$ = 6.6, H3′-A); 5.74 (ddd, 1H, $J_{2',1'}$ = 8.7, $J_{2',3'}$ = 4.0, $J_{2',P}$ = 3.8, H2′-G); 5.93 (d, 1H, $J_{1',2'}$ = 8.7, H1′-G); 6.27 (s, 1H, H1′-A); 6.26 (dd, 1H, $J_{4,3}$ = 3.5, $J_{4,5}$ = 1.8, H4-furyl); 6.28 (dd, 1H, $J_{3,4}$ = 3.5, $J_{3,5}$ = 0.8, H3-furyl); 7.42 (dd, 1H, $J_{5,4}$ = 1.8, $J_{5,3}$ = 0.8, H5-furyl); 7.75 (s, 1H, H8-G); 8.00 (s, 1H, H6-A); 8.19 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.48 (d, $J_{C,P}$ = 4.0, C5′-A); 68.56 (d, $J_{C,P}$ = 5.2, C5′-G); 72.66 (d, $J_{C,P}$ = 5.3, C3′-A); 73.70 (C3′-G); 76.92 (C2′-A); 77.59 (br, C2′-G); 82.34 (t, $J_{C,P1}$ = $J_{C,P2}$ = 11.5, C4′-A); 85.82 (d, $J_{C,P}$ = 10.2, C4′-G); 88.84 (d, $J_{C,P}$ = 15.0, C1′-G); 92.50 (C1′-A); 102.40 (C5-A); 106.58 (C3-furyl); 109.14 (C4a-A); 114.75 (C4-furyl); 119.79 (C5-G); 120.95 (C6-A); 142.52 (C8-G); 143.61 (C5-furyl); 150.48 (C2-furyl); 150.75 (C7a-A); 154.03 (C2-A); 154.47 (C4-G); 155.82 (C2-G); 159.59 (C4-A); 161.11 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.16 and −1.18. ESI MS m/z (rel. %): 368 (100) $[M-2H]^{2-}$, 379 (6) $[M-3H-Na]^{2-}$, 738 (14) $[M-H]^-$, 760 (14) $[M-2H + Na]^-$. HR MS (ESI): for $C_{25}H_{26}O_{14}N_9P_2$ $[M-H]^-$, calcd 738.10799; found, 738.10760.

*Cyclo-4-amino-7-β-D-ribofuranosyl-5-(thiophen-2-yl)-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5o**).* NTP **3o** (2 μmol) and GTP (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **5o** (266 nmol, 13%). ^{1}H NMR (600 MHz, D_2O): 4.15 (ddd, 1H, $J_{5'b,4'}$ = 1.8, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.2, H5′b-G); 4.29 (ddd, 1H, $J_{5'a,4'}$ = 2.8, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 4.9, H5′a-G); 4.31 (ddd, 1H, $J_{5'b,5'a}$ = 12.0, $J_{5'b,4'}$ = 3.1, $J_{5'b,P}$ = 1.2, H5′b-A); 4.44 (ddd, 1H, $J_{4',5'a}$ = 2.8, $J_{4',5'b}$ = 1.8, $J_{4',P}$ = 3.6, H4′-G); 4.49 (dm, 1H, $J_{4',3'}$ = 9.2, H4′-A); 4.52 (dm, 1H, $J_{5'a,5'b}$ = 12.0, H5′a-A); 4.66 (d, 1H, $J_{3',4'}$ = 4.0, H3′-G); 4.79 (overlap, H2′-A); 5.00 (ddd, 1H, $J_{3',2'}$ = 4.2, $J_{3',4'}$ = 9.2, $J_{3',P}$ = 6.5, H3′-A); 5.70 (dt, 1H, $J_{2',1'}$ = 8.7, $J_{2',3'}$ = 4.0, $J_{2',P}$ = 4.0, H2′-G); 5.99 (d, 1H, $J_{1',2'}$ = 8.7, H1′-G); 6.28 (s, 1H, H1′-A); 6.82 (dd, 1H, $J_{5,4}$ = 3.5, $J_{5,3}$ = 1.2, H5-thienyl); 6.98 (dd, 1H, $J_{4,3}$ = 5.2, $J_{4,5}$ = 3.5, H4-thienyl); 7.24 (dd, 1H, $J_{3,4}$ = 5.2, $J_{3,5}$ = 1.2, H3-thienyl); 7.65 (s, 1H, H6-A); 7.87 (s, 1H, H8-G); 8.24 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.53 (d, $J_{C,P}$ = 4.2, C5′-A); 68.66 (d, $J_{C,P}$ = 5.2, C5′-G); 73.03 (d, $J_{C,P}$ = 5.6, C3′-A); 73.86 (C3′-G); 76.84 (C2′-A); 77.74 (C2′-G); 82.62 (t, $J_{C,P}$ = 11.1, C4′-A); 85.96 (d, $J_{C,P}$ = 9.8, C4′-G); 88.78 (d, $J_{C,P}$ = 9.8, C1′-G); 92.20 (C1′-A); 103.70 (C5-A); 112.08 (C4a-A); 120.00 (C5-G); 123.16 (C6-A); 128.07 (C3-thienyl); 128.26 (C5-thienyl); 130.70 (C4-thienyl); 137.46 (C2-thienyl); 142.56 (C8-G); 151.16 (C7a-A); 154.12 (C2-A); 154.69 (C4-G); 155.97 (C2-G); 160.00 (C4-A); 161.29 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.24 and −0.93. ESI MS m/z (rel. %): 376 (100) $[M-2H]^{2-}$, 387 (4) $[M-H + Na]^{2-}$, 754 (4) $[M-H]^-$, 776 (10) $[M-2H + Na]^-$. HR MS (ESI): for $C_{25}H_{26}O_{13}N_9P_2S$ $[M-H]^-$, calcd 754.08515; found, 754.08502.

*Cyclo-4-amino-5-(benzofuran-2-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5p**).* CDN **5p** was prepared as described for **5g** from iodinated CDN **5a** (15 mg, 17.8 μmol) and benzofuran-2-ylboronic acid (14.4 mg, 88.9 μmol). After two HPLC purifications (5−25% MeCN in 0.1 M TEAB and 5−50% MeOH in 0.1 M TEAB) and conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle), CDN **5p** (8.1 mg, 55%) was obtained as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.07 (ddd, 1H, $J_{5'b,4'}$ = 1.8, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.4, H5′b-G); 4.20 (ddd, 1H, $J_{5'a,4'}$ = 2.9, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 4.2, H5′a-G); 4.35 (ddd, 1H, $J_{5'b,4'}$ = 1.0, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.6, H5′b-A); 4.38 (ddd, 1H, $J_{4',5'a}$ = 2.9, $J_{4',5'b}$ = 1.8, $J_{4',P}$ = 3.6, H4′-G); 4.51 (dm, 1H, $J_{4',3'}$ = 9.5, H4′-A); 4.59 (d, 1H, $J_{2',3'}$ = 3.8, H2′-A); 4.65 (bdd, 1H, $J_{5'a,4'}$ = 2.0, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ < 1, H5′a-A); 4.67 (d, 1H, $J_{3',4'}$ = 3.8, H3′-G); 4.93 (ddd, 1H, $J_{3',2'}$ = 3.8, $J_{3',4'}$ = 9.5, $J_{3',P}$ = 6.5, H3′-A); 5.62 (um, H2′-G); 5.90 (d,

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1H, $J_{1',2'}$ = 8.5, H1′-G); 6.18 (s, 1H, H1′-A); 6.65 (d, 1H, $J_{3,4}$ = 3.8, H3-benzofuryl); 7.22 (td, 1H, $J_{5,6}$ = 6.8, $J_{5,4}$ = 6.8, $J_{5,7}$ = 1.3, H5-benzofuryl); 7.24 (ddd, 1H, $J_{6,5}$ = 6.8, $J_{6,7}$ = 7.1, $J_{6,4}$ = 1.6, H6-benzofuryl); 7.42 (m, 2H, H4-benzofuryl + H7-benzofuryl); 7.47 (s, 1H, H8-G); 8.09 (s, 1H, H2-A); 8.10 (s, 1H, H6-A). ^{13}C NMR (150.9 MHz, D_2O): 65.53 (C5′-A); 68.59 (d, $J_{C,P}$ = 3.7, C5′-G); 72.68 (d, $J_{C,P}$ = 4.3, C3′-A); 73.74 (C3′-G); 78.30 (d, $J_{C,P}$ = 5.4, C2′-G); 78.86 (C2′-A); 82.33 (t, $J_{C,P1}$ = $J_{C,P2}$ = 10.6, C4′-A); 85.83 (d, $J_{C,P}$ = 6.3, C4′-G); 88.02 (d, $J_{C,P}$ = 12.0, C1′-G); 92.25 (C1′-A); 102.44 (C4a-A); 102.51 (C3-benzofuryl); 108.58 (C5-A); 113.48 (C7-benzofuryl); 119.31 (C5-G); 122.77 (C6-A); 123.46 (C4-benzofuryl); 125.99 (C5-benzofuryl); 126.32 (C6-benzofuryl); 131.49 (C3a-benzofuryl); 141.30 (C8-G); 150.77 (C7a-A); 152.57 (C2-benzofuryl); 153.71 (C2-A); 154.26 (C4-G); 155.77 (C2-G); 156.02 (C7a-benzofuryl); 159.60 (C4-A); 160.54 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.10 and −1.10. ESI MS m/z (rel. %): 393 (100) $[M-2H]^{2-}$, 788 (44) $[M-H]^-$, 810 (21) $[M-2H + Na]^-$. HR MS (ESI): for $C_{29}H_{28}O_{14}N_9P_2$ $[M-H]^-$, calcd 788.12364; found, 788.12413.

*Cyclo-4-amino-5-(benzothiophen-2-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5q**).* CDN **5q** was prepared as described for **5g** from iodinated CDN **5a** (12 mg, 14.2 μmol) and benzothiophen-2-ylboronic acid (12.6 mg, 71.1 μmol). After two HPLC purifications (5−25% MeCN in 0.1 M TEAB and 9−24% MeCN in 0.1 M TEAB) and conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle), CDN **5q** (4.8 mg, 40%) was obtained as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.12 (ddd, 1H, $J_{5'b,4'}$ = 1.6, $J_{5'b,5'a}$ = 11.8, $J_{5'b,P}$ = 2.5, H5′b-G); 4.25 (ddd, 1H, $J_{5'a,4'}$ = 2.6, $J_{5'a,5'b}$ = 11.8, $J_{5'a,P}$ = 4.4, H5′a-G); 4.35 (ddd, 1H, $J_{5'b,4'}$ ∼ 1.0, $J_{5'b,5'a}$ = 12.0, $J_{5'b,P}$ = 2.5, H5′b-A); 4.44 (ddd, 1H, $J_{4',5'a}$ = 2.6, $J_{4',5'b}$ = 1.6, $J_{4',P}$ = 3.8, H4′-G); 4.52 (dm, 1H, $J_{4',3'}$ = 9.3, H4′-A); 4.57 (dm, 1H, $J_{5'a,5'b}$ = 12.0, H5′a-A); 4.71 (d, 1H, $J_{3',4'}$ = 4.0, H3′-G); 4.77 (overlap, H2′-A); 4.95 (ddd, 1H, $J_{3',2'}$ = 4.0, $J_{3',4'}$ = 9.3, $J_{3',P}$ = 6.7, H3′-A); 5.60 (um, 1H, H2′-G); 6.03 (d, 1H, $J_{1',2'}$ = 8.7, H1′-G); 6.30 (s, 1H, H1′-A); 7.10 (s, 1H, H3-benzothienyl); 7.36 (ddd, 1H, $J_{6,4}$ = 1.3, $J_{6,5}$ = 7.0, $J_{6,7}$ = 8.0, H6-benzothienyl); 7.41 (ddd, 1H, $J_{5,4}$ = 8.0, $J_{5,6}$ = 7.0, $J_{5,7}$ = 1.2, H5-benzothienyl); 7.72 (dd, 1H, $J_{4,5}$ = 8.0, $J_{4,6}$ = 1.3, H4-benzothienyl); 7.75 (s, 1H, H6-A); 7.78 (s, 1H, H8-G); 7.82 (dd, 1H, $J_{7,6}$ = 8.0, $J_{7,5}$ = 1.2, H7-benzothienyl); 8.23 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.61 (b, C5′-A); 68.75 (d, $J_{C,P}$ = 4.3, C5′-G); 72.96 (d, $J_{C,P}$ = 5.4, C3′-A); 73.90 (C3′-G); 76.89 (C2′-A); 78.91 (C2′-G); 82.52 (t, $J_{C,P}$ = 11.3, C4′-A); 86.08 (d, $J_{C,P}$ = 9.6, C4′-G); 87.84 (C1′-G); 92.23 (C1′-A); 103.70 (C5-A); 112.55 (C4a-A); 119.66 (C5-G); 123.70 (C6-A); 124.04 (C3-benzothienyl); 124.89 (C7-benzothienyl); 126.22 (C4-benzothienyl); 126.99 (C6-benzothienyl); 127.37 (C6-benzothienyl); 141.14 (C8-G); 138.36 (C2-benzothienyl); 141.78 (C7a-benzothienyl); 142.94 (C3a-benzothienyl); 151.46 (C7a-A); 154.26 (C2-A); 154.59 (C4-G); 156.00 (C2-G); 160.18 (C4-A); 160.87 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.16 and −0.88. ESI MS m/z (rel. %): 436 (100) $[M-2H]^{2-}$, 874 (16) $[M-H]^-$, 896 (14) $[M-2H + Na]^-$. HR MS (ESI): for $C_{29}H_{28}O_{14}N_9P_2$ $[M-H]^-$, calcd 874.17568; found, 874.17462.

*Cyclo-4-amino-5-(dibenzo[b,d]furan-4-yl)-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (2′ → 5′) Sodium Salt (**5r**) and Open Isomer **16**.* CDN **5r** was prepared as described for **5g** from iodinated CDN **5a** (15 mg, 17.8 μmol) and dibenzofuran-4-ylboronic acid (19 mg, 89.6 μmol). After two purifications (5−25% MeCN in 0.1 M TEAB) and conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle), CDN **5r** (6.3 mg, 40%) was obtained as a white lyophilizate (water). As a byproduct, compound **16** was isolated and converted to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle). Open isomer **16** (2.0 mg, 13%) was obtained as a white lyophilizate (water). CDN **5r**: ^{1}H NMR (600 MHz, D_2O): 4.10 (ddd, 1H, $J_{5'b,4'}$ = 2.1, $J_{5'b,5'a}$ = 11.9, $J_{5'b,P}$ = 2.4, H5′b-G); 4.22 (ddd, 1H, $J_{5'a,4'}$ = 2.5, $J_{5'a,5'b}$ = 11.9, $J_{5'a,P}$ = 5.8, H5′a-G); 4.33 (m, 1H, H5′b-A); 4.38 (ddd, 1H, $J_{4',5'a}$ = 2.5, $J_{4',5'b}$ = 2.1, $J_{4',P}$ = 3.4, H4′-G); 4.48 (m, 1H, H5′a-A); 4.49 (dm, 1H, $J_{4',3'}$ = 9.0, H4′-A); 4.64 (d, 1H, $J_{3',4'}$ = 3.5, H3′-G); 4.85 (d, 1H, $J_{2',3'}$ = 4.6, H2′-A); 5.10 (ddd, 1H, $J_{3',2'}$ = 4.6, $J_{3',4'}$ = 9.0, $J_{3',P}$ = 6.2, H3′-A); 5.84 (ddd, 1H, $J_{2',1'}$ = 8.6, $J_{2',3'}$ = 3.5, $J_{2',P}$ = 3.7, H2′-G); 5.92 (d, 1H, $J_{1',2'}$ = 8.6, H1′-G); 6.40 (s, 1H, H1′-A); 7.04 (dd, 1H, $J_{3,2}$ = 7.5, $J_{3,1}$ = 1.3, H3-dibenzofuryl); 7.14 (dd, 1H, $J_{2,3}$ = 7.5, $J_{2,1}$ = 7.8, H2-dibenzofuryl); 7.40 (td, 1H, $J_{8,9}$ = 7.4, $J_{8,7}$ = 7.4, $J_{8,6}$ = 1.2, H8-dibenzofuryl); 7.44 (ddd, 1H, $J_{7,6}$ = 8.2, $J_{7,8}$ = 7.4, $J_{7,9}$ = 1.5, H7-dibenzofuryl); 7.49 (s, 1H, H6-A); 7.59 (ddd, 1H, $J_{6,7}$ = 8.2, $J_{6,8}$ = 1.2, $J_{6,9}$ = 0.7, H6-dibenzofuryl-A); 7.68 (s, 1H, H8-G); 7.76 (dd, 1H, $J_{1,2}$ = 7.8, $J_{1,3}$ = 1.3, H1-dibenzofuryl); 8.00 (ddd, 1H, $J_{9,8}$ = 7.4, $J_{9,7}$ = 1.5, $J_{9,6}$ = 0.7, H9-dibenzofuryl); 8.19 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 65.59 (d, $J_{C,P}$ = 4.9, C5′-A); 68.80 (d, $J_{C,P}$ = 4.9, C5′-G); 73.79 (d, $J_{C,P}$ = 5.5, C3′-A); 74.22 (C3′-G); 77.14 (C2′-A); 77.50 (d, $J_{C,P}$ = 4.5, C2′-G); 82.84 (t, $J_{C,P1}$ = $J_{C,P2}$ = 11.2, C4′-A); 85.76 (d, $J_{C,P}$ = 9.5, C4′-G); 88.79 (d, $J_{C,P}$ = 12.3, C1′-G); 91.31 (C1′-A); 104.21 (C4a-A); 113.84 (C5-A); 114.61 (C6-dibenzofuryl); 119.57 (C4-dibenzofuryl); 119.60 (C5-G); 122.52 (C1-dibenzofuryl); 123.72 (C9-dibenzofuryl); 124.05 (C6-A); 125.81 (C8-dibenzofuryl); 126.08 (C2-dibenzofuryl); 126.44 (C9a-dibenzofuryl); 127.01 (C9b-dibenzofuryl); 130.15 (C7-dibenzofuryl); 130.40 (C3-dibenzofuryl); 142.45 (C8-G); 151.79 (C7a-A); 154.22 (C4-G); 154.27 (C2-A); 155.08 (C4a-dibenzofuryl); 155.88 (C2-G); 158.29 (C5a-dibenzofuryl); 160.13 (C4-A); 160.59 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.19 and −0.44. ESI MS m/z (rel. %): 418 (100) $[M-2H]^{2-}$, 838 (28) $[M-H]^-$, 860 (13) $[M-2H + Na]^-$. HR MS (ESI): for $C_{33}H_{30}O_{14}N_9P_2$ $[M-H]^-$, calcd 838.13929; found, 838.13778. Open isomer **16**: ^{1}H NMR (600 MHz, D_2O): 3.34 (dd, 1H, $J_{5'b,4'}$ = 4.5, $J_{5'b,5'a}$ = 12.8, H5′b-G); 3.46 (dd, 1H, $J_{5'a,4'}$ = 2.5, $J_{5'a,5'b}$ = 12.8, H5′a-G); 3.50 (dd, 1H, $J_{3',2'}$ = 4.6, $J_{3',4'}$ = 7.8, H3′-G); 3.76 (ddd, 1H, $J_{4',3'}$ = 7.8, $J_{4',5'a}$ = 2.5, $J_{4',5'b}$ = 4.5, H4′-G); 4.12 (ddd, 1H, $J_{5'b,4'}$ = 2.6, $J_{5'b,5'a}$ = 11.7, $J_{5'b,P}$ = 4.2, H5′b-A); 4.19 (ddd, 1H, $J_{5'a,4'}$ = 2.8, $J_{5'a,5'b}$ = 11.7, $J_{5'a,P}$ = 5.1, H5′a-A); 4.42 (ddd, 1H, $J_{2',1'}$ = 2.1, $J_{2',3'}$ = 4.6, $J_{2',P}$ = 7.4, H2′-G); 4.58 (m, 1H, $J_{4',3'}$ = 3.2, $J_{4',5'a}$ = 2.8, $J_{4',5'b}$ = 2.6, $J_{4',P}$ = 2.5, H4′-A); 5.19 (td, 1H, $J_{3',2'}$ = 6.6, $J_{3',4'}$ = 3.2, $J_{3',P}$ = 6.6, H3′-A); 5.42 (ddd, 1H, $J_{2',1'}$ = 4.3, $J_{2',3'}$ = 6.6, $J_{2',P}$ = 11.6, H2′-A); 5.60 (d, 1H, $J_{1',2'}$ = 2.1, H1′-G); 6.34 (d, 1H, $J_{1',2'}$ = 4.3, H1′-A); 7.00 (dd, 1H, $J_{3,2}$ = 7.4, $J_{3,1}$ = 1.3, H3-dibenzofuryl); 7.25 (dd, 1H, $J_{2,3}$ = 7.4, $J_{2,1}$ = 7.7, H2-dibenzofuryl); 7.39 (ddd, 1H, $J_{8,9}$ = 7.7, $J_{8,7}$ = 7.2, $J_{8,6}$ = 1.0, H8-dibenzofuryl); 7.47 (s, 1H, H8-G); 7.49 (s, 1H, H6-A); 7.50 (ddd, 1H, $J_{7,6}$ = 8.2, $J_{7,8}$ = 7.2, $J_{7,9}$ = 1.3, H7-dibenzofuryl); 7.56 (ddd, 1H, $J_{6,7}$ = 8.2, $J_{6,8}$ = 1.0, $J_{6,9}$ = 0.7, H6-dibenzofuryl); 7.74 (dd, 1H, $J_{1,2}$ = 7.7, $J_{1,3}$ = 1.3, H1-dibenzofuryl); 7.92 (ddd, 1H, $J_{9,8}$ = 7.7, $J_{9,7}$ = 1.3, $J_{9,6}$ = 0.7, H9-dibenzofuryl); 8.01 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 62.76 (C5′-G); 67.72 (d, $J_{C,P}$ = 4.1, C5′-A); 71.28 (C3′-G); 79.98 (d, $J_{C,P}$ = 5.4, C2′-G); 80.78 (d, $J_{C,P}$ = 1.5, C3′-A); 84.00 (d, $J_{C,P}$ = 1.1, C2′-A); 85.52 (C4′-G); 86.02 (d, $J_{C,P}$ = 13.6, C4′-A); 89.95 (d, $J_{C,P}$ = 6.7, C1′-G); 90.99 (d, $J_{C,P}$ = 2.8, C1′-A); 103.36 (C4a-A); 114.12 (C5-A); 114.35 (C6-dibenzofuryl); 118.64 (C5-G); 119.57 (C4-dibenzofuryl); 122.29 (C1-dibenzofuryl); 123.51 (C9-dibenzofuryl); 124.43 (C6-A); 125.85 (C8-dibenzofuryl); 126.16 (C2-dibenzofuryl); 126.20 (C9a-dibenzofuryl); 127.03 (C9b-dibenzofuryl); 129.87 (C3-dibenzofuryl); 130.34 (C7-dibenzofuryl); 139.26 (C8-G); 152.46 (C7a-A); 152.86 (C4-G); 154.06 (C2-A); 154.96 (C4a-dibenzofuryl); 155.58 (C2-G); 158.12 (C5a-dibenzofuryl); 159.44 (C4-A); 160.77 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): 0.66 and 20.92. ESI MS m/z (rel. %): 418 (100) $[M-2H]^{2-}$, 838 (12) $[M-H]^-$, 860 (17) $[M-2H + Na]^-$, 882 (4) $[M-3H+2Na]^-$ HR MS (ESI): for $C_{33}H_{30}O_{14}N_9P_2$ $[M-H]^-$, calcd 838.13929; found, 838.13853.

*Cyclo-4-amino-5-phenyl-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) 2-Amino-5-phenyl-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidin-4(3H)-one 5′-O-phosphate (2′ → 5′) Sodium Salt (**6f**).* NTPs **2f** (2 μmol) and **3f** (2 μmol) were enzymatically cyclized using mcGAS. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **6f** (128 nmol, 6%). ^{1}H NMR (600 MHz, D_2O): 4.14 (ddd, 1H, $J_{5'b,4'}$ = 1.2, $J_{5'b,5'a}$ = 11.6, $J_{5'b,P}$ = 3.0, H5′b-G); 4.26 (ddd, 1H, $J_{5'a,4'}$ = 2.1, $J_{5'a,5'b}$ = 11.6, $J_{5'a,P}$ = 2.7, H5′a-G); 4.36 (ddd, 1H, $J_{5'b,4'}$ = 1.2, $J_{5'b,5'a}$ = 12.2, $J_{5'b,P}$ = 3.2, H5′b-A); 4.46 (m, 1H, H4′-G); 4.475 (dm, 1H, $J_{4',3'}$ = 9.4, H4′-A); 4.52 (d, $J_{2',3'}$ = 4.2, H2′-A); 4.53

(m, 1H, H5′a-A); 4.76 (d, 1H, $J_{3',4'}$ = 3.8, H3′-G); 4.99 (ddd, 1H, $J_{3',2'}$ = 4.2, $J_{3',4'}$ = 9.4, $J_{3',P}$ = 7.2, H3′-A); 5.28 (um, 1H, H2′-G); 6.22 (s, 1H, H1′-A); 6.34 (d, 1H, $J_{1',2'}$ = 8.4, H1′-G); 6.98 (m, 3H, *m*- and *p*-Ph-G); 7.11 (m, 2H, *o*-Ph-A); 7.26 (m, 3H, *m*- and *p*-Ph-A); 7.31 (m, 2H, *o*-Ph-G); 7.37 (s, 1H, H6-G); 7.54 (s, 1H, H6-A); 7.98 (s, 1H, H-2-A). ^{13}C NMR (150.9 MHz, D_2O): 66.04 (C5′-A); 69.04 (C5′-G); 73.11 (C3′-A); 74.29 (C3′-G); 77.29 (C2′-A); 81.50 (C2′-G); 82.55 (C4′-A); 85.90 (C1′-G); 86.40 (C4′-G); 92.42 (C1′-A); 104.12 (C5-A); 117.34 (C6-G); 119.53 (C4a-A); 122.67 (C6-A); 123.20 (*p*-Ph-G); 124.93 (C4a-G); 129.29 (*o*-Ph-G); 129.85 (*o*-Ph-A); 130.10 (*m*-Ph-G); 131.27 (*m*-Ph and *p*-Ph-A); 151.02 (C7a-A); 153.39 (C2-A); 155.51 (C7a-G); 160.00 (C4-A); 163.10 (C4-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): 0.28 and −1.33. ESI MS *m*/*z* (rel. %): 411 (100) $[M-2H]^{2-}$, 422 (13) $[M-3H + Na]^{2-}$, 823 (11) $[M-H]^-$, 845 (19) $[M-2H + Na]^-$. HR MS (ESI): for $C_{34}H_{33}N_8O_{13}P_2$ $[M-H]^-$, calcd 823.16478; found, 823.16454.

*Cyclo-4-amino-5-iodo-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]-pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (3′ → 5′) Sodium Salt (**7a**).* NTP **3a** (13 μmol) and GTP (13 μmol) were enzymatically cyclized using DncV. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **7a** (5.3 mg, 48%) as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.11 (m, H5′b-A); 4.12 (m, 1H, H5′b-G); 4.36 (m, 1H, H5′a-A); 4.39 (m, 1H, H4′-A); 4.42 (m, 2H, H4′-G + H5′a-G); 4.55 (dd, 1H, $J_{2',1'}$ = 1.0, $J_{2',3'}$ = 4.6, H2′-A); 4.73 (overlap water, H3′-A); 4.79 (overlap water, H2′-G); 4.85 (m, 1H, H3′-G); 6.01 (d, 1H, $J_{1',2'}$ = 1.0, H1′-G); 6.24 (d, 1H, $J_{1',2'}$ = 1.0, H1′-A); 7.75 (s, 1H, H6-A); 8.05 (s, 1H, H8-G); 8.10 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 53.44 (C5-A); 64.77 (d, $J_{C,P}$ = 5.0, C5′-A); 64.92 (d, $J_{C,P}$ = 4.9, C5′-G); 73.32 (d, $J_{C,P}$ = 4.8, C3′-A); 73.34 (d, $J_{C,P}$ = 4.8, C3′-G); 76.57 (C2′-A); 76.74 (C2′-G); 81.85 (t, $J_{C,P1}$ = $J_{C,P2}$ = 10.4, C4′-A); 82.43 (t, $J_{C,P1}$ = $J_{C,P2}$ = 10.8, C4′-G); 91.90 (C1′-A); 92.86 (d, $J_{C,P}$ = 12.0, C1′-G); 106.75 (C4a-A); 119.66 (C5-G); 129.22 (C6-A); 139.65 (C8-G); 151.10 (C7a-A); 153.43 (C4-G); 154.33 (C2-A); 158.62 (C2-G); 159.91 (C4-A); 161.62 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.56 and −0.89. ESI MS *m*/*z* (rel. %): 398 (100) $[M-2H]^{2-}$, 798 (45) $[M-H]^-$, 820 (27) $[M-2H + Na]^-$. HR MS (ESI): for $C_{21}H_{22}O_{13}N_9IP_2$ $[M-2H]^{2-}$, calcd 398.49340; found, 398.49324.

*Cyclo-4-amino-5-phenyl-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]-pyrimidine 5′-O-phosphate (3′ → 5′) Guanosine 5′-O-phosphate (3′ → 5′) Sodium Salt (**7f**).* CDN **7f** was prepared as described for **5g** from iodinated CDN **7a** (13 mg, 15.4 μmol) and phenylboronic acid (9.4 mg, 77.1 μmol). The final product was purified using HPLC (0−15% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **7f** (8.1 mg, 66%) as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.10 (ddd, 1H, $J_{5'b,4'}$ = 0.9, $J_{5'b,5'a}$ = 12.0, $J_{5'b,P}$ = 4.4, H5′b-A); 4.13 (dd, 1H, $J_{5'b,5'a}$ = 11.0, $J_{5'b,P}$ = 4.3, H5′b-G); 4.37 (bdd, 1H, $J_{5'a,4'}$ = 2.5, $J_{5'a,5'b}$ = 12.0, $J_{5'a,P}$ = <1, H5′a-A); 4.41 (m, 1H, H4′-A); 4.43 (m, 1H, H4′-G); 4.44 (dd, 1H, $J_{5'a,5'b}$ = 11.0, $J_{5'a,P}$ = 2.8, H5′a-G); 4.52 (d, 1H, $J_{2',3'}$ = 4.4, H2′-G); 4.61 (d, 1H, $J_{2',3'}$ = 4.4, H2′-A); 4.78 (overlap, H3′-A); 4.80 (overlap, 1H, H3′-G); 5.98 (s, 1H, H1′-G); 6.42 (s, 1H, H1′-A); 7.18 (m, 3H, 2× *m*-Ph + *p*-Ph); 7.28 (m, 2H, 2× *o*-Ph); 7.47 (s, 1H, H6-A); 8.03 (s, 1H, H8-G); 8.18 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 64.73 (d, $J_{C,P}$ = 5.9, C5′-A); 64.77 (d, $J_{C,P}$ = 4.7, C5′-G); 72.94 (d, $J_{C,P}$ = 4.8, C3′-A); 73.55 (d, $J_{C,P}$ = 5.0, C3′-G); 76.74 (C2′-A); 76.86 (C2′-G); 81.62 (t, $J_{C,P1}$ = $J_{C,P2}$ = 10.8, C4′-A); 82.11 (t, $J_{C,P}$ = 11.5, C4′-G); 91.51 (C1′-A); 92.46 (C1′-G); 103.77 (C4a-A); 119.48 (C5-G); 120.80 (C5-A); 122.27 (C6-A); 129.37 (*p*-Ph); 130.68 (2× *o*-Ph); 131.16 (2× *m*-Ph); 135.81 (*i*-Ph); 138.74 (C8-G); 152.17 (C7a-A); 152.78 (C4-G); 154.17 (C2-A); 156.67 (C2-G); 160.35 (C4-A); 161.40 (C6-G). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.36 and −0.75. ESI MS *m*/*z* (rel. %): 373 (100) $[M-2H]^{2-}$, 748 (6) $[M-H]^-$, 770 (5) $[M-2H + Na]^-$. HR MS (ESI): for $C_{27}H_{28}O_{13}N_9P_2$ $[M-H]^-$, calcd 748.12873; found, 748.12726.

*Cyclic-di-4-amino-5-iodo-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]-pyrimidine 5′-O-phosphate (3′ → 5′) Sodium Salt (**8a**).* NTP **3a** (26 μmol) was enzymatically cyclized using DisA. The final product was purified by HPLC (5−30% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **8a** (7.0 mg, 57%) as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.15 (dd, 1H, $J_{5'b,5'a}$ = 11.7, $J_{5'b,P}$ = 5.1, H5′b-A); 4.42 (dt, 1H, $J_{4',3'}$ = 9.8, $J_{4',5'a}$ = 2.7, $J_{3',P}$ = 2.8, H4′-A); 4.47 (bdd, 1H, $J_{5'a,4'}$ = 2.7, $J_{5'a,5'b}$ = 11.7, $J_{5'a,P}$ < 1, H5′a-A); 4.60 (ddd, 1H, $J_{3',2'}$ = 4.0, $J_{3',4'}$ = 9.8, $J_{3',P}$ = 6.2, H3′-A); 4.69 (d, 1H, $J_{2',3'}$ = 4.0, H2′-A); 6.28 (s, 1H, H1′-A); 7.91 (s, 1H, H6-A); 8.12 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 52.38 (C5-A); 64.69 (d, $J_{C,P}$ = 5.1, C5′-A); 73.45 (d, $J_{C,P}$ = 5.3, C3′-A); 77.15 (C2′-A); 81.80 (t, $J_{C,P1}$ = $J_{C,P2}$ = 10.9, C4′-A); 92.67 (C1′-A); 107.78 (C4a-A); 128.45 (C6-A); 150.57 (C7a-A); 154.11 (C2-A); 160.03 (C4-A). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −2.00. ESI MS *m*/*z* (rel. %): 452 (100) $[M-2H]^{2-}$, 906 (28) $[M-H]^-$, 928 (35) $[M-2H + Na]^-$. HR MS (ESI): for $C_{22}H_{23}O_{12}N_8I_2P_2$ $[M-H]^-$, calcd 906.90055; found, 906.90021.

*Cyclic-di-4-amino-5-phenyl-7-β-D-ribofuranosyl-7H-pyrrolo[2,3-d]pyrimidine 5′-O-phosphate (3′ → 5′) Sodium Salt (**8f**).* CDN **8a** (15 mg, 15.8 μmol), phenylboronic acid (19.2 mg, 157.5 μmol), and cesium carbonate (30.8 mg, 94.5 μmol) were mixed with MeCN−H_2O (1:2 v/v, 1.065 mL) in an argon-purged vial. In a separate vial, $Pd(OAc)_2$ (1.0 mg, 4.45 μmol) and TPPTS (12.6 mg, 22.2 μmol) were dissolved in MeCN−H_2O (1:2 v/v, 1.0 mL), and the solution was sonicated under argon atmosphere for 30 s. Then, 353 μmol of this solution was transferred into the mixture containing CDN **8a**, and the reaction was stirred at 100 °C for 30 min. Then, the reaction mixture was cooled to rt, diluted with water to approx. 3 mL, and filtered through a 5 μm nylon syringe filter. The filtrate was directly applied on HPLC for purification (0−17.5% MeCN in 0.1 M TEAB). The conversion to a sodium salt form on a Dowex 50WX8 (in a Na^+ cycle) provided CDN **8f** (10.5 mg, 78%) as a white lyophilizate (water). ^{1}H NMR (600 MHz, D_2O): 4.18 (ddd, 1H, $J_{5'b,5'a}$ = 11.3, $J_{5'b,4'}$ = 1.5, $J_{5'b,P}$ = 4.8, H5′b-A); 4.28 (d, 1H, $J_{2',3'}$ = 3.9, H2′-A); 4.45 (m, 2H, H4′-A + H5′a-A); 4.79 (overlap, H3′-A); 6.37 (s, 1H, H1′-A); 6.90 (m, 4H, *o*-Ph + *m*-Ph); 7.04 (m, 1H, *p*-Ph); 7.61 (s, 1H, H6-A); 8.16 (s, 1H, H2-A). ^{13}C NMR (150.9 MHz, D_2O): 64.78 (d, $J_{C,P}$ = 5.0, C5′-A); 73.41 (d, $J_{C,P}$ = 5.1, C3′-A); 77.32 (C2′-A); 81.62 (t, $J_{C,P}$ = 10.9, C4′-A); 91.84 (C1′-A); 103.96 (C4a-A); 120.61 (C5-A); 121.74 (C6-A); 129.62 (*p*-Ph); 130.32 (*o*-Ph); 130.85 (*m*-Ph); 135.28 (*i*-Ph); 151.20 (C7a-A); 153.87 (C2-A); 159.85 (C4-A). ^{31}P NMR (^{1}H-dec, 202.4 MHz, D_2O): −0.84. ESI MS *m*/*z* (rel. %): 403 (100) $[M-2H]^{2-}$, 807 (3) $[M-H]^-$, 829 (4) $[M-2H + Na]^-$. HR MS (ESI): for $C_{34}H_{33}O_{12}N_8P_2$ $[M-H]^-$, calcd 807.16986; found, 807.16888.

*4-Benzamido-5-iodo-7-[5-O-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-7H-pyrrolo[2,3-d]pyrimidine (**10**).* 7-Iodo-7-deazaadenosine (**9**, ref 55, 500 mg, 1.28 mmol) was co-evaporated with anhydrous pyridine (2 × 3 mL) and suspended in anhydrous pyridine (5 mL). The suspension was cooled to 0 °C, and trimethylsilyl chloride (728 μL, 5.74 mmol) was added dropwise over a period of 30 min. Then, benzoyl chloride (222 μL, 1.91 mmol) was added, and the mixture was stirred at rt overnight. The reaction mixture was then cooled to 0 °C, and water (1.25 mL) was added. After stirring for 15 min, aqueous ammonia (25%, 2.5 mL) was added, and the stirring was continued for another 30 min. Solvents were then evaporated, and the residue was co-evaporated with anhydrous pyridine (2 × 3 mL) and dissolved in anhydrous pyridine (5 mL). DMTrCl (432 mg, 1.25 mmol) was added in two portions over 1 h, and the reaction mixture was stirred overnight. Then, DMTrCl (432 mg, 1.25 mmol) was added again in two portions over 1 h, and the reaction mixture was stirred for another 1.5 h. The solvent was removed in vacuo, the residue was suspended in DCM (35 mL), and the solid phase was filtered off through a pad of Celite. The filtrate was washed with saturated aqueous $NaHCO_3$, dried over magnesium sulfate, and evaporated. Flash chromatography on silica (0−10% MeOH in DCM + 1% Et_3N) provided nucleoside **10** (689 mg, 68%) as a white amorphous solid. ^{1}H NMR (500.0 MHz, CD_3OD): 3.39 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 2.9, H-5′b); 3.42 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 3.7, H-5′a); 3.757, 3.760 (2 × s, 2 × 3H, CH_3O-DMTr); 4.19 (ddd, 1H, $J_{4',3'}$ = 4.1, $J_{4',5a'}$ = 3.7, $J_{4',5b'}$ = 2.9, H-4′); 4.46 (dd, 1H, $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 4.1, H-3′); 4.72 (dd, 1H, $J_{2',1'}$ = 5.4, $J_{2',3'}$ = 5.0, H-2′); 6.37 (d, 1H,

$J_{1',2'}$ = 5.4, H-1′); 6.81−6.88 (m, 4H, H-*m*-C_6H_4-DMTr); 7.21 (m, 1H, H-*p*-C_6H_5-DMTr); 7.25−7.32 (m, 2H, H-*m*-C_6H_5-DMTr); 7.30−7.37 (m, 8H, H-*o*-C_6H_4-DMTr); 7.42−7.48 (m, 2H, H-*o*-C_6H_5-DMTr); 7.54−7.59 (m, 2H, H-*m*-Bz); 7.64 (m, 1H, H-*p*-Bz); 7.86 (s, 1H, H-6); 8.08−8.12 (m, 2H, H-*o*-Bz); 8.65 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): 52.84 (C-5); 55.75, 55.76 (CH_3O-DMTr); 64.74 (CH_2-5′); 72.42 (CH-3′); 76.35 (CH-2′); 85.43 (CH-4′); 88.01 (C-DMTr); 89.41 (CH-1′); 114.23 (CH-*m*-C_6H_4-DMTr); 114.68 (C-4a); 127.99 (CH-*p*-C_6H_5-DMTr); 128.96 (CH-*m*-C_6H_5-DMTr); 129.23 (CH-*o*-Bz); 129.41 (CH-*o*-C_6H_5-DMTr); 129.90 (CH-*m*-Bz); 131.29, 131.40 (CH-*o*-C_6H_4-DMTr); 133.30 (CH-6); 133.78 (CH-*p*-Bz); 134.94 (C-*i*-Bz); 136.87, 137.15 (C-*i*-C_6H_4-DMTr); 146.04 (C-*i*-C_6H_5-DMTr); 152.24 (CH-2); 152.41 (C-4); 153.87 (C-7a); 160.17, 160.19 (C-*p*-C_6H_4-DMTr); 169.21 (C-*i*-Bz). ESI MS *m/z* (rel. %): 799 (23) [M + H]$^+$, 821 (100) [M + Na]$^+$, 837 (9) [M + K]$^+$. HR MS (ESI): for $C_{39}H_{36}O_7N_4I$ [M + H]$^+$, calcd 799.16232; found, 799.16154.

4-Benzamido-5-iodo-7-[2-O-tert-butyldimethylsilyl-5-O-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-7H-pyrrolo[2,3-d]pyrimidine (11). Nucleoside **10** (555 mg, 0.70 mmol) was co-evaporated with anhydrous pyridine (2 × 5 mL) and dissolved in anhydrous THF (10 mL). Anhydrous pyridine (485 μL) and silver nitrate (195 mg, 1.15 mmol) were added. The reaction flask was covered with aluminum foil, and the mixture was stirred at rt for 15 min. Then, TBDMSCl (183 mg, 1.21 mmol) was added, and the mixture was stirred overnight in the dark. The reaction mixture was then filtered through a pad of Celite. The filtration pad was washed with DCM, and the filtrate was evaporated. Flash chromatography on silica (5−50% EtOAc + 1% Et_3N in cyclohexane) provided nucleoside **11** (251 mg, 40%) as a white foam. ^{1}H NMR (500.0 MHz, CD_3OD): −0.13, 0.01 (2 × s, 2 × 3H, CH_3Si); 0.81 (s, 9H, $(CH_3)_3C$); 3.43 (dd, 1H, J_{gem} = 10.7, $J_{5'b,4'}$ = 3.0, H-5′b); 3.46 (dd, 1H, J_{gem} = 10.7, $J_{5'a,4'}$ = 2.7, H-5′a); 3.779, 3.782 (2 × s, 2 × 3H, CH_3O-DMTr); 4.29 (ddd, 1H, $J_{4',3'}$ = 3.1, $J_{4',5'a}$ = 2.7, $J_{4',5'b}$ = 3.0, H-4′); 4.38 (dd, 1H, $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 3.1, H-3′); 4.80 (dd, 1H, $J_{2',1'}$ = 5.9, $J_{2',3'}$ = 5.0, H-2′); 6.39 (d, 1H, $J_{1',2'}$ = 5.9, H-1′); 6.85−6.90 (m, 4H, H-*m*-C_6H_4-DMTr); 7.24 (m, 1H, H-*p*-C_6H_5-DMTr); 7.28−7.33 (m, 2H, H-*m*-C_6H_5-DMTr); 7.34−7.39 (m, 8H, H-*o*-C_6H_4-DMTr); 7.45−7.49 (m, 2H, H-*o*-C_6H_5-DMTr); 7.55−7.60 (m, 2H, H-*m*-Bz); 7.66 (m, 1H, H-*p*-Bz); 7.91 (s, 1H, H-6); 8.09−8.13 (m, 2H, H-*o*-Bz); 8.65 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): −4.94, −4.68 (CH_3Si); 18.99 ($(CH_3)_3\mathbf{C}$); 26.15 ($(\mathbf{C}H_3)_3C$); 53.05 (C-5); 55.80 (CH_3O-DMTr); 64.80 (CH_2-5′); 72.98 (CH-3′); 78.48 (CH-2′); 86.02 (CH-4′); 88.27 (C-DMTr); 89.24 (CH-1′); 114.32 (CH-*m*-C_6H_4-DMTr); 114.57 (C-4a); 128.11 (CH-*p*-C_6H_5-DMTr); 129.05 (CH-*m*-C_6H_5-DMTr); 129.23 (CH-*o*-Bz); 129.35 (CH-*o*-C_6H_5-DMTr); 129.93 (CH-*m*-Bz); 131.33, 131.43 (CH-*o*-C_6H_4-DMTr); 133.16 (CH-6); 133.82 (CH-*p*-Bz); 134.94 (C-*i*-Bz); 136.74, 136.99 (C-*i*-C_6H_4-DMTr); 145.99 (C-*i*-C_6H_5-DMTr); 152.29 (CH-2); 152.55 (C-4); 153.92 (C-7a); 160.25, 160.26 (C-*p*-C_6H_4-DMTr); 169.22 (C-*i*-Bz). ESI MS *m/z* (rel. %): 913 (100) [M + H]$^+$. HR MS (ESI): for $C_{45}H_{50}O_7N_4ISi$ [M + H]$^+$, calcd 913.24880; found, 913.24853.

4-Benzamido-5-iodo-7-[2-O-tert-butyldimethylsilyl-β-D-ribofuranosyl]-7H-pyrrolo[2,3-d]pyrimidine 3′-O-Phosphonate Triethylammonium Salt (12). Nucleoside **11** (525 mg, 0.58 mmol) was co-evaporated with anhydrous pyridine (2 × 5 mL) and dissolved in anhydrous pyridine (5 mL), and diphenyl phosphite (216 μL, 1.44 mmol) was added. The reaction mixture was stirred for 1 h, and then, water (8 mL) was added. The reaction mixture was stirred for 5 min, and it was then diluted with EtOAc and washed with brine. Aqueous phase was extracted twice with EtOAc. Combined organic phases were dried over $MgSO_4$ and evaporated. The residue was dissolved in DCM (7 mL), and then, water (104 μL) and a solution of dichloroacetic acid in DCM (6%, 6.58 mL, 5.0 mmol) were added. The solution was stirred at rt for 15 min, and then, triethylsilane (919 μL, 5.75 mmol) was added. The reaction mixture was stirred for another 30 min. The reaction was quenched with pyridine (823 μL), and solvents were removed in vacuo. Flash chromatography on C18 column (gradient 5−100% MeCN in 0.1 M TEAB) provided phosphonate **12** (354 mg, 77%) as a white foam. ^{1}H NMR (500.0 MHz, CD_3OD): −0.24, 0.01 (2 × s, 2 × 3H, CH_3Si); 0.77 (s, 9H, $(CH_3)_3C$); 1.31 (t, 9H, J_{vic} = 7.3, $C\mathbf{H_3}CH_2N$); 3.20 (q, 6H, J_{vic} = 7.3, $CH_3C\mathbf{H_2}N$); 3.87 (d, 2H, $J_{5',4'}$ = 2.6, H-5′); 4.33 (q, 1H, $J_{4',3'} = J_{4',5'}$ = 2.6, H-4′); 4.73−4.79 (m, 2H, H-2′,3′); 6.33 (d, 1H, $J_{1',2'}$ = 6.1, H-1′); 6.96 (d, 1H, $J_{H,P}$ = 630.1, HP); 7.54−7.59 (m, 2H, H-*m*-Bz); 7.65 (m, 1H, H-*p*-Bz); 8.07 (s, 1H, H-6); 8.10−8.15 (m, 2H, H-*o*-Bz); 8.67 (s, 1H, H-2). ^{13}C NMR (125.7 MHz, CD_3OD): −5.22, −4.60 (CH_3Si); 9.22 ($\mathbf{C}H_3CH_2N$); 18.84 ($(CH_3)_3\mathbf{C}$); 26.13 ($(\mathbf{C}H_3)_3C$); 47.86 ($CH_3\mathbf{C}H_2N$); 52.61 (C-5); 62.66 (CH_2-5′); 75.43 (d, $J_{C,P}$ = 5.1, CH-3′); 77.09 (d, $J_{C,P}$ = 3.0, CH-2′); 87.07 (d, $J_{C,P}$ = 3.4, CH-4′); 89.77 (CH-1′); 114.84 (C-4a); 129.24 (CH-*o*-Bz); 129.89 (CH-*m*-Bz); 133.74 (CH-*p*-Bz); 133.93 (CH-6); 135.03 (C-*i*-Bz); 152.05 (CH-2); 152.68 (C-4); 153.65 (C-7a); 169.10 (C-*i*-Bz). ^{31}P{^{1}H} NMR (202.4 MHz, CD_3OD): 5.24. ESI MS *m/z* (rel. %): 673 (100) [M−H]$^-$. HR MS (ESI): for $C_{24}H_{31}O_7N_4IPSi$ [M−H]$^-$, calcd 673.07498; found, 673.07515.

Differential Scanning Fluorimetry. Measurements were performed with wt hSTING as described earlier.[47] Briefly, thermal denaturation was performed in a 20 μL mixture consisting of 0.1 mg/mL wt hSTING, 150 μM CDN, 100 mM Tris−HCl [pH 7.4], 150 mM NaCl, and 1:500 (v/v) SYPRO Orange (Thermo Fisher Scientific, USA). Measurements were taken in a 96-well format on a LightCycler480 Instrument II (Roche, Switzerland). Thermal shift (ΔT_m) for each CDN was determined by subtracting the melting temperature (T_m) of the reference sample (ligand-free STING) from the melting temperature of the STING−CDN complex.

Standard 293T Cell-Based Reporter Assay. The generation of 293T reporter cells expressing different full-length STING protein allelic variants and the assay procedure itself was described previously.[20] Briefly, the day before testing, the cells were seeded into 96-well transparent plates in a DMEM High glucose medium containing 10% (v/v) heat inactivated fetal bovine albumin (FBS). The medium was removed the next day, and the cells were incubated for 7 h (37 °C, 5% CO_2) with serially diluted compounds. After incubation, the collected cell culture medium was mixed with Bright-Glo Luciferase Assay System reagent (Promega, USA) in white 96-well plates (Corning, USA). Luminescence readout was performed on a SPARK instrument (Tecan, Austria), and the values were used to calculate EC_{50} values using nonlinear regression and software Prism (GraphPad, USA).

Digitonin 293T Cell-Based Reporter Assay. Cells were plated as described above for a standard 293T cell-based reporter assay. The following day, the medium was removed, and serially diluted compounds were incubated with cells for 30 min at 37 °C, 5% CO_2 in buffer containing 50 mM HEPES [pH 7.0], 100 mM KCl, 3 mM $MgCl_2$, 0.1 mM DTT, 85 mM sucrose, 0.2% (w/w) BSA, 1 mM ATP, 0.1 mM GTP, and 10 μg/mL digitonin A. Next, the buffer was removed, and the cells were washed with a cell culture medium twice and then incubated for 5 h (37 °C, 5% CO_2) with fresh medium. Finally, the levels of Lucia Luciferase in medium were determined using Bright Glo (Promega, USA), and EC_{50}s were calculated as described above.

PBMC Assay. Buffy coats from healthy individuals were obtained from the Institute of Hematology and Blood Transfusion (IHBT, Prague, Czech Republic). An informed written consent was obtained from each individual enrolled. The study was approved by the Institutional Review Board of IHBT (ev. nb. 13/06/2012). The assay was performed as previously described.[54] Briefly, the isolation of PBMCs was performed using Ficoll density gradient centrifugation (Ficoll Paque Plus, GE Healthcare) in SepMate tubes (SepMate PBMC Isolation Tubes, Stemcell Technologies). Next, isolated PBMCs were seeded into a 96-well U-shape plate in RPMI 1640 media containing 10% (v/v) heat inactivated fetal bovine serum (Capricorn Scientific, Germany). Serially diluted CDNs were added, and the plates were incubated for a predetermined 16 h (37 °C, 5% CO_2). Finally, secreted IFNα, IFNγ, and TNFα were determined in the collected medium using ProcartaPlex Assays using a MAGPIX System (Merck, Germany) according to manufacturer's instruction. The cytotoxic effect of CDNs on PBMCs was determined using

CellTiter-Glo Luminescent Cell Viability Assay (Promega Corporation, USA) according to manufacturer's instruction.

Protein Expression and Purification. The expression and purification of mouse cGAS were performed as previously described[20] with minor changes. The recombinant plasmid was transformed in *Escherichia coli* BL21 (DE3) cells (Thermo Fisher, USA). The bacteria were grown at 37 °C until OD_{600nm} of 0.6−0.8 units and cooled down to 20 °C, and then, cGAS expression was induced with 0.4 mM IPTG for 16 h in an orbital shaker. After cell collection, the pellet was resuspended in ice-cold lysis/wash buffer (50 mM Tris−HCl [pH 8], 300 mM NaCl, 20 mM imidazole, 10% (w/v) glycerol, 3 mM *β*-mercaptoethanol). The cells were lysed by sonication, and cell debris was removed by centrifugation. The supernatant was incubated with equilibrated Ni-NTA beads (Macherey-Nagel, Germany). The beads were loaded into a gravity flow column and washed with lysis/wash buffer. Next, the resin was washed with lysis/wash buffer supplemented with NaCl to 1 M concentration, then washed with lysis/wash buffer. The mcGAS was eluted with lysis/wash buffer supplemented with imidazole to a 300 mM concentration. The collected protein was further purified on a size-exclusion chromatography column HiLoad 26/60 Superdex 200 pg in buffer containing 50 mM Tris−HCl [pH 7.4] and 150 mM NaCl. The eluate was concentrated to about 5 mg/mL and stored in 20% glycerol at −80 °C.

Expression and purification of DncV and DisA proteins were performed as described previously.[33] Briefly, proteins were overexpressed in *E. coli* BL21 (DE3) cells (ThermoFisher, USA). Purification steps consisted of cell disintegration, Ni-NTA chromatography, and SEC chromatography using HiLoad 26/60 Superdex 200 pg. The DncV and DisA proteins were concentrated using an Amicon Ultra-15 10K device (Merck Millipore Ltd), and enzymes were flash-frozen in liquid nitrogen.

The expression and purification of wt hSTING were performed as described previously.[47] Briefly, the expression, lysis, and purification using Ni-NTA resin were done following mcGAS protocol. Eluate from the Ni-NTA resin was supplied with Ulp1 protease and incubated for 2 h at 4 °C. Digested wt hSTING protein was further purified by size exclusion with HiLoad 26/60 Superdex 200 pg (GE Healthcare, USA) equilibrated in buffer containing 50 mM Tris−HCl [pH 7.4] and 150 mM NaCl. Finally, the purified protein was concentrated to about 5 mg/mL and stored in 20% glycerol at −80 °C, for usage in DSF. For the crystallography experiments, wt hSTING was further purified on HiTrap Capto S (GE Healthcare, USA), equilibrated in 50 mM Tris−HCl [pH 8] buffer, and eluted with a gradient of 50−1000 mM NaCl in the same buffer. Fractions corresponding to wt hSTING were concentrated to about 20 mg/mL and stored at −80 °C without glycerol supplement.

Crystallization and Structure Determination. Crystallizations were done using our previously optimized protocol.[52] wt hSTING-**5f** was co-crystalized at 18 °C using sitting drop vapor diffusion in a condition composed of 200 mM ammonium nitrate, 20% (w/v) PEG 3350, with additive 10 mM EDTA. Crystals were cryoprotected in mother liquor supplemented with 20% (v/v) glycerol. A complete diffraction data set was collected for a single crystal to 2.69 Å. wt hSTING-**5k** was co-crystalized at 18 °C using sitting drop vapor diffusion in a condition composed of 100 mM tri-sodium citrate [pH 5.0] and 20% (w/v) PEG 6000, with additives of 10 mM EDTA and 100 mM tri-sodium citrate. Crystals were cryoprotected in mother liquor supplemented with 20% (v/v) ethylene glycol. A complete diffraction data set was collected for a single crystal to 2.32 Å. wt hSTING-**5l** was co-crystalized at 18 °C using sitting drop vapor diffusion in a condition composed of 100 mM citric acid, 20% (w/v) PEG 6000, with additive 10 mM EDTA. Crystals were cryoprotected in mother liquor supplemented with 20% (v/v) ethylene glycol. A complete diffraction data set was collected for a single crystal to 2.16 Å. wt hSTING-**5m** was co-crystalized at 18 °C using sitting drop vapor diffusion in a condition composed of 100 mM PIPES [pH 7.0], 100 mM magnesium formate dihydrate, 100 mM rubidium chloride, 25% (w/v) PEG smear high, with additives 10 mM EDTA and 100 mM tri-sodium citrate. Crystals were cryoprotected in mother liquor supplemented with 20% (v/v) ethylene glycol. A complete diffraction data set was collected for a single crystal to 1.89 Å. Measurements for hSTING-**5f** and hSTING-**5k** were carried out at an in-house diffractometer (Rigaku, Japan) at 100 K. Measurements for hSTING-**5l** and hSTING-**5m** were carried out at MX14.1 operated by the Helmholtz-Zentrum Berlin (HZB) at the BESSY II electron storage ring (Berlin-Adlershof, Germany) at 100 K.[56] Data were reduced and processed by *XDS*.[57] The structures were solved by the molecular replacement method with *MOLREP*[58] using the structure of human STING (PDB entry 4KSY) as the search model. Model refinement was done using *REFMAC* from *CCP4 suite*,[59] *PHENIX*,[60] and *Coot*.[61] Parameters for structures and their geometry are summarized in Table S1.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c01305.

Synthesis of compound **3e**, crystal data and diffraction data collection and refinement statistics, UPLC purity of CDNs including UPLC traces, top signals and CC_{50}s for compounds tested on PBMCs, 2*Fo*−*Fc* map of loops over ligand-binding sites, and interaction scheme of 2′3′-*c*GAMP in STING ligand-binding sites (PDF)

Molecular formula strings (CSV)

Accession Codes

PDB codes for human STING with bound **5f** (8A2H), **5k** (8A2J), **5l** (8A2I), and **5m** (8A2K). Authors will release the atomic coordinates upon article publication.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CDN, cyclic dinucleotide; cGAS, cyclic GMP−AMP synthase; DAMP, damage-associated molecular pattern; DisA, diadenylate cyclase from *Bacillus thuringiensis*; DMOCP, 2-chloro-5,5-dimethyl-1,3,2-dioxaphosphorinane 2-oxide; DMTr, 4,4′-dimethoxytrityl; DncV, dinucleotide cyclase from *Vibrio cholerae*; DSF, differential scanning fluorimetry; ETT, ethylthio-1*H*-tetrazole; FBS, fetal bovine albumin; IRF, interferon regulatory factor; *J*, coupling constant in Hz; mcGAS, mouse variant of cyclic GMP−AMP synthase; NF-κB, nuclear factor κ-light-chain enhancer of activated B-cells; NMR, nuclear magnetic resonance; NTP, nucleoside triphosphate; PAMP, pathogen-associated molecular pattern; rt, room temperature; SAR, structure−activity relationship; STING, stimulator of interferon genes; TBAF, tetrabutylammonium fluoride; TFA, trifluoroacetic acid; TLC, thin layer chromatography; TPPTS, triphenylphosphan-3,3′,3″-trisulfonate; UV, ultraviolet; wt hSTING, wild type variant of human stimulator of interferon genes

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